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Allosteric Interactions and QSAR: On the Role of Ligand Hydrophobicity

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Abstract—A study of a very large database of QSAR (9100) has uncovered a few unusual examples where as one increases the hydrophobicity of the members of a set of congeners, activity decreases until at a certain point, activity begins to increase. Obviously a change in mechanism is involved. The only way we have found to rationalize this unusual event is by a change in the structure of the receptor. We have found this to occur with hemoglobin, a substance first used to define allosteric reactions.

Introduction

Since the pioneering work of Koshland[1] and Monod et al.,[2] interest in allosteric interactions has grown steadily. The word allosteric now appears in 5682 abstracts in *Chemical Abstracts*. The early work in this fascinating field has been reviewed by Koshland.[3] Since then, other efforts[4,5] have been made to review the voluminous reports, but like so many other areas of science, the subject is now too large for anything like complete coverage.

We have recently observed several instances where QSAR based on inverted parabolic relationships dependent on Clog*P*, CMR, and molar volume correlate such data.[7–10] That is, at first activity decreases as the values of these parameters increase, but then at a specific point turns around and increases. Obviously a change in the mechanism of the *reaction* occurs at the inversion point. What is this to be attributed to? We have come to attribute this to a change in the structure of the receptor that occurs with ligand binding. Allosteric interactions are said to be due to a change in the structure of the receptor. An interesting aspect of our finding is that despite rather different changes occurring in more than one position on the ligands, correlation is often with a single parameter (e.g., $-\log P + \log P^2$). In a few instances an additional electronic or steric term appears.

After finding the examples cited above, we decided to make a careful study of our current database of 9000 biological QSAR to see how many such examples we could uncover. Over the last 40 years, as we have developed our database, we have entered the 'best' QSAR we could derive for each dataset, the fact that we had collected a number of such inverted parabolic relationships suddenly dawned on us.

Allosteric effects have been considered to occur when the interaction of a ligand and a receptor results in a change in structure of the receptor. In the early work where hemoglobin was the receptor, the results were deduced by rather complex data analysis. The first precise understanding came from the studies of Perutz's[5] X-ray analysis of the exact location of ligand on the hemoglobin subunits. Hemoglobin is an incredibly complex substance that can, apparently, be composed of possibly many subunits as Royer et al. have discussed.[6] Thus we have much to learn from other ways of approaching the subject. Of course, we have no way of knowing exactly what the structure of a receptor is in a cell, much less a whole animal. Hence we believe that QSAR can serve as a useful role in gaining an indirect view of what we might learn about its in situ properties. The conclusions we draw from our QSAR may not be the final word, but we do believe that they are an important start on an extremely complex problem. As in the case with all of life's chemical processes, the more carefully we examine them, the more fearfully complex they appear.

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Results

We report here the QSAR where we find the inverted parabolic relationship associated with a hydrophobic interaction correlated by Clog*P*. First of all, it is important to establish a connection with the fundamental studies with hemoglobin by Monod et al.[2] From data of Reisberg and Olson[11] we have developed QSAR 1

Rate constants for the binding of RN=C to the alpha subunit of human hemoglobin at pH 7.0, 20 °C (Table 1)[11]

$$\text{Log } k = -0.77(\pm 0.44)\text{Clog}P + 0.35(\pm 0.23) \quad \text{Clog}P^2 \; 1.72(\pm 0.44)\text{B1}_\text{R} + 4.76(\pm 0.78) \tag{1}$$

$n = 12, \quad r^2 = 0.949, \quad s = 0.188, \quad q^2 = 0.833$

inversion point = 1.1 (0.8−1.7)

outlier: CH_2CHMe_2

In the above expression, the sterimol parameter $B1_R$ brings out a negative effect of four examples of the more complex R groups. First, activity decreases with increasing hydrophobicity, as brought by −0.77 Clog*P*, but as substituents become more hydrophobic, the ClogP^2 term takes over and activity starts to increase (inversion point). Clearly a change in mechanism has occurred. This is a sharp correlation with good 95% confidence limits on the inversion point. B1 is one of three sterimol parameters to assess steric properties of substituents.[12] It accounts for the steric effect of the first atom of the substituent attached to the parent compound.

In the example of QSAR 1, Clog*P*, CMR and MgVol are collinear so one might wonder which to choose. The following equation sheds some light on the problem.

0.5–1 binding of a set of miscellaneous chemicals to bovine hemoglobin (Table 2)[13]

$$\text{Log } 1/C = 0.66(\pm 0.12)\text{Clog}P + 1.95(\pm 0.30) \tag{2}$$

$n = 16, \quad r^2 = 0.912, \quad s = 0.145, \quad q^2 = 0.886$

outlier: 4-Br−C_6H_4OH

Hence, one would expect allosteric effects with hemoglobin to depend on hydrophobicity, as defined by the octanol/water partition coefficient. Clog*P* is the calculated partition coefficient.

From a different point of view, Reisberg and Olson[11] studied the equilibrium binding of RN=C to human hemoglobin, the results of which yielded QSAR 3 (Table 3)[11]

Table 1. Rate constants for the binding of RN=C to the alpha subunit of human hemoglobin at pH 7.0, 20 °C[11]

No.	Substituents (R)	Log *k*				
		Obsd	Calcd (eq 1)	Δ	Clog*P*	$B1_R$
1	Me	2.59	2.55	0.04	−0.44	1.52
2	C_2H_5	2.15	2.08	0.07	0.09	1.52
3	C_3H_7	1.60	1.80	−0.20	0.62	1.52
4	C_4H_9	1.77	1.71	0.06	1.15	1.52
5	C_5H_{11}	1.92	1.82	0.10	1.68	1.52
6	C_6H_{13}	2.08	2.13	−0.05	2.21	1.52
7	$CHMe_2$	1.08	1.24	−0.16	0.40	1.90
8	CH_2CHMe_2[a]	0.87	1.72	−0.85	1.02	1.52
9	(+)$CH(Me)C_2H_5$	1.00	1.07	−0.07	0.93	1.90
10	(−)$CH(Me)C_2H_5$	1.00	1.07	−0.07	0.93	1.90
11	CMe_3	0.08	−0.11	0.19	0.80	2.60
12	cy-C_6H_{11}	0.89	1.12	−0.23	1.59	1.91
13	$CH_2C_6H_5$	2.04	1.73	0.31	1.33	1.52

[a]Data point not included in deriving equation.

$$\text{Log } 1/C = 1.14(\pm 0.28)\text{Es}_\text{R} - 0.44(\pm 0.54) \tag{3}$$

$n = 11, \quad r^2 = 0.902, \quad s = 0.190, \quad q^2 = 0.820,$

outliers: Me; cy-C_6H_{11}

This is not a very sharp correlation and is entirely different from eq 1. It seems likely that covalent bonding is required to produce the results seen in QSAR 1. The C=N moiety is quite reactive and might, for example, react with the SH or NH_2 units in hemoglobin. In other experiments, they studied the binding to the beta subunit.

Binding of RN=C to human hemoglobin dimer of beta subunit at pH 7.0, 20 °C (Table 4)[11]

$$\text{Log } K = 1.47(\pm 0.44)\text{Es}_\text{R} + 4.78(\pm 0.86) \tag{4}$$

$n = 10, \quad r^2 = 0.879, \quad s = 0.296, \quad q^2 = 0.802$

outlier: C_3H_7

Table 2. Data for 0.5–1 binding of miscellaneous compounds to bovine hemoglobin[13]

No.	Substituents	Log 1/*C*			
		Obsd	Calcd (eq 2)	Δ	Clog*P*
1	2-COMe-naphthalene	3.58	3.81	−0.24	2.82
2	Naphthalene	4.19	4.18	0.01	3.37
3	4-NO_2-chlorobenzene	3.33	3.53	−0.20	2.39
4	4-NO_2-aniline	2.98	2.86	0.12	1.37
5	3-NO_2-aniline	2.78	2.87	−0.09	1.39
6	4-Br-aniline	3.24	3.30	−0.06	2.03
7	4-Cl-aniline	3.22	3.16	0.06	1.83
8	4-NH_2-azobenzene	4.08	4.17	−0.09	3.36
9	4-F-phenol	3.15	3.12	0.03	1.77
10	4-I-phenol	3.94	3.88	0.06	2.91
11	4-C_6H_4-phenol	4.52	4.32	0.20	3.58
12	4-Cl-phenol	3.70	3.53	0.17	2.39
13	3-Cl-phenol	3.39	3.61	−0.22	2.50
14	4-Br-phenol[a]	4.00	3.67	0.34	2.59
15	3-CF_3-phenol	3.94	3.90	0.04	2.95
16	1-naphthol	3.75	3.74	0.01	2.70
17	1-NH_2-naphthalene	3.62	3.43	0.19	2.23

[a]Data point not included in deriving equation.

Binding of RN=C to human hemoglobin single chain beta subunit at pH 7.0, 20 °C (Table 5)[11]

$$\text{Log } K = 0.43(\pm 0.27)\text{Clog}P + 1.12(\pm 0.33)\ \text{Es}_R + 3.82(\pm 0.75) \quad (5)$$

$n = 10, \quad r^2 = 0.926, \quad s = 0.202, \quad q^2 = 0.870$

outliers: Me; C_3H_7; cy-C_6H_{11}

In QSAR 3, 4, and 5 there is no evidence of an allosteric interaction. In each instance only a negative steric effect is seen. Recall that the steric parameter Es[12] values (eqs 3, 4, and 5) are negative so that the positive coefficient in QSAR implies a negative steric effect.

Possibly the most interesting study of hemoglobin binding is the interaction of nitrobenzenes and anilines with hemoglobin in rats. From Sabbioni's[14] data we have derived QSAR 6.

Binding of X–$C_6H_4NO_2$ to hemoglobin in Wistar rats (Table 6)[14]

$$\text{Log } HBI = 3.62(\pm 1.44)\sigma^+ - 11.1(\pm 5.64)\ \text{Clog}P + 1.97(\pm 1.00)\text{Clog}P^2\ +1.51(\pm 1.00)\text{B1}_4 + 14.20(\pm 7.86) \quad (6)$$

$n = 14, \quad r^2 = 0.874, \quad s = 0.507, \quad q^2 = 0.743$

inversion point: 2.8(2.6−3.1)

outlier: 2, 4-di-F

In the above equation, *HBI* is the hemoglobin index (mmol compound/mol·*HB*/mmol/kg body weight). This equation is not as sharp as one would like and it is complicated by the σ^+ and B1 terms so that one cannot obtain a meaningful plot of the results. Nevertheless, the inversion point is well defined.

Table 3. Data for equilibrium binding of RN=C to human hemoglobin[11]

No.	Substituents (R)	Log 1/*C*			
		Obsd	Calcd (eq 3)	Δ	Es_R
1	Me[a]	−2.52	−1.85	−0.67	−1.24
2	C_2H_5	−1.72	−1.93	0.21	−1.31
3	C_3H_7	−2.13	−2.07	−0.06	−1.43
4	C_4H_9	−2.55	−2.29	−0.26	−1.63
5	C_5H_{11}	−2.40	−2.30	−0.09	−1.64
6	C_6H_{13}	−2.05	−2.19	0.14	−1.54
7	$CHMe_2$	−2.48	−2.38	−0.10	−1.71
8	CH_2CHMe_2	−2.77	−2.91	0.14	−2.17
9	(+)$CH(Me)C_2H_5$	−2.89	−3.13	0.25	−2.37
10	(−)$CH(Me)C_2H_5$	−3.00	−3.13	0.13	−2.37
11	CMe_3	−3.85	−3.60	−0.25	−2.78
12	cy-C_6H_{11}[a]	−3.30	−2.50	−0.80	−1.81
13	$CH_2C_6H_5$	−2.25	−2.16	−0.10	−1.51

[a]Data points not included in deriving equation.

Table 4. Data for binding of RN=C to human hemoglobin dimer of beta subunit at pH 7.0, 20 °C[11]

No.	Substituents (R)	Log *K*			
		Obsd	Calcd (eq 4)	Δ	Es_R
1	Me	3.38	2.96	0.42	−1.24
2	C_2H_5	2.45	2.86	−0.42	−1.31
3	C_3H_7[a]	1.90	2.69	−0.78	−1.43
4	C_4H_9	2.20	2.39	−0.19	−1.63
5	C_5H_{11}	2.53	2.38	0.15	−1.64
6	C_6H_{13}	2.69	2.53	0.17	−1.54
7	$CHMe_2$	2.08	2.28	−0.20	−1.71
8	CH_2CHMe_2	1.40	1.60	−0.20	−2.17
9	(+)$CH(Me)C_2H_5$	1.73	1.31	0.42	−2.37
10	(−)$CH(Me)C_2H_5$	1.23	1.31	−0.08	−2.37
11	CMe_3	0.63	0.71	−0.08	−2.78

[a]Data point not included in deriving the equation.

Table 5. Data for binding of RN=C to human hemoglobin single chain beta subunit at pH 7.0, 20 °C[11]

No.	Substituents (R)	Log *K*				
		Obsd	Calcd (eq 5)	Δ	Es_R	Clog*P*
1	Me[a]	2.90	2.24	0.66	−1.24	−0.44
2	C_2H_5	2.48	2.39	0.09	−1.31	0.09
3	C_3H_7[a]	1.92	2.48	−0.56	−1.43	0.62
4	C_4H_9	2.53	2.48	0.05	−1.63	1.15
5	C_5H_{11}	2.65	2.69	−0.04	−1.64	1.68
6	C_6H_{13}	3.00	3.03	−0.03	−1.54	2.21
7	$CHMe_2$	1.86	2.07	−0.21	−1.71	0.40
8	CH_2CHMe_2	1.49	1.82	−0.33	−2.17	1.02
9	(+)$CH(Me)C_2H_5$	1.81	1.56	0.26	−2.37	0.93
10	(−)$CH(Me)C_2H_5$	1.76	1.56	0.20	−2.37	0.93
11	CMe_3	0.94	1.04	−0.10	−2.78	0.80
12	cy-C_6H_{11}[a]	1.60	2.46	−0.86	−1.81	1.59
13	$CH_2C_6H_5$	2.78	2.69	0.09	−1.51	1.33

[a]Data points not included in deriving equation.

Table 6. Data for binding of X-$C_6H_4NO_2$ to hemoglobin in wistar rats[14]

No.	Substituents	Log *HBI*					
		Obsd	Calcd (eq 6)	Δ	σ^+	Clog*P*	$B1_4$
1	4-Me	−0.37	0.08	−0.44	−0.31	2.38	1.52
2	4-C_2H_5	−0.92	−0.25	−0.67	−0.30	2.91	1.52
3	4-C_6H_5	2.25	2.24	0.01	−0.18	3.77	1.71
4	H	1.78	1.76	0.01	0.00	1.89	1.00
5	4-F	1.60	1.55	0.05	−0.07	2.03	1.35
6	4-Cl	2.33	1.74	0.59	0.11	2.60	1.80
7	4-Br	2.35	2.02	0.33	0.15	2.75	1.95
8	2-Me	−0.14	−0.56	0.42	−0.31	2.30	1.00
9	2-C_2H_5	−0.59	−1.05	0.46	−0.30	2.83	1.00
10	3-Me	0.01	0.16	−0.15	−0.07	2.38	1.00
11	3,5-di-Me	−0.20	−0.46	0.26	−0.14	2.88	1.00
12	2-Cl	0.32	0.79	−0.47	0.11	2.40	1.00
13	3-Cl	1.73	1.48	0.26	0.37	2.60	1.00
14	3-Cl-4-F	1.00	1.66	−0.66	0.30	2.74	1.35
15	2,4-di-F[a]	0.36	1.84	−1.48	−0.14	1.87	1.35

[a]Data point not included in deriving equation.

The σ^+ term is strong and interesting as it suggests that the NO_2 group is reduced to a radical that then leads to binding to hemoglobin. Actually, in the present case σ^+ and σ^- are quite collinear ($r^2 = 0.929$) so that using σ^- in place of σ^+ yields a correlation almost as good as σ^+ ($r^2 = 0.854$). We have extensive data that shows σ^-

Table 7. Reduction of 4-X-$C_6H_4NO_2$ by CH_3CHOH in N_2O saturated solution at 20 °C[16]

No.	Substituents	Log k			
		Obsd	Calcd (eq 7)	Δ	σ^-
1	NO_2	9.38	9.33	0.05	1.27
2	CN	9.08	9.10	−0.02	1.00
3	SO_2NH_2	8.90	9.05	−0.15	0.94
4	CHO	9.26	9.13	0.13	1.03
5	CF_3	8.63	8.81	−0.17	0.65
6	COMe	9.00	8.97	0.03	0.84
7	CO_2Me	8.93	8.89	0.04	0.75
8	SO_3^-	8.78	8.75	0.03	0.58
9	$CONH_2$	8.63	8.77	−0.14	0.61
10	CO_2^-	8.77	8.52	0.25	0.31
11	H[a]	8.52	8.26	0.26	0.00
12	Me	8.18	8.11	0.06	−0.17
13	OMe	8.04	8.04	0.00	−0.26
14	OH	7.85	7.94	−0.10	−0.37

[a]Data point not included in deriving equation.

normally correlates the radical reduction of aromatic NO_2.[15]

From this we infer that a radical reduction of the nitro group occurs in the rat followed by binding to hemoglobin. We have found many examples showing that the toxicity of the aromatic nitro group is related to σ^- of the substituents. The following few examples are illustrative.

Table 8. Reduction of X-$C_6H_4NO_2$ by milk xanthine oxidase under anaerobic condition[17]

No.	Substituents	Log k				
		Obsd	Calcd (eq 8)	Δ	σ^-	$B5_2$
1	4-NH_2	1.36	1.16	0.20	−0.63	1.00
2	4-OMe	1.42	1.53	−0.11	−0.26	1.00
3	4-OH	1.46	1.42	0.05	−0.37	1.00
4	4-SH	1.46	1.93	−0.47	0.15	1.00
5	4-Me	1.46	1.61	−0.15	−0.17	1.00
6	4-SO_3^-[a]	1.58	2.35	−0.77	0.58	1.00
7	H	1.68	1.78	−0.10	0.00	1.00
8	4-C_6H_5	1.78	1.80	−0.02	0.02	1.00
9	4-CH_2Cl	1.80	1.90	−0.10	0.12	1.00
10	4-CH_2OH	1.89	1.86	0.03	0.08	1.00
11	4-COO^-	1.90	2.09	−0.18	0.31	1.00
12	4-Cl	2.20	1.97	0.23	0.19	1.00
13	4-Br	2.23	2.03	0.21	0.25	1.00
14	4-SO_2NH_2[a]	2.25	2.71	−0.46	0.94	1.00
15	4-CN	2.68	2.77	−0.09	1.00	1.00
16	4-$CONH_2$	2.71	2.38	0.33	0.61	1.00
17	4-COOMe	2.72	2.52	0.20	0.75	1.00
18	4-NO_2	2.80	3.03	−0.23	1.27	1.00
19	4-COMe	2.82	2.61	0.21	0.84	1.00
20	4-CHO	2.84	2.79	0.04	1.03	1.00
21	4-COC_6H_5	2.85	2.60	0.26	0.83	1.00
22	3-Cl	1.84	2.15	−0.31	0.37	1.00
23	2-Cl	1.64	1.69	−0.04	0.19	1.80
24	3-CHO	2.16	2.13	0.03	0.35	1.00
25	2-CHO[a]	1.92	2.32	−0.40	1.03	2.36
26	3-NO_2	2.29	2.48	−0.19	0.71	1.00
27	2-NO_2	2.53	2.53	0.01	1.27	2.44
28	3-NH_2	1.80	1.62	0.18	−0.16	1.00
29	2-NH_2	0.85	0.82	0.02	−0.63	1.97

[a]Data points not included in deriving equation.

Table 9. LD_{50} for Fathead minnows by X-$C_6H_4NO_2$[17]

No.	Substituents	Log $1/C$				
		Obsd	Calcd (eq 9)	Δ	σ^-	L_3
1	2-Me	3.57	3.74	−0.17	−0.17	2.06
2	3-Me	3.63	3.58	0.05	−0.07	2.87
3	4-Me	3.76	3.74	0.02	−0.17	2.06
4	2-NO_2	5.45	5.32	0.13	1.27	2.06
5	3-NO_2	4.38	4.25	0.13	0.71	3.44
6	4-NO_2	5.22	5.32	−0.10	1.27	2.06
7	2-NO_2, 3-Me	5.01	4.98	0.03	1.20	2.87
8	2-Me, 5-NO_2	3.75	4.06	−0.31	0.54	3.44
9	3-Me, 4-NO_2	5.15	4.98	0.17	1.20	2.87
10	2-Me, 3-NO_2	3.99	4.06	−0.07	0.54	3.44
11	2-NO_2, 5-Me	5.08	5.25	−0.17	1.20	2.06
12	3-NO_2, 5-Me	3.91	4.17	−0.26	0.64	3.44
13	3,5-di-NO_2	5.29	5.03	0.26	1.42	3.44
14	3-Cl	3.94	3.85	0.09	0.37	3.52
15	H[a]	3.02	3.92	−0.90	0.00	2.06
16	2-NH_2	3.70	3.23	0.47	−0.63	2.06
17	2-NH_2, 5-NO_2	4.07	4.01	0.06	0.08	2.06
18	4-OH	3.36	3.52	−0.16	−0.37	2.06
19	4-F	3.70	3.89	−0.19	−0.03	2.06

[a]Data point not included in deriving equation.

Reduction of 4-X–$C_6H_4NO_2$ by CH_3CHOH in N_2O saturated solution at 20 °C (Table 7)[16]

$$\text{Log } k = 0.85(\pm 0.15)\sigma^- + 8.26(\pm 0.11) \quad (7)$$

$$n = 13, \quad r^2 = 0.932, \quad s = 0.125, \quad q^2 = 0.911,$$

outlier: H

Eq 7 shows that radical reduction of NO_2 is correlated with σ^- as are biological reductions (eqs 8 and 9).

Table 10. Data for binding of anilines to hemoglobin in wistar rats[14]

No.	Substituents	Log *HBI*					
		Obsd	Calcd (eq 10)	Δ	σ	F_2	$B1_3$
1	4-Me	0.63	0.85	−0.21	−0.17	0.00	1.00
2	4-C_2H_5	0.76	0.92	−0.15	−0.15	0.00	1.00
3	4-C_6H_5[a]	2.54	1.42	1.11	−0.01	0.00	1.00
4	H[a]	1.34	1.46	−0.12	0.00	0.00	1.00
5	4-F	1.52	1.68	−0.16	0.06	0.00	1.00
6	4-Cl	2.76	2.29	0.46	0.23	0.00	1.00
7	4-Br	2.53	2.29	0.24	0.23	0.00	1.00
8	2-Me	0.60	0.77	−0.17	−0.17	0.01	1.00
9	2-C_2H_5	0.71	0.92	−0.21	−0.15	0.00	1.00
10	2,4-di-Me	0.36	0.16	0.20	−0.34	0.01	1.00
11	3,6-di-Me	0.04	0.09	−0.05	−0.34	0.02	1.00
12	3-Me	0.69	0.28	0.41	−0.07	0.00	1.52
13	3,4-di-Me	−0.16	−0.34	0.18	−0.24	0.00	1.52
14	3,5-di-Me[a]	1.15	0.03	1.12	−0.14	0.00	1.52
15	2,4,6-tri-Me	−0.70	−0.53	−0.17	−0.51	0.02	1.00
16	2-Cl	−0.30	−0.68	0.38	0.23	0.42	1.00
17	2,4-di-Cl	−0.22	0.15	−0.37	0.46	0.42	1.00
18	3-Cl	1.10	1.38	−0.28	0.37	0.00	1.80
19	3-Cl-4-F	1.49	1.59	−0.11	0.43	0.00	1.80
20	2,4-di-F[a]	1.51	−1.29	2.80	0.12	0.45	1.00

[a]Data points not included in deriving equation.

Table 11. Data for oxidation of X-$C_6H_4NH_2$ by bromate in aqueous 10% acetic acid at 30 °C[19]

No.	Substituents	Log k			
		Obsd	Calcd (eq 12)	Δ	σ
1	H	−2.01	−2.00	−0.01	0.00
2	3-NO_2[a]	−1.98	−1.25	−0.73	0.71
3	3-Cl	−1.61	−1.61	0.00	0.37
4	4-Cl	−1.75	−1.76	0.01	0.23
5	3-Me	−2.09	−2.07	−0.01	−0.07
6	4-Me	−2.17	−2.18	0.01	−0.17

[a]Data point not included in deriving equation.

Reduction of X–$C_6H_4NO_2$ by milk xanthine oxidase under anaerobic condition (Table 8)[17]

$$\text{Log } k = 0.98(\pm0.16)\sigma^- - 0.35(\pm0.23)\text{B5}_2 + 2.13(\pm0.27) \quad (8)$$

$n = 26, \quad r^2 = 0.884, \quad s = 0.201, \quad q^2 = 0.865$

outliers: 4-SO_3^-; 4-SO_2NH_2; 2-CHO

LD_{50} for Fathead minnows by X-$C_6H_4NO_2$ (Table 9)[18]

$$\text{Log } 1/C = 1.10(\pm0.18)\sigma^- - 0.33(\pm0.18)\text{L}_3 + 4.61(\pm0.46) \quad (9)$$

$n = 18, \quad r^2 = 0.923, \quad s = 0.211, \quad q^2 = 0.881$

outlier: H

In eq 8, B5 is the sterimol parameter for 2-substituent bulk. In eq 9, L_3 is the sterimol parameter for the length of substituents in the 3-position.

Sabbioni also studied the binding of X–$C_6H_4NH_2$ to hemoglobin in rats.

Binding of anilines to hemoglobin in wistar rats (Table 10)[14]

$$\text{Log } HBI = 3.62(\pm0.73)\sigma - 7.09(\pm1.48)\text{F}_2 - 1.78(\pm0.67)\text{B1}_3 + 3.24(\pm0.84) \quad (10)$$

$n = 16, \quad r^2 = 0.923, \quad s = 0.300, \quad q^2 = 0.814$

outliers: 4-C_6H_5; H; 3, 5-di-Me; 2, 4-di-F

QSAR 10 shows ortho substituents have a negative field/inductive effect (F_2), however, σ is the most important electronic parameter. If all compounds with *ortho* substituents are removed, we obtain eq 11.

$$\text{Log } HBI = 3.75(\pm1.02)\sigma - 1.87(\pm0.78)\text{B1}_3 + 3.37(\pm0.99) \quad (11)$$

$n = 10, \quad r^2 = 0.919, \quad s = 0.310, \quad q^2 = 0.832$

Eq 11 suggests a possible radical activation of aniline that provides the means for hemoglobin binding as in the case of the nitrobenzenes. However, no allosteric effect is seen with anilines. A different binding site is implied.

Oxidation of X–$C_6H_4NH_2$ by bromate in aqueous 10% acetic acid at 30 °C (Table 11)[19]

$$\text{Log } k = 1.06(\pm0.09)\sigma - 2.00(\pm0.02) \quad (12)$$

$n = 5, \quad r^2 = 0.998, \quad s = 0.012, \quad q^2 = 0.994$

outlier: 3-NO_2

Bromate promotes radical formations.[20]

Hemoglobin is indeed a complex set of subunits. Using X-ray crystallography, Perutz[5] demonstrated that hemoglobins can exist in alternate quaternary structures depending on the state of the ligand interaction. More recently, Royer et al.,[6] have discussed the fact that hemoglobin can range in oligomeric state from dimers to complexes of many hemoglobin subunits. Thus it is surprising that we can obtain simple QSAR to rationalize binding of ligands to such a complex of possible structures.

The apparent radical reaction of nitrobenzenes with hemoglobin finds support in the well known radical reaction of nitric oxide (NO) with hemoglobin to yield Fe (II)–Hb. NO is a well known vascular signaling agent. Hemoglobin appears to be susceptible to radical reactions.[21]

We find seven other instances showing inverted parabolic correlation with ClogP.

I_{50} for inhibition of specific binding of [^{3}H]-*N*-ethylcarboxamideadenosine (NECA) to A_{2A} adenosine receptor in rat cortical membranes by I in rat cortical membranes by I (Fig. 1) (Table 12)[22]

$$\text{Log } 1/C = -4.15(\pm1.17)\text{Clog}P + 0.52(\pm0.22)\text{Clog}P^2 + 13.5(\pm3.3) \quad (13)$$

$n = 8, \quad r^2 = 0.889, \quad s = 0.135, \quad q^2 = 0.717$

inversion point: 4.0(3.8−4.2)

outliers: See Table 12.

This data were taken from Kuroda et al.[22] It is of interest that one of the two stereo isomers (**I-5**) is well fit by QSAR 12, but the other is poorly fit (**I-4**). The equation is not as sharp as one would like, however, it was a surprise to us that a correlation of any kind could be formulated with such a complex set of 'congeners'.

From the data of Sami et al.,[23] we have derived QSAR 14, 15, and 16.

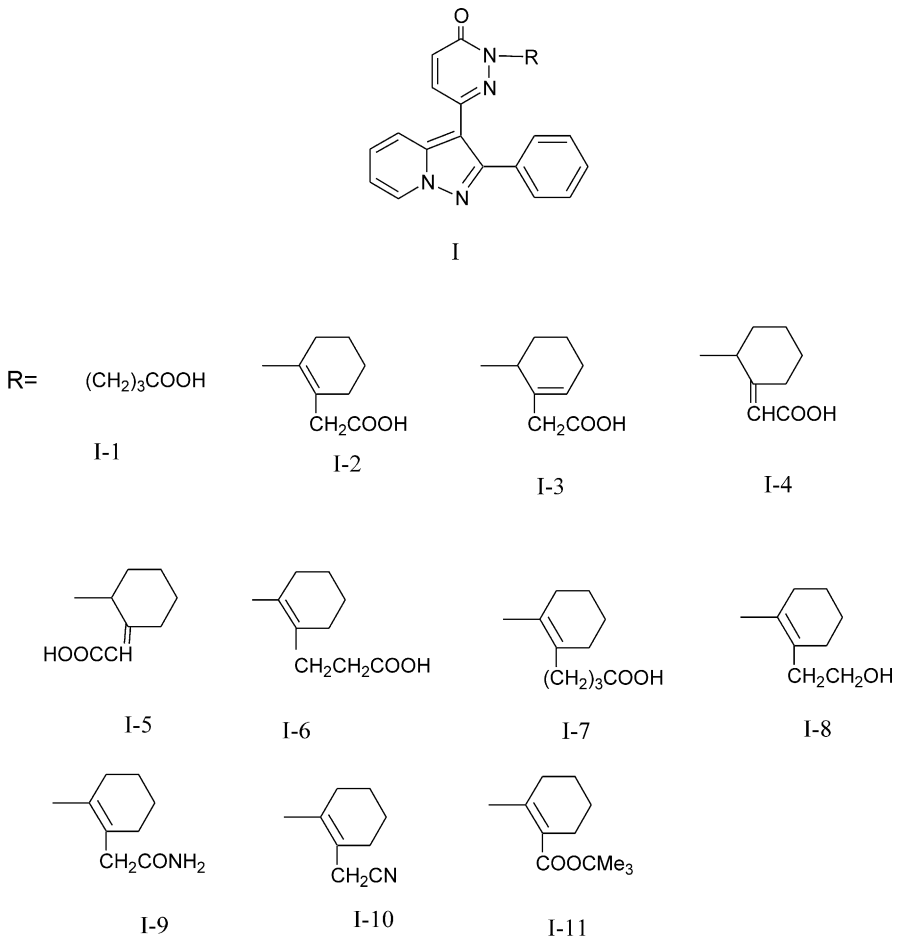


Figure 1. Structures of compounds in Table 12.

Table 12. I_{50} data for inhibition of specific binding of [^{3}H]-N-ethylcarboxamideadenosine (NECA) to A_{2A} adenosine receptor in rat cortical membranes by I[22]

No.	Substituents	Log 1/*C*			
		Obsd	Calcd (eq 13)	Δ	Clog*P*
1	$(CH_2)_3COOH$[a]	5.23	7.37	−2.14	1.94
2	2-CH_2COOH-cyclohexen-1-yl	5.21	5.29	−0.08	3.55
3	2-CH_2COOH-cyclohex-2-en-1-yl[a]	5.13	5.56	−0.43	3.15
4	2-CH_2COOH-cyclohexylidene-1-yl (trans)[a]	5.03	5.34	−0.31	3.45
5	2-CH_2COOH-cyclohexylidene-1-yl (cis)	5.18	5.34	−0.16	3.45
6	2-$(CH_2)_2COOH$-cyclohexen-1-yl	5.12	5.20	−0.08	3.88
7	2-$(CH_2)_3COOH$-cyclohexen-1-yl	5.41	5.29	0.12	4.41
8	2-$(CH_2)_2OH$-cyclohexen-1-yl	5.60	5.43	0.17	3.31
9	2-CH_2CONH_2-cyclohexen-1-yl	6.13	6.15	−0.02	2.63
10	2-CH_2CN-cyclohexen-1-yl	5.48	5.40	0.08	3.35
11	2-$CH_2COOCMe_3$-cyclohexen-1-yl	5.77	5.80	−0.03	5.07

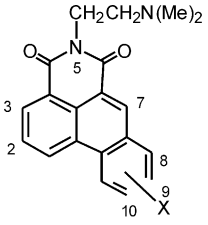
[a]Data points not included in deriving equation.

Table 13. I_{50} for inhibition of A549 non-small-cell drug resistant lung carcinoma cells by isoquinoline-4,6-diones II[23]

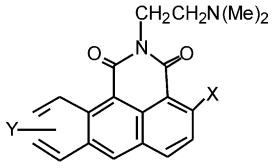
No.	Substituents	Log 1/C			
		Obsd	Calcd (eq 14)	Δ	ClogP
1	H	6.66	6.34	0.32	3.97
2	8-NO_2[a]	6.81	6.29	0.53	3.76
3	8-NH_2[a]	6.55	7.49	−0.94	2.87
4	11-NO_2	6.02	6.29	−0.27	3.76
5	11-NH_2	7.43	7.49	−0.06	2.87
6	8-Cl	7.66	7.49	0.16	4.70
7	8-OH	6.38	6.46	−0.08	3.44
8	8-OMe	6.06	6.33	−0.27	3.97
9	11-Cl	7.35	7.49	−0.15	4.70
10	11-OH	6.80	6.46	0.34	3.44

[a]Data points not included in deriving equation.

I_{50} of A549 non-small-cell drug resistant lung carcinoma cells by isoquinoline-4,6-dione analogues II (Table 13)[23]



II



III

$$\text{Log } 1/C = -10.89(\pm 5.65)\text{Clog}P + 1.44(\pm 0.73)\,\text{Clog}P^2 + 26.9(\pm 10.81) \quad (14)$$

$n = 8, \quad r^2 = 0.849, \quad s = 0.290, \quad q^2 = 0.615$

inversion point: 3.8(3.6−4.0)

outliers: X = 8-NO_2; 8-NH_2

Eq 14 based on **II** yield Clog*P* based QSAR. The subtle effects involved in allosteric interactions are brought to light by the fact that derivatives of **III** tested on the same system showed no inverse parabolic relationships (unpublished results).

Table 14. I_{50} data for inhibition of L1210 leukemia sensitive cells by II[23]

No.	Substituents	Log 1/C			
		Obsd	Calcd (eq 15)	Δ	ClogP
1	H[a]	6.71	6.03	0.68	3.97
2	8-NO_2[a]	7.30	6.10	1.21	3.76
3	8-NH_2	7.27	7.22	0.05	2.87
4	11-NO_2	6.12	6.10	0.03	3.76
5	11-NH_2	7.27	7.22	0.05	2.87
6	8-Cl	6.11	6.39	−0.27	4.70
7	8-OH	6.09	6.34	−0.25	3.44
8	8-OMe	6.28	6.03	0.26	3.97
9	11-Cl	6.59	6.39	0.21	4.70
10	11-OH	6.27	6.34	−0.07	3.44

[a]Data points not included in deriving equation.

Table 15. I_{50} data for inhibition of L1210 leukemia resistant cell by II[23]

No.	Substituents	Log 1/C			
		Obsd	Calcd (eq 16)	Δ	ClogP
1	H[a]	6.78	5.90	0.89	3.97
2	8-NO_2[a]	7.30	5.95	1.35	3.76
3	8-NH_2	7.27	7.25	0.02	2.87
4	11-NO_2	6.30	5.95	0.35	3.76
5	11-NH_2	7.30	7.25	0.05	2.87
6	8-Cl	6.29	6.47	−0.18	4.70
7	8-OH	5.87	6.22	−0.35	3.44
8	8-OMe	5.81	5.90	−0.09	3.97
9	11-Cl	6.64	6.47	0.16	4.70
10	11-OH	6.27	6.22	0.05	3.44

[a]Data points not included in deriving equation.

I_{50} of L1210 leukemia sensitive cells by II (Table 14)[23]

$$\text{Log } 1/C = -6.97(\pm 4.09)\text{Clog}P + 0.86 \times (\pm 0.54)\text{Clog}P^2 + 20.14(\pm 7.62) \quad (15)$$

$n = 8, \quad r^2 = 0.853, \quad s = 0.227, \quad q^2 = 0.586$

inversion point: 4.1(3.9−4.5)

outliers: H; 8-NO_2

I_{50} of L1210 leukemia resistant cell by II (Table 15)[23]

$$\text{Log } 1/C = -8.77(\pm 4.52)\text{Clog}P + 1.10(\pm 0.59)\,\text{Clog}P^2 + 23.36(\pm 8.45) \quad (16)$$

$n = 8, \quad r^2 = 0.860, \quad s = 0.251, \quad q^2 = 0.683$

inversion point: 4.0(3.8−4.3)

outliers: H; 8-NO_2

Table 16. I_{50} data for inhibition of monoamine oxidase B (MAO B) in rat brain mitochondria by X-$OCONHCH_2CH_2NH_2$[24]

No.	Substituents	Log $1/K_i$			
		Obsd	Calcd (eq 17)	Δ	ClogP
1	C_6H_5	3.86	3.92	−0.07	0.63
2	4-Me-C_6H_4	3.40	3.37	0.03	1.13
3	$CH_2C_6H_5$	3.30	3.41	−0.11	1.07
4	CH_2-(4-Cl-C_6H_4)	3.46	3.40	0.06	1.78
5	CH_2-(2-Cl-C_6H_4)	3.40	3.40	0.00	1.78
6	CH_2-(2,4-di-Cl-C_6H_3)[a]	3.80	4.41	−0.62	2.49
7	CH_2-(3,4-di-Cl-C_6H_3)	4.15	4.17	−0.02	2.37
8	CH_2-(4-NO_2-C_6H_4)	3.90	3.67	0.23	0.81
9	CH_2-(4-OMe-C_6H_4)	3.35	3.48	−0.13	0.99
10	Adamant-1-yl[a]	6.00	3.61	2.39	2.01

[a]Data points not included in deriving equation.

Table 17. K_i data for the inhibition of bovine milk xanthine oxidase by IV[25]

No.	Substituents	Log 1/C			
		Obsd	Calcd (eq 18)	Δ	ClogP
1	H[a]	6.77	6.64	0.13	0.40
2	F[a]	6.70	6.81	−0.11	0.55
3	Cl	5.72	5.61	0.11	1.12
4	Br	5.60	5.67	−0.07	1.27
5	I	5.42	5.83	−0.41	1.53
6	Me	5.62	5.60	0.02	0.90
7	C_2H_5	5.96	5.76	0.20	1.43
8	C_3H_7	6.28	6.22	0.07	1.96
9	$CHMe_2$	6.30	6.09	0.21	1.83
10	CMe_3	6.35	6.50	−0.15	2.23
11	OMe	5.82	5.86	−0.04	0.44
12	C_4H_9	6.82	6.78	0.04	2.49
13	OH	6.52	6.51	0.01	−0.02

[a]Data points not included in deriving equation.

It is fascinating that QSAR 14–16 show very similar results for three different types of cancer cells. This means that the receptor site must be the same in each case. Possibly DNA?

The other six QSAR derived for the data of Sami et al.[23] on different cell lines showed variations in B1 or MR based on substituents at the 8-position only, with no apparent effect by substituents in the eleven position.[10]

A study by Pozdnev et al.,[24] on mitochondria provided data with which we derived QSAR 17.

I_{50} of monoamine oxidase B (MAOB) in rat brain mitochondria by X–OCONHCH$_2$CH$_2$NH$_2$ (Table 16).[24]

$$\text{Log } 1/C = -2.91(\pm1.39)\text{Clog}P + 1.01(\pm0.46)\ \text{Clog}P^2 + 5.36(\pm0.92) \quad (17)$$

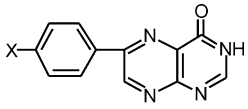
$n = 8, \quad r^2 = 0.871, \quad s = 0.135, \quad q^2 = 0.683$

inversion point: 1.4(1.3−1.5)

outliers: CH_2-(2, 4-di-Cl-C_6H_3); adamant-1yl

There are a few instances where we have found that using the bilinear model rather than the parabola yields better results.

K_i for the inhibition of bovine milk xanthine oxidase by IV (Table 17).[25]



IV

$$\text{Log } 1/K_i = -2.39(\pm1.07)\ \text{Clog}P + 3.55(\pm1.44)\log(\beta\cdot10^{\text{Clog}P} + 1) + 6.17(\pm0.36) \quad (18)$$

$n = 11, \quad r^2 = 0.849, \quad s = 0.206, \quad q^2 = 0.786, \quad \beta = -0.65$

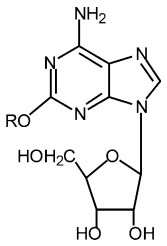
outliers: H; F inversion point: 0.90(±0.36)

Table 18. EC_{50} data for prolongation of the stimulus-QRS interval of guinea pig A_1 adenoreceptor by V[26]

No.	Substituents	Log 1/C			
		Obsd	Calcd (eq 19)	Δ	ClogP
1	Me	5.03	4.69	0.34	−1.74
2	C_2H_5	4.10	4.20	−0.10	−1.21
3	C_3H_7	3.83	3.99	−0.16	−0.68
4	C_4H_9	4.43	4.06	0.37	−0.15
5	C_5H_{11}	4.34	4.31	0.03	0.38
6	C_6H_{13}	4.86	4.63	0.23	0.91
7	$CHMe_2$	3.65	4.04	−0.39	−0.90
8	CH_2CHMe_2	3.97	4.02	−0.05	−0.28
9	$CH_2CH_2CHMe_2$	4.18	4.24	−0.06	0.25
10	$CH_2CH_2CH_2CHMe_2$	4.64	4.55	0.09	0.78
11	cy-C_6H_{11}	4.00	4.27	−0.27	0.30
12	CH_2-cy-C_6H_{11}	4.30	4.64	−0.34	0.91
13	CH_2CH_2-cy-C_6H_{11}	5.06	4.98	0.08	1.44
14	$(CH_2)_3$-cy-C_6H_{11}	5.43	5.33	0.10	1.97
15	$(CH_2)_4$-cy-C_6H_{11}	5.58	5.68	−0.10	2.50
16	$CH(Me)C_2H_5$[a]	3.05	3.48	−0.43	−0.37
17	cy-C_5H_9	4.10	4.03	0.07	−0.26
18	CH_2CH_2-cy-C_5H_9	4.85	4.62	0.23	0.88
19	$CH_2CH(C_2H_5)_2$	4.45	4.55	−0.10	0.78
20	$CH_2CH_2CH(C_2H_5)_2$	4.80	4.89	−0.09	1.31
21	$CH_2CH_2C{\equiv}CMe$	4.20	3.98	0.22	−0.63
22	CH_2CH_2OH	5.66	5.76	−0.10	−2.61

[a]Data point not included in deriving equation.

EC_{50} for prolongation of the stimulus-QRS interval of guinea pig A_1 adenosine receptor by V (Table 18).[26]



V

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$$\text{Log } 1/C = -1.34(\pm 0.32)\,\text{Clog}P + 2.01(\pm 0.42)\log\left(\beta \cdot 10^{\text{Clog}P} + 1\right) + 2.24(\pm 0.48) \quad (19)$$

$n = 21,\ r^2 = 0.871,\ s = 0.224,\ q^2 = 0.803,\ \beta = -0.88$

outliers: $CH(Me)C_2H_5$

inversion point: $-0.32(\pm 0.65)$

The bilinear model[12] (obtained by nonlinear regression analysis) means that activity first decreases linearly up to the inversion point and then increases linearly. Although this is a better way to define an inversion point a wide range in the data points is necessary.

Methodology

All of the standard Hammett parameters, calculated octanol/water ClogP values and the sterimol parameters have been discussed and applications illustrated.[12]

We have more apparent inverted parabolic relationships based on CMR[9] or Clog*P* than now reported. The reason is that one can calculate minimum or optimum inversion points in a parabola without actually having data points on the up and down side of the parabola. The ideal way to determine the minimum or maximum is to use nonlinear regression analysis that yields a bilinear result. However, this requires more data and a better spread in the datapoints.[12] What needs to be done when establishing a solid QSAR is to obtain a data set with a good range in the parameters of interest. In most instances, we have been able to plot the data to be sure that the inversion point is well established. Of course we have placed 95% confidence limits on each inversion point.

Discussion

Our method of uncovering allosteric interactions is unique as far as we can ascertain. The traditional approach is to carefully study a single molecule and by a rather complex analysis to establish a structural change with concentration changes. It must be kept in mind that QSAR is a very complex empirical science. A single new QSAR standing alone cannot be taken very seriously. Only as one obtains lateral support for it from as many directions as possible can one begin to place confidence in it.

We now have enough examples[7–10] to be sure that we have uncovered a general phenomenon that we have not been able to explain by any other logic than an allosteric effect. The term 'allosteric' (from Greek origin) means 'another shape'. Thus, the term allosteric as it is presently used is so general that the concept of induced fit can be covered by it. The concept of induced fit was first proposed by Koshland[27] and then analyzed by Jencks.[28] Jencks pointed out that the active site of an enzyme or receptor may not be in a position in which it can exert the most effective activity. When a good substrate is bound to it, the binding forces between them are utilized to force the active site into an energetically less favorable state, but a more active state catalytically. Actually, studies of induced fit are different from what we are considering. We find that at first a given class of congeners shows a decreasing potency as log*P* (or CMR) increases often over considerable range. Then suddenly activity changes and continues to increase as log*P* or CMR increase.

Another way of explaining our result is that there could be another binding site. As molecules become larger in log*P* or CMR and be limited in binding to the 'normal' site they are forced in this secondary site.

One of the most encouraging discoveries are QSAR 1 and 6 for studies with hemoglobin. In both of these examples the ligands would likely bind to hemoglobin covalently. The isocyanide group is quite active chemically and could react with –SH or NH_2 groups in hemoglobin. Most interesting is QSAR 6 where we have deduced that an allosteric interaction can be observed in vivo with rats. QSAR is the only means for identifying such a reaction. It should be noted that we have hundreds of QSAR with a $+\log P - \log P^2$ set of terms for a normal parabolic relation. We have no way of inferring whether or not allosteric reactions could be involved. Other reasons have been advanced for such QSAR.[12]

Another interesting finding[7] is that tyrosine kinase exhibits (four examples) an allosteric reaction according to QSAR based on CMR. CMR is the calculated molar refractivity that is related to size and polarizability of a molecule. The tyrosine kinase receptor is most interesting in that it can exist as a dimmer or a tetramer.

Thus we have intriguing evidence for a new means of uncovering allosteric reactions that can be of great importance in drug development. It is likely that congeners on the up side of our curve would have a more unique activity for a given receptor that could be more specific.

The importance of understanding allosteric interactions for drug discovery and the enormous complexity of the work in this area has been extensively reviewed by Christopoulos.[29]

We hope our results will encourage others to look for allosteric effects via QSAR.

Acknowledgements

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