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New efficient imidazolium aldoxime reactivators for nerve agent-inhibited acetylcholinesterase

Zhao Wei [a], Yan-qin Liu [b], Xin-bo Zhou [a], Yuan Luo [b], Chun-qian Huang [b], Yong-an Wang [b,*], Zhi-bing Zheng [a,*], Song Li [a]

[a] Laboratory of Computer-Aided Drug Design & Discovery, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China
[b] Department of Military Toxicology and Biochemical Pharmacology, Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China

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ABSTRACT

Herein, we described a new class of uncharged non-pyridinium reactivators for nerve agent-inhibited acetylcholinesterase (AChE). Based on a dual site binding strategy, we conjugated the imidazolium aldoxime to different peripheral site ligands (PSLs) of AChE through alkyl chains. Compared with the known quaternary pyridinium reactivators, two of the resulting conjugates (**7g** and **7h**) were highlighted to be the first efficient non-pyridinium oxime conjugates exhibiting similar or superior ability to reactivate sarin-, VX- and tabun-inhibited AChE. Moreover, they were more broad-spectrum reactivators.

Organophosphate (OP) nerve agents (e.g., sarin, VX, tabun, and soman) are highly toxic compounds and pose potential threats to the military and the public (such as the terrorist attacks in Tokyo subway in 1995 and the recent Syria civil war).[1] Acetylcholinesterase (AChE) is a hydrolase which terminates cholinergic neurotransmission by hydrolyzing the neurotransmitter acetylcholine (ACh).[2] The acute toxic effect of OPs stems from irreversible inhibition of AChE by forming a covalent P–O bond in the active site (A-site) of the enzyme, and the resulting accumulation of unhydrolyzed ACh in synapse would lead to cholinergic crisis, respiratory distress, convulsive seizures and ultimately death.[3]

Current available drugs for OP-poisoning include an AChE reactivator, such as one of the standard pyridinium oximes (e.g., pralidoxime or 2-PAM, trimedoxime or TMB-4, obidoxime, HI-6, and MMB-4 Fig. 1),[4,5] a muscarinic receptor antagonist (e.g., atropine) and an anticonvulsant (i.e., diazepam).[6,7] It is generally believed that the highly nucleophilic oximes could break the P–O bond and restore the enzyme's activity.[8] Whereas one drawback for these quaternary reactivators is that they provide little or no protection against neurological effects of OP exposure in the central nervous system (CNS), because the permanent charges seriously limit their blood–brain barrier (BBB) penetration,[9] while the brain is a major target of nerve agents.[10] Another weakness is that there is no

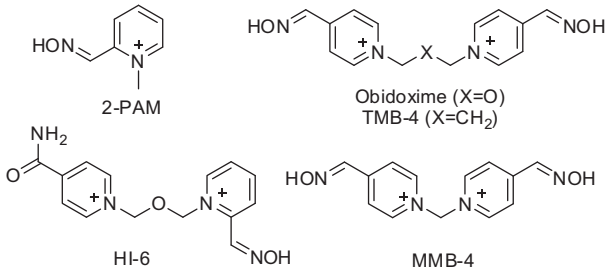

Figure 1. Current available pyridinium oximes in the treatment of OP poisoning.

universal broad-spectrum oxime suitable for the antidotal treatment of various OP-poisoning, especially in the cases of tabun and soman. For instance, HI-6, the best available reactivators to date, is inefficient in reactivating tabun-inhibited hAChE.[4,5] So it remains a challenge to develop effective antidotes for OP exposure.

Over the past several decades, a number of strategies have been developed to overcome the problems mentioned above.[11–13] It was proven that nonionic reactivators (e.g., monoisonitrosoacetone or MINA, amidine-oximes, Fig. 2) would facilitate the BBB penetration as a result of increased lipophilicity, and they showed obvious superiority to charged 2-PAM as antidotes for CNS poisoning.[14–17] Nevertheless, the absence of charge will cause decreasing reactivation potency because the uncharged reactivators don't properly bind

* Corresponding authors. Tel.: +86 01066931642.
E-mail address: zzbcaptain@aliyun.com (Z.-b. Zheng).

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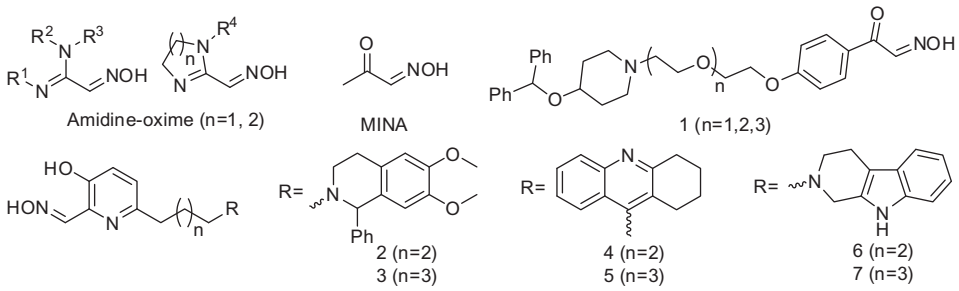

Figure 2. Chemical structures of some reported nonionic oximes.

to the aromatic moiety of the A-site,[18] which buries at the bottom of a deep active gorge into the AChE.[19] However, there is a peripheral site (P-site) at the entrance of the active gorge, serving as binding site for distinctive substrates.[20,21] Accordingly, a dual site binding strategy was proposed, a peripheral site ligand (PSL) was conjugated to the oxime group to contribute extra affinity for AChE at the P-site.[22,23] Based on this strategy, a series of promising reactivators were synthesized (conjugates **1**–**7**, Fig. 2), and among these compounds, conjugate **3** outperformed all others. But all these conjugates are pyridyl aldoximes and they are difficult to prepare due to a long synthetic route, moreover, their reactivation potencies against sarin-inhibited hAChE are still unknown.[24–27] Hence we sought to develop alternatives of the pyridyl aldoximes.

From the beginning, a kind of imidazolium aldoximes drew our attention, whose chemical structures are similar to the amidine oximes mentioned above.[16,17] Early in 1990s, quaternary imidazolium aldoximes had been intensively investigated as reactivators,[28–31] and recently, a series of nonquaternary imidazolium aldoximes (Fig. 3) were reported as antidotes for OP poisoning.[32–35] Nevertheless, the reactivation abilities of these oximes are still not satisfying and they need further modification for more efficient reactivators.

Therefore, enlightened by the dual site binding strategy, the PSL (phenyl-tetrahydroisoquinoline, PIQ) from conjugates **3** was connected to the imidazolium oxime through flexible alkyl chains, aiming at developing more efficient tertiary reactivators (Fig. 3). The designed conjugates were expected to possess enhanced BBB penetration due to dramatic improvement of calculated lipophilicity (a higher value of $S + \log P$ indicates higher lipophilicity; values are listed in Table 1). In this study, we prepared eight new tertiary imidazolium reactivators (Fig. 3), the in vitro screening essays demonstrated that conjugates **7g** and **7h** were efficient and relatively broad-spectrum reactivators.

Two different approaches were utilized for the synthesis of conjugates **7a**–**7h**. They are outlined in Schemes 1 and 2. In both approaches, the starting materials tetrahydroisoquinoline (TIQ) **1a** and PIQ **1b** were firstly synthesized. In path A, it was started with N-alkylation of **1a** and **1b** with two different halide **2a** and **2b** to give the intermediates **3a**, **3d**, **3e** and **3h**. They were treated with TsCl in pyridine to produce the tosylates **4a**, **4d**, **4e** and **4h.** Then N-alkylation of imidazole-2-carboxaldehyde **5a** with the tosylates provided compounds **6a**, **6d**, **6e** and **6h.** Finally, treatment of the latters with hydroxylammonium chloride afforded the target conjugates **7a**, **7d**, **7e** and **7h**, whose methylene length is either 3 or 6 units. Unfortunately, efforts to obtain conjugates with methylene length of 4 or 5 units using method A failed, because unexpected compounds were produced in the N-alkylation of **5a**. Thus, we turned to path B and synthesized the desired compounds successfully. The starting materials **2b** and **2c** were converted to tosylates **3b** and **3c** in a similar way as in path A. In comparison with halide moiety in **3b** and **3c**, the tosylate moiety was much easier to undergo an N-alkylation reaction with **5a** to obtain the intermediates **4b** and **4c**, and then they were readily converted to oximes **6b** and **6c** by treating with hydroxylammonium chloride. At last, N-alkylation was conducted again between **6b**, **6c** and **1a**, **1b** to give the desired compounds 7**b**, **7c**, **7f** and **7g**. In addition, compound **3** (Fig. 3), one of the most promising uncharged reactivators in the literature, was prepared in a similar way as described by Mercey et al.[26] In contrast to **7a**–**7h**, the synthetic route of pyridine-aldoxime **3** was long as ten steps from the starting material **1b**. Furthermore, the synthesis of **3** involved a Sonogashira coupling reaction and two protection and deprotection reactions, which were complex, costly and poor yield, while the synthesis of conjugates **7a**–**7h** was simple, economical and high-yield.

The in vitro experiments were conducted with fresh human whole blood serving as enzyme source. The enzyme activity was measured using a similar method of Ellman et al.[36] and the experimental detail was described in the section of supporting information. Two oxime concentrations (1 mM and 0.1 mM) were selected

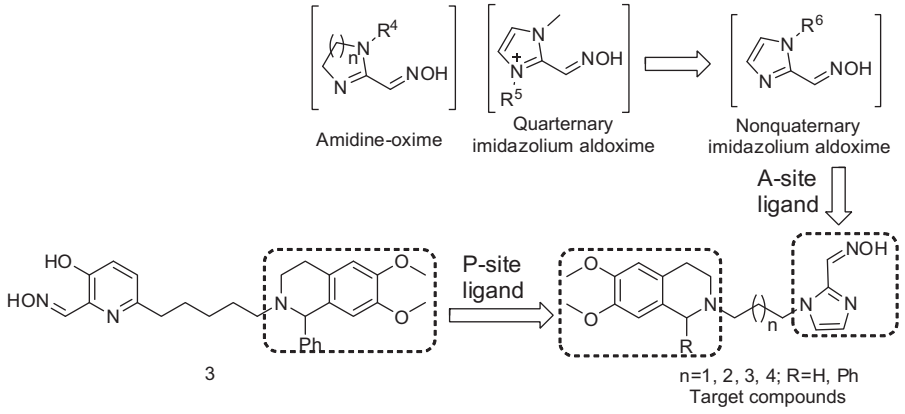

Figure 3. Chemical structures of the designed target compounds based on a dual site binding strategy.