Quantitative Structure-Activity Relationships for Commercially Available Inhibitors of COX-2

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Abstract: Quantitative structure activity relationship (QSAR) studies of selective COX-2 inhibitors of commercial interest (drugs in market and on clinical trials) were performed. The COX-2 inhibitory activity (pIC_{50}=-logIC_{50}) of these twelve compounds was correlated with nineteen descriptors including steric, electronic and constitutional parameters. pIC_{50} activity showed high positive correlation with both volume and HOMO (Highest occupied molecular orbital). A Bi-parametric model was developed that included both these descriptors. The predictive capability (q^2= 0.66) of this equation was satisfactory. So it can be used to design newer templates or modify existing templates. Volume is an important parameter for the selective COX-2 inhibitory activity, because the secondary pocket in the active site of this enzyme is bigger than the active site of COX-1 enzyme (by 17%). HOMO is a measure of the nucleophilicity of the molecule and a molecule with high HOMO energy is ready to donate its electrons and thus is more reactive than molecule with low values. Binding studies were performed between the COX-2 enzyme and these molecules. The inhibitory activity increased with decrease in binding energy (or interaction energy) between the compounds with the COX-2 enzyme (with a correlation coefficient = -0.65). Calculated Log BBB (Blood Brain barrier), Log P (octonol water partition) and HBD (hydrogen bond donor) values were in the acceptable range (i.e., BBB = -1 to 0.3; LogP= 0 to 5; HBD < 5).

Key Words: COX-2, QSAR, HOMO, binding energy.

INTRODUCTION

Cyclooxygenase is an enzyme involved in part in the conversion of arachidonic acid to eicosanoids such as prostaglandin E_2 (PGE_2), and thromboxane B2 (TXB2). There are at least two COX isoforms in mammals. COX-1 is a constitutive isoform, and its inhibition is undesirable while, COX-2 is an inducible isoform, and its inhibition is responsible for therapeutic effect [1, 2]. Non-selective inhibition of isoforms of cyclooxygenase (COX) enzyme by the traditional NSAIDs causes gastric ulceration and renal insufficiency [3]. To overcome the adverse effects with classical NSAIDs, selective COX-2 inhibitors like Celecoxib and Rofecoxib as first generation and Valdecoxib and Etoricoxib, etc as second-generation inhibitors are introduced in the market and third generation inhibitors are about to be introduced in the market for the treatment of rheumatoid arthritis and osteoarthritis [4]. Apart from the beneficial gastrointestinal protection, COX-2 inhibitors have several therapeutic applications in the treatment of various cancers [5], neurodegenerative disorders like Parkinson's disease [6], and Alzhemier's disease [7], etc which further triggers the research in the field of COX-2 inhibition. Coxibs are a class of nonsteroidal anti-inflammatory drugs thought to have fewer side effects than traditional NSAIDs as well as specifically inhibit cyclooxygenase -2 enzyme. Research is still proceeding to improve the solubility of Coxibs which are hydrophobic because of their methyl sulfonyl moiety [8].

84 million people worldwide and 20 million in U.S alone have used Rofecoxib (Vioxx), a major COX-2 inhibitor and its nearest rival Valdecoxib (Bextra). Both have been recently withdrawn from the market due to their increased cardiovascular complications such as heart attacks, chest pain, stroke, sudden death and blood clots, necessitating the need for design of novel COX-2 inhibitors without these problems. It is important to emphasize that researchers have found correlation between the inhibition of cyclooxygenase and the redox properties of anti-inflammatory agents before the discovery of COX-2 enzyme. That study is based on sixty-three phenolic compounds which showed that the inhibition activity increased with electron donating substitutions [9]. The researchers also found relationship between the COX-2 enzyme inhibition and the redox properties of benzoates and salicylates. Hammett substituent constants, energy of the highest occupied molecular orbital (E_{HOMO}) and thermodynamic parameters are usually used for the expression of redox properties [10-14]. Both structure based (QSAR) and target based (docking studies) approaches are in progress to design new lead molecules. Recent papers that deal with QSAR and docking have also discussed the importance of designing newer COX-2 inhibitors [15-17].

Computational studies which include developing quantitative structure activity relationship and binding studies of commercial COX-2 inhibitors that are available in the market and those which are under clinical investigations are carried out in this paper. The two main objectives are to find a relationship between their selective inhibitory activity (1) with structural parameters and, (2) with the binding properties to the enzyme, in order to design new COX-2 inhibitor with optimal activity. The current attempt will be useful for drug designers to fill the void created by the withdrawal of Celecoxib and Rofecoxib. For this study twelve commercial COX-2 inhibitors reported in journals are considered, where

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ten of the compounds have diarylheterocycle and two of the compounds have 2-phenoxyaryl methanesulfonamide as scaffolds [18].

MATERIALS AND METHODS

The structure of the twelve molecules is built and their energy is minimized using Hyperchem® software (Hypercube Inc. USA, version 7). Initial geometry optimization is performed using MM+ force field [19], which comprises of terms corresponding to bond stretching, angle bending, torsion, and non-bonded interactions such as electrostatic and hydrogen bond. MM+ is generally a robust method suitable for small organic molecules, but some caution must be used when dealing with conjugated molecules, particularly with heteroaromatics. For the final geometry optimization PM3, semi-empirical quantum mechanics, is adopted [20]. Semi-empirical methods are simplified versions of Hartree-Fock theory using empirical relations and correlations from experimental data. To facilitate our attempt, IC_{50} (μM) values that are reported in the reference for COX-2 are converted to pIC_{50} (which is = -log IC_{50}). ClogP values are calculated from Chem Draw Ultra 7.0® (CambridgeSoft Corporation, USA) and, polar surface area (PSA) and hydrogen bond donor (HBD) from Dragon software® ((Milano Chemometerics, Italy). The logP values for all the twelve compounds lie between –5 to +5 which is in the acceptable range [21].

Regression analysis is performed using SYSTAT® software (SPSS Inc., USA) and cluster analysis which shows the relative distance between the compounds based on their pIC_{50} is carried out using Ky plot® (USA) software. Cluster analysis groups similar compounds into groups. Hence compounds in the same group may have similar properties and compounds in different groups may have different properties. The goodness of the linear model fit is evaluated using several statistical parameters such as, r = co-efficient of correlation, R^2 = squared co-efficient of correlation, R^2_{adj} = adjusted R^2, F-value = ratio of variance of the regression line to the variance of the error, and q^2 = predicted R^2. Same statistical software is used to determine these parameters. The twelve compounds chosen for QSAR studies are listed in Table **1**.

Leave-One-Out method is meant to test the predictive capability of the QSAR. The procedure consists of leaving one data point and developing the regression equation with the remaining points. The developed regression equation (which has left one data point out) is used to predict the activity of the point which is left out. This is repeated for all points and the sum of squares of the difference between the model value and the actual activity value is estimated. This predicted sum of squares is an indication of the predictive capability of the model.

Enzyme Ligand Binding

The 3-D X-ray crystallographic structure of COX-2 enzyme (1CX2) is obtained from the RCSB protein data bank. The enzyme has a diarylheterocycle ligand, namely SC-558 bound to it. Docking study is performed by superimposing commercial COX-2 inhibitors instead of the SC-558 in the active site. MM+ force field is used to minimize the energy of the combined enzyme-ligand system. The interaction energy of the enzyme-ligand is estimated as

MM+ Energy of the Combined System – (Energy of the Free Enzyme + Energy of the Ligand)

The active site of the enzyme (murine) comprises of sixteen amino acids namely, Leu^{359}, $Ileu^{345}$, Leu^{531}, Ser^{530}, Ala^{527}, Val^{349}, Tyr^{385}, Trp^{387}, Leu^{352}, Val^{523}, Gln^{192}, Arg^{513}, His^{90}, Arg^{120}, Tyr^{355}, Phe^{518}. The ligand, diarylheterocycle,

Table 1. COX-2 Inhibitors Considered for the QSAR Studies

S. No	Compound Name	pIC_{50}*	Predicted pIC_{50}(LOO+)	HOMO	Volume	Reference No.
1	Celecoxib	1.1549	1.4096	-9.5896	968.27	18(a)
2	Rofecoxib	0.301	0.5587	-9.6426	856.38	18(a)
3	Valdecoxib	0.7375	0.3603	-9.7542	871	18(b)
4	Nimesulide	-0.1139	0.0093	-9.7576	817.28	18(c)
5	DFP	0.5228	0.8484	-9.6507	905.2	18(d)
6	DUP697	1.2218	1.6638	-9.4123	935.45	18(e)
7	DFU	0.5228	0.7502	-9.805	953.09	18(f)
8	SC58125	1	0.7693	-9.7678	940.82	18(g)
9	SC57666	1.585	1.8225	-9.2302	885.67	18(h)
10	NS398	1	0.4262	-9.6692	846.48	18(h)
11	L768, 277	2	1.7958	-9.3458	928.91	18(i)
12	SC58451	2.9586	2.4759	-9.259	1000.1	18(j)

* pIC_{50}- COX-2 inhibitory values in μM

+ LOO- Leave-One-Out method.

will interact with the enzyme through the sulfonyl and methyl sulfonyl at the p-substitution of one of the benzene rings in the diarylheterocycle with the secondary pocket of the COX-2 enzyme [25].

Predicting Human Effective Permeability (P_{eff})

The effective permeability through the intestine is calculated by the following equation [22];

$LogP_{eff} = -2.546 - 0.011 \times PSA - 0.278 \times HBD$

where PSA=polar surface area in A^2 and

HBD=number of hydrogen bond donor.

Blood Brain Barrier (BBB) Penetration [22]

Log BBB is a measure of the permeability of a drug into the brain and it is the ratio between the steady state concentration of the drug in the brain and in the blood. Penetration of drug, which is aimed at other sites of action, incorrectly through the brain may lead to undesirable side effects

$Log\ BBB = -0.0148 \times PSA + 0.152 \times ClogP + 0.139$

Compounds with logBB > 0.3 are characterized as BBB penetrating whereas compounds with logBB < -1.0 are poorly distributed to the brain. ClogP is the calculated octanol-water partition coefficient.

RESULTS & DISCUSSION

COX-1 and COX-2 active sites have very small differences. The isleucine present in the positions 434 and 523 in COX-1 is replaced by valine in COX-2. Also histidine present in the position 513 in COX-1 is replaced by arginine in COX-2. Val 523 is responsible for the insertion of secondary pocket in the active site of COX-2, which is important for the binding of selective COX-2 inhibitors. Moreover phenylalanine residue at 503 in COX-1 is replaced by leucine in COX-2 which makes the latter more flexible. Majority of the selective COX-2 inhibitors fall in the class of diarylheterocycles, and the latter consists of two vicinal diaryl moieties in a heterocyclic system. 4-methylsulfone or sulfonamide substitution in the phenyl rings of the vicnal diaryl moiety leads to selectivity towards COX-2, whereas sulfoxide and sulfide do not. Due to the larger size of the coxibs, they have selectivity towards the COX-2 enzyme. Coxibs can enter the COX-2 active site but cannot enter the COX-1 active site, due to the narrow entrance of the latter enzyme.

Fig. (**1**) shows the results of cluster analysis relating the activity of the compounds. Cluster analysis is used to group compounds based on their similarity in the activity. It is assumed that the compounds in the same clusters will have similar characteristics. Compounds without any similarity will generally be in different clusters. This technique can be used to interpret heterogeneous data set and identify structural similarity. There appears to be about four clusters (see dotted lines separating the compounds into four clusters) with nimesulide having the lowest activity (pIC_{50} = -0.11) when compared to the other compounds and it is separate from the rest. The compounds which are in the clinical trails make up the high activity cluster (pIC_{50} between 1.0 to 2.9). The second cluster contains Celecoxib and a few more compounds that are undergoing clinical trails (pIC_{50} between 1 to1.2) and the third cluster contains Rofecoxib, Valdecoxib and a few others (pIC_{50} between 0.3 to 0.7).

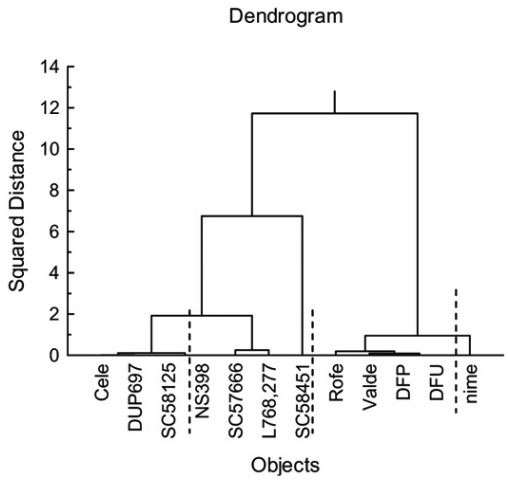

Fig. (**1**). Dendrogram (Cluster analysis) based on the pIC_{50} activity of the twelve compounds.

Comparison between the 19 descriptors with the biological activity (pIC_{50}) shows that HOMO (highest occupied molecular orbital), volume, and refractivity (a descriptor related to the molecular size and polarizability of the molecules) are reasonably correlated with the activity (having correlation coefficient of 0.813, 0.665 and 0.723 respectively). Figs. (**2**-**4**) plot the pIC_{50} with these three descriptors and the corresponding linear regression equation. The contribution of molar refractivity has been already observed by

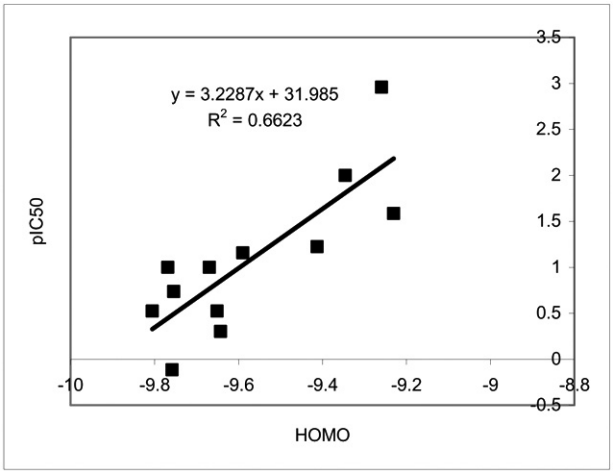

Fig. (**2**). Correlation between HOMO and pIC_{50}.

Wilkerson *et al.* in the QSAR of COX enzymes [26]. Cross correlation coefficient between HOMO and volume, HOMO and refractivity and volume and refractivity are 0.37, 0.53 and 0.89 respectively, indicating a high correlation exists between volume and refractivity, and low correlation between volume and HOMO. Hence it is sufficient to use either volume or refractivity as one of the independent variables during the development of the multilinear regression model.

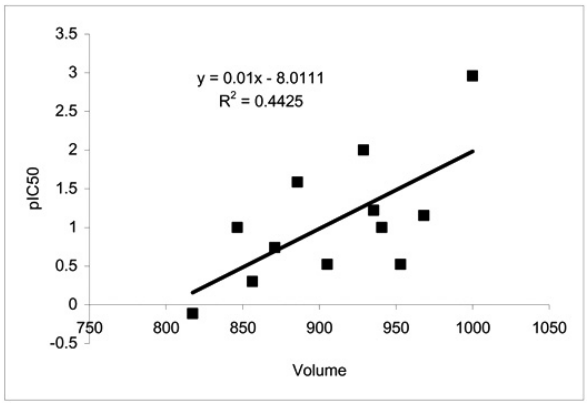

Fig. (**3**). Correlation between Volume and pIC_{50}.

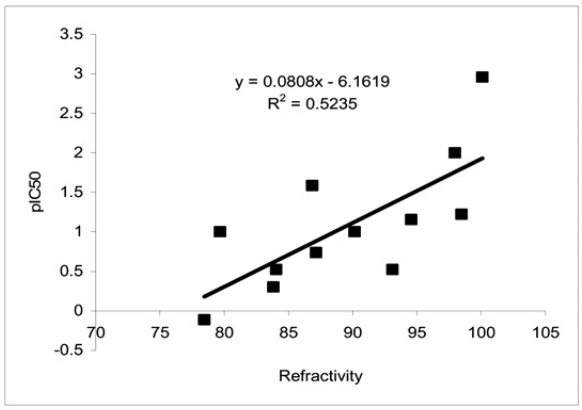

Fig. (**4**). Correlation between Refractivity and pIC_{50}.

Moreover both these descriptors relate to the size of the molecule, hence for the current study HOMO and volume are considered as independent variables. A biparametric equation with these two independent variables as shown below fits the data well ($R^2 = 0.818$, $R^2_{adj} = 0.777$). The predictive capability of the model ($q^2 = 0.616$) is also good. So it can be used to design newer molecules. A large F-ratio (=20.2) indicates a good regression fit.

$$pIC_{50} = 20.295 + 2.612\ (HOMO) + 0.006\ (Volume)$$

Fig. (**5**) compares the experimental pIC_{50} data with model predictions. A biparametric model with HOMO and refractivity gave a poor R^2 and F-ratio, and hence is not discussed here. Table **1** lists the values of the descriptors as well as the estimated pIC_{50} values. Fig. (**6**) is a three dimensional representation of the effect of the two descriptors namely, HOMO and volume on the pIC_{50}. It also compares the experimental with model predictions.

Volume is an important parameter for the selective COX-2 inhibitory activity, because of the presence of the secondary pocket in the active site of this enzyme. Hence the overall active site is bigger than the active site of COX-1 enzyme (by 17%). So if COX-2 by COX-1 activity ratio has to be high then the volume of the molecule has to be high.

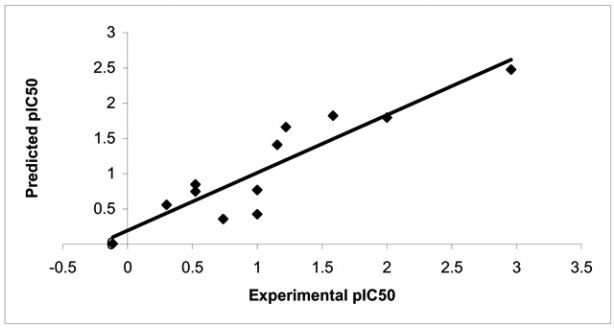

Fig. (**5**). Comparison of pIC_{50} data and model predictions based on a biparametric model.

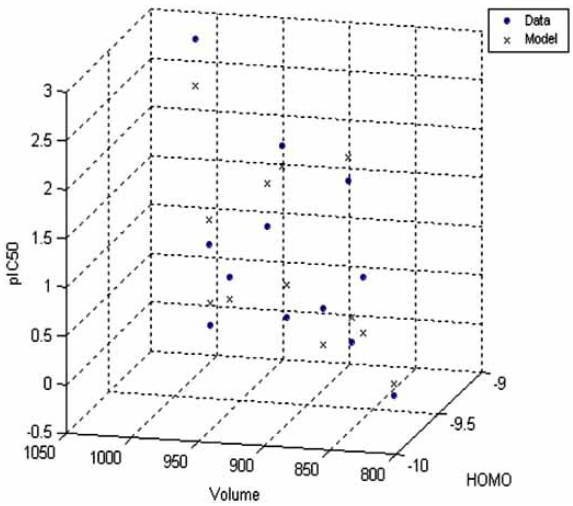

Fig. (**6**). Effect of HOMO and volume of the COX-2 inhibitors on pIC_{50}.

Our calculations indicate that classical NSAIDs such as Ibuprofen and Naproxen have molecular volumes of 702.03 and 706.38Å^3 respectively, which are much less than the twelve selective COX-2 inhibitors (which have an average volume of about 850Å^3 – see Table **1**). So as expected, these two compounds have lower COX-2 selectivity**.** Other researchers also found that properties that describe molecular size such as MR (molecular refractivity) and sterimol (molecular steric factor) appear in the QSAR describing the COX inhibitory activity of 4, 5-diarylpyrrole analogues [26].

HOMO is a measure of the nucleophilicity of the molecule. This means that molecule with high HOMO energy is ready to donate its electrons and thus is more reactive than molecule with low value. Moreover HOMO can also be related to the ionization and oxidation potentials of the molecule. Hence the anti-inflammatory and antioxidant properties of molecules depend on their HOMO energy values [12-14].

Our current findings that the COX-2 inhibitory activity depends on HOMO energy and volume match with the findings of Ranatunge *et al.* [23] who explained the sensitivity of COX-2 active site towards electronic and steric parameters.

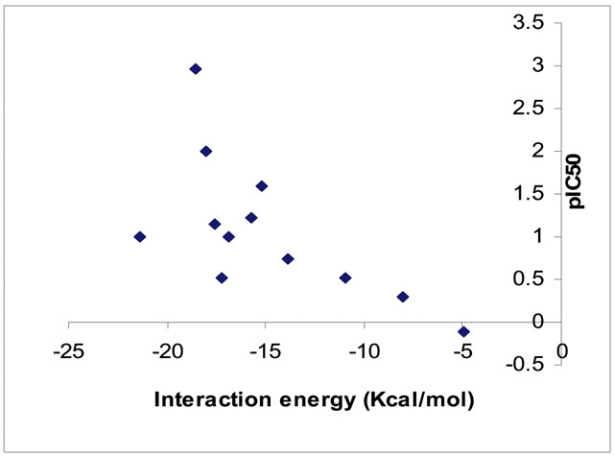

Fig. (7). Relationship between interaction energy and pIC_{50}.

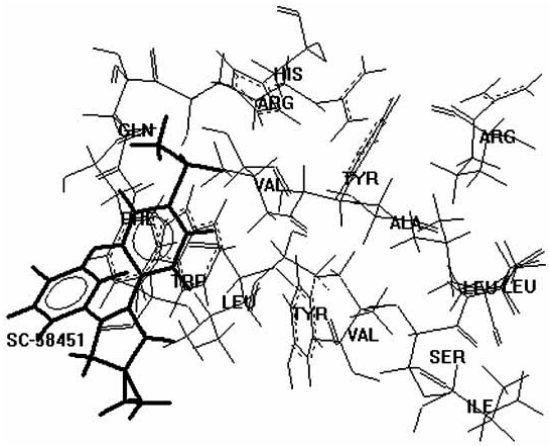

Fig. (8). Interaction between the active site and the most active ligand SC-58451.

Zoete *et al.* [24] also reported the positive contribution of HOMO towards COX-2 selectivity. The positive sign of the coefficients in the regression equation means that the activity will decrease for increasing HOMO energy (the numerical values are negative) and decreasing volume. SC58451, the most active compound in this series and nimesulide, the least active compound, have volumes of 1000.1 and 817.28 $Å^3$ respectively. They have HOMO energy of – 9.259 and -9.7576 respectively (Table **1**).

Fig. (**7**) plots the interaction energy of these compounds with COX-2 and pIC50 values (the negative logarithm of inhibitory concentration). The inhibitory activity increases with decrease in the binding energy (or interaction energy) between the compounds with COX-2 enzyme with a correlation coefficient of -0.65. Fig. (**8**) shows the ligand SC58451 surrounded by the amino acids in the active site of the COX-2 enzyme.

The drug likeliness properties such as PSA, Log P, CLogP, HBD, LogBBB and Log Peff of these twelve compounds are listed in Table **2**. Log BBB for all the twelve compounds is in the range of – 0.8 and 0.15 and it is reported that if log BBB is > 0.3 drugs will cross the Blood Brain Barrier easily and if Log BBB is < -1.0 then the drug is poorly distributed in the brain [22]. All the twelve compounds have their Log BBB in the optimal range and so they are naturally safe. P_{eff} (effective permeability through the intestine) decreases when the number of hydrogen bond donors in the molecule and the polar surface area are increased. For these twelve compounds P_{eff} it is estimated to be between –3.0 and –3.6. The LogP and CLogP values of all the compounds are between 1.1 and 5 and hence they can be assumed to have a reasonable liphophilic – hydrophilic balance. The LogP and ClogP values are calculated by the method reported by Ghose *et al.* [27-28]. Lipinski's rule states that LogP should be in the range of 0 to 5 for a com-

Table 2. The Drug Likeness Properties of the Molecules

S. No	Compound	PSA	LogP	CLogP	HBD	LogBBB	Log Peff
1	Celecoxib	46.93	4.03	4.372	2	0.10898	-3.61823
2	Rofecoxib	68.82	2.24	1.798	0	-0.60624	-3.30302
3	Valdecoxib	55.66	2.47	1.9911	0	-0.38212	-3.15826
4	Nimesulide	85.89	2.67	3.212	0	-0.64395	-3.49079
5	DFP	78.05	1.12	1.7382	0	-0.75193	-3.40455
6	DUP697	70.76	3.68	4.6032	0	-0.20856	-3.32436
7	DFU	68.82	2.87	3.019	0	-0.42065	-3.30302
8	SC58125	46.93	3.96	4.1293	2	0.07209	-3.61823
9	SC57666	42.52	3.75	3.959	0	0.111472	-3.01372
10	NS398	85.89	2.58	3.1956	0	-0.64644	-3.49079
11	L768,277	72.73	2.17	2.2345	0	-0.59776	-3.34603
12	SC58451	42.52	4.01	4.273	0	0.1592	-3.01372

pound to have an ideal liphophilic – hydrophilic balance [29]. In addition the same rule states that HBD should be less than 5. All the commercial COX-2 inhibitors here have their LogP and HBD in the acceptable range and hence posses the drug likeness property.

CONCLUSIONS

QSAR studies of twelve structurally different commercial COX-2 inhibitors clearly suggest that there is a relationship between the pIC_{50} activity with the steric (volume) and electronic (HOMO) parameters. Even though researchers reported the relationship between pIC_{50} and steric and electronic parameters with COX-2 inhibitors of the same family, the studies carried out in this paper on commercial compounds is unique since the structures considered here are very diverse. The study will be useful for designing ideal COX-2 inhibitors since two drugs have been withdrawn from the global market due to side effects, creating a vacuum. Optimized structures were generated using the MM+ molecular mechanics and PM3 semi empirical quantum mechanical methods. From a list of 19 molecular descriptors two were short listed because they had high correlation with the activity.

Increasing the volume of the molecule leads to better COX-2 selectivity, since its active site has 17% higher volume than the active site of COX-1. So larger molecules can enter the COX-2 and not the COX-1 active site. The general approach for designing selective COX-2 inhibitor is by the introduction of a diarylheterocycle, since it leads to an increase in the molecular volume. The contribution of HOMO (a measure of the electron donating capacity of the molecule) to the COX-2 inhibitory activity is also observed by other researchers. Enzyme ligand binding studies indicate that higher is the interaction energy higher is the activity. Diarylheterocyclic moiety in the ligand can provide higher interaction in the active site. The oxygen atom in $MeSO_2$ interacts with the amino acid Phe^{518} and the other oxygen atom interacts with Arg^{513}. A weak polar interaction is also observed between the ligand and the Gln^{192} residue.

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