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Original Research

Indole Metabolites as Modulators of Human Cholinesterases: A Kinetic and Molecular Docking Study with Comparison to Alzheimer's Drugs

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ABSTRACT

Objective: Alzheimer's disease (AD) is characterised by the gradual decline of cognitive function and impaired cholinergic neurotransmission due to reduced brain acetylcholine levels. AChE and BChE regulate acetylcholine degradation and are important targets in AD. This research investigated the inhibitory effects of indole-3-propionic acid (IPA) and indole-3-butyric acid (IBA) on human cholinesterases, using enzyme kinetics and molecular docking.

Methods: We assessed the inhibitory effects of purified human BChE and commercially sourced erythrocyte-derived AChE using a modified Ellman spectrophotometric assay in a 96-well microplate. Kinetic parameters (Km, Vmax, and Ki) were determined by nonlinear regression and the Michaelis–Menten model, with Lineweaver–Burk plots used to analyse inhibition patterns. IC_{50} values were calculated by nonlinear regression analysis. Molecular docking simulations were performed to predict the binding positions of the indole derivatives and the reference Alzheimer's drugs donepezil and tacrine analogue in the active sites of AChE and BChE.

Results: According to docking simulations, both indole derivatives bind to the catalytic gorge of cholinesterases, but with lower predicted affinity than reference inhibitors. Enzyme inhibition assays showed that IBA exhibited weak inhibitory activity against both enzymes, with IC_{50} values of 15.61 mM for BChE and 54.28 mM for AChE. IPA exhibited stronger inhibition, particularly against BChE, with an IC_{50} of 2056 µM. Kinetic analysis indicated that IPA displays mixed-type inhibition of BChE (Km = 170.1 µM, Ki = 1774 µM), while its inhibition of AChE is competitive (IC_{50} = 3773 µM, Km = 268.5 µM, Ki = 6727 µM).

Conclusion: These findings suggest that short-chain indole metabolites can interact with cholinesterases, but are not potent inhibitors. Compared to IBA, IPA showed higher activity and distinct inhibition profiles for BChE and AChE. The development of new indole-based cholinesterase inhibitors may prove to be potent modulators of cholinergic enzymes.

Keywords: Alzheimer's disease, Cholinesterase inhibition, Enzyme kinetics, Indole metabolites, Molecular docking

INTRODUCTION

Alzheimer's disease (AD) is a neurological condition that mainly affects elderly individuals. AD causes abnormal behaviours and a progressive decline in cognitive function [1]. Worldwide, more than 50 million people are currently affected by this condition, and this number is predicted to triple by 2050 according to the World Health Organization (WHO) [2]. Reduction of brain acetylcholine (ACh) levels is a key sign of Alzheimer's disease, even though the exact causes of the disease are unknown [3]. As a result, extensive research over the past two decades has focused on acetylcholinesterase (AChE), the enzyme responsible for acetylcholine hydrolysis. This focus led to the development of AChE inhibitors as therapeutics for AD [4,5].

Acetylcholine is hydrolyzed into choline and acetic acid by cholinesterases, which belong to the esterase family of enzymes. The main types of cholinesterase enzymes are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [6]. Although these enzymes are structurally homologous glycoproteins, they differ in tissue distribution, catalytic properties, and substrate specificity [7]. AChE is abundant in the central nervous system (CNS), on skeletal muscle neurons, and on erythrocyte membranes. In contrast, BChE is primarily associated with glial cells and is predominantly found in blood plasma [7]. In addition to treating AD, cholinesterase inhibitors are also a standard therapy for other dementias [8].

The amino acid tryptophan (Trp) undergoes several metabolic alterations in the gastrointestinal tract. Three main pathways are involved in the intestinal metabolism of Trp. In this context, Trp serves as a precursor for serotonin synthesis by enterochromaffin cells and the gut microbiota, for kynurenine production by immune and epithelial cells, and for the generation of various bioactive metabolites – mainly indole derivatives – by intestinal microorganisms [9,10]. Indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), and indole-3-butyric acid (IBA) are among these metabolites [11,12]. Studies indicate that indole derivatives may play a role in regulating metabolic, immune, cardiovascular, and neurological functions [13]. The potent antioxidant and neuroprotective properties of IPA have been well studied, with research models in metabolic, inflammatory, and neurodegenerative alterations [14,15]

IBA is a plant-derived natural compound and an indole derivative with a butyric acid side chain. Similar to IPA, IBA exhibits various biological effects, such as anticancer, antiviral, neuroprotective, anti-inflammatory, antidiabetic, and antioxidant activities. These findings suggest that indole derivatives could be beneficial in medical treatments [16].

This study aimed to elucidate the function of short-chain indole derivatives, IBA and IPA, as inhibitors or regulators of BChE and AChE. In addition to enzyme kinetic experiments, the differences in enzymatic activity between the indole derivatives IPA and IBA were revealed through molecular docking studies.

Main Points

- The study examined how IPA and IBA affect human AChE and BChE using kinetic assays and docking analysis.
- Docking simulations showed that the compound binds within the catalytic gorge, but its affinity is lower than that of standard Alzheimer's drugs.
- IPA showed notable inhibition, especially for BChE, while IBA displayed only slight activity.
- Indole metabolites appear to act more as mild modulators than as strong cholinesterase inhibitors.

MATERIALS AND METHODS

Enzyme Source of Human BChE and AChE

Human butyrylcholinesterase was extracted from expired 0 Rh (–) human serum obtained from the Hacettepe Hospital Blood Bank [17]. Purification followed previously established protocols. In brief, serum samples underwent acid dialysis, then enzyme separation by Sephadex G-50 gel filtration chromatography. Final purification was achieved using procainamide–Sepharose 4 Fast Flow column affinity chromatography. Acetylcholinesterase from human erythrocytes was purchased commercially (Sigma-Aldrich, C0663) and used according to the supplier's instructions.

Molecular Docking

Ligands were modelled and optimized using LigPrep (2025-1, Schrödinger LLC, NY) and MacroModel (2025-1, Schrödinger LLC, NY) according to the OPLS5 (2025-1, Schrödinger LLC, NY) forcefield parameters [18]. Crystallographic structures of human AChE in complex with donepezil (PDB ID: 7e3h [19]) and BChE in complex with a tacrine analogue (PDB ID: 6i0b [20]) were downloaded from the RCSB Protein Data Bank

(www.rcsb.org) and prepared for docking using the Protein Preparation Workflow panel of Maestro (2025-1, Schrödinger LLC, NY). In this process, redundant molecules were removed, missing atoms were added, bond orders and partial charges were assigned, protonation states and hydrogen (H) bonds were set, and restrained forcefield minimization was performed. Receptor active site grids were generated using Maestro. The active sites were defined as a cube of 27.000 $Å^3$ volume with central coordinates -43.57, 37.98, -30.22 for AChE and 135.05, 112.48, 40.61 for BChE. Molecular docking was performed using these grids with Glide (2025-1, Schrödinger LLC, NY) [21] in standard precision mode with 100 runs per ligand, and the generated ligand poses were visually evaluated in Maestro.

Determination of IC_{50} Values for IPA and IBA

The IC_{50} values of indole-3-propionic acid and indole-3-butyric acid were determined using a modified 96-well plate Ellman assay with a total volume of 200 µL [22]. Indole derivatives for the test were dissolved in 1% DMSO, and solutions of IPA or IBA at varying final concentrations (1-750000 nM) were added to the wells. BChE assays were prepared in 96-well plates with IPA or IBA and 50 mM Tris/HCl buffer at pH 8.0. The plates also contained 0.25 mM DTNB (ε412, DTNB=14.2 mM^{-1} cm^{-1}) and 0.5-1.0 µg BChE. The mixtures were preincubated for 5 minutes in the dark at 37 °C while stirring on a plate shaker. The modified Ellman technique was used to measure enzyme activity, initiating the reaction by adding 0.5 mM butyrylthiocholine iodide (BTCh) substrate to the activity mixture. For AChE assays, the procedure used 0.5-1.0 µg of AChE and 0.5 mM acetylthiocholine iodide (ATCh) instead of BChE and BTCh. After adding the appropriate substrate to initiate enzyme reactions, activity was monitored for 10 minutes with kinetic measurements at 412 nm on a Multiskan Go (Thermo Fisher, USA) microplate reader.

We calculated enzyme activity for each inhibitor concentration as a percentage relative to the control wells, where enzyme activity was set at 100%. IC_{50} values were determined using nonlinear regression analysis in GraphPad Prism (version 8.4). Enzyme activity was plotted against the logarithm of inhibitor concentration. Mean values are reported for each experiment, which was conducted independently at least three times.

Enzyme Inhibition Kinetics of IPA

Enzyme kinetic analysis was conducted to investigate the inhibitory mechanism of IPA on cholinesterases using a modified Ellman method in a 96-well plate [22]. Three IPA concentrations, ranging from 250-1000 µM, and a control group without inhibitor were selected based on preliminary IC_{50} results. Michaelis-Menten kinetic data were obtained by measuring cholinesterase activity at various substrate concentrations. Butyrylthiocholine (BTCh) was used as the substrate for BChE activity, while acetylthiocholine (ATCh) was used for AChE assays. Substrate concentrations ranged from 1.0 mM to 0.05 mM to study enzyme kinetics. The enzyme–inhibitor mixtures, purified enzyme (0.5-1.0 µg AChE or BChE), 50 mM Tris/HCl pH 8.0, and the specified IPA concentration were incubated for five minutes at 37 °C in the dark. DTNB (final concentration 0.25 mM) was added, and the reaction was initiated with either ATCh or BTCh substrate. Absorbance was continuously recorded for 10 minutes at 412 nm using a Multiskan Go microplate reader. Each kinetic measurement was performed in triplicate. Enzyme activity was expressed in milliunits (mU), defined as the hydrolysis of one nmol of substrate per minute under the specified assay conditions. Kinetic parameters, including Km, Vmax, and Ki, were calculated using nonlinear regression analysis in GraphPad Prism. Inhibition patterns were assessed using Lineweaver–Burk double reciprocal plots.

RESULTS

Molecular Docking Analysis

The cholinesterase active site forms a deep catalytic gorge of approximately 20 Å that supports the hydrolysis of ACh and other esters through peripheral and acylation sites (P-site and A-site, respectively). P-site residues guide the substrate toward the A-site, where it is stabilized and hydrolyzed through an acylation-deacylation process involving the catalytic triad of serine, histidine, and glutamate. While AChE is almost exclusive to ACh, BChE can hydrolyse a wide range of substrates thanks to a larger acyl-binding pocket in A-site that accommodates diverse structures [23]. Molecular docking was performed to predict the affinity and binding modes of IPA and IBA in AChE and BChE active sites and to gain mechanistic insights at molecular level. Before the analysis, the docking protocol was validated by redocking the co-crystallized inhibitor in each receptor active site to determine whether the experimental binding modes could be reproduced. The RMSD values between the co-crystallized and predicted binding modes were 0.2109 and 0.5902 Å for donepezil and the tacrine analogue in AChE and BChE active site, respectively, indicating good predictive capacity for the method. Docking scores indicated that IPA and IBA showed similar affinity to the cholinesterases. Although

both compounds obtained better scores for BChE, their predicted affinities were markedly lower than those of the co-crystallized inhibitors (Table 1). In Table 1, cross-target docking values for donepezil with BChE and the tacrine analogue with AChE are indicated as not calculated, because these reference ligands were used only for redocking validation in their corresponding co-crystallized target enzymes.

Crystallographic study shows that donepezil occupies both A-site and P-site. The benzylpiperidine moiety stacks with the sidechain of Trp86, a choline-binding pocket residue of the A-site, which stabilizes the choline of ACh during hydrolysis [19]. At the same time, donepezil makes key interactions with Phe295, an acyl-binding pocket residue of A-site, Asp74 and Trp286, both P-site residues (Figure 1a).

The tacrine analogue is reported to span A-site and P-site of BChE active site making key interactions, such as π-stacking with Trp82 (corresponds to Trp86 of AChE), H bonding with His438, one of the catalytic triad, π-stacking with Phe329, a key acyl-binding pocket residue, and H bonding with Pro285 and Ser287, both acyl-binding pocket residues (Figure 1c) [20].

The selective inhibition of AChE and BChE by IPA

The inhibitory effects of IPA and IBA on cholinesterase activity were evaluated using enzyme kinetic experiments. As previously reported, the purified human BChE enzyme exhibited a specific activity of 15.1 Units/mg protein [17]. The entire process resulted in an overall yield of 38.3% and approximately 151-fold purification. Preliminary IC_{50} screening showed that IBA had limited inhibitory capacity on both enzymes, requiring high concentrations to produce significant effects. The IC_{50} values were found to be 15.61 mM for BChE and 54.28 mM for AChE, based on the activity percentage versus concentration graph (Figure 2a, d). Therefore, subsequent kinetic studies focused on IPA.

IBA and IPA were predicted in deprotonated form regarding the carboxylic acid moiety in physiological pH. The carboxylate was located close to the catalytic residues of AChE making H bond with Tyr133 (IBA) or Glu202 (IPA), while the indole ring stacked against Tyr337 sidechain, a key residue of the choline-binding pocket and made H bond with Asp74 at the same time (Figure 2e and Figure 4c; IBA and IPA for AChE, respectively). IBA was predicted with additional π-π interaction with Trp86 (Figure 2e).

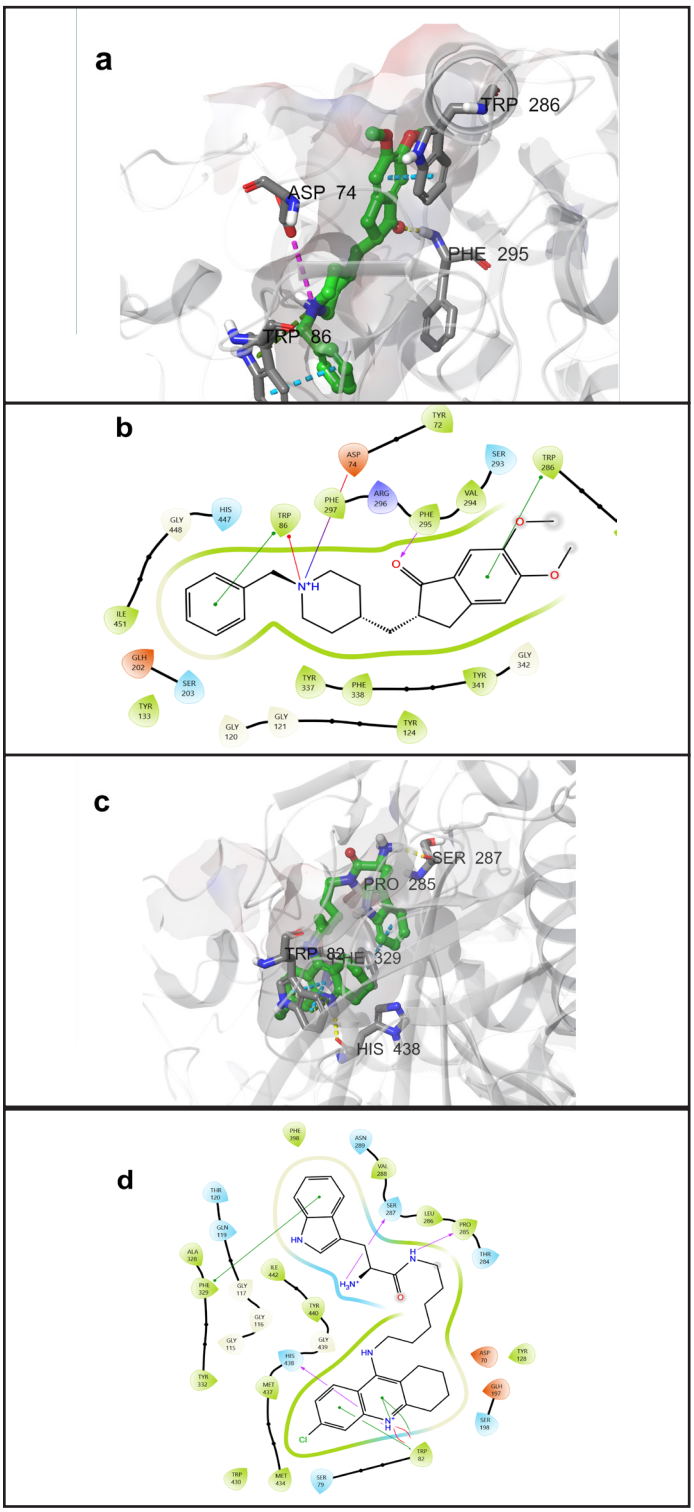
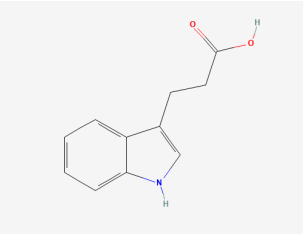
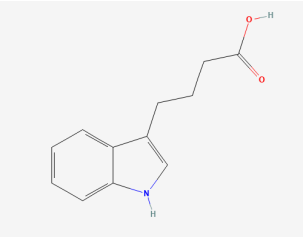
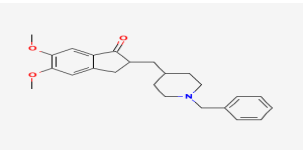
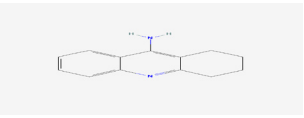


Figure 1. Co-crystallized binding of donepezil (a) and tacrine analogue (c) in AChE and BChE active sites, respectively. Ligands are shown in colored stick-and-ball representation, amino acid residues as grey sticks, receptor backbones as cartoons, and electrostatic interactions as colored dashed lines. The receptor molecular surface is rendered for AChE (a) and BChE (c). The 2D interactions diagrams of these binding modes are provided (b and d, respectively).

Table 1. Docking scores (kcal/mol) of IPA, IBA and reference drugs with Human AChE and BChE

Compound	Molecular structure	Binding Energy for AChE (kcal/mol)	Binding Energy for BChE (kcal/mol)
IPA		**-6.6**	**-7.7**
IBA		**-6.9**	**-7.4**
Donepezil		**-13.2**	**NC**
Tacrine		**NC**	**-11.1**

Co-crystallized inhibitor[a]: [a]The co-crystallized inhibitor is donepezil for AChE and a tacrine analogue for BChE. NC: Not Calculated

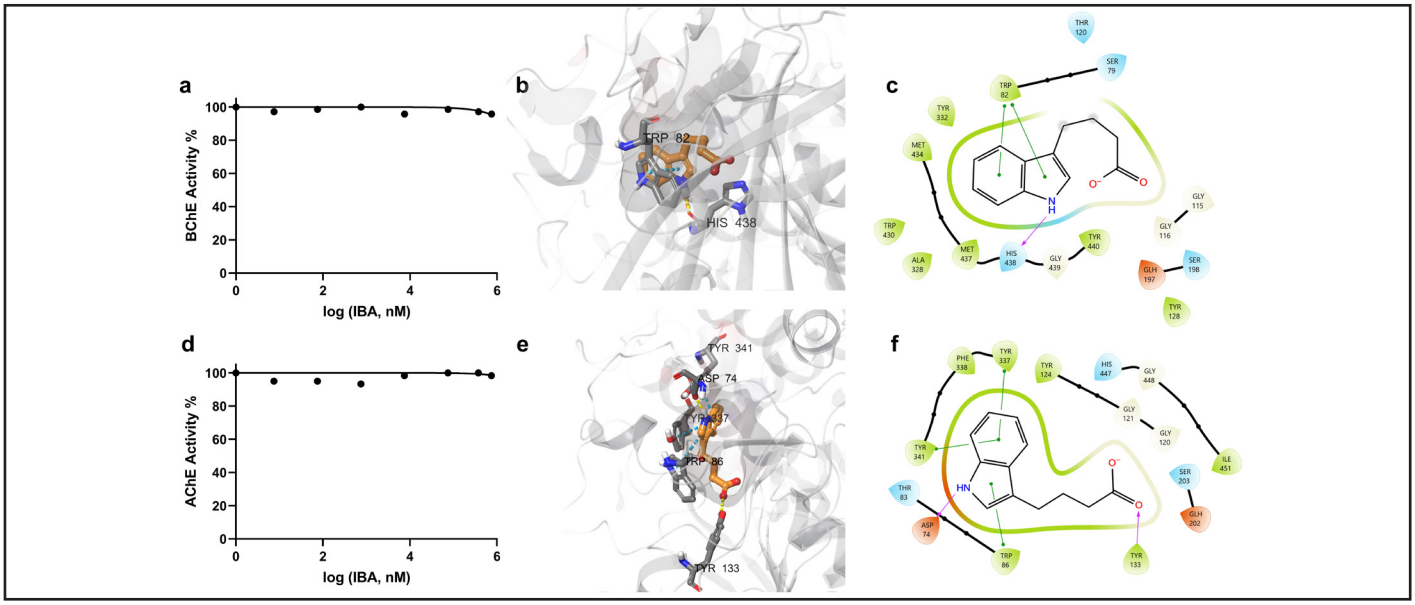


Figure 2. IC_{50} values of IBA with BChE and AChE: IC_{50} values for BChE and AChE are 15.61mM and 54.28 mM, respectively (a, d). Predicted binding of IBA in the active sites of BChE and AChE: ligands are shown in colored stick-and-ball representation, amino acid residues as grey sticks, receptor backbones as cartoons, and electrostatic interactions as colored dashed lines. The receptor molecular surface is rendered (b, e). 2D interactions diagrams of IBA for its predicted binding modes with BChE and AChE are shown (c, f).

The study evaluated IPA's inhibitory effects on AChE and BChE using initial velocity experiments with varying substrate and inhibitor concentrations. Based on the activity percentage versus concentration graph (Figure 3a), the IC_{50} value for BChE was determined to be 2056 µM. Data were analyzed with GraphPad Prism using nonlinear regression and inhibition models. The inhibition of BChE by IPA was tested at three concentrations (500–750 µM). Kinetic analysis confirmed a mixed-type inhibition model for BChE (Figure 3b). The Michaelis constant (Km) for the uninhibited enzyme was determined to be 170.1 µM, with a maximal velocity (Vmax) of 1505 µmol min^{-1} mg $protein^{-1}$. The inhibitory constant (Ki) was 1774 µM, while the alpha (α) value was 1.881. As α is greater than 1, IPA interacts with both the free enzyme and the enzyme–substrate complex, showing a preference for the free BChE enzyme. A strong fit ($R^2 = 0.995$) between IPA concentration and enzyme inhibition was observed through Lineweaver-Burk plot analysis. In these plots, increasing IPA concentrations resulted in simultaneous increases in both the slope and y-intercept, with the lines intersecting away from the y-axis, indicating mixed inhibition.

A different pattern was observed with AChE inhibition. The IC_{50} value for AChE, determined from the activity percentage versus concentration graph (Figure 4a), was 3773 µM. The experimental data was best fitted using nonlinear regression with a competitive inhibition model (Figure 4b). This model yielded a Km of 268.5 µM and a Vmax of 6240 µmol min^{-1} mg $protein^{-1}$. The obtained Ki of 6.73 mM indicated a relatively low inhibitor affinity. Lineweaver-Burk plot analysis showed a strong fit ($R^2 = 0.997$) between IPA concentration and AChE enzyme inhibition. The double reciprocal plots showed that increasing IPA concentrations caused the slope to increase while the y-intercept remained nearly constant, indicating that Vmax was unaffected and Km increased. This behaviour is consistent with competitive inhibition. According to the predicted poses of IPA and IBA with BChE, the indole ring was positioned similarly to the tricyclic core of the tacrine analogue and stacked with the Trp82 side chain. Additionally, the indole NH formed a critical hydrogen bond with His438 (Figure 3c and Figure 2b). Unlike in AChE, the carboxylate group of both compounds faced the acyl-binding pocket without significant contact.

DISCUSSION

Inhibiting cholinesterase is a primary pharmaceutical strategy for the symptomatic treatment of neurodegenerative diseases, especially AD. The hydrolysis of acetylcholine by AChE and BChE is essential for controlling synaptic levels and cholinergic signalling [24]. Cognitive impairment in AD is characterized by reduced acetylcholine and the degeneration of cholinergic neurons [7]. Rivastigmine, galantamine, and donepezil are clinically used cholinesterase inhibitors, but they provide limited effectiveness and frequently cause adverse effects [25]. Given these limitations, researchers have continually sought novel compounds that can modulate cholinesterase activity through diverse structural modifications or interaction mechanisms.

Indole compounds are of significant interest due to their diverse biological activities, including neuroprotective, anti-inflammatory, and antioxidant effects [26]. Microbial degradation of tryptophan leads to the production of IPA [27]. IPA has been shown to be a potent antioxidant, providing protection against oxidation-related neuronal damage [28]. While the biological functions of these indole metabolites are well established, their direct interaction with cholinesterase enzymes remains poorly understood. This research investigates whether short-chain indole derivatives such as IBA and IPA can act as inhibitors or regulators of BChE and AChE. Through molecular docking and enzyme kinetic experiments, it is demonstrated that IPA and IBA, indole derivatives differing by one carbon, affect enzyme activities differently.

Recent studies have highlighted that microbiota-derived Trp metabolites may also influence host physiology through activation of the aryl hydrocarbon receptor (AhR) signalling pathway [29]. Several bacterial Trp catabolites, including tryptamine, indole-3-acetic acid (IAA), indole-3-lactic acid (ILA), and IBA, have been identified as ligands of AhR. AhR is a ligand-activated transcription factor widely expressed in immune cells and within the central nervous system, and its activation has been shown to modulate both innate and adaptive immune responses in a ligand-specific manner [30]. Although IPA was primarily investigated in this study for its direct interaction with cholinesterases, activation of AhR signalling may provide an additional mechanism by which indole metabolites influence neuronal physiology. As AhR functions as a transcriptional regulator, its activation can alter the expression of various metabolic and regulatory enzymes and modulate inflammatory and oxidative stress pathways [31]. Since neuroinflammation and oxidative stress are closely linked to cholinergic dysfunction in neurodegenerative diseases, AhR-mediated signalling may indirectly influence cholinesterase activity by altering the cellular environment that regulates enzyme function [32]. Thus, indole metabolites from the

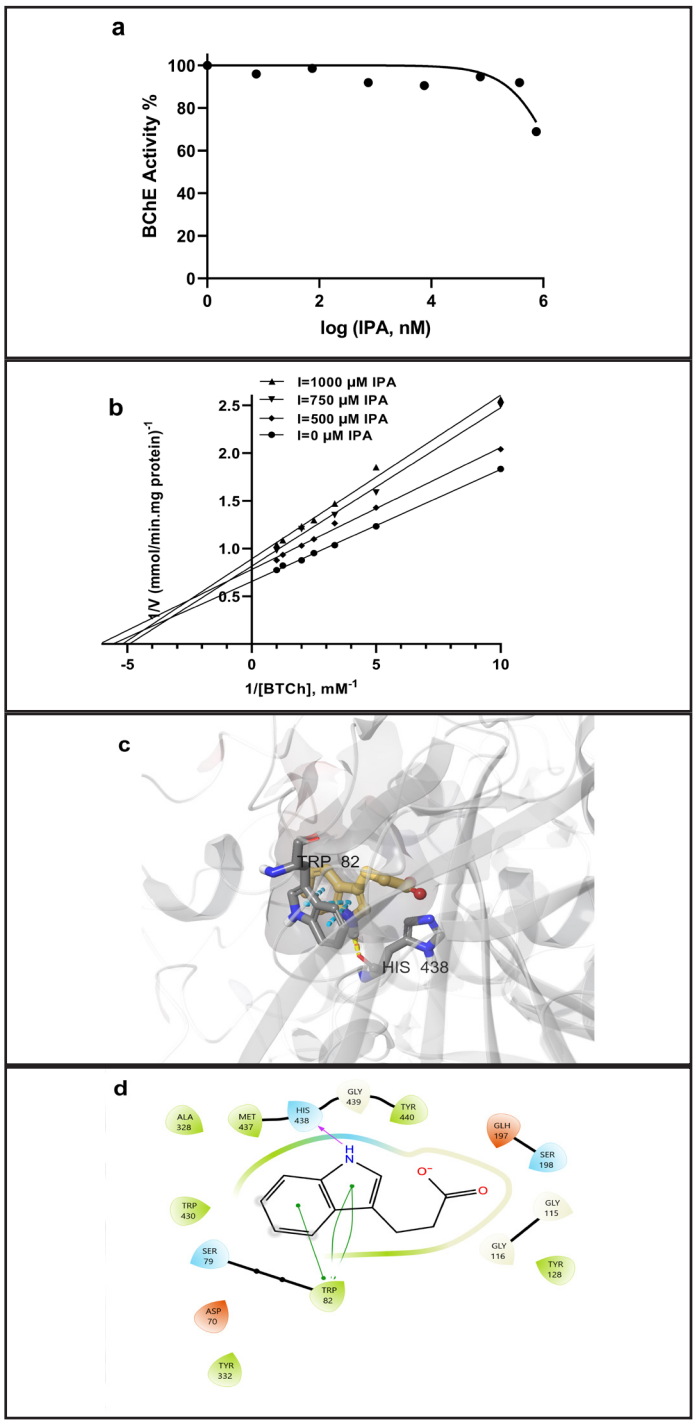


Figure 3. The kinetic features and anticipated interactions between IPA and BChE. IPA exhibits mixed inhibition of BChE, with an IC_{50} of 2056 µM and a Ki of 1774 µM (a, b). According to docking studies, IPA binds in a specific orientation to the BChE active site. The protein backbone is shown as a cartoon, the ligand as a colored stick-and-ball model, and interacting residues as grey sticks. Dashed lines indicate electrostatic interactions, and the receptor surface is displayed for structural context (c). Key contacts between IPA and BChE residues are shown in a two-dimensional interactions diagram in panel (d).

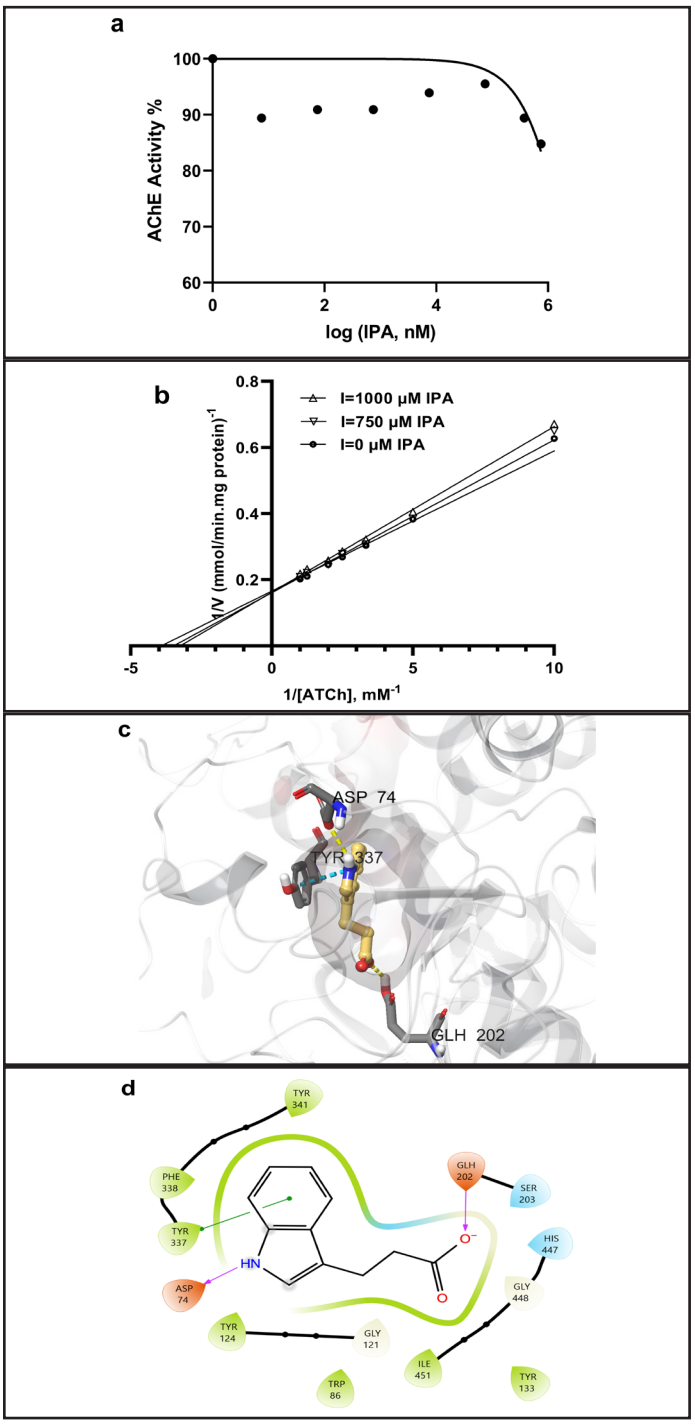


Figure 4. Kinetic characteristics and predicted binding interactions of IPA with AChE. IPA inhibits AChE through competitive inhibition, with an IC_{50} value of 3773 µM and a Ki of 6727 µM (a, b). Predicted binding of IPA in the active site of AChE is shown. Ligands are depicted in colored stick-and-ball representation, amino acid residues as grey sticks, receptor backbones as cartoons, and electrostatic interactions as colored dashed lines. The receptor molecular surface is rendered (c). Two-dimensional interactions diagram of IPA for its predicted binding mode with AChE is shown in panel (d).

microbiota, such as IPA, may influence the cholinergic system through direct enzymatic actions and broader receptor-mediated regulation involving AhR signalling.

Docking scores confirmed that cholinesterases have similar affinity for both IPA and IBA. Docking studies indicate that IPA and IBA may serve as useful scaffolds for designing cholinesterase inhibitors. Due to their small size, unlike known inhibitors that can occupy key regions of the active site, IPA and IBA are predicted to interact mainly with parts of the A-site, while regions such as the P-site remain inaccessible, likely resulting in relatively low affinity and stabilization. Both compounds achieved higher BChE scores, but their performance was significantly lower than that of co-crystallized inhibitors. Effective inhibitors such as donepezil bind throughout the entire catalytic gorge, interacting with various residues in both regions [33], thereby achieving stronger binding affinity and increased inhibitory potency.

Kinetic analysis revealed a significant difference in the inhibitory behavior of IPA and IBA. According to initial findings, IBA exhibited weak inhibitory activity against AChE and BChE. In parallel with the docking findings, this indicates that while the indole structure can engage with amino acids within the catalytic gorge, IBA does not lead to strong binding at the enzyme's active site. Conversely, IPA showed more potent inhibition, especially against BChE. The IC50 value for BChE inhibition indicates moderate affinity, as clinical cholinesterase inhibitors work at lower concentrations [25].

Kinetic modelling provided a deeper understanding of the inhibition mechanism. IPA exhibited mixed-type inhibition of BChE, indicating that it binds to the enzyme in both its free and substrate-bound forms, thereby affecting catalysis and substrate modification. Ligands binding to various sites within the catalytic gorge, beyond the substrate-binding site, often result in mixed inhibition [23]. As α is greater than 1, IPA binds to both the free enzyme and the enzyme-substrate complex, with a preference for the free BChE enzyme. The cholinesterase active site features a deep gorge composed of aromatic residues. This gorge contains both the catalytic and peripheral anionic sites, which are essential for recognising and stabilizing substrates [34]. Therefore, compounds interacting with residues in these regions may also influence catalytic activity and substrate binding.

According to Michaelis–Menten kinetic analysis, IPA affected AChE activity through a pattern distinct from that observed for BChE. The kinetic results indicated competitive inhibition, characterized by an increased Km and an unchanged Vmax. These results suggest that IPA binds to the enzyme's active site, competing with the substrate for its binding region. According to the Ki value, IPA had a moderate effect on inhibiting cholinesterases. Although IPA is a weaker inhibitor than existing treatments, its interaction suggests it may act as a mild, natural regulator of cholinesterases.

A recent study examined AChE activity in brain tissue from an LPS animal model treated with IPA. Compared to the LPS group, AChE activity decreased following IPA treatment. This suggests that IPA helps reduce negative effects on the cholinergic system and supports its continued function. Therefore, IPA could offer therapeutic benefits for cognitive impairment through cholinergic inhibition [35].

A key finding from the research is the differing strength of inhibition against AChE and BChE. Compared to AChE, BChE has a wider and more flexible acyl binding pocket, enabling it to accommodate a greater variety of substrates and inhibitors [36,37]. This structural feature may partially explain the greater inhibition of BChE by IPA observed in our current experiments. BChE is attracting attention as a potential therapeutic target, particularly for late-stage AD, as AChE activity decreases while BChE activity increases [38]. Future therapeutic strategies may benefit from compounds that selectively target BChE.

IPA and other indole metabolites can affect neuronal physiology beyond their direct interaction with cholinesterases. IPA is known to reduce oxidative stress, provide neuronal protection from β-amyloid, and reduce inflammatory signalling [28,39]. Compounds that influence both oxidative damage and cholinergic dysfunction may offer combined neuroprotection, as these issues often occur together in neurodegenerative diseases [40]. Although IPA's ability to inhibit cholinesterases is not very strong, its various biological effects could still help protect the nervous system under normal conditions.

Limitations

This study used purified enzymes, which may not reflect the complex biochemistry of actual neuronal systems. In addition, the inhibitory potency of IPA and IBA was modest compared to cholinesterase inhibitors, and docking analyses provide only a

theoretical perspective on possible ligand-enzyme interactions. Further studies are required to examine structural modifications of indole derivatives and to test their activity in cellular or in vivo models. This approach will provide a better understanding of their potential in cholinergic regulation and neuroprotective drug development.

CONCLUSIONS

The study investigated the interaction of indole-3-propionic acid and indole-3-butyric acid with human cholinesterases, using molecular docking and enzyme kinetics. The results indicate that both compounds can bind to the catalytic gorge of AChE and BChE, but their enzyme inhibition is significantly weaker than that of established cholinesterase inhibitors. IPA exhibited greater inhibitory effects than IBA, showing mixed-type inhibition of BChE and primarily competitive inhibition of AChE. The findings suggest that microbiota-derived indole metabolites may have a moderate impact on cholinesterase activity, rather than strongly inhibiting it. The structural features of indole derivatives may still be useful for designing new cholinesterase-targeting compounds with improved pharmacological properties.

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Conflict of Interest: The authors declare no conflicts of interest.

Informed Consent: NA.

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Ethical Approval: Not required, as this study did not involve human or animal subjects.

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Author Statement Regarding the Use of Artificial Intelligence-Enhanced Tools:

The authors declare that artificial intelligence (AI)–assisted tools were used solely to support language editing and improve the clarity and readability of the manuscript. These tools were not involved in the generation, analysis, or interpretation of scientific data, nor in the formulation of the study's conclusions. All scientific content, including study design, data analysis, and interpretation of results, was developed independently by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the work.

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