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In Silico and *Ex Vivo* Analyses of the Inhibitory Action of the Alzheimer Drug Posiphen and Primary Metabolites with Human Acetyl- and Butyrylcholinesterase Enzymes

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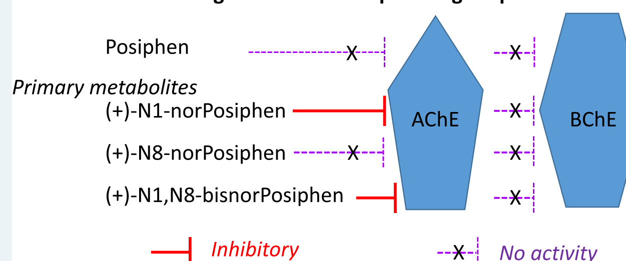
ABSTRACT: Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide. Ongoing research to develop AD treatments has characterized multiple drug targets including the cholinergic system, amyloid-β peptide, phosphorylated tau, and neuroinflammation. These systems have the potential to interact to either drive or slow AD progression. Promising agents that simultaneously impact many of these drug targets are the AD experimental drug Posiphen and its enantiomer phenserine that, currently, are separately being evaluated in clinical trials. To define the cholinergic component of these agents, the anticholinesterase activities of a ligand dataset comprising Posiphen and primary metabolites ((+)-N1-norPosiphen, (+)-N8-norPosiphen, and (+)-N1,N8-bisnorPosiphen) were characterized and compared to those of the enantiomer phenserine. The "target" dataset involved the human cholinesterase enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Binding interactions between the ligands and targets were analyzed using Autodock 4.2. The computationally determined inhibitory action of these ligands was then compared to *ex vivo* laboratory-measured values versus human AChE and BChE. While Posiphen lacked AChE inhibitory action, its major and minor metabolites (+)-N1-norPosiphen and (+)-N1,N8-bisnorPosiphen, respectively, possessed modest AChE inhibitory activity, and Posiphen and all metabolites lacked BChE action. Phenserine, as a positive control, demonstrated AChE-selective inhibitory action. In light of AChE inhibitory action deriving from a major and minor Posiphen metabolite, current Posiphen clinical trials in AD and related disorders should additionally evaluate AChE inhibition; particularly if Posiphen should be combined with a known anticholinesterase, since this drug class is clinically approved and the standard of care for AD subjects, and excessive AChE inhibition may impact drug tolerability.



KEYWORDS: *Posiphen, acetylcholinesterase, butyrylcholinesterase, Alzheimer's disease, cholinesterase inhibitors, molecular docking, (+)-N1-norPosiphen, (+)-N8-norPosiphen, (+)-N1, N8-bisnorPosiphen, phenserine*

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1. INTRODUCTION

The hydrolysis of synaptic acetylcholine (ACh) to terminate its physiologic actions is central to the optimal regulation of cholinergic neurotransmission. This is achieved by the cholinesterase (ChE) enzymes that cleave ACh into choline and acetic acid.[1] Two classes of cholinesterase enzymes coexist throughout the body and play a range of both cholinergic and non-cholinergic roles that are determined by their time and volume of expression, location, and particular subtype.[2,3] Within the healthy human brain, acetylcholinesterase (AChE; EC 3.1.1.7.) dominates and accounts for some 90% of cholinesterase activity, with butyrylcholinesterase (BChE; EC 3.1.1.8) providing the remainder. While AChE is primarily localized to neurons, BChE is largely associated with and secreted from glial cells, although studies by Darvesh and colleagues[4,5] have demonstrated that some 10−15% of neurons in the hippocampus and amygdala, key areas associated with cognition, possess BChE in lieu of AChE. Though these two enzymes share some 65% amino acid sequence homology, despite being encoded by disparate genes on different chromosomes (AChE: 7q22; BChE: 3q26), they possess slightly different substrate preferences and kinetics. Their precise levels and proximity, together with the expression of choline acetyltransferase (ChAT), the rate-limiting enzyme

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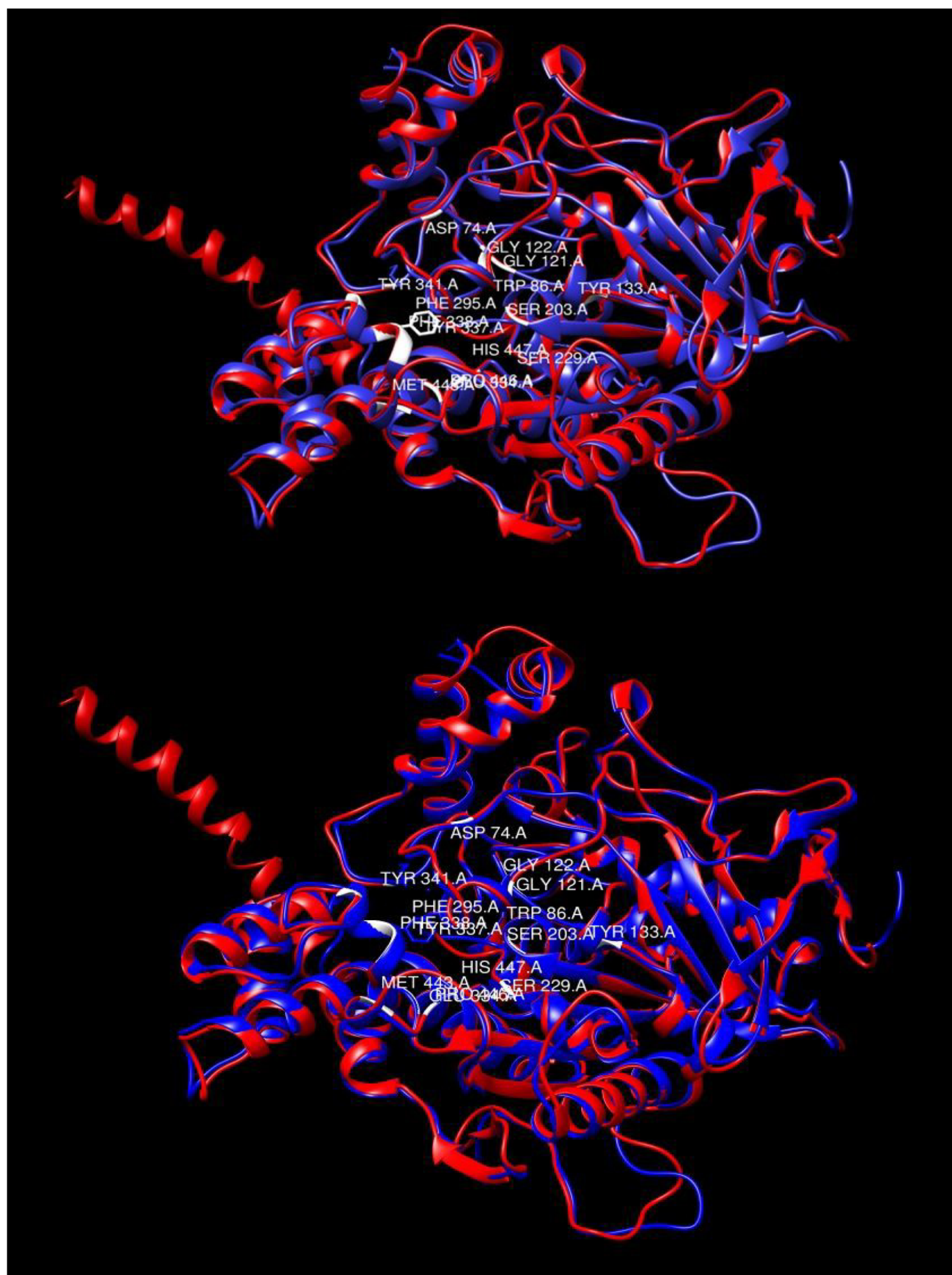

Figure 1. Superimposed structure of mouse and human AChE showing binding site residues. (a) Residues highlighted on hAChE (red). (b) Residues highlighted on mAChE (blue).

that catalyzes the re-synthesis of ACh, coregulate cholinergic function to optimize brain action throughout life.[6]

The dysfunction and death of cholinergic neurons that arise within the basal forebrain and project into the cerebral cortex and hippocampus lead to the cognitive decline that ensues during aging and, more severely, in Alzheimer's disease (AD).[6,7] Such impaired cortical cholinergic neurotransmission, characterized by a loss of classical cholinergic markers (e.g., levels of ACh, AChE, ChAT, and nicotinic/muscarinic receptors) may additionally influence the hallmark histopathological cortical and neocortical amyloid plaque and neurofibrillary tangle pathology that develop in the AD brain, by impacting the expression and processing of amyloid-β (Aβ) precursor protein (APP) and thereby Aβ generation or the level of tau hyperphosphorylation.[6,8,9] Likewise, elevated levels of soluble Aβ and hyperphosphorylated tau can impair cholinergic synaptic function and decrease ACh.[6] Furthermore, as ACh levels play a key role in regulating the peripheral and brain immune system via the "cholinergic anti-inflammatory pathway", ACh deficits, in addition to Aβ-induced oxidative stress and tau pathology, can upregulate pro-inflammatory cytokines and lead to neuroinflammation, a further classical

Figure 2. Structure of BChE showing key binding site residues (red).

hallmark of AD.[6,10,11] In light of these considerations, cholinesterase inhibitors have become the standard treatment for AD, although the etiology of AD is not completely understood and clearly involves the potential interaction of multiple environmental and genetic factors that contribute to the initiation and advancement of the disease.[12,13] Although numerous treatment strategies have been proposed and evaluated in AD clinical trials, so far, the majority have failed, and hence, the available regulatory-approved ones are primarily for symptomatic treatment.[14−17] These are predominantly focused on the cholinergic hypothesis of restoring ACh levels in the brain. The hAChE (*human* AChE) inhibitors donepezil (Aricept), rivastigmine (Exelon), and galanthamine (Reminyl) are currently approved and widely used AD drugs.[6,12,18] Of these, rivastigmine co-inhibits BChE, which has been receiving increasing attention in its role in comodulating ACh levels in cholinergic neurons under normal conditions[19] and when AChE activity is decreased.[4,20] Consequently, both enzymes are important targets in AD treatment.[2,21−23]

A particularly interesting category of cholinesterase inhibitors is carbamic acid derivatives that are *N*-alkyl and *N*,*N*-dialkyl carbamates. The natural carbamate (−)-physostigmine[24] falls within this drug class and, although short-acting, was evaluated in a controlled-release oral formulation in AD patients.[25] Phenyl carbamoyl analogues of (−)-physostigmine were developed to provide longer acting and better tolerated AD clinical candidates, namely, Posiphen and phenserine [aka (+)-phenserine (Posiphen—*sometimes termed as ANVS401*) and (−)-phenserine (phenserine)].[26−30] Posiphen, developed as a APP- and Aβ-lowering drug that additionally mitigates neuroinflammation, is in current clinical trials in AD and Parkinson's disease (PD) and generates three primary metabolic products following its administration to humans and preclinical animal models,[30] specifically, (+)-N1 norPosiphen,[31] (+)-N8-norPosiphen,[32] and (+)-N1,N8-bisnorPosiphen.[33] Unlike several other drug classes, there is no "chiral switching" on the core hexahydropyrroloindole structure that forms the tricyclic backbone of Posiphen and phenserine, and hence, all generated metabolites retain the enantiomeric purity of their parent compound, and these two opposite isomers, together with all metabolites, remain as completely separate drugs with different ranges of pharmacological actions.

The focal point of this study is the *in silico* analysis together with "real-world" wet laboratory evaluation of the interaction between the human cholinesterase enzymes (AChE and BChE) and Posiphen together with its three primary metabolic products (specifically, (+)-N1 norPosiphen, (+)-N8-norPosiphen, and (+)-N1,N8-bisnorPosiphen) and the enantiomer, the clinical AD drug candidate phenserine, to aid in defining whether there is a cholinergic component of Posiphen administration. This is of both scientific and translational interest, as anticholinesterases are approved and routinely used in AD and sometimes in PD and might be combined with Posiphen, a drug in current clinical evaluation for these disorders. An unexpected, excessive AChE inhibition could potentially impact patient health.

2. RESULTS AND DISCUSSION

We used the crystallographic structures of hAChE and hBChE[34] as targets for our receptor−ligand docking studies. The 3D structures were downloaded from PDB.[35] The structural information regarding the binding site of currently known inhibitors to hAChE and hBChE was then collected by literature search.[36] The active site of AChE forms a deep and sterically restricted cavity that serves as the binding site for ligands, both natural and synthetic.[37,38] The active site and the structure of AChE are evolutionarily conserved among the extensively studied organisms *Mus musculus* (mAChE),[39] *Torpedo californica* AChE,[37] and *Homo sapien* (hAChE).[36] It contains common regions similar to the other serine hydrolases. The catalytic site is situated at the base of the gorge and contains the catalytic triad (H447, E334, and S203 in *human* AChE). A second or peripheral site extends beyond Y337 (*human* AChE) at the catalytic/peripheral site interface to the entrance of the gorge and contains numerous aromatic side chains. Kinetic and thermodynamic studies indicate that inhibitors can interact with either or both of the two binding sites found in AChE.[40−42] Residues of the hAChE binding site include Tyr337, Trp86, Ser203, Gly122, His447, Gly121, Tyr133, Ser229, Pro446, Tyr341, Met443, Phe295, Phe338,

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Glu334, and Asp74. Binding site residues for hBuChE include Tyr128, Glu197, Ser198, Ser224, Glu325, Ala328, Met434, Tyr332, Trp430, Asp70, Trp82, His438, and Tyr440.[36] Figure 1 is a visual representation of the 3D structures of AChE with interacting residues marked. A visual representation of the 3D structure of BChE with interacting residues marked is depicted in Figure 2.

A ligand dataset was prepared from Posiphen, its three primary metabolic products, (+)-N1-norPosiphen, (+)-N8-norPosiphen, and (+)-N1,N8-bisnorPosiphen, and from Posiphen's opposite enantiomer phenserine. Figure 3 shows

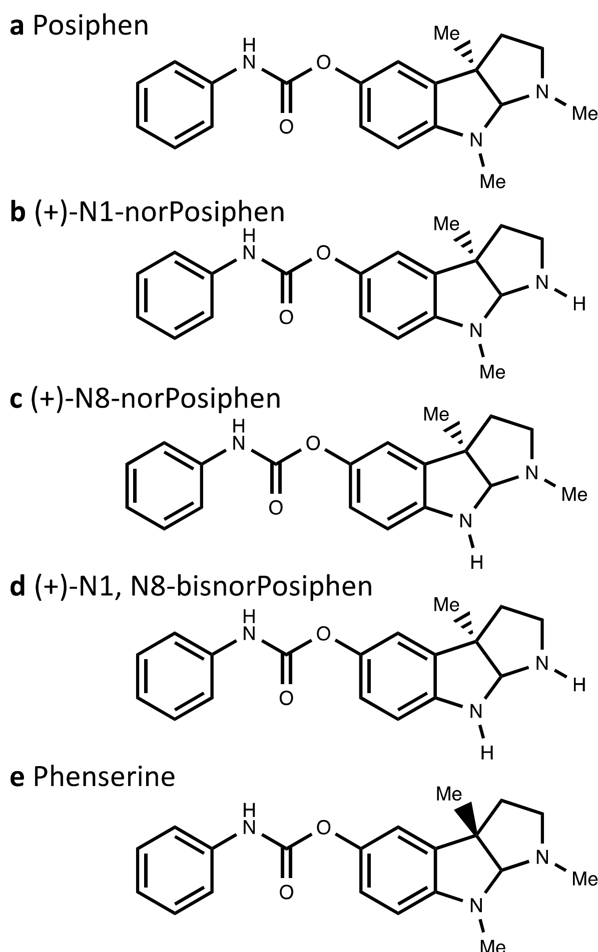

Figure 3. 2D structural representation of the ligand dataset. (a) Posiphen, (b) (+)-N1-norPosiphen, (c) (+)-N8 norPosiphen, (d) (+)-N1,N8-bisnorPosiphen, and (e) Phenserine. Note (a–d) all exist solely as (+)-enantiomers, whereas Phenserine exists as the natural (−)-enantiomer.

a 2D representation of the ligand dataset. After docking studies, a detailed evaluation was performed on the analysis of binding interactions between ligands and both cholinesterase enzymes. Table 2 shows binding energy (kcal/mol) along with K_i (μM) and IC_{50} (nM) values. For hAChE, the binding energy values ranged from −4.95 to −7.53 kcal/mol. We observe that the binding energy and K_i values for Posiphen and its enantiomer phenserine are dissimilar (−5.25 vs −6.94 kcal/mol, respectively) in line with their unalike K_i values (142.96 vs 8.22 μM, respectively). This is in accordance with the lack of Posiphen-associated AChE inhibition determined in wet lab studies (IC_{50} >10,000 nM) and the potency of phenserine as

Table 1. List of Grid and Docking Parameters Used to Perform Docking Studies

grid parameters		docking parameters	
spacing	0.375 Å	energy evaluations	2.5 × 106
grid center	80*X* Å	iterations	27,000
	80*Y* Å	mutation rate	0.02
	80*Z* Å	crossover rate	0.80
		elitism value	1
		RMS tolerance	1.0 Å

an AChE inhibitor (IC_{50} 18.6 nM). For BChE enzyme binding, the range of energy values was narrower, from −4.86 to −5.59 kcal/mol, with Posiphen and phenserine showing similar values (−5.53 and −5.59 kcal/mol, respectively), which is in accordance with their alike lack of BChE inhibitory action, as determined by both predicted K_i and experimentally determined IC_{50} values. Parenthetically, phenserine demonstrates pharmacologically valuable AChE inhibition when administered to animals and humans,[38,43−45] in addition to useful non-cholinergically mediated actions.[26,46] However, it is AChE inhibition that likely also underpins phenserine's dose-limiting actions.[44] In contrast, Posiphen lacks AChE inhibitory action and hence can be escalated to a higher dose in both humans and rodents.[30,47] Notable in Table 2, (+)-N1-norPosiphen and (+)-N1,N8-bisnorPosiphen both possess modest AChE inhibitory action, as reflected in their predicted binding energy and K_i values and in their wet lab evaluation. In human and animal studies, the metabolite (+)-N1-norPosiphen is generated in slightly lower amounts compared to (+)-N8-norPosiphen, and levels of (+)-N1,N8-bisnorPosiphen are very low following Posiphen administration.[30] In published human studies, following a Posiphen 40 mg dose, plasma concentrations had a C_{Max} of Posiphen: 118.5 ng/mL, (+)-N1-norPosiphen: 25.6 ng/mL, (+)-N8-norPosiphen: 31 ng/mL, and (+)-N1,N8-bisnorPosiphen: 3.8 ng/mL,[30] and thus, there is quite possibly some level of AChE inhibition and cholinergic action at high but potentially clinically relevant Posiphen doses. Finally, BChE inhibition is not a feature of any of the ligand set members.

Table 3 shows binding analysis of ligands with both enzymes. The data constitute information regarding the respective atoms involved in hydrogen bonding along with distances and hydrophobic interacting residues.

Binding analysis with hAChE revealed that the ligands bind to the gorge binding site residues. All the ligands possess hydrogen binding with aromatic rings of either or both Tyr124 and Trp286, except (+)-N8-norPosiphen. (+)-N1,N8-bisnorPosiphen appears to interact with all three residues of the catalytic triad, Ser203, Glu202, and His447. Hydrophobic interactions for most ligands are seen with Pro290, Trp286, Val361, Phe295, Tyr341, Tyr337, Val294, and Tyr396 residues. In the case of BChE, none of the ligands show hydrogen bonding with the exception of (+)-N1,N8-bisnorposiphen, which exhibits interactions with the residue Asn83. The majority of hydrophobic interactions are observed with Val529, Val361, Trp522, Phe526, Tyr396, Cys400, and Pro401. As can be seen in Table 2, the binding energies for AChE and BChE and the corresponding IC_{50} values are correlated. In synopsis and comparison, the ligands show low IC_{50} values (indicative of higher potency/inhibition) with AChE, whereas those of BChE are higher (indicative of low or no potency/inhibition). The IC_{50} values and their respective

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Table 2. Energy Values for Docking Results of (a) Acetylcholinesterase and (b) Butyrylcholinesterase

ligands	binding energy (kcal/mol)	K_i (μM)	inter-molecular energy (kcal/mol)	vdW + H bond + desolv energy (kcal/mol)	electrostatic energy (kcal/mol)	final total internal energy/unbound system's energy (kcal/mol)	torsional free energy (kcal/mol)	IC_{50} (nM)
				(a) Acetylcholinesterase				
Posiphen	−5.25	142.96	−6.14	−6.19	−0.05	0.0	0.89	>10,000
(+)-N1-norPosiphen	−7.07	6.59	−7.96	−7.93	−0.04	0.0	0.89	46 ± 6.0
(+)-N8-norPosiphen	−4.95	236.54	−5.84	−5.74	−0.11	0.0	0.89	>10,000
(+)-N1,N8-bisnorPosiphen	−7.53	3.03	−8.42	−8.32	−0.11	0.0	0.89	83 ± 9.0
Phenserine	−6.94	8.22	−7.83	−7.81	−0.03	0.0	0.89	18.6 ± 0.3
				(b) Butyrlcholinesterase				
Posiphen	−5.53	88.6	−6.42	−6.39	−0.03	0.0	0.89	>10,000
(+)-N1-norPosiphen	−4.86	275.67	−5.75	−5.59	−0.17	0.0	0.89	>10,000
(+)-N8-norPosiphen	−5.38	114.49	−6.27	−6.19	−0.09	0.0	0.89	>10,000
(+)-N1,N8-bisnorPosiphen	−5.22	149.99	−6.11	−5.98	−0.13	0.0	0.89	>10,000
Phenserine	−5.59	79.86	−6.49	−6.49	0.01	0.0	0.89	1380 ± 240

Table 3. Tabular Representation of Residues Involved in Binding Interactions During Docking with the Inhibitor Data Set

ligand name	hydrophobic interactions	hydrogen bonding interactions
	Acetylcholinesterase Interactions	
Posiphen	Pro290, Glu292, Gln291, Leu289, Ser293, Arg296, Val294, Tyr337, Phe338, Tyr341, Trp286, Tyr124	none
(+)-N1-norPosiphen	Tyr337, Phe338, Tyr72, Asp74, Phe295, Leu289, Tyr341, Trp286	Tyr124 (O−N1)
(+)-N8-norPosiphen	Pro290, Leu289, Glu292, Gly291, Ser293, Val294, Phe338, Phe295, Arg296, Phe297, Trp286, Tyr341	none
(+)-N1,N8-bisnorPosiphen	Try133, Trp86, Phe338, Tyr337, Phe295, Val294, Trp286	Tyr124 (OH−N3), Glu202 (OE−N1), Ser203 (OG−N2)
Phenserine	Asp74, Phe295, Phe338, Leu289, Tyr337, Val294, Tyr341, Tyr124	Trp286 (O−N3)
	Butyrylcholinesterase Interactions	
Posiphen	Gly360, Val529, Val361, Phe526, Pro527, Tyr396, Pro401, Trp522, Thr523, Cys400	none
(+)-N1-norPosiphen	Val361, Val529, Phe526, Pro527, Trp522, Cys400, Thr523, Tyr396, Pro401	none
(+)-N8-norPosiphen	Gly439, Tyr440, Trp82, Ala328, Met437, Tyr332, Pro285, Phe329, Thr284	none
(+)-N1, N8-bisnorPosiphen	Ile69, Pro84, Glu80, Asn85, His126, Met81, Leu125	Asn83 (O−N3)
Phenserine	Gly360, Val529, Val361, Pro527, Phe526, Tyr396, Trp522, Cys400, Pro401, Thr523	none

binding energies are, in large part, agreeable with previously conducted studies.[35] Figure 4 graphically represents individual ligand binding patterns with active site residues of AChE.

Figure 4 shows the interaction of each ligand with the hAChE enzyme. It is observed that the ligands Posiphen and (+)-N8-norPosiphen exhibit no hydrogen bonding with the enzyme. However, weak hydrophobic interactions are observed with the protein's residues, which include those involved in the binding site interaction such as Trp286, Tyr341, and Phe338 in the case of Posiphen and Tyr341and Phe295 in the case of (+)-N8-norPosiphen. Apart from these two ligands, the other three form hydrogen bonds with the binding site residues Tyr124 and Ser203. Similarly, Figure 5 individually shows the binding pattern of each ligand with BChE. The ligands are found to be involved in forming weak interactions with the binding site residues, such as Ala328. Only the ligand (+)-N1,N8-bisPosiphen is observed to undergo hydrogen bonding with the Asn83 residue. Despite this, its IC_{50} value is in line with a lack of BChE inhibitory action, in accordance with the other ligands.

3. CONCLUSIONS

The data presented in this study provide an extension of our previous wet laboratory experiments.[32] *In silico* analysis revealed that for hAChE, the IC_{50} values are observed to be lower (i.e., associated with binding high potency), which corresponds to the respective binding energies. In contrast, the IC_{50} values for all the ligands interacting with BChE are higher (i.e., low binding potency), when compared to those of AChE, owing to the presence of only weak hydrophobic interactions. As noted earlier, AChE inhibitory potency can mitigate AD-associated central cholinergic impairments to potentially symptomatically improve cognition and augment the cholinergic anti-inflammatory pathway to potentially ameliorate AD-associated inflammation.[6] Phenserine, initially developed as an oral, immediate release, AChE inhibitor that proved well tolerated in human studies (645 subjects for up to 1 year) and demonstrated an efficacy signal in AD,[27−29,45,48] has in recent studies demonstrated far more interesting pharmacological action by mitigating programmed neuronal cell death, synaptic loss, and neuroinflammation across multiple cellular and animal neuronal injury models at clinically translatable doses.[26,46,49−53] As a consequence and to optimize these more recent cholinergically and non-cholinergically mediated pharmacological actions, phenserine has re-entered clinical development as an extended controlled-release oral tablet formulation to maintain steady-state therapeutic drug levels and AChE inhibition in AD and traumatic brain injury human clinical trials.[54] In contrast, Posiphen was originally developed as a "cholinergically inert" APP synthesis inhibitor to lower Aβ generation and subsequent tau phosphorylation and associated neuroinflammation,[47] which have been confirmed by recent

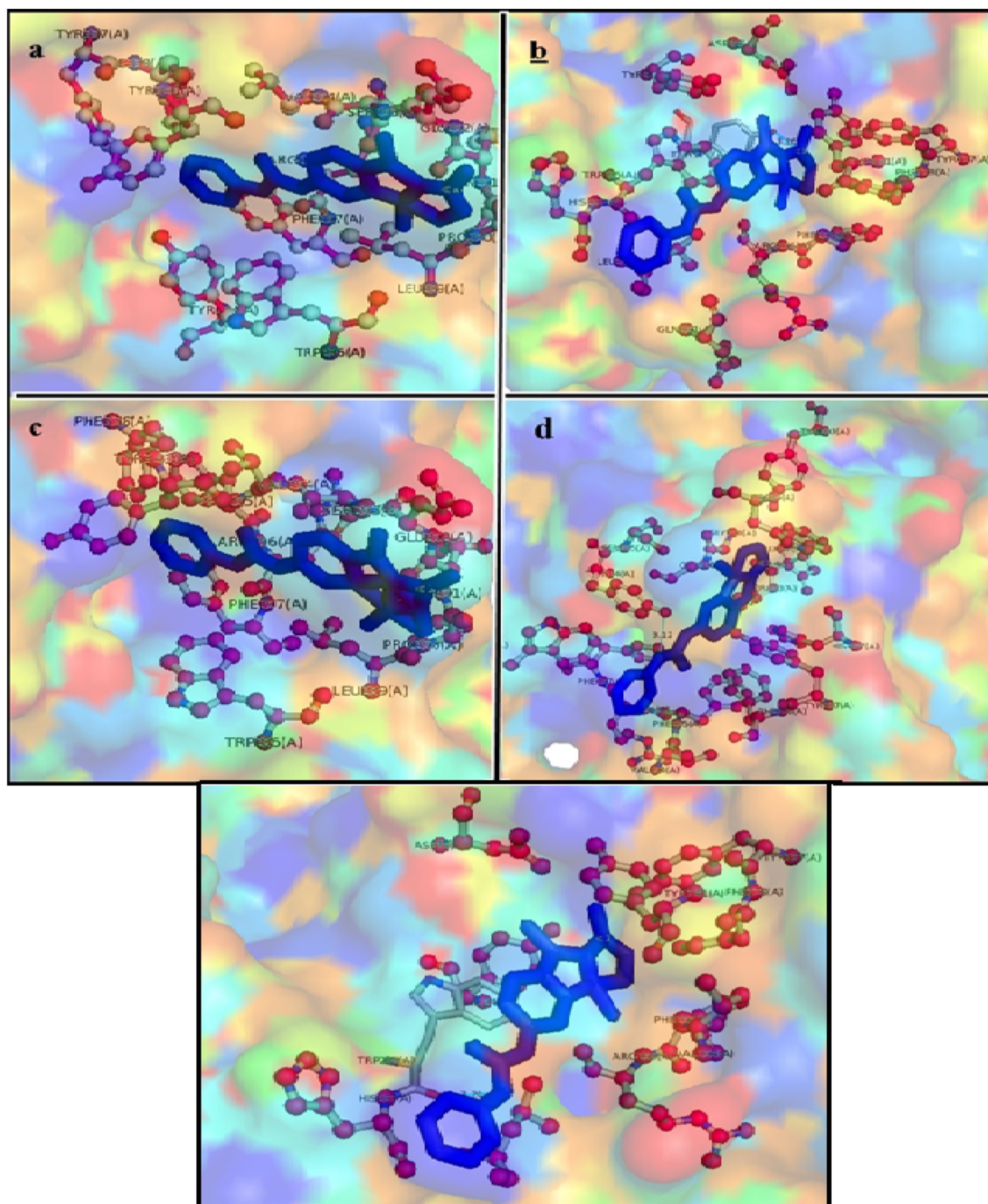

Figure 4. Acetylcholinesterase interactions, (a) Posiphen, (b) (+)-N1-norPosiphen, (c) (+)-N8-norPosiphen, (d) (+)-N1,N8-bisnorPosiphen, and (e) Phenserine. Ligands are shown in sticks, while target residues involved in interactions are represented as balls and sticks.

studies by others.[55,56] Likewise, these actions and others[57,58] are achieved at concentrations of clinical relevance,[30] and the release of recent Posiphen clinical development data, although highly preliminary and from a small patient number, is promising.[59] Our *in silico* analysis of these ligands with hAChE and BChE proves to be in accordance with experimental data in relation to their cholinergic actions. Our studies reaffirm AChE inhibitory action for phenserine, in line with current clinical studies that are optimizing it via its new extended controlled-release formulation,[54] and importantly demonstrate that two Posiphen metabolites possess AChE inhibitory action [(+)-N1-norPosiphen and (+)-N1,N8-bisnorPosiphen]. In light of this, ongoing Posiphen clinical trials should evaluate erythrocyte AChE inhibition to define any potential cholinergic component of the drug, particularly if Posiphen is administered to any patient already taking an anticholinesterase—as these anticholinesterases are routinely administered to AD patients, and an unexpected addition of Posiphen's metabolite cholinergic actions might potentially result in untoward dose-limiting actions.

4. MATERIALS AND METHODS

4.1. Docking Studies. Docking of AChE and BChE with the ligand dataset was performed using Autodock 4.2.[60] In brief, polar hydrogen atoms and Kollman charges were assigned to the target proteins. For ligands, Gasteiger partial charges were designated, and non-polar hydrogen atoms were merged. All torsions for ligands were allowed to rotate during the docking procedure. The program AutoGrid was used to generate the grid maps. Each grid was centered at the structure

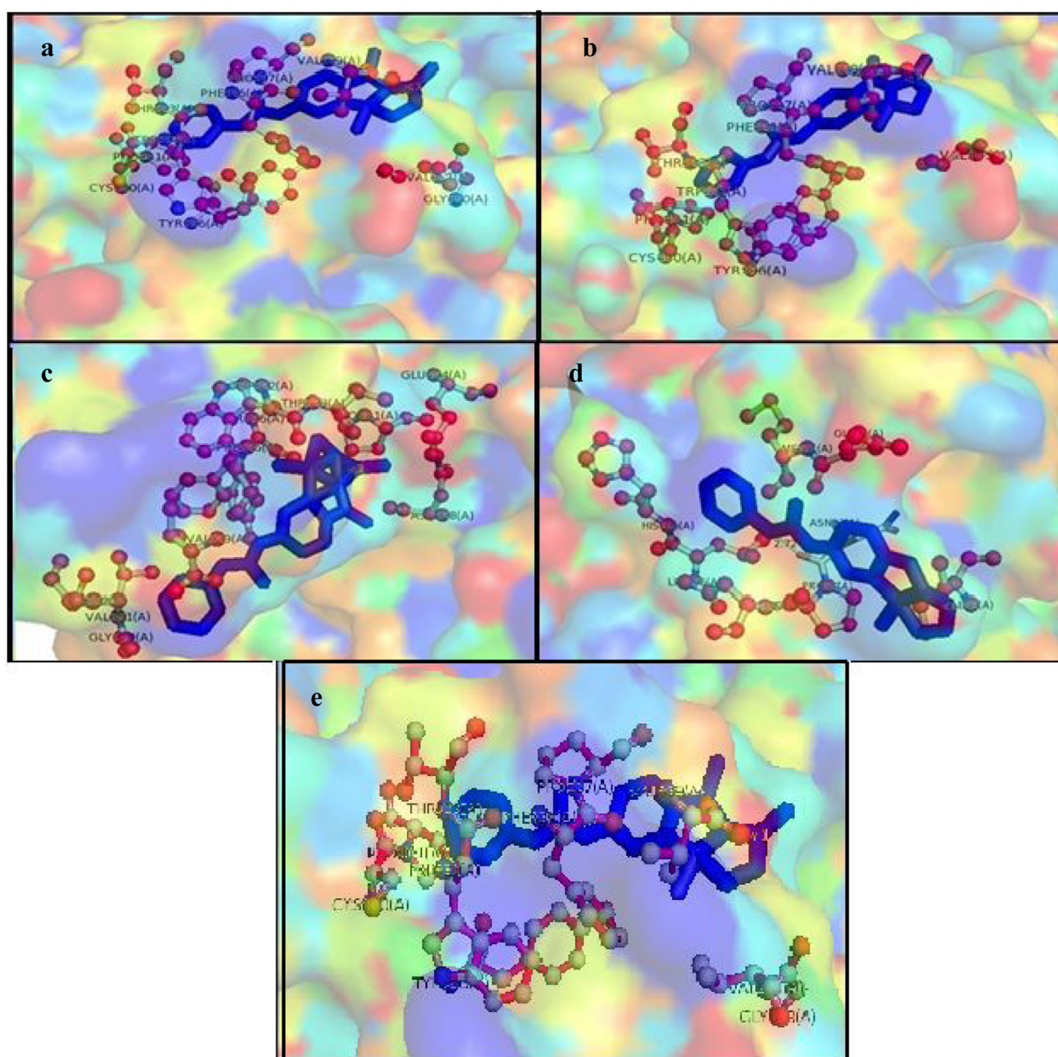

Figure 5. Butyrylcholinesterase interactions, (a) Posiphen, (b) (+)-N1-norPosiphen, (c) (+)-N8-norPosiphen, (d) (+)-N1,N8-bisnorPosiphen, and (e) Phenserine. Ligands are shown as sticks enveloped in the surface, while target residues involved in interactions are represented by lines.

of the corresponding enzyme. For all ligands, the starting positions, orientations, and torsions used were random. The translation, quaternion, and torsion steps were selected from default values available in AutoDock. The Lamarckian genetic algorithm was used for minimization using the default parameters. The parameters for the docking experiments are shown in Table 1.

4.2. Anticholinesterase Activity. The cholinesterase inhibitory activity of the ligand set was assessed by quantifying its ability to inhibit freshly prepared hAChE and BChE to enzymatically cleave their respective selective substrates, acetyl-(*β*-methyl)thiocholine and *s*-butyrylthiocholine (0.5 mmol/L) (Sigma Chemical Co., St. Louis, MO), as detailed previously,[32] using the same synthesized batch of agents detailed by Yu and colleagues.[32] Samples of AChE and BChE were prepared from freshly collected human erythrocytes and plasma, respectively. Compounds were dissolved in and then were diluted in 0.1 M Na_3PO_4 buffer (pH 8.0) in half-log concentrations to provide a final concentration range that spanned from 0.3 to 10,000 nM.

Briefly, hBChE was separated from fresh plasma (10,000*g*, 10 min, 4 °C) and diluted 1:125 with 0.1 M Na_3PO_4 buffer (pH 7.4). hAChE was prepared from erythrocytes washed (×5) in isotonic saline and lysed in nine volumes of 0.1 M Na_3PO_4 buffer (pH 7.4) containing 0.5% *Triton-X* (Sigma) and, thereafter, diluted with 19 volumes of buffer to a final dilution of 1:200.

Evaluation of anticholinesterase activity was performed on a 25 *μ*L sample of each enzyme preparation at pH, 8.0 in 0.1 M Na_3PO_4 buffer (0.75 mL total volume), using physostigmine as an external control. Preincubation time with enzymes was 30 min (21 °C); incubation with their respective substrates and with 5,5′-dithiobis-2-nitrobenzoic acid was for 25 min (37 °C). The substrate/enzyme interaction was halted by addition of excess physostigmine (1×10^{-5} M), and generation of a yellow thionitrobenzoate anion was then measured using a spectrophotometer at 412 nm *λ*. Correction for non-specific substrate hydrolysis was performed under conditions of absolute enzyme inhibition (achieved by 1×10^{-5} M physostigmine). All agents were analyzed for a minimum of three times, in duplicate. Mean enzyme activity at each compound concentration was expressed as a percent of the activity in the absence of the compound. This was then transformed into a logit format [logit = ln(% activity/100 − % activity)] and then was plotted as a function of compound log concentration to provide an IC_{50} value, defined as the

concentration of the compound (nM) required to inhibit 50% of enzymatic activity, as determined from a correlation between log concentration and logit activity (with correlation coefficients of $r^2 \geq -0.98$ considered acceptable).

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All *in silico* data can be obtained from SB, and all *ex vivo* binding data can be obtained from NHG on request.

ABBREVIATIONS

Ach acetylcholine
AChE acetylcholinesterase
BChE butyrylcholinesterase
hAChE human acetylcholinesterase
mAChE mouse acetylcholinesterase
AD Alzheimer's disease
PD Parkinson's disease
PDB Protein Data Bank

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