J. Enzyme Inhibition, 1998, Vol. 13, pp. 409–417
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NOVEL INHIBITORS OF CYCLOOXYGENASE-2: THE SULFONES AND SULFONAMIDES OF 1,2-DIARYL-4,5-DIFLUOROBENZENE. ANALYSIS OF QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP

P. SINGH* and R. KUMAR

Department of Chemistry, S. K. Government College, Sikar-332001, India

(Received 19 February 1998)

By a quantitative structure–activity relationship (QSAR) study, the inhibitory activity of a novel series of sulfones and sulfonamides of 1,2-diaryl-4,5-difluorobenzene, against the inducible form of cyclooxygenase (COX-2) was shown to be significantly correlated with the electronic constant (σ) and some suitable indicator parameters. Considering the results of derived correlations, the substitutional requirement at different positions in the aromatic rings is discussed. In addition, a similar substitutional pattern for these congeners is found through a Fujita–Ban study. The latter approach also assigns higher (positive) activity contributions to those substituents that were guided by the former QSAR study.

Keywords: Quantitative structure–activity relationship (QSAR); Inhibitors of cyclooxygenase; COX-2; Sulfones and sulfonamides of 1,2-diaryl-4,5-difluorobenzene; Terphenyls; Fujita–Ban approach

INTRODUCTION

The presently available nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of inflammatory conditions.[1–3] These drugs, however, disrupt the production of beneficial prostaglandin through a mechanism-based toxicity mainly in the gastrointestinal tract and kidney.[4–6]

* Corresponding author.

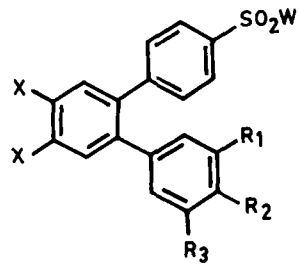

FIGURE 1 Structures of 1,2-diaryl-4,5-difluorobenzene sulfones and sulfonamides.

This restricts their therapeutic utilisation especially when long-term treatment is prescribed. Recently an inducible form of cyclooxygenase, known as COX-2, has been discovered which is expressed mainly in inflammed tissues.[7–10] This useful finding has prompted many pharmaceutical industries to identify selective and orally active COX-2 inhibitors as these may provide the desired anti-inflammatory and analgesic activities without the harmful side effects which are often associated with the commonly available NSAIDs.

More recently Li *et al.*[11] have successfully identified a novel series of 1,2-diaryl-4,5-difluorobenzene sulfones and sulfonamides (Figure 1) which possess greater *in vitro* COX-1/COX-2 enzyme selectivity and much superior *in vivo* activity than that observed previously for other series of compounds.

The *in vitro* inhibitory activities of these congeners were evaluated against both the constitutive (COX-1) and inducible (COX-2) forms of the human recombinant enzymes.[12] The inhibitory activity profile, IC_{50} represents the minimum concentration of a compound required to bring about 50% inhibition of the enzyme under consideration. As the COX-1 inhibitory activities are reported only for a few compounds, the present study is, therefore, aimed at establishing the quantitative structure–activity relationship (QSAR) between the COX-2 inhibitory activities and relevant physicochemical parameters governing the binding forces at the site on the enzyme. Such a QSAR study not only provides the rationale for drug design but also illuminates the mechanism of action of a congeneric series.

MATERIALS AND METHODS

The QSAR study was made on a series of sulfones and sulfonamides of 1,2-diaryl-4,5-difluorobenzene. These compounds, also known as the terphenyls, are novel and selective inhibitors of an inducible form of the human

recombinant enzyme system, the cyclooxygenase-2 (COX-2). Their inhibitory actions against this enzyme are expressed as $-\log IC_{50}$ on a molar basis. The most important physicochemical parameter was found to be the electronic constant, σ whose values were taken directly from the literature.[13,14] The title compounds, their $-\log IC_{50}$ values for the COX-2 enzyme system and the correlative parameter, σ are listed in Table I. Additionally,

TABLE I Novel terphenyls studied by Li *et al.*[11] and their COX-2 inhibitory activities and physicochemical parameters (Figure 1 for structures)

Compound no.	R_1	R_2	R_3	W	X	$\sigma(R_2)$	$-\log IC_{50}(M)$[a]		
							Obsd.[c]	*Calcd. Eq.* (4)	*Calcd. F.B.*
1	H	F	H	Me	F	0.06	7.85	8.04	8.02
2	H	F	H	NH_2	F	0.06	8.40	8.57	8.55
3	H	F	Cl	Me	F	0.06	8.00	8.04	8.11
4	H	F	Cl	NH_2	F	0.06	8.70	8.57	8.64
5	H	F	Me	Me	F	0.06	8.30	8.04	8.05
6	H	F	Me	NH_2	F	0.06	8.70	8.57	8.58
7	H	OMe	F	Me	F	−0.27	7.68	7.80	7.52
8	H	OMe	F	NH_2	F	−0.27	7.89	8.34	8.05
9	H	OMe	Cl	Me	F	−0.27	7.72	7.80	7.61
10	H	OMe	Cl	NH_2	F	−0.27	7.89	8.34	8.14
11	Cl	OMe	Cl	NH_2	F	−0.27	7.68	7.50	7.52
12	H	OMe	Me	Me	F	−0.27	7.89	7.80	7.55
13	H	OMe	Me	NH_2	F	−0.27	8.30	8.34	8.08
14	H	OMe	OMe	Me	F	−0.27	6.47	6.58	6.56
15	H	OMe	OMe	NH_2	F	−0.27	7.19	7.12	7.10
16	H	Me	H	Me	F	−0.17	8.15	7.88	7.85
17	H	Me	H	NH_2	F	−0.17	8.40	8.41	8.38
18	H	Me	Cl	Me	F	−0.17	7.89	7.88	7.93
19	H	Me	Cl	NH_2	F	−0.17	8.52	8.41	8.46
20	H	Me	Me	Me	F	−0.17	7.64	7.86	7.88
21	H	Me	Me	NH_2	F	−0.17	8.30	8.41	8.41
22	H	Cl	Me	Me	F	0.23	8.22	8.16	8.39
23	H	Cl	Me	NH_2	F	0.23	8.52	8.70	8.93
24	H	NMe_2	Cl	Me	F	−0.72[b]	8.10	7.48	7.89
25	H	NMe_2	Cl	NH_2	F	−0.72	8.22	8.02	8.43
26	H	F	H	Me	H	0.06	6.59	6.74	6.64
27	H	F	H	NH_2	H	0.06	6.79	7.27	7.17
28	Cl	F	H	Me	H	0.06	6.44	5.90	6.02
29	Cl	F	H	NH_2	H	0.06	7.77	—[d]	—[d]
30	H	Cl	H	Me	H	0.23	6.85	6.86	6.98
31	H	Cl	H	NH_2	H	0.23	8.22	7.40	7.51
32	Cl	OMe	H	Me	H	−0.27	4.94	5.66	5.52
33	Cl	OMe	H	NH_2	H	−0.27	7.72	—[d]	—[d]
34	H	$-OCH_2O-$		Me	F	−0.16	7.92	7.88	7.89
35	H	$-OCH_2O-$		NH_2	F	−0.16	8.40	8.42	8.43
36	H	$-OCH_2CH_2O-$		Me	F	−0.12[b]	6.47	6.69	6.71
37	H	$-OCH_2CH_2O-$		NH_2	F	−0.12	7.49	7.23	7.25

[a] IC_{50} represents the minimum concentration of a compound, required to bring about 50% inhibition of COX-2 enzyme. [b] Taken from Ref. [14]. [c] Taken from Ref. [11]. [d] The 'outlier' of present study.

some indicator variables are also used in connection with some specific alterations. Both the Hansch and the Fujita–Ban approaches were used for obtaining meaningful results.

RESULTS AND DISCUSSION

The listed compounds of Table I have alterations at R_1, R_2, R_3, W and X positions in the aromatic rings but three of these have binary variations only. These positions, being R_1, W and X contain either H, Me and H or Cl, NH_2 and F respectively. Such variations are easily accounted for by considering three different positional indicator variables, I_1, I_W and I_X. The resulting, correlation between $-\log IC_{50}$ and the indicator variables alone is:

$$-\log IC_{50} = -0.471(\pm 0.340)I_1 + 0.675(\pm 0.193)I_W + 0.816(\pm 0.282)I_X + 6.813,$$
$$n = 37, \quad r = 0.710, \quad s = 0.583, \quad F(3,33) = 11.211. \qquad (1)$$

In this equation, n is the number of data points, r is the correlation coefficient, s is the standard deviation, F is the F-ratio between the variances of calculated and observed activities, and the data within parentheses are 95% confidence intervals. Here the indicator variable I_1 characterising R_1-position, is indicated by a value 1 for Cl and 0 for H. Analogously, I_W is taken as 1 when there is NH_2, and 0 for Me at the position W. The variations of X-position are either F or H, which are described by I_X with a value of 1 for the former and 0 for the latter. As seen from Eq. (1), the three indicator variables have alone accounted for 50% ($r^2 = 0.504$) of variance in observed activity values. Further improvement of the statistical parameters of the above equation is possible if suitable parameters, accounting for the substitutional variations of R_2 and R_3 are also considered. A large number of important parameters pertaining to electronic, hydrophobic and steric effects have been attempted for these two positions but only the electron-withdrawing effect, described by σ for R_2 and none for R_3 could provide slightly better results. The derived correlation is given by Eq. (2).

$$-\log IC_{50} = 0.786(\pm 0.468)\sigma(R_2) - 0.309(\pm 0.345)I_1 + 0.671(\pm 0.188)I_W + 1.033(\pm 0.303)I_X + 6.718,$$
$$n = 37, \quad r = 0.738, \quad s = 0.568, \quad F(4,32) = 9.577. \qquad (2)$$

All the statistical parameters have now slightly improved and this equation as such is in favour of electron-withdrawing substituents at the R_2-position. Further, in four compounds, viz. **14**, **15**, **36** and **37**, both the R_2- and R_3-positions are substituted symmetrically either by $-OMe$ ($R_2 = R_3 = OMe$) or by $-OCH_2CH_2O-$ ($R_{2,3} = -OCH_2CH_2O-$). Such symmetric substitutions often display unusual results and are commonly reflected through the consideration of dummy variables in QSAR studies. Thus a value 1 is assigned to the dummy variable I_S if both R_2- and R_3-positions are substituted symmetrically and 0 otherwise. Inclusion of this additional variable has significantly improved the results and the derived correlation is shown by Eq. (3):

$$\begin{aligned} -\log IC_{50} = {} & 0.647(\pm 0.344)\sigma(R_2) - 0.393(\pm 0.253)I_1 - 1.202(\pm 0.225)I_S \\ & + 0.666(\pm 0.137)I_W + 1.135(\pm 0.223)I_X + 6.766, \\ n = 37, \quad & r = 0.874, \quad s = 0.416, \quad F(5, 31) = 19.975. \end{aligned} \tag{3}$$

Further, compounds **29** and **33** have shown the 'outlier' behaviour from the remaining compounds. Their calculated activity values, using Eq. (3), were largely deviating from the observed ones. At present no reason seems to be appropriate that could explain such an out of trend behaviour. Perhaps, these two compounds entail errors in the determination of their biological activities. Ignoring both these congeners, the resulting highly significant correlation was given by Eq. (4).

$$\begin{aligned} -\log IC_{50} = {} & 0.713(\pm 0.277)\sigma(R_2) - 0.841(\pm 0.224)I_1 - 1.220(\pm 0.178)I_S \\ & + 0.535(\pm 0.112)I_W + 1.300(\pm 0.180)I_X + 6.696, \\ n = 35, \quad & r = 0.928, \quad s = 0.329, \quad F(5, 29) = 36.082. \end{aligned} \tag{4}$$

The r^2-value now accounts for 86% of variance and F-value stands significant at 99% level [$F_{5,29}(0.01) = 3.73$] has also increased. The s-value, in comparison with earlier equations, is also lowered and the $\pm$ data associated with the coefficients of descriptor variables are found to be suitably adjusted. This highly significant correlation equation was, therefore, used to calculate the theoretical activity value for 35 data points; these were found to be in close agreement with the observed ones (Table I). Further, the variables used in the above equation have no mutual correlations as shown in Table II. The correlation equation (4) in five independent variables is further cross-checked by considering a random sample of 25 compounds in four different training sets.

TABLE II Mutual correlation of the variables used in deriving Eq. (4)

	$\sigma(R_2)$	I_l	I_S	I_W	I_X
$\sigma(R_2)$	1.000	0.053	0.118	0.023	0.374
I_l		1.000	0.110	0.093	0.402
I_S			1.000	0.010	0.163
I_W				1.000	0.139
I_X					1.000

TABLE III Derived correlations using $-\log IC_{50} = b_1\sigma(R_2) + b_2I_l + b_3I_s + b_4I_W + b_5I_X + c$ (for $n = 25$) for random training sets[a] to cross-validate the correlation in Eq. (4)

Equation no.	b_1	b_2	b_3	b_4	b_5	c	r	s	$F_{5,19}$
(4a)	0.504 (±0.242)	−0.607 (±0.249)	−1.250 (±0.177)	0.413 (±0.098)	2.327 (±0.345)	5.683	0.964	0.234	50.116
(4b)	0.632 (±0.295)	−0.649 (±0.227)	−1.253 (±0.234)	0.415 (±0.130)	1.621 (±0.215)	6.425	0.949	0.312	34.433
(4c)	0.618 (±0.335)	−0.980 (±0.328)	−1.284 (±0.202)	0.575 (±0.151)	1.287 (±0.227)	6.787	0.942	0.358	29.960
(4d)	0.662 (±0.321)	−0.659 (±0.223)	−1.283 (±0.194)	0.489 (±0.127)	1.630 (±0.214)	6.412	0.954	0.307	38.184

[a] Compounds considered for correlation (4a) are **1–24, 32**; for (4b) are **4, 6–28, 32**; for (4c) are **4, 6, 13–37**; and for (4d) are **3–7, 10–14, 17–21, 24–28, 31–35**.

In each of these sub-sets, the congeners of highest (compounds **4** and **6**) and lowest (compound **32**) activity values were retained and subjected to MRA. The derived correlations (Eqs. (4a)–(4d)) for them are summarised in Table III. These equations gave highly significant results and followed the similar statistical trends to that of Eq. (4), which cross-validates the best correlation obtained. From Eq. (4), the following conclusions may now be drawn:

1. The position R_1 better remains unsubstituted, possibly due to steric reasons.
2. The positions W and X, having respectively the NH_2- and F-substituents, improve the activity of a compound.
3. Substituents capable of withdrawing electron density more efficiently from the phenyl ring and are present at R_2 are advantageous.
4. Symmetrical substituents, like –OMe or $-OCH_2CH_2O-$ present at the neighbouring positions, R_2 and R_3 are unfavourable.

These findings are supported further through the study of the Fujita–Ban approach in which the biological activity, BA_i of ith compound is

expressed as

$$BA_i = \sum a_{ij}X_{ij} + \mu, \tag{5}$$

where a_{ij} is the group contribution of the substituent X_i in the position j and μ is the calculated biological activity value of the reference compound. Depending upon the presence or absence of the substituent X_i in the position j a value 1 or 0, in that order is taken for X_{ij}. For the present work, Eq. (5) has resulted into 37 linear equations in 14 unknowns including the contribution of the parent moiety. Compound **26** is considered as the reference compound. Tabulation of the matrix of these equations is avoided here for the sake of brevity and are solved by the method of least squares for a_{ij} and μ. The results obtained are summarised in the first column of Table IV and the statistical parameters of the study were:

$$n = 37, \quad r = 0.895, \quad s = 0.453, \quad F(14, 22) = 6.302.$$

In addition the study has also accounted for 67% of variance which is a slightly low value to justify the true significance of the derived results. Further, compounds **29** and **33** which were 'outliers' of the Hansch study were also ignored here. In doing so, the corresponding rows were removed from the Fujita–Ban matrix and the regression analysis of the resulting matrix lead to the contributions of parent moiety and various substituents, summarised in the second column of Table IV. The statistical parameters were:

$$n = 35, \quad r = 0.950, \quad s = 0.333, \quad F(14, 20) = 13.168.$$

The variance obtained now was steeply increased to 83%. All these statistical parameters were in tune with highly significant results and the contributions of various substituents obtained may provide the compounds with increased activity profiles. The substituents that are making a positive contribution to activity in the parent moiety have the following pattern:

R_1	R_2	R_3	W	X
H	Cl	Cl	NH_2	F
	F	Me		
	Me			

TABLE IV Parent and substituent contributions to COX-2 inhibition activities of novel terphenyls (Figure 1 for structures)

Positions	*Substituents*	*Contributions*	
		$n = 37, \mu = 6.716$	$n = 35, \mu = 6.635$
R_1	–Cl	–0.176	–0.616
R_2	–Cl	0.271	0.344
	–Me	–0.132	0.175
	–OMe	–0.456	–0.499
	$-NMe_2$	–0.108	–0.211
R_3	–F	–0.025	–0.004
	–Cl	0.002	0.084
	–Me	0.047	0.029
	–OMe	–0.980	–0.959
$R_{2,3}$	$-OCH_2O-$	–0.106	–0.127
	$-OCH_2CH_2O-$	–1.286	–1.307
W	$-NH_2$	0.667	0.532
X	–F	1.217	1.387

These conclusions regarding substitutional requirements were in accordance with the results obtained through the Hansch analysis. The position R_1 is required to be unsubstituted whereas the positions W and X have demanded, respectively, the NH_2- and F-substitutions. Likewise, the substituents that are either better electron acceptors (Cl and F) or poor electron donor (Me) at R_3 are desirable. In addition, the Fujita–Ban study has emphasised the suitability of substituents such as Cl and Me at R_3 and none bridging the R_2- and R_3-positions. The substituents at these positions such as $-OCH_2O-$ and $-OCH_2CH_2O-$ have their negative contributions (Table III) and thus lead to a decrease in activity values. In corroboration of the results of both approaches, the theoretical activity of a simple compound with substituents such as $R_1 = H$, $R_2 = Cl$, $R_3 = H$, $W = NH_2$ and $X = F$ (the compound being outside the training set) is nearly 1.2 orders of magnitude higher than the highest active compound of the present series. This activity value may further be increased by incorporating better electron acceptors at R_2, in addition to the substituents of R_1-, R_3-, W- and X-positions of the above suggested compound. The calculated $-\log IC_{50}$ values obtained by adding the requisite substituents contribution to μ, are in close agreement with the observed values (last column of Table I).

In conclusion it can be stated that the present QSAR study provides a rationale for the *in vitro* inhibition of the inducible form of the human recombinant enzyme, COX-2, and opens up new direction for the drug–receptor interaction at the molecular level. The study also serves as a basis for substituent-selection in drug-design strategy.

Acknowledgements

This work was supported by the UGC, Central Regional Office, Bhopal through a minor research grant sanctioned to P.S.

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