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QSAR of Apoptosis Induction in Various Cancer Cells

Corwin Hansch,[a,*] Ali Jazirehi,[b] Suresh Babu Mekapati,[a] Rajni Garg[a] and Benjamin Bonavida[b,*]

[a]*Pomona College, Department of Chemistry, Claremont, CA 91711, USA*
[b]*UCLA, Department of Microbiology, Immunology and Molecular Genetics, Los Angeles, CA 90095, USA*

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Abstract—In continuing our QSAR study of apoptosis, we consider in this report the action of phenolic compounds on Ramos cells (non-Hodgkins B-cell lymphoma): the effect of O-8-thapsigargin analogues on human prostate cancer cells, Tsu-Pr-1 and the induction of apoptosis of a complex set of congeners on human fibrosarcoma cells HT 1080. The human prostate cancer cells activity is very similar to that of the Ramos cells. While the QSAR for the fibrosarcoma cells resembles that of our earlier study with L1210 leukemia cells. The two different types of QSAR suggest at least two quite different types of receptors for the induction of apoptosis.

Introduction

Programmed or physiological cell death, also known as apoptosis, is a unique type of cell death characterized by cytoskeletal disruption, cellular shrinkage, membrane blebbing, nuclear condensation, and internucleosomal DNA fragmentation. Being a genetically controlled process, apoptosis is susceptible to mutations, and dysregulation of the apoptotic machinery is frequently observed in numerous types of cancers.

Because of the close correlation between tumorigenesis and dysregulation of apoptosis, any therapeutic strategy aimed at specifically triggering apoptosis in tumor cells might have potential therapeutic applications. It is well established that exposure of cells to chemical toxicants, chemotherapeutic drugs or radiation perturbs cellular homeostasis including the induction of nucleotide excision and DNA double strand breaks. These events disturb normal cell cycle checkpoints and trigger the apoptotic machinery.

The emergence of resistance to conventional therapeutic strategies has encouraged the design and/or exploitation of novel chemicals with anti-cancer properties. The objective of the present study was to obtain quantitative structure–activity relationships (QSAR) using an array of phenol compounds to analyze their ability to induce apoptosis in L1210 murine lymphoma cell line.

*Corresponding author. Tel.: +1-909-621-8445; fax: +1-909-607-7726; e-mail: atessier@pomona.edu

In our studies to help elucidate the various mechanisms of action of phenols on living systems, or their parts,[1–3] it was decided to test a set of phenols on L1210 leukemia cells. From this study, QSAR 1 was formulated.[2]

$$\log 1/C = -0.19\ (\pm 0.02)\ \mathrm{BDE} + 0.21\ (\pm 0.03)\ \log P + 3.11(\pm 0.10) \quad (1)$$

$$n = 52, \quad r^2 = 0.920, \quad s = 0.202, \quad q^2 = 0.909$$

BDE is the calculated homolytic bond dissociation energy for the OH bond. Using the Hammett parameter σ^+ gave a somewhat less satisfactory result: $r^2 = 0.895$, $n = 51$. This was due, at least in part, to some unusual phenols such as estradiol, estriol, diethylstilbestrol, etc. for which σ^+ values are not known and had to be estimated.

Actually this study was inspired by a publication from the EPA on the deformation of rat embryos in vitro by phenols.[3] We found the data to be correlated by σ^+. We decided that the rapidly growing embryo cells were producing large amounts of ROS (reactive oxygen species) and that these were converting the phenols to radicals that caused the initial damage. It was well known from studies in physical organic chemistry that radical formation from substituted phenols is correlated by σ^+. Hence we decided to study the action of phenols on fast growing cancer cells.

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With this background, it was decided to study some of the phenols on which QSAR 1 was based for their ability to cause apoptosis.[4]

$$\log 1/C = -3.61\ (\pm 1.0)\ \text{Clog P} + 2.77\ (\pm 0.65)\ \text{CMR} - 3.76\ (\pm 1.3) \quad (2)$$

$n = 11, \quad r^2 = 0.939, \quad s = 0.630, \quad q^2 = 0.892$

outlier : Bisphenol A

C is the molar concentration causing 25% apoptosis in 24 h. In QSAR 1, C is the concentration causing 50% decrease in cell growth in 48 h. We were astonished that no electronic term could be found for QSAR 2. Obviously we have *much* to learn about how chemicals affect living systems. It is interesting that a period of 24 h seems to be enough to separate the two processes.

One interesting fact about QSAR 2 is that the most potent inducer of apoptosis was the normal female hormone estradiol. One wonders if one of its functions in women might be that of inhibiting the growth of abnormal cells such as cancer. After menopause the production of estradiol decreases and the incidence of cancer increases. Women are often given a supplement of female hormones to offset the decline.

Methodology

The experimental technique for QSAR 3 has been previously discussed.[4] The regression analysis including autoloading of all parameters was accomplished with the C-QSAR program.[5]

Results

As far as we can ascertain, QSAR 2 is the first published example for apoptosis, despite the fact that there is enormous interest in the subject, one of which is the search for anticancer drugs. Eq 2 supports this possibility. After the study on L1210 leukemia cells it was decided to study other types of cells. The first effort was made with 2F cells with the same phenolic compounds, however, no satisfactory QSAR could be obtained. We now report results obtained using Ramos cells (Table 1) from which QSAR 3 was developed.

$$\log 1/C = 0.67\ (\pm 0.21)\ \text{Clog P} + 0.37\ (\pm 0.63)$$

$n = 8, \quad r^2 = 0.910, \quad s = 0.201, \quad q^2 = 0.863$

outliers : 4-$OCOCH_3$-Phenol; 4-SMe Phenol; diethylstilbesterol (3)

There is a startlingly different result from QSAR 2 and indicates that interaction with a hydrophobic receptor is involved. However, there is no explanation for three of the four outliers. It is not surprising that diethylstilbesterol is an outlier, what was surprising is that it is well fit by QSAR 1 and 2. Of the well fit compounds, estradiol is also potent as in the case of QSAR 2.

In searching the literature for other data suitable for QSAR construction, that of Jakobsen et al.[6] attracted our attention. They reported the concentrations of 0-8-Thapsigargin analogues causing 50% loss of clonogenic survival of human prostate cancer TSU-PR-1 cells, that is, the ability to induce apoptosis, from which we formulated QSAR 4 (Table 2).

Table 1. Data for QSAR 3

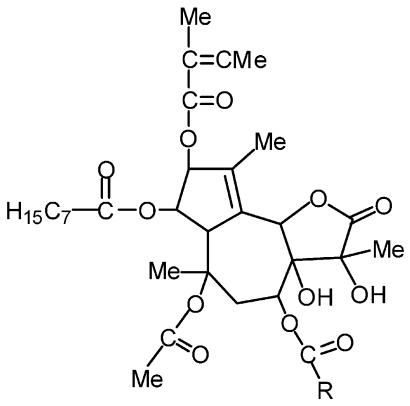
	Compd	Log 1/C	Pred log 1/C	Dev	Clog P
1	Estradiol	2.79	2.88	−0.09	3.78
2	4-MeO-phenol	1.27	1.41	−0.14	1.57
3	4-C_6H_5O-phenol	2.67	2.74	−0.07	3.57
4	4-CH_3COO-phenol[a]	3.01	1.33	1.68	1.46
5	Bisphenol A	2.84	2.81	0.03	3.67
6	4-$(Me)_3C$-phenol	2.65	2.56	0.09	3.30
7	4-CN-phenol	1.44	1.43	0.01	1.60
8	Diethylstilbestrol[a]	2.89	3.66	−0.77	4.96
9	4-I-phenol	2.08	2.29	−0.21	2.90
10	Phenol[a]	3.10	1.35	1.75	1.48
11	4-MeS-phenol[a]	2.60	1.72	0.88	2.03
12	4-C_3H_7O-phenol	2.51	2.12	0.39	2.63

[a]Data points not used in deriving QSAR 3.

Table 2. Data for QSAR 4

	Substituent	Log 1/C	Pred log 1/C	Dev	Clog P′
1	C_3H_7[a]	7.52	5.26	2.27	2.66
2	$(CH_2)_2C_6H_4$-4-$NHCOCH(NH_2)CH_2CHMe_2$	6.06	5.95	0.10	3.57
3	$(CH_2)_6NH_2$	4.96	5.03	−0.07	2.36
4	$(CH_2)_7NH_2$	5.42	5.43	−0.17	2.89
5	$(CH_2)_{10}NH_2$	6.13	6.64	−0.52	4.47
6	$(CH_2)_{11}NH_2$[a]	5.94	7.04	−1.11	5.00
7	$(CH_2)_{11}NHCOCH(NH_2)CHMe_2$	7.52	7.27	0.25	5.30
8	$(CH_2)_{11}NHCOCH(NH_2)Me$	6.55	6.56	−0.01	4.37
9	$(CH_2)_{11}NHCOCH(NH_2)CH_2OH$	6.05	5.79	0.26	3.36
10	$(CH_2)_{11}NHCOCH(NH_2)CH_2C_6H_5$[a]	6.68	7.65	−0.97	5.79

[a]Not used in the derivation of QSAR 4.



$$\log 1/C = 0.76\ (\pm 0.30)\ \text{Clog P}' + 3.23\ (\pm 1.15)$$

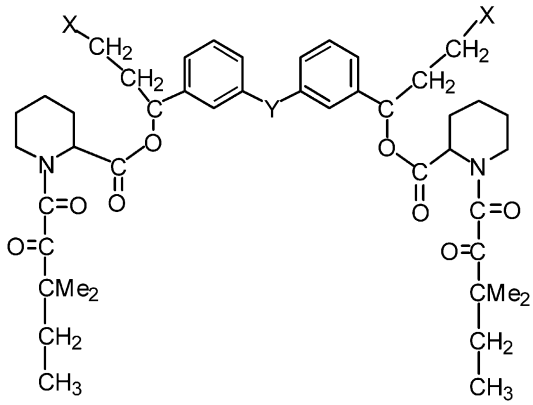
$$n = 7, \quad r^2 = 0.897, \quad s = 0.287, \quad q^2 = 0.786$$

$$\textit{outliers}: \text{R} = \text{C}_3\text{H}_7; \qquad (4)$$

$$(CH_2)_{11}NH_2;\ NHCO(CH_2)_{11}COCHCH_2C_6H_5$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad NH_2$$

The Clog P values are very large, ranging from 5.33 to 8.8 while the statistics are good, three data points could not be included. All of the R except C_3H_7 contained an amino function that would be protonated under experimental conditions. For this reason the C_3H_7 cannot be expected to fit. P′ indicates that we have corrected the calculated log P by subtracting 3.5 from the calculated value. The figure 3.5 is the difference between Clog P and log P of RNH_2 measured at pH 7.4. This correction results in a more reasonable value for the intercept. It is the log P terms in equations 3 and 4 that are essentially identical, indicating similar receptors in the two different types of cancer cells. It is generally assumed that it is the perturbation of DNA that induces apoptosis.[7–9]

Next from the data of Keenan et al.[10] we have developed QSAR 5 for induction of apoptosis of human fibrosarcoma cells HT1080 (Table 3).



$$\log 1/C = -0.45\ (\pm 0.17)\ \text{Clog P} + 0.35 \times (\pm 0.13\ )\ \text{CMR} + 0.56\ (\pm 3.9) \qquad (5)$$

$$n = 12, \quad r^2 = 0.886, \quad s = 0.240, \quad q^2 = 0.717$$

outliers: see Table 3

C is the molar concentration of chemical inducing 50% apoptosis.

It is of interest that QSAR 5 is based on the same parameters with the same signs of the coefficients as QSAR 2 suggesting the same type of reaction center despite the grossly different chemical structures that are involved as well as the different type of cells. However, the size of the coefficients is much smaller.

From data from Christensen et al.[11] on the induction of apoptosis in human prostate cancer cells TSU-PR-1, QSAR 6 was formulated (Table 4).

Table 3. Data for QSAR 5

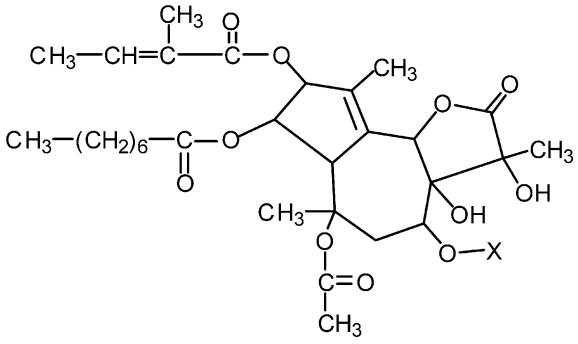
	Compd						
	X	Y	Log 1/C	Pred log 1/C	Dev	Clog P	CMR
1[a]	3,4-di-OCH_3-C_6H_3	$CH_2CONH(CH_2)_2NHCOCH_2$	8.22	7.41	0.81	9.95	32.33
2	4-OCH_3-C_6H_4	$CH_2CONH(CH_2)_2NHCOCH_2$	6.40	6.74	−0.34	10.47	31.10
3	3-C_5H_4N	$CH_2CONH(CH_2)_2NHCOCH_2$	7.52	7.44	0.09	7.64	29.44
4[a]	N-C_4H_8NO	$CH_2CONH(CH_2)_2NHCOCH_2$	6.00	7.78	−1.78	6.71	29.24
5	3,4-di-OCH_3-C_6H_3	$CH_2CONH(CH_2)_2NHCOCH_2$	7.22	7.41	−0.19	9.95	32.33
6	3,4-di-OCH_3-C_6H_3	$CH_2CONH(CH_2)_3NHCOCH_2$	7.70	7.48	0.22	10.15	32.80
7	3,4-di-OCH_3-C_6H_3	$CH_2CONH(CH_2)_2O(CH_2)_2NHCOCH_2$	8.00	7.76	0.24	10.01	33.41
8	3,4-di-OCH_3-C_6H_3	$CH_2CONH(CH_2)_2O(CH_2)_2O(CH_2)_2NHCO$	7.82	8.11	−0.28	10.07	34.49
9	3,4-di-OCH_3-C_6H_3	$(CH_2)_2NHCOCONH(CH_2)_2$	7.10	7.10	0.00	10.64	32.33
10[a]	3,4-di-OCH_3-C_6H_3	$(CH_2)_2NHCONH(CH_2)_2$	8.22	6.66	1.56	11.22	31.83
11	3,4-di-OCH_3-C_6H_3	$CH_2CH(OH)CH_2$	6.15	6.44	−0.29	10.51	30.29
12	3,4-di-OCH_3-C_6H_3	$(CH_2)_2O(CH_2)_2$	6.46	6.20	0.25	11.40	30.75
13	3,4-di-OCH_3-C_6H_3	$(CH_2)_2O(CH_2)_2O(CH_2)_2$	6.70	6.55	0.15	11.46	31.83
14	3,4-di-OCH_3-C_6H_3	$(CH_2)_2O(CH_2)_2O(CH_2)_2O(CH_2)_2$	6.86	6.90	−0.05	11.52	32.91
15	3,4-di-OCH_3-C_6H_3	$CH_2CONH(CH_2)_2NHCOCH_2$	7.60	7.41	0.19	9.95	32.33

[a]Not used in the derivation of QSAR 5.

Table 4. for QSAR 6

	Substituent	log 1/C	Pred log 1/C	Dev	MgVol
1	COC_3H_7	7.52	7.51	0.01	4.98
2	H[a]	5.00	8.26	−3.26	4.40
3	$CO(CH_2)_5CONH$-(C_6H_3-3-NH_2-4-Me)	5.51	5.70	−0.19	6.36
4	$CO(CH_2)_3CONH$-(C_6H_3-3-NH_2-4-Me)[a]	4.78	6.07	−1.29	6.08
5	CO-C_6H_4-4-NH_2[a]	5.40	7.14	−1.74	5.26
6	$CO(CH_2)_2$-C_6H_4-4-NH_2	6.55	6.77	−0.22	5.54
7	$COCH=CH$-C_6H_4-4-NH_2	6.96	6.83	0.13	5.50
8	$CO(CH_2)_3$-C_6H_4-4-NH_2	6.64	6.59	0.05	5.68
9	$CO(CH_2)_2$-C_6H_4-4-$NHCO_2C(Me)_3$	6.06	5.76	0.30	6.32
10	$COCH=CH$-C_6H_4-4-$NHCO_2C(Me)_3$	5.72	5.81	−0.09	6.28

[a]Not used to derive QSAR 6.



$$\log 1/C = -1.30\ (\pm 0.41)\ \mathrm{MgVol} + 14.0\ (\pm 2.36)$$

$$n = 7, \quad r^2 = 0.932, \quad s = 0.203, \quad q^2 = 0.870$$

$$\textit{outliers}: \quad X = H;\ CO(CH_2)_3CONH\text{-}C_6H_3\text{-}3\text{-}NH_2,\ 4\text{-}Me;\quad COC_6H_4\text{-}4\text{-}NH_2 \qquad (6)$$

Again we find a very complex set of 'congeners' correlated by a simple QSAR with no sign of an electronic or hydrophobic interaction. MgVol is a parameter for the molecular volume of the molecule. The larger the substituent, the less effective it is in inducing apoptosis. Using CMR in place of MgVol yields a similar QSAR, with $r^2 = 0.914$.

Discussion

It is well established that chemotherapeutic drugs eradicate tumor cells via the induction of apoptosis, a genetic process of programmed cell death.[13] The effector cells of the immune system also utilize various apoptotic pathways (e.g., Fas ligand, TRAIL, and TNF-α) in killing their target cells.[13] Altered expression or mutation of genes encoding key apoptotic proteins can provide cancer cells with an intrinsic survival advantage and inherent resistance to apoptotic machinery. As a corollary to this, tumor cells become resistant to apoptosis induced by chemotherapeutic drugs as well as the immune system, which will result in their growth and expansion.[14] This has spurred the intriguing idea of exploitation of alternative chemical compounds capable of the induction of apoptosis in otherwise resistant tumor cells. In the present study we have evaluated the apoptotic attributes of an array of phenol compounds on murine lymphoma cell line L1210. In addition, using quantitative structure function relationship (QSAR) equations, we have established a close correlation between the chemical structure of the compounds and their ability to induce apoptosis.

The most amazing aspect of QSAR 2 to 6 is that such heterogeneous 'congeners' can be correlated at all by simple QSAR. This was unexpected in the findings of QSAR 1 and 2, but it was even more surprising in QSAR 4 and 5. A perusal of the substituents in Tables 2, 3 and 4 finds that these are not at all simple. There is no way to employ Hammett parameters or steric parameters such as the sterimol group B1, B5 and L. Thus we are left with the general descriptors log P, CMR and MgVol.

The parameter CMR that is the calculated molecular refractivity is complex. It is based on the Lorentz–Lorenz equation:

$$\mathrm{MR} = n^2 - 1/n^2 + 2\ (\mathrm{MW/d})$$

In this expression, n represents index of refraction, MW stands for molecular weight and d is density. Thus, n is a measure of the interaction of the electrons with light (polarizability) and MW/d is molar volume. Thus two properties are involved in CMR. Despite this complexity, we have found CMR[5] to be essential in the formulation of 1200 biological QSAR. We have recently discussed its properties in some detail.[15]

Over the years it is our experience in developing 9300 biological QSAR that one cannot take a newly formed equation very seriously. Only as one develops lateral validation by means of related biological QSAR or by establishing relationships with equations from physical organic chemistry can confidence be placed in a new QSAR. We have found such support in our current study. QSAR 3 and 4 both point to a hydrophobic receptor site, possibly DNA. Equations 2 and 5 contain the same terms with the same signs and thus point to a different hydrophilic site. Previously we noted[4] that a QSAR with −log P and +CMR terms was associated with mutagenic activity. No doubt DNA was involved.

Obviously none of the data sets considered so far are ideal for QSAR studies. That is structural changes are

so gross that there is little reason to assume that all members of a set are binding in the same mode to the same site. However, we now see that apoptosis can be treated via relatively simple equations. We hope that our results will encourage others to start the huge amount of work that will be necessary to support new ideas in anticancer drug design. However, in doing so one needs a relatively simple parent structure on which relatively simple substituents with good variation in hydrophobic, electronic and steric parameters are present when the first QSAR is established one can then make structural changes with some confidence.

Regulation of apoptosis is accomplished at multiple levels, including the initiation, transduction, amplification and execution stages and mutations that disrupt each of these stages have been detected in tumor cells.[12] Alternatively, altered expression of crucial apoptotic regulatory gene products confers different levels of resistance to apoptosis-inducing stimuli in different tumor cell lines. Because these alterations necessarily produce a selective advantage to emerging tumor cells, the identification of altered/ mutated components highlights critical regulatory points in survival and proliferation processes that merits further investigation.

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