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QSAR analysis of 6-methylsulfonylindoles as selective cyclooxygenase-2 inhibitors

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ABSTRACT

The use of quantitative structure activity relationship, since its advent, has become increasingly helpful in understanding many aspects of biochemical interactions in drug research. This approach was utilized to explain the relationship of structure with biological activity of selective Cox-2 inhibitors. QSAR study on derivatives of 6-methylsulfonylindoles as selective cyclooxygenase-2 inhibitors was carried out by using chem. office and VALSTAT program allowed obtaining a quite simple equation capable of correlating the structural features of these ligands to their activity towards Cox-2 inhibition. The model was investigated for reliability and stability by using statistical analysis criteria stricter than usual. Particular care was put in defining the chemical space where the model gave reliable prediction. The model allowed the identification of relevant structural features required for the interactions with Cox-2 specific activity, enabling the prediction of activity of molecules belonging to focuses virtual libraries.

KEYWORDS

QSAR;
Cox-2 inhibition;
6-methylsulfonyl indoles.

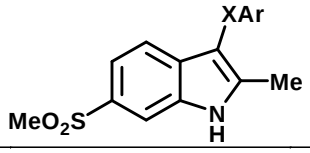
INTRODUCTION

The therapeutics benefits of inhibitors of the enzymes of the arachidonic acid cascade (AAC)[1] have been well established for a number of pathological conditions that involve inflammation, bronchial asthma, allergy and thrombi-embolic disease[2]. Non-steroidal anti-inflammatory drugs [NSAIDs] are among the most widely used prescriptions and over the counter medications used primarily for the treatment of the pain and inflammation particularly, rheumatoid arthritis.

The inhibition of cyclooxygenase (Cox) or prostaglandin-H Syntheses (PGHS), the enzymes that catalyst the conversion of arachidonic acid (AA) into prostaglandins and thromboxane, was considered for a long-time to be responsible for both the therapeutic and the adverse effects of NSAIDs. In 1990 the existence of a second Cox-enzyme, also named PGHS-2, was described[3-5]. The discovery and characterization of cyclooxygenase-2 isoform as mitogeninducible enzymes, associated with physiopathological states, such as inflammation, opens a new perspective for therapeutically use of NSAIDs[6-9]. The design of safer anti-inflammatory agents, acting as selective Cox-2 inhibitors, has drawn the attraction of several industrial and academic research groups[10-12].

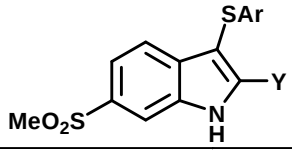
Classical NSAIDs are non selective PGHS inhibitors that reduce the formation of prostaglandins pro-

TABLE 1 : Cyclooxygenase activity of various substituted 2-methyl, 6-methylsulfonylindole analogues



Comp. No.	XAr	IC_{50}	pIC_{50}
1	OPh(4-F)	0.03	7.52
2	OPh(2,4-Di-F)	0.11	6.95
3	OPh(4-Cl)	0.30	6.52
4	OPh(4-OMe)	0.08	6.69
5	OPh(2,4-Di-Cl)	0.06	6.69
6	(C = O) Ph(4-F)	0.22	6.95
7	(C = O) Ph(4-OMe)	0.43	6.17
8	S Ph(4-F)	0.37	5.96
9	S Ph(2,4-Di-F)	0.26	7.69
10	S Ph(4-OMe)	0.28	8
11	S(2-Pyridyl)	0.14	6.32
12	CH_2Ph(4-F)	0.17	5.74
13	CH_2Ph(2,4-Di-F)	0.15	7.09
14	CH_2Ph(4-Cl)	0.11	6.58
15	CH_2Ph(4-OMe)	0.10	6.58
16	CH_2Ph(2-Cl)	0.20	6.56
17	S(= O) Ph(4-F)	0.13	7.15
18	SO_2 Ph(4-F)	0.16	4.39

TABLE 2 : Cyclooxygenase activity of 2-substituted -6-methylsulfonyl-3-thioarylloxyindole analogues



Comp. No.	Ar	Y	IC_{50}	pIC_{50}
19	Ph(4-F)	CO_2Me	0.03	7.52
20	OPh(2,4-Di-F)	CO_2H	0.11	6.95
21	OPh(4-Cl)	$CONH_2$	0.30	6.52
22	OPh(4-OMe)	CN	0.08	6.69
23	Ph(2,4-Di-F)	CO_2Me	0.06	6.69
24	Ph(2,4-Di-F)	$CONH_2$	0.22	6.95
25	Ph(2,4-Di-F)	CONHMe	0.43	6.17
26	Ph(2,4-Di-F)	CN	0.37	5.96
27	Ph(2-Cl)	CN	0.26	7.69
28	Ph(2-Cl) 4-OMe	CN	0.28	8
29	Ph(4-F)	CH_2OH	0.14	6.32
30	Ph(2-Cl)	CH_2CH_2OH	0.17	5.74
31	Ph(2-Cl)	CH_2OAc	0.15	7.09
32	Ph(2-Cl)	CH_2SO_2Me	0.11	6.58

duced by the "house keeping" isoform of prostaglandin-H synthase-1 (Cox-1) which is constitutively expressed in several tissues, including the gastrointestinal tract and kidney[13,14]. On the other hand, Cox-2 is induced significantly under inflammatory condition.

NSAIDS block the Cox-1 about halfway down the channel X-ray crystallography suggested that this blocking occurs by hydrogen bonding with Arg-120 which it is also present in Cox-2. Although both isoforms are very similar in most aspects, 3-D structure analysis and amino acid sequencing have shown slight difference in the Cox-1 and Cox-2[15]. In the Cox-1 isoform, the 523 position is occupied by isoleucine while in Cox-2 the same position is occupied by valine residue which is smaller by a single methyl group. The smaller valine residue in Cox-2 produces a larger gap in the wall of channel, giving access to side pocket, which is through to be the binding site of many selective agents[16].

The distinct kinetic mechanism of inhibition may give insight towards to Cox isoforms selectivity. Cox-1 inhibition is instantaneous and competitively reversible, as would be expected from a process based on hydrogen bonding. In contrast Cox-2 inhibition is a time dependent mechanism, resulting in the formation of an irreversibly inhibited enzyme[15,17].

Classical NSAIDs have carboxylic acid moiety located in a favorable position for interaction with the guanidine group of Arg-120 in both Cox isoforms. Since, many of the different classes of NSAIDs carboxylic acid groups, these interactions may prove to be a general binding feature for these drugs[18]. However, selective Cox-2 inhibitors have this important pharmacophore changed by a metylsulfone or sulfonamide group.

Selective inhibitors of the Cox-2 isoforms result a new generation of NSAIDs with a superior safely profile[19]. Several compounds classified as selective Cox-2 inhibitors do not present the typically deleterious effect of conventional NSAIDs on the gastrointestinal tracts and renal system[20]. In fact, this class of NSAIDs does not process a mechanism-based toxic profile and therefore has therapeutical utility especially in long term treatment of chronic inflammatory states.

In the quest for search of selective Cox-2 inhibitors, the concept of QSAR was exploited in modifying conventionally available NSAIDs in the hope of de-

TABLE 3 : Descriptors calculated for QSAR study

Sr. No.	Descriptor	Type
1	Heat of formation (HF)	Thermodynamic
2	Boiling point (BP)	Thermodynamic
3	Critical pressure (CP)	Thermodynamic
4	Critical temperature (CT)	Thermodynamic
5	Critical volume (CV)	Thermodynamic
7	Henry's law constant (HLC)	Thermodynamic
8	Ideal Gas thermal capacity (IGTC)	Thermodynamic
9	Log P	Thermodynamic
10	Melting point (MP)	Thermodynamic
11	Molar refractivity (MR)	Thermodynamic
12	Standard gibbs free energy (SGFE)	Thermodynamic
13	Connolly accessible area (CAA)	Steric
14	Connolly molecular area (CMA)	Steric
15	Connolly Solvent–Excluded Volume (CSEV)	Steric
16	Ovality (OVA)	Steric
17	Principal moment of inertia – X (PMI–X)	Steric
18	Principal moment of inertia – Y (PMI–Y)	Steric
19	Principal moment of inertia – Z (PMI–Z)	Steric
20	Dipole moment (D)	Electronic
21	Dipole moment –X Axis (DX)	Electronic
22	Dipole moment –Y Axis (DY)	Electronic
23	Dipole moment –Y Axis (DZ)	Electronic
24	Electronic energy (EE)	Electronic
25	HOMO energy (HOMO)	Electronic
26	LUMO energy (LUMO)	Electronic
27	Repulsion energy (RE)	Electronic
28	Bend energy (E_b)	Thermodynamic
29	Charge–charge energy (CCE)	Thermodynamic
30	Charge–dipole energy (CDE)	Thermodynamic
31	Dipole–dipole energy (DDE)	Thermodynamic
32	Non–1, 4 VDW energy (E_v)	Thermodynamic
33	Stretch energy (SE)	Thermodynamic
34	Stretch–bend energy (SBE)	Thermodynamic
35	Torsion energy (E_t)	Thermodynamic
36	Total energy (E)	Thermodynamic
37	Van der waals e 1,4 energy (VDWE)	Thermodynamic
38	VDW 1,4 energy (VDWE)	Thermodynamic
39	Partition coefficient	Thermodynamic

TABLE 4 : Calculated descriptor values for the given series of compounds

Comp. No.	Log P	MR	CAA	P-Y	P-Z	HOMO
1	2.311	82.280	522.683	4369.35	4895.48	-0.9.6
2	2.470	82.496	529.686	4738.60	5205.25	-0.957
3	2.712	86.868	539.706	5046.24	5660.04	-0.896
4	2.027	88.526	561.311	4985.63	5598.55	-0.801
5	3.270	91.673	559.653	5799.10	6299.74	-0.937
6	2.027	88.526	531.223	3162.09	3871.79	-0.768
7	1.984	86.614	554.276	6427.70	6574.95	-1.428
8	1.700	92.861	564.703	6102.64	6156.30	-0.825
9	2.877	88.723	530.416	4639.93	5163.69	-0.903
10	3.036	88.939	535.799	4972.05	5492.28	-0.970
11	2.593	94.970	570.493	5007.75	5671.73	-0.826
12	1.942	89.787	522.182	4051.20	4595.21	-0.794
13	2.867	85.776	522.203	4796.89	5148.43	-0.749
14	3.025	85.992	522.615	4777.05	5379.64	-0.781
15	3.267	90.364	540.681	5131.24	5716.21	-0.758
16	2.582	92.023	560.958	5277.09	5729.37	-0.778
17	3.267	90.364	530.135	4729.36	5188.38	-0.717
18	1.619	90.095	522.131	4982.82	5240.70	-0.842
19	1.666	89.571	525.230	4662.51	5139.61	-1.076
20	2.417	94.979	577.964	5110.86	6233.43	-1.474
21	2.154	90.210	538.208	5052.15	5859.97	-1.569
22	1.503	92.032	539.482	4480.97	5646.17	-1.354
23	2.630	89.189	532.273	4461.33	5348.19	-1.516
24	2.575	95.195	582.376	5531.39	6541.01	-1.534
25	1.661	92.248	534.934	5087.14	6104.68	-1.318
26	11.897	97.145	567.926	5323.44	6531.13	-1.291
27	2.788	89.405	537.722	4964.64	5704.06	-1.590
28	3.030	93.777	554.005	4958.28	5466.58	-1.484
29	2.904	100.242	589.125	5586.75	6446.08	-1.466
30	2.022	90.267	539.441	4625.01	5515.14	-0.995
31	2.652	100.008	614.271	8797.31	9363.54	-1.464
32	1.423	106.254	623.734	5447.42	7965.03	-1.449

Log P- Log partition coefficient, MR- Molar refractivity, CAA- Connelly accessible area, P-Y - Principal moment of inertia –Y axis, P-Z- Principal moment of inertia- Z axis, HOMO- Highest occupied molecular orbit

veloping them as powerful, nonulerogenic, anti-inflammatory agents. QSAR studies of meclofenamic acid analogus, oxazoles, pyrazoles, imidazoles, thiophenoles and furanones as selective Cox-2 inhibitors, have also been reported, no QSAR work has been reported so far for 6-methylsulfonyl indoles.

MATERIAL AND METHODS

The Cox-1 and Cox-2 inhibitory activity data of 6-methylsulfonyl indoles analogous were takes from the reported work of cambellet et al[21]. The activity data have been given as IC_{50} values. The biological activity values [IC_{50} (μM)] reported in the literature was con-

TABLE 5 : Predicted activity data of model 1

Sr.No.	Observed pIC_{50}	Predicted pIC_{50}	Calculated pIC_{50}
1	7.52	7.594	6.892
2	6.95	6.924	6.927
3	6.52	6.610	6.696
4	6.69	6.516	6.247
5	6.69	6.676	6.515
6	6.95	6.899	6.289
7	6.17	6.167	5.748
8	5.96	5.970	5.186
9	7.69	7.657	7.307
10	8.00	8.211	7.327
11	6.32	6.369	6.618
12	5.74	5.819	6.653
13	7.09	7.073	7.074
14	6.58	6.485	7.124
15	6.58	6.579	6.840
16	6.56	6.451	6.263
17	7.15	7.181	6.888
18	4.39	4.562	5.429
19	4.39	4.238	5.045
20	6.09	6.207	6.198
21	4.39	-	-
22	5.91	5.981	5.554
23	5.99	5.953	6.891
24	6.42	6.495	6.210
25	5.64	5.689	5.236
26	4.39	4.355	4.891
27	6.02	6.055	6.901
28	6.88	6.816	6.639
29	6.48	6.490	6.006
30	6.37	6.404	6.400
31	4.39	4.350	4.831
32	4.39	4.496	4.071

verted to molar units and then further to -log scale and subsequently used as the response variable for the QSAR analysis. The -log values of IC_{50} along with the structure of compounds in the series are presented in TABLE 1 and TABLE 2.

All the computations in the present study were performed on PIV workstation. The molecular structures of the training set were sketched using Chem. Draw Ultra module of CS Chem-Office software version 6.0 (Cambridge Soft)[22]. The structures of all compounds were sketched using the builder module of the program.

TABLE 6 : Correlation matrix for parameters in model 1

Parameters	Log P	MR	CAA	PMI-Z
LP	1.000			
MR	0.152	1.000		
CAA	0.090	0.872	1.000	
PMI-Z	0.062	0.769	0.854	1.000

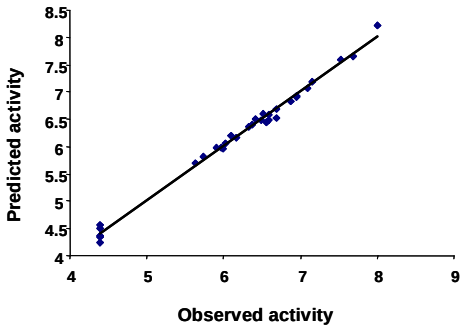


Figure 1 : Graph between observed activity and predicted activity of model 1

These structures were subjected to energy minimization by using Allinger's MM2 force field by fixing Root Mean Square Gradient (RMS) to 0.1 Kcal/mol/A°. Further geometry optimization was done using semiemperical AM1 (Austin Model) Hamiltonian method, closed shell restricted wave function available in the MOPAC module until the RMS value becomes smaller than 0.001 Kcal/mol/A°.

The low energy conformers obtained from the aforementioned procedure were used for the calculation of the ChemSAR descriptors. The ChemSAR descriptors include thermodynamic, electronic and spatial descriptors available in the 'Analyze' option of the Chem3D package (TABLE 3). The descriptors calculated for the present study accounts four important properties of the molecules: thermodynamic, electronic and steric, as they represent the possible molecular interactions between the receptor and 6-methylsulfonyl indoles (value of only those descriptors occurring in different equation is given in TABLE 4).

Stepwise multiple linear regression analysis method was used to perform QSAR analysis employing the statistical program VALSTAT[23]. The best model was selected on the basis of statistical parameters viz., correlation coefficient (r), observed squared correlation coefficient (r^2), standard error of estimate (s), and sequential Fischer test (F). Z score (absolute difference be-

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TABLE 7 : Predicted activity data of model 2

Sr.No.	Observed pIC_{50}	Predicted pIC_{50}	Calculated pIC_{50}
1	7.52	7.418	7.019
2	6.95	7.002	7.160
3	6.52	6.630	6.835
4	6.69	6.712	6.708
5	6.69	6.650	6.961
6	6.95	6.911	6.923
7	6.17	6.261	5.997
8	5.96	5.840	5.538
9	7.69	7.723	6.793
10	8.00	8.010	6.867
11	6.32	6.241	6.667
12	5.74	5.819	5.866
13	7.09	6.991	6.909
14	6.58	6.527	6.886
15	6.58	6.555	6.911
16	6.56	6.597	6.687
17	7.15	6.989	6.932
18	4.39	4.328	5.054
19	4.39	4.497	5.343
20	6.09	6.077	6.347
21	4.39	4.351	5.660
22	5.91	5.824	4.977
23	5.99	5.947	6.422
24	6.42	6.401	6.403
25	5.64	5.564	4.630
26	4.39	4.479	5.012
27	6.02	6.008	6.479
28	6.88	6.892	6.549
29	6.48	6.414	6.352
30	6.37	6.336	5.807
31	4.39	4.400	4.395
32	4.39	4.418	4.195

tween values of model and activity field, divided by the square root of mean square error of data set) was taken as a measure of outlier detection. To assess the self-consistency of derived models, they were validated using leave-one-out (LOO) and the predictive ability was checked using cross-validated squared correlation coefficient (r^2_{cv} or q^2), bootstrapping squared correlation coefficient (r^2_{bs}), chance statistics (evaluated as the ratio of the equivalent regression equations to the total number of randomized sets; a chance value of 0.001 corresponds to 0.1% chance of fortuitous correlation),

TABLE 8 : Correlation matrix for parameters in model 2

Parameters	Log P	MR	HOMO
Log P	1.000		
MR	0.156	1.000	
HOMO	0.218	0.307	1.000

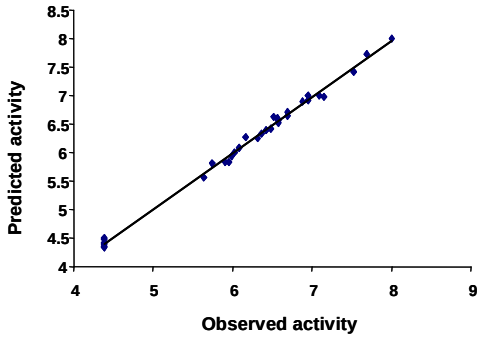

Figure 2 : Graph between observed activity and predicted activity of model 2

and outliers (on the basis of Z-score value). The ±data within parentheses are the standard deviation, associated with the coefficient of descriptors in regression equations. Each of the statistical parameters mentioned above were used for assessing the statistical significance of QSAR. Additionally the developed QSAR models were also checked for significance of the regression coefficients in the model and for multicolinearity problem by the calculation of Student's t-test values (t-value) using statistical software SYSTAT[24].

The generated QSAR models were validated for predictive ability inside the model (leave one out method) by using VALSTAT. The statistical program which is tailored specifically for QSAR statistics estimates the predictive potential of model by calculating the validation parameters squared cross-correlation coefficient (q^2), standard deviation of sum of square of difference between predicted and observed values (S_{PRESS}) and standard deviation of error of prediction (S_{DEEP}).

RESULT AND DISCUSSION

When the data set was subjected to stepwise multiple linear regression analysis, in order to develop a 2D-QSAR model between inhibitory activity as dependent variables and substituent constant as independent variables, several equation were obtained. The statically significant equation (eq. 1) with coefficient of corre-

lation (r = 0.840) was considered as model-1 (TABLE 5, TABLE 6 and Figure 1).

Model 1

pIC_{50} = 22.252(±7.464) + 0.755 (±0.449) LP- 0.112 (±0.041) MR - 0.870 (±0.549) HOMO (1)

n = 31, r = 0.845, r^2 = 0.715, V = 0.303, S = 0.550, F = 22.653, r^2bs = 0.695, q^2 = 0.670, S_{PRESS} = 0.670, S_{DEEP} = 0.615, chance<0.01.

Equation (1) explains 70.7% of the variance in activity with low standard error of estimation. The model showed overall internal statistical significance level better than 99.9% as it exceeded tabulated F (3, 19α 0.001). The study revealed that the series also subjected to molecular modeling using 3D QSAR; all the descriptors values (TABLE 4) for molecules calculated as independent variables and inhibitory concentration data (pIC_{50}) were taken as dependent variables. Various multivalent equations with significant coefficient correlation r = 0.887 by regression analysis were considered. The equation showed overall internal statistical significance level better than 99.9% as it exceeded the tabulated F (4 18 α 0.001).

Model 2

pIC_{50} = 1.676 (±6.875) + 0.977 (±0.395) LP - 0.131 (±0.086) MR + 0.033 (±0.026) CAA -0.007 (±0.004) P-Z (2)

n = 32, r = 0.887, r^2 = 0.757, variance = 0.354, S = 0.595, F = 15.869, r^2bs = 0.767, q^2 = 0.729, S_{PRESS} = 0.439, S_{DEEP} = 0.579, chance<0.01.

Equation (2) explains 75.7% of the variance in activity with low standard error of estimation. To ascertain the predictivity of model, internal validation using leave one out (LOO) method of cross validation process (TABLE 7, TABLE 8 and Figure 2) bootstrapping techniques and randomized test performed. The equation was further subjected to cross validation to confirm the internal consistency; the cross validated squared correlation coefficient (q^2 = 0.729) Standard deviation of error (S_{PRESS} = 0.439), Standard deviation of error of prediction (S_{DEEP} = 0.579) suggested good predictive ability of the activity. The robustness and wide pragmatism of the equation was further supported by r^2bs = 0.767, chance <0.01. At per value of bootstrap squared correlation coefficient (r^2bs) with conventional correlation coefficient (r^2), suggested that the model is a proper representative of analogs.

The study of model-2 reveals that thermodynamic parameter i.e. logP (Log P) and steric parameters i.e., molar refractivity (MR), Connolly accessible area (CAA) and principal moment of inertia-Z (P-Z) are associated with Cox-2 inhibitor activity.

In model-2 Log P and CAA positively contribute to biological activity where as MR and P-Z negatively contributes to biological activity. Log P and MR play a significant role in inhibition of Cox-2 enzymes. Log P the partition coefficient calculated using atom based approach and represents the hyderophobicity of the molecule. This property assumes significance in the present case because of the fact that the molecules under study contain lipophilic groups. MR is a "corrected" from of the molar volume, it reflects the effect of size and polariazability, as indicated by equation. 2, suggesting that MR plays a significant role towards expressed biological activities, which is probably due to steric interaction occurring in polar spaces. It has generally been assumed that a positive coefficient with MR term in a correlation equation suggest a binding action via dispersion forces. Such binding could produce a concomitant conformational change in a macromolecular binding site; however, if negative coefficient could result for the MR term. Negative coefficients with MR have also been assumed to reflect steric hindrance of one kind or another. The value of principle of inertia depends on the total mass of the molecule, the distribution within the molecule and position of axis rotation of the molecule. Equation shows inverse relation of P-Z of molecule on their biological activity.

CONCLUSION

QSAR analysis was performed on a series of Cox-1 and Cox-2 inhibitory activity data of 6-methylsulfonyl indoles analogous using molecular modeling program Chemoffice 2004. QSAR models were proposed for Cox-2 inhibitory activity of the indole using ChemSAR descriptors employing sequential multiple regression analysis method. The selected models were checked for multicolinearity and autocorrelation with Durbin Watson statistics values. The predictive power of each model was estimated with bootstrapping r^2 method and

leaves one-out cross validation method. The result of the study suggests involvement of LP and MR play a significant role in inhibition of Cox-2 enzymes of indole decreases in molar volume and increases lipophilicity conducive for Cox-2 inhibition. Thus, the discussed models could be explored further to design potent anti-inflammatory agents.

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