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Anti-cholinergic alkaloids as potential therapeutic agents for Alzheimer's disease: An *in silico* approach

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Alzheimer's disease (AD), a progressive neurodegenerative disorder with many cognitive and neuropsychiatric symptoms is biochemically characterized by a significant decrease in the brain neurotransmitter acetylcholine (ACh). Plant-derived metabolites, including alkaloids have been reported to possess neuroprotective properties and are considered to be safe, thus have potential for developing effective therapeutic molecules for neurological disorders, such as AD. Therefore, in the present study, thirteen plant-derived alkaloids, namely pleiocarpine, kopsinine, pleiocarpamine (from *Pleiocarpa mutica*, family: Annonaceae), oliveroline, noroliveroline, liridonine, isooncodine, polyfothine, darienine (from *Polyalthia longifolia,* family: Apocynaceae) and eburnamine, eburnamonine, eburnamenine and geissoschizol (from *Hunteria zeylanica,* family: Apocynaceae) were analyzed for their anti-cholinergic action through docking with acetylcholinesterase (AChE) as target. Among the alkaloids, pleiocarpine showed promising anti-cholinergic potential, while its amino derivative showed about six-fold higher anti-cholinergic potential than pleiocarpine. Pleiocarpine and its amino derivative were found to be better inhibitors of AChE, as compared to commonly used drugs tacrine (brand name: Cognex) and rivastigmine (brand name: Exelon), suggesting development of these molecules as potential therapeutics in future.

Keywords: Anti-cholinergic, Anti-cholinesterase, Alkaloids, Alzheimer's disease, Pleiocarpine, Molecular docking, Pleiocarpine

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with many cognitive and neuropsychiatric symptoms, including dementia[1]. Though AD is considered as a multi-factorial disease, oxidative stress leading to the imbalance between production and detoxification of reactive oxygen species (ROS) is considered as one of the important factors in the development of this neurodegenerative disease[2]. Furthermore, the ROS-mediated neurodegenerative progression also leads to genetic damage[3]. Biochemically, development of AD has been related to a significant decrease in the brain neurotransmitter acetylcholine (Ach)[4]. Therefore, augmentation of the central cholinergic function by the inhibition of acetylcholinesterase (AChE) is one of the effective approaches for the treatment of AD[5,6]. Thus, search for AChE inhibitors is important in management of AD.

The two commonly used drugs against AD, namely tacrine (brand name, Cognex) and rivastigmine (brand name, Exelon), having AChE inhibitory action, are reported to have several side effects[7]. Both of these drugs have been reported to produce liver toxicity, nausea and diarrhea[8]. Therefore, there is a need to identify and develop novel drugs from natural sources, which are considered as relatively safer[9].

The alkaloids, derived from plants have also been reported to possess neuroprotective properties by virtue of their interaction with the receptors at the nerve endings, such as huperzine[10], thus making them important as therapeutic molecules for neurological disorders, including AD [11]. Many alkaloids have been reported to act as AChE inhibitors. For example, based on *in vitro* and *in vivo* studies, the alkaloids such as galanthamine, montanine, arecoline, bulbocapnine and corydalis etc. have been reported to possess anti-cholinergic activity and thus used for

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Abbreviations: Ach, acetylcholine; AChE, acetylcholinesteras; AD, Alzheimer's disease.

treatment of AD in many countries[12-16]. The effective and long-lasting, anti-cholinergic action of alkaloids has encouraged researchers to look for other alkaloids having AChE inhibitory action. The alkaloids pleiocarpine, kopsinine and pleiocarpamine from *Pleiocarpa mutica* (family: Annonaceae), oliveroline, noroliveroline, liridonine, isooncodine, polyfothine and darienine from *Polyalthia longifolia* (family: Apocynaceae) and eburnamonine, eburnamine, eburnamenine and geissoschizol from *Hunteria zeylanica* (family: Apocynaceae) have also been reported to possess neuroprotective properties[17-22].

In the present study, the above-mentioned thirteen neuroprotective alkaloids have been analyzed through *in silico* molecular docking approaches for their anti-cholinergic potential as AChE inhibitors. Furthermore, the theoretical derivatives of the best screened out alkaloid were generated and analyzed for their anti-cholinergic action. Also, the most potent alkaloid and its theoretical derivatives have been compared with commonly used drugs against AD to find out the relative potential of their anti-cholinergic action as AChE inhibitors.

Materials and Methods

Preparation of protein structure

The crystal structure of acetylcholinesterase (PDB: 1B41) in complex with snake venom toxin fasciculin-II solved by X-ray crystallography at 1.009 Å was retrieved from the Protein Data Bank (http://www.rcsb.org/). All the necessary changes like removal of extra chains and connect were done with CHIMERA software[23].

Preparation of ligand structures

A number of AChE inhibitors belonging to the secondary metabolites class alkaloids were selected on the basis of available literature. The mol or sdf files were retrieved from Chemspider (http://www.chemspider.com/) and PubChem (http://pubchem.ncbi.nlm.nih.gov/). The files were then changed to PDB format with the help of an online tool Molecular File Converter (http://www.webqc.org/moleculerformatsconverter.php).

Molecular docking

The binding region for the docking study by Autodock was defined at a 0.375 Å radius sphere centred on the active site. A grid box of dimensions 88 Å × 88 Å × 88 Å was constructed around the binding site, based on the co-crystallized ligand. Ten genetic algorithm (GA) runs were performed for each compound and 10 ligand bumps were allowed in an attempt to account for mutual ligand/target fit. Each of the GA run was performed on a population of 150 individuals. The rates of mutation and crossover were set to 0.02 and 0.8, respectively. AutoDock 4.0 included Lamarckian Genetic Algorithm search engine[24] and an experimental free energy function for estimating binding energy, inhibitory constant, docking energy, inter-molecular energy, internal energy and torsional energy. The binding free energy was empirically calculated based on these energy terms and a set of co-efficient factors[24]. The value of binding energy was used to rank the docking positions of the molecules. The clusters with lowest binding energy were selected.

All the docking interactions between target molecule and ligands were visually examined in Chimera[23] and hydrogen bonds within the 0.4 Å radius interaction spaces of active site and near the substrate atoms were analyzed.

Active site identification of AChE

The binding pocket analysis of AChE for active site identification along with area and volume of binding pocket was performed with Computed Atlas of Surface Topography of Proteins (CASTp) program (http://cast.engr.uic.edu)[25].

Results and Discussion

Active site identification of AChE

AChE active site prediction was performed using CASTp program for binding pocket analysis. The predicted ligand binding pocket for AChE is shown in Fig. 1. The ligand binding pocket containing active site consisted of 34 residues, namely Gln71, Tyr72, Asp74, Gly82, Thr83, Trp86, Asn87, Pro88, Gly120, Gly121, Gly122, Tyr124, Ser125, Gly126, Leu130,Tyr133, Glu202, Glu202, Ser203, Ala204, Trp236, Trp286, Leu289, Ser293, Val294, Phe295, Arg296, Phe297, Tyr337, Phe338, Tyr341, Trp439, His447, Gly448 and Tyr449. The result of CASTp active site identification was further validated through docking analysis.

Docking studies on AChE with selected alkaloids

In view of promising use of alkaloids, namely montanine, arecoline and galanthamine[14,15,26] for treatment of AD, in the present study, thirteen

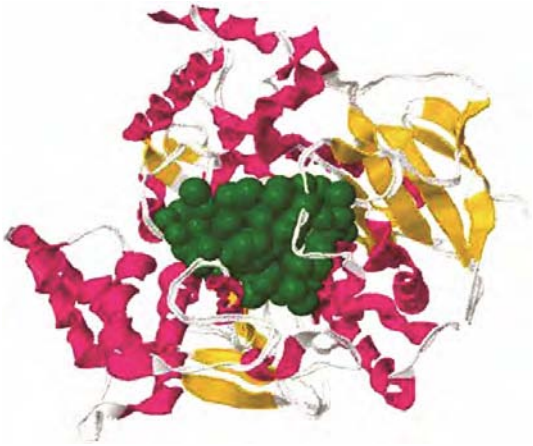

Fig. 1—Active site of AChE predicted using CASTp program

Table 1—Results of docking of various alkaloids (ligands) with AChE as target

Interacting ligands	Binding energy (kcal/mol)	Inhibition constant (K_i) (μM)
Pleiocarpine	-12.50	6.90×10^{-4}
Kopsinine	-11.12	7.07×10^{-3}
Oliveroline	-10.84	1.12×10^{-2}
Noroliveroline	-10.77	1.26×10^{-2}
Eburnamine	-10.61	1.67×10^{-2}
Eburnamenine	-10.48	2.07×10^{-2}
Pleiocarpamine	-10.35	2.57×10^{-2}
Geissoschizol	-10.35	2.60×10^{-2}
Eburnamonine	-8.56	5.34×10^{-2}
Liriodenine	-9.36	0.13
Isooncodine	-7.10	6.23
Polyfothine	-8.37	9.10
Darienine	-6.57	15.25

neuroprotective alkaloids were analyzed for their anti-cholinergic potential using AChE as target through *in silico* docking approach. Results are presented in Table 1. Based on the docking parameters, namely binding energy and inhibitory constant (*K*i), the alkaloid pleiocarpine was found to be the best inhibitor of AChE with binding energy of -12.50 kcal/mol and *K*i of 6.90×10^{-4} μM.

To the best our knowledge, there are no reports available in the literature for anti-cholinergic activity of these alkaloids. However, there are reports suggesting that extracts of plants belonging to Apocynaceae family being considered as rich source of alkaloid class eburnamine/vincamine, showing neuroprotective properties[19]. The *in silico* predictions in the present study revealed promising anti-cholinergic activity of the alkalloids eburnamine, eburnamenine and eburnamonine from *Hunteria zeylanica*. The alkaloids oliveroline and noroliveroline from *Polyalthia longifoliai* have been used to treat Parkinson disease, a neurological disorder[22] and other alkaloids from the same plant, such as liridonine, isooncodine, polyfothine and darienine have shown neuroprotective properties[19,21,]. Thus, results of the present docking study suggested that these alkaloids having AChE activity might be effective for treatment of AD.

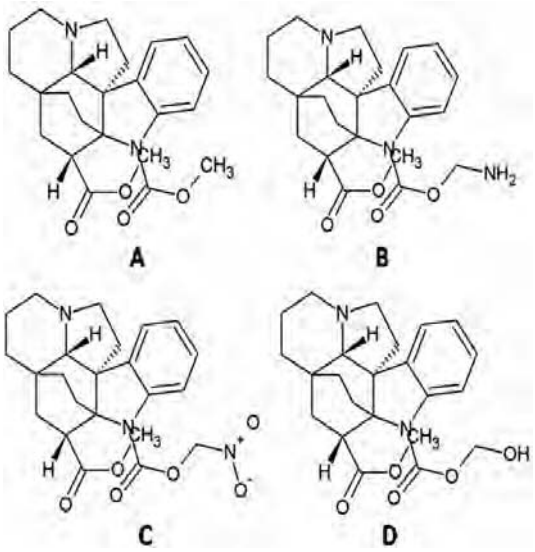

Fig. 2—Chemical structures of pleiocarpine (A), its amino (B) nitro (C) and hydroxyl (D) derivatives, respectively

Docking studies of pleiocarpine, its derivatives and known drugs with AChE

In order to improve the AChE activity of pleiocarpine, a number of pleiocarpine derivatives were generated by substituting the methyl hydrogen of carboxylate group linked with nitrogen with various other functional groups, such as -NH_2, -NO_2 and -OH using Chemsketch Software version 11.0. The chemical structures of pleiocarpine and its derivatives are shown in Fig. 2.

The amino-, nitro- and hydroxyl- derivatives of pleiocarpine were used for docking analysis with AChE as target and the results are presented in Table 2. Interestingly, based on the docking parameters, namely binding energy and inhibitory constant, amino- derivative of pleiocarpine (Fig. 2B) was found to be promising, showing binding energy of -13.34 kcal/mol and *K*i of 1.65×10^{-4} μM. An estimate of the area and volume of binding pocket of various ligands at binding site of AChE was also done using CASTp program by molecular surface model

Table 2—Docking result of pleiocarpine and its derivatives and common anti-cholinergic drugs (Cognex and Exelon) with AChE

Interacting ligands	Binding energy (kcal/mol)	Inhibition constant (*Ki*) (μM)	Ligand binding pocket area (A^2)	Ligand binding pocket volume (A^3)
Pleiocarpine	-12.50	6.90×10^{-4}	14.00	7.00
Amino-derivative of pleiocarpine	-13.34	1.65×10^{-4}	15.02	6.70
Hydroxyl-derivative of pleiocarpine	-12.51	6.74×10^{-4}	14.21	7.06
Nitro-derivative of pleiocarpine	-12.29	9.78×10^{-4}	25.14	11.80
Cognex	-7.20	5.30	26.00	12.00
Exelon	-7.19	5.35	24.00	11.00

Table 3—Interacting amino acid residues of AChE with ligands: pleiocarpine, its amino-, nitro-, hydroxyl- derivatives and common anti-cholinesterase drugs (Cognex and Exelon) at the active site.

Ligands	H-bonding		Interacting residues
	Inter-molecular	Intra-molecular	
Pleiocarpine	------	Trp86-Tyr449 (3.204 Å) Tyr337-Tyr224 (4.788 Å)	Asp74, Gly82, Thr83, Trp86, Gly120, Gly121, Tyr124, Ser125, Glu202, Ser203, Tyr337, Tyr341, Trp439, His447, Gly448, Tyr449
Amino derivative of pleiocarpine	Thr83-ligand (3.100 Å)	Trp86-Tyr449 (3.204 Å) Tyr337-Tyr224 (4.788 Å) Tyr341-Asp74 (2.497 Å) Trp439-Tyr449 (2.73 Å)	Asp74, Gly82, Thr83, Trp86, Gly120, Gly121, Tyr124, Ser125, Glu202, Ser203, Tyr337, Tyr341, Trp439, His447, Gly448, Tyr449,
Nitro derivative of pleiocarpine	------	Trp86-Tyr449 (3.204 Å) Tyr337-Tyr341 (3.129 Å) Tyr341-Asp74 (2.497 Å) Trp439-Tyr449 (2.73 Å)	Asp74, Gly82, Thr83, Trp86, Gly120, Gly121, Tyr124, Ser125, Glu202, Ser203, Tyr337, Tyr341, Trp439, His447, Gly448, Tyr449
Hydroxyl derivative of pleiocarpine	------	Trp86-Tyr449 (3.204 Å) Trp439-Tyr449 (2.73 Å)	Asp74, Gly82, Thr83, Trp86, Gly120, Gly121, Tyr124, Ser125, Glu202, Ser203, Tyr337, Tyr341, Trp439, His447, Gly448, Tyr449
Cognex	------	------	Gly82, Trp86, Gly120, Gly121, Ser125, Tyr133, Glu202, Ser203, Tyr337, Trp439, His447, Gly448, Tyr449, Ile451
Exelon	------	Trp86- Tyr449 (3.204 Å) Trp439-Tyr449 (2.73 Å)	Gly82, Thr83, Trp86, Gly120, Gly121, Tyr124, Ser125, Glu202, Ser203, Tyr337, Trp439, His447, Gly448, Tyr449

(Connolly's surface). Results are presented in Table 2. Binding of pleiocarpine and its amino derivative to AChE were found to be more compact than those of drugs, in agreement with the docking results.

The inhibitory potential of pleiocarpine and its derivatives was also compared with those of commonly used anti-cholinergic drugs, namely Cognex and Exelon and data are presented in Table 2. As compared to these drugs, nearly all the selected alkaloids and in particular pleiocarpine and its amino derivative were found to be promising inhibitors of AChE.

Regiospecificity of binding of pleiocarpine, its derivative and drugs (Cognex and Exelon) with AChE

The amino acids residues of AChE involved in binding and interaction with the ligands (pleiocarpine, its derivatives and drugs Cognex and Exelon) at the active site are presented in Fig. 3A-F and Table 3. Analysis of regiospecificity of interacting ligands with AChE revealed that pleiocarpine and its amino-, nitro- and hydroxyl derivatives and the drugs (Cognex and Exelon) interacted in the similar manner (except Tyr341 and Asp74 missing in case of Cognex and Exelon). On the other hand, Tyr133 interacted only with the drugs, but not with pleiocarpine and its

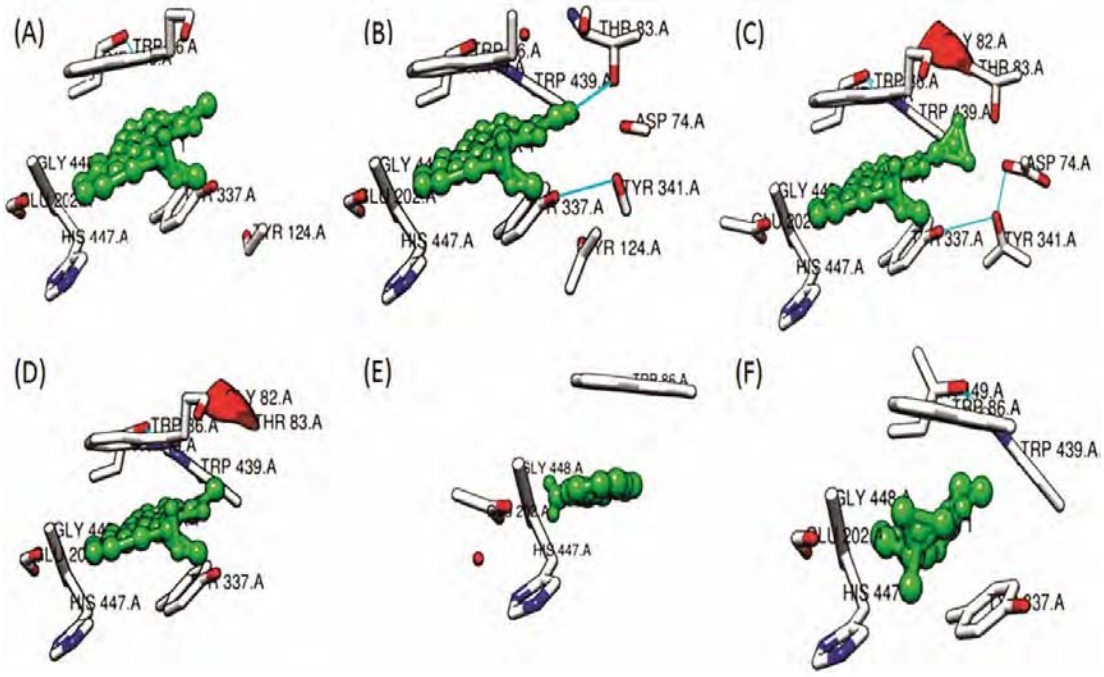

Fig. 3—Comparison of the residues (amino acids) of AChE, involved in binding and interaction with the ligands (A) pleiocarpine, (B) amino-derivative of pleiocarpine, (C) nitro-derivative of pleiocarpine, (D) hydroxyl-derivative of pleiocarpine and the commonly used drugs (E) Cognex and (F) Exelon at the active site

derivatives. The Thr83 and Tyr124 residues interacted with pleiocarpine and its derivative as well as Exelon, but not with Cognex. The Ile451 was the only residue which interacted with Cognex, but not with any of the other ligands analyzed. Nine residues, namely Trp86, His447, Gly448, Gly120, Gly82, Gly121, Tyr337, Glu202 and Tyr449 were the common residues which interacted with all the ligands, including drugs. These common nine residues were also evident at the active site of AChE as predicted using CASTp program.

In vivo and *in vitro* analyses have shown that the active site of AChE consists of aromatic amino acids[27-29]. Among them, Trp is reported to be conserved and crucial for AChE activity[30]. Another study has also reported that the active site of human AChE consists of serine, histidine and glutamate[31]. In agreement to these reports, in the present study also, Trp, Ser, His and Glu residues were found to be involved in the interaction of pleiocarpine, its derivatives and drugs with AChE.

It was also found that none of these ligands (pleiocarpine and its nitro- and hydroxyl- derivatives and the drugs Cognex and Exelon) had any inter-molecular H-bonding with target, however, amino-derivative of pleiocarpine exhibited only one inter-molecular H-bonding between Thr83 and ligand with a distance of 3.1 Å. On the other hand, analysis of intra-molecular H-bonding revealed 2-4 hydrogen bonding in all the cases, except in case of Cognex. Thus, the residues Trp86-Tyr 449 (3.204 Å) and Tyr337-Tyr224 (4.788 Å) were involved in intra-molecular H-bonding in case of binding of pleiocarpine and its amino- and nitro derivatives, whereas in case of hydroxyl derivative and Exelon Trp439-Tyr449 (2.73 Å) were involved in intra-molecular H-bonding, in addition to Trp86-Tyr449 (3.204 Å). The residues Tyr341-Asp74 (2.497 Å) and Trp439-Tyr449 were involved in forming two additional intra-molecular H-bonding only in case of interaction with amino- and nitro derivatives (Table 3).

Conclusion

Thirteen alkaloids were analyzed for their anti-cholinergic potential through docking with AChE as a target and compared with the commonly used drugs, such as Cognex and Exelon. Based on the various docking parameters, pleiocarpine was found to be the most potent anti-cholinergic alkaloid, which can be used as potential therapeutic molecule against AD. Furthermore, as anti-cholinergic agent, the amino derivative of pleiocarpine was found to be the best

among the various pleiocarpine derivatives. The present work can be further extended for analyzing the absorption, distribution, metabolism and excretion (ADME) properties and 3D-QSAR studies of pleiocarpine and its amino derivative for proposing as better putative therapeutic molecules for the treatment of AD from natural sources.

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