Available online on 15.12.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

In silico pharmacological study of lacourtianal, a new terpenoid isolated from the stem bark of *Chrysophyllum lacourtianum* De Wild (Sapotaceae) against Alzheimer's disease

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Article Info:

Article History:

Received 18 Sep 2023
Reviewed 07 Nov 2023
Accepted 24 Nov 2023
Published 15 Dec 2023

Cite this article as:

Ambamba Akamba BD, Piebeng CQN, Kenassi MBN, Ebouel FLE, Nanhah JVK, Ella FA, Ngoumen DJN, Talla RM, Mandob DE, Ngondi JL, *In silico* pharmacological study of lacourtianal, a new terpenoid isolated from the stem bark of *Chrysophyllum lacourtianum* De Wild (Sapotaceae) against Alzheimer's disease, Journal of Drug Delivery and Therapeutics. 2023; 13(12):84-90

DOI: http://dx.doi.org/10.22270/jddt.v13i12.6322

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Abstract

Urgent need to treat, prevent, delay the onset, slow the progression and reduce the symptoms of Alzheimer's disease (AD) is dictated by the growing number of people affected by the disease. This research requires an *in silico* approach. The aim of this work was to carry out an *in silico* pharmacological study of lacourtianal, a new terpenoid against Alzheimer's disease. The structure of lacourtianal and reference drugs were drawn on chemdraw 2D. Energy minimization was performed using the molecular mechanics force field (MM2) and saved in the PDB using *chemdraw 3D*. The compound's Smiles format was entered into the *pk-CSM* web servers for ADME/Tox parameter prediction. Conformational site analysis and docking parameters such as binding energy, interaction profiles with AD target residues (AchE, BuchE, β-secretase and GSK-3β) were determined using *AutoDock 4.2* and *Discovery Studio visualizer*. Lacourtianal is a valuable oral drug candidate. It crosses the Blood-brain barrier (BBB) and shows considerable docking scores for AD targets. These scores were higher for BuchE and β-secretase compared with reference compounds. It binds to residues Phe297, Phe 338 and Tyr31, Trp286 in the acyl pocket and peripheral site of AchE, respectively. Importantly, it establishes low-energy interactions (hydrogen and pi) with the His438 residue of the Butyrylcholinesterase catalytic triad. It also establishes favorable interactions with residues Tyr71 and Tyr216, which are residues controlling substrate accessibility to the active site of the BACE1 and GSK 3β enzymes respectively. These results show that lacourtianal has promising therapeutic potential for the treatment of Alzheimer's disease.

Keywords: Lacourtianal, *in silico*, AchE, BuchE, β-secretase, GSK-3β and AD

1. INTRODUCTION

According to the National Institute on Aging and Alzheimer's Disease, it is estimated that more than 50 million people worldwide are living with Alzheimer's disease, and this number is expected to reach 152 million by 2050 [1]. AD is much more common in the United States, with a current prevalence of around 6.5 million cases and an estimated increase to 13.8 million by 2050. Africa, Europe and Asia are not spared, and pooled data from population studies in Europe suggest that age-standardized prevalence among 65-year-olds is 4.4 %. This compares with prevalences of 4.0 % in China and the Western Pacific, 4.6 % in Latin America, 5.4 % in Western regions and 1.6 % in Africa [2]. Particular attention is being paid to AD in developing countries because, due to the absence of a real marker, AD is most often confirmed unambiguously post mortem, whereas the first cerebral lesions appear 10 to 15 years before the onset of clinical symptoms [3]. Meanwhile, the lack of a curative treatment, as well as the ever-increasing ageing of the population, make it a real major public health problem [3]. AD is multifactorial and not all the pathophysiological mechanisms are known, despite numerous efforts to combat it. These have focused mainly on treating

clinical symptoms. To date, more than 400 clinical trials have failed [4], due to the heterogeneity of the disease and the selectivity of the BBB, and 187 trials are ongoing [5]. Recently, two monoclonal antibodies have been approved by the FDA. These antibodies are capable of reducing the accumulation of amyloid plaques, one of the central features of the pathogenesis of the disease. However, there is considerable controversy because they do not improve patients' quality of life, and a confirmatory post-approval trial will not be completed before 2030 [6,7]. This is why the research challenge remains the development of new anti-AD drugs that can both reduce symptoms and act on the key factors in the pathogenesis of the disease. There are many key enzyme targets for the treatment of Alzheimer's disease, such as β-secretase, GSK-3β [8]. Cholinesterases (AchE and BuchE) are the targets for memory loss, which is the main symptom of AD. In advanced AD, AChE activity falls by 90 % and BuChE activity reaches 105 to 165 % of normal [9]. For this reason, the selective AChE inhibitors approved have a very limited effect on severe AD. It would therefore make sense to find new compounds that can inhibit both AchE and BuchE to reduce the symptoms of the disease and inhibit the key targets of the disease (β-secretase and GSK-3β) to slow the progression of the disease. This research involves the *in silico* approach, which has become indispensable in current research because it saves time, money and consumables and provides a favorable framework for assessing the affinity and deciphering the interactions between a ligand and a target protein [10]. Terpenoids have attracted a great deal of interest in the search for neuroprotective compounds because they can easily cross the BBB and interact as scaffolds in various signaling pathways [11]. Lacourtianal is a new terpenoid isolated from the stem bark of *Chrysophyllum lacourtianum* De Wild (Sapotaceae) highlighted by [12], which has antibacterial potential. To date, no study has investigated its neuroprotective potential against AD. In this research paper, we present the *in silico* pharmacology of lacourtianal against Alzheimer's disease.

2. MATERIALS AND METHODS

2.1. ADME/Tox evaluation

Compound was designed in the *pk-CSM* interactive platform, and the pharmacokinetics as well as the toxicological parameters including the potential to cross the BBB were evaluated [13].

2.2. Molecular docking

2.2.1. Software used

Python 2.5, Python 2.7, *Molecular Graphics Lab Tools* (MGL), *AutoDockTools-1.5.7*, *Discovery Studio Visualizer 2.5.5*, and *ChemDraw* were used. The Python 2.7 was downloaded from www.python.com, Cygwin (a data store) c:\program; and Python 2.5 were downloaded simultaneously from www.cygwin.com. Molecular Graphics Lab Tools (MGL) and *AutoDockTools-1.5.7* were downloaded from www.scripps.edu; *Discovery Studio Visualizer 2.5.5* was downloaded from www.accelerys.com; and *ChemDraw* was downloaded from www.clubic.com. Online smile scoring was performed using cactus.nci.nih.gov/translate/ [14].

2.2.2. Preparation of the target enzyme

The crystal structures of AchE, BuchE, β-secretase, GSK-3β proteins were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database. Preparation of the target proteins with *AutoDock* tools involved the addition of all hydrogen atoms to the macromolecule, a necessary step for the correct calculation of partial atomic charges. Three-dimensional affinity grids (which close the amino acids of the active site of each protein) sized with a spacing of 0.375 Å were centered on the geometric center of the proteins and were calculated for each of the following atom types (HD, C, A, N, OA and SA), representing all possible atom types in a protein (Table 1) [15].

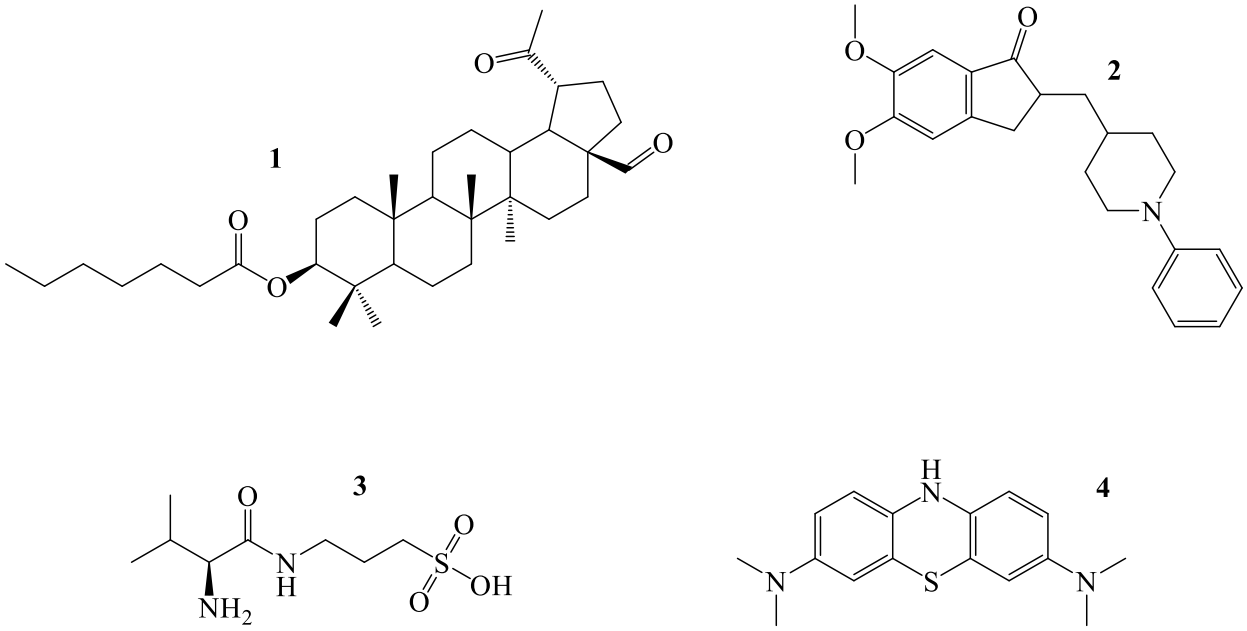
Table 1: Grid center coordinates

Protein	AchE (5hcu)	BuchE (5dyw)	β-secretase (2WJ0)	GSK-3β (4acc)
Grid point (X, Y, Z)	(60, 58,68)	(72, 108,72)	(110, 90,92)	(40, 40,40)
Grid center dimension (x, y, z)	(1.844, 9.360, -15.328)	(-41.056, 1.41, -7.139)	(20.278, 38.927, 47.389)	(28.689, -1.395, 14.195)

AchE: *Acetylcholinesterase,* ***BuchE:*** *Butyrylcholinesterase,* ***GSK-3β:*** *Glycogen synthetase kinase 3 beta*

2.2.3. Preparation of the ligand

Two dimensional structures (2D) of lacourtianal and reference drug (donepezil, valiltamiprosate and hydromethylthionine) compounds were drawn using *ChemDraw* software (figure 1). Three-dimensional structures (3D) were obtained using chemdraw 3D software and energy minimization was performed using the Molecular Mechanics (MM2) force field and saved in PDB format with the same software.



1: lacourtianal, 2: Donepezil, 3: valiltamiprosate , 4: hydromethylthionine

Figure 1: lacourtianal and reference drugs

2.2.4. In silico inhibition

Lamarckian genetic algorithm (LGA) was used for ligand conformation search, which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first constructs a population of individuals (genes), each having a different random conformation of the anchored molecule. Each individual is then mutated to acquire a slightly different translation and rotation, and the local search algorithm then performs energy minimizations on a user-specified proportion of the population of individuals. The individuals with the lowest resulting energy are transferred to the next generation and the process is repeated. The algorithm is called Lamarckian because each new generation of individuals is allowed to inherit the local search adaptations of its parents. Gasteiger charges are calculated for each atom of the macromolecule in *AutoDock 4.2* instead of the Kollman charges that were used in previous versions of this program [16]. The docking calculation in *AutoDock 4.2* was performed using the refined protein and the desired ligand in .pdb format. The execution of the *AutoGrid* calculation was performed using the .glg file. The execution of *AutoDock* is performed by the dlg file. Finally, 10 different conformations were studied for a single compound. For each ligand, 25 best docking simulations were obtained against the target molecule [17]. The results were visualized using *the Discovery Studio visualizer*.

3. RESULTS

3.1. Prediction of ADME/Tox parameters

Table 2 shows the prediction of ADME/Tox parameters.

Pharmacokinetic and toxicological prediction of the compound of interest shows its good permeability through its predictive value of Caco-2 permeability (Log Papp in 10-6 cm/s 1.386 > 0.9). This molecule has good absorption in the intestine via its predictive value (80.289 > 30). Nevertheless, it has a low volume of distribution. It readily crosses the BBB, reflected by a predictive value of log BBB = 0.349 > 0.3. It is permeable to the CNS, with a predictive value of Log PS = -1.214 > -2. It inhibits only the CYP1A2 isoform, with a total clearance predictive value of -1.402. The compound doesn't inhibit hERG I and II. The predictive value for acute oral toxicity is 2.482 mol/kg, implying that it isn't hepatotoxic.

Table 2: Prediction of ADME/Tox parameters for Lacourtianal

Model Name	Predicted Value	Unit
Caco2 permeability	1.386	Numeric (log Papp in 10-6 cm/s)
Intestinal absorption (human)	80.289	Numeric (% Absorbed)
P-glycoprotein substrate	Yes	Categorical (Yes/No)
P-glycoprotein I inhibitor	No	Categorical (Yes/No)
P-glycoprotein II inhibitor	No	Categorical (Yes/No)
VDss (human)	-2.123	Numeric (log L/kg)
Fraction unbound (human)	0.286	Numeric (Fu)
BBB permeability	0.349	Numeric (log BB)
CNS permeability	-1.214	Numeric (log PS)
CYP2D6 substrate	No	Categorical (Yes/No)
CYP3A4 substrate	No	Categorical (Yes/No)
CYP1A2 inhibitor	Yes	Categorical (Yes/No)
CYP2C9 inhibitor	No	Categorical (Yes/No)
CYP3A4 inhibitor	No	Categorical (Yes/No)
total Clearance	-1.402	Numeric (log ml/min/kg)
Renal OCT2 substrate	No	Categorical (Yes/No)
AMES toxicity	Yes	Categorical (Yes/No)
Max. tolerated dose (human)	1.165	Numeric (log mg/kg/day)
hERG I inhibitor	No	Categorical (Yes/No)
hERG II inhibitor	No	Categorical (Yes/No)
Oral Rat Acute Toxicity (LD50)	2.482	Numeric (mol/kg)
Hepatotoxicity	No	Categorical (Yes/No)
Skin Sensitization	No	Categorical (Yes/No)
T. Pyriformis toxicity	0.285	Numeric (log ug/L)
Minnow toxicity	5.598	Numeric (log mM)

3.2. Molecular docking scores of lacourtianal AD therapeutic targets

3.2.1. Docking scores of compound of interest on Alzheimer's disease therapeutic targets

Table 3 shows the docking scores of compound of interest on Alzheimer's disease therapeutic targets.

According to *AutoDock* software, compounds likely to bind with negative energy (ΔG) highlight an existential affinity between a compound and its target. With this in mind, a higher negative value indicates a better docking score and better binding power. Lacourtianal showed docking scores between -6.09 and -11.30 kcal/mol against the four selected targets associated with Alzheimer's disease. Similarly, compounds used as standards presented docking scores between -6.79 and -10.91 kcal/mol on the same targets studied respectively. On the other hand, lacourtianal showed the best activities on BuChE (PDB ID: 5dyw), β-secretase (PDB ID: 2wjo) compared to the clinical trial standards with binding energies of -11.30Kcal/mol (Table 3).

Table 3: Molecular docking scores (Kcal/mol) of lacourtianal with pipeline drugs against four different targets proteins associated with AD

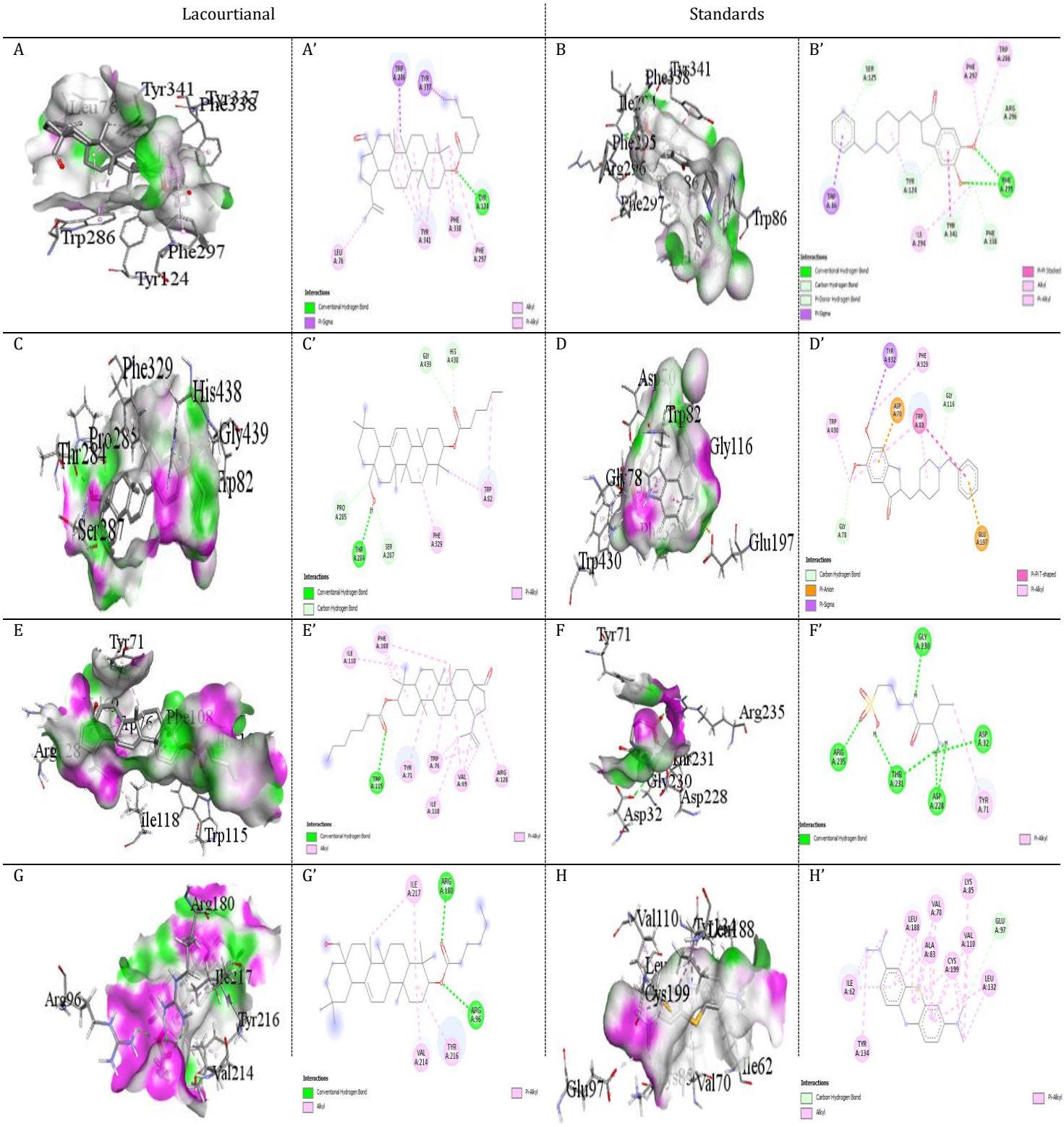
Lacourtianal / Standard	Selected target			
	AChE	**BuChE**	**BACE1**	**GSK3β**
Lacourtianal	-9.18	-10.68	-11.30	-6.09
Donepezil	-10.91	- 9.02	/	/
Valiltamiprosate	/	/	-6.80	/
hydromethylthionine	/	/	/	-6.79

AchE: *Acetylcholinesterase,* ***BuchE:*** *Butyrylcholinesterase,* ***GSK-3β:*** *Glycogen synthetase kinase 3 beta*

3.2.2. Interaction profiles of compounds of interest and reference drugs with AD therapeutic targets

Figure 2 shows the interactions between various active site residues, the compound of interest, as well as reference compounds.

Analysis of 2D and 3D diagrams of the established interactions showed that, lacourtianal penetrates the active site of Acetylcholinesterase by binding to residues Phe297, Phe 338 and Tyr31, Trp286 respectively of the acyl pocket and the peripheral site (Figure 2). Even better than donepezil, it establishes low energy interactions (hydrogen and pi) with the His438 residue of the catalytic triad of Butyrylcholinesterase. In this momentum, we also obtained favorable interactions with residues Tyr71 and Tyr216 which are residues controlling the accessibility of the substrate to the active site of the enzymes BACE1 and GSK 3β respectively (Figure 2).



(A, A' and B, B'): represent interactions with Acetylcholinesterase; (C, C' and D, D'): represent interactions with Butyrylcholinesterase; (E, E' and F, F'): represent interactions with BACE1; (G, G' et H, H'): represent interactions with GSK 3β.

Standards (AchE and BuchE): Donepezil, Standard BACE-1 : Valiltamiprosate, Standard GSK 3β : hydromethylthionine

All standards are compounds either approved by the FDA or listed in the pepiline of AD drugs in clinical trials [5].

Figure 2: 3D and 2D views of molecular interactions of Lacourtianal and drugs in development on AD protein targets

4. DISCUSSION

The need to treat, prevent, delay the onset, slow the progression and improve the symptoms of Alzheimer's disease is dictated by the growing number of people with Alzheimer's disease and the growing public health crisis posed by the disease [18]. Conventional drug discovery strategies in the past were time-consuming and costly. Recently, *in silico* approaches have attracted considerable interest because of their potential to accelerate drug discovery in terms of time, labor and cost [10]. The aim of our study was to investigate *in silico* pharmacological study of lacourtianal, a new terpenoid isolated from the stem bark of *Chrysophyllum lacourtianum* De Wild (Sapotaceae) against Alzheimer's disease. Pharmacokinetics (PK) plays an important role in drug discovery, as it helps define the absorption, distribution, metabolism and excretion (ADME) properties of candidates, with the ultimate aim of achieving a clinical candidate that achieves an adequate concentration-time profile in the body for the desired efficacy and safety profile [19]. It has been reported that 50 % of drug candidates fail due to a poor ADMET profile. Terpenoids have aroused great interest because of their neuroprotective effect and their potential to cross the BBB [20]. This potential to cross is a positive point as many drugs fail in clinical trials due to BBB selectivity. Predictive values for ADME/Tox parameters showed good intestinal mucosal permeability and intestinal absorption, thus predicting good oral bioavailability of this compound. The latter crosses the BBB and penetrates the CNS, according to predictive values, and would be a good candidate to act as a scaffold and modulate brain protein activity [21]. This ADME/Tox prediction is in line with that of the terpenoids studied by [22]. Lacourtianal has a docking score similar to that of donepezil on AchE, and higher on BuchE. Furthermore, the interaction profile (with the exception of Trp 86) with AchE active pocket residues is similar to that of donepezil. Importantly, lacourtianal interacts with His 438 of the BuchE catalytic triad via the pi-hydrogen bond. This effect highlights a potential candidate to correct the limitations of the cholinesterase targets approved for the treatment of AD. These do not effectively inhibit BuchE, despite the fact that in severe AD it is the main enzyme involved in acetylcholine hydrolysis. Accumulation of senile plaques is central to AD, occurring as a result of fibrillation of amyloid β-peptide, which is produced by the action of β-secretase on the β-amyloid precursor protein. β-secretase is a key target in AD. We recorded a higher docking score than valiltamiprosate (a drug in phase 3 clinical trial): -11.3 versus -6.8 Kcal/mol. This high score reflects lacourtianal's interaction with the residue Tyr71, which controls substrate accessibility to the active site of BACE1 enzymes [23]. Hyperphosphorylation of the tau protein is one of the main alterations leading to AD. Tau protein is mainly phosphorylated by GSK 3β [24]. The results show that, lacourtianal has a docking score similar to that of the reference compounds (hydromethylthionine) [25]. The latter interacts with Tyr216 which are residues controlling the accessibility of the substrate to the active site of the enzymes GSK 3β [26].

5. CONCLUSION

In silico investigation of lacourtianal showed that, this compound has a promising therapeutic potential for the treatment of AD, so it would be a goldmine as it has a multi-target action by inhibiting AchE and BuchE, which could considerably reduce AD symptoms. It also inhibits β-secretase and GSK 3β, which could limit disease progression. Further *in vitro* and *in vivo* studies are required to confirm this potential.

Competing Interests

The authors have declared that no competing interests exist.

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