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Original Article

New Molecular Screening Approach for Discovery of Novel *CycloOxygenase-2* Inhibitors

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Abstract

Introduction: Inflammation is basically caused through the conversion of Arachidonic acid into Prostaglandin H_2 by CycloOxygenase. In this study, a new algorithmic procedure is applied in order to screen molecules, not only with high affinity to *COX-2*, but also different from their ancestor compounds.

Materials and Methods: *NSAIDs*, *COX-1* and *COX-2* molecules were acquired from Drug Bank and Protein Data Bank. Drugs were docked with both proteins, using *FlexX* software. Top 10 molecules with lowest *COX-2* interaction energies and highest differences between *COX-2* and *COX-1* IEs were selected for structural similarity searches in *PUBCHEM* and ENCANCED NCI databases. Second generation molecules were docked with proteins once again. Compounds with lower *IEs* than parents were collected. Bioactivities and bio-availabilities of compounds were analyzed through *PASS* software and Lipinski rules. A best multi linear regression model was developed based on some physicochemical descriptors for further studies.

Results: Fifty NSAIDs were selected and 2000 similar molecules were gathered. Screening the molecules based on Lipinski rules, bioactivities and drug likenesses, a trustable BMLR model was developed with more than 80% accuracy including following descriptors: Log P, Log D, Molar Refractivity, Polarity Number, and Aromaticity Ratio. Finally, 6 compounds were selected as best structurally new compounds for further *in vitro* analysis.

Conclusion: The final molecules, being highly drug-like with affinity and structurally different from their ancestors, can be used in order to develop new lead compounds with higher selectivity.

Keywords: CycloOxygenase, Anti Inflammatory Drugs, FlexX software, PASS, MLR Model

1. Introduction

Nowadays, utilization of new borne drugs called Non Steroidal Anti-Inflammatory Drugs (*NSAIDs*) has been increased drastically. These molecules not only may inhibit activity of *CycloOxygenase-2* in prostaglandin synthesis pathway for pain signaling and inflammation initiations, but also reduce action of *Cox-1* which is a constitutive protein expression in brain, kidney and other critical body organs [1]. Prostaglandins are classified as the main mediators of pain and inflammations which are driven from Arachidonic acid by means of a cycling reaction catalyzed by *CycloOxygenase* isozymes [2]. Figure 1 presents the prostaglandin biosynthesis pathway [3].

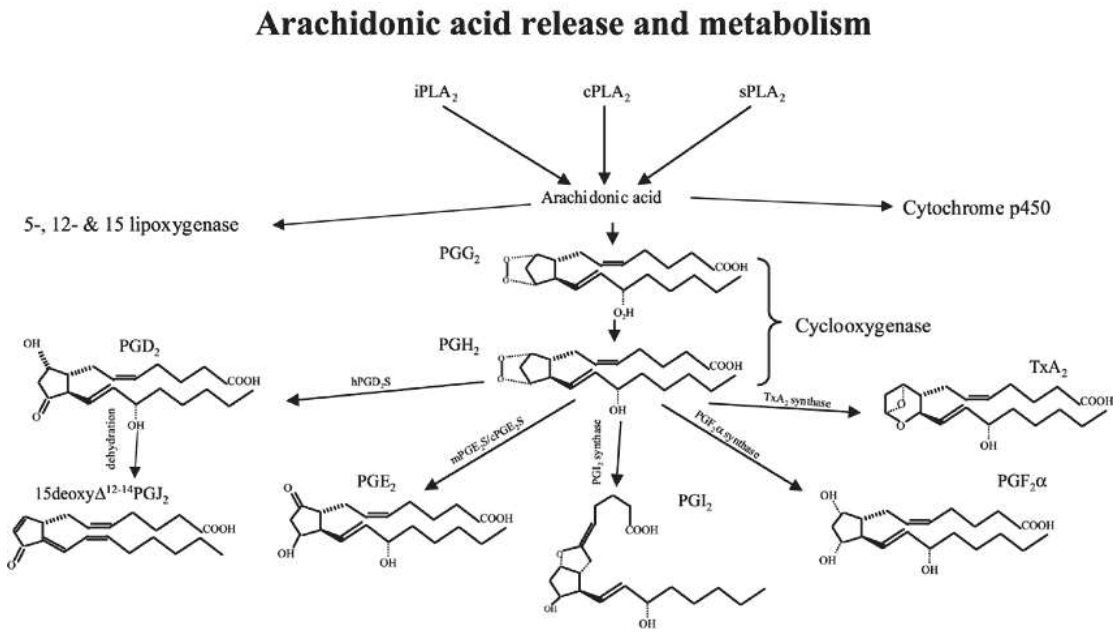


Figure 1. Scheme of prostaglandins biosynthesis pathway

The inhibition of *COX-2* may cause undesired reduction in activity of *COX-1,* which may cause serious injuries to the mentioned organs. Progression of new drug discovery strategies is followed by development of novel drugs that has fewer side effects on human body [4]. Figure 2 specifies the organs in which different isozymes of *COX* are expressed.

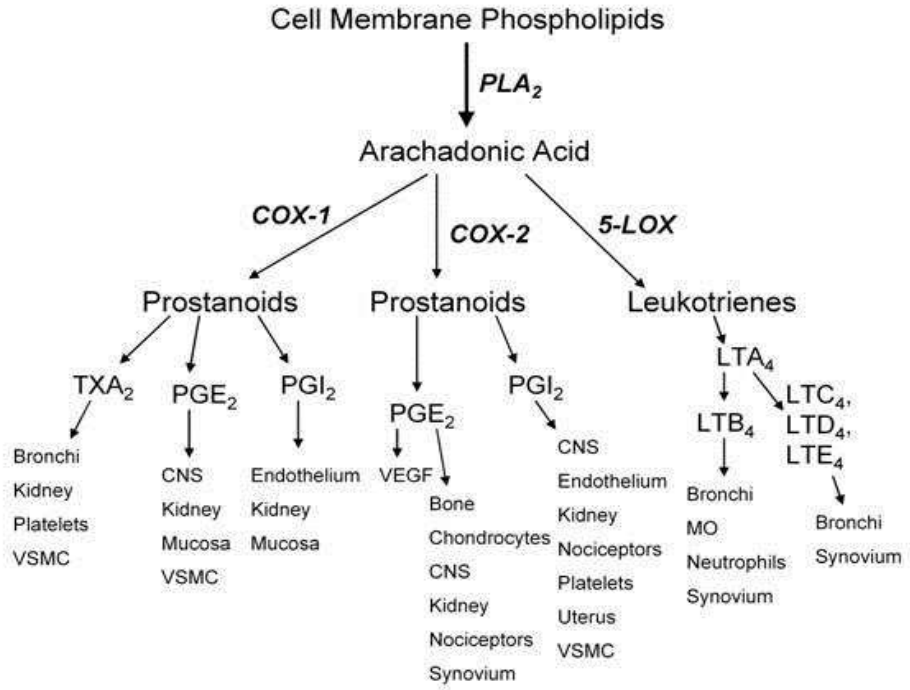


Figure 2. COX-1 & 2 expression organs and their importance

Thus far, there are three generations of *NSAIDs* including *Aspirin*, *Diclofenac* and *Celecoxib* representing first, second, and third generations, respectively. Discovery

and design of new generations of such drugs are needed because new inflammation states are generated for engendered alterations in life styles [5].This study presents an algorithmic approach through screening of new compounds for future synthesis and in-*vitro* tests. Here, different filtering steps are applied such as estimation of interaction energies of molecules with *fast flexible docking algorithms* [6], online similarity searches for structure and function relationship analysis, various bioactivity predictions of similar molecules and eventually field studies. Finally, this is presumed that the driven molecules are the key structures for design of new generations of anti inflammatory drugs.

2. Materials and Methods

In this study a new screening strategy has been used in order to find similar compounds in activity and target, to *Food-and-Drug-Administration*-approved *NSAIDs*. Molecular structures of all approved NSAIDs were downloaded from Drug Bank database (*www.drugbank.com*), redrawn in *MarvinSkech, ChemAxon, version 5.0.4* and optimized in the *Hyperchem, Hypercube, version 8.0.6* program by *AM1* algorithm along with addition of hydrogen atoms in order to normalize valances [7]. *Sdf Library* files were generated with *Marvin* and modified for structural defects. Crystallized structures of *COX-1* and *Cox-2* proteins were obtained from *Protein Data Bank* database (*http://www.pdb.org/*). All *NSAIDs* were docked with both structures by *FlexX, Biosolveit, version 3.1.4*. The derived Interaction Energies (*IE*) from *Cox-1* and *Cox-2* were compared within all molecules and top ones with the highest gaps between Cox-1 and 2 were collected for web-based molecular similarity search (*http://pubchem.ncbi.nlm.nih.gov*). The new similar compounds were docked with both proteins for more accurate selection. Similar molecules with *anti-inflammatory, COX and COX-2 inhibition* bioactivities of less than 0.7, which has been predicted by *PASS Prediction, Proikov et. al*, version 1.0.9 software, were ignored [8]. In addition, molecules were screened for no more than one violation of *Lipinski rules* [9]. All molecules were docked in a reversed manner with an online Reverse Molecular Docking Server *TarFisDock* (*http://www.dddc.ac.cn/tarfisdock/*) for elimination of false positive results which could possibly interact with other proteins and enzymes. Whole screening flowchart is presented in figure 3 comprehensively.

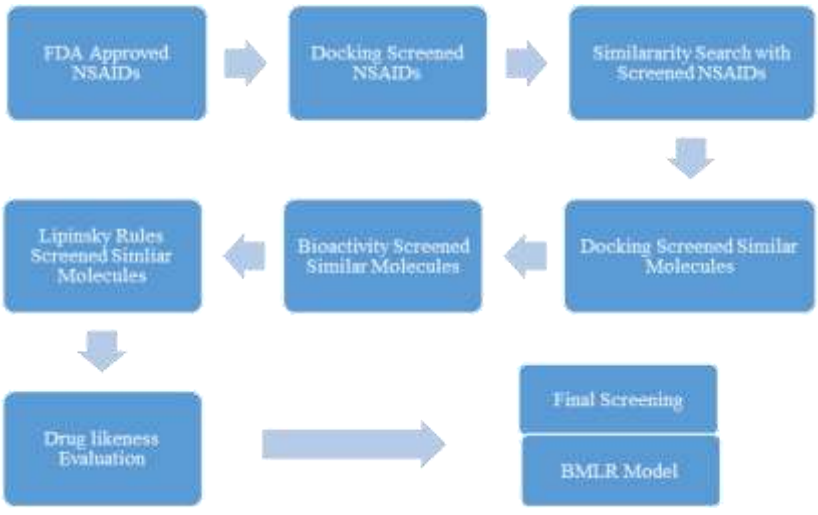


Figure 3. Whole flowchart of screening process

The remaining molecules were classified into a *CAS-numbered* group for probable future purchase and synthesis. Although, special well studied molecular and physicochemical descriptors of final screened molecules including: *MW, H Acceptor/Donor, Log P, Log D (pH=6.5), Polar Surface Area, Molar Refractivity, Aromaticity Index, Polarity Number, Aromatic Ratio, Global Topological Charge, Elemental Analysis, and etc.* were extracted with *Dragon, Talete, version 5.4* and *MarvinViewer, ChemAxon, version 5.0.4* for a simple handy theoretical model preparation based on *Multiple Linear Regression* Method. The reliability of these descriptors is evaluated with correlation and best multi linear regression tests by *SPSS version 16* and *Matlab, MathWorks, version 8.0* [10].

3. Results

About 50 different *NSAIDs* were gathered from *DRUG BANK* database which were *FDA* approved and widely used in across the world. All molecules were docked with *PDB* driven protein structures of *COX-1* and *COX-2*, which are respectively represented as *1U67* and *1PXX* at *PDB* database, out of which 10 were selected for structural and sub-structural similarity searches because they have the highest differences between *COX-2* and *COX-1* IEs. These molecules are listed in Table 1.

Molecule Name	Docking Score of COXII	Docking Score of COXI	IE COX II – IE COX I
(4044) mefenamic acid	-25.9779	-17.6315	-8.3464
(6927361) niflumic acid	-23.9812	-16.3618	-7.6194
(23663959) meclofenamic acid	-22.5076	-15.4295	-7.0781
(23667642) diclofenac	-21.5161	-11.8022	-9.7139
(24188576) indomethacin	-19.8167	-11.5175	-8.2992
(119607) valdecoxib	-16.4327	-8.6269	-7.8058
(123619) etoricoxib	-14.6197	-2.2789	-12.3408
(31508) proquazone	-14.5438	-6.0009	-8.5429
(5388987) meloxicam	-10.5976	-3.0729	-7.5247
(23715649) parecoxib	-9.3627	-1.457	-7.9057

Table 1. Interaction energies of Best Docked NSAIDs and their subtractions

The mentioned compounds were used for similarity searches at *Enhanced NCI* and *PubChem* molecular databases. More than 2000 molecules were achieved through similarity searches. At the next step of selection procedure, similar molecules were docked with target proteins and screened according to *COX-2* IE thresholds of their parents. This means that for example a similar molecule to *Diclofenac* is going to be chosen for further processing if its docking energy with *COX-2* would be lower than -21.5161 *kJol/Mol* which is IE of Diclofenac. Two hundred molecules remained to precede the screening steps. All monitored second generation molecules were inspected for their possible favorite bioactivities such as *COX* and *COX-2 Inhibitory, Anti-Inflammatory* and *Prostaglandin-E2 9 Reductase Inhibitory*, etc via *PASS* software. The filtering thresholds for different bioactivities were 0.7 (70% possible bioactivity). In other words, molecules with bioactivities less than 0.7 were prohibited from further analysis. Figure 4 shows the platform of *PASS Prediction* software with a sample molecule inside.

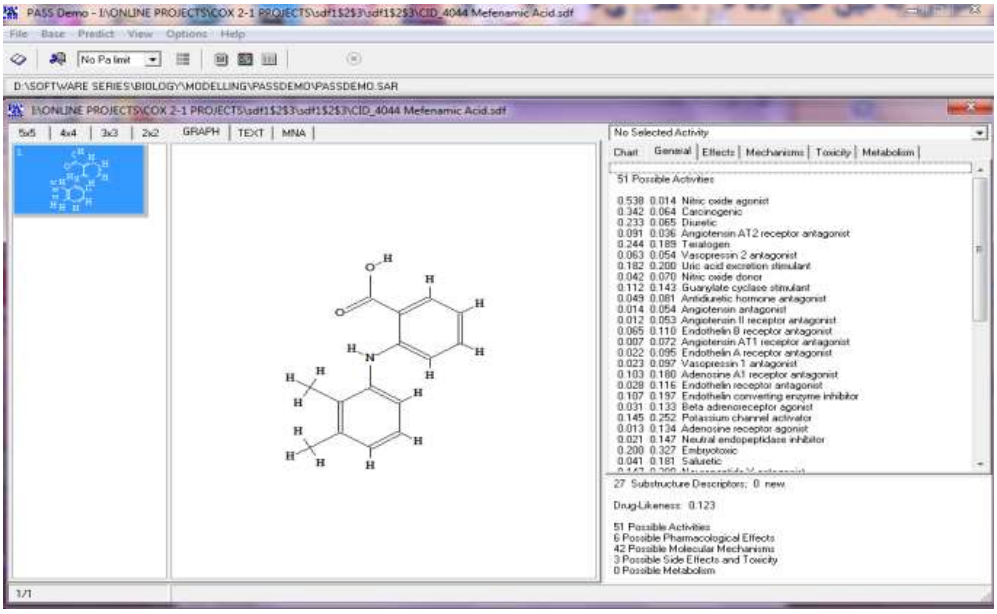
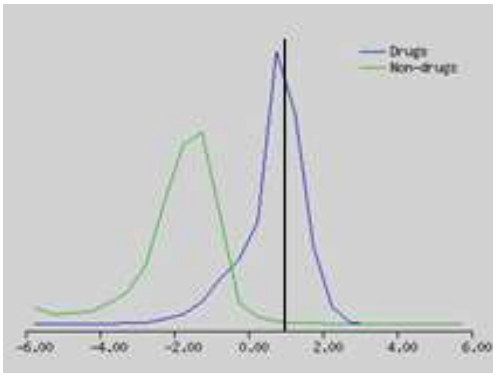


Figure 4. Pass prediction platform for a sample molecule

More than 120 molecules were filtered according to thresholds and the remaining compounds were studied for drug-likelihood online and offline within *MOLSOFT* server (*http://www.molsoft.com/mprop*) and *PASS* software (*http://www.pharmaexpert.ru/passonline*), respectively. An online-analyzed sample molecule with normalized results is presented in Figure 5 along with its molecular structure.



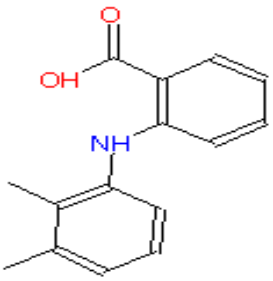


Figure 5. (A) Normalized results of Molsoft drug-likeness prediction server, (B) Sample molecule structure. The drug-likelihood of this compound is about 0.97 and is shown with a black horizontal line.

At the next step, *Lipinski's Rules* related descriptors including *MW, LogP, H Donor/Acceptor, Molar Refractivity and Polar Surface Area* as well as other previously mentioned critical operational descriptors were extracted by *Dragon* software for proper monitoring of compatible molecules. Finally, 17 molecules remained which are completely new in research area of inflammation. Some well selected and scored molecular structures are shown in Figure 6. Filtering with Lipinski rules, PASS activity prediction followed by reverse docking algorithmic steps, just 6 molecules were left for future purchase or syntheses which are supposed to be the best new probable *Cox-2* selective inhibitors or lead compounds and are highlighted in a CAS-Numbered manner with extracted descriptors.

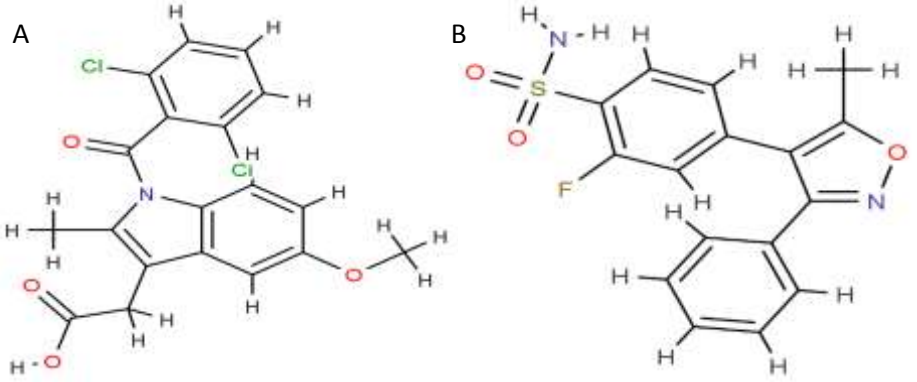


Figure 6. Some best selected and scored molecules (A) 15292266, (B) 9858791

In order to make a user-friendly and simple model for future molecular designs and predictions, some practical statistical softwares such as *SPSS* and *EasyNN-Plus* were used. Reciprocal coefficients are calculated and classified in Table 4. This data shows impacts of selected descriptors on predicted bioactivities of molecules. As this is visible, only *LogP*, *LogD*, *Aromaticity Ratio*, *Polarity Number*, and *Molar Refractivity* descriptors are properly related to bioactivities. Best Multi linear regression studies of 10 mentioned descriptors represent a model with higher than 84% precision which is supported with ANOVA tests and could be seen below at Tables 2 and 3. The descriptors could be classified into 3 main groups including *Hydropathical*, *Charge*, *and Electrochemical*. However, ignoring one or two of these descriptors in order to make the model easier would not decrease the R square of the models drastically. Some descriptors were eliminated from first model because of their low coefficients. It is evident that the *R Square* and *Adjusted R Square* do not fluctuate too much and 84% precision is high enough to support the accuracy of model. The final formula of built model is written below in Equation 1.

Anti-Inflammatory Bioactivity= -3.686 + 4.824 (***AR***) + .045 (***LP***) + .047 (***PN***) - .004 (***MR***) - .039 (***LD***)

Equation1. Final built model with best descriptors, AR= Aromaticity Ratio, LP= Log P, PN= Polarity Number, MR= Molar Refractivity, LD= Log D

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-3.686	1.180		-3.124	.010
Aromaticity Ratio	4.824	1.261	6.611	3.825	.003
Log P	.045	.030	.717	1.521	.157
Polarity Number	.047	.011	5.732	4.254	.001
Log D	-.039	.018	-1.530	-2.210	.049
Molar Refractivity	-.004	.002	-.723	-1.977	.074

Table 2. Coefficients of each one of selected descriptors on Anti Inflammatory activity, Dependent Variable: NSAID Activity

Correlation matrix of descriptors of final model in Table 5 presents no significant interaction between descriptors.

Correlation matrix:					
Variables	**Aromaticity Ratio**	**Log P**	**Polarity Number**	**Log D**	**Molar Refractivity**
Aromaticity Ratio	1.000	---	---	---	---
Log P	0.379	1.000	---	---	---
Polarity Number	-0.009	0.864	1.000	---	---
Log D	0.337	0.868	0.958	1.000	---
Molar Refractivity	0.535	0.519	0.305	0.212	1.000

Table 3. Demonstrates correlation matrix of predicted descriptors

Figure 7 and 8 are prediction and scoring plots which verify accuracy and monodispersity of data used in model preparation
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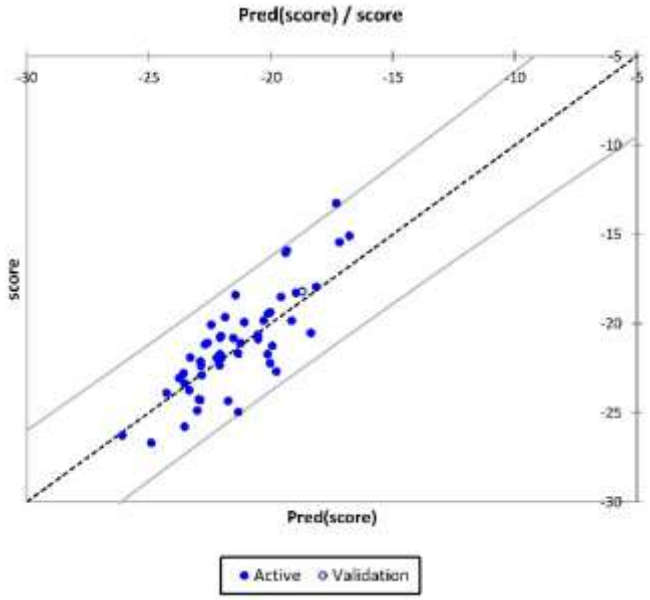

Figure 7. Score/Score prediction table shows monodispersity of data

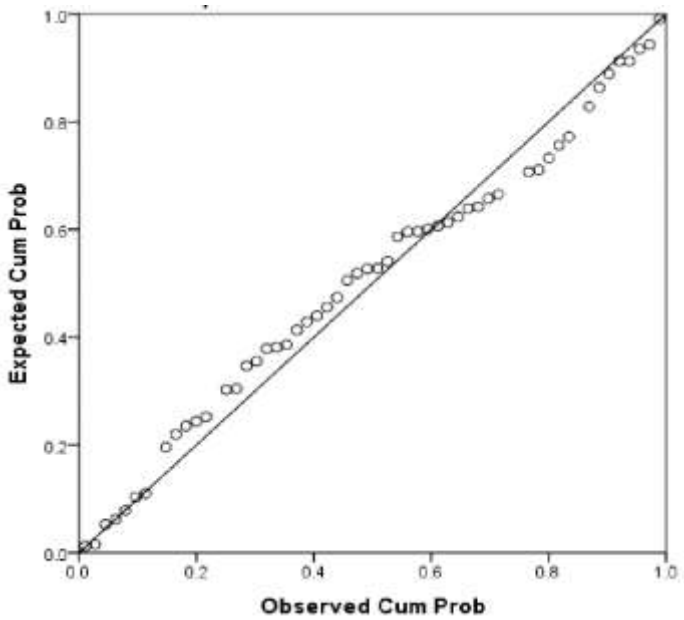

Figure 8. P-P plot demonstrates reliability of predictions

The correlation between Non-Steroidal Anti-Inflammatory bioactivity and Interaction Energies was higher than 80%. However, hydrogen bond donor and acceptor properties were also studied but as they were statistically meaningless, the data was omitted from analysis. This means that the interaction energy is important in filtering and design process but not as a molecular descriptor (at least in the present case).

A close scheme of what is done in screening process is visualized in table 4 and Figure 9

.

Molecule Name	I.E (Cox2)	NSAID Act*	COX Ac	COX2 Act*	D.L (PASs)+	D.L (Molsoft)+	H Acceptor+	H Donor+	LogP+	MW+	LogD (6.5)+	P.S.A+	P.R+	%Halogen+	%N+	%O+	%C+	%S+	A.R+	P.N+	G.T.C+	A.I+
9968255	-18.4474	0.942	0.894	0.603	0.091	-0.07	5	1	3.11	350.34	3.4	86.19	85.14	10.85	8	13.7	54.85	9.15	0.654	40	0.538	0.966
11674669	-18.0095	0.918	0.872	0.529	0.034	1.07	4	1	3.43	348.804	3.43	86.19	89.51	10.16	8.03	13.76	55.09	9.19	0.68	36	0.541	0.965
9858791	-18.5112	0.966	0.925	0.721	0.059	0.27	4	1	2.97	332.349	2.96	86.19	84.92	5.72	8.43	14.44	57.82	9.65	0.68	37	0.518	0.966
22643585	-17.6119	0.91	0.88	0.576	0.066	1.11	4	1	2.97	332.349	2.97	86.19	84.92	5.72	8.53	14.44	57.82	9.65	0.68	36	0.538	0.965
22746139	-18.8327	0.95	0.908	0.647	0.085	-0.31	7	1	3.39	386.321	3.33	86.19	85.57	19.67	7.25	12.42	49.74	8.3	0.607	44	0.608	0.965
22746389	-19.2116	0.934	0.875	0.56	0.074	0.13	5	1	3.62	364.366	3.56	86.19	90.16	10.43	7.69	13.17	56.04	8.8	0.63	42	0.582	0.965
22746454	-18.3898	0.942	0.899	0.625	0.089	-0.01	6	1	3.25	368.33	3.19	86.19	85.36	15.47	7.61	13.03	52.17	8.71	0.63	42	0.538	0.965
22747003	-16.635	0.925	0.862	0.521	0.078	0.08	5	1	3.62	364.366	3.56	86.19	90.18	10.43	7.69	13.17	56.04	8.8	0.63	42	0.576	0.965
412155	-24.2219	0.884	0.619	0.172	0.823	1.16	5	2	2.62	339.342	-0.07	87.86	91.99	0	4.13	23.57	67.25	0	0.593	43	0.477	0.98
127942	-22.1381	0.892	0.634	0.187	0.638	1.52	7	1	4.74	426.678	2.01	68.53	104.42	24.93	3.28	15	53.48	0	0.552	48	0.538	0.98
15292266	-22.6776	0.883	0.799	0.328	0.665	1.22	6	1	4.13	392.233	1.5	68.53	99.62	18.08	3.57	16.32	58.18	0	0.571	46	0.498	0.98
19849808	-24.1944	0.884	0.642	0.227	0.696	1.08	5	1	3.07	341.333	0.51	68.53	90.22	5.57	4.1	18.75	66.86	0	0.593	43	0.477	0.98
19849812	-23.1324	0.895	0.665	0.247	0.679	1.35	6	1	3.21	359.324	0.49	68.53	90.44	10.57	3.9	17.81	63.51	0	0.571	45	0.524	0.98
20342259	-22.344	0.877	0.616	0.189	0.579	1.17	5	1	3.53	357.788	0.96	68.53	94.81	9.91	17.89	3.91	63.78	0	0.593	43	0.477	0.98
19608748	-17.48	0.875	0.868	0.553	0.062	0.68	4	1	3.48	346.376	3.48	86.19	89.97	5.48	8.09	13.86	58.95	9.26	0.654	39	0.569	0.965
22086816	-21.5513	0.856	0.771	0.364	0.048	-0.65	5	1	4.32	314.359	4.32	70.51	85.71	0	8.91	10.2	61.13	12	0.708	32	0.344	0.965

Table4. Final best scored similar screened molecules along with Activities* and Descriptors+, Highlighted molecules are the best selected compounds for future lead compound discovery

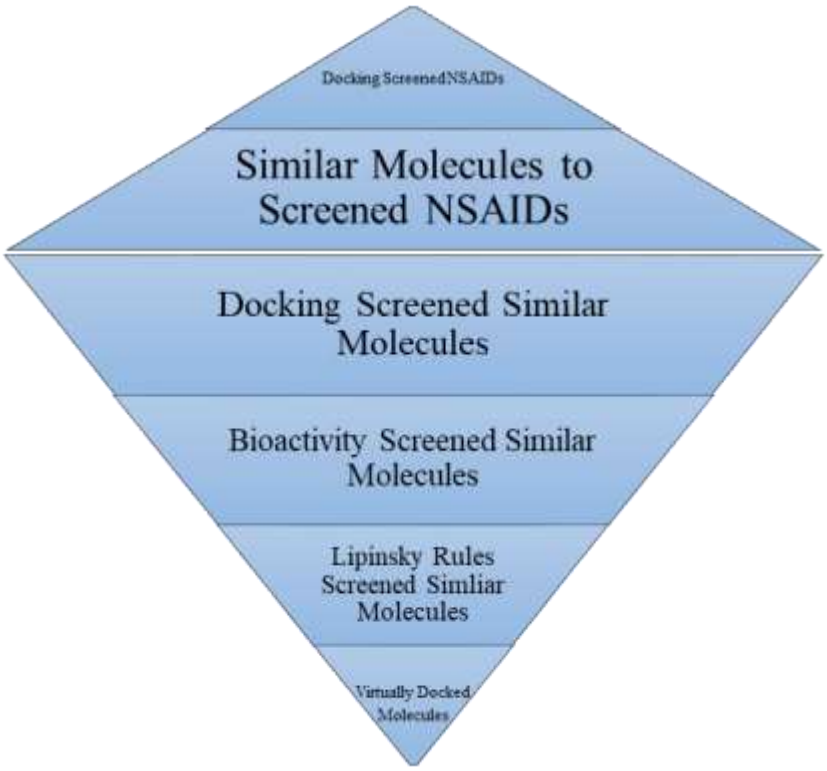

Figure 9. A scheme of molecular screening approach, Text size represents the numbers of molecules in each filtering, searching, Docking and analysis step.

4. Discussion

This article not only introduces new series of available and hypothetical molecules in order to synthesize or purchase for enzymology in vitro experiments, but also describes a new unique screening methodology which combines proper web-based resources with software ones.

This study statistically approves that H Donor and Acceptor properties of molecules do not extremely impact their bioactivity and drug-likelihood, because all good molecules have the same range of hydrogen bonds and such bonds are vital in solubility and Pr-Ligand interactions. Any drug must have a hydrophobic and a hydrophilic side in order to have better interactions with protein docking sites. Bipolarity in drugs is much more important than number of Hydrogen bonds. Quantitative Structure and Activity Relationships (QSAR) are practically mentioned and utilized in this study for proper prediction of bioactivities of molecules [11]. These obtained molecules which are ranked by screening procedures and other filtering approaches not only could selectively inhibit Cox II much more than Cox I, but also may have no interactions with other enzymes or body proteins.

5. Conclusions:

This built model is going to be used in future developments of selected lead compounds and creation of new generations of NSAIDs. This study underlines that some Lipinski elements in contrast with reports are not so important in drug discovery [12].

Conflict of interest

The authors declare no conflict of interest.

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