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Scientific paper

N-(9,10-Dioxo-9,10-dihydroanthracen-1(2)-yl)-2-(R-thio) Acetamides: Synthesis, Antioxidant and Antiplatelet Activity

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Abstract

The synthesis of new *N*-(9,10-dioxo-9,10-dihydroanthracen-1(2)-yl)-2-(R-thio) acetamides was carried out using reaction of 2-chloro-*N*-(9,10-dioxo-9,10-dihydroanthracene-1(2)-yl)acetamides with functionalized thiols in the presence of potassium carbonate in *N,N*-dimethylformamide (DMF) at room temperature. Evaluation of the synthesized compounds on such indicators of radical scavenging activity as lipid peroxidation (LP) and oxidative modification of proteins (OMP) *in vitro* in rat liver homogenate was carried out. It was determined that the compounds with a substituent in the first position of anthracedione core showed better antioxidant properties than their isomers with a substituent in the second position. The compounds **6** and **7** with the best indicators of radical-scavenging activity were determined. Antioxidant effect in OMP processes was also determined for compound **10**. The antiplatelet activity study *in vitro* revealed compound **10** with the inhibited effect of ADP-induced aggregation.

Keywords: *N*-(9,10-dioxo-9,10-dihydroanthracen-1(2)-yl)-2-(R-thio) acetamides; free-radical oxidation markers; antioxidant activity; antiplatelet activity; structure-activity relationship

1. Introduction

Arterial thrombosis is one of the critical factors determining the outcome of cardiovascular and oncological diseases,[1–4] which share the first place among all diseases, both in Ukraine and in the world.[5] They cause sudden death in myocardial infarction, vascular complications of diabetes mellitus, cancer chemotherapy.[6] Also, it lowers the effectiveness of surgical treatment of coronary artery disease, etc. There are several mechanisms of thrombosis formation:[7–9] the activation of platelet and coagulation chains of homeostasis, disruption of synthesis for some blood coagulation factors II (Prothrombin), VII (Proconvertin), IX (Christmas factor), and X (Stuart–Prower factor), a decrease of fibrinolytic activity of blood, activation of lipid peroxidation, disruption of endothelium functional activity, etc.[10] Modern antiplatelet and anticoagulant drugs influence the thrombocyte aggregation and blood coagulation system. However, their effectiveness often does not satisfy clinicians. Numerous clinical studies show that the use of modern antiplatelet drugs is often accompanied by such side effects as resistance to their action, an increased risk of uncontrolled bleeding, as well as the development of serious systemic complications.[11] The high cost and the listed side effects of these drugs indicate the need for further research in the pursue for new, more effective, and safe substances and the development of antiplatelet drugs based on them.

In recent years, the important role of lipid peroxidation in the pathogenesis of thrombus formation has

been shown. The influence of free radical mechanisms on the development of various types of cancer, atherosclerosis and its thrombonecrotic consequences (heart attack, stroke), diabetes mellitus, chronic nonspecific lung diseases, diseases of the reproductive system, as well as radiation injury, hepatitis, decreased cellular and humoral immunity, etc. has been studied.[12–14] Therefore, there has been a constant search for antioxidants, both natural and synthetic.[15–16]

The quinoid system is the structural block of many natural biologically active molecules, such as vitamins K and E, as well as compounds directly involved in oxidative metabolism, such as coenzyme Q10. Many antioxidants contained in food products are quinones (for example, flavonoids). The derivatives from quercetin (contained in vegetables and fruits), resveratrol (red vine), catechins and epicatechin (chocolate and tea) and also compounds obtained from tyrosine and tryptophan aminoacids (hydroxytyrosol, 5-hydroxytryptophan and pyrroloquinoline quinone) are considered to be quinone compounds.[17] The main advantage of quinones is their aromatic nature, which implies the stability necessary for functioning in an oxidative environment and actively participating in redox reactions.[18]

Many natural anthracenediones extracted from plants demonstrate antioxidant properties.[19–21] Among the synthetic derivatives of 9,10-anthracenedione were discovered compounds with antioxidant properties[22–24] and oxidative stress and cytotoxicity ability.[25] It was demonstrated that the amount and position of substituents in the anthracenedion's ring influence antioxidant properties.[26–28] The scientists[29,30] discovered some compounds among anthracenedione derivatives with antiplatelet and anticoagulant action. Dutch scientists obtained 1,4- and 1,8-derivatives of 9,10-anthracenedione included in oligodeoxynucleotides to investigate the influence on coagulation time of fibrinogen in the blood. The investigation showed better anticoagulation properties for 1,8-anthracenedione products.[31] Some sulfur-containing derivatives of 9,10-anthracenedione demonstrated high antioxidant activity.[32] There are studies dedicated to researching antithrombotic drugs in Ukraine as well.[33,34]

Therefore, the purpose of the present work is to carry out the synthesis of new derivatives of 2-chloro-*N*-(9,10-dioxo-9,10-dihydroanthracen-1(2)-yl)-acetamides using functionalization by thio fragments and *in vitro* study of obtained derivatives for antioxidant and antiplatelet effects.

2. Experimental

2. 1. Chemistry

Melting points were measured in open to air-glass capillaries using a Büchi B-540 melting point apparatus and are uncorrected. Elemental analysis was performed on Perkin–Elmer 2400 CHN-analyzer, and the results were found to be in good agreement with the calculated values. ^{1}H and ^{13}C NMR spectra in DMSO-d_6 were recorded on Varian Mercury-400 spectrometer with TMS as an internal standard. Mass spectra were recorded on Agilent 1100 Series G1956B LC/MSD SL LCMS system (Zorbax SBC18 column, 4.6×15 mm, 1.8 μm (PN 82(c) 75-932); solvent dimethylsulfoxide), using electrospray ionization at atmospheric pressure (70 eV). Infrared spectra were recorded on a Perkin–Elmer Spectrum Two FT-IR Spectrometer equipped with an UATR (HR Single-Reflection with a diamond sensor) using Perkin–Elmer Spectrum 10 Spectroscopy Software with an ATR absorbance correction for Spectrum Two UATR spectra. All chemicals were of reagent grade and used without further purification. The solvents were purified according to the standard procedures.[35]

2-Chloro-*N*-(9,10-dioxo-9,10-dihydroanthracen-1(2)-yl)-acetamides **1** and **2** were obtained by published methods.[36,37]

General Procedure for the Preparation of *N*-(9,10-Dioxo-9,10-dihydroanthracen-1(2)-yl)-2-(R-thio) Acetamides 3–20

To 0.5 g (1.668 mmol) of chloroacetamide **1** or **2** in 40 mL of DMF, 1.835 mmol of the corresponding thiol and 0.507 g (3.67 mmol) of potassium carbonate were added under stirring. The reaction mixture was kept under stirring and at room temperature for 12 hours. Then, the reaction mixture was poured into 200 mL of water, acidified with 10% HCl solution to pH 6, and left overnight. The mixture was filtered off. The precipitate was washed with 20 mL of cold water and dried. As a result, target sulfide derivatives **3–20** were obtained with 60–93% yields.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)acetic Acid (3). Yield 0.551 g (93%), mp 217 °C dec. FT-IR (UATR diamond) ν_{max} 3196.07, 2928.41, 1751.23, 1670.84, 1652.32, 1590.18, 1517.03, 1420.10, 1345.29, 1281.09, 1169.65, 1021.33, 801.68, 705.48 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.70 (br. s, 1H, OH), 12.43 (s, 1H, NH), 8.88 (d, J = 7.7 Hz, 1H$_{Ar}$), 8.08 (dd, J = 23.9, 6.1 Hz, 2H$_{Ar}$), 7.84 (d, J = 10.0 Hz, 3H$_{Ar}$), 7.79 (d, J = 7.4 Hz, 1H$_{Ar}$), 3.67 (s, 2H, CH$_2$), 3.46 (s, 2H, CH$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.45, 182.35, 171.26, 169.08 (C=O), 141.28, 135.97, 135.10, 135.02, 134.01, 133.87, 132.48, 127.40, 126.79, 125.83, 122.41, 118.29 (C$_{Ar}$), 37.65, 34.35 (CH$_2$). LC-MS, m/z (I_{rel}): 356 (M+H, 100). Anal. Calcd for $C_{18}H_{13}NO_5S$: C, 60.84; H, 3.69; N, 3.94; S, 9.02. Found: C, 60.77; H, 3.60; N, 3.88; S, 9.12.

Methyl 2-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)acetate (4). Yield 0.554 g (90%), mp 120 °C dec. FT-IR (UATR diamond) ν_{max} 3193.96, 2955.61, 1754.54, 1695.98, 1652.48, 1594.73, 1516.00, 1411.04, 1340.64, 1280.24, 709.26 cm^{-1}. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 12.41 (br. s, 1H, NH), 8.87 (d, J = 7.7 Hz, 1H_{Ar}), 8.07 (dd, J = 22.8, 6.6 Hz, 2H_{Ar}), 7.82 (dd, J = 18.2, 7.6 Hz, 4H_{Ar}), 3.68 (s, 2H, CH_2), 3.59 (s, 3H, CH_3), 3.56 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.48, 182.32, 170.40, 168.96 (C=O), 141.26, 136.01, 135.11, 135.04, 134.03, 133.85, 132.50, 127.37, 126.81, 125.77, 122.44, 118.24 (C_{Ar}), 52.55 (CH_3), 37.78, 33.84 (CH_2). LC-MS, m/z (I_{rel}): 370 (M+H, 100). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.78; H, 4.09; N, 3.79; S, 8.68. Found: C, 61.69; H, 4.20; N, 3.60; S, 8.77.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)propanoic Acid (5). Yield 0.566 g (92%), mp 182 °C dec. FT-IR (UATR diamond) ν_{max} 3135.98, 2985.20, 2933.81, 1749.97, 1673.73, 1652.70, 1593.52, 1520.90, 1283.63 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (br. s, 1H, OH), 12.46 (s, 1H, NH), 8.84 (d, J = 7.3 Hz, 1H_{Ar}), 8.04 (d, J = 6.5 Hz, 1H_{Ar}), 7.98 (d, J = 6.7 Hz, 1H_{Ar}), 7.85–7.79 (m, 2H_{Ar}), 7.74 (q, J = 7.5, 7.0 Hz, 2H_{Ar}), 3.73–3.63 (m, 2H, CH_2), 3.57 (q, J = 6.7 Hz, 1H, CH), 1.41 (d, J = 6.9 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.29, 182.20, 173.84, 169.06 (C=O), 141.16, 135.90, 135.03, 134.95, 133.88, 133.74, 132.34, 127.32, 126.72, 125.70, 122.36, 118.14 (C_{Ar}), 41.62 (CH), 36.88 (CH_2), 17.38 (CH_3). LC-MS, m/z (I_{rel}): 370 (M+H, 100). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.78; H, 4.09; N, 3.79; S, 8.68. Found: C, 61.71; H, 4.22; N, 3.63; S, 8.81.

3-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)propanoic Acid (6). Yield 0.443 g (72%), mp 227 °C dec. FT-IR (UATR diamond) ν_{max} 3183.89, 1739.22, 1679.84, 1655.50, 1594.52, 1521.37, 1339.16, 1282.47, 709.32 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (br. s, 1H, OH) 12.46 (s, 1H, NH), 8.87 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 6.8 Hz, 1H), 8.02 (d, J = 7.4 Hz, 1H_{Ar}), 7.84 (q, J = 6.5, 4.0 Hz, 2H_{Ar}), 7.78 (q, J = 8.5 Hz, 2H_{Ar}), 3.59 (s, 2H, CH_2), 2.84 (t, J = 7.1 Hz, 2H, CH_2), 2.62 (t, J = 7.1 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.39, 182.27, 173.27, 169.69 (C=O), 141.25, 135.95, 135.05, 134.97, 133.97, 133.81, 132.42, 127.37, 126.74, 125.67, 122.37, 118.17 (C_{Ar}), 37.43, 34.43, 27.86 (CH_2). LC-MS, m/z (I_{rel}): 370 (M+H, 100). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.78; H, 4.09; N, 3.79; S, 8.68. Found: C, 61.75; H, 4.01; N, 3.72; S, 8.77.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)succinic Acid (7). Yield 0.613 g (89%), mp 167 °C dec. FT-IR (UATR diamond) ν_{max} 3193.78, 3014.84, 1992.55, 1739.80, 1677.70, 1654.32, 1593.35, 1575.24, 1281.58, 1242.10, 708,99 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (br. s, 1H, OH), 12.57 (br. s, 1H, OH), 12.45 (s, 1H, NH), 8.87 (d, J = 7.9 Hz, 1H_{Ar}), 8.06 (dd, J = 23.3, 7.8 Hz, 2H_{Ar}), 7.93 (s, 1H_{Ar}), 7.86–7.78 (m, 3H_{Ar}), 3.76 (d, J = 3.8 Hz, 2H, CH_2), 3.70 (dd, J = 10.2, 5.1 Hz, 1H, CH), 2.85–2.76 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.38, 182.30, 172.64, 172.15, 168.84 (C=O), 141.16, 135.94, 135.07, 135.00, 133.98, 133.85, 132.43, 127.37, 126.76, 125.84, 122.44, 118.32 (C_{Ar}), 42.19 (CH), 36.97, 36.23 (CH_2). LC-MS, m/z (I_{rel}): 414 (M+H, 100). Anal. Calcd for $C_{20}H_{15}NO_7S$: C, 58.11; H, 3.66; N, 3.39; S, 7.76. Found: C, 58.16; H, 3.76; N, 3.48; S, 7.65.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)benzoic Acid (8). Yield 0.64 g (92%), mp 250–252 °C dec. FT-IR (UATR diamond) ν_{max} 3254.37, 1711.64, 1675.22, 1646.37, 1593.72, 1540.41, 1339.99, 1276.95 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (br. s, 1H, OH), 12.62 (s, 1H, NH), 8.92 (d, J = 8.1 Hz, 1H_{Ar}), 8.15 (d, J = 7.5 Hz, 1H_{Ar}), 8.11 (d, J = 6.5 Hz, 1H_{Ar}), 7.93–7.87 (m, 4H_{Ar}), 7.84 (t, J = 8.0 Hz, 1H_{Ar}), 7.50 (t, J = 7.6 Hz, 1H_{Ar}), 7.43 (d, J = 7.7 Hz, 1H_{Ar}), 7.20 (t, J = 7.5 Hz, 1H_{Ar}), 4.16 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.46, 182.48, 169.09, 167.90 (C=O), 141.05, 139.90, 136.05, 135.09, 134.24, 133.99, 132.97, 132.63, 131.51, 129.03, 127.42, 126.84, 125.84, 125.04, 122.57, 118.52 (C_{Ar}), 37.59 (CH_2). LC-MS, m/z (I_{rel}): 418 (M+H, 100). Anal. Calcd for $C_{23}H_{15}NO_5S$: C, 66.18; H, 3.62; N, 3.36; S, 7.68. Found: C, 66.05; H, 3.65; N, 3.44; S, 7.84.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)nicotinic Acid (9). Yield 0.635 g (91%), mp 192 °C dec. FT-IR (UATR diamond) ν_{max} 3492.52, 3217.87, 3085.59, 2926.78, 1698.96, 1672.50, 1643.98, 1581.67, 1558.28, 1519.99, 1466.38, 1411.38, 1338.67, 1313.37, 1274.00, 1246.80, 1236.69, 1157.46, 1068.96, 708.93 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.57 (br. s, 1H, OH), 12.44 (s, 1H, NH), 8.86 (d, J = 5.4 Hz, 1H_{Ar}), 8.60 (d, J = 3.1 Hz, 1H_{Ar}), 8.24 (d, J = 5.9 Hz, 1H_{Ar}), 8.02 (t, J = 9.2 Hz, 2H_{Ar}), 7.96–7.91 (m, 1H_{Ar}), 7.83 (t, J = 7.1 Hz, 2H_{Ar}), 7.78–7.76 (m, 1H_{Ar}), 7.23 (dd, J = 7.8, 4.7 Hz, 1H_{Ar}), 4.10 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.26, 182.32, 169.50, 166.76 (C=O), 159.59, 152.44, 141.29, 139.61, 135.88, 134.98, 134.92, 134.04, 133.86, 132.48, 127.22, 126.72, 125.76, 124.23, 122.20, 120.06, 118.13 (C_{Ar}), 35.89 (CH_2). LC-MS, m/z (I_{rel}): 419 (M+H, 100). Anal. Calcd for $C_{22}H_{14}N_2O_5S$: C, 63.15; H, 3.37; N, 6.70; S, 7.66. Found: C, 63.17; H, 3.37; N, 6.59; S, 7.73.

***N*-(9,10-Dioxo-9,10-dihydroanthracen-1-yl)-2-((2-hydroxyethyl)thio)acetamide (10)**. Yield 0.467 g (82%), mp 145 °C dec. FT-IR (UATR diamond) ν_{max} 3460.15, 2945.84, 1689.32, 1652.84, 1590.36, 1521.72, 1414.92, 1342.11, 1277.93, 1171.80, 707.98 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H, NH), 8.92 (d, J = 7.9 Hz, 1H_{Ar}), 8.13 (d, J = 8.1 Hz, 1H_{Ar}), 8.06 (d, J = 6.5 Hz, 1H_{Ar}), 7.89–7.79 (m, 4H_{Ar}), 4.87 (t, J = 5.1 Hz, 1H, OH), 3.65–3.58 (m, 4H, 2CH_2), 2.73 (t, J = 6.6 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.07, 182.00, 169.55 (C=O), 140.91, 135.57, 134.68, 134.59, 133.70, 133.52, 132.12, 126.98, 126.38, 125.30, 121.97, 117.91 (C_{Ar}), 60.46 (CH_2-OH), 37.27, 34.80 (CH_2). LC-MS, m/z (I_{rel}): 342 (M+H, 100).

Anal. Calcd for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43; N, 4.10; S, 9.39. Found: C, 63.32; H, 4.29; N, 4.01; S, 9.47.

2-((2,3-Dihydroxypropyl)thio)-*N*-((9,10-dioxo-9,10-dihydroanthracen-1-yl) Acetamide (11). Yield 0.446 g (72%), mp 162 °C dec. FT-IR (UATR diamond) ν_{max} 3380.05, 3190.26, 3106.35, 2988.43, 2934.29, 2845.55, 2780.01, 1674.85, 1647.56, 1591.37, 1575.92, 1515.35, 1476.80, 1408.66, 1337.05, 1280.48, 1238.14, 1172.97, 1021.33, 706.82 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.61 (s, 1H, NH), 8.96 (dd, J = 14.4, 6.6 Hz, $1H_{Ar}$), 8.21 (dd, J = 5.1, 2.4 Hz, $1H_{Ar}$), 8.16–8.12 (m, $2H_{Ar}$), 7.95–7.89 (m, $3H_{Ar}$), 4.89 (t, J = 6.1 Hz, 1H, OH), 4.66–4.60 (m, 2H, CH_2), 4.25 (dd, J = 13.6, 4.2 Hz, 1H, CH), 4.12–3.90 (m, 2H), 3.61 (d, J = 3.0 Hz, 2H, CH_2), 2.78 (dd, J = 13.3, 4.6 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 186.51, 182.52, 170.04 (C=O), 141.37, 136.03, 135.18, 135.07, 134.15, 133.99, 132.55, 127.47, 126.83, 125.78, 122.40, 118.41 (C_{Ar}), 71.67 (CH-OH), 64.92 (CH_2-OH), 36.51, 34.43 (CH_2). LC-MS, *m/z* (I_{rel}): 372 (M+H, 100). Anal. Calcd for $C_{19}H_{17}NO_5S$: C, 61.44; H, 4.61; N, 3.77; S, 8.63. Found: C, 61.40; H, 4.52; N, 3.68; S, 8.72.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino) -2-oxoethyl)thio)acetic Acid (12). Yield 0.432 g (73%), mp 136 °C dec. FT-IR (UATR diamond) ν_{max} 3589.84, 3516.18, 3296.49, 3203.80, 3102.15, 3064.54, 2957.96, 1719.69, 1675.77, 1656.88, 1644.88, 1589.23, 1574.78, 1548.17, 1425.65, 1332.17, 1302.35, 1226.20, 1192.26, 1133.81, 712.54 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.36 (br. s, 1H, OH), 10.69 (s, 1H, NH), 8.28–8.26 (m, $1H_{Ar}$), 8.05 (d, J = 7.8 Hz, $2H_{Ar}$), 8.01 (d, J = 8.4 Hz, $1H_{Ar}$), 7.94 (d, J = 7.7 Hz, $1H_{Ar}$), 7.83–7.79 (m, $2H_{Ar}$), 3.48 (d, J = 11.6 Hz, 4H, $2CH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.66, 181.58, 171.50, 168.81 (C=O), 144.79, 134.88, 134.54, 134.40, 133.42, 133.38, 128.81, 128.35, 127.08, 127.01, 124.12, 124.04, 116.24 (C_{Ar}), 36.46, 34.27 (CH_2). LC-MS, *m/z* (I_{rel}): 356 (M+H, 100). Anal. Calcd for $C_{18}H_{13}NO_5S$: C, 60.84; H, 3.69; N, 3.94; S, 9.02. Found: C, 60.79; H, 3.64; N, 3.88; S, 9.10.

Methyl 2-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)acetate (13). Yield 0.486 g (79%), mp 180 °C dec. FT-IR (UATR diamond) ν_{max} 3328.94, 3297.88, 3237.78, 3104.62, 2966.01, 2921.55, 1726.79, 1709.91, 1670.16, 1651.28, 1583.47, 1538.40, 1345.28, 1293.99, 1171.15, 1126.35, 713.52 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1H, NH), 8.35–8.31 (m, $1H_{Ar}$), 8.13–8.05 (m, $3H_{Ar}$), 7.98 (d, J = 7.5 Hz, $1H_{Ar}$), 7.86–7.83 (m, $1H_{Ar}$), 3.67–3.60 (m, 3H, CH_3), 3.56–3.54 (m, 2H, CH_2), 3.52–3.48 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.69, 181.61, 170.60, 168.67 (C=O), 144.77, 134.91, 134.57, 134.44, 133.45, 133.41, 128.86, 128.39, 127.09, 127.02, 124.12, 116.24 (C_{Ar}), 52.59 (CH_3), 36.49, 33.68 (CH_2). LC-MS, *m/z* (I_{rel}): 370 (M+H, 100). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.78; H, 4.09; N, 3.79; S, 8.68. Found: C, 61.93; H, 4.00; N, 3.78; S, 8.57.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)propanoic Acid (14). Yield 0.462 g (75%), mp 138 °C dec. FT-IR (UATR diamond) ν_{max} 3563.85, 3480.16, 3175.69, 3102.14, 3056.23, 2990.48, 2942.47, 1729.97, 1676.97, 1589.49, 1576.80, 1548.81, 1423.12, 1349.47, 1333.52, 1303.10, 1180.68, 851.93, 713.74 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.70 (s, 1H, OH), 10.74 (s, 1H, NH), 8.29–8.27 (m, $1H_{Ar}$), 8.06–8.03 (m, $2H_{Ar}$), 8.00 (d, J = 8.5 Hz, $1H_{Ar}$), 7.94 (d, J = 9.1 Hz, $1H_{Ar}$), 7.82–7.79 (m, $2H_{Ar}$), 3.62 (q, J = 6.7 Hz, 1H, CH), 3.56 (d, J = 3.3 Hz, 2H, CH_2), 1.38 (d, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.16, 181.08, 173.64, 168.34 (C=O), 144.32, 134.37, 134.03, 133.90, 132.93, 132.89, 128.30, 127.85, 126.58, 126.51, 123.64, 115.76 (C_{Ar}), 41.12 (CH), 35.42 (CH_2), 17.28 (CH_3). LC-MS, *m/z* (I_{rel}): 370 (M+H, 100). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.78; H, 4.09; N, 3.79; S, 8.68. Found: C, 61.69; H, 4.17; N, 3.82; S, 8.80.

3-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)propanoic Acid (15). Yield 0.542 g (88%), mp 149 °C dec. FT-IR (UATR diamond) ν_{max} 3066.41, 3014.57, 2980.94, 2934.81, 1745.47, 1724.84, 1667.40, 1651.21, 1644.45, 1589.56, 1573.68, 1511.50, 1417.25, 1385.74, 1332.89, 1279.43, 1239.20, 1162.90, 1081.52, 707.49 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H, OH), 10.90 (s, 1H, NH), 8.42–8.40 (m, $1H_{Ar}$), 8.14–8.12 (m, $2H_{Ar}$), 8.10–8.09 (m, $1H_{Ar}$), 8.04 (d, J = 8.4 Hz, $1H_{Ar}$), 7.87 (t, J = 8.0 Hz, $2H_{Ar}$), 3.43–3.42 (m, 2H, CH_2), 2.84 (t, J = 7.0 Hz, 2H, CH_2), 2.59 (t, J = 7.0 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.77, 181.70, 173.32, 169.56 (C=O), 144.95, 134.95, 134.62, 134.52, 133.50, 133.47, 128.87, 128.41, 127.13, 127.05, 124.20, 116.27 (C_{Ar}), 35.98, 34.51, 27.54 (CH_2). LC-MS, *m/z* (I_{rel}): 370 (M+H, 100). Anal. Calcd for $C_{19}H_{15}NO_5S$: C, 61.78; H, 4.09; N, 3.79; S, 8.68. Found: C, 61.71; H, 4.10; N, 3.80; S, 8.72.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)succinic Acid (16). Yield 0.386 g (56%), mp 185 °C dec. FT-IR (UATR diamond) ν_{max} 3335.94, 3008.21, 2786.81, 1717.18, 1675.41, 1589.10, 1543.68, 1468.67, 1417.92, 1339.34, 1299.14, 1235.29, 1177.40, 711.15 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.81 (br. s, 1H, OH), 12.51 (br. s, 1H, OH), 10.98 (s, 1H, NH), 8.47 (d, J = 8.1 Hz, $1H_{Ar}$), 8.16–8.08 (m, $3H_{Ar}$), 7.97–7.94 (m, $1H_{Ar}$), 7.83–7.76 (m, $2H_{Ar}$), 3.91–3.89 (m, 1H, CH), 3.69–3.58 (d, J = 7.6 Hz, 2H, CH_2), 2.70–2.59 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.18, 181.22, 171.68, 171.29, 168.72 (C=O), 142.77, 133.92, 133.44, 133.35, 132.62, 127.58, 127.11, 123.53, 116.01 (C_{Ar}), 42.30 (CH), 36.17, 34.78 (CH_2). LC-MS, *m/z* (I_{rel}): 414 (M+H, 100). Anal. Calcd for $C_{20}H_{15}NO_7S$: C, 58.11; H, 3.66; N, 3.39; S, 7.76. Found: C, 58.18; H, 3.59; N, 3.27; S, 7.70.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)benzoic Acid (17). Yield 0.557

g (80%), mp 237 °C dec. FT-IR (UATR diamond) ν_{max} 3568.41, 3506.01, 3244.18, 3173.85. 3101.99, 3061.49, 2931.53, 2882.55, 1671.01, 1643.70, 1590.20, 1576.63, 1541.84, 1466.10, 1331.88, 1299.86, 1259.74, 1159.88, 1119.05, 710.97 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.73 (br. s, 1H, OH), 10.94 (s, 1H, NH), 8.37–8.32 (m, $1H_{Ar}$), 8.13–8.04 (m, $2H_{Ar}$), 7.98 (d, J = 8.7 Hz, $2H_{Ar}$), 7.92 (d, J = 7.7 Hz, $2H_{Ar}$), 7.87–7.80 (m, $2H_{Ar}$), 7.57–7.52 (m, $2H_{Ar}$), 7.24 (t, J = 6.6 Hz, $1H_{Ar}$), 3.96–3.93 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.25, 181.20, 167.88, 167.45 (C=O), 144.24, 140.26, 134.47, 134.13, 134.03, 133.01, 132.97, 132.52, 131.02, 128.44, 128.05, 127.91, 126.65, 126.58, 125.74, 124.40, 123.75, 115.83 (C_{Ar}), 36.71 (CH_2). LC-MS, m/z (I_{rel}): 418 (M+H, 100). Anal. Calcd for $C_{23}H_{15}NO_5S$: C, 66.18; H, 3.62; N, 3.36; S, 7.68. Found: C, 66.05; H, 3.65; N, 3.44; S, 7.84.

2-((2-((9,10-Dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)nicotinic Acid (18). Yield 0.523 g (75%), mp 180 °C dec. FT-IR (UATR diamond) ν_{max} 3588.77, 3528.34, 3241.81, 3174.17, 3102.68, 3000.36, 2636.05, 1670.57, 1648.96, 1632.10, 1590.54, 1575.51, 1556.74, 1543.53, 1419.68, 1389.13, 1331.30, 1301.77, 1252.08, 1233.62, 1156.54, 1129.87, 1070.10, 710.97 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 12.60 (br. s, 1H, OH), 10.90 (s, 1H, NH), 8.57 (s, $1H_{Ar}$), 8.37 (s, $1H_{Ar}$), 8.23 (d, J = 7.1 Hz, $1H_{Ar}$), 8.10–7.99 (m, $5H_{Ar}$), 7.83 (s, $2H_{Ar}$), 7.26–7.21 (m, $1H_{Ar}$), 4.09 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.71, 181.59, 168.67, 166.96 (C=O), 160.38, 152.02, 145.04, 139.45, 134.84, 134.50, 134.45, 133.41, 128.81, 128.19, 127.05, 126.98, 124.42, 124.07, 119.67, 116.17 (C_{Ar}), 35.74 (CH_2). LC-MS, m/z (I_{rel}): 419 (M+H, 100). Anal. Calcd for $C_{22}H_{14}N_2O_5S$: C, 63.15; H, 3.37; N, 6.70; S, 7.66. Found: C, 63.09; H, 3.23; N, 6.78; S, 7.77.

***N*-(9,10-Dioxo-9,10-dihydroanthracen-2-yl)-2-((2-hydroxyethyl)thio)acetamide (19)**. Yield 0.484 g (85%), mp 187 °C dec. FT-IR (UATR diamond) ν_{max} 3589.84, 3516.18, 3296.49, 3203.80, 3102.15, 3064.54, 2957.96, 1719.69, 1675.77, 1656.88, 1644.88, 1589.23, 1574.78, 1548.17, 1425.65, 1352.14, 1332.17, 1302.35, 1226.20, 1192.26, 1167.14, 1151.73, 1133.81, 931.71, 712.54 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 10.70 (s, 1H, NH), 8.40–8.37 (m, $1H_{Ar}$), 8.14–8.12 (m, $2H_{Ar}$), 8.11–8.09 (m, $1H_{Ar}$), 8.01 (d, J = 8.4 Hz, $1H_{Ar}$), 7.87 (t, J = 7.6 Hz, $2H_{Ar}$), 4.88 (t, J = 5.3 Hz, 1H, OH), 3.60 (q, J = 6.1 Hz, 2H, CH_2), 3.40–3.39 (m, 2H, CH_2), 2.74 (t, J = 6.6 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.34, 181.25, 169.21 (C=O), 144.48, 134.51, 134.17, 134.10, 133.08, 133.05, 128.47, 127.99, 126.69, 126.61, 123.73, 123.65, 115.82, 115.74 (C_{Ar}), 60.44 (CH_2-OH), 35.90, 34.62 (CH_2). LC-MS, m/z (I_{rel}): 342 (M+H, 100). Anal. Calcd for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43; N, 4.10; S, 9.39. Found: C, 63.20; H, 4.30; N, 4.09; S, 9.29.

2-((2,3-Dihydroxypropyl)thio)-*N*-((9,10-dioxo-9,10-dihydroanthracen-2-yl) Acetamide (20). Yield 0.501 g (81%), mp 163 °C dec. FT-IR (UATR diamond) ν_{max} 3335.95, 3104.86, 3069.88, 2927.97, 1675.70, 1589.96, 1542.74, 1419.12, 1340.62, 1299.62, 1237.15, 933.59, 711.60 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ 11.06 (s, 1H, NH), 8.45 (d, J = 2.3 Hz, $1H_{Ar}$), 8.20–8.12 (m, $2H_{Ar}$), 8.04 (dd, J = 8.6, 2.3 Hz, $1H_{Ar}$), 7.91–7.86 (m, $3H_{Ar}$), 5.21 (dd, J = 19.7, 5.4 Hz, 1H, CH) 4.83 (t, J = 6.3 Hz, 1H, OH), 4.09 (t, J = 12.6 Hz, 1H, OH), 3.43 (d, J = 5.0 Hz, 2H, CH_2), 3.24–3.00 (m, 2H, CH_2), 2.99–2.77 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.65, 181.64, 165.00 (C=O), 144.34, 134.96, 134.63, 134.46, 133.41, 128.87, 128.68, 127.10, 127.03, 124.30, 116.38 (C_{Ar}), 66.76 (CH-OH), 59.23 (CH_2-OH), 36.27, 34.66 (CH_2). LC-MS, m/z (I_{rel}): 372 (M+H, 100). Anal. Calcd for $C_{19}H_{17}NO_5S$: C, 61.44; H, 4.61; N, 3.77; S, 8.63. Found: C, 61.32; H, 4.57; N, 3.69; S, 8.52.

2. 2. Antioxidant Activity

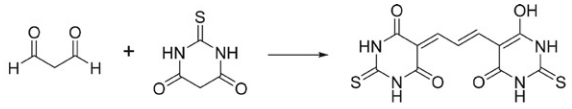
2. 2. 1 Method of Study of Lipid Peroxidation (LP) and Oxidative Modification of Proteins (OMP) of Thioether Acetamides

The LP and OMP processes study was performed *in vitro* on rat's liver homogenate according to the Lushchak's method.[38] The amount of protein in the sample was determined due to the Lowry method. This method is based on LP activation by ferrous iron ions to a level recorded spectrophotometrically by reaction with thiobarbituric acid. The degree of OMP was evaluated calculating the amount of additional CGs formed in the side chains of aminoacids under the reaction with 2,4-dinitrophenylhydrazine. The methanol solutions served as control, while the standard of measurement was 10^{-6} M solution of quercetin. Experimental data were analyzed considering the arithmetic mean M and standard error m in the form of ($M \pm m$), n = 5. Differences between experimental data were determined via Tukey's test of one-way analysis (ANOVA), and the differences were considered to be statistically significant at $P < 0.05$.[39]

At the beginning of our experiment, to 0.3 g of rat liver homogenate 0.3 mL methanol solutions of sulfur-containing derivatives of 2-chloro-*N*-(9,10-dioxo-9,10-dihydroanthracen-1(2)-yl)-acetamides (10^{-6} M concentration) was poured. Iron(II) sulfate solution (0.3 mL of 2.8%) was introduced to the obtained solution. The reaction of LP was induced within 10 minutes. Then 0.3 mL of 4% solution of hydrogen peroxide was added, and the solution was incubated for 2 hours. The reaction was stopped after introducing 1.2 mL of 40% trichloroacetic acid, precipitating polypeptides. Reaction mixture was centrifuged for 10 minutes at $5{\cdot}10^3$ RPM. Bioactive compounds' influence analyses on FRO were conducted for a single sample. Carbonyl groups were determined in the sediment, and products of lipids interaction with TBA were determined from the supernatant.

2. 2. 2. Determination of the Content of Products of Lipids Interaction with TBA in the Supernatant

1.5 mL of 0.8% TBA was added to the supernatant separated from the sediment. The reaction between TBA and MDA proceeds during the heating of this solution to 100 ºC for about 1 hour. The formation of the pinky colored complex allows determining the content of TBA-active products:



After cooling, 3 mL of butanol was added to the reaction mass and was left for 2 hours. After, the formation of two phases was observed. Determination of the extinction coefficient was determined from the butanol fraction at λ = 532 nm.

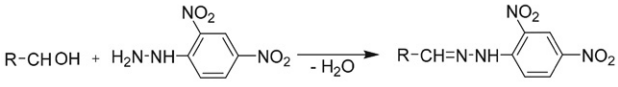
Calculation of TBA-active products was performed according to:

$$[\text{TBA} - \text{active products} = \frac{E \cdot V_1 \cdot V_2}{\varepsilon \cdot V \cdot C}, \ \mu\text{mol/mg of protein} \quad (1)$$

where E – extinction coefficient of the test sample; ε – millimolar extinction coefficient (ε = 156 $cm^2/\mu mol$); V_1 – a volume of butanol (mL); V_2 – sample volume (mL); V – supernatant volume (mL); C – protein concentration in the supernatant (μmol).

2. 2. 3. Determination of CGs Proteins in the Precipitate

To the formed precipitate 1 mL of 1% 2,4-dinitrophenylhydrazine solution was added.



The resulting solution was incubated for an hour, then centrifuged at $5 \cdot 10^3$ RPM for 10 minutes. The precipitate was washed three times with the addition of 1 mL of ethanol-ethyl acetate solution (1:1) and centrifuged. Then 3 mL of 50% urea solution was added to the precipitate, centrifuged, and the additional CG was determined using a spectrophotometer (λ = 370 nm).

2. 3. Anti-platelet Activity

2. 3. 1. Preparation of Rabbit Platelet-rich Plasma

Platelet-rich plasma (PRP) was obtained from at least 3 different healthy adult rabbits. The Scientific Ethics Committee of Taras Shevchenko National University of Kyiv, Ukraine approved the study protocol.

Rabbit blood was collected from the ear artery into a polyethylene tube with 3.8% sodium citrate in the ratio 9:1. PRP was obtained by centrifugation of stabilized blood at 300 g for 10 min. The supernatant (PRP) was carefully separated and used further in the aggregation assay. Platelet-pure plasma (PPP) was prepared by further centrifugation of the remained stabilized blood at 1,500 g for 30 min.

2. 3. 2. Platelet Aggregation Assay

Platelet aggregation was assessed within the first 3 h after blood sampling using photo-optical aggregometer AT-02 (Medtech, Russia). Before the assessment, the platelet count in PRP was adjusted with PPP to about 230×10^3–250×10^3 cells/μL.

The studied compounds were dissolved in pure dimethyl sulfoxide (DMSO) and their test concentrations were prepared using distilled water. The final DMSO concentration in all experiments was fixed at 1% to minimize the effect of DMSO on the platelet aggregation.

Primary screening for anti-aggregation activity of compounds **3**–**20** was performed *in vitro*: PRP was incubated with studied compounds (final concentration was 50 μM) in a cuvette for 2 min at 37 ºC with constant stirring (500 rpm). PRP incubated with DMSO alone was used as a control. ADP (Sigma, USA) in the final concentration of 5×10^{-6} M was added to the sample, and the change of light transmission was monitored for 8 minutes ADP to induce platelet aggregation. In this experiment the level of spontaneous aggregation induced by addition of the tested compounds to PRP was studied. The degree of platelet aggregation (the maximal level of light transmission of PRP after addition of inducer) was evaluated. The degree of inhibition of ADP-dependent aggregation under the action of obtained derivatives **3**–**20** relative to control, which was taken as 100%, was calculated.

3. Results and Discussion

3. 1. Synthesis of *N*-(9,10-Dioxo-9,10-dihydroanthracen-1(2)-yl)-2-(R-thio) Acetamides

In continuation of the previous studies of our group on the functionalization of 9,10-anthracenedione,[36,37,40–46] a structural modification of 2-chloro-*N*-(9,10-dioxo-9,10-dihydroanthracene-1(2)-yl)acetamides **1**, **2** by pharmacophore fragments, namely, sulfur-containing substituents was carried out. In this work, the reaction of chloroacetamides **1**, **2** with a number of alkyl(aryl/hetaroaryl)thiols, additionally functionalised with mercapto, hydroxy, carboxy and carboxylate groups was carried out (Scheme 1). It can be a key aspect for improving biological activity, including antiplatelet and antioxidant actions, as well as bioavailability due to an increase in the polarity of the obtained compounds and better water solubility.

Modification of chloroacetamides **1**, **2** with the corresponding thiols was carried out using their interaction at

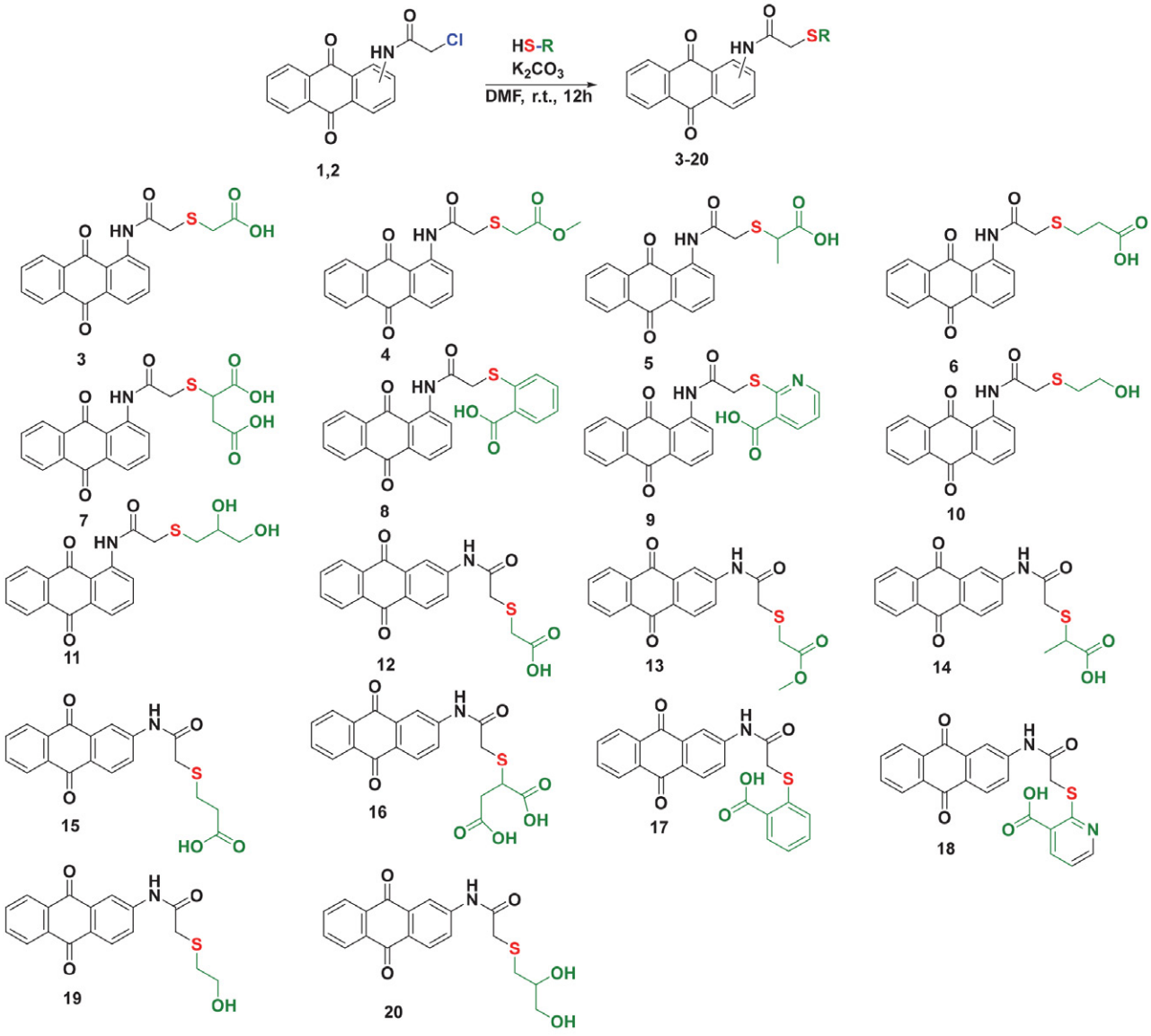
room temperature in the presence of potassium carbonate in DMF (Scheme 1). As a result, the corresponding sulfide derivatives **3**–**20** were obtained in good and high yields of up to 93%.

The structures of new thioether acetamides **3**–**20** were reliably confirmed by modern physicochemical analysis methods, namely ^{1}H and ^{13}C NMR, LC-MS, IR-Fourier spectroscopy and elemental analysis. In particular, the secondary amino group resonates at 12.41–12.62 ppm for sulfides **3**–**11**, and in the case of sulfides **12**–**20** at 10.69–10.94 ppm in the ^{1}H NMR spectra.

In turn, the methylene group of the oxoethyl fragment of acetamide in ^{1}H NMR observed for compounds **3**–**7**, **10**, **11** at 3.59–3.76 ppm, and for compounds **12**–**16**, **19**, **20** at 3.40–3.56 ppm. In the case of the aromatic thioether substituent of **8**, **9**, **17**, **18**, the CH_2 group shifts downfield and resonates within 3.94–4.16. In ^{13}C NMR spectra, the appearance of a signal of the carbon atom of the carboxyl group at 167.88–173.84 ppm is characteristic for sulfide derivatives **4**, **6**–**9**, **12**, **14**–**18**. For sulfide derivatives **9** and **18** containing a fragment of nicotinic acid in their structures, the appearance of signals of the quaternary carbon atom of the N-C-S group of the nicotine fragment was determined at 159.59 and 160.38 ppm, respectively.

3. 2. Antioxidant Properties

The free-radical oxidation (FRO) research was conducted *in vitro* using a rat liver homogenate. LP and OMP as two markers of oxidative stress were used for the evaluation of antioxidant properties of the compounds.



Scheme 1. Synthesis of *N*-(9,10-dioxo-9,10-dihydroanthracene-1(2)-yl)-2-(R-thio) acetamides **3**–**20**

This method is based on the LP activation by ferrous ions to a level registered spectrometrically by reaction with thiobarbituric acid (TBA-active products content). The degree of OMP was determined by the amount of additional carbonyl groups formed (CG content) in the side chains of amino acids using the reaction with 2,4-diphenylhydrazine.

The results of this investigation, in particular the content of TBA-active products and CGs, found from a comparison of isomers (compounds where the substituent is introduced in position 1 or 2). Data are presented in Figures 1 and 2. All compounds were compared with control, as well as with reference antioxidant quercetin. Quercetin in the LP processes acted as a control, and in the processes of OMP it showed antioxidant properties, reducing the level of CG relative to the control by 41.5%.

The comparison antioxidant activity of isomers **3**–**11** and **12**–**20** are as follows. Compound **8** contained the residue of 2-((2-amino-2-oxoethyl)thio)benzoic acid in the position 1, showed minor antioxidant properties (8.3% content of TBA-active products) compared to the control as presented in Figure 1. The derivative **17** increased the content of TBA-active products by 8.6%, i.e., it had prooxidant properties relative to control. The compounds **8** and **17** demonstrated antioxidant effect in OMP processes decreasing the CGs level by 15.7% and 26.4%, respectively. The compound **17** contained the residue of 2-((2-amino-2-oxoethyl)thio)benzoic acid in the position 2, decreased the level of CGs by 10.7% more than the derivative **8** in the free radical processes of protein oxidation. Therefore, 2-((2-((9,10-dioxo-9,10-dihydroanthracen-1-yl)amino)-2-oxoethyl)thio)benzoic acid **8** exhibited antioxidant properties on two FRO markers.

The compounds **10** and **19** contained 2-((2-hydroxyethyl)thio)acetamide residue in positions 1 or 2 of anthracenedione ring demonstrated similar results. In this case, derivative **10** reduced the content of TBA-active products relative to control slightly, namely by 8.7%. Compound **19** showed high prooxidant properties and significantly (by 41.0%) increased the content of TBA-active products compared to control (Fig. 1). In contrast, compound **19** showed better results on OMP processes than compound

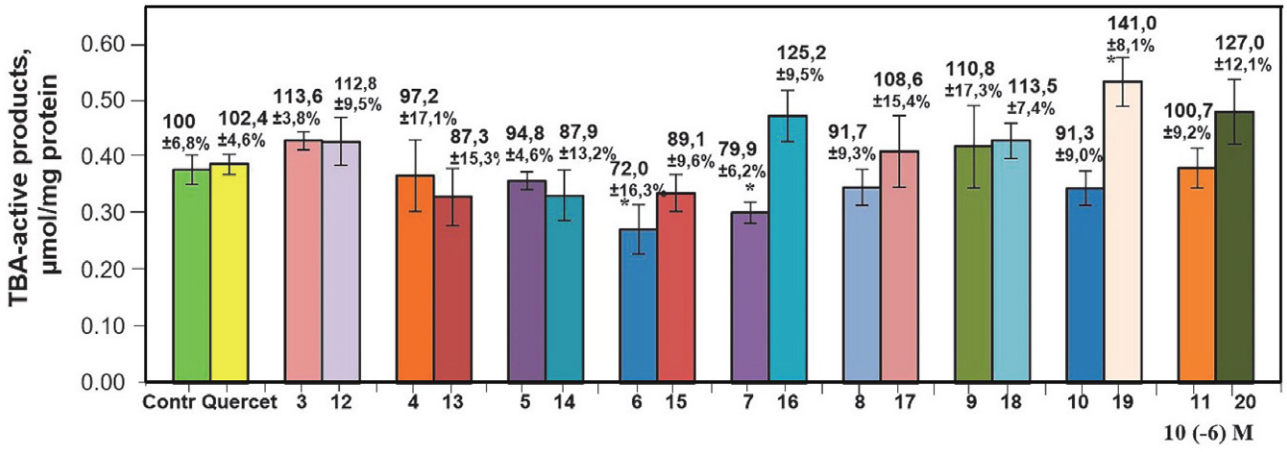


Figure 1. Influence of compounds **3**–**20** on amount of TBA-active products in rat liver homogenate (*- $p \le 0,05$; $M \pm m$; $n = 5$)

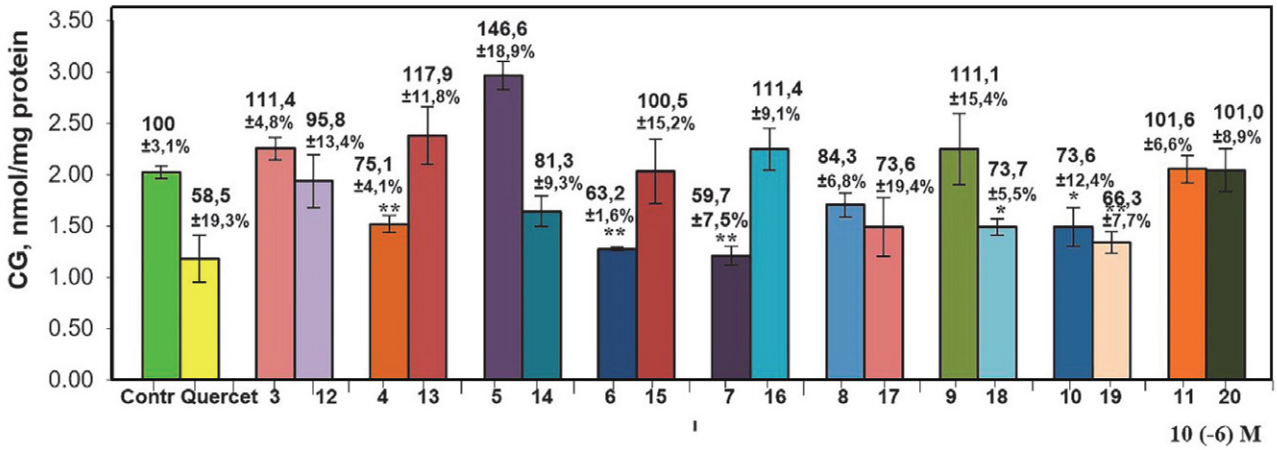


Figure 2. Influence of compounds **3**–**20** on amount of CGs of proteins in rat liver homogenate (*- $p \le 0,05$; **- $p \le 0,01$; $M \pm m$; $n = 5$)

10 and reduced the CG content by 33.7% compared to the control (Fig. 2). In turn, derivative **10** also exhibited antioxidant properties in OMP processes, reducing the level of CG proteins relative to control by 24.4%.

Moreover, compounds **6** and **15** show antioxidant activity in LP processes (Fig. 1). Derivative **6** reduces the content of TBA-active products by 28.0% relative to the control ($p \leq 0.05$), whereas derivative **15** decreases the value by 10.1%. The studied compound **6** statistically significantly ($p \leq 0.01$) decreases the formation of CGs in proteins in FRP of OMP processes by 37.8% relative to the control (Fig. 2). Meanwhile, the content of CGs under the action of compound **15** shows no difference to the control with a value at 100.5 ± 15.2%.

Comparing compounds, which contain in anthracenedione nuclei the residue of succinic acid in the first (**7**) or second (**16**) position, demonstrates that compound **7** has antioxidant properties to lipids and lowers the content of TBA-active products by 21.1% (Fig. 1). Compound **16**, in contrast to compound **7**, exhibits pro-oxidant properties and increases the content of TBA-active products by 25.2% relative to control. In addition, compound **7** (Fig. 2) shows antioxidant effect in OMP processes and statistically significantly ($p \leq 0.01$) decreases the level of CGs proteins by 40.3% compared to the control. In turn, 2-((2-((9,10-dioxo-9,10-dihydroanthracen-2-yl)amino)-2-oxoethyl)thio)succinic acid **16** increases the CGs content by 11.3%.

The LP study of compound **11**, which contains (dihydroxypropyl)thio)acetamide residue in the structure, has not shown antioxidant properties due to no difference in the TBA-active products content to the control (Fig. 1). Compound **20** with the ((dihydroxypropyl)thio)acetamide residue in the structure at position 2 of anthracenedione exhibits prooxidant properties and increases the content of the TBA-active product by 27.0% relative to control (Fig. 1). Compounds **11** and **20**, based on the results of the study of OMP (Fig. 2), showed that the content of protein CGs does not differ from the control. Such contents for derivatives **11** and **20** are 101.6 ± 6.6% and 101.0 ± 8.9%, respectively. Thus, these compounds are neutral to the oxidation process.

In turn, compounds **3** and **12** almost equally increase the content of TBA-active products compared to the control, namely, by 13.6% and 12.8%, respectively (Fig. 1). The compound **3** in the OMP processes, as well as for LP, demonstrates a prooxidant effect due to an increase of CGs for 11.4% compared to the control (Fig. 2). In contrast, compound **12** has shown minor antioxidant properties and decreased CGs by 4.2%.

Compound **4**, due to the results of LP investigation, has the same effect as the control (Fig. 1), decreasing the level of TBA-active products only by 2.8%. Compound **13**, which has in structure the aminooxoethylthioacetate residue at the second position of anthraquinone, showed better antioxidant properties compared to its isomer **4** and reduced the content of TBA-active products relative to control by 12.8%. Furthermore, compound **4**, according to the results of OMP (Fig. 2), exhibits antioxidant properties and reduces the content of CGs by 24.9% in comparison with the control. Moreover, compound **13** shows the opposite effect due to the increasing content of CGs relative to the control by 17.9%, which indicates an increase in free radical processes in the proteins (Fig. 2).

Hence, summarizing the obtained results, the studied compounds with a substituent in the first position of anthracenedione fragment exhibit higher antioxidant properties than their isomers with a substituent in the second position. Compounds with a substitution in the first position **6**, **7**, **8** and **10** demonstrate the antioxidant properties concerning oxidative stress markers POL and OMP. Moreover, compound **6** reduces the content of TBA-active products by 28.0% and content of CGs by 36.8%, whereas derivative **7** decreases by 21.1% and 41.3%, respectively. Test compounds **4**, **10**, **17**, **19**, as well as quercetin, showed antioxidant properties only in OMP processes.

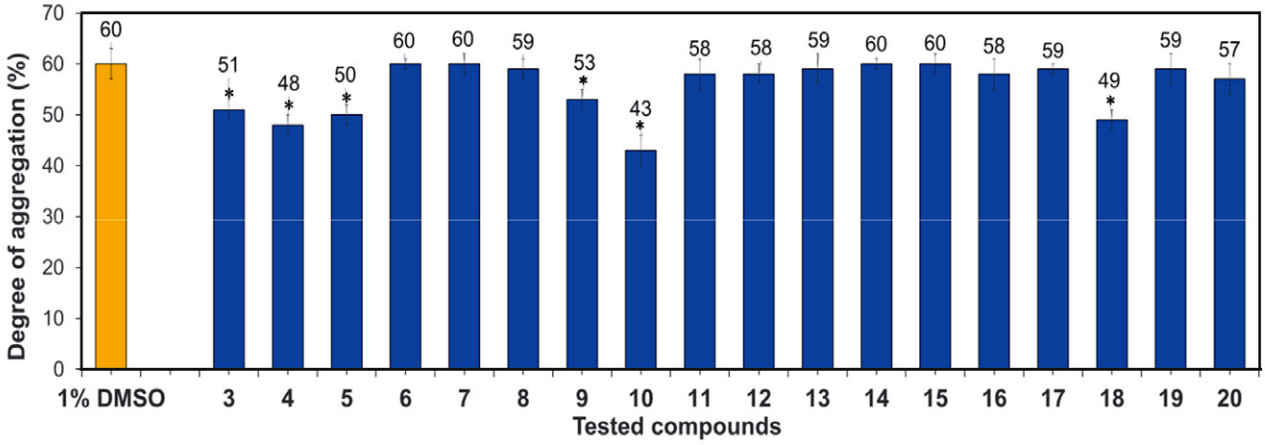


Figure 3. Effect of derivatives **3**–**20** at a concentration of 50 μM on ADP-induced platelet aggregation in rabbit PRP (M ± SEM; $n = 6$, * $p \leq 0.05$ changes are statistically significant compared to the control 1% DMSO)

3. 3. Antiplatelet Activity

Antiplatelet activity of derivatives **3**–**20** was studied *in vitro* using rabbit PRP. As can be seen from the results (Fig. 3), among the 20 tested compounds, only six (**3**, **4**, **5**, **9**, **10**, **18**) showed moderate antiplatelet activity.

The most active compound, namely compound **10**, inhibited ADP-induced aggregation by 28%, while the inhibitory effect of others ranged from 12% to 20%. The latter is associated with the structure of the thio fragment and the inhibitory effect is characteristic for five compounds (**3**, **4**, **5**, **9**, **10**), and increases in the following order of substituents (Fig. 4):

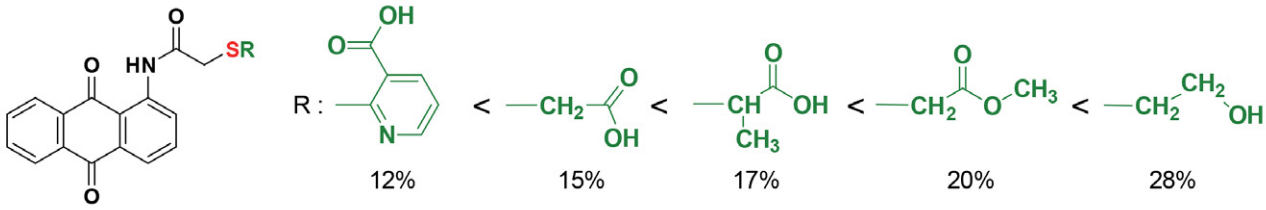


Figure 4. Correlation of substituent of the thio fragment and the inhibitory effect for five compounds **3**, **4**, **5**, **9**, **10**

Analysis of the influence of the structure of the thio fragment on the manifestation of the antiplatelet activity of compounds **3**–**20** showed that anthracenedione derivatives containing this residue in the first position of the anthracenedione ring (**3**, **4**, **5**, **9**, **10**) can inhibit platelet aggregation. It was found that the presence of a less branched 2-((2-hydroxyethyl)thio)acetamide residue (compound **10**) causes the highest percentage of the degree of inhibition. At the same time, derivatives **12**–**20** with a thio substituent in position 2 of the anthracenedione nucleus and compounds **6**–**8**, **11** with branched and bulky substituents near the sulfur atom do not affect the degree of inhibition.

4. Conclusions

A convenient way to obtain new sulfide derivatives with a 9,10-anthracenedione ring has been proposed. It includes the interaction of 2-chloro-*N*-(9,10-dioxo-9,10-dihydroanthracen-1(2)-yl)-acetamides **1**, **2** with a number of alkyl(aryl/hetaroaryl)thiols at room temperature in the presence of potassium carbonate in DMF. The study of antioxidant activity in terms of lipid peroxidation and oxidative modification of proteins in rat liver homogenate *in vitro* identified compounds **6** and **7** with the best properties of radical scavenging activity in terms of the content of TBA-active products and CGs in the corresponding range 21,1–28 % and 36.8–41.3%. An *in vitro* study of the antiplatelet activity using rabbit PRP revealed derivative **10**, exhibiting the highest degree of inhibition of platelet aggregation among the synthesized compounds. Sulfide derivative **10** also demonstrated antioxidant properties in OMP processes, which manifested in lowering CGs protein level compared to control for 24.4%. The structure-activity relationships for the obtained *N*-(9,10-dioxo-9,10-dihydroanthracene-1(2)-yl)-2-(R-thio) acetamides were determined. The data obtained are the basis for further studies on molecular design and the search for new compounds with antioxidant and antiplatelet activity in a series of new derivatives of 9,10-anthracenedione.

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Supplementary Data

^{1}H and ^{13}C NMR spectra of compounds **3**–**20** are provided in supplementary material via the "Supplementary Content" section of this article's webpage.

Conflicts of Interest

The authors declare no conflict of interest.

5. References

1. H. Ten Cate, *Front Cardiovasc. Med*. **2021**, *8*, 637005. **DOI:**10.3389/fcvm.2021.637005
2. A. E. A. Dahm, *Med. Sci. (Basel)*. **2021**, *9*, 41. **DOI:**10.3390/medsci9020041
3. L. Gervaso, H. Dave, A. A. Khorana, *JACC CardioOncol.* **2021**, *3*, 173–190. **DOI:**10.1016/j.jaccao.2021.03.001
4. E. Grilz, F. Posch, S. Nopp, O. Königsbrügge, I. M. Lang, P. Klimek, S. Thurner, I. Pabinger, C. Ay, *Eur. Heart J.* **2021**, *42*, 2299–2307. **DOI:**10.1093/eurheartj/ehab171
5. J. Gregson, S. Kaptoge, T. Bolton, L. Pennells, P. Willeit, S. Burgess, T. Meade, *JAMA Cardiology.* **2019**, *4*, 163–173. **DOI:**10.1001/jamacardio.2018.4537
6. J. Hippisley-Cox, M. Patone, X. W. Mei, D. Saatci, S. Dixon, K. Khunti, *BMJ* **2021**, *374*, n1931. **DOI:**10.1136/bmj.n1931
7. K. Stark, S. Massberg, *Nat. Rev. Cardiol.* **2021**, *18,* 666–682. **DOI:**10.1038/s41569-021-00552-1

8. E. M. Page, R. A. S. Ariëns, *Thromb. Res*. **2021**, *200*, 1–8. **DOI:**10.1016/j.thromres.2021.01.005
9. H. Y. Lin, C. Y. Lin, M. C. Shen, *Thrombosis J.* **2021**, *19,* 43. **DOI:**10.1186/s12959-021-00296-5
10. C. Jerjes-Sánchez (Ed.): Mechanisms of Thrombosis. In Thrombolysis in Pulmonary Embolism, Springer, Cham, Switzerland, **2015**, pp. 1–17. **DOI:**10.1007/978-3-319-19707-4
11. D. Shoichiro, *Ther. Apher. Dial*. **2019**, *23*, 32–37. **DOI:**10.1111/1744-9987.12744
12. M. Martín-Fernández, R. Aller, M. Heredia-Rodríguez, E. Gómez-Sánchez, P. Martínez-Paz, H. Gonzalo-Benito, L. Sánchez-de Prada, Ó. Gorgojo, I. Carnicero-Frutos, E. Tamayo, Á. Tamayo-Velasco, *Redox Biol*. **2021**, *48*, 102181. **DOI:**10.1016/j.redox.2021.102181
13. L. Liao, M. Zhou, J. Wang, X. Xue, Y. Deng, X. Zhao, C. Peng, Y. Li, *Front Pharmacol*. **2021**, *12*, 742954. **DOI:**10.3389/fphar.2021.742954
14. K. Wang, T. Shang, L. Zhang, L. Zhou, C. Liu, Y. Fu, Y. Zhao, X. Li, J. Wang, *ACS Appl. Mater. Interfaces*. **2021**, *13*, 35431–35443. **DOI:**10.1021/acsami.1c08880
15. J. Flieger, W. Flieger, J. Baj, R. Maciejewski, *Materials (Basel)*. **2021**, *14*, 4135. **DOI:**10.3390/ma14154135
16. A. Varesi, S. Chirumbolo, G. Ricevuti, *Intern. Emerg. Med.* **2021**, 1–4. **DOI:**10.1007/s11739-021-02865-y
17. K. R. Olson, Y. Gao, K. D. Straub, *Int. J. Mol. Sci*. **2021**, *22*, 961. **DOI:**10.3390/ijms22020961
18. N. Q. Trung, N. M. Thong, D. H. Cuong, T. D. Manh, L. P. Hoang, N. K. Hien, P. C. Nam, D. T. Quang, A. Mechler, Q. V. Vo, *ACS Omega*, **2021**, *6*, 13391–13397. **DOI:**10.1021/acsomega.1c01448
19. G. Greco, E. Turrini, E. Catanzaro, C. Fimognari, *Mar. Drugs.* **2021**, *19*, 272. **DOI:**10.3390/md19050272
20. J. A. Duke (Ed.): Handbook of phytochemical constituents of GRAS herbs and other economic plants, CRC Press, Boca Raton, **2001**, pp. 143–144. **DOI:**10.1201/9780203752623
21. N. M. Storozhok, A. Drulle, I. Login, I. Dregeris, N. G. Khrapova, E. B. Burlakova, *Vopr. Med. Khim.* **1995**, *41*, 16–21.
22. G. Yen, P. Duh, D. Chuang, *Food Chem*. **2000**, *70*, 437–441. **DOI:**10.1016/S0308-8146(00)00108-4
23. Z. Marković, M. Filipović, N. Manojlović, A. Amić, S. Jeremić, D. Milenković, *Chem. Pap.* **2018**, *72*, 2785–2793. **DOI:**10.1007/s11696-018-0534-3
24. N. Liu, G. Sun, *Ind. Eng. Chem. Res.* **2011**, *50*, 5326–5333. **DOI:**10.1021/ie101423v
25. L. Lin, H. Du, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2018**, *202*, 314–318. **DOI:**10.1016/j.saa.2018.05.058
26. Z. Marković, S. Jeremić, J. Dimitrić Marković, M. Stanojević Pirković, D. Amić, *Comput. Theor. Chem.* **2016**, *1077*, 25–31. **DOI:**10.1016/j.comptc.2015.10.004
27. V. Zvarych, M. Stasevych, V. Lunin, N. G. Deniz, C. Sayil, M. Ozyurek, K. Guclu, M. Vovk, V. Novikov, *Monatsh. Chem.* **2016**, *147*, 2093–2101. **DOI:**10.1007/s00706-016-1839-y
28. M. Stasevych, V. Zvarych, V. Lunin, N. Kopak, O. Komarovska-Porokhnyavets, N.G. Deniz, C. Sayil, M. Ozyurek, K. Guclu, M. Vovk, V. Novikov, *Monatsh. Chem.* **2018**, *149*, 1111–1119. **DOI:**10.1007/s00706-018-2157-3
29. A. Kaur, S. Kaur, M. Kaur, A. Mahajan, S. Bose, *World J. Pharm. Res.* **2014**, *4*, 1892–1902. **DOI:**10.2174/2210303113036660017
30. Z. Memariani, R. Moeini, S. Hamedi, G. Narjes, S. Mozaffarpur, *J. Thromb. Thrombolysis.* **2018**, *45*, 158–179. **DOI:**10.1007/s11239-017-1580-3
31. A. Gouda, M. S. Amine, E. B. Pedersen, *Helv. Chim. Acta*. **2016**, *99*, 116–124. **DOI:**10.1002/hlca.201500207
32. V. Zvarych, M. Stasevych, V. Lunin, N. G. Deniz, C. Sayil, M. Ozyurek, K. Guclu, M. Vovk, V. Novikov, *Monatsh. Chem.* **2016**, *147*, 2093–2101. **DOI:**10.1007/s00706-016-1839-y
33. T. I. Halenova, I. V. Nikolaeva, M. V. Stasevych, V. I. Zvarych, V. V. Lunin, V. P. Novikov, O. M. Savchuk, *Res. J. Pharm. Biol. Chem. Sci*. **2017**, *8*, 1626–1632
34. T. Halenova, I. Nikolaeva, A. Nakonechna, V. Lubenets, *Res. Pract. Thromb. Haemost*. **2017**, *1*, 1276–1277. **DOI:**10.1002/rth2.12012
35. W. L. F. Armarego, C. Chai, Purification of Laboratory Chemicals, 4th ed., Elsevier, Oxford, **2003**, pp. 8–13. **DOI:**10.1016/B978-075067571-0/50003-X
36. M. V. Stasevych, V. I. Zvarych, V. P. Novikov, *Biointerface Res. Appl. Chem.* **2021**, *11*, 8818–8824. **DOI:**10.33263/BRIAC112.88188824
37. V. I. Lushchak, T. V. Bagnyukova, L. I. Luzhna, *Ukr. Biochem. J.* **2006**, *78*, 113–119.
38. G. A. Morgan, N. L. Leech, G. W. Gloeckner, K. C. Barrett, IBM SPSS for Introductory Statistics. Use and Interpretation, 4th ed., Taylor & Francis Group, New York, USA, **2012**, p. 256. **DOI:**10.4324/9780203127315
39. V. I. Zvarich, M. V. Stasevich, O. V. Stanko, E. Z. Komarovskaya-Porokhnyavets, V. V. Poroikov, A. V. Rudik, V. P. Novikov, *Pharm. Chem. J.* **2014**, *48*, 584–588. **DOI:**10.1007/s11094-014-1154-z
40. M. V. Stasevych, V. I. Zvarych, V. P. Novikov, M. V. Vovk, *Biointerface Res. Appl. Chem.* **2021**, *11*, 7725–7734. **DOI:**10.33263/BRIAC111.77257734
41. V. Zvarych, M. Stasevych, V. Novikov, E. Rusanov, M. Vovk, P. Szweda, K. Grecka, S. Milewski, *Molecules.* **2019**, *24*, 4581. **DOI:**10.3390/molecules24244581
42. V. I. Zvarych, M. V. Stasevych, V. V. Lunin, M. V. Vovk, V. P. Novikov, *Chem. Heterocycl. Compd*. **2016**, *52,* 421–423. **DOI:**10.1007/s10593-016-1904-9
43. T. Strobel, Y. Schmidt, A. Linnenbrink, A. Luzhetskyy, M. Luzhetska, T. Taguchi, E. Brötz, T. Paululat, M. Stasevych, O. Stanko, V. Novikov, A. Bechthold, *Appl. Environ. Microbiol*. **2013**, *79*, 5224–32. **DOI:**10.1128/AEM.01652-13
44. M. V. Stasevich, V. I. Zvarich, V. P. Novikov, S. D. Zagorodnya, O. Yu. Povnitsa, M. A. Chaika, M. V. Nesterkina, I. A. Kravchenko, D. S. Druzhilovskiy, V. V. Poroikov, *Pharm. Chem. J*. **2020**, *53*, 905–913. **DOI:**10.1007/s11094-020-02098-x
45. M. Stasevych, V. Zvarych, R. Musyanovych, V. Novikov, M. Vovk, *Chem. Chem. Technol*. **2014**, *8*, 135–140. **DOI:**10.23939/chcht08.02.135

46. M. V. Stasevych, M. Yu. Plotnikov, M. O. Platonov, S. I. Sabat, R. Ya. Musyanovych, V. P. Novikov, *Heteroatom Chem*. **2005**, *16*, 205–211. **DOI:**10.1002/hc.20112

Povzetek

S pomočjo reakcije 2-kloro-*N*-(9,10-diokso-9,10-dihidroantracen-1(2)-il)acetamidov s funkcionaliziranimi tioli v prisotnosti kalijevega karbonata v *N,N*-dimetilformamidu (DMF) pri sobni temperaturi smo izvedli sintezo serije novih *N*-(9,10-diokso-9,10-dihidroantracen-1(2)-il)-2-(R-tio) acetamidov. Za nove spojine smo s pomočjo *in vitro* testov na osnovi lipidne peroksidaze (LP) in oksidativne modifikacije proteinov (OMP) v homogenizatu jeter podgan določili sposobnost delovanja v vlogi lovilcev radikalov. Ugotovili smo, da spojine, ki imajo vezane substituente na položaju 1 v antracendionskem skeletu, kažejo boljše antioksidacijske lastnosti kot njihovi izomeri s substituenti na položaju 2. Kot najbolj učinkovita lovilca radikalov sta se izkazali spojini **6** in **7**. Antioksidacijske lastnosti v OMP procesu smo določili tudi za spojino **10**; za to spojino smo izvedli tudi *in vitro* študijo delovanja proti agregaciji krvnih ploščic, kjer smo ugotovili inhibitorno delovanje na agregacijo, povzročeno z ADP.