Evaluation of the antimicrobial and fungicidal activity of nucleoside derivatives

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Abstract. On the background of the growing antibiotic resistance problem, the search for new antimicrobial therapy agents is of special interest. As sources of such compounds, it is reasonable to consider substances with other known target activity, like antiviral, and even the "waste heaps" content left from research on their development. In this study, we evaluated the antimicrobial, antibiofilm, mutagenic and cytotoxic properties of 83 nucleosides derivatives. All investigated agents did not inhibit the growth of *E. coli* ATCC 25922, *S. typhimurium* TA 100, *S. aureus* ATCC 35591, *S. epidermidis*, did not show any cytotoxicity towards A549 cells in MTT test, but 20 of them had a confirmed ability to inhibit the growth of *C. albicans*. 11 agents tested suppressed the development of *C. albicans* biofilms, and 5 caused the disruption of preformed yeast biofilms. The most active was compound 202, an N6-substituted adenosine derivative with a furan moiety (MIC = 32 μg/mL; BPC = 8 μg/mL). There was no mutagenic activity in the agents studied except for compounds 384 and 392. The results obtained indicate the possibility of nucleosides derivatives application as agents for the *Candida* species biofilm control.

1 Introduction

In the context of the COVID-19 pandemic, research to develop new agents for etiotropic antiviral therapy is of urgent interest. The researchers pay special attention to promising molecules - derivatives and analogs of nucleotides and nucleosides, which are more likely to affect the process of viral genome replication in the infected cells, disrupting the work of virus RNA-dependent RNA polymerase. The application of such scientific search strategy is valid not only for SARS-CoV-2 but also for other RNA viruses. Successful drugs with identified antiviral activity from this lineup include favipiravir [1]. The class of antitumor agents belonging to nucleosides is also widely represented [2]. At the same time, nucleosides and their analogs exhibit a wide range of biological activities in addition to antiviral and antitumor ones, including antimicrobial [3], antibiofilm and antifouling [4], fungicidal and fungistatic [5]. Moreover, the set of nucleoside derivatives with known antiviral and cytotoxic activity is considered as a source of new antimicrobial agents, therefore, these

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compounds are also gaining interest as a promising object of biotechnology especially considering the fact that some nucleosides can be obtained in the process of nucleic acid hydrolysis by nucleases, as shown, for example, for guanyl-specific RNase *Bacillus pumilus*, under the action of which 2',3' cGMP is formed [6]. Therefore, it is reasonable to combine screening for antiviral and antitumor activity of nucleosides and their analogs with evaluation of antimicrobial and antimycotic properties of these agents. In connection with the above, the aim of the present study was to evaluate the genotoxic, antibacterial, antimycotic and cytotoxic potential of a panel of nucleosides and their ana-logs from the groups of N6-substituted purine nucleosides, cytokinin nucleosides, uracil and barbituric acid derivatives, anhydro-nucleosides, glucosides, modified purine and pyrimidine nucleosides

2 Materials and methods

2.1 Tested compounds

83 nucleosides and their derivatives (Table S1) kindly provided by the Laboratory of Design and Synthesis of Biologically Active Compounds, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, were used as objects of our study. All the studied compounds can be categorized into three large groups: synthetic nucleosides and their analogues containing the D-ribose fragment and without it, and barbituric acid derivatives, which belong to the class of ureides, close in properties to uracil. Groups of synthetic nucleosides containing the D-ribose fragment and without it are represented by N6-substituted purine nucleosides, cytokinin nucleosides, uracil derivatives, which include alkyl, propargyl, benzyl, aryl, polyphenol, isopentenyl, furfuran, thiophene substituents, as well as residues of acrylic acid derivatives, hydroxy- and amino groups. The group of synthetic barbituric acid derivatives is represented by 5-substituted barbiturates that containing alkyl, furan, anisole, phenol, and pyrogallol moiety. The synthesis procedures and some properties of the compounds have been described previously [6, 7]. The compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma- Aldrich) at an initial concentration of 5 mg/mL.

2.2 Biological investigations

2.2.1 Microbial strains and cell cultures

Salmonella typhimurium TA100, *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 35591 as well as clinical isolates of *Staphylococcus epidermidis* and *Candida albicans* obtained from the Kazan Institute of Epidemiology and Microbiology (Kazan, Russia) were used in experiments. Bacterial cultures were grown in LB-broth (tryptone – 10 g/L; yeast extract – 5 g/L; NaCl – 5 g/L; pH 7.5); *Candida albicans* clinical isolate was cultured in Sabouraud dextrose broth (Merck). The day before the experiments, cultures were inoculated into fresh medium to maintain the exponential growth phase.

Human lung adenocarcinoma A549 cells (Russian Collection of Cell Cultures of Vertebrates (CCCV)) were cultured in DMEM (Gibco) supplemented with 10% FBS (BioSera), 2 mM L-glutamine, 100 mg/L penicillin and 100 mg/L streptomycin in a humid atmosphere with 5% CO_2 at 37°C.

2.2.2 Determination of the Minimal Inhibitory Concentrations

The MICs of the agents tested were determined by resazurin test in 96-well microtiter plates (SPL Lifesciences) according to [8]. Briefly, after overnight culture in the broth, the microorganisms were harvested and washed twice with phosphate-buffered saline (PBS, pH 7.2) then suspended in fresh broth to 1×10^6 cells/mL. Then 100 µL of the microbial suspensions were added to wells of 96-well flat-bottom culture plates (SPL Lifescienses) and when incubated for 24 hours at 37°C in humidified atmosphere. After incubation the resazurin solution (10 µL, 1 %) was added to wells and plates incubated for several hours at 37°C. A change in color from purple to pink or discoloration was considered as evidence of the microorganism's development in the well. The MIC was estimated based on the results of three independent experiments. Wells containing culture medium with the addition of an appropriate amount of solvent (DMSO), where microorganisms were added similarly to the experimental variants, were used as comparison variants.

2.2.3 Biofilm formation and nucleosides inhibitory effect examination

C. albicans biofilms were assessed using sterile, flat-bottom 96-well microtiter plates (SPL Lifesciences). The effects of compounds on biofilms were evaluated by two ways. In the first one, nucleosides were added to the wells together with *C. albicans* suspension (5×10^5 cells/mL) to evaluate the ability of the agents to prevent biofilm formation. Plates were incubated at 37°C for 24 h and biofilm formation was assessed. In the second one, *C. albicans* biofilms were pre-formed in wells by adding 100 µL of cell suspension (5×10^5 cells/mL) and incubated at 37°C for 24 h. The wells were then washed twice with sterile PBS and 100 µL of Sabouraud broth containing nucleosides in serially double-diluted concentrations from 0.5 to 128 µg/mL was added. Afterwards the plates were incubated at 37°C for an additional 24 h. In both treatments, the wells where only microorganisms were applied and the wells where only culture medium was added were used as positive and negative controls. Biofilm formation was quantified by a crystal violet assay [9]. The wells with biofilms were washed twice from planktonic cells with PBS and then air dried overnight. 100 µL of the crystal violet 0.1 % solution (Sigma) in 96% ethanol was added to wells and the plates were incubated for 20 min. Afterwards the crystal violet solution was removed and the plates were washed 3 times with PBS. The plates were 30 min air dried and 200 µL of 96% ethanol to wells was added to destain the biofilms. After 45 min of destaining, 100 µL of solution from wells was transferred to a new plate and the absorbance was measured at 570 nm with the BIO-Rad xMark Microplate spectrophotometer. The absorbance values for the controls (wells with adding of the broth only) were subtracted from the values for the test wells to minimize background interference.

2.2.4 Ames test

The mutagenicity of nucleosides was assessed using the Ames test with *Salmonella typhimurium* TA100 strain as described [10]. The strain is a histidine auxotroph due to a point mutation in the histidine operon. Under the influence of mutagenic factors, the strain reverts to prototrophy. Bacterial suspensions (2×10^9 cells/mL) were exposed to different doses (1-100 µg/mL) of the compounds. The negative control was a suspension of bacteria, with the addition of the required amount of solvent (DMSO). Sodium azide (2 µg/plate) was used as a positive control. Based on the results obtained, the mutation induction factor (IF) was calculated as the ratio of the number of revertant colonies in the variants with the addition of the tested compounds to the same indicator in the negative control. If IF exceeded 2 it was concluded that there was a mutagenic effect.

2.2.5 Cytotoxicity

The cytotoxic activity of nucleosides and their derivatives was evaluated in the MTT test, according to [11] using A549 cells. Briefly, cells were seeded in 96-well plates with the final density of 1×10^4 cells per well and incubated for 24 h at 37°C and 5% CO_2 in humidified atmosphere. The medium from the wells was then aspirated and replaced with fresh one containing the test compounds at a final concentration of 100 μg/mL or an appropriate amount of solvent (DMSO). After incubation, the medium in the wells was changed to MTT (0.5 mg/mL final concentration) containing medium for 3 h. Then the medium was completely aspirated. Formazan crystals formed during the reduction of tetrazolium by living cells were dissolved by adding 100 μl of DMSO to the wells. The optical density of the formazan solution in the wells was measured using BioRad xMarkTM Microplate Spectrophotometer at a wavelength of 570 nm.

2.3 Statistical analysis

All experiments were performed in biological triplicates (i.e. newly prepared cultures and medium) with at least three repeats in each run. For all tests significant differences were reported at $p < 0.05$ using the nonparametric Mann-Whitney U-test. The data are presented as the mean ± standard deviation.

3 Results and Discussion

3.1 Antimicrobial activity, mutagenicity and cytotoxicity

The MICs of nucleosides and nucleoside derivatives were evaluated for *Salmonella typhimurium* TA100**,** *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 35591 as well as clinical isolates of *Staphylococcus epidermidis* and *Candida albicans* (Table 1, Table S2).

In the whole range of concentrations (0.5 μg/mL - 128 μg/mL) for the tested compounds the ability to inhibit the growth of bacterial cultures was not established, which indicates the potentially low toxicity of these agents against prokaryotes and the probable absence of their antibacterial properties. At the same time, the nontoxicity of nucleosides and their derivatives at doses of 128 μg/mL and lower towards *S. typhimurium* TA100 makes it impossible to obtain false-negative results in the Ames test. For 2 of the 83 agents tested, significant mutagenic activity was recorded at a concentration of 100 μg/mL, which was expressed as an increase in mutation induction factor (IF) to 2.65 ± 0.24 and 2.57 ± 0.11 for compounds **384** and **392**, respectively, allowing these modified purine nucleosides to be evaluated as weak mutagens (Table S3). Cytotoxicity properties of the agents were characterized in the MTT test and it was established that at a concentration of 100 μg/mL none of the studied compounds inhibited the viability of human lung adenocarcinoma A549 cells including the analogue of intermediate of the RNA cleavage by guanyl-specific RNase - 2',3' cGMP. At the same time, 20 of the 83 agents tested had the ability to inhibit the development of *C. albicans* yeast with MICs ranged from 32 to 128 μg/mL. The highest ability to inhibit yeast growth was observed for compound **202**.

Table 1. Minimum inhibitory concentration of the selected nucleosides, μg/mL.

N	Code, molecular formula	*E. coli* ATCC 25922	*S. typhimurium* TA 100	*S. epidermidis*	*S. aureus* ATCC 35591	*C. albicans*
1	**202,** $C_{15}H_{17}N_5O_5$	> 128	> 128	> 128	> 128	32
2	**282,** $C_{17}H_{18}FN_5O_4$	> 128	> 128	> 128	> 128	64
3	**335,** $C_{25}H_{23}N_5O_4$	> 128	> 128	> 128	> 128	128
4	**344,** $C_{11}H_{14}N_6O_4$	> 128	> 128	> 128	> 128	128
5	**372,** $C_{10}H_{14}N_6O_4$	> 128	> 128	> 128	> 128	64
6	**384,** $C_{10}H_{14}N_6O_4$	> 128	> 128	> 128	> 128	64
7	**392,** $C_{11}H_{15}N_5O_5$	> 128	> 128	> 128	> 128	64
8	**422,** $C_{17}H_{18}ClN_5O_4$	> 128	> 128	> 128	> 128	128
9	**429,** $C_{18}H_{21}N_5O_5$	> 128	> 128	> 128	> 128	128
10	**452,** $C_{15}H_{17}N_5O_4S$	> 128	> 128	> 128	> 128	128
11	**466,** $C_7H_{10}N_2O_3$	> 128	> 128	> 128	> 128	128
12	**467,** $C_{12}H_{12}N_2O_4$	> 128	> 128	> 128	> 128	128
13	**510,** $C_9H_{10}N_2O_5$	> 128	> 128	> 128	> 128	128
14	**511,** $C_{10}H_{11}N_5O_5$	> 128	> 128	> 128	> 128	128
15	**512,** $C_{16}H_{14}N_2O_6$	> 128	> 128	> 128	> 128	128
16	**513,** $C_{35}H_{53}N_5O_{11}$	> 128	> 128	> 128	> 128	128
17	**514,** $C_{18}H_{22}N_2O_{11}$	> 128	> 128	> 128	> 128	128
18	**515,** $C_9H_{10}N_2O_4$	> 128	> 128	> 128	> 128	128
19	**518,** $C_5H_6N_2O_2$	> 128	> 128	> 128	> 128	128
20	**519,** $C_5H_6N_2O_3$	> 128	> 128	> 128	> 128	128

3.2 Antibiofilm activity

Candida species are opportunistic human yeasts that can cause various infections in susceptible patients. *Candida* commonly exists as part of biofilms on a variety of surfaces, including mucosal as well as medical devices surfaces [12]. In this light, the search for agents capable to inhibit the formation of *Candida* biofilms or to lead to their destruction is particularly interesting. For nucleosides that can suppress *C. albicans* growth, we determined the ability to inhibit yeast biofilm formation and established biofilm preventing concentration (BPC) when the agents were applied to the wells directly together with the microbial suspension (Table 2).

11 out of 20 tested agents had a differently expressed ability to inhibit the formation of *C. albicans* biofilms. The most pronounced activity was characteristic of compounds **202** and **392**, for which it was possible to establish BPC, which reached 8 μg/ml and 32 μg/ml, respectively. But we cannot hypothesise specific antibiofilm activity in these nucleosides due to insufficient MIC and BPC difference. The other compounds in the range of concentrations investigated did not lead to complete deprivation of yeast ability to form biofilms under the experimental conditions (Table 2).

The next step of our study was to evaluate the ability of compounds that exhibited an inhibitory effect on biofilm formation to induce the destruction of *C. albicans* biofilms. Substances with such properties have high potential for use in biomedical applications. For this purpose, agents were applied to wells with mature biofilms and then after incubation their ability to hold crystal violet was evaluated in comparison with the untreated variant. Nucleoside concentrations of 1 μg/mL and 2 μg/mL were excluded from the study as they had no significant effect on biofilm formation for all agents tested.

Table 2. Effect of nucleosides on *C. albicans* biofilm formation.

N	Code, molecular formula	Biofilm formation, %*				
		Concentration of nucleoside, μg/mL				
		4	8	16	32	64
1	**202,** $C_{15}H_{17}N_5O_5$	44.1 ± 12.6**	0**	0**	-***	-
2	**282,** $C_{17}H_{18}FN_5O_4$	99.6 ± 7.8	87.4 ± 17.2	57.4 ± 18.3**	34.8 ± 8.2**	-
3	**335,** $C_{25}H_{23}N_5O_4$	117.8 ± 21.2	102.7 ± 18.1	106.5 ± 16.8	83.1 ± 8.6**	68.7 ± 16.3**
4	**344,** $C_{11}H_{14}N_6O_4$	104.1 ± 13.7	118.1 ± 19.5	105.1 ± 8.6	107.4 ± 12.8	88.9 ± 11.2
5	**372,** $C_{10}H_{14}N_6O_4$	103.3 ± 7.1	104.8 ± 8.5	92.4 ± 14.1	87.2 ± 5.8**	-
6	**384,** $C_{10}H_{14}N_6O_4$	97.2 ± 9.4	93.3 ± 6.5	76.1 ± 17.4**	52.8 ± 11.3**	-
7	**392,** $C_{11}H_{15}N_5O_5$	21.2 ± 5.4**	17.2 ± 3.3**	5.7 ± 1.2**	0**	-
8	**422,** $C_{17}H_{18}ClN_5O_4$	107.3 ± 10.5	98.5 ±15.4	100.4 ± 12.2	95.8 ± 13.2	93.5 ± 8.1
9	**429,** $C_{18}H_{21}N_5O_5$	108.1 ± 17.2	91.9 ± 24.6	88.4 ± 15.1	71.2 ± 27.8	33.8 ± 6.6**
10	**452,** $C_{15}H_{17}N_5O_4S$	107.6 ± 11.8	98.5 ± 12.1	103.3 ± 10.5	108.6 ± 17.3	101.7 ± 8.5
11	**466,** $C_7H_{10}N_2O_3$	103.3 ± 9.6	108.3 ± 14.4	94.7± 9.6	88.6 ± 16.8	92.1 ± 12.8
12	**467,** $C_{12}H_{12}N_2O_4$	101.7 ± 6.1	94.4 ± 17.8	91.5 ± 6.2	53.8 ± 8.3**	6.1 ± 2.4**
13	**510,** $C_9H_{10}N_2O_5$	113.9 ± 14.8	126.4 ± 21.5	105.6 ± 13.4	88.5 ± 10.1	83.1 ± 4.6**
14	**511,** $C_{10}H_{11}N_5O_5$	100.2 ± 15.8	106.3 ± 19.3	113.9 ± 10.8	95.2 ± 16.6	106.8 ± 19.6
15	**512,** $C_{16}H_{14}N_2O_6$	103.3 ± 9.6	98.4 ± 8.8	102.4 ± 11.3	94.7 ± 9.1	98.5 ± 14.1
16	**513,** $C_{35}H_{53}N_5O_{11}$	109.4 ± 7.1	104.7 ± 8.5	103.8 ± 8.2	89.8 ± 15.1	91.3 ± 9.6
17	**514,** $C_{18}H_{22}N_2O_{11}$	98.4 ± 28.6	103.8 ± 21.3	101.7 ± 14.1	102.4 ± 11.7	90.7 ± 18.2
18	**515,** $C_9H_{10}N_2O_4$	77.1 ± 23.9	12.1 ± 7.2**	5.3 ± 3.6**	2.8 ± 0.5**	2.4 ± 1.2**
19	**518,** $C_5H_6N_2O_2$	94.9 ± 18.3	101.2 ± 9.4	99.6 ± 16.0	107.0 ± 12.4	62.1 ± 13.8**
20	**519,** $C_5H_6N_2O_3$	109.5 ± 11.4	94.4 ± 6.5	103.1 ± 13.5	108.1 ± 19.1	81.5 ± 14.2

* - biofilm in control (untreated with nucleosides wells) was taken for 100%;
** - statistically significant differences with control, $p < 0.05$;
*** - the value was not determined due to MIC

Treatment of mature yeast biofilms with compounds **202**, **282**, **392**, **467** and **515** resulted in their partial disruption (Table 3). The action of all compounds showed a concentration dependence. The compound with the highest ability to disrupt *Candida* biofilm was the compound with code **202**, which is an adenosine derivative. Treatment of mature *C. albicans* biofilm with this agent at a concentration of 64 μg/mL (2 × MIC) resulted in its degradation to 14.6 ± 2.8 %. 8 μg/mL of compound **202** (BPC) reduced biofilm to 61.5 ± 17.2 % during 24 h incubation. Compounds **282**, **392** (adenosine derivatives) and **467** (barbituric acid derivative), exhibited the ability to disrupt *C. albicans* biofilms only at concentrations of 32 μg/mL or more, while agent **515** (pyrimidine derivative) was able to mediate biofilm disruption up to a concentration of 8 μg/mL similar to **202**.

Table 3. Effect of nucleosides on pre-formed *C. albicans* biofilms.

N	Code, molecular formula	Biofilm, %*				
		Concentration of nucleoside, μg/mL				
		4	8	16	32	64
1	**202,** $C_{15}H_{17}N_5O_5$	106.8 ± 23.1	61.5 ± 17.2**	34.8 ± 10.6**	32.7 ± 13.1**	14.6 ± 2.8**
2	**282,** $C_{17}H_{18}FN_5O_4$	103.1 ± 22.4	99.5 ± 28.8	81.1 ± 24.5	66.3 ± 12.6**	41.3 ± 10.4**
3	**335,** $C_{25}H_{23}N_5O_4$	111.0 ± 27.3	95.7 ± 21.6	101.9 ± 18.0	98.1 ± 28.7	106.5 ± 21.6
4	**372,** $C_{10}H_{14}N_6O_4$	114.2 ± 18.3	118.1 ± 26.8	98.4 ± 19.8	101.9 ± 28.2	86.5 ± 20.3
5	**384,** $C_{10}H_{14}N_6O_4$	102.0 ± 17.1	109.8 ± 19.2	104.2 ± 20.6	96.3 ± 25.4	98.1 ± 14.9
6	**392,** $C_{11}H_{15}N_5O_5$	117.0 ± 21.5	94.6 ± 18.9	87.2 ± 23.5	47.1 ± 15.6**	38.2 ± 8.8**
7	**429,** $C_{18}H_{21}N_5O_5$	103.9 ± 21.4	99.5 ± 22.7	110.1 ± 21.8	103.3 ± 20.6	88.3 ± 27.6
8	**467,** $C_{12}H_{12}N_2O_4$	98.2 ± 23.5	91.3 ± 21.4	106.9 ± 22.7	88.1 ± 16.5	61.5 ± 14.8**
9	**510,** $C_9H_{10}N_2O_5$	107.2 ± 21.7	118.6 ± 24.7	101.2 ± 21.5	87.1 ± 22.6	83.7 ± 21.4
10	**515,** $C_9H_{10}N_2O_4$	98.6 ± 19.1	76.8 ± 12.5**	55.8 ± 15.2**	51.1 ± 19.3**	49.6 ± 11.3**
11	**518,** $C_5H_6N_2O_2$	97.4 ± 20.6	96.9 ± 23.6	106.1 ± 24.7	102.2 ± 23.5	91.8 ± 27.1

* - biofilm in control (untreated with nucleosides wells) was taken for 100%;
** - statistically significant differences with control, $p < 0.05$

Biological activity characterization of the nucleoside set allowed us to determine that most often these compounds exhibited antimycotic activity, which was established for 24% of the compounds studied. Indeed, to date, a number of nucleosides of natural origin with a similar

type of activity have been described, the main mechanism of action being the disruption of the cell wall component biosynthesis, in particular by inhibiting the activity of fungal chitin synthases and protein-synthesizing system [13, 14]. Inhibition of bacterial cell wall and protein biosynthesis by natural and engineered nucleosides occurs, which explains their antimicrobial properties. We suggest a similar mechanism of the detected antimicrobial action towards *C. albicans*.

We applied two methods to assess the effect of nucleosides on *C. albicans* biofilm formation. Agents added directly with microorganisms allow us to evaluate the yeasts potential for biofilm formation in the presence of nucleosides, and the addition of compounds to wells with pre-formed biofilms allows to characterize their ability to biofilm disruption. This approach is justified because microorganisms within the formed biofilm acquire increased resistance to the drugs and has been used previously, in particular, to characterize the antibiofilm properties of the natural nucleoside tunicamycin [15]. A number of agents we investigated had comparable antibiofilm activity to tunicamycin. Compounds **202** and **515** inhibited biofilm development and caused biofilm destruction (100% for **202** and 87.9 ± 7.2% for **515**) at concentrations of 8 μg/mL, while tunicamycin significantly inhibited *C. albicans* biofilm formation at concentrations starting at 0.844 μg/mL but did not cause 50% destruction of mature biofilms at concentrations of 108.03 μg/mL or less [15].

4 Conclusions

Overall, this study indicates that in the analysed set of nucleosides and their derivatives there are agents with significant fungicidal activity. Agent **202**, which is an N6-substituted adenosine derivative with a furan moiety, had the greatest ability to inhibit the growth and biofilm formation of a *C. albicans* clinical isolate, as well as to cause the disruption of mature yeast biofilms. The antimycotic properties of such agents are demonstrated for the first time. Taking into account the absence of cytotoxic activity and mutagenicity, it is possible to consider this compound as a promising antifungal agent, especially in the context of its antibiofilm properties.

Acknowledgments

This work was carried out with the support of a grant from the Academy of Sciences of the Republic of Tatarstan, provided to young candidates of science (postdoctoral researchers) for the purpose of defending their doctoral dissertations, conducting scientific research, and performing work duties in scientific and educational organizations of the Republic of Tatarstan within the framework of the State Program of the Republic of Tatarstan “Scientific and Technological Development of the Republic of Tatarstan.”

References

1. U. Agrawal, R. Raju, Z.F. Udwadia, Favipiravir: A new and emerging antiviral option in COVID-19. Med. J. Armed. Forces India **76**, 370-376 (2020**)**. https://doi.org/10.1016/j.mjafi.2020.08.004
2. W.B. Parker, Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer. Chem. Rev. **109**, 2880-2893 (2009). https://doi.org/10.1021/cr900028p

3. T.D.H. Bugg, R.V. Kerr, Mechanism of action of nucleoside antibacterial natural product antibiotics. J. Antibiot. (Tokyo). **72**, 865-876 (2019). https://doi.org/10.1038/s41429-019-0227-3
4. G. Di Bonaventura, V. Lupetti, S. De Fabritiis, A. Piccirilli, A. Porreca, M. Di Nicola, A. Pompilio, Giving Drugs a Second Chance: Antibacterial and Antibiofilm Effects of Ciclopirox and Ribavirin against Cystic Fibrosis Pseudomonas aeruginosa Strains. Int. J. Mol. Sci. **23**, 5029 (2022)**.** https://doi.org/10.3390/ijms23095029
5. M. Yang, Q. Wei, L. Shi, Z. Wei, Z. Lv, N. Asim, K. Zhang, B. Ge, Wuyiencin produced by Streptomyces albulus CK-15 displays biocontrol activities against cucumber powdery mildew. J. Appl. Microbiol. **131**, 2957-2970 (2021). https://doi.org/10.1111/jam.15168
6. Y.V. Sokurenko, P.V. Zelenikhin, V.V. Ulyanova, A.I. Kolpakov, D. Muller, O.N. Ilinskaya, Identification of 2',3' cGMP as an Intermediate of RNA Catalytic Cleavage by Binase and Evaluation of Its Biological Action. Russ. J. Bioorg. Chem. **41**, 31-36 (2015). https://doi.org/10.1134/S1068162015010136
7. E.M. Savelieva, V.E. Oslovsky, D.S. Karlov, N.N. Kurochkin, I.A. Getman, S.N. Lomin, G.V. Sidorov, S.N. Mikhailov, D.I. Osolodkin, G.A. Romanov, Cytokinin activity of N6-benzyladenine derivatives assayed by interaction with the receptors in planta, in vitro, and in silico. Phytochemistry. **149**, 161-177 (2018). https://doi.org/10.1016/j.phytochem.2018.02.008
8. S.D. Sarker, L. Nahar, Y. Kumarasamy, Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. **42**, 321-324. (2007). https://doi.org/10.1016/j.ymeth.2007.01.006
9. J.H. Merritt, D.E. Kadouri, G.A. O'Toole, Growing and analyzing static biofilms. Curr. Protoc. Microbiol. Chapter 1: Unit 1B.1. (2005). https://doi.org/10.1002/9780471729259.mc01b01s00
10. K. Mortelmans, E. Zeiger, The Ames Salmonella/microsome mutagenicity assay. Mutat. Res. **455**, 29-60 (2000). https://doi.org/10.1016/s0027-5107(00)00064-6
11. T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods**. 65**, 55-63 (1983). https://doi.org/10.1016/0022-1759(83)90303-4
12. M. Gulati, C.J. Nobile, Candida albicans biofilms: development, regulation, and molecular mechanisms. Microbes Infect. **18**, 310-321 (2016). https://doi.org/10.1016/j.micinf.2016.01.002
13. M. Winn, R.J. Goss, K. Kimura, T.D. Bugg, Antimicrobial nucleoside antibiotics targeting cell wall assembly: recent advances in structure-function studies and nucleoside biosynthesis. Nat. Prod. Rep. **27**, 279-304 (2010). DOI: https://doi.org/10.1039/b816215h
14. C.T. Walsh, W. Zhang, Chemical logic and enzymatic machinery for biological assembly of peptidyl nucleoside antibiotics. ACS Chem. Biol. **6**, 1000-1007 (2011). DOI: https://doi.org/10.1021/cb200284p
15. Q. Yu, X. Ding, N. Xu, X. Cheng, K. Qian, B. Zhang, L. Xing, M. Li, In vitro activity of verapamil alone and in combination with fluconazole or tunicamycin against Candida albicans biofilms. Int. J. Antimicrob. Agents. **41**, 179-182 (2013). DOI: https://doi.org/10.1016/j.ijantimicag.2012.10.009