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Possible Allosteric Effects in Anticancer Compounds

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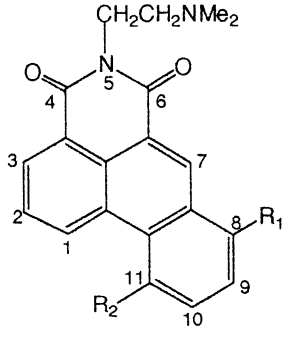
Abstract—We have found in this report, and an earlier one, that in a variety of instances an inverted parabolic relationship between biological activity and CMR or logP is observed. That is, activity first decreases as CMR or logP increases and then turns about and increases. This could be attributed to the ligands causing a change in the receptor structure. The present report considers QSAR for a variety of resistant and sensitive cancer cells.

Introduction

In continuing to develop our database of QSAR, that now contains over 16,250 equations of which 7850 are for chemical–biological interactions, we have very recently been surprised to uncover a few instances where we find an inverted parabolic relationship between parameters in which size of the substituent varies significantly.[1] In the first discovery we found 11 clear examples based on CMR and CMR[2] in which the coefficient with the first term was negative and the squared term was positive. That is, as size increased activity first decreased up to a minimum and then increased with further increase in size. CMR is the calculated molar refractivity: $MR = (\eta^2 - 1/\eta^2 + 2)\ (MW/d)$ where η is the refractive index, MW is the molecular weight and d is the density of the molecule. We could think of no way to rationalize this result except to propose that at a certain point increased bulk and polarizability (modeled by η) caused a change in structure that allowed larger sized molecules to produce increased activity possibly by opening up a new binding site in an allosteric mode.[3]

We have now discovered another intriguing example from data from Remers laboratory[2] on anticancer activity of the following class of anticancer agents 5-[2′-(dimethylamino)ethyl]-5,6-dihydro-4H-dibenz [de,g]isoquinoline-4,6-dione (**I**) acting on 10 murine and human tumor cells in culture.



(I)

Results

We have formulated the following QSAR (for the data see Tables 1a–c) that with the exception of one example (no. 10) have the parabolic relationship. Interestingly QSAR 1–7 have the inverted parabola.

I_{50} of A549 non-small cell lung drug-resistant cells

$$\log 1/C = -10.9(\pm 5.65)\ \mathrm{ClogP} + 1.44\ (\pm 0.73)\ \mathrm{ClogP}^2 + 26.9\ (\pm 10.8) \quad (1)$$

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

inversion point of ClogP = 3.78 (3.57 to 3.95);

outliers: 8-NO_2, 8-NH_2

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I_{50} of L1210 leukemia sensitive cells

$$\log 1/C = -6.97(\pm 4.10)\ \mathrm{ClogP} + 0.86 \times (\pm\ 0.54)\ \mathrm{ClogP}^2 + 20.1(\pm 7.62) \quad (2)$$

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

inversion point of ClogP = 4.05 (3.87 to 4.50)

outliers: H, 8-NO_2

I_{50} of L1210 leukemia resistant cells

$$\log 1/C = -8.77(\pm 4.52)\ \mathrm{ClogP} + 1.10 \times (\pm\ 0.59)\ \mathrm{ClogP}^2 + 23.4(\pm 8.4) \quad (3)$$

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

inversion point of ClogP = 3.98 (3.83 to 4.27)

outliers: H, 8-NO_2

I_{50} of MCF 7 human breast cells

$$\log 1/C = -12.71(\pm 4.83)\ B1_8 + 4.76(\pm 1.77)\ B1_8^2 + 14.6(\pm 3.16) \quad (4)$$

$n = 9, r^2 = 0.885, s = 0.148, q^2 = 0.790$

inversion point of $B1_8 = 1.33$ (1.27 to 1.37)

outlier: 11-OH

I_{50} of MCF 7 human breast cells resistant to doxorubicin (D40)

$$\log 1/C = -7.20(\pm 4.18)\ B1_8 + 2.92(\pm 1.52)\ B1_8^2 + 10.58(\pm 2.73) \quad (5)$$

$n = 9, r^2 = 0.890, s = 0.128, q^2 = 0.796$

inversion point of $B1_8 = 1.23$ (1.07 to 1.30)

Outlier: 11-Cl

I_{50} of WiDr human colon sensitive cells

$$\log 1/C = -11.6(\pm 6.10)\ B1_8 + 4.62(\pm 2.25)\ B1_8^2 + 13.6(\pm 3.97) \quad (6)$$

$n = 8, r^2 = 0.896, s = 0.177, q^2 = 0.730$

inversion point of $B1_8 = 1.25$ (1.13 to 1.32)

outliers: 11-NO_2, 8-NO_2

I_{50} of OVCAR3 human ovarian cells

$$\log 1/C = -4.53(\pm 2.18)MR_8 + 7.36(\pm 2.54)\ MR_8^2 + 6.66(\pm 0.29) \quad (7)$$

$n = 8, r^2 = 0.975, s = 0.113, q^2 = 0.929$

inversion point of $MR_8 = 0.308$ (0.24 to 0.34)

outliers: 11-NH_2, 8-Cl

I_{50} of MCF 7 human breast cells resistant to mitoxanthrone (MITOX)

$$\log 1/C = 13.96(\pm 5.93)\ MR_8 - 16.50(\pm 7.01)\ MR_8^2 + 5.33(\pm 0.62) \quad (8)$$

$n = 8, r^2 = 0.880, s = 0.204, q^2 = 0.723$

opt. $MR_8 = 0.423$ (0.403 to 0.445)

outliers: 8-NH_2, 8-OH

I_{50} of UA375 human melanoma cells

$$\log 1/C = 3.47(\pm 0.47)\ B5_{11} - 1.04(\pm 0.14)\ B5_{11}^2 + 3.84(\pm 0.34) \quad (9)$$

$n = 7, r^2 = 0.991, s = 0.026, q^2 = 0.644$

inversion point of $B5_{11} = 1.67$ (1.65 to 1.69)

outliers: 8-NO_2, 8-NH_2, 11-OH

I_{50} of WiDr resistant human colon cells

$$\log 1/C = 1.65(\pm 0.52)\ B1_8 + 3.96(\pm 0.72) \quad (10)$$

$n = 7, r^2 = 0.931, s = 0.151, q^2 = 0.903$

outliers: H, 11-NH_2, 11-Cl

Discussion

It is fascinating to see how QSAR brings out the differences in the receptor sites in the various cells. To be sure of the significance we must be sure of lack of collinearity

among the various parameters. This can be seen as follows where 8 represents the number of data points considered.

Correlation matrix:

	ClogP	CMR	MgVol	$B5_{11}$	$B1_8$	$B5_8$	MR_8
ClogP	—	0.114	0.05	−0.10	0.194	0.024	0.123
CMR	8	—	0.947	0.09	0.065	0.129	0.208
MgVol	8	8	—	0.065	0.056	0.240	0.281
$B5_{11}$	8	8	8	—	0.420	−0.408	−0.405
$B1_8$	8	8	8	8	—	0.397	0.638
$B5_8$	8	8	8	8	8	—	0.865
MR_8	8	8	8	8	8	8	—

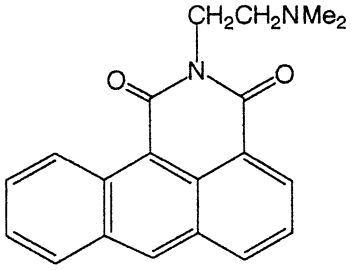
In general, except for CMR Vs MgVol and $B5_8$ Vs MR_8 the collinearity is quite low. Another way to view the consistency is via the optimum inversion points. The three QSAR with ClogP [(1),(2) and (3)] show very good agreement in the points of inversion, as do the three equations based on $B1_8$ [(4),(5) and (6)]. The two QSAR based on MR_8 [(7) and (8)] are also close. There is nothing to compare QSAR (9) and (10) with.

Our constant concern in formulating a new QSAR is to find support for it in as many ways as possible. In fact we believe that a single QSAR standing alone can not be taken seriously until one can in one way or another show it has some generality. The consistency in the present instance is reassuring even though the number of data points is small and we had to omit two points in a number of examples. Unfortunately with so few points we could not attempt to use additional parameters. However, the present results will be valuable in the further study of this class of antitumor agents and allosteric problems.

The sterimol parameters B1 and B5 were calculated by Verloop and his colleagues. We have discussed their use in QSAR.[4] B1 is largely a measure of size of the first atom of a substituent attached, in this case to an aromatic ring. B5 is an attempt to define the volume of the whole substituent. In our QSAR B5 is found to be significant for the substituents at the 11-position [QSAR (9)]. While B1 is found significant for 8-position substituents as evidenced by QSAR (4),(5),(6) and (10). Importance of substituents at 8-position in receptor binding is also pointed out by QSAR (7) and (8) where MR (molar refractivity) is found significant. The variation in the importance of the parameters points to different ways in which a part of the molecule appears to

Table 1a. Physicochemical parameters of compound **I** derivatives used in deriving QSAR 1-10[2,4]

S No.	Substituent	ClogP	$B1_8$	MR_8	$B5_{11}$
1	H	3.973	1.000	0.103	1.000
2	8-NO_2	3.756	1.700	0.736	1.000
3	8-NH_2	2.873	1.350	0.542	1.000
4	11-NO_2	3.756	1.000	0.103	2.440
5	11-NH_2	2.873	1.000	0.103	1.970
6	8-Cl	4.704	1.800	0.603	1.000
7	8-OH	3.441	1.350	0.285	1.000
8	8-OMe	3.967	1.350	0.787	1.000
9	11-Cl	4.704	1.000	0.103	1.800
10	11-OH	3.441	1.000	0.103	1.930



(II)

Table 1b. I_{50} data of compound **I** derivatives against human and murine tumor cells in culture[2]

S. No.	Substituent	Log1/C									
		A549 (res)		L1210 (sen)		L1210 (res)		MCF7 (sen)		MCF7 (res to D40)	
		Obsd.[a]	Calcd [eq (1)]	Obsd.[b]	Calcd [eq (2)]	Obsd.[c]	Calcd [eq (3)]	Obsd.[d]	Calcd [eq (4)]	Obsd.[e]	Calcd. [eq (5)]
1	H	6.658	6.335	6.706*	6.026	6.783*	5.898	6.658	6.696	6.301	6.305
2	8-NO_2	6.812*	6.288	7.301*	6.095	7.301*	5.954	6.824	6.823	6.770	6.785
3	8-NH_2	6.553*	7.489	7.268	7.215	7.268	7.251	6.260	6.177	6.229	6.187
4	11-NO_2	6.022	6.288	6.124	6.095	6.300	5.954	6.444	6.696	6.208	6.305
5	11-NH_2	7.432	7.489	7.268	7.215	7.301	7.251	6.886	6.696	6.538	6.305
6	8-Cl	7.658	7.493	6.114	6.387	6.290	6.473	7.222	7.222	7.097	7.087
7	8-OH	6.377	6.459	6.092	6.341	5.870	6.220	6.204	6.177	6.252	6.187
8	8-OMe	6.060	6.332	6.284	6.026	5.807	5.898	6.066	6.177	6.086	6.187
9	11-Cl	7.347	7.493	6.592	6.387	6.636	6.473	6.796	6.696	6.544*	6.305
10	11-OH	6.796	6.459	6.268	6.341	6.268	6.220	6.204*	6.696	6.171	6.305

*Data points not included in deriving the respective equations.[a]Non-small-cell, drug-resistant cell line.
[b]L1210 drug-sensitive MDR strain.
[c]L1210 drug-resistant MDR strain.
[d]Human mammary carcinoma sensitive cell line.
[e]Human mammary carcinoma cells resistant to doxorubicin (D40).

Table 1c. I_{50} data of compound **I** derivatives against human and murine tumor cells in culture[2]

S. No.	Substituent	Log1/C									
		WiDr (sen)		OVCAR 3		MCF 7 (res to mitox))		UA 375		WiDr (res)	
		Obsd.[f]	Calcd [eq (6)]	Obsd.[g]	Calcd [eq (7)]	Obsd.[h]	Calcd [eq (8)]	Obsd.[i]	Calcd [eq (9)]	Obsd.[j]	Calcd [eq (10)]
1	H	6.620	6.621	6.249	6.273	6.553	6.593	6.249	6.273	6.377*	5.606
2	8-NO_2	6.870*	7.241	7.301	7.314	6.745	6.667	6.757*	6.273	6.757	6.758
3	8-NH_2	6.553	6.364	6.267	6.368	6.260*	8.049	5.965*	6.273	6.280	6.182
4	11-NO_2	6.097*	6.621	6.203	6.273	6.357	6.593	6.124	6.119	5.620	5.606
5	11-NH_2	6.620	6.621	7.167*	6.273	6.824	6.593	6.613	6.643	6.337*	5.606
6	8-Cl	7.699	7.699	6.290*	6.606	7.721	7.748	6.290	6.273	6.959	6.923
7	8-OH	6.495	6.364	6.092	5.968	6.469*	7.968	6.268	6.273	6.301	6.182
8	8-OMe	6.046	6.364	7.699	7.655	6.046	6.098	6.284	6.273	5.886	6.182
9	11-Cl	6.658	6.621	6.415	6.273	6.824	6.593	6.747	6.720	6.420*	5.606
10	11-OH	6.585	6.621	6.171	6.273	6.409	6.593	6.393*	6.667	5.638	5.606

[f]Human colon carcinoma sensitive cell line.
[g]Human ovarian cancer cell line.
[h]Human mammary carcinoma cell line resistant to mitoxantrone (mitox).
[i]Human melanoma cell line.
[j]Human colon carcinoma MDR resistant cell line.

distort the binding cavity. This would suggest different allosteric actions.

The critical nature of the ligand fit to the site of action can be seen from an earlier study by Remers group[5] of variations of 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3H-dibenz [de,h]isoquinoline-1,3-dione (**II**) on various tumor cells.

For these no indication of an allosteric effect could be found.[6] This is another example of the special nature of the ligand-receptor interaction needed to produce the inverse parabolic relationship.

It is of interest to consider the nature of the outliers. In five instances the NO_2 group is poorly fit. We have presented evidence[7] that the aromatic NO_2 often exhibits its toxic effects in a variety of situations by ready conversion to a radical. Further unpublished results support this fact. Why it is not an outlier in every instance in not clear. Unfortunately we do not have enough data to explore for electronic effects. The amino function is an outlier in five instances, this like nitro group, has a strong electronic effect that we can not take into consideration.

Looking for ways to increase potency besides the electronic effects we can not see any leads from QSAR (1)–(3), other than increasing the size of the first atom of substituent 8. The following substituents would be of interest (I, SMe, CMe_3).

We hope that our findings about allosteric effects will encourage others to be on the look out for these unusual relations. We believe the best place to search is with the study of more or less purified receptors.

Acknowledgements

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