Journal of Computer-Aided Molecular Design, 5 (1991) 441–453
ESCOM

J-CAMD 129

The structure–activity relationship of inhibitors of serotonin uptake and receptor binding

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Received 3 July 1990
Accepted 19 February 1991

Key words: 5-Hydroxytryptamine receptors; Serotonin inhibitors; QSAR of serotonin inhibition and uptake

SUMMARY

An analysis of five different datasets of inhibitors of serotonin uptake has yielded quantitative structure/activity relationships (QSARs) which delineate the role of steric and hydrophobic properties essential for inhibition by phenylethylamine-type analogues.

INTRODUCTION

Serotonin (5-hydroxytryptamine or 5-HT) is essential to many physiological processes and is of special interest as a neurotransmitter. It is present in the blood and intestines of virtually every animal species: the venom of amphibians, wasps, scorpions, the saliva of octopi, and the nematocysts of sea anemones. A large effort is under way to develop drugs to modulate the action of serotonin in an effort to control depression, anxiety, psychosis, overeating, alcohol dependency and migraine headaches. Despite the great practical interest in obtaining such drugs, there has been very little published work on quantitative structure/activity relationships (QSARs) as an aid in drug design and understanding of their mechanism of action at the molecular level. We have therefore decided to make a review of the data which is suitable for QSAR work to evaluate what has been done, as well as to see what the potential of QSAR in this area might be.

METHODOLOGY

Since the first general model for QSAR was formulated [1], the methodology has been applied to almost every type of biologically active chemical [2], and has been used to design bioactive com-

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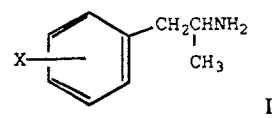
pounds now used commercially [3]. An extensive database of parameters for such analyses has been published [4], and the methodology has been extensively discussed [5,6]. The present study has been somewhat limited by the availability of structure/activity data in which systematic variation of a parent structure has been made. In fact, we have found only five datasets for work on in-vitro systems which seemed suitable for our analysis.

RESULTS

From the data in Table 1 [7] for the 50% inhibition of 5-HT uptake by platelet-rich plasma by substituted β-phenylethylamines (I) we have derived Eqs. 1–3.



I

$$\log 1/C = 2.79\,(\pm 1.2)MR_4 + 3.86\,(\pm 0.38) \quad (1)$$
$$n = 19,\ r = 0.773,\ s = 0.497,\ F_{1,17} = 25.3$$

$$\log 1/C = 3.04\,(\pm 0.72)MR_4 + 1.06\,(\pm 0.41)\,\pi_3 + 3.77\,(\pm 0.24) \quad (2)$$
$$n = 19,\ r = 0.928,\ s = 0.302,\ F_{1,16} = 30.0$$

$$\log 1/C = 2.76\,(\pm 0.55)MR_4 + 0.89\,(\pm 0.32)\,\pi_3 + 0.48\,(\pm 0.26)\,I + 3.49\,(\pm 0.23) \quad (3)$$
$$n = 19,\ r = 0.965,\ s = 0.219,\ F_{1,15} = 15.4$$

In these equations, C is the molar concentration of drug producing 50% inhibition, MR_4 is the molar refractivity of the substituent scaled by 0.1 (to make it roughly equiscalar with π) and π is a parameter for the hydrophobicity of 3-substituents [4]. The subscript of 4 with MR indicates that only 4-substituents are parameterized for this property. Since MR is primarily a measure of the bulk of the substituent, the positive coefficient with this term indicates that 4-substituents are contacting polar space in the receptor [8], not hydrophobic space. Adding a term in π_4 does not improve the correlation. 3-Substituents on the other hand appear to contact hydrophobic space since it is π_3 which accounts for their inhibitory potency. The larger the value of π, the more hydrophobic the substituent. The indicator variable I is assigned the value of 1 for amphetamines (and one example where an αEt group is present (#1) instead of an αMe) which have a methyl group on the side chain, and 0 for five examples of β-phenylethylamines which lack the methyl group. The positive coefficient with I shows that, on average, these compounds are about three times as potent as the phenylethylamines (antilog of 0.48). There are three instances where there are *two* side-chain methyl groups. No parameterization has been made for the second methyl; since these congeners are reasonably well fitted, we assume only one methyl group can contact the receptor. Compound 17 in Table 1, containing the cyclopropyl moiety, is unique in not having a simple H or CH_3 on carbon alpha to the crucial nitrogen atom. We have treated this substance as

TABLE 1
DATA USED TO DERIVE EQS. 1–3 FOR THE INHIBITION OF 5-HT UPTAKE BY HUMAN PLATELET-RICH PLASMA OF AMPHETAMINES I

	X	Obs. log 1/C	Calc[a] log 1/C	|Δlog 1/C|	0.1 MR_4	π_3	I
1	4-Cl-$C_6H_4CH_2CH$ (Et) NHMe	5.77	5.63	0.14	0.60	0.00	1
2	4-CF_3-$C_6H_4CH_2CH$ (Me) NH_2	5.64	5.36	0.28	0.50	0.00	1
3	4-Cl-$C_6H_4CH_2C$ (Me) $_2NH_2$	5.59	5.63	0.04	0.60	0.00	1
4	4-Cl-$C_6H_4CH_2CH$ (Me) NHMe	5.51	5.63	0.12	0.60	0.00	1
5	4-Cl-$C_6H_4CH_2CH$ (Me) NH_2	5.50	5.63	0.13	0.60	0.00	1
6	3-CF_3-$C_6H_4CH_2CH$ (Me) NHC_2H_5	5.23	5.04	0.19	0.10	0.88	1
7	3-CF_3-$C_6H_4CH_2CH_2NHC_2H_5$	4.99	5.04	0.05	0.10	0.88	1
8	4-$HOC_6H_4CH_2CH$ (Me) NH_2	4.70	4.78	0.08	0.29	0.00	1
9	$C_6H_5CH_2CH$ (Me) NHC_2H_5	4.53	4.25	0.28	0.10	0.00	1
10	$C_6H_5CH_2CH$ (Me) NHMe	4.27	4.25	0.02	0.10	0.00	1
11	4-HO-$C_6H_4CH_2CH_2NH_2$	4.25	4.29	0.04	0.29	0.00	0
12	3-CF_3-$C_6H_4CH_2CH_2NHR$[b]	4.14	3.77	0.37	0.10	0.88	0
13	$C_6H_5CH_2CH$ (Me) $_2NHMe$	4.10	4.25	0.15	0.10	0.00	1
14	$C_6H_5CH_2CH$ (Me) NH_2	4.06	4.25	0.19	0.10	0.00	1
15	3-HO-$C_6H_4CH_2CH$ (Me) NH_2	3.90	3.66	0.25	0.10	−0.67	1
16	$C_6H_5CH_2C$ (Me) $_2NH_2$	3.87	4.25	0.38	0.10	0.00	1
17	C_6H_5  NH_2 (*trans*)	3.73	3.77	0.04	0.10	0.00	0
18	3,4-$(HO)_2C_6H_3CH_2CH_2NH_2$	3.64	3.70	0.06	0.29	−0.67	0
19	$C_6H_5CH_2CH_2NH_2$	3.54	3.77	0.23	0.10	0.00	0

[a] Calculated using Eq. 3.
[b] R = $CH_2CH_2OCOC_6H_5$.

though it were a phenylethylamine (i.e., I = 0) since this produced a better fit than I = 1. This implies that the CH_2 moiety in the cyclopropyl group does not bind in the same fashion as a methyl group. It should be noted that no parameterization has been made for congeners having a substituent on the amino group. Seven of the eight examples in this class are well fitted, indicating that such groups do not contact the receptor. Although there are only five examples of substances having *meta* substituents and only two classes of substituents, we feel that the reasonably good fit of the very different OH and CF_3 groups, and especially the good fit of dopamine (# 18), means that the π_3 is justified even beyond the significance of the F test. Another aspect of the π_3 term which is reassuring is that its coefficient is near 1, a commonly observed value for in-vitro systems.

The figures in parentheses are for the construction of the 95% confidence limits, n represents the number of data points used to derive the equation, r is the correlation coefficient, s is the standard deviation from the regression equation and F is the F ratio for the significance of each additional term in the stepwise development of Eq. 3. All of the equations are highly significant since, for the lowest level of significance $F_{1,15}$, $\alpha.01 = 8.68$. The squared correlation matrix of the key variables associated with Eq. 3 is:

	MR_3	MR_4	π_3	π_4	I
MR_3	1	0.09	0.23	0.04	0.01
MR_4		1	0.02	0.39	0.05
π_3			1	0.02	0.07
π_4				1	0.22

The parameters are reasonably orthogonal.

From perusal of the structures in Table 1, it is inferred that there are ways in which the potency of these compounds might be improved. One might use a more hydrophobic group in the 3-position, but care should be taken not to increase the size of the substituent greatly beyond CF_3 or one may go beyond bulk tolerance of the receptor. A conservative choice might be 3-I. Equation 3 predicts that, if no steric hindrance occurs, such derivatives would be about twice as potent as the chloro analogues.

Unfortunately, there is some collinearity between MR_4 and π_4, which means some uncertainty in the coefficients of these terms in Eq. 3. The central message of Eq. 3 is that 4-substituents contact nonhydrophobic space, while 3-substituents contact hydrophobic space. If π_4 is used in place of MR_4 in Eq. 3, a much poorer correlation is obtained ($r = 0.804$), and if MR_3 is used in place of π_3, $r = 0.905$. We cannot be as sure of 3-substituents as of the 4-substituents, the reason being that there are only five examples containing 3-substituents. However, compounds 15 and 18, which contain the very hydrophilic hydroxy group in the 3-position are both well fitted by Eq. 3 and their low activity brings out the deleterious effect of the polar group in the 3-position.

The large coefficient with MR_4 in Eq. 3 brings out the sensitivity of the receptor to steric effects. There are three examples in Table 1 of 4-OH substituents which are all well fitted by the MR_4 term. Since two of these compounds are more active than the corresponding parent compound without a 4-OH, one might attribute the effect to hydrogen bonding. Such rationalization would not explain the even greater effect of 4-Cl or 4-CF_3. Although Eq. 3 is an encouraging result, this SAR study attempts to cover five changes on the parent phenylethylamine (1 or 2 side-chain alkyl groups, substitution on N and substitution on two ring positions) with 19 derivatives. Consider-

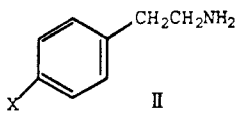
TABLE 2
DATA USED TO DERIVE EQ. 4 FOR THE INHIBITION OF BINDING OF TRYPTAMINE IN RAT CORTICAL MEMBRANE BY PHENYLETHYLAMINES II

X	Obs. log 1/C	Calc.[a] log 1/C	|Δlog1/C|	0.1 MR_4
H	6.47	6.53	0.06	0.10
OCH_3	8.30	7.99	0.31	0.79
Br	7.85	8.20	0.35	0.89
CH_3	7.72	7.53	0.19	0.57
Cl	7.62	7.59	0.03	0.60
OH	6.74	6.93	0.19	0.29
F	6.59	6.51	0.08	0.09

[a] Calculated using Eq. 4.

ing that steric, electronic, and hydrophobic properties of the substituents might be operative, this is a small set.

Using the data in Table 2 [9] Eq. 4 has been derived from congeners II inhibiting binding of [^{3}H]tryptamine in rat frontal/parietal cortical membrane.



II

$$\log 1/C = 2.12(\pm 0.81)MR_4 + 6.32(\pm 0.45) \quad (4)$$
$$n = 7, r = 0.949, s = 0.247, F_{1,5} = 45.4$$

For congeners II, all substituents are in the 4-position, so that only one parameter is needed for the correlation. The parameters π and MR show some collinearity ($r^2 = 0.305$), but π yields a very poor equation ($r = 0.506$). Adding a term in σ to Eq. 4 does not improve the correlation so that electronic effects of X appear unimportant as in the case of Eq. 3.

The coefficient with MR_4 is similar to that in Eq. 3 which suggests that in each case, 4-substituents are behaving in a similar fashion and that they are not binding in hydrophobic space. This is especially interesting since Eq. 3 was developed using platelet-rich plasma as the test system,

TABLE 3
DATA USED TO DERIVE EQS. 5–7 FOR INHIBITION OF 5-HT UPTAKE BY MOUSE BRAIN OF CONGENERS III

	X	R1	R2	Obs. log 1/C	Calc.[a] log 1/C	|Δlog1/C|	MR_N	MR_4
1	H	Me	Me	5.43	5.21	0.22	1.13	0.10
2	F	Me	Me	5.39	5.20	0.19	1.13	0.09
3	F	H	Me	5.85	6.29	0.44	0.57	0.09
4	Cl	Me	Me	5.68	5.56	0.12	1.13	0.60
5	Cl	H	Me	6.70	6.65	0.05	0.57	0.60
6	Br	Me	Me	5.77	5.76	0.01	1.13	0.89
7	Br	H	Me	7.00	6.84	0.16	0.57	0.89
8	Br	H	Et	5.70	5.95	0.25	1.03	0.89
9	Br	H	Pr	4.62	5.05	0.43	1.50	0.89
10	I	Me	Me	6.00	6.10	0.10	1.13	1.39
11	CF_3	Me	Me	5.70	5.49	0.21	1.13	0.50
12	Me	Me	Me	5.62	5.54	0.08	1.13	0.57
13	$Si(Me)_3$	Me	Me	4.60[b]	6.86	2.26	1.13	2.50
14	OMe	Me	Me	6.10	5.69	0.41	1.13	0.79
15	SMe	Me	Me	6.30	6.09	0.21	1.13	1.38
16	CN	Me	Me	5.13	5.58	0.45	1.13	0.63

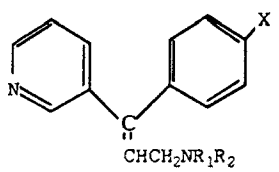
[a] Calculated using Eq. 7.

[b] This data point is not used in the derivation of Eq. 7.

while the data for Eq. 4 was obtained from studies of binding to the frontal/parietal cortical membranes of rats.

Equation 4 reinforces our confidence in Eq. 3 in another mode since substituent variation in Table 2 is different from that of 4-substituents in Table 1; still, this set of seven congeners is not ideally selected. Larger substituents and more hydrophilic substituents should have been included.

From the data in Table 3 [10] for the inhibition of the accumulation of 5-[^{14}C]HT in mouse brain in vitro Eqs. 5 and 6 were formulated. Although 30 compounds (III) were studied, we have considered only the *cis*-isomers (as shown in III) with substituents in the *para*-position as indicated in III. There were insufficient 3-substituents to attempt a meaningful correlation and only two *trans*-isomers. Also, too few *ortho*-substituents were available to attempt to define substituent effects in this position.



III

$$\log 1/C = -1.81(\pm 1.0)MR_N + 10.61\ (\pm 1.1) \quad (5)$$
$$n = 16,\ r = 0.711,\ s = 0.468,\ F_{1,14} = 14.3$$

$$\log 1/C = -1.93(\pm 0.76)MR_N + 1.50\ (\pm 0.90)\ MR_4 - 0.64(\pm .35)\ (MR_4)^2 + 10.16\ (\pm 0.83) \quad (6)$$
$$n = 16,\ r = 0.888,\ s = 0.331,\ F_{2,12} = 8.00$$
$$\text{ideal } MR_4 = 1.17$$

In these equations, MR_N refers to R_1 and R_2, while MR_4 refers to X. The exponential term in Eq. 6 with the negative coefficient shows that, after a certain size (MR = 1.2), activity no longer increases with MR but instead declines. There are only three substituents with MR values larger than 1.2, and of these only $Si(Me)_3$ is much larger than 1.2. Dropping this point, Eq. 7 is obtained:

$$\log 1/C = -1.94(\pm 0.67)MR_N + 0.69(\pm 0.44)MR_4 + 10.34(\pm 0.72) \quad (7)$$
$$n = 15,\ r = 0.886,\ s = 0.294$$

Equation 7 is actually a better correlation than Eq. 6 (compare values of s), showing that until larger groups are tested, one cannot place much confidence in Eq. 6. The role of X in congeners III is similar to that in congeners I and II in that MR, not π, is the significant variable; however, the coefficient with MR in Eq. 7 is much smaller than that in Eqs. 3 and 4 showing that congeners III interact with the receptor in a somewhat different manner. The following squared correlation matrix shows some collinearity between π_4 and MR_4.

	π_4	MR_4	MR_N
π_4	1	0.29	0.00
MR_4		1	0.05

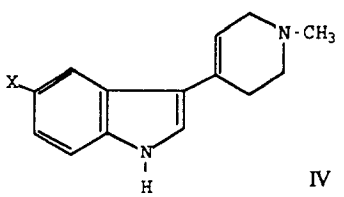
Adding a term in π_4 to Eq. 7 does not improve the correlation, and the coefficient with π_4 is only 0.07. Replacing MR_4 with π_4 in Eq. 7 yields a poorer correlation ($r = 0.805$). Thus, it seems safe to say that 4-substituents do not contact hydrophobic space.

Perusing the results in Table 3 in the light of Eq. 7, it is interesting to note that large 4-substituents (4-I, 4-SCH_3), which contribute strongly to activity, were present only in congeners with *N,N*-dimethyl groups which inhibit activity. Since these two congeners are well predicted by Eq. 7, it would be interesting to incorporate them into mono *N*-methyl analogues or simple NH_2 analogues. The negative steric effect is obvious at $MR_4 = 2.50$ for $Si(Me)_3$, but there is interesting space to explore between 4-I ($MR_4 = 1.39$) and $Si(Me)_3$, avoiding of course bulky groups on N.

As with the other examples, this dataset is not ideal for exploration of 4-X (the usual over-emphasis on halogens and only a single substituent (CN) with a negative π value). The OCH_3 group has a π of -0.02, but this does not differ enough from 0 for H to make it interesting.

The large coefficient with MR_N not only brings out the deleterious effect of substitution on N, but when compared with Eq. 3, which shows no effect of substitution on N, clearly indicates a different binding mode or a different receptor for these congeners compared with congeners I and II.

Recently, Taylor et al. [11] have formulated QSAR for congeners IV inhibiting 5-HT2 sites in a rat brain preparation. Their dataset contains a much better selection of substituents than sets I–III.



IV

From their data in Table 4 they derived Eq. 8:

$$\log K_i = 1.01 \log P - 0.0332\, V_5 + 5.0946\, Q_5 + 1.47\, R + 4.123 \quad (8)$$
$$n = 15,\ r = 0.937,\ s = 0.361$$

where log P represents calculated log P values, V_5 represents substituent volume, and Q_5 and R are electronic parameters. Equation 8 may be a bit over-determined, since there are only four data points/variable.

We have obtained Eqs. 9 and 10 from their data.

$$\log 1/K_i = -0.52(\pm 0.49)MR + 6.87(\pm 0.68) \quad (9)$$
$$n = 15,\ r = 0.540,\ s = 0.783,\ F_{1,13} = 5.34$$

$$\log 1/K_i = -0.86(\pm 0.34)MR + 0.90(\pm 0.41)\,\pi + 7.16\,(\pm 0.44) \quad (10)$$
$$n = 15,\ r = 0.869,\ s = 0.478,\ F_{1,12} = 22.8$$

While Eq. 10 is not as sharp a correlation as Eq. 8, it contains two fewer variables and fits the pattern found for datasets I–III, in that no role could be found for an electronic effect using either σ or σ^-. Since X in IV is quite remote from the crucial basic nitrogen atom, we would not expect an electronic term to be significant.

TABLE 4
DATA USED TO DERIVE EQS. 8–10 FOR THE INHIBITION OF TRYPTAMINE BINDING TO RAT CORTICAL MEMBRANES (5-HT2 RECEPTOR) BY CONGENERS IV

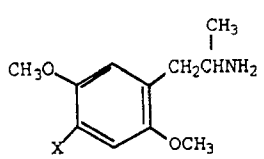
	X	Obs. log $1/K_i$	Calc.[a] log $1/K_i$	$\lvert\Delta \log 1/K_i\rvert$	0.1 MR	π
1	H	7.17	7.08	0.09	0.10	0.00
2	OCH_3	6.05	6.47	0.42	0.79	−0.02
3	Br	7.32	7.17	0.14	0.89	0.86
4	Cl	7.29	7.29	0.00	0.60	0.71
5	F	7.35	7.21	0.14	0.09	0.14
6	$CONH_2$	4.77	4.99	0.22	0.98	−1.49
7	CO_2CH_3	5.44	6.05	0.61	1.29	−0.01
8	$CO_2C_2H_5$	5.36	6.13	0.77	1.75	0.51
9	$OCOCH_3$	6.25	5.52	0.73	1.25	−0.64
10	CH_3	6.90	7.18	0.28	0.57	0.56
11	NO_2	7.06	6.28	0.78	0.74	−0.28
12	CN	6.02	6.11	0.09	0.63	−0.57
13	OH	6.45	6.31	0.14	0.29	−0.67
14	$OCH_2C_6H_5$	6.40	5.90	0.50	3.22	1.66
15	Phthalimido	4.97	4.93	0.14	2.9	0.27

[a] Calculated using Eq. 10.

The π values used in Eq. 10 are from the benzene system, while the ring to which X is attached in congeners IV is somewhat more closely related to aniline, but π values from the aniline system did not correlate the data as well as those from benzene. Delocalization of the lone-pair electrons on the indole nitrogen in the aromatic system apparently make them less susceptible to electronic effects of substituents [12]. Further work needs to be done with congeners IV to sort out the different conclusions implied by Eqs. 8 and 10. However, it is clear, from either equation, that hydrophobicity improves potency and bulk decreases it. This is in contrast to Eqs. 3, 4, and 7 where bulky substituents at least up to $Si(CH_3)_3$ in size increase potency.

The finding by Taylor et al. [11] that X of IV affects activity by a combination of a positive hydrophobic effect and a negative steric effect, and our confirmation of their discovery, is important for the SAR of this class of compounds, and, if properly followed up, should do much to clarify our understanding of the 5-HT receptors. Our view that electronic effects are unimportant is in general agreement with Taylor et al., since they were well aware of the weakness of the electronic terms in Eq. 8.

Finally, Glennon et al. [13] obtained the data in Table 5 for congeners V from which we have developed Eqs. 11 and 12.



V

TABLE 5
DATA USED TO DERIVE EQS. 11 AND 12 FOR THE INHIBITION OF 5-HT AFFINITY IN RAT FUNDUS BY CONGENERS V

	X	Obs.	Calc.[a]	|Δlog 1/C|	π	σ
1	Br	7.35	7.36	0.01	0.86	0.23
2	OCH_3	6.81	6.77	0.04	−0.02	−0.27
3	I	7.49	7.44	0.05	1.12	0.18
4	NO_2	7.07	7.13	0.06	−0.28	0.78
5	H	6.83	6.90	0.07	0.00	0.00
6	C_2H_5	7.18	7.25	0.07	1.02	−0.15
7	CH_3	7.13	7.05	0.08	0.56	−0.17
8	OC_2H_5	6.78	6.95	0.17	0.38	−0.24
9	F	7.20	6.98	0.22	0.14	0.06

[a] Calculated using Eq. 12.

$$pA_2 = 0.34(\pm 0.32)\pi + 6.95(\pm 0.20) \quad (11)$$
$$n = 9,\ r = 0.687,\ s = 0.193,\ F_{1,7} = 6.25$$

$$pA_2 = 0.41(\pm 0.23)\pi + 0.45(\pm 0.35)\sigma + 6.90(\pm 0.15) \quad (12)$$
$$n = 9,\ r = 0.894,\ s = 0.128,\ F_{1,16} = 9.77$$

In these expressions, pA_2 (log 1/C) is for the 5-HT receptor affinity of the inhibitors in the isolated rat stomach fundus assay.

Equation 12 appears to be an exception to the other QSARs in that an electronic term is definitely present; however, since the amino group is well insulated from the substituents, we believe that σ represents the effect of X on the OCH_3 groups, increasing their hydrophobicity. It is very well established that when electron-attracting groups are placed on a benzene ring carrying substituents with lone-pair electrons, there is an increase in hydrophobicity over normal π values [14]. Hence, in this instance it seems likely that only the hydrophobicity of the substituent is involved. The rather small substituents considered in this study, and the limited size of the dataset, do not allow us to uncover a steric effect. Adding an MR term to Eq. 12 does give a better correlation (r = 0.930) and a −0.18 coefficient with MR, but this is not justified by the F test (F = 2.39) for the small dataset. There is also some collinearity between MR and π as seen in the following squared correlation matrix:

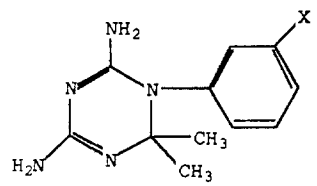
	MR	π	σ
MR	1	0.35	0.00
π		1	0.06

Like the other datasets, we find the problem so often associated with SAR studies, that is, selection of a set of substituents which precludes complete separation of steric and hydrophobic effects because of the collinearity problem.

Glennon et al. [13] concluded that hydrophobicity of X of V is unimportant, but we feel that the result is not out of line with the findings of Eqs. 8 and 10. A better selection of substituents with *measured* log P values is needed to clarify the situation.

DISCUSSION

This report considers the QSAR of ligands which bind to a receptor or an uptake system (a transport receptor) or both. The question is, does the binding site on the uptake receptor resemble that on the receptor? That is, could one expect to see similarities between two different types of binding sites? We do not know, but one way to gain some insight about the two binding sites is to do comparative QSAR. Actual reflection on the problem suggests that it is not a far-fetched idea that both receptors must be somewhat similar, and it is not inconceivable that they could both employ the same basic receptor. Studies with dihydrofolate reductase (DHFR) provide some evidence for this line of reasoning. Equations 13 and 14 are QSARs for the inhibition of pure L1210 DHFR and for L1210 leukemia cells [15] by triazines VI.



VI

Inhibition of L1210 DHFR by VI.

$$\log 1/K_i = 0.98\,\pi' - 1.14\log(\beta\cdot 10^{\pi'} + 1) + 0.79\,\sigma + 6.12 \qquad (13)$$
$$n = 58,\ r = 0.900,\ s = 0.264,\ \pi_0' = 1.76$$

Inhibition of sensitive L1210 cells by VI.

$$\log 1/C = 1.13\,\pi - 1.20\log(\beta\cdot 10^{\pi} + 1) + 0.94\,\sigma + 0.66\,I_R - 0.32\,I_{OR} + 6.72 \qquad (14)$$
$$n = 61,\ r = 0.890,\ s = 0.241,\ \pi_0 = 1.45$$

In these equations, π' represents π for X, with the reservation that for substituents of the type 3-$ZCH_2C_6H_4$-Y, π for Y is set to zero. Studies with molecular graphics of VI bound to DHFR show that Y projects beyond the enzyme into aqueous space and, hence, shows no hydrophobic effect. In Eq. 14, normal π values have been used in which the hydrophobicity of Y is included. In Eq. 14, I_R is an indicator variable which takes the value of 1 when X = normal alkyl and I_{OR} takes the value of 1 for alkoxy substituents. I_{OR} is of marginal value. Thus, the only real difference between Eqs. 13 and 14 is the I_R term, showing that alkyl groups enhance activity of this class of inhibitors in cells compared to isolated enzyme. C in Eq. 14 is the molar concentration producing 50% inhibition of tumor cell growth. Although K_i and C are not strictly comparable, note that the intercepts of the two equations are close, implying that about the same intrinsic activity is shown by the triazines in each system.

These equations were unexpected since the two test systems are so different. For the pure DHFR, the inhibitor simply partitions onto the receptor site from the aqueous phase. In the cells there are three factors which must be considered: (1) active transport of the inhibitor into the cell; (2) movement through cellular material from the point of departure from the transport system to the cellular DHFR; and (3) inhibition of the cellular DHFR. We believe that π serves better in Eq. 14 than π' because, in movement through the cell, Y exerts some hydrophobic effects, albeit not large. Note that the hydrophobic terms and π_0 are very similar in Eqs. 13 and 14, as are the coefficients with σ. What the role of I_R might be is not yet clear.

Equations 13 and 14 become more significant when they are compared with Eqs. 15–17 for cellular systems where active transport does not appear to be significant.

Inhibition of L1210 cells resistant to methotrexate by VI.

$$\log 1/C = 0.42\,\pi - 0.15\,MR + 4.83 \qquad (15)$$
$$n = 62,\ r = 0.941,\ s = 0.220,\ \pi_0 \sim 6$$

Inhibition of *E. coli* cells by VI.

$$\log 1/C = 0.60\,\pi - 1.89 \log(\beta \cdot 10^{\pi} + 1) + 2.84 \qquad (16)$$
$$n = 66,\ r = 0.963,\ s = 0.344,\ \pi_0 = 5.86$$

Inhibition of *S. aureus* cells by VI.

$$\log 1/C = 0.59\,\pi - 1.52 \log(\beta \cdot 10^{\pi} + 1) + 2.83 \qquad (17)$$
$$n = 23,\ r = 0.986,\ s = 0.218,\ \pi_0 = 5.79$$

The dramatic difference between Eqs. 14 and 15 clearly shows the importance of the nature of the active site in the transport system on the SAR, as current evidence suggests that the transport system in the resistant L1210 cells is seriously impaired, if not completely ineffective. Neither of the two bacterial cells contains transport systems. The initial slopes with Eqs. 15–17 are similar, and the dependence on hydrophobicity overrides all other structural features. It should be noted that the DHFR used to derive Eq. 13 comes from the resistant cell line.

While π_0 cannot be precisely determined for Eq. 15, it is clear that it is near 6, which agrees well with the two other systems, and is wildly different from that for Eqs. 13 and 14. The negative MR term in Eq. 15 is of minor importance; it is a measure of the bulk of X including Y. That is, large groups hinder the entrance of VI into the cell, which seems to be a normal effect shown by a variety of resistant mammalian cells [17].

The QSAR of Eq. 15 is much simpler than that of Eq. 13. Since π is by far the more important parameter, it would seem that the simple linear relationship (up to $\pi = 6$) is associated with the passive diffusion of the compounds to the active site. Small structural features (e.g. σ) influencing how the inhibitor reacts with the enzyme are overshadowed by the penetration problem. With the isolated enzyme, and with cells having a transport system, only substituents with $\pi < \pi_0$ make full hydrophobic contact. Long alkyl groups show no improved activity. This implies that, in the transport process, that part of the substituent beyond π_0 does not contact the receptor.

The conclusion from these studies is that the binding site on the transport protein must be rather similar to that on the DHFR. In fact, it is not inconceivable that the transport system could somehow utilize a molecule of DHFR.

While our QSARs on serotonin are not comparable to those for DHFR, we have been interested to see similarities which suggest in a small way that binding to the two receptors may be similar. The situation with binding and transport with serotonin is not exactly parallel to that with DHFR. With serotonin receptor, binding is occurring on one surface while uptake occurs on another; however, in each instance the active sites must have the ability to recognize serotonin (i.e. there must be characteristics in common for the two active sites). The common thread of MR in the QSAR suggests that steric effects are more likely to be of importance than hydrophobic ones. To our knowledge, evidence for such a view has not been previously advanced.

The five examples of serotonin agonists suitable for QSAR analysis show that, even though none of the data are from ideally designed sets, one can derive QSARs which provide considerable insight about how physicochemical properties of these drugs affect interaction with presumed 5-HT receptors. Dataset V (Eq. 12) comes from a rather different rat stomach fundus assay, and this QSAR is different from the others in that no MR term is involved. In the four other examples, MR modeling the bulk of substituents plays a crucial role. In three of the four examples (sets I–III), the coefficient with MR_4 is positive, suggesting a positive steric effect of substituents. It would appear that X in these examples may produce a conformational change in the receptor, since the hydrophobic effect of X can be ruled out. Equations 3 and 7 for datasets I and II show a similar effect for X, but have about 3 log units difference in their intercepts. The lower intercept (weaker inhibitors) of Eq. 1 is for inhibitors of 5-HT, while the higher intercept (Eq. 4) is for the inhibition of tryptamine binding. Such inhibition is more easily accomplished, which brings out the importance of the 5-OH group in 5-HT binding.

The most sophisticated study (dataset IV) with the best variation in substituents shows a combined positive hydrophobic and negative steric effect in receptor binding. This is a complex effect which cannot be resolved without regression analysis and explains some of the confusion surrounding early SAR studies. Either Eq. 8 or 10 show that there is little to be gained in studying more substituents in the 5-position of structure IV.

QSAR for dataset I provides a most interesting lead in that it shows that 3- and 4-substituents contact different types of space and by proper substitution in the two positions, it should be possible to make more effective drugs.

A final point which needs consideration is, why are hydrophobic terms missing in three of the five equations? It is generally found that, for sets of congeners containing a reasonable range in hydrophobicity, penetration of cells involves hydrophobic interactions which must be accounted for by hydrophobic terms in the QSAR [1]. If active transport is involved, one could expect the QSAR to contain terms which are necessary for reaction at the receptor and for reaction with the transport proteins. For the present study, it is possible that the protonated amino group is recognized by the transport system without assistance from hydrophobic interactions. Hence, if interactions at the receptor do not involve hydrophobic interactions, no such terms would appear in the QSAR. Hence, the results from data I–IV could be rationalized by involving active transport and assuming that the π term in Eq. 10 is related to a hydrophobic site on the receptor.

Dataset V has different implications. No MR term is present, so that if we assume that only hydrophobic effects are being modeled by this equation, then it would seem that these are associated with movement of the drugs through cellular material to the site of action.

It is, of course, appreciated that different test systems with different end points are involved with the datasets considered in this report. In undertaking the analysis, we had no idea of what the outcome might be and were, in fact, surprised to find the MR term so prominent. There was no reason to expect 5-HT uptake by platelet-rich plasma (Eqs. 1–3) to parallel binding of tryptamine in rat cortical membrane (Eq. 4) or 5-HT uptake in mouse brain (Eqs. 5–7) or 5-HT binding to HT2 receptors in rat brain, except that the two types of receptors might be similar, as in the case of DHFR.

One might elect to discuss the QSAR from the point of view of their differences or focus on similarities. We have tended to take the latter view, since we find the importance of MR in all QSARs except 12 to be of special interest.

The interpretation of the MR terms can be complex [8]. If substituent effects at a given position are correlated only by MR (addition of a term in π does not improve the result), one assumes contact is not occurring with hydrophobic space and polar space is involved. The coefficient with the MR may be either positive or negative. Since MR is primarily a measure of bulk, a negative term suggests steric hindrance, either directly or through a conformational change in the receptor. A positive coefficient might suggest an interaction, depending on the polarizability of the substituent, although there is little concrete evidence for the importance of such an effect. It might also be interpreted to mean that substituents provide a buttressing effect in holding the ligand in the most favorable position in the receptor [8]. Such an effect might be a direct result, or it might come about through a conformational change in the receptor.

Our analysis of the current data, suitable for QSAR treatment, raises as many problems as it answers; however, it does delineate many problems associated with the SAR of the inhibition of serotonin uptake or binding, which must be considered in the next round of investigations. What is now needed is a comparative study along the lines of that discussed for DHFR, and QSAR is at present about the only way to compare the structure of the receptor and the transport binding sites.

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