ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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DISCOVERY OF DONEPEZIL-LIKE COMPOUNDS AS POTENTIAL ACETYLCHOLINESTERASE INHIBITORS DETERMINED BY PHARMACOPHORE MAPPING-BASED VIRTUAL SCREENING AND MOLECULAR DOCKING

FARMAKOFOR HARİTALAMA-ESASLI SANAL TARAMA VE MOLEKÜLER YERLEŞTİRME İLE BELİRLENEN POTANSİYEL ASETİLKOLİNESTERAZ İNHİBİTÖRLERİ OLARAK DONEPEZİL-BENZERİ BİLEŞİKLERİN KEŞFİ

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Öz

Amaç

Alzheimer hastalığı yaşlı insanlarda kolinerjik sistemdeki anormalliklerden dolayı bunamanın en yaygın nedenidir. Asetilkolinesteraz kolinerjik sistemin düzenlenmesinde önemli bir role sahiptir. Bu nedenle, AChE'yi hedeflemek AH tedavisi için en umut verici stratejilerden biridir. AH tedavisi için onaylanmış birkaç ilaç olmasına rağmen potansiyel inhibitör adaylarının keşfedilmesine halen ihtiyaç vardır. Bu nedenle, bu çalışmanın amacı asetilkolinesteraz enzimini (AChE) hedef alan yeni donepezil benzeri doğal bileşiklerin ve bunların sentetik türevlerinin keşfedilmesidir.

Gereç ve Yöntem

Bilinen bir ilaç olan donepezilin farmakofor modeli oluşturulmuştur. Discovery Studio 2021 programının farmakofor haritalama modülü kullanılarak doğal ürün ve sentetik türevlerini içeren kimyasal kütüphanesi taranmıştır. Taranan bileşiklerin farmakokinetik ve ilaca benzer özellikleri ADMET ve Lipinski ve Veber kuralı ile tahmin edilmiştir. Filtre olarak bazı kriterler kullanılmıştır. Ayrıca veri tabanının biyoaktif bileşikleri taranmıştır. Daha sonra, potansiyel molekülleri belirlemek için Maestro Glide/SP (Schrödinger, Inc.) kullanılarak moleküler yerleştirme çalışması yapılmıştır.

Bulgular

Moleküler modelleme çalışmalarının ardından öncü bileşikler için bağlanma enerjileri belirlendi. Ayrıca, protein-ligand kompleksi arasındaki H-bağ, pi-pi istifleme, pi-katyon ve pi-alkil etkileşimleri, Tyr, Asp, His, Trp, Arg gibi çeşitli amino asit kalıntıları ile tanımlanmıştır. Sonuçlar, potansiyel bileşiklerin donepezil ile karşılaştırıldığında bağlanma enerjisi ile umut verici bir aday olduğunu göstermektedir. Moleküler modelleme sonuçları, yeni yapı iskelelerinin standart ilaca kıyasla yeni AChE inhibitörlerinin keşfedilmesine katkıda bulunabileceğini belirtmektedir.

Sonuç

Bu çalışma daha ileri çalışmalara yol açabilir ve in vitro analizlerle incelenmesine katkı sağlayabilir. Yapı iskeleleri, yeni ve etkili inhibitörlerin tasarlanması için kullanılabilir.

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Abstract

Objective
Alzheimer's disease (AD) is the most common cause of dementia in older people due to abnormalities in the cholinergic system. Acetylcholinesterase has an important role in the regulation of the cholinergic system. Therefore, targeting AChE is one of the most promising strategies for the treatment of AD. Although several approved drugs to treat AD, it is still needed to develop potential inhibitor candidates. Therefore, the aim of this study is to discover newly donepezil-like natural compounds and their synthetic derivatives targeting acetylcholinesterase enzyme (AChE).

Material and Method
A pharmacophore model of a known drug, donepezil was generated. Using the pharmacophore mapping module of the Discovery Studio 2021 program, the chemical library containing natural products and synthetic derivatives was screened. The pharmacokinetics and drug-likeness properties of the screened compounds were predicted by ADMET and Lipinski and Veber's rule. Some criteria were used as a filter. In addition, bioactive compounds of the database were screened. Then, molecular docking study was performed by using Glide/SP of Maestro (Schrödinger, Inc.) to determine the potential molecules.

Results
The binding energies were determined for hit compounds after molecular modeling studies. Furthermore, H-bonding, pi-pi stacking, pi-cation, and pi-alkyl interactions between the protein-ligand complex have been identified by various amino acid residues such as Tyr, Asp, His, Trp, Arg. The results show that the potential compounds are a promising candidate with binding energy compared to donepezil. The molecular modeling results indicate that new scaffolds may contribute to the discovery of new AChE inhibitors compared to a reference drug.

Conclusion
This study may lead to further studies and contribute to examination with in vitro analysis. The scaffolds can be used to design novel and effective inhibitors.

Keywords: Alzheimer's Disease, Pharmacophore, Acetylcholinesterase, Molecular Docking, Donepezil

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, and it is known as a widespread form of dementia (1-4). AD is observed on more than 15 million people worldwide and it is estimated that this number may increase concerning life span (5-7). The cholinergic hypothesis that proposes the low levels of acetylcholine (ACh) in the brain are related to the functions of patients, are widely accepted by the researchers (5, 8). The efficacy of AD treatment may be increased by the inhibition of acetylcholinesterase (AChE) (5). Up to date, there have been many approved drugs as cholinesterase inhibitors (ChEIs) such as donepezil, galantamine, rivastigmine, and tacrine to be used in the treatment of AD (5, 9). These inhibitors improve ACh level in the brain by decreasing the hydrolysis of ACh (1). Although tacrine was the first synthetic drug due to its hepatotoxic effect, its usage was restricted (10). However, the discovery of novel ChEIs inhibitors is still needed because low bioavailability, high toxicity and narrow therapeutic effects are indicated for known drugs (5).

Several bioactive components from plants are known with their anticholinesterase activities to be used in the treatment of traditional medicine. For instance, galantamine is an alkaloid compound and has a selectivity against AChE. Besides, many studies have indicated that coumarin compounds are identified as AChE inhibitors (11). This circumstance may lead to discovery of new and effectively natural, synthetic and/or hybrid compounds as anti-Alzheimer therapeutics.

In the last decades, the discovery of potential targeted drugs with computer-aided drug discovery methods has gained importance. Thus, it is possible to explain the activity of biomolecules to identify the interactions with drug targets and ligands, and to develop more efficient drug candidates by using computational approaches such as pharmacophore modeling, molecular docking, and virtual screening studies (12). Pharmacophore-based techniques are an important tool of computer-aided drug design. This technique is widely used for rational design of novel drug candidates (13). Virtual screening is an advantageous method and used to determine the new and potential candidates against related targets (12). The pharmacophore features of known and effective drug supply an advantage because of chemical and functional properties. According to usage of pharmacophore features of specific drugs, the

best compounds can obtain with a combined study including virtual screening and molecular docking (14, 15). Donepezil was approved because of its high selectivity against AChE. Until today, the studies have focused on the discovery of donepezil like compounds as cholinesterase inhibitors (16-23).

The present study is based on the pharmacophore-based study of a reference drug donepezil. The overview of workflow is given in Figure 1. Accordingly, a virtual screening study was performed to find new compounds against target enzyme. The obtained model was used to screen over 68.000 natural compound (NC) collection from InterBioScreen (IBS) database including natural compounds and their derivatives to determine potential compounds. In addition, IBS bioactive compounds (BAC) were analyzed to find potentials. Then, molecular docking studies were performed following in silico pharmacokinetic properties with mapped compounds. Therefore, the findings reveal that selected ligands show potential binding abilities against AChE and good pharmacokinetic, physicochemical, and drug-likeness properties. Throughout these computer-assisted studies, H-bond, pi-pi stacking, and pi-pi cation interactions with main amino acid residues such as Phe295, Tyr341, Phe338, and Arg296 were observed between the target enzyme and natural-synthetic hybrid compounds in the binding pocket of AChE. In conclusion, these results indicate that new structures may be used as potential candidates to develop novel therapeutics and can contribute to in vitro studies.

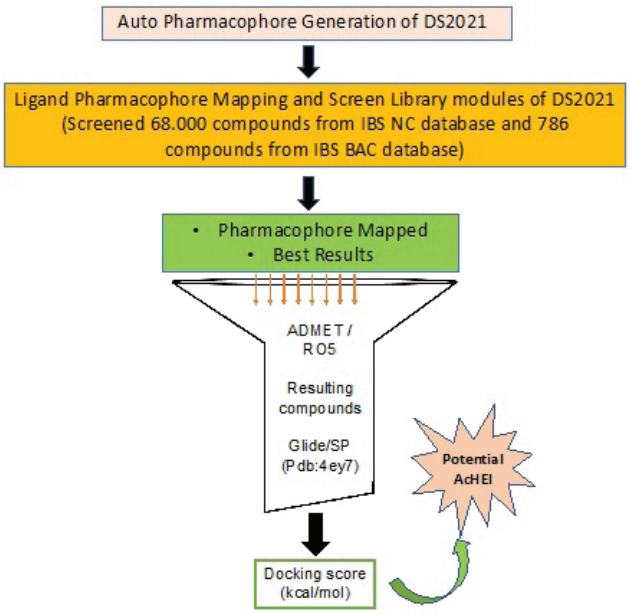


Figure 1:
A schematic overview of workflow

Material and Method

In Silico Pharmacophore Modeling

In this study, clinically known drug donepezil was used to build a model. It was determined the chemical features of the drug by using the Auto Pharmacophore Generation module of Discovery Studio 2021 (DS 2021) (24). Maximum features and minimum feature distance were determined as 6 and 2.5 in this module, respectively. Among the obtained 10 pharmacophores, the best pharmacophore model was determined that was selected with the Genetic Function Approximation model prediction. In addition, many chemical features including hydrogen bond donor and acceptor (HBD and HBA), aromatic ring, and ionizable feature were determined to find potential compounds (25, 26).

Virtual Screening from Chemical Database and Prediction of Pharmacokinetic Properties

Approximately 68.000 compounds were downloaded from InterBioScreen (IBS) natural compound (NC) collection database (https://www.ibscreen.com/). The compounds were screened by using the best selective pharmacophore model of donepezil with the Ligand Pharmacophore Mapping module of DS2021. The resulting compounds were filtered in accordance with ADMET and Lipinski and Veber rules by using ADMET descriptors, Filter by Lipinski and Veber Rule modules, respectively, to determine drug-likeness properties (25, 26). Then, these compounds were prepared for molecular docking study. In addition, approximately 786 IBS BAC library were screened by using a pharmacophore model through the Screen Library module of DS. Eight compounds were analyzed following ADMET and RO5 analysis. Then resulting compounds were docked against AChE.

Ligand Preparation

To prepare filtered ligands, LipPrep module of Maestro was used (Schrödinger Release 2022-1 Schrödinger, LLC, New York, NY, 2022) (27). According to Epik, ionization state was calculated at neutral pH (28). The structural optimization of compounds was provided with the OPLS-2005 force field (29).

Protein Preparation

The crystal structure of recombinant human acetylcholinesterase complexed with donepezil was downloaded from Protein Data Bank (PDB) (4EY7). Protein preparation module was used to prepare target protein (30). Missing loops and missing side chains were filled by using the Prime module. H-bond assignment was provided by PROPKA at neutral pH

(27). The target protein was optimized by using the same force field.

Molecular Docking

Molecular docking study was performed by using the Glide module of Maestro with SP (Standard Precision) protocol (26, 31).

Results

Pharmacophore Model Generation

Pharmacophore model of donepezil was generated to determine its key features such as acceptor, hydrophobic, ionizable etc. These features were determined by obtaining 10 pharmacophore models and the first model was used for further studies. Hydrophobic, ring aromatic, hydrogen bond acceptor (HBA), and ionizable features of donepezil are indicated as colored in Figure 2.

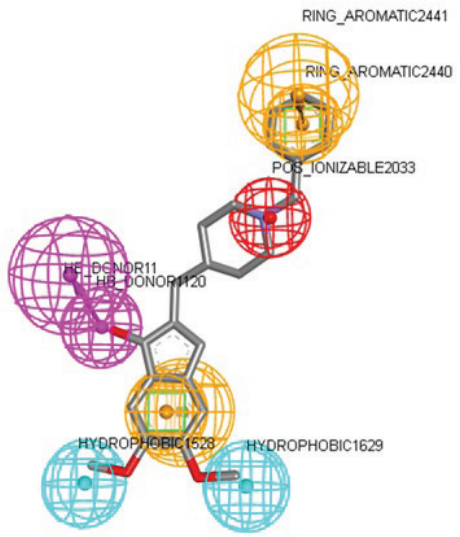


Figure 2:
Pharmacophore features of donepezil.

Virtual Screening, ADMET Prediction, Drug-Likeness, and Pharmacokinetic Properties of Potential Compounds

It is known that initial prediction of *in silico* ADMET and drug-likeness properties of studied ligands is a useful tool for the design and discovery of new candidates (32). Therefore, the ligand pharmacophore mapping module was used to identify the potential candidates in this study. For this purpose, a NC library of IBS that contain natural compounds and their derivatives, was screened using a 3D pharmacophore model. *In silico* pharmacokinetic properties were determined by ADMET (Adsorption, Distribution, Metabolism, Excretion, Toxicity) descriptors and Lipinski and Veber's rule, respectively. Hereby, totally, 216 compounds mapped and then filtered to 31 compounds to determine less than value of 500 for molecular weight. *In silico* ADMET descriptors, drug-likeness and pharmacokinetic properties including ADMET solubility, blood brain barrier (BBB) penetration, CYP2D6 binding, hepatotoxicity, intestinal absorption, plasma protein binding (PPB), number of H acceptors and donors, and molecular weights of the selected compounds were given in Table 1. These resulting compounds were evaluated for molecular docking study to understand the interaction diagram between the target enzyme and ligands.

Herein, as given in Table 1, all ligands displayed good ADMET solubility, BBB level, CYP2D6, PPB, and hepatotoxicity (33). The level of ADMET solubility indicates that thirteen compounds are soluble in water at room temperature. All ligands have a good human intestinal absorption level. Two ligands, STOCK1N-94304 and STOCK1N-90335 have acceptable values to bind with plasma protein. The enzyme, CYP2D6 takes a part in drug metabolism and no ligands was predicted to be an inhibitor of CYP2D6. All ligands were found to be non-toxic in terms of hepatotoxic properties. BBB level indicates that the results of six ligands range from very high penetration to moderate. H bond acceptors and donors must be below or equal 10 and 5, respectively. Thus, each ligand is proper for Lipinski's rule. According to values given in Table 1, the results showed that filtered compounds have an acceptable range with their *in silico* predictions for their pharmacokinetic and physicochemical properties, and drug-likeness. Consequently, all ligands were determined in accordance with for BBB permeation, absorption, Lipinski and Veber's rule, and molecular weight (<500 g/mol). Thus, these compounds may be potential candidates or can be improved according to in vitro/in vivo efficacy for the treatment of AD.

Molecular Docking

To understand the binding pattern of the potential compounds, molecular docking study was performed by Glide/SP with flexible ligand sampling (Schrödinger, Inc.). The ligands and reference drug were prepared by the LigPrep module of Maestro and were docked against the AChE. According to these results, binding scores of co-ligand donepezil and first candidate (STOCK1N-94304) were calculated as -13.603 kcal/mol and -11.907 kcal/mol, respectively. The docking scores for the selected ligands were given in the range of binding energies as kcal/mol in Table 2. Furthermore, these ligands exhibited

Table 1 *In silico* predicted pharmacokinetic and drug-likeness properties of potential donepezil-like compounds

No	Database Code	Molecular weight	[a]ADMET solubility level	[b]BBB levels	[c]Absorption level	[d]CYP2D6	[e]Hepato-toxicity	[f]PPB level	Num H Acceptors	Num H Donors
1	STOCK1N-94304	490.548	2	2	0	0	0	0.6	7	1
2	STOCK1N-69824	427.497	3	3	0	0	0	0	6	3
3	STOCK1N-90335	428.521	3	2	0	0	0	0.6	6	1
4	STOCK1N-79076	462.538	2	2	0	0	0	0	7	1
5	STOCK1N-90395	414.495	3	2	0	0	0	0.3	6	1
6	STOCK1N-94680	493.505	3	3	0	0	0	0	9	1
7	STOCK1N-69982	427.497	3	3	0	0	0	0	6	3
8	STOCK1N-69983	487.615	3	3	0	0	0	0	7	3
9	STOCK1N-70138	487.615	3	3	0	0	0	0	7	3
10	STOCK1N-94909	483.514	3	4	0	0	0	0	9	1
11	STOCK1N-96773	493.552	3	4	0	0	0	0	8	3
12	STOCK1N-94100	485.477	2	2	0	0	0	0	7	1
13	STOCK1N-69649	427.497	3	3	0	0	0	0	6	3
14	STOCK1N-05902	403.520	2	1	0	0	0	0	4	1
15	STOCK1N-93564	423.462	3	3	0	0	0	0	7	1

[a]Aqueous-solubility level: 0 (extremely low); 1 (very low, but possible); 2 (low); 3 (good). bBlood Brain Barrier level: 0 (very high penetrant); 1 (high); 2 (medium); 3 (low); 4 (undefined). [e]Human-intestinal absorption level: 0 (good); 1 (moderate); 2 (poor); 3 (very poor). [d]Cytochrome P450 2D6 level: 0 (non-inhibitor); 1 (inhibitor). eHepatotoxicity: 0 (nontoxic); 1 (toxic). [f]Plasma Protein Binding: 0 (absorbent weak); 1 (absorbent strong) (27).

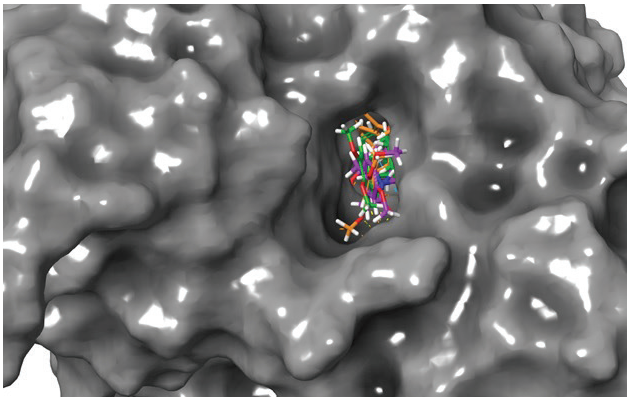


Figure 3:
Binding cavity of AChE. STOCK1N-94304, Bio-0939, and donepezil were shown in orange, magenta, and green colors, respectively.

expected protein-ligand interactions such as H-bond, pi-pi stacking, pi-cation, pi-alkyl, and salt-bridge with Phe295, Tyr341, Phe338, Trp86, Tyr337, Trp286, Arg296 amino acid residues of AChE active sites. The binding energy scores were found as -10.825 kcal/mol and -8.325 kcal/mol for galantamine and rivastigmine, respectively.

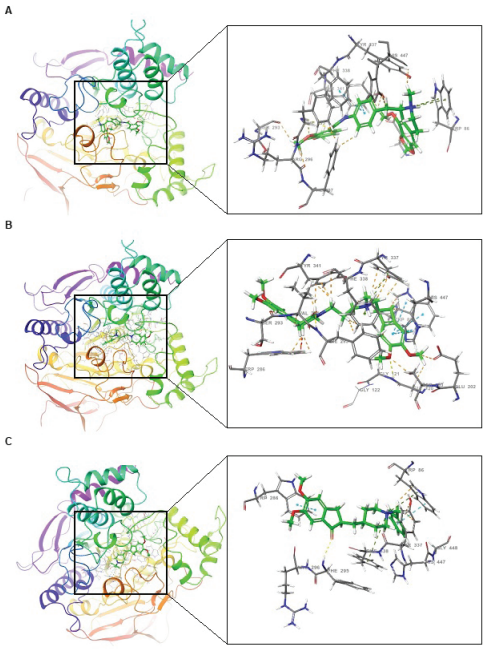


Figure 4:
Binding mode prediction of STOCK1N-94304 (A), Bio-0939 (B), and donepezil (C). Compounds were given in stick mode colored in green and key residues were labeled.

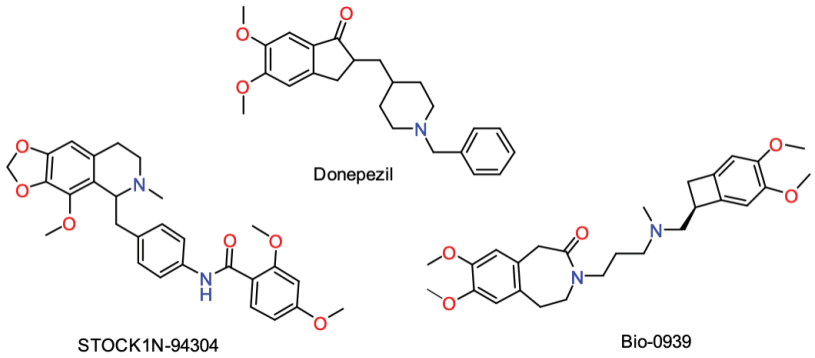
Table 2 Glide/SP docking scores and protein ligand interactions of potential donepezil-like ligands

No	Database code	Protein-ligand interactions	Docking score (kcal/mol)
	Donepezil	Trp286, Phe295, Trp86, Phe338, Tyr337, H-bond, pi-pi stacking, pi-cation	-13.603
1	STOCK1N-94304	Phe295, Tyr341, Tyr337, Phe338, Trp86, Asp86, Arg296, His447, Ser293, H-bond, pi-pi stacking, pi-cation,	-11.907
2	STOCK1N-69824	Phe295, Trp286, Trp86, Glu202, Tyr133, Tyr124, Ser293, Tyr337, His447, H-bond, pi-pi stacking, pi-cation	-11.798
3	STOCK1N-90335	Phe338, Tyr337, Trp86, Tyr341, Trp286, Gly120, Gly126, pi-cation, pi-pi stacking	-11.634
4	STOCK1N-79076	Tyr341, Tyr337, Tyr124, Trp286, Trp86, pi-pi stacking, pi-cation,	-11.564
5	STOCK1N-90395	Arg296, Phe295, Phe297, Leu130, Phe338, Tyr337, Gly126, Glu202, Trp86, H-bond, pi-pi stacking, pi-cation	-11.162
6	STOCK1N-94680	Arg296, Phe295, Tyr341, Phe338, Tyr337, Trp286, Tyr124, His447, Gly121, H-bond, pi-pi stacking, pi-cation	-11.161
7	STOCK1N-69982	Phe295, Phe338, Trp286, Trp86, Tyr341, Tyr133, Glu202, Ser293, Gly121, H-bond, pi-pi stacking, pi-cation, salt bridge	-11.118
8	STOCK1N-69983	Phe295, Phe297, Phe338, Trp286, Trp86, Tyr72, Tyr124, Glu202, Ser203, Ser293, Gly448, H-bond, pi-pi stacking, pi-cation	-11.056
9	STOCK1N-70138	Trp86, Trp286, Arg296, Phe295, Phe297, Tyr72, Tyr124, Tyr337, Glu202, His447, H-bond, pi-pi stacking, pi-cation	-11.039
10	STOCK1N-94909	Phe297, Arg296, Phe295, Phe338, Tyr124, Tyr341, Trp286, Tyr72, Tyr337, Gly122, His447 H-bond, pi-pi stacking, pi-cation	-11.022
11	STOCK1N-96773	Phe295, Phe338, Tyr124, Tyr337, Tyr341, Trp286, Trp86, Glu202, His447 H-bond, pi-pi stacking, pi-cation	-10.818
12	STOCK1N-94100	Phe295, Tyr341, Trp86, Trp286, Phe338, Tyr124, Tyr72, Tyr337, Asp74, His447 H-bond, pi-pi stacking, pi-cation	-10.731
13	STOCK1N-69649	Phe295, Phe338, Trp286, Trp86, Glu202, His447, Ser293, Val294, H-bond, pi-pi stacking, pi-cation, salt bridge	-10.565
14	STOCK1N-05902	Trp286, Tyr337, Tyr341, Tyr124, Trp86, Tyr72, Phe297, Ser293, Gly448, H-bond, pi-pi stacking, pi-cation	-10.516
15	STOCK1N-93564	Trp86, Trp286, Tyr72, Tyr124, Tyr341, Phe338, His447, Gly448, Glu202, pi-pi stacking, pi-cation	-10.263

Additionally, a screening study was performed throughout the IBS BAC library including 789 compounds by a Screen Library module of DS2021. The results showed that one of known drugs, brand name Corlanor (database code Bio-0939) that was approved by Food and Drug Administration (FDA) to be used in the treatment of chronic heart failure in patients, exhibited high binding score as -12.257 kcal/mol with its H-bond, pi-pi stacking, pi-cation interactions including Phe295, Phe338, Tyr337, Trp86, Gly121, His447 amino acid residues (Table 3). Furthermore, the top docking scores of selected bioactive compounds were given in Table 3. It is seen that these compounds were located in the active site

Table 3 Glide/SP docking scores and protein ligand interactions of potential donepezil-like bioactive compounds

No	Database Code	Protein-ligand interactions	Docking score (kcal/mol)
1	Bio-0939 Ivabradine hydrochloride (Corlanor)	Phe295, Phe338, Tyr337, Tyr341, Trp86, Trp286, Gly120, Ser203, Glu202, Ser293, His447, H-bond, pi-pi stacking, pi-cation	-12.257
2	Bio-0051 (Imatinib mesylate)	Phe295, Trp286, Tyr341, Trp86, Glu202, H-bond, pi-pi stacking, pi-cation	-10.836
3	Bio-0717 (Sulpiride)	Trp286, Tyr341, Tyr337, Phe338, Trp86, Tyr72, Tyr124, Asp74 H-bond, pi-pi stacking, pi-cation	-10.354
4	Bio-0889 (Doxazosin Mesylate)	Trp286, Tyr341, Tyr337, Tyr124, Gly448, Phe297, Phe338 H-bond, pi-pi stacking, pi-cation	-10.119



Scheme 1:
Structures of the best candidates of donepezil-like compounds.

and interacted with important residues of AChE (34-36). As a result, it is thought that this known drug Corlanor may evaluate as potential AChE inhibitor candidate for drug reporpositioning studies as a new donepezil-like compound with its 4,5-dihydro-1H-benzo[d]azepin-2(3H)-one scaffold in place of 1-indanone scaffold of donepezil. Superpositioned docking poses of STOCK1N-94304, Bio-0939 and donepezil are given in the active site cavity of AChE in Figure 3. Furthermore, the binding mode predictions can be seen in Figure 4. The structures of a few potential candidates are given in Scheme 1.

According to protein-ligand interactions of donepezil, carbonyl group and phenyl ring of 1-indanone show H-bond and pi-pi stacking interaction with Phe295 and Trp286, respectively. *N*-Benzyl moiety and piperidine ring of donepezil interact with Trp86 and Phe338 through pi-pi stacking and pi-cation. The compound STOCK1N-94304 displays similar interactions with amino acid residues like donepezil. Thus, the carbonyl group of 5,6,7,8-tetrahydro-[1,3] dioxolo[4,5-g]isoquinoline interacts with Phe295 via H-bonding. Phenyl and piperidine ring exhibit pi-cation interaction with Trp86 amino acid residue. The substituted phenyl ring of ligand shows pi-cation interaction with Phe338 and Tyr341. Corlanor shows similar interactions like H-bonding, pi-pi stacking and pi-cation between carbonyl group of 4,5-dihydro-1H-benzo[d]azepin-2(3H) scaffold, substituted amine group, phenyl ring and related residues such as Gly121, Phe295, Tyr337, Phe338, His447, and Trp86.

Discussion

Although there have been many studies including AChE inhibitors for AD treatment, the researchers have been still ongoing to studies to find new, potent, and effective candidates through computational approaches in recent years (37, 38). Among them, in one of the reported studies, *in silico* molecular properties and ADME predictions with various parameters such as number of hydrogen bond acceptors and donors (HBA and HBD), Lipinski rules, and plasma protein binding was performed to the synthesized compounds as AChEI compared to donepezil (34). According to a similar study, a comparison was performed between natural compound geraniol, and it showed the same ADME result like tacrine (39). In a pharmacophore and molecular docking-based study, similar pharmacophore features were reported as given in Figure 1. The pharmacokinetic properties of hit compounds were analyzed by ADMET descriptors and Lipinski and Veber rules (26). In addition, ADMET and pharmacokinetic properties including solubility, BBB level, hepatotoxicity, CYP2D6, absorption level, and PPB were resulted with good *in silico* predictions for a targeted inhibitor by using DS 4.5 in a previously reported study (33).

According to virtual screening studies, the researchers have used various algorithms such as HypoGen to obtain 3D-QSAR pharmacophore model of donepezil by DS (40, 41). Then, pharmacophore- and structure-based virtual screening and molecular docking studies have been performed through IBS and other databases and different software packages against AChE (pdb: 1B41, 4EY7) (40-43). There have been no identified similar potentials compared to this study results.

Throughout molecular docking studies, binding energies of donepezil were given in the changing values such as -12.2 kcal/mol (34), -11.9 kcal/mol (37), -10.46 kcal/mol (44) calculated by AutoDock Vina and Glide/SP. However, in this study, the screened ligands displayed near docking scores to donepezil against AChE drug target. According to a reported study, binding energies were determined as -12.2 kcal/mol, -9.6 kcal/mol, and -10.4 kcal/mol for reference drug donepezil, reported two ligands, respectively. Moreover, the highest IC50 values against AChE were calculated with these reported ligands and donepezil. It was indicated that the active site of AChE contains subsites that have various amino acid residues such as Phe295, Trp86, Trp286, Tyr337 etc. (34, 39, 45). We indicated that many interactions including residues Tyr337, Trp86, Phe338, Tyr341, Asp74, Gly120 in the binding pocket of AChE for coumarin-based compounds were obtained in MD simulations (11). Furthermore, significant interactions such as pi-pi, pi-alkyl and pi-sigma with various residues such as Tyr, His, Trp, Phe, Arg, and Ser residues in protein-ligand complex were detected. It was seen that docking analysis displayed similar interactions such as donepezil (34). Besides, a ligand interacted with PAS aromatic amino acid residues including Trp286 and Trp341 to form H-bonding and pi-pi stacking, was reported with its lowest binding energy (-10.8 kcal/mol) than donepezil (-11.9 kcal/mol) (37).

In a previously reported study, molecular docking scores of donepezil and rivastigmine were given as -10.46 kcal/mol and -7.64 kcal/mol, respectively, throughout ZINC15 ligands the scores were found between -8.02 kcal/mol and -15.10 kcal/mol by using Glide/SP (44). Thus, the resulted 11 new inhibitors were interacted with various amino acid residues including Phe335, His444, Asp71, Trp83, Phe295, Asp74, Tyr341, Tyr337, Tyr334 Gly119, Gly118, and Ala201 in the binding pocket of AChE with H-bond, pi-pi stacking, pi-cation, and salt bridge interactions compared to rivastigmine (44).

In general, AChE inhibitors bind to the catalytic active site (CAS) that contains a catalytic triad of Ser200, Glu327, and His440 (39, 46). It is reported that Trp84 and Phe330 has an important role in the catalytic reaction. Furthermore, secondary noncholinergic function of AChE that is related with peripheral anionic site (PAS) including Tyr70, Asp72, Tyr121, Trp279, and Tyr334 amino acid residues. As is indicated in previously reported studies, both PAS and CAS can be targeted for discovering innovations for AD (39, 45, 46, 47). In addition, the binding modes of some ligands were obtained in CAS and PAS sites of AChE and one of the ligands interacted with key residues (26, 48).

According to given results in this study, it can be seen that 5,6,7,8-tetrahydro-[1,3]dioxolo[4,5-g]isoquinoline and 3,4-dihydroisoquinolin-2(1H) scaffolds and their functional groups such as dimethoxy, aliphatic -CH2 chain, amide, phenyl ring, and 4H-pyran-4-one may increase potency in vitro AChE activity. Consequently, tetrahydroquinolines and quinoline compounds are known with their important biological properties. Also, substituted tetrahydroquinolines were successfully evaluated *in silico* drug-likeness properties and molecular docking studies against AChE (49). Thus, the proposed structures may represent the design of new classes of AChE inhibitors.

In conclusion, this study is based on the discovery of new scaffolds and functional groups, and potential candidates for the treatment of AD. All compounds were tested for the prediction of *in silico* pharmacokinetic activity. In molecular docking study, binding energies between the target enzyme and ligands were determined and several compounds displayed the similar binding affinity compared to a reference drug. These compounds may be potential AChE inhibitor candidates and used in in vitro studies. In addition, as far as is known, novel structures were identified for the first time by using this generated pharmacophore features of donepezil in this study. Overall, these lead compounds may guide the design and development of novel AD-based therapeutics.

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The authors have no conflicts of interest to declare.

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