CHAPTER 8

PREDICTION OF pK_a FROM BASICITY OF ATOMIC SITES OF DRUGS

SUMMARY

The pK_a values of various acridine carboxamides have been evaluated to understand the acid-base behaviors of these drugs. The pK_a values are determined from the gas phase proton affinities with consideration from zero point energies and solvation energies. The *local* basic sites were taken for calculating pK_a. The pK_a values of these drugs are very close and approximately range from 8-9. There are some differences between the pK_a values of nitrogen atoms present in chromophore and carboxamide side chain.

8.1 INTRODUCTION

The hydrogen bonds and the role of proton in biological system are believed to be the physical ground of numerous enzymatic reactions as well as transport phenomena through membranes ***[1-4]***. Obviously, knowledge of such physical property is indirectly used in drug designing, because the exact description of physio-chemical behavior of drugs is likely to influence the efficiency of cell penetration occurred within biological system.

A large number of acridine-4-carboxamides with increased DNA binding ability have been reported and subsequently the relationship between the binding ability and anticancer properties are examined so that the information may be used for designing new drugs with more biological property. However, all these drugs have varying range of pK_a values ***[5-11]***. The chromophore itself forms charged dication and also acquires low lipophilicity. Drugs having low pK_a values act as uncharged chromophore. The formation of dication in these drugs occurs only at low pH, (in strong acidic solution). Moreover uncharged species are more lipophilic, and the anticancer property has been observed to be dependent on the lipophilic behavior and on pK_a values ***[9-11]***. There are evidences that in some cases alteration of the cationic side chain of the drugs lead to reduced activity ***[7-9]***. Although many studies indicate the relation between the strength of drug-DNA binding and their biological properties ***[11-15]***, some DNA binding drugs do not acquire sufficient biological properties. However one of the distinguishable observations found in these drugs is the

variation of pK_a values. In such situation monitoring physio-chemical properties rather than monitoring the DNA binding ability of drugs may be necessary. As such the DNA binding ability of a drug may be dominated by the chromophores intercalation, where the charge acceptor capacity of chromophores play major role. The charge acceptor capacity is related to the type of substituents and the position and nature of side chain in chromophore. Various studies on the DNA binding model of acridine-4-carboxamide shows that strong binding of drug within DNA alone do not ensure *in vivo* anticancer properties ***[11-13]***. In other words, for this class of drugs, the DNA binding ability is not the unique property. Among the acridine-4-carboxamides, the drug with carboxamides side chain at C4 in chromophore shows efficient *in vivo* potency, whereas the carboxamides with side chain at C1, C2 and C3 positions acquire significantly low potency ***[7-10]***. The existence of unusually stable DNA-drug complex is found due to the presence of intramolecular hydrogen bond between the chromophore nitrogen and carboxamide side chain that is observed only in the carboxamide having side chain at C4 position. If the charge accepting ability of chromophore is very important, then the pH and the pK_a of drugs appear to be critical for these acridine-4-carboxamides.

Such behavior is likely to be more prominent in the azaacridine-4-carboxamides because of the presence of additional basic sites in the ring. The fact that the presence of more basic sites generally makes the drug to be strongly influenced by the Ions and protons present in the environment. Here, we aim to determine the basicities of these molecules, and thereby to predict the pK_a values of these drugs. The prediction of absolute pK_a is a topic of recent interest ***[16]***. Like other methods used in determining pK_a, such as thermodynamics parameters, we attempt to use *ab initio* method in computing absolute pK_a values of drugs, so that the relative change in pK_a values with respect to DNA binding ability of drugs can be analysed. Understanding the pK_a of a compound, that can be characterized as a Bronsted acid, is very important for gaining more details of reaction in solution. Moreover many proton transfer reactions occur in biological systems generally depend on the intracellular and extra cellular pK_a values. Indeed determination of absolute pK_a value for the dissociation of ion is necessary in many reactions. Thus most of the related phenomena involved in the reaction system can be obtained from the acid-base equilibrium structures.

8.2 THEORY

The acidity or basicity of a molecule is defined by Bronsted theory based on the ability of generating H^+. Similarly, Lewis's concept of explaining acidity or basicity depends on the ionization ability of atoms or molecules. In such cases, K_a is the equilibrium constant for the following reaction,

$$(AZ)H(aq) + H_2O \longrightarrow H_3O^+ + AZ^-(aq) \quad \text{.....(1)}$$

$$(AZ)H \longrightarrow AZ^-(aq) + H^+(aq) \quad \text{.....(2)}$$

Then the deprotonation of H^+ from the active site of the base may be used for determining pK_a. The experimental pK_a values are not the absolute value, and for complex system it is difficult to determine exact value. Sometimes the pK_a of a molecule depends on specific site of ion dissociation and such basic sites may be multiple in some molecules. Here the calculation of pK_a values consist of two steps,

(1) A thorough analysis of all the active sites of protonation by H^+ in gas phase protonation is to be performed, and the specific site or ionizable center in the molecule to be found. Knowledge of specific site is important than determining the global ionization potential of the molecule. The most basic site of molecule may be determined from the computed proton affinities (PA) for different sites. The additional contributions from the solvent environment as well as zero point vibrational energies are considered.

$$E_m = E + ZPE + S,$$

where E_m , ZPE, S are the energies of the reaction-1 (above), zero point energies and solvation energies of drug at the point of dissociation of the proton.

(2) In the second step we employ the mechanism of deprotonation from the most active site (most basic site), that is the site with highest PA value. The magnitude of minimum dissociation energy of H+ from this site was determined. The energy of deprotonation from this site and corresponding zero point energy and solvation energies (absolute value) were computed.

$$E_{min} = (E(AZ)H^+ - EAZ) + ZPE_{min} + S_{min}$$

$$pK_a = -\text{Log } K_a = E_{min} / 2.303 \cdot RT$$

The pK_a values are correlated with the ability of dissociating of H^+, herein easily ionizable acid acquires low pK_a.

For computing solvation energies, Self Consistent Reaction Field method has been used. Complete geometry optimizations were carried out for both the acid and its conjugate base *[17]*.

8.3 RESULTS AND DISCUSSION

8.3.1 SITES OF PROTONATION

There are four major sites for protonation in 9-oxoazaacridone-4-carboxamide (AZO) and 9-chloroazaacridine-4-carboxamide (AZCl). They are N10, N16, N19 and Nx (x = 5, 6, 7 and 8 depending on the position of Nx) (**Figure 8.1a-d** and **8.2a-d**). Of these N10 and Nx are the basic sites in the chromophore, and N16 and N19 are additional basic sites located in the side chain. Similarly, the protonation sites in 9-aminoazaacridine-4-carboxamide (AZN) are N10, N17, N20,-NH_2 and Nx (**Figure 8.3a-d**). Of these N10 and Nx are the basic sites in the chromophore, and N17 and N20 lie in the side chain. The ionization of proton from these sites is used to determine the overall pK_a values of these molecules. The proton affinities of these sites are calculated, and the site having maximum PA is found for all the drugs (**Table 8.1, 8.2** and **8.3**). In the AZOs and AZCls, the N19 at the side chain is the most basic site. However the differences in the PA values of the atomic sites located in chromophore and side chains are very significant (**Table 8.1, 8.2** and **8.3**). In this case pK_a value of this drug is measured by the ionizability of proton from the sites located in chromophore and side chain. Again in case of all five atomic sites of 9-aminoazaacridine-4-carboxamides (AZN), N20 acquires highest basicity than the other sites. As we have seen in AZN, the N20 nitrogen in the side chain is highly basic but the PA value is not much different from that of N10 site of chromophore where as for the acridine analogues 9-chloroazaacridine-4-carboxamides (AZCl), the basicities of N19 is much more than chromophore nitrogen (**Table 8.2** and **8.3**). If we consider only the chromophore of these drugs, the differences in the basicities of N10 and Nx in the ring are distinct, and the Nx is found to be less basic than N10 in AZN and AZCl except in AZO where N10 is more basic. Earlier investigation shows that the electron withdrawing and the electron donating groups

like -Cl, $-NH_2$ and $-OCH_3$ influences the basicities of chromophore nitrogen of acridine-4-carboxamides. Similarly in substituted azaacridine-4-carboxamide there observed change in proton effinities of basic sites in chromophore. On this ground it is expected that by changing substituents in chromophore of this drug it is possible to adjust pK_a values of these classes of carboxamides.

8.3.2 GEOMETRIES OF PROTONATED DRUGS

While computing the basicities of these molecules, there observed contrasting geometries of the protonated molecules compared to unprotonated species (**Figure 8.4a-l**). It is found that in the protonated structure the formation of intramolecular hydrogen bond between H30 and N10, and another hydrogen bond between O28 (in carboxamide) and proton is indicated (**Figure 8.4a-d**). Unlike acridine-4-carboxamide, in the unprotonated azaacridine the oxygen atom of the carboxamide side chain is strictly planar. However the geometry of protonated azaacridine-4-carboxamide is different from free molecule. Structural changes are shown in **Figure 8.4a-d**, and the H-bond lengths are shown in **Table 8.4**. Similar observation is also noticed in the protonated AZOs (at position N19), where H-bond formation is in between H25 to O29 and O29 to proton (**Figure 8.4e-h** and **Table 8.5**). Similarly the intramolecular H-bond persists in 9-aminoazaacridine-4-carboxamide (**Figure 8.4i-l** and **Table 8.6**). As evident from the above results, the substituent at position 9 as well as the position of Nx affects the intramolecular hydrogen bond formation in these molecules. It is worth explaining the ionization of H+ from the ring protonated nitrogen. In this case the proton is well embedded between the ring N and O of carboxamide side chain at equilibrium position. In this case, the dissociation of proton will not be easy under high pH, while the dissociation of other protons attached to side chain, and at N6 or N7 or N8 of ring may easily occur at high pH. Hence it is necessary to examine the reasonable pK_a values for these sites. We have computed the pK_a values from the dissociation energies of H+ of the protonated species of these drugs.

8.3.3 ESTIMATION OF pK_a

The effective sites for proton binding are considered for computing the pK_a where the ionization of H+ from these sites may give some idea of drug's pK_a value. The pK_a of these sites are given in **Tables 8.1**, **8.2** and **8.3**. The pK_a of N19 in 9-oxoaza(7)acridone-4-carboxamide (AZO7) is found to be higher than the pK_a values of other sites. This may be

because of the electron withdrawing nature of -CO group attached at position 9 in the ring. But the proton is well embedded in between oxygen and nitrogen, hence the dissociation of proton from this region may result marked increase in the pK_a value, and the intramolecular H-bond may be considered as another factor for the changes in pK_a values of different drugs.

Similarly, in order to estimate absolute pK_a values of 9-aminoazaacridine-4-carboxamides (AZNs), the various sites for protonation are found, the PA values are given in **Table 8.3**. Here N20 nitrogen is found to be most basic site in this molecule. The variation of proton affinities of ring nitrogen Nx and N10 is not much. Here, the difference between the proton affinities of 9-aminoazaacridine-4-carboxamide and 9-chloroazaacridine-4-carboxamide is quite significant. Hence the electronic properties of substituents at position 9 may alter pK_a values of these drugs. Here, there observed differences in the pK_a of 9-aminoazaacridine-4-carboxamide and 9-chloroazaacridine-4-carboxamides (**Table 8.2** and **8.3**). Not only the variations in the geometries of these drugs compared to unprotonated counter parts, the pK_a values of these drugs differ (**Figures 8.1a-d, 8.2a-d, 8.3a-d** and **8.4a-l**). In that case the ability of these drugs for penetrating inside the cell membrane, which normally depend on the pK_a values, can be assessed for use in physiological environment. Herein, either the protonated form or the unprotonated form might target DNA for intercalating between sequences. The intercalating ability of the protonated and unprotonated form of drug may determine the efficiency of DNA interaction. As per our findings, both protonated form and unprotonated forms might intercalate easily due to the planarity of chromophore whereas the conformation of the side chain in unprotonated form differ from protonated form. Thereafter, the information on the geometries, pK_a values and intercalating abilities might be useful for analyzing the optimal condition essential for designing a class of this anticancer drug.

8.3.4 COMPLEMENTARITY OF pK_a VALUES OF DRUGS WITH PHYSIOLOGICAL ENVIRONMENT

Besides the DNA binding ability of drugs, it is necessary to know the transportability of drug towards the binding site (DNA) sequences, herein multiple association of drug molecule with protons or H2O molecules present in biological environment may occur. The pK_a values of these drugs should be approximately 7.4, so that undissociated drug

could be transferred towards the receptor site. However in most cases such basic sites are also target for other H bonding systems in the environment, and if the pK_a of drug is very less, then these sites may interact with other bio-molecules rather than interacting with DNA. The oxidation-reduction reactions and formation of hydrogen bonds normally occur in biological environment. Hence, the primary importance should be given for adjusting the pK_a values of drugs in addition to enhancing DNA binding ability. In this case the pK_a values of most azaacridine-4-carboxamide significantly differ from physiological pH. Comparison of pK_a values of these drugs are shown in **Table 8.1, 8.2** and **8.3**, where the pK_a values of 9-oxoaza(7)acridine-4-carboxamide is found to be more than those of other drugs

8.4 CONCLUSION

The pK_a values of specific atomic sites are clearly indicated, and the predicted pK_a is higher than physiological pH. Thus the computed pK_a values of these drugs may reflect some idea of physio chemical behavior of this class of drug.

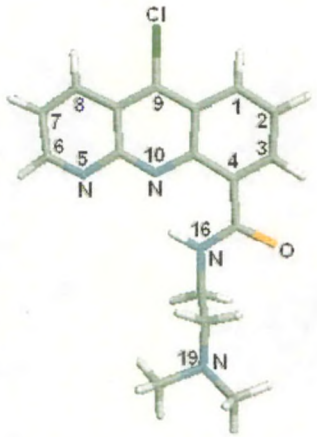

Figure 8.1a – Unprotonated AZCL5

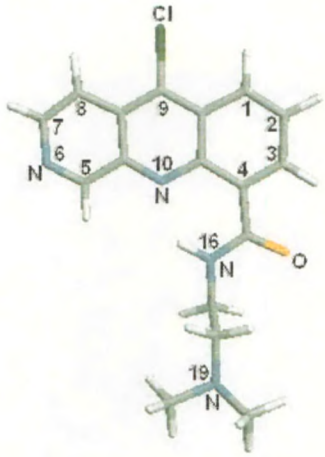

Figure 8.1b – Unprotonated AZCL6

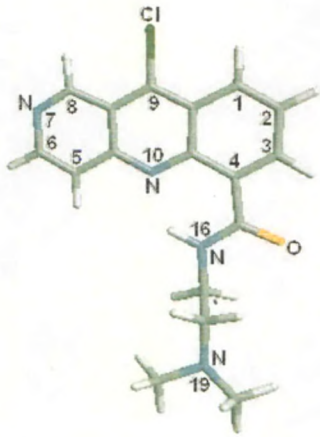

Figure 8.1c – Unprotonated AZCL7

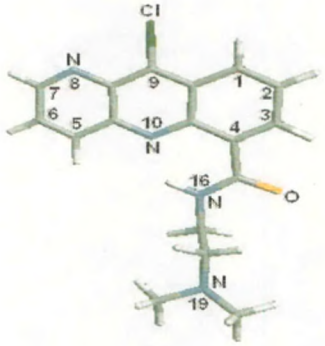

Figure 8.1d – Unprotonated AZCL8

Figure 8.1a-d- Structure of Unprotonated AZCl and location of probable protonation site.

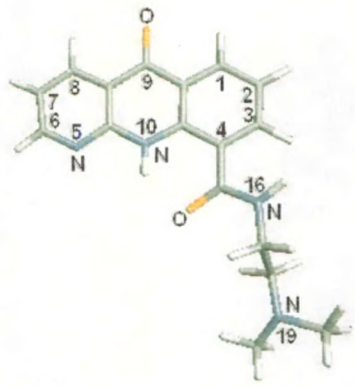

Figure 8.2a – Unprotonated AZO5

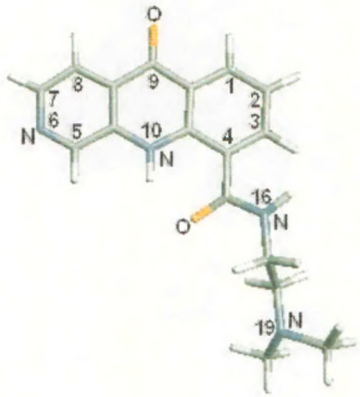

Figure 8.2b – Unprotonated AZO6

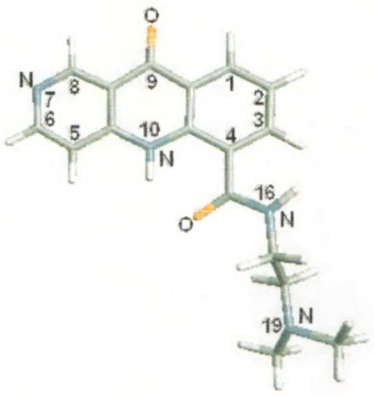

Figure 8.2c – Unprotonated AZO7

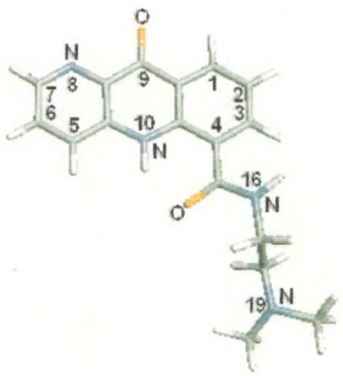

Figure 8.2d – Unprotonated AZO8

Figure 8.2a-d- Structure of Unprotonated AZO and location of probable protonation site.

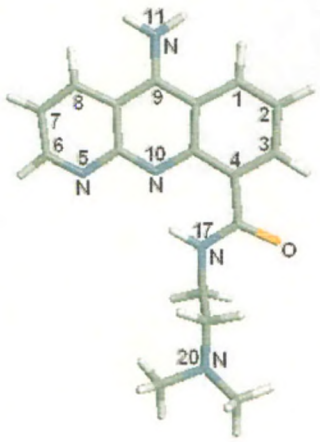

Figure 8.3a – Unprotonated AZN5

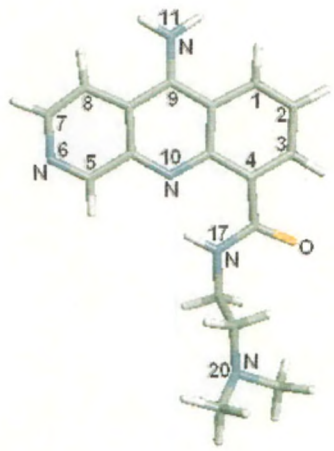

Figure 8.3b – Unprotonated AZN6

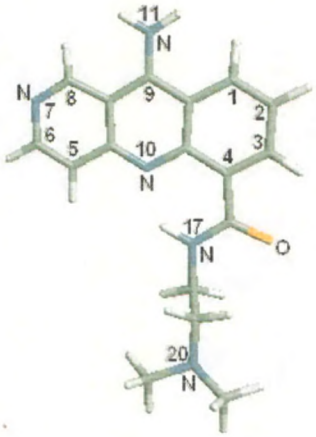

Figure 8.3c – Unprotonated AZN7

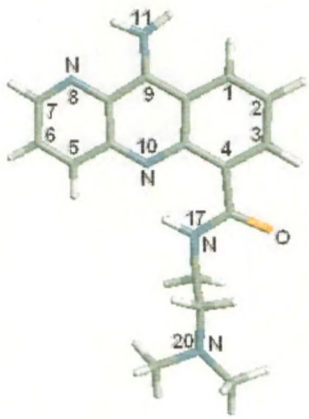

Figure 8.3d – Unprotonated AZN8

Figure 8.3a-d- Structure of Unprotonated AZN and location of probable protonation site.

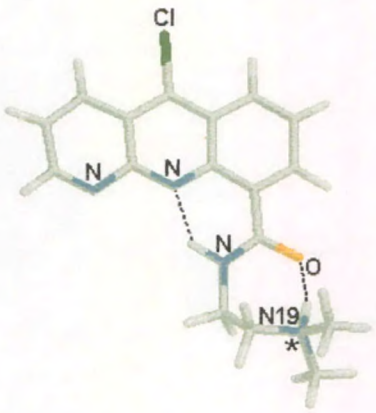

Figure 8.4a – Optimised AZCL5-19+

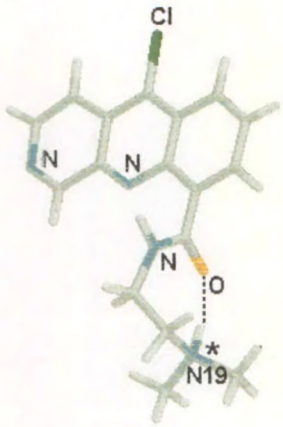

Figure 8.4b – Optimised AZCL6-19+

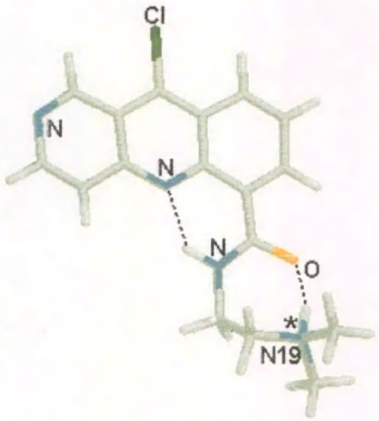

Figure 8.4c – Optimised AZCL7-19+

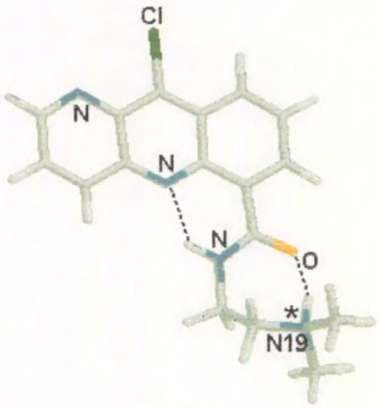

Figure 8.4d – Optimised AZCL8-19+

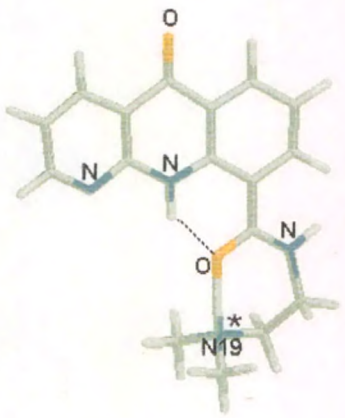

Figure 8.4e – Optimised AZO5-19+

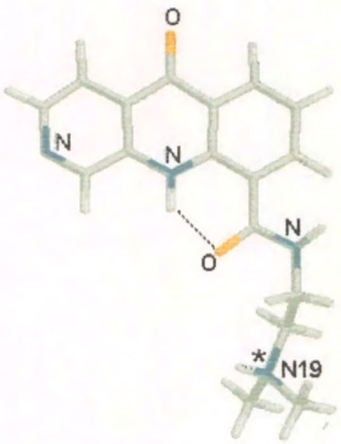

Figure 8.4f – Optimised AZO6-19+

Figure 8.4g – Optimised AZO7-19+

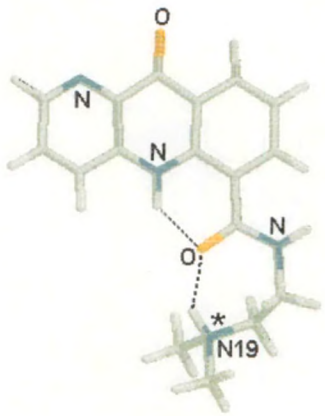

Figure 8.4h – Optimised AZO8-19+

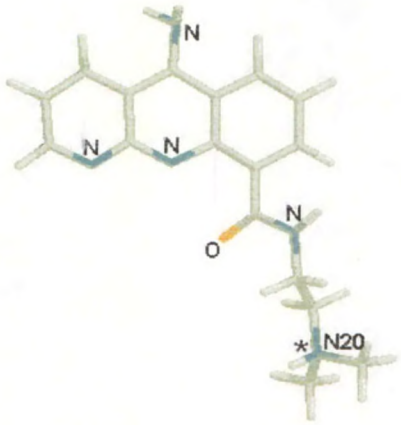

Figure 8.4i – Optimised AZN5-20+

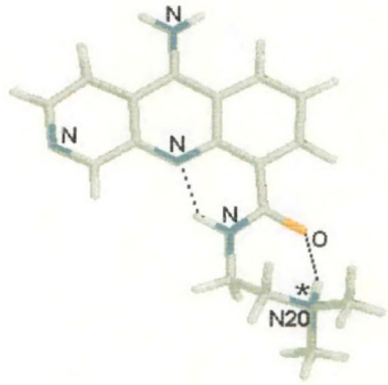

Figure 8.4j – Optimised AZN6-20+

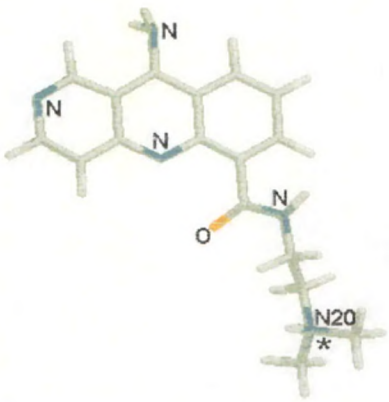

Figure 8.4k – Optimised AZN7-20+

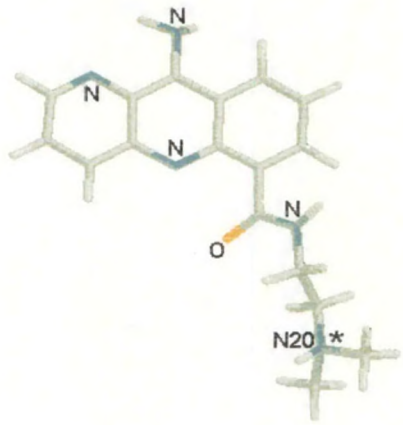

Figure 8.4l– Optimised AZN8-20+

Figure 8.4a-l- Structure of protonated AZO, AZN and AZCl (protonated at the most basic site.)

Table 8.1- Computed proton affinities (PA) and corresponding pK_a's for the most basic sites of 9-oxoazaacridone-4-carboxamide (AZO).

Drug molecules	Protonation Site	P. A. (in a. u.)	pK_a
AZO5	N5	0.388615	8.93
	N10	0.354312	
	N16	0.331249	
	N19	**0.410069**	
AZO6	N6	0.388942	9.06
	N10	0.349885	
	N16	0.330303	
	N19	**0.408756**	
AZO7	N7	0.406784	9.17
	N10	0.348216	
	N16	0.328707	
	N19	**0.406984**	
AZO8	N8	0.400033	8.98
	N10	0.346707	
	N16	0.327286	
	N19	**0.403607**	

Table 8.2- Computed proton affinities (PA) and corresponding pK_a's for the most basic site of 9-chloroazaacridine-4-carboxamide (AZCl).

Drug molecules	Protonation Site	P. A. (in a. u.)	pK_a
AZCL5	N5	0.381851	8.81
	N10	0.386261	
	N16	0.382215	
	N19	**0.427487**	
AZCL6	N6	0.374016	8.86
	N10	0.377968	
	N16	0.364756	
	N19	**0.420805**	
AZCL7	N7	0.373220	8.86
	N10	0.379952	
	N16	0.370587	
	N19	**0.420927**	
AZCL8	N8	0.372428	8.91
	N10	0.382768	
	N16	0.371969	
	N19	**0.422124**	

Table 8.3- Computed proton affinities (PA) and corresponding pK_a's for most basic site of 9-aminoazaacridine-4-carboxamide (AZN).

Drug molecules	Protonation Site	P. A. (in a. u.)	pK_a
AZN5	N5	0.397110	9.05
	N10	0.426332	
	N11	0.323765	
	N17	0.378665	
	N20	**0.435279**	
AZN6	N6	0.379941	8.99
	N10	0.413934	
	N11	0.317757	
	N17	0.367322	
	N20	**0.428501**	
AZN7	N7	0.383156	8.99
	N10	0.417789	
	N11	0.312199	
	N17	0.368528	
	N20	**0.428704**	
AZN8	N8	0.363810	9.05
	N10	0.422157	
	N11	0.336589	
	N17	0.371655	
	N20	**0.430547**	

Table 8.4- The distance of proton from the most basic site and intra molecular H-bond that observed in some protonated form, along with out of plane angle of O atom in protonated AZCl

Drug molecules	H-bond length		Out of plane angle of O atom
	H30-N10	O28-proton	
AZCl5	1.845 Å	1.614 Å	1.0°
AZCl6	–	1.702 Å	22.7°
AZCl7	1.899 Å	1.636 Å	-0.8°
AZCl8	1.903 Å	1.629 Å	-1.8°

Table 8.5- The distance of proton from the most basic site and intra molecular H-bond that observed in some protonated form, along with out of plane angle of O atom in protonated AZO

Drug molecules	H-bond length		Out of plane angle of O atom
	H25-O29	O29-proton	
AZO5	1.791 Å	1.128 Å	-0.1°
AZO6	1.886 Å	–	4.8°
AZO7	1.805 Å	–	-30.3°
AZO8	1.921 Å	1.883 Å	36.2°

Table 8.6- The distance of proton from the most basic site and intra molecular H-bond that observed in some protonated form, along with out of plane angle of O atom in protonated AZN

Drug molecules	H-bond length		Out of plane angle of O atom
	N7-H32	O30-proton	
AZN5	–	–	14.5°
AZN6	1.954 Å	1.794 Å	-4.3°
AZN7	–	–	3.8°
AZN8	–	–	61.3°

Table 8.7– The computed Zero point energies and Solvation energies of Free and Protonated 9-chloroazaacridine-4-carboxamides (AZCl) for the most basic sites.

Molecules	Most Basic Protonation site	Solvation energies (a. u.)		Zero Point energies (a. u.)	
		Free drug	Protonated drug	Free drug	Protonated drug
AZCl5	N19	0.005710	0.021202	0.345806	0.362641
AZCl6	N19	0.001689	0.030639	0.345729	0.362549
AZCl7	N19	0.000451	0.029737	0.345718	0.362482
AZCl8	N19	0.002373	0.036182	0.345708	0.362490

Table 8.8– The computed Zero point energies and Solvation energies of Free and Protonated 9-oxoazaacridone-4-carboxamides (AZO) for the most basic sites.

Molecules	Most Basic Protonation site	Solvation energies (a. u.)		Zero Point energies (a. u.)	
		Free drug	Protonated Drug	Free drug	Protonated Drug
AZO5	N19	0.002105	0.033699	0.362007	0.378965
AZO6	N19	0.006345	0.054531	0.362444	0.378638
AZO7	N19	0.007382	0.070694	0.362714	0.378812
AZO8	N19	0.007561	0.052788	0.361684	0.378518

Table 8.9 – The computed Zero point energies and Solvation energies of Free and Protonated 9-aminoazaacridone-4-carboxamides (AZN) for the most basic sites.

Molecules	Most Basic Protonation site	Solvation energies (a. u.)		Zero Point energies (a. u.)	
		Free drug	Protonated drug	Free drug	Protonated drug
AZN5	N20	0.015840	0.006938	0.374258	0.391202
AZN6	N20	0.006765	0.014730	0.374325	0.391226
AZN7	N20	0.006472	0.014052	0.374529	0.391382
AZN8	N20	0.006011	0.019406	0.374966	0.391799

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