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Original article

Docking and quantum mechanic studies on cholinesterases and their inhibitors

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Abstract

Docking studies and density functional theory (DFT) calculations were made for 88 *N*-aryl derivatives and for some acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) residues. Based on this information, some compounds were synthesized and tested kinetically in vitro as AChE inhibitors.

Finally, some chemical properties of the *N*-aryl derivatives were calculated: partition coefficient (π) and molecular electrostatic potentials (MESPs) whereas their electronic effects (ρ) were taken from the literature. The results showed that all compounds act inside the AChE gorge, making $\pi-\pi$ interactions and hydrogen bonds with Trp86 and Ser203 and by high HOMO energies of Ser2003 and high LUMO energies of *N*-aryl derivatives. These theoretical calculations for AChE are in agreement with the experimental data, whereas such calculations for BChE do not show the same behavior which could be due to in spite of both cholinesterase enzymes displaying similar functional activities they do possess important structural differences at their catalystic sites.

Keywords: Alzheimer's disease; Butyrylcholinesterase; Acetylcholinesterase; *N*-aryl derivatives; HOMO–LUMO densities; Docking

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative alteration characterized by a low concentration of acetylcholine (ACh) in hippocampus and cortex [1]. ACh is a neurotransmitter that is hydrolyzed by acetylcholinesterase (AChE, E.C. 3.1.1.7) and butyrylcholinesterase (BChE, E.C. 3.1.1.8) [2,3]. Recently, the genesis of amyloid protein plaques has been associated with some alterations of both ChEs (AChE and BChE), given that by using ChE inhibitors such plaques decrease considerably in patients with AD [4,5]. Therefore, it is not surprising that ChE inhibitors (ChEIs) have shown better results in the treatment of AD than any other strategy explored [6,7]. However, their clinical use is limited due to their side effects and

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some pharmacokinetic parameter disadvantages [8–12]. On this basis, our work group has focused on the synthesis and evaluation of new AChEIs [13–15], which have been developed by taking several structural properties into account. On the other hand, both ChEs have a catalytic triad (Ser, His, Glu), while on the other, in AChE Phe295 and Phe297 residues are near the catalytic triad and in BChE the equivalent residues are Leu286 and Val288 near the same. The catalytic triad and such residues are located at the bottom of a narrow gorge, where there is an anionic binding site formed by Trp82 and Trp86 for BChE and AChE, respectively, which is the binding site for the quaternary nitrogen of their substrates and some ligands [16,17]. The active site of both ChEs is characterized by having a highly negative electrostatic potential [18]. From our results, it can be said that to be recognized by the active site of the ChEs, the ligands should have a N atom which could carry a positive partial charge generated by resonance effects through an aromatic system.

In this contribution, docking studies and quantum chemistry calculations were done for 88 *N*-aryl substituted derivatives (44 amides and 44 imides) in order to explore their recognition by some amino acids involved in the binding site of these ChEs, paying special attention to the electronic effects, which are related to HOMO–LUMO energies and Hammet effects (ρ). And also, other chemical properties (partition coefficient and steric effects) of the compounds were taken into account. Given that the substituent-effects play an important role in the electronic effects, compounds with electron withdrawing (**1b**, **1i** and **3b**) or donating (**1q** and **3q**) groups, or those without effects (**1a** and **3a**), were selected to be synthesized and tested as AChE inhibitors.

2. Chemistry

2.1. Synthesis of amides (*1a*, *1b*, *1i* and *1q*)

The synthesis of amides was performed under solventless conditions by mixing an equimolar amount of monosubstituted anilines and succinic anhydride (see Scheme 1). This mixture was vigorously stirred at room temperature (r.t.) from 2 to 4 h. The products obtained were suspended and washed with H_2O (3×30 mL) at pH ≅ 3. The resulting suspensions were filtered and the corresponding products were dried at 40 °C for 24 h. All compounds were obtained with short reaction times and high yields.

2.2. Synthesis of imides (*3a*, *3b* and *3q*)

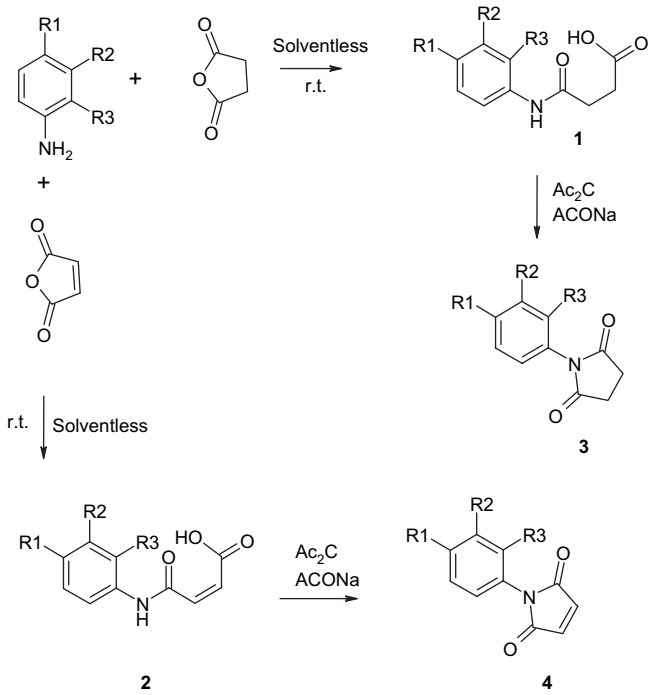
Two grams of the starting materials **1a**, **1b** and **1q** were mixed with an equimolar amount of sodium acetate in acetic anhydride, which were then heated for 4 h at 80–85 °C, resulting in the corresponding *N*-arylimides (**3a**, **3b** and **3q**). The resulting mixtures were cooled and the products were washed with H_2O (3×30 mL) at pH ≅ 3. The solids were dried at 40 °C. These reactions were monitored by *TLC* (acetone/ethanol 1:1, SiO_2). All the physico-chemical properties of the synthesized compounds were compared with those previously reported by our work group [19,20].

3. Kinetic assays

The inhibitory effects of the compounds obtained here were tested on AChE in vitro by using the modified Bonting and Featherstone's colorimetric method [21]. AChE from bovine erythrocytes and iodide acetylcholine were purchased from Sigma–Aldrich. Five units of AChE from bovine erythrocytes were used after diluting them in 0.04 mL of phosphate buffer (0.1 M, pH = 8). One unit of this enzyme hydrolyzes 1.0 μmol of ACh per min at pH = 8.0 and at 37 °C. ACh iodide was used as a substrate at several concentrations (0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8 and 25.6 mM), which are near the K_m of AChE (≅ 12 mM). The ligands were dissolved in a phosphate buffer (0.1 M, pH = 8). The enzymatic inhibition measurements were carried out with and without ligands at the different substrate concentrations mentioned. At the end of each incubation, the reaction was stopped by adding 0.2 mL of hydroxyl amine (0.47 mmol) and 1.5 mL of 0.5 N HCl. Then, 0.08 mL aliquots were mixed with 1.6 mL of ferric chloride (0.086 mmol), and finally the absorption measurements were made at $\lambda = 540$ nm.

4. Computational procedure

In order to gain insight into the recognition partners between the ChEs and the ligands, docking simulations were done on the three-dimensional structures of human AChE and BChE (PDB codes: 1B41 and 1POI, respectively) since they represent the pharmacological target for the development



Scheme 1. The scheme of synthesis of the compounds.

of new drugs to treat the AD. Missing residues were built and hydrogen atoms were added to the amino acids of the protein, followed by a minimization procedure by using 500 steps of steepest descents' protocol. These procedures were carried out by using the program Swiss-PdbViewer version 3.7 [22].

The minimum energy structure of the compounds and some selected ChE residues (from the catalytic triad: Ser, His, Glu of both ChEs; Phe295 and Phe297 for AChE as well as Val288 and Leu286 for BChE, which were taken from the original PDB file) was obtained by means of DFT calculations at the B3LYP/6-31G+(*d*,*p*) level, with the aid of the Gaussian 98 package [23].

For docking studies, the latest version of AutoDock (3.0.5) was chosen because its algorithm allows full flexibility of small ligands [24]. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical binding free energy evaluation. Docking to both ChEs was carried out by using the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals and a maximum number of 1.0×10^7 energy evaluations. Resulting docked orientations within a root-mean square deviation of 1.5 Å were clustered together. The lowest energy cluster returned by AutoDock for each compound was used for further analysis. All other parameters were maintained at their default settings.

Before starting the docking evaluations, the partial atomic charges were assigned using the Gasteiger–Marsili formalism while all possible rotable bonds of the ligands and Kollman charges for all atoms in ChEs were assigned by using the AutoDock Tools.

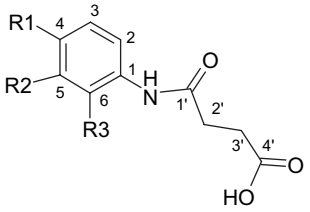
Finally, for all the ligands the electronic effects were taken up from the literature as was reported by Skagerber et al. [25], whereas their partition coefficients and steric effects were calculated. The partition coefficients were calculated online by using the Molinspiration site (Molinspiration, Bratislava, Slovak Republic) [26]. Finally, the steric effects were calculated by using the electrostatic potential as was reported by Suresh [27].

5. Results and discussion

Theoretical studies (docking and quantum chemistry) were performed for 88 *N*-aryl derivatives (Tables 1 and 2), considering that several compounds with related structures have activities as AChE inhibitors (see Table 3) [13–15,28]. The docking studies suggest that all the tested compounds bind at the active site of both ChEs. This could be due to the fact that they have an aromatic ring and a nitrogen atom, like other ChE inhibitors [28,29]. However, there are several functional groups that modify the electronic density on the aromatic ring and the N atom, which might change the affinity between the ligands and the enzymes. Docking calculations allow predicting the structure of all the complexes between the enzymes and the ligands, thus suggesting the kind of interaction. The inhibitors are depicted as balls in Figs. 1 and 2. They are located in the active site gorge of both AChE and BChE so as to maximize the favorable contacts. The hydrogen bonds and the van der Waals forces are the main features of the interactions of **1n**, **2e**, **3i** and **4i** with the Trp86 and Ser203 of AChE (see Fig. 1) as well as of **1p**, **2p**, **3o** and **4f** with the Trp82 and Ser198 of BChE (see Fig. 2). However, the π–π interaction plays an important role, giving the ligand–AChE complexes high stability and at the same time improving the recognition process between this enzyme and the compounds. The π–π interaction is formed between the aromatic ring of the ligands and the aromatic ring from the Trp86 of AChE [30]. And also, the π–π interactions increase for compounds **2** and **4** due to their sp^2 carbons located in the amide and imide fractions, which could explain in part their best affinity in comparison to **1** and **3** derivatives (see Tables 1 and 2). Such interactions have not been observed clearly for BChE, as can be seen in Fig. 2, where are shown four compounds which display the complex with the best affinity on this enzyme. All the compounds are bound to AChE between Trp86 and Ser203, making direct contact with the two residues. Nevertheless, such compounds are bound to BChE in a more disperse form than to AChE, despite the fact that in both cases the interactions are at the bottom of the ChE gorges. The structural differences in the complexes formed by the compounds with each of the enzymes could be due to the fact that the ligand affinity is less favored in BChE than AChE. This could explain the low inhibitory activity on the former enzyme (see Tables 1–3). The low affinity of the ligands at the active site of BChE could be due to the larger space at the bottom of the gorge as well as due to the greater distance (9.02 Å) between the two important residues (Ser198 and Trp82) of this enzyme than Ser203 and Trp86 of AChE (7.92 Å). In addition, the electrostatic potential of Ser198 could not influence Trp82 or the ligands. These features could explain why BChE recognizes ligands with diverse chemical properties [31].

In recent years, the pathogenesis of AD has been associated with both ChEs, resulting in several studies that have targeted these two enzymes [32,33]. The fact that both ChEs have some different structural characteristics and the anionic site and the catalytic triad are conserved at the gorge led us to hypothesize that aryl derivatives could act in the recognition site of both ChEs. Thus, drug design efforts were made with the initial idea that they would act on both ChEs with similar affinity. Nevertheless, this was not the case (see Tables 1 and 2), given that the most potent AChE inhibitors were **1n**, **2e**, **3i** and **4i** of each family, judged by the theoretical calculations (see Fig. 1). Except **1n**, such inhibitors are characterized by having an electron withdrawing group (CO_2H and Cl at the *m*- or *p*-position, respectively). On the other hand, the least potent inhibitors were **1d**, **2o**, **3a** and **4a**. Compound **1d** has a NO_2 group at *ortho*-position, **2o** has an electron-donating group (NH_2) at *meta*-position, while **3a** and **4a** do not have any substituents (see Table 2). These results suggest the importance of the electronic effects on ligand recognition given that the compounds with the most affinity on AChE were those that have an electron withdrawing effect group at *m*-or *p*-position except for **1n**. However, despite of the **1n** the latter compound does not have an electron withdrawing group and it is characterized by having high LUMO energy and low HOMO energy (see Table 1).

Table 1
The HOMO–LUMO energies and inhibitory effects of aryl derivatives of compounds **1** and **2** on AChE and BChE activities

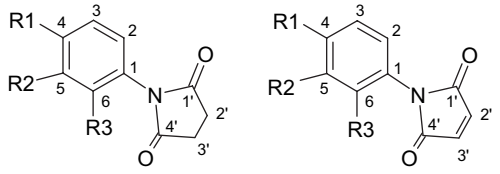


1 **2**

Compounds	R1	R2	R3	AchE (K_i, μM)	BchE (K_i, μM)	HOMO energies	LUMO energies	Energy (Hartrees)	ρ	π	MESP steric (kcal/mol)
1n	NH_2	H	H	1.93	12.30	−0.00807	0.00767	−723.5126	−0.66	−0.924	0.043
1o	H	NH_2	H	2.10	24.40	−0.00385	0.01010	−723.5120	−0.16	−0.948	0.085
1c	H	NO_2	H	2.42	9.08	−0.00389	0.00333	−872.6635	0.71	−0.065	−0.049
1e	CO_2H	H	H	2.43	8.06	−0.02094	0.00217	−856.7323	0.45	−0.089	−0.047
1l	H	OH	H	2.53	6.19	−0.01570	0.00030	−743.3812	0.12	−0.503	−0.034
1f	H	CO_2H	H	2.74	6.15	−0.02333	0.00489	−856.7411	0.37	−0.113	−0.041
1g	H	H	CO_2H	3.48	2.68	−0.02199	0.00641	−856.7429	–	0.043	−0.036
1i	H	Cl	H	3.80	7.17	−0.02055	0.00471	−1127.7593	0.37	0.654	−0.064
1h	Cl	H	H	4.03	10.80	−0.02133	0.00267	−1127.7596	0.23	0.678	−0.009
1p	H	H	NH_2	4.39	0.93	−0.00321	0.01505	−723.4656	–	−0.903	0.127
1v	H	H	F	4.53	7.70	−0.00767	0.01715	−767.3787	–	0.116	−0.063
1q	OCH_3	H	H	4.87	13.80	−0.00319	0.00767	−782.6784	−0.27	0.057	−0.038
1j	H	H	Cl	4.93	6.40	−0.01097	0.00116	−1127.6434	–	0.630	−0.115
1m	H	H	OH	5.61	4.15	−0.00633	0.00768	−743.3560	–	−0.267	−0.065
1r	H	OCH_3	H	6.59	10.20	−0.01395	0.00070	−782.6778	0.12	0.033	−0.074
1b	NO_2	H	H	7.69	17.80	−0.00279	0.01513	−872.6659	0.78	−0.041	−0.054
1u	H	F	H	8.14	8.52	−0.00905	0.00527	−767.3956	0.34	0.140	−0.047
1k	OH	H	H	8.26	15.00	−0.10209	0.00209	−743.3795	−0.37	−0.479	−0.012
1t	F	H	H	8.62	21.40	−0.01366	0.00420	−767.3960	0.06	0.164	−0.017
1s	H	H	OCH_3	9.59	9.98	−0.01304	0.00470	−782.6522	–	0.009	−0.023
1a	H	H	H	15.4	13.60	−0.00695	0.00700	−668.1657	0	0	0
1d	H	H	NO_2	17.0	3.64	−0.00003	0.01990	−872.6607	–	−0.089	0.004
2e	CO_2H	H	H	1.44	8.54	−0.01821	0.00797	−855.4992	0.45	−0.089	−0.011
2h	Cl	H	H	1.65	10.70	−0.02316	0.01755	−1126.5152	0.23	0.678	−0.005
2n	NH_2	H	H	1.65	8.24	−0.00883	0.02489	−722.2685	−0.66	−0.924	−0.021
2k	OH	H	H	1.78	8.62	−0.00279	0.02804	−742.1409	−0.37	−0.479	−0.033
2j	H	H	Cl	2.81	8.45	−0.02298	0.01724	−1126.5106	–	0.630	0.050
2p	H	H	NH_2	2.84	1.65	−0.00774	0.03060	−722.2655	–	−0.565	−0.024
2g	H	H	CO_2H	2.95	7.48	−0.02442	0.03007	−855.4845	–	0.044	0.006
2f	H	CO_2H	H	3.00	5.22	−0.02626	0.01643	−855.4977	0.37	−0.113	0.002
2i	H	Cl	H	3.05	4.67	−0.02230	0.01512	−1126.5139	0.37	0.654	−0.014
2c	H	NO_2	H	3.68	9.80	−0.00510	0.01056	−871.4147	0.71	−0.071	0.008
2m	H	H	OH	4.18	6.16	−0.01905	0.00032	−742.1367	–	−0.267	0.012
2t	F	H	H	4.20	10.40	−0.01589	0.02131	−766.1516	0.06	0.164	−0.042
2q	OCH_3	H	H	4.29	11.20	−0.00607	0.02681	−781.4344	−0.27	0.057	−0.073
2l	H	OH	H	4.47	6.49	−0.00039	0.02377	−742.1415	0.12	−0.511	−0.035
2r	H	OCH_3	H	4.55	5.68	−0.01707	0.00042	−781.4321	0.12	0.033	−0.036
2u	H	F	H	4.75	8.36	−0.00988	0.01947	−766.1511	0.34	0.139	−0.018
2v	H	H	F	5.61	6.95	−0.00971	0.02288	−766.1510	–	0.116	−0.017
2a	H	H	H	5.65	10.50	−0.00897	0.02460	−669.9215	0	0	0
2s	H	H	OCH_3	7.87	6.71	−0.01205	0.00487	−781.3892	–	0.009	−0.066
2d	H	H	NO_2	9.74	5.81	−0.00195	0.01870	−871.4186	–	−0.089	0.028
2b	NO_2	H	H	35.4	23.10	−0.02214	0.00936	−871.4218	0.78	−0.041	−0.007
2o	H	NH_2	H	483.0	2.81	−0.00569	0.02524	−722.2679	−0.16	−0.948	−0.023

Such results showed that the electronic effects could not be explained totally by the Hammet effects as was reported for the peripheral anionic site of AChE in another study [34]. The compounds with more affinity for AChE were those that have electron withdrawing groups, but they do not have the same behavior with BChE. As can be seen from the docking studies, **1p**, **2p**, **3o** and **4f** are the most potent and **1o**, **2b**, **3d** and **4d** the least potent BChE inhibitors (See Fig. 2). Thus, we can infer that BChE inhibition is not clearly dependent on electronic effects, because these compounds do not

Table 2
The HOMO–LUMO energies and inhibitory effects of aryl derivatives of compounds **3** and **4** on AChE and BChE activities



3 **4**

Compounds	R1	R2	R3	AchE (K_i, μM)	BchE (K_i, μM)	HOMO energies	LUMO energies	Energy (Hartrees)	ρ	π	MESP steric (kcal/mol)
3i	H	Cl	H	0.23	59.00	−0.01565	0.02205	−1051.3221	0.37	0.654	0.014
3j	H	H	Cl	0.38	113.00	−0.01439	0.02756	−1051.2968	–	0.630	−0.015
3h	Cl	H	H	1.34	115.00	−0.01634	0.02477	−1051.3207	0.23	0.678	−0.005
3o	H	NH_2	H	2.19	12.50	−0.00224	0.03357	−647.0768	−0.16	−0.948	0.298
3n	NH_2	H	H	2.34	26.50	−0.00304	0.03409	−647.0769	−0.66	−0.924	0.289
3l	H	OH	H	6.42	32.80	−0.00217	0.03099	−666.9411	0.12	−0.503	0.074
3g	H	H	CO_2H	6.66	106.00	−0.01052	0.03317	−780.2390	–	0.042	0.109
3c	H	NO_2	H	7.38	17.70	−0.00326	0.02138	−796.2235	0.71	−0.089	0.137
3e	CO_2H	H	H	7.50	216.00	−0.01545	0.00913	−780.2980	0.45	−0.089	0.133
3p	H	H	NH_2	7.97	33.90	−0.02053	0.00358	−647.0614	–	−0.565	0.335
3m	H	H	OH	8.07	56.50	−0.02292	0.00659	−666.9333	–	−0.267	0.033
3f	H	CO_2H	H	8.35	27.30	−0.02668	0.02032	−780.2997	0.37	−0.113	0.091
3k	OH	H	H	8.47	44.50	−0.21903	0.08269	−666.9495	−0.37	−0.479	0.040
3r	H	OCH_3	H	8.95	34.20	−0.00200	0.03120	−706.2414	0.12	0.033	0.022
3b	NO_2	H	H	12.90	40.00	−0.01594	0.02329	−796.2246	0.78	−0.041	0.176
3q	OCH_3	H	H	14.80	32.50	−0.00561	0.03763	−706.2430	−0.27	0.052	−0.004
3d	H	H	NO_2	16.70	255.00	−0.02378	0.00743	−796.1315	–	1.025	0.179
3s	H	H	OCH_3	18.60	80.00	−0.01215	0.00959	−706.1948	–	0.009	−0.031
3u	H	F	H	18.80	65.10	−0.00438	0.02857	−690.9592	0.34	0.140	−0.011
3v	H	H	F	23.00	87.30	−0.00471	0.03045	−690.9531	–	0.116	−0.010
3t	F	H	H	26.80	83.00	−0.01329	0.03087	−690.9601	0.06	0.164	−0.017
3a	H	H	H	29.60	115.00	−0.00193	0.03352	−591.7290	0	0	0
4i	H	Cl	H	0.17	54.20	−0.01714	0.02433	−1050.0828	0.37	0.865	0.005
4j	H	H	Cl	0.33	119.00	−0.01625	0.02584	−1050.0589	–	0.841	0.003
4h	Cl	H	H	0.47	130.00	−0.01787	0.02531	−1051.0536	0.23	0.678	0.001
4p	H	H	NH_2	6.01	13.80	−0.01338	0.00207	−645.8231	–	−0.354	0.257
4g	H	H	CO_2H	6.22	56.40	−0.02148	0.04581	−778.9990	–	0.678	0.071
4o	H	NH_2	H	6.93	13.70	−0.00334	0.05834	−645.8375	−0.16	−0.737	0.242
4e	CO_2H	H	H	7.13	18.40	−0.01692	0.02610	−779.0591	0.45	0.098	0.072
4f	H	CO_2H	H	7.46	9.70	−0.02323	0.03692	−779.0603	0.37	−0.265	0.041
4c	H	NO_2	H	7.50	15.20	−0.00134	0.04475	−794.9841	0.71	0.146	0.076
4n	NH_2	H	H	8.30	26.60	−0.00146	0.05906	−645.8375	−0.66	−0.924	0.232
4r	H	OCH_3	H	8.93	30.00	−0.00248	0.05635	−705.0021	0.12	0.244	−0.004
4l	H	OH	H	9.24	34.00	−0.00280	0.05524	−665.7019	0.12	−0.292	0.001
4k	OH	H	H	11.10	66.50	−0.00132	0.06245	−665.7100	−0.37	−0.479	−0.012
4b	NO_2	H	H	11.80	191.00	−0.00780	0.04418	−794.9848	0.78	−0.041	0.110
4q	OCH_3	H	H	14.10	44.30	−0.00409	0.06155	−705.0035	−0.27	0.056	−0.001
4d	H	H	NO_2	14.80	243.00	−0.03640	0.00593	−794.8915	–	−0.878	0.115
4m	H	H	OH	14.90	56.10	−0.01661	0.00625	−665.6943	–	−0.061	0.038
4s	H	H	OCH_3	18.30	84.40	−0.00899	0.00929	−705.9508	–	0.220	−0.013
4u	H	F	H	18.50	70.50	−0.00526	0.05377	−689.7200	0.34	0.351	−0.005
4v	H	H	F	21.90	79.10	−0.0058	0.05676	−689.7134	–	0.327	0.008
4t	F	H	H	25.00	84.50	−0.01460	0.05547	−689.7206	0.06	0.163	0.000
4a	H	H	H	29.50	122.00	−0.00334	0.05876	−590.4896	0	0	0

have related substituents as was mentioned above for AChE. Although the docking scoring does not consider several variables, this study showed that all compounds have a higher affinity for AChE than for BChE, which was corroborated by the experimental data (see Table 3) [13]. In this sense, the experimental data showed that the most active compounds as inhibitors on AChE activity were those that have an electron withdrawing group, such as **1e**, **1f**, **3f** and **4e**. Thus, the higher affinity could be due to their electronic effects. However, in spite of the fact that **3n** has strong electronic effects, it was not a good inhibitor and also in Tables 1 and 2 are shown the electronic effects which do not reflect that. This is probably because the interaction is mediated by other factors such as the conformational changes of the enzyme that allow the

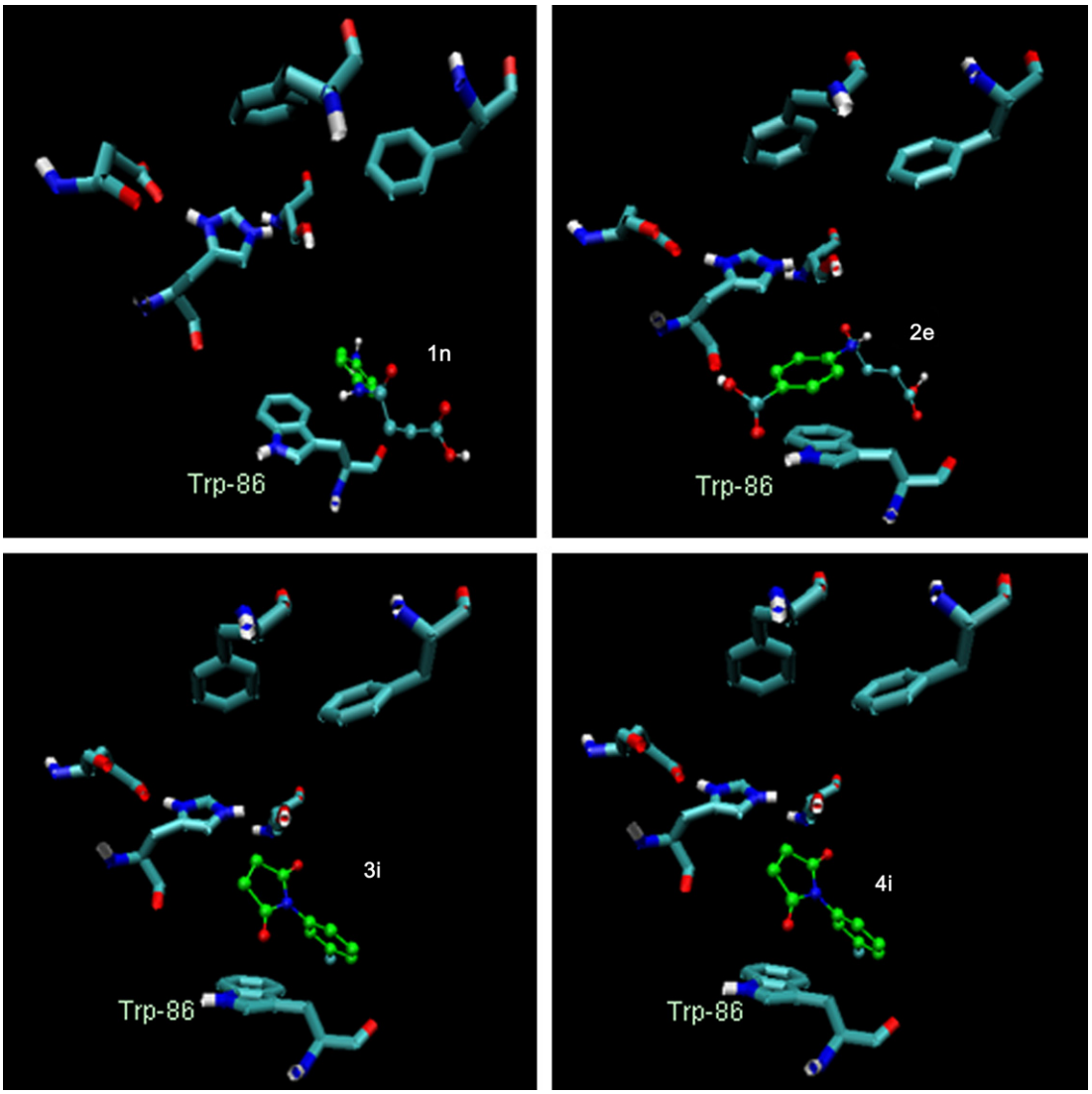


Fig. 1. Ligands with the highest potency (**1n**, **2e**, **3i**, and **4i**) on AChE activity.

ligand to pass through the gorge and interact in the active site. This effect could be explored by mixing docking and molecular dynamic as was reported by Alonso et al. [35]. Furthermore, it is evident that compounds with electron withdrawing groups showed a better correlation with the inhibition of AChE than BChE, which is reflected in the calculated and experimental K_i values. This could be explained by two factors: (1) the hindrance effects inside the active site of AChE in comparison to BChE, and (2) the electrostatic potential, which increases at the bottom of the AChE but not considerably for BChE gorge [36]. This might be due to the fact that BChE has hydrophobic residues (Val288 and Leu286) instead of the two aromatic residues (Phe275 and Phe277 for AChE). Such aromatic residues are close to either the catalytic triad or Trp86 and thus they modulate the ligand recognition, as was reported for the aromatic rings located in the peripheral anionic site [34]. In addition, these Phe residues of AChE possibly lead to the enzyme being less promiscuous with its ligands and have a stereoselective behavior [37], unlike what has been observed with the hydrophobic residues of BChE [38].

Given that the electronic effects play a very important role in the drug recognition process [39], and despite the fact that AChE catalytic activity has been studied in several *ab initio* calculations [40–42], some quantum-chemical descriptors such as HOMO and LUMO energies have suggested that there is a correlation between these frontier orbitals and biological activity [43]. The HOMO and LUMO energies were evaluated for the 88 compounds (see Tables 1 and 2) as well as for the principal residues involved in the ligand recognition at the bottom of both ChE gorges (see Fig. 3). As can be seen in this figure there is a significant interaction between these orbitals, which were cleared by the HOMO densities of some residues of both cholinesterases and LUMO densities of the ligands. However, such energy data do not evidence to be strongly related to the inhibitory effects of the compounds on BChE activity, which could be explained by the fact that the HOMO of Ser198 is far from the Trp82 of this enzyme,

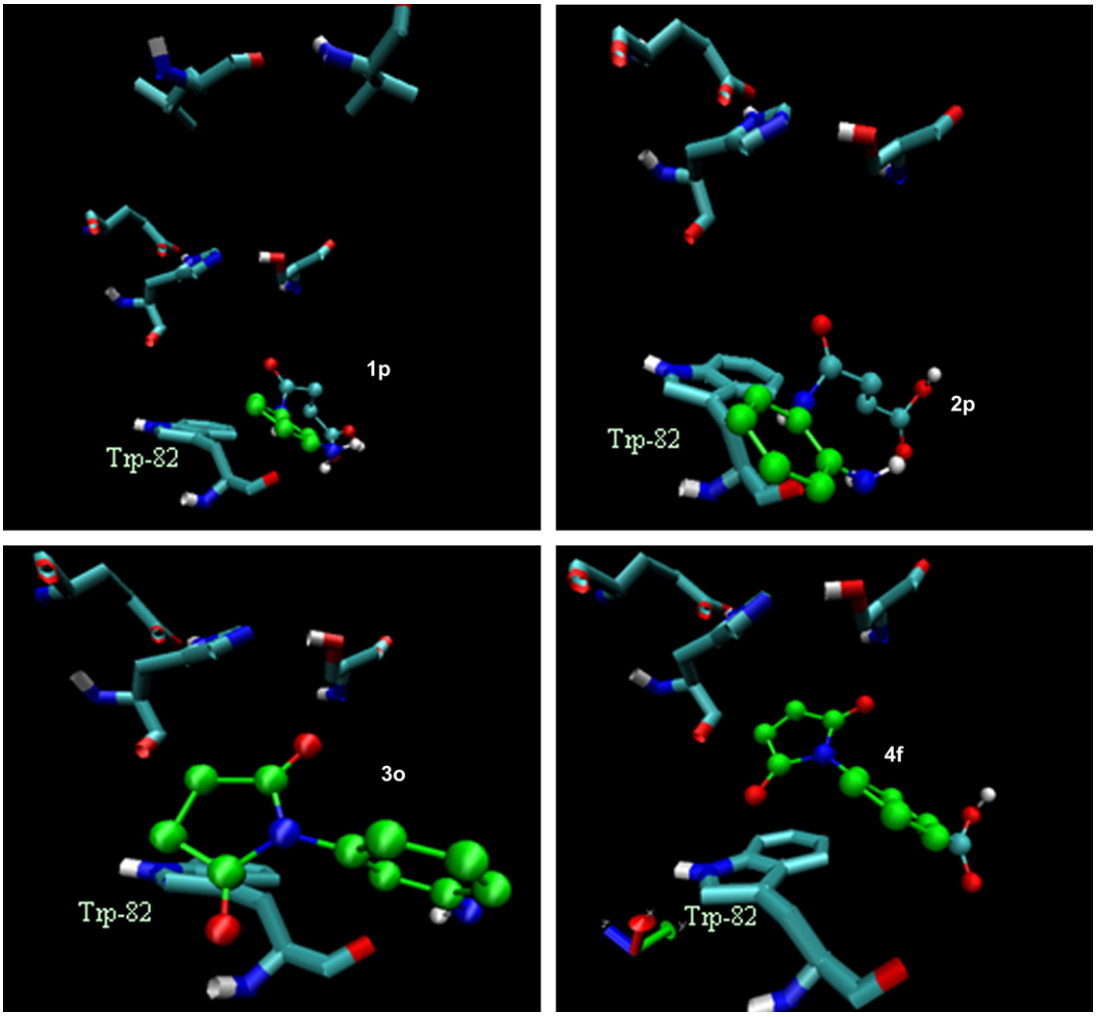


Fig. 2. Ligands with the highest potency (**1p**, **2p**, **3o** and **4f**) on BChE activity.

whereas the HOMO of Ser203 is close to the Trp86 of AChE. It appears that the LUMO energies of both AhEs do not influence importantly with inhibitory effects, whether by docking calculations or experimental data. This is contrary to the data observed for the LUMO of the physostigmine analogs, which showed a good relationship with the inhibitory effects [44]. However, with physostigmine derivatives there is not any experimental or theoretical data that suggest a recognition at the active site of AChE.

Another parameter that has been scarcely explored is that of the energy values of the optimized structure of the compounds [30], which showed to have a very important role in the inhibition of AChE activity obtained theoretically. The results showed that the low optimized energies values of the ligands are the most potent inhibitors of AChE activity but not BChE activity (data not shown).

The principal molecular physico-chemical parameters involved in the ligands' recognition are the electronic, partition coefficient and the steric effects. For this reason such parameter were taken into account (see Tables 1 and 2). The ChEs are dependent of these parameters over all in the ligand recognition process observing that the electronic effects, the hydrophobicity and the low steric effects as was observed for **1n** (−0.924 and 0.043, respectively) are very important to improve the affinity of the compounds on AChE (see Tables 1 and 2).

Comparing these results with other reports, it is evident that compounds that have small size [45,46], low HOMO or high LUMO energies, low optimized energies values of the ligands as well as an electron withdrawing group in the aromatic ring show a better recognition for the AChE active site, particularly at Trp86 and Ser203 by π−π interactions and hydrogen bonds [47,48]. Such factors were not observed in the recognition of BChE. Thus, the chemical structure and electronic properties as well as energies are very important parameters for designing selective and potent ChE inhibitors. With this evidence, it is important to mention that the interaction between AChE and ligands is by HOMO−LUMO energies [49]. In the other words, the unequal distribution of the electron density in bonds produces a bond dipole, the magnitude of which is expressed by the dipole moment, having the units of charge times distance, which could have a cyclic behavior, given the ligand and the recognition site have an aromatic system increasing the stability between the enzyme−ligand complexes.

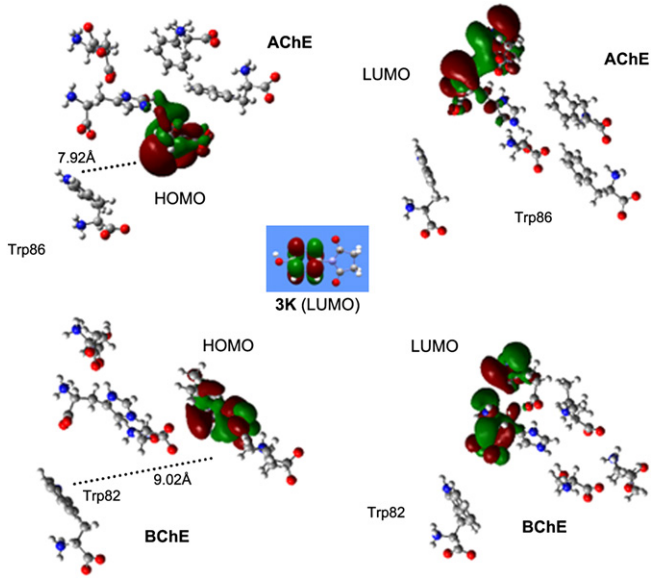


Fig. 3. HOMO (left) and LUMO (right) 3D orbital shapes for AChE (A and B) and BChE (C and D); ligand **3k** is located at the center of the shapes represented as LUMO density.

6. Conclusions

In summary, the compounds reported here are easily synthesized and active site directed for both ChEs. They bind predominantly with the Trp86 of AChE as can be seen by the experimental data and docking calculations. The Ser203 residue located at the bottom of the AChE gorge increases the HOMO energies close to Trp86, which in turn increases the recognition by ligands which are poor in electrons or have high LUMO energy.

7. Experimental protocols

7.1. General

The reactions were monitored by *TLC* using Whatman precoated plates (silica gel 60 F_{254}, 0.25 mm). The synthesized compounds (**1b**, **1i**, **3b**, **1q**, **3q**, **1a**, and **3a**) were observed at $\lambda = 254$ nm wavelength on an Epi Chemi II Darkroom Transilluminator. The molecules obtained were identified by ^{1}H and ^{13}C NMR spectra recorded at 300 MHz and 75.4 MHz, respectively, on a Jeol GSX-270 spectrometer, using DMSO-d_6 as a solvent and TMS as an internal reference. Melting points were obtained in open-ended capillary tubes with an Electrothermal 9300 digital apparatus and are uncorrected. AChE and BChE activities were measured by using UV–Vis on a Beckman Coulter DU-650 spectrophotometer.

*7.2. Synthesis of amides (**1a**, **1b**, **1i** and **1q**)*

The synthesis of amides was performed under solventless conditions by mixing an equimolar amount of monosubstituted anilines (2 g, 14.59 mmol) and succinic anhydride (weights were according to the type of aniline at 14.59 mmol). This mixture was vigorously stirred at room temperature from 2 to 4 h. The products obtained were suspended and washed with H_2O (3×30 mL) at pH ≅ 3. The resulting suspensions were filtered and the corresponding products were dried at 40° C for 24 h. All compounds were obtained with short reaction times and high yields [19]. This efficient and green-chemical alternative could be used for synthesizing many other related compounds.

*7.2.1. 2′-Oxo-2′(aminophenyl) butanoic acid (**1a**)*

Yield: 90%; m.p.: 140 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm 2.52 (2H, t, $J = 6.2$ Hz, H-2′), 2.56 (2H, t, $J = 6.2$ Hz, H-3′), 7.01 (1H, t, $J = 7.4$ Hz, H-4), 7.28 (1H, t, $J = 8.3$ Hz, H-3,5), 7.58 (1H, d, $J = 8.3$ Hz, H-2,6); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ/ppm 29.3 (C-2′), 31.6 (C-3′), 119.4 (C-2,6), 123.4 (C-4), 129.2 (C-3,5), 139.9 (C-1), 170.6 (C-1′), 174.4 (C-4′).

*7.2.2. 2′-Oxo-2′(4-nitroaminophenyl) butanoic acid (**1b**)*

Yield: 85%; m.p.: 117 ± 2 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm 1.82 (2H, t, $J = 6.2$ Hz, H-2′), 2.8 (2H, t, $J = 8.4$ Hz, H-3′), 7.63 (1H, d, $J = 8.4$ Hz, H-2,6), 8.35 (1H, d, $J = 5.4$ Hz, H-3,5); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ/ppm 22.80 (C-1′), 28.64 (C-2′), 124.18 (C-2,6), 127.94 (C-3,5), 138.42 (C-4), 146.47 (C-1), 176.52 (C-4′).

*7.2.3. 2′-Oxo-2′(5-chloroaminophenyl) butanoic acid (**1i**)*

Yield: 95%; m.p.: 148 ± 2 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm 2.52 (1H, t, $J = 7.4$ Hz, H-4), 7.02 (2H, t, $J = 6.2$ Hz, H-2′), 7.30 (2H, t, $J = 6.2$ Hz, H-3′), 7.38 (1H, d, $J = 8.3$ Hz, H-2), 7.78 (1H, t, $J = 8.3$ Hz, H-3); ^{13}C NMR (75.4 MHz, DMSO-d_6) δ/ppm 28.73 (C-10), 31.13 (C-5),

Table 3
Comparison of experimental and theoretical data on the inhibition constant of ChEs catalytic activities

Compounds	K_i[a] (μM) AChE	K_i[b] (μM) AChE	K_i[a] (μM) BChE	K_i[b] (μM) BChE
1a	3.471	15.4	nd	13.60
1b	nd	7.69	nd	17.80
1e	0.150[c]	2.43	0.736[c]	8.06
1f	0.092.5[c]	2.74	nd	6.15
1i	4.880	3.80	nd	7.17
1l	46.000[c]	2.53	nd	6.19
1k	1.300[c]	8.26	nd	15.00
1q	nd	4.87	nd	13.80
2e	0.100[c]	1.44	0.411[c]	8.54
2f	0.357[c]	3.00	nd	5.20
2l	2.300[c]	4.47	nd	6.49
2k	1.200[c]	1.78	nd	8.62
3a	22.126	29.6	nd	115.00
3b	107.308	12.9	nd	40.00
3e	0.243[c]	7.50	0.117[c]	21.60
3f	0.033.4[c]	8.35	nd	27.30
3k	1.800[c]	8.47	nd	–
3q	952.690	14.8	nd	32.50
4a	2.700[c]	29.5	nd	122.00
4e	0.052.6[c]	7.13	0.083[c]	18.90
4f	0.256[c]	7.46	nd	9.70

[a] Experimental data.
[b] Calculated by using AutoDock.
[c] Obtained from literature [13–15].

117.30 (C-1), 118.43 (C-4), 122.71 (C-7), 130.45 (C-6), 133.16 (C-3), 140.78 (C-9), 170.64 (C-2), 173.91 (C-8).

*7.2.4. 2′-Oxo-2′(4-metoxyaminophenyl) butanoic acid (**1q**)*

Yield: 90%; m.p.: 134 ± 2 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm 2.74 (2H, t, $J = 6.2$ Hz, H-2′), 3.77 (2H, d, $J = 9$ Hz, H-3′), 7.00 (1H, d, $J = 8.3$ Hz, H-3,5), 7.17 (1H, d, $J = 9$ Hz, H-2,6); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ/ppm 28.5 (C-1′), 55.34 (C-2′), 114.06 (C-4), 125.30 (C-2,6), 128.34 (C-3,5), 158.80 (C-1), 177.19 (C-4′).

*7.3. Synthesis of imides (**3a**, **3b** and **3q**)*

Two grams of the starting materials **1a**, **1b** and **1q** were mixed with an equimolar amount of sodium acetate in 5 mL of acetic anhydride, which was then heated for 4 h at 80–85 °C, resulting in the corresponding *N*-arylimides (**3a**, **3b** and **3q**). These reactions were monitored by *TLC* (acetone/ethanol 1:1, SiO_2). The resulting mixtures were cooled and the products were washed with H_2O (3 × 30 mL) at pH ≅ 3. The solids were dried at 40 °C. All syntheses were done as reported by our work group for some aryl derivatives.

*7.3.1. N-Phenyl-pyrrolidine-1′,4′-dione (**3a**)*

Yield: 62%; m.p.: 325 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm 2.30 (3H, s, H-6), 6.63 (1H, d, $J = 5.4$ Hz, H-2), 7.12 (2H, d, $J = 8.6$ Hz, H-3′,5′), 7.34 (1H, d, $J = 5.4$ Hz, H-3), 7.47 (2H, d, $J = 8.6$ Hz, H-2′,6′); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ/ppm 21.1 (C-6), 121.9 (C-3′,5′), 124.7 (C-2′,6′), 130.8 (C-2), 141.1 (C-1′), 143.3 (C-3), 148.2 (C-4′), 150.1 (C-4), 167.0 (C-5), 169.3 (C-1).

*7.3.2. N-(4-Nitro-phenyl)-pyrrolidine-1′,4′-dione (**3b**)*

Yield: 46%; m.p.: 162 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm 2.30 (3H, s, H-6), 6.63 (1H, d, $J = 5.4$ Hz, H-2), 7.12 (2H, d, $J = 8.6$ Hz, H-3′,5′), 7.34 (1H, d, $J = 5.4$ Hz, H-3), 7.47 (2H, d, $J = 8.6$ Hz, H-2′,6′); ^{13}C NMR (DMSO-d_6, 75.4 MHz) δ/ppm 21.1 (C-6), 121.9 (C-3′,5′), 124.7 (C-2′,6′), 130.8 (C-2), 141.1 (C-1′), 143.3 (C-3), 148.2 (C-4′), 150.1 (C-4), 167.0 (C-5), 169.3 (C-1).

*7.3.3. N-(4-Metoxy-phenyl)-pyrrolidine-1′,4′-dione (**3q**)*

Yield: 49%; m.p.: 279 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ/ppm 2.30 (3H, s, H-6), 6.63 (1H, d, $J = 5.4$ Hz, H-2), 7.12 (2H, d, $J = 8.6$ Hz, H-3′,5′), 7.34 (1H, d, $J = 5.4$ Hz, H-3), 7.47 (2H, d, $J = 8.6$ Hz, H-2′,6′); ^{13}C NMR (DMSO-d_6, 74.4 MHz) δ/ppm 21.1 (C-6), 121.9 (C-3′,5′), 124.7 (C-2′,6′), 130.8 (C-2), 141.1 (C-1′), 143.3 (C-3), 148.2 (C-4′), 150.1 (C-4), 167.0 (C-5), 169.3 (C-1).

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