*Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii*
*Vol. 33, issue 2, 2023, pp. 49 - 55*


# DISCOVERY OF NOVEL ACETYLCHOLINESTERASE INHIBITORS FOR ALZHEIMER'S DISEASE USING MOLECULAR DOCKING TECHNIQUE

**Brahim BENAICHA[1,2], Salim GASMI[2]*, Chaima HELIMI[2], Ouissal HADFI[2], Soufian SAADI[2]**

[1]Laboratory of Toxicology and Ecosystems Pathologies, Echahid Cheikh Larbi Tebessi University, Tebessa 12002, Algeria
[2]University of Tebessa, Faculty of Sciences and Natural and Life Sciences, Tebessa, 12000, Algeria

**Abstract:** Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and changes in social and behavioral functions. In this study, we screened 90 phytochemical compounds as potential inhibitors of acetylcholinesterase (AChE) using molecular docking techniques to identify candidates with high binding affinity and favorable pharmacokinetic properties. Molecular docking simulations were conducted using the MOE (Molecular Operating Environment) software, identifying 11 top candidates with binding scores superior to Donepezil. Among these, Tangeritin was highlighted for its favorable ADME (Absorption, Distribution, Metabolism, and Excretion) profile and low toxicity predictions, suggesting it is a promising lead for further development. This research underscores the potential of in silico methods in the efficient discovery of new therapeutic agents for AD.

**Keywords:** Alzheimer's disease, behavioral, acetylcholinesterase, molecular docking, Donepezil, in silico.

## INTRODUCTION

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder globally, particularly among older populations, and represents a significant health and societal burden. It is marked by progressive cognitive decline, including memory impairment, diminished judgment, and eventual loss of behavioral and social abilities (Carrillo et al., 2012). These symptoms arise due to the breakdown of acetylcholine (Ach), a key neurotransmitter involved in memory and cognition. The situation is exacerbated by the rapid enzymatic degradation of Ach by acetylcholinesterase (AChE), leading to reduced cholinergic transmission in the brain (Huang et al., 2022; Chen., 2022). AChE inhibitors have emerged as a therapeutic approach to AD by preventing the enzymatic breakdown of Ach, thereby enhancing cholinergic transmission. Donepezil, Rivastigmine, and Galantamine are commonly used AChE inhibitors; however, they exhibit limited efficacy, significant side effects, and fail to halt disease progression (Vecchio et al., 2021; Tan et al., 2014). This highlights the urgent need for novel AChE inhibitors with better efficacy, higher selectivity, and reduced toxicity (Benaicha et al., 2024; Zhang et al., 2019). Molecular docking, an advanced computational approach, is widely employed to predict ligand-receptor interactions. It evaluates binding affinities based on spatial complementarity and interaction energies. By modeling how molecules bind to AChE at the atomic level, docking enables the efficient screening of numerous compounds, prioritizing those with promising therapeutic potential for experimental validation (Guedes & Dardenne, 2014). This cost-effective method significantly streamlines drug discovery by identifying promising candidates prior to in vitro or in vivo testing (Bai et al., 2018). This study investigates 90 phytochemicals as potential AChE inhibitors, employing molecular docking to evaluate their binding affinities while predicting pharmacokinetic and toxicity profiles. Our goal is to identify novel inhibitors with improved therapeutic properties for AD and to provide a foundation for future research in this area. The introduction will also include a brief overview of the relevance and advantages of the in silico method used.

## MATERIALS AND METHODS

### Software and Databases

The Molecular Operating Environment (MOE) was selected for its robust molecular docking capabilities, enabling the simulation of ligand-protein interactions within the active site of acetylcholinesterase (AChE) (Kader et al., 2024). The protein structure of AChE was obtained from the Protein Data Bank (PDB), ensuring accurate 3D conformations for precise docking studies (Ganeshpurkar et al., 2020). Additionally, a total of 90 phytochemical ligands were retrieved in 3D format from PubChem, offering a diverse collection of compounds recognized for their medicinal properties. This combination of tools and resources facilitated a comprehensive analysis of ligand interactions with the AChE active site.

### Protein and Ligands Preparation

The AChE protein (PDB ID: 4EY7) was processed by removing all water molecules, ions, and irrelevant co-factors to ensure a clean active site. Hydrogen atoms were added to both the protein and ligands to optimize binding interactions (Kader et al., 2024). Ligands were energy-minimized to their most stable configurations, facilitating accurate docking simulations.

### Docking Protocol

Docking simulations were conducted to predict the binding affinity of each ligand within AChE's active

***Correspondence**: Dr Salim Gasmi, University of Tebessa, Faculty of Sciences and Natural and Life Sciences, Tebessa, Algeria. TEL: (+213)673203709, email: Salim.gasmi@univ-tebessa.dz*

site. We focused on critical residues known to influence AChE inhibition, such as Trp86, Tyr341, and Phe 295 Figure 1. These residues are essential for stabilizing ligands through hydrophobic, hydrogen-bonding, and cation-π interactions. Each ligand was docked multiple times to confirm the stability and consistency of the binding conformation, with RMSD values used as a measure of structural reliability (Pantsar & Poso, 2018).

### ADME and Toxicity Predictions

The top-performing ligands from the docking studies were subjected to further evaluation to ensure their pharmacokinetic suitability and safety. SwissADME was utilized to analyze key pharmacokinetic properties, including solubility, membrane permeability, and drug-likeness, providing insights into the compounds' potential as viable drug candidates. Additionally (Ojuka et al., 2023), Protox-II was employed to predict toxicity levels, expressed as LD50 values, offering an initial safety assessment of the ligands to gauge their suitability for further development (Laskar et al., 2023).

## RESULTS AND DISCUSSION

### Docking scores and binding affinities

Out of the 90 ligands screened, 11 exhibited docking scores indicating stronger binding affinities than Donepezil (reference score: -8.1263 kcal/mol) Figure 1. These ligands were selected based on their ability to form stable interactions within the AChE active site. The results of the simulation by MOE of these inhibitors are represented below in Table 1.

**Table 1.**

The Interaction energy (kcal/mol) of AChE and studied inhibitors obtained by molecular docking.

| N° | Ligands | Score (Kcal/mol) | RMSD |
|---|---|---|---|
| | **Donepezil** | **-8.12** | **0.84** |
| 1 | 6-Shogaol | -7.25 | 1.06 |
| 2 | α-Zingiberen | -5.98 | 0.76 |
| 3 | Andrographolid | -6.55 | 0.66 |
| 4 | Apigenin | -5.92 | 0.89 |
| 5 | Bassic acid | -5.30 | 2.54 |
| 6 | Linoleic acid | -7.12 | 1.34 |
| 7 | Berberine | -7.56 | 0.89 |
| 8 | Chlorogenic acid | -6.87 | 1.08 |
| 9 | Chrysin | -5.97 | 2.47 |
| 10 | Colchicin | -7.68 | 1.07 |
| 11 | Corydin | -7.54 | 1.43 |
| 12 | Crocetin | -6.95 | 2.35 |
| **13** | **Curcumin** | **-8.41** | **2.12** |
| 14 | Curdion | -6.14 | 0.77 |
| 15 | Cyanidin | -5.96 | 1.72 |
| 16 | Decursinol | -5.69 | 2.63 |
| 17 | Ellagic acid | -6.05 | 0.71 |
| 18 | Emodin | -6.08 | 0.98 |
| 19 | Epicatechin | -6.49 | 1.95 |
| 20 | Epigallocatechin | -5.88 | 1.08 |
| 21 | Eriodyctiol | -6.47 | 2.59 |
| **22** | **Etoposide** | **-9.84** | **1.81** |
| 23 | Eugenol | -5.13 | 1.04 |
| 24 | Fisetin | -6.29 | 0.69 |
| 25 | Genkwanin | -6.04 | 1.03 |
| **26** | **Ginkgetin** | **-8.54** | **2.35** |
| 27 | Isorhamnetin | -6.43 | 1.59 |
| 28 | Kaempferol | -5.93 | 1.34 |
| 29 | Licochalcone A | -7.53 | 1.19 |
| 30 | Luteolin | -6.05 | 0.99 |
| 31 | Hispidulin | -6.09 | 0.63 |
| 32 | Nectandrin B | -7.34 | 1.89 |
| 33 | Niaziminin | -7.78 | 2.95 |
| 34 | Nimbolide | -7.65 | 1.31 |
| 35 | Oleanolic acid | -5.76 | 1.44 |
| 36 | Panaxadiol | - 4.72 | 1.17 |
| 37 | Panaxatriol | -5.69 | 1.28 |
| 38 | Plumbagin | -4.86 | 1.48 |
| 39 | Podophyllotoxin | -7.49 | 1.33 |
| 40 | Quercetin | -5.92 | 1.89 |
| 41 | Salvicine | -7.32 | 3.89 |
| **42** | **Silibinin** | **-8.36** | **1.58** |
| **43** | **Tetrandrine** | **-8.22** | **2.00** |
| 44 | Theaflavin | -7.62 | 1.94 |
| 45 | Tylophorine | -7.53 | 0.65 |

| N° | Ligands | Score (Kcal/mol) | RMSD |
|---|---|---|---|
| 46 | Ursolic acid | -4.34 | 5.53 |
| 47 | Withaferin A | -7.08 | 1.25 |
| **48** | **Yuanhuanin** | **-8.19** | **1.95** |
| 49 | Hecogenin | -5.80 | 1.42 |
| 50 | Gallic acid | -4.13 | 1.19 |
| 51 | Naringin | -8.00 | 1.66 |
| 52 | Capsaicin | -8.06 | 0.89 |
| 53 | Psychotrine | -7.36 | 2.12 |
| 54 | Plicamine | -7.60 | 1.11 |
| 55 | Narciclasine | -6.68 | 1.83 |
| 56 | Catechin | -6.34 | 1.65 |
| 57 | Lycoricidine | -7.03 | 1.41 |
| 58 | Caffeic ac | -4.72 | 3.30 |
| 59 | Coumaric ac | -4.16 | 1.65 |
| 60 | Menthol | -5.14 | 0.75 |
| 61 | Feulic ac | -5.11 | 0.95 |
| 62 | Rosmarinic ac | -7.04 | 1.51 |
| 63 | Sinapic ac | -5.17 | 0.57 |
| 64 | Vitamin C | -4.91 | 3.87 |
| 65 | Vitamin E | -8.06 | 1.18 |
| 66 | Melatonin | -6.35 | 2.30 |
| **67** | **Alpha carotene** | **-8.81** | **1.42** |
| **68** | **Astaxanthin** | **-9.20** | **2.65** |
| 69 | Beta carotene | -8.94 | 1.50 |
| **70** | **Canthaxanthin** | **-8.85** | **1.45** |
| **71** | **Lutein** | **-9.53** | **2.78** |
| 72 | Lycopene | -9.23 | 2.15 |
| 73 | Zeaxanthin | -8.74 | 2.35 |
| **74** | **Tangeritin** | **-8.09** | **1.02** |
| 75 | Myricetin | -6.11 | 1.22 |
| 76 | Hesperetin | -6.69 | 0.79 |
| 77 | Gallocatechin | -6.94 | 1.46 |
| 78 | Daidzein | -6.08 | 1.00 |
| 79 | Ganistein | -5.84 | 3.46 |
| 80 | Glycitein | -6.13 | 1.78 |
| 81 | Resveratrol | -5.56 | 0.76 |
| 82 | Pterostilbene | -6.41 | 1.29 |
| 83 | Delphinidin | -6.55 | 1.75 |
| 84 | Malvidin | -6.70 | 2.26 |
| 85 | Pelargonidin | -6.07 | 1.89 |
| 86 | Peonidin | -5.95 | 2.99 |
| 87 | Petunidin | -6.47 | 0.70 |
| 88 | Cichoric acid | -7.29 | 1.46 |
| 89 | Cinnamic acid | -4.52 | 0.79 |
| 90 | Salicyclic acid | -4.13 | 1.38 |

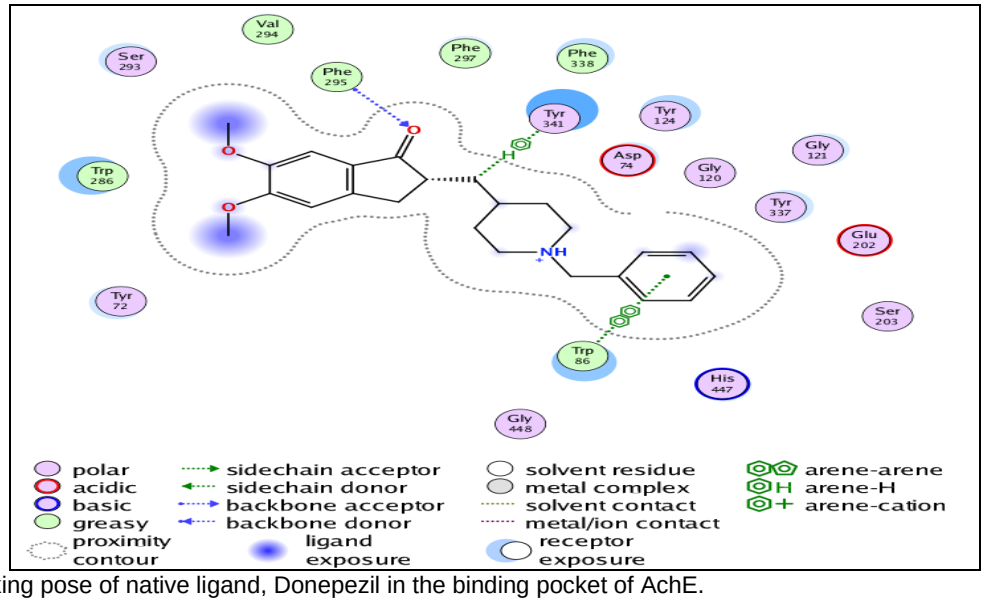


**Fig. 1.** Docking pose of native ligand, Donepezil in the binding pocket of AchE.

### ADME Profile and Toxicity Evaluation

### ADME Screening

Physicochemical properties such as solubility and lipid saturation play a key role in developing a drug into an effective drug candidate, and these calculations were performed using the Swiss ADME website (Daina et al., 2017). The parameters considered to evaluate the outcome include lipophilicity (X Log P ≤ 5), molecular weight (MW) (MW ≤ 500 g/mol), polarity (TPSA < 130), solubility (0.7 < 0 < log S < -6), saturation (0.25 < fraction Csp3 < 1), and flexibility (NROT < 10). Table 2 presents the physicochemical properties of the 11 ligands and the reference compound. Analysis Based on Table 2, the ligands L13 (Curcumin), L22 (Etoposide), L42 (Silibinin), L48 (Yuanhuanin), and L74 (Tangeretin) have log P values less than 5, indicating good water solubility, efficient renal elimination, and good permeability across the cell membrane. The remaining ligands have log P values greater than 5. The molecular weight of the compounds L13 (Curcumin), L42 (Silibinin), L48 (Yuanhuanin), and L74 (Tangeretin) is less than 500 g/mol, which allows them to easily cross cell membranes. In contrast, the remaining compounds have a molecular weight greater than 500 g/mol. Based on the results obtained, it is observed that all ligands except L22 (Etoposide), L26 (Ginkgetin), L42 (Silibinin), and L48 (Yuanhuanin) have TPSA values above 140 Å². This indicates a good prediction for oral bioavailability and transport across membranes (Asano et al., 2023; Varma et al., 2010). The number of rotatable bonds for all ligands is less than 15, meaning these compounds are capable of exhibiting biological activity without encountering issues with oral absorption (Asano et al., 2023).

**Table2.**

Physicochemical properties of the ligands

| Ligands | MW | HBA | HBD | NROT | Log P | MR | Log S | TPSA | CSp3 |
|---|---|---|---|---|---|---|---|---|---|
| **Donepezil** | 379.49 | 4 | 0 | 6 | 4.28 | 115.31 | -4.81 | 38.77 | 0.46 |
| **L 13** | 368.38 | 6 | 2 | 8 | 3.20 | 102.80 | -3.94 | 93.06 | 0.14 |
| **L 22** | 588.56 | 13 | 3 | 5 | 0.60 | 139.11 | -3.75 | 160.83 | 0.55 |
| **L 26** | 566.51 | 10 | 4 | 5 | 5.69 | 155.91 | -7.17 | 159.80 | 0.06 |
| **L 42** | 482.44 | 10 | 5 | 4 | 1.90 | 120.55 | -4.14 | 155.14 | 0.24 |
| **L 43** | 622.75 | 8 | 0 | 4 | 6.66 | 186.07 | -8.02 | 61.86 | 0.37 |
| **L 48** | 462.40 | 11 | 6 | 5 | 1.23 | 112.60 | -3.51 | 179.28 | 0.32 |
| **L 67** | 536.87 | 0 | 0 | 10 | 13.65 | 184.43 | -11.11 | 0.00 | 0.45 |
| **L 68** | 596.84 | 4 | 2 | 10 | 10.27 | 187.16 | -9.35 | 74.60 | 0.40 |
| **L 70** | 564.84 | 2 | 0 | 10 | 11.38 | 184.83 | -9.85 | 34.14 | 0.40 |
| **L 71** | 568.87 | 2 | 2 | 10 | 11.01 | 186.76 | -9.64 | 40.46 | 0.45 |
| **L 74** | 372.37 | 7 | 0 | 6 | 3.04 | 100.38 | -4.11 | 76.36 | 0.25 |

**MW:** Molecular Weight, **HBA**: Num. H-bond Acceptors, **HBD:** Num. H-bond Donors, **NROT**: Num. Rotatable Bonds, **LogP**: Log Po/w (XLOGP3), **TPSA**: Topological Polar Surface Area, **MR**: Molar Refractivity, **CSp3**: Fraction Csp3, **Log S**: Topological method implemented from Delaney.

Bioavailability Score Analysis Table 3 in terms of bioavailability scores, the following observations can be made: The compounds L22 (Etoposide), L48 (Yuanhuanin), L67 (Alpha-carotene), L68 (Astaxanthin), L70 (Canthaxanthin), and L71 (Lutein) share the same score of 0.17, indicating relatively low predicted oral bioavailability. On the other hand, the compounds Ginkgetin, Silibinin, and Tetrandrine have a higher score of 0.55, suggesting better oral absorption potential. Notably, L13 (Curcumin) and L74 (Tangeritin) also show a bioavailability score of 0.55 and comply with several drug-likeness rules, including Lipinski's Rule of Five (molecular weight, lipophilicity, hydrogen bond donors, and acceptors). Veber's Rule (TPSA and rotatable bonds). Muegge's Filter (designed to assess drug-likeness). Ghose Filter (size, lipophilicity, and molar refractivity). Egan's Rule (predicting membrane permeability). These findings indicate that L74 (Tangeritin), in particular, demonstrates good bioavailability and is a promising candidate for further drug development. Drug Absorption and Membrane Barriers Analysis. When a drug is absorbed into the system, it encounters several membrane barriers, including epithelial cells, gastrointestinal membranes, hepatocyte membranes, blood capillary walls, restrictive organ barriers (e.g., blood-brain barrier), the glomerulus, and target cells. A molecule is considered less skin-permeable when its log Kp value is more negative. Observations from ADME Table 3: Skin Permeability, all compounds are less skin-penetrating except for the ligand L67 (Alpha-carotene).

Gastrointestinal Absorption (GI): High GI absorption was observed for L13 (Curcumin), L43 (Tetrandrine), and L74 (Tangeritin), indicating strong intestinal absorption (HIA). Blood-Brain Barrier (BBB): All ligands, except for the reference molecule and L74 (Tangeritin), show a negative response for BBB permeability, meaning they are unlikely to cross the blood-brain barrier. P-glycoprotein Substrate: P-glycoprotein (P-gp) is a key membrane transporter involved in the kinetics of many xenobiotics. It facilitates the efflux of drugs from cells and is present in organs responsible for drug absorption and excretion. The ligands L13 (Curcumin), L26 (Ginkgetin), L42 (Silibinin), L43 (Tetrandrine), and L74 (Tangeritin) are identified as P-gp substrates, indicating their potential interaction with this

transporter. Cytochrome P450 Enzymes (CYP): Within the CYP family, CYP3A4 is the most significant, metabolizing approximately 50% of all drugs, while CYP2C9 metabolizes many clinically used drugs (Manikandan & Nagini, 2018; Zhao et al., 2021; Zhou et al., 2007).

The involvement of these enzymes indicates the importance of understanding their role in drug metabolism for these ligands. From the ADME properties, L74 (Tangeritin) demonstrates excellent results, including high GI absorption, BBB permeability, and compatibility with P-gp and CYP enzymes. These findings highlight L74 (Tangeritin) as a promising drug candidate for further development Figure 2.

**Table 3.**

Druglikenss and Bioavailability of ligands.

| Composants | Lipinski | Ghose | Veber | Egan | Muegge | Bioavailability score |
|---|---|---|---|---|---|---|
| **Donepezil** | 0 | 0 | 0 | 0 | 0 | 0.55 |
| **L 13** | 0 | 0 | 0 | 0 | 0 | 0.55 |
| **L 22** | 2 | 3 | 1 | 1 | 2 | 0.17 |
| **L 26** | 1 | 3 | 1 | 1 | 2 | 0.55 |
| **L 42** | 0 | 1 | 1 | 1 | 1 | 0.55 |
| **L 43** | 1 | 4 | 0 | 0 | 2 | 0.55 |
| **L 48** | 2 | 0 | 1 | 1 | 3 | 0.17 |
| **L 67** | 2 | 4 | 0 | 1 | 2 | 0.17 |
| **L 68** | 2 | 4 | 0 | 1 | 1 | 0.17 |
| **L 70** | 2 | 4 | 0 | 1 | 1 | 0.17 |
| **L 71** | 2 | 4 | 0 | 1 | 1 | 0.17 |
| **L 74** | 0 | 0 | 0 | 0 | 0 | 0.55 |

**Table 4.**

Pharmacokinetics of ligands

| Ligands | Abs GI | BBB Perméant | P-gp substrate | CYP1A2 Inhibitor | CYP2C19 inhibitor | CYP2C9 Inhibitor | CYP2D6 inhibitor | CYP3A4 Inhibitor | log Kp (cm /s) |
|---|---|---|---|---|---|---|---|---|---|
| **Donepezil** | High | Yes | Yes | No | No | No | Yes | Yes | -5.58 |
| **L 13** | High | No | No | No | No | Yes | No | Yes | -6.28 |
| **L 22** | Low | No | Yes | No | No | No | Yes | No | -9.46 |
| **L 26** | Low | No | No | No | No | Yes | No | No | -5.72 |
| **L 42** | Low | No | No | No | No | No | No | Yes | -7.89 |
| **L 43** | High | No | No | No | No | No | No | No | -5.37 |
| **L 48** | Low | No | Yes | No | No | No | No | Yes | -8.25 |
| **L 67** | Low | No | Yes | No | No | No | No | No | 0.12 |
| **L 68** | Low | No | Yes | No | No | No | No | No | -2.65 |
| **L 70** | Low | No | Yes | No | No | No | No | No | -1.67 |
| **L 71** | Low | No | Yes | No | No | No | No | No | -1.95 |
| **L 74** | High | Yes | No | No | No | Yes | No | Yes | -6.41 |

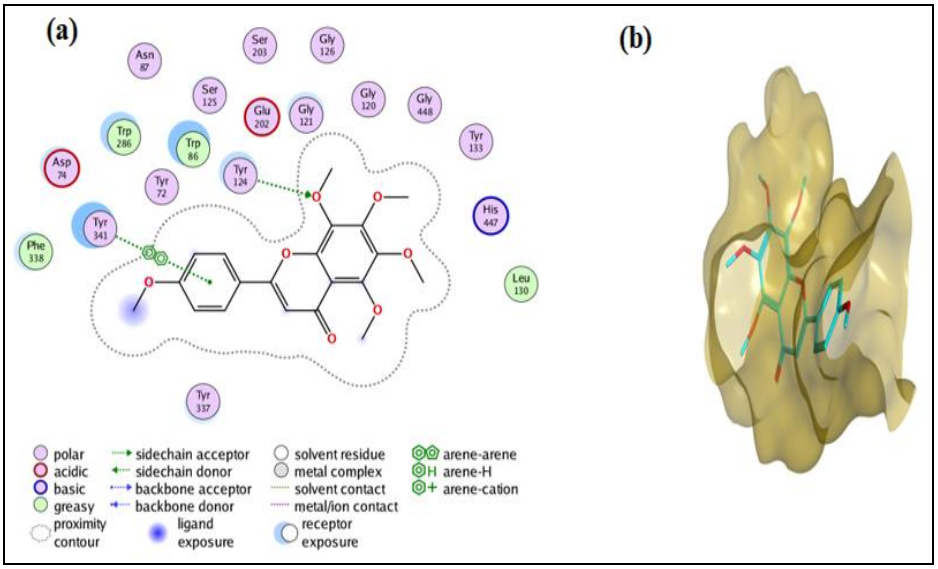


**Fig. 2.** (a) Docking pose of Tangeritin in the binding pocket of AchE, (b) Illustration of the positioning of Tangeritin in the binding site of AchE.

### Toxicity prediction

Table 5. After predicting the toxicity of 11 molecules that passed the ADME tests, we selected molecule number 74 Tangeritin, because the LD50 of this molecule compared to the reference molecule is high, which is 505, and as it is known that the higher the LD50, the less toxicity and vice versa. Secondly, this molecule does not cause any kind of toxicity (Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, Cytotoxicity, Neurotoxicity).

**Table 5.**

Toxicity prediction

| N° | Ligand | Classe | $DL_{50}$ | Hepatotoxicity | Carcinogenicity | Immunotoxicity | Mutagenicity | Cytotoxicity | Neurotoxicity |
|---|---|---|---|---|---|---|---|---|---|
| 13 | Curcumin | 4 | 2000 | Inactive | Inactive | Active | Inactive | Inactive | Inactive |
| 22 | Etoposide | 3 | 215 | Inactive | Inactive | Active | Inactive | Inactive | Inactive |
| 26 | Ginkgetin | 5 | 4000 | Inactive | Inactive | Active | Inactive | Inactive | Inactive |
| 42 | Silibinin | 4 | 2000 | Inactive | Inactive | Active | Inactive | Inactive | Inactive |
| 43 | Tetrandrine | 4 | 1700 | Inactive | Active | Active | Active | Inactive | Active |
| 48 | Yuanhuanin | 5 | 5000 | Inactive | Inactive | Active | Inactive | Inactive | Inactive |
| 67 | Alpha-carotene | 4 | 1510 | Inactive | Inactive | Inactive | Active | Inactive | Active |
| 68 | Astaxanthin | 5 | 4600 | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| 70 | Canthaxanthin | 6 | 10000 | Inactive | Inactive | Inactive | Inactive | Inactive | Inactive |
| 71 | Lutein | 2 | 10 | Inactive | Inactive | Active | Inactive | Inactive | Inactive |
| **74** | **Tangeritin** | **5** | **5000** | **Inactive** | **Inactive** | **Inactive** | **Inactive** | **Inactive** | **Inactive** |
| | Donepezil | 4 | 505 | Inactive | Active | Active | Inactive | Active | Active |

## CONCLUSION

This study underscores the utility of molecular docking as an efficient method for identifying potential AChE inhibitors in the search for new treatments for Alzheimer's disease. Tangeritin, with its strong binding affinity, favorable ADME profile, and low predicted toxicity, has emerged as a promising candidate for further development. Notably, similar molecular docking studies on compounds like galantamine have successfully predicted their efficacy as AChE inhibitors, which was later confirmed through experimental studies. These results not only validate the approach but also lay a foundation for future research that could lead to more effective and safer therapies for AD patients.

## ACKNOWLEDGEMENTS

The authors would like kindly to thank the Ministry of High Education and Scientific Research of Algeria, via the University of Tebessa for supporting this work.

## AUTHORS CONTRIBUTIONS

Conceptualization, BB and SG; methodology, BB, CH, OH, and SS; formal analysis and investigation, CH, OH, and SG; writing original draft preparation, BB and SS; conceptualization, BB, SG, and OH; writing review and editing, BB, CH and SS. All authors read and approved the final manuscript.

## FUNDING

This research received no external funding.

## CONFLICT OF INTEREST

The authors do not have any competing financial, professional, or personal interests from other parties.

## REFERENCES

Asano, D., Takakusa, H., & Nakai, D. (2023). Oral Absorption of Middle-to-Large Molecules and Its Improvement, with a Focus on New Modality Drugs. Pharmaceutics, 16(1), 47.

Bai, Q., Li, L., Liu, S., Xiao, S., & Guo, Y. (2018). Drug design progress of in silico, in vitro, and in vivo research. In-vitro In-vivo In-silico Journal, 1(1), 16-37.

Benaicha, B., Gasmi, S., Mujwar, S., & Rebiai, E. (2024). Effects of Diazinon in endocrine disruption: molecular docking and dynamics simulation on hormonal receptors in the context of the extensive use. Studies in engineering and exact sciences, 5(2), e11815.

Carrillo, M. C., Bain, L. J., Frisoni, G. B., & Weiner, M. W. (2012). Worldwide Alzheimer's disease neuroimaging initiative. Alzheimer's & Dementia, 8(4), 337-342.

Chen, Z. R., Huang, J. B., Yang, S. L., & Hong, F. F. (2022). Role of cholinergic signaling in Alzheimer's disease. Molecules, 27(6), 1816.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules. Scientific reports, 7(1), 42717.

Ganeshpurkar, A., Singh, R., Gore, P. G., Kumar, D., Gutti, G., Kumar, A., & Singh, S. K. (2020). Structure-based screening and molecular dynamics simulation studies for the identification of potential acetylcholinesterase inhibitors. Molecular Simulation, 46(3), 169-185.

Guedes, I. A., de Magalhães, C. S., & Dardenne, L. E. (2014). Receptor–ligand molecular docking. Biophysical reviews, 6, 75-87.

Huang, Q., Liao, C., Ge, F., Ao, J., & Liu, T. (2022). Acetylcholine bidirectionally regulates learning and memory. Journal of Neurorestoratology, 10(2), 100002.

Kader, D. A., Aziz, D. M., Mohammed, S. J., Maarof, N. N., Karim, W. O., Mhamad, S. A., ... & Qurbani, K. (2024). Green synthesis of ZnO/catechin nanocomposite: Comprehensive characterization, optical study, computational

analysis, biological applications, and molecular docking. Materials Chemistry and Physics, 319, 129408.

Manikandan, P., & Nagini, S. (2018). Cytochrome P450 structure, function and clinical significance: a review. Current drug targets, 19(1), 38-54.

Laskar, Y. B., Mazumder, P. B., & Talukdar, A. D. (2023). Hibiscus sabdariffa anthocyanins are potential modulators of estrogen receptor alpha activity with favourable toxicology: a computational analysis using molecular docking, ADME/Tox prediction, 2D/3D QSAR and molecular dynamics simulation. Journal of Biomolecular Structure and Dynamics, 41(2), 611-633.

Ojuka, P., Kimani, N. M., Apollo, S., Nyariki, J., Ramos, R. S., & Santos, C. B. (2023). Phytochemistry of plants in the genus Vepris: A review and in silico analysis of their ADMET properties. South African Journal of Botany, 157, 106-114.

Pantsar, T., & Poso, A. (2018). Binding affinity via docking: fact and fiction. Molecules, 23(8), 1899.

Tan, C. C., Yu, J. T., Wang, H. F., Tan, M. S., Meng, X. F., Wang, C., ... & Tan, L. (2014). Efficacy and safety of donepezil, galantamine, rivastigmine, and memantine for the treatment of Alzheimer's disease: a systematic review and meta-analysis. Journal of Alzheimer's Disease, 41(2), 615-631.

Varma, M. V., Obach, R. S., Rotter, C., Miller, H. R., Chang, G., Steyn, S. J., ... & Troutman, M. D. (2010). Physicochemical space for optimum oral bioavailability: contribution of human intestinal absorption and first-pass elimination. Journal of medicinal chemistry, 53(3), 1098-1108.

Vecchio, I., Sorrentino, L., Paoletti, A., Marra, R., & Arbitrio, M. (2021). The state of the art on acetylcholinesterase inhibitors in the treatment of Alzheimer's disease. Journal of Central nervous system disease, 13, 11795735211029113.

Zhang, P., Xu, S., Zhu, Z., & Xu, J. (2019). Multi-target design strategies for the improved treatment of Alzheimer's disease. European journal of medicinal chemistry, 176, 228-247.

Zhao, M., Ma, J., Li, M., Zhang, Y., Jiang, B., Zhao, X., ... & Qin, S. (2021). Cytochrome P450 enzymes and drug metabolism in humans. International journal of molecular sciences, 22(23), 12808.

Zhou, S. F., Xue, C. C., Yu, X. Q., Li, C., & Wang, G. (2007). Clinically important drug interactions potentially involve mechanism-based inhibition of cytochrome P450 3A4 and the role of therapeutic drug monitoring. Therapeutic drug monitoring, 29(6), 687-710.