CHAPTER 3

Ab initio CALCULATIONS ON THE STACKING OF 9-AMINOACRIDINE WITH NUCLEOBASES AND WATSON-CRICK BASE PAIRS

SUMMARY

The intercalation by tricyclic chromophore of anticancer drugs within sequences of DNA has been found to be related to the therapeutic values. Hence the stacking of simple tricyclic molecule with nucleobases and Watson Crick base pair has been studied for demonstrating intercalative mode of binding. Various stacked geometries of 9-aminoacridine (AD) with nucleobases and Watson Crick base pairs are analysed for understanding the sequence specificity of this molecule. In the optimum stacked structures obtained from MNDO calculation, the position of 9-aminoacridine is not totally outside the stacking region. The interaction energies obtained from *ab initio* method demonstrate favourable,stacking of this drug with various nucleobases and base pairs. The acridine is found well stacked within base pair in the optimum structure. The stacking energies of AD-GC and AD-AT are quite different, and AD stacks preferably with AT sequence.

3.1 INTRODUCTION

The study in nucleic acid targeted drug design aims to determine drug binding site within DNA sequences. In this context the sequence recognition by a small drug molecule, an intercalator, may be addressed. Some intercalators bind at a particular sequence in DNA whereas some do not show such selectivity ***[1-6]***. Therefore these molecules may possess certain factor for being specific or nonspecific intercalator. Denny et al analysed the binding of a number of acridine analogues with DNA, and it has been found that most of these drugs intercalate within GC rich sequences in DNA ***[7-11]***. There are very few AT specific intercalator of acridine derivatives. Hence the DNA binding ability of these molecules can be assessed from the study on the intercalation model with GC or AT sequence. At the same time intercalation of small drugs rather than big molecules attracts interest of many researchers for understanding the mechanism of sequence specific recognition of drugs ***[10-17]***. The study is chosen to probe the binding affinity of AD with sequences of DNA for extracting information on the selective binding of tricyclic

ring. Herein it is necessary to know why most of the acridines intercalate with GC sequence of DNA but not so preferred for AT sequence. Moreover in designing potentially effective anticancer drugs of acridine analogues thorough knowledge of binding characteristic and selectivity of this drug become important. In other words, based on the ideas of selectivity and nature of binding by simple acridine molecule it may be possible to design better intercalators. Accordingly, before studying all the acridine analogues, it is necessary to examine the binding of simple molecules like 9-aminoacridine so that a comparison can be made with other acridine analogues having multiple DNA binding groups. In general the drugs that bind preferably with GC will definitely acquire more binding affinity within GC rich sites of DNA *[5-7]*. The extent of helix unwinding occurred after intercalation by acridine analogues might also be related to the effectiveness of sequence specific intercalation, and the information may be used in the logical approach of designing new intercalator. The resultant change in unwinding angles due to intercalation might be correlated with the binding ability of drug with nucleobases or base pairs, and probably produce local stiffening of the sugar backbone due to induction by drug when it penetrates within sequence. Hence understanding of sequence specificity of 9-aminoacridine may be taken up before studying other acridine analogue.

The *ab initio* calculations are applied in most of the major research areas, and used in studying stacking of nucleobases and H-bonded structures of DNA. The importance of correlation energy as well as the inclusion of large basis set in *ab initio* calculation is also suggested. Hence we have taken up *ab initio* HF and MP2 methods for studying the stacking of AD with nucleobases and base pairs.

3.2 METHODOLOGY

(a) Initially the calculation were carried out by taking the crystal structure of amino acridine and base pairs, and the optimum stacked models were searched within the region of base pairs by using MNDO calculation*[2-5]*. We have used such approximate method to justify that stacking interactions cannot be studied by this method. The optimum stacked geometries were taken for computing interaction energies at *ab initio* level.

(b) Again we have used *ab initio* method for finding the optimum stacked structures within sequences. For constructing the stacked models of 9-aminoacridine (AD)

with a base pair, we have taken the completely optimized geometries of these molecules. Complete geometry optimization were performed with 6-31G* basis set *[18]*. The stacked models of AD and base pair were constructed by placing AD above the base pair at the vertical separation of 3.6 Å, since most acridine analogues intercalate between base pair at this distance.

The interaction energies of various stacked models were calculated using different basis sets in the HF calculation, and the optimum stacked structures are determined. Again DFT and MP2 calculations were performed for the optimum structures. In order to probe the optimum stacked models, AD was rotated above the nucleobase and base pair along XY plane without changing the vertical separation. The interaction energy, ΔE is obtained from the equation -

$$\Delta E = E_S - E_B - E_{AD}$$

Where E_S, E_B and E_{AD} are the total energies of stacked models, nucleobase or base pairs and 9-aminoacridine (AD) respectively.

It is well known that HF method is not sufficient for studying stacking problems, hence we have further computed interaction energies values of using MP2/6-31G level only for the optimum stacked structures.

3.3 RESULTS AND DISCUSSIONS

(a) Frist the stacked configuration of AD with nucleobases and base pairs were optimized by using MNDO study in spite of its limitation for studying such systems. Then the *ab initio* calculations with large basis set were used to estimate interaction energies of optimum stacked structures (**Table 3.2a-b**). The use of MNDO study indicates the region where the AD resides in and around the sequences of DNA (**Figure 3.6a-b**). The interaction energies for the stacked models of AD with adenine, guanine, cytosine and uracil are given in **Table 3.1**. The stacked geometry of AD-A corresponding to minimum interaction energies is shown in **Figure 3.1**. We have found two favorable stacked structures of AD-C with small variation in the interaction energies (**Figure 3.2a-b**). Likewise among different configurations of AD-G, only two stacked models are observed to be stable (**Figure 3.3a-b**). The interaction energies for AD-U are slightly lower than those of AD-G (**Table 3.1**). On the basis of these results, the interaction energies of stacked AD and nucleobases is

arranged in decreasing order as :

AD-G>AD-C>AD-U>AD-A

As we know that acridine-4-carboxamide intercalate between the GC base pairs of DNA, and the mode of binding by acridine chromophore may be either specific or nonspecific and also perhaps controlled by the side chain ***[4, 15-17]***. Besides, there are quite a number of acridine analogues that bind specifically in GC sequences ***[5-7]***. The computed interaction energies may be taken to monitor the sequence specificity of acridine chromophore through intercalation. For instance, among the stacked models, AD-C and AD-G stacking are found to be quite stable and we expect high specificity of this drug for cytosine and guanine.

Further studies on the stacking of AD with Watson Crick base pairs AU and GC have been performed to examine any change in specific binding of AD with base pairs (**Figure 3.5a-b** and **Figure 3.6a-b**). The interaction energies of various stacked AD-AU and AD-GC are given in **Table 3.2a-b**. In the optimum structure of AD-GC, AD is found shifted towards guanine, conforming with the fact that this molecule interacts preferentialy with guanine than cytosine nucleobase. Hence the inclusion of hydrogen-bonded region between nucleobases produces no difference in the nature of stacking which appeared in case of individual nucleobases. Similarly in the stacked structure of AD with AU, AD resides more towards U than A. The results agree with the nature of stacking of AD with Individual nucleobases where the preference for U than A is observed. The results imply that variation of stacking interaction of individual nucleobases from those in base pairs are consistent, and the results may be useful for demonstrating specificity of AD. In all cases we have taken the rigid molecules where the relaxation of the geometrical parameters of AD and base pairs after stacking is not considered. One can consider the crystal structure of intercalated AD in sequences of DNA where the Watson Crick hydrogen bonds still persist at the intercalation site ***[2-5]***. Hence the specific binding of AD with base pairs may be taken for analyzing sequence specificity rather than individual nucleobase.

As shown in **Figure 3.5a-b** and **Figure 3.6a-b**, the optimum structures of AD-GC and AD-AU show partial overlapping of AD and base pair, hence the stabilization of these geometries are contributed from the partial stacking of AD. In this case the issue of sequence specificity cannot be tested because the AD is almost outside the region of

base pairs. However the MNDO method used in searching the optimum stacked structure can at least locate a stable stacked geometry of AD with sequences, which may be due to local polarizability of nucleobases in AU and GC sequences, and thereby produces preference for G and U in AD-GC and AD-AU. Moreover the aminoacridine is not totally outside the stacking region of sequences.

As shown in **Table 3.2a-b**, the interaction energies changes with basis sets used in the SCF method. However with the improving basis set from 6-31G to 6-31G**, the values are slightly improved. It is worth mentioning that interaction energies computed with MP2/STO-3G method and the values obtained may be used for comparison with those of SCF method. **Table 3.2a-b** shows that the difference between results obtained from MP2/STO-3G and SCF/6-31G is not quite large. Considering this discrepancy in the present study, the interaction energies obtained from these methods give reliable pictures of stacked AD with nucleobases and base pairs.

(b) We have also used *ab initio*, DFT and MP2 (for stacked region only) methods for determining the optimum stacked structures of AD with sequences. We have constructed the stacking models from some arbitrarily chosen positions in base pairs with 9-aminoacridine. The plots of various models versus interaction energies are shown in **Figure 3.7** and **3.8**. The geometries corresponding to minimum energy configuration with AT and GC are shown in **Figure 3.9-3.10**. As we can see that the stacking energies of this molecule for all possible stacking of drugs within base pairs were used to locate the optimum structure shown in **Figure 3.9-3.10**. We can compare these optimum stacked structures with those obtained from MNDO method shown in **Figure 3.5a-b** and **3.6a-b**, there are wide variations in these structures. However the drug is almost outside the stacking region of sequences in the optimum stacked geometries of MNDO studies, whereas in the optimum stacked geometries obtained from *ab initio* method shows AD within the stacking region of base pairs. In fact the importance of accurate *ab initio* method and the calculations beyond HF have been indicated in dealing with stacking problem. In that way MNDO calculation should not locate any local favourable stacked structure within the stacking distance of 3.6 Å. Moreover the stacked structure for the local minimum detected by 6-31G** method correspond to higher energy level than the corresponding structure obtained from MNDO method. Again we have checked the entry of drug either from minor groove or major groove in DNA (**Table 3.3a-b, 3.4a-b** and **3.5**). There are significant variations of

interaction energies depending on the position of acridine through minor or major groove. The present study indicates some variation in the stacking energies of 9-aminoacridine with sequence of DNA.

3.4 CONCLUSION

From the present study, it has been found that 9-aminoacridine stacks favorably with guanine and uracil nucleobases. The differences between the interaction energies of this molecule with AT and GC sequences are not so much.

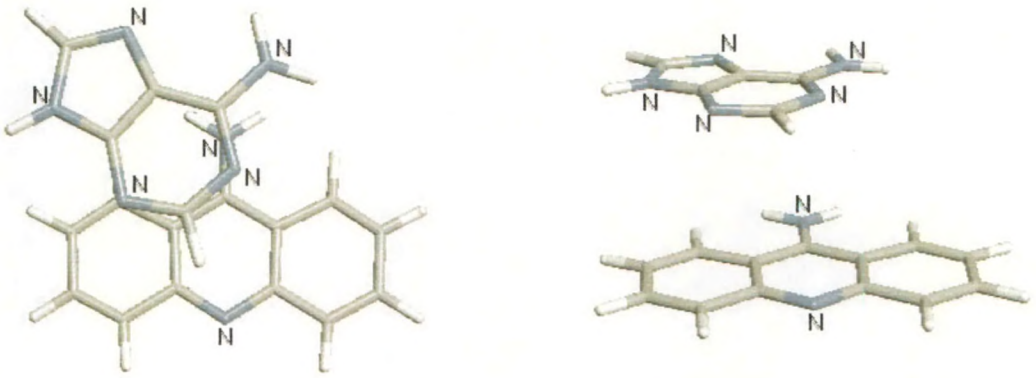

Figure 3.1 – The optimum stacked structure of 9-aminoacridine and Adenine (AD-A) corresponding to MNDO method of calculation.

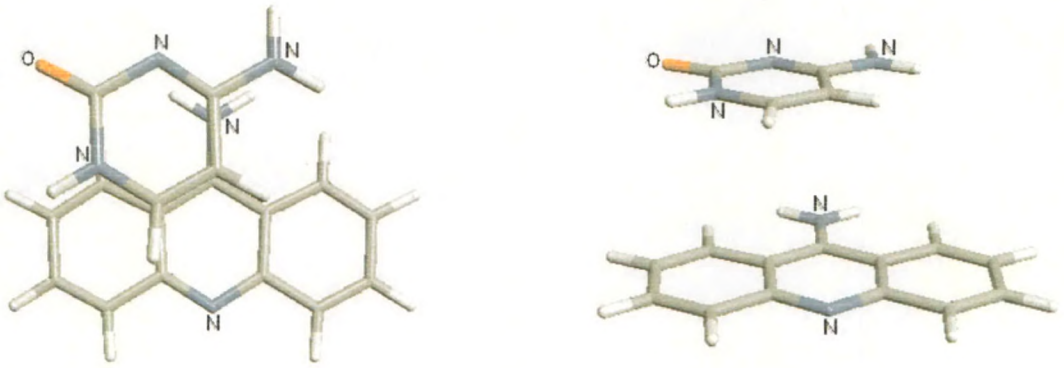

Figure 3.2a – The optimum stacked structure of 9-aminoacridine and Cytosine (AD-C1) corresponding to MNDO method of calculation.

Figure 3.2b – The optimum stacked structure of 9-aminoacridine and Cytosine (AD-C2) corresponding to MNDO method of calculation.

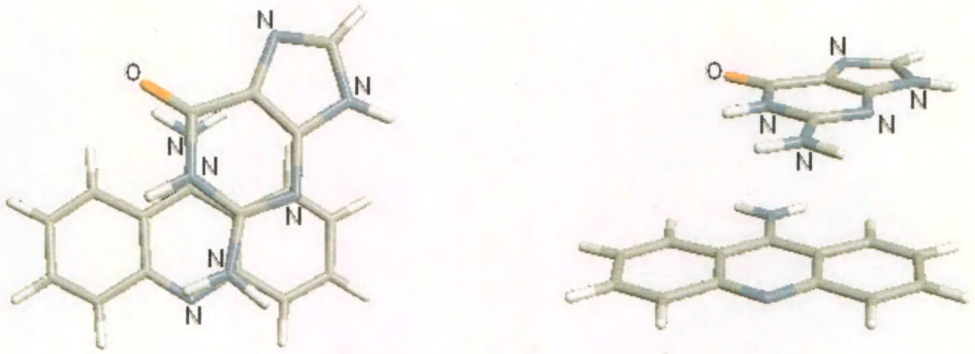

Figure 3.3a – The optimum stacked structure of 9-aminoacridine and Guanine (AD-G1) corresponding to MNDO method of calculation.

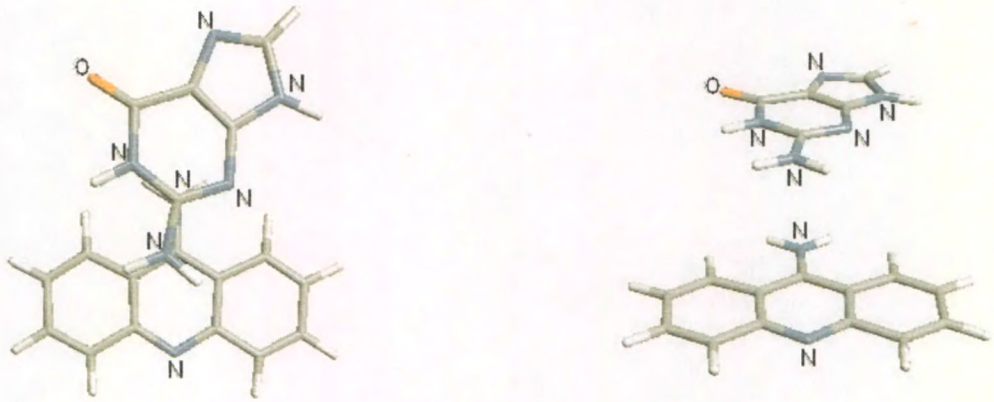

Figure 3.3b – The optimum stacked structure of 9-aminoacridine and Guanine (AD-G2) corresponding to MNDO method of calculation.

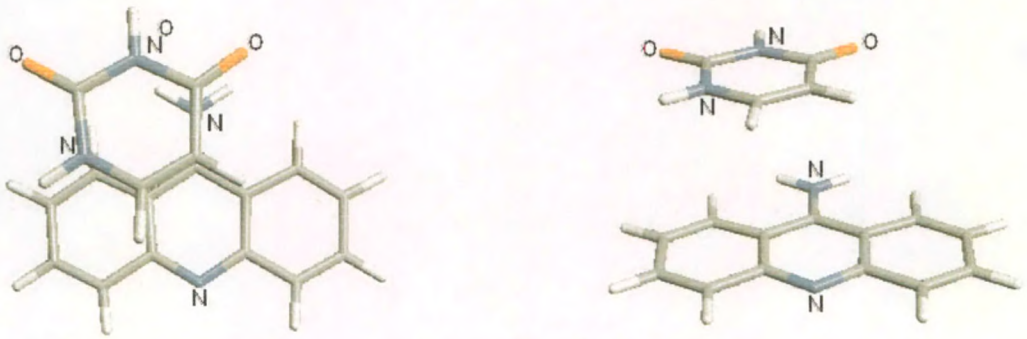

Figure 3.4a – The optimum stacked structure of 9-aminoacridine and Urasil (AD-U1) corresponding to MNDO method of calculation.

Figure 3.4b – The optimum stacked structure of 9-aminoacridine and Urasil (AD-U2) corresponding to MNDO method of calculation.

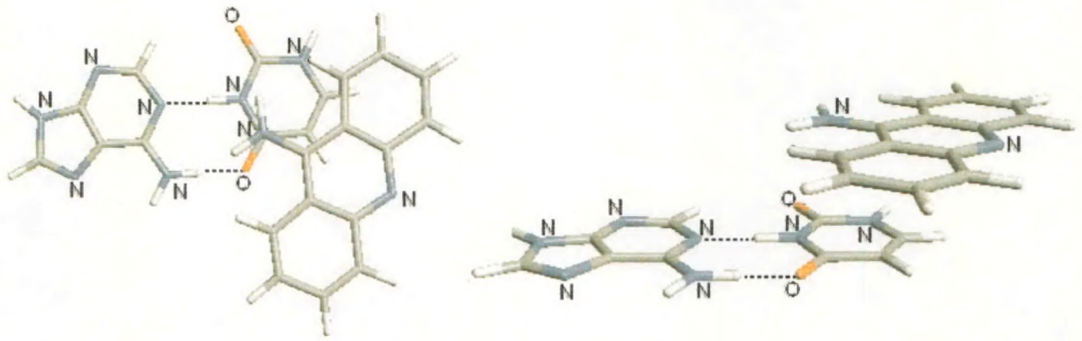

Figure 3.5a – The optimum stacked structure of 9-aminoacridine and AU base pair (AT-AD2) corresponding to MNDO method of calculation.

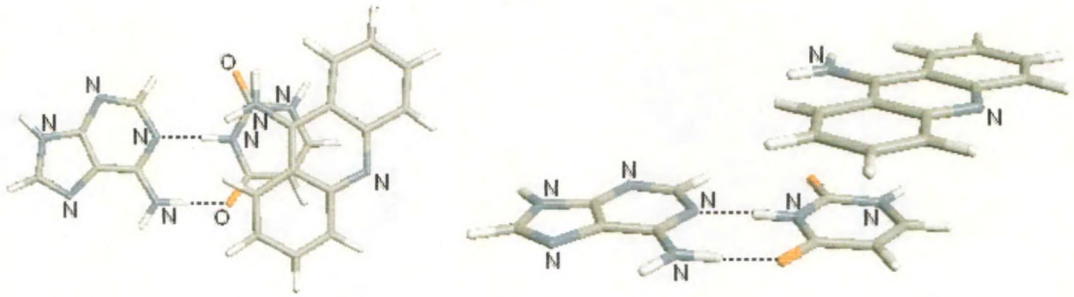

Figure 3.5b– The optimum stacked structure of 9-aminoacridine and AU base pair (AT-AD3) corresponding to MNDO method of calculation.

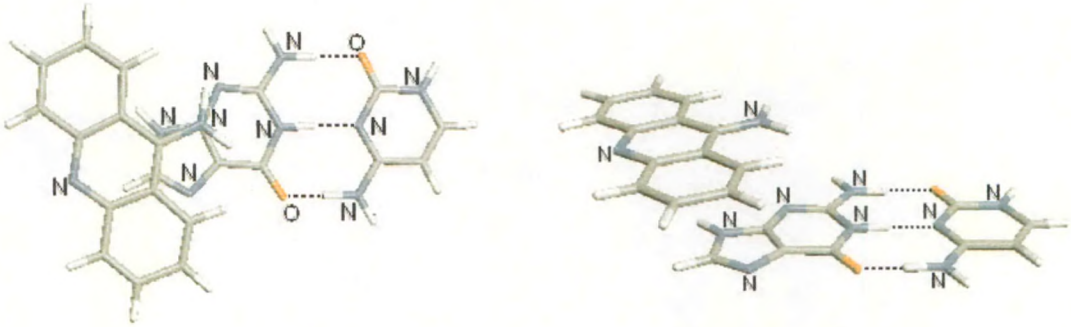

Figure 3.6a– The optimum stacked structure of 9-aminoacridine and GC base pair (GC-AD6) corresponding to MNDO method of calculation.

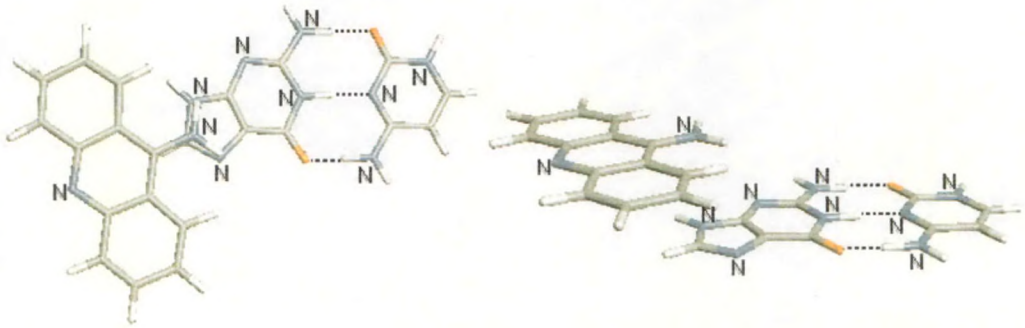

Figure 3.6b– The optimum stacked structure of 9-aminoacridine and GC base pair (GC-AD7) corresponding to MNDO method of calculation.

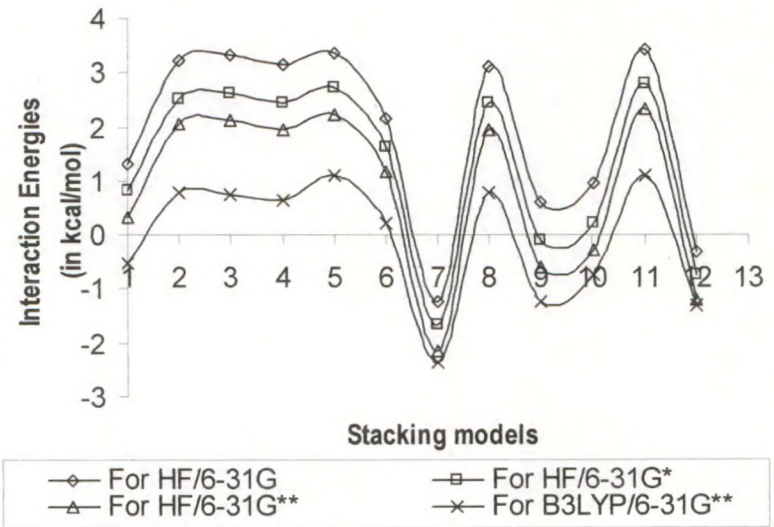

Figure 3.7 – Plot of stacking models versus Interaction energies of ð-ð interaction of AT base pair and 9-aminoacridine in different levels of theory.

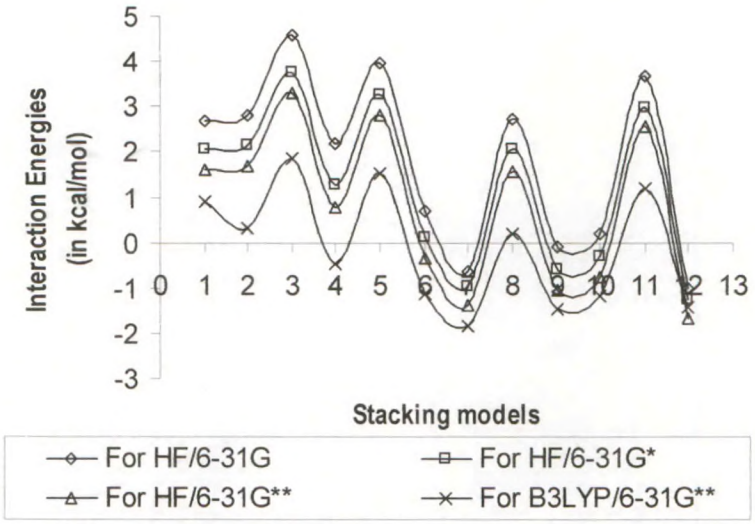

Figure 3.8- Plot of stacking models versus Interaction energies of ð-ð interaction of GC base pair and 9-aminoacridine in different levels of theory.

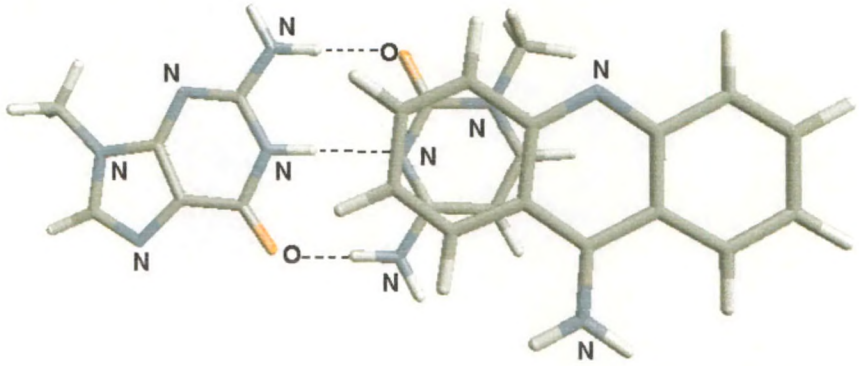

Figure 3.9: Optimum stacked structure of 9-aminoacridine and GC corresponding to HF/6-31G** calculation. (GC-acri-12)

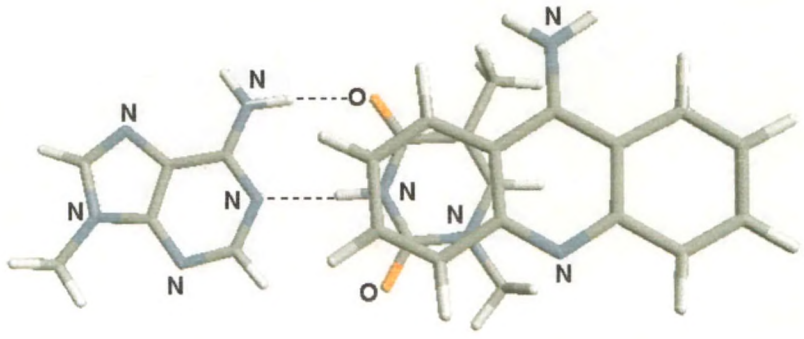

Figure 3.10: Optimum stacked structure of 9-aminoacridine and AT corresponding to HF/6-31G** calculation. (AT-acri-7)

Table 3.1- Computed Interaction Energies of some stacked models of 9-aminoacridine (AD) with individual bases (A, C, G and U)

Stacked Models	Interaction energies (in k cal/mol)			
(X-ADn)	HF/6-31G	HF/6-31G*	HF/6-31G**	MP2/STO-3G
A-AD1	1.415	1.208	1.190	0.799
C-AD1	-3.028	-2.900	-2.897	-1.015
G-AD1	**-2.998**	**-3.119**	**-3.137**	**-1.422**
G-AD2	-2.502	-2.722	-2.711	-0.877
U-AD1	-2.615	-2.565	-2.569	-0.672
U-AD2	-2.889	-2.815	-2.815	-0.767

X= A, C, U or G; AD = 9-aminoacridine; n = stacking location.

Table 3.2a- Computed Interaction Energies of some stacked models of 9-aminoacridine (AD) with DNA basepair AU

Stacked Models	Interaction energies (in k cal/mol)			
(AT-ADn)	HF/6-31G	HF/6-31G*	HF/6-31G**	MP2/STO-3G
AU-AD1	0.248	-0.067	-0.099	0.430
AU-AD2	**-1.927**	**-2.024**	**-2.025**	**-0.470**
AU-AD3	**-2.913**	**-2.868**	**-2.872**	**-0.767**
AU-AD4	0.470	0.320	0.267	0.338
AU-AD5	2.332	2.013	1.955	1.165
AU-AD6	2.393	2.223	2.175	1.185
AU-AD7	-1.668	-1.698	-1.705	-0.288

n = stacking location.

Table 3.2b- Computed Interaction Energies of some stacked models of 9-aminoacridine (AD) with DNA basepair GC

Stacked Models	Interaction energies (in k cal/mol)			
(GC-ADn)	HF/6-31G	HF/6-31G*	HF/6-31G**	MP2/STO-3G
GC-AD1	3.434	2.999	2.988	2.444
GC-AD2	3.284	3.094	3.040	2.031
GC-AD3	0.648	0.473	0.490	0.586
GC-AD4	0.996	0.880	0.859	0.620
GC-AD5	-0.713	-0.907	-0.909	-0.159
GC-AD6	**-1.110**	**-1.271**	**-1.275**	**-0.303**
GC-AD7	**-2.126**	**-2.205**	**-2.200**	**-0.791**
GC-AD8	3.372	2.949	2.909	2.248
GC-AD9	2.803	2.523	2.477	1.929

n = stacking location.

Table 3.3a- Computed Interaction Energies for stacked models of 9-aminoacredine binding through minor groove to AT base-pair at different levels of theory (π-π interaction)

Stacked Models (AT-AD-n)	Interaction energies (in k cal/mol)			
	HF/ 6-31G	HF/ 6-31G*	HF/ 6-31G**	B3LYP/6-31G**
AT-AD-1	1.330	0.825	0.333	**-0.530**
AT-AD-3	3.329	2.616	2.112	0.751
AT-AD-5	3.380	2.722	2.248	1.090
AT-AD-7	**-1.220**	**-1.669**	**-2.137**	**-2.377**
AT-AD-9	0.608	**-0.117**	**-0.611**	**-1.229**
AT-AD-11	3.437	2.809	2.348	1.100

n = stacking location.

Table 3.3b- Computed Interaction Energies for stacked models of 9-aminoacredine, binding through major groove to AT base-pair at different levels of theory (π-π interaction)

Stacked Models (AT-AD-n)	Interaction energies (in k cal/mol)			
	HF/ 6-31G	HF/ 6-31G*	HF/ 6-31G**	B3LYP/6-31G**
AT-AD-2	3.207	2.526	2.068	0.776
AT-AD-4	3.148	2.434	1.936	0.655
AT-AD-6	2.152	1.643	1.167	0.226
AT-AD-8	3.124	2.427	1.951	0.775
AT-AD-10	0.973	0.214	-0.280	**-0.748**
AT-AD-12	**-0.313**	**-0.753**	**-1.195**	**-1.293**

n = stacking location.

Table 3.4a- Computed Interaction Energies for stacked models of 9-aminoacredine binding through major groove to GC base-pair at different levels of theory (π-π interaction)

Stacked Models (GC-AD-n)	Interaction energies (in k cal/mol)			
	HF/ 6-31G	HF/ 6-31G*	HF/ 6-31G**	B3LYP/6-31G**
GC-AD-1	2.665	2.075	1.621	0.901
GC-AD-3	4.595	3.741	3.283	1.849
GC-AD-5	3.954	3.270	2.818	1.513
GC-AD-7	**-0.654**	**-0.976**	**-1.399**	**-1.844**
GC-AD-9	**-0.081**	**-0.592**	**-1.065**	**-1.446**
GC-AD-11	3.662	2.982	2.542	1.174

n = stacking location.

Table 3.4b- Computed Interaction Energies for stacked models of 9-aminoacredine binding through minor groove to GC base-pair at different levels of theory (π-π interaction)

Stacked Models (GC-AD-n)	Interaction energies (in k cal/mol)			
	HF/ 6-31G	HF/ 6-31G*	HF/ 6-31G**	B3LYP/6-31G**
GC-AD-2	2.787	2.148	1.685	0.335
GC-AD-4	2.189	1.287	0.790	**-0.452**
GC-AD-6	0.689	0.107	-0.360	**-1.124**
GC-AD-8	2.726	2.043	1.573	**0.171**
GC-AD-10	0.195	**-0.318**	**-0.782**	**-1.162**
GC-AD-12	**-0.999**	**-1.252**	**-1.685**	**-1.404**

n = stacking location.

Table 3.5- Calculated Interaction energies for stacked portion of optimum models of 9-aminoacridine stacked with AT and GC base pair using MP2/6-31G.

Structure (only the fully stacked portion of optimum models)	Observed binding direction	Interaction energies (MP2/6-31G)
AT-ACR-7	Minor groove	-8.71166
AT-ACR-12	Major groove	-7.53323
GC-ACR-7	Major groove	-7.05541
GC-ACR-12	Minor groove	-6.13557

Reference

1. Wilson W. D., Wang Y Hwa, Kusuma S., Chandraslkharan S., Yang N. C., Boykin D. W.; *J. Am. Chem. Soc., 107(1985)4989-4994.*

2.(a) Leonard G. A., Hambley T. W., Katherine Mc Auley, Brown T., Hunter W.; *Acta Cryst.* D49(1993)458-468.

(b) Voet D. and Rich A. P.; *Nucleic Acid Res, Mol. Biol.* 10(1970)183.

3. Chew K. X., Gresh N., Pullman B.; *Molecular Pharmacology*, 30(1986)279-286.

4.(a) Pullman B., *Anticancer Drug Des,*

(b) Hobza P, Sandorfy C, *J Am Chem Soc*, **1987**, 109, 1302.

(c) Hobza P, Sponer J, Polasek M, *J Am Chem Soc*, **1995**, 117, 792.

(d) Parajuli R, Medhi C, *Ind J Chem (SecA)*, 45A, **2006**, 146

5. Denny W. A., Atwell G. J., Baguley, B. C., Cain B. F., *J. Med. Chem.*, 22(1979)134.

6. Arwell G. J., Cain B. F., Baguley B. C., Finlay G. J. and Denny W. A.; *J. Med. Chem.* 27(1984)1481-1485.

7. Waring J., Colson P., Haussler C., Bailey C.; *Biochemistry*, 33(1994)9865-9874.

8. Baguley B. C.; *Anticancer Drug Des* (1991)6-35.

9. Bailey C. and Hemichart J. P.; *Bioconjugate Chem.* 2(1991)379-383.

10. Capranico G., Palumbo M., Tinelli S., Mabella M., Pozzan A., Zumuno F.; *J. Mol. Biol.* 235(1994)1218-1230.

11. Jensen P. B., Sarensen S. B., Schested M., Demant H. H.; *Biochem Pharmacol* 45(1993)2025-2035.

12. Zwelling L. A., Mitchell M. J., Satitpunwaycha P., Mayes J., Altochuler E., Hinds M., Baguley B.; *Cancer Research* 52(1992)209-217.

13. Spicer J. A., Gamage S. W., Atwell G. J., Finlay G. J. Baguley B. C., Denny W. A.; *J. Med. Chem.* 40(1997)1919-1929.

14. Finlay G. J., Riou J. F., Baguley B. C.; *Eur. J. of Cancer,* 32A(1996)708-714.

15. Chen Q., Deady L. W., Baguley B. C., Denny W. A.; *J. Med. Chem.* 37(1994)593-597.

16. Gamage S. A., Spicer J. A., Rewcastle G. W., Denny W. A.; *Tetrahedron Lett.* 38(1997)699-702.

17. Balmer B. D., Rewcastle G. W., Baguley B. C., Denny W. A.; *J. Med. Chem.* 31(1988)707-712.

19. Frisch M J, Trucks G W, Schlegel H B, Gill P M W, Johnson B G, Robb M A, Cheeseman J R, Keith T, Petersson G A, Montgomery J A, Raghavachari K, Al- Laham, M A, Zakrzewaki V G, Ortiz J V, Foresmann J B, Ciolowski J, Stefanov B B, Namayakkara A, Challacombe M, Peng CY, Ayala P Y, Chen W, Wong M W, Andres J L, Replogle E S, Gomperts R, Martin R L, Fox D J, Binkley J S, Defrees D J, Baker J, Stewart J P, Head-Gordon M, Gonzalez C & Pople J A, Gaussian **94**; Gaussian Inc, Pittsburgh PA, **1995.**