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Quantitative structure-activity relationship study of orally active cyclooxygenase-2 (COX-2) inhibitors of derivatives of 3-phenoxypyran-4-one

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The cyclooxygenase (COX) inhibition activities of the derivatives of 3-phenoxypyran-4-one were analyzed through multiple-regression analysis (MRA). Appropriate physicochemical parameters, identified for the substitutents of phenyl ring, attached to 3-phenoxypyran-4-one moiety were quantitatively correlated with COX-2 and COX-1 inhibition activities of these compounds. The derived significant correlation equation for COX-2 inhibition suggested that the *ortho*-substituent with negative resonance parameter, the *para*-substituent with lower dipole moment and the *meta*-substituent having higher resonance parameter were advantageous for the activity. The derived correlation equation for COX-1 inhibition suggested the significance of resonance effect for *ortho*-substituents and electron-donating effect for *para*-substituent. A few potential congeners were also suggested for further synthesis.

Keywords: Cyclooxygenase-2 inhibitors, 3-phenoxypyran-4-one derivatives, Quantitative structure-activity relationship

The characterization of cyclooxygenase-2 (COX-2)[1] led to the hypothesis that selective inhibitors of this isoform would exhibit similar clinical efficacy, but reduced ulcerogenicity than traditional non-steroidal anti-inflammatory drugs (NSAIDs), which were non-selective COX-1 and COX-2 inhibitors. Thus, a new class of anti-inflammatory agents celecoxib[2] and rofecoxib[3] were discovered. These drugs were extensively used in the management of pain and inflammation[4,5], with improved ulcerogenic profile[6]. However, rofecoxib, a selective COX-2 inhibitor could lead to thrombotic cardiovascular events through inhibition of prostacyclin formation in the infracted heart, which resulted in its withdrawal from the clinical practice[7]. The concern about the potential increased risk of cardiovascular events, associated with other coxibs still remains to be addressed and would determine the future use of these compounds. In addition to their use in analgesia and inflammation, therapeutic applications of coxibs were also expanded. The studies mainly targeted at the inhibition of COX-2 and focused on the treatment of various cancers[8-11] and neurodegenerative disorders, such as the Parkinson[12] and Alzheimer[13] diseases.

The second generation COX-2 inhibitors such as valdecoxib[14,15], etoricoxib[16] and lumiracoxib[17] were more selective for COX-2 than COX-1, compared to celecoxib and rofecoxib. Nevertheless, these drugs displayed an overall pharmacological profile, similar to first generation coxibs. Thus, efforts are being made to explore new compounds with better COX-2 inhibition activity profiles and minimal or no side effects. Earlier, a few quantitative structure-activity relationship (QSAR) studies[18-20] were reported on different classes of COX-2 inhibitors, with a view to rationalize the physicochemical or topological properties in relation to their inhibition actions.

Recently, a new coxib series, the 3-phenoxypyran-4-ones, a family of orally active, potent and very selective COX-2 inhibitors, with one of the best anti-inflammatory profiles in animal model, was reported[21]. However, this study was aimed only at the alterations of substituents at different positions of the 3-phenoxy ring and provided no rationale to reduce the trial-and-error factors. Hence, in the present communication, a QSAR study on these analogues (Fig. 1, Table 1) was conducted, in order to provide the rationale for drug design and to explore the possible mechanism of their action.

Materials and Methods

The 3-phenoxypyran-4-one derivates (Fig. 1 and listed in Table 1) reported by Caturla *et al.*[21] were subjected to QSAR analysis. The inhibitory effects against the human COX-2 and COX-1 enzymes and most appropriate quantifying parameters are also

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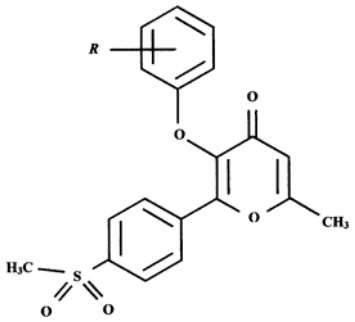

Fig. 1 — Derivatives of 3-phenoxypyran-4-one used in the present study

given in Table 1. The activity data was based on IC_{50} values (concentration of a compound to accomplish 50% inhibition of the enzyme) and were expressed as –log IC_{50} on the molar basis. The quantifying parameters — the resonance, dipole moment and Hammett´s electronic constant of different substituents were taken from the literature[22,23].

To derive the QSAR, multiple-linear regression (MLR) analysis following the method of least squares was considered. A number of statistical parameters were obtained in conjunction with such calculations to access the significance of the derived results. These

Table 1 — QSAR parameters and COX inhibition activity of derivatives of 3-phenoxypyran-4-one

S. No.	*R*	R_2	R_3	μ_4	σ_4	–log IC_{50}(M)[a]					
						Obsd.[b]	Cald. Eq. (4)	Prctd. LOO	Obsd.[c]	Cald. Eq. (5)	Prctd LOO
1	4-F	0.00	0.00	1.43	0.06	6.01	6.20	6.22	u.a.	4.18	--
2	4-Cl	0.00	0.00	1.59	0.23	6.49	6.15	6.12	3.68	3.98	4.10
3	4-Br	0.00	0.00	1.57	0.23	6.44	6.16	6.13	3.92	3.98	4.01
4	4-I	0.00	0.00	1.36	0.18	6.39	6.22	6.20	u.a.	4.04	--
5	4-CF_3	0.00	0.00	2.61	0.54	5.61	5.88	5.92	u.a	3.62	--
6	4-OCF_3	0.00	0.00	2.36	0.35	5.84	5.94	5.96	u.a.	3.84	--
7	4-NO_2	0.00	0.00	4.13	0.78	5.40	5.46	5.52	u.a.	3.34	
8	2,4-diF	–0.34	0.00	1.43	0.06	7.10	6.95	6.91	4.65	4.89	4.96
9	3,4-diF	0.00	–0.34	1.43	0.06	5.35	5.14	4.44	n.t.	--	--
10	3,4-diCl	0.00	–0.15	1.59	0.23	5.47	5.69	5.73	u.a.	3.98	--
11	2-F, 4-Cl	–0.34	0.00	1.59	0.23	6.70	6.91	6.97	4.73	4.69	4.68
12	2-F, 4-Br	–0.34	0.00	1.57	0.23	6.82	6.91	6.94	4.64	4.69	4.71
13	2-Cl, 4-Br	–0.15	0.00	1.57	0.23	6.51	6.49	6.49	4.45	4.30	4.27
14	4-F, 2-CH_3	–0.13	0.00	1.43	0.06	6.74	6.48	6.47	4.67	4.45	4.43
15	2-Cl, 4-CH_3	–0.15	0.00	0.36	–0.17	6.62	6.82	6.85	4.78	4.77	4.76
16	4-Cl, 2-CH_3	–0.13	0.00	1.59	0.23	6.72	6.44	6.43	4.44	4.26	4.22
17	2-Cl, 4-OCH_3	–0.15	0.00	1.30	–0.27	6.74	6.56	6.55	4.86	4.88	4.89
18	H	0.00	0.00	0.03	0.00	6.00	–[d]	–[d]	u.a.	--	--
19	2-CH_3	–0.13	0.00	0.03	0.00	6.77	6.86	6.88	4.64	4.53	4.51
20	3-CH_3	0.00	–0.13	0.03	0.00	5.87	6.17	6.27	u.a.	--	--
21	4-CH_3	0.00	0.00	0.36	–0.17	6.68	6.49	6.44	4.41	4.45	4.47
22	2-F,4-CH_3	–0.34	0.00	0.36	–0.17	7.22	7.24	7.25	5.16	5.16	5.16
23	4-NH_2	0.00	0.00	1.53	–0.66	5.83	6.17	6.20	u.a.	5.03	--

[a]IC_{50} = concentration of a compound to accomplish 50% inhibition of enzyme; [b]For COX-2; [c]For COX-1

u.a., uncertain activity value; n.t., not tested

[d]'outlier' compound in present study

Obsd., observed; Cald., calculated; Pretd., predicted

were the multiple correlation coefficient R, standard error of estimates s and F-value representing the ratio of the variance of calculated to observed activities. The ± data within parentheses, associated with coefficient of the quantifying parameters in a regression equation were the 90% confidence intervals.

The derived most significant QSAR equations were further subjected to a validation test[24] by the leave-one-out (LOO) method. This method created a number of modified data sets by taking away one compound from the present data set in such a way that each observation was taken away once only. Then, one model was developed for each reduced data set and the response values of the deleted observations were predicted from the model. The squared differences between predicted and actual values were added to give the predictive residual sum of squares (PRESS). The cross-validation index q^2 was calculated as (SSY– PRESS)/SSY, where SSY represents the variance of observed activities of compounds around the mean value. To be a reasonable QSAR model, q^2 should be >0.6 and a value >0.9 for this index indicated an excellent model. The required softwares for MLR and cross-validation were developed in our laboratory.

Results and Discussion

Initially, a data set comprising of substituent constants such as hydrophobicity π, hydrogen-bond donor HD, hydrogen-bond acceptor HA, electronic (*meta* and *para*) σ, field F, resonance R, dipole moment μ, Taft´s steric Es, molar refraction MR, molecular weight MW, and van der Waals volume Vw for each of three varying positions at 3-phenoxy ring of pyran-4-one moiety, was considered for the compounds in Table 1. This led to a large number of QSAR equations, which were then subjected to various statistical tests. The correlation equations, which provided the highest R and F and lowest s values were only retained for further consideration. Following this strategy of variable selection, the MRA in a step-wise manner gave the correlation Eqs. (1) to (3) for COX-2 inhibition activity in the variables describing resonance effect and dipole moment

$$-\log IC_{50} = -3.177\ (\pm 1.07)R_2 + 6.015$$

n=23, R=0.746, s=0.377, F(1,21)=26.285 …(1)

$$-\log IC_{50} = -2.772(\pm 0.97)R_2 + 2.527(\pm 1.58)R_3 + 6.122$$

n=23, R=0.824, s=0.329, F(3,19)=21.086 …(2)

$$-\log IC_{50} = -2.464(\pm 0.77)R_2 + 2.844(\pm 1.24)R_3 - 0.224(\pm 0.10)\ \mu_4 + 6.465$$

n = 23, R=0.902, s=0.256, F(3,19)=27.762, q^2=0.669 …(3)

The subscripted numeral associated to the identified variables were indicative of different positions of substituents in 3-phenoxy ring. The derived statistical parameters R, s and F of these equations were improved over in succession. The q^2-index was, however, obtained for Eq. (3) only to ascertain the significance of final statistical model. All the statistical parameters of Eq. (3) were in favor of significantly sound results. Both the R^2 and the F-values accounted for 81% of variance and 99% level of significance [$F_{3,19}(0.01) = 5.01$] respectively. The q^2-index, in addition, was also in support of statistical sound model. Eq. (3) was further improved by ignoring compound **18**, which was a lone unsubstituted compound in the series. Possibly, 3-phenoxy ring alone might not be proper for interaction at the receptor site. The resulting correlation is shown in Eq. (4):

$$-\log IC_{50} = -2.219(\pm 0.58)\ R_2 + 3.100(\pm 0.92)\ R_3 - 0.272(\pm 0.08)\mu_4 + 6.584$$

n = 22, R=0.923, s=0.234, F(3,18)=34.307, q^2=0.683 …(4)

The statistical parameters of this equation were improved over to that of Eq. (3). The s-value and ± confidence intervals (within parentheses) were both lowered. The increased R and F values accounted respectively for 85% (R^2 = 0.852) of variance and 99% level of significance [$F_{3,18}(0.01) = 5.09$]. The q^2-index, representing a reasonable statistical sound model was also improved. That the independent variables were mutually orthogonal is shown in Table 2. This equation was used to calculate the activity values of all the compounds and the same were found in close agreement with the observed ones (Table 1).

The plot showing the variation of observed *versus* calculated and predicted –log IC_{50} values, obtained respectively through Eq. (4) and LOO approach is shown in Fig. 2. Such a demonstration may help to

Table 2 — Intercorrelation matrix[a] amongst the descriptors of Eq. (4)

	R_2	R_3	μ_4
R_2	1.000	0.278	0.225
R_3		1.000	0.099
μ_4			1.000

[a]Matrix elements are the simple correlation coefficient, (r) values

understand the goodness of fit and to identify systematic variation of observed *versus* calculated/predicted activities for the compounds under present study. In addition to LOO approach, the study was also carried out to leave-many-out congeners, following the external validation method (EVM). In this approach, the sets of four compounds (18% of total data points) were selected as the test set, while remaining 18 members were considered in the predictive set to derive correlation equations to further validate the outcomes. These results are given in Table 3, along with the predicted activities of compounds in a test set. All derived correlations remained statistically sound and the calculated activities were in close agreement with the observed ones.

From Eq. (4), it appeared that the resonance parameter of *ortho*-substituents and dipole moment of *para*-substituents were adding negatively to the activity values, whereas the resonance parameter of *meta*-substituents was augmenting it. Thus, for a higher activity, *ortho*-substituent having a more negative resonance parameter, and *para*-substituent with a lower dipole moment were advantageous. Additionally, *meta*-substituent having higher resonance parameter was advantageous for the activity.

In addition to COX-2 activity, 13 of these compounds with substituents only at *ortho*- and *para*-positions had also shown the COX-1 inhibition activity[21] (Table 1). A large number of quantifying parameters were attempted to derive correlation equations, however, only the resonance R and the electronic σ parameters respectively for *ortho*- and *para*-substitutents could exhibit a significant correlation, shown in Eq. (5)

$$-\log IC_{50} = -2.089(\pm 0.66)\, R_2 - 1.173(\pm 0.45)\sigma_4 + 4.254$$

$$n=13,\ R=0.919,\ s=0.166,\ F(2,10)=27.148,\ q^2 = 0.751 \quad \ldots(5)$$

This equation accounted for 84% of variance in the observed activities and expressed a reasonable statistical sound model. In addition, the F-value

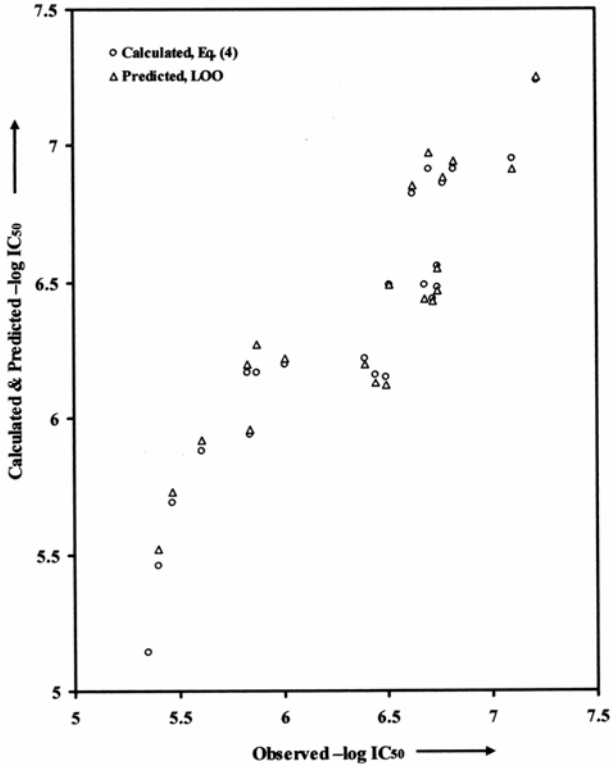

Fig. 2 — Plot of observed *versus* calculated and predicted –log IC_{50} values

remained significant at 99% level [$F_{2,10}(0.01) = 7.56$]. Both descriptors correlating inhibition actions of the compounds for COX-1 enzyme were found to be poorly intercorrelated (R_2 *vs.* σ_4; r = 0.011). The –log IC_{50} values, calculated using above equation and predicted from LOO approach were in close agreement with the observed ones.

From Eq. (5), it may be concluded that for a higher potency compound, an *ortho*-substituent should have more negative resonance parameter, while a *para*-substituent, in addition, should be more electron-donor. Following above guidelines, new analogues with improved activity may be designed for further synthesis. Thus, substituents to be selected at different positions in 3-phenoxylpyran-4-one moiety that may enhance the inhibition activity towards COX-2 and COX-1 enzymes now become evident from the model Eqs (4) and (5). While selecting the substituents at *ortho*-, *meta*- and *para*- positions of 3-phenoxy ring, it was noted that the selected pattern should not deviate too much from what was reported in the original data set. A few compounds, suggested for further synthesis with improved activity values are listed in Table 4. The present study, therefore, may be

Table 3 — Test set, derived correlations on predictive set (n = 18) by external validation method and predicted activities of compounds in test set

Test set[a]	Equation: $-\log IC_{50} = a_1R_2 + a_2R_3 + a_3\mu_4 + a_0$				R	s	F (3,14)	Prctd. $-\log IC_{50}$
	a_1	a_2	a_3	a_0				(Test set)
1,3,5,7	–2.113 (±0.79)	3.220 (±1.20)	–0.206 (±0.15)	6.535	0.921	0.231	25.942	6.24, 6.21, 6.00, 5.68
9,11,13,15	–2.246 (±0.79)	5.375 (±2.23)	–0.306 (±0.11)	6.677	0.936	0.219	32.930	4.41, 6.95, 6.53, 6.90
17,19,21,23	–2.160 (±0.81)	3.142 (±1.25)	–0.266 (±0.15)	6.586	0.931	0.236	30.289	6.56, 6.86, 6.49, 6.18
2,4,6,8	–2.293 (±0.87)	2.923 (±1.22)	–0.267 (±0.11)	6.533	0.930	0.235	29.841	6.11, 6.17, 5.90, 6.93
10,12,14,16	–2.205 (±0.82)	2.789 (±1.24)	–0.270 (±0.11)	6.561	0.925	0.237	27.829	5.71, 5.89, 6.46, 6.42
1,4,7,10	–2.163 (±0.82)	2.901 (±1.32)	–0.243 (±0.14)	6.565	0.905	0.245	21.161	6.22, 6.23, 5.56, 5.74
13,16,19,22	–2.192 (±0.86)	3.092 (±1.28)	–0.285 (±0.12)	6.596	0.912	0.251	23.085	6.48, 6.43, 6.87, 7.24
2,6,10,14	–2.284 (±0.75)	2.738 (±1.21)	–0.263 (±0.10)	6.540	0.931	0.227	30.343	6.12, 5.92, 5.71, 6.46
2,7,12,17	–2.415 (±0.86)	2.920 (±1.22)	–0.255 (±0.14)	6.517	0.922	0.239	26.339	6.11, 5.47, 6.94, 6.55
3,8,13,19	–2.025 (±0.84)	2.831 (±1.19)	–0.311 (±0.11)	6.645	0.933	0.230	31.432	6.16, 6.89, 6.46, 6.27

[a]For numbers see Table 1

Table 4 — Suggested substituents of 3-phenoxypyran-4-one moiety, quantifying parameters and predicted activity values from model equations for inhibition of COX-2 and COX-1

S. No.	*R*	R_2	R_3	μ_4	σ_4	$-\log IC_{50}$(M)	
						Prctd (COX-2)	Prctd (COX-1)
						Eq. (4)	Eq. (5)
1	2-NH_2, 3-CF_3	–0.68	0.19	0.03	0.00	8.67	5.67
2	2-NH_2, 3-NO	–0.68	0.49	0.03	0.00	9.60	5.67
3	2-$NHCH_3$, 3-CF3	–0.74	0.19	0.03	0.00	8.81	5.80
4	2-$NHCH_3$, 3-NO	–0.74	0.49	0.03	0.00	9.74	5.80
5	2-$NH(CH_3)_2$, 3-CF3	–0.92	0.19	0.03	0.00	9.21	6.18
6	2-$NH(CH_3)_2$, 3-NO	–0.92	0.49	0.03	0.00	10.14	6.18
7	2-NH_2,3-CF_3, 4-C_2H_5	–0.68	0.19	0.39	–0.55	8.58	6.32
8	2-NH_2,3-NO, 4-C_2H_5	–0.68	0.49	0.39	–0.55	9.50	6.32
9	2-$NHCH_3$, 3-CF_3, 4-C_2H_5	–0.74	0.19	0.39	–0.55	8.71	6.45
10	2-$NHCH_3$, 3-NO, 4-C_2H_5	–0.74	0.49	0.39	–0.55	9.64	6.45
11	2-$NH(CH_3)_2$, 3-CF_3, 4-C_2H_5	–0.92	0.19	0.39	–0.55	9.11	6.82
12	2-$NH(CH_3)_2$, 3-NO, 4-C_2H_5	–0.92	0.49	0.39	–0.55	10.04	6.82

used for rationalizing the substituents in the design of new congeners.

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