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Design, synthesis and biological evaluation of novel tetrahydroacridine pyridine- aldoxime and -amidoxime hybrids as efficient uncharged reactivators of nerve agent-inhibited human acetylcholinesterase

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ABSTRACT

A series of new uncharged functional acetylcholinesterase (AChE) reactivators including heterodimers of tetrahydroacridine with 3-hydroxy-2-pyridine aldoximes and amidoximes has been synthesized. These novel molecules display *in vitro* reactivation potencies towards VX-, tabun- and paraoxon-inhibited human AChE that are superior to those of the mono- and bis-pyridinium aldoximes currently used against nerve agent and pesticide poisoning. Furthermore, these uncharged compounds exhibit a broader reactivity spectrum compared to currently approved remediation drugs.

1. Introduction

Organophosphorus compounds (OP) include the extremely toxic chemical warfare agents (CWA) (sarin, soman, cyclosarin, tabun, methylphosphonothioate VX) and pesticides (paraoxon, parathion, tetraethyl pyrophosphate (TEPP)) (Fig. 1). Their acute toxicity results from the irreversible inhibition of acetylcholinesterase (AChE) through phosphylation of its catalytic serine [1]. Accumulation of neurotransmitter acetylcholine (ACh) at cholinergic synapses ensues, leading to nervous and respiratory failures. Depending on the class of OP and on the administrated dose, death can occur within minutes [2].

Due to the similarity between the chemical precursors of CWA and pesticides, and to the relatively simple chemistry involved in their synthesis, efforts to control the proliferation of these agents have proved of limited success [3]. Illustrative examples include the terrorist attack in the Tokyo subway in 1995, the bombing of Kurd civilians during the Iraq–Iran war in 1988, and that of civilians in Syria, as reported in August 2013. Additionally, despite the international efforts aimed at regulating and lessening the use of these environmentally toxic compounds, ca. 100 different OP are still used intensively as pest control agents, with only anecdotal monitoring. This results in about 3,000,000 acute intoxications per year, 200,000 of which lead to death [4,5]. Therefore, the development of effective measures to counteract OP poisoning remains a challenging issue to protect and treat both civilian and military populations [6].

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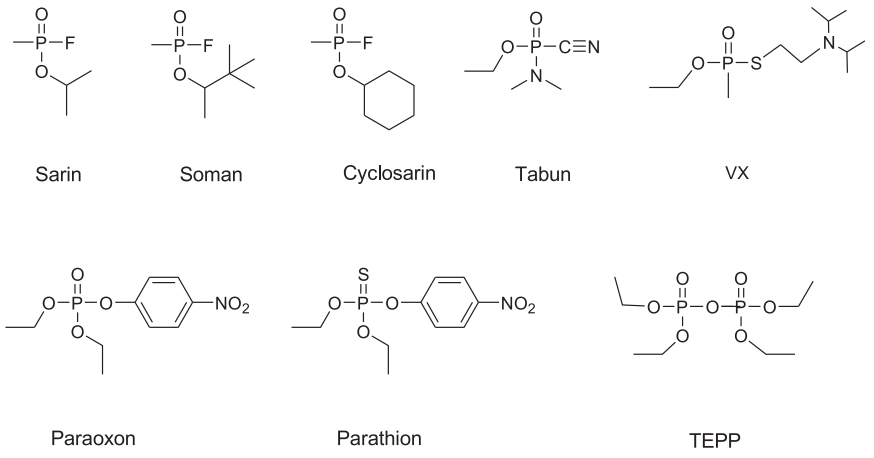

Fig. 1. Structures of organophosphorus CWA and pesticides.

The current treatment against OP poisoning consists in the administration of a combination of atropine (antimuscarinic agent) and diazepam (anticonvulsant drug), to limit convulsions, and of a standard pyridinium oxime (pralidoxime, trimedoxime, HI-6, obidoxime, and HLö-7, Fig. 2) to reactivate AChE. Oximes exert their action on OP-inhibited AChE by attacking the phosphorus atom of the phosphylated serine, yielding to the removal of the phosphonate and recovery of the enzyme's catalytic activity. To this end, pyridinium oximes must display high nucleophilicity, which is generally attained by the formation of an oximate anion at physiological pH. As of today, however, not a single oxime has proven equally effective against all types of OP-inhibited AChE, and most are ineffective for the reactivation of tabun-inhibited hAChE [6].

Another weakness of currently approved pyridinium aldoximes is their difficulty in crossing the blood–brain barrier (BBB) owing to the permanent charge carried by the oximate [7]. For example, it was estimated using *in vivo* rat brain microdialysis coupled with HPLC/UV, that the BBB penetration of the most commonly used oxime, 2-PAM, is only 10% [8]. Therefore, oximes reactivating AChE in the peripheral nervous system are not effective in the brain and, consequently, do not protect against the neurological effects of OP-exposure.

To overcome this obstacle, we recently reported the synthesis of a series of uncharged oxime-based compounds, both able to cross the BBB and to reactivate OP-inhibited AChE in the CNS [6]. Monoisonitrosoacetone (MINA) and diacetylmonooxime (DAM) respectively bearing the α-ketoaldoxime and ketoxime moieties (Fig. 3) were also reported to cross the BBB, yet their *in vitro* reactivation potency towards OP-inhibited AChE was lower than that exhibited by 2-PAM [9]. Connection of an α-ketoaldoxime function to a piperidine-derived peripheral site ligand (PSL) allowed increasing the affinity for AChE, resulting in higher reactivation rates of sarin- and VX- inhibited AChE. Yet, these compounds were still less efficient than currently used pyridinium oximes towards the latter, and totally inefficient towards tabun-inhibited AChE [10]. Hydroxyimino acetamides **1a**–**b** showed *in vitro* reactivation efficacy superior to MINA and DAM, but remained less effective than 2-PAM, except for cyclosarin [11]. Likewise, amidine-oxime reactivators **2a,b** were less potent than 2-PAM in reactivating AChE *in vitro* [12]. Low reactivation potency of these new uncharged reactivators is likely due to their higher pKa, which results in inefficient deprotonation at physiological pH and thus, in a reduced nucleophilicity (for instance, for MINA, pKa = 8.3) [13].

Recently we found that 3-hydroxy-2-pyridine aldoximes **3** and amidoxime **4** (Fig. 4) exhibited high reactivation first order rate constant towards VX-inhibited hAChE ($k_r = 0.5 \pm 0.1$ min^{-1} and 0.08 ± 0.1 min^{-1}, respectively) [14,15], *i.e.* values either similar or one order of magnitude larger than that displayed by 2-PAM ($k_r = 0.06 \pm 0.01$ min^{-1}). Owing to the lower affinity of **3** and **4** for VX-hAChE (second order reactivation rate constants $k_{r2} = k_r/K_D$ are 0.015 and 0.0026 mM^{-1} min^{-1} for **3** and **4**, respectively, to compare to 0.28 mM^{-1} min^{-1} for 2-PAM), much higher concentrations are however required to reach the desirable reactivation of the enzyme.

We have shown that connection of 3-hydroxy-2-pyridinaldoxime reactivators with a PSL ligand, such as phenyltetrahydroisoquinoline (PIQ) [16], results in a dramatically improved affinity for the enzyme and, consequently, in increased

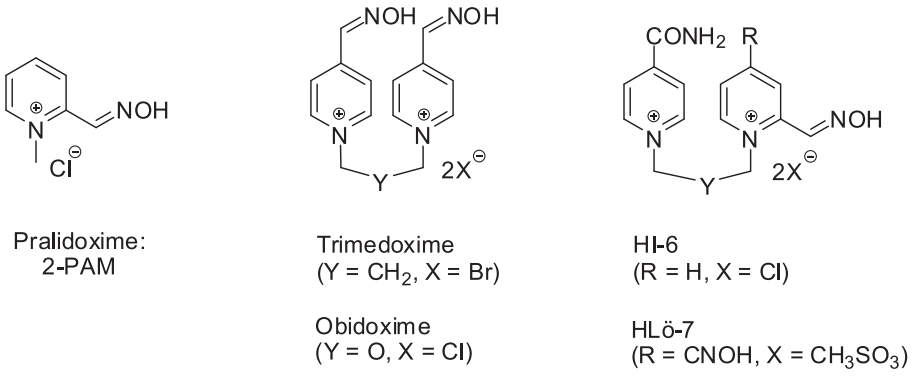

Fig. 2. Structures of developed pyridinium aldoxime reactivators.