CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 ACRIDINECARBOXAMIDE, A CLASS OF ANTICANCER DRUG

A major problem of anticancere drug designing is our insufficient understanding of the nature of DNA-drug binding. In the pursuit of new therapeutic agents, most approaches focus on the "DNA binding features" of drugs and are used for correlating to the anticancer property *[1-7]*. So researchers thoroughly exploit the selectivity of anticancer agents for DNA sequences and the information have been used in the design of new therapeutic agents with improved antitumour properties. It has been well established that the main cellular target of anticancer drugs related to their activity is DNA *[8-14]*. The ideas of using various models to represent real molecular systems have been extensively used in describing their chemical properties. The anticancer drugs used in chemotherapy acquires different modes of binding with DNA, and the binding of these drugs within biological system are too complex to understand their structural details relating to biological activity *[15-17]*. However all types of anticancer drugs have pharmacophore that measures the biological activity of a drug.

The anticancer drug acridine-4-carboxamides (**Figure 1**) have emerged from a drug development program, where the utility of tricyclic chromophore and the cationic side chain is found to be useful in enhancing anticancer property. The nature and the positioning of carboxamide side chain in the chromophore are critical, and attachment of side chain at C4 position in tricyclic ring is found to be neccessary for acquiring high potency of this class of drug.

1.2 DESCRIPTION OF DNA-DRUG BINDING

DNA binding ability of these drugs can be analysed from two different ways -

(a) intercalation by chromophore and

(b) groove binding by carboxamide side chain.

There are many reports on the structure activity relationship with respect to

chromophore intercalation as well as side chain binding *[17-20]*. In this aspect derivatives of acridine-4-carboxamides have been synthesized by modifying the chromophore with different substituents. For obtaining new drugs of enhanced anticancer property, electron donating and electron withdrawing groups are inserted in the ring. It has been found that the intercalative ability of drugs and the resultant unwinding of DNA helix depend on the type of substituents used in chromophore. All these compounds results helix unwinding angles in the range of 12° - 20° approximately and most of these drugs intercalate preferentially in between the GC sequences *[20-25]*. Again the nature of the intercalative binding of these drugs may correlate with the variation in cytotoxic potency. The variation in the helix unwinding angle may be related to the strength of DNA binding ability of these drugs. Thus the study on the binding of drug within the sequence of DNA may be useful for giving some information related to their anticancer property.

Alternatively the carboxamide side chain is further restricted only at C4 position in the chromophore, whereas the drugs with side chain at C1 and C2 positions are much less active than the C4 substituted carboxamides. In a sense that the drugs like acridine-4-carboxamides having chromophore peri to heavy atom acquires high anticancer property *[20-30]*.

The necessity of co-planarity of the chromophore ring has been emphasized in most of the intercalating drugs *[10-25]*. This flat ring molecular fragment preferably intercalates between the sequences whereas the side chain binds in the grooves of DNA. However, from the existing crystallographic data it is not possible to determine the side chain binding ability or chromophore intercalating ability separately *[25-37]*. It is necessary to assess whether the contribution towards DNA binding is through chromophore intercalation or side chain binding. It has been shown that modification of chromophore by various substituents results broad spectrum of change in anticancer property of these drugs *[5-17]*. The only important observation found in most of the crystallography studies is the change in the unwinding angles after intercalation by different types of modified chromophore *[5-20]*.

1.3 AZAACRIDINECARBOXAMIDE, A NEW ANTICANCER AGENT

Subsequently Denny and coworkers have reported another class of potent anticancer drugs, azaacridine-4-carboxamides (**Figure 2**). In most of these drugs also, the placement

of carboxamide side chain has been reported to be crucial, and the anticancer property of these drugs are quite significantly different from other acridine-4-carboxamides *[1-10]*. The structure activity relationship of this class of anticancer drug has been given much importance in the exploration of new drug depending on chromophore type and position of carboxamide side chain. The only reason for selecting carboxamide as side chain in chromophore is to increase the DNA binding ability of this drug, and consequently the information are used for relating with the antitumour activity.

The aza analogues of acridine-4-carboxamide is formed by inserting additional N in the acridine ring (ring A), and the other derivatives have different types of side chain at the different positions in the ring, particularly C4 and C9 positions are found to be important for positioning any type of substituents *[5-6]*. Hence the azaacridine-4-carboxamides, formed by changing the additional N atom to different positions in the ring is shown in **Figure-2**, may be taken up for study.

Indeed, side chain modification also shows quite distinct change in anticancer property. However, the carboxamide side chain appears to bind efficiently within the groove of DNA, and in accordance with the literature, modification of this side chain is less important than modifying chromophore by different substituents. Hence, emphasis has been given to design good intercalator by modifying in different ways. The electron withdrawing as well as electron donating groups have been used as substituents in various position of ring in order to change the electronic properties in the chromophore for enhancing intercalation as well as sequence specificity within the GC rich region of DNA.

1.4 IMPORTANCE OF DRUG pK_a

The diffusion of drug inside the cell depends on physicochemical properties of drug molecule. Generally a drug molecule containing groups such as amino, carbonyl or aromatic groups etc are absorbed in their unionized forms. Again the ionized form cannot pass through hydrophobic region of the cell membrane. So as per the pH partition hypothesis in pharmacology, the knowledge of pK_a is necessary in monitoring the absorption of drug and the degree of ionization of drug molecule inside the biological system *[38-40]*. Suppose, if the drug is highly acidic and it will be absorbed in the stomach (pH=2), but it ionizes on reaching the blood stream and cannot pass back again. Thus pH and ionization ability are two important physical properties taken as parameters in drug designing and also the

knowledge of Ionization energy is required for comparing the electronic properties of drugs. The biological activity of some molecule depends on their pK_a value.

The relationship between ionization ability and biological activity of this class of drugs has been known from different studies on acridine-4-carboxamides. Here the drug may interact with receptor in the ionic form. Thus ionization ability of acridine-4-carboxamides may be considered as another physiochemical property used in designing new drugs. In this case the ionization from the local sites of drug has been taken into consideration before studying any sort of basic site in the drug molecule. The ionization ability of drug is again related to drug solubility as well as pK_a value. Generally the drugs are weak acid or base and the dependence of drugs affinity for DNA on pK_a value has been noticed ***[30-32]***. The relative change of anticancer properties of this class of drugs having different pK_a has been evidenced. As we know that the penetration through cell membrane is associated with the unionized form of drug. Moreover partition between organic and aqueous solution clearly depend on the ionization ability of drug. Hence the use of distribution coefficient (log D) has been used to determine the biological activity. In addition to this when a molecule is equilibrated between organic and aqueous medium, pH of the aqueous solution also determines the partition coefficient. In this case pH of aqueous solution and pK_a of drug are quite related in stabilizing one particular molecular form (unionized) ***[30-38]***. The DNA binding ability of this molecular form may determine the biological property. Hence pK_a of drug must also be taken as another important factor for determining biological properties. It is extremely important to understand the electronic factor related to variation of pK_a and the structure of molecule. Moreover intramolecular hydrogen bonding, steric effect and geometry of molecules are necessarily considered in predicting the value of pK_a.

Among the acridine-4-carboxamides, the 9-aminoacridine-4-carboxamide exists as dication in contrast to monocation form of most acridine. The pK_a of these drugs vary quite distinctly where C1, C2 and C3 substituted carboxamides acquire pK_a values different from 9-aminoacridine-4-carboxamide ***[5-12]***. There is additional H-bond between the ring nitrogen and carboxamide side chain. It has been suggested that free base binds differently from the cationic form. Hence it has been prompted that in the design of most effective acridine 4 carboxamide it is necessary to take proper consideration of both intercalating ability as well as pK_a.

Prediction of pK_a value is necessary in determining *in vivo* biological activity of a drug, particularly with very strong acid and very weak bases. Theoretical approach of predicting pK_a is a topic of interest ***[39-45]***. Here experimentally obtained pK_a always give some error and understanding of exact pK_a is necessary while examining biologically active molecules. On the other hand, the theoretically computed pK_a also have some deviation from experimentally found value, but the experimental values may not be the absolute pK_a.

There are many studies of predicting pK_a such as thermodynamic parameters, hybrid QM/MM method and semi-empirical level of theory ***[32-36]***. Occasionally the values obtained from these methods are exactly equal to the experimental values, while large deviation from the experimental values is also found in most cases. In another case, *ab initio* method of calculation with continuum model for studying solvent effect is sometimes used for computing pK_a. However all these methods, including the *ab initio* MP2 calculation with cluster continuum model, results some discrepancy ***[34-36]***.

1.5 OBJECTIVE OF STUDY

The substituents like -CO, -Cl and -NH2 etc have been used in many drug and the -CO substituted compounds are less potent than other carboxamides. However the nature of intercalation of these drugs has been studied and the helix unwinding angles are determined to assess the intercalating ability. The -CO substituted compounds acquire significant unwinding angles and intercalates within GC sequences. Figure 2 shows the structure of drugs where the position of additional nitrogen atom in the ring has changed. Again the dependence of carboxamide chain position in chromophore on anticancer property rather than the type of side chain have been evidenced. The structure activity relationship clearly indicates high potency in drugs with carboxamide side chain at C4 position. The anticancer properties of C4 substituted carboxamide are much better than those of C1 and C2 substituted carboxamides. Hence the position of side chain at C4 position remains intact in studying the aza analogues of carboxamide. However the electronic effect on azaacridine chromophore by -NH2, -Cl and -C=O may also be required for further study as the compounds was reported by Denny. We aim to explore the stacking ability of these drugs for understanding sequence specific interaction of azaacridinecarboxamides. Again intercalation mode of binding by chromophore having substituent at C9 may be looked for comparison, since this compound acquires significantly different biological property.

Another important property of drugs is the distributive property when used in physiological environment. Hence the drug molecule should be efficient to pass through the specialized cell membrane. Rather than acting as just a good intercalator, in order to pass through the cell membrane i.e. to take the molecule towards the real chemical environment, a detailed knowledge of structure and physiochemical behavior of the drug (pK_a) under physiological environment is essential.

Generally, the minimum local ionization energies are used for computing pK_a of several compounds. Similarly, choosing the most basic site for predicting absolute pK_a may be meaningful. Hence alternative method of estimating pK_a from quantum mechanical method with proper consideration of zero point energy as well as solvation energies may be considered.

As we know that the basic feature that control the pK_a of any molecule depends how the dissociation of acid (strong or weak) explicitly taken in pK_a calculation. In such cases there may be multiple acidic sites. In addition the solvent dielectric field also contributes the dissociation of proton from the acidic site. Therefore the basic idea of predicting deprotonation energy from the most acidic site, and using the energy value for computing pK_a quantity may be logical. In fact the experimentally determined pK_a of a molecule depends on the medium taken for the experiment. Here the ionization of proton from the local site that controls the global acid-base equilibrium in conjugation with solvent dielectric field that can assist ionization may not be distinctly known. Hence the absolute value of pK_a may be computed from the local dissociation energy of proton. Hence, we also aim to calculate pK_a of this drug by using quantum mechanical method from the most basic site of these drugs in addition to studing stacking of drugs with base pair of DNA.

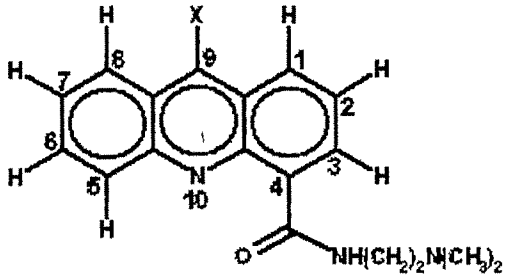

Figure 1.1- General Structure of drug Acridine-4-carboxamide.

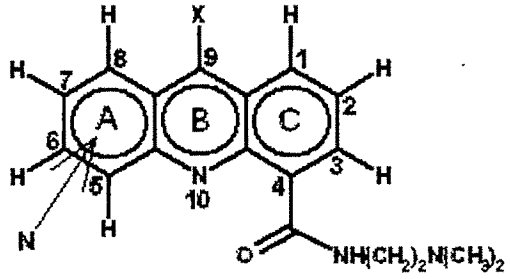

Figure 1.2- General Structure of drug Azaacridine-4-carboxamide. N of the ring A is attached to location 5, 6, 7 or 8 for forming different aza derivatives. X= -CO, $-NH_2$, -Cl.

Reference

1. Hay M P, Wilson W R, Moselen J W, Palmer H D, Denny W A, *J Med Chem*, **1994**, 37, 381.
2. Hoy C A, Thompson L H, Mooney C L, Salazar E P, *Cancer research*, **1985**, 45, 1737.
3. Konopa J, Koldej K, Pawlak J W, *Chemico-Biological Interactions*, **1976**, 13, 99.
4. Lee H H, Denny W A, *J Chem Soc Perkins Transactions*, **1999**, 1, 2755.
5. Lee H H, Wilson W R, Ferry D M, Van Zigl P, Pullen S M, Denny W A, *J Med Chem*, **1996**, 39, 2508.
6. Rauth A M, Melo T, Mistra V, *Int J of Radiation Oncology, Biology and Physics*, **1998**, 42, 755.
7. Siim B G, Atwell G J, Wilson W R, *British J of Cancer*, **1994**, 70, 596.
8. Wilson W R, Van Zigl P, Denny W A, *Int J of Radiation Oncology, Biology and Physics*, **1992**, 22, 693.
9. Wilson W R, Thompson L H, Anderson R F, Denny W A, *J Med Chem*, **1989**, 32, 31.
10. Chelplak P, Rao S N, Grootenhuis P D J, and Kollman P, *Biopolymers*, **1990**, 29, 717.
11. Gresh N, Claverie P and Pullman A, *Theoritica Chimica Acta*, **1984**, 66, 1.
12. Gresh N, Pullman A, and Claverie P, *Theoritica Chimica Acta*, **1985**, 67, 11.
13. Levery R, Olson W, Sunderlingam M, Sarma M H, and Sarma R H, *In DNA Binding and Curvature*, Adenine Press, New York, **1988a**, P-191.
14. Philips D R, Cullinane C, Treest H A, White R J, *in molecular basis of Specificity in Nucleic Acid-Drug Interaction*, Pullman B, and Jrotner J, Kluwer Academic Press, Dardrecht / Holland, **1990**, 6, 137.
15. Feigon J, Denny W A, Leupin W, Kearns D R, *J Med Chem*, **1984**, 27, 450.
16. Brana M F, Sanz A M, Castellano J M, Roldan M, Roldan C, *Eur J Med Chem*, **1981**, 16, 207.
17. Baguley B C, Denny W A, Atwell G J, Cain B F, *J Med Chem*, **1981**, 24, 170.
18. Wright R D McR, Wakelin L P G, Fields A, Acheson R M, Waring M J, *Biochemistryl*, **1980**, 19, 5825.
19. Finlay G F, Baguley B C, *J Cancer Clin Oncol*, **1984**, 20, 947.
20. Atwell G J, Cain B F, Baguley B C, Finlay G J, Denny W A, *J Med Chem*, **1984**, 27, 1481.
21. Rewcastle G W, Denny W A, *Synthesis*, **1985**, 217.
22. Denny W A, Atwell G J, Rewcastle G W, Baguley B C, *J Med Chem*, **1987**, 30, 658-663.
23. Atwell G J, Rewcastle G W, Baguley B C, Denny W A, *J Med Chem*, **1987**, 30, 664-669.
24. Finlay G J, Marshall E S, Matthews J H L, Paull K D, Baguley B C, *Cancer Chemother Pharmacol*, **1993**, 31, 404-406.
25. Haldane A, Finlay G J, Gavin J B, Baguley B C, *Cancer Chemother Pharmacol*, **1992**, 29, 475-479.
26. Palmer B D, Rewcastle G W, Baguley B C, Denny W A, *J Med Chem*, **1988**, 31, 707-712.
27. Wakelin L P G, Atwell G J, Rewcastle G W, Denny W A, *J Med Chem*, **1987**, 30, 855-862.

28. Rewcastle G W, Denny W A, Baguley B C, *J Med Chem*, **1987**, 30, 843-851.
29. Atwell G J, Rewcastle G W, Banguley B C, Denny W A, *J Med Chem*, **1987**, 30, 664-669.
30. Finlay G J, Marshall E S, Matthews J H L, Paull K D, Banguley B C, *Cancer Chemother Pharmacol*, **1993**, 31, 401-406.
31. Haldane A, Finlay G J, Gavin J B, Banguley B C, *Cancer Chemother Pharmacol*, **1992**, 29, 475-479.
32. Palmer B D, Rewcastle G W, Banguley B C, Denny W A, *J Med Chem*, **1988**, 31, 702-712.
33. Rewcastle G W, Denny W A, Banguley B C, *J Med Chem*, **1987**, 30, 843-851.
34. Chen Q, Deady L W, J Heterocycle Chem, **1992**, 29, 1179-1201.
35. Denny W A, Atwell G J, Cain B F, Leo A, Panthenanekal A, Hanoch C, *J Med Chem*, **1982**, 25, 276-316.
36. Marshall E S, Finlay G J, Matthers J H L, Shaw J H F, Nixon J, Baguley B C, *J Natt Cancer Int*, **1992**, 84, 340-345.
37(a) Lin S, Struve W S, *J Phys Chem*, **1991**, 95, 2251.
(b) Charles A Smith, Hai-Chou Chang, Waller S Struve, Graham J Atwell and William A. Denny, *J Phys Chem*, **1995**, 99, 8929-8935.
38(a) Silva C O, Silva E C da, Nascimento M A C; *J. Phys. Chem.* A **2000** 104, 2402-2409
(b) Lopaz X, Schaefer M, Dejaegere A, Karplus M; J, Am. Chem. Soc., 124, 18, **2002**, 5011
(c) Pliego J R Jr., Riveros J M; *J. Phys. Chem.* A **2002**, 106, 7434-7439.
39. Li H, Hains A W, Everts J E, Rovertson A D, Jensen J H; *J. Phys. Chem.*, B **2002**, 106, 3486-3494.
40. Jang Y H, Sowers L C, Cagin T, Goddard W A, III, *J. Phys. Chem.*, A **2001**, 105, 274.
41. Pliego J R Jr., Riveros J M, *J. Phys. Chem.*, A **2001**, 105, 7241.
42. Barone V, Cossi M, Tomasi J, *J. Phys. Chem.*, **1997**, 107, 3210.
43. Pliego J R Jr., Riveros J M, *J. Phys. Chem.*, A **2000**, 112, 4045.
44. Pitarch J, Ruiz-Lopez M F, Silla E, Pascual Ahuir J L, Tunon, *J. Am. Chem. Soc.*, **1998**, 120, 2146.
45. Foresman J B, Keith T A, Wiberg K B, Snoonian J, Frisch M J; *J. Phys. Chem.*, **1996**, 100, 16098.