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# Antioxidant and Antiinflammatory Activity of New Water-Soluble Sulfur-Containing Phenolic Compounds

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**Abstract**—We synthesized a series of structurally related water-soluble alkyl phenols – sodium 4-hydroxyphenyl propyl sulfonates and thiosulfonates with different number of tert-butyl groups at the *ortho*-position. In experimental systems of transient metal-induced ethyl oleate and low-density lipoprotein oxidation the antioxidant activity of the compounds increased when the tert-butyl group number at the *ortho*-position increased and when the sulfonate group was replaced with thiosulfonate. Compounds containing thiosulfonate group in *para*-propyl substituent also more effectively inhibited reactive oxygen metabolites generated in xanthine–xanthine oxidase system and during morpholinosydnonimine decomposition compared to sulfonate-containing analogs. Phenols with one tert-butyl group at the *ortho*-position have been shown to exhibit the highest antiinflammatory activity in the model of carrageenan-induced rat paw inflammation, as well as with regard to the expression of the glutathione S-transferase P1-1 gene in HepG2 human hepatoma cell line. Thus, it can be reasonably speculated that the antiinflammatory activity of sulfur-containing phenolic antioxidants *in vivo* is mediated by their effect on redox-sensitive transcription factors.

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*Key words*: oxidative stress, phenolic antioxidants, antioxidant responsive element

Development of the oxidative stress reflecting disturbance of the balance in "prooxidant–antioxidant" system is implicated in more than 100 pathological states and diseases including inflammatory process and atherosclerosis [1]. Inverse dependence between consumption of antioxidant-rich vegetables and fruits and the cardiovascular and oncological death rate has been revealed [2, 3]. One of the main antioxidant components of plant extracts including extracts from medicinal herbs are phenolic compounds [4]. Positive therapeutic effect of phenolic antioxidant administration was shown for inflammatory processes of different localization [2, 5].

Structural peculiarities of natural phenols and polyphenols, which determine their antioxidative properties in the experimental systems, have been thoroughly investigated [2, 6]. However, direct relation between antioxidative characteristics and biological systemic activity is frequently hard to designate [7]. Many polyphenols are effective bioregulators and are able to activate MAP-kinases, redox-sensitive transcription factors, or to affect hormone receptors [1, 5, 8]. In recent years, researchers note that systemic biological effect of phenolic antioxidants is often determined not by their antioxidative prop-

*Abbreviations*: ARE) antioxidant responsive element; GSTP1-1) glutathione S-transferase P1-1; HBSS) Hanks' balanced salt solution; LDL) low-density lipoproteins; ROS) reactive oxygen species; RT-PCR) reverse transcription-polymerase chain reaction; S-9) sodium 3-(4′-hydroxyphenyl)propyl sulfonate; S-10) sodium 3-(4′-methoxyphenyl)propyl sulfonate; S-13) sodium 3-(3′-tert-butyl-4′-hydroxyphenyl)propyl sulfonate; S-17) sodium 3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)propyl sulfonate; SIN-1) morpholinosydnonimine; TBARS) thiobarbituric acid-reactive substances; tBHQ) tert-butylhydroquinone; TS-9) sodium 3-(4′-hydroxyphenyl)propyl thiosulfonate; TS-10) sodium 3-(4′-methoxyphenyl)propyl thiosulfonate; TS-13) sodium 3-(3′-tert-butyl-4′-hydroxyphenyl)propyl thiosulfonate; TS-17) sodium 3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)propyl thiosulfonate.

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erties but by their regulatory action; this is the case even for "classic" antioxidants such as vitamin E [9]. Important intracellular targets for the action of exogenous phenolic antioxidants are redox-sensitive transcription factors, and above all – antioxidant responsive element (ARE) [10].

In this study, we investigated the antioxidative properties of several new sulfur-containing phenolic compounds synthesized on the basis of bromine substituted alkyl phenols. These compounds are highly hydrophilic and demonstrate bifunctional effect, since in spite of phenol hydroxyl group they contain sulfurous fragments with anti-peroxidative activity [2]. Antioxidative and antiradical properties of the new compounds were explored in different experimental systems *in vitro*: oxidation of ethyl oleate in water emulsion, accumulation of thiobarbituric acid-reactive substances (TBARS) during the incubation of low-density lipoproteins (LDL) with transient metal ions, generation of reactive oxygen species (ROS) following the stimulation of blood neutrophils, synthesis of superoxide anion-radical in the xanthine–xanthine oxidase system, and generation of peroxynitrite anion ($ONOO^-$) during decomposition of morpholinosydnonimine (SIN-1). Antiinflammatory effect was investigated using the carrageenan-induced rat paw inflammation model. Ability of the synthesized compounds to affect the expression of the ARE-controlled glutathione S-transferase P1 gene (*GSTP1*) was tested in the human hepatoma HepG2 cell culture.

## MATERIALS AND METHODS

S-Alkylthiosulfonates and S-alkylsulfonates with different number of *ortho*-substituents were obtained by the interaction of halogen alkanes with sodium thiosulfate or sulfite correspondingly in aqueous-alcoholic solution (Fig. 1). Initial bromoalkanes were synthesized on the basis of 3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)propanol-1 by the methods developed previously [11]. To obtain S-alkylthiosulfonates and S-alkylsulfonates, 20 mmol of $Na_2S_2O_3 \cdot 5\ H_2O$ (or 23 mmol of $Na_2SO_3$) in 7 ml of water were added to 15 mmol of appropriate bromoalkane dissolved in 20 ml of ethanol. The reaction mixture was boiled for 8 h in an argon atmosphere, then the reaction mass was cooled, the solvent was evaporated from the sample, pentane was added to the pellet, and precipitated crystals were filtered, washed with warm pentane, recrystallized from acetone, and dried. Reaction yield for desired thiosulfonates (TS-9 (3-(4′-hydroxyphenyl)propyl thiosulfonate sodium salt), TS-10 (3-(4′-methoxyphenyl)propyl thiosulfonate sodium salt), TS-13 (3-(3′-tert-butyl-4′-hydroxyphenyl)propyl thiosulfonate sodium salt), TS-17 (3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)propyl thiosulfonate sodium salt)) and sulfonates (S-9 (3-(4′-hydroxyphenyl)propyl sulfonate sodium salt), S-10 (3-(4′-methoxyphenyl)propyl sulfonate sodium salt), S-13 (3-(3′-tert-butyl-4′-hydroxyphen-yl)propyl sulfonate sodium salt), S-17 (3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)propyl sulfonate sodium salt)) was 60-95%. $^1$H-NMR spectra were obtained on a Bruker DR 500 spectrometer (Germany) ($D_2O$ as a solvent, external standard – $Si(CH_3)_4$) (Table 1). Infrared spectra were recorded with a Vector 22 Fourier spectrometer (Bruker) in KBr (150 : 1). UV spectra were obtained with a Specord HP-8453 spectrometer (Hewlett Packard, Germany) (solvent is $H_2O$). Melting points were determined on an Electrothermal melting point apparatus in an open capillary tube (Khimlaborpribor, Russia) and

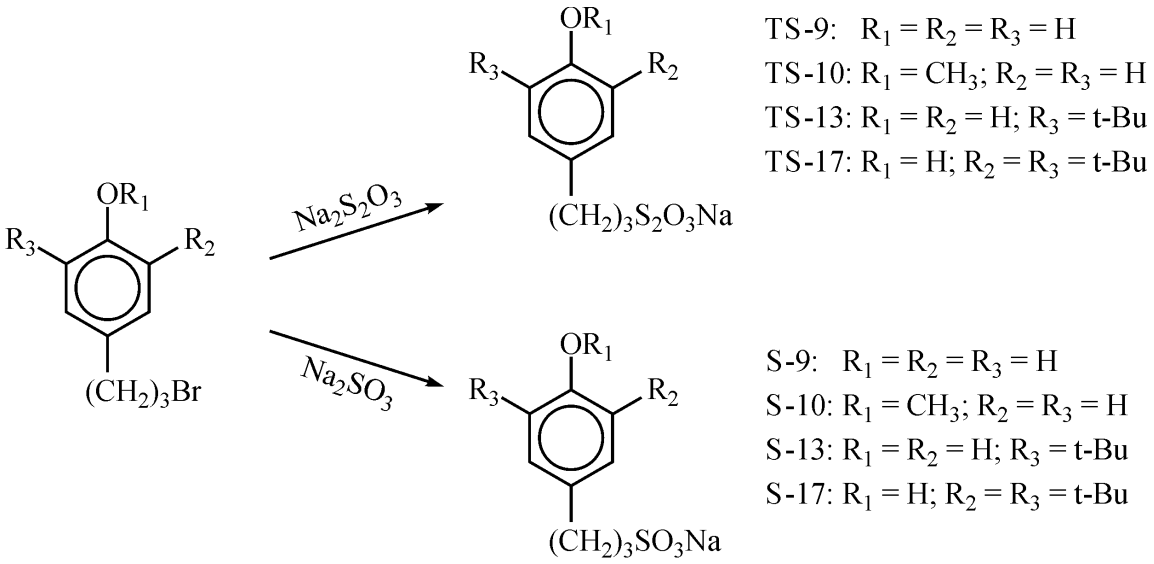


**Fig. 1.** Scheme of the S-alkylthiosulfonate (TS-9, TS-10, TS-13, TS-17) and S-alkylsulfonate (S-9, S-10, S-13, S-17) synthesis.

**Table 1.** Properties of the synthesized compounds

| Compound | Melting point, °C | Elemental analysis, found/calculated, % | | | | Molecular formula | $^1$H-NMR spectrum, δ, ppm | IR spectrum, $\nu_{max}$, $cm^{-1}$ | UV spectrum, λ, nm (log ε) |
|---|---|---|---|---|---|---|---|---|---|
| | | C | H | S | Na | | | | |
| S-9 | 263 | 45.6/ 45.37 | 4.96/ 4.65 | 13.70/ 13.46 | 9.64/ 9.65 | $C_9H_{11}O_4SNa$ | 2.035-2.066 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.702-2.732 t (2H, Ar-$CH_2$), 2.909-2.940 t (2H, $CH_2$-S), 6.901-6.918 d (2H, Ar-H), 7.207-7.224 d (2H, Ar-H) | 3588.0 (PhOH), 2966.2 (CH), 1192.2 and 1053.8 ($-SO_3^-$) | 221 (0.45), 276 (0.1) |
| TS-9 | 252 | 40.18/ 39.99 | 4.30/ 4.10 | 24.0/ 23.72 | 8.70/ 8.50 | $C_9H_{11}O_4S_2Na$ | 2.030 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.666 t (2H, Ar-$CH_2$), 3.067-3.096 t (2H, $CH_2$-S), 6.852-6.869 d (2H, Ar-H), 7.070 d (2H, Ar-H) | 3528.8 (PhOH), 2932.8 (CH), 1182.4 and 1031.3 ($-SO_3^-$) | 198 (2.74), 277 (0.14) |
| S-10 | ⩾300 (decomp.) | 47.80/ 47.61 | 5.30/ 5.19 | 12.90/ 12.71 | 8.90/ 9.11 | $C_{10}H_{13}O_4SNa$ | 2.034-2.065 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.720-2.750 t (2H, Ar-$CH_2$), 2.894-2.925 t (2H, $CH_2$-S), 3.849 s (3H, O-$CH_3$), 6.993-7.011 d (2H, Ar-H), 7.270-7.284 d (2H, Ar-H) | 3594.9 (PhOH), 2936.5 (CH), 1155.1 and 1034.3 ($-SO_3^-$) | 197 (2.7), 222 (0.93), 275 (0.17) |
| TS-10 | 232 | 42.50/ 42.24 | 4.59/ 4.60 | 22.47/ 22.55 | 8.05/ 8.10 | $C_{10}H_{13}O_4S_2Na$ | 2.034-2.093 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.693-2.723 t (2H, Ar-$CH_2$), 3.095-3.124 t (2H, $CH_2$-S), 3.802-3.843 s (3H, O-$CH_3$), 6.970-6.987 d (2H, Ar-H), 7.240-7.257 d (2H, Ar-H) | 3454.7 (PhOH), 2957.0 (CH), 1178.8 and 1060.1 ($-SO_3^-$) | 202 (8.5) |
| S-13 | 235 | 53.0/ 53.05 | 6.40/ 6.52 | 10.50/ 10.89 | 7.50/ 7.80 | $C_{13}H_{19}O_4SNa$ | 1.430 s (9H, *t*-Bu), 2.095-2.110 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.659 t (2H, Ar-$CH_2$), 2.979 t (2H, $CH_2$-S), 6.912-6.928 d (1H, Ar-H), 7.049-7.069 d (1H, Ar-H), 7.183-7.187 s (1H, Ar-H) | 3511.8 (PhOH), 2958.1 (CH), 1196.2 and 1056.1 ($-SO_3^-$) | 198 (2.81), 277 (0.16) |
| TS-13 | 207 | 47.80/ 47.84 | 5.60/ 5.88 | 19.71/ 19.65 | 6.90/ 7.04 | $C_{13}H_{19}O_4S_2Na$ | 1.430 s (9H, *t*-Bu), 2.070 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.725 t (2H, Ar-$CH_2$), 2.949 t (2H, $CH_2$-S), 6.889 d (1H, Ar-H), 7.060 d (1H, Ar-H), 7.254 s (1H, Ar-H) | 3512.5 (PhOH), 2957.0 (CH), 1196.3 and 1055.9 ($-SO_3^-$) | 203 (3.28), 278 (0.28) |
| S-17 | ~273 (decomp.) | 58.12/ 58.27 | 7.50/ 7.76 | 9.0/ 9.15 | 6.02/ 6.56 | $C_{17}H_{27}O_4SNa$ | 1.430 s (18H, *t*-Bu), 2.134 m (2H, Ar-$CH_2$-$\underline{CH_2}$), 2.734 t (2H, Ar-$CH_2$), 3.005 t (2H, $CH_2$-S), 7.175 s (2H, Ar-H) | 3644.2 (PhOH), 2875.0 (CH), 1184.7 and 1056.1 ($-SO_3^-$) | 200 (2.91), 275 (0.14) |
| TS-17 | 154 | 53.02/ 53.39 | 6.89/ 7.10 | 16.9/ 16.77 | 5.80/ 6.0 | $C_{17}H_{27}O_4S_2Na$ | 1.430 s (18H, *t*-Bu), 2.116 m (2H, Ar-$CH_2$-$\underline{CH_2}$-), 2.708 t (2H, Ar-$CH_2$), 3.221 t (2H, $CH_2$-S), 7.108 s (2H, Ar-H) | 3640.2 (PhOH), 2957.0 (CH), 1215.2 and 1043.2 ($-SO_3^-$) | 200 (2.88), 275 (0.12) |

using a Kofler's heating table (Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences).

To evaluate the antioxidative activity of the synthesized compounds three model test systems, based on the oxidation of ethyl oleate in water emulsion, TBARS production during the incubation of isolated LDLs with transition metal ions ($Cu^{2+}$, $Fe^{2+}$), and generation of reactive oxygen metabolites by stimulated blood neutrophils (respiratory "burst") were used. Ionol (2,6-di-tert-butyl-4-methyl phenol) and potassium phenosan (3-(3′,5′-di-tert-butyl-4′-hydroxyphenyl)propionate potassium salt) were used as standards.

Oxidation of ethyl oleate in water emulsion was carried out in the presence of catalytic additions of copper (II), cetavlon being used as a surface active substance. Working concentrations of the components in the sample were: ethyl oleate, 0.67 M; $CuCl_2$, 3 mM; cetavlon, 30 mM; tested compounds, 2 mM; total volume of the sample, 5 ml; oxidation temperature, 60°C. A volumetric Warburg vessel was used in studies; $O_2$ pressure in the system was 1 atm. The induction period ($\tau$) was determined graphically as the point of intersection of two tangents to the kinetic curve, with tangents of the tilt 0.5 and 0.75 from the tangent of the tilt angle on the line of the uninhibited reaction.

Isolation of LDL (1.019-1.063 g/ml) from donor plasma was performed by the method of sequential ultracentrifugation in KBr density gradient (1,050,000*g*, 20 h and 105,000*g*, 24 h) according to [12], purification of LDL from KBr was carried out by chromatography on the Sephadex G-25. LDL protein content was determined by the Lowry method [13]. LDL oxidation was induced by 5 μM $CuSO_4$ or 25 μM $FeSO_4$ at 37°C for 30 min; working concentrations of the tested compounds were 1, 10, 100, and 500 μM; ionol was used as a standard. TBARS formation was evaluated by the fluorescence method (Hitachi P3000 fluorimeter; Hitachi, Japan) in 2 h dynamics [14].

Ability of the chemicals to scavenge superoxide anion radical was determined by chemiluminescence inhibition in the lucigenin–xanthine oxidase–xanthine system [15] as follows: 10 μM lucigenin (Sigma, USA) was placed into a thermostatted (at 37°C) cuvette containing 50 μM xanthine (Sigma) dissolved in colorless Hanks' balanced salt solution (HBSS), after 5 min the reaction was started by addition of the xanthine oxidase solution in HBSS (final concentration $5 \cdot 10^{-3}$ U/ml; Sigma). Xanthine oxidase introduction lead to the rapid increase in chemiluminescence, which reached a plateau in 3 min and remained practically unchanged during the following 15 min. Efficacy of the compound was evaluated by the change in chemiluminescence intensity 7 min after the reaction start, and it was calculated using the formula:

$$((I_0 - I_c)/I_0) \cdot 100\%, \quad (1)$$

where $I_0$ and $I_c$ are the chemiluminescence intensity before and 30 sec after the addition of the compound, respectively.

Scavenging of $ONOO^-$ by tested compounds was estimated using the SIN-1–lucigenin system [16]: 100 μM lucigenin and 20 μM SIN-1 (Sigma) were introduced into a thermostatted (at 37°C) cuvette containing HBSS, and after 5 min tested compound solutions in HBSS or equal HBSS volume (control samples) were added. Efficacy was evaluated by the chemiluminescence intensity 5 min after the reaction start and was expressed as a percentage relative to the corresponding value in control samples.

Blood granulocyte oxidative metabolism was measured in samples of whole heparinized blood, diluted for 20 times, by the chemiluminescence with luminol or lucigenin following stimulation by zymosan according to the method of Tono-Oka et al. with our own modifications [17]. Rat blood containing heparin (20 U/ml) was diluted by HBSS in 1 : 20 ratio and thermostatted for 5 min in a chemiluminometer cuvette, then 10 μM luminophore and zymosan suspension in HBSS (final concentration 2 mg/ml) were added and chemiluminescence intensity was registered until reaching maximum. Measurements were carried out using a Foton chemiluminometer (USSR). Tested compound, diluted in proper concentration in the saline, was introduced into the measuring cuvette before addition of zymosan. Efficacy was evaluated by the maximum chemiluminescence intensity and was expressed as a percentage relative to the corresponding value in the absence of the tested compound.

Expression level of the *GSTP1* gene mRNA in human hepatoma HepG2 cells was determined by the reverse transcription-polymerase chain reaction method (RT-PCR). HepG2 cells were cultivated in DMEM (Sigma) supplemented with 10% fetal bovine serum and 1% of antibiotic and antimycotic solution (10,000 μg/ml streptomycin, 10,000 U/ml penicillin, 25 μg/ml amphotericin B; MP Biomedicals, Germany) at 37°C in 5% $CO_2$ atmosphere. One day before incubation with the tested compounds cells were plated on 24-well plates ($2 \cdot 10^5$ cells per well), after that adhered cells were washed and fresh medium containing the tested compounds in 10, 20, 50, or 100 μM concentration was added. After 24-h incubation, total cellular RNA was isolated from treated cells by SDS/phenol extraction according to [18], and cDNA synthesis was performed. PCR was run using a Hybaid OMN-E thermocycler (Hybaid, UK) in buffer (67 mM Tris-HCl, pH 8.0, 16 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.001% Tween-20) with synthesized cDNA, deoxynucleoside triphosphates mixture, specific primers, and Taq-DNA-polymerase. For amplification of *GSTP1* gene product, the following primers were used: 5′-ATGACTATGTGAAGGCACTGC-3′ and 5′-TGTTTCCCGTTGCCATTGAT-3′ (the product length is 280 bp). The β-

actin gene cDNA was used in PCR as "internal standard". Primers for β-actin gene cDNA amplification were: 5′-AGCCATGTACGTAGCCATCCA-3′ and 5′-TCTCCGGAGTCCATCACAATG-3′ (the product length is 81 bp); they were added to the reaction mixture after the 15th cycle. Reaction was run for 40 cycles under the following conditions: 95°C for 1 min, 54°C for 1 min, 72°C for 1 min. PCR products were analyzed in 8% polyacrylamide gel with the acrylamide/bis-acrylamide ratio 20 : 1 in Tris-borate buffer at 20°C. pBR322 DNA/*Bsu*(*Hae*III) was applied as molecular size marker. Gels were stained with ethidium bromide and photographed in UV-light visualization with subsequent computer densitometry of obtained images. To evaluate relative mRNA content, the integral optical density of the bands, which corresponded to the gene-specific PCR products, was normalized to the optical density of the β-actin bands.

Investigation of the antiinflammatory effect of tested phenolic compounds was carried out using the local inflammation of the rat paw. The inflammation was evoked by intraplantar injection of 0.9% saline solution of carrageenan (0.1 ml 10% carrageenan; Sigma) in the right hind paw of Wistar rats weighing 280-300 g. The paw volume as indicator of the inflammatory response was measured 5 h after carrageenan injection using a plethysmometer, and the results were expressed as:

$$((V_r - V_l)/V_l)\cdot 100\%, \quad (2)$$

where $V_r$ is a volume of the right paw and $V_l$ is a volume of the left paw. Tested compounds were given in dose 100 mg/kg body weight (1 ml solution in distilled water) *per os* by intragastric gavage 1 h before carrageenan injection.

**Statistical analysis.** Inhibitory concentration 50% ($IC_{50}$) values were calculated by interpolation from the dose–response curves obtained from at least four significant concentrations of the tested compounds. Final RT-PCR data on the level of *GSTP1* gene expression are represented as percentage related to *GSTP1/β-actin* ratio in cells, incubated with 20 μM tert-butylhydroquinone (tBHQ); experimental error, determined as standard deviation, obtained from the *GSTP1/β-actin* values of three separate repeats of one experimental point, did not exceed 10.1%. Inflammation model data were expressed as mean ± standard error of the mean and analyzed statistically by one-way analysis of variance (ANOVA) followed by one-sided Dunnett's test. Differences were considered statistically significant when $p < 0.05$.

## RESULTS AND DISCUSSION

Study of the synthesized compounds in different experimental systems showed that all compounds containing hydroxyl and/or thiosulfonate group display antioxidative and antiradical properties to more or less extent (Table 2). Methylation of the functional hydroxyl group of S-10 imparted prooxidant properties to the compound, i.e. its introduction in the experimental system either enhanced the oxidation of ethyl oleate and LDL or raised radical generation and chemiluminescence in other systems. In the course of the ethyl oleate and LDL oxidation experiments, the antioxidant activity of the compounds increased with increasing number of tert-butyl substituents at the *ortho*-position. At the same time, thiosulfonates (TS-10, TS-9, TS-13, and TS-17) had more pronounced effect than respective sulfonates (S-10, S-9, S-13, and S-17), which can be related to an antioxi-

**Table 2.** Antioxidative activity of the tested phenolic antioxidants ($IC_{50}$, μM)

| Compound | $Cu^{2+}$-induced oxidation of ethyl oleate* | Blood LDL oxidation | | Granulocyte respiratory burst | | $O_2^-$ (xanthine–xanthine oxidase system) | $ONOO^-$ |
|---|---|---|---|---|---|---|---|
| | | $Cu^{2+}$ | $Fe^{2+}$ | with luminol | with lucigenin | | |
| S-10 | 10 | Dose-dependent stimulation | | | | | |
| TS-10 | 13 | 11 800 | $> 1 \cdot 10^6$ | 74 | 141 | 1285 | 16.5 |
| S-9 | 38 | 2770 | 2650 | 214 | 245 | 2878 | 5.9 |
| TS-9 | 53 | 398 | 381 | 27 | 219 | 577 | 2.9 |
| S-13 | 59 | 43 | 32 | 1170 | 1600 | 1064 | 10.1 |
| TS-13 | 64 | 30 | 18 | 525 | 1800 | 1214 | 9.7 |
| S-17 | 200 | 15 | 3.6 | 631 | 724 | 207 | 24.7 |
| TS-17 | 258 | 10 | 1.5 | 123 | 68 | 1044 | 10.7 |
| Phenosan K | 93 | 13 | 1.8 | 800 | 1400 | 1170 | 21.3 |

* Length of induction period (min).

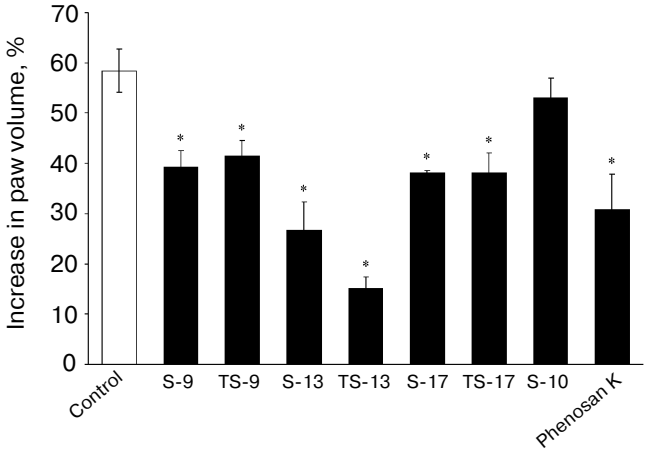


**Fig. 2.** Influence of the phenolic antioxidants on the intensity of inflammation reaction (* $p < 0.05$, significantly different from control).

dant effect of the bivalent sulfur of the thiosulfonate fragment. As to antioxidant activity, the TS-17 compound with two tert-butyl *ortho*-substituents exceeded the structure-corresponding potassium phenosan.

In the stimulated granulocyte-based radical production systems, in the xanthine–xanthine oxidase system, or during the SIN-1 decay, no distinct dependence was observed between the compound inhibitory activity and hydroxyl group screening (Table 2). In the chemiluminescent systems with luminol (granulocytes and SIN-1), thiosulfonates exceeded corresponding sulfonate in their activity. This may be connected with thiosulfonate fragment-based inactivation of hydrogen peroxide and peroxynitrite, which are known to initiate luminol chemiluminescence [2]. Results showed that antioxidant properties of the studied phenolic compounds in the model conditions strongly depend both on the phenol OH group screening mode and on the nature of the ionogenic fragment in the *para*-propyl substituent. In order to exclude the possibility of cell-derived ROS inhibition by the tested compounds due to their toxic effect on the blood granulocytes, we evaluated compound cytotoxicity by trypan blue exclusion test. One-hour incubation with each of the tested compound in 1 mM concentration did not reduce the cells viability by more than 4%, which was not significantly different from the control measurements obtained in the course of incubation in the absence of compound. This shows that in the studied range of concentrations (1-1000 μM) phenols do not affect cell viability.

Investigation of the antiinflammatory effect of the synthesized phenolic compounds demonstrated that all of them to a greater or lesser extent diminished the carrageenan-induced rat hind paw edema (Fig. 2); the partially-hindered phenol with thiosulfonate group in *para*-propyl substituent TS-13 was the most effective. Direct interrelation between the ability of a compound to reduce edema and the number of *ortho*-substituents was not observed, in contrast to dependence revealed in the experimental systems with ethyl oleate and LDL oxidation (Table 2). An important role in the inflammatory process belongs to radicals, particularly $NO^{\bullet}$, which is considered to be the key inducing element in the rat paw swelling [19]. However, a correlation between antiinflammatory effect of tested compounds and their ability to inhibit granulocyte oxidative metabolism, ROS production in xanthine–xanthine oxidase system, or NO radicals was not revealed. Therefore, we assumed that the antiinflammatory effect of exogenous phenolic antioxidants can be realized through their influence on redox-sensitive transcription factors and expression of respective inflammatory genes, which contain promoter regulatory sites controlled by these transcription factors, above all the antioxidant-responsive element. In order to test this hypothesis, we studied the ability of the synthesized compounds to enhance the *GSTP1* gene transcription, which is known [20] to be ARE-driven, in the human hepatoma HepG2 cell culture.

Human glutathione S-transferase P1 (EC 2.5.1.18) is a phase II detoxification enzyme. Two NF-κB binding sites, two SP-1 binding regions, one AP-1 regulatory element, three ARE sequences, and ARE-like element are revealed in the promoter of the gene encoding GSTP1-1 [20]. The studied phenolic compounds increased *GSTP1* gene expression in concentration 20 μM (Fig. 3 and Table 3). The largest apparent effect was displayed by the less hindered phenol TS-13, whose activity in concentrations 10-100 μM was on average 1.5 times greater than those of "classical" ARE inducer tBHQ. The fact that this compound also to the largest extent diminished the car-

**Table 3.** Level of *GSTP1* gene mRNA expression in HepG2 cells incubated with tested phenolic antioxidants

| Compound | Concentration, μM | | | |
|---|---|---|---|---|
| | 10 | 20 | 50 | 100 |
| S-9 | 28.0* | 106.6* | 84.9* | 5.2 |
| TS-9 | 4.1 | 124.5* | 3.3 | 6.9 |
| S-10 | 14.8* | 138.8* | 101.8* | 13.0* |
| TC-10 | 3.7 | 161.9* | 97.8* | 33.6* |
| S-13 | 0 | 128.9* | 3.1 | 15.4* |
| TS-13 | 12.8* | 186.2* | 151.7* | 165.6* |
| S-17 | 2.3 | 151.1* | 0 | 50.3* |
| TS-17 | 57.4* | 33.7* | 88.4* | 0 |
| tBHQ | 16.7* | 100.0* | 53.4* | 192.6* |
| Control | 0 | 0 | 0 | 0 |

Note: Results are expressed in %, the value obtained from the treatment by 20 μM tBHQ is counted as 100%.
* $p < 0.05$, significantly different from control.

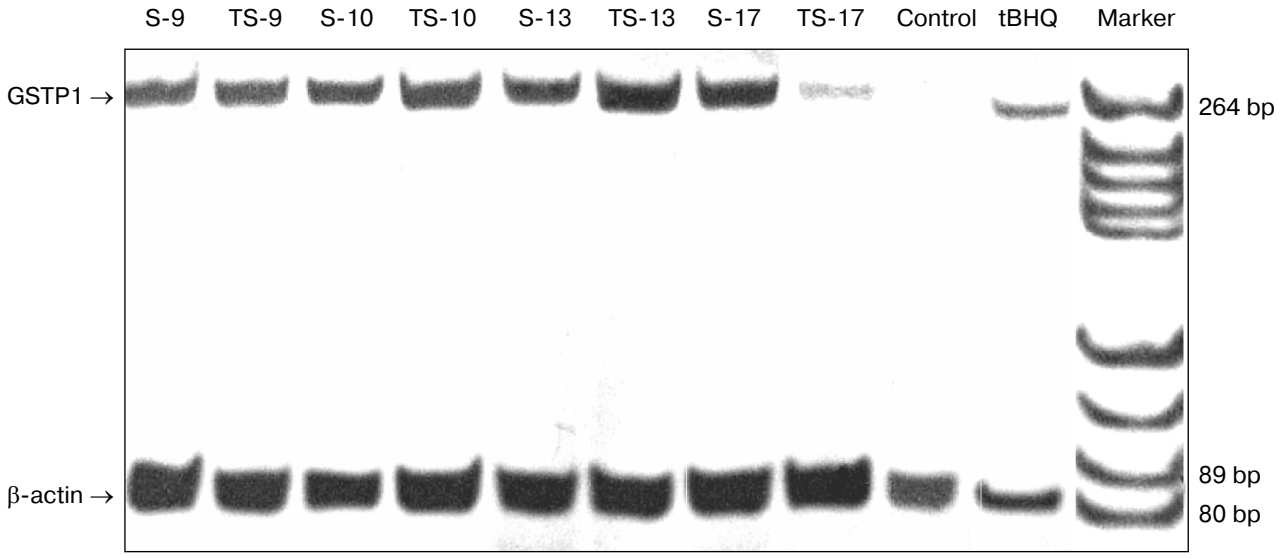


**Fig. 3.** Induction of *GSTP1* gene expression in HepG2 cells by the tested compounds; the concentration of each compound in the medium was 20 μM. Control, non-treated cells; marker, molecular size marker. Sizes of the specific PCR products: *GSTP1*, 280 bp; *β-actin*, 81 bp.

rageenan-induced edema (Fig. 2) suggests that the ability of the synthesized phenolic antioxidants to induce the expression of ARE-driven inflammatory genes underlies their antiinflammatory effect.

The study showed that less hindered phenols with one tert-butyl *ortho*-substituent (S-13 and TS-13) are the most active ARE inducers and possess high antiinflammatory activity. It is interesting to note that methylated phenols (S-10 and TS-10) also significantly enhanced the *GSTP1* gene expression. The substitution of sulfonate group in the *para*-position of the tested compounds with thiosulfonate group increased ARE-inducing activity of less hindered phenols. Investigation of the phenolic antioxidant BO-653 (2,3-dihydro-5-hydroxy-2,2-dipentyl-4,6-di-tert-butylbenzofuran) and its analogs showed that ARE induction requires functional hydroxyl group and tert-butyl substituents at the *ortho*-position [9]. However, in the cited study the effect of less hindered compounds with one tert-butyl *ortho*-substituent was not investigated. Our results demonstrate that phenols with one *ortho*-tert-butyl substituent possess the greatest activity, whereas the availability of an active OH group is not a determinative factor.

Using the animal models of pathological processes, antiinflammatory, anti-carcinogenic, anti-atherosclerotic, and anti-diabetic activities of the phenolic antioxidants were shown [2, 5, 21, 22]. A broad spectrum of biological effects of antioxidants implies target plurality of their action. As shown in the cell cultures, polyphenol compounds of tea and red grape are able either to directly inhibit the activation of NF-κB and AP-1 transcription factors or to affect them through kinase signaling [5, 8, 23]. ARE is the most preferable target for phenolic antioxidants [9, 10]. Therefore, it is not surprising that cytoprotective and antiinflammatory effect of the synthesized phenols can be realized through modulation of ARE-regulated gene expression.

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