

## The Length Dependent Activity of Oximes on Reactivation of Tabun Inhibited Acetylcholinesterase; A Theoretical Study

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In this work, the effect of ligand length on interaction energy of six oximes, A [2-(hydroxyimino)-N-((1-(2-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)ethyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide], B [2-(hydroxyimino)-N-((1-(3-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)propyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide], C [2-(hydroxyimino)-N-((1-(4-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)butyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide], D [2-(hydroxyimino)-N-((1-(5-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)pentyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide], E [2-(hydroxyimino)-N-((1-(6-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)hexyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide] and F [2-(hydroxyimino)-N-((1-(7-(2-((hydroxyimino)methyl)-1H-imidazol-1-yl)heptyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide] with tabun inhibited acetylcholinesterase (t-AChE) is investigated by using docking methods. The results show that the length size of ligands, due to changing the position of interactive groups of oximes in active site of enzyme, can regulate the oxime-enzyme interaction energy for better reactivation of inhibited cholinesterases by oximes. Indeed, increment of oxime-enzyme interaction energy has paradoxical effects; *i.e.*, increasing the oxime affinity toward t-AChE, in one hand, and decreasing the rate of the oxime access to phosphorus atom within tabun binding to the acetylcholinesterase active site, on the other hand. The obtained results show that to design an oxime as a drug, the length of oxime should be chosen in a size that the interaction of oxime and enzyme is neither too weak to couple, nor too strong to limit oxime mobility in reactivation t-AChE after coupling.

**Keywords:** Acetylcholinesterase, Oximes, Tabun

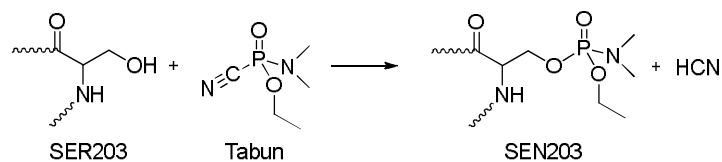
### INTRODUCTION

The highly toxicity of organophosphate nerve agents (OPNAs) is mostly due to their inhibitory role of Cholinesterase enzymes. OPNAs such as the methylfluorophosphonate agent (*e.g.* sarin, cyclosarin and soman), the cyanophosphoramidate agent (*e.g.* tabun) and the methylphosphothioate agents, *etc.* exhibit their toxicity by binding the serine residue to the active site of acetylcholinesterase (AChE) through phosphorylation hydroxyl group of serine [1,2]. These compounds illustrate a range of effects in AChE inhibition. AChE is one of the cholinesterases that quickly hydrolyzes the neurotransmitter acetylcholine into acetate and choline and terminates

cholinergic neurotransmission in the chemical synapses of the central and peripheral nervous systems [3-5]. Inhibition of AChE causes aggregation of acetylcholine that leads to respiratory arrest and to death eventually [6].

One of the strategies for treatment of OPNAs poisoning is using oximes. Diverse oximes were developed as therapeutic agents for reactivation inhibited cholinesterases [7-9]. Common reactivators, quaternary pyridinium aldoximes, used to the reactivation of inhibited AChE are limited to the peripheral tissues. These compounds such as Pralidoxime, Obidoxime, Asoxime, *etc.* have positive charge, so they cannot traverse the blood brain barrier (BBB) and reach the central nervous system (CNS). Therefore, more than one hundred uncharged oximes including a tertiary amine or imidazole protonable functional groups were designed that can traverse the BBB

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*Scheme 1.* Inhibition of SER203 by tabun and SEN203 formation

[10]. To find the more efficient reactivator for AChE and BChE (Buthylcholinesterase) inhibited with tabun in the CNS, 29 diverse uncharged oximes were studied in an experimental work and it was shown that the geometry of oximes plays an important role in an efficient reactivation [11]. In this study, we chose six ligands of oximes, RS182A and RS184A, and four other ligands which are different in number of CH<sub>2</sub> between imidazole and triazole rings (ligands with various lengths; A, B, C, D, E and F ligands; Fig. 2) to investigate the effect of the length of ligands in their activity as reactivator for tabun-inhibited AChE (t-AChE) in a computational method.

## COMPUTATIONAL DETAILS

### Protein

The crystal structure of human acetylcholinesterase inhibited by aged tabun and complexed with fasciculin-II (PDB code 2X8B) was obtained using X-ray Diffraction, downloaded from RCSB Protein Data Bank [12]. This structure left some missing residues including the amino acids Pro259, Gly260, Gly261, The262, Gly263 and Gly264. After adding and optimizing the missing residues by using Molegro Virtual Docker (MVD) [13], the missing ethyl of tabun was added and fasciculin-II coordinates was removed. Furthermore, the pdb file of AChE was changed into mol2 file with AMBER ff12SB charge for standard residues and AM1-BCC charge for other residues by using UCSF chimera v1.8.1 package [14].

### Ligands

All ligands were optimized at the HF/6-31G\* level using GAUSSIAN 09 package [15]. Then, a mol2 file with AM1-BCC charge using UCSF chimera was prepared for each ligand.

### The Simulation Details

Protein-Ligand docking was performed by using MVD.

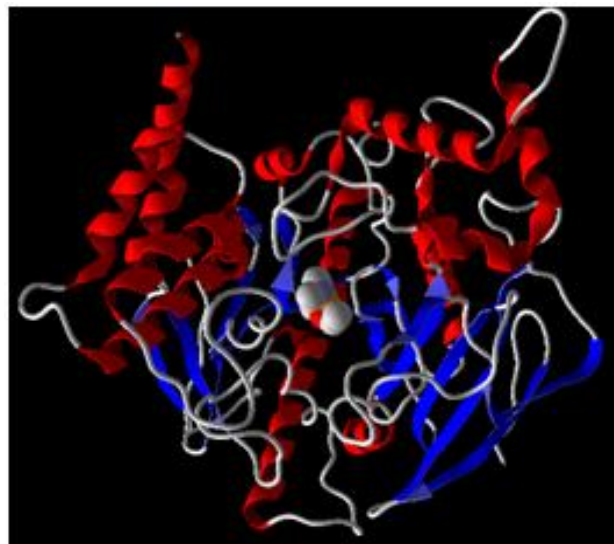
Therefore, the mol2 files of protein and ligand imported to MVD. In order to maintain more proper partial charges determined by UCSF chimera and to avoid partial charge calculation by MVD, we set 'Assign charge' to 'Never' option in 'Preparation' panel of 'Import Molecules' window. In addition the non-standard residue of protein, SEN, was defined as a cofactor.

Due to stochastically nature of searching algorithm in ligand-protein docking, in three docking implementations, 300 runs (about 100 runs in each implementation) were conducted in MolDock Optimizer algorithm for each individual ligand and 150 poses (ligand conformations) with higher interaction energy were returned for each one. In any implementation, we calculated mean interaction energy (MIE) of poses. Each pose at the active site of protein was investigated at ligand map window again and the various interaction energies ( $E_{\text{Pair}}$ ,  $E_{\text{Elec}}$ , ...) between any atom of ligand and protein were calculated. Hence, we could calculate interaction energy of any part of the ligand with protein.

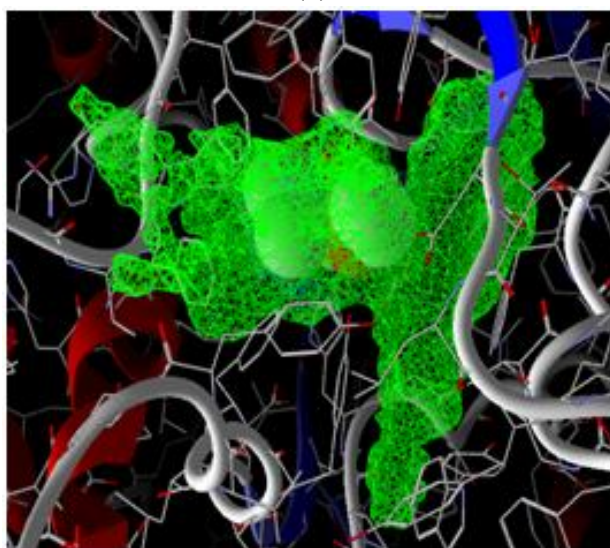
## RESULTS AND DISCUSSIONS

The residue SER203 in active site of AChE plays a basic role in acetylcholine hydrolysis. Binding of OPNAs as tabun to SER203 causes to lose its capability (Scheme 1). The secondary structure of tabun-inhibited AChE (t-AChE) and the cavity of its blocked active site calculated by MVD are shown in Fig. 1.

We chose six structurally optimized oximes, A to F with different lengths (Fig. 2), and investigated the effect of the number of methylene group (-CH<sub>2</sub>-) on the reactivity of oxime molecules in a computational method. The reactivation of t-AChE by second and third ligands, RS184A and RS182A, were compared together in an experimental method previously [11] and the results indicated larger  $k_{\text{obs}}$  for RS182A.



(a)



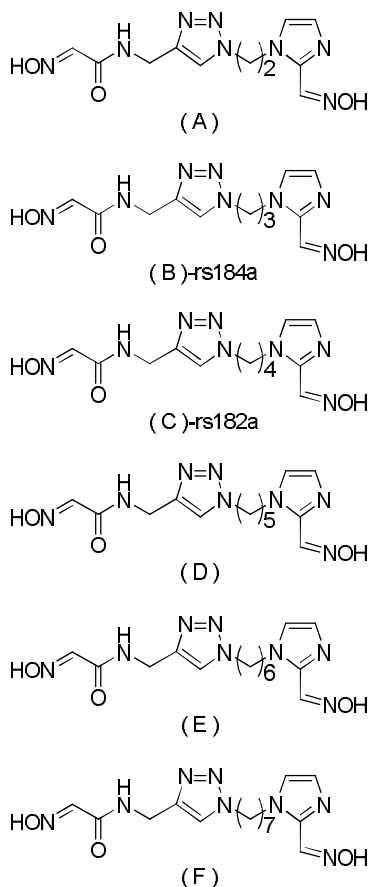
(b)

**Fig. 1.** (a) The secondary structure of t-AChE in which SEN residue is illustrated in spacefill view. (b) The cavity (green mesh) of the t-AChE blocked active site was calculated by MVD with  $463.75 \text{ \AA}^3$ .

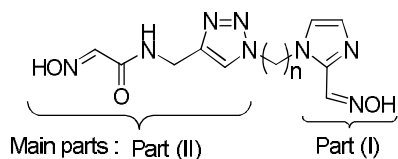
Herein, reactivation of t-AChE by six above mentioned oximes was assessed in a docking method. The results of the ligands and protein docking as mean interaction energies (MIEs) are shown in Table 1. Owing to existence of OH and NH groups in oximes structures and similar groups in enzyme, a large contribution of interaction energy related to hydrogen bonding. Increment of number of  $\text{CH}_2$  between

imidazole and triazole rings caused a decrease in MIE from ligand A to ligand C and an increase in MIE from ligand D to ligand F. It seems that changing of oximes' length regulates the interaction of oximes with the cavity inner wall of the enzyme.

According to the kinetics of the inhibited AChE reactivation, the process is comprised of two steps: first, the



**Fig. 2.** Structure of oximes with different number of CH<sub>2</sub> between imidazole and triazole rings.

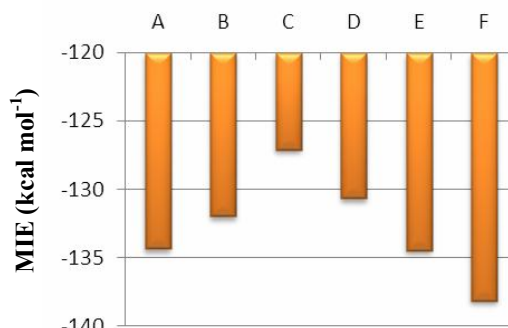


**Fig. 3.** Main parts of ligands (whole ligand expects CH<sub>2</sub> chain).

coupling of oxime and enzyme, and secondly, reactivation of inhibited AChE that is shown in the following Scheme:



in which EP is inhibited enzyme, OX is oxime, EP.OX is



**Diagram 1.** Comparing interaction energies between Oximes and t- AChE.

oxime and enzyme complex and E and POX are products [16].  $K_D$  is the dissociation constant and  $k_r$  is the reactivation first order rate constant.  $K_D$  represents the affinity of oximes to enzyme. The stronger binding between oximes and enzyme causes the larger  $K_D$ , but it can reduce  $k_r$ . The stronger binding reduces the mobility of oxime and delays the reactivation of inhibited enzyme.

As noted previously [11] the ligand C (RS182A) is better than ligand B (RS184A) in reactivation of t-AChE, whereas interaction energy of ligand B is significantly larger than that of ligand C. In other words, increasing of chain length can play a significant role in biological activity of ligands. So, extra attraction can reduce dynamism of the ligands leading to decreasing their activity.

Although, ligand and protein interactions are not uniquely determinative factors in reactivation of inhibited AChE by oximes, they should be evaluated as an effective factor that can be kept in an appropriate extent.

According to the Diagram 1, it can presumably be concluded that the reactivity of ligands A, E and F should be less than the reactivity of ligand C. This result is more valid for ligands E and F owing to longer chains can decrease their dynamism and their displacement rate. The interaction energy of ligand D is intermediate of interaction energies of ligands B and C, so, interaction of ligand D with protein may be more appropriate than ligand C. Interaction energy of any pose and protein was calculated in 'ligand map' window to extract MIEs of parts (I) and (II) of poses (Fig. 3) with protein. These values are shown in Table 2.

According to Table 2, increasing the length of ligand

**Table 1.** The MIEs of Oximes with t-AChE in Three Runs and their Averages

Ligands	MIE (kcal mol <sup>-1</sup> )			
	Run1	Run2	Run3	Average
A	-134.35	-135.53	-133.268	-134.38
B(rs184a)	-130.55	-133.74	-131.701	-132
C(rs182a)	-127.07	-126.37	-127.914	-127.12
D	-130.66	-130.84	-130.601	-130.7
E	-133.75	-134.48	-135.275	-134.5
F	-138.69	-138.87	-136.97	-138.18

**Table 2.** The MIEs of Main part of Oximes with Enzyme Calculated by the Ligand Map

Ligands	MIEs of main part (kcal mol <sup>-1</sup> )		
	Total	Part (I)	Part (II)
A	-126.695	-74.757	-51.937
B(rs184a)	-119.025	-71.682	-47.343
C(rs182a)	-105.440	-58.013	-47.427
D	-105.138	-59.928	-45.210
E	-101.916	-60.056	-41.860
F	-100.471	-59.387	-41.084

may take distance its main parts from their proper location. This effect can reduce the contribution of these parts in MIE. However, increase in the number of methylene groups in ligands D, E and F increases the contribution of  $-(CH_2)_n$ -chain in the total interaction. MIE of part I for ligands A and B is considerably more than the same part for other ligands. This indicates that the size of ligands A and B allows them to be located in appropriate positions with the strongest

interaction, while MIE of part I for other ligands are less and do not show considerable changes with increment of  $CH_2$  group.

## CONCLUSIONS

The length of oximes plays an important role in the reactivation of inhibited cholinesterases. This factor can

regulate the intensity of oximes interaction with enzyme due to changing the position of interactive groups of oximes in active site of enzyme. As shown for oximes B and C, an intense interaction reduces the activity of oximes because of decreasing the mobility of molecules and reduces the rate of t-AChE reactivation, as the increment of the oxime size can decrease mobility due to limited volume of the cavity in active site of enzyme. The type and the number of interactive groups of oximes are noticeable determinants for choosing the size of oximes. To design an oxime as a drug, the length of oxime should be chosen in a size that the interaction of oxime and enzyme is neither too weak to couple, nor too strong to limit oxime mobility in reactivation t-AChE after coupling.

## ACKNOWLEDGMENTS

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