Quantitative Structure–Activity Relationship Studies on Anticancer Drugs

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Contents

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I. Introduction

Cancer is one of the most formidable diseases of the world. In fact, if there is any disease which the mankind is still most afraid of it is cancer. Cancer is not one disease, but a group of diseases affecting different organs and systems of the body. It develops due to the abnormal and uncontrolled cell division, frequently at a rate greater than that of the most normal body cells. Although cancer mortality is second to heart diseases, the first is steadily increasing, while the latter is leveling off. The agony of cancer patients and the financial burden to families add to the dread of cancer.

Although it is commonly said that prevention is better than cure, efforts to provide preventive measures to cancer have not been very effective, as variety of chemicals and environmental factors can cause cancer. Thus once the cancer has developed, one has to resort to its treatment. There are four major modalities for the treatment of the cancer: (1) surgery, (2) radiation therapy, (3) immunotherapy, and (4) chemotherapy. Surgery cannot be applied when the disease is spread throughout the body, and radiation therapy damages not only the cancerous cells but also the normal cells. Thus in this situation, the only treatment for the disseminated cancer is chemotherapy, although immunotherapy—the manipulation of immune response—holds encouraging promise, but it is still in its infancy.

Chemotherapy is today providing increasing cure rates in many forms of human cancer.[1-3] Because of our day by day progress in understanding the effects of drugs on cells, both normal and cancerous, there has been a continued improvement in this mode of cancer treatment. For the immediate future, the combined modalities—surgical adjuvant therapy, radiation, and chemotherapy—seem sure to provide better responses and response rates. However, it cannot be denied that for continued advances more effective drugs still need to be found.

The ease and success of finding the better drugs for any disease depend upon how best we can rationalize the design of the drugs. The rational design of an agent with specific activity toward a selected target requires that this target be so precisely defined that it can be hit selectively in the presence of other identical or similar targets. With regard to cancer, there is little information on unique characteristics of cancer cells that may be exploited in the investigation of new agents. Nonetheless, the useful anticancer drugs are being produced but mostly based on empiricism. The mechanism by

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which the anticancer drugs selectively kill cancer cells has not been clearly established, but evidence points out that these drugs might interfere with the synthesis or function of nucleic acids or with the mitotic process itself. The advances in quantitative structure–activity relationship (QSAR) studies have widened the scope of rationalizing the drug design and finding the mechanisms of drug actions. It is, therefore, expected that a critical review of QSAR studies on anticancer drugs would be of immense help in providing a greater prospective to the rationalization of designing better agents and to the understanding of their modes of action. QSARs have proven their worth in the interpretation of mechanisms of inhibition of a number of enzyme systems[4] and in elucidating the modes of actions of local anesthetics[5] and a variety of drugs acting at the central nervous system.[6,7]

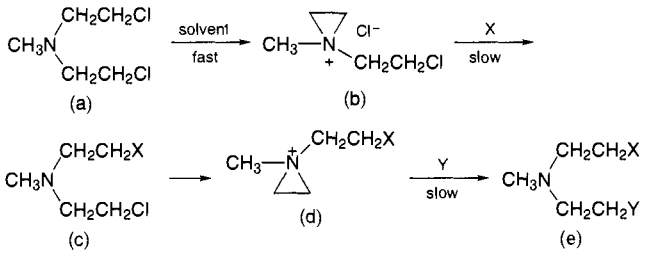
II. Classification of Anticancer Drugs

Anticancer drugs belong to different categories of chemicals and follow different modes of action. They are, therefore, usually classified on the basis of their modality of action and thus are put in the following categories.

A. Chemically Reactive Drugs Having Nonspecific Action

A variety of chemicals elicit their anticancer activity by alkylating nucleic acids. Alkylating agents can combine covalently with nucleophilic centers and thus can attack nonselectively at any nucleophilic center available in vivo. These centers can be nitrogen, sulfur, or oxygen atoms of biologically important functional groups, such as amino groups, thiolate anions of proteins, and ring nitrogen atoms and phosphate anions of nucleic acids. Evidence, however, indicates that DNA is the critical target of biological alkylating agents and that the 7-position of guanine is the primary site of attack. Scheme 1 explains how any two nucleophiles X and Y can be alkylated by a bifunctional alkylating agent, such as mechlorethamine (a). Such a bifunctional alkylation may cross-link two guanine molecules of opposite strands in DNA (Figure 1), which results finally in cross-linking the two strands. This interstrand cross-linking of DNA leads to the impaired template function for further replication.[8] Rapidly proliferating cells are supposed to be more sensitive to cross-linking than normal cells, because they are not able to repair damaged DNA, while the latter do so by enzymatic excision of alkylated bases.

Scheme 1. Bifunctional Alkylation with Mechlorethamine



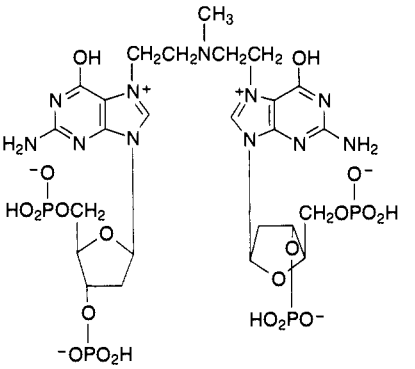


Figure 1. Bifunctionally alkylated guanine bases in DNA strands.

The bifunctional alkylation may also lead to interstrand cross-linking of DNA or to the binding of DNA to protein molecules.[9,10] Monofunctional alkylation may also take place, but that would be less cytotoxic than the bifunctional one. A possible consequence of monofunctional alkylation would be mispairing of bases in DNA, e.g., hydrogen bonding of the keto form of guanine with cytosine is normal, but after N-7 alkylation the enol form of guanine would be favored which can pair atypically with thymine.[11] Such mispairing could lead to miscoding and mutation. However, on a theoretical basis, it is assumed that alkylations at O-6 and N-3 would be more effective than at N-7.[12]

The important alkylating agents are nitrogen mustards, aziridines, esters of sulfonic acids, nitrosoureas, and triazenes. Although bifunctionality is not a prerequisite for significant anticancer activity, the most active agents are usually bifunctional. Although the cross-linking in DNA is more cytotoxic than monofunctional alkylation, there is no general explanation of the basic mechanism of anticancer action of alkylating agents.[13]

B. Mitotic Inhibitors

Certain chemicals exert their anticancer effects by producing metaphase arrest through microtubule interactions. These interactions lead to spindle dissolution, interference with some phases of phagacytosis, and changes in morphology and motility. Such chemicals include *Vinca* alkaloids, colchicine derivatives, podophyllotoxins, and some miscellaneous compounds. Although a number of other effects of these compounds, such as inhibition of RNA synthesis, have been observed, the mitotic arrest is supposed to be the most important for their anticancer activity.[14,15]

C. Cellular Respiration Inhibitors

From the detailed examinations of glycolysis and respiration pathways, it has been found that some cancer cells exhibit abnormal levels or activities of certain enzymes like malate and lactate dehydrogenases.[16–19] The selective inhibition of these enzymes in abnormal cells may lead to the inhibition of cellular respiration, resulting in the death of the cells. Certain copper(II) chelates[20,21] and derivatives of 4-hydroxyquinoline-3-carboxylic acids[22,23] have been

found to have direct effect on the inhibition of cellular respiration.

D. Hypoxia-Selective and Radiosensitizing Agents

Hypoxia (oxygen deficient) cells in solid tumors are an important target for cancer chemotherapy.[24,25] Hypoxia is more pronounced in solid tumors than in normal tissues, creating an environmental difference exploitable in the design of novel antitumor agents. Hypoxic cells may be activated only in the absence of oxygen, providing selective bioactivation within tumor tissues.[26] Compounds that have been shown to be hypoxia selective are generally nitroaromatics and nitroheterocyclics.[27]

Since hypoxic cells are refractive to radiation, they limit the clinical efficacy of radiotherapy.[28] Hence, attempts to develop agents that can selectively sensitize cancer cells to the effects of ionizing radiation may represent a novel approach to cancer chemotherapy. Many nitroaromatics and nitroheterocyclics have been found to act as radiosensitizers also.[29,30]

E. Agents Binding to DNA

Since DNA is an important cellular receptor, many chemicals exert their anticancer effects through binding to DNA, and their effectiveness depends upon the mode and intensity of the binding. There are mainly three types of binding: (1) covalent binding, (2) nonintercalative groove binding, and (3) intercalation. Out of these, the first kind of binding, i.e., the covalent binding, takes place when the drugs are bifunctional alkylating agents (section II.A). The other two types of binding are not so strong but involve weak forces like van der Waals force or hydrogen bonding. In DNA molecule, each base pair provides two grooves: a minor groove and a major groove (Figure 2).[31] Typically groove-binding molecules are composed of several heteroaromatic rings linked together through amide or other functional groups, or directly through single bonds.[32,33] The important representatives of such molecules are shown in Figure 3. These molecules, being relatively long and flexible and having a number of proton donor and acceptor groups and positively charged end(s), are supposed to have the specificity for the minor groove of the DNA. They may bind with the minor groove of A–T or G–C base pair through the hydrogen bond formation between their NH groups and N^3 atom of adenine/guanine and/or O^2 atom of thymine/cytosine.[34-37] The charged end groups of drugs are supposed to be involved in the interaction generally with the phosphate groups of the DNA.

The stronger attachment of drug molecules with DNA is however caused by intercalation. In this mode of interaction, a molecule is inserted between two adjacent layers of base pairs and is held there primarily through van der Waals forces. If there is any side chain present in the molecule, it can interact with the phosphates of the DNA backbone.[31] Outstanding among the intercalating antitumor drugs are anthracyclines. They are derivatives of anthraquinone, a planar chromophore particularly well adapted for intercalation between nucleic acid base

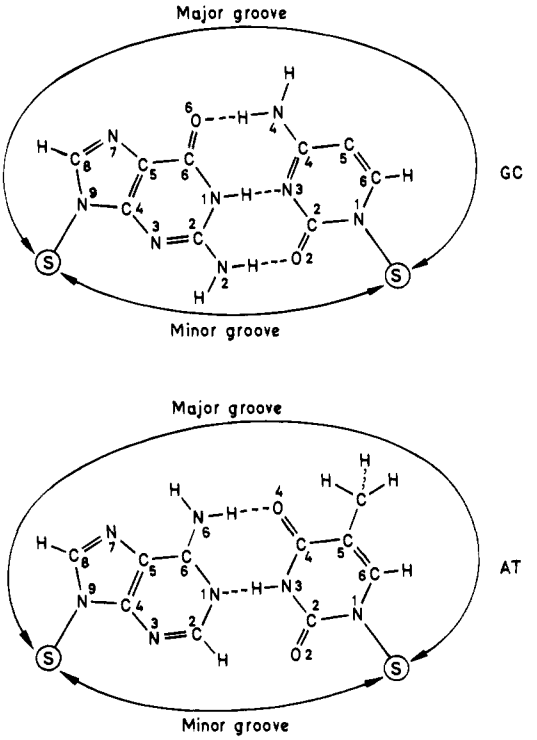


Figure 2. Sites in the major and minor grooves of DNA.[31]

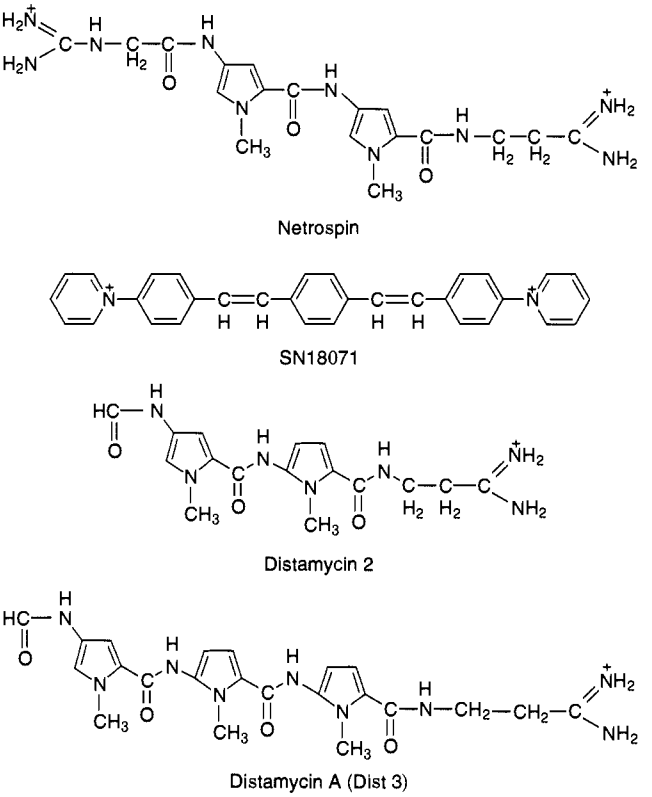


Figure 3. Important representatives of nonintercalating groove-binding molecules.[31]

pairs, its surface matching closely the surface of these pairs.[38,39] Anthracyclines are highly potent and chemotherapeutically useful agents. Some of them are in clinical use today. Drugs belonging to acridine series have also been extensively studied for their intercalative properties and anticancer activity. Some miscellaneous compounds have also been found to exert their anticancer effects through intercalation.

F. Antimetabolites

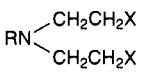
Antimetabolites interfere with the formation or utilization of a normal cellular metabolite. The interference may result from the inhibition of an enzyme or enzymes or from incorporation, as a fradulent building unit, into macromolecules such as proteins or nucleic acids. Several types of antimetabolites have been investigated for anticancer activity and many have been found to have some effect on cancers. The most important of them have been the analogues of the metabolites involved in the biosynthesis of nucleic acids and the purine- and pyrimidine-containing cofactors.

III. QSAR Results and Discussions

A. Chemically Reactive Drugs Having Nonspecific Action

1. Nitrogen Mustards

Aliphatic and aromatic nitrogen mustards having simple general structures as I and II, respectively, form an important class of alkylating agents—drugs that have nonspecific action. Among the variety of



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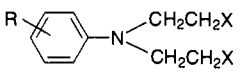


II

alkylating agents, nitrogen mustards are widely studied and better exploited for chemotherapy.[40] A QSAR study was first made on them by Lien and Tong.[41] Using the data of Bardos et al.[42] for a series of aniline mustards (Table 1) acting against Walker 256 carcinoma in rats, these authors obtained eqs 1 and 2. Equation 1 correlates the antitumor activity of the first nine compounds with hydrophobic constant π and the electronic parameter σ (Hammett constant) of the substituents. Equation 2, however, obtained for the next six compounds correlates the activity with only the hydrophobic constant of the substituents. In these equations, n refers to the

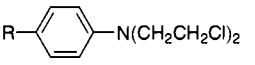
$$\log(1/ED_{50}) = 3.660 - 0.263\pi - 1.623\sigma$$
$$n = 9, r = 0.839, s = 0.449 \quad (1)$$

$$\log(1/ED_{50}) = 5.070 + 0.441\pi - 0.648\pi^2$$
$$n = 6, r = 0.980, s = 0.160, \pi_o = 0.34 \quad (2)$$

number of data points, r is the correlation coefficient, and s is the standard deviation. Since eq 2, representing a parabolic correlation, is based only on six data points, little confidence can be attached to it. Therefore, one would find from eq 1 that the hydrophobic character of the substituents will have the negative effect on the anticancer activity but an electronic property, the electron-donating nature, will have the positive effect. (Positive value of σ denotes the electron-withdrawing character, and negative value, the electron-donating character of the substituent.)

The toxicity data also of these two sets of compounds were found to be well correlated with π and σ. The LD_{50} for the first set of compounds (1–9) had exactly similar correlation with π and σ (eq 3) as ED_{50} (eq 1), but for the second set of compounds (10–16), it, unlike ED_{50}, had only a negative linear relationship with π (eq 4). The negative effect of π on toxicity, however, seems to be unusual.

Table 1. Activity (ED_{50}) of Aniline Mustards against Walker 256 Carcinoma in Rats and Their Toxicity (LD_{50}) in Rats[41]



compd	R	log-(1/ED_{50})	log-(1/LD_{50})	π	σ
1	H	3.40	3.44	0.00	0.00
2	COOH	3.30	3.04	−0.28	0.27
3	SO_2NH_2	2.82	2.95	−1.82	0.62
4	OH	4.49	4.13	−0.67	−0.36
5	NH_2	4.70	4.82	−1.23	−0.66
6	$NHCOCH_3$	4.47	3.99	−0.97	−0.02
7	$NHCOCH_2NH_2$	4.47	4.47	−2.16	−0.02
8	$NHCOCH_2NHCOCH_3$	4.80	4.17	−1.94	−0.02
9	$NHCOCH_2NHCOOCH_3$	3.85	3.70	−2.43	−0.02
10	OH(HCl)	4.46	4.09	−1.23	−0.66
11	$OCOCH_3$	4.58	4.26	−0.44	0.08
12	OCOPh	4.82	4.03	0.39	0.08
13	$OCOC_6H_3$-2,6-$(CH_3)_2$	3.27	3.07	0.11	0.08
14	$OCOC_6H_4$-2-CH_3	4.51	3.68	0.11	0.08
15	$OCONHC_6H_4$-4-COOH	5.05	3.82	0.38	0.08
16	$PhOCONHC_6H_4$-4-COOH		2.93	0.38	0.08

$$\log(1/LD_{50}) = 3.476 - 0.269\pi - 1.577\sigma$$
$$n = 9, r = 0.927, s = 0.273 \quad (3)$$

$$\log(1/LD_{50}) = 4.021 - 0.373\pi$$
$$n = 7, r = 0.904, s = 0.241 \quad (4)$$

Lien and Tong did not consider an earlier study by Bordos et al.,[43] where the aniline mustards (II) had the variation in X substituent also. For that set of compounds, Panthananickal et al.[44] obtained the following equations:

$$\log(1/ED_{50}) = 3.84(\pm0.33) - 1.00(\pm0.87)\pi - 0.53(\pm0.55)\pi^2 - 1.19(\pm0.51)\sigma^- + 0.75(\pm0.41)I$$
$$n = 14, r = 0.940, s = 0.291, \pi_o = -0.95 \quad (5)$$

$$\log(1/LD_{50}) = 3.78(\pm0.33) - 0.90(\pm0.86)\pi - 0.45(\pm0.55)\pi^2 - 1.31(0.51)\sigma^- + 0.59(0.41)I$$
$$n = 14, r = 0.941, s = 0.289, \pi_o = -1.00 \quad (6)$$

In the above equations, σ^- denotes the ability of the substituents to withdraw the electron from a negative site by direct resonance interaction and I is an indicator parameter which was used to account for the variation in X substituent. It was given a value of zero when X = Cl or I and 1 when X = Br. The data within parentheses are 95% confidence intervals. (If they are written without ± sign, they would refer to the standard error of the coefficient of the variables.) Equations 5 and 6 are very much similar to each other, showing that antitumor activity parallels toxicity.

For another series of aniline mustards (Table 2) studied by Vickers et al.,[45] Panthananickal et al.[44] obtained eqs 7 and 8,which are also quite similar to

$$\log(1/ED_{90}) = 4.69(\pm0.26) + 0.35(\pm0.29)\pi - 0.14(\pm0.13)\pi^2 + 0.40(\pm0.18)E_{s,2} - 0.40(\pm0.36)\sigma + 0.36(\pm0.21)I$$

$$n = 26, r = 0.862, s = 0.245, \pi_o = 1.25 \quad (7)$$

$$\log(1/LD_{50}) = 4.13(\pm0.15) + 0.15(\pm0.14)\pi - 0.08(\pm0.07)\pi^2 + 0.30(\pm0.11)E_{s,2} + 1.10(\pm0.11)I$$

$$n = 26, r = 0.979, s = 0.14, \pi_o = 0.96 \quad (8)$$

each other except that the latter has no electronic term. The indicator parameter I in both the equations had the value of 1 for X = Br and 0 for X = Cl, and the Taft steric constant E_s was used to describe the steric effect of the substituent at the ortho position.

Continuing their study, these authors made QSAR studies on NCI (National Cancer Institute) data on L1210 leukemia for many groups of congeners of II. For three different series, each having X = Cl, they had obtained the equations

$$\log(1/C) = 4.07(\pm0.21) - 0.31(\pm0.10)\pi - 0.96(\pm0.54)\sigma^- + 0.86(\pm0.37)I_o$$

$$n = 19, r = 0.926, s = 0.313 \quad (9)$$

$$\log(1/C) = 4.59(\pm0.13) - 0.40(\pm0.08)\pi - 1.00(\pm0.35)\sigma$$

$$n = 19, r = 0.949, s = 0.240 \quad (10)$$

$$\log(1/C) = 4.17(\pm0.20) - 0.35(\pm0.11)\pi - 1.38(\pm1.0)\sigma$$

$$n = 16, r = 0.897, s = 0.325 \quad (11)$$

where C is the dose (in mol/kg) required to produce a desired therapeutic ratio (T/C) (100 × ratio of survival times of treated and control animals). For eqs 9 and 10, (T/C) = 125 (25% increase in life span), and for eq 11, (T/C) = 180. In these series, the substituents of the aryl ring were at varying positions. Only a few compounds were disubstituted, otherwise a majority of them were monosubstituted. The indicator parameter I_o was used to denote the substituent at 2-position. It was given a value of unity for the presence of any substituent at 2-position, otherwise it was zero. This parameter was found found to be significant only in eq 9. In the above three equations, π and σ or σ^- refer to sum of their values for all the substituents present at the aryl ring of a compound.

For another set of aniline mustards (II), where X was either OSO_2CH_3 or $OSO_2C_6H_4CH_3$, the NCI data against L1210 leukemia (T/C = 125) were found to be correlated with only electronic parameter and two indicator parameters I_1 and I_2 (eq 12).[44] The parameter I_1 was given a value of 1 for X = $OSO_2C_6H_4CH_3$ and 0 for X = OSO_2CH_3. A number of compounds in the series had a nitroso group at 4-position of the aryl ring. The parameter I_2 with a value of 1 was used to account for the exclusive effect of this group.

Table 2. Activity (ED_{90}) of $RO-C_6H_4-N(CH_2CH_2X)_2$ against Walker 256 Rat Tumor and Toxicity (LD_{50}) in Rats[44]

compd	R	X	log-(1/ED_{90})	log-(1/LD_{50})	π^a	σ^a	$E_{s,2}$
1	H	Cl	4.44	4.07	−0.67	−0.37	0.00
2	H	Br	5.00	5.00	−0.67	−0.37	0.00
3	OC_6H_5	Br	5.45	5.45	1.46	0.13	0.00
4	OC_6H_4-2-CH_3	Br	4.96	4.96	2.02	0.03	−1.24
5	OC_6H_3-2,6-$(CH_3)_2$	Br	3.81	3.58	2.58	−0.07	−2.48
6	OC_6H_4-2-Et	Br	4.96	4.64	2.48	−0.02	−1.31
7	OC_6H_4-2-*i*-Pr	Br	4.39	4.66	2.99	−0.03	−1.71
8	OC_6H_4-3-CH_3	Br	5.18	5.16	2.02	0.06	0.00
9	OC_6H_4-4-CH_3	Br	4.94	5.32	2.02	−0.04	0.00
10	OC_6H_4-3-Cl	Br	4.96	5.11	2.17	0.50	0.00
11	OC_6H_4-4-Cl	Br	4.66	5.18	2.17	0.36	0.00
12	OC_6H_4-4-OCH_3	Br	5.12	5.21	1.44	−0.14	0.00
13	OC_6H_4-3-CF_3	Br	4.99	5.11	2.34	0.56	0.00
14	OC_6H_4-3-NO_2	Br	4.67	5.07	1.18	0.84	0.00
15	OC_6H_4-4-NO_2	Br	4.86	5.37	1.18	0.91	0.00
16	OC_6H_5	Cl	4.86	4.06	1.46	0.13	0.00
17	OC_6H_4-2-CH_3	Cl	4.55	3.71	2.02	0.03	−1.24
18	OC_6H_3-4-CH_3	Cl	4.78	4.20	2.02	−0.04	0.00
19	OC_6H_3-2,6-$(CH_3)_2$	Cl	3.31	3.10	2.58	−0.07	−2.48
20	OC_6H_4-3-Br	Cl	4.22	3.84	2.32	0.52	0.00
21	OC_6H_4-4-Br	Cl	4.84	4.19	2.32	0.36	0.00
22	OC_6H_4-3-Cl	Cl	4.55	3.95	2.17	0.50	0.00
23	OC_6H_4-3-F	Cl	4.88	4.38	1.60	0.47	0.00
24	OC_6H_4-4-F	Cl	4.90	4.21	1.60	0.19	0.00
25	OC_6H_4-4-OCH_3	Cl	4.89	4.11	1.44	−0.14	0.00
26	OC_6H_4-3-NO_2	Cl	4.81	4.40	1.18	0.84	0.00

[a] For complete −OR moiety.

$$\log(1/C) = 4.96(\pm0.45) - 1.04(\pm0.66)I_1 + 3.11(\pm0.87)I_2 - 1.11(\pm0.59)\sigma^-$$

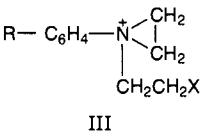
$$n = 17, r = 0.932, s = 0.426 \quad (12)$$

In a later study,[46] Panthananickal et al. found the activity of a series of aniline mustards [R-$C_6H_4N(CH_2CH_2Cl)_2$] against B-16 melanoma in mice (T/C = 125) to be correlated with electronic and hydrophobic parameters (eq 13)almost in the same manner as

$$\log(1/C) = 4.20(\pm0.19) - 0.17(\pm0.11)\pi - 0.14(\pm0.06)\pi^2 - 1.84(\pm0.44)\sigma$$

$$n = 21, r = 0.931, s = 0.323, \pi_o = -0.62 \quad (13)$$

Bordos data of some congeners against Walker 256 carcinoma in rats (eq 5). From all these studies by Panthananickal et al. and the one by Lien and Tong, the foremost thing that has emerged is the dominant role played by the electronic factor in the anticancer activity of aniline mustards, be it against Walker 256 tumor, L1210 leukemia, or B-16 melanoma. Although the exact mechanism of alkylation by aniline mustards has not been yet established, according to many studies[47] they are supposed to form first an unstable intermediate, cyclic ethylene immonium ion (III), just like aziridinium ion (b) formed by mechlorethamine in Scheme 1. The formation of this ion would be highly dependent upon the electron density on nitrogen. Consequently, the antitumor activity of aromatic mustards should be the function of electron



III

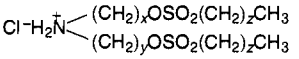
density on the nitrogen, which can be affected by electron-releasing substituents at the aryl ring. This proposition is fully supported by the negative coefficient of σ or σ^- in all QSAR equations discussed so far. A QSAR study, however, made by Lewis[48] on Bordos et al.'s data[42,43] indicated that electronic properties of the ring atoms will also affect the activities of aniline mustards, although no definite correlations were obtained between the activities and the quantum mechanical parameters used. Equations 5–8 with positive coefficient of indicator variable I express that the formation of immonium ion would be easier with X = Br than with X = Cl/I in aniline mustards (II), but eqs 7 and 8 with positive coefficient of $E_{s,2}$ indicate that there would be steric hindrance in the formation of this ion by 2-substituent of aryl ring. This steric effect by the 2-substituent was however not corroborated by further studies. Equation 9 obtained for activity against L1210 leukemia suggests, having positive value of variable I_o, that any substituent at the 2-position will increase the activity. The parameter I_o was however not found to be significant when introduced in eqs 10 and 11 which were also obtained for L1210 leukemia, but the reason was that there were very few ortho-substituted compounds in the related series. The positive effect of the ortho substituent was also observed in the hydrolysis of aniline mustards (eq 14). To find the importance of substituent effects on

$$\log \% \text{ hyd} = 1.19(\pm 0.07) - 1.49(\pm 0.22)\sigma + 0.46(\pm 0.18)I_o + 0.74(\pm 0.14)I$$

$$n = 43, r = 0.939, s = 0.190 \qquad (14)$$

the nucleophilic character of nitrogen, Ross made an extensive study on the hydrolysis of a series of aniline mustards,[49] and the hydrolysis data were found[44] to be correlated with Hammett constant and indicator parameters I_o (used for ortho substituent) and I (used for X = Br) as shown by eq 14. This equation shows that the ortho substituent will increase the rate of hydrolysis, presumably by twisting the nitrogen out of conjugation with π electrons, thus making nitrogen a better nucleophile. That an exclusive positive effect on activity can be produced by nitroso group present at 4-position has been exhibited by eq 12 which has a high positive coefficient of indicator variable I_2 used for 4-nitroso group. These QSAR studies, however, do not give a clear picture of the role played by the hydrophobic character of substituents. While eq 12 does not exhibit any role of π in anticancer activity, eqs 1 and 9–11 show a typically negative role and other equations lead to an optimization of π value with significant differences from equation to equation. Therefore we have to look forward for other studies.

In their study, Lien and Tong and also Panthananickal et al. ignored the ionizing effect of compounds, but when Cain and Denny[50] attempted a QSAR study on antitumor data of dialkanolamine bis(alkanesulfonic ester)s (IV),analogous to nitrogen



IV

mustards, they found that incorporation of ionic factor in the π value had a considerable effect on the correlation. While the use of unmodified $\sum\pi$ revealed a poor correlation (eq 15), a modification in it,

$$\log(\text{ILS}_{max}) = 1.85 \pm 0.17(\pm 0.18)\sum\pi - 0.088(\pm 0.065)(\sum\pi)^2$$

$$n = 15, r = 0.67, s = 0.17 \qquad (15)$$

$$\pi_c = \sum\pi + \log(H^+/K_a + H^+) \qquad (16)$$

$$\log(\text{ILS}_{max}) = 1.92 + 0.11(\pm 0.07)\pi_c - 0.080(\pm 0.031)\pi_c^2$$

$$n = 15, r = 0.85, s = 0.12 \qquad (17)$$

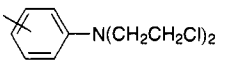
$$\log(\text{ILS}_{max}) = 1.86 - 0.51(\pm 0.09)R_c - 0.46(\pm 0.07)R_c^2$$

$$n = 15, r = 0.97, s = 0.05 \qquad (18)$$

according to eq 16, led to a significantly improved correlation (eq 17), and a similar modification in reversed-phase chromatographic parameter (R_c), also a measure of hydrophobic character, had led to a still better correlation (eq 18). In these equations, ILS_{max} refers to the maximum increase in life span at LD_{10} in L1210 leukemia test. In eq 16, K_a refers to the ionization constant of the compound.

Denny and co-workers, however, tried to show that anticancer activity of aniline mustards can depend only upon the electronic factor.[51,52] For a series of compounds (Table 3) synthesized and studied for

Table 3. The Growth Inhibition Potency of Aniline Mustards against UV_4 Cells under Aerobic conditions with a Drug Exposure of 1 h[51]



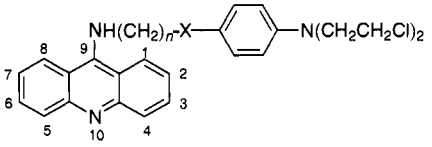
compd	R	IC_{50}, μM	σ
1	H	1.2	0.0
2[a]	4-NO_2	15.8	0.78
3	3-NO_2	96	0.71
4	4-SO_2Me	174	0.72
5	3-SO_2Me	76	0.63
6	4-$CONMe_2$	14.6	0.36
7	3-$CONMe_2$	7.6	0.35
8	4-SMe	1.62	0.0
9	4-CH_3	0.40	−0.17
10	3-CH_3	1.2	−0.07
11	4-OCH_3	0.45	−0.27
12	3-OCH_3	2.7	0.12
13	4-NH_2·HCl	0.071	−0.66
14	3-NH_2·HCl	0.38	−0.16

[a] This compound was not included in the derivation of eq 19, as it behaved an outlier. The compound appears to bind to plastic surfaces, which may reduce the efficiency of drug washout after 1-h exposure.[51]

growth inhibitory potency (IC_{50}) against Chinese hamster ovary derived UV_4 cell lines, the correlation obtained by these authors was as

$$\log(1/IC_{50}) = -0.21(\pm 0.25) - 2.50(\pm 0.41)\sigma$$
$$n = 13, r = 0.97, s = 0.35 \quad (19)$$

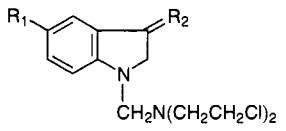
However, when these simple aniline mustards were attached to classical DNA-affinic intercalator 9-aminoacridine (V),their cytotoxicity was found to be less



V: X = O, CH_2, S, CONH, NHCO, CO, SO_2

dependent upon σ, but their hydrolysis data were observed to depend upon this parameter in similar manner as those of unattached mustards.[52] This attachment had led to a drastic increase in biological activity of mustard[52] and an increase in the chain length (value of n) was found to alter the cross-linking ability, associating the maximal activity with $n = 4$.[53] These studies of Denny et al. led to the suggestion that less reactive alkylating agents can be useful by linking them to DNA-targeting acridine chromophore.

A series of nitrogen mustards of isatins (VI) was



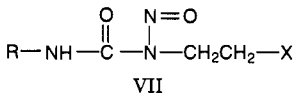
VI

synthesized and studied for their HeLa cell growth inhibition activity by Maysinger et al.[54] These authors attempted to correlate this activity with log P and obtained eq 20 neglecting three compounds where $R_1 = NO_2$. This exclusion and consequently obtaining a parabolic correlation based on only seven data points do not allow much confidence to be placed in eq 20.

$$\log(1/IC_{50}) = 3.154 + 1.162 \log P - 0.256(\log P)^2$$
$$n = 7, r = 0.851, s = 0.182, \log P_o = 2.19 \quad (20)$$

2. Nitrosoureas

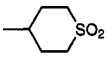
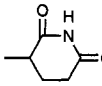
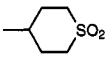
Nitrosoureas (VII) constitute another important class of alkylating agents possessing a variety of biologically significant effects.[55] As anticancer agents, they have been shown to have significant activity against a variety of transplanted tumors, but are of major interest due to their antileukemia activity.[56,57]



VII

Most QSAR studies suggest that their activity is strongly dependent upon hydrophobicity, e.g., for

Table 4. Activity of Nitrosoureas (VII) against L1210 Leukemia in Mice ($T/C = 175$)

compd	R	X	log(1/C)	log P
1	4-*tert*-butylcyclohexyl	Cl	3.03	4.51
2	2-adamantyl	Cl	3.24	3.62
3	*cis*-2-chlorocyclohexyl	Cl	3.73	2.73
4	4-methylcyclohexyl	F	3.78	2.70
5	3-methylcyclohexyl	Cl	3.79	3.30
6	norbornyl	Cl	3.91	2.98
7	cyclopentyl	Cl	3.94	2.19
8		F	3.94	1.54
9	cyclohexyl	Cl	3.97	2.83
10	*trans*-4-methylcyclohexyl	Cl	4.00	3.30
11	cyclohexyl	F	4.01	2.23
12	*trans*-2-chlorocyclohexyl	Cl	4.03	2.73
13	norbornyl	F	4.06	2.38
14		Cl	4.06	2.07
15	2-chloroethyl	Cl	4.07	1.53
16		Cl	4.20	2.08
17		F	4.23	1.43
18	4-(acetyloxy)cyclohexyl	Cl	4.23	2.56
19		F	4.43	−0.41
20		Cl	4.52	0.37
21	H	Cl	4.58	0.57
22	2-fluoroethyl	F	4.66	0.33
23		Cl	4.55	0.19

compounds in Table 4, the correlation obtained was as[58]

$$\log(1/C) = 4.527(\pm 0.17) - 0.069(\pm 0.17) \log P - 0.057(\pm 0.042)(\log P)^2$$
$$n = 22, r = 0.992, s = 0.163, \log P_o = -0.6 \quad (21)$$

and for another similar set of compounds, the correlation obtained was as[59]

$$\log(1/C) = 1.31(\pm 0.19) + 0.038(\pm 0.12) \log P - 0.061(\pm 0.04)(\log P)^2 - 0.62(\pm 0.42)D$$
$$n = 13, r = 0.904, s = 0.199, \log P_o = 0.31 \quad (22)$$

In eq 21, the activity was against L1210 leukemia ($T/C = 175$) and in eq 22, it was against Lewis lung carcinoma in mice (concentration delaying tumor growth by 4 days). Further in eq 22, D is a dummy parameter which was used to account for the effect of COOH group present in R substituent adjacent to NH moiety (VII) in two compounds. Compound 8 was not included in the derivation of eq 21 in order to have slightly better correlation. Even for a large series of nitrosoureas, the activity against L1210 leukemia was shown to be correlated with log P (eq 23).[60] In eq 23, the indicator variable I_1 was used with a value of 1 for compounds where R substituent in the series VII had COOEt, CH_3, or COOH group at its α-position, and $I_2 = 1$ was used for the compounds

$$\log(1/C) = 1.78(\pm0.09) - 0.13(\pm0.07)\log P - 0.014(\pm0.15)(\log P)^2 - 0.76(\pm0.15)I_1 + 0.33(\pm0.17)I_2 - 0.24(\pm0.11)I_3$$

$$n = 90, r = 0.868, s = 0.206, \log P_0 = -4.4 \quad (23)$$

where this substituent had oxidizable S (not SO_2) in the ring. The variable I_3 was used for X substituent in the series VII. It was given a value of 1 for X = F and zero for X = Cl.

The above three equations (eqs 21−23) express parabolic correlations in log P, but in eqs 21 and 22 the log P term is not very significant, hence deletion of it will lead to $\log P_0 = 0$. On the other hand in eq 23, the $(\log P)^2$ term is not significant so the exclusion of this term will lead to no optimization of $\log P$. In another QSAR study on an equally large series, Montgomery[57] obtained for antileukemic activity $\log P_0 = 0.63$, although the correlation was very poor. If we do not count Montgomery's finding, we find from other equations that anticancer activity of nitrosoureas will be determined by low hydrophobicity or in other words by hydrophilicity of compounds. But how hydrophilicity in fact works to affect the activity is a question. Hansch et al.[60] have observed that even for those compounds which can completely ionize at physiological pH, the $\log P$ or π of their neutral form yields better correlation than that of ionized form. But it does not mean that such compounds are not ionized in body fluids. In fact, the higher activity which one might expect from the ionized form, because of its low $\log P$, may be offset by loss of drug binding to serum albumin or by increased difficulty of charged particles crossing biomembranes. Hansch et al.,[60] however, concluded that prospecting among more hydrophilic neutral congeners of VII might yield analogues with a better therapeutic index.

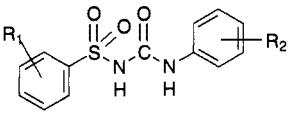
The above QSAR studies, although not providing many clues to the mechanism of drug action, suggested that a separation of active and toxic compounds is possible. For example, for the same set of compounds for which the activity was found to be correlated with $\log P$ as shown by eq 23, the toxicity data were correlated as[60]

$$\log(1/LD_{10}) = 1.01(\pm0.06) - 0.041(\pm0.007)(\log P)^2 - 0.62(\pm0.15)I_1$$

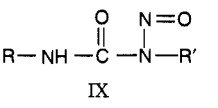
$$n = 96, r = 0.829, s = 0.221, \log P_0 = 0 \quad (24)$$

Since in eq 23, the $(\log P)^2$ term is not significant and, since in eq 24, only this term appears and not $\log P$, one should search for better antileukemia drugs only among more hydrophilic analogues of VII. The positive coefficient of I_2 in eq 23 suggests that an oxidizable sulfur in R group may increase the activity, but this parameter was not found to be significant in the case of the toxicity. Similarly, the parameter I_3 describing the effect of the X substituent was not found to be important for the toxicity, but indicated that X = F will give a less active compound than X = Cl. However, a substituted R group is indicated by I_1 parameter to reduce both the activity and the toxicity.

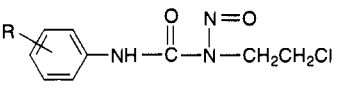
The lipophilicity of substituents was also found to be the dominant determinant of anticancer activity in case of a large series of *N*-aryl-*N'*-(arylsulfonyl)-ureas (VIII).[61] In a cluster significance analysis of



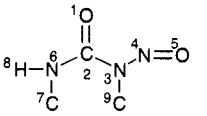
VIII



IX



X



XI

these agents, Howbert et al.[61] found that π produced clustering much superior to any electronic parameter. But by some molecular orbital calculations on two different series of nitrosoureas, *N*-alkylnitrosoureas (IX) studied by Johnston et al.[62,63] and some of *N*-cyclohexyl-*N'*-(chloroethyl)nitrosoureas (X) already treated by Hansch et al.,[60] Lewis[64] tried to show that some electronic factors may also be important in anticancer activities of this class of agents. For his MINDO/3 (modified intermediate neglect of differential overlap) treatment, Lewis considered only the central moiety (XI) of molecules and obtained eq 25 for congeners of IX and eq 26 for congeners of X.

$$\log \text{ILS}(\%) = 3.0 - 0.725S_6^N - 8.8f_1(\text{LEMO})$$

$$n = 18, r = 0.65, s = 0.50 \quad (25)$$

$$\log(1/ED_{50}) = -0.012H_f + 7.24Q_7 - 16.01f_6(\text{LEMO})$$

$$n = 27, r = 0.89, s = 0.20 \quad (26)$$

In both the equations, the activity is against L1210 leukemia. The parameters S_r^N, f_r(LEMO), and Q_r refer to nucleophilic superdelocalizability, frontier orbital density in LEMO (lowest empty molecular orbital), and atomic charge, respectively, at atom r, and the parameter H_f is the calculated heat of formation (in kcal/mol). However, since eq 25 does not represent a very significant correlation and since H_f of eq 26 was found to have good correlation with $\log P$ ($r = 0.71$), the electronic parameters seem to be of only secondary importance. Further, since separate parameters of varying atoms have been shown to be correlated in the two equations, it seems difficult to draw any conclusion regarding the mechanistic aspect of the compounds.

3. Triazenes

Triazenes, $R-N=N-NR_1R_2$ belong to that class of alkylating agents which require activation by a metabolizing enzyme of the host. A common belief regarding their mechanism of action is that they produce, by microbial oxidation, a carbonium ion (CH_3^+), which finally alkylates DNA (Scheme 2).[65] But the involvement of CH_3^+ in DNA alkylation is more favored for carcinogenic action of triazenes rather than their antitumor activity.[66] Thus these

Scheme 2. Microsomal Activation of Phenyltriazenes

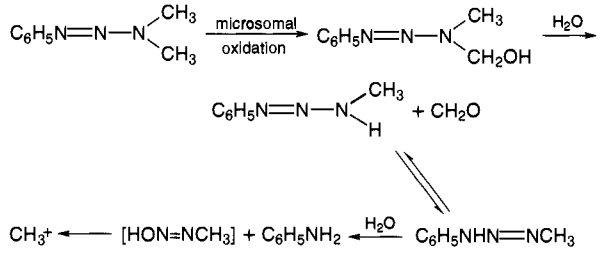
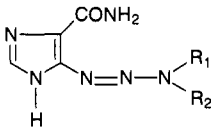


Table 5. Activity of Triazenoimidazole Carboxamides (XII) against L1210 Leukemia in Mice (T/C = 150)[58]

compd	R_1	R_2	log(1/C)	log P
1	H	C_2H_5	2.96	−0.39
2	CH_3	CH_2CH_2OH	2.99	−0.79
3	CH_3	CH_3	3.26	−0.24
4	CH_3	C_5H_{11}	3.44	1.76
5	CH_3	c-C_6H_{11}	3.70	1.77
6	CH_3	$CH_2C_6H_5$	3.70	1.54
7	CH_3	$CH_2CH(CH_3)_2$	3.75	1.06
8	CH_3	$CH_2CH_2CH_3$	3.75	0.79
9	CH_3	$CH(CH_3)CH_2CH_3$	3.84	1.08
10	CH_3	$(CH_2)_3CH_3$	3.90	1.26
11	CH_2CH_2Cl	CH_2CH_2Cl	4.33	1.54

compounds are highly carcinogenic and consequently there is little support to design triazenes as anticancer drugs. Assuming that QSAR might help, some workers attempted this direction.

The first series of triazenes that was subjected to a QSAR study belonged to XII (triazenoimidazolecarboxamide). For the compounds as given in Table



XII

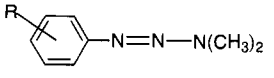
5, the activity against L1210 leukemia (T/C = 150) was found to be significantly correlated with log P as[58]

$$\log(1/C) = 3.454(\pm0.18) + 0.586(\pm0.24)\log P - 0.276(\pm0.19)(\log P)^2$$

$$n = 10,\ r = 0.929,\ s = 0.146,\ \log P_o = 1.1 \quad (27)$$

The last compound was not included in the derivation of eq 27 as it had considerably high activity compared to the predicted one. In this compound, both the substituents were CH_2CH_2Cl; hence it can be assumed that it acted as a nitrogen mustard which would not require metabolic activation.

For a series of 1-phenyl-3,3-dimethyltriazenes (XIII,



XIII

Table 6), the activity against Sarcoma-180 ascitic tumor (T/C = 130) was found to be correlated with electronic parameter σ of substituent at phenyl ring (eq 28).[67] The toxicity was, however, also found to be correlated with σ (eq 29)[67] but not as significantly

Table 6. Activity of Phenyltriazenes (XIII) against Sarcoma-180 Ascitic Tumor in Mice (T/C = 130) and Their Toxicity and Electronic Constants[67]

compd	R	log-(1/C)	log-(1/LD_{50})	σ	F	R
1	H	3.42	2.84	0.00	0.00	0.00
2	3-CH_3	3.37	2.91	−0.07	−0.04	−0.13
3	4-CN	2.91	2.56	0.66	0.51	0.19
4	3-Cl	3.16	2.68	0.37	0.41	−0.15
5	3-CF_3	3.18	2.80	0.43	0.38	0.19
6	3-COOH	3.01	2.55	0.37	0.33	0.15
7	3-SCH_3	3.33	2.76	0.15	0.20	−0.18
8	4-F	3.34	2.99	0.06	0.43	−0.34
9	4-n-C_3H_7	3.56	2.91	−0.13	−0.06	−0.08
10	3-$NHCOCH_3$	3.45	2.70	0.21	0.28	−0.26
11	4-COC_6H_5	3.16	2.82	0.43	0.30	0.16
12	4-C_6H_5	3.43	2.66	−0.01	0.08	−0.08
13	4-CH=CHCOOH	2.79	2.57	0.90	−0.15	1.04
14	4-NO_2		3.00	0.78	0.67	0.16

as the activity. The considerable differences in slopes and in significance of eqs 28 and 29 gave some hope

$$\log(1/C) = 3.41(\pm0.03) - 0.69(\pm0.09)\sigma$$

$$n = 13,\ r = 0.92,\ s = 0.09 \quad (28)$$

$$\log(1/LD_{50}) = 2.84(\pm0.04) - 0.33(\pm0.10)\sigma$$

$$n = 14,\ r = 0.69,\ s = 0.11 \quad (29)$$

to separating the toxic compounds from the active compounds, but when the Swain–Lupton resonance and field constants R and F were used instead of σ, the correlations obtained for activity and toxicity were not much different (eqs 30 and 31),suggesting

$$\log(1/C) = 3.42(\pm0.05) - 0.77(\pm0.18)F_p - 0.68(\pm0.11)R_p - 0.63(\pm0.20)F_m$$

$$n = 13,\ r = 0.91,\ s = 0.11 \quad (30)$$

$$\log(1/LD_{50}) = 2.87(\pm0.04) - 0.55(\pm0.13)F_p - 0.68(\pm0.11)R_p - 0.41(\pm0.18)F_m$$

$$n = 14,\ r = 0.82,\ s = 0.10 \quad (31)$$

that separation of toxic and antitumor congeners would be difficult. Equations 30 and 31 indicate that electron-releasing substituent at any position will equally affect both the toxicity and the antitumor activity.

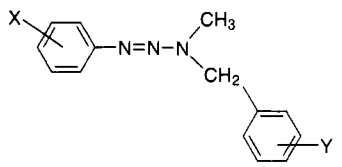
For a few of 4-substituted analogues of Table 6, the antitumor activity was shown by Lepsi et al.[68] to be correlated with Kier's first-order valence molecular connectivity index ($^1\chi^v$),[69] a parameter that signifies the degree of branching or connectivity in a molecule, as exhibited by eq 32. Although this parameter was

$$\log(1/C) = 14.125 - 7.809(^1\chi^v) + 1.331(^1\chi^v)^2$$

$$n = 6,\ r = 0.89,\ s = 0.041 \quad (32)$$

found by Lepsi et al.[68] to be related to log P, a parabolic correlation (eq 32) based on only six data points cannot be conclusive.

Dunn and Greenberg[70] synthesized a series of 1-phenyl-3-benzyl-3-methyltriazenes (XIV)and found by a discrimant analysis that their activity against



XIV

Sarcoma-180 ascitic tumor was governed by the electron-releasing nature of the X substituent and the electron-withdrawing and lipophilic characters of the Y substituent. These findings of Dunn and Greenberg were in agreement with their earlier findings[67] regarding the role of electronic character of substituent at 1-phenyl (eq 28) and with the findings of Hansch et al.[58] regarding the lipophilic character of substituent at 3-nitrogen (eq 27). For such a series of compounds, where substituents vary at both 1-phenyl and 3-nitrogen, the electron-releasing characteristic of the substituent at the former and the lipophilic character of the whole molecule were also found to significantly affect the toxicity (eq 33).[66] In

$$\log(1/\mathrm{LD}_{50}) = 3.490(\pm0.12) - 0.264(\pm0.16)\sigma^{+} - 0.024(\pm0.013)(\log P)^2$$

$$n = 11,\ r = 0.913,\ s = 0.11,\ \log P_o = 0 \quad (33)$$

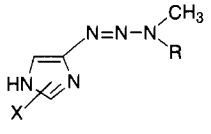
eq 33, the change in log P that really affected the toxicity was obviously due to the change in substituent at 3-nitrogen. σ^{+} denotes electron-releasing effect by direct resonance. For a small series of imidazolyltriazenes (XII) where variation was only at 3-nitrogen ($R_1 = R_2 = CH_3$ to C_5H_{11}),[71] Hansch et al. have shown how the toxicity was the function of log P only (eq 34).[66] In eq 34, the additional statistical parameter

$$\log(1/\mathrm{LD}_{50}) = 2.46(\pm0.42) + 0.23(\pm0.19)\log P$$

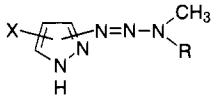
$$n = 5,\ r = 0.912,\ s = 0.185,\ F_{1,3} = 19.8 \quad (34)$$

F is F ratio between the variances of calculated and observed activities. For such a correlation where a very small number of data points has been used, this parameter has its own importance. Its high value shows the validity of the correlation. In eq 34, it is significant at 95% level [$F_{1,3}(0.05) = 10.13$].

Just like eq 27 obtained for the congeners of XII, Hatheway et al.[72] obtained eq 35 for another series of imidazolyltriazenes belonging to XV and acting



XV



XVI

against L1210 leukemia. For a series of pyrazolyltriazenes (XVI) also, the correlation obtained (eq 36) was almost of the same type except that an

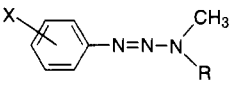
$$\log(1/C) = 3.507(\pm0.09) + 0.180(\pm0.10)\log P - 0.096(\pm0.04)(\log P)^2$$

$$n = 21,\ r = 0.780,\ s = 0.154,\ \log P_o = 0.93 \quad (35)$$

$$\log(1/C) = 3.610(\pm0.12) + 0.350(\pm0.17)\log P - 0.173(\pm0.09)(\log P)^2 - 0.349(\pm0.18)I$$

$$n = 13,\ r = 0.895,\ s = 0.131,\ \log P_o = 1.01 \quad (36)$$

indicator parameter I was also used for compounds where X was an ester group.[72] In both eqs 35 and 36, C was the concentration of drug producing $T/C = 140$. These studies and the earlier study of Hansch et al.[58] (eq 27) show that imidazolyl- or pyrazolyltriazenes acting against L1210 leukemia have their activity mainly dependent upon log P and that there is a common optimum log P around 1. For these compounds no electronic characteristic of X substituent at the ring was found to play any role. But as shown by Dunn et al.[67] (eqs 28 and 30), Hatheway et al.[72] also found that in case of the phenyltriazenes the electronic property of substituents at phenyl ring did play a difinite role in the activity of compounds and that, as observed by Dunn and Greenberg,[70] the lipophilicity of the molecule also governed the activity. Thus for a large series of congeners of XVII



XVII

acting against L1210 leukemia, these authors derived eq 37, where C was again drug concentration produc-

$$\log(1/C) = 4.124(\pm0.27) - 0.312(\pm0.11)\sum\sigma^{+} - 0.100(\pm0.08)\log P - 0.042(\pm0.02)(\log P)^2 - 0.178(\pm0.08)\mathrm{MR\text{-}2,6} + 0.391(\pm0.18)E_s\text{-R}$$

$$n = 61,\ r = 0.836,\ s = 0.191,\ \log P_o = 1.18 \quad (37)$$

ing $T/C = 140$. In this equation, the presence of molar refractivity index MR for substituents at ortho positions of 1-phenyl and the presence of Taft's steric parameter E_s for substituent at 3-nitrogen indicate that these substituents would produce steric effects.

That a substituent at 3-nitrogen in triazenes affects the activity through its hydrophobic character was also shown by Wilman et al.[73] by correlating the activity against TLX/5 lymphoma in mice of a small set of congeners of XVII, having X = p-$CONH_2$ and R = varying alkyl group or a benzyl group, with log P as

$$\log A_c = 0.447(\pm0.098) + 0.305(\pm0.028)\log P$$

$$n = 11,\ r = 0.965,\ s = 0.137 \quad (38)$$

where A_c = % ILS/drug concentration (μM/Kg). A connectivity index analysis of these triazenes by Srivastava et al.[74] had further indicated the importance of hydrophobic character of molecules in their antitumor activity. They had obtained eq 39 and shown that $^1\chi^v$ had a good correlation ($r = 0.93$) with chromatographic R_m values that measure the hydrophobicity of the compounds. In the derivation of eq 39,the authors did, however, not include the propyl

$$\log A_c = 0.23(^1\chi^v) - 0.07$$

$$n = 9, r = 0.84, s = 0.19 \quad (39)$$

and dodecyl derivatives and gave no explanation for this exclusion.

For some of these compounds, Wilman et al. also studied demethylation, alkylation (R substituent), and microsomal metabolism and found that data on these processes were also correlated with log P as

$$\% \text{ demethylation} = 37.5(\pm 2.2) - 6.18(\pm 0.67) \log P$$

$$n = 7, r = 0.972, s = 1.77 \quad (40)$$

$$\% \text{ dealkylation} = 39.2(\pm 2.6) - 1.68(\pm 0.22)(\log P)^2$$

$$n = 7, r = 0.957, s = 3.56 \quad (41)$$

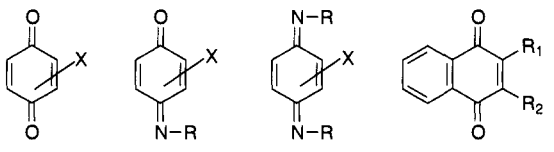
$$\% \text{ metabolism} = 65.2(\pm 3.3) - 2.35(\pm 0.28)(\log P)^2$$

$$n = 8, r = 0.959, s = 5.37 \quad (42)$$

These findings led the authors to suggest that antitumor activity of triazenes must be related with their oxidative metabolism. However, one should not rely much upon such equations where dependent variables are not in the log form and where the number of data points used are so small.

4. Quinones

Naturally occurring quinones have important biological functions including a role in oxidative phosphorylation and electron transfer.[75] Some natural quinones and many synthetic ones have the ability to inhibit the growth of tumors.[76] Quinones that act against animal tumors are thought to function as bioreductive alkylating agents.[77,78] They are supposed to undergo bioreduction in vivo to form *o*-quinone methides that alkylate DNA and other vital cellular components.[79] A variety of 1,4-benzoquinones (XVIII), their nitrogen analogues (XIX and XX), and 1,4-naphthoquinones (XXI) have been synthesized and tested for their antitumor activities, but QSAR studies on them are limited. A detailed QSAR



XVIII XIX XX XXI

study was made by Yoshimoto et al.[80] on a series of 2,5-bis(1-aziridinyl)-*p*-benzoquinones (Table 7) acting against lymphoid leukemia L1210 in BDF_1 mice. Two types of test were used (1) chronic treatment with daily injection for 12 days and (2) single injection on day one only. And for each type of test, two activity parameters were obtained (1) minimum effective dose (MED), i.e., the dose giving a 40% increase in life span (ILS), and (2) optimal dose (OD), i.e., the dose giving maximum ILS. In each case the dose reported was in moles per kilogram and with the use of data as given in Table 7, the best correlations obtained were as[80]

chronic injection

$$\log(1/\text{MED}) = 6.092(\pm 0.26) - 0.521(\pm 0.10)\pi_2 - 0.344(\pm 0.14)\text{MR}_1 - 1.784(\pm 1.12)F - 0.825(\pm 0.54)R$$

$$n = 37, r = 0.921, s = 0.262, F_{4,32} = 44.8 \quad (43)$$

$$\log(1/\text{OD}) = 5.383(\pm 0.25) - 0.352(\pm 0.09)\pi_2 - 0.290(\pm 0.13)\text{MR}_1 - 2.075(\pm 1.05)F - 1.165(\pm 0.51)R$$

$$n = 37, r = 0.894, s = 0.247, F_{4,32} = 31.7 \quad (44)$$

single injection

$$\log(1/\text{MED}) = 5.305(\pm 0.20) - 0.487(\pm 0.09)\pi_{1,2} - 3.954(\pm 1.05)F - 1.490(\pm 0.49)R$$

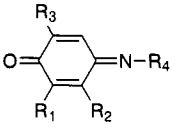
$$n = 35, r = 0.907, s = 0.288, F_{3,31} = 48.0 \quad (45)$$

$$\log(1/\text{OD}) = 4.707(\pm 0.18) - 0.366(\pm 0.07)\pi_{1,2} - 3.253(\pm 0.83)F - 1.391(\pm 0.43)R$$

$$n = 37, r = 0.913, s = 0.257, F_{3,33} = 54.8 \quad (46)$$

All of the above equations show that electron-releasing substituents at both the 3- and the 6-positions will increase both MED and OD through their field and resonance effects irrespective of the kind of the test performed. However, in chronic treatment the substituent at 6-position is found, because of negative coefficient of MR_1, to produce the steric effect, while in 1-day treatment substituents at both the 3- and the 6-positions are observed to have lipophilic effects. As for the most of the other alkylating agents discussed, the activities here also are found to have negative dependence on lipophilicity and this dependence was shown to be quite significant in all the cases (correlations with $\pi_{1,2}$ alone had $r = 0.85$, 0.79, 0.67, and 0.68, respectively).

The lipophilic and electronic parameters were also found to play important roles in the antitumor activity of some nitrogen analogues of 1,4-benzoquinones. Hodnett et al.[81] synthesized a series of derivatives of XXII and tested their activity against Sarcoma-180 ascitic tumors in Swiss mice. A dis-



XXII

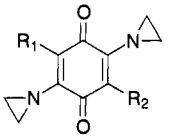
criminant analysis performed on 16 compounds had revealed eq 47,where the discriminant function y may

$$y = -6.30\sum\pi + 15.1\sum F + 0.66\sum R + 75.1E_{1/2} - 44.2$$

$$F_{4,11} = 7.43, F_{4,11}(0.005) = 6.88 \quad (47)$$

be used to discriminate highly active compounds (*T/C*

Table 7. Antitumor (Lymphoid Leukemia L1210 in BDF_1 Mice) Data of 2,5-Bis(1-aziridinyl)-*p*-benzoquinones[80]



			chronic inj		single inj	
compd	R_1	R_2	log(1/MED)	log(1/OD)	log(1/MED)	log(1/OD)
1	CH_3	$COCH_3$			3.94	3.48
2	C_6H_5	C_6H_5	4.43	4.14		3.53
3	CH_3	$(CH_2)_3C_6H_5$	4.47	4.21	3.93	3.60
4	C_5H_{11}	C_5H_{11}	4.63	4.52	4.07	3.62
5	$CH(CH_3)_2$	$CH(CH_3)_2$	4.77	4.59	4.36	4.14
6	CH_3	$CH_2C_6H_5$	4.85	4.69	4.74	4.26
7	C_3H_7	C_3H_7	4.92	4.44	4.32	4.14
8	CH_3	$CH_2OC_6H_5$	5.15	4.71	4.68	3.89
9	$(CH_2)OCON(CH_3)_2$	$(CH_2)_2OCON(CH_3)_2$	5.16	4.85		4.62
10	C_2H_5	C_2H_5	5.46	5.09	4.94	4.79
11	CH_3	$(CH_2)_2OCH_3$	5.57	5.42	5.19	5.12
12	OCH_3	OCH_3	5.59	5.17	4.81	4.32
13	CH_3	$CH(CH_3)_2$	5.60	5.21	4.96	4.69
14	C_3H_7	$CH(OCH_3)CH_2OCONH_2$	5.63	5.07	5.01	4.64
15	CH_3	$(CH_2)_2CON(CH_3)_2$			5.09	4.84
16	CH_3	CH_3	5.66	5.36	5.36	4.79
17	H	$CH(CH_3)_2$	5.68	5.37	5.16	4.59
18	CH_3	$CH(OCH_3)CH_2CH_3$	5.68	5.33	5.26	4.84
19	C_3H_7	$(CH_2)_2OCONH_2$	5.68	5.23	4.90	4.42
20	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	5.69	5.31	5.18	4.71
21	C_2H_5	$CH(OC_2H_5)CH_2OCONH_2$	5.76	5.24	5.40	4.64
22	CH_3	$(CH_2)_2OCOCH_3$	5.78	5.78		
23	CH_3	$(CH_2)_3$ dimer	5.82	5.39		
24	CH_3	C_2H_5	5.86	5.37	5.16	4.52
25	CH_3	$CH(OCH_2CH_2OCH_3)CH_2OCONH_2$	6.03	5.39	5.45	4.96
26	CH_3	$CH_2CH(CH_3)OCONH_2$	6.14	5.79	5.86	5.18
27	C_2H_5	$CH(OCH_3)CH_2OCONH_2$	6.16	5.22	5.62	4.92
28	CH_3	$CH(C_2H_5)CH_2OCONH_2$	6.18	5.66	6.03	5.20
29	CH_3	$CH(OC_2H_5)CH_2OCONH_2$	6.18	5.22	5.53	4.62
30	CH_3	$(CH_2)_3OCONH_2$	6.18	5.93	5.55	5.48
31	CH_3	$(CH_2)_2OCONH_2$	6.21	5.75	5.03	5.46
32	C_2H_5	$(CH_2)_2OCONH_2$	6.25	5.48	5.98	4.88
33	CH_3	$(CH_2)_2OH$	6.39	5.79	5.89	5.25
34	CH_3	$CH(CH_3)CH_2OCONH_2$	6.41	5.71	5.93	5.31
35	CH_3	$CH(OCH_3)CH_2OCONH_2$	6.41	5.66	5.81	5.03
36	H	$N(CH_2)_2$	6.45	6.19	6.02	5.74
37	$(CH_2)_2OH$	$(CH_2)_2OH$	6.54	6.05	5.93	5.60
38	CH_3	$N(CH_2)_2$	6.77	6.21	6.54	5.69
39	CH_3	$CH(OCH_3)CH_2OH$	6.90	5.75	6.05	5.27

> 175) from moderately active compounds (T/C < 175). But since the equation uses four independent variables, including a new parameter, redox potential ($E_{1/2}$), for just 16 data points and has a very poor value for F test, its validity may be questionable. However, for an extended series of these compounds, Hodnett and Wacharayothin[82] quantitatively correlated the antitumor data as

$$\log(1/ED_{25}) = 0.99(\pm0.12) + 0.57(\pm0.06)f_{R_4} + 0.58(\pm0.18)R_{R_4}$$

$$n = 23, r = 0.90, s = 0.27, F_{2,20} = 41.8 \quad (48)$$

$$\log(1/OD) = 0.85(\pm0.11) + 0.55(\pm0.06)f_{R_4} + 0.53(\pm0.17)R_{R_4}$$

$$n = 23, r = 0.90, s = 0.26, F_{2,20} = 42.1 \quad (49)$$

where f is Rekker's fragment value for hydrophobicity.[83] The two equations represent almost parallel correlations and exhibit that only the R_4 substituent attached to the nitrogen will affect the activity and that its hydrophobic and electron-withdrawing nature would have a positive effect. These properties of the R_4 substituent were, however, shown to equally affect the toxicity too (eq 50).[82]

$$\log(1/LD_{50}) = 1.45(\pm0.11) + 0.50(\pm0.06)f_{R_4} + 0.76(\pm0.17)R_{R_4}$$

$$n = 23, r = 0.88, s = 0.26, F_{4,20} = 9.95 \quad (50)$$

Again for a series of 1,4-naphthoquinones (XXI) acting against Sarcoma-180 of Swiss mice, a discriminant analysis was performed and eq 51 was obtained,[84] which meets the same criticism as eq 47.

$$y = -18.6\sum\pi + 7.30\sum MR + 6.30(\sum\pi)^2 - 392\,E_{1/2} - 52.8$$

$$F_{4,10} = 4.38, F_{4,10}(0.05) = 3.48 \quad (51)$$

But in a quantitative analysis for a series of similar

Table 8. Biological and Chemical Data of Some 1,4-Naphthoquinones (XXI) Acting against Sarcoma-180 Ascitic Tumor in Mice[85]

compd	R_1	R_2	ED_{25} (mg/kg)[a]	LD_{50} (mg/kg)[a]	$E_{1/2}$ (V)
1	H	H	1.90	5.5	−0.164
2	OH	C_5H_9	141.0	400	−0.347
3	Cl	NHC_6H_5	94.0	1600	−0.272
4	Cl	Cl	4.89	30	−0.146
5	Cl	NH_2	25.5	800	−0.382
6	Cl	OCH_3	2.49	14	−0.184
7	Cl	OC_2H_5	1.97	16	−0.192
8	Cl	$NHCOCH_3$	2.97	11	−0.165
9	OH	H	33.0	100	−0.330
10	CH_3	H	30.0	150	−0.224
11	OCH_3	H	49.3	320	−0.283
12	Cl	O-*n*-C_3H_7	2.45	20	−0.195

[a] Converted to millimoles per kilogram (mM/kg) for regression.

compounds (Table 8), Hodnett et al.[85] showed that activity of such compounds could be well correlated with only the redox potential (eq 52). However, the toxicity of these compounds was also found to be well correlated with $E_{1/2}$ in the same manner (eq 53) but

$$\log(1/ED_{25}) = 5.82(\pm1.65) + 31.0(\pm13.7)E_{1/2} + 45.7(\pm26.1){E_{1/2}}^2$$

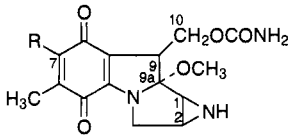
$$n = 12, r = 0.86, s = 0.406, F_{2,9} = 12.73 \quad (52)$$

$$\log(1/LD_{50}) = 4.84(\pm1.84) + 28.3(\pm15.3)E_{1/2} + 38.4(\pm29.2){E_{1/2}}^2$$

$$n = 12, r = 0.86, s = 0.454, F_{2,9} = 10.11 \quad (53)$$

the occurrence of squared term of $E_{1/2}$ in both eqs 52 and 53, particularly with the same sign as that of $E_{1/2}$, seems meaningless. In fact there was no significant effect in the correlations on the deletion of this term. In both the cases the linear correlations obtained had $r = 0.81$, suggesting simply that an increase in the redox potential will lead to an increase in both anticancer activity as well as toxicity.

A number of attempts have been made to study the structure−activity relationship of mitomycins (XXIII). The effectiveness of mitomycin C (XXIII, R



XXIII

= NH_2) as a clinical antitumor agent has stimulated the preparation and evaluation of hundreds of mitomycins,[86] but initial attempts on QSAR study on them did not give encouraging results.[87-90] However, in one of their studies, Sami et al.[88] were able to find a correlation (eq 54) between the antitumor activity

$$\log(1/C) = 5.69 - 0.96\pi$$

$$n = 13, r = 0.92, s = 0.33 \quad (54)$$

of some N^7-aryl-substituted mitomycin C analogues (XXIII, R = XC_6H_4NH) and π of the para substituent (X) of the aryl ring. The activity was against P388 leukemia in mice and the concentration C was for $T/C = 125$. Later, in a recent study, Kunz et al.[91] obtained the following equations for compounds in Table 9:

mitomycin C analogues (compounds **1**−**19**)

$$\log(1/C) = 11.50 + 9.45E_{1/2}$$

$$n = 19, r = 0.73, s = 0.48, F_{1,17} = 19.4 \quad (55)$$

mitomycin A (R = CH_3O) analogues (compounds **20**−**30**)

$$\log(1/C) = 8.58 + 0.44 \log P$$

$$n = 11, r = 0.80, s = 0.46, F_{1,9} = 15.9 \quad (56)$$

combined group (all compounds)

$$\log(1/C) = 10.1 + 6.59E_{1/2} + 0.35 \log P$$

$$n = 30, r = 0.83, s = 0.46, F_{2,27} = 30.3 \quad (57)$$

In these equations C was the average of in vitro assays (IC_{50}) against three different human tumor cell lines, WiDr colon, 2780 ovarian, and MCF-7 breast cancer. For a comparative QSAR study, these authors however also evaluated the activity of these compounds against P388 leukemia in culture[91] and performed the regression analysis to obtain the

Table 9. Activities of Mitomycin Analogues (XXIII) against Human Tumor and P388 Leukemia Cells in Culture and Their Physicochemical Parameters[91]

[91] compd	R	log-(1/C)[a]	log-(1/C)[b]	log P	$E_{1/2}$ (V)	σ
	Mitomycin C Analogues					
1	NH_2	7.70	7.29	−0.38	−0.45	
2	$HO(CH_3)NH$	6.98	6.34	0.10	−0.42	
3	$HC{\equiv}CCH_2NH$	8.46	8.01	0.24	−0.41	
4	tetrahydrofurfuryl-NH	7.13	7.46	0.21	−0.41	
5	2-furyl-$(CH_2)_2NH$	7.34	7.58	1.90	−0.42	
6	2-pyridyl-$(CH_2)_2NH$	7.38	7.27	1.23	−0.41	
7	C_6H_5NH	8.78	8.77	1.30	−0.33	
8	4-NH_2-C_6H_4NH	7.83	7.73	0.07	−0.38	0.00
9	4-F-C_6H_4NH	8.67	8.52	1.44	−0.32	−0.66
10	4-Br-C_6H_4NH	8.72	8.72	2.16	−0.32	0.06
11	3-I-C_6H_4NH	8.90	8.85	2.42	−0.31	0.23
12	4-I-C_6H_4NH	8.77	8.79	2.42	−0.33	0.35
13	4-OH-C_6H_4NH	7.88	8.23	0.63	−0.37	0.18
14	4-NO_2-C_6H_4NH	9.07	9.36	1.02	−0.28	−0.37
15	3-I-4-OH-C_6H_3NH	7.76		1.75	−0.33	0.73
16	4-OH-3-NO_2-C_6H_3NH	7.71		0.51	−0.32	
17	5-indolyl-NH	8.75		2.45	−0.36	
18	4-methylthiazolyl-NH	8.69	9.30	1.52	−0.26	
19	3-pyrazolyl-NH	7.38	7.81	0.56	−0.38	
	Mitomycin A Analogues					
20	CH_3O	9.52	9.61	0.26	−0.21	
21	*c*-C_3H_5O	9.20	9.20	0.83	−0.21	
22	*c*-$C_3H_5CH_2O$	9.43	9.51	1.35	−0.21	
23	*c*-$C_4H_7CH_2O$	9.66	9.39	2.47	−0.20	
24	$C_6H_5CH_2O$	9.21	9.75	1.94	−0.21	
25	$HO(CH_2)_2O$	8.31	9.15	−1.10	−0.21	
26	$C_6H_5O(CH_2)_2O$	9.48	10.08	1.74	−0.21	
27	$HO(CH_2)_2O(CH_2)_2O$	7.32	7.73	−1.08	−0.21	
28	$CH_3O(CH_2)_2O(CH_2)_2O$	8.24	8.39	−0.46	−0.21	
29	$C_6H_5S(CH_2)_2O$	9.16	8.92	2.38	−0.21	
30	$HO(CH_2)_2SS(CH_2)_2O$	8.65	8.73	0.36	−0.21	

[a] Against human tumor cells in culture. [b] Against P388 leukemia cells in culture.

following equations which are closely parallel to those obtained for human tumor cells:

mitomycin C analogues (compounds **1**–**14**, **18**, and **19**)

$$\log(1/C) = 13.3 + 14.4E_{1/2}$$

$$n = 16, r = 0.91, s = 0.36, F_{1,14} = 63.7 \quad (58)$$

mitomycin A analogues (compounds **20**–**30**)

$$\log(1/C) = 8.88 + 0.32 \log P$$

$$n = 11, r = 0.62, s = 0.55, F_{1,9} = 5.52 \quad (59)$$

combined group (all compounds)

$$\log(1/C) = 10.84 + 8.63E_{1/2} + 0.32 \log P$$

$$n = 27, r = 0.87, s = 0.46, F_{2,24} = 36.2 \quad (60)$$

Kunz et al.[91] were also able to correlate their earlier data[89,90] on mitomycin A analogues (Table 9) against P388 leukemia in mice with a better estimate of log P as given in Table 9, while in their previous studies they had not found any correlation.[89,90] Now the equation obtained was

$$\log(1/C) = 7.07 - 0.56 \log P$$

$$n = 11, r = 0.84, s = 0.50, F_{1,9} = 22.0 \quad (61)$$

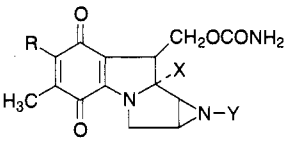
which shows the negative dependence of activity on log P. On the other hand, not withstanding eq 54, Kunz et al.[91] found that activity of N^7-aryl-substituted mitomycin C analogues against human tumor cells in culture was correlated with σ and not with π of the substituent at the aryl ring, and for compounds 7–14 (Table 9) the equation obtained was

$$\log(1/C) = 8.46 + 0.92\sigma$$

$$n = 8, r = 0.89, s = 0.22, F_{1,6} = 23.4 \quad (62)$$

This difference in the correlations of two assay systems is surprising and suggests that the uptake and the mechanism of action of drugs in the two systems may be entirely different. Further, the factors determining the activity is found to be dependent upon the type of mitomycin analogues (C or A). Equations 55–59 show that while the activity of mitomycin C analogues will depend upon reduction potential, that of mitomycin A analogues will depend upon lipophilicity. The reason for this difference may be that since mitomycin A analogues are readily reduced, it would be their ability to reach the receptor site that would only matter for their activity, and since mitomycin C analogues are not so susceptible to bioreduction, it would be their reduction potential that would really matter for them more than their ability to reach the site of action. There was another report on the relationship of reduction potential of mitomycin C analogues and their cytotoxicity to HCT-116 human colon carcinoma cells.[92] However, when mitomycin A analogues act against leukemia cells in mice, the activity is found to have a negative dependence upon log P (eq 61), suggesting that hydrophilicity rather than hydrophobicity would be a determining factor. This may be due to the suspension of mice leukemia cells in ascities, which is an aqueous environment confined to abdominal cavity. Therefore it would be desired that for effectiveness of the drug, a large portion of its concentration applied remains in the aqueous environment, which is possible for only highly hydrophilic drugs.

For mitomycin analogues having substituents at more than one position (XXIV),a discriminant analysis was performed on their activity against Sarcoma-180 tumor in mice by Moriguchi and Komatsu.[93] For a set of 16 compounds the discriminant function obtained was as shown by eq 63. In this equation,



XXIV

$$y = -4.33F_R - 2.64V_{w,R} + 0.77E_{s\text{-}Y} + 2.48I_1 + 2.28I_2 + 1.42 \quad (63)$$

V_w is the van der Waals volume, and I_1 and I_2 are two dummy parameters with a value of 1 each for X = OCH_3 and OH, respectively, and 0 for any other X substituent. Although this equation is not based on any large number of data points, its goodness of fit was highly significant at 0.001 level by χ^2 test and was able to give 93.8% correct prediction. On the basis of this equation, therefore, Moriguchi and Komatsu indicated that while electron-releasing character of R group may increase the activity, its bulkiness may also produce the steric effect. The steric effect was indicated to be produced by Y substituent also, but OCH_3 and OH groups at X position were shown to give positive effect. Through a pattern recognition analysis of these mitomycin analogues, using first the adaptive least-squares approach[94] and then Fuzzy adaptive least-squares approach,[95] Moriguchi et al. suggested that electron-donating and less bulky substituents for R, electron-withdrawing groups for X and thin substituents for Y were favorable for the antitumor activities of these compounds, and it was further verified by a recent study made by Liu et al.[96] using functional-link net in QSAR.

The functional-link net is a neural computing method, which is derived from the simplified concept of the brain in which a number of nodes called processing elements or neurons are interconnected in a netlike structure. The characteristics of the neural network have been found to be suitable for data processing in which the relationship between the cause and its results cannot be exactly defined. Thus, its use in biologically-related responses is strongly suggested.[97] The functional-link net developed by Liu et al.[96] could be applied to 2,5-bis(1-aziridinyl)-*p*-benzoquinones (Table 7) also, and the QSAR equation obtained was as[96]

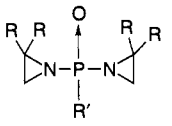
$$I_q = 1.894 \cos(\pi\pi_{1,2}) + 0.757 \cos(\pi MR_1) + 2.438 \cos(\pi F) - 3.938R$$

$$n = 37, r = 0.951, s = 0.169, F_{4,32} = 76.4 \quad (64)$$

which seems to be better than eq 44 obtained by simple multiple regression analysis. In this equation, I_q stands for the internal value of the node, the activity parameter scaled into the range of 0.0 to 1.0, and π is the usual angle constant (180°).

5. Miscellaneous

For a series of [bis(1-aziridinyl)phosphonyl]carbamates (XXV)acting against Walker 256 carcinoma



XXV

in rats, the anticancer activity was shown[41] to be correlated with Taft's electronic parameter (σ^*) of aliphatic substituent (eq 65). The toxicity of these compounds was also found to be correlated with σ^* (eq 66) but not as significantly as the activity.

$$\log(1/ED_{50}) = 2.74\sigma^*_R + 3.34$$

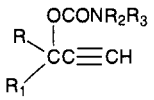
$$n = 10, r = 0.945, s = 0.26 \quad (65)$$

$$\log(1/LD_{50}) = 1.73\sigma^*_R + 3.03$$

$$n = 10, r = 0.77, s = 0.39 \quad (66)$$

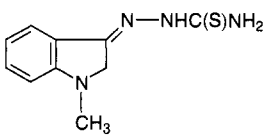
Equations 65 and 66 show that only the R substituent will affect the activity through its electronic property, but unfortunately this substituent was only H or CH_3. Thus even an indicator parameter would have given the same results; hence, it is difficult to say that it is only the electronic property of the substituent that is determining the activity.

By a Free–Wilson analysis[98] on a series of acetylenic carbamates (XXVI) studied by Dillard et al.,[99] Purcell and Clayton[100] observed that 2-naphthyl, 4-fluorophenyl, or phenyl groups or a combination of them at nitrogen, and cyclopentyl, cyclohexyl, or cycloheptyl groups or a combination of them at 1,1-(2-propynyl) positions will provide highly active compounds.



XXVI

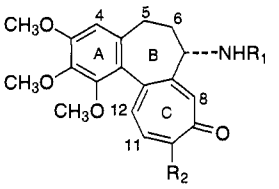
N-Methylisatin-3-thiosemicarbazone (XXVII), an antiviral compound, was used as a model for the design of nitrogen mustards.[54] A QSAR study on a small series of such nitrogen mustards has been already discussed (eq 20). But the correlation was obtained for a very small number of compounds excluding three of nitro-substituted derivatives and including a few compounds that did not have alkylating potential. Hence, the validity of this correlation is questionable.



XXVII

B. Mitotic Inhibitors

Colchicine (XXVIII; $R_1 = CH_3CO$, $R_2 = CH_3O$) which occurs naturally in the autumn crocus, *Colchicum autumnale L*, is a potent mitotic inhibitor. Its



XXVIII

antitumor property long since has been recognized, but its use in the treatment of neoplasms is still limited.[101] Its activity is thought to be due to its ability to bind to a cysteine residue in tubulin polypeptide chain, thus preventing cell division.[102] It has been suggested that for antimitotic activity cholchicines should possess at least one methoxy group on ring A, the amine group on ring B, and a methoxy, alkylamino, or alkylthio group on ring C, which should be necessarily a seven-membered ring.[103] In order to find optimum characteristics of antitumor colchicines, Quinn and Beisler[104] attempted a QSAR on a series of colchicine analogues (Table 10). The NCI data for the first 16 compounds against P388

Table 10. Activity (against P388 Leukemia in Mice)[104] and Toxicity[105] Data of Colchicines (XXVIII)

compd	R_1	R_2	log-(1/*C*)	log-(1/LD_{50})	log *P*
1	CH_3CO	CH_3O	6.46	5.46	1.03
2	CH_3CO	CH_3O	5.82	5.82	0.93
3	H	CH_3O	4.69	3.71	1.10
4	CH_3	CH_3O	4.66	3.63	1.53
5[a]		CH_3O	4.03	4.21	2.07
6	$ClCH_2CO$	CH_3O	6.78	5.54	1.71
7	FCH_2CO	CH_3O	7.13	5.69	1.19
8	HCO	CH_3O	6.63	5.16	1.02
9	C_6H_5CO	CH_3O	5.76	4.24	2.94
10	CH_3CO	$CH_3OCOCH(NH_2)-(CH_2)_4NH$	3.61	3.00	−2.10
11	$ClCH_2CO$	CH_3S	6.74	5.65	2.34
12	$HOCH_2CO$	CH_3S	6.94	5.66	1.50
13	C_2H_5OCO	CH_3S	6.24	4.19	3.12
14	α-L-arabinosyl	CH_3S	4.39	3.98	1.40
15	β-D-glucosyl	CH_3S	4.39	3.71	1.25
16	β-D-glucosyl, $(CH_3CO)_4$	CH_3S	3.81	3.81	4.61
17	CH_3CO	CH_3S	6.77	5.62	1.66
18	CH_3CO	$C_6H_5CH_2S$	4.76	4.22	3.21
19	$Cl(CH_3)_2NHCO$	CH_3S	4.93	4.93	3.53
20	H	CH_3S	4.57	3.75	1.73
21	CH_3CO	$(C_2H_5)_2N$	5.43	4.64	2.56
22	CF_3CO	CH_3O	6.36	5.73	2.36
23	*p*-$NO_2C_6H_4CO$	CH_3O	5.29	4.41	2.96
24	CH_3CO	CH_3NH	6.52	5.72	0.58
25	CF_3CO	CH_3S	6.23	5.51	2.99

[a] In this compound the C_7 carbon is substituted with a dimethylamino group, having the same stereochemical configuration as colchicine.

leukemia in mice were found to be correlated with log P as[104]

$$\log(1/C) = 4.06(\pm0.48) + 0.73(\pm0.22)\log P - 0.27(\pm0.08)(\log P)^2 + 2.15(\pm0.54)I$$
$$n = 15, r = 0.954, s = 0.385, \log P_o = 1.32 \quad (67)$$

where C is the molar concentration to give a 40% increase in life span and I is an indicator parameter used with a value of 1 for $R_1 = CH_3O$. In deriving eq 68, compound **16** was not included, because it was thought to be a prodrug of **15**, hydrolyzing rapidly to the latter in the presence of esterases. Its inclusion had given a slightly inferior correlation (eq 68).

$$\log(1/C) = 4.13(\pm0.62) + 0.67(\pm0.28)\log P - 0.19(\pm0.07)(\log P)^2 + 1.17(\pm0.59)I$$
$$n = 16, r = 0.927, s = 0.499, \log P_o = 1.76 \quad (68)$$

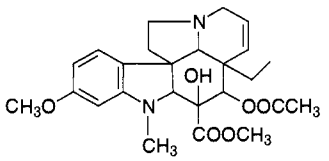
To test the validity of eq 67, Quinn and Beisler synthesized and tested[104] some additional compounds (**17**–**25**) and found that they could be easily included in this equation. Thus, again excluding compound **16**, the equation obtained was

$$\log(1/C) = 4.11(\pm0.42) + 0.70(\pm0.22)\log P - 0.30(\pm0.08)(\log P)^2 + 2.16(\pm0.44)I$$
$$n = 24, r = 0.932, s = 0.412, \log P_o = 1.17 \quad (69)$$

Equations 67 and 69 suggested that the principal determining factor of antitumor activity of colchines is log P with an optimum value of 1.0 to 1.5 and that the presence of an acyl group at R_1 position will be beneficial. However, in a later study, Quinn et al.[105] found that the toxicity of these compounds was as significantly correlated with log P (eq 70) as the antitumor activity. Equation 70 closely parallels eq 69, suggesting that activity would be highly correlated with toxicity. Consequently, the use of col-

$$\log(1/LD_{50}) = 3.54(\pm0.41) + 0.58(\pm0.22)\log P - 0.24(\pm0.08)(\log P)^2 + 1.71(\pm0.43)I$$
$$n = 24, r = 0.904, s = 0.401, \log P_o = 1.19 \quad (70)$$

chicine as an antitumor drug will remain limited. So was the case with *Vinca* alkaloids of which vindoline (XXIX) is an example. For a series of such alkaloids,



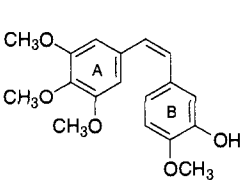
XXIX

eq 71 obtained for the activity (against P388) luekemia in mice) and eq 72 obtained for the toxicity[106] were closely parallel, and they reproduced almost equal log P_o values. K_d in these equations is the drug–tubulin binding constant.

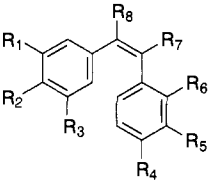
$$\log(1/C) = 1.06\log P - 0.22(\log P)^2 + 0.52\log K_d - 5.37$$
$$n = 10, r = 0.85, \log P_o = 2.4 \quad (71)$$

$$\log(1/LD_{50}) = 0.52\log P - 0.13(\log P)^2 + 0.48\log K_d - 4.65$$
$$n = 10, r = 0.80, \log P_o = 2.0 \quad (72)$$

A novel class of antimitotic agents, combretastin (XXX) and its analogues (XXXI), were also studied for their antitumor activity.[107] In XXXI, substituents



XXX



XXXI

were only H, OH, OCH_3, or $OCOCH_3$. A QSAR study on the antitumor activity of these analogues against L1210 murine leukemia revealed the following equation:[108]

$$\log(1/C) = -1.29(\pm0.59)\sum\mu_b + 1.39(\pm0.48)B_t - 0.89(\pm0.64)N_{OH} - 3.27(\pm1.05)(\sum\pi_b)^2 - 4.67(\pm1.56)\sum\pi_b - 1.84$$
$$n = 25, r = 0.91, s = 0.50, F_{5,19} = 37.82 \quad (73)$$

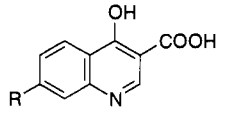
where $\sum\mu_b$ is the vector summation of the group dipole moments of ring B, B_t is the bond type (single or double) of the spacer between the two phenyl rings, N_{OH} is the number of free hydroxy groups on ring A, and $\sum\pi_b$ is the sum of the hydrophobicity values of the functional group on ring B. Of all these parameters, only B_t, which has been given a value of unity for the double bond and zero for the single bond, is shown to have the positive effect on the activity, suggesting that the unsaturation of the linker will favor the activity. The unsaturation of the linker will restrict the free rotation of the two phenyl rings and thus keeping them in the proper orientation toward the active sites of tubulin. It was observed that cis isomers were comparatively more active than the trans ones.[107] The cis isomers of combretastins, where $R_1 = R_2 = R_3 = OCH_3$, have some structural similarity with colchicine, hence they are thought to fit the binding sites on the receptor in the same manner as the colchicine.

Like mitotic inhibitors, the inducers of leukemia cell differentiation may also be useful as antitumor agents. A number of alkyl derivatives of formamide, acetamide, and urea have been found to promote the terminal differentiation of a number of animal and human solid and leukemia tumor cells. In a study by Langdon and Hickman[109] on a series of these derivatives, a linear correlation ($r = -0.937$) was observed between the molecular weight of the compounds and the logarithm of their concentration required to bring about the differentiation of the

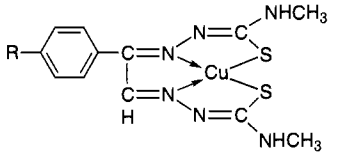
greatest number of cells, and a recent study on these compounds by Harpalani et al.[110] suggested that there was almost an even contribution toward activity from steric interactions, electrostatic potential, and molecular weight.

C. Cellular Respiration Inhibitors

7-Substituted 4-hydroxyquinoline-3-carboxylic acids (XXXII) and some copper(II) chelates of structure XXXIII have been studied for their ability to inhibit the respiration of Ehrlich ascites cells.[20-23] In their



XXXII



XXXIII

preliminary study on 7-substituted 4-hydroxyquinoline-3-carboxylic acids (Table 11, compounds **1**−**15**), Shah and Coats[22] found that the cell inhibition activity of these compounds had a significant correlation with π (eq 74). In the derivation of eq 74,compound **15** was excluded because it was poorly

$$\log(1/C_{50}) = 3.22(\pm0.16) + 0.46(\pm0.11)\pi$$
$$n = 14, r = 0.933, s = 0.28 \quad (74)$$

fit in the equation. The reason for this may be a poor estimate of its π value. While for all other compounds π was estimated by authors themselves, for compound **15** it was taken from the literature.

However, for an extended series (Table 11), Coats et al. found[23] that π was not able to afford a good correlation; rather, another parameter of lipophilicity, the HPLC retention index (RI), showed good correlation (eq 75). Compound **15** was not found to

$$\log(1/C_{50}) = 2.05(\pm0.35) + 0.21(0.05)\mathrm{RI}$$
$$n = 19, r = 0.92, s = 0.31 \quad (75)$$

be well fit in eq 75 also, and hence it was excluded.

Since some cancer cells have been found to exhibit abnormal levels of lactate and malate dehydrogenases,[16-19] the compounds of Table 11 were also studied for the inhibition of these enzymes.[20-23] The enzyme inhibition activities were then shown to be well correlated with molar refractivity index (MR)[22,23] or with van der Waals volume (V_w) or molecular connectivity index (χ)[111,112] and not with π. This shows that interaction of drugs with enzymes may involve dispersion interaction, but inhibition of cell respiration would totally depend upon the transport of the drug across the cell membrane. The QSAR of enzyme inhibition will be presented later.

A principal component analysis on some polar compounds of this series (Table 11, compounds **2**, **5**, **6**, **9**, and **11**−**14**) indicated that activity against the whole cell test system cannot be directly attributed to the inhibition of the enzymes.[113] The enzyme systems were reflected by the first component that could be identified with only polar and steric parameters, the hydrophobic effect being almost absent, and the second component was entirely due to the inhibition of the ascites cell that depended primarily on the hydrophobicity.[113]

Table 11. Physicochemical Properties and Ehrlich Ascites Cell Respiration Inhibition Activity of 7-Substituted 4-Hydroxyquinoline-3-carboxylic Acids (XXXII)

compd	R	log(1/C)	π	RI	MR[a]
1	H	2.98	0.00	4.80	0.103
2	Cl	3.84	0.55	6.18	0.603
3	F	3.30	0.06	5.67	0.092
4	OCH_3	3.28	0.49	5.87	0.787
5	$COCH_3$	3.10	−0.39	4.48	1.118
6	$N(CH_3)_2$	3.33	1.10	6.79	1.555
7	$OCH_2C_6H_5$	4.41	1.81	8.51	3.174
8	$OCH_2C_6H_3$-3,4-Cl_2	4.82	3.23	10.79	4.174
9	NO_2	3.24	−0.40	5.32	0.736
10	$CONH_2$	2.24	−1.18	2.84	0.981
11	COOH	2.24	−2.80	1.81	0.605
12	SO_2CH_3	2.75	−1.39	3.25	1.349
13	OH	3.04	0.06	3.89	0.285
14	SO_2NH_2	2.47	−1.36	3.08	1.228
15[b]	SO_3	2.88	−4.76	1.21	0.971
16	$OCH_2C_6Cl_5$	4.37	5.34	13.70	5.664
17	OCH_2-α-$C_{10}H_7$	4.07	3.13	9.26	4.715
18	$OCH_2C_6H_4$-4-F	3.78	1.95	8.70	3.163
19	$OCH_2C_6H_4$-4-Br	4.27	2.67	9.86	3.959
20	$OCH_2C_6H_4$-4-OC_6H_5	4.00	3.89	11.23	5.839

[a] Scaled by 0.1. [b] Not included in the correlations.

For copper(II) chelates, however, Coats et al.[20,21] found the negative dependence of cell respiration inhibition activity on hydrophobicity. These authors also studied the ability of chelates to inhibit the respiration of rat liver slices as a normal cell model[20,21] and found, for a set of 14 analogues of XXXIII, a combined set of their two studies, that liver cell and ascitic cell inhibition activities will have the correlation with hydrophobic and electronic parameters as[21]

$$\log(1/C_{50})_{\mathrm{liver}} = 2.60(\pm0.16) - 0.18(\pm0.08)\pi$$
$$n = 14, r = 0.82, s = 0.26 \quad (76)$$

$$\log(1/C_{50})_{\mathrm{ascites}} = 5.09(\pm0.22) - 0.48(\pm0.19)\pi - 0.11(\pm0.05)\pi^2 - 1.22(\pm0.56)\sigma_p$$
$$n = 14, r = 0.91, s = 0.28, \pi_o = -2.18 \quad (77)$$

$$\log(1/C_{50})_{\mathrm{ascites}} - \log(1/C_{50})_{\mathrm{liver}} = 2.27(\pm0.27) - 0.26(\pm0.24)\pi - 0.10(\pm0.07)\pi^2 - 0.65(\pm0.44)\sigma_p^+$$
$$n = 14, r = 0.81, s = 0.37, \pi_o = -1.30 \quad (78)$$

In eq 78, the resonance electron-donating parameter σ^+ was found to be better than the simple Hammett constant σ. However, in their first study on a small set of compounds, Coats et al.[20] obtained eq 79 which uses σ only and is free from π^2 term.

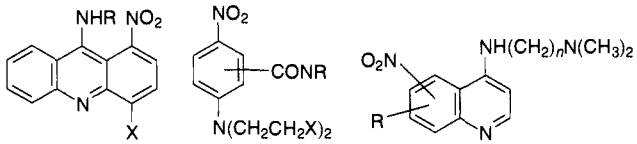
$$\log(1/C_{50})_{\mathrm{ascites}} - \log(1/C_{50})_{\mathrm{liver}} = 2.42 - 0.54(\pm0.36)\pi - 1.03(\pm0.81)\sigma_p$$
$$n = 8, r = 0.92, s = 0.30 \quad (79)$$

From these studies, Coats et al. suggested that the desired selective cytotoxicity against Ehrlich ascites

tumor cells with minimal cytotoxicity to rat liver slices could be achieved by introducing substituents which would enhance water solubility, while stabilizing the chelate against premature dissociation by electron donation through conjugated ring system, and predicted from eq 78 that π_0 of -1.30 would provide the greatest selectivity and minimize liver cell toxicity.

D. Hypoxia-Selective and Radiosensitizing Agents

Recently, a variety of compounds were studied for their hypoxia-selective antitumor activity.[114-118] These compounds were substituted nitracrines (XXXIV),[114-116] nitroaniline mustards (XXXV),[117] and 4-(alkylamino)nitroquinolines(XXXVI).[118] Through all



XXXIV XXXV XXXVI

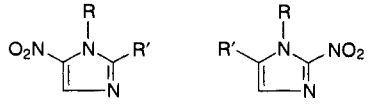
these studies, it was pointed out that cytotoxicity of these compounds was due to nitro group reduction and subsequent molecular adduct formation. Since rapid metabolism of these compounds may prevent their activity against hypoxic cells in solid tumors, it was shown that electron-donating substituent can be used to increase metabolic stability in vitro. Such stabilization may enhance the therapeutic utility of these compounds in cancer chemotherapy. However, Denny et al. did not show any direct correlations existing between hypoxia-selective cytotoxicity of these agents and any physicochemical parameter of substitutents.

Analogues of XXXVI were also studied for their radiosensitizing potency.[118] For a set of 12 compounds with substituent(s) CH_3, OCH_3, or $NHCH_3$ along with NO_2 at varying positions, this potency was shown to be correlated with a one-electron reduction potential E_1 of nitro group as[118]

$$\log(1/C_{1.3}) = 6.41 + 5.55(\pm 2.13)E_1$$

$$n = 12, r = 0.63 \qquad (80)$$

where $C_{1.3}$ is concentration (μM) required to increase radiation sensitivity by a factor of 1.3 when Chinese hamster ovary cells (subline AAB) are exposed to the drug for 30 min before and during irradiation under hypoxic conditions. Although this correlation is not very significant, it suggests that the reduction potential of nitro group would be an important factor in radiosensitizing potency of the compounds. In a study on nitroimidazoles (XXXVII and XXXVIII)



XXXVII XXXVIII

against hypoxic bacterial cells (*Escherichia coli* and *S. lactis*), it was shown[30] that this potential along with log P was able to afford very significant correlation as shown by eqs 81 and 82 for *E. coli* and *S. lactis*, respectively, and in a study on a large series of nitroaromatics and nitroheterocyclics against Chinese hamster cells, Adams et al.[29] found that reduc-

$$\log(1/C_{1.7}) = 9.32(\pm 1.06)E_1 + 0.25(\pm 0.05)\log P + 6.71(\pm 0.41)$$

$$n = 9, r = 0.97, s = 0.16 \qquad (81)$$

$$\log(1/C_{1.7}) = 9.53(\pm 2.47)E_1 + 1.49(\pm 0.12)\log P + 6.74(\pm 0.97)$$

$$n = 9, r = 0.91, s = 0.36 \qquad (82)$$

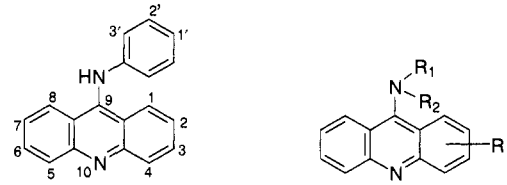
tion potential alone was able to account for about 74% of the variance in radiosensitizing activity of compounds (eq 83).

$$\log(1/C_{1.4}) = 7.01(\pm 0.64)E_1 + 6.48(\pm 0.26)$$

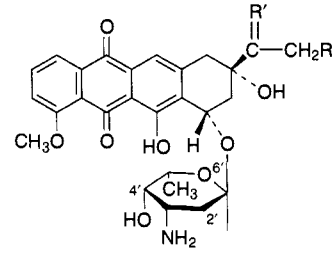
$$n = 42, r = 0.86, s = 0.28 \qquad (83)$$

E. Agents Binding to DNA

Majority of drugs that bind to DNA bind by intercalation, and widely studied intercalators are either the derivatives of 9-anilinoacridine (XXXIX) or 9-aminoacridine (XL), or analogues of anthracycline (XLI).[31] Some bleomycins (XLII) and certain



XXXIX XL

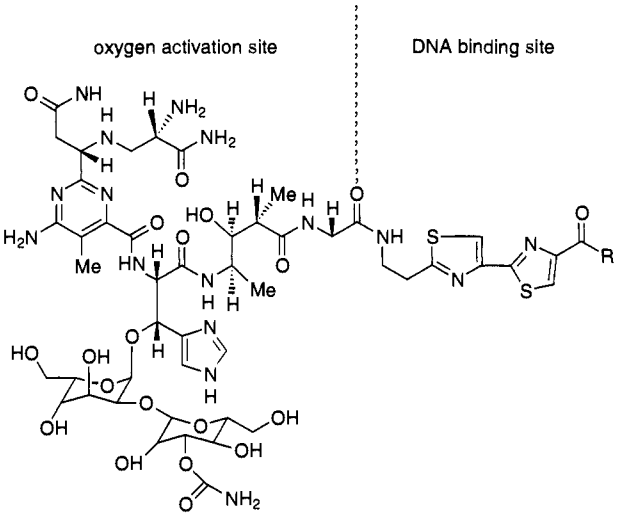


XLI

miscellaneous compounds have also been studied for their intercalating ability.[31] These intercalators have also been widely subjected to QSAR studies which are described below.

1. 9-Anilinoaridines

A large number of derivatives of 9-anilinoacridines have been prepared and tested for their antitumor activities by Denny and co-workers. These authors compiled the data of their several studies, and for a



XLII: bleomycin A_2; R = $NH(CH_2)_3\overset{+}{S}Me$

bleomycin B_2; R = $NH(CH_2)_4NH{-}\overset{\overset{NH_2}{\|}}{C}{-}NH_2$ (+)

set of 509 compounds obtained the equation[119]

$$\log(1/ED_{50}) = 3.73(\pm0.07) - 0.14(\pm0.03)\sum\pi - 0.01(\pm0.006)(\sum\pi)^2 - 1.08(\pm0.09)\sum\sigma - 1.25(\pm0.37)R_{BS} - 0.32(\pm0.16)MR_2 + 1.04(\pm0.13)MR_3 - 0.25(\pm0.05)(MR_3)^2 - 0.77(\pm0.13)I_{3,6} - 1.68(\pm0.21)E_{s,3'} - 1.60(\pm0.22)(E_{s,3'})^2 + 0.78(\pm0.13)I_{NO_2} + 0.70(\pm0.32)I_{DAT} + 0.50(\pm0.18)I_{BS}$$

$$n = 509,\ r = 0.878,\ s = 0.323,\ \sum\pi_o = -6.04,\ (MR_3)_o = 2.09,\ (E_{s,3'})_o = -0.53 \quad (84)$$

In this series of compounds, there were variety of substituents at varying positions of all the rings of XXXIX and in the above equation $\sum\pi$ and $\sum\sigma$ refer to the sum of π values and the sum of σ values, respectively, of all substituents in a compound. I_{NO_2}, I_{DAT}, and I_{BS} are three indicator parameters used with a value of 1 each for 3-NO_2, 3,3-dialkyltriazene, and 1′-$NHSO_2C_6H_5$, respectively, and R_{BS} is Swain and Lupton parameter for group at the para position of the latter. The other variables occurring in the equation have their usual meanings. The activity was against L1210 leukemia in mice, where ED_{50} refers to the drug dose in moles per kilogram per day providing a 50% increase in life span. A bilinear model was also obtained[119] with only a slight improvement in the correlation ($r = 0.893$). Both eq 84 and bilinear model exhibited hydrophobic, electronic, and steric effects in the antitumor activity of 9-anilinoacridines and also exhibited specific role played by 3-NO_2, 3,3-dialkyltriazene, and 1′-$NHSO_2C_6H_5$ substituents.

Compounds with 1′-$NHSO_2CH_3$, i.e., derivatives of 4′-(9-acridinylamino)methanesulfonanilide (AMSA), were found to act against a number of animal tumor systems and one member of this class, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulfonamide (m-AMSA, amsacrine) (XXXIX 1′-$NHSO_2CH_3$, 3′-OCH_3) was selected by NCI as NSC 249992 for preclinical evaluation[120] and has now finally come into clinical use. For this member and a number of its analogues, the antitumor activities (L1210 assay) were found to be governed mainly by lipophilic–hydrophilic balance measured by chromatographic R_m values (eqs 85 and 86).[121] In eq 85 the indicator

$$\log(ILS_{max}) = 2.05 - 0.55(\pm0.08)R_m^2 + 0.08(\pm0.04)I$$

$$n = 78,\ r = 0.83,\ s = 0.09,\ F_{2,75} = 82.9 \quad (85)$$

$$\log(1/ED_{40}) = 4.46 - 0.34(\pm0.28)R_m - 1.47(\pm0.48)R_m^2 + 0.91(\pm0.15)\log(t_{1/2})$$

$$n = 50,\ r = 0.92,\ s = 0.23,\ F_{3,46} = 83.7 \quad (86)$$

parameter I is equal to 1 for 3-NO_2 or 4-CONHR and zero for all other substituents. In the derivation of eq 86, all 3-NO_2 derivatives were excluded, as they were showing behavior which was probably due to their concomitant in vivo reduction. The $t_{1/2}$ in this equation is the half-life of the compounds. The toxicity of these compounds was found to be poorly correlated with the variables used in eq 86.

In a subsequent QSAR study on a smaller set of AMSA analogues, Ferguson and Denny[122] derived eqs 87 and 88 and for a slightly bigger series of amsacrine analogues, where the substituents were only at 1-, 2-, or 3-position, Baguley et al.[123] derived eq 89,where

$$\log(ILS_{max}) = 2.07 - 0.83(\pm0.38)R_m^2$$

$$n = 18,\ r = 0.73,\ s = 0.12,\ F_{1,16} = 18.6 \quad (87)$$

$$\log(1/ED_{40}) = 0.85 - 3.06(\pm2.14)R_m^2 + 0.55(\pm0.28)pK_a$$

$$n = 23,\ r = 0.76,\ s = 0.54,\ F_{2,20} = 13.6 \quad (88)$$

$$\log(1/ED_{40}) = 1.32(\pm0.35)\log K_{AT} - 2.64(\pm1.14)R_m^2 - 3.34$$

$$n = 48,\ r = 0.80,\ s = 0.44,\ F_{2,45} = 39.0 \quad (89)$$

K_{AT} is the drug–DNA binding constant measured for the binding of the compound to poly[d(A-T)]. While eqs 87 and 88 are only marginally significant, eq 89 suggests that role of R_m values in the activity of amsacrines cannot be ignored.

Attempts were made to find the physicochemical parameters related to the drug–DNA binding constant. For only 3-substituted analogues of amsacrine, Baguley et al.[123] obtained eq 90, correlating K_{AT} with σ_p and MR, but this equation expressed a very poor correlation. However, for a series of anilino-ring-substituted analogues, these authors obtained[124] a better correlation (eq 91) between the binding constant and σ and MR, but eq 91 included only 1′- and 2′-substituted derivatives and not the 3′-substituted ones. For the latter, the K_{AT} was found to have some correlation with only steric parameter E_s (eq 92),

suggesting that 3′-substituent may produce some steric effect in the binding.

$$\log K_{AT} = 5.56 - 0.34(\pm 0.34)\sigma_p + 0.036(\pm 0.031)\mathrm{MR}$$

$$n = 18, r = 0.68, s = 0.30, F_{2,15} = 6.5 \quad (90)$$

$$\log K_{AT} = 6.00 - 0.49(\pm 0.07)\sigma + 0.70(\pm 0.28)\mathrm{MR}_{1'}$$

$$n = 42, r = 0.92, s = 0.10, F_{2,39} = 112.4 \quad (91)$$

$$\log K_{AT} = 0.16(\pm 0.11)E_s + 5.33$$

$$n = 10, r = 0.71, s = 0.11, F_{1,9} = 8.1 \quad (92)$$

In eq 91, while σ values were used for both the substituents, MR values were used only for 1′-substituents. $MR_{2'}$ was not found to be significant. Thus electron-donating substituents at 1′- and 2′-positions appear to strengthen the binding and also the former may be involved in some dispersion interaction. For a series of 3- and 5-substituted amsacrine derivatives studied by Denny et al.,[125] the dispersion interaction appeared to be a major factor in the DNA binding when the binding constants were found to be correlated with van der Waals volume V_w (eqs 93 and 94).[126] However, it is obvious from eqs

$$\log K_{AT} = 2.524(\pm 1.225)V_{w,3} + 5.423$$

$$n = 20, r = 0.714, s = 0.238, F_{1,18} = 18.75 \quad (93)$$

$$\log K_{GC} = 1.770(\pm 0.635)V_{w,3} + 1.064(\pm 0.215)V_{w,5} + 5.567$$

$$n = 20, r = 937, s = 0.120, F_{2,17} = 16.20 \quad (94)$$

93 and 94 that while binding to poly[d(G-C)] through dispersion interactions will depend upon the size of both 3- and 5-substituents, the binding to poly[d-(A-T)] will depend only upon the size of the former.

For this series of compounds, the in vitro antitumor activity (inhibition of growth of cultured L1210 cells) was shown by Denny et al.[125] to be correlated with only pK_a ($r = 0.80$), but Gupta et al.[126] found that hydrophobic property of 5-substituent was also an important factor in the cell inhibition and the correlation obtained by them was

$$\log(1/IC_{50}) = 0.197(\pm 0.127)pK_a + 0.308(\pm 0.148)\pi_5 + 6.039$$

$$n = 20, r = 0.90, s = 0.279, F_{2,17} = 36.40 \quad (95)$$

In one of their studies, Atwell et al.[127] showed that in vivo activities of amsacrine analogues against P388 leukemia and Lewis lung (LL) carcinoma were well correlated with in vitro activities (inhibition of growth of cultured L1210 or human colon tumor (HCR-8) cells). The equations obtained by them were

$$\log(1/ED_{50})_{P388} = 7.07 + 1.08(\pm 0.16)\log(1/IC_{50})_{L1210} - 1.17(\pm 0.41)R_m$$

$$n = 31, r = 0.80, s = 0.36 \quad (96)$$

$$\log(1/ED_{50})_{LL} = 1.97 + 0.44(\pm 0.10)\log(1/IC_{50})_{L1210} + 1.41(\pm 0.41)R_m$$

$$n = 22, r = 0.86, s = 0.19 \quad (97)$$

$$\log(1/ED_{50})_{P388} = 6.04 + 0.52(\pm 0.14)\log(1/IC_{50})_{HCT\text{-}8}$$

$$n = 34, r = 0.54, s = 0.50 \quad (98)$$

$$\log(1/ED_{50})_{LL} = 6.03 + 0.72(\pm 0.07)\log(1/IC_{50})_{HCT\text{-}8}$$

$$n = 22, r = 0.92, s = 0.14 \quad (99)$$

These equations exhibit that, in combination with R_m values, the in vitro L1210 assay can be a good predictor of in vivo activities in both the systems, but HCT-8 assay can be a good predictor of in vivo activity against only Lewis lung carcinoma (eq 99) and not against P388 leukemia (eq 98). The R_m values were of no consequence in eqs 98 and 99. In all the equations ED_{50} stands for the dose leading to 50% ILS.

Denny and Cain[128] also made a very systematic study on antitumor activity of some carboxylic acid derivatives of 9-anilinoacridine and found that, for homologous 1′-$(CH_2)_nCOOH$ congeners of XXXIX, there was a very good parabolic correlation between maximum increase in life span (ILS_{max}) in L1210 tests and the R_m values (eq 100). The corresponding carboxamides [1′-$(CH_2)_nCONH_2$] also provided a similar parabolic correlation (eq 101)[128] and so did some 1′-$NHSO_2(CH_2)_nCH_3$ congeners and their 3-NH-$COCH_3$ derivatives.[129] When these compounds and

$$\log(ILS_{max}) = 8.86(\pm 2.22)R_m - 7.50(\pm 1.94)R_m^2 - 0.28$$

$$n = 9, r = 0.96, s = 0.27, R_{m,o} = 0.59 \quad (100)$$

$$\log(ILS_{max}) = 0.32(\pm 0.30)R_m - 1.32(\pm 0.56)R_m^2 + 2.04$$

$$n = 8, r = 0.93, s = 0.09, R_{m,o} = 0.12 \quad (101)$$

some sulfonamides [1′-$(CH_2)_nSO_2NH_2$] were treated along with carboxamides, eq 102 was obtained[128] which was exactly identical to eq 101 showing that all these derivatives can be put into one group.

$$\log(ILS_{max}) = 0.32(\pm 0.15)R_m - 1.32(\pm 0.26)R_m^2 + 2.04$$

$$n = 25, r = 0.93, s = 0.07, R_{m,o} = 0.12 \quad (102)$$

In a different study on 9-anilinoacridine bearing 1′-$NH((CH_2)_nCH_3$, or 1′-$NHSO_2(CH_2)_nCH_3$ with or without 3′-OCH_3/3-$NHCOCH_3$/4-$CONH_2$, Ferguson and Denny[130] found that toxicity to bacteria (*Salmonella typhimurium*) also of these compounds had a good relation with R_m (eq 103) and that compounds having variation at only 1′-position had their mutagenicity related to the toxicity (eq 104). In these equations, D_{50} was the molar concentration causing

$$\log D_{50} = 1.56 - 1.65(\pm 0.48)R_m$$

$$n = 28, r = 0.79, s = 0.40 \quad (103)$$

$$\log M_{50} = 1.73(\pm 0.54) \log D_{50} + 0.23$$

$$n = 11, r = 0.91, s = 0.43 \quad (104)$$

50% inhibition of bacterial growth and M_{50} was mutation frequency at this dose.

Some of the QSAR works mentioned here were reviewed by Denny and co-workers themselves[131] and the conclusion drawn was that, since SAR for antileukemic activity parallels SAR for intercalative DNA binding, useful substitution patterns for one tumor class may carry over to another, given the common mode of action. However, the differences seen between P388 and LL activities for some compounds suggest that drug design for antitumor agents is still far from an exact science.

2. 9-Aminoacridines

A great number of nitro derivatives of 9-aminoacridines (XL) were synthesized and studied for their biological activities. Among the various derivatives studied, 1-nitro-9-aminoacridines were found to possess high cytotoxic activity and antitumor properties.[132-134] One of these compounds, 1-nitro-9-[[3'-(dimethylamino)propyl]amino]acridine [XL; R = 1-NO_2, R_1 = H, R_2 = $CH_2CH_2CH_2N(CH_3)_2$], has been used clinically in Poland under the name of nitracrine.

Investigations on the mode of action of 1-nitro-9-aminoacridines demonstrated that nitracrine is a latent form of the drug which upon metabolic activation[135] binds covalently to DNA and other cellular macromolecules.[136] The ability of this compound to produce covalent interstrand cross-links has been postulated to represent the crucial event responsible for its antitumor properties.[137,138] However, nitracrine also reveals undesirable side effects,[139-141] which limit its use in human therapy. For that reason new 9-aminoacridines were searched out and subjected to QSAR studies.[142,143]

In an initial QSAR study on a series of N-substituted 1-nitro-9-[(3-aminopropyl)amino]acridine derivatives, Mazerska and Ledochowsky[142] found the antitumor activity of these compounds against Sarcoma-180 in mice to be correlated with the hydrophobicity of the substituents (eq 105). The series was

$$\log(1/ED_{50}) = 0.083(\pm 0.066)\pi - 0.090(\pm 0.033)\pi^2 + 2.470(\pm 0.098)$$

$$n = 14, r = 0.887, s = 0.107, F_{2,11} = 20.31 \quad (105)$$

later extended[143] and studied for four in vitro activities, antitumor activity against S-180 in mice, and toxicities on healthy as well as tumor-bearing mice. The in vitro activities were antimicrobial activity against *Saccharomyces cerevisie* FL5991B activity against plant sprouts, inhibition of the dehydrogenase enzyme, and the activity against HeLa tissue culture. All these activities were subjected to principal components PC1 and PC2. The PC1 was formed by all seven different activities and was thus called general biological activity. The PC2, on the other hand, was formed only by the therapeutic index, log (LD_{50}/ED_{50}), where LD_{50} refers to the toxicity data in healthy mice and ED_{50} to the antitumor data. Thus PC2 refers to the selectivity.

When principal component scores ($PC1_{sc}$ and $PC2_{sc}$) were subjected to multiple regression analysis, the following correlations were obtained:

$$PC1_{sc} = 0.30(\pm 0.11) - 0.80(\pm 0.08) \log P - 0.25(\pm 0.03)(\log P)^2$$

$$n = 28, r = 0.970, s = 0.23, F_{2,25} = 201 \quad (106)$$

$$PC2_{sc} = 0.028(\pm 0.018)({}^2K) - 0.122(\pm 0.07)I_{N2N} - 0.124(\pm 0.101)$$

$$n = 28, r = 0.762, s = 0.07, F_{2,25} = 17.33 \quad (107)$$

In eq 107, ${}^2K$ is a second-order shape index of the substituent, which increases for large substituents but decreases for branched ones,[144] and I_{N2N} is an indicator variable defining the presence or absence of a two carbon atom chain between proximal and distal nitrogens in the side chains. It was given a value of 1 for two carbon atom chain and 0 for three or more carbon atom chain. Now eq 106 is in agreement to eq 105, but while eq 105 correlates the hydrophobicity with only one biological activity, eq 106 correlates the same property with the general activity suggesting that the transport through the lipid barrier is a significant factor in general activity, eq 107, however, suggests that the selectivity, i.e., the therapeutic index will be favored by high ${}^2K$ value, necessitating a large and preferably unbranched substituent at 9-position, and by a three or more methylene spacers between proximal and distal nitrogen atoms in this substituent. Equation 106 gives that the optimum value of log P for the general activity should be between −1 and −2 (log P_o = −1.60).

3. Anthracyclines

Anthracyclines (XLI) are the most outstanding intercalating antitumor drugs. Among them, daunorubicin (XLI; R = H, R′ = O) and adriamycin (XLI; R = OH, R′ = O) have become important drugs for cancer chemotherapy.[145-147] These drugs are also called daunomycin and doxorubicin, respectively. Their usefulness has generated considerable interest in developing analogues with improved properties. Among these analogues, rubidazone (XLI; R = H, R′ = $NNHCOC_6H_5$) has received considerable attention. People were encouraged by its decreased cardiotoxicity relative to adriamycin.[148,149] Tong et al.[150] therefore studied the physical and biological properties of a series of rubidazone analogues (Table 12). They attempted a QSAR study also on them and found that there was no substituent effect on in vivo or in vitro cytotoxicity potency of the compounds but their cardiotoxicity was closely correlated with the electronic character of phenyl substituents.[150] The correlation obtained for all 11 compounds of Table 12 was as shown by eq 108, which had further improved (eq 109) with the exclusion of the last two

Table 12. Physicochemical and Biological Data for Rubidazone Analogues (XLI; R = H, R′ = NNHCOC$_6$H$_4$-X)[150]

compd	X	DNA, RNA synth inhibn[a] (ED_{50}, μM) DNA	RNA	MCCD,[b] μM/kg	σ_p
1	4-NMe_2	2.6	2.5	16	−0.83
2	4-OH	3.6	1.9	29	−0.37
3	4-OMe	3.1	1.6	34	−0.27
4	4-Et	2.6	1.1	28	−0.15
5	4-C_6H_5	1.9	1.0	53	−0.01
6	H	2.2	1.2	35	0.0
7	4-Cl	1.9	1.0	67	0.23
8	3,4-Cl_2	2.1	1.7	53	0.23
9	3-NO_2	3.2	2.3	44	0.0
10[c]	H (R = OH)	14	8.8	46	0.0
11[c]	3,4-Cl_2 (R = OH)	6.6	3.6	20	0.23

[a] In cultured L1210 cells. [b] Minimum cumulative cardiotoxic dose. [c] For these compounds R = OH. Not included in the derivation of eq 109.

compounds. These two compounds were in fact adriamycin phenylhydrazones and not exactly rubidazone analogues. In these equations, MCCD stands

$$\log(\text{MCCD}) = 0.43(\pm 0.23)\sigma_p + 1.6(\pm 0.30)$$

$$n = 11, r = 0.77, s = 0.12 \qquad (108)$$

$$\log(\text{MCCD}) = 0.53(\pm 0.07)\sigma_p + 1.63(\pm 0.19)$$

$$n = 9, r = 0.94, s = 0.065 \qquad (109)$$

for minimum cumulative cardiotoxic dose in rat. These equations show that an electron-withdrawing group on the phenyl ring will reduce the cardiotoxicity of the compounds. However, for a different series of anthracyclines, where whole C(R′)CH_2R groups at 9-position varied and some of the compounds had at 3′-position substituents other than NH_2 group (XLI), the cardiotoxicity was shown by Fink et al.[151] to be affected by the lipophilicity of the molecule. Equation 110 obtained by them expresses that a less lipophilic molecule will be more cardiotoxic. In this equation, the indicator variable I_o

$$\log(1/\text{MCCD}) = 4.82(\pm 0.22) - 0.30(\pm 0.11)\log P + 1.01(\pm 0.25)I_o + 0.69(\pm 0.33)I_1 + 0.74(\pm 0.34)I_2$$

$$n = 21, r = 0.934, s = 0.181 \qquad (110)$$

was used, with a value of 1, for congeners in which 4-OCH_3 was converted to OH or H; $I_1 = 1$ indicated compounds having a very lipophilic hydrazone substituent at position 9, and $I_2 = 1$ was meant for an alkylated amino group at 3′-position. For these compounds antitumor activity (B-16 melanoma) was also found to have similar dependence on the lipophilicity and the indicator variables (eq 111).[151] In eq 111, C is the dose (in mol/kg) producing a T/C of 125 against B-16 melanoma in mice.

$$\log(1/C) = 6.57(\pm 0.32) - 0.41(\pm 0.13)\log P + 0.48(\pm 0.35)I_o + 0.81(\pm 0.38)I_1$$

$$n = 23, r = 0.874, s = 0.288 \qquad (111)$$

The positive effect of the I_o parameter on both the cardiotoxicity and the antitumor activity was attributed to the electronic effect of H and OH group at the 4-position. As is obvious from the equations, this effect was, however, more significant in cardiotoxicity than in antitumor activity. The parameter I_1, on the other hand, indicated that large lipophilic hydrazone moieties at 9-position will produce almost equal effect on both the activities, but it was not clear whether this effect was due to really lipophilicity of the substituents or their volume, as the two properties were collinear. Since the I_2 parameter did not appear in eq 111, it was suggested that an alkylated amino group in place of NH_2 at 4-position would increase only the cardiotoxicity and not the antitumor activity. However, the two equations show that an increase in the overall lipophilicity of the molecule will lead to an equivalent decrease in both the activities. From these correlations, therefore, the authors suggested that separation of antitumor and cardiotoxic compounds might not be feasible.

For just three anthracyclines, Kessel[152] had observed that their cytotoxicity to L1210 cells in culture was parallel to log P. An attempt was made to substantiate this observation recently by Hoffman et al.[153] These authors examined cytotoxic activities of several natural and semisynthetic anthracyclines against L1210 leukemia and two human colon tumor cells (Colon 4, HT 29) in vitro after short (1 h) and long (7 days) incubation times and correlated first the activities against L1210 cells with log P as shown by eqs 112 and 113. Of these equations, eq 113

$$\log(1/IC_{50})_{1h} = 1.40\log P - 0.63(\log P)^2 + 1.15$$

$$n = 11, r = 0.80, s = 0.32 \qquad (112)$$

$$\log(1/IC_{50})_{7d} = 0.91\log P - 0.29(\log P)^2 + 1.64$$

$$n = 11, r = 0.67, s = 0.37 \qquad (113)$$

expresses a poor correlation, but it was significantly improved when the DNA-binding constant (K) was also introduced (eq 114). This binding constant, however, did not produce much improvement in the correlation expressed by eq 112 (eq 115). Since

$$\log(1/IC_{50})_{7d} = 0.81\log P - 0.11(\log P)^2 + 0.41\log K - 1.29$$

$$n = 11, r = 0.90, s = 0.22 \qquad (114)$$

$$\log(1/IC_{50})_{1h} = 1.41\log P - 0.64(\log P)^2 + 0.23\log K - 0.48$$

$$n = 11, r = 0.85, s = 0.28 \qquad (115)$$

authors have not reported t-test or confidence limits for variables, it cannot be said how significant the variables really are. For colon tumor cells, the correlations obtained were

Colon 4

$$\log(1/IC_{50})_{7d} = 0.83 \log P - 0.30(\log P)^2 + 0.46 \log K - 1.49$$
$$n = 11, r = 0.86, s = 0.25 \quad (116)$$

$$\log(1/IC_{50})_{1h} = 0.86 \log P - 0.32(\log P)^2 + 0.24 \log K - 0.75$$
$$n = 11, r = 0.87, s = 0.18 \quad (117)$$

HT 29

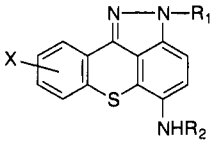
$$\log(1/IC_{50})_{7d} = 1.16 \log P - 0.43(\log P)^2 + 0.35 \log K - 0.90$$
$$n = 11, r = 0.79, s = 0.35 \quad (118)$$

$$\log(1/IC_{50})_{1h} = 0.83 \log P - 0.30(\log P)^2 + 0.20 \log K - 0.93$$
$$n = 11, r = 0.71, s = 0.28 \quad (119)$$

All these correlation studies of Hoffmann et al. suggest that some additional factors are to be sought to significantly account for the variation in cytotoxic activities. This suggestion of Hoffmann et al. was in full agreement to the finding of Nakata and Hopfinger,[154] who had reexamined the correlation obtained by Fink et al. (eq 111). They calculated the global minimum intercalating energy (IE) and incorporated this in the regression to obtain eq 120 which expresses better correlation than eq 111 even after including those compounds which were misfit in eq 111 and so excluded. This shows that intercalation

$$\log(1/C) = 0.246 \log P - 0.030(\log P)^2 - 0.423(IE \times 10) - 0.27$$
$$n = 29, r = 0.901, s = 0.289, \log P_o = 4.17 \quad (120)$$

energy is an important parameter for the antitumor activity of anthracyclines. For a series of benzothiopyranoindazoles (XLIII),the free-space (no solvent)



XLIII

intercalation binding energy (E), and the aqueous desolvation energy of the intercalation complex (ΔE_D) were found to be the prime factors for the antitumor activity as shown by eq 121.[155]

$$\log(1/C) = -0.46(\pm 0.02)(E + \Delta E_D) - 0.46$$
$$n = 14, r = 0.97, s = 0.13 \quad (121)$$

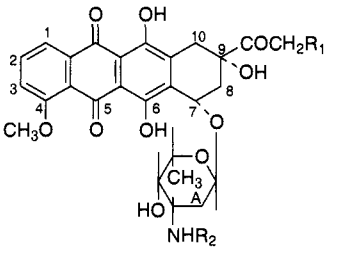
In a recent study, Fachetti et al.[156] attempted to correlate the antitumor activities of some anthracyclines, having the substituents at both chromophore and sugar moiety, with reverse-phase HPLC retention index (RI). The cytotoxicities of compounds in doxorubicin-sensitive (LoVo) and doxorubicin-resistant (LoVo/Dx) human cell lines were shown to be correlated with RI as

$$\log(1/ID_{50})(LoVo) = 6.44(\pm 1.05) \log RI - 1.83(\pm 0.30)(\log RI)^2 - 6.48(\pm 0.81)$$
$$n = 17, r = 0.853, s = 0.451, F_{2,14} = 18.69 \quad (122)$$

$$\log(1/ID_{50})(LoVo/Dx) = 5.67(\pm 1.26) \log RI - 1.45(\pm 0.36)(\log RI)^2 - 7.47(\pm 0.97)$$
$$n = 17, r = 0.817, s = 0.539, F_{2,14} = 14.08 \quad (123)$$

Since the parameter RI is a measure of hydrophobicity, eqs 122 and 123 show that hydrophobicity may be a major factor in the activity of anthracyclines but not a sole factor, corroborating Hoffman's observation that some additional factors are to be sought to significantly account for the variation in the activities of anthracyclines.

However, the studies by various other authors[157-160] on the relationship of biological activities with DNA affinity and lipophilicity of anthracyclines were not substantiated.[161,162] Rather, Prabhakar et al.[162] found that in vitro antitumor activity (inhibition of human lymphoblastic leukemia cells) of some adriamycin analogues (XLIV) had a significant correlation with



XLIV

van der Waals volume as shown by eq 124. In this

$$\log(1/IC_{50}) = 7.621 - 1.632(\pm 0.369)V_w(NHR_2) - 1.115(\pm 0.251)I_1 - 0.632(\pm 0.293)I_2$$
$$n = 22, r = 0.930, s = 0.218, F_{3,18} = 38.32 \quad (124)$$

equation, $V_w(NHR_2)$ represents the volume of NHR_2 group. The volume of R_1 group was not found to be important. Instead, an indicator variable I_1, indicating whether R_1 was H/OH/OR or SR/SeR, was found to be significant. It was given a value of 0 for the former and 1 for the latter. The other indicator variable I_2 was used to signify if the glycoside ring A was a natural six-membered ring or a five-membered or any other kind of ring. There were many compounds which did not have the natural six-membered glycoside ring. For all such compounds, $I_2 = 1$, and for the remaining, $I_2 = 0$. Thus eq 124 exhibits that any drastic modification in the structure of the two potent anthracyclines, adriamycin (R_1 = OH, R_2 = H) and daunorubicin (R_1 = H, R_2 = H), will lead to a decrease in the activity.

4. Bleomycins

Bleomycins (XLII) are a family of metal-chelating glycopeptides and are well recognized for their an-

ticancer activity.[163] It is believed that the ferrous chelate is mainly responsible for their activity in vivo and that the DNA is the target for them. The ferrous complex is supposed to be activated in the presence of molecular oxygen and then to bind with DNA causing its degradation.[164] Although several other modes of antitumor action of bleomycins have been suggested[163] and although their several other metal complexes are known to bind with and cause degradation of DNA,[163] it is strongly believed that ferrous ion-induced chemistry is of the greatest importance in vivo.

The cytotoxic effect of bleomycins can be potentiated in vitro and in vivo by agents that may not be active alone. The enhancement effect is believed due to (i) increasing the intracellular level of the drug,[165] (ii) inhibition of the DNA repair and/or synthesis,[166] (iii) enhancement of the efficiency with which Fe^{2+} is recruited in the drug,[167] (iv) direct action of the amplifying agents on DNA,[168] and (v) other less understood mechanisms of action.[169] In order to understand better the amplification phenomenon, Strekowski et al.[170-172] analyzed the amplification activities of many DNA binding and structurally related compounds using QSAR. In their first such study,[171] they treated polyamines having formula $H_2N(CH_2)_xNH_2$ or $H_2N(CH_2)_xNH(CH_2)_yNH_2$ and found that bleomycin amplification activity of these amines had a good relation with Kier's molecular connectivity index of third-order and path type ($^3\chi^v$)[69] as shown by eq 125. In this equation, k_{amine} and k_{blank} are the

$$\log(k_{amine}/k_{blank}) = 0.770 - 1.518(^3\chi^v) + 0.928(^3\chi^v)^2$$
$$n = 7, r = 0.99, s = 0.02 \qquad (125)$$

rate constants for bleomycin-mediated degradation of DNA with and without amine, respectively.

Polyamines are present in virtually all living systems and their biological roles are well studied.[173] Many studies have shown that they strongly interact with DNA and stabilize it against thermal denaturation, shear breakage, and radiation damage,[174] but induce conformational changes in it.[171] The extent of the conformational change depends upon the number of cationic sites on polyamine, distances separating them, and the strength of the binding of the molecule with DNA. It has been speculated that a distorted helix is a better target for bleomycin than the native form,[175] and hence it has been suggested that amplification effect should be related to the extent of conformational perturbation of DNA.[171] In the simplest amplification model, a dication interacts with two phosphates of DNA and perturbs the DNA conformation. Although the correlation with the molecular connectivity index does not directly give any idea about the nature of interaction, eq 125 led Strekowski et al.[171] to suggest that electrostatic interaction between DNA and polyamine was important for amplification and that the activity of compounds was governed by a stereochemical factor. The same conclusion was drawn by these authors for a set of polyheteroaromatic compounds substituted with flexible cationic groups and of similar molecular size,[172] when they obtained eqs 126 and 127,correlating the amplification activity of these compounds

$$\log(k_{compd}/k_{blank}) = 1.139(^1\chi^v) - 0.068(^1\chi^v)^2 - 4.485$$
$$n = 12, r = 0.93, s = 0.03 \qquad (126)$$

$$\log(k_{compd}/k_{blank}) = 1.453(^1\chi^v) - 0.065(^1\chi^v)^2 - 7.678$$
$$n = 10, r = 0.98, s = 0.02 \qquad (127)$$

with first-order valence molecular connectivity index, $^1\chi^v$. These two equations divided the set of compounds into two groups, despite the close structural similarities among all the compounds, and this division led Strekowski et al.[172] to suggest that there can be two different modes of binding with DNA, inducing different stereochemical changes in the helix. Although the index $^1\chi^v$ encodes the additive and constitutive nature of the complex molecules including their basic electronic and stereochemical properties, drawing such unequivocal conclusions based on so simple correlations reflects simply the overzealous attitude of the authors. However, these conclusions are not to be totally discarded, rather they demand further in-depth study.

That an amplifier molecule induces stereochemical changes in DNA helix was also shown in another study by Strekowski et al.[170] Increase in DNA viscosity upon intercalation of the amplifier molecule in the absence of the bleomycin was taken to be a measure of the extent of this change. For some unfused heterobiaromatic and biphenyl compounds substituted with an amino side chain, the amplification activity was found to be correlated with the viscosity changes in DNA (eq 128). In eq 128, η_o is

$$k_{compd}/k_{blank} = 1.594(\eta/\eta_o) - 0.678$$
$$n = 9, r = 0.96 \qquad (128)$$

the initial reduced specific viscosity of the DNA before the addition of bleomycin and η is the reduced specific viscosity at reaction time t. In this study, Strekowski et al. concluded that the enhancement effect was related to the extent of conformational perturbations of the double helix induced by intercalating compounds and that the polarity of the latter was of primary importance for the effective binding of the molecule with native DNA and, at the same time, for its amplification activity. In another study on unfused heterobi- and heterotriaromatic compounds (Figure 4) including some of those of previous study,[170] Strekowski et al.[176] reaffirmed the importance of the polarity of the molecule in DNA binding. For compounds **1**–**3**, **5**, and **7**–**9**, they obtained a good correlation between their DNA binding constant K and the dipole moment μ (eq 129). For compounds

$$\log K = 2.711(\pm 0.173) + 0.272(\pm 0.033)\mu$$
$$n = 7, r = 0.97, s = 0.10 \qquad (129)$$

1–**9**, the dipole moment was studied earlier,[170] but no quantitative correlation was obtained. Compounds with an amino linkage between the cationic side chain and pyrimidine (**4** and **6**) proved outliers in eq 129. For compounds **1**–**15**, the binding constant was found to be linearly correlated with $^1\chi^v$ (eq 130). Kier has shown that $^1\chi^v$ permits quantitative

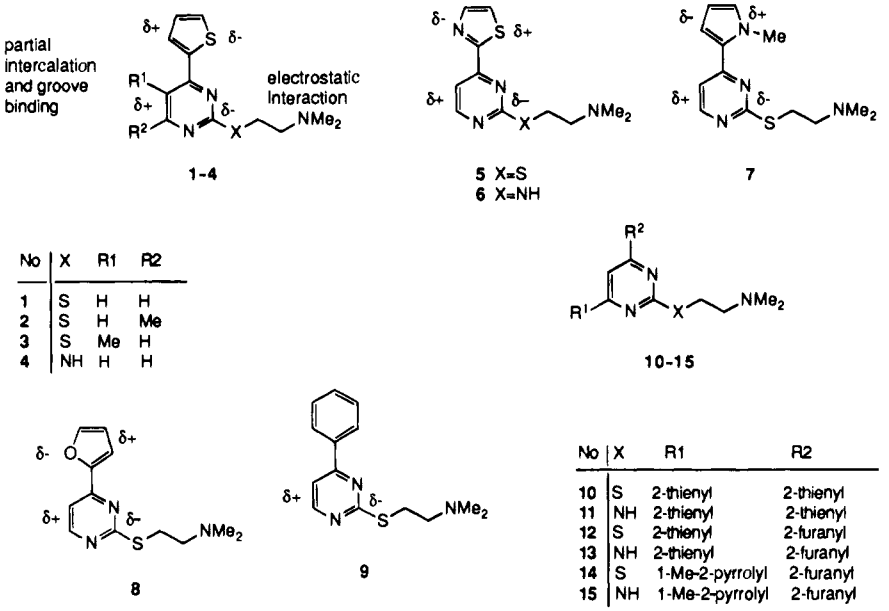


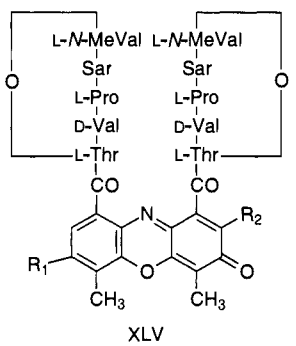
Figure 4. Unfused heterobi- and heterotriaromatic compounds studied for bleomycin amplification.[176]

$$\log K = 0.603(\pm 0.033)(^1\chi^v) - 0.186(\pm 0.250)$$
$$n = 15, r = 0.98, s = 0.12 \qquad (130)$$

evaluation of the polarity of compound directly from its structural formula. Thus Strekowski et al. assumed that DNA binding involves the interaction of molecular dipole with the negative electrostatic potential of the grooves of DNA and suggested that it is actually the groove-binding interaction of unfused aromatic compounds that accounts for quantitative correlations between their binding constants and molecular properties.[176] However, these authors noted that classical fused-ring planar intercalators do not follow the polarity–DNA affinity correlation, presumably because the intercalative forces depend more strongly on polarizability than on polarity of aromatic systems.[176]

5. Actinomycin D (AMD) Analogues

Actinomycin D (XLV; R_1 = H, R_2 = NH_2) is an important antibiotic that binds to double-stranded DNA and selectively inhibits RNA synthesis. It binds



XLV

precisely to d(G-C) base pairs in the helix by intercalation of its chromophore and by hydrogen bonding and hydrophobic interactions of its peptide functions.[177-181] Because of this, it has been established to be a very important agent in the study of RNA metabolism. Further, it is known to cure two different tumors: Wilms' tumor[182] and gestational choriocarcinoma.[183] However, its poor uptake by several tumors and its acute and cumulative toxicity due to lack of detoxification in, and insufficient elimination from, the human system narrow down the spectrum of its use in patients.[184-188] Consequently, its analogues were studied in order to find compounds possessing a broader range of antitumor activity and reduced toxicity. One of such studies[189] was subjected to QSAR analysis by Prabhakar et al.[190] There were two different series: one in which only the R_1 group varied and the other in which only the R_2 group changed. For both the series, the in vitro antitumor activity (inhibition of human lymphoblastic leukemia cells) was found to be significantly correlated with van der Waals volume. For the first series, where $R_2 = NH_2$ and R_1 = H, OC_nH_{2n+1} (n = 1–6), O(p-NO_2)-Bzl, O(p-Cl)Bzl, O(3′,4′-Cl_2)Bzl, $OCOC_6H_5$, OCO-β-naphthyl, and OCO(3′,4′-Cl_2)Bzl, the correlation obtained was as shown by eq 131 and for the second series where R_1 = H and R_2 = NH_2, $NH(CH_2)_nCH_3$ (n = 2–5, 8), or $NH(CH_2)_nNH_2$ (n = 3–5), the correlation obtained was as shown by eq 132. Both

$$\log(1/IC_{50}) = 7.213 - 0.935V_{w,R}$$
$$n = 13, r = 0.82, s = 0.32, F_{1,11} = 21.70 \qquad (131)$$

$$\log(1/IC_{50}) = 7.614 - 1.594V_{w,R}$$
$$n = 9, r = 0.96, s = 0.20, F_{1,7} = 76.13 \qquad (132)$$

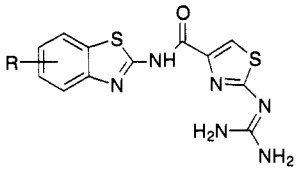
eqs 131 and 132 simply show that the substituents produce the steric effects in the cell inhibition. This may be obviously due to the hindrance in binding of drug with DNA base pairs. For the first series, the apparent binding constant measured for some compounds was shown to be correlated with V_w as[190]

$$\log K_{app} = 7.771 - 2.488V_{w,R}$$
$$n = 9, r = 0.98, s = 0.25, F_{1,7} = 173.97 \qquad (133)$$

The binding sites in actinomycin D are actually supposed to be 4- and 6-positions.[191] On the basis of crystalline AMD–DNA complex, Sobell and Jain[192] proposed that phenoxazone ring of AMD intercalates between adjacent base pairs of the double helix with cyclic pentapeptide groups in the minor groove of the double helix (for grooves see Figure 2). This proposition of Sobell and Jain was consistent with the intercalation model of Müller and Crothers,[177] proposed on the basis of kinetic and hydrodynamic studies. In the crystalline complex, Sobell et al.[179,192,193] observed that a guanine ring of DNA formed two hydrogen bonds with L-threonine residue of one of the pentapeptide rings. Thus it was proposed that there is a general requirement for the presence of a guanine base when AMD binds to DNA. According to Wells and Larson,[194] actinomycin D does bind strongest to poly(dG-dC)–poly(dG-dC) but it can bind almost as tightly to other DNA helices that contain only one guanine base at the intercalation site. However, Wells and Larson[194] also observed that poly d(A-T-C)–poly d(G-A-T) did not bind to AMD even though it contained guanine bases. From this observation, Wells and Larson concluded that the binding of AMD was dependent upon the conformation of the DNA molecule, which in turn was a function of the base sequence at the binding site.

6. Miscellaneous

a. Guanidinothiazolecarboxamides. Guanidinothiazolecarboxamides (GTCs) (XLVI) are a novel class of antitumor agents found to be systematically active against experimental pulmonary metastases 3LL Lewis lung carcinoma. Schnur et al.[195] therefore



XLVI

synthesized a series of GTCs and made a QSAR study of them. For 6-substituted analogues of XLVI (Table 13), the probit transform of the drug-induced ILS was shown to be correlated with field and hydrophobic constants (eq 134). With an additional parameter MR, eq 135 was obtained for some 5-substituted analogues, and for a combined set of 5- and 6-monosubstituted and 5,6-disubstituted analogues, eq 136 was obtained, which included the resonance term R also for 6-substituent. No significant correlation could

$$\log[100/(100 - \mathrm{ILS})] = 0.193F + 0.128\pi + 0.071$$
$$n = 18, r = 0.75, p = 0.0021 \quad (134)$$

$$\log[100/(100 - \mathrm{ILS})] = 0.401F + 0.183\pi - 0.016\mathrm{MR} + 0.045$$
$$n = 9, r = 0.94, p = 0.01 \quad (135)$$

be found for 4-substituted analogues and even 4,5- and 4,6-disubstituted analogues afforded poor correlations. An insufficient number of 7-substituted analogues were prepared. Their inclusion in the larger set resulted in poor correlation and they were too small in number to afford a separate correlation.

Table 13. Anticancer Efficacy and Substituent Constants for 6-Substituted GTCs (XLVI)[195]

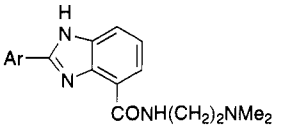
compd	R	ILS, %	F^a	π^a
1	H	15.0	0.00	0.00
2	NO_2	51.7	1.09	0.11
3	Cl	58.0	0.68	0.77
4	F	38.8	0.68	0.22
5	OCH_3	7.5	0.41	0.12
6	$CONH_2$	1.0	0.40	−1.51
7	OCH_2CH_3	6.0	0.36	0.62
8	CH_3	37.3	−0.05	0.52
9	CN	53.0	0.83	−0.31
10	$CH(CH_3)_2$	38.0	−0.07	1.33
11	SO_2NH_2	1.0	0.67	−1.86
12	SCH_3	25.5	0.33	0.64
13	CH_2CH_3	33.0	−0.06	0.99
14	phenyl	67.0	0.14	1.92
15	$O(CH_2)_3CH_3$	59.0	0.40	1.62
16	$C(CH_3)_3$	61.0	−0.10	1.70
17	$(CH_2)_2CH_3$	21.5	−0.03	1.45
18	$SO_2(CH_2)_4CH_3$	30.0	0.88	0.15

[a] Taken for meta position.

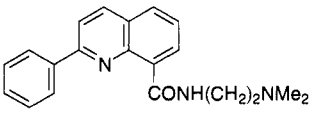
$$\log[100/(100 - \mathrm{ILS})] = 0.131F_6 + 0.151\pi_6 + 0.861R_6 + 0.303F_5 + 0.177\pi_5 - 0.017\mathrm{MR}_5 + 0.107$$
$$n = 35, r = 0.85, p = 0.0000007 \quad (136)$$

For the use of values of variables in eqs 134–136, the 6-position was treated as a meta position and the 5-position as para. In all these equations the coefficients of π and MR are very small as compared to those of electronic parameters. Hence the electronic effects seem to be the dominant factor in the activity of GTCs. However, since no confidence limits are given for the variables in any equation, the level of significance of the electronic parameters can also not be judged.

b. 2-Arylbenzimidazole-4-carboxamides. In search of "minimal" DNA intercalating agents with the lowest possible binding constants, Denny et al.[196] studied a series of 2-arylbenzimidazole-4-carboxamides (XLVII). Such a "2–1" tricyclic chromophores of



XLVII



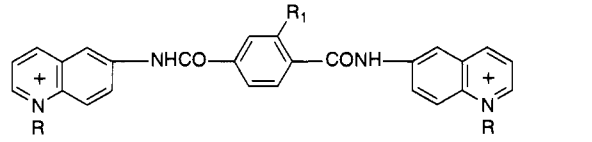
XLVIII

lower aromaticity than the structurally similar 2-phenylquinoline system (XLVIII) have the lowest DNA binding affinity yet seen in the broad series of tricyclic carboxamide intercalating agents. But a QSAR study on 30 congeners of XLVII including 2-furyl, 2-thienyl, 3-thienyl, 2-pyrrolyl, and several mono- and disubstituted phenyl analogues failed to reveal any significant correlation between in vitro activity of compounds against wild-type P388 leukemia cells and their DNA binding constant (eq 137).

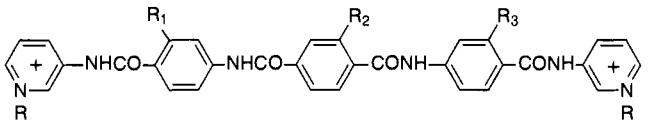
$$\log(1/\mathrm{IC}_{50}) = 0.68(\pm 0.34)\log K + 1.24(\pm 0.93)$$
$$n = 30, r = 0.61, s = 0.31 \quad (137)$$

Denny et al. therefore suggested that the mechanism of cytotoxicity of these compounds either might not involve inhibition of topoisomerase II, an enzyme contained by P388 leukemia cells, or might be via the altered enzyme. Amsacrine-resistant cell line (P388/A) has been found to have a structurally altered topoisomerase enzyme.[197]

c. Bisquaternary Ammonium Heterocycles and Bis(guanylhydrazones). Bisquaternary ammonium heterocycles (BQAHs) are the compounds that contain a number of aromatic rings bridged by groups like NHCO, CONH, or NH, and possess necessarily quaternized heterocyclic bisbases at terminals, as exemplified by XLIX, and L. Following demonstra-



XLIX



L

tion of significant antitumor (L1210) activity with a BQAH,[198] Atwell's group synthesized a large number of congeners and screened their antitumor activity.[198-206] These authors also attempted to analyze the structure–activity relationships using ILS of L1210 screening test of these congeners. But their initial SAR study was only qualitative.[203] A quantitative study was made much later.[207] In that study, Denny et al.[207] first tried to correlate three different activity parameters: ILS_{max} (maximum % increase in life span at LD_{10} dose), D_{40} (the drug dose necessary to provide 40% ILS), and CI (= LD_{10}/D_{40}), with chromatographic R_m values, and for a set of 174 congeners obtained the following equations:

$$\log(ILS_{max}) = 2.14 - 0.23(\pm 0.06)R_m - 0.44(\pm 0.14)R_m^2$$

$$n = 174,\ r = 0.59,\ s = 0.19,\ F_{2,171} = 46 \quad (138)$$

$$\log(1/D_{40}) = 5.32 - 0.02(\pm 0.19)R_m - 0.74(\pm 0.42)R_m^2$$

$$n = 169,\ r = 0.26,\ s = 0.53,\ F_{2,166} = 6.0 \quad (139)$$

$$\log(CI) = 0.76 - 0.23(\pm 0.13)R_m - 0.60(\pm 0.29)R_m^2$$

$$n = 169,\ r = 0.37,\ s = 0.36,\ F_{2,166} = 13.6 \quad (140)$$

In the last two equations, five compounds were deleted, because their D_{40} values could not be measured. All the three equations represent very poor correlations; hence, another term C_{50}, a measure of drug–DNA interaction, was also included in the regression. The C_{50} refers to the micromolar concentration of drug necessary to displace 50% of DNA-bound ethidium as monitored by fluorimetry. BQAH agents have been found to bind more strongly to adenine–ethymine (A-T)-rich DNAs than to their guanine–cytosine (G-C)-rich counterparts, and there was a limited covariance between the C_{50} values observed for binding to poly[d(A-T)] and poly[d(G-C)]. Interaction of these agents with DNA could be then specified in two ways: (1) by their level of interaction with a particular DNA and (2) by their discriminatory ability for different DNAs. The latter was quantified by the ratio of C_{50} values measured for two different sequenced DNAs, e.g., C_{50}-poly[d(A-T)]/C_{50}-poly[d(G-C)]. Incorporation of this discriminatory factor in eqs 138–140 had led to significant improvements in the correlations as shown by eqs 141–143. These

$$\log(ILS_{max}) = 1.90 - 0.33(\pm 0.06)R_m - 0.38(\pm 0.10)R_m^2 + 0.39(\pm 0.07)\log[C_{50}(G\text{-}C)/C_{50}(A\text{-}T)]$$

$$n = 174,\ r = 0.79,\ s = 0.14,\ F_{3,170} = 93.4 \quad (141)$$

$$\log(1/D_{40}) = 4.72 - 0.27(\pm 0.18)R_m - 0.62(\pm 0.36)R_m^2 + 0.97(\pm 0.28)\log[C_{50}(G\text{-}C)/C_{50}(A\text{-}T)]$$

$$n = 169,\ r = 0.62,\ s = 0.43,\ F_{3,165} = 34.5 \quad (142)$$

$$\log(CI) = 0.39 - 0.42(\pm 0.12)R_m - 0.56(\pm 0.25)R_m^2 + 0.62(\pm 0.16)\log[C_{50}(G\text{-}C)/C_{50}(A\text{-}T)]$$

$$n = 169,\ r = 0.62,\ s = 0.31,\ F_{3,165} = 33.7 \quad (143)$$

equations suggest that the selective antitumor properties of BQAHs result from their liopophilic–hydrophilic balance and ability to distinguish certain DNA sites.

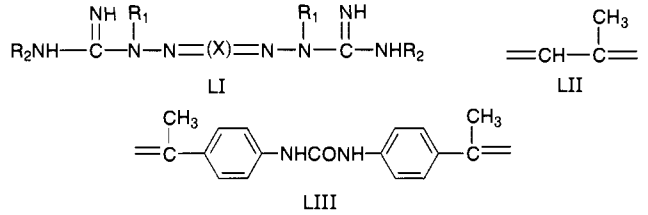
Judging from the goodness of fit of various equations derived for three measures of biological activity, i.e., ILS_{max}, D_{40}, and CI, it is apparent that ILS_{max} is the superior parameter and that in order to satisfactorily account for the variance in each activity parameter, some more physicochemical properties must be attempted.

The sequence selectivity of DNA binding of BQAHs invariably favors better binding to poly[d(A-T)]. Similar binding distinctions have been observed with distamycin and it has been suggested that there is selective hydrogen-bond formation between polar functions of the drug and the A-T pairs distinguished.

However, a space-filling model suggested that a dominant steric inhibition of drug binding would be expected by the 2-NH_2 group of guanine, particularly exacerbated if this function has associated H-bonded water molecule(s).[207] On such steric grounds, the binding distinction between d(A-T) and d(G-C) would depend primarily on how far the skeletal framework between the two charged functions of any drug extended into the minor groove, just impinging against the guanine amino groups. Both qualitative[203] and quantitative[207] SARs are now compatible with lodging BQAH agents in the minor groove of twin helical DNAs.

Certain L1210-active bis(guanylhydrazones) were also found[208] to act like BQAHs. They are structur-

ally similar to the latter and bind with minor groove of DNA and, just like BQAHs, bind more strongly to poly[d(A-T)] than to poly[d(G-C)]. Their general structure may be as LI, where X may be either some aliphatic moiety as, for example, LII, or some aromatic moiety as, for example, LIII.[208] The correspon-



LI LII

LIII

dence between the BQAH and the aromatic bis(guanylhydrazone) (BGH) agents—both possessing L1210 activity, charge separation of greater than 18 Å, structural aromatic components permitting lodgement in a slotlike annular site, and DNA as a putative site of action—suggested that the two groups of agents might be considered congeneric and that QSAR developed for BQAHs[207] might then be applicable to aromatic BGHs. Denny and Cain[209] however made a separate QSAR study on a series of aliphatic and aromatic BGHs studied by Dave et al.[208] and found that drug concentration necessary to inhibit L1210 DNA-dependent DNA polymerase in vitro by 50% (IC_{50}) was linearly related to the measure of drug–DNA binding (C_{50}) with no preference for a particular primary sequence of DNA being evident (eqs 144 and 145). The parameter IC_{50} was

$$\log(IC_{50}) = 2.00 - 0.49(\pm 0.12)\log[1/C_{50}(\text{A-T})]$$

$$n = 13, r = 0.92, s = 0.22, F_{1,11} = 64.6 \quad (144)$$

$$\log(IC_{50}) = 1.74 - 0.47(\pm 0.11)\log[1/C_{50}(\text{G-C})]$$

$$n = 13, r = 0.91, s = 0.24, F_{1,11} = 51.1 \quad (145)$$

used because it was found qualitatively to have a relationship with the magnitude of the interaction of BGHs with calf thymus DNA.[208] The mammalian toxicity (optimal dose) was also found to be related to IC_{50}, of course, in combination with R_m values (eq 146) and since IC_{50} was correlated with C_{50} (eq 144 and 145), the toxicity could as well be correlated to C_{50} (eqs 147 and 148). Thus while it has been

$$\log(\text{OD}) = 0.21 \pm 0.59(\pm 0.30)\log(IC_{50}) - 0.87(\pm 0.44)R_m - 0.74(\pm 0.45)R_m^2$$

$$n = 16, r = 0.83, s = 0.30, F_{3,12} = 9.2 \quad (146)$$

$$\log(\text{OD}) = 1.30 - 0.37(\pm 0.13)\log[1/C_{50}(\text{A-T})] - 1.00(\pm 0.39)R_m - 0.68(\pm 0.39)R_m^2$$

$$n = 16, r = 0.89, s = 0.25, E_{3,12} = 15.5 \quad (147)$$

suggested that the antitumor activity of aromatic BGHs is related to their ability to inhibit DNA-dependent DNA polymerase (DDP) in vivo,[208-210] the study of Denny and Cain shows that this property is related to in vivo toxicity of general class of BGHs

$$\log(\text{OD}) = 1.10 - 0.37(\pm 0.13)\log[1/C_{50}(\text{G-C})] - 0.92(\pm 0.34)R_m - 0.58(\pm 0.36)R_m^2$$

$$n = 16, r = 0.90, s = 0.23, F_{3,12} = 17.5 \quad (148)$$

and may indeed be a useful predictor of the toxicity. However, if the aromatic BGHs are congeneric with BQAH, then it would be expected that the antitumor selectivity is dependent on in vivo binding to an as yet undefined, alternating A-T-rich site(s) in the tumor cell DNA.

d. Antitumor Agents in Multidrug Resistance. Resistance of tumor cells to multiple cytotoxic agents is one of the major causes of treatment failure in cancer chemotherapy. This resistance is generally acquired due to exposue of drug-sensitive malignant cells to various antitumor drugs. This general phenomenon of "pleotropic drug resistance" is now addressed as multidrug resistance (MDR).

Beidler and Reihm were the first to describe the MDR phenomenon.[211] They observed that exposure of several sublines of chinese hamster ovary (CHO) cells to increasing concentrations of actinomycin D resulted in resistance to a broad range of structurally varied agents (Table 14) and that the cross resistance (CR) of the agents was correlated with their molecular weight (MW). A QSAR study was made of their data by Selassie et al.[212] and eq 149 was obtained.In

$$\log(\text{CR}) = 3.65(\pm 1.1)\log \text{MW} - 8.54(\pm 2.9)$$

$$n = 13, r = 0.915, s = 0.470, F_{1,11} = 56.9 \quad (149)$$

this equation, CR represents the cross resistance to actinomycin D, defined as $ED_{50}R/ED_{50}S$, where $ED_{50}R$ and $ED_{50}S$ are the molar concentrations of drug inducing 50% inhibition of growth in resistance and sensitive cells, respectively. Similarly, eq 150 was obtained[212] for the data on cross resistance of CCRF-CEM cells resistance to vincristine (Table 15) studied by Conter and Beck.[213] Both eqs 149 and 150 exhibit

$$\log(\text{CR}) = 6.89(\pm 3.1)\log \text{MW} - 17.4(\pm 8.7)$$

$$n = 9, r = 0.893, s = 0.468, F_{1,17} = 27.5 \quad (150)$$

Table 14. Data on Cross Resistance of Chinese Hamster Cells Resistant to Actinomycin D[211]

compd	antitumor agent	log CR	log MW	log *P*
1	mithramycin	2.83	3.04	−0.25
2	vincristine	2.28	2.97	2.57
3	puromycin	1.92	2.67	0.86
4	daunomycin	1.46	2.72	0.66
5	demecolsine	1.26	2.57	1.37
6	mitomycin C	0.49	2.52	−0.38
7	proflavin	0.46	2.49	1.10
8[a]	novobiocin	0.28	2.80	1.58
9	bromodeoxyuridine	0.08	2.49	−0.29
10	nitroquinoline *N*-oxide	0.04	2.28	1.02
11	amethopterin	0.04	2.66	−2.52
12	6-mercaptopurine	−0.30	2.23	0.01
13[a]	hydrocortisone	−0.40	2.69	1.20
14[a]	nitrogen mustard	−0.40	2.28	−2.00
15	actinomycin D	2.58	3.10	3.21
16	vinblastine	2.38	2.96	3.69

[a] Not included in the derivation of eq 149.

Table 15. Data on Cross Resistance of CCRF-CEM Cells Resistance to Vincristine[213]

no.	antitumor agent	log CR	log MW	log P
1	vindesine	3.00	2.93	0.67
2	maytansine	2.85	2.84	1.99
3	vincristine	2.76	2.97	2.57
4	teniposide	1.56	2.82	1.22
5	etoposide	1.51	2.77	0.60
6	doxorubicin	1.46	2.75	0.10
7[a]	vinblastine	1.28	2.96	3.69
8	daunorubicin	1.20	2.72	0.66
9	colchicine	1.08	2.60	1.03
10	podophyllotoxin	0.07	2.62	2.01

[a] Not included in the derivation of eq 150.

simple linear correlation between effectiveness of drugs in resistant cells and their molecular weight. But for the data on cross resistance of L1210/R71 cells resistant to methotrexate (Table 16), a bilinear equation (eq 151) was obtained,[212] which even then did not express as good a correlation as eqs 149 and 150; hence, the hydrophobic parameter was also included (eq 152). A bilinear equation (eq 153) in both

$$\log(\mathrm{CR}) = 12.10(\pm 4.37)\log \mathrm{MW} - 17.20(\pm 5.90)\log(\beta 10^{\log \mathrm{MW}} + 1) - 19.51(\pm 7.77)$$

$$n = 29,\ r = 0.781,\ s = 0.491,\ F_{3,25} = 13.06,\ \log \beta = -2.13,\ \log \mathrm{MW}_o = 2.50 \quad (151)$$

$$\log(\mathrm{CR}) = 7.44(\pm 2.10)\log \mathrm{MW} - 14.97(\pm 3.94)\log(\beta 10^{\log \mathrm{MW}} + 1) - 0.13(\pm 0.06)\log P - 13.13(\pm 4.22)$$

$$n = 29,\ r = 0.871,\ s = 0.394,\ F_{4,24} = 14.68,\ \log \beta = -2.60,\ \log \mathrm{MW}_o = 2.60 \quad (152)$$

$$\log(\mathrm{CR}) = 5.67(\pm 3.55)\log \mathrm{MW} - 52.05(\pm 42.39)\log(\beta_1 10^{\log \mathrm{MW}} + 1) + 1.03(\pm 1.31)\log P - 1.12(\pm 1.48)\log(\beta_2 10^{\log P} + 1) - 10.17(\pm 8.25)$$

$$n = 19,\ r = 0.828,\ s = 0.486,\ F_{6,12} = 4.36,\ \log \beta_1 = -3.72,\ \log \beta_2 = 1.33,\ \log P_o = -0.30,\ \log \mathrm{MW}_o = 2.81 \quad (153)$$

log MW and log P was obtained[212] for the data on cross resistance of CHO cells (CH^RC5) resistance to colchicine (Table 17) studied by Bech-Hansen et al.[214] The log P term was found to be of little significance, when included linearly or parabolically in eqs 149 and 150.

The MDR phenomenon has been shown to be associated with reduced drug accumulation, which has been attributed to two different phenomena. One involves drug influx via normal channels but drug extrusion by an energy-dependent efflux pump that is more effective in resistance cells.[215] The other mode of action suggests the existence of an energy-dependent permeability barrier which effectively restricts drug entry into the cells.[216] On the basis of QSAR studies Selassie et al. suggested two modes of entry into the resistance cells.[212] One involves normal diffusion governed by a rate constant k_1, while the other includes entry via endocytosis controlled by k_3. Efflux out of the cell (the simple reverse of entry) would be governed by k_2. Exocytosis is assumed to be negligible compared to endocytosis. For small molecules $k_1 \gg k_3$, so that simple diffusion takes place, but for large molecules (MW > 400), $k_3 \gg k_1$ and endocytosis route prevails.

In deriving eqs 149–153, some compounds in each case were excluded (see footnote of the respective tables). They were excluded because of their aberrant behavior, and their this behavior was hard to

Table 16. Data on Cross Resistance of L1210/R71 Cells Resistant to methotrexate[212]

no.	antitumor agent	log CR	log MW	log P
1	3-H[a]	2.18	2.40	−3.00
2	3-$CONH_2$[a]	1.85	2.47	−4.49
3	3-$COCH_3$	2.20	2.47	−3.55
4	3-OH[a]	1.86	2.43	−3.67
5	3-Br[a]	2.06	2.52	−2.14
6	3-$C_{12}H_{25}$[a]	0.52	2.62	3.41
7	3-$CH_2OC_6H_4$-3′-C_6H_5[a]	1.22	2.64	0.69
8	hydroxyurea	0.30	1.88	−1.27
9	guanazole	0.08	2.00	−1.61
10[b]	5-fluorouracil	−0.04	2.11	−0.89
11	6-mercaptopurine	0.60	2.23	0.01
12	azacytidine	1.54	2.39	−2.17
13	cytosine arabinoside	1.61	2.44	−2.13
14	metropine	1.70	2.43	2.56
15	etoprine	1.69	2.45	2.93
16	mitomycin C	1.89	2.52	−0.38
17	DAMP	1.63	2.57	2.64
18	piritrexin	1.89	2.64	1.77
19	methotrexate	2.55	2.66	−2.52
20	puromycin	1.82	2.67	0.86
21	trimetrexate	1.75	2.70	0.84
22	bakers antifol I	2.18	2.73	−1.84
23	bakers antifol II	1.12	2.79	2.42
24	tamoxifen	0.59	2.75	4.03
25	maytansine	1.23	2.84	1.99
26	mithramycin	0.66	3.04	−0.25
27	valinomycin	0.59	3.05	3.24
28	actinomycin D	0.12	3.10	3.21
29	bleomycin	0.24	3.15	−2.38
30	liblomycin	0.29	3.31	−1.11

[a] Substituent X in 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3-X-phenyl)-*s*-triazine. [b] Not used in the derivation of eqs 151 and 152.

Table 17. Data on Cross Resistance of CHO Cells (CH^RC5) Resistant to Colchicine[214]

no.	antitumor agent	log CR	log MW	log P
1	colchicine	2.26	2.60	1.30
2	puromycin	2.02	2.67	0.86
3	daunomycin	1.88	2.72	0.66
4	emetine	1.46	2.74	3.24
5	ethidium bromide	1.04	2.60	1.15
6	acriflavin	0.84	2.41	1.60
7	cytochalastin B	1.04	2.68	3.37
8	erythromycin	0.70	2.98	1.26
9	colcemid	1.20	2.57	1.37
10	vinblastin	1.46	2.91	3.69
11[a]	gramicidin D	2.16	3.27	0.05
12	adriamycin	1.40	2.75	0.10
13	proflavin	0.60	2.32	1.10
14	melphalan	1.18	2.53	−0.52
15	mechlorethamine	0.48	2.28	0.91
16	chlrorambucil	0.30	2.48	1.47
17	ara-C	0.00	2.44	−2.13
18	bleomycin	0.00	3.15	−2.38
19	5-fluorouracil	0.00	2.11	−0.89
20	thiotepa	0.00	2.28	0.53

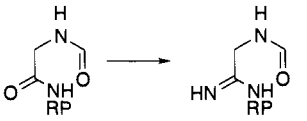
[a] Not included in the derivation of eq 153.

justify as all compounds in any group were noncongeneric.

F. Antimetabolites

1. Glutamine Antagonists

Glutamine antagonists derive their biological activity from interference with the various metabolic processes in which glutamine is involved as a cofactor, such as, for example, the conversion of formylglycinamide ribonucleotide (LIV) to formylglycinamidine ribonucleotide (LV). Jha et al.[217-219] subjected

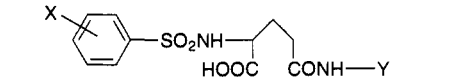


LIV LV

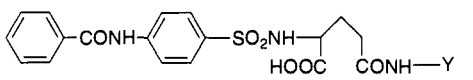
R = β-D-ribofuranose

P = phosphate

a series of glutamine analogues like LVI and LVII, synthesized in their own laboratory, to QSAR studies.



LVI



LVII

In these analogues, the X substituent was either a halogen or an NO_2 group and the Y substituent was an alkyl group. The QSAR studies produced a significant correlation in the case of only LVII derivatives (eq 154).[219] This correlation suggests that

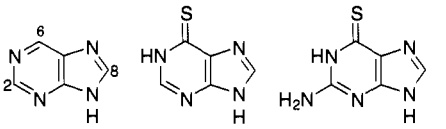
$$\log BA = 0.26(\pm 0.12)\pi_{X,p} + 0.3(\pm 0.17)MR_{X,p} + 1.44(\pm 0.08)$$

$$n = 14, r = 0.906, s = 0.079, F_{2,11} = 15.74 \quad (154)$$

only the para substituents of the phenyl ring will produce some effect on activity and that this effect would be due to their hydrophobic character as well as their size which is measured by the molar refractivity index MR. The hydrophobicity here may be responsible for the transport of the drug to the receptor site and the size may be responsible for the dispersion interaction of the drug with the receptor. Such a correlation could not be found in the case of LVI derivatives, suggesting that for the activity substituents must be at proper distance from the sulfonyl group. The activity of these compounds was against Ehrlich ascites carcinoma cell line and BA in eq 154 refers to % ascites fluid weight inhibition.

2. Purine Antagonists

Analogues of purine (LVIII) comprise an important class of potential anticancer agents. Synthetic, unnatural purines can be administered exogeneously and utilized by the intact animal to meet its requirements for nucleotides. These analogues then may be incorporated directly into RNA and DNA, eventually



LVIII LIX LX

producing cell death. These considerations led to the synthesis[220] and testing of thousands of purine derivatives for their anticancer properties, but only two purine analogues, 6-mercaptopurine (LIX) and its guanine analogue, i.e., 6-thioguanine (LX), have found general clinical use in the treatment of human cancer.

To investigate the mode of action of these drugs, Neiman and Quinn[221] performed a QSAR study on a series of 2- and 6-mono- and disubstituted purine derivatives and correlated their activity against murine solid tumor adenocarcinoma CA 755 with molar refractivity and the resonance parameter as

$$\log(1/C) = 4.26 - 0.47MR_2 + 1.18R_6$$

$$n = 22, r = 0.815, s = 0.372 \quad (155)$$

suggesting that the activity would be predominantly determined by the resonance effect of the 6-substituents. However, in a recent study by Mekenyan et al.,[222] who derived eq 156 for the same series, the

$$\log(1/C) = 3.69(\pm 0.14) + 0.51(\pm 0.14)S_6^E + 0.24(\pm 0.14)\pi_6$$

$$n = 17, r = 0.920, s = 0.265, F_{2,14} = 39.67 \quad (156)$$

essential electronic process taking place was assumed to be the charge transfer from position 6 on the purine ring to the biomacromolecule. This assumption of Mekenyan et al. was based on a fairly large coefficient of electrophilic superdelocalizability index (S^E) for the 6-position. The substituents at this position are, however, shown to have their effect through their hydrophobic character. Thus the conclusion was that before the charge transfer could take place, the purine would be fixed to the receptor site by means of a preliminary hydrophobic interaction. In the derivation of eq 156, five of the derivatives listed in the original set and used to derive eq 155 were not included: three iodo derivatives, owing to the lack of reliable CNDO parameters for iodine, and two larger purine derivatives, due to the microcomputer quantum mechanical program limitations concerning the size of the molecule.

Unlike eq 155, eq 156 however did not exhibit any effect of the substituents at 2-position, but in a successive study Mercier et al.[223] derived a DARC/PELCO model (eq 157) to show a negative effect of some substituents at this position. This model is

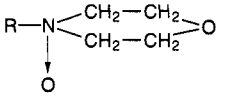
$$\log(1/C) = 3.20(\pm 0.51)[N,O]_6 + 4.20(\pm 0.50)[S,Cl,Br,SO_2]_6 - 0.83(\pm 0.26)[Br,SO_2F]_2$$

$$n = 17, r = 0.979, s = 0.237, F = 110 \quad (157)$$

based upon the exhaustive generation of all topochromatic sites around the reference structure and

the evaluation of their contribution to the property.[224,225] In eq 157, the square brackets refer to the activity contributions of the atoms/groups, written within them, at specific positions indicated by subscript.

A series of 4-alkylmorpholine *N*-oxides (LXI)were



LXI: R = an alkyl group

also found to behave as purine antagonists and studied for their ability to inhibit the incorporation of [^{14}C]adenine into nucleic acids and [^{14}C]valine into proteins in Ehrlich ascites carcinoma cells.[226] A QSAR study showed that these inhibition activities of morpholines were significantly correlated to the number of carbon atoms (m) in the alkyl chain (C_mH_{2m+1}) in a bilinear fashion as[226]

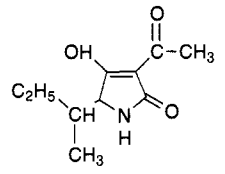
$$\log(1/IC_{50})_{ad} = 0.218(\pm0.032)m - 0.881(\pm0.132)\log(\beta 10^m + 1) + 0.925(\pm0.378)$$
$$n = 11, r = 0.932, s = 0.221, \log\beta = -15.59 \quad (158)$$

$$\log(1/IC_{50})_{val} = 0.222(\pm0.028)m - 1.087(\pm0.156)\log(\beta 10^m + 1) + 0.931(\pm0.347)$$
$$n = 11, r = 0.944, s = 0.213, \log\beta = -16.18 \quad (159)$$

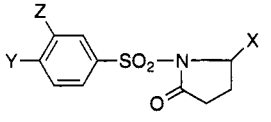
The above equations show that the activity increases with the length of the chain to a certain value of m (15−16) and then starts decreasing. This effect of the length of the chain on inhibition phenomena is however not well understood.

3. Inhibitors of Protein Synthesis

Certain compounds resembling tenuazonic acid (LXII) were studied for their anticancer activity.[227]



LXII



LXIII: X = COOH, $COOC_2H_5$, or $CONHCONH_2$; Y = H, Cl, or CH_3; Z = H or Cl

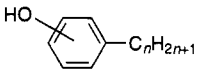
Tenuazonic acid inhibits the growth of human adenocarcinoma growing in the embryonated egg by inhibiting the protein synthesis.[228,229] Assuming that compounds resembling tenuazonic acid may be useful as anticancer drugs, Purkayastha et al.[227] synthesized a series of esters and urides of 1-(substituted benzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid (LXIII) and studied their activity against Ehrlich ascites carcinoma cells in mice. Through a QSAR study then, these authors showed that the anticancer activity of these compounds had a good correlation with the molar refractivity of the substituents (eq 160). Since the Z substituent was either H or Cl only,

$$BA = 22.9(2.85)MR_X + 51.6(8.01)MR_Y - 1.13(0.39)I_Z - 6.34(0.55)$$
$$n = 12, r = 0.964, s = 0.546, F_{3,8} = 35.43 \quad (160)$$

an indicator parameter I_Z was used for it with a value of 0 for the former and 1 for the latter. However, since X and Y substituents also did not have much variation, no fruitful conclusion can be drawn from eq 160. The biological activity parameter BA was calculated, using eq 161, from the % inhibition of ascitic fluid weight.

$$BA = \left(\frac{mw}{dose}\right)\log\left(\frac{\%\ inhbn}{100 - \%\ inhbn}\right) \quad (161)$$

For a series of long-chain phenols (LXIV) interact-



LXIV

ing with proteins and possessing antitumor activity,[230] Itokawa et al.[230] found that their activity against Chinese hamster V79 cell could be well correlated with log P and the quantum mechanical (MNDO) parameter E_{LUMO} as

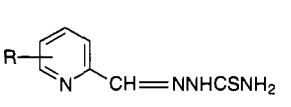
$$\log(1/ED_{50}) = 0.278(\pm0.132)\log P - 0.017(\pm0.001)(\log P)^2 - 3.485(\pm0.458)E_{LUMO} + 0.818(\pm0.062)$$
$$n = 29, r = 0.845, s = 0.227, F_{3,25} = 20.74 \quad (162)$$

Equation 162 thus suggested that the activity of the compounds will not only depend upon their ability to cross the cell membrane but also upon their ability to participate in the charge-transfer phenomenon with the receptor, where they will act as an electron acceptor.

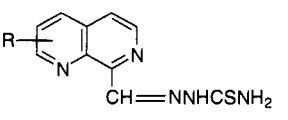
4. Inhibitors of Specific Enzymes

a. Ribonucleoside Diphosphate (Ribonucleotide) Reductase. Ribonucleoside diphosphate reductase (RDR) is a key enzyme in the conversion of ribonucleotides to deoxyribonucleotides, a rate-limiting step in DNA synthesis. This enzyme is therefore a potential target for the development of anticancer agents.

A number of α-*N*-formylheteroaromatic thiosemicarbazones such as LXV and LXVI are known to inhibit RDR.[231] Dunn and Hodnett[232] made a QSAR



LXV



LXVI

study on these inhibitors for the enzyme extracted from rat Novikoff tumor and H.Ep.-2 tumor of human origin. Equation 163 was obtained for the inhibition of RDR of H.Ep.-2 tumor by 2-formylpyridine thi-

osemicarbazones (LXV) and eqs 164 and 165 were obtained, respectively, for the inhibition of H.Ep.-2 and rat Novikoff tumor enzymes by 1-formylisoquinoline thiosemicarbazones (LXVI). While eq 163 exhibits inductive, hydrophobic, and steric effects, other equations exhibit only the steric effect. The

$$\log(1/IC_{50}) = 6.30 - 0.81\sum F_{3,5} + 0.29\sum \pi_{3,5} - 0.24MR_5$$

$$n = 28, r = 0.88, s = 0.33 \quad (163)$$

$$\log(1/IC_{50}) = 6.70 - 1.81MR_5$$

$$n = 13, r = 0.80, s = 0.37 \quad (164)$$

$$\log(1/IC_{50}) = 7.67 - 0.44(MR_5)^2$$

$$n = 12, r = 0.93, s = 0.35 \quad (165)$$

dominance of the steric effect of the 5-substituent in these inhibitors was also shown by Gupta et al.[233] by correlating the inhibition activities with van der Waals volume. Equation 166 was obtained for a series of 2-formylpyridine thiosemicarbazones (Table 18) studied by French et al.[231] against RDR of H.Ep.-2 cells and eq 167 was obtained for a series of 1-formylisoquinoline thiosemicarbazones (Table 19) studied by Agrawal et al.[234] against RDR of rat Novikoff ascites tumor cells. In eq 167, RA stands for relative

$$\log(1/IC_{50}) = 6.360 - 1.070(0.158)V_w$$

$$n = 23, r = 0.827, s = 0.39 \quad (166)$$

$$\log RA = 0.087 - 1.409(0.162)V_w$$

$$n = 15, r = 0.923, s = 0.32 \quad (167)$$

activity, defined as the ratio of IC_{50} for the parent molecule to that for the derivative. A few smaller series of these two types of inhibitors were also treated and similar equations were obtained.[233] All these studies did indicate the steric effects produced by 5-substituent but could not throw any light on the mode and nature of drug–enzyme interaction. The ring nitrogen may be expected to be involved in some electronic interaction with the enzyme, which is hindered by the substituent at the 5-position. Treating the data of French et al.[231] on some 3,5-disubstituted analogues of LXV, Miertus et al.[235] postulated the involvement of C=N bond of the chain in the interaction with the enzyme where a nucleophilic attack may take place at the carbon atom. These authors had calculated some quantum mechanical parameters, using Pariser–Parr–Pople method, for these analogues in free state as well in a complex state with Fe^{2+} and found that the inhibition activity was well correlated with nucleophilic superdelocalizability of carbon as

$$\log(1/IC_{50}) = 18.6S_C^N - 2.5$$

$$n = 10, r = 0.914 \quad (168)$$

From this correlation, Miertus et al. speculated that

Table 18. RDR (H. Ep.-2 Cells) Inhibitory Activities[231] of 2-Formylpyridine Thiosemicarbazones (LXV)

compd	5-R	log(1/IC50)	V_w, 10^2 Å^3
1	H	6.55	0.056
2	CH_3	6.51	0.245
3	C_2H_5	6.66	0.399
4	F	5.92	0.115
5	Cl	6.25	0.244
6	Br	6.30	0.287
7	I	6.39	0.388
8	CF_3	5.62	0.383
9[a]	OH	5.17	0.137
10	OCH_3	5.92	0.304
11	OCF_3	5.60	0.442
12	OC_2H_5	6.07	0.458
13	$OC_2H_4N(CH_3)_2$	4.62	0.881
14	$O(C_2H_4O)_2C_2H_5$	5.69	0.847
15	$OOCCH_3$	5.44	0.479
16	$OOCC_2H_5$	5.28	0.633
17	n-$OOCC_3H_7$	5.17	0.787
18	n-$OOCC_{15}H_{31}$	3.96	2.635
19	$OOCCH_2OCH_3$	5.30	0.714
20	$OOCCH_2OC_2H_5$	5.25	0.868
21	$OOCCH_2N(CH_3)_2$	5.24	0.902
22	$OOCCH_2OC_6H_5$	4.89	1.254
23	$NHCOCH_3$	5.92	0.514
24	$N(CH_3)_2$	6.40	0.501

[a] Not included in the derivation of eq 166 as it was reported not to exist purely in the phenolic form at physiological pH but as a mixture of zwitterionic and phenolic forms.

Table 19. RDR (Rat Novikoff Ascites Cells) Inhibitory Activities[234] of 1-Formylisoquinoline Thiosemicarbazones (LXVI)

compd	5-R	log(1/RA)	V_w, 10^2 Å^3
1	H	0.00	0.056
2	$NHSO_2CH_3$	0.59	0.637
3	$NHCOC_6H_4(m\text{-}SO_2F)$	1.62	1.398
4	$NHCOC_6H_4(p\text{-}SO_2F)$	2.15	1.398
5	OSO_2CH_3	1.03	0.597
6	$OCO_2C_2H_5$	0.61	0.714
7	OH	0.63	0.137
8	$OCOC_6H_4(m\text{-}SO_2F)$	1.00	1.363
9	NH_2	0.00	0.177
10	$OCO_2C_6H_5$	1.54	1.100
11	$OCOC_6H_4(p\text{-}SO_2F)$	1.93	1.363
12	$OSO_2C_6H_4(o\text{-}SO_2F)$	2.11	1.451
13	$OSO_2C_6H_4(m\text{-}SO_2F)$	2.18	1.451
14	$OSO_2C_6H_4(p\text{-}SO_2F)$	2.20	1.451
15	$N(CH_2CH_2Cl)_2$	1.62	1.079

before the compound interacts with the enzyme, it should form a complex with Fe^{2+}.

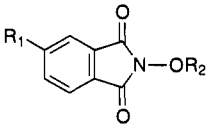
A group of arylhydroxamic acids (RCONHOH; R being a substituted aryl moiety) were also studied for their RDR inhibition activity.[236,237] The IC_{50} values obtained for them against rat Novicoff hepatoma were then correlated by van't Riet et al.[238] with molecular connectivity indices as shown by eq 169.

$$\log(1/IC_{50}) = 2.36(\pm0.04)({}^3\chi_p) - 3.98(\pm0.53)({}^0\chi^v) + 0.97(\pm0.05)({}^1\chi^v)^2 + 9.20(\pm5.50)$$

$$n = 28, r = 0.943, s = 0.21 \quad (169)$$

This equation also, however, does not throw any light on the inhibition mechanism. When some RDR inhibitors were studied for cell growth inhibition, the hydrophobic and electronic properties were found to play important roles. For a series of 16 2-hydroxy-1*H*-isoindole-1,3-diones (Table 20) studied for the

Table 20. L1210 Cell Growth Inhibitory Activity and Physicochemical Parameters of 2-Hydroxy-1*H*-isoindole-1,3-diones[239]



compd	R_1	R_2	log(1/IC_{50})	R_m	σ	μ
1	NH_2	H	3.456	0.10	−0.66	1.53
2	NH_2	SO_2CH_3	5.481	−0.17	−0.66	1.53
3	NH_2	$SO_2CH(CH_3)_2$	6.000	−0.15	−0.66	1.53
4	NH_2	$SO_2C_6H_5$	5.509	−0.15	−0.66	1.61
5	$(CH_3)_2N$	H	4.620	−0.04	−0.83	1.61
6	$(CH_3)_2N$	SO_2CH_3	7.036	−0.29	−0.83	1.61
7	$(CH_3)_2N$	$SO_2CH(CH_3)_2$	6.721	−0.37	−0.83	1.61
8	$(CH_3)_2N$	$SO_2C_6H_5$	6.538	−0.31	−0.83	1.61
9	$(CH_3)_2N$	$SO_2C_6H_4CH_3$	6.638	−0.27	−0.83	1.61
10	$(CH_3)_2N$	$SO_2C_6H_4NO_2$	6.721	−0.19	−0.83	1.61
11	C_2H_5HN	SO_2CH_3	6.468	−0.21	−0.61	1.61
12	C_2H_5HN	$SO_2CH(CH_3)_2$	6.149	−0.27	−0.61	1.61
13	C_2H_5HN	$SO_2C_6H_4CH_3$	6.167	−0.29	−0.61	1.61
14	NO_2	SO_2CH_3	4.745	−0.40	0.78	−4.13
15	NO_2	$SO_2CH(CH_3)_2$	4.658	−0.37	0.78	−4.13
16	H	SO_2CH_3	5.530	−0.25	0.00	0.03

growth inhibition of L1210 cells,[239] the activity was found[239] to be correlated with R_m values and Hammett constant σ as shown by eq 170. The σ was used for only R_1 substituent. However, a very much similar correlation (eq 171) was obtained, when σ was replaced by the dipole moment μ of the compounds.

$$\log(1/IC_{50}) = 3.595(\pm0.501) - 10.800(\pm3.151)R_m - 13.597(\pm9.440)R_m^2 - 1.312(\pm0.525)\sigma$$

$$n = 16, r = 0.959, s = 0.313, (R_m)_o = -0.397 \quad (170)$$

$$\log(1/IC_{50}) = 4.901(\pm0.501) - 10.247(\pm3.630)R_m - 10.990(\pm11.317)R_m^2 + 0.376(\pm0.143)\mu$$

$$n = 16, r = 0.948, s = 0.354 \quad (171)$$

Thus while eqs 170 and 171 show that an optimum balance of lipophilicity and hydrophobicity is needed for antitumor action of diones, they do not clearly tell in what way the electronic properties of molecules will affect the activity. There is a high covariance between σ and μ ($r^2 = 0.95$) and there is little variation in the types of R_1 and R_2 substituents.

From a study on the derivatives of *N*-hydroxyurea, $H_2NC(O)NHOH$, Chou et al.[240] concluded that substantially higher molecular weight and polar functional group providing low lipophilicity would favor the anticancer activity of these compounds. Hydroxyurea is used in the treatment of leukemias and head and neck cancers. It is the only drug used clinically whose primary mode of action is the inhibition of ribonucleotide reductase. The essential pharmacophore in the hydroxyurea molecule is the hydroxamic acid moiety, =CNHOH.[241] Guanidine, $H_2NC(=NH)NH_2$, is an antiviral agent. Hydroxyguanidine, $H_2NC(=NH)NHOH$, contains features of both guanidine (imino group) and hydroxyurea (hydroxyamino group) and possesses, therefore, both antiviral and anticancer activities. In order to enhance these activities, Tai et al.[242] synthesized a series of hydroxyguanidine derivatives of the structure R=NNHC(=NH)NHOH, where R = aromatic or heterocyclic aldehyde. The anticancer activity of these compounds was tested against cultured L1210 cells and the antiviral activity against Rous sarcoma virus (RSV).[242] These compounds were prepared in order to alter lipophilic–hydrophilic balance as well as electronic and steric properties, but when a QSAR analysis was performed[243] the anticancer activity was found to be correlated with only molar refractivity index (eq 172) and the antiviral activity with molecular weight and R_m values (eq 173). But excluding

$$\log(1/ID_{50}) = 0.752 \log MR + 3.587$$

$$n = 11, r = 0.83, s = 0.21 \quad (172)$$

$$\log(1/ID_{50}) = 1.181 \log MW + 1.896 R_m + 22.71$$

$$n = 11, r = 0.92, s = 0.22 \quad (173)$$

some compounds Pandey et al.[244] showed that both anticancer as well as antiviral activities could be well correlated with van der Waals volume alone as shown by eqs 174 and 175, respectively. Thus the dispersion interaction seems to be the main interaction involved in the drug action. All the equations here suggest that the drug molecule should be sufficiently large so that it fits in the receptor site. The parameter R_m in eq 173 suggests that for antiviral activity, the membrane permeability would also be important.

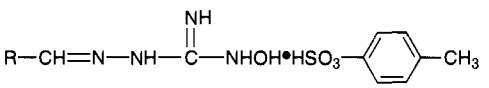
$$\log(1/ID_{50}) = 0.65V_w + 3.92$$

$$n = 9, r = 0.91, s = 0.16 \quad (174)$$

$$\log(1/ID_{50}) = 0.72V_w + 3.77$$

$$n = 9, r = 0.82, s = 0.27 \quad (175)$$

For a different series of hydroxyguanidines belonging to LXVII,the L1210 leukemia cell growth inhibition activity was again found to be predominantly correlated with molar refractivity index (eq 176).[245]



LXVII

$$\log(1/ID_{50}) = 5.020 \log MR - 0.175\pi - 2.866$$

$$n = 10, r = 0.88, s = 0.25 \quad (176)$$

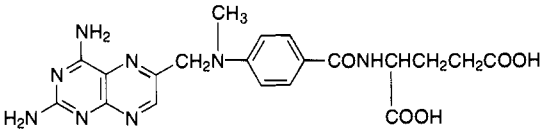
This correlation further supports the involvement of dispersion interaction in the antitumor activity of hydroxyguanidines. The small negative coefficient of π suggests that a less hydrophobic (more polar) group may simply favor the interaction. An additional support to the dispersion interaction can be derived from eq 177 which relates the activity of 15 different anticancer drugs with the molecular weight.[246]

$$\log(1/C) = 3.411 \log \mathrm{MW} - 2.559$$
$$n = 15, r = 0.79, s = 0.84 \qquad (177)$$

Since, for the derivatives of LXVII, Lien et al.[245] found a good correlation existing between the anticancer activity and the RDR inhibition potency (r = 0.90) but failed to find any correlation existing between the antiviral activity and the latter, they concluded that for anticancer activity the inhibition of RDR may be the major mechanism but a different mechanism may be involved in the antiviral action.

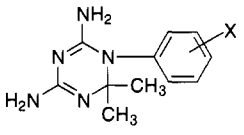
b. Dihydrofolate Reductase. Dihydrofolate reductase (DHFR) or tetrahydrofolate dehydrogenase is an enzyme of central importance in biochemistry and medicinal chemistry. It catalyzes the reduction of dihydrofolate to tetrahydrofolate, a substance only one step short of the coenzyme for thymine synthesis. The inhibition of this enzyme provides a very important ground for designing anticancer drugs.

Methotrexate, a pteridine derivative (LXVIII),was

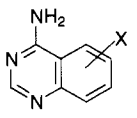


LXVIII

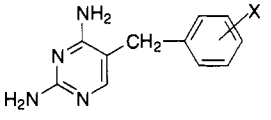
found to be the most effective DHFR inhibitor. It was synthesized in 1949 by Seegar et al.,[247] and since then it has been the most widely used antitumor drug[248] and even today, after more than 40 years of clinical use, it remains the most effective antifolate antitumor drug.[249] However, although methotrexate (MTX) remains supreme in the area of cancer chemotherapy, there is still an enormous effort to find better antifolates. Many variations of LXVIII, hundreds of triazines (LXIX), quinozolines (LXX), and pyrimidines (LXXI) have been tested as inhibitors of DHFR in cells and animals. DHFR inhibitors have also been useful as antimicrobial agents. Trimethoprim (LXXII), which developed out of the work of Roth et al.,[250] created a storm of publications on the search for better antimicrobial agents.



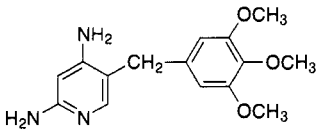
LXIX



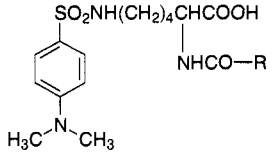
LXX



LXXI



LXXII

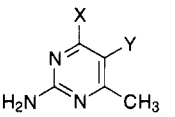


LXXIII

A number of DHFR inhibitors carrying fluorescent labels have been synthesized,[251,252] and some fluorescent compounds such as derivatives of LXXIII have been found almost as potent inhibitors of DHFR as MTX. This makes them excellent means for labeling and identifying DHFR.[252]

Enormous structure–activity relationship studies, including molecular graphics, have been made on all kinds of DHFR inhibitors. A review written on these studies 10 years ago covered 75 pages of the literature,[253] and since then dozens of papers have further appeared.[254] Thus the work on DHFR inhibitors is too voluminous to be presented here, but a detailed implication of the work will be presented in the latter section.

c. Thymidylate Synthetase. Thymidylate synthetase (ThS) belongs to the group of enzymes that are important in DNA synthesis. It catalyzes the formation of thymidylate from 2′-deoxyuridylate with transfer and reduction of one carbon unit of 5,10-methylenetetrahydrofolate. Hence this enzyme is also a popular target for antitumor agents. A variety of compounds were studied for their ThS inhibition activity and subjected to QSAR studies. For a series of 2-amino-6-methylpyrimidines (LXXIV),a QSAR



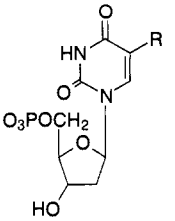
LXXIV

study revealed eq 178,correlating the enzyme (*E. coli*)

$$\log(S/I)_{50} = 0.255(\pm 0.05)\mathrm{MR_Y} + 0.905(\pm 0.29)I_1 - 0.664(\pm 0.23)I_2 - 2.910(\pm 0.32)$$
$$n = 41, r = 0.914, s = 0.299 \qquad (178)$$

inhibition activity mainly with the molar refractivity of the Y substituents.[255] The indicator parameter I_1 was used for X substituents. It was given a value of 1 for X = SH and 0 for other substituents. The parameter I_2 was used, with a value of 1, to indicate a Y substituent of the type $(CH_2)_3NR_1R_2$ with R_2 = $COCH_3$, COC_6H_5, or $COOC_6H_5$. Thus eq 178 suggests that while molar refractivity of the Y substituent in general would produce a positive affect on the activity, a substituent of the type as indicated by I_2 would give negative effect and that an SH group at 4-position will enhance the activity. The activity parameter $(S/I)_{50}$ refers to the ratio of the concentration of substrate to that of inhibitor which gives 50% inhibition of the enzyme.

The negative influence of bulky Y substituents at 5-position in LXXIV may be due to steric effects. This kind of steric effect of 5-substituent was clearly accounted for by the MR parameter in *Lactobacillus casei* ThS inhibition by some 5-substituted 2′-deoxyuridylates (LXXV)(eq 179).[256] Equation 179 also



LXXV: R = H, F, Cl, Br, I, CH_3, CF_3, CHO, CH_2OH

indicates a positive effect of electron-withdrawing nature of 5-substituent.

In another study[257] on *L. casei* ThS inhibition by the congeners of LXXV, where R = C_6H_4-4-X, the

$$\log(1/K_i) = 1.58(\pm1.17)\sigma^- + 3.49(\pm2.33)F - 1.43(\pm1.11)\mathrm{MR} + 5.88(\pm0.84)$$

$$n = 9, r = 0.953, s = 0.461 \qquad (179)$$

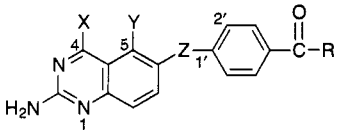
activity was found to be affected by electronic parameters of X substituents of aryl ring but not by their MR values (eq 180). Instead their hydrophobic

$$\log(1/K_i) = 0.25(\pm0.17)\pi - 1.13(\pm0.82)F - 0.39(\pm0.46)R - 0.08(\pm0.30)$$

$$n = 12, r = 0.912, s = 0.218 \qquad (180)$$

constant was observed to be of some value. The inhibition activities of these compounds against enzymes obtained from other sources, like L1210 and human lymphoblast cells, were not found to be so well correlated with physicochemical parameters.

In the case of a series of quinazolines (LXXVI),the



LXXVI

ThS inhibition activities were found to be related to only some indicator parameters defined as follows. $I_1 = 1$ for X = OH or SH and 0 for X = NH_2; $I_2 = 1$ for Y = CH_3 and 0 for Y = H; $I_3 = 1$ for Z = $NHCH_2$ and 0 for all others; $I_4 = 0$ for Z = CH_2NH or $NHCH_2$ and 1 for all others; $I_5 = 0$ for R = Glu or Glu(Et)$_2$ and 1 for R = OH or OC_2H_5; $I_6 = 0$ for R = Glu and 1 for all others. With these parameters, Chen et al.[258] correlated the L1210 mouse leukemia cell ThS inhibition activity as shown by eq 181 and *L. casei* ThS inhibition activity as shown by eq 182. In eq 182, π_6

$$\log(1/\mathrm{IC}_{50}) = 5.98(0.14) + 0.75(0.23)I_4 - 2.01(0.20)I_5$$

$$n = 29, r = 0.905, s = 0.525 \qquad (181)$$

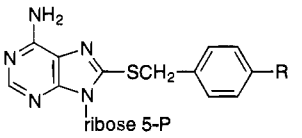
$$\log(1/\mathrm{IC}_{50}) = 4.638(0.173) - 0.395(0.110)I_1 + 0.391(0.105)I_3 - 0.809(0.113)I_6 + 0.426(0.093)\pi_6$$

$$n = 28, r = 0.911, s = 0.246 \qquad (182)$$

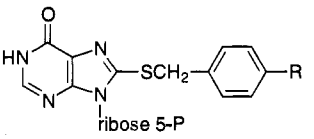
is the hydrophobic constant of Z substituent. Since these two equations are based mostly on indicator parameters and since many compounds were outlier to them, no mechanistic conclusion could be drawn from them. Even cluster, factor, and discriminant analyses performed[258] on these compounds did not throw any light on the mechanistic aspect of ThS inhibition by these compounds.

d. Inosinic Acid Dehydrogenase. Inosine monophosphate (inosinic acid) dehydrogenase (IMPDH) catalyzes the conversion of IMP to xanthosine monophosphate (XMP). The conversion of IMP to XMP is the first step of the biochemical conversion of IMP to GMP (guanosine monophosphate). Hence, the enzyme IMPDH is of vital importance to rapidly growing cells, and therefore, the inhibition of this enzyme will lead to the inhibition of cell growth. This may be an important aspect of the design of anticancer drugs.

Some AMP (adenosine monophosphate) and IMP analogues were found to act as IMPDH inhibitors,[259,260] and for a small series of 8-(para-substituted benzylthio)AMP and IMP[LXXVIIa, R = H, F, Cl, OCH_3, CN, NO_2, $C(CH_3)_3$, COO^- and LXXVIIb, R = H, Cl, OCH_3, CN, NO_2, $C(CH_3)_3$],Skibo and Meyer[259]



LXXVIIa



LXXVIIb

were able to correlate the inhibition constant with the field constant F as shown by eq 183, where $I = 1$ for IMP analogues and zero for AMP analogues. No other parameter was found to be correlated with K_i.

$$\log(1/K_i) = 3.87(\pm0.11) + 0.75(\pm0.25)F + 0.32(\pm0.14)I$$

$$n = 14, r = 0.933, s = 0.116 \qquad (183)$$

Since only F gave a significant correlation, Skibo and Meyer assumed that some type of charge-transfer interaction with an electron-rich site in the active site was involved.

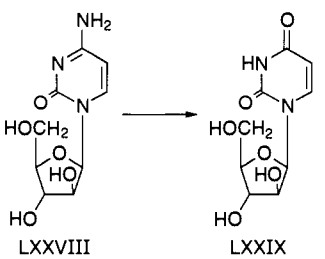
The K_i values of these compounds were, however, also found to be well correlated with van der Waals volume (eq 184).[261] Since F was not found to be

$$\log(1/K_i) = 3.924 + 2.123(0.767)V_w - 3.720(0.993)V_w^2 + 0.136(0.074)I$$

$$n = 13, r = 0.907, s = 0.132 \qquad (184)$$

correlated with V_w and since inclusion of F did not make any significant improvement in the correlation expressed by eq 184, Gupta and Handa[261] assumed that there might be some dispersion interaction between the substituent and the active site of the enzyme and that steric hindrance by a comparatively bulkier group would limit this interaction.

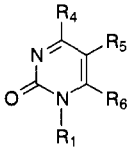
e. Deaminases. Inhibitors of certain deaminases, such as cytosine nucleoside deaminase, guanine deaminase, and adenosine deaminase, can be exploited to design antitumor drugs. The cytosine nucleoside deaminase catalyzes the deamination of nucleoside such as ara-*C* (LXXVIII) to ara-*U* (LXXIX). Since ara-*C*, but not ara-*U*, has been



LXXVIII LXXIX

effective against certain types of cancer, it was worthwhile to find inhibitors of cytosine nucleoside deaminase that could be effective against the tumor

enzyme but not against the human enzyme. The initial study was done by Baker and Kelley[262] and their inhibition data on uracil analogues (LXXX) were



LXXX

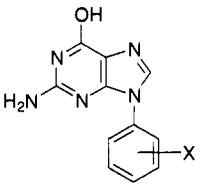
correlated by Yoshimoto and Hansch[255] as shown by eq 185. There were a variety of substituents in LXXX and the parameter I_1 was used with a value of 1 for 4-NH_2, 4-SH, and 4-NHOH functions. These groups

$$\log(1/IC_{50}) = 0.283(\pm0.06)\pi_5 + 0.188(\pm0.07)\pi_{1,6} + 0.265(\pm0.16)I_1 + 2.257(\pm0.24)$$

$$n = 71, r = 0.927, s = 0.227 \qquad (185)$$

are thus shown to produce a positive effect on the activity and the substituents of three consecutive positions 1, 6, and 5 are supposed to be involved in hydrophobic interactions with the enzyme.

Guanine deaminase hydrolytically deaminates guanine to xanthine. Hence, the selective inhibitors of this enzyme can also be exploited as anticancer drugs. A QSAR study[263] on a large series of 9-(X-phenyl)guanines (LXXXI) had revealed eq 186 for



LXXXI

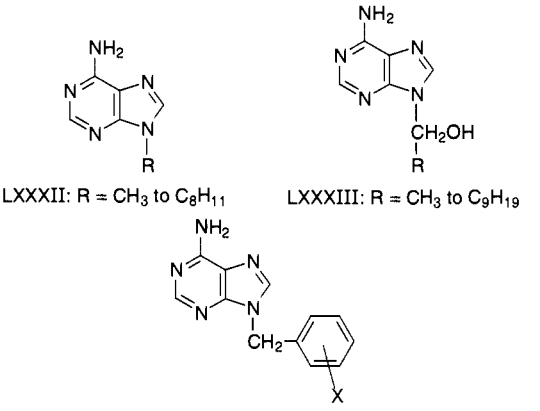
their guanine deaminase inhibition activity. In this equation, I was used with a value of 1 for the presence of 4-OCH_3 as a second substituent and I_2, with a value of 1, to account for the presence of an SO_2F group in the substituents. The third parameter

$$\log(1/IC_{50}) = 1.176(\pm0.25)MR_3 + 0.403(\pm0.11)\pi_4 - 0.127(\pm0.05)(MR_3)^2 - 3.417(\pm0.44)I_1 - 0.613(\pm0.25)I_2 + 1.608(\pm0.29)I_3 + 0.994(\pm0.43)E_{s,2} + 3.659(\pm0.50)$$

$$n = 92, r = 0.941, s = 0.366 \qquad (186)$$

I_3 was given a value of 1 for a 4-OR group, where R was a variety of functions. An obvious interpretation of this equation was that while the 4-substituents may be involved in hydrophobic interaction, the 3-substituents may be involved in dispersion interaction to the extent of bulk tolerance. Further, a 4-OR group appears to produce a positive effect on the activity, a 4-OCH_3 group present as a second substituent appears to decrease the activity, and the presence of an SO_2F function in the substituents is also shown to produce a negative effect.

Like guanine deaminase, adenosine deaminase hydrolytically deaminates adenosine to inosine. The selective inhibitors of this enzyme, therefore, would also be of importance to the treatment of tumors. Schaeffer et al. studied 9-alkyladenines (LXXXII),[264] 9-(1-hydroxy-2-alkyl)adenines (LXXXIII),[265] and 9-benzyladenines (LXXXIV),[266]for their adenosine deaminase inhibition activity and made a QSAR study[266] on them. In their QSAR study, they obtained eq 187 for LXXXII, eq 188 for LXXXIII with R = CH_3-C_6H_{13}, eq 189 for LXXXIII with R = C_7H_{15}-C_9H_{19}, and eq 190 for meta substituted analogues of LXXXIV. QSAR was also tried for para-substituted analogues of LXXXIV, but no significant correlation was obtained.



LXXXII: R = CH_3 to C_8H_{11} LXXXIII: R = CH_3 to C_9H_{19}

LXXXIV

$$\log(S/I)_{50} = 0.452(\pm0.06)\pi - 1.194(\pm0.15)$$

$$n = 8, r = 0.992, s = 0.078 \qquad (187)$$

$$\log(S/I)_{50} = 0.932(\pm0.21)\pi - 0.483(\pm0.41)$$

$$n = 6, r = 0.987, s = 0.157 \qquad (188)$$

$$\log(S/I)_{50} = 0.19\pi + 1.66$$

$$n = 3, r = 0.996, s = 0.012 \qquad (189)$$

$$\log(S/I)_{50} = 0.296(\pm0.17)\pi + 1.096(\pm0.431)\sigma - 0.093(\pm0.17)$$

$$n = 9, r = 0.963, s = 0.132 \qquad (190)$$

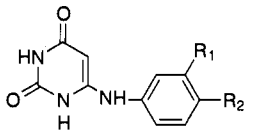
The coefficient of π in eq 188 is much larger than that in eq 187. This led Schaeffer et al. to suggest that the hydrophobic interactions of two different types of compounds are different. This difference in interactions was attributed to the possibility of conformational change in the enzyme brought out by congeners of LXXXIII, so that the hydrophobic region of the enzyme was more accessible to these congeners. But a much smaller coefficient of π in eq 189 derived for another set of LXXXIII congeners possessing bigger substituents indicated the limitation of the bulk tolerance.

Equation 190 derived for meta-substituted 9-benzyladenines suggests that, since the coefficient of π is much smaller than that of σ, the electronic nature of substituents will dominate over their hydrophobic property, and hence, there can be strong electrostatic interaction between the inhibitor and the enzyme.

f. DNA Polymerase. DNA polymerase is important in the DNA replication. Replication can begin at any

point in the double helix. The enzyme deoxyribonuclease splits open one strand by catalyzing the hydrolysis of a 3′-phosphate ester bond so that a deoxyribose with a free 3′-OH group is exposed. DNA polymerase then catalyzes the addition of nucleotides to 3′-OH end, pairing appropriate nucleotides against the unbroken parent strand, which acts as a template. The selective inhibitors of this enzyme may be useful as anticancer and antibacterial drugs.

A QSAR study was made[267] on a series of 6-anilinouracils (LXXXV) for their inhibitory activity against



LXXXV

the wild-type DNA polymerase III (pol III) and a mutant enzyme, pol III/azp-12, derived from *Bacillus subtilis*. For the two enzymes, the concentrations of inhibitors required to achieve 50% inhibition of enzyme activity were found to be correlated with π and MR as

$$\log(1/IC_{50}) = 1.00(0.13)\pi_m + 0.71(0.12)\pi_p + 3.87(0.48)MR_m - 2.30(0.27)(MR_m)^2 + 2.86(0.54)MR_p - 2.18(0.26)(MR_p)^2 + 2.50(0.30)$$

$$n = 37, r = 0.942, F_{6,30} = 40.01 \quad (191)$$

$$\log(1/IC_{50}) = 1.26(0.13)\pi_m + 1.13(0.12)\pi_p + 2.51(0.46)MR_m - 1.67(0.26)(MR_m)^2 + 3.48(0.52)MR_p - 2.42(0.25)(MR_p)^2 + 2.39(0.29)$$

$$n = 37, r = 0.948, F_{6,30} = 45.35 \quad (192)$$

These equations simply show that the inhibition of either of the enzymes would involve hydrophobic interaction with both the meta and para substituents of the inhibitors but that the interaction would be controlled by the relatively bigger size of the substituents. However, as it is obvious from the coefficients of the variables, the extent of the interaction of substituents from the two different positions and the extent of the tolerance for the size of the substituents by the hydrophobic pockets of the enzymes are little different in the two enzymes.

The inhibition of RNA-instructed DNA polymerase by some rifamycin derivatives was shown[268] qualitatively to be a function of the lipophilicity as measured by a reversed-phase thin-layer chromatographic technique.

g. Lactate and Malate Dehydrogenases. Lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) are both oxidoreductases utilizing NAD^+ (nicotinamide adenine dinucleotide) as acceptor. LDH converts lactate to pyruvate in the glycolytic process, and MDH plays a role in the Krebs cycle by the conversion of malate to oxalacetate. Some cancer cells have exhibited abnormal levels or activities of these enzymes.[16-19] Selective inhibition of these enzymes in cancerous cells would, therefore, increase the prospects for chemotherapy of solid tumor systems. Inhibitors of these enzymes may be useful in the treatment of tumors that are slow in dividing and hence are resistant to inhibitors of DNA synthesis. Inhibition of either of LDH or MDH will ultimately lead to cessation of cellular respiration.

7-Substituted 4-hydroxyquinoline-3-carboxylic acids (Table 11) were studied not only for their cellular respiration inhibition activity but also for LDH and MDH inhibition activities.[22,23] For compounds listed in Table 11 and a few more compounds, the mitochondrial MDH (m-MDH) inhibition activity was found to be significantly correlated with MR (eq 193),[23] and so cytoplasmic MDH (s-MDH) and skeletal muscle LDH (LDH-M_4) inhibition activities were studied for some of these compounds (eqs 194 and 195).[23] These inhibition activities were also shown to

$$\log(1/IC_{50})(\text{m-MDH}) = 0.58MR + 2.46$$

$$n = 29, r = 0.961, s = 0.34 \quad (193)$$

$$\log(1/IC_{50})(\text{s-MDH}) = 0.29MR + 2.61$$

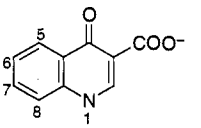
$$n = 17, r = 0.87, s = 0.38 \quad (194)$$

$$\log(1/IC_{50})(\text{LDH-M}_4) = 0.36MR + 2.79$$

$$n = 11, r = 0.96, s = 0.20 \quad (195)$$

be linearly well correlated with van der Waals volume,[112] having $r = 0.96$, 0.89, and 0.95, respectively, and with molecular connectivity index ($^1\chi^v$)[112] having $r = 0.96$, 0.89, and 0.96, respectively.

Since the cell respiration inhibition activity of these compounds was found to be correlated with lipophilic parameters (eqs 74 and 75) and not with MR, it becomes difficult to say if these enzymes are principal intracellular target sites in ascitic cells. In the case of enzymes, the role of molar refractivity was also demonstrated for a large series of 1,4-dihydro-4-quinoline-3-carboxylates (LXXXVI) studied by Baker's group.[269] Equations obtained by Yoshimoto and



LXXXVI

Hansch[255] for m-MDH and s-LDH inhibition by these compounds were

$$\log(1/IC_{50})(\text{m-MDH}) = 0.699(\pm0.06)\pi_5 + 0.290(\pm0.08)MR_{6,7,8} - 1.121(\pm0.37)I_5 + 3.156(\pm0.18)$$

$$n = 75, r = 0.943, s = 0.385 \quad (196)$$

$$\log(1/IC_{50})(\text{s-LDH}) = 0.08(\pm0.02)MR_{1,5,6,8} + 0.487(\pm0.16)I_5 - 0.114(\pm0.09)I_1 + 3.853(\pm0.11)$$

$$n = 79, r = 0.836, s = 0.173 \quad (197)$$

Equation 196 shows that there is some hydrophobic effect only from the 5-position, but simultaneously the negative coefficient of the indicator parameter I_5, indicative of the presence of 5-$O(CH_2)_nOC_6H_5$ with n

= 3 or 4, shows the steric effect produced by bulky 5-substituents. Equation 197 derived for cytoplasmic LDH does not show any apparent hydrophobic effect, but since the coefficient of I_5, which is defined for the presence of 5-$(CH_2)_nC_6H_5$ and takes the value of unity for $n = 2-6$, is positive, it was assumed[255] that such groups at the 5-position might be involved in the hydrophobic interaction. However, since I_5 defines the bulkiness of the group, it is more proper to assume only the dispersion interaction from the 5-position. This is in accordance with the correlation of MR_5 with the activity. The parameter I_1 accounts for 1-H, which has a small negative effect on the activity.

h. Glyceraldehyde 3-Phosphate and Glutamate Dehydrogenases. Congeners of LXXXVI were also found to inhibit glyceraldehyde 3-phosphate dehydrogenase (GPDH) and glutamate dehydrogenase (GDH).[269] Like MDH and LDH, GPDH and GDH are also important in the glycolytic pathway, and therefore, their inhibition will also lead to the inhibition of cell growth. Yoshimoto and Hansch[255] obtained correlations for their inhibition by congeners of LXXXVI as shown by eqs 198 and 199. In eq 198, I_5

$$\log(1/IC_{50})(GPDH) = 0.091(\pm0.02)MR_{1,5,6,8} + 0.498(\pm0.18)I_5 - 0.149(\pm0.10)I_{1,5} + 3.127(\pm0.10)$$

$$n = 72, r = 0.849, s = 0.172 \qquad (198)$$

$$\log(1/IC_{50})(GDH) = 0.491(0.04)\pi_5 + 0.233(\pm0.05)MR_6 - 0.553(\pm0.17)I_5 + 3.355(\pm0.08)$$

$$n = 87, r = 0.948, s = 0.253 \qquad (199)$$

takes the value of 1 for 5-$(CH_2)_2C_6H_4$-3′-X or -4′-X. This is however not an important parameter, as there were only four such groups. $I_{1,5}$ takes the value of 1 for congeners having H at both the 1- and 5-positions. This parameter suggests only a small negative effect. In totality, eq 198 is very similar to eq 197 derived for LDH; hence, the inhibition of GDPH can also be said to involve dispersion interaction.

Likewise, eq 199, where I_5 takes the value of 1 for 5- or 6-$O(CH_2)_nC_6H_5$ ($n = 2-5$), is remarkably similar to eq 196 derived for MDH. The negative coefficient of I_5 shows a steric disturbance in the hydrophobic interaction, which otherwise will take place between the 5-substituent and the enzyme. The substituent at the 6-position appears to involve dispersion interaction. Substituents at other positions were not shown to be important in this case.

IV. An Overview

All structure–activity relationship studies, qualitative or quantitative, made so far on antitumor drugs have primarily established that the main cellular target of these drugs is DNA. These drugs either inhibit the synthesis of DNA or stop its proliferation in the cancerous cells. However, in either case their modes of interaction with the receptors are highly diversified. The antitumor drugs that directly bind with the DNA and stop its proliferation involve mainly three types of binding: (1) nonintercalative groove binding, (2) intercalation, and (3) covalent bond formation. This last kind of binding is the strongest one and is also responsible for the activity of carcinogens.[270] The mode of interaction of carcinogens with DNA involves almost exclusively the covalent bond formation, even if for some of them it may involve a preliminary intercalative step[271-274] and even if the structure of the covalent adduct may possibly involve a partial intercalation of the drug.[275-277]

Both antitumor drugs and carcinogens act upon DNA in the form of electrophilic attacking agents and frequently bind with the same type of bases in it. These common features in the covalent mode of interaction of certain antitumor drugs and carcinogens seem to hinder the progress in the design of potent antitumor drugs, but there exists base sequence specificity for all the indicated types and species of antitumor drugs in their interaction with DNA. Important recent achievements in the analysis of such sequence specificities open prospects for rapid progress in the development of rationally conceived and improved new generation of effective and specific chemotherapeutic agents.

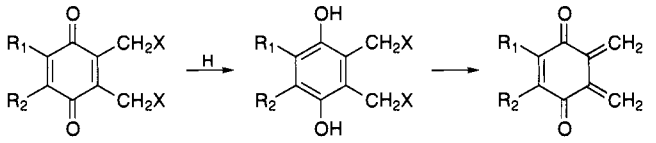
QSAR studies on antitumor drugs have thrown light on how physicochemical properties of these drugs would help them to have better interaction with the targets and thus produce better effects. For alkylating agents that bind covalently with nucleophilic centres in vivo, QSARs have pointed out the prime importance of the electronic factors. The lipophilicity which is a fundamental property of drug molecules describing their ability to cross the cell membrane and reach the receptor sites has been shown of secondary importance and several inconsistencies have been observed in its role. Alkylating agents have to be first converted to an electrophile and the ease of this conversion will decide the efficiency of the drug. Hence the electronic properties that can facilitate this conversion need to be investigated. QSARs have very clearly exhibited that, among the various electronic properties, the electron-releasing ability of the substituents that can be described by the negative value of the parameters like Hammett constant σ or σ^-, Taft constant σ^*, field constant F, or resonance constant R would be of prime importance. This observation is based upon eqs 1, 5–7, 9–13, and 19 obtained for the nitrogen mustards and eqs 28, 30, 31, and 33 obtained for triazenes. Nitrogen mustards are supposed to first form the aziridinium ion (b, Scheme 1) or cyclic ethylene immonium ion (III). The formation of these ions will be highly dependent upon the electron density on the nitrogen, which can be affected by electron-releasing substituents.

The microsomal activation of phenyltriazenes leading to the formation of the reactive species, carbonium ion, may also require high electron density at the 3-nitrogen, which obviously can be affected by the electron-releasing substituents at the phenyl ring and that is what has been corroborated by the regression equations mentioned in the preceding paragraph for triazenes. Since triazenes could also be substituted at their 3-N, the substituents of this position affected the activity mostly by their hydro-

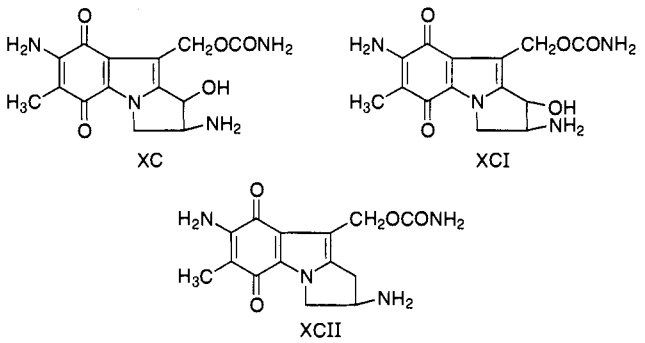
phobic property as exhibited by eqs 27 and 33–39. The hydrophobicity of substituents obviously helped the transport of the drug with a common optimum log $P \approx 1$. The bulky substituents at phenyl ring as well as at 3-N position are however indicated to produce some steric effects in the action of triazenes (eq 37).

In the case of nitrosoureas, the lipophilicity was found to be the main factor governing the activity. However, except eq 22, all other equations (eqs 21, 23, and 24) obtained for nitrosoureas had negative coefficients for both log P and $(\log P)^2$. This raises a question as to how the lipophilicity will affect the activity. One answer to this question can be given by assuming that the effectiveness of the drug demands that a larger portion of its concentration applied remains in the aqueous medium where the actual drug–receptor interaction takes place.

Quinones that act against animal tumors are thought to function as bioreductive alkylating agents. The bioreductive agents are metabolically reduced by some enzymes. Quinones are reduced presumably by an NADPH-dependent quinone reducing system. This reduction converts them into a dihydroquinone (LXXXVIII) which spontaneously generates the reactive species, an *o*-quinone methide (LXXXIX). This



reactive species is then visualized to be capable of alkylating DNA or other vital cellular components. Hence the electronic factors are supposed to play important roles in the action of quinones. Equations 43–46 corroborate this idea by incorporating the electronic factors like F and R. These factors with negative coefficients indicate that the electron-releasing effects of the substituents will have direct bearing over the activity of quinones. Notwithstanding this, eqs 48 and 49 suggest that electron-withdrawing and not electron-releasing substituents will produce the positive effect on the antitumor activity. But since these equations exhibit the role of only R_4 substituent, which is attached to the nitrogen atom, it may be assumed that its electron-withdrawing nature may attract the electron from the ring toward nitrogen and may make the latter susceptible to the reduction. That the ease of reduction may have the direct bearing over the action of quinones is well supported by eqs 52 and 53, which relate the antitumor activity and toxicity, respectively, of a series of 1,4-naphthoquinones with half-wave reduction potential ($E_{1/2}$). The latter was also found to have a linear relationship with the antitumor activities of certain mitomycin C analogues (eqs 55 and 58). However, for mitomycin A analogues, the lipophilicity and not any electronic property was found to be important (eqs 56, 59, and 61). But, while eq 56 obtained for the activity against human tumor cells shows the positive effect of lipophilicity on activity, eq 61 obtained for the activity against leukemia cells in mice shows the negative dependence. Equation 59 obtained for the same does represent a positive correlation between the activity and lipophilicity but is not very significant. This difference has been assumed[91] to be due to the suspension of mice leukemia cells in ascites which is an aqueous environment confined to abdominal cavity. It would be desired therefore that for the effectiveness of the drug, a larger portion of its concentration applied remains in the aqueous environment, which is possible for only highly hydrophilic drugs. And the reason as to why mitomycin A analogues base their activity mainly on lipophilicity and not on reduction potential lies in the fact that they, unlike mitomycin C analogues, are readily reduced, so they need only to reach the receptor site, which can be facilitated only by their lipophilicity. Since mitomycin C analogues are not so susceptible to bioreduction, their activity would certainly be based on the ease of the reduction and hence on the reduction potential. The ultimate products of the reduction of mitomycin C (XXIII) are *cis*- and *trans*-2,7-diamino-1-hydroxymitosene (XC and XCI) and 2,7-diaminomitosene (XCII).



The yields of these compounds were found to be the same when the rate of reduction was varied by 11 orders of magnitude.[278] However, Moriguchi et al.[93,94] have concluded from their QSAR studies that electron-releasing and less bulky substituents at the 7-position of mitomycin analogues will favor the antitumor activity but the substituents at other positions, such as X and Y in XXIV, will affect the activity by other characteristics. The activity may increase when X substituent is electron withdrawing and Y is thin.

Correlations obtained for miscellaneous alkylating agents such as XXV and XXVI have not been very conclusive.

For mitotic inhibitors, the lipophilicity appears to be an important factor, particularly for colchicines and *Vinca* alkaloids, but both their antitumor activity and toxicity were found to be equally affected by this property (eqs 67–72). This showed a parallelism between the antitumor activity and the toxicity of these compounds and thus limited their use as antitumor drugs. However, for another class of antimitotic agents, analogues of combretastin (XXXI), some additional factors including the electronic ones were also found to affect the antitumor activity (eq 73), but since nothing was available regarding the toxicity of these compounds, it was hard to say if these factors would help in the design of useful antitumor drug in this class.

The lipophilicity was found to be important for cellular respiration inhibitors also. But for one class

of compounds studied, i.e. 7-substituted 4-hydroxyquinoline-3-carboxylic acids, it was found to produce the positive effect on the activity (eqs 74 and 75) and for another class of compounds, i.e., copper(II) chelates, it was found to produce the negative effect (eq 77). Thus while in the case of former the cell permeability may be the sole governing factor for the inhibition of the cell respiration, in the case of the latter some important interaction seems to take place between the compound and receptor in the aqueous medium, so that the compounds needs to have the greater water solubility. Since copper chelates were also observed to inhibit the respiration of the normal cells and since the lipophilicity was found to have significant effect on this inhibition also (eq 76) a selectivity was sought from eqs 78 and 79. These equations suggested that the desired selective cytotoxicity to Ehrlich ascites tumor cells with minimal cytotoxicity to normal cells could be achieved by introducing substituents which would enhance water solubility, while stabilizing the chelate against permature dissociation by electron donation through conjugated ring system. Equation 78 predicted that $\pi = -1.30$ may provide the greatest selectivity and minimize the normal cell toxicity.

7-Substituted 4-hydroxyquinoline-3-carboxylic acids were also found to inhibit the enzymes lactate and malate dehydrogenases, which are found to be abnormally present in some cancer cells. The QSAR studies however indicated that the activity against the whole cell system cannot be directly attributed to the inhibition of the enzymes.

For hypoxia-selective antitumor agents, the activity could not be directly correlated with any physicochemical parameters, but since it was pointed out that their cytotoxicity was due to the nitro group reduction, the radiosensitizing potency studied for some of them was found to be well correlated with one-electron reduction potential (eqs 83). This potential was not found to be so significant in all the cases, but along with log P it gave significant correlations (eqs 81 and 82). However, these correlations were based on so small a number of data points that nothing conclusive could be drawn from them.

With regard to drugs binding to DNA, there is a much greater variety in the mode of their specific binding than usually admitted. The assumption that the narrow minor groove and not the wider major groove (Figure 2) is the preferred locus of binding for purely groove-binding drugs as well as intercalators that extend a nonplanar chain or group down into an adjacent groove[279] is not completely valid at least for the latter. Today a significant number of intercalators seem to bind with the major groove, but such observations were made particularly for GC-specific intercalators.[31] According to Pjura et al.[279] GC intercalators bind with the groove by forming hydrogen bonds with N-2 amino group of guanine, but this statement is concerned with minor groove GC intercalators and neglects the increasing number of major groove GC intercalators in which the specificity is due to preferential hydrogen bonding to N^7 of guanine rather than to N^7 of adenine.

Usually, the specificity of a groove-binding drug can be described in terms of a sequence of base pairs, but that of an intercalator can only be described in terms of two base pairs, and for some intercalators it seems essential to define their specificity in terms of triplets of base pairs.[31] The microheterogeneity of the DNA and the continuous or abrupt variation of its structure as a function of base sequence represent a fundamental reality of the major importance for the specificity and the mechanism of action of antitumor drugs. However, it should be kept in mind that antitumor action is a complex phenomenon in which the binding of a drug to DNA represents only one aspect of a multifaceted problem and QSAR studies unravel little about this complex phenomenon. In some cases it appears that DNA intercalation is a precursor catalyst to alkylation and/or cross-linking reactions of a ligand to DNA.[280] The number of molecular-modeling studies of ligand–DNA interactions, whether the intermolecular process is intercalation or otherwise, is small in comparison to the number of ligand–protein interaction modeling investigations.[281] From merely QSAR studies one can simply find what physicochemical or structural properties of molecules can enhance the drug–DNA binding and produce biological response which is ultimately desired.

As far biological response is concerned, for both acridine and anthracycline series of drugs binding to DNA the major role in it has been found to be played by hydrophilic–lipophilic balance (eqs 85–89, 100–103, 105, 106, and 111–121). However, since many of the equations mentioned in the parentheses involve the drug–DNA binding constant to exhibit better correlation and since this constant is shown to depend upon the electronic property and/or the polarizability of the substituents (see for example eqs 91, 93, and 94), the electronic factors too appear to have their roles in overall biological effect. Instead of the binding constant, Nakata and Hopfinger found that the intercalating energy was a more suitable parameter to be incorporated in the correlation along with the hydrophobic parameter (eq 120). Since most of the correlations exhibited the negative dependence of activity on lipophilicity, it can be assumed that for greater biological response the drug must stay for a sufficient period of time in the aqueous medium.

For bleomycin amplifiers the polarity of the molecules seems to be important. Equation 129 directly correlates the DNA binding constant with the dipole moment and other equations like eqs 125–127 and 130 that involve the molecular connectivity indices suggest indirectly the involvement of molecular dipole in the interaction. On the basis of a number of theoretical and experimental studies, Strekowski et al.[176] suggested that DNA binding of unfused aromatic compounds involves the dipole interaction with the groove of the DNA and that of classical fused-ring planar intercalators the dispersion interaction.

For actinomycin D analogues, the DNA binding was found to depend more upon the conformation of the DNA than any other factors, and the bulky substituents at the aromatic rings were shown to produce the steric hindrance in the binding (eq 133). Consequently, the antitumor activity was also found

to be affected by this steric hindrance (eqs 131 and 132).

In the miscellaneous category of the DNA intercalators, the GTCs (XLVI) were shown to involve both electronic and hydrophobic factors in eliciting their biological response (eqs 134–136), but for 2-arylbenzimidazole-4-carboxamides (XLVII) nothing could be said about their mode of action as no significant correlation could be obtained for them. For bisquaternary ammonium heterocycles (XLIX and L), eqs 141–143, although not very significant, could hint that their antitumor properties result from their lipophilic–hydrophilic balance and ability to distinguish certain DNA sites. With regard to the mode of action of bis(guanylhydrazones) (LI) no clear picture could emerge.

In a recent communication on drugs binding to DNA, Hopfinger and Cardozo[280] pointed out that (1) the majority of intercalating ligands show no base-pair sequence specificity or exhibit a preference for GC base pairs, (2) DNA intercalation is a necessary, but not a sufficient condition for anticancer activity, (3) intercalating binding strength of a ligand shows a positive correlation with cytotoxic potency in several, but not in all, intercalating ligand analogue series, and (4) there exists a correlation between anticancer activity and ligand–DNA residence time. The idea of using calculated intercalation properties along with other molecular descriptors, usually derived from the ligand, to develop a QSAR as a part of a modeling study has been rare.

In multidrug resistance phenomenon, the cross resistance of antitumor agents was shown to depend primarily on molecular weight (eqs 149–153). QSAR results suggested two modes of entry of drugs into the resistant cells. Small molecules may involve the normal diffusion and those having MW > 400 may adopt endocytosis route.

With regard to antimetabolites, QSAR studies suggest that the activity of any kind of antimetabolites would be largely affected by their electronic properties. However, in the case of inhibitors of protein synthesis no consistency was observed in the role of physicochemical parameters.

Drugs that elicit their antitumor activity by inhibition of specific enzymes do not involve any complex mechanisms. Enzymes are normally flexible and generally possess both polar and hydrophobic sites. Thus the inhibitors may involve either both polar (or dispersion) and hydrophobic interactions together or only one of them. For example inhibitors of all the dehydrogenases discussed were shown to involve primarily the dispersion interaction (eqs 193–199). It was true even for the inhibitors of inosinic acid dehydrogenase (eq 184) which interfere entirely different cellular reactions than those interfered by inhibitors of other dehydrogenases. Although the study made by Skibo and Meyer (eq 183) indicated the involvement of the charge-transfer phenomenon in the inhibition of the inosinic acid dehydrogenase, it could not be corroborated by the QSAR analysis made by Gupta et al. who derived eq 184.

On the other hand, the majority of inhibitors of deaminases were found to involve only hydrophobic interaction (eqs 185 and 187–189). Exceptions to this were a series of guanine deaminase inhibitors (LXXXI), for which eq 186 showed that both hydrophobic and dispersion interaction can be involved, and a series of adenosine deaminase inhibitors (LXXXIV), where eq 190 showed that electrostatic interaction may dominate.

Similarly, in the inhibition of the DNA polymerase, only hydrophobic interaction was suggested to take place (eqs 191 and 192) but the hydrophobic pocket in the enzyme was indicated to possess the limited bulk tolerance. No definite conclusion however could be drawn from QSARs regarding the mode of interaction of thymidylate mynthetase inhibitors.

Some interesting observations were made regarding the inhibition of ribonucleoside diphosphate reductase (RDR). Different kinds of RDR inhibitors were shown to have different modes of action. Some RDR inhibitors were studied for their activity against the growth of cancer cells. Among them, the diones were found to base their activity basically on hydrophilic–lipophilic balance (eqs 170 and 171). Although eqs 170 and 171 also involved the electronic parameters σ and μ, respectively, a high covariance between them did not permit any mention about how the electronic properties would affect the activity. However, for another class of RDR inhibitors, that were tested for their tumor cell growth inhibition activity, i.e. hydroxyguanidines, the activity was found to depend upon the molar refractivity (eqs 172 and 176) or van der Waals volume (eq 174), suggesting that these inhibitors will involve the dispersion interaction in their activity. The dispersion interaction seemed to be important in the anticancer activity of even a series of miscellaneous RDR inhibitors (eq 177).

From a study on the derivatives of *N*-hydroxyurea ($H_2NC(O)NHOH$) it was concluded[240] that substantially higher molecular weight and polar functional groups providing low lipophilicity would favor the anticancer activity of these compounds. This conclusion simply indicates the dominance of the dispersion interaction. Hydroxyurea is used in the treatment of leukemias and head and neck cancers. It is the only drug used clinically whose primary mode of action is the inhibition of RDR. A good correlation was shown to exist between the antitumor potency and RDR inhibition activity of hydroxyguanidines also.[245]

With regard to the inhibition of RDR by thiosemicarbazones and aryl hydroxamic acids, no clear mechanism of interaction could emerge. From eq 168, obtained for only 10 thiosemicarbazones, one can simply speculate that thiosemicarbazones may undergo some nucleophilic reaction with the enzyme.

A careful examination of molecular models for several classes of DHFR inhibitors shows that many of the congeners incorporate a bridged aromatic substituent which can attain a conformation sufficiently similar to that of bound methotrexate to allow beneficial van der Waals interaction within the hydrophobic pocket. It has been observed from the DHFR–MTX complex that there are a number of hydrophobic amino acids which interact with the *p*-aminobenzoyl side chain of MTX (LXVIII). Therefore, the observed significance of hydrophobic param-

eters and of indicator variables for the various bridges between parent nucleus and aromatic substituent in correlations is not surprising. The nearly identical shapes and the optima of parabolic relationships obtained for pteridines, pyrimidines, and triazines,[253] support the conclusion derived from the molecular model that substituents can and do interact with the same region of *L. casei* DHFR. It has been discussed[282] that the *N*-phenyl of the triazines (LXIX), especially when accompanied by $-CH_2O-$ bridge to a second ring, provides appropriate positioning of substituents to allow interactions with the DHFR hydrophobic pocket, similar to those of pteridines and pyrimidines. For quinazolines, however, it is only anticipated that they interact with DHFR in much the same manner as pteridines as well as pyrimidines and triazines.[282] Although a comparative molecular shape analysis[283-286] of triazines, pyrimidines, and quinazolines tends to support this anticipation, the QSAR results for the latter do not match with the hypothesis.[282]

Using the distance geometry approach, a three-dimensional QSAR methodology, Ghose and Crippen[287-290] postulated an active-site model for ligand–DHFR interaction. When this model was compared with the X-ray crystal structure of methotrexate bound to *E. coli* DHFR, three interesting features were observed: (i) all the site pockets were sterically accessible, i.e., they did not overlap with the receptor site atoms, (ii) three site points portrayed as hydrogen bonding were surrounded by groups capable of hydrogen bonding, and (iii) the pockets showing high correlation with the hydrophobic property of the ligand were surrounded by various hydrophobic groups. In a recent communications, Bradley and Crippen however pointed out that different regions of the receptor bind with different ligands.[291]

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VI. References

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