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Molecular modeling, docking and ADMET studies applied to the design of a novel hybrid for treatment of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common form of dementia in adults, which is characterized by senile plaquets and cholinergic deficit as the disease progresses. Improvement of cholinergic neurotransmission is the basis of some drugs currently used in the treatment of AD. It is achieved by acetylcholinesterase (AChE) inhibition, the enzyme responsible for acetylcholine hydrolysis. Molecular modeling techniques were of utmost importance to design a new pharmaceutical against Alzheimer's disease, with potential inhibitory activity over AChE, since the inhibition of human plasma butyrylcholinesterase (BChE) may cause side effects. Some of the drugs currently used in the treatment of AD are capable of increasing the cholinergic transmission through the AChE inhibition. In this work we proposed molecular hybrids of tacrine with donepezil (fusion of these structures), in order to suggest new proposals of AChE inhibitors for future treatment of AD. We have analyzed all the structures by docking, density functional studies and drug like properties.

Keywords: Molecular modeling; Acetylcholinesterase inhibitor; Alzheimer's disease; Docking

1. Introduction

Molecular modeling and docking techniques are becoming increasingly important in drug design for therapy treatment of AIDS, cancer, Alzheimer and other diseases [1–4]. The Alzheimer's disease is the most common cause of dementia in elderly people. It affects 10% of the population over the age of 65 and 50% over the age of 85 [5]. This disease is microscopically characterized by the presence of senile plaques and neurofibrillary tangles in the brain, associated with the cognitive dysfunction and progressive deterioration of memory and learning processes, resultant from a cholinergic deficit [6]. The progression of the disease symptoms is associated with structural changes in cholinergic synapses in certain brain regions and consequent impairment of cholinergic neurotransmission [7].

Increasing the cholinergic neurotransmission capacity constitutes the fundamental mechanism of the drugs utilized for the treatment of Alzheimer's disease. It is possible through the inhibition of the acetylcholinesterase enzyme (AChE), which functions in cholinergic synapses of the central and peripheral nervous systems, where its principal biological role is termination of impulse transmission by rapid hydrolysis of acetylcholinesterase [7]. Thus, the anticholinesterase agents represent the drugs of choice for the treatment of Alzheimer's disease [8].

Among the drugs currently used for the treatment of the Alzheimer's disease are tacrine (Cognex®) and donepezil (Aricept®). Tacrine was the first inhibitor of AChE licensed by FDA (Food and Drug Administration). It is a reversible inhibitor, noncompetitive and nonselective to the AChE, possessing the same type inhibition as acetylcholinesterase and butyrylcholinesterase (BChE) enzymes. Its usage triggers hepatotoxic side effects and, due to its short plasmatic half-life (3–5 h), it requires doses four times per day (120–160 mg/day) [5]. Donepezil is currently the most prescribed agent for

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Alzheimer's disease, accounting for approximately 38% of prescriptions. It is a reversible and noncompetitive inhibitor and, contrary to tacrine, is selective to AChE. The adverse effects resulted from its use are of gastrointestinal nature. Considering the high plasmatic half-life (70 h) of Donepezil, a once-daily dosing (5–10 mg/day) is sufficient for the treatment [5].

The function of acetylcholinesterase (AChE) is rapid and efficient to catalyze the hydrolysis of Ach. The significance of a the rapid destruction of Ach is to deactivate the neurotransmitter after it binds to the receptor so as not to accumulate to levels that produce a continuous barrage of impulses by repetitive interactions. After all, the depolarization produced by the Ach-receptor binding must be terminated so that the excitability of the postsynaptic membrane and its permeability can be restored by re-polarization. The enzyme AChE is a molecule of about 320,000 Da, consisting of two types of polypeptide chains, which are each present twice ($\alpha_2\beta_2$). AChE is actually a general ester-hydrolyzing enzyme that is highly effective against Ach. In this respect it is more specific than other esterases such as pseudo or butyrylcholinesterase [9].

The active site of AChE is mainly composed of two subsites, one esterasic and the other that is a link to choline, binding, respectively, to the carbonyl group and quaternary ammonium of acetylcholine. There is also a second binding site of choline, called peripheric, because it is distant from the active site. Bisquaternary inhibitors of AChE derive their enhanced potency, relative to homologous monoquaternary ligands, from their ability to span these two 'anionic sites', which are 14 Å apart. The 3D structure of *Torpedo californica* (*Tc*) AChE reveals that the active site is located at the bottom of a deep and narrow cavity, named the *aromatic gorge*, composed in more than 50% of its volume by aromatic rings of specific amino acids [10]. In the active site of the AChE, the amino acids Ser200, His440 and Glu327 are considered essential for the catalytic enzymatic activity, being known as the 'catalytic triad' of AChE [11].

Based on the orientations of the compounds linked to the receptor, it is possible to suggest molecular modifications with the aim of optimizing them, considering their pharmacodynamic and pharmacokinetic properties, synthetic viability, among others. The molecular modifications based on the molecular hybridization process have permitted the elaboration of new therapeutic derivatives, potentially more active, by the optimization of a prototype [12]. The molecular hybridization is represented by the conjugation of defined structural characteristics of two distinct bioactive compounds in a unique new molecule [13].

Analysis of the interactions between the AChE enzyme and the drugs tacrine and donepezil led us to the elaboration of three different proposals of molecular hybridization involving the drugs above cited. The focus of the present study is the design, by molecular modeling and docking, of a new molecule with higher selectivity and enzymatic inhibitory activity. Toxicity and metabolism predictions are also reported. The three proposals were subsequently validated by docking. The objective of this work involves the designing of new substances, with potential inhibitory activity over AChE, using rational drug design strategies.

2. Methodology

AChE complexes with tacrine and donepezil were analyzed using the Insight II 2004 [14], Pymol 0.95 [15] and SPDBViewer 3.7 [16] softwares. The structures of the molecular hybridization proposals described in this work were built and initially optimized by molecular mechanics—Universal Force Field (UFF)—using the ArgusLab 4.0 software [17]. For calculation of log *P* and parameters of the 'Rule of Five', we used the Tsar 3.3 software [18]. This program calculates log *P* by two methods [19]: 'substitute values' and 'atomic values'. The program has log *P* data for all the standard organic elements. Flexible docking simulations were performed with GOLD 2.1.1 software [20], which contains a genetic algorithm. Default parameters have been used, which were optimized for a set of 305 different complexes with solved structure in the Protein Data Bank (PDB). We used a population of 100 conformers, 100 000 operations, 95 mutations and 95 crossovers. Docking calculations were performed inside a sphere with radius of 15 Å and origin at the side chain oxygen of Tyr121 of AchE. This residue was selected since it is situated at the center of the AChE active site. The ten orientations of highest score obtained for each compound were thus selected through of the GoldScore score function. Based on this function, GOLD classifies the orientations of the molecules by a decreasing affinity (the scores) with the binding site of the receptor. Previous to the docking calculations and after the removal of the ligand and crystallographic waters inside the AChE–donepezil (PDB code 1EVE) active site [10], hydrogen atoms of the residues side chains were added and oriented. Charges and atomic potentials of the AMBER force field were added to the protein structure using the Pymol program. We have not used human AChE complexes because these structures do not contain donepezil in the active site, and it is desirable to dock molecular hybrids of donepezil in an enzyme structure that is very similar inside this binding pocket, considering the induced adjustment that occurs between ligand and receptor. Sequences of human and *Torpedo californica* AChE share more than 50% of sequence identity and their structures are highly similar, especially in the active site, thus supporting the use of TcAChE for structure-based drug design [10,21]. We have not used the complex structure of TcAChE–tacrine (PDB code 1ACJ) [11] as well, due to the unsuitable conformation of the Phe330 side chain in this complex, which perform bad contacts with the piperidine ring of donepezil, and that is also present in the proposals when the two complexes are superimposed. Conformational search was performed on the three proposed structures using the MONTE CARLO method with the MMFF molecular mechanics model. The proposals were then fully optimized in the gas phase at the B3LYP/6-31G* level, using the Spartan 04 1.0.1 software [22]. Toxicity and metabolism predictions were

performed with DEREK and METEOR programs, respectively [23].

3. Results and discussion

3.1. Donepezil–acetylcholinesterase (AChE) interactions

The three moieties of donepezil [*R*,*S*-1-benzyl-4-(5,6-dimethoxy-1-indanon-2-yl)-methyl] interact with various residues of the AChE active site, performing specific interactions, whereas some are intermediated by water molecules. Donepezil interacts with the enzyme through the following groups: (i) benzyl; (ii) piperidinic nitrogen and (iii) dimethoxy-indanone. This pharmaceutical has pi-stacking interactions with aromatic aminoacids of the active site as well as hydrophobic interactions.

Interaction with the catalytic choline-binding site occurs between the benzyl group of the donepezil molecule and the indole group of Trp84, which is present in the enzymatic cavity. Donepezil does not interact directly with the catalytic triad (Ser200, His440 and Glu327), but via water molecules. In the region of the enzymatic cavity located between the choline binding site and the peripheral anionic site, the piperidinic nitrogen of the ligand, which contains a positive charge, interacts by cation-pi with the phenyl group of Phe330 of the enzymatic cavity, and Tyr334 forms a ''calix-domain'' with Phe330 and the piperidinic ring of donepezil. Hence, the interaction with Phe330 represents an additional site of quaternary binding, with functional significance, inside the active site. In the peripheral anionic site, the indanone ring interacts with the indolic group of Trp279, via a classical parallel pi–pi interaction. The binding of donepezil with AChE is extremely dependent on the interactions with Trp279 and Phe330, which are not present in the butyl cholinesterase enzyme (BChE); this could explain the high specificity of donepezil for AChE and not for BChE [10].

3.2. Tacrine–acetylcholinesterase interactions

The pharmaceutical tacrine (1,2,3,4-tetrahydroacridine) has strong interactions with the choline binding site of AChE, however it does not interact with the peripheral anionic site. There is a pi-stacking interaction between the quinoline ring of the tautomer of tacrine and the indolic ring of Trp84. The nitrogen atom of the quinoline ring of this tautomer has hydrogen bond with the carbonyl group of the main chain of His440, of the catalytic triad, and the quinoline ring is parallel and has contacts with the phenyl group of Phe330. When the complexes AChE–tacrine and AChE–donepezil are compared via structural superposition, it is possible to observe a conformational change of Phe330 of the former complex, in order to avoid a steric hindrance between its side chain and tacrine, as well as to favor a pi-stacking interaction between its aromatic ring and the phenyl group of the quinoline system of tacrine. Thus, tacrine is flanked by Trp84, establishing a strong pi-stacking interaction with it, and by Phe330, establishing a strong pi–pi interaction with the quinoline ring [11].

3.3. Molecular hybridization of donepezil with tacrine

Three proposals of molecular hybridization involving the pharmaceuticals donepezil and tacrine were performed in order to build new molecules, hybrids of these two anticholinesterasic agents, containing drug properties and capable of maintaining the original interactions of tacrine and donepezil. We calculated for all the proposals, as well as tacrine and donepezil, the parameters that define the 'Rule of Five' [24], which the drugs in general follow: molecular weight lower than 500, number of hydrogen bond donors lower or equal than 5, number of hydrogen bond acceptors lower or equal than 10 and log *P* lower than 5. None of our proposals violated this rule (Table 1), which were further investigated by docking with AChE.

The structural and energetic viabilities of the compounds were verified in each case by the minimum stationary configuration. Previously to the studies involving the proposed hybrids, we performed docking studies between donepezil and AChE in order to validate the method with this enzyme. The good superposition between the donepezil structure oriented with GOLD and the same molecule in the crystallographic orientation (PDB code 1eve) suggest the method used is appropriate (Fig. 1).

In Proposal 1, the piperidinic nitrogen of donepezil was connected to the quinoline ring of tacrine, through the aromatic carbon bound to the free amino group. The objective of this modification was to intensify the pi-stacking interaction of the aromatic ring of tacrine with the indole group of the Trp84 residue and to provide a hydrogen bond interaction between the nitrogen atom of that quinoline ring and the carbonyl group of the His440 residue of AChE. This latter interaction is not present in the donepezil–AChE complex (Scheme 1a).

The first proposal of molecular hybridization, shown in Scheme 1, maintains the molecule oriented in order to allow the pi-stacking interaction between the indanone group of donepezil and the indole group of Trp279, which corresponds

Table 1
Absorption, distribution, metabolism and excretion (ADME) parameters of the 'Rule of Five' (RO5) for donepezil, tacrine and Proposals 1–3

ADME properties	Ligands				
	Donepezil	Tacrine	Proposal 1	Proposal 2	Proposal 3
Molecular weight	379.54	198.29	435.62	445.61	
Number of Hbond acceptors	4	1	5	5	5
Number of Hbond donors	0	1	2	1	2
Log *P*	3.69	2.83	3.31	3.39	2.46

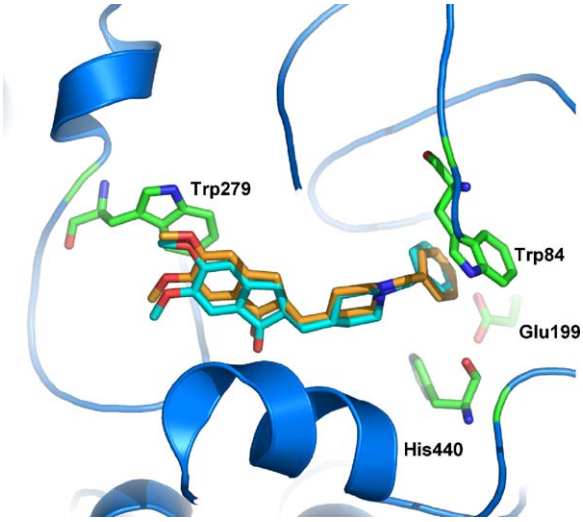


Fig. 1. Details of the AChE active site in which the superposition of the crystallographic orientation of donepezil (carbon atoms in cyan) with the top-ranked solution suggested by GOLD (carbon atoms in orange) is shown.

to Trp286 in the human sequence [25]. However, we had no success in maintaining the pi-stacking interaction between the aromatic ring of tacrine and the indole group of the Trp84 residue of AChE. It was also not possible to maintain the hydrogen bond interaction between the nitrogen atom of the original quinoline ring and the carbonyl group of the main chain of His440. This is due to the fact that, after the flexible docking, the conformation with highest score (score = 57) obtained with GOLD indicates two rings originating from tacrine oriented toward the opposite direction as compared to the equivalent rings of this pharmaceutical in its original complex (PDB code 1ACJ) (Fig. 2).

In order to avoid a possible steric hindrance between the side chain of the Phe330 residue of AChE (Tyr337 in the human sequence) and the piperidinic ring of the Proposal 1, which is condensed to an aromatic ring, we proposed to open this ring and simplify it by removing two carbon atoms (Proposal 2, Scheme 1b). In this new hybrid, besides the pi-stacking interaction involving the indanone group of the hybrid, it was

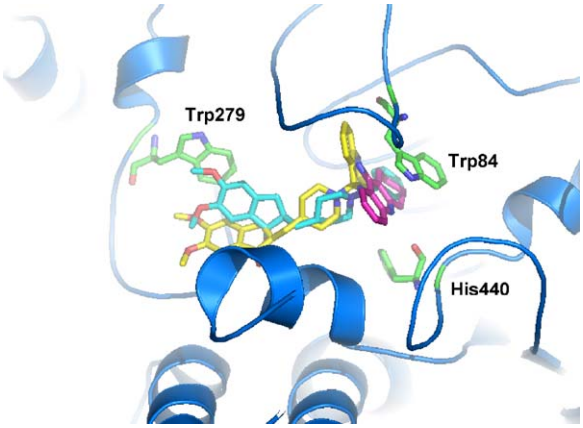


Fig. 2. Superposition of the structures of tacrine (carbon atoms in magenta), donepezil (carbon atoms in cyan) and Proposal 1 (carbon atoms in yellow).

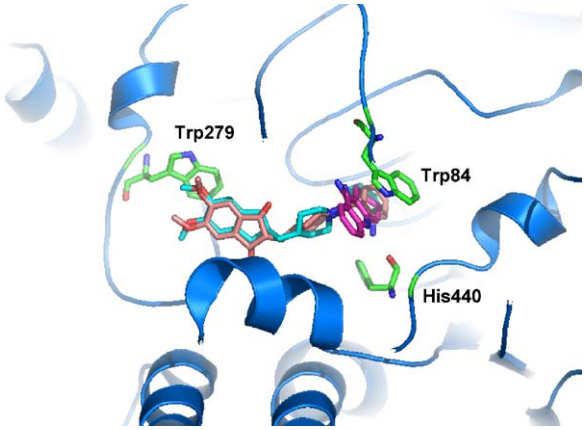
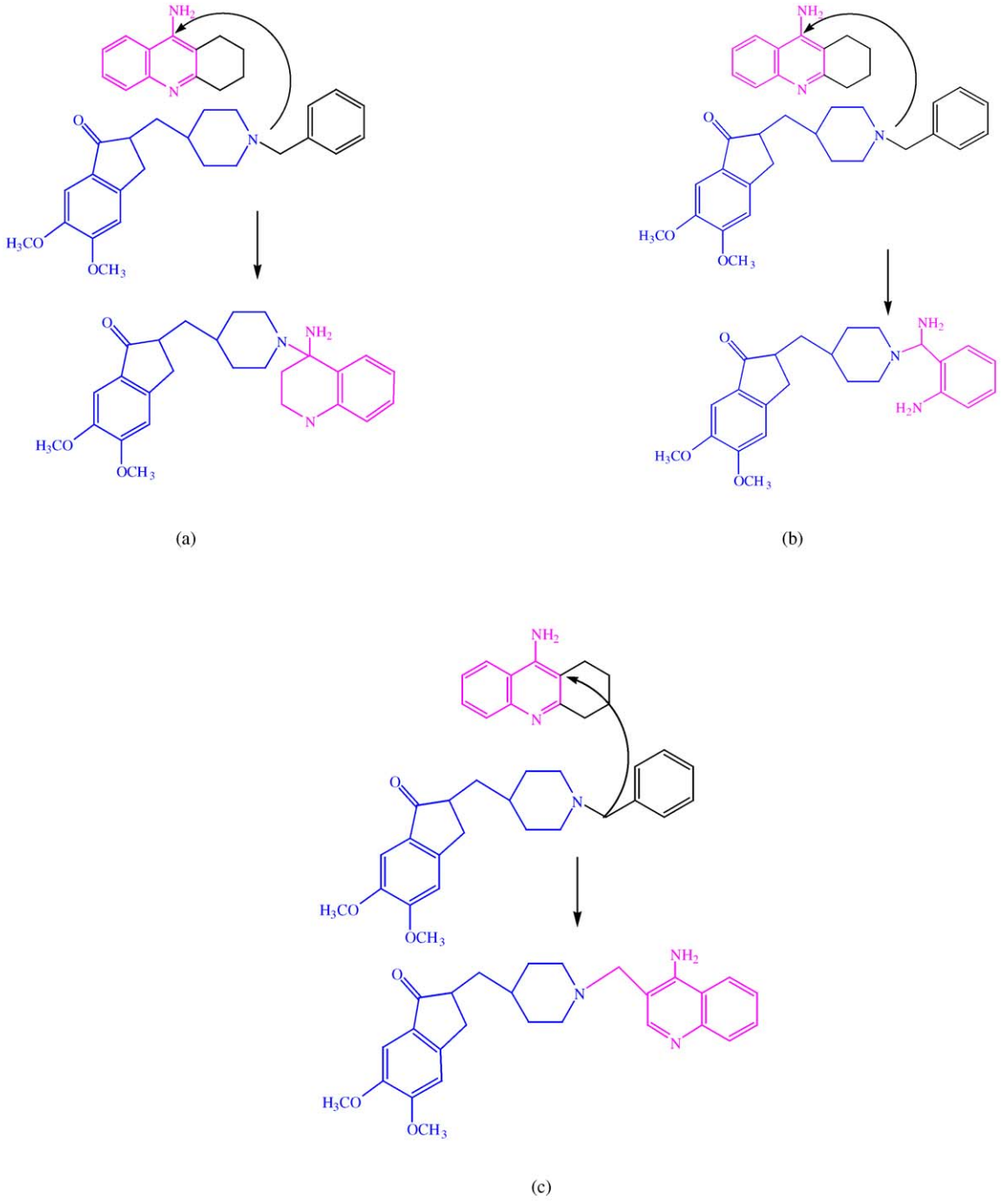


Fig. 3. Superposition of the structures of tacrine (carbon atoms in magenta), donepezil (carbon atoms in cyan) and Proposal 2 (carbon atoms in light orange).

also possible to maintain, in the final molecule, an hydrogen bond interaction between the amino group of aniline and the carbonyl group of the main chain of His440. This aromatic ring also maintained the charge transfer interaction with the phenyl ring of the indole group of Trp84. Our docking results indicate a score of 66 for this Proposal 2. The top-ranked orientation suggested by GOLD maintain the interactions above cited and indicates that, likely, the opening of the ring of Proposal 1 resulted in an orientation of lowest energy for the ligand inside the enzymatic active site, which can be represented by enhancing the score of Proposal 2 with respect to Proposal 1 (Fig. 3).

Since the scores obtained for the above two cited proposals were lower than that obtained for the complex donepezil–AChE (score = 78), and considering the difficulties of preparation of these two hybrids that contain a hemi-aminal function, we proposed a third modification by molecular hybridization. In this new proposal, the methylenic carbon bound to the piperidine nitrogen of donepezil was connected to the quinoline ring of tacrine, maintaining in the derivative hybrid, the quinoline ring originating from tacrine (Proposal 3, Scheme 1c).

The conformation of highest score obtained with GOLD for this Proposal 3 (score = 88) maintains the molecule oriented allowing the pi-stacking interaction involving the indanone group of the hybrid, as well as the pi-stacking interaction with Trp84 and hydrogen bond with the His440 residue of AChE, besides a possible additional hydrogen bond interaction between the free amino group of the resulting molecule and the side chain of Glu199 (Fig. 4). The atomic NBO (natural bond order) charges calculated for the Proposal 3 indicate the nitrogen of this free amino group as the atom that contains the most negative charge (−0.891) amongst all atoms, supporting the possibility of this ligand to establish a strong hydrogen bond with Glu199 and His440 (via carbonyl group of its main chain). The orientation suggested by GOLD for this molecule inside the AChE active site is very close to the minimum global conformation obtained for the same unbound molecule, using the ab initio method and the conformational search performed in this work. Our results suggest that the energetic cost for



Scheme 1. (a) Proposal 1 of molecular hybridization of tacrine with donepezil; (b) proposal 2 of molecular hybridization of tacrine with donepezil; (c) proposal 3 of molecular hybridization of tacrine with donepezil.

Proposal 3 to achieve the bioactive conformation would be low. For comparison, the superposition between the two minimum global structures, calculated by DFT and molecular mechanics (MMFF force field), and the top-ranked solution obtained with GOLD are shown in Fig. 5.

Toxicity prediction performed with DEREK for Proposal 1 indicates alpha-2-mu-globulin nephropathy as well as carcinogenicity plausible in humans and skin sensitization due to secondary amine. Secondary amines may react to form nitrosamines, either in vivo or in the environment. Nitrosamines are a major class of chemical carcinogens and mutagens. The presence of a skin sensitization structural alert within a molecule using DEREK indicates the molecule has the potential to cause skin sensitization. Whether or not the molecule will be a skin sensitizer will also depend upon its percutaneous absorption. Generally, small lipophilic molecules

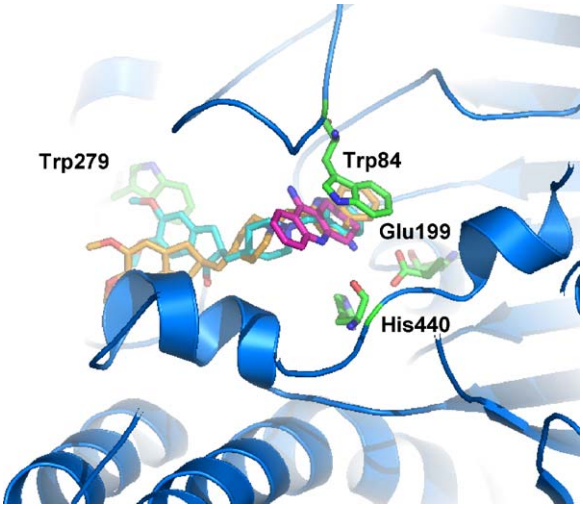


Fig. 4. Superposition of the structures of tacrine (carbon atoms in magenta), donepezil (carbon atoms in cyan) and Proposal 3 (carbon atoms in orange).

are more readily absorbed into the skin and are therefore more likely to cause sensitization.

Toxicity predictions were made with DEREK and METEOR. Proposal 2 indicates alpha-2-mu-globulin nephropathy as well as carcinogenicity plausible in humans and skin sensitization due to a primary aromatic amine. Cellular metabolism is required for activity. The best evidence indicates that hydroxylamino compounds have near carcinogenic forms. The aromatic amine can be converted to hydroxylamine by hydrolases, oxidases, or reductases endogenous to most tissues. Proposal 3 indicates alpha-2-mu-globulin nephropathy as well as carcinogenicity plausible in humans and skin sensitization due to an aromatic amine, similar to Proposal 2. The predictions for tacrine are the same as those obtained for our proposals 2 and 3, except the alpha-2-mu-globulin nephropathy. Our results indicate that no additional toxicity was introduced in any of our proposals. Plausible bio transformations for our proposed inhibitors include the same reactions predicted for donepezil or tacrine, without addition of any other plausible biotransformation. Sequences of human and *Torpedo californica* AChE share high sequence identity and their structures are highly similar in the binding pocket, supporting Proposal 3 as a starting point to design a novel AChE inhibitor for treatment of Alzheimer's disease.

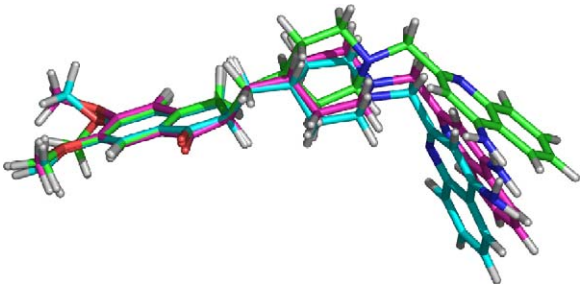


Fig. 5. Superposition of the structures of donepezil calculated by three different methods: molecular mechanics—MMFF force field (carbon atoms in green), ab initio—B3LYP/6-31G* (carbon atoms in cyan) and docking (carbon atoms in magenta).

4. Conclusions

In this work, we proposed and evaluated theoretically three novel molecular hybrids of the pharmaceuticals tacrine and donepezil, used in the Alzheimer's disease (AD), for further investigation and experimental validation. Based on this work, the hybrids herewith proposed have shown orientations very close to the original pharmaceuticals. Our results suggest that the Proposal 3, with highest synthetic viability, has more interactions with the AChE than the other two proposals. This molecule is an interesting pharmaceutical candidate to be prepared and investigated, since it incorporates the interactions originating from tacrine and donepezil with AChE, and also maintain their drug properties. Molecular modeling, docking and ADMET predictions can be important initial steps toward the development of novel pharmaceuticals in the fight against Alzheimer's disease.

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