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Searching for Allosteric Effects Via QSAR. Part II

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Abstract—Allosteric interactions have in the past been established by means of X-ray crystallography or careful study of a single molecule at a variety of concentrations. Here we report a method for using QSAR to establish a change in reaction mechanism by establishing an inversion point. That is, as polarizability of a member of a congeneric set of compounds is increased (as measured by CMR), activity at first decreases until, at the inversion, activity turns around and increases. Out of 23 examples, 14 have inversion points of 10 ± 1. This includes a wide variety of receptors such as thrombin, 5-HT, dopamine, and tyrosine kinase acting with a variety of ligands.
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Introduction

We have recently discovered a unique type of QSAR that we have used to prospect for allosteric interactions. Using *C*Log *P*, CMR, and molar volume (MgVol) we occasionally find equations in which biological activity decreases as one of these parameters increases in size then at a certain point (inversion point) turns around and begins to increase.

Obviously, a change in mechanism of *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. These changes in the structure of the receptor are said to be allosteric interactions. Interest in allosteric interactions has grown steadily since the pioneering work of Koshland[1] and Monod et al.[2] The early work in this field has been reviewed by Koshland.[3] The classic means for uncovering allosteric reactions is by carefully evaluating a single molecule at a time, eventually using X-ray crytallography.[27] It has recently been reviewed by Changuex and Edelstein.[5] Taking advantage of allosteric interactions has become important in drug discovery.[6]

Since our first publication on allosteric effects via QSAR,[7] we have found more examples.[8–10] In this report, we consider new examples mostly based on CMR (calculated molecular refractivity) and compare these with our earlier studies using this parameter.[7,8]

For the present report, CMR is the most important parameter to comprehend. It is based on the Lorentz-Lorenz equation: $MR = (n^2 - 1)/(n^2 + 2)\ (MW/d)$ where n is the index of refraction, MW represents molecular weight of the compound and d the density. Since the index of refraction has a small range for most organic compounds (1.35–1.60), its contribution will be less than the volume term (MW/d). Early on attempts were made to use it to rationalize chemical–biological interactions,[11a,b] but not in the terms we are now considering. We first assumed many years ago that MR or calculated CMR would be largely a measure of volume and a measure of a kind of steric effect.[12] That is, one would usually expect a negative coefficient with such a term. However, this is not what we have found. To gain a better understanding of CMR, we have built into our program[13a] a method for calculating molar volume according to Abraham and McGowan (MgVol).[13b,c]

The fascinating aspect of CMR is that although it does depend on polarizability as defined by the index of refraction, it also depends on molecular volume. Even so using molecular volume rarely can replace it.[2]

*C*Log *P* is calculated partition coefficient in octanol/water and is a measure of hydrophobicity. Cπ is the calculated hydrophobic parameter for substituents attached to benzene. *C*Log *P* and CMR are calculated for the neutral form of partially ionized compounds using C-QSAR program of BioByte Corp.[13a]

We believe that QSAR can serve a useful role in gaining an indirect view of what we might learn about receptor–ligand

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interaction. 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.

An interesting aspect of our findings is that despite rather different changes occurring in more than one position on the ligands, correlation is often with a single parameter (e.g., $-CMR + CMR^2$).

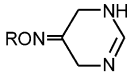
In QSAR equations, n is the number of data points, r is the correlation coefficient, s is the standard deviation, q is the measure of quality of fit and calculated as described by Cramer et al.,[14] and the data within the parentheses are for the 95% confidence intervals.

Results

The following QSAR based on the inverted parabolic relationship with CMR and MgVol (McGowan's volume[13b]) have been formulated using data taken from the literature mentioned with the respective equation. We have only included the examples with satisfactory statistics with respect to the inversion point. The QSAR have been arranged in order of the increasing value of inversion point.

QSAR derived using CMR

Agonistic activity of M_3 muscarinic receptor in guinea pig ileum by I (Table 1).[15]

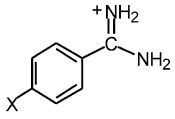


I

$$pD_2 = -20.63(\pm5.54)CMR + 2.17(\pm0.58)CMR^2 + 0.47(\pm0.37)I + 53.83(\pm3.05) \quad (1)$$

$n = 15$, $r^2 = 0.868$, $s = 0.265$, $q^2 = 0.668$ *inversion point:* 4.8 (4.7–4.9) *outliers:* Me; $CH_2C{\equiv}CCH_3$; (*E*)-$CH_2CH{=}C(Me)C{\equiv}CH$; (*E*)-$CH_2CH{=}CHC_6H_5$; $CH_2CH{=}CHC{\equiv}CH$ $I = 1$ for presence of unsaturation on β-carbon of the *R*-substituents.

Inhibition of human plasma serine protease by II (Table 2).[16]

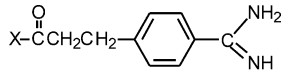


II

$$\text{Log } 1/K_i = -0.98(\pm0.46)CMR + 0.10(\pm0.04)CMR^2 + 2.78(\pm1.36) \quad (2)$$

$n = 21$, $r^2 = 0.905$, $s = 0.139$, $q^2 = 0.820$ *inversion point:* 4.9 (4.1–5.3) *outliers:* NH_2; $OC_{11}H_{23}$; $OC_{12}H_{25}$; Br; COOH

Inhibition of thrombin by III (Table 3).[17]



III

Table 1. Data for agonistic activity of M_3 muscarinic receptor in guinea pig ileum by **I**[15]

No.	Substituents (R)	Obsd	pD_2 calcd (eq 1)	Δ	CMR	I
1	Me[a]	5.47	8.94	−3.47	3.38	0
2	C_2H_5	6.73	6.63	0.10	3.84	0
3	C_3H_7	5.20	5.26	−0.06	4.30	0
4	C_4H_9	4.61	4.83	−0.22	4.77	0
5	$CHMe_2$	5.50	5.26	0.24	4.30	0
6	CH_2-cy-C_3H_5	4.66	4.86	−0.20	4.63	0
7	CH=CHMe	5.45	5.31	0.14	4.28	0
8	(*E*)-$CH_2CH{=}CHCH_3$	5.60	5.29	0.31	4.74	1
9	(*Z*)-$CH_2CH{=}CHCH_3$	5.51	5.29	0.22	4.74	1
10	$CH(Me)CH{=}CH_2$	5.24	5.29	−0.05	4.74	1
11	$CH_2C(Me){=}CH_2$	4.80	5.29	−0.49	4.74	1
12	$CH_2CH{=}CMe_2$	6.04	5.74	0.30	5.21	1
13	$CH_2C{\equiv}CH$	5.98	6.21	−0.23	4.10	1
14	$CH_2C{\equiv}CCH_3$[a]	5.89	5.32	0.57	4.64	1
15	$CH_2C{\equiv}CCH_2CH_3$	5.74	5.56	0.18	5.10	1
16	(*E*)-$CH_2CH{=}CHC{\equiv}CH$[a]	5.10	5.64	−0.54	5.15	1
17	(*E*)-$CH_2CH{=}C(Me)C{\equiv}CH$[a]	5.83	6.92	−1.09	5.62	1
18	(*Z*)-$CH_2CH{=}C(Me)C{\equiv}CH$	6.75	6.92	−0.17	5.62	1
19	$CH(Me)C{\equiv}CH$	5.31	5.37	−0.06	4.56	1
20	(*E*)-$CH_2CH{=}CHC_6H_5$[a]	4.64	14.99	−10.35	6.87	1

[a]Data points not included in deriving equation.

Table 2. K_i data for inhibition of human plasma serine protease by **II**[16]

No.	Substituents	Obsd	Log $1/K_i$ calcd (eq 2)	Δ	CMR
1	H	0.46	0.50	−0.04	3.80
2	Me	0.52	0.42	0.10	4.27
3	Cl	0.60	0.42	0.18	4.29
4	OMe	0.35	0.41	−0.06	4.42
5	NH_2[a]	0.89	0.44	0.45	4.17
6	COOH[a]	−0.18	0.40	−0.58	4.45
7	C_2H_5	0.38	0.39	−0.01	4.73
8	C_3H_7	0.40	0.40	0.01	5.19
9	C_4H_9	0.52	0.45	0.07	5.66
10	C_5H_{11}	0.46	0.54	−0.08	6.12
11	OC_2H_5	0.35	0.39	−0.04	4.88
12	OC_3H_7	0.30	0.41	−0.11	5.35
13	OC_4H_9	0.40	0.47	−0.07	5.81
14	OC_5H_{11}	0.60	0.58	0.02	6.27
15	OC_6H_{13}	0.70	0.73	−0.03	6.74
16	OC_7H_{15}	1.10	0.93	0.17	7.20
17	OC_8H_{17}	1.40	1.17	0.23	7.67
18	OC_9H_{19}	1.60	1.45	0.15	8.13
19	$OC_{10}H_{21}$	1.46	1.77	−0.31	8.59
20	$OC_{11}H_{23}$[a]	0.92	2.14	−1.22	9.06
21	$OC_{12}H_{25}$[a]	0.85	2.55	−1.70	9.52
22	$CO_2C_2H_5$	0.30	0.41	−0.11	5.38
23	$CO_2CH_2C_6H_5$	1.10	1.04	0.06	7.43
24	$OCH_2C_6H_5$	0.60	0.81	−0.21	6.93
25	$O(CH_2)_3C_6H_5$	1.35	1.28	0.07	7.86
26	Br[a]	0.82	0.40	0.43	4.58

[a]Data points not included in deriving equation.

Table 3. K_i data for inhibition of thrombin by **III**[17]

No.	Substituents	Obsd	Log $1/K_i$ calcd (eq 3)	Δ	CMR
1	Me	1.10	1.16	−0.06	5.60
2	C_6H_5[a]	1.82	0.99	0.83	7.65
3	4-Cl-C_6H_4	0.92	1.08	−0.16	8.14
4	4-Br-C_6H_4	1.40	1.15	0.25	8.43
5	4-Me-C_6H_4	1.08	1.07	0.01	8.12
6	4-OMe-C_6H_4	1.04	1.11	−0.07	8.27
7	4-C_6H_5-C_6H_4	1.72	1.96	−0.24	10.16
8	4-OC_6H_5-C_6H_4	2.26	2.06	0.20	10.32
9	Naphth-1-yl[a]	2.17	1.50	0.67	9.34
10	OMe	1.18	1.12	0.06	5.76
11	OC_2H_5	1.04	1.02	0.02	6.22

[a]Data points not included in deriving equation.

$$\text{Log } 1/K_i = -1.44(\pm 0.96)\,\text{CMR} + 0.10(\pm 0.06)\text{CMR}^2 + 6.02(\pm 3.69) \quad (3)$$

$n=9$, $r^2=0.869$, $s=0.180$, $q^2=0.627$ *inversion point:* 7.0 (5.6–7.5) *outliers:* C_6H_5; naphth-1-yl

Hydrolysis of $C_6H_5CONHCH_2COOCH(R)COO^-$ by carboxypeptidase A (Table 4).[18]

$$\text{Log } k_3 = -16.58(\pm 5.46)\,\text{CMR} + 1.15(\pm 0.40)\,\text{CMR}^2 + 61.66(\pm 18.33) \quad (4)$$

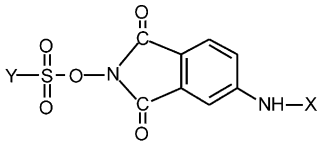
$n=7$, $r^2=0.982$, $s=0.134$, $q^2=0.873$ *inversion point:* 7.2 (7. 1–7.4) *outlier*: C_6H_{13}

Table 4. Hydrolysis of $C_6H_5CONHCH_2COOCH(R)COO^-$ by carboxypeptidase A[18]

No.	Substituents (R)	Obsd	Log $1/K_3$ calcd (eq 4)	Δ	CMR
1	H	4.40	4.32	0.08	5.79
2	Me	2.91	3.07	−0.16	6.25
3	C_2H_5	2.30	2.32	−0.02	6.72
4	C_3H_7	2.08	2.06	0.02	7.18
5	C_4H_9	2.32	2.30	0.02	7.65
6	C_6H_{13}[a]	2.85	4.28	−1.43	8.57
7	$CHMe_2$	2.23	2.06	0.17	7.18
8	CH_2CHMe_2	2.20	2.30	−0.10	7.65

[a]Data point not included in deriving equation.

Rate of inhibition of human leukocyte elastase by IV (Table 5).[19]

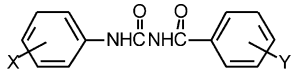


IV

$$\text{Log } k = -1.31(\pm 0.47)\,\text{CMR} + 0.07(\pm 0.03)\,\text{CMR}^2 + 10.19(\pm 2.03) \quad (5)$$

$n=9$, $r^2=0.889$, $s=0.148$, $q^2=0.645$ *inversion point:* 9.0 (8.6–9.5) *outliers:* X = $CO(CH_2)_3COOMe$, Y = Me; X = COCOOMe, Y = C_2H_5; X = $CO(CH_2)_3COOMe$, Y = C_2H_5

Inhibition of murine soluble epoxide hydrolase by benzoyl phenylureas V (Table 6).[20]



V

$$\text{Log } 1/C = -5.41(\pm 2.39)\,\text{CMR} + 0.28(\pm 0.12)\,\text{CMR}^2 + 0.19(\pm 0.09)\,C\log P + 29.22(\pm 11.35) \quad (6)$$

$n=12$, $r^2=0.840$, $s=0.092$, $q^2=0.622$ *inversion point:* 9.7 (9.5–9.8) *outliers:* X = 4-C_3H_7, Y = 2,6-di-F; X = 4-C_8H_{17}, Y = 2,6-di-F; X = 4-CN, Y = 2,6-di-F

Table 5. Data for inhibition of human leukocyte elastase by **IV**[19]

No.	Substituents		Obsd	Log *k* calcd (eq 5)	Δ	CMR
	X	Y				
1	H	Me	5.04	5.04	0.00	5.74
2	CO-[4-SO_2-(4-Me-C_6H_4)C_6H_4]	Me	5.20	5.17	0.04	12.60
3	$CO(CH_2)_2C_6H_5$	Me	3.99	4.28	−0.29	9.68
4	COCH=CH-C_6H_5(*trans*)	Me	4.30	4.31	−0.01	9.95
5	COC_6H_5	Me	4.40	4.25	0.14	8.75
6	CO-naphth-1-yl	Me	4.40	4.39	0.01	10.44
7	COCOOMe	Me	4.48	4.46	0.02	7.35
8	$CO(CH_2)_3COOMe$	Me[a]	4.76	4.26	0.50	8.74
9	H	C_2H_5	4.78	4.84	−0.06	6.20
10	COCOOMe	C_2H_5[a]	4.75	4.36	0.39	7.82
11	$CO(CH_2)_2COOMe$	C_2H_5	4.40	4.26	0.14	8.74
12	$CO(CH_2)_3COOMe$	C_2H_5[a]	5.34	4.25	1.09	9.21

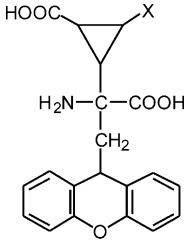
[a]Data points not included in deriving equation.

Table 6. I_{50} data for murine soluble epoxide hydrolase by benzoyl phenylureas **V**[20]

No.	Substituents		Obsd	Log 1/*C* calcd (eq 6)	Δ	*C*log *P*	CMR
	X	Y					
1	4-C_3H_7	2,6-di-F[a]	4.24	4.44	−0.20	4.69	8.36
2	4-C_4H_9	2,6-di-F	4.32	4.27	0.06	5.21	8.82
3	4-C_8H_{17}	2,6-di-F[a]	4.11	4.76	−0.65	7.33	10.68
4	4-OC_6H_5	2,6-di-F	4.06	4.07	−0.01	5.23	9.63
5	4-CN	2,6-di-F[a]	4.25	5.04	−0.79	3.09	7.45
6	4-C_6H_5	2,6-di-F	4.14	4.04	0.10	5.02	9.48
7	4-Cl	NMe_2	4.18	4.23	−0.05	4.77	8.72
8	4-C_3H_7	2,6-di-Cl	3.93	4.06	−0.13	4.99	9.31
9	4-C_4H_9	2,6-di-Cl	4.04	4.13	−0.09	5.51	9.77
10	4-C_6H_{13}	2,6-di-Cl	4.68	4.63	0.05	6.57	10.70
11	4-OC_2H_5	2,6-di-Cl	4.07	3.94	0.13	3.90	9.00
12	4-$SO_2N(C_2H_5)_2$	2,6-di-Cl	4.37	4.36	0.01	4.04	11.02
13	4-SO_2-pyrrolidin-1-yl	2,6-di-Cl	4.12	4.16	−0.04	3.62	10.84
14	2-Cl	2,6-di-Cl	4.15	4.22	−0.07	3.71	8.41
15	2-NO_2	2,6-di-Cl	4.17	4.13	0.04	3.64	8.53

[a]Data points not used in deriving equation.

Inhibition of cyclic-AMP formation in rat forebrain membranes by group 2 metabotropic glutamate (MGLUR3) receptor by VI (Table 7).[21]

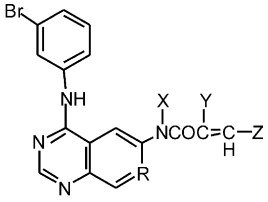


VI

$$\text{Log } 1/C = -15.94(\pm 9.56)\text{CMR} + 0.76(\pm 0.45)\ \text{CMR}^2 + 91.06(\pm 50.69) \quad (7)$$

$n = 7$, $r^2 = 0.854$, $s = 0.214$, $q^2 = 0.567$ *inversion point:* 10.6 (10.3–10.8) *outliers:* C_6H_{13}; $(CH_2)_2C_6H_5$

Inhibition of the autophosphorylation of epidermal growth factor receptor (EGFR) tyrosine kinase in A431 cells by VII (Table 8).[22]



VII

$$\text{Log } 1/C = -10.23(\pm 2.78)\text{CMR} + 0.44(\pm 0.12)\ \text{CMR}^2 + 0.93(\pm 0.46)\ C\pi_Z + 65.82(\pm 15.63) \quad (8)$$

$n = 20$, $r^2 = 0.836$, $s = 0.337$, $q^2 = 0.737$ *inversion point:* 11.7 (11.6–11.9) *outliers:* R = C, X = H, Y = H, Z = (=CH_2); R = N, X = H, Y = H, Z = C_6H_5; R = C, X = H, Y = H, Z = COOH; R = N, X = H, Y = H,

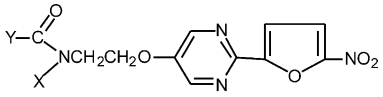
Table 7. I_{50} data for inhibition of cyclic-AMP formation in rat forebrain by MGLUR3 by **VI**[21]

No.	Substituents	Obsd	Log 1/*C* calcd (eq 7)	Δ	CMR
1	H	7.80	7.74	0.06	9.50
2	Me	7.00	7.16	−0.16	9.96
3	C_2H_5	7.12	6.90	0.22	10.42
4	C_3H_7	6.71	6.97	−0.26	10.89
5	C_4H_9	7.54	7.36	0.18	11.35
6	CH_2CHMe_2	7.40	7.36	0.04	11.35
7	C_5H_{11}	8.00	8.08	−0.08	11.81
8	C_6H_{13}[a]	6.91	9.12	−2.20	12.28
9	$(CH_2)_2C_6H_5$[a]	6.90	11.14	−4.24	12.93

[a]Data points not included in deriving equation.

$Z = CONH(CH_2)_3NMe_2$; R = N, X = H, Y = H,
$Z = CONH(CH_2)_3N(C_2H_5)_2$; R = N, X = H, Y = H,
$Z = CONH(CH_2)_3N$-imidazolyl

MIC of *Escherichia coli* growth by VIII (Table 9).[23]



VIII

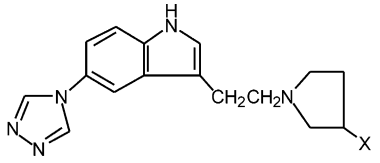
$$\text{Log } 1/C = -0.92(\pm 0.58)\text{CMR} + 0.71(\pm 0.73) \log(\beta \cdot 10^{\text{CMR}} + 1) + 12.0(\pm 4.55) \quad (9)$$

$n = 18$, $r^2 = 0.815$, $s = 0.185$, $q^2 = 0.713$, $\beta = -8.33$ *inversion point*: 8.0 (±5.35) *outliers:* X = $CHMe_2$, Y = Me; X = $CH(Me)C_2H_5$, Y = Me; X-CH_2CH_2O-Y; X-$CH_2CH_2OC(=O)$-Y

Bilinear QSAR are obtained by nonlinear regression and have been discussed by Kubinyi.[12]

QSAR derived using MgVol

EC_{50} for stimulation of [^{35}S]GTPγs binding to human 5-HT_{1D} receptor in CHO cells by IX (Table 10).[24]

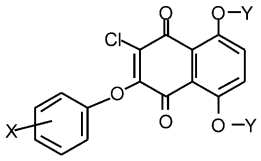


IX

$$\text{Log } 1/C = -39.42(\pm 27.10)\text{MgVol} + 7.44(\pm 5.10) \text{MgVol}^2 + 59.06(35.04) \quad (10)$$

$n = 7$, $r^2 = 0.805$, $s = 0.295$, $q^2 = 0.459$ *inversion point:* 2.7 (2.6–2.7) *outlier:* $CH_2N(Me)CH_2C_6H_5$ (*R*)

Relative activity causing 50% topoisomerase II mediated DNA cleavage of linear 8.4-kb YE_PTG DNA by X (Table 11).[25]



X

$$\text{Log } k_{\text{rel}} = -48.83(\pm 35.29)\text{MgVol} + 9.60(\pm 7.14) \text{MgVol}^2 + 63.52(\pm 43.23) \quad (11)$$

$n = 8$, $r^2 = 0.770$, $s = 0.479$, $q^2 = 0.591$ *inversion point:* 2.5 (2.5–2.8) *outliers:* X = 3-OH, Y = H; X = 3,5-di-OMe, Y = COMe

Using CMR in place of MgVol yields QSAR with $r^2 = 0.755$.

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 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 some assurance from a variety of sources about our method of ascertaining allosteric interactions. Most important of all is that we have found our approach to work with hemoglobin,[26] the molecule that was first used to define the allosteric interaction.[4,5] Also, one would expect it to occur with tyrosine kinase as we have found.[8] This enzyme exists as a dimer and tetramer. In our present database of 8700 biological QSAR we have found only a few such examples mostly based on CMR.

Further support of a common mechanism in all these examples comes from Table 12, where we have organized the results of all the QSAR based on CMR with respect to the size of the inversion point. The majority of the inversion points fall in the range of 10 ± 1. Included in this group are five examples based on 5-HT receptor data, four on tyrosine kinase and three on dopamine. Except for one example all of the QSAR for 5-HT receptor fall in the range 10 ± 1, even though the ligands are rather different. The same holds true for dopamine. Tyrosine kinase is of special interest since it can exist in the form of two or four units. To some degree, this mimics hemoglobin, the cornerstone of the

Table 8. I_{50} data for the inhibition of autophosphorylation of epidermal growth factor receptor (EGFR) tyrosine kinase in A431 cells by **VII**[22]

No.	R	Substituents			Obsd	Log 1/*C* calcd (eq 8)	Δ	CMR	$C\pi_Z$
		X	Y	Z					
1	N	H	H	H	8.47	8.59	−0.12	9.25	0.00
2	C	H	H	H	8.57	8.15	0.41	9.46	0.00
3	N	Me	H	H	7.89	7.69	0.20	9.71	0.00
4	N	H	Me	H	7.36	7.69	−0.33	9.71	0.00
5	C	H	Me	H	7.80	7.34	0.46	9.92	0.00
6	N	H	H	Me	8.11	8.39	−0.28	9.71	0.76
7	C	H	H	Me	8.06	8.04	0.02	9.92	0.76
8	N	H	H	*cis*-Cl	7.70	8.09	−0.39	9.74	0.48
9	C	H	H	CF_3	7.46	7.42	0.04	9.97	0.17
10	N	H	H	$CH{=}CH_2$	7.57	7.44	0.13	10.45	0.86
11	C	H	H	$=CH_2$[a]	6.92	7.80	−0.88	9.94	0.53
12	N	H	H	C_6H_5[a]	7.11	8.30	−1.18	11.99	2.48
13	C	H	H	COMe	5.98	6.33	−0.35	10.50	−0.28
14	C	H	H	COOH[a]	—	6.76	—	10.19	−0.20
15	C	H	H	$COOC_2H_5$	7.19	6.90	0.30	11.11	0.85
16	N	H	H	$COOC_2H_5$	7.29	7.03	0.27	10.90	0.85
17	N	$(CH_2)_2NMe_2$	H	H	5.64	5.98	−0.34	11.47	0.00
18	N	$(CH_2)_3$-N-morpholinyl	H	H	6.81	6.52	0.28	12.84	0.00
19	C	$(CH_2)_3$-N-morpholinyl	H	H	6.71	6.75	−0.04	13.05	0.00
20	C	H	H	$COO(CH_2)_3NMe_2$	6.97	7.32	−0.36	12.87	0.83
21	C	H	H	$CONH(CH_2)_3NMe_2$	7.23	6.88	0.35	13.09	0.08
22	N	H	H	$CONH(CH_2)_3NMe_2$[a]	7.24	6.64	0.61	12.88	0.08
23	N	H	H	$CONH(CH_2)_3N(C_2H_5)_2$[a]	7.68	8.96	−1.28	13.81	1.14
24	N	H	H	$CONH(CH_2)_3$N-morpholinyl	8.06	7.82	0.23	13.78	−0.03
25	N	H	H	$CONH(CH_2)_3$-N-imidazolyl[a]	7.85	6.74	1.11	13.31	−0.37
26	N	Me	H	$CONH(CH_2)_3NMe_2$	6.71	7.21	−0.49	13.34	0.08

[a]Data points not included in deriving equation.

early allosteric studies.[27] One might have anticipated allosteric QSAR for this enzyme. It is satisfying to see that the two slightly different sets of ligands for PDGFR tyrosine kinase have the same inversion points. Also, the two QSAR for molar volume, although based on quite different structures, have similar inversion points of 2.7 and 2.5. Thus we are gaining support for a new approach to search for allosteric interactions. Taken with all of the other examples, we believe that our approach to the elucidation of allosteric effects must be taken seriously.

Table 9. MIC data for inhibition of *E. coli* growth by **VIII**[23]

No.	Substituents		Obsd	Log 1/*C* calcd (eq 9)	Δ	CMR
	X	Y				
1	Me	Me	5.10	5.16	−0.07	7.48
2	C_2H_5	Me	4.87	4.80	0.07	7.94
3	C_3H_7	Me	4.57	4.51	0.06	8.41
4	C_4H_9	Me	4.42	4.30	0.11	8.87
5	C_3H_7	C_3H_7	4.35	4.16	0.19	9.34
6	$CHMe_2$	Me[a]	3.96	10.24	−6.28	8.41
7	$CH(Me)C_2H_5$	Me[a]	3.82	10.14	−6.33	8.87
8	C_5H_9	Me	4.05	4.21	−0.16	9.16
9	C_6H_{13}	Me	4.05	4.09	−0.04	9.62
10	CH_2CH_2OH	Me	5.02	4.70	0.32	8.10
11	$CH_2CH(Me)OCOMe$	Me	4.32	4.11	0.21	9.52
12	CH_2CH_2OMe	Me	4.19	4.43	−0.24	8.56
13	$CH_2CH_2OCOC_2H_5$	C_2H_5	3.96	4.00	−0.04	9.99
14	$CH_2CH_2OCOC_3H_7$	C_3H_7	3.60	3.80	−0.20	10.92
15	$CH(C_2H_5)CH_2OCOMe$	Me	4.05	4.00	0.05	9.99
16	$CH_2CH(CH_2OCOMe)OCOMe$	Me	4.05	3.86	0.19	10.64
17	CH_2CH_2OH	OMe	4.35	4.60	−0.25	8.25
18	$–CH_2CH_2O–$[a]		4.57	10.44	−5.87	7.46
19	CH_2CH_2OCOMe	Me	4.11	4.24	−0.13	9.06
20	$CH_2CH_2CH_2OCOMe$	Me	4.19	4.11	0.09	9.52
21	$CH(Me)CH_2OCOMe$	Me	3.96	4.11	−0.15	9.52
22	$–CH_2CH_2OC(=O)–$[a]		3.75	10.33	−6.59	7.96

[a]Data points not included in deriving equation.

Table 10. EC_{50} for stimulation of [^{35}S]GTPγs binding to human 5-HT_{1D} receptor in CHO cells by **IX**[24]

No.	Substituents	Obsd	Log 1/*C* calcd (eq 10)	Δ	Mg Vol
1	H	8.64	8.57	0.07	2.17
2	$OCH_2C_6H_5$ (*R*)	7.82	7.68	0.15	2.98
3	OH (*R*)	8.11	8.18	−0.07	2.23
4	$OCH_2C_6H_5$ (*S*)	7.51	7.68	−0.17	2.98
5	$CH_2OCH_2C_6H_5$ (*R*)	8.68	8.51	0.17	3.12
6	$CH_2N(Me)CH_2C_6H_5$ (*R*)[a]	8.82	10.03	−1.21	3.30
7	$CH_2NHCH_2C_6H_5$ (*R*)	9.10	8.81	0.29	3.16
8	$CH_2NHCH_2C_6H_5$ (*S*)	8.39	8.81	−0.43	3.16

[a]Data point not included in deriving equation.

Table 11. Data for 50% topoisomerase II mediated DNA cleavage by **X**[25]

No.	Substituents		Obsd	Log k_{rel} calcd (eq 11)	Δ	Mg Vol
	X	Y				
1	H	H	3.60	3.61	−0.01	2.07
2	3-OH	H[a]	2.00	3.10	−1.10	2.13
3	3,5-di-OH	H	2.30	2.66	−0.36	2.18
4	3-OMe	H	2.90	2.16	0.75	2.27
5	3,5-di-OMe	H	1.70	1.48	0.22	2.47
6	3-OH	Me	1.00	1.60	−0.60	2.41
7	3-OMe	Me	1.30	1.42	−0.12	2.55
8	3,5-di-OMe	Me	2.00	1.81	0.19	2.75
9	3-OMe	COMe	2.30	2.38	−0.08	2.86
10	3,5-di-OMe	COMe[a]	1.70	3.98	−2.28	3.06

[a]Data points not included in deriving equation.

Table 12. Summary of inversion points of QSAR based on CMR

S. no.	QSAR no.	Inversion point	System
1	1[a]	4.8	Guinea pig ileum muscarinic receptor
2	2[a]	4.9	Human plasma serine protease
3	1[b]	6.8	Cyclooxygenase from Bovine seminal vescicles
4	3[a]	7.0	Thrombin
5	4[a]	7.2	Carboxypeptidase A
6	2[b]	7.7	5-HT_{1D} receptor from pig caudate membrane
7	9[a]	8.0	*E. coli*
8	5[a]	9.0	Human leukocyte elastase
9	3[b]	9.1	A_1 adenosine receptor from rat brain cortical membrane
10	6[a]	9.7	Epoxide hydrolase
11	4[b]	9.7	5-HT transporter
12	5[b]	9.9	Dopamine D_2 receptor from rat striatal membrane
13	6[b]	9.9	Dopamine transporter
14	7[b]	10.0	Dopamine transporter
15	8[b]	10.0	5-HT_{1D} receptor expressed in CHO cells
16	21[c]	10.3	PDGFR tyrosine kinase
17	9[b]	10.4	5-HT_{1D} receptor expressed in CHO cells
18	10[b]	10.4	5-HT_{1B} receptor expressed in CHO cells
19	20[c]	10.4	PDGFR tyrosine kinase
20	7[a]	10.6	Group 2 metabotropic glutamate receptor
21	11[b]	10.8	Bovine tyrpsin
22	8[a]	11.7	EGFR tyrosine kinase
23	11[c]	12.0	EGFR tyrosine kinase

[a]QSAR from this report.
[b]QSAR from ref 7.
[c]QSAR from ref 8.

We were astonished to find the large number of QSAR with inversion points that fell into the range of 10% of 10. Could there be some advantage to receptors avoiding binding with unwanted ligands that evolved over the eons of time?

The term 'allosteric' comes from the Greek and means another shape. The inversion point clearly establishes a definite change in mechanism. We assume that the structure of the receptor is forced into a new shape at the inversion point that results in a new kind of substrate interaction. We have not been able to think of any other very convincing mechanism. A possibility would be for the protein to have more than one binding site; in that case, it would not have continued binding in the same site according to the same parameter that defines the first half of the QSAR.

One must keep in mind that in all of the studies from which we have formulated our QSAR, the authors had not conducted their research with anything in mind such as double binding sites or allosteric possibilities. We believe that our results will encourage others to construct QSAR extended to the limit on each side of the inversion point. Still, the only way to be absolutely sure of what is actually occurring is to do the necessary crystallography.

We have two means for checking the quality of our conclusions. First, we have not included QSAR for which a plot of the data did not show a good range of data points on both sides of the parabola. Second, we have selected examples where good 95% confidence could be placed on the point of inversion.

We hope that this review will encourage others to look for allosteric effects via QSAR. An understanding of this phenomenon could have importance in drug design that would not be easy to uncover without the inverted parabolic relationship in mind. While it would be relatively easy to observe in the isolated receptor it could be difficult to sort out in whole organisms as we have been able to do.[26] However, care must be taken in the design of sets of congeners to be sure that molecules have a wide enough range of CMR values to firmly establish the inversion point.

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