Archives of Advances in Biosciences

Original Article:

Investigation of the Interaction of Some Alkaloids Derived from Marine Algae's with Human Acetylcholinesterase: *In Silico* Study

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Cite this article as: Zarrabi Ahrabi N, Sarve Ahrabi Y, Nejati Khoei S. Investigation of the Interaction of Some Alkaloids Derived from Marine Algae's with Human Acetylcholinesterase: *In Silico* Study. 2022; 13:E38273. https://doi.org/10.22037/aab.v13i2.38273

https://journals.sbmu.ac.ir/aab/article/view/38273

Article info:
Received: 27 Apr 2022
Accepted: 19 Jun 2022
Published: 07 Oct 2022

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Abstract

Introduction: Nowadays, acetylcholinesterase inhibitors are commonly used to improve the symptoms of Alzheimer's disease and central nervous system disorders. Marine algae's contain compounds like alkaloids that have many biological activities. We aimed to investigate the inhibitory effects of some alkaloids with human acetylcholinesterase using molecular docking.

Materials and Methods: In this study, the interaction of 19 alkaloid compounds with human acetylcholinesterase (hAChE) was investigated using molecular docking. The initial structure of the compounds was optimized using mm2 method by Chem3D software; the compounds were evaluated as inhibitors on the gap of the active site of the enzyme by AutodackVina software, and the output results were analyzed by Discovery Studio software.

Results: The results showed that most of these compounds act as inhibitors with good binding energy (-7.7 to -9.8 kcal/mol). The best performance is related to the three compounds including Caulerpin, Martefragin A and N-Acetyl tyramine. Caulerpin was involved in hydrogen bonding with an affinity of -9.6 with the amino acids Histidine: 405, Arginine: 296, and Asparagine: 233. Martefragin A was involved in hydrogen bonding with an affinity of -9.6 with the amino acids Serine: 293, Phenylalanine: 295, and Arginine: 296, respectively. N-Acetyl tyramine was involved in hydrogen bonding with an affinity of -9.8 with the amino acids Asparagine: 186, Lysine: 53, and Glycine: 14.

Conclusion: The results of this study suggest that most of these alkaloid compounds can inhibit this enzyme by binding to the cleavage site of the active site of hAChE, and thus are suitable options for further studies to design new anticholinesterase drugs.

Keywords: Alkaloids, Acetylcholinesterase, Alzheimer disease, Molecular docking simulation

1. Introduction

Alzheimer's disease is a progressive neurodegenerative disorder that ultimately leads to death. This disease is the most common cause of dementia in people over 65 years [1]. Based on the cholinergic hypothesis of the pathogenesis of Alzheimer's disease, the clinical features of dementia observed in this disease are attributed to decreased levels of acetylcholine, a neurotransmitter involved in memory and learning, in the hippocampus and cortex [2]. To date, no definitive treatment for this disease is known. However, acetylcholinesterase inhibitors are commonly used to partially improve the symptoms of mild to moderate Alzheimer's disease [3]. These inhibitors are also used to manage other forms of dementia and central nervous system disorders such as

Parkinson's disease, Lewy body-related dementia, mild cognitive impairment, Down syndrome, Korsakov disease, and vascular dementia [4]. Today, researchers around the world are looking to design, synthesize, and evaluate new drugs for the treatment of Alzheimer's that, in addition to being more effective, have the fewest side effects [5]. Also, the study of natural resources in order to find new drugs for this disease is also receiving a lot of attention. Studies on the structure of the enzyme acetylcholinesterase (hAChE) show that the active site of this enzyme consists of several different parts and in the form of a deep and narrow gap (gorge) in the protein surface [6]. The strategic site, which consists of the three amino acids serine, glutamate and histidine (catalytic triad), is located deep in this gap. There is also a position called the oxyanion cavity in the depth of the gap, which plays an important role in the catalysis process [7]. Site Anionic Catalytic (CAS) and acyl envelope, located near the depth of the fissure, is involved in the binding to the choline and acetyl substrate (acetylcholine) sections, respectively. Near the slit entrance, there is an allosteric site consisting of several conserved aromatic amino acids called the Peripheral Anionic Site (PAS) [8]. In addition to the allosteric regulation of the catalytic activity of the enzyme, this site is involved in several other functions [9]. Alkaloids are produced by many organisms, including bacteria, fungi, plants, and animals. Alkaloids are a group of natural compounds known as secondary metabolites [10]. The role of alkaloids is still unclear in living organisms; at first, it was thought that alkaloids were the last product of nitrogen metabolism in plants, but this theory was rejected [11]. The term alkaloid was first used by Meissner in 1819 to describe alkaline compounds derived from plants, but nowadays the definition has changed and alkaloid is a compound that has a nitrogen atom in a cyclic ring. Therefore, many bio amines and compounds containing halogenated cyclic nitrogen are classified as alkaloid compounds [12]. Alkaloids, in addition to carbon, hydrogen, and nitrogen atoms, may contain oxygen, sulfur, and more broadly elements such as chlorine, bromine, and phosphorus. Even these compounds may have groups with very weak acidic properties [13]. The study of medicinal compounds derived from medicinal algae is expanding rapidly. Structurally, most alkaloids extracted from marine algae belong to the indole and phenyl ethylamine groups [14]. The biological properties of these alkaloids have not been fully studied. Alkaloids from marine algae are rare compared to alkaloids in terrestrial plants [15]. The aim of this study was to investigate the interaction of some alkaloids derived from marine algae with human acetylcholinesterase using molecular docking.

2. Materials and Methods

Ligands preparation

The crystallized structure of 19 alkaloid compounds as ligands was downloaded from the PubChem database in SDF format. The PubChem ID, Chemical Formula, Molecular Weight and 2D structure are reported in Table 1 and Two-dimensional (2D) structures of compounds are reported in Figure 1. The structures were converted from .SDF

Table 1. Structures information

Compounds	Name	Compound CID	Molecular Formula	Molecular Weight (g/mol)
1	Caulersin	10593388	C21H14N2O3	342.3
2	Caulerpin	5326018	C24H18N2O4	398.4
3	Martensin A	23426920	C18H25N3O2	315.4
4	Martensin B	23426921	C18H23N3O2	313.4
5	Denticin A	91991248	C30H34N3O7S+	580.7
6	Fragilamide	101248179	C25H31N3O2	405.5
7	Martefragin A	9841578	C20H25N3O3	355.4
8	Amazolon	139291572	C14H11BrN2O2	319.15
9	Bromo Indole A	636777	C9H6Br3N	367.86
10	Bromo Indole B	334778	C9H6Br3N	367.86
11	Bromo Indole C	11212652	C8H3Br4N	432.73
12	1-Phenylethylamine	7408	C8H11N	121.18
13	N-Phenethylacetamide	70143	C10H13NO	163.22
14	Tyramine	5610	C8H11NO	137.18
15	N-Acetyltyramine	121051	C10H13NO2	179.22
16	Hordenine	68313	C10H15NO	165.23
17	Dopamine	681	C8H11NO2	153.18
18	Lophocladine A	11708368	C14H10N2O	222.24
19	Lophocladine B	11665726	C14H11N3	221.26

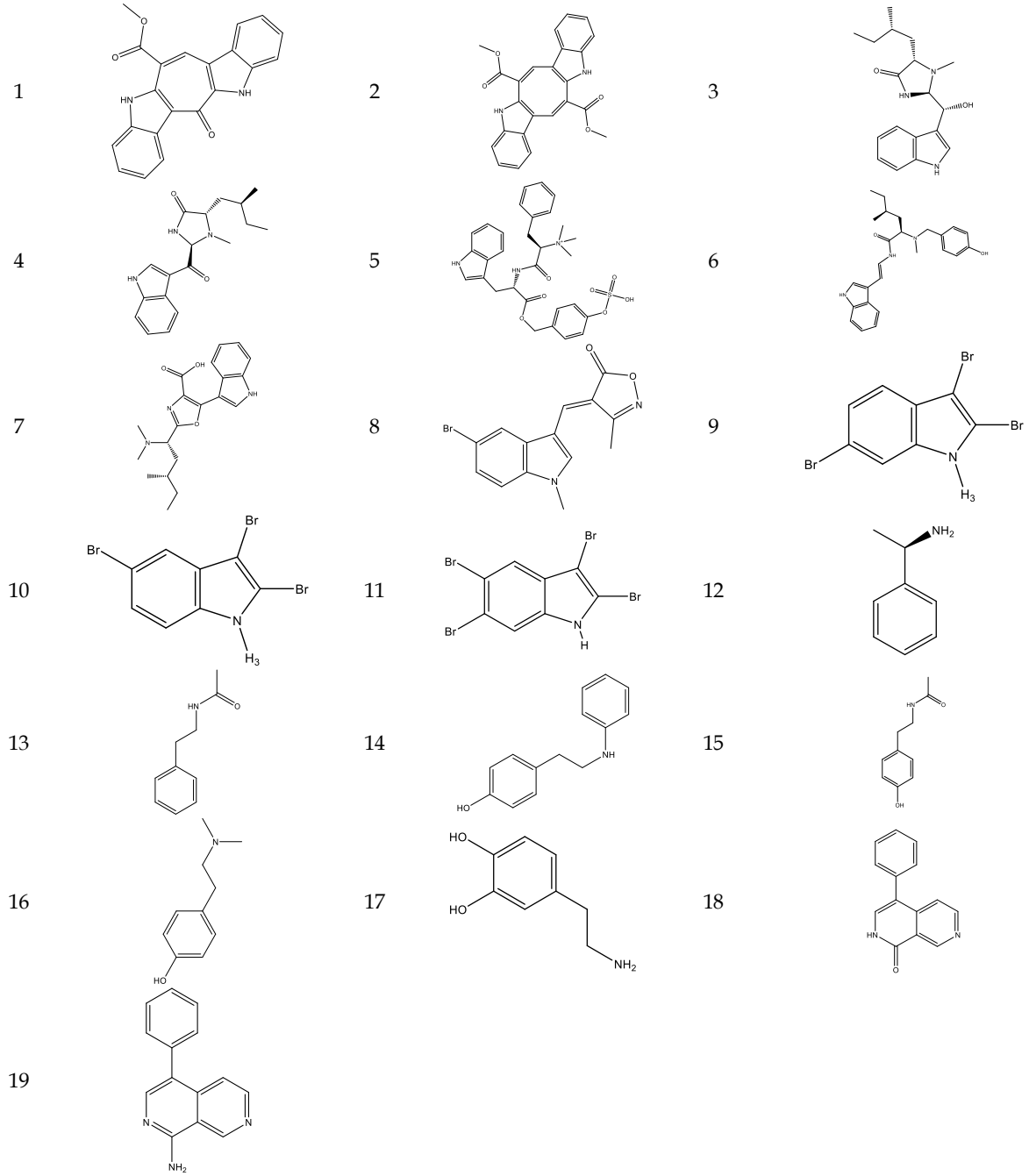


Figure 1. 2D Structures of all compounds (1-19)

to .pdb by ChemDraw 19.01 software. The ligands were optimized with MM^2 Job command by Chem3D v20.1.1.125 software. After optimization, the Total Energy, Stretch (i.e., the energy required to stretch the two bonds involved in a bond angle when that bond angle is severely compressed), Bend (i.e., the energy associated with any bond angles that are deformed from their optimal values) and Torsion (i.e., the energy associated with deforming torsional angles (dihedral angles) in the molecule) of all compounds were reported. Then, AutoDockTools 1.5.6 software was used to convert the initial format of the ligands from .pdb to .pdbqt format [16].

Enzyme preparation

The crystallized structure of human acetyl-cholinesterase (hAChE) (PDB ID: 4M0E), was downloaded from Protein Data Bank (PDB) with a resolution of 2°A (Angstrom) and .pdb format. Then,

with Discovery Studio 4.5 Client software, the ligands and water molecules were separated from the protein and the A and B chains were stored separately as a .pdb file. Given that the number of unspecified amino acids in the B chain proportional to Chain A is less, chain B was used in later stages. Chain B lacked the two amino acids arginine 493 and aspartate 494. These two amino acids, which were not present at the active site of the enzyme, were added to the protein structure using Modeller software and using B-chain as a template. Finally, the chain was prepared with the addition of polar hydrogens using AutoDockTools-1.5.6 software [17].

Molecular docking

At this stage, the config files were prepared which were required for docking. For this purpose, the size and location of the grid box were determined using AutoDockTools-1.5.6 software. Dimensions and centers _X, _Y and _Z of the grid box were considered in such a way that covered all parts of the enzyme. Then, the required information was recorded and stored in the config file and a specific config file was prepared for each ligand. In order to perform docking, each ligand was assigned a folder containing the .pdbqt format of the enzyme and config file. Finally, using the prompt command section and the information of these folders, docking operations were performed for all 19 alkaloid compounds with AutoDockVina software [18]. The overall results obtained from molecular docking were analyzed using AutoDockTools-1.5.6 software. In order to study the results more precisely, for each alkaloid compound, the ligand-receptor complex related to conformation with the highest binding energy was prepared and examined using Discovery Studio 4.5 Client software.

3. Results

As shown in Table 2, all affinities of the compounds were calculated. The affinity of all compounds was

Table 2. Chem 3D and AutoDockVina results of compounds (1-19)- (SER: Serine, VAL: Valine, TYR: Tyrosine, TRP: Tryptophan, PHE: Phenylalanine, HIS: Histidine, ARG: Arginine, ASN: Asparagine, PRO: Proline, ASP: Aspartic acid, THR: Threonine, LEU: Leucine, GLU: Glutamic acid, LYS: Lysine, GLY: Glycine)

Compounds	Total Energy (KCal/Mol)	Stretch	Bend	Torsion	Affinity - ΔGbind (KCal/Mol)	H-Bonds	H-C Bonds	Pi-Pi
1	100.8892	2.6655	44.8929	26.0241	-8.7	SER:293	VAL:294	TYR:341 TRP:286 PHE:297
2	119.9350	4.6321	44.1106	33.2522	-9.6	HIS:405 ARG:296 ASN:233	-	PRO:235
3	20.8351	2.2090	23.3993	0.2524	-8.1	ASP:74 THR:75	-	LEU:76 TYR:341 TRP:286
4	35.7760	2.6841	26.0453	0.9907	-7.7	TYR:72	ASP:74	TRP:286 LEU:76
5	201.5844	3.6439	60.3016	21.4463	-7.9	TRP:532 PRO:232	PRO:235 PRO:312	GLU:313 LEU:536 PRO:410 HIS:405 ARG:296
6	7.3441	1.8408	19.6359	-16.4184	-8.9	SER:293	ASP:74	TYR:341 TYR:124 PHE:297 TRP:286 HIS:287
7	52.4279	2.0188	32.3486	0.3414	-9.6	PHE:295 ARG:296 SER:293	-	LEU:289 TRP:286 TYR:341
8	42.7877	1.1380	34.7344	-11.4836	-7.7	-	-	-
9	16.3675	0.3196	19.4945	-10.1500	-7.2	-	-	-
10	16.3727	0.3159	19.5084	-10.1500	-6.7	-	-	-

Table 2. Continued

11	13.9342	0.5748	14.5979	-10.2300	-6.7	-	-	-
12	-0.3485	0.3451	0.3410	-5.8062	-6.3	-	-	TYR:341 PHE:297
13	-4.0988	0.4198	1.5522	-5.1764	-8.2	TYR:341 SER:293	-	-
14	-0.4704	0.6531	3.0619	-14.1466	-7.2	-	-	TRP:86 ASP:74
15	-5.1224	0.4245	2.2760	-5.1765	-9.8	LYS:53 ASN:186 GLY:14	-	PRO:52 LEU:178
16	5.1931	0.8058	2.2496	-5.5634	-5.5	-	-	TRP:286 TYR:341
17	-6.8233	0.4068	2.8195	-8.5908	-8.5	TYR:124 GLU:202	-	TRP:86 SER:125
18	32.4868	1.5621	12.2153	8.3798	-8.5	PHE:295	SER:293 TYR:341	TRP:286
19	25.1518	1.5313	4.2686	-2.7654	-7.6	-	SER:293 TYR:341 ASP:74	TYR:124 TRP:286

reported between -5.5 and -9.8. Among these, compounds 2, 7 and 15 showed the highest amount of H-bonds; also, these three compounds created the highest amount of variety and number of hydrogen bonds (bonds with 3 types of amino acids). In the next category, compounds 3, 5, 13 and 17 also inhibited this enzyme by creating a hydrogen bond with two amino acids. Compounds 8, 9, 10, 11, 12, 14, 16 and 19 did not form any hydrogen bonds with any amino acids. As shown in Figure 2, Compound 2 (Caulerpin) was involved in hydrogen bonding with an affinity of -9.6 with the amino acids Histidine: 405, Arginine: 296, Asparagine: 233. The compound was also involved with hydrophilic bonds with the amino acid Proline: 235. Compound 7 (Martefragin A) was involved in hydrogen bonding with an affinity of -9.6 with the amino acids Serine: 293, Phenylalanine: 295, Arginine: 296. The compound was also involved in hydrophilic bonds with the amino acids Tyrosine: 341, Tryptophan: 286, Leucine: 289. Compound 15 (N-Acetyl tyramine) was involved in hydrogen bonding with an affinity of -9.8 with the amino acids Asparagine: 186, Lysine: 53, Glycine: 14. The compound was also involved in hydrophilic bonds with the amino acids Proline: 52, Leucine: 178.

4. Discussion

Molecular docking is a bioinformatic method that is widely used nowadays in rational methods of structural drug design. Docking programs simulate how a biological macromolecule (receptor, enzyme, or nucleic acid) interacts with small ligands such as substrates, inhibitors, or other drug candidates [19]. Alkaloids are a group of low molecular weight secondary metabolites that are made up of amino acids or transamination processes and have one or more nitrogen atoms in their heterocyclic structure [20]. These compounds are one of the largest groups of natural compounds from which various pharmaceutical products are produced. The number of known alkaloids is 12,000. Alkaloids have been one of the most widely used compounds used by humans since ancient times [21]. In this research, the interaction of 19 alkaloid compounds isolated from algae was investigated with hAChE active site using molecular docking. The results of this study showed that 11 of the 19 alkaloid compounds have the ability to interact with this enzyme and their best affinity varies between -7.7 to -9.8 kcal/mol. The highest and lowest affinities are respectively related to N-Acetyl tyramine and Martensin B, which were involved in hydrogen bonding with the active site of the enzyme. In a study, Romeiro et al. (2020) examined the effects of anti-acetylcholinesterase on halogenated marine natural products from Laurencia dendroidea. The results of their study showed that (−)Elatol-1 was able to inhibit acetylcholinesterase, thus becoming a suitable skeleton in the search for new anti-Alzheimer drugs of natural and semi-synthetic origin [22]. A study by Cinar et al. (2019) investigated the anti-acetylcholinesterase effects of some Turkish seaweed. Their study showed that crude extract of P. fascia possessed the highest inhibition against AChE [23]. As mentioned earlier in the introduction, the active site of the enzyme acetylcholinesterase, which is a deep and narrow gap, consists of several sections. The amino acids for each segment are as follows: strategic position (catalytic triad: Serine: 203, Glutamate: 334 and Histidine: 447), Oxyanion hole (Glycine: 121,

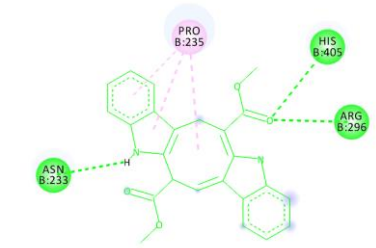
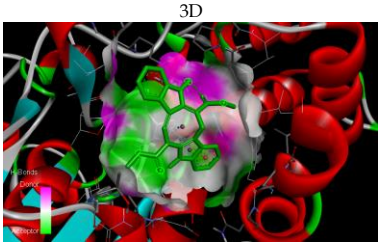
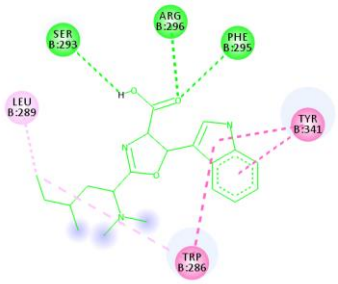
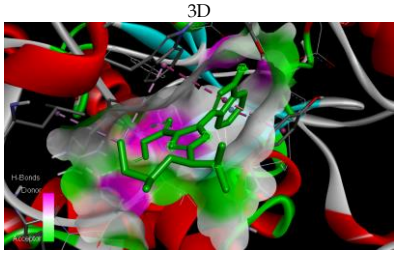
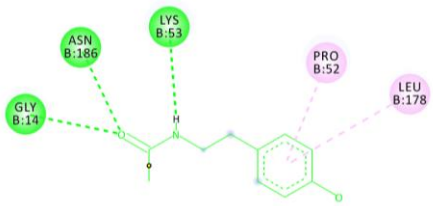
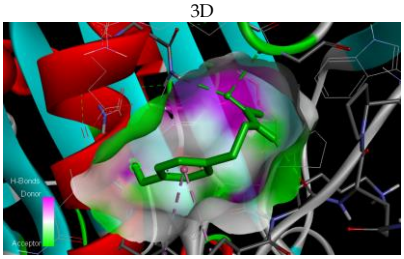
Compound		
2	2D	
	3D	
7	2D	
	3D	
15	2D	
	3D	

Figure 2. 2D and 3D results of AutoDockVina (compounds 2, 7 and 15) - Interaction of compounds 2, 7 and 15 with hAChE active site provided by Discovery Studio 4.5 Client software. Hydrogen bonds are shown as green circles in 2D and 3D images. The amino acids that make hydrophobic bonds are marked as non-green circles.

Glycine: 122 and Alanine: 204), Acyl packets (Tryptophan: 236, Phenylalanine: 295, Phenylalanine: 297 and Phenylalanine: 338), CAS (Tryptophan: 86, Glutamate: 202 and Tyrosine: 337), PAS (Tyrosine: 72, Aspartate: 74, Tyrosine: 124, Tryptophan: 286 and Tyrosine: 341) [24, 25, 26, 27, 28]. As the results show, many of the amino acids involved in the binding process are similar. All of the compounds that make up the hydrogen bond interact with the PAS portion and the acyl pack. The PAS section is an allosteric station located near the slot entrance of the active site and the acyl envelope located near the depth of the slot is involved in the connection to the steel substrate group. But only three alkaloid compounds, including Caulerpin, Martefragin A, and N-Acetyl tyramine, interact with the deeper part of the active site cleft. These three compounds interact with CAS, which is deeper than the acyl envelope, and also with the oxyanion cavity, which is located deep in the gap. Caulerpin contains two indole groups attached to a cyclic ring containing 8 carbons and 2 carboxy groups. The structure of Caulerpin-1 was first discovered. The crystal structure of this compound was studied and two structural analogues of Caulerpin including Caulerpin-2 and Caulerpin-3 were also extracted from the Caulerpa racemosa Algae [29]. The anti-diabetic, analgesic, anti-inflammatory, anti-tumor, anti-larval, anti-herpes, anti-tuberculosis, antimicrobial and immune-stimulant activities of caulerpin have been identified [30]. As shown in Figure 2, Caulerpin penetrates the active site slit through various parts of its structure and interacts with hAChE by forming three hydrogen bonds (Histidine, Arginine, and Asparagine) and one hydrophobic bond (Proline). Martefragin A has also been extracted from the Martensia fragilis algae. Martifrage A has the structure of oxazolindole and this compound has inhibitory effects against NADPH-dependent lipid peroxidation in rat liver microsomal [31]. As shown in Figure 2, Martefragin A penetrates the active site slit through various parts of its structure and interacts with hAChE by forming three hydrogen bonds (Serine, Phenylalanine, Arginine) and three hydrophobic bonds (Tyrosine, Tryptophan, Leucine). N-Acetyl tyramine is found in the seaweed Phyllophora crispa and Gelidium crinale and is also produced by many microorganisms and terrestrial plants [32]. As shown in Figure 2, N-Acetyl tyramine penetrates the active site slit through various parts of its structure and interacts with hAChE by forming three hydrogen bonds (Asparagine, Lysine, Glycine) and two hydrophobic bonds (Proline, Leucine).

Evidence from scientific data suggests that PAS has the ability to accelerate the formation of amyloid-beta fibrils in the brains of people with Alzheimer's disease [33]. This function occurs through a non-catalytic mechanism by binding to the non-amyloidogenic form of the amyloid-beta protein. Thus, inhibitors that have the ability to bind simultaneously to the catalytic site of the enzyme (catalytic triad) or to the binding site of the substrate (CAS and packet acyl); PAS can improve Alzheimer's symptoms through two mechanisms [34], because they can also slow down the formation of beta-amyloid fibrils in addition to increasing the level of acetylcholine in the brain of patients. This group of inhibitors is considered a new therapeutic agent for the more effective treatment of Alzheimer's [35]. Due to the fact that most of the alkaloid compounds studied in this study had the ability to simultaneously bind to the PAS region as well as the substrate-binding site (acyl envelope), they are in the group of these inhibitors. As mentioned above, the compounds Caulerpin, Martefragin A, and N-Acetyl tyramine have the ability to bind to CAS and the oxyanion cavity in addition to interacting with these two regions.

5. Conclusion

The results of this study indicate that most alkaloid compounds especially compounds 2, 7 and 15 isolated from seaweed samples with good binding energy through the active site of hAChE inhibit the interaction and activity of this enzyme. These results are consistent with published scientific data that indicate the anticholinesterase activity of alkaloids. According to the results of the present study, these alkaloid compounds, especially Caulerpin, Martefragin A, and N-Acetyl tyramine, can be suggested as suitable candidates for more extensive research to design new drugs for the treatment of Alzheimer's and other central nervous system disorders. Ours comes about Caulerpin, Martefragin A, and N-Acetyl tyramine will be a supportive structure for conceivable improvement of unused drugs, but this result needs to be affirmed by other broad clinical trials.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-forprofit sectors.

Author's contributions

Conceptualization: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi; Methodology: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi, Sarina Nejati khoei; Investigation: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi, Sarina Nejati khoei; Writing – original draft: Yasin SarveAhrabi; Writing – review & editing: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi; Funding acquisition: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi; Resources: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi; Supervision: Yasin SarveAhrabi, Nakisa Zarrabi Ahrabi.

Conflict of interest

The authors declared no conflict of interests.

Acknowledgments

The authors appreciate all professors who participated voluntarily in this investigation.

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