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Ludmila Bobkova

SYNTHESIS AND RESEARCH OF REACTIVATION OF PHOSPHORYLATED CHOLINESTERASE BY QUATERNARY PYRIDINIUM-ALDOXIME SALTS

State Institution "Institute of Pharmacology and Toxicology of National Academy of Sciences of Ukraine", 14, Eugene Potie str., 03680 Kyiv, Ukraine; ift-bobkova@rambler.ru

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Abstract. The interaction of pyridine-4(-3,-2)aldoxime has been studied whit 3-bromo-*N*,*N*,*N*-trialkyl-2-oxo(-hydroxyimino)-1-propanaminium bromide. The structure of all synthesized compounds was determined by elemental analysis as well as by physical and chemical methods. All synthesized compounds were tested for reactivation of phosphorylated acetylcholinesterase. Using fragments of the molecules (an oxime group, 3-bromo-*N*,*N*,*N*-trimethyl(or triethyl)ammonium [(or *N*-methyl-morpholinium)-2-oxo(or 2-hydroxyimino)]propan bromides), a new series of pyridinium-aldoxime salt (cholinesterase reactivator) was obtained.

Keywords: synthesis, quaternary pyridinium-aldoxime salt, cholinesterase reactivator.

1. Introduction

An important direction of modern chemistry is to find new compounds for development of drugs for the treatment of acute poisonings including very dangerous organophosphate (PO) poisonings [1-3]. Therefore, of great importance among biologically active compounds are reactivators of phosphorylated acetylcholinesterase (AChE) – known bis-quaternary salts of pyridine-aldoxime, as well as methods for their preparation [4]. Quaternary salts of pyridine-aldoxime are a promising group of compounds for the treatment of organophosphate intoxication [4-11]. Such salts can then be used for the treatment of cholinesterase inhibitors effect [5-8, 12]. Reactivators of phosphorylated AChE (*e.g.* pralidoxime, HI-6, obidoxime, trimedoxime 1-4; Fig. 1) are commonly used in the treatment of organophosphate intoxication [2, 6]. They contain a nucleophilic oxime group capable of cleaving the phosphorylated fragment AChE and restoring the function of the enzyme [13].

Presently known reactivators of phosphorylated AChE have several defects. These include high toxicity, low stability and limited antidote activity in poisonings by various types of organophosphorus agents, including sarin, soman and VX. Thus, changes in the active site of AChE caused by the action of GA are leading to partial deactivation of the toxicity compared to the oxime 2 [2]. This article presents a synthesis of quaternary salts of pyridine-aldoxime containing *N*,*N*,*N*-trialkyl-2-oxo-1-propanammonium fragment and its modification to *N*,*N*,*N*-trialkyl-2-(hidroxyimino)-1-propanammonium fragment (Fig. 2). This fragment is close to the structure of the choline moiety of acetylcholine that active center so that only some reactivators (3, 4) can resist this effect [2].

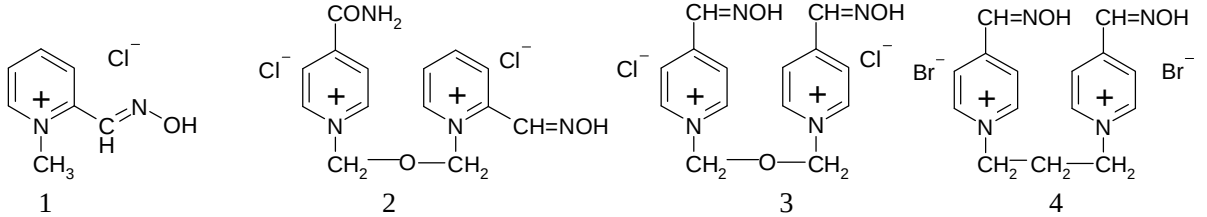
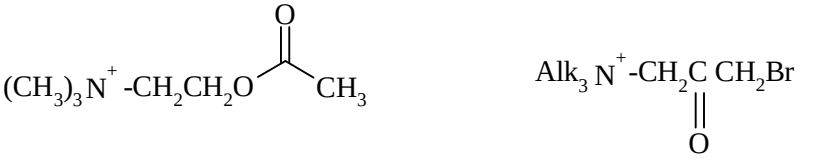


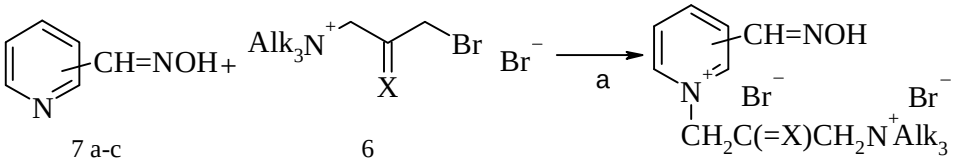
Fig. 1. Commercially available acetylcholinesterase reactivators



5 6

Fig. 2. Structures of fragments of acetylcholine molecules (5) and of alkylating agent – *N,N,N*-trialkylammonium-2-oxo-1-propan bromide (6)

Quaternary salts of pyridinium-aldoxime are received as follows:



7 a-c 6 8a-k

where X=O, Alk_3=$(CH_3)_3$, 4-CH=N-OH (a); 3-CH=N-OH (b);
X=O, Alk_3=$(C_2H_5)_3$, 4-CH=N-OH (c); 3-CH=N-OH (d);
X=O, Alk_3=CH_3 and $(CH_2)_4O$ (e); 3-CH=N-OH (f);
X=N-OH, Alk_3=$(CH_3)_3$, 4-CH=N-OH (g); 3-CH=N-OH (h); 2- CH=N-OH (i)
X=N-OH, Alk_3=$(C_2H_5)_3$, 4-CH=N-OH (j); 3-CH=N-OH (k).

However oximes 3 and 4 show an increased can enhance reactivation of phosphorylated cholinesterase in lesions of various types of PO compounds. Reactivation activity of the synthesized compounds was determined *in vitro* on enzyme preparations – human blood erythrocyte acetylcholinesterase with specific activity of 2.9 U/ml, suppressed by organophosphorus agent VX by 95–99.9 %, and evaluated by the largest concentration of the compound at which reactivation of phosphorylated enzyme by 50 % takes place, by the method [14].

2. Experimental

2.1. Experimental Chemistry

The ^{1}H NMR spectra were recorded by "Tesla BS-485, Bruker" (80 MHz, 200 MHz) spectrometer in a DMSO-d6 using tetramethylsilane (TMS) as an internal standard (chemical shift values are reported in ppm units, coupling constants *J* are in Hz). Abbreviations are as follows: s – singlet; d – doublet; t – triplet; m – multiplet. IR spectra (pellets KBr) were recorded by the "Perken Elmer" instrument. Progress of the reaction and purity of the synthesized compounds are monitored by thin-layer chromatography on "Silufol UV-254" sheets in the system of *n*-propanol : acetate acid : water = 5:2:5. All solvents and reagents were used after additional purification. Melting points were measured on a small-sized heating table with PHMK 05 device (VEB Analytik, Dresden).

2.2. Synthesis

2.2.1. General procedure for the synthesis of oximes: 4(or 3)-[(hydroxyimino)methyl]-1-[2-oxo-3-trialkyl)ammonium)propyl] pyridinium dibromides 8a-f

4(or 3-)-[(hydroxyimino)methyl]pyridine (0.01 mol) in absolute alcohol (15 ml) was added to a solution of 3-bromo-2-oxo-*N,N,N*-trialkylammonium-1-propan bromide **6** (0.01 mol) in absolute alcohol (ethanol or propanol (50 ml) under stirring and refluxed for 2 h. The reaction mixture was stirred at a room temperature (293 ± 5 K) for 12–24 h. The formed crystalline solid was filtered off, washed with ethanol and diethyl ether on a filter, dried and crystallized.

4-[(Hydroxyimino)methyl]-1-[2-oxo-3-(trimethylammonium)propyl]pyridinium dibromide (**8a).** Yield 77 %, m.p. 481–483 K (EtOH), (R_f·100) 0.16 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{12}H_{19}Br_2N_3O_2$: N 10.58, Br 40.24; Found: N 10.38, Br 40.12. NMR ^{1}H (DMSO-d6, 80 MHz) δ (ppm):12.81 (c., 1H, OH), 9.07 (d., 2H, *J* = 7 Hz, H-2+H-6), 8.41 (d, 2H, *J* = 7 Hz, H-3 + H-5), 8.51 (c., 1H, CH=N), 6.18 (c., 2H, CH_2), 4.32 (c., 2H, CH_2), 3.43 (c., 9H, $3CH_3$).

3-[(Hydroxyimino)methyl]-1-[2-oxo-3-(trimethylammonium)propyl]pyridinium dibromide (**8b**). Yield 69 %, m.p. 476–477 K (EtOH:*i*-PrOH = 1:1), (R_f·100) 4, (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{12}H_{19}Br_2N_3O_2$: N 10.58, Br 40.24; Found: N 10.41, Br

39.96. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 10.54 (c., 1H, OH), 9.47 (c., 1H, H-2), 8.87 (c., 1H, H-6), 8.13 (c., 1H, CH=N), 8.02 (m., 2H, H-4+ H-6), 6.01 (c., 2H, CH_2), 4.33 (c., 2H, CH_2), 3.23 (c., 9H, $3CH_3$).

4-[(Hydroxyimino)methyl]-1-[2-oxo-3-(triethylammonium)propyl]pyridinium dibromide (**8c**). Yield 24 %, m.p. 459–461 K (*i*-PrOH), (R_f·100) 6, (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{15}H_{25}Br_2N_3O_2$: N 9.57, Br 36.39; Found: N 9.55, Br 36.23. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 12.81 (c., 1H, OH), 9.07 (2H, H-2 + H-6), 8.4 (2H, H-3 + H-5), 8.51 (c., 1H, CH=N), 6.18 (c., 2H, CH_2), 4.32 (c., 2H, CH_2), 3.42 (q., 6H, J = 7 Hz, $3CH_2$), 1.3 (t., 9H, J = 7 Hz, $3CH_3$).

3-[(Hydroxyimino)methyl]-1-[2-oxo-3-(triethylammonium)propyl]pyridinium dibromide (**8d**). Yield 23 %, m.p. 456–458 K (*i*-PrOH), (R_f·100) 5, (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{15}H_{25}Br_2N_3O_2$: N 9.57, Br 36.39; Found: N 9.7, Br 36.5. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 10.54 (c., 1H, OH), 9.47 (c., 1H, H-2), 8,86 (c.,1H, H-6), 8.13 (c., 1H, CH=N), 8.01 (m, 2H, H-4 + H-5), 6.01 (c., 2H, CH_2), 4.32 (c., 2H, CH_2), 3.43 (q., 6H, J = 7 Hz, $3CH_2$), 1.29 (t., 9H, J = 7 Hz, $3CH_3$).

4-[3-[4-[(Hydroxyimino)methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methyl-morpholinium dibromide **(8e).** Yield 95 %, m.p. 468–469 K (EtOH), (Rf·100) 7 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $_{14}H_{21}Br_2N_3O_3$: Br 36.39; N 9.56; Found: Br 36.58; N 9.33. NMR ^{1}H (DMSO-d6, 80 MHz) δ (ppm): 11,3 (1H, c., OH), 9,22 (d., 2H, J= 4 Гц, H2 + H-6); 8,5 (c., 1H, CH=N); 8,34 (d., 2H, J = 4 Hz, H-3 + H-5); 6,1 (c., 2H, CH_2); 4.34 (c., 2H, CH_2); 3.9 (4H, CH_2OCH_2); 3.6 (4H, CH_2NCH_2), 3.39 (c., 3H, CH_3). IR-spectrum (KBr, см$^{-1}$): 3485 (υ_{OH}); 1740 ($\upsilon_{C=O}$); 1630 ($\upsilon_{C=N}$); 1605, 1520 (υ_{Py}).

4-[3-[3-[(Hydroxyimino)methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methyl-morpholinium dibromide (**8f**). Yield 57 %, m.p. 481–482 K (EtOH-MeOH), (R_f·100) 4 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{14}H_{21}Br_2N_3O_3$: N 9.56, Br 36.39; Found: N 9.46, Br 36.52. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 10.54 (c., 1H, OH), 9.47 (1H, H-2), 8,86 (1H, H-6), 8.13 (c., 1H, CH=N), 8.02 (m., 2H, H-4 + H-6), 6.01 (c., 2H, CH2), 4.34 (c., 2H, CH2), 3.9 (4H, CH_2OCH_2), 3.6 (4H, CH_2NCH_2), 3.39 (c., 3H, CH_3).

2.2.2. General procedure for the synthesis of oximes: 4(or 3, or 2)-[(hydroxyimino)methyl]-1-[2-hydroxyimino-3-trialkylammonium)propyl] pyridinium dibromides 8g-k

3-Bromo-*N*, *N*, *N*-trialkylamonium-2-(hydroxyimino) propane bromide (0.01 g-mol) was dissolved in 50 ml of ethanol with heating and 4 (or 3 or 2)-(hydroxyiminomethyl) pyridine (0.01 g-mol) was added. The reaction mixture was heated for 2.5 h (or 4 h., or 8–15 h., respectively), cooled and stirred for 12 h at 293 K. Colorless crystalline precipitate is filtered off, dried and crystallized. For substances **8g–k** solvent is evaporated, getting the oily residue with grout crystalline solid which is filtered, dried and washed with ether and crystallized.

4-[(Hydroxyimino)methyl]-1-[2-(hydroxyimino)-3-(trimethylammonium)propyl]pyridinium dibromide (**8g**). Yield 36.5 %, m.p. 465–467 K (EtOH), (R_f·100) 9 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{12}H_{20}Br_2N_4O_2$: N 27.19, Br 38.77; Found: N 27.33, Br 38.61. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 11.59 (c., 1H, OH), 11.33 (c., 1H, OH), 9.24 (d., 2H, J = 6 Hz, H-2 + H-6), 8.34 (d., 2H, J = 6 Hz, H-3 + H-5), 8.48 (c., 1H, CH=N), 5.77 (c., 2H, CH_2), 4.40 (c., 2H, CH2), 3.39 (c., 9H, $3CH_3$). ^{13}C NMR (DMSO-d6, 200 MHz) δ (ppm): 54.20, 58.00,61.62, 123.77, 143.75, 145.27,146.61, 149.24.

3-[(Hydroxyimino)methyl]-1-[2-(hydroxyimino)-3-(trimethylammonium)propyl]-pyridinium dibromide (**8h**). Yield 43 %, m.p. 477–478 K (EtOH), (R_f·100) 1 (n-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{12}H_{20}Br_2N_4O_2$: N 27.19, Br 38.77; Found: N 27.28, Br 38.58. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 12.05 (c., 1H, OH), 10.54 (c., 1H, OH), 9.22-8.81 (2H, H-2 + H-6), 8.01 (2H, H-4 + + H-5), 8.13 (c., 1H, CH=N), 5.34 (c., 2H, CH_2), 4.67 (c., 2H, CH_2), 3.15 (c., 9H, $3CH_3$).

*2-[(Hydroxyimino)methyl]-1-[2-(hydroxyimino)-3-(trimethylammonium)propyl]-pyridinium dibromide (***8i)**. Yield 32.7 %, m.p. 458–460 K, (R_f·100) 1 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{12}H_{20}Br_2N_4O_2$: N 27.19, Br 38.77; Found: N 27.28, Br 38.67. NMR ^{1}H (D_2O, 200 MHz) δ (ppm): 8.67-8.69 (d, 1H, J = 6 Hz, H-6), 8.61 (s, 1H, CH=N), 8.40-8.45 (t, 1H, J = 7 Hz, H-4), 8.29-8.32 (d, 1H, J = 7 Hz, H-3), 7.89-7.94 (t, 1H, J = 6 Hz, H-5), 5.67 (c., 2H, CH_2), 4.67 (c., 2H, CH_2), 3.15 (c., 9H, $3CH_3$).

4-[(Hydroxyimino)methyl]-1-[2-(hydroxyimino)-3-(triethylammonium)propyl]-pyridinium dibromide (**8j**). Yield 34 %, m.p. 451–453 K (*i*-PrOH), (R_f·100) 13 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{15}H_{26}Br_2N_4O_2$: N 12.33, Br 35.18; Found: N 12.24, Br 35.34. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 11.6 (c., 1H, OH), 11.3 (c.,1H, OH), 8.40 (d., 2H, J = 6 Hz, H-2 + H-6), 7.45 (d., 2H, J = 6 Hz, H-3 + H-5), 5.51 (c., 2H, CH_2), 4.66 (c., 2H, CH_2), 3.5 (q, 6H, J = 7 Hz, $3CH_2$), 1.36 (t., 9H, J = 7 Hz, $3CH_3$).

3-[(Hydroxyimino)methyl]-1-[2-(hydroxyimino)-3-(triethylammonium)propyl]-pyridinium dibromide (**8k**). Yield 53 %, m.p. 445–448 K (EtOH), (R_f·100) 4 (*n*-PrOH:AcOH:H_2O, 5:2:5). Anal.calcd. for $C_{12}H_{20}Br_2N_4O_2$: N 12.33, Br 35.18; Found: N 12.43, Br 35.10. NMR ^{1}H (DMSO-d6, 200 MHz) δ (ppm): 11.8 (c., 1H, OH), 10.54 (c., 1H, OH), 9.2 (c., 1H, H-2), 8.8 (c., 1H, H-6), 8.02 (m.,

2H, H-4 + H-3), 8.13 (c.,1H, CH=N), 5.3 (c., 2H, CH_2),4.7 (c., 2H, CH_2),3.5 (q., 6H, J = 7 Hz, $3CH_2$), 1.36 (t., 9H, J = 7 Hz., $3CH_3$).

2.3. Biological Activity

2.3.1. Reactivation of phosphylated cholinesterases by oximes 8 a-k

In vitro reactivation ability at the concentration of oximes **C** (10^{-5}–10^{-3} M/l) was studied in human erythrocytes cholinesterase from phosphorylation VX. The reduced enzyme activity was determined (in percent) at 310 K, pH 7.8 for 30 min. The method of continuous potentiometric titration of acetate acid formed during the hydrolysis of acetylcholine (Ach·HCl) solution of alkali (NaOH, 0.05 M) at a constant temperature of 310 K and pH 7.8 was used. The enzyme activity was determined by potentiometric method [14, 15].

For calculation of the enzyme activity we determined slope angles of alkali consumption depending on hydrolysis time of ACh ($\Delta V/\Delta t$).

Calculation of the reactivators relative ability in concentration C is performed using the formula:

$$A = [(\varphi_c - \varphi_d)/(\varphi_0 - \varphi_d)]\cdot 100, \%$$

where φ_0 – $\Delta V/\Delta t$ for active AChE; φ_c – $\Delta V/\Delta t$ for reactivator in concentration C, M; φ_d – $\Delta V/\Delta t$ for the depressed AChE.

Reactivation ability of the compounds was evaluated by the size of the maximum recovery of phosphorylated enzyme in the concentration range of 10^{-5}–10^{-3} M/l and the largest concentration of compound that restores the depressed enzyme activity by 50 % for 30 h, 310 K and pH 7.8. According to the schedule of reactivation ability (A, %) dependence on the concentration C, parameter C50 was determined. In Table 1 the index of reactivation ability of the synthesized compounds is presented.

2.3.2. Determining reactivation ability *in vitro*

Reactivation ability was determined at human acetylcholinesterase (specific activity 2.9 E/ml). Acetylcholine chloride (initial concentration $2\cdot10^{-2}$ M) was determined for substrate. Enzyme has been deactivated by inhibitor VX (10^{-1} M) by 95–99 %. The inhibitor was incubated with a solution of acetylcholinesterase (3 mg/ml) in phosphate buffer (1/15 M) for 3 min at 298 K. Excess of inhibitor is removed by dialysis in phosphate buffer (1/15 M, pH 7.6). Solutions of tested compounds are prepared in ethanol (initial concentration $2\cdot10^{-3}$ M). Working concentration of tested compounds is prepared by the method of cultivation by the phosphatic buffer.

8 ml of water, 1 ml of KCl (0.1 N), 0.4 ml substrate ($2\cdot10^{-2}$ M), 0.6 ml sample: 0.3 ml phosphylated acetylcholinesterase, and 0.3 ml reactivator (tested compound) in phosphate buffer (pH 7.8, 1/15 M) were placed in a thermostated cuvette. Total reaction volume was 10 ml.

Table 1

Ability of tested compounds 8 a-k to reactivate VX-inhibited acetylcholinesterase *in vitro*

Compounds	Reactivation , % (concentration, M/l)					A_{max}, %	C_{50}
	10^{-5}	$5\cdot10^{-5}$	10^{-4}	$5\cdot10^{-4}$	10^{-3}	–	(10^{-4}), M/l
8a	7.45	31.73	44.4	67.15	58.3	67.15	2
8b	2.58	3.41	5.17	9.33	12.1	12.1	–
8c	21	30.6	37.5	57.54	59.43	59.43	3.4
8d	0.5	1.1	1.8	14.9	22.7	22.7	–
8e	19.49	34.9	48.74	61.63	68.96	68.96	1.6
8f	0.37	1.8	2.8	2.84	3.7	3.7	–
8g	8.4	20.23	30.35	41.71	32.6	41.71	5
8h	2.14	6.98	13.23	24.88	20.96	24.88	–
8i	2.42	3.09	6.62	7.72	8.16	8.16	–
8j	20	25.55	32.81	13	4.84	32.81	–
8k	1.93	7.11	14.12	21.54	12.9	21.54	–
TMB4	11.7	27.1	30.3	34.1	36.1	36.1	–

Note: A_{max} – maximal reactivation ability among all the tested compounds at concentrations: 10^{-5}–10^{-3} M; C50– concentration of compounds for reactivation ability of 50 %; TMB4 – trimedoxime

3. Results and Discussion

3.1. Synthesis and Characteristics

Pyridinium-aldoxime quaternary salts **8a-k** were obtained by reaction of 4(or 3)- [(hydroxyimino)methyl]pyridine of 3-bromo-2-oxo(or 2-hydroxyimino)-*N,N,N*-trialkylammonium-1-propane bromide in ethanol. Alkylation reaction of 3-bromo-2-oxo-*N,N,N*-trialkylammonium-1-propane bromide occurs with higher yields than similar reaction of 3-bromo-2-hydroxyimino-*N,N,N*-trialkylammonium-1-propane bromide. Yield of compounds **8a-k** is 23–95 %. According to H^1 NMR of pyridinium-aldoxime salts **8a-k** were formed. One of protons of iminomethyl-fragment of 4-substituted pyridinium-aldoxime salts can be seen in H^1 NMR spectrum as a singlet at 8.5 ppm. Similarly, the singlet at 8.13 ppm characterizes the proton of iminomethyl-fragment for 3-substituted pyridinium-aldoxime salts. The position of the signal proton of hydroxy group for aldoxime and ketoxime fragments is located in the weak field in the range of 12–10.5 ppm. The study of H^1 NMR spectra in D_2O shows the deuterium exchange of hydroxy group protons of oxime substituent.

3.2. Biological Activity

Reactivation ability of the salts **8a-f** is largely determined by the position of hydroxyiminomethyl group in the pyridine ring. 4-(Hydroxyimino)methyl]-1-[2-oxo-3-(trialkylammonium)propyl]pyridinium dibromides are characterized by considerably higher reactivating activity than 3-[(hydroxyimino)methyl]-1-[2-oxo-3-(trialkylammonium)propyl]pyridinium dibromides. A similar dependence was observed in the series of **8g-k** with a ketoxime group in the alkyl chain. Compounds **8a** and **8e** show high reactivation ability (**8a**: A_{max} = 67.15 % at the concentration of $5\cdot10^{-4}$ M; **8e**: A_{max} = 68.96 % at the concentration of $1\cdot10^{-3}$ M). Thus, the concentrations of compounds **8a** and **8e** which reduce activity of the suppressed enzyme by 50 % are $2\cdot10^{-4}$ and $1.6\cdot10^{-4}$ M, respectively. In a series of compounds **8g-k** compound **8g** restores deactivated enzyme activity by 50 % at the concentration of $5\cdot10^{-4}$ M. Compound **8j** has A_{max} = 32.81 % at the concentration of $1\cdot10^{-4}$ M (TMB4: A_{max} is 36.1 % at the concentration of $1\cdot10^{-3}$ M and activity 30.3 % at the concentration of $1\cdot10^{-4}$ M).

Investigated compounds **8a,g,h,k** and **8b,c,d,e,f,j** display maximum reactivation activity at the concentrations of 0.001 and 0.0005 M/l, respectively (Fig. 3). Compounds **8a,c,e** reduced activity of phosphorylated acetylcholine esterase by $A > 50$ %. Activity of compounds **8a,c,e** is higher than activity of trimedoxime. Dependence of reactivation activity of the compounds on the concentration is shown in Figs. 4 and 5.

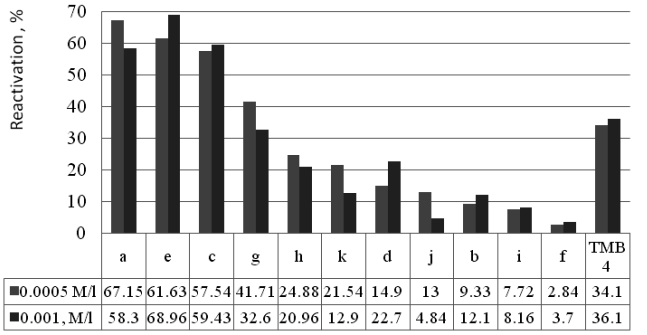


Fig. 3. Bar graph of reactivation activity of compounds **8a-k** and TMB4 in the concentrations of 0.0005 M/l and 0.001 M/l

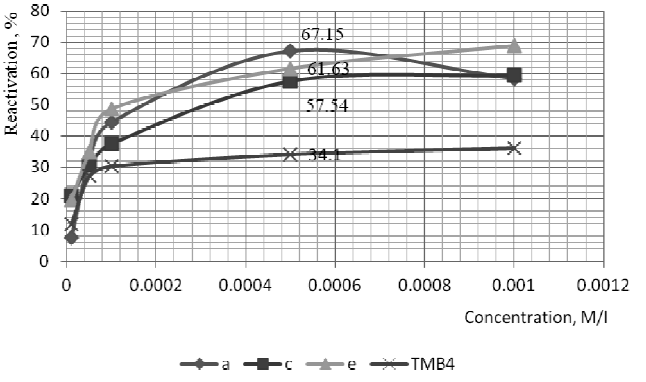


Fig. 4. Dependence of reactivation activity of compounds **8a,c,e** ($A > 50$ %) and TMB4 on concentration

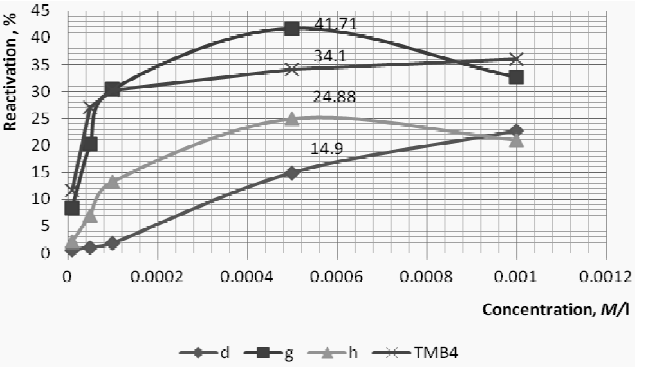


Fig. 5. Dependence of reactivation activity of compounds **8d,g,h** ($A < 50$ %) and TMB4 on concentration

For compounds **8a,c,e** kinetic parameters (p*C*50, p*Kr*), which characterize the process of reactivation (*Kr* – reactivators efficiency ratio in the equilibrium stage at concentration of reactivators 1 M and n = [p*Kr*/p*C*50] – Hill coefficient) can be determined. For this we studied the dependence of the p(100-*A*)/*A* from p*C* (Fig. 6). On the basis of approximating curve (linear) of each of the dependencies, corresponding kinetic parameters were received (Table 2) graphically or by calculations (Eqs. (1-3)).

$$\mathbf{p(100\text{-}A)/A} = -0.64484\ \mathbf{pC} - 2.31415\ (\mathbf{8a}) \qquad (1)$$

R = 0.9368, R^2 = 0.8776, $F(1,3)$ = 21.512, p < 0.01889, Std. error of estimate: .22192.

p(100-*A*)/*A* = -0.39584 **p*C*** - 1.38069 **(8c)** (2)

R = 0.9924, R^2 = 0.9848, $F(1,3)$= 193.94, p < 0.00080, Std. error of estimate: 0.04537.

p(100-*A*)/*A* = -0.47762 **p*C*** -1.80108 **(8e)** (3)

R = 0.9918, R^2 = 0.9837, $F(1,3)$= 181.21 p < 0.00089, Std. error of estimate: 0.05664.

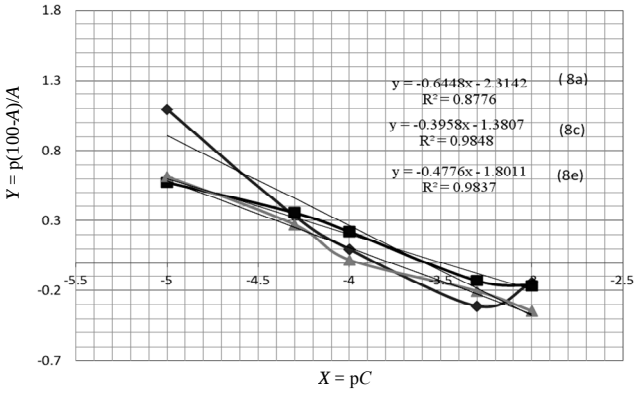


Fig. 6. Dependence of p(100-*A*)/*A* from p*C* for compounds **8a,c,e** (if У = 0, then X = p*C*50; if X = 0, then У = [p*Kr*])

Table 2

Kinetic parameters of process of reactivation compounds 8 a,c,e

Compound	p*C*50	p*Kr*	*n*
8a	-3.58	2.31	0.65
8c	-3.49	1.38	0.40
8e	-3.77	1.8	0.48

4. Conclusions

A simple method for the synthesis of pyridinium-aldoxime salts **8a-k** by alkylation reaction of pyridine-aldoxime from bromides, containing 3-bromo-*N,N,N*-trialkyl-2-oxo(or 2-hydroxyimino)-1-propanammonium fragments was developed. All synthesized compounds were tested for reactivation of phosphorylated acetylcholinesterase. Using fragments of the molecules (an oxime group, 3-bromo-*N,N,N*-trimethyl (or triethyl) ammonium bromides (or *N*-methyl-morpholinium)-2-oxo- or 2-hydroxyimino)-1-propane bromides), a new series of pyridinium-aldoxime salt (reactivators of cholinesterase) was obtained.

Compounds 4-[(hydroxyimino)methyl]-1-[2-oxo-3-(trimethylammonium)propyl]pyridinium dibromide (**8a),** 4-[(hydroxyimino)methyl]-1-[2-oxo-3-(triethylammonium)propyl]pyridinium dibromide (**8c**) and 4-[3-[4-[(hydroxyimino)methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methyl-morpholinium dibromide **(8e)** reduced activity of phosphorylated acetylcholine esterase by the value of A > 50 % (p*C*50 = -3.58; -3.49 and 3.77 respectively).

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СИНТЕЗ І ДОСЛІДЖЕННЯ РЕАКТИВАЦІЇ ФОСФОРИЛЬОВАНОЇ ХОЛІНЕСТЕРАЗИ ЧЕТВЕРТИННИМИ СОЛЯМИ ПІРИДИНІЙ-АЛЬДОКСИМУ

Анотація. *Вивчена взаємодія піридин-4(-3,-2)альдоксиму із 3-бром-N,N,N-тріалкіл-2-оксо(-гідроксіміно)-1-пропанамоній броміду. Склад і структура синтезованих сполук 4(-2,-3)-(гідроксіміометил)-1-[(2ℓ-оксо(гідроксіміно)-3ℓ-тріалкіламоній)пропіл)]піридиній дибромідів встановлені з використанням елементного аналізу, фізичних та хімічних методів. Синтезовані сполуки випробувані на їх активність як реактиватори фосфорильованої ацетилхолінестерази. З використанням молекулярних фрагментів (оксимна група, 3-бром-N,N,N-тріметил(або тріетил)амоній(або N-метил-морфолініій)-2-оксо(або 2-гідроксіміно)пропан броміди), отримані нові реактиватори холінестерази в ряду солей піридину.*

Ключові слова. *синтез, четвертинна сіль піридиній-альдоксиму, реактиватор фосфорильованої ацетилхолінестерази.*