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Pyridoxal oxime derivative potency to reactivate cholinesterases inhibited by organophosphorus compounds

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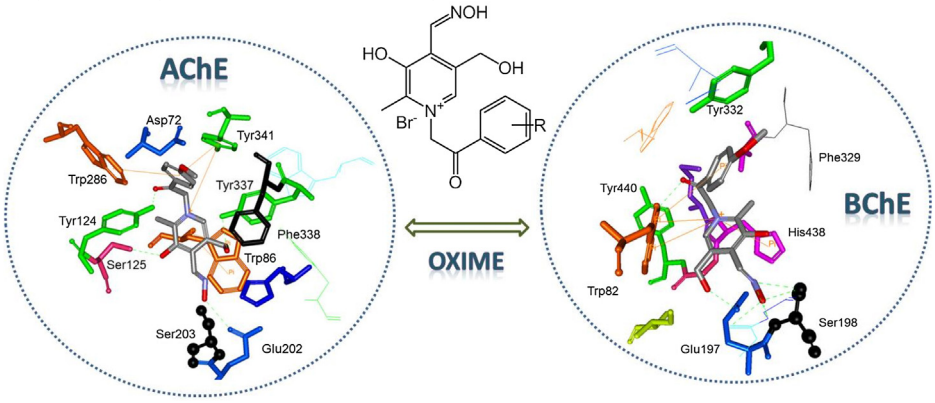
HIGHLIGHTS

- Nine pyridoxal oximes were tested as reactivators of inhibited ChEs.
- Tested oximes were more efficient in the reactivation of inhibited BChE than AChE.
- Substituents at the vicinity of the oxime moiety presented a steric hindrance for reactivation.
- A positive correlation between affinity for an oxime and its reactivation efficacy was not observed.

GRAPHICAL ABSTRACT

Pyridoxal oximes as reactivators of inhibited hAChE and hBChE show a similar binding pattern within the enzymes creating interactions close to the targeted active site serine.



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ABSTRACT

Organophosphorus (OP) nerve agents (sarin, tabun VX and soman) inhibit the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) by binding to its active site while preventing neurotransmission in the cholinergic synapses. The protection and treatment of this kind of poisoning are still a challenge as we are yet to discover an antidote that would be effective in all cases of poisoning. To aid the search for more efficient antidotes, we evaluated the ability of nine pyridoxal oxime derivatives, prepared by a novel synthetic pathway, to reactivate recombinant human AChE and the related purified human plasma butyrylcholinesterase (BChE, EC 3.1.1.8) inhibited by VX, tabun and paraoxon. Oximes are derivatives of vitamin B6 bearing a phenacyl moiety attached to the quaternary nitrogen atom and having various substituents on the phenyl ring. As the results have shown, the tested oximes were in general more efficient in the reactivation of OP-inhibited BChE than AChE. The highest observed rate was in the case of VX-inhibited BChE reactivation, where k_{obs} was 0.0087 min^{-1} and the reactivation maximum of 90% was achieved within 5 h. The cholinesterases displayed a binding affinity for these derivatives in a μmolar range no matter the substituent on their rings which was in accordance with the molecular modelling results showing a similar binding pattern for all oximes within the active site of both AChE and BChE. Such a positioning reveals also that hydroxy and a metoxy substituents at the vicinity of the oxime moiety present a possible steric hindrance explaining the reactivation results.

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1. Introduction

Numerous, structurally different organophosphorus compounds (OPs) constitute a heterogeneous category of chemicals specifically designed for the control of pests, weeds or plant diseases (Delfino et al., 2009). Though they are highly toxic to humans, their application is still an accepted means for the plant protection, and has contributed significantly to enhanced agricultural productivity and crop yields (Bolognesi, 2003). OPs are also used as plasticizers, stabilizers in lubricating and hydraulic oils, flame retardants, and gasoline additives (Kumar et al., 2010). In addition to these beneficial uses, some highly potent OPs, including tabun, sarin, and VX have been used as nerve agents in chemical warfare.

OP nerve agents inhibit acetylcholinesterase (AChE 3.1.1.7) forming a covalent bond with the active site catalytic serine. The inhibition prevents AChE's essential role, a hydrolysis of the neurotransmitter acetylcholine (ACh), which causes its accumulation, resulting in overstimulation of nicotinic and muscarinic receptors in both the central and peripheral nervous systems leading to severe symptoms in the victim (including death). The phosphylated AChE is relatively stable, and depending on the groups attached to the phosphorus atom, becomes irreversibly inhibited. Modern therapy utilises compounds bearing the oxime group capable of reactivating inhibited enzymes by a nucleophilic attack on the phosphylated serine. Unfortunately, none of the currently used oximes is sufficiently effective against all nerve agents and pesticides, meaning that they are not able to reactivate inhibited enzymes regardless of the structure of the conjugated phosphorus moiety (Kumar et al., 2010; Dawson, 1994; Žunec et al., 2015). For example, one of the most problematic nerve agents to counteract is tabun, since the existence of a lone electron pair located on its amino group makes the nucleophilic attack required for reactivation very difficult (Eto, 1976; Koplovitz and Stewart, 1994; Čalić et al., 2006; Carletti et al., 2009). Furthermore, oximes used in medical practice today lack the ability to cross the blood-brain barrier and reactivate AChE in the central nervous system in order to diminish the life threatening symptoms (Kalász et al., 2015; Katalinić et al., 2015). In this field of research, certain progress has been made by the synthesis of novel oximes demonstrating the entry to the brain and up to 35% reactivation of VX or sarin inhibited AChE (Chambers et al., 2013, 2016a, 2016b).

On the other hand, OP pesticides present a somewhat different problem. Although less lethal than chemical weapons, OP pesticides are more accessible to general population both in greater amounts and at higher concentrations. The World Health Organization reports about three million cases per year of intoxication with OP pesticides in the world. These intoxications usually result from pesticides misuses or suicidal attempts (Bardin et al., 1994; Carlton et al., 1998).

In the search for more efficient OP poisoning treatments, we synthesized nine oxime derivatives of vitamin B6 continuing our previous study (Gašo-Sokač et al., 2010), and tested them as reactivators of human AChE and BChE inhibited by tabun, VX and paraoxon (Fig. 1). The vitamin B6 scaffold was chosen since it shares a similar structure to classical monopyridinium oximes and contains a template to facilitate an optimum binding and efficient attack on the phosphylated active site serine. To be more precise, the neighbouring substituents to feasible oxime group, like $-OH$ and $-CH_2OH$, should prevent reinhibition of the reactivated enzyme by phosphorylated oxime due to the accelerated decomposition of phosphorylated oximes via elimination of the phosphoric group, and may improve enzyme affinity through additional bonding (Kliachyna et al., 2014). Several previous studies employed a similar hypothesis as well (Kliachyna et al., 2014; Radić et al., 2013a).

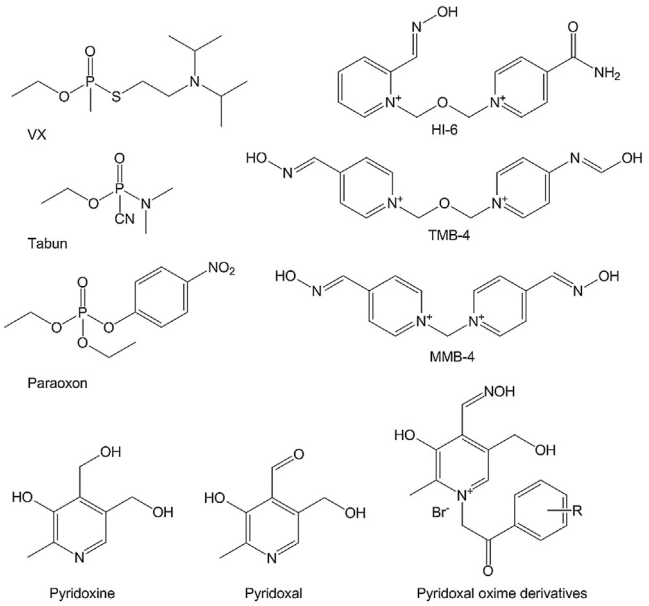

Fig. 1. Structure of organophosphorus compounds (VX, tabun and paraoxon), conventional oximes (MMB-4, TMB-4, HI-6), vitamin B6 forms (pyridoxine, pyridoxal) and nine pyridoxal oxime derivatives as potential antidotes. R denotes substituent and its position on the phenyl ring: **1** (R = 4-H), **2** (R = 4-F), **3** (R = 4-Cl), **4** (R = 4-Br), **5** (R = 4-NO_2), **6** (R = 4-CH_3), **7** (R = 2-OCH_3), **8** (R = 4-OCH_3) and **9** (R = 4-Ph).

We approached the quaternary salts of pyridoxal oxime derivatives synthesized here through a novel environmentally friendly and facile method. Pyridoxal oxime derivatives contain quaternary nitrogen on the pyridinium ring; the oxime group at the position 4 on the heteroaromatic ring; and acyl moiety for accommodation at the acyl pocket of the enzyme (Fig. 1).

2. Materials and methods

2.1. Chemicals

The general procedure for the synthesis of compounds **1**–**9** has been given in our previous study (Gašo-Sokač et al., 2014; Bušić et al., 2013). The structure of the compounds is given in Fig. 1, which were named accordingly: **1** (R = 4-H), **2** (R = 4-F), **3** (R = 4-Cl), **4** (R = 4-Br), **5** (R = 4-NO_2), **6** (R = 4-CH_3), **7** (R = 2-OCH_3), **8** (R = 4-OCH_3) and **9** (R = 4-Ph). Purity of compounds was higher than 95% according to their spectral analysis (Gašo-Sokač et al., 2014; Bušić et al., 2013). The synthesized oximes were dissolved in DMSO as 100 mM solutions. Paraoxon (*O*,*O*-diethyl-*O*-(4-nitrophenyl)phosphate; CAS number 311-45-5; Pestanal®) was purchased from Sigma-Aldrich, St. Louis, MO, USA (purity 90%). VX (*S*-[2-(diisopropylamino)ethyl]-*O*-ethyl methylphosphonothioate; CAS number 50782-69-9) and tabun (*O*-ethyl-*N*,*N*-dimethylphosphoramidocyanidate; CAS number 77-81-6) were purchased from NC Laboratory, Spiez, Switzerland (purity >95%). The stock solution of paraoxon was prepared in ethanol while VX and tabun in isopropyl alcohol. Further dilutions were done in distilled water. Acetylthiocholine iodide (ATCh) and thiol reagent 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich, St. Louis, MO, USA. Stock solutions were prepared in water or 0.1 M sodium phosphate buffer pH 7.4, respectively. The final concentration of DTNB was 0.33 mM in all of the experiments. All of the experiments were done in 0.1 M sodium phosphate buffer pH 7.4, at 25 °C.

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