

COMPUTATIONAL ANALYSIS OF N-FERROCENYLMETHYL-N-PHENYLBENZOHYDRAZIDE: MOLECULAR DOCKING AND DYNAMIC STABILITY WITH BSA

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ABSTRACT

This study provides a comprehensive computational analysis of N-ferrocenylmethyl-N-phenylbenzohydrazide (FHD) as a potential bioactive compound by employing density functional theory (DFT), molecular docking, and molecular dynamics (MD) simulations. DFT calculations, including frontier molecular orbital and molecular electrostatic potential analyses, reveal the electronic structure and reactive regions of FHD. Reduced density gradient analysis highlights weak intermolecular interactions, confirming molecular stability. Molecular docking studies show strong binding of FHD to BSA (BSA, PDB ID: 6QS9), with a binding energy comparable to the anti-inflammatory drug diclofenac (DIF). MD simulations demonstrate that the 6QS9-FHD complex exhibits superior stability over 100 ns, lower root RMSD, and consistent compactness compared to 6QS9-DIF. Radius of gyration (Rg) and solvent-accessible surface area (SASA) analyses further confirm the stability of the FHD complex. These findings suggest FHD's potential as a candidate for anti-inflammatory applications.

Keywords: N-ferrocenylmethyl-N-phenylbenzohydrazide; Molecular docking; MD simulations; BSA

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1. INTRODUCTION

With the rise of computational tools, bioinformatics has become a reliable approach for accurately predicting the physicochemical and pharmacological characteristics of various compounds. This advancement is primarily driven by the growing field of *in silico* studies, which utilize computer-aided drug design software and integrated collaborative platforms [1].

This computational approach proves particularly valuable as a preliminary step before performing *in vitro* and *in vivo* experiments [2]. The integration of *in silico* techniques, including molecular docking, has significantly advanced various research areas, such as genetic and evolutionary biology [3]. These methods enable the prediction of molecular interactions with specific receptors at the atomic scale, relying on energy transfer mechanisms [4]. Such insights assist researchers in developing more efficient therapeutic strategies, particularly for combating inflammation.

Human health is continuously challenged by various agents, including microorganisms, chemicals, radiation, and physical factors, which can also cause detrimental effects at the genetic level [5,6]. However, the human body employs several natural defense mechanisms to preserve its physiological balance. Among these, inflammation serves as a critical immune response, primarily driven by immune cells that release key mediators like cytokines, chemokines, and interferons to facilitate tissue healing and recovery [7]. Nevertheless, under pathological conditions, immune system dysfunction may trigger an excessive inflammatory response, contributing to the onset of chronic diseases such as ulcers, asthma, tuberculosis, and rheumatoid arthritis [8].

Ferrocenylmethyl derivatives have gained considerable attention due to their remarkable stability under aqueous and aerobic conditions, as well as their diverse biological applications [9–16]. Extensive research has shown that specific ferrocene-based derivatives display potent activities against a wide range of diseases, including fungal and bacterial infections [17–21], cancer [22,23], diabetes [24], malaria [16,25], and inflammation [26].

In this context, we investigated the interaction of N-ferrocenylmethyl-N-phenylbenzohydrazide (FHD) with bovine serum albumin (BSA) using molecular docking studies. To further assess its molecular stability and behavior, molecular dynamics (MD) simulations were performed for a duration of 100 ns.

2. RESULTS AND DISCUSSION

2.1. Docking analysis

The docking protocol was assessed by reintroducing the co-crystallized ligand into the binding site of its respective protein, as outlined in the methodology. The results from the validation experiments indicated a good alignment between the docked conformation of the ligand and its original structure, with an RMSD value of 1.315 Å, Figure1.

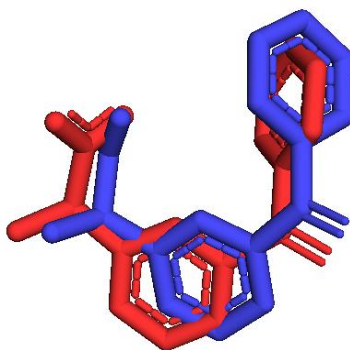


Fig.1. Structures of (Blue) native co-crystal and (Red) dock pose in the active site of 6QS9

As presented in Table 1, FHD exhibited a higher binding affinity to BSA with a binding free energy (ΔG) of -9.21 kcal/mol, compared to the standard anti-inflammatory drug diclofenac (DIF), which showed a ΔG of -7.79 kcal/mol.

Both FHD and DIF formed two hydrogen bonds with key amino acids in the active site, namely Tyrosine (TYR A:149) and Arginine (ARG A:256), as detailed in Table 1 and Figure 2.

DIF was further stabilized by five hydrophobic interactions involving Leucine (LEU A:218, LEU A:259), Alanine (ALA A:260), and Isoleucine (ILE A:263, ILE A:289).

FHD exhibited similar hydrophobic interactions with the amino acids as observed with DIF, including LEU A:218 (two bonds), LEU A:259, ALA A:260, and ILE A:289 (two bonds). In addition to other hydrophobic bonds with ARG A:198, ARG A:217, LYS A:221, PHE A:222, ARG A:256, and ALA A:290 (four bonds). Moreover, FHD also displayed one electrostatic interaction with ARG A:298, which contributed to its enhanced binding affinity and overall stability within the active site.

These findings highlight FHD's enhanced binding interactions compared to DIF, suggesting its potential as a candidate for anti-inflammatory applications.

Table 1. Interaction type, and Binding interaction of ligands with BSA receptors

Ligand	Binding interaction	Residues
DIF	Conventional H-Bond	TYR A:149, ARG A:256
	Pi-Sigma	LEU A:218, ILE A:289
	Pi-Alkyl	LEU A:259, ALA A:260, ILE A:263
FHD	Conventional H-Bond	TYR A:149, ARG A:256
	Electrostatic	ARG A:198
	Pi-Sigma	ALA A:290
	Alkyl	ARG A:217, LEU A:218, LYS A:221, ILE A:289, ALA A:290
	Pi-Alkyl	PHE A:222, ARG A:256, LEU A:259, ALA A:260, ILE A:289, ALA A:290

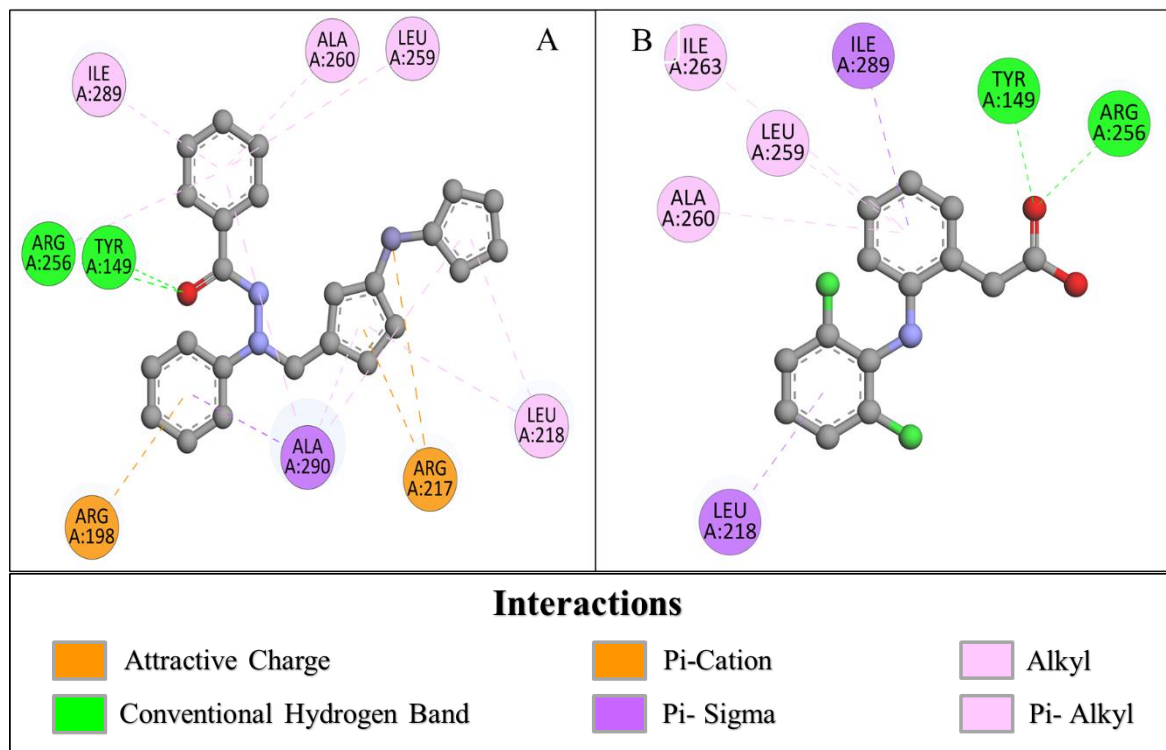


Fig.2. 2D illustration of possible interactions of FHD and DIF with 6QS9 protein

2.2. DFT calculations studies

2.2.1. FMO and MEP surface

The HOMO-LUMO energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$) provides valuable insights into a compound's reactivity, with HOMO acting as the electron donor and LUMO as the electron acceptor [27–30]. The MEP surface helps identify reactive regions by showing the distribution of electronic charges. Blue regions indicate electron-deficient areas, making them favorable for nucleophilic attack, while red regions, being electron-rich, are suited for electrophilic attack [27–30]. The HOMO-LUMO energy and MEP surfaces of the FHD compound are shown in Figure 3. The HOMO is mainly concentrated on the ferrocene moiety, whereas the LUMO is predominantly associated with the benzo hydrazide group. The energy gap indicates the presence of intramolecular charge transfer interactions [31,32]. Smaller ΔE values suggest greater charge transfer, while larger energy gaps are linked to increased stability and reduced reactivity [33,34]. As depicted in Figure 3, the HOMO and LUMO energies for FHD are -5.586 eV and -1.588 eV, respectively, resulting in an $\Delta E_{\text{L-H}}$ of 3.998 eV. The most negative electrostatic potential is found around the oxygen atom of the carbonyl group (C=O) and the aniline ring, while the positive regions are centered on the hydrogen atoms of the amine group (NH), as well as the hydrogen atoms of the ferrocene moiety and benzo hydrazide ring.

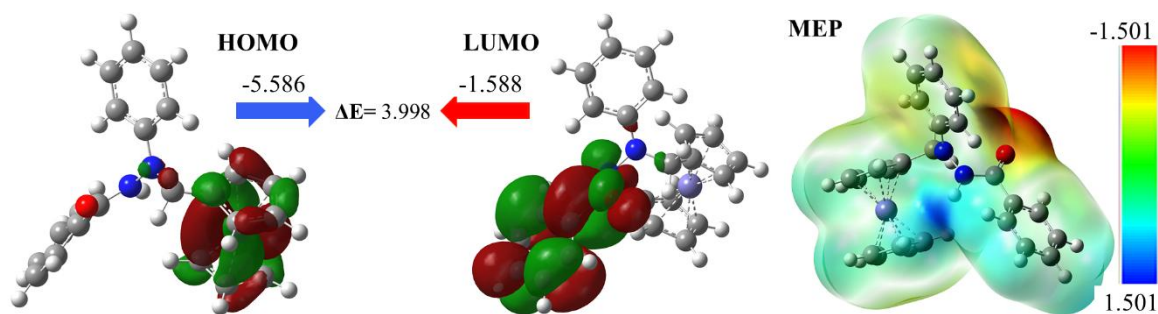


Fig.3. HOMO-LUMO and MEP surfaces of FHD

2.2.2. RDG Analysis

The stability of the FHD was largely due to weak intermolecular and intramolecular interactions, analyzed using the reduced density gradient (RDG) method, focusing on non-covalent forces (NCI). The λ_2 sign differentiate between bound ($\lambda_2 < 0$) and unbound ($\lambda_2 > 0$) interactions. Red peaks indicate steric repulsion within the ring structure, green peaks corresponded to Van der

Waals interactions, and blue spikes represented electrostatic forces, such as hydrogen and halogen bonding [35,36].

The 2D and 3D RDG plots (Figure 4) reveal that steric effects, represented by red color, were observed within the range of 0.01 a.u. to 0.05 a.u., particularly at the centers of the phenyl rings, cyclopentadiene rings, and around the ferrocene atom. Van der Waals interactions are indicated by red-green mixed spikes, found in the range of -0.015 a.u. to 0.01 a.u., particularly between the hydrogen atoms and the carbonyl (C=O) group or the carbon atoms in the phenyl ring.

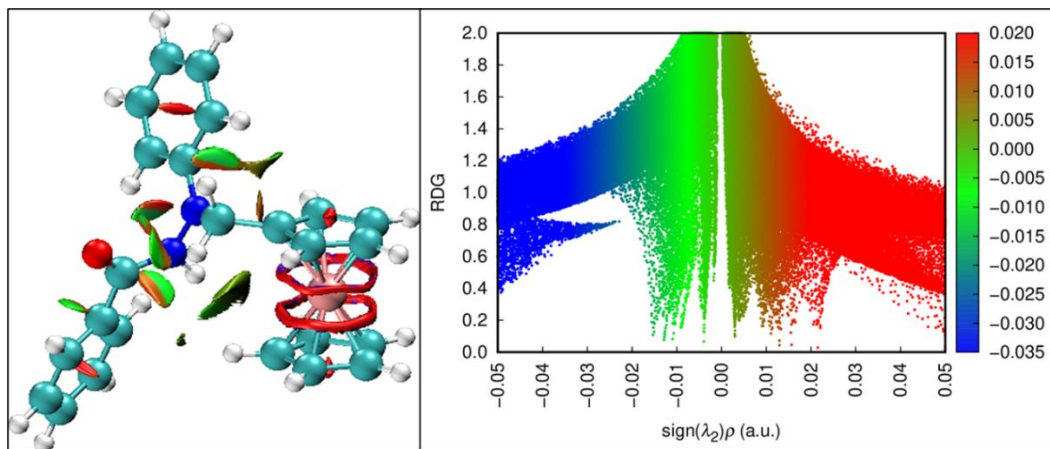


Fig.4. RDG (right) and NCI (left) plot of FHD

2.3. MD Simulations

The stability of the molecular configurations throughout a 100 ns MD simulation was evaluated using RMSD, RMSF, Rg and SASA analysis (Table 2, Figure 4).

Table 2. The average value obtained from molecular dynamics simulations for FHD and DIF

Metrics	6QS9-FHD	6QS9-DIF
RMSD (nm)	0.141	0.411
RMSF (nm)	0.175	0.166
Rg (nm)	2.741	2.715
SASA (nm ²)	292.24	290.53

As shown in Figure 5, the initial analysis of RMSD values indicates that the 6QS9-FHD complex maintains significant conformational stability within the protein's active site, with an average RMSD of 0.141 nm. This stability is evident as the RMSD remains constant throughout the simulation period. In contrast, the standard drug diclofenac (6QS9-DIF) shows a higher average RMSD of 0.411 nm over 100 ns. After an initial increase to 0.510 nm by 22 ns, the RMSD remains stable for the remainder of the simulation.

The RMSF analysis highlights the flexibility and mobility of individual amino acid residues in the 6QS9-FHD and 6QS9-DIF complexes (Figure 5). The average RMSF value for the 6QS9-FHD complex was found to be 0.175 nm, which is comparable to the 6QS9-DIF complex (0.166 nm), indicating lower flexibility in both complexes.

The Rg was analyzed to evaluate the compactness and molecular stability of the docked complexes, while SASA analysis was performed to examine ligand-induced changes in the solvent-accessible surface area. Both the FHD and DIF complexes exhibited comparable Rg and SASA fluctuations. The Rg values were 2.741 nm for 6QS9-FHD and 2.715 nm for 6QS9-DIF. Similarly, the average SASA values were 292.24 nm² and 290.53 nm² for 6QS9-FHD and 6QS9-DIF, respectively.

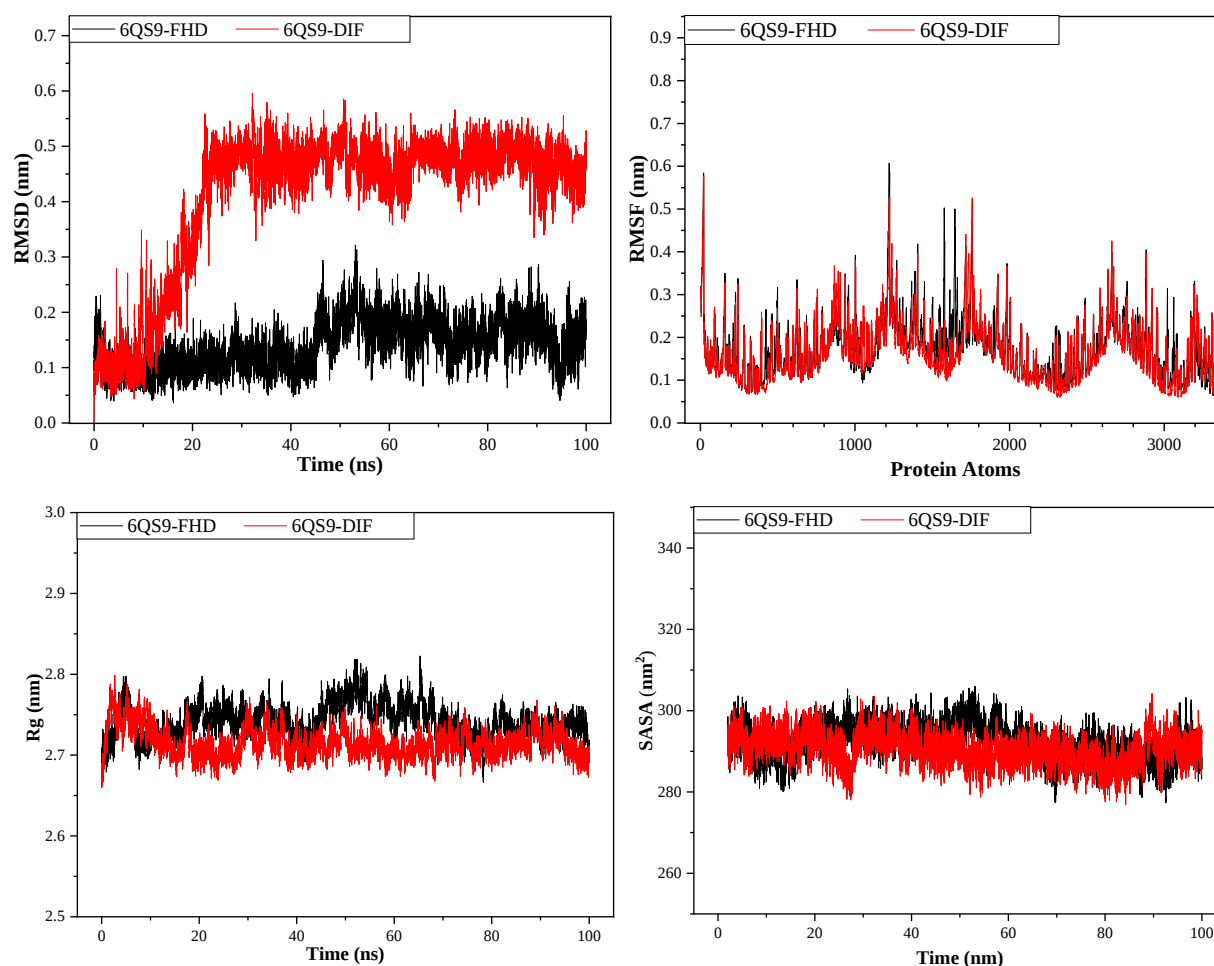


Fig.5. stability evaluation through 100 ns of MD simulations for each system

2.4. Theoretical methodology

2.4.1. Computational details

The structural optimization of FHD was carried out using Gaussian 16 W software [37], applying the DFT method at the B3LYP/6-311++G (d,p) level of theory [38]. Essential parameters, including the energy of the highest occupied molecular orbital (E_{HOMO}), the energy of the lowest unoccupied molecular orbital (E_{LUMO}), the energy gap (ΔE) between HOMO and LUMO, molecular electrostatic potential (MEP) and reduced density gradient (RDG) were calculated.

2.4.2. Docking studies:

The crystallographic structure of the target protein BSA (PDB ID: 6QS9) was retrieved from the RCSB PDB database [39]. Preprocessing was carried out using AutoDock Tools 1.5.7 (ADT) software [40], where water molecules and heteroatoms were removed. Additionally, Kollman and

Gasteiger charges, along with polar hydrogens, were added to the protein to prepare it for docking simulations.

The geometry of FHD was derived from its DFT-optimized structure and saved in PDB format. The 3D structure of the reference drug diclofenac (DIF) was acquired from PubChem [41], and MMFF charges were assigned using ADT.

Key amino acids in the active site were identified with Discovery Studio Visualizer (DSV) software [42] based on the co-crystallized ligand. Docking simulations were performed within a grid box of dimensions $50 \times 50 \times 50$ Å (xyz points), with a grid spacing of 0.375 Å. The grid center coordinates were set to X = -11.134, Y = -8.424, and Z = 14.203.

The docking protocol's validity was verified by re-docking the co-crystallized ligand into its protein's binding site. According to the literature [43], docking results are considered reliable if the root mean square deviation (RMSD) value is below 2.0 Å compared to the experimental structure. Docking calculations were performed using Lamarckian genetic algorithms, with each experiment consisting of 20 runs. These runs produced various ligand binding energies and corresponding conformations. The conformation with the lowest binding energy was identified as the optimal pose and used for further docking analysis [44–48].

2.4.3. Molecular dynamics (MD) simulations:

The stability of biomolecular complexes was evaluated using 100 ns MD simulations with Gromacs-2023 on a GPU system [49]. The initial conformation was taken from the docked complex. Protein topologies were generated with the AMBERGS force field, and ligand parameters were assigned using the general AMBER force field (GAFF).

The systems were solvated in a TIP3P water cubic box, neutralized with Na⁺ and Cl[−] ions, and energy-minimized using steepest descent and conjugate gradient methods until forces dropped below 10.0 kJ/mol. NVT equilibration was conducted at 300 K for 0.1 ns using the v-rescale thermostat, followed by 0.1 ns NPT equilibration with Berendsen pressure coupling [50,51].

To explore the biomolecular behavior of FHD compound within target protein active sites and its impact on essential biological functions, several key parameters were analyzed. The root mean square deviation (RMSD) was used to monitor changes in the backbone atoms of target proteins, while root mean square fluctuation (RMSF) quantified the flexibility of individual amino acids. The radius of gyration (Rg) was calculated to evaluate the compactness of the complexes, and the

solvent-accessible surface area (SASA) was determined to assess the overall stability of the systems.

3. CONCLUSION

The computational evaluation of ferrocenylmethyl-N-phenylbenzohydrazide (FHD) binding to bovine serum albumin (BSA) demonstrated its strong binding affinity, as confirmed by molecular docking. DFT calculations, including FMO, MEP, and RDG analyses, established the electronic and structural features of FHD, highlighting its molecular stability and regions prone to interactions. Comparative studies with diclofenac (DIF), an anti-inflammatory drug, revealed that the 6QS9-FHD complex exhibited greater conformational stability and reduced fluctuations during MD simulations. Additionally, the Rg and SASA analyses indicated that FHD induces minimal structural perturbations in the protein. suggest FHD as a promising anti-inflammatory agent, warranting further in vitro and in vivo studies.

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