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QSAR Analysis of Indomethacin Derivatives as Selective COX–2 Inhibitors

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Abstract

Motivation. Selective inhibition of cyclooxygenase–2 (COX–2) is an important strategy in design of potent anti–inflammatory compounds with significantly reduced side effects. We selected ester and amide derivatives of indomethacin to explore the structural requirement of these analogues necessary for selective COX–2 inhibition.

Method. In the present investigation, a QSAR study was performed using 66 ester and amide derivatives using Dragon 3.0 structural descriptors. Cluster analysis technique was applied to generate training and test sets. The relationship between inhibitory activity and various descriptors is established by step–wise multiple regression analysis using SYSTAT 10.2 and VALSTAT.

Results. The analyses have produced good predictive and statistically significant QSAR models. These models were cross–validated with the leave–one–out (LOO) method. The values of statistical data are: R = 0.908, F = 37.45, SEE = 0.317 and R^2_{CV} = 0.765 for COX–2 inhibition; R = 0.958, F = 45.00, SEE = 0.312 and R^2_{CV} = 0.836 for COX–1 inhibition; and R = 0.949, F = 39.7, SEE = 0.392 and R^2_{CV} = 0.711 for selectivity. The predicted activity shows a linear relationship with the observed activity.

Conclusions. The present study suggests that hydrogen bonding from amide nitrogen to a protein acceptor is an important determinant of the receptor binding. Lipophilicity and topological distance indices are important for COX–2 inhibition. Also, the Geary autocorrelation and eigenvalue descriptors modulate COX–1 and COX–2 inhibition and selectivity. These studies are promising for the development of novel compounds, which may have potent anti–inflammatory activity devoid of side effects like gastric ulcer and renal failures.

Keywords. QSAR; quantitative structure–activity relationships; cyclooxygenase–2; COX–2; COX–2 inhibitors; NSAIDs; nonsteroidal anti–inflammatory drugs.

1 INTRODUCTION

The biosynthesis of prostaglandins (PGs) involves conversion of arachidonic acid to PGG_2 and then to PGH_2, a reaction catalyzed by sequential action of prostaglandin H_2 endoperoxide synthase (PGHS) or cyclooxygenase (COX) [1]. COX enzyme exists as two related but distinct isoforms designated as COX–1 and COX–2 [2]. Distinct genes on separate chromosomes encode these enzymes [3–4]. COX–1 is found in most tissues, such as gastric mucosa, kidneys, platelets, and

Dedicated on the occasion of the 75th birthday to Professor Lemont B. Kier.

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many other tissues, as a constitutive enzyme. It is responsible for the production of "housekeeping" prostaglandins critical to the maintenance of normal renal function, gastric mucosal integrity, vascular hemostasis and the autocrine response to circulating hormones. The COX–2 isoform is the inducible form, expressed in response to inflammatory stimulus. It is upregulated 20–fold in macrophages, monocytes, synoviocytes, chondrocytes, fibroblasts, osteoblasts and endothelial cells in response of various inflammatory stimuli [5]. This knowledge led to the hypothesis that side effects such as ulcers, renal failure associated with clinically useful NSAIDs are caused by homeostatic COX–1 enzyme inhibition, whereas the anti–inflammatory properties result from inhibition of the inducible COX–2 [6]. Selective inhibition of COX–2 provides a new class of anti–inflammatory compounds and analgesic drugs with significantly reduced side effects. Researchers suggest that the inhibition of COX–2 may suppress carcinogenesis by affecting a number of pathways: inhibiting angiogenesis, invasiveness of tumors and promoting apoptosis [7]. References estimate that highly selective COX–2 inhibitors may get a role in the treatment of cancer [8] as an adjuvant therapy or as a co–chemotherapeutic agent [9].

Sustained efforts have been made regarding the identification of COX–2 inhibitors with an attractive pharmacological profile: NS–398, *N*(2–cyclohexyloxy–4–nitrophenyl) methane–sulphonamide; Dup–697, 5–bromo–2–(4–flurophenyl)–3–(4–methylulphonylphenyl)thiophene; SC–58635 (celecoxib), 4–[5–(4–methylphenyl)–3–trifluoromethyl–1*H*–1–pyrozolyl]–1–benzene–sulphonamide), 2–acetoxyphenyl alkyl sulphides and diarylisoxazoles, have been developed as highly selective COX–2 inhibitors [10–14]. QSAR studies of meclofenamic acid analogues, oxazoles, pyrazoles, imidazole, thiophenes and furanones as selective COX–2 inhibitors, have also been reported [15–17].

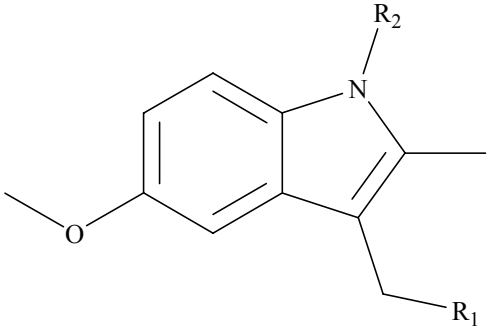
Indomethacin is a nonselective inhibitor of both COX–1 and COX–2, but its ester, amide and thiazole analogues are selective COX–2 inhibitors [18–20]. In view of the above and to explore the necessary structural requirement of indomethacin analogues for selective COX–2 inhibition, quantitative structure activity relationship (QSAR) studies have been performed and are presented in this paper.

2 MATERIALS AND METHODS

2.1 Data Set

The COX–1 and COX–2 inhibition of indomethacin ester and amides have been reported [20] in terms of inhibitory concentration 50% of enzyme (IC_{50} in micromoles). The enzyme inhibition data were converted to negative logarithmic values (concentration in moles) and selectivity (COX–1/COX–2 enzyme inhibition ratio) was converted to logarithmic value. These values were used for subsequent QSAR analyses as response variable. The structures of all indomethacin analogues with their COX–2, COX–1 inhibitory activity and selectivity are presented in Table 1.

Table 1. Structural features of all the indomethacin analogues studied and their observed COX–2, COX–1 inhibitory activity and selectivity



1–66

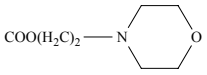
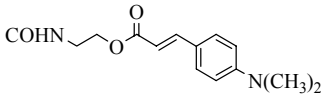
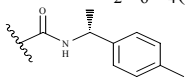
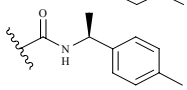
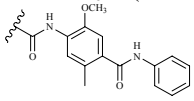
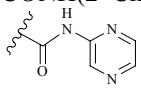
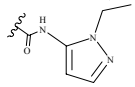
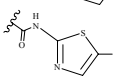
No	R_1	R_2	COX–2 [a]		COX–1 [a]		Selectivity [c]
			IC_{50} (μM)	pC_2 [b]	IC_{50} (μM)	pC_1 [b]	
1	COOH	COC_6H_4Cl	0.75	6.12	0.05	7.30	–1.18
2	$COOCH_3$	COC_6H_4Cl	0.25	6.60	33.00	4.48	2.12
3	$COOC_2H_5$	COC_6H_4Cl	0.10	7.00	#	#	#
4	$COOC_3H_7$	COC_6H_4Cl	0.10	7.00	#	#	#
5	COO–i–C_3H_7	COC_6H_4Cl	0.25	6.60	37.00	4.43	2.17
6	$COOC_4H_9$	COC_6H_4Cl	0.05	7.30	#	#	#
7	$COOC_5H_{11}$	COC_6H_4Cl	0.05	7.30	#	#	#
8	$COOC_6H_{13}$	COC_6H_4Cl	0.06	7.22	#	#	#
9	COO–*cyc*C_6H_{11}	COC_6H_4Cl	0.12	6.92	#	#	#
10	$COO(CH_2)_2$–*cyc*C_6H_{11}	COC_6H_4Cl	1.00	6.00	#	#	#
11	$COOC_7H_{15}$	COC_6H_4Cl	0.04	7.40	#	#	#
12	$COO(CH_2)_2O(CH_2)_3$ CH_3	COC_6H_4Cl	0.06	7.22	#	#	#
13	COO–*trans*–$CH_2CHCH(CH_2)_3CH_3$	COC_6H_4Cl	0.05	7.30	#	#	#
14	$COOCH_2C{\equiv}C(CH_2)_3CH_3$	COC_6H_4Cl	0.25	6.60	#	#	#
15	$COOCH(CH_3)CH_2C{\equiv}CCH_2CH_3$	COC_6H_4Cl	0.12	6.92	#	#	#
16	$COOC_8H_{17}$	COC_6H_4Cl	0.09	7.05	#	#	#
17		COC_6H_4Cl	0.68	6.17	#	#	#
18	$COO(CH_2)_2NHCOOC(CH_3)_3$	COC_6H_4Cl	0.05	7.35	#	#	#
19	$COOC_6H_5$	COC_6H_4Cl	0.40	6.40	#	#	#
20	COO–α–$C_{10}H_7$	COC_6H_4Cl	5.00	5.30	#	#	#
21	$COO(CH_2)_2C_6H_5$	COC_6H_4Cl	0.04	7.40	#	#	#
22	$COOC_6H_4$(4–SCH_3)	COC_6H_4Cl	0.30	6.52	3.00	5.52	1.00
23	$COOC_6H_4$(2–SCH_3)	COC_6H_4Cl	0.06	7.22	#	#	#
24	$COOC_6H_4$(4–OCH_3)	COC_6H_4Cl	0.04	7.40	#	#	#
25	$COOC_6H_4$(4–$NHCOCH_3$)	COC_6H_4Cl	0.05	7.30	66.00	4.18	3.12
26	$COOC_6H_4$(4–F)	COC_6H_4Cl	0.08	7.12	#	#	#
27	COO[3–pyridyl]	COC_6H_4Cl	0.05	7.30	2.50	5.60	1.70
28	$CONHCH_3$	COC_6H_4Cl	0.70	6.15	#	#	#
29	$CON(CH_3)_2$	COC_6H_4Cl	18.00	4.74	#	#	#
30	$CON(C_2H_5)_2$	COC_6H_4Cl	25.00	4.60	#	#	#
31	$CONHC_8H_{17}$	COC_6H_4Cl	0.04	7.40	66.00	4.18	3.22
32	$CONHC_9H_{19}$	COC_6H_4Cl	0.04	7.40	17.00	4.77	2.63
33	$CONH(CH_2)_3Cl$	COC_6H_4Cl	0.05	7.30	45.00	4.35	2.95
34	$CONH(CH_2)_2OH$	COC_6H_4Cl	0.25	6.60	#	#	#
35		COC_6H_4Cl	0.19	6.72	#	#	#
36	$CONHCH_2COOCH_3$	COC_6H_4Cl	4.00	5.40	#	#	#
37	CO–(D)–$NHCH(CH_3)COOCH_3$	COC_6H_4Cl	0.40	6.40	#	#	#

Table 1. (Continued)

No	R_1	R_2	COX–2 [a]		COX–1 [a]		Selectivity [c]
			IC_{50} (μM)	pC_2 [b]	IC_{50} (μM)	pC_1 [b]	
38	CO–(L)–NHCH(CH_3)$COOCH_3$	COC_6H_4Cl	0.19	6.72	#	#	#
39	$CONH(CH_2)_2C_6H_5$	COC_6H_4Cl	0.06	7.22	#	#	#
40	$CONH_2$	COC_6H_4Cl	0.70	6.15	#	#	#
41	$CONHCH_2C_6H_4$(2–CH_3)	COC_6H_4Cl	0.15	6.82	#	#	#
42	$CONHCH_2C_6H_4$(4–CH_3)	COC_6H_4Cl	0.06	7.22	8.00	5.10	2.12
43		COC_6H_4Cl	0.06	7.22	4.00	5.40	1.82
44		COC_6H_4Cl	0.20	6.70	4.00	5.40	1.30
45	$CONHCH_2C_6H_4$(4–$COCH_3$)	COC_6H_4Cl	0.08	7.10	#	#	#
46	$CONHC_6H_4$(4–F)	COC_6H_4Cl	0.06	7.22	#	#	#
47	$CONHC_6H_4$(4–Cl)	COC_6H_4Cl	0.06	7.26	#	#	#
48	$CONHC_6H_4$(4–SCH_3)	COC_6H_4Cl	0.12	6.92	#	#	#
49	$CONHC_6H_4$(3–SCH_3)	COC_6H_4Cl	0.22	6.66	#	#	#
50	$CONHC_6H_4$(4–OCH_3)	COC_6H_4Cl	0.06	7.25	#	#	#
51	$CONHC_6H_4$(3–OC_2H_5)	COC_6H_4Cl	0.65	6.19	53.00	4.28	1.91
52	$CONHC_6H_4$(4–$NHCOCH_3$)	COC_6H_4Cl	0.12	6.92	#	#	#
53	$CONHC_6H_4$(4–CH_2COOCH_3)	COC_6H_4Cl	0.06	7.24	#	#	#
54	$CONHC_6H_4$(4–$CONH_2$)	COC_6H_4Cl	0.14	6.85	#	#	#
55		COC_6H_4Cl	0.60	6.22	17.00	4.77	1.45
56	$CONHC_6H_4$(4–C_6H_5)	COC_6H_4Cl	0.50	6.30	#	#	#
57	CONH(3–Pyridyl)	COC_6H_4Cl	0.05	7.28	#	#	#
58	CONH(5–Chloro–3–Pyridyl)	COC_6H_4Cl	0.05	7.33	#	#	#
59	CONH(2–Chloro–3–Pyridyl)	COC_6H_4Cl	0.05	7.30	45.00	4.35	2.95
60		COC_6H_4Cl	4.00	5.40	#	#	#
61		COC_6H_4Cl	0.70	6.15	#	#	#
62		COC_6H_4Cl	4.00	5.40	#	#	#
63	$CONHOCH_2C_6H_5$	COC_6H_4Cl	0.05	7.30	0.06	7.22	0.08
64	$CONHOCH_2C_6H_4$(4–NO_2)	COC_6H_4Cl	0.06	7.22	4.00	5.40	1.82
65	$CONHNHCH_2C_6H_5$	COC_6H_4Cl	2.50	5.60	#	#	#
66	COOH	$CH_2C_6H_4Br$	2.50	5.60	#	#	#

[a] IC_{50} values were determined by incubating several concentration of inhibitors in DMSO with human COX–2 or ovine COX–1 (ref. 20).
[b] Negative logarithmic value of IC_{50} (in moles)[$pC_1 = -\log_{10}IC_{50}$ (for COX–1) and $pC_2 = -\log_{10}IC_{50}$ (for COX–2)]
[c] $\text{Log}_{10}[IC_{50}(\text{COX–1})/IC_{50}(\text{COX–2})]$
Not available

COX–2 inhibitory data have been available for 66 compounds. This compounds set was first divided into two subsets based on hierarchical clustering: one training set composed of 49 compounds and one test set composed of 17 compounds. Models for COX–2 inhibition were constructed based on the training set and the generated models were then validated: internally (using the leave one out technique) and externally (predicting the activities of the test set). The

models for COX-1 inhibition and selectivity were constructed based on available biological data and the models thus generated were internally validated using the leave one out technique.

All of the molecular modeling studies reported here used structural descriptors computed with Dragon 3.0 (Milano Chemometrics) [21]. Molecular structures were generated with ChemDraw Ultra 6.0 and optimized in CS Chem3D Ultra (Cambridge soft) [22], first by molecular mechanics (MM2) and re–optimized by MOPAC–AM1 until the root mean square (RMS) gradient value becomes smaller than 0.0001 kcal/mol. Å. [23–24]. Energy minimized molecules were saved as MDL MolFiles for computing various molecular descriptors using Dragon 3.0 [21].

Constitutional descriptors, functional groups, atom centered fragments, empirical descriptors, properties, topological descriptors, molecular walk counts, BCUT descriptors, Galvez topological charge indices, 2D autocorrelations were computed and variable exclusion was done for constant variable and near–constant variable at paired correlation. As the total number of descriptors involved in the study is high for each set of compounds, only significant descriptors are presented in the discussion. The descriptors considered in this study along with their definitions are presented in Table 2. Physicochemical descriptors and COX–2 inhibition for compounds of the training set and test set are presented in Table 3 and Table 4, respectively. COX–1 inhibitory/selectivity data have been available for 17 compounds which are presented along with descriptors in Table 5.

Table 2. Molecular descriptors selected that significantly influence COX–2, COX–1 inhibition and selectivity

Descriptors	Definition	Class
nCONR2	number of tertiary amides (aliphatic)	Functional groups
BEHm2	highest eigenvalue n. 2 of Burden matrix / weighted by atomic masses	BCUT descriptors
MLOGP	Moriguchi octanol–water partition coeff. (logP)	Properties
T(Cl..Cl)	Sum of topological distances between Cl..Cl	Topological descriptors
GATS7v	Geary autocorrelation – lag 7 / weighted by atomic van der Waal volume	2D autocorrelation
BELm6	lowest eigenvalue n. 6 of Burden matrix / weighted by atomic masses	BCUT descriptors
GATS1e	Geary autocorrelation – lag 1 / weighted by atomic Sanderson electronegativities	2D autocorrelations
GATS7e	Geary autocorrelation – lag 7 / weighted by atomic Sanderson electronegativities	2D autocorrelations
GATS8e	Geary autocorrelation – lag 8 / weighted by atomic Sanderson electronegativities	2D autocorrelations

2.2 Statistical Computation

The relationship between response variable (as a dependent variable) and various physicochemical as well as structural descriptors (as independent variables), were established by step–wise linear multiple regression analysis using SYSTAT 10.2 [26] and VALSTAT [27] running on a Pentium 4 processor (CPU 3.00 GHz HT). Significant descriptors were chosen on the basis of statistical data of analysis. The intercorrelation (Pearson correlation) between these descriptors was checked for independence of the variables.

Table 3. Descriptors, observed, calculated and predicted COX–2 inhibition of compounds of training set

No	Descriptors					pC_2		
	nCONR2	BEHm2	MLOGP	T(Cl..Cl)	GATS7v	Obs [a]	Cal [b]	LOO [b]
1	0	3.92	3.16	0	1.042	6.12	6.85	6.88
2	0	3.92	3.38	0	1.137	6.60	6.57	6.57
3	0	3.92	3.60	0	1.124	7.00	6.65	6.62
5	0	3.92	3.81	0	1.114	6.60	6.72	6.73
7	0	3.92	4.23	0	1.085	7.30	6.89	6.86
9	0	3.92	4.44	0	1.018	6.92	7.15	7.17
10	0	3.97	4.84	0	1.045	6.00	6.18	6.21
12	0	3.92	3.66	0	1.062	7.22	6.87	6.86
13	0	3.92	4.56	0	1.029	7.30	7.14	7.13
14	0	3.92	4.56	0	1.043	6.60	7.09	7.12
16	0	3.92	4.84	0	1.013	7.05	7.24	7.26
17	0	3.92	2.91	0	1.149	6.17	6.45	6.49
18	0	3.92	3.91	0	1.079	7.35	6.86	6.83
19	0	3.92	2.22	0	0.986	6.40	6.87	6.95
20	0	4.00	5.23	0	0.966	5.30	5.94	6.14
21	0	3.92	4.62	0	1.061	7.40	7.04	7.01
23	0	3.93	4.42	0	0.936	7.22	7.23	7.23
24	0	3.92	3.65	0	0.956	7.40	7.22	7.20
25	0	3.92	3.43	0	0.970	7.30	7.14	7.12
26	0	3.92	4.32	0	0.993	7.12	7.21	7.22
27	0	3.92	3.24	0	1.025	7.30	6.92	6.90
28	0	3.92	2.97	0	1.121	6.15	6.55	6.59
29	1	3.92	3.19	0	1.195	4.74	4.59	4.45
30	1	3.92	3.62	0	1.172	4.60	4.75	4.89
32	0	3.92	4.63	0	1.002	7.40	7.24	7.23
33	0	3.92	3.62	15	1.061	7.30	7.36	7.38
34	0	3.92	2.42	0	1.102	6.60	6.52	6.51
35	0	3.92	3.53	0	1.113	6.72	6.68	6.67
36	0	3.92	2.62	0	1.105	5.40	*	*
37	0	3.92	2.83	0	1.096	6.40	6.61	6.63
42	0	3.92	4.21	0	0.964	7.22	7.29	7.30
44	0	3.92	4.41	0	1.024	6.70	7.13	7.15
45	0	3.92	3.53	0	1.043	7.10	6.91	6.90
47	0	3.93	4.02	16	0.974	7.26	7.56	7.70
49	0	3.97	4.02	0	0.964	6.66	6.31	6.27
50	0	3.92	3.24	0	0.971	7.25	7.10	7.09
51	0	3.92	3.44	0	0.966	6.19	*	*
53	0	3.92	3.62	0	0.994	7.24	7.09	7.08
54	0	3.92	2.79	0	0.982	6.85	6.98	7.00
56	0	3.97	4.74	0	0.955	6.30	6.46	6.48
57	0	3.92	2.84	0	1.015	7.28	6.88	6.85
58	0	3.93	3.04	15	1.041	7.33	7.14	7.07
59	0	3.94	3.04	14	0.986	7.30	7.10	7.03
61	0	3.92	1.28	0	1.028	6.15	6.56	6.71
62	0	4.02	3.11	0	0.975	5.40	5.16	5.03
63	0	3.92	4.06	0	1.005	7.30	7.13	7.12
64	0	3.92	3.84	0	1.012	7.22	7.07	7.06
65	0	3.92	3.84	0	1.012	5.60	*	*
66	0	4.02	3.50	0	0.932	5.60	5.37	5.26

[a] Observed value
[b] Calculated (Cal.) and predicted (LOO) values of pC_2 from Model 2.
* Compounds removed as outliers

Table 4. Descriptors, observed and predicted COX–2 inhibition of compounds of test set

S	Descriptors					pC_2	
No.	nCONR2	BEHm2	MLOGP	T(Cl..Cl)	GATS7v	Obs	[b]Eq. 2
4	0	3.91	3.81	0	1.055	7.00	7.07
6	0	3.92	4.02	0	1.073	7.30	6.86
8	0	3.92	4.44	0	1.003	7.22	7.16
11	0	3.92	4.64	0	1.018	7.40	7.15
15	0	3.93	4.56	0	1.034	6.92	6.89
22	0	3.95	4.42	0	0.938	6.52	6.83
31	0	3.92	4.43	0	1.005	7.40	7.16
38	0	3.92	2.83	0	0.996	6.72	6.90
39	0	3.92	4.21	0	1.047	7.22	6.98
40	0	3.92	2.75	0	1.030	6.15	6.80
41	0	3.92	4.21	0	1.032	6.82	7.03
43	0	3.92	4.41	0	1.024	7.22	7.09
46	0	3.92	3.92	0	0.985	7.22	7.13
48	0	3.97	5.02	0	0.852	6.92	6.87
52	0	3.92	3.02	0	0.990	6.92	6.96
55	0	3.96	3.35	0	0.881	6.22	6.68
60	0	3.92	1.28	0	1.028	5.40	6.53

[a]Observed value
[b]Predicted values of pC_2 from Eq. (2).

Table 5. Descriptors and observed, calculated and predicted COX–1 inhibition and selectivity of compounds

S	Descriptors				pC_1			Selectivity		
No.	BELm6	GATS7e	GATS8e	GATS1e	[a]Obs.	[b]Calc	[b]LOO	[a]Obs.	[c]Calc	[c]LOO
1	1.19	0.63	0.83	0.73	7.30	7.12	6.53	–1.18	–0.70	0.08
2	1.34	0.65	0.68	0.70	4.48	4.77	4.87	2.12	1.48	1.38
5	1.39	0.64	0.72	0.69	4.43	4.32	4.29	2.17	2.28	2.30
22	1.40	0.82	0.79	0.69	5.52	5.78	5.82	1.00	0.90	0.89
25	1.45	0.57	0.90	0.70	4.18	4.05	3.95	3.12	3.51	3.67
27	1.40	0.67	0.72	0.70	5.60	*	*	1.70	1.94	1.96
31	1.54	0.78	0.92	0.68	4.18	4.57	4.72	3.22	2.98	2.93
32	1.59	0.85	0.91	0.68	4.77	4.40	4.20	2.63	3.07	3.27
33	1.41	0.64	0.73	0.67	4.35	4.21	4.18	2.95	2.64	2.58
42	1.44	0.81	0.78	0.67	5.10	5.24	5.26	2.12	1.57	1.53
43	1.45	0.80	0.77	0.67	5.40	5.02	4.99	1.82	1.77	1.76
44	1.45	0.80	0.77	0.67	5.40	5.02	4.99	1.30	1.77	1.80
51	1.46	0.74	0.85	0.72	4.28	4.84	4.90	1.91	1.94	1.94
55	1.48	0.83	0.75	0.72	4.77	4.75	4.74	1.45	1.50	1.52
59	1.41	0.64	0.76	0.68	4.35	4.33	4.33	2.95	2.55	2.49
63	1.42	1.00	0.86	0.65	7.22	7.16	7.10	0.08	–0.07	–0.19
64	1.42	0.88	0.69	0.53	5.40	5.52	5.56	1.82	2.09	3.16

[a]Observed value
[b]Calculated (Cal.) and predicted (LOO) values of pC_2 from Eq. (2).
*Compound removed as outliers

The statistical quality of the developed equations was judged by the parameters like correlation coefficient (*R*), explained variance (*%EV*), standard error of estimate (*SEE*), variance ratio (*F*) at specified degrees of freedom (df), 95% confidence intervals of the regression coefficients. The predictive power of equations were validated by leave one out (*LOO*) cross–validation method (R^2_{CV} values), standard deviation based on predicted residual sum of squares (S_{PRESS}) and standard deviation of error of prediction (S_{DEP}).

3 RESULTS AND DISCUSSION

The number of developed equations was high, so further analysis was based on statistically significant parameters, namely R, R^2_{CV}, F, SEE and inter–correlation among parameters. Here we report the results for the QSAR study for COX–1 and COX–2 inhibition and selectivity.

3.1 COX–2 Inhibition

Regression analysis of training set generated model 1 that contain NCONR2, BEHM2, MLOGP, T(Cl..Cl) and GATS7v descriptors, which is able to explain 69.2% of variance of COX2 inhibition.

$$\begin{gathered} pC2 = [78.724\ (\pm 22.993)] + NCONR2\ [-1.695\ (\pm 0.727)] + BEHM2\ [-17.746\ (\pm 5.577)] + \\ MLOGP\ [0.216\ (\pm 0.169)] + T(Cl..Cl)\ [0.039\ (\pm 0.030)] + GATS7v\ [-2.984\ (\pm\ 2.502)] \\ n = 49 \quad R = 0.832 \quad \%EV = 69.2 \quad p<0.001 \quad F = 19.370 \quad SEE = 0.432 \\ R^2_{CV} = 0.625 \quad S_{PRESS} = 0.477 \quad S_{DEP} = 0.447 \end{gathered} \tag{1}$$

This model has three outliers (compounds **36**, **51** and **65**) because their residual values exceeded twice the standard error of estimate. When these outliers have been removed from the dataset, a highly significant Eq. (2) has been found which is able to explain 82.4% of variance of COX2 inhibition. This equation has a high internal predictivity as shown by the good Q^2 value of 0.765.

$$\begin{gathered} pC2 = [84.506\ (\pm 17.254)] + NCONR2\ [-1.748\ (\pm 0.542)] + BEHM2\ [-19.065\ (\pm 4.169)] + \\ MLOGP\ [0.175\ (\pm\ 0.127)] + T(Cl..Cl)\ [0.033\ (\pm 0.023)] + GATS7v\ [-3.339\ (\pm 1.918)] \\ n = 46 \quad R = 0.908 \quad \%EV = 82.4 \quad p<0.001 \quad F = 37.450 \quad SEE = 0.317 \\ R^2_{CV} = 0.765 \quad S_{PRESS} = 0.367 \quad S_{DEP} = 0.342 \end{gathered} \tag{2}$$

The parameters used in the equation are almost independent, which can be seen from the Pearson correlation matrix (Table 6).

Table 6. Pearson correlation matrix for descriptors influencing COX–2 inhibition

	NCONR2	BEHM2	MLOGP	T(Cl..Cl)	GATS7v
NCONR2	1.00				
BEHM2	0.09	1.00			
MLOGP	0.07	0.19	1.00		
T(Cl..Cl)	0.07	0.01	0.09	1.00	
GATS7v	0.49	0.41	0.23	0.09	1.00

The coefficient corresponding to the number of tertiary amide bears a negative sign in model 2 which indicates that absence of it (tertiary amide) or that the presence of primary or secondary amide is favorable for COX–2 inhibition. This suggests that hydrogen bonding from amide nitrogen to a protein acceptor is an important determinant of receptor binding. This study supports and reconfirms previous SAR work reported by Kalgutkar *et al*. [20]. When atomic properties combine with the Burden matrix, the resultant eigenvalues encode global structure–property characteristics of a molecule. The coefficient the descriptor BEHM2 has a negative sign in model 2 which indicates that the lower is the highest eigenvalues the higher is the COX–2 inhibition.

COX–2 enzyme is a membrane–based enzyme and entry of the inhibitor in the enzyme requires that molecule should be lipophilic in nature. Thus an increase in lipophilicity increases COX–2

inhibition as suggested by positive sign of MLOGP. The sum of topological distances between Cl..Cl measures the position of chlorine atoms with respect to each other. The coefficient the descriptor T(Cl..Cl) has a positive sign in model 2 which indicates that an increase in distance is favorable to COX–2 inhibition. GATS7v is the volume–weighted Geary graph spatial autocorrelation coefficient of the seventh lag. Strong autocorrelation produces low values of this index; moreover, positive autocorrelation translates into values between 0 and 1 whereas negative autocorrelation produces values larger than 1. The coefficient corresponding to the descriptor GATS7v has a negative sign in model 2 which indicates that low values for this descriptor are favorable for COX–2 inhibition. The model 2 was tested for 17 compounds as a test set (Table 4) and the predicted activity shows linear relationship (Figure 1) with observed activity in the test set (R = 0.88) showing the robustness of the model.

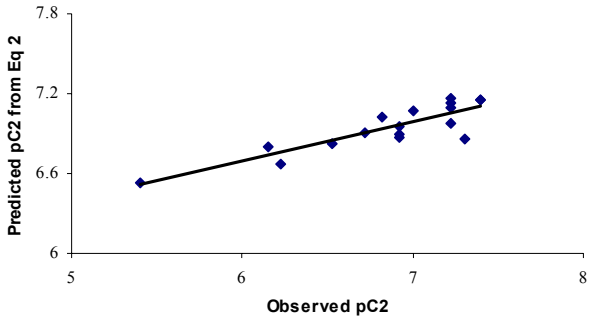


Figure 1. Observed versus Calculated pC_2 from Eq (2) for COX–2 inhibition.
Predicted pC_2 = 0.297 (Observed pC_2) + 4.909, R^2 = 0.7714, R = 0.88.

3.2 COX–1 Inhibition

Data set of all compounds having COX–1 inhibitory activity was chosen for regression analysis and model 1 has been obtained that contain BELm6, GATS7e and GATS8e descriptors, which is able to explain 84.7% of variance of COX–1 inhibition.

$$pC_1 = [13.126\ (\pm 3.948)] + \text{BELm6}\ [-11.152\ (\pm 3.190)] + \text{GATS7e}\ [6.545\ (\pm 2.181)] + \text{GATS8e}\ [3.743\ (\pm 3.241)]$$
$$n = 17 \quad R = 0.920 \quad \%EV = 84.7 \quad p<0.001 \quad F = 24 \quad SEE = 0.414$$
$$R^2_{CV} = 0.740 \quad S_{PRESS} = 0.540 \quad S_{DEP} = 0.473 \qquad (3)$$

This equation has one outlier (compound **27**) as its residual value exceeded twice the standard error of estimate. When this outlier has been removed from the dataset, a highly significant Eq. (4) has been found which is able to explain 91.8% of variance of COX–2 inhibition. This equation has high internal predictivity as shown by good Q^2 value of 0.836, and the predicted activity showed linear relationship with the observed activity (Figure 2, R = 0.92).

$$pC_1 = [12.442\ (\pm 3.039)] + \text{BELm6}\ [-11.416\ (\pm 2.434)] + \text{GATS7e}\ [6.946\ (\pm 1.681)] + \text{GATS8e}\ [4.622\ (\pm 2.535)]$$
$$n = 16\ R = 0.958\ \%EV = 91.8\ p<0.001\ F = 45.000 \quad SEE = 0.312$$
$$R^2_{CV} = 0.836\ S_{PRESS} = 0.443\ S_{DEP} = 0.383 \qquad (4)$$

The parameters used in the equation are almost independent, as can be seen from the Pearson correlation matrix (Table 7).

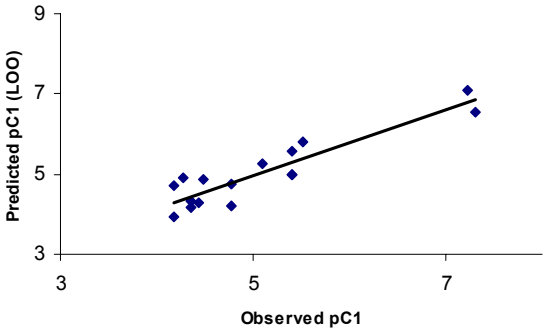


Figure 2. Observed versus predicted (LOO) pC_1 for COX–2 inhibition.

Table 7. Pearson correlation matrix for descriptors influencing COX–1 inhibition

	BELm6	GATS7e	GATS8e
BELm6	1.00		
GATS7e	0.44	1.00	
GATS8e	0.39	0.16	1.00

The lowest eigenvalue no. 6 of the Burden matrix is negatively correlated and the Geary autocorrelation lag 7 and lag 1 indices are positively correlated with the COX–1 inhibition.

3.3 Selectivity

Selectivity is important to increase the therapeutic effect and to decrease the side effects. In this context Eq (5) was developed.

$$\begin{gathered}\text{Selectivity} = [-3.666\ (\pm 5.640)] + \text{BELm6}\ [12.814\ (\pm 2.821)] + \text{GATS7e}\ [-8.542(\pm 2.306)] + \\ \text{GATS1e}\ [-9.340\ (\pm 5.476)] \\ n = 17\ R = 0.949\ \%EV = 90.1\ p<0.001\ F = 39.7 \qquad SEE = 0.392 \\ R^2_{CV} = 0.711\ S_{PRESS} = 0.671\ S_{DEP} = 0.587\end{gathered} \tag{5}$$

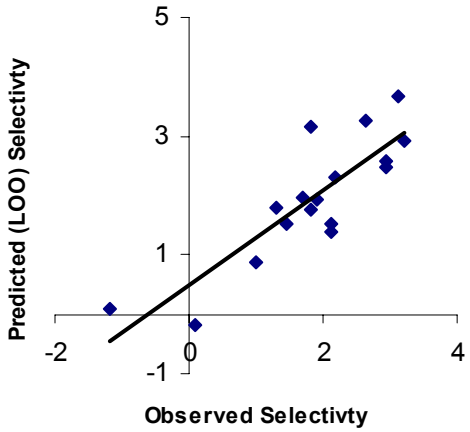


Figure 3. Observed versus predicted (LOO) selectivity for COX–2 inhibition.

Eq (5) is able to explain 90.1% of variance of COX2 inhibition. This equation has a high internal predictivity as shown by a good Q^2 value of 0.711, and the predicted activity has a good linear relationship with the observed activity (Figure 3). The parameters used in the equation are almost independent, as can be seen from the Pearson correlation matrix (Table 8).

Table 8. Pearson correlation matrix for descriptors influencing selectivity

	BELm6	GATS7e	GATS1e
BELm6	1.00		
GATS7e	0.44	1.00	
GATS1e	0.17	0.47	1.00

The lowest eigenvalue no. 6 of the Burden matrix is positively correlated and the Geary autocorrelation lag 7 and lag 1 indices are negatively correlated with the selectivity.

4 CONCLUSIONS

Selective inhibition of cyclooxygenase–2 (COX–2) is an important strategy in the design of potent anti–inflammatory compounds with significantly reduced side effects. In view of this, ester and amide derivatives of indomethacin were selected to explore the necessary structural requirement of these analogues for selective COX–2 inhibition.

In the present investigation, a QSAR study was performed using 66 ester and amide derivatives using Dragon 3.0. The cluster analysis technique was applied for the generation of training set and test set. The relationship between the inhibitory activity and various descriptors is established by step–wise multiple regression analysis using SYSTAT 10.2 and VALSTAT. The analyses have produced good predictive and statistically significant QSAR models. These models were cross–validated with the leave–one–out (LOO) method.

The values of statistical data are: R = 0.908, F = 37.45, SEE = 0.317 and R^2_{CV} = 0.765 for COX–2 inhibition; R = 0.958, F = 45.00, SEE = 0.312 and R^2_{CV} = 0.836 for COX–1 inhibition; and R = 0.949, F = 39.7, SEE = 0.392 and R^2_{CV} = 0.711 for selectivity. The predicted activity shows linear relationship with observed activity.

The present studies suggest that hydrogen bonding from amide nitrogen to a protein acceptor is an important determinant for receptor binding. Lipophilicity and topological distance indices are correlated to COX–2 inhibition. Also, Geary autocorrelation and eigenvalues indices modulate COX–1 and COX–2 inhibition and selectivity. These studies are promising for the development of novel compounds, which may have potent anti–inflammatory activity devoid of side effects like gastric ulcer and renal failures.

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