CHAPTER 4

A MODEL STUDY ON THE STACKING INTERACTION OF 1, 10-PHENANTHROLINE LIGAND WITH NUCLEIC ACID BASE PAIRS: AN *AB INITIO*, MP2 AND DFT STUDIES
**SUMMARY**

The stacking interaction of 1,10-phenanthroline (phen) ligand within base pairs of DNA is one of the important factors for the stabilization of metal-phen complex within DNA. The stacking ability of this ligand has been assessed to identify the base pair selectivity within intercalation site. Several studies have been used to predict the favorable regions for stacking interactions of phen ligand with base pair. The results of MP2/6-31+G(d,p) is found to be reasonably good for monitoring such interactions.
4.1 INTRODUCTION:

The interactions between aromatic ligands and base pairs of DNA are important characteristics of certain potential anticancer agents, and the sequence specificity of ligand in DNA binding has been the major concern as shown in many literatures [1-7]. There are wide ranges of anticancer drugs, such as acridine derivatives, m-AMSA and daunomycin etc, that intercalate within sequences of DNA [8-10]. The tris 1,10-phenanthroline (phen) metal complexes have been known as important compounds for their antitumour activity. Most phen metal complexes bind with DNA either through non-covalent interaction within base pairs or within major and minor grooves (Figure 4.1). The charge transfer band from metal to ligand of some complexes found in experimental studies is an important characteristic of ligand intercalation [11-17]. Such non covalent interactions also partly contribute to the stabilization of metal complex within DNA.

It has been evidenced that some complexes can bind better with d(CGCGCG)$_2$ oligonucleotide than d(GTGCAC)$_2$, and the $^1$H NMR chemical shift of drug-DNA adduct of these oligonucleotides may be taken as an indirect implication of phen ligand intercalation with d(CGCGCG)$_2$, but there is no concrete evidence to distinguish the groove binding and intercalation within these oligonucleotides [4-16]. Although, the structural disposition of intercalated drug and conformational changes of double helical DNA can be analyzed by experimental methods like, $^1$H NMR and X-Ray diffraction studies, it may be necessary to understand insight into the energetic of weak interactions between phen ligand and base pair. As we know that $^1$H NMR chemical shift can only infer the structural information of metal complex-oligonucleotide adduct [17-20]. Nevertheless, it is likely that this ligand may also intercalate within GC sequences of d(CGCGCG)$_2$ unlike the binding within minor groove of d(GTGAC)$_2$ due to the presence of AT base pair. Also, the drug may not easily access to oligonucleotide for intercalation, and the drug can be stabilized within the grooves of DNA.

In certain crystal structures the phen ligand is found partially intercalated within two thymine nucleobases [18]. As we know that the stacking interaction depends on the charge transfer capability of phen ligand and base pair. The stability of intercalated metal coordinated-phen complexes within base pair sequences may be either due to the
stacking interactions with base pairs or perhaps depend on the conformational accessibility of ligand towards minor groove. In view of this, it is noteworthy to compare the stacking interactions between phen ligand and base pairs of DNA. In the sense that the stacking interactions of phen ligand with AT may be different from that of GC base pairs, thereby produces AT or GC specificity during ligand intercalation.

*Ab initio* calculations have been found useful for computing weak non-bonded interactions. In most cases, the accurate *ab initio* methods are recommended for studying such type of interactions, where inclusion of large basis set and electron correlation is always necessary [21-27]. The stacking interactions of aromatic molecules can be estimated from the dispersion forces, short-range exchange repulsion and electrostatic interactions, while the intermolecular electron correlation between aromatic rings is the core factor for calculating the dispersion forces. However, it is often found that the less accurate force field methods and even the density functional method have been successfully applied to large molecules [25-26]. Most DFT methods are not appropriate tools for studying stacking interactions because of its failure to estimate dispersion energies of stacked molecules. Moreover, the *ab initio* calculations with the inclusion of correlation effect at least at the MP2/6-31G* level have been successfully used in some cases [27]. The applications of correlated *ab initio* methods are limited to small molecules, and the Moller-Plesset perturbation theory (MP2) is used for medium size molecules, but consumes lots of time for large molecules. As we know that the *ab initio* methods like CCSD(T) is not so popular for large molecules, and in some cases the DFT method may describe the π-π stacking of large molecules [24-29]. Although most DFT method cannot be used for calculating the dispersion energies required for the stabilization of stacked structures, the additional empirical terms included in this method may sometimes be useful for computing some dispersion forces. In this context, there are many other concerns over the limitations of high level *ab initio* methods for studying non-bonded interactions particularly for large molecules [23-29]. Also, systematic analysis of various stacked models is always necessary, since certain configurations might produce strong repulsion, which is not suitable for calculating weak stacking interactions. On the other hand, the stacking pattern of charged drugs with base pair may again hamper in describing the non bonded π-π interactions for some charged intercalators [23]. For such molecules, comparison of
results obtained from different level of theories may not particularly explain the stacking interactions perfectly. To rationalize such issues, the phen ligand, which has been used in many potential metal-based anticancer drugs has been taken up for assessing the stacking interactions with base pairs.

4.2 METHODOLOGY:

The geometries of AT, GC and phen ligand were completely optimized with HF/6-31G** method. The completely optimized geometries were taken for constructing the stacked models. The optimum structures of stacked phen ligand with base pair were identified from various stacked structures by translating the phen ligand over AT and GC, and stacking energies were computed with different methods. All models are constructed at the intermolecular separation of 3.6 Å, since the available crystal structure of metal-phen complex, and also most aromatic ligands intercalate within base pairs approximately at this distance [10-12, 18]. We have used rigid geometries of the stacked molecules i.e. the relaxation of the geometries of drug and base pair after interaction was not considered, and single point calculations [MP2/6-31G(d,p)] have been carried out on the stacked molecules. The stacking energies were also computed with several methods with different basis set just to check the variation of energies values.

In order to visualize the structural characteristic of phen ligand and base pair interaction, we have analyzed the different model structures, and the most favored structure so obtained was compared with the available crystal structure [18]. The stacking energies for mutual orientations (both horizontal and axial shifting) of phen ligand have been carefully analyzed by constructing several stacked models with the help of self developed programme package (JoinMolecule) [30]. Accountable error in the stacking energies might occur due to small deviation of certain parameter from the uniformity in the model construction. So, the Join Molecule package has been developed for constructing desired models accurately.

The stacking energies were calculated from the following equation,

$$\Delta E = E_{ST} - E_B - E_{PH}$$

$E_{ST}$, $E_B$ and $E_{PH}$ are the energies of stacked structure, base pair and phen ligand respectively. It is extremely important to choose appropriate basis set in the \textit{ab initio}
calculations [29]. In the present study, different types of basis set, which are tested in several calculations, have been used. The stacking energies and the most favored stacked phen ligand and base pairs obtained from various calculations are compared.

4.3 RESULTS AND DISCUSSION:

The variation of stacking energies calculated with MP2/6-31+G(d,p) for some arbitrarily chosen stacked structures of phen ligand with GC and AT are shown in Figures 4.3 and 4.4. The optimum structures are identified from the energy minima in the plots (Figure 4.2), and the corresponding stacking energies are given in Table 4.1. Similarly, the variation of stacking energies with different basis set in the DFT calculations is also analyzed. The stacking energies of most stable structures are given in Table 4.2, where the values of SVP, LANL2DZ, and cc-PVDZ are too small for the analysis of stacked phen with base pair. The plots in Figures 4.3 and 4.4 may be used for illustrating the relative change of stacking energies with the position of stacked phen within AT and GC. The stacking energies with the diffused function (6-31+G(d,p) basis set) in the MP2 calculations are found better than the 6-31G** basis set with the improved stacking energies of ~10 kcal/mol, and the dispersion interaction included in the calculations may be useful for analyzing the stabilization of stacked structures. The method has been found useful for analyzing similar type of chemical issues [20-24].

Although different level of theories and basis set have been taken up in the study, the results of MP2/6-31+G(d,p) are mainly used for demonstrating base pair specificity of phen ligand. In fact, inclusion of diffused function and the electron correlation in this method may be appropriate for analyzing the stacking interactions. It has been known from various investigations on weak interactions that the inclusion of diffused function, such as 6-31+G(d,p) in the MP2 calculation lead to the cancellation of errors in combination with that of MP2 methods [20-21]. The HF calculations have been carried out for several stacked structures, and the variation of stacking energies are slightly basis set dependent (Table 4.1). The stacking energies obtained from HF calculations are all positive, which clearly demonstrate the weakness of the level of theory for studying stacked molecules.

The most favorable positions of phen ligand in stacked AT and GC can be analyzed from the plots of MP2/6-31+G(d,p) calculations. The optimum structures are
shown in Figures 4.2(a) and 4.2(b), where the phen ligand is found preferably towards T of AT, and C of GC. The theoretically predicted structure of phen-AT is found complementary to the reported position of phen in crystal structure of phen coordinated metal complexes shown in Figure 4.2(c) [18]. In this case, the two oxygen atoms in thymine could result better interaction with phen ligand, whereas the hydrogen bonded region in AT is not a preferable region for ligand stacking. Tables 4.1 and 4.2 show the stacking energies of the most stable structures obtained from different level of theories and basis set. The stacking energies obtained from MP2 method may be compared with that of DFT and HF methods, where the MP2/6-31+G(d,p) energies are within the range of -23 to -21 kcal/mol. Although high level calculations may be required for such systems, the MP2/6-31+G(d,p) may still be used for understanding stacking interactions. In order to check the effect of diffused function in the basis set of MP2 calculation, we have taken various basis set in the calculations. The stacking energies are found consistently more negative with the inclusion of diffused function than that of without diffused function in the basis set. Moreover, the limitation of DFT method for computing dispersion interaction is distinctly shown, in spite of its applicability for some large molecules as reported in literatures [24-27]. We have also carried out extensive calculations of various stacked models with HF and DFT methods for comparison with MP2 method, but the stacking energies of HF calculations are uniformly positive and small negative values are found for DFT calculations. Tables 4.1 and 4.2 demonstrate the trend of stacking energies of MP2, HF and DFT for some chosen basis set. Hence, the electron correlation effect included in the MP2/6-31+G(d,p) may be useful for explaining the stabilization of stacked phen with base pairs, and the ordering of HF and MP2 energies is not very drastically contradictory (Table 4.1). Comparison of the MP2/6-31G+G(d,p) results with that of more accurate ab initio method is rather difficult for such large systems. But the different sets of calculations with HF, DFT and MP2 may give some information on the stacking stabilization of phen ligand and base pair.

However, the optimum structures obtained from different methods are not exactly similar, and highly basis set dependent. The stacking energies of most favorable structure with MP2/6-31+G(d,p) calculation is found to be approximately -23 kcal/mol compared to -9 kcal/mol for MP2/6-31G calculation. There are no other reported ab
*initio* calculations on stacked phen with base pair, and some calculations on the stacking interaction of small molecules are available [20,21,29]. Moreover the position of phen predicted by MP2/6-31+G(d,p) agrees well with that of crystal structure, but the stacking energies of experimentally reported stacked geometry is higher by 10 kcal/mol [Figure 4.2(c) and Table 4.1] [18]. The molecular geometry of crystal structure is not equal to optimized geometry, and it may be due to other factors like crystal packing and additional effect from accompanied solvent molecules as well as ions in the crystal. The stacking energy of phen ligand with GC is found more negative compared to that of AT, and it shows that the phen ligand intercalation within GC rich oligonucleotide is possible as evidenced in some studies [20].

### 4.4 Conclusion:

The stacking interaction of phen ligand with base pair is clearly explained in this work. Both AT and GC base pairs may stabilize phen ligand, but preferably with GC base pair. The predicted stacked structure of phen ligand with AT base pair is found complementary to the available crystal structure. The study demonstrates that the stacking energies of MP2/6-31+G(d,p) may be useful for studying such systems, although the accurate *ab initio* method is necessary. The DFT calculation cannot be used to demonstrate the energetic of aromatic ring stacking in spite of the inclusion of diffused function in the basis set. Even the stacking energies of MP2 method with smaller basis set are reasonably good for application to large molecules when the calculation with accurate *ab initio* method is not possible.

### 4.5 References:

Table 4.1. The stacking energies (kcal/mol) of stacked phen with base pairs computed with HF and MP2 calculations.

<table>
<thead>
<tr>
<th>Base pair</th>
<th>Basis sets</th>
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<tbody>
<tr>
<td></td>
<td>6-31G</td>
</tr>
<tr>
<td>GC</td>
<td>-10.29</td>
</tr>
<tr>
<td></td>
<td>(1.82)</td>
</tr>
<tr>
<td>AT</td>
<td>-9.44</td>
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<td></td>
<td>(3.40)</td>
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(*) Bracketed values are for HF method and the value with star is for crystal structure.

Table 4.2. The stacking energies (kcal/mol) of stacked phen with base pairs computed with DFT calculations.

<table>
<thead>
<tr>
<th>Base Pair</th>
<th>Basis sets</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LANL2DZ</td>
</tr>
<tr>
<td>GC</td>
<td>-0.42</td>
</tr>
<tr>
<td>AT</td>
<td>2.10</td>
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</table>
Figure 4.1. Intercalation of 1,10-phenanthroline with base pairs.
Figure 4.2. The most stable structures of stacked phen with (a) AT, (b) GC and (c) T (crystal structure).
Figure 4.3. Plots of stacking energies (kcal/mol) vs. distances (Å) of GC sequence for MP2 method with different basis set, (a) 6-31+G(d,p)  (b) 6-31G**.
Figure 4.4. Plots of stacking energies (kcal/mol) vs. distances (Å) of AT sequence for MP2 method with different basis set, (a) 6-31+G(d,p) (b) 6-31G**.