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Quantitative Structure Activity Relationship Analysis of Ester and Amide Derivatives of Indomethacin

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Quantitative structure activity relationship analysis of 41 amide and ester derivatives of indomethacin using Hansch's extra thermodynamic multi-parameter approach and receptor surface analysis has been performed to determine the factors required for selective inhibition of second isoform of cyclooxygenase enzyme by these derivatives. The substrate's conformation was abstracted from Cambridge crystallographic data bank. For the Hansch approach, special emphasis was laid on various electronic, spatial and thermodynamic descriptors at the minimum energy conformation. Receptor surface analysis was performed taking most selective molecule as a reference compound. Statistical analysis was performed using multiple regression analysis for simple quantitative structure activity relationship analysis and genetic function approximation for receptor surface analysis. The quantitative structure activity relationship analysis model derived from receptor surface analysis was found to be significant as compared to those produced from using conventional physico-chemical descriptors. These results provide the tools for predicting the affinity of related compounds and for guiding the design and synthesis of new selective cyclooxygenase-2 inhibitors with predetermined affinity.

The discovery of cyclooxygenase-2 (COX-2) isoform has opened a new way for the development of anti-inflammatory and analgesic agents with minimum gastric side effects of traditional nonsteroidal antiinflammatory drugs (NSAIDs)[1]. Numerous agents that inhibit this isoform, can also delay or prevent certain forms of cancer[1] and are reported to be beneficial in Alzheimer's disease[2].

Co-crystallization and site directed mutagenesis studies have revealed that ion-pairing of carboxylic group of NSAIDs and arachidonic acid with positively charged arginine 120 residue of cyclooxygenase-1 (COX-1) is necessary for both inhibition and catalysis while such ion pairing is not necessary for the catalysis in case of COX-2[3,4]. On the basis of these observations Kalgutkar *et al*[5] have hypothesized that derivatization of carboxylic acid moiety of NSAIDs would eliminate inhibition of COX-1 without significantly affecting their COX-2 inhibitory properties. By using this hypothesis, they proposed a general strategy for the conversion of conventional, nonselective NSAIDs to selective COX-2 inhibitors and thus have taken the advantage of structural class with well established safety profile.

Analogue based 3D QSAR analysis of these facile conversions of carboxylic acid containing NSAIDs may provide some structural insight of COX-2 to develop more selective inhibitors. In the present study, we have performed the quantitative structure activity relationship analysis of a series of ester and amide derivatives of indomethacin by conventional Hansch's extra thermodynamic multi-parameter approach[6] and receptor surface analysis (RSA)[7]. In Hansch's approach, structural features of drug molecules are quantified in terms of different parameters and these

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structural features are correlated to quantified biological activity through equation using regression analysis. RSA represent essential information about hypothetical receptor site as a 3D surface with associated properties mapped on to the surface model. The location and shape of the surface represent information about the steric nature of the receptor site; while the associated properties represent other information of interest such as hydrophobicity, partial charge, electrostatic potential and hydrogen bonding propensity. The RSA model conveys important information in an intuitive manner and provides predictive capability for evaluating new compounds.

MATERIAL AND METHODS

Data Set:

A data set of 41 molecules has been taken from published results[8]. The structure and COX-2 inhibition data of amide and ester derivatives of indomethacin along with their corresponding biological activity data, used in training set for QSAR analysis, are given in Table 1. The biological data have been converted to -log molar concentration to make all the data positive.

Molecular structure generation:

All the molecular modeling and statistical analysis were performed using Cerius[2] version 4, SPSS and SMRAIL[9] software. The structures of the compounds were built using molecular sketcher facilities provided in the modeling environment of Cerius[2] version 4. Initial conformation of compound 1 was extracted from Cambridge crystallographic structure data bank. Other molecules of the series were built by making the modification in the structure of molecule 1. The energy minimization of these molecules were performed by employing the universal force field[10] and a conjugate gradient method[11] to refine the structure with convergence 0.1 kcal/mole. Partial atomic charges were calculated using semi empirical program MOPAC and applying the AM1 Hamiltonian method.

Alignment:

Initially all the molecules were manually aligned to a molecule having highest potency and selectivity towards COX-2 (1800, compound no.14) by considering the significant common structure, fig. 1. Further refinement in the alignment was carried out by automated approach in Cerius[2]. A stereo view of aligned molecules is observed in fig. 2.

Hansch approach:

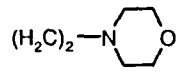
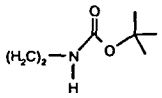
The thermodynamic, spatial and electronic parameters were calculated for QSAR analysis. Thermodynamic parameters describe free energy change during drug receptor complex formation and include desolvation free energy for water (F_{H2O}), desolvation free energy for octanol (F_{OCT}), log of partition coefficient (LogP), molecular refractivity (MR). Spatial parameters are the quantified steric features of drug molecules required for it's complimentary fit with receptor and include number of rotatable bonds (ROTBOND), molecular surface area (AREA), molar volume (V_m), principal moment of inertia (PMI) and it's X ,Y and Z components (PMI-X, PMI-Y, PMI-Z). Electronic parameters describe weak non-covalent bonding between drug molecules and receptor and include sum of atomic polarizability (APOL), dipole moment (DIP) and it's X, Y and Z components (DIP-X, DIP-Y, DIP-Z), energy of highest occupied molecular orbital (HOMO) and energy of lowest unoccupied molecular orbital (LUMO)[12-14].

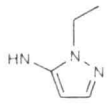
Stepwise multiple regression analysis was used to generate QSAR equations. Statistical measures used were: n-number of sample in the regression, r-correlation coefficient, r^2- squared correlation coefficient (coefficient of determination), s-standard deviation, F-test (Fischer's value) for statistical significance and correlation-matrix to show mutual correlation among the parameters (Table 2)[13].

RSA:

According to the technique described by Hahn[7], hypothetical receptor surface was generated. Compounds 2, 14, 26 and 29 were selected as a template to build the receptor surface model. The interaction energy of all the molecules was evaluated within this receptor surface. The receptor surface descriptors, expressed as 3D field descriptors, were derived from van der Waals and electrostatic interaction energies between the receptor surface and CH_3 and H^+ groups as probes. Fig. 3 is a stereo view of receptor surface also showing the template molecules inside the receptor surface. These descriptors were used as independent variables for the generation of QSAR equations. Regression analysis was carried out using genetic function approximation (GFA) method where genetic operations have been performed over 50,000 generations. From the multiple QSAR equations generated, the one with highest r^2, r^2_{cv} and F-test value and lowest s and $PRESS$ value was considered for further discussion.

TABLE 1: STRUCTURE AND COX-2 INHIBITION DATA OF INDOMETHACIN ESTER AND AMIDE DERIVATIVES ALONGWITH PREDICTED BIOLOGICAL ACTIVITY FROM RSA MODEL.

Comp. No	R	IC_{50}[a]	pIC_{50}[b]	Pred.pIC_{50}[c]	DIP-X	PMI-M	MR
1	OH	0.75	6.125	6.47	0.04	315.6	92.9
2	OC_7H_{15}	0.04	7.397	6.99	1.655	602.8	125.3
3	OC_8H_{17}	0.09	7.045	7.09	1.712	645.7	126.9
4		0.68	6.17	5.96	2.035	564.6	124.8
5		0.045	7.35	7.02	2.629	721.2	130.5
6	OC_2H_5	0.10	7.00	6.49	1.551	380.7	102.4
7	a-$C_{10}H_7$	5.0	5.3	5.23	1.735	502.7	133.9
8	OC_6H_4(4-$NHCOCH_3$)	0.05	7.30	7.40	2.050	585.6	130.5
9	OC_6H_4(4-F)	0.075	7.12	6.57	2.253	524.6	117.7
10	3-Pyridyl	0.05	7.30	6.94	2.328	570.7	115.3
11	$NHCH_3$	070	6.15	6.39	3.585	340.0	99.6
12	$N(CH_3)_2$	18.0	4.74	4.87	2.324	362.9	104.5
13	$N(C_2H_5)_2$	25.0	4.60	4.60	2.244	413.4	114.0
14	NHC_8H_{17}	0.04	7.397	7.24	1.840	629.5	131.9
15	$NH(CH_2)_3Cl$	0.05	7.30	6.94	2.670	518.5	113.5
16	$NH(CH_2)_2OH$	0.25	6.6	6.94	3.364	421.5	105.9
17	(D)-$NHCH(CH_3)CO_2CH_3$	0.40	6.397	6.63	1..761	545.8	115.0
18	(L)-$NHCH(CH_3)CO_2CH_3$	0.19	6.72	6.61	2.241	525.6	115.0
19	NH $(CH_2)_2C_6H_5$	0.06	7.22	6.94	1.640	557.8	129.0
20	NH_2	0.70	6.15	6.44	2.110	312.3	94.7
21	$NHCH_2C_6H_4$(4-CH_3)	0.06	7.22	7.05	1.440	675.2	129.3
22	$NHCH(CH_3)C_6H_4$(4-CH_3)	0.20	6.698	7.10	1.871	587.0	133.7
23	$NHCH_2C_6H_4$(4-$COCH_3$)	0.08	7.096	7.21	1.841	620.5	134.6
24	OC_4H_9	0.05	7.30	6.81	1.662	474.2	111.5
25	NHC_6H_4(4-F)	0.06	7.22	7.16	3.855	565.7	119.6
26	NHC_6H_4(4-Cl)	0.05	7.259	7.07	3.767	618.5	124.3
27	NHC_6H_4(4-SCH_3)	0.12	6.92	6.98	2.680	651.9	132.3
28	NHC_6H_4(3-SCH_3)	0.22	6.657	6.87	3.931	697.0	132.3

29	$NHC_6H_4(4\text{-}OCH_3)$	0.056	7.25	7.11	4.034	627.1	125.9
30	$NHC_6H_4(3\text{-}OC_2H_5)$	0.65	6.19	6.56	2.620	722.0	130.6
31	$NHC_6H_4(4\text{-}CH_2CO_2CH_3)$	0.058	7.24	7.08	1.309	684.6	135.5
32	OC_5H_{11}	0.05	7.30	7.09	1.638	535.1	116.1
33	NH(3-Pyridyl)	0.052	7.28	6.83	2.880	519.4	117.2
34	NH(2-Cl-3-Pyridyl)	0.050	7.30	7.46	1.722	575.8	123.1
35		0.70	6.15	6.58	4.300	532.8	122.0
36	$NHOCH_2C_6H_5$	0.050	7.30	7.34	3.554	576.8	125.6
37	$NHOCH_2C_6H_4(4\text{-}NO_2)$	0.06	7.22	6.90	6.564	574.6	132.9
38	OC_6H_{13}	0.06	7.22	7.02	1.689	466.4	120.7
39	O-*cyc*-C_6H_{11}	0.12	6.92	7.00	1.313	588.7	118.7
40	$C_6H_4(2\text{-}SCH_3)$	0.06	7.22	6.63	-1.208	526.3	130.3
41	O-*i*-C_3H_7	0.25	6.60	6.99	1.520	423.4	111.5

IC_{50} [a] Dose in micro molar (mM) required to produce 50% inhibition (from Ref. 8) pIC_{50} [b] =-log , molar dose, Pred. pIC_{50} [c]=Predicted pIC_{50} from receptor surface model (Eqn. 3).

RESULTS AND DISCUSSION

Out of several QSAR equations, the best two are, pIC_{50}=-0.2345 DIP-X+0.01218 PMI M+ 0.9161 (1), where, n=41, r^2=0.650, r=0,806, s =0.419, F=35.28 and pIC_{50}=-0.259DIP-X+0.0022 PMI M+ 0.0354 MR+1.91612 (2), where n=41, r^2=0.701, r=0.836, s=0.393, F=28.70.

Moment of inertia is a steric parameter. The value of PMI depends on the total mass of the molecule, mass distribution within the molecule and position of axis of rotation of the molecules. Equations show positive effect of PMI of molecules on the biological activity. This suggests that the substitution, which does not facilitate the rotatory motion of the molecules around the principal axis, produces the derivatives with better inhibition of COX-2.

Molecular refractivity is a measure of polarizability and steric bulk of molecules. Equations show positive correla-

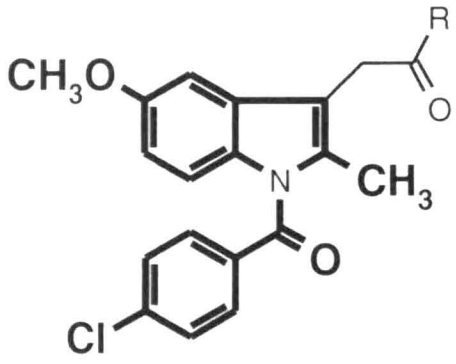


Fig. 1. Significant common structure for molecular alignment showing in bold face.

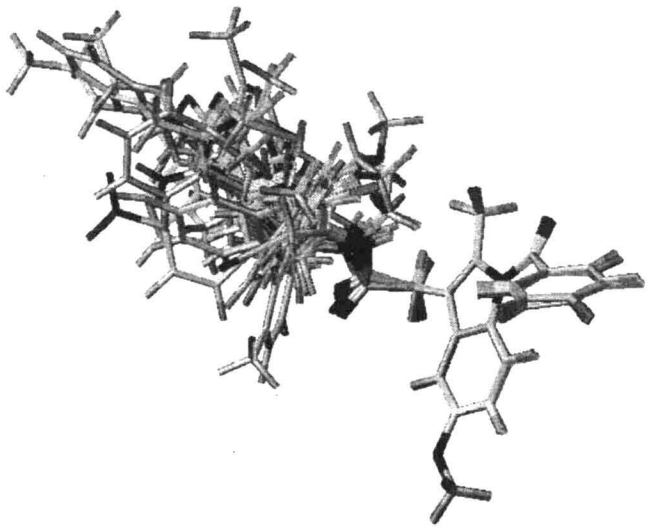


Fig. 2: A stereo view of aligned molecules.

TABLE 2: CORRELATION MATRIX FOR THE PARAMETERS IN EQUATION 2.

	pIC_{50}	DIP-X	PMI-M	MR
pIC_{50}	1			
DIP-X	0.015	1		
PMI-M	0.495	0.084	1	
MR	0.352	0.105	0.0865	1

tion between biological activity and molecular refractivity. This infers that the biological activity would increases with increase in molecular refractivity i.e. increase in polarizability and steric bulk of the compounds within the series.

Dipole moment reflects the strength and orientation behavior of a molecule in electrostatic field. Equations reveal negative correlation of X-components of dipole moment of the molecules (DIP-X).

Although results obtained from Eqns. 1 and 2 are not very significant statistically, these equations indicate that steric and electronic effects of substituents might have a role to pay in determining the COX-2 inhibition. On the basis of these results, more advanced and accurate RSA approach was further considered for this data set.

In the RSA, receptor surface was generated around the selected templet molecules (Compounds 2, 14, 26 and 29) at 0.01A° surface fit and 50% transparency level. All the interaction energies and pIC_{50} of these 41 derivatives were subjected to GFA and the following equation was obtained as the best equation in the population of 100 equations: pIC_{50}=6.340–0.890*TOT/2633–1.425*TOT/2534 – 0.892*vdw2265 +0.761*vdw2255-0.224*ele2083 (3), where n=41,r^2=0.816, r=0.903, s=0.316, F =23.724, r^2_{CV} =0.653, *PRESS*=7.597, where TOT/2633 and TOT/2534 are the total interaction energies at 2633rd and 2534th receptor surface points, respectively. vdw2265 and vdw2255 are steric interaction energy at 2265th and 2255th points, respectively. ele2083 is electrostatic interaction energy at 2083rd point. Total number of receptor surface points generated is 350. The above equation explains 81.6% variance in the activity with respect to steric and electrostatic interaction energies between receptor points and molecules in data set. The statistical measures, r^2, s and F determine the estimation power of model for the same data from which it has been determined and evaluate it only internally. On the other hand cross-validated parameters, r^2_{CV} and *PRESS*, determine the prediction power for the data not included in deriving the model and evaluate the model externally to avoid chance correlation completely. It can be observed that overall statistics of the equation generated in RSA are excellent and their prediction ability is also significant which is evident from their cross-validated r^2_{cv} value.

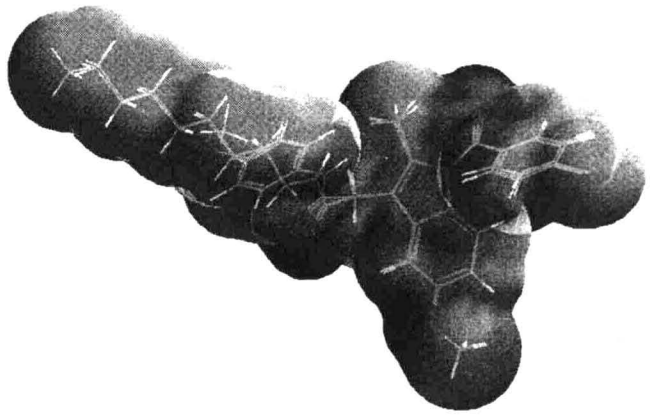


Fig. 3: Receptor Surface Model (RSA) generated at 0.01A° surface fit and 50% transparency level.

On the basis of the above studies it can be concluded that selective COX-2 inhibition by the amide and ester derivative of indomethacin is strongly influenced by the steric and electrostatic nature of substituents. Pattern of substitution can be extracted from the developed model. Consequently this study may prove to be helpful in development and optimization of existing selective COX-2 inhibitors of this class of compounds.

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