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Quantitative Structure–Activity Relationships of Phenolic Compounds Causing Apoptosis

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Abstract—A study of a variety of phenolic compounds (simple phenols, estradiol, bisphenol A, diethylstilbesterol) on their action on L1210 leukemia cells led to the formulation of the following QSAR for apoptosis:

$$\log 1/C = -3.16\ C\log P + 2.77\ \mathrm{CMR} - 3.76$$
$$n = 11,\ r^2 = 0.939,\ s = 0.630,\ q^2 = 0.892$$

C is the molar concentration causing 25% apoptosis, $C\log P$ is the calculated octanol/water partition coefficient and CMR is the calculated molecular refractivity. Our results imply the significance of characterization of the phenolic compounds with apoptotic activity and the development of new agents for cancer therapy.

Introduction

Apoptosis or programmed cell death (PCD) is an innate mechanism by which unwanted, defective, or damaged cells are rapidly and selectively eliminated from the body. It occurs during tissue remodeling, embryonic development, and immune regulation.[1–5] Apoptosis is the principle mechanism employed by the immune system and chemotherapeutic drugs in eradicating tumor cells. Resistant tumor cells evade the action of anticancer agents by increasing their apoptotic threshold. This has spurred the development of novel chemical compounds capable of inducing apoptosis in chemo/immune-resistant tumor cells.

Apoptosis (programmed cell death, or 'use it or lose it') has received a huge amount of attention in recent years.[1] Programmed cell death occurs in all multicellular organisms including embryos and insects as well as the adult organisms. If a cell is not involved in its usual function, apoptosis can be induced by other cells which results in self destruction of the cell. What has become of unusual interest is the finding that many chemicals can induce the process. A Google internet search makes 240,000 hits for apoptosis. Like so many other areas of science, we are being swamped with information. Our interest in the subject stems from the curiosity of understanding chemical–biological reactions of all possible types.[6,7] Our hope is that large amounts of information can be compressed into mathematical equations that can be compared with each other. These equations are now referred to as Quantitative Structure–Activity Relationships (QSAR).

One of the most interesting chemical functions is the aromatic OH. There are so many beneficial chemicals such as the flavanoids[8] that contain this group as well as many seriously toxic compounds.[9,10] A study of phenols that provided the interest for this report is that embodied in eq 1.

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Toxicity of phenols to L1210 leukemia cells[9]

$$\log 1/C = -0.19(\pm 0.02)\mathrm{BDE} + 0.21(\pm 0.03)\log P + 3.11(\pm 0.10)$$
$$n = 52,\ r^2 = 0.920,\ s = 0.202,\ q^2 = 0.909 \quad (1)$$

C is the molar concentration of chemical necessary to cause 50% reduction in cell growth in 48 h, P is the experimental octanol/water partition coefficient and BDE is the quantum chemically calculated homolytic bond dissociation energy for the following reaction:

$$\mathrm{X{-}C_6H_4OH} + \mathrm{C_6H_5O\bullet} \rightarrow \mathrm{X{-}C_6H_4O\bullet} + \mathrm{C_6H_5OH}$$

Using the Hammett parameter in place of BDE gives a slightly poorer correlation: $n = 51$, $r^2 = 0.895$. This is due, at least in part, to the lack of experimental σ^+ values for the more complex phenols: bisphenol A, diethylstilbestrol, estradiol, estriol, equilin, equilenin. For these compounds, σ^+ values were estimated. The way BDE was calculated is strong evidence for a radical reaction that causes the toxic effect. That is, we assume the phenols are first oxidized by metabolically formed reactive oxygen species (ROS) to radicals that then attack DNA. This provides an explanation for the carcinogenicity of phenols with electron releasing substituents and explains the carcinogenicity of the female hormones in the drug Premarin.[11] Substituents having electron attracting groups do not fit this expression, but are simply correlated by log P alone. This explains the fact that phenol is not carcinogenic but 4-methoxyphenol is. With the above background in mind, it was decided to investigate the programmed cell death of some of the phenols used to develop QSAR 1.

Methods

Cell line. The murine lymphoma cell line L1210 was purchased from the American Type Culture Collection (ATCC, Bethesda, MD) and was maintained in sterile 75 cm^2 tissue-culture flasks in RPMI-1640 medium (Life Technologies, Bethesda, MD), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1% (v/v) penicillin (100 U/mL), 1% (v/v) streptomycin (100 U/mL), 1% (v/v) L-glutamine, 1% Na-pyruvate, 1% non-essential amino acids (Life Technologies, Bethesda, MD). Cell culture was incubated in a controlled atmosphere incubator at 37 °C with saturated humidity and an atmosphere of 95% air and 5% CO_2. The cells were sub-cultured every 2 days.

Determination of apoptosis by flow cytometric analysis. The percentage of apoptotic cells was determined by evaluating propidium iodide (PI) stained preparations of L1210 tumor cells treated with various concentrations of different phenol derivatives for 24 h.[12] Briefly, 2×10^6 cells were washed twice with 1 mL ice cold 1×PBS/0.1% BSA. The supernatant was aspirated and the cells were fixed and permeabilized with 1 mL ice cold 75% ethanol and cells were incubated at −20 °C for 1 h. Thereafter, the cells were washed with 1 mL of ice cold 1×PBS/0.1% BSA twice. After the last wash, 100 μL of PI solution (50 μg/mL PI + 0.05 mg/mL RNAse) was added and the cells were incubated at room temperature for at least 1 h prior to analysis (light protected). Then, the volume was brought up to 1 mL by adding 0.9 mL of PBS. Apoptosis was determined using an Epic XL flow cytometer (Coulter Electronic, Inc., Miami, FL). A minimum of 6000 events was collected on each sample and acquired in listmode by a PC Pentium computer. The percentage of apoptotic cells is represented as the percentage of hypodiploid cells accumulated at the sub-G0 phase of the cell cycle.

The molar concentration of phenol compounds that results in 25% apoptosis was determined by analyzing the logarithmic equation derived from Cricket graphs.

Results

From the data in Table 1 on leukemia cells, we have derived QSAR 2:

$$\log 1/C = -3.16(\pm 1.0)\ C\log P + 2.77(\pm 0.65)\mathrm{CMR} - 3.76(\pm 1.3)$$
$$n = 11,\ r^2 = 0.939,\ s = 0.630,\ q^2 = 0.892,\ \text{outlier: bisphenol A} \quad (2)$$

C is the molar concentration causing 25% apoptosis. $C\log P$ is the calculated log P value. MR is a measure of molecular volume and polarizability of a compound defined as: $MR = (n^2-1)/(n^2+2)\ (MW/d)$ where n = refractive index, MW = molecular weight and d = density. This term depends on volume and polarizability (n). CMR is the calculated MR value. MR was first proposed for correlating chemical–biological interactions by Pauling and Pressman.[13] Our values were calculated using the Clog P program from BioByte Corporation.

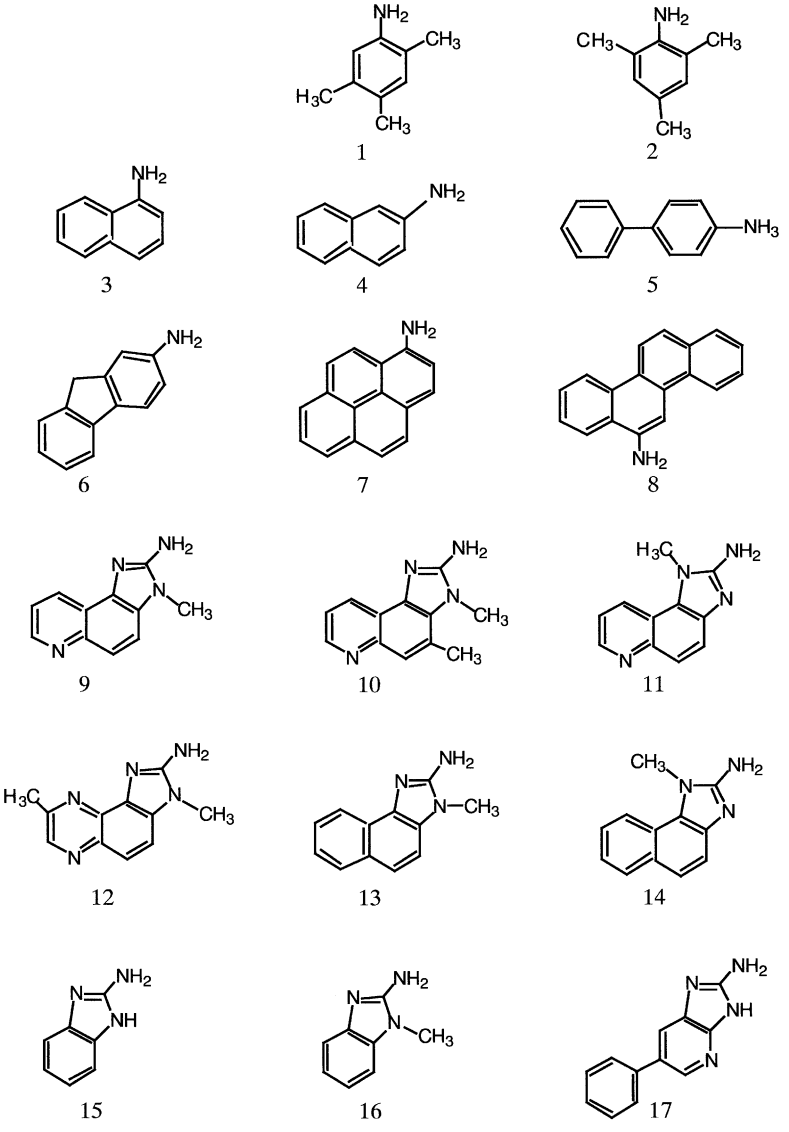
Table 1. Data used to derive QSAR 2

	Substituents	Log 1/C	Log 1/C		CLog P	CMR
			Pred	Dev		
1	Estradiol	6.380	6.034	0.346	3.784	7.862
2	4-MeO-Phenol	0.029	0.832	−0.803	1.574	3.459
3	4-C_6H_5O-Phenol	−0.741	0.179	−0.920	3.573	5.506
4	4-MeCO-Phenol	1.460	1.346	0.114	1.581	3.652
5	Bisphenol[a]	−0.326	3.715	−4.041	3.673	6.897
6	4-Me_3C-Phenol	−0.963	1.20	0.237	3.301	4.697
7	4-CN-Phenol	−0.315	0.375	−0.690	1.597	3.319
8	Diethylstilbestrol	3.770	3.859	−0.089	4.956	8.415
9	4-I-Phenol	−1.170	−1.435	0.265	2.895	4.148
10	Phenol	0.190	−0.562	0.752	1.475	2.842
11	4-MeS-Phenol	1.40	1.187	0.213	2.034	4.112
12	4-C_3H_7O-Phenol	0.630	0.054	0.576	2.632	4.386

[a]Outlier.

Table 2. Data used to derive QSAR 3

Compd	Log Mut	Pred	Dev	*C*Log *P*	CMR
1	−0.677	−0.234	−0.443	2.312	4.449
2	−0.259	−0.234	−0.025	2.312	4.449
3	0.380	0.776	−0.396	2.089	4.745
4	1.206	0.776	0.430	2.089	4.745
5	1.307	1.273	0.034	2.803	5.569
6	2.840	1.951	0.889	2.848	5.928
7	3.540	3.168	0.372	3.723	7.225
8	2.971	3.818	−0.847	4.437	8.121
9	4.127	4.548	−0.421	1.318	5.904
10	4.593	4.661	−0.068	1.817	6.368
11	4.643	4.548	0.095	1.318	5.904
12[a]	3.420	5.504	−2.084	1.073	6.157
13	3.041	3.000	0.041	2.470	6.115
14	3.635	3.000	0.635	2.470	6.115
15	0.204	0.603	−0.399	1.238	3.964
16	1.579	1.478	0.101	1.296	4.427
17[a]	2.780	4.281	−1.501	1.802	6.175



[a]Outliers.

We have recently found it to be very important in explaining allosteric reaction mechanisms.[14] *MR* can be collinear with log *P* as in the case of QSAR 2 ($r^2 = 0.835$). However, they do clearly have independent roles. If we use only *C*log *P* to correlate the data the resulting QSAR has an r^2 of 0.210. Using only CMR yields an equation with $r^2 = 0.570$.

The statistics of QSAR 2 are good and only one compound was so poorly fit that it had to be omitted. It is much less potent than QSAR 2 would suggest. We were astonished, in the light of eq 1, to find that adding electronic terms of BDE or σ^+ did not improve the correlating eq 2.

Discussion

What is impressive about eqs 1 and 2 is that both equations correlate diethylstilbesterol and estradiol that are so grossly different from the simple phenols and phenol itself. The implications of our results are that the interaction occurs in polar space (negative log *P* term) and that volume and polarizability favor apoptosis (CMR). This suggests that reaction might be occurring with DNA that is not generally hydrophobic. We have found a number of QSAR for chemicals reacting with DNA that either have no hydrophobic terms or negative hydrophobic terms.[15] DNA fragmentation is a hallmark of apoptosis.[1–5] There have been many studies on DNA fragmentation during apoptosis.[1] Therefore, we speculate that chemical-induced apoptosis is due to perturbation in DNA structure or function.

Another way of approaching the problem is via comparative QSAR. Turning now to our database of 8700 QSAR,[7] we can seek out equations with the same parameterization as QSAR 2. We find 13 equations for cells that contain a negative log *P* term and a positive CMR term. Many of these are associated with mutagenicity or carcinogenicity, processes that normally involve DNA. Eq 3, derived from the data of Sabbioni,[16] is a good example.

Mutagenicity of aromatic or heteroaromatic amines activated by UV radiation (Table 2)

$$\log 1/C = -1.73(\pm 0.45)$$
$$C\log P + 2.10(\pm 0.35)\mathrm{CMR} - 5.60(\pm 1.4) \quad (3)$$
$$n = 15,\ r^2 = 0.934,\ s = 0.496,\ q^2 = 0.866,$$

outliers: **12** and **17** in Table 2

What is especially interesting about the molecules on which QSAR 3 is based is the very wide variation in structure. Yet they are well fit by two general parameters like the phenols of eq 3. We were amazed to find equations that did not contain an electronic term seen in QSAR. We believe that shorter QSAR time of incubation for apoptosis, 24 h versus 48 h for QSAR 2, may be responsible for this.

It is generally expected that chemical perturbation of DNA induces apoptosis.[1–5] The equations derived from our data emphasize a correlation between the chemical structure of various phenolic compounds with their ability to induce apoptosis in L1210 murine leukemia cells. These results are of paramount significance in the development of new compounds with structural similarities, yet varying in their apoptosis-inducing capacity, in cancer therapy. However, it is unclear whether the observed apoptosis is the result of a direct interaction of the compounds with DNA (e.g., hindering DNA repair mechanisms, inhibition of topoisomerases, etc.) or is due to the activation of death-inducing signaling pathways such as JNK/SAPK, p38 MAPK and/or caspase cascades.

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