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Synthesis of a New Reactivator of Tabun-Inhibited Acetylcholinesterase

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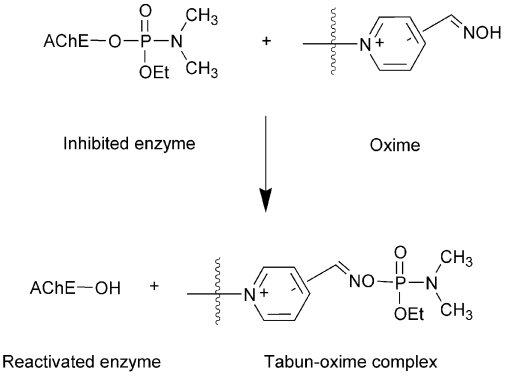
Abstract—Synthesis of a new asymmetric bisquaternary reactivator of tabun-inhibited acetylcholinesterase-1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide is described. Reactivation potency of this oxime is compared to the currently used reactivators—pralidoxime, obidoxime and H-oxime HI-6.

Introduction

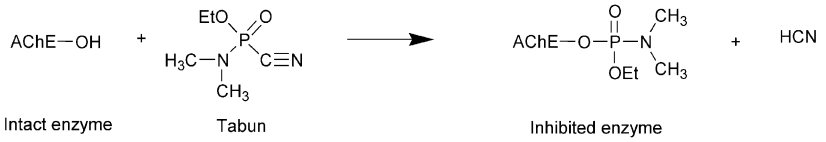
Neurotoxic organophosphorus compounds used at the present time as chemical warfare agents (sarin, soman, tabun, cyclosarin or VX agent) belong among irreversible inhibitors of the enzyme acetylcholinesterase (AChE; EC 3.1.1.7). Their inhibitory effect is based on phosphorylation or phosphonylation of serine hydroxy group at the esteratic site of the enzyme active site (Scheme 1).[1,2]

Tabun [GA agent, *O*-ethyl-*N*,*N*-dimethyl phosphoramidocyanidate] is the chemical warfare agent that some US troops may have been exposed to in the Gulf War operations.[3,4] Commonly used reactivators of phosphonylated acetylcholinesterase based on monopyridinium (pralidoxime) and bispyridinium oximes (obidoxime, methoxime and the oxime HI-6) are able to reactivate organophosphate-inhibited AChE. They attack phosphorylated or phosphonylated serine group and liberate the enzyme as shown in Scheme 2.[5]

Currently used AChE reactivators are not able to counteract the toxic effects of tabun because of very low reactivating efficacy.[6] The lack of their efficacy to reactivate tabun-inhibited AChE was explained by difficulties with nucleophilic attack.[7] According to the recent



Scheme 2. Oxime-induced reactivation of tabun-inhibited acetylcholinesterase.



Scheme 1. Inhibition of acetylcholinesterase by tabun.

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research results, the oxime HI-6 seems to be the most promising AChE reactivator.[8–10] Unfortunately, its reactivating efficacy for tabun-inhibited AChE is not so high.[11–14] The chemical structures of reactivators are shown in Figure 1.

In the present study, we have synthesized a new reactivator of tabun-inhibited acetylcholinesterase-1-(4-hydroxyiminomethylpyridinium) - 4 - (4 - carbamoylpyridinium) butane dibromide **4**, and then, we have compared its reactivating efficacy with currently used oximes (pralidoxime, obidoxime, and H-oxime HI-6) by in vitro methods.

Experimental

Preparation of the new oxime **4** is shown in Scheme 3. The synthesis of 1-(4-bromobutane)-4-carbamoylpyridinium bromide (**7**): Isonicotinamide **5** (7.4 g, 0.06 mol) was dissolved in acetonitrile (260 mL) and mixed with 1,4-dibrombutane **6** (65 g, 0.3 mol). Mixture was kept at 65–70 °C for 18 h. White solid product **7** was collected by filtration. Yield, 19.55 g (96%), mp after crystalization from acetonitrile 160–163 °C.

Synthesis of 1-(4-hydroxyiminomethylpyridinium)-4-(4-carbamoylpyridinium) butane dibromide **4**: A mixture of the quaternary salt **7** (8.5 g, 0.025 mol) and 4-pyridinaldoxime **8** (4.6 g, 0.038 mol) in 200 mL dimethylformamide was kept at 65–70 °C for 20 h. The yellow crystaline product **4** was collected by filtration and washed with acetone. Yield 7.65 g (66%), mp after recrystalization from ethanol 252–256 °C; pK_a = 8.24.

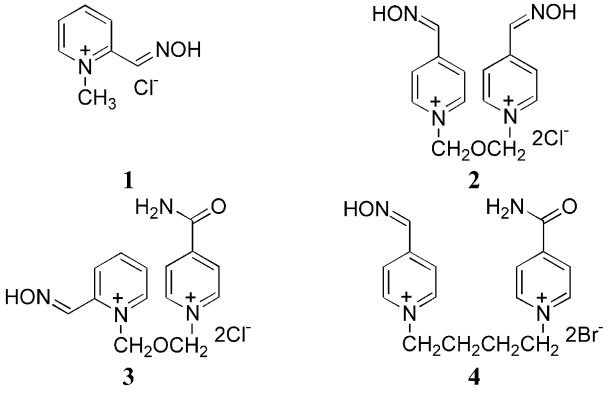


Figure 1. Structures of the oximes.

Purity of this product was evaluated by HPLC (Spectra Physics instrument equipped with a UV 1000 detector, and Purospher RP-18E column), ^{1}H NMR (Varian Gemini 300; 300 MHz) and elemental analysis (Perkin-Elmer CHN Analyser 2400 Serie II).[15]

Pralidoxime (**1**; 2-PAM, 2-hydroxyiminomethyl-1-methylpyridinium bromide; mp 228–230 °C; pK_a = 7.98), obidoxime (**2**; toxogonine; 1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxa-propane dibromide; mp 210–212 °C; pK_{a1} = 7.46 and pK_{a2} = 8.22), and H-oxime HI-6 (**3**; 1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride; mp 139–142 °C; pK_a = 7.50) were prepared in our laboratory earlier.

GA agent was obtained from Technical institute, Brno (Czech Republic) and was 89% pure. All other chemicals obtained from commercial sources were of reagent grade.

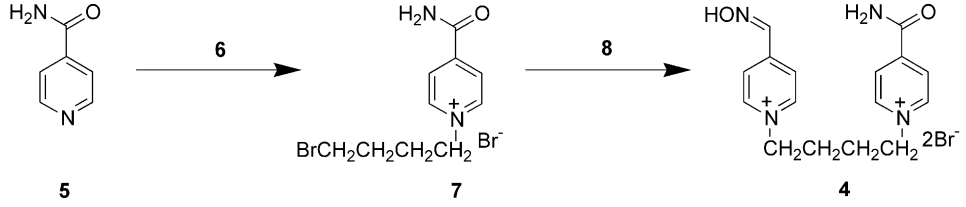
In vitro experiments

In vitro testing of oximes involved a standard collection of experimental procedures.[16] The reactivating efficacy of oximes was evaluated in 10% rat brain homogenate that was incubated with tabun for 30 min and then, the tested oxime was added for 10 min. The activity of brain AChE was measured by potentiostatic method with the help of automatic titrator RTS 822 (Radiometer, Denmark).[16] The data about initial rate of enzyme reaction with substrate made possible the calculation of the dissociation constant of enzyme-reactivator complex (K_{DIS}), the dissociation constant of enzyme–inhibitor–reactivator complex (K_R) and the first-order rate constant (k_R). Bimolecular constants of reactivation (k_r), which represent overall reactivation ability, were calculated from the equation ($k_r = k_R/K_R$).[17] The ability of oxime to reactivate tabun-inhibited AChE was calculated as the percentage of increase in the activity of reactivated enzyme in the reaction mixture.[18]

Table 1. Kinetics parameters of the reactivation

Reactivator	K_{DIS} (μM)	K_R (μM)	k_R (min^{-1})	k_r (min^{-1} M^{-1})
1	210	—[a]	—[a]	—[a]
2	280	3.2	0.020	6250
3	24	6.3	0.007	1111
4	228	93	0.0324	348

[a]We were not able to measure values of the kinetics constants because of very low ability of this oxime to reactivate tabun-inhibited AchE.



Scheme 3. Synthesis of the oxime **4**.

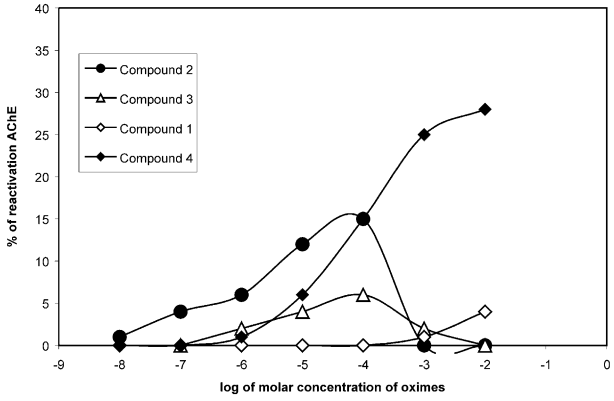


Figure 2. Concentration–reactivation relationship of the oximes to tabun-inhibited AChE.

Results and Discussion

Kinetics parameters (dissociation constants: K_{DIS} and K_R; rate constants: k_R and k_r) shown in Table 1 characterize the ability of the studied oximes to reactivate tabun-inhibited AChE in vitro. The first constant, K_{DIS}, indicates the affinity of oximes toward the non-inhibited AChE. The affinity of new oxime **4** to the intact AChE is comparable with the affinity of oximes **1**, **2** and lower than the affinity of oxime **3**. The dissociation constant K_R, which characterizes affinity of oximes to the inhibited AChE, indicates that the affinity of the compound **4** to the enzyme–inhibitor complex is lower compared to all other measured compounds. The values of the constant k_R express the breakdown of the intermediate complex. It is the highest for compound **4**. The value of bimolecular constant of reactivation (k_r), representing overall reactivation ability, is for compound **4** lower compared to compounds **3** (3 times) and **2** (18 times) thanks to high value of the constant $K_R(k_r = k_R/K_R)$.

The concentration–reactivation relationship is demonstrated in Figure 2. Compound **2** is efficacious to reactivate tabun-inhibited AChE in vitro in concentration range from 10^{-5} to 10^{-4} M. 10^{-2} M concentration of the oxime **4** is necessary to reach 28% increase in the activity of tabun-inhibited AChE. The other oximes tested showed very little or no potency to reactivate tabun-inhibited AChE.

In conclusion, we have developed a new promising reactivator of tabun-inhibited AChE-1-(4-hydroxyiminomethylpyridinium) - 4 - (4 - carbamoylpyridinium)-butane dibromide. Its potency to reactivate tabun-inhibited AChE measured by in vitro method was considered to be higher than the reactivation efficacy of pralidoxime (**1**) and HI-6 (**3**) and comparable with the reactivation potency of obidoxime (**2**).[6,11–14]

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15. New compounds gave satisfactory ^{1}H NMR spectra and elemental analysis: (a) **7**-EA: calcd 35.53% C, 4.17% H, 8.29% N, 47.27% Br; found 35.34% C, 4.12% H, 8.10% N, 47.31% Br; ^{1}H NMR (DMSO-d_6): 1.80 m, 2H, $(\underline{CH_2}CH_2Br)$; 2.03 m, 2H, $(\underline{CH_2}CH_2N')$; 3.53 t, 2H, $J = 6.60$, $(CH_2\underline{CH_2}Br)$; 4.71 t, 2H, $J = 7.15$ Hz, $(CH_2\underline{CH_2}N')$; 8.26 s, 1H, $(CO\underline{NH_2})$; 8.45 d, 2H, $J(3',2') = J(5',6') = 6.60$ Hz, (H-3′, H-5′); 8.70 s, 1H, $(CO\underline{NH_2})$; 9.30 d, 2H, $J(2',3') = J(6',5') = 6.88$ Hz, (H-2′, H-6′); (b) **4**-EA: calcd 41.76% C, 4.38% H, 12.18% N, 34.73% Br; found 41.65% C, 4.36% H, 11.90% N, 33.66% Br; ^{1}H NMR (DMSO-d_6): 1.97 m, 4H, $(CH_2\underline{CH_2}\underline{CH_2}CH_2)$; 4.67 t, 2H, $J = 7.15$ Hz, $(CH_2\underline{CH_2}N)$; 4.76 t, 2H, $J = 7.15$ Hz, $(CH_2\underline{CH_2}N')$; 8.24 m, 3H, (H-3, H-5, $CO\underline{NH_2}$); 8.45 m, 3H, (H-3′, H-5′, $\underline{CH}{=}NOH$); 8.73 s, 1H, $(CO\underline{NH_2})$; 9.12 d, 2H, $J(2,3) = J(6,5) = 6.60$ Hz, (H-2, H-6); 9.34 d, 2H, $J(2',3') = J(6',5') = 6.32$ Hz, (H-2′, H-6′).
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