

Antibacterial Activity of Synthesized Derivatives of Purpurin Containing Cyclopropane and Cyclobutane Fragment

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Abstract

The present study is a continuation of the scientific research works for the synthesis of anthraquinone-containing derivatives with cyclic ring systems by the interaction of 1,2,4-trihydroxyanthraquinone (purpurin) with cyclopropanecarboxylic acid chloride and cyclobutanecarboxylic acid chloride by using the ultrasonic treatment. Esters of purpurin, studied for antibacterial activity in museum test strains of microorganisms (*Staphylococcus aureus* ATCC 6538-P, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231) in vitro with the determination of the minimum inhibitory concentration (MIC) value. The study revealed that the cyclopropane substituent exhibits moderate antibacterial activity against *Bacillus subtilis* ATCC 6633. While with the cyclobutane moiety it had a weak effect with respect to *Pseudomonas aeruginosa* ATCC strain. It has been found that the compounds with the cyclopropane and cyclobutane fragment have possessed antimicrobial activity in relation to strains of microorganisms *Staphylococcus aureus* ATCC 6538 for which the MIC value was 62.5 µg/ml.

1. Introduction

The search for novel compounds with antimicrobial action refers to a priority direction in the development of new anti-infectious drugs. Currently, infectious diseases rank 3–4 in the ranking of causes of death [1] and are becoming a global health problem. The need to search for new antibacterial drugs, despite the large range of available drugs, is associated primarily with the high adaptability of pathogens to them, including antibiotics, with the emergence of new strains of pathogens, multi-resistant forms [2–4]. Antibiotic resistance is present worldwide and new resistance mechanisms are continuing to emerge, strongly increasing the risk of spread of resistant strains. Thus, antibiotic resistance poses a threat to public health worldwide and represents an important economic challenge due to higher medical costs of essential treatments and increased duration of disease, treatment, and potential hospitalization compared to non-resistant common infections.

Nature is a nearly inexhaustible source of novel therapeutic compounds. The discovery of new antibacterial molecules plays a key role in solving the current problem of the antibiotic crisis. From the earliest times, many plants have been known to exhibit healing properties against human infections due to their content of secondary metabolites, which have recently been found to act as antimicrobial agents against human pathogens. Over the past decade, much attention has been paid to the study of phytochemicals for their antibacterial activity, especially against gram-negative and gram-positive multidrug-resistant bacteria [5, 6]. In recent years, many studies have shown that phytochemicals exhibit their antibacterial activity through various mechanisms of action, such as damage to the bacterial membrane and suppression of virulence factors, including inhibition of enzyme and toxin activity and formation of bacterial biofilm [7].

Currently, great attention is paid to ethnopharmacological research [5] and the search for new phytochemicals with antimicrobial activity [8–11] among secondary plant metabolites, including

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alkaloids, terpenoids, phenolic acids, flavonoids, tannins, quinones [8–14], as well as lichen derivatives [15], marine organisms [16], etc. Anthraquinones are a group of polycyclic organic natural compounds consisting of three rings with two keto groups located at the central ring. They comprise a class of colorful, biologically abundant secondary metabolites in plants, bacteria and fungi [17]. Pharmacological effects of anthraquinones include among others a range of antiviral antibacterial, pro-apoptotic, anti-oxidant, pro-oxidative, phototoxic, anti-proliferative, and anti-cancer effects [18, 19]. These classes of compounds represent promising sources of antimicrobial agents [20].

2. Experimental part

2.1. Experimental chemical part

2.2.1. Materials and instrumentation

The progress of the reaction and the purity of the obtained compounds were monitored by TLC on Silufol UV-254 plates in various solvent systems. IR spectrum was recorded on a Nicolet 5700 spectrometer in KBr pellets. ^1H NMR spectra were recorded on spectrometer MSL-400 «Bruker» with an operating frequency of 400 MHz for protons at room temperature. The melting point is determined on the device “Boetius”. The reaction products were separated on silica gel of the Silica Gel 60 brand (Merck, Germany). Ultrasonic activation was carried out on a Cole-Parmer ultrasonic cleaner (100 W, 42 KHz). The reagents were used as received from commercial suppliers unless otherwise stated (Aldrich).

2.2.2. The method of synthesis derivatives 4, 5

Purpurin (1.2.4-trihydroxy-9.10-anthraquinone) (1) (0.001 mol) was dissolved in 50 ml of pyridine. After that, in the reaction flask during 15 min 0.001–0.003 mol and cyclopropanecarboxylic acid chloride (2) or cyclobutanecarboxylic acid (3) was added. The reaction was carried out with vigorous stirring while heating the reaction mixture (30–65 °C) by monitoring the progress of the reaction using TLC. The reaction was performed by using ultrasonic activation. In various experiments, the temperature regime and the ratio of reactants varied. To select the technique, we used three temperature regimes of 30 °C, 45 °C and 65 °C, as well as a different ratio of reactants 1:1, 1:2 and 1:3

(purpurin (1):chloride (2 or 3)). After the reaction (2–2.5 h), part of the solvent was removed, the resulting concentrated solution was treated with water, acidified with HCl, the precipitate was filtered and dried. The reaction products were isolated by silica gel column chromatography. It was suitable hexane with a gradual transition to a mixture of hexane-ethyl acetate (with a gradient from 100:1 to 1:5, v/v). Recrystallization was carried out from a mixture of hexane-ethyl acetate (1:5). The physical characterization and elemental analysis data are presented in Table 1.

2-O-cyclopropanecarbonyloxy-1,4-dihydroxy-9,10-anthraquinone 4 was prepared by reacting 1 (2.56 g, 0.01 mol) with cyclopropanecarboxylic acid chloride (2, 1.82 mL, 0.02 mol). Yield 67%. M.p. 124–126 °C. IR spectrum (KBr, ν , cm^{-1}): 1761 ($\text{C}=\text{O}_{\text{sub}}$), 1670, 1627 ($\text{C}=\text{O}_{\text{anth}}$), 1586 ($\text{C}=\text{C}$, Ar). ^1H NMR spectrum (400 MHz, DMSO-d_6 , δ , ppm): 6.41 (s, H-3), 7.68 (m, H-6,7), 7.91 (m, H-5,8), 1.08 (m, 2H), 1.25 (m, 2H), 2.10 (m, 1H), 13.24 (s, α -OH), 13.28 (s, α -OH).

2-O-cyclobutanecarbonyloxy-1,4-dihydroxy-9,10-anthraquinone 5 was prepared by reacting 1 (2.56 g, 0.01 mol) with cyclobutanecarboxylic acid chloride (3, 2.28 mL, 0.02 mol). Yield 48%. M.p. 134–136 °C. IR spectrum (KBr, ν , cm^{-1}): 1762 ($\text{C}=\text{O}_{\text{sub}}$), 1668, 1627 ($\text{C}=\text{O}_{\text{anth}}$), 1586 ($\text{C}=\text{C}$, Ar). ^1H NMR spectrum (400 MHz, DMSO-d_6 , δ , ppm): 6.40 (s, H-3), 7.69 (m, H-6,7), 7.91 (m, H-5,8), 1.98 (m, 2H), 2.22 (m, 2H), 2.64 (m, 1H), 3.43 (m, 2H), 13.25 (s, α -OH), 13.30 (s, α -OH).

2.2. Experimental biological part

The compounds 1, 4, 5 were studied for antibacterial activity in relation to the museum strains of microorganisms, the effects of these preparations *in vitro* concerning *Staphylococcus aureus* ATCC 6538-P, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and fungus *Candida albicans* ATCC 10231 were studied. The test strains used in the study were obtained from the American Type Culture Collection. The antimicrobial activity of the compounds was determined by methods of diffusion into agar (wells) and by two-fold serial dilutions in the nutrient broth – determination of a minimum inhibitory concentration of the studied substance. More detailed information and biological research methodology can be found in the publication [21].

3. Results and discussion

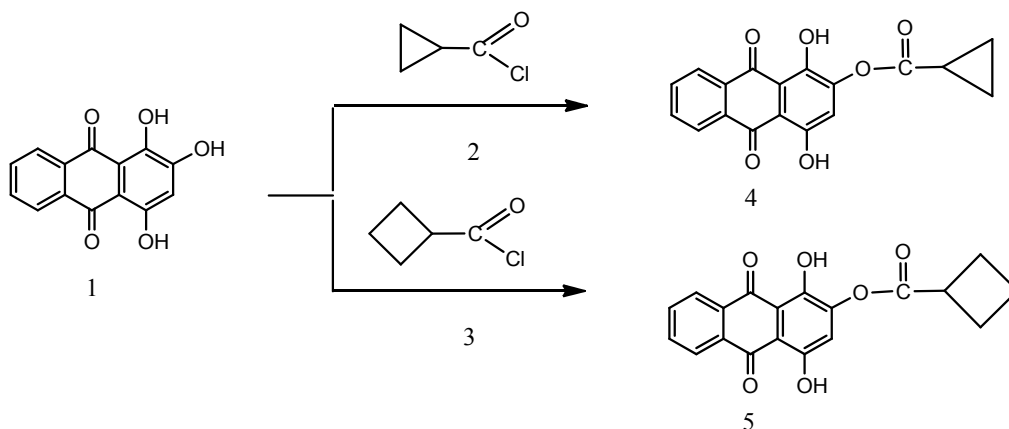
The rational way of searching for effective biologically active compounds (BAS) is the directed construction of new molecules from pharmacophore structural fragments based on natural biologically active compounds, which have established themselves as convenient reactive building blocks in organic synthesis.

The present study is a continuation of the scientific research works for synthesis and of derivatives of 9,10-antraquinone derivatives [22–24] and is devoted to the study of the antimicrobial activity of purpurin derivatives containing cyclopropane- and cyclobutane fragments.

An interest in the chemistry of 9,10-antraquinone derivatives has been aroused by the fact, that they have pharmacological activity and they are included in the structure of many natural and

synthetic medicinal preparations. Compounds of particular interest were anthraquinone derivatives with different functional groups (carboxy, hydroxy, amino, etc.), whose presence causes different biological effects. Attention is drawn to the interaction of a thin chemical structure of a substance with its activity, which is an important stage of the directed synthesis of physiologically active preparations [18]. Recently, there has been an increased interest in compounds containing fragments of saturated cyclic carboxylic acids in the structure due to biological and pharmaceutical applications [25–27].

Purpurin (1,2,4-trihydroxy-9,10-antraquinone) (1) is a biologically active anthraquinone derivative which found in the roots of the madder (*Rubia tinctorum*) and the Asian madder (*Rubia akane*) [28]. For the synthesis of derivatives (4, 5) we have used purpurin (1) as the starting material:



In recent years, great successes have been achieved in the process of extracting various biologically active substances from natural materials [33, 34], as well as in intensifying and increasing the effectiveness of various chemical reactions using physical factors, including the use of ultrasound [29, 30]. The use of ultrasound in chemical synthesis is one of the promising methods since ultrasound has a significant effect on the speed and direction of reactions, and in some cases, the use of ultrasound improves the selectivity of chemical processes [31–34].

Synthesis of compounds 4, 5 has been performed in one stage. To activate the process, ultrasound was used. The reaction mixture was heated at a temperature range of 30–65 °C. To select the technique, we used three temperature regimes of 30 °C, 45 °C and 65 °C, as well as a different ratio of reactants 1:1, 1:2 and 1:3 (purpurin (1):chloride (2 or 3)). The synthesis was carried out with vig-

orous stirring and heating of the reaction mixture. The results of the study showed that the use of ultrasound reduces the synthesis time. Thus, if we use the conventional reaction variant, the synthesis time varies within 3–3.5 h, whereas during ultrasonic activation the process was completed within 2–2.5 h. After each synthesis, the reaction mixture was separated to isolate the monosubstituted derivative. Column chromatography was used for this purpose. The column was eluted with a saturated yellow colored zone corresponding to the monosubstituted derivative. The results showed that the best synthesis option was achieved using a 1:2 ratio of reactants and heating to 45 °C. The yields of products 4 and 5 were 57 and 41% without ultrasound and 67 and 48% with ultrasonic activation, respectively. An increase in the ratio of reactants to temperature conditions leads to the formation of polysubstituted compounds, which makes it difficult to isolate monosubstituted products 4 or 5.

Table 1
Physical characterization of compounds

Compound code	R_f	Color	Molecular formula/ molecular weights	Elemental analysis			
				Found, %		Calculated, %	
				C	H	C	H
4	0.62 (hexane-ethyl acetate, 2:1)	Orange	$C_{18}H_{12}O_6$ M.w. 324.12	60.62	3.52	60.70	3.70
	0.74 (CCl ₄ -acetone, 7:3)						
5	0.65 (hexane-ethyl acetate, 2:1)	Orange	$C_{19}H_{14}O_6$ M.w. 338.13	64.34	3.95	64.49	4.14
	0.76 (CCl ₄ -acetone, 7:3)						

The physical and spectral characteristics of the compounds obtained in the traditional synthesis, as well as using the ultrasonic treatment, were identical [35]. The physical characteristics and elemental analysis data are presented in Table 1.

The compounds 4, 5 were studied for antibacterial activity concerning the museum strains of microorganisms. The effects of these preparations *in vitro* concerning *Staphylococcus aureus* ATCC 6538-P, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 were studied. The results of the study are presented in Table 2.

According to the data from Table 2 purpurine has a moderately pronounced antimicrobial activity at a concentration of 1 mg with the test strains of *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231. It has been found, these compounds (4) and (5) have antimicrobial activity concerning the museum strain of microorganisms *Staphylococcus aureus* ATCC 6538 with a growth inhibition zone of 17 ± 0.1 and 16 ± 0.1 , respectively. The presence of a cyclopropane fragment in the structure of purpurin (compound 4) slightly increases

the activity to *Bacillus subtilis* ATCC 6633 with a growth inhibition zone of 16 ± 0.2 . Of all the tested samples, only compound (5) showed weak activity to *Pseudomonas aeruginosa* ATCC 27853 strains (12 ± 0.2). All other derivatives were inactive both to *Pseudomonas aeruginosa* ATCC 27853 strains and to *Escherichia coli* ATCC 25922.

The synthesis of purpurin esters containing the cyclopropane and cyclobutane fragment did not affect the activity of the yeast *Candida albicans* ATCC 10231. Thus, according to the obtained experimental data, the growth inhibition zone was 15 ± 0.1 (0.2) for all tested compounds.

A comparative analysis of the antibacterial activity of other purpurin esters containing a cyclic fragment showed these compounds containing a cyclopropane, cyclobutane, cyclopentane and cyclohexane fragment showed activity against *Staphylococcus aureus* ATCC 6538, the most active substance with a cyclohexane fragment for which the growth inhibition zone was 19 ± 0.1 [21]. Derivatives containing the cyclopropane (4), cyclobutane (5) fragment showed activity concerning *Candida albicans* ATCC 10231, while substances with the cyclopentane and cyclohexane fragment were inactive [21].

Table 2
The results of studies of antimicrobial activity compounds

Compound code	Concentration	Bacteria strains and diameter of the zone of inhibition of growth of test cultures, mm				
		<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6633	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Candida albicans</i> ATCC 10231
1	1 mg	17 ± 0.1	13 ± 0.1	-	-	15 ± 0.1
4	1 mg	17 ± 0.2	16 ± 0.2	-	-	15 ± 0.1
5	1 mg	16 ± 0.1	-	-	12 ± 0.2	15 ± 0.2
Benzylpenicillin sodium salt	1 mg	16 ± 0.1	15 ± 0.1	15 ± 0.1	14 ± 0.1	-
Nystatin	1 mg	-	-	-	-	21 ± 0.2

* «-» No activity

Table 3
The results of studies of MIC compounds

Compound code	MIC, µg/ml				
	<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6633	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Candida albicans</i> ATCC 10231
1	62.5	250	-	-	125
4	62.5	125	-	-	125
5	62.5	-	-	500	125
Benzyl-penicillin sodium salt	62.5	125	125	250	-
Nystatin	-	-	-	-	31.25

* «-» No activity

Also, samples (4, 5) of derivatives of 9,10-anthraquinone were tested to determine the minimum inhibitory concentration (MIC). The results of the experiment are presented in Table 3. A study of the antimicrobial activity to the presented compounds (4, 5) concerning the reference strains: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and to the yeast fungus *Candida albicans* ATCC 10231, demonstrated these compounds (4) and (5) showed a moderately pronounced antibacterial effect to the reference strain of *Staphylococcus aureus* ATCC 6538, the MIC for this testing culture was 62.5 µg/ml. The activity of the sample (4) was also observed with the *Bacillus subtilis* ATCC 6633 strain, and the MIC value was 125 µg/ml. The results of the study of the antimicrobial activity of the compounds (4) and (5) showed the efficiency to the test strain with *Candida albicans* ATCC 10231 MIC = 125 µg/ml.

Comparative analysis of the MIC values of (4, 5) compounds and derivatives containing cyclopentane and cyclohexane fragment [21] showed that for the compound containing cyclopentane fragment, the MIC value relative to the strain *Staphylococcus aureus* ATCC 6538 was 125 µg/ml, and against the strain of *Bacillus subtilis* ATCC 6633 MIC = 62.5 µg/ml. The results of the study of the antimicrobial activity of the compound with cyclohexane moiety [21] showed the high efficiency of this compound to the test strain *Staphylococcus aureus* ATCC 6538 with MIC = 31.25 µg/ml. While the MIC value to the strain *Escherichia coli* ATCC 25922 was 250 µg/ml.

4. Conclusions

This study is the first study of the antimicrobial

activity of purpurine derivatives containing cyclopropane- and cyclobutanecarboxylic acid fragments (4, 5). It was shown that purpurin (1) and its derivatives (4, 5) have a moderately pronounced antimicrobial activity against *Staphylococcus aureus* ATCC 6538 for which the MIC value was 62.5 µg/ml. Compound (4), in comparison with purpurine (1), was also active against the strain of *Bacillus subtilis* ATCC 6633 strains, which can be associated with the presence of a cyclopropane moiety. At the same time, compound (5) containing the cyclobutane fragment had a weak effect against the *Pseudomonas aeruginosa* ATCC 27853 strain.

These compounds can be recommended for the next stage of the study to assess their acute toxic effect. In the presence of favorable toxicological characteristics, compounds (4) and (5) can be used as pharmacological substances for the development of antimicrobial agents.

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