Synthesis and biological evaluation of some new 2-oxazoline and salicylic acid derivatives

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Abstract

Starting from methyl salicylate and 2-amino-2-(hydroxymethyl)propane-1,3-diol **1a**, or 2-amino-2-methylpropane-1-ol **1b**, the 2-oxazoline derivatives **2a**, **2b** or **3,** as well as mono- **4a** and **4b** and bis- **5a** and **5b** derivatives of salicylic acid were synthesized. Reactions were performed by microwave irradiation in the presence of tetrabutylammonium bromide or metallic sodium as catalyst, as well as by conventional heating. Microwave-induced reaction of some diols, diamines and amino alcohols with methyl salicylate gave mono- and/or bis- derivatives of salicylic acid **4c, 5c, 5d, 6c, 8c, 7a, 7b, 8a** and **8b**. The mono- and bis-salicyloyl derivatives **4c**, **5c** and **5d** were transformed to the corresponding phenyl-azo derivatives **9**, **10c** and **10d**. The structure of compound **3** was proved by the X-ray analysis and the R-configuration on its stereocenter was confirmed. The antioxidant and cytotoxic activities of the synthesized derivatives were evaluated in a series of *in vitro* tests. Compounds **5d**, **8b** and **8c** exhibited very strong activity against hydroxyl radical. Six **4c**, **5d**, **8a-c**, **10c** of 16 tested compounds inhibited growth of MDA-MB-231 cells at a nanomolar concentration. Compounds **8c** and **10c** showed high cytotoxicity against MCF7 cells, whereas compounds **4c**, **5d**, **8a-c** and **10d** showed high activity against K562 cells.

Keywords: 2-Oxazoline derivatives, Salicylic acid derivatives, Microwave-assisted synthesis, Antioxidant activity, In vitro cytotoxicity, X-ray structural analysis

Introduction

The complex sequence of cellular and molecular changes that take place during cancer formation are mediated by the different endogenous and exogenous stimuli.[1] Among endogenous stimuli are intermediates of oxygen reduction, i.e. *oxygen free radicals* (OFR), or more generally, *reactive oxygen species* (ROS), which interact with DNA, forming various aducts. [2a-c] OFRs are important in the pathogenesis of many different diseases.[2a-d] ROS are also involved in the processes of aging. Because of that, the recent research activities have been directed to discovering new efficient antiradical and antioxidant compounds. Many phenol substances of plant origin or synthetic products and some salicylic acid derivatives as aspirin exhibit certain antioxidant and antiproliferative activities.[3-14]

Bearing the above in mind, one part of the present research was directed to the microwave-induced synthesis of some new derivatives of salicylic acid, starting from different amino alcohols, diamines and diols, as well as to the determination of their antioxidant and antiproliferative activities. Microvawe irradiation is an alternative to conventional heating for introducing energy into reactions. One of the main advantages of microwave-assisted organic synthesis is the drastic decrease of reaction times. There is a considerable interest in the rapid synthesis of pharmacologically active compounds, such as 2-oxazolines. Namely, 2-oxazolines represent a very interesting class of heterocyclic compounds that is widely used in synthetic organic chemistry. There are numerous methods of forming 2-oxazoline ring, especially the microwave-assisted synthesis in which different amino alcohols, N-(β-hydroxy) amides yielded differently substituted 2-oxazolines.[15-17] Recently, it has been reported on the one-pot synthesis of 2-hydroxyphenyl-4-methyloxazoline-4-carboxamide substructures, found also in some natural cytotoxic agents such as brasilibactin A.[18]

Because of that, the other part of the present research was directed towards finding a suitable way for the microwave-catalyzed synthesis of some new 2-(2-hydroxyphenyl)-4-substituted derivatives of 2-oxazoline. Since the structures of these compounds are similar to those of some bioactive natural products such as the brasilibactin A, it was interesting to investigate their antioxidant and cytotoxic activities against selected human cancer cell lines.[19] Some azo-salicylic acids show biological activity and also azo derivatives of some phenols are useful precursors for the synthesis of anticarcinogenic, antiviral, antimicrobial and antimalaric agents.[20] Hence, our research was also concerned with the synthesis of phenyl-azo-salicyloyl derivatives of diamines and amino alcohols and with testing of their biological activity.

Results and discussion

Chemistry

The 2-substituted oxazoline derivatives **2a**[21], **2b**[22] and **3** were synthesized starting from methyl salicylate and amino alcohols **1a** or **1b**, using microwave irradiation under conditions described

in the literature.[23,24] Namely, microwave-assisted phase transfer catalyzed (Bu_4NBr) reaction in basic medium (K_2CO_3) of methyl salicylate with 2-amino-2-(hydroxymethyl)propane-1,3-diol **1a**, at the mole ratio methyl salicylate/alcohol **1a** 2:1 during 15 min, yielded the oxazoline derivative **2a**. However, when the mole ratio metyl salicylate/alcohol **1a** was 6:1 and the reaction time extended to 30 min, then, in addition to compound **2a,** the product was also salicyloyloxymethyl derivative **3** (Table 1, Table 2). 2-Amino-2-methylpropan-1-ol **1b** gave the oxazoline derivative **2b**.

Table 1. Synthesized compounds from methyl salicylate and starting compounds **1a-1d** and **6a-6**

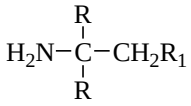
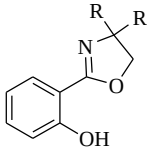
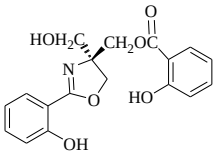
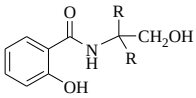
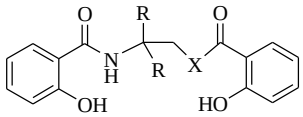
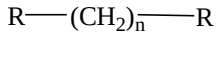
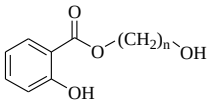
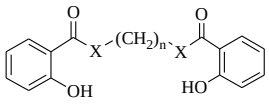
Starting compounds	Synthesized compounds			
 **1**	 **2**	 **3**	 **4**	 **5**
1a, R=CH_2OH, R_1=OH	**2a**, R=CH_2OH	**3**	**4a**, R=CH_2OH	**5a**, R=CH_2OH, X=O
1b, R=CH_3, R_1=OH	**2b**, R=CH_3		**4b**, R=CH_3	**5b**, R=CH_3, X=O
1c, R=H, R_1=OH			**4c**, R=H	**5c**, R=H, X=O
1d, R=H, R_1=NH_2				**5d**, R=H, X=N
 **6**	 **7**	 **8**		
6a, R=OH, n=2	**7a**, n=2	**8a**, X=O, n=2		
6b, R=OH, n=8	**7b**, n=8	**8b**, X=O, n=8		
6c, R=NH_2, n=3		**8c**, X=NH, n=3		

Table 2. Microwave-assisted synthesis catalyzed by Bu_4NBr in basic conditions (K_2CO_3)

Amino alcohol	Methyl salicylate/ amino alcohol	Time (min.)	Temp. (^oC)	MW power (W)	Products (yield, %)
1a	2 : 1	15	160	130	**2a** (42)
	6: 1	30	135	140	**2a** (18), **3** (30)
1b	2 : 1	15	160	160	**2b** (29)
	4 : 1	15	160	160	**2b** (78)

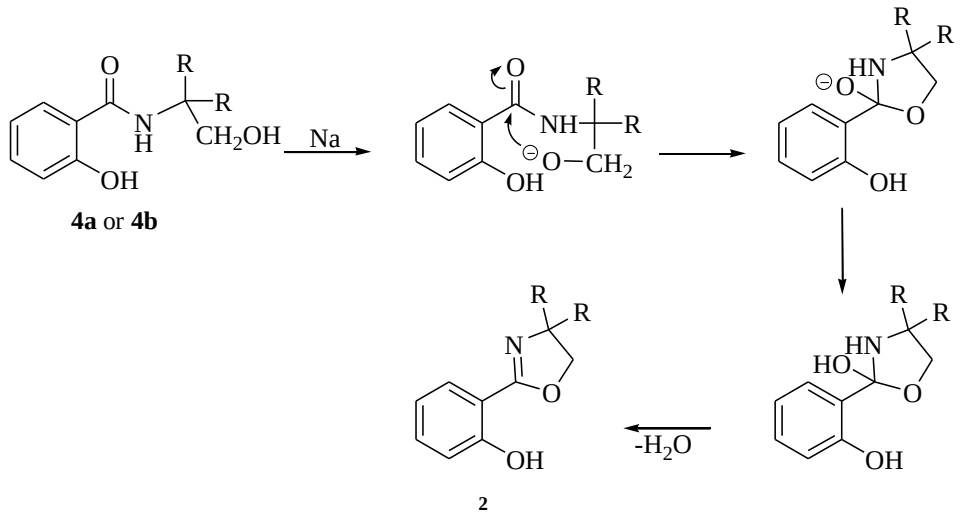
When the microwave-assisted reaction of compounds **1a** and **1b** with methyl salicylate was carried out in the presence of sodium as catalyst, the products were also oxazolines **2a, 2b** and **3** (Table 3). Oxazolines **2a**, **2b** and **3** were also formed at conventional heating of methyl salicylate and amino alcohol **1a** and **1b** in the presence of sodium as catalyst at 150 ^{o}C during 2h (Table 3). However, under these reaction conditions, 2-amino-2-(hydroxymethyl)propane-1,3-diol **1a**, apart from compounds **2a** and **3**, afforded also the mono **4a**[25] and bis **5a** derivatives of salicylic acid, whereas 2-amino-2-methylpropane-1-ol **1b**, in addition to the oxazoline derivative **2b**, gave also the mono **4b** and bis **5b** derivatives of salicylic acid.

Table 3. Comparison of the results of the synthesis of salicylic acid derivatives obtained by conventional heating and microwave irradiation, in the presence of sodium as catalyst

Starting material	Products (isolated yield, %)	
	Thermal heating[a]	MW[b]
1a	**2a** (42), **3** (6), **4a** (8), **5a** (1.3)	**2a** (45), **3** (6)
1b	**2b** (16), **4b** (7), **5b** (17)	**2b** (37)
1c[c]	**4c** (38), **5c** (12)	**4c** (65), **5c** (7)
1d[c]	**5d** (23)	**5d** (20)
6a	**8a** (23)	**7a** (16), **8a** (22)
6b	**8b** (20)	**7b** (11), **8b** (19)
6c[c]	**8c** (58)	**8c** (35)

[a]Temperature 150^{o}C, time 2 h. [b]Power applied 170W (except for **1a**, 150 W), temperature 100 – 160 ^{o}C, time 15 min (except for **1b**, 20 min.). [c]The microwave-assisted synthesis was performed in the absence of sodium.

It can be supposed that the oxazoline derivatives **2a** and **2b** are formed by cyclization of the amides **4a**[25] and **4b**,[26] generated primarly in the reaction. Salicyloyl-oxazoline **3** is probably formed in a similar way from the salicyloyl-amide **5a**, or by transesterification of methyl salicylate with oxazoline **2a**. Cyclization of compounds **4a** and **4b** into a heterocyclic ring proceeds probably according to the mechanism presented in Scheme 1.

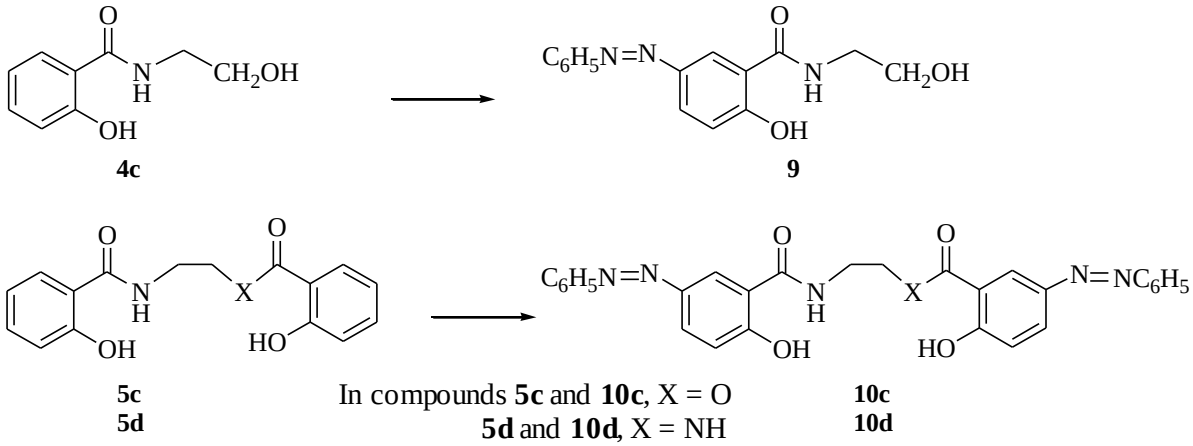


Scheme 1. Probable mechanism of cyclization of **4a** and **4b** to a heterocyclic ring.

Microwave-assisted reactions of 2-aminoethanol **1c**, ethane-1,2-diamine **1d**, ethane-1,2-diol **6a**, octane-1,8- diol **6b** and propane-1,3-diamine **6c** with methyl salicylate were also carried out in present work; the microwave power, temperature, and reaction time being the same as above (Table 3). Under these conditions, amino alcohol **1c** as well as diols **6a** and **6b** gave mono **4c, 7a**,[27] **7b** and bis **5c,**[25] **8a,**[28] **8b**[29] derivatives of salicylic acid, whereas the diamines **1d** and **6c** gave only the respective bis derivatives **5d**[28] and **8c**[30].

In our previous works, compounds **4c**,[25] **5c**,[25] **5d**,[28] as well as **8a**-**8c**[28-30] were prepared from the corresponding starting substrates in the presence of sodium as catalyst by conventional heating at 150 °C, for 2 h, the corresponding yields are given in Table 3. In the present work, at the same mole ratio of the reactants as in the above works [25,28-30] the reaction time under microwave irradition was shortened to 15 min (Table 3). The microwave irradiation gave significantly higher yield of compound **4c** compared to that obtained by conventional heating. The yield of compound **5c** was somewhat lower, but the overall yield of **4c** and **5c** in the microwave-assisted reaction was significantly higher. It can be noticed that 2-aminoethanol **1c** did not produce oxazoline derivative, either at conventional heating or microwave irradiation.

Coupling reactions of compunds **4c**, **5c** and **5d** with benzenediazonium chloride at 0 °C yielded azo derivatives **9**, **10c** and **10d** (Scheme 2).



Scheme 2. Reagents and reaction conditions: benzenediazonium chloride solution, 10% NaOH, 0 ºC, 30 min.

Molecular and crystal structure of compound **3** was solved using X-ray structural analysis (Figure 1),[31] showing the R-configuration at the stereocenter (C4) of the molecule.

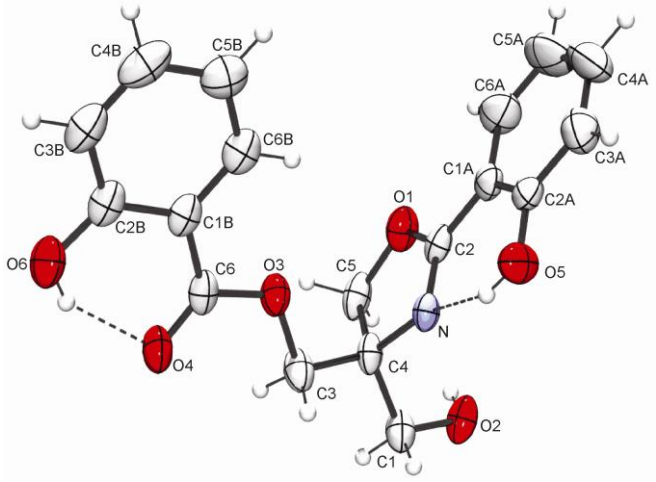


Figure 1. ORTEP presentation of structure **3** with the labeling of non-H atoms. Displacement ellipsoids are shown at the 50% probability level and H atoms are drawn as spheres of arbitrary radii. Intramolecular hydrogen bonds are shown as dashed lines.

As can be seen from Figure 1, the compound **3** contains intramolecular O-H...O and O-H…N hydrogen bonds. Crystal packing of compound **3** (Figure 2) shows that the molecules in the crystal are connected by the intermolecular O-H...O hydrogen bond along the c-axis. The hydrogen bond parameters are given in Table 4.

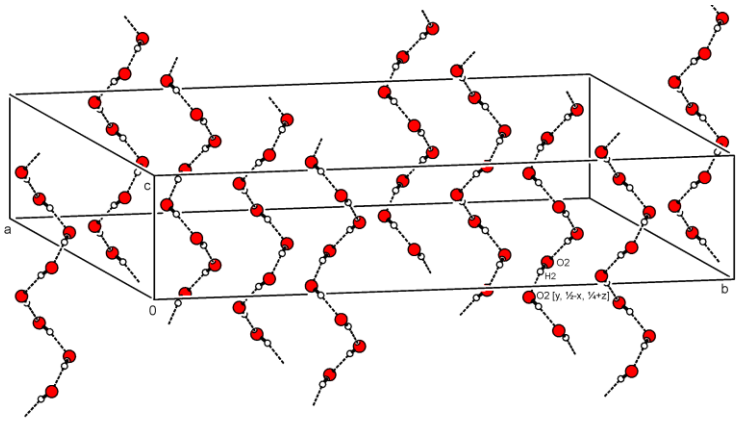


Figure 2. PLATON drawing showing the crystal packing of compound **3**; Intermolecular hydrogen bond O2-H2...O2 is shown as dashed line (atoms that are not involved in O2-H2...O2 intermolecular hydrogen bond are not presented because of clarity).

Table 4. Intramolecular and intermolecular O−H…O hydrogen-bond parameters

D–H…A	**D–H** (Å)	**H…A** (Å)	**D…A** (Å)	**D–H…A** (°)
O5-H5…N	0.85(3)	1.82(3)	2.588(3)	150(3)
O6-H6…O4	0.81(3)	1.87(3)	2.609(2)	152(3)
O2-H2…O2 [y, ½-x, ¼+z]	0.80(3)	1.82(3)	2.607(3)	169(3)

Biological properties

Antioxidant activity. The antioxidant activities of the synthesized derivatives of salicylic acid were evaluated in a series of *in vitro* tests. In the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, the ability of tested compounds to act as donors of hydrogen atoms or electrons in transforming DPPH• into its reduced form, DPPH-H, was measured by spectrophotometric method.[32] All tested compounds were able to reduce the stable, purple-colored radical DPPH• into yellow-colored DPPH-H form (Table 5). Commercial synthetic antioxidants, 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) and 3-*tert*-butyl-4-hydroxyanisole (BHA) were used as positive controls. The strongest DPPH-scavenging activity showed the phenylazo derivatives **9** and **10c,** which exhibited much stronger activity than their precursors **4c** and **5c**, which indicates a positive influence of the introduced phenylazo groups. By comparing the activities of mono derivatives **4a** and **4c** with the corresponding bis derivatives **5a** and **5c** it can be seen that the latter are more active, indicating that the introduction of the new salicyloyl group increased the DPPH-scavenging effect. This could be observed with oxazoline derivatives, too. Namely, by introducing the salicyloyl group into the oxazoline **2a**, the more active salicyloyl oxazoline **3** is obtained. Also, it can be noted that the length of methylene chain in the esters **8a** and **8b** and amides **5d** and **8c** does not influence essentially the IC_{50} values. Commercial synthetic

antioxidants BHT and BHA exhibited higher DPPH scavenging properties than those observed for the examined compounds.

Table 5. IC_{50} Values for radical scavenging activities of the tested compounds and commercial antioxidants BHA and BHT

Comp.	DPPH (IC_{50}, mM/1h)	$HO^{\bullet}$ (IC_{50}, mM)	LP (IC_{50}, mM)
2a	7.65	0.660	/
2b	3.20	1.98	/
3	3.98	0.35	/
4a	6.52	4.400	/
4b	2.87	2.300	/
4c	9.37	0.180	6.89
5a	2.40	0.920	/
5b	3.40	1.500	/
5c	3.19	0.260	4.00
5d	4.31	0.066	na[a]
8a	4.03	0.150	2.31
8b	4.61	0.012	na[a]
8c	4.04	0.018	3.02
9	0.20	0.580	0.14
10c	0.17	0.180	0.02
BHT	0.040	1.940	0.210
BHA	0.012	2.130	0.048

[a]50% inhibition not achieved.

The hydroxyl radical scavenging activity of the tested compounds (Table 5) was measured by the deoxyribose assay.[32] Protective effect of the tested compounds was followed as their ability to remove hydroxyl radicals from the test solution and prevent the degradation. High inhibition of degradation was observed for all tested compounds, especially for compounds **8c**, **8b** and **5d**. Comparison of the IC_{50} values for the tested compounds with the IC_{50} values for BHT and BHA indicates that salicylic acid derivatives are very strong scavengers of OH radicals (generated in Fenton`s reaction). It is evident that the activity is essentially influenced by the length of methylene chain in the ester and amide bis-derivatives. Namely, the bis-ester **8b** and bis-amide **8c** are more active than the corresponding compounds with smaller number of methylene groups, **8a** and **5d**. Of the 2-oxazoline derivatives **2a**, **2b** and **3** the most active is oxazoline **3**, which has a salicyloyl group at C-4 position.

The inhibition of lipid peroxidation (LP) was determined by measuring the formation of MDA, using liposomes as an oxidizable substrate.[32] Some of the examined compounds showed

notable inhibition of the Fe^{2+}/ascorbate induced LP in liposomes, compound **10c** expressing the highest inhibition. Comparison of the IC_{50} values for tested compounds with the IC_{50} values for BHT and BHA shows that azo-derivatives **9** and **10c** could be classified as promising inhibitors of LP.

Cytotoxicity

The synthesized compounds **2a**, **2b**, **3**, **4a-c**, **5a-d**, **7a**, **7b**, **8a-c**, **9**, **10c**, and **10d** were evaluated for their *in vitro* cytotoxicity against MCF7, human breast adenocarcinoma ER+, MDA-MB-231, human breast adenocarcinoma ER-, PC3, prostate cancer, HeLa S3, cervix epithelioid carcinoma, Hs 294T, human melanoma, K562, chronic myelogenous leukemia, as well as MRC-5, normal fetal lung fibroblasts. Cytotoxic activity was determined by standard SRB assay, after exposure of cells to the tested compounds for 48 h.[33] The results are presented in Table 6.

Table 6. IC_{50} Values for *in vitro* cytotoxicity of the tested compounds and Dox

Comp.	IC_{50} (μM)						
	MCF7	MDA-MB-231	PC3	HeLa S3	Hs 294T	K562	MRC-5
2a	>100	43.66	19.43	60.04	>100	>100	88.06
2b	>100	2.37	8.78	4.67	>100	34.44	>100
3	41.01	20.32	13.45	1.06	30.04	56.36	98.31
4a	>100	10.37	11.96	43.01	74.05	>100	>100
4b	2.25	40.94	40.04	13.71	>100	>100	>100
4c	1.46	0.06	19.09	0.04	0.59	0.38	>100
5a	22.25	56.31	>100	40.58	>100	>100	>100
5b	10.85	0.53	13.45	9.87	19.43	30.85	78.62
5c	>100	9.77	>100	0.05	24.58	>100	99.48
5d	0.12	0.01	0.20	9.87	0.28	0.29	>100
7a	16.12	>100	12.12	/	/	/	>100
7b	56.34	>100	15.54	/	/	/	>100
8a	2.28	0.0012	>100	0.06	0.58	0.10	>100
8b	19.97	0.0004	19.97	4.67	23.33	0.55	86.99
8c	0.08	0.03	0.08	0.21	0.04	0.83	>100
9	3.02	12.29	0.55	2.06	2.24	>100	>100
10c	0.02	0.04	0.02	1.42	0.45	5.66	>100
10d	0.17	0.10	0.06	0.36	0.44	0.45	>100
Dox	0.75	0.12	95.61	1.17	15.39	0.36	0.12

It is important to point out that eight **4c**, **5c**, **5d**, **8a-8c**, **10c** and **10d** out of eighteen tested compounds were active at nanomolar concentrations. Estrogen receptor negative MDA-MB-231 cells were most sensitive of all of the examined cell lines. Six **4c**, **5d**, **8a-8c**, **10c** out of sixteen

tested compounds inhibited growth of MDA-MB-231 cells at nanomolar concentrations (IC_{50} 0.0004-0.06 μM).

The bis derivatives appeared to be very active antiproliferative agents. Thus, bis-salicyloyl derivative with eight methylene groups, **8b**, exhibited the most potent activity of all the tested compounds in the case of MDA-MB-231 cells. Its analog with two methylene groups, compound **8a**, showed three times weaker activity against the mentioned cells, but still at the nanomolar level. Of the bis-amides **5d** and **8c** higher activity showed compound **8c**, having three methylene groups. The bis-derivatives with phenylazo groups, **10c** and **10d**, showed also strong cytotoxicity, especially against three cell lines: MCF7, MDA-MB-231 and PC3. By examining the differences of the following pairs of compounds **2a** and **3**, **4a** and **5a**, **4b** and **5b**, **4c** and **5c**, **9** and **10c**, **7a** and **8a**, as well as **7b** and **8b**, it can be noticed that the introduction of salicyloyl or phenylazo-salicyloyl group into the corresponding starting compound changed significantly cytotoxicity against all of examined cell lines of human tumors. In the majority of cases, these groups increased the cytotoxicity of the synthesized compounds.

The established antitumor drug doxorubicin (Dox) was used as reference, and its activity was in the range of IC_{50} from 0.12 to 95.61 μM. The synthesized salicyloyl derivatives **4c**, **5c**, **5d**, **8a**-**8c**, **9**, **10c** and **10d** were significantly more active against particular cell lines compared to Dox. A markedly higher cytotoxicity compared to Dox was observed with compounds **8a**-**8c**. Namely, compound **8a** was by 100 and compound **8b** by 300 times more active against MDA-MB-231 cells than Dox. The most pronounced increase in the antiproliferative activity compared to Dox (i.e. by 385 times) showed compound **8c** against Hs 294T cells. It should be noticed that compounds **5d**, **8c**, **9**, **10c**, and **10d** exhibited strong cytotoxicity against PC3, in contrast to Dox, which was not practically toxic against these cells. Only compounds **2a**, **3**, **5b**, **5c**, and **8b** were slightly toxic to normal fetal lung fibroblasts cells, MRC-5, unlike doxorubicin which was very toxic.

By comparing the antioxidant and antiproliferative activities it can be concluded that bis-ester **8b**, which showed the highest activity as a scavenger of OH radicals (IC_{50} 0.012 mM), exhibited also the highest antiproliferative activity (MDA-MB-231 cell lines, IC_{50} 0.0004 μM). Similarly, the most potent LP inhibitor, bis-azo derivative **10c** (IC_{50} 0.02 mM), showed cytotoxicity at a nanomolar concentration against three cell lines (MCF7, MDA-MB-231 and PC3).

It can be concluded that mono and bis derivatives of salicylic acid, with salicyloyl- and phenylazo-salicyloyl groups, as well as some new oxazoline derivatives, showed very potent cytotoxicity against K562, Hs 294T, HeLa S3, MCF7, PC3 and MDA-MB-231 cell lines, and strong antioxidant activity as scavengers of OH radicals and inhibitors of LP.

Experimental Section

General. Melting points were determined using a Büchi SMP 20 apparatus and are uncorrected. The infrared spectra (wave numbers in cm^{-1}) were taken on a Nexus 670 FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 250 apparatus operating at 250 MHz (proton) and 62.9 MHz (carbon), using standard Bruker software, with tetramethylsilane as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (*J*) are given in Hz. High resolution mass spectra (TOF) were recorded on a 6210 Time-of-Flight LC/MS Agilent Technologies (ESI+) instrument. GC/MS analyses were performed on an Agilent Technologies GC 7890A instrument with Mass Selective Detector 5975C. Absorbances of the reaction mixtures in free radical scavenging tests were recorded on a CECIL CE2021 spectrophotometer. The microwave reactor was a monomode system (Microwave Synthesis Sistem – Discover Bench Mate from CEM) with focused waves. Organic solutions were dried over Na_2SO_4 and evaporated on a rotary evaporator under reduced pressure. Column chromatography was performed on Merck grade 60 silica gel (0.063-0.2 mm).

Chemical synthesis

Compounds **4c**, **5c**, **5d**, **8a-c** were obtained in our previous studies[25, 28-30] by conventional heating of methyl salicylate with corresponding diols, diamines or amino alcohols, in the presence of sodium as a catalyst. Benzendiazonium chloride was prepared as described in literature.[34]

General procedure for the microwave-assisted reaction of amino alcohols 1a and 1b with methyl salicylate, catalyzed by Bu_4NBr, to obtain compounds (2a)[21], (2b)[22] and (3)

Amino alcohols **1a** and **1b** (5 mmol), methyl salicylate (10 mmol and 30 mmol for **1a** and 20 mmol for **1b**), K_2CO_3 (10 mmol), Bu_4NBr (0.2 mmol) and DMF (2 mL) were mixed and exposed to microwave irradiation under the indicated conditions (see Table 2). After cooling, the reaction mixture was poured into water, 1 M NaOH was added to pH 9, and extracted with CH_2Cl_2. The organic extracts were dried and evaporated to dryness. Purification by column chromatography (30 g silica gel, toluene – EtOAc, 9:1 for **3** then 2:1 for **2a**, 20:1 for **2b**), afforded the corresponding products in yields given in Table 2.

(R)-4-Hydroxymethyl-2-(2-hydroxyphenyl)-4-salicyloyloxymethyl-4,5-dihydro- oxazole (3). Colorless crystals (mp 85 ºC after recrystallization from chloroform – *n*-hexane). IR (KBr): 3426 (br), 3194 (br), 1682 (vs), 1641 (vs), 1615 (vs), 1586 (s), 1486 (vs), 1402 (s), 1370 (s), 1298 (s), 1257 (s), 1210 (m), 1161 (s), 1071 (s), 769 (s), 749 (s), 700 (m). ^{1}H NMR (DMSO-D6): 3.64 (bs, 2H, CH_2OH); 4.32-4.54 (m, 4H, CH_2O_2C and C-5); 5.40 (bs, 1H, OH); 6.72-7.60 (m, 8H, H-Ar); 10.38 (bs, 1H, HO-Ar); 12.92 (bs, 1H, HO-Ar). ^{13}C NMR (DMSO-D6): 63.87 (CH_2OH); 66.95 (**C**H_2O_2C); 71.05 (C-4); 74.50 (C-5); 110.26 (C-1, Ar); 113.17 (C-1', Ar); 116.81 (C-3, Ar); 117.76 (C-3', Ar); 119.36 (C-5, Ar); 119.73 (C-5', Ar); 128.18 (C-6, Ar); 130.09 (C-6', Ar); 134.30 (C-4, Ar); 136.15 (C-4', Ar); 159.48 (C-2, Ar); 160.36 (C-2', Ar); 165.83 (C-2); 168.66 (C=O). HRMS (TOF) *m/z*: $C_{18}H_{17}NO_6$ $[M+H]^+$ calculated: 343.11286, found: 344.11403.

General procedure for the microwave-assisted reaction of amino alcohols 1a or 1b or diols 6a or 6b with methyl salicylate, catalyzed by Na, to obtain compounds (2a,[21] 2b,[22] 3, 7a,[27] 7b, 8a,[28] 8b[29])

Amino alcohols **1a** or **1b** (4 mmol), or diols **6a** or **6b** (12 mmol), methyl salicylate (24 mmol) and metallic Na (1 mmol) were heated at 110 ºC for 10 min. The reaction mixture was cooled to room temperature, transfered to the microwave reactor and the reaction performed under conditions given in Table 3. Then, the reaction mixture was diluted with water, 1 M NaOH was added to pH 9, and extracted with CH_2Cl_2. The combined extracts were dried, evaporated to dryness, and the crude product was purified by column chromatography (toluene – EtOAc, 9 : 1 for **3**, then 2:1 for **2a** or toluene – EtOAc, 20 : 1 for **2b** or petrol ether – acetone 12 : 1 for **7a** and **8a**, i.e. **7b** and **8b**). The yields of obtained products are given in Table 3.

8-Hydroxyoctyl salicylate (7b). Colorless oil. IR (film): 3341 (br), 1674 (vs), 1614 (vs), 1586 (s), 1486 (s), 1396 (m), 1302 (s), 1251 (s), 1215 (s), 1158 (s), 1090 (s), 758 (s), 701 (s). ^{1}H NMR ($CDCl_3$): 1.23-1.79 (m, 12H, $6CH_2$); 2.44 (bs, 1H, OH); 3.58 (t, 2H, CH_2OH, J = 6.5 Hz); 4.29 (t, 2H, CO_2CH_2, J = 6.5 Hz); 6.81-7.83 (m, 4H, H-Ar); 10.85 (s, 1H, HO-Ar). ^{13}C NMR ($CDCl_3$): 25.44, 25.63, 28.24, 28.94, 29.04 and 32.38 ($6CH_2$); 62.35 (CH_2OH); 65.21 (CO_2CH_2); 112.31 (C-1); 117.23 (C-3); 118.85 (C-5); 129.59 (C-6); 135.31 (C-4); 161.30 (C-2); 169.96 (C=O). HRMS (TOF) *m/z*: $C_{15}H_{22}O_4$ $[M+H]^+$calculated: 267.15909, found: 267.16005; $[M+Na]^+$ calculated: 289.14103, found: 289.14086.

General procedure for the microwave-assisted reaction of amino alcohol 1c or diamines 1d and 6c with methyl salicylate, without catalyst, to obtain compounds (4c,[25] 5c,[25] 5d,[28] 8c[30])

The corresponding amine (10 mmol) was added carefully to methyl salicylate (20 mmol) cooled to 0 – 5 ºC, and the reaction mixture was left at the same temperature for 30 min. Then, the mixture was irradiated with microwaves under the conditions given in Table 3. The reaction mixture was diluted with water, 1 M NaOH was added to pH 8, and then extracted with CH_2Cl_2 and EtOAc. The joint extracts were dried and the solvent removed. The obtained crude mixture was separated on silica gel column (toluene, then toluene – EtOAc 4:1). The resulting products were **4c** (mp 117 ºC after recrystallization from chloroform, mp 117 ºC [23]), **5c** (mp 97 ºC after recrystallization from CH_2Cl_2 – *n*-hexane, lit [23] mp 98 ºC), **5d** (mp 184 ºC after recrystallization from 95% ethanol, mp 185 ºC [24]), and **8c** (mp 183 ºC after recrystallization from 95% ethanol, mp 184-185 ºC [26]) in yields given in Table 3.

General procedure for conventional synthesis catalyzed by metallic sodium, to obtain compounds (2a, 2b, 3, 4a,[25] 4b,[26] 5a, 5b)

Methyl salicylate (10 mmol), amino alcohols **1a** or **1b** (4 mmol) and metallic Na (1 mol) were boiled at 150 ºC for 2 h. The reaction mixture was diluted with water, HCl (1:1) was added to pH 7, and extracted with EtOAc. The joint extracts were dried and the desiccant and solvent were removed. The product mixture was separated on silica gel column [toluene, then toluene – EtOAc, 9 : 1 for **2a**, **3**, **4a** (mp 122-123 ºC after recrystallization from EtOAc, mp 122-123 ºC [23])

and **5a**; toluene, then toluene – EtOAc, 20 : 1 for **2b** and **5b**, then EtOAc for **4b**]; the yields are given in Table 3.

3-Hydroxy-2-(2-hydroxybenzamido)-2-(hydroxymethyl)propyl-2-hydroxybenzoate (5a). Yellow oil. IR (film): 3335 (br), 1681 (vs), 1640 (vs), 1614 (vs), 1539 (vs), 1487 (vs), 1455 (m), 1365 (m), 1302 (m), 1248 (m), 1214 (m), 1159 (s), 755 (s), 700 (s). ^{1}H NMR (acetone-D6): 3.97 (bs, 4H, $2CH_2OH$); 4.77 (s, 2H, CH_2O_2C); 6.84-7.89 (m, 8H, H-Ar); 10.66 (bs, 1H, HO-Ar). ^{13}C NMR (acetone-D6): 62.17 and 64.46 ($2CH_2OH$); 65.31 (CH_2O_2C); 71.17 (N-Cq); 113.24 (C-1, Ar); 116.61 (C-1', Ar); 118.16 (C-3, Ar); 118.45 (C-3', Ar); 119.57 (C-5, Ar); 120.12 (C-5', Ar); 128.71 (C-6, Ar); 130.93 (C-6', Ar); 134.74 (C-4, Ar); 136.74 (C-4', Ar); 161.28 (C-2, Ar); 162.31 (C-2', Ar); 170.57 (C=O, amide); 171.03 (C=O, ester). HRMS (TOF) m/z: $C_{18}H_{19}NO_7$ $[M+H-H_2O]^+$calculated: 344.11286, found: 344.11253; $[M+H]^+$calculated: 362.12343, found: 362.12358; $[M+Na]^+$ calculated: 384.10537, found: 384.10550.

2(2-Hydroxybenzamido)-2-methylpropyl-2-hydroxybenzoate (5b). Yellowish oil. IR (film): 3385 (br), 1676 (s), 1643 (s), 1614 (s), 1537 (s), 1488 (s), 1455 (sh), 1376 (m), 1302 (m), 1249 (m), 1212 (m), 1159 (m), 750 (s), 698 (s). ^{1}H NMR ($CDCl_3$): 1.59 (s, 6H, $2CH_3$); 4.59 (s, 2H, CH_2O_2C); 6.57 (s, 1H, CONH); 6.80-7.83 (m, 8H, H-Ar); 10.64 (s, 1H, HO-Ar); 12.29 (s, 1H, HO-Ar). ^{13}C NMR ($CDCl_3$): 24.03 ($2CH_3$); 54.51 (N-Cq); 69.93 (CH_2O_2C); 112.14 (C-1, Ar); 114.64 (C-1', Ar); 117.81 (C-3, Ar); 118.66 (C-3', Ar); 118.73 (C-5, Ar); 119.36 (C-5', Ar); 125.36 (C-6, Ar); 129.69 (C-6', Ar); 134.18 (C-4, Ar); 136.19 (C-4', Ar); 161.67 (C-2, Ar); 161.81 (C-2', Ar); 170.21 (C=O, amide); 170.28 (C=O, ester). HRMS (TOF) *m/z*: $C_{18}H_{19}NO_5$ $[M-H]^-$ calculated: 328.11905, found: 328.11815.

General procedure for preparation of azo-derivatives (9, 10c and 10d)

To NaOH solution (10%; 10 mmol for **4c**; 20 mmol for **5c** and **5d**) the compounds **4c**, **5c** or **5d** (5 mmol) were added and the solution was cooled to 0 – 5 ^{o}C. After that the cold freshly prepared benzenediazonium chloride solution [31] (5 mL for **4c**, 12.5 mL for **5c** and **5d**) was added very slowly (the pH 8-9). When all the diazonium salt solution was added, the mixture was left in an ice bath with stirring for 30 min. After that, distilled water and HCl (1:1 to make the pH 5-6) were added, and the content was stirred at the same temperature for 1 h. After the reaction was finished, the reaction mixture was filtered and crude products were recrystallyzed to obtained pure compounds **9**, **10c** or **10d**.

2-Hydroxy-N-(2-hydroxyethyl)-5-phenylazobenzamide (9). Orange crystals (81%, mp 152-153 ^{o}C after recrystallization from acetone – *n*-hexane). IR (KBr): 3407 (br), 1641 (vs), 1590 (vs), 1549 (vs), 1496 (vs), 1378 (m), 1358 (s), 1251 (m), 1060 (s), 836 (m), 802 (m), 764 (m), 685 (m). ^{1}H NMR (acetone-D6): 3.59-3.62 (m, 2H, H-1'); 3.74 (t, 2H, H-2', J = 6.7 Hz); 4.24 (bs, 1H, OH); 7.07 (d, 1H, H-3, J = 8.9 Hz); 7.44-7.92 (m, 5H, H-Ar); 8.01 (dd, 1H, H-4, $J_{3,4}$ = 8.9 Hz, $J_{4,6}$ = 2.2 Hz); 8.48 (d, 1H, H-6, $J_{6,4}$ = 2.2 Hz); 8.65 (bs, 1H, NH). ^{13}C NMR (acetone-D6): 43.14 (C-1'); 61.00 (C-2'); 115.48 (C-1, Ar); 119.55 (C-3, Ar); 123.17, 130.02 and 131.53 (CH from PhN=N); 125.26 (C-6, Ar); 127.06 (C-4, Ar); 145.60 and 153.29 (C-5, Ar and Cq from

PhN=N); 165.32 (C-2, Ar); 170.79 (C=O, amide). HRMS (TOF) m/z: $C_{15}H_{15}N_3O_3$ $[M+H]^+$ calculated: 286.11862, found: 286.11959; $[M+Na]^+$ calculated: 308.10056, found: 308.10048.

5,5'-Bis(phenylazo)-2-(2-hydroxybenzamido)ethyl-2-hydroxybenzoate (10c). Orange crystals (65%, mp 223-224 ^oC after recrystallization from acetone). IR (KBr): 3422 (br), 1674 (s), 1644 (vs), 1589 (vs), 1540 (vs), 1492 (vs), 1412 (s), 1372 (m), 1288 (m), 1207 (m), 1075 (s), 836 (s), 794 (s), 765 (m), 686 (m). ^1H NMR (DMSO-D6): 3.89 (t, 2H, $CONHCH_2$, J = 6.7 Hz); 4.52 (t, 2H, $COOCH_2$, J = 6.7 Hz); 6.88 (d, 1H, H-3, Ar, J = 9.0 Hz); 7.15 (d, 1H, H-3', Ar, J = 8.9 Hz); 7.39-7.86 (m, 10H, from PhN=N); 7.99 (dd, 1H, H-4, Ar, $J_{3,4}$ = 9.0 Hz, $J_{4,6}$ = 2.6 Hz); 8.03 (dd, 1H, H-4', Ar, $J_{3',4'}$ = 8.9 Hz, $J_{4',6'}$ = 2.5 Hz); 8.36 (d, 1H, H-6', Ar, J = 2.5 Hz); 8.48 (d, 1H, H-6, Ar, J = 2.6 Hz); 10.09 (bs, 1H, NH). ^{13}C NMR (DMSO-D6): 37.91 (CH_2NH); 64.28 ($COOCH_2$); 114.40 (C-1, Ar); 116.80 (C-1', Ar); 118.78 (C-3, Ar); 120.01 (C-3', Ar); 121.93, 121.39, 129.36 (6CH from 2PhN=N); 125.52 (C-4, Ar); 126.89 (C-4', Ar); 128.02 (C-6, Ar); 129.36 (C-6', Ar); 127.17; 129.29; 131.05; 142.56; 144.41; 151.83; 152.29; 162.55; 167.07; 167.69; 168.38 (2C=O). HRMS (TOF) m/z: $C_{28}H_{23}N_5O_5$ $[M+H]^+$ calculated: 510.17720, found: 510.17674.

5,5'-Bis(phenylazo)ethane-1,2-diyl-bis(2-hydroxybenzamide) (10d). Yellow crystals (50 %, mp > 310 ^oC after recrystallization from dioxane). IR (KBr): 3443 (br), 1650 (vs), 1598 (vs), 1550 (vs), 1505 (vs), 1394 (s), 1304 (s), 1262 (s), 1207 (s), 841 (s), 785 (s), 698 (s). ^1H NMR (DMSO-D6): 3.54 (t, 4H, $2CONHCH_2$, J = 6.7 Hz); 7.08 (d, 2H, H-3 and H-3', Ar, J = 9.0 Hz); 7.48-7.84 (m, 10H, from 2PhN=N); 7.84 (dd, 2H, H-4 and H-4', Ar, $J_{3,4} = J_{3',4'}$ = 9.0 Hz, $J_{4,6} = J_{4',6'}$ = 2.6 Hz); 8.52 (d, 2H, H-6 and H-6', Ar, J = 2.6 Hz); 9.31 (bs, 2H, NH). ^{13}C NMR (DMSO-D6): 38.50 ($2CH_2NH$); 115.86 (C-1 and C-1', Ar); 118.70 (C-3 and C-3', Ar); 122.26, 125.45, 126.18 (6CH from 2PhN=N); 125.45 (C-4, Ar); 129.50 (C-6, Ar); 131.00; 144.44; 152.00; 163.22; 168.84 (2C=O). MS: *m/z* 507 $[M-H]^+$ (100); 283 $[M-H-C_{13}H_8N_2O_2]^+$ (68); 240 $[M-H-C_{15}H_{13}N_3O_2]^+$ (7). Anal. Calcd for $C_{28}H_{24}N_6O_4$: C, 66.13; H, 4.76. Found: C, 66.59; H, 5.09.

Biological methods

Antioxidant activity: free radical scavenging activity

Free radical scavenging activity of tested compounds was evaluated by measuring their ability to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH) and OH radicals.

DPPH assay. The DPPH-assay was performed as described before.[32] The different aliquots (0.1 - 2 mL) of 0.01 M sample solution in methanol were added to 1 mL of 90 μM $DPPH^•$ in methanol (Sigma; St. Louis, MO) and filled up with 95 % (v/v) methanol to a final volume of 4 mL. The same reaction mixture without tested compound was used as the control. Absorbances of the reaction mixtures and control were recorded at 515 nm after 1 h. Commercial synthetic antioxidants, 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.

DPPH scavenging activity was expressed as radical scavenging activity (DPPH RSC). The percentage of DPPH RSC was calculated using the following equation:

RSC (%) = ($A_{blank} - A_{sample} / A_{blank}$) × 100.

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.

Hydroxyl-radical scavenging assay. Hydroxyl-radicals scavenging capacity ($HO^{\bullet}$ RSC) of the tested compounds was evaluated by measuring the degradation of 2-deoxy-D-ribose (Aldrich; Taufkirchen, Germany) in the reaction with OH radicals, generated *in situ* in Fenton's reaction.[32] These radicals attack the sugar 2-deoxy-D-ribose and degrade it into a series of fragments, some or all of which react on heating with 2-thiobarbituric acid (TBA) (Sigma; St. Louis, MO) at low pH 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 test tubes (final concentration ranged from 0.01 to 8 mM), each containing 0.1 mL of 5 mM H_2O_2, 0.1 mL of 10 mM $FeSO_4$ and 0.1 mL of 0.05 M 2-deoxy-D-ribose and 0.067 M KH_2PO_4-K_2HPO_4 buffer pH 7.4 to a final volume of 3 mL. The same reaction mixture without sample was used as the control. After an incubation period of 1 h 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 M EDTA (Sigma; St. Louis, MO) were added to the reaction mixture, and the tubes were heated at 100 °C for 20 min. After cooling, absorbance of the reaction mixtures and control were recorded at 532 nm.

Percentage of $HO^{\bullet}$ RSC was calculated using the following equation:

RSC (%) = ($A_{blank} - A_{sample} / A_{blank}$) × 100.

Three replicates were recorded for each sample; BHT and BHA were used as reference compounds.

Determination of lipid peroxidation (LP). The extent of LP was determined by measuring the absorbance of adduct produced in the reaction between TBA and malondialdehyde (MDA) as an oxidation product in the peroxidation of phospholipids from liposomes, by the TBA assay.[32]

The commercial preparation of liposomes "PRO-LIPO S" (Lucas-Meyer, Hamburg, Germany), pH 5-7, was used as a model system of biological membranes. The liposomes, 225-250 nm in diameter, were obtained by dispersing the commercial preparation in demineralized water (1:10) in an ultrasonic bath.

In the Fe^{2+}/ascorbate-induced lipids peroxidation, a 60 μL suspension of liposomes was incubated with 20 μL of 0.01 M $FeSO_4$, 20 μL of 0.01 M ascorbic acid, and 10 μL of tested compound and filled with 0.05 M KH_2PO_4-K_2HPO_4 buffer, pH 7.4 to a final volume of 3 mL. Samples were incubated at 37 °C for 1 h, and LP was terminated by adding 2 mL of TBA reagent and 0.2 mL of EDTA, and heating the test tubes at 100 °C for 20 min. After centrifugation (4000 rpm for 10 min), the content of the MDA (TBARS) was determined by measuring the absorbance of the product at 532 nm.

Analyses were compared with the commercial synthetic antioxidants BHT and BHA as a positive control. All reactions were carried out in triplicate.

The percentage of LP inhibition was calculated by the following equation:

I (%) = ($A_o - A_1$) / A_o × 100

where A_o was the absorbance of the control (without the test compound) and A_1 was the absorbance in the presence of the inhibitor.

Antiproliferative activity

Cell lines. Six human tumor cell lines and one human non-tumor cell line were used in the study: human breast adenocarcinoma ER+, MCF7, human breast adenocarcinoma ER-, MDA-MB-231, prostate cancer PC3, cervix epithelioid carcinoma, HeLa S3, human melanoma, Hs 294T, chronic myelogenous leukemia, K562, and normal fetal lung fibroblasts, MRC-5.

The cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 4.5% of glucose (MCF7, MDA-MB-231, PC3, HeLa S3, Hs 294T and MRC-5) or in RPMI 1640 (K562). Media were supplemented with 10% of fetal calf serum (FCS, NIVNS) 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 the 100% humidity atmosphere and 5% of CO_2. Only viable cells were used in the assay. Viability was determined by dye exclusion assay with trypan blue.

SRB assay. Cytotoxicity was evaluated by colorimetric sulforhodamine B (SRB) assay.[33] Briefly, single cell suspension was plated into 96-well microtiter plates (Costar, flat bottom): 5×10^3 cells (MCF7, MDA-MB-231, PC3, HeLa S3, Hs 294T and MRC-5) or 10^4 (K562) cells per 180 mL of medium. Plates were pre-incubated 24 h at 37 ^oC, 5% CO_2. Tested substances at concentrations ranging from 10^{-8} to 10^{-4} M were added to all wells except for the control ones. After incubation period (48 h /37 ^oC /5% CO_2) SRB assay was carried out as follows: 50 μL of 80% trichloroacetic acid was added to all wells; an hour later the plates were washed with distilled water, and 75 μL 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 Tris (pH 10.5) was added to all wells. Absorbance (A) was measured on the microplate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells, containing complete medium only, served as the blank.

Cytotoxicity was calculated according to the formula:

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

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

Crystal structure determination

A single crystal of compound **3** was mounted on a glass fiber and measured on an Oxford Diffraction Gemini S system. The diffraction data for compound **3** were collected at the temperature of 200 K with graphite-monochromated MoKα radiation (λ = 0.7107 Å). The data reduction was performed with the program package CrysAlis RED.[35] The space group determination was based on an analysis of the Laue class and the systematically absent reflections. The structure of compound **3** was solved by direct methods using SIR92 [36] and refined using full-matrix least-squares. Non-hydrogen atoms were refined anisotropically; the C—H hydrogen atoms were included at calculated positions riding on their attached atoms with fixed distances of 0.93 (CH) or 0.97 Å (CH_2) and all O—H hydrogen atoms were identified on

difference electron density maps and isotropically refined. The data were subjected to the removal of the scattering contribution from heavily disordered solvent molecules, which must be *n*-hexane molecules, using the Squeeze routine as implemented in the program PLATON.[37]
All calculations were performed using SHELXL97,[38] PARST [39] and PLATON,[37] as implemented in the WINGX [40] system of programs. The crystal data and refinement parameters are summarized in Table 7.

Table 7. The crystal data and refinement parameters

Crystal data	
Chemical formula	$C_{18}H_{17}NO_6$
M_r	343.33
Crystal system, space group	Tetragonal, $I4_1cd$
Temperature (K)	200
a, c (Å)	33.3098 (12), 6.9315 (4)
V (Å^3)	7690.8 (6)
Z	16
F(000)	2880
Radiation type	Mo $K\alpha$
μ (mm^{-1})	0.09
Crystal shape	Prism
Colour	Colorless
Crystal size (mm)	0.60 × 0.18 × 0.15
Data collection	
Absorption correction	Multi-scan *CrysAlis RED*, Oxford Diffraction Ltd., Version 1.171.32.24 Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.866, 1.000
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	11071, 4156, 2961
R_{int}	0.028
θ values (°)	θ_{max} = 29.3, θ_{min} = 3.2
No. and frequency of standard reflections	h = -45→25, k = -42→29, l = -8→8 every 60 min
Refinement	
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.049, 0.107, 1.02
No. of reflections	4156
No. of parameters	238

No. of restraints	1
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.23, -0.20

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References

1. Valko, M.; Izakovic, M.; Mazur, M.; Rhodes, Ch. J.; Telser J. *Mol. Cell. Biochem.* **2004**, *266*, 37.
2. (a) Dröge, W. *Physiol. Rev.* **2002**, *82*, 47. (b) Valko, M.; Rhodes, C. J.; Moncol, J.; Izakovic, M.; Mazur, M. *Chem.-Biol. Interact.* **2006**, *160*, 1. (c) Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44. (d) Soler, C.; Espin, J.C.; Wichers, H. *Phytochem. Anal.* **2000**, *11*, 1.
3. Al-Dabbas, M. M.; Suganuma, T.; Kitahara, K.; Hou, D. X.; Fujii, M. *J. Ethnopharmacol.* **2006**, *108*, 287.
4. Ljubincic, P.; Azaizeh, H.; Portnaya, I.; Cogan, U.; Said, O.; Abu Saleh, K.; Bomzon, A. *J. Ethnopharmacol.* **2005**, *99*, 43.
5. Fujisawa, S.; Atsumi, T.; Kadoma, Y.; Sakagami, H. *Toxicology* **2002**, *177*, 39.
6. Sestili, P.; Diamantini, G.; Bedini, A.; Cerioni, L.; Tommasini, I.; Tarzia, G.; Cantoni, O. *Biochem. J.* **2002**, *364*, 121.
7. Grosser, N.; Schröder, H. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 1345.
8. Soltermann, A. T. ; de la Peña, D.; Nonell, S.; Amat-Guerri, F.; García, N. A. *Polym. Deg. Stab.* **1995**, *49*, 371.
9. Fujimori, T. ; Yamada, S.; Yasui, H.; Sakurai, H.; Tu, Y.; Ishida, T. *J. Biol. Inorg. Chem.* **2005**, *10*, 831.
10. Rotem, R.; Tzivony, Y.; Flescher, E. *Prostate* **2000**, *42*, 172.
11. 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.; dos Santos, M. L. *J. Braz. Chem. Soc.* **2005**, *16*, 1217.
12. Erickson, K. L.; Beutler, J. A.; Cardellina II, J. H.; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8188.
13. (a) Ott, I. ; Schmidt, K.; Kircher, B.; Schumacher, P.; Wiglenda, T.; Gust, R. *J. Med. Chem.* **2005**, *48*, 622. (b) Marzotto, A.; Clemente, D. A.; Gerols, T.; Valle, G. *Polyhedron* **2001,**

20, 1079. (c) Etcheverry, S. B.; Williams, P. A. M.; Barrio, D. A.; Sálice, V. C.; Ferrer, E. G.; Cortizo, A. M. *J. Inorg. Biochem.* **2000**, *80*, 169.
14. Singh, N. K.; Singh, N.; Prasad, G. C.; Sodhi, A.; Shrivastava, A. *Bioorg. Med. Chem.* **1997**, *5*, 245.
15. Bandgar, B. P.; Pandit, S. S. *Tetrahedron Lett.* **2003**, *44*, 2331.
16. (a) Cwik, A.; Hell, Z.; Hegedüs, A.; Finta, Z.; Horváth, Z. *Tetrahedron Lett.* **2002**, *43*, 3985. (b) Vorbrüggen, H.; Krolikiewicz, K. *Tetrahedron* **1993**, *49*, 9353. (c) Kamata, K.; Agata, I. *J. Org. Chem.* **1998**, *63*, 3113. (d) Jnaneshwara, G. K.; Deshpande, V. H., Lalithambika, M.; Ravindranathan, T.; Bedekar, A.V. *Tetrahedron Lett.* **1998**, *39*, 459. (e) Clarke, D. S.; Wood, R. *Synth. Commun.* **1996**, *26*, 1335. (f) Bolm, C.; Weickhardt, K.; Zehnder, M.; Ranff, T. *Chem. Ber.* **1991**, *124*, 1173. (g) Oussaid, B.; Berlan, J.; Soufiaoui, M.; Garrigues, B. *Synth. Commun.* **1995**, *25*, 659. (h) Katritzky, A. R.; Cai, C.; Suzuki, K.; Singh, S. K. *J. Org. Chem.* **2004**, *69*, 811. (i) Fukuhara, T.; Hasegawa, C.; Hara, S. *Synthesis* **2007**, 1528. (j) Schwekendiek, K.; Glorius, F. *Synthesis* **2006**, 2996.
17. Crogsinani, S.; Young, A. C.; Linclau, B. *Tetrahedron Lett.* **2004**, *45*, 9611.
18. Fan, L.; Lobkovsky, E.; Ganem, B. *Org. Lett.* **2007**, *9*, 2015.
19. Nigović, B.; Komorsky-Lovrić, Š.; Šimunić, B. *Electroanal.* **2005**, *17*, 839.
20. Przybylski, P.; Huczynski, A.; Pyta, K.; Brzezinski, B.; Barth, F. *Curr. Org. Chem.* **2009**, *13*, 124.
21. Wiggins, L. F.; Beard, C. C.; James, J. W. British Patent 953 427, 1964; *Chem. Abstr.* **1964**, 60**,** 15875.
22. Clarke, D. S.; Wood, R., *Synthetic Communications* **1996**, *26*, 1335.
23. Bao, K.; Zhang, W.; Zhang, C.; Qu, Y.; Tian, L.; Wu, L.; Zhao, X.; Cheng, M. *Molecules* **2007**, *12*, 2123.
24. Limousin, C.; Cleophax, J.; Loupy, A.; Petit, A. *Tetrahedron* **1998**, *54*, 13567.
25. Djurendić, E. A.; Vastag, Gy. Gy.; Surányi, T. M.; Popsavin, M. M.; Penov-Gaši, K. M. *J. Serb. Chem. Soc.* **2000**, *65*, 681.
26. Zhou, P.; Natale, N. R. *Synthetic Communications* **1998**, *28*, 3317.
27. Bayer and Co., D. R. P. 164128; *C.***1905** II,1476; *B.*10.81, I 38, II, 53
28. Miljković, D. A.; Djurendić, E. A.; Šuranji, T. M. *Review of Research*, Faculty of Sciences-University of Novi Sad, **1983**, *13*, 5.
29. Djurendić, E. A.; Surányi, T. M.; Miljković, D. A. *Coll. Czech. Chem. Comm.* **1991**, *56*, 1446.
30. Djurendić, E. A.; Surányi, T. M.; Miljković, D. A. *Coll. Czech. Chem. Comm.* **1990**, *55*, 1763.
31. Farrugia, L. J. *J. Appl. Cryst.* **1997**, *30*, 565.
32. Mimica-Dukić, N.; Božin, B.; Sokolović, M.; Simin, N. *J. Agric. Food Chem.* **2004**, *52*, 2485.
33. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, T. J.; Bokesch, H.; Kenney, S.; Boyd, R. M.; *J. Natl. Cancer Inst.* **1990**, *82*, 1107.

34. *Vogel's Textbook of Practical Organic Chemistry*, 5th Ed., Longman Group Limited, London, 1989, pp. 920-921.
35. Oxford Diffraction CrysAlis CCD and CrysAlis RED. Versions 1.171., Oxford Diffraction Ltd, Abingdon, Oxfordshire, England, 2008.
36. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Cryst.* **1993**, *26*, 343.
37. Sheldrick, G. M.; SHELX97, Programs for Crystal Structure Analysis, University of Göttingen, Germany, 1997.
38. Nardelli, M. J. *J. Appl. Cryst.* **1995**, *28*, 659.
39. Spek, A. L.; PLATON, A Multipurpose Crystallographic Tool, University of Utrecht, The Netherlands, 1998.
40. Farrugia, L. J. *J. Appl. Cryst.* **1999**, *32*, 876.

