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Quantum Mechanical Study of some Intercalating and Groove Binding Anticancer Drugs with AT and GC Base Pair of DNA Nucleobase

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Anticancer drugs bind to DNA nucleobase pairs (AT and GC) through different binding modes such as intercalation, groove binding, covalent binding *etc*. Quantum mechanical method such as, density functional theory (DFT) is quite useful for computing the interaction energies of anticancer drug-DNA nucleobase complexes. In our study, we have selected some candidate anticancer drugs to investigate the interaction energies of drug-DNA complexes. Among the different binding modes of anticancer drugs, minor and major groove binding to DNA base pair are important ones; therefore, some anticancer drugs may be minor groove specific and some may be major groove specific. Such sequence-specific experimental studies for drug-DNA nucleobase complexes are very complicated; hence, theoretical calculations based on quantum mechanical theories are helpful. Here, we performed DFT calculations using M062X method and 6-311++G(d,p) basis set. Our results reveal that the stacked models of anticancer drugs-DNA nucleobase (AT and GC) complexes all show negative interaction energy values. Among all such complexes, the complex with the most negative interaction energy value indicates the most stable and favoured stacked system. The stacking interaction energies for anticancer drugs-DNA nucleobase (AT and GC) complexes could easily be reflected in the interaction energy plots.

Keywords: Anticancer drugs, DNA nucleobase, AT, GC, DFT Methods, M062X *etc*.

INTRODUCTION

Anticancer drug is a kind of drug which is efficient in the treatment of malignant or cancerous disease. Chemotherapy is the treatment of cancer with anticancer drugs that can destroy cancer cells. These drugs could generate their anticancer activities either by inhibiting or modifying the growth of cancer cells, or by killing those cells [1-2]. It is well established that DNA is the main target of all anticancer agents. An empirical study has shown that compounds with anticancer activity target the DNA nucleuobase by inhibiting the enzymes; this can control the DNA integrity and provide building blocks for DNA nucleobase [3]. Resent research on anticancer agent has established several therapeutic modalities targeting DNA antimetabolites, which deplete nucleotides, including folic acid antagonists such as methotrexate. Alkylating agents cause direct DNA damage (*e.g.* nitrogen mustard and its derivatives) and intercalators, such as actinomycins, bind to DNA and inhibit the activity of many enzymes that use DNA nucleobase as a substrate. Now a days, the most widely used anticancer agents are nonspecific DNA-damaging chemicals, inhibitors of topoisomerases (TOPO) I and II, antimetabolites, alkylating agents, and agents that cause covalent modification of DNA nucleobase [3-4].

The anticancer agents can be divided into two broad categories: those with covalent and those with non-covalent interaction with DNA nucleobase [5]. The mechanism of covalent binding of anticancer agents to DNA (*e.g. cis*-platin binding to guanine bases) is irreversible causing permanent stall of transcription and may lead to cell death. On the other hand, the non-covalent interaction between anticancer agent and DNA nucleobase is usually reversible

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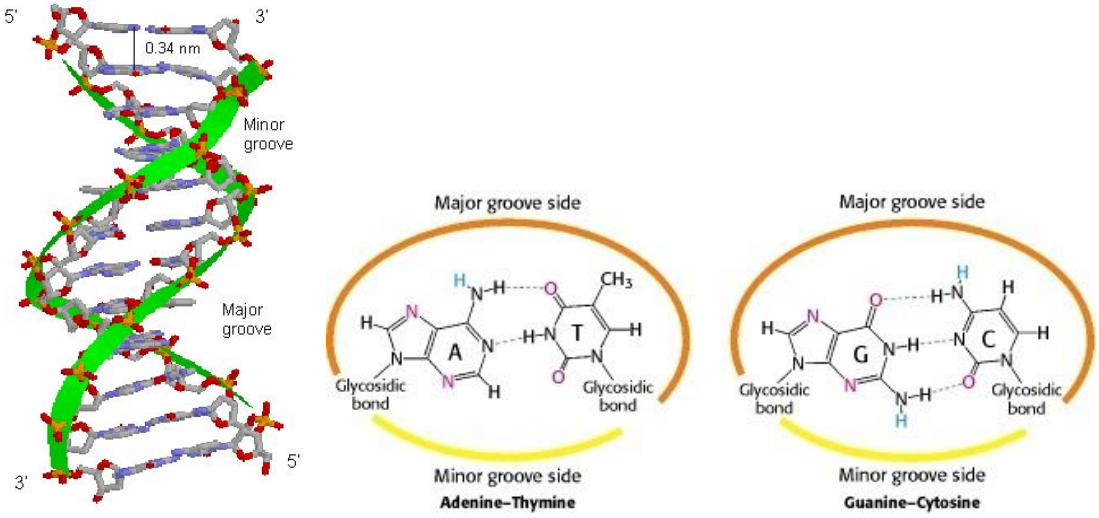


Fig. 1. Major and Minor Groove of DNA base pair (AT and GC).

and such anticancer agents can be classified as intercalators or groove binders. Natural and designed molecules that display multivalency in DNA recognition by binding at more than one recognition sites *i.e.* minor or major groove have been reported [6-7]. Moreover, other mode of non-covalent reversible interaction between DNA and anticancer agent is called DNA intercalation. DNA intercalators are often used as good chemotherapeutic agents [8-9]. Generally, DNA intercalators are composed of planar aromatic ring or heteroaromatic groups, which is capable of stacking between the adjacent base pairs of DNA nucleobase. These drug-DNA complexes are stabilized by weak van der Waals force, π-π stacking interaction, charge transfer force or hydrophobic interaction [10]. Several DNA intercalating drugs have been identified over the years, including daunomycin (trade name Cerubidine), doxorubicin (trade name Adriamycin), epirubicin (anthracycline family), dactinomycin (trade name Cosmegen), ditercalinium, bleomycin, elsamicin A, m-AMSA, mitoxantrone, acridines, ethidium bromide, *etc.* [11-14].

Groove binding is a kind of standard lock-and-key type mechanism for ligand-macromolecular binding and it does not induce large conformational changes in DNA nucleobase. Groove binding anticancer drugs are usually crescent-shaped molecules that bind to the minor or major groove of DNA nucleobase. It happens because such groove binders are stabilized by intermolecular interactions and typically have larger association constants compared to intercalators (approximately 10^{11} M^{-1}), as the free energy is found to be more negative at binding site [15]. Groove binding drugs are classified into two categories, minor groove and major groove binders as shown in Fig. 1.

All biological macromolecules interact with the major or minor groove of DNA nucleobase via hydrogen bonding interaction. A detailed review on natural products and DNA major groove binders such as pluramycins, aflatoxins, azinomycins, leinamycins, aminosugars, and neocarzinostatins was reported, including their binding mechanisms and sequence specificity [16]. These drug molecules bind to the edges of the base pairs of the DNA duplex (usually GC sites in the major groove, and AT sites in the minor groove) by reversible non-covalent interactions. Such binding interactions reduce the conformational freedom of the molecules and usually are opposed to an unfavourable entropic cost. However, these energetic costs and hydrophobic interactions in drug-DNA complexes are well balanced through equilibrium. Examples of such minor groove binding drugs are netropsin, distamycin, pentamidine, DAPI, *etc.* [17-18].

Anticancer drugs such as 9-aminoacridine(AA), Acriflavine (ACF), Niclosamide(NA), Proflavine(PF), Pyridoacridine (PY), and Phenanthridine (PT), are found as good intercalators and they may also bind to the minor or

Table 1a. Different Types of Anticancer Drugs

Sl. No.	Drug name	Common name	IUPAC name
1	9-Aminoacridine (AA)	Aminacrine	Acridin-9-amine
2	Acriflavine (ACF)	Acriflavinium chloride	3,6-Diamino-10-methylacridin-10-ium chloride
3	Niclosamide (NA)	Niclocide	5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hyroxybenzamide
4	Proflavine (PF)	Diaminoacridine or proflavin	Acridine-3,6-diamine
5	Phenanthridine (PT)	Benzo[c]quinoline	3,4-Benzoisoquinoline
6	Pyridoacridine (PY)	-	11*H*-pyrido[4,3,2-*mn*]acridine

major grooves of DNA nucleobase (Table 1a).

Aminoacridine is an essential class of acridine derivatives which represents most of the acridine drugs and dyes. 9-Aminoacridine (AA) undergoes the most significant history in comparison to all of the monoamine isomers; Quinacrine is possibly the best known 9-amino containing acridine drug which was used as the first known clinically tested antimalarial drug; but recently, amsacrine was developed as a good anticarcinogenic agent [19-22]. Acriflavine (ACF) was first reported by the Nobel Prize winner, Paul Ehrlich, as an antiseptic; it was used to kill parasites and it has been broadly studied as a fluorescent molecule in detecting bacteria due to its intercalating properties, and it is also identified as a strong antitumoral molecule in colorectal cancers (CRC) by high-throughput drug screening [23-24]. Interestingly, Proflavine (PF) has a planar structure with monocationic aromatic ring that intercalates between the DNA nucleobase pairs and can block the replication in cancer cells. This intercalation ability makes PF a good anticancer agent [25-28]. Recently, Phenanthridine (PT) derivatives with non-flat 3D structure showed great interest in their relevant biological and medicinal fields. In addition, many synthetic non-flat PT molecules have been also found to exhibit important bioactivities such as antibacterial, antitumor, antileukemic, and anti-HIV activities [29-35]. Pyridoacridine (PY) is a class of marine-derived alkaloids and used in many respective therapeutic categories. It was observed as a DNA binding molecule and mostly identified on the basis of its cytotoxicity. It also showed a wide range of biological activities related to anticancer, anti-HIV, antimicrobial, antiparasitic, anti-viral and insecticidal activities [36-37].

In recent years, extensive quantum mechanical TD-DFT and molecular dynamics simulation studies on the bioactivity of 9-aminoacridine arene-based metallodrug complexes were reported [38]. Force field method was also used to study the dynamical effects of intercalation of anticancer agent into DNA. To this end, the transmission coefficient transition state theory for the reaction rate constant was calculated by examination of the recrossing events at the transition state [39]. Furthermore, molecular docking study using Glide (Schrodinger) is very useful for studying the anticancer agent containing natural anticancer pigments. Among many different cell cycle pathways, CDK-6 was found to be the most suitable anticancer target for the pyridoacridine [40]. Further analysis using molecular docking showed that anticancer agents binds to B-DNA and TOP2B, and VEGFR2 protein targets; this study revealed that phenanthridine derivative is a promising candidate with potential anticancer and DNA nuclease activity [41]. In the present work, we have considered six numbers of anticancer drugs (Table 1a) based on their compatibility. The interactions of mentioned drugs with DNA base pairs (AT and GC) and also single nucleobases, adenine (A), thymine (T), guanine (G), and cytosine (C) have been investigated to understand the activity of the drug molecule in DNA which can predict the drug modification with enhanced DNA affinity or selectivity.

METHODOLOGY

Herein, all the anticancer drugs and DNA base pairs (AT and GC) were constructed and then, the geometries were optimized. Furthermore, these optimized geometries were used to build the various stacked models for anticancer drugs and DNA base pairs using the joinMolecule software package. Moreover, Arguslab was also used to visualize and observe the different anticancer drugs and DNA base pairs stacked models. For studying the long-range non-covalent interaction, such as van der Waals, π-π interaction, *etc*. in the anticancer drugs and DNA base pair systems, the quantum mechanical density functional theory (DFT) method is the most useful one. All the stacked models have been computed using M062X method. GaussView5.0 and Gaussian09 software packages were used. Basis set, 6-311++G(d,p) was used for the optimization of models and also for the calculation of single point energies of the stacked models at 298 K and 1 atmospheric pressure. Details of stacking and model construction are explained in the result and discussion part.

The interaction energies for the stacked models of anticancer drugs and DNA base pairs are calculated by the following equation:

$$\text{Interaction energy} = E_{\text{Drug-Base pair}} - E_{\text{Drug}} - E_{\text{Base pair}}$$

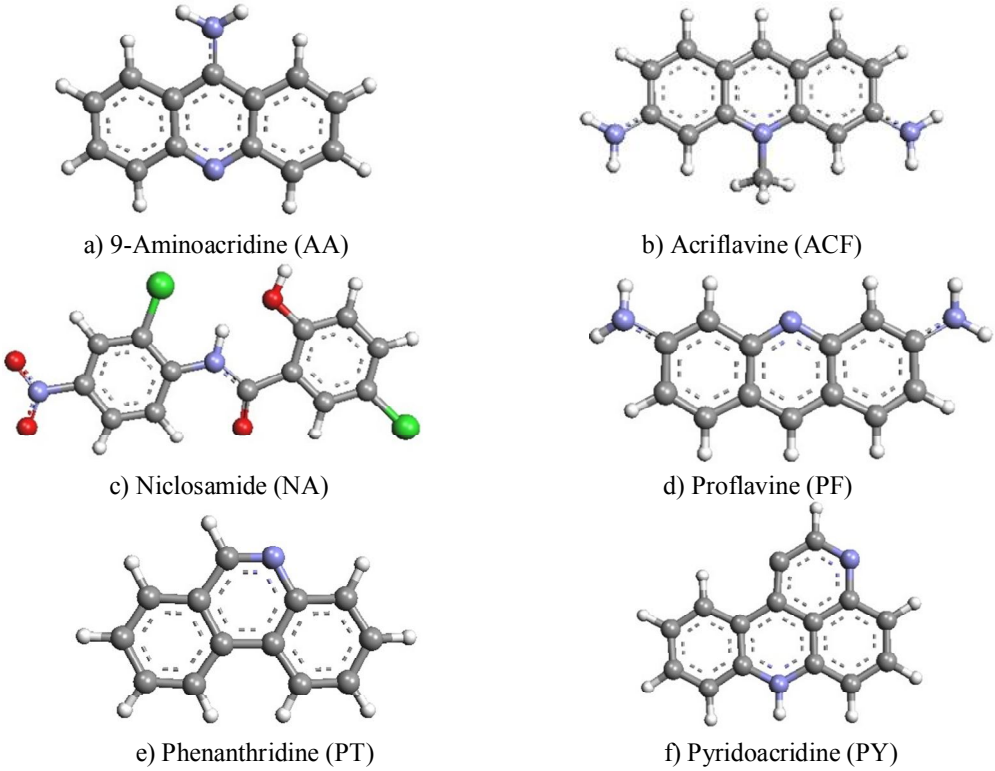
In above equation, $E_{\text{Drug-Base pair}}$ is single point energy of the stacked anticancer drugs and DNA base pairs complex, E_{Drug} is single point energy of the anticancer drug molecule, and $E_{\text{Base pair}}$ is single point energy of the DNA base pairs (AT and GC). All the calculations were computed by using Gaussian09 software package [42].

RESULT AND DISCUSSIONS

In this study, the non-covalent stacking interactions of some of the intercalating anticancer drugs and DNA nucleobase (AT and GC) stacked models (Figs. 2-3) were studied. Intercalation of anticancer drugs into DNA nucleobase pair is a very important factor in cancer research. It has been reported in literature that an anticancer drug may intercalate into the DNA base pair through different paths. In our study, we have considered only the favoured intercalating sites of the anticancer drugs as shown in Table 1b.

It is well known that the standard intermolecular distance for any long range non-covalent interaction of DNA nucleobase lies within a range of 3.4-3.6 Å; therefore, while constructing the stacked models of anticancer drugs-DNA nucleobase (AT and GC), the intermolecular separation was kept constant at 3.6 Å. The stacking interaction of anticancer drug-DNA nucleobase pair (AT and GC) complexes were studied by horizontal shifting of anticancer drug above the DNA nucleobase pairs, AT and GC, keeping the base pairs at a constant position. The horizontal shifting of anticancer drugs above the base pairs of DNA was done both in positive and negative direction along x or y-axis. Herein, the horizontal shifting of anticancer drugs and DNA nucleobase (AT and GC) stacked models was done from 0 to +1.5 Å, and from 0 to -1.5 Å directions along the x-axis. All the stacked models of anticancer drugs and DNA nucleobase pairs (AT and GC) stacked models were studied only in gas phase.

DNA sequence-specific study is the most important part of the interaction of anticancer drugs with DNA nucleobase pairs (AT and GC). Some anticancer drugs may bind to minor groove and some other drugs may bind to major groove of DNA nucleobase. The interaction energy of anticancer drug-DNA nucleobase complex is greatly influenced by the mode of binding of anticancer drugs with AT and GC base pairs. In our investigation, we considered some anticancer drugs as shown in Table 1a and Fig. 2 and studied the DNA sequence-specific binding with DNA nucleobase pairs (AT and GC). Also, we studied the DNA minor and major groove binding interaction of anticancer drug with AT and GC base pairs. It is well known that the interaction between anticancer drugs and DNA nucleobase pair (AT and GC) is very complex and difficult to understand; therefore, we investigated the drug-DNA base pair interactions in different ways. First, we investigated the interaction of anticancer drugs and single base of DNA nucleobase, *i.e.*, Adenine, Thymine, Guanine and Cytosine; such study is essential to observe how effectively the anticancer drugs bind to a single nucleobase of DNA. The computed interaction energy for interaction of anticancer drugs with single base of DNA nucleobase reveals that the most of anticancer drugs effectively bind with a single DNA

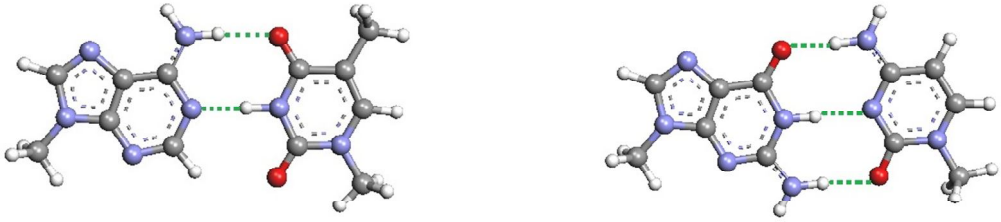


a) 9-Aminoacridine (AA) b) Acriflavine (ACF)

c) Niclosamide (NA) d) Proflavine (PF)

e) Phenanthridine (PT) f) Pyridoacridine (PY)

Fig. 2. Optimized models of anticancer drugs.



AT base pair GC base pair

Fig. 3. Optimized models of AT and GC base pair of DNA.

Table 1b. Intercalating Sites of the Anticancer Drugs

Sl. No.	Drugs	Intercalating sites	
		Favored	Unfavored
1	AA	Ring nitrogen	-NH_2 group
2	ACF	Aromatic ring	-CH_3 group
3	NA	-Cl and -OH group	-Cl group
4	PF	Ring nitrogen	Aromatic ring
5	PT	Ring nitrogen	Aromatic ring
6	PY	Ring nitrogen (3 Membered ring)	Ring nitrogen (2 Membered ring)

Table 2. Minimized Stacked Interaction Energy (kcal mol^{-1}) of Anticancer Drugs with Individual Nucleobase (A, T, G and C)

Drug	Interaction energies (kcal mol^{-1})			
	A	T	G	C
AA	-6.94	-8.80	-8.61	-10.24
ACF	-10.35	-10.30	-11.07	-11.14
NA	-10.52	-8.33	-12.42	-9.17
PF	-6.86	-8.70	-11.54	-11.08
PT	-6.08	-6.02	-10.78	-9.05
PY	-9.47	-10.47	-13.54	-10.19

nucleobase, as the interaction energy values for these interactions have negative values (Table 2).

Since anticancer drug directly binds to AT and GC base pairs, therefore, the computed interaction energy value is found to be more negative for drug-DNA complexes compared to that for a single DNA nucleobase (A, T , G and C). During the interaction of anticancer drug with DNA nucleobase pair (AT and GC), the anticancer drug may predominantly bind to the A nucleobase of AT base pair (A specific), or it may predominantly bind to the T nucleobase of AT base pair (T specific). Similarly, for GC base pair interaction the anticancer drug may be G specific or it may be C specific.

In computational calculations, it is well established that the more negative the interaction energy value for a stacked model, the more stable it is. Therefore, the anticancer drugs and DNA nucleobase pair (AT and GC) stacked models with more negative interaction energy value has the most stable and favoured geometry. All such interaction energies for anticancer drugs and DNA nucleobase pairs (AT and GC) can easily be reflected by the minima of interaction energy plots, *i.e*., interaction energy vs. horizontal shifting, as shown in the Figs. 4-9.

According to our results, for minor groove interaction, the sequences of stability of anticancer drug-DNA nucleobase pair complexes based on their interaction energy values (Table 3) are:

For AT base pair, A specific: AA < PT < PY < PF < ACF < NA

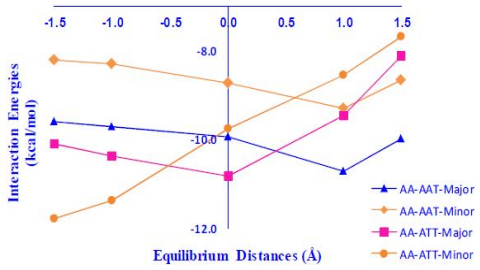


Fig. 4a. Interaction energy *vs*. equilibrium distance plot for AA-AT base pair (AAT: A specific and ATT: T specific).

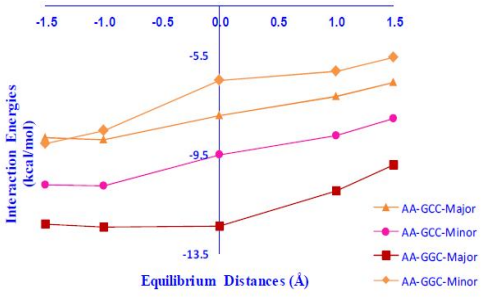


Fig. 4b. Interaction energy *vs*. equilibrium distance plot for AA-GC base pair (GCC: C specific and GGC: G specific).

For AT base pair, T specific: NA < PT < PF < AA < ACF < PY

For GC base pair, C specific: ACF < NA < PF < AA < PT < PY

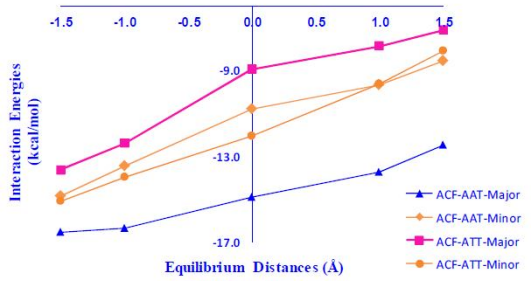


Fig. 5a. Interaction energy *vs*. equilibrium distance plot for ACF-AT base pair (AAT: A specific and ATT: T specific).

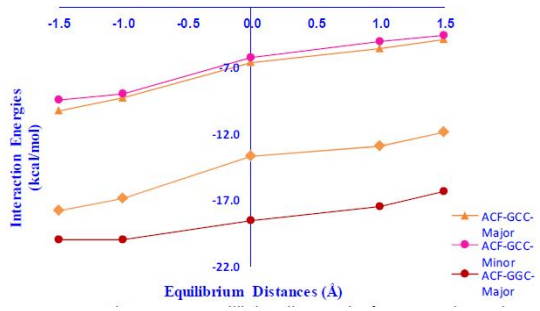


Fig. 5b. Interaction energy *vs*. equilibrium distance plot for ACF-GC base pair (GCC: C specific and GGC: G specific).

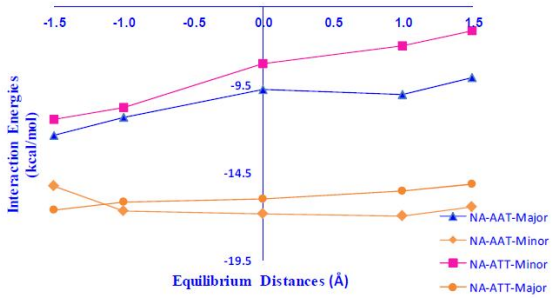


Fig. 6a. Interaction energy *vs*. equilibrium distance plot for NA-AT base pair (AAT: A specific and ATT: T specific).

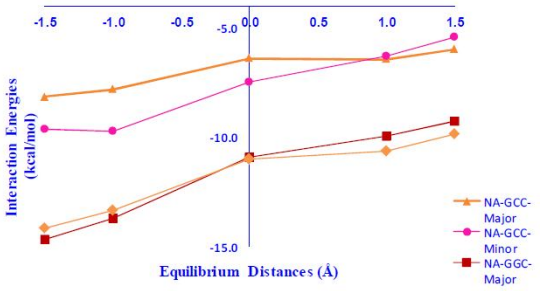


Fig. 6b. Interaction energy *vs*. equilibrium distance plot for NA-GC base pair (GCC: C specific and GGC: G specific).

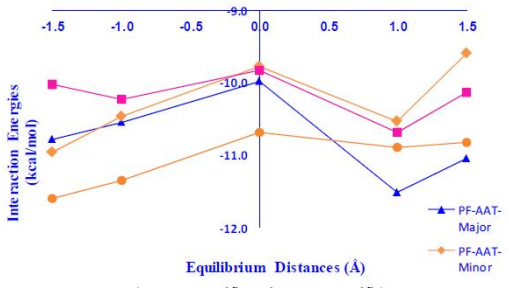


Fig. 7a. Interaction energy *vs*. equilibrium distance plot for PF-AT base pair (AAT: A specific and ATT: T specific).

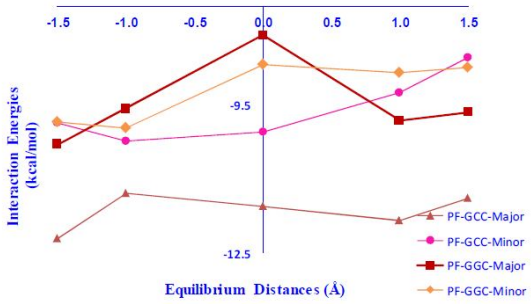


Fig. 7b. Interaction energy *vs*. equilibrium distance plot for PF-GC base pair (GCC: C specific and GGC: G specific).

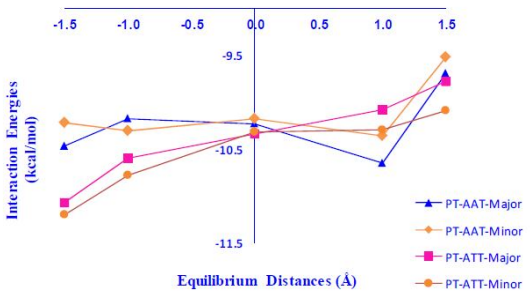


Fig. 8a. Interaction energy *vs*. equilibrium distance plot for PT-AT base pair (AAT: A specific and ATT: T specific).

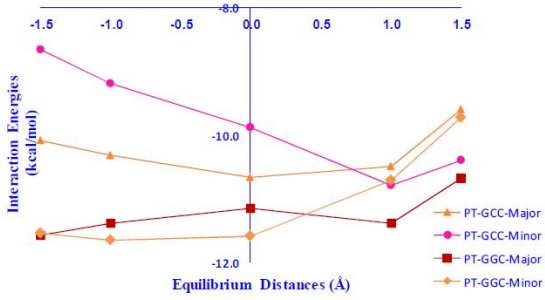


Fig. 8b. Interaction energy *vs* equilibrium distance plot for PT-GC base pair (GCC: C specific and GGC: G specific).

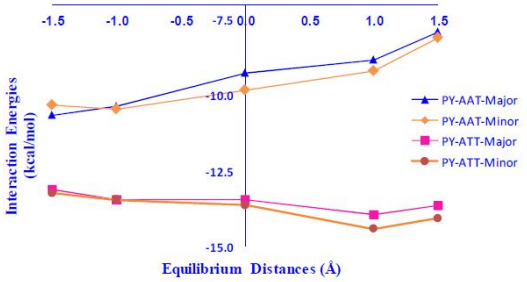


Fig. 9a. Interaction energy *vs*. equilibrium distance plot for PY-AT base pair (AAT: A specific and ATT: T specific).

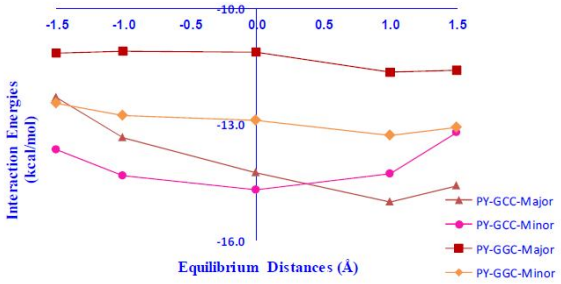


Fig. 9b. Interaction energy *vs*. equilibrium distance plot for PY-GC base pair (GCC: C specific and GGC: G specific).

For GC base pair, G specific: AA < PF < PT < PY < NA < ACF

From the above sequence of stability for minor groove interaction of anticancer drug-DNA nucleobase pair complex, it has been observed that AT minor groove binding anticancer drug Niclosamide (NA) is highly A specific in the AT base pair of DNA nucleobase pair, with interaction energy value of -16.97 kcal mol^{-1}, which is higher than that of other anticancer drugs. Furthermore, Pyridoacridine (PY) is highly T specific in the AT base pair of DNA nucleobase, with interaction energy value of -14.44 kcal mol^{-1}, which is higher than that of other anticancer drugs (Table 3). On the other hand, for GC minor groove binding, the anticancer drug Pyridoacridine (PY) is highly C specific in the GC base pair of DNA nucleobase, with interaction energy value of -14.71 kcal mol^{-1}, which is higher than that of other anticancer drugs; whereas Acriflavine (ACF) is highly G specific in the GC base pair of DNA nucleobase, with interaction energy value of -16.96 kcal mol^{-1}, which is higher than that of other anticancer drugs (Table 3). All the minimized stacked models for anticancer drug-DNA nucleobase pair complexes are shown in Figs. 10-15.

In addition, the sequences of stability of anticancer drug-DNA nucleobase pair complexes for major groove interaction, based on interaction energies (Table 4) are shown below:

For AT base pair, A specific: PY < PT < AA < NA < PF < ACF

For AT base pair, T specific: PT < PF < AA < ACF < PY < NA

Table 3. Minimized Stacked Interaction Energy (kcal mol^{-1}) of Anticancer Drugs with AT and GC Nucleobase Pair (Minor Groove Interaction)

Drug	Interaction energies (kcal mol^{-1})			
	AT		GC	
	A-Specific	T-Specific	C-Specific	G-Specific
AA	-9.31	-11.39	-10.78	-8.55
ACF	-13.46	-13.98	-9.06	-16.96
NA	-16.97	-10.75	-9.71	-13.34
PF	-10.54	-11.36	-10.24	-9.97
PT	-10.36	-10.78	-10.79	-11.66
PY	-10.45	-14.44	-14.71	-13.30

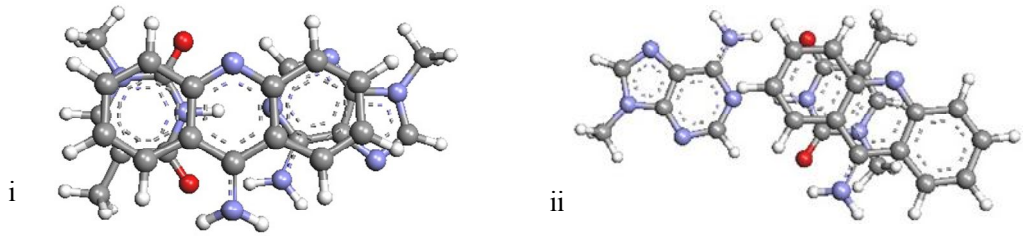


Fig. 10a. Minimized stacked model for AA-AT complex (i) Major groove and (ii) Minor groove interaction.

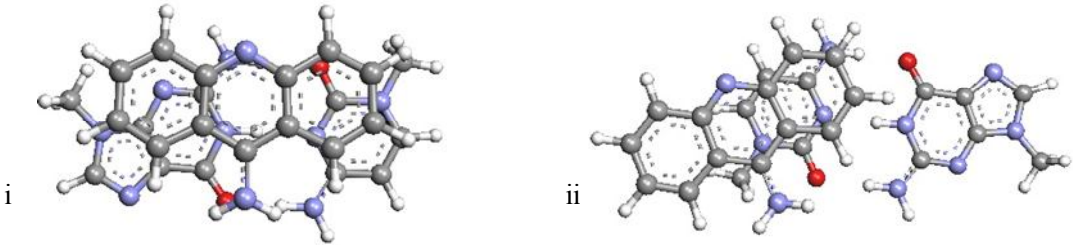


Fig. 10b. Minimized stacked model for AA-GC Complex (i) Major groove and (ii) Minor groove interaction.

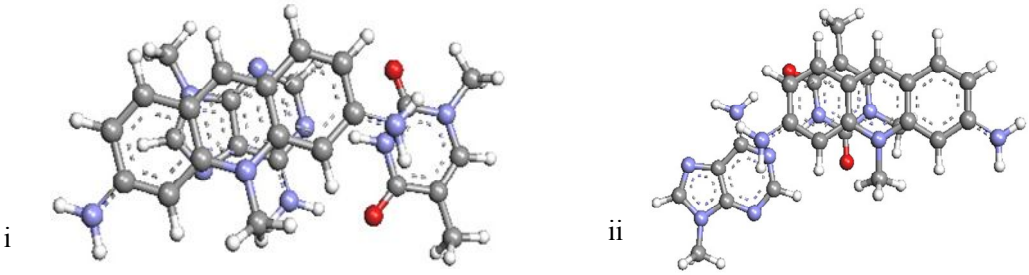


Fig. 11a. Minimized stacked model for ACF-AT complex (i) Major groove and (ii) Minor groove interaction.

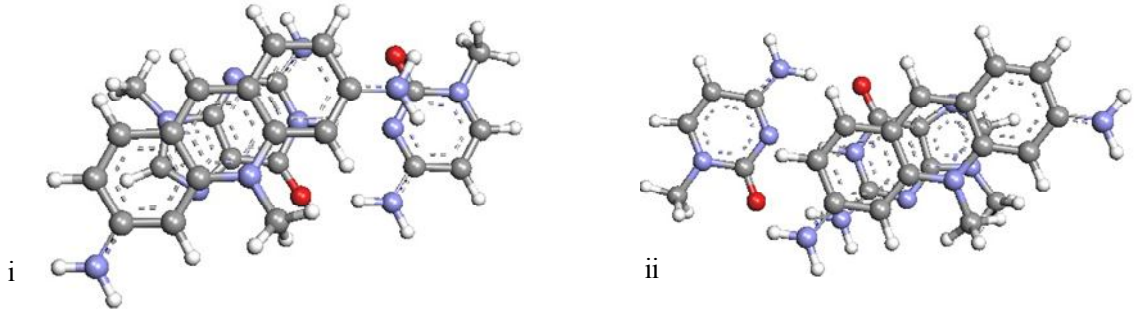


Fig. 11b. Minimized stacked model for ACF-GC Complex (i) Major groove and (ii) Minor groove interaction.

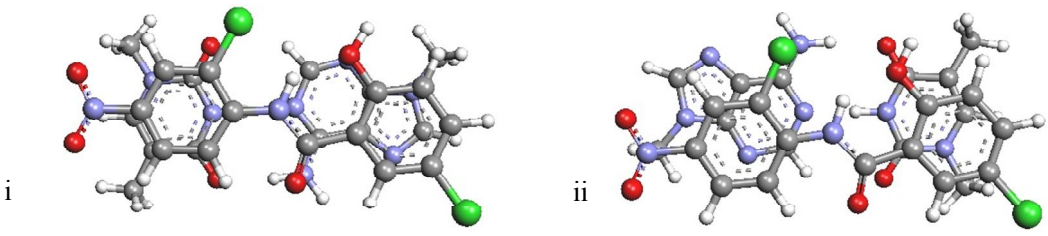


Fig. 12a. Minimized stacked model for NA-AT complex (i) Major groove and (ii) Minor groove interaction.

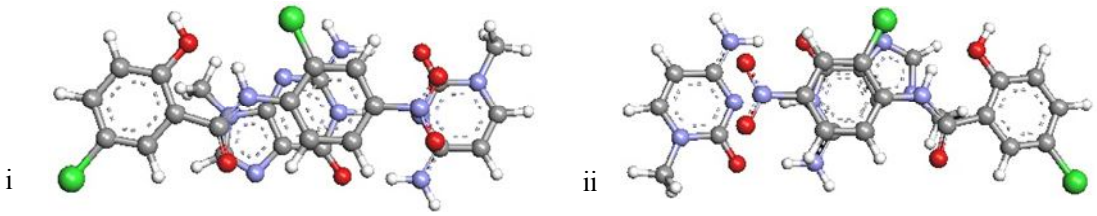


Fig. 12b. Minimized stacked model for NA-GC Complex (i) Major groove and (ii) Minor groove interaction.

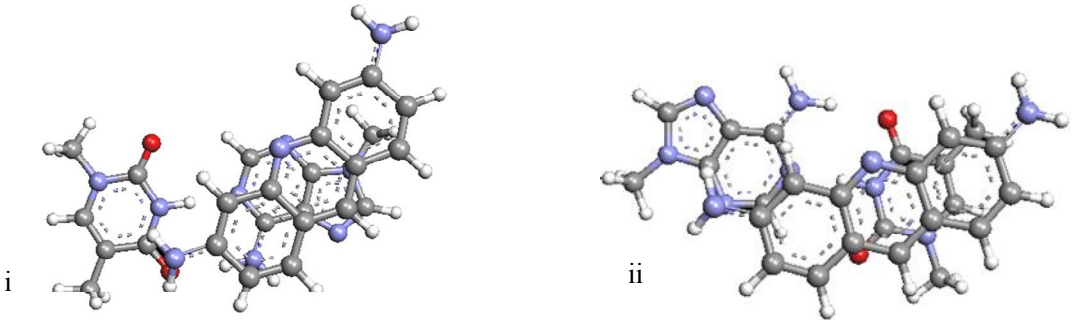


Fig. 13a. Minimized stacked model for PF-AT Complex (i) Major groove and (ii) Minor groove interaction.

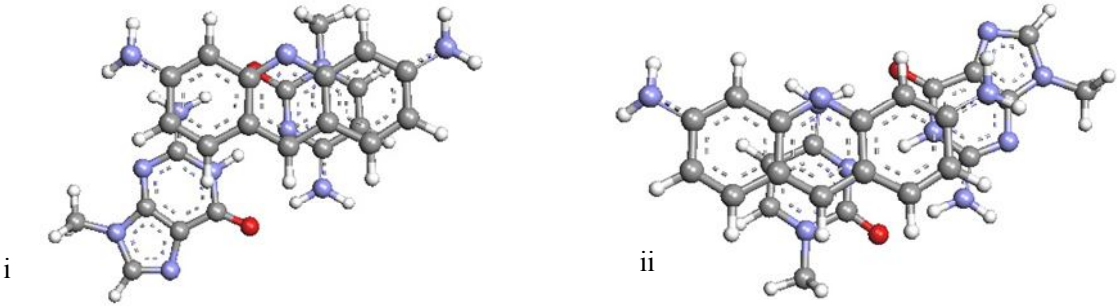


Fig. 13b. Minimized stacked model for PF-GC Complex (i) Major groove and (ii) Minor groove interaction.

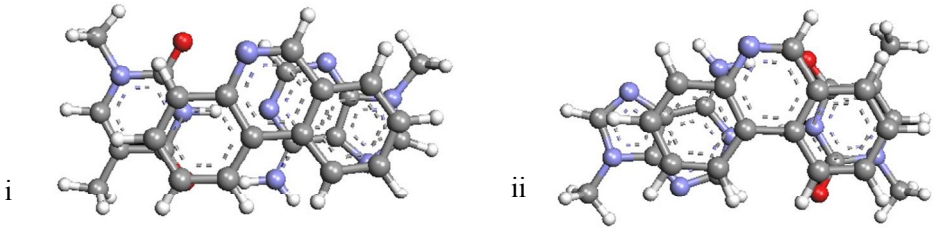


Fig. 14a. Minimized stacked model for PT-AT Complex (i) Major groove and (ii) Minor groove interaction.

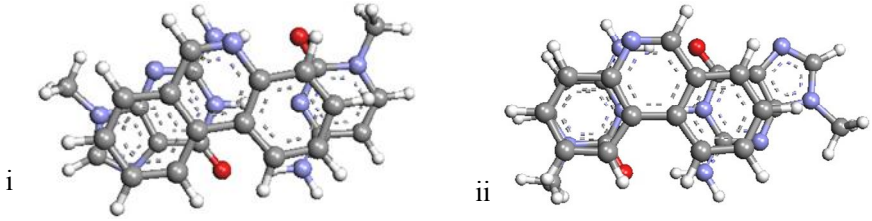


Fig. 14b. Minimized stacked model for PT-GC Complex (i) Major groove and (ii) Minor groove Interaction.

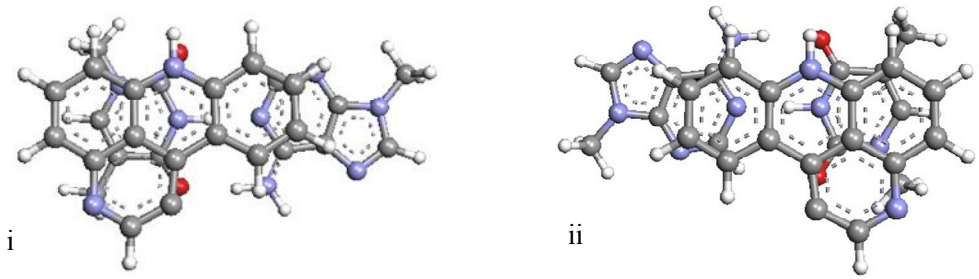


Fig. 15a. Minimized stacked model for PY-AT Complex (i) Major groove and (ii) Minor groove interaction.

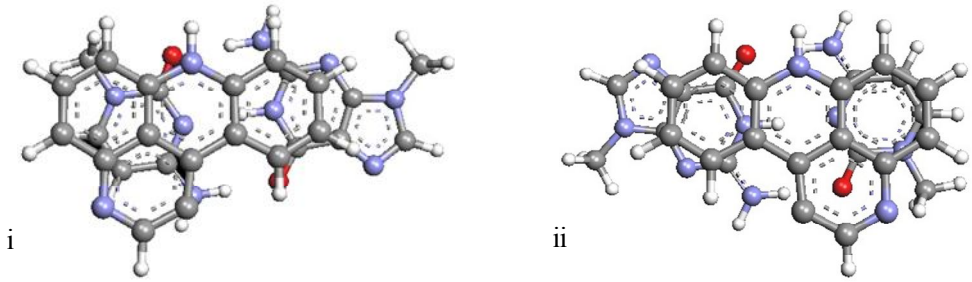


Fig. 15b. Minimized stacked model for PY-GC Complex (i) Major groove and (ii) Minor groove Interaction.

Table 4. Minimized Stacked Interaction Energy (kcal mol^{-1}) of Anticancer Drugs with AT and GC Nucleobase Pair (Major Groove Interaction)

Drug	Interaction energies (kcal mol^{-1})			
	AT		GC	
	A-specific	T-Specific	C-Specific	G-Specific
AA	-10.73	-10.84	-8.90	-12.46
ACF	-16.35	-12.40	-9.37	-20.05
NA	-11.37	-16.19	-7.81	-13.68
PF	-11.52	-10.68	-11.86	-9.83
PT	-10.66	-10.60	-10.66	-11.40
PY	-10.35	-13.95	-15.01	-11.66

For GC base pair, C specific: NA < AA < ACF < PT < PF < PY

For GC base pair, G specific: PF < PT < PY < AA < NA < ACF

Similarly, for major groove interaction of anticancer drug-DNA nucleobase complexes, it has been observed that, AT major groove binding anticancer drug Acriflavine (ACF) is highly A specific in the AT base pair of DNA nucleobase, with interaction energy value of -16.35 kcal mol^{-1}, which is higher than that of other anticancer drugs; whereas Niclosamide (NA) is highly T specific in the AT base pair of DNA nucleobase pair, with interaction energy value of -16.19 kcal mol^{-1}, which is higher than that of other anticancer drugs (Table 4). On the other hand, for GC major groove binding, the anticancer drug pyridoacridine (PY) is highly C specific in the GC

Table 5. Minimized Stacked Interaction Energy (kcal mol^{-1}) of Anticancer Drugs with AT and GC Nucleobase Pair

Drug	Interaction energies (kcal mol^{-1})			
	Major groove		Minor groove	
	AT	GC	AT	GC
AA	-10.84	-12.46	-11.39	-10.78
ACF	-16.35	-20.05	-13.98	-16.96
NA	-16.19	-13.68	-16.97	-13.34
PF	-11.52	-11.86	-11.36	-10.24
PT	-10.66	-11.40	-10.78	-11.66
PY	-13.95	-15.01	-14.44	-14.71

base pair of DNA nucleobase pair, with interaction energy value of -15.01 kcal mol^{-1}, which is higher than that of other anticancer drugs; whereas Acriflavine (ACF) is highly G specific in the GC base pair of DNA nucleobase, with interaction energy value of -20.05 kcal mol^{-1}, which is higher than that of other anticancer drugs (Table 4). All the minimized stacked models for drug-DNA nucleobase complexes are shown in Figs. 10-15.

If we compare the anticancer drug-DNA nucleobase pair interaction energies for AT and GC base pairs, related to minor and major groove, we can conclude that the sequence of interaction energies (Table 5) are as followings:

AT minor: PT < PF < AA < ACF < PY < NA

AT major: PT < AA < PF < PY < NA < ACF

GC minor: PF < AA < PT < NA < PY < ACF

GC major: PT < PF < AA < NA < PY < ACF

For overall anticancer drug-DNA nucleobase pair (AT and GC) complexes, it has been observed that Niclosamide (NA) is highly AT minor groove specific, with interaction energy value of -16.97 kcal mol^{-1}, which is higher than that of other anticancer drug-DNA nucleobase complexes (Table 5). Acriflavine (ACF) is highly AT major groove specific, with interaction energy value of -16.35 kcal mol^{-1}, which is higher than that of other anticancer drugs. Moreover, Acriflavine (ACF) shows the most negative interaction energy values for both GC minor and major groove binding, with interaction energy values -16.96 and -20.05 kcal mol^{-1}, respectively, higher than that of other anticancer drugs (Table 5). From the above results it is clear that only the Acriflavine (ACF) can bind effectively to the both minor and major groove of DNA nucleobase. The reason is related to the molecular structure of Acriflavine (ACF) drug, as it can directly enter into the AT and GC base pairs through the planer side of the ring, rather than the bulky $-CH_3$ and $-NH_2$ groups. Therefore, for both minor and major groove, it is quite easy for Acriflavine (ACF) to enter the AT and GC base pairs (Fig. 1b).

The calculation of interaction energy for drug-DNA nucleobase pair complexes may also be justified by computing their HOMO-LUMO energy. The frontier orbitals of a molecule, *i.e*., HOMO and LUMO, are the most important orbitals in a molecule or molecular complex. The highest occupied energy of HOMO characterizes the electron donating ability of a molecule, while the lowest unoccupied LUMO energy determines the ability to accept an electron. The computed energies of these orbitals determine the way that a molecule interacts with other species, and it provides the information about the stability or reactivity of specific regions of the molecule. Moreover, from the HOMO-LUMO energy of a molecular system, we can determine the chemical reactivity descriptors such as chemical potential (μ), electronegativity (χ), hardness (η), softness (S), electrophilicity index (ω), *etc*. The HOMO-

Table 6. HOMO-LUMO Energy (ev) for Minimized Stacked Models of Anticancer Drugs with AT and GC Base Pairs (Major and Minor Grooves)

Drug	HOMO-LUMO energy (ev)			
	Major groove		Minor groove	
	AT	GC	AT	GC
AA	2.27	2.01	2.28	1.95
ACF	2.61	2.60	2.34	2.59
NA	2.58	2.28	2.62	2.27
PF	2.28	1.95	2.28	1.88
PT	2.19	1.89	2.20	2.01
PY	2.29	2.29	2.61	2.28

LUMO energies for minimized stacked models were obtained at the level of M062X theory and the values for drug-DNA nucleobase complexes, for minor and major grooves, are shown in Table 6.

The higher values of HOMO and LUMO energy (ev) indicates that the molecule is chemically stable, while a small HOMO-LUMO gap represents the low excitation energies for transition to the manifold of excited states. Therefore, we can compare the computed interaction energies of the above studied anticancer drug-DNA nucleobase complexes with HOMO-LUMO energy to justify their calculated interaction energy. It was found that for all those complexes which have more negative interaction energy values, the HOMO-LUMO energy gap is also higher. Although we could not compute the accurate HOMO-LUMO energy for all such molecular complexes, still in most cases, the higher HOMO-LUMO energy was related to the more stable drug-DNA nucleobase complexes (with more negative interaction energy).

According to the computed HOMO-LUMO energy of anticancer drug-DNA nucleobase, related to minor and major groove, the following sequence was obtained, which is almost similar to the interaction energies that we observed in Table 5.

AT minor: PT < PF ~ AA < ACF < PY ~ NA

AT major: PT< AA ~ PF ~ PY < NA < ACF

GC minor: PF < AA < PT < NA < PY < ACF

GC major: PT< PF < AA < NA ~ PY < ACF

CONCLUSIONS

In this work, we have investigated the stacking interaction of anticancer drug-DNA nucleobase pair complexes by using quantum mechanical calculation. According to our observations, some of the anticancer drugs interact significantly and show effective stacking interactions, either through major or minor groove of DNA nucleobase. For each of the anticancer drug-DNA nucleobase pair complexes, the most negative stacking interaction energy was computed for the most stable minimized stacked model. From our investigation, Niclosamide (NA) is highly AT minor groove specific, with more negative interaction energy value of -16.97 kcal mol^{-1}, which is higher than that of other anticancer drug-DNA nucleobase pair complexes. On the other hand, Acriflavine (ACF) was found to be highly AT major groove specific, with interaction energy value of -16.35 kcal mol^{-1}, which is higher than that of other anticancer drugs. However, Acriflavine (ACF) was interestingly the only anticancer drug that showed the most negative interaction energy values for both GC minor and major groove interaction, *i.e.*, -16.96 and -20.05 kcal mol^{-1}, respectively, higher than that of other anticancer drugs**.** All the minimized and the most

stable models of anticancer drug-DNA base pair complexes could be computed from the minima of each of the interaction energy plots. The computed HOMO-LUMO energies of drug-DNA nucleobase pair also justified the interaction energy of the minimized complexes.

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