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Original scientific paper

ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF MONO- AND BIS-SALICYLIC ACID DERIVATIVES

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*A simple synthesis of mono- and bis-salicylic acid derivatives **1-10** by the transesterification of methyl salicylate (methyl 2-hydroxybenzoate) with 3-oxapentane-1,5-diol, 3,6-dioxaoctane-1,8-diol, 3,6,9-trioxaundecane-1,11-diol, propane-1,2-diol or 1-aminopropan-2-ol in alkaline conditions is reported. All compounds were tested in vitro on three malignant cell lines (MCF-7, MDA-MB-231, PC-3) and one non-tumor cell line (MRC-5). Strong cytotoxicity against prostate PC-3 cancer cells expressed compounds **3**, **4**, **6**, **9** and **10**, all with the IC_{50} less than 10 μmol/L, which were 11-27 times higher than the cytotoxicity of antitumor drug doxorubicin. All tested compounds were not toxic against the non-tumor MRC-5 cell line. Antioxidant activity of the synthesized derivatives was also evaluated. Compounds **2**, **5** and **8** were better OH radical scavengers than commercial antioxidants BHT and BHA. The synthesized compounds showed satisfactory scavenger activity, which was studied by QSAR modeling. A good correlation between the experimental variables IC_{50}^{DPPH} and IC_{50}^{OH} and MTI (molecular topological indices) molecular descriptors and CAA (accessible Connolly solvent surface area) for the new compounds **1**, **3**, and **5** was observed.*

KEY WORDS: salicylic acid derivatives, antioxidant activity, cytotoxic activity, QSAR study

INTRODUCTION

Active oxygen, originating from metabolic or external sources, could causes tissue damages and, consequently, various human diseases (1-3). Also, it attacks biomolecules, causing cell or tissue injury and promoting different diseases. It is known that radical reactions play a significant role in the development of many life-limiting chronic diseases - cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, cataracts and others (4-7). Therefore, creation or the isolation from natural sources of new molecules with

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antioxidant properties has become a very popular research area (1-6). The compounds with antioxidant potency may play a significant role in the prevention or alleviation of such diseases by reducing oxidative damage to cellular components caused by reactive oxidant species (8-11).

Derivatives of salicylic acid and many phenolic substances of plant origin and synthetic products belong to powerful antioxidants (12-20). Some phenol substances were recognized as effective in reducing the incidence of various types of carcinomas (12, 14, 21, 22). Namely, numerous newly synthesized salicylic acid derivatives exhibited cytotoxic activity against human prostate, breast and colon adenocarcinoma and leukemia cell lines (17, 18, 23-25).

In view of their pharmacological potential, in this article we present a simple and efficient synthesis of salicylic acid mono- and bis-derivatives obtained from methyl salicylate and appropriate diols or amino alcohol in the presence of metallic sodium as catalyst. Since the structures of these compounds are similar to those of some bioactive natural products, it was interesting to study their antioxidant potency by measuring their DPPH and OH radical scavenging capacities and cytotoxic activity against three human cancer cell lines: PC-3 (AR- prostate cancer), MCF-7 (human breast adenocarcinoma ER+), MDA-MB-231 (human breast adenocarcinoma ER-) and one non-cancerous cell line (MCR-5). Also, we describe the relationship between the antioxidant activity and the structures of the synthesized compounds (QSAR).

EXPERIMENTAL

Reagents and instruments

Melting points were determined using a Büchi SMP 20 apparatus and the results are uncorrected. IR spectra were recorded on a Nexus 670 FT-IR spectrometer. NMR spectra were recorded using a Bruker AC 250E spectrometer operating at 250 MHz (proton) and 62.5 MHz (carbon) with tetramethylsilane as the internal standard. Chemical shifts are given in ppm. Mass spectra were recorded on a Finnigan MAT 8230 instrument, using chemical ionization (isobutane) technique. Absorbance of the reaction mixtures in free radical scavenging tests was recorded on a CECIL CE2021 spectrophotometer. Absorbance in the cytotoxicity test was measured on the microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. Organic solutions were dried with anhydrous Na_2SO_4. Chromatographic separations were performed on silica gel column (Kieselgel 60, 0.063-0.20 mm, Merck). All reagents used were of analytical grade.

General procedure for the synthesis of compounds 1-10

A mixture consisting of methyl salicylate (10 - 40 mmol), the corresponding alcohol: 3-oxapentane-1,5-diol, 3,6-dioxaoctane-1,8-diol, 3,6,9-trioxaundecane-1,11-diol or propane-1,2-diol, 1-aminopropan-2-ol (10 - 100 mmol) and sodium (1 - 22 mmol) was heated on an oil bath at 60^{o}C to 150^{o}C under atmospheric pressure with the continuous removal of methanol. The total reaction time was from 30 min to 6 h. Afterwards, distilled

water (50 mL) and HCl (1 : 1) to pH 6-7 were added to the residue. The crude product was extracted with ethyl acetate or dichloromethane (5 × 50 mL), dried, and evaporated. Pure compounds were obtained after column chromatography.

3-Oxapentane-1,5-diyl bis(2-hydroxybenzoate) (1) and 3-oxa-5-hydroxypentyl-2-hydroxybenzoate (2). According to the general procedure, a mixture of methyl salicylate (4.56 g, 30 mmol), 3-oxapentane-1,5-diol (diethylene glycol, 1.06 g, 10 mmol) and sodium (0.046 g, 2 mmol) was treated as described above, at 150°C for 2 h. After column chromatography (25 g silica gel, petroleum ether-acetone 9 : 1 and 6 : 1) pure compounds **1** (mp 80°C, after recrystallization from hexane) and **2** were obtained.

Compound **1**: White crystals, yield: 2.69 g, 52 %. IR ν_{max} (KBr) cm^{-1}: 3400, 1678, 1613, 1583, 1249, 1216, 1165, 1154, 1087, 772, 732, 695. ^{1}H NMR ($CDCl_3$): 3.89 (t, J = 4.7 Hz, 4H, 2COOCH$_2$C**H$_2$**); 4.53 (t, J = 3.4 Hz, 4H, 2COOC**H$_2$**CH$_2$); 6.84 (td, $J_{(5,3)}$ = 0.9 Hz, $J_{(5,4)}$ = 7.8 Hz, $J_{(5,6)}$ = 8.0 Hz, 2H, 2H-5 from A,B rings); 6.97 (dd, $J_{(3,4)}$ = 8.4 Hz, $J_{(3,5)}$ = 0.9 Hz, 2H, 2H-3); 7.44 (m, 2H, 2H-4); 7.83 (dd, $J_{(6,4)}$ = 1.6 Hz, $J_{(6,5)}$ = 8.0 Hz, 2H, 2H-6); 10.69 (s, 2H, 2OH from A,B rings). ^{13}C NMR ($CDCl_3$): 64.12 (2COOCH$_2$**C**H$_2$); 68.91 (2COO**C**H$_2$CH$_2$); 112.18 (2C-1 from A,B rings); 117.52 (2C-3 from A,B rings); 119.15 (2C-5 from A,B rings); 129.93 (2C-6 from A,B rings); 161.58 (2C-2 from A,B rings); 169.93 (2C = O). MS (*m*/*z*) %: 346 (20, M^+); 165 (42); 121 (100); 120 (26); 93 (17); 92 (22); 65 (26). Analytical data for $C_{18}H_{18}O_7 \times 0.5\ H_2O$ (M Wt 355.33): Calc. C, 60.84; H, 5.35 %. Found C, 60.70; H, 5.47 %.

Compound **2**: Colorless oil, yield: 0.298 g, 4.4 %. IR ν_{max} (film) cm^{-1}: 3415, 3200, 1674, 1614, 1585, 1250, 1215, 1159, 1129, 1090, 759, 701. ^{1}H NMR ($CDCl_3$): 2.54 (bs, 1H, CH$_2$O**H**); 3.55 (m, 2H, C**H$_2$**OH); 3.70 (m, 2H, C**H$_2$**CH$_2$OH); 3.82 (m, 2H, OC**H$_2$**CH$_2$OCO); 4.45 (m, 2H, OCH$_2$C**H$_2$**OCO); 6.86 (m, 1H, H-5, from A ring); 6.96 (dd, $J_{(3,4)}$ = 7.9 Hz, $J_{(3,5)}$ = 1.0 Hz, 1H, H-3 from A ring); 7.44 (m, 1H, H-4 from A ring); 7.86 (dd, $J_{(6,4)}$ = 1.7 Hz, $J_{(6,5)}$ = 8.1 Hz 1H, H-6 from A ring); 10.67 (s, 1H, OH from A ring). ^{13}C NMR ($CDCl_3$): 61.55 (O**C**H$_2$CH$_2$OH); 64.18 (**C**H$_2$CH$_2$OCO); 68.76 (**C**H$_2$OCO); 72.44 (**C**H$_2$OH); 112.14 (C-1); 117,52 (C-3); 119.13 (C-5); 129.89 (C-6); 135.73 (C-4); 161.45 (C-2); 169.89 (C = O). MS (*m*/*z*) %: 226 (26, M^+); 138 (29); 121 (100); 92 (26); 65 (12); 45 (31).

3,6-Dioxaoctane-1,8-diyl bis(2-hydroxybenzoate) (3) and 3,6-dioxa-8-hydroxyoctanyl-2-hydroxybenzoate (4). According to the general procedure, compounds **3** and **4** were obtained in the transesterification of methyl salicylate (2.72 g, 20 mmol) with 3,6-dioxaoctane-1,8-diol (triethylene glycol, 1.50 g, 10 mmol) in the presence of sodium (0.023 g, 1 mmol), after column chromatography (25 g silica gel, petroleum ether-acetone 8 : 1).

Compound **3**: Colorless oil, yield: 0.60 g, 10 %. IR ν_{max} (film) cm^{-1}: 3430-3100, 1680, 1620, 1590, 1250, 1220, 1170, 1150, 1100, 760, 700. ^{1}H NMR ($CDCl_3$): 3.73 (s, 4H, OC**H$_2$CH$_2$**O); 3.84 (m, 4H, 2C**H$_2$**CH$_2$OCO); 4.49 (m, 4H, 2C**H$_2$**OCO); 6.86 (td, $J_{(5,3)}$ = 0.9 Hz, $J_{(5,4)}$ = 7.8 Hz, $J_{(5,6)}$ = 8.0 Hz 2H, 2H-5 from A,B rings); 6.97 (dd, $J_{(3,4)}$ = 8.4 Hz, $J_{(3,5)}$ = 0.9 Hz 2H, 2H-3 from A,B rings); 7.44 (m, 2H, 2H-4 from A,B rings); 7.86 (dd, $J_{(6,4)}$ = 1.6 Hz, $J_{(6,5)}$ = 8.0 Hz 2H, 2H-6 from A,B rings); 10.70 (s, 2H, 2OH from A,B rings). ^{13}C NMR ($CDCl_3$): 64.25 (O**C**H$_2$**C**H$_2$O); 68.96 (2O**C**H$_2$CH$_2$OCO); 70.72 (2**C**H$_2$OCO); 112.27 (2C-1 from A,B rings); 117.48 (2C-3, from A,B rings); 119.09 (2C-5, from A,B rings); 129.98 (2C-6, from A,B rings); 135.71 (2C-4, from A,B rings);

161.53 (2C-2, from A,B rings); 169.91 (2C = O). MS (*m*/*z*) %: 390 (100, M^+); 165 (37); 121 (61).

Compound **4**: Colorless oil, yield: 2.47 g, 54 %. IR ν_{max} (film) cm^{-1}: 3430, 3210, 1680, 1620, 1590, 1250, 1220, 1170, 1140, 1100, 760, 710. ^{1}H NMR ($CDCl_3$): 2.81 (bs, 1H, $CH_2O\mathbf{H}$); 3.57 (m, 4H, $2C\mathbf{H_2}OH$); 3.67 (m, 6H, $OC\mathbf{H_2}C\mathbf{H_2}OC\mathbf{H_2}CH_2OH$); 3.82 (m, 2H, $C\mathbf{H_2}CH_2OCO$); 4.49 (m, 2H, $CH_2C\mathbf{H_2}OCO$); 6.86 (m, 1H, H-5 from A ring); 6.94 (dd, $J_{(3,4)}$ = 8.4 Hz, $J_{(3,5)}$ = 0.9 Hz 1H, H-3 from A ring); 7.43 (m, 1H, H-4 from A ring); 7.86 (dd, $J_{(6,4)}$ = 1.6 Hz, $J_{(6,5)}$ = 8.0 Hz 1H, H-6 from A ring); 10.64 (s, 1H, OH from A ring). ^{13}C NMR ($CDCl_3$): 61.59 ($O\mathbf{C}H_2CH_2OH$); 64.16 ($\mathbf{C}H_2OCO$); 68.82 ($\mathbf{C}H_2CH_2OCO$); 70.23 and 70.64 ($OCH_2\mathbf{C}H_2O$); 72.49 ($\mathbf{C}H_2OH$); 112.24 (C-1 from A ring); 117.45 (C-3 from A ring); 119.14 (C-5 from A ring); 130.01 (C-6 from A ring); 135.72 (C-4 from A ring); 161.42 (C-2 from A ring); 169.82 (C = O). MS (*m*/*z*) %: 270 (45, M^+); 121 (100).

3,6,9-Trioxaundecane-1,11-diyl bis(2-hydroxybenzoate) (5) and 3,6,9-trioxa-11-hydroxyundecyl-2-hydroxybenzoate (6). Compounds **5** and **6** were obtained under analogous experimental conditions as described in the general procedure by the rection of methyl salicylate (4.56 g, 30 mmol), 3,6,9-trioxaundecane-1,11-diol (tetraethylene glycol, 1.94 g, 10 mmol) and sodium (0.46 g, 20 mmol). The crude product was purified by column chromatography (25 g silica gel, petroleum ether-acetone 9 : 1 and 3 : 1) giving pure compounds **5** and **6**.

Compound **5**: Colorless oil, yield: 0.79 g, 13 %. IR ν_{max} (film) cm^{-1}: 3187, 1674, 1614, 1585, 1301, 1250, 1215, 1159, 1134, 1089, 758, 701. ^{1}H NMR ($(CH_3)_2CO$-d_6): 3.62 (m, 8H, $OCH_2CH_2OCH_2CH_2O$); 3.83 (m, 4H, $2OC\mathbf{H_2}CH_2OCO$); 4.53 (m, 4H, $2CH_2OCO$); 6.93 (td, $J_{(5,3)}$ = 1.0 Hz, $J_{(5,4)}$ = 7.6 Hz, $J_{(5,6)}$ = 8.2 Hz 2H, 2H-5 from A,B rings); 7.50 (m, 2H, 2H-4 from A,B rings); 7.53 (dd, $J_{(3,4)}$ = 7.8 Hz, $J_{(3,5)}$ = 1.0 Hz 2H, 2H-3 from A,B rings); 7.87 (dd, $J_{(6,4)}$ = 1.8 Hz, $J_{(6,5)}$ = 8.2 Hz 2H, 2H-6 from A,B rings); 10.72 (s, 2H, 2OH from A,B rings). ^{13}C NMR ($(CH_3)_2CO$-d_6): 65.44 (4C, $OCH_2CH_2OCH_2CH_2O$); 69.31 ($\mathbf{C}H_2CH_2OCO$); 71.24 ($\mathbf{C}H_2OCO$); 113.21 (2C-1 from A,B rings); 118.10 (2C-3 from A,B rings); 120.03 (2C-5 from A,B rings); 130.80 (2C-6 from A,B rings); 136.67 (2C-4 from A,B rings); 162.39 (2C-2 from A,B rings); 170.71 (2C = O). MS (*m*/*z*) %: 434 (4, M^+); 121 (100); 165 (64).

Compound **6**: Colorless oil, yield: 0.94 g, 10 %. IR ν_{max} (film) cm^{-1}: 3430, 3200, 1674, 1614, 1585, 1250, 1216, 1159, 1129, 1089, 760, 702. ^{1}H NMR ($(CH_3)_2CO$-d_6): 2.95 (bs, 1H, $CH_2O\mathbf{H}$); 3.49 (m, 8H, $OCH_2CH_2OCH_2CH_2O$); 3.55 (m, 2H, $C\mathbf{H_2}OH$); 3.67 (m, 2H, $C\mathbf{H_2}CH_2OH$); 3.86 (m, 2H, $C\mathbf{H_2}CH_2OCO$); 4.52 (m, 2H, $CH_2C\mathbf{H_2}OCO$); 6.95 (m, 1H, H-5 from A ring); 6.99 (dd, $J_{(3,4)}$ = 7.8 Hz, $J_{(3,5)}$ = 0.9 Hz, 1H, H-3 from A ring); 7.52 (m, 1H, H-4 from A ring); 7.90 (dd, $J_{(6,4)}$ = 1.5 Hz, $J_{(6,5)}$ = 7.9 Hz, 1H, H-6 from A ring); 10.72 (s, 1H, OH from A ring). ^{13}C NMR ($(CH_3)_2CO$-d_6): 61.84 ($O\mathbf{C}H_2CH_2OH$); 65.45 ($\mathbf{C}H_2CH_2OCO$); 69.28 ($\mathbf{C}H_2OCO$); 70.95, 71.11, 71.14 and 71.17 (4C, $OCH_2CH_2OCH_2CH_2O$); 73.41 ($\mathbf{C}H_2OH$); 111.18 (C-1 from A ring); 118.11 (C-3 from A ring); 120.05 (C-5 from A ring); 130.80 (C-6 from A ring); 136.69 (C-4 from A ring); 162.34 (C-2 from A ring); 170.71 (C = O). MS (*m*/*z*) %: 314 (5, M^+); 226 (17); 165 (47); 121 (100); 89 (21); 45 (52).

Propane-1,2-diyl bis(2-hydroxybenzoate) (7) and 2-hydroxypropyl 2-hydroxybenzoate (8). Compounds **7** and **8** were obtained as described in the general procedure by

the reaction of methyl salicylate (4.56 g, 30 mmol), propan-1,2-diol (0.76 g, 10 mmol) and sodium (0.14 g, 6 mmol). After column chromatography (15 g silica gel, petroleum ether-acetone 8 : 1), compound **7** was obtained in a yield of 13 % (0.587 g, mp 76-76.5°C after recrystallization from 95 % ethanol; mp lit. (26) 76-76.5°C) and compound **8** in a yield of 1 % (0.037 g, mp 78.5-79°C after recrystallization from water; mp lit. (26) 78.5-79°C). When methyl salicylate (1.52 g, 10 mmol) and propan-1,2-diol (3.81 g, 50 mmol) reacted in a ratio 1 : 5 (150°C for 2h) in the presence of sodium (0.23 g, 10 mmol), compound **7** was obtained in a yield of 2 % (0.042 g, mp 76-76.5°C after recrystallization from 95 % ethanol) and compound **8** was obtained in a yield of 56 % (1.11 g, mp 78.5-79°C after recrystallization from water). When methyl salicylate (1.52 g, 10 mmol) and propan-1,2-diol (7.62 g, 100 mmol) reacted in the ratio 1 : 10 (60°C for 30 min) in the presence of sodium (0.596 g, 22 mmol), compounds **7** and **8** were obtained in yields of 1.4 % (0.022 g, mp 76-76.5°C after recrystallization from 95 % ethanol) and 57 % (1.13 g, mp 78.5-79°C after recrystallization from water), respectively. Compound **7** was also obtained from methyl salicylate (1.22 g, 8 mmol), compound **8** (0.29 g, 1.5 mmol) and sodium (0.046 g, 2 mmol) as described in the general procedure. Pure compound **7** was obtained in a yield of 69.5 % (0.448 g, mp 76-76.5°C after recrystallization from 95 % ethanol).

2-(2-Hydroxybenzamido)-1-methylethyl-2-hydroxybenzoate (9) and N-(2-hydroxypropyl)-2-hydroxybenzamide (10). According to the general procedure, the pure compounds **9** and **10** were obtained when methyl salicylate reacted with 1-aminopropane-2-ol in a ratio of 40 mmol : 10 mmol (5.45 g : 0.75 g) at 150°C for 2h in the presence of sodium (0.046 g, 2 mmol) and 20 mmol : 10 mmol (2.72 g : 0.75 g) at 150°C for 6 h in the presence of sodium (1 mmol). After column chromatography (25 g silica gel, toluene-ethyl acetate 9 : 1) pure compound **9** was obtained in a yield of 13 % (0.402 g, for ratio 4 : 1) and 12 % (0.386 g, for ratio 2 : 1) in a form of oil. Pure compound **10** was obtained in a yield of 40.5 % (0.781 g, for the ratio 4 : 1) and 44.5 % (0.867 g, for ratio 2 : 1) in the form of oil. When methyl salicylate (10 mmol) and 1-aminopropane-2-ol (10 mmol) reacted at 150°C during 5h, in the presence of *p*-toluenesulfonic acid (1.72 g, 10 mmol), only compound **10** was obtained in a yield of 7 % (0.137 g).

BIOLOGICAL TESTS

Free radical scavenging assays

Free radical scavenging capacity (RSC) of the synthesized compounds was evaluated by measuring their ability to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) and hydroxyl ($^{\bullet}$OH) radicals. The final concentrations of the tested compounds were in the range of 0.01 – 8 mmol/L.

The DPPH assay was performed as described before (17). The different aliquots (0.10 - 2.00 mL) of 0.01 M sample solution in methanol were added to 1.00 mL of 90 μmol/L DPPH$^{\bullet}$ in methanol (Sigma; St. Louis, MO) and filled up with 95 % (v/v) methanol to a final volume of 4.00 mL. The same reaction mixture without the tested compounds was used as the control. Absorbances of the reaction mixtures and control were recorded at 515 nm (CECIL CE2021 spectrophotometer) after 1 hour. Commercial synthetic antioxi-

dants, 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) (Aldrich; Taufkirchen, Germany) and 3-*tert*-butyl-4-hydroxyanisole (BHA) (Fluka; Taufkirchen, Germany) were used as positive controls. For each sample, three replicates were recorded. The percentage of the DPPH radical scavenging capacity ($RSC_{DPPH\bullet}$) was calculated using the following equation:

$$RSC_{DPPH\bullet}\ (\%) = 100 \times (A_{control} - A_{sample} / A_{control}).$$

The IC_{50} values (the concentration of the tested compound in the reaction mixture which causes 50 % of RSC) were determined by linear regression analysis from the obtained RSC values.

The hydroxyl radicals scavenging capacity ($RSC_{\bullet OH}$) of the tested compounds was evaluated by measuring the degradation of 2-deoxy-D-ribose (Aldrich; Taufkirchen, Germany) in the reaction with hydroxyl radicals, generated in situ in Fenton's reaction (17). These radicals attack 2-deoxy-D-ribose and degrade it into a series of fragments, some or all of which react with 2-thiobarbituric acid (TBA) (Sigma; St. Louis, MO) at low pH and high temperature to give a pink chromogen, which can be determined spectrophotometrically at 532 nm. Different aliquots (0.005-0.5 mL) of sample solution in methanol were added to the test tubes (final concentration ranged from 0.01 to 8 mmol/L), each containing 0.1 mL of 5 mmol/L H_2O_2, 0.1 mL of 10 mmol/L $FeSO_4$ and 0.1 mL of 0.05 mol/L 2-deoxy-D-ribose and 0.067 mol/L KH_2PO_4-K_2HPO_4 buffer pH 7.4 to a final volume of 3.00 mL. The same reaction mixture without sample was used as the control. After an incubation period of 1 hour at 37°C, 2 mL of TBA reagent (10.4 mL of 60 % (v/v) $HClO_4$, 3 g TBA and 120 g of trichloroacetic acid (Sigma; St. Louis, MO) and 0.2 mL of 0.1 mol/L EDTA (Sigma; St. Louis, MO) were added to the reaction mixture, and the tubes were heated at 100°C for 20 min. After cooling, the absorbances of the reaction mixtures and of the control were recorded at 532 nm. The percentage of $RSC_{\bullet OH}$ was calculated using the following equation:

$$RSC_{\bullet OH}\ (\%) = 100 \times (A_{control} - A_{sample} / A_{control}).$$

Three replicates were recorded for each sample. BHT and BHA were used as reference compounds. The IC_{50} values (the concentration at which 50 % of $^{\bullet}OH$ is neutralized) were determined by linear regression analysis from the obtained RSC values.

Cytotoxicity assay

The cytotoxicity of the synthesized compounds was evaluated using a previously described method (18). The chemotherapy drug doxorubicin (DOX), used as control, was tested under the same experimental conditions.

The cytotoxicity was evaluated by the colorimetric sulforhodamine B (SRB) assay (27). Briefly, single cell suspension (5 x 10^3 cells) was plated into 96-well microtitar plates (Costar, flat bottom). The plates were pre-incubated 24 h at 37°C in a 5 % CO_2 incubator. The tested substances (at the final concentrations ranging from 10^{-8} M to 10^{-4} M) were added to all wells except for the control ones. After the incubation period (48 h / 37°C / 5 % CO_2) the cytotoxicity assay was carried out as follows: 50 μL of 80 % trichlo-

roacetic acid (TCA) was added to all wells; an hour later the plates were washed with distilled water, and 75 mL of 0.4 % SRB was added to all wells; half an hour later the plates were washed with citric acid (1 %) and dried at room temperature. Finally, 200 μL of 10 mmol/L Tris (pH 10.5) basis was added to all wells. The absorbance (A) was measured on the microplate reader. The wells without cells, containing complete medium only, acted as the blank. The cytotoxicity was calculated according to the formula:

$$CI\ (\%) = (1 - A_{sample} / A_{control}) \times 100.$$

Two independent experiments were set out in quadruplicate for each concentration of the compound. The IC_{50} (value that defines the dose of compound that inhibits cell growth by 50 %) of compounds was determined by Median effect analysis.

Three human tumor: estrogen receptor positive human breast adenocarcinoma (ER+, MCF-7), estrogen receptor negative human breast adenocarcinoma (ER-, MDA-MB-231), human prostate cancer (PC-3) and one normal cell lines, fetal lung fibroblasts (MRC-5) were used in this study. These cells were grown in Dulbecco's modified Eagle's medium with 4.5 % of glucose. The media were supplemented with 10 % of fetal calf serum and antibiotics (100 IU/mL of penicillin and 100 μg/mL of streptomycin; ICN Galenika). All cell lines were cultured in flasks (Costar, 25 cm^2) at 37^oC in 100 % humidity atmosphere and 5 % of CO_2 incubator. Only viable cells were used in the assay. The viability was determined by the dye exclusion assay with trypan blue.

PRELIMINARY QSAR STUDIES

These studies were performed using http://www.cambridgesoft.com software.

RESULTS AND DISCUSSION

Chemistry

The derivatives **1**-**10** were synthesized in the reaction of methyl salicylate and selected diols: 3-oxapentane-1,5-diol, 3,6-dioxaoctane-1,8-diol, 3,6,9-trioxaundecane-1,11-diol, propane-1,2-diol or 1-aminopropan-2-ol, in the presence of metallic sodium, during 0.5 h to 6 h, at a temperature from 60^oC to 150^oC. The structures of the synthesized mono- and bis-salicylic acid derivatives are presented in Table 1.

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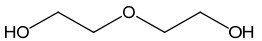
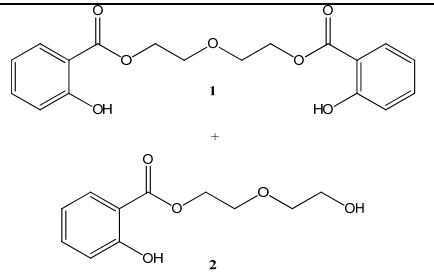
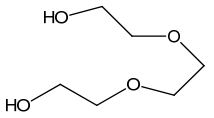
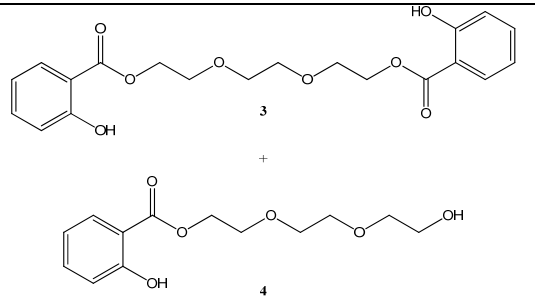
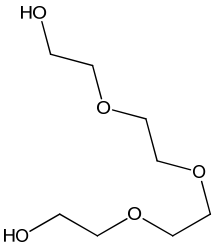
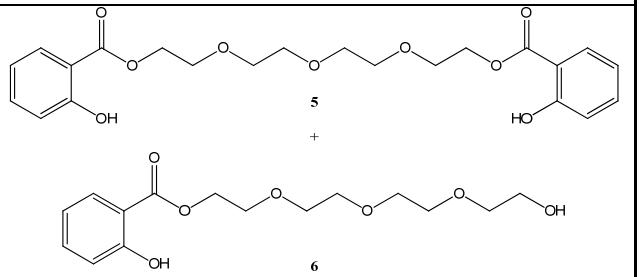
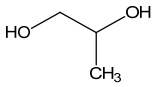
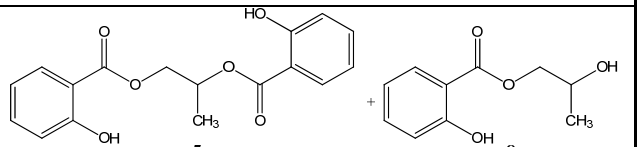
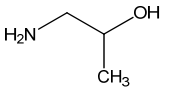
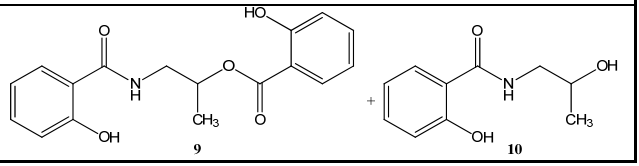
Table 1. Products from reaction of methyl salicylate with alcohols

Alcohols	Products
	
	
	
	
	

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The molar ratio of the reactants, reaction times, temperatures and the catalysts were varied in order to obtain better yields of the corresponding mono-and bis-salicyloyl derivatives **1**-**10** (Table 2).

Table 2. Reaction conditions and yields of compounds **1**-**10**

Compound	Methyl salicylate/alcohol	Reaction time (h)	Yield (%)
1	3 : 1	2	52
2	3 : 1	2	4.4
3	2: 1	2	10
4	2: 1	2	54
5	3 : 1	2	13
6	3 : 1	2	10
7	3 : 1 1 : 5 1 : 10 8 : 1.5 (from **8**)	2 2 0.5 (60°C) 2	13 2 1.4 69.5
8	3 : 1 1 : 5 1 : 10	2 2 0.5 (60°C)	1 56 57
9	4 : 1 2 : 1 1 : 1	2 6 5 (*p*-TsOH)	13 12 /
10	4 : 1 2 : 1 1 : 1	2 6 5 (*p*-TsOH)	40.5 44.5 7

When the transesterification was carried out for 2 h in a molar ratio of 3 : 1 of methyl salicylate and the corresponding alcohol (3-oxapentane-1,5-diol, 3,6-dioxaoctane-1,8-diol or 3,6,9-trioxaundecane-1,11-diol), the bis-salicylic acid derivatives **1** and **5** were obtainned in the yields 52 % and 13 % respectively and the mono-salicylic acid derivatives **2** and **6** in the yields 4.4 % and 10 % respectively.

Compounds **3** and **4** were obtained in the yields of 10 % and 54 %, respectively, when a molar ratio of methyl salicylate and 3,6-dioxaoctane-1,8-diol was 2 : 1.

In a molar ratio 3 : 1, 1 : 5 or 1 : 10 of methyl salicylate and propane-1,2-diol, during 2 h, i.e. 30 min, compounds **7** and **8** were obtained in different yields. The reaction time was shorter (30 min instead of 2 h) if the molar ratio was 1 : 10, and the reaction temperature was lower. Further, the bis-derivative **7** was obtained from the mono-derivative **8** and methyl salicylate in a yield of 69.5 %.

When the reaction of transesterification of methyl salicylate was carried out in a molar ratio of methyl salicylate and 1-aminopropane-2-ol 4 : 1 (2 h) or 2 : 1 (6 h), the bis-derivative **9** was obtained in the of yields 13 %, i.e. 12 %, respectively, while the mono-derivative **10** was obtained in the of yields of 40.5 % and 44.5 %, respectively. When the ratio of the reactants was 1 : 1 (with *p*-toluenesulfonic acid as catalyst), only the mono-derivative **10** was obtained in a yield 7 %.

It can be concluded that the products could be obtained in different yields by varying molar ratios of the reactants, reaction times and/or catalyst used.

Antioxidant activity

The *in vitro* antioxidant activity of the synthesized salicyloyl derivatives was evaluated and compared with those of the commercial antioxidants BHT and BHA (Table 3). In the DPPH assay, the ability of the tested compounds to act as donors of hydrogen atoms or electrons in transforming DPPH$^{\bullet}$ into its reduced form, DPPH-H, was measured by the spectrophotometric method (17). The hydroxyl radical scavenging activity of the tested compounds was measured in the deoxyribose assay (17), where the protective effects of the compounds were assessed as their ability to remove hydroxyl radicals (formed in the Fenton reaction) from the test solution and prevent sugar degradation. The hydroxyl radical scavenging activity of the tested compounds was determined indirectly by measuring the absorbance of the pink-colored solutions.

The results of the antioxidant capacity of the synthesized compounds **1-10** are presented in Table 3. The synthesized compounds **1-10** expressed the DPPH$^{\bullet}$ and $^{\bullet}$OH scavenger activity, but the commercial antioxidants BHT and BHA showed higher activities towards DPPH$^{\bullet}$. Comparing the hydroxyl radical scavenger capacity of salicyloyl derivatives **2**, **5** and **8** with those of the commercial antioxidants BHT and BHA, it comes out that the synthesized compounds **2**, **5** and **8** were better hydroxyl radical scavengers. Also, compounds **7** and **10** were better hydroxyl radical scavengers than BHA. In a comparison of the hydroxyl radical scavenger activity of the mono-salicyloyl derivatives **2** and **8** with the bis-salicyloyl derivative **5**, it can be seen that the introduction of another salicyloyl group increased the hydroxyl radical scavenging activity.

Table 3. Radical scavenging activities of the synthesized and the reference compounds

Compound	IC_{50} (mmol/L)	
	DPPH$^{\bullet}$	$^{\bullet}$OH
1	4.00	3.20
2	2.40	1.50
3	2.75	2.20
4	3.55	2.72
5	1.80	1.31
6	7.50	4.25
7	3.05	2.12
8	2.45	1.40
9	5.50	3.40
10	2.90	2.00
BHT	0.04	1.94
BHA	0.01	2.13

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QSAR studies

In order to study the relationship between the structures of the synthesized compounds **1-10** and their radical scavenging activities, we performed preliminary QSAR studies. Hierarchical analysis (dendrogram or two-dimensional vector space variable IC_{50}^{DPPH} and IC_{50}^{OH}, the difference function: Euclidean, connection method: Ward) shows that on the basis of the IC_{50}^{DPPH} and IC_{50}^{OH} values the molecules from the initial set (10 different objects) are not classified into one chemical congeneric group (Fig. 1). Therefore, the IC_{50}^{DPPH} and IC_{50}^{OH} variable do not correlate with the *in silico* molecular descriptors (descriptors of the software package ChemBio3D Draw 10, www.cambridgesoft.com). To obtain multiple regression equations is not possible due to the small number of the molecules ("over-fitting effect").

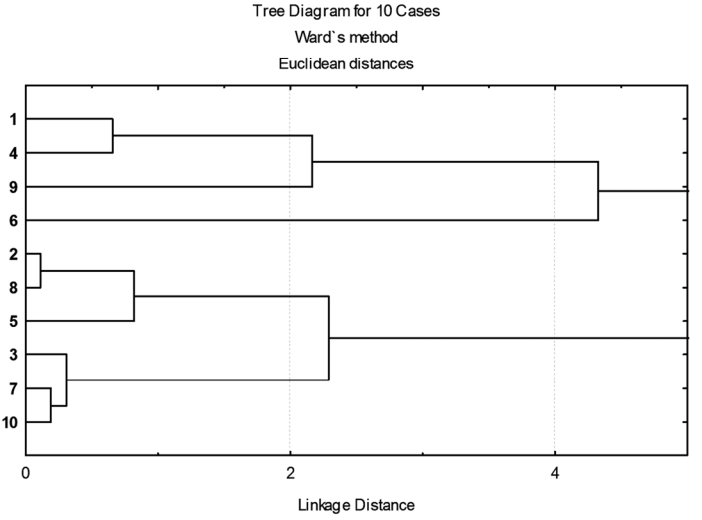

Figure 1. Dendrogram based on the experimental IC_{50}^{DPPH} and IC_{50}^{OH} values

However, in the chemical congeneric group of the bis-salicylate esters with relatively long spacers between two salicylic acid residues (**1**, **3**, and **5**) there is a good correlation between the experimental variables IC_{50}^{DPPH} and IC_{50}^{OH} and the MTI molecular descriptors (molecular topological indices) and CAA (accessible Connolly solvent surface area) (www.cambridgesoft.com):

$$IC_{50}^{DPPH} = 5.9496 - 0.001\ MTI \quad \text{(Eq. 1)}$$
$$n = 3;\ R^2 = 0.972;\ F = 45.89;\ sd = 0.092$$

$$IC_{50}^{DPPH} = 12.3691 - 0.0131\ CAA \quad \text{(Eq. 2)}$$
$$n = 3;\ R^2 = 0.998;\ F = 513;\ sd = 0.028$$

Both regression equations predict the IC_{50}^{DPPH} decline, i.e. increased antioxidant activity in the congeneric group of bis-salicylate molecules with the increase of the number

of oxyethylene units. The topological molecular descriptor MTI increases with increasing the length of the spacer between the active sites of the molecules (salicyloyl groups).

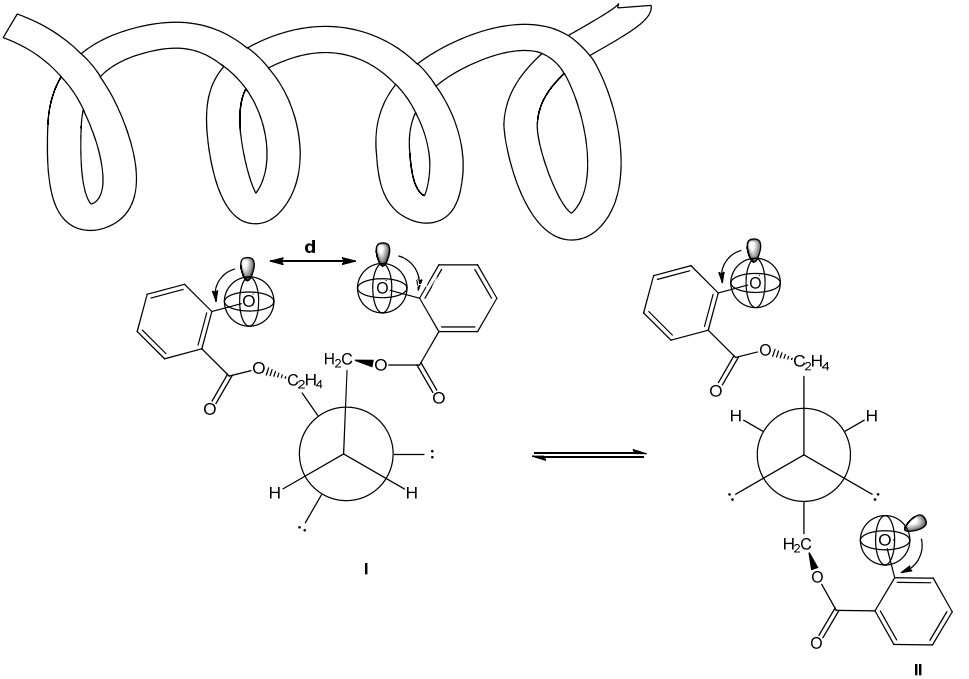

Figure 2. Conformation (**I**) of the bis-salicylate **1** during the simultaneous takeover of electrons: The higher is molecular descriptor MTI, greater is the distance **d**, and the more effective is the stabilization of conformation (**I**) comparing to conformation (**II**).

The correlation between the IC_{50}^{DPPH} and MTI can be explained if we consider the takeover of the electron from the damaged molecules by bis-salicylate (Fig. 2). Namely, in the collision of bis-salicylate and damaged biomolecules both salicyloyl groups could simultaneously be involved in accepting electrons only if they both are oriented towards the biomolecule. This is possible if the salicyloyl groups in the corresponding Newman projection formula are in a sin-periplanar position (Fig. 2 **I**), i.e. the bis-salicylate is in an energetically unfavorable conformation. However, with the spacer length increase (higher value of MTI), the repulsive interaction between the salicyloyl groups decreases, i.e. the unfavorable sin-periplanar conformation is stabilized. This is confirmed by the regression equation (Eq. 2). Namely, if CAA has a higher value, the higher number of water molecules (or solvent) is in the bis-salicylates hydratation shell, which means that the effect of electrons shielding between salicyloyl groups is larger (Fig. 2 **II**), i.e. the energetically unfavorable conformation is stabilized.

In the mono-salicylate compounds congeneric group (**2**, **4**, **6**) there is a correlation between the IC_{50}^{DPPH} and IC_{50}^{OH} and MTI descriptor:

$$IC_{50}^{DPPH} = -0.8054 + 0.0008\ MTI \quad \text{(Eq. 3).}$$
$$n = 3;\ R^2 = 0.957;\ F = 22.43;\ sd = 0.132$$

Along with the increase in the number of oxyethylene units, the IC_{50}^{DPPH} and MTI grow as well. A longer oxyethylene chain probably sterically hinders acceptance of electrons by the phenolic OH group.

As the correlation between the IC_{50}^{DPPH} and IC_{50}^{OH} is high (Pearson correlation: 0.99) in both congeneric groups, these equations (Eq. 1-3) are also valid for describing the IC_{50}^{OH}, but with other parameters.

In the second group of molecules (**7**, **8**, **9** and **10**), the correlation between the IC_{50}^{DPPH} and IC_{50}^{OH} and the analyzed molecular descriptors could not be found.

Cytotoxicity

The synthesized compounds **1-10** were evaluated for their antiproliferative activity against MCF-7 (ER+ breast adenocarcinoma), PC-3 (AR- prostate cancer) and MDA-MB-231 (ER- breast adenocarcinoma), as well as the control non-cancerous MRC-5 (normal fetal lung fibroblast) cells. Antiproliferative activity was evaluated *in vitro* using the SRB assay (27), following a 48-h treatment with test compounds. The cytotoxicity of the compounds and the non-selective antiproliferative drug doxorubicin were compared, and the results are presented in Table 4 as the IC_{50} values (μmol/L).

Table 4. Cytotoxic activity of the synthesized and the reference compounds against a panel of human cancer cell lines

Compound	IC_{50} (μmol/L)			
	MCF-7	**MDA-MB-231**	**PC-3**	**MRC-5**
1	>100	35.45	75.31	>100
2	>100	41.87	45.32	>100
3	>100	>100	5.46	>100
4	>100	>100	7.02	>100
5	83.53	57.31	41.13	>100
6	11.54	22.45	8.64	>100
7	>100	>100	18.64	>100
8	>100	>100	22.87	>100
9	8.34	>100	3.54	>100
10	21.62	13.99	4.35	>100
DOX	0.75	0.12	95.61	0.12

The results presented in Table 4 show that all of the synthesized compounds **1-10** selectively inhibit growth of some tumor cells.

Considering the fact that five of ten synthesized compounds have an IC_{50} less than 10 μmol/L, it may be concluded that the PC-3 cells are the most sensitive to these compounds. The most potent cytotoxic agent against prostate cancer (PC-3) cells were the

bis- and mono-salicyloyl derivatives **9** (IC_{50} = 3.54. μmol/L) and **10** (IC_{50} = 4.35 μmol/L). The derivative **9** was even 27 times more potent than doxorubicin. A strong cytotoxic activity against the PC-3 cells also showed the salicyloyl derivatives of triethylene glycol **3** (IC_{50} = 5.46 μmol/L) and **4** (IC_{50} = 7.02 μmol/L), as well as the mono-derivative of tetraethylene glycol **6** (IC_{50} = 8.64 μmol/L), while a satisfactory cytotoxicity was observed for the compounds **7** (IC_{50} = 18.64 μmol/L) and **8** (IC_{50} = 22.87 μmol/L).

The MCF-7 cells were less sensitive to these compounds, since only compound **9** (IC_{50} = 8.34 μmol/L) showed a strong cytotoxic activity against this ER+ breast adenocarcinoma cell line, while the compounds **6** and **10** displayed a satisfactory cytotoxicity (IC_{50} = 11.54 μmol/L and IC_{50} = 21.62 μmol/L, respectively).

A satisfactory cytotoxicity against the MDA-MB-231 (ER- breast adenocarcinoma) cells expressed compound **10** (IC_{50} = 13.99 μmol/L) and compound **6** (IC_{50} = 22.45 μmol/L).

All of the synthesized compounds were non-toxic to the normal MRC-5 cells, whereas doxorubicin was highly toxic to these cells, which is consistent with the severe side effects associated with doxorubicin chemotherapy.

CONCLUSION

In this paper we reported a simple and efficient synthesis of salicylic acid mono- and bis-derivatives from methyl salicylate and appropriate diols or amino alcohol in the presence of metallic sodium in the different reaction conditions: the temperature range from 60°C to 150°C and the reaction time from 0.5 h to 6 h. It can be concluded that products can be obtained in different yields by varying molar ratios of the reactants, reaction time and/or the catalyst. Also, we described the assaying of the antioxidant capacity and cytotoxic activity of the mono- and bis-salicylic acid derivatives and the relationship between the antioxidant activity and their structures (QSAR).

The salicylic acid derivatives **2**, **5** and **8** expressed higher OH radical scavenger activity compared to the commercial antioxidants BHT and BHA. In the preliminary QSAR studies, a good correlation between the experimental variable IC_{50}^{DPPH} and IC_{50}^{OH} and the MTI molecular descriptors (molecular topological indices) and CAA (accessible Connolly solvent surface area) was observed in the group of the bis-salicylate derivatives with relatively long spacers between two salicylic acid residues (**1**, **3** and **5**).

On comparing the cytotoxic activities of the synthesized compounds and doxorubicin it may be concluded that the prostate cancer cells (PC-3) were the most sensitive to these compounds. Namely, the compounds **3**, **4**, **6**, **9** and **10** displayed strong cytotoxicity against these cells. The most potent cytotoxic agent was bis-salicyloyl derivative **9**, which showed strong cytotoxicity against the MCF-7 cells as well. A satisfactory cytotoxicity against MDA-MB-231 cells exspressed compounds **6** and **10**. All of the synthesized compounds were non-toxic to the normal MRC-5 cells, while doxorubicin was highly toxic against the these cells.

Acknowledgements

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REFERENCES

1. Halliwell, B., Gutteridge, J.M.C. and Cross, C.E.: Free radicals, antioxidants and human disease: where are we now? J. Lab. Clin. Med. **119** (1992) 598-620.
2. Yen, G. and Chen, H.: Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agr. Food Chem. **43**, 1 (1995) 27-32.
3. Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J. and Telser, J.: Role of oxygen radicals in DNA damage and cancer incidence. Mol. Cell. Biochem. **266**, 1-2 (2004) 37-56.
4. Dröge, W.: Free radicals in the physiological control of cell function. Physiol. Rev. **82**, 1 (2002) 47-95.
5. Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M. and Mazur, M.: Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem.-Biol. Interact. **160**, 1 (2006) 1-40.
6. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J.: Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. **39**, 1 (2007) 44-84.
7. Soler-Rivas, C., Espin, J. C. and Wichers, H.J.: An easy fast test to compare total free radical scavenger capacity of food stuffs. Phytochem. Analysis **11**, 5 (2000) 330-338.
8. Hollman, P.C.H.: Evidence for health benefits of plant phenols: local or systemic effects? J. Sci. Food Agr. **81**, 9 (2001) 842-852.
9. Hatanaka, M., Takahashi, K., Nakamura, S. and Mashino T.: Preparation and antioxidant activity of α-pyridoin and its derivatives. Bioorgan. Med. Chem. **13**, 24 (2005) 6763-6770.
10. Bailly, F., Maurin, C., Teissier, E., Vezin, H. and Cotelle, P.: Antioxidant properties of 3-hydroxycoumarin derivatives. Bioorgan. Med. Chem. **12**, 21 (2004) 5611-5618.
11. Lertvorachon, J., Kim, J., Soldatov, D. V., Boyd, J., Roman, G., Cho, S. J., Popek, T., Jung, Y., Lau, P. C. K. and Konishi, Y.: 1,12-Substituted tetracyclines as antioxidant agents. Bioorgan. Med. Chem. **13**, 15 (2005) 4627-4637.
12. Al-Dabbas, M.M., Suganuma, T., Kitahara, K., Hou, D. and Fujii, M.: Cytotoxic, antioxidant and antibacterial activities of *Varthemia iphionoides* boiss. extracts. J. Ethnopharmacol. **108**, 2 (2006) 287-293.
13. Ljubuncic, P., Azaizeh, H., Portnaya, I., Cogan, U., Said, O., Abu Saleh, K. and Bomzon, A.: Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. J. Ethnopharmacol. **99**, 1 (2005) 43-47.
14. Fujisawa, S., Atsumi, T., Kadoma, Y. and Sakagami, H.: Antioxidant and prooxidant action of eugenol-related compounds and their cytotoxicity. Toxicology **177**, 1 (2002) 39-54.

15. Sestili, P., Diamantini, G., Bedini, A., Cerioni, L., Tommasini, I., Tarzia, G. and Cantoni, O.: Plant-derived phenolic compounds prevent the DNA single-strand breakage and cytotoxicity induced by *tert*-butylhydroperoxide via an iron-chelating mechanism. Biochem. J. **364** (2002) 121-128.
16. Rastkari, N., Abdollahi, M., Ahmadkhaniha, R. and Shafiee, A.: Syntheses and biological activities of benzimidazolo[2,1-b] benzo[e]thiazepin-5(10h)-ones. Arch. Pharm. **341**, 1 (2008) 49-54.
17. Djurendić, E.A., Dojčinović-Vujašković, S.V., Sakač, M.N., Jovin, E.D., Kojić, V.V., Bogdanović, G.M., Klisurić, O.R., Stanković., S.M., Lazar, D.V., Fabian, L. and Penov-Gaši, K.M.: X-Ray structural analysis, antioxidant and cytotoxic activity of newly synthesized salicylic acid derivatives. Struct. Chem. 21, 1 (2010) 67-78**.**
18. Djurendić, E.A., Dojčinović-Vujašković, S.V., Sakač, M.N., Ajduković, J.J., Gaković, A.R., Kojić, V.V., Bogdanović, G.M., Klisurić, O.R. and Penov-Gaši, K.M.: Synthesis and biological evaluation of some new 2-oxazoline and salicylic acid derivatives. ARKIVOC Part II (2011) 83-102.
19. Grosser, N. and Schröder, H.: Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway. Arterioscl. Throm. Vasc. **23**, 8 (2003) 1345-1351.
20. Fujimori, T., Yamada, S., Yasui, H., Sakurai, H., In, Y. and Ishida, T.: Orally active antioxidative copper(II) aspirinate: Synthesis, structure characterization, superoxide scavenging activity, and in vitro and in vivo antioxidative evaluations. J. Biol. Inorg. Chem. **10**, 8 (2005) 831-841.
21. Simpson, J., Forrester, R., Tisdale, M. J., Billington, D. C. and Rathbone, D. L.: Effect of catechol derivatives on cell growth and lipoxygenase activity. Bioorg. Med. Chem. Lett. **13**, 15 (2003) 2435-2439.
22. Zheng, L.F., Wei, Q.Y., Cai, Y.J., Fang, J.G., Zhou, B., Yang, L. and Liu, Z.L.: DNA Damage induced by resveratrol and its synthetic analogues in the presence of Cu(II) ions: Mechanism and structure-activity relationship. Free Radical Biol. Med. **41**, 12 (2006) 1807-1816.
23. Rotem, R., Tzivony, Y. and Flescher, E.: Contrasting effects of Aspirin on prostate cancer cells: Suppression of proliferation and induction of drug resistance. Prostate **42**, 3 (2000) 172-180.
24. Lograd, L.P.L., Silveira, D., Romeiro, L.A.S., De Moraes, M.O., Cavalcanti, B.C., Costa-Lotufo, L.V., Do Ó Pessoa, C. and Dos Santos, M.L.: Synthesis and biological evaluation of new salicylate macrolactones from anacardic acids. J. Brazil Chem. Soc. **16**, 6a (2005) 1217-1225.
25. Ott, I., Kircher, B., Schumacher, P., Schmidt, K. and Gust, R.: Antitumor-active cobalt-alkyne complexes derived from acetylsalicylic acid: Studies on the mode of drug action. J. Med. Chem. **48**, 2 (2005) 622-629.
26. Djurendić, E.A., Vastag, G.G., Surányi, T.M., Popsavin, M.M. and Penov-Gaši, K.M.: Synthesis of some bis- and mono-2-hydroxybenzoic acid derivates and the determination of their acidity constants. J. Serb. Chem. Soc. **65**, 10 (2000) 681-689.
27. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, T.J., Bokesch, H., Kenney, S. and Boyd, R.M.: New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. **82**, 13 (1990) 1107-1112.

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АНТИОКСИДАТИВНА И ЦИТОТОКСИЧНА АКТИВНОСТ МОНО- И БИС- ДЕРИВАТА САЛИЦИЛНЕ КИСЕЛИНЕ

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У овом раду је описана ефикасна и једноставна синтеза моно- и бис-деривата салицилне киселине **1-10**, који су добијени трансестерификацијом метил салицилата (метил 2-хидроксибензоат) са 3-оксапентан-1,5-диолом, 3,6-диоксаоктан-1,8-диолом, 3,6,9-триоксаундекан-1,11-диолом, пропан-1,2-диолом или 1-аминопропан-2-олом у алкалним условима. Цитотоксичност свих једињења је тестирана *in vitro* на три малигне (MCF-7, MDA-MB-231, PC-3) и једну здраву ћелијску линију (MRC-5). Снажну цитотоксичност према ћелијама канцера простате PC-3 показала су једињења **3**, **4**, **6**, **9** и **10**, са IC_{50} вредностима мањим од 10 μmol/L, а која је 11-27 пута већа од цитотксичности за антитуморски лек доксорубицин. Тестирана једињења нису била токсична према здравим ћелијама (MRC-5), док је доксорубицин показао изузетну цитотоксичност према овим ћелијама. Антиоксидативна активност синтетизованих деривата је такође испитивана. Једињења **2**, **5** и **8** су показала већу *OH*-скевинџер активност од комерцијалних *BHT* и *BHA* антиоксиданата. Код једињења **1**, **3** и **5** постоји добра корелација између експерименталних варијабли IC_{50}^{DPPH} и IC_{50}^{OH} и *MTI* молекуларних дескриптора *(molecular topological indices)* и *CAA (accessible Connolly solvent surface area).*

Кључне речи: деривати салицилне киселине, антиоксидативна активност, цитотоксична активност, QSAR

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