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Computational Evaluation of Molecular Docking and Molecular Dynamic, Study of New Acyloxyalkyl Carbamates Prodrug Derivatives of Fluoxetine

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Abstract: Several candidates were suggested to evaluate the possible actions of five novel fluoxetine prodrug derivatives. The prodrug approach aims to enhance biopharmaceutical drug delivery, pharmacokinetics, and bioavailability. This approach provides additional advantages, such as therapeutic targeting through enzyme-triggered drug release. The extent of butyrylcholinesterase-mediated activation was predicted using computational models, which also aided in prodrug development. Various computational techniques, such as molecular docking and molecular dynamics simulations, were employed to determine the binding affinities of the five fluoxetine prodrug derivative molecules to the enzyme binding site. Computer simulations were performed using the GOLD program [CCDC] version 5.43, while the compounds were created with ChemDraw version 22.2 [professional version]. They were then evaluated for selectivity with BChE, using butyrylthiocholine as a control for comparison. For a 1-nanosecond duration, the SiBioLead server was used to run molecular dynamics simulations of compound A1 complexes with the BChE enzyme. During this time, enzyme energy, RG results, SASA results, RMSD, and RMSF were estimated. The results demonstrated that the BChE enzyme was responsible for the prodrug's activation, as evidenced by the maximum binding energy within the enzyme's active site. Compounds A1, A2, and A4 yielded PLP fitness values of 86.65, 80.81, and 82.819, respectively. This work shows that prodrug molecular design for fluoxetine can be more mechanistic and less empirical when led by computational simulations. In the future, prodrugs' mechanisms of action ought to be predicted utilizing molecular modeling methods as early in the development process as feasible.

Keywords: fluoxetine prodrug, acyloxy alkyl carbamate, molecular docking, molecular dynamic simulation.

1. Introduction

Major depressive disorder (MDD) impacts twice as many women as males [1]. It has an estimated frequency of 15% in the general population, making it one of the most prevalent and severe mental disorders. Major Depressive Disorder significantly elevates the chance of suicide and other common medical illnesses, including vascular disorders that may lead to premature mortality [2]. Two notable consequences of depression are a profound hindrance to occupational performance and the execution of daily responsibilities. Depression in primary care, including severe cases, frequently remains misdiagnosed and untreated due to the general practitioner's potential inability to recognize it. Conversely, the majority of patients exhibit a favorable response to treatment. This essay will examine the effects of comorbidity, the debilitating nature of depression, and the current understanding and treatment options for this condition [3]. The monoamine hypothesis is a significant theory regarding the pathophysiology of depression. It suggests that changes in serotonin [5-HT], norepinephrine [NE], and dopamine [DA] levels are the fundamental cause of depression [4]. The development of selective serotonin reuptake inhibitors (SSRIs) represents significant therapeutic progress in psychopharmacology. A

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variety of anxiety disorders and mood disorders have been shown to implicate serotonin [5-HT] in their pathophysiology. These findings revealed for the first time that inhibiting neurotransmitter reuptake is a crucial aspect of treatment [5]. Fluvoxamine, paroxetine, sertraline, citalopram, escitalopram, and fluoxetine are the six primary SSRIs now accessible in the United States. These drugs are classified as a group of chemical substances with distinct structures yet analogous mechanisms of action. Despite all SSRIs possessing a similar fundamental mechanism of action, each drug exhibits distinct pharmacokinetic, pharmacodynamic, effectiveness, and adverse effect profiles, rendering them variably suitable for specific clinical contexts. The patient will select the appropriate SSRI if the side effects are perceived as a secondary advantage. All SSRIs have specific common side effects, albeit to varying extents. xerostomia, extended QT intervals, gastrointestinal discomfort, and sexual dysfunction [6].

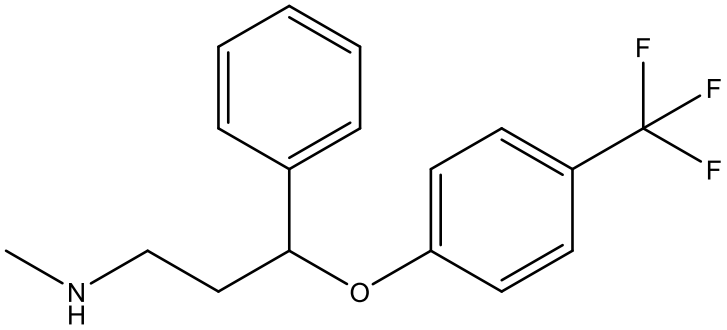


Figure 1. Fluoxetine structure

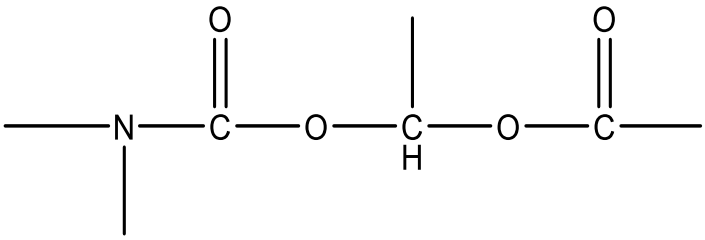


Figure 2. N-[acyloxy] alkyl carbamate

Fluoxetine, one of the first approved selective serotonin reuptake inhibitors [SSRIs], is widely recognized as a revolutionary drug for treating depression [7]. It has been acknowledged as a significant treatment for depression ever since. SSRIs, including fluoxetine, remain among the most commonly prescribed antidepressant drugs today due to their high selectivity for serotonin transporter (SERT) compared to tricyclic antidepressants [TCAs] [8,9]. Prodrugs have garnered significant attention as a promising technique to optimize drug delivery and pharmacological performance. This approach involves converting active drug moieties into prodrugs, which are then converted by the body to release the active parent drug, thus achieving the desired therapeutic effects [10]. The prodrug strategy has gained popularity as a means of overcoming the physicochemical, biopharmaceutical, pharmacokinetic, and pharmacodynamic challenges associated with pharmacologically active compounds [11-14]. The primary concept behind prodrug development is to improve the drug-like properties of parent compounds, such as distribution, metabolism, excretion, and absorption [14,15]. These improvements are particularly important because undesirable characteristics can present significant obstacles in the drug development process. Prodrugs also offer pathways to better formulations, increased therapeutic efficacy, reduced toxicity, easier administration, and enhanced site specificity [15]. These improvements are particularly important because undesirable characteristics can present significant obstacles in the drug development process. Prodrugs also offer pathways to better formulations, increased

therapeutic efficacy, reduced toxicity, easier administration, and enhanced site specificity [16].

Amine prodrugs improve lipophilicity and water solubility, aiding drug administration by promoting effective membrane penetration [17]. For parent drugs—particularly those undergoing intermolecular aminolysis—such prodrugs can also enhance chemical and metabolic stability. Additionally, amine prodrugs can be used to achieve targeted drug delivery [16]. For amino drugs that require penetration of the blood-brain barrier to be pharmacologically effective, their performance is limited by their ionization tendency under physiological conditions. N-[acyloxy] alkyl carbamate derivatives [Figure 2] have drawn attention as potential amine prodrugs [18-21]. These compounds possess an esterase-sensitive terminal group that, upon hydrolysis, undergoes spontaneous breakdown, releasing the parent amine. This unique pathway allows for prodrug activation via enzymatic bioconversion [18,19]. The [acyloxy] alkoxy promoiety was initially selected due to its susceptibility to esterase metabolism. This approach successfully addresses the relative enzymatic stability of simple N-acyl groups. The enzymatic stabilities of prodrugs with [acyloxy] alkyl ester linkers show that increasing steric hindrance around the linker improves the ester's hydrolytic stability while maintaining its stability in anhydrous acidic conditions [22].

Molecular docking, a method that simulates molecular interactions and calculates the binding mechanism and affinity between ligands and receptors, is a prominent structure-based drug design technique [23,24]. In recent years, this technology has been extensively utilized in drug design research. Researchers can use chemical databases to screen for potential pharmacophores, facilitating the purchase, synthesis, and subsequent pharmacological testing of compounds. This strategy not only significantly boosts productivity but also reduces research costs. To further enhance the ability to predict therapeutic targets and understand the underlying molecular mechanisms for drug design, reverse molecular docking technology may be developed [25]. The aim of the in silico methodologies in this work is to enhance the enzyme-mediated prodrug activation process. Various enzymes can aid in prodrug activation, including oxidoreductases such as CYP450 and hydrolytic enzymes like phospholipase A2 [PLA2], human valacyclovirase, paraoxonase, butyrylcholine esterase, acetylcholinesterase, matrix metalloproteinase, alkaline phosphatase [ALP], and others [26, 27]. The two subfamilies of cholinesterase that are easily identified are acetylcholinesterase [AChE] and butyrylcholinesterase [BChE], which are characterized by their preference for specific substrates and inhibitors [28]. Butyrylcholinesterase [BChE], also known as pseudocholinesterase or plasma cholinesterase, is a serine hydrolase found in almost all mammalian organs, with the highest concentrations in plasma and liver [29, 30]. BChE hydrolyzes anything with an ester bond large enough to fit in the active site pocket, including acetylcholine [31-34]. This includes drugs such as procaine, heroin, cocaine, and succinylcholine, as well as anesthetics. Before the determination of BChE's structure, a homology model based on electric ray acetylcholinesterase was used for structural investigations due to the close relation between BChE and AChE [35]. BChE's catalytic site, located at the bottom of a 20-deep valley, is where the hydrolysis reaction occurs. The components of this site include conserved aromatic amino acids. Glu325, His438, and Ser198 constitute the catalytic triad in human BChE. Among the aromatic amino acids essential for the hydrolysis process are Trp84 and Phe330 [36]. Both enzymes are classified as serine hydrolases due to their shared possession of the three amino acids that form the catalytic triad. The neighboring histidine, by activating the adjacent serine, generates an intermediate acyl-enzyme that breaks the ester bond and acts as a nucleophile against the electrophilic carbon of the substrate. Subsequently, the nucleophilic hydroxyl group of water replaces the cleaved substrate, releasing it and converting it to an acid [37].

Molecular dynamics [MD] simulations estimate the movements of individual atoms within proteins and other molecular systems over time. These simulations are based on a general description of the physics governing interatomic interactions. They can capture a wide range of important biomolecular processes, including protein folding, ligand binding, and conformational changes, and they provide the locations of all atoms with temporal precision down to femtoseconds. Most

importantly, computer simulations can predict, at the atomic level, how biomolecules would respond to various perturbations such as the addition or removal of ligands, phosphorylation, protonation, or mutations [38]. This work aims to investigate the possibility of enhancing the pharmacokinetics and bioavailability of fluoxetine through five new prodrug derivatives activated by the esterase enzyme (butyrylcholinesterase). The binding affinities, structural stability, and activation mechanisms of these prodrugs will be assessed computationally using molecular docking and molecular dynamic simulation methods.

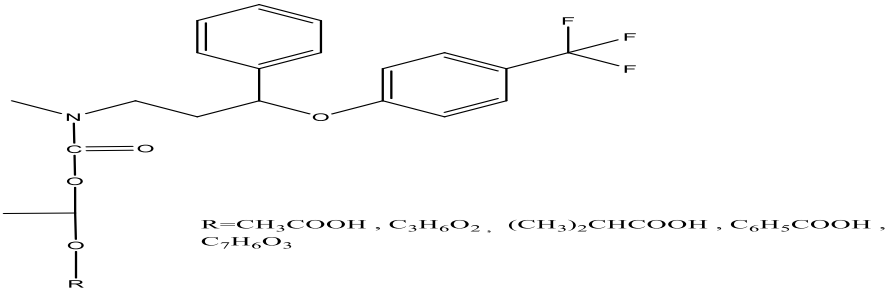
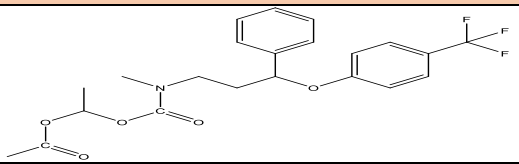
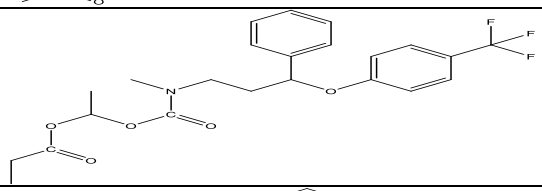
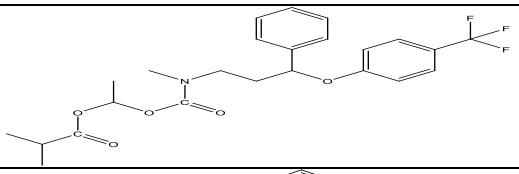
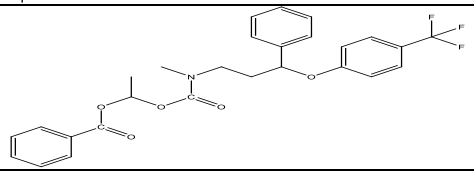


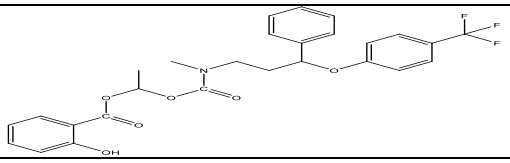
Figure 3. General structure of the suggested compounds

2. Computational Method

The team's extensive literature review informed the creation of the proposed compounds [fluoxetine derivative prodrugs], as illustrated in Figure 3 and Table 1. An in-silico modeling analysis was conducted to evaluate the potential impact of the proposed chemicals on the BChE enzyme [Protein Data Bank ID: 1p0p]. Following the assessment of the compounds' potential activity relative to butyrylthiocholine by computer docking, the interaction between the ligand and proteins will be examined. Initially, the BChE enzyme was employed in molecular docking to evaluate its compatibility with the enzyme's active site, including the establishment of hydrogen bonds and other transient interactions inside that site.

Table 1. The suggested prodrug derivative's structures

COMP.	STRUCTURE	IUPAC NAME
A1		**[1S]-1-[[methyl[3-phenyl-3-[4-[trifluoromethyl]phenoxy]propyl]carbamoyl]oxy]ethyl acetate**
A2		**[1S]-1-[[methyl[3-phenyl-3-[4-[trifluoromethyl]phenoxy]propyl]carbamoyl]oxy] ethyl propionate**
A3		**[1S]-1-[[methyl[3-phenyl-3-[4-[trifluoromethyl]phenoxy]propyl]carbamoyl]oxy] ethyl isobutyrate**
A4		**[1S]-1-[[methyl[3-phenyl-3-[4-[trifluoromethyl]phenoxy]propyl]carbamoyl]oxy] ethyl benzoate**

A5		**[1S]-1-[[methyl[3-phenyl-3-[4-[trifluoromethyl]phenoxy]propyl]carbamoyl]oxy] ethyl 2-hydroxybenzoate**

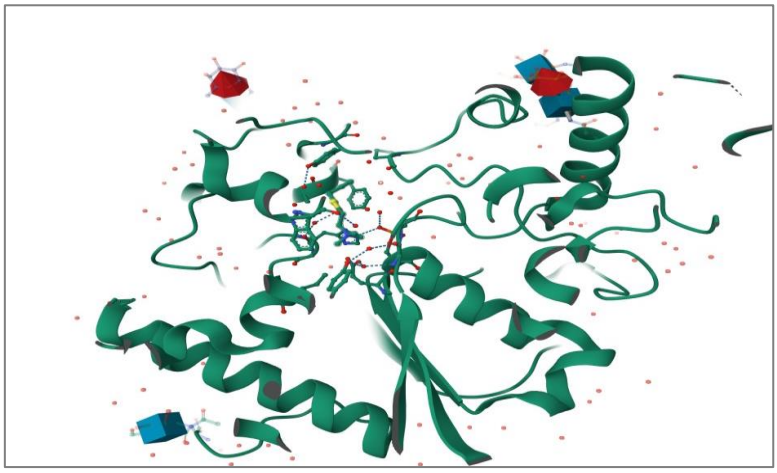


Figure 4. Crystal structure of human butyryl cholinesterase in complex with the substrate analog butyrylthiocholine

2.1. Preparation of compounds

To prepare for the docking process, use ChemOffice software [ChemDraw 22.2.0] to draw the chemical structures of the ligands [A1 to A5] and use Chem3D to minimize the energy.

2.2. Molecular Docking [Preparation of protein receptor for docking]

The docking procedure commences with the acquisition of the BChE enzyme [1p0p] from the Protein Data Bank [Figure 4]. The BChE enzyme interacts with butyrylthiocholine in the crystal structure denoted as 1p0p. Following the elimination of water molecules from the target enzyme, hydrogen atoms were introduced to confirm that the amino acid residues were in the appropriate tautomeric forms and to address ionization. The ligand docking process was conducted using GOLD software [Hermes 2022.3.0] from the Cambridge Crystallographic Data Centre [CCDC] [39]. The full license version was utilized to set up the receptor, prepare it for docking, and generate multiple configurations for each drug. The binding site and all protein residues within a 20 Å radius of the reference ligands in the enzyme structure were included in the GOLD docking process [40].

Early termination was turned off, and the docking process was allowed to complete. Using ChemScore kinase configuration guide, ChemPLP fitness as the scoring function, and the default setting, the top-ranked solution was kept. Windows 10 OS, an Intel Core i7 processor, 12 GB RAM, and an ASUS laptop were used to run the software.

2.3. Molecular dynamics simulation

The dynamic behavior of the higher-binding complexes, as predicted by molecular docking studies, was further investigated by MD simulations [41, 42]. Molecular docking investigations produced the compound of [1S]-1[[methyl [3-phenyl-3-[4-[trifluoromethyl] phenoxy] propyl] carbamoyl]oxy]ethyl acetate [A1], which was then utilized in molecular dynamics simulations. Butyrylcholinesterase [BChE] complexes with butyrylthiocholine were utilized as a reference. This strategy facilitated the comprehension of the protein and the docked complexes within a biological context. Molecular dynamics simulations were conducted using the SiBioLead server to assess the binding stability of the top candidate molecule [A1], ligand-induced structural modifications, and interaction mechanisms. The reference ligand-enzyme combination of 1P0P control was utilized to compare the results. Compound A1, exhibiting elevated PLP fitness, was the focus of the MD simulation. The PDB file of Compound A1 was

preprocessed by removing crystal water molecules utilizing the BIOVIA Discovery Studio Visualizer [Dassault Systems BIOVIA, 2024]. Subsequently, this file was saved in PDB format and processed using the Swiss-Pdb Viewer [SPDBV] to minimize its energy use. The system was subsequently created with an OPLS4 force field and an SPC water model, implemented directly in the MD simulation via the SiBioLead server. A 0.15 M NaCl solution was utilized to achieve a neutral pH in the system. The temperature was consistently maintained at 300 Kelvin throughout the one-nanosecond molecular dynamics simulations.

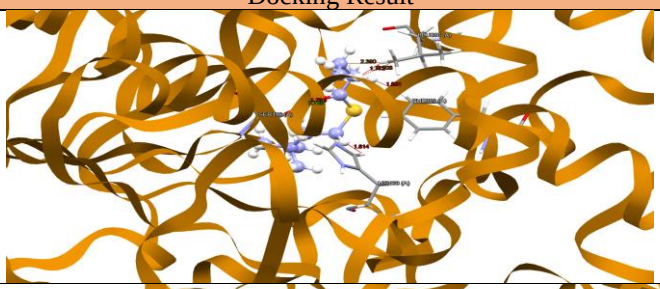
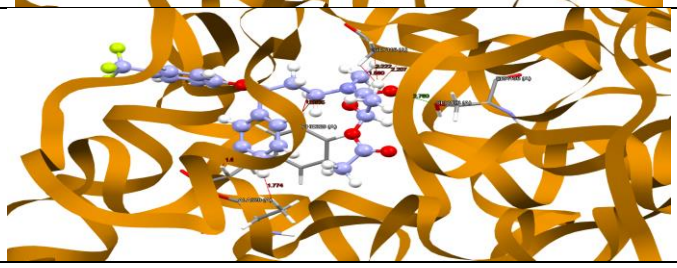
3. Results and discussion

3.1. Molecular Docking and Virtual Screening

Virtual screening (VS) is the methodology for discovering chemicals by aligning them with the target receptor through computational software. The docking data ascertain the hydrogen bond and short contact distances between the enzyme and ligands. A significant quantity of hydrogen bonds and hydrophobic interactions augments the biological activity necessary for substrate binding to the active site [40, 41]. The data acquired from molecular docking indicates the binding energies related to ligand-receptor interactions. Table 2 and Figure 5 display each chemical's average resultant binding affinity [PLP fitness] score. Table [2] presents the PLP fitness of butyrylthiocholine and the docked compounds with butyrylcholinesterase. The docking findings demonstrate that all produced compounds show enhanced binding energies with the enzyme's active site. This corresponds with the expected favorable activity of butyrylcholinesterase, which engages with its amino acid residue through hydrophobic interactions, hydrogen bonds, and other transient contacts. The docking analysis results indicate that the amino acid residues at the butyrylcholine esterase active site, detailed in table [2], engage with the final ligand library via hydrogen bonding and short contacts with ASP70, TRP82, TYR128, GLY116, SER198, LEU286, SER287, THR120, GLY121, LEU125, SER287, PHE329, ALA328, TYR332, and HIS438.

Table 2. The docked compounds' PLP fitness on the butyrylcholine esterase enzyme

Compound	PLP fitness	H-bond	Short contacts
Butyrylthiocholine	60.9	SER198	SER198, LEU286 ,PHE 329, HIS438
A1	86.65	SER198	GLY116 ALA328 PHE329
A2	80.81	SER287	ASP70 TYR128 SER198 LEU286 SER287
A3	79.28	SER198	TRP82 SER198 GLY121 LEU125 TYR 128 ALA328
A4	82.819	THR120	THR120 GLY121 TYR128 ALA328 TYR332 HIS438
A5	75.54		SER287 PHE 329 HIS438 GLY439

Docking Result	Compound
	Butyrylthiocholine
	A1

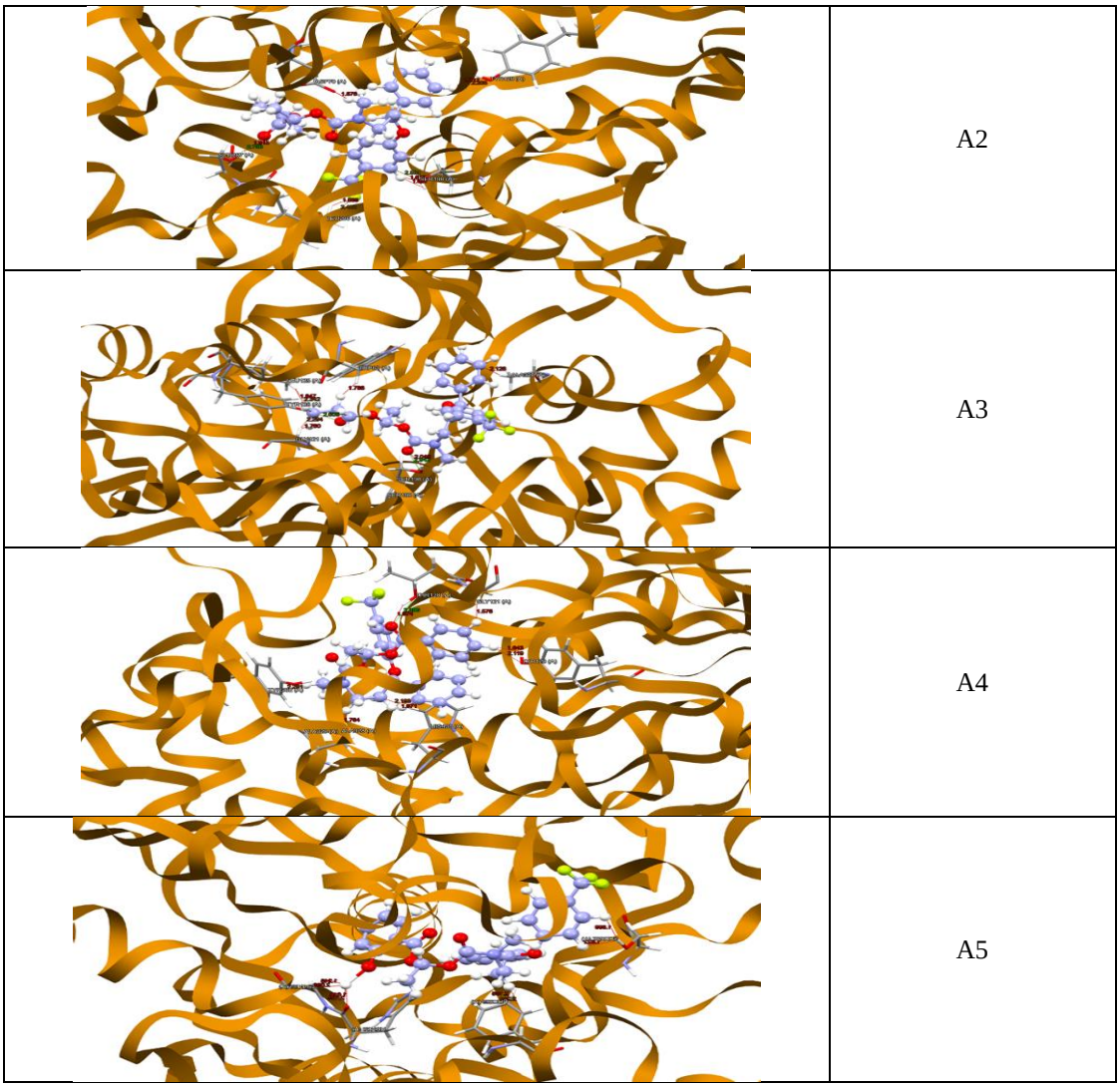


Figure 5. 3D intermolecular interactions of the best-docked complexes between proposed compounds and BChE [PDB: 1P0P] using gold software

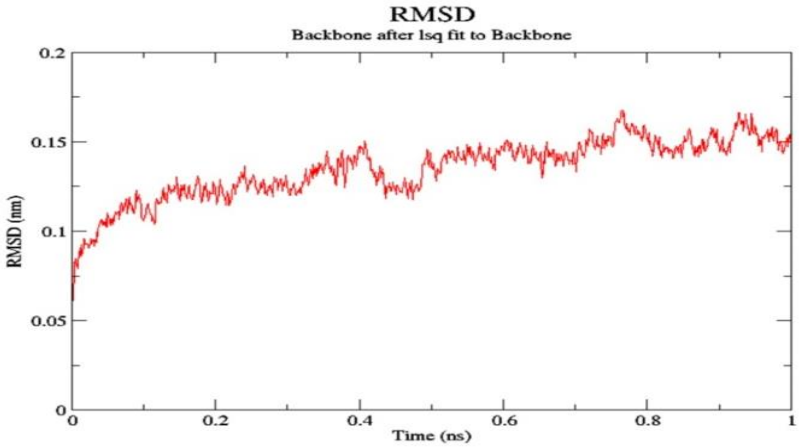


Figure 6. RMSD of the atoms of protein over time.

Each compound exhibits significant binding affinity for butyrylcholinesterase. Compounds [A1, A2, A3, A4, and A5] demonstrate the greatest PLP fitness values [86.65, 80.81, 79.28, 80.819, and 79.54], signifying robust interaction bonds for the ultimately produced compounds. Conversely,

butyrylthiocholine exhibits a PLP fitness value of 60.9.

Table 2 illustrates that butyrylthiocholine established a hydrogen bond with SER198 and exhibited brief interactions with SER198, LEU286, PHE329, and HIS438. The PLP fitness value obtained was 60.9. Table 2 indicates that Compound A1 exhibited robust docking fitness, establishing an H-bond with SER198 and creating close contacts with GLY116, ALA328, and PHE329, yielding an average PLP fitness value of 86.65. This amino acid is also present in the binding of butyrylthiocholine to the 1P0P active site. These results suggest that the compounds may effectively engage with the active site of the BChE enzyme, which is crucial for ensuring the full activation of prodrugs.

3.2. Molecular Dynamics

MD is an *in silico* technique that employs experimentally derived structural data to extrapolate potential configurations of molecular systems and the many pathways connecting them. Computational simulations of confined molecular systems were originally employed to examine models with a restricted number of atoms. However, remarkable technological advancements have facilitated a significant enhancement in computing power and data storage capacity during the last decade. Currently, research of extensive systems, such as receptors fully integrated within their biological context, are easily and quickly performed [43].

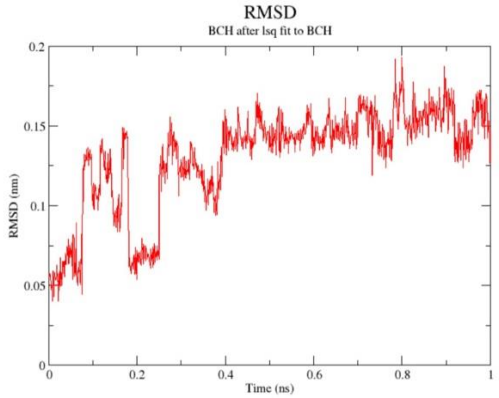
3.2.1. Root mean square deviations (RMSD)

It calculates the frame-by-frame root mean square deviations (RMSD) of the target ligand and target protein in relation to a reference structure. Generally, a stable protein's RMSD should range from 0.1 to 0.3 nm with no observable fluctuations. In the simulation, the protein RMSD ranged from 0.1 to 0.15 nm. This signifies that very minor structural alterations exist, indicating that the structure is relatively stable as it remains within the usual range of 0.1-0.3 nm, suggesting stability. The protein structure maintained its overall fold and conformation, which is crucial for ensuring the active site remains functional throughout the simulation. This is evidenced by the slight variations and the relatively low RMSD value [Figure 7].

3.2.2. Ligand Root mean square deviations (Ligand RMSD)

The stability of the protein during ligand binding during the specified simulation period is evaluated using RMSD analysis of the protein-ligand complex. The study indicates that the ligand exhibits a certain level of flexibility inside the binding region, evidenced by the low RMSD [0.1 to 0.17 nm] found. The interaction between compound A1 and BChE remained predominantly steady during the 1 ns simulation. The RMSD results indicate that the ligand maintains a very stable binding location throughout the simulation, albeit minor alterations. The prodrug A1 demonstrates an RMSD range comparable to that of the reference ligand, BChE, ranging from 0.1 to 0.15 nm, signifying analogous stability and interaction characteristics. The relatively low RMSD values for both the protein and ligand in Figure [7] indicate that the prodrug A1 is likely binding stably within the enzyme's active site.

a



b

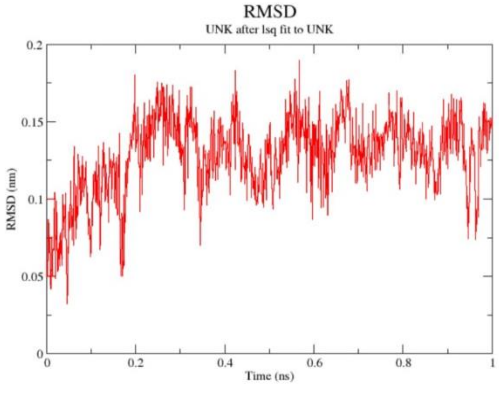


Figure 7. [a] is the RMSD result of reference ligand with BChE. [b] is the RMSD result of A1 compound with BChE

3.2.3. Root mean square fluctuation (RMSF)

The alterations in the protein backbone resulting from ligand binding are characterized by the RMSF [44]. After aligning with the reference frame, the standard deviation of the atomic positions in the trajectory is divided by the root mean square fluctuation (RMSF) to ascertain the atomic fluctuations of the target protein's amino acid residues. Both the prodrug and the reference ligand exhibited RMSF values ranging from 0.1 to 0.2 nm for most residues. This indicates that the enzyme's overall structure remained constant and that the A1 ligand did not alter its general shape. Conversely, the peak of the reference ligand was approximately 0.3 nm, while the peak of the A1 compound reached 0.4 nm, as indicated by the RMSF. This suggests that while both ligands enhanced flexibility at residue 400, the A1 region exhibited a greater degree of flexibility. The enhanced flexibility indicates that this region has experienced greater structural modifications than the reference ligand, potentially due to changes in the binding site's environment or the interaction mechanism of the protein and A1 [Figure 8].

3.2.4. Radius of gyration (RG)

To assess the structural compactness of the protein post-ligand binding, variations in the radius of gyration [RG] among the MD trajectories were examined. In simulated trajectory analysis, the radius of gyration is a frequently utilized parameter for predicting the structural behavior of macromolecules. The radius of gyration may vary due to a conformational change induced by the binding of a lead or ligand to a protein. The compactness of the protein structure, shown by RG, is strongly associated with the rate of protein folding. The RG values of the A1 molecule varied from 2.31 to 2.32 nm, slightly above those of the reference ligand, which exhibited RG values between 2.28 and 2.32 nm. This indicates that the enzyme's overall structure remains largely intact and is not substantially destabilized by the A1 molecule. The binding introduces a minor degree of flexibility, while the enzyme remains predominantly rigid. Moreover, compound A1 induces a conformational change in the enzyme, facilitating its adaptation to A1, which is essential for the enzyme's activity and optimal functioning. This elucidates the minor enhancement in adaptability. The enzyme's structural compactness is largely unaffected by compound A1, despite its ability to induce a conformational shift [Figure 9].

3.2.5. Surface area accessible to solvents (SASA)

The solvent-accessible surface area (SASA) is computed to ascertain the surface area of a macromolecule that is reachable by a solvent. SASA is derived from the non-solvent atoms on the entire surface and is utilized to assess the solvent-accessible areas. Furthermore, the molecule's volume and density are quantified, alongside approximations of solvent-free energies obtained from per-atom solvation energies. SASA can be employed to forecast aggregation-prone sequences in proteins. The modeling findings indicated that the SASA values fluctuated between 225 and 230 nm^2, exhibiting a progressive increase over time. This suggests that the enzyme was gradually revealing an increased surface area to the solvent, maybe due to little amounts of previously concealed residues becoming revealed or undergoing mild unfolding. In comparison to the reference ligand, which exhibited SASA values between 218 and 227 nm^2, the results for compound A1 were notably comparable. The alterations indicate that the enzyme predominantly maintains stability during the simulation, but with a somewhat increased interface exposed to the solvent, potentially affecting its interactions with other molecules. The A1 molecule does not substantially impair the enzyme, suggesting that the enzyme is largely stable. This indicates that the interaction is predominantly advantageous and that the prodrug A1 is probably efficacious in its designated function [Figure 10].

3.2.6. Hydrogen bonds binding

The average number of hydrogen bonds is calculated and evaluated using donor and acceptor criteria, as well as distance and angle cutoffs. The results of several calculations, including the quantity, angle, and distance of hydrogen bonds, together with the number of donors and acceptors, can be analyzed using Raman spectroscopy. The SiBioLead MD simulation module calculates intramolecular and intermolecular hydrogen bonding. The enzyme's structural dynamics are evidenced by the intramolecular hydrogen bonds, which fluctuate between 350 and 390 [Figure 11].

These connections enhance the enzyme's tertiary structure, contributing to its flexibility and stability. The consistent number of intramolecular bonds signifies a stable protein structure, despite slight fluctuations in transitory interactions between the ligand and the enzyme, as evidenced by the intermolecular hydrogen bonds, which range from 0 to 1 [Figure 11]. This indicates that hydrogen bonds may be less significant in the binding of the A1 molecule to the enzyme compared to other forces, such as hydrophobic interactions. The A1 molecule establishes three transient interactions with GLY116, ALA328, and PHE329, along with one hydrogen bond with SER198.

a b

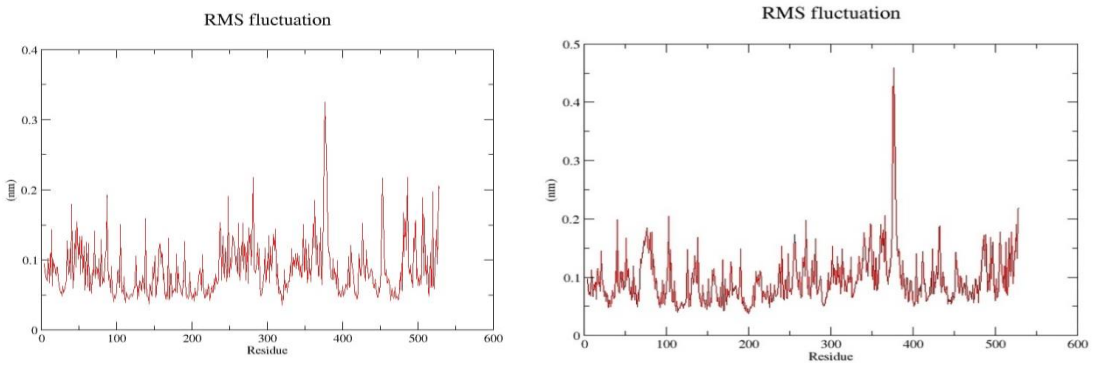


Figure 8. [a] is the RMSF result of reference ligand with BChE [b] is the RMSD result of A1 compound with BChE

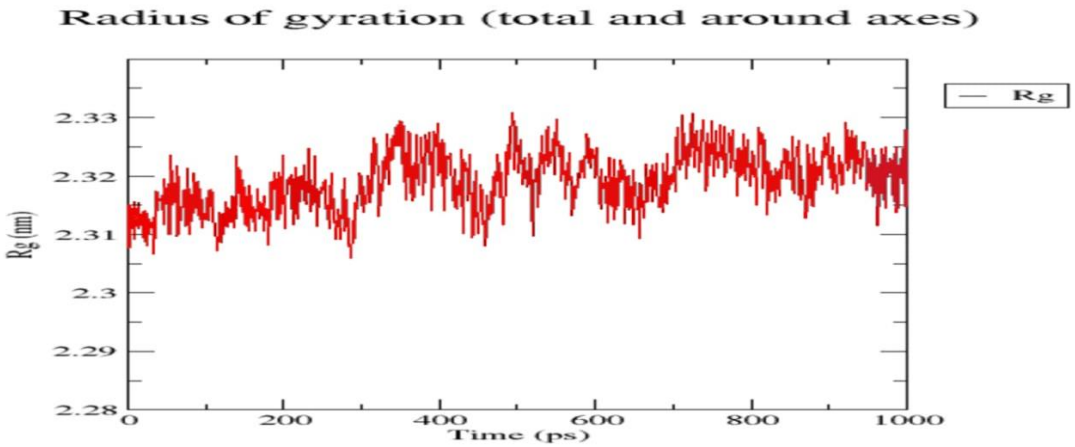


Figure 9. RG of A1coumpond with enzyme

a b

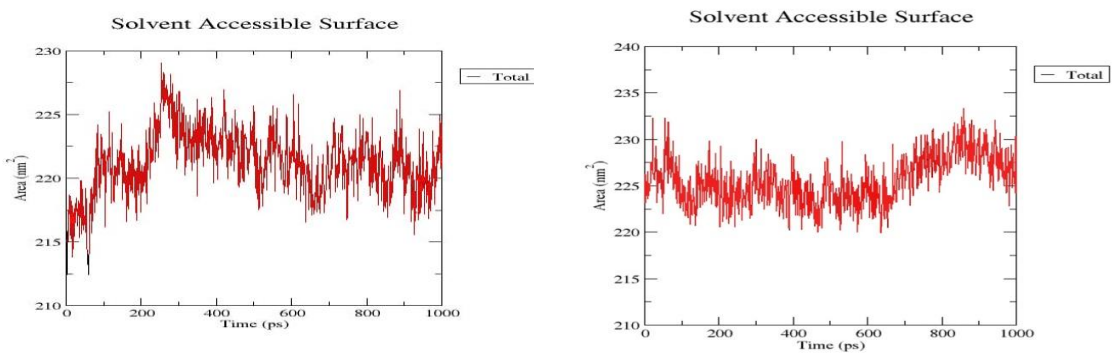


Figure [10]. [a] is the SASA result of reference ligand with BChE [b] is the SASA result of A1 compound with BChE

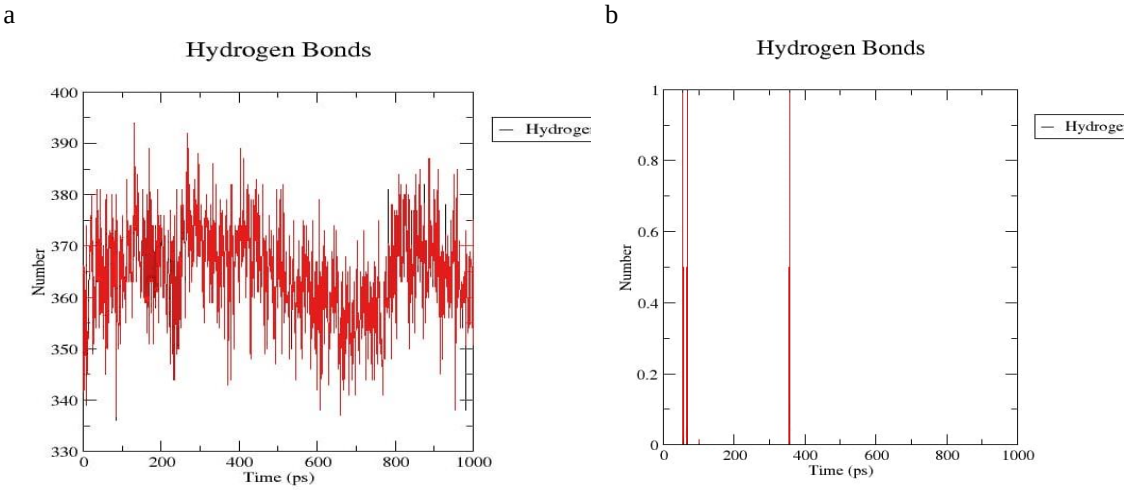


Figure 11. [a] enzyme's intramolecular H bond, [b] intermolecular H bond between the ligand and the BChE enzyme

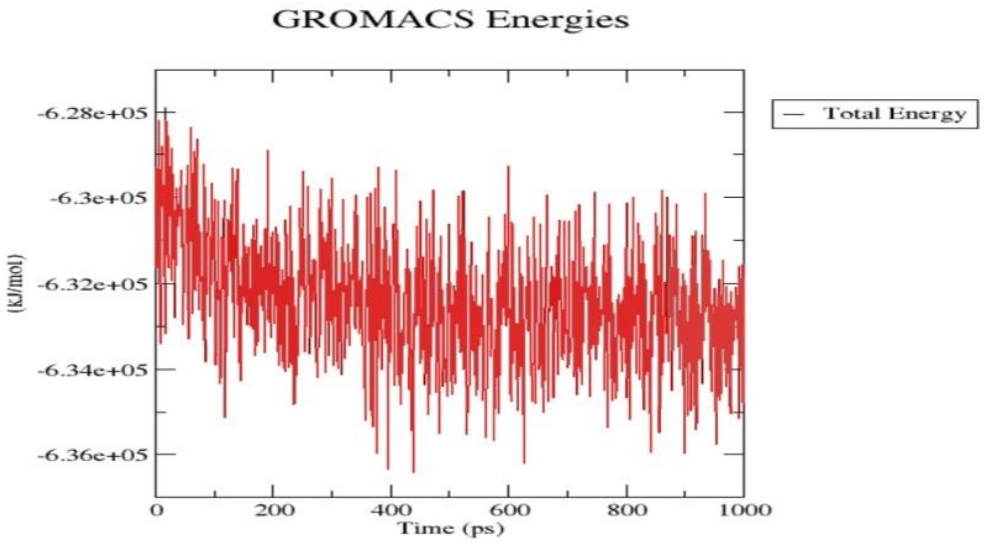


Figure 12. Binding free energy of the ligand –enzyme complex.

3.2.7. Energy of enzyme

The ligand's affinity for the target receptor in the ligand-protein complex, as predicted by molecular docking studies, was validated by assessing the binding free energy of the complex. The results indicate that the system stabilizes within 100–200 ps [Figure 12], demonstrating that the prodrug is ideally situated for enzymatic hydrolysis, as the A1 molecule interacts with the enzyme and assumes a favorable shape by induced fit interaction. Following stabilization, the energy levels exhibited constant fluctuations around the mean value, indicating that the enzyme-prodrug complex maintained stability throughout the simulation. This consistent activity verifies that the prodrug remains adequately bound to the enzyme and is ready for activation. After stabilization, the prodrug's binding free energy, ranging from -630,000 to -636,000 kJ/mol, further substantiates the stability of the enzyme-prodrug complex. The consistent binding energy suggests that the prodrug exhibits a favorable affinity for the enzyme, while its binding strength is inferior to that of a robust inhibitor. The moderate binding energy indicates that the enzyme maintains a shape conducive to its optimal operation.

4. Conclusions

Molecular docking is a highly effective computational method for identifying new medicines with improved potency and affinity for enzyme binding. This study's innovation is the design of several fluoxetine prodrug molecules and

the assessment of their potency profiles. The proposed compounds [A1, A2, and A4] demonstrate enhanced binding scores in the BChE binding area relative to butyrylthiocholine, the reference ligand, indicating efficient prodrug activation. Compound A1 was subjected to a comprehensive MDS analysis, resulting in outstanding outcomes for RG, SASA, RMSD, RMSF, and enzyme energy metrics. Molecular docking and MD simulations give a comprehensive investigation of the key enzymatic barriers and yield essential insights into the parameters required for the efficient identification and development of prodrugs. To decre`ase the number of trials and lower expenses, preliminary modeling studies should be performed prior to prodrug manufacturing and in vitro assessment. The integration of novel in silico simulations of prodrug/enzyme complexes with strategic prodrug design offers a viable avenue for the future advancement of the prodrug method.

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