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QSAR of the Inhibition of Angiogenesis by TNP-470 and Ovalicin Analogues: Another Example of an Allosteric Interaction

Suresh Babu Mekapati and Corwin Hansch*

Department of Chemistry, Pomona College, Claremont, CA 91711, USA

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Abstract—QSAR have been formulated for variations of TNP-470 and Ovalicin on various cell lines. In the examples of mouse lymphocyte cells and bovine endothelial cells the results suggest an allosteric interaction. These results are compared with the binding of nitrobenzene to hemoglobin in rats in vivo. Such a reaction does not occur with methionine aminopeptidase.

Introduction

Angiogenesis is the formation of new blood vessels that are important in the development of cancer.[1,2] Preventing angiogenesis can stop cancer cell growth. Although this does not normally cure cancer it is attracting interest as a tool in the treatment of cancer. In this report, we examine structure–activity studies on the inhibition of angiogenesis from the laboratory of Liu and his associates[3] on a variety of cell lines.

There is evidence that the inhibition of angiogenesis is due to the inhibition of the enzyme methionine aminopeptidase-2 (MetAP-2).[4] Our results reveal an unusual negative hydrophobic interaction with the analogues of ovalicin and TNP-470. Of greatest interest are QSAR (1) and (1a) whose inverted parabolas we have previously associated with allosteric interactions.[5]

Results

The following QSAR were derived by us from the data in ref 3 and are unusual for such a varied set of complex ligands. Eqs (1)–(5) are based on the compounds shown in Figure 1.

I_{50} (50% inhibition of cell proliferation) against mixed mouse lymphocyte cell cultures (Table 1)

$$\log 1/C = -3.98(\pm 1.46)\mathrm{ClogP} + 0.95(\pm 0.39)\ \mathrm{ClogP}^2 + 0.92(\pm 0.72)\mathrm{I} + 10.47(\pm 1.50) \quad (1)$$

$$n = 11,\ r^2 = 0.941,\ s = 0.375,\ q^2 = 0.812$$

Outliers: **5** and **10** in Table 1. Inversion point 2.09 (1.92–2.35).

The indicator variable I = 1 for congeners having two epoxide units.

$$\log 1/C = -3.38(\pm 1.10)\mathrm{ClogP} + 5.05(\pm 1.84)\log(\beta.10^{\mathrm{ClogP}} + 1) + 0.87(\pm 0.67)\mathrm{I} + 10.40(\pm 1.30) \quad (1a)$$

$$n = 11,\ r^2 = 0.960,\ s = 0.335,\ q^2 = 0.894$$

Outliers **5** and **10**. Inversion point 1.93(±0.27), $\log\beta = -1.62$

Eq. (1a) agrees well with (1) and is a better correlation. However, the important point is that its slopes can be compared with other linear equations.

*Corresponding author. Tel.: +1-909-621-8410; fax: +1-909-607-7726; e-mail: atessier@pomona.edu

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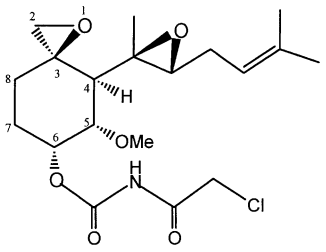
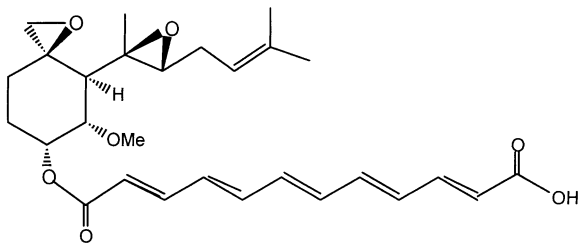


1 (TNP-470)



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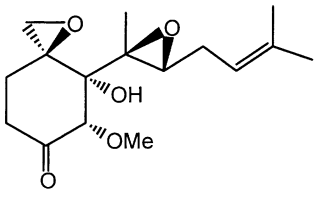
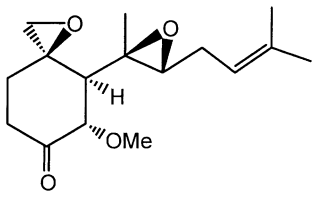


3 (Ovalicin)



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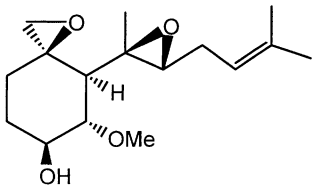

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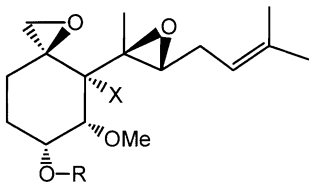
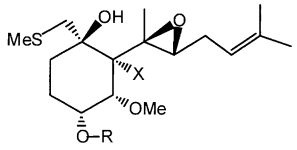


6 - 10

	X	R
6	OH	$CONHCOCH_2Cl$
7	OH	$CO(CH_2)_3Cl$
8	H	$CO(CH_2)_3Cl$
9	OH	H
10	H	H



11 - 14

	X	R
11	OH	H
12	H	H
13	OH	$CONHCOCH_2Cl$
14	H	$CONHCOCH_2Cl$

Figure 1. Analogues of TNP-470 and Ovalicin.

Table 1. I_{50} (50% inhibition of cell proliferation) against mixed mouse lymphocyte cell cultures[3]

Compd no.	log1/C					ClogP	I
	Obsd	Calcd [eq (1)]	Δ	Calcd [eq (1a)]	Δ		
1	8.79	8.88	−0.09	8.91	−0.12	0.77	1
2	8.64	8.81	−0.18	8.73	−0.09	3.38	1
3	9.66	9.13	0.53	9.18	0.48	0.68	1
4	9.89	10.10	−0.20	10.15	−0.26	0.35	1
5[a]	8.01	9.68	−1.67	12.04	−4.03	0.49	1
6	8.14	8.08	0.06	8.00	0.14	1.15	1
7	7.68	7.56	0.12	7.70	−0.03	2.67	1
8	7.36	7.27	0.09	7.31	0.05	2.30	1
9	7.22	7.55	−0.32	7.39	−0.17	1.51	1
10[a]	6.70	9.68	−2.98	12.04	−5.34	0.49	1
11	6.97	6.40	0.57	6.52	0.45	2.39	0
13	6.09	6.32	−0.22	6.29	−0.20	2.02	0
14	6.06	6.40	−0.34	6.31	−0.25	1.78	0

[a]Data points not included in deriving equation.

Table 2. I_{50} (50% inhibition of cell proliferation) against Human mixed lymphocyte cell cultures[3]

Compd no.	log1/C			MgVol
	Obsd	Calcd [eq (2)]	Δ	
1	8.85	8.18	0.67	2.91
2	9.66	9.94	−0.28	3.80
3	6.22	6.88	−0.66	2.25
4[a]	9.54	6.76	2.77	2.19
5[a]	9.39	6.85	2.54	2.30
6	8.28	8.30	−0.02	2.96
9	7.05	6.96	0.08	2.29
10	7.05	6.85	0.20	2.30

[a]Data points not included in deriving equation.

I_{50} (50% inhibition of cell proliferation) against human mixed lymphocyte cell cultures (Table 2)

$$\log 1/C = 1.97(\pm 1.00)\mathrm{MgVol} + 2.46(\pm 2.80)$$
$$n = 6,\ r^2 = 0.881,\ s = 0.50,\ q^2 = 0.655 \quad (2)$$

Outliers **4** and **5**. MgVol, McGowan molar volume. It is interesting that eq (2) is not similar to eq (1). ClogP and MgVol are not at all collinear ($r^2 = 0.749$, $q^2 = -0.222$); that is, eq (2) is clearly telling us that a different mechanism is involved. We have no idea what it might be.

I_{50} (50% inhibition of cell proliferation) against human umbilical vein endothelial cell line (Table 3)

$$\log 1/C = -2.07(\pm 0.41)\mathrm{ClogP} + 12.74(\pm 0.55)$$
$$n = 6,\ r^2 = 0.980,\ s = 0.243,\ q^2 = 0.926 \quad (3)$$

Outliers: **2** and **10**

Compound **2** contains a carboxyl group. The ClogP value is for the unionized form of the molecule. Its distribution coefficient at pH 7.4 would be considerably lower. In the case of QSAR (1) the good fit of this molecule is probably accidental.

Table 3. I_{50} (50% inhibition of cell proliferation) against Human umbilical vein endothelial cell line[3]

Compd no.	log1/C			ClogP
	Obsd	Calcd [eq (3)]	Δ	
1	11.07	11.14	−0.07	0.77
2[a]	10.21	5.74	4.48	3.38
3	11.60	11.34	0.26	0.68
4	11.70	12.01	−0.32	0.35
6	10.53	10.37	0.16	1.15
9	9.73	9.61	0.12	1.51
10[a]	8.97	11.74	−2.77	0.49
11	7.63	7.79	−0.16	2.39

[a]Data points not included in deriving equation.

I_{50} (50% inhibition of cell proliferation) against bovine aortic endothelial cells (Table 4)

$$\log 1/C = -3.44(\pm 2.55)\mathrm{ClogP} + 0.94(\pm 0.80)\mathrm{ClogP}^2 + 2.03(\pm 0.89)\mathrm{I} + 10.22(\pm 2.09)$$
$$n = 10,\ r^2 = 0.939,\ s = 0.448,\ q^2 = 0.810 \quad (4)$$

Outliers: **10** and **12**. Inversion point 1.83 (1.56–3.54)

$$\log 1/C = -3.01(\pm 2.24)\mathrm{ClogP} + 4.33(\pm 3.77)\log(\beta \times 10^{\mathrm{ClogP}} + 1) + 2.06(\pm 0.94)\ \mathrm{I} + 10.02 \times (\pm 2.03)$$
$$n = 10,\ r^2 = 0.948,\ s = 0.456,\ q^2 = 0.833 \quad (4a)$$

Outliers: **10** and **12**. Inversion point $1.65(\pm 0.89)$, $\log\beta = -1.38$

As in the case of eqs (1) and (1a), we find that congener (2) is reasonably well fit inspite of the problem of calculating logP.

I_{50} (50% inhibitory activity) against methionine aminopeptidase-2 (Table 5)

$$\log 1/C = 0.66(\pm 0.55)\mathrm{ClogP} + 3.49(\pm 0.84)\mathrm{I} + 4.63(\pm 1.20)$$
$$n = 11,\ r^2 = 0.925,\ s = 0.491,\ q^2 = 0.875 \quad (5)$$

Outlier: **8**.

Table 4. I_{50} (50% inhibition of cell proliferation) against Bovine aortic endothelial cells[3]

Compd no.	log1/C					ClogP	I
	Obsd	Calcd [eq (4)]	Δ	Calcd [eq (4a)]	Δ		
1	10.43	10.16	0.28	10.17	0.27	0.77	1
3	10.75	10.36	0.39	10.38	0.36	0.68	1
4	10.89	11.15	−0.27	11.18	−0.29	0.35	1
6	9.34	9.55	−0.21	9.50	−0.16	1.15	1
7	9.51	9.77	−0.26	9.75	−0.24	2.67	1
8	9.92	9.31	0.61	9.37	0.55	2.30	1
9	8.66	9.20	−0.54	9.15	−0.49	1.51	1
10[a]	8.02	10.81	−2.79	12.71	−4.69	0.49	1
11	7.19	7.36	−0.18	7.39	−0.21	2.39	0
12[a]	5.55	7.18	−1.63	11.99	−6.44	1.49	0
13	7.40	7.11	0.29	7.12	0.28	2.02	0
14	6.96	7.07	−0.12	7.04	−0.08	1.78	0

[a]Data points not included in deriving equation.

Table 5. I_{50} (50% inhibitory activity) against Methionine aminopeptidase-2[3]

Compd no.	log1/C			ClogP	I
	Obsd	Calcd [Eq. (5)]	Δ		
1	9.00	8.62	0.38	0.77	1
3	9.40	8.56	0.84	0.68	1
4	8.22	8.34	−0.12	0.35	1
6	8.70	8.87	−0.17	1.15	1
7	10.00	9.88	0.12	2.67	1
8[a]	8.46	9.64	−1.18	2.30	1
9	8.40	9.11	−0.71	1.51	1
10	8.10	8.43	−0.33	0.49	1
11	6.40	6.22	0.18	2.39	0
12	5.30	5.62	−0.32	1.49	0
13	6.40	5.97	0.43	2.02	0
14	5.52	5.81	−0.29	1.78	0

[a]Data point not included in deriving equation.

Discussion

Although the correlations are not as satisfactory as one would like, we were surprised that the results are as good as they are. These complex structures are not the kind of congeners that one feels comfortable studying via our type of QSAR analysis.[6] However, there is interesting uniformity. Eqs (1) and (4) are extremely interesting to us because of their inverted parabolic nature. Activity first decreases with increasing values of ClogP and then turns around and increases. The same holds true using the bilinear models. The inversion points are similar. We have come to associate this type of effect with an allosteric change in structure. The fact that the structure of the enzyme has been established may be of help in elucidating the reaction mechanism.[4]

In these three examples, the initial negative slope is much larger than we normally find.[7] It is rare to find coefficients out side of ± 1.20. This suggests something other than a simple hydrophobic interaction. In a first report,[5] we developed 11 QSAR with inverted parabolic structures for a variety of reactions of different ligands with various biological systems based on the parameter Calculated Molar Refractivity (CMR). Molar refractivity is a measure the polarizability of a ligand and how this affects interaction with a receptor.[7] Perusing our database of 7700 bio QSAR we have found an additional 13 examples based on CMR and six examples based on ClogP besides the two examples in this report. We plan to publish these shortly.

The term allostery is of Greek origin and means another shape. Since the first use of this concept by Monod et al. in 1965,[8] there has been a constantly increasing interest in the subject. Reviews by Changeux and Edelstein[9] and Koshland et al.[10] and others[11] discuss the current state of the subject. The Koshland and Monod models focus on changes in protein receptors containing sub units. It seems possible to us that a ligand could induce a change in a receptor that might open another mode of binding. Lodish et al.[11] define allosteric transition as "change in the tertiary and/or quarternary structure of a protein induced by binding of a small molecule to a specific regulatory site, causing a change in the proteins activity. Allosteric regulation is particularly prevalent in multi subunit enzymes".

In our present database, we have 480 QSAR based on logP–logP^2 terms where activity first rises to a peak and then begins to fall. In addition, we have 170 such bilinear equations. In the case of CMR we have 78 such 'normal' QSAR. Thus inverted parabolic relationships are much more common with CMR. The inverted parabolic relationship occurs much more often with more or less purified receptors than with enzymes. These inverted relationship are more or less continuous processes so that it is not easy to visualize just what is occurring.

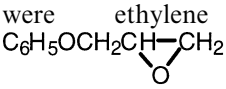
However, we believe that such QSAR are uncovering a gradual change in the structure of a protein or a set of proteins. In any case, it is a phenomena that is worthy of serious study.

Eq (2) is unique in that all members of this set contain two epoxide units. It is not clear why compounds **4** and **5** are not fitted into the equation. We have no idea why the correlation equation is so simple.

In the case of eq (3) compounds **2** and **10** are outliers; the former contains a COOH moiety. The ClogP program calculates logP for the unionized form of the acid. There are not enough data points to obtain a clear picture of possibilities beyond ClogP.

Eq (5) involves the isolated enzyme. The indicator variable for the presence of two epoxide functions is of overwhelming importance. Note the standand deviation on the logP term. This contrasts greatly with QSAR 1 and 4 and suggests that the epoxide terms are the only important factor in the reaction with the purified enzyme. This suggests a different kind of structure for the enzyme in vivo.

There is evidence that the epoxide containing fumagillin (TNP-470) binds covalently to inhibit MetAp-2[12] and

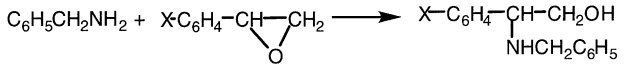
that this is related to inhibition of cell growth. Consequently we have examined data for information on the reactivity of epoxides. We have no satisfactory data for diepoxides, but there are interesting results for mono epoxides. In an extensive survey Gold et al.[13] listed over 400 compounds causing cancer in rats or mice. Included were ethylene oxide, methylene oxide,  and the insecticide dieldrin that contains an epoxide ring.

What kind of chemical reaction is involved? Barbin and Bartsch[14] listed a set of carcinogenic compounds causing cancer in rodents. From their data, we have formulated (ref 6, p 365) the following QSAR.

$$\log TD_{50} = 5.12(\pm 1.40)S - 0.64(\pm 1.08)$$
$$n = 15,\ r^2 = 0.819,\ s = 0.560 \quad (6)$$

TD_{50} (mg/kg/day) $= d.t^2/\ln[(1-n_c/N_c)/(1-n_e/N_e)]$, where d is the daily dose in mg, t is the time of the experiment, n_c is the incidence of tumors among controls, N_c is the total number of controls, n_e is the number of tumors among experimental animals. The Swain Scott parameter, S, is defined as $n.s = \log k/k_o$. The nucleophilicity of a reagent is represented by n and s is a measure of the sensitivity of the substrate to the nucleophilicity of the reagent.[15] The above equation covers four examples of epoxides and implies that carcinogenicity depends on the reactivity of epoxides and nucleophilicity of the attacking chemical moiety. In our case, n is essentially constant.

Turning now to our physical database, we find 5 QSAR for the reaction of styrene epoxides with nucleophiles of which the following is representative.[16]



$$\log k = -1.19(\pm 0.54)\sigma^+ - 4.67(\pm 0.14)$$
$$n = 7,\ r^2 = 0.938,\ s = 0.093,\ q^2 = 0.891 \quad (7)$$

Outlier: 3-OMe

Eq (7) suggests that a nucleophilic function in the enzyme reacts with the epoxide to block enzymatic action that is we assume that the epoxide moieties are crucial in inhibiting angiogenesis. This could be the result of reaction with nucleophilic entity in the enzyme methionine aminopeptidase-2. We do not mean to imply that it would be like benzylamine. As we state, fumagillin appears to bind covalently to inhibit the enzyme. There is considerable work showing that epoxides react covalently with nucleophiles.

The above results suggest that TNP-470 and Ovalicin analogues are likely to be carcinogenic and that they react with nucleophiles. This offers an explanation as to why two epoxide rings result in more potent inhibitors of the essential enzyme. There may be another factor. When the epoxide ring is on the cylohexane ring it might be less sterically hindered than when it is part of the side chain and hence more reactive. We do not have the data to rationalize this.

Turning now to a much different system, binding of alkylisonitriles (RN=C) to Hemoglobin. From the work of Reisberg and Olson[17] we have derived QSAR 8.

Rate constants for the binding of RN = C to the alpha subunit of human hemoglobin (Table 6).[17]

$$\log k = -0.88(\pm 0.34)\mathrm{ClogP} + 0.39(+0.16)\mathrm{ClogP}^2 - 1.61(\pm 0.35)B1_R + 4.57(\pm 0.61) \quad (8)$$
$$n = 12,\ r^2 = 0.971,\ s = 0.142,\ q^2 = 0.902$$

Outlier: $R = CH_2CH(CH_3)_2$

Inversion point: 1.32 (0.97–1.40)

In the above expression, the sterimol parameter brings out the negative effect of four of the more complex substituents. The confidence interval is reasonable. The beta subunit shows no such effect.

The result is most important because Monod et al.[8] used hemoglobin to first establish the allosteric effect. Since then, others have studied hemoglobin confirming the nature of allosteric reactions.[9] However, the most interesting finding is that QSAR can detect allosteric interactions in animals as shown in the following example.

Binding of X-$C_6H_4NO_2$ to hemoglobin in wistar rats (Table 7).[18]

$$\log HBI = 3.62(\pm 1.40)\sigma^+ - 11.10(\pm 0.56)\mathrm{ClogP} + 1.97(\pm 1.00)\mathrm{ClogP}^2 + 1.51(\pm 1.00)B1_4 + 14.20(\pm 7.90) \quad 9$$
$$n = 14,\ r^2 = 0.874,\ s = 0.507,\ q^2 = 0.743$$

Outlier: 2,4-di-F; Inversion point : 2.82(2.61–3.10)

In the above expression, HBI = hemoglobin binding index, that is (mmol compound/mol HB)/(mmol compound/kg body weight).

Although the above equation is not as sharp as one would like and the ratio of data points to variables is not as good as one would want, the inversion point is rather well defined. The $B1_4$ sterimol parameter indicates the presence of a positive steric effect by 4-substituents.

Table 6. Rate constants for the binding of RN=C to the alpha subunit of human hemoglobin[17]

S. no.	R	logk			ClogP	$B1_R$
		Obsd	Calcd	Δ		
1	CH_3	2.59	2.58	0.01	−0.44	1.52
2	C_2H_5	2.15	2.05	0.10	0.09	1.52
3	C_3H_7	1.60	1.73	−0.13	0.62	1.52
4	C_4H_9	1.77	1.63	0.14	1.15	1.52
5	C_5H_{11}	1.92	1.74	0.18	1.68	1.52
6	C_6H_{13}	2.08	2.07	0.01	2.21	1.52
7	$CHMe_2$	1.08	1.23	−0.15	0.40	1.90
8[a]	CH_2CHMe_2	0.87	1.63	−0.77	1.02	1.52
9	$(+)CHMeC_2H_5$	1.00	1.03	−0.03	0.93	1.90
10	$(-)CHMeC_2H_5$	1.00	1.03	−0.03	0.93	1.90
11	CMe_3	0.08	−0.07	0.15	0.80	2.60
12	C_6H_{11}	0.89	1.08	−0.20	1.59	1.91
13	$CH_2C_6H_5$	2.04	2.08	−0.04	2.21	1.52

[a]Data point not included in deriving equation.

Table 7. Binding of X-$C_6H_4NO_2$ to hemoglobin in wistar rats.[18]

S. no.	X	logHBI			σ^+	ClogP	$B1_4$
		Obsd	Calcd	Δ			
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	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-Cll	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[a]	2,4-di-F	0.36	1.84	−1.48	−0.14	1.87	1.35

[a]Data point not included in deriving equation.

The electronic term σ^+ suggests that the nitro group is reduced to a radical that then binds to hemoglobin. We normally find that σ^- is a better descriptor for this process, however, in this example it yields a slightly poor result ($r^2 = 0.854$). The two parameters σ^- and σ^+ are highly collinear in the present instance ($r^2 = 0.964$). A better choice of substituents would clarify the situation. Sabbioni calculated LUMO values, using these in QSAR 9 yield significantly lower result ($r^2 = 0.723$).

At this point, we can say that QSAR can be used to uncover allosteric interactions in hemoglobin, enzymes, cells or animals. We believe that this information will be helpful to those involved in drug development.

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References and Notes

1. Folkman, J. *N. Engl. J. Med.* **1971**, *285*, 1182.
2. Hanahan, D.; Folkman, J. *Cell* **1996**, *86*, 353.
3. Turk, B. E.; Su, Z.; Liu, J. O. *Bioorg. Med. Chem.* **1998**, *6*, 1163.
4. Liu, S.; Widom, J.; Kemp, C. W.; Crews, C. M.; Clardy, J. *Science* **1998**, *282*, 1324.
5. Hansch, C.; Garg, R.; Kurup, A. *Bioorg. Med. Chem.* **2001**, *9*, 283.
6. Hansch, C.; Hoekman, D.; Gao, H. *Chem. Rev.* **1996**, *96*, 1045.
7. Hansch, C.; Leo, A. *Exploring QSAR*; American Chemical Society: Washington, DC, 1995.
8. Monod, J.; Wyman, J.; Changeux, J. P. *J. Mol. Biol.* **1965**, *12*, 88.
9. Changeux, J. P.; Edelstein, S. I. *Neuron* **1998**, *21*, 959.
10. Koshland, D. E.; Nemethy, G.; Filmer, D. *Biochemistry* **1966**, *5*, 365.
11. Lodish, H., Berk, A., Zibursky,S. L., Matsudaria, P., Baltimore, D., Darnell, J. *Molecular Cell Biology*, 4th ed.; W.H. Freeman: New York, 2000.
12. Sin, N.; Meng, L.; Wang, M. Q. W.; Wen, J. J.; Bornman, W. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6099.
13. Gold, L. S.; Slone, T. H.; Bernstein, L. *Environ. Health Persp.* **1989**, *79*, 259.
14. Barbin, A.; Bartsch, H. *Mutat. Res.* **1989**, *215*, 95.
15. Swain, C. G.; Scott, C. B. *J. Am. Chem. Soc.* **1953**, *75*, 141.
16. Laird, A. M.; Parker, R. E. *J. Am. Chem. Soc.* **1961**, *83*, 4277.
17. Reisberg, P. I.; Olson, J. S. *J. Biol. Chem.* **1980**, *255*, 4151.
18. Sabbioni, G. *Chem. Res. Toxicol* **1994**, *7*, 267.