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Quantitative Structure–Activity Relationship Study on Tetrahydro-β-carboline Antagonists of the Serotonin 2B ($5HT_{2B}$) Contractile Receptor in the Rat Stomach Fundus

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The antagonist actions of three sub-series of tetrahydro-β-carbolines at the serotonin 2B ($5HT_{2B}$) contractile receptor in the rat stomach fundus are analyzed in relation to the physicochemical properties of the molecules. Significant correlations are obtained between the $5HT_{2B}$ receptor antagonist affinity and the hydrophobic, steric, electronic, hydrogen bond acceptor and some indicator variables of substituents. Based on these findings, the mode of actions of these congeneric series and future strategy to synthesize more potential compounds are discussed.

Keywords: Antagonists; Serotonin 2B ($5HT_{2B}$) contractile receptor; Tetrahydro-β-carboline antagonists; QSAR analysis; Physicochemical properties

INTRODUCTION

Serotonin (5HT) is a potent agonist that can produce a contraction effect in isolated smooth muscle from most tissues. One of the tissues most sensitive to 5HT-induced contraction, with nanomolar concentrations producing a pronounced effect,[1–8] is the rat stomach fundus. A considerable amount of work on this subject in the recent past has resulted in the characterization of the stomach fundal receptor, currently designated as the $5HT_{2B}$ receptor.[9] The rat $5HT_{2B}$ receptor and its human homologue have been recently cloned, allowing for examination of the binding affinity of receptor agonists and antagonists as well as studies of receptor localization and effector coupling.[10–14] Such studies have established the correlation for a series of ligands between the cloned $5HT_{2B}$ receptor and the receptor mediating the contraction in response to 5HT in the rat stomach fundus.[15] The receptor agonists and antagonists, identified recently help to differentiate among the family of closely related $5HT_2$ receptors.[16–19] Increasingly selective and high-affinity agents may prove important as tools for further study of the rat receptor and its human homologue, for which the message has been identified in human brain,

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liver, heart, kidney, retina and GI tract.[20,21] To that end, structure–activity relationship (SAR) studies have been targeted at the development of new $5HT_{2B}$ receptor antagonists based upon the tetrahydro-β-carboline alkaloid, yohimbine. Among its multiple pharmacologic effects, this alkaloid has been shown to antagonize 5-HT-induced contractions in the rat stomach fundus.[22] In order to identify the key structural elements necessary for potent antagonism of $5HT_{2B}$ receptor-mediated contraction in the rat stomach fundus, the structure of yohimbine was successively simplified further by Audia *et al.*[23] and a substantial number of partial structural analogues were reported. Their initial SAR studies on these compounds were, however, only directed towards altering of the substituents at the various position of the structure and no rationale has yet been provided to reduce the trial-and-error factors. Hence, a quantitative SAR (QSAR) on these drugs was conducted since QSAR not only provides the rationale for drug design but also illuminates the mechanism of their action.

MATERIALS AND METHOD

The QSAR study was made on recently reported[23] tetrahydro-β-carbolines. Their antagonist activity data for serotonin-induced contraction via. the $5HT_{2B}$ receptor in the rat stomach fundus smooth muscle strips are compiled in Tables I–III and subjected to multiple regression analysis (MRA) using the appropriate physicochemical parameters. One of the most important part of a QSAR study involves selecting the representations of the

TABLE I QSAR parameters and serotonin 2B receptor affinity of tetrahydro-β-carboline derivatives

S. No.	X	Y	I_X	$\pi_{2'}$	$HA_{3'}$	$HA_{4'}$	$MR_{3'+4'}$	$MR_{5'}$	$-\log K_B$	
									Obsd.[a]	Calc.[b]
1	H	H	0	0.00	0	0	0.206	0.103	7.61	7.90
2	H	4′-Me	0	0.00	0	0	0.668	0.103	7.84	8.07
3	H	4′-OMe	0	0.00	0	1	0.890	0.103	8.09	8.14
4	H	3′-OMe	0	0.00	1	0	0.890	0.103	8.04	8.14
5	H	3′,4′-Cl_2	0	0.00	0	0	1.206	0.103	8.22	8.26
6	H	2′,5′-OMe_2	0	−0.02	0	0	0.206	0.787	7.75	7.33
7	H	3′,4′,5′-OMe_3	0	0.00	1	1	1.574	0.787	8.10	7.83
8	H	3′,4′-OCH_2O-	0	0.00	1	1	0.896	0.103	8.40	8.15
9	H	3′,4′-OEt_2	0	0.00	1	1	2.494	0.103	8.57	8.71
10	H	2′-Cl,3′,4′-OMe_2	0	0.71	1	1	1.574	0.103	9.27	8.75
11	H	3′,4′-Benzo	0	0.00	0	0	1.748	0.103	7.84	8.45
12	Me	3′-OH,4′-OMe	1	0.00	1	1	1.072	0.103	9.23	9.18
13	Me	2′-Cl,3′,4′-OMe_2	1	0.71	1	1	1.574	0.103	9.80	9.72
14	Me	2′-Br,3′,4′-OMe_2	1	0.86	1	1	1.574	0.103	9.70	9.80
15	Me	2′-NO_2,3′,4′-OMe_2	1	−0.28	1	1	1.574	0.103	9.16	9.22
16	Me	2′-NH_2,3′,4′-OMe_2	1	−1.23	1	1	1.574	0.103	9.02	8.73
17	Me	5′-I,3′,4′-OMe_2	1	0.00	1	1	1.574	1.394	8.12	8.30
18	Me	5′-NO_2,3′,4′-OMe_2	1	0.00	1	1	1.574	0.736	8.55	8.84
19	Me	5′-NH_2,3′,4′-OMe_2	1	0.00	1	1	1.574	0.542	8.89	9.00
20	Me	3′,5′-F_2	1	0.00	0	0	0.195	0.092	9.05	8.88
21	Me	3′,4′-F_2	1	0.00	0	0	0.184	0.103	8.47	8.87
22	Me	3′-F,4′-OMe	1	0.00	0	1	0.879	0.103	9.42	9.11
23	Me	3′-CF_3	1	0.00	0	0	0.605	0.103	8.91	9.02
24	Me	3′,4′-Me_2	1	0.00	0	0	1.130	0.103	9.06	9.20
25	Me	3′,4′-OMe_2	1	0.00	1	1	1.574	0.103	9.86	9.36
26	H	3′,4′-OMe_2	0	0.00	1	1	1.574	0.103	9.17	–[c]

a Binding affinity for the $5HT_{2B}$ receptor in the rat stomach fundus; taken from Ref. 23.
b Calculated using Eq. (4).
c The "outlier" compound of the present study.

molecules that can explain the activity induced in the biological system. In general, these representations can be divided into physicochemical, theoretical and structural parameters. One representation, the physicochemical model of the biological activity, assumes that the activity of a compound is a function of three separable factors: electronic effects, steric effects and solvent-partitioning or hydrophobic effects. A variety of parameters for each type of such effect are available in the literature. The most important of these parameters, for present study, are found to be the hydrophobic constant π, the molar refraction constant *MR* (scaled to 0.1), the hydrogen bond acceptor parameter *HA*, the electron withdrawing effect, σ and the field effect, *F*. The values of these quantifying parameters are taken directly from the compilation of Hansch *et al*.[24] Additionally, an indicator variable was also used to describe the effect of some specific alteration. In order to overcome the problem of intercorrelations amongst the independent variables used in various correlation equations, descriptors satisfying the orthogonality conditions ($r < 0.250$), in a partial least squares (PLS) approach were only retained in MRA. The $5HT_{2B}$ receptor antagonist dissociation constant, K_B was determined[23] according to the following equation:

$$K_B = [B]/(\text{dose ratio} - 1) \quad (1)$$

where [B] is the concentration of antagonist and dose ratio is the ED_{50} of the agonist in the presence of the antagonist divided by the control ED_{50}. These results were then expressed as the negative logarithm of the K_B (i.e. $-\log K_B$).

RESULTS AND DISCUSSION

The compounds in Table I have substituent variations both at *Y* on the phenyl ring and *X* on the indole portion of the tetrahydro-β-carboline. However, the substituents at *X* are binary in nature and may be accounted for by considering an indicator variable, I_X. A value of unity for $X = 6\text{-Me}$ and zero for $X = H$ was arbitrarily chosen for it. For the substituents at *Y*, a large number of descriptors amongst the physicochemical and the structural parameters were successively attempted. In this effort, the $\pi_{2'}$ and the $MR_{5'}$ parameters, respectively for 2′- and 5′-positions emerged as the best quantifying parameters. However, for the substituents collectively at 3′- and 4′-positions both the *HA* and *MR* parameters seem to be appropriate. This is apparent from the following correlation equations. Employing the data set in Table I, the MRA gave the regression Eq. (2):

$$\begin{aligned} -\log K_B = {} & 0.452(\pm 0.273)\pi_{2'} \\ & + 0.320(\pm 0.110)HA_{3'+4'} \\ & - 0.966(\pm 0.310)MR_{5'} \\ & + 0.809(\pm 0.199)I_X + 8.106 \end{aligned} \quad (2)$$

$$n = 26,\ r^2 = 0.845,\ s = 0.287,\ F(4, 21) = 28.338$$

The statistical parameters *n*, *r*, *s* and *F* in this and subsequent equations represent, respectively, the number of data points, the correlation coefficient, the standard deviation and the *F*-ratio of the variances of calculated to observed activity values. The ± data within parentheses are 90% confidence intervals. The *F*-value, obtained for the above equation, is significant at 99% level [$F_{4,21}(0.01)=4.37$] and the r^2-value accounts for 84% of the variance in observed activity values. Although the above equation yielded statistically sound results, the inclusion of $HA_{3'+4'}$ variable, obtained by adding two indicator type of variables ($HA_{3'}$ and $HA_{4'}$) may lose its physical significance. The added values are now ternary in nature (0, 1 and 2) and this may mislead the QSAR results. Even consideration of $HA_{3'}$ and $HA_{4'}$ as separate variables was not justified as the two were not mutually orthogonal (r^2=0.582). This led us to select an alternative parameter such as $MR_{3'+4'}$ in place of $HA_{3'+4'}$ as the two, in the present data set, are significantly correlated

with each other (regression Eq. (3)).

$$MR_{3'+4'} = 0.442(\pm 0.153)HA_{3'+4'} + 0.649 \qquad (3)$$

$$n = 26,\ r^2 = 0.503,\ s = 0.421,\ F(1,24) = 24.327$$

Obviously, Eq. (3) has shown that the substituents of 3′- and 4′-positions are collectively involved in a steric and/or polar type of interaction. The derived correlation with this new parameter, on ignoring compound **26**, is shown in regression Eq. (4):

$$\begin{aligned} -\log K_B = {} & 0.508(\pm 0.296)\pi_{2'} \\ & + 0.354(\pm 0.187)MR_{3'+4'} \\ & - 0.818(\pm 0.333)MR_{5'} \\ & + 0.973(\pm 0.217)I_X + 7.914 \end{aligned} \qquad (4)$$

$$n = 25,\ r^2 = 0.823,\ s = 0.310,\ F(4,20) = 23.065$$

The *F*-value obtained above, stands significant at 99% level [$F_{4,20}(0.01)$=4.43] and the r^2-value accounted for 82% of the variance in observed affinity values. This equation was, therefore, used to obtain the theoretical values of $-\log K_B$. The same, included in Table I, are found to be in close agreement with the observed ones. In addition, a plot of observed versus predicted $-\log K_B$ values is also shown in Fig. 1 emphasize the goodness of fit as well as any systematic variations. From Eq. (4), it appears that the substituent *X*=6-Me rather than *X*=*H* is beneficial in improving the affinity of a compound. Likewise, the highly polar bulky substituents at the 3′- and 4′-positions and more hydrophobic substituents at the 2′-position of the aryl ring are also essential. The bulkier substituents at 5′-position of this ring are least preferred. The "outlier" compound **26** with substituents *X*=*H* and *Y*=3′,4′-OMe_2 does not follow the trend similar to other analogues of Table I. The mode of action of this compound may, however, be more similar to the congeners in Table II. Thus compounds **26** and **25** both possessing structural similarities with other compounds (*Y*=3′,4′-OMe_2 is fixed and *X* is varied at 5-, 6-, 7- and 8-positions of indole moiety) are also included in Table II.

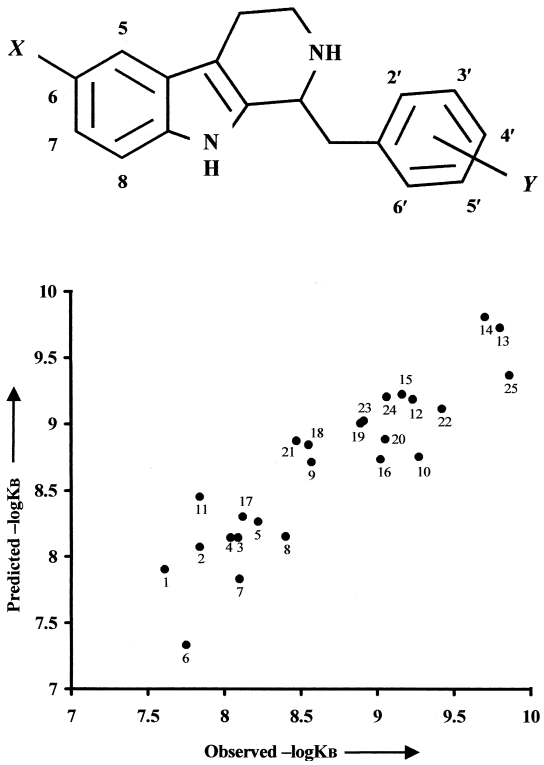

FIG. 1 Plot of observed versus predicted $-\log K_B$ values (Eq. 4).

The MRA on the data set in Table II has revealed the correlation shown in Eq. (5):

$$\begin{aligned} -\log K_B = {} & -0.481(\pm 0.272)\pi_6 \\ & - 1.477(\pm 0.506)HA_6 \\ & - 2.167(\pm 0.705)HA_7 \\ & - 1.280(\pm 1.373)\sigma_8 + 9.477 \end{aligned} \qquad (5)$$

$$n = 23,\ r^2 = 0.684,\ s = 0.490,\ F(4,18) = 9.729$$

in which the subscripted numeral associated with descriptor variables stands to indicate various positions of *X*-substitents in the indole moiety. None of the physicochemical or structural parameter was found to be suitable for the 5-position of this ring. Thus, it is better to have this position unsubstituted. The slightly low r^2-value obtained for the above equation may not be tuned to a statistically sound correlation and required further improvement. This was achieved by considering the "out-of-trend"

TABLE II QSAR parameters and serotonin 2B receptor affinity of tetrahydro-β-carboline derivatives

S No.	X	π_6	HA_6	HA_7	σ_8	$-\log K_B$	
						Obsd[a]	Calc[b]
25	6-Me	0.56	0	0	0.00	9.86	9.37
26	H	0.00	0	0	0.00	9.17	9.68
27	6-OMe	−0.02	1	0	0.00	8.48	7.82
28	6-OBn	1.66	1	0	0.00	6.73	6.89
29	6-F	0.14	0	0	0.00	8.49	–[c]
30	6-Cl	0.71	0	0	0.00	8.36	–[c]
31	6-Br	0.86	0	0	0.00	8.80	9.20
32	6-I	1.12	0	0	0.00	9.52	9.06
33	6-SMe	0.61	0	0	0.00	9.56	9.34
34	6-Et	1.02	0	0	0.00	9.46	9.11
35	6-n-Pr	1.55	0	0	0.00	8.64	8.82
36	6-i-Pr	1.53	0	0	0.00	8.50	8.83
37	7-OMe	0.00	0	1	0.00	7.31	7.81
38	8-Me	0.00	0	0	−0.17	9.00	–[c]
39	5-F,6-Me	0.56	0	0	0.00	9.30	9.37
40	5,7-Me_2	0.00	0	0	0.00	9.34	9.68
41	6,7-Me_2	0.56	0	0	0.00	9.71	9.37
42	6,8-Me_2	0.56	0	0	−0.17	9.61	9.74
43	7,8-Me_2	0.00	0	0	−0.17	10.12	10.05
44	7,8-Benzo	0.00	0	0	0.22	9.22	9.20
45	6,8-F_2	0.14	0	0	0.06	9.60	9.47
46	6-Me,8-Br	0.56	0	0	0.23	8.92	8.87
47	7-Me,8-Br	0.00	0	0	0.23	9.02	9.18

a Binding affinity for the $5HT_{2B}$ receptor in the rat stomach fundus; taken from Ref. 23.
b Calculated using Eq. (7).
c The "outlier" compound of the present study.

behavior of certain congeners. The calculated affinity values of three compounds, **29**, **30** and **38**, was largely deviating from their observed ones, for no specific reason known at present. These compounds rather require further intense experimental study to draw any meaningful conclusion. These compounds were, however, ignored and the MRA resulted into a highly significant correlation Eq. (6):

$$-\log K_B = -0.631(\pm 0.258)\pi_6 - 1.603(\pm 0.458)HA_6 - 2.415(\pm 0.642)HA_7 - 2.259(\pm 1.354)\sigma_8 + 9.725 \quad (6)$$

$$n = 20,\ r^2 = 0.861,\ s = 0.346,\ F(4, 15) = 23.421$$

Further, the substituents in the 6- and 7-positions are both nearly equally sensitive to hydrogen bond acceptor property and add negatively to the $-\log K_B$. Consideration of the HA_6 and HA_7 as one variable, $HA_{6,7}$, still maintaining binary values (0 or 1) for this data set, have further helped us in reducing one more independent variable. The resulting correlation is given by Eq. (7):

$$-\log K_B = -0.556(\pm 0.265)\pi_6 - 1.871(\pm 0.407)HA_{6,7} - 2.193(\pm 1.445)\sigma_8 + 9.682 \quad (7)$$

$$n = 20,\ r^2 = 0.830,\ s = 0.371,\ F(3, 16) = 26.156$$

The r^2-value for the correlation equation mentioned above in three descriptor variables, derived out of 20 data points, now accounts for 83% of variance in observed activity values. The F-value, significant at 99% level [$F_{3,16}(0.01)$=5.29] has steeply increased. In addition, the calculated $-\log K_B$ values, listed in Table II, are in agreeable limits to the observed ones. From Eq. (7) it appears that a more electron-donor substituent

such as Me (having negative σ value) at the 8-position and a less hydrophobic substituent at the 6-position helps in improving the affinity of a compound. The substituent either at the 6- or 7-position, prone to hydrogen bond acceptor property, however, adds negatively to it. This equation may, therefore, be used to design ligands with a high affinity profile.

The limited number of compounds listed in Table III, have variations only in the indole ring while the other part of these molecules, containing a 1-naphthyl ring, is fixed. The MRA on this data sub-set gave correlation Eq. (8):

$$-\log K_B = -1.064(\pm 0.736)F_6 - 0.747(\pm 0.195)\pi_{7+8} + 9.598 \quad (8)$$

$$n = 9,\ r^2 = 0.906,\ s = 0.153,\ F(2,6) = 28.868$$

Here also, the 5-position remained insensitive to substitutional variation. The r^2-value accounts for 91% of variance and the F-value remained significant at 99% level [$F_{2,6}(0.01)$=10.92]. The calculated and observed affinity values have reached parity (Table III). Equation (8) reveals the important role of hydrophobic and electronic parameters on the affinity of a compound. The substituents at the 6-position are engaged in electronic interaction while that of the 7- and 8-positions are involved in hydrophobic interaction. The substituents at the 6-position, expressing more negative field effect and the substituents at the 7- and 8-position having smaller hydrophobic characters are advantageous in raising the affinity of a compound. In conclusion, it may be stated that:

1. The substituent variations in two parts (i.e. *X* in the indole ring and *Y* in the phenyl ring) of these molecules are significantly influencing each other, which in turn leads to increase or decrease in $5HT_{2B}$ receptor antagonist affinity value of a compound.
2. For compounds in Table I, the variation *X*=6-Me in the indole ring is advantageous. For variation *Y* in the phenyl ring, the 2′-substituents are participating in hydrophobic interaction while the 3′-, 4′- and 5′-substituents are engaged in a polar/steric type of interaction.
3. For compounds in Tables II and III, the substitutions in the phenyl ring are differently fixed. In the former case the fixed portion is *Y*=3′,4′-OMe_2 while in the later case it is *Y*=2′,3′-benzo. These fixed substitutions (differing in their structural features) in the phenyl ring then transmit varying effects on *X*, present in another part of a molecule and are responsible for the different mode of actions of these two congeneric series. For example, the hydrogen bond acceptor property for the 6- and/or 7-substituents, hydrophobic interaction for the 6-substituents and electronic effect for the 8-substituents are important for the compounds in Table II while electronic effect (field effect) for 6-substituents and the hydrophobic effect for 7- and 8-substituents combined, play a significant role for the compounds in Table III.
4. The substitutions at the 5-position of the indole ring seems to be redundant in all compounds in the present study.

These guidelines are helpful in directing synthesis towards potential compounds that may selectively antagonize serotonin 2B contractile receptor in the rat stomach fundus.

TABLE III QSAR parameters and serotonin 2B receptor affinity of tetrahydro-β-carboline derivatives

S. No.	*X*	F_6	π_{7+8}	$-\log K_B$	
				Obsd[a]	Calc[b]
48	H	0.00	0.00	9.35	9.60
49	6-Cl	0.41	0.00	9.16	9.16
50	6-Me	−0.04	0.00	9.75	9.64
51	6-Et	−0.05	0.00	9.65	9.65
52	8-OMe	0.00	−0.02	9.72	9.61
53	8-Br	0.00	0.86	9.10	8.96
54	5-F,6-Me	−0.04	0.00	9.69	9.64
55	6-Me,8-Br	−0.04	0.86	8.82	9.00
56	7-Me,8-Br	0.00	1.42	8.56	8.54

a Binding affinity for the $5HT_{2B}$ receptor in the rat stomach fundus; taken from Ref. 23.
b Calculated using Eq. (8).

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