Synthesis and Biological Activity of Hydrophilic Alkylphenols

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Abstract

Synthesis of hydrophilic derivatives from 3-(4-hydrixyaryl)propyl series with sulphur-containing ionogenic fragments has been performed. The laws of toxic effect variation have been investigated for the compounds synthesized depending on their structure with respect to laboratory animals (mice) and bacterial cultures (*Photobacterium phosphoreum*).

Key words: hydrophilic polyfunctional antioxidants, hydroxyarylalkylsulphonates, hydroxyarylalkylthiosulphates, hydroxyarylalkyl isothiuronium halogenides, biological activity, toxicity of phenols, bioluminescence

INTRODUCTION

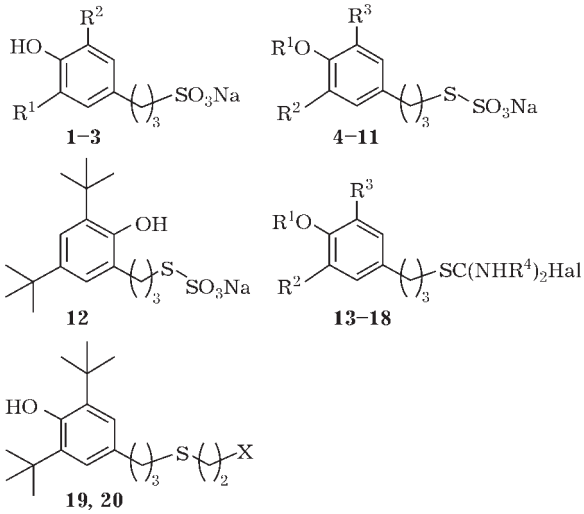
The development of oxidative stress under acute pathological states (infarction, stroke, ischemia/reperfusion, those caused by surgical procedures, traumas, *etc.*) requires for the creation of highly efficient antioxidative preparations with a high level of bioavailability. Of a doubtless interest from this standpoint are hydrophylic derivatives of alkylphenols with sulphur-containing ionogenic fragments (SO_3Na, SSO_3Na, $SC(NH_2)_2Cl(Br)$) within *n*-propyl substituent, those were earlier demonstrated to exhibit a pronounced antioxidative activity *in vitro* [1–3] as well as hepatoprotection and cardioprotection [2, 4], anti-inflammatory [3] and immunomodulation [5, 6] properties *in vivo*.

In the estimation of practical use prospects for synthetic compounds in biology and medicine alongside with their biological activity one should take into account the safety of application, too. The present work is devoted to the studies on toxic effects of structurally related hydrophylic alkylphenols **1–20** with respect to laboratory animals (mice) and bacterial cultures *(Photobacterium phosphoreum)* (Scheme 1).

EXPERIMENTAL

We presented earlier the synthesis of sulphonates **1–3** and thiosulphates **4–12** in [3, 7]; the synthesis of isothiuronium salts **14–17** in was presented [8]. Compound **13** we obtained *via* the reaction between 3-(3,5-dimethyl-4-hydroxyphenyl)-1-bromopropane **21** with thiourea; the derivatives **18–20** were obtained basing on chloropropane **22** according to Scheme 2.

S-[3-(3,5-dimethyl-4-hydroxyphenyl)-propyl]isothiuronium bromide (13). To an ampoule made of heat-resistant glass were placed 5 g (20 mmol) of bromopropane **21** [7] and 1.29 g (17 mmol) of thiourea, then 6 ml of ethanol was added. The ampoule was sealed, placed into



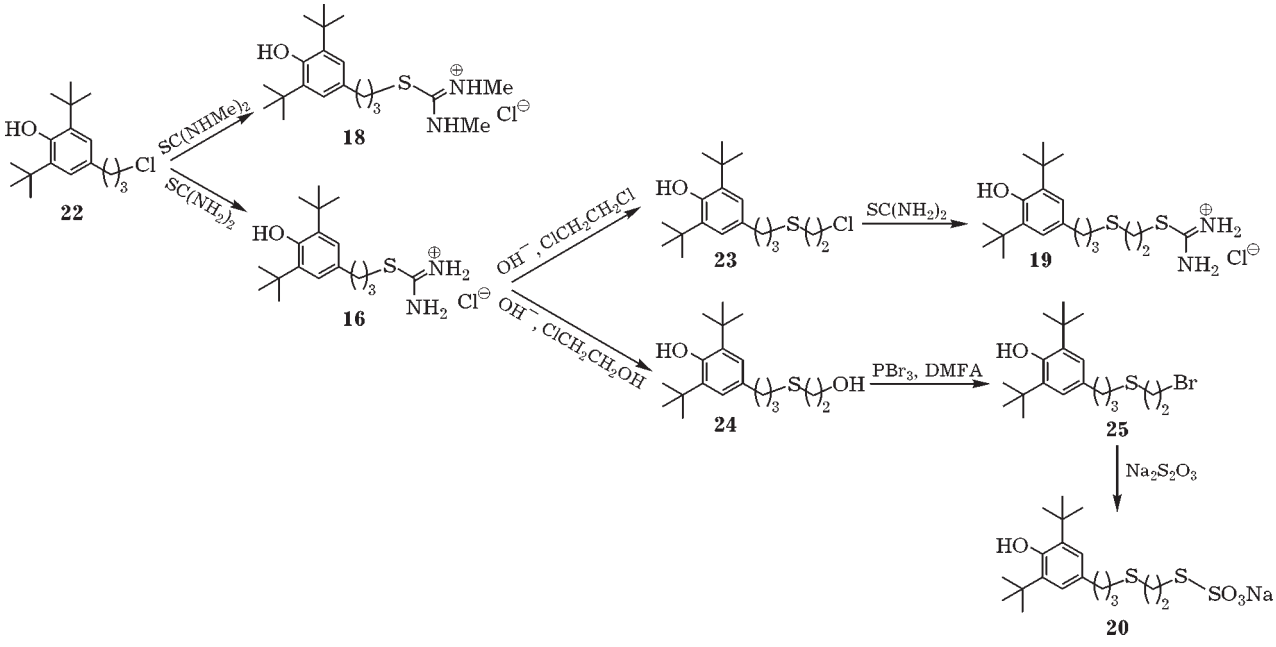
$R^1 = R^2 = H$ (**1**), *t*-Bu (**2**); R^1 = H, R^2 = *t*-Bu (**3**); R^1 = Me, $R^2 = R^3 = H$ (**4**); R^1 = H: $R^2 = R^3 = H$ (**5**), Me (**6**), *t*-Bu (**7**), *cyclo*-C_6H_{11} (**8**), R^2 = Me, R^3 = *t*-Bu (**9**); $R^1 = R^2 = H$: R^3 = *t*-Bu (**10**), *cyclo*-C_6H_{11} (**11**); $R^1 = R^4 = H$, $R^2 = R^3$ = Me, Hal = Br (**13**); R^1 = Me, $R^2 = R^3 = R^4 = H$, Hal = Cl (**14**); $R^1 = R^4 = H$, Hal = Cl: $R^2 = R^3 = H$ (**15**); *t*-Bu (**16**); R^2 = H, R^3 = *t*-Bu (**17**), R^1 = H, $R^2 = R^3$ = *t*-Bu, R^4 = Me, Hal = Cl (**18**); X = $SC(NH_2)_2Cl$ (**19**), SSO_3Na (**20**).

Scheme 1.

a thermostat supplied with a shaker and held during 7 h at 120–125 °C. After cooling the ampoule was opened, the solvent was distilled away. The residue obtained was treated by warm hexane. Crystals precipitated were filtered, washed on the filter with warm hexane three times and then dried. We obtained 5.32 g (98 %) of isothiuronium bromide **13**, m.p. being of 165–167 °C. ^{1}H NMR spectrum, δ, ppm: 1.98 m (2H, $ArCH_2C\underline{H}_2$), 2.21 s, (6H, Me), 2.64 t (2H, $ArC\underline{H}_2$), 3.13 t (2H, CH_2S), 6.79 s (2H, H_{arom}). IR spectrum, ν_{max}, cm^{-1}: 3274 and 3009 (NH_2^+), 1653 (NH_2^+). Elemental analysis: Found, %: C 45.02, H 6.03, Br 25.23, N 4.99, S 9.95. $C_{12}H_{19}BrN_2OS$. Calculated for $C_{12}H_{19}BrN_2OS$, %: C 45.15, H 6.00, Br 25.03, N 5.01, S 10.04.

S-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propyl]-N,N′-dimethylisothiuronium chloride (18) was obtained from chloropropane **22** [9] and N,N′-dimethylthiourea in a manner similar to the previous compound. The yield amounted to 79 %, m.p. being of 101–103 °C. ^{1}H NMR spectrum, δ, ppm: 1.44 s (18H, *t*-Bu), 2.04 m (2H, $ArCH_2C\underline{H}_2$), 2.72 t (2H, $ArC\underline{H}_2$), 3.03–3.11 d (6H, NMe), 3.17 t (2H, CH_2S), 7.01 s (2H, H_{arom}). IR spectrum, ν_{max}, cm^{-1}: 3639 (PhOH), 3119 и 2295 (N^+H), 1639 (N^+H). UV spectrum, λ_{max}, nm (log ε): 207 (2.29), 277 (0.19). Elemental analysis: Found, %: C 61.80, H 9.02, Cl 9.25, N 7.48, S 8.35. $C_{20}H_{35}ClN_2OS$. Calculated for $C_{20}H_{35}ClN_2OS$, %: C 62.07, H 9.11, Cl 9.16, N 7.24, S 8.28.

2-Chloroethyl-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl]sulphide (23). To a solution of 5 g (13.9 mmol) isothiuronium chloride **16** in 15 mL of ethanol were added 1.23 g (30.8 mmol) NaOH in 15 mL of water and then 5.5 mL (69.5 mmol) of 1,2-dichloroethane, in an



atmosphere of argon. The reaction mixture was stirred during 3 h at 60–65 °C, cooled and treated with toluene. An extract was washed with water, dried by means of Na_2SO_4. Toluene was evaporated; the residue obtained was distilled under vacuum. The yield of target chloro derivative **23** amounted to 3.53 g (74 %), b.p. being of 183–184 °C (1 Torr). ^{1}H NMR spectrum, δ, ppm: 1.43 s (18H, *t*-Bu), 1.86–1.88 m (2H, $ArCH_2C\underline{H}_2$), 2.50–2.58 m (4H, $C\underline{H}_2SC\underline{H}_2$), 2.66–2.69 t (2 H, $ArC\underline{H}_2$), 3.55–3.58 t (2H, CH_2Cl), 4.95 s (1H, OH), 6.89 s (2H, H_{arom}). Elemental analysis: Found, %: C 66.21, H 9.22, Cl 10.18, S 9.09. $C_{19}H_{31}ClOS$. Calculated for $C_{19}H_{31}ClOS$, %: C 66.54, H 9.11, Cl 10.33, S 9.35.

S-(β-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propylthio]ethyl)isothiuronium chloride (19) was obtained in a similar manner as it was done for isothiuronium bromide **13** from chloro derivative **23** and $SC(NH_2)_2$. The yield amounted to 94 %, m.p. being of 148–151 °C. ^{1}H NMR spectrum, δ, ppm: 1.43 s (18H, *t*-Bu), 1.89 m (2H, $ArCH_2C\underline{H}_2$), 2.62 t (2H, $Ar(CH_2)_2C\underline{H}_2$), 2.65 t (2H, $ArC\underline{H}_2$), 2.89 t (2H, $(CH_2)_3SC\underline{H}_2$), 3.41 t (2H, $C\underline{H}_2S(NH_2)_2^+$), 6.99 s (2H, H_{arom}). IR spectrum, ν_{max}, cm^{-1}: 3642 (PhOH), 3182 and 3006 (NH_2^+), 1644 (NH_2^+). UV spectrum, λ_{max}, nm (log ε): 207 (2.64), 277 (0.18). Elemental analysis: Found, %: C 57.20, H 8.72, Cl 8.30, N 6.47, S 15.35. $C_{20}H_{35}ClN_2OS_2$. Calculated for $C_{20}H_{35}ClN_2OS_2$, %: C 57.32, H 8.42, Cl 8.46, N 6.68, S 15.30.

2-Hydroxyethyl-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propyl]sulphide (24). 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propanethiol-1 in the amount of 10 g (35.7 mmol) [10], 3.44 g (43 mmol) of 2-chloroethanol and 1.71 g (43 mmol) NaOH were dissolved in 30 mL of ethanol, the mixture was heated and boiled in an inert gas atmosphere during 2.5 h. The reaction mixture was cooled, neutralized with HCl and treated with toluene. The extract obtained was washed with water, dried by means of Na_2SO_4, then the solvent was distilled off and the residue obtained was distilled under vacuum. The yield of the target product **24** amounted to 10.3 g (86 %), b.p. being of 184–186 °C (1 Torr). ^{1}H NMR spectrum, δ, ppm: 1.45 s (18H, *t*-Bu), 1.87 m (2H, $ArCH_2C\underline{H}_2$), 2.30 s (1H, $CH_2O\underline{H}$), 2.53 t (2H, $C\underline{H}_2S$), 2.62 t (2H, $ArC\underline{H}_2$), 2.69 t (2H, $C\underline{H}_2S$), 3.66 t (2 H, $C\underline{H}_2OH$), 4.97 s (1H, $ArO\underline{H}$), 6.91 s (2H, H_{arom}). Elemental analysis: Found, %: C 70.24, H 9.86, S 10.02. $C_{19}H_{31}ClOS$. Calculated for $C_{19}H_{31}ClOS$, %: C 70.32, H 9.94, S 9.88.

2-Bromoethyl-[3-(3,5-di-*tert*-butyl-4-hydroxophenyl)propyl]sulphide (25). In 40 mL of toluene were dissolved 10.3 g (31.7 mmol) of hydroxyethylsulphide 24 and 2.4 mL DMFA, then at 50 °C was dropwise added 1.65 ml (17.5 mmol) of PBr_3. The mixture was heated and stirred during 2.5 h at 80 °C. Further the reactionary mixture was cooled down to 60 °C; 10 mL of water was added. Then the mixture was heated again and stirred during 0.5 h at 80 °C, cooled and treated with toluene. The extract obtained was washed with water, dried by means of Na_2SO_4, and then the solvent was distilled off. The residue obtained was chromatographed on silica gel, hexane/diethyl ether mixture (5 : 1) being used as an eluent. The yield of target bromo derivative **25** amounted to 6.4 g (52 %). ^{1}H NMR spectrum, δ, ppm: 1.44 s (18H, *t*-Bu), 1.87 m (2H, $ArCH_2C\underline{H}_2$), 2.50–2.56 m (4H, $C\underline{H}_2SC\underline{H}_2$), 2.68 t (2H, $ArC\underline{H}_2$), 3.37 t (2 H, CH_2Br), 5.00 s (1H, OH), 6.90 s (2H, H_{arom}). Elemental analysis: Found, %: C 59.05, H 8.17, Br 20.49, S 8.14. $C_{19}H_{31}BrOS$. Calculated for $C_{19}H_{31}BrOS$, %: C 58.90, H 8.06, Br 20.62, S 8.28.

Sodium S-2-[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propylthio]ethylthiosulphate (20). To 6.4 g (16.5 mmol) of bromo derivative **25** dissolved in 20 mL of ethanol was added 5.7 g (23.1 mmol) $Na_2S_2O_3 \cdot 5H_2O$ in 5 mL of water. The mixture was boiled during 6 h in an argon atmosphere, cooled, treated with diethyl ether. The extract obtained was dried with Na_2SO_4, the solvent was distilled off. The residue obtained was washed with warm (~40 °C) hexane and recrystallized from toluene. The yield of the target thiosulphonate **20** was of 2.77 g (38 %), m.p. being of 162–164 °C. ^{1}H NMR spectrum, δ, ppm: 1.45 s (18H, *t*-Bu), 1.88 m (2H, $ArCH_2C\underline{H}_2$), 2.59–2.66 m (4H, $ArC\underline{H}_2CH_2C\underline{H}_2S$), 2.97 m (2H, CH_2S), 3.25 m (2H, CH_2SSO_3Na), 6.99 s (2H, H_{arom}). Elemental analysis: Found, %: C 51.49, H 7.00, S 21.84. $C_{19}H_{31}BrOS$.Calculated for $C_{19}H_{31}BrOS$, %: C 51.56, H 7.06, S 21.73.

^{1}H NMR spectra were registered using a Bruker DRX500 spectrometer with operation frequency of 500.13 MHz: in $CDCl_3$ with $CHCl_3$ reference for compounds **23–25**, in D_2O with

$Si(CH_3)_4$ reference for compounds **13**, **18**, in CD_3OD with $Si(CH_3)_4$ reference for compounds **19** and **20**. Melting point values were determined using PTP apparatus and Kofler warm stage. IR spectra were registered in KBr (150 : 1) with the use of a Vektor 22 Fourier transform spectrometer; UV spectra were registered in EtOH using a Specord HP-8453 spectrophotometer.

In order to study the acute toxicity of compounds **1–20** we used male C57B1/6 mice with the mass of 22–28 g. The mean lethal doze values (LD_{50}) were determined using a commonly known method described in [11]. The compounds under investigation were dissolved in physiological salt solution according to 10 various dozes (from 20–40 up to 3000 mg/kg), with the subsequent intraperitoneal introduction. The observation and animal death rate calculation was carried out during 3 days after introducing of the preparations.

The toxic effect of the compounds synthesized on *Photobacterium phosphoreum* was estimated basing on ID_{50} value numerically equal to the concentration of the preparation whose presence caused the intensity of bacterial bioluminescence of bacteria to exhibit a 50 % decrease with respect to the reference solution.

A lyophilized preparation of luminous bacteria "Microbiosensor B17-677E" from the collection of the Institute of Biophysics, SB RAS (Krasnoyarsk) was activated during 15 min in 1.5 % NaCl solution at 20 °C. In order to determine the value of ID_{50}, to a cellular suspension containing 10^9–10^{10} cells/mL (in 3 % NaCl solution) were added solutions with various concentrations of compounds 1–20. The intensity of bacterial luminescence was measured at 20 °C using a BLM 3606M bioluminescent analyzer (Russia).

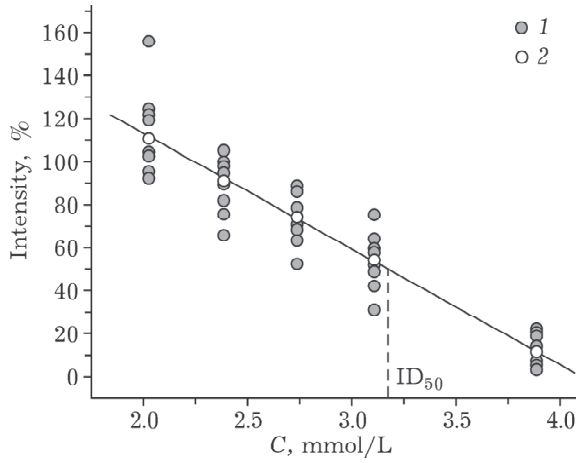


Fig. 1. Intensity of bacteria luminescence depending on the concentration of sodium 3-(4-hydroxyphenyl)propylthiosulphate **5**: *1* – for a separate sample, *2* – average for a given concentration.

The luminescence intensity data obtained were plotted against the concentration the connection under investigation wherefrom the ID_{50} values were determined (Fig. 1).

RESULTS AND DISCUSSION

The results of the studies on biological activity of compounds **1–20** are presented in Table 1. One can see that for the compounds synthesized the level of toxic effect on laboratory animals and *Ph. phosphoreum* depends both on the nature of an ionogenic fragment, and on

TABLE 1

LD_{50} and ID_{50} values for hydrophilic derivatives of alkylphenols under investigation

Compound	LD_{50}, mg/kg	ID_{50}, mmol/L	Compound	LD_{50}, mg/kg	ID_{50}, mmol/L
1	1800	0.21	**11**	300	0.96
2	275	1.34	**12**	150	1.07
3	860	0.51	**13**	110	208
4	975	5.87	**14**	600	670
5	800	3.14	**15**	175	235
6	1000	4.68	**16**	30	0.02
7	175	0.65	**17**	112	46.4
8	320	0.37	**18**	50	38.1
9	288	1.38	**19**	30	0.02
10	450	1.84	**20**	280	0.45

the number and the structure of *o*-substituents in the aromatic nucleus.

For sulphonates **1–3** and thiosulphates **4–12**, **20** LD_{50} = = 275–1800 and 150–1000 mg/kg, respectively, for isothiuronium salts **13–19** this value amounted to 30–600 mg/kg. According to the classification by the author of [12], the most part of the compounds synthesized belongs to the IV class of toxicity (low-toxic substances), and only spatially shielded isothiuronium salts **16**, **18**, **19** belong to III class (moderately toxic substances), whereas *o*-substituted sulphonate **1** could be related to V class of toxicity (almost non-toxic substances).

In the series of compounds **1–5–15**, **2–7–16**, **3–10–17** with the identical structure of an arylalkyl substituent the acute toxicity grows with the substitution of the sulphonate group by the thiosulphate one and further by the isothiuroium group. At the same time, irrespective of the nature of an ionogenic fragment, LD_{50} value decreases with switching from *o*-di-*tert*-butyl substituted compounds to less shielded analogues. The maximum values of LD_{50} are inherent in *o*-unsubstituted compounds, as well as in *o*-dimethyl and O-methyl substituted derivatives. At first sight, these data seem to be contradictory with respect to commonly known concepts of the fact that the toxicity level of spatially hindered phenols is lower as compared to the toxicity level of unsubstituted analogues [13]. However, it should be noted that a low toxicity level was demonstrated earlier only for hydrophobic 2,6-di-*tert*-butylphenols and it could be caused by a considerably lower (in comparison with non-alkylated phenols) solubility of the latter in biological media of living organisms.

For compounds **1–20** the values of ID_{50} vary within the range of 0.02–670 mmol/L, *i.e.* within a wider range as compared to LD_{50} values. In addition, the variation of the structure in the series of the compounds under investigation is ambiguously reflected in the values of ID_{50}. So, with the transition from sulphonate to thiosulphate and further to isothiuroium chloride in the series of di-*tert*-butyl substituted compounds **2–7–16** the values of ID_{50} exhibit a decrease from 1.34 to 0.02 mmol/L. At the same time for similar series of di- and mono-*o*-unsubstituted compounds **1–5–15** and **3–10–17** one can observe an increase in the values of ID_{50}. In the series of isothiuronium and thiosulphate derivatives **16–17–15** and **7–10–5** with a consecutive removal of *tert*-butyl substituents the ability of compounds to inhibit bacterial bioluminescence decreases, whereas in the series of sulphonates **2–3–1**, this property, on the contrary, increases.

The methylation of the phenolic OH group and the nitrogen atoms of the isothiuronium fragment results in a decrease in toxic effect of compounds **4**, **14**, **18** as compared to the toxic action of the compounds **5**, **15**, **16**, both with respect to laboratory animals and regarding *Ph. phosphoreum*.

Comparative analysis of LD_{50} and ID_{50} values for compounds **1–20** has allowed us to reveal two reaction series within the range of those the mentioned parameters exhibit a reliable correlation with each other (Fig. 2).

Using the correlation equation derived from the values of LD_{50} and ID_{50} for 3-(4-

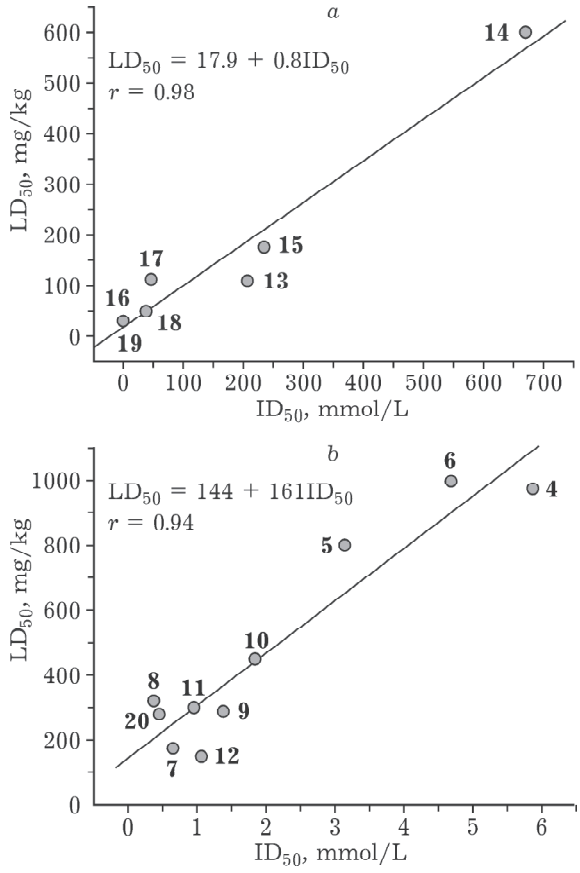


Fig. 2. Correlation between LD_{50} and ID_{50} values for chlorides 3-(4-hydroxyaryl) propylisothiuronium chlorides (*a*) and 3-(4-hydroxyaryl)propylthiosulphated (*b*).

hydroxyaryl)propylthiosulphates **4**, **6–10**, we have calculated LD_{50}values from experimentally determined ID_{50} parameters for compounds **5** and **11**. The LD_{50} values calculated for mentioned thiosulphates amounted to 627 and 286 mg/kg, respectively, which is in a good agreement with LD_{50} values determined from the experiments (800 and 300 mg/kg, respectively). It should be noted that the present approach has allowed us to reduce the number of laboratory animals involved into acute experiment aimed at determining the values of LD_{50} for compounds **5** and **11**.

CONCLUSION

The results of the studies carried out indicate the fact that the level of toxic influence of hydrophilic derivatives from 3-(4-hydroxyaryl)propyl series upon laboratory animals and bacterial cultures depends on the two structural factors: the nature of a polar fragment in the *p*-substituent, as well as on the number and structure of alkyl *o*-substituents within the aromatic nucleus. In separate reaction series the values of LD_{50} and ID_{50} correlate with each other, which could be used for the practical purposes.

As far as the level of toxic influence upon animal organisms is concerned, the hydrophilic derivatives under investigation belong to low-toxic compounds, which in the aggregate with the pronounced antioxidation and protection activity [1–7] allows one to consider them to be promisinge bioantioxidants.

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