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Molecular docking studies on binding specificity of 3,6- and 2,7-carbazoles with DNA duplexes

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ABSTRACT: Molecular docking is a widely used computational technique used to find the probabilistic binding sites of drugs in the vicinity of macromolecules. The drugs produce their working effect only when they bind and interact with the target macromolecule. The potential drugs can only be identified by their relative binding affinities and corresponding binding modes. Availability of huge numbers of such drugs has made the estimation of their relative potency, a difficult task. In the present work, carbazoles (3,6 and 2,7) and their analogs were studied for their DNA binding abilities using molecular docking calculations. Since the docked ligands had planar structures, it allowed them to adopt crescent shape and thus minor groove binding with DNA was preferred by all. However, it was found that a single molecule (Mol-6) (2,7-carbazole) showed promising results with all the selected DNA sequences also its results were exactly verified with those in the reported literature and therefore it can be said that its in-vivo studies could possibly produce some exciting results. This study also revealed that DNA binding energies of 3,6- and 2,7-carbazoles followed the same trend as their thermal melting values.

Keywords: Carbazoles; Docking; DNA; Minor Groove.

1. INTRODUCTION

Deoxyribonucleic acid (DNA) is a double helical, twisted strand that serves as the main ingredient by acting as the carrier of all the genetic information [1]. Almost all the anti-cancer therapies involve the interaction of drugs with the target DNA. Therefore drug-DNA interaction studies are amongst the top rated in the biomedicine and related fields. Drug-DNA interactions can be broadly classified into two major categories viz., intercalation and groove binding. Groove binding in DNA takes place via two modes, viz., major groove binding and minor groove binding [2, 3]. However, intercalation involves the insertion of planar molecules into the DNA base pairs.

In the present study, we prefer to analyse the minor groove binding tendency of carbazoles and its analogs with DNA. The ability to predict the geometrical conformation as well as the associated energy of binding of drugs in that conformation with DNA has played a crucial role in the field of biology and allied sciences. Due to the presence and wide use of various computational tools, the design of new drugs and test of their potency has been very easy since past few years. Molecular docking technique has proven itself to be of great importance in predetermination of binding site, in the approximation of associated binding energy and in

the identification of those macromolecule base pairs/residues that interact with the drug, in such a field of research.

Since, almost all the anti-cancer and anti-microbial drugs preferentially bind itself to the DNA for their therapeutic action with DNA to begin [4], our study also adds to the same. Minor groove binders are found preferentially either to have crescent shape or to have adopted the crescent shape during the simulation and therefore they complement the shape of the minor groove of DNA [5-8] resulting in better binding and raised binding affinities. The binding mechanism of ligand/drug to the minor groove of the DNA can be mainly described via following two steps: in the first step, the ligand gets itself transferred to the minor groove of the DNA via electrostatic interactions and hydrophobic interactions whereas in the second step, numerous covalent interactions are formed in between ligand/drug and DNA base pairs. These interactions include van-der Waal's contacts, hydrogen bonds, electrostatic interactions and hydrophobic interactions. It has been observed that most of the minor groove binders preferentially bind themselves to AT-rich regions of the DNA [9].

Various experimental studies have been performed worldwide in order to explain and understand the binding mechanism of anti-cancer, anti-bacterial and various other diseases. The results obtained from these experiments serve as an excellent database for theoretical scientists to carry out and test the authenticity of their simulations [10-14].

In the present work, carbazoles and its analogs (3,6- and 2,7-carbazoles), were computationally docked to selected DNA sequences, for a deeper and better insight of their binding affinities as well as their binding sites. Literature [15] reported that selected carbazoles possess antimicrobial tendencies and therefore computational modelling of their binding with DNA was studied to understand their bindings with DNA. There are many predefined models to understand protein-DNA interactions but these models are not reliable in the case of drug-DNA interactions; the reason behind this is that unlike in the case proteins and enzymes there is no predetermined active binding site in DNA. Therefore, for such cases, molecular modelling techniques have proven themselves to be of great importance in understanding various types of non-covalent interactions existing between drug/ligand and DNA [16].

2. MATERIALS AND METHODS

2.1. System preparation and data set

The crystal structures of the selected eight DNA sequences (1BNA [17], 1DNE [18], 1QSX [19], 1RMX [20], 195D [21], 2MNB [22], 2MNE [22], 4AH0 [23]) were downloaded from Protein Data Bank (PDB) [24] and are listed in Table 1. Fig. 1 represents the generalized structure showing the fused rings and the position of the substituent derivatives that were docked with the selected DNA sequences were obtained from literature [15].

2.2. Geometry optimization

Water molecules from each of these DNA sequences were removed using UCSF Chimera [25]. Chemical structures of all the ligands/drugs were selected from the literature [15]. These chemical structures were then put to geometry optimization using Gaussian 09 software package [26]. Fig. 2 shows the chemical structures of the ligands that went under geometry optimization.

In the present work, the geometry optimization of selected ligands was carried out using Gaussian 09 software using B3LYP hybrid functional at 6-31G** level of theory for their potentials to attain a local minimum. These optimized ligands were then docked to the selected DNA sequence.

Table 1. PDB Id's and sequence of the selected DNA sequences.

S. No.	PDB Id.	DNA Sequence
1	1BNA	5'-CGCGAATTCGCG-3'
2	1DNE	5'-CGCGATATCGCG-3'
3	1QSX	5'- CTTTTGCAAAAG-3'
4	1RMX	5'- CGACTAGTCG-3'
5	195D	5'-CGCGTTAACGCG-3'
6	2MNB	5'- CGACGCGTCG-3'
7	2MNE	5'- CGACTAGTCG-3'
8	4AH0	5'- CGCAAATTTGCG-3'

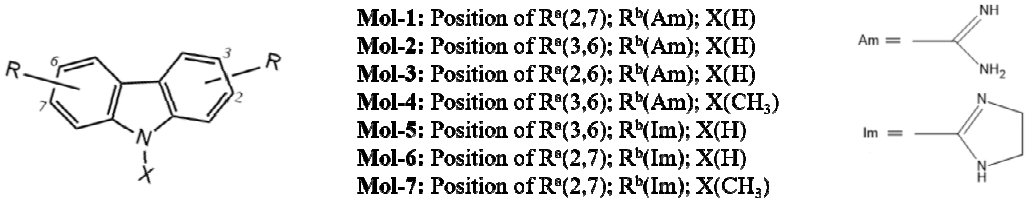

Figure 1. A generalised structure of the fused carbazole ring along with the position where the substituents are to be attached.

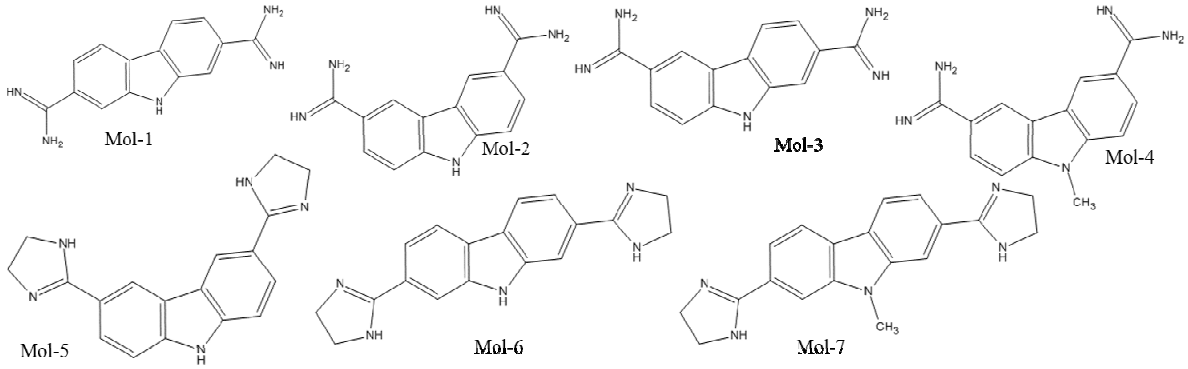

Figure 2. Figure showing the chemical structure of ligands before geometry optimization.

2.3. Molecular docking setup

Molecular docking was performed using Autodock4 software package [27]. Gasteiger charges were added to the drug-DNA complex using Autodock Tools (ADT) before starting the docking calculations. A grid box, having different dimensions, was prepared for each drug-DNA complex that enclosed the entire macromolecule. This helped the drug/ligand in finding the most preferential binding site while the docking calculations were performed [28-30]. Calculations were set up using the classical Lamarckian Genetic Algorithm (LGA). A 20 LGA run with a maximum cycle of 2500000 energy evaluations was performed for each of the drug-DNA complex. The docked pose with the lowest binding affinity was extracted and aligned with the receptor for further analysis [31-33].

3. RESULTS AND DISCUSSION

The results as obtained by the docking studies can be summarized and discussed as follows:

3.1. Optimized geometries

Optimizing the ligand geometry before docking and other computational calculations is a very crucial step, as it brings the entire system into its lowest possible energy state; having least steric hindrances and charge based repulsions. The reason behind this is because then the system would then have attained a maximized distance between the bonds, the angles and the dihedrals [34]. Further, the electrostatic charges also tend to get well distributed throughout the system leading to the electron transfer between definite pair of atoms; eventually no distortions in the chemical structures are offered. Fig. 3 shows the optimized geometries of the selected ligands.

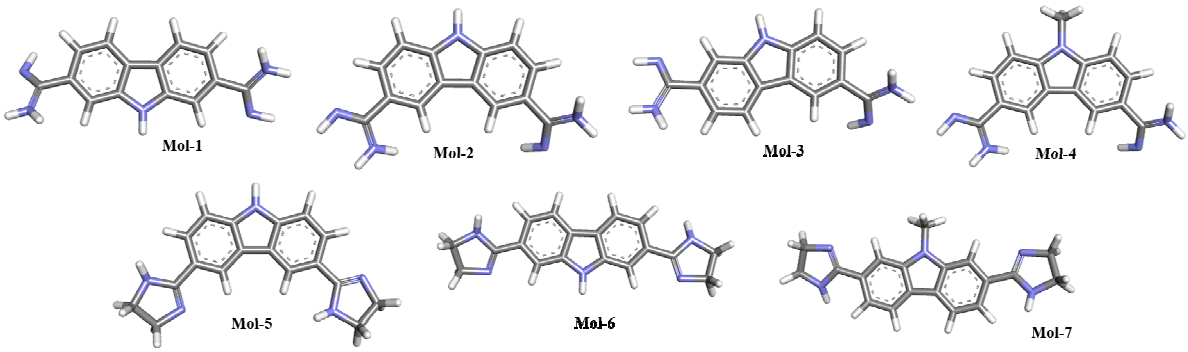

Figure 3. Figure showing the optimized geometrical structures of selected ligands.

3.2. Molecular docking studies

The binding energies obtained by computational docking for the selected ligands are listed below in Table 2, the compounds having the least binding affinities are shown in bold; these represent the formation of stable complexes [2] with corresponding mentioned DNA sequence. Fig. 4 represents the best docked poses corresponding to compounds having least binding affinities whereas Fig. 5 represents the interaction profile in 2D obtained using Discovery studio visualizer [35] reveals various types of interactions taking place between the DNA bases and the selected drugs eventually leading to stabilization or de-stabilization of the complexes thus formed. These figures also reveal that the active binding site of the drug to be the AT-rich region of the selected DNA sequences.

Clearly it can be seen from the above Table 2 that Mol-6 (2,7-carbazole) has highest reported (ΔT_m) value and the docking binding affinity values of Mol-6 with all the selected DNA sequences verify the results obtained from docking studies that the geometrical structure of Mol-6 is apt for binding with DNA sequences providing least binding energy values. Also, the literature [15] shows that 2,7-carbazoles bind with DNA having their carbazole nitrogen pointing down into the groove, similar results were obtained from our docking simulations also. Further, Fig. 4 shows that binding of Mol-6 with 1RMX, 2MNB & 2MNE has least binding affinities as the ligand (Mol-6) turns out to get aligned vertically during the docking process and thus having maximum binding affinities. And therefore, the results obtained from our study are of significant importance.

This can also be verified from Fig. 6 that the variations in docking affinities with corresponding change in thermal melting values (ΔT_m) are maximum for Mol-6, as represented in Table 2.

Table 2. DNA binding affinities of ligands obtained by computational docking along with their change in thermal melting values (ΔT_m).

S.No.	Exp. (ΔTm)	1BNA (kCal/mol)	1DNE (kCal/mol)	1QSX (kCal/mol)	1RMX (kCal/mol)	195D (kCal/mol)	2MNB (kCal/mol)	2MNE (kCal/mol)	4AH0 (kCal/mol)
Mol-1	10.9	-7.12	-8.11	-8.10	-6.97	-7.89	-6.92	-6.74	-7.26
Mol-2	8.2	-7.42	-7.20	-7.70	-6.60	-7.53	-6.41	-6.75	-7.10
Mol-3	6.5	-7.80	-7.68	-7.96	-7.08	-7.90	-6.94	-6.47	-8.13
Mol-4	7.5	-6.91	-6.90	-7.85	-6.56	-8.01	-5.86	-6.14	-7.28
Mol-5	7.8	-7.77	-8.05	-8.54	-6.46	-8.03	-6.68	-7.01	-8.09
Mol-6	12.0	-8.76	-10.19	-9.43	-8.36	-10.30	-8.32	-7.36	-10.03
Mol-7	6.3	-8.40	-8.38	-8.21	-7.35	-9.16	-7.44	-7.14	-9.30

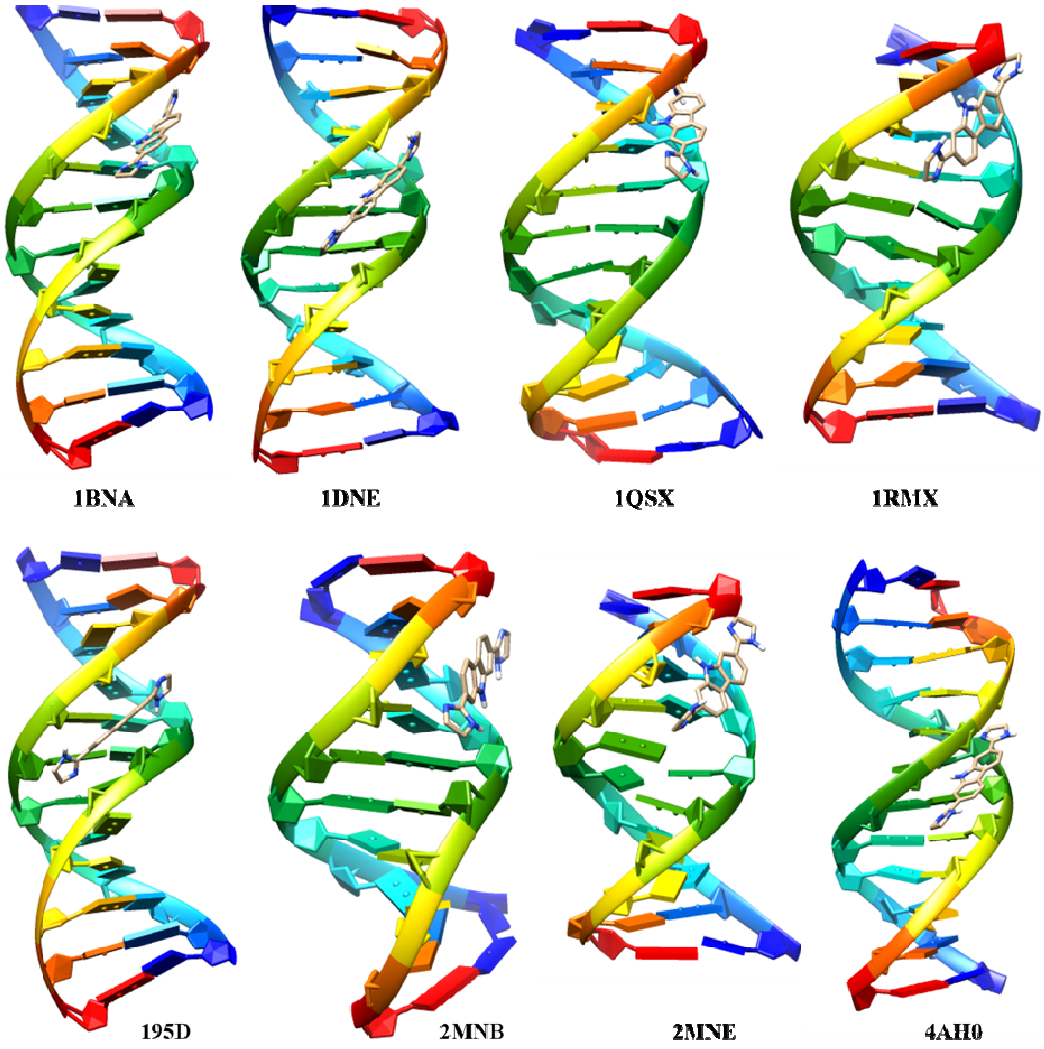

Figure 4. Figure representing the best docked posed complexes.

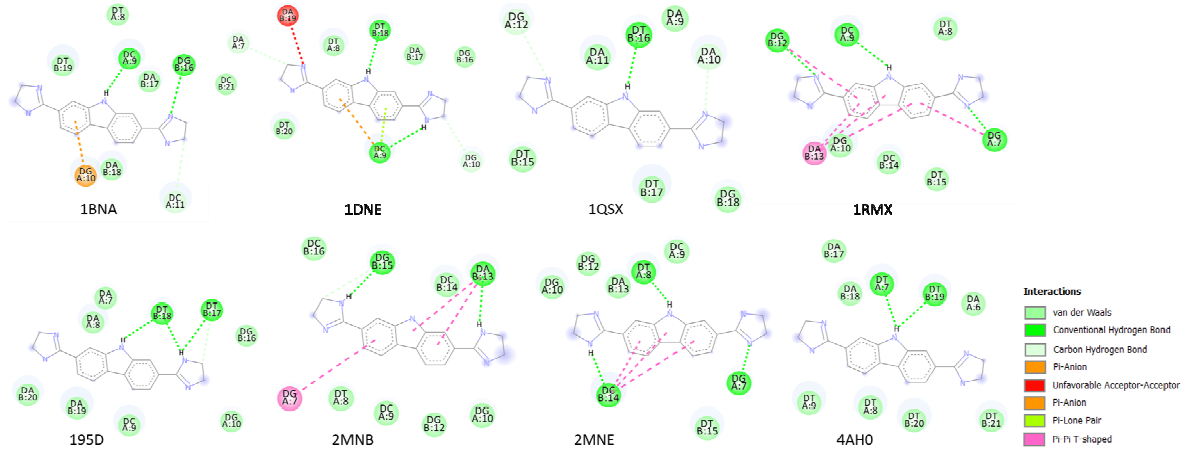

Figure 5. Figure representing the interaction profile for the best docked posed complexes.

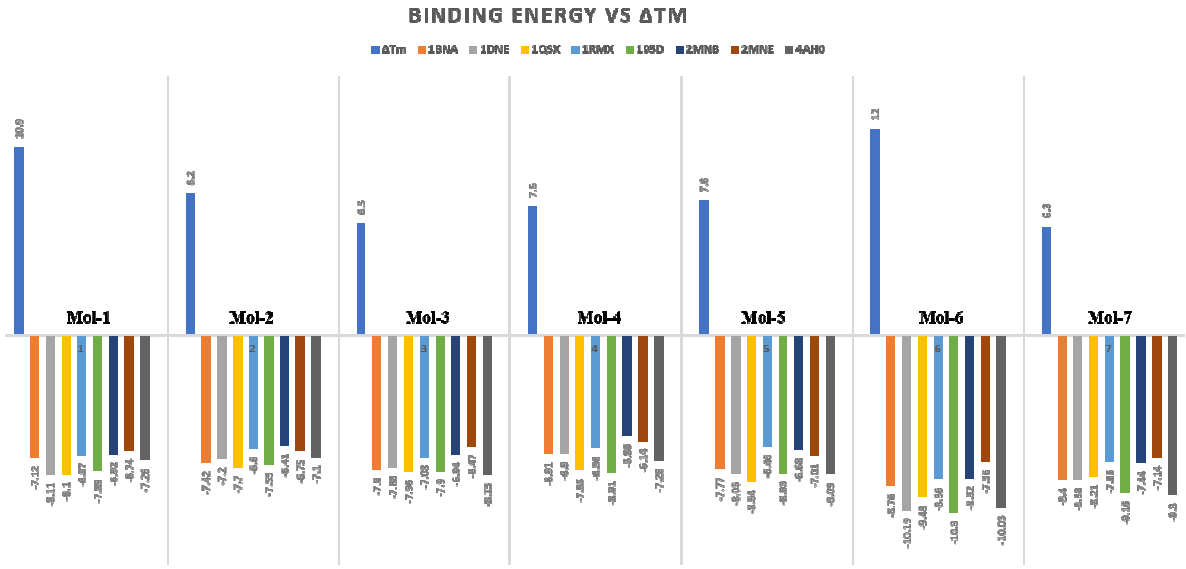

Figure 6. Figure depicting the binding energy trend along with the thermal melting values (ΔT_m).

3.3. Hydrogen bonding analysis

Formation of hydrogen bond owes solely to the complex stability [2]. Fig. 7 shown below, gives the 3D representation of the hydrogen bonds formed between the DNA bases and Mol-6 corresponding to the best docked posed complexes whereas Table 3 represents the donor and the acceptor bases of the DNA involved in the formation of hydrogen bond and corresponding length and number of hydrogen bonds formed.

The acceptor and donor regions as shown in Fig. 8; assist in demonstrating the electron rich and electron deficient sites in the drug-DNA complexes and thus helps in the prediction of possible hydrogen binding sites within the system. These regions also represent the atoms which have the tendency to donate/accept electrons so as to achieve stability [36] during the docking calculations. It can be seen that due to the carbazole nitrogen in Mol-6 pointing down into the groove, there are more donor regions in the grooves of 1BNA, 1DNE, 1QSX, 195D and 4AH0 than that in that of 1RMX, 2MNB and 2MNE, eventually leading to stability and higher binding affinities.

Table 3. Following table represents the donor and the acceptor species and H-bond length formed.

S. No.	Complexes	Number of H-bonds	Interacting species	H-bond length (Å)
1	1BNA + Mol-6	2	DG16:N2 - UNK0:N UNK0:H - A:DC9:O2	2.835594 2.039468
2	1DNE + Mol-6	2	UNK0:H - A:DC9:O3' UNK0:H - B:DT18:O2	2.459272 2.189784
3	1QSX + Mol-6	3	UNK0:H - B:DT16:O2 UNK0:H - B:DT18:O2 UNK0:H - A:DC9:O3'	2.081030 2.189784 2.459272
4	1RMX + Mol-6	3	DG12:H22 - UNK0:N UNK0:H - A:DC9:O2 DG7:H22 - UNK0:N	2.334379 2.483855 1.737888
5	195D + Mol-6	3	UNK0:H - B:DT18:O2 UNK0:H - B:DT17:O2 UNK0:H - B:DT18:O4'	2.187745 2.659172 1.948403
6	2MNB + Mol-6	2	UNK0:H - B:DG15:O4' UNK0:H - B:DA13:O3'	2.032501 2.152659
7	2MNE + Mol-6	3	DG7:H22 - UNK0:N UNK0:H - A:DT8:O2 UNK0:H - B:DC14:OP1	1.750523 2.968755 2.182582
8	4AH0 + Mol-6	2	UNK0:H - B:DT19:O2 UNK0:H - A:DT7:O2	2.159898 1.897104

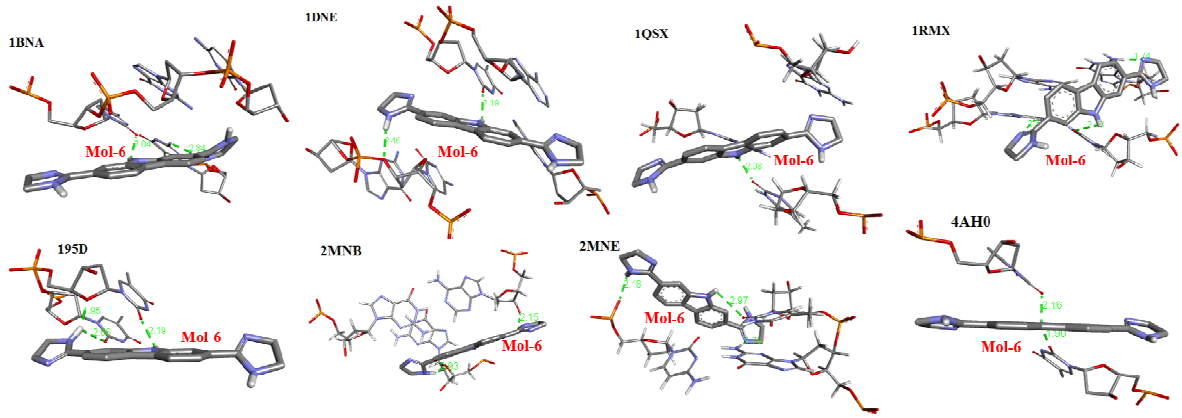

Figure 7. Figure showing 3D representations of the H-bonds formed corresponding to the best docked posed complexes.

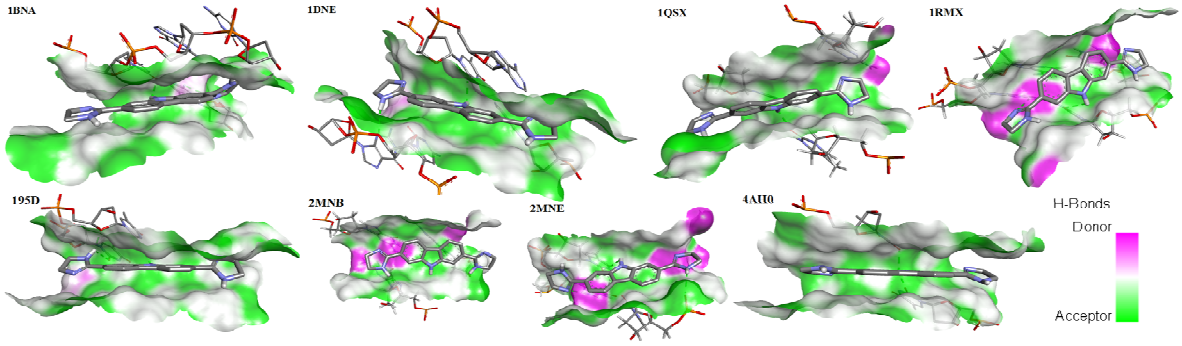

Figure 8. Figure showing H-bond donor and acceptor regions of the H-bonds formed corresponding to the best docked posed complexes.

4. CONCLUSIONS

The computational study performed here was done in order to analyse and evaluate the DNA binding affinities of the ligands. The major focus was to get a detailed perspective of DNA minor groove binders at atomistic levels. The experimental studies revealed that all these ligands preferred to bind in the minor groove of the DNA rather than intercalating between the DNA base pairs; as expected due to the presence of fused rings in their chemical structures.

Clearly it can be seen from the obtained results that Mol-6 (2,7-carbazole) has highest reported (ΔT_m) value; as well as the docking binding affinity values of Mol-6 with all the selected DNA sequences are also higher than the others. These findings conclude that the geometrical structure of Mol-6 is apt for binding with DNA sequences providing least binding energy values. Results reported in the previous works show that 2,7-carbazoles bind with DNA having their carbazole nitrogen pointing down into the groove, similar results were obtained from our docking simulations also. Further, it was also observed from the obtained results that binding of Mol-6 with 1RMX, 2MNB & 2MNE has least binding affinities (amongst those of the higher ones) as the ligand (Mol-6) gets aligned vertically during the docking process and thus offers steric hindrances eventually leading to decrease in binding affinities. Since our findings match with that of previous works, therefore, the results obtained from our study hold significant importance.

Thus, we conclude that geometrical factors were the reasons why Mol-6 showed favourable results with all the selected DNA sequences and binding site was AT-rich regions as favoured by most of the minor groove binders. Therefore, our study helps in getting a deeper insight regarding the DNA binding mechanism and binding affinity of carbazoles and its analogs. This analysis will certainly help in improvement of the existing minor groove binders and would also prove helpful in the design of new and potent drugs with anti-microbial activity. This study also fulfils its aim of complementing the experimental techniques and serves as a good database for structure-energy relationship of drug-DNA complexes.

Authors' Contributions: AP did the simulations. AP & MM wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The author has no conflict of interest to declare.

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