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ORIGINAL ARTICLE

Docking studies and effects of *syn-anti* isomery of oximes derived from pyridine imidazol bicycled systems as potential human acetylcholinesterase reactivators

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Summary
In order to contribute to a better understanding of the mechanism of action of oximes, we evaluated the affinities of 10 new oximes, derived from pyridine-imidazol bicycled systems, for human acetylcholinesterase (*Hss*AChE) complexed with tabun, by estimating their docking energy values and comparing of the values obtained to known oximes using the software Molegro Virtual Docker (MVD)®. We evaluated the influence of the position of the oxime group as substituent in the structures and, also, the influence of the oxime group *syn-anti* isomery on the docking score values for all the molecules studied. Results suggest that: the affinities of the 10 new oximes for the tabun inhibited *Hss*AChE active site are better than pralidoxime's and similar to trimedoxime's; the *meta*-pralidoxime could have more affinity for the *Hss*AChE active site and the oximes' *anti* isomers could present slightly better affinities for the *Hss*AChE active site than the *syn* isomers.

Key words: acetylcholinesterase; docking studies; oximes; neurotoxic agents; theoretical calculation

INTRODUCTION

Despite the existence of many different oximes in use today against intoxication with neurotoxic agents, the literature has not yet reported one able to act effectively against all the existing neurotoxic agents and oximes usually effective with one specific nerve agent can be completely ineffective with another. This probably happens because their mechanisms of action are not yet well elucidated (Ekström et al. 2007). In addition, other relevant factors: the adequate orientation of the phosphoryl bond inside the active site, the most suitable oxime charge, the most adequate angles for attacking the phosphylated serine, the influence of the oxime's isomery, and the chemical environment of the oxime group, remain unknown despite the fact that they are recurrent issues in the literature (Bay et al. 1958, Smirnova et al.

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1975, Castro and Figueroa-Villar 2002, Bartosova et al. 2005, Gonçalves et al. 2006, 2010, Ekström et al. 2007, Worek et al. 2007, Delfino and Figueroa-Villar 2009, Delfino et al. 2009, Ramalho et al. 2010).

In the present work, in order to contribute to a better understanding of the mechanism of action of oximes (Fig. 1), we proposed the structures and evaluated *in silico* the affinities of 10 new oximes, derived from pyridine-imidazol bicycled systems, for the *Hss*AChE active site inhibited by the neurotoxic agent tabun. The softwares MVD® and Spartan® were used to estimate the values of the oximes' affinity (measured by the docking scores). The 10 oximes were studied together with the standard oximes pralidoxime (2-PAM), trimedoxime (TMB-4), and obidoxime as references. Additionally the reactivation constants of 2PAM and its *ortho*, *meta* and *para* isomers were calculated according to a procedure formerly established by Ramalho et. al. (2010). We evaluated the influence of the oxime group position as a substituent in the structures for 2-PAM and the 10 new oximes and, also, the influence of the oximes *syn-anti* isomery in the docking scores values for all the molecules studied. Our results suggest that the affinities of the 10 new oximes for the *Hss*AChE active site are better than all 2PAM's isomers and some were quite similar to TMB-4's. It was also observed that *meta*-2-PAM could present better affinities for the *Hss*AChE active site than *para* and *ortho*-2-PAM, and that 62.5% of the *anti* isomers presented slightly better affinities for the *Hss*AChE active site when compared to the *syn* isomers.

MATERIALS AND METHODS

Docking energy calculations

The structure of the *Hss*AChE used was that phosphonylated by tabun proposed and optimized by Gonçalves et al. (2006) and complexed with toxogonine, using as template the structure reported by Kryger et al. (2000) deposited in the Proteins Data Bank (Bernstein et al. 1977, Berman et al. 2000) under the PDB code IB41. The water molecules were removed using the program MVD®, the three-dimensional structures of the oximes (Fig. 2) were built and optimized with the software Spartan Pro 5® and their partial atomic charges calculated by the PM3 semi-empirical method. The compounds were docked into the *Hss*AChE binding site using MVD® (Thomsen et al. 2006) according to instructions which considered all the protein residues as flexible. Binding sites were restricted within spheres with radius of 8 Å, centered at the toxogonine binding site in the protein complex and enclosing all the active site residues. Due to the stochastic nature of the ligand-protein docking search algorithm, about 10 runs were conducted and 30 docking solutions were retained for each ligand. The best superimposing poses related to toxogonine, were chosen to the analysis performed in this work.

DFT Studies

QM/MM techniques were performed to determine the preferred route for the reactivation process. On the technical side, we applied a procedure combining docking technique and DFT calculations at the QM/MM interface for the enzymatic mechanism. The QM calculations were carried out in the Spartan08 (Hehre et al. 1999) and Gaussian98 (Frisch et al. 2001) packages. The QM region, which consisted of residues, neighbouring peptide bonds, link atoms, crystallographic water molecules, ligand and inhibitor, was confined into a sphere with a radius of 15 Å, centered at each oxime.

The initial coordinates for the heavy atoms were taken from the *Hss*AChE 3D structure proposed by Gonçalves et al. (2006). All the transition states, intermediates and precursors involved were calculated. Each conformer was fully optimized at the DFT level with B3LYP/6-31G (Ramalho and Taft 2005). Furthermore, after each optimization, a force constant calculation was made in order to verify whether the optimized structures were indeed local minima (no imaginary frequencies) or transition states (one imaginary frequency).

RESULTS AND DISCUSSION

Docking results

The cavity (Fig. 3) of the *Hss*AChE active site was calculated by MVD® as having 949,696 $Å^3$. The results of the docking studies of the oximes studied inside this cavity, allowed us to identify the relevant H-Bonds that occur between each oxime and the amino acid residues of the active site in order to obtain the conformations adopted with these molecules, compare them to the conformations of toxogonine and thus get subsidies for the investigations performed in this work.

The values of the docking energies obtained for the best poses of the *syn-anti* isomers of the 10 new oximes, the *ortho*, *meta* and *para* isomers of 2-PAM, TMB-4 and obidoxime are presented in Fig. 4. Table 1 reports the H-bond energy values obtained for each ligand in the *Hss*AChE active site and, also, the amino acid residues involved in H-bonds with them.

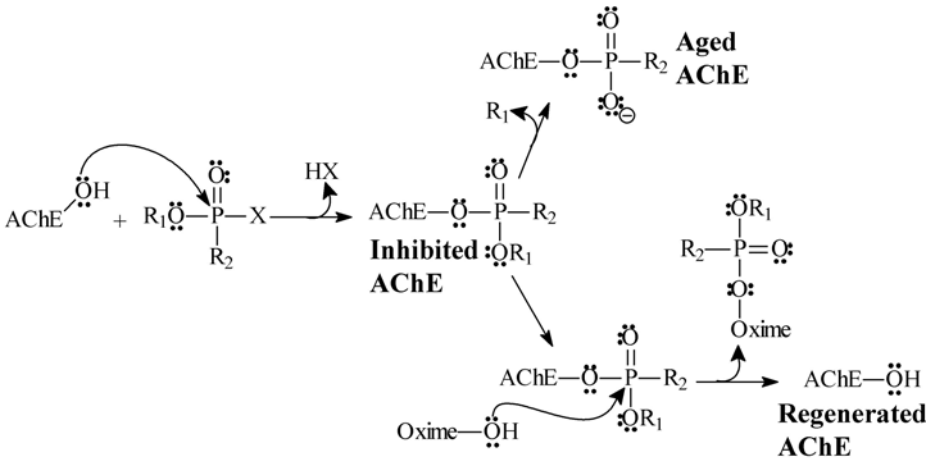

Fig. 1. **Inhibition, desinhibition and ageing of acetylcholinesterase. X is the leaving group.**

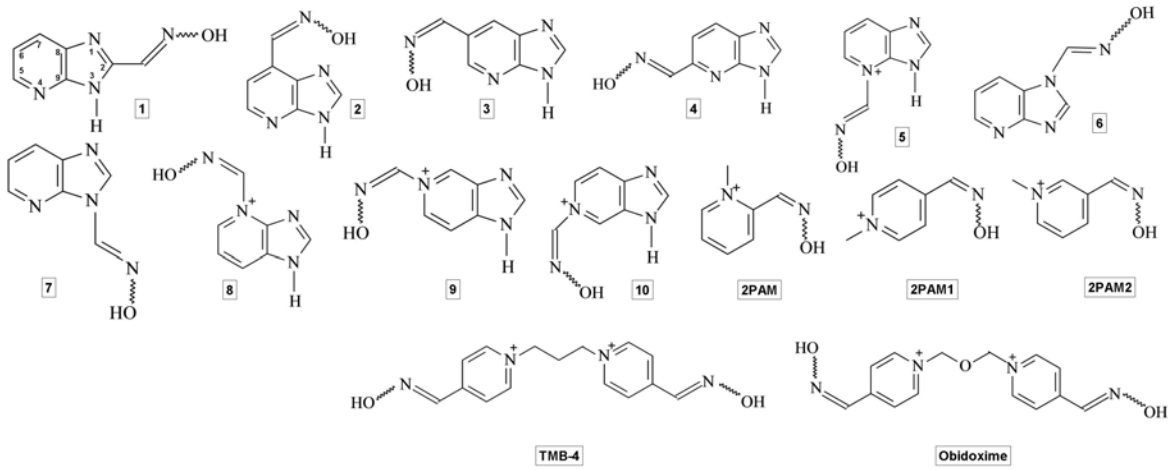

Fig. 2. **Structures of the oximes studied.**

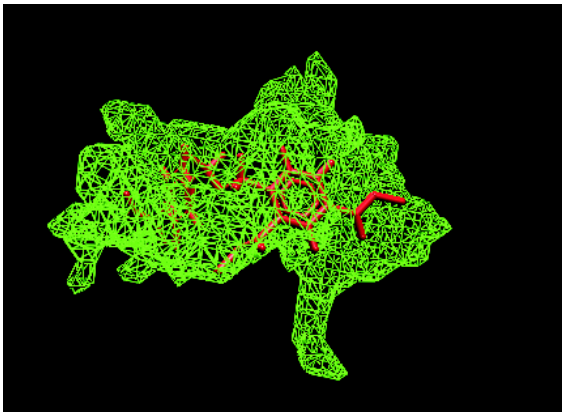

Fig. 3. **Cavity of the active site of *Hss*AChE complexed with obidoxime.**

An analysis of Fig. 4 indicates that all the 10 new oximes isomers presented docking energies better than the 2PAM isomers but worse than TMB-4 and obidoxime isomers. This result reflects the smaller size of 2PAM and the new oximes, avoiding a full superposition to obidixime and interactions with the totality of residues in the cavity (Figs 5 and 6). However, oximes 2, 3, 5, 6 and 8 presented at least one isomer with similar docking values to TMB-4 isomers.

H-bond energy values obtained for most of the new oximes isomers are better than for 2PAM and quite similar to the values obtained for TMB-4 and obidoxime isomers (see Table 1). All the aminoacid residues observed forming H-bonds with 2PAM were also observed for the new oximes. The new oximes also were able to form H-bonds with some additional

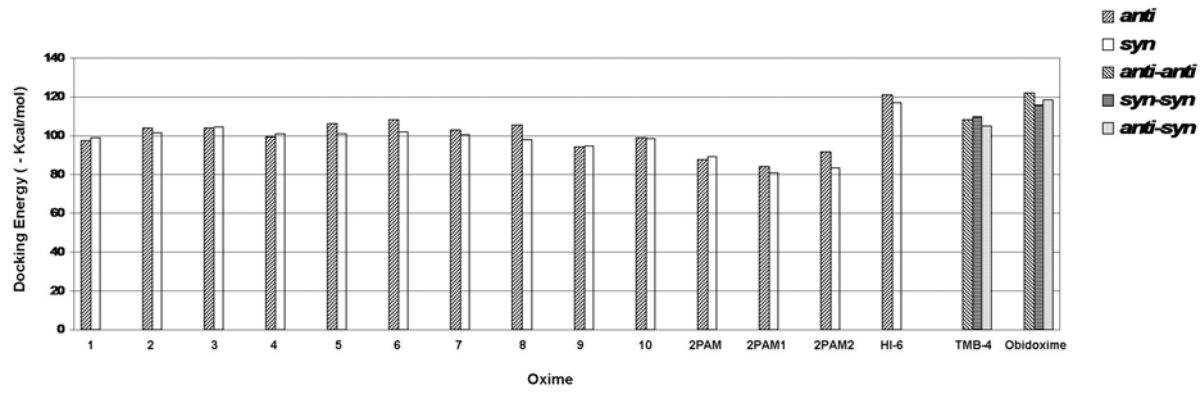

Fig. 4. **Comparative docking energies of the *syn-anti* isomers of the new oximes, 2PAM isomers, HI-6, TMB-4 and obidoxime.**

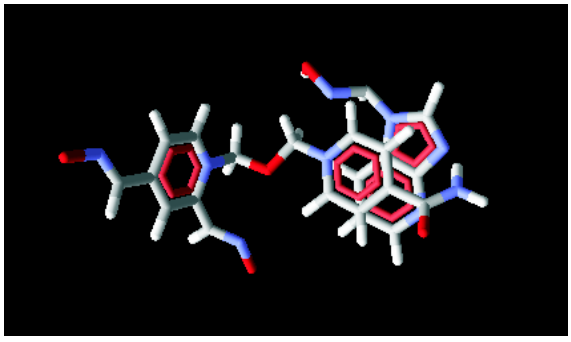

Fig. 5. **Superposition of oxime 6 to obidoxime.**

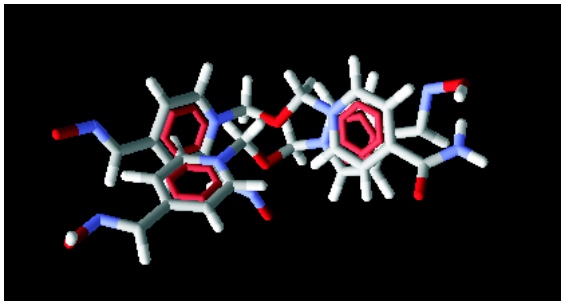

Fig. 6. **Superposition of TMB-4 to obidoxime.**

residues not observed for 2PAM (Tyr68, Asp70, Tyr120, Glu281 and Phe291). All the residues observed making H-bonds with the new oximes were observed in TMB-4 and obidoxime isomers which were, also, able to make additional H-bonds with the residues: Gly117, Gly118, Glu198, Ser199, Ala200 and His443 (see Table 1).

Concerning the position of the oxime group in 2PAM, the *anti* isomer of *meta*-2PAM presented a slightly better docking energy value than *para* and *ortho*-2PAM isomers but similar H-bond value to *ortho*-2PAM. However the best result among the 2PAM *syn* isomers was observed for *ortho*-2PAM.

The presence of these amino acids with 2PAM, 2PAM1 and 2PAM2 compounds in *anti* and *syn* conformations, suggests that they have a direct influence on the mechanism of reactivation of *Hss*AChE inhibited by the organophosphorus agent Tabun.

Finally, analysis of the effect of the *syn-anti* isomery on the oximes' affinities for the *Hss*AChE active site showed that for 10 of the 16 oximes studied (62.5%), the *anti* isomers presented better docking values than the *syn* isomers. For the H-bonds energy values the result pointed to 50%.

Reaction

The kinetics of the AChE reactivation process is believed to occur in two steps: (1) the association of the oxime to the inhibited AChE and (2) the reactivation of AChE by the leaving of the oxime complexed to the neurotoxic agent (Fig. 1). The process of kinetic reactivation of AChE could be illustrated by Equation 1:

$$EI + Ox \underset{}{\overset{K_R}{\rightleftharpoons}} EIOx \xrightarrow{k_R} E + I\text{-}Ox \qquad \text{Eq. 1}$$

Where EI is the organophosphate-inhibited enzyme, Ox is the reactivator (oxime), E is the

Table 1. **H-bond energy values in kcal/mol obtained and main aminoacid residues interacting with the ligands.**

Oxime	*Anti*	*Syn*	Oxime	*Anti*	*Syn*
1	–7.2209 Phe291 (1) Arg292 (2) Ser289 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–10.7584 Phe291 (1) Arg292 (4) Ser294 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	8	–12.0939 Trp282 (1) Ser294 (1) Arg292 (3) Glu281 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–3.9584 Tyr 120 (1) Ser294 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)
2	–10.3404 Glu281 (1) Arg292 (3) Trp282 (1) Ser294 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–8.6853 Trp282 (1) Tyr68 (1) Arg292 (3) Ser 294 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	9	–7.9259 Tyr68 (1) Ser294 (1) Arg292 (2) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	0.0528 Trp282 (2) Ser294 (1) Glu281 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)
3	–7.7793 Trp282 (1) Ser289 (1) Arg292 (3) Ser294 (3) Glu281 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–7.7927 Trp282 (1) Ser289 (1) Arg292 (3) Ser294 (3) Glu281 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	10	–9.7289 Asp70 (1) Tyr120 (1) Ser294 (2) Gly118 (1) Tyr129 (1) Glu198 (1)	–10.7966 Arg292 (3) Ser294 (2) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)
4	–6.7650 Asp70 (1) Phe291 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–9.7872 Tyr120 (2) Ser294 (2) Gly118 (1) Tyr129 (1) Glu198 (1)	2PAM	–5.9830 Trp282 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–5.4829 Trp282 (1) Arg292 (3) Ser294 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)
5	–7.4288 Asp70 (1) Trp282 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–1.7457 Trp282 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	2PAM1	–1.6366 Trp282 (1) Ser289 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	3.4180 Trp282 (1) Arg292 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)

(Continues)

(Continues)

Oxime	*Anti*	*Syn*	Oxime	*Anti*	*Syn*
6	–11.0145 Asp70 (2) Ser294 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–8.1143 Trp282 (1) Ser294 (3) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	2PAM2	–5.7507 Trp282 (1) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–5.8037 Trp282 (2) Arg292 (2) Ser294 (1) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)
7	–9.0249 Trp282 (2) Ser294 (2) Arg292 (2) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)	–7.3808 Trp282 (1) Ser294 (2) Arg292 (3) Gly118 (1) Tyr120 (1) Tyr129 (1) Glu198 (1)			

Oxime	*anti-anti*	*syn-syn*	*anti-syn*
TMB4	–10.4772 Ala200 (1) Ser199 (1) Gly118 (1) Tyr120 (1) Ser294 (1) Glu281 (1) Gly118 (1) Tyr129 (1) Glu198 (1)	–5.0099 Tyr120 (1) Ser294 (3) Gly118 (1) Tyr129 (1) Glu198 (1)	–9.2889 Gly118 (2) Tyr120 (1) Ser294 (3) Gly118 (1) Tyr129 (1) Glu198 (1)
Obidoxime	12.3490 Ser294 (2) His443 (1) Tyr120 (1) Glu198 (1) Glu281 (1) Ser199 (1) Gly118 (1) Tyr129 (1)	–9.1658 Tyr68 (1) Tyr120 (2) Gly117 (1) Gly118 (1) Ser199 (1) Tyr129 (1) Glu198 (1)	–15.5061 Ser294 (4) Gly118 (1) Tyr120 (3) Ser199 (1) Tyr129 (1) Glu198 (1)

reactivated enzyme, EIOx is the intermediate complex and I-Ox is the product. K_R and k_R are the dissociation constants, which represent the affinity of oximes for tabun-inhibited AChE, and the rate constant for the decomposition of the stable enzyme-inhibitor-reactivator complex, respectively.

Nowadays, despite the recent efforts at both theoretical and experimental levels to elucidate the reaction mechanism involved in the reactivation process, some relevant facts, such as the influence of the oxime's isomery, still need to be clarified. Fig. 7 displays the proposed reaction mechanism considering 2PAM with *syn* and *anti* conformation.

2PAM was selected for the mechanism study due to experimental evidence, which suggest that this oxime could be used as a lead compound in order to propose structures of new oximes, such as 2PAM1 and 2PAM2.

The *syn* and *anti* conformations present the hydroxyl group close to the phosphate group of tabun, with distance values around 3.14Å and 3.24Å respectively (Fig. 8). For 2PAM1 and 2PAM2, similar distances were obtained for amino acid residues from the protein, favouring then, the reaction process.

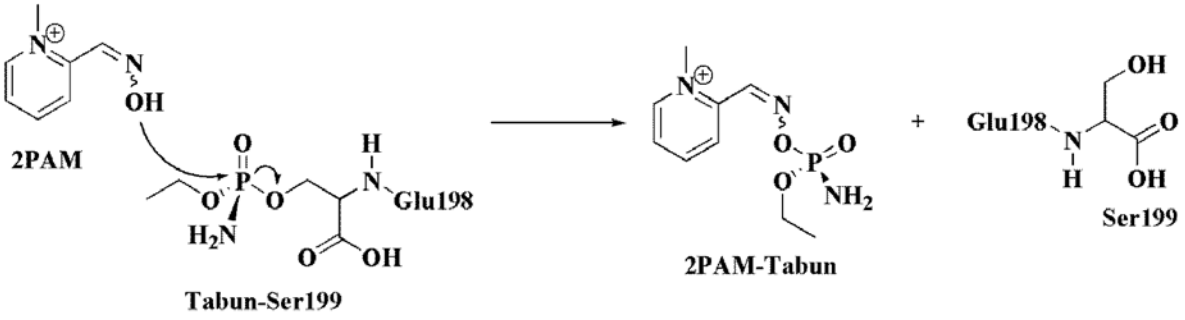

Fig. 7. **Scheme of the reactivation mechanism of the acetylcholinesterase enzyme.**

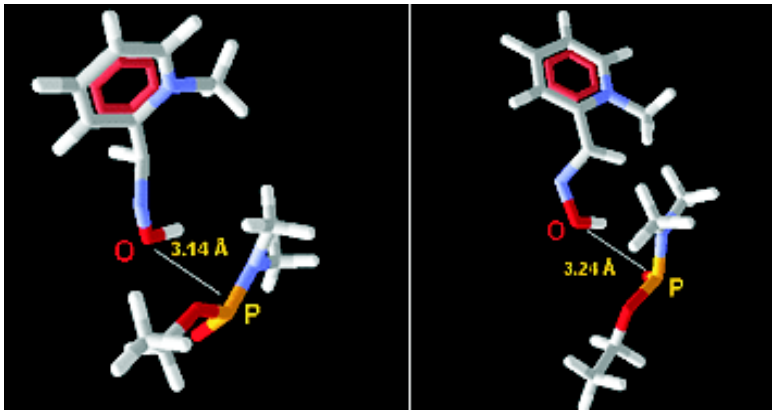

Fig. 8. **Compound 2PAM in the *anti* and *syn* conformations, respectively.**

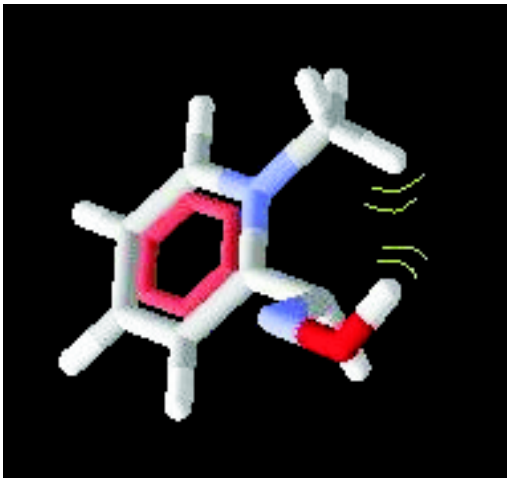

Fig. 9. **Intermolecular steric effect in the compound *anti*-2PAM.**

From our theoretical calculations of the catalytic mechanism, we obtained the relative activation energy of the three isomers 2PAM, 2PAM1 and 2PAM2, in different conformations, *syn* and *anti*. Theoretical data from Table 2 show that 2PAM and 2PAM2 in the *syn* conformation perform lower activation energy values than the compounds in *anti* conformations. This means that those conformations revealed a lower energetic barrier for the reaction pathway.

The lower thermodynamic stability of the 2PAM isomers with *anti* conformation, can be rationalized due to the lower number of hydrogen interactions with the residues close to the active site (Trp282, Arg292, Gly118, Tyr120, Tyr129, Glu198). Regarding the compounds in the *syn* conformation (Trp282, Arg292, Ser294, Gly118, Tyr120, Tyr129, Glu198), we can observe a lower stability inside the active site, suggesting a less stable transition state (higher activation energy).

Table 2. **Relative activation energy values of the studied oximes.**

Compounds	$\Delta\Delta E^{\#}$ (kcal mol^{-1})
2PAM *syn*	31.98
2PAM *anti*	34.05
2PAM1 *syn*	10.30
2PAM1 *anti*	0.00
2PAM2 *syn*	23.88
2PAM2 *anti*	32.48

Turning now to the 2PAM1, our theoretical data point out that the *syn* conformation presents a steric hindrance between the aromatic hydrogen and the hydroxyl group in the same compound. The same scenario did not occur with 2PAM1 in the *anti* conformation, leaving the hydroxyl group free to interact with the phosphate group of the inhibitor. This molecule, then, possesses significant internal degrees of freedom, leading consequently to a lower activation energy value for the compound in the *anti* conformation.

Besides *anti* and *syn* conformations, the *ortho*, *meta* and *para* substituent orientations in the compounds, can also affect the transition state stability. In this way, we noticed that the compounds 2PAM and 2PAM2 have a methyl group in *ortho*-orientation, generating a higher steric hindrance with the hydroxyl group in the *anti* conformation, resulting in a higher activation energy value in relation to all other compounds, as reported in Fig. 9 and Table 2.

CONCLUSION

From the results obtained and discussed here it is possible to conclude that: 1) oximes derived from pyridine imidazol bicycled systems are worth synthesizing and testing *in vitro* as *Hss*AChE reactivators and are expected to present similar experimental results as TMB-4; 2) the position of the oxime group as substituent, in the six or the five membered ring, on these molecules seems not to have a determinant influence on their affinities for the *Hss*AChE active site; 3) *meta*-2PAM should be considered further as an *Hss*AChE reactivator in experimental studies and 4) it is important, also, to consider a deeper investigation of the influence of the *anti* isomers in experimental studies of oximes as *Hss*AChE reactivators.

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