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QSAR, docking, dynamic simulation and quantum mechanics studies to explore the recognition properties of cholinesterase binding sites

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ABSTRACT

A set of 84 known *N*-aryl-monosubstituted derivatives (42 amides: series 1 and 2, and 42 imides: series 3 an 4, from maleic and succinic anhydrides, respectively) that display inhibitory activity toward both acetylcholinesterase and butyrylcholinesterase (ChEs) was considered for Quantitative structure–activity relationship (QSAR) studies. These QSAR studies employed docking data from both ChEs that were previously submitted to molecular dynamics (MD) simulations. Donepezil and galanthamine stereoisomers were included to analyze their quantum mechanics properties and for validating the docking procedure. Quantum parameters such as frontier orbital energies, dipole moment, molecular volume, atomic charges, bond length and reactivity parameters were measured, as well as partition coefficients, molar refractivity and polarizability were also analyzed. In order to evaluate the obtained equations, four compounds: 1a (4-oxo-4-(phenylamino)butanoic acid), 2a ((2*Z*)-4-oxo-4-(phenylamino)but-2-enoic acid), 3a (2-phenylcyclopentane-1,3-dione) and 4a (2-phenylcyclopent-4-ene-1,3-dione) were employed as independent data set, using only equations with $r^2_{m(test)}$>0.5. It was observed that residual values gave low value in almost all series, excepting in series 1 for compounds 3a and 4a, and in series 4 for compounds 1a, 2a and 3a, giving a low value for 4a. Consequently, equations seems to be specific according to the structure of the evaluated compound, that means, series 1 fits better for compound 1a, series 3 or 4 fits better for compounds 3a or 4a. Same behavior was observed in the butyrylcholinesterase (BChE). Therefore, obtained equations in this QSAR study could be employed to calculate the inhibition constant (*Ki*) value for compounds having a similar structure as *N*-aryl derivatives described here. The QSAR study showed that bond lengths, molecular electrostatic potential and frontier orbital energies are important in both ChE targets. Docking studies revealed that despite the multiple conformations obtained through MD simulations on both ChEs, the ligand recognition properties were conserved. In fact, the complex formed between ChEs and the best *N*-aryl compound reproduced the binding mode experimentally reported, where the ligand was coupled into the choline-binding site and stabilized through π–π interactions with Trp82 or Trp86 for BChE and AChE, respectively, suggesting that this compound could be an efficient inhibitor and supporting our model.

1. Introduction

Alzheimer's disease (AD) is the most widespread form of human dementia among elderly people worldwide, and this illness is characterized by a low concentration of acetylcholine (ACh) in the hippocampus and cortex [1,2], giving rise to symptoms such as loss of cerebral capability, cognition deterioration and a diversity of neuropsychiatric conditions [1–4]. ACh is a neurotransmitter that plays a role in the modulation of memory function in normal and neurodegenerative conditions [5,6]. ACh is hydrolyzed and degraded by acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BChE, E.C. 3.1.1.8) [7–9]. AChE has been characterized as being the only target identified in the design of several drugs for AD treatment [6–9].

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There is considerable three-dimensional information about both cholinesterases (ChEs) in the Protein Data Bank (PDB, http://www.pdb.org/), which also reveals structural details of both conformational states. In their free state, these enzymes are monomers whose structural topology is comprised of a 12-stranded mixed beta sheet surrounded by 14 alpha helices with a molecular weight of approximately 60 kDa; these monomers often form aggregates (dimers) that possess catalytic activity [10] as monomer. In the bound state, both ChEs have been found to form complexes with ACh, their natural inhibitor.

The knowledge of the interactions that stabilize the AChE–ACh complex has been exploited for the initial coordinates in docking and molecular dynamics (MD) simulation studies for the purpose of localizing multiple binding sites for ACh and other known inhibitors, as well as for the development of new acetylcholinesterase inhibitors (AChEIs) [11]. Both the three-dimensional data and MD simulations confirm that the ChEs share a catalytic triad (Ser, His, Glu) and an anionic subsite (Trp). However, a comparison between human AChEs and BChE reveals that aromatic residues Phe295 and Phe297 in the former are exchanged for the aliphatic residues Leu286 and Val288 in the latter. Furthermore, it is worth mentioning that these residues are in close proximity to the catalytic triad in both enzymes and contribute to their specificity and selectivity [12].

The catalytic triad and other important residues that constitute functional subsites are located in a deep narrow gorge (approximately 20 Å) with an oxyanion hole (Gly121, Gly122, Ala204) and an acyl binding pocket (Trp286, Phe295, Phe297, Phe338). An anionic subsite is found at the bottom of this gorge, which is formed by Trp82 and Trp86 in human AChE and BChE, respectively, and binds the quaternary nitrogen of their substrates and other ligands [13]. This active site is characterized by a highly negative electrostatic potential. In addition, it has recently been reported that recognition processes can be achieved by another important site, known as the peripheral anionic subsite, which is located in the deep surface of this hole (Asp74, Tyr124, Ser125, Trp286, Tyr337, Tyr341) [14].

The quantitative structure–activity relationship (QSAR) has been one of the principal strategies to predict the activity of new molecules by correlating structural or property descriptors of compounds through mathematical equations [15–21]. Furthermore, QSAR methodology has led to the design of several AD inhibitors such as phenylpentenone derivatives [22], physostigmine analogs [23], indanone and tacrine [24,25].

Recently, Correa-Basurto et al. through docking and quantum mechanics studies described the activity of 88 *N*-aryl derivatives as inhibitors of AChE and BChE [26], where density functional theory (DFT) calculations at the B3LYP/6-31G+(*d,p*) level were employed to obtain the energy value of the optimized structure and the energies of the frontier orbitals to correlate them with the inhibitory effects of the compounds. However, a conformational description for the optimized structures was not obtained, nor were other quantum descriptors determined [26].

Meanwhile, Solomon et al. applied a QSAR study that derived the models for 53 compounds bound to AChE and 61 compounds bound to BChE with the aid of genetic function approximation (GFA) techniques using the logarithm of the partition coefficient (logP), the sum of chemical bonds between atoms (Wiener), the molecular Shape Kappa indices (KAPPA-1-AM), the dipole moment (μ), and the molecular connectivity indices (CHI-1) [27].

In this contribution, a QSAR study for a series of 84 known *N*-aryl derivatives that display inhibitory activity towards AChE and BChE was performed using docking and quantum mechanics to explore the recognition properties of both. Furthermore, in order to evaluate the obtained equations, compounds 1a (4-oxo-4-(phenylamino)butanoic acid), 2a ((2*Z*)-4-oxo-4-(phenylamino)but-2-enoic acid), 3a (2-phenylcyclopentane-1,3-dione) and 4a (2-phenylcyclopent-4-ene-1,3-dione) were employed as independent data set [26]. ChEs. Donepezil and galanthamine were included to analyze their quantum mechanics properties and for validating the docking procedure. Furthermore, before performing the docking studies, both ChEs were submitted to MD simulations with the aim of taking into account the target flexibility properties. Therefore, our findings may provide quantum chemical details that can be used for drug design by combining different computational tools.

2. Computational procedure

2.1. Molecular dynamics

Classical MD simulations were performed using the NAMD 2.6 program [28] employing the CHARMM27 force field [29]. The initial ChE coordinates were obtained from the PDB (PDB IDs: 1B41 and 1P0I). The co-crystallized ligands and water molecules of the crystal structure were removed. Hydrogen atoms were added using the psfgen program included in the VMD package [30]. Afterwards, these structures were neutralized and solvated with TIP3P water molecules. The equilibration protocol consisted of 1500 minimization steps, followed by 30 ps of MD simulations at 10 K with fixed protein atoms. Subsequently, the entire system was minimized over 1500 steps (at 0 K), followed by gradual heating from 10 to 310 K using temperature reassignment during the initial 60 ps of the 100 ps equilibration dynamics without restraints.

The final step involved a 30 ps NTP simulation using the Nose–Hoover Langevin piston pressure control at 310 K and 1.0 bars for density (volume) fitting [31]. After this point, the simulation was continued using the NTV ensemble for 10 ns. Periodic boundary conditions and the particle mesh Ewald method [32,33] were applied for a complete electrostatics calculation. The dielectric water constant was used, and the temperature was maintained at 310 K using Langevin dynamics. Nonbonded interactions were calculated by applying a 10 Å cutoff with a switching function at 8 Å. The nonbonded list generation was terminated at 11.5 Å. The SHAKE method [34] was employed to provide an integration time step of 2 fs while keeping all bonds to the hydrogen atoms rigid. The trajectory was stored every 1 ps and was further analyzed with the VMD program [30]. The MD simulation output over 10 ns provided several ChE structures, which were sampled every 0.5 ns to evaluate the energetics of ligand recognition and binding modes of the target compounds (Table 1).

Some average geometrical properties, such as the root-mean squared deviation (RMSD), root-mean squared fluctuation (RMSF) and radius of gyration (Rg), were evaluated using Carma software [35].

2.2. Docking simulations

For docking studies, we utilized several protein conformations previously obtained through the MD simulation procedures mentioned above. First, the initial geometry optimization of ligands was performed with HYPERCHEM (Version 7.0, Hypercube, USA, http://www.hyper.com) at the MM+ level [36]. Then, the compound was optimized at the AM1 and DFT (B3LYP/6-31G(*d,p*)) levels using the Gaussian 09 program [37,38]. The AutoDock (4.2) program was selected for docking studies because this algorithm maintains a rigid macromolecule while allowing ligand flexibility [39]. This program has been widely used because it displays good free energy correlation values between docking simulations and experimental data [40]. A GRID-based procedure was utilized to prepare the structural inputs and to define all of the binding sites