

# Mechanistical Approach Through Discovery of New Generations of Anti Inflammatory Drugs

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## Abstract

Non-Steroid-Anti-Inflammatory-Drugs (NSAIDs) are the main focal points of linkage between inflammation and cancer. These compounds affect the catalytic conversion of *Arachidonic Acid* to the main molecules of prostaglandin family. These exchanges are catalyzed by *CycloOxygenase* isozymes called *COX I & II* which are constitutive proteins in important organs like liver or kidney and inducible one in inflamed tissues or tumors, respectively. This study discusses on a new mechanistic approach through discovery of 4th generation of *NSAIDs*. Here utilizing combinations of online databases including *DRUG BANK* and *PUBCHEM* in conjunction with computational algorithms like *Fast Flexible Docking* as in *FLEXX* software followed by applying new filtering processes including extraction of structural descriptors by means of *PASS* software. Also, correlation between docking scores and bioactivities were analyzed by *SPSS* software. However, important molecular substructures were analyzed with *Library MCS* software for detailed constructional engineering of the compounds. All molecules and substructures were searched for their potential anti inflammatory activity in databases and searching engines such as *ISI*, *GOOGLE* and etc. Consequently, new drug candidates were appeared in novel resources in order to synthesize, buy or extract for future in vitro assays.

**Keywords:** Non-Steroid-Anti-Inflammatory-Drugs; *PASS* software; *CycloOxygenase* isozyme; *FLEXX* software

## Introduction

Drugs are of the most important concerns of human societies. Every year new drug generations are needed in order to cope with new diseases and drug resistances. Among all diseases, cancer and inflammation are the most time consuming issues in world of science and health. However, inflammation is a severe or undesired human body immune system response to foreign unfamiliar particles [1]. These two are potentially different in mechanisms but similar in some aspects. The main proteins which play a central role in both processes are *CycloOxygenase* (PGH2 Synthase) isozymes that mediate conversion of *Arachidonic acid* to prostaglandin compounds. *Arachidonic acid* is oxidized and peroxidized to *PGG2* and *PGH2* respectively [2]. The active site of this enzyme consists of a long channel coated with hydrophobic residues that ends with Tyr 385 and Ser 530 which could be affected by some of *NSAIDs* during inhibition [3]. *COX I* is a constitutive protein that is apparently expressed in all important human tissues such as brain, liver, kidney, stomach and etc and protects them from shocks and stresses. On the other hand, *COX II* only expresses in stressed tissues, inflamed organs and tumors to enhance blood circulations or improved immune responses [4]. Inhibition of *COX II* not only stops mentioned processes but also affects *COX I* expressed organs and harms them. Various ranges of compounds could affect inflammation states such as steroids or cytokines [5]. The main compounds used to suppress *COX II* catalytic activity are called Non Steroid Anti Inflammatory Drugs (*NSAIDs*). These molecules directly interact with active site of enzyme and inhibit them from enzymatic reactions [3]. There have been three generations of *NSAIDs* till now that could be used in treatments of inflammations. Aspirin, diclofenac and gelophen are belonged to first, second and third generations, respectively [6]. This study discusses about possible new generation of *NSAIDs* which more specifically tend to inhibit *COX II* rather than *COX I*. Here some mechanistic bioinformatics approaches are applied

in order to reach new mother and lead compounds that are practically accessible though chemical synthesis or natural extraction. We believe that this would be the nearest answer to problems of inflammations because every suitable and practical online softwares and databases are utilized along with best approved computerized algorithmic softwares.

## Materials and Methods

As any molecular bioinformatics project, a practical database is needed for future project steps. Our database is generated by addition of all FDA approved *NSAIDs* downloaded from *DRUG BANK* database to a library file. Then library file were docked by FlexX version 3.1.4, BioSolveIt, software, uses Fast Flexible Docking Algorithm, with *COX I* and *COX II* proteins. The crystallographic structures of these proteins were downloaded from *PROTEIN DATA BANK* (*PDB*) as 1U67 and 1PXX. Interaction energies were derived for both proteins and subtracted *COX II* from *COX I* in order to gain the highest energy differences. Then top molecules with highest energy differences were selected and searched for their similarities in *PUBCHEM* databases with 90% threshold. On the other hand, top structurally different molecules were sub-structurally searched in mentioned databases too. Then similar molecules were filtered by virtual screening mechanisms to decrease the likeliness between similar molecules and compounds with drug specifications. At the next phase these molecules were docked with both proteins yet again for better screening of affecting molecules

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in order to enhance COX II specificity. Similar filtered molecules were studied for their availability in natural resources in databases and references. At the second phase all NSAIDs were analyzed for their main comprising fundamental and organic functional groups along with second generation similar molecules by Library version 0.7, ChemAxon software series to recognize most affecting groups in ligand-protein interactions. IUPAC names were generated for these organic functional groups by ACD lab/ChemSketch version 11.01, Advanced Chemistry Development Group. More guaranteeing the activities of similar molecules PASS software, V.poroikov et al, version 1.917, was utilized to predict their anti inflammatory tendencies. Filtered molecules were literately searched for their and derivatives presence at natural resources in books, databases and websites such as Flore of Iranica, ILVIS and CHCD and ISI, GOOGLE and Science Direct, respectively. On the other hand the relationships of docking scores and predicted activities were studied for their rationality.

## Results

About 53 molecules were gathered from DRUG BANK databank and all combined in a library file in order to dock with proteins by FlexX software. Figure 1 demonstrates the workbench of FlexX with COX I active site which is designed as docking site for all ligand types.

Table 1 shows interaction energies of both proteins and their subtractions. The highest differences between IEs are highlighted in table. Top 10 molecules were selected to progress the project.

PubChem database were searched for similar compounds of these energetically top molecules with higher than 90% threshold. More than 1000 compounds were gathered as structurally similar molecules. About 500 molecules were reached from the same database by substructure similarity searches of fundamentally diverse top docked compounds. All structurally and sub-structurally similar compounds were analyzed for comprising functional fragments by Library MCS software. This analysis shows specific organic functional groups in molecules that structurally relate molecules and may have the same activity in any of all compounds. Important functional groups of some molecules are presented in Figure 2.

Benzene, 5-amino-2, 3-dihydro-1H-inden and etc are of the most important functional groups of similar molecules. Next phase

specifies the focal screening step of new anti inflammatory compounds. Bioactivity filtering was performed by applying PASS software for structure and activity relationship prediction of all concluding molecules. Compounds with higher than 60% anti inflammatory activity were selected for final literature searches and analyzes. Figure 3 shows the platform of PASS software with anti inflammatory analysis of a similar molecule.

Some top rated molecules of different sources are represented in Table 2 along with their docking scores and bioactivities. Cyclooxygenase Inhibitory and Anti Inflammatory bioactivities of all gathered compounds were analyzed for more precision in molecule selection. However, these predictions could be accomplished in Enhanced NCI database too [7]. In addition, drug likenesses were driven by PASS software in order to decrease cytotoxicity of elected compounds [8]. About 1000 molecules gathered and looked for their availability in natural resources such as plants or aquatic animals.

It is obvious that best predicted molecules have high docking scores compared with their source molecules. Graph 1(included as supplementary data), represents docking scores of selected molecules with COX II in detail.

Also functional groups were explored by their generated names for any possible anti inflammatory activity in any compound. These searches accomplished in ISI and Science Direct databases, GOOGLE searching engine and ILVIS and CHCD databases. It was interesting that the 5 out of 9 molecules have similar structures in databases. In addition, pharmacophores were studied with visual and analytical means of FlexX software and revealed that the active site is a completely hydrophobic cavity covered with some hydrophilic residues at its entrance in order to absorb the carboxyl group of Arachidonic acid. These functional groups are going to be docked with proteins in future projects for more detailed analysis and sketch of a novel new mother lead compound.

## Discussion

NSAIDs are the most highly used drugs among human nations, in order to reduce different kinds of symptoms of various diseases.

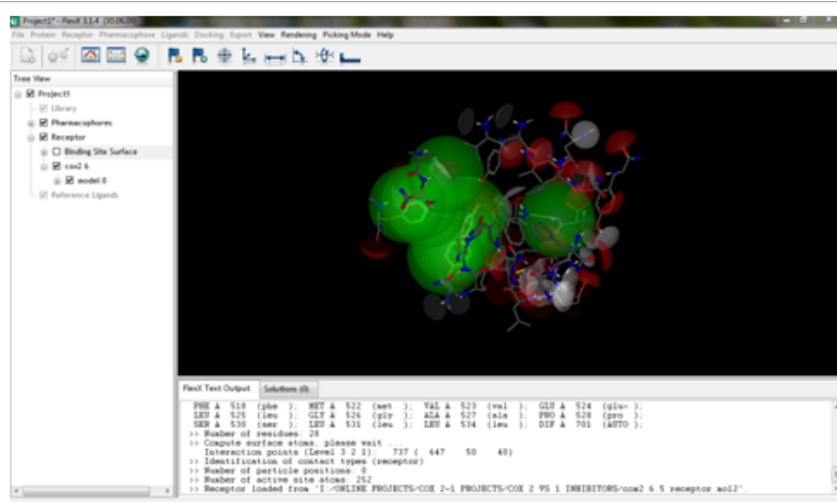


Figure 1: Working platform of FlexX software along with the binding site of a target protein (COX I).

| Molecule Name                      | Score of COXII | Score of COXI | IE COX II – IE COX I |
|------------------------------------|----------------|---------------|----------------------|
| (4044) mefenamic acid              | -25.9779       | -17.6315      | -8.3464*             |
| (181818) ketorolac                 | -24.6539       | -19.849       | -4.8049              |
| (6927361) niflumic acid            | -23.9812       | -16.3618      | -7.6194*             |
| (23663959) meclofenamic acid       | -22.5076       | -15.4295      | -7.0781*             |
| (10265888) indoprofen              | -21.625        | -16.5147      | -5.1103              |
| (23667642) diclofenac              | -21.5161       | -11.8022      | -9.7139*             |
| (11777117) ketoprofen              | -21.4358       | -26.5477      | 5.1119               |
| (189556) indobufen                 | -21.2416       | -16.6798      | -4.5618              |
| (5468) tiaprofenic acid            | -20.3595       | -23.2333      | 2.8738               |
| (24188576) indomethacin            | -19.8167       | -11.5175      | -8.2992*             |
| (23677829) tolmetin                | -18.9846       | -19.6516      | 0.667                |
| (6918975) fenbufen                 | -18.0567       | -23.8689      | 5.8122               |
| (60726) bromfenac                  | -17.8499       | -17.7812      | -0.0687              |
| (2581) carprofen                   | -17.4266       | -17.9461      | 0.5195               |
| (44119558) fenoprofen              | -17.0201       | -21.3646      | 4.3445               |
| (23681059) naproxen                | -16.6501       | -21.8954      | 5.2453               |
| (119607) valdecoxib                | -16.4327       | -8.6269       | -7.8058*             |
| (10378029) suprofen                | -16.431        | -18.2949      | 1.8639               |
| (11610600) celecoxib               | -16.2502       | -9.9018       | -6.3484              |
| (23706216) flurbiprofen            | -15.8982       | -16.8741      | 0.9759               |
| (3434975) aspirin                  | -15.5944       | -13.5838      | -2.0106              |
| (71246) flusolid                   | -14.6285       | -14.436       | -0.1925              |
| (123619) etoricoxib                | -14.6197       | -2.2789       | -12.3408*            |
| (31508) proquazone                 | -14.5438       | -6.0009       | -8.5429*             |
| (23711828) tenidap                 | -14.4817       | -12.274       | -2.2077              |
| (4614) oxaprozin                   | -14.3879       | -21.0796      | 6.6917               |
| (4495) nimesulide                  | -13.6915       | -11.7593      | -1.9322              |
| (11681277) rofecoxib               | -12.7897       | -10.3964      | -2.3933              |
| (7090) phenidone                   | -12.5521       | -10.6521      | -1.9                 |
| (4306515) SC 560                   | -12.4513       | -8.8809       | -3.5704              |
| (688461) etodolac                  | -12.4089       | -9.1605       | -3.2484              |
| (522325) dipyron                   | -11.9369       | -6.2648       | -5.6721              |
| (151166) lumiracoxib               | -11.5956       | -13.365       | 1.7694               |
| (4409) nabumetone                  | -11.5354       | -13.365       | 1.8296               |
| (22723678) piroxicam               | -10.7097       | -6.2079       | -4.5018              |
| (5388987) meloxicam                | -10.5976       | -3.0729       | -7.5247*             |
| (23662458) ibuprofen               | -10.2982       | -10.5233      | 0.2251               |
| (9799453) L745337                  | -9.7468        | -3.4825       | -6.2643              |
| (23715649) parecoxib               | -9.3627        | -1.457        | -7.9057*             |
| (40466911) sulindoc                | -9.3017        | -7.8325       | -1.4692              |
| (4237) mofezolac                   | -8.9338        | -7.54         | -1.3938              |
| (4781) phenyl butazon              | -8.8359        | -4.7399       | -4.096               |
| (688036) Nordihydroguaiaretic Acid | -8.1563        | -11.8401      | 3.6838               |
| (4641) oxyphenbutaz                | -7.4646        | -1.518        | -5.9466              |
| (6931410) salicin                  | -7.3571        | -5.758        | -1.5991              |
| (10523508) sulindoc sulfone        | -4.5268        | -1.4543       | -3.0725              |
| (5312154) tenoxicam                | -2.6391        | -3.4647       | 0.8256               |
| (30869) ethonium                   | 16.2745        | 20.2632       | -3.9887              |

Table 1: Interaction energies of Docking and their subtraction.

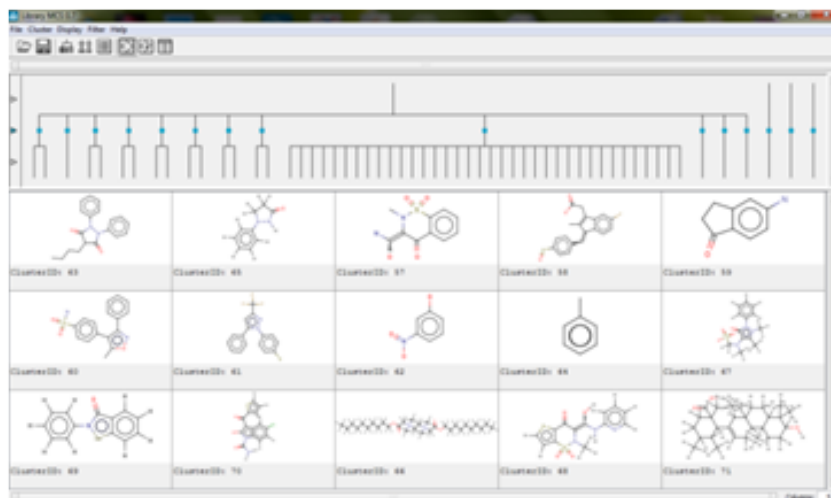


Figure 2: 2 Working platform of Library MCS software along with some major functional groups of similar molecules.

| Molecule Name | Source Molecule   | Pass Activity (NSAID agent) | Pass Inactivity (NSAID agent) | Pass Activity (CycloOxygenase inhibitor) | Pass Inactivity (CycloOxygenase inhibitor) | Drug likeness |
|---------------|-------------------|-----------------------------|-------------------------------|--|--|---------------|
| 23353722      | Diclofenac        | 0.735                       | 0.007                         |  |  | 0.076         |
| 443378        | Etoricoxib        | 0.605                       | 0.01                          |  |  | 0.087         |
| 19849808      | Indomethacin      | 0.884                       | 0.006                         |  |  | 0.696         |
| 231510        | Meclofenamic Acid | 0.604                       | 0.010                         | 0.442                                    | 0.014                                      | 0.040         |
| 12166238      | Mefenamic Acid    | 0.742                       | 0.007                         | 0.467                                    | 0.012                                      | 0.147         |
| 1432576       | Niflumic Acid     | 0.703                       | 0.007                         | 0.472                                    | 0.012                                      | 0.366         |
| 22961167      | Parecoxib         | 0.799                       | 0.006                         | 0.772                                    | 0.005                                      | 0.035         |
| 23340381      | Proquazone        | 0.786                       | 0.006                         | 0.453                                    | 0.013                                      | 0.494         |
| 22086816      | Valdecoxib        | 0.856                       | 0.006                         | 0.771                                    | 0.005                                      | 0.048         |

Table 2: Pass predictions of top rated molecules of different sources and their docking scores.

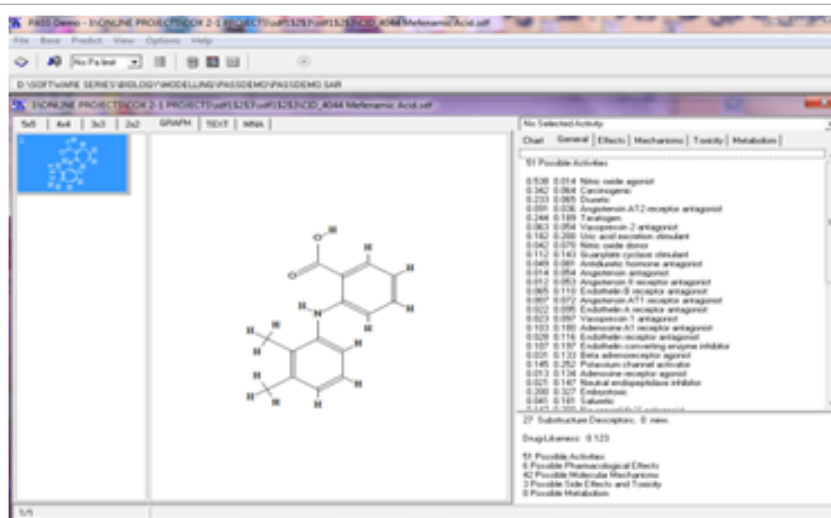


Figure 3: Platform and analysis of a molecule for its anti inflammatory activity with PASS Prediction.

Over dosage of these drugs or long time consumption may cause side effects such as ulcer and different gastrointestinal problems. Any drug used for reduction of inflammations has at least little side

effects in accordance with their partial tendency to COX I. The more it tends COX I than COX II, the higher side effects will be. Selection of molecules according to their docking scores is a semi-empirical

| Correlations |                     |        |          |
|--------------|---------------------|--------|----------|
|              |                     | Score  | Activity |
| Score        | Pearson Correlation | 1      | .650**   |
|              | Sig. (2-tailed)     |        | .000     |
|              | N                   | 159    | 159      |
| Activity     | Pearson Correlation | .650** | 1        |
|              | Sig. (2-tailed)     | .000   |          |
|              | N                   | 159    | 159      |

\*\* . Correlation is significant at the 0.01 level (2-tailed).

**Table 3:** Correlation between Scores and Activities of similar compounds.

idea because drugs are somehow specialized for different tissues. For instance gelofen has more effect on muscle pains than headaches. So the selection of molecules just has been done based on their best interaction energies with proteins. This was the only way for evaluation of better structures in order to draw a new lead compound. As first, 10 major compounds were selected for similarity searches but just 9 molecules were accomplished the procedure [9]. This happened for inability of FlexX software to dock the meloxicam similar compounds for unknown reasons. Using FlexX software ensures the user about the docking scores for they are the based on analytical calculation of bonds and interactions such hydrogenic, hydrophobic and ionic bonds or aromatic and lypophallic interactions. First equation shows the main concept of the energy calculations.

$$\Delta G = \Delta G_0 + \Delta g_{rot} \times N_{rot} \quad (1)$$

$$+ \Delta G_{hb} \sum f(\Delta R, \Delta \alpha) \quad \text{Neutral H-bonds} \quad (2)$$

$$+ \Delta G_{io} \sum f(\Delta R, \Delta \alpha) \quad \text{Ionic bonds.} \quad (3)$$

$$+ \Delta G_{aro} \sum f(\Delta R, \Delta \alpha) \quad \text{Aro. Int.} \quad (4)$$

$$+ \Delta G_{lipo} \sum f(\Delta R) \quad \text{Lipo. cont.} \quad (5)$$

2<sup>nd</sup> & 3<sup>rd</sup> equations refer to molecular bonds while 4<sup>th</sup> & 5<sup>th</sup> show the impacts of interactions on total  $\Delta G$  [10].

To understand the impact of docking scores on the direction of mother compound discovery procedure, correlation between predicted activities and docking scores were analyzed based on Pearson Correlation method by SPSS, version 16.0.0, IBM, and results are presented below in Table 3.

The total number of properly docked similar molecules that have higher than 0.6 anti-inflammatory activities were 174 with positive correlation coefficient of about 0.50. However, after negligence of similar molecules of Proquazone, the correlation increased to 0.65. This omission seems to be logic because the docking scores of Proquazone similar compounds were too low like their source molecule and this significant difference makes it possible not to interfere these compounds in calculations. Also, analysis of data revealed that the ratio of interactions to activities seem to be a constant value. Following graphs represent this idea that the new lead compound needs to have the same score to activity ratio for proper *In-vivo* activity.

After all, the best predicted molecules of table 2 seem to be good enough in order to found the creation of new lead compounds with higher affinity to COX II. Apparently, for their low drug likenesses, the screening procedures suppose to be reached new unknown compounds with the same activity of mother molecules but different structure.

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