QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDIES ON SOME ANTI- CANCER DRUGS

THESIS

Submitted in Partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

By

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Under the Supervision of

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To

My Reverent Parents

Late Smt. Ganga Devi and Dr. S.K. Porwal whose ardent desire for higher studies inspired me to complete this work

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI (RAJASTHAN)

CERTIFICATE

This is to certify that the thesis entitled "**QUANTITATIVE STRUCTURE - ACTIVITY RELATIONSHIP STUDIES ON SOME ANTI-CANCER DRUGS**" by **ALKA KURUP**, IDNO 92PHXF012, for award of Ph.D. Degree of the Institute, embodies original work done by her under my supervision.

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C O N T E N T S

CHAPTER - I

ANTI-CANCER DRUGS

ANTI-CANCER DRUGS

1.1 Introduction

Cancer is one of the most formidable disease of the world. In medical term it is known as Neoplasm, which means a relatively autonomous growth of tissue. It can be found as tumor or in disseminated form as leukemia. It can be best regarded as a group of diseases characterised by (i) abnormal and uncontrolled growth, (ii) ability to invade adjacent tissues and even distant organs, and (iii) the death of the patient, if the malignant or benign tumor has reached the stage when it can not be removed from the body.[1] The main difference between the normal tissues and the tumor is that in most normal tissues the rate of proliferation equals the rate of cell death, but in cancer, proliferation exceeds the death rate. Also in normal cells, proliferation is in response to the subtle signals, when cell division is needed for repair, regeneration or growth and development, but cancer cells seems to lack such an auto regulation of proliferation.[2] Although cancer is the 2nd leading cause of death, next to cardiovascular diseases in developed world, and cancers rank 4th as a cause of death in developing countries,[3] it is rising, especially because of changes in environment and life style.

1.2 Causes of Cancer

The etiology of cancer is still not well understood,

but continuous efforts of scientists in this direction has given ample information about causes of some forms of cancer and their prevention.[1]

Environmental Factors:

80-90% of the different types of human cancers are believed to be due to different environmental factors. The major factors established so far include :-

(a) Tobacco: In various forms of usage (e.g. smoking, chewing) is the major cause of cancer of lung, larynx, mouth, pharynx, bladder, oesophagus, pancreas.

(b) Alcohol: Excessive intake of alcoholic beverages is associated with oesphageal and liver cancer.

(c) Dietary Factors: e.g. Smoked fish is related to stomach cancer, beef consumption to bowel cancer, a high fat diet to breast cancer.

(d) Occupational Exposures: Exposure to chemicals like cadmium, chromium, benzene, asbestos, polycyclic hydrocarbons, arsenic etc.

(e) Viruses: Hepatitis B virus is related to hepatocellular carcinoma, the Epstein-Barr virus (EBV) is associated with Burkitt's Lymphoma and nasopharyngeal carcinoma. Human papilloma virus (HPV) is a chief suspect in cancer of cervix. Virus is also believed to be responsible for Hodgkin's disease.

(f) Others: There are numerous other environmental factors such as radiations, air and water pollution, pesticides etc.

To this must be added the life style and habits which may be associated with an increased risk of certain cancers.

Genetic Factors:

Though these factors are less prominent and more difficult to identify, it appears that probably there is an inter-relationship between hereditary susceptibility and environmental carcinogenic stimuli in the causation of number of cancers.

In fact, genetic influences have long been suspected. It has been observed that retinoblastoma occurs in the children of the same parent. Mongols are more likely to develop leukemia than normal children.

1.3 Problems Faced in the Development of Chemotherapeutic Agents

Though cell biologists have been able to establish some sort of relationship between the gene action and cancer, where it is assumed that alteration of a protein by a carcinogen is reflected at the level of transformation of information from DNA or RNA, the heterogeneity of cancers impose a lot of problems. Because the individual tumor contain many subpopulations of neoplastic cells that differ in crucial characteristics such as (i) karyo type,

(ii) morphology, (iii) immunogenicity, (iv) rate of growth, (v) the capacity to metastasize, and significantly (vi) responsiveness to antineoplastic agents.

There are cogent reasons for why cancer is more difficult to cure than bacterial infections.[4] Human cells and bacterial cells differ qualitatively, whereas difference between human normal cells and cancer cells is only quantitative. Because of the basic differences at cellular level in the former, the host's immune system plays significant role in killing foreign (bacterial) cells, but is not very effective in killing cancer cells. Further, the antineoplastic agents kill the cells by 1st order kinetics, i.e., they kill only a constant fraction of cells rather than a constant number, e.g., if a patient has a trillion leukemia cells, then a potent anticancer drug might reduce population by 10,000 fold, when symptoms would be alleviated and the patient would be in a state of remission, but the remaining hundred million leukemia cells would readily increase to the original number after cessation of therapy. There may be a higher portion of resistant cells which means the same drug may not prove very effective. So multiple regimens have to be used to reduce the number of neoplastic cells drastically.

Another complication in chemotherapy is the relative unresponsiveness of slow-growing solid tumors. Current antineoplastic agents are most effective against cells with a high growth fraction. This is because their action is due to

blocking of the biosynthesis or transcription of nucleic acids or by preventing cell division by interfering with mitotic spindles. So cells in the synthetic or mitotic phases are highly susceptible to these agents, while cells in the resting state are resistant to many agents. Slow growing tumors characteristically have many cells in the resting state.[5]

Most antineoplastic drugs are highly toxic to the patient and must be administered with extreme caution. Some of them require clinical setting where supportive care is available. The toxicity usually involves rapidly proliferating tissues such as bone marrow and intestinal epithelium. However, individual drugs produce distinctive toxic effects on heart, lungs, kidneys and other organs.

1.4 Therapies Used For Treatment of Cancer

There are five modalities of treatment of cancer.

(i) Surgery

(ii) Photoradiotherapy

(iii) Radiotherapy

(iv) Immunotherapy

(v) Chemotherapy

(i) Surgery:

The most widely used method of treatment at present is surgery, i.e., the total removal of the tumor by mechanical means. Even an extremely large tumor can be removed by

surgery with lasting benefit if it has not metastasized. Conversely, a small tumor that has dispersed even a few cells to other organs such as the lungs, liver, or brain cannot be treated successfully by removing the primary tumor alone.[6] Disseminated forms of cancer such as leukemia cannot be treated surgically. Other forms of treatment such as radiotherapy and chemotherapy are necessary in conjunction with surgery.

(ii) Photoradiotherapy:

Tumors can be localized and destroyed by making use of the selectivity and fluorescence of hematoporphyrin derivative (HPD), a complex mixture of porphyrins resulting from treatment of hematoporphyrin with a mixture of acetic and sulphuric acids.[7] To locate tumors, HPD is injected into the cancer patient. After a 24 to 72 hour interval, fluorescence can be observed in tumor tissues by the use of probes with fiber optics. Porphyrin also accumulates in certain normal organs of the host, including liver, kidney, spleen, and skin, the latter accounting for transient skin photosensitization. To destroy tumors, light from a tunable dye laser of 620 to 640 nm wavelength is directed onto the tumors, using fiber optic extensions where necessary. Normal tissues, which have minimal absorption of 620 to 640 nm of light, are minimally damaged.

(iii) Radiotherapy:

Treatment with radiant energy is a successful rival of

surgery and it is used alone or in conjunction with other techniques in treating many forms of localised cancer.[6] Radiation therapy causes only minimal damage to the surrounding normal tissues. Γ-rays from radio isotopes such as Co-60 and X-rays are usually used in this kind of treatment. The effects of radiation are:

(i) direct destruction of the cells;

(ii) inhibition of developing mitosis followed by the death of the cells;

(iii) loss of proliferative capacity only in successive generations.

(iv) Immunotherapy:

By immunotherapy it is possible to destroy last few cancer cells remaining after surgery, radiotherapy, or chemotherapy.[7] This delays sometimes for long periods the spread or reappearance of cancer. As for example, BCG immunotherapy, begun after chemotherapy, produces remissions of much greater duration than chemotherapy alone in some forms of childhood leukemia, adult leukemia, Hodgkin's disease, and head, neck, breast, skin, and colon cancers. Used before chemotherapy, BCG does not increase the remission rate.

Early research in humans involved immunization of patients with extracts of their own tumor cells. With few exceptions, these early attempts at active specific

immunotherapy failed. Nevertheless, subsequent work in trials of patients with leukemia, melanoma, and lung cancer has given favourable results, and specific Immunotherapy may eventually be successful.[10]

(v) Chemotherapy:

The only treatment for the disseminated cancer is chemotherapy. Also it is less likely to have more effective method of treatment from further improvement in surgery or radiotherapy alone.

The increased understanding of cell cycle kinetics, tumor biology, recent advances in clinical techniques and improved preliminary screening are enhancing the emergence of newer, more potent compounds. Chemotherapy is providing increasing cure rates in 10 -15 forms of human cancer.[11-13] With increased understanding of the drug at molecular level, there has been continued improvement in this modality of cancer treatment. Today the research is directed towards development of not only newer, potent and less toxic chemotherapeutic agents but efforts are also made to design more effective regimens for concurrent administration of drugs and increased use of adjuvant therapies, which seem sure to provide better responses and response rates for immediate future. However, more effective drugs are yet to be discovered.

The design of a more effective drug depends on how best can it be rationalised. Because rational design of an

agent to have a specific activity on the selected target depends upon how best the target can b· defined so that it can be hit selectively in the presence of other similar targets. Unfortunately, in the case of cancer cells there is little information about the unique characteristics of the cancer cells, that may be exploited in the investigation of new agents. Nonetheless, useful anticancer drugs are being produced but mostly based on empiricism. The mechanism by which the anticancer drugs kill cancer cells selectively has not been clearly established, but evidences show that these drugs might interfere with the synthesis or function of nucleic acid or with mitotic process itself. The advances in quantitative structure activity relationship (QSAR) studies have widened the scope of rational drug design and finding the mechanism of drug actions. QSARs have proven their worth in the interpretation of mechanisms of inhibition of a number of enzyme systems[14] and in elucidating the modes of action of local anesthetics[15] and a variety of drugs acting at the CNS.[16,17] A recent review on QSAR of anticancer drugs[18] has provided valuable information about their rational design and has aroused further interest in their QSAR studies.

1.5 Classification of Anticancer Drugs

Anticancer drugs belong to different classes of chemical compounds and follow different modes of action. Therefore, these drugs can also be classified on the basis of their mode of action as given below.

(i) Chemically Reactive Drugs Having Nonspecific Action

Different types of chemicals exhibit their anticancer activity by alkylating nucleic acid. Alkylating agents can combine covalently with nucleophilic centre and hence can attack at any nucleophilic centre, available in vivo, nonselectively. These centres can be N, S or O atoms of biologically important functional groups such as amino groups, thiolate anions of proteins, and ring nitrogen atoms and phosphate anions of nucleic acids. However, it has been evidenced that the DNA, and in that also, 7-position of the guanine is the prime site of attack of biological alkylating agents. Scheme 1 explains how any two nucleophiles Y and Z can be alkylated by a bifunctional alkylating agent such as mechlorethamine(a). It cyclizes readily to the highly active ethyleneimmonium ion in polar solvents, like water, at physiological pH. The three membered ring reacts readily with replaceable hydrogen atoms in the above mentioned groups, 7-position of guanine in particular, in each of the double strands of DNA (Fig. 1.1) causing cross linking of the strands which hampers the DNA replication and other cell functions.[1,9] Cell multiplying rapidly are more sensitive to cross linking than normal cells because they are not able to repair damaged DNA, while latter do so by enzymatic excision of alkylated bases.

The bifunctional alkylation may also lead to interstrand cross linking of DNA or to the binding of DNA to

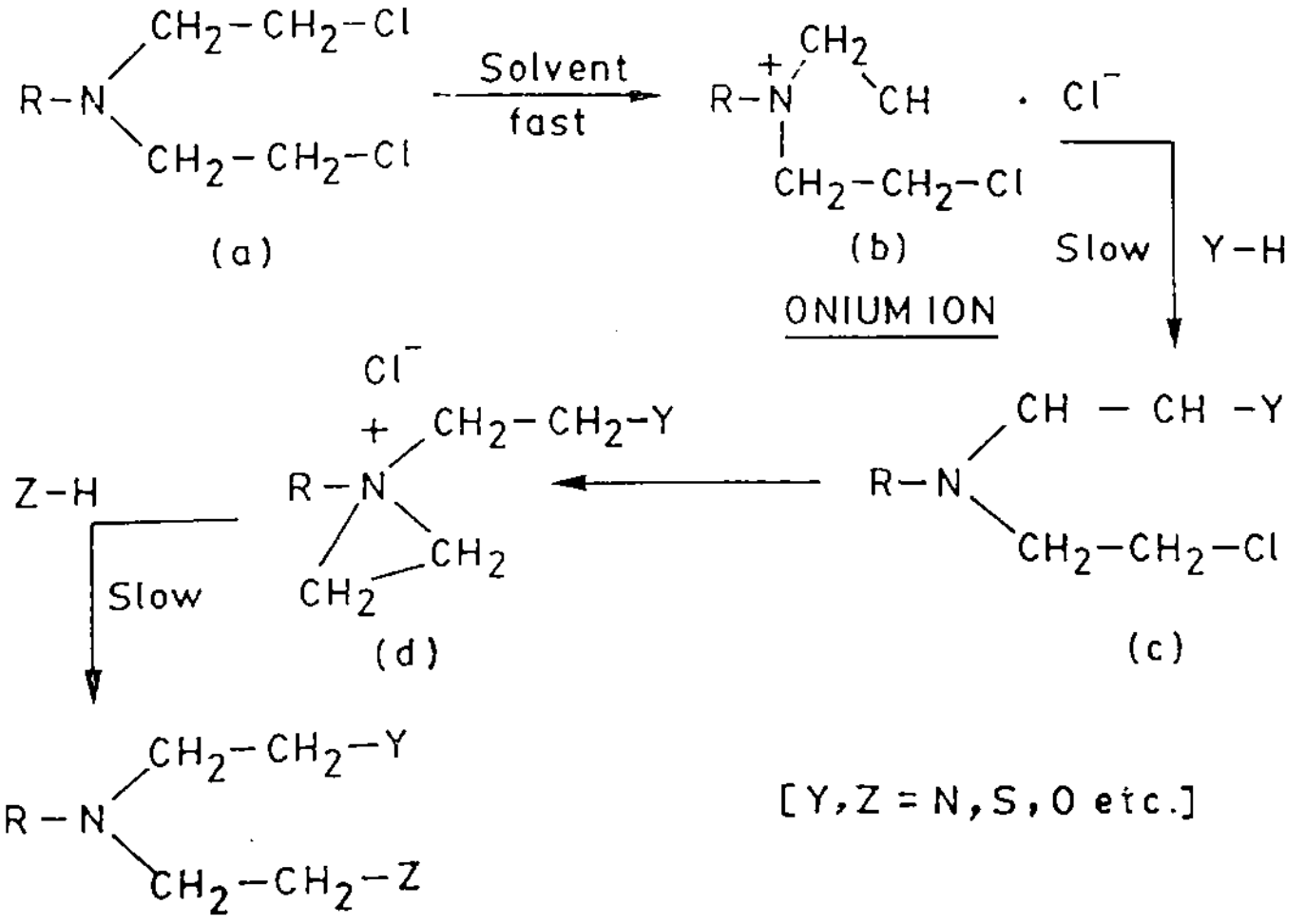
protein molecules.[20,21] Monofunctional alkylation may also take place but that would be less cytotoxic. It may result in mispairing of bases in DNA, e.g., normally the keto form of guanine forms hydrogen bond with cytosine, but after N-7 alkylation the enol form of guanine would be favoured which can pair with thymine [22] (Fig. 1.2a) Such mispairing could lead to miscoding and mutation. Monofunctional alkylation may also lead to opening of imidazole ring causing serious damage to DNA (Fig. 1.2b). However, theoretically alkylation at O-6 and N-3 would be more effective than at N-7.[23]

The important alkylating agents are nitrogen mustards, esters of sulfonic acids (alkyl sulfonates), nitrosoureas, triazenes and aziridines.

(ii) Mitotic Inhibitors

These are agents which arrest metaphase by interacting with microtubules, thus blocking mitosis.[24,25] Hence cell division gets arrested in metaphase. In the absence of intact mitotic spindle, the chromosomes may get dispersed throughout the cytoplasm (exploded mitosis) or may occur in unusual grouping, which are difficult to segregate correctly resulting in death of the cell.

These are usually compounds of natural origin and include vinca alkaloids, colchicine derivatives, podophyllotoxins and some other miscellaneous compounds.



Scheme.1. Bifurctional Alkylation with mechlorethamine.

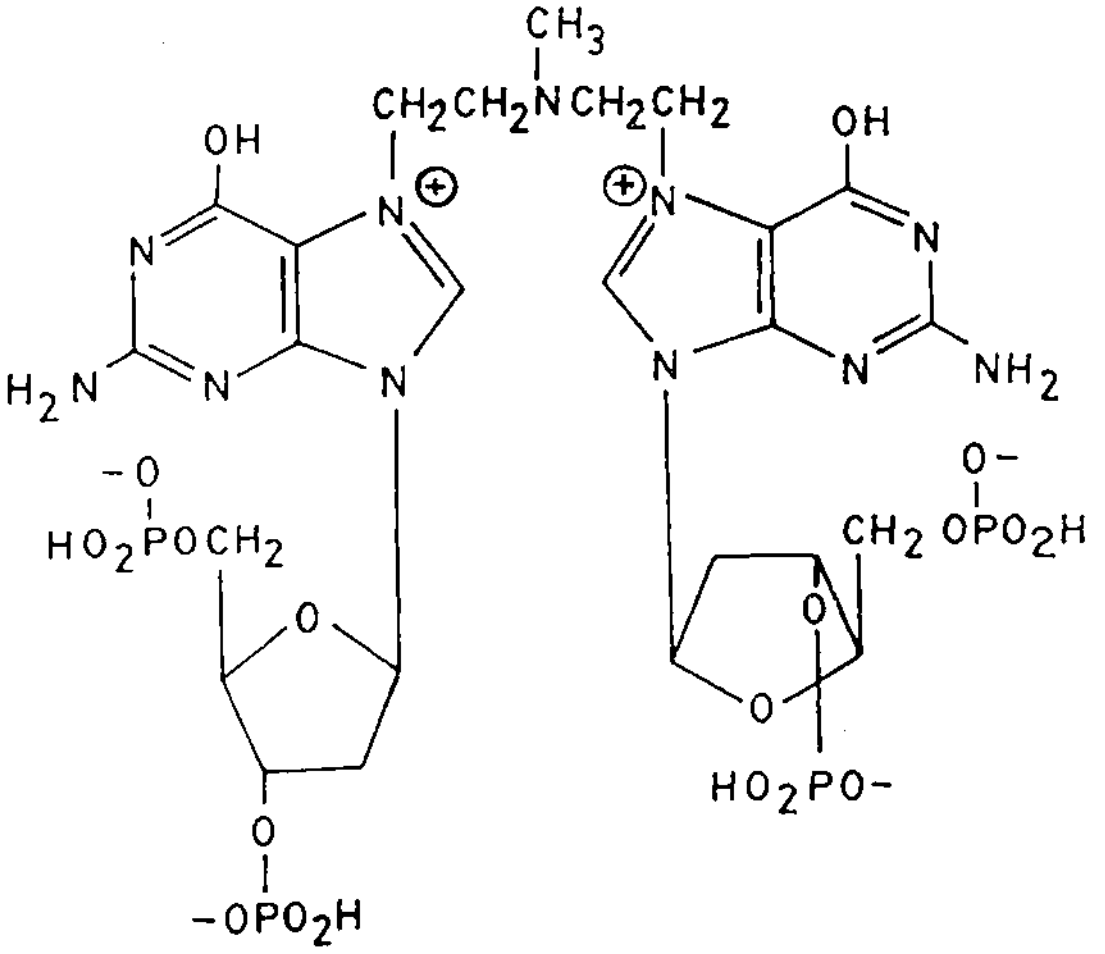


FIG. 1.1. Bifunctionally alkylated guanine bases in DNA strands.

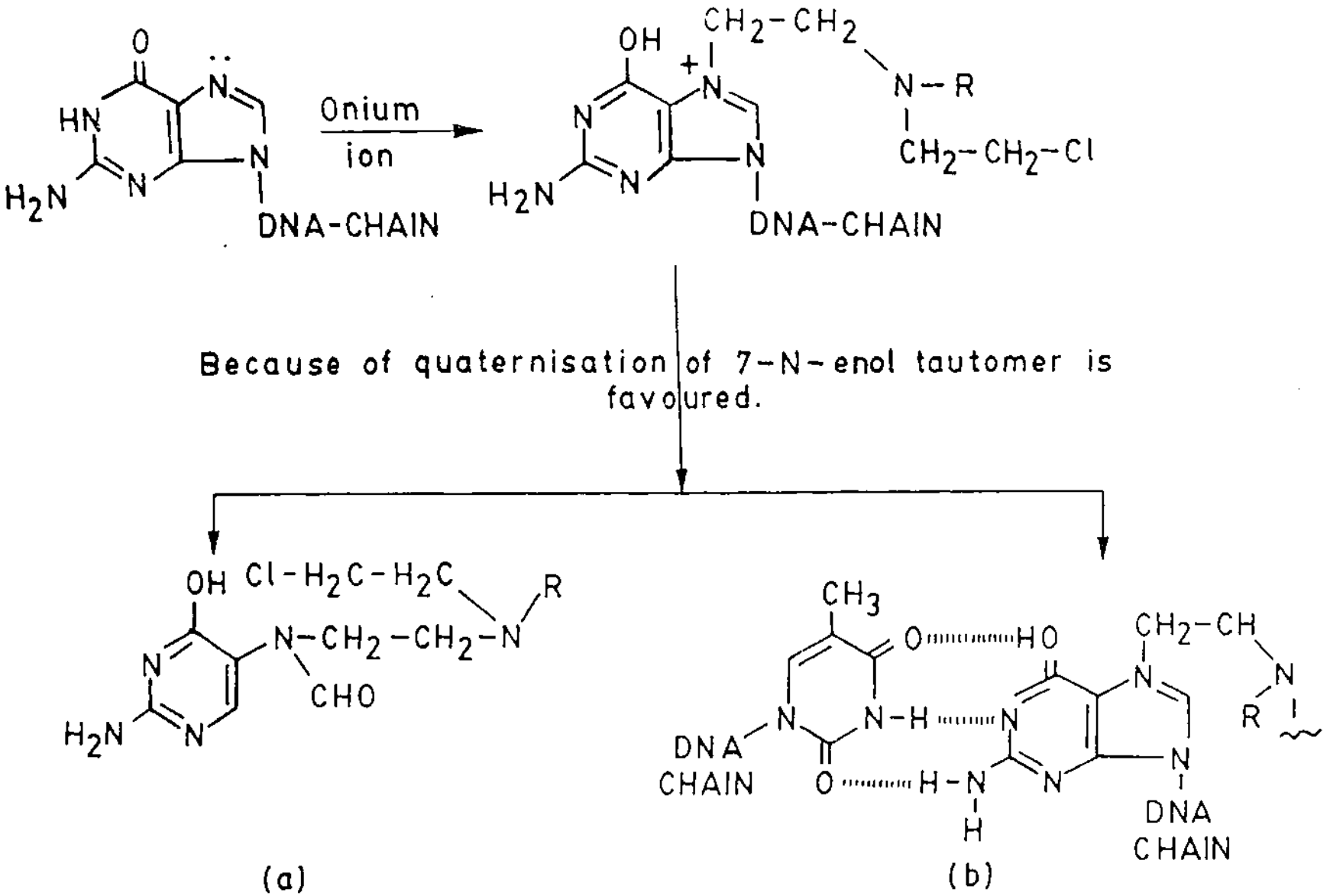


FIG.1.2. (a) Imidazole ring opens causing serious damage to DNA.
(b) Pair with thymidine thus miscoding will result.

(iii) Cellular Respiration Inhibitors

The detailed study of glycolytic and respiratory path ways in cancer cells has revealed that some of the cancer cells show abnormal levels or activities of certain enzymes like malate and lactate dehydrogenases.[26-29] Hence cellular respiration can be inhibited by selectively inhibiting these enzymes, which would result in the death of the cells. Certain copper (II) chelates[30,31] and derivatives of 4-hydroxyquinoline-3-carboxylic acids[32,33] have been found to have direct effect on the inhibition of cellular respiration.

(iv) Hypoxia-Selective and Radiosensitizing Agents

Hypoxia (oxygen deficient) is more prominent in solid tumors than in normal tissues, hence it serves as an important target for developing novel anticancer agents,[34,35] as hypoxia creates an environmental difference, which can be exploited for the purpose. Hypoxia cells may be activated only in the absence of oxygen, providing selective bioactivation within tumor tissues.[36] In general, nitroaromatics and nitro hetrocyclics have been found hypoxia selective.[37]

Since hypoxic cells are refractory to radiation, they limit the clinical efficacy of radiotherapy.[38] This fact can be utilized to develop newer chemotherapeutic agents where attempts should be made to develop agents which can

selectively sensistize hypoxic cancer cells to the effects of ionizing radiations. Many of the above compounds have been found possessing radiosensitizing activity.[37,40]

(v) Agents Binding to DNA

DNA is a vital target for anticancer agents and many chemicals exert their action through binding to DNA. Their potency depends upon the mode and intensity of binding. There are three main types of binding:

(a) Covalent binding,

(b) Intercalation and

(c) Non-intercalative groove binding.

Covalent binding takes place when the drugs are bifunctional alkylating agents. The other two bindings involve weak forces like van der Waals force or hydrogen bonding and are not very strong. In DNA molecule, each base pair provides two grooves:

(a) Minor groove and

(b) Major groove (Fig. 1.3).[41]

Typical groove-binding molecules are composed of several hetroarmatic rings linked through amide or other functional groups, or directly through single bonds.[42,43] Some of the important molecules of this type are shown in Fig. 1.4. These molecules are relatively long and flexible and have a number of proton donor and acceptor groups, have positively charged end(s), and 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.[44-47] The charged end groups of drugs are supposed to be involved in the interaction generally with the phosphate groups of DNA.

In the case of the intercalation where the attachment of the drug molecules with DNA is stronger, the drug molecule is inserted between two adjacent layers of base pairs and is held there primarily through van der Waals forces. The side chain, if any, of the molecule can interact with the phosphates of the backbone of the the DNA molecule.[41] The potent and useful intercalating anticancer drugs are anthracyclines which are derivatives of anthraquinone, this being a planar chromophore is well adapted for intercalation between the nucleic acid base pairs, as its surface matches closely the surface of these pairs.[48,49] Different series of acridines have also been studied for their intercalating properties. Some miscellaneous compounds have also been found to exert their anticancer effects through intercalation.

(vi) Antimetabolites

These are compounds usually closely related in structure to the metabolite, that is antagonised. They interfere with the formation or utilization of normal cellular metabolite by inhibiting an enzyme or enzymes. In this capacity they may combine with the active site as if

they are substrate or cofactor. Alternatively, they may bind to an allosteric regulatory site, especially when they resemble the end product of biosynthetic path way under feedback control.[30] Interference also results 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 a few of them have been found effective in treating cancer. The important drugs among these are analogues of the metabolites involved in the biosynthesis of nucleic acids and the purine and pyrimidine-containing co-factors.

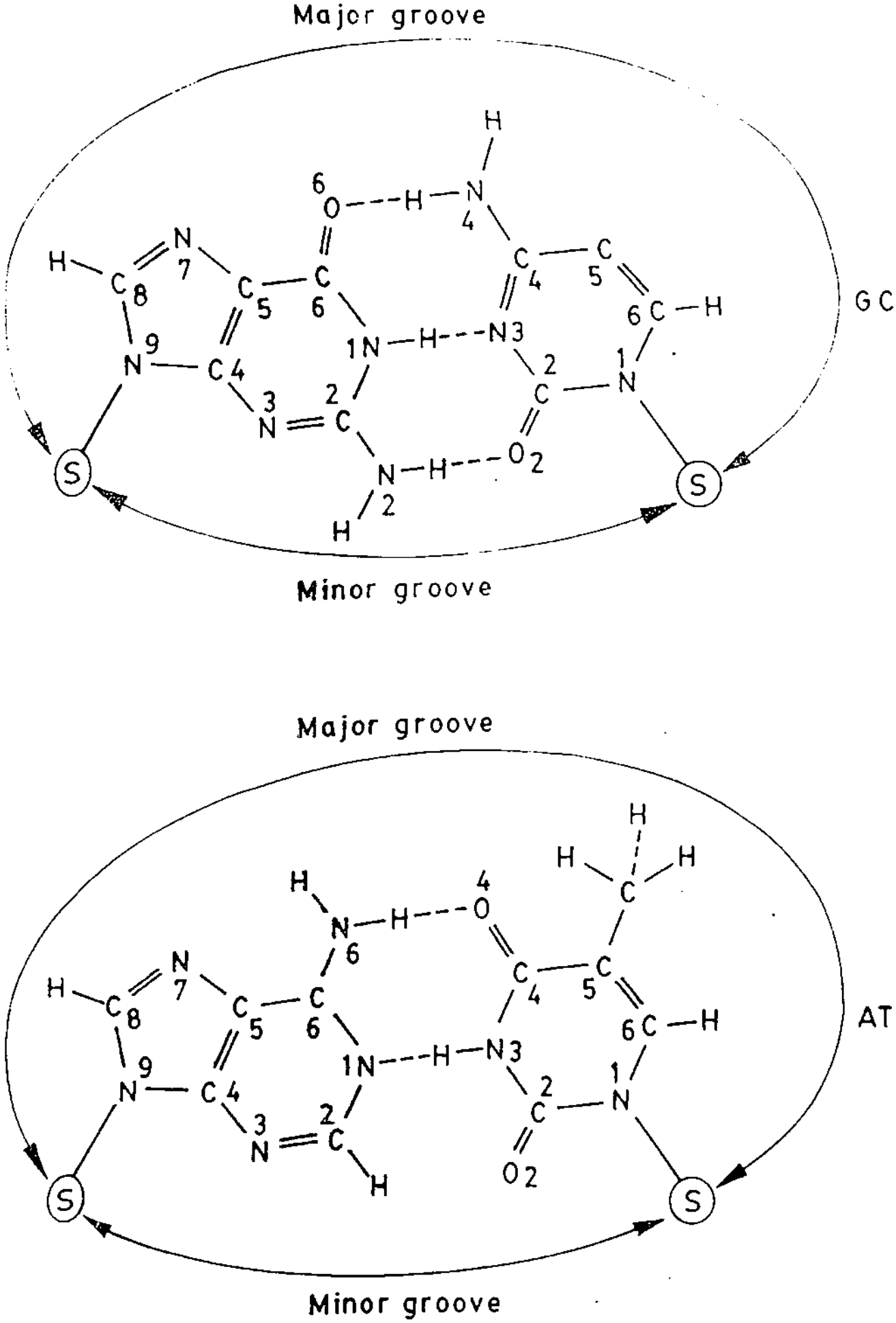
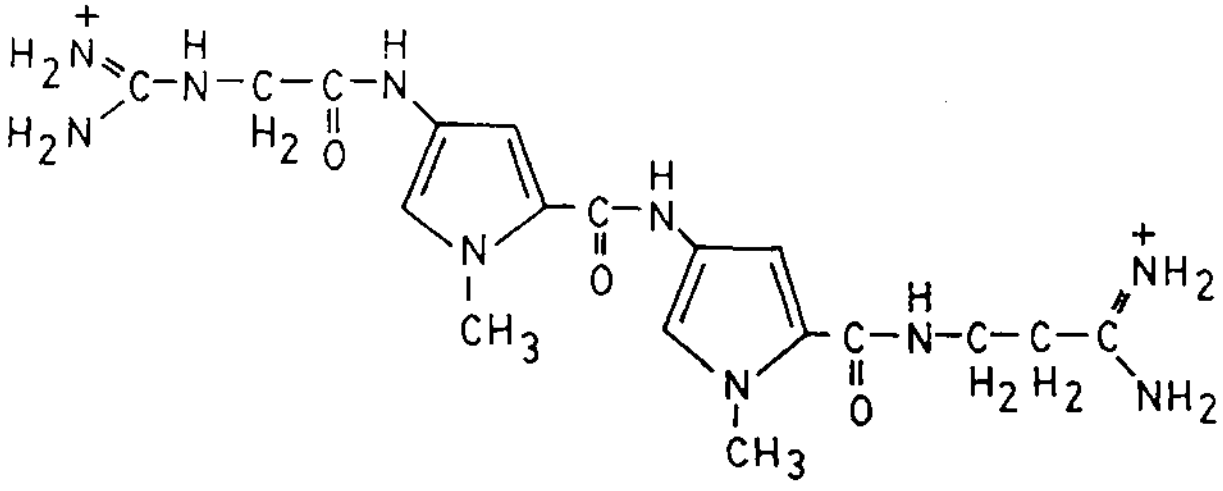


FIG.1.3. Sites in the major and minor grooves of DNA.[41]



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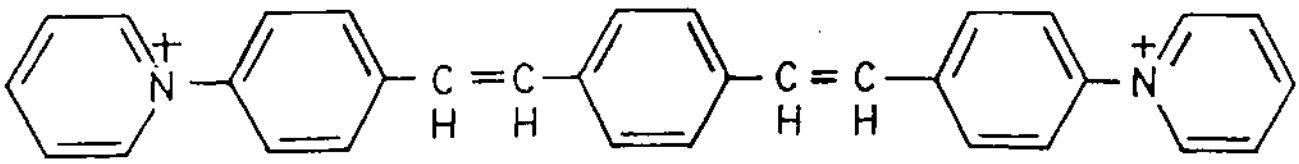

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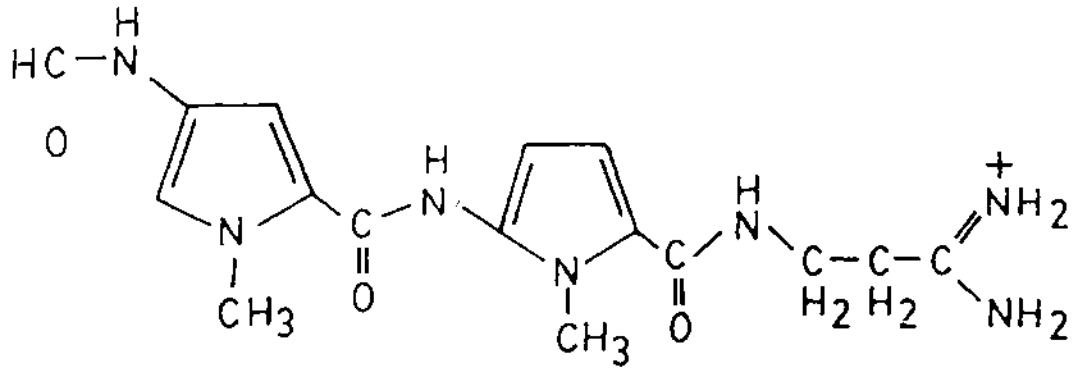

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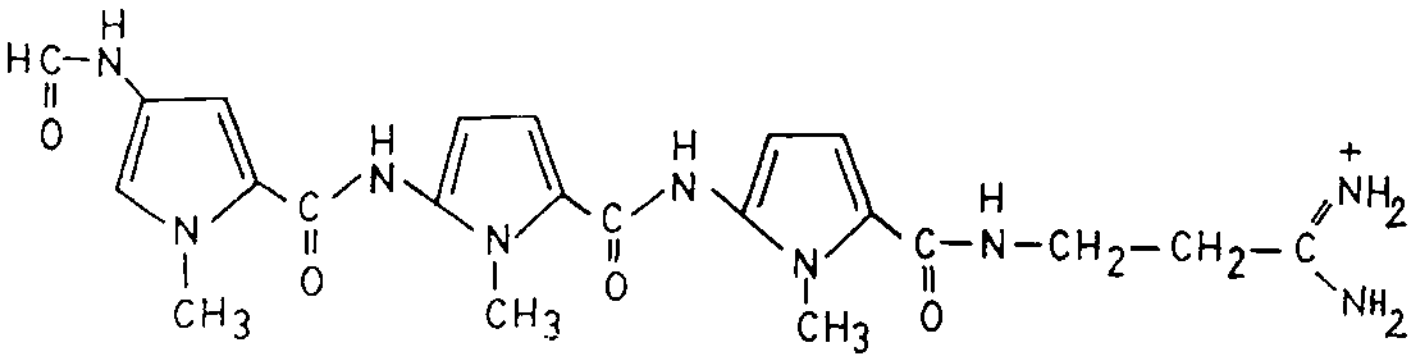

Distamycin A (Dist 3)

FIG. 1.4. Important representatives of non-intercatating grooves-binding molecules.[41]

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CHAPTER - II

QSAR METHODOLOGY

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2.1 Introduction

Quantitative Structure Activity Relationship (QSAR) and Rational Drug Design (RDD) are two inseparable terms. They are almost synonymous and can hardly be demarcated. However, if any one insists to draw a demarcation line, it may be said that QSAR is the way to RDD. With a high demand of newer and better drugs on one side, their discovery is a challenging process on the other side due to complexity of the various biological systems. In fact, most of the discoveries of drugs so far have been either by sheer luck or creativity or a combination of these.

Drug research is rather a multistep process involving synthesis, isolation, random or intuitive selection and screening (Scheme 2.1). The conventional drug research before 70's, as evident from Scheme 2.1 was simply random, intuitive, trial & error method. But design, development and commercialization of a drug by trial and error methods usually employed for the development of a new drug is a tedious, time consuming and cost intensive process, as this requires various predictions like pharmacokinetic, pharmacodynamic and toxic properties before the synthesis of a chemical compound. And moreover after synthesis, these compounds must be tested on a suitable biological system. By a reasonable estimate it takes upto 12 years before any product materialises from the process' and upto 15 years

before it comes to market. In this process a very small fraction of the compounds characterized in the discovery are commercialized[2] and the estimate cost is to the tune of $7,500 per compound.[3]

To avoid all this, recent advances made in various branches of science have been employed in designing new chemical leads and optimization of activities with the congeneric series of compounds. Computers also have been used for this purpose and it is observed that computer aided techniques have been useful in reducing random synthesis and screening of various chemical compounds.

Long ago it was proposed that the biological activity of a compound is a function of its chemical structure. Today, biological or therapeutic activity is considered to be a function of physicochemical properties. With this concept, structure-activity relationships are developed, when a set of physicochemical properties of a group of congeners is found to explain the variations in biological responses of those compounds. This has resulted in the discovery, examination, and interpretation of structure-activity relationships in a more systematic way, which has led to the introduction of quantitative structure-activity relationship (QSAR) studies.

DRUG RESEARCH

Random biological screening of natural and synthetic compounds in test animals to assess therapeutic activity and toxicity.

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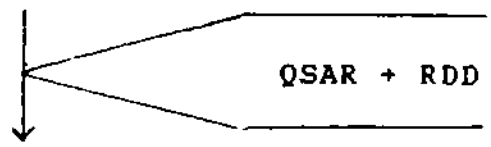
Active member(s) with low toxicity are selected, and a number of homologues and analogues are prepared and biologically evaluated (therapeutic and toxic)



Compounds are further tailored to provide a potential drug of high therapeutic index

↓

Subacute, acute and chronic toxicity studies

↓

Clinical Trial

↓

MARKET

Scheme 2.1

The correlation of molecular structure with biological activity is at the heart of modern medicinal chemistry, being fundamental both to our understanding of how drugs act, and to the rational design of more effective analogues. Over the last decade, considerable advances have been made in studies of structure-activity relationship, largely because of the trend towards expressing all aspects of "structure" in quantitative terms relative to some standard. The most significant contributions to this endeavour have been made by an organic chemist, Prof. C. Hansch and co-workers.[4]

2.2 Applications

The quantitative approach to understand drug action depends upon the ability to express structure by numerical values, and then to relate these values to corresponding changes in activity. The response is going to be determined by the structure, i.e., by the physicochemical properties of the compound, and within a closely related or so-called congeneric series of compounds, changes in structure can be related to changes in biological activity.

The QSAR study tries to explain the reasons of observed variations in biological activities of a group of congeners in terms of molecular modifications or variations caused by the change of the substituents. QSAR studies generally have two important aspects: (i) the predictive aspect and (ii) the diagnostic aspect. The predictive aspect, as the name

implies, deals with the extrapolation and interpolation of a correlation study to identify synthesis of more active derivatives and to avoid the synthesis and testing of derivatives of same or equivalent activity, minimizing the time needed to find a better derivative. The diagnostic aspect, on the other hand, answers mechanistic aspects of the reaction, i.e., it helps to obtain the information about the type of binding forces involved and about the mode of actions of drugs. Results of both these aspects can lead to tailor-made design of new drug of better activity with lesser or no side effects.

Several approaches used in QSAR studies are: the non-parametric methods like Free-Wilson approach[3] and Fujita-Ban approach,[6] the parametric methods developed by Hansch,[4] discriminant analysis,[7] and the pattern recognition technique.[8] Various factors such as quality of the biological data, number of compounds tested, degree of variance in the results, and ratio of the time required for synthesis and biological testing dictate the choice of approach for the QSAR study.

The most popular and widely used approach continues to be the so called Hansch approach,[4] where the variance in biological effect (ΔBE) is explained by the variance of certain linear free-energy related substituent constants which describe the changes in lipophilic/hydrophilic ($\Delta L/\Delta H$), electronic (ΔE_1), steric (ΔE_s) and other properties of

the parent molecule induced by the substituents. This model can be expressed as follows:

$$BE = f(\Delta L/\Delta H, \Delta El, \Delta E_s, \ldots)$$

The change in lipophilicity can be described by the partition coefficient (log P)[9] or the hydrophilic constant π defined[10] as:

$$\pi = \log P_x - \log P_H,$$

where X refers to the substituted derivative and H to the parent compound. The hydrophobic constant is measured in terms of octanol-water partition coefficient of the compounds. Lipophilicity can also be described by Rm values obtained from reverse-phase chromatography and by log K obtained from HPLC. The change in electronic properties can be expressed by Hammett constant,[11] charge densities, spectroscopic properties like chemical shift from IR or UV spectra, field constant (F) and resonance constant (R). The steric influence of the substituents can be described by the Taft steric constant (E_s),[12] molar volume (MV) and molar refractivity (MR).[13,14]

Besides many a drug activities have been found to depend exclusively upon the molecular size,[15-23] which can be described by the van der Waals volume (Vw), and upon the molecular graph which is delineated by molecular connectivity index ($^1\chi$).[24] In this thesis the extensive use has been made of these parameters.

In a stepwise linear multiregression analysis, the biological activity (BA) can be related to various physicochemical, electronic, and steric parameters as:

$$BA = a\pi(\text{or } \log P) + b\pi^2(\text{or } [\log P]^2) + c\sigma + dE_s + k \qquad (2.1)$$

Where a, b, c and d are the regression coefficients and k the intercept obtained by least square method. Biological activity can be expressed by negative logarithmic of the concentration of drug leading to a desired response. Equation 2.1 shows a nonlinear, i.e., a parabolic dependence of activity on the hydrophobic character of molecules. Actually, Hansch had assumed a "random walk" of the molecules, where hydrophilic molecules tend to remain in aqueous phase, while hydrophobic molecules tend to go into lipid phase. Only those molecules that have an optimal hydrophilic/hydrophobic balance tend to reach their goal in reasonable time and concentration. The nonlinear dependence of activity on π or logP value for in vivo system is due to the nonlinear dependence of the rate constant of drug transport through aqueous and bio-organic phases on lipophilicity whereas for in vitro systems, like drug-binding inhibition, such nonlinear relationships result from equilibrium distribution of the drug toward different areas at the receptor surface, from limited binding space at the active site or from limited solubility of more lipophilic congeners.

However, in many cases the relationships between

activity and lipophilicity were found to be strictly linear[4] and although the parabolic model proved to be extremely useful for practical purposes, there was an inconsistency between it and the linear model. Although much less is known about the dependence of biological activities on lipophilic character beyond the point of optimal lipophilicity ($\log P_o$ or π_o), most often a linear relationship is observed with a negative slope beyond it. To overcome such inconsistencies between the linear and nonlinear models, a number of different models [29-34] were proposed, out of which Kubinyi's bilinear model was found, after Hansch's parabolic model, to be the most useful model [35-38] to describe the nonlinear relationships.

2.3 Limitations of QSAR

Though QSAR studies can be successfully utilized to predict the activity of new analogues and discuss the mechanisms of drug-receptor interactions, they have some drawbacks and limitations as described below.[39]

The substituent effect on hydrophobicity is characterized by logP based on an octanol-water system; hence, even a very significant correlation can not represent a true model for hydrophobic interaction between a drug molecule and the receptor. The value of logP also depends on the electronic characters and the hydrogen bonding properties of the substituents.[40,41] Thus, if one gets a correlation with logP only, one can not conclude that there is only

hydrophobic interaction between drug and receptor and that no electronic interaction or hydrogen bonding takes place. Another factor that may influence logP values is steric effect that can prevent the access of water to a hydrophilic group.[42] Steric interactions are extremely difficult to extrapolate from system to system. The use of parameters like MR, MW, Vw, etc., do not give any idea in what way steric effects would affect the drug-receptor interaction. A more serious problem arises with the electronic parameters. The Hammett constants do not reflect which portion of the drug molecule would be actually involved in the interaction with the receptor. Quantum mechanical calculations can provide some help in this, but they are time consuming and expensive.

Although molecules are represented as rigid structures on paper, they may in fact be quite different in solution and their dynamic nature should be recognized. There is considerable evidence that macromolecules, even in crystalline state, exhibit a wide spectrum of motion.[43-47] These motions may be involved in some molecular conformational changes on substrate or group binding. Both drugs and biomolecules are three dimensional objects whose chemical features are related to their three dimensional structures. The interaction between them involves a complementarity or fit between the two objects. Even a successful QSAR study will provide only indirect information about the three-dimensional aspects of drug-biomolecule interaction.

Many structural features that affect the activity but can not be parametized by the usual variables like π, σ, E_s, etc., are accounted for by the use of indicator variables. These indicator variables are arbitrarily assigned two values: one to indicate the presence of the specific structural feature and other to indicate its absence. If the entire series of congeners is divided into two sets, one with and one without the specific structural feature, one would obtain two equations almost parallel, with a difference in their intercepts only. An indicator variable thus can be pictured simply as a constant that adjusts two parallel equations into one. If two sets are far apart in data space described by the usual parameters, one builds on a large amount of variance with the indicator variable leading to a much higher correlation coefficient(r).[46] Despite the better r, the new correlation may be a poorer one, and thus, one can be misled if other statistical parameters are not available.

Another serious problem in QSAR analysis is the problem of collinearity.[47] For example, π and MR most often turn out to be so collinear that it becomes impossible to tell whether one or both are involved in SAR. Over and above all, a QSAR study may be incorrectly interpreted if the biological property of interest is not correctly measured. A measured biological response may be a complex result of several processes, and an in vitro model of drug-receptor interaction does not always represent the true in vivo model.

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CHAPTER - III

PARAMETERS USED
AND
THEIR CALCULATIONS

PARAMETERS USED AND THEIR CALCULATIONS

This Chapter discusses the methodology of calculation of various distinct parameters, on which most of the biological activities are found to depend. They have been found to be very useful in QSAR studies.

3.1. Hydrophobic Parameter (log P)

The fragment method suggested by Hansch and Leo[1] for calculating logP, where P is the partition coefficient of the solute in octanol-water system, is known as constructionist or synthetic approach. Experimentally determined logP values can often be reproduced or approached theoretically with the help of this approach. The basic assumption of this approach is: the logP of a solute can be expressed as a linear sum of fundamental structural constants known as fragments (f) and factors (F) that affect the partitioning equilibrium.

$$\log P = \sum_n a_n f_n + \sum_m b_m F_m \qquad (3.1)$$

Carefully conducted partitioning experiment and statistical survey of the then available partition data have been used in assigning values to the fragments and Factors. The working principle is summarized in the following paragraphs.

In this approach carbon atoms are divided into two

categories: isolating carbons (IC) and nonisolating carbons (NIC). ICs are those having either four single bonds (at least two of which are to nonheteroatoms) or are multiply bonded to other carbon atoms. NIC atoms are carbon atoms multiply bonded to hetero atoms. For example -C= in $CH_2=CH_2$ is an IC but not in $H_2C=O$. Fragments are of two types:

(i) Fundamental fragments: Fragments whose free valency will lead to isolating carbons,

(ii) Derived fragments: A derivative of fundamental fragments (e.g. CF_3).

A fundamental fragment can be either a single atom or a group of multiple atoms (e.g. -C=O, -C≡N, etc.). A single-atom fundamental fragment can be either an isolating carbon atom or a hydrogen or a hetero atom all of which are bonded to ICs. Depending on its nature a fragment will come under one of the following classes:

(i) Non-polar fragments: These are simple ICs and hydrogens attached to ICs.

(ii) H-polar fragments: A fragment that can be expected to form H bonds either by accepting or donating an electron pair e.g. -OH, -COOH, $-NH_2$ etc., and

(iii) S-polar fragments: A fragment that is strongly electron withdrawing with little tendency to form H-bonds (e.g. halogens).

In expressing fragments, the structural formulae (or WLN

code) of the respective fragments will be written as subscripts of "ƒ" for example as $f_{-NH-CO-NH}$ for expressing the fragment -NH-CO-NH- present in $CH_3NHCONHCH_3$. Various Factors (F) are designed to account for the intramolecular forces and factors that affect the partitioning equilibrium of the solute. All these Fs are identified with the help of different subscripts and superscripts. The subscripts are mentioned in the Factors table. The superscripts are applicable also to fragments. They are listed as:

(i) None = aliphatic structural attachment

(ii) Ø = attachment to aromatic ring, if bivalent the attachment is from left as written

(iii) 1/Ø = as 2 but attachment from right as written

(iv) ØØ = two aromatic attachments

(v) X = aromatic attachment, value enhanced by second, electron-withdrawing substituent ($\sigma_I \geq \pm 0.35$)

(vi) IR = benzyl attachment.

Underlining any symbol means it is present in a ring system. Whenever halogens and H-polar fragment are separated by only one IC an additional Factor will come into operation.

In calculating the logP of any compound, the first step is dividing that compound into 'well defined' fragments based on the above discussion and then searching for different factors operating in between the fragments within the structure of the molecule. Now the sum of all these fragments and Factors will give the calculated logP of that compound.

It is always safe to break any compound, especially compound containing hetero atoms, into fundamental fragments rather than into derived fragments. Some important fragment values and Factor values are listed in Tables 3.1 and 3.2, respectively. A simple example for logP calculation is shown below.

Example, Toluene ($C_6H_5-CH_3$) : This can be treated as a compound comprising six aromatic carbons, one aliphatic carbon and eight hydrogens. The fragments can be expressed as:

$$6f^{\circ}_{C} + f_{C} + 8f_{H} = \log P \text{ (Toluene)}$$

$$6(0.13) + 0.20 + 8(0.23) = 2.82 \text{ (Calcd.); } 2.80 \text{ (obsd.)}$$

Since aromatic ring is excluded from bond Factor, there is no F_b term in the above equation. And here aliphatic chain length is one ($-CH_3$), so (n-1) F_b is equal to zero (C-H bonds are excluded from Factors). The logP of this compound can also be calculated from two derived fragments as:

$$f^{\circ}_{C_6H_5} + f_{CH_3} = \log P(\text{toluene})$$

$$1.9 + 0.89 = 2.79 \text{ (calcd.)}$$

Sometimes calculated logP values of compounds deviate very much from the experimentally determined values. For example, observed logP of 1,2-methylenedioxybenzene is 2.08, but the calculated value comes out to be 1.34 only. This

large difference may be due to Factors beyond the control of this method. However, since it is an additive model, it will serve the purpose of drug design when used in a congeneric series of compounds. Further details are given in the literature.[1]

Table 3.1: Some common fragment constants[a]

Without Carbon	f	f[a]	f[aa]	with Carbon	f	f[a]	f[aa]
-Br	0.20	1.09		C	0.20	0.20	
-Cl	0.06	0.94		-CF_3[b]		1.11	
-F	-0.38	0.37		-CN	-1.27	0.34	
-I	0.59	1.35		-CON	-3.04	-2.80	-1.93
-N	-2.18	-0.93	-1.13	-C(O)-	-1.90	-1.09	-0.50
-NO_2	-1.16	-0.03		-CO2	-1.49	-0.56	-0.09
-O-	-1.82[c]	-0.61	0.53	-CO_2[c,d]	-5.19	-4.13	
-H	0.23	0.23		-COH	-1.10	-0.42	
-NH-	-2.15	-1.03	-0.09	-CO_2H	-1.11	-0.03	
-NH_2	-1.54	-1.00		-$CONH_2$	-2.18	-1.26	
-OH	-1.64	-0.44		-CONH-	-2.71	-1.81	-1.06
-SH	-0.23	0.62		-NHCONH-	-2.18	-1.57	-0.82

Fused in Aromatic Ring

Without Carbon	f[a]	without Carbon	f[a]	with Carbon	f[a]	With Carbon	f[a]
-N=	-1.12	-N=N-	-2.14	C	0.13	-CH-	0.35[e]
-N	-1.60	-O-	-0.08	C*	0.25[e,d]	-C(O)-	-0.59
-N[a]	-0.56	-NH-	-0.65	C	0.44[a]	-OC(O)-	-1.40

[a] Taken from reference 1
[b] Derived fragment
[c] For methyl ethers and ethylene oxide, use -1.54
[d] For ring fusion carbon
[e] For ring fusion - hetero

Table 3.2: List of some factors[a]

Involving bonds				
	Unsaturation		Geometric	
	Double	Triple	Proportional to Length: x(n-1) short Chains: 1-time	
Normal	$F(=) = -0.55$	$F(\equiv) = -1.42$	Chain: $F_b = -0.12$	Alkane Chain: $F_{cBr} = 0.13$
Conjugate to Ø	$F^{ø}(=) = -0.42$		Ring[b]: $F_b = -0.09$	H-polar Fragment: $F_{pBr} = -0.22$
Conjugate to 2Ø	$F^{øø}(=) = 0.0$	$F^{øø}(\equiv) = 0.0$	Branching: $F_{bYN} = -0.20$[c]	Ring Cluster = $F_{rCl} = -0.45$
			$F_{bYP} = -0.31$[d]	

Involving multiple halogenation[e]		
		On adjacent Carbon
On same	(n=2) = 0.30	
Carbon (geminal)	(n=3) = 0.53	(vicinal) $F_{aHal} : 0.28(n-1)$
F_{aHGm}	(n=4) = 0.72	

Involving H-polar Proximity			
$F_{p1} = -0.42\ \Sigma f_1+f_2$	Aliphatic	$F_{p1} = -0.32\ \Sigma f_1+f_2$	Aromatic : $F^{ø}_{p1} = -0.16\ \Sigma f_1+f_2$
Chain: $F_p^2 = -0.26\ \Sigma f_1+f_2$	ring	$F_{p2} = -0.20\ \Sigma f_1+f_2$	$F^{ø}_{p2} = -0.08\ \Sigma f_1+f_2$
$F_p^3 = -0.10\ \Sigma f_1+f_2$			

Involving intramolecular H-bond	
$F_{HBN} = 0.60$ for nitrogen	$F_{HBO} = 1.0$ for oxygen

[a] Taken from ref. 1
[b] Aromatic rings are excluded
[c] For amine
[d] For Phosphorus esters
[e] Value per halogen atom

3.2. Hydrophobic Constant (π) of Substituents

Although $\log P$ can be used as a measure of the hydrophobicity of a whole molecule, one often works with a set of derivatives of a parent compound in which a large portion of the structure remains constant. In such a case, knowing the relative hydrophobicity of substituents can be sufficient for correlation analysis. Sometimes it has been found that only substituents in certain positions interact hydrophobically with a given biosystem.[2,3] To enable one to work with the relative hydrophobicity of substituents and in this way separate hydrophobic character from electronic and steric effects of substituents, the parameter π has been defined analogous to σ as

$$\pi_x = \log P_x - \log P_H \qquad (3.2)$$

In this expression, P_x is the partition coefficient of a derivative and P_H that of the parent compound, for example,

$$\pi_{Cl} = \log P_{C_6H_5Cl} - \log P_{C_6H_6} \qquad (3.3)$$

$$= 2.84 - 2.13$$

$$= 0.71$$

A positive value for π means that relative to H the substituent favours the octanol phase. A negative value indicates its hydrophilic character relative to H. The value of π varies somewhat from system to system. Certain π values are given in Table 3.3.

Table 3.3: Data on physicochemical parameters of some important substituents*

SNo.	Substituent	π	σ_m	σ_p	MR
1.	H	0.0	0.0	0.0	1.03
2.	CH_3	0.56	-0.07	-0.17	5.65
3.	C_2H_5	1.02	-0.07	-0.15	10.30
4.	C_3H_7	1.05	-0.07	-0.13	14.96
5.	$i-C_3H_7$	1.53	-0.07	-0.15	14.96
6.	$n-C_4H_9$	2.13	-0.08	-0.16	19.61
7.	F	0.14	0.34	0.06	0.92
8.	Cl	0.71	0.37	0.23	6.03
9.	Br	0.86	0.39	0.23	8.88
10.	I	1.12	0.35	0.18	13.94
11.	OCH_3	-0.02	0.12	-0.27	7.87
12.	NH_2	-1.23	-0.16	-0.66	5.42
13.	OH	-0.67	0.12	-0.37	2.85
14.	COOH	-0.32	0.37	0.45	6.93
15.	$COOCH_3$	-0.01	0.37	0.45	12.87
16.	CF_3	0.88	0.43	0.54	5.02
17.	NO_2	-0.28	0.71	0.78	7.36
18.	CHO	-0.65	0.35	0.42	6.88
19.	C_6H_5	1.96	0.06	-0.01	25.36
20.	CN	-0.57	0.56	0.66	6.33
21.	N_3	0.46	0.27	0.15	10.20
22.	NHOH	-1.34	-0.04	-0.34	7.22
23.	$CH=CH_2$	0.82	0.05	-0.02	10.99
24.	$COCH_3$	-0.55	0.38	0.50	11.18
25.	$COOC_2H_5$	0.51	0.37	0.45	17.47
26.	$COOC_3H_7$	1.07	0.37	0.45	22.17
27.	CH_2OH	-1.03	0.0	0.0	7.19
28.	$CHOHCH_3$	-0.86	0.0	-0.07	11.82
29.	CH_2OCH_3	-0.78	0.02	0.03	12.07
30.	SCH_3	0.61	0.15	0.0	13.82
31.	NHCHO	-0.98	0.19	0.0	10.31
32.	$OCOCH_3$	-0.64	0.39	0.31	12.47
33.	$OCH(CH_3)_2$	0.85	0.10	-0.45	17.06
34.	OC_3H_7	1.05	0.10	-0.25	17.06
35.	$N(CH_3)_2$	0.18	-0.15	-0.83	15.55

* Taken from reference 1

3.3. Electronic Parameter (σ)

The development of electronic parameter is one of the most important breakthroughs for mechanistic organic chemistry which came in 1935 when L.P. Hammett proposed[4] the following equation to define an electronic parameter σ.

$$\sigma = \log K_X - \log K_H \qquad (3.4)$$

In Equation 3.4, K_H is the ionisation constant of benzoic acid in water at 25°C and K_X is the ionisation constant for the meta or para derivative under the same experimental conditions. Positive values of σ represent the electron-withdrawing and the negative ones electron-donating character of substituents in the aromatic ring. For certain substituents, σ values are given in Table 3.3.

3.4. Molar Refractivity (MR)

In various organic reactions, dispersion forces play an important role and these could be modeled by the molar refractivity (MR) of substituents. Experimentally, MR is usually obtained via the Lorentz-Lorenz equation.

$$MR = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{MW}{d}$$

Where n is the index of refraction, d is the density, and MW is the molecular weight of a compound. Since MR is an additive constituent property of molecules, fragment values have been calculated for many common groups of atoms. It has

generally been assumed that a positive coefficient with an MR term in a correlation equation suggests a binding action via dispersion forces. Such binding could produce a concomitant conformational change in a macromolecular binding site. If the conformational change favoured the process under study, one would certainly expect a positive coefficient with the MR term. However, if the conformational change were detrimental, a negative coefficient could result for the MR term. Negative coefficient with MR have also been assumed to reflect steric hindrance of one kind or another. Some MR value used are tabulated in Table 3.3.

3.5. van der Waals Volume

The van der Waals volume (V_w) has been found to be one of the most fundamental characteristics of the drug structure controlling biological activity. This determines the molecular size and shape of the compounds which are very important in the aspect of drug-receptor interactions.

To calculate V_w of molecules, spherical shapes are assumed for all atoms according to Bonds[3] because of the absence of generally accepted pear shapes. The values of the van der Waals radii used and calculated volume of atoms are listed in Table 3.4. Since van der Waals radii are greater than covalent radii, a correction for sphere overlapping due to covalent bonding between atoms is needed for the calculation of V_w of polyatomic molecules. The covalent bond lengths and correction values are tabulated in Table 3.5. A

correction for branching in the molecule is also included in the V_w calculation. Such correction is also mentioned in the Table 3.5. All these values have been taken from the literature.[6]

Table 3.4: van der Waals radius and volume of atoms*

Atom	Radius (A°)	Sphere Volume (10^2 A°^{3})
C	1.7	0.206
H	1.1	0.056
N	1.5	0.141
O	1.4	0.115
S	1.8	0.244
F	1.4	o.115
Cl — aliphatic	1.7	0.206
Cl — aromatic	1.8	0.244
Br — aliphatic	1.8	0.244
Br — aromatic	1.9	0.287
I — aliphatic	2.0	0.335
I — aromatic	2.1	0.388
B	2.1	0.388
He	1.2	0.072
Ne	1.6	0.171
Ar	1.9	0.287
Kr	2.0	0.335
Xe	2.2	0.446

* Taken from reference 6

Table 3.5: Correction values of van der Waals volume, for sphere overlapping due to covalent bonding and branching*

Bond	Bond length (A°)	Correction value (10^2 A°^{3})
C-C	1.5	-0.078
C-H	1.1	-0.043
C-N	1.4	-0.060
C-O	1.4	-0.056
C-S	1.8	-0.066
C-F	1.4	-0.056
C-Cl (aliphatic)	1.8	-0.058
C-Cl (aromatic)	1.8	-0.066
C-Br (aliphatic)	1.9	-0.060
C-Br (aromatic)	1.9	-0.068
C-I (aliphatic)	2.1	-0.063
C-I (aromatic)	2.1	-0.072
C-B	1.6	-0.113
B-H	0.7	-0.030
N-H	1.0	-0.038
N-N	1.4	-0.050
N-O	1.4	-0.042
N-S	1.6	-0.061
O-H	1.0	-0.034
O-B	1.5	-0.079
S-H	1.3	-0.040
S-S	2.0	-0.062
S-F	1.6	-0.052
C=C	1.3	-0.094
C=N	1.3	-0.072
C=O	1.2	-0.068
C=S	1.6	-0.081
N=N	1.2	-0.061
N=O	1.2	-0.053
S=O	1.5	-0.057
C≡C	1.2	-0.101
C≡N	1.2	-0.079
C≡C (aromatic)	1.4	-0.086
Branching for saturated bond except bonding with H		-0.050

* Taken from reference 6

3.6. Molecular Connectivity Index

Kier and Hall[7] introduced this additive topological parameter to drug design. Here the molecular connectivity index, χ, signifies the degree of branching or connectivity in a molecule. Different versions of χ are calculated from the hydrogen-suppressed graph of the molecule. For this purpose the hydrogen-suppressed graph will be decomposed, depending on the χ considered, into uniform parts called as subgraph(s). Here two types of connectivity indices, simple molecular connectivity index ($^m\chi$) and valence molecular connectivity index ($^m\chi^v$) are discussed. The superscript m is known as order of the connectivity index and is numerically equal to the number of non hydrogenic sigma bonds present in the subgraph of the particular χ.

A simple version of simple molecular connectivity index is first-order connectivity index, $^1\chi$, and it is computed by

$$^1\chi = \Sigma C_{i,j} = \Sigma(\delta_i \delta_j)^{-1/2} \qquad (3.5)$$

where the summation extends to all connections or edges ($C_{i,j}$) of the hydrogen-suppressed graph and δ_i and δ_j are integers assigned to each atom indicating the number of atoms adjacent or connected to atoms i and j which are formally bonded. Here, in deriving this index, only the number of non-hydrogenic adjacent atoms are considered but not the nature of the atoms and the unsaturation in the molecule.

The valence molecular connectivity index, in contrast

to the simple molecular connectivity index, takes into account the nature of the atoms as well as the unsaturation present in the molecules. Here the connectivity term, δ^v, is defined as:

$$\delta^v = Z^v_i - N_H \qquad (3.6)$$

in which Z^v_i is the number of valence electrons present in atom i and N_H is the number of hydrogens attached to it. A simple version of valence molecular connectivity index is first-order valence molecular connectivity index, $^1\chi^v$, and is formulated as:

$$^1\chi^v = \Sigma C_{ij} = \Sigma(\delta^v_i \, \delta^v_j)^{-1/2} \qquad (3.7)$$

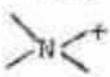
The application of Equation 3.6 for atoms beyond the second row in the periodic table leads to the same δ^v value for each family member, for example, seven for each halogen and six for each chalcogen. Consideration of valence electrons (Z^v_i) of atom i together with its atomic number (Z_i) and the number of hydrogen atoms (h_i) attached to that atom will give appropriate δ^v value for atoms beyond second row in the periodic table.[8] The mathematical expression for this is:

$$\delta^v = (Z^v_i - h_i)/(Z_i - Z^v_i - 1) \qquad (3.8)$$

According to this equation $\delta_{Cl} = 0.78$ and $\delta_{Br} = 0.26$. The δ^v value for some heteroatoms including halogens are listed in Table 3.6.

Only the above discussed connectivity indices are used in our studies. Higher order connectivity indices are discussed by Kier and Hall in their monograph.[7]

Table 3.6: Valance delta (δ^v) values for heteroatoms[a]

Group	δ^v	Group	δ^v
NH2	3	OH	5
NH	4	O	6
N	5	C=O	6
C≡N	5	Furan O	6
C=NH	4	O=NO	6
Pyridine N	5	H_2O	4
Nitro N	6	H_3O^+	3
NH_3	2	F	7
NH_4^+	1	Cl	0.78[b]
	6	Br	0.26[b]
$=NH_2^+$	3	I	0.16[b]
		S	0.67[b]

[a] Taken from reference 7
[b] Obtained from Equation 3.8.

3.7. Steric Parameter (E_s)

Steric effect of substituents in organic reactions are very important. The first generally successful numerical definition of steric effects in organic reactions was

proposed by Taft.[9,10] Following a suggestion of Ingold, Taft defined the steric constant E_s as:

$$E_s = \log(K_X/K_H) \qquad (3.9)$$

where K refers to the rate constant for the acid hydrolysis (denoted by A) of esters of type $X\text{-}CH_2COOR$.

The size of X will affect attainment of the transition state, which is an essential step for acid hydrolysis by water.

For QSAR studies in this thesis, standard values for different parameters for various substituents have been taken from literature.[9]

3.8 References

1. Hansch, C. and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, John Wiley: New York (1979).
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CHAPTER - IV

QSAR STUDIES:
RESULTS AND DISCUSSIONS

QSAR STUDIES: RESULTS AND DISCUSSION

As discussed in Chapter 1 cancer chemotherapy is yet to receive a spectacular breakthrough of the kind that discovery of penicillin provided for the antibacterial chemotherapy. However, there has been substantial progress in many aspects of cancer research, particularly because of an increased understanding of tumor biology which has led to elucidation of the mechanisms of action of antineoplastic agents. It has also provided a basis for the more rational design of new agents, and with improved chemical screening system there has been enhancement in the emergence of newer and more potent compounds. Many anticancer drugs are being synthesised by scientists and tested for their biological activity.

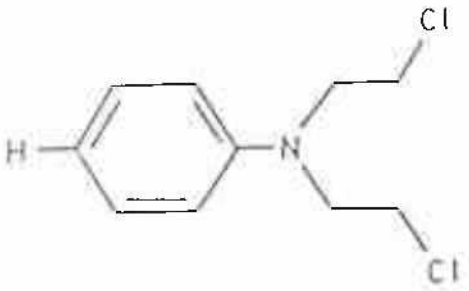
A quantitative analysis of the biological activity and the physicochemical properties of the compounds will precisely determine the extent of role played by different physicochemical properties of the compounds for the drug-receptor binding. Further, the correlation equations may be exploited to design a better compound.

Hence a detailed QSAR study has been carried out on the following categories of drugs having anticancer action in order to understand their mode of action and to rationalize the selection of substituents. Thus aiming at optimizing the best potent analogues for some series of anticancer drugs considering the physicochemical parameters of the drug structure, the QSAR studies have been performed.

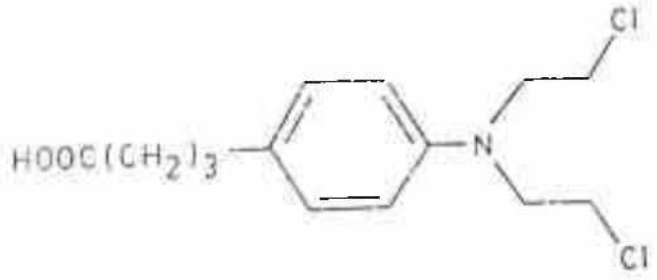
4.1 DNA-Directed Alkylating Agents

Gourdie et al.[6] made quantitative structure-activity relationship (QSAR) study on a series of antitumor aniline mustards and a series of acridine-linked aniline mustards. While cytotoxic activities of the former are found to depend only upon the electronic parameter, those of the latter are found to depend upon the lipophilicity of the linker chain and its connectivity index. This led to suggest that the linker chain probably facilitates the transport of the compounds to the DNA and helps them bind with the DNA.

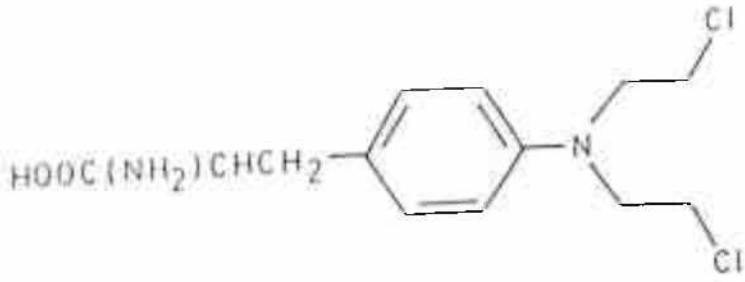
The simplest bifunctional alkylating agent that has long been recognized for its experimental antitumor activity[1] and has also shown useful clinical results[2] is aniline mustard(I). However, the most commonly used aniline mustards[3] are its two derivatives chlorambucil(II) and melphalan(III). Though these derivatives remain important as anticancer drugs,they possess a few drawbacks. They exhibit high chemical reactivity and, although they act by alkylating DNA, they have no particular affinity for it. Thus there can be a significant loss in the potency of the drug before it reaches the DNA, because a high proportion of the drug may be lost by hydrolysis and by the interactions with other cellular macromolecules. Further, a proportion of the drug may loose its one arm, thus leading to only monoalkylation events which are considered to be genotoxic rather than cytotoxic.[4,5]


Cl
H
N
Cl

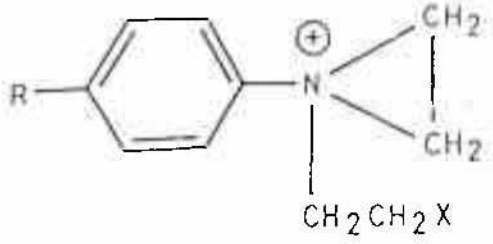
I


Cl
HOOC(CH2)3
N
Cl

II


Cl
HOOC(NH2)CHCH2
N
Cl

III


R
N
CH2
CH2
CH2CH2X

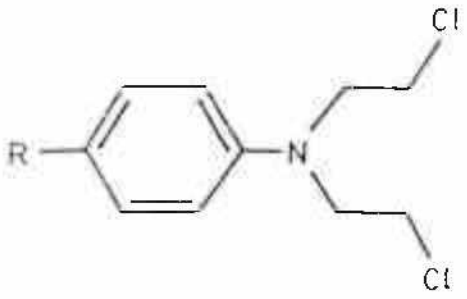
IV

Many of these drawbacks can be overcome by attaching the mustard to a DNA-affinic carrier, which may target it specifically to the DNA without any loss in its potency or in its structure. With this logic, a series of aniline mustards (Table 4.1)[6] and a corresponding group of compounds, where the mustards are linked by a chain of defined length and geometry to the DNA-intercalating chromophore, 9-amino-acridine, (Table 4.2),[6,7] were synthesized and their cytotoxic activities and the effect on the activity of variation in the length of the linker chain studied. Now the question as to how targeting a mustard to the DNA by a DNA-affinic carrier leads to an increase in the cytotoxic activity of the mustards warrants a structure-activity relationship (SAR) study. A quantitative SAR (QSAR) gives a better picture of the situation, hence we report here QSAR study on the problem.

The cytotoxic activities reported for the mustards were the in-vitro measurements against murine leukemia P388 and Chinese hamster ovary-derived cell lines AA8 and UV4. These measurements were in terms of the IC_{50}, the molar concentration of the drug producing 50% effect on the cells.[6,7] The HF in Tables stands for the hypersensitivity factor and is defined as the ratio of IC_{50}(AA8) to IC_{50}(UV4), but the values reported are actually the means of intra-experiment ratios and therefore are not identical to the ratio of reported IC_{50}'s of AA8 and UV4. The very large values of this factor suggest cross-linking ability and

smaller but significant ones the formation of the bulky monoadduct.

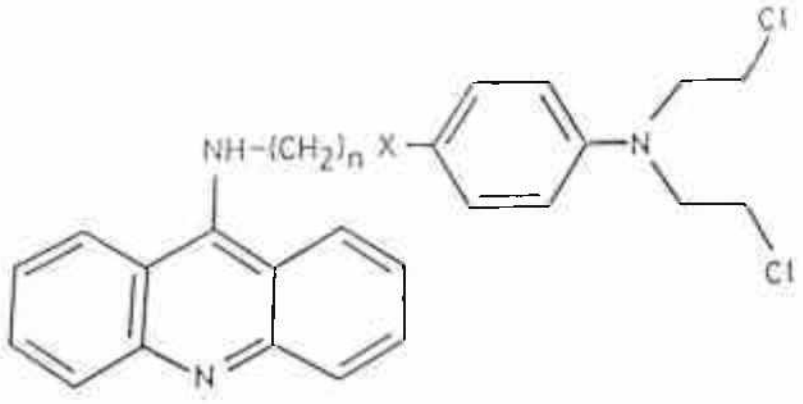
Table 4.1: Physicochemical and Biological Properties of Aniline Mustards



SNo.	R	σ*	χ	Cytotoxicity log(1/IC$_{50}$)*			LogHF
				P388	AA8	UV4	
1.	CH_3O	-0.27	0.61	6.20	5.54	6.47	1.62
2.	CH_3	-0.17	0.50	6.39	5.49	6.77	1.66
3.	CH_3S	0.00	1.83	5.83	5.28	6.80	1.52
4.	CH_3CONH	0.00	1.20	5.09	5.51	7.00	1.49
5.	$(CH_3)_2NCO$	0.36	1.57	4.72	4.55	5.92	1.72
6.	CH_3CO	0.50	0.95	<4.40	<4.40	5.26	>0.90
7.	CH_3SO_2	0.72	2.83	4.41	3.84	4.38	0.54

* Taken from reference 6.

Table 4.2: Physicochemical and Biological Properties of Acridine-linked Aniline Mustards



SNo.	X	n	π*	(π)ᵇ	Cytotoxicity log(1/IC₅₀)ᶜ P388	Cytotoxicity log(1/IC₅₀)ᶜ AA8	logHF
1.	O	2	-0.02	1.59	7.14	6.57	1.73
2.	O	3	0.26	2.09	7.33	6.42	1.73
3.	O	4	0.64	2.59	7.20	6.55	1.74
4.	O	5	1.18	3.09	7.30	6.68	1.66
5.	CH_2	2	0.67	1.95	7.21	6.51	1.63
6.	CH_2	3	1.21	2.45	7.07	6.36	1.56
7.	CH_2	4	1.75	2.95	7.09	6.51	1.77
8.	CH_2	5	2.29	3.45	6.87	6.28	1.56
9.	S	2	0.42	2.57	6.43	5.92	1.38
10.	S	3	0.79	3.07	6.44	5.96	1.26
11.	S	4	1.22	3.57	6.31	5.86	1.32
12.	S	5	1.76	4.07	6.42	6.15	1.28
13.	SO_2	2	-1.17	3.57	5.64	4.74	0.52
14.	SO_2	3	-1.14	4.07	5.58	5.01	0.71
15.	SO_2	4	-0.92	4.57	5.72	5.77	0.15
16.	SO_2	5	-0.38	5.07	6.12	6.24	0.00

ª Contribution to the lipophilicity of the whole molecule by the variable linker chain, $-NH(CH_2)_nX-$, estimated by Valu et al.[7] using Hansch-Leo fragment constants.

ᵇ Calculated for the whole linker chain including the aromatic carbons linked on both sides.

ᶜ Taken from reference 7.

For the simple mustards (Table 4.1), Gourdie et al.[6] had found that cytotoxicities against P388 and UV4 cell lines had significant correlations with the electronic parameter σ (Hammett constant) (Eqns. 4.1, 4.2), having the correlation coefficient r = -0.91 and -0.89, respectively. In these correlations, n is the number of data points, r is the multiple correlation coefficient, s is the standard error of estimates, F is the F-ratio between the variances of calculated and observed activities, and data within parentheses are 95% confidence intervals. In the derivation of Eqn. 4.1, the uncertain data point of compound 6 was not included. In our study, we correlated the activity against AA8 cell line also and found that it too is well correlated with σ (r= -0.97), even better than those against P388 and UV4 (Eqn.4.3). However, the hypersensitivity factor (HF) was not found so well correlated with it σ (r= -0.76)(Eqn. 4.4). Now the negative dependence of cytotoxicities on σ points out that electron-releasing substituents would greatly affect them. Although the exact mechanism of alkylation of nucleic acids by aniline mustards has not yet been established, according to many studies[6] they are supposed to form first an unstable intermediate, cyclic ethyleneimmonium ion (IV). The formation of this ion would be highly dependent upon the electron density on the nitrogen atom. Consequently, the antitumor activity of aromatic mustards should be the function of the electron density on this atom, which can be

affected by an electron-releasing substituent at the aryl ring. This proposition is in full accordance to the negative dependence of cytotoxic activities on σ.

$$Log(1/IC_{50})_{PSCC} = 5.66 - 2.01(\pm 0.38)\sigma \qquad (4.1)$$

$n = 6,\ r = -0.91,\ s = 0.31,\ F_{1,4} = 19.27\ (7.71)$

$$Log(1/IC_{50})_{UVA} = 6.42 - 2.30(\pm 0.52)\sigma \qquad (4.2)$$

$n = 7,\ r = -0.89,\ s = 0.46,\ F_{1,5} = 19.05\ (16.26)$

$$Log(1/IC_{50})_{AAA} = 5.23 - 1.83(\pm 0.59)\sigma \qquad (4.3)$$

$n = 6,\ r = -0.97,\ s = 0.18,\ F_{1,4} = 63.68\ (21.20)$

$$Log\ HF = 1.52 - 0.92\ (\pm 1.07)\sigma \qquad (4.4)$$

$n = 6,\ r = -0.76,\ s = 0.32,\ F_{1,4} = 5.62\ (7.71)$

The σ was however not found so important in the case of acridine-linked mustards. The activities of these mustards (Table 4.2) were rather found to be correlated with the lipophilicity (π) and Kier's first-order valence molecular connectivity index $^1\chi^v$ of the linker chain (Eqns.4.5-4.7).

$$log(1/IC_{50})_{AAA} = 0.55(\pm 0.24)\pi - 0.24(\pm 0.18)\pi^2 + 6.14 \qquad (4.5)$$

$n = 16,\ r = 0.81,\ s = 0.34,\ F_{2,13} = 12.40\ (6.70)$

$$\log(1/IC_{50})_{P388} = 0.27(\pm 0.17)\pi - 0.41(\pm 0.19)^{1}\chi^{v} + 7.76 \quad (4.6)$$

$n = 16,\ r = 0.88,\ s = 0.32,\ F_{2,13} = 22.15\ (6.70)$

$$\log HF = 0.28(\pm 0.09)\pi - 0.42(\pm 0.10)^{1}\chi^{v} + 2.44 \quad (4.7)$$

$n = 16,\ r = 0.97,\ s = 0.16,\ F_{2,13} = 89.20\ (6.70)$

In Eqns. 4.5-4.7 the dependence of activities of lipoplhilicity of the linker chain suggests that linking probably affects the activity by facilitating the transport of the compounds to the DNA site. A parabolic correlation between $\log(1/IC_{50})_{ADR}$ and π, however, points out that for this activity of mustards, the π will have an optimum value equal to 1.15, meaning thereby that a linker chain having a π-value greater than this would not be favourable.

The dependence of the activity against P388 and of HF on $^{1}\chi^{v}$ too (Eqns. 4.6 and 4.7) suggests that they may also be affected by the nature and connectivity of atoms in the linker chain. Since there was found no mutual correlation existing between π and $^{1}\chi^{v}$ ($r=0.26$), these two properties of atoms seem to play the role independent of the lipophilicity of the chain. Since the value of $^{1}\chi^{v}$ increases as the electronegativity and unsaturation of atoms decrease[7], the negative coefficient of it in both Eqns.4.6 and 4.7 points out that atoms of low electronegativity and low

unsaturation will not be beneficial. Therefore, for high activity, the highly electronegative and highly unsaturated atoms would be crucial. Such atoms may probably affect the polarity of the chain, which in turn may help in some way in binding of the molecules with the DNA. The connectivity index was not found to play any role in the case of simple mustards of Table 4.1. The $^1\chi^v$ was calculated using a unified definition[10] of vertex connectivity index (δ^v) for second and third row atoms (Eqn.4.8). Thus, Eqn.4.8 in which Z^v_i is the count of valence electrons of atom i, hi is the count of hydrogen atoms bonded to it, and Z_i is its atomic number, gives a value of δ^v for sulfur equal to 0.67, irrespective of its oxidation state.

$$\delta^v = \frac{Z^v_i - hi}{Z_i - Z^v_i - 1} \qquad (4.8)$$

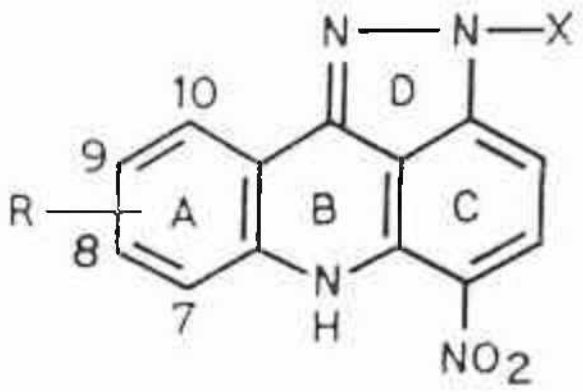
The use of this value of δ^v_s has been reported to accurately reproduce the molar refractivity (MR) data for all classes of sulfur compounds.[10] We have also used the same value of δ^v_s for all oxidation states of sulfur and the $^1\chi^v$ thus calculated has been found to give better correlations than the use of empirical values of δ^v_s. The reported $^1\chi^v$ values are for the whole linker chain including the aromatic carbons linked on both sides.

4.2 Some Acridines Binding to DNA.

A large number of antitumor agents are known to elicit their activity by binding to DNA. The binding to DNA occurs principally either by covalent bond formation or by intercalation. The bifunctional alkylating agents such as nitrogen mustards, nitrosoureas, triazenes, etc., enter into the former kind of binding and the compounds having planar, polycyclic nucleus have been found usually to bind by intercalation. Outstanding among the intercalating antitumor drugs are anthracyclines. They are highly potent and chemotherapeutically useful agents. Drugs belonging to acridine series have also been extensively studied for their intercalating properties and anticancer activity.

Analogues of both anthracycline and acridine were also subjected to quantitative structure-activity relationship studies in order to find out the role of physicochemical properties of compounds in their activity.[11] The dominant role was observed to be played by hydrophobic and electronic parameters. The present communication presents a QSAR analysis on some new series of acridines (V-VII)

The synthesis and anticancer activity of acridine series belonging to V (Table 4.3), VI (Table 4.4), and VII (Table 4.5) were reported by Capps et al.[12], Sugaya et al.[13], and Colody et al.[14], respectively. The physicochemical parameters used were taken from the literature.[15]


N
N
X
10
D
9
R
A
B
C
8
N
H
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NO2

V


N
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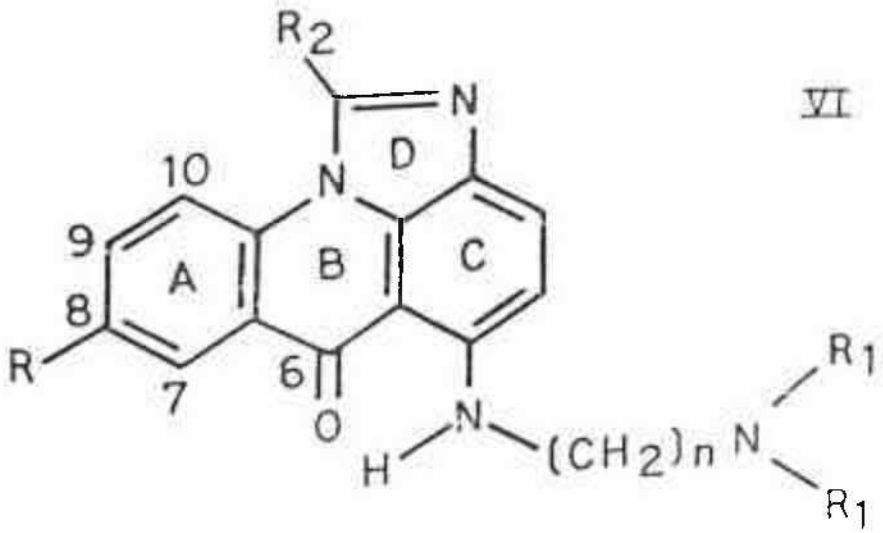
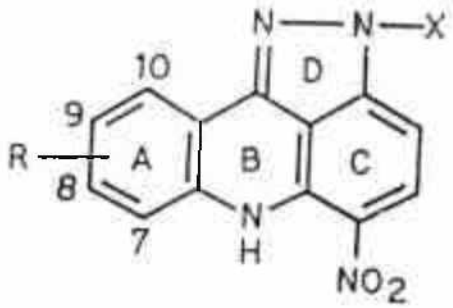
Z
O
Y

VI


R2
N
D
10
N
9
A
B
C
8
R
6
7
O
R1
N
H
(CH2)n
N
R1

VII

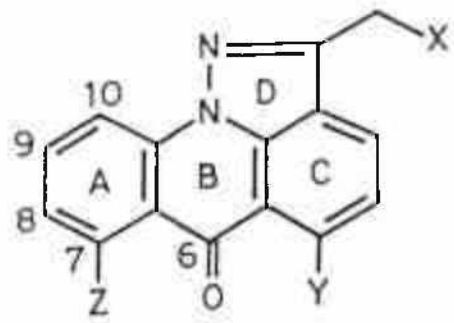
Table 4.3: A series of 2-(aminoalkyl)-5-nitropyrazolo[3,4,5-kl]- acridines and their anticancer activity and physicochemical parameters.

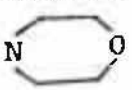


SNo.	R	π_7	log(1/ID_{50}), L1210 Leukemia	
			Obsd.[a]	Calcd.[b]
		X = $(CH_2)_2NMe_2$		
1.	9-OH	-0.67	8.57	8.84
2.	9-OCH_3	-0.02	6.40	6.45
3.	9-OC_2H_5	0.38	6.57	6.61
4.	9-$N(CH_3)_2$	0.18	6.22	6.38
5.	7,9-$(OCH_3)_2$	-0.02	6.68	6.45
6.	9-$OCOCH_3$	-0.64	8.92	8.66
7.	9-$OCOC(CH_3)_3$	0.87	8.54	8.51
		X = $(CH_2)_3NMe_2$		
8.	H	0.00	6.72	6.62
9.	8-OH	0.00	7.17	6.62
10.	9-OH	-0.67	8.75	8.89
11.	9-OCH_3	-0.02	6.38	6.64
12.	9-OC_2H_5	0.38	6.66	6.58
13.	9-$OCH_2CH=CH_2$	0.37	5.66	6.57
14.	9-OCH_3, 10-Cl	-0.02	7.00	6.64
15.	9-$OCOCH_3$	-0.64	8.72	8.73
16.	9-$OCOC(CH_3)_3$	0.87	8.11	7.87

[a] Taken from reference 12.
[b] Using Eqn. 4.9 for Group 1 and Eqn. 4.10 for Group 2.

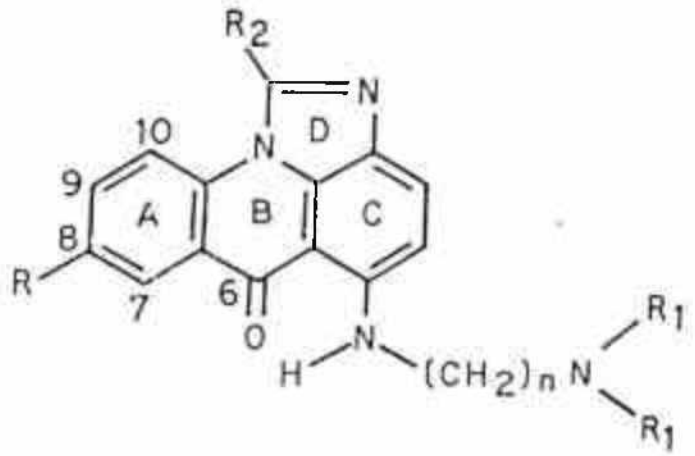
Table 4.4: A series of 6-H-Pyrazolo[4,5,1-de]acridin-6-ones and their antitumor activity.



SNo.	X	Y	Z	log(1/ID$_{50}$), HeLa S$_3$ Cells	
				Obsd.*	Cald. Eqn.4.13
1.	$N(CH_2)_2NH_2$	$N(CH_2)_2NH_2$	OCH_3	4.62	4.59
2.	$N(CH_2)_2N(CH_3)_2$	$N(CH_2)_2N(CH_3)_2$	OCH_3	4.55	4.59
3.	$N(CH_2)_2NH_2$	$N(CH_2)_2NH_2$	H	6.08	5.89
4.	$N(CH_2)_2N(CH_3)_2$	$N(CH_2)_2N(CH_3)_2$	H	5.70	5.89
5.	$N(CH_2)_2NH_2$	$N(CH_2)_2NH_2$	OH	7.68	7.70
6.	$N(CH_2)_2N(CH_3)_2$	$N(CH_2)_2N(CH_3)_2$	OH	7.70	7.70
7.	NCH_2CH_3	$N(CH_2)_2N(CH_3)_2$	OH	8.12	7.70
8.	$N(CH_2)_2OH$	$N(CH_2)_2NH_2$	OH	8.47	7.70
9.	$N(CH_2)_2OH$	$N(CH_2)_3NH_2$	OH	7.28	7.70
10.	$N(CH_2)_2OH$	$N(CH_2)_4NH_2$	OH	6.96	7.70
11.	$N(CH_2)_2OH$	$N(CH_2)_2N(CH_3)_2$	OH	8.23	7.70
12.	$N(CH_2)_2OH$	$N(CH_2)_2NH(CH_2)_2OH$	OH	8.10	7.70
13.	$N(CH_2)_2OH$	$N(CH_2)_3OH$	OH	7.02	7.70
14.	$N(CH_2)_3OH$	$N(CH_2)_2NH_2$	OH	8.11	7.70
15.	$N(CH_2)_4OH$	$N(CH_2)_2NH_2$	OH	7.85	7.70
16.	$N[(CH_2)_2OH]_2$	$N(CH_2)_2N(CH_3)_2$	OH	7.10	7.70
17.		$N(CH_2)_3NH_2$	OH	7.42	7.70

* Taken from reference 13.

Table 4.5: A series of substituted 5-amino-6H-imidazo[4,5-1-de] acridine-6-ones and their antitumor activity.



SNo.	R	R_1	R_2	n	log(1/ID_{50}), L1210 Leukemia	
					Obsd.*	Calcd., Eqn.4.14
1.	OCH_3	CH_3	H	2	5.78	6.03
2.	OCH_3	CH_3	CH_3	2	6.11	5.80
3.	OCH_3	C_2H_5	H	2	5.75	5.92
4.	OCH_3	C_2H_5	CH_3	2	5.81	5.69
5.	OCH_3	CH_3	H	3	5.09	5.68
6.	OCH_3	CH_3	CH_3	3	5.49	5.45
7.	OCH_3	C_2H_5	H	3	5.60	5.57
8.	OCH_3	C_2H_5	CH_3	3	5.85	5.34
9.	OH	CH_3	H	2	7.32	7.25
10.	OH	CH_3	CH_3	2	6.87	7.01
11.	OH	C_2H_5	H	2	7.51	7.14
12.	OH	C_2H_5	CH_3	2	6.60	6.91
13.	OH	CH_3	H	3	7.47	6.89
14.	OH	CH_3	CH_3	3	6.64	6.66
15.	OH	C_2H_5	H	3	6.75	6.79
16.	OH	C_2H_5	CH_3	3	6.05	6.56

* Taken from reference 14.

The compounds of Table 4.3 can be put into two different groups depending upon X-substituent of the side chain. Group 1 (compounds 1-7) has X = $(CH_2)_2NMe_2$ and Group 2 (Compounds 8-16) has X = $(CH_2)_3NMe_2$. For both the groups, the activities of the compounds against L1210 leukemia cells were found to have the excellent correlations with the hydrophobic constant π of substituents at the 9-position.

Group 1

$$\log(1/ID_{50}) = 6.43 - 1.00(\pm 0.50)\pi_9 + 3.88(\pm 0.93)(\pi_9)^2 \quad (4.9)$$

$n = 7,\ r = 0.99,\ s = 0.24,\ F_{2,4} = 74.70\ (18.00)$

Group 2

$$\log(1/ID_{50}) = 6.62 - 1.29(\pm 0.88)\pi_9 + 3.14(\pm 1.58)(\pi_9)^2 \quad (4.10)$$

$n = 9,\ r = 0.92,\ s = 0.49,\ F_{2,6} = 16.24\ (10.92)$

In the above equations, ID_{50} refers to the molar concentration of the compound leading to 50% inhibition of the leukemia cells. The values of the statistical parameters in both the equations exhibit highly significant correlations. Eqn 4.9 accounts for 98% of the variance in the activity ($r^2 = 0.98$) and Eqn. 4.10 for 85% of that and in both of them the F-value is significant at 99% level (data within parenthesis following the F-values are the F-values at 99% level). However, since both the equations exhibit an inverted parabola, the worst value of π_9 giving the lowest

activity in each case is obtained. Eqn. 4.9 gives $(\pi_7)_{worst}$ = 0.13 and Eqn. 4.10 gives $(\pi_7)_{worst}$ = 0.21.

A combined correlation of Group 1 and Group 2 can be had if an indicator parameter I is used to account for the variation in their X-substituent. Thus using I=0 for X = $(CH_2)_2NMe_2$ (Group 1) and I=1 for X = $(CH_2)_3NMe_2$ (Group 2) we get the correlation as:

$$\log(1/ID_{50}) = 6.55 - 1.15(\pm 0.46)\pi_7 + 3.48(\pm 0.84)(\pi_7)^2 + 0.01(\pm 0.44)I \qquad (4.11)$$

$n = 16,\ r = 0.95,\ s = 0.40,\ F_{3,12} = 33.37\ (5.95)$

This correlation exhibits that the indicator parameter is completely insignificant, suggesting that the variation in X-substituent is of no consequence. Thus without the indicator parameter the correlation obtained is as follows:

$$\log(1/ID_{50}) = 6.54 - 1.15(\pm 0.44)\pi_7 + 3.48(\pm 0.80)(\pi_7)^2 \qquad (4.12)$$

$n = 16,\ r = 0.95,\ s = 0.38,\ F_{2,13} = 54.22\ (6.70)$

which is in fact somewhat better than that expressed by Eqn. 4.11. Eqn. 4.12 gives $(\pi_7)_{worst}$ = 0.17, which is exactly the mean of those given by Eqns. 4.9 and 4.10. Thus, the minimum of the activity seems to lie around $\pi_7 = 0.17 \pm 0.04$. A change on either side of this value of π_7 will lead to an

increase in the activity. Thus both highly hydrophilic and highly hydrophobic group at the 9-position appear to favour the activity, suggesting the possibility of both hydrophobic and electrostatic interactions of 9-substituents with the receptor. Since no physicochemical parameters of substituents at any other positions were found to be related with the activity, it can be said that the 9-position substituents have the best orientation with respect to the active site of the receptor. An OH group at this position seems to have an special effect through the hydrogen bonding with the receptor, as the 9-OH derivative (compound 10) possesses much higher activity than the 8-OH derivative (compound 9). This effect of 9-OH, however, has been accounted for by its quite low value of π.

The hydrogen bonding seems to dominate the activity of compounds of Table 4.4, as the only parameters that we could significantly correlate with the activity in this case were the hydrogen-bond donor and hydrogen-bond acceptor parameters for the 7-substituents (Eqn. 4.13). No other physicochemical or structural parameters of any substituents were found to have any correlation with the activity. Eqn. 4.13 represents a very significant correlation that accounts for more than 86% of the variance in the activity ($r^2 > 0.86$). In this equation H_a is the hydrogen-bond acceptor parameter which is equal to 1 for all such groups that can accept a hydrogen bond such as OCH_3 and zero for others, and H_d is a hydrogen-bond donor parameter which

is equal to 1 for all such groups that can donate a hydrogen bond such as OH and zero for others. Since H_d has a positive coefficient in the equation, a hydrogen-bond donor at the 7-position will be very conducive to the activity. Such a substituent will strongly bind with a hydrogen-bond acceptor site at the receptor, that would strongly repel a hydrogen-bond acceptor group at this position, leading to an adverse effect as reflected by the negative coefficient of H_a in Eqn. 4.13.

$$\log(1/ID_{50}) = 5.89 - 1.31(\pm 1.01)H_a + 1.81(\pm 0.77)H_d \qquad (4.13)$$

$$n = 17,\ r = 0.93,\ s = 0.47,\ F_{2,14} = 45.57 \quad (6.51)$$

In Table 4.5, all the substituted positions have only two substituents; therefore the use of any physicochemical parameter for the substituents at any position will be identical to using any dummy parameter for them. Hence it was thought better to find out the activity contribution of each individual substituent at different positions, using the Free-Wilson approach[16] in which it is assumed that:

$$\text{Biological response} = \mu + \Sigma \text{ subs. contribution} \qquad (4.14)$$

where μ represents the activity contribution of the parent structure. Eqn 4.14 will generate 16 linear equations for the compounds of Table 4.5 with 8 substituent variables and one parent structure variable. The substituent variables will

however reduce to only 4, as the contributions of all the substituents at a position sum to zero and thus one of them can be treated as an independent variable. Thus, in total, there would be only 5 variables for 16 linear equations. These linear equations were solved by least square method and the activity contributions obtained are shown in Table 4.6. In this approach, the contribution of the parent structure comes out to be equal to the average of the activity values of all the compounds.

Table 4.6: Individual substituent contribution in acridine series of Table 4.5.

R	R_1	R_2	n	μ
OCH_3=-0.61	CH_3=0.05	H=0.12	2 → 0.18	6.29
OH=0.61	C_2H_5=-0.05	CH_3=-0.12	3 → -0.18	

r = 0.90, s = 0.39, $F_{4,11}$ = 11.05, [$F_{4,11}(0.01)$ = 5.67]

From Table 4.6 we find that the highest contribution to the activity of the compounds of Table 4.5 would be made by an 8-OH group and the lowest by an 8-OCH_3 group. Thus, as in the case of the compounds of Table 4.4, we can assume here

too that this is merely the result of strong hydrogen bonding in which the OH group will participate in the interaction with the hydrogen-bond acceptor site of the receptor and the OCH_3 will be repelled by it.

Thus the intercalation mechanism of DNA binding of acridine series seems to predominantly involve the hydrogen bonding or the hydrophobic interaction. In the series of VII (Table 4.5), the next best contribution is shown by Table 4.6 to be made by the 5-amino side chain that contains a smaller number of $-CH_2-$ groups (n=2). The R_1-substituents in this chain are found to have negligible effect, but an R_2-substituents at ring D, being a CH_3 group, seems to produce a steric effect.

All the statistical parameters given in Table 4.6 are quite significant, indicating the validity of the substituents contribution analysis. If the activities of compounds are calculated using the substituents contribution in Eqn. 4.14, they are found to agree well with the observed ones (Table 4.5).

4.3 Human Steroid 5α-Reductase and Human Adrenal 3β-Hydroxy-Δ^5-Steroid Dehydrogenase/3-Keto-Δ^5-Steroid Isomerase Inhibitors

Among the various types of neoplastic disease, the benign prostatic hyperplasia (BPH) is the most common in the aging male.[17] The hyperplastic growth of the prostrate has been found to be supported by dihydrotestosterone (DHT)[17-19]

and thus DHT is supposed to be the causative factor of BPH. Therefore, for the treatment of this disease, attempts have been made to investigate the potent inhibitors of the enzyme steroid 5α-reductase (EC1.3.1.30) that is responsible for the production of DHT from testosterone.[20] The involvement of DHT in BPH is supported by the study on DHT-deficient males. These males are born pseudohermaphroditic, lacking well-developed external genitalia. Although, at puberty, they have phallic growth as well as the development of normal male sexual orientation, libido and performance, they lack hairline regression and acne and possess small, often undetectable, prostrates, and never develop the symptomatology of BPH.[21]

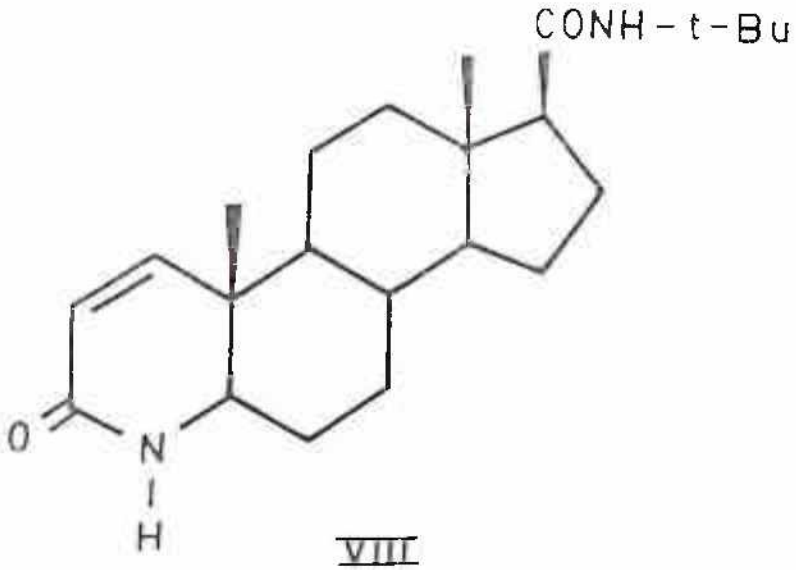
The enzyme, steroid 5α-reductase (5AR), has two isozymes called type 1 5AR and type 2 5AR. The relative roles of these two isozymes in developmental physiology and in patho-physiology of BPH and other androgen related disorders are the subject of much current research.[19,22-24] A number of 5AR inhibitors have been identified including finasteride (MK-906,VIII) and epristeride (SK & F 105657, IX) which have been assessed clinically.[25] The dual inhibitors of type 1 and type 2 5AR have been found to be very effective in the circulating DHT in human.

However, while studying the dual inhibition of type 1 and type 2 5AR, it was also found essential to study the selectivity of the inhibitors against human adrenal 3β-hydroxy-Δ^5-steroid dehydrogenase/3-keto-Δ^5-steroid iso-

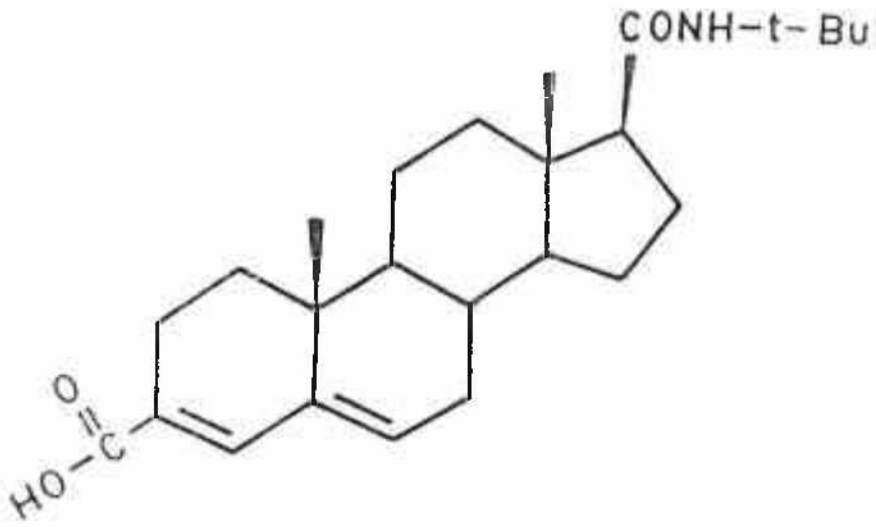
merase (3BHSD), the enzyme which plays critical role in steroid biosynthesis. With all these considerations, Frye et al.[27] recently synthesized some 6-azasteroids and studied their human 5AR (both type 1 and type 2) inhibition potency with selectivity against human 3BHSD. However, the whole study of Frye et al. was merely based on trial-and-error factors. In order to provide a rational basis to design the potent 5AR inhibitors, a quantitative structure-activity relationship study on these steroids has been attempted.

Three different series of 6-azasteroids studied by Frye et al.[27] are listed in Tables 4.7 - 4.9 along with their 5AR and 3BHSD inhibition activities. Table 4.10 lists another series of steroids along with their 5AR inhibition activity studied by Holt et al.[28] In Tables, K_i refers to the enzyme inhibition constant and IC_{50} to the minimum molar concentration leading to 50% inhibition of the enzyme.

Tables also list the physicochemical parameters of the compounds that were found to be correlated with their activities. The values of these physicochemical parameters were taken from the literature.[29] In addition to these physicochemical parameters, some dummy parameters were also used to describe the effects of some specific alterations in the molecules. To find the structure-activity relationships, a multiple regression analysis was performed, using the least square method.[29]

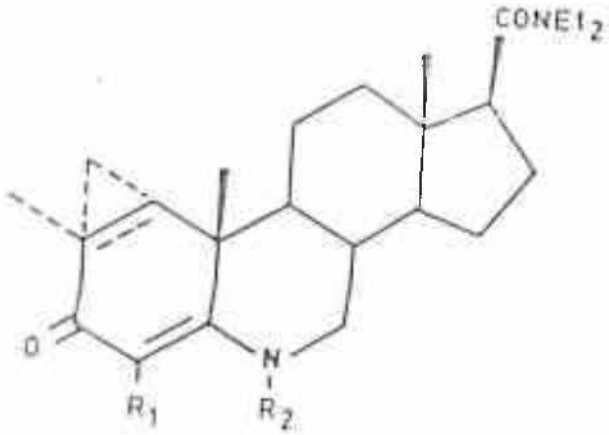


VIII



IX

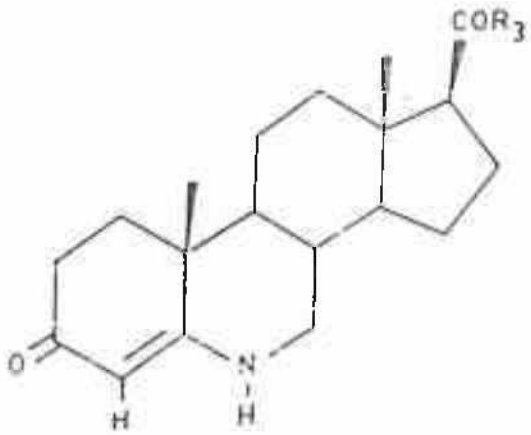
Table 4.7: 6-Azaandrost-4-en-3-ones and their 5α-Reductase and 3βHSD inhibition potencies studied by Frye et al.[27] and physicochemical parameters.



Sno	R_1	R_2	Other	σ_1	σ_2	MR_1	MR_2	θ_1	θ_2	I	$\log(1/K_i)_{1-5\alpha R}$ obsd*	$\log(1/K_i)_{1-5\alpha R}$ Calcd Eqn.4.15	$\log(1/IC_{50})_{2-5\alpha R}$ obsd*	$\log(1/IC_{50})_{2-5\alpha R}$ Calcd Eqn.4.16	$\log(1/K_i)_{3\beta HSE}$ obsd*	$\log(1/K_i)_{3\beta HSE}$ Calcd Eqn.4.17
1.	H	H		0.00	0.00	0.103	0.103	0.00	0.00	0	6.12	5.80	8.82	8.50	7.22	7.20
2.	H	H		0.00	0.00	0.103	0.103	0.00	0.00	0	5.24	5.80	8.46	8.50	7.10	7.20
3.	H	H	Δ[4]	0.00	0.00	0.103	0.103	0.00	0.00	1	4.85	4.91	8.74	8.50	7.02	7.20
4.	H	H	b	0.00	0.00	0.103	0.103	0.00	0.00	0	5.46	5.80	8.47	8.50	6.29	7.20
5.	H	$COCH_3$	c	0.00	-0.55	0.103	1.118	0.00	0.50	0	4.40	4.70	5.52	6.25	4.47	5.35
6.	H	CN		0.00	-0.57	0.103	0.633	0.00	0.66	0	5.08	4.70	7.38	6.96	5.82	4.80
7.	H	CH_2CO_2H		0.00	-0.72	0.103	1.188	0.00	-0.07	0	5.00	5.42	5.82	5.93	6.74	7.13
8.	H	Me		0.00	0.56	0.103	0.565	0.00	-0.17	0	6.74	5.82	8.64	8.51	8.44	7.59
9.	H	Et		0.00	1.02	0.103	1.030	0.00	-0.15	0	5.89	5.60	8.46	8.39	7.31	7.10
10.	H	Pr		0.00	1.55	0.103	1.496	0.00	-0.13	0	4.62	5.37	8.38	8.36	6.28	6.23
11.	H	i-Pr		0.00	1.53	0.103	1.496	0.00	-0.15	0	5.47	5.40	8.31	8.33	5.96	6.34
12.	H	Bu		0.00	2.13	0.103	1.961	0.00	-0.16	0	5.11	5.21	7.54	8.39	5.44	5.09
13.	H	Bn		0.00	2.01	0.103	3.001	0.00	-0.09	0	5.00	4.67	7.40	6.66	4.80	5.14
14.	H	Me	Δ[4]	0.00	0.56	0.103	0.565	0.00	-0.17	0	5.89	5.82	8.24	8.51	7.66	7.59
15.	H	Me	b	0.00	0.56	0.103	0.565	0.00	-0.17	1	5.00	4.94	8.30	8.51	8.00	7.59
16.	Cl	H		0.71	0.00	0.603	0.103	0.23	0.00	0	7.29	7.04	8.72	8.85	7.57	7.54
17.	Br	H		0.86	0.00	0.888	0.103	0.23	0.00	0	7.01	6.73	8.68	8.63	7.89	7.61
18.	I	H		1.12	0.00	1.394	0.103	0.18	0.00	0	6.16	5.73	8.22	7.93	7.82	7.67
19.	CH_2NMe_2	H		-0.15	0.00	1.874	0.103	0.01	0.00	0	5.00	4.96	6.77	6.75	5.92	5.95
20.	Me	H		0.56	0.00	0.565	0.103	-0.17	0.00	0	7.68	7.15	8.41	7.95	7.75	7.38
21.	Me	H		0.56	0.00	0.565	0.103	-0.17	0.00	0	6.55	7.15	8.09	7.95	7.04	7.38
22.	Et	Me	Δ[4]	1.02	0.00	1.030	0.103	-0.15	0.00	0	5.39	6.19	6.74	7.62	7.33	7.79
23.	Me	H		0.56	0.56	0.565	0.565	-0.17	-0.17	0	7.09	7.17	8.35	7.97	7.96	7.77

* Taken from reference 27.
b = 1,2-α-methano
c = 2α,β-Me

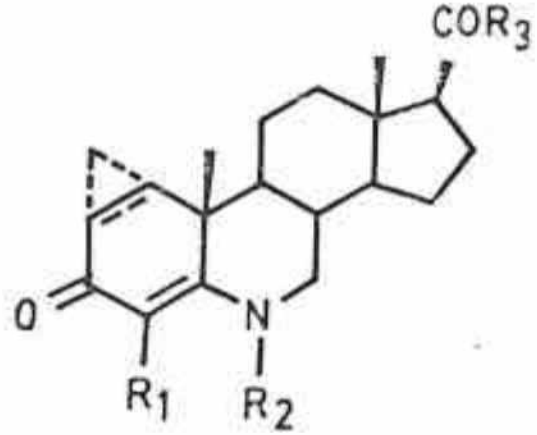
Table 4.8: 6-Azaandrost-4-en-3-ones and their 5α-Reductase and 3βHSD inhibition potencies studied by Frye et al.[27] and physicochemical parameters.



No.	R_3	π^3	I_1	I_2	$\log(1/K_i)_{5\alpha\text{-}3OR}$ Obsd.	$\log(1/K_i)_{5\alpha\text{-}3OR}$ Calcd* Eqn.4.21	$\log(1/IC_{50})_{5\alpha\text{-}3OR}$ Obsd.	$\log(1/IC_{50})_{5\alpha\text{-}3OR}$ Calcd Eqn.4.22	$\log(1/K_i)_{3\beta HSD}$ Obsd.	$\log(1/K_i)_{3\beta HSD}$ Calcd Eqn.4.23
1.	OCH_3	0.612	0	0	6.82	6.77	8.49	8.49	7.92	7.70
2.	O-2-Ad	5.292	0	0	8.16	8.72	-	-	6.74	7.01
3.	N-MeOMe	1.262	0	1	5.64	6.35	8.62	8.89	7.30	7.32
4.	Piperazine	2.563	1	0	5.07	5.54	7.48	8.42	6.66	7.01
5.	Morpholine	2.433	1	0	5.66	5.48	8.15	8.37	6.72	7.02
6.	Thiomorpholine	3.584	1	0	6.24	5.95	8.89	8.67	7.51	6.83
7.	Piperidine	2.856	1	0	7.07	5.68	9.30	8.67	6.96	6.99
8.	$NHCH(4\text{-fluorophenyl})_2$	4.535	0	1	7.70	7.73	9.80	9.81	6.49	6.87
9.	$NHCH(4\text{-Chlorophenyl})_2$	5.963	0	1	7.70	7.86	9.92	9.65	6.29	5.97
10.	$NHNPh_2$	4.742	0	1	7.85	7.77	9.64	9.80	6.80	6.76
11.	NOH-t-Bu	2.147	0	1	7.42	6.88	9.22	9.32	7.21	7.43
12.	$NHCH(Cyclohexyl)_2$	6.838	0	1	7.70	7.77	9.40	9.35	5.40	5.16
13.	$NHCPh_3$	6.982	0	1	8.09	7.75	-	-	7.00	5.01
14.	n-Pr	1.561	0	0	7.92	7.43	9.52	9.05	7.96	7.95
15.	n-Octyl	4.061	0	0	9.00	8.52	-	-	8.12	7.65
16.	$CH_2(Cyclohexyl)$	2.992	0	0	8.40	8.16	-	-	7.80	7.94
17.	2,6-difluorophenyl	1.770	0	0	7.41	7.55	-	-	7.96	7.97
18.	1-Naphityl	3.571	0	0	7.82	8.38	-	-	8.11	7.82
19.	2,4,6-tri-isopropylphenyl	6.235	0	0	6.55	8.73	9.30	9.56	5.72	6.29

* taken from reference 27.

Table 4.9: 6-Azaandrost-4-en-3-ones and their 5α-reductase and 3BHSD inhibition potencies studied by Frye et al.[87] and physicochemical parameters.



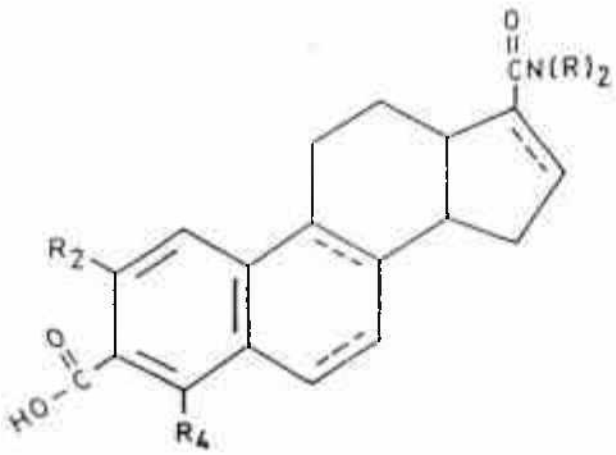
SNo.	R_1	R_2	R_3	other	π_1	π_2	I_1	I_2	I_3	I_4	$\log(1/K_i)_{1\text{-}5\alpha R}$ Obsd*	$\log(1/K_i)_{1\text{-}5\alpha R}$ Calcd Eqn.4.24	$\log(1/IC_{50})_{2\text{-}5\alpha R}$ Obsd*	$\log(1/IC_{50})_{2\text{-}5\alpha R}$ Calcd Eqn.4.25	$\log(1/K_i)_{3BHSD}$ Obsd*	$\log(1/K_i)_{3BHSD}$ Calcd Eqn.4.26
1.	H	H	NH-t-Bu		0.00	0.00	0	0	1	0	6.09	6.88	9.06	9.14	6.82	7.06
2.	H	H	NH-t-Bu	b	0.00	0.00	1	0	1	0	5.62	5.71	8.75	8.75	5.66	6.08
3.	H	H	NH-t-Bu	c	0.00	0.00	0	1	1	0	4.31	4.31	8.36	8.36	6.52	7.06
4.	H	Me	NH-t-Bu		0.00	0.56	0	0	1	0	7.06	6.88	8.77	8.73	8.18	7.93
5.	H	Me	NH-t-Bu	b	0.00	0.56	1	0	1	0	5.85	5.71	8.17	8.34	7.21	6.95
6.	Me	H	NH-t-Bu		0.56	0.00	0	0	1	0	7.92	7.29	8.85	8.56	8.04	7.48
7.	Me	Me	NH-t-Bu		0.56	0.56	0	0	1	0	7.22	7.29	8.07	8.15	7.64	8.11
8.	H	H	t-Bu		0.00	0.00	0	0	0	1	8.05	8.05	-	-	8.00	8.15
9.	H	Me	t-Bu		0.00	0.56	0	0	0	1	8.51	8.05	10.00	9.88	8.96	9.02
10.	Br	H	t-Bu		0.86	0.00	0	0	0	1	8.50	8.68	9.40	9.39	8.64	8.79
11.	Me	H	t-Bu		0.56	0.00	0	0	0	1	9.40	8.46	-	-	8.92	8.57
12.	H	H	NH-1-Ad		0.00	0.00	0	0	0	0	7.96	8.05	-	-	7.10	7.06
13.	H	H	NH-1-Ad	b	0.00	0.00	1	0	0	0	7.13	6.88	-	-	6.00	6.08
14.	H	Me	NH-1-Ad		0.00	0.56	0	0	0	0	8.08	8.05	-	-	8.57	7.93
15.	H	Me	NH-1-Ad	b	0.00	0.56	1	0	0	0	6.55	6.88	9.53	9.49	7.21	6.95
16.	Br	H	NH-1-Ad		0.86	0.00	0	0	0	0	8.35	8.68	-	-	7.89	7.70
17.	Me	H	NH-1-Ad		0.56	0.00	0	0	0	0	8.96	8.46	-	-	8.05	7.48
18.	Br	Me	NH-1-Ad		0.86	0.56	0	0	0	0	8.06	8.67	8.77	8.98	8.08	8.57
19.	Me	Me	NH-1-Ad		0.56	0.56	0	0	0	0	8.21	8.46	9.40	9.30	7.75	8.35
20.	H	H	NH-CH-Ph$_2$		0.00	0.00	0	0	0	0	7.52	8.05	-	-	6.82	7.06
21.	H	H	NH-CH-Ph$_2$	b	0.00	0.00	1	0	0	0	7.32	6.88	-	-	6.00	6.08
22.	H	Me	NH-CH-Ph$_2$		0.00	0.56	0	0	0	0	8.19	8.05	9.70	9.88	7.96	7.93
23.	H	Pr	NH-CH-Ph$_2$	b	0.00	1.55	1	0	0	0	6.47	6.88	8.89	8.77	6.75	6.69
24.	Me	H	NH-CH-Ph$_2$		0.56	0.00	0	0	0	0	8.44	8.46	-	-	7.75	7.48

* taken from reference 27

b = Δ^1

c = 1,2-α-methano

Table 4.10: A Ring carboxylic acids and their 5α-reductase inhibition potencies studied by Holt et al.[28] and physicochemical parameters.



SNo	R_2	R_4	$(R)_2$	Others	MR_2	MR_4	π_4	I_M	I_{un}	$\log(1/K_i)_{rat}$ Obsd*	$\log(1/K_i)_{rat}$ Calcd. Eqn.4.27	$\log(1/K_i)_{human}$ Obsd*	$\log(1/K_i)_{human}$ Calcd. Eqn.4.28
1.	H	H	i-Pr		1.03	1.03	0.00	0	0	6.45	6.39	7.70	7.78
2.	H	H	t-Bu,H	Δ^6	1.03	1.03	0.00	1	0	6.82	6.76	7.37	7.43
3.	H	H	i-Pr	Δ^6	1.03	1.03	0.00	0	1	6.35	6.39	7.52	7.51
4.	H	H	i-Pr	Δ^6, Δ^8	1.03	1.03	0.00	0	1	6.46	6.39	7.44	7.51
5.	H	H	t-Bu,H	Δ^{16}	1.03	1.03	0.00	1	1	6.70	6.76	7.22	7.16
6.	H	F	i-Pr		1.03	0.92	0.06	0	0	6.30	6.55	8.00	7.82
7.	Cl	H	i-Pr		6.03	1.03	0.00	0	0	6.70	6.39	7.46	7.32
8.	H	Cl	i-Pr		1.03	6.03	0.23	0	0	6.05	6.11	6.82	6.69
9.	Br	H	i-Pr		8.88	1.03	0.00	0	0	6.59	6.39	7.12	7.06
10.	H	Br	i-Pr		1.03	8.88	0.23	0	0	5.72	5.64	6.67	6.69
11.	CN	H	i-Pr		6.33	1.03	0.00	0	0	6.02	6.39	7.19	7.29
12.	H	CN	i-Pr		1.03	6.33	0.66	0	0	–	–	6.70	6.67
13.	COOH	H	i-Pr		6.93	1.03	0.00	0	0	–	–	5.30	7.24
14.	CH_3	H	i-Pr		5.65	1.03	0.00	0	0	6.47	6.39	7.22	7.36
15.	H	CH_3	i-Pr		1.03	5.65	-0.17	0	0	5.16	5.23	6.59	6.73

* taken from reference 28.

For the inhibition potencies of compounds in Table 4.7, the best correlations obtained were as follows:

$$\log(1/K_i)_{T\text{-}3AM} = 4.89(\pm 2.10)\pi_1 - 4.42(\pm 2.21)(\pi_1)^2 - 1.32(\pm 1.14)\sigma_2 - 0.43(\pm 0.34)MR_2 - 0.88(\pm 0.82)I + 5.839 \quad (4.15)$$

$$n = 23,\ r = 0.88,\ s = 0.50,\ F_{5,17} = 12.07 \quad (4.34)$$

$$\log(1/IC_{50})_{2\text{-}3AM} = 1.28(\pm 0.37)\pi_2 - 1.52(\pm 0.40)MR_2 - 0.51(\pm 0.26)(MR_1)^2 + 2.29(\pm 1.91)\sigma_1 + 8.661 \quad (4.16)$$

$$n = 23,\ r = 0.90,\ s = 0.45,\ F_{4,18} = 19.32 \quad (4.58)$$

$$\log(1/K_i)_{3\text{-}HMAP} = 0.93(\pm 0.74)(\pi_1)^2 - 0.36(\pm 0.32)(MR_1)^2 - 0.58(\pm 0.20)(\pi_2)^2 - 3.36(\pm 1.19)\sigma_2 + 7.204 \quad (4.17)$$

$$n = 23,\ r = 0.90,\ s = 0.52,\ F_{4,18} = 19.05 \quad (4.58)$$

In these equations the subscripts to parameters 1 and 2 refer to the substituents R_1 and R_2, respectively. The values of all these parameters have been taken from the literature.[29] A dummy parameter I in Eqn. 4.15 has been used, with a value of unity, to describe the effect of 1,2-α-methano moiety.

All these equations exhibit the significant correlations but indicate that the patterns of dependency on the

variables of all the three different enzyme systems are not all the same. The hydrophobic property of R_1-substituent is shown to affect the inhibition of type 1 5AR and 3BHSD but not of type 2 5AR. In both the former cases, the inhibition potency is found to have a parabolic correlation with π_1, but while for 1-5AR there would be an optimum value of $\pi_1(\pi_{1,opt})$ equal to 0.55, suggesting that the activity will not be favoured beyond this value, for 3BHSD any value of π_1, positive or negative, will always increase the activity. Similarly, an R_2-substituent, whether hydrophobic or hydrophilic, is found to always decrease the inhibition of 3BHSD. The R_2 also produces the negative effect on the inhibition of 1-5AR, but in this case its effect is described by molar refractivity index and not by hydrophobic parameter. The latter was found to have poorer correlation with the activity than the former in this case, but the reverse was true in the case of 3BHSD. However, in both the cases, the electronic effect was found to be quite consistent. Both Eqns. 4.15 and 4.17 exhibit that an electron-releasing R_2-substituent will enhance the inhibition potency. This increase in the potency may be due to the increase in electronic charge by the donation of R_2-substituent at some crucial position of the compound that needs the highest negative charge for the strongest interaction with 1-5AR and 3BHSD enzymes.

Such commonalties were, however, not observed in the case of 2-5AR inhibition. In this case, Eqn. 4.16 suggests

that a hydrophobic R_2-substituent will be conducive to the activity, but if it is highly polarizable there would be an adverse effect, as the molar refractivity index for this substituent has a negative coefficient and it is a measure of polarizability, which of course is the function of molecular size. Regarding the R_1-substituent, Eqn. 4.16 tells that while the size of the substituent may produce the negative effect, its electron-withdrawing nature will lead to an increase in the activity. By withdrawing the electron, the substituent probably reduces the electronic charge of the crucial position of the compound, which ought to be least negatively (or highly positively) charged for the strongest interaction with the enzyme.

Any other changes at any other positions were found to be of little value for the inhibition of any enzyme, except that 1,2-α-methano group, for which the dummy parameter I has been used in Eqn. 4.15, is found to have some negative effect on the inhibition of 1-5AR.

We also analysed the mutual correlations among the inhibition potencies (Eqns. 4.18-4.20) and found that there exists a noticeable correlation between type 1 5AR inhibition and 3BHSD inhibition (Eqn. 4.18) and this explains the commonalties of physicochemical properties governing them. Such noticeable correlations were not found between 1-5AR and 2-5AR inhibitions (Eqn. 4.19) or between 2-5AR and 3BHSD inhibitions (Eqn. 4.20)

$$\log(1/K_i)_{1\text{-}5AR} = 0.60(\pm 0.29)\log(1/K_i)_{3BHSD} + 1.641 \qquad (4.18)$$

$n = 23,\ r = 0.69,\ s = 0.69,\ F_{1,21} = 18.61(8.02)$

$$\log(1/K_i)_{1\text{-}5AR} = 0.54(\pm 0.39)\log(1/IC_{50})_{2\text{-}5AR} + 1.468 \qquad (4.19)$$

$n = 23,\ r = 0.54,\ s = 0.80,\ F_{1,21} = 8.48\ (8.02)$

$$\log(1/IC_{50})_{2\text{-}5AR} = 0.52(\pm 0.32)\log(1/K_i)_{3BHSD} + 4.370 \qquad (4.20)$$

$n = 23,\ r = 0.60,\ s = 0.76,\ F_{1,21} = 11.70\ (8.02)$

Table 4.8 lists the compounds, where the study was made on the effect of variations in the substituent of 17-position.[28] For these compounds, the inhibition potencies were found to be correlated with Kier's first order valence molecular connectivity index($^1\chi^v$)[30] of the varying R moiety at the 17-position. This index signifies the degree of branching, saturation, and the molecular size of a molecule or a group. The correlations obtained were as follows.

$$\log(1/K_i)_{1\text{-}5AR} = 0.85(\pm 0.58)\,^1\chi^v - 0.07(\pm 0.08)(^1\chi^v)^2 - 2.43(\pm 0.70)I_1 - 0.89(\pm 0.61)I_2 + 6.282 \qquad (4.21)$$

$n = 17,\ r = 0.93,\ s = 0.45,\ F_{4,12} = 19.71(5.41)$

$$\log(1/IC_{50})_{5\alpha\text{-}5AR} = 0.78(\pm 0.54)\,{}^{1}\chi^{v} - 0.09(\pm 0.07)({}^{1}\chi^{v})^{2} - 1.06(\pm 0.61)I_1 + 8.043 \quad (4.22)$$

$n = 11,\ r = 0.91,\ s = 0.29,\ F_{3,7} = 11.02(8.45)$

$$\log(1/K_i)_{3\beta HSD} = 0.50(\pm 0.51)\,{}^{1}\chi^{v} - 0.11(\pm 0.07)({}^{1}\chi^{v})^{2} - 0.98(\pm 0.52)I_1 - 0.57(\pm 0.45)I_2 + 7.438 \quad (4.23)$$

$n = 18,\ r = 0.92,\ s = 0.37,\ F_{4,13} = 17.22(5.20)$

In the derivation of all these equations, however, some compounds that were found misfit in them were excluded. Compounds 7 and 19 were excluded from Eqn. 4.21, 3 and 6 from Eqn. 4.22, and 13 was excluded from Eqn. 4.23. Since these compounds were not common, it was found difficult to explain their aberrant behaviour.

In all these equations, the two dummy parameters I_1 and I_2 were used for the groups that contained nitrogen. The former was for a group that had nitrogen in the ring and the latter was for a group that had nitrogen anywhere. Both the parameters were assigned a value of unity for the group for which they stand and zero for others. In Eqns. 4.21 and 4.23, both I_1 and I_2 are present with a negative coefficient, quite significant at 95% confidence level, suggesting that groups containing nitrogen will not be conducive to the inhibition of 1-5AR and 3BHSD enzymes. However, in Eqn. 4.22, I_2 was not found to be significant, hence groups containing nitrogen

anywhere but not in any ring were not so detrimental for the inhibition of 2-5AR. The parabolic correlation of the inhibition potency with 'χ' in each case, however, suggested that only a limited bulk and saturation of the group will favour the activity. Table 4.9 lists the compounds where the variation were made in A and B rings with different C_{17} groups. The best correlations obtained for the inhibition potencies for these compounds were as follows:

$$\log(1/IC_{50})_{1\text{-}5AR} = 0.73(\pm0.67)\pi_1 - 1.17(\pm0.45)I_3 - 1.17(\pm0.51)I_4 - 2.57(\pm1.05)I_2 + 8.05 \quad (4.24)$$

$$n = 24,\ r = 0.94,\ s = 0.45,\ F_{4,17} = 35.24 \quad (4.50)$$

$$\log(1/IC_{50})_{2\text{-}5AR} = -1.04(\pm0.40)\pi_1 - 1.15(\pm0.27)I_3 - 0.78(\pm0.47)I_2 - 0.40(\pm0.29)I_1 - 0.73(\pm0.35)\pi_2 + 10.29 \quad (4.25)$$

$$n = 14,\ r = 0.97,\ s = 0.18,\ F_{5,8} = 25.71 \quad (6.63)$$

$$\log(1/IC_{50})_{3BHSD} = 0.75(\pm0.59)\pi_1 + 1.09(\pm0.49)I_4 - 0.98(\pm0.48)I_1 + 2.22(\pm1.07)\pi_2 - 1.18(\pm0.85)(\pi_2)^2 + 7.06 \quad (4.26)$$

$$n = 24,\ r = 0.92,\ s = 0.41,\ F_{5,17} = 20.03 \quad (4.34)$$

In these equations the dummy parameters I_1, I_2, I_3 and I_4 were used with a value of unity to show the effect of Δ^1 unsaturation, 1,2-α-methano moity, and NH-tertiary Butyl (NH-tBu) and iso-Butyl (i-Bu) moities at C_{17} position, respectively.

Once again all the equations were found to exhibit significant correlations, but here also patterns of dependency of variables of all the three enzymes systems are not same. The hydrophobic property of R_1-substituent is conducive to the inhibition of type 1 5AR and 3BHSD. A negative co-efficient of π_1 and π_2 in Eqn. 4.25 suggests that the hydrophobicity of both R_1 and R_2-substituents decrease the activity for 2-5AR. For 3BHSD, inhibition potency has parabolic correlation with π_2. The π_2 has an optimum equal to 0.94, implying that the activity will not be favoured if π_2 exceeds this value. But π_2 does not play any role against 1-5AR. In both 1-5AR and 2-5AR, the negative co-efficient of the dummy parameters I_1, I_2 and I_3 (Eqns. 4.24, 4.25) suggests that Δ^1 unsaturation, 1,2-α-methano and NH-tBu subtituents decrease the activity. I_1 has detrimental effect even on inhibition of 3BHSD and I_2, I_3 were found to be insignificant. However, positive co-efficient of I_4 shows that i-Bu moiety at C_{17} is favourable and adds to the activity. Any other change in the molecule was found to be of little value for the inhibition of any type of the enzyme.

The steroids studied by Holt et al.[28] (Table 4.10) were actually steroidal A ring aryl carboxylic acids. Their inhibition potencies were studied against human 5AR as well as rat 5AR without distinguishing the type of isozyme. For this series of compounds, the best correlations that had surfaced by the regression analysis were:

$$\log(1/K_i)_{rat} = 2.36(\pm 1.50)\sigma_4 - 0.16(\pm 0.06)MR_4 + 0.37(\pm 0.36)I_R + 6.560 \qquad (4.27)$$

$$n = 13,\ r = 0.92,\ s = 0.20,\ F_{3,9} = 17.32(6.99)$$

$$\log(1/K_i)_{human} = 8.271 - 0.41(\pm 0.17)MR_4 + 0.03(\pm 0.02)(MR_4)^2 - 0.09(\pm 0.04)MR_2 - 0.35(\pm 0.26)I_R - 0.27(\pm 0.24)I_{un} \qquad (4.28)$$

$$n = 14,\ r = 0.97,\ s = 0.13,\ F_{5,8} = 23.60(6.63)$$

In these equations, the dummy parameter I_R has been used for the R group of $CONR_2$ moiety at the 17-position. It is equal to 1 if R_2 = t-Bu, H, and zero otherwise. The other dummy parameter I_{un} has been used for the compounds 3-5 that have unsaturation not only in ring A but also in some other rings. It is equal to unity for these compounds and zero for the rest.

Now these equations exhibit that rat 5AR inhibition potency of the compounds will be predominantly governed by the electronic property and the molecular size of the 4-

substituent. Its electron-withdrawing ability will be very conducive to the activity but as its size would increase the activity will decrease, because there is a negative coefficient of MR_4 in Eqn.4.27 However, the positive coefficient of I_{4t} in this equation suggests that a tertiary butyl group in COR_2 moiety will have an additive effect. The 2-position substituent and the unsaturation in rings other than ring A were not found to have any effect on rat 5AR inhibition. On the other hand, these two plus the t-Bu group in COR_2 are shown by Eqn. 4.28 to have negative effect on the human 5AR inhibition. Also no electronic property of the 4-substituent was found to play any role in human 5AR inhibition. However, its size was not that detrimental to the potency in this case. Since Eqn. 4.28 expresses a parabolic correlation between the potency and MR_4, the potency will decrease only upto a limited value of MR_4 = 7.63, after that it will increase. Thus a substituent having MR_4 larger than 7.63 will always be favourable to the activity against human 5AR.

These differences between rat and human 5AR inhibitions may be attributed to the structural differences between the enzymes. There was found a very poor correlation to exist between the activities of compounds against these two enzymes ($r = 0.56$).

Table 4.11: Mutual correlations (r-values) of the variables used in deriving equations

Eqn.4.15

	π_1	σ_2	MR_2	I
π_1	1.0	0.020	0.416	0.185
σ_2		1.000	0.139	0.114
MR_2			1.000	0.138
I				1.000

Eqn. 4.16

	π_2	MR_2	$(MR_1)^2$	σ_1
π_2	1.0	0.730	0.214	0.050
MR_2		1.000	0.318	0.062
$(MR_1)^2$			1.000	0.173
MR_2				1.000

Eqn. 4.17

	$(\pi_1)^2$	$(MR_1)^2$	$(\pi_2)^2$	σ_2
$(\pi_1)^2$	1.0	0.453	0.305	0.000
$(MR_1)^2$		1.000	0.258	0.013
$(\pi_2)^2$			1.000	0.274
σ_2				1.000

Eqn. 4.27

	σ_4	MR_4	I_n
σ_4	1.0	0.513	0.115
MR_4		1.000	0.223
I_n			1.000

Eqn. 4.28

	MR_4	MR_2	I_n	I_{un}
MR_4	1.0	0.371	0.248	0.317
MR_2		1.000	0.250	0.319
I_n			1.000	0.284
I_{un}				1.000

If we compare Eqn. 4.27 with Eqn. 4.16 we find that both the equations exhibit almost the similar effects of the 4-position substituent (see the parameters related to R_4-substituent in Eqn.4.16). Thus we can say that the rat 5AR enzyme may be of type 2. However, nothing can be said about the nature and type of the human 5AR studied by Holt et al., as no similarities exists between Eqn. 4.28 and any of Eqns. 4.15-4.17.

4.4 Antimitotic Chalcones

The mechanism by which the anticancer drugs selectively kill cancer cells has not been clearly established, but evidences point out that these drugs might interfere with the synthesis or function of nucleic acids or with the mitotic process itself. The interference in the mitotic process can be brought by inhibiting the microtubule assembly with binding in an irreversible manner to tubulin.[3]

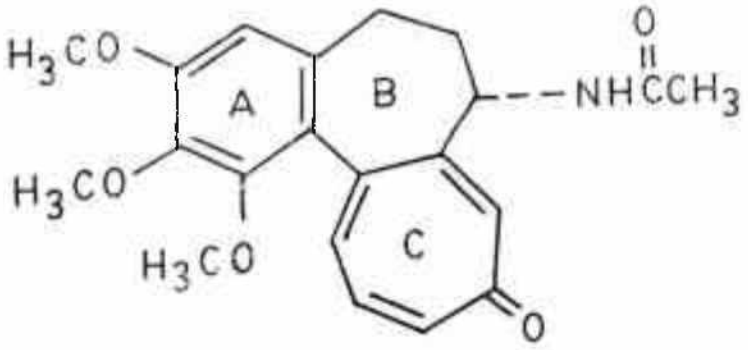
The chemicals that exert their anticancer effects through the inhibition of mitotic process include vinca-alkaloids, colchicine derivatives, podophyllotoxin, and some miscellaneous compounds. With the exception of colchicine (X), reversible inhibitors of microtubule assembly are generally not clinically useful. Colchicine's reversible binding site on tubulin has been found to be different from that of vinca alkaloids.[3] A recent review on structure-

activity relationships of anticancer drugs has delineated the important structural features of antimitotic agents.[11] For colchicine, these features were suggested to be the methoxy groups on ring A, the amine group on ring B, and the carbonyl and methoxy groups on ring C. However the combretastin (XI)[32] and a similar compound XII[33], with no B ring, were found to exhibit tubulin binding of the same order as that of colchicine.

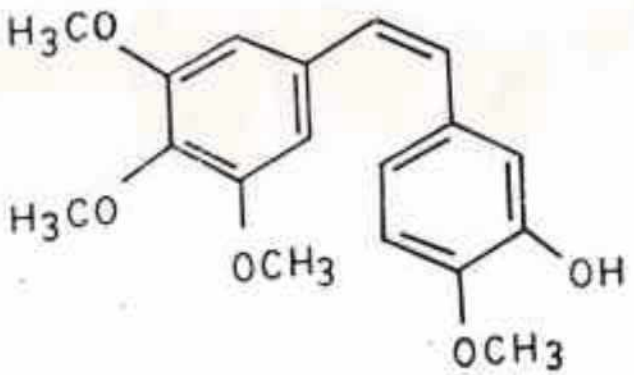
A recent study[34] has shown that appropriately substituted chalcones, as typified by compound XIII, can also bind with tubulin and act as antimitotic agents.

The chalcone series of Edwards et al.[34] that have been subjected to QSAR analysis are listed in Tables 4.12-4.14 along with their antimitotic activity. the ED (equivalent dose) in the tables refers to the concentration of the test compound (ug/mL) that gave the same mitotic index as 0.05 ug/mL colchicine in an in vitro assay system (Hela cells).[34] The physicochemical parameters, namely the hydrophobic constant π and electronic constant σ (Hammett constant) which were found to be useful in the the correlations, were taken from the literature.[35] A least square method was applied to find the correlation.

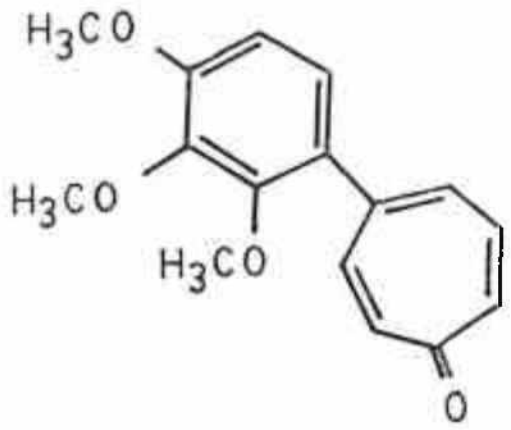
For the compounds of Table 4.12, the best correlation that was obtained is as shown by Eqn. 4.29.



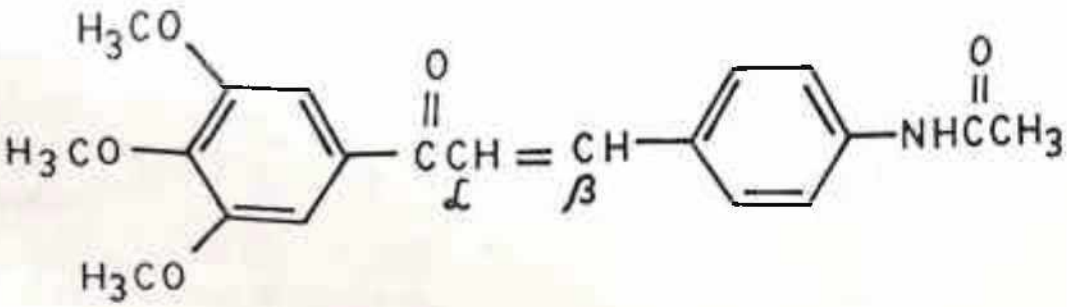
X



XI

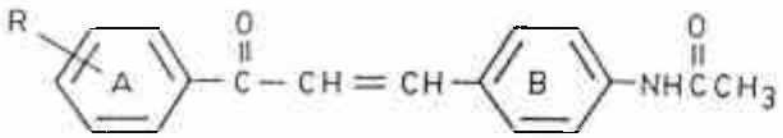


XII



XIII

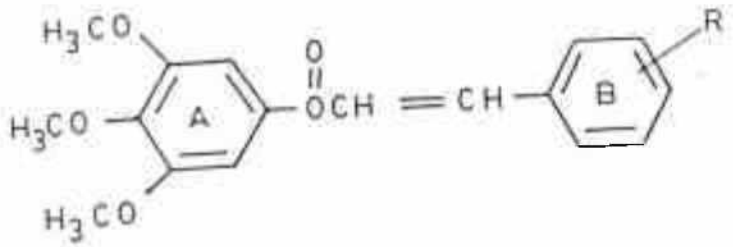
Table 4.12: The Antimitotic Activity of Chalcones and Related physicochemical Constants.



SNo	R	π_4	π_3	σ_4	log(1/ED)	
					Obsd.*	Cald., Eqn.4.29
1.	3,4,5-$(OCH_3)_3$	-0.02	-0.04	-0.27	2.51	2.52
2.	4 - OCH_3	-0.02	0.00	-0.27	2.51	2.50
3.	3 - CF_3	0.00	0.88	0.00	1.60	1.60
4.	4 - t-Bu	1.98	0.00	-0.20	3.60	3.60
5.	4 - CN	-0.57	0.00	0.66	1.60	1.60
6.	4 - $C(O)NH_2$	-1.49	0.00	0.36	2.51	2.50
7.	2,5-$(OCH_3)_3$	0.00	0.00	0.00	2.20	2.21
8.	2,4,6-$(OCH_3)_3$	-0.02	0.00	-0.27	2.51	2.50
9.	2,3,4-$(OCH_3)_3$	-0.02	-0.02	-0.27	2.51	2.51

* taken from reference 34.

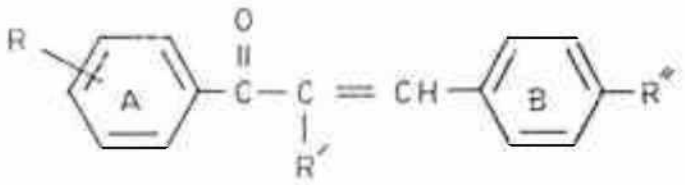
Table 4.13: The Antimitotic Activity of Chalcones and Related Physicochemical Constants.



SNo	R	π_R	π_4	σ	log(1/ED) Obsd.*	log(1/ED) Cald. Eqn.4.30
1.	4-$NHCOCH_3$	0.00	-0.97	0.00	2.51	2.55
2.	4-t-Bu	0.00	1.98	-0.20	2.60	2.66
3.	H	0.00	0.00	0.00	3.82	3.40
4.	4-SCH_3	0.00	0.61	0.00	3.52	3.47
5.	4-$N(CH_3)_2$	0.00	0.18	-0.83	4.82	4.95
6.	4-NH_2	0.00	-1.23	-0.66	3.82	3.35
7.	4-CN	0.00	-0.57	0.66	2.51	1.82
8.	4-Br	0.00	0.86	0.23	4.82	2.98
9.	4-CF_3	0.00	0.88	0.54	2.20	2.42
10.	4-NO_2	0.00	-0.28	0.78	1.60	1.84
11.	2-CF_3	0.88	0.00	0.54	1.00	1.00
12.	3-NH_2	0.00	0.00	-0.16	3.82	3.69
13.	4-N O	0.00	-1.53	-0.50	2.20	2.53
14.	4-NHC_4H_9	0.00	1.16	-0.51	2.20	4.14
15.	4-$NHCOC_2CH_3$	0.00	-0.37	-0.15	2.51	3.45
16.	4-OC_2H_5	0.00	0.38	-0.24	2.82	3.92
17.	4-$N(C_2H_5)_2$	0.00	1.18	-0.90	5.12	4.82

* taken from reference 34.

Table 4.14: The Antimitotic Activity of Chalcones and Useful Parameters



SNO	R'	R"	R	Ip	Im	Io	D	Fr (R)	log(1/ED) Obsd.*	log(1/ED) Cald. Eqn.4.32
1.	H	$N(CH_3)_2$	3,4,5-$(OCH_3)_3$	1	2	0	0	0.0	4.82	5.22
2.	H	$N(CH_3)_2$	2-OCH_3	0	0	1	0	0.0	3.82	3.55
3.	H	$N(CH_3)_2$	2,4,6-$(OCH_3)_3$	1	0	2	0	0.0	1.90	2.16
4.	H	$N(CH_3)_2$	2,5-$(OCH_3)_2$	0	1	1	0	0.0	5.12	5.08
5.	H	$N(CH_3)_2$	3,4-$(OCH_3)_2$	1	1	0	0	0.0	2.51	3.69
6.	H	$N(CH_3)_2$	2,4-$(OCH_3)_2$	1	0	1	0	0.0	2.20	2.16
7.	H	$N(CH_3)_2$	2,3,4-$(OCH_3)_3$	1	1	1	0	0.0	4.82	3.69
8.	H	$N(CH_3)_2$	3,5-$(OCH_3)_2$,4-OH	0	2	0	0	0.0	2.51	6.56
9.	H	$N(C_2H_5)_2$	2,3,4-$(OCH_3)_3$	1	1	1	1	0.0	3.82	2.86
10.	CH_3	$N(C_2H_5)_2$	2,3,4-$(OCH_3)_3$	1	1	1	1	-0.04	1.60	2.86
11.	CH_3	$N(C_2H_5)_2$	3,4,5-$(OCH_3)_3$	1	2	0	1	-0.04	5.12	4.39
12.	CH_3	$N(C_2H_5)_2$	2,5-$(OCH_3)_2$	0	1	1	1	-0.04	3.82	4.25
13.	Br	$N(CH_3)_2$	2,5-$(OCH_3)_2$	0	1	1	0	0.44	5.12	5.08
14.	Br	$N(CH_3)_2$	2,3,4-$(OCH_3)_3$	1	1	1	0	0.44	3.82	3.69
15.	Br	$N(CH_3)_2$	3,4,5-$(OCH_3)_3$	1	2	0	0	0.44	5.42	5.22
16.	Cl	$N(CH_3)_2$	2,5-$(OCH_3)_2$	0	1	1	0	0.41	4.82	5.08
17.	Cl	$N(CH_3)_2$	2,3,4-$(OCH_3)_3$	1	1	1	0	0.41	4.82	3.69
18.	Cl	$N(CH_3)_2$	3,4,5-$(OCH_3)_3$	1	2	0	0	0.41	2.51	5.17
19.	C_2H_5	$N(CH_3)_2$	2,3,4-$(OCH_3)_3$	1	1	1	0	-0.05	3.82	3.69
20.	CH_3	$N(CH_3)_2$	2,5-$(OCH_3)_2$	0	1	1	0	-0.04	5.42	5.08
21.	CH_3	$N(CH_3)_2$	2,3,4-$(OCH_3)_3$	1	1	1	0	-0.04	2.82	3.69
22.	CH_3	$N(CH_3)_2$	3,4,5-$(OCH_3)_3$	1	2	0	0	-0.04	4.72	5.22

* taken from reference 34.

In this equation data within the parenthesis are 99% confidence intervals.

$$\log(1/ED) = 2.21 - 1.07\ (\pm 0.04)\sigma_4 - 0.69(0.04)\pi_3 + 0.30(\pm 0.01)(\pi_4)^2 \qquad (4.29)$$

$n = 9,\ r = 1.00,\ s = 0.01,\ F_{3,5} = 5203.46\ (12.06)$

This equation exhibits a very very high correlation and suggests that among the substituents at ring A of chalcones (XIII), the dominant effect on the activity would be produced by the electron-releasing substituents at the 4-position, the para position. The occurrence of $(\pi_4)^2$ in the equation with a small positive coefficient indicates that the para - substituents may also affect the activity to some extent by their lipophilic character. However, a comparatively large negative coefficient of π_3 in the equation suggests that the substituents at the 3-position, the meta-position, may produce the steric effects.

For the compounds of Table 4.13, where the substitution varies at ring B, the best correlation obtained was:

$$\log(1/ED) = 3.40 - 1.80(\pm 0.58)\sigma + 0.41(\pm 0.31)\pi_4 - 0.49(\pm 0.27)(\pi_4)^2 - 1.63(\pm 1.28)\pi_B \qquad (4.30)$$

$n = 15,\ r = 0.94,\ s = 0.46,\ F_{1,13} = 19.75\ (9.07)$

In this equation, σ is meant for the substituent at any

position and thus its high negative coefficient indicates that an electron - releasing substituent at any position will highly favour the antimitotic activity of compounds. However, the substituents of 4-position appear to have a small positive effect on the activity by their lipophilic character also, but since the correlation is parabolic in π_4, this effect is limited, and Eqn.4.30 gives an optimum value of π_4 equal to 0.42, suggesting that any para - substituent having a π-value higher than this may be detrimental to the activity. Similarly, a substituent at 2 - position, de facto the ortho-position, would also be detrimental to the activity. However, since there is only one substituent at this position, one can not say what property of the substituent at this position, would negate the activity. in the place of π_2, one might use a dummy parameter as well.

For the chalcone series of Table 4.14, where only one kind of substituent is present at ring A but at different positions and where the ring B is substituted only at the para - position, we became interested first in quantifying the positional effect of a particular kind of substituent, i.e., OCH_3 group at ring A and then studying the effect of dialkylamino group at para - position of ring B. Since the chalcones of Table 4.14 have also substituents (R') at C_α of the propene chain, their effect was also analysed. Since there were not much variations in the substituents at any position in the molecules, we used only some dummy parameters, as defined below, for the QSAR analysis of this

series of compounds.

For the ring A, the three parameters Ip, Im and Io were given a value of 1 each to account for the presence of OCH_3 group at p-, m-, and o- positions, respectively. If both the m-positions or both the o-positions were substituted then the corresponding parameter had a value of 2. For the ring B, a dummy parameter D was used to account for the effect of $N(CH_3)_2$ and $N(C_2H_5)_2$ with a value of 0 and 1, respectively. For the propene chain substituents R_1 which had some variations, we used the fragment constant (Fr) of the lipophilicity.[15] The use of this Fr and all the dummy parameters revealed Eqn. 4.31, which shows that the two parameters Io and Fr are highly insignificant at 95% confidence level. Hence when we dropped them, there was little effect on the significance of the correlation (Eqn. 4.32). Though parameter D also appears to be insignificant at 95% level, its deletion led to a considerable decrease in the significance of the correlation (Eqn. 4.33).

$$\log(1/ED) = 2.89 - 1.31(\pm 0.83)Ip + 1.73((\pm 1.09)Im + 0.38(\pm 1.23)Io - 0.74(\pm 1.00)D + 0.79(\pm 2.06)Fr(R_1) \quad (4.31)$$

$n = 20,\ r = 0.85,\ s = 0.75,\ F_{5,14} = 7.14\ (4.69)$

$$\log(1/ED) = 3.55 - 1.39(\pm 0.79)Ip + 1.53(\pm 0.62)Im - 0.83(\pm 0.89)D \quad (4.32)$$

$n = 20,\ r = 0.83,\ s = 0.74,\ F_{3,16} = 11.86\ (5.29)$

$$\log(1/ED) = 3.49 - 1.40(\pm 0.85)\pi + 1.43(\pm 0.66)I_m \qquad (4.33)$$

$$n = 20,\ r = 0.78,\ s = 0.80,\ F_{2,17} = 13.53\ (6.11)$$

Thus the correlation expressed by Eqn. 4.32 appears to be the best one and can be therefore used to draw some conclusions. It indicates that a methoxy group at the para - positions of ring A would lead to a decrease in the activity, while the same at the meta positions may lead to, almost of an equal order, an increase in the activity. Regarding the effect of a dialkylamino group at the para-position of ring B, it is indicated that a bigger group will reduce the activity. These findings about this series of compounds do not corroborate with those observed for compounds of Tables 4.12 and 4.13, where the electron - releasing substituents at para - positions of both rings A and B were found to enhance the activity. The one reason to this disagreement can be attributed to the substituents R' at C_α of the propene chain, which is a linker chain between the two rings. These substituents may affect in an irregular manner the flexibility of the molecule about the C(O)-Cα bond, so that the orientation of the phenyl rings and of their substituents become unpredictable. For a series of analogues of combretastin (XI), it was observed that the restriction in the free rotation of the two phenyl rings was essential for their antimitotic activity. [11,35] The other reason of this disagreement may be the use of only dummy parameters for the

compounds of Table 4.14. The dummy parameters hardly reflect a reliable SAR analysis, but the present situation provided no better alternative.

In the derivation of Eqn. 4.30 for Table 4.13 and Eqns. 4.31-4.33 for Table-4.14, one or two compounds marked with asterisk were not included as they had exhibited the aberrant behavior, which were hard to explain.

From the above QSAR analysis, it however appears that the binding of chalcones with tubulin requires the planarity of the molecules and that it involves both the phenyl rings and their substituents predominantly in some electronic interaction. And the participation of OCH_3 group of ring A and $NHCOCH_3$ type of group at ring B in hydrogen bonding with the receptor can not be ruled out.

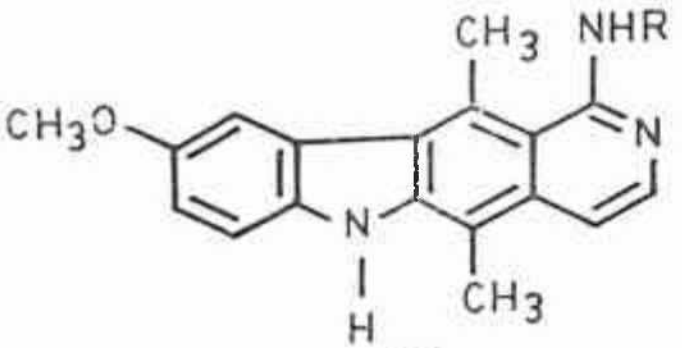
4.6 1[[(Dialkylamino)alkyl]amino]-4-methyl-5H-pyrido[4,3-b]benzo[e]-and-benzo[g] indoles

The polycyclic DNA-intercalating system having antitumor properties showed enhanced biological activity by adjunction of a [(dialkylamino)alkyl]amino side chain. 10-[(3-Diethyl amino)propyl]amino]-6-methyl-5H-pyrido[3',4':4,5]pyrolo [2,3-g] isoquinoline (XIV, BD40) and 1-[[3-(diethylamino)propyl] amino] -9-methoxy-5,11-dimethyl-tH-pyrodo [4,3-b] carbazole (XV,BD84) were found to have high anti-neoplastic activity[36-38] and are currently undergoing clinical trials. Promising results have been obtained in phase I trials with compound XIV.[39]

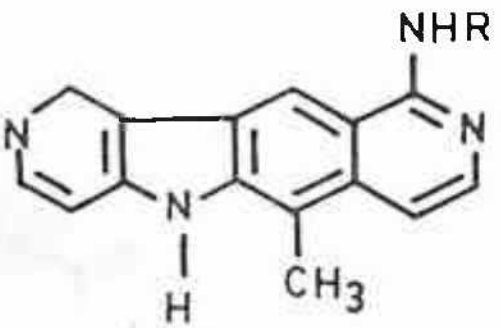
Further studies done with tricyclic series XVI[40,41] and XVII[42] where one aromatic ring was deleted with respect to XIV and XV showed that DNA affinities of XVI and XVII were lower than XIV and XV[43,44] and 4-CH_3 group played a significant role for the activity. Also with R_1 = $(CH_2)_3N(Me)_2$ or $-N(Et)_2$ and R_2 = H/CH_3, R_3 = OH, compounds showed better activity in series XVII than in XVI. So on the basis of these results, new compounds related to series XVII with an additional aromatic ring in the angular position viz 5H-pyrido [4,3-b]benzo[e]- and -benzo[g] indole derivatives corresponding to structures XVIII and XIX were synthesised by Chi Hung Nguyen et al.[45]

In the present work a QSAR study has been carried out for the derivatives of XVIII and XIX to find the future prospects so that better antineoplastic agents belonging to this class may be designed.

The two series studied by Chi Hung Nguyen are listed in Tables 4.15 and 4.16 along with their in vitro activity against P388 cells. The physicochemical parameters that were found correlated with the activity are also included in these Tables. These parameters were taken from the literature.[15] When a multiple regression analysis was performed, Eqn.4.34 emerged for the compounds of Table 4.15 and Eqn. 4.35 for the compounds of Table 4.16.

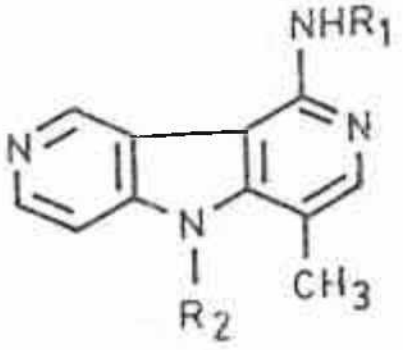


XV

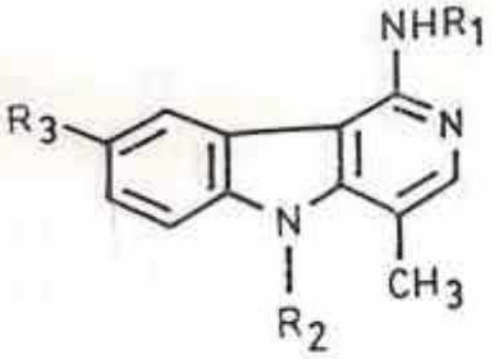


XIV

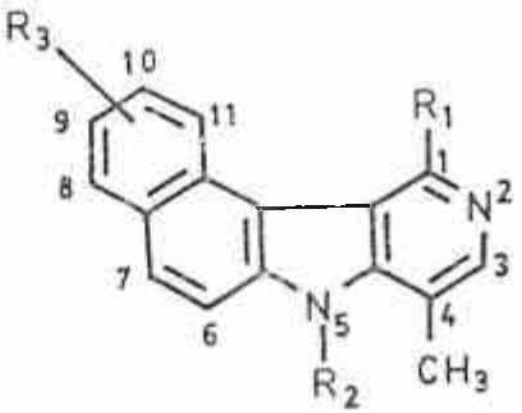
$R = CH_2CH_2N(CH_2CH_3)_2$



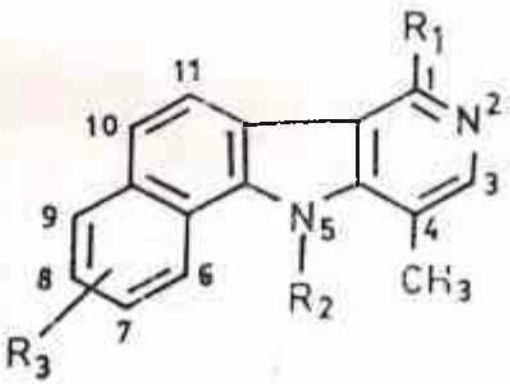
XVI



XVII

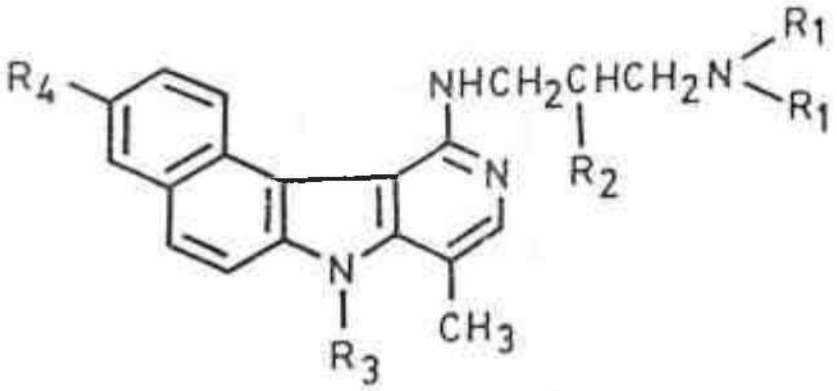


XVIII



XIX

Table 4.15: 1-[[3-(Dialkylamino) propyl] amino]-4-methyl-5H-pyrido[4,3-b]benzo[e]indoles studied by Nguyen, C.H. et al.[45] and physicochemical parameters.



SNo	R_1	R_2	R_3	R_4	MR_4	log(1/ID_{50}), Leukemia	
						Obsd.*	Cald. Eqn.4.34
1.	CH_3	H	H	H	0.103	4.00	4.01
2.	CH_3	H	H	OCH_3	0.787	3.13	3.16
3.	CH_3	H	H	OH	0.285	4.60	4.46
4.	C_2H_5	H	H	H	0.103	3.75	4.01
5.	C_2H_5	H	H	OCH_3	0.787	3.00	3.16
6.	C_2H_5	H	H	OH	0.285	4.30	4.46
7.	CH_3	CH_3	H	OCH_3	0.787	3.26	3.16
8.	CH_3	CH_3	H	OH	0.285	4.00	4.46
9.	CH_3	H	CH_3	H	0.103	4.30	4.01
10.	CH_3	H	CH_3	OCH_3	0.787	3.30	3.16
11.	CH_3	H	CH_3	OH	0.285	4.82	4.46
12.	C_2H_5	H	CH_3	H	0.103	4.00	4.01
13.	C_2H_5	H	CH_3	OCH_3	0.787	3.13	3.16
14.	C_2H_5	H	CH_3	OH	0.285	4.60	4.46

* Taken from reference 45.

SNo	R_1	R_2	R_3	R_4
1.	CH_3	H	H	H
2.	CH_3	H	H	OCH_3
3.	CH_3	H	H	OH
4.	C_2H_5	H	H	H
5.	C_2H_5	H	OCH_3	H
6.	CH_3	CH_3	H	H
7.	CH_3	CH_3	H	OCH_3
8.	CH_3	CH_3	H	OH
9.	C_2H_5	CH_3	H	H
10.	C_2H_5	CH_3	OH	H
11.	C_2H_5	CH_3	H	OCH_3
12.	C_2H_5	CH_3	H	OH

* Taken from reference 45.

MR_3	MR_4	$\log(1/ID_{50})$, Leukemia	
		Obsd.[a]	Cald. Eqn. 4.34
0.103	0.103	3.43	3.36
0.103	0.787	3.82	3.36
0.103	0.285	4.30	4.40
0.103	0.103	3.13	3.36
0.787	0.103	3.30	3.30
0.103	0.103	3.43	3.36
0.103	0.787	3.13	3.36
0.103	0.285	4.60	4.40
0.103	0.103	3.43	3.36
0.285	0.103	4.00	4.00
0.103	0.787	3.13	3.36
0.103	0.285	4.30	4.40

(4.34)

$$\log(1/IC_{50}) = 5.38(\pm 3.21)MR_4 - 7.44(\pm 3.38)(MR_4)^2$$

$$n = 14,\ r = 0.94,\ s = 0.24,\ F_{2,11} = 38.81\ (7.20)$$

$$\log(1/IC_{50}) = 10.21(\pm 4.26)MR_4 - 11.46\ (\pm 4.59)(MR_4)^2 + 6.37(\pm 6.41)MR_3 - 7.25\ (\pm 7.19)(MR_3)^2 + 1.834 \qquad (4.35)$$

$$n = 12,\ r = 0.92,\ s = 0.25,\ F_{4,7} = 9.95\ (7.85)$$

The Eqn. 4.34 shows that there exists a parabolic correlation between the potency and the molar refractivity (MR) of the substituents at the 4th position. This suggests the involvement of 4-position substituents in dispersion interaction. However the parabolic correlation implies that there is an optimum value of MR, $(MR_{opt}) = 0.76$, which suggests that activity will not increase with an increase in MR beyond this value. All other substituents at R_1, R_2 and R_3 did not show any significant effect on the activity.

The Eqn. 4.35 for the compounds of Table 4.16 again exhibits parabolic correlation of the activity with MR_4. In addition, in this series a few derivatives had another substitution in the angular ring as R_3. The MR for these substituents also has a parabolic correlation with the activity. For both MR_4 and MR_3 there is an optimum value as given below:

$MR_{4(opt)} = 0.45$ $\qquad$ $MR_{3(opt)} = 0.44$

Hence the molar refractivity of both the R_3- and R_4-substituents is conducive to the activity only upto their optimum values. Beyond these values, it will show detrimental effect. Also as given by Eqn. 4.35, MR_3 has poor correlation with the activity but it does show an additive effect in presence of R_4-substituents. This shows that R_4-substituent has more significant contribution towards the activity but R_3-substituent also enhances the activity.

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2. Some Acridines Binding to DNA, Quant. Struct.-Act. Relat., communicated.
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4. Antimitotic Chalcones, in preparation.
5. 1[[(Dialkylamino) alkyl] amino]-4-Methyl-5H-pyrido [4,3-b] benzo[a]- and-benzo[c] indoles, in preparation.