

Synthesis of Bisquaternary Symmetric – χ,δ -Bis(2-Hydroxyiminomethylpyridinium) Alkane Dibromides and Their Reactivation of Cyclosarin-Inhibited Acetylcholinesterase

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Abstract: Preparations of the symmetric bisquaternary oximes – 1,4-bis(2-hydroxyiminomethylpyridinium) butane dibromide and 1,3-bis(2-hydroxyiminomethylpyridinium)propane dibromide, and their abilities to reactivate cyclosarin-inhibited acetylcholinesterase are described. Common reactivator (pralidoxime) was used as a standard for comparison of the reactivation efficacy. The first oxime seems to be very good reactivator of cyclosarin-inhibited acetylcholinesterase.

Keywords: Oximes, reactivation, acetylcholinesterase, cyclosarin, *in vitro*.

Highly toxic organophosphorus compounds considered as chemical warfare agents (sarin, soman, tabun, cyclosarin or VX) belong to irreversible inhibitors of acetylcholinesterase (AChE; EC 3.1.1.7) [1], an enzyme playing an important role in cholinergic transmission in the nervous system. Therefore, its inhibition is life-limiting factor. Inhibitory effect is based on phosphorylation or phosphonylation of serine hydroxy group at the esteratic site of the active center of the enzyme [2].

For the treatment of toxic effects of these agents, parasympatolytics and AChE reactivators are commonly used [3]. Monoquaternary pralidoxime (**1**; 2-PAM, 2-hydroxyiminomethyl-1-methylpyridinium chloride) [3, 4] or more extended bisquaternary compounds, such as trimedoxime (TMB-4, 1,3-bis(4-hydroxyiminomethylpyridinium) propane dibromide) [5], toxogonine (obidoxime, 1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxa-propane dibromide) [6] and H-oxime HI-6 (1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride) [7] belong to the fundamental representatives of these aldoximes.

After the Gulf war, Iraq was found to incorporate a chemical warfare agent cyclosarin (GF; cyclohexyl methylphosphonofluoridate) in its chemical weapons stocks [8]. Recently used reactivators of AChE have not sufficient efficacy to reactivate by cyclosarin-inhibited acetylcholinesterase [9]. Goal of our investigation was to synthesize symmetric bisquaternary oximes – 1,4-bis(2-hydroxyiminomethyl-pyridinium)butane dibromide **2** and 1,3-bis(2-hydroxyiminomethyl-pyridinium)propane dibromide **3** and evaluate *in vitro* their reactivation efficiency against cyclosarin-inhibited AChE. We have chosen only these both compounds because of the highest reactivation potency of the tri and tetramethylene analogs, respectively [10]. Their reactivation ability was compared with pralidoxime **1** (Fig. 1) [11].

Both of the potential reactivators of cyclosarin-inhibited AChE were synthesized in the same way [12] using the modification of the synthesis mode by Proffitt and Kruger and Berry *et al.* [13]. Using our new synthetic approach (solvent - dimethylformamide, time of the reaction - 20 hours), we have improved final reaction yield in the case of the compound **2** to 21 % and in the case of the compound **3** to 28 % in comparison with the published data (i.e. **2** – 12.2%; **3** – 7.4%).

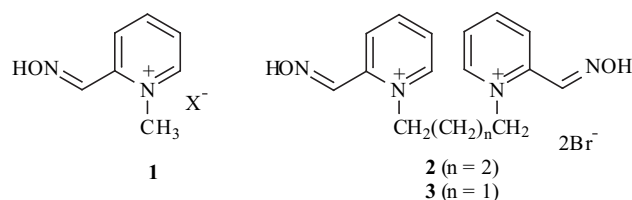


Fig. (1). Reactivators of OP-inhibited AChE.

The action of the 1,4-dibrombutane **4** or 1,3-dibrompropane **5**, respectively with 2-hydroxyiminomethylpyridine **6** of appropriate amount in dimethylformamide gives desired compounds **2** or **3**. The whole synthetic procedure is described in reference [12] and the reaction scheme is outlined in Fig. 2. Purity of the synthesized products was evaluated by HPLC (Spectra Physics instrument equipped with a UV 1000 detector, and Purospher RP-18E column) and $^1\text{H-NMR}$ (Varian Gemini 300; 300 MHz). pKa values of the oximes were determined by a potentiometric titration of 0.05 M oxime solution using 0.1 M sodium hydroxide. Melting points were measured on Boetius block and were uncorrected.

Reactivation effectivity of the oximes were tested *in vitro* on the model of AChE inhibited by cyclosarin using standard reactivation test with electrometric instrumentation [14]. The GF agent was obtained from the Military facility (VOZ 072) Zemianské Kostolany, Slovak Republic (98 % purity). As a source of AChE, a homogenate of the whole brain of rats (Wistar strain) of both sexes, weighing 200-240 g, was used. The whole biochemical procedure is described in reference [15]. The activities of intact (a_0) and GF-

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Relationship between concentration of the oximes and the reactivation ability is shown in Fig 3.

According to our results, the compound **2** should be sufficiently effective to reactivate cyclosarin-inhibited AChE *in vivo* in concentrations from 10^{-6} to 10^{-4} M, i.e. concentrations attainable following oxime administration *in vivo* [14]. Compound **3** does not reach high reactivation percentage in comparison with the compound **2**. On the other hand, the compound **1** is able to reactivate cyclosarin-inhibited AChE *in vitro* in very high concentrations, and therefore, it would be toxic for the use *in vivo*.

In conclusion, we have developed a new method for the preparation of the symmetric bisquaternary oximes – 1,4-bis(2-hydroxyiminomethylpyridinium)butane dibromide **2** and 1,3-bis(2-hydroxyiminomethylpyridinium)propane dibromide **3**. Using this new method, we have improved reaction yields of the final compounds compared to the earlier data mentioned [13]. From the biochemical point of view, compound **2** seems to be very good reactivator of cyclosarin-inhibited AChE. According to the value of the constant k_{tr} , characterizing reactivation as the whole process, ability of the compound **2** to reactivate cyclosarin-inhibited AChE is about 1600 times higher than that for the reactivation potency of the commonly used pralidoxime. Further studies dealing with the evaluation of reactivation efficacy of this compound against nerve agents, such as tabun, sarin, soman or VX are currently under our consideration.

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 [11] Pralidoxime **1** (2-PAM, 2-hydroxyiminomethyl-1-methylpyridinium bromide) was purchased from Léciva (Czech Rep). Its melting point was –228-230 °C, and pKa = 7.96.
 [12] **Synthetic procedure:** a) 1,4-bis(2-hydroxyiminomethylpyridinium)butane dibromide **2**: 2-pyridinealdoxime (10 g, 82 mmole) was dissolved in dry dimethylformamide (50 ml) and mixed with a solution of 1,4-dibromobutane (6.9 g, 32 mmol) in dry dimethylformamide (10 ml). Mixture was kept at 80°C degree for 20 hours. Solid product was collected by filtration (3.1 g, 21 %). Melting point of the product was –233-235 °C, pKa 1 = 7.12; pKa 2 = 8.07. ¹H NMR Spectra (300 MHz, DMSO-*d*₆): δ 2.49 (m, 4H, NCH₂CH₂); 4.76 (m, 4H, CH₂CH₂N); 8.09 (dd, *J*(3,2) = *J*(3,4) = 6.87 Hz, 2H, H-3 and H-3'); 8.41 (d, *J*(2,3) = 7.43 Hz, 2H, H-2 and H-2'); 8.54 (dd, *J*(4,3) = *J*(4,5) = 7.98 Hz, 2H, H-4 and H-4'); 8.80 (s, 2H, CH=NOH); 9.03 (d, *J*(6,5) = 6.33 Hz, 2H, H-6 and H-6'). b) 1,3-bis(2-hydroxyiminomethylpyridinium)propane dibromide **3**: This compound was prepared using the same way as above mentioned compound **2** (28 %). Melting point of the product was –253-265 °C, pKa 1 = 7.13; pKa 2 = 8.55. ¹H NMR Spectra (300 MHz, DMSO-*d*₆): δ 2.48 (2H, m, CH₂CH₂CH₂), 4.88 (t, *J* = 7.56 Hz, 4H, CH₂CH₂N); 8.13 (dd, *J*(3,2) = *J*(3,4) = 6.88 Hz, 2H, H-3 and H-3'); 8.43 (d, *J*(2,3) = 8.25 Hz, 2H, H-2 and H-2'); 8.56 (dd, *J*(4,3) = *J*(4,5) = 7.84 Hz, 2H, H-4 and H-4'); 8.90 (s, 2H, CH=NOH); 9.11 (d, *J*(6,5) = 6.05 Hz, 2H, H-6 and H-6').
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 [15] **Biochemical procedure:** Narcotised animals were killed by bleeding from a carotid artery and the brains were removed, rinsed in physiological saline and homogenized in an Ultra-Turrax (Germany) homogenizer in a distilled water to make a 10 % homogenate. The AChE homogenate (0.5 ml) was mixed with 0.5 ml of 0.01 μM cyclosarin in dry isopropanol and incubated for 30 min (25 °C). Then 2.5 ml of 3M NaCl was added and supplied by distilled water to a volume of 23 ml. After that, 2 ml of 0.02 M acetylcholine bromide was added and enzyme activity was assayed titrimetrically at pH 8.0 and 25 °C on the Autotitrator RTS 822 (Radiometer, Denmark).