CHAPTER 4

Ab Initio STUDY OF THE NATURE OF STACKING BETWEEN AZA ANALOGUES OF ACRIDINE-4-CARBOXAMIDES WITH SEQUENCES OF DNA

SUMMARY

Various 9-aminoazaacridine-4-carboxamide having nitrogen atom at different position of chromophore are used for analyzing recognition of base pairs by chromophore. The interaction energies of these chromophores with base pairs of DNA changes with the variation of nitrogen position in chromophore. The presence of nitrogen atoms within the stacking region contributes to the stability of this chromophore within sequences of DNA. So the recognition of sequences by these aza analogues appear to occur from two interactions, one of which is π-π type and the other is σ-π type of interaction.

4.1 INTRODUCTION

The intercalation mode of DNA binding by chromophore of acridine-4-carboxamides has been known from many experimental and theoretical studies. In this context the intercalation of chromophore within specific sequences of DNA has been studied for relating the DNA binding ability and biological properties ***[1-5]***. The acridine-4-carboxamides have two important DNA binding molecular parts such as chromophore (tricyclic ring) and carboxamide side chain. Attempts have been made to design new intercalator by modifying the chromophore with different substituents so that the altered electronic properties of chromophore would enhance the intercalation mode of binding with GC sequences. Another way of modifying the chromophore for similar reason has been observed in aza analogue of this drug where a nitrogen atom is found in place of a carbon atom. It has been known that the chromophore group of most carboxamides intercalate within GC rich region of DNA ***[4-10]***. Here the absolute requirements for acquiring enhanced intercalative binding may be contributed from the π–π, σ–π and lone pair-lone pair interactions. Thus the interactions between chromophore and sequences resulted from the electronic effect of the aza analogues having altered N position (Nx) in the ring (chromophore) at the long range interaction distance of 3.6Å may be examined for understanding sequence specific intercalation.

The relation between DNA binding ability and biological properties of these aza analogues of acridine-4-carboxamide with poly (A-T) oligonucleotides using ethidium bromide displacement method has been well studied ***[7-12]***. Although some acridine carboxamides binds with AT sequences, most of these drugs preferably bind with GC sequences in DNA ***[5-13]***. The general investigation on the sequence specificity of these aza analogues in comparison to parent N-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (DACA) has not been properly explained. Hence the investigations on the sequence specific binding of these drugs, contributed from chromophore intercalation are essential.

The use of quantum mechanical methods in analyzing the stacked structures of DNA bases has been known. The dispersion forces that stabilize stacking interaction should be included in the calculation that can be obtained by considering intermolecular electron correlation. For studying intercalation model of chromophore and sequences, It is necessary to perform *ab initio* calculations with sufficient basis set and electron correlation ***[13-18]***. In addition, various empirical studies are also applicable in describing conformational variability of DNA structures ***[14-15]***. As the high level *ab initio* calculations have some limitations, therefore the empirical calculations using Lennard-Jones potential are also used to reproduce *ab initio* results ***[15]***. Here we extend the investigation on the stacking of aza analogues of acridine-4-carboxamides with sequences of DNA by using *ab initio* methods.

4.2 METHODOLOGY

4.2.1 MODELS OF STACKED STRUCTURES:

Initially the aza analogues of acridine-4-carboxamides and all the base pairs were completely optimized with 6-31G basis sets by using Gaussian program code ***[19]***. The drug and base pairs were allowed to stack at optimum vertical separation of 3.6Å, where the methyl groups representing sugar and carboxamide side chain lie on the same side as well as on opposite side (**Figure 4.1a-b**). The stacking distance of 3.6Å was chosen for constructing all the stacked models of drug and sequence, since most drugs intercalates at this distance ***[7]***. We have selected some positions along the horizontal plane of base pair for stacking with drug chromophore. Here the three rings of the chromophore are allowed to stack with various aromatic rings of base pairs, so that all the possible π–π intercalations between the aromatic rings are properly included in the study.

4.2.2. CALCULATION OF STACKING INTERACTION

Further the position of Nx was changed in chromophore (C5-C8), and stacking energies of these azaacridine-4-carboxamides at various positions of base pairs were computed. We have used *ab initio*/6-31G** level of theory for calculating stacking energies of the optimum structure, and corresponding values for 6-31G**/B3LYP rout were also computed to examine the variability of the interaction energies. Further, the interaction energies (only for the stacked portions of drug) and base pairs are computed using MP2/6-31G level of theory.

We know that the choice of basis set as well as inclusion of electron correlation is extremely important for quantitative estimation of stacking energies. However for such large system the use of MP2 level of calculation with large basis set is difficult. So in order to test the feasibility of less expensive calculation in dealing such problems, we have studied stacking of benzene-benzene and benzene-pyridine combinations at the vertical separation of 3.6Å, because such systems contain similar aromatic rings as observed in the stacking of azaacridine-4-carboxamide and base pairs. The stacking energies for the complete orientation of benzene over benzene and pyridine over benzene are studied with different methods. It has been observed that the optimum stacked structures obtained from HF/6-31G, HF/6-31G*, HF/6-31G** and MP2/6-31G** are exactly equal (**Figure 4.6a-h** and **Table 4.2a-b**). In view of these findings we have directly used HF/6-31G** for locating optimum stacked structures of aza-analogues with base pairs. The interaction energies were computed from equation 1.

$$\Delta E = E_{st} - E_{d} - E_{seq} \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

In this equation E_{st}, E_{d} and E_{seq} are the total energies of stacked model, drug and sequences.

4.3 RESULTS AND DISCUSSION

The stacking of chromophore and base pairs are modeled by taking them at vertical separation of 3.6Å, as found in the crystal structure of some acridine-4-carboxamides (**Figure 4.2**). At this distance we have constructed various stacked models of chromophore and base pair so that the aromatic rings (A, B and C of drug) are placed over the aromatic ring of the sequence (ring 1 and 2 of base pair). Here we changed the drug chromophore laterally along XY plane (**Figures 4.3, 4.4** and **4.7a-b**, **Table 4.1a-c**). The interaction

energies of these stacked models are computed by using various levels of theory.

Similarly the interaction energies of stacked models for the carboxamide having N atom at position 5, 6, 7 and 8 are analysed (**Figure 4.3**). The computed interaction energies for optimum stacked models of the 9-aminoaza(N6)acridine-4-carboxamides, 9-aminoaza(N7)acridine-4-carboxamides and 9-aminoaza(N8)acridine-4-carboxamides with GC sequence are given in **Table 4.3** and the corresponding structures are shown in **Figure 4.7a-d**. Likewise the detectable optimum stacked structures of azaacridines having Nx at 6, 7 and 8 positions with AT base pairs are obtained (**Figures 4.8a-d**). Here the interaction energies are also computed at the stacking distance of 3.6 Å, and the interaction energies of the optimum stacked structures of drug and AT sequences are significantly different from those of GC. Indeed 9-aminoazaacridine-4-carboxamides stacks preferably with GC sequences (**Table 4.3 and 4.4**).

In optimum stacked model of azaacridine-4-carboxamide (AZN) and GC base pair, the acridine chromophore lie towards the guanine nucleobase, and the stacking of this drug above the cytosine nucleobase is least stable except in the optimum stacked model of AZN8 with GC where the drug lie towards cytosine (**Figure 4.7a-d**). However we consider only the optimum stacked structure that may be stabilized by π-π interaction. On the other hand AT specificity of drug 9-aminoazaacridine-4-carboxamide is also examined from the stacked structures of chromophore with AT base pair (only π–π) at the vertical separation of 3.6Å (**Table 4.4**). In this case also various arbitrary positions along XY plane of AT base pair are selectively considered. In the optimum stacked structures for AZN6 and AZN7 with AT base pairs, the acridine chromophore is located towards adenine nucleobase whereas in those of AZN5 and AZN8, the drug lie towards thymine (**Figure 4.8a-d**).

We further examined the structure of optimum stacked models of chromophore (having Nx at different position) so that the factor causing to shifting of chromophore in the optimum stacked structure can be sorted out. **Figures 4.7a-d** and **Figure 4.8a-d** show the optimum stacked structures of various azaacridine chromophore (Nx at 5, 6, 7 and 8 position) with GC and AT sequences respectively. As a consequence of changing the position of Nx, the positions of chromophore in the optimum stacked structures vary. Indeed the variation of interaction energies of the optimum stacked structures of various azaacridines having Nx at 5, 6, 7 and 8 positions are different, and the values are shown in **Table 4.5a-b** and **4.6a-b**. It is worth examining the orientation of drug in the optimum

stacked model with respect to the positions of Nx atoms. The relative differences in the interaction energies of optimum stacked structures obtained from HF/6-31G** do not differ much, and similar observation are found for 6-31G**/B3LYP route (**Table 4.3** and **4.4**). At the beginning we have characterized only the $\pi-\pi$ stacking in all these stacked models, but the contribution from the N atoms present in stacked regions (at 5-8 positions) is revealed.

In view of this the positions of N atoms in all these optimum stacked structures are crucial and the contribution of heavy atoms to the stability of these stacked models cannot be ruled out. As we can see that among the aza analogues, the 9-aminoaza(7)acridine-4-carboxamide (with N at position C7) gives rise to the most favorable stacking with AT, and the 9-aminoaza(5)acridine-4-carboxamide stacks most favorably with GC. The values of interaction energies with respect to the position of N are shown in **Table 4.5a-b** and **4.6a-b**. The plot of the optimum stacked structure for different Nx position versus interaction energies of drug and sequence are shown in **Figure 4.9a-b**.

We know that the twisting or unwinding of sequences at the site of interaction may depend on how strongly the drug stacks with the sequences and the resultant unwinding angles after intercalation by various chromophores with DNA has been evidenced ***[1-8]***. Hence the variation of stacking energies with respect to the position of N atoms is an indication how these heavy atoms involve in the stabilization of stacked structures. Heavy atoms contributed to the improvment of interaction energies, as observed in certain models, where the models thet lacks such heavy atom stacking, acquires less stacking energies (**Figure 4.7a-d** and **Figure 4.8a-d**).

In all the optimum structures where the aromatic rings of base pairs tend to stack with the aromatic rings of drug chromophore, the involvement of more N atoms within the stacking regions has been observed. It should be noted that the change in the net charges on Nx atomic sites of chromophore in stacked models compared to free drug may infer the involvement of this atomic sites in some of the stable stacked structures. So we have computed the net charges of N atoms of drug before and after stacking with base pairs (**Table 4.7a-b**).

Initially we focused on the various $\pi-\pi$ stacked models of the aromatic rings present in drug and DNA sequences, but additional contribution from Nx in the stabilization

of these structure is also observed in some cases. **Table 4.7a-b** reveals the variation of net charges obtained from Mulliken population analysis. However no correlation was observed between the net charge on N atoms and interaction energies. This might be due to the coupling of π- π and lone pair-π interactions in the stacking (**Figure 4.10a-d**).

The necessity of electron correlation in the calculation directed to carry out MP2 level of calculation at least for the stacked portions of models having least interaction energies (**Tables 4.3** and **4.4**). Here we have computed the MP2 interaction energies and the values are slightly lower than the values obtained for other methods. However the interaction energies obtained from all other methods show similar variability with MP2 results.

4.4 CONCLUSION

The interaction energies between chromophore and sequences are small but there observed some variations for understanding sequence specificity of this chromophore. The stacking of chromophore with sequences of DNA are demonstrated and the preference of these chromophore for AT or GC sequences are well represented. The contribution of N atoms of both chromophore and base pair on the stacking energies is observed. Also the improved interaction energies have been observed how these heavy atoms try to accommodate within the optimum stacked structures. The interaction energies of various aza analogues of acridinecarboxamides (at 5-8 positions) do not vary much. The coupling of π–π and σ -π interaction might contribute to the stabilization of optimum stacked structures.

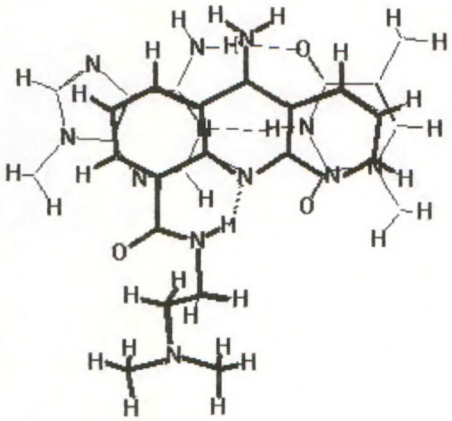

Figure 4.1a

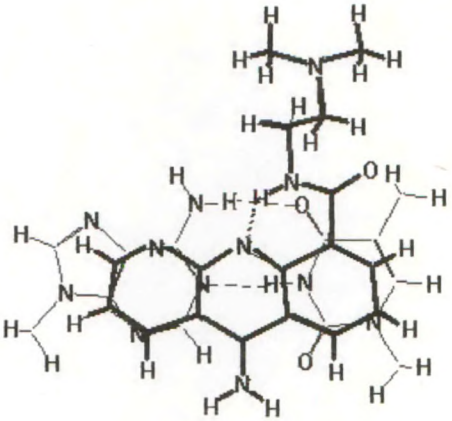

Figure 4.1b

Figure 4.1 a-b – The configuration of sugar and carboxamide side chain with respect to methyl group of base pair.

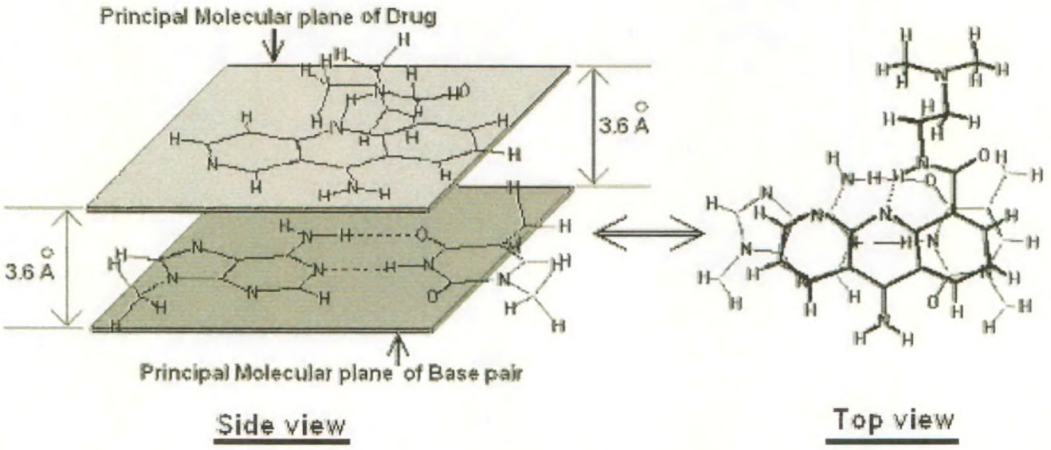

Figure 4.2 – Model of DNA base pair and drug stacking.

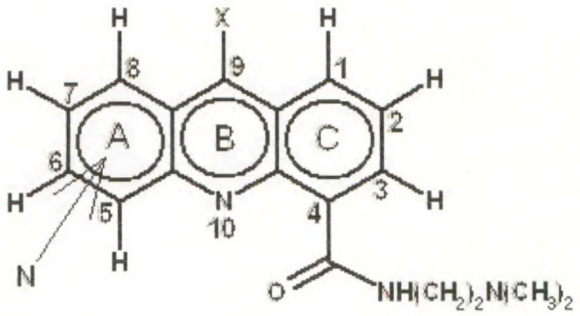

Figure 4.3 - Structure of Azaacridine-4-carboxamide. N at the ring A is attached to location 5, 6, 7 or 8 for forming different aza derivatives. X= -CO, -NH_2, -Cl.

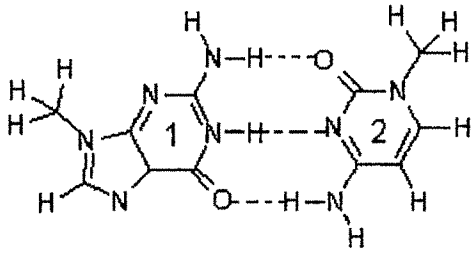

Figure 4.4 - GC Base pair.

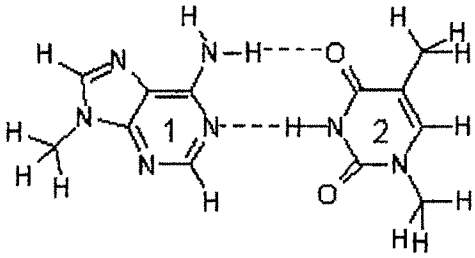

Figure 4.5 - AT Base pair.

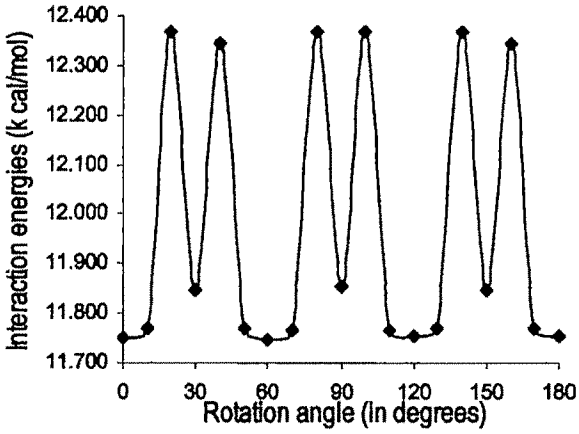

Figure 4.6a – Plot of angle of rotation of two stacked Benzene versus Interaction energies using HF/6-31G.

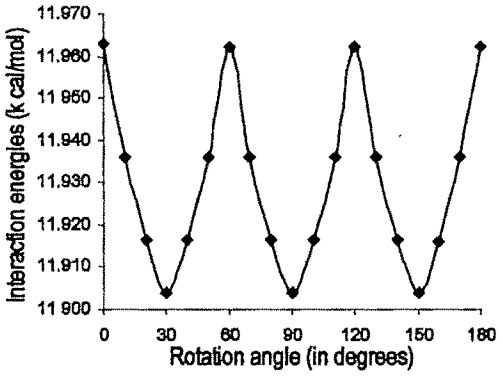

Figure 4.6b – Plot of angle of rotation of two stacked Benzene versus Interaction energies using HF/6-31G*.

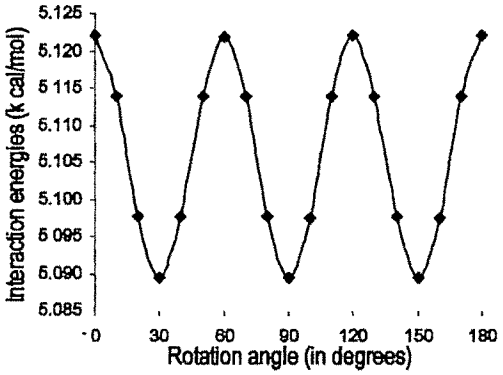

Figure 4.6c – Plot of angle of rotation of two stacked Benzene versus Interaction energies using HF/6-31G**.

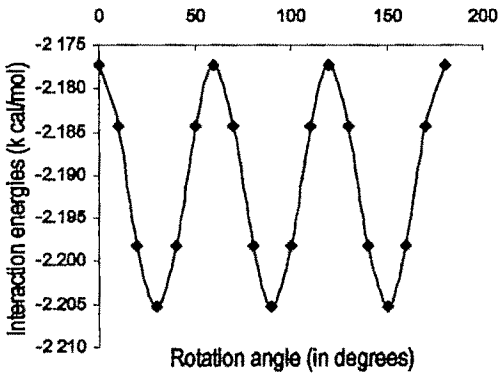

Figure 4.6d – Plot of angle of rotation of two stacked Benzene versus Interaction energies using MP2/ 6-31G**.

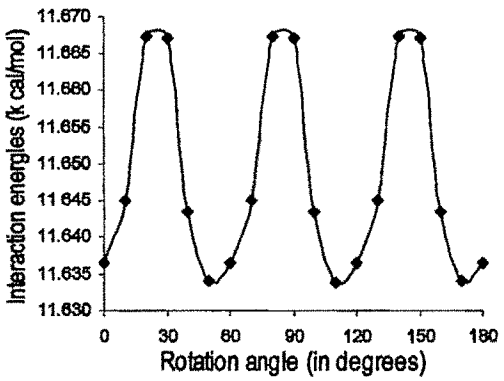

Figure 4.6e – Plot of angle of rotation of stacked Benzene and Pyridine versus Interaction energies using HF/6-31G.

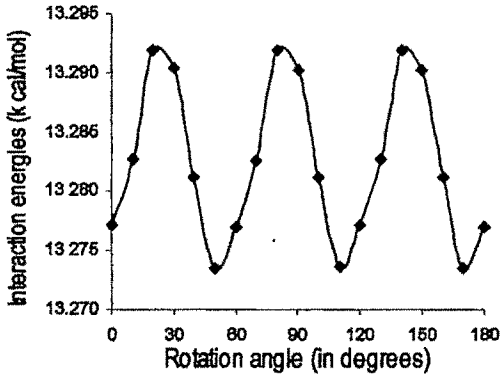

Figure 4.6f – Plot of angle of rotation of stacked Benzene and Pyridine versus Interaction energies using HF/6-31G*.

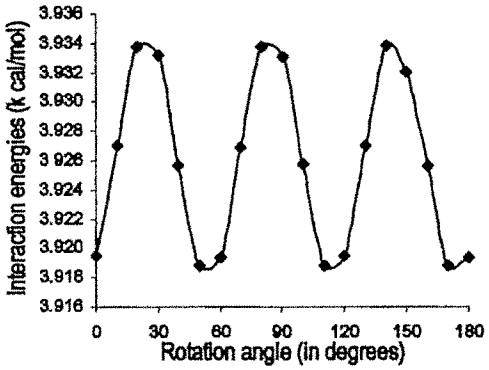

Figure 4.6g – Plot of angle of rotation of stacked Benzene and Pyridine versus Interaction energies using HF/6-31G**.

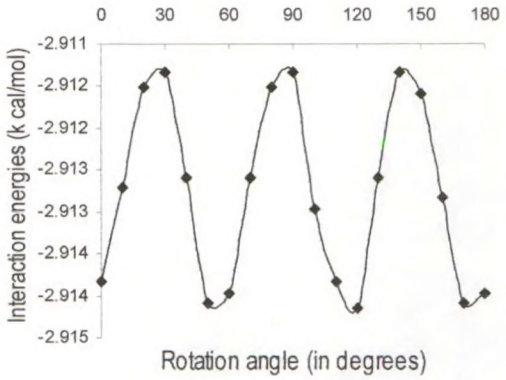

Figure 4.6h – Plot of angle of rotation of stacked Benzene and Pyridine versus Interaction energies using MP2/6-31G**.

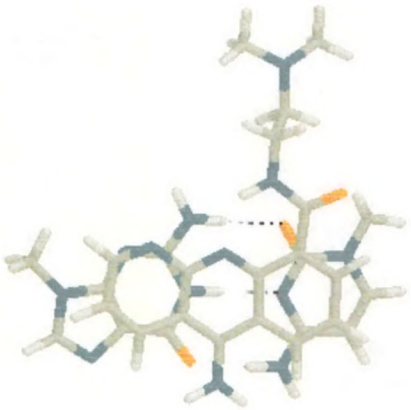

Figure 4.7a- Optimum AZN5-GC Stacking.

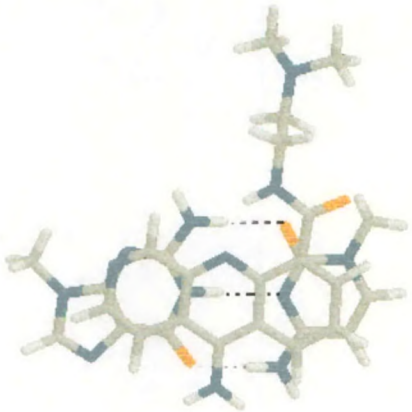

Figure 4.7b- Optimum AZN6-GC Stacking.

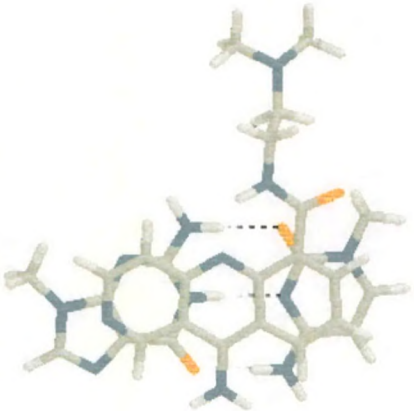

Figure 4.7c- Optimum AZN7-GC Stacking.

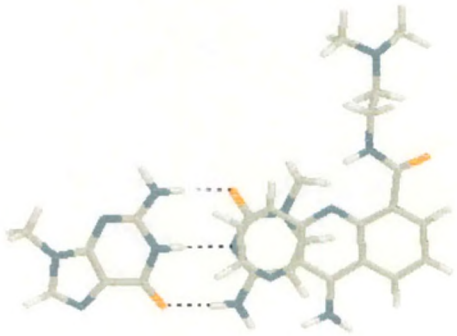

Figure 4.7d- Optimum AZN8-GC Stacking.

Figure 7(a), (b), (c), (d)- Optimum stacked structure of GC and drugs (AZN5, AZN6, AZN7 and AZN8.)

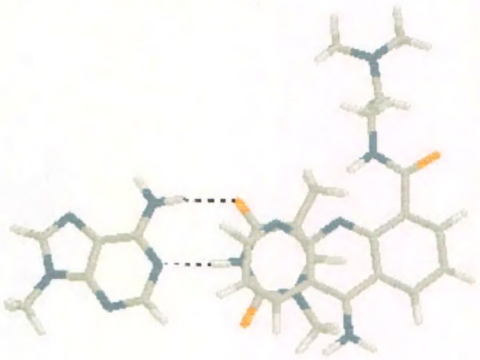

Figure 4.8a- Optimum AZN5-AT Stacking.

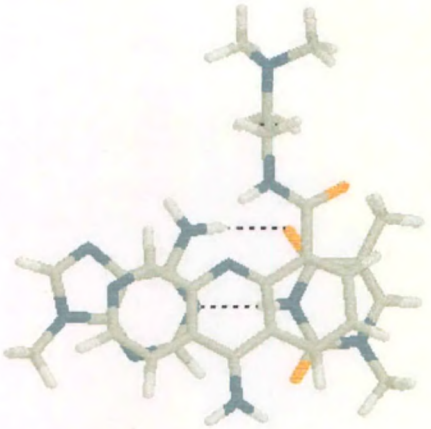

Figure 4.8b- Optimum AZN6-AT Stacking.

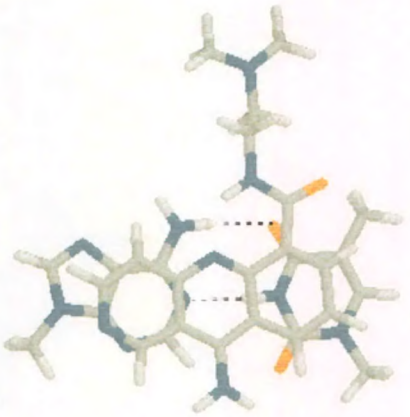

Figure 4.8c- Optimum AZN7-AT Stacking.

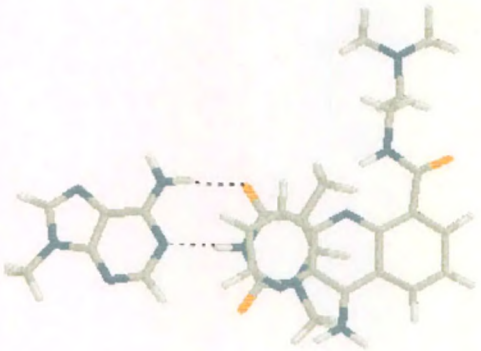

Figure 4.8d- Optimum AZN8-AT Stacking.

Figure 8(a), (b), (c), (d) - Optimum stacked structure of AT and drug (AZN5, AZN6, AZN7 and AZN8.)

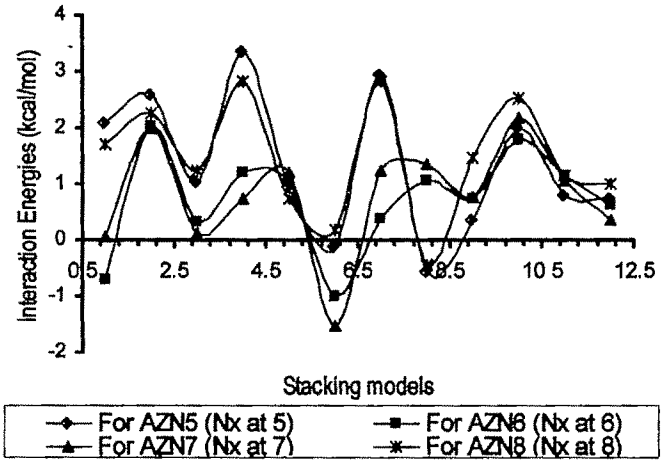

Figure 4.9a - Plot for stacking models versus variation of Interaction energies of 9-aminoazaacridine-4-carboxamide (AZN) with different position of Nx and AT.

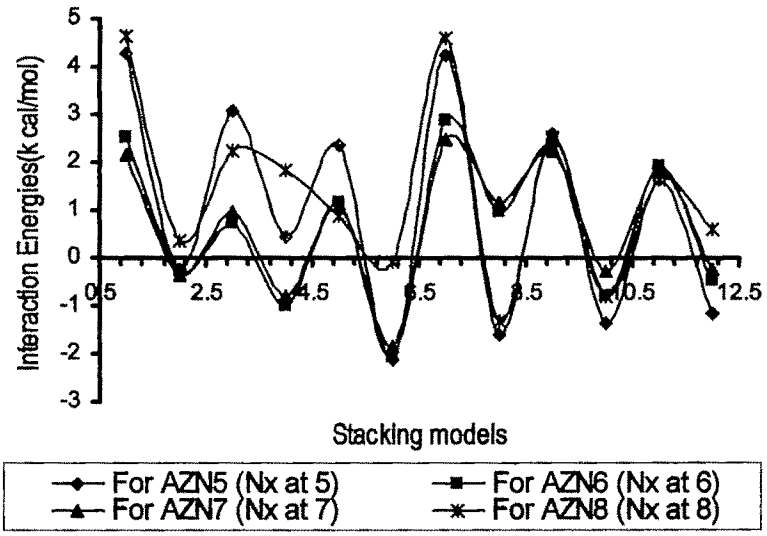

Figure 4.9b - Plot for stacking models versus variation of Interaction energies of 9-aminoazaacridine-4-carboxamide (AZN) with different position of Nx and GC.

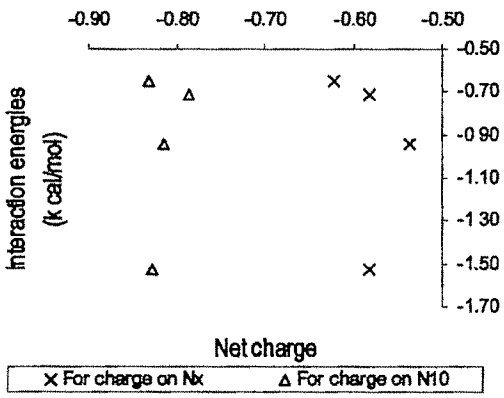

Figure 4.10a - Net charge on Nx and N10 versus Interaction energy variation of AZN and AT base pair stacking. (HF/6-31g**)

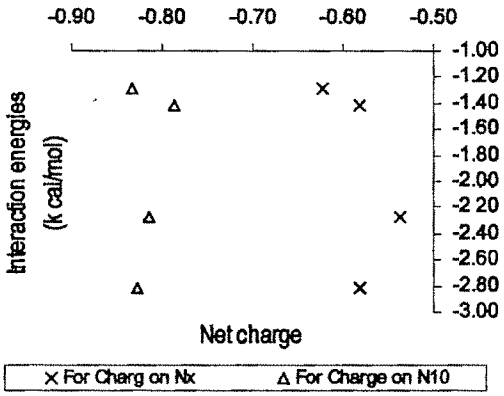

Figure 4.10b - Net charge on Nx and N10 versus Interaction energy variation of AZN and AT base pair stacking. (B3LYP/6-31g**)

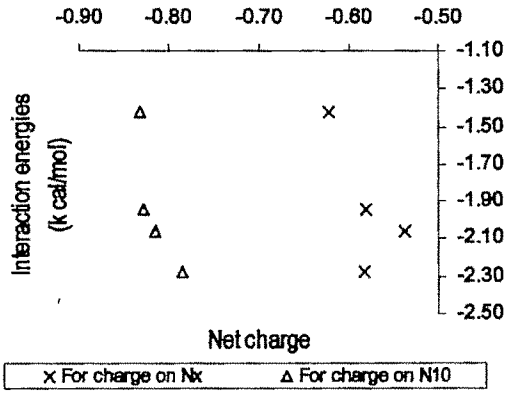

Figure 4.10c - Net charge on Nx and N10 versus Interaction energy variation of AZN and GC base pair stacking. (HF/6-31g**)

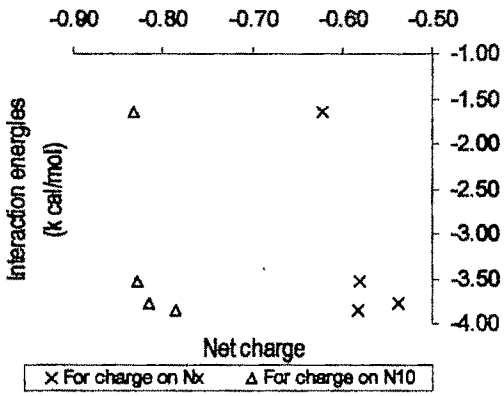

Figure 4.10d - Net charge on Nx and N10 versus Interaction energy variation of AZN and GC base pair stacking. (B3LYP/6-31g**)

Table 4.1a – Construction of various stacking models (π - π stacking) of 9-aminoazaacridine-4-carboxamide (AZN) and AT base pair.

Structure name (AT-AZNx-n)	Stacking location of Base pair	Stacking location of Drug
AT-AZNx-1	Ring-1	Ring-C (minor)
AT-AZNx-2	Ring-1	Ring-C (major)
AT-AZNx-3	Ring-1	Ring-B (minor)
AT-AZNx-4	Ring-1	Ring-B (major)
AT-AZNx-5	Ring-1	Ring-A (minor)
AT-AZNx-6	Ring-1	Ring-A (major)
AT-AZNx-7	Ring-2	Ring-A (minor)
AT-AZNx-8	Ring-2	Ring-A (major)
AT-AZNx-9	Ring-2	Ring-B (minor)
AT-AZNx-10	Ring-2	Ring-B (major)
AT-AZNx-11	Ring-2	Ring-C (minor)
AT-AZNx-12	Ring-2	Ring-C (major)

x = position of Nx; n = stacking location. ; (minor) = minor groove; (major) = major groove

Table 4.1b – Construction of various stacking models (π - π stacking) of 9-aminoazaacridine-4-carboxamide (AZN) and GC base pair.

Structure name (GC-AZNx-n)	Stacking location of Base pair	Stacking location of Drug
GC-AZNx-1	Ring-1	Ring-C (major)
GC-AZNx-2	Ring-1	Ring-C (minor)
GC-AZNx-3	Ring-1	Ring-B (major)
GC-AZNx-4	Ring-1	Ring-B (minor)
GC-AZNx-5	Ring-1	Ring-A (major))
GC-AZNx-6	Ring-1	Ring-A (minor)
GC-AZNx-7	Ring-2	Ring-A (major)
GC-AZNx-8	Ring-2	Ring-A (minor)
GC-AZNx-9	Ring-2	Ring-B (major)
GC-AZNx-10	Ring-2	Ring-B (minor)
GC-AZNx-11	Ring-2	Ring-C (major)
GC-AZNx-12	Ring-2	Ring-C (minor)

x = position of Nx; n = stacking location. ; (minor) = minor groove; (major) = major groove

Table 4.1c – Construction of various stacking models of 9-aminoazaacridine-4-carboxamide (AZN) and base pair Stacking.

Structure name (XX-AZNx-n)	Stacking location of Base pair	Stacking location of Drug
AT-AZNx-S1	Ring-2	substituents at C9 (minor)
AT-AZNx-S2	Ring-2	substituents at C9 (major)
AT-AZNx-S3	Ring-1	substituents at C9 (minor)
AT-AZNx-S4	Ring-1	substituents at C9 (major)
GC-AZNx-S1	Ring-2	substituents at C9 (major)
GC-AZNx-S2	Ring-2	substituents at C9 (minor)
GC-AZNx-S3	Ring-1	substituents at C9 (major)
GC-AZNx-S4	Ring-1	substituents at C9 (minor)

XX= AT or GC; x = position of Nx; n = stacking location. ; (minor) = minor groove; (major) = major groove

Table 4.2a– Interaction energies of Benzene-Benzene stacking in different basis sets and theory at vertical separation of 3.6Å.

Angle of rotation	Interaction energies (in k cal/mol)				
	HF/STO-3G	HF/6-31G	HF/6-31G*	HF/6-31G**	MP2/6-31G**
30°	7.881	11.847	11.904	5.089	-2.205
90°	7.881	11.854	11.904	5.089	-2.205
150°	7.881	11.847	11.904	5.089	-2.205

Table 4.2b – Interaction energies of Benzene-Pyridine stacking in different basis sets and theory at vertical separation of 3.6Å.

angle of rotation	Interaction energies (in k cal/mol)				
	HF/STO-3G	HF/6-31G	HF/6-31G*	HF/6-31G**	MP2/6-31G**
0	6.966	11.637	13.277	3.919	-2.914
50	6.966	11.634	13.274	3.919	-2.914
110	6.965	11.634	13.274	3.919	-2.914
170	6.966	11.634	13.274	3.919	-2.914

Table 4.3 – The computed Interaction Energies of the optimum stacked models of 9-aminoazaacridine-4-carboxamide (AZN) and GC base pair at different theory level. (in kcal/mol)

Stacking Geometry (GC-AZNx-n)	HF/6-31G*	HF/6-31G**	B3LYP/ 6-31G**	MP2/ STO-3G
GC-AZN5	-2.213	-2.277	-3.850	-2.832
GC-AZN6	-1.990	-2.058	-3.766	-2.847
GC-AZN7	-1.866	-1.942	-3.522	-2.584
GC-AZN8	-1.415	-1.419	-1.638	-1.919

p = position of Nx; n = stacking location

Table 4.4 - The computed Interaction Energies of the optimum stacked models of 9-aminoazaacridine-4-carboxamide (AZN) and AT base pair at different theory level. (In kcal/mol)

Stacking Geometry (AT-AZNx-n)	HF/ 6-31G*	HF/ 6-31G**	B3LYP/ 6-31G**	MP2/ STO-3G
AT-AZN5	-0.684	-0.717	-1.413	-1.353
AT-AZN6	-0.908	-0.942	-2.271	-2.708
AT-AZN7	-1.493	-1.524	-2.813	-2.962
AT-AZN8	-0.622	-0.649	-1.284	-1.338

p = position of Nx; n = stacking location.

Table 4.5a – Comparison of Interaction Energies for different Nx position on 9-aminoazaacridine-4-carboxamide (AZN) stacked with AT base-pair with side chain at **minor grooves** (HF/6-31G) (in k cal/mol)

Stacking Geometry (AT-AZN-n)	AZN5 (Nx at 5)	AZN6 (Nx at 6)	AZN7 (Nx at 7)	AZN8 (Nx at 8)
AT-AZN-1	2.077	**-0.720**	0.049	1.715
AT-AZN-3	1.040	0.336	0.128	1.243
AT-AZN-5	0.936	1.043	1.219	0.721
AT-AZN-7	2.938	0.391	1.240	2.822
AT-AZN-9	0.342	0.729	0.768	1.472
AT-AZN-11	0.792	1.146	1.047	1.137

n = stacking location.

Table 4.5b - Comparison of interaction energies for different Nx position on 9-aminoazaacridine-4-carboxamide (AZN) stacked with AT base-pair with side chain at **major grooves** (HF/6-31G) (in k cal/mol)

Stacking Geometry (AT-AZN-n)	AZN5 (Nx at 5)	AZN6 (Nx at 6)	AZN7 (Nx at 7)	AZN8 (Nx at 8)
AT-AZN-2	2.585	2.031	1.998	2.266
AT-AZN-4	3.356	1.204	0.723	2.829
AT-AZN-6	**-0.115**	**-0.995**	**-1.531**	0.181
AT-AZN-8	**-0.554**	1.062	1.356	**-0.431**
AT-AZN-10	1.986	1.806	2.169	2.539
AT-AZN-12	0.745	0.628	0.351	0.991

n = stacking location.

Table 4.6a - Comparison of Interaction energies for different Nx position on 9-aminoazaacridine-4-carboxamide (AZN) stacked with GC base-pair with side chain at **major grooves** (HF/6-31G) (in k cal/mol)

Stack Geometry (GC-AZN-n)	AZN5 (Nx at 5)	AZN6 (Nx at 6)	AZN7 (Nx at 7)	AZN8 (Nx at 8)
GC-AZN-1	4.279	2.520	2.172	4.621
GC-AZN-3	3.082	0.7268	0.943	2.251
GC-AZN-5	2.348	1.153	1.123	0.862
GC-AZN-7	4.254	2.890	2.468	4.591
GC-AZN-9	2.613	2.362	2.232	2.537
GC-AZN-11	1.654	1.904	1.842	1.643

n = stacking location.

Table 4.6b - Comparison of interaction energies for different Nx position on 9-aminoazaacridine-4-carboxamide (AZN) stacked with GC base-pair with side chain at **minor grooves** (HF/6-31G) (in k cal/mol)

Stack Geometry (GC-AZN-n)	AZN5 (Nx at 5)	AZN6 (Nx at 6)	AZN7 (Nx at 7)	AZN8 (Nx at 8)
GC-AZN-2	**-0.227**	**-0.272**	**-0.358**	0.368
GC-AZN-4	0.437	**-1.007**	**-0.797**	1.822
GC-AZN-6	**-2.132**	**-2.078**	**-1.834**	**-0.099**
GC-AZN-8	**-1.602**	0.952	1.168	**-1.336**
GC-AZN-10	**-1.350**	**-0.796**	**-0.289**	**-0.818**
GC-AZN-12	**-1.176**	**-0.465**	**-0.256**	0.611

n = stacking location.

Table 4.7a – Variation of Net Charge on Nx of optimum stacked structure of AT and 9-aminoazaacridine-4-carboxamide (AZN).

Optimum Stacked Structure (AT-AZNx-n)	Total Atomic Charge on Nx	
	Free Drug	Interacted Drug
AT-AZN5	-0.525	-0.530
AT-AZN6	-0.513	-0.520
AT-AZN7	-0.552	-0.565
AT-AZN8	-0.584	-0.587

p = position of Nx; n = stacking location

Table 4.7b – Variation of Net Charge on Nx of optimum stacked structure of GC and 9-aminoazaacridine-4-carboxamide (AZN).

Optimum Stacked Structure (GC-AZNx-n)	Total Atomic Charge on Nx	
	Free Drug	Interacted Drug
GC-AZN5-6	-0.525	-0.533
GC-AZN6-6	-0.513	-0.515
GC-AZN7-6	-0.552	-0.562
GC-AZN8-8	-0.584	-0.592

p = position of Nx; n = stacking location.

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