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Quantitative structure-activity relationship study on N-(pyridin-4-yl)-(indol-3-yl) alkylamides as antiallergic agents

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The antihistamine activity of N-(pyridin-4-yl)-(indol-3-yl) alkylamides has been analyzed using Fujita-Ban and Hansch approaches. The analyses have helped to ascertain the role of different substituents in explaining the antiallergic actions of these analogues. From both approaches it is revealed that the small size substituents at R and R_2 and non-hydrogen bond acceptor substituent at R improve histamine antagonist activity of a compound. Likewise, a small incision such as $-CH_2CONH-$ serving as the spacer between pyridinyl and indolyl rings and a bigger substituent like 4-FBn at R_1 are also desirable for inhibitory activity.

A new series of N-(pyridin-4-yl)-(indol-3-yl)alkylamides (Fig. 1) as the potent antiallergic agents have recently been reported by Menciu *et al.*[1]. Antiallergic acitivity of these compounds was evaluated using three *in vitro* assays i.e., inhibition of interleukins, IL-4 and IL-5 production in Th-2 cells and inhibition of ovalbumin-induced histamine release in guinea pig peritoneal mast cells. Results were expressed in per cent inhibition of IL-4 and IL-5 and by IC_{50} for histamine antagonism. The per cent inhibition of IL-4 and IL-5 at 10 μM concentrations was reported only for a few compounds, reflecting the minimum scope to draw any meaningful conclusion. The *in vitro* inhibition of allergically induced histamine release in rat peritoneal mast cells was, however, determined for a fairly large number of compounds and the same is considered for present work.

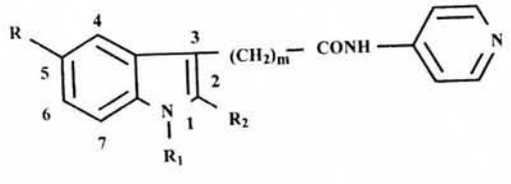

Fig. 1—Structures of N-(pyridin-4-yl)-(indol-3-yl)alkylamides

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The aim of present communication was to establish quantitative structure-activity relationship (QSAR), similar to Hansch analysis, between inhibition activity of histamine and relevant parameters quantifying the structural variations in a compound. In addition, the Fujita-Ban approach was also followed to calculate the contributions of parent moiety and various substituents mounted on it. These studies further helped to predict the antagonist activities of untested compounds of the series.

Hansch and co-workers[2-7] have proposed that the molar concentration (in logarithmic scale) that elicits a constant biological response of a drug molecule is the linear function of its physico-chemical parameters governing various types of interactions. Among others, three major physico-chemical factors, namely, the hydrophobic, the steric and the electronic, are recognized to play the pivotal role in drug-receptor interaction. The multiple regression analysis[8,9] (MRA) is used to derive the QSARs between the activity and various parameters governing the aforesaid interactions and the resulting correlations are assessed through a number of statistics obtained in conjunction with such calculations.

The observation that in a congeneric series the substituents ought to contribute constant increments or decrements to biological activity, led Free and Wilson[10] to develop an alternative QSAR approach, which was based on an additive principle. This is most conveniently expressed by Eq. (1)

$$BA_i = \Sigma a_j X_{ij} + \mu \qquad \ldots (1)$$

where X_j is the jth substituent with a value of 1 if present and 0 if not, a_j is the contribution of the substituent to biological activity, and μ is the overall average activity. All activity contributions at each position of substituents must sum to zero. The series of linear equations so generated is solved by the method of least squares for a_j and μ. Fujita and Ban[11] later modified this approach in which symmetric equations were not required and μ was interpreted as the theoretical biological activity value of the reference compound of a series. The reference compound is usually (but not necessarily) the unsubstituted congener.

The van der Waals' volume, *Vw* a structural parameter that accounts for the bulk of a molecule/substituent has emerged the most appropriate quantifying parameter for this series. Its method of calculation has been described in one of our earlier publications[12] and was based on the original suggestion of Bondi[13]. Further, the hydrogen bond acceptor, property for certain substituents was also found as the additional appropriate quantifying parameter.

The histamine antagonist activity, IC_{50} represents the concentration of a compound to bring out the half-maximal inhibition of induced histamine release. This activity, further expressed as $-\log IC_{50}$ on molar scale, and the explaining variables of the substituents are given in Table 1. The best fit between $-\log IC_{50}$ and these explaining variables (or predictors) was found through MRA employing the method of least squares.

The compounds listed in Table 1 were used in the construction of Fujita-Ban matrix with compound 3 as the reference congener. Tabulation of this matrix of 26 linear equations in 10 unknowns including the contribution of parent compound is avoided here for the sake of brevity. These equations were solved by the method of least squares for the unknowns, μ and a_j. The contributions of various substituents obtained thereby are summarized in Table 2 along with 95% confidence intervals (the ± data within parentheses). The resulting statistical parameters of the study are:

$n = 26, R = 0.886, s = 0.418, F(10,15) = 5.502$

The above derived statistical parameters, at first instance, are not statistically impressive to draw any conclusion regarding the selection of substituents at varying sites in a compound. The close inspection of calculated activity values of all the data points in the training set, however, revealed that the compound 24 is the only congener whose calculated activity value (4.84) was found to be lower than the observed one. This data point was, therefore, ignored in the follow

Table 1—QSAR parameters, observed and calculated histamine inhibition activity values of N-(pyridin-4-yl)-(indol-3-yl) alkylamides (Fig. 1 for structures)

Compound no.	m	R	R_1	R_2	R_2 *HA* (R)	ΣVw 10^2 Å^3	$Vw(R_1)$ 10^2 Å^3	$-\log IC_{50}$(M) Obsd[a]	Cald F.B.	Cald Eq.(3)
1	1	H	4–FBn	H	0	0.344	0.995	7.80	7.46	7.13
2	2	H	4–FBn	H	0	0.498	0.995	6.66	6.99	6.69
3	3	H	H	H	0	0.652	0.056	5.54	5.57	5.53
4	3	H	4–FBn	H	0	0.652	0.995	6.29	6.26	6.24
5	1	OMe	Bn	H	1	0.592	0.939	5.55	5.82	5.74
6	2	OMe	Bn	H	1	0.746	0.939	5.41	5.35	5.29
7	1	OMe	4–FBn	Me	1	0.781	0.995	5.30	5.09	5.23
8	1	Cl	H	H	0	0.532	0.056	5.35	5.84	5.88
9	2	Cl	H	H	0	0.686	0.056	5.84	5.37	5.43
10	2	Cl	Bn	H	0	0.686	0.939	6.13	6.03	6.10
11	1	Cl	4–FBn	H	0	0.532	0.995	6.64	6.53	6.59
12	2	Cl	4–FBn	H	0	0.686	0.995	5.89	6.07	6.15
13	2	F	H	H	0	0.557	0.056	5.66	5.78	5.81
14	1	F	Bn	H	0	0.403	0.939	7.22	6.90	6.92
15	2	F	Bn	H	0	0.557	0.939	6.38	6.44	6.48
16	2	F	4–FBn	H	0	0.557	0.995	6.42	6.48	6.52
17	1	F	H	Me	0	0.592	0.056	4.99	5.48	5.71
18	1	F	Bn	Me	0	0.592	0.939	6.32	6.13	6.38
19	1	F	4–FBn	Me	0	0.592	0.995	6.40	6.17	6.42
20	1	^{i}Pr	H	H	0	0.791	0.056	5.31	5.31	5.13
21	2	^{i}Pr	H	H	0	0.945	0.056	5.51	4.84	4.69
22	2	^{i}Pr	Bn	H	0	0.945	0.939	5.22	5.49	5.36
23	2	^{i}Pr	4–FBn	H	0	0.945	0.995	5.27	5.53	5.40
24	1	^{i}Pr	H	Me	0	0.980	0.056	5.55[b]	4.84	4.59
25	1	^{i}Pr	Bn	Me	0	0.980	0.939	5.12	5.19	5.26
26	1	^{i}Pr	4–FBn	Me	0	0.980	0.995	5.16	5.23	5.30

[a]Molar concentration required for *in vitro* study to produce half-maximal inhibition of allergically induced histamine release with rat peritoneal mast cells; taken from ref. 1; [b]Outlier compound of the present study

up QSAR analysis. At present, there is no specific reason known that may account for its 'outlier' behaviour. Possibly this compound entails an error in the experimental determination of its activity value. While eliminating this data point, the corresponding row was removed from the Fujita-Ban matrix and the MRA leads to the results summarized in the last column of Table 2. The improved statistical parameters of the study are:

n = 25, R = 0.919, s = 0.368, F(10,14) = 7.557

The squared value of R now accounts for 84% of the variance and the F-value obtained is significant at 99% level [$F_{10,14}(0.01) = 3.94$]. The ± data, the 95% confidence intervals, associated to the regression coefficient terms in Table 2 (last column) have indicated that, except the spacer $-(CH_2)_2-$ and R = F, all the others are statistically significant. The calculated values of $-\log IC_{50}$, listed in Table 1, are also in close agreement with the observed ones. The substituents to be incorporated at various positions of the parent moiety, that make higher positive contributions to activity may be used to design more active compounds of the series in future. The predicted $-\log IC_{50}$ values of a few reported and untested compounds are given in Table 3.

It is important to note that the Fujita-Ban (or the Free-Wilson) approach cannot extrapolate beyond the substituent used in the training set whereas the Hansch approach, attempted next for the same training data set, can do so.

Thus, the listed data in Table 1 were subjected to MRA and the derived correlation due to them is shown by Eq. (2)

$$-\log IC_{50} = 7.280 - 0.653(\pm 0.50)HA(R) - 2.581(\pm 0.86)\Sigma Vw + 0.645(\pm 0.38)Vw(R_1)$$

$$n = 26,\ R = 0.861,\ s = 0.379,\ F(3,22) = 21.053 \quad \ldots (2)$$

in which ΣVw denotes the added values of van der Walls' volume for the structural segments, m, R and

Table 2—Substituent contribution to histamine release inhibitory activities of N-(pyridin-4-yl)-(indol-3-yl) alkylamides

Variation	Substitution	Substituent contribution	
		n = 26	n = 25
m = 1	$-CH_2-$	1.261(±0.94)	1.198(±0.84)
= 2	$-(CH_2)_2-$	0.804(±0.96)	0.730(±0.85)
R	Cl	– 0.987(±0.79)	–0.926(±0.70)
	F	– 0.634(±0.82)	–0.516(±0.74)
	OMe	–1.668(±0.90)	–1.599(±0.80)
	^{i}Pr	–1.447(±0.82)	–1.459(±0.73)
R_1	Bn	0.544(±0.49)	0.654(±0.44)
	4-FBn	0.558(±0.46)	0.695(±0.43)
R_2	Me	–0.612(±0.56)	–0.771(±0.52)
		μ =5.636(±0.67)	5.567(±0.60)

Table 3—Predicted activity values of reported compounds that were not tested for inhibition of histamine (Fig. 1 for structures)

Compound no.	m	R	R_1	R_2	$-\log IC_{50}$(M)	
					F.B.	Eq.(3)
1	1	OMe	H	H	5.17	5.20
2	2	OMe	H	H	4.70	4.74
3	1	OMe	4-FBn	H	5.86	5.79
4	2	OMe	4-FBn	H	5.39	5.33
5	1	OMe	H	Me	4.40	4.63
6	1	Cl	Bn	H	6.49	6.57
7	1	Cl	H	Me	5.07	5.45
8	1	Cl	4-FBn	Me	5.76	6.04
9	1	F	H	H	6.25	6.40
10	1	F	4-FBn	H	6.94	6.99
11	2	F	4-FBn	H	6.48	6.53
12	1	^{i}Pr	4-FBn	H	6.00	5.83

R_2 (Fig. 1). Likewise, the other two descriptors stand to quantify the substituents indicated within the parentheses right to them. The statistical parameters s and F obtained for this equation, tune to statistically significant result. The F-value is significant at 99% level [$F_{3,22}(0.01) = 4.82$] and the s value is sufficiently low. The slightly low R^2-value, accounting for 74% of variance in observed activity values, however, demands for further improvement of Eq. (2). This is achieved by ignoring compound 24 (the 'outlier' of Fujita-Ban study) again from the data set. The resulting correlation is given by Eq. (3)

$$-\log IC_{50} = 7.371 - 0.638(\pm 0.45)HA(R) - 2.885(\pm 0.81)\,\Sigma Vw + 0.759(\pm 0.35)Vw(R_1)$$

$$n = 25,\ R = 0.895,\ s = 0.340,\ F(3,21) = 28.059 \quad \ldots(3)$$

All the statistical parameters of Eq. (3) are now improved, in statistical sense, over to that of Eq. (2). The R^2-value, obtained for an equation in 25 data-points, is accounting for 80% of variance in observed activity values. Also the s-value and ± 95% confidence intervals, associated to the regression coefficients are further lowered. In addition, the F-value, significant at 99% level [$F_{3,21}(0.01) = 4.87$], is also increased. The above derived correlation equations are the best alternatives amongst a large number of QSAR equations, obtained by considering a variety of correlative parameters pertaining to the electronic, hydrophobic and steric interactions. But none of these parameters, except the *Vw*, could explain more lucidly the variation in biological activity of these compounds. Even before finalizing Eq. (3), separate *Vw* parameters for m, R and R_2, the summed values such as $\Sigma Vw(m + R)$, $\Sigma Vw(m + R_2)$ and $\Sigma Vw(R + R_2)$ were successively included in the multiple regression analysis, but none of these was superior to Eq. (3). The rationale of summing *Vw* parameters of m, R and R_2 segments become apparent on the statistical ground as the derived regression coefficients of *Vw*(m), *Vw*(R) and *Vw*(R_2) and their 95% confidence intervals (in a 5 variable regression equation) were almost equal in magnitude and similar in sign. In addition, the statistical significance of leading equation was also similar to Eq. (3). Such outcomes, therefore, enabled us to club these variables into one that have further reduced the number of predictors, implicit in a statistically sound correlation Eq. (3). This equation is used to calculate the activity values of all the data-points (Tables 1 and 3). The same, listed in Table 1 (for the training set), are in close agreement to the observed ones. The listed r-values in Table 4 satisfy the orthogonality requirement among the predictor variables of Eq. (3). In addition, the plot between observed and predicted $-\log IC_{50}$ values is also given in Fig. 2 to show a better impression of the goodness of fit and the systematic variations in the present congeneric series. From Eq. (3), it appears that the varying sites (*i.e.*, m, R, R_1 and R_2) of the present series are all engaged in steric interactions. The substituents at m, R and R_2 combined, having lower molecular bulk and that of at R_1 having higher molecular bulk, are advantageous to improve the inhibition potency of a compound.

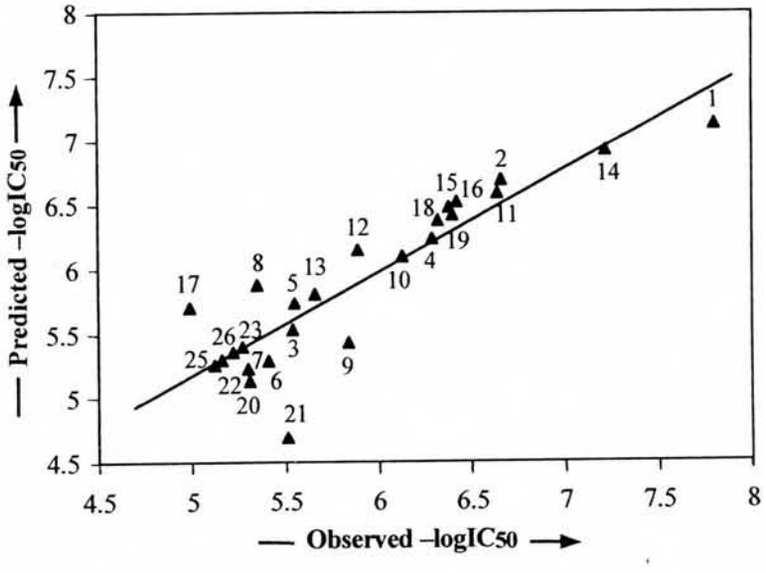

Fig. 2—Plot of observed $-\log IC_{50}$ *versus* predicted $-\log IC_{50}$ values

Table 4 — The intercorrelation matrix among the predictor variables of Eq. (3)

	HA(R)	ΣVw	$Vw(R_1)$
HA(R)	1.000	0.071	0.219
ΣVw		1.000	0.027
$Vw(R_1)$			1.000

Likewise, the hydrogen bond acceptor property of R-substituents, if present, leads to decrease it. These findings may, therefore, be helpful in the selection of the substituents to be incorporated at the varying sites of a molecule leading to the synthesis of better potency compounds.

From both the approaches, the following conclusions may now be drawn: (i), a small size spacer group such as $-(CH_2)_mCONH-$, where m = 1, present between pyridinyl and indolyl rings is helpful in raising the activity of a compound. The Fujita-Ban study, in conformity with this, assigned higher substitutional contribution to this variation; (ii), the less bulky substituents both at R and R_2 are also essential for incremental effect to the activity. But, if the substituent at R also possesses a hydrogen bond acceptor property in addition then it may further add to the detrimental effect. The contributions obtained for the substituents of R- and R_2-positions are all negative. This corroborates to the demand of the smaller substituents at these positions. But, if the subtituent is small in size and also possesses hydrogen bond acceptor property then its contribution to activity, is found to be lowered further. For example, $-OCH_3$ is hydrogen bond acceptor and smaller in size compare to ^{i}Pr. It has the contribution equal to –1.599 while ^{i}Pr has slightly higher contribution equal to –1.459 (Table 2); (iii), the substituent, 4-FBn at R_1 having a higher positive substituent contribution relative to Bn, enhances the activity of a compound. The same is also reflected by the positive regression coefficient associated with the variable $Vw(R_1)$ in Eq. (3).

In conclusion, the two analyses in the present study provide the ground for rationalizing the substituent selection in designing more potent compounds of the series.

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