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In silico research on Novel Derivatives of N-(Acetylphenyl)-N-Ferrocenylmethyl-3-nitroaniline as DNA Binding Agents: Using Diverse Computational Methods, including Molecular Docking and ADME/Toxicity Assessment

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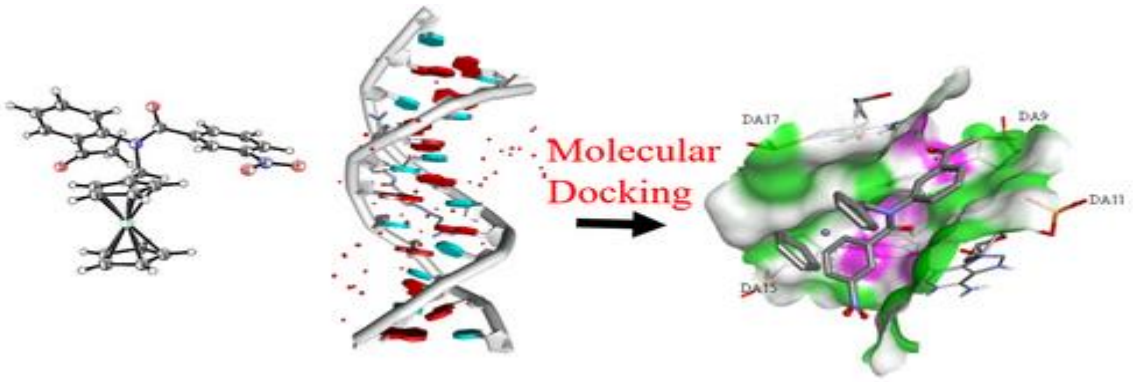
Abstract: This study presents an in silico investigation into the potential DNA binding properties of novel derivatives of N-(Acetylphenyl)-N-Ferrocenylmethylnitroaniline using different computational techniques, including molecular docking and ADME/Toxicity assessment, we explored the interaction between these derivatives and DNA. The results reveal promising candidates with strong binding affinities to DNA, substantiated by robust electrostatic interactions. Furthermore, our study sheds light on the ADME and toxicity profiles of these compounds, providing insights into their pharmacological potential. These findings offer valuable insights into the design and development of DNA-binding agents with potential applications in various biomedical fields.

Keywords: DNA Binding Agents, Computational Methods, ADME/Toxicity Evaluation, Drug Design, DFT Method.

Graphical Abstract:



1. Introduction

The exploration of novel compounds with the ability to selectively bind to DNA has gained substantial significance in the fields of molecular biology and drug design [1–5]. In this context, we embark on an in silico investigation to assess the DNA binding properties of N-(acetylphenyl)-N-ferrocenylmethylnitroaniline derivatives. This investigation utilizes a diverse computational strategy, incorporating molecular docking, as well as an assessment of ADME (Absorption,

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Distribution, Metabolism, Excretion), and toxicity [6,7].

The main aim of this study is to clarify the capability of these compounds as agents binding to DNA. By using computational techniques, we delve into the intricate interactions occurring at the molecular level between these compounds and DNA strands [8–10]. Our investigation not only quantifies the binding affinities but also delves into the structural conformations and dynamic behaviors governing these interactions [11–15]. Specifically noteworthy are the electrostatic forces that assume a crucial role in the binding mechanism, contributing to the stability of the complexes formed between DNA and the derivatives [16].

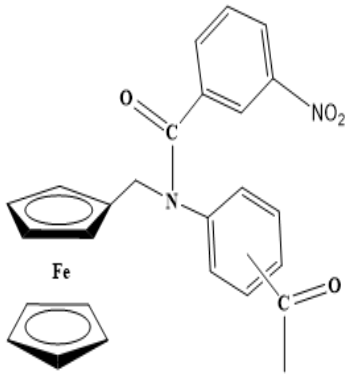
Furthermore, this study extends its scope to include an assessment of the ADME properties and toxicity profiles of the compounds under scrutiny [17–19]. This comprehensive evaluation provides essential insights into the pharmacological potential and safety considerations of these derivatives [20,21].

The implications of our findings extend beyond fundamental research, as they hold the promise of advancing the development of DNA-binding agents. These agents, with their distinctive properties, could find applications in a multitude of biomedical fields, including drug delivery, gene therapy, and cancer treatment [22,23]. Therefore, our exploration of these novel N-(Acetylphenyl)-N-Ferrocenylmethylnitroaniline derivatives contributes significantly to the ongoing pursuit of innovative molecular tools and therapeutic strategies in the realm of DNA-targeted interventions.

2. Computational Method

2.1. Material and method

N-(Acetylphenyl)-N-ferrocenylmethyl-3-nitroaniline derivatives investigated in this study are depicted in Figure 1.



Fc2Ac : 2-Ac
Fc3Ac : 3-Ac
Fc4Ac : 4-Ac

Figure 1. Molecular structures of N-(2-acetylphenyl)-N-ferrocenylmethyl-3-nitroaniline (Fc2Ac), N-(3-acetylphenyl)-N-ferrocenylmethyl-3-nitroaniline (Fc3Ac), and N-(4-acetylphenyl)-N-ferrocenylmethyl-3-nitroaniline (Fc4Ac)

2.2. Screening For Toxicity properties

The ProTox-II website was used to predict the toxicity of the compounds, incorporating assessments for acute toxicity, cytotoxicity, hepatotoxicity, carcinogenicity, immunotoxicity and mutagenicity. This information can help identify probable toxicity mechanisms and rank compounds for upcoming toxicity evaluations.

2.3. Structural optimization

To acquire a deeper understanding of the binding behavior, the structures of all compounds were thoroughly optimized utilizing the DFT (Density Functional Theory) method. This optimization process was conducted using the Gaussian 09 package [24] , applying the B3LYP theoretical level. For the optimization of the iron atom, the LanL2DZ basis set was employed [25–27], while the carbon, hydrogen, oxygen, and nitrogen atoms were optimized using the 6-311G+(d) basis set [28–30]. Figure 2 displays the optimized ground state geometries of these compounds. The figure reveals that the ferrocene segment of these molecules exhibits a sandwich-like structure, with two cyclopentadienyl moieties enclosing the Fe^{2+} ion between them.

2.4. Molecular docking studies

Molecular docking analysis of the three N-(acetylphenyl)-N-ferrocenylmethyl-3-nitroaniline derivatives with DNA were performed using AutoDockVina 1.1.2 docking software [31]. The 3D crystal structure of the dodecamer DNA

sequence d(CGCGATATCGCG) (ID: 1DNE), as illustrated in Figure 3, was retrieved from the online Protein Data Bank (https://www.rcsb.org/pdb) and employed as the target molecule[32]. The output files from the geometry optimization of N-(acetylphenyl)-N-ferrocenylmethyl-3-nitroaniline derivatives were converted into pdb format and subsequently employed in the docking procedure. The target DNA receptor underwent a preparation process involving the removal of small compounds, water molecules, and ions. Additionally, to enhance the accuracy of the simulation, polar hydrogen atoms and Gasteiger charges were incorporated into the target structure. Subsequently, the Lamarckian genetic algorithm method was employed to explore the optimal binding position within the DNA target. The grid box center and size were chosen as indicated in table 1.

while all other parameters were maintained at their default settings[33,34]. After the docking process, the optimal binding positions of the compounds with DNA were visualized using Discovery Studio 4.1 [35].

3. Results and discussion

3.1. Toxicity study

According to the globally harmonized system of categorization and labeling of chemicals, the LD_{50} values of the investigated compounds, including the control cisplatin, were found to range between 1502 and 200 mg/kg. This places them within classes 4 and 2 of the system. From 0.58 to 0.99, all of the substances had a high chance of prediction that they were immunotoxic active. As can be seen in Table 2, all substances were expected to be neither cytotoxic nor hepatotoxic. Furthermore, cisplatin showed a better toxicity profile, with predictions that it would not be mutagenic, hepatotoxic, immunotoxic, carcinogenic, or cytotoxic.

3.2. Molecular docking investigation

The lowest energy conformation obtained from the ligand-target complex was selected as the predicted binding mode. The output data from the docking studies were then visualized using Discovery Studio[35]. As illustrated in Figure 4, this visualization allows to observe the lowest energy docking poses of the compounds.

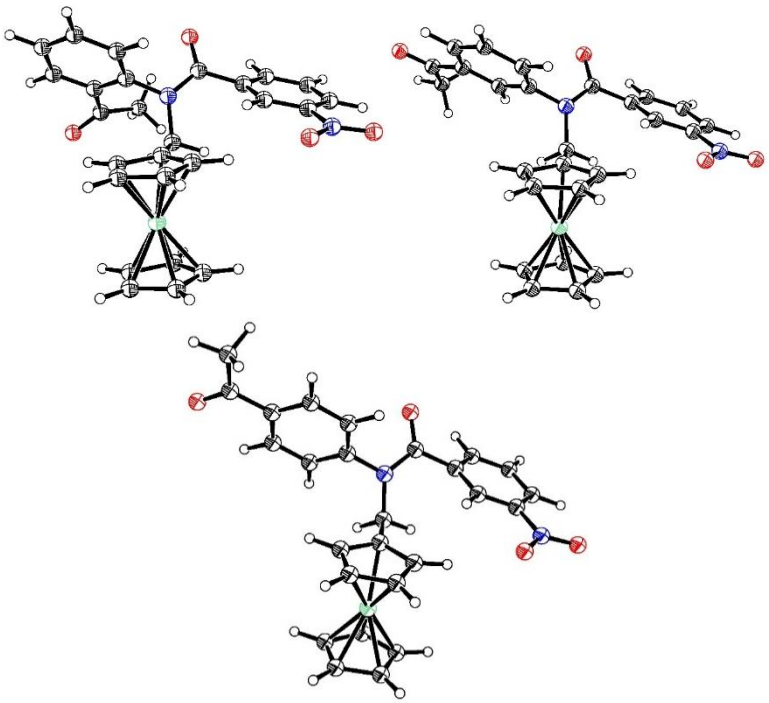


Figure 2. The fully optimized three-dimensional structures of ligand FcAc (ORTEP View 03, V1.08); thermal ellipsoids are plotted at the 50% probability level

Table 1. Grid parameters for molecular docking simulation

Grid Centre			Grid Size (Å)	Spacing (Å)
x	y	z	60 × 60 × 60	0.375
11.664	23.101	78.864		

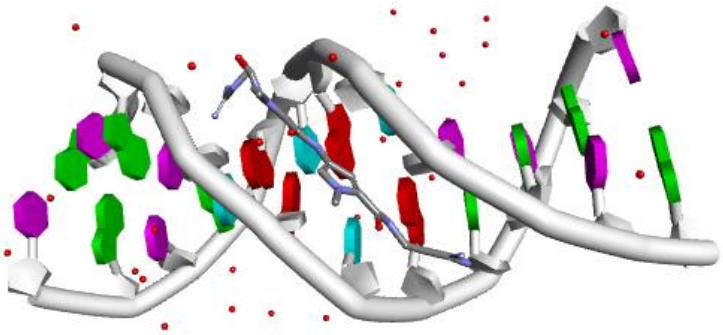
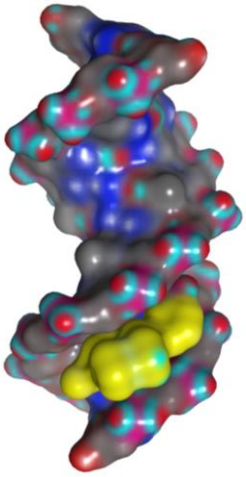


Figure 3. 3D-crystal structure of DNA sequence d(CGCGATATCGCG) 2 dodecamer (ID: 1DNE)

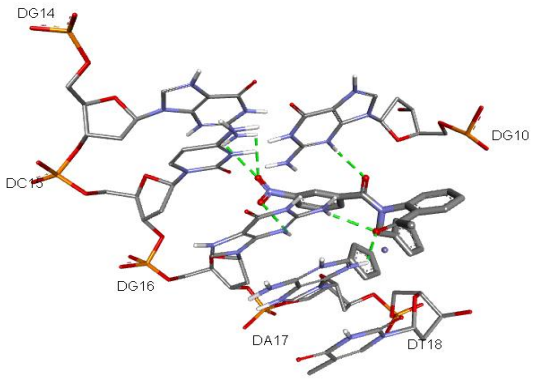
Table 2. Toxicity prediction and probability of prediction.

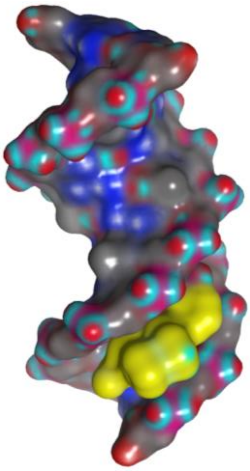
Molecule	Class	LD_{50}	Hepato	Carcino	Immuno	Muta	Cyto
Fc2Ac	4	1502	-0.63	+0.56	+0.89	+0.76	-0.61
Fc3Ac	4	1500	-0.65	+0.60	+0.58	+0.79	-0.64
Fc4Ac	2	538	-0.65	+0.60	+0.88	+0.79	-0.64
Cis-p	3	200	-0.90	-0.78	-0.99	-0.80	-0.73

LD50 (mg/kg), - (Inactive toxic class (probability score)), + (Active toxic class (probability score))

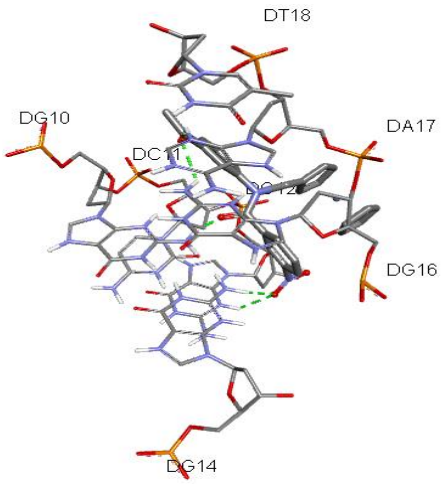


Fc2Ac





Fc3Ac



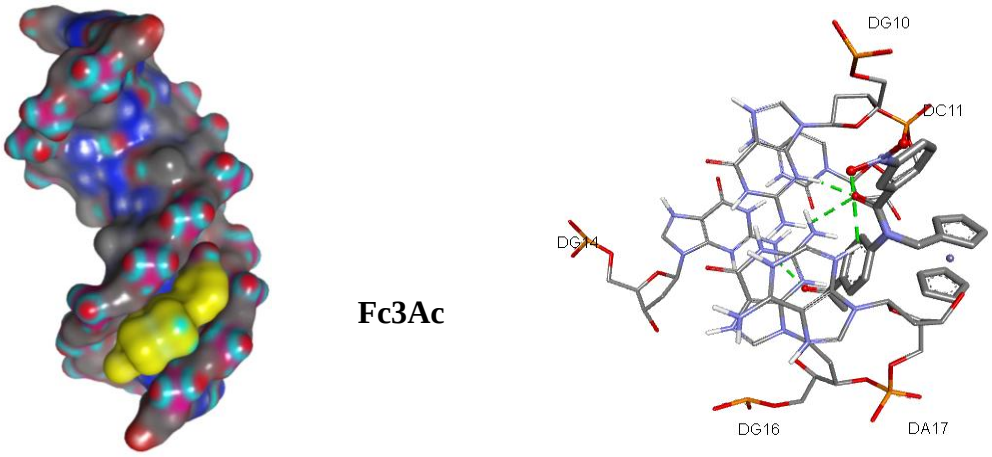


Figure 4. Surface view and 3D binding mode of the docked poses of FcAc (yellow color) with DNA (PDB ID: 1DNE) illustrating the insertion of FcAc in the minor groove of DNA

The findings from our molecular docking analyses indicate a significant involvement of hydrogen bonding interactions in the binding process. Remarkably, all molecules exhibited negative binding energy higher than 8 kcal.mol^{-1}, establishing interactions with the target DNA (1DNE) through conventional hydrogen bonds with the nucleotides DG10, DC11, DG14, DG16, DA17, and DT18. Table 3 provides a concise summary of the interacting nucleotides, their respective bond types, bond lengths, and the associated binding energy.

To assess the affinity of FcAc for various base pairs, a mutant target was generated using the 1DNE sequence [37–39]. Two new DNA sequences, d(GCGCGCGCGCGC)2 dodecamer and poly AT sequence d(ATATATATATAT)2 dodecamer, were designed using PyMOL 1.2r3pre [12] (Figure 5). Then, the docking simulation at the same condition was carried out for both sequences. All ligands are more inclined to the mutant poly GC and poly AT sequence, but this tendency is not very different and it can be concluded that all ligands have almost the same tendency to both mutant and non-mutant sequences.

Conducting molecular docking studies on mutant targets enables the evaluation of the resilience of ligand binding across diverse DNA sequences, potentially enhancing the predictive capability of the investigation. This approach serves as a guide for crafting compounds that exhibit optimized binding to distinct DNA motifs, thereby augmenting the prospects for precise therapeutic interventions. Additionally, such studies facilitate an exploration of how flexible ligands adapt to varying DNA sequences, a critical aspect in comprehending the dynamic nature of ligand-DNA interactions.

Docking analysis was performed using the stable conformation that has the lowest binding energy, the interactions were visualized using Discovery Studio[35]. The results indicated that all compounds are more inclined to poly GC sequence and Fc4Ac is more inclined to both sequences, but this tendency is not very different and it can be concluded that all compounds have the same tendency to both mutant and non-mutant sequences. Figures 6 and 7 depict the 3D hydrogen binding mode of FcAc with the neighboring nucleotides in the active site of mutant poly GC and poly AT DNA.

In the binding model of the FcAc ligands with the mutant poly AT and poly GC sequence, the estimated free binding energy and binding constant values are listed in Table 4.

Table 3. Hydrogen bonding, binding constant, and binding free energy values of FcAc-DNA adducts obtained from molecular docking analysis data

Adduct	nucleotide	Distance (Å)	$K_b(M^{-1})$	-ΔG (kcal/mol)
Fc2Ac-DNA	DG10 DG14 DG16 DA17	2.43 2.76 and 2.80 2.73 2.50	2.87×10^6	8.8

	DT18	2.24		
Fc3Ac-DNA	DC11 DG12 DC14 DG16 DC17	1.81 2.46 1.43 2.32 2.31	1.23×10^6	8.3
Fc4Ac-DNA	DG10 DC11 DG14 DA17	2.02 3.00 2.47 3.02	3.40×10^6	8.9

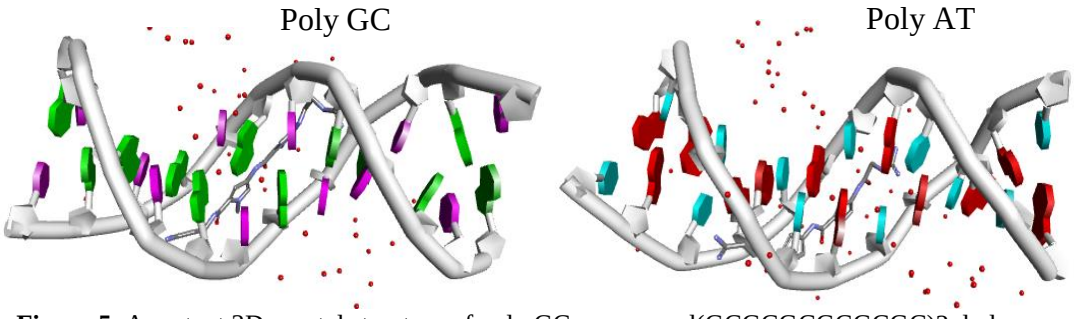


Figure 5. A mutant 3D-crystal structure of poly GC sequence d(GCGCGCGCGCGC)2 dodecamer and poly AT sequence d(ATATATATATAT)2 dodecamer

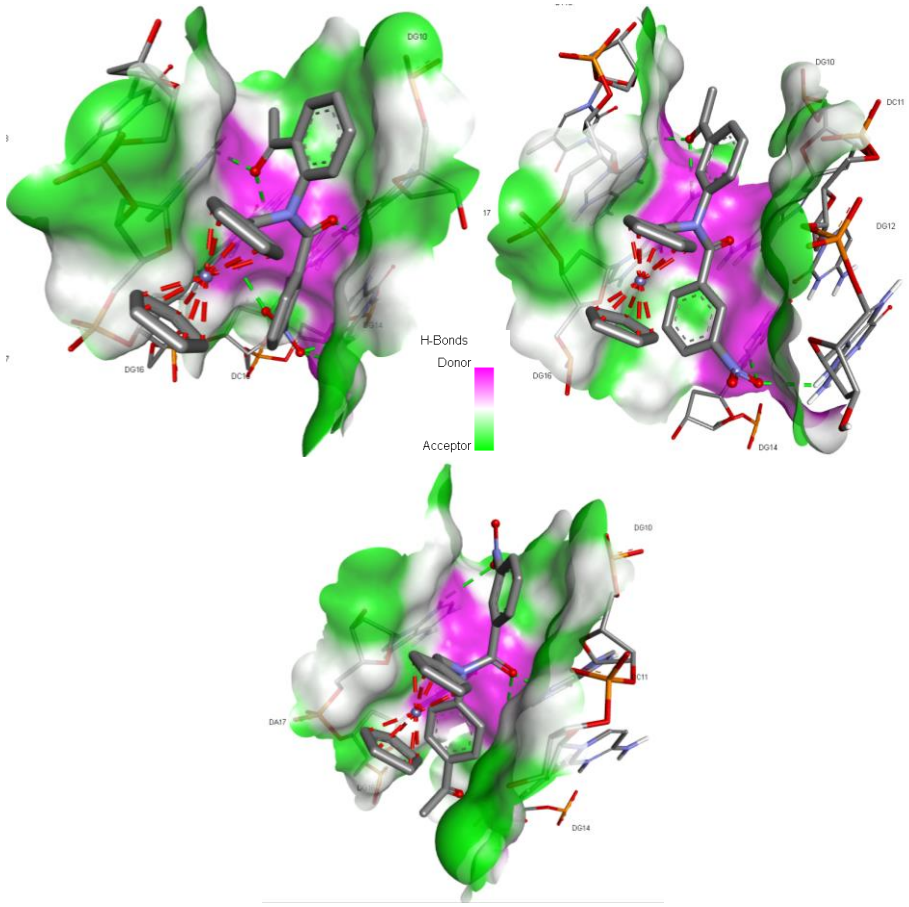


Figure 6. 3D Hydrogen binding mode of the FcAc with poly AT sequence d(ATATATATATAT)2 dodecamer, hydrogen bonds are denoted by green dashed line

Table 4. Binding constant and binding free energy values of the interaction of ligands FcAc with mutants poly AT and poly GC obtained from molecular docking analysis data

Adduct	K(M^{-1})	-ΔG (kcal/mol)
Fc2Ac-poly AT	8.81×10^{5}	8.1
Fc3Ac-poly AT	3.40×10^{6}	8.9
Fc4Ac-poly AT	6.69×10^{6}	9.3
Fc2Ac-poly GC	1.04×10^{5}	8.2
Fc3Ac-poly GC	9.39×10^{6}	9.5
Fc4Ac-poly GC	1.11×10^{7}	9.6

3.3. Docking validation

The validation of our docking procedure was ensured by conducting a redocking experiment with the co-crystallized ligand netropsin, using the same methodology applied to FcAc. Netropsin was initially detached from the DNA structure and then repositioned within the DNA utilizing identical grid settings. The resulting redocked conformation was superimposed onto the reference co-crystallized ligand. Figure 8 provides a comparative view, showcasing the redocked conformation (highlighted in red) alongside the original ligand (highlighted in green). The calculated RMSD value between them is 1.533 Å. The remarkable alignment and the RMSD value below 2 Å underscore the efficacy of the AutoDock Vina algorithms in executing a robust molecular docking protocol.

4. Conclusions

In conclusion, our in silico study exploring the DNA binding characteristics of novel N-(Acetylphenyl)-N-Ferrocenylmethylnitroaniline derivatives presents promising candidates with

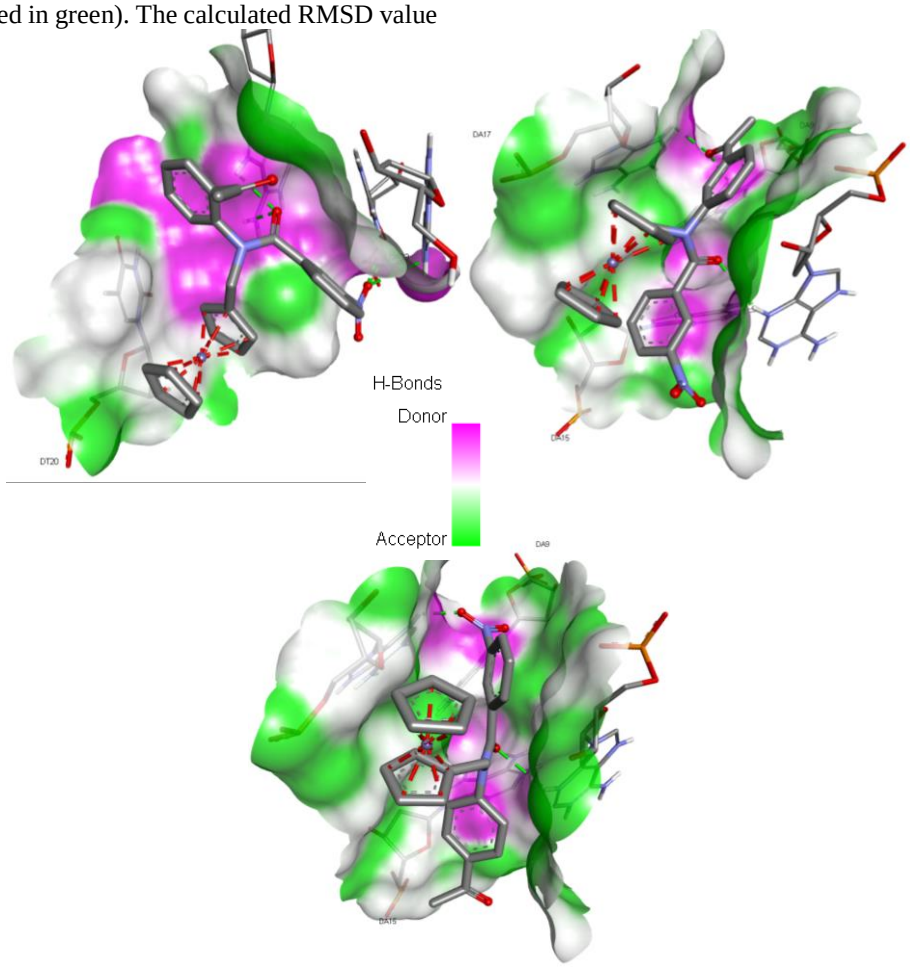


Figure 7. 3D Binding mode of the FcAc with poly GC sequence d(GCGCGCGCGCGC)2 dodecamer, hydrogen bonds are denoted by green dashed line

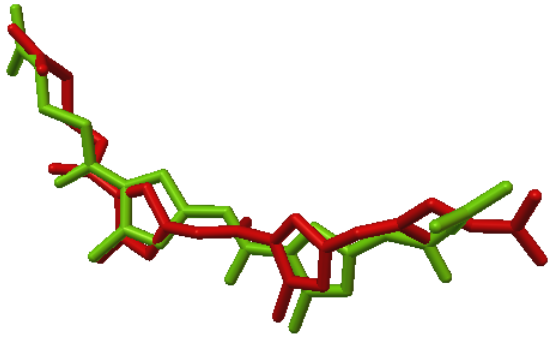


Figure 8. Comparison between the redocked conformation and original ligand; red: redocked conformation; green: original ligand with an RMSD value of 1.533 Å

robust affinities for DNA, as evidenced by strong electrostatic interactions. The extensive computational techniques used, such as ADME/toxicity evaluation and molecular docking, gave important new information about the safety profiles and binding potential of these drugs. The results of the toxicity investigation indicated favorable profiles that placed these compounds in certain hazardous groups. The importance of hydrogen bonding interactions in the binding process was brought to light by molecular docking studies, where all drugs showed negative binding energies, which are a sign of strong interactions with the target DNA. The evaluation of these chemicals' affinity for various base pairs highlighted their tendency to remain consistent across both mutant and non-mutant sequences. The efficiency of the AutoDock Vina algorithms was proven by redocking the co-crystallized ligand netropsin to validate the docking process. All things considered, our research adds important information to the design of DNA-binding substances that may find use in a variety of biomedical domains, such as medication delivery, gene therapy, and cancer treatment.

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