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IN SILICO STUDY OF β-CARBOLINE ALKALOIDS AS ACETYLCHOLINESTERASE INHIBITORS: ALTERNATIVE OF NATURAL PRODUCTS FOR THE THERAPEUTIC TREATMENT OF ALZHEIMER'S DISEASE

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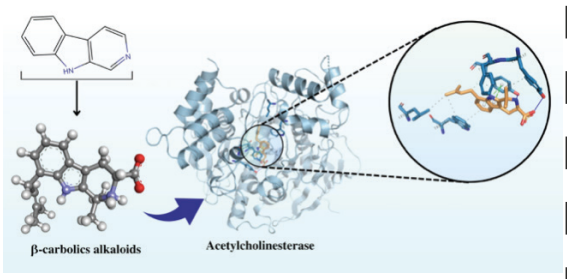
Abstract: Alzheimer's disease is a neurodegenerative disease that mainly affects the elderly population. The cholinergic hypothesis is based on the decrease in the synthesis of the neurotransmitter acetylcholine, compromising the cholinergic function. Thus, a therapeutic strategy adopted for this hypothesis is the use of drugs that inhibit acetylcholinesterase, increasing acetylcholine levels, favoring neurotransmission. The present work aims to carry out the study of β-carboline alkaloids as acetylcholinesterase inhibitors, through molecular docking studies and analysis of their ADME/Tox properties. For this, 92 alkaloids from 14 different subclasses were analyzed. Among them, alkaloid penipaline B showed important interactions with the peripheral anionic subsite of AChE, evidenced by docking studies. The data obtained indicate that the alkaloid penipaline B is the most promising inhibitor among the studied ligands due to its important interactions with the protein and its ADME/Tox properties.

Keywords: acetylcholinesterase, β-carboline, alkaloids, molecular docking.

GA Figure:



GA Text: Docking studies and ADME/Tox analysis studies showed β-carbolines alkaloids as potential acetylcholinesterase inhibition

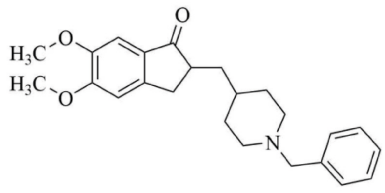
INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia that commonly affects the elderly population. This brain disorder gets worse over time. It's characterized by changes in the brain that lead to loss of memory, trouble understanding visual images and spatial relationships, and impaired reasoning or judgment. Over the time, symptoms become more severe and include increased confusion and behaviour changes.[1]

A person with AD has low levels of acetylcholine (ACh). This neurotransmitter helps to send messages between certain nerve cells and also some of the nerve cells that use acetylcholine are also lost.[2,3] ACh works using synapses in the ganglia of the visceral motor system, and at a variety of sites within the central nervous system.

Acetylcholinesterase (AChE) is an enzyme that catalyzes the breakdown of acetylcholine, disrupting the neurotransmissions signalization.4,5 AChE is a serine hydrolase that creates a tetrahedral intermediate through acid-base reactions with a catalytic triad (SER203, GLU334, e HIS447).[6–8] It appears that AChE may directly interact with amyloid-beta in a manner that increases the deposition of this peptide into insoluble plaques. Also, cholinergic modulation and other functional consequences of AChE inhibition may affect amyloid precursor protein processing and protect neurons against a variety of insults.[9,10]

AChE inhibitors might be able to act as disease-modifying agents rather than as mere palliatives. Currently drugs, such as donepezil (**1**), physostigmine (**2**) and galantamine (**3**), all prevent AChE from breaking down acetylcholine. This means there is a higher concentration of acetylcholine in the brain, which leads to better communication between nerve cells.[11–14]



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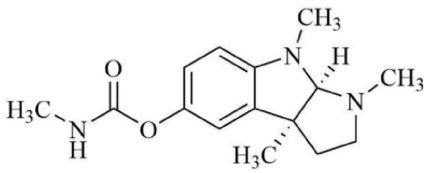

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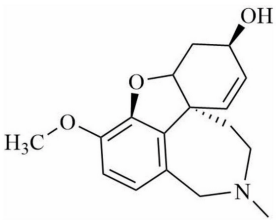

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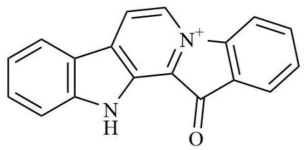
Figure 1. Donepezil (1), Physostigmine (2) and Galantamine (3)

Natural products (NPs) are considered an excellent source for the discovery and development of new drug molecules. A significant number of natural drugs are available in the market. NPs have the potential to yield novel drugs having improved pharmacokinetic properties and biological activities, which provide therapeutic benefits in treating human brain diseases; specially their mechanism of action against AD pathologies.[15–18]

Significant pharmacological properties like neuroprotective, anti-oxidant, anti-inflammatory, anti-apoptotic, etc., demonstrated by phytonutrients like tannins, alkaloids, phenols, carotenoids can be inspected to devise potential drugs.

β-carboline alkaloids have been reported as a class of compounds with anticholinesterase activity.[19,20] These inhibitors act in neurodegenerative-disorder including neuroprotection, neuroinflammation, neurogenesis, tau pathology and amyloid beta accumulation. Therefore, natural-product-based alkaloids with polypharmacology modulation properties are potentially useful for further drug development or, to a lesser extent, as nutraceuticals to manage neurodegeneration.[21,22] It seems likely that new AChE inhibitors, which capitalize on all these strengths would be excellent candidates for future Alzheimer's disease therapy. As examples, fascaplysin derivatives (**5**) can penetrate the blood–brain barrier (BBB) to exert their functions;[19,23,24] 4-oxoprunifoleine (**6**)[25] and prunifoleine (**7**)[25] showed activity against *Electrophorus electricus* (EeAChE).



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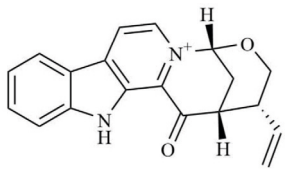

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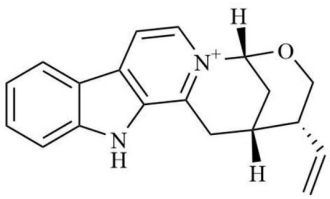

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Figure 2. Structures of β-carbolinic alkaloids, (**5**) fascaplysin, (**6**) 14-oxoprunifoleine, (**7**) prunifoleine, AChE inhibitors.

Guided by this background, we can employ rational design using docking to gain insight into key interactions involved in the molecular recognition of the β-carboline alkaloids AChE inhibitors, evaluate in vitro ADMET properties, and as a result, this present work aims to pave the road for the discovery of new and improved AChE inhibitors.

EXPERIMENTAL

SELECTION OF LIGAND AND PROTEIN STRUCTURES

The choice of the protein structure for the molecular docking studies was based on crystallographic parameters (Å, R, and R_{free}). The crystallographic structure of hAChE complexed with donepexil with PDB code 4EY7[26] and resolution of 2,35Å, was retrieved from *Research Collaboratory for Structural Bioinformatics:* Protein Data Bank. It was chosen due to its good crystallographic parameters and the presence of co-crystallized ligand occupying the catalytic site.

The data set found and used in this work consists of a serie of β-carboline alkaloids isolated from different natural sources, such as, plants, marine organisms and micro-organisms reported elsewhere.[22] About 92 compounds of 14 different subclasses were selected to be evaluated as hAChE ligands. Thus, the 3D coordinates of the commercial drugs donepezil, fisostigmina e galantamina were used to validate the predicted Mode of Binding (pMOB) by molecular docking.

PREPARATION AND OPTIMIZATION OF THE LIGANDS

The 2D structures of the selected compound with due stereochemistry assignments were built using the software *MarvinSketch* v22.19[27] and the protonation state and tautomers of these molecules at a pH of 7.4 (which is the same pH used in biochemical assays). Following, the chemical scaffolds had their geometries optimized using the software *Avogrado* v1.2.0[28] (classic force field: MMFF94) and saved as mol2. The same procedure was adopted for the reference compounds.

VALIDATION BY REDOCKING

Applying GOLD v.2022.2 as a docking software, the re-docking was used to evaluate the accuracy of docking protocols and to assess the ability of the docking program to recreate the original cocrystallized poses of the ligands (PDB: 4EY7). Thus, the set of compounds and the initial pose of the cocrystallized ligand donepezil (E20) were compared to each other and final RMSD (Root Mean Square Deviation) values were obtained. We defined RMSD thresholds values under or equal 2 Å were good. Discovery Studio Visualizer v.21.1.0 software was used for the visualization of the superposition of the compounds with the E20 reference.

MOLECULAR DOCKING PROTOCOL

Using the software GOLD v.2022.2 to prepare the protein, the coordinates of the active site was set up (x = -14.108464 y = -43,832714 z = 27,669929) as a 20 Å cubic box centered at the centroid of the chain A. All water molecules, co-crystallized ligand and crystallography residues were deleted were removed from the ternary complex and hydrogen atoms were added to the crystal structures.

Gold software is able to reproduce bioactive conformations; we therefore used the program in its default configuration to generate 100 conformations for each molecule of the dataset for the docking procedure. Next, an automated ligand docking GoldScore and ASP that uses a genetic algorithm to explore the full range of ligand conformational were applied to range the β-carboline alkaloids according to the score and rescore values. The reference ligands were prepared using the same protocol to compare the score and pMOB. Bioactive conformations with better score than reference ligands were selected for ADME/Tox evaluations.

ADME/TOX PROPERTIES

A preliminary ADME/Tox evaluation can be carried out using SwissADME[29] to give insight into the drug-likeness, physicochemistry and pharmacokinetics of the compounds. In silico studies with selected compounds were used to evaluate the absorption in the gastrointestinal tract (GIT), permeability of the blood-brain-barrier (BBB) and the inhibition of cytochromes P450 (CYPs).[30,31] In addition, the carcinogenicity in rats and mice, as well as inhibition of the hERG gene, were performed through a PreADMETox plataform.[32]

RESULTS AND DISCUSSIONS

VALIDATION BY REDOCKING

In order to evaluate the parameters used for docking and the consistency of the generated model, the E20 reference was re-docked into the active site of AChE. The superposition of co-crystallized and re-docked E20 (donepezil) is shown in Figure 3.

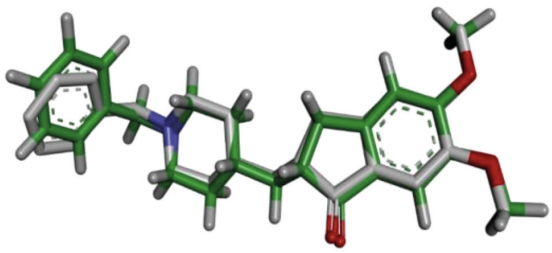

Figure 3. Overlay of redocked donepezil (green) with crystallographic donepezil (white).

The method used for pose selection at the target's active site with a known conformation and orientation of the donepezil, is considered to have performed successfully because the RMSD values obtained is 0.41Å. As observed in Figure 3, only the benzyl moiety adopted a different conformation, as reflected in the RMSD value. Thus, the method is considered validated because it was able to return donepezil to co-crystallized pose.[33]

MOLECULAR DOCKING ANALYSIS

Significant binding affinities were observed for B121, B81 and B132 alkaloids, the scores are better than the reference drugs. Remaining alkaloids showed scores only above physostigmine and galantamine, which suggests that these compounds can have good affinities with AChE. The best results for each subclass are shown in Table 1.

ADME/TOX ANALYSIS

After all calculations completed, a BOILED-Egg grapic is displayed in the Figure 4. The graphical output predicts simultaneously two key ADME parameters, the white region is for high probability of passive absorption by the gastrointestinal tract, and the yellow region (yolk) is for high probability of brain penetration. Yolk and white areas are not mutually exclusive. The outside grey region stands for molecules with properties implying predicted low absorption and limited brain penetration.[29,34] Also, active efflux from the CNS or to the gastrointestinal lumen by colour-coding: blue dots for P-gp substrates (PGP+) and red dots for P-gp non-substrate (PGP−).

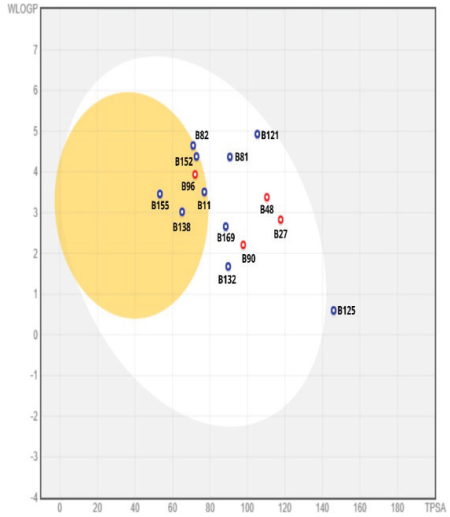

Figure 4. Parâmetros de absorção dos alcaloides β-carbolínicos apresentados no modelo BoiledEgg.

	GoldScore	*ASP*	Subclasse
B121	98.19	72.76	Dimers
B81	86.29	48.57	Tricyclic indole
B132	83.50	46.95	Basic tetrahydro
Donepezil	83.28	62.79	-
B48	82.61	39.37	Acyl-amide tricyclic
B152	81.52	56.85	Cyclic and tetracyclic tetrahydro
B125	80.67	49.31	Dihydro-β-carboline
B169	79.07	64.51	Divergent
B11	76.85	52.54	Basic Tricyclic
B96	73.88	51.95	Cyclic and tetracyclic
B138	72.43	45.36	Tetrahydro acyl
B90	71.75	49.21	Tricyclic imidazole
B82	71.55	56.19	Tricyclic quinoline
B155	71.36	46.76	Tetrahydro dimers
B27	68.89	28.51	Simple acyl tricyclic
Galantamine	66.31	40.49	-
Physostigmine	60.25	42.63	-

Table 1. Docking score obtained by GOLD program using GoldScore and ASP fuctions

	Log Po/w	**TPSA**	**TGI/BHEa**	**CYP[b]**	**Carcinogenicity**		**hERG**
					Rats	Mice	
B11	2.47	76.99Å²	+	1A2, 2C19, 2D6, 3A4	-	-	High Risk
B82	3.67	71.03Å²	+	1A2, 2C19, 2D6, 3A4	-	-	Medium risk
B96	3.30	72.05Å²	+	1A2, 2C19, 2C9, 3A4	-	-	Medium Risk
B138	2.31	65.12Å²	+	-	-	-	Medium Risk
B152	3.49	72.80Å²	+	1A2, 2C9, 2C19, 2D6, 3A4	-	-	Medium Risk
B155	3.46	53.28Å²	+	1A2, 2C9, 2C19, 2D6, 3A4	-	+	Medium Risk

Table 2. ADME/Tox results for the best alcaloides

[a] Positive for absorption in the gastrointestinal tract and permeable through the blood-brain barrier; b CYP450 isoforms that demonstrated inhibition.

As a result, B125 is predicted as not absorbed and not brain penetrant (outside the Egg), B121, B81, B169 and B132 are predicted well-absorbed but not accessing the brain (in the white) and PGP+ (blue dot), B82, B152, B11, B138 and B155 are predicted as passively crossing the BBB (in the yolk), but pumped-out from the brain (blue dot), and B96 is predicted as brain-penetrant (in the yolk) and not subject to active efflux (red dot).

In summary, eight compounds are out of the ideal predicted absorption properties; because it is a requiment for anticholinesterase drugs to cross the BBB to act in the central nervous system (CNS).[35–37]

B121, B81 and B132 obtained the best scores but are located in the white region of the graph. Besides the great TPSA values for these compounds, they won't be absorbed by BBB. On the other hand, the six compounds located in the yellow region are potential candidates as anticholinesterase drugs.[35–37]

Also essential is the knowledge about interaction of alkaloids with cytochromes P450 (CYP) to evaluate drug elimination through metabolic biotransformation. The predicted results for the set of compounds against CYP enzymes are shown in Table 2.

Only compound B138 did not exhibit inhibition for all the superfamily of isoenzymes CYP. Inhibition of these isoenzymes is certainly

one major cause of pharmacokinetics-related drug-drug interactions leading to toxic or other unwanted adverse effects due to the lower clearance and accumulation of the drug or its metabolites.[29,38]

Among the most frequent adverse effects that lead to the failure of drugs, cardiac arrhythmias, is one of the major causes. It can be caused by the inhibition of the cardiac potassium channel (K+) encoded by the human ether-à-go-go-related gene (hERG), and consequently have received increasing regulatory agencies attention.[39–41] Using *in silico* models to predict the potential of the alkaloids to act as hERG inhibitor, only B11 showed high risk of interactions with this gene. Furthermore, any of the selected alkaloids under this study showed carcinogenicity in rats and mice, except, compound B155 was considered carcinogenic to mice.

ENZYME-INHIBITOR INTERACTIONS WITH ACHE BINDING SITE

According to the results of docking and the ADMET profile, alkaloid B138 (Figure 4) proved to be the most promising β-carboline alkaloid compared to AChE. B138 exhibited a higher score than drugs already approved for the therapeutic treatment of AD, showed the best ADME/Tox profile, it is permeable by the BBB, absorbed by the TGI, not a inhibitor for CYP enzymes and showed low toxicity.

This alkaloid showed some important interactions to act as an AChE inhibitor. TYR337 interacts with the ligand through hydrogen bonding with the ligand's carboxylate group, acting as a hydrogen acceptor for this residue. Furthermore, TYR337 also interacts with the positive charged quaternary amino group by π-cation interaction. This residue is part of the peripheral anionic subsite and its main function is to guide and orient the positive part of the substrate to the active site of the enzyme[13], acting as an oscillating door at the entrance of substrates, being an important interaction for the inhibition of AChE.[42] A Interaction with this residue is also observed in all reference drugs.

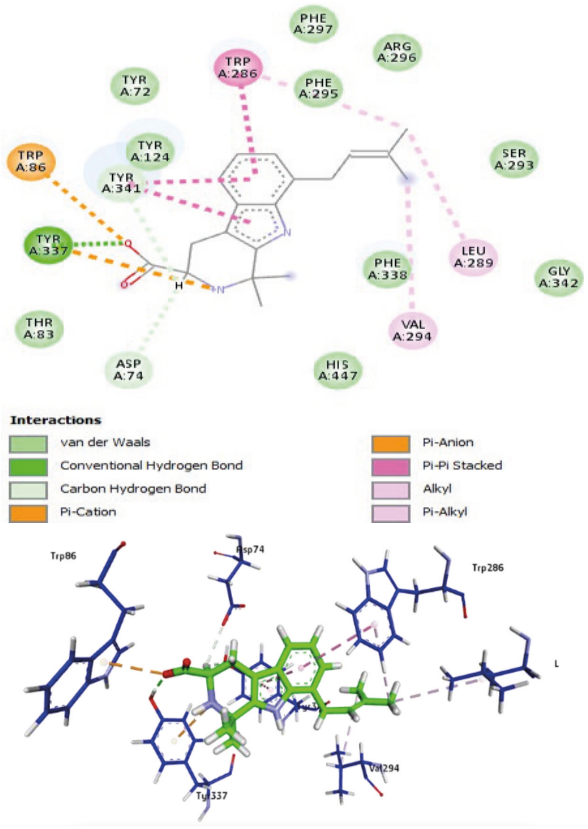

Figure 5. B138 alkaloid interactions with AChE.

Other important interactions take place with TRP86 from the anionic subsite, TYR341 and TRP286 of the PAS, through π-anion and π stacking, respectively. These residues contribute to the potential inhibition of ligands, especially for TRP86. Inhibitors such as donepezil, this interaction takes place by π-cation between protonated groups,[8] however, it was not possible to obtain this mode of interaction with the alkaloid B138, because this interaction with TRP86 takes place through a π-anion with the deprotonated oxygen of the alkaloid carboxylate group (Figure 4). In general, this alkaloid showed interactions that are also observed in reference drugs, binding to more than one subsite,

which may indicate that this ligand can act in the inhibition of AChE. These interactions are found with greater similarity in the drugs donepezil, galantamine and physostigmine.

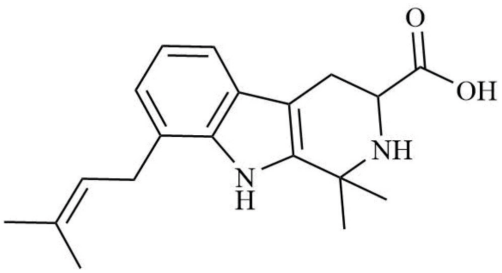

Figure 6. Structure of the alkaloid Penipaline B.

Penipaline B (Figure 5)[22] is a β-carboline alkaloid of the tetrahydro acyl subclass, where it was first isolated from the fungus Penicillium paneum and its cytotoxic activity against two types of tumor cells was reported.[43]

CONCLUSIONS

The in silico study carried out here provided the evaluation of several β-carboline alkaloids against AChE, which may act as a therapeutic alternative for Alzheimer's disease. With molecular docking it was possible to fit the ligands in different conformations, showing their affinities with AChE, and to compare with some drugs that are known to inhibit this enzyme. In addition, the results obtained by SwissADME and PreADMETox, pointed out that the alkaloid Penipaline B, demonstrated the best ADME/Tox profile, being easily absorbed by the GI tract and permeable by the BBB, which is consistent with the activity of drugs that act in the CNS. This alkaloid also showed low toxicity and carcinogenicity. Finally, it was possible to visualize important interactions of Penipaline B with AChE, being similar to the interactions also found in the reference drugs that is important for the inhibition of this enzyme, evidencing its anticholinesterase potential. It is also important to point out that more accurate in vitro analyzes and clinical tests are essential to experimentally evaluate the enzymatic inhibitory behavior of this alkaloid.

The in silico study carried out here provided the evaluation of several β-carboline alkaloids, against AChE, which may act as a therapeutic alternative for Alzheimer's Disease. With molecular docking, it was possible to fit the ligands into different conformations, highlighting their affinities with AChE, comparing some drugs that are known to inhibit this enzyme. Furthermore, the results obtained by SwissADME and PreADMETox showed that the alkaloid Penipalina B demonstrated the best ADME/Tox profile, being easily absorbed by the GIT and permeable by the BBB, which is consistent with the activity of drugs that act on the CNS, in addition to having low toxicity and carcinogenicity. Finally, it was possible to notice important interactions between Penipalin B and AChE, similar to the interactions also found in reference drugs, said to be important for the inhibition of this enzyme, highlighting its anticylinesterase potential, which is the best candidate analyzed here.

It is also important to point out that more accurate in vitro analyzes and clinical tests are essential to experimentally evaluate the enzymatic inhibitory behavior of this alkaloid.

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