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Acetylcholinesterase reactivation potential of a novel oxime: *in silico* and *in vitro* studies

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Abstract

Organophosphate (OP) pesticide poisoning is a major global health issue, endangering millions of lives. These compounds cause toxicity primarily by inhibiting cholinesterase enzymes. The standard treatment involves atropine, with oximes used as adjunct therapy to reactivate acetylcholinesterase. Pralidoxime (PAM) is the first and only oxime approved by the U.S. Food and Drug Administration (FDA), yet its clinical effectiveness remains disputed. The ongoing controversy over the efficacy of existing oximes underscores the urgent need to develop more potent and widely available alternatives. This study aimed to evaluate the reactivation potential of a novel experimental oxime, K727, against acetylcholinesterase (AChE) irreversibly inhibited by organophosphates (OPs). Its efficacy was compared with pralidoxime (PAM) against methyl paraoxon (POX)-inhibited AChE using both *in silico* and *in vitro* approaches. The *in vitro* assessment involved measuring red blood cell AChE activity in sheep by Ellman's method. The in-silico analysis for human AChE using molecular docking, revealed that K727 exhibited the highest binding affinity to the receptor, followed by PAM. However, *in vitro* studies revealed higher intrinsic toxicity and reactivate AChE at very lower concentration but did not reach the highest efficacy in comparison to PAM which was effective at higher doses. Further studies with other animal models and human blood are suggested.

Key words: Organophosphates; Oxime; Paraoxon; Pralidoxime; K727

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1. Introduction

Organophosphorus (OP) compounds, a diverse class of potent poisons, have been known since the 18th century. The first OP synthesized was triethyl pyrophosphate (TEPP) by Lassaigne in 1820, followed by tetraethyl pyrophosphate by de Clermont in 1854. Nearly a century later, German chemist Gerhard Schrader, while researching new insecticides, identified TEPP's insecticidal properties. Schrader and his colleagues subsequently developed several highly toxic OP compounds, including tabun, sarin, soman, and parathion (Chambers & Levi, 2013). The primary toxicological effect of organophosphate (OP) poisoning is the inhibition of acetylcholinesterase (AChE) within the nervous system. OP compounds exert their toxicity by irreversibly binding to the enzyme's catalytic site, thereby reducing cholinesterase (ChE) activity. In clinical practice, oximes are employed to reactivate inhibited AChE. Pralidoxime, introduced in the mid-20th century, was the first pyridinium oxime to be used therapeutically (Wilson & Ginsburg, 1955a).

The toxicity of organophosphates (OPs) primarily arises from their inhibition of acetylcholinesterase (AChE), a serine hydrolase responsible for hydrolyzing the neurotransmitter acetylcholine (ACh). ACh is a key chemical messenger that mediates numerous physiological processes in both the peripheral and central nervous systems. AChE is an exceptionally rapid enzyme, operating near the catalytic speed limit of biological systems, with a turnover rate (k_cat) exceeding 10^4 s^{-1} and completing the hydrolysis of ACh in less than a millisecond (Mercey et al., 2012). The active site of AChE contains a catalytic triad of serine, histidine, and glutamate residues, which mediate ACh hydrolysis through a two-step mechanism: Acylation and diacylation**.** OP inhibition closely mimics the natural acylation step. The nucleophilic serine instead attacks the phosphorus atom of the OP, displacing the leaving group and forming a stable phosphyl-serine complex. Unlike the acetylated intermediate, this adduct is hydrolyzed extremely slowly—over hours to days—due to steric hindrance that prevents efficient activation of water molecules (Mercey et al., 2012).

Oximes are essential adjuncts in the treatment of organophosphate (OP) poisoning. The first oxime was synthesized in 1950, nearly a century after the discovery of the earliest OP compound (Wilson & Ginsburg, 1955). Pralidoxime—the first clinically available oxime and the only one approved by the U.S. Food and Drug Administration (FDA) for OP poisoning—was introduced in Japan in 1957 for the treatment of parathion poisoning (Namba & Hiraki, 1958).

Despite decades of use, there remains a scarcity of published clinical trials evaluating oxime efficacy, including optimal dosing regimens, choice of oxime, and clinical outcomes. A broad-spectrum oxime effective against structurally diverse OP compounds is still needed. Evidence suggests that obidoxime may be more effective than pralidoxime for pesticide- or insecticide-related OP poisoning, whereas HI-6 is particularly suited for nerve agent exposure. Considerable efforts have been made to develop oximes with enhanced antidotal activity against different classes of OP acetylcholinesterase inhibitors, leading to structural modifications and improved pharmacological properties (Jokanović & Stojiljković, 2006)).

Notably, research led by Kamil Kuca and Kamil Musilek in the Czech Republic has yielded a series of so-called K-oximes, initially designed to target tabun and other OP nerve agents (Kassa et al., 2007)**.** Subsequent studies expanded their evaluation to pesticide-induced AChE inhibition, showing promising reactivation potential. Since 2003, over 200 structurally distinct K-oximes have been synthesized, with K-027 emerging as the most promising candidate (J. Kassa et al., 2008; (Peter & Cherian, 2000). The present study was undertaken with one of the K-oxime in comparison to standard oxime, pralidoxime (PAM).

2. Methodology

Molecular docking soft wares

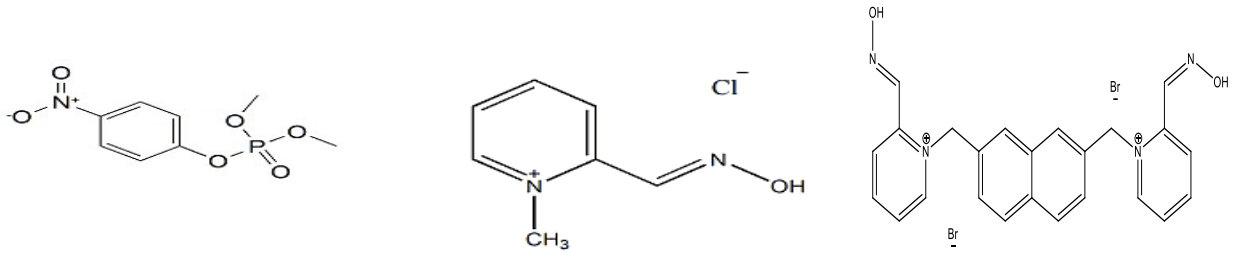
In molecular docking three-dimensional pharmacophore models were constructed to predict the binding affinity of Paraoxon-methyl, Pralidoxime, and K727. Chemdraw ultra 8.0 was used to draw structures and Autodock 4.1 was used for molecular docking,USF Chimera (for the interactive visualization and analysis of molecular structures and Pymol for 3d visualization and editing of organic molecules.

LogP determination software

The LogP, or octanol-water partition coefficient, is a measurement of a molecule's hydrophilicity or hydrophobicity. It shows how easily an analyte partitions between the aqueous and organic phases. A more polar, hydrophilic chemical will have a lower logP (even negative), indicating that it prefers to "reside" in the aqueous phase and vice versa. Two sources have been employed in the present study to calculate Log*P* of the oximes. Chemdraw, Pallas 3413 software based on the Pro log*P* module of the Pallas system. (A free web tool used to evaluate and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery).

Experimental Chemicals

Paraoxon-methyl, also known as O,O-dimethyl O-(4-nitrophenyl) phosphate or dimethyl (p-nitrophenyl) phosphate, is an organophosphate and the active metabolite of the insecticide parathion; in this study, Paraoxon-methyl (Sigma-Aldrich, Cat. No. D9286, CAS No. 950-35-6) was kindly provided by Prof. Georg Petroian (Florida International University, USA). Pralidoxime chloride, an organic pyridinium salt (C7H9N2OCl), functions as a reactivator of phosphorylated acetylcholinesterase inhibited by organophosphates by breaking the enzyme–agent bond, thereby restoring normal enzyme activity; purchased from Sigma-Aldrich Chemie (Germany), it is an odorless, water-soluble, crystalline powder with a melting point of 215–225°C and is typically administered with atropine and diazepam for organophosphate poisoning. K-727, a bisquaternary symmetric oxime containing two functional groups, was synthesized at the Department of Toxicology, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic, by Kamil Musilek and colleagues, and obtained through the courtesy of Dr. Kamil Kuca and Dr. Kamil Musilek.



a. Paraoxon **b. Pralidoxime** **c. K727**

Figure 1: Structures of Paraoxon-methyl, Pralidoxime Chloride and K727

Table 1: Physico-chemical properties of Paraoxon-ethyl and tested oximes

Properties	Paraoxon-methyl	Pralidoxime	K727
Molecular weight	275.20 g/mol	172.6 g/mol	558.27g/mol
Molecular Formula	$C_8H_{10}NO_6P$	$C_7H_9N_2O.Cl$	….
Physical State	Reddish-yellow Oily Liquid	Powder	Powder
Solubility	Organic Solvents	Organic Solvents	Organic Solvents
Density at 25 °C	1.383 g/cm3	0.94 g/ml	NA
Melting Point	0-4° C	NA	NA
Boiling Point	315°C at 760mmHg	NA	NA

Molecular Docking Studies

Molecular interaction between oximes/Organophosphates with human and was explored by using molecular docking technique.

Construction of the models

The protein structures were taken from protein data bank (PDB) and UniProt.The X-ray crystal structure of human AChE, PDB entry 4PQE, having resolution of 2.90 Å and human BChE PDB entry 4P0P, having resolution of 2.30 Å. The X-ray crystal structure of sheep AChE, UniProt entry P23795 and sheep BChE UniProt entry P32749, were used for molecular docking studies as shown in the Tables below. The selection criteria for protein 3D structures included

1) Non-covalent protein-ligand complex,

2) The crystallographic resolution was less than or equal to 2.5Å

3) No or minimum missing residues

4) Known experimental binding data.

Table 2: Target proteins obtained from PDB.

Name of the target protein (Receptor)	Source	PDB ID	STRUCTURE OF TARGET PROTEINS	M.W
Acetylcholinesterase	Homo sapiens	4PQE		59.58 kDA

Table 3: List of Target proteins obtained from UniProt.

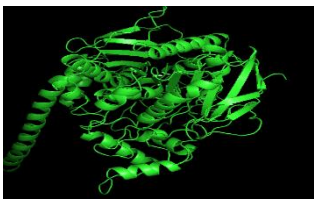
Name of the target protein (Receptor)	Source	UniProt ID	STRUCTURE OF TARGET PROTEINS
Acetylcholinesterase	Bos Taurus (bovine)	P23795	

Table 4: Determination of Binding pockets residues of the target receptors: Binding pockets of receptors were determined through literature, using CASTp v 3.0 and PyMOL.

Amino acid Binding pockets residues of the receptor		
Sr no.	Receptors	Amino acid binding pockets

1	4PQE (AChE)	Active site consists of catalytic triad (Ser203, His447, Glu334) oxyanion hole (Gly121, Gly122, Ala204) anionic subsite (Trp86, Tyr133, Glu202, Glu450, Gly448, Ile451) acyl binding pocket (Trp236, Phe295, Phe297, Phe338), peripheral anionic subsite (Asp74, Tyr124, Ser125, Trp286, Tyr337, Tyr341) omega loop (Thr83, Asn87, Pro88) other residues: Cys69 , Cys96, Leu76, Gly342, Leu289
3	P23795 (AChE)	Active site consists of: Glu232,Pro476,His477,Ser233,Tyr163 Trp116,Tyr367,Gly151,Ser155,Phe368 Gly152,Tyr154 ,Tyr371,Phe325,Phe327

Molecular Docking

Molecular modeling investigations were carried out using an Autodock4 molecular docking program. The structure of Paraoxon-Methyl was retrieved from the chemical databases Drug Bank. While the structure of Oximes were sketched in ChemDraw followed by geometry optimization at RM1 methods in Gaussian 03 using GaussView. The final optimized structures were saved in PDB format. The PDB files of proteins and ligands were converted into PDBQT files using Autodock Tools.

***In silico* LogP Determination**

Log*P* of K727 was calculated by using chemdraw (in chemdraw the structure was sketched and Log*P* was determined by viewing chemical structure analysis) and pallas system (in which SMILES format) was used to find out LogP.

Table 5: Smiles file format used for Log*P* determination in Pallas system

Oximes	SMILES
K727	O/N=C/C1=CC=CC=[N+]1CC2=CC3=C(C=C2)C=CC(=C3)C[N+]4=C(C=CC=C4)\C=N/O

IC50 Of oximes and methyl paraoxon

IC50 is the methyl paraoxon concentration necessary to inhibit RBC AChE activity by 50% (IC50) of human was used according to (Lorke & Petroianu, 2019)

RBC-AChE measurement

The RBC-AChE was measured in diluted whole blood samples in the presence of the selective butyryl- cholinesterase inhibitor, ethopropazine, as previously described (Worek et al., 1999) assay which is based on Ellman's method, measures the reduction of dithiobis-nitrobenzoic acid (DTNB) to nitrobenzoate (TNB) by thiocholine, the product of acetylthiocholine hydrolysis (Ellman et al., 1961).

Table 6 Procedure for the determination of AChE activity

Chemicals	Concentrations	Volume
Phosphate Buffer	0.1 mol/l, pH7.4	2000µL
DTNB	10mmol/l	100 µL
Ethopropazine	6mmol/l	10 µL
Hemolysate	whole blood 1:100 (dilution)	1000 µL
Ach	28.4 mmol/l	50 µL
Phosphate Buffer	0.1 mol/l, pH7.4	2000µL
DTNB	10mmol/l	100 µL
Plasma	Undiluted	10 µL
BCh	28.4mmol/l	L

Statistical analysis

Statistical analysis and calculations were done using SPSS 19.0, USA by Graph Pad Prism 5.0, Graph Pad Software, Inc. USA and Microsoft Excel. Non-parametric Manwhitney test was used to determine the significance at $p < 0.05$.

3. Results

In silico studies

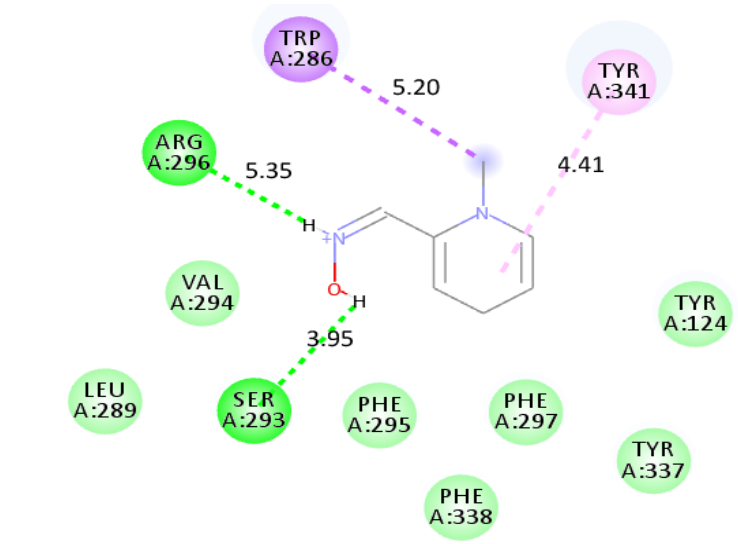
Molecular Docking Studies

To understand the ligand-protein interaction molecular docking was carried out against human AChE . For human AChE binding energy value of PAM found to be greater than Methyl POX inhibitor which shows that it has less binding affinity with that of receptor compared to inhibitor Methyl POX. Binding energy values of PAM was**:** -5.6 kcal/mol**.** K727 was found to have good binding affinity with binding energy value of -9.2.

Table 8 Binding energy of paraoxon methyl and novel oximes in blind docking against human RBC-AChE

Structures	Molecular weight g/mol	Binding Energies kcal/mol
Paraoxon methyl	275.20 g/mol	-6.2
PAM	172.6 g/mol	-5.6
K727	558.72 g/mol	-9.2

PAM



K727

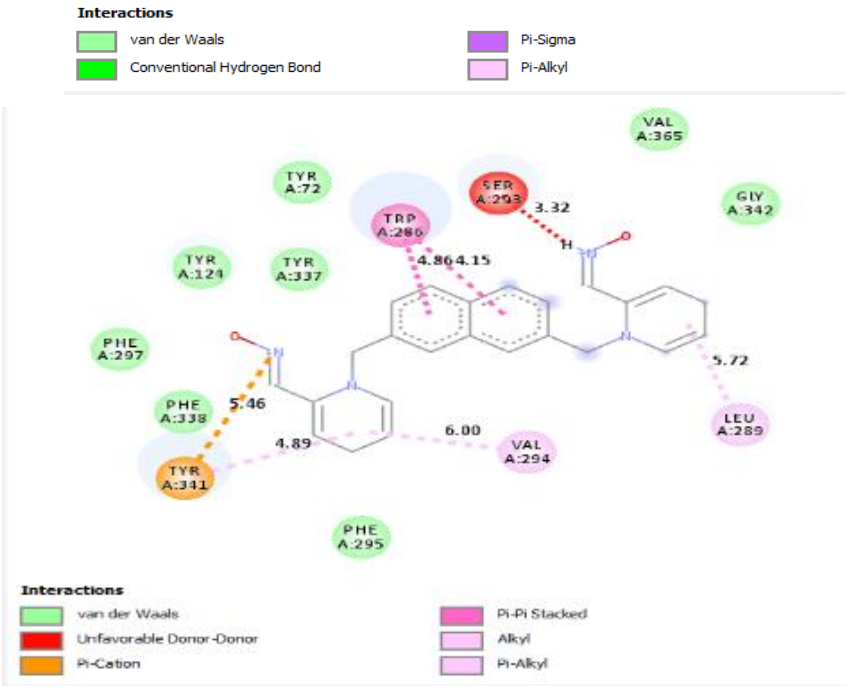


Figure 2: a and b Diagrammatic representation of the binding interaction (H-bonding and π-π interaction) between human Acetylcholinesterase and ligands.

Log*P* Calculation

The LogP, or octanol-water partition coefficient, is a measurement of a molecule's hydrophilicity or hydrophobicity. A more polar, hydrophilic chemical will have a lower logP (even negative), indicating that it prefers to "reside" in the aqueous phase and vice versa. Calculated Log*P* values are shown in Table 5. K727 possess greater log*P* value which is +3.28 indicating that it possesses higher permeability to BBB compared to PAM.

Determination of Sheep RBC-AChE activity

The efficacy of novel oxime K727, and one key oxime PAM pralidoxime was determined against IC_{50} of methyl POX. RBC- Acetylcholinesterase (RBC-AChE) was inhibited by IC_{50} of POX. The mean activity of RBC-AChE (mU/µmolHb) with no treatment (baseline) and after treatment with POX (≈IC_{50}) and subsequent-application of different concentrations of oximes is shown in **Table 3.18-3.29** alongwith descriptive statistics in all three studies. Dosage level of oximes was selected based on IC_{50} of the oximes. IC_{50} of oximes PAM is

reported earlier by (Lorke, Hasan, et al., 2008; Lorke, Nurulain, et al., 2008) while k727 was taken from (Arshad et al., 2018). Mean of the three studies, conducted to calculate the sheep RBC-AChE activity when co-applied with the four tested oximes and 70nM methyl Paraoxon is given below.

Table 9 LogP values of tested oximes

Name	log*P* Chemdraw	log*P* Pallas system
PAM	-2.31	-2.27
K727	+3.28	+3.89

Table 10 shows the activity of RBC-AChE (mU/µmolHb) by PAM (paralidoxime**)** at IC10 and IC5 which are 60µM and 120µM respectively. The mean RBC-AChE activity of PAM at different concentration in three different studies is shown in Table 10. % reactivation was best achieved by PAM at IC5 (120µM) compared to k727 which is 65.408% as shown in Table 3.65

Table 11 shows activity of RBC-AChE (mU/µmolHb) by K727. Less Enzyme activity was observed after the application of K727 oxime compared to all other oximes. The mean RBC-AChE activity of K727 at different concentration in three different studies is shown in Table 11. % reactivation was best achieved by k727at IC5 (0.2 µM) which is still less than all other tested oximes.

Table 10 Descriptive analysis of percent Inhibition of sheep RBC-AChE with IC50 of methyl paraoxon and subsequent application of IC5 and IC10 of oxime pralidoxime.

		N	Mean (%)	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Study 1	Methyl-POX	6	53.01	10.24	4.18	42.27	63.75
	PAM 60microM	4	17.13	21.69	10.84	-17.38	51.65
	PAM 120 microM	2	16.86	4.88	3.45	-26.98	60.70
Study 2	Methyl-POX	6	60.31	7.98	3.26	51.93	68.68
	PAM 60microM	5	36.24	14.47	6.47	18.27	54.21
	PAM 120 microM	4	17.97	8.85	4.43	3.88	32.05
Study 3	Methyl-POX	6	60.30	17.78	7.26	41.63	78.96
	PAM 60microM	6	32.49	9.09	3.71	22.94	42.03
	PAM 120 microM	6	15.16	9.68	3.95	5.00	25.32

Table 11 Descriptive analysis of percent Inhibition of sheep RBC-AChEwith IC50 of methyl paraoxon and subsequent application of IC5 and IC10 of oxime K727.

		N	Mean (%)	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
study 1	Methyl-POX	6	63.42	8.393	3.426	54.61	72.23
	K727 0.1microM	6	55.07	11.925	4.868	42.56	67.58
	k727 0.2 microM	6	35.80	27.222	11.113	7.23	64.37
study 2	Methyl-POX	6	63.42	8.393	3.426	54.61	72.23
	K727 0.1microM	6	50.17	4.408	1.800	45.54	54.79
	k727 0.2 microM	6	44.93	14.764	6.027	29.43	60.42
study3	Methyl-POX	6	63.42	8.393	3.426	54.61	72.23
	K727 0.1microM	6	53.58	14.869	6.070	37.97	69.18
	k727 0.2 microM	6	41.61	17.291	7.059	23.47	59.76

4. Discussion

Organophosphate (OP) poisoning, most often resulting from exposure to OP-based insecticides and pesticides, remains a major global health concern and one of the leading causes of chemical intoxication worldwide. Each year, an estimated 3 million cases of OP poisoning occur, leading to approximately 200,000 deaths. Despite decades of research, there has been little tangible progress in improving treatment outcomes. Current standard therapy includes the administration of atropine, oxime-based acetylcholinesterase reactivators such as pralidoxime, and diazepam, supported by general interventions like oxygen supplementation and intravenous fluids(Alozi & Rawas-Qalaji, 2020). Gastric decontamination using activated charcoal or similar methods has not been shown to provide significant benefit.

The continued risk of terrorist use of OP agents, as documented in past incidents (Okudera, 2002) underscores the urgent need for the development of more effective antidotes. OP pesticides act by phosphorylating the serine hydroxyl group of acetylcholinesterase (AChE), irreversibly inactivating the enzyme. This leads to the accumulation of acetylcholine at synapses, causing overstimulation of nicotinic and muscarinic receptors, which can ultimately result in death (Robb et al., 2025).

Over the past two decades, several strategies for managing OP poisoning have been proposed (Aman et al., 2021; Nahum et al., 2021; Nurulain et al., 2009; Poirier et al., 2021; Yesilbas et al., 2016, 2016). However, the combination of atropine—used to counteract muscarinic symptoms—and oximes—used to reactivate inhibited AChE—remains the most widely accepted and effective therapeutic approach. Atropine sulfate is approved by the U.S. FDA specifically for the management of organophosphate and muscarinic poisoning, whereas pralidoxime and other oximes serve to restore suppressed AChE activity Organophosphates encompass a diverse group of chemical compounds and are formed through esterification between phosphoric acid and alcohol. Currently, organophosphates have common applications in pesticides and herbicides, as well as nerve agents in chemical warfare. Therefore, most patients exposed to organophosphates typically encounter these compounds through the use of insecticides and herbicides. When introduced into the body, organophosphates inhibit the enzyme acetylcholinesterase (AChE), resulting in an overabundance of the neurotransmitter acetylcholine. This surplus of acetylcholine in the body manifests with cholinergic toxidrome, which includes effects on nicotinic and muscarinic receptors, as well as the central nervous system (CNS). The onset of symptoms, which varies

based on the specific compound, frequently occurs within minutes, and resolution can take several weeks. Although developed nations have experienced a decline in organophosphate poisoning cases due to stricter regulations on the use of these chemicals, developing countries have continued to grapple with clinical concerns related to this condition in recent years. Pesticides are frequently used as a means of self-harm due to their lethality and widespread availability in the developing world. Therefore, developing nations that heavily depend on agriculture and often have less stringent pesticide regulations result in the majority of cases of organophosphate toxicity. Research indicates that deliberate poisoning leads to a higher mortality rate than accidental exposure to these compounds. Respiratory failure resulting from bronchorrhea and bronchospasm is the leading cause of death in cases of organophosphate toxicity. Chronic toxicity and neurological complications, such as the intermediate syndrome, are also well documented. In industrial or developed nations, healthcare professionals must possess the capability to recognize this toxicity, given the potential for its utilization as a weapon in acts of warfare and terrorism. Antidotal therapy and comprehensive supportive care are necessary for the effective treatment of organophosphate toxicity to prevent morbidity and mortality. History of Organophosphates Use The first organophosphate insecticide was developed in the mid-1800s, but it only gained widespread usage after World War II. Initially, in the 1930s, these compounds were used as insecticides before finding application as neurotoxins by the German military. The organophosphate chemicals sarin and VX were utilized by the Japanese cult Aum Shinrikyo in 1994 and 1995, marking the initial reported instances of VX's use as a terrorist agent. In February 1997, the first reported murder involving VX occurred with the assassination of Kim Jong-nam at a Malaysian airport. In March 2018, the poisoning of Sergei and Yulia Skripal took place in England, leading to the hospitalization of a police officer who was also poisoned during this assassination attempt. The compound used in this event was the organophosphorus agent known as (Robb et al., 2025).

The aim of this study was to compare the efficacy and potency of the novel experimental oxime K727 with the standard oxime pralidoxime (PAM) in reactivating red blood cell acetylcholinesterase (RBC-AChE) inhibited by methyl paraoxon (POX). Both *in silico* and *in vitro* approaches were employed. The *in silico* analysis involved molecular docking to assess the binding affinities between oximes (ligands) and acetylcholinesterase receptors. The *in vitro* evaluation measured RBC-AChE activity in sheep blood following exposure to approximately 50% inhibitory doses of POX, with subsequent treatment using K727 or PAM. Enzyme activity was determined by Ellman's method to identify oximes with superior reactivation potential. Our *in silico* work revealed a good molecular interaction of K727 with receptor, predicting good efficacy but *in vitro* study showed pralidoxime as a better oxime than K727. The study concludes that *in silico* study is not sufficient to establish conclusion. However, further studies with other models are suggested for a tangible conclusion.

Conclusion and Recommendations

Organophosphate poisoning continues to pose a major global health threat, especially in regions with high agricultural pesticide use and limited regulation. Although oximes such as pralidoxime are widely used to restore acetylcholinesterase activity, there remains a need for improved therapeutic agents. In this study, the performance of a novel oxime, K727, was compared to that of pralidoxime. While molecular docking suggested that K727 had a strong binding affinity to acetylcholinesterase, experimental data from *in vitro* tests showed that pralidoxime was more effective in reactivating enzyme activity in red blood cells exposed to methyl paraoxon. These results highlight the gap between computational predictions and actual biological outcomes. Therefore, even though molecular docking revealed good molecular interaction the novel Oxime K727 was found to be less effective in reactivation of sheep RBC Acetylcholinesterase. Further studies with human RBC-AChE and other models like in vivo study is recommended or tangible

conclusion.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

Alozi, M., & Rawas-Qalaji, M. (2020). Treating organophosphates poisoning: Management challenges and potential solutions. *Critical Reviews in Toxicology*, *50*(9), 764–779. https://doi.org/10.1080/10408444.2020.1837069

Aman, S., Paul, S., & Chowdhury, F. R. (2021). Management of Organophosphorus Poisoning: Standard Treatment and Beyond. *Critical Care Clinics*, *37*(3), 673–686. https://doi.org/10.1016/j.ccc.2021.03.011

Arshad, M., Fatmi, M. Q., Musilek, K., Hussain, A., Kuca, K., Petroianu, G., Kalasz, H., & Nurulain, S. M. (2018). *In silico* and *in vitro* evaluation of two novel oximes (K378 and K727) in comparison to K-27 and pralidoxime against paraoxon-ethyl intoxication. *Toxicology Mechanisms and Methods*, *28*(1), 62–68. https://doi.org/10.1080/15376516.2017.1357777

Chambers, J. E., & Levi, P. E. (2013). Organophosphates Chemistry, Fate, and Effects: Chemistry, Fate, and Effects. Elsevier.

Ellman, G. L., Courtney, K. D., Andres, V., & Feather-Stone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*, 88–95.

Jokanović, M., & Stojiljković, M. P. (2006). Current understanding of the application of pyridinium oximes as cholinesterase reactivators in treatment of organophosphate poisoning. *European Journal of Pharmacology*, *553*(1), 10–17. https://doi.org/10.1016/j.ejphar.2006.09.054

Kassa, J., Kuca, K., Karasova, J., & Musilek, K. (2008). The Development of New Oximes and the Evaluation of their Reactivating, Therapeutic and Neuroprotective Efficacy Against Tabun. *Mini-Reviews in Medicinal Chemistry*, *8*(11), 1134–1143. https://doi.org/10.2174/138955708785909871

Kassa, T., Gebre-Selassie, S., & Asrat, D. (2007). Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. *Veterinary Microbiology*, *119*(1), 82–87. https://doi.org/10.1016/j.vetmic.2006.08.011

Lorke, D. E., Hasan, M. Y., Arafat, K., Kuča, K., Musilek, K., Schmitt, A., & Petroianu, G. A. (2008). *In vitro* oxime protection of human red blood cell acetylcholinesterase inhibited by diisopropyl-fluorophosphate. *Journal of Applied Toxicology*, *28*(4), 422–429. https://doi.org/10.1002/jat.1344

Lorke, D. E., Nurulain, S. M., Hasan, M. Y., Kuča, K., Musilek, K., & Petroianu, G. A. (2008). Eight new bispyridinium oximes in comparison with the conventional oximes pralidoxime and obidoxime: In vivo efficacy to protect from diisopropylfluorophosphate toxicity. *Journal of Applied Toxicology*, *28*(7), 920–928. https://doi.org/10.1002/jat.1359

Lorke, D. E., & Petroianu, G. A. (2019). The Experimental Oxime K027—A Promising Protector From Organophosphate Pesticide Poisoning. A Review Comparing K027, K048, Pralidoxime, and Obidoxime. *Frontiers in Neuroscience*, *13*. https://doi.org/10.3389/fnins.2019.00427

Mercey, G., Verdelet, T., Renou, J., Kliachyna, M., Baati, R., Nachon, F., Jean, L., & Renard, P.-Y. (2012). Reactivators of Acetylcholinesterase Inhibited by Organophosphorus Nerve Agents. *Accounts of Chemical Research*, *45*(5), 756–766. https://doi.org/10.1021/ar2002864

Nahum, V., Nili, U., Bloch-Shilderman, E., Smolkin, B., & Ashkenazi, N. (2021). Towards catch-up therapy: Evaluation of nucleophilic active pharmaceutical ingredients for the treatment of percutaneous VX poisoning,

in-vial and in-vitro studies. *International Journal of Pharmaceutics*, *603*, 120689. https://doi.org/10.1016/j.ijpharm.2021.120689

Namba, T., & Hiraki, K. (1958). PAM (PYRIDINE-2-ALDOXIME METHIODIDE) THERAPY FOR ALKYLPHOSPHATE POISONING. *Journal of the American Medical Association*, *166*(15), 1834–1839. https://doi.org/10.1001/jama.1958.02990150030007

Nurulain, S. M., Lorke, D. E., Hasan, M. Y., Shafiullah, M., Kuča, K., Musilek, K., & Petroianu, G. A. (2009). Efficacy of Eight Experimental Bispyridinium Oximes Against Paraoxon-Induced Mortality: Comparison with the Conventional Oximes Pralidoxime and Obidoxime. *Neurotoxicity Research*, *16*(1), 60–67. https://doi.org/10.1007/s12640-009-9048-7

Okudera, H. (2002). Clinical features on nerve gas terrorism in Matsumoto. *Journal of Clinical Neuroscience*, *9*(1), 17–21. https://doi.org/10.1054/jocn.2001.1020

Peter, J. V., & Cherian, A. M. (2000). Organic Insecticides. *Anaesthesia and Intensive Care*, *28*(1), 11–21. https://doi.org/10.1177/0310057X0002800102

Poirier, L., Jacquet, P., Plener, L., Masson, P., Daudé, D., & Chabrière, E. (2021). Organophosphorus poisoning in animals and enzymatic antidotes. *Environmental Science and Pollution Research*, *28*(20), 25081–25106. https://doi.org/10.1007/s11356-018-2465-5

Robb, E. L., Regina, A. C., & Baker, M. B. (2025). Organophosphate Toxicity. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK470430/

Wilson, I. B., & Ginsburg, S. (1955a). A powerful reactivator of alkylphosphate-inhibited acetylcholinesterase. *Biochimica et Biophysica Acta*, *18*, 168–170. https://doi.org/10.1016/0006-3002(55)90040-8

Wilson, I. B., & Ginsburg, S. (1955b). A powerful reactivator of alkylphosphate-inhibited acetylcholinesterase. *Biochimica et Biophysica Acta*, *18*, 168–170. https://doi.org/10.1016/0006-3002(55)90040-8

Worek, F., Mast, U., Kiderlen, D., Diepold, C., & Eyer, P. (1999). Improved determination of acetylcholinesterase activity in human whole blood. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *288*(1–2), 73–90.

Yesilbas, O., Kihtir, H. S., Altiti, M., Petmezci, M. T., Balkaya, S., Bursal Duramaz, B., Ersoy, M., & Sevketoglu, E. (2016). Acute severe organophosphate poisoning in a child who was successfully treated with therapeutic plasma exchange, high-volume hemodiafiltration, and lipid infusion. *Journal of Clinical Apheresis*, *31*(5), 467–469. https://doi.org/10.1002/jca.21417