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New bispyridinium oximes: *In vitro* and *in vivo* evaluation of their biological efficiency in soman and tabun poisoning

Suzana Berend[a,*], Ana Lucić Vrdoljak[a], Božica Radić[a], Kamil Kuča[b]

[a] *Institute for Medical Research and Occupational Health, Ksaverska c. 2, P.O. Box 291, HR-10001 Zagreb, Croatia*
[b] *Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic*

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ABSTRACT

Improving the efficacy of antidotal treatment of poisonings with nerve agents is still a challenge for the scientific community. This study investigated the interactions of four bispyridinium oximes with human erythrocyte acetylcholinesterase (AChE) and their effects on soman- and tabun-poisoned mice. Oximes HI-6 and TMB-4 were used for comparison. These oximes inhibited AchE with inhibitory potency (IC_{50}) ranging from 0.02 to 1.0 mM. The best reactivating potency (%R) was obtained with K074, when AChE was inhibited by tabun. The protective potency (P_{50}) of all oximes in human erythrocyte AChE inhibited by soman and tabun could not be determined. In tabun-poisoned mice very good antidotal efficacy was obtained with K027, K048, and K074, which makes them interesting for future investigation. The combination of HI-6 and atropine is the therapy of choice for soman poisoning.

1. Introduction

Organophosphorous (OP) compounds are widely used as pesticides, as drugs in the treatment of cholinergic disorders, and as nerve agents in chemical warfare [1,2]. Irreversible inhibition of acetylcholinesterase (AChE, EC 3.1.1.7) by these compounds results in acethylcholine accumulation in the synaptic cleft with consequences to the central and peripheral nervous system. The clinical signs of AChE inhibition manifest as hypersalivation, lacrimation, diarrhea, tremor, respiratory distress, convulsions, and seizures. Signs are dose-dependent, leading to severe incapacitation and rapid death. Presently, OP poisoning is treated with a combination of an antimuscarinic agent, e.g. atropine and an AChE reactivator oxime [3,4]. Pyridinium compounds, with or without the oxime group, are reversible inhibitors of AChE; they bind to either the catalytic or allosteric (substrate inhibition) enzyme binding site or both, and they also protect AChE from phosphorylation [5]. Their primary action is attributed to the nucleophilic displacement of the compounds' moiety from the phosphorylated enzyme [6]. Unfortunately, OP such as soman phosphorylate AChE and rapidly "age" into a form that cannot be reactivated by oximes [7]. On the other hand, tabun-phosphorylated AChE is resistant to oxime reactivation due to an electron pair located on the amidic group that makes the nucleophilic attack almost impossible [8–10]. The inability of the standard therapy to provide adequate protection against these compounds calls for a synthesis of new compounds with the characteristic oxime group. This study investigated four oximes with a similar basic structure, but differing in the length of the linker between two pyridinium rings and in the position of the oxime group in the pyridinium ring. The aim was to determine their *in vitro* toxicity and protective and reactivating potency in human erythrocyte AChE. We also tested their *in vivo* antidotal efficacy in soman- and tabun-poisoned mice. Currently used oximes were included for comparison; HI-6 for soman and TMB-4 for tabun poisoning.

* Corresponding author. Tel.: +385 1 46 73 188; fax: +385 1 46 73 303.
E-mail address: suzana@imi.hr (S. Berend).

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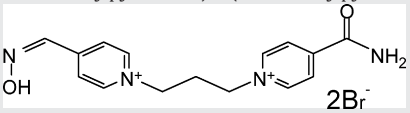
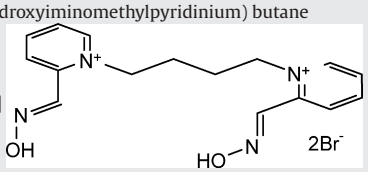
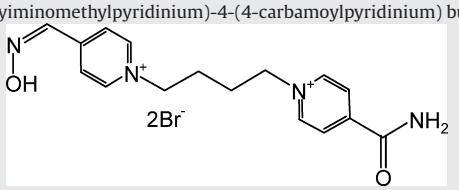
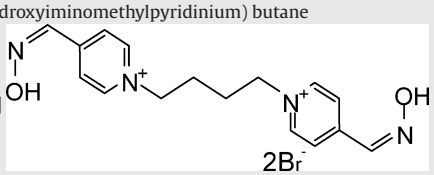
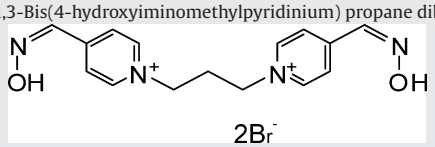
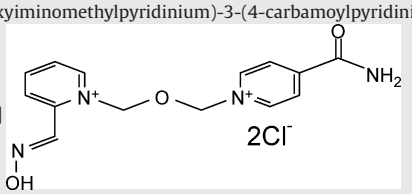
Table 1
Chemical name, structure, and LD_{50} of the tested oximes in male mice

Compound	Code	LD_{50} (mg/kg) (95% confidence limits)
[1-(4-Hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide] 	K027	672.8 (599.0–755.3)
[1,4-Bis(2-hydroxyiminomethylpyridinium) butane dibromide] 	K033	33.4 (29.7–37.5)
[1-(4-Hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide] 	K048	224.9 (154.2–328.0)
[1,3-Bis(4-hydroxyiminomethylpyridinium) butane dibromide] 	K074	21.4 (19.0–24.0)
[1,3-Bis(4-hydroxyiminomethylpyridinium) propane dibromide] 	TMB-4	73.5 (56.0–96.5)
[(1-(2-Hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride)] 	HI-6	448.4 (380.8–527.1)

2. Materials and methods

The enzyme source were native human erythrocytes. The oximes were K027 [1-(4-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium) propane dibromide], K033 [1,4-bis(2-hydroxyiminomethylpyridinium) butane dibromide], K048 [1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide], K074 [1,3-bis(4-hydroxyiminomethylpyridinium), HI-6 [(1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride)] and TMB-4 [1,3-bis(4-hydroxyiminomethylpyridinium) propane dibromide].

We determined *in vitro* inhibitory potency (IC_{50}) and reactivating (%R) and protective (P_{50}) capacities with respect to soman- and tabun-inhibited AChE for all tested oximes. The experiments were performed in 0.1 mol/l phosphate buffer, pH 7.4, and enzyme activities were measured in the presence of 1.0 mmol/l acetylthiocholine iodide (ATCh) as substrate. AChE activity was determined spectrophotometrically using the method of Ellman et al. [11]. Enzyme activities were corrected for substrate hydrolysis due to the reaction of ATCh with the oxime.

Acute intraperitoneal toxicity (LD_{50}) was based upon 24 h mortality rates calculated according to Thompson [12] and Weil [13]. Each LD_{50} was evaluated from the results obtained with four to six doses of a given compound (dissolved in water). Four male BALB-C mice (18–25 g body weight) were injected per dose. Whenever the results of the experiment allowed, 95% confidence limits were estimated from tables described elsewhere [12,13]. The oximes (1/4 of their LD_{50} dose) were given together with atropine