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QSAR Analysis of Some Fused Pyrazoles as Selective Cyclooxygenase-2 Inhibitors: A Hansch Approach

Quantitative structure activity relationships (QSAR) for two unique series of centrally fused pyrazole ring systems have been studied for selective cyclooxygenase-2 inhibitory activity. Several statistically significant QSAR models were developed and suggest that hydrophobicity of entire molecules and a fluorine atom substitution at position 8 of the non benzene sulphonyl ring fused with central pyrazole core of series 1 compounds is crucial for improved COX-2 selectivity. Various structural and physicochemical stipulations to improve the inhibitory activities of the enzymes among individual series of compounds are also discussed. The conclusions derived may serve as an example to advance the design of new selective COX-2 inhibitors.

Keywords: QSAR; COX-1; COX-2; Selectivity; Hydrophobicity

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Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain and inflammation especially arthritis or arthritis-associated disorders [1, 2]. The rate-limiting step in the synthesis of prostaglandins and thromboxanes is the conversion of arachidonate to prostaglandin H_2 which is catalyzed by cyclooxygenase (COX) enzymes [3]. The classical NSAIDs act by nonselective inhibition of COX enzymes [4–6]. COX exists in two isoforms namely COX-1 and COX-2. The constitutive enzyme, COX-1, is associated with general housekeeping functions and the inducible enzyme, COX-2, is responsible for production of proinflammatory prostaglandins [7]. The severity of gastrointestinal toxicity associated with the chronic use of classical NSAIDs is due to the inhibition of COX-1 whereas inhibition of COX-2 is primarily responsible for their clinical efficacy. Some of the selective COX-2 inhibitors with proven therapeutic utility for the treatment of inflammation include Celecoxib, Rofecoxib, Valdecoxib, and Etoricoxib [8–11]. X-ray crystallographic studies [12, 13] suggest that it is a single amino acid difference that is primarily responsible for the selectivity of most selective COX-2 inhibitors: at position 523 is an isoleucine molecule in COX-1 and valine in COX-2. The amino acid valine, which is smaller than isoleucine by a single methyl group in COX-2, allows access to a side pocket, the binding site of most selective COX-2 inhibitors, whereas the bulkier isoleucine in COX-1 blocks access to that side pocket.

Compounds with a central heterocyclic or carbocyclic core bearing two vicinal aryl rings have been studied in a greater extent for selective COX-2 inhibition. Substitution of one of the aromatic rings with a sulphonamido or methyl sulphonyl group is crucial for selective COX-2 inhibition. The central heterocyclic core is essential in properly orienting the aromatic rings in the COX binding site. The common heterocycles used as the central core includes pyrrole, thiazole, oxazole, furan, imidazole, isooxazole, pyrimidine, thiophene, and so on. Among these, pyrazole derivatives are of special interest, ever since the discovery of 1,5 diaryl pyrazole derivative Celecoxib as a promising lead structure for selective COX-2 inhibition. A recent QSAR review reported by Hansch et al. [14] provides plethora of information regarding the most of the important classes of COX inhibitors. Unfortunately a search of literature for fused heterocylces as template revealed only few citations for selective COX-2 inhibition. Hence it is considered worthwhile to study the fused heterocyclic ring systems with physicochemical and structural pertinence for selective COX-2 inhibition. In view of this, two series of compounds with a fused pyrazole core reported as selective COX-2 inhibitors were selected from the literature.

In this communication, we report the QSAR analysis of the afore-mentioned two series of compounds. Series 1 comprises of 15 compounds [15] with a central pyrazole ring fused to the nonsulphonyl benzene

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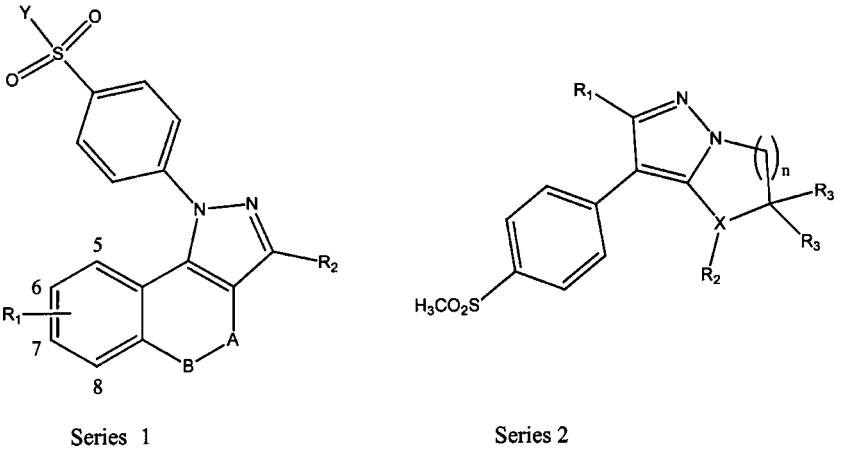

Figure 1. Fused pyrazole central core of series 1 and 2 compounds.

ring of common diaryl heterocylces type. Series 2 consists of 9 compounds of bicyclic pyrazoles recently reported by Ranatunge et al. [16] as selective COX-2 inhibitors. Structures of both the series are depicted in Figure 1. Despite the smaller number of compounds to be considered for QSAR analysis, the latter series is considered, as it is being the first time to introduce such compounds after the imidazo [1,2-a] pyridines and pyrazole [1,5-b] pyridazines under the bicyclic core for selective COX-2 inhibition.

Results and discussion

For series 1 compounds, COX-1 and COX-2 *in vitro* inhibitory activity are reported as IC_{50} in μM units, where IC_{50} is the drug concentration required to inhibit 50% of the enzymes. For the present QSAR study, the reported IC_{50} was converted to negative logarithm (pIC_{50}) in molar units. The *in vitro* human whole blood COX-2 inhibition by bicyclic pyrazoles of series 2 was reported as % inhibition (P). For the QSAR study, the data were converted to log P/(100-P). Compounds of both the series with observed activity are enlisted in Tables 1 and 2. QSAR models were built using the regression analysis module of Systat. The correlation matrix was used to correlate the biological activity with the various physicochemical and structural predictor variables. Descriptors with intercorrelation above $r > 0.5$ are not considered while deriving QSAR models. The predictor variables with p values greater than 0.05 were eliminated whilst deriving the QSAR models in order to assure their statistical reliability. The QSAR models were evaluated by using the statistical parameters viz., correlation coefficient (r), or coefficient of determination (r^2), adjusted r^2 (r^2_{Adj}), standard error of estimate (s), Fischer F-value and student's t-distribution. The latter is used to assess the significance of the individual regression terms. The figures within the parentheses following the coefficient terms are the standard error of the regression terms and the constants. Durbin-Watson (DW) test was employed to check the serial correlation in residuals. Since the DW values in all our derived models are greater than 1.4, there is probably not any serious autocorrelation in the residuals. A data point is considered as an outlier if it has a large magnitude (when the residual value exceeds twice the standard error of estimate of the model). Self-consistency of the derived models is ensured using the leave-one-out (Loo) process and the predictability of each model was assessed using cross-validated r^2 or q^2. Statistically significant QSAR models are discussed below:

Model for COX-2 inhibition

Model-1

$pIC_{50} = 1.440\ (\pm 0.252)\ I_{S\text{-}CH2} + 0.692\ (\pm 0.161)\ ClogP + 3.201\ (\pm 0.700)$
$n = 15,\ r = 0.899,\ r^2 = 0.807,\ r^2_{Adj} = 0.775,\ s = 0.390,$
$F = 25.14,\ p = 0.000,\ q^2 = 0.6463,$
$DW = 1.833.$

Model-1 was developed for COX-2 inhibitory activity for the series 1 compounds. The indicator variable $I_{S\text{-}CH2}$ denotes the presence of isothiochromanone ring fusion assuming a value of 1 for A = S, B = CH_2, and 0 for others. The positive contribution of the indicator variable $I_{S\text{-}CH2}$ suggests a isothiochromanone ring

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Table 1. Series 1 compounds with physicochemical, structural descriptors, observed and predicted activities through various models.

Comp No.	A	B	R_1	R_2	Y	Observed activity COX-2 pIC_{50} (Molar)	Observed activity COX-1 pIC_{50} (Molar)	Observed activity Log (COX-1 /COX-2)	ClogP	$I_{S\text{-}CH2}$	I_{8F}	Predicted activity (Loo) Model-1	Model-2	Model-3	Model-4
1	S	CH_2	H	CF_3	NH_2	7.6990	6.3979	1.3010	4.2555	1	0	7.57712	6.32828	1.08600	1.15465
2	CH_2	CH_2	H	CF_3	NH_2	5.9586	4.8665	1.0934	4.3309	0	0	6.32418	5.08098	1.11566	1.23072
3	CH_2	S	H	CF_3	NH_2	6.3372	5.0132	1.3222	4.2422	0	0	6.03778	5.00762	1.08297	1.14430
4	CH_2	O	H	CF_3	NH_2	5.8761	5.1487	0.7243	3.8028	0	0	5.81122	4.93986	1.16838	1.00449
5	S	CH_2	$7CH_3$	CF_3	NH_2	7.6576	6.1024	1.5563	4.7545	1	0	7.98525	6.40216	1.04953	1.34973
6	S	CH_2	$7OCH_3$	CF_3	NH_2	7.8860	6.5229	1.3617	4.1745	1	0	7.49908	6.29703	1.07733	1.10211
7	S	CH_2	$7OCH_3$, 8F	CF_3	NH_2	8.0458	5.5638	2.4814	4.2575	1	1	7.54514	5.24433	2.63147	2.46997
8	S	CH_2	7F	CF_3	NH_2	7.3979	6.5686	0.8451	4.3986	1	0	7.71886	6.28559	1.15113	1.32039
9	S	CH_2	$7CH_3$, 8F	CF_3	NH_2	7.7959	4.9547	2.8407	4.8976	1	1	8.08942	5.44738	2.51171	2.75622
10	S	CH_2	8F	CF_3	NH_2	7.6778	5.2048	2.4728	4.3986	1	1	7.68793	5.36401	2.63434	2.56533
11	S	CH_2	7,8diF	CF_3	NH_2	8.2218	5.5735	2.5809	4.4716	1	1	7.67878	5.24111	2.59830	2.57583
12	S	CH_2	$7OCH_3$, 8F	CF_2H	NH_2	7.4948	–	–	3.4197	1	1	6.91682	–	–	–
13	S	CH_2	$7OCH_3$, 8F	CF_3	CH_3	7.1249	–	–	4.4955	1	1	7.83280	–	–	–
14	S-O	CH_2	$7OCH_3$, 8F	CF_3	NH_2	5.8239	–	–	2.4814	1	1	6.94712	–	–	–
15	S	CH_2	7F	CN	NH_2	6.8539	6.1192	0.6990	3.0755	1	0	6.74187	6.39795	1.17200	0.38809

Table 2. Series 2 compounds with physicochemical descriptors, observed and predicted activities through model-5.

Comp No.	X	n	R_1	R_2	R_3	Observed activity Log P/(100-P)	CMR	Predicted activity (Loo) Model-5
1	O	1	Cyclohexyl	–	H	0.9542	9.2632	0.5823
2	O	1	Phenyl	–	H	0.9542	9.1690	0.7289
3	N	1	Cyclohexyl	H	H	0.6021	9.4788	0.3845
4	N	1	Cyclohexyl	$COCH_3$	H	−0.9542	10.4421	−0.6821
5	N	1	Cyclohexyl	CH_3	H	−0.2126	9.9426	−0.1366
6	N	1	Cyclohexyl	CH_2COO^tBu[#]	H	0.0871	12.4504	–
7	N	1	Cyclohexyl	CH_2COOH	H	−0.2881	10.5952	−1.4019
8	O	1	Cyclohexyl	–	CH_3	−0.8451	10.1908	−0.3655
9	O	2	Cyclohexyl	–	H	−0.4771	9.7270	0.21144

[#] Data point considered as outlier.

structure in fusing nonbenzene sulphonyl ring with the central pyrazole core for enhanced activity. The absence of other indicator variables $I_{CH2\text{-}S}$, $I_{CH2\text{-}CH2}$, $I_{CH2\text{-}O}$ shows that the thiochromanone, chromanone, and tetralone ring type fusion as detrimental for COX-2 inhibitory activity. The contribution of ClogP corroborates the presence of a hydrophobic binding pocket in the binding site of COX-2. In the derived Model-1, the t value of the descriptors $I_{S\text{-}CH2}$ and ClogP are 5.726 and 4.311, respectively. Therefore, the chosen descriptors are statistically significant above 99% level of significance (two tailed test). Table 3 illustrates the orthogonal nature of the descriptors.

Model for COX-1 inhibition

Model-2

pIC_{50} = 1.333 (±0.173) $I_{S\text{-}CH2}$ − 1.018 (±0.159) I_{8F} + 5.009 (±0.137)
n = 12, r = 0.943, r^2 = 0.889, r^2_{Adj} = 0.865, s = 0.237, F = 36.09, p = 0.000, q^2 = 0.8080,
DW = 2.340.

Model-2 was developed for COX-1 inhibitory activity reported for 12 compounds of series 1. Model-2 is a di-parametric equation explaining 88.9% variance of COX-1 inhibitory activity. The fairly high variance ratio

Table 3. Inter correlation matrix for descriptors of model-1, 2, and 4.

	$I_{S\text{-}CH2}$	ClogP	I_{8F}
$I_{S\text{-}CH2}$	1.000		
ClogP	−0.023	1.000	
I_{8F}	0.408	0.401	1.000

shows the overall statistical significance of the derived model. The positive coefficient of the indicator variable once again necessitates the importance of isothiochromanone ring fusion even for improving COX-1 inhibitory activity. Hence, it can be concluded that the isothiochromanone ring type fusion is conducive both for COX-1 as well as COX-2 inhibitory activities. I_{8F} is another structural variable assigned a value of 1 for compounds with 8F substitution and 0 for others. Its negative contribution suggests an untoward effect of a fluorine atom at position 8 for COX-1 inhibitory activity.

Models for COX-2 selectivity

Model-3

Log (COX-1/COX-2) = 1.481 (±0.175) I_{8F} + 1.113 (±0.101)
n = 12, r = 0.936, r^2 = 0.877, r^2_{Adj} = 0.865, s = 0.286, F = 71.29, p = 0.000, q^2 = 0.833,
DW = 2.328.

Model-3 was developed for 12 compounds in order to explain the selectivity of COX-2 inhibition over COX-1. Log (COX-1/COX-2) in Model-3 denotes the logarithm of the reported selectivity index (COX-1/COX-2). Model-3 is a mono-parametric equation with 87.7% explained variance. The positive contribution of I_{8F} suggests a fluorine atom substitution at position 8 for improved enzyme selectivity among series 1 compounds. This is attributed to the high lipophilic nature and ability to form a strong hydrogen bond by the fluorine atom with the amino acid residues of active site of COX-2 enzyme. Since a good electron donating group in para position of the nonbenzene sulphonyl ring is crucial for selectivity, we attempted to correlate the indicator variable I_{7OCH3} with the selectivity index. Surprisingly, our investigation reveals no such significant correlation. The indistinctness of the results is probably due to the fusion of the nonbenzene sulphonyl ring with the central pyrazole core among these congeners.

Model-4

pIC_{50} = 1.298 (±0.133) I_{8F} + 0.485 (±0.141) ClogP − 0.891 (±0.586)
n = 12, r = 0.973, r^2 = 0.947, r^2_{Adj} = 0.935, s = 0.198, F = 80.26, p = 0.000, q^2 = 0.9102,
DW = 2.475.

Model-4 was developed upon including the ClogP term to Model-3. The inclusion of ClogP improves all the statistical parameters. The observed t value of 3.44 of the added parameter ClogP shows it is significant above the 99% confidence interval. The positive contribution of the ClogP term in both Model-1 and Model-4 suggests hydrophobicity of the entire molecules as key factor in governing the potency and selectivity of COX-2 inhibition over COX-1. The predictability of the model is excellent as reflected from the q^2 value.

Model-5

Log P/(100-P) =−1.236 (±0.305) CMR + 12.143 (±3.010)
n = 8, r = 0.856, r^2 = 0.732, r^2_{Adj} = 0.688, s = 0.431, F = 16.41, p = 0.007, q^2 =0.456,
DW = 1.669.

Model-5 was developed upon omitting data point 6 as outlier for bicyclic pyrazoles of series 2 compounds. Residual analysis shows compound 6 as outlier and the outlying behavior is probably due to the steric hindrance caused by the bulky tertiary butyl ester group. The negative coefficient of the CMR term in model-5 indicates that with increasing polarizability, larger molecules decrease the COX-2 inhibitory activity.

In conclusion, our QSAR investigations on two series of fused pyrazoles ring systems reveal important structural insights. In series 1 compounds, isothiochromanone ring fusion between the nonbenzene sulphonyl ring and the central pyrazole core is crucial for cycloxygenase enzyme inhibition. Hydrophobicity of entire molecules also plays an important role in promoting both COX-2 inhibitory activity and selectivity. A fluorine atom substitution at position 8 of the nonbenzene sulphonyl ring fused with central pyrazole core is decisive for improved COX-2 selectivity. In series 2 compounds increasing size and polarizability of individual molecules is detrimental to COX-2 inhibitory activity. The epitome of current study provides important structural and physicochemical requirements that can be used in designing more selective COX-2 inhibitors among these congeners.

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Experimental

For QSAR analysis, COX-1, COX-2, inhibitory activity, and COX-1/COX-2 selectivity ratio were considered as dependent variables and the calculated physicochemical properties and indicator variables as independent variables. The linear regression analysis was performed using Systat version 10.2 on a Compaq PC. The physicochemical properties such as ClogP and CMR were calculated using ChemDraw ultra 6.0.1 software. ClogP is the calculated partition coefficient of compounds in octanol/water. It is a measure of hydrophobicity of compounds. CMR is the calculated molar refractivity based on the Lorentz-Lorentz equation, MR = $(n^2\text{-}1)/(n^2\text{+}2)$ MW/d, where n is the index of refraction, MW represents molecular weight of the compound and d is the density. It is a measure of volume and polarizability of the whole molecules. The other physicochemical descriptors were derived from the literature [17].

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