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Novel series of bispyridinium compounds bearing a (*Z*)-but-2-ene linker—Synthesis and evaluation of their reactivation activity against tabun and paraoxon-inhibited acetylcholinesterase

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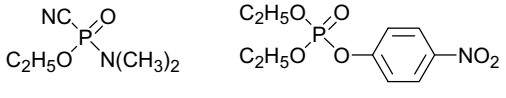
Abstract—Six novel AChE reactivators with a (*Z*)-but-2-ene linker were synthesized using the known synthetic pathways. Their ability to reactivate AChE, which had been previously inhibited by nerve agent tabun or pesticide paraoxon, was tested in vitro and compared to pralidoxime, HI-6, obidoxime, and K075. The novel synthesized compounds were found to be ineffective against GA-inhibited AChE but the ability of (*Z*)-1,4-bis(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide to reactivate paraoxon-inhibited AChE was comparable with that of oxime K075. Notably, the oxime group in position four substantially increased the ability of the novel compounds to reactivate paraoxon-inhibited AChE.

Acetylcholinesterase (AChE, EC 3.1.1.7) is a well-known enzyme studied for various reasons, for example, Alzheimer's disease, Parkinson disease, an eco-toxicology marker.[1–3] The enzyme occurs throughout invertebrates and vertebrates species.[4,5] Many inhibitors of AChE exist both in natural and artificial compounds.[5,6] The organophosphorus inhibitors of AChE (OPI) are some of the oldest artificial inhibitors synthesized (Fig. 1), for example the first members of this group were synthesized as military nerve agents (NA; e.g., sarin, soman, tabun).[5] Afterwards many similar compounds with decreased toxicity were prepared. These are currently used in agricultural production as pesticides (e.g., parathion, chlorpyrifos, diazinon) or for industrial purposes as softening agents and flame retardants.[7] Therapeutically, metrifonate was also proved for treatment of Alzheimer's disease.[8]

All OPI irreversibly inhibit AChE through binding to a serine hydroxyl within the active site of the enzyme. Subsequently, the AChE is not able to fulfill its physiological role in cholinergic transmission and so leads to over-stimulation by acetylcholine with the resultant possibility of respiratory failure and death.[5]

The AChE reactivators (e.g., pralidoxime, obidoxime, HI-6; Fig. 2) in combination with atropine have been used to counteract the poisonous effects of OPI. The reactivator is able to cleave the covalent bond between the OPI and AChE, restoring the activity of the enzyme. This reactivation process consists of an attack of the nucleophilic oxime group (in the form of oximate anion) on the covalent bond. However, there is no reactivator able to coun-



tabun paraoxon

Figure 1. Examples of organophosphorus inhibitors of acetylcholinesterase.

Keywords: Acetylcholinesterase; Reactivation; Nerve agent; Tabun; Pesticide; Paraoxon; Reactivator; Oxime.
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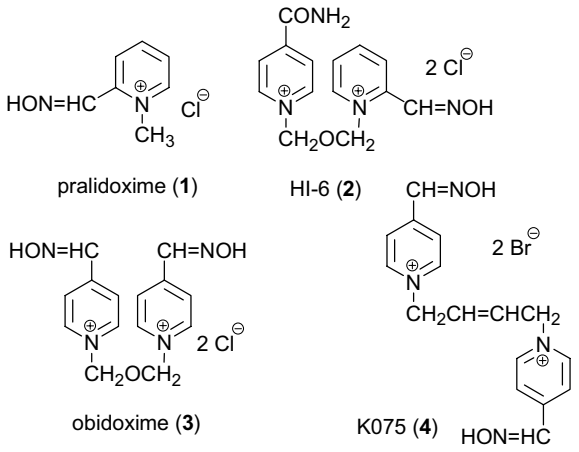
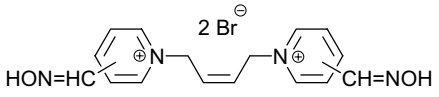


Figure 2. Examples of AChE reactivators used and tested.

teract the full spectrum of OPI.[9] Moreover, the covalent bond present between OPI and the enzyme is very stable and is subjected to intramolecular modifications called 'aging'.[10] For example, the nerve agent tabun (GA; *O*-ethyl-*N*,*N*-dimethylphosphoramidocyanidate) undergoes one of the fastest aging processes that makes GA-inhibited AChE almost impossible for reactivation.[11] Ekström et al. described this modification by changes within the cavity of GA-inhibited AChE, especially at the hydrogen bonds of His447.[12,13] Additionally, the conformational change of Pro338 partially closes the narrow AChE cleft. Consequently the phosphoramidoyl group of GA is replaced by a molecule of water and the rest of GA molecule is coordinated in the enzyme's cavity.



Compound	Oxime position
5	2,2′-CH=NOH
6	3,3′-CH=NOH
7	4,4′-CH=NOH
8	2,3′-CH=NOH
9	2,4′-CH=NOH
10	3,4′-CH=NOH

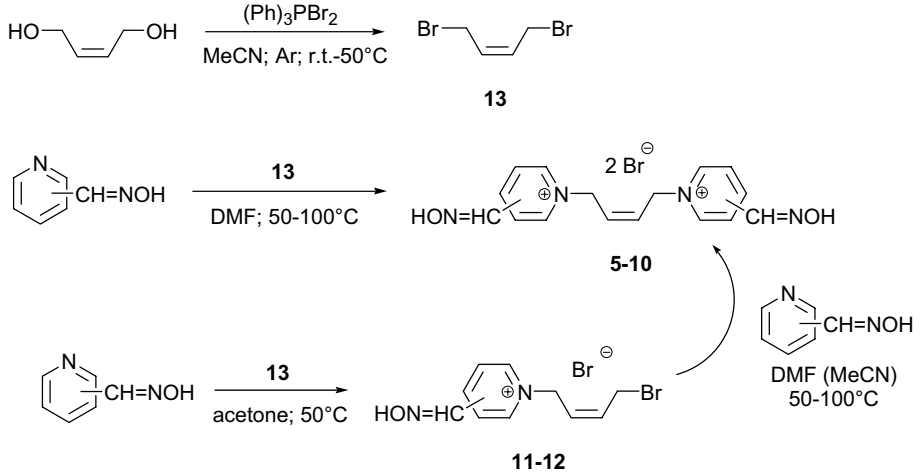
Figure 3. Six oxime reactivators tested against tabun and paraoxon-inhibited AChE.

The reactivators of AChE bearing a (*E*)-but-2-ene linker have been published previously.[14,15] Some of these reactivators (e.g., K075; Fig. 2) showed very promising activity in the reactivation of GA in vitro and so were subjected to further in vivo testing.[16] Owing to the in vitro results, the idea to modify the (*E*)-formation of the double bond for related (*Z*)-but-2-ene linker was used. Consequently, six novel reactivators (**5**–**10**) were prepared in an appropriate yield and purity (Fig. 3). At first, a novel synthetic approach for preparation of (*Z*)-1,4-dibromobut-2-ene (**13**) from a corresponding diol was used.[17] Second, monoquaternary substances (**11**–**12**) were synthesized in the presence of excess alkylating agent. Finally, the bisquaternary compounds were produced (**5**–**10**) (Scheme 1).[18] Their reactivation activity was measured using a model of GA and paraoxon-inhibited rat brain AChE.

In vitro testing of synthesized oximes involved standard experimental procedures and is described in full by the work of Kuca and Cabal.[19] Briefly, a 10% rat brain homogenate (the source of AChE) in water was inhibited by GA or paraoxon. After 30 min of incubation with the OPI moiety, this achieved 95% inhibition of AChE. Next the reactivator was added to the solution for a further 10 min. Activities of intact AChE (a_0), inhibited AChE (a_i), and reactivated AChE (a_r) were deduced from the rate of consumption of a NaOH solution (0.01 M). The percentage of reactivation (%) was calculated from the measured data according to the formula:

$$x = \left(1 - \frac{a_0 - a_r}{a_0 - a_i}\right) \cdot 100[\%]$$

Pralidoxime, HI-6, obidoxime, and K075 (**1**–**4**) of HPLC purity were synthesized in our laboratory and used as references. Collected data are summarized in Table 1.



Scheme 1. Preparation of bisquaternary substances with (*Z*)-but-2-ene linker.