



Unexplored Natural Compounds as Dual Inhibitors of Cholinesterase and Ferroptosis: A Neuroprotective Perspective on Seven Bioactive Phytochemicals

Kolinesteraz ve Ferroptosis'in Çift İnhibitörü Olarak Keşfedilmemiş Doğal Bileşikler:
Yedi Biyoaktif Fitokimyasal Üzerine Nöroprotektif Bir Bakış Açısı

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Unexplored Natural Compounds as Dual Inhibitors of Cholinesterase and Ferroptosis: A Neuroprotective Perspective on Seven Bioactive Phytochemicals

ABSTRACT

Objective: This study investigated the dual-target binding potential of seven natural compounds celastrol, pachypodol, hinokitiol, withanone, isobavachalcone, magnolol, and xanthorrhizol against AChE and BChE, using donepezil and rivastigmine as references.

Material and Method: Molecular structures were obtained from PubChem and energy-minimised with the MMFF94 force field. Docking was performed using AutoDock Vina with human AChE (PDB ID: 4M0E) and BChE (PDB ID: 6QAA). Binding affinities and interaction profiles were assessed with Discovery Studio Visualizer.

Results: Isobavachalcone (-9.2 kcal/mol) and magnolol (-8.4 kcal/mol) showed high affinity for AChE, while withanone (-9.7 kcal/mol) and celastrol (-9.6 kcal/mol) strongly bound BChE, comparable to donepezil. These compounds established stabilising interactions, including hydrogen bonds, π - π stacking, and hydrophobic contacts, with minimal steric conflicts, supporting structural compatibility with target enzymes.

Conclusion: Isobavachalcone, magnolol, celastrol, and withanone are identified as promising multifunctional candidates. Their dual cholinesterase inhibition and reported antioxidant properties suggest potential to address both cholinergic dysfunction and ferroptosis-associated neurodegeneration, warranting further experimental validation.

Keywords: Alzheimer's Disease, Natural compounds, Ferroptosis, Multi-target cholinesterase inhibitors, Molecular docking, Neuroprotection.

Kolinesteraz ve Ferroptosis'in Çift İnhibitörü Olarak Keşfedilmemiş Doğal Bileşikler: Yedi Biyoaktif Fitokimyasal Üzerine Nöroprotektif Bir Bakış Açısı

ÖZET

Amaç: Bu çalışmada, celastrol, pachypodol, hinokitiol, withanone, isobavachalcone, magnolol ve xanthorrhizol olmak üzere yedi doğal bileşiğin AChE ve BChE'ye karşı çift hedefli bağlanma potansiyeli, donepezil ve rivastigmin referans alınarak incelendi.

Gereç ve Yöntem: Moleküler yapılar PubChem veritabanından elde edilerek MMFF94 kuvvet alanı ile enerji minimizasyonu yapıldı. Moleküler kenetleme (docking) analizleri AutoDock Vina kullanılarak insan AChE (PDB ID: 4M0E) ve BChE (PDB ID: 6QAA) üzerinde gerçekleştirildi. Bağlanma afiniteleri ve etkileşim profilleri Discovery Studio Visualizer ile değerlendirildi.

Bulgular: Isobavachalcone (-9,2 kcal/mol) ve magnolol (-8,4 kcal/mol) AChE için yüksek afinite gösterirken, withanone (-9,7 kcal/mol) ve celastrol (-9,6 kcal/mol) BChE'ye güçlü bağlanma potansiyeli sergilemiş ve donepezil ile karşılaştırılabilir sonuçlar vermiştir. Bu bileşikler, hidrojen bağları, π - π etkileşimleri ve hidrofobik temaslar gibi stabilizasyon sağlayan etkileşimler oluşturmuş, minimal sterik çatışma ile enzimlerin aktif bölgelerine yapısal uyumluluk göstermiştir.

Sonuç: Isobavachalcone, magnolol, celastrol ve withanone, umut verici çok işlevli adaylar olarak belirlenmiştir. Çift yönlü kolinesteraz inhibisyonu ve bildirilen antioksidan özellikleri, hem kolinerjik disfonksiyon hem de ferroptozis ile ilişkili nörodejenerasyonu hedefleyen yeni tedavi stratejileri için potansiyel taşımaktadır.

Anahtar Sözcükler: Alzheimer hastalığı, Doğal bileşikler, Ferroptozis, Çok hedefli kolinesteraz inhibitörleri, Moleküler kenetlenme, Nöroproteksiyon.

Introduction

Neurodegenerative diseases, especially Alzheimer's and Parkinson's, are characterized by progressive neuronal dysfunction caused by multifactorial pathological cascades. A key pathological element in these diseases is the cholinergic deficit, which underlies cognitive decline due to impaired acetylcholine transmission. Consequently, pharmacological inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) has become a cornerstone of symptomatic treatment, albeit with recognised limitations such as modest efficacy and adverse effects (1, 2).

In parallel, ferroptosis, a particular iron-dependent form of regulated cell death characterized by the accumulation of lipid peroxides, has attracted considerable attention as a mechanism contributing to neurodegeneration (3). The intersection of cholinergic dysfunction and ferroptotic cell death pathways provides a compelling basis for therapeutic strategies that are dual-targeted, particularly those that can modulate both enzymatic activity and oxidative homeostasis.

Natural substances remain a largely untapped reservoir in this context. Despite the extensive pharmacognostic research into phytochemicals, many bioactive molecules with favourable neurochemical profiles have yet to be investigated for their cholinesterase inhibition or ferroptosis modulation. The present study highlights seven such compounds, celastrol, pachypodol, hinokitiol, withanone, isobavachalcone, magnolol, and xanthorrhizol, which were selected on the basis of their structural suitability and preliminary pharmacological evidence.

Celastrol, a quinone methide triterpenoid from *Tripterygium wilfordii*, is known for its anti-inflammatory and neuroprotective properties, but its interaction with cholinesterases and ferroptotic machinery has not been systematically studied (4). Similarly, pachypodol, a polymethoxylated flavonoid, exhibits properties favorable to cholinesterase inhibition, although empirical verification is still limited (5). Hinokitiol,

a naturally occurring derivative of tropolone, possesses notable metal-chelating capacity, a property relevant to the modulation of ferroptosis, yet its interaction spectrum with enzymatic targets remains insufficiently defined (6).

Withanone, classified as a withanolide lactone and isolated from *Withania somnifera*, has been examined in experimental models of neurodegeneration; however, direct assessments of its inhibitory profile against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are scarce (7). Similarly, isobavachalcone and magnolol, representatives of chalcone and biphenolic structural classes, respectively, exhibit well-documented antioxidant and anti-inflammatory activities, but have yet to be rigorously evaluated in the combined context of AChE/BChE modulation and ferroptosis regulation (8, 9). Xanthorrhizol, obtained from *Curcuma xanthorrhiza*, stands out as the most unconventional member of this set, given the near absence of research linking it to cholinesterase inhibition or ferroptotic pathways (10).

Against this background, the present study seeks to explore the therapeutic relevance of these seven molecules as potential bifunctional agents capable of modulating both cholinesterase activity and susceptibility to ferroptosis. Through this dual-focus evaluation, the work aspires to enrich the emerging evidence base for multifunctional neuroprotective strategies aimed at tackling the multifaceted pathophysiology of complex neurodegenerative disorders.

Material and Method

Ligand Preparation

The molecular structures of seven natural test compounds, Celastrol, Pachypodol, Hinokitiol, Withanone, Isobavachalcone, Magnolol, and Xanthorrhizol, alongside the reference inhibitors Donepezil and Rivastigmine, were retrieved from the PubChem database (Celastrol: CID 122724; Pachypodol: CID 5281677; Hinokitiol: CID 3611; Withanone: CID 21679027; Isobavachalcone: CID 5281255; Magnolol: CID 72300; Xanthorrhizol: CID

93135; Donepezil: CID 3152; Rivastigmine: CID 77991). Prior to molecular docking, all ligands were subjected to geometric optimization in Avogadro (v1.2.0) using the MMFF94 force field. Energy minimization was performed under default convergence settings, ensuring that electrostatic and steric factors were balanced to produce stable, low-energy conformations (11).

Protein Preparation and Docking Protocol

High-resolution crystallographic models of human acetylcholinesterase (AChE; PDB ID: 4MOE, 2.00 Å) (Cheung et al., 2013) and human butyrylcholinesterase (BChE; PDB ID: 6QAA, 1.90 Å) (Meden et al., 2019) were downloaded from the RCSB Protein Data Bank. Non-essential heteroatoms and crystallographic water molecules were removed, hydrogen atoms were added to simulate physiological protonation states, and Gasteiger partial charges were assigned.

Docking experiments were carried out with AutoDock Vina (v1.2.5), defining the binding search space according to the coordinates of the co-crystallized ligands. For AChE, the grid was centered at $x = -17.27$, $y = -42.32$, $z = 25.90$, while for BChE, the grid center was set at $x = 18.99$, $y = 42.61$, $z = 40.81$. A cubic search box of 20 Å per side was used for both enzymes to encompass the full active site. The Lamarckian Genetic Algorithm was applied with default parameters to explore ligand binding poses and estimate binding energies (12, 13).

Interaction Analysis

Post-docking, the resulting ligand–protein complexes were examined using Discovery Studio Visualizer. Key molecular interactions—including hydrogen bonding, π – π stacking, hydrophobic contacts, and electrostatic interactions—were annotated. The binding orientations of each compound were compared with those of the standard inhibitors Donepezil (AChE) and Rivastigmine (BChE) to verify docking accuracy and active-site targeting efficiency.

Statistical Evaluation

Binding energies (kcal/mol) were compared across all ligands and interpreted in relation to the reference inhibitors. Interaction patterns were qualitatively assessed for both stabilizing and destabilizing contacts, and binding orientations were analyzed for structural complementarity.

Results

Active Site-Based Docking Simulations

Docking simulations were carried out using the crystallographic structures of human acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The predicted binding configurations of the reference ligands, Donepezil for AChE and Rivastigmine for BChE, are shown in Figure 1 (panels A and B, respectively). In both cases, the ligands were positioned within the predefined grid boundaries, centered on the catalytic pockets. These results indicate that the docking parameters were appropriately defined and that the ligands adopted plausible orientations within the active sites.

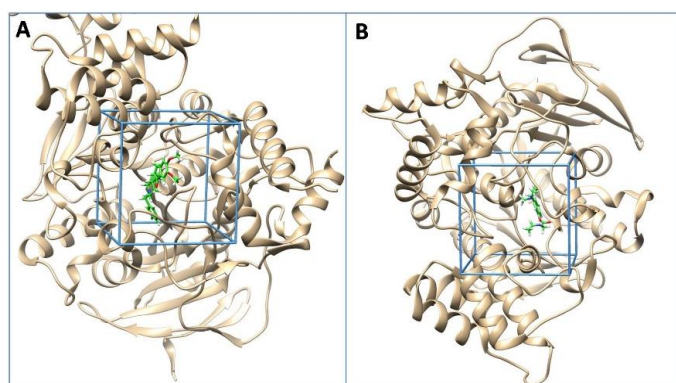


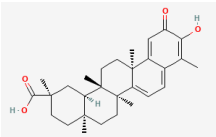
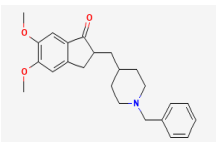
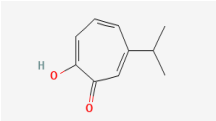
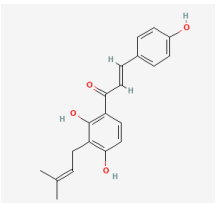
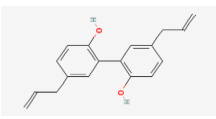
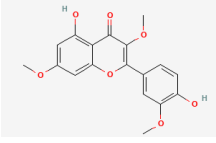
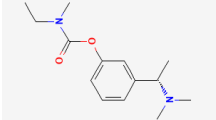
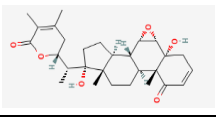
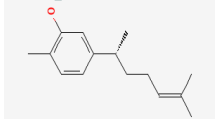
Figure 1. Predicted docking configurations of reference inhibitors within the catalytic sites of AChE and BChE. (A) Binding orientation of Donepezil in the active site of acetylcholinesterase (PDB ID: 4MOE). (B) Binding orientation of Rivastigmine in the active site of butyrylcholinesterase (PDB ID: 6QAA).

Docking Results

The docking analysis demonstrated that the tested compounds exhibited a range of binding affinities toward AChE and BChE. Calculated binding energy values for all ligands are presented in Table I. For AChE, Donepezil showed

the strongest affinity (-9.5 kcal/mol). Among the natural compounds, Isobavachalcone (-9.2 kcal/mol) and Celastrol (-7.9 kcal/mol) displayed notable binding potential, while Magnolol also ranked highly with a score of -8.4 kcal/mol.

Table I. Comparative binding energy values (kcal/mol) for the investigated natural compounds and reference inhibitors (Donepezil and Rivastigmine) against human acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).

Compound	Molecular structure	Binding Energy for AChE (kcal/mol)	Binding Energy for BChE (kcal/mol)
Celastrol		-7.9	-9.6
Donepezil		-9.5	-9.7
Hinokitiol		-7.0	-6.2
Isobavachalcone		-9.2	-9.0
Magnolol		-8.4	-8.2
Pachypodol		-7.3	-8.4
Rivastigmine		-6.2	-6.6
Withanone		-7.2	-9.7
Xanthorrhizol		-8.0	-7.5

For BChE, Celastrol achieved the most favorable binding score (-9.6 kcal/mol), closely matched by Donepezil (-9.7 kcal/mol). Isobavachalcone (-9.0 kcal/mol) and Withanone (-9.7 kcal/mol) also showed strong affinities. In contrast, Rivastigmine and Hinokitiol exhibited comparatively lower binding strengths for both enzymes. Overall, these results suggest that Isobavachalcone, Magnolol, and Celastrol may be promising AChE inhibitors, whereas Celastrol, Isobavachalcone and Withanone hold potential as BChE inhibitors.

The docking poses for Donepezil and the three highest-affinity AChE ligands, Isobavachalcone, Magnolol, and Celastrol are depicted in Figure II. Donepezil is a well-established AChE inhibitor frequently used as a reference in docking analyses. The selected natural compounds were chosen based on their superior docking scores, indicating strong binding potential within the enzyme's catalytic site. The figure illustrates the conformations of each ligand within the active pocket of AChE.

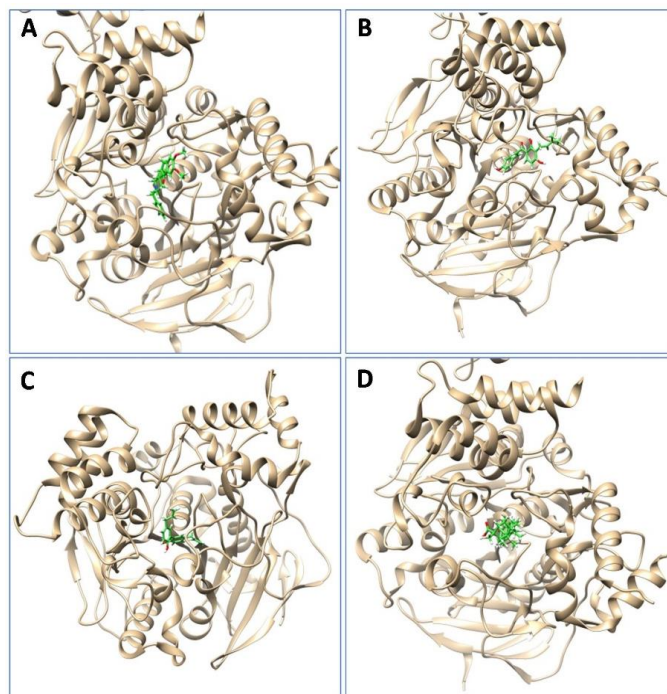


Figure II. Representative docking conformations of top-performing ligands in the AChE active site: (A) Donepezil, (B) Isobavachalcone, (C) Magnolol, and (D) Celastrol.

Similarly, Figure III shows Rivastigmine, the reference ligand for BChE, alongside Withanone, Celastrol, and Isobavachalcone, the three compounds with the highest docking scores for this enzyme. Rivastigmine, a clinically approved BChE inhibitor, is often used as a standard in molecular docking studies. The selected compounds were prioritized for visualization due to their favorable predicted binding affinities, suggesting meaningful interaction potential within the BChE catalytic site. The figure displays the spatial arrangement and orientation of each ligand in the binding cavity.

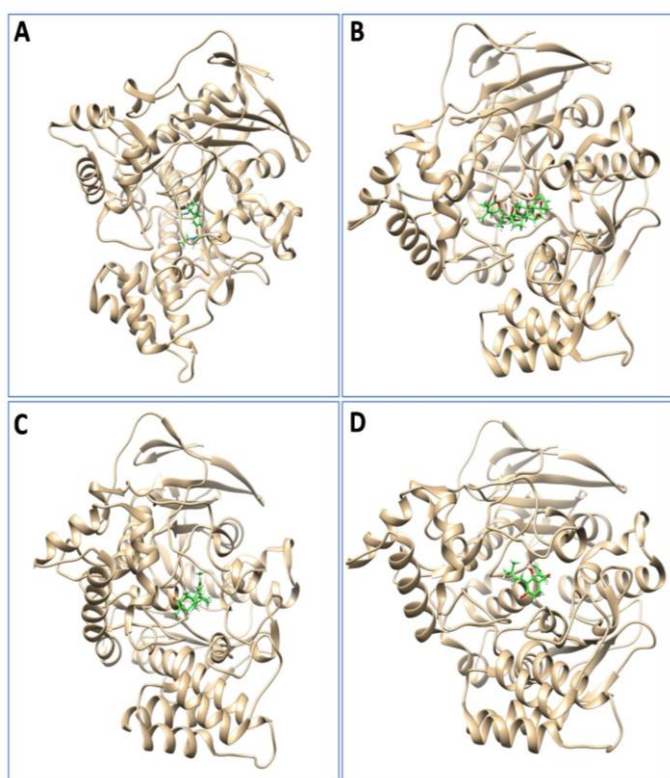


Figure III. Representative docking conformations of selected ligands in the BChE active site: (A) Rivastigmine, (B) Withanone, (C) Celastrol, and (D) Isobavachalcone.

Analysis of Molecular Interactions

As shown in Figure IV, the reference inhibitor donepezil exhibited strong binding interactions within the active site of acetylcholinesterase (AChE), forming multiple key contacts including π -anion interactions with ARG486 and π -alkyl interactions with ARG224. A carbon hydrogen bond was observed with HIS223, further stabilizing the complex. In addition to favorable interactions, several unfavorable contacts such as

steric clashes (unfavorable bumps) and electrostatic repulsion (attractive charge) with residues like LEU352 and PHE338 were also present, indicating spatial strain in certain regions of the binding site (Figure IV.A).

Among the tested compounds, isobavachalcone, magnolol, and celastrol demonstrated the highest binding affinities after donepezil. Isobavachalcone formed π -alkyl interactions with TYR341 and TRP286, a conventional hydrogen bond with PHE295, and a π -anion interaction, suggesting a favorable binding conformation supported by both polar and hydrophobic forces. It showed a well-fitted interaction network with minimal unfavorable contacts (Figure IV.B). Magnolol primarily engaged in π -alkyl interactions with TRP286 and TYR341 and exhibited an unfavorable negative-negative electrostatic interaction with PHE338, which may reduce overall stability despite its otherwise hydrophobic nature (Figure IV.C). Celastrol displayed a conventional hydrogen bond with TYR124 and a strong π -alkyl interaction with TRP286, but also presented an unfavorable electrostatic interaction with GLU292, suggesting some repulsive forces exist alongside its stabilizing interactions (Figure IV.D).

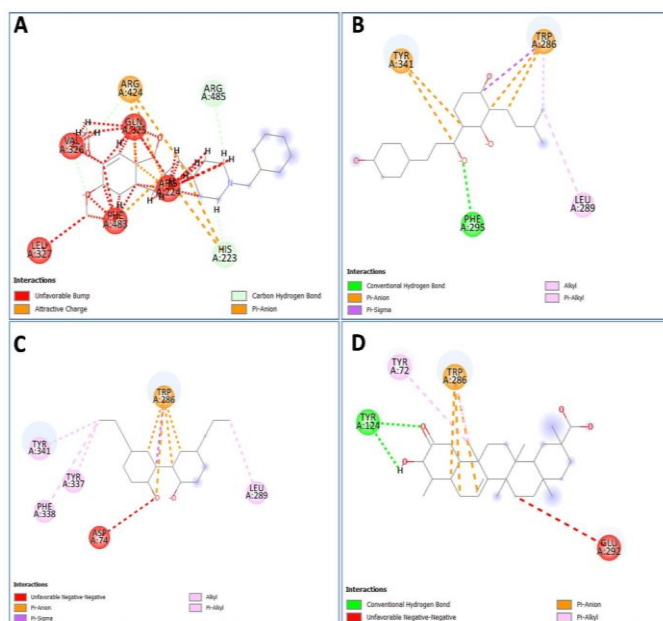


Figure IV. Molecular interactions of the compounds within the active site of acetylcholinesterase (AChE): A) Donepezil, B) Isobavachalcone, C) Magnolol, D) Celastrol.

As shown in Figure V, the reference inhibitor rivastigmine interacted with the active site of butyrylcholinesterase (BChE) through a combination of hydrogen bonds and electrostatic contacts. It formed conventional hydrogen bonds with THR120 and TYR332, a carbon hydrogen bond with TRP82, and a π -anion interaction with ASP70. However, an unfavorable negative-negative interaction was also observed with ASP70, indicating potential repulsion that may slightly reduce overall binding stability. The relatively low docking scores observed for rivastigmine may be attributed to its pseudo-irreversible covalent binding mechanism, which cannot be fully captured by conventional non-covalent docking simulations.

Among the tested compounds, withanone, celastrol, and isobavachalcone exhibited the strongest binding affinities to BChE. Withanone showed multiple π -anion interactions with key aromatic residues such as TRP82 and PHE329, contributing to strong π -stacking and hydrophobic stabilization. However, the presence of an unfavorable negative-negative interaction with ASP70 suggests some electrostatic repulsion within the binding pocket (Figure V.B).

Celastrol engaged in several stabilizing hydrogen bonds with SER198, GLU197, and ALA199, in addition to a π -anion interaction with TRP82. Despite these favorable contacts, unfavorable interactions were observed with ASP70 and SER287, reflecting possible steric or electrostatic strain (Figure V.C).

Isobavachalcone formed conventional hydrogen bonds with TYR128, TYR332, and PRO285, along with carbon hydrogen bond interactions involving ALA328 and PHE329. Additionally, π -alkyl and alkyl interactions were observed with TRP82. Although an unfavorable negative-negative interaction was detected with ASP70, the overall interaction profile suggests a relatively stable and well-accommodated binding conformation within the BChE active site (Figure V.D).

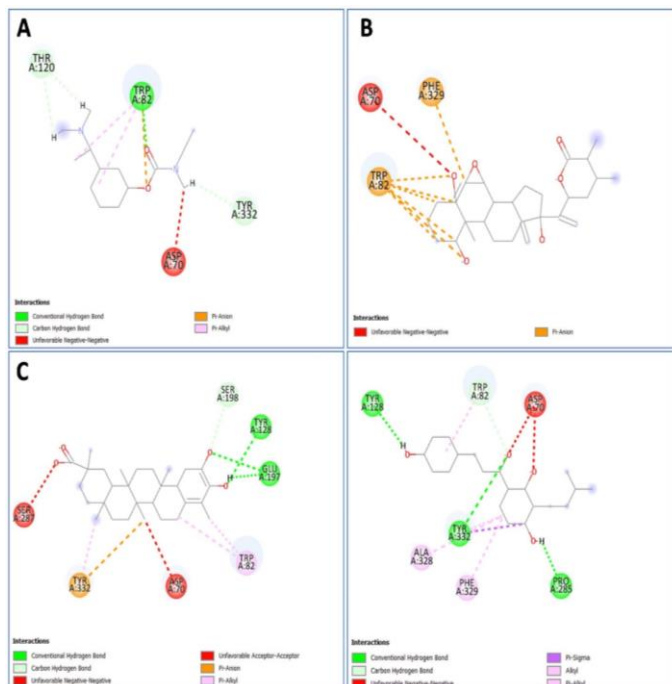


Figure V. Molecular interactions of the compounds within the active site of butyrylcholinesterase (BChE): A) Rivastigmine, B) Withanone, C) Celastrol, D) Isobavachalcone.

Discussion

The results of this docking-based study provide significant insight into the dual inhibitory potential of previously unexplored natural products against AChE and BChE, enzymes that play an important role in the pathophysiology of neurodegeneration. Among the molecules tested, isobavachalcone, magnolol, celastrol and withanone showed promising binding affinities that in some cases rivalled or came close to those of clinically validated drugs such as donepezil and rivastigmine.

The strong binding of isobavachalcone to AChE (-9.2 kcal/mol) with interaction patterns involving π -alkyl interactions and hydrogen bonding with key catalytic residues is consistent with the findings of (14), who showed that chalcone derivatives have high affinity for cholinesterases due to their planarity and conjugated systems. In their study, isobavachalcone analogues also inhibited AChE activity in vitro, with IC_{50} values in the low micromolar range, consistent with the high docking values observed here. Remarkably, our results extend this finding to BChE (-9.0

kcal/mol), suggesting a broader inhibitory spectrum than previously reported.

Magnolol showed remarkable binding to both enzymes with docking values of -8.4 kcal/mol (AChE) and -8.2 kcal/mol (BChE). This confirms previous findings by Xian et al. (15), who reported that magnolol exerted neuroprotective effects via AChE inhibition in a scopolamine-induced memory impairment model. The results of their enzymatic assays showed that magnolol has the ability to inhibit AChE with moderate efficacy and they observed an improvement in behavioural performance, supporting its cognition-enhancing and neuroprotective properties. Our study confirms the enzyme-binding potential of magnolol by elucidating key molecular interactions, such as π - π stacking with TRP286 and TYR341, and confirms its compatibility with the catalytic pocket of BChE. Magnolol and honokiol have been shown to possess strong acetylcholinesterase inhibitory activity (IC_{50} : 0.069 and 0.057 mM) and potential as natural insecticides. These findings support the feasibility of discovering cholinesterase-targeting inhibitors from natural sources using rapid screening methods, which may also be relevant for neuroprotective applications (16).

Celastrol, a compound best known for its anti-inflammatory and antioxidant effects, exhibited considerable binding affinity for BChE (-9.6 kcal/mol), which has not been previously reported in this context. Although there are limited data on the direct interaction of celastrol with cholinesterases, Hooda et al. (17) reported that celastrol ameliorates $A\beta$ -induced cognitive deficits and reduces neuroinflammation in APP/PS1 transgenic mice. These effects were partly attributed to the suppression of lipid peroxidation and NF- κ B signalling, mechanisms that converge with ferroptosis pathways (18). The results of the present study thus provide new structural evidence that inhibition of cholinesterase may be an additional mechanism of action through which celastrol exerts neuroprotective effects.

Perhaps most remarkably, withanone has a binding energy of -9.7 kcal/mol to BChE,

equivalent to that of donepezil. This finding is consistent with recent data suggesting that withanolides, particularly withanone and withaferin A, modulate neuroinflammatory and neurodegenerative cascades (19). However, the direct interaction between withanone and cholinesterases has not yet been reported in the literature. Thus, our study fills a critical gap by providing the first *in silico* evidence that withanone can serve as a BChE inhibitor through strong π -anion and hydrophobic interactions, particularly with TRP82 and HIS438. Withaferin A (WA), a withanolide isolated from *Withania adpressa*, has demonstrated significant acetylcholinesterase and butyrylcholinesterase inhibitory activity (IC_{50} : 45.5 ± 0.14 and 68.2 ± 0.27 μ g/mL, respectively) and improved learning and memory performance in a scopolamine-induced mouse model. These neuroprotective effects, supported by molecular docking results showing strong binding affinity to both enzymes, highlight WA as a promising natural candidate for the treatment of Alzheimer's disease and other cholinergic dysfunction-related disorders (20).

In contrast, rivastigmine, despite being a standard treatment for Alzheimer's disease, showed lower docking values (-6.2 kcal/mol for AChE; -6.6 kcal/mol for BChE). These results are not necessarily inconsistent with its known pharmacological profile, as rivastigmine acts as a pseudo-irreversible inhibitor and forms covalent bonds that are not captured by docking simulations based on reversible binding thermodynamics (21).

Compounds such as hinokitiol and pachypodol showed weaker binding values (-6.2 to -7.3 kcal/mol) and less stabilising interactions, suggesting limited cholinesterase inhibition despite their known antioxidant or metal chelating activity. Although hinokitiol has been previously described as a modulator of ferroptosis through iron binding (22), its role in cholinergic regulation remains minimal, consistent with our docking results.

Xanthorrhizol, which has shown anti-cancer and anti-inflammatory effects in previous studies (10), showed moderate interactions with AChE

(-8.0 kcal/mol) and BChE (-7.5 kcal/mol). To date, no studies have investigated its effect on cholinesterases. The results presented here indicate a structural potential, but emphasise the need for further biochemical validation.

The ferroptosis literature highlights that lipid peroxidation, iron accumulation, and GPX4 depletion form the biochemical core of ferroptotic cell death (23). Notably, natural products with antioxidant properties such as celastrol, magnolol, and withanone may protect neurons by both preserving cholinergic neurotransmission and interrupting ferroptotic cascades. This aligns with emerging therapeutic paradigms advocating for multi-target agents capable of modulating both neurotransmitter systems and redox balance (24, 25). Each selected phytochemical exhibits distinct mechanistic features related to ferroptosis modulation, such as iron chelation capacity, suppression of lipid peroxidation, or preservation of GPX4 activity, which collectively suggest their potential to mitigate oxidative cell death. However, these predictions are currently limited to in silico evidence and require experimental validation in cellular and animal models.

Taken together, these results argue in favour of a two-target therapeutic approach. In Alzheimer's disease, BChE activity increases with disease progression and its inhibition becomes increasingly important in later stages (26). Therefore, compounds such as withanone and celastrol, which have a strong BChE affinity, may be particularly useful in advanced stages of the disease. Moreover, several of these phytochemicals, including celastrol and magnolol, possess intrinsic antioxidant properties that may enable simultaneous modulation of cholinergic signalling and ferroptotic susceptibility, an emerging axis of interest in neurodegeneration research (24, 25).

In summary, this study provides new structural insights into natural products as multifunctional neuroprotective agents. The docking affinities and interaction profiles of isobavachalcone, magnolol, celastrol and withanone provide a basis for further pharmacological and preclinical

investigations. In the future, experimental validation in enzyme inhibition assays, ferroptosis models and animal studies will be crucial to determine their therapeutic feasibility and mechanistic specificity.

Conclusion

The present study provides compelling preliminary evidence for the potential of selected natural compounds, in particular isobavachalcone, magnolol, celastrol and withanone, as dual inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), enzymes critically involved in the cholinergic deficit observed in neurodegenerative diseases. These compounds exhibited a binding affinity comparable or approaching that of clinically approved drugs, accompanied by favorable interaction profiles within the catalytic pockets of both enzymes.

In addition to their cholinesterase inhibitory potential, several of the compounds tested are structurally designed to modulate oxidative stress pathways, including ferroptosis, further reinforcing their relevance in the context of multifactorial neurodegeneration. Although these results were obtained by in silico docking simulations, they provide a good basis for subsequent in vitro and in vivo studies to validate the biological efficacy, target specificity and therapeutic feasibility of these phytochemicals.

Overall, this work emphasizes the value of previously under-researched natural products as promising scaffolds for the development of multifunctional agents to alleviate the complex pathophysiology of diseases such as Alzheimer's and Parkinson's disease. Future research should focus on pharmacokinetic profiling, toxicity assessment and mechanistic studies to facilitate the translational advancement of these compounds towards clinical application. In particular, incorporating well-designed animal model studies alongside pharmacokinetic and toxicity evaluations will be essential to confirm their therapeutic relevance and in vivo efficacy.

References

- Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics. *Molecular medicine reports* 2019;20(2):1479-1487.
- Giacobini E. Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacological research* 2004;50(4):433-440.
- Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012;149(5):1060-1072.
- Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor- κ B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. *Molecular cancer research* 2008;6(6):1059-1070.
- Kim HP, Son KH, Chang HW, et al. Anti-inflammatory plant flavonoids and cellular action mechanisms. *Journal of pharmacological sciences* 2004;96(3):229-245.
- Abdul Ghani MA, Ugusman A, Latip J, et al. Role of terpenophenolics in modulating inflammation and apoptosis in cardiovascular diseases: a review. *International journal of molecular sciences* 2023;24(6):5339.
- Pandey A, Bani S, Dutt P, et al. Multifunctional neuroprotective effect of Withanone, a compound from *Withania somnifera* roots in alleviating cognitive dysfunction. *Cytokine* 2018;102:211-221.
- Michalkova R, Mirossay L, Gazdova M, et al. Molecular mechanisms of antiproliferative effects of natural chalcones. *Cancers* 2021;13(11):2730.
- Zhang J, Chen Z, Huang X, et al. Insights on the multifunctional activities of magnolol. *BioMed research international* 2019;2019(1):1847130.
- Oon SF, Nallappan M, Tee TT, et al. Xanthorrhizol: A review of its pharmacological activities and anticancer properties. *Cancer cell international* 2015;15(1):100.
- Hanwell MD, Curtis DE, Lonie DC, et al. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of cheminformatics* 2012;4(1):17.
- Eberhardt J, Santos-Martins D, Tillack AF, et al. AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. *Journal of chemical information and modeling* 2021;61(8):3891-3898.
- Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry* 2010;31(2):455-461.
- Wang F-C, Peng B, Cao S-L, et al. Synthesis and cytotoxic activity of chalcone analogues containing a thieno [2, 3-d] pyrimidin-2-yl group as the A-ring or B-ring. *Bioorganic Chemistry* 2020;94:103346.
- Xian Y-F, Qu C, Liu Y, et al. Magnolol ameliorates behavioral impairments and neuropathology in a transgenic mouse model of Alzheimer's disease. *Oxidative medicine and cellular longevity* 2020;2020(1):5920476.
- Ye Y-H, Li C, Yang J, et al. Construction of an immobilised acetylcholinesterase column and its application in screening insecticidal constituents from *Magnolia officinalis*. *Pest Management Science* 2015;71(4):607-615.
- Hooda P, Malik R, Bhatia S, et al. Phytoimmunomodulators: A review of natural modulators for complex immune system. *Heliyon* 2024;10(1).
- Xie Y, Hou W, Song X, et al. Ferroptosis: process and function. *Cell Death & Differentiation* 2016;23(3):369-379.
- Syed AA, Reza MI, Singh P, et al. *Withania somnifera* in neurological disorders: Ethnopharmacological evidence, mechanism of action and its progress in delivery systems. *Current drug metabolism* 2021;22(7):561-571.
- Ben Bakrim W, El Bouzidi L, Manouze H, et al. Anti-amnesic effects of withaferin A, a steroidal lactone isolated from *Withania adpressa*, on scopolamine-induced memory impairment in mice. *Arabian Journal of Chemistry* 2022;15(1):103529.
- Birks JS, Harvey RJ. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database of systematic reviews* 2018;6(6): CD001190.
- Xi J, Zhang Z, Wang Z, et al. Hinokitiol functions as a ferroptosis inhibitor to confer neuroprotection. *Free Radical Biology and Medicine* 2022;190:202-215.
- Li J, Cao F, Yin H-I, et al. Ferroptosis: past, present and future. *Cell death & disease* 2020;11(2):88.
- Wang F, Wang J, Shen Y, et al. Iron dyshomeostasis and ferroptosis: a new Alzheimer's disease hypothesis? *Frontiers in aging neuroscience* 2022;14:830569.
- Skouta R, Dixon SJ, Wang J, et al. Ferrostatins inhibit oxidative lipid damage and cell death in diverse disease models. *Journal of the american chemical society* 2014;136(12):4551-4556.
- Darvesh S, Hopkins DA, Geula C. Neurobiology of butyrylcholinesterase. *Nat Rev Neurosci* 2003;4(2):131-138.