

Interaction of novel imidazole-based CO₂ sorbents with cholinesterases: an in vitro and in silico evaluation

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Abstract

The increasing demand for efficient and sustainable CO₂ capture technologies has driven the development of new ionic liquids (ILs) as potential alternatives to established sorbents. However, before large-scale industrial adoption, a comprehensive understanding of the toxicological profiles of these new ILs is essential to ensure human and environmental safety. Thus, in this study, we conducted a toxicological screening of nine imidazolium-based ILs (five previously reported and four newly synthesized analogs), focusing on their inhibitory effects on the activity of human cholinesterase enzymes (acetylcholinesterase, AChE; and butyrylcholinesterase, BuChE). Initial screening at 40 μ M revealed that, among the nine ILs, only IL 1-Dodecyl-3-methyl-2-(4-hydroxyphenyl) imidazolium iodide (C9), the dodecyl-substituted analog, inhibited human erythrocyte AChE (IC_{50} = 3.64 μ M) and plasma BuChE (IC_{50} = 0.87 μ M). When compared with compounds edrophonium and sodium dodecyl sulfate (SDS), only edrophonium inhibited AChE activity (IC_{50} = 3.03 μ M). Molecular docking simulations suggested that the long alkyl moiety of C9 stabilizes its interaction near the active sites of both AChE and BuChE. While shorter-chain ILs demonstrated no inhibitory effect, the potent cholinesterase inhibition exhibited by C9 indicates that elongating the alkyl chain introduces significant neurotoxic risks. Consequently, future designs of imidazole-based CO₂ sorbents must carefully balance these structural modifications to ensure environmental and occupational safety.

Introduction

Ionic liquids (ILs) are chemical compounds made up solely of cations and anions, with melting points typically below 100°C. Their structure can be highly customized by varying the types of cations and anions, resulting in a vast number of possible combinations and properties. Most ILs are based on organic cations (such as imidazolium, pyridinium, ammonium, or phosphonium) paired with organic or inorganic anions [1, 7, 24]. ILs are used as green solvents, electrolytes in batteries and supercapacitors, catalysts, and in separation techniques. Their design flexibility allows for applications in pharmaceuticals, materials science, energy storage, and analytical chemistry [1, 7, 24, 25].

For example, ILs can act as catalysts or as media that stabilize and immobilize catalytic species, and their broad solvation range and ability to stabilize reactive intermediates enhance reaction rates, selectivity, and recyclability. Task-specific ILs can even be engineered to facilitate particular catalytic transformations [7, 24]. In electrochemistry and energy applications, ILs are valued for their high ionic conductivity, wide electrochemical windows, and excellent thermal stability, making them effective electrolytes for batteries, supercapacitors, and fuel cells, as well as heat-transfer media and phase-change materials for thermal energy storage [1, 24, 25]. ILs also play an important role in separation and extraction technologies. Their adjustable solubility and selectivity enable efficient separation of organic, inorganic, and chiral species in processes such as liquid–liquid extraction, gas chromatography, and membrane-based techniques [1, 24, 25]. In materials science, ILs are incorporated into polymers, gels, and composites to enhance ionic conductivity, thermal stability, and interfacial behavior, features that are

useful in sensors, smart materials, and functional coatings [24, 25]. Biological and pharmaceutical applications have emerged as well, with some ILs demonstrating antimicrobial or cytotoxic activity [31]. These properties have encouraged the development of ILs for drug delivery, pharmaceutical synthesis, and even as active pharmaceutical ingredients [24, 25, 31].

Additionally, ILs are being explored for environmental applications, including CO₂ capture, pollutant removal, and biomass processing, contributing to cleaner and more sustainable industrial practices [1, 2, 7, 24, 25]. These features make them attractive, environmentally friendly alternatives to traditional organic solvents, reducing emissions and improving safety [1, 2]. Consequently, the synthesis of new ILs with low molecular mass and high-water solubility has become an important objective in the search for improved CO₂ sorbents. Recently, Aydos et al. [3] reported a new family of imidazole-derived zwitterionic compounds that represent a significant advancement in this area (Fig. 1). These novel water-soluble zwitterions are formed by covalently attaching hydroxy benzene groups to a 1,3-dimethylimidazolium cation, resulting in compounds specifically designed to overcome limitations associated with both conventional amine solutions and traditional ILs. This new class of imidazole-based zwitterions is of considerable environmental and industrial relevance, as it offers a promising alternative to the solutions currently used for CO₂ capture. Acting as basic compounds, they chemisorb CO₂ in aqueous media primarily by bicarbonate formation. They capture CO₂ efficiently, with an absorption enthalpy estimated to be about 30% lower than that of industrial N-methyl-diethanolamine solutions, suggesting that less energy is required for regeneration [3].

In view of the potential release of new chemicals into the environment, as well as laboratory and occupational exposure to newly developed ILs, it is imperative to evaluate their *in vitro* toxicity as an early step in assessing their safety for humans and the environment [23, 26, 27]. Accordingly, the toxicity of different classes of ILs has been widely reported in the literature [4, 5, 6]. For instance, some classes of ILs have been described as inhibitors of the activity of cholinesterase enzymes, both *in vitro* [7, 8] and *in vivo* following the exposure of invertebrates to high concentrations of imidazolium-containing ILs [9, 10]. Also, imidazolium-containing ILs have been reported to be weak or moderate inhibitors of cholinesterase from both invertebrates and fish [7–10]. Consequently, human cholinesterases (e.g., acetylcholinesterase and butyrylcholinesterase) can be potential targets for imidazolium-containing IL toxicity. Of note, the imidazole-containing IL compounds synthesized by Aydos et al. [3] share some chemical similarities with the therapeutic agent edrophonium (the phenol bound to a tertiary N) (Fig. 1), which is a reversible inhibitor of cholinesterases, used in the diagnosis of myasthenia gravis, and to accelerate muscle recovery after the use of non-depolarizing neuromuscular blocking agents in surgeries (e.g., curare-like drugs, gallamine, etc.) [11–14].

As noted above, the toxicity of various IL classes indicates that they can act as inhibitors of essential enzymes, with acetylcholinesterase (AChE) inhibition recognized as a potential biomarker of IL toxicity [28]. Accordingly, human cholinesterases are considered potential targets of imidazolium-based IL toxicity. In this study, we conducted an early-stage toxicological screening of nine novel imidazole-derived ILs to assess their potential human and environmental safety. To achieve this, we first evaluated

their *in vitro* inhibitory effects on human erythrocyte AChE and plasma butyrylcholinesterase (BuChE). Subsequently, we determined the half-maximal inhibitory concentrations (IC_{50}) for the most potent inhibitor, compound C9. The inhibitory activity of these ILs was compared with both the therapeutic agent edrophonium and sodium dodecyl sulfate (SDS) to evaluate the influence of structural moieties like the aliphatic side chain. Finally, their binding modes and molecular interactions within the active sites of both cholinesterase enzymes were further characterized using *in silico* molecular docking.

Material and Methods

Chemicals and synthesis

Solvents and reagents were purchased from Aldrich and used as received. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III HD 400 spectrometer, equipped with BBO 5mm probe with z gradients (^1H NMR: 400 MHz; ^{13}C NMR: 101 MHz). Edrophonium chloride [ethyl(m-hydroxyphenyl)dimethylammonium chloride, European Pharmacopoeia (EP) Reference Standard] was purchased from Merck.

Compounds 1 to 5 (C1–C5) were synthesized and characterized as previously described by Aydos et al. (2023). The remaining compounds (C6–C9) and their respective precursors were synthesized and characterized in-house. The detailed synthetic procedures, reaction conditions, purification methods, product yields, and complete structural characterization data (^1H and ^{13}C NMR spectra) for all compounds are provided in the Supplementary Material (Figures S1–S16).

Erythrocytes and plasma separation

Blood samples from healthy volunteers were centrifuged at $800 \times g$ for 10 minutes to separate the plasma from the erythrocytes. The erythrocyte pellet was subsequently washed twice with physiological saline (0.9% NaCl) by centrifugation at $800 \times g$ for 10 minutes. The washed erythrocytes were then resuspended in physiological saline to achieve a hematocrit of approximately 50% [17]. Immediately prior to cholinesterase quantification, the plasma and erythrocyte suspensions were diluted 80-fold and 300-fold, respectively, in distilled Milli-Q water [15].

Initial inhibitory evaluation of imidazole-derivatives on human plasma (BuChE) and erythrocyte (AChE) cholinesterases

As an initial step to determine the potential inhibitory effects of the new compounds, we tested all of them at a high concentration of 40 μM (compounds 1 to 9). The classical colorimetric methodology of Ellman [16] was adapted to a final volume of 200 μL to determine cholinesterase activities spectrophotometrically at 412 nm in a 96-microplate reader (SpectraMax M5). The medium consisted of a potassium phosphate buffer (50 mM, pH 7.4), 1 mM of 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent or DTNB).

The diluted plasma or erythrocytes (20 μ L) was pre-incubated with the inhibitors, H₂O (control for compounds 1 to 8, edrophonium and SDS) or DMSO (final concentration of 0.25%, control for compound 9) for 30 min at 25°C. The enzymatic reactions were started by adding acetylthiocholine to a final concentration of 0.8 mM. The reaction was monitored for 30 min at 412 nm.

As indicated in Fig. 2, among the 9 new imidazole-derivatives of edrophonium, only the compound 9 inhibited both activities in human blood in vitro. Subsequently, we performed a set of experiments to determine the IC₅₀ values (concentrations of the compound 9 and edrophonium that inhibited 50% of AChE or BuChE). Since compound 9 has an aliphatic lateral group with 12 carbons, we investigated whether the inhibitory effectiveness could be related to this aliphatic portion by testing the influence of SDS on both cholinesterases.

AChE and BuChE inhibitory potency of compound 9, SDS, and edrophonium

Inhibitory activity assays for C9, SDS, and edrophonium were conducted in triplicate. Inhibitory curves for C9 (from 0.78 to 25 μ M), edrophonium (1.25 to 10 μ M), and SDS (5 to 25 μ M) were performed. C9 was diluted with DMSO and edrophonium, and SDS was diluted with water. After 30 min of pre-incubation, the enzymatic reactions were started by adding acetylthiocholine. The raw data of absorbance values were normalized utilizing the formula below ($\bar{x}$ is the mean of the triplicates, and n is the time in seconds):

$$Z_n = \bar{x}_n - \bar{x}_0$$

The inhibition rate (IR, %) was calculated using the following equation:

$$IR = [1 - (Z_n / Z_{\text{control}})] \times 100$$

Dose-response non-linear regression was calculated in GraphPad Prism software to obtain IC₅₀ (95% confidence interval). DMSO was used as control for C9 (final concentration of DMSO 0.25%) and H₂O was the control for edrophonium and SDS.

Docking simulations

The software AutoDock Vina 1.1.1 was applied for the molecular docking simulations, according to previous studies [15, 18]. The grid box of AChE (PDB 4EY6) was placed on x = -13.2, y = -46.7, and z = 33.5 (size: 30 $\times$ 30 $\times$ 30 Å). Structural essential water molecules (HOH) were considered in the simulations, such as 704, 705, 707, 773, 803, 805, and 815. For the BuChE (PDB 4BDS), it was centered on the coordinates x = 138.8, y = 111.5 and z = 39.9 (size: 30 $\times$ 30 $\times$ 30 Å), taking into account the water molecules 2064, 2089, 2090, 2093, 2131, and 2140 [15, 19]. The 3D model of the ligands was created using the Avogadro 1.1.1 [20] and MOPAC (PM6 method) software [21], and the partial charges from the semiempirical method were used. Only the organic moiety of the ligands was in silico tested against the cholinesterases. Edrophonium was also used to validate the protocol, which demonstrated similar interactions and binding pose when compared with the crystallographic structure of the *Tetronarce californica* AChE complexed with edrophonium (PDB ID 2ACK) [22]. For each molecule, 20 conformers

were generated and analyzed in the Accelrys Discovery Studio Visualizer program (<https://www.3ds.com/>). As the possible binding mode, the conformers with the most favorable predicted binding free energy were selected (ΔG , kcal/mol) from the most populated cluster (RMSD cutoff of 2Å) [18].

Statistical analysis

The data from the study were presented as mean with standard error (SE). GraphPad Prism software was used for t-test statistical analysis, and $P < 0.05$ was considered significant.

Results

The chemical characterization of compounds C6 to C9 is presented in the supplementary material.

The initial screening of all imidazole-containing ionic liquids at 40 μM is depicted in Fig. 2 for AChE (erythrocytes) and BuChE (plasma). C9 was the only compound that almost completely inhibited AChE and caused an inhibition of about 90% in BuChE. C8 caused a 30% inhibition of AChE and 20% of BuChE. At 40 μM , C1 to C7 caused a small but significant inhibition of AChE varying from 5 to about 15% (Fig. 2). However, at 25 μM (data not shown), only compound C9 caused inhibition of AChE or BuChE. Since the IC_{50} for C1 to C8 was higher than 40 μM , we did not further test them as inhibitors of AChE or BuChE.

Since C9 specifically caused a marked and significant inhibition of AChE and BuChE, we performed a concentration-dependent inhibitory curve to calculate the IC_{50} values for both enzyme activities (Table 1). First, we compared its inhibitory potency with the reversible AChE inhibitor edrophonium (Fig. 3A and B). The C9 inhibited both erythrocyte AChE and plasma BuChE, but edrophonium inhibited only AChE. The inhibitory potencies of C9 and edrophonium for AChE were similar (Fig. 3A and Table 1). In contrast, edrophonium did not inhibit the BuChE, and C9 had a lower IC_{50} in BuChE compared to AChE (Table 1).

Since the C9 has an aliphatic side chain of 12 C atoms, we hypothesized that this large hydrophobic moiety could disrupt the tertiary structures of AChE and BuChE and be the molecular moiety responsible for cholinesterase inhibition. However, SDS had only a small inhibitory effect at 25 μM in human AChE but no effect in human BuChE (Fig. 3A and B).

The general poses of the interactions of ligands (C1 to C9 and edrophonium) with the active sites of AChE (Fig. 6A and B) and of BuChE (Figs. 6C and D) indicated that all the ligands interacted close to the active site of both enzymes.

The energetics and the chemical nature of the interaction of C1 to C9 and edrophonium with AChE are presented in Table 2 (the main residues of the enzyme interacting with the compounds are also indicated in Table 2). All the compounds exhibited stable interaction with the active site of human AChE, and the ΔG varied from -7.3 (for C8 and C9) to $-6.2 \text{ kcal.mol}^{-1}$ (edrophonium). In fact, edrophonium (Fig. 7A)

and the ligands C1 to C7 (Fig. 7B to 7G) interacted with the active site of AChE mainly by hydrophobic, electrostatic, cation- π , and H-bonds intermolecular forces. (Fig. 7 and Table 2).

Compounds 8 and 9 interacted near the entrance of the AChE active site, in the peripheral anionic subsite (PAS). Overall, the tested compounds interacted near the catalytic triad (CT: Ser203, His447, and Glu334), particularly with Trp86, Tyr124, Glu202, Tyr337, and some molecules of H₂O (Fig. 7 and Table 2).

The results are somewhat similar to the interaction of other inhibitors with AChE [15, 19]. Edrophonium, a reversible inhibitor of AChE, also interacted close to the catalytic triad but with a distinct conformation from compounds 1 to 9. In addition, the meta-O(H) compounds C1 and C2 demonstrated a different molecular orientation when compared with the para-O(H) compounds 3,4, 6, and 7, indicating that the position of the hydroxyl group interfered in the binding mode, while the protonation state of the hydroxyl group does not (Fig. 7).

For compounds 1 and 2 (Fig. 7B and C), the benzene moiety interacted with the Trp86, while for compounds 3,4,6, and 7 it was the imidazolium group that interacted with this Trp86. The additional interaction of compounds 8 and 9 with the PAS region was due to the presence of the bulky aromatic and aliphatic groups linked to the imidazole moiety.

The thermodynamic stability (ΔG) and the chemical nature of the interaction of C1 to C9 and edrophonium with BuChE are presented in Table 3. The main residues of the enzyme contributing to the interaction of the enzyme with the ligands and the nature of the interactions are also indicated in Table 3 and Fig. 8. All the compounds exhibited stable interaction with the active site of human BuChE, and the ΔG varied from -8.4 kcal.mol⁻¹ (for C8) followed by C7 and C9 (-7.1 kcal.mol⁻¹), C4 (-7.0 kcal.mol⁻¹), C6 (-6.5 kcal.mol⁻¹), C1 and C2 (-6.4 kcal.mol⁻¹), C3, C5 and edrophonium (-6.2 kcal.mol⁻¹).

For BuChE, all the in silico tested molecules bound close to the bottom of the gorge (i.e., close to the catalytic triad Ser198, His438, and Glu325), and among the Trp82, Gly116, Glu197, Ser198, Leu286, and Phe329 residues. Overall, all of them have similar binding poses to the edrophonium (Fig. 8). For BuChE, the imidazolium moiety of the ligands binds practically in the same region as the alkylammonium group of edrophonium. The position and protonation state of the hydroxyl groups, as well as the type of organic moiety linked to the imidazole ring (methyl, ethyl, benzyl, and dodecyl), do not appear to interfere with the binding position of the compounds significantly. Possibly, the distinct binding mode of interaction between the cholinesterases may be attributed to the BuChE active site being less restrictive than that of AChE, as previously observed [15, 19].

Discussion

The growing demand for efficient and sustainable technologies has led to the development of new ILs for diverse applications, including their use as green solvents, electrolytes, as well as CO₂ capture. Due to their design flexibility and favorable properties, ILs are increasingly considered environmentally

friendly alternatives to conventional organic solvents. However, widespread industrial use increases the risk of environmental contamination through accidental spills or effluents, resulting in the accumulation of pollutants in surface waters and soils [29]. Consequently, comprehensive toxicological assessments are essential before large-scale industrial implementation to ensure human and environmental safety.

Here, our initial screening revealed that among the nine (C1-C9) tested imidazole-derivatives, the compound C9, characterized by a dodecyl aliphatic side chain, was the only one to exhibit substantial inhibition of both human AChE and BuChE activities at relatively low concentrations (near 1 μ M). This observation aligns with previous reports that have characterized imidazolium-containing ILs as moderate to weak inhibitors of cholinesterases (IC_{50} greater than 10 or even greater than 100 μ M) [5, 6]. The inhibitory effectiveness of C9 against AChE activity was found to be comparable to that of edrophonium, a known reversible AChE inhibitor (Table 1, Fig. 3A) [18]. This finding supports the initial hypothesis that most of these imidazole-containing compounds, sharing some structural similarity with edrophonium, could indeed toxically target cholinesterases. However, C9 demonstrated potent inhibition of BuChE, with an even lower IC_{50} value than for AChE, whereas edrophonium showed no significant BuChE inhibition when tested at 10 μ M (Table 1, Fig. 3B). Our results agree with the data of Rosenberry et al. [19], who demonstrated that the K_D determined by the isothermal titration calorimetry for edrophonium with AChE and BuChE were 1.4 and 27.2 μ M, respectively. Likewise, the authors [19] reported that the K_I for inhibition of AChE (0.82 μ M) by edrophonium was much lower than that found for BuChE (49 μ M), and in accordance with the IC_{50} obtained in this study for AChE. The differential selectivity of C9 towards BuChE over AChE, and its distinct profile compared to edrophonium, suggests a unique mechanism of interaction influenced significantly by its structural features, particularly the long alkyl chain. Indeed, the long alkyl (dodecyl) moiety of C9 contributed to further stabilize the interaction of the ligand near the active site of AChE and BuChE (Tables 2 and 3 and Figs. 7 and 8).

To investigate whether the C12 aliphatic chain of C9 is a contributing factor to cholinesterase inhibition, SDS was used as a control. At concentrations up to 25 μ M, SDS exhibited only minimal inhibitory effects on AChE and no effect on BuChE (Fig. 3A, B). This suggests that although the hydrophobicity conferred by the long alkyl chain plays a role in C9 activity, a dodecyl chain does not appear to be the primary mechanism for the observed potential cholinesterase inhibition. Instead, the entire molecular structure of C9, including the imidazolium group and the specific arrangement of the alkyl chain, likely contributes to its specific binding and inhibitory action on the cholinesterases activities. While a pure alkyl chain like SDS does not cause significant inhibition, the combination of a lipophilic tail and a cationic imidazolium headgroup creates a potent surfactant-like effect. As noted in the literature, the "side chain effect" in imidazolium-based ILs dictates that an increase in the cation side chain length drastically enhances lipophilicity, which facilitates the disruption of folded protein structures and biological membranes. Therefore, the C12 chain of C9 likely allows it to penetrate and anchor more stably into the hydrophobic regions of the cholinesterases compared to its shorter-chain analogs [31].

Consequently, the assumption that newly developed "green" ILs are uniformly safe must be continuously reevaluated. In vitro toxicity screening provides a critical first step in assessing their safety, and the

cholinesterase inhibition observed for C9 highlights the need for ongoing toxicological evaluation before any newly synthesized IL is considered for widespread industrial use. While cholinesterase inhibition poses a clear toxicological concern for industrial solvents, it also points to a potential pharmacological application. Targeted inhibition of AChE and BuChE remains a potential therapeutic strategy for managing cognitive decline in neurodegenerative diseases, particularly Alzheimer's disease [30]. Thus, the structural features of these compounds could provide valuable scaffolds for both the development of novel neurotherapeutic agents and the design of safer ILs for environmental applications, such as CO₂ capture.

Conclusion

The present results indicate that while most of the newly synthesized imidazole-derived ILs exhibit little to no inhibitory potential against human cholinesterases, the compound C9, featuring a dodecyl side chain, is a potent inhibitor of both human AChE and, notably, BuChE. Its inhibitory potency is comparable to, or even exceeds, that of the classic cholinesterase inhibitor edrophonium. Consequently, for the future design and application of imidazole-based CO₂ sorbents, structural modifications should favor shorter alkyl chains (such as those in C1-C7) to maintain environmental and occupational safety, as the elongation to a 12-carbon chain (C9) clearly crosses a threshold into potential neurotoxicity. Ultimately, these findings emphasize that comprehensive in vitro and in vivo toxicological screenings are indispensable to accurately determine the safety profile of novel imidazole-containing ILs prior to their industrial application.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by M.M.B., J.B.T.R., P.A.N., G.M., G.A., and G.E.. Resources and essential laboratory supplies were provided by D.O.G.S. and J.D. The first draft of the manuscript was written by M.M.B., D.O.G.S., and J.B.T.R., and all authors commented on previous versions and reviewed the manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Tables

Table 1

Inhibitory potency of compound C9, edrophonium, and sodium dodecyl sulfate (SDS) against human acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The half-maximal inhibitory concentration (IC_{50}) values are expressed in μM and were calculated using a non-linear regression model.

Enzyme	C9 IC_{50}	CI 95%	Edrophonium IC_{50}	CI 95%	SDS IC_{50}	CI 95%
AChE	3.64	2.73 to 4.80	3.03	2.54 to 3.62	> 25.0	—
BuChE	0.87	0.49 to 1.24	> 10.0	—	> 25.0	—

Table 2

Predicted binding energies (ΔG , kcal/mol) and primary molecular interactions of edrophonium and compounds C1–C9 within the active site of human acetylcholinesterase (AChE). Compounds 3 and 5 are identical in the docking simulations because the counter anions (Br^- and Cl^-) were not included in the simulations.

Molecule (ΔG)	Residue	Interaction	Distance ($\text{\AA}$)
Edrophonium (−6.2)	Trp86	cation– π	4.46
	Trp86	cation– π	4.45
	Glu202	electrostatic	5.51
	Tyr337	hydrophobic (σ – π)	3.81
	Phe338	hydrophobic (π – π)	5.26
C1 (−6.9)	Trp86	hydrophobic (π – π)	4.84
	Trp86	hydrophobic (π – π)	4.13
	Gly121	H-bond	2.87
C2 (−7.1)	Trp86	hydrophobic (π – π)	4.84
	Trp86	hydrophobic (π – π)	4.15
	HOH707	H-bond	1.97
C3 or C5 (−6.3)	Trp86	cation– π	4.55
	Trp86	cation– π	3.87
	Trp86	hydrophobic (π – π)	5.15
	Tyr124	H-bond	1.92
	Glu202	electrostatic	3.29
	HOH803	CH-bond	3.55
C4 (−6.5)	Trp86	cation– π	4.48
	Trp86	cation– π	3.76
	Tyr124	H-bond	1.84
	Glu202	electrostatic	3.23
	HOH803	CH-bond	3.58
C6 (−6.5)	Trp86	cation– π	4.51

Molecule (ΔG)	Residue	Interaction	Distance (Å)
	Trp86	cation- π	3.76
	Trp86	hydrophobic (π - π)	5.09
	Tyr337	hydrophobic (σ - π)	3.47
	Glu202	electrostatic	3.25
	HOH803	CH-bond	3.46
C7 (-6.9)	Trp86	cation- π	4.51
	Trp86	cation- π	3.76
	Tyr337	hydrophobic (σ - π)	3.53
	Glu202	electrostatic	3.18
	HOH803	CH-bond	3.50
C8 (-7.3)	Trp286	cation- π	4.86
	Trp286	hydrophobic (π - π)	4.82
	Trp286	H-bond	2.19
C9 (-7.3)	Trp86	hydrophobic (alkyl- π)	5.45
	Trp86	hydrophobic (σ - π)	3.73
	Tyr124	hydrophobic (alkyl- π)	5.37
	Phe297	hydrophobic (alkyl- π)	5.14
	Trp286	hydrophobic (π - π)	5.42
	Trp286	cation- π	4.63
	Tyr337	hydrophobic (alkyl- π)	5.34
	Tyr337	hydrophobic (alkyl- π)	5.30
	Phe338	hydrophobic (alkyl- π)	4.99
	Tyr341	hydrophobic (alkyl- π)	4.60
	Tyr341	hydrophobic (π - π)	4.96

Table 3

Predicted binding energies (ΔG , kcal/mol) and primary molecular interactions of edrophonium and compounds C1–C9 within the active site of human butyrylcholinesterase (BuChE). Compounds 3 and 5 are identical in the docking simulations because the counter anions (Br^- and Cl^-) were not included in the simulations.

Molecule (ΔG)	Residue	Interaction	Distance ($\text{\AA}$)
Edrophonium (–6.2)	Trp82	hydrophobic (π – π)	3.86
	Trp82	hydrophobic (σ – π)	3.80
	Trp82	cation– π	4.22
	HOH2090	H-bond	2.69
	HOH2093	H-bond	2.97
C1 (–6.4)	Trp82	hydrophobic (π – π)	4.80
	Trp82	hydrophobic (π – π)	4.14
	HOH2090	H-bond	2.57
C2 (–6.4)	Trp82	hydrophobic (π – π)	4.80
	Trp82	hydrophobic (π – π)	4.19
	HOH2089	H-bond	2.12
	HOH2090	H-bond	2.81
C3 or C5 (–6.2)	Trp82	hydrophobic (σ – π)	3.80
	Trp82	cation– π	4.72
	Gly115	H-bond	2.60
	HOH2089	H-bond	2.11
C4 (–7.0)	Trp82	hydrophobic (π – π)	4.60
	Trp82	hydrophobic (π – π)	4.17
	Trp82	hydrophobic (σ – π)	3.75
	Trp82	cation– π	4.68
	HOH2089	H-bond	2.01
C6 (–6.5)	Trp82	hydrophobic (alkyl– π)	4.37
	Trp82	cation– π	4.87

Molecule (ΔG)	Residue	Interaction	Distance (Å)
	Ala328	hydrophobic (alkyl– alkyl)	4.10
	Met437	hydrophobic (alkyl– alkyl)	5.25
	HOH2090	H-bond	2.68
C7 (–7.1)	Trp82	hydrophobic (alkyl– π)	4.36
	Trp82	cation– π	4.75
	Trp82	hydrophobic (π – π)	4.62
	Trp82	hydrophobic (π – π)	4.20
	Ala328	hydrophobic (alkyl– alkyl)	4.15
	Met437	hydrophobic (alkyl– alkyl)	5.15
	Trp430	hydrophobic (alkyl– π)	5.41
	Trp430	hydrophobic (alkyl– π)	5.08
	HOH2089	H-bond	2.01
C8 (–8.4)	Gly115	hydrophobic (π –amide)	4.31
	Gly116	hydrophobic (π –amide)	5.10
	Glu197	H-bond	2.90
	Leu286	hydrophobic (π –alkyl)	5.37
	Phe329	hydrophobic (π – π)	4.73
	HOH2090	H-bond	2.64
C9 (–7.1)	Trp82	hydrophobic (alkyl– π)	3.90
	Trp82	cation– π	4.16
	Trp82	hydrophobic (π – π)	4.07
	Trp82	hydrophobic (π – π)	3.87
	Glu197	H-bond	2.23
	Trp231	hydrophobic (alkyl– π)	4.39
	Trp231	hydrophobic (alkyl– π)	4.16
	Leu286	hydrophobic (alkyl– alkyl)	4.71
	Phe329	hydrophobic (alkyl– π)	5.31
	Phe329	hydrophobic (alkyl– π)	5.21

Figures

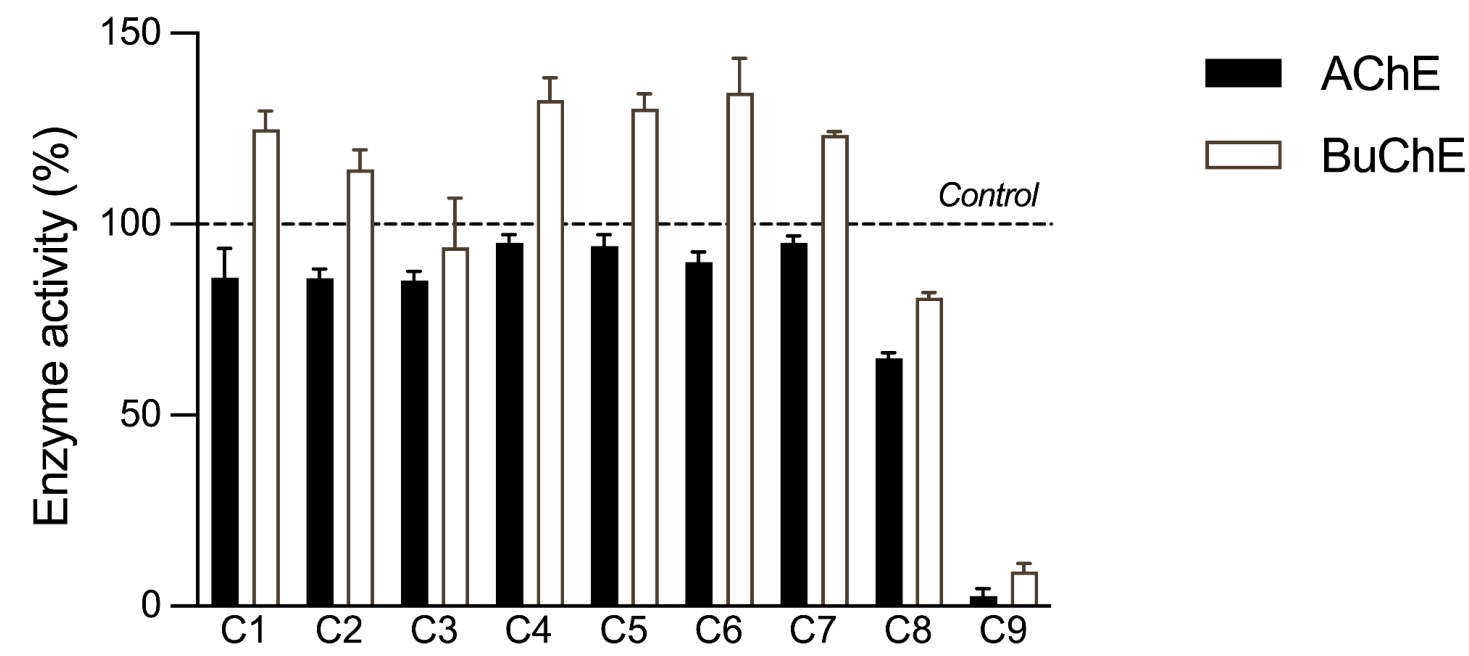


Figure 1

Chemical structures of the imidazole-derived ionic liquids (compounds C1–C9) and edrophonium. C1: 1,3-Dimethyl-2-(3-hydroxyphenyl) imidazolium iodide, C2: 1,3-Dimethyl-2-(3-hydroxyphenyl) imidazolium zwitterionic salt, C3: 1,3-Dimethyl-2-(4-hydroxyphenyl) imidazolium iodide, C4: 1,3-Dimethyl-2-(4-hydroxyphenyl) imidazolium zwitterionic salt; C5: 1,3-Dimethyl-2-(4-hydroxyphenyl) imidazolium chloride; C6: 1-Ethyl-3-methyl-2-(4-hydroxyphenyl) imidazolium iodide; C7: 1-Ethyl-3-methyl-2-(4-oxyphenyl) imidazolium zwitterionic salt; C8: 1-Methyl-3-benzyl-2-(4-hydroxyphenyl) imidazolium bromide; C9: 1-Dodecyl-3-methyl-2-(4-hydroxyphenyl) imidazolium iodide. Compounds C1 to C5 were synthesized as described by Aydos et al. (2023). The synthesis procedures for compounds 6 to 9 are described in Material and Methods.

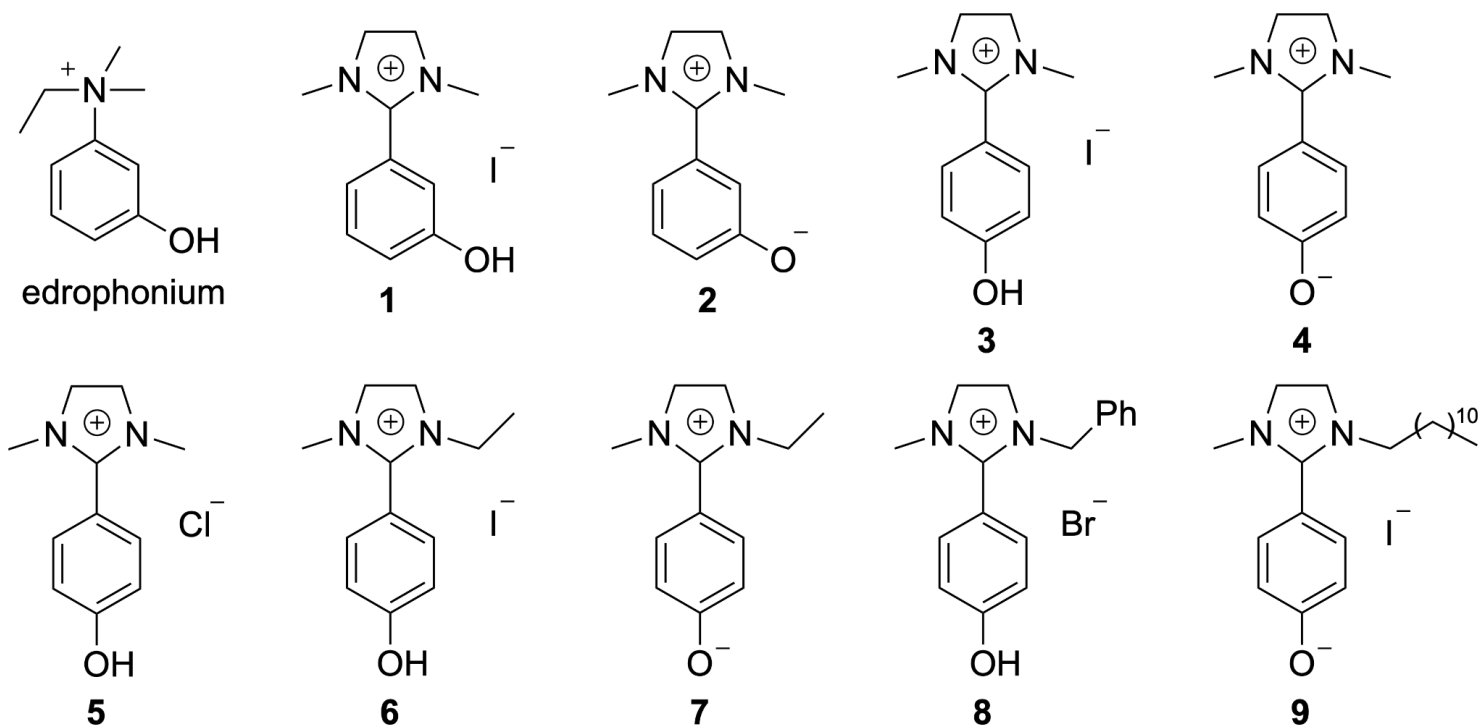


Figure 2

Inhibition of human erythrocyte acetylcholinesterase (AChE; black columns) and plasma butyrylcholinesterase (BuChE; white columns) by imidazole-derived ionic liquids. All compounds (C1–C9) were tested at a concentration of 40 μ M. Enzymatic activity was calculated relative to their respective controls (H_2O for C1–C8; 0.25% DMSO for C9). Data are expressed as mean $\pm$ standard error (SE) of 3–5 independent experiments.

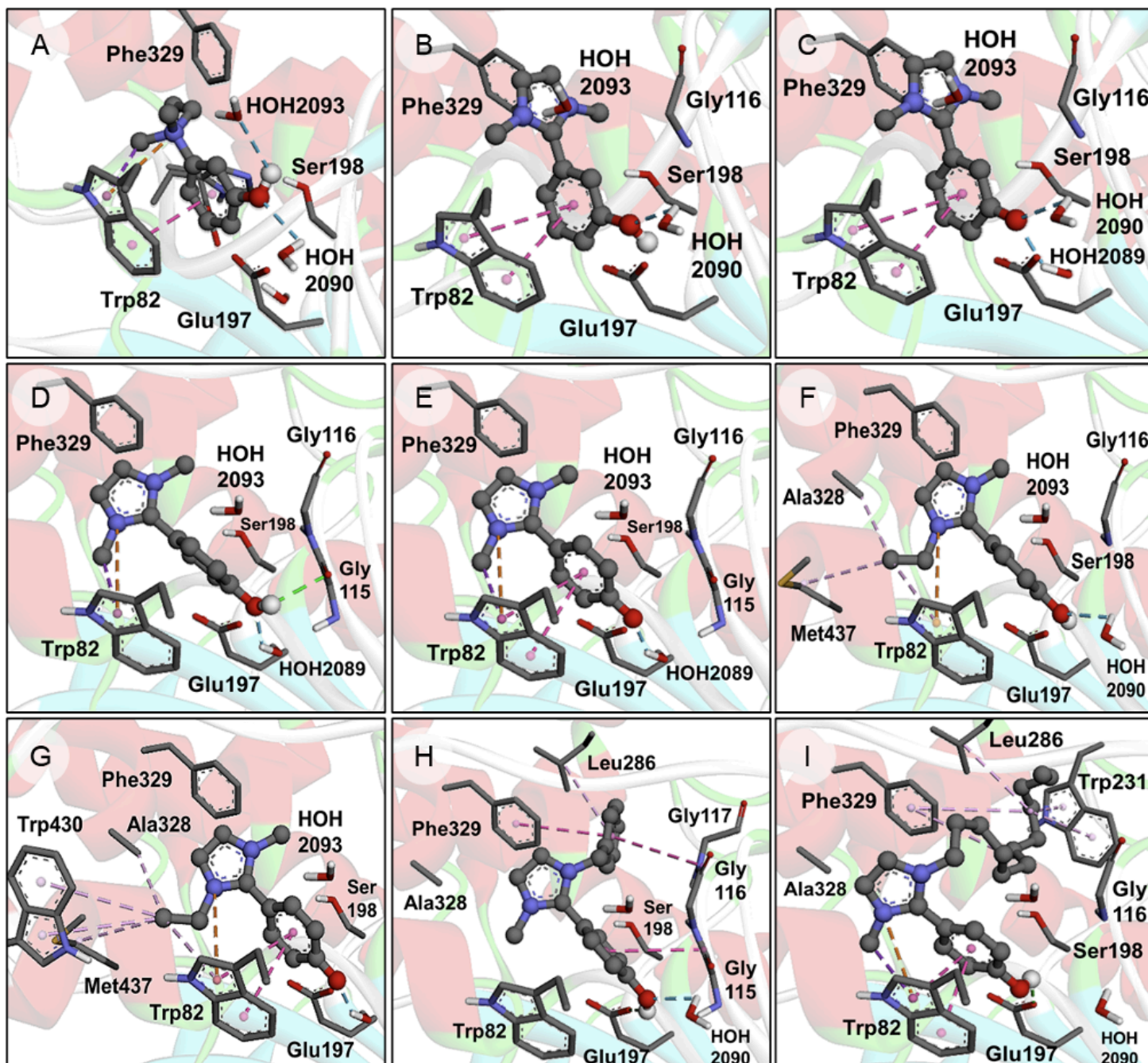


Figure 3

Concentration-response inhibitory curves for (A) human acetylcholinesterase (AChE) and (B) human butyrylcholinesterase (BuChE). C9 is indicated by the circle, edrophonium by the square, and sodium dodecyl sulfate (SDS) by the triangle. The standard deviation was less than 20% of the respective means and was not indicated for the sake of clarity (N = 2 to 5).

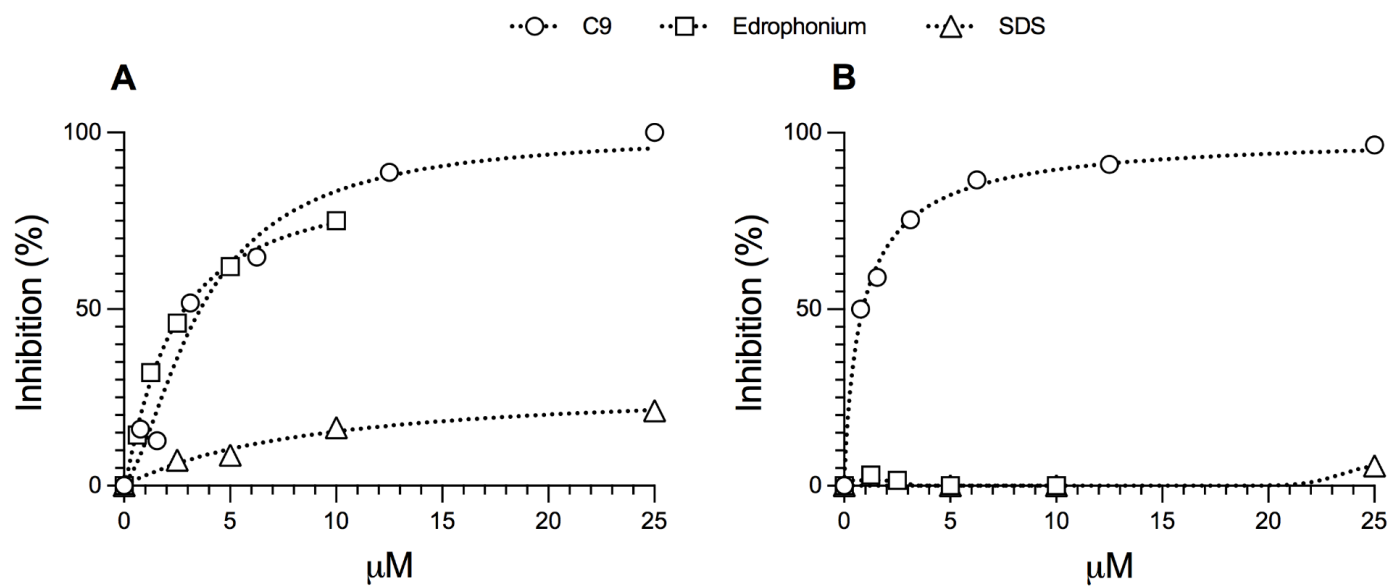


Figure 4

Molecular interactions of the ligands (edrophonium and compounds C1–C9) within the active sites of (A, B) human acetylcholinesterase (AChE) and (C, D) human butyrylcholinesterase (BuChE).

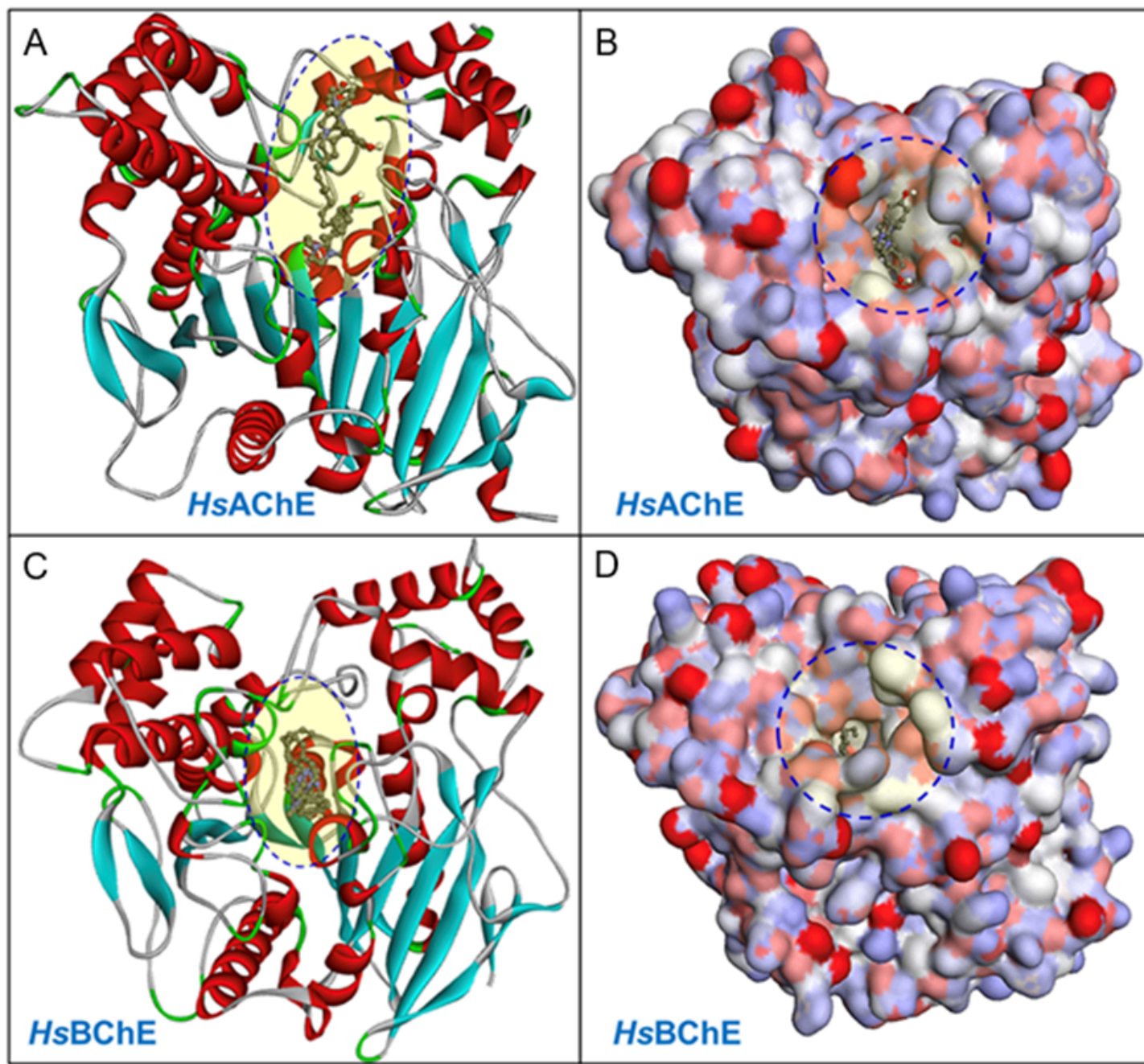


Figure 5

Molecular docking simulations of (A) edrophonium and (B–I) compounds C1–C9 within the active site of human acetylcholinesterase (AChE). The ligands are represented as balls and sticks with the carbon atoms in grey. Main residues of AChE interacting with the ligands are displayed as sticks (carbon in grey). H-bonds, water H-bonds, electrostatic, and hydrophobic interactions are displayed as green, light blue, orange and pink dashed lines, respectively. Compounds 3 and 5 are identical because the counter anions (Br^- and Cl^-) were not included in the simulations.

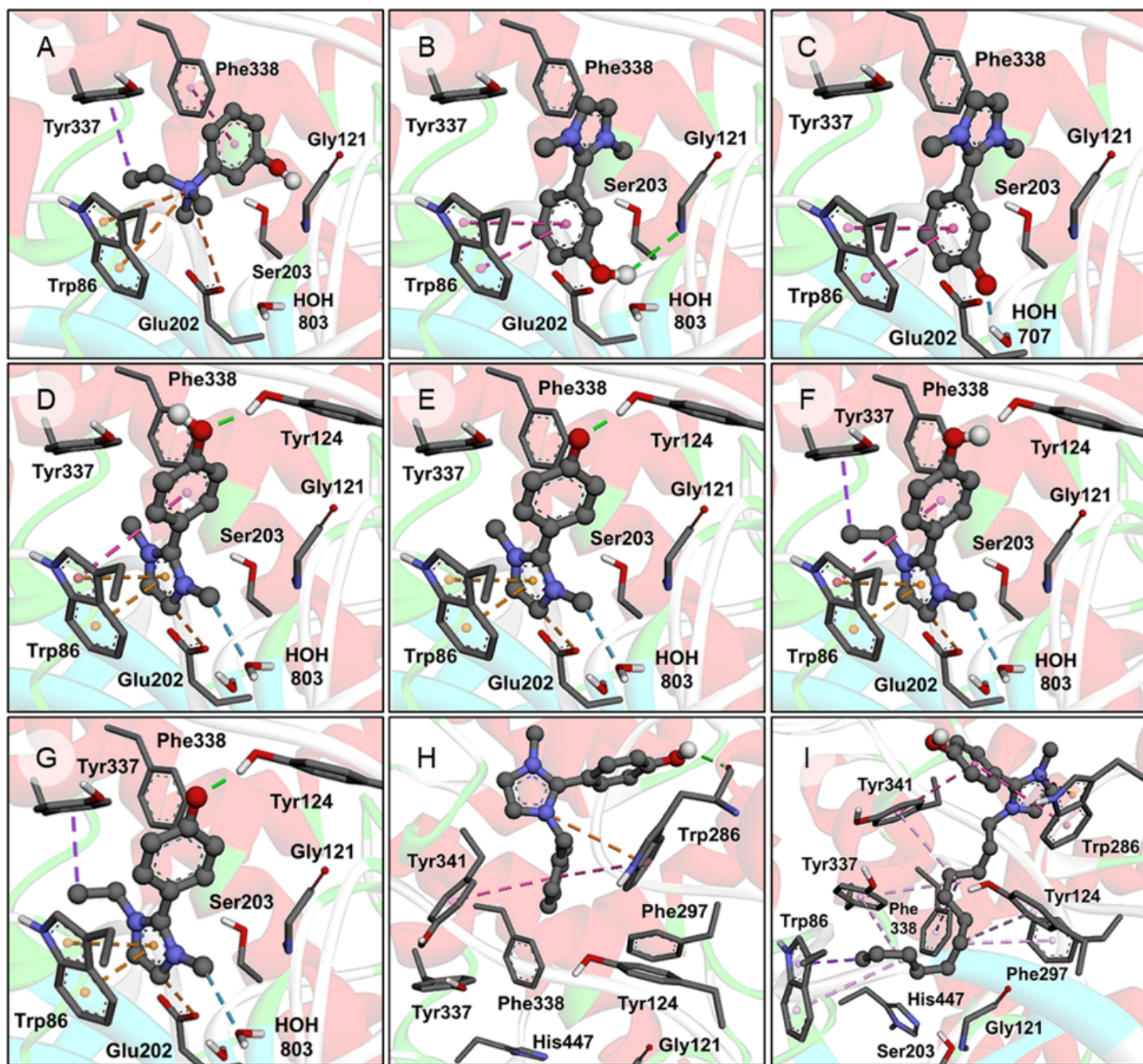


Figure 6

Molecular docking simulations of (A) edrophonium and (B–I) compounds C1–C9 within the active site of human butyrylcholinesterase (BuChE). The ligands are indicated by balls and sticks with the carbon atoms in grey. Main residues of BuChE interacting with the ligands are displayed as sticks (carbon in grey). H-bonds, water H-bonds, electrostatic, and hydrophobic interactions are displayed as green, light blue, orange and pink dashed lines, respectively. Compounds 3 and 5 are identical because the counter anions (Br^- and Cl^-) were not included in the simulations.

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