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Synthesis of asymmetrical bispyridinium compounds bearing cyano-moiety and evaluation of their reactivation activity against tabun and paraoxon-inhibited acetylcholinesterase

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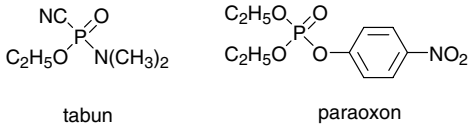
Abstract—Three asymmetrical AChE reactivators with cyano-moiety and propane linker were synthesized using modification of currently known synthetic pathways. Their potency to reactivate AChE inhibited by nerve agent tabun and insecticide paraoxon was tested in vitro and compared to pralidoxime, HI-6, obidoxime, K027, and K048. According to the results, three compounds seem to be promising against paraoxon-inhibited AChE. Better results were obtained for bisquaternary substances at least with one oxime group in position four. None of tested substances was able to satisfactorily reactivate tabun-inhibited AChE at concentration applicable for in vivo experiments.

Enzyme acetylcholinesterase (AChE, EC 3.1.1.7) plays a very important role in human body. It controls cholinergic transmission by decomposition of neuromediator acetylcholine. The blockade of its physiological function by various inhibitors could be used for treatment of Alzheimer disease (competitive inhibitors) or misused for military or terrorist activity (irreversible inhibitors).[1–5] The well-known irreversible inhibitors are organophosphorus compounds (OC).[6] They are thio- or oxo-derivates of phosphonic and phosphoric acid.[6] They are used massively in agriculture as pesticides (e.g., parathion, chlorpyrifos, and diazinon), for industrial purposes (e.g., tributylphosphate), and they were also misused as nerve agents (e.g., sarin, soman, tabun, and VX) in wars and by terrorists (Fig. 1).[7–10]

The mechanism of AChE inhibition by OC depends in the blockade of serine hydroxyl in the enzyme's cavity.[6] This narrow 20 Å deep gorge was studied in detail by ligand binding and crystallographic studies for various species.[11–14] It has one catalytic (acylation, A) site on the bottom and one peripheral site (P) at the lip of the cavity.[15,16] The A-site contains the catalytic triad (for human AChE, S203, E334, and H447) which together with W86 is responsible for binding of trimethylammonium group of acetylcholine as acyl transfer to Ser203 is initiated.[14] The P-site involves other residues including W286.[11] The ligands bound to the P-site can affect the A-site by steric blockade or allosteric activation. Moreover, some ligands can span the 12–15 Å distance between these two sites.[16]

Besides, the enzyme's gorge changes during the OC-inhibition.[14] After inhibition, the conjugate OC-AChE may undergo further intramolecular modifications called



tabun paraoxon

Figure 1. Examples of organophosphorus compounds.

Keywords: Acetylcholinesterase; Reactivation; Nerve agent; Tabun; Pesticide; Paraoxon; Reactivator; Oxime.
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'aging' that involves dealkylation or deamidation.[17] The aged enzyme is truly irreversibly inhibited and it cannot be reactivated. Nerve agent tabun (GA) causes one of the most resistant inhibitions.[18,19] The structural basis for resistance of tabun conjugates is unknown. Nevertheless, the crystal structures of murine AChE showed that non-aged tabun conjugate induces structural changes in H447 and its hydrogen bonds.[14] Moreover, the conformational change of P338 position partially closes the narrow AChE gorge.[14] After aging reaction, the tabun molecule is coordinated in the AChE gorge and phosphoroamidoyl group is replaced by a water molecule.[14] Therefore, GA belongs to the worst reactivated nerve agents.

The AChE reactivator in its dissociated form is able to cleave the covalent bond OC-enzyme by nucleophilic reactive group (oximate anion) and to restore the activity of AChE. Nevertheless, every type of OC needs a specific structure of AChE reactivator and there is none broad-spectrum reactivator after more than 50 years of investigations.[1,17–19] Therefore, the development and selection of new effective reactivators of AChE-like antidotes of OC are very important.

For these reasons, we decided to develop new potent structure relative to promising reactivators K027 (**1**) and K048 (**2**) (Fig. 2) against GA-inhibited AChE.[20,21]

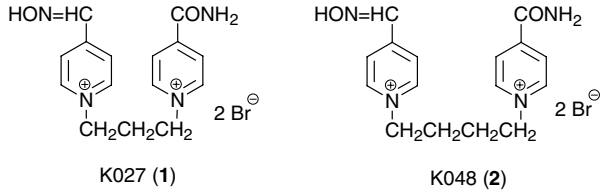
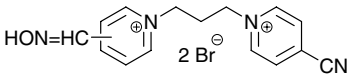


Figure 2. Promising oximes tested on tabun-inhibited AChE.



Compound	O	xime position
6		2-CH=NOH
7		3-CH=NOH
8		4-CH=NOH

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

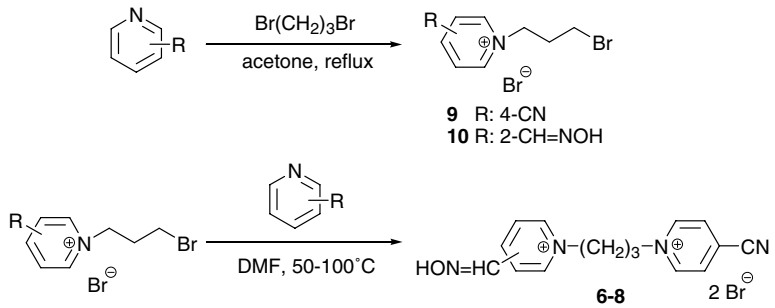
The asymmetrical model with one oxime group and three carbon spacer was chosen according to similarity both with promising reactivators (K027) and reactivators currently used (obidoxime). The cyano group was used as more rigid variant of carbamoyl group (K027 and K048). Three compounds (**6**–**8**) were prepared using conventional synthetic procedures; two of them (**6**–**7**) were not previously described in the literature (Fig. 3). First, monoquaternary salts (**9**–**10**) were synthesized using excess of five equivalent of 1,3-dibromopropane in acetone, where by-products occurs only in minor yields (Scheme 1). The mono-salts were purified by recrystallization from acetonitrile (MeCN), where bis-salts were almost insoluble.[22] Second, the bisquaternary substances were completed in DMF using excess of corresponding hydroxyiminomethylpyridine (Scheme 1).[23] The yields and reaction conditions described in the literature were exceeded and purity of the compounds was also estimated using NMR and MS analysis, where foregoing literature data were not available.[24–26,37] Afterwards, the compounds were tested in vitro on tabun-(GA) and paraoxon-inhibited AChE Figure 4.

In vitro testing of synthesized oximes involved a standard collection of experimental procedures. The 10% rat brain homogenate (source of AChE) in water was inhibited by GA or paraoxon. After 30 min of incubation with OC to achieve 95% inhibition of AChE, the reactivator was added to the solution for the next 10 min. Activities of intact AChE (a_0), inhibited AChE (a_i), and reactivated AChE (a_r) were deduced from the influence of consumption of NaOH solution (0.01 M) on time. The percentage of reactivation (%) was calculated from the measured data according to the formula:

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

The whole method is in detail described in the work of Kuca and Cabal.[27] Pralidoxime, HI-6, and obidoxime (**3**–**5**, Fig. 3) of HPLC purity previously synthesized in our laboratory were used as references. Obtained data are summarized in Table 1.

First of all, the reactivation potency suitable for in vivo experiments should exceed 10% ability in vitro.[1] No newly prepared reactivator is able to fulfill this rule for tabun at both concentrations. Only three compounds (**1**, **2**, and **5**) showed some applicable results at concen-



Scheme 1. Two-step synthesis of asymmetrical bisquaternary compounds.

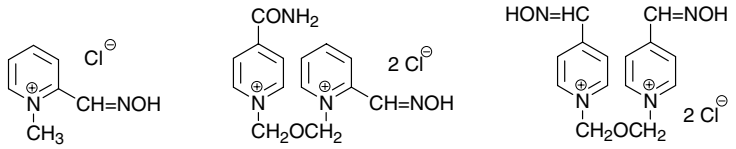


Figure 4. Oxime reactivators used as reference compounds.

Table 1. Reactivation potencies of tested oximes (%, mean value of three independent determinations)—time of inhibition—30 min; time of reactivation by AChE reactivators—10 min; pH 7.6; temperature 25 °C

Inhibitor	Reactivation (%)			
	Tabun		Paraoxon	
	Concentration (M)			
	10^{-3}	10^{-5}	10^{-3}	10^{-5}
Reactivator				
K027 (**1**)	11 ± 0	1 ± 0	59 ± 3	21 ± 1
K048 (**2**)	25 ± 0	0	57 ± 3	5 ± 2
Pralidoxime (**3**)	4 ± 1	0	42 ± 1	0
HI-6 (**4**)	2 ± 1	4 ± 1	35 ± 2	0
Obidoxime (**5**)	11 ± 0	0	76 ± 2	37 ± 2
6	0	0	0	3 ± 1
7	0	0	4 ± 0	0
8	0	0	32 ± 3	25 ± 0

tration 10^{-3} M for tabun-inhibited AChE. Moreover, concentration 10^{-3} M is not attainable for in vivo experiments.[28] No tested compound showed satisfactory results at concentration 10^{-5} M.

Second, pesticides are known as weaker inhibitors of AChE than nerve agents.[29] In the past, the design of AChE reactivators was focused on preparation of potent substances against nerve agents. By the time, it was found out that commonly used 'nerve agent's' reactivators are not suitable for pesticide intoxications' treatment.[30,31] At concentration 10^{-3} M, all reference compounds and one newly prepared compound (**8**) showed promising results for paraoxon-inhibited AChE. Nevertheless, the measured data for concentration 10^{-5} M marked only three promising substances (**1**, **5**, and **8**). Currently used substances pralidoxime (**3**) and HI-6 (**4**) had no efficacy at concentration 10^{-5} M for paraoxon-inhibited AChE.

In addition, the structural factors appropriate for reactivation can be recommended.[32] The oxime functional group breaks down the bond OC inhibitor-enzyme and is essential for activity of the reactivator.[21–23,32] Our results confirm the hypothesis that one aldoxime group in position four on heteroaromatic ring is sufficient for reactivation of tabun- or paraoxon-inhibited AChE (**1** and **2**). The other substituent at second heteroaromatic ring also influences the reactivation ability by different affinity to AChE. Carbamoyl functional group (**1** and **2**) gave better results for paraoxon-inhibited AChE than cyano functional group in newly prepared substances (**8**). It means that presence of more rigid cyano group (sp hybridization) compared to carbamoyl group (sp^2 hybridization) decreases reactivation potency of tested compounds. If second oxime group is presented (**5**), reactivation potency increases only partially and this fact can be caused by double plausibility of steric orientation of symmetrical molecule in the cavity of enzyme. Length and structure of connecting chain is another important factor for activity of the reactivator.[21–23,32–35] The propane and butane connecting chain (**1–2**, **5–8**) was earlier found to be promising for tabun-inhibited AChE.[36] Moreover, quaternary nitrogens are important for affinity to the enzyme.[1,18–23,32–36]

In conclusion, a series of three reactivators was prepared in satisfactory yield and purity. Their ability to reactivate GA- and paraoxon-inhibited AChE was measured in vitro. One compound was found to be promising against paraoxon-inhibited AChE at physiological concentration. Pralidoxime and HI-6 were found to be improper for pesticide poisonings. The reactivation potency of these compounds depends on structural factors such as position of the functional oxime group at the pyridinium ring, presence of quaternary nitrogen, and the constitution of the linking chain.

Acknowledgments

The authors express their appreciation to Mrs. M. Hrabinova for her technical assistance. The work was supported by the grant of Grant Agency of Charles University No. 302/2005/B-CH/FaF and by the grant of Ministry of Defence of Czech Republic No. FVZ0000501.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.08.011.

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37. 1-(2-Hydroxyiminomethylpyridinium)-3-(4-cyanopyridinium)-propane dibromide (**6**). Prepared by method D via (**10**). Yield 0.29 g (24%), TLC R_f 0.15, mp 214–216 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.50 (d, 2H, J = 6.2 Hz, PyrH), 9.18 (d, 1H, J = 6.0 Hz, PyrH), 8.89–8.73 (m, 3H, PyrH + –*CH*═NOH), 8.65–8.53 (m, 1H, PyrH), 8.44 (d, 1H, J = 8.0 Hz, PyrH), 8.21–8.08 (m, 1H, PyrH), 5.06–4.75 (m, 4H, N–CH_2–), 2.71–2.53 (m, 2H, –CH_2–). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 147.14, 146.41, 145.76, 145.40, 141.29, 131.02, 127.46, 126.92, 125.73, 114.76, 58.28, 54.31, 31.19.EA: calculated 42.08% C, 3.77% H, 13.09% N; found 41.66% C, 4.06% H, 12.75% N. ESI-MS: m/z 267.1 $[M\text{-}H]^+$ (calculated for $[C_{15}H_{16}N_4O\text{–}H]^+$ 267.13).

    1-(3-Hydroxyiminomethylpyridinium)-3-(4-cyanopyridinium)-propane dibromide (**7**). Prepared by method C via (**9**). The reaction mixture was stirred in MeCN (reflux) and stopped after 20 h. Yield 0.15 g (33%), TLC R_f 0.15, mp 188–190 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.53 (d, 2H, J = 6.2 Hz, PyrH), 9.39 (s, 1H, PyrH), 9.17 (d, 1H, J = 6.0 Hz, PyrH), 8.86–8.70 (m, 3H, PyrH), 8.38 (s, 1H, –*CH*═NOH), 8.27–8.16 (m, 1H, PyrH), 4.89 (t, 2H, J = 7.0 Hz, N–CH_2–), 4.82 (t, 2H, J = 7.0 Hz, N–CH_2–), 2.81–2.62 (m, 2H, –CH_2–). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 146.49, 144.51, 143.23, 142.66, 141.78, 133.43, 131.03, 128.19, 126.95, 114.77, 58.25, 57.45, 31.46. EA: calculated 42.08% C, 3.77% H, 13.09% N; found 38.98% C, 4.07% H, 11.94% N. ESI-MS: m/z 267.1 $[M\text{-}H]^+$ (calculated for $[C_{15}H_{16}N_4O\text{–}H]^+$ 267.13).

    1-(4-Hydroxyiminomethylpyridinium)-3-(4-cyanopyridinium)-propane dibromide (**8**). Prepared by method C via (**9**). The reaction mixture was stirred in DMF (100 °C) and stopped after 6 h. Yield 0.39 g (84%), TLC R_f 0.15, mp 214–218 °C (reported 225–226 °C[25]). ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.32 (d, 2H, J = 6.0 Hz, PyrH), 8.94 (d, 2H, J = 6.2 Hz, PyrH), 8.62 (d, 2H, J = 6.0 Hz, PyrH), 8.28 (s, 1H, –*CH*═NOH), 8.10 (d, 2H, J = 6.2 Hz, PyrH), 4.67 (t, 2H, J = 6.7 Hz, N–CH_2–), 4.56 (t, 2H,J = 6.7 Hz, N–CH_2–), 2.38–2.28 (m, 2H, –CH_2–). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 148.55, 146.47, 145.15, 145.06, 131.04, 126.97, 124.09, 114.77, 58.28, 56.69, 31.47. EA: calculated 42.08% C, 3.77% H, 13.09% N; found 41.69% C, 3.90% H, 13.05% N. ESI-MS: m/z 267.1 $[M\text{-}H]^+$ (calculated for $[C_{15}H_{16}N_4O\text{–}H]^+$ 267.13).

    1-(3-Bromopropyl)-4-cyanopyridinium bromide (**9**). Prepared by method A. Yield 1.39 g (47%), TLC R_f 0.5, mp 172–174 °C (reported 171–172.5 °C[25], 167–168 °C[26]). ^{1}H NMR spectrum (300 MHz, DMSO-d_6) is consistent with the literature.[26] ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 146.45, 130.97, 126.96, 114.76, 60.41, 32.94, 29.91. EA: calculated 35.33% C, 3.29% H, 9.15% N; found 35.29% C, 3.37% H, 9.17% N. ESI-MS: m/z 224.9 $[M^+]$ (calculated for $[C_9H_{10}BrN_2^+]$ 225.00).

    1-(3-Bromopropyl)-2-hydroxyiminomethylpyridinium bromide (**10**). Prepared by method B. Yield 0.86 g (8%), TLC R_f 0.5, mp 162–165 °C. ^{1}H NMR spectrum (300 MHz, DMSO-d_6): δ (ppm) 9.30 (d, 1H, J = 5.6 Hz, PyrH), 9.07 (d, 1H, J = 6.0 Hz, PyrH), 8.76 (s, 1H, –*CH*═NOH), 8.53–8.45 (m, 1H, PyrH), 8.35–8.24 (m, 1H, PyrH), 6.27–6.20 (m, 2H, –CH_2–Br), 5.48–5.32 (m, 4H, N–CH_2–CH_2). ^{13}C NMR spectrum (75 MHz, DMSO-d_6): δ (ppm) 163.19, 148.66, 145.87, 145.04, 130.45, 125.87, 60.81, 60.27, 34.39. EA: calculated 33.36% C, 3.73% H, 8.65% N; found 33.59% C, 3.82% H, 8.98% N. ESI-MS: m/z 243.1 $[M^+]$ (calculated for $[C_9H_{12}BrN_2O^+]$ 243.01).