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ANTIMICROBIAL ACTIVITY OF HYBRIDS OF COUMARIN'S DERIVATIVES WITH NEUROTRANSMITTERS

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Abstract: In this study, the antimicrobial activity of five hybrids of 3-acetyl-4-hydroxy coumarin with neurotransmitters was determined: dopamine (1), tyramine (2), octopamine (3), norepinephrine (4) and methoxy-tyramine (5). Antimicrobial activity was assessed using the microdilution method incorporating resazurin, with determination of MIC/MMC concentrations. Testing involved twelve microorganisms, five of which were sourced from mine wastewater. The best activities were shown by substances (1) and (4) on *Bacillus pumilus* and *Pantoea agglomerans*, where the MIC was 31.8 μg/mL. *Pantoea agglomerans* showed the highest sensitivity to all tested substances, except (5). Tested substances didn't show activity on standard strains of bacteria and yeast. In general, substances act above the range of positive controls and have selective and limited activity.

Keywords: antimicrobial activity, standard strains, natural isolates, coumarin hybrids

Introduction

Resistance patterns of pathogenic bacteria to commonly used drugs and antibiotics have become commonplace today, creating disruptions in healthcare worldwide. Natural products, especially phytochemicals, such as curcumin and several others, are often used as antibacterial, antifungal, and anticancer agents in medicinal chemistry. For example, coumarin carboxamide derivative - "novobiocin" and chlorobiocin, aminocoumarin and several others are

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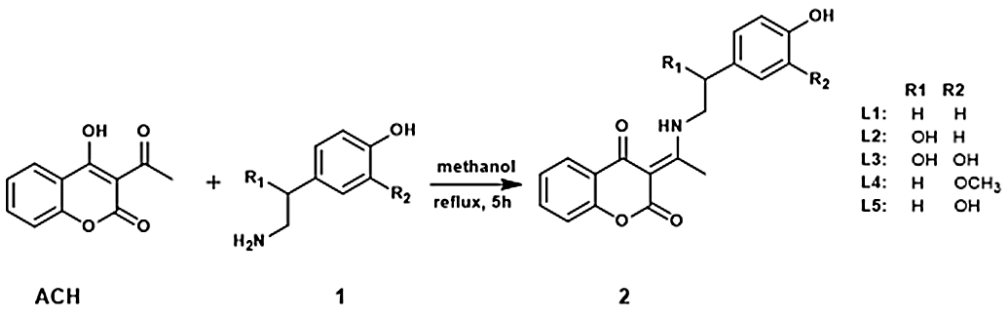
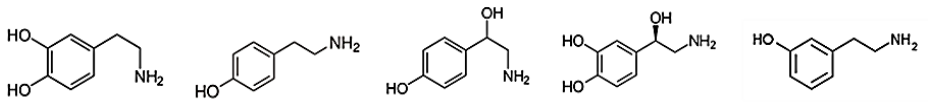
commercial antibiotics. A certain number of antibacterial candidates, coumarin derivatives, were developed by attaching several substituents (for example as functional groups, by incorporating and complexing metal ions in different positions of coumarin, etc.) (Sahoo et al., 2021). Therefore, the aim of this research is to examine the antimicrobial activity of synthesized neurotransmitter-coumarin derivatives.

Materials and methods

All chemicals, 4-hydroxycoumarin, acetic acid, methanol, ethanol, toluene, dimethyl sulfoxide, phosphoroxy chloride, dopamine hydrochloride, tyramine hydrochloride, norepinephrine hydrochloride, octopamine hydrochloride, 3-methoxy-tyramine hydrochloride were obtained from Sigma Aldrich, Germany. The first step is the synthesis of the heterocyclic compound 3-acetyl-4-hydroxycoumarin (Avdović et al. 2021). In the next step, in the reaction of 3-acetyl-4-hydroxycoumarin (AHC) (1 mmol) with different neurotransmitters 1 (2 mmol) (dopamine hydrochloride, tyramine hydrochloride, norepinephrine hydrochloride, octopamine hydrochloride, 3-methoxy-tyramine hydrochloride) were heated in methanol for 5 h (Dimić et al. 2022). The course of the reaction was monitored by thin-layer chromatography. When the reaction was complete, the resulting mixture was cooled to room temperature and the precipitate was collected by filtration. The resulting products 2 were subsequently purified from ethanol (Scheme 1). The neurotransmitters used in this work are shown in Figure 1.



Scheme 1. Synthesis of neurotransmitter-coumarin derivatives



dopamine (1) tyramine (2) octopamine (3) norepinephrine (4) methoxy-tyramine (5)

Figure 1. Neurotransmitters used in this work for the synthesis of coumarin derivatives

In vitro antimicrobial test

Test substances, microorganisms and preparation of the suspension

The antimicrobial activity of the tested hybrid of 3-acetyl-4-hydroxy coumarin with neurotransmitters: dopamine (1), tyramine (2), octopamine (3), norepinephrine (4) and methoxy-tyramine (5) was tested on twelve microorganisms: six standard strains (two gram-positive, three gram-negative bacteria, and one yeast) as well as six isolates from nature (four gram-positive and two gram-negative bacteria) (Table 1). All clinical isolates were a generous gift from the Institute of Public Health Kragujevac. Other microorganisms were provided from the collection of the Microbiology Laboratory of the Faculty of Science, University of Kragujevac. Five isolates originated from mine wastewater. Isolation and identification is described in the work of Branković et al (2022).

Bacterial suspensions were prepared by the direct colony method. The turbidity of the initial suspension was adjusted using a densitometer (DEN-1, BioSan, Latvia). When adjusted to a turbidity of 0.5 McFarland standard (Andrews, 2005), the bacterial suspension contains about 10^8 colony forming units (CFU/mL), and the yeast suspension contains 10^6 CFU/mL. Tenfold dilutions of the initial suspension were additionally prepared in sterile 0.85% saline. Bacterial suspensions were obtained from bacterial cultures incubated for 24 h at 37°C on Mueller–Hinton agar. Yeast suspensions were prepared from two-day-old yeast cultures grown at 30°C on Saburo dextrose agar.

Microdilution method

Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and the minimum microbicidal concentration (MMC) using the microdilution method with resazurin (Sarker et al. 2007). 96-well plates were prepared by adding 100 μL of Mueller–Hinton broth for bacteria

and Saburo dextrose broth for fungi. An aliquot of 100 μL of the stock solution of the tested compounds (concentration 2000 μg/μL) was added to the first row of the plate. Two-fold serial dilutions were then performed using a multichannel pipette. The concentration range obtained was from 1000 to 7.8 μg/μL. The method is described in detail in the published paper (Radić et al. 2012).

Doxycycline and fluconazole were used as positive controls. Each test included a growth control and a sterility control. It was noted that 10% DMSO (as a control test solvent) did not inhibit the growth of microorganisms. All assays were performed in triplicate and the MICs were constant (Figure 2).

Minimum microbicidal concentrations were determined by placing 10 μL samples from wells in which no indicator color change was noted, or mycelial growth was not noted, on agar medium. At the end of the incubation period, the lowest concentration without growth (no colony) was defined as the minimum microbicidal concentration.

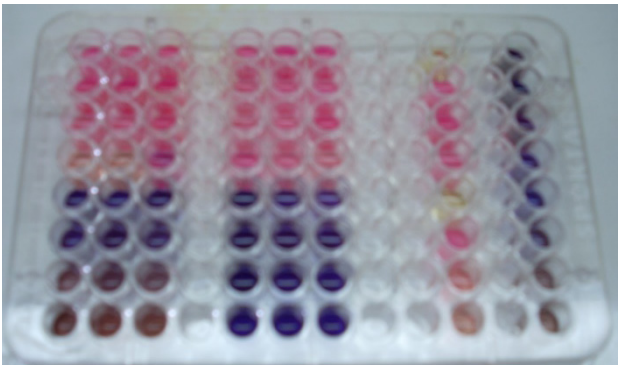


Figure 2. Microdilution method - example of results

Results and discussion

The results of antimicrobial activity testing are shown in Table 1. Coumarin derivatives with selected neurotransmitters have significant antimicrobial activity on isolates from mine waste water. The same tested substances have no activity on the tested standard strains (both gram-positive and gram-negative bacteria, as well as yeast). The minimum inhibitory concentrations and the minimum microbicidal concentrations for standard strains are in the range of 1000/>1000 μg/mL. The best activity was demonstrated by substances 1

(coumarin derivative with dopamine) and 4 (coumarin derivative with norepinephrine) on *Bacillus pumilus* and *Pantoea agglomerans* where the MIC was 31.8 μg/mL. The same substances also work well on isolates of *Bacillus altitudinis* and *Bacillus cereus*. *Pantoea agglomerans* showed the highest sensitivity to all tested substances with the exception of test substance 5 (coumarin derivative with methoxy-tyramine). Generally, the substances act above the range of positive controls and have selective and limited activity.

Table 1. Antimicrobial activity of tested compounds and positive controls

Species	1		2		3		4		5		D/F	
	MIC[1]	MMC[2]	MIC	MMC	MIC	MIC	MIC[1]	MMC[2]	MIC	MMC	MIC	MMC
Bacillus subtilis ATCC 6633	>1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	31.3	62.5
Sarcina lutea	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	<0.5	7.8
Staphylococcus aureus ATCC 25923	1000	1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	2	15.6
Pseudomonas aeruginosa ATCC 27853	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	1000	1000	7.812	62.5
Proteus mirabilis ATCC 12453	1000	1000	>1000	>1000	>1000	>1000	1000	1000	1000	1000	125	125
Escherichia coli ATCC 25922	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	>1000	>1000	1	4
Escherichia coli	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	2	4
Bacillus altitudinis	31.8	31.8	1000	1000	1000	>1000	125	500	62.50	>1000	nt	nt
Bacillus pumilus	31.8	31.8	1000	>1000	>1000	>1000	31.8	500	125	>1000	nt	nt
Pantoea agglomerans	31.8	31.8	62.50	500	31.8	31.8	31.8	250	1000	1000	nt	nt
Bacillus cereus	125	125	1000	>1000	1000	>1000	125	500	250	>1000	<0.5	3.9
Pseudomonas veronii	500	500	1000	>1000	>1000	>1000	1000	1000	>1000	>1000	nt	nt
Candida albicans ATCC 10231	>1000	>1000	1000	>1000	>1000	>1000	>1000	>1000	1000	>1000	0.5	2

[1]MIC values (μg/ml) – means inhibitory activity. [2]MMC values (μg/ml) – means microbicidal activity. nt – not tested; D/F – positive controls.

Acknowledgement

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