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Quantitative structure activity relationship studies of diaryl thiophen derivatives as selective COX-2 inhibitors

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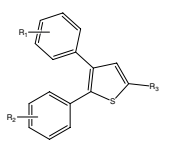
QSAR studies were carried out on a series of diaryl thiophens that act as selective COX-2 inhibitors using Molecular Operating Environment (MOE). The studies were carried out on a training set of 20 analogs. These studies produced good predictive models and indicated that partial Charge Descriptors (Q_VSA_FPNEG), shape descriptor (STD_DIM 3); hydrophobic descriptor (LOG P) and electronic descriptor (TPSA-Topological positive surface area) contribute to selective cyclooxygenase-2 inhibitory activity. Pharmacophoric query generation showed the pharmacophores required for selective COX-2 inhibitory activity.

1. Introduction

Selective COX-2 inhibitors hold promise as a new generation of anti-inflammatory agents with reduced side effects, particularly gastrointestinal toxicity, compared to current non-steroidal anti-inflammatory drugs (Prasit et al. 1997) This is based upon the observation that current NSAIDs inhibit both constitutive cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2). COX-1 is responsible for normal physiological functions such as platelet aggregation, cyto-protection in the stomach and maintenance of normal kidney function and COX-2 is associated with inflammatory conditions. (Vane et al. 1995; Herchman et al. 1996; Jouzeau et al. 1997). This has led to intense efforts in the search for potent and selective COX-2 inhibitors, as the next generation of anti-inflammatory agents. The improved safety profile of COX-2 inhibitors may allow the use of these new agents for long-term prophylactic use in certain chronic diseases (Kergman et al 1995). Several classes of compounds have been reported as being selective COX-2 inhibitors. Among diaryl heterocyclics, celecoxib (Penning et al. 1997) with a diaryl pyrazole nucleus, rofecoxib (Prasit et al. 1999) with a diaryl furanone nucleus and valdecoxib (Talley et al. 2000) with a diaryl isoxazole nucleus have been used.

Thus, this study aims at designing specific inhibitors of COX-2 with a different heterocyclic ring system, a thiophen ring system (**A**, **B**) (Pinto et al. 1996), using the quantitative structure activity technique.



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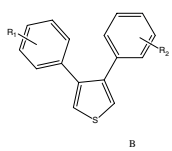
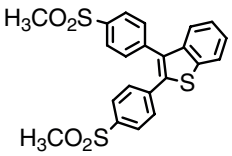


B

2. Investigations, results and discusion

All computational work was performed on a Compaq PC Pentium IV workstation using Molecular Operating Environment (MOE) by Chemical Computing Group Inc. A total of 20 compounds were selected for the present study. The biological activity data for these diaryl thiophens (Pinto et al. 1996) were taken from the literature (Table 1). All the compounds were drawn using the builder module of MOE. The compounds were then subjected to conformational analysis and energy minimization using stochastic conformation search with an RMS gradient of 0.001 and an iteration limit of 10000. The lowest energy conformer of all the compounds was transferred to the database viewer and descriptors calculated for the compounds. MOE calculates 193 descriptors from three classes – 2D descriptors (use the atoms and connection information of the molecule for the calculation), i3D – internal 3D descriptors (use 3D coordinate information about each molecule and they are invariant to rotations and translations of the conformation) and x3D – external 3D descriptors which use 3D coordinate information but also require an absolute frame of reference. The correlation between the biological activity (–log ic50) as dependent variables and the MOE descriptors as independent variables was calculated on Compaq PC using SYSTAT 10.2 © Copyright 2002, SYSTAT Software Inc. The following statistical measures were used: N = number of samples, multiple R = coefficient of correlation, F-test for quality of fit, t-test, standard error of estimate, spress = standard devia-

Table 1: Training set with experimental, calculated and predicted structure activity data for COX-2 inhibition

S. NO.	R1	R2	R3	ic50[a]	−log ic50	Calculated −log ic50	Residual (Experimental-Calculated activity)	Predicted (Leave one out) −log ic50	Residual (Experimental − predicted activity)
1A	SO_2CH_3	4-F	H	8	−0.903	−0.94	0.037	−0.94	0.04
2A	SO_2CH_3	4-F	Br	7	−0.845	−0.68	−0.165	−0.63	−0.22
3A	SO_2CH_3	4-F	SO_2CH_3	200	−2.301	−1.87	−0.431	−1.73	−0.57
4A	SO_2CH_3	4-F	NO_2	14	−1.146	−1.60	0.454	−1.98	0.83
5A	SO_2CH_3	4-F	$NH_2.HCl$	3	−0.477	−0.50	0.023	−0.51	0.03
6A	SO_2CH_3	4-F	$CH_2CH_2COO-C_2H_5$	130	−2.11	−2.19	0.08	−2.27	0.16
7A	SO_2CH_3	4-F	CH_3	10	−1	−1.09	0.09	−1.10	0.10
8A	SO_2CH_3	4-F	CH_2CN	49	−1.69	−1.65	−0.04	−1.64	−0.05
9A	SO_2CH_3	4-F	SC_6H_5	66	−1.81	−1.82	0.01	−1.86	0.05
10A	SO_2CH_3	H	H	4	−0.602	−1.19	0.588	−1.29	0.69
11A	SO_2CH_3	4-CH_3	H	13	−1.113	−0.93	−0.183	−0.91	−0.20
12A	SO_2CH_3	4-OCH_3	H	5	−0.698	−1.14	0.442	−1.21	0.51
13A	SO_2CH_3	4-OH	H	86	−1.934	−1.42	−0.514	−1.33	−0.60
14B	SO_2CH_3	H	–	4	−0.602	−0.68	0.078	−0.69	0.09
15B	SO_2CH_3	4-F	–	8	−0.903	−0.63	−0.273	−0.59	−0.31
16B	SO_2CH_3	4-CH_3	–	4	−0.602	−0.55	−0.052	−0.54	−0.06
17B	SO_2CH_3	4-OCH_3	–	5	−0.698	−0.67	−0.028	−0.67	−0.03
18B	SCH_3	4-SCH_3	–	101	−2	−1.92	−0.08	−1.78	−0.22
19B	SCH_3	4-SO_2CH_3	–	6	−0.778	−1.01	0.232	−1.08	0.30
20B				19	−1.278	−1.03	−0.248	−0.96	−0.32

[a]: ic50 determined using human recombinant enzyme

Table 2: QSAR Model

Effect	Coefficient	Std Error	Std Coef	Tolerance	t	P (2 Tail)
CONSTANT	6.576	1.114	0.000	.	5.901	0.000
Q_VSA_FPNEG	−18.031	4.439	−1.033	0.233	−4.062	0.001
LOGP	0.068	0.141	0.081	0.528	0.480	0.638
STD_DIM3	−5.869	1.020	−0.959	0.541	−5.752	0.000
TPSA	0.022	0.010	0.593	0.221	2.272	0.038

Dep Var: −log ic50 N: 20 Multiple R: 0.880 Squared multiple R: 0.774
Adjusted squared multiple R: 0.714 Standard error of estimate: 0.312

tion of prediction and S dep = standard error of prediction and P as the probability of exceeding the F ratio when the group means are equal. The leave one out method and bootstrapping were employed for cross-validation of the equation using qsar_val developed in the Shri Govindram Seksariya Institute of Technology and Science, Indore. The 20 compounds were aligned using the flexible alignment module of MOE. Nonlinear optimization was carried out with the MOE Truncated Newton optimizer preceded by two steps of Steepest Descent and terminated when the RMS gradient fell below 0.001. The MOE implementation of MMFF94 was used to measure the internal strain of each molecule. Chirality was preserved using signed volume restraints on all chiral centers. Pharmacophore atom type assignment was performed using the MOE implementation of the Daylight SMARTS pattern matching language. The determination of the RMSD between structures used the MOE Superpose functionality, which calculates an optimal global superposition by minimizing a weighted least squares error function. The most active

Table 3: Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
Regression	4.998	4	1.250	12.867	0.000
Residual	1.457	15	0.097		

Durbin-Watson D Statistic 2.821
First Order Autocorrelation −0.432

Table 4: Statistical results

R^2	0.774
Variance	0.097
F-TEST	12.86
Bootstrapping r^2	0.81
Chance <	0.01
Q^2	0.595
Spress	0.417
Sdep	0.361

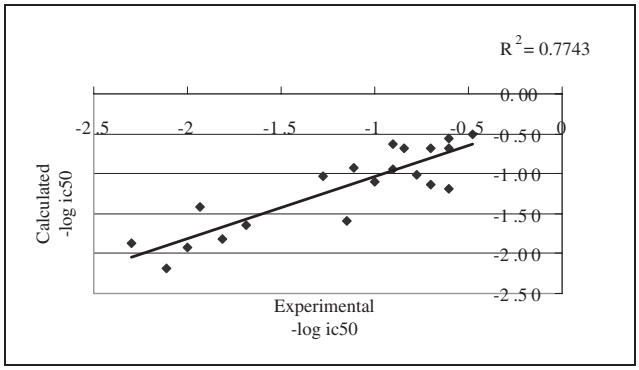


Fig. 1: Relationship between experimental and calculated biological activity

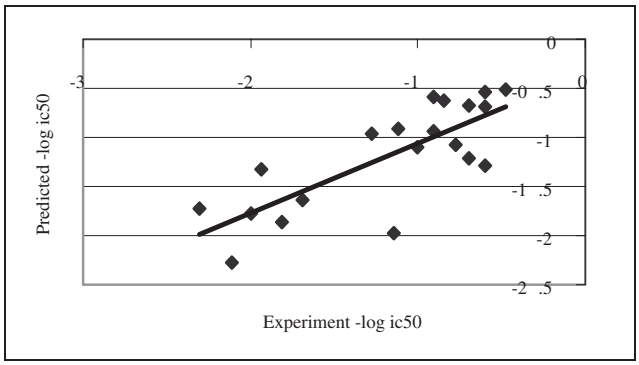


Fig. 2: Relationship between experimental and predicted (leave one out) biological activity

molecule was also used for identification of pharmacophores using pharmacophoric query generation.

The correlation between different descriptors as independent variables and biological activity as the dependent variable was carried out on SYSTAT 10.2 software and the best statistical model is shown in Table 2. Table 3 contains the analysis of variance and Table 4 contains the regression parameters, which explain the validity of the model. The equation gives a correlation coefficient ($R = 0.880$) of more than 95% statistical significance. ($F_{4,\ 15\alpha\ 0.05} = 3.06$, $F_{4,\ 15} = 12.867$) The equation was used to calculate the biological activity of all the molecules (Table1) and Fig. 1 shows a graph of calculated against experimental activity. The lower residual values indicate the robustness of the model. The predicted −log ic50 is in good agreement with the experimental data as is indicated by the graph between experimental activity and predicted activity model shown in Fig. 2.

The cycooxygenase-2 inhibitory concentration (−log ic50) shows a negative correlation with the partial charge descriptor (Q_VSA_FPNEG) and shape descriptor (STD_DIM 3). It also shows positive correlation with the hydrophobic descriptor (LOG P) and electronic descriptor (TPSA-Topological positive surface area).

The negative contribution of the partial charge descriptor, Q_VSA_FPNEG, which is the fractional negative polar Van der Waals surface area, indicates that the selective cyclooxygenase inhibitory activity increases as the fractional negative polar Van der Waals surface area decreases. The shape descriptor (STD_DIM 3), the square root of the third largest eigen value of the covariance matrix of the

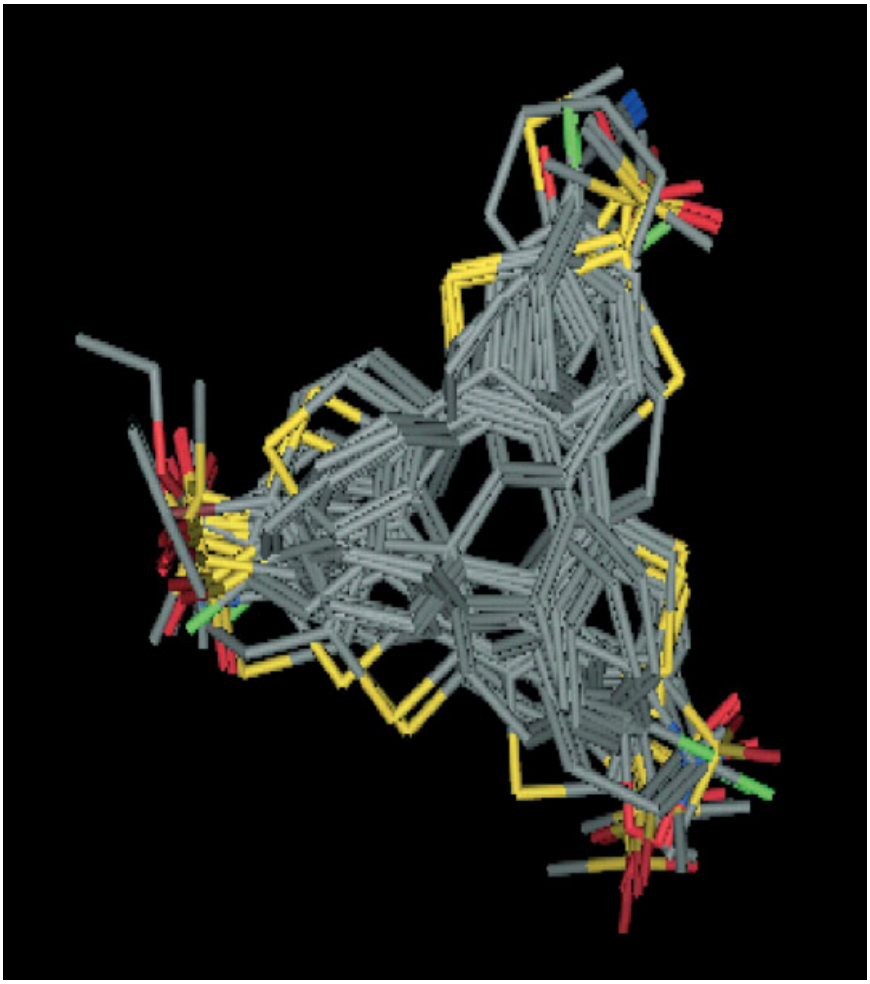


Fig. 3:
Alignment of all molecules of training set

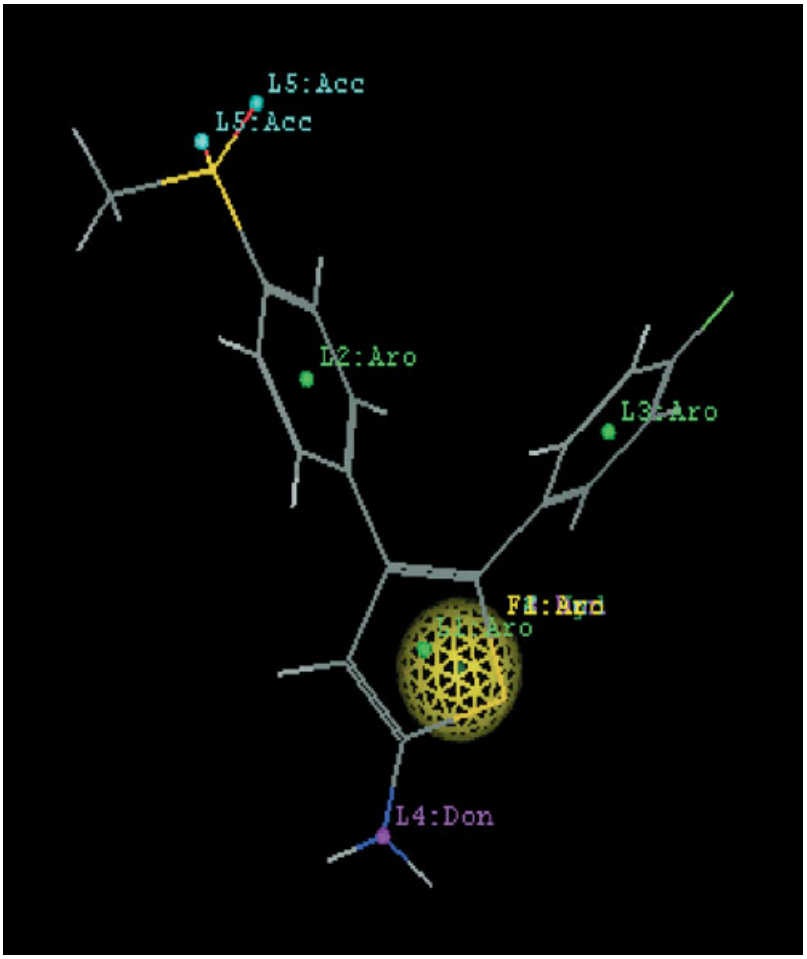


Fig. 4: Pharmacophores from pharmacophore query module of MOE

atomic coordinates which is equivalent to the standard deviation along a principal component axis, also contributes negatively showing that shape of the molecules affects the inhibitory activity. The shape descriptor and fractional negative polar Van der Waals surface area are both steric descriptors, whose contribution to inhibitory activity justifies the importance of proper orientation of the ligand molecule for the inhibition of COX-2 enzyme. X-ray crystallographic study of the COX enzyme reveals the flexibility of COX-2 in accepting the ligand molecule as the total NSAID binding site is 17% larger than COX-1 and has an additional side pocket due to the replacement of isoleucine at position 523 in COX-1 with valine in COX-2. (Picot et al. 1994; Hinz et al. 2002).

The hydrophobic parameter LOG P contributes positively towards COX-2 inhibitory activity, suggesting that increase in lipophilicity of the molecule increases the inhibitory activity. X-ray crystallographic study of the COX-2 enzyme implies that the COX-2 enzyme is a membrane-based enzyme, which requires sufficient lipophilicity in the ligand molecule to enter the membrane channel and have an inhibitory effect (Picot et al. 1994; Kurumbail et al. 1996). A positive contribution by topological positive surface area indicates that there might be an anionic site in the cyclooxygenase enzyme where the molecule participates in electrostatic interaction to show an inhibitory effect. This is supported by the fact that the cyclooxygenase enzyme has an arginine amino acid at position 120, which might be involved in electrostatic interaction. (Picot et al. 1994).

MOE was used to perform alignment of all the molecules. The best-aligned model is given in Fig. 3. Fig. 4 shows the pharmacophores required for cyclooxygenase-2 inhibitory activity on the most active compound of the series. The pharmacophores which might be responsible for cyclooxygenase-2 inhibitory action are the heterocyclic ring, two hydrogen acceptors on the methylsulphonyl group which might be involved in hydrogen binding and an electron donor amine on thiophen moiety for ionic binding with the enzyme. It can be assumed that the heterocycle is responsible for the appropriate orientation of the aromatic rings in space and finally for binding to the enzyme (Gans et al. 1990). The methyl sulfonylphenyl group is believed to interact in the hydrophilic side pocket of the COX-2, which is produced as a result of substitution of isoleucine at 523 position of COX-1 with valine in COX-2 (Kurumbail et al. 1996).

This study gives us a perspective regarding the physicochemical descriptors, which contribute to the cyclooxygenase-2 inhibitory activity along with better understanding of the pharmacophores required for designing novel selective COX-2 inhibitors.

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References

Chavatte P, Yous S, Marot C, Baurin N, Lesieur D (2001) Three-dimensional quantitative structure-activity relationships of cyclo-oxygenase-2 (COX-2) inhibitors: a comparative molecular field analysis. J Med Chem 44: 3223–3230.

Gans KR, Galbraith W, Roman RJ, Haber SB, Kerr JS, Schmidt WK, Smith C, Hewew WE, Ackerman NR (1990) Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor. J Pharmacol Exp Ther 254: 180–187.

Herchman HR (1996) Prostaglandin synthase-2 Biochem. Biophys Acta 1299: 125–140.

Hinz B, Brune K (2002) Cyclooxygenase-2–10 years later. J Pharmacol Exp Ther 300: 367–375.

Jouzeau JY, Terlain B, Abid A, Nedelec E, Netter P (1997) Cyclo-oxygenase isoenzymes. How recent findings affect thinking about non-steroidal anti-inflammatory drugs Drugs. 53: 563–582.

Kargman S, O'Neil GP, Vickers PJ, Evans JF, Mancini JA, Jothy S (1995) Expression of prostaglandinG/H synthase-1 and -2 in human colon cancer. Cancer Res 55: 2556–2559.

Kurumbail RG, Stevens AM, Gierse JK, Mc Donald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC (1996) Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents Nature 384: 644–648.

Penning TD, Talley JJ, Carter SR, Collins PW, Doctor S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogrs RS, Rogiers D, Yu SS, Anderson GD, Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veehuizen AW, Zhang YY, Isakson PC (1997) Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: Identification of 4-[5-(4-methylphenyl)-3-(trifluromethyl)-1H-pyrazol-1-yl] benzenesulfonamide (SC-58635, Celecoxib) J Med Chem 40: 347–357

Pinto DJP, Copeland RA, Covington MB, Pitts WJ, Batt DG, Orwat MJ, Lam GN, Joshi A, Charn YC, Wang S, Trzaskos JM, Magolda RL, Kornhauser DM (1996) Chemistry and pharmacokinetics of diaryl thiophens and terphenyls as selective Cox-2 inhibitor. Bioorg Med Chem Lett 24: 2907–2912.

Prasit P, Riendeau D (1997) Selective cyclooxygenase-2 inhibitors. Annu Rep Med Chem 32: 211–220.

Prasit P, Wang Z, Brideau C, Chan C, Gauthier JY, Gordon R, Charleson S, Cromlish WA, Ethier D, Evans JF, Ford-Hutchinson AW, Guay J, Gresser M, Kargman S, Kennedy B, Leblanc Y, Leger J, Mancini J, O'Neill GP, Ouellet M, Percival MD, Perrier H, Riendeau D, Rodger D, Tagari P, Therien P, Vickers P, Wong E, Xu L, Young RN, Zamboni R, (1999) The discovery of rofecoxib, [MK 966, VIOXX, 4-(4′-methylsulfonylphenyl)-3-phenyl-2(5H)-furanone] an orally active cyclooxygenase-2 inhibitor. Bioorg Med Chem Lett 9: 1773–1778.

Picot D, Loll PJ, Garavito M (1994) The X-ray crystal structure of the membrane protein prostaglandin H_2 synthase-1 Nature 367: 243–249.

Vane J, Botting RM (1995) New insights into the mode of action of anti-inflammatory drugs. Inflamm Res 44: 1–10.