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Muhammad Mohsin Ejaz

Ghazi University

Hamza Iftikhar

Ghazi University

K.S. Al-Namshah

Imam Mohammad ibn Saud Islamic University

Saira Khan

Ghazi University

Sadaf Ashraf

University of Balochistan

Nazia Meer

University of Balochistan

Musarat Riaz

Sardar Bahadur Khan Women's University

Muhammad Saleem

`msaleem@gudgk.edu.pk`

Ghazi University

Muhammad Shar Jhahan Khan

Ghazi University

Research Article

Keywords: Alzheimer's disease, DFT, TD-DFT, Molecular docking, and ADMET

Posted Date: October 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11082767/v1>

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Additional Declarations: No competing interests reported.

Substituent Effects on Electronic Properties and Acetylcholinesterase Inhibitory Potential of Donepezil Analogues: A DFT, Molecular Docking, and ADMET Study with Dynamics Validation of the Lead Candidate

Muhammad Mohsin Ejaz^a, Hamza Iftikhar^a, K.S. Al-Namshah^b, Saira Khan^{*a}, Sadaf Ashraf^c, Nazia Meer^c, Musarat Riaz^d, Muhammad Saleem^{*a}, Muhammad Shar Jhahan Khan^a

^a Department of Chemistry, Faculty of Science, Ghazi University, Dera Ghazi Khan, 32200, Pakistan

^b Department of Chemistry, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, 11623, Saudi Arabia.

^c Department of Chemistry, University of Balochistan, Quetta, Balochistan, 83700, Pakistan

^d Department of Chemistry, Sardar Bahadur Khan Women's University, Quetta, Balochistan, 83700, Pakistan

*Correspondance: msaleem@gudgk.edu.pk ; hod.chem@gudgk.edu.pk; sairaakhan692@gmail.com

Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is the leading cause of dementia in older people. Donepezil is an acetylcholinesterase inhibitor used for symptomatic management of AD. Due to its high efficacy, low hepatic toxicity, prolonged half-life, and enhanced bioavailability, it was used as a reference. In this study, a series of donepezil derivatives (**MH-1 to MH-5**) were designed through structural modification at the benzyl moiety and systematically investigated along with the reference using density functional theory, time-dependent DFT, molecular docking, and in silico pharmacokinetics analysis. The geometry optimization and electronic structure calculations were performed using the B3LYP/6-311G (d, p) level. Frontier molecular orbital analysis indicated reduced energy in **MH-1** and **MH-2**, while the optical study shows that all derivatives (**MH-1 to MH-5**) demonstrate a bathochromic shift. The molecular docking of all derivatives and the **reference** against the acetylcholinesterase receptor 4EY7 demonstrates strong affinities, where **MH-1**, **MH-2**, and **MH-4** show binding energy equivalent to the reference (-12.2 kcal/mol). A 300 ns molecular dynamics simulation of the AChE **MH-4** complex showed a stable trajectory, as indicated by RMSD/RMSF profiles. Binding free energy calculated using MM-GBSA combined with the Interaction Entropy approximation yielded $\Delta G = -13.04 \pm 3.73$ kcal/mol, confirming favourable and thermodynamically stable binding. Moreover, the results of the ADME analysis indicated good oral absorption and blood-brain barrier penetration. Toxicity assessment predicted favourable safety profiles for most of the derivatives, except for a few that show potential concerns. In summary, all these results indicate that **MH-4** has encouraging physicochemical and biological properties for anti-Alzheimer drug development.

Keywords

Alzheimer's disease, DFT, TD-DFT, Molecular docking, and ADMET.

1. Introduction

Globally, Alzheimer's disease (AD) is the most commonly occurring form of dementia and primarily affects older people. It is an age-related neurodegenerative disorder that is increasing each year in the aging population. It is estimated that approximately 55 million people worldwide are suffering from dementia; this number is projected to grow to 131 million by the year 2050 (Ortiz-Islas et al. 2025). It was first discovered in 1906 by a neurologist named Dr Alois Alzheimer. While examining the brain tissue of a dead AD patient, he published his findings in 1907 (Joodi et al. 2025). The exact cause of AD is yet to be determined. Several hypotheses are proposed to explain it, but these predominantly discuss the Amyloid hypothesis, Tau Hypothesis, Neuroinflammation hypothesis, and Cholinergic Hypothesis.(Zhang et al. 2024)

The hypothesis that AD could be caused by amyloid- β ($\alpha\beta$) first originated in the 1980s.(Kepp et al. 2023) The amyloid cascade hypothesis suggests that the leading cause of AD is the aggregation of $\alpha\beta$ plaques in the brain. According to the hypothesis, in AD, normal clearance mechanisms are disrupted, thus leading to the formation of $\alpha\beta$ plaques (Nasb et al. 2024).

The tau hypothesis, which develops on the amyloid cascade idea, suggests that in AD patients, the normal balance of glycogen synthase kinase-3 β (GSK3 β) and cyclin-dependent kinase 5 (CDK5) is disrupted. This leads to detachment of tau protein from microtubules, alteration in conformation, and aggregation of different pathological structures. Ultimately, all this leads to cell death (Ivanova et al. 2025).

Recent evidence suggests that microglial-mediated neuroinflammation plays a key role in disease onset and progression (Erdag and Haskologlu 2025). The inflammatory response can assist rapid clearance of $\alpha\beta$, but it can also cause neurodegeneration and synapse loss due to an increase in production of tau and $\alpha\beta$ (AmeliMojarad and AmeliMojarad 2024).

The formulation of the "Cholinergic Hypothesis of Alzheimer's disease" originated from scientific data collected over the last 40 years, suggesting that the loss of cholinergic innervation in the cerebral cortex of Alzheimer's disease patients is the early event associated with cognitive decline (Hampel et al. 2019). Based on the Cholinergic Hypothesis, acetylcholinesterase (AChE) inhibitors offer an effective therapeutic strategy for treating AD symptoms (Makarian et al. 2022).

Donepezil (DNP) was the first revolutionary drug for treating neurodegenerative disorders, especially Alzheimer's disease (Patel et al. 2024). It is an acetylcholinesterase inhibitor (AChEI) which is reversible and non-competitive in its mechanism of action (Saeedi and Mehranfar 2022). Dubey et al.(Dubey et al. 2010) synthesized Donepezil hydrochloride by reacting 2-bromo-5,6-dimethoxy-1-indanone with pyridine-

4-carboxaldehyde in the presence of a strong nucleophilic base, such as n-BuLi. DNP acts by preventing the hydrolysis of acetylcholine. DNP binds to both the peripheral anionic site (PAS) and catalytic sites located within the enzyme's active-site gorge. The inhibition of the acetylcholinesterase enzyme leads to an increase in cholinergic neurotransmission.(Eissa et al. 2023) Development of the drug is very complex, time-consuming, and risky. Many drugs fail at the final stages of drug development due to low efficacy and toxicity, which cause loss of valuable resources.(Wu et al. 2020) Due to these limitations, computational approaches for drug development have gained significant interest in the last few years.(Sadybekov and Katritch 2023) In the last decade, different computational techniques have been employed for the investigation and development of anticancer drugs, antiviral drugs, antibacterial drugs, (Abdulqahar et al. 2023) and inhibitors.(Sharma et al. 2023)

In this study, donepezil was selected ((2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one) as our standard molecule (**R**) due to its efficacy, low hepatic toxicity, prolonged half-life, and enhanced bioavailability.(Jon et al. 2025; Swain et al. 2025) In this study, series of DNP analogues (**MH-1** to **MH-5**) named as 2-((1-(4-aminobenzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one, 5,6-dimethoxy-2-((1-(4-nitrobenzyl)piperidin-4-yl)methyl)-2,3-dihydro-1H-inden-1-one, 2-((1-(4-chlorobenzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one, 2-((1-(4-hydroxybenzyl)piperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one, and 5,6-dimethoxy-2-((1-(4-methoxybenzyl)piperidin-4-yl)methyl)-2,3-dihydro-1H-inden-1-one were studied. **Figure 1** shows the structures of **R** and its analogues. The active site of DNP, containing the 5,6-dimethoxyindanone ring attached to a piperidine moiety, was retained in its original form, so the key engagement with acetylcholinesterase should have been maintained. Structural alterations were implemented at the benzyl group joined to the piperidine nitrogen by incorporating different para-substituted reactive groups, including chloro, nitro, amino, methoxy, and hydroxy groups. Two methods were used to hit the mark: Time-dependent density functional theory (TD-DFT and DFT). The theoretical value of **R** was analysed by four functionals. By comparing the theoretical and experimental values of absorption maxima, the appropriate functional to use for consecutive calculation was identified. Multiple analyses were performed to evaluate many parameters, Such as frontier molecular orbital (FMO) analysis, energy band gap, absorption maxima, non-covalent interaction (NCI), electron localization function, localized orbital locator (LOL), DOS graphs, NBO, light harvesting efficacy, binding energies, and MEP Graph. The Potent and well-known drug was determined by the outcomes. *In silico* analysis was also done for both the standard and its derivatives.

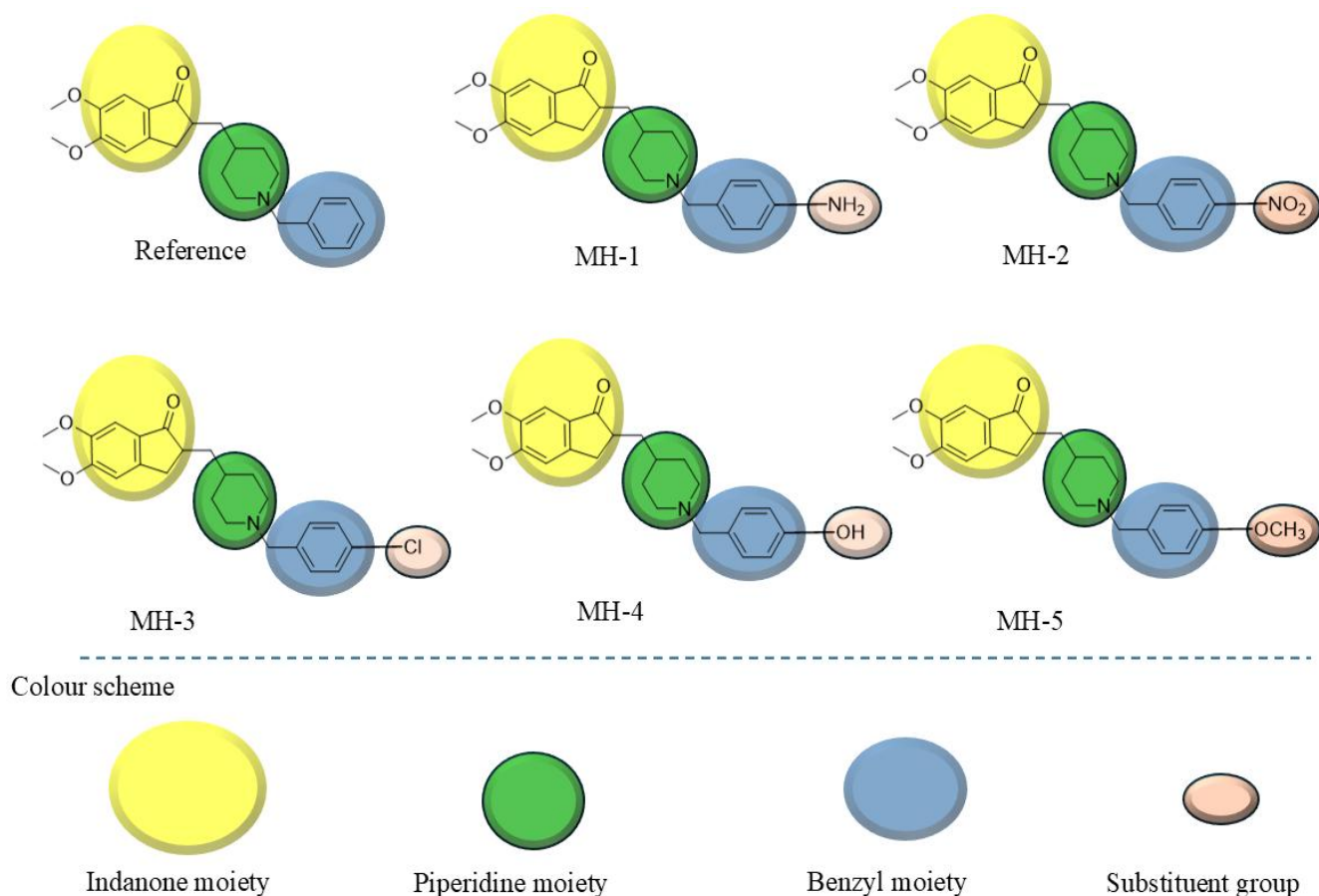


Fig.1 Representation of the reference (R) for all donepezil derivatives (DDs).

2. Computational Methodology

2.1. Level Selection

First, the reference compound (Donepezil) was optimized using four different levels, including B3LYP, CAM-B3LYP, ω B97XD, and MPW1PW91, along with the basis set 6-311G (d, p). After optimizing the reference compound, UV/Visible analysis was performed at the same functionalities mentioned above in methanol solvent. The absorption maximum values for the reference in methanol solvent were 316.49, 298.26, 298.91 nm, and 311.20 at the above-mentioned functionalities coupled with the 6- 311 G (d, p) level (Fig. 2). The experimental value of λ_{max} for the reference was 314nm.(Tanuja et al. 2015) The B3LYP level, along with 6- 311 G (d, p), was selected for further evaluation as it gives the result closest to the experimental λ_{max} value.

2.2. DFT and TD-DFT Calculation

Molecular properties were comprehensively investigated using advanced computational techniques. Molecular behavior was analyzed via quantum chemical calculations in Gaussian 09W (Akhtar et al. 2025), and to visualize the results derived from the complex input, Gauss View 6.0 was employed as a key tool. To investigate the electronic properties of molecules for a broad analysis, the Density functional theory

(DFT) computational simulation approach was employed for all molecules at the B3LYP functional level, combined with the 6-311G (d, p) basis set. The main aim was to investigate the electronic properties of the reference molecule, as well as a series of customized molecules. The absorption maximum of each molecule was calculated in its gaseous state as well as when dissolved in a solvent, utilizing a conductor polarizable continuum model (CPCM) in performing the computational calculations in an appropriate solvent.(Khan 2025) Every donepezil derivative was investigated using molecular electrostatic potential plots (MEP), transition density analysis (TDM), densities of states (DOS),electron-hole overlapping graphs, ELF, LOL, and non-covalent interactions. The ELF, LOL, and NCI were analyzed by Multiwfn (Lu 2024), while VMD 1.9.3(Humphrey et al. 1996) was used for NCI visualization.

2.3. Molecular Docking

For the molecular docking analysis, the crystallographic structure of human acetylcholinesterase (AChE) complexed with donepezil (PDB ID: 4EY7)(Cheung et al. 2012) was obtained from the RCSB Protein Data Bank (<https://www.rcsb.org/>). The co-crystallized ligand, chain B, solvent, and unnecessary molecules were removed using PyMOL(Schrodinger 2015) for docking studies. ChemDraw 20.1.1 was used to draw the ligand structures, and was converted into 3D geometries using Chem3D.(Mendelsohn 2004) Geometry optimization and energy minimization were performed before starting docking calculations. In SDF format, the optimized ligand structure was saved and imported into PyRx.(Dallakyan and Olson 2015) AutoDock Vina(Trott and Olson 2010) engine integrated in PyRx was used to perform the Molecular docking simulation. The active site grid box was defined based on the binding region of the co-crystallized ligand using PyMol. Docking calculations were performed using an exhaustiveness value of 64. The docked complexes and intermolecular interactions were additionally analysed and displayed using Discovery Studio Visualizer 2025(BIOVIA 2025).

2.4. Molecular Dynamics Simulation

The best-docked pose of MH-4 in complex with acetylcholinesterase (AChE; PDB ID: 4EY7, chain A) was utilized as the starting model for molecular dynamics (MD) simulations. The ligand was prepared using the CHARMM-GUI Ligand Reader & Modeler, with the CHARMM General Force Field (CGenFF, version 5.0)(Brooks et al. 2009; Lee et al. 2015) used for atom typing and partial charge assignment.

The protein was parameterized using the CHARMM36 additive force field within GROMACS. The protein–ligand system was placed at the center of a rhombic dodecahedral simulation box, ensuring a minimum distance of 1.0 nm between the solute and the box boundaries. The system was solvated with TIP3P water molecules, and Na⁺ and Cl[−] ions were added using the GROMACS(Abraham 2025; Abraham et al. 2015; Bekker et al. 1993) genion utility to neutralize the system's net charge and maintain a physiological ionic concentration of 150 mM NaCl.

Energy minimization was subsequently performed using the steepest-descent algorithm. Following energy minimization, the system underwent two sequential equilibration stages. The first stage consisted of 100 ps of equilibration under the NVT ensemble at 300 K using a velocity-rescaling thermostat. This was followed by 100 ps of equilibration under the NPT ensemble at 300 K and 1 bar using the Berendsen barostat. After equilibration, an unrestrained production MD simulation was performed for 300 ns using a 2-fs integration timestep. Bonds involving hydrogen atoms were constrained using the LINCS algorithm. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method with an electrostatic interaction cutoff of 1.2 nm. Van der Waals interactions were smoothly switched off over the range of 1.0–1.2 nm. Periodic boundary conditions were applied throughout the simulations. Trajectory coordinates were saved at 10-ps intervals for subsequent structural and dynamical analyses. XMGRACE was used for plotting graphs.

2.5. Binding Free Energy Calculations (MM-GBSA):

Binding free energy calculations were conducted using the Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) method as implemented in gmx_MMPBSA (v1.6.5)(Valdés-Tresanco et al. 2021). Fifty snapshots were extracted at 3-ns intervals from the 150–300 ns segment of the production pathway to confirm that calculations were based on the structurally equilibrated system. The single-trajectory strategy was used, with polar solvation energies determined through the Generalized Born model (igb = 5, salt concentration = 150 mM) and non-polar involvements estimated from the solvent-accessible surface area. The entropic contribution to binding was estimated using the Interaction Entropy (IE) approach, and per-residue energy decomposition was carried out for residues within 4 Å of the ligand.

2.6. ADMET

The pharmacokinetic (ADMET) and toxicity of the investigated compounds were evaluated using ADMETlab 3.0(Fu et al. 2024) and ProTox 3.0(Banerjee et al. 2024), respectively.

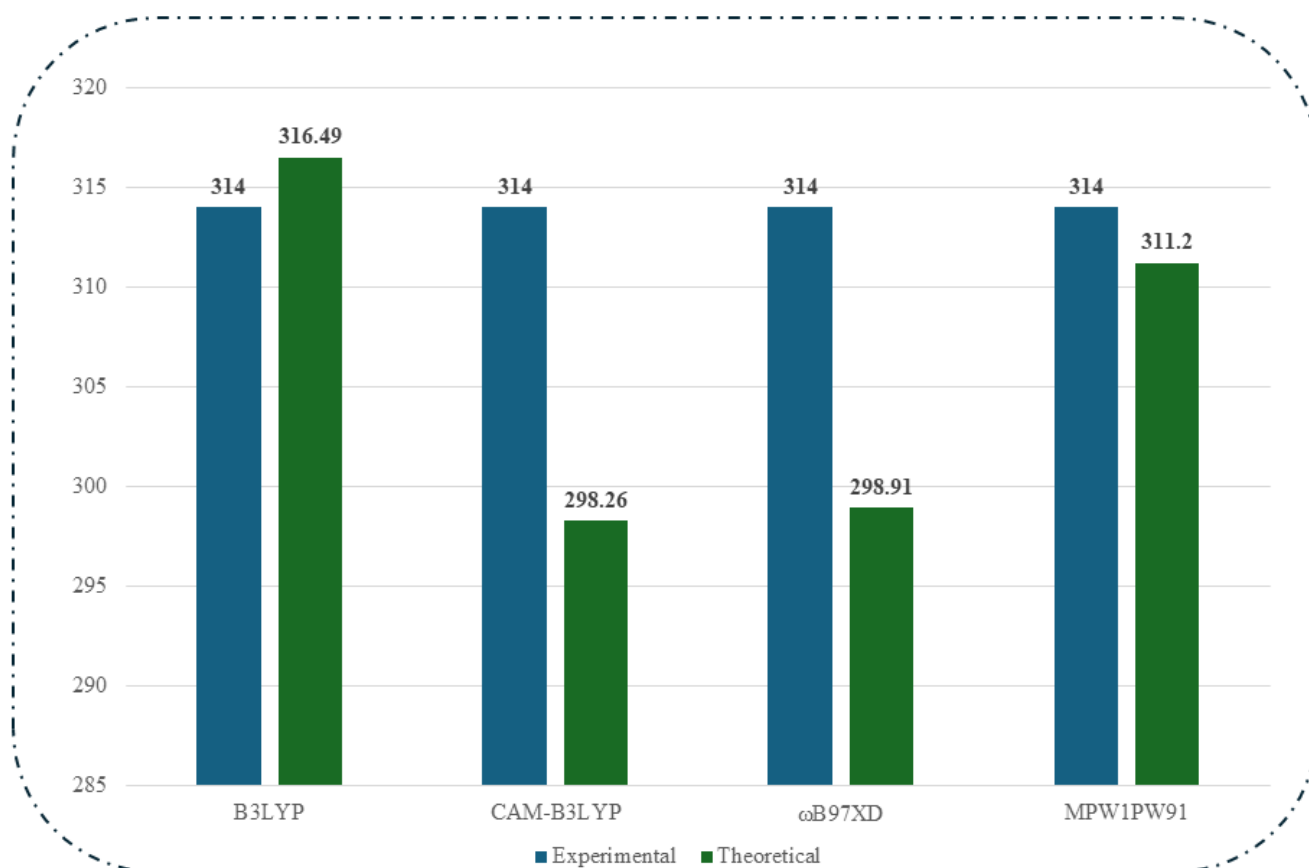


Fig. 2 Comparative analysis of $\lambda_{\max}$ values for reference at different functionalities along with 6-311G (d, p).

3. Results and Discussion

3.1. Frontier Molecular Orbital (FMO) Analysis:

Frontier Molecular Orbital (FMO) assessment gives key insights into molecular reactivity and stability. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) represent the electron-donating and electron-accepting capability of molecules. The energy gap (ΔE) can be defined as the difference in energy between HOMO and LUMO.(Hussein and Fadhil 2023) It is a key parameter to predict stability and reactivity. A smaller ΔE suggests higher reactivity and lower stability of molecules, whereas a larger gap reflects less reactivity and higher stability.(Bakheit et al. 2024) FMO calculations were performed for all donepezil derivatives (DDs) **MH-1**, **MH-2**, **MH-3**, **MH-4**, **MH-5**, and **R** by utilizing B3LYP level along with the 6-311G (d, p) basis set. DFT-optimized structures of **R** and **MH-1** to **MH-5** are shown in **Figure 3**.

The LUMO distribution of all DDs was consistent with **R**. The acceptor region remains in the indanone moiety except for **MH-2**. In this case, the LUMO shift to the benzyl moiety due to the strong electron-withdrawing nature of the NO₂ group. The HOMO of **R** and DDs was mainly localized on the piperidine and substituted benzyl moiety, suggesting this region serves as an electron-donating site. In **MH-2**, the

HOMO was only on the piperidine moiety owing to the electron-withdrawing ability of the nitro group. The fading orders for LUMO and HOMO were **MH-1>R>MH-4>MH-5>MH-3<MH-2** and **MH-1>R>MH-4>MH-5>MH-3>MH-2** respectively. The energy band gap (ΔE_g) for R and DDs (**MH-1** to **MH-5**) was 4.33, 3.89, 3.94, 4.56, 4.34, and 4.47 eV, respectively (Table 1). The distribution of electron density in the HOMO and LUMO of the reference and all DDs is exhibited in **Figure (4)**.

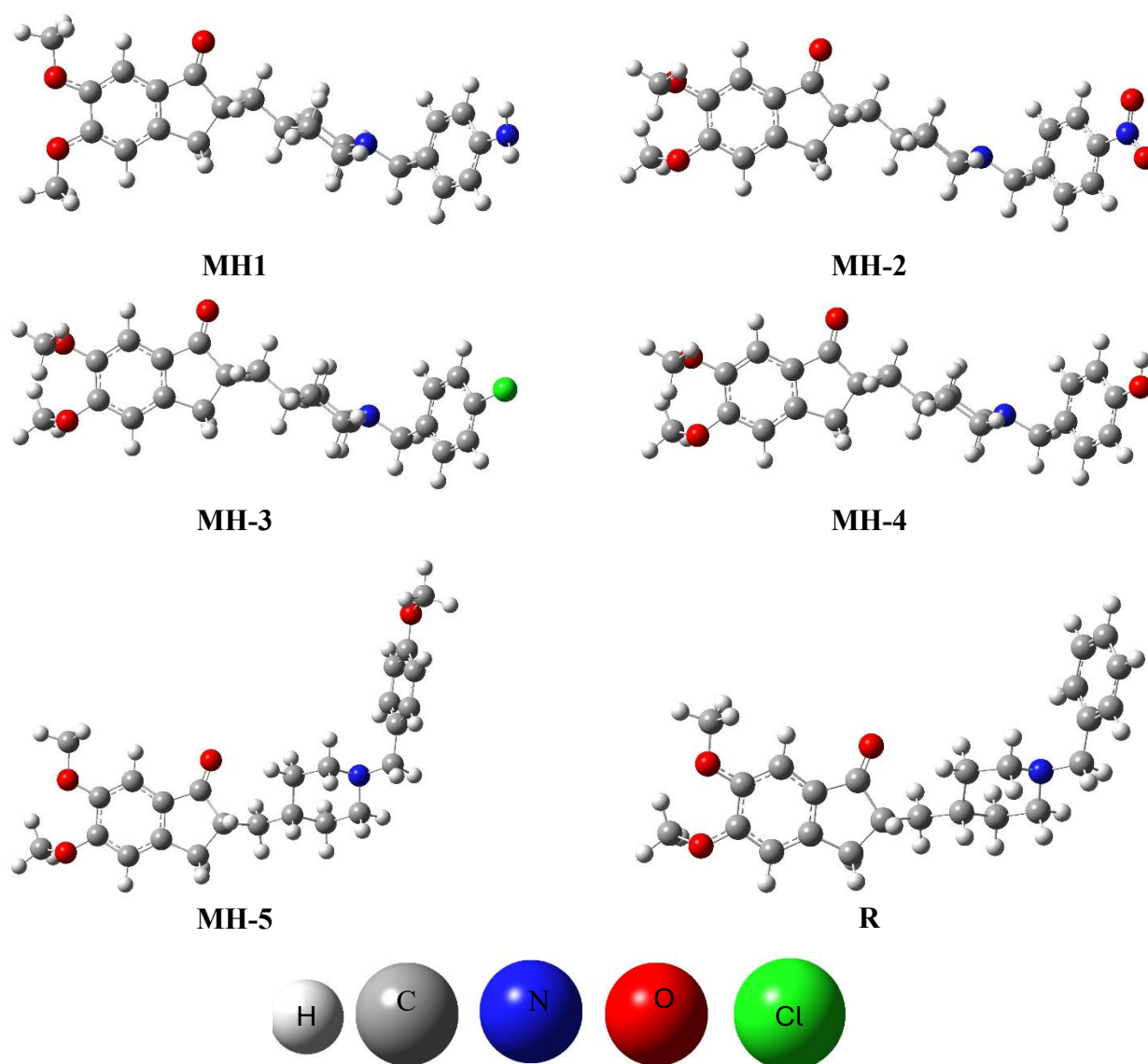


Fig. 3 Illustration of the optimized 3D structures of R and all DDs.

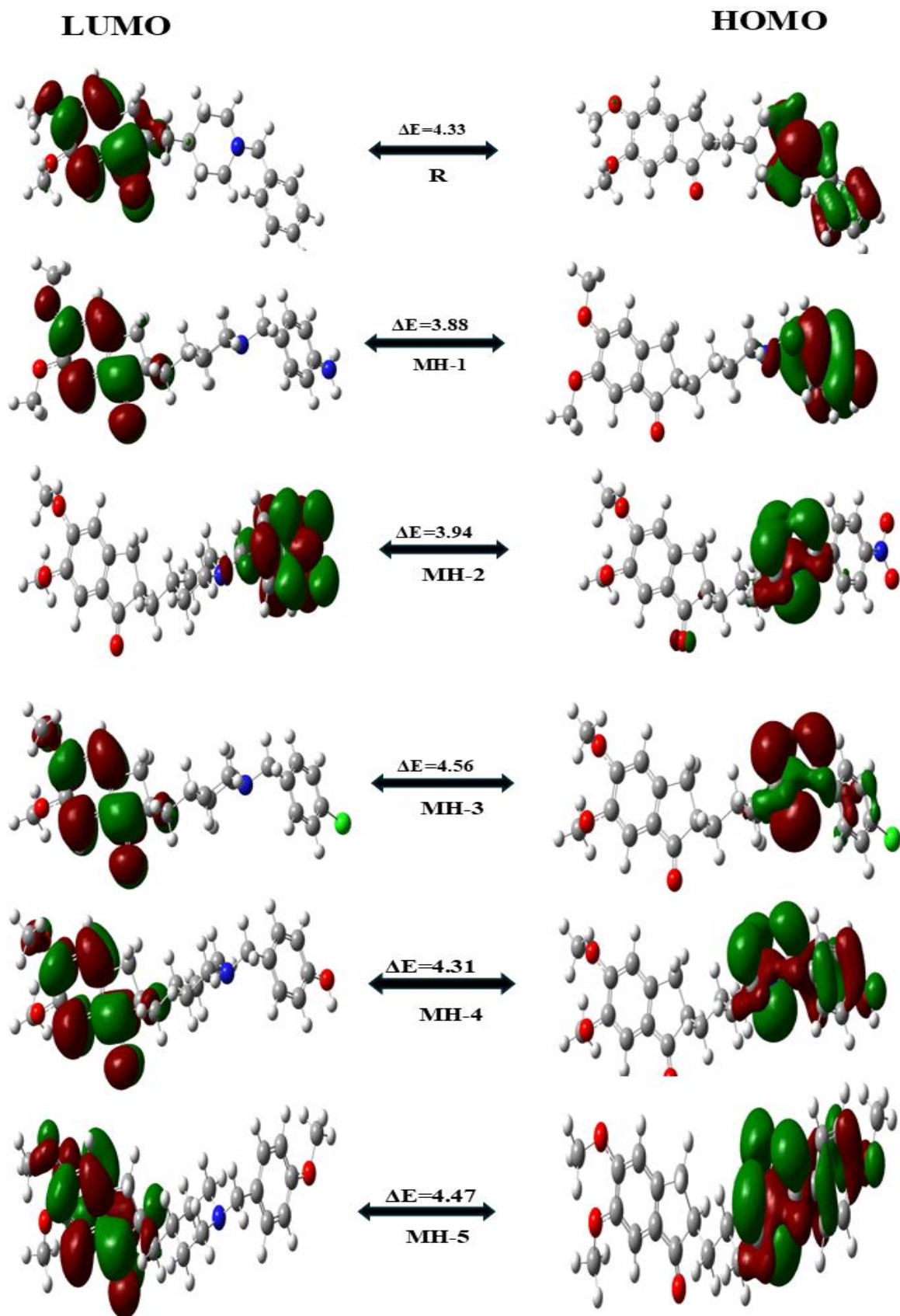


Fig. 4 Electronic density arrangement pattern on HOMO/LUMO of MH-1 to MH-5 including R.

Table 1. Estimated HOMO, LUMO and energy gap values for reference and MH-1 to MH-5.

Compounds	HOMO	LUMO	ΔE_g
Reference	-5.85	-1.52	4.33
MH-1	-5.38	-1.50	3.89
MH-2	-6.42	-2.48	3.94
MH-3	-6.19	-1.62	4.57
MH-4	-5.92	-1.58	4.34
MH-5	-6.07	-1.60	4.47

*eV ~ electron Volt.

3.2. Global Reactivity Descriptors:

The frontier molecular orbital (FMO) analysis provides major findings on the electronic reactivity and stability of the reference and its **MH-1–MH-5** derivatives (Table 2). Among the investigated molecules, **MH-1** display the smallest HOMO-LUMO energy gap ($\Delta E_g = 3.88$ eV), comparable to **MH-2** (3.94 eV), both of which were smaller than the reference (4.33 eV), suggesting improved chemical reactivity and greater electron mobility related to the parent compound. In line with this pattern, **MH-1** and **MH-2** exhibit the lowest chemical hardness ($\eta = 1.940$ and 1.970 eV, respectively) and correspondingly the highest softness ($S = 0.258$ and 0.254 eV⁻¹), additionally confirming their increased polarizability and susceptibility to charge transfer interactions. On the other hand, **MH-3** exhibits the largest energy gap (4.57 eV) and highest hardness (2.285 eV), indicating greater kinetic stability and reduced reactivity compared to the other derivatives. Importantly, MH-2 exhibited the most negative chemical potential ($\mu = -4.450$ eV) and the highest electrophilicity index ($\omega = 5.026$ eV), significantly above all other compounds, including the reference ($\omega = 3.136$ eV), suggesting a distinctly stronger electron-accepting capacity and improved potential for electrophilic interactions with nucleophilic residues in the target active site. The remaining derivatives, **MH-4** and **MH-5**, displayed moderate electronic properties ($\Delta E_g = 4.34$ and 4.47 eV, respectively), closely comparable to the reference compound. Overall, these results indicate that substitution in **MH-1** and **MH-2** significantly increases electronic reactivity and electrophilic character in contrast to the reference molecule. All the formulas used for calculation are as follows:

$$\text{Electronegativity } (\chi) = \frac{\text{Ionization potential}(IP) + \text{Electron affinity (EA)}}{2} \quad \text{Eq.1}$$

$$\text{Chemical potential } (\mu) = -\text{Electronegativity } (\chi) \quad \text{Eq.2}$$

$$\text{Hardness } (\eta) = \frac{\text{Ionization potential}(IP) - \text{Electron affinity (EA)}}{2} \quad \text{Eq.3}$$

$$\text{Softness (S)} = \frac{1}{2\eta} \quad \text{Eq.4}$$

$$\text{Electrophilicity index } (\omega) = \frac{\mu^2}{2\eta} \quad \text{Eq.5}$$

Table 2. Computed conceptual DFT descriptors: chemical potential (μ), electronegativity (χ), electrophilicity index (ω), and for reference (R) and MH-1 to MH-5

Compound	ΔE_g (eV)	η (eV)	S (eV ⁻¹)	μ (eV)	χ (eV)	ω (eV)
Reference	4.33	2.165	0.231	-3.685	3.685	3.136
MH-1	3.88	1.940	0.258	-3.440	3.440	3.055
MH-2	3.94	1.970	0.254	-4.450	4.450	5.026
MH-3	4.57	2.285	0.219	-3.905	3.905	3.337
MH-4	4.34	2.170	0.230	-3.750	3.750	3.240
MH-5	4.47	2.235	0.224	-3.835	3.835	3.290

3.3. Optical Properties

To measure various optoelectronic characteristics, such as absorption maxima (λ_{max}), oscillator strength, excitation energy, dipole moment, and MO assignment, the DFT approach was used. The CPCM was employed to calculate absorption maxima (λ_{max}) for R and DDs (MH-1 to MH-5) using B3LYP, together with 6-311G (d, p) was utilized for this purpose. Methanol was selected as the solvent for calculations. The experimental value for donepezil was reported as 314nm.(Tanuja et al. 2015) The theoretical value of donepezil using DFT is reported as 316 nm, which align well with the experimental value (**Table 3**). The λ_{max} value for DDs was observed within the range of 322-571nm. All DDs exhibited a bathochromic shift in the UV-Visible region relative to R. **MH-1**, **MH-4**, and **MH-5** show a redshift due to the electron-donating resonance effect of attached substituents, which enhanced π -electron delocalization and facilitated the electronic transition. The redshift in **MH-3** was because the -Cl resonance with the conjugated π -system leads to excited state stabilization. Among all DDs, **MH-2** showed the highest bathochromic shift owing to the strong electron-withdrawing nature of the nitro group attached to an electron-rich benzyl moiety, which creates a strong donor–acceptor interaction. It also intensifies intramolecular charge transfer interactions, significantly reducing the energy band gap between HOMO and LUMO, resulting in a redshift. The increasing order of absorption maxima (λ_{max}) for R and DDs was **R<MH-5<MH-3<MH-4<MH-1<MH-2**. The excitation energies and oscillator strength (f) for DDs (**MH-1 to MH-5**) and **R** are shown in Table 3. The “f” for all the analogues was very small, which suggests weakly allowed electronic transitions with low absorption intensity. Among all the DDs, the **MH-1** “f” value, while **MH-2** had the highest value of oscillator strength, which was still quite low compared to **R**. Major molecular orbital (MO) assignments

are also a key parameter for understanding the nature of electronic transitions involving FMO. **MH-1** and **MH-2** exhibited 100% contribution from HOMO to LUMO. while **MH-3** to **MH-5** involved transitions from lower occupied orbitals such as H-1, H-2, and H-3 to the LUMO.

Table 4 summarizes the results attained in the gaseous phase. The results revealed that **R** was highly solvent-dependent, as the oscillator strength reduced to nearly zero in the gaseous phase. The **MH-1** and **MH-2** showed bathochromic shifts toward longer wavelengths, while the remaining derivatives showed hypsochromic shifts toward slightly smaller wavelengths than in the solvent phase. Oscillator strength for all derivatives remains very small, suggesting weakly allowed electronic transitions.

Table 3. Analysis of the photodynamic behaviour of the R compound and all DDs in the solvent phase.

Compounds	Absorption $\lambda_{\max}$ (nm)	Experimental $\lambda_{\max}$ (nm)	Ex (eV)	f	MO assign	Dipole solvent
Reference	316	314	3.97	0.0203	H-5->LUMO (30%)	3.42
MH-1	363	-	3.42	0.0000	HOMO->LUMO (100%)	5.35
MH-2	571	-	2.17	0.0013	HOMO->LUMO (100%)	10.16
MH-3	332	-	3.73	0.0002	H-1->LUMO (95%)	6.81
MH-4	332	-	3.73	0.0002	H-2->LUMO (95%)	3.95
MH-5	325	-	3.82	0.0006	H-3->LUMO (96%)	5.20

Table 4. Estimated values of the photodynamic behaviour of R and all DDs in the gas phase.

Compounds	Absorption $\lambda_{\max}$ (nm)	Experimental $\lambda_{\max}$ (nm)	Ex (eV)	f	MO assign	Dipole gaseous
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Reference	331	314	3.75	0.0000		2.96
MH-1	349	-	3.55	0.0000	HOMO->LUMO (100%)	3.71
MH-2	565	-	2.19	0.0000	H-3->LUMO (99%)	7.82
MH-3	346	-	3.58	0.0001	H-1->LUMO (97%)	4.95
MH-4	346	-	3.58	0.0001	H-2->LUMO (97%)	2.61
MH-5	339	-	3.66	0.0002	H-3->LUMO (96%)	3.57

3.4. Molecular Electrostatic Potential:

The Molecular electrostatic potential is a property of molecules that is generated in the area around a molecule by its nuclei and electrons. This is a very beneficial characteristic for investigating and estimating molecular reactive behaviour. It is precisely elaborate and can be measured experimentally as well as computationally.(Poltzer et al. 1985) In addition, it also supports the assessment of electrostatic interactions, acting as a valuable tool for interpreting how the interaction between the active site and drug occurs.(Ullah et al. 2022) MEP graphs of donepezil and all DDs are exhibited in **Figure 5**. The negative extremes were displayed in the red regions, which were most extreme around the nitro group in **MH-2** and the carbonyl groups across the series. In **MH-4**, the negative potential was clearly aggregated around the hydroxyl group, while in **MH-5**, it was localized near the methoxy and carbonyl oxygens. These oxygen-rich regions indicate strongly nucleophilic sites, making them the most probable candidates for electrophilic attack. On the other hand, positive extremes are illustrated by the blue colour, which was most noticeable in the nitrogen-containing regions of **MH-1** and the areas surrounding the chlorine atom in **MH-3**, making them prone to attack by electron-rich (nucleophilic) centers.

These findings from MEP suggest that these oxygen-rich nucleophilic sites can interact with the key catalytic and oxyanion hole residues of acetylcholinesterase via electrostatic and H-bonding interactions, encouraging stable complex formation. This substituent-facilitated polarization arrangements can align with the differential inhibitory potencies.

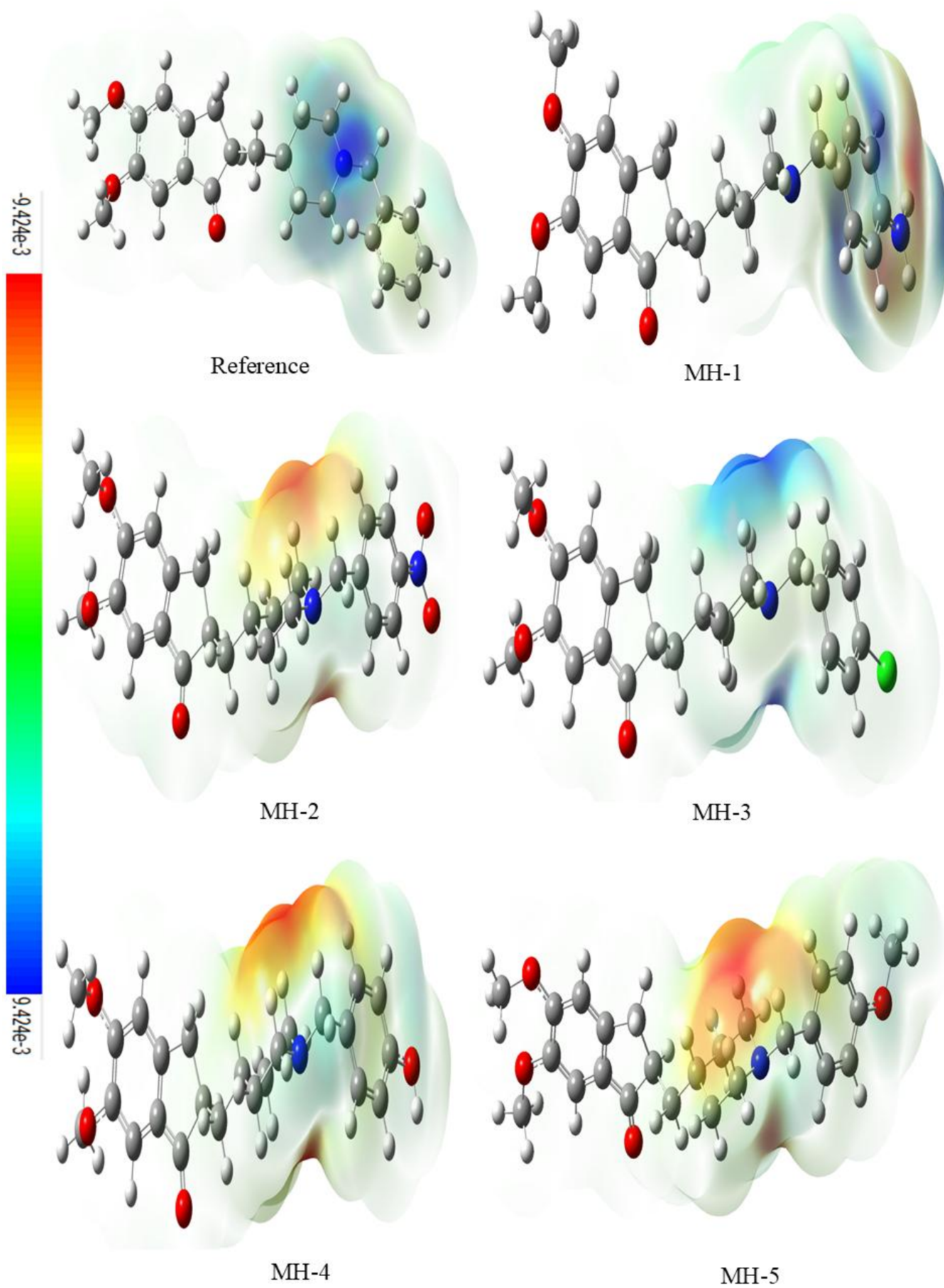


Fig. 5 MEP plots for R and MH-1 to MH-5 molecules calculated at B3LYP functional.

3.5. Dipole Moment:

The degree of molecular dispersion within a polar solution is directly indicated by the molecule's dipole moment. These findings suggest that polar solvents boost the solubility of pharmaceutical drugs possessing higher dipole moments (Liu et al. 2022). The impact of the dipole moment is explained by charge motion between HOMO and LUMO. The higher the dipole moment, the quicker the movement of electrons within the molecule. As derivatives are stimulated, their polarity rises. Since, this was only for a limited duration, earlier than the de-excitation occurs (Kavitha and Alivelu 2023). The dipole moments for the DNP derivatives were simulated at the **B3LYP/6-311G (d, p)** level of theory using the **CPCM** model to represent the aqueous environment. The analysis of all derivatives and the reference molecules is shown below.

Table 5: Theoretically evaluated dipole moment values for reference and all DDs in solvent and gas phase

Compounds	Dipole solvent (Debye))	Dipole gaseous (Debye)	Difference
Reference	3.42	2.96	0.46
MH-1	5.35	3.71	1.64
MH-2	10.16	7.82	2.34
MH-3	6.81	4.95	1.86
MH-4	3.95	2.61	1.34
MH-5	5.20	3.57	1.63

The reference molecule R acts as the baseline with a dipole moment of 3.4247 D in the solvent phase and 2.9650 D in the gaseous phase. The overview of various functional groups produced the following observations: **MH-2** exhibited the highest dipole moment in both media (10.168 D in solvent and 7.8155 D in gas), showing the strongest molecular polarization. **MH-3**: Demonstrated a high solvent dipole moment of 6.8118 D and a gaseous value of 4.9454 D. This derivative showed the largest difference between phases (2.6416 D). **MH-1**: Displayed a dipole moment of 5.3526 D in the solvent phase and 3.7082 D in the gaseous phase. **MH-5**: Showed moderate polarity with a dipole moment of 5.1959 D in the solvent phase and 3.5709 D in the gaseous phase. **MH-4**: Recorded a solvent dipole moment of 3.9479 D. Notably, it was the only derivative with a gaseous dipole moment (2.6143 D) lower than that of the reference R. Every molecule displayed a higher dipole moment in the solvent phase, contrasted with the gaseous phase. This trend appears because the dielectric medium (water) interacts with the solute, rising the electronic polarization

and causing higher μ values. These interactions were missing in the gaseous phase, resulting in lower dipole moments. The resulting declining order of the dipole moments for solvent phase (aqueous) was **MH-2 > MH-3 > MH-1 > MH-5 > MH-4 > R** and for gaseous phase fading order was **MH-2 > MH-3 > MH-1 > MH-5 > R > MH-4**. Table 5 lists the dipole moment for each derivative.

3.6. Density of States

The Density of states (DOS) is potentially the key concept for insight into the physical properties of a substance. It offers a simple way to describe complicated electronic structures. The capability to compute a high-quality DOS that indicates the true electronic structure of a material significantly enhances the capacity to interpret and tune multiple material properties (Toriyama et al. 2022). The Density of states backed the frontier molecular orbital energies. For **R** and all other DDs (**MH1–MH5**), the FMO inspection was carried out using the 6-311G (d, p) basis set with the B3LYP functional.

The Density of States graph was made up of two sections. The HOMO region was indicated by the right-hand region of the graph, while the LUMO region refers to the left side of the plot. The zone between HOMO and LUMO where no electron exists is termed the energy band gap. Bonding as well as dispersion of orbitals of the whole molecule can be witnessed and examined by the density of states graph. The analysis demonstrated in fig 6 that all DDs, with R, show a spatial arrangement of a small shift in the region of HOMO. With the changing number of states, the difference occurred specifically in the LUMO region. To reveal the impact of the functional groups, the following order was used for the DOS plot for **R** and all other DDs. **MH-2 > MH-3 > MH-1 > MH-5 > MH-4 > R**. The Findings demonstrated that the region of the LUMO of the **MH-2** molecule has a higher density of States compared to other regions. Within the therapeutic agent, the electro-retraction exerted by the nitro group stimulates long-term conjugation due to the presence of this strong electron-withdrawing substituent.

The DOS-based sequence (**MH-2 > MH-3 > MH-1 > MH-5 > MH-4 > R**) indicates changes in the electronic distribution near the HOMO-LUMO region among the derivatives, resulting from the distinct functional groups attached. These alterations in frontier orbital character suggest varying degrees of electronic reactivity across the series, which can influence their interaction with the enzyme's active site.

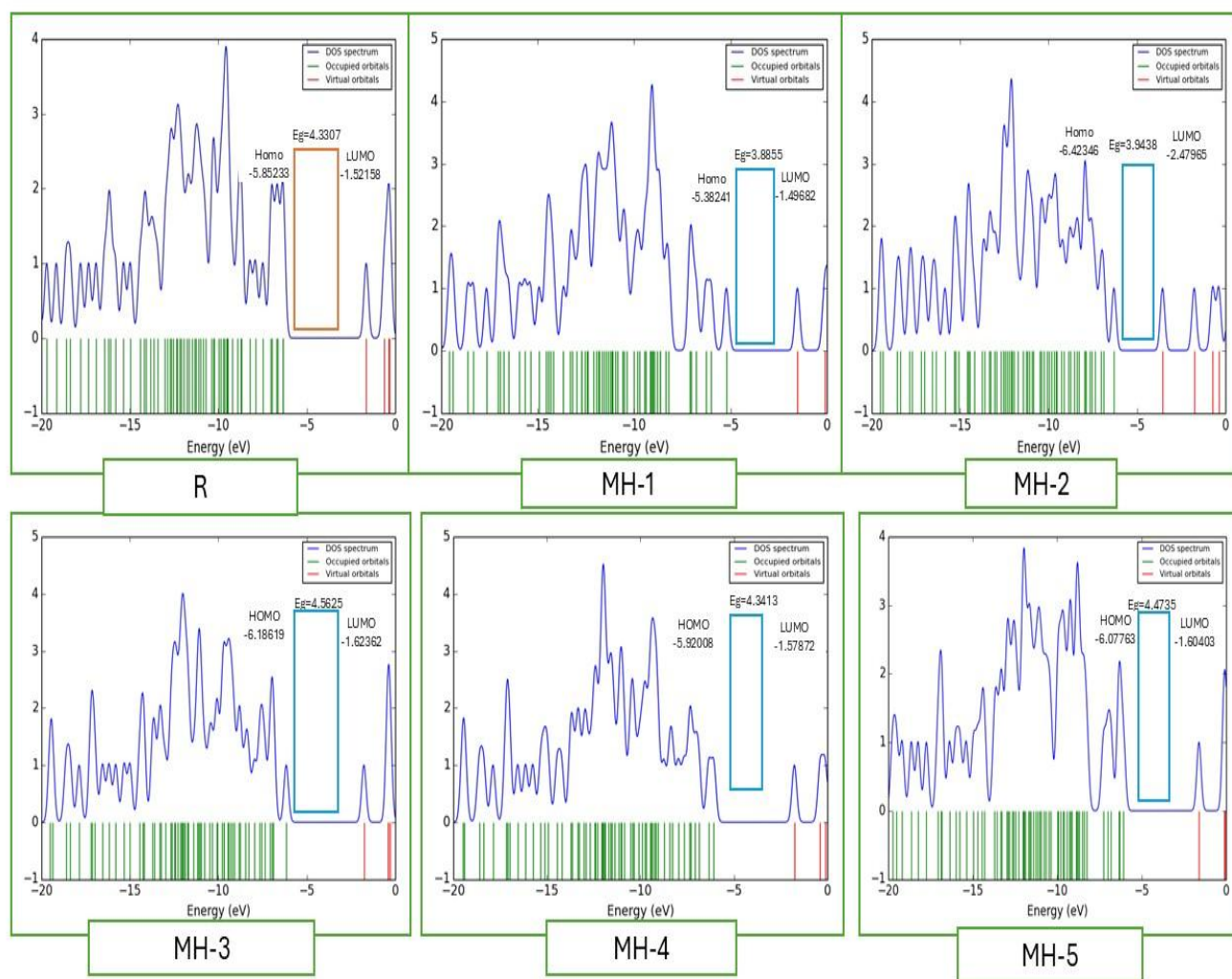


Fig. 6 For reference and all DDs, D.O.S. performed at B3LYP/6-311G (d, p).

3.7. Natural Bond Orbital (NBO)

One of the most effective approaches to analyse the electronic charge distribution of each atom is described through Natural Bond Orbital (NBO). It makes a molecule's electronic structure more accessible by turning complex, spread-out molecular orbitals from quantum calculations into localized bonds, lone pairs, and core shells that match chemical intuition.(Nayaka and Pushpavathi 2026)

All optimized DDs (**MH-1–MH-5**) were examined via full NBO calculations to explore the distribution of electronic charge (**Table 6**). For the reference molecule (**R**), an NBO charge of 0.205 e was localized on carbon (C2), whereas the hydrogen atom (H29) exhibited a charge of 0.197 e. In comparison, the derivatives **MH-1**, **MH-2**, **MH-3**, **MH-4**, and **MH-5** exhibited NBO charges of 0.152, -0.002, -0.029, 0.320, and 0.310 e on the carbon atoms (C13) at the substitution site, respectively (**Table 6**). Furthermore, the NBO charges on the heteroatoms (Atom 29) bound to carbon in **MH-1**, **MH-2**, **MH-3**, **MH-4**, and **MH-5** were -0.743, 0.476, -0.021, -0.687, and -0.539 eV, respectively. The results demonstrate that **MH-2** shows the highest

positive charge localization among all derivatives. NBO charge on the substituent atom, contrasted with the reference and the other derivatives, is ascribed to the strong electron-withdrawing nature of the nitro group. These changes in atomic charge profile suggest differences in electron-donating/withdrawing character among the substituents, which can exert an impact on the sites capable of electrostatic or hydrogen-bonding interactions with active-site residues.

Table 6. Calculated NBO for R and all DDs.

Compounds	Atom No	Atom Type	NBO charges
Reference	2	C	0.205
	29	H	0.197
MH-1	13	C	0.152
	29	N(NH ₂)	-0.743
MH-2	13	C	-0.002
	29	N(NO ₂)	0.476
MH-3	13	C	-0.029
	29	Cl	-0.021
MH-4	13	C	0.320
	29	O(OH)	-0.687
MH-5	13	C	0.310
	29	O(OCH ₃)	-0.539

3.8. TDM and Binding Energy Analysis

The transition density matrix (TDM) is an ongoing assessment that is utilized to determine the spatial distribution and the hole of electrons. It shows a high spatial overlap of the electron-hole pair. By evaluating these specific charge-carrier variables (e⁻ and h⁺), the overall performance of the drugs can be systematically analyzed. To better understand electronic excitation, TDM is useful for evaluating how the donor and acceptor regions interact within the molecular framework. (Ans et al. 2018) We carried out TDM analysis on **R** and the DDs using the DFT method with the B3LYP functional and the 6-311G (d, p) basis set. In Figure. 7 TDM graphs are displayed. The effect of hydrogen atoms on these transitions was ignored because their role is very small. To make the analysis simpler, the molecule was split into two parts: a donor (D) and an acceptor (A). The electron-withdrawing groups in the acceptor help with charge transfer, creating an electron coherence that runs corner-to-corner between the donor and acceptor. Donor and end-point acceptor units have the highest charge density. Charge locations on extremity acceptors demonstrate the

comparatively favourable charge-accepting capabilities of end-cap groups. The graph showed that the **MH-1** and **MH-2** molecules' nitrogen-containing functional groups give rise to a notable transition, driving the highest electron cohesiveness at the terminal edges caused by strong inductive and resonance effects of the nitrogen atom. The graph also elaborated that the structural variations in **MH-3** and **MH-4** provide their acceptor regions with a geometrically uniform, evenly distributed electron cohesiveness across the corners and the 19–22 region. At the same time, the **MH-5** alteration concentrates its electron cohesiveness strongly in the lower-left corner (regions 4–10). Consequently, we can achieve higher charge transfer and easier exciton dissociation during the $S_0 \rightarrow S_1$ transition. The distribution of charge locations in the reference molecule (R) and all DDs reflects their overall electronic performance.

Analyzing the efficiency of molecules was also done by another factor called binding energy. (Adnan et al. 2017) Sufficient binding energy was necessary to overcome the Coulombic forces holding the exciton together. (Hussain et al. 2020a) The exciton binding energy was the primary force keeping the electron-hole pair bound. (Dkhissi 2011) Generally, a higher charge density and lower binding energy correspond to enhanced efficiency rates. Molecules. Compounds showed excellent electron-hole overlaps with reduced binding energies for better charge transfer. Using the formula given below, the binding energy can be calculated:

$$E_b = E_{H-L} - E_{opt} \quad \text{Eq 6}$$

This symbol indicates an energy bandgap. E_{L-H} in the equation mentioned above. (Liang et al. 2016) The small amount of energy required to generate the first excitation is known as E_{opt} . (Hussain et al. 2020b) Using the B3LYP and 6-311G (d, p) level of DFT, the binding energies of R and all DDs were determined. The corresponding initial excitation and binding energies are listed in Table 7. The data show that the molecules (**MH-1–MH-5**) possess binding energies of 0.466, 1.772, 0.832, 0.612, and 0.655 eV, respectively; on the other hand, the R molecule exhibits a lower binding energy of 0.359 eV. Compared to the reference R molecule, all modified derivatives reported an increase in binding energy, with **MH-2** showing the highest value among all systems studied. This exceptional performance of **MH-2** was primarily due to the presence of the nitrogen atom in its strong electron-withdrawing nitro group (NO_2), which significantly increases the molecular interaction stability. Among the modified systems, the binding energy of **MH-1** was the lowest owing to the absence of hydrogen bonding and the dominance of hydrophobic interactions with the target pocket. In contrast, **MH-4** exhibited the second lowest binding energy among all the derivatives because its specific functional groups form reliable hydrogen bonds with the target molecule, as verified by docking simulations. The calculated binding energy follows this specific order, starting with the highest value: **MH-2** > **MH-3** > **MH-5** > **MH-4** > **MH-1** > **R**. To understand how charge moves and merges, this part also looks at electron-hole overlapping. It gives additional evidence for the TDM heat map analysis from the study. (Mehboob et al. 2022) The overlap graph displays how many holes

and electrons overlap. Non-hydrogen atoms are listed on the x-axis. Different colours show where electrons and holes overlap. The red colours show where the overlap was best and strongest. The findings suggested that **MH-2** achieved the most optimal results across all studied systems, demonstrating a highly concentrated, maximum peak intensity in the hole contribution at the terminal edge. This localized charge aggregation was directly produced by the powerful electron-withdrawing nature of its nitrogen-containing nitro group (NO₂), which heavily polarizes the electron-hole distribution. Conversely, **MH-4** shows a much lower maximum intensity scale but shows a highly distributed, uniform charge spreading across a wider range of atoms. Meanwhile, **MH-1**, **MH-3**, and **MH-5** display standard charge transport landscapes, with their major hole contributions localized near the central fragments. The evaluated molecules exhibit electron-hole transport efficiency in the following diminishing order: **MH-2 > MH-4 > MH-3 > R > MH-1 > MH-5**

Overall, the electron-hole transport analysis, TDM distribution, and DFT-derived binding energies suggest that **MH-2** display the most suitable electronic characteristics, linking strong charge-transfer efficiency with a stable binding energy (0.48 kcal/mol), supporting its capability for effective enzyme interaction. **MH-1**, **MH-3**, and **MH-4** also show comparable findings like **MH-2**, displaying reasonable charge cohesiveness and binding stability, with binding energies of 0.65, 0.83, and 0.61 kcal/mol, respectively, suggesting also suitable outcomes for active-site engagement. Meanwhile, **MH-5**, although displaying localized charge concentration, shows the weakest overall binding energy (1.77 kcal/mol), pointing to a relatively lower capacity for stable enzymatic interaction within the series.

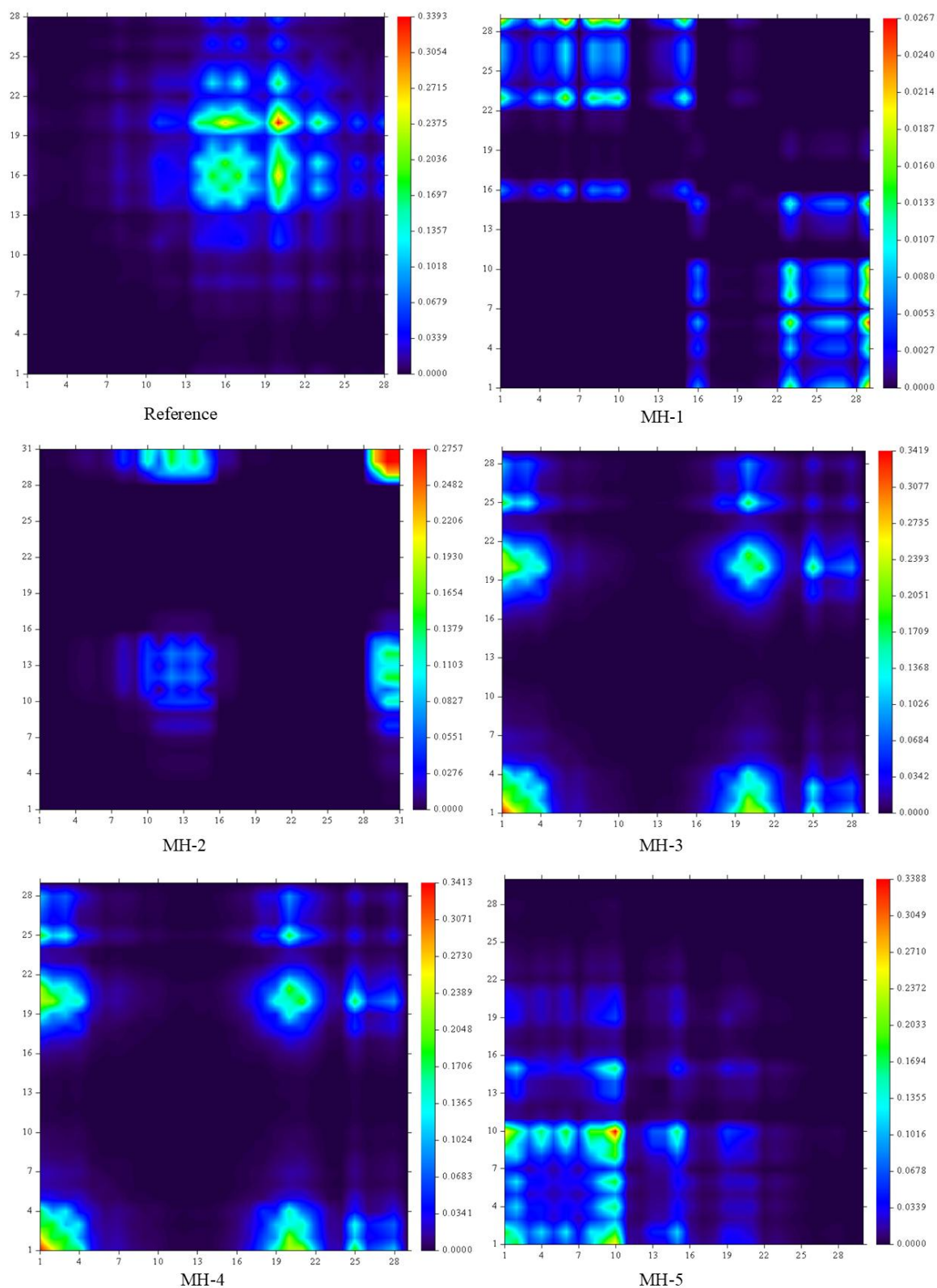


Fig. 7 Display of transition density matrix plots for reference and MH-1 to MH-5 at B3LYP.

Table 7. Estimated binding energy values for all compounds using the DFT method.

Compounds	$E_{H-L}(\text{eV})$	$E_{\text{opt}}(\text{eV})$	$E_b(\text{eV})$
Reference	4.33	3.97	0.36
MH-1	3.89	3.41	0.48
MH-2	3.94	2.17	1.77
MH-3	4.56	3.73	0.83
MH-4	4.34	3.73	0.61
MH-5	4.47	3.82	0.65

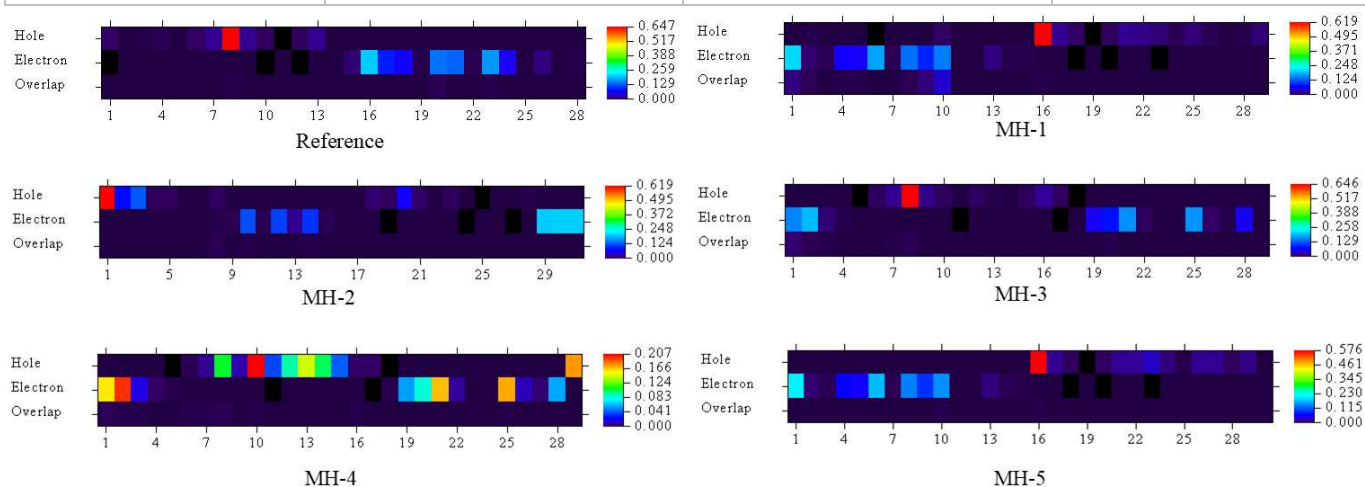
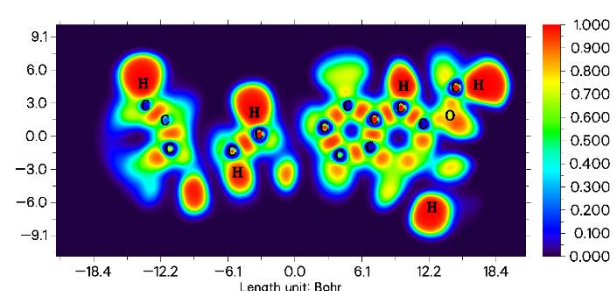


Fig.8 Demonstration of electron-hole overlapping graphs for reference and all DDs.

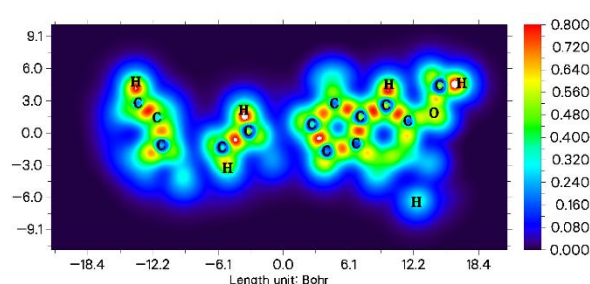
3.9. Electron Localized Function/Localized Orbital Locator:

DFT-based molecular parameters, Electron Localization Function (ELF), and Localized Orbital Locator (LOL) help to analyze and interpret how localized and delocalized electron density is spread within a molecule. They display core electrons, covalent bonding regions, and lone pairs that are unclear from just the molecule's structure. Enhancing ELF, LOL maps are often used together to find chemically active heteroatom areas and to cross-validate bonding areas identified by ELF (Elangovan and Arumugam 2025). To map the electron density profiles of the reference along with derivatives to find out the areas relevant to non-covalent binding, ELF and LOL analyses were conducted. Both descriptions showed highly localized density (red) near 1: core electrons, σ -bonding pairs, and heteroatom lone pairs; intermediate values (green) display delocalized, π -conjugated density; and values assessing 0 (blue) showed electron-poor regions between non-bonded atoms. In both maps, all six compounds, the reference, along with derivatives, showed a continuous green band of delocalized density across the entire benzofuran–pyridine–phenyl backbone,

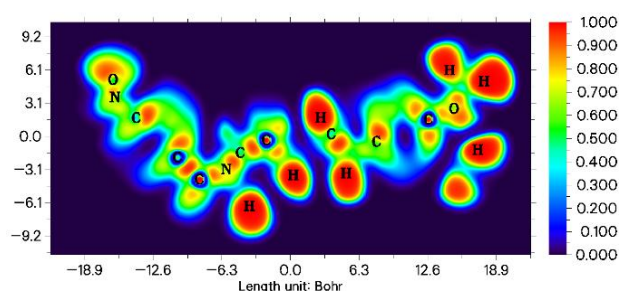
which indicates that π -conjugation is sustained across all three rings and supports the aromatic surface area required for π -stacking with the Trp residues lining the AChE catalytic site. Superimposed on this backbone, discrete high-intensity red discs were uniformly localized at the benzofuran oxygen, pyridine nitrogen, and terminal substituent heteroatom, respectively, corresponding to the attractive regions determined in the RDG-NCI analysis and cross-validating these atoms as candidate hydrogen-bond-acceptor sites from two independent parameters. The effect of the terminal localization reflected the substituent identity: the unsubstituted reference displayed localized density limited to the terminal ring carbons with no added heteroatom influence while in the substituted derivatives; the nitro oxygens in **MH-2** showed tight, significantly localized density uniform with strong but geometrically specific acceptor character; the hydroxyl and amino groups in **MH-4** and **MH-1** maintained comparable localized lone-pair density while further providing an N–H/O–H donor vector; chlorine in **MH-3** produced a evident larger, more dispersed region of localized density; and the methoxy oxygen in **MH-5** distributed its localized density between the ether oxygen and close methyl carbon. Overall, the ELF and LOL findings throughout the series uniformly show well-defined localized and delocalized electron regions, supporting a favourable electronic feature for all derivatives and providing additional evidence of their expected capability as suitable AChE-inhibitory compounds. Figure 9 shows ELF and LOL graphs for all compounds.



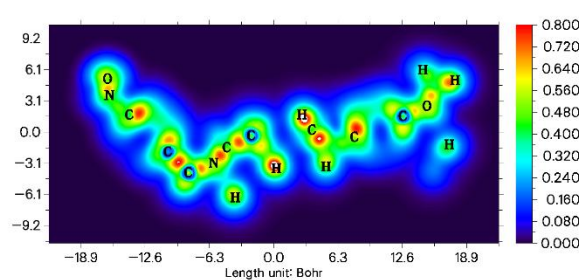
MH-1



MH-1



MH-2



MH-2

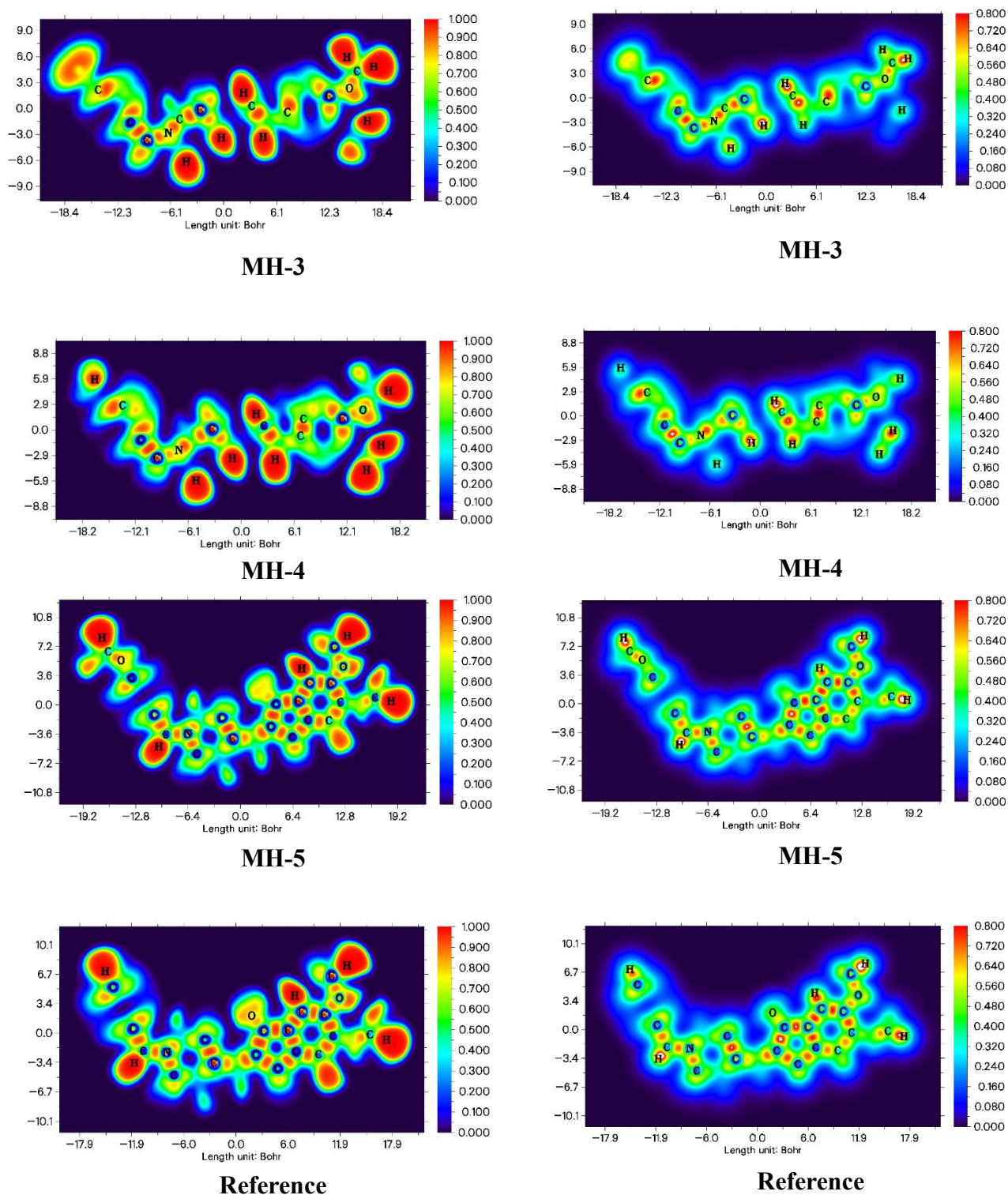
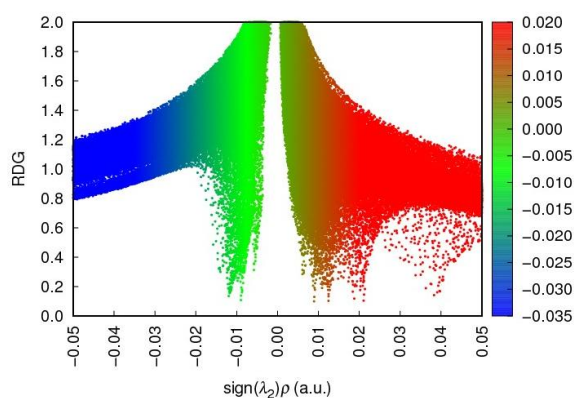


Fig. 9 Maps of ELF and LOL of Reference and MH-1 to MH-5.

3.10. Reduced Density Gradient and Non-covalent Interactions:

RDG analysis in DFT helps to analyze chemical bonding in molecules. The arrangement of electrons in a molecular space can be considered as its electron density. To find areas of electron aggregation and depletion, RDG analysis calculates and explores the gradient ($\nabla\rho$) of this electron density (ρ), thus revealing

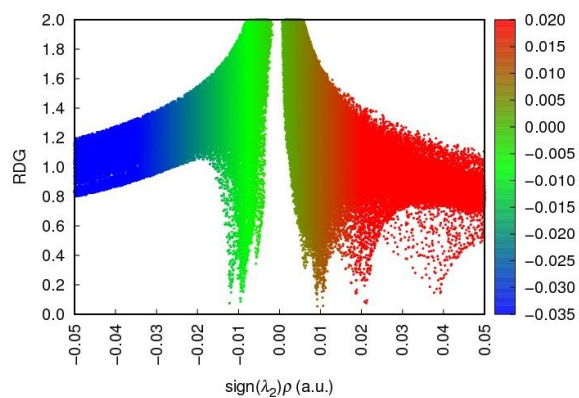
the types of chemical connections and associations between atoms in a molecule. Non-Covalent Interaction (NCI) analysis builds on this by recognizing low-gradient, low-density areas because of the sign of the second Hessian eigenvalue (λ_2), hence splitting attractive hydrogen bonds from weak van der Waals interactions and repulsive steric repulsion within a single real-space representation. (Sumathi et al. 2025) Intramolecular RDG-NCI analysis was done on all the compounds along with the reference to assess conformational stability before docking. The compounds with resolved scatter plots **Reference**, **MH-1**, **MH-2**, **MH-3**, **MH-4**, **MH-5** all displayed the same three-region profile: a strong attractive (blue) spike at $\text{sign}(\lambda_2)\rho < -0.02$ a.u. localized at the benzofuran oxygen and pyridine nitrogen; a weak van der Waals (green) area close to $\text{sign}(\lambda_2)\rho \approx 0$; and a repulsive steric (red) spike at $\text{sign}(\lambda_2)\rho > 0.01$ a.u. Figure 10 shows a discrete red island ($\text{sign}(\lambda_2)\rho \approx 0.015\text{--}0.045$ a.u., $\text{RDG} \approx 0.1\text{--}0.6$) at the pyridine–phenyl linkage, indicating localized, not global, strain and retention of the extended geometry required for dual-site AChE attachment. The size of this red Island and the intensity of the blue spike, along with the green region, clearly showed the substituent effects. **MH-3** (Cl) shares this same signature given its steric/electronic profile at the same location. **MH-2** (NO_2) showed the biggest, most scattered red island, therefore reflect it steric and electron-withdrawing bulk of the nitro group. **MH-1** (NH_2) and **MH-4** (OH) revealed tighter red islands with more attractive character from their H-bond-donor groups, hence suggesting cleaner extended shapes. The Reference and **MH-5** (OCH_3) tracked each other well, demonstrating moderate steric character and no added attractive enhancement. Overall, the maintained extended topology of the derivatives supports the structural concept of a dual-binding-site AChE inhibitor, and all compounds showed a conformational profile suitable for prospective AChE-inhibitory action.



MH-1



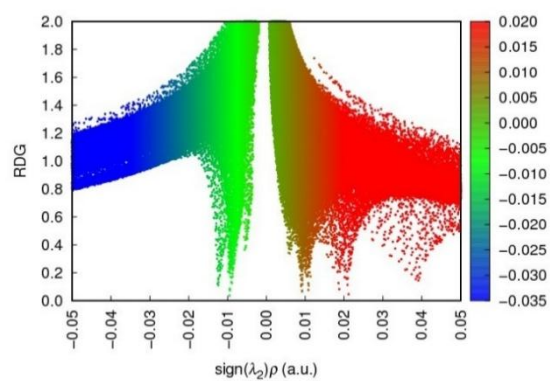
MH-1



MH-3



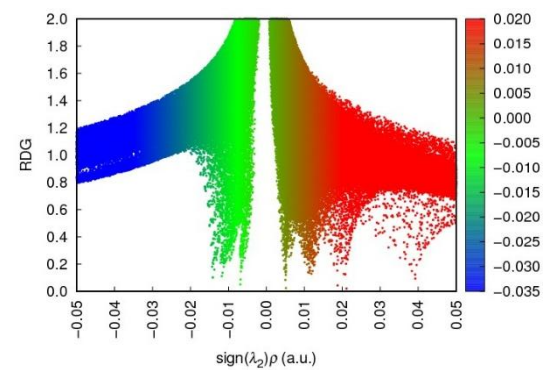
MH-3



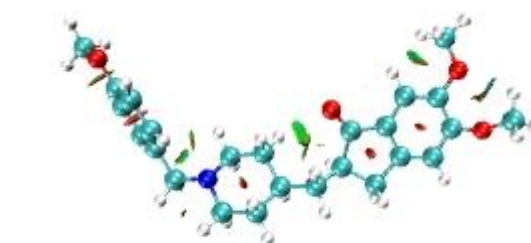
MH-4



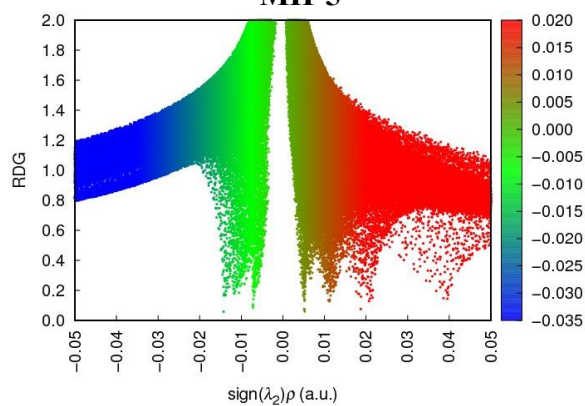
MH-4



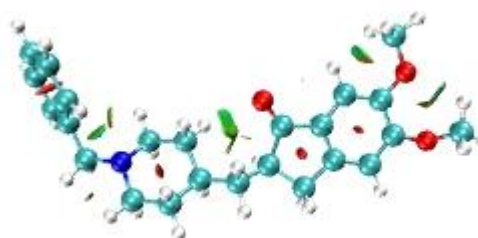
MH-5



MH-5



Reference



Reference

3.11. *In Silico* Docking Results

The docking analysis of all DDs with 4EY7 is shown in **Table 8**. The results indicated that all the designed donepezil derivatives retained strong affinity toward the active gorge of the receptor, with binding energy ranging from -11.6 to -12.2 kcal/mol. For the docking protocol validation, the co-crystallized ligand was redocked into the active site of the acetylcholinesterase receptor (**PDB ID 4EY7**). The redocked pose reproduced the experimental orientation with an RMSD value of **1.36 Å**, showing good reliability and accuracy of the docking methodology.

The values were highly comparable to the reference, which demonstrates a docking score of -12.2 kcal/mol, forming characteristic interactions within the active site of the receptor. A conventional hydrogen bond interaction with PHE295 was seen at 1.85 Å, pointing to very strong stabilization. A carbon-hydrogen bond interaction with SER293 was observed at 3.58 Å. Moreover, π -sigma interactions were established with TYR341, TRP286, and TRP86 at distances of 3.66 Å, 3.64 Å, and 3.91 Å, respectively. Aromatic stabilization was further achieved through π - π stacked interactions involving TRP86 at 4.08 Å and TRP286 at 3.71 Å and 5.42 Å, while TYR341 contributed another π - π stacked interaction at 4.04 Å. Hydrophobic stabilization included an alkyl interaction with LEU289 at 4.97 Å. Several π -alkyl interactions were also formed with TYR72 at 4.49 Å, TRP286 at 4.61 Å, TYR337 at 4.99 Å, PHE338 at 5.07 Å, and TYR341 at 5.07 Å. These interactions confirmed that the binding conformation for R was within the catalytic and peripheral active regions of the receptor. Among all DDs, **MH-1**, **MH-2**, and **MH-4** show the highest binding energy (-12.2 Kcal/mol), equivalent to R, suggesting a highly stable interaction network within the active site of the receptor. **MH-1** demonstrate a conventional hydrogen bond was observed with GLU202 at a bond distance of 2.30 Å, indicating strong electrostatic stabilization. In addition, a carbon-hydrogen bond interaction with TYR72 was formed at 3.70 Å. The ligand further exhibited a π -sigma interaction with TRP286 at 3.68 Å. Extensive aromatic stabilization was observed through multiple π - π stacked interactions involving TRP86 at distances of 4.50 Å and 3.79 Å, while TYR341 also contributed a π - π stacked interaction at 4.92 Å. Hydrophobic stabilization was further enhanced by an alkyl interaction with LEU289 at 5.41 Å. Moreover, several π -alkyl interactions were detected with TYR72 at 4.65 Å, TRP286 at 4.67 Å and 3.90 Å, TYR337 at 5.22 Å, and PHE338 at 4.65 Å. In **MH-2**, a conventional hydrogen bond was formed with PHE295 at 2.17 Å, indicating strong anchoring of the ligand within the active site. A carbon-hydrogen bond interaction with TYR72 was observed at 3.70 Å. The ligand also established a π -sigma interaction with TRP286 at 3.63 Å. Multiple π - π stacked interactions were formed with TRP86 at 4.53 Å and 4.39 Å, as well as TRP286 at 5.26 Å and 3.99 Å. TYR341 further contributed a π - π stacked interaction at 5.09 Å. Hydrophobic stabilization involved π -alkyl interactions with TYR72 at 4.70 Å, TRP286 at 4.06 Å and 4.83 Å, TYR337 at 4.99 Å, PHE338 at 4.12 Å, and TYR341 at 5.44 Å. **MH-4**

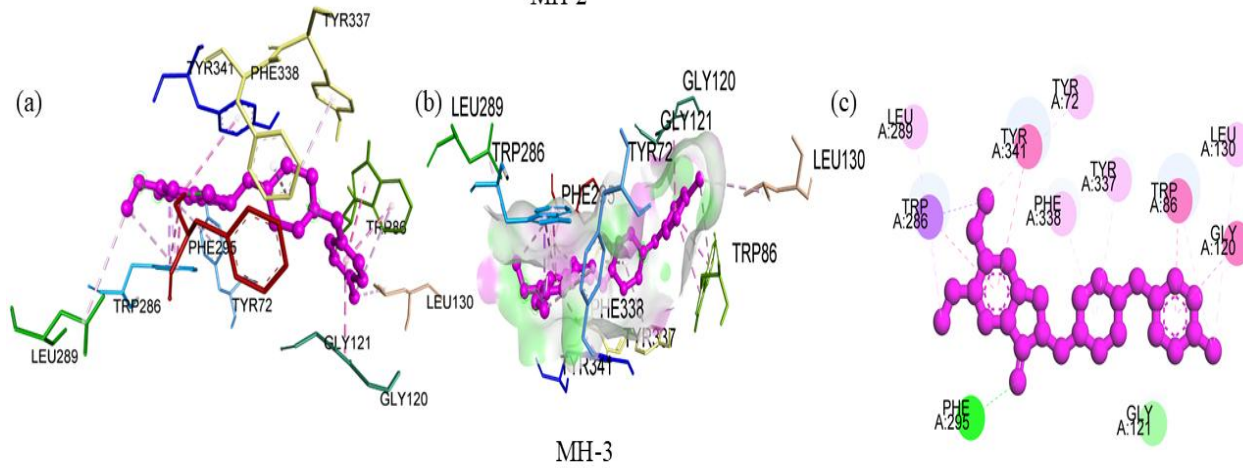
exhibits one of the most favourable interaction profiles among all DDs. Conventional Hydrogen bonds were formed between the ligand and two residues, TYR133 at 2.88 Å and GLU202 at a distance of 2.01 Å, suggesting electrostatic stabilization. The carbon-hydrogen bond interaction was seen between the ligand and TYR72 at 3.74 Å. Additionally, MH-4 established a π -sigma interaction with TRP286 at 3.70 Å. Multiple π - π stacked interactions were formed with TRP86 at distances of 4.50 Å and 3.78 Å, while TYR341 contributed another π - π stacked interaction at 4.91 Å. Interaction with LEU289 was also noted through the alkyl group at a distance of 5.37 Å. Moreover, several π -alkyl interactions were detected with TYR72 at 4.69 Å, TRP286 at 4.64 Å and 3.89 Å, TYR337 at 5.21 Å, and PHE338 at 4.67 Å. The presence of dual hydrogen-bond interactions together with extensive aromatic stabilization suggested that **MH-4** possessed highly stable accommodation within the receptor binding pocket. **MH-3** shows a binding affinity of -11.8 kcal/mol, which was lower than the other three derivatives but retained many key interactions responsible for ligand stabilization within the receptor cavity. A conventional hydrogen bond interaction was formed with PHE295 at 2.29 Å. Furthermore, a π -sigma interaction with TRP286 occurred at 3.81 Å. Aromatic stabilisation was achieved through multiple π - π stacked interactions between TRP86 and TRP286 with distances of 4.60 Å and 4.29 Å, 5.25 Å and 3.93 Å, respectively, and finally through another π - π stacked interaction between TYR341 with a distance of 5.15 Å. Amide- π stacked interaction was observed at a distance of 4.37 Å with GLY120 and GLY121. Hydrophobic contacts were achieved with LEU289 and LEU130 having distances of 5.30 Å and 5.25 Å, respectively. Additional π -alkyl interactions were established with TYR72 at 5.04 Å, TRP86 at 5.19 Å, TRP286 at 4.18 Å and 4.75 Å, TYR337 at 5.0 Å, and PHE338 at 4.16 Å. The slight effect on binding affinity is probably due to the steric effect of the larger chloro group. **MH-5** demonstrated a docking score of -11.6 kcal/mol, which was the lowest among all DDs. Forming a conventional hydrogen bond interaction with PHE295 at 2.41 Å, while a carbon-hydrogen bond interaction with TRP86 was found at 3.57 Å. The ligand also established a π -sigma interaction with TRP286 at 3.64 Å. Aromatic stabilization was mediated through π - π stacked interactions involving TRP86 at 4.63 Å and 4.20 Å, as well as TRP286 at 5.20 Å and 3.95 Å, while TYR341 contributed another π - π stacked interaction at 5.09 Å. Hydrophobic interactions included an alkyl interaction with LEU130 at 5.24 Å. Additional π -alkyl interactions were observed with TYR72 at 4.84 Å, TRP86 at 5.18 Å, TRP286 at 4.13 Å and 4.86 Å, TYR337 at 5.01 Å, PHE338 at 4.10 Å, and TYR341 at 5.45 Å. The comparatively low docking score of **MH-5** may be attributed to bulky group attached to the benzyl moiety, resulting in a steric effect. Figure 11 shows results for all DDs and R.

Table 8: Docking analysis of receptor 4EY7 with reference ad all DDs

Receptor Interaction with compounds	Amino Acid Residues involved in binding	Nature of Bonding Interactions	Residues-ligand Distance (Å)	Predicted Binding Energy (Kcalmol ⁻¹)
MH-1	GLU202	Conventional Hydrogen Bond	2.30	-12.2
	TYR72	Carbon Hydrogen Bond	3.70	
	TRP286	Pi-Sigma	3.68	
	TRP86	Pi-Pi Stacked	4.50	
	TRP86	Pi-Pi Stacked	3.79	
	TRP286	Pi-Pi Stacked	5.52	
	TRP286	Pi-Pi Stacked	4.13	
	TYR341	Pi-Pi Stacked	4.92	
	LEU289	Alkyl	5.41	
	TYR72	Pi-Alkyl	4.65	
	TRP286	Pi-Alkyl	4.67	
	TRP286	Pi-Alkyl	3.90	
	TYR337	Pi-Alkyl	5.22	
	PHE338	Pi-Alkyl	4.66	
MH-2	PHE295	Conventional Hydrogen Bond	2.17	-12.2
	TYR72	Carbon Hydrogen Bond	3.70	
	TRP286	Pi-Sigma	3.63	
	TRP86	Pi-Pi Stacked	4.53	
	TRP86	Pi-Pi Stacked	4.39	
	TRP286	Pi-Pi Stacked	5.26	
	TRP286	Pi-Pi Stacked	3.99	
	TYR341	Pi-Pi Stacked	5.09	
	TYR72	Pi-Alkyl	4.70	
	TRP286	Pi-Alkyl	4.06	
	TRP286	Pi-Alkyl	4.83	
	TYR337	Pi-Alkyl	4.99	
	PHE338	Pi-Alkyl	4.12	

	TYR341	Pi-Alkyl	5.44	
MH-3	PHE295	Conventional Hydrogen Bond	2.29	-11.8
	TRP286	Pi-Sigma	3.81	
	TRP86	Pi-Pi Stacked	4.60	
	TRP86	Pi-Pi Stacked	4.29	
	TRP286	Pi-Pi Stacked	5.25	
	TRP286	Pi-Pi Stacked	3.93	
	TYR341	Pi-Pi Stacked	5.15	
	GLY120, GLY121	Amide-Pi Stacked	4.37	
	LEU289	Alkyl	5.30	
	LEU130	Alkyl	5.25	
	TYR72	Pi-Alkyl	5.04	
	TRP86	Pi-Alkyl	5.19	
	TRP286	Pi-Alkyl	4.18	
	TRP286	Pi-Alkyl	4.75	
	TYR337	Pi-Alkyl	5.08	
	PHE338	Pi-Alkyl	4.16	
MH-4	TYR133	Conventional Hydrogen Bond	2.88	-12.2
	GLU202	Conventional Hydrogen Bond	2.01	
	TYR72	Carbon Hydrogen Bond	3.74	
	TRP286	Pi-Sigma	3.70	
	TRP86	Pi-Pi Stacked	4.50	
	TRP86	Pi-Pi Stacked	3.78	
	TRP286	Pi-Pi Stacked	5.56	
	TRP286	Pi-Pi Stacked	4.15	
	TYR341	Pi-Pi Stacked	4.91	
	LEU289	Alkyl	5.37	
	TYR72	Pi-Alkyl	4.69	
	TRP286	Pi-Alkyl	4.64	
	TRP286	Pi-Alkyl	3.89	
	TYR337	Pi-Alkyl	5.21	
	PHE338	Pi-Alkyl	4.67	
MH-5	PHE295	Conventional Hydrogen Bond	2.41	-11.6

	TRP86	Carbon Hydrogen Bond	3.57	
	TRP286	Pi-Sigma	3.64	
	TRP86	Pi-Pi Stacked	4.63	
	TRP86	Pi-Pi Stacked	4.20	
	TRP286	Pi-Pi Stacked	5.20	
	TRP286	Pi-Pi Stacked	3.95	
	TYR341	Pi-Pi Stacked	5.09	
	LEU130	Alkyl	5.24	
	TYR72	Pi-Alkyl	4.84	
	TRP86	Pi-Alkyl	5.18	
	TRP286	Pi-Alkyl	4.13	
	TRP286	Pi-Alkyl	4.86	
	TYR337	Pi-Alkyl	5.01	
	PHE338	Pi-Alkyl	4.10	
	TYR341	Pi-Alkyl	5.45	
Reference	PHE295	Conventional Hydrogen Bond	1.85	-12.2
	SER293	Carbon Hydrogen Bond	3.58	
	TYR341	Pi-Sigma	3.66	
	TRP286	Pi-Sigma	3.64	
	TRP286	Pi-Sigma	3.91	
	TRP86	Pi-Pi Stacked	4.08	
	TRP86	Pi-Pi Stacked	3.71	
	TRP286	Pi-Pi Stacked	5.42	
	TRP286	Pi-Pi Stacked	4.04	
	TYR341	Pi-Pi Stacked	4.97	
	LEU289	Alkyl	5.38	
	TYR72	Pi-Alkyl	4.49	
	TRP286	Pi-Alkyl	4.61	
	TYR337	Pi-Alkyl	4.99	
	PHE338	Pi-Alkyl	5.07	
	TYR341	Pi-Alkyl	5.08	



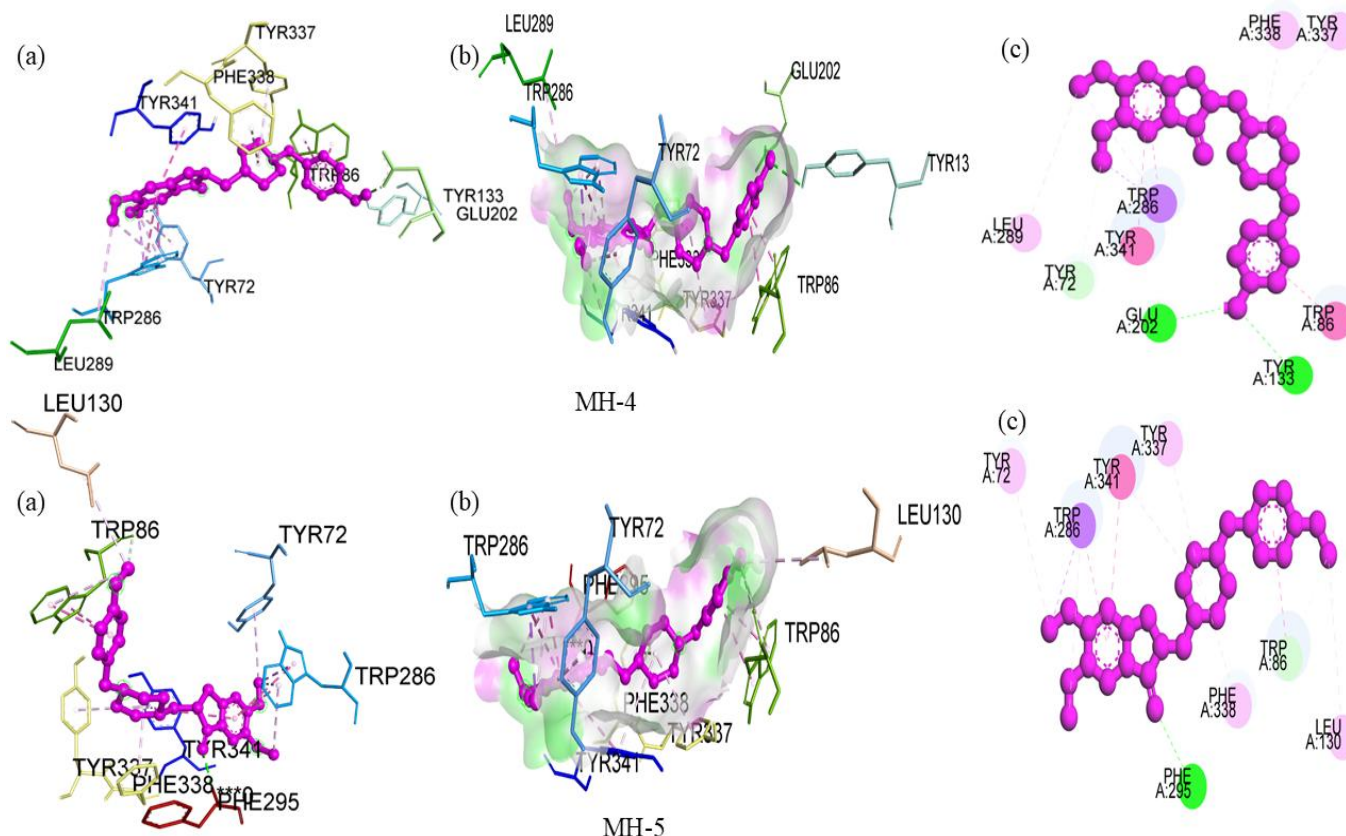


Fig.11 (a) 3D view of amino acid interaction; (b) Hydrogen Bond interaction view; (c) 2D amino acid interaction view of 4EY7 with MH-1, MH-2, MH-3, MH-4, MH-5 and reference.

3.12. Molecular Dynamics (MD) Simulation

The **MH-4** compound was selected for MD simulation due to its good docking score (-12.2Kcal/mol) and acceptable AMDET profile. The AChE MH-4(OH) complex was assessed over the full 300 ns trajectory by tracing backbone RMSD with respect to the minimized starting structure. After an initial rise during the first ~10–20 ns of the simulation, the anticipated settling-in period as the system relaxes away from its crystallographic starting point, the backbone RMSD remained constant between roughly 0.15 and 0.22 nm for the rest of the run. A single deviation up to 0.25 nm appears near 180–190 ns before the trajectory returns to its determined range within a few nanoseconds. As AChE is a fairly large, multi-domain enzyme, the variation of this size at a single point in an otherwise 300 ns run is unremarkable on its own and does not indicate any loss of the natural conformation; it most likely indicates a brief, local rearrangement (a loop shifting position, or a temporary adjustment near the gorge entrance) rather than any general instability. The ligand revealed a more interesting story. When **MH-4(OH)**'s RMSD was calculated relative to itself, distinguishing how much the ligand's own shape changes, unrelated to where it sits in the pocket. It does not settle down at all. Rather it moves between a series of different bands over throughout the simulation: an increased state around 0.20–0.26 nm for roughly the first 70 ns, a drop to 0.11–0.17 nm between about

70 and 100 ns, a revert to the higher band from approximately 100 to 140 ns, a further drop to its lowest range (0.10–0.15 nm) between about 140 and 175 ns, an Sustained intermediate stable region near 0.14–0.19 nm covering almost a hundred nanoseconds (175–270 ns), and a final rise toward 0.18–0.26 nm in the closing ~30 ns. Each of these shifts happens steadily over a few nanoseconds, and each steady state remained for tens of nanoseconds before the next switch. The marker of a molecule's transport between a small number of individuals, suitably stable conformations rather than losing its structural stability or displaying random motion.

You would expect flexible donepezil-type scaffolds set in the AChE gorge to act just like this. More than one rotatable bond connects the aromatic and saturated ring systems of the molecule; various rotamers about those bonds can sit tightly inside the aromatic-lined walls of the gorge without pushing the ligand out of the pocket or causing the protein to alter around it. Few flexible ligands do, but rather whether the complex stays intact as it examines these states, few flexible ligands do. Here it does: at the same point as the ligand's conformational changes, the protein backbone RMSD doesn't show any connected jumps.

Beyond the backbone RMSD, we looked at per-residue flexibility to see whether the stability we were seeing was constant across the protein or concentrated in confined regions, which turned out to be quite informative. The RMS fluctuation pattern is exactly what you'd expect from a well-folded, correctly Stabilized globular enzyme: most of the sequence fluctuates by no more than 0.05–0.15 nm, and the few positions that stand out above this baseline map onto surface loops rather than the structural core. The most significant of these, a broad peak reaching roughly 0.28 nm in the residue-70-to-80 region, sits right where AChE's well-known Ω -loop (omega loop) is located, a mobile section near the entry to the active-site gorge that has consistently been involved in gating access to the catalytic framework in structural and simulation studies of this enzyme, so seeing it appear as one of the more flexible stretches here is favourable rather than surprising. A second, sharper peak near residue 260–270 increases to about 0.38 nm, the highest interior value in the whole profile, and a further peak around residues 385–390 reaches roughly 0.26 nm; both fall in surface-located loop regions away from the gorge itself. The very large variations right at the two ends of the plot, up to 0.8 nm at the extreme N-terminus and 0.55 nm at the C-terminus, are generally observed in these calculations, since chain ends are only attached by a single connection to the rest of the protein and have comparatively little to limit their movement; they're not reflective of any problem with the simulation. What stands out most, and what matters for the binding story, is that the residues lining the catalytic gorge itself stay relatively quiet throughout; the flexibility is being retained by the loops around it rather than by the pocket the ligand is sitting in.

The radius of gyration supports this picture in the simplest way possible: across all 300 ns it sits in an extremely narrow band, essentially 2.30 to 2.33 nm without any drift up or down. For a protein of this size, that's a moderately flat line; it rules out any large-scale compression, swelling, or limited loss of structure

over the course of the run. Whatever local loop motion is happening in the RMSF data, it isn't translating into any change in the protein's overall dimensions. Taken together, these results indicate that MH-4 is not tightly locked into a single pose but is nonetheless maintained within the active site, sampling a limited set of stable conformations while maintaining stable, Lower-energy contact with the surrounding residues throughout the simulation.

Hydrogen bonding between the ligand and the protein followed a pattern that fits clearly with the conformational behaviour assessed for the ligand's internal RMSD. Rather than holding a fixed number of contacts, the count changes continuously between zero and four over the trajectory but spends the great majority of its time in the one-to-two range, with short-lived changes to three and only occasional, short-lived spikes to four; for example, a small cluster of four-bond frames appears early in the run around 40–50 ns, and isolated spikes repeat later without ever becoming the standard. Rather than reading this as noisy or variable, it's more accurately described as a dynamic hydrogen-bonding network: the ligand isn't locked into one specific donor-acceptor pairing for the full 300 ns, but it's essentially never without at least one hydrogen bond to the protein for any long interval, and the moments where the H-bond count shifts track reasonably well with the periods where the ligand's own RMSD was found to shift between conformational states. Taken together with the Enthalpy-favoured binding free energy calculated separately, this suggests a bound ligand that alters its exact orientation and hydrogen-bonding partner somewhat over time, without ever detaching from the pocket.

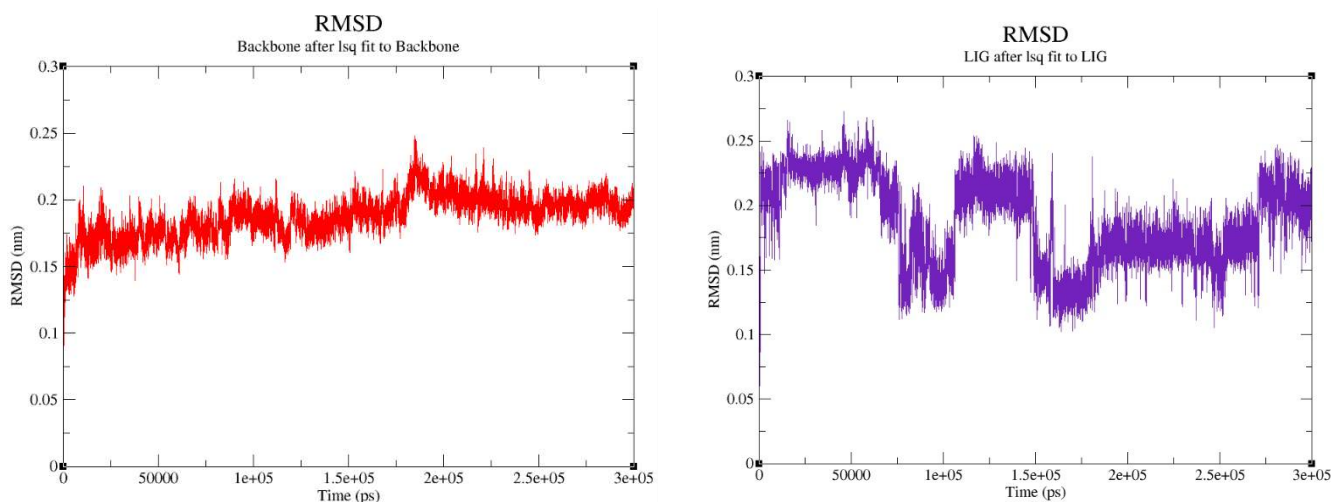


Fig. 12 The RMSD plot of backbone and ligand (MH-4).

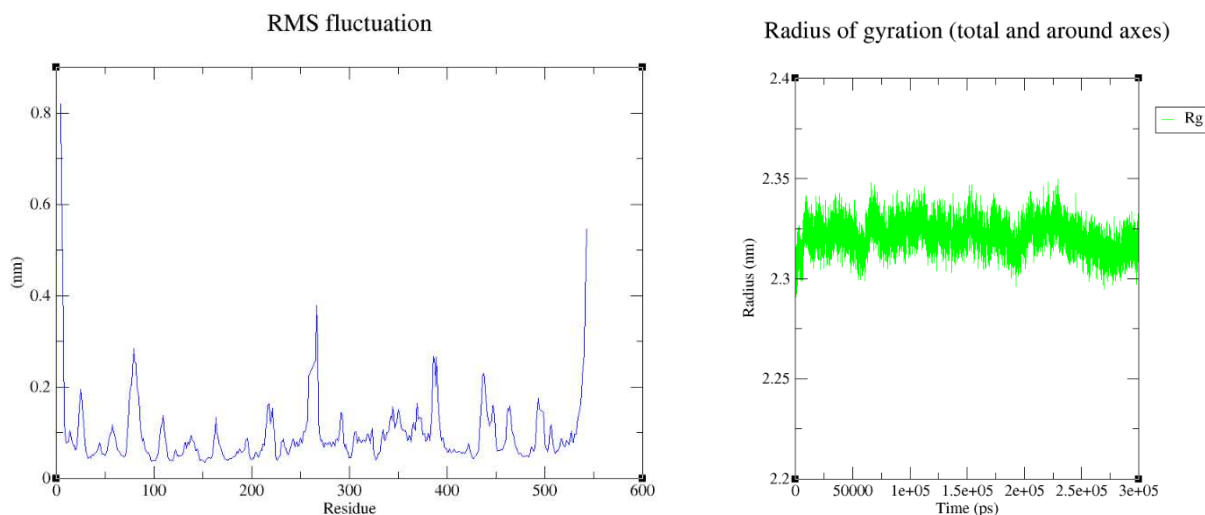


Fig. 13 RMSF and radius of gyration plot.

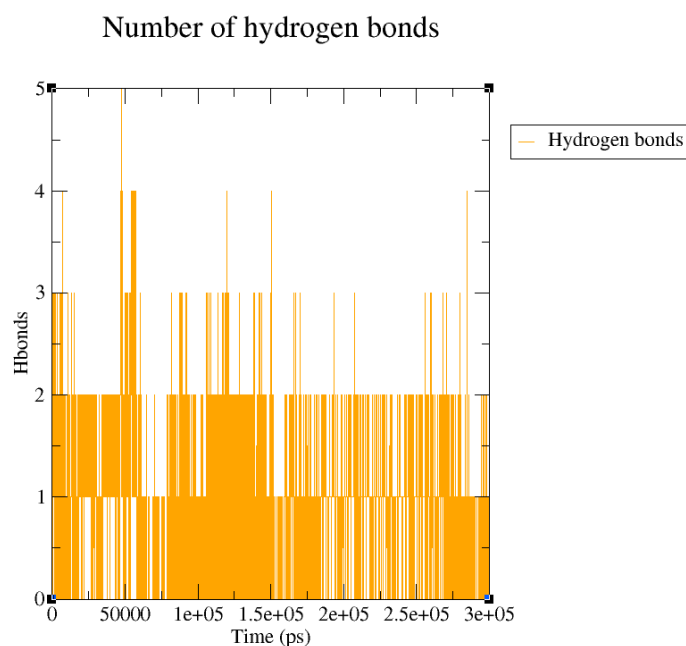


Fig. 14 H-Bond graph of the MH-4 complex

3.13. MM/GBSA

Binding free energies for the AChE–MH-4(OH) complex was calculated from the steady segment of the 300 ns MD trajectory using the MM-GBSA method as applied in gmx_MMPBSA, with entropic involvement calculated using the Interaction Entropy (IE) approximation. Energy decomposition (Table 9) shows that van der Waals interactions ($\Delta\text{VDWAALS} = -42.52 \pm 0.38$ kcal/mol) provide the major favourable contribution to binding, consistent with broad hydrophobic and well-matched in-shape contacts within the enzyme's aromatic gorge. Electrostatic interactions in the gas phase were also favourable ($\Delta\text{EEL} = -8.11 \pm 0.52$ kcal/mol), but this input was largely offset by an unfavourable polar solvation term ($\Delta\text{GGB} = +31.42 \pm 0.28$ kcal/mol), representing the desolvation energy required for burial of polar groups at the binding interface. The nonpolar solvation contribution ($\Delta\text{GSA} = -5.33 \pm 0.08$ kcal/mol) added a small

favourable term. Together, these components gave a gas-phase energy (ΔG_{GAS}) of -50.63 ± 0.79 kcal/mol and a solvation free energy (ΔG_{SOLV}) of $+26.09 \pm 0.29$ kcal/mol, giving a total binding enthalpy (ΔH) of -24.53 ± 0.85 kcal/mol. Including the entropic penalty from the IE approximation ($-T\Delta S = +11.49$ kcal/mol) gave a final binding free energy of $\Delta G_{\text{binding}} = -13.04 \pm 3.73$ kcal/mol. This favourable and thermodynamically stable $\Delta G_{\text{binding}}$, together with the stable RMSD/RMSF/Rg profiles observed over the production run, confirms that **MH-4(OH)** forms a stable complex within the AChE active site, with van der Waals packing rather than electrostatic complementarity as the principal driver of binding.

Table 9: MM-GBSA binding free energy and its energy components(kcal/mol) for MH-4 complex.

Energy Component	ΔG (kcal/mol)
ΔV_{DWAALS}	-42.52 ± 0.38
ΔE_{EL}	-8.11 ± 0.52
ΔG_{GAS}	-50.63 ± 0.79
ΔG_{GB}	$+31.42 \pm 0.28$
ΔG_{SA}	-5.33 ± 0.08
ΔG_{SOLV}	$+26.09 \pm 0.29$
ΔH ($\Delta G_{\text{GSA}} + \Delta G_{\text{SOLV}}$)	-24.53 ± 0.85
$-T\Delta S$ (IE)	$+11.49$
$\Delta G_{\text{binding}}$	-13.04 ± 3.73

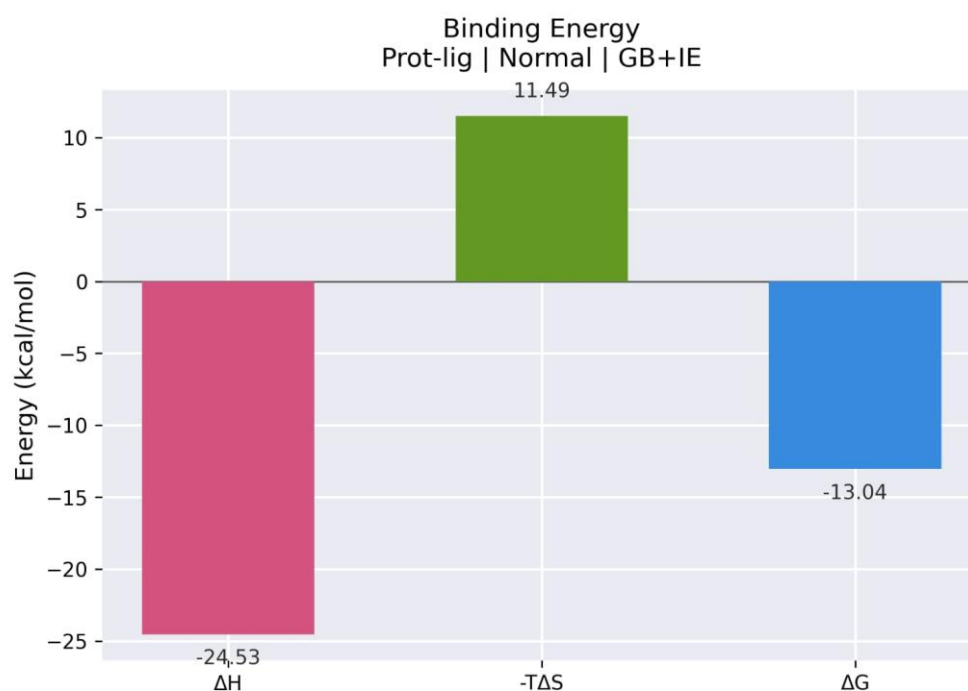


Fig. 15 Representation of Binding Energy values.

3.14. In Silico ADME Study:

ADMETlab 3.0 online tool was used to subjected in silico ADME screening of all designed derivatives. Specific molecular, pharmacokinetic, and physicochemical properties were analysed relative to the reference anti-Alzheimer drug (Table 10). These included Caco-2 permeability, P-gp modulation, human intestinal absorption (HIA), blood-brain barrier (BBB) permeation, plasma protein binding (PPB), MDCK permeability, volume of distribution, cytochrome P450 inhibition profiles, clearance, and circulatory half-life. The predicted human intestinal absorption (HIA) values exceeded 0.91 for all designed compounds, indicating favourable oral absorption and systemic exposure. The blood-brain barrier (BBB) permeation values were in the range of 0.88 to 0.95, suggesting efficient blood–brain barrier penetration and favourable central nervous system distribution. In all derivatives, the cellular Caco-2 permeability values were consistently bounded between -4.43 and -4.85. Most CNS-active compounds exhibit high plasma protein binding, and more than 80% binding was established throughout the entire series of compounds, comparable to the reference drug. The metabolic profiling of drug candidates was primarily associated with the interaction of major hepatic Cytochrome P450 isoenzymes. Thus, the probability of avoiding drug-drug interactions increases when candidates show no inhibition of major metabolic isoforms. The analysis revealed inactive inhibition profiles for the CYP1A2, CYP2C19, CYP2C9, and CYP3A4 enzymes across the standard baseline set. The plasma clearance rate was a major physiological factor related to the metabolic excretion and systemic clearance of compounds. The biological time of drugs was strongly influenced by their clearance profiles, and the rate at which they remain active in circulation was directly proportional to their physiological half-life. Circulatory half-lives between 0.40 and 0.85 hours were established for these neurological scaffolds, where higher values show a prolonged systemic retention. From the obtained data, we found that compound **MH-1** (10.370 mL/min/kg) had the highest plasma clearance value among the synthesized derivatives, followed by the remaining series, which clustered uniformly at 3.518 mL/min/kg, both sets of values flanking around that of the control reference (5.289 mL/min/kg). Most of the functionalized molecules showed acceptable half-life values peaking at 0.81 hours, except for a few molecules with a lower, accelerated excretion profile, such as **MH-1** ($t_{1/2}$ = 0.428 h).

Table 10. The ADME results for R and DDs

ADME Parameter	Reference	MH-1	MH-2	MH-3	MH-4	MH-5
Human Intestinal Abs. (HIA)	High	High	High	High	High	High
Caco-2 Permeability	-4.685	-4.435	-4.689	-4.689	-4.689	-4.847

MDCK Permeability	-4.453	-4.512	-4.689	-4.689	-4.689	-4.689
Blood-Brain Barrier (BBB)	Permeant	Permeant	Permeant	Permeant	Permeant	Permeant
Plasma Protein Binding (PPB)	94.62%	83.16%	92.74%	92.74%	92.74%	92.74%
CYP2D6 Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor	Inhibitor
CYP3A4 Substrate	Yes	No	No	No	No	No
CYP1A2 Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Clearance	5.289	10.370	3.518	3.518	3.518	3.518
Half-life	0.697	0.428	0.812	0.812	0.812	0.812

3.15. Predicted Toxicity:

In Table (11), the results of the toxicity prediction experiment benchmarked with a proposed reference and all other derivatives are displayed. The predicted organ toxicities and toxicity endpoints were evaluated and presented along with the corresponding value of the probability. The predictive results revealed that almost all compounds were defined as Inactive regarding hepatotoxicity, nephrotoxicity, and cardiotoxicity. A range of activity with a relatively low level of likelihood was established for specific parameters across the derivatives. In terms of carcinogenicity, most compounds were determined to be active with low probability levels between 0.52 and 0.62. Only two of the tested compounds, **MH-3** and **MH-5**, showed an inactive profile in the carcinogenicity study with probability levels of 0.52 and 0.53, respectively. Conversely, in the immunotoxicity study, all derivatives exhibited high predicted immunotoxicity probabilities ranging from 0.98 to 0.99, closely matching the reference drug itself (0.95). Furthermore, in the mutagenicity profiling, **MH-1** (0.60) and **MH-2** (0.92) were flagged as active, whereas **MH-3**, **MH-4**, and **MH-5** maintained an inactive status, with probability scores peaking at 0.99 for derivatives **MH-2** and **MH-3**, consistently mirroring or outperforming the reference drug itself (0.98).

Table 11: Toxicity profile for R and all DDs.

Toxicity Parameter	Reference	MH-1	MH-2	MH-3	MH-4	MH-5
Hepatotoxicity	Inactive (0.98)	Inactive (0.84)	Inactive (0.73)	Inactive (0.79)	Inactive (0.97)	Inactive (0.98)

Neurotoxicity	Active (0.90)	Active (0.83)	Inactive (0.71)	Active (0.87)	Active (0.76)	Active (0.74)
Nephrotoxicity	Inactive (0.67)	Inactive (0.59)	Inactive (0.58)	Inactive (0.62)	Inactive (0.61)	Inactive (0.57)
Respiratory toxicity	Active (0.96)	Active (0.94)	Active (0.77)	Active (0.95)	Active (0.95)	Active (0.95)
Cardiotoxicity	Inactive (0.67)	Inactive (0.66)	Inactive (0.63)	Inactive (0.69)	Inactive (0.72)	Inactive (0.68)
Immunotoxicity	Active (0.95)	Active (0.98)	Active (0.99)	Active (0.99)	Active (0.99)	Active (0.98)
Mutagenicity	Inactive (0.53)	Active (0.60)	Active (0.92)	Inactive (0.58)	Inactive (0.57)	Inactive (0.58)
Nutritional toxicity	Inactive (0.53)	Inactive (0.63)	Inactive (0.53)	Inactive (0.55)	Inactive (0.50)	Inactive (0.52)
Carcinogenicity	Active (0.50)	Active (0.62)	Active (0.53)	Inactive (0.52)	Active (0.52)	Inactive (0.53)
Cytotoxicity	Active (0.63)	Active (0.53)	Active (0.62)	Active (0.59)	Active (0.59)	Active (0.61)

4. Conclusion:

In this study, a series of donepezil derivatives (**MH-1** to **MH-5**) was designed by introducing substituents at the benzyl moiety. All molecules were computationally investigated using DFT, TD-DFT, molecular docking, and in silico pharmacokinetics. The FMO analysis reveals a reduced energy gap for **MH-1** and **MH-2** as compared to the reference. Optical analysis revealed that all derivatives show a bathochromic shift. Among them, **MH-2** exhibited the highest λ_{max} value due to the strong donor-acceptor interaction induced by the nitro group. MEP, DOS, NBO, TDM, and electron-hole overlap analyses further indicated the influence of substituents on the electronic properties of derivatives. In silico docking analysis against the acetylcholinesterase receptor (PDB ID:4EY7) revealed a good docking score for all derivatives. Especially, **MH-1**, **MH-2**, and **MH-4** have binding energies equivalent to the reference (-12.2 kcal/mol). The 300ns MD simulation for the **MH-4** complex with the receptor exhibited stable trajectories. Moreover, ADME analysis shows favourable oral absorption, blood-brain barrier permeability and decent

pharmacokinetic characteristics for all derivatives. Despite a few concerning toxicity predictions, most derivatives demonstrated acceptable toxicity profiles. **MH-2** predicted mutagenic and carcinogenic liabilities, which require further validation. Among the designed derivatives, MH-4 showed the most balanced electronic properties, strong receptor interactions, and suitable pharmacokinetic profiles. Collectively, the present findings suggest these derivatives may serve as a potential lead for future Alzheimer's drug development.

Acknowledgements

This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2601).

Author contributions

Conceptualization Muhammad Mohsin Ejaz, and Hamza Iftikhar; **original draft writing and editing:** Muhammad Mohsin Ejaz, Hamza Iftikhar, and Sadaf Ashraf, Reviewing Saira Khan, Nazia Meer, and Musarat Riaz **Formal analysis, Funding acquisition** K.S. Al-Namshah, Muhammad Saleem, **Resources:** Saira Khan and Muhammad Shar Jhahan Khan **Data validation, data curation:** Muhammad Saleem.

Funding

The authors are thankful to the Higher Education Commission (HEC) for the NRPU Research Grant No. 15788.

Conflict of interest

The authors declare no competing interests.

Data availability

The data presented in the current study will be available on demand.

Ethical approval

Not applicable.

Statement of human and animal rights

Not applicable.

Statement of informed consent

Not applicable.

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