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Synthesis of a potential reactivator of acetylcholinesterase—1-(4-hydroxyiminomethylpyridinium)-3-(carbamoylpyridinium)-propane dibromide

Kamil Kuča,* Jiří Bielavský, Jiří Cabal and Marcela Bielavská

Purkyně Military Medical Academy, Department of Toxicology, PO Box 35/T, 500 01, Hradec Králové, Czech Republic

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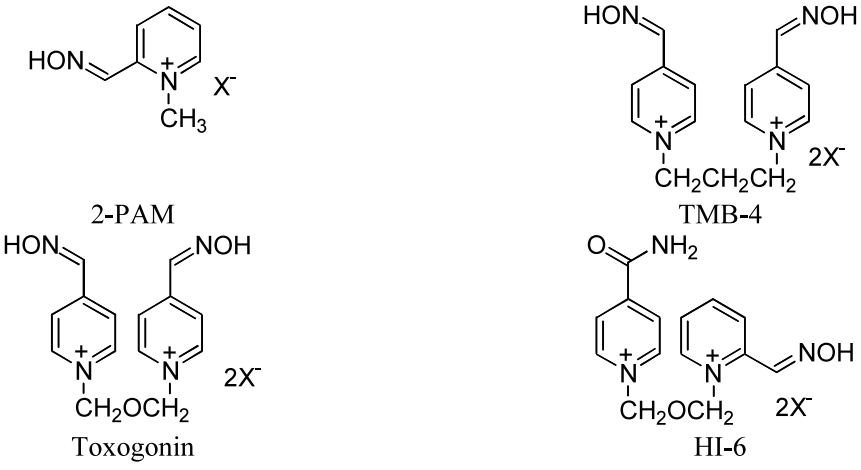
Abstract—Two methods for the synthesis of a new unsymmetric bispyridinium oxime-1-(4-hydroxyiminomethylpyridinium)-3-(carbamoylpyridinium)propane dibromide are described. In vitro efficacy of this new oxime to reactivate sarin-inhibited acetylcholinesterase has been evaluated.

Highly toxic organophosphorus compounds (OP), for example the nerve agents (sarin, soman, tabun), inactivate the enzyme acetylcholinesterase (AChE) by phosphorylation or phosphonylation of its active site.[1] The inhibition of the AChE depends on the chemical structure of the inhibitors whereas reactivation of inhibited AChE depends not only on the inhibitors used but also on the chemical structure of the reactivator. There are many commonly used reactivators of organophosphate- or organophosphonate-inhibited AChE such as 2-PAM (pralidoxime),[2] TMB-4 (trimedoxime),[3] toxogonin (obidoxime)[4] and the oxime HI-6 (Scheme 1).[5]

Due to the high variability of AChE inhibitors, there is no single AChE reactivator having the ability to sufficiently reactivate inhibited enzyme regardless of the chemical structure of the inhibitor. Therefore, many laboratories throughout the world have decided to synthesize new reactivators of OP-inhibited AChE, in order to reactivate OP-inhibited AChE regardless of the chemical structure of the organophosphorus inhibitor.

According to the latest research results, the oxime HI-6 (1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoyl-



Scheme 1. Currently used reactivators of OP-inhibited AChE.

Keywords: acetylcholinesterase; oxime; reactivation; sarin; synthesis.

* Corresponding author. Tel.: +420-49-5251506; fax: +420-49-5518094; e-mail: kucakam@pmfhk.cz

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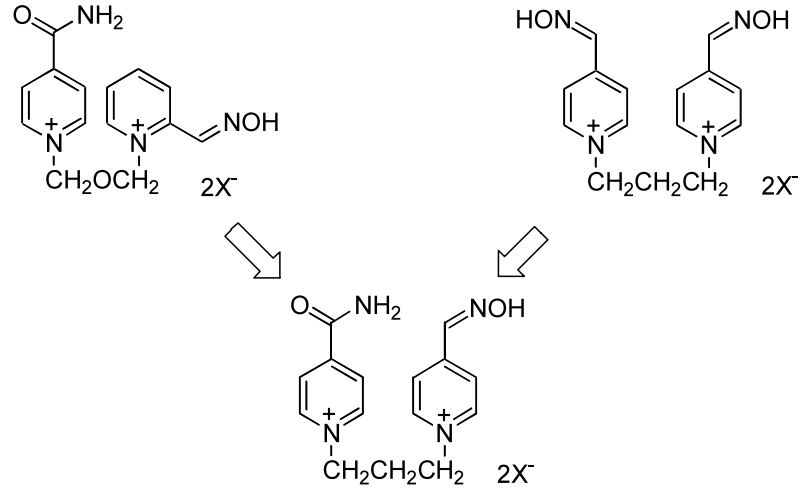
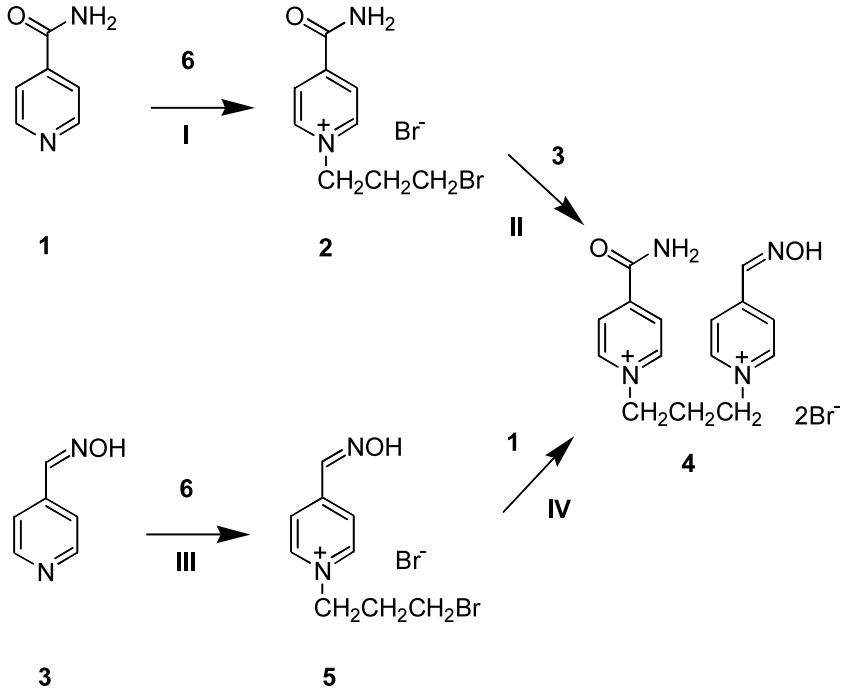
pyridinium)-2-oxapropane dichloride) seems to be the most promising AChE reactivator.[5] Nevertheless, the human use of this oxime is limited by the lack of stability due to the oxapropane bridge and low efficacy in reactivating tabun-inhibited AChE.[5] On the other hand, a reactivator of OP-inhibited AChE-TMB-4 (1,3-bis(4-hydroxyiminomethylpyridinium)propane dibromide) is sufficiently stable.[3] Therefore, we decided to synthesize a new oxime involving part of the chemical structures of both oximes (oxime HI-6 and TMB-4) (Scheme 2).

There are two methods of synthesising the new oxime. The first step of the first method, as outlined in Scheme 3, is quaternization of the isonicotinamide **1** using an excess of 1,3-dibromopropane **6**. In the next step, intermediate **2** is reacted with an equivalent amount of 4-hydroxyiminomethylpyridine **3**. Finally, the quaternary product is recrystallized from acetonitrile. The second method—reaction of 4-hydroxyiminomethylpyridine **3** with an equivalent amount of 1,3-dibromopropane **6** gives 1-(3-bromopropane)-4-hydroxyiminopyridinium bromide **5**. The bisquaternary pyridinium salt **4** is obtained by alkylation of isonicotinamide **1** with intermediate **5**.

All reaction conditions and yields are described in Table 1. The intermediates **2** and **5** and salt **4** were identified by their ^{1}H NMR spectra and elemental analysis.[6]



Scheme 2. Chemical structure of the new oxime.



Scheme 3. Two methods for synthesis of the new oxime **4**.

Table 1. Reaction conditions and yields

Reaction	Conditions of the reaction	Yield (%)
I	CH_3CN, 70°C, 40 h	73
II	CH_3CH_2OH, reflux, 36 h	72
III	$CHCl_3$, reflux, 144 h	55
IV	Dimethylformamide, 80°C, 72 h	34

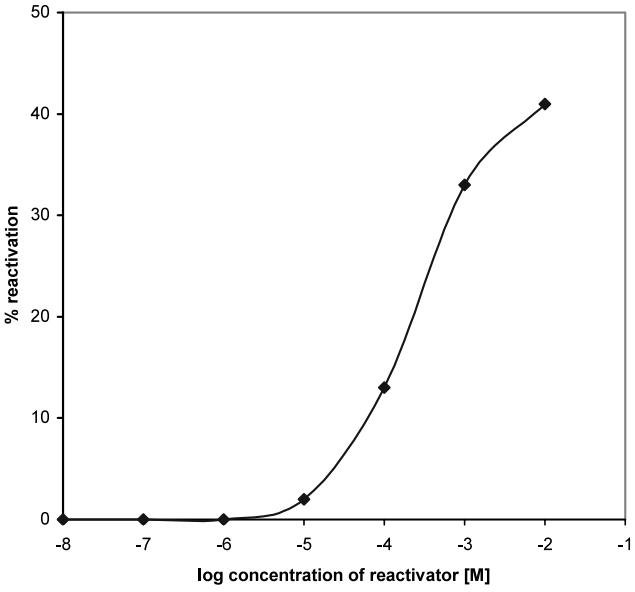


Figure 1. Concentration–reactivation relationship of the oxime **4** to sarin-inhibited AChE, semilogarithmic transformation.

The reactivation efficacy of the newly synthesized compound **4** was evaluated by the standard reactivation test with electrometric instrumentation.[7] Rat brain homogenate in distilled water (10%, w/v) was used as a source of AChE. Measurements were taken at 25°C, pH 8, and the concentrations of the AChE reactivators were varied from 10^{-8} to 10^{-2} M. The activity of AChE was determined by pH static titration of acetic acid released from acetylcholine iodide.[8] The data from the initial rate of the enzyme reaction with the substrate made the calculation of the dissociation constant of the enzyme–reactivator complex (K_R) possible. The ability of the oxime to reactivate sarin-inihibited AChE was calculated as the percentage of the increase in the reactivation of sarin-inhibited AChE.[9] A concentration of 10^{-3} M of the oxime **4** was necessary to acheive 35% increase in the reactivation of sarin-inhibited AChE (Fig. 1).

In conclusion, we have developed a new reactivator of OP-inhibited AChE. Its ability to reactivate sarin-inhibited AChE in vitro is characterized by k_R (0.054 min^{-1}), K_R (281 μM) and k_r (200 M^{-1} min^{-1}).[10] The concentration–reactivation relationship is expressed in Figure 1. Further studies dealing with the evaluation of the reactivation efficacy of this compound against nerve agents such as tabun, cyclosarin, soman and VX are currently under investigation.

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6. (a) **2**: m.p. 179–182°C; EA: For $C_9H_{12}N_2Br_2O$ (324.01) calc. 33.36% C, 3.73% H, 8.65% N, 49.32% Br; found 33.53% C, 3.76% H, 8.53% N, 49.10% Br; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.49 (m, 2H, $CH_2\underline{CH_2}CH_2'$); 3.57 (t, J=6.60 Hz, 2H, $CH_2\underline{CH_2}Br'$); 4.73 (t, J=7.15 Hz, 2H, $CH_2\underline{CH_2}N'$); 8.27 (s, 1H, $CO\underline{NH_2}$); 8.42 (d, J (3,2)=J(5,6)=6.60, 2H, H-3′ and H-5′); 8.66 (s, 1H, $CO\underline{NH_2}$); 9.22 (d, J (2,3)=J(6,5)=6.88 Hz, 2H, H-2′ and H-6′); (b) **5**: m.p. 178–181°C; EA: For $C_9H_{12}N_2Br_2O$ (324.01) calc. 33.36% C, 3.73% H, 8.65% N, 49.32% Br; found 33.42% C, 3.72% H, 8.43% N, 49.26% Br; ^{1}H NMR (DMSO-d_6): δ 2.48 (m, 2H, $CH_2\underline{CH_2}CH_2$); 3.57 (t, J=6.60 Hz, 2H, $CH_2\underline{CH_2}Br$); 4.68 (t, J=7.15 Hz, 2H, $CH_2\underline{CH_2}N$); 8.23 (d, J(3,2)=J(5,6)=6.87 Hz, 2H, H-3 and H-5); 8.43 (s, 1H, $\underline{CH}$=NOH); 9.06 (d, J(2,3)=J(6,5)=6.00 Hz, 2H, H-2 and H-6); (c) **4**: m.p. 214–217°C; EA: For $C_{15}H_{18}N_4Br_2O_2\cdot1.5H_2O$ (473.15) calc. 38.08% C, 4.47% H, 11.84% N, 33.77% Br; found 38.16% C, 4.49% H, 11.61% N, 34.14% Br; ^{1}H NMR (DMSO-d_6): δ 2.68 (m, 2H, $CH_2\underline{CH_2}CH_2$); 4.74 (t, J=7.15 Hz, 2H, $CH_2\underline{CH_2}N$); 4.82 (t, J=7.15, 2H, $CH_2\underline{CH_2}N'$); 8.27 (m, 3H, H-3 and H-5 and $\underline{CH}$=NOH); 8.48 (m, 3H, H-3′ and H-5′ and $CO\underline{NH_2}$); 8.74 (s, 1H, $CO\underline{NH_2}$); 9.12 (d, J(2,3)=J(6,5)=6.61 Hz, 2H, H-2 and H-6); 9.34 (d, J(2′,3′)=J(6′,5′)=6.32 Hz, 2H, H-2′ and H-6′).
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10. K_R, dissociation constant of inhibited enzyme reactivator complex; k_R, the first-order rate constant of reactivation; k_r, the second rate constant of reactivation.