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ORIGINAL PAPER

Synthesis and investigation of antimicrobial and antioxidant activity of anthraquinonylhydrazones

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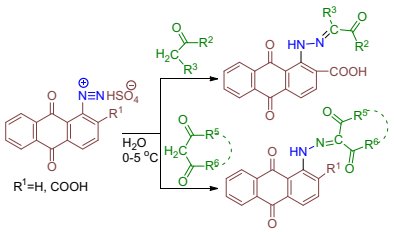
Abstract

The new anthraquinonylhydrazones were obtained by the interaction of 9,10-dioxoanthracene-1-diazonium sulfates with a number of α- and β-carbonyl-containing compounds under modified conditions of the Japp–Klingemann reaction, and a probable mechanism of the formation has been proposed. It was found that hydrazones, unsaturated in the second position of the anthraquinone ring, containing acetyl or ethoxycarbonyl moieties in the ylidene part of the molecule, are capable of eliminating these fragments. It has been experimentally established that hydrazones, free rotation around the N=C bond of which is possible, exist as one isomer due to the presence of an intramolecular hydrogen bond in the molecule. The anthraquinonylhydrazone of dimedone with action against the bacteria strains of *Staphylococcus aureus 209-P*, *Mycobacterium luteum B-917,* and fungus *Candida tenuis VKM Y-70* was found. The hydrazones of dimedone and barbituric acid with a higher trolox equivalent antioxidant coefficients of antioxidant action were found using CUPRAC assay. In addition, the hydrazones of dimedone and barbituric acid exhibited better activity against catalase enzyme. Correlations between the structure of the synthesized hydrazones and their antioxidant activity have been defined.

Graphical abstract



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Keywords 9,10-Dioxoanthracene-1-diazonium sulfates · Hydrazones · Antimicrobial activity · Antioxidant activity · TEAC · Catalase enzyme activity

Introduction

Hydrazones are valuable objects in the organic synthesis due to their ability to interact with electrophile and nucleophile reagents [1]. Compounds with anti-inflammatory, analgesic, anticonvulsant, antituberculosis, antitumor, anti-HIV, antimicrobial, and other activity have been found among them [2].

The synthesis of hydrazones based on anthrone or its derivatives is presented in works [3–5]. In addition, the arylation of phenylacetylene and 2-naphthol with diazonium salts of 9,10-anthracenedione was studied, and several representatives of hydrazones were obtained [6]. Until recently, the interaction of diazonium salts of 9,10-anthracenedione with methylene-active compounds was represented by the synthesis of only one derivative, an intermediate, anthraquinonylhydrazone of ethyl pyruvate [7], obtained by the reaction of the corresponding 2-diazonium chloride 9,10-anthracenedione with ethyl 2-methylacetoacetate under classical conditions of the Japp–Klingemann reaction. At the same time, the anthraquinonylhydrazones of ketones and aldehydes are not described, that could possibly due to the tendency of (anthraquinone-1-yl)hydrazine to an intramolecular cyclocondensation [8].

Previously [9], we proposed an effective way of obtaining of anthraquinonylhydrazones **4a**–**4e** (Scheme 1) containing acyl and/or alkoxycarbonyl moieties in the ylidene part of the molecule, which are convenient for further chemical transformations [10].

Taking into account the limited number of publications on the synthesis and transformation of hydrazones that contain the 9,10-anthraquinone fragment in the hydrazone part, it seems promising to obtain new derivatives by the coupling reaction of 9,10-dioxoanthracene-1-diazonium sulfates with α- and *β*-carbonyl-containing compounds for further chemical conversions to obtain biologically active substances.

Results and discussion

Chemistry

Continuing research in a direction of the synthesis and modification of anthraquinonylhydrazones, in this paper, we reported a synthesis of new hydrazones by the interaction of freshly prepared diazonium salt **2** obtained by the method of [11] with acetone (**3a**), ethylmethyl ketone (**3b**), acetylacetone (**3c**), malonic esters **3d**, and acetoacetate **3e** esters under the modified conditions of the Japp–Klingemann reaction in water (Scheme 1).

The formation of hydrazones **4a**–**4e** and **5a**–**5e** probably occurs according to the following mechanism (Scheme 1). The initial step of the reaction is the azo coupling of diazonium sulfate **1** or **2** with the enol form of compound **3** in an aqueous medium, which leads to intermediate **A**, which then, as a result of the thermodynamically favorable 1,5-hydrogen migration, is converted to hydrazone **4** or **5**.

The hydrazone structures of **4a**–**4e** [9] and **5a**–**5e** are confirmed by the presence of corresponding signals of the protons and carbon atoms of the carbonyl-containing groups in the ^{1}H and ^{13}C NMR spectra, as well as by the presence of corresponding molecular peaks in the spectra of liquid chromatography–mass spectrometry (LC–MS). In the ^{1}H and ^{13}C NMR spectra of compounds **4a**, **4b**, **4e**, **5a**, **5b**, and **5e**, for which rotation around a bond is possible, only one set of resonance signals is present, which indicates the existence of these derivatives in the form of one geometric isomer. This behavior is explained by the presence of an intramolecular hydrogen bond between the NH group of the hydrazone fragment and the proton acceptor in the ylidene part of the molecule (C=O of the ester group of the substituent [12, 13]), which is confirmed by the corresponding weak-polar placement of the proton signal of the NH group [14–16] in ^{1}H NMR spectra (12.29–14.48 ppm).

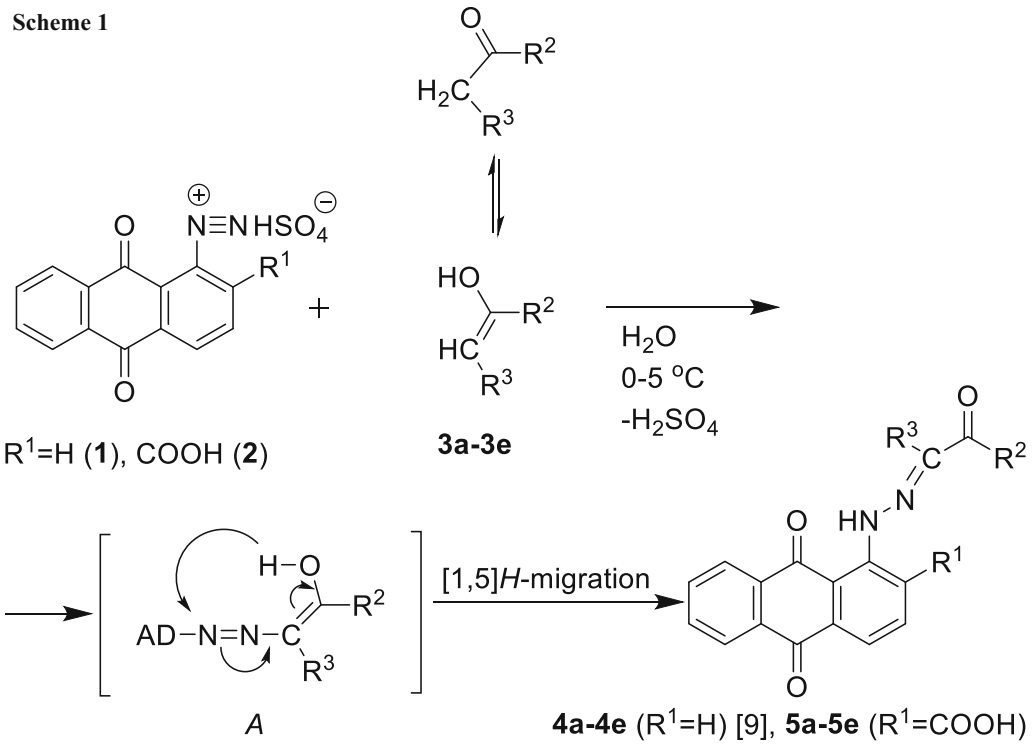
In turn, the presence of an electron-withdrawing conjugated quinone substituent in the hydrazone fragment in combination with an intramolecular hydrogen bond causes the bifurcation of proton of the NH group in the hydrazone fragment (Fig. 1) [17], which lead to the complete predominance of only one geometrical isomer.

The signals of the ylidene proton in compounds **4a**, **4b** and **5a**, **5b** are superimposed with the protons of the anthraquinone fragment in the range from 7.71 to 7.99 ppm. The proton signal of the NH group of the anthraquinonylhydrazones **4a**–**4e** and **5a**–**5e** resonates as a wide singlet within the range of 12.29–14.48 ppm.

It was found that the formation of the **4d**, **4e** compounds is accompanied by the by-product **4f** in an amount of 10 and 13%, respectively, which was separated from the main product by chromatography on silica gel (eluent benzene) (Scheme 2).

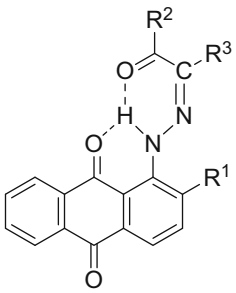
It should be noted that in the case of the presence of a carboxyl group in the second position of the anthraquinone

Scheme 1



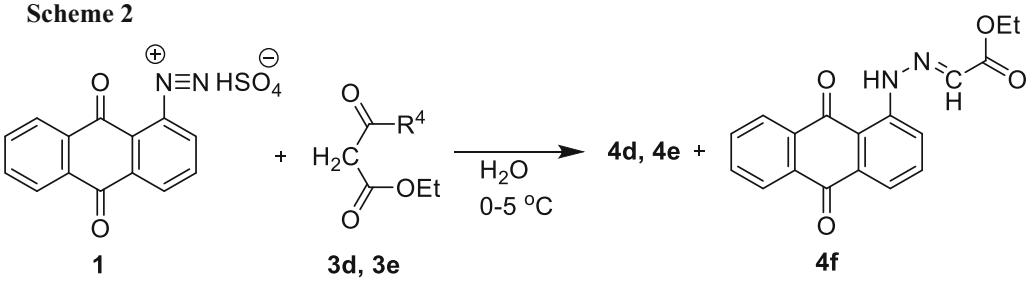
R^2=Me, R^3=H (**a**); R^2=Et, R^3=H (**b**); R^2=Me, R^3=C(O)Me (**c**);
R^2=OEt, R^3=C(O)OEt (**d**); R^2=OEt, R^3=C(O)Me (**e**)

Fig. 1 Bifurcated hydrogen in hydrazones



ring in the compounds **5d**, **5e**, the formation of the by-product of elimination of the acetyl or ethoxyl fragment is not observed. It is probably explained by the stabilizing effect of the COOH group on an intramolecular hydrogen bond in the formed hydrazones **5d**, **5e**. The formation of hydrazone **4f** was confirmed by the data of ^{1}H NMR, ^{13}C NMR, and LC–MS. In particular, in the ^{1}H NMR spectrum of **4f**, the proton signal of the methylidene group superimposes with the protons of the anthraquinone fragment. The ethoxyl fragment is represented by multiplets of two

Scheme 2



R^4=OEt (**d**); Me (**e**)

protons of the methylene group at 4.35 ppm and a triplet of three protons of the methyl group at 1.30 ppm. In the ^{13}C NMR spectrum, in addition to the signals of the carbon in anthraquinone and methylidene fragments, is characterized by the presence of carbon signals of one ethoxycarbonyl group. The LC–MS spectrum contains the corresponding molecular peak with a mass of 322 [M^+].

Functionalization of the ylidene part with carbocyclic and heterocyclic fragments has been carried out by coupling of the 9,10-dioxoanthracene-1-diazonium sulfates **1** and **2** with cyclic β-dicarbonyl compounds **6a–6c** (5,5-dimethyl-1,3-cyclohexanedione, 2,4,6-pyrimidinetrione, and 2-thioxopyrimidine-4,6-dione) with obtaining the hydrazones **7a–7c** and **8a–8c** (Scheme 3).

Analysis of the data of ^1H and ^{13}C NMR spectra of the obtained hydrazones **7a–7c**, **8a–8c** showed that compounds **7a**, **7b**, **8a**, **8b** exist in the ketone form and hydrazones **7c**, **8c** in the thioketone form in DMSO-d_6 solution. In the ^1H NMR spectra of compounds **7b**, **7c** and **8b**, **8c,** the singlet signals of the proton of NH group of the hydrazone fragment are presented in the range from 13.45 to 15.98 ppm and amide NH protons of a heterocyclic fragment are observed in the range 11.61–11.90 ppm, respectively.

Antimicrobial activity

The synthesized compounds **4a–4e**, **5a–5e**, **7a–7c**, **8a–8c** were evaluated for their antibacterial and antifungal activity against strains of *Escherichia coli B-906*, *Staphylococcus aureus 209-P*, *Mycobacterium luteumB-917*, *Candida tenuis VKM Y-70*, and *Aspergillus niger VKM F-1119* by the diffusion technique [18] and by the serial dilution technique (determination of minimal inhibition concentrations MIC) [19]. Their activities were compared with those of the known antibacterial agent vancomycin and antifungal agent nystatin (control C).

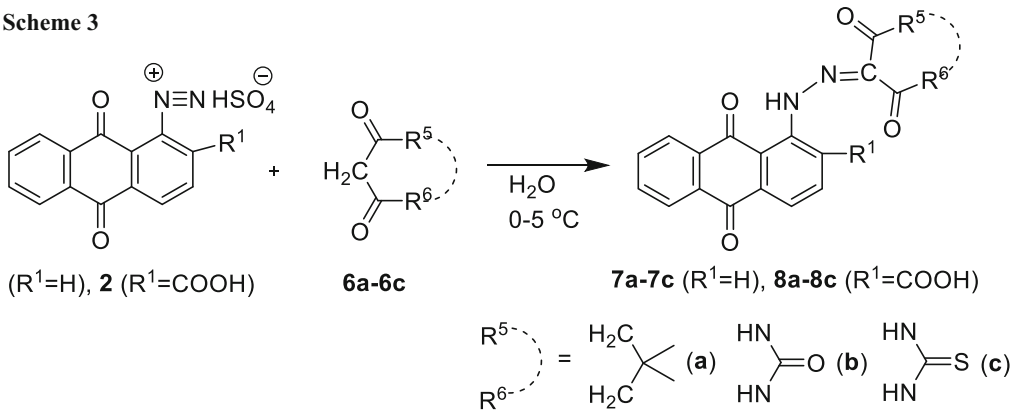
The strain of bacteria *E. coli* appeared not to be sensitive to the tested hydrazones **4a–4e**, **5a–5e**, **7a–7c**, **8a–8c** investigated by the diffusion technique at concentrations of 0.1 and 0.5% (Table 1). *S. aureus* was highly sensitive to compound **8a** and moderately sensitive to derivative **8b** (the diameter of the inhibition zone was 20.0 and 14.0 mm, respectively) at a concentration of 0.5%. The test culture of bacteria *M. luteum* was highly sensitive to hydrazone **8a** at the same concentration (the diameter of the inhibition zone was 21.0 mm). Other compounds were not active against these strains of bacteria. Strains of fungi *C. tenuis* and *A. niger* were slightly sensitive to compounds **5c**, **5e**, **8b** at a concentration of 0.5% (the diameter of the inhibition zone was 7.0–15.0 mm).

Evaluation of the antibacterial activity of synthesized compounds using the serial dilution technique (Table 2) showed that only compounds **7c**, **8a**, **8b** have low antibacterial action at MIC 250.0 and 500.0 μg/cm^3 against strain *E. coli*. The hydrazones **4a**, **4b**, **5c**, **8a**, **8b** have MIC 125–500 μg/cm^3 against test-culture bacteria *S. aureus*. Strain *M. luteum* was sensitive at MIC 62.5–500.0 μg/cm^3 to the compounds. Derivatives **4a**, **4b**, **5e**, **7c**, **8a**, **8b** show antifungal action against test-culture *C. tenuis* at MIC 62.5–250.0 μg/cm^3. Only compound **8b** had inhibitory effect against *A. niger* (MIC 500.0 μg/cm^3). The other hydrazones did not show any antifungal activity at the studied concentration against fungi *C. tenuis* and *A. niger*.

CUPRAC antioxidant capacity

Antioxidant capacity of anthraquinonylhydrazones **4a–4e**, **5a–5e**, **7a–7c**, **8a–8c** was determined by comparing with trolox (TR) as the standard reference compound using CUPRAC assay (at room temperature) [20]. The CUPRAC molar absorption coefficient (ε) of the tested compound divided by that of TR under the same conditions gave the TEAC-CUPRAC values (Fig. 2). Amongst the compounds screened for antioxidant capacity, **8a** and **8b** exhibited the highest antioxidant capacities, and the trolox equivalent antioxidant coefficients (TEAC) of these compounds were

Scheme 3



1 (R^1=H), **2** (R^1=COOH) **6a-6c** **7a-7c** (R^1=H), **8a-8c** (R^1=COOH)

Table 1 Antimicrobial activity of the synthesized compounds determined by diffusion method (only compounds with positive results are included in the table)

Compound	Concentration/%	Inhibition diameter of microorganism growth/mm				
		Bactericidal activity			Fungicidal activity	
		E. coli	*S. aureus*	*M. luteum*	*C. tenuis*	*A. niger*
5c	0.5	0	0	0	0	15.0
	0.1	0	0	0	0	10.0
5e	0.5	0	0	0	7.0	10.0
	0.1	0	0	0	0	6.0
8a	0.5	0	20.0	21.0	0	0
	0.1	0	15.0	0	0	0
8b	0.5	0	14.0	0	0	7.0
	0.1	0	10.0	0	0	0
C[a]	0.5	14.0	15.0	18.0	19.0	20.0

[a]Vancomycin was used as a control in the tests of antibacterial activity of the synthesized compounds and nystatin was used in the tests of antifungal action

Table 2 Bactericidal and fungicidal activity of the synthesized compounds determined by serial dilution method (only compounds with positive results are included in the table)

Compound	Minimal inhibition concentration MIC/μg/cm^3				
	E. coli	*S. aureus*	*M. luteum*	*C. tenuis*	*A. niger*
4a	+	250.0	500.0	250.0	+
4b	+	500.0	+	250.0	+
5c	+	500.0	+	+	+
5e	+	+	125.0	125.0	+
7c	500.0	+	+	62.5	+
8a	250.0	125.0	62.5	62.5	+
8b	250.0	125.0	125.0	250.0	500.0

+, growth of microorganisms

0.89 and 0.87, respectively (Fig. 2). Based on the experimental testing of hydrazone derivatives, the effect of substituents on the side of the ylidene fragment on the antioxidant activity of the compounds has been established. Antioxidant activity of **5d** and **8a**, **8b** approximates to the level of the reference preparation. Replacement of the acetyl fragments in compound **5c** with ethoxycarbonyl (compound **5d**) increases the TEAC coefficient, and replacing one of ethoxycarbonyl substituents (**5c**) with acetyl fragment (**5e**) decreases this index. The presence of the carboxyl group in the second position of the anthraquinone ring in compounds **5a**–**5e** and **8a**–**8c** increases the TEAC coefficient in comparison with hydrazones **4a**–**4e** and **7a**–**7c**.

All synthesized compounds were tested in vitro for their catalase activity [21], which is an integral part of antioxidant protection in cells, using the CUPRAC method. The inhibition values are given in Fig. 3.

The results (Fig. 3) showed that the highest activity was characteristic for compounds **4d** (0.77 U/cm^3), **5e** (0.75 U/cm^3), **8a** (0.79 U/cm^3), **8b** (0.78 U/cm^3), and **8c** (0.76 U/cm^3). The presence of the carboxyl group in the second position of the anthraquinone ring in compounds **8a**–**8c** increases the catalase enzyme activities in comparison with hydrazones **7a**–**7c**. Replacement of one ethoxycarbonyl fragment in compound **5d** with acetyl fragment (compound

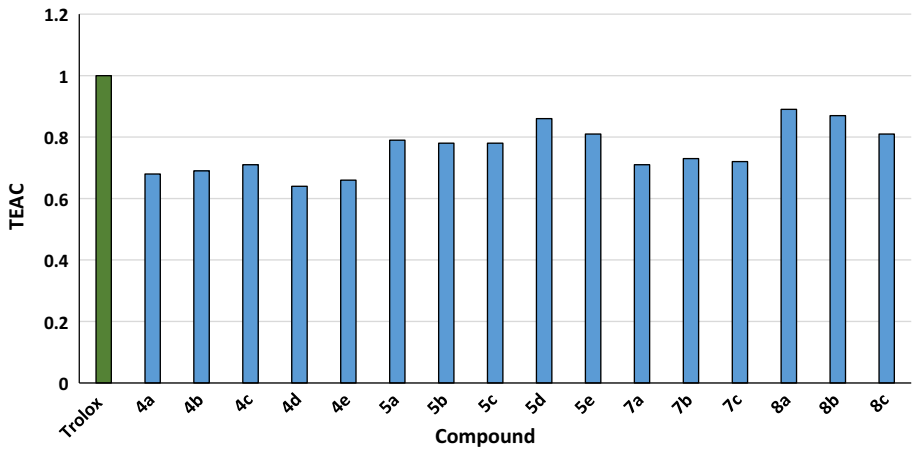


Fig. 2 TEAC coefficients ($N = 3$) of the hydrazones with respect to the CUPRAC assay

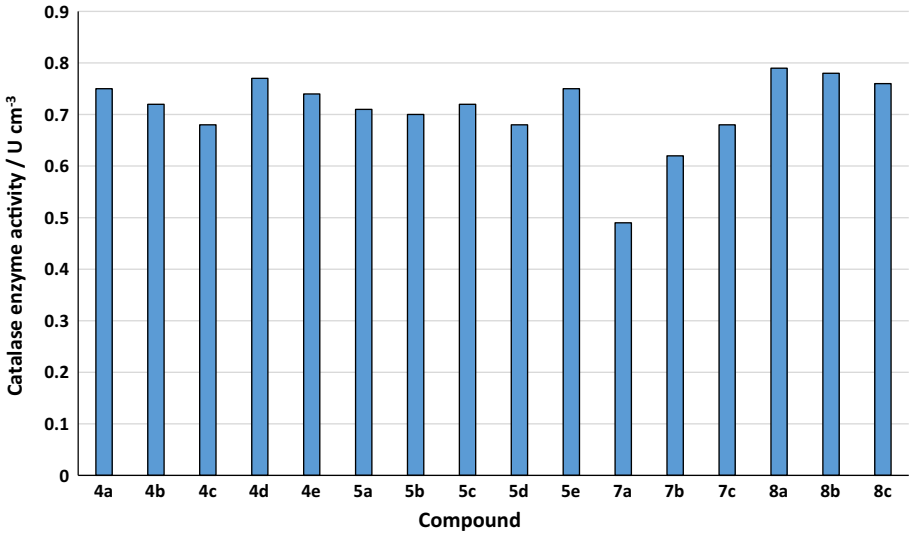


Fig. 3 Catalase enzyme activities (U/cm^3) of the hydrazones

5e) increases the TEAC coefficient. The results of catalase enzyme activity of hydrazones **8a**, **8b** are consistent with the data of TEAC investigation.

Conclusion

The synthesis and probable mechanism of formation of new anthraquinonylhydrazones by the coupling reaction of 9,10-dioxoanthracene-1-diazonium sulfates with an active α-ketones and β-carbonyl compounds in neutral aqueous medium under mild conditions are presented. It was determined that the obtained hydrazones exist in the form of a single geometric isomer due to the presence of an intramolecular hydrogen bond between the NH group of the hydrazone fragment and the proton acceptor in the ylidene part of the molecule. All the synthesized compounds were tested for their antibacterial, antifungal, and antioxidant activity. The anthraquinonylhydrazone **8a** exhibited a promising antimicrobial activity against bacteria *S. aureus*, *M. luteum,* and antifungal effect against fungus *C. tenuis*. All compounds were tested for their antioxidant capacity using the CUPRAC method and for inhibitory activities against catalase enzyme. The synthesized compounds **8a**, **8b** exhibited better antioxidant capacity than the other compounds. The results revealed that **8a**, **8b** exhibited high catalase enzyme inhibition activity compared to the other hydrazones.

Experimental

Melting points were measured using a Buchi B-540 melting point apparatus. Elemental analysis was performed on a Perkin Elmer 2400 CHN-analyzer, and their results were found to be in good agreement with the calculated values. ^{1}H NMR spectra (in DMSO-d_6) and ^{13}C NMR spectra (in CF_3COOD) were recorded on a Varian Mercury-400 spectrometer with TMS as internal standard. Mass spectra were recorded on an Agilent 1100 Series G1956B LC/MSD SL LCMS system, using electrospray ionization at atmospheric pressure (70 eV). Thin-layer chromatography (TLC) was performed on Merck silica gel plates (60F254) in benzene, and detection was carried out with ultraviolet light (254 nm).

All chemicals were of reagent grade and used without further purification. Diazonium salts **1** and **2** were obtained according to a published method [9] from 1-aminoanthracene-9,10-dione and 1-amino-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Sigma-Aldrich). Hydrazones **4a**–**4e** were obtained by the method described in [9].

General method of obtaining of hydrazones 5a–5e, 7a–7c, 8a–8c 9,10-Dioxoanthracene-1-diazonium sulfate (**1** or **2**) (9 mmol) was added to a solution of appropriate compound containing active methylene group (**3a**–**3e**, **6a**–**6c**) (30 mmol) in 150 cm^3 water at 0–5 °C. The reaction mixture was stirred for 1 h at 0–5 °C. The precipitate was filtered off, washed with water (2 × 100 cm^3) and dried. If necessary, the products can be purified by recrystallization from acetic acid.

Ethyl 2-[2-(9,10-dioxo-9,10-dihydroanthracene-1-yl)hydrazineylidene]acetate (4f, $C_{18}H_{14}N_2O_4$) Compound **4f** was separated from the main product (**4d** or **4e**) by column chromatography on silica gel (Merck silica gel 60 H), eluent benzene. Yield 10% (from compound **4d**), 13% (from compound **4e**); m.p.: 238–239 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.31$ (t, 3H, $J = 7.2$ Hz, CH_3), 4.35 (m, 2H, CH_2), 7.78–7.88 (m, 5H, $4H_{Ar}$ and CH=),

8.03–8.15 (m, 3H, H_{Ar}), 12.55 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 12.31$ (CH_3), 63.60 (CH_2), 114.63, 121.52, 122.75, 128.02, 128.66, 130.04, 130.57, 133.13, 133.32, 134.82, 135.48, 135.88, 147.14 (C_{Ar}), 163.30, 185.31, 185.82 (C=O) ppm; LC–MS (70 eV): $m/z = 322$ (M^+).

9,10-Dioxo-1-[2-(2-oxopropylidene)hydrazinyl]-9,10-dihydroanthracene-2-carboxylic acid (5a, $C_{18}H_{12}N_2O_5$) Yield 62%; m.p.: 260–261 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.07$ (s, 3H, CH_3), 7.92–7.99 (m, 5H, $4H_{Ar}$ and CH=), 8.16–8.21 (m, 2H, H_{Ar}), 12.98 (br s, 1H, OH), 14.01 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 22.78$ (CH_3), 114.70, 121.86, 122.85, 127.19, 127.43, 127.64, 132.05, 133.45, 135.00, 135.62, 136.22, 138.33, 145.69 (C_{Ar}), 169.40, 185.41, 187.06, 204.79 (C=O) ppm; LC–MS (70 eV): $m/z = 336$ (M^+).

9,10-Dioxo-1-[2-(2-oxobutylidene)hydrazinyl]-9,10-dihydroanthracene-2-carboxylic acid (5b, $C_{19}H_{14}N_2O_5$) Yield 56%; m.p.: 278–280 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.14$ (t, 3H, CH_3, $J = 7.2$ Hz), 2.42 (m, 2H, CH_2), 7.68–7.76 (m, 5H, $4H_{Ar}$ and CH=), 7.87–8.14 (m, 2H, H_{Ar}), 13.07 (br s, 1H, OH), 14.03 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 7.34$ (CH_3), 22.27 (CH_2), 114.51, 121.82, 122.39, 127.45, 127.71, 132.23, 133.36, 133.87, 134.75, 135.57, 136.15, 145.73, 146.55 (C_{Ar}), 170.09, 186.20, 187.48, 203.69 (C=O) ppm; LC–MS (70 eV): $m/z = 350$ (M^+).

1-[2-(2,4-Dioxopentan-3-ylidene)hydrazinyl]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid (5c, $C_{20}H_{14}N_2O_6$) Yield 68%; m.p.: > 310 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 2.30$ (s, 6H, CH_3), 7.83–7.93 (m, 4H, H_{Ar}), 8.15–8.23 (m, 2H, H_{Ar}), 12.74 (br s, 1H, OH), 13.22 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 24.68$ (CH_3), 30.24 (CH_3), 121.01, 124.75, 127.49, 127.81, 127.89, 131.75, 133.78, 135.30, 135.76, 135.85, 136.09, 136.17, 141.92 (C_{Ar}), 172.90, 184.78, 185.29, 200.39, 203.04 (C=O) ppm; LC–MS (70 eV): $m/z = 378$ (M^+).

1-[2-(1,3-Diethoxy-1,3-dioxopropan-2-ylidene)hydrazinyl]-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (5d, $C_{22}H_{18}N_2O_8$) Yield 67%; m.p.: 295–297 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.36$ (s, 6H, CH_3), 4.28–4.41 (m, 4H, CH_2), 7.82–7.85 (m, 4H, H_{Ar}), 7.89–8.13 (m, 2H, H_{Ar}), 13.11 (br s, 1H, OH), 14.52 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 13.08$, 13.13 (CH_3), 63.46, 63.98 (CH_2), 116.88, 122.64, 123.44, 124.30, 127.36, 132.11, 133.53, 133.49, 133.67, 134.84, 135.54, 135.86, 144.45 (C_{Ar}), 166.80, 167.91, 169.65, 186.04, 186.71 (C=O) ppm; LC–MS (70 eV): $m/z = 438$ (M^+).

1-[2-(1-Ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl]-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (5e, $C_{21}H_{16}N_2O_7$) Yield 60%; m.p.: 224–226 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.39$ (t, 3H, $J = 7.2$ Hz, CH_3), 2.37 (s, 3H, CH_3), 4.45 (q, 2H, $J = 7.2$ Hz, CH_2), 7.90–7.92 (m, 4H, H_{Ar}), 8.12–8.17 (m, 2H, H_{Ar}), 13.43 (br s, 1H, OH), 14.08 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 12.25$ (CH_3), 21.83 (CH_3), 64.17 (CH_2), 124.15, 127.39, 127.57, 127.99, 128.05, 131.82, 133.15, 133.55, 133.57, 135.51, 135.58, 136.02, 141.00 (C_{Ar}), 172.20, 173.35, 184.60, 186.71, 201.80 (C=O) ppm; LC–MS (70 eV): $m/z = 408$ (M^+).

1-[2-(4,4-Dimethyl-2,6-dioxocyclohexylidene)hydrazinyl]anthracene-9,10-dione (7a, $C_{22}H_{18}N_2O_4$) Yield 55%; m.p.: 243–245 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.09$ (s, 6H, CH_3), 2.67 (s, 2H, CH_2), 2.75 (s, 2H, CH_2), 7.93–8.02 (m, 4H, H_{Ar}), 8.15–8.22 (m, 2H, H_{Ar}), 8.36–8.38 (m, 1H, H_{Ar}), 16.05 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 26.54$, 26.65 (CH_3), 30.16 ($\underline{C}(CH_3)_2$), 50.58, 50.71 (CH_2), 120.07, 123.49, 123.95, 127.36, 127.62, 130.12, 131.09, 131.95, 133.48, 133.97, 134.18, 135.73, 142.33 (C_{Ar}), 185.39, 186.13, 198.64, 201.90 (C=O) ppm; LC–MS (70 eV): $m/z = 374$ (M^+).

5-[2-(9,10-Dioxo-9,10-dihydroanthracene-1-yl)hydrazinylidene]pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-*trione* (7b, $C_{18}H_{10}N_4O_5$) Yield 66.5%; m.p.: > 300 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.89$–7.99 (m, 4H, H_{Ar}), 8.05–8.19 (m, 3H, H_{Ar}), 11.66 (br s, 1H, NH), 11.90 (br s, 1H, NH), 15.90 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 119.59$, 120.72, 122.55, 124.65, 126.85, 131.56, 132.21, 133.64, 133.72, 134.09, 135.97, 137.02, 144.15 (C_{Ar}), 150.35, 159.14, 160.09, 184.52, 185.30 (C=O) ppm; LC–MS (70 eV): $m/z = 362$ (M^+).

5-[2-(9,10-Dioxo-9,10-dihydroanthracene-1-yl)hydrazono]-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (7c, $C_{18}H_{10}N_4O_4S$) Yield 67%; m.p.: > 300 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.88$–7.92 (m, 4H, H_{Ar}), 8.15–8.21 (m, 3H, H_{Ar}), 11.41 (br s, 1H, NH), 11.61 (br s, 1H, NH), 15.78 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 114.47$, 119.67, 124.67, 125.72, 126.70, 132.56, 133.11, 133.23, 133.30, 134.43, 134.86, 137.48, 142.44 (C_{Ar}), 159.68, 160.21 (C=O), 174.58 (C=S), 185.07, 185.89 (C=O) ppm; LC–MS (70 eV): $m/z = 378$ (M^+).

1-[2-(4,4-Dimethyl-2,6-dioxocyclohexylidene)hydrazinyl]-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (8a, $C_{23}H_{18}N_2O_6$) Yield 56%; m.p.: 274–276 °C; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.07$ (s, 6H, CH_3), 2.62 (s, 2H, CH_2), 2.76 (s, 2H, CH_2), 7.94–7.99 (m, 3H, H_{Ar}), 8.08 (m, 1H, H_{Ar}), 8.17–8.23 (m, 2H, H_{Ar}), 13.30 (br s, 1H, OH), 16.02 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 26.74$, 26.84 (CH_3), 30.53 ($\underline{C}(CH_3)_2$), 43.50,

43.63 (CH_2), 121.05, 126.04, 127.50, 127.87, 128.72, 131.16, 131.75, 133.56, 135.12, 135.43, 135.69, 135.99, 140.71 (C_{Ar}), 173.14, 184.16, 185.20, 199.05, 200.33 (C=O) ppm; LC–MS (70 eV): $m/z = 418$ (M^+).

9,10-Dioxo-1-[2-(2,4,6-trioxotetrahydropyrimidin-5(2*H*)-ylidene)hydrazinyl]-9,10-dihydroanthracene-2-carboxylic acid (8b, $C_{19}H_{10}N_4O_7$) Yield 57%; m.p.: > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.91$–7.99 (m, 3H, H_{Ar}), 8.04–8.05 (m, 1H, H_{Ar}), 8.14–8.20 (m, 2H, H_{Ar}), 11.39 (br s, 1H, NH), 11.66 (br s, 1H, NH), 13.45 (br s, 1H, OH), 15.70 (1H, br s, NH) ppm; ^{13}C NMR (100 MHz, CF_3-COOD): $\delta = 114.23$, 119.31, 124.69, 125.89, 126.85, 127.10, 131.60, 133.80, 134.56, 135.36, 135.72, 137.31, 142.46 (C_{Ar}), 150.23, 159.82, 160.09, 170.20, 184.62, 185.07 (C=O) ppm; LC–MS (70 eV): $m/z = 406$ (M^+).

1-[2-(4,6-Dioxo-2-thioxotetrahydropyrimidin-5(2*H*)-ylidene)hydrazinyl]-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (8c, $C_{19}H_{10}N_4O_6S$) Yield 59%; m.p.: > 300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.90$–7.93 (m, 4H, H_{Ar}), 8.17–8.23 (m, 2H, H_{Ar}), 11.66 (br s, 1H, NH), 11.88 (br s, 1H, NH), 13.49 (br s, 1H, OH), 15.98 (br s, 1H, NH) ppm; ^{13}C NMR (100 MHz, CF_3COOD): $\delta = 117.58$, 119.86, 123.27, 124.92, 126.11, 131.82, 132.46, 133.14, 133.47, 136.31, 137.40, 137.86, 143.49 (C_{Ar}), 159.31, 160.06, 169.49 (C=O), 175.22 (C=S), 184.58, 185.15 (C=O) ppm; LC–MS (70 eV): $m/z = 422$ (M^+).

Antimicrobial activity

Diffusion method

Antibacterial activity of compounds was evaluated by diffusion in peptone on nutrient medium (meat-extract agar for bacteria; wort agar for fungi). The microbial loading was 10^9 cells (spores)/1 cm^3. The required incubation periods were as follows: 24 h at 35 °C for bacteria and 48–72 h at 28–30 °C for fungi. The results were recorded by measuring the zones surrounding the disk. Control disk contained vancomycin (for bacteria) or nystatin (for fungi) as a standard.

Serial dilution method

Testing was performed in a flat-bottomed 96-well tissue culture plate. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to the necessary concentration. The exact volume of solution of compounds was brought in nutrient medium. The inoculum of bacteria and fungi was in nutrient medium (meat-extract agar for bacteria; wort agar for fungi). The duration of incubation was 24–72 h at 37 °C for bacteria and 30 °C for fungi. The results were estimated according to the presence or absence of microorganism growth.

Antioxidant capacity

All reagents and solvents were of analytical reagent grade. Neocuproine (2,9-dimethyl-1,10-phenanthroline) was purchased from Sigma Chemical Co. (Steinheim, Germany). Copper(II) chloride dihydrate, ammonium acetate (NH_4-Ac), absolute ethanol (EtOH), methanol (MeOH), and dimethyl sulfoxide (DMSO) were purchased from Merck (Darmstadt, Germany). The spectra and absorption measurements were recorded in matched Helma quartz cuvettes of 1 cm thickness using a Perkin Elmer Lambda 35 UV–Vis spectrophotometer.

Preparation of solutions

The standard solutions of synthesized compounds were prepared in DMSO at a concentration of 1.0 mM. All standard solutions were stored at + 4 °C prior to analysis. The $CuCl_2$ solution (10.0 mM) and ammonium acetate buffer solution (1 M, pH 7.0) were prepared in distilled water and neocuproine (Nc) solution (7.5 mM) in absolute ethanol.

CUPRAC antioxidant capacity

The CUPRAC method, as described by Apak et al. [20], is based on the reduction of a cupric neocuproine complex (Cu(II)-Nc) by compounds having antioxidant capacity to the yellow–orange coloured cuprous chelate (Cu(I)-Nc). The CUPRAC reaction mixture consisted of 1 cm^3 $CuCl_2{\cdot}2H_2O$ (10 mM), 1 cm^3 Nc (7.5 mM), 1 cm^3 pH 7 NH_4Ac buffer solution (1.0 M), × cm^3 synthesized compound, and (1.1-×) cm^3 DMSO. The mixture (4.1 cm^3) was then incubated at 25 °C for 30 min, and afterwards, the absorbance at 450 nm was recorded against a reagent blank. Under the described experimental conditions, the calibration curves (absorbance versus concentration graphs) of each compound were constructed, and their TEAC coefficients were calculated.

Catalase enzyme activity

The method described by Bekdeşer et al. was employed for the determination of catalase enzyme activity [21]. The reaction mixture consisted of 0.5 cm^3 H_2O_2 (1.0 mM), 1.8 cm^3 H_2O, 0.1 cm^3 catalase solution (3.691 U/cm^3), and 0.2 cm^3 synthesized compound (1.0 mM). This mixture (total volume 2.6 cm^3) was then incubated at 25 °C. After 30 min incubation period, the optical CUPRAC sensor [Cu(II)-Nc-impregnated membrane] was taken out and

immersed in a test tube consisted of 2.0 cm^3 of the incubation reaction mixture + 6.2 cm^3 of EtOH. After 30 min agitation, the coloured membrane was taken out and its absorbance was recorded at 450 nm against that of a blank membrane without tested sample.

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