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3D-QSAR Analysis of Substituted 1,3,4-triaryl-3-pyrrolin-2-ones as Selective Cyclooxygenase-2-Inhibitors

P. SENTHIL KUMAR, M. AROCKIA BABU, M. ELIZABETH SOBHIA[2], A. K. SAXENA[1] AND S. G. KASKHEDIKAR*
Department of Pharmacy, S.G.S.I.T.S, Indore-452 003
[1]Medicinal Chemistry Division, C.D.R.I., Lucknow-116 001
[2]Medicinal Chemistry Division, N.I.P.E.R., S.A.S Nagar (Mohali)-160 062

Three dimensional quantitative structural activity relationship studies have been performed on a series of 17 compounds of 1,3,4 triaryl-3-pyrrolin-2-ones, for their cyclooxygenase-2 inhibitory activities by using the logico-structural based pharmacophore mapping approach employing APEX 3D software program. Among several biophoric models, two models were selected based on statistical consideration; chance ≤0.15, correlation coefficient >0.75 and match >0.65. Both models showed three common biophoric sites, one being oxygen atom, second being it's lone pair and third being center of phenyl ring at 1st position and center of phenyl ring at 4th position for model No. 1 and 2 respectively. In model No. 2, three secondary sites are indicated. At one site, the steric refractivity contributes positively, whereas at the remaining sites both steric refractivity and total refractivity contribute negatively. Among the two models, model No.1 has better acceptability having r^2=0.91, Root mean square error of approximation=0.10, Root mean square error of prediction "leave one out"=0.11 and n=17. In this model there are four secondary sites, which are total refractivity, H-acceptor, hydrophobicity and steric refractivity and all these found to contribute negatively.

New types of non-steroidal antiiflammatory drugs (NSAIDs) are being developed based on new understanding of their mechanism of action and the pathogenesis of inflammation[1]. These include a new class of NSAIDs called selective cyclooxygenase-2 (COX-2) inhibitors. These agents preserve the cyclooxygenase-1 (COX-1) that is responsible for the production of cytoprotective prostaglandins in the stomach and selectively inhibit COX-2 resulting in the same analgesic and antiinflammatory effects as the existing NSAIDs with less toxicity[2]. No QSAR studies have been attempted so far on 1,3,4-triaryl pyridin-2-ones. Hence, it was thought worthwhile to carry out 3D-QSAR analysis for one such reported series with COX-2 inhibitory activities, in order to identify the necessary structural and physicochemical requirements for binding with COX-2.

*For correspondence
E-mail: babuphd2001@yahoo.co.in

MATERIAL AND METHODS

APEX-3D was used to identify common biophoric structural pattern for a series of 1,3,4-triaryl-3-pyrrolin-2-ones, as new selective cyclooxygenase-2 inhibitors reported by Bosch *et al.*[3] (Table 1 and fig. 1). All molecular modeling and 3D-QSAR studies were performed on silicon graphics, INDY R-4000 workstation employing Molecular Simulation Incorporation (MSI) software[4] (Insight II, Builder, Search_compare, Discover and Apex 3D). All structures of 1,3,4-triaryl-3-pyrrolin-2-one derivatives were constructed using the sketch program in builder module Insight II software[5] and minimized for the energy using steepest descent, conjugative gradient and Newton-Raphson algorithms in sequence followed by Quasi Newton-Raphson (Va09a)[6], optimization techniques implemented in the discover module by using energy tolerance value of 0.001 kcal/mol and maximum number of iteration set to 1000. All these molecules

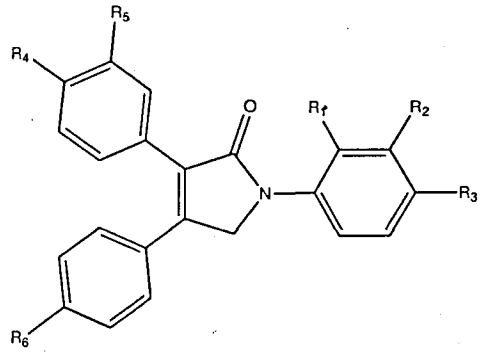


Fig. 1: Substituted 1,3,4-tri aryl –3-pyrrolin-2-ones used in this study.

were stored in MDL format and were used for computation of different physico-chemical properties including atomic charge, pi-population, hydrogen donor and acceptor indices, HOMO and LUMO coefficient and hydrophobicity and molar based atomic contribution[7,8] by the MOPAC version 6.0 (MNDO Hamiltonian) version[9]. Apex 3D expert system is based on logico structural approach to drug design developed by Golender *et al.*[10] and is used for prediction of biological activity. The data were used by APEX-3D programme for automated biophore identification and 3D-QSAR model building.3D-QSAR equation was derived with site radius at 1.20, the occupancy at 10, and the sensitivity at 1 and the randomization at 100. The total hydrophobicity and total refractivity were selected as global properties. The

TABLE 1: *IN VITRO* COX-2 INHIBITORY ACTVITY DATA OF THE SUBSTITUTED 1,3,4-TRIAARYL-3-PYRROLIN-2-ONES.

C. No.	R_1	R_2	R_3	R_4	R_5	R_6	Exp. Act. log $1/IC_{50}$	Model 1		Model 2	
								Calc.	Pred.	Calc.	Pred.
1	H	H	H	H	H	SO_2CH_3	0.55	0.46	0.41	0.50	0.44
2	F	H	H	H	H	SO_2CH_3	0.05	0.16	0.20	0.14	0.18
3	H	Cl	H	H	H	SO_2CH_3	0.00	-0.10	-0.13	0.01	0.01
4	H	H	F	H	H	SO_2CH_3	0.46	0.31	0.28	0.32	0.28
5	H	H	CH_3	H	H	SO_2CH_3	0.00	-0.11	0.15	0.00	0.00
6	H	H	CF_3	H	H	SO_2CH_3	0.01	-0.01	-0.01	0.16	0.17
7	H	H	H	OCH_3	H	SO_2CH_3	0.47	0.47	0.47	0.41	0.37
8	H	H	H	F	H	SO_2CH_3	0.51	0.31	0.27	0.51	0.50
9	F	H	H	F	H	SO_2CH_3	0.49	0.47	0.47	0.50	0.50
10	Cl	H	H	OCH_3	H	SO_2CH_3	0.66	0.49	0.35	0.62	0.58
11	OCH_3	H	H	F	H	SO_2CH_3	-0.02	0.12	0.14	-0.11	-0.13
12	H	F	H	OCH_3	H	SO_2CH_3	0.55	0.60	0.62	0.58	0.59
13	H	H	CH_3	F	H	SO_2CH_3	-0.02	0.03	0.03	-0.07	-0.08
14	H	H	OCH_3	F	H	SO_2CH_3	-0.08	0.09	0.16	0.06	0.13
15	H	H	CF_3	F	H	SO_2CH_3	0.02	-0.03	-0.03	-0.09	-0.12
16	H	H	F	OCH_3	H	SO_2CH_3	0.52	0.60	0.63	0.58	0.60
17	H	H	H	F	F	SO_2CH_3	0.00	0.30	0.35	0.07	0.09

Exp.Act.= Experimental Activity, Calc.= Calculated, Pred.= Predicted. PGE_2 level in lipopolysaccharide (LPS) challenged human whole blood was measured as biochemical index for COX-2 IC_{50}.

biophoric sites were set to pi-population, charge HOMO, LUMO, hydrogen acceptor, hydrogen donor and hydrophobicity. The secondary sites were set to hydrogen acceptor, presence; hydrogen donor, presence; heteroatom, presence; hydrophobic, hydrophobicity; steric, refractivity; ring, presence. All these were selected as independent variable and biological activity considered as dependent variable and the stepwise regression was performed to derive equation for 3D-QSAR models.

RESULTS AND DISCUSSION

A series of 17 compounds were considered for analysis of COX-2 inhibitory activity on APEX 3D system. Out of the several biophoric models for analysis of COX-2 for all the 17 compounds, only two models were considered based on statistical criteria; $R^2 \geq 0.75$, chance<0.15, superimposition match>0.65. Both the models 1 and 2 have high statistical significance >99.9% with F-values $F_{(3,13)}$= 14.612 and $F_{(4,12)}$=30.892 against the value for 99.9% significance ($F_{3,13}\alpha_{0.001}$=10.2) and ($F_{4,12}\alpha_{0.001}$=9.63), respectively. The common key structural features have been identified (fig. 3), as biophoric sites (A_1, B_1, C_1 and A_2, B_2, C_2) and secondary sites (SSa_1, SSb_1, SSc_1, SSd_1 and SSa_2, SSb_2, SSc_2) for model 1 and 2, respectively.

The three biophoric sites A_1, B_1 and C_1 are common to all molecules in the model-1, which correspond to ketooxygen, its lone pair and center of phenyl ring attached at 1st position, respectively. The distance matrix (in A°) evolved from this special disposition of the centers of these

TABLE 2: PARAMETER'S VALUES FOR THE SECONDARY SITES (SS) IN MODELS 1 AND 2.

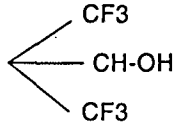
Comp. No.	Model 1			Model 2			
	Total Refractivity (SSa_1)	H-Acceptor (SSb_1)	Hydrophobicity (SSc_1)	Steric Refractivity (SSd_1)	Total Refractivity (SSa_2)	Steric Refractivity (SSb_2)	Steric Refractivity (SSc_2)
1.	110.8	1.00	-	-	110.8	-	-
2.	111.0	1.00	-	3.00	111.0	3.00	-
3.	115.6	1.00	-	3.00	115.6	3.00	-
4.	111.0	1.00	-	1.45	111.0	1.45	-
5.	115.8	1.00	-	3.00	115.8	3.00	-
6.	116.7	1.00	-	1.45	116.7	1.45	-
7.	117.2	1.00	-1.10	-3.00	117.2	3.00	3.00
8.	111.0	-	0.60	1.45	111.0	1.45	-
9.	111.2	-	0.60	1.45	111.2	1.45	0.80
10.	122.0	1.00	-1.10	-	122.0	-	3.00
11.	117.5	1.00	0.60	1.45	117.5	1.45	0.80
12.	117.5	1.00	-1.10	1.45	117.5	1.45	3.00
13.	116.0	1.00	0.60	1.45	116.0	1.45	-
14.	117.5	1.00	0.60	-	117.5	-	-
15.	117.0	1.00	0.60	1.45	117.0	1.45	-
16.	117.5	1.00	-1.10	1.45	117.7	1.45	3.00
17.	111.2	1.00	0.60	1.45	111.2	1.45	-

*(-) indicates absence of property. Secondary sites such as SSa_1, SSb_1, SSc_1, SSd_1 and SSa_2, SSb_2, SSc_2 for model 1 and 2, respectively.

biophoric sites are given below: A_1B_1=3.00, B_1C_1=5.8, C_1A_1=4.12. The physicochemical characteristics of the biophoric center corresponding to sites are in terms of charge at heteroatom DON-01 (8.294) for A_1, the hydrogen site (1.0) for B_1, cycle size (6) and pi-population(6) for C_1.

The following 3D-QSAR equation (Eqn 1) was generated by multiple regression analysis for model 1. Log1/IC_{50} (COX-2)=-0.029 (±0.009), Total refractivity at SSa_1–0.426 (±0.082), Hydrogen acceptor presence at SSb_1–0.405 (±0.041), Hydrophobicity at SSc_1–0.117 (± 0.025) and Steric

TABLE 3: NEW PROPOSED AND THEORETICALLY PREDICTED COMPOUNDS FOR COX-2 INHIBITION.

Comp. No.	R_1	R_2	R_3	R_4	R_5	R_6	*Model 1 $IC_{50}\mu m$	*Model 2 $IC_{50}\mu m$
1	H	H	H	H	H	H	0.04	0.05
2	F	H	H	H	H	H	0.04	0.06
3	H	Cl	H	H	H	H	0.06	0.10
4	H	H	F	H	H	H	0.04	0.06
5	H	H	CH_3	H	H	H	0.06	0.11
6	H	H	CF_3	H	H	H	0.07	0.12
7	H	H	H	CH_3O	H	H	0.02	0.03
8	H	H	H	F	H	H	0.08	0.06
9	F	H	H	F	H	H	0.08	0.04
10	Cl	H	H	CH_3O	H	H	0.03	0.12
11	CH_3O	H	H	F	H	H	0.12	0.09
12	H	F	H	CH_3O	H	H	0.02	0.03
13	H	H	CH_3	F	H	H	0.11	0.14
14	H	H	CH_3O	F	H	H	0.10	0.14
15	H	H	CF_3	F	H	H	0.12	0.13
16	H	H	F	CH_3O	H	H	0.02	0.03
17	H	H	H	F	F	H	0.08	0.06
18	Cl	H	H		H	SH	2.81	0.35
19	Cl	H	H	-C $(CH_3)_3$	H	SH	1.90	0.20
20	Cl	H	H	-CH $(CH_3)_2$	H	SH	3.46	0.20
21	Cl	H	H	CF_3	H	SH	2.36	0.21
22	H	H	H	CF_3	H	SO_2NH_2	0.58	1.04
23	H	H	H	-C $(CH_3)_3$	H	SO_2NH_2	0.95	0.48
24	H	H	H	-CH $(CH_3)_2$	H	SO_2NH_2	0.60	0.80

*IC_{50} Values were predicted using model 1 and 2, respectively.

refractivity at SSd_1+4.135 (Eqn. 1). n =17, r=0.955 F=30.892, RMSA=0.10 and RMSP=0.11. From Eqn.1, it is indicated that secondary sites SSa_1, SSb_1, SSc_1, and SSd_1 contribute negatively for COX-2 inhibitory activity (Table 2).

Similar to model 1, the model 2 also contain three common biophoric sites A_2, B_2 and C_2 for all 17 compounds which correspond to keto oxygen, its lone pair and center of phenyl ring bearing no substitution respectively. The mean distance (in A°) between these biophore sites are A_2B_2=3.00, B_2C_2=9.10, A_2C_2=6.29. The physicochemical characteristic of the biophoric center corresponding to sites, are in terms of DON-01 (8.35) for A_1, electron donor capability in the presence of hydrogen site (1.0) for B_1, cycle size (6) and pi-population(6) for sites for C_2. In addition to this biophoric center there are three independent parameter corresponding to secondary sites SSa_2, SSb_2, SSc_2.

The following 3D QSAR equation (Eqn. 2) was obtained by multiple regression analysis for model 2. $Log1/IC_{50}$ (COX-2)=-0.056 (±0.014) Total refractivity at SSa_2–0.096 (±0.038) Steric refractivity at SSb_2+0.219 (±0.035) Steric refractivity at SSc_2+6.633 (Eqn. 2), n=17, r=0.878, F=14.612, RMSA=0.15 and RMSP=0.17. Secondary sites SSa_2, SSb_2 contribute negatively, while secondary site SSc_2 as steric refractivity in the R_4 bearing substitution contribute positively for the activity as shown in model 2.

Considering the models, it is revealed that there is a common biophore comprising of the three-biophoric sites. The sites A_1 and B_1 (model1) and A_2 and B_2 (model 2) may involved in hydrogen bond interaction or electrostatic interactions. Similarly, the sites C_1 (model 1) and C_2 (model 2) may probably contribute to the pi-pi interactions. In model 1, all the four secondary sites contribute negatively. In model 2, the site SSc_2 contributes positively, and SSa_2 and SSb_2 contribute negatively, implying that these sites probably participate in the intermolecular interaction with the residues of the active site.

Based on the understanding of 3D-QSAR Eqns. 1 and 2, a few new selective cyclooxygenase inhibitors were proposed and their activities were predicted. It has been noticed in the literature that methyl sulphonyl and suphonamide group moieties play a pivotal role in binding to the active site and hence these groups have been considered important in synthesizing the selective COX-2 inhibitors. However in the recent past, quite a few series of diaryl heterocycles without methyl sulphonyl group have been reported[11-14] as selective COX-2 inhibitors. In the present study, in model 1, all the four secondary sites contribute negatively, by intuition we removed the sulphonyl methyl moiety, and predicted the activity. To our surprise, we found that the predicted activities of the molecules were found to be higher than the reported ones (1-17, Table 3 and fig. 1). Based on this, we

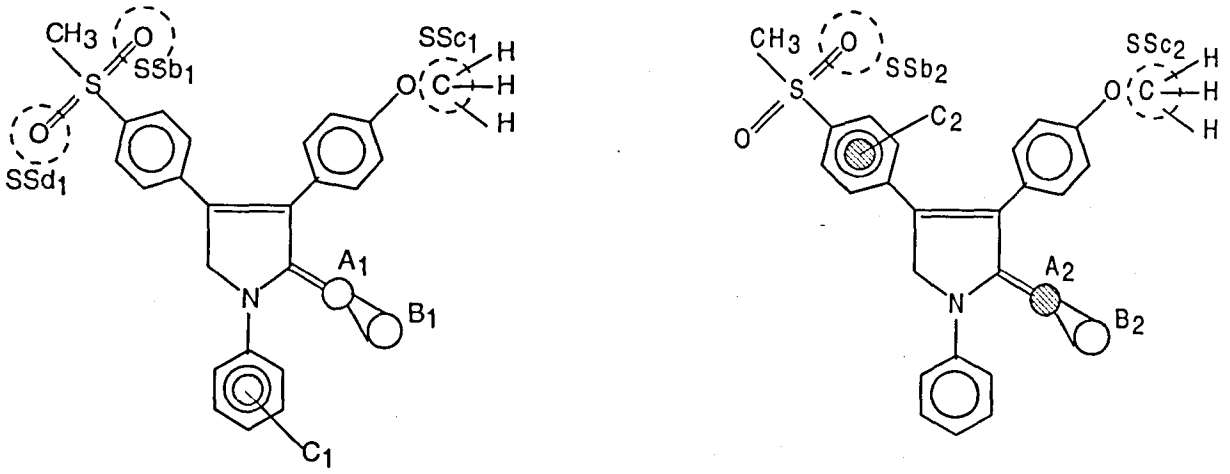


Fig. 2: Biophoric ⊘ and ◌ secondary sites represented on the single compound.

A_1, B_1 and C_1 correspond to carbonyl oxygen, its lone pair and center of phenyl ring for model 1. A_2, B_2 and C_2 correspond to carbonyl oxygen, its lone pair and center of phenyl ring for model 2. Total refractivity at SSa_1, hydrogen acceptor at SSb_1, hydrophobicity at SSc_1 and steric refractivity at $SSd_1$for model 1.Total refractivity at SSa_2, steric refractivity at SSb_2 and steric refractivity at SSc_2 for model 2. SSa_1 and SSa_2 have not been shown in the figure 2, because both are total refractivity.

have substituted different groups and predicted their activity. The steric bulky groups introduced in the position of R_4 with thiol group (19-21, Table 3 and fig. 1) are also found to have good activity. We also substituted suphonamide group in the place of methyl sulphonyl group and found that the activity decreased than the reported compounds (22-24, Table 3 and fig. 1). The same substitutions were carried for both models 1 and 2, the results were found to be similar.

The three-dimensional quantitative structure activity relationship studies have been carried out on a series of 17 compounds of pyrrolin-2-ones derivatives as COX-2 inhibitors to search for the best pharmacophore model. It brings important structural insights to aid the design of selective COX-2 inhibitors. The results of this study have successfully been applied to propose new molecules and this study presumably forms a valuable basis for future drug design against COX-2 research.

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