QSAR Analysis of 4,5-Diarylpyrroles with Cyclooxygenase-2 Inhibitory Activity

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A series of antiinflammatory 4,5-diarylpyrroles was subjected to quantitative structure activity relationship analysis. The effect of structural modification in 4,5-diarylpyrroles on their COX-2 inhibition potential was analysed by quantitative structure activity relation (QSAR) analysis using the software Cerius² 3.5. Special emphasis was laid on various electronic, spatial and thermodynamic descriptors at the minimum energy conformation. Out of several electronic, spatial and thermodynamic descriptors investigated on the COX-2 inhibitory potential of substituted 4,5-diarylpyrroles, PMI, ROG and MR showed appreciable correlation with COX-2 inhibitory potential. These investigations will further help in rationalizing the design of new molecules.

Nonsteroidal antiinflammatory drugs (NSAIDs) are important agents for the treatment of acute and chronic inflammatory disorders such as osteoarthritis and rheumatoid arthritis. The chronic use of these agents is often limited by some common side effects like gastrointestinal hemorrhage and ulceration. A major mechanism of action of these drugs is the lowering of prostaglandin synthesis through inhibition of cyclooxygenase (COX) enzyme. COX exists in two isoforms, COX-1 and COX-2, which are encoded by two distinct genes[1]. COX-1 is thought to provide cytoprotection; COX-2 expression is up regulated by pain and inflammatory mediators and downregulated by corticosteroids. This regulated expression suggests that a selective inhibitor of COX-2 might possess antiinflammatory properties without the troublesome GI side effects.

The hypothesis that selective COX-2 inhibitors could offer therapeutic advantages over conventional NSAIDs coupled with work suggested by Wilkerson *et al.*[2] directed us to investigate some finer details of QSAR analylsis of 4,5-diarylpyrroles, which are selective COX-2 inhibitors. These studies will further help in the selection of new compounds for developmental studies.

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The antiinflammatory activity (AA) data of 4,5-diarylpyrroles reported by Wilkerson *et al.*[2] were taken (Table 1 and fig.1). These AA data were originally expressed in terms of a specific dose in mg/kg required to produce 50% inhibition of COX-2. These were converted to μM/kg for further calculation. The biological activity (BA) data, reciprocal of AA was taken for QSAR analysis. The QSAR analysis was performed using the software Cerius² 3.5 (Biosym/MSI)[3] on a Silicon Graphics[4] workstation. The structures of the compounds were built using molecular sketcher facilities provided in the modeling environment of Cerius². The energy of the molecules was minimized by conjugate gradient algorithm[5] working under universal force field[6].

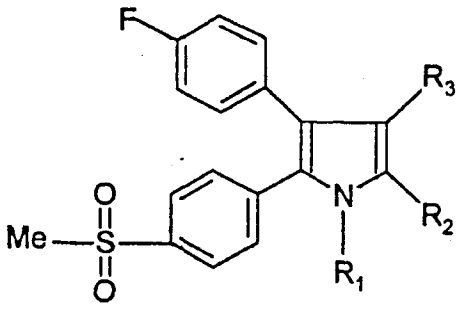

Fig. 1: 4,5-Diarylpyrroles

TABLE 1: STRUCTURES AND BIOLOGICAL ACTIVITY DATA FOR 4,5-DIARYLPYRROLES

Comp. No.	R_1	R_2	R_3	Biological Activity $IC_{50}(\mu M)$* COX-2	LOG (BA)
1	H	H	H	12.65	-1.10278
2	Me	H	H	20.20	-1.30103
3	Me	Cl	Cl	25.00	-1.39794
4	H	SMe	H	34.5	-1.53782
5	Me	Br	H	35.5	-1.55023
6	H	Br	H	40.00	-1.60206
7	H	Cl	H	42.00	-1.62325
8	Me	Br	Br	55.67	-1.74562
9	H	NO_2	H	63.00	-1.79934
10	H	CN	H	64.40	-1.80889
11	H	Cl	Cl	69.00	-1.83885
12	H	SCN	H	72.00	-1.85733
13	H	I	H	92.30	-1.9652
14	H	SO_2Me	H	267.00	-2.42651
15	H	$COCF_3$	H	298.2	-2.47451

*$IC_{50}(\mu M)$= micromolar dose (μ Moles/litre) required to produce 50% inhibition of COX-2

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 (FH_2O)[7], desolvation free energy for octanol (F_{OCT})[8], log of partition coefficient (LOGP)[8] and molecular refractivity (MR)[9]. 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)[10], molecular surface area (AREA)[10], molar volume (V_m)[10], principal moment of inertia (PMI)[11] and it's X, Y, and Z components (PMI-X, PMI-Y and PMI-Z). Electronic parameters describe weak non-covalent bonding between drug molecules and receptor and include sum of atomic polarizability (APOL)[12], dipole moment (DIP)[12] and it's X,Y and Z components (DIP-X, DIP-Y and DIP-Z), energy of highest occupied molecular orbital (HOMO)[13] and energy of lowest unoccupied molecular orbital (LUMO)[14].

Stepwise multiple regression analysis method[15,16] was used to generate QSAR equations. Statistical measures used were; n-number of sample in the regression, r-correlation coeficient, r^2 - squared correlation coefficient (coefficient of determination), s-standard deviation, t-student's test and F-test (Fischer's value) for statistical significance.

Structures and biological activity data for the compounds considered in the analysis are given in Table 1. The calculated parameters subjected to stepwise multiple linear regression analysis[16] are given in Table 2. Parameters were selected on the basis of correlation matrix (Table 3). Only those parameters having the intercorrelaton coefficient below 0.6-0.7 were considered to select the best equation.

The following equations were generated.

LOG (BA) = -1.1476101 ROG-0.00284 PMI-X + 4.089961 1
n = 15, r=0.819, r^2= 0.671, F = 6.79, t = 2.672, s = 0.251

LOG(BA) = -1.1476101 ROG - 0.032973 DIP-X + 4.43505 2
n = 15, r = 0.826, r^2 = 0.682, F = 7.139, t = 2.672, s = 0.247

LOG(BA) = -1.832372 ROG - 1.731942 DENSITY + 8.105965 3
n = 15, r= 0.840, r^2 = 0.706, F=8.808, t= 2.83, s = 0.237

LOG(BA)= -1.25986 ROG-0.001218 PMI M + 0.035344 MR + 1.91612. 4
n = 15, r=0.982, r^2 = 0.796, F = 8.801, t =2.967, s=0.208

TABLE 2: CALCULATED DESCRIPTORS AND PREDICTED ACTIVITY FOR 4,5-DIARYLPYRROLES

Comp. No.	ROG	DEN.*	PMI-M	MR	PMI-X	DIP-X	EQN.1*	EQN.2*	EQN.3*	EQN.4*
1	4.00	1.17	985.66	83.9	229.20	5.98	-1.37	-1.266	-1.285	-1.161
2	4.04	1.17	1011.53	88.7	281.36	6.91	-1.27	-1.325	1.301	-1.346
3	4.06	1.27	1426.67	99.4	374.74	-6.43	-1.42	-1.533	-1.770	-1.633
4	4.39	1.18	1348.25	97.1	363.44	3.98	-1.83	-1.992	-1.922	-1.987
5	4.06	1.34	1543.74	97.2	416.20	-0.87	-1.65	-1.668	-1.598	-1.760
6	4.04	1.37	1515.34	93.3	413.53	-1.83	-1.73	-1.676	-1.594	-1.725
7	4.03	1.24	1515.34	89.7	344.46	-2.53	-1.49	-1.442	-1.610	-1.523
8	4.06	1.51	1869.85	104.8	440.21	-4.70	-1.78	-1.957	-1.720	-1.825
9	4.17	1.23	1317.66	91.5	377.10	-3.64	-1.72	-1.676	-1.849	-1.773
10	4.12	1.29	1184.69	89.2	333.60	2.41	-1.57	-1.681	-1.569	-1.587
11	4.03	1.29	1405.62	94.5	349.41	-7.68	-1.54	-1.525	-1.777	-1.535
12	4.24	1.22	1465.06	97.6	363.80	0.216	-1.77	-1.789	-1.826	-1.817
13	4.05	1.5	1924.12	96.9	441.50	0.302	-1.99-	-1.9985	-1.537	-1.815
14	4.55	1.23	1581.19	97.7	448.11	1.865	-2.3	-2.3781	-2.233	-2.414
15	4.41	1.28	1797.73	94.9	401.25	10.70	-2.48	-2.2008	-2.434	-2.116

*Density *Activity calculated from respective equations

In the data set, 15 compounds (n) were taken. The generated QSAR equations (from 1-4) show that steric parameters play dominating role in producing variation in biological activity. Equations 1-3 contain two different parameters. These equations depict the effect of PMI-X, DIP-X and DENSITY with that of ROG respectively. However, these equations are not very significant in terms of statistical measurements.

Equation 4 shows the effect of MR along with ROG and PMI-M. ROG describes distribution of mass within the molecule and also shows negative correlation of ROG with respect to biological activity. This suggests that substitution at 1,2 and 3 positions of pyrrole ring should be made in such a manner so that the centre of gravity shifts towards the principle rotatory axis of the molecule.

TABLE 3: CORRELATION MATRIX FOR THE PARAMETERS IN EQUATION 4

	LOGBA	ROG	PMI-M	MR
LOGBA	1			
ROG	0.168	1		
PMI-M	0.170	0.216	1	
MR	0.387	0.278	0.087	1

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. Equation shows inverse effect of PMI of molecules on their biological activity. This suggests that substitution, which facilitates the rotatory motion of the molecules around principal axis, produce the derivatives with better inhibition of COX-2.

Molecular refractivity is a measure of polarizability and steric bulk of molecules. In equation 4, a positive correlation between biological activity and molecular refractivity was observed. This infers that biological activity increases with increase in molecular refractivity i.e increase in polarizability and steric bulk of the compounds within the series.

Although results obtained from equations 1-3 are not very significant in terms of statistical measurements but indicate that these descriptors might have a role to pay in determining the COX-2 inhibition potential at different

experimental conditions. However in the experimental set up chosen, equation 4 describes various descriptors, which may play an important role in rationalizing the design of new pyrrole molecules with COX-2 inhibitory activity.

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UV Spectrophotometric Methods for the Determination of Celecoxib and Tizanidine Hydrochloride

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Two UV spectrophotometric methods have been developed for the determination of celecoxib and tizanidine hydrochloride in pure and in its pharmaceutical formulations. Celecoxib having absorption maximum at 251.2 nm in 0.1 N sodium hydroxide, where as tizanidine HCl exhibiting maximum absorption at 228 nm in distilled water.

Celecoxib (CXB) is a new NSAID, Which is a specific COX-2 inhibitor, and chemically it is benzenesulfonamide, 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-l-yl). Tizanidine hydrochloride (TZN) is chemically 2,1,3-benzothiadiazol-4-amine 5-chloro-N-(4,5-dihydro-1H-imidazole-2-yl) monohydrochloride and is used as a central muscle relaxant[1]. It acts presynaptically on the excitatory spinal interneurons and specifically on the polysynaptic pathways. Both the drugs are not official in any pharmacopoeia. So for, only a HPLC method[2] has been reported for the estimation of CXB in human plasma, where as for TZN cyclic voltammetric[3] and visible sectrophotometric methods

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