# CHAPTER 9

# IS PROTONATED CHROMOPHORE NECESSARY FOR INTERCALATION?


## SUMMARY

The study on the sequence specificity and $pK_a$ values of acridine-4-carboxamides prompts to examine the nature of chromophore during intercalation. The stacking of protonated and unprotonated forms of chromophores with sequences has been studied. The protonated form stacks with base pairs favourably than the unprotonated form.


## 9.1 INTRODUCTION

Studies on the intercalation of DNA by chromophore of acridine-4-carboxamide having distinguished electronic property prompted further investigation on the nature of the chromophore (protonated or unprotonated form) during intercalation. Indeed the biological property may be correlated with the DNA intercalation ability of chromophore inspite of side chain binding ***[1-5]***. **Figure 9.1a-b** presents the structures of various types of chromophores, amino acridine and aza analogues of acridine. We have already found the contribution of $\pi$–$\pi$ type of interaction along with the $\sigma$–$\pi$ type of interaction for the stabilization of stacked structures of acridine-4-carboxamides and its aza analogues ***[6-8]***. In some cases the $pK_a$ value of drugs and the biological properties correlate with the new drug, azaacridine-4-carboxamide, which acquires much reduced biological property compared to the parent carboxamides, however the $pK_a$ values of these are quite different.

Furthermore in most cases enhancing the intercalative binding within DNA sequences cannot ensure better biological property ***[7-12]***. In some drugs there observed fair correlation between $pK_a$ value and biological properties, where the drug having $pK_a$ close of biological pH act as efficient anticancer drug. Then the chromophore may enter within sequences of DNA either in its protonated or unprotonated form. Alternatively the drugs having low $pK_a$ may intercalate in unprotonated form and if the drug acquires high $pK_a$ then it may be less selective towards DNA because of its preference for other non nucleic acid molecules in biological systems.

We have determined $pK_a$ values of several acridine-4-carboxamides and the $pK_a$ value of side chain is quite different from the $pK_a$ of chromophore. Most of the acridine-4-carboxamide acquires $pK_a$ ~5 whereas in some cases it goes up to 8 ***[8]***. In view of this it is not sure whether the drug remains ionized or unionized under physiological condition, then how these forms are related to the intercalative mode of binding. Hence we intend to examine the comparative study on the intercalative mode of binding in the free and protonated drugs by constructing various stacked models of this drug.

## 9.2 METHODOLOGY

The protonated and free aminoacridine and 9-aminoaza(N3)acridine are chosen for the study. The geometries of these molecules were optimized by using 6-31G** basis set ***[12]***, and the proton affinities at ring chromophore were estimated. There are two sites for the aza analogues and the ring nitrogen is found to be more basic than the other nitrogen. Hence the PA of this more basic site was taken for computing $pK_a$. The $pK_a$ of these ring nitrogen were computed by using the equation given in chapter 8. Again the stacking energies of protonated and unprotonated form of 9-aminoacridine and its aza analogue were computed.

## 9.3 RESULTS AND DISCUSSION

The computed $pK_a$ values of these molecules are shown in **Table 9.1**. Hence from the $pK_a$ values may indicate whether the chromophore occur as free drug or protonated drug in the physiological pH. It has been found that the $pK_a$ value of 9-azaacridine and 9-aminoacridine is more than 7. Therefore these molecules will be in free state in the biological system(**Table 9.1**). So the chances of intercalating by 9-aminoaza(N3)acridine in protonated form may be less. In such situation the free molecule is likely to occur in physiological environment. So we have made a comparison on the intercalative mode of binding for the protonated and unprotonated form. The energies of the optimum structures are shown in **Table 9.2**. The free molecule stacks better than the protonated form (**Table 9.2**). In view of this the DNA binding ability as well as the biological property depends on how efficiently the free molecule goes into intercalation. **Table 9.2** shows wide differences between the interaction energies of protonated and unprotonated form of 9-aminoacridine and 9-aminoaza(N3)acridine. Hence the state of the molecule during intercalation may

also be considered in analyzing the stability of these drugs within DNA. Then the other conditions like the efficiency of drug entering inside the cell for interacting with DNA might be necessary for consideration. Hence the anticancer property of azaacridine may depend on other physio-chemical property rather than intercalative mode of binding.

## 9.4 CONCLUSION

Significant differences between the interaction energies of protonated and unprotonated chromophore with sequences of DNA has observed. Therefore the nature of the chromophore during intercalation may be important for explaining wide variation of biological properties of intercalators.

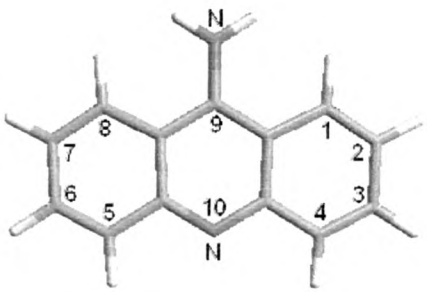


**Figure 9.1-** Structure of 9-aminoacridine.

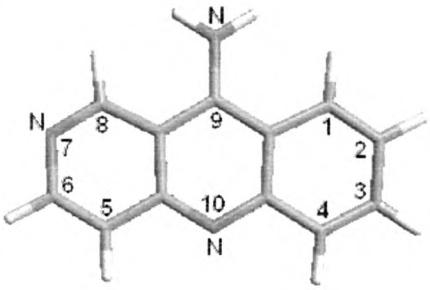


**Figure 9.2-** Structure of 9-aminoazaacridine.

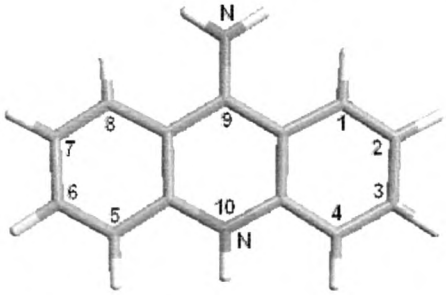


**Figure 9.3a-** Structure of 9-aminoacridine protonated at N10.

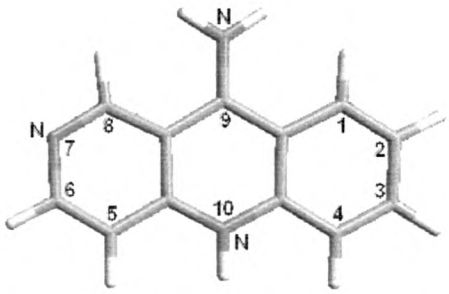


**Figure 9.3b-** Structure of 9-aminoazaacridine protonated at N10.

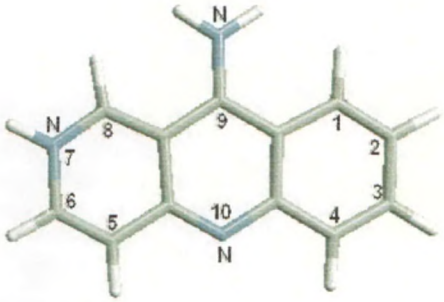


**Figure 9.3c-** Structure of 9-aminoazaacridine protonated at N7.

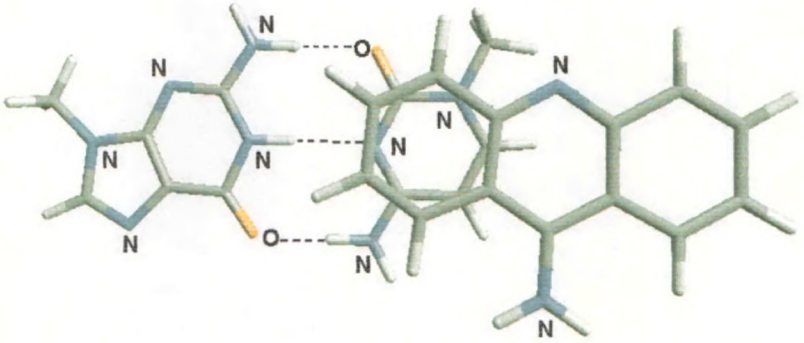


**Figure 9.4a:** Optimum stacked structure of GC and unprotonated 9-aminoacridine.

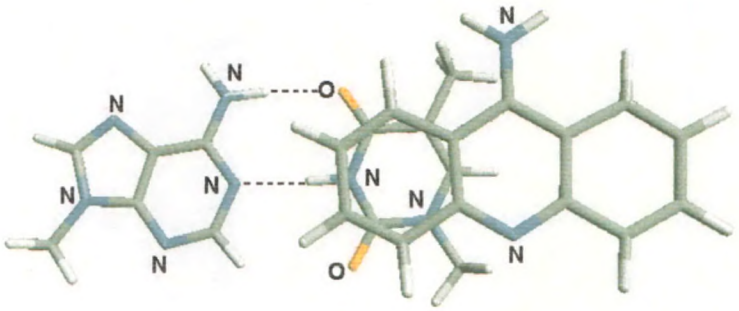


**Figure 9.4b:** Optimum stacked structure of AT and unprotonated 9-aminoacridine.

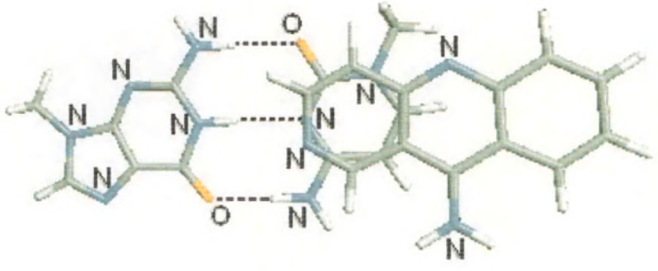


**Figure 9.4c:** Optimum stacked structure of GC and unprotonated 9-aminoaza(6)acridine.

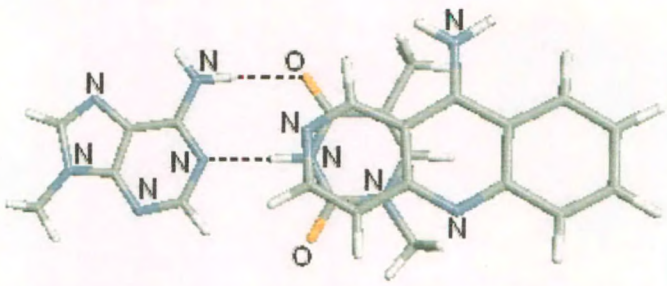

**Figure 9.4d:** Optimum stacked structure of AT and unprotonated 9-aminoaza(6)acridine.

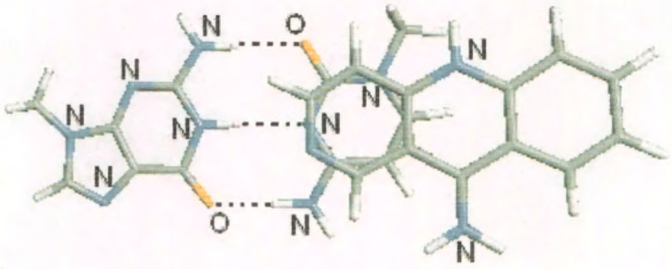

**Figure 9.5a:** Optimum stacked structure of GC and 9-aminoaza(6)acridine (protonated at N10).

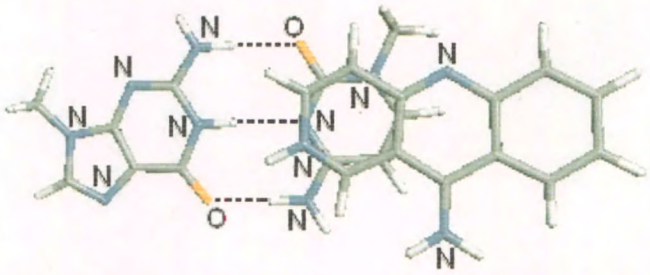

**Figure 9.5b:** Optimum stacked structure of GC and 9-aminoaza(6)acridine (protonated at N7)

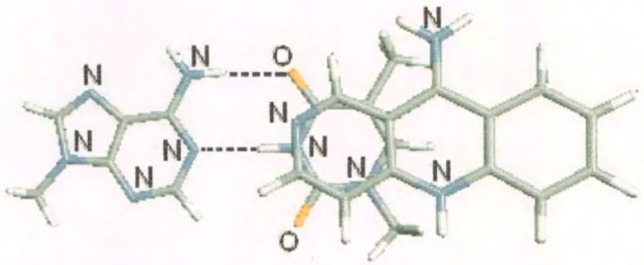

**Figure 9.5c:** Optimum stacked structure of AT and 9-aminoaza(6)acridine (protonated at N10)

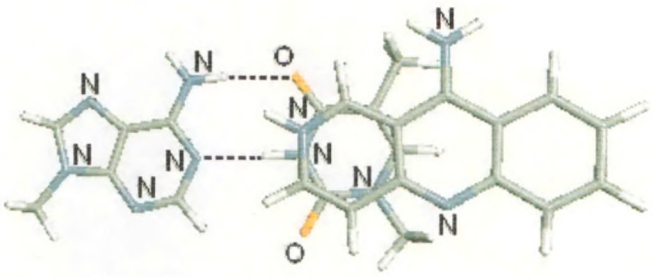


**Figure 9.5d:** Optimum stacked structure of AT and 9-aminoaza(6)acridine (protonated at N7)

**Table 9.1**– The computed proton affinities (PA), pKa values and solvation energies of 9-aminoacridine and 9-aminoazaacridine.

| Drug | Protonation site | PA (au) | pKa | Solvation energy (au) |
|---|---|---|---|---|
| 9-aminoacridine | N10 | 0.502447 | 8.40 | 0.001595 |
| 9-aminoazaacridine | N10 | 0.491947 | 8.21 | 0.001162 |
| 9-aminoazaacridine | N7 | 0.465524 | 8.12 | 0.017068 |

**Table 9.2**– Variation of interaction energies with protonation of Acridine and base pair stacking.

| Stacking geometry | Interaction energies (with un-protonated drug) (kcal/mol) | | Interaction Energies (with protonated drug) (kcal/mol) | | |
|---|---|---|---|---|---|
| | ACR | AZCR | ACR protonated at N10 | AZCR protonated at N10 | AZCR protonated at N7 |
| AT-AR-7 | -1.220190 | 0.035549 | 1.359734 | 3.0492551 | -2.884039 |
| GC-AR-12 | -0.999198 | 0.783973 | 4.124578 | 6.182035 | -0.999376 |

ACR= 9-aminoacridine; AZCR=9-aminoazaacridine; AR = ACR or AZCR

**References**


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