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QSAR on Heteroaryl-Phenyl-Substituted Pyrazole Derivatives as Selective and Potent Canine COX-2 Inhibitors

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ABSTRACT

A series of heteroaryl-phenyl-substituted pyrazole derivatives was subjected to QSAR analysis and the equations generated thereof showed good correlation between COX-2 inhibitory activity and physico-chemical as well as steric properties of the derivatives. One of the significant equations generated has a r value of 0.859 and cross validated r^2 (q^2) value of 0.534. The contributing parameters include Connolly Accessible Area (CAA), Principal Moment of Inertia at z-axis (PMZ) and Partition Coefficient (PC).

Keywords: COX-2, QSAR, steric parameters, physicochemical properties, regression, statistical validation

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and inflammatory swelling by blocking prostaglandin (PG) synthesis at the step of cyclooxygenase (COX) enzyme. However, most of the NSAIDs, which are clinically used to date, inhibit the production of PGs not only associated with inflammatory processes but also involved in maintaining normal physiological processes. The most common NSAIDs frequently show side effects including life-threatening ulcer and kidney dysfunction, which limit their therapeutic value for safe and long-term use[1,2]. The enzyme responsible for PG synthesis is PG endoperoxide synthase or COX. Cyclooxygenase is a bifunctional intracellular membrane bound haem-protein that catalyzes the bisoxygenation of arachidonic acid to PGG_2 and the reduction of PGG_2 to PGH_2 [3]. This enzyme, COX exists as two isoforms, COX-1 and COX-2 [4,5]. COX-1 is constitutively expressed in most cells and tissues and is the major isoform of gastrointestinal tissue [6], whereas COX-2 is an inducible isozyme, the expression of which is elevated in response to inflammatory cytokines and endotoxin [7]. Since COX-1 is concerned with the maintenance of the normal physiological functions of the body, therefore non-selective inhibition of COX results in untoward side-effects. Thus selective inhibition of inducible form of COX i.e., COX-2 may provide an anti-inflammatory/analgesic agent without the side-effects currently associated with NSAIDs.

Progressive degenerative joint disease, or osteoarthritis, is the most common cause of chronic pain in dogs [8]. It is estimated that out of every five adult dogs, or approximately 8 million animals, has osteoarthritis, yet nearly half (48%) of these patients are untreated [9]. As with humans chronic use of NSAIDS in dogs is often associated with GI side effects [10]. Many agents with fewer side-effects are marketed for the treatment of inflammation in dogs [11,12] and some are under clinical investigation[13]. In the same context hetero-aryl-phenyl substituted pyrazole derivatives have shown to be highly selective and potent inhibitors of canine COX-2 enzyme [14]. In this paper, we report the 3D QSAR analysis carried out on a series of hetero-aryl-phenyl substituted pyrazole derivatives.

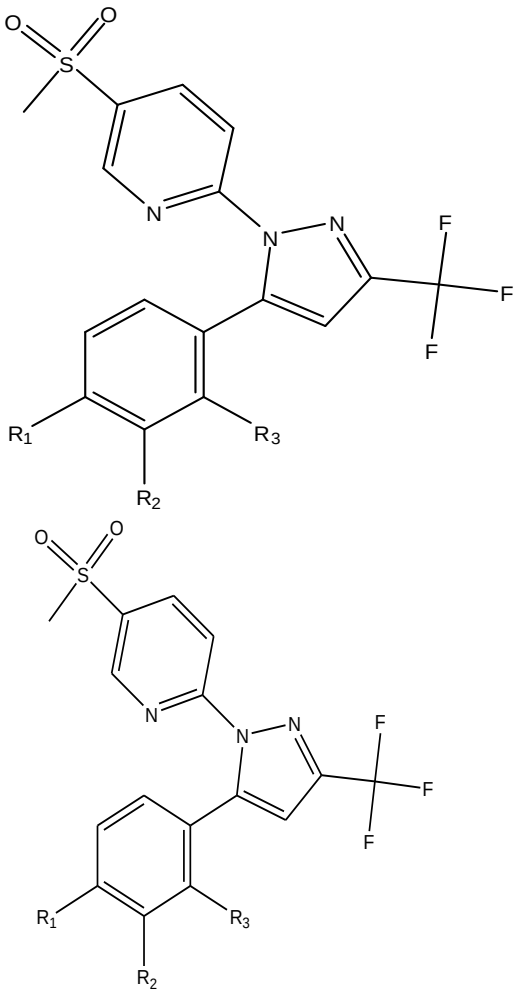


Fig 1: Structure of pyrazole analogs used in the study

2. MATERIALS AND METHODS

Data Set

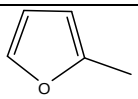
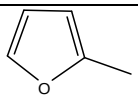
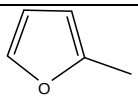
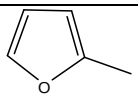
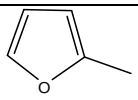
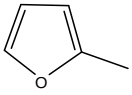
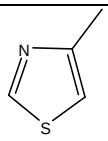
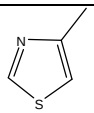
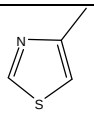
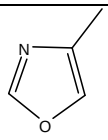
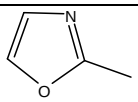
The studied 22 compounds are a series of hetero-aryl-phenyl-substituted pyrazoles, whose structures are shown in Fig. (1) and Table (1). The biological activity expressed as IC_{50} (in-vitro whole blood for COX-2) was taken from literature. The IC_{50} values range from 0.01 to >0.5 µM. QSAR analysis was performed on all the compounds leaving four compounds from the data set due to lack of defined activity. For analysis purposes, -logIC_{50} (pIC_{50}) values were used as the dependent variables.

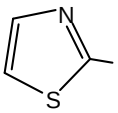
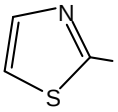
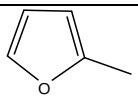
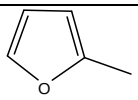
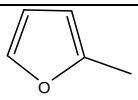
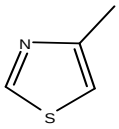
Descriptor generation

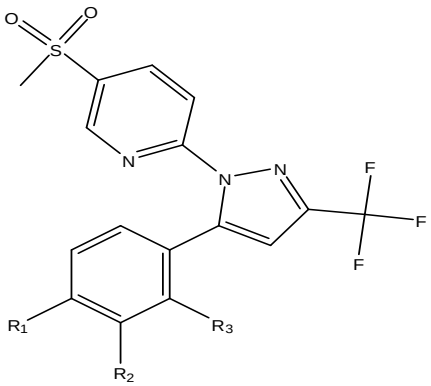
The molecular modeling (3D) studies were carried out using CS Chem Office Ultra 2006 version 10.0 loaded on a P4 computer. The two dimensional structures of the reported compounds were drawn in CS Chem Draw Ultra10.0 and saved with similar names as in the reported series. These structures were transferred to CS Chem 3D Ultra 10.0 molecular modeling window. Each structure is subjected to energy minimization by using calculation module in the active window of CS Chem 3D Ultra 10.

The minimization of energy and geometry optimization of all the molecules were carried out first by using Allinger's Molecular Mechanics (MM2) method keeping the RMS gradient at 0.1 and then by Gamess interface by using Austin Mechanics (AM1) at unrestricted open shell wave function and POPLE as exponent. The energy minimized structures thus generated were used for the calculation of physico-chemical, electronic and steric properties by using compute properties module available in CS Chem 3D active window. Various thermodynamic, steric, electronic and topological parameters calculated are reported in Table (2), which were further used in QSAR analysis.

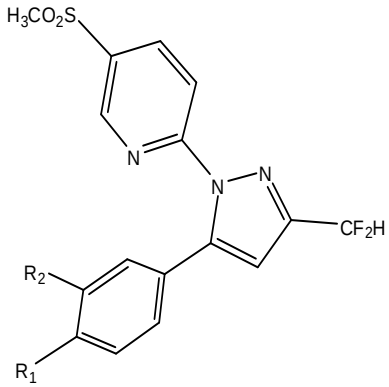
Once the descriptors are generated, multiple linear regression analysis was performed on the generated data using the calculated descriptors as independent variables and pIC50 values as dependent variables taken from the reported series [14]. First of all, the descriptors are checked to ensure: (a) that values of each descriptor are available for each structure; and (b) that there is a variation in these values. Descriptors for which values are not available for every structure in the data set in question are discarded. Descriptors having a constant value for all structures in the data set are also discarded. As a next step, pair correlation matrix of all the descriptors was generated using 'data analysis' tool in Excel 2003. This helped in eliminating highly correlated descriptors and thus reducing the descriptor pool for multiple regression analysis.

Compound Entry No.	Compound Name	Substituents			IC_{50} (μM) for COX-2 Inhibition	pIC_{50} ($-\log IC_{50}$)
		R_1	R_2	R_3		
1	1		Cl	H	0.012	1.7212
2	14		H	H	0.020	1.2218
3	15		CN	H	0.013	2
4	16		F	H	0.050	0.886
5	17		Me	H	0.01	0.7447
6	18		$CONH_2$	H	0.41	0.6777
7	20		CN	H	0.11	1.187
8	21		H	H	0.16	1.522
9	22		Me	H	0.09	0.5376
10	24		H	F	0.29	1.045
11	25		Me	H	0.03	0.7958
12	26		Me	H	0.065	0.9586

13	28		H	H	0.21	0.3872
14	29		H	H	0.18	2
15	31		H	H	0.130	1.301
16	32		Cl	H	0.010	1.886
17	33		F	H	0.060	1.6989
18	34		Me	H	0.019	1.9208



(Comp. Entry No. 1-14)



(Comp. Entry No. 15-18)

Table 1: Structures and Activities of Pyrazole Derivatives used in the study

The minimization of energy and geometry optimization of all the molecules were carried out first by using Allinger's Molecular Mechanics (MM2) method keeping the RMS gradient at 0.1 and then by Gamess interface by using Austin Mechanics (AM1) at unrestricted open shell wave function and POPLE as exponent. The energy minimized structures thus generated were used for the calculation of physico-chemical, electronic and steric properties by using compute properties module available in CS Chem 3D active window. Various thermodynamic, steric, electronic and topological parameters calculated are reported in Table (**2**), which were further used in QSAR analysis.

Once the descriptors are generated, multiple linear regression analysis was performed on the generated data using the calculated descriptors as independent variables and pIC50 values as dependent variables taken from the reported series [14]. First of all, the descriptors are checked to ensure: (a) that values of each descriptor are available for each structure; and (b) that there is a variation in these values. Descriptors for which values are not available for every structure in the data set in question are discarded. Descriptors having a constant value for all structures in the data set are also discarded. As a next step, pair correlation matrix of all the descriptors was generated using 'data analysis' tool in Excel 2003. This helped in eliminating highly correlated descriptors and thus reducing the descriptor pool for multiple regression analysis.

The multiple linear regression analysis was carried out using VALSTAT program [15]. All possible random combinations of the parameters were considered for the QSAR analysis. The validation of the statistical models generated above was done by using Leave-One-Out (LOO) cross validation method [16, 17].

The most important statistical parameters that were considered for cross validation of the generated models are Q^2 (cross validated r^2), Spress (Predicted residual sum of squares), and Sdep (Standard deviation error of Prediction). The predictive power of the models is ascertained by various significant regression parameters like r^2, r, standard deviation (sd), variance ratio (F-test) at 99.9% confidence level.

3. RESULT AND DISCUSSION

Multiple linear regression analysis performed on the data generated during molecular modeling of the 18 compounds for COX-2 inhibition generated two statistically significant equations (Table (**3**)). Both the equations show correlation coefficient values (r)> 0.8 with low values of standard deviations. In model 1, Connolly Solvent Accessible Area (CAA) and Partition Coefficient (PC) contribute positively to the model and Principal Moment of Inertia at Z-axis (PMZ) contributing negatively. The overall contribution of these parameters to the model (model 1) is 71.83%, 4.36% and 23.80% respectively. Similarly in model 2, it is depicted that PC and Connolly Molecular Area (CMA) contribute positively to the model and PMZ contributing negatively again. The overall contribution of these parameters to the model (model 2) is 8.15%, 63.23% and 28.62% respectively. The above models were cross validated using Leave one out method and the results of cross validation summarized in Table (**3**) depict the acceptability and good predictivity of the two models. The various cross validation parameters considered were q^2 which is more than the values of Spress and Sdep in both the equations and having values more than 0.5 in both the models. Both the models pass the Fischer's F-test for 99.9% confidence levels and show very small standard deviations, thus acknowledging the acceptability and predictivity.

In both the equations, we observed that partition coefficient (PC) is contributing positively to biological activity. This can be easily understood from the early observations of the Authors [14] that changing CF_3 to CF_2H on the pyrazoles ring decreases COX-2 selectivity of the pyrazoles derivatives owing to more hydrophilicity of CF_2H group. So our study illustrated the contribution of partition coefficient to the biological activity, which can be further utilized to design more potent and selective derivatives. Principal Moment of Inertia at Z-axis (PMZ) contributes negatively to both the equations showing that less bulky groups should enhance the biological activity if substituted at z-axis direction when the molecule is aligned with its z-axis coordinate as the principal coordinate. Connolly Solvent accessible area and Connolly molecular area contributing positively to the biological activity in both the equations relate the accessibility of a solvent to the molecule. Their positive contribution describes that the more the Surface area of the molecules the greater their biological effect. As a matter of fact, introduction of such groups with long chains and large surface area will enhance the biological effect.

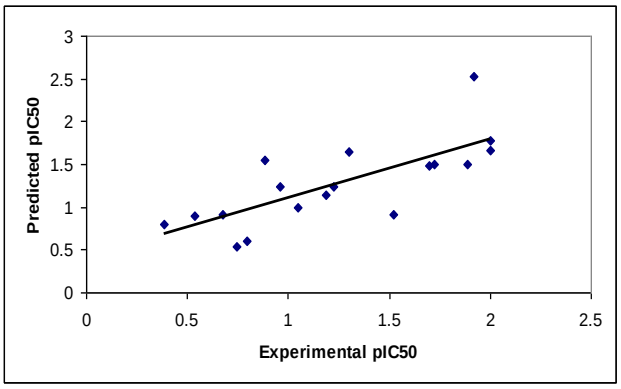


Fig. 2 : Experimental pIC_{50} Vs Predicted pIC_{50}

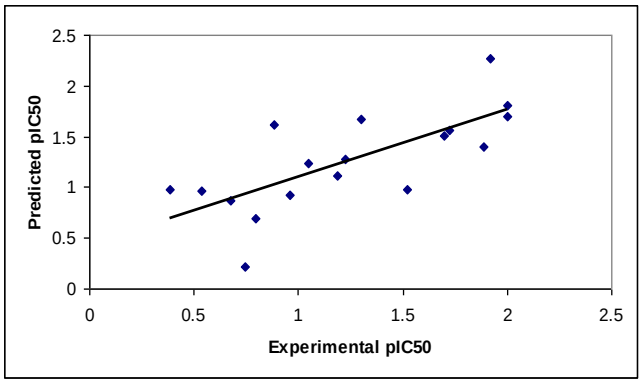


Fig. 3: Experimental pIC_{50} Vs Predicted pIC_{50} for COX-2 inhibition (Model 2)

Moreover the experimental and predicted pIC_{50} values (Table (4)) are showing good correlation as evidenced from the plot of experimental Vs predicted pIC_{50} for both the models which showed linear relationship (Fig. 2 &3).

Table 2: List of various descriptors used in the study

S. No.	Descriptor Symbol	Type of Descriptor	Description (Units)
1	LogP	Thermodynamic	Logarithmic Partition Coefficient
2	MR	Thermodynamic	Molecular Refractivity
3	CAA	Steric	Connolly Accessible Surface Area (Å)
4	CMA	Steric	Connolly Molecular Area (Å)
5	CSEV	Steric	Connolly Solvent Excluded volume (Å)
6	EM	Steric	Exact Mass
7	Oval	Steric	Ovality
8	PMX	Steric	Principal Moment of Inertia at X-axis
			(g/moles Å)
9	PMY	Steric	Principal Moment of Inertia at Y-axis (g/moles Å)
10	PMZ	Steric	Principal Moment of Inertia at Z-axis (g/moles Å)
11	PC	Thermodynamic	Partition Coefficient
12	BI	Steric	Balaban Index
13	CC	Steric	Cluster Count
14	MI	Steric	Molecular Topological Indices
15	PSA	Steric	Polar Surface Area (Å)
16	SA	Steric	Sum of Attributes
17	SOD	Steric	Sum of Degrees
18	SOVD	Steric	Sum of Valence Degrees
19	TC	Steric	Topological Indices
20	WI	Steric	Wiener Index
21	HLC	Thermodynamic	Henry's Law Constant

Table 3: Summary of Multiple Linear Regression Analysis with statistical equations and parameters

QSAR Models		Statistical Parameters					Validation Parameters			
		n	R	r^2	Sd	F	q^2	r^2_{bs}	Spress	Sdep
Model 1	BA = [-11.7259(± 7.73852)] +CAA [0.0264277(± 0.0133777)] +PMZ [-0.000621147(± 0.000388252)] +PC [0.302966(± 0.277248)]	18	0.859	0.738	0.298	13.199	0.533	0.791	0.399	0.352
Model 2	BA = [-6.26669(± 5.7256)] +PC [0.402161(± 0.278014)] +PMZ [-0.000530262(± 0.000381145)] +CMA [0.0302183(± 0.0162681)]	18	0.85	0.72	0.31	12.03	0.50	0.74	0.413	0.364

BA= Biological Activity, CMA= Connolly Molecular Area, PMZ= Principal Moment of Inertia at Z-axis, CAA= Connolly Accessible Area & PC= Partition Coefficient.

QSAR Model Abbreviations: Correlation Coefficient (r), Squared Correlation Coefficient (r^2), Standard deviation (sd), Fischer Value (F) at specified degree of freedom (Limit of confidence, 99.9%), Leave-one-out cross validation coefficient (q^2), boot strapping r^2 (r^2bs), standard deviation error of prediction (SDEP), Predictive Residual Error Sum of Squares.

Table 4: Experimental and Predicted pIC_{50} values of the Pyrazole Derivatives

Compound Name	Model 1		Model 2	
	Experimental pIC_{50}	Predicted pIC_{50}	Experimental pIC_{50}	Predicted pIC_{50}
1	1.7212	1.50722	1.7212	1.56262
14	1.2218	1.24439	1.2218	1.27731
15	2	1.77142	2	1.70353
16	0.886	1.54911	0.886	1.61167
17	0.7447	0.534904	0.7447	0.221111
18	0.6777	0.915078	0.6777	0.866316
20	1.187	1.14303	1.187	1.11092
21	1.522	0.906254	1.522	0.984264
22	0.5376	0.904191	0.5376	0.966535
24	1.045	0.998277	1.045	1.23611
25	0.7958	0.60454	0.7958	0.687615
26	0.9586	1.2372	0.9586	0.925527
28	0.3872	0.799771	0.3872	0.977179
29	2	1.66563	2	1.81313
31	1.301	1.64269	1.301	1.66777
32	1.886	1.49409	1.886	1.40191
33	1.6989	1.48472	1.6989	1.51302
34	1.9208	2.52292	1.9208	2.26995

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