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 (HssAChE) 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 HssAChE active site are better than pralidoxime’s and similar to trimedoxime’s; the meta-pralidoxime could have more affinity for the HssAChE active site and the oximes’ anti isomers could present slightly better affinities for the HssAChE 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 phosphorylated 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.
1975, Castro and Figueroa-Villar 2002, Bartosova et
2007, Worek et al. 2007, Delfino and Figueroa-Villar
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 HssAChE 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
influenced the reactivation 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 HssAChE
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 HssAChE active site than para and
ortho-2-PAM, and that 62.5% of the anti isomers
presented slightly better affinities for the HssAChE
active site when compared to the syn isomers.

MATERIALS AND METHODS
Docking energy calculations
The structure of the HssAChE 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 HssAChE 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 HssAChE 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 HssAChE active site was
calculated by MVD® as having 949.696 Å³. 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 HssAChE active site and, also, the
amino acid residues involved in H-bonds with them.
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.

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 HssAChE inhibited by the organophosphorus agent Tabun.

Finally, analysis of the effect of the syn-anti isomery on the oximes’ affinities for the HssAChE 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:

\[
K_R \quad k_R \\
EI + Ox \leftrightarrow EI\text{Ox} \rightarrow E + I-Ox
\]

Eq. 1

Where EI is the organophosphate-inhibited enzyme, Ox is the reactivator (oxime), E is the...
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<tr>
<th>Oxime</th>
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<th>Syn</th>
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<th>Anti</th>
<th>Syn</th>
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</table>

(Continues)
reactivated enzyme, EIOx is the intermediate complex and I-Ox is the product. \( K_a \) 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.
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.

<table>
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<th>Compounds</th>
<th>ΔΔE° (kcal mol⁻¹)</th>
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<tr>
<td>2PAM syn</td>
<td>31.98</td>
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<tr>
<td>2PAM anti</td>
<td>34.05</td>
</tr>
<tr>
<td>2PAM1 syn</td>
<td>10.30</td>
</tr>
<tr>
<td>2PAM1 anti</td>
<td>0.00</td>
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<tr>
<td>2PAM2 syn</td>
<td>23.88</td>
</tr>
<tr>
<td>2PAM2 anti</td>
<td>32.48</td>
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</tbody>
</table>

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 HssAChE 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 HssAChE active site; 3) meta-2PAM should be considered further as an HssAChE 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 HssAChE reactivators.

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