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Antimicrobial Activity of the Structure Derivates of Purines, Pyrimidines and Boronic Acid against Selected Microorganisms

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REFERENCES

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ABSTRACT

The doctoral thesis was focused on determination of antimicrobial effects of structural analogs of purines, pyrimidines and boronic acid derivatives on selected strains of bacteria and yeasts. The individual structural analogs come from the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic. Testing was carried out at BSL-3 laboratories at the Biological Protection Center in Těchonin (OBO). In detecting the antimicrobial effects of structural analogs, we first verified their ability to inhibit the growth of test microorganisms. Subsequently, we determined the minimum inhibitory concentration for each active substance by the microdilution method. Due to the unique nature and origin of test compounds, we have investigated the inhibitory effects of antibiotics introduced to compare the efficacy of structural analogs. Based on the results, we evaluated potential candidate substances with antimicrobial activity. The growth of test organisms best inhibited boronic acid structural analogs. On the other hand, the smallest spectrum of efficacy was observed for analogs of purine compounds that inhibited growth only in the *Bordetella pertussis* and *Francisella tularensis* strains. No compound with antimicrobial activity was found in any of these groups for all tested bacteria and yeast species. The lowest inhibitory effects of structural analogs against tested microorganisms were detected for *Acinetobacter baumannii* and *Bacillus subtilis*. None of the tested compounds inhibited the growth of *Pseudomonas aeruginosa* and EHEC O104:H4.

ABSTRAKT

Disertační práce byla zaměřena na stanovení antimikrobiálních účinků strukturních analogů purinů, pyrimidinů a derivátů kyseliny boronové na vybrané kmeny bakterií a kvasinek. Jednotlivé strukturní analogy pocházely z Ústavu organické chemie a biochemie, Akademie věd České republiky. Testování probíhalo v laboratořích úrovně BSL-3 Odboru biologické ochrany v Těchoníně. Při zjišťování antimikrobiálních účinků strukturních analogů byla nejprve ověřována jejich schopnost inhibovat růst testovaných mikroorganismů. Následně byla pro jednotlivé účinné sloučeniny stanovena mikrodiluční metodou jejich minimální inhibiční koncentrace. Vzhledem k unikátní povaze a původu testovaných sloučenin byly inhibiční účinky zjišťovány i pro zavedená antibiotika a léčiva ze skupiny pyrimidinových sloučenin, pro možnost srovnání účinnosti strukturních analogů. Na základě výsledků byly vyhodnoceny potenciální kandidátní sloučeniny s antimikrobiální aktivitou. Růst testovaných mikroorganismů nejlépe inhibovaly strukturní analogy kyseliny boronové. Naopak

nejmenší spektrum účinnosti bylo zaznamenáno u analogů purinových sloučenin, které inhibovali růst pouze *Bordetella pertussis* a *Francisella tularensis*. V žádné z uvedených skupin nebyla zjištěna sloučenina s antimikrobiálním účinkem na všechny testované druhy bakterií a kvasinek. Nejnižší inhibiční účinky použitých strukturních analogů byly detekovány na druhy *Acinetobacter baumannii* a *Bacillus subtilis*. Žádná z testovaných sloučenin neinhibovala růst *Pseudomonas aeruginosa* a EHEC O104:H4.

KEYWORDS

Antimicrobial activity, boronic acid, purines, pyrimidines, structure derivate, antimicrobial susceptibility testing

KLÍČOVÁ SLOVA

Antimikrobiální účinky, kyselina boronová, puriny, pyrimidiny, strukturní deriváty, testování citlivosti

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1. INTRODUCTION

The use of antibiotics (ATB), possibly antimycotic, is an integral part of the therapy of bacterial or yeast infections. Since the discovery of penicillin in 1928, a number of other antimicrobial products have been developed and introduced and the hope has been that they will completely eradicate one infectious disease. However, even today, the treatment of infectious diseases remains a serious global problem. The reason is the increasing resistance of microorganisms to antimicrobial agents.

Due to this fact, it is necessary to have attention to the presence of resistant bacteria, both in human and veterinary, food and environmental practice. Dissemination of resistant strains is currently a global problem requiring a common strategy at global level. The solution of problems associated with increasing resistance to antimicrobial agents is coordinated by the introduction of special programs called surveillance where epidemiological analysis of resistance and consumption of ATB is established.

In addition to microorganisms resistant to various ATB at the same time, the so-called multi-resistant strains, we also encounter microorganisms called "pan-resistant" that do not respond to treatment from any currently available ATB. A number of research centers around the world are therefore intensively engaged in the development of new antimicrobial agents that could replace existing ineffective drugs to prevent the spread of resistant strains, or even eliminate them.

One of the possibilities of finding new effective antimicrobial products is the application of already established compounds, or their structural analogs. The aim of the doctoral thesis was to determine the antimicrobial effects of compounds derived from the structural bases of purine, pyrimidine and boronic acid on selected microorganisms.

2. AIMS

- To determine inhibition activity of structure analogs of purines, pyrimidines and boronic acid against selected strains of bacteria and yeasts.
- To determine minimal inhibition concentration of compounds with observed antimicrobial activity on bacterial and yeasts strains by microdilution method.
- To determine minimal inhibition concentration of control antibiotics against selected strains of bacteria and yeasts.
- To analyse results and possibilities of their using in praxis

3. MATERIAL AND METHODS

3.1 Tested Microorganisms

The antimicrobial activity of structure derivates was tested on the reference strains of selected species of bacteria and yeasts from the Czech Collection of

Microorganisms in Brno (CCM), or from the American Collection of Microorganisms (ATCC). The antimicrobial susceptibility testing was also performed for EHEC O104: H4 strain isolated from the patient (imported infection from Germany) in 2011 and was provided by an employee of the State Health Institute in Prague.

Bacterial and yeast strains were stored and further propagated on blood agar, McLeod's soil, Sabourad agar, and Bordet-Gengou agar. The media was prepared in a 90 mm diameter Petri dish directly by LabMedia Service and delivered weekly (LabMedia Service, s.r.o., Jaroměř).

Table 1: List of tested microorganisms

Microorganism	Označení kmene
Acinetobacter baumannii	CCM 7031, CNCTC 5558
Bacillus subtilis	ATCC 6051
Bordetella pertussis	ATCC 9797
Candida albicans	ATCC 90028
Escherichia coli	ATCC 11775
EHEC O104:H4	Clinical isolate
Francisella tularensis LVS	ATCC 29884
Listeria monocytogenes	ATCC 19111, CNCTC Li 14/57
Pseudomonas aeruginosa	ATCC 27853
Salmonella Enteritidis	ATCC 49222
Staphylococcus aureus	ATCC 10832

3.2 Chemical and Reagents

3.2.1 Tested Compounds and Antibiotics

Compounds tested for their antimicrobial activity were obtained from the Institute of Organic Chemistry and Biochemistry (UOCHB), Academy of Sciences of the Czech Republic in Prague. These substances were from the group of structural analogs of purine and pyrimidine bases and derivatives of boronic acid. At the beginning of the study, these substances were labeled as a cover name. The chemical

composition of test compounds was provided to UOChB workers after confirmation of their antimicrobial effects.

In order to compare the inhibitory effects of the test compounds, antimicrobial activity was also tested in selected ATB and in the clinical practice of the introduced compounds from the group of structural analogs which also originated from UOChB.

3.3 The Antimicrobial Susceptibility Testing methods

3.3.1 Preparation of Working Solutions of Tested Compounds

Tested compounds were delivered from UOChB and dissolved in 1% DMSO (dimethylsulfoxide). These solutions containing varying concentrations of the active compound were then diluted with PBS buffer 1:10 to obtain so-called working solutions, or the first dilution of the effective concentration with which further testing was performed.

3.3.2 Preparation of Solution of Tested Antibiotics

Solutions of selected antimicrobial agents dissolved in a 1% DMSO were supplied from UOChB. As with the test compounds, these solutions were then diluted with 1:10 PBS buffer to give the initial concentration of working solutions to determine the inhibitory effects.

3.3.3 Preparation of Microbial Suspension

A suspension of tested microorganisms was prepared from 24, eventually 72-hour culture of individual strains grown on broth. The required number of colonies was removed from the bacteriological loop and suspended in a liquid Mueller Hinton medium (MH), or another fluid medium suitable for a particular microorganism.

The turbidity of the suspension was measured with a nephelometer and its density was adjusted to 0.5 degree McFarland turbidity scale, which is approximately 1.5×10^8 cells per ml. The prepared inoculum was used over a time horizon of about 30 minutes to avoid a possible decrease in the number of vital microbial cells.

3.3.4 Testing Antimicrobial Activity of Substances

The tested compounds were first analysed for their ability to inhibit the growth of tested microorganisms. To each well in the microtiter plate was pipetted 180 μl of MH broth. Then 20 μl of the initial concentration of test compound was added. All substances were tested in triplets.. The last three wells of line G (G10-G12) served to control the growth of the test microorganism without the presence of inhibitory substances. The H-wells (columns 1-6) contained gentamicin or amphotericin B (for yeast), and after inoculation served as a positive control. Each part of microtiter plate also included sterility control of culture media and microbial suspension.

To the wells containing the individual test compounds, including growth control and positive control, 5 µl suspension of the microorganism was subsequently transferred by 96-microtiter plate inoculator. The plate was then covered with a sterile lid and inserted into a foil to prevent dryness. Plates were incubated at 37 ° C or, if desired, 30 ° C (*C. albicans*), for 24, 48 and 72 hours. Incubation time for *B. pertussis* and *Fr. tularensis* was prolonged for five days. Evaluation of the antimicrobial activity of the compounds was performed by subtracting the growth of the test microorganisms in the individual wells, which resulted in turbid or sediment formation. The contents of the wells without apparent growth were seeded on a suitable culture medium to verify growth inhibition.

3.3.5 Microdilution Method

Compounds with proven inhibitory activity were subsequently tested by the microdilution method to determine the minimum inhibitory concentration.

To each well of the microtiter plate in columns 1 - 12 was pipetted 100 µl of liquid medium. Subsequently, 100 µl of tested compound solution was added to the A-wells. Then, 100 µl of solution from the first line was transferred via the multichannel pipette to the second line, reassembled and discharged, the solution was mixed and the procedure repeated until the penultimate row. From the G-wells, 100 µl of the solution was transferred to disinfection. The H-wells contained clean medium which, after inoculation, was used as growth control of the tested microorganism. In this way, the solutions of the compounds were binary diluted (dilution 2x-128x). Since the initial concentrations of test compounds were different, the concentration range for each substance was different.

Subsequently, 5 µl of the inoculum of the test microorganism was added to the plate. The plate was then covered with a sterile lid and covered by parafilm foil to prevent drying. The plates were incubated at 37 ° C or 30 ° C (*C. albicans*), for 24, 48 and 72 hours. *B. pertussis* and *Fr. tularensis* were incubated for five days.

After incubation, the growth of microorganisms in the individual wells was assessed and the minimal inhibitory concentration (MIC) was determined as the lowest effective concentration of the tested compound that visibly inhibited the growth of the microorganism.

4. RESULTS AND DISCUSSION

Increasing resistance of microorganisms to antimicrobial drugs is a serious global problem that makes it difficult to treat infectious diseases and represent significant risk of lacking effective medicines in the future. The impact of the increasing incidence of multidrug-resistant microbes on current medicine can be seen in many areas. These strains are a frequent cause of the failure of antibiotic therapy, resulting in higher mortality. At the same time, these microorganisms are significant etiologic agents of nosocomial infections. Last but not least, the financial costs of antimicrobial treatment are increased, due to the need to use more efficient and often more expensive products. In recent years, therefore, a number of research centers around the world have been looking into new effective compounds with antimicrobial effects.

The research and development of new antimicrobial products is a very lengthy and costly process requiring a comprehensive collaboration not only between microbiologists but also toxicologists and other disciplines. One possibility of preparing new therapeutically useful substances is the use of the synthesis of structural analogs of compounds with previously proved therapeutic effects.

This dissertation was created within the joint project UOChB and OBO Těchonín. Our goal was to test antimicrobial activity and to determine effective concentrations of purine, pyrimidine and boronic acid compounds for selected clinically significant bacterial and yeast species. These substances have previously found usefull in human medicine for the possible treatment of some malignant or autoimmune diseases.

Due to the unique nature of the test compounds, it has not been possible to obtain recent information on antimicrobial properties and MIC values in the available literature for the same compounds as in this work. For the purposes of comparison and discussion of these parameters, therefore, the study was expanded with the panel of antimicrobial agents that were introduced in clinical practice and the published data on structurally similar compounds were used.

Overall, 658 compounds were included in the testing of antimicrobial effects on selected bacterial and yeast species. The inhibitory activity was shown in 135 (20.5%) of these compounds, which were divided into groups according to the chemical structure. The first group consisted of analogs of purine bases (18), a second analog of pyrimidine bases (19), and boronic acid derivatives (46) were included in the third group. The other active compounds (52) could not be structurally included in any of these groups and were therefore not processed for the purposes of this doctoral thesis.

In terms of efficacy on the tested microorganisms, the broadest range of antimicrobial activity was recorded in the group of structural boronic acid derivatives. On the other hand, the narrowest spectrum of efficacy exhibited analogs of purine compounds that inhibited only *B. pertussis* and *F. tularensis* growth. No compound with antimicrobial activity inhibited all bacteria and yeast tested species.

Table 2: Antimicrobial activity of tested compounds against selected microorganisms

SPECIES	PURINES	PYRIMIDINES	BORONIC ACID
A. baumannii	-	1	-
Bac. subtilis	-	2	1
B. pertussis	18	16	41
C. albicans	-	4	4
E. coli	-	1	3
EHEC *O104:H4*	-	-	-
F. tularensis	6	4	24
L. monocytogenes	-	2	2
Ps. aeruginosa	-	-	-
Sal. Enteritidis	-	-	1
St. aureus	-	-	2

B. pertussis and *F. tularensis* strains showed the highest susceptibility to the structure analogs. For these bacterial species, the efficacy of the compounds from each group of tested substances was demonstrated. In contrast, none of the compounds used in this study inhibited growth of *Ps. aeruginosa* and EHEC O104: H4. The low inhibitory effect of the tested substances was further reported for *A. baumannii*, *Bac. subtilis, E. coli*, *Sal.* Enteritidis and *L. monocytogenes*.

From the point of view the efficacy of the test compounds on individual microbial species, the inhibitory activity in the purine analogue group was recorded only on *B. pertussis* and *F. tularensis* strains. This result differs from the conclusion of the study by Krajewski et al. (2017) describing the antimicrobial activity of 6-N-hydroxylaminopurines on *L. monocytogenes*. This bacterium was also tested in this doctoral thesis, but the compounds did not inhibit its growth. A similar phenomenon was observed for *E. coli*, *St. aureus* and *Bac. subtilis*, to which purine analogs have previously been tested with antimicrobial activity (Kim et al., 2009). As a mechanism of action of these compounds on bacteria, these studies show the ability of purines to inhibit microbial metabolism through "riboswitch" elements. Kinali-Demirci et al (2012) synthesized new amino and thiotetrazole purine derivatives. They subsequently detected their antimicrobial activity on *St. aureus, Bac. subtilis, Ps. aeruginosa, E. coli* and *C. albicans* by the broth dilution method.. The authors have shown that tetrazole-containing compounds in the structure show stronger antimicrobial effects. Studies conducted by Amer et al. (2013) studied new purine complexes and investigated their antimicrobial activity against *E. coli*, *St. aureus* and *C. albicans*. The authors observed that all of them tested compounds except L1 (empirical formula C17H12N8) showed moderate antibacterial activity for both test species compared to tetracycline. The antifungal effect was observed only for two compounds. Hu et al. (2010) synthetized 6-substituted-purine derivatives and studied their biological properties. They verified the antimicrobial effects of their compounds on *Bac. subtilis*, *Aspergillus niger* and *C. tropicalis* by the disk diffusion method. Altogether, they tested 17 structural analogs.

The strongest inhibitory activity on the above strains has been shown for 7 (6N- [3-(trifluoromethyl) phenyl] -9H-purine-2,6-diamine), 8- [(2-fluoro-6-trifluoromethylpurine), 15 (2-amino-6-trifluoroethylpurine), 16 fluoro-6-trifluoroethylpurine), 17 (2-hydroxyl-6-trifluoroethylpurine). In all cases, the inhibition zone was greater than 30 mm, similar to the control fuconazole.

In the group of derivatives of pyrimidine, an inhibitory activity of a total of 16 compounds against *B. pertussis* was detected. Other bacterial strains with antimicrobial effect of these derivatives were *A. baumannii* (1), *Bac. subtilis* (2), *F. tularensis* (4), *L. monocytogenes* (2) and *E. coli* (1). In contrast to the previous group of purine compounds, inhibitory effect was observed on *C. albicans* (4). Several studies with the same topic are available in the literature. Abunada et al. (2008) tested pyrazolo [4,3-e] [1,2,4] triazolo [1,5-c] pyrimidine for selected bacterial, fungi and yeast species by the disc diffusion method. They observed weak inhibitory activity against *E. coli* and *St. aureus* in comparison with tetracycline. The authors also noted a moderate inhibitory effect against *C. albicans* compared to amphotericin B. The agar diffusion method was used to demonstrate the antimicrobial activity of the pyrimidine analogs by Ghorab et al. (2004). The results were compared with chloramphenicol for bacteria and terbinafine for yeast and fungi. At a concentration of 2.5 mg/ml, the authors demonstrated inhibitory effects on *Ps. aeruginosa*, *S. aureus*, *E. coli* and *C. albicans.* In available publications, no studies have been found dealing with the mechanism of antimicrobial action of pyrimidines and their derivatives. Sharma et al. (2012) synthesized several structural analogs of pyrimidines, respectively pyrimidine-2,4-diones. Their ability is reversible inhibition of thymidine phosphorylase. The authors also tested the action of these analogs against selected viruses, tumors, or their effects as antimalarials or as anticoagulants. Antibacterial activity was verified against G^+ bacteria *Bac. subtilis* and *E. coli* G^-bacteria by the disc diffusion method. The discs contained test compounds at a concentration of 50 μg/ml or 100 μg/ml, respectively. Significant inhibition of growth by these newly synthesized compounds was reported by the authors on *B. subtilis* and *E. coli*. The substance designated OBP-07 (C17H12N4O4S molar) consisted of a 100 μg/ml inhibition zone for *Bac. subtilis* 17 mm and for *E. coli* then 16 mm. The OBP-10 substance (C19H16N4O4S molar) was formed at a concentration of 100 μg/ml of the inhibition zone in the diameter 18 mm for *Bac. subtilis* and 15 mm for *E. coli*.

Kaur et al. (2012) tested antimicrobial activity of synthesized pyrimidine derivatives at a concentration of 10, 50, 100, 250, 500 and 750 μg/ml against *Bac. subtilis* and *Ps. aeruginosa* by agar diffusion method. The ciprofloxacin (concentration 10 μg/ml) was used as a reference ATB to compare the efficacy of the compounds. The authors demonstrated a moderate inhibitory activity on *Bac. subtilis* of the compounds labeled 3b, 3i, 3j, which induced zones of inhibition of 3-6 mm for the bacterial species at a concentration of 250 μg/ml. The control ATB created inhibition zone in a diameter of 22 mm for the same species. The authors observed the strongest antimicrobial effect of compound 3i on *Ps. aeruginosa*. The inhibition zone was 2 mm at the same concentration (250 μg/ml). The ciprofloxacin inhibition zone for the same species was 30 mm.

The structure derivatives of boronic acid showed the broadest spectrum of antimicrobial activity against selected microbial species. The most sensitive species to these compounds were *B. pertussis* and *F. tularensis*. Compared with the previous

groups of compounds, the inhibitory activity was observed against *Sal.* Enteritidis (1) and *St. aureus* (2). Boronic acid analogs were tested by Adamczyk-Woźniak (2012) against selected microorganisms (*E. coli*, *St. aureus*). Antimicrobial activity was verified by both, disk diffusion and dilution method for 12 new boronic acid analogs. The inhibitory activity of tested compounds was recorded for *St. aureus* in 10 substances, whereas for the *E. coli* strain it was demonstrated in 6 substances. In the available literature is only limited verified information about mechanism of antibacterial action of boronic acid derivatives. However many studies have demonstrated the ability of these compounds to inhibit the class A and C serine beta-lactamases. Santucci et al. (2017) even suggested using benzo- [b] -thiophene-2-boronic acid analogs as a diagnostic tool for distinguishing serine beta-lactamases from metal-beta-lactamases.

Table 3: Minimal inhibitory concentrations of purine analogs

COMPOUND	SPECIES	MIC (µg/ml)	µM
1/C6	*B. pertussis*	17,45	62,50
2/A12	*B. pertussis*	7,88	41,66
2/B4	*B. pertussis*	2,19	7,84
	F. tularensis	4,37	15,67
2/H10	*B. pertussis*	20,03	62,50
4/D6	*B. pertussis*	112,64	249,46
5/H12	*B. pertussis*	10,80	62,59
6/A12	*B. pertussis*	12,85	31,25
6/D1	*B. pertussis*	5,89	31,19
	F. tularensis	23,88	124,80
8/D7	*B. pertussis*	75,73	250,11
8/D9	*B. pertussis*	0,64	12,91
	F. tularensis	0,82	3,30
8/E5	*B. pertussis*	0,16	0,40
8/E7	*B. pertussis*	79,75	239,58
8/F1	*B. pertussis*	14,62	55,86
	F. tularensis	7,30	27,90
8/G1	*B. pertussis*	81,33	250,00

Table 3: Minimal inhibitory concentrations of purine analogs (continue)

COMPOUND	SPECIES	MIC (μg/ml)	μM
8/G2	*B. pertussis*	2,28	7,82
5/H12	*F. tularensis*	1,14	3,91
8/G4	*B. pertussis*	0,87	2,48
8/G9	*B. pertussis*	2,93	7,83
	F. tularensis	5,87	15,67
8/G10	*B. pertussis*	5,90	15,63

Compounds from the purine base analog group inhibited the growth of *B. pertussis* in the concentration range of 0.16 - 112.64 μg/ ml and 0.82 - 23.88 μg/ml for *F. tularensis*. The most effective compounds against *B. pertussis* were 8/E5 (0.16 μg/ml), 8/D9 (0.64 μg/ml) and 8/G4 (0.87 μg/ml). Compound 8/D9 also showed a strong antimicrobial effect on *F. tularensis* (0.82 μg/ml). The values of the inhibitory concentrations determined were further compared with those obtained for the control ATB. Compounds 8/E5, 8/D9 and 8/G4 showed greater inhibitory activity than nalidixic acid (7.28 μg/ml) or isoniazid (4.28 μg/ml) for *B. pertussis*. In contrast, ciprofloxacin and rifamycin inhibited growth of the tested strain of *Bordetella* at a lower concentration of 0.13 μg/ml and 0.016 μg/ml, respectively. Compound 8/D9 exhibited the most potent inhibitory effect on *F. tularensis* from the purine analogs tested. Its effect was comparable to rifamycin, which had a similar inhibition concentration - 0.79 μg/ml. However, it was lower antimicrobial effect compared to ciprofloxacin (0.3 μg/ml) and streptamine (0.016 μg/ml).

Because of the unique nature and origin of the compounds included in this study does not make it possible to find MIC values for identical substances in available literature. The obtained results are compared for the structurally closest compounds. Vitali et al. (2012) tested the antibacterial activity of adenosine structural analogs on the reference strains of *St. aureus* (RN 4200); *St. epidermidis* (ATCC 35984), *Str. pneumoniae* (ATCC 9619), *E. coli* (ATCC 25922) and *Ps. aeruginosa* (ATCC 27853). The authors have observed that the presence of a halogen or a lipophilic alcynyl chain at the position of the 2-substituted purine skeleton results in inactivation of the antimicrobial activity of the tested compounds. This finding was not observed during the testing in this work, when the inhibitory activity was also recorded for halogen compounds in the same position for the *B. pertussis* or *F. tularensis* tested strains. The authors also noted that none of the tested compounds inhibited the growth of *E. coli* or *Ps. aeruginosa*, as in our observation.

Table 4: Minimal inhibitory concentrations of pyrimidine analogs

COMPOUND	SPECIES	MIC (µg/ml)	µM
1/A4	*B. pertussis*	8,36	41,67
1/B6	*B. pertussis*	6,88	31,25
1/D3	*B. pertussis*	15,51	125,00
	F. tularensis	7,75	31,25
1/E8	*B. pertussis*	5,72	31,25
	F. tularensis	15,63	2,86
1/F2	*C. albicans*	20,65	125,00
1/F4	*B. pertussis*	7,58	33,33
1/G12	*Bac. subtilis*	9,48	31,25
	B. pertussis	0,24	0,78
2/B5	*B. pertussis*	30,77	125,00
2/B11	*A. baumannii*	12,60	62,50
	B. pertussis	3,15	15,63
	E. coli	12,60	62,50
2/H7	*B. pertussis*	8,75	41,67
	C. albicans	52,50	250,00
2/H8	*B. pertussis*	0,34	1,25
	F. tularensis	0,84	3,13
	L. monocytogenes	8,44	31,25
2/H11	*B. pertussis*	1,15	2,15
3/E5	*B. pertussis*	11,63	41,74
3/E8	*C. albicans*	5,06	20,88
3/G6	*B. pertussis*	10,83	41,64
5/F7	*C. albicans*	1,04	7,69
5/F9	*B. pertussis*	19,88	124,19

Table 4: Minimal inhibitory concentrations of pyrimidine analogs (continue)

COMPOUND	SPECIES	MIC (μg/ml)	μM
8/E9	*Bac. subtilis*	3,99	15,75
	B. pertussis	3,99	15,75
	F. tularensis	3,99	15,75
	L. monocytogenes	15,98	62,99
8/H8	*B. pertussis*	14,45	62,64

Structurally derived compounds from pyrimidine inhibited in this study the growth of *B. pertussis* in the concentration range 0.24 and 30.77 μg/ml. Compared to control compounds, the substance 1/G12 exhibited a lower concentration (0.24 μg/ml) compared to nalidixic acid (7.28 μg/ml), isoniazid (4.28 μg/ml) and formicin B (4.19 μg/ml). However, the inhibitory effect on the bacterial species was lower compared to ciprofloxacin (0.13 μg/ml) and rifamycin (0.016 μg/ml). For compounds that inhibited the growth of *F. tularensis*, the MIC was determined in the range of 0.84 - 15.63 μg/ml. In the group of structural analogs of pyrimidine, a single compound inhibiting *A. baumannii* (12.6 μg/ml) and *E. coli* (12.6 μg/ml) was further identified in this study. It was a substance named 2/B11 that simultaneously suppressed *B. pertussis* (3.15 μg/ml). Only compounds 1/G12 and 8/E9 from the whole group inhibited growth of *Bac. subtilis*. However, a lower antimicrobial effect was seen in comparison with controls. The two pyrimidine structural analogs (2/H8 and 8/E9) then suppressed the growth of *L. monocytogenes*. For the total of four compounds, antifungal activity was shown to *C. albicans*, the strongest effect of which was observed for substance 5/F7 at a concentration of 1.04 μg/ml. For control amphotericin B, a lower inhibitory concentration of 0.04 μg/ml was found.

Narwal et al. (2017) dealt with the synthesis of novel pyrimidin-2-ol/thiol/amine derivatives, which subsequently investigated their antimicrobial properties. The MIC values were determined for *St. aureus* (MTCC 3160), *Bac. subtilis* (MTCC 441), *E. coli* (MTCC 443), *Ps. aeruginosa* (MTCC 3542), Sal. Enterica (MTCC 1165) and *C. albicans* (MTCC 227), by the broth dilution method. Antibacterial activity was compared to cefadroxil and antifungal effects against fluconazole. The authors demonstrated antimicrobial potential for all test compounds. The compounds with the strongest inhibitory activity include compounds No. 2 (4- (2-chlorophenyl) -6- (4-nitrofenyl) pyrimidine-2-thiol), 5- (4- nitrophenyl) pyrimidin-2-amine and 12 (4- (2,4-dichlorophenyl) -6- (4-nitrophenyl) pyrimidin-2-amine) suppress the growth of all tested microorganisms with the lowest MIC compared to other test compounds.

Sreenivas et al. (2012) synthesized structural analogs of pyrimidines, which subsequently examined their antimicrobial activity against selected microorganisms,

including *E. coli*, *Bac. subtilis*, *Kl. pneumoniae* and *C. albicans*. The inhibitory activity was determined by disc diffusion and broth dilution methods. In total, they tested 8 different pyrimidine structural derivatives, including the compound 2-amino-4,6-dichloropyrimidine, which is found among the tested compounds under the designation 5/F7 in this doctoral thesis. The authors of the study noted inhibitory activity for *C. albicans* at 50 μg/ml, followed *Bac. subtilis* and *E. coli* at 200 μg/ml. In the testing performed in this work, the substance inhibited only growth of *C. albicans* at a lower concentration (2.1 μg/ml) compared to the quoted study. At the same time, inhibitory activity was not shown for compound 5/F7 for the tested strains of *E. coli* and *Bac. subtilis*, which were also included in this doctoral thesis. Among the most effective compounds, 4-chloro-5-carboxypyrimidine was included in the study, in which the authors demonstrated antimicrobial activity against *Bac. subtilis* at a concentration of 200 μg/ml and *E. coli* 500 μg/ml. This compound further inhibited the growth of *C. albicans* with MIC of 10 μg/ml.

Table 5: Minimal inhibitory concentrations of boronic acid analogs

Označení látky	Druh	MIC (μg/ml)	μM
2/C4	*B. pertussis*	2,00	15,63
2/C6	*B. pertussis*	2,78	15,63
	C. albicans	22,25	125,00
	F. tularensis	11,13	62,50
2/C12	*Sal.* Enteritidis	19,64	124,79
2/D6	*B. pertussis*	24,71	124,79
	F. tularensis	6,18	31,20
2/D7	*B. pertussis*	0,70	3,91
	E. coli	44,50	250,00
	F. tularensis	11,13	62,50
2/D9	*B. pertussis*	1,11	7,81
	F. tularensis	35,50	250,00
2/E4	*B. pertussis*	2,17	7,81
2/E7	*B. pertussis*	11,13	62,50
2/E8	*F. tularensis*	49,51	250,00
2/E9	*B. pertussis*	18,07	62,50
	F. tularensis	0,56	1,95

2/E11	*F. tularensis*	21,62	125,00

Table 5: Minimal inhibitory concentrations of boronic acid analogs (continue)

COMPOUNDS	SPECIES	MIC (µg/ml)	µM
2/E12	*B. pertussis*	1,78	7,82
	C. albicans	3,57	15,65
2/F2	*B. pertussis*	21,50	125,00
	F. tularensis	43,00	250,00
2/F3	*B. pertussis*	6,94	31,25
2/F4	*B. pertussis*	0,19	0,78
	F. tularensis	0,48	1,95
2/F5	*B. pertussis*	6,16	31,12
	F. tularensis	12,33	62,25
2/F6	*B. pertussis*	26,75	125,00
	F. tularensis	1,67	7,81
2/F7	*Bac. subtilis*	6,94	31,25
	B. pertussis	3,47	15,63
	C. albicans	3,47	15,63
	E. coli	55,51	250,00
	L. monocytogenes	1,73	7,81
2/F9	*B. pertussis*	43,00	250,00
2/F11	*B. pertussis*	2,03	7,81
2/F12	*B. pertussis*	2,17	7,81
	F. tularensis	8,69	31,25
2/G4	*B. pertussis*	6,19	31,25
2/G5	*B. pertussis*	6,63	31,25
	C. albicans	13,25	62,50
3/A2	*B. pertussis*	13,91	83,33
	F. tularensis	41,73	250,00
3/A3	*B. pertussis*	2,62	15,71
	F. tularensis	20,98	125,71

3/A6	*B. pertussis*	2,31	15,61
	F. tularensis	18,47	124,85

Table 5: Minimal inhibitory concentrations of boronic acid analogs (continue)

COMPOUNDS	SPECIES	MIC (µg/ml)	µM
3/A7	*B. pertussis*	26,49	125,00
3/A10	*B. pertussis*	10,99	62,50
	E. coli	43,98	250,00
	F. tularensis	21,99	125,00
3/A11	*B. pertussis*	18,47	124,84
3/B1	*B. pertussis*	2,43	15,78
	F. tularensis	38,89	252,49
3/B2	*B. pertussis*	0,26	1,55
	F. tularensis	2,60	15,50
	L. monocytogenes	20,58	123,99
	St. aureus	20,56	123,90
3/B3	*B. pertussis*	2,75	15,63
3/B6	*B. pertussis*	15,19	124,59
3/B7	*B. pertussis*	41,78	250,28
3/B8	*B. pertussis*	12,85	62,39
3/B9	*B. pertussis*	4,30	31,19
	F. tularensis	4,30	31,19
	St. aureus	4,30	31,19
3/B10	*B. pertussis*	11,87	62,50
3/D4	*F. tularensis*	20,59	124,80
4/A11	*B. pertussis*	3,57	31,31
4/A12	*B. pertussis*	1,29	7,78
	F. tularensis	10,33	62,26
4/B3	*B. pertussis*	1,14	6,25
	F. tularensis	11,40	62,50
4/B4	*B. pertussis*	30,15	250,51
	F. tularensis	15,07	125,26

4/B8	*B. pertussis*	14,69	62,50
4/H6	*B. pertussis*	29,56	125,14

Table 5: Minimal inhibitory concentrations of boronic acid analogs (continue)

COMPOUNDS	SPECIES	MIC (μg/ml)	μM
5/B8	*B. pertussis*	2,05	15,52
5/F11	*F. tularensis*	10,82	62,52

The inhibitory activity of structural boronic acid analogs against *B. pertussis* was reported for total 41 compounds in the concentration range of 0.19 - 43 μg/ml. The most active compound against this bacterium with a MIC of 0.19 μg ml was 2/F4, which simultaneously inhibited the growth of *F. tularensis* with the lowest MIC of 0.48 μg/ml. In the group of compounds derived from boronic acid, the only compounds of the whole test set of substances acting inhibitory on *St. aureus* and *Sal.* Enteritidis. Specifically, compounds 3/B2 and 3/B9 that inhibited the growth of *St. aureus* in a concentration of 20.56 and 4.3 μg/ml. Substance 2/C12 then as the only compound inhibited the growth of *Sal.* Enteritidis with MIC of 19.64 μg/ml. The inhibitory effects on *L. monocytogenes* growth were also observed for substances 2/F7 and 3/B2, where the highest activity at a concentration of 1.73 μg/ml was demonstrated for structural analog 2/F7. Antifungal activity was confirmed for a total of 4 compounds in the range of 3.47-22.25 μg/ml. None of the antimicrobial compounds on yeast exhibited superior efficacy compared to amphotericin B control. In the group of structural boronic acid analogs, compounds with the broadest spectrum of antimicrobial activity were found in the test, 2/F7 and 3/B2. Compound 2 / F7 was the only one in the whole doctoral thesis to have antimicrobial action against G^+ and G^- bacteria, including yeasts.

In the available literature, the results of the antimicrobial effects of boron acid investigated by Yilmaz (2012) have been obtained. The MIC values were determined by the broth dilution method. For reference strains *St. aureus* (ATCC 25923) and *Acinetobacter septicus* (DSM 19415), the author reported a MIC of 3.8 mg/ml, whereas for *E. coli* (ATCC 35218) and *Ps. aeruginosa* (ATCC 27853) obtained 7.6 mg/ml. During the testing conducted in this work, no compound from the group of structural boronic acid analogs was effective on *Ps aeruginosa* or *A. baumannii*. In the case of *E. coli* and *St. aureus*, however, a significantly higher inhibitory activity was observed in the substances used in this work, where, in the case of staphylococcus, the MIC values of 4.3 and 20.56 μg/ml were detected in the active compounds. For *E. coli*, the concentration range was 43.98 - 55.51 μg/ml.

Table 6: Minimal inhibitory concentrations of control compounds

COMPOUNDS	Mw	MIC (µg/ml)										
		1	2	3	4	5	6	7	8	9	10	11
TRIMETHOPRIME	*290,31*	**18,14**	**N**	**N**	**N**	**18,4**	**N**	**N**	**N**	**N**	**0,28**	**N**
SULFAMETHOXAZOLE	*235,27*	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
CIPROFLOXACINE	*331,34*	**0,52**	**0,13**	**0,13**	**N**	**0,013**	**0,3**	**N**	**0,26**	**0,06**	**0,004**	**N**
FORMYCINE B	*268,22*	**N**	**N**	**4,19**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
5-FLUOROURACILE	*130,07*	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**32,54**	**N**
5-FLUORO-2'-DEOXYURIDIN	*246,19*	**N**	**1,92**	**N**	**N**	**N**	**0,005**	**N**	**0,19**	**N**	**N**	**3,85**
METRONIDAZOLE	*171,15*	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
PYRAZINAMIDE	*123,11*	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
CYKLOHEXIMIDE	*281,34*	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
D-STREPTAMINE	*545,64*	**N**	**0,14**	**0,28**	**N**	**0,57**	**0,016**	**0,06**	**0,113**	**0,14**	**0,28**	**0,14**
FOSFOMYCINE	*182,02*	**N**	**N**	**N**	**N**	**2,83**	**N**	**N**	**N**	**N**	**0,18**	**N**
RIFAMYCINE	*822,94*	**0,4**	**3,2**	**0,016**	**N**	**3,2**	**0,798**	**N**	**6,4**	**6,4**	**3,2**	**0,4**
ISONIAZIDE	*137,13*	**N**	**N**	**4,28**	**N**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
AMPHOTERICIN B	*924,07*	**N**	**N**	**N**	**0,04**	**N**	**N**	**N**	**N**	**N**	**N**	**N**
NALIDIXIC ACID	*232,23*	**N**	**N**	**7,28**	**N**	**7,28**	**14,56**	**N**	**N**	**N**	**N**	**N**

LEGEND: 1 – *A. baumannii***, 2 –** *Bac. subtilis***, 3 –** *B. pertussis*, **4 –** *C. albicans***, 5 –** *E. coli***, 6 -** *F. tularensis* LVS**, 7 -** EHEC O104:H4**, 8 –** *L. monocytogenes***, 9 –** *Ps. aeruginosa***, 10 –** *Sal.* Enteritidis, 11 **–** *St. aureus***, N** – non-inhibited

5. CONCLUSION

Given the dangerous increase in the resistance of microorganisms to antimicrobial products together, research and development of new efficacious drugs is now necessary. One of the ways how to speed up this lengthy and demanding process is to use structural analogues of already existing and in practice compounds.

In this doctoral thesis the antimicrobial activity of compounds structurally derived from purines, pyrimidines and boronic acid was investigated. These substances are already widely used in current clinical practice, especially for their antitumor activity. However, recent observations have also demonstrated the potential ability of these compounds in the field of antimicrobial therapy.

In the doctoral thesis was demonstrated the ability of the test compounds to inhibit the growth of selected clinically significant microorganisms. The greatest antimicrobial effect on a broad spectrum of microorganisms was found mainly for compounds derived from boronic acid. The presented results confirm the possible use of some compounds tested in this work as potential antimicrobial drugs. However, for their successful implementation, it is necessary to clarify and describe the mode of action on the target structures of bacterial cells. Recently, this mechanism has been satisfactorily explained only for a group of purine analogues. To confirm of these obtained results, it would be appropriate to supplement and extend this group to strains isolated from clinical material, including strains with already proven resistance. Due to the ability of some boronic acid derivatives to inhibit the activity of bacterial beta-lactamases, it would be very interesting to verify this ability in the compounds used in this work. For active compounds, it would also be useful to perform their assays in combination. Beta-lactamase production is a serious and very common mechanism of resistance in bacteria. Research and possible introduction of these compounds into practice could reverse this unfavorable situation.

The presented results confirm the high potential of the test compounds to inhibit the growth of clinically significant pathogens. At the same time, the work has opened up a lot of new interesting topics that are essential for their future application in the future.

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Results of the thesis have been presented in 2 posters at international and national conferences by the author since 2016.