# Compatibility of molecular structures of Diphenyl diamines drugs with minor groove of DNA and the importance of cationic amidine group in DNA binding

**Bipul Bezbaruah, Rituraj Kalita, Pankaj Hazarika, Rajib L Sarma and C Medhi***

Department of Chemistry, Gauhati University (P.O.), Guwahati–781014, INDIA.

Email: chitrani@sify.com

**Abstract.** The binding of Diphenyl diamine drugs within the minor groove of DNA has been investigated by using force field and *ab initio* MO methods. Four drugs of different molecular sizes are taken to examine the compatible sizes of drugs for binding within minor groove of DNA. It is one of the essential characteristic of minor groove binding drugs. In addition the energetic of cationic amidine group in DNA binding is also analysed to differentiate the dominating factors for minor groove binding. The crescent sized molecules that fit to the minor groove of DNA can bind effectively within the minor groove. The role of cationic amidine groups of these drugs in DNA binding is significant. These charged groups interact with various atomic sites present within minor groove. The formation of stable drug-DNA complexes depends on the types of intermolecular hydrogen bonds formed in the complexes. The hydrogen bonds between amidine groups of drugs and thymine nucleobase can stabilize the drug within AT sequences, and it is one of the reasons for AT sequence selectivity of this drug.

# 1  Introduction

The Diphenyl diamine (DB) drugs are known for their antimicrobial as well as antitumour properties. The interactions of these drugs with biological molecules inhibit many biochemical reactions [1, 2, 3, 4, 5]. Also these drugs are capable of penetrating within the cisplatin resistant cell lines that could be related to the physio-chemical behavior of cationic amidine group. It has been evidenced that the dicationic drugs acquire more anticancer property than the monocationic drugs [6, 7, 8, 9, 10, 11, 12]. There are many minor groove binding DB drugs, and very few drugs can bind within the major groove of DNA [5, 13, 14]. Moreover the presence of aromatic rings in some DB drugs drastically affect the anticancer property and that could be due to the decrease of cell uptake [6, 15, 16]. The interaction ability of cationic amidine group with various atomic sites in minor groove may be another important aspect of forming stable drug-DNA complexes. There are many other donor-acceptor sites in DNA for forming hydrogen bonds in DB-DNA complexes [3, 15, 17]. The hydrogen bonds formed in DB-DNA complexes could stabilize the drug molecule within the minor groove of DNA. In the sense, those drugs that can access completely within the minor groove may form hydrogen bonds effectively. All these minor groove binders must acquire basic structural compatibility with that of minor groove. Although there are several reasons for binding drugs within minor groove, but the compatibility of molecular structures of drug molecule with the binding region may be the basic requirement to form stable DB-DNA complexes. The molecules having compatible structures with the crescent width of minor groove may favorably form hydrogen bonds with the donor-acceptor sites present in the minor groove of DNA. It is rather crucial to determine the concrete correlation between molecular size and DNA binding ability of these drugs. So the present study focuses on the nature of DNA binding groove binding ability of few DB drugs having different molecular structures and sizes (Figs. 1(a) and 1(b). As such the forma-

tion of stable drug-DNA complex arises from the van der Waals interactions between donor-acceptor sites of a particular region. It has been known that the distribution of electron density within various parts of DNA is different. Accumulation of maximum electron density within the minor groove of DNA has been shown. This region may attract the cationic group of DB drugs that result favourable binding of this drug within minor groove. Therefore it is rather important to study the nature of drug binding within various sequences of DNA. The study may be useful to understand the minor groove selectivity of DB drugs. The applications of accurate quantum mechanical methods to large molecules are limited. Force field calculations have certain advantages than the quantum mechanical studies for its application to large molecules. The formation of DB-DNA complexes can be understood from the local minimum electrostatic energies obtained from force field studies [18, 19]. It is not feasible to carry out complete geometry optimization of such large molecules with quantum mechanical methods. Hence the force field method has been used in the present investigation.

## 2   Methodology

The geometries of DB drugs are initially optimized with HF/6-31G method before proceeding to force field calculations on drug and DNA complexes [18, 19]. The electrostatic stabilization energies of DB-DNA complexes are calculated with CHARMm force method [18]. The structures of DB-DNA complexes are completely minimized with CHARMm force field minimization protocol. The electrostatic interaction (stabilization) energies are computed from (1).

$$\Delta E = E_{\text{COM}} - (E_{\text{NU}} + E_D) \tag{1}$$

The $E_{\text{COM}}$, $E_{\text{NU}}$ and $E_D$ are the electrostatic energies of DB-DNA complex, DNA and drug. Energy minimization was performed with steepest decent technique up to the energy convergence of 0.001. The dielectric constant of surrounding solvent was taken as 80 to represent aqueous medium. However we have checked the binding of these drugs within the minor groove of various sequences with receptor-ligand docking protocol The binding mode of these DB drugs is again evaluated from the large number of ligand-receptor docking poses. First we have taken the possibility of docking within

the particular sequences of DNA . The receptor ligand docking algorithm implemented in CDocker has been used to find the interaction energies.

# 3    Construction of models

The pentamidine(PN), propamidine(PR), stilbamidine(ST) and berenil(BN) drugs have been chosen in the present study (Fig. 1). The structure of oligonucleotide d(ACCGACGTCGGT) is obtained from crystallographic database [20]. We have chosen certain regions of DNA having different sequence combinations. The positions of DB drugs within various regions of the minor groove are changed to locate the most favorable regions of drug selected for binding. The models of DB-DNA are constructed similar to some reported crystal structures [14, 21, 22]. In these models, the interaction between amidine group (cationic) and thymine nucleobase is particularly considered to analyze the contribution of cationic group in DNA binding. Here the positions of drugs are adjusted with respect to the interaction distances between cationic amidine group and thymine nucleobase, and subsequently the structures are minimized. The DB drugs bind with thymine within AT rich regions of DNA. So the selectivity of thymine by cationic amidine group of DB drugs within AT sequence is particularly taken for analysis. The structures of DB drugs are curve with typical crescent shape. Here in all DB-DNA complexes, the matching of drug crescent with that of minor groove is also carefully examined. In this investigation we have examined the different types of hydrogen bonds formed in DB-DNA complexes.

# 4    Results and Discussion

In order to understand the binding of DB drugs within minor groove of DNA, we have computed the electrostatic stabilization energies of these drugs. The electrostatic stabilization energies of BN, PR, PN and ST are given in Table 1. The energies are obtained from the minimized structures of DB-DNA complexes. The most favorable orientations of BN for entering inside the minor groove are obtained from the correlation plots of interaction energies versus positions of drugs within this groove (Fig. 2). The variation

of the energies in this plot is due to steric hindrance from different sites in the minor groove. Similarly the plots for PR, PN and ST are shown in Figs. 3, 4 and 5. The energy minima so identified have been used to locate the most favorable position of drug within the minor groove. The inner hydrogen bonds formed in minimized complexes of BN-DNA complexes can be visualized from the structures given in Fig. 6. Table 2 displays the nature of the intermolecular hydrogen bonds formed in this complex. The minimized ST-DNA complex shown in Fig. 7 clearly indicates the position of ST within the minor groove as well as the nature of hydrogen bonds in this complex. The electrostatic stabilization energy of BN is found more negative than that of ST, and the hydrogen bonds formed in these two complexes are not exactly similar. One of the two amidine groups of ST and BN interact with thymine nucleobase present in minor groove whereas the other amidine group is involved in hydrogen bonding with different atomic sites of DNA (Table 2). These drugs can form hydrogen bonds in a different manner within the minor groove, and also the corresponding electrostatic stabilization energies are found different (Tables 3 and 4). Among the four drugs, BN and ST molecules acquire almost equal crescent sizes. So the variations of interaction energies of these two drugs can be compared to distinguish the factors responsible for the binding of drugs within minor groove of DNA. These drugs hydrogen bonds formed either by amidine groups or by other donor-acceptor sites of drugs within minor groove. The electrostatic stabilization energy of BN is somewhat more negative than that of ST. It may be due to the presence of additional donor atoms (nitrogen atoms) in between the rings of BN (Table 1). As mentioned above, the interaction between amidine group and thymine is mainly considered in constructing the models of DB-DNA complexes as reported in crystal structure [20]. The BN molecule can bind perfectly within the minor groove of DNA , but no prominent hydrogen bonds between amidine group of drug and thymine nucleobase are observed. The nature of the hydrogen bonds formed by these two drugs within minor groove of DNA is not exactly equal. The molecular sizes of PR and PN are larger than BN and ST. We have compared the binding of these drugs within minor groove. The electrostatic stabilization energy of PR-DNA is found more negative than PN-DNA (Table 1). It may be due to the differences in the hydrogen bonds formed in these complexes. The amidine group of PN forms hydrogen bond with thymine nucleobase from the exterior side of the minor groove. In the

minimized structures of PN-DNA and PR-DNA, the drug molecules are found outside the minor groove (Figs. 8 and 9). Also PN binds with DNA less efficiently than PR. It may be due to the interaction of only one amidine group with thymine in PN-DNA complex. Both these molecules acquire larger crescent size than that of minor groove and it may be the reason why these drugs bind with DNA from the exterior position. Among these drugs BN and ST acquire compatible molecular sizes to the crescent size of minor groove, hence these drugs can bind favorably within the minor groove. So the formation of stable complexes within the minor groove depends on the crescent sized molecular structures of drugs. In fact both the amidine groups of ST and BN form hydrogen bonds within minor group resulting stable drug-DNA complexes. The hydrogen bonds formed by these molecules are given in Table 2. Hence the interaction between cationic amidine groups and thymine of DNA is one of the factors for stabilizing drug-DNA complex but the drug molecule may not bind within the minor groove. The compatibility of crescent sized drugs with minor groove size is an essential criterion for minor groove binding drugs. Such information is also a crucial part in the rational drug design. Moreover the cationic drugs could be advantageous in other physio-chemical aspects like cell penetration ability of drugs [24]. It is rather important to examine the stability of proton in the cationic form of drugs. Indirectly, the stability of cationic form of amidine groups can be estimated from the dissociation energies (DE) of proton from this group. The DE of proton from amidine groups of these drugs and the Mulliken net charges on the respective atoms of amidine groups are shown in Table 5. The DE of BN is ∼1.1 kcal/mol more than that of ST. This may be due to the presence of additional basic atoms (nitrogen in between aromatic rings) in berenil. Hence the amidine group of BN acquires more cationic behavior than that of ST. As shown in Table 1, the difference of electrostatic stabilization energies of ST and BN is small. The DE of proton for PN is more than that of PR. Such variation of DE might be useful for understanding the cationic nature of amidine groups. The interaction energies calculated from the docked ligands within three different sequence combinations are found different(Table 6). Among these drugs the ST molecule can interact with all sequences better than other drugs.

# 5    Interaction sites and AT specificity of drugs

It has been shown that the BN, ST, PR and PN can form hydrogen bonds with various atomic sites of DNA. The number of inter molecular hydrogen bonds in the complexes have been analyzed to understand the binding of drugs with DNA (Table 1). It has been found that the amidine group of BN forms hydrogen bond in a different manner than that of ST (Figs. 6 and 7). The two amidine groups of PN form hydrogen bonds with certain sites of DNA from the exterior side of minor groove. In fact hydrogen bonding pattern for PR is different from PN(Table 2). Hence the nature of hydrogen bonds formed between drug and DNA is quite different, and also the formation of drug-DNA complexes depends on the energetic of amidine group and on the crescent sized molecules. The number of hydrogen bonds in all these drug-DNA complexes ranges from 1 to 3. For the minor groove binding drugs the crescent sized molecule should fit perfectly within the minor groove of DNA, and also the formation of hydrogen bonds between amidine group and thymine is observed in the minimized complexes. So the minor groove binding drugs must acquire compatible molecular size with the size of the minor groove. The large sized drugs can bind effectively with DNA from the exterior region of DNA. but the position of molecule is not within the minor groove. The presence of AT sequences in minor groove is necessary for interaction between amidine group and thymine of DNA. It may be the basis of AT sequence selective binding of drugs in some crystal structures [3, 12, 15, 23]. However the other atomic sites of drug can also form hydrogen bond with DNA. Hence the type of hydrogen bond formation strictly depends on the positions of drug within the minor groove. Only the accessible atomic sites can form hydrogen bonds within minor groove. Thus the binding ability of PN, PR, ST and BN depends entirely on the types of hydrogen bonds. The differences in the electrostatic stabilization energies of small and large molecules are $\sim$60 kcal/-mol. It appears that the interaction ability of the cationic amidine groups with thymine nucleobases is one of the factors for stabilizing drug within the minor groove. However the other interaction sites cannot be ignored. The structures of DB-DNA complexes formed by these drugs within minor groove of DNA oligomer are not uniform in terms of hydrogen bonds. The two positively charged amidine groups provide the potential binding sites of interaction with available thymine nucleobases in the minor groove. In

this respect the thymine nucleobase should be the preferred site for binding with two cationic amidine groups. In the sense that the AT specificity of these drugs basically depend on the presence of thymine nucleobase for interaction with amidine groups of drugs.

# 6   Conclusion

The structural aspects and molecular sizes of these drugs are important basis for binding of drugs within minor groove of DNA. The interaction between cationic amidine groups and thymine nucleobase is one of the factors for stabilizing drug-DNA complex. However the formation of other hydrogen bonds is also found in some drug-DNA complexes. Apparently the stabilization of drug within DNA depends on the nature of the hydrogen bonds formed within that region. The AT sequence selectivity of DB drugs is found to be due to the interaction of amidine group and thymine nucleobase.

### Acknowledgements

Table 1: Computed electrostatic energies of Drug-DNA complexes, DNA drugs and the corresponding electrostatic stabilization energies.

| Most stable Drug-DNA complexes | Energies of Drug-DNA complexes (kcal/mol) | Electrostatic energies of DNA oligomer (kcal/mol) | Electrostatic energies of drugs (kcal/mol) | Electrostatic stabilization Energies (kcal/mol) |
|---|---|---|---|---|
| BN | -3427.74 |  | -38.59 | -2011.05 |
| PR | -3463.48 | -1378.09 | -42.76 | -2042.62 |
| PN | -3333.20 |  | -41.61 | -1913.49 |
| ST | -3382.71 |  | -34.35 | -1970.27 |

Table 2: The formation of H-bonds in the minimized structures of Drug-DNA complexes.

| Complexes of cationic drug with DNA | Number of H-bond between amidine group and O of Thymine | Number of H-bond between amidine group and O of Sugar | Number of other H- bonds | Total H-bonds |
|---|---|---|---|---|
| BN-DNA | 1 | – | 1 (with C) | 2 |
| PR-DNA | – | – | 1 (with C) | 1 |
| PN-DNA | – | – | 2 (with A & $PO_4$) | 2 |
| ST-DNA | 1 | – | 1 (with $PO_4$) | 2 |

C = cytosine and A = Adenine



(a)                                                    (b)

(c)                                                    (d)
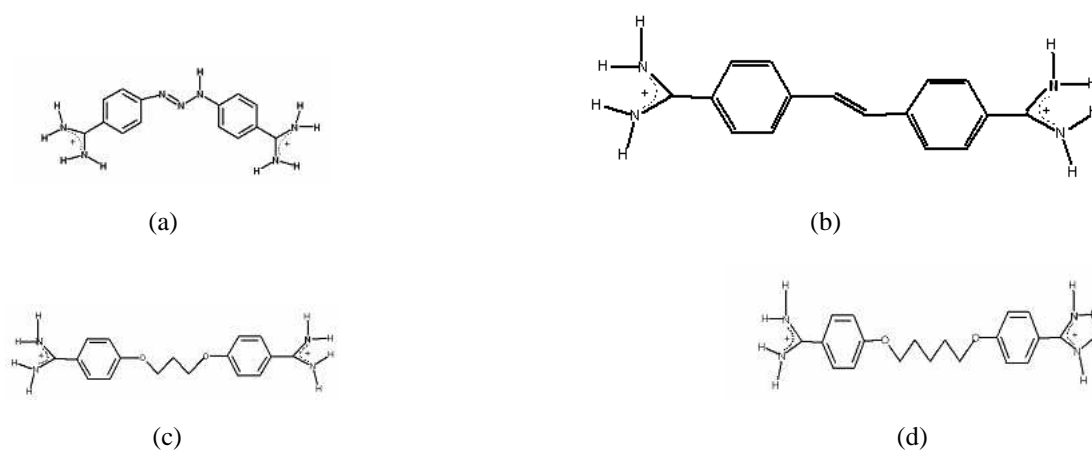
Figure 1: Structures of (a) Berenil (BN), (b) Stilbamidine (ST) (c) Propamidine (PR), (d) Pentamidine(PN).

Table 3: Computed electrostatic stabilization energies of Drug-Oligonucleotide complex at various positions.

| Positions of drug within minor groove | Electrostatic energies of the minimised DB-DNA complexes (kcal/mol) | Electrostatic energies of DNA oligomer (kcal/mol) | Electrostatic energies of drugs (kcal/mol) | Electrostatic stabilization energies (kcal/mol) |
|---|---|---|---|---|
| | | BN | | |
| 1 | -3110.07 | | | -1693.39 |
| 2 | -3161.85 | -1378.09 | -38.59 | -1745.17 |
| 3 | -3148.32 | | | -1731.64 |
| 4 | -3107.75 | | | -1691.07 |
| | | PR | | |
| 1 | -3199.39 | | | -1778.54 |
| 2 | -3307.19 | -1378.09 | -42.76 | -1886.33 |
| 3 | -3310.96 | | | -1890.11 |
| 4 | -3160.07 | | | -1739.21 |
| | | PN | | |
| 1 | -3311.52 | | | -1891.82 |
| 2 | -3314.26 | -1378.09 | -41.61 | -1894.56 |
| 3 | -3156.83 | | | -1737.12 |
| 4 | -3112.03 | | | -1692.32 |
| | | ST | | |
| 1 | -3078.26 | | | -1665.81 |
| 2 | -3129.45 | -1378.09 | -34.35 | -1717.00 |
| 3 | -3107.43 | | | -1694.99 |
| 4 | -3069.31 | | | -1656.87 |

Table 4: The formation of hydrogen bonds between cationic drug and DNA oligomer and H-bond distances at different positions.

| Drugs | Positions of drug within minor groove | Number of H-bond between amidine group and O of Thymine | Number of H-bond between amidine group and O of Sugar | Number of H-bonds of other atomic sites | Total H-bonds |
|---|---|---|---|---|---|
| BN | 1 | 1 | 1 | 1(With C) | 3 |
| | 2 | 1 | – | 1(With C) | 2 |
| | 3 | 1 | 1 | – | 2 |
| | 4 | – | 1 | – | 1 |
| PR | 1 | 1 | – | 1(With $PO_4$) | 2 |
| | 2 | – | – | 2(With C & $PO_4$) | 2 |
| | 3 | – | – | 2(With C & $PO_4$) | 2 |
| | 4 | – | 1 | 1(With G) | 2 |
| PN | 1 | 1 | – | 1(With $PO_4$) | 2 |
| | 2 | – | – | 2(with A & $PO_4$) | 2 |
| | 3 | 1 | – | – | 1 |
| | 4 | 1 | – | – | 1 |
| ST | 1 | 1 | 1 | 1(With $PO_4$) | 3 |
| | 2 | 1 | 1 | 1(With $PO_4$) | 3 |
| | 3 | – | – | 1(With $PO_4$) | 1 |
| | 4 | – | 1 | – | – |

Table 5: The computed Mulliken net charges and dissociation energies (DE) of proton from amidine group.

| Cationic drugs | Mulliken net charges on two amidine groups | | Mulliken net charges on the hydrogen atoms of amidine group | | Dissociation energies (DE) of proton (kcal/mol) |
|---|---|---|---|---|---|
| | N(-NH$_2$) | N(-NH$_2$) | H | H | |
| BN | -0.707 | -0.708 | 0.373 | 0.365 | 486.439 |
| PN | -0.717 | -0.719 | 0.359 | 0.368 | 501.393 |
| PR | -0.717 | -0.715 | 0.369 | 0.361 | 495.819 |
| ST | -0.709 | -0.708 | 0.372 | 0.365 | 485.352 |

Table 6: The computed interaction energies obtained from receptor ligand docking protocol.

| Drugs | Interaction energies (kcal/mole) within various sequences | | |
|---|---|---|---|
| | TCGGT | ACGTC | ACCGA |
| BN | -4.058 | -4.155 | -4.155 |
| ST | -6.631 | -7.526 | -6.587 |
| PR | -3.776 | -3.824 | -4.106 |
| PEN | -3.306 | -4.564 | -3.132 |
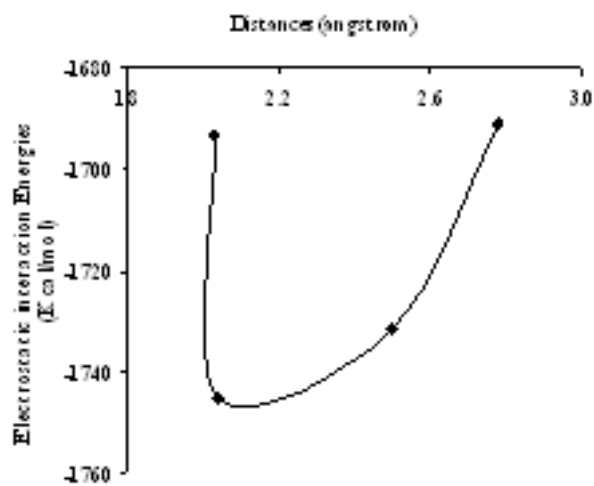
Figure 2: Plot of interaction energies versus positions of BN within the minor groove.
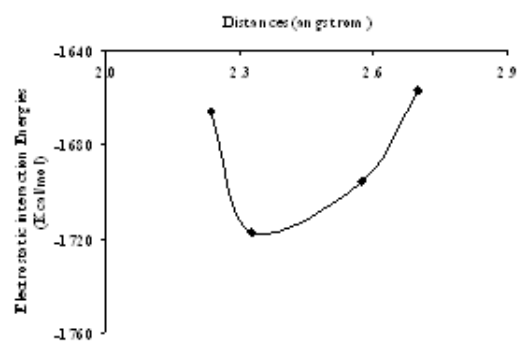


Figure 3: Plot of interaction energies versus positions of ST within the minor groove.
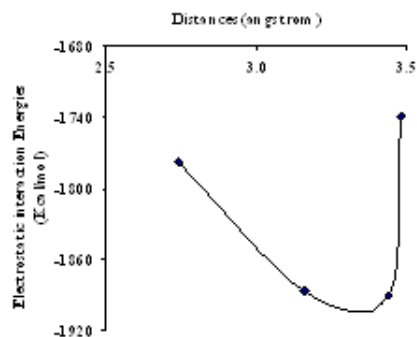
Figure 4: Plot of interaction energies versus positions of PR within the minor groove.
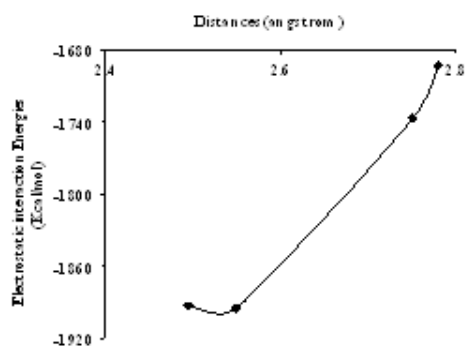


Figure 5: Plot of interaction energies versus positions of PN within the minor groove.
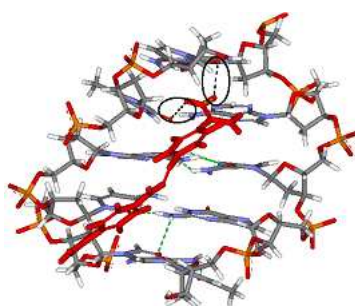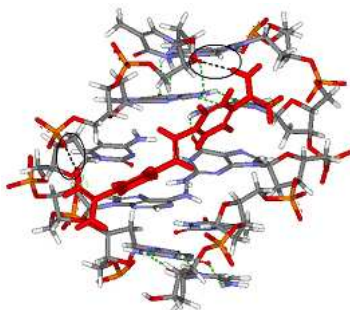


Figure 6: Minimized structure of BN-DNA.

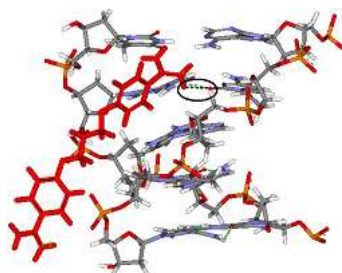Figure 7: Minimized structure of ST-DNA.
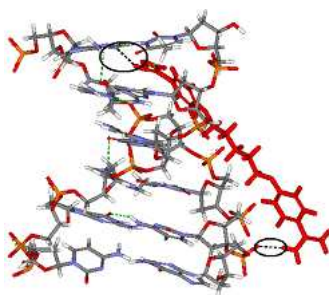


Figure 8: Minimized structure of PR-DNA.



Figure 9: Minimized structure of PN-DNA.

# References

[1] L.Amélie, T.Farial, M.Zohar, D.Laurent, K.Arvind, E. S.Chad, H.Qiyue., W.W. David, W.B. David, B. Christian, *Distribution of Furamidine Analogues in Tumor Cells: Targeting of the Nucleus or Mitochondria Depending on the Amidine Substitution*, Cancer Research, **62** No.15 (2002) 7219–7229.

[2] A.Y. Chen, C. Yu, B. Gatto, L.F. Liu, *DNA minor groove binding ligands: A different class of mammalian DNA topoisomerase I inhibitors*, Proc. Natl. Acad. Sci. USA, Pharmacology. **9** (1993) 8131–8135.

[3] A. L.Crowell, C. E.Stephens, A.Kumar, D. W.Boykin, W. E.Secor, *Activities of dicationic compounds against Trichomonas vaginalis*, Antimicrob, Agents Chemother. **48** No.9 (2004) 3602–3605.

[4] M. de S. Elen, M.O. Gabriel, W.B. David, K. Arvind, H. Qiyue, C.S. Maria De Nazaré, *Trypanocidal activity of the phenyl-substituted analogue of furamidine DB569 against Trypanosoma cruzi infection in vivo*, J of Antimicrobial Chemotherapy. **58** (2006) 610–614.

[5] S. Erikson, S.K. Kim, M. Kubista, B. Nordden *Binding of 4′,6-diamidino-2-phenylindole (DAPI) to AT regions of DNA: Evidence for an allosteric conformational change*, Biochemistry **32** (1993) 2987–2998.

[6] Y. Kubota, K. Kubota, S. Tani, *DNA binding properties of DAPI (4′,6-diamidino-2-phenylindole) analogs having an imidazoline ring or a tetrahydropyrimidine ring: Groove-binding and intercalation*, Nucleic Acids Symposium Series No. Oxford University Press **44** (2000) 53–54.

[7] K. Jansen, B. Norden, M. Kubista, *Sequence dependence of 4′,6-diamidino-2-phenylindole (DAPI)-DNA interactions*, J. Am. Chem. Soc. **115** (1993) 10527–10530.

[8] G. Manzini, L. Xodo, M.L. Barcellonal, F. Quadrifoglio, *Interaction of DAPI with double-stranded ribonucleic acids*, Nucleic Acids Research, **24** No.13 (1985) 8955–8967.

[9] A.M. Mathis, J.L. Holman, L.M. Sturk, M.A. Ismail , D.W. Boykin, R.R. Tidwell, J.E. Hall, *Accumulation and Intracellular Distribution of Antitrypanosomal Diamidine Compounds DB75 and DB820 in African Trypanosomes*, Antimicribial Agents and Chemotherapy.No. 50, **6** (2006) 2185–2191.

[10] U. Schlstedt, S.K. Kim, B. Norden, *Binding of 4′,6-diamidino-2-phenylindole to [poly(dI-dC)]2 and [poly(dG-dC)]2: the exocyclic amino group of guanine prevents minor groove*

*binding*, J. Am .Chem. Soc. **115** 1(993) 12258–12263.

[11] S. Mazur, F.A. Tanious, D. Ding, A. Kumar, D.W. Boykin, I.J. Simpson, S. Neidle, W.D. Wilson, *Large Hydrophobic Contributions Are Involved in DNA Minor-Groove Complex Formation with a Series of Diphenylfuran Dications: Surface Plasmon Resonance and Isothermal Titration Calorimetry Studies*, Journal of Molecular Biology. **300** No.2 2000 321–337.

[12] S. Mohan and N. Yattindra, *Flexibility of DNA in 2:1 drug-DNA complexes simultaneous binding of two DAPI molecules to DNA*, J Biomol Struct. Dyn, **11** (1994) 849–867.

[13] E.D. Trotta, E. Ambrosio, N. Del Grosso, G. Ravagnan, M.Cirilli, M. Paci, *1H NMR study of [d(GCGATCGC)]2 and its interaction with minor groove binding 4′,6-diamidino-2-phenylindole*, J. Biol. Chem. **268** (1993) 3944–3951.

[14] J.J. Welch, F.J. Rauscher, T.A. Beerman, *Targeting DNA-binding drugs to sequence-specific transcription factor DNA complexes. Differential effects of intercalating and minor groove binding drugs*, J. Biol. Chem. **69** No.2 (1994) 31051–31058.

[15] A. M.Mathis, A.S. Bridges, M.A. Ismail, A. Kumar, I. Francesconi, M. Anbazhagan, Q. Hu, F.A. Tanious, T. Wenzler, J. Saulter, W.D. Wilson, R. Brun, D.W. Boykin, R.R. Tidwell, J.E. Hall, *Diphenyl Furans and Aza Analogs: Effects of Structural Modification on In Vitro Activity, DNA Binding, and Accumulation and Distribution in Trypanosomes*, Antimicrob. Agents Chemother. **51** No 8 (2007) 2801–2810.

[16] K. Storl, J. Storl, C.H. Zimmer, J.W. Lown, *Minor-groove binders are inhibitors of the catalytic activity of DNA gyrases*, FEBS Lett. **317** 1 (993) 157–167.

[17] J. Kapuscinski, B. Skoczylas, *Fluorescent complexes of DNA with DAPI 4′,6-diamidine-2-phenyl indole 2HCl or DCI 4′,6-dicarboxyamide-2-pnenyl indole*, Nucleic Acids Research, **10** No.5, (1978), 3775–3799.

[18] Accelrys inc, San Diego, U.S.A.

[19] Gaussian Inc, Pittsburgh P.A, 2003.

[20] H. Rozenberg, D. Rabinovich, F. Frolow, R.S. Hegde, Z.Shakked, *Structural code for DNA recognition revealed in crystal structures of papillomavirus E2-DNA targets*, Proc. Natl Acad.Sci.USA. **95** (1998) 15194–15199.

[21] F.A. Tanious, J.M. Veal, H. Buczak, I.S. Ratneyer, W.D. Wilson, *DAPI 4′,6-diamidino-2-phenylindole) binds differently to DNA and RNA: minor groove binding at AT sites and intercalation at AU sites*, Biochemistry. **31** (1992) 3103–3112.

[22] D. Vlieghe, J. Sponer, L.V. Meervelt, *Crystal Structure of d(GGCCAATTGG) Complexed with DAPI Reveals Novel Binding Mode*, Biochemistry. **38** (1999) 16443–16451.

[23] C. Parolin, G. Zanotti, G. Palu, *A Model for the Sequence-Dependent DNA Binding of 4′,6-Diamidino-2-Phenylindole (DAPI).*, Biochem. Biophys. Res. Commun. **208** (1995), 332–338.

[24] E.M. De.Souza, A. Lansiaux, C. Baily, W.D. Wilson, Q. Hu, D.W. Boykin, M.M. Batista, T.C. Araujo-Jorge, M.N. Soerio, *Phenyl substitution of furamidine markedly potentiates its anti-parasitic activity against Trypanosoma cruzi and Leishmania amazonensis*, Biochem. Pharmacol. **68** No.15 2004, 593–600.