

Synthesis and Antimicrobial Activity Of Nitrogen-Containing Anthraquinone Derivatives

Shupeniuk V.I.^{*1}, Taras T.N.^{*}, Sabadakh O.P.^{*}, Luchkevich E.R.^{*},
Matkivsky M.P.^{*} and Kutsyk R.V.^{**}

^{*}Vasyl Stefanyk Precarpathian National University, Shevchenko Street 57, Ivano-Frankivsk, 76018, Ukraine

^{**}Ivano-Frankivsk national medical university, Halytska Street 57, Ivano-Frankivsk, 76018, Ukraine

Abstract

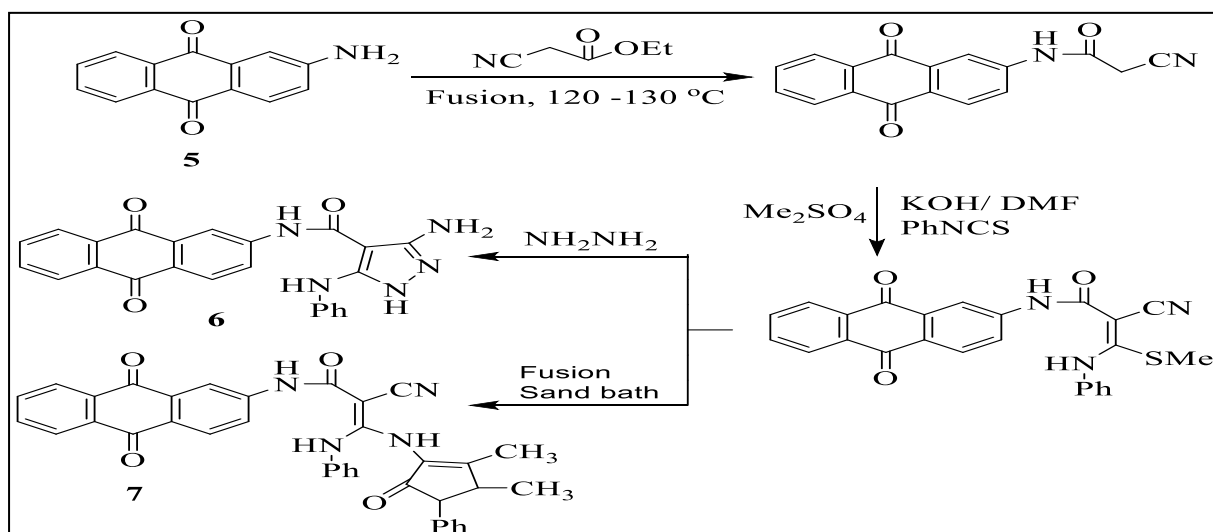
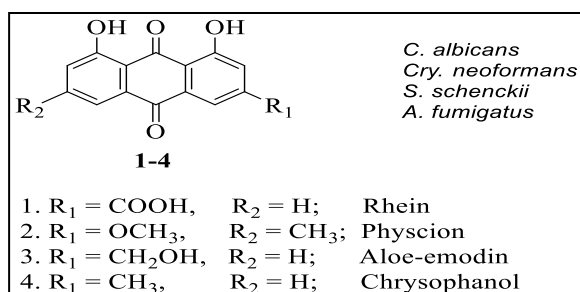
New substituted anthraquinones with amino derivations fragments were synthesized through the substitution of bromine atom by different amines using the Ullmann coupling reaction. Obtained compounds based on anthraquinone used for experimental antimicrobial studies. The structure of the synthesized compounds was confirmed by LC-MS and ¹H, ¹³C NMR spectroscopy. Studies on planktonic microorganisms have shown that the first synthesized anthraquinone derivatives have an inhibitory effect against bacteria and fungi. The triazene 1-(3-(benzoic acid(triaz-1-en-1-ol(-4-(1H-imidazol-1-yl(-9,10-dioxo-9,10-dihydroanthracene -2-sulfonic acid, have wide spectrum of activity, growth retardation zones against gram-positive microorganisms in the range of 8.41-11.5 mm, gram-negative bacteria 5.87-8.18 mm, fungi of the genus *Candida* 5.81-7.48 mm. The high antimicrobial activity of this compound is probably due to the presence of benzoic acid in its molecule.

Key words: Antimicrobial activity, Ullmann reaction, Anthraquinone derivatives, Triazene, Bacteria.

Introduction

Anthraquinone derivatives of both natural and synthetic origin are well-known for their wide range of biological effects^(1,2). Of particular interest are compounds with antibacterial effect⁽³⁻⁵⁾. Many literatures described antibacterial and antifungal activity of anthraquinone compounds isolated from natural components⁽⁶⁾. Also known antifungal activity of rhizomes of *Rheum emodi*, which contain four active components of anthraquinone **1-4**. The antimicrobial activity of anthraquinone amino derivatives synthesized by various methods was studied. In particular, on the basis of 2-aminoanthraquinone **5** synthesized derivatives **6-7**, which showed activity against

gram-positive bacteria *Staphylococcus epidermidis* (*S. epidermidis*) and gram-negative bacteria *Pseudomonas aeruginosa* (*P. auregenosa*) and fungicidal activity *Alternaria solani* (*A solani*) and *Fusarium solani* (*F. solani*)⁽⁷⁾.



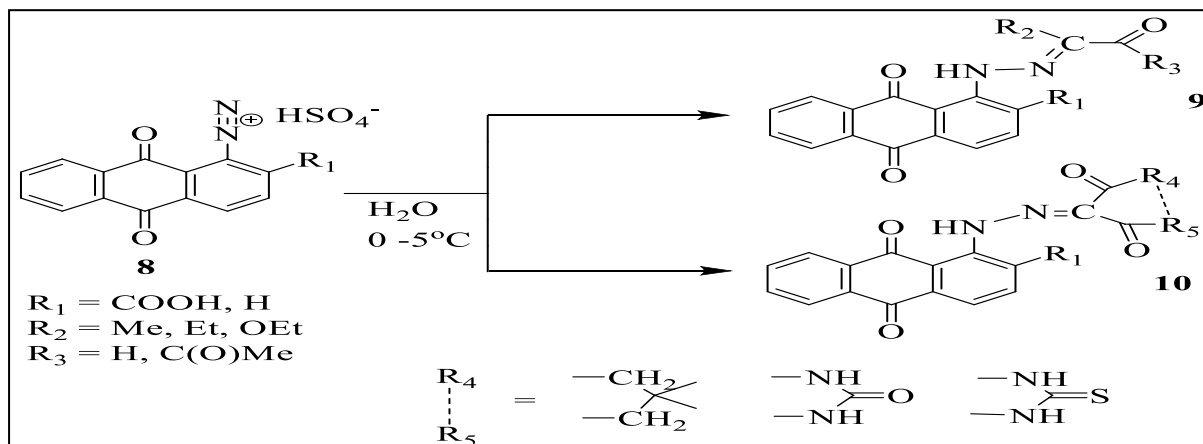
¹Corresponding author E-mail: vasyi.shupeniuk@pnu.edu.ua

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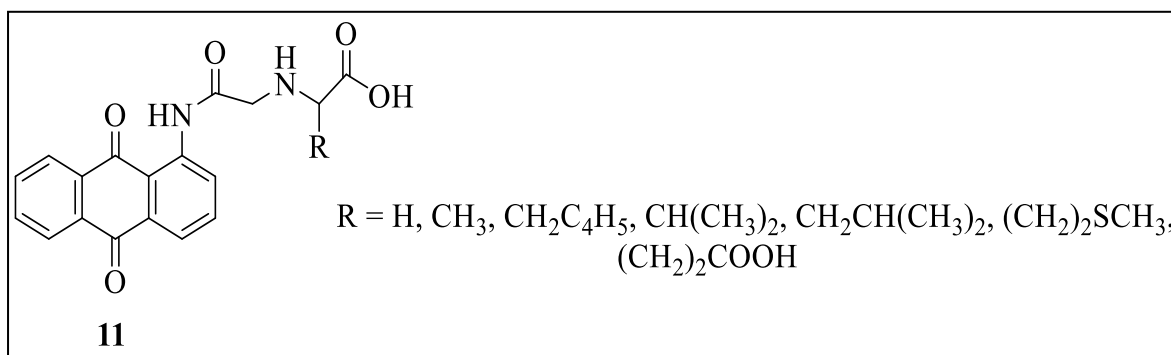
Anthraquinone hydrazones **9**, **10** synthesized on the basis of diazo derivatives of 1-aminoanthraquinone **8** by the Japp-Klingeman reaction showed activity against bacteria

Staphylococcus aureus (*S. aureus*) 209-P, *Mycobacterium luteum* B-917 and fungicidal activity of *Candida tenuis* VKM Y-70^(8,9).



Using the computer program Prediction of Activity Spectra for Substances (PASS), two possible mechanisms of antimicrobial activity were predicted for the amino acid derivatives of

anthraquinone **11**, which are realized by inhibition of histidine kinase and antagonists of membrane integrity⁽¹⁰⁾.



Current study evaluated the antimicrobial activity of twenty (11 new) synthesized nitrogen-containing derivatives of 9,10-anthraquinone and identified substituent fragments whose greatest influence on antimicrobial activity.

Materials and Methods

Studies of the antimicrobial activity of anthraquinone derivatives were performed by the method of diffusion into agar according to the generally accepted method with the determination of the diameters of the growth retardation zones. Working solutions of the compounds were prepared in 12.5 % aqueous Dimethyl sulfoxide (DMSO) solution (concentration 20 mg/ml). One-day cultures of microorganisms were used in the experiments. In the agar layer on the Petri dish, wells with a diameter of 4.0 mm were prepared and the cups were inoculated with standardized suspensions of test cultures (1×10^7). 20 μl of solutions of test compounds were added to all wells, and 12.5 % aqueous DMSO solution was added to control wells. The plates were incubated for 24 h at $(36 \pm 1)^\circ\text{C}$. To

determine the diameters of the zones of growth retardation of microorganisms, digital images of crops on cups were analyzed using the computer program UTHSCSA ImageTool 3.0 (The University of Texas Health Science Center in San Antonio, ©1995-2002). The obtained results were processed by the methods of variation statistics.

Clinical isolates of microorganisms isolated from patients with purulent-septic diseases (*Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Pseudomonas aeruginosa*) and candidal stomatitis (*Candida*) were used as test strains. Clinical strains of bacteria were identified by morphological, cultural properties according to using biochemical microtests "STAPHYtest 16", "ENTEROtest 24", "NEFERMtest 24" (Lachema, Czech Republic). Identification of fungi was performed using the VITEK 2 system using a VITEK 2 YST ID card (biomerieux, France). Test strains of staphylococci included a strain of *S. aureus*, sensitive to all groups of antibiotics, *S. aureus* and *S. haemolyticus*, resistant to methicillin,

macrolides, tetracyclines and fluoroquinolones. Gram-negative bacteria included extended-spectrum β -lactamase (ES β L) producers. Cultures of yeast-like fungi of the genus *Candida* showed moderate sensitivity to polyenes (nystatin, amphotericin B), resistance or moderate dose-dependent sensitivity to imidazoles (ketoconazole) and triazoles (fluconazole, itraconazole, voriconazole).

^1H and ^{13}C NMR spectra of the synthesized compounds were obtained on a Varian Mercury-400 spectrometer (400 and 100 MHz, respectively) in DMSO- d_6 solutions, and a mixture of DMSO- d_6 + CCl_4 , the internal standard of Tetramethylsilane (TMS). Chromato-mass spectrometry data were obtained on a high-performance Agilent 1100 Series liquid chromatograph equipped with a diode array with a mass-selective Agilent LC\MSD SL detector, ionization method - chemical ionization at atmospheric pressure (APCI). Elemental analysis was performed on a PerkinElmer CHN-Analyzer series 2400. The individuality of the compounds was monitored by Thin-layer chromatography (TLC) on plates (DC-Fertigfolien ALUGRAM Xtra SIL G/UV254, Germany) using eluents of different composition.

General procedure for preparation of compounds 13-16, 30-31, 36-39.

1-Amino-2-methyl-4-bromoanthraquinone (0.01 mol), was dissolved in 40 ml dimethylformamide (DMF) (70-100 °C), amino derivatives (0.015 mol), acid binding agent sodium bicarbonate (0.02 mol), copper sulfate (0.05 g) and ferrous sulfate (0.05 g) catalysts were then added to it. The reaction mixture stirred and heated to 90 °C. Maintained temperature 90 °C for 4 h under stirring. The product salted out by adding sodium chloride, cooled to room temperature, filtered and washed with 10 % w/v brine solution. The dried product was recrystallized from toluene.

1-amino-4-[(morpholin-2-yl)amino]-2-methylantracene-9,10-dione (13). Blue solid; Yield 40 %, m.p. >300 °C. $\text{C}_{19}\text{H}_{19}\text{O}_3\text{N}_3$, ^1H NMR (DMSO- d_6) δ , ppm: 2.03 s (3H, CH_3), 3.00-4.01 m (8H, CH_2), 4.50 s (2H, NH_2), 7.70 s (1H, H_{ap}), 8.01-8.20 m (4H, H_{ap}), 9.00 s (1H, NH). LC/MS spectrum: Found, m/z: 344 $[\text{M}+\text{H}]^+$; $\text{C}_{19}\text{H}_{19}\text{O}_3\text{N}_3$; Calculated m/z: 343.

1-amino-4-[(2-hydroxyethyl)amino]-2-methylantracene-9,10-dione (14). Blue solid; Yield 50 %, m.p. >300 °C. $\text{C}_{17}\text{H}_{17}\text{O}_3\text{N}_2$; ^1H NMR (DMSO- d_6) δ , ppm: 2.30 s (3H, CH_3), 3.40-3.50 m (2H, CH_2), 3.67 d (2H, CH_2 , J 5.2 Hz), 4.00 s (1H, OH), 7.34 s (1H, H^3), 7.77 d (2H, H_{ap} , J 6.8 Hz), 7.86 t (2H, H_{ap} , J 6.8 Hz), 8.60 s (1H, NH). LC/MS spectrum: Found, m/z: 297 $[\text{M}+\text{H}]^+$; $\text{C}_{17}\text{H}_{17}\text{O}_3\text{N}_2$; Calculated m/z: 296.

1-amino-4-(butylamino)-2-methylantracene-9,10-dione (15). Blue solid; Yield 60 %, m.p. >300 °C. $\text{C}_{19}\text{H}_{20}\text{O}_2\text{N}_2$; ^1H NMR

(DMSO- d_6) δ , ppm: 1.01 t (3H, CH_3), 1.50 – 1.55 m (6H, CH_2), 2.28 s (3H, CH_3 ar.), 3.00 s (2H, NH_2), 7.80 - 8.20 m (5H, H_{ap}), 8.80 s (1H, NH). FT-IR (KBr, ν_{max} , cm^{-1}): 1300 (CH_2), 1450, 2900 (CH_3), 1500-1600 (NH), 1630 (C=O). LC/MS spectrum: Found, m/z: 312 $[\text{M}+\text{H}]^+$; $\text{C}_{19}\text{H}_{20}\text{O}_2\text{N}_2$; Calculated m/z: 312.5.

1-amino-4-[[[furan-2-yl)methyl]amino]-2-methylantracene-9,10-dione (16). Yield 30 %, m.p. >300 °C. $\text{C}_{19}\text{H}_{14}\text{O}_6\text{N}_2$; ^1H NMR (DMSO- d_6) δ , ppm: 2.27 t (3H, CH_3), 4.74 s (2H, CH_2), 5.00 s (2H, NH_2), 7.35-8.20 m (8H, H_{ap}), 9.10 s (1H, NH). ^{13}C NMR, δ , ppm.: 42.24 (CH_2), 49.47, 53.22 (C-O); 113.48, 114.19, 123.18, 125.18, 126.41, 131.91, 133.05, 134.73, 140.98, 143.98, 144.34, 145.34, 148.43 (C_{ar}); 154.95 (C-NH $_2$); 181.43, 186.69 (C=O). LC/MS spectrum: Found, m/z: 333.2 $[\text{M}+\text{H}]^+$; $\text{C}_{19}\text{H}_{14}\text{O}_6\text{N}_2$; Calculated m/z: 332.1.

1-Amino-4-[(2-hydroxyethyl)amino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (30). Yield 90 %, mp 288-289 °C (methanol-acetone); Ref.¹¹ mp 287-290 °C.

1-amino-4-[1H-imidazol-1-yl]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (31). Yield 80 %, mp 253.5-255.5 °C (methanol-acetone); Ref.¹¹ mp 255-257 °C.

1-amino-4-(propylamino)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (36). Yield 83 %, mp 261.5 °C (methanol); Ref.¹² mp 262 °C.

1-Amino-4-(butylamino)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (37). Yield 91 %, mp 287-288 °C (methanol); Ref.¹² mp 290-292 °C.

1-Amino-4-[(propan-2-yl)amino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (38). Yield 65 %, mp 261-262 °C (methanol-acetone); Ref.¹² mp 260-262 °C.

1-Amino-4-[(morpholin-4-yl)amino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (39). Yield 75 %, mp 280-281 °C (methanol); Ref.¹² mp 282 °C.

General procedure for preparation of diazo derivatives.

Aminoanthraquinone **18-19** (0.01 mol) was mixed with 100 ml of glacial acetic acid and heated to boiling. In the cooled mixture was added 100 ml of conc. Hydrochloric acid and diazotized with an aqueous solution of 0.7 g of sodium nitrite at a temperature of 0-5 °C and kept for one hour. The anthraquinone diazonium chloride solution **20-21** thus obtained was used without isolation to obtain the triazene.

General procedure for preparation of triazens 22-29, 32-35.

The amino derivative (0.015 mol) was dissolved in water (20 ml) and cooled to 0-5 °C in an ice bath. With stirring, diazonium salt **20-21** was added over 10-15 minutes, and Na_2CO_3 solution (10 %) was added to maintain a pH of 7.5-8. The

temperature of the reaction mixture was raised to 60 °C for 1 hour and filtered.

1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-hydroxyanthracene-9,10-dione (22). Yield 61 %, m.p. 260 °C. C₂₀H₂₂N₄O₈S; ¹H NMR (DMSO-d₆) δ, ppm: 2.10 t (4H, CH₂), 3.20 t (4H, CH₂), 4.10 s (2H, OH), 7.60-8.50 m (6H, H_{ap.}), 9.50 s (1H, OH). FT-IR (KBr, ν_{max}, cm⁻¹): 3260, 2950, 2845, 1697, 1670, 1590, 1535, 1433; LC/MS spectrum: m/z 356.0 [M+H]⁺.

1-[(1E)-3-(5-methyl-1,2-oxazol-3-yl)triaz-1-en-1-ol]anthracene-9,10-dione (23). Yield 65 %, m.p. 250 °C. C₁₈H₁₂N₄O₃; ¹H NMR (DMSO-d₆) δ, ppm: 2.50 s (3H, CH₃), 6.55 s (1H, CH), 7.20-8.70 m (7H, H_{ap.}), 9.50 s (1H, NH). LC/MS spectrum: m/z 333.0 [M+H]⁺.

1-[(1E)-3-(1,3,4-thiadiazol-2-yl)triaz-1-en-1-ol]anthracene-9,10-dione (24). Yield 71 %, m.p. 270 °C. C₁₆H₉SN₅O₂; ¹H NMR (DMSO-d₆) δ, ppm: 7.01-8.50 m (7H, H_{ap.}), 9.10 s (1H, CH), 10.10 s (1H, NH). LC/MS spectrum: m/z 336.0 [M+H]⁺.

1-[(1E)-3-(1-ethylpiperidin-4-yl)triaz-1-en-1-ol]anthracene-9,10-dione (25). Yield 85 %, m.p. 280 °C. C₂₀H₂₁N₅O₂; ¹H NMR (DMSO-d₆) δ, ppm: 2.00 t (3H, CH₃), 3.00-3.50 m (10H, CH₂), 7.20-8.50 m (7H, H_{ap.}), 11.02 s (1H, NH). LC/MS spectrum: m/z 363.1 [M+H]⁺.

1-[(1E)-3-(2-hydroxyethyl)triaz-1-en-1-ol]-4-(1H-imidazol-1-ol)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (26). Yield 95 %, m.p. 250 °C. C₁₉H₁₅N₅O₆S; ¹H NMR (DMSO-d₆) δ, ppm: 3.38 t (6H, CH₃), 3.58 q (4H, CH₂), 7.89 - 8.20 m (8H, H_{ap.}), 9.33 s (1H, OH).

1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-(1H-imidazol-1-ol)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (27). Yield 62 %, m.p. 265 °C. C₂₁H₁₉N₅O₇S; ¹H NMR (DMSO-d₆) δ, ppm: 2.99 t (4H, CH₂), 3.65 t (4H, CH₂), 4.03 s (2H, OH), 7.88 - 8.17 m (8H, H_{ap.}), 9.40 s (1H, OH). ¹³C NMR, δ, ppm: 49.49 (CH₂); 56.81 (CH₂-OH); 113.37, 120.16, 121.03, 124.12, 124.21, 126.65, 126.93, 132.90, 133.11, 133.73, 134.42, 135.15, 135.24, 137.04, 137.83 (C_{ar}); 182.58, 184.60 (C=O). FT-IR (KBr, ν_{max}, cm⁻¹): 1450 (N=N), 1600 (C=N), 1680 (C=O).

4-(1H-imidazol-1-ol)-1-[(E)-(morpholin-4-yl)diazenyl]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (28). Yield 94 %, m.p. 270-271 °C. C₂₃H₂₂N₅O₇S; ¹H NMR (DMSO-d₆) δ, ppm: 3.70-3.77 m (8H, CH₂), 7.70 - 8.20 m (8H, H_{ap.}), 9.33 s (1H, OH).

1-[3,3-Bis(2-hydroxyethyl)triaz-1-en-1-yl]-4-[(2-hydroxyethyl)amino]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (29). Yield 75 %, mp 280 °C (methanol); Ref.¹².

2-[3,3-Bis(2-hydroxyethyl)-1-triaz-1-en-1-yl]-anthracene-9,10-dione (32). Yield 83%, mp 165°C. ¹H NMR (DMSO-d₆) δ, ppm: 3.77 t (4H,

CH₂), 3.94 t (4H, CH₂), 7.58 d (1H, H¹, J 7.3 Hz), 7.75 t (1H, H³, J 7.7 Hz), 7.85 t (2H, H^{6,7}, J 7.4 Hz), 8.04 d (1H, H⁴, J 7.7 Hz), 8.14 d (2H, H^{5,8}, J 8 Hz). Found, %: C 62.87; H 5.10; N 11.87. C₁₈H₁₇N₃O₄. Calculated, %: C 63.72; H 5.01; N 12.39.

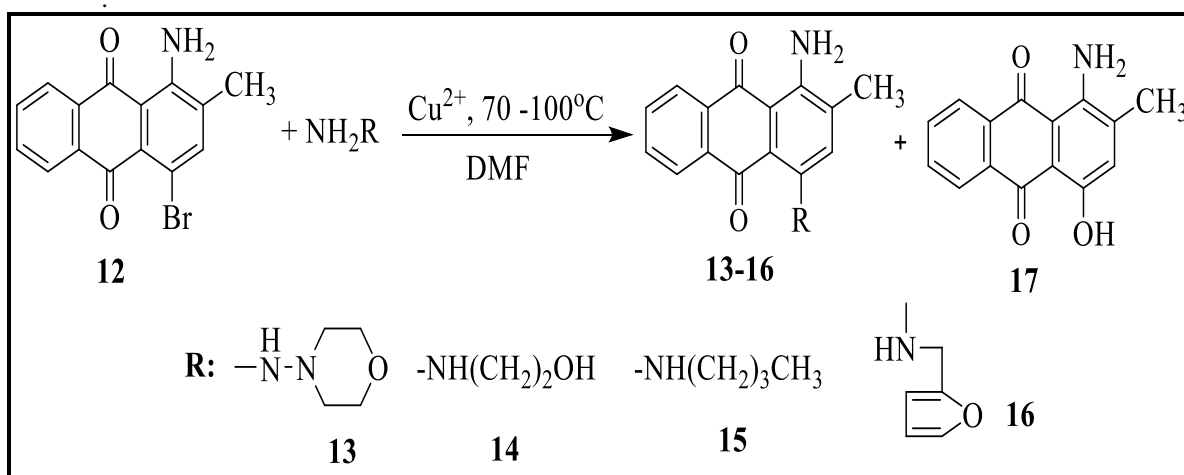
1-[3,3-Bis(2-hydroxyethyl)-1-triaz-1-en-1-yl]-anthracene-9,10-dione (33). Yield 91 %, mp 157-158 °C (methanol); Ref.¹³ mp 160 °C.

1-(Morpholinodiazenyl)anthracene-9,10-dione (34). Yield 80 %, mp 167-168 °C (methanol); Ref.¹³ mp 169 °C.

1-[3-(Benzoic acid)triaz-1-en-1-ol]-4-[1H-imidazol-1-yl]-9,10-dioxo-9,10-dihydroanthracene-2-sulfonic acid (35). Yield 74 %, m.p. 275-276 °C. ¹H NMR (DMSO-d₆) δ, ppm: 7.22 - 8.22 m (12H, H_{ap.}), 9.25 s (1H, OH), 9.69 s (1H, NH), 12.50 s (1H, COOH); ¹³C NMR, δ, ppm.: 124.13, 124.18, 124.21, 126.69, 126.74, 131.61, 131.68, 135.06, 135.11, 135.20, 137.04, 137.12, 137.89, 138.00, 149.79, 149.84, 152.73, 152.84 (C_{ar}); 167.95, 182.58, 184.59 (C=O). LC-MS: m/z 493 [M]⁺.

Results and Discussion

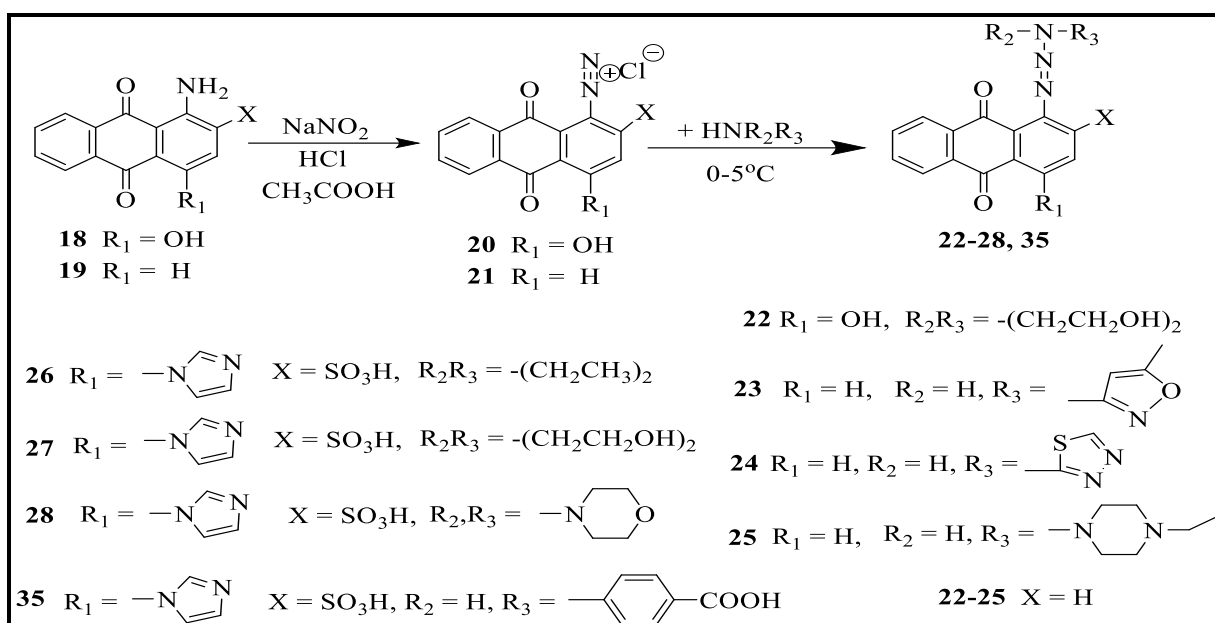
Biogenic amines of different classes (with potentially high antimicrobial activity) were introduced into the 4-position of commercially available bromaminic acid and its 2-methyl derivative. 4-Substituted derivatives based on bromaminic acid were synthesized and described by us earlier^(11, 12). Nucleophilic substitution of bromine in the 2-methyl derivative **12** was performed in DMF. The yield of compounds **13-16** was not high (30-65 %) the main undesirable product was 4-hydroxy derivative **17**, the structure was confirmed by ¹H NMR spectra



Scheme 1. General synthesis of 4-substituted derivatives of 9,10-anthraquinone

In compounds **13-15** in ^1H NMR spectra there are chemical shifts of methylene groups at 2-4 ppm. In the case of furfurylamine **16** at high temperatures (70-100 °C) its polymerization took place, and at room temperature the reaction took a very long time with a yield of 30%. In the ^1H NMR

spectra of compound **16**, furfurylamine protons are present at 7-9 ppm and chemical shift of CH_2 at 2.5 ppm. In the ^{13}C NMR spectrum of the methylene group with a chemical shift at 39.08 ppm, as well as carbon signals of two carbonyl groups at 181.43 and 186.69 ppm.

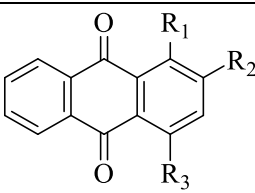



Scheme 2. General synthesis of anthraquinone triazenes

Synthesis of triazenes of the anthraquinone series, the most pressing problems were described in (12, 13). New triazenes were obtained by the reaction of diazotization of aminoanthraquinone derivatives **18, 19** in acetic and hydrochloric acid with sodium nitrite. The final stage is the reaction of addition of amino compound to diazo derivatives **20, 21**, as a result were obtained triazenes **22-25**. The structure of the obtained triazenes **22-25** was confirmed by ^1H NMR spectra. In particular, triazene **22** in the ^1H -

NMR spectrum (DMSO- d_6 + CCl_4) has proton signals of two methylene groups, broad singlets at 2.1 ppm. and 3.2 ppm, as well as shifts in the aromatic region of 7.6-8.5 ppm. six aromatic protons and singlet of protons of phenolic group at 9.5 ppm and hydroxyl groups at 11.3 ppm. And in triazenes **23** and **25** in the ^1H -NMR spectrum (DMSO- d_6), there are signals of protons of the methyl group at 1-2 ppm, as well as shifts in the aromatic region of 7-8.5 ppm.

Table 1. Synthesized compounds for antimicrobial studies

№ Compound	Formula		
			
	R ₁	R ₂	R ₃
14*	NH ₂	CH ₃	NHCH ₂ CH ₂ OH
16*	NH ₂	CH ₃	
22*	N=N-N(CH ₂ CH ₂ OH) ₂	H	OH
23*	N=N-NH-C=N-O-C(CH ₃)=CH (Het)	H	H
24*	N=N-NH-C=N-N=CH-S (Het)	H	H
25*	N=N-NH(CH ₂) ₂ N(CH ₂) ₂ (CH ₂ CH ₃)	H	H
26*	N=N-N(CH ₂ CH ₃) ₂	SO ₃ H	Ar, Het NCH=CH-N=CH (imidazole)
27*	N=N-N(CH ₂ CH ₂ OH) ₂	SO ₃ H	Ar, Het NCH=CH-N=CH (imidazole)
28*	N=N-N(CH ₂) ₂ O(CH ₂) ₂ (cyclic)	SO ₃ H	Ar, Het NCH=CH-N=CH (imidazole)
29	N=N-N(CH ₂ CH ₂ OH) ₂	SO ₃ H	NHCH ₂ CH ₂ OH
30	NH ₂	SO ₃ H	NHCH ₂ CH ₂ OH
31	NH ₂	SO ₃ H	Ar, Het NCH=CH-N=CH (imidazole)
32*	H	N=N-N(CH ₂) ₂ O(CH ₂) ₂ (cyclic)	H
33	N=N-N(CH ₂) ₂ O(CH ₂) ₂ (cyclic)	H	H
34	N=N-N(CH ₂ CH ₂ OH) ₂ (cyclic)	H	H
35*	N=N-NH- <i>n</i> -COOH-C ₆ H ₄	SO ₃ H	Ar, Het NCH=CH-N=CH (imidazole)
36	NH ₂	SO ₃ H	NHCH ₂ CH ₂ CH ₃
37	NH ₂	SO ₃ H	NHCH ₂ CH ₂ CH ₂ CH ₃
38	NH ₂	SO ₃ H	NHCH ₂ (CH ₃) ₂
39	NH ₂	SO ₃ H	NHN(CH ₂) ₂ O(CH ₂) ₂

* - new derivatives

Antibacterial activity

Evaluation of the antimicrobial activity of twenty-three synthesized nitrogen-containing derivatives of anthraquinone showed that Gram-positive microorganisms are more sensitive to the action of compounds (Table 2).

The most pronounced activity against Gram-positive bacteria is shown by compound **35** with a substituent benzoic acid in position 1 of the triazene group and an imidazole fragment in position 4 of the sulfoanthraquinone. Zone of growth retardation of Methicillin-sensitive *Staphylococcus aureus* (MSSA) under the action of compound **35** is (8.98 ± 1.09) mm (p < 0.01 compared to control),

Methicillin-resistant *Staphylococcus aureus* (MRSA) - (8.41 ± 0.43) mm (p < 0.05), *Staphylococcus haemolyticus* (*S. haemolyticus* MS) - (11.50 ± 0.64) mm (p < 0.01), *Staphylococcus epidermidis* (*S. epidermidis* MS) - (9.36 ± 0.22) mm (p < 0.01). The introduction into the position 1 of the triazene group of the substituent diethanolamine (compound **27**) led to a slight decrease in antistaphylococcal activity against *S. aureus* (MSSA), and the introduction of diethyl (**26**) and morpholine (**28**) into the triazene group had no antimicrobial effect. These data allowed us to assume that *S. aureus* (MRSA) is more sensitive to our compounds than *S. aureus* (MSSA).

Table 2. Antimicrobial effect of synthesized compounds on Gram-positive bacteria.

Spp. of bacteria	<i>S. aureus</i> MSSA	<i>S. aureus</i> MRSA	<i>S. haemolyticus</i> MS	<i>S. epidermidis</i> MS	<i>E. faecalis</i>
DMF №	4,70±0,18	6,66±0,35	-*	4,80±0,32	5,16±0,17
14	-	5,33±0,49	-	4,64±0,38	-
16	-	-	-	5,90±0,34 [†]	6,06±0,27 [†]
22	-	6,41±0,45	-	6,50±0,14 [†]	5,62±0,50
23	-	6,42±0,21	-	6,18±0,40 [†]	6,61±0,39 [†]
24	-	5,47±0,65	4,92±0,34	5,11±0,27	6,09±0,45 [†]
25	-	-	4,70±0,22	5,38±0,16	5,32±0,25
26	-	-	-	5,22±0,58	-
27	6,07±0,40 [†]	5,63±0,38	-	5,60±0,21	5,00±0,59
28	-	5,75±0,44	-	-	-
29	-	-	-	-	-
30	-	-	-	-	-
31	4,67±0,38	6,11±0,39	-	4,49±0,65	5,13±0,29
32	5,29±0,30	6,19±0,49	-	4,42±0,38	-
33	4,92±0,39	6,04±0,31	-	4,94±0,09	-
34	-	5,31±0,29	-	-	-
35	8,98±1,09 ^{††}	8,41±0,43 [†]	11,50±0,64 ^{††}	9,36±0,22	9,57±1,28 ^{††}
36	-	-	-	6,11±0,32 [†]	-
37	-	5,92±0,43	5,10±0,47	5,73±0,25 [†]	-
38	-	5,16±0,17	-	4,96±0,40	5,46±0,65
39	5,81±0,66 [†]	4,97±0,36	5,43±0,41	4,86±0,16	4,91±0,31

Notes: * - no zones of growth inhibition; [†] - p < 0,05, ^{††} - p < 0,01 compared with the control.

It was found (Table 2) that in the presence of diethanolamine residue in position 2 of the triazene group of the molecule anthraquinone (compound **22**) there is a pronounced antimicrobial activity against Gram-positive microorganisms, regardless of antibiotic susceptibility of strains (p < 0,05). Moving the diethanolamine substituent to position 1 of the triazene group and the introduction of the hydroxyl radical to position 4 (**22**) does not affect the severity of antimicrobial action against *S. epidermidis* (MS) (p < 0,05). Methicillin-resistant epidermal staphylococci are sensitive to compounds with an amino group in position 1 of the sulfoanthraquinone framework (**36**, **37**) and methylantraquinone ⁽¹⁶⁾ (p < 0,05).

The introduction of a fragment of methyloxazole in position 1 of the triazene group of the anthraquinone molecule **23** leads to antimicrobial action against *S. epidermidis* (MS) and *Enterococcus faecalis*, growth retardation zones are (6.18 ± 0.40) mm and (6.61 ± 0,39) mm, respectively (p < 0,05). Similar activity is shown by compound **16** with a furan derivative at position 4. Antimicrobial activity against *Enterococcus faecalis* was recorded in compounds **35** with a benzoic acid substituent and

24 with a thiodiazole fragment at position 1 of the triazene group.

The results of the experiments show (Table 3) that the test compounds are inactive against Gram-negative bacteria, compound **25** and **35** (is higher effectiveness) inhibits the growth of planktonic microorganisms *E. coli*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, the values of the diameters of the growth retardation zones are (6.54 ± 0.37) mm, (6.46 ± 0.22) mm, (5.87 ± 0.48) mm and (8.18 ± 0.19) mm, respectively (p < 0,05). The introduction in position 4 of the sulfoanthraquinone framework of the aminomorpholine substitute **39** does not affect the severity of antibacterial activity against test strains of *E. coli* (7.10 ± 0.35 mm) and *K. pneumoniae* (5.66 ± 0.35 mm), p < 0,05 compared to control (probably due to the low activity of the aminomorpholine fragment). Compound **29** with a diethanolamine substituent at position 1 of the triazene group and a fragment of ethanolamine at position 4 of the sulfoanthraquinone and **24** with a thiodiazole substituent at position 1 of the triazene group show little activity against *E. coli* plankton cells probably due to the lack of a sulfo group that imparts solubility.

Table 3. Antimicrobial effect of synthesized compounds on Gram-negative bacteria

Spp. of bacteria	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Providencia stuartii</i>	<i>P. aeruginosa</i>
DMF				
No	5,03±0,18	-*	4,46±0,47	6,84±0,57
14	5,74±0,67	-	-	5,06±0,29
16	5,31±0,14	4,53±0,37	-	-
22	-	-	-	-
23	5,68±0,41	4,93±0,34	-	4,81±0,17
24	6,53±0,16 [†]	5,02±0,36	-	4,57±0,21
25	5,50±0,27	4,76±0,24	5,50±0,41	4,73±0,43
26	5,16±0,35	-	-	-
27	4,61±0,37	-	5,11±0,21	-
28	-	-	4,80±0,40	-
29	5,33±0,18 [†]	-	-	6,0±0,24 [†]
30	5,69±0,51	-	-	5,91±0,52
31	5,28±0,28	-	-	-
32	5,02±0,14	-	4,79±0,17	-
33	5,46±0,32	-	-	-
34	6,10±0,38 [†]	-	-	-
35	6,54±0,37 [†]	6,46±0,22 [†]	5,87±0,48 [†]	8,18±0,19 [†]
36	4,53±0,27	-	-	-
37	5,36±0,32	5,31±0,30	-	5,09±0,80
38	5,34±0,20	5,11±0,18	5,35±0,35	-
39	7,10±0,35 [†]	5,66±0,35 [†]	4,97±0,32	-

Notes: * - no zones of growth inhibition; [†] - p < 0,05, ^{††} - p < 0,01 compared with the control.

Fungicidal properties were found highest in compound **35** (Table 4), the diameters of the growth retardation zones increased by 2.5 mm (*C. albicans*)

and 1.6 mm (*C. tropicalis*) compared to the control (p < 0.05).

Table 4. Antimicrobial effect of synthesized compounds on fungi activity

Spp. of fungi	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. lipolytica</i>
DMF			5,27±0,29
No	5,80±0,31	5,45±0,6	
14	-	-	-
16	4,61±0,57	4,93±0,46	6,04±0,16
22	4,99±0,34	4,93±0,28	4,98±0,37
23	4,95±0,24	5,18±0,57	-
24	6,13±0,37	4,45±0,33	5,15±0,58
25	-	-	-
26	-	-	-
27	5,57±0,49	-	-
28	5,13±0,73	-	-
29	5,57±0,85	-	-
30	5,79±0,52	-	-
31	5,00±0,12	-	-
32	6,08±0,32	-	-
33	-	-	-
34	5,24±0,48	-	-
35	7,48±0,17 [†]	7,06±0,69 [†]	5,81±0,51
36	5,54±0,63	5,01±0,37	4,96±0,39
37	4,88±0,54	4,54±0,28	-
38	-	4,68±0,46	-
39	5,63±0,46	-	-

Notes: * - no zones of growth inhibition; [†] - p < 0,05, ^{††} - p < 0,01 compared with the control.

Conclusion

4-Substituted derivatives based on bromaminic acid and new triazenes based on anthraquinone were synthesized. Experimental data from the study of new nitrogen-containing anthraquinone derivatives have established some patterns of "structure-activity", and identified a leader compound with antimicrobial and fungicidal action.

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