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Efficacy of Beta-Secretase-1 Enzyme Inhibitors in Alzheimer's Disease

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ABSTRACT

Background and Objectives: A number of BACE1 inhibitors have been tested in clinical trials but have been discontinued due to lack of efficacy or unacceptable side effects. In this study, we investigate the physicochemical properties of some of these ligands and their binding affinity for the molecular model of the BACE1 enzyme [PDB ID: 6EQM]. **Materials and Methods:** The molecular model of the human BACE-1 enzyme [PDB ID: 6EQM] and the ligands studied were obtained from www.rcsb.org and the PubChem database. The physicochemical properties, the possibility of gastrointestinal absorption and blood-brain barrier crossing of the studied compounds were taken from the Swiss Adam database. After preparing the enzyme model and its ligands by molegro visual docker software (v5), we performed the molecular docking process for all studied ligands. **Results:** According to the results of Swiss Adam software, of the compounds studied, only AZD-3293 can cross the blood-brain barrier. This compound also has a high affinity for the active site of the enzyme. PF -06751979 had the highest affinity based on the docking score for the active site of the enzyme, but it also has low gastrointestinal absorption and cannot cross the blood-brain barrier. **Discussion:** Many clinical trials in which beta-secretase-1 inhibitors were administered resulted in successful inhibition of beta-secretase activity and a reduction in beta-amyloid production and beta-amyloid concentration in serum and cerebrospinal fluid. However, most of these trials were discontinued in the long term due to the ineffectiveness of this treatment method and did not improve disease symptoms in Alzheimer's patients. **Conclusion:** A BACE1 inhibitor that cannot cross the blood-brain barrier cannot affect neurons. For agents that can cross the blood-brain barrier, such as AZD-3293, the results of previous studies have shown no improvement in memory loss and other cognitive disorders associated with Alzheimer's disease, and the agent has very severe side effects.

Keywords: *Beta-secretase-1, Molecular docking, Alzheimer's.*

INTRODUCTION

Loss of memory, mood swings, demotivation, and behavioral disturbances are common in Alzheimer's disease patients. Alzheimer's disease (AD) is an irreversible, progressive neurological disorder characterized by memory loss, impaired cognition and thinking, and personality and behavioral changes. It seriously threatens the physical and mental health of older people. The biggest risk factor for this disease,

whose incidence doubles every five years after age 65, is getting older. Worldwide, approximately 40 million people over the age of 60 suffer from Alzheimer's disease, and the number of patients is increasing, doubling every 20 years [1, 2].

The β-secretase1 (BACE1) cleaves the amino terminus of the amyloid precursor protein (AβPP) between amino acids 671 and 672, whereupon the Aβ-peptide is released by cleavage through the γ-secretase. The therapeutic method based on inhibition of BACE1 activity prevents the initial enzymatic cleavage of AβPP, resulting in inhibition of AβPP processing and promotion of beta-amyloid production. Several natural and synthetic BACE1 inhibitors have been used as drugs in clinical trials. There is a need for a compound that has good absorption in the digestive tract, good solubility in blood and body fluids, strong binding to enzymes, and the ability to cross the cell membrane and blood-brain barrier so that it is well tolerated for long-term use [3].

A number of BACE1 inhibitors have been tested in clinical trials but have been discontinued due to lack of efficacy or unacceptable side effects. These compounds include Atabecestat (JNJ-54861911), LY2886721, LY3202626, Lanabecestat, LY3314814, and PF 06751929. In this study, we investigate the physicochemical properties of some of these ligands and their binding affinity for the molecular model of BACE1 enzyme [PDB ID: 6EQM] based on molecular docking results [4].

MATERIAL AND METHODS

Molecular model of beta-secretase enzyme and ligands studied

The molecular model of the human enzyme BACE-1 [PDB ID: 6EQM] with a resolution of 1.35 Å, crystallized in interaction with the inhibitor CNP520, was obtained from the database www.rcsb.org. This model contains water molecules, the inhibitor CNP520, and a 385 amino acid sequence of the human BACE-1 enzyme (5). We obtained the molecular model of the ligands studied from the PubChem database. These compounds are known inhibitors of the beta-secretase enzyme studied in Alzheimer's disease [6].

Structural and physicochemical properties of each ligand

Structural and physicochemical features of the individual ligands were obtained from the PubChem online database. For additional investigation of physicochemical properties, possibility of gastrointestinal absorption, and passage through the blood-brain barrier of the studied compounds, we used the software available in the Swiss Adam database [6-8].

Performing molecular docking

To prepare the molecular model of beta-secretase enzyme, we removed the CNP520 inhibitor compound and water molecules from the model [PDB ID: 6EQM]. After preparing the enzyme model and its ligands using molegro visual docker software (v5) and identifying the holes in the protein model with a minimum size of 1 Angstrom, we performed molecular docking of the studied ligands with the human BACE-1 protein model. The results of the compounds with the highest affinity to the enzyme active site in the [PDB ID: 6EQM] molecular model of the enzyme were extracted [9].

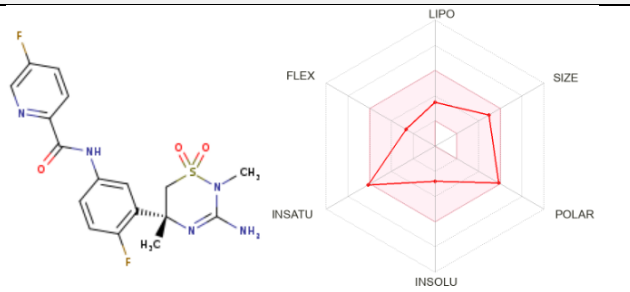
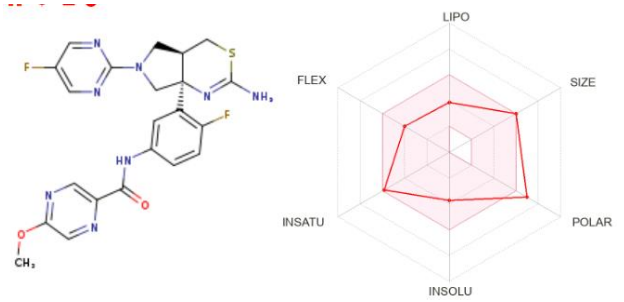
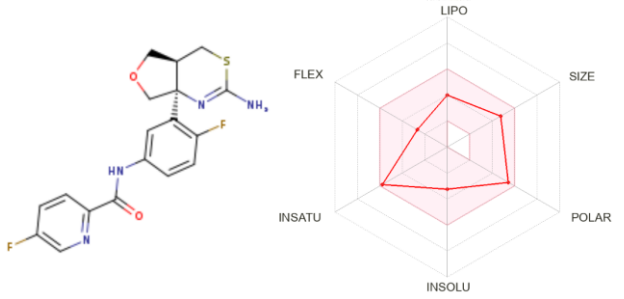
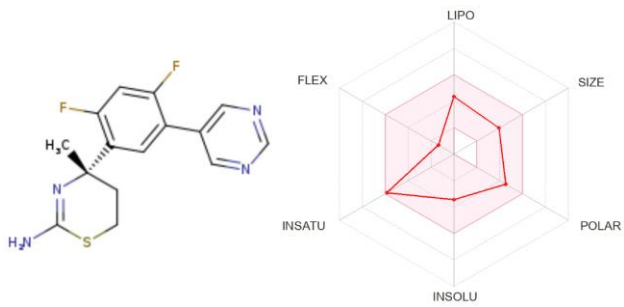
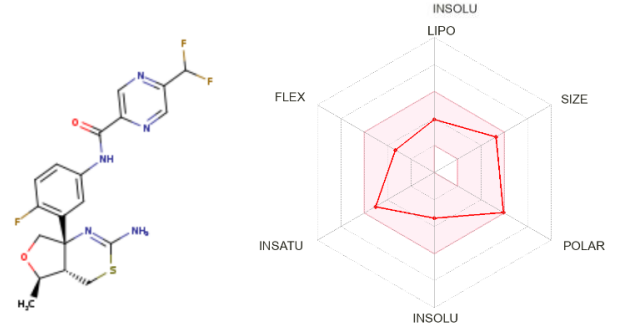
RESULTS

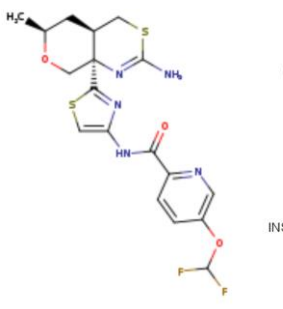
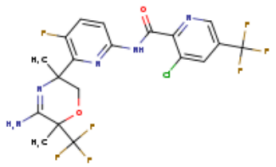
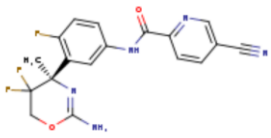
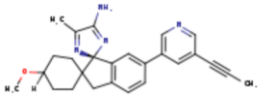
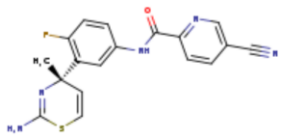
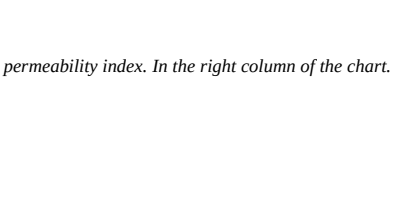
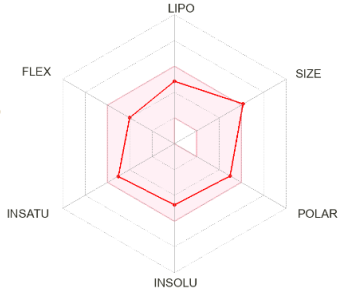
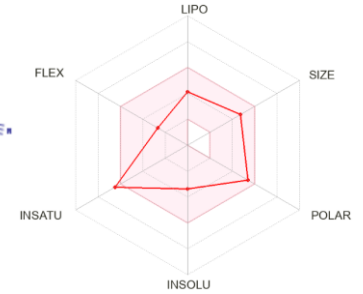
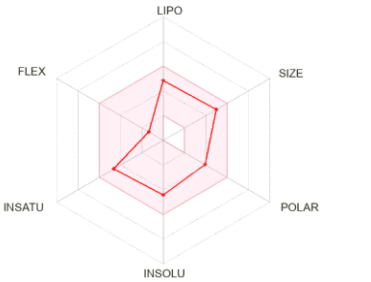
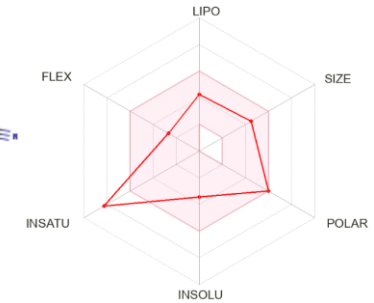
The compounds studied have a molecular weight between 320 and 513 Daltons. The heaviest compound is CNP-520 (Umibecestat) and the lightest compound is LY2811376. According to the results of the software available on the SwissADME website, the compounds LY3202626, Cnp-520, PF -06751979 have low absorption in the digestive tract and are heavier than other compounds. These compounds had molecular weights ranging from 450 to 513 Daltons. RO5508887, LY2886721, LY2811376, Verubecestat (MK -8931), Lanabecestat (AZD-3293), Elenbecestat (E2609), Atabecestat (JNJ-54861911) on the other hand have high gastrointestinal absorption. Among the studied compounds, CNP-520 (Umibecestat) has the highest molecular weight, which has low gastrointestinal absorption. According to the results of the software available on the Swiss Adam website, of the compounds studied, only AZD-3293 can cross the blood-brain barrier (**Table 1**).

Most of studied compounds are polar. The total polar surface area of these compounds is in a variable range between 72.9 and 165 (Å²). LY3202626, PF -06751979 have the highest polar surface area. Lanabecestat and LY2811376 have the lowest polar surface area. According to the docking results, LY3202626 forms the strongest hydrogen bond among the compounds with the active site of the enzyme in the molecular model of beta secretase-1 enzyme, showing the relationship between the overall polarities of the molecules and the strength of hydrogen bonds between the ligand and the protein (**Table 2**).

Based on the Log S index (ESOL), the solubility of the studied compounds in aqueous solutions is in a highly variable range (-2.06 to -9.32). The closer this value is to zero, the higher their solubility in water. The absolute insolubility is -10. AZD-3293 and CNP-520 have the highest hydrophobicity and the lowest solubility among these compounds in aqueous liquids.

Table 1. Physicochemical properties of some of well-known beta secretase 1 inhibitors.

CID (traditional name)	IUPAC Name	X.LOGP	Log S (ESOL)	GI absorption	BBB permeant	Chemical structure and Physicochemical properties scale
51352361 (Verubecestat)	N-[3-[(5R)-3-amino-2,5-dimethyl-1,1-dioxo-6H-1,2,4-thiadiazin-5-yl]-4-fluorophenyl]-5-fluoropyridine-2-carboxamide	0.6	-2.80	HIGH	NO	
78210254 (LY3202626)	N-[3-[(4aR,7aS)-2-amino-6-(5-fluoropyrimidin-2-yl)-4,4a,5,7-tetrahydropyrrolo[3,4-d] [1,3] thiazin-7a-yl]-4-fluorophenyl]-5-methoxypyrazine-2-carboxamide	1.3	-3.72	Low	NO	
49837968 (LY2886721)	N-[3-[(4aS,7aS)-2-amino-4,4a,5,7-tetrahydrofuro[3,4-d][1,3]thiazin-7a-yl]-4-fluorophenyl]-5-fluoropyridine-2-carboxamide	1.47	-3.25	High	No	
44251605 (LY2811376)	(4S)-4-(2,4-difluoro-5-pyrimidin-5-ylphenyl)-4-methyl-5,6-dihydro-1,3-thiazin-2-amine	2.1	-3.43	High	No	
57827330 (Elenbecestat)	N-[3-[(4aS,5R,7aS)-2-amino-5-methyl-4,4a,5,7-tetrahydrofuro[3,4-d] [1,3] thiazin-7a-yl]-4-fluorophenyl]-5-(difluoromethyl) pyrazine-2-carboxamide	1.4	-3.39	HIGH	NO	

118435360 (PF-06751979)	**57524525 (Cnp-520)**	**53241828 (RO5508887)**	**67979346 (Lanabecestat)**	**68254185 (Atabecestat)**
N-[2-[(4aR,6S,8aR)-2-amino-6-methyl-4a,5,6,8-tetrahydro-4H-pyrano[3,4-d][1,3]thiazin-8a-yl]-1,3-thiazol-4-yl]-5-(difluoromethoxy)pyridine-2-carboxamide	N-[6-[5-amino-3,6-dimethyl-6-(trifluoromethyl)-2H-1,4-oxazin-3-yl]-5-fluoropyridin-2-yl]-3-chloro-5-(trifluoromethyl) pyridine-2-carboxamide	N-[3-[(4R)-2-amino-5,5-difluoro-4-methyl-6H-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopyridine-2-carboxamide	N-[2-[(4aR,6S,8aR)-2-amino-6-methyl-4a,5,6,8-tetrahydro-4H-pyrano[3,4-d][1,3]thiazin-8a-yl]-1,3-thiazol-4-yl]-5-(difluoromethoxy)pyridine-2-carboxamide	N-[3-[(4S)-2-amino-4-methyl-1,3-thiazin-4-yl]-4-fluorophenyl]-5-cyanopyridine-2-carboxamide
2.4	2.9	1.70	3	2
-4.04	-4.74	-3.38	-4.43	-3.44
Low	Low	High	HIGH	High
No	NO	No	YES	No
				
				

X.LOGP: hydrophobicity index, Log S (ESOL): solubility index in aqueous solution, BBB permeant: blood-brain barrier permeability index. In the right column of the chart. The diagram shows the physicochemical parameters.

Table 2. Molecular docking results of beta-secretase enzyme inhibitors.

CID (name)	IUPAC	Name	MolDock Score	Torsion bonds	H-Bond (kcal/mol)	Heavy Atoms	MW	LE1	XLogP3-AA	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor	Total Polar Surface Area
118435360 (PF-06751979)	*N*-[2-[(4a*R*,6*S*,8a*R*)-2-amino-6-methyl-4a,5,6,8-tetrahydro-4*H*-pyrano[3,4-*d*][1,3]thiazin-8a-yl]-1,3-thiazol-4-yl]-5-(difluoromethoxy)pyridine-2-carboxamide	118435360	-130.1	4	-3.0	30	455.5	-4.3	2.4	2	10	165 Å^2
57524525 (Cnp-520)	*N*-[6-[5-amino-3,6-dimethyl-6-(trifluoromethyl)-2*H*-1,4-oxazin-3-yl]-5-fluoropyridin-2-yl]-3-chloro-5-(trifluoromethyl)pyridine-2-carboxamide	57524525	-122.4	5	-6.7	34	513.7	-3.6	2.9	2	12	103 Å^2
53241828 (RO5508887)	N-[3-[(4R)-2-amino-5,5-difluoro-4-methyl-6H-1,3-oxazin-4-yl]-4-fluorophenyl]-5-cyanopyridine-2-carboxamide	53241828	-91.7	3	-2.2	28	392.3	-3.2	1.7	2	8	113 Å^2
67979346 (Lanabecesta)	N-[2-[(4aR,6S,8aR)-2-amino-6-methyl-4a,5,6,8-tetrahydro-4H-pyrano[3,4-d][1,3]thiazin-8a-yl]-1,3-thiazol-4-yl]-5-(difluoromethoxy)pyridine-2-carboxamide	67979346	-128.9	4	-2.7	31	414.5	-4.1	3	1	4	72.9 Å^2
68254185 (Atabecestat)	*N*-[3-[(4*S*)-2-amino-4-methyl-1,3-thiazin-4-yl]-4-fluorophenyl]-5-cyanopyridine-2-carboxamide	68254185	-118.1	3	-5.1	26	370.4	-4.5	2	2	6	130 Å^2
57827330 (Elenbecestat)	*N*-[3-[(4a*S*,5*R*,7a*S*)-2-amino-5-methyl-4,4a,5,7-tetrahydrofuro[3,4-d][1,3]thiazin-7a-yl]-4-fluorophenyl]-5-(difluoromethyl)pyrazine-2-carboxamide	57827330	-111.6	3	-4.4	30	437.4	-3.7	1.4	2	9	128 Å^2
44251605 (LY2811376)	(4S)-4-(2,4-difluoro-5-pyrimidin-5-ylphenyl)-4-methyl-5,6-dihydro-1,3-thiazin-2-amine	44251605	-81.1	2	-2.1	22	320.3	-3.6	2.1	1	6	89.5 Å^2
49837968 (LY2886721)	*N*-[3-[(4a*S*,7a*S*)-2-amino-4,4a,5,7-tetrahydrofuro[3,4-d][1,3]thiazin-7a-yl]-4-fluorophenyl]-5-fluoropyridine-2-carboxamide	49837968	-103.8	2	-2.0	27	390.4	-3.8	1.47	2	7	115 Å^2
78210254 (LY3202626)	*N*-[3-[(4a*R*,7a*S*)-2-amino-6-(5-fluoropyrimidin-2-yl)-4,4a,5,7-tetrahydropyrrolo[3,4-d][1,3]thiazin-7a-yl]-4-fluorophenyl]-5-methoxypyrazine-2-carboxamide	78210254	-124.5	4	-9.3	35	498.5	-3.5	1.3	2	11	157 Å^2
51352361 (Verubecestat)	*N*-[3-[(5*R*)-3-amino-2,5-dimethyl-1,1-dioxo-6*H*-1,2,4-thiadiazin-5-yl]-4-fluorophenyl]-5-fluoropyridine-2-carboxamide	51352361	-119.7	2	-6.2	28	409.4	-4.2	0.6	2	7	126 Å^2

MV: Molecular weight, MolDock Score: ligand-protein affinity score, LE: MolDock Score/ Molecular weight, XLogP3-AA: Hydrophobicity index.

Verubecestat has the lowest hydrophobicity and the highest solubility in aqueous liquids among the compounds we studied. The compounds we studied often have low hydrophobicity (Xlog p) (0.6 to 3.0). The affinity of these compounds ranges from -81.1 to -130.1, based on molecular docking results (MolDock score) using Molegro software. Lanabecestat, LY3202626, PF -06751979 had the highest docking scores among the compounds studied (**Table 2**).

DISCUSSION

Based on clinical trials, our studied compounds were frequently able to reduce beta-amyloid levels in blood plasma and cerebrospinal fluid. Clinical trials of most of these compounds were discontinued because of lack of efficacy or unacceptable side effects [10, 15, 17, 18].

Verubecestat (MK -8931) was administered orally, and its blood-brain barrier permeability and cell permeability were high. Its solubility in water was high at neutral pH. Long-term treatment with Verubecestat in animals can significantly reduce β-amyloid Aβ40, Aβ42, and soluble amyloid precursor protein (sAPPβ) in cerebrospinal fluid (CSF) and brain. Published study results showed no benefit of Verubecestat in reducing cognitive/functional decline in patients with mild to moderate Alzheimer's disease and were even associated with cognitive decline and reduced brain volume. In 2022, the use of Verubecestat for the treatment of Alzheimer's disease was discontinued. Our results showed that this compound does not cross the blood brain barrier but has good absorption in the digestive tract.

It has low hydrophobicity and high water solubility (**Table 1**)[10, 17-20].

CNP-520 (Umibecestat) is jointly provided by Novartis and Amgen. It was shown to be safe and well tolerated in animal models and in early studies, resulting in a strong reduction in beta-amyloid (Aβ) in CSF and proportional to dose. The use of CNP-520 for the treatment of Alzheimer's disease has since been discontinued. Our study showed that this compound dissolves well in body fluids and has low hydrophobicity. It's absorption in the digestive tract is low and does not pass the blood-brain barrier (10, 18-21). LY2811376 was developed by Eli Lilly. In a phase 1 clinical trial, this drug produced significant reductions in plasma and cerebrospinal fluid (CSF) Aβ concentrations. Because of toxicological data indicating damage to the pigmented epithelium of the iris in rats, the studies were discontinued. According to our study, this compound was lighter than other compounds and was well absorbed by the digestive tract, but did not cross the blood-brain barrier. Among the compounds we studied, it had the lowest affinity for the enzyme active site, based on docking results [10, 19-22].

LY2886721 was developed by Eli Lilly. Results from Phase I studies have shown that LY2886721 generally reduces Aβ-isoforms and is well tolerated. The results of later studies showed abnormal increases in liver enzymes, which led to the discontinuation of the studies. According to our study, it is well absorbed by the digestive tract and cannot cross the blood and brain barriers [10, 15, 20, 21].

RG7129 (RO5508887) is manufactured by Roche. At the end of 2013, Roche decided to discontinue the development of RG7129. Hepatotoxicity was cited as the reason for discontinuing clinical trials with this drug. According to the results of this study, it has a low molecular weight and is well absorbed by the digestive tract, but cannot cross the blood-brain barrier. According to the molecular docking results, it has low affinity for the active site of the enzyme [10, 15, 20-22].

JNJ-54861911 (Atabecestat) is manufactured by Janssen. The effect of Atabastat on reducing CSF Aβ levels was proportional to dose, and a long-term study confirmed its pharmacodynamic effect in reducing Aβ production by centrally inhibiting BACE1 cleavage APP. Hepatotoxicity was reported in some patients, suggesting an immune-based mechanism for the observed increase in liver enzymes. In 2022, the use of JNJ-54861911 in clinical trials was discontinued. According to the results of our study, it does not cross the blood-brain barrier, in contrast to its low molecular weight (**Table 1**) (10, 11, 21-23).

LY3314814 (AZD3293, Lanabecestat) is a BACE1 and BACE2 inhibitor developed in collaboration between AstraZeneca and Eli Lilly. Results from Phase I studies showed that this drug is generally safe and well tolerated. A dose of 50 mg of Lanabastat as a solution or tablet was well tolerated. It causes a dramatic decrease in Aβ levels in plasma and cerebrospinal fluid. The results of phase III studies of treatment with Lanabecestat showed that this drug did not reduce memory loss and other cognitive disorders. It did not change disease progression and was even associated with worsening of cognitive processes as well as brain volume reduction. Currently, Lanabecestat has a phase-out status for AD. Lanabecestat was the only drug we studied that could cross the blood-brain barrier. It had high gastrointestinal absorption and high affinity for the enzyme active site based on molecular docking results, and interestingly, more severe side effects were reported (**Table 1)** [12-14]. E2609 (Elenbecestat) has specificity (3.53-fold) for BACE1 over BACE2 and is being developed by Biogen and Eisai Co. Ltd. Preliminary preclinical studies in animal models and experimental results showed that administration of Elenbecestat reduced the levels of beta-amyloids in plasma and cerebrospinal fluid. This ligand is well tolerated, and no serious side effects were reported, and there was no need to limit or adjust the dose. In 2022, the use of E2609 in Alzheimer's disease

trials was discontinued. According to our study, it is well absorbed by the digestive tract and cannot cross the blood-brain barrier [15, 20-24].

PF-06751979 has higher specificity for BACE1 compared to BACE2 and is manufactured by Pfizer. It was well tolerated and showed a concentration-proportional reduction of Aβ in CSF and plasma when administered once daily. As of 2022, the use of this drug in studies related to Alzheimer's disease was discontinued. This compound had the highest affinity based on the docking score for the active site of the enzyme, but it has low absorption in the digestive tract and cannot cross the blood-brain barrier [15, 16, 20-24].

CONCLUSION

Based on the software available in PubChem and SwissADME online databases and molecular docking software, we attempted to analyze some known drug compounds that inhibit beta-secretase 1. We investigated the physicochemical, pharmacological, and affinity of the studied compounds with the active site of beta-secretase 1 enzyme based on molecular docking. These drugs were tested in clinical trials in patients [6, 7].

Based on the results of the online software on the SwissADME website, it was found that, with the exception of Lanabecestat, none of the investigated compounds can cross the blood-brain barrier. Although these compounds are well absorbed by the digestive tract, they often cannot cross the blood-brain barrier. They enter the blood and tissues and cause side effects by inhibiting the enzyme beta-secretase 1. They can also stimulate the immune response or damage liver tissue cells, but they do not reduce the production of beta-amyloids in brain neurons. And if, like Lanabecestat, they can cross the blood-brain barrier, they have even more serious side effects [6, 7, 10].

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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