KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 2): 307-315, 2022
KSU J. Agric Nat 25 (Suppl 2): 307-315, 2022
https://doi.org/10.18016/ksutarimdoga.vi.1092038

Computational Approaches for Drug Repurposing in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease is a progressive age-related brain disorder. It causes gradual memory loss, changes in personality traits, confusion, impaired thinking, and mood changes Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors have been chosen for the treatment of Alzheimer's disease. Dual cholinesterase inhibitors have become a new hotspot in the investigation of anti-Alzheimer's drugs. The current study was designed to identify inhibitors for both AChE and BuChE enzymes using computational approaches to accelerate the process of identifying an effective treatment for Alzheimer. From the available drugs, we selected families of the aspirin and imatinib. After the adoption of molecular docking, we found that fendosal from aspirin group and Flumatinib from the Imatinib group are the most active compounds. The docking scores for fendosal was -8.160 kcal/mol against AChE while Flumatinib had -9.433 kcal/mol and -9.541 kcal/mol scores with BuChE and AChE, respectively. The 10 ns molecular dynamics simulation for fendosal and flumatinib against AChE and BuChE was performed to evaluate the drug's ability to remain stable within the binding sites of AChE and BuChE with the aid of RMSD and RMSF plots. These results revealed that Flumatinib and fendosal are good inhibitors for both BuChE and AChE, which could be used in vivo and in vitro studies to improve outcomes.

Biochemistry

Research Article

Article History
Received : 23.03.2022
Accepted : 09.05.2022

Keywords
Aspirin
Imatinib
Acetylcholinesterase
Butyrylcholinesterase
Molecular docking

Alzheimer Hastalığında İlacın Yeniden Kullanım İçin Hesaplamalı Yaklaşımlar

ÖZET

Alzheimer hastalığı, ilerleyici, yaşa bağlı bir beyin hastalığıdır. Kademeli hafıza kaybına, kişilik özelliklerinde değişikliklere, kafa karışıklığına, düşünme bozukluğuna ve ruh hali değişikliklerine neden olur. Alzheimer hastalığının tedavisi için asetilkolinesteraz (AChE) ve butirilkolinesteraz (BuChE) inhibitörü seçilmiştir. Çift kolinesteraz inhibitörleri, anti-Alzheimer ilaçlarının araştırılmasında yeni bir etkin nokta haline geldi. Bu çalışma, Alzheimer için etkili bir tedavi tanımlama sürecini hızlandırmak için hesaplama yaklaşımlarını kullanarak AChE ve BuChE enzimlerine yönelik inhibitörlerin belirlemesi için tasarlanmıştır. Mevcut ilaçlardan aspirin ve imatinib aileleri seçilmiştir. Moleküler yerleştirmenin benimsenmesinden sonra, aspirin grubundan fendosalın ve Imatinib grubundan flumatinib'in en etkin bileşikler olduğu bulunmuştur. Fendosal için kenetlenme skorları AChE'ye karşı -8,160 kcal/mol iken Flumatinib BuChE ve AChE ile sırasıyla -9,433 kcal/mol ve -9.541 kcal/mol skorlarına sahip olmuştur. AChE ve BuChE'ye karşı fendosal ve flumatinib için 10 ns moleküler dinamik simülasyonu, RMSD ve RMSF grafiklerinin yardımıyla ilacın AChE ve BuChE'nin bağlanma bölgeleri içinde stabil kalma kabiliyetini değerlendirmek için yapılmıştır. Bu sonuçlar, Flumatinib ve fendosal'ın hem BuChE hem de AChE için iyi bir inhibitör olduğunu ortaya koymuş olup sonuçların geliştirilmesi için in vivo ve in vitro çalışmaların kullanılabilir.

Biyokimya

Araştırma Makalesi

Makale Tarihçesi
Geliş Tarihi : 23.03.2021
Kabul Tarihi : 09.05.2022

Anahtar Kelimeler
Aspirin
Imatinib
Asetilkolin esteraz
Butirilkolin esteraz
Moleküler yerleştirme

Atıf Şekli: Al-Nuaimi ZJA, Çelekli A, Tok TT 2022. Alzheimer Hastalığında İlacın Yeniden Kullanım İçin Hesaplamalı Yaklaşımlar . KSÜ Tarım ve Doğa Derg 25 (Ek Sayı 2): 307-315. https://doi.org/10.18016/ksutarimdoga.vi.1092038

To Cite : Al-Nuaimi ZJA, Çelekli A, Tok TT 2022. Computational Approaches for Drug Repurposing in Alzheimer's Disease. KSU J. Agric Nat 25 (Suppl 2): 307-315. https://doi.org/10.18016/ksutarimdoga.vi.1092038

INTRODUCTION

Alzheimer's disorder is the most prominent dementia condition and its global incidence has increased in recent years. Alzheimer's disease (AD) is described as synaptic dysfunction, oxidative stress, neuroinflammation, mitochondrial dysfunction, and disruption of the blood-brain barrier (Mendiola-Precoma et al., 2016). In 2021, more than 55 million people worldwide suffered from AD and dementia leading to a large number of deaths, but the data in 2020 cannot be stated as a precise number due to the COVID-19 outbreak (Gauthier et al., 2021). The disease has most frequently been observed in individuals over the age of 65, while about 4-5 percent of cases are early-onset. Down syndrome is a common form of early-onset dementia. Adults with down syndrome, after the age of 40, consistently display oncoming cognitive decline and dementia superimposed on their baseline cognitive limitations. They gather amyloid, neurofibrillary tangles, and cell depletion similar to sporadic AD (Gauthier et al., 2021). Alzheimer's disease is an age-related neurodegenerative disorder that leads to a rapid decline of physical, cognitive, and behavioral abilities (Jellinger, 2010).

Pathophysiologically, Alzheimer's disease is marked by the extracellular accumulation of Aß protein in amyloid plaques and the generation of neurofibrillary tangles resulting from the hyperphosphorylation of the tau protein linked with cellular microtubules (Brunton et al., 2011). Abnormalities in acetylcholine (ACh) and butyrylcholine (BCh) levels, which act as neurotransmitters, have been observed in the brains of patients with Alzheimer's disease. Inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE)enzymes degrade ACh and BCh neurotransmitters and so it is accepted as one of the therapy choices for AD (Kim et al., 2018). Especially in patients with Alzheimer's disease, cholinesterase inhibitors are thought to i) enhance cognition and indirectly aid function and behavior, ii) improve cognition and overall outcome, and iii) ameliorate some stabilization of function during this time (Birks and Evans, 2015). Cholinesterase is a family of esters that degrade choline-based esters and many of which act as neurotransmitters (Colović et al., 2013). Thus, it is either of the two enzymes that stimulate the hydrolysis of these cholinergic neurotransmitters, like acetylcholine breakdown into choline and acetic acid (Colović et al., 2013). As part of the evaluation of the effects of aspirin and iminatip family compounds on cholinesterase enzymes, it is known that Alzheimer's disease affects many neurotransmitter systems, especially ACh and neurotransmission deficiency (Corbett et al., 2012).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitor have been chosen for the treatment of Alzheimer's disease (Colović et al., 2013). Dual cholinesterase inhibitors have become a new hotspot in the investigation of anti-Alzheimer's drugs. Related families of aspirin and imatinib drugs are commonly used in the market. Our aim is to determine more economical and rapid anti-Alzheimer's inhibitors by evaluating the interactions amid commonly used drug groups with cholinesterase target structures ACh and BCh. Based on this main purpose, the effects of aspirin and imatinib family drugs as anti-Alzheimer inhibitors were assessed by using computational approaches to accelerate the process of an effective treatment and identifyng.

MATERIALS and METHODS

Molecular docking analysis

Molecular docking studies of the selected compounds from aspirin and imatinib families with AChE and BuChE were carried out with AutoDock Vina (Trott and Olson, 2010). The targeted protein structures (PDB ID: 1GQS for AChE and 4XII for BuChE) were obtained via the RCSB PDB database, and were prepared before the docking, including water removing, adding hydrogen atoms and missing residues and charges with help of Autodock Tools 1.5.6. The related ligands were optimized using the DFT/B3LYP/6-31G(d) basis set in Gaussian09 (Gaussian 09, Revision E.01, 2009). Autodock Tools was also utilized to define the grid box with the dimensions of 40 × 40 × 40 sizes. During the docking procedure, 100 conformations for each ligand were left flexible, while the protein was held rigid. At end of the docking processes, the docking conformations were ranked and determined the fendosal and flumatinib compounds according to their lowest binding energies, for each target

Molecular dynamics simulation

Molecular dynamics (MD) simulation is now considered a decision-making step in the computational investigations of drug discovery (Al-Khafaji and Taskin Tok 2021a). In the present study, two ligands obtained from the docking were subjected to the ligand-free and ligand-based simulations of the target enzymes via MD simulation on a 10-ns time scale. We used ‹GROMACS 2018.1 package'

(Abraham et al., 2015) to operate MD simulations. The three-point transferable intermolecular potential (TIP3P) was selected as solvent and the charge of the targets was adjusted by adding (Na^+ or Cl^- ions). The energy was reduced by using the steepest descent algorithm at a tolerance value of 1000 kJ/mol nm. In the next phase, the amount of substance's volume, temperature, moles, pressure, and temperature ensembles counterpoised the complexes with position restraint on molecules of each target protein for 0.1 ns. The particle-mesh Ewald (PME) was appointed for dealing with nonbonded interactions (Essmann et al., 1995). Now the MD simulation was carried out to determine stability for 10 ns, with no restraint on the protein molecules or ligands.

Both fendosal and flumatinib were carried out by MD simulation to determine stability for 10 ns without any restraint on the protein. The related ligands also were compared to the docking of both fendosal and flumatinib againt BuChE and AChE. The results of MD simulations give us the root mean square deviation (RMSD) of AChE and BuChE backbone atoms and also measure the root-mean-square fluctuation (RMSF) values for the assessment of chosen comparable drugs against BuChE and AChE..

Statistical Analyses

Shapiro-Wilk test was used to test the normality of data. Multiple comparisons were performed using one-way variance analysis, ANOVA or the Kruskal-Wallis test. Student t-test and Mann-Whitney U test were used for normal and non-normally distributed data in comparison of treatment groups, respectively. Results are expressed as mean ± SEM or median ± interquartile ranges (Q1 and Q3). A cut off level 0.05 was used for the statistical importance of the results. OriginPro 2021b program was used for statistical analysis (OriginPro, Version 2021b. OriginLab Corporation, Northampton, MA, USA.).

RESULTS

Results of Aspirin family

Results of the binding energy of the Aspirin family and ref ligand against to BuChE and ref ligand are summarized in Table 1. Diflunisal, fendosal, and salsalate had the lower binding energy against BuChE, which indicates that they have higher docking scores against BuChE. Salicylic acid had a higher binding energy against BuChE, which refers that it has the lowest docking score against BuChE. The fendosal displayed a significant interaction than the rest of chosen drugs against BuChE because it had the lowest binding energy, the highest docking score (Table 1), and the suitable RMSD value.

A docking comparison of aspirin family against AChE are given in Table 1. The same approach was carried out for the same compounds (Aspirin, Diflunisal, Fendosal, Phosphosal, Salicylic acid, Salsalate and reference ligand.) against acetylcholinesterase as a target. Fendosal had the lower binding energy against AChE, which indicates that it has the highest docking score against AChE. Salicylic acid had higher binding energy against AChE, which indicates that it has the lowest docking score against AChE. Fendosal significantly has stronger interaction than those of chosen remain drugs against AChE because it has the lowest binding energy, the highest docking score, and the suitable RMSD value, as given in Table 1.

Results of Imatinib Family

Results of the binding energy of ref ligand and the Imatinib family against to BuChE are given in Table 2. Imatinib, Flumatinib, and Nilotinib had the lower binding energy against BuchE, which indicates that they have higher docking scores against BuchE. Mocetinostat had the higher binding energy against BuchE. This shows that it's has the lowest docking score against BuchE. The Flumatinib significantly has stronger interaction than those of chosen comparable drugs against BuChE because it has the lowest binding energy, the highest docking score (Table 2), and the suitable RMSD value. The binding energy of ref ligand acetylcholinesterase AChE = -8.16 kcal/mol and the binding energy of AChE results with Imatinib family were exhibited in Table 2 . Imatinib, Flumatinib, and Nilotinib indicated the lower binding energy against AChE, which means that they have the highest docking scores against AChE. Mocetinostat had the higher binding energy against AChE and the lowest docking score against AChE. Flumatinib significantly has stronger interaction than those of chosen remain drugs against AChE because it has the lowest binding energy, the highest docking score and the suitable RMSD value. Results revealed that Flumatinib has the highest affinity to act as a dual inhibitor against AChE and BuChE (Table 2).

Docking results of the selected medicinal group was higher than the docking scores of the ref ligands when compared with the native ligand, diflunisal, fendosal, and salsalate. It is important to note that fendosal had significantly stronger interaction than that of chosen comparable drugs against BuChE (Table 1). A comparision of the selected Aspirin family drugs against the AChE for targeting Alzheimer's showed that fendosal has a binding affinity higher than the native ligand of AChE (Table 1). The rest drugs of the selected group had a binding affinity less than the ref ligand. Molecular docking results revealed that flumatinib, imatinib, and nilotinib have good binding affinities to interact with both AChE and BuChE as dual targets for targeting Alzheimer's.

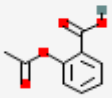
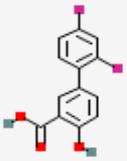
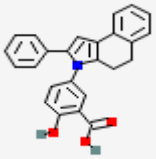
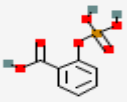
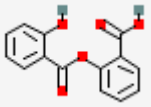
Molecular dynamics simulation results

The impact of ligand-protein interactions on the

dynamics of a biological system is crucial in drug discovery. The RMSD was used to explore the influence of fendosal and flumatinib drugs upon the stability of AChE protein and compare it with the apo form of AChE. We used Gromacs to perform the MD simulations of 10 ns for four drug-protein systems besides apo AChE protein. RMSD fluctuations for apo and holo forms were measured and presented in Figure 1. It was apparent that the AChE protein's backbone has indistinguishable-stable RMSD values (Figure 1) when NadR in apo, NadR-flumatinib, and NadR- fendosal forms. Fendosal had a similar fashion of RMSD backbone atoms when compared with apo AChE form. The RMSD values for flumatinib showed little fluctuations.

The RMSD average values of BuChE- fendosal were lower than the BuChE-flumatinib (Figure 2). Both of them fluctuated nearly to the RMSD values of BuChE apo form. According to the dynamic results in Figure 2, fendosal rather than flumatinib has RMSD values closer to the BuChE apo form, and therefore fendosal compound has priority.

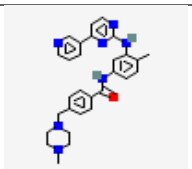
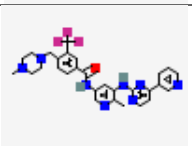
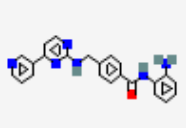
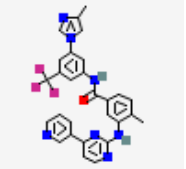
Table 1. Docking results of aspirin family against BuChE and AChE.
Çizelge 1. Aspirin ailesinin BuChE ve AChE'ye karşı kenetlenme sonuçları

No	Name of ligand *Ligandın adı*	PubChem CID *PubChem KID*	Structure of ligand (2D) *Ligandın yapısı (2D)*	Molecular Formula *Moleküler formül*	against BuChE (kcal/mol) *BuChE'ye karşı (kcal/mol)*	against AChE (kcal/mol) *AChE'ye karşı (kcal/mol)*
1	Aspirin *Aspirin*	2244		$C_9H_8O_4$	-5.340	-5.340
2	Diflunisal *Diflunisal*	3059		$C_{13}H_8F_2O_3$	-5.991	-5.990
3	Fendosal *Fendosal*	40821		$C_{25}H_{19}NO_3$	-7.836	-8.240
4	Fosfosal *Fosfosal*	3418		$C_7H_7O_6P$	-5.470	-5.631
5	Salicylic acid *Salisilik asit*	338		$C_7H_6O_3$	-4.610	-4.730
6	Salsalate *Salsalate*	5161		$C_{14}H_{10}O_5$	-6.560	-6.0130
7	Ref ligand *(ref ligandı)*	-	-		-5.490	-8.160

Root mean square fluctuation (RMSF) values explored the effect of fendosal and flumatinib upon the flexibility of AChE and BuChE backbone atoms were presented in Figure 3 and Figure 4, respectively. The binding of fendosal and flumatinib to the AChE conserved the binding site regions and whole proteins from fluctuation (Figure 3). Especially in the range of 2500-3000, 4500-5500 and 7000-8000, fluctuations in AChE backbone atoms are quite evident in the phendosal compound. On the other hand, Fendosal

and flumatinib increased the flexibility of BuChE in different regions. This indicates that both selected drugs effectively block the binding site of BuChE from different sites.

Table 2. Docking results of Imatinib family against BuChE and AChE.
Çizelge 2. Imatinib ailesinin BuChE ve AChE'ye karşı kenetlenme sonuçları

No.	Name of ligand *Ligandın adı*	PubChem CID *PubChem KID*	Structure of ligand *Ligandın yapısı*	Molecular Formula *Moleküler formül*	against BuChE (kcal/mol) *BuChE'ye karşı (kcal/mol)*	against AChE (kcal/mol) *AChE'ye karşı (kcal/mol)*
1	Imatinib *Imatinib*	5291		$C_{29}H_{31}N_7O$	-9.100	-9.390
2	Flumatinib *Flumatinib*	46848036		$C_{29}H_{29}F_3N_8O$	-9.433	-9.541
3	Mocetinostat *Mocetinostat*	9865515		$C_{23}H_{20}N_6O$	-8.0700	-7.980
4	Nilotinib *Nilotinib*	644241		$C_{28}H_{22}F_3N_7O$	-9.570	-9.340
5	Ref ligand *Ref ligandı*	-	-		-5.490	-8.160

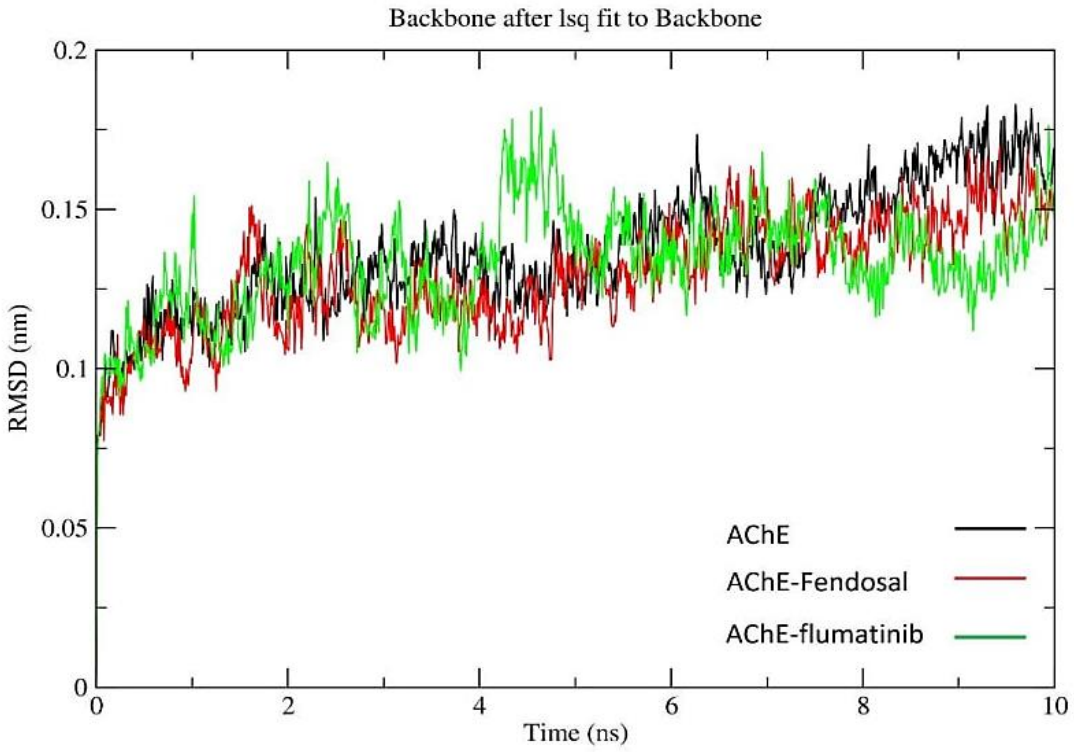


Figure 1. Analysis of RMSD of AChE backbone atoms
Şekil 1. AChE omurga atomlarının RMSD analizi

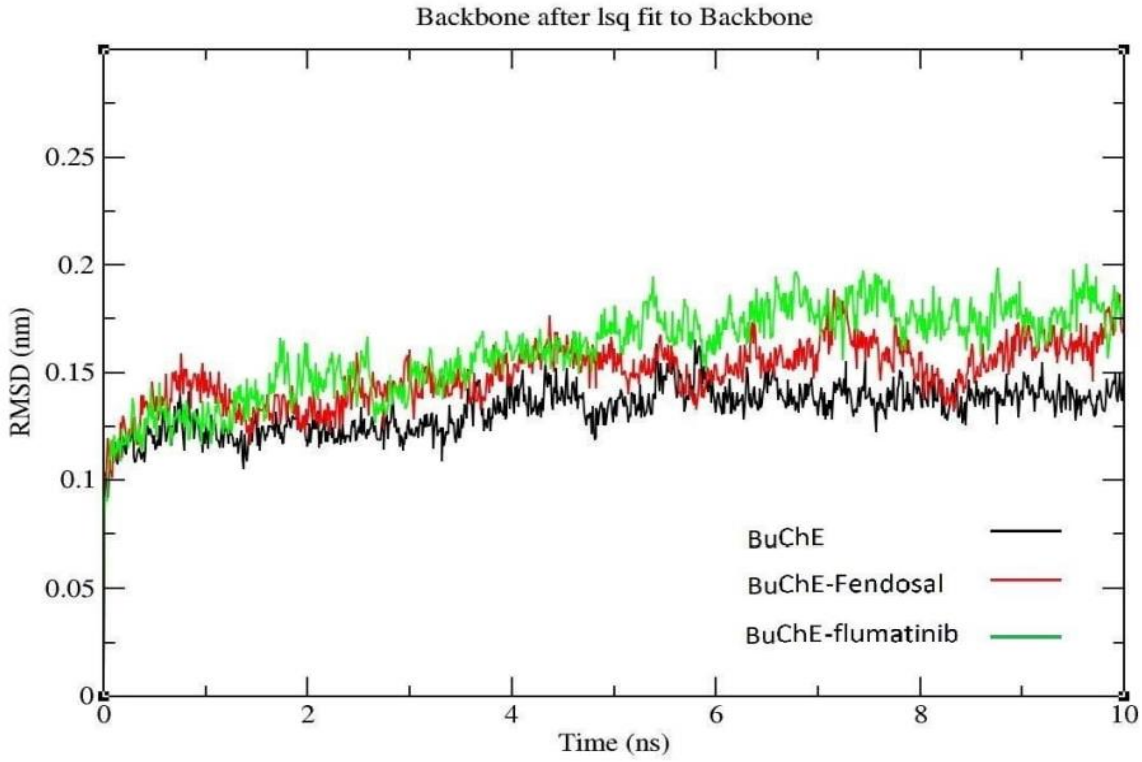


Figure 2. Analysis of RMSD of BuChE backbone atoms.
Şekil 2. BuChE omurga atomlarının RMSD analizi

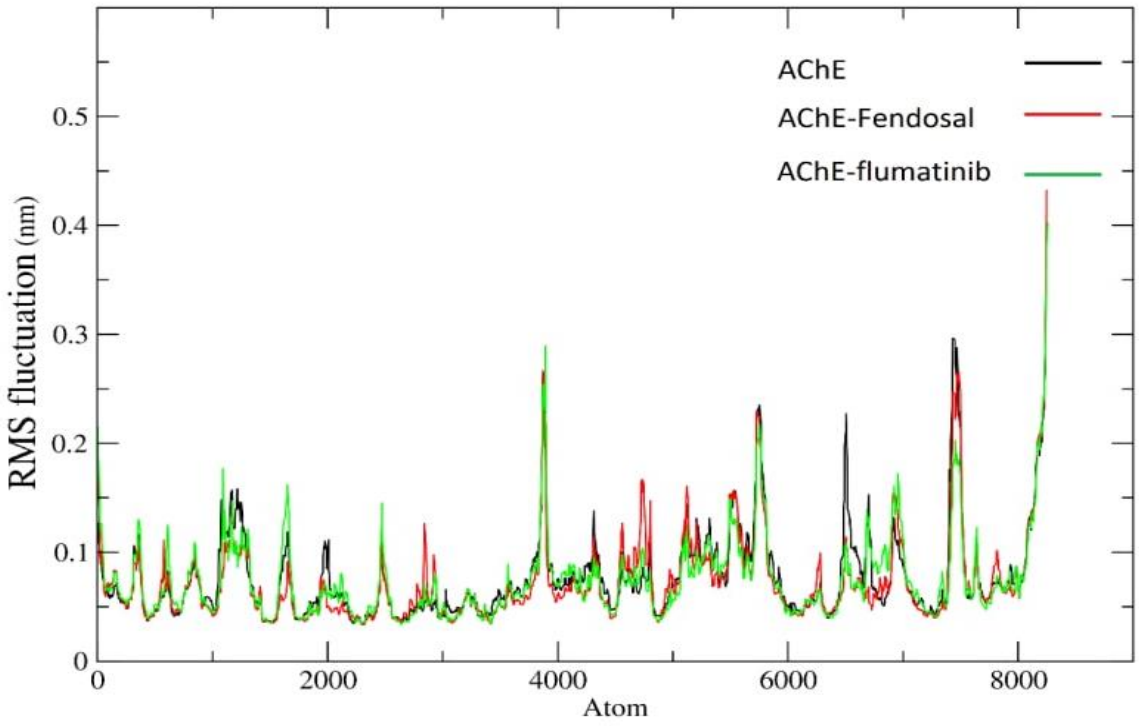


Figure 3. RMSF evaluations of AChE backbone atoms.
Şekil 3. AChE omurga atomlarının RMSF değerlendirmeleri

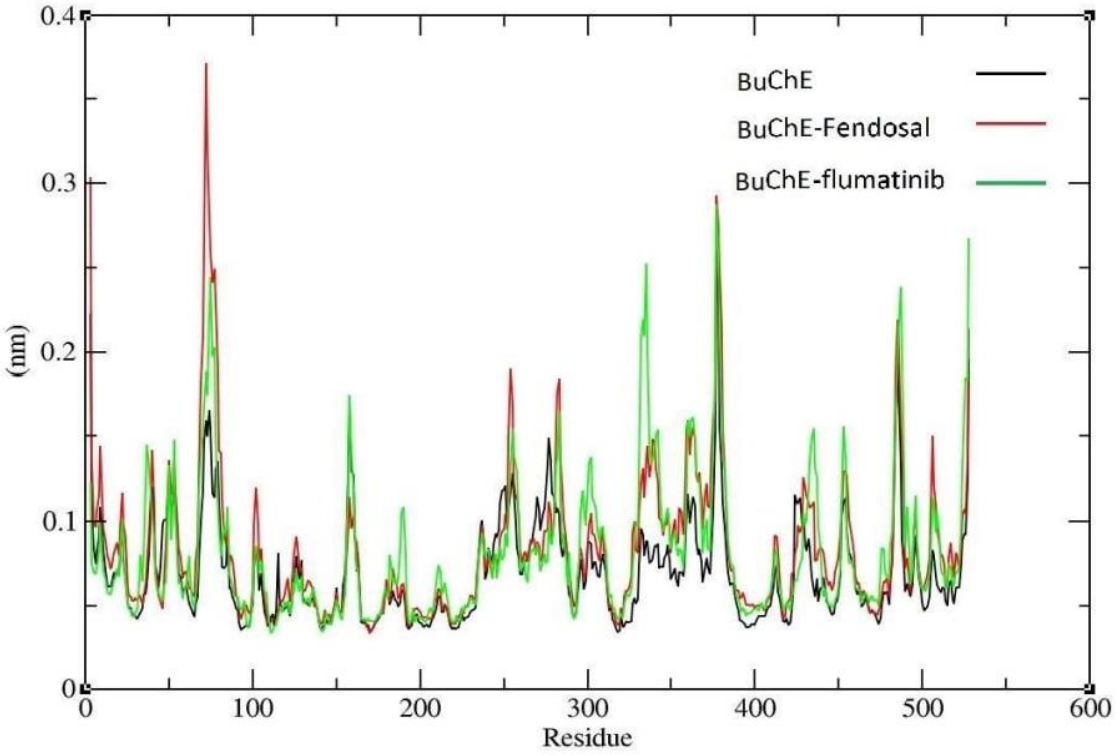


Figure 4. RMSF evaluations of BuChE backbone atoms.
Şekil 4. BuChE omurga atomlarının RMSF değerlendirmeleri

DISCUSSION

Alzheimer's illness is a retrogressive brain complication of unknown etiology, which is the most popular kind of dementia. Alzheimer's generally develops in middle age or old age, rises in gradual memory damage, defective thoughts, disorder, and personality and mood shifts (Wittenauer and Smith, 2013; Birks and Evans, 2015). The magnitude of computational tools has been investigated to identify effective treatment in a short time and the most promising targets are AChE (Al-Khafaji et al., 2021b) and BuChE as promising targets to stop or reduce the progression of Alzheimer's. We took the advantage of the possibility of Food and Drug Administration (FDA) available medicines that could be warheads to inhibit the AChE and BuChE proteins. Inhibition of cholinesterase in approximately 50 % of Alzheimer's patients stabilizes the cognitive function at a steady level during a 1-year period of treatment (Giacobini, 2003). The growing evidence revealed that both AChE and BuChE are significant proteins in the development and progression of AD (Greig et al., 2002).

Results of the present study indicated that Aspirin and imatinib have biological activities against Alzheimer's. The most interesting finding was that the fendosal compound has the binding affinities toward AChE and BuChE better than native ligands of both AChE and BuChE. Where the docking score of fendosal against BuChE (-7.836 kcal/mol) was smaller than the docking score of AChE's ref ligand (-5.490 kcal/mol). Further, fendosal had -8.240 kcal/mol which is lower than the ref ligand of AChE. This shows that only fendosal can act as a dual inhibitor from the relative drugs.

Contrary to this result, in the imatinib family, Nilotinib showed the best binding tendency with a binding energy value of -9.570 kcal/mol against BuChE, while Flumatinib compound took its place as the compound with the best interaction tendency against the AChE target with a binding energy of -9.541 kcal/mol. Based on docking scores, we nominated flumatinib and fendosal for further investigation by running a molecular dynamics simulation.

One interesting finding is the RMSD of protein of apo AChE's backbone has a similar fashion of fluctuation of AChE-fendosal and AChE-flumatinib. This reflects that both fendosal and flumatinib have a relaxed mode to bind efficiently with AChE. Whereas the binding of fendosal and flumatinib with BuChE increased the fluctuations in RMSD averages. Another remarkable notice was that the RMSF of the protein's backbone is more flexible when compared to an apo form of BuChE. It is possible to use fendosal and flumatinib in two ways: either alone or in a combined way. These findings suggest the potential using of nominated medicines against Alzheimer's in a short time. As well as the declared results are

noteworthy in at least showing the clinicians to adopt these safe remedies to suspend the evolution of Alzheimer's and secondly suggesting available drugs as a powerful therapy.

CONCLUSION

Based on molecular docking and molecular dynamics studies, the most prominent findings in the estimation of the interactions and activities of compounds selected from the aspirin and imatinib family against AChE and BuChE enzymes are that Fendosal compound exhibits strong inhibitory properties for both targets, while Flumatinib compound is more active against AChE enzyme than BuChE target. The other important result of this study is that Fendosal and Flumatinib can form stable interactions within the binding sites of AChE and BuChE with the help of MD simulation analysis. Comprehensively, both respective drugs show computationally good affinity for inhibition of AChE and BuChE enzymes. The results may suggest Fendosal and Flumatinib for extra clinical studies to evaluate inhibitory activities against AChE and BuChE enzymes.

ACKNOWLEDGEMENTS

Authors thank the Scientific Research Projects Executive Council of Gaziantep University. We would like to thank Dr. Khattab Al-Khafaji for his support and assistance.

Financial support

There was no financial disclosure.

Statement of Conflict of Interest

The authors declare that there are no conflicts of interest.

Author's Contributions

The contribution of the authors is equal.

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