

Reactivators of Acetylcholinesterase Inhibited by Organophosphorus Nerve Agents

GUILLAUME MERCEY,^{§,†} TRISTAN VERDELET,^{§,†}
JULIEN RENO,^{§,†} MARIA KLIACHYNA,^{||} RACHID BAATI,^{||}
FLORIAN NACHON,[⊥] LUDOVIC JEAN,^{§,†} AND
PIERRE-YVES RENARD*,^{§,†,‡}

[§]Equipe de Chimie Bio-Organique, COBRA - CNRS UMR 6014 & FR 3038, Rue Lucien Tesnière, 76131 Mont-Saint-Aignan, France, [†]Université de Rouen, Place Emile Blondel, 76821, Mont-Saint-Aignan, France, ^{||}Faculté de Pharmacie, Université de Strasbourg, CNRS UMR 7199, Laboratoire des Systèmes Chimiques Fonctionnels, 74 route du Rhin, BP 60024, 67401 Illkirch, France, [⊥]Département de Toxicologie, Institut de Recherche Biomédicale des Armées, 24 Avenue des Maquis du Grésivaudan, BP87, 38702 La Tronche, France, and [‡]Institut Universitaire de France, 103 Boulevard Saint Michel, 75005 Paris, France

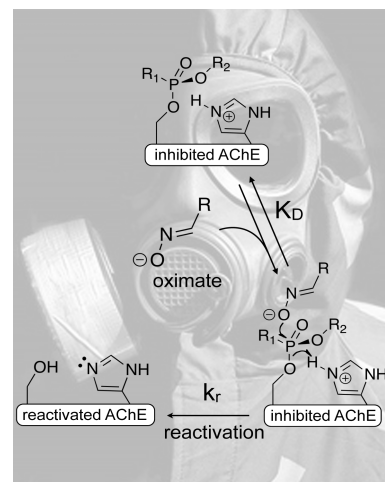
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CONSPECTUS

Since the September 11, 2001, terrorist attacks in the United States, the specter of a chemical threat against civilian populations has renewed research interest in chemical warfare agents, their mechanisms of action, and treatments that reverse their effects. In this Account, we focus specifically on organophosphorus nerve agents (OPNAs). Although some OPNAs are used as pest control, the most toxic chemicals in this class are used as chemical warfare agents in armed conflicts. The acute toxicity of OPNAs results from the irreversible inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) via the formation of a covalent P–O bond at the serine hydroxyl group in the enzyme active site. AChE breaks down the neurotransmitter acetylcholine at neuronal synapses and neuromuscular junctions. The irreversible inhibition of AChE causes the neurotransmitter to accumulate in the synaptic cleft, leading to overstimulation of cholinergic receptors, seizures, respiratory arrest, and death.

The current treatment for OPNA poisoning combines an antimuscarinic drug (e.g., atropine), an anticonvulsant drug (e.g., diazepam), and an AChE reactivator of the pyridinium aldoxime family (pralidoxime, trimepridoxime, obidoxime, HI-6, HLö-7). Because of their high nucleophilicity, oximes can displace the phosphyl group from the catalytic serine, thus restoring the enzyme's catalytic activity. During 50 years of research in the reactivator field, researchers have synthesized and tested numerous structural modifications of monopyridinium oximes and bispyridinium oximes. In the past decade, medicinal chemists have focused their research on the more efficient bispyridinium reactivators, but all known reactivators have several drawbacks. First, due to their permanent positive charge, they do not cross the blood–brain barrier (BBB) efficiently and do not readily reactivate AChE in the central nervous system. Second, no single oxime is efficient against a wide variety of OPNAs. Third, oximes cannot reactivate “aged” AChE.

This Account summarizes recent strategies for the development of AChE reactivators capable of crossing the BBB. The use of nanoparticulate transport and inhibition of P-glycoprotein efflux pumps improves BBB transport of these AChE reactivators. Chemical modifications that increased the lipophilicity of the pyridinium aldoximes, the addition of a fluorine atom and the replacement of a pyridyl ring with a dihydropyridyl moiety, enhances BBB permeability. The glycosylation of pyridine aldoximes facilitates increased BBB penetration via the GLUT-1 transport system. The development of novel uncharged reactivators that can move efficiently across the BBB represents one of the most promising of these new strategies.



Introduction

Poisoning by organophosphorus-based pesticides is a serious public health issue with over 200 000 fatalities annually worldwide.¹ Organophosphorus warfare agents present a persistent threat to the general population as a consequence of armed conflicts (e.g., Gulf War) and terrorist attacks (e.g., subway attacks in Japan in 1995). These compounds irreversibly inhibit acetylcholinesterase (AChE, EC 3.1.1.7), which plays an essential role in neurotransmission. Over the last 60 years, pyridinium oxime compounds have been widely used as antidotes to treat these intoxications.^{2,3} Despite decades of research in this field, there is no efficient and general reactivator for organophosphorus-inhibited AChE. Interest in this field has increased since the September 2001 terrorist attacks in the U.S.A. The purpose of this Account is to highlight the important and recent advances in research on organophosphorus-inhibited AChE.

We will focus most of the discussion on the reactivation of human AChE (hAChE) inhibited by the highly toxic organophosphorus chemical warfare agents. Details on the reactivation of AChE after organophosphorus pesticide poisoning have been recently reviewed extensively.⁴ In the first section, the organophosphorus nerve agents will be discussed, as well as the mechanism responsible for AChE irreversible inhibition and the effects caused by this inhibition. In the next section, the main pyridinium oxime reactivators and their mechanism of reactivation will be discussed. Then, the structural modifications of pyridinium and bis-pyridinium oximes, developed in the past decade, will be summarized. Finally, new concepts focused on AChE reactivation in the brain will be discussed.

Inhibition of AChE by Organophosphorus Nerve Agents

The first generation of organophosphorus (OP) nerve agents, called G-agents (German agents), share a common $O=P^V(O-R)$ moiety. They include the cyanophosphoramidate, tabun (GA), and the methylfluorophosphonates, sarin (GB), soman (GD), and cyclosarin (GF) (Figure 1). After WWII, methylphosphothioates called V-agents (venomous agents) were invented: VX (Great Britain), RVX (Russian isomer), and CVX (Chinese isomer) (Figure 1). V-agents differ from G-agents by their lower volatility, their higher persistency in the environment, and their higher toxicity.⁵

The acute toxicity of OPs is due to their rapid inhibition of AChE. This enzyme is a serine hydrolase and is responsible for the breakdown of the neurotransmitter acetylcholine at

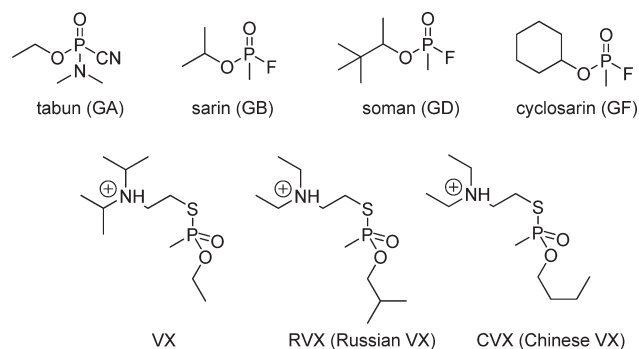


FIGURE 1. Chemical structures of the main organophosphorus nerve agents.

neuronal synapses and neuromuscular junctions.⁶ The inhibition of AChE leads to an accumulation of acetylcholine, resulting in permanent saturation of muscarinic and nicotinic receptors and ultimately a system-wide cholinergic crisis. Among the many symptoms that appear upon hyperstimulation of the cholinergic system are paralysis, seizures, and respiratory failure causing death.

During normal function of AChE, a serine–histidine–glutamate triad, located in the active site of the enzyme, catalyzes the hydrolysis of acetylcholine (Figure 2A). The catalytic mechanism consists of two steps: (1) the nucleophilic serine attacks acetylcholine to form a tetrahedral transition state that collapses to the acetyl-enzyme with release of choline; (2) a water molecule, activated by the nearby histidine, attacks the acetylserine leading to the formation of a second tetrahedral transition state that collapses to the free enzyme and acetic acid. This mechanism is extremely efficient; AChE hydrolyzes more than 10^4 molecules of acetylcholine per second.⁷

The mechanism of AChE inhibition by OPs is similar to the initial step of hydrolysis. Once the OP has reached the bottom of the active site gorge, the nucleophilic serine attacks the phosphorus atom, forming a bipyramidal transition state, which is followed by the departure of the leaving group and the formation of the phosphorylserine (Figure 2B). The phosphoryl adduct is a remarkable mimic of the transition state of the initial step of hydrolysis. However, in the second step, the catalytic histidine cannot fulfill the role of water activation because it is either forced into a nonproductive conformation (e.g., VX⁸ and tabun conjugates⁹) or shielded from water (e.g., soman conjugate¹⁰). Therefore the spontaneous hydrolysis of the phosphorylserine is extremely slow, varying from hours for dimethylphosphoryl conjugates¹¹ to days for V-agent AChE conjugates.¹²

Spontaneous hydrolysis of the conjugate is in competition with a time-dependent intramolecular reaction yielding

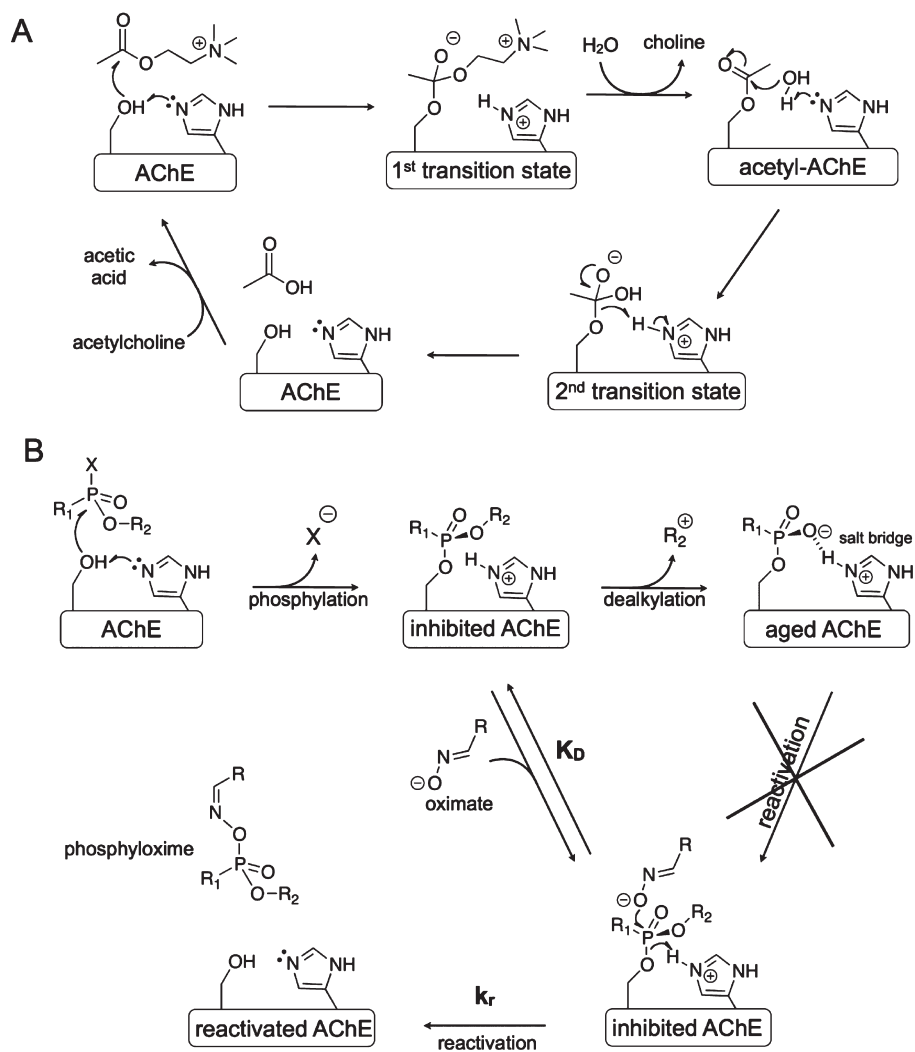


FIGURE 2. (A) Mechanism of acetylcholine hydrolysis by AChE. (B) Mechanism of AChE inhibition by organophosphorus nerve agents, aging, and reactivation by oximes.

an “aged” form of the conjugate.¹³ The aging reaction of AChE conjugates is generally a dealkylation of the alkoxy substituent present on the phosphorus atom, and it yields a phosphonate adduct (Figure 2B). The aging half-time are 2–4 min for soman, 5 h for sarin, 46 h for tabun, and 48 h for VX.¹⁴ The resulting phosphonic oxyanion forms a salt bridge with the protonated triad histidine¹⁵ that strongly stabilizes the conjugate.⁹ Moreover, the phosphonic oxyanion prevents any negatively charged nucleophile from approaching the phosphorus atom. Consequently, aged phosphyl-AChE conjugates do not get hydrolyzed.

Known Antidotes against OP Poisoning

In the 1950s, Wilson showed that hydroxylamine¹⁶ and nicotinic acid¹⁷ were able to reactivate diethylphosphoryl AChE. These initial findings quickly led to the

discovery that oximes,¹⁸ 2-oxoaloximes,¹⁹ and especially 2-pyridinium aldoxime (2-PAM; Figure 3)²⁰ were powerful reactivators.

The efficiency of reactivators can be estimated by the second-order rate constant for reactivation, k_{r2} , which is the ratio of the reactivation rate constant (k_r) and the approximate dissociation constant of the reactivator/phosphyl-AChE complex (K_D) (Figure 2B).¹⁵ The good activity of 2-PAM was attributed to strong binding of the positively charged pyridinium to the enzyme active site and proper orientation of the oxime group for displacement of the phosphyl moiety. The corresponding oxime, 4-PAM, was less efficient than 2-PAM because its orientation was improper. It was hypothesized that combining 4-PAM with a ligand that is able to strongly bind to the enzyme could yield a compound with both better affinity and proper orientation

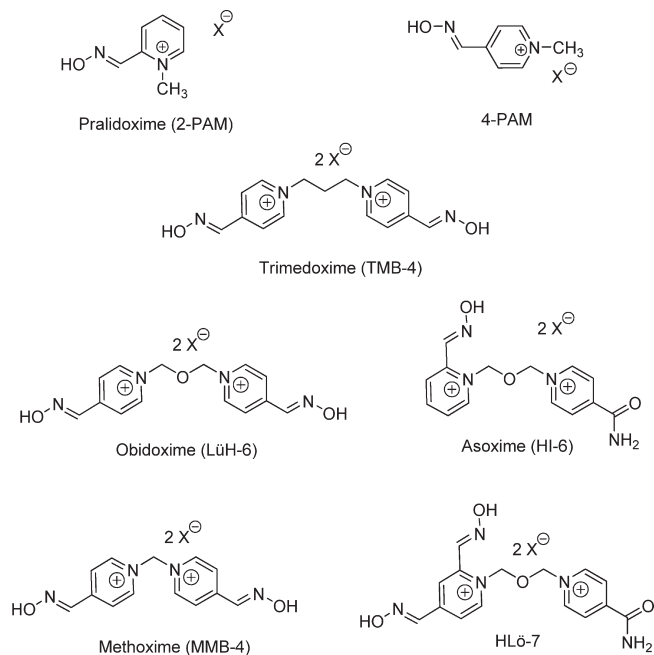


FIGURE 3. Chemical structures of the main pyridinium aldoxime reactivators.

of the oxime group in the AChE catalytic site. Following this reasoning, the first bispyridinium aldoxime, TMB-4 (Figure 3), was prepared. TMB-4 proved to be superior to both 2-PAM and 4-PAM due to an improved affinity.²¹ Subsequently, other aldoximes were synthesized based on the TMB-4 structure: LüH-6,²² HI-6,²³ and HLö-7²⁴ (Figure 3). Though the bispyridinium aldoximes are effective reactivators, none is a universal reactivator. Their efficiency of reactivation varies greatly with the nature of the phosphyl group on the inhibited AChE.¹⁵ LüH-6 is generally considered as the best reactivator for pesticides (dialkylphosphoryl-AChE).²⁵ HI-6 is active against soman and VX¹⁵ but is inefficient against tabun.²⁶ 4-Substituted oximes like TMB-4, HLö-7, and LüH-6 are efficient against tabun inhibition,²⁷ but their reactivation rates are very slow compared with those obtained for VX-inhibited AChE.¹⁵ This poor reactivity is related to the weak electrophilicity and steric hindrance of the phosphoramidyl-AChE adduct created by tabun.²⁷

What is worse, all aged conjugates are completely refractory toward oxime reactivation. The only viable strategy for reactivation of aged adducts seems to be modification of the phosphonic moiety by realkylation *in situ*, using powerful and specific alkylating agents.¹³

The pK_a of oximes is also of pivotal importance since the reactive species is the oximate. To be effective, the oxime must remain partially deprotonated in the range of physiological pH; full deprotonation is unwanted because the

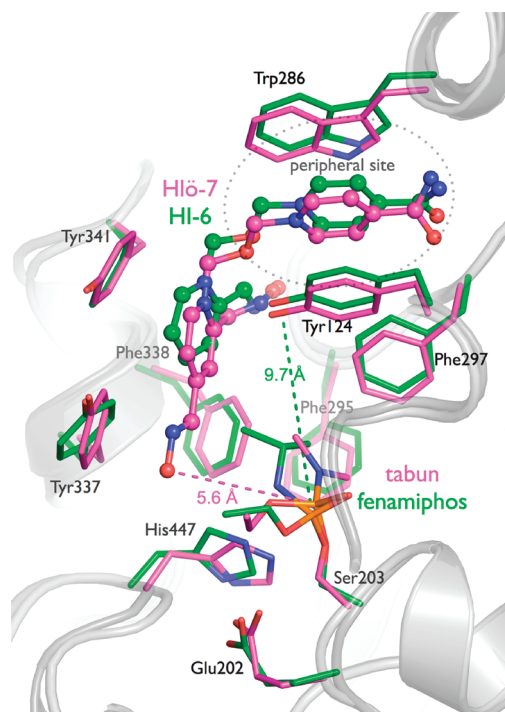


FIGURE 4. Active site view of HLö-7–tabun–mouse AChE (carbon atoms in magenta) and HI-6–fenamiphos–mouse AChE (carbon atoms in green). Ser203 and His447 are components of the catalytic triad. Tyr124 and Trp286 are components of the peripheral site at the entrance of the active site gorge.

reactivity is compromised by the cost in the desolvation energy for formation of the oximate anion.²⁸ The conjugated ring systems of 2- and 4-alkylpyridinium aldoximes (e.g., 2-PAM, HI-6, and obidoxime) increase the acidity of these oximes yielding pK_a values ranging between 7.3 and 8.0.²⁸

The recent X-ray structures of HLö-7 and HI-6 complexed with phosphoramidyl-AChE illustrate the prototypic binding of a bisquaternary oxime to tabun-inhibited enzyme. Association is predominantly via π - π and cation- π interactions.^{29,30} One pyridinium moiety is stacked between the aromatic residues of the peripheral site at the entrance of the active site gorge, and the second pyridinium interacts with tyrosines in the middle of the active site gorge (Figure 4). The phosphorus–oximate distance is 5.6 Å for HLö-7 and 9.7 Å for HI-6. The oxime functions are neither in a proper orientation nor at a proper distance to attack the phosphorus atom. These observations suggest that the structure of oxime reactivators could be substantially improved. One such improvement might involve coupling a peripheral site ligand to a nucleophilic function.³¹ A major difficulty in designing new reactivators is that the details of the reactivation mechanism are not yet well understood. Some structural work suggests that deprotonation of the oxime is assisted by the catalytic histidine³² or by a

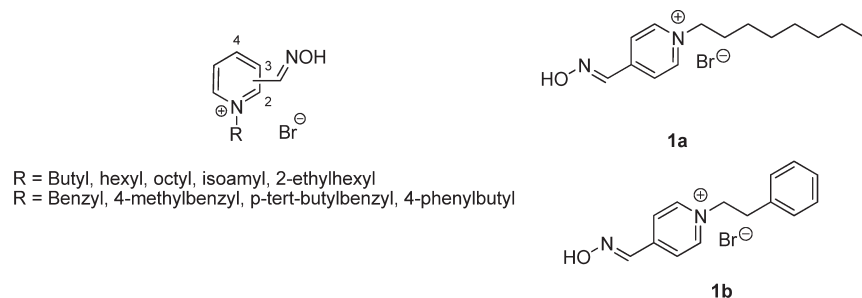


FIGURE 5. PAM analogues.

bridging water molecule.³³ It has also been suggested that the nucleophile does not have to attack the face of the phosphyl group opposite to the serine (apical position), but it could also attack the vicinal face if it is open.³⁴ The wealth of new structural information on complexes of inhibited-AChE and oximes must be considered when designing new generations of reactivators.

Structural Modifications of Monoquaternary and Bisquaternary Pyridinium AChE Reactivators

Monoquaternary pyridinium reactivators are known to be weak AChE reactivators compared with the bisquaternary reactivators. Still, analogues of monoquaternary pralidoxime (PAM) have been synthesized in an effort to improve their reactivity. Their reactivation abilities were evaluated *in vitro* on organophosphorus inactivated human AChE (hAChE) (Figure 5).³⁵ These studies have shown that elongation of the side chain (to improve lipophilicity) or the presence of an aromatic group in the side chain (to increase interactions with AChE residues via π - π interactions) did not improve their reactivation ability compared with 2-PAM (the relative reactivation activity to 2-PAM is 46% and 44% for **1a** and **1b**, respectively). These lipophilic derivatives **1a** and **1b** were shown to penetrate the blood-brain barrier (BBB) with a penetration ratio of 30% and 3%, respectively.³⁶ However, the usefulness of these compounds is limited due to their significant toxicity. Monopyridinium oximes were studied less frequently following the realization that bispyridinium oximes were better reactivators.

To date, the principal modifications carried out on bispyridinium structures have included modifying the position of the oxime on the pyridinium ring, the introduction of various substituents, and especially alterations to the nature and the length of the linker between the pyridinium rings.

To test the effect of the linker chain length on the reactivation of tabun-inhibited AChE, analogues of trimedoxime

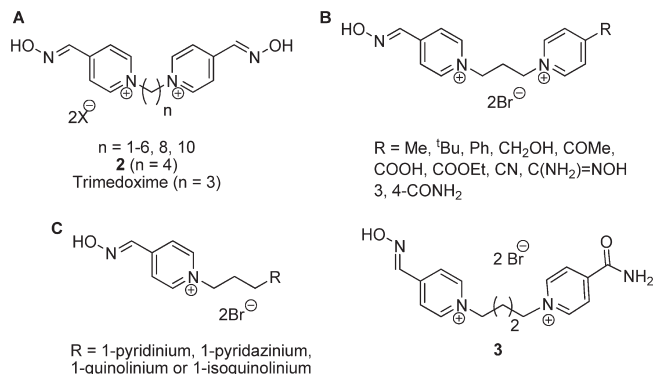


FIGURE 6. Analogues of trimedoxime (TMB-4) evaluated in the reactivation of tabun-inhibited hAChE.

(TMB-4) were synthesized. It was determined that the optimal distance between two 4-pyridinium aldoximes was three or four carbons (Figure 6A).³⁷ Mono-oxime bispyridinium analogues of TMB-4 were also evaluated as potential hAChE reactivators. The nonoxime pyridinium ring was substituted in position 4 with various groups (Figure 6B) or was replaced with a heteroaromatic ring (Figure 6C). Some of these analogues (R = COMe, Ph, or C(NH₂)=NOH, refer to Figure 6B) had a slightly lower reactivation potency for tabun-inhibited hAChE than trimedoxime.³⁸ Of all the synthesized and evaluated analogues, only compound **3** (Figure 6)³⁹ was more efficient than trimedoxime at reactivating tabun-inhibited hAChE *in vitro* (5-fold more efficient).⁴⁰ Interestingly, the presence of a carbamoyl group influences the reactivation ability of the analogue by increasing its affinity for the organophosphorus-inhibited enzyme. Affinity is improved via hydrogen bond interactions with residues at the peripheral site of the enzyme. For example, compound **3** is 6-fold more efficient than compound **2** (Figure 6A) due to its increased affinity.

Modifications to the HI-6 and obidoxime structures have also been evaluated. The research teams of K. Kuča and J. Acharya have independently studied the effects on reactivation of introducing additional heteroatoms into the linker. It was hypothesized that a pair of oxygen atoms in the linker

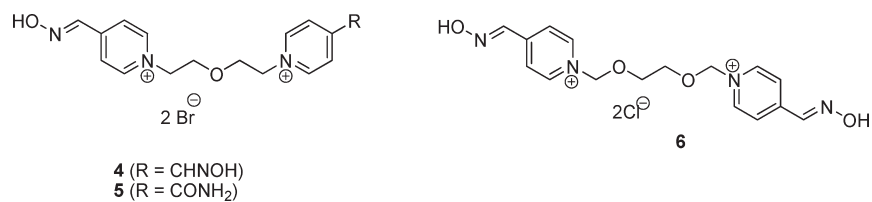


FIGURE 7. Structures of reactivators bearing one or two oxygen atoms in the linker between the two pyridinium rings.

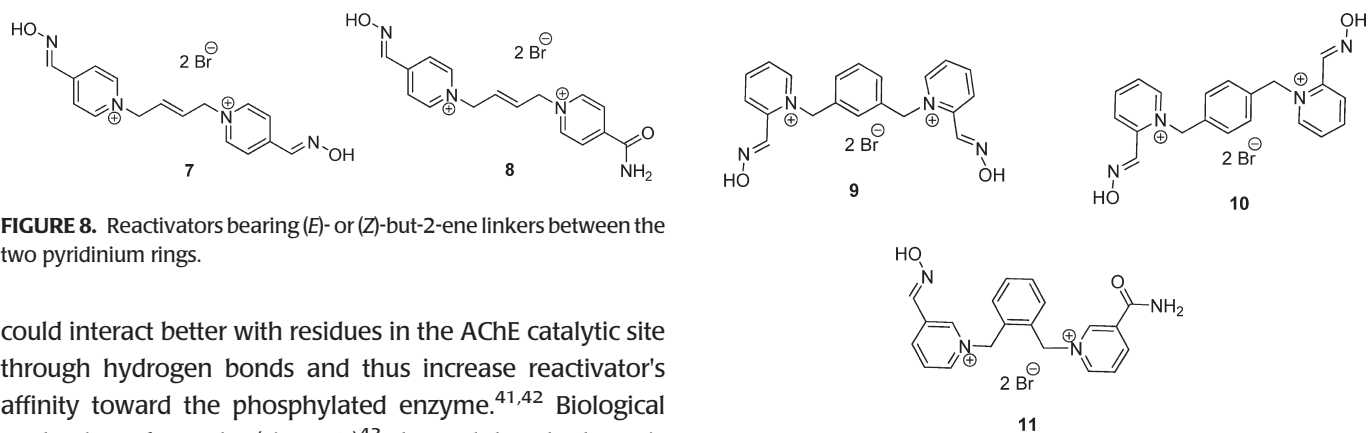


FIGURE 8. Reactivators bearing (*E*)- or (*Z*)-but-2-ene linkers between the two pyridinium rings.

could interact better with residues in the AChE catalytic site through hydrogen bonds and thus increase reactivator's affinity toward the phosphorylated enzyme.^{41,42} Biological evaluation of **4** and **5** (Figure 7)⁴³ showed that the latter is 2-fold more efficient than trimedoxime for the reactivation of tabun-inhibited hAChE. On the other hand, Acharya et al. have prepared and evaluated symmetrical bispyridinium aldoximes with longer linkers bearing two oxygen atoms.⁴⁴ Among these compounds, **6** (Figure 7) showed a slightly better ability to reactivate sarin-inhibited hAChE than 2-PAM.

The inclusion of an unsaturated chain to connect the two pyridinium rings has also been evaluated. In comparison with its saturated analogue, **7** (Figure 8) is 2.5-fold more efficient at reactivating tabun-inhibited AChE due to a better affinity and reactivity, and it is 2-fold more efficient than trimedoxime.⁴⁰ Interestingly, the substitution of one of the oxime functions by a carbamoyl group dramatically increased the reactivation potency of **8** (5-fold more efficient than **7**).⁴⁵ Molecular docking studies have shown that the improved reactivity of **8** (compared with **3**) could be due to supplementary interactions of **8** with AChE, specifically edge-to-face interactions between the double bond and AChE aromatic residues.⁴⁶

With the intention of increasing the reactivator's affinity toward the inhibited enzyme through cation- π or π - π interactions, bispyridinium compounds with xylene connecting linkers were synthesized and evaluated. Compounds **9** and **10** (Figure 9) were 6-fold less efficient than trimedoxime at reactivating tabun-inhibited AChE.⁴⁶ However, **10** reactivated 45% of sarin-inhibited hAChE in comparison to, respectively, 34% and 24% reactivation by 2-PAM and obidoxime at the concentration 10^{-3} M.⁴⁷ A

FIGURE 9. Reactivators bearing xylene-connecting linkers between the two pyridinium rings.

total of 26 xylene-modified, monooxime-monocarbamoyl bispyridinium compounds were tested, and only structure **11** (Figure 9) displayed substantial reactivation potency, but it was 1.6-fold less efficient than trimedoxime at reactivating tabun-inhibited AChE. Moreover, it was 1.5-fold more toxic than trimedoxime.⁴⁶

Strategies for Blood–Brain Barrier Penetration

The blood–brain barrier (BBB) is composed of an endothelial cell layer, which separates the circulating blood and the brain's extracellular fluid. Tight junctions (TJ) between endothelial cells and limited pinocytotic activity⁴⁸ make the BBB nearly impenetrable to viruses, bacteria, proteins, and polar molecules.⁴⁹ OP nerve agents, being small lipophilic molecules, can easily penetrate the BBB by free diffusion and thereby inhibit AChE in the central nervous system (CNS). However, commonly used reactivators are permanently charged cationic compounds that have difficulty in crossing the BBB.⁵⁰ For instance, the BBB penetration of 2-PAM (striatal extracellular/blood concentration ratio) has been estimated to be only approximately 10% by *in vivo* rat brain microdialysis technique with HPLC/UV.⁵¹ Therefore, oximes reactivate AChE in peripheral sites, but they are not effective in the CNS. Consequently they provide little to no protection against the neurological effects of OP exposure, which

includes seizures, convulsions, and behavioral and psychological changes. This dilemma prompted the development of oxime-based agents that can cross the BBB and reverse the effects of OP on AChE in the CNS.

A number of strategies have been developed to circumvent or disrupt the BBB. Direct injections into the brain,⁵² local exposure to high-intensity focused ultrasound (HIFU),⁵³ and osmotic TJ opening (by hypertonic mannitol)⁵⁴ represent the most painful and invasive BBB disruption methods. Significant progress in BBB penetration has been achieved by using targeted nanoparticulate drug delivery. Obidoxime dichloride and both HI-6 dichloride monohydrate and HI-6 dimethanesulfonate bound to biodegradable human serum albumin (HSA) nanoparticles were able to cross an *in vitro* BBB model.⁵⁵ In general, the oximes transported in nanoparticles exhibited a better reactivation of paraoxon-ethyl and sarin-inhibited AChE than free oximes, resulting from higher BBB crossing. For example, the concentration of HI-6 dimethanesulfonate loaded on NP-ApoE nanoparticles, measured across the BBB, was 44.6 μM compared with 15.2 μM for the free oxime (transport difference +193.16%).

Safe and effective modulation of transport across the BBB also represents an attractive approach for targeting drugs into the brain. Inhibition of the active efflux transporter P-glycoprotein (Pgp) located in the endothelial cell membranes was shown to improve the BBB permeability of HI-6.⁵⁶ Administration of tariquidar, a specific noncompetitive Pgp pump inhibitor, resulted in a 2-fold increase in HI-6 levels in the brain and, subsequently, twice as much AChE activity after 1 h of treatment, while HI-6 concentration in the blood was not affected. More recently, adenosine receptor (AR) signaling was shown to modulate BBB permeability *in vivo*, facilitating the entry of dextrans and antibodies to β -amyloid into the brain.⁵⁷ AR signaling may be a promising strategy for improvement in the BBB permeability of therapeutically important oximes.

Introduction of a fluorine atom into the heterocyclic ring of pyridinium oximes should enhance their lipophilicity. Increased lipophilicity would enable the oxime to more readily diffuse across the BBB increasing its AChE reactivation potency. According to computer-aided calculations, fluorinated 4-PAM analogues **12** and **13** (Figure 10) are more lipophilic than nonfluorinated 4-PAM. These predictions were confirmed by AChE reactivation experiments⁵⁸ and by assessment of BBB permeability using the parallel artificial membrane permeation assays (PAMPA) method.⁵⁹ In the PAMPA experiment, the fluorinated *N*-methyl-4-pyridinium oxime **12** exhibited higher permeability than

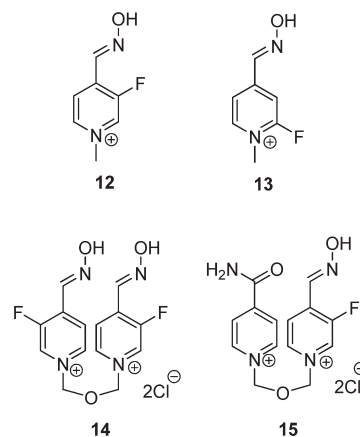


FIGURE 10. Fluorinated mono- and bisquaternary pyridinium aldoximes.

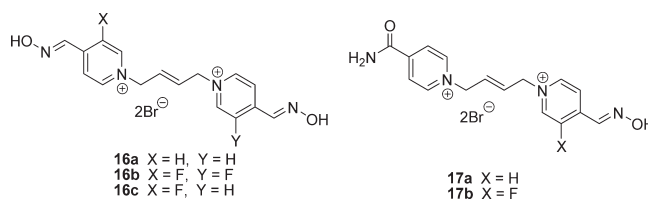


FIGURE 11. Fluorinated analogues of **7** and **8**.

4-PAM (log $P_e = -7.2$ and -6.4 for 4-PAM and **12**, respectively, where P_e is effective permeability). In the AChE reactivation experiments, compound **12** showed a reactivation potency toward the paraoxon-inhibited housefly AChE and bovine RBC (red blood cell) AChE that was 2.5-fold and 2.2-fold higher than 4-PAM, respectively.

Fluorinated oximes **14** and **15** both exhibited higher reactivation potencies toward paraoxon-inhibited housefly AChE than obidoxime and HI-6. However, toward paraoxon-inhibited bovine RBC AChE, obidoxime and HI-6 were more active than their fluorinated analogs **14** and **15**.⁵⁸ Membrane permeability measurements showed that the BBB permeability increased in proportion to the number of fluorine atoms. However, for the bis-pyridinealdoximes **16a–c** and **17a,b** (Figure 11) the permeability data did not correlate with the *in vitro* reactivation results.⁵⁹

The modification of pyridine aldoxime with a glucose moiety was proposed to facilitate its BBB penetration. This was confirmed by Heldman et al. These sugar–oxime conjugates are thought to penetrate the BBB due to recognition of the glucose moiety by the facilitative glucose transporters.⁶⁰ The most active sugar–oxime, compound **18** (Figure 12), had a reactivation potency toward diisopropyl phosphorofluoridate (DFP)- and paraoxon-inhibited hAChE that was similar to that of 2-PAM.⁶¹ Moreover, the sugar-derivative **19** showed lower toxicity than 4-PAM.

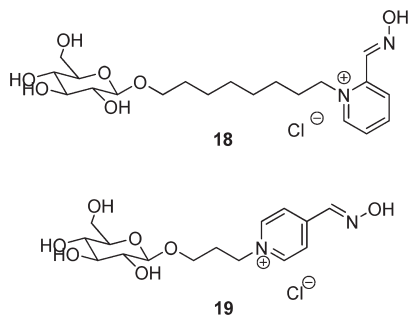
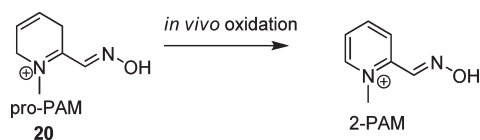
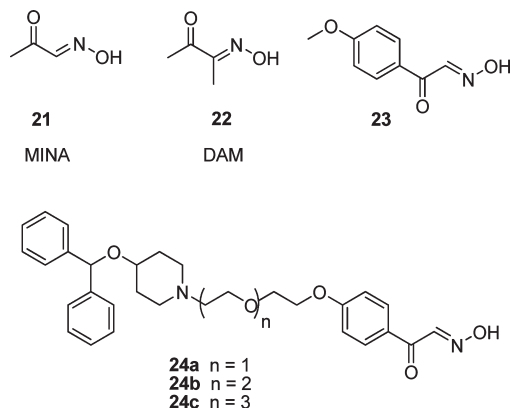


FIGURE 12. Sugar–Oximes.

FIGURE 13. *In vivo* oxidation of pro-PAM.FIGURE 14. Neutral reactivators: α -ketoaldoximes and ketoxime.

Yet another approach is based on the use of a prodrug of 2-PAM, in which the highly charged pyridine ring is replaced with a significantly less charged dihydropyridyl moiety.^{62,63} Once it has penetrated the BBB, pro-PAM **20** (Figure 13) rapidly undergoes oxidation in the brain to produce a functionally active quaternary oxime 2-PAM, which can then reactivate OP-inhibited AChE in the CNS. Disadvantages of this approach include the difficult synthesis of pro-PAM and its rather low stability due to autoxidation.

A novel strategy used to improve the BBB permeability is the synthesis of uncharged reactivators, which are capable of diffusing across the BBB and reactivating AChEs within the CNS. The neutral oximes monoisonitrosoacetone **21** (MINA) and diacetylmonooxime **22** (DAM) bearing the ketoaldoxime or ketoxime moiety as a reactivator function (Figure 14) are reported to cross the BBB, but their *in vitro* reactivation potency toward OP-inhibited AChE is much lower than that of 2-PAM and other quaternary oximes.^{64,65}

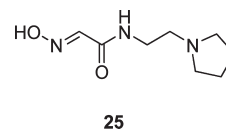
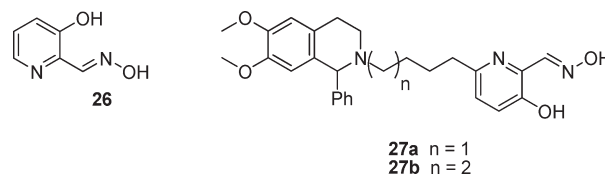
FIGURE 15. Uncharged hydroxyiminoacetamide **25**.

FIGURE 16. Phenyl-tetrahydroisoquinoline–pyridinaldoxime conjugates.

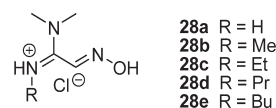


FIGURE 17. Amidine-oxime reactivators.

M. de Koning et al. proposed linking the reactivating α -ketoaldoxime moiety to a piperidine-derived peripheral site ligand (PSL) in order to increase the affinity for AChE (Figure 14).⁶⁶ The hybrids **24a–c** displayed a remarkable increase in reactivation potency (about 25–36% reactivation of sarin-inhibited hAChE) compared with the reference compound **23** (5% reactivation of sarin-inhibited hAChE), but they still remained inefficient reactivators compared with the commonly used pyridinium oximes. Replacing the ketone moiety with an amide one resulted in compound **25** (Figure 15), which showed reactivation kinetics superior to the reference uncharged compounds **21** and **22**, but in comparison with 2-PAM, this analogue still requires the further refinement.⁶⁷

Nonquaternary pyridinealdoxime, compound **26** (Figure 16), exhibited a high potency for reactivation of VX-inhibited hAChE, but a low affinity toward inhibited enzyme.⁶⁸ Linking this oxime to phenyl-tetrahydroisoquinoline (a peripheral site ligand) to create compounds **27a,b** enhanced the affinity toward the enzyme, and increased reactivation of VX and tabun-inhibited hAChE. Rates of reactivation equaled and even exceeded those of HI-6, obidoxime, and HLö-7.⁶⁹ For example, **27b** is as efficient at reactivating VX–hAChE as HLö-7, which is currently the best bispyridinium oxime reactivator for VX–hAChE. Compound **27b** is also 5-fold more efficient at reactivating tabun-inhibited AChE than trimedoxime, which is currently the best bispyridinium oxime reactivator for tabun–hAChE.

Reactivation of inhibited AChE with pyridinium aldoximes (especially 4-pyridinium aldoximes) results inevitably in the formation of highly reactive phosphoxyoximes, which

in turn may inhibit AChE (recapture phenomenon).⁷⁰ This complication could be limited with oximes **27** due to the presence of the phenol moiety, which takes part in the formation of an isoxazole by a subsequent intramolecular and irreversible reaction.⁷¹

Amidine–oxime reactivators **28a–e** (Figure 17) are expected to possess increased lipophilicity.⁷² Although these compounds were found to be less potent than 2-PAM in reactivation of AChE *in vitro*, they have the advantage of being more lipophilic than 2-PAM and were expected to be found at much higher concentrations in the brain. Two amidine-oximes, **28c** and **28d**, were efficacious *in vivo* and protected animals from CNS toxicity of nerve agent model compounds.

Conclusion and Outlook

During the three decades since the discovery of monopyridinium and bispyridinium oximes as reactivators for OP-inhibited AChE, hundreds of variations have been synthesized and evaluated. All of those reactivators have three major drawbacks: (1) Their permanent positive charge prevents them from crossing the BBB to reactivate brain AChE. (2) They exhibit unequal reactivation abilities against AChE inhibited with different types of OP. (3) they are inefficient at reactivating “aged” AChE. Recent research has developed new and efficient uncharged reactivators that are able to cross the BBB. However, further research is necessary to discover a broad-spectrum reactivator suitable for the whole range of OPs. None of existing pyridinium oximes is a true broad-spectrum reactivator.⁷³ In the short term, a solution to the broad-spectrum reactivator issue would be to combine two or more oximes that have complementary activities. In this regard, combining obidoxime with HI-6 is a promising approach.⁷⁴ Regarding “aged” AChE, further research is necessary, since no existing reactivator is able to reactivate it. Further developments in this field will lead to better protection for the public from OPs used in both pest control and warfare.

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BIOGRAPHICAL INFORMATION

Guillaume Mercey received his Ph.D. from the University of Caen (France) in 2009 with Dr. Mihaela Gulea. He is a postdoctoral fellow at the University of Rouen (France) with Professor Pierre-Yves Renard studying syntheses of uncharged AChE reactivators.

Tristan Verdelet and **Julien Renou** are completing their Ph.D. programs in bioorganic chemistry at the University of Rouen with Professor Pierre-Yves Renard. They are studying syntheses of uncharged AChE reactivators.

Maria Kliachyna received her Ph.D. in 2010 from the Institute of Organic Chemistry, NASU (Ukraine). She is a postdoctoral fellow with Dr. Rachid Baati and Dr. Alain Wagner at the University of Strasbourg (France) working on the syntheses of AChE reactivators.

Rachid Baati is a CNRS Researcher at the University of Strasbourg. His research is dedicated to the study of organic chemistry, bio-orthogonal and bio-organic chemistry (AChE reactivation) through methodology development and catalysis.

Florian Nachon is the leader of the structural enzymology team at the Department of Toxicology in the Army Institute of Biomedical Research in Grenoble (IRBA, France). His present research interests are focused on the development of nerve agent bioscavengers and the design of new generations of reactivators for inhibited cholinesterases.

Ludovic Jean is an assistant professor at the University of Rouen since 2008 in the research team of professor Pierre-Yves Renard. His research topics are focused on organophosphorus nerve agent and reversible AChE inhibitors.

Pierre-Yves Renard is a professor in bioorganic chemistry at the University of Rouen and has been the head of the bioorganic chemistry team at the Institut de Recherche en Chimie Organique Fine (IRCOF) since 2003. His research topics are focused on (1) organophosphorus nerve agent and reversible AChE inhibitors and (2) chemical tools for the understanding of biological mechanisms (bioconjugate chemistry, fluorescence, chemiluminescence, self-immolative linkers, probes for *in vivo* imaging).

FOOTNOTES

*To whom correspondence should be addressed. Fax: (+)33 2 35 52 29 59. E-mail: pierre-yves.renard@univ-rouen.fr.
The authors declare no competing financial interest.

REFERENCES

- Eddleston, M.; Buckley, N. A.; Eyer, P.; Dawson, A.-H. Management of acute organophosphorus pesticide poisoning. *Lancet* **2008**, *371*, 597–607.
- Jokanović, M.; Prostran, M. Pyridinium oximes as cholinesterase reactivators. Structure–activity relationship and efficacy in the treatment of poisoning with organophosphorus compounds. *Curr. Med. Chem.* **2009**, *16*, 2177–2188.
- Jung, Y.-S.; Kuča, K. Current study on the development of bis-pyridinium oxime reactivators for the antidotal treatment of poisoning by toxic organophosphorus agents. *Curr. Org. Chem.* **2011**, *15*, 433–444.
- Musilek, K.; Dolezal, M.; Gunn-Moore, F.; Kuča, K. Design, evaluation and structure–activity relationship studies of the AChE reactivators against organophosphorus pesticides. *Med. Res. Rev.* **2009**, *31*, 548–575.
- Watson, A.; Opresko, D.; Young, R.; Hauschild, V.; King, J.; Bakshi, K. Organophosphate Nerve Agents. In *Handbook of Toxicology of Chemical Warfare Agents*; Gupta, R. C., Ed.; Academic Press Inc: London, 2009; Chapter 6.
- Maxwell, D. M.; Brecht, K. M.; Kopolovitz, I.; Sweeney, R. E. Acetylcholinesterase inhibition: Does it explain the toxicity of organophosphorus compounds? *Arch. Toxicol.* **2006**, *80*, 756–760.
- Quinn, D. M. Acetylcholinesterase: Enzyme structure, reaction dynamics, and virtual transition states. *Chem. Rev.* **1987**, *87*, 955–979.
- Millard, C. B.; Koellner, G.; Ordentlich, A.; Shafferman, A.; Silman, I.; Sussman, J. L. Reaction products of acetylcholinesterase and VX reveal a mobile histidine in the catalytic triad. *J. Am. Chem. Soc.* **1999**, *121*, 9883–9884.
- Carletti, E.; Colletier, J.-P.; Dupeux, F.; Trouvilet, M.; Masson, P.; Nachon, F. Structural evidence that human acetylcholinesterase inhibited by tabun ages through O-dealkylation. *J. Med. Chem.* **2010**, *53*, 4002–4008.

- 10 Sanson, B.; Nachon, F.; Colletier, J.-P.; Froment, M.-T.; Toker, L.; Greenblatt, H. M.; Sussman, J. L.; Ashani, Y.; Masson, P.; Silman, I.; Weik, M. Crystallographic snapshots of nonaged and aged conjugates of soman with acetylcholinesterase, and of a ternary complex of the aged conjugate with pralidoxime. *J. Med. Chem.* **2009**, *52*, 7593–7603.
- 11 Worek, F.; Diepold, C.; Eyer, P. Dimethylphosphoryl-inhibited human cholinesterases: inhibition, reactivation, and aging kinetics. *Arch. Toxicol.* **1999**, *73*, 7–14.
- 12 Trovaslet-Leroy, M.; Musilova, L.; Renault, F.; Brazzolotto, X.; Misik, J.; Novotny, L.; Froment, M.-T.; Gillon, E.; Loiodice, M.; Verdier, L.; Masson, P.; Rochu, D.; Jun, D.; Nachon, F. Organophosphate hydrolases as catalytic bioscavengers of organophosphorus nerve agents. *Toxicol. Lett.* **2011**, *206*, 14–23.
- 13 Masson, P.; Nachon, F.; Lockridge, O. Structural approach to the aging of phosphorylated cholinesterases. *Chem. Biol. Interact.* **2010**, *187*, 157–162.
- 14 Worek, F.; Thiermann, H.; Szinicz, L.; Eyer, P. Kinetic analysis of interactions between human acetylcholinesterase, structurally different organophosphorus compounds and oximes. *Biochem. Pharmacol.* **2004**, *68*, 2237–2248.
- 15 Segall, Y.; Waysbort, D.; Barak, D.; Ariel, N.; Doctor, B. P.; Grunwald, J.; Ashani, Y. Direct observation and elucidation of the structures of aged and nonaged phosphorylated cholinesterases by ³¹P NMR spectroscopy. *Biochemistry* **1993**, *32*, 13441–13450.
- 16 Wilson, I. B. Acetylcholinesterase. XI. Reversibility of tetraethyl pyrophosphate. *J. Biol. Chem.* **1951**, *190*, 111–117.
- 17 Wilson, I. B.; Meislich, E. K. Reactivation of acetylcholinesterase inhibited by alkylphosphates. *J. Am. Chem. Soc.* **1953**, *75*, 4628–4629.
- 18 Childs, A. F.; Davies, D. R.; Green, A. L.; Rutland, J. P. The reactivation by oximes and hydroxamic acids of cholinesterase inhibited by organo-phosphorus compounds. *Br. J. Pharmacol.* **1955**, *10*, 462–465.
- 19 Green, A. L.; Smith, H. J. The reactivation of cholinesterase inhibited with organophosphorus compounds. I. Reactivation by 2-oxalodioximes. *Biochem. J.* **1958**, *68*, 28–31.
- 20 Wilson, I. B.; Ginsburg, B. A powerful reactivator of alkylphosphate-inhibited acetylcholinesterase. *Biochim. Biophys. Acta* **1955**, *18*, 168–170.
- 21 Wilson, I. B.; Ginsburg, S. Reactivation of alkylphosphate inhibited acetylcholinesterase by bis quaternary derivatives of 2-PAM and 4-PAM. *Biochem. Pharmacol.* **1959**, *1*, 200–206.
- 22 Luettringhaus, A.; Hagedorn, I. Quaternary hydroxyiminomethylpyridinium salts. The dischloride of bis-(4-hydroxyiminomethyl-1-pyridinium-methyl)-ether (lueh6), a new reactivator of acetylcholinesterase inhibited by organic phosphoric acid esters. *Arzneimittelforschung* **1964**, *14*, 1–5.
- 23 Hagedorn, I.; Gundel, W. H.; Schoene, K. Reactivation of phosphorylated acetylcholinesterase with oximes: contribution to the study of the reaction course. *Arzneimittelforschung* **1969**, *19*, 603–606.
- 24 Eyer, P.; Hagedorn, I.; Klimmek, R.; Lippstreu, P.; Löffler, M.; Oldiges, H.; Spöhrer, U.; Steidl, I.; Szinicz, L.; Worek, F. HLö-7 dimethanesulfonate, a potent bispyridinium-dioxime against anticholinesterases. *Arch. Toxicol.* **1992**, *66*, 603–621.
- 25 Worek, F.; Eyer, P.; Aurbek, N.; Szinicz, L.; Thiermann, H. Recent advances in evaluation of oxime efficacy in nerve agent poisoning by in vitro analysis. *Toxicol. Appl. Pharmacol.* **2007**, *219*, 226–234.
- 26 Worek, F.; Aurbek, N.; Koller, M.; Becker, C.; Eyer, P.; Thiermann, H. Kinetic analysis of reactivation and aging of human acetylcholinesterase inhibited by different phosphoramidates. *Biochem. Pharmacol.* **2007**, *73*, 1807–1817.
- 27 Carletti, E.; Aurbek, N.; Gillon, E.; Loiodice, M.; Nicolet, Y.; Fontecilla-Camps, J. C.; Masson, P.; Thiermann, H.; Nachon, F.; Worek, F. Structure-activity analysis of aging and reactivation of human butyrylcholinesterase inhibited by analogues of tabun. *Biochem. J.* **2009**, *421*, 97–106.
- 28 Terrier, F.; Rodriguez-Dafonte, P.; Le Guével, E.; Moutiers, G. Revisiting the reactivity of oximate α -nucleophiles with electrophilic phosphorus centers. Relevance to detoxification of sarin, soman and DFP under mild conditions. *Org. Biomol. Chem.* **2006**, *4*, 4352–4363.
- 29 Ekström, F.; Astot, J. C.; Pang, Y. P. Novel nerve-agent antidote design based on crystallographic and mass spectrometric analyses of tabun-conjugated acetylcholinesterase in complex with antidotes. *Clin. Pharmacol. Ther.* **2007**, *82*, 282–293.
- 30 Hörnberg, A.; Artursson, E.; Wärme, R.; Pang, Y. P.; Ekström, F. Crystal structures of oxime-bound fenamiphos-acetylcholinesterases: reactivation involving flipping of the His447 ring to form a reactive Glu334-His447-oxime triad. *Biochem. Pharmacol.* **2010**, *79*, 507–515.
- 31 Taylor, P.; Kovarik, Z.; Reiner, E.; Radić, Z. Acetylcholinesterase: Converting a vulnerable target to a template for antidotes and detection of inhibitor exposure. *Toxicology* **2007**, *233*, 70–78.
- 32 Hörnberg, A.; Artursson, E.; Wärme, R.; Pang, Y. P.; Ekström, F. Crystal structures of oxime-bound fenamiphos-acetylcholinesterases: reactivation involving flipping of the His447 ring to form a reactive Glu334-His447-oxime triad. *Biochem. Pharmacol.* **2010**, *79*, 507–515.
- 33 Ekström, F.; Hörnberg, A.; Artursson, E.; Hammarström, L. G.; Schneider, G.; Pang, Y. P. Structure of HI-6/sarin-acetylcholinesterase determined by X-ray crystallography and molecular dynamics simulation: Reactivator mechanism and design. *PLoS One* **2009**, *4*, e5957.
- 34 Nachon, F.; Carletti, E.; Worek, F.; Masson, P. Aging mechanism of butyrylcholinesterase inhibited by an N-methyl analogue of tabun: Implications of the trigonal-bipyramidal transition state rearrangement for the phosphorylation or reactivation of cholinesterases. *Chem. Biol. Interact.* **2010**, *187*, 44–48.
- 35 Ohta, H.; Ohmori, T.; Suzuki, S.; Ikegaya, H.; Sakurada, K.; Takatori, T. New Safe method for preparation of sarin-exposed human erythrocytes acetylcholinesterase using non-toxic and stable sarin analogue isopropyl p-nitrophenyl methylphosphonate and its application to evaluation of nerve agent antidotes. *Pharm. Res.* **2006**, *23*, 2827–2833.
- 36 Okuno, S.; Sakurada, K.; Ohta, H.; Ikegaya, H.; Kazui, Y.; Akutsu, T.; Takatori, T.; Iwadate, K. Blood-brain barrier penetration of novel pyridinealdoxime methiodide (PAM)-type oximes examined by brain microdialysis with LC-MS/MS. *Toxicol. Appl. Pharmacol.* **2008**, *227*, 8–15.
- 37 Cabal, J.; Kuča, K.; Kassa, J. Specification of the structure of oximes able to reactivate tabun-inhibited acetylcholinesterase. *Basic Clin. Pharmacol. Toxicol.* **2004**, *95*, 81–86.
- 38 Musilek, K.; Komloova, M.; Holas, O.; Horova, A.; Pohanka, M.; Gunn-Moore, F.; Dohnal, V.; Dolezal, M.; Kuča, K. Mono-oxime bisquaternary acetylcholinesterase reactivators with prop-1,3-diyll linkage—Preparation, in vitro screening and molecular docking. *Biorg. Med. Chem.* **2011**, *19*, 754–762.
- 39 Kuča, K.; Bielavský, J.; Cabal, J.; Kassa, J. Synthesis of a new reactivator of tabun-inhibited acetylcholinesterase. *Biorg. Med. Chem. Lett.* **2003**, *13*, 3545–3547.
- 40 Kassa, J.; Kuča, K.; Karasová, J.; Musilek, K. The development of new oximes and the evaluation of their reactivating, therapeutic and neuroprotective efficacy against tabun. *Mini Rev. Med. Chem.* **2008**, *8*, 1134–1143.
- 41 Artursson, E.; Akfur, C.; Homborg, A.; Worek, F.; Ekström, F. Reactivation of tabun-hAChE investigated by structurally analogous oximes and mutagenesis. *Toxicology* **2009**, *265*, 108–114.
- 42 Ekström, F.; Pang, Y.-P.; Boman, M.; Artursson, E.; Akfur, C.; Börjegen, S. Crystal structures of acetylcholinesterase in complex with HI-6, Ortho-7 and obidoxime: Structural basis for differences in the ability to reactivate tabun conjugates. *Biochem. Pharmacol.* **2006**, *72*, 597–607.
- 43 Kuča, K.; Cabal, J.; Jung, Y. S.; Musilek, K.; Soukup, O.; Jun, D.; Pohanka, M.; Musilova, L.; Karasová, J.; Novotný, L.; Hrabivova, M. Reactivation of human brain homogenate cholinesterases inhibited by tabun using newly developed oximes K117 and K127. *Basic Clin. Pharmacol. Toxicol.* **2009**, *105*, 207–210.
- 44 Acharya, J.; Dubey, D. K.; Srivastava, A. K.; Raza, S. K. In vitro evaluation of bis-pyridinium oximes bearing methoxy alkane linker as reactivators of sarin inhibited human acetylcholinesterase. *Toxicol. in Vitro* **2010**, *24*, 1797–1802.
- 45 Musilek, K.; Jun, D.; Cabal, J.; Kassa, J.; Gunn-Moore, F.; Kuča, K. Design of a potent reactivator of tabun-inhibited acetylcholinesterase synthesis and evaluation of (E)-1-(4-Carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene Dibromide (K203). *J. Med. Chem.* **2007**, *50*, 5514–5518.
- 46 Musilek, K.; Holas, O.; Misik, J.; Pohanka, M.; Novotny, L.; Dohnal, V.; Opletalova, V.; Kuča, K. Monooxime-monocarbamoyl bispyridinium xylene-linked reactivators of acetylcholinesterase—Synthesis, in vitro and toxicity evaluation, and docking studies. *ChemMedChem* **2010**, *5*, 247–254.
- 47 Acharya, J.; Dubey, D. K.; Srivastava, A. K.; Raza, S. K. In vitro reactivation of sarin-inhibited human acetylcholinesterase (AChE) by bis-pyridinium oximes connected by xylene linkers. *Toxicol. in Vitro* **2011**, *25*, 251–256.
- 48 Ballabh, P.; Bruan, A.; Nedergaard, M. The blood-brain barrier: An overview structure, regulation, and clinical implications. *Neurobiol. Dis.* **2004**, *16*, 1–13.
- 49 Abbott, N. J.; Ronnback, L.; Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* **2006**, *7*, 41–53.
- 50 Lorke, D. E.; Kalasz, H.; Petroianu, G. A.; Tekes, K. Entry of oximes into the brain: A review. *Curr. Med. Chem.* **2008**, *15*, 743–753.
- 51 Sakurada, K.; Matsubara, K.; Shimizu, K.; Shiono, H.; Seto, Y.; Tsuge, K.; Yoshino, M.; Sakai, I.; Mukoyama, H.; Takatori, T. Pralidoxime iodide (2-PAM) penetrates across the blood-brain barrier. *Neurochem. Res.* **2003**, *28*, 1401–1407.
- 52 Cook, A. M.; Mieux, K. D.; Owen, R. D.; Pesaturo, A. B.; Hatton, J. Intracerebroventricular administration of drugs. *Pharmacotherapy* **2009**, *29*, 832–845.
- 53 Bradley, W. G., Jr. MR-guided focused ultrasound: A potentially disruptive technology. *J. Am. Coll. Radiol.* **2009**, *6*, 510–513.
- 54 Neuwelt, E. A.; Frenkel, E. P.; Diehl, J. T.; Maravilla, K. R.; Vu, L. H.; Clark, W. K.; Rappoport, S. I.; Barnett, P. A.; Hill, S. A.; Lewis, S. E.; Ehle, A. L.; Beyer, C. W., Jr.; Moore, R. J. Osmotic blood-brain barrier disruption: A New means of increasing chemotherapeutic agent delivery. *Trans. Am. Neurol. Assoc.* **1979**, *104*, 256–260.
- 55 Wagner, S.; Kuffelthner, J.; Zensi, A.; Dadparvar, M.; Wien, S.; Bungert, J.; Vogel, T.; Worek, F.; Kreuter, J.; von Briesen, A. Nanoparticulate transport of oximes over an in vitro blood-brain barrier model. *PLoS One* **2010**, *5*, No. e14213.
- 56 Joosen, M. J. A.; van der Schans, M. J.; van Dijk, C. G. M.; Kuijpers, W. C.; Wortelboer, H. M.; van Helden, H. P. M. Increasing oxime efficacy by blood-brain barrier modulation. *Toxicol. Lett.* **2011**, *206*, 67–71.

- 57 Carman, A. J.; Mills, J. H.; Krenz, A.; Kim, D. G.; Bynoe, M. S. Adenosine receptor signaling modulates permeability of the blood-brain barrier. *J. Neurosci.* **2011**, *31*, 13272–13280.
- 58 Jeong, H. C.; Kang, N. S.; Park, N. J.; Yum, E. K.; Jung, Y. S. Reactivation potency of fluorinated pyridinium oximes for acetylcholinesterases inhibited by paraoxon organophosphorus agent. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1214–1217.
- 59 Jeong, H. C.; Park, N. J.; Chae, C. H.; Musilek, K.; Kassa, J.; Kuca, K.; Jung, Y. S. Fluorinated pyridinium oximes as potential reactivators for acetylcholinesterases inhibited by paraoxon organophosphorus agent. *Bioorg. Med. Chem.* **2009**, *17*, 6213–6217.
- 60 Heldman, E.; Ashani, Y.; Raveh, L.; Rachaman, E. S. Sugar conjugates of pyridinium aldoximes as antidotes against organophosphonate poisoning. *Carbohydr. Res.* **1986**, *151*, 337–347.
- 61 Garcia, G. E.; Campbell, A. J.; Olson, J.; Moorad-Doctor, D.; Morthole, V. I. Novel oximes as blood-brain barrier penetrating cholinesterase reactivators. *Chem. Biol. Interact.* **2010**, *187*, 199–206.
- 62 Shek, E.; Higuchi, T.; Bodor, N. Improved delivery through biological membranes. 2. Distribution, excretion, and metabolism of N-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride, a pro-drug of N-methylpyridinium-2-carbaldoxime chloride. *J. Med. Chem.* **1976**, *19*, 108–112.
- 63 Demar, J. C.; Clarkson, E. D.; Ratcliffe, R. H.; Campbell, A. J.; Thangavelu, S. G.; Herdman, C. A.; Leader, H.; Schulz, S. M.; Marek, E.; Medynets, M. A.; Ku, T. C.; Evans, S. A.; Khan, F. A.; Owens, R. R.; Nambiar, M. P.; Gordon, R. K. Pro-2-PAM therapy for central and peripheral cholinesterases. *Chem. Biol. Interact.* **2010**, *187*, 191–198.
- 64 Shih, T. M.; Skovira, J. W.; O'Donnell, J. C.; McDonough, J. H. Central acetylcholinesterase reactivation by oximes improves survival and terminates seizures following nerve agent intoxication. *Adv. Stud. Biol.* **2009**, *1*, 155–196.
- 65 Skovira, J. W.; O'Donnell, J. C.; Kopolovitz, I.; Kan, R. K.; McDonough, J. H.; Shih, T. M. Reactivation of brain acetylcholinesterase by monoisobutylacetone increases the therapeutic efficacy against nerve agents in guinea pigs. *Chem. Biol. Interact.* **2010**, *187*, 318–324.
- 66 de Koning, M. C.; van Grol, M.; Noort, D. Peripheral site ligand conjugation to a non-quaternary oxime enhances reactivation of nerve agent-inhibited human acetylcholinesterase. *Toxicol. Lett.* **2011**, *206*, 54–59.
- 67 Sit, R. K.; Radić, Z.; Gerardi, V.; Zhang, L.; Garcia, E.; Katalinić, M.; Amitai, G.; Kovarik, Z.; Fokin, V. V.; Sharpless, K. B.; Taylor, P. New structural scaffolds for centrally acting oxime reactivators of phosphorylated cholinesterases. *J. Biol. Chem.* **2011**, *286*, 19422–19430.
- 68 Saint-André, G.; Klichyna, M.; Kodepelly, S.; Louise-Lerliche, L.; Gillon, E.; Renard, P.-Y.; Nachon, F.; Baati, R.; Wagner, A. Design, synthesis and evaluation of new α -nucleophiles for the hydrolysis of organophosphorus nerve agents: application to the reactivation of phosphorylated acetylcholinesterase. *Tetrahedron* **2011**, *67*, 6352–6361.
- 69 Mercey, G.; Verdelet, T.; Saint-André, G.; Gillon, E.; Wagner, A.; Baati, R.; Jean, L.; Nachon, F.; Renard, P.-Y. First efficient uncharged reactivators for the dephosphorylation of poisoned human acetylcholinesterase. *Chem. Commun.* **2011**, *47*, 5295–5297.
- 70 Ashani, Y.; Bhattacharjee, A. K.; Leader, H.; Saxena, A.; Doctor, B. P. Inhibition of cholinesterases with cationic phosphoryl oximes highlights distinctive properties of the charged pyridine groups of quaternary oxime reactivators. *Biochem. Pharmacol.* **2003**, *66*, 191–202.
- 71 Louise-Lerliche, L.; Păunescu, E.; Saint-André, G.; Baati, R.; Romieu, A.; Wagner, A.; Renard, P.-Y. A HTS assay for the detection of organophosphorus nerve agents scavengers. *Chem.—Eur. J.* **2010**, *16*, 3510–3523.
- 72 Kalisiak, J.; Ralph, E. C.; Zhang, J.; Cashman, J. R. Amidine-oximes: Reactivators for organophosphate exposure. *J. Med. Chem.* **2011**, *54*, 3319–3330.
- 73 Antonijević, B.; Stojiljković, M. P. Unequal efficacy of pyridinium oximes in acute organophosphate poisoning. *Clin. Med. Res.* **2007**, *5*, 71–82.
- 74 Worek, F.; Aurbek, N.; Thiermann, H. Reactivation of organophosphate-inhibited human AChE by combinations of obidoxime and HI 6 in vitro. *J. Appl. Toxicol.* **2007**, *27*, 582–588.