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Original Article

Synthesis, antioxidant activity and QSAR studies of some pyridine derivatives

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ABSTRACT

New quaternary pyridine salts and bases was synthesized and characterized in the presence of iminomethyl-fragment containing the hydroxyl group or the hydroxy-ethyl group or the hydroxyphenyl group with imine nitrogen atom. The synthesized compounds were tested *in vitro* for an antioxidant activity with a stable radical 2.2-diphenyl-1-picrylhydrazyl and reactivation ability of Sarin-inhibited AChE. The influence of antioxidant activity parameters on reactivation ability and toxicity of the studied compounds was analyzed.

Key words: pyridine derivative, antioxidant activity**,** reactivation ability, QSAR studies

INTRODUCTION

The overproduction of cell free radicals affects significantly on the development of many pathological states. Protection against its excess ensures the functioning of the cellular antioxidant system [1]. The appearance of cancer, arteriosclerosis, Alzheimer's disease is often associated whit the action of free radicals [9].

The antioxidant activity of natural and synthetic substances now is subject of very intensive studies and considered as their important description. This is evidenced by the large number of publications in the scientific periodical literature. This is due, in particular, with the fact that, as follows from the literature data, it is antioxidant properties are crucial in many aspects of the biological activity of substances [5].

Reducing the toxicity of drugs can be achieved through sharing with them the drugs of a class of antioxidants, because oxidative stress is accompanied by toxic effects of most xenobiotics [7]. For example, one of the mechanisms of toxicity of Adriamycin is a violation of pro - and antioxidant balance and oxidative stress [15].

Antioxidants display a wide spectrum of pharmacological activity and have anticancer, anti-inflammatory, and regenerating properties [3, 4]. For example, the antioxidant activity of Dibunole plays a role in the mechanism of its anti-cancer and local anti-inflammatory action [16].

Found that many drugs with antioxidant properties cause a pronounced anti-inflammatory effect. As a probable mechanism of anti-inflammatory and immunomodulatory effects may be assumed ability of drugs to regulate the processes of free-radical oxidation.

It is known that Uracil and its derivatives are biologically active agents and are widely used in medicine. One of the most important properties of Uracil and its derivatives is their ability to slow down the radical-chain process. To the present time has been studied quite extensively reactivity of a number of Uracil derivatives in the reactions of chain breakage on peroxyl radicals [14].

By this time developed many different methods for determination of antioxidant activity *in vitro* [11]. Methods for determination of antioxidant activity of medicinal compounds using colored stable radicals, based on the determination of the reactivity of drugs by spectrophotometric method or by the EPR method. Usually use stable free radicals such as hydrazyl, nitroxyl, isolated in the free state [13].

To study the activity of natural antioxidants is commonly use the DPPH (2.2-diphenyl-1-picrylhydrazyl) [4]. PubMed database shows that

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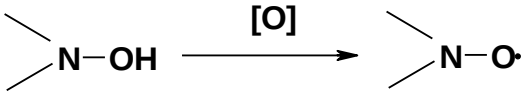
this radical has been used in more than 850 researches, since 1969 [11].

DPPH is intensely colored crystalline substance (λ_{max} 520 nm), stable in alcohol solutions. When storing it in ethanol solution in the dark for 240 hours the intensity of the maximum absorption of its electronic spectrum remains unchanged. It is important that the DPPH is stable to air oxygen [13]. These properties are important when choosing a method of determining the antioxidant activity of medicinal compounds for their reactivity with DPPH.

Numerous studies have shown that the antioxidant activity of a substance fundamentally depends on the used test system. For example, the rate constants of the interaction of phenolic antioxidants (gallic acid, epicatechin, pyrocatechole, chrysin, p-oxybenzoic acid) with DPPH approximately 5 orders of magnitude smaller than the rate constants of the interaction of these substances with alkylperoxide radicals in the lipophilic environment [14, 15].

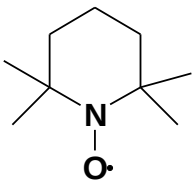
But, in both cases, there is a similar relationship between the logarithm of the rate constants of the reaction of antioxidants with the corresponding radical and enthalpy of dissociation of the phenolic hydroxy group. The same correlation between the values of rate constants of reactions (k_7), measured by the method of chemiluminescence and method with DPPH, was established for more than 20 compounds belonging to different classes of inhibitors of radical processes (various alkyl substituted phenols, polyphenols, 3-oxypyridine, tocopherol). These data confirm the correct use of DPPH radical for the initial evaluation of antiradical activity of antioxidants [14].

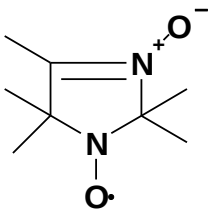
It is known that the oxidation of substituted hydroxylamine leads to the formation of nitroxyl radicals:

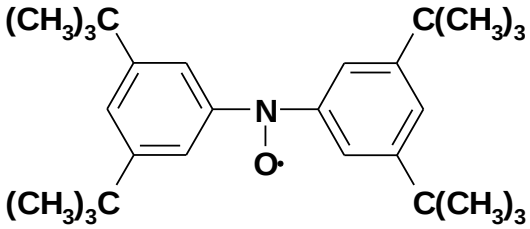


The reaction proceeds very easily, even when standing on the air. The stability of these substances depends on the degree of delocalization of the unpaired electron, and also of steric hindrance in the molecule. Nitroxyl radicals are representatives of different ranks: piperidine, pyrroline, pyrrolidine, piperazine, isoindoline, carboline, azetidine, imidazoline and others.

Examples:







Nitroxyl radicals, which are characterized by the presence of steric hindrance near the N-O group (for example, due to the tertiary carbon atoms), are highly stable and can be isolated in the free state. It replaced hydroxylamines are oxime reaktivators (Dipiroxime and its analogues). The main biological activity of Dipiroxime and its analogues is the ability to dephosphorylate locked acetylcholinesterase. We can expect that the antioxidant properties of oximes reactivators due to the presence of N-OH fragment may play a role in the mechanism of their reactivation ability and toxic effects.

In connection with the above, we studied the reactivity and antioxidant activity some derivatives of pyridine and their analogues and QSAR analysis.

RESULTS AND DISCUSSION

Chemistry. Some pyridinium salts known as acetylcholinesterase reactivators and as effective antidotes in poisoning by organophosphates. The most important oximes are Toxogonin, Dipiroxime, Pralidoxime and Alloxime (Figure-1). In this article the structure of pyridinium salts are characterized by the presence of iminomethyl fragment containing hydroxyl group, hydroxy-ethyl group or hydroxyl-phenyl group about the imine nitrogen atom. Pyridinium salts (**a-c**) were obtained by the reaction of 1-brom-2-oxo-3-(N-methyl morpholinium)propane bromide with 4-pyridinealdoxime, 4-(2-hydroxyethyl)imino-methyl)-pyridine, and 3-(or 2- (or 3-, or 4-)-(hydroxyphenyl) imino)methyl)pyridine in ethanol or isopropanol, and all reaction products were

obtained as (2-oxo-4-methylmorpholinium)propyl derivatives.

Biological activity. Reactivation of phosphorylated cholinesterase by the tested compounds. Reactivation ability of the synthesized compounds at a concentration of 5×10^{-4}, mol/L were studied in experiments *in vitro* on human erythrocyte cholinesterase, phosphorylated with Sarin. The reduced enzyme activity was determined (in percent) at 37 ° C, pH 7.8 for 30 min used the method of continuous potentiometric titration of acetic acid formed during the hydrolysis of acetylcholine (ACh×HCl) by solution of alkali (NaOH, 0.05 mol). Calculation of the enzyme activity was determined by the angles (tgά) curves of alkali consumption inclination amounts by hydrolysis of ACh by time (ΔV/Δt).

Calculation of the relative potency in reactivators concentration C was performed using the formula:

$$A\ (\%) = [(\varphi_c - \varphi_{пр.})/(\varphi_0 - \varphi_{пр.})]\times100,$$

φ_0 – ΔV/Δt for active AChE; φ_c – ΔV/Δt for reactivator in concentration C (M); $\varphi_{пр.}$ – ΔV/Δt for the depressed AChE.

Reactivation ability (A, %) of mono-pyridinium salts (a-c, pralidoxime, alloxime) and bis-pyridinium salts (toxogonin and dipiroxime) is determined by the presence aldoxime group in pyridine ring. Salts (**a,c)** and, with the bases of the pyridine (**d-i)** did not show reactivation ability in relation to acetylcholinesterase, phosphorylated with Sarin, at a concentrations of 5×10^{-4} and 10^{-5} mol/L. The compound (**b**) restores the activity acetylcholinesterase, phosphorylated Sarin, the value of 50.2 %. Reactivation ability of oxime drugs comparison in the experiment was two times higher in comparison with compound (**b**) (Table-1).

Antioxidant activity. The antiradical activity of the tested compounds was evaluated using the spectrophotometric method *in vitro*. To investigate the antiradical activity of the studied compounds the ethanol solution of DPPH (0.25×10^{-3} mol/L) was incubated in the presence of the tested compound (0.25×10^{-3} mol/L) at room temperature. The optical density of the alcohol solution tested compounds with DPPH free radical was measured at 520 nm immediately after mixing.

The Table-2 demonstrates the antiradical activity of the tested compounds as effective reaction rate constant (k_2) with DPPH free radical, and the time of half-transformation radical DPPH in the non-radical form (t_{50}). The formation energy of the radical (ΔE_{rad}) was calculated as the difference between the sum of the binding energies of the formed radicals ($E_{rad}O + E_{rad}H$) and the energy of binding for the parent molecule (E_{mol}): $\Delta E_{rad} = (E_{rad}O + E_{rad}H) - E_{mol}$. This dependence takes into consideration the homolytic cleavage of the connection between the oxygen atom and the hydrogen of the hydroxyl group for the optimized molecules of the tested compounds.

The reaction of the tested compounds with DPPH obeys the kinetic equation of the second order, since there is a linear relationship in the coordinates (1/[DPPH] - time t) (Figures-2,3,4).

The kinetic equation has the form:

$$-(d[DPPH])/dt = k_{eff} \times [DPPH]^2, \qquad (1)$$

where [DPPH] is the concentration of DPPH, k_{eff} is the effective reaction rate constant equal to the slope of a straight line to the *x*-axis.
Upon integrating the equation (1) converted to equation (2):

$$1/[DPPH] = 1/[DPPH]_0 + k_{eff} \times t_{50}, \qquad (2)$$

where $[DPPH]_0$ – is the initial concentration of DPPH in the reaction mixture.

From equation (2) we can calculate the time of half-transformation (t_{50}) of DPPH radical in the non-radical form:

$$1/[DPPH]_0 = k_{eff} \times t_{50} \text{ or } b = k_{eff} \times t_{50};\ t_{50} = b/k_{eff},$$

where b - segment, k_{eff} is the slope of the straight.

The tested compounds are characterized by different levels of antioxidant activity. It follows from the obtained results that the reaction of the tested compounds with radical DPPH method depends on the nature of the reacting compounds. Comparison of the effective rate constants (k_2), and of the time of half-transformation radical DPPH in the non-radical form shows that the rate of reaction of salts of the higher reaction rate of the relevant the base. So, in the presence of (2-hydroxyethyl)imino]methyl) substituent in the pyridine nucleus reactivity salt (**a)** (k_2=139.90 molˆ-1×L×minˆ-1) exceeds the reactivity of the corresponding Schiff bases (**d)** (k_2 = 2.70 molˆ-1×L×minˆ-1). In the presence of 3-[4-hydroxyphenyl)imino]methyl] the substituent at the pyridine nucleus reactivity salt (**c)** ($k_2 \approx 1014$ molˆ-1×L×minˆ-1) exceeds the reactivity of the corresponding Schiff bases (**h)** ($k_2 \approx 24$ molˆ-1×L×minˆ-1). Among 4-R-1-pyridiniumyl-2'-oxopropyl-4'-methyl-morpholinium dibromides reactivity decreases in the series: **c > a > b**.

Quaternary salt (Pralidoxime, Toxogonin, Dipiroxime, **b**, Alloxime) are characterized by the values of the effective rate constants of the reaction with DPPH radical in a narrow interval of values (k_2 from 12 to 37 molˆ-1×L×minˆ-1). Among the bases **(f, g, h**) base (**f**) is the most active compound in the interaction with the DPPH.

QSAR studies. In Table-3 shows the results of QSAR analysis. Correlation matrix contains data for reactivation ability (A1, A2), acute toxicity (Ld_{50}) (A1, A2), antioxidant activity (k_2, ΔE_{rad}) (Table-2), as well as lipophilicity and the volume molecules of the tested compounds (Table-4). The logP value, the volume (V_{mol}) was carried out using the Hyperchem Molecular package [17].

The model (1, 2) describes the relationship reactivation ability of the tested compounds with their lipophilicity (logP) and antioxidant activity (k_2). Analysis of the obtained models (1, 2) showed that reactivation ability of the tested compounds increases with increasing lipophilicity, and the contribution of lipophilicity in equations (1, 2) significant (beta-coefficient of 0.87, and 0.84 for A1, A2, respectively). Antioxidant properties, which are represented by the rate constant of the reaction with DPPH, affect reactivation ability of test compounds to a lesser extent. Their contribution to the equations (1, 2) is characterized by the value of beta coefficient -0.228, and -0.225 (for A1, and A2, respectively).

Model (3) describes the relationship of acute toxicity of the tested compounds with their lipophilicity (logP) and antioxidant activity (k_2, ΔE_{rad}). Negative values of the regression coefficients for these descriptors indicate that to reduce acute toxicity (increase in LD_{50} values) these descriptors should have smaller values. The contribution of the descriptors (logP, k_2, ΔE_{rad}) for acute toxicity (model 3) is characterized by the beta- coefficients -0.95, -0.51, -0.28 respectively.
Thus, the results of QSAR analysis shows a slight influence of antioxidant activity, represented by the rate constant of the reaction of the tested compounds with DPPH and the formation energy of the oxygen-centered radical of the tested molecules, on their reactivation ability and toxicity.

Experimental

Chemistry. All compounds were fully characterized by elemental analysis and spectroscopic data. ^{1}H NMR spectra were recorded on a Tesla BS-485 80 MHz, Varian Gemini 200 MHz, Bruker 300 MHz spectrometers. Deuterated DMSO was used as a solvent. The chemical shifts are expressed in δ (ppm) and the coupling constants (J) in herz (Hz). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet and br, broad. The IR spectra were recorded by Perkin Elmer spectrometers in KBr pellets. The UV spectra were recorded by Shimadzu MPS-5000 (Japan) spectrophotometer in quartz cuvettes with optical path of 10 mm. Progress of the reaction and purity of the synthesized compounds are monitored by thin-layer chromatography using "Silufol UV-254" sheets in the n- propanol - acetic acid - water (5:2:5). All solvents and reagents were used after further purification. Melting points were determinated on a small-sized table with a heating device supervisory PHMK 05 (VEB Analytik, Germany).

The Pralidoxime (2-hydroxyiminomethyl-1-methylpyridinium chloride), Dipiroxime (1.3-bis(4-hydroxyiminomethyl) pyridinium-propane] dibromide), Toxogonin (1.3-bis(4-hydroxy iminomethyl) pyridinium-2-oxapropane dichloride) and Alloxime (2-hydroxyiminomethyl-1-(2-propenyl) pyridinium bromide) are commercially available oxime reactivators. Quaternary pyridinium salt (**a-c**) were obtained by interaction of 4-(or 3-) substituted pyridine with [1-bromo-2-oxo-3-(N-methylmorpholiniume]propane bromide in equimolar ratio in boiling ethanol.

Preparation of compound (**b)**.
Step 1):
Pyridine-4-aldehyde oxime. Yield 90 %, m.p. 129-131 ºC, ^{1}H NMR (DMSO-d6): 10.3 (s, 1H, OH), 8.3 (s., 1H, CH), 8.5 -8.4 (d, 2H, J=9 Hz, PyH), 7.8 -7.7 (d, 2H, J=9 Hz, PyH).

Step 2):
[1-Bromo-2-oxo-3-(N-methyl morpholiniume] propane bromide. To a solution of 6 g (0.027 mol) of dibromacetone in 20 ml of acetone slowly add drops of 1.4 g (0.013 mol) of methylmorpholine in 15 ml of acetone. The reaction mixture was stirred for 2 h and incubated for 10 h at a temperature of (20±5) ºC. Then the precipitate was filtered, washed with acetone, dried, and crystallized from ethanol. Yield 2.1 g (47.9 %); m.p. (180-182) ºC (decomposition). Anal. calcd for $C_8H_{15}Br_2NO_2$: Br 50.41; N 4.41. Found: Br 50.56; N 4.31; (R_f×100) 22 (n-propanol-acetic acid-water 5:2:5).

Step 3):
4-[3-[4-[(Hydroxyimino)methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methyl-morpholinium dibromide (**b**). To 0.01 mol [1-bromo-2-oxo-3-(N-methylmorpholiniume]propane bromide in absolute ethanol is added 0.01 mol 4-[(hydroxyimino)methyl]pyridine in absolute ethanol. The reaction mixture is boiled for 2 h, allowed to stand at a temperature of (20±5) ° C for

10 h. Then ½ of the solvent is evaporated in water jet pump and sediment drops out, is filtered off and crystallized from ethanol. Yield 95 %; m.p. (195-196) ºC. (R_f×100) 7 (n-propanol-acetic acid-water 5:2:5). Anal. Calcd. for $C_{14}H_{21}Br_2N_3O_3$: Br 36.39; N 9.56. Found: Br 36.58; N 9.33. NMR ^{1}H (DMSO-d6) δ (ppm): 11.3 (s, 1H, OH), 9.22 (d, 2H, J=7 Hz, PyH); 8.5 (s, 1H, CHN); 8.34 (d, 2H, J=7 Гц, PyH); 6.1 (s, 2H, CH2), 4.34 (s, 2H, CH_2), 3.9 (4H, CH_2OCH_2), 3.6 (4H, CH_2NCH_2), 3.39 (c., 3H, CH_3); IR (KBr, cm^{-1}): 3485(υ_{OH}); 1740 ($\upsilon_{C=O}$); 1630 ($\upsilon_{C=N}$); 1605, 1520 (υ_{Py}).

Preparation of compound (**a**).

Step 1):

4-(2-Hydroxyethyl)iminomethyl)pyridine. Yield 98 %; m.p. 49-50 ºC, (R_f ×100) 53 (mobile phase ethanol), ^{1}H NMR (DMSO-d6): 8.5 (s., 1H, CH), 8.9 (d, 2H, J=7 Hz, PyH), 7.9 (d, 2H, J=7 Hz, PyH), 5.6 (s, 1H, OH), 3.9 (4H, $2CH_2$).

Step 2):

4-[3-[4-[(2-Hydroxyethyl)iminomethyl]-1-pyridiniumyl]-2-oxopropyl]-4-methyl-morpholinium dibromide (**a**). To 0.01 mol [1-bromo-2-oxo-3-(N-methylmorpholiniume]propane bromide in absolute ethanol is added 0.01 mol 4-[(2-hydroxyethyl)iminomethyl]pyridine in absolute ethanol. The reaction mixture is boiled for 2 h, allowed to stand at a temperature of (20±5) °C for 10 h. Then ½ of the solvent is evaporated in water jet pump, and residue drops out, is filtered off and crystallized from ethanol. Yield 76 %; m.p. (107-110) ºC (decomposition). (R_f×100) 5 (n-propanol-acetic acid-water 5:2:5). Anal. calcd for $C_{16}H_{25}N_3O_3Br_2$: N 8.97, Br 34.13. Found: N 8.76; Br 34.03. NMR ^{1}H (DMSO-d6) δ (ppm): 9.22 (d, 2H, J=7 Hz, PyH); 8,5 (s, 1H, CHN); 8.34 (d, 2H, J=7 Hz, PyH); 6.1 (s, 2H, CH_2), 5.6 (s, 1H, OH), 4.34 (s, 2H, CH_2), 3.9 (4H, CH_2OCH_2), 3.6 (4H, CH_2NCH_2), 3.39 (s, 3H, CH_3).

Preparation of compound (**c**).

Step 1):

3-[(4′-Hydroxyphenyl)iminomethyl]pyridine (h). To 0.1 mol of 4-aminophenol in 200 ml of absolute methanol at reflux, with stirring, was added 0.1 mol of pyridine-3-aldehyde in 100 ml of absolute methanol. The reaction mixture is boiled for 1-1.5 h. The precipitate was filtered, washed with methanol, diethyl ether and crystallized. Yield 98 %; m.p. (204-206) ºC. R_f×100 = 81 (aqueous ammonia (25 %) - ethanol 1:10). Anal. calcd for $C_{12}H_{10}N_2O$: N 14.1. Found: N 14.4. UV (DMSO): λ_{max} 355 нм. NMR ^{1}H (DMSO-d6, δ (ppm): 9.0 (s, 1H, PyH), 8.8 (d, J=4.7 Hz, 1H, PyH), 8.5 (s, 1H, CHN), 7.6 (t, J=7.7 Hz, 1H, PyH), 8.2 (d, J=7.7 Hz, 1H, PyH), 10.5 (s, 1H, OH), 7.2 (d, J=7.8 Hz, 2H, ArH), 6.9 (d, J=7.8 Hz, 2H, ArH).

Step 2) **3-[(4′-Hydroxyphenyl)iminomethyl]-1-pyridiniumyl]-2-oxopropyl]-4-methyl-morpholinium dibromide (c).** To 0.05 mol [1-bromo-2-oxo-3-(N-methyl-morpholiniume]propane bromide in 50 ml isopropanol is added 0.05 mol 3-[(4′-hydroxyphenyl)iminomethyl]pyridine (**h**) in 30 ml isopropanol. Yield 40 %; m.p. (204-206) ºC (in sealed capillary, with decomposition). Anal. calcd for $C_{20}H_{25}Br_2N_3O$: N 8.16, Br 31.02; Found: N 8,00; Br 31.20. NMR ^{1}H (DMSO-d6, δ (ppm): 9.1 (d, 2H, J=7 Hz, PyH); 8,2 (s., 1H, CHN); 8,7 (d., 2H, J=7 Hz, PyH); 7.6 (d, J=8.6 Hz, 2H, ArH), 7.4 (d, J=8.6 Hz, 2H, ArH), 6.2 (s, 2H, CH2), 4.34 (s, 2H, CH_2), 3.9 (4H, CH_2OCH_2), 3.6 (4H, CH_2NCH_2), 3.39 (s., 3H, CH_3).

Preparation of compounds (**f, g, i).** Similar to the synthesis of compound (**h).**

3-[(2′-Hydroxyphenyl)iminomethyl]pyridine (**f**). Yield 98 %; m.p. (96-98) ºC (hexane-methanol). Anal. Calcd. for $C_{12}H_{10}N_2O$: N 14.1; Found: N 14.00. IR (KBr, cm^{-1}): ν1630 (C=N). R_f×100 = 84 (aqueous ammonia (25 %) - ethanol 1:10). UV (DMSO): λ_{max} 360 nm. NMR ^{1}H (DMSO-d6, δ (ppm): 9.0 (s, 1H, PyH), 8.8 (d, J=4.7 Hz, 1H, PyH), 8.5 (s, 1H, CHN), 7.6 (t, J=7.7 Hz, 1H, PyH), 8.2 (d, J=7.7 Hz, 1H, PyH), 10.1 (s, 1H, OH), 7.3 (d, J=8 Hz, 1H, ArH),7.1 (t, J=8 Hz, 1H, ArH), 6.95 (t, J=8 Hz, 1H, ArH), 6.8 (d, J=8 Hz, 1H, ArH).

3-[(3′-Hydroxyphenyl)iminomethyl]pyridine (**g**). Yield 98 %; m.p. 315 ºC. Anal. calcd for $C_{12}H_{10}N_2O$: N 14.1; Found: N 13.77. IR (KBr, cm^{-1}): ν1630 (C=N). R_f×100 = 20 (aqueous ammonia (25 %) - ethanol 1:10). NMR ^{1}H (DMSO-d6, δ (ppm): 9.0 (s, 1H, PyH), 8.8 (d, J=4.7 Hz, 1H, PyH), 8.5 (s, 1H, CHN),7.6 (t, J=7.7 Hz, 1H, PyH), 8.2 (d, J=7.7 Hz, 1H, PyH), 7.6 (s, 1H, OH), 7.1 (t, J=8.8 Hz, 1H, ArH), 6.8 (d, J= 8.8 Hz, 1H, ArH), 6.5 (d, J=8.8 Hz, ArH), 6.1 (s, 1H, ArH).

3-[(4′-Methoxyphenyl)iminomethyl]pyridine (i). Yield 70 %; m.p. (55-56) ºC. Anal. calcd for $C_{13}H_{12}N_2O$: N 13.20; Found: N 13.27. IR (KBr, cm^{-1}): ν1630 (C=N). R_f×100 = 78 (aqueous ammonia (25 %) - ethanol аміак-еганол 1:10). UV (DMSO): λ_{max} 340 nm. NMR ^{1}H (DMSO-d6, δ (ppm): 9.0 (s, 1H, PyH), 8.8 (d, J=4.7 Hz, 1H, PyH), 8.5 (s, 1H, CHN), 7.6 (t, J=7.7 Hz, 1H, PyH), 8.2 (d, J=7.7 Hz, 1H, PyH), 7.2 (d, J=8.7 Hz, 1H, ArH), 6.9 (d, J=8.7 Hz, 1H, ArH), 3.7 (s, 3H, CH_3).

Reactivation ability. Reactivation ability was determined at human acetylcholinesterase (specific activity 2.9 u/ml). Acetylcholine chloride (initial

concentration 2×10^{-2} M) was determined for substrate. Enzyme has been deactivated by inhibitor Sarin on 95 - 99 %. The inhibitor was incubated with a solution of acetylcholinesterase (3 mg/ml) in phosphate buffer (1/15 M) for 30 min at 25 °C. Excess of inhibitor delete by dialysis in phosphate buffer (1/15 M, pH 7.6). Dilution of tested compounds was prepared in ethanol (initial concentration 2×10^{-3} M). Working concentration of tested compounds was prepared by method of cultivation in the phosphate buffer.

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

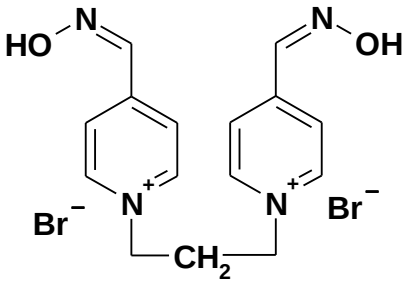
Antioxidant activity. Antiradical properties of pyridine derivatives were evaluated in the test with DPPH using spectrophotometric method [3-5]. The optical density measurement was carried out on a spectrophotometer Shimadzu MPS-5000 (Japan) in quartz cuvettes with optical path of 10 mm. The reaction of the tested compounds with DPPH was carried out in ethanol solution in equimolar doses and was determined by optical density at 520 nm immediately after mixing, then held definitions of certain intervals of time.

QSAR analysis. Multiple linear regression (MLR) analyses were used to determine regression equations, correlation coefficients R, R^2 and Standard Error of estimate.

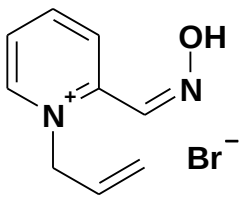
CONCLUSION

By the reaction of 1-bromo-2-oxo-3-(N-methylmorpholinium)propane bromide with 4-pyridinealdoxime or 4-(2-hydroxyethyl)imino)methyl)-pyridine, or 3-(2-(or 3-, or 4-)-(hydroxyphenyl)imino)methyl)pyridine obtained the corresponding salts. The reaction of the studied compounds with DPPH passes at different speeds. It is shown that the speed of the reactions of salts with the free radical was higher than the speed of reaction of the corresponding Schiff bases with a free radical. Quaternary salt pyridinium oxime (pralidoxime, toxogonin, dipiroxime, alloxime, and compound (**b**) are characterized by the values of the effective rate constants of the reaction with DPPH in a narrow interval of values. The results of QSAR analysis shows a slight influence of antioxidant activity (represented by the rate constant of the reaction of the tested compounds with DPPH, and the formation energy of the oxygen-centered radical of the tested molecules), on reactivation ability and toxicity of the tested compounds.

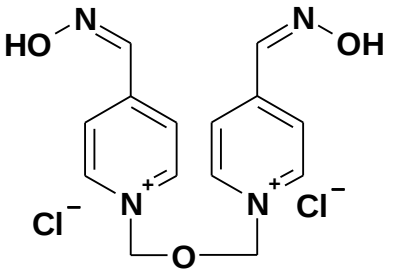
Competing interests: The authors declare no conflict interest.



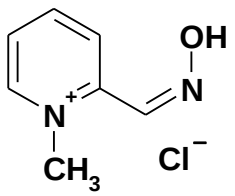
Dipiroxime



Alloxime



Toxogonin



Pralidoxime

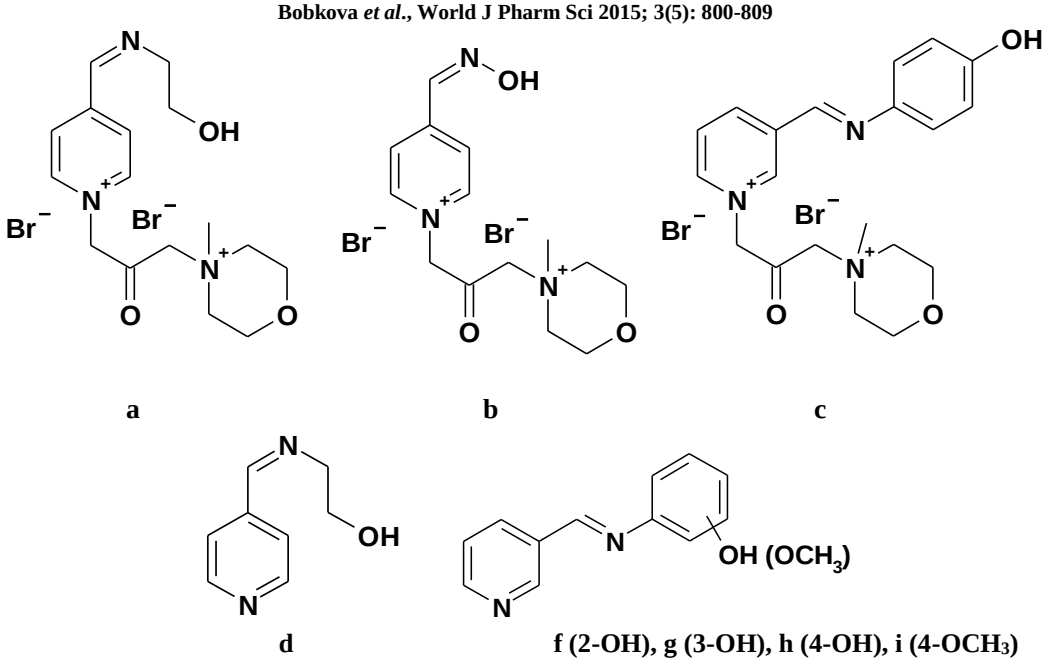


Figure-1: Structural formulas of Dipiroxime, Alloxime, Toxogonin, Pralidoxime, 4-[3-[4-[[(2-hydroxyethyl)imino]methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methylmorpholinium dibromide (**a**), 4-[3-[4-[(hydroxyimino)methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methylmorpholinium dibromide (**b**), 4-[3-[3-[[(4-hydroxyphenyl)imino]methyl]-1-pyridiniumyl]-2-oxopropyl]-4-methylmorpholinium dibromide (**c**), 4-[[(2-hydroxyethyl)imino]methyl]pyridine (**d**), 3-[[2-(or 3- or 4-) hydroxyphenyl)imino]-methyl]pyridine (**f-h**), 3-[[4-methoxyphenyl)imino]methyl]pyridine (**i**)

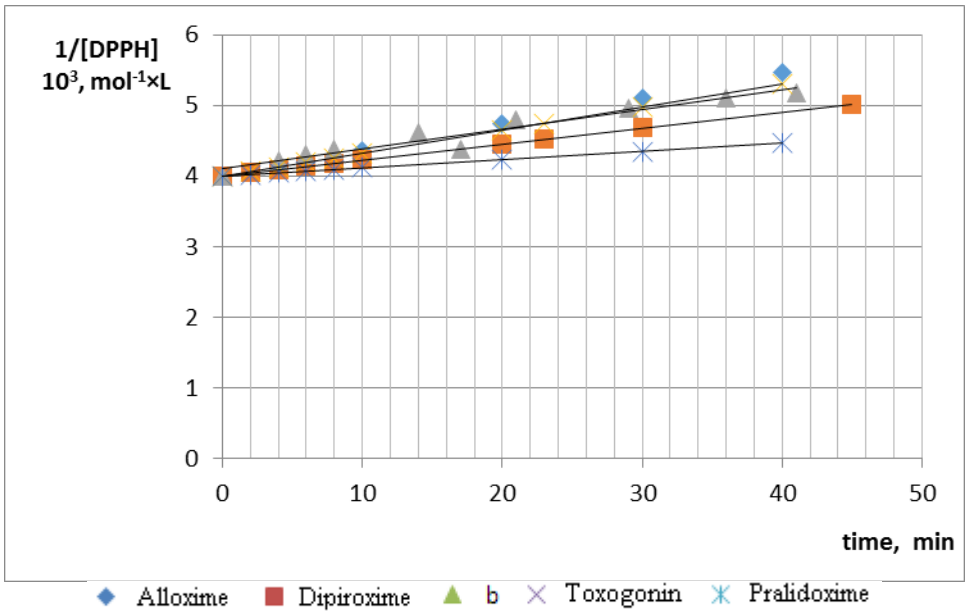


Figure-2: The inverse dependence of the concentration of DPPH (y) against time (x) for the reaction of DPPH with compounds Alloxime, Dipiroxime, **b**, Toxogonin, Pralidoxime

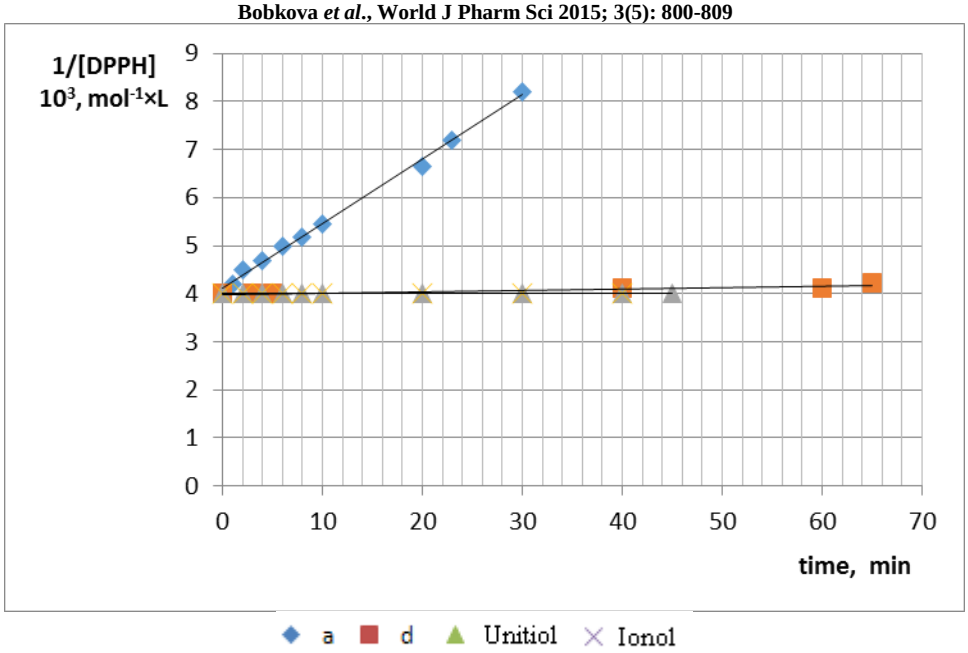


Figure-3: The inverse dependence of the concentration of DPPH (y) against time (x) for the reaction of DPPH with compounds **a, d,** Unitiol, Ionol

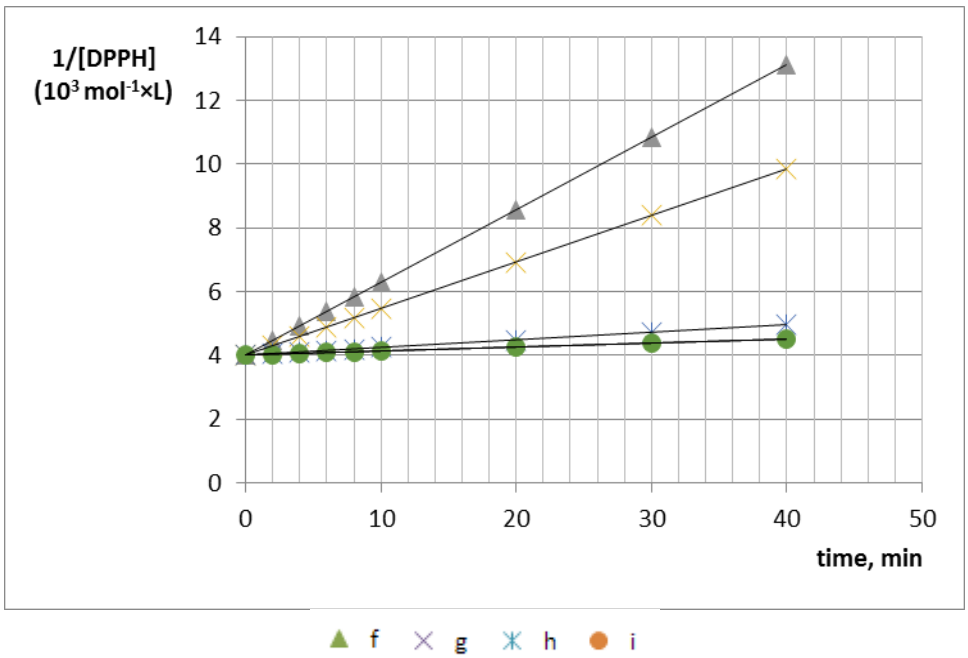


Figure-4: The inverse dependence of the concentration of DPPH (y) against time (x) for the reaction of DPPH with compounds **f, g, h, i**

Table-1: Reactivation ability and acute toxicity of the tested compounds

Compounds	A1, % (C=5×10^{-4}, mol/L)	A2, % (C=10^{-5}, mol/L)	Ld_{50}, mM/kg
b	50.2	29	0.9906
a	0	0	0.5779
c	0	0	0.547
Toxogpnin	84.5	69	0.309
Dipiroxime	94	50.2	0.2015
Pralidoxime	95.9	58.1	0.5793
Alloxime	96	54.4	0.2633

d	0	0	0.547
f	0	0	1.42
g	0	0	0.94
h	0	0	2.26
* A1, A2 – reactivation ability (A) of the tested compounds at different concentrations (C_{A2}= 0.00001; C_{A1}= 0.0005 mol/L).			

Table-2: Antioxidant activity of the tested compounds

Compounds	K_2, molˆ-1×L×minˆ-1	T_{50}, min	R^2	ΔErad; kkal/mol
b	31.70±0.84	126.2	0.94	72.025
a	139.90±3.80	28.6	0.92	93.897
c	1013.81±25.60	3.9	0.99	73.943
d	2.70±0.08	1481.5	0.86	92.163
f	227.93±5.76	17.5	0.99	73.230
g	146.35±3.73	27.3	0.98	77.144
h	24.06±0.61	166.3	0.99	73.982
i	12.91±0.35	309.8	0.91	-
Toxogonin	22.70±0.57	176.2	0.99	72.917
Dipiroxime	32.40±0.82	123.5	0.99	71.892
Alloxime	36.60±0.93	109.3	0.98	69.521
Pralidoxime	11.67±0.29	342.8	0.99	71.843
Unitiol	0.41±0.02	-	0.85	-
Ionol	0.33±0.02	-	0.86	-

Table-3: Statistical results of QSAR generated by MLR (method - forward stepwise)

№	Model	Statisical Parameter					
		R	**R^2**	**F**	**p**	**Std. Error of estimate**	**n**
1	A1 (%)=(25.5579±6.4577)+(29.1691±3.9276)× **logP**--(0.0350±0.01826)×**k2;**	0.95	0.89	33.5	0.0001	16.661	**11**
2	A2(%)=(16.2160±5.1495)+(17.6227±3.1320)×**logP**--(0.0223±0.0146)×**k2;**	0.91	0.83	19.5	0.0009	13.286	**11**
3	Ld_{50} (mM/kg)=(3.9562±1.3459)--(0.4192±0.1050)×logP-(0.0368±0.01692)×**ΔE**rad--(0.0006±0.0004)×**k2;**	0.84	0.70	5.4	0.03	0.398	**11**

Table-4: Additional QSAR properties of the tested molecules

№	Compounds	V_{mol}, $Å^3$	logP
1	b	924.35	0.96
2	a	1022.38	0.27
3	c	1130.61	0.31
4	Toxogpnin	947.12	1.56
5	Dipiroximt	942.77	1.64
6	Pralidoxime	520.47	2.08
7	Alloxime	574.29	3.13
8	d	746.58	-0.47
9	f	633.90	-0.89
10	g	630.95	-0.89
11	h	635.06	-0.89

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