ISBN 978-9940-611-06-4

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INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY

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PROCEEDINGS

II INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY
COAST 2023

Book title:
Proceedings COAST 2023

Publisher:
Faculty of Management Herceg Novi

Design and Computer processing:
Sanja Samardžić, MSc, Jelena Poznanović, MSc, Zvonko Perušina, BSc

CIP - Каталогизација у публикацији
Национална библиотека Црне Горе, Цетиње

INTERNATIONAL conference on advances in science and technology (II ; 2023 ; Herceg Novi)
Proceedings/International conference on on advances in science and technology, Herceg Novi, 31 May - 03 June, 2023 = Zbornik radova / Međunarodna konferencija o savremenim dostignućima u nauci i tehnologiji, Herceg Novi, 31 maj - 03 jun 2023. godine : Fakultet za menadžment, 2023 (Herceg Novi). - 1421 стр. : илустр.

Радови на срп. и енгл. језику. - Текст ћир. и лат. - Напомене и библиографске референце уз текст. - Библиографија уз сваки рад. - Сажеци на енгл. и срп. језику уз радове.

ISBN **978-9940-611-06-4**
COBISS.CG-ID **27152388**

ANTIMICROBIAL ACTIVITY OF OS(II) COMPLEXES CONTAINING *N,N,N*-INERT LIGANDS DERIVATES OF PYRAZYL-PYRIDINE

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ABSTRAC

Complexes of transition metals, including osmium complexes, are recognized for their good antitumor activity. Osmium complexes are considered to be potential anticancer agents with some of them also having potential as antimicrobial agents. Therefore, we have examined the antimicrobial activity of Os(II) complexes with N,N,N-inert ligands derivates of pyrazyl-pyridine that showed good anticancer activity. Antimicrobial activity was tested by determining the minimum inhibitory concentrations (MIC) and minimum microbicidal concentration (MMC) using the microdilution plate method for four gram-positive and three gram-negative bacteria, one probiotic, and one yeast. Gram-positive bacteria showed the highest sensitivity except for the isolate S. aureus. MIC was in the range of <3.9 to >1000 μg/mL. The highest resistance was shown by the yeast Candida albicans ATCC 10231. Os3 is distinguished from the examined complexes by the strength of the antimicrobial activity. In the activity of this complex, there are no differences in the action between Gram-positive and Gram-negative bacteria. Os3 on bacteria operates in the range of <7.8 for MIC to 250 μg/mL for MMC

Keywords: Os(II) complexes, antibacterial, antifungal

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1. INTRODUCTION

According to the World Health Organization, [1] after cardiovascular disease, various cancers are the leading cause of death, with lung cancer having the highest

mortality rate. Cancer-related deaths are followed by the number of HIV-related deaths. Some researches suggest that the increasing occurrence of various viruses such as Covide-19, H1N1, SARS, MERS, etc., which are claiming more and more lives, should be taken into account, and viral infections may be the main cause of death in the future. [2] Every year, billions of Euros are spent on the treatment of these diseases worldwide, which indicates that research in the field of inventing more effective drugs with fewer side effects and more economical drugs is necessary in the present era. The current situation shows that the major challenges in modern medicine are: 1) the resistance of microorganisms, which has become a worldwide problem, 2) the use of drugs, which is increasing dramatically, while the discovery of new drugs is slowing down drastically, 3) the diagnosis of an increasing number of diseases for which new drugs must be found. It is estimated that the number of deaths caused by resistant microorganisms will increase to 10 million by 2050, exceeding the number of deaths from cancer (WHO, 2014).

What is the role of transition metals in this story? It is known that iron compounds have been used since ancient times to treat anemia, silver is known for its antibacterial effects, and lithium compounds are used to treat various forms of depression. After the discovery of the platinum(II) complex known as cisplatin, which has anticancer activity (today, most chemotherapy protocols contain cisplatin), research expanded into the use of transition metal compounds for medical purposes.[3-5] In addition to the intensive use of certain transition metal compounds in chemotherapy, research has shown that transition metal compounds are effective as anti-HIV and anti-malarial agents, and also have antiviral and antibacterial activity[6,7] Research into the use of transition metals is widely justified because they are effective against diseases that cause millions of deaths worldwide each year.

Thus, in the present study, we investigated the antimicrobial activity of Os(II)complexes with N,N,N-inert ligands, which are pyrazyl-pyridine derivatives.

2. Experimental Section

2.1. Chemicals and solutions

Preparation of Os1, Os2 and Os3 complexes were, according to published procedures.[8] Dimethyl sulfoxide (DMSO) was purchased from Acros Organics (New Jersey, USA).

2.1.9. In vitro antimicrobial assay

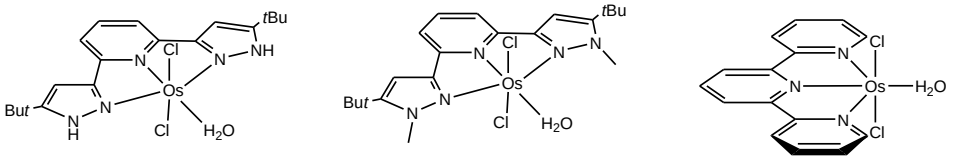
Test substances, microorganisms, and suspension preparation the tested compounds were dissolved in DMSO and then diluted in a liquid culture medium to reach a concentration of 10%. The antibiotic doxycycline (Galenika A.D., Belgrade) was dissolved in a liquid culture medium, Mueller-Hinton broth (Torlak, Belgrade), while the antifungal fluconazole (Pfizer Inc., USA) was dissolved in Sabouraud dextrose broth (Torlak, Belgrade). The antimicrobial activity of the complexes was tested against four Gram-positive and three Gram-negative bacteria, one probiotic and one yeast (Table 1). The 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 the turbidity of the 0.5-McFarland standard [9], the bacterial suspension contained approximately 10^8 colony-forming units (CFU)/mL, and the yeast suspension contained 10^6 CFU/mL. Tenfold dilutions of the initial suspension were also prepared in sterile 0.85% saline. Bacterial inoculi were obtained from bacterial cultures incubated for 24 hours at 37°C on Mueller-Hinton agar substrate and brought to approximately 10^6 CFU/mL by dilution according to the 0.5-McFarland standard. Yeast inoculi were incubated for 48 h at 26 ◦C on tryptone soya agar substrate and brought up to approximately 10^4 CFU/mL by dilution according to the 0.5 McFarland standard.

Microdilution procedure: Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) using the microdilution plate method with resazurin. [10] The 96-well plates were prepared by adding 100 µl of a nutrient broth, Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for yeast, to each well. An aliquot of 100 µl of stock solution of the tested compound (with a concentration of 2000 µg/ml) was added to the first row of the plate. Then, twofold dilution series were performed using a multichannel pipette. The concentration range obtained was between 1000 and 7.8 µg/mL. The method is described in detail in the report [11]. Doxycycline and fluconazole were used as positive controls. it was found that 10% DMSO (as solvent control) did not inhibit the growth of microorganisms. Each test included a growth control and a sterility control. All tests were performed in duplicate, and MICs were constant. Minimum bactericidal and fungicidal concentrations were determined by plating 10 µl of samples from wells in which no change in indicator color or mycelial growth was detected onto nutrient agar medium. At the end of the incubation period, the lowest concentration with no growth (no colony) was defined as the minimum microbicidal concentration.

3. RESULTS AND DISCUSSION

The antimicrobial activity of Os(II) complexes with *N,N,N*-inert ligands derivates of pyrazyl-pyridine, that showed good anticancer activity, was investigated. The structure of investigated complexes have shown in Fig. 1., H_2L^{tBu} = 2,6-bis(5-tert-butyl-1H-pyrazol-3-yl)pyridine, Me_2L^{tBu} = 2,6-bis(5-tert-butyl-1methyl-pyrazol-3-yl)pyridine, terpy = 2,2′:6′,2″-terpyridine.



$[Os^{II}(H_2L^{tBut})Cl_2H_2O]$ (Os1) **$[Os^{II}(Me_2L^{tBut})Cl_2H_2O]$ (Os2)** **$[Os^{II}(terpy)Cl_2H_2O]$ (Os3)**

Figure 1. The structure of investigated complexes

The results of *in vitro* antimicrobial activity of studied compounds against four Gram-positive and three Gram-negative bacteria, one probiotic, and one yeast, were determined by the microdilution method. The results are presented in Table 1. Inhibitory effects of 10% DMSO on microorganism's growth was not observed. Generally, the compounds showed different degrees of antimicrobial activity.

Gram-positive bacteria showed the highest sensitivity except for the isolate *Staphylococcus aureus*. MIC was in the range of <3.9 to >1000 μg/mL. The highest resistance was shown by the yeast *Candida albicans* ATCC 10231.

Os3 is distinguished from the examined complexes by the strength of the antimicrobial activity. In the activity of this complex, there are no differences in the action between Gram-positive and Gram-negative bacteria. Os3 on bacteria operates in the range of <7.8 for MIC to 250 μg/mL for MMC. For Gram-negative bacteria, Os3 acts at the same level as positive control doxycycline. Looking at the effect of the positive control, we can see that Os3 acts better than doxycycline on *Proteus mirabilis* ATCC 12453.

Table 1. The results of *in vitro* antimicrobial activity of studied Os1, Os2 and Os3 complexes.

Species / compounds		**OS1**	**OS2**	**OS3**	**Doxycycline/ Fluconazole**
Lactobacillus plantarium	**MIC** [a]	15.6	250	<7.8	0.45
	MMC [b]	15.6	1000	15.6	7.8
Staphylococcus aureus ATCC 25923	**MIC**	<3.9	125	<7.8	0.22
	MMC	375	250	250	3.75
Staphylococcus aureus	**MIC**	500	>1000	15.6	0.45
	MMC	>1000	>1000	31.25	7.8
Bacillus subtilis ATCC 6633	**MIC**	15.6	125	<7.8	1.95
	MMC	125	250	15.6	31.25
Bacillus subtilis	**MIC**	<7.8	250	<7.8	0.11
	MMC	250	250	11.7	1.95
Escherichia coli	**MIC**	>1000	1000	15.6	15.6
	MMC	>1000	>1000	46.8	31.25
Salmonella enterica	**MIC**	1000	500	<7.8	15.6
	MMC	>1000	>1000	<7.8	31.25
Proteus mirabilis ATCC 12453	**MIC**	15.6	31.25	<7.8	250
	MMC	47.3	93.75	<7.8	>250
Candida albicans ATCC 10231	**MIC**	>1000	>1000	500	31.25
	MMC	>1000	>1000	1000	1000

[a] MIC values (μg/mL) – means inhibitory activity
[b] MMC values (μg/mL) – means microbicidal activity

Previous research shows different antimicrobial activities of osmium complexes. Some studies show the activity of Os(II) complexes in both Gram-positive and Gram-negative bacteria [12]. The antimicrobial activity of other osmium complexes is very significant as it has been demonstrated against highly resistant strains of both Gram-positive and Gram-negative bacteria such as *E. coli* (EC958), *S. aureus* (MRSA), *A. baumannii* (AB184), and *P. aeruginosa* (PA2017). Antimicrobial activity was demonstrated in MRSA cells, with an MIC was 32 μg/mL [13]. In contrast to our study, one study shows that the osmium complex exhibits significant activity on Gram-positive bacteria and *Candida albicans*, whereas it shows no activity in selected Gram-negative bacteria [14]. Some osmium complexes show no activity on standards of Gram-negative bacteria (*Pseudomonas aerugonis* ATCC27853, *Escherichia coli* ATCC25922) or clinical isolates of *Acinetobacter baumannii* or yeasts (*Candida albicans* SC5314, *Candida auris* ATCC21092). Conversely, Gram-positive *Staphylococcus aureus* ATCC11007 and *Enterococcus faecalis* ATCC29112 are sensitive to the same complexes. The same complexes show activity against isolates of highly resistant MSSA, MRSA, VSE, and VRE, where they exhibit MIC values in the low micromolar range (MIC<10 μM) [15]. Osmium(II) complex showed antinicrobal activity against *M. smegmatis* and bactericidal activity against drug-resistant *E. faecalis* and methicillin-resistant *S. aureus* ATCC 43300 (Gichumbi et al 2018).

3. CONCLUSION

The obtained data showed that the studied complexes exhibited significant antimicrobial activity. A significant number of Gram-positive bacteria showed the highest sensitivity, with the exception of the isolate *S. aureus*. The highest resistance was shown by the yeast *Candida albicans* ATCC 10231. Os3 differs from the studied complexes in the strength of antimicrobial activity. There are no differences in the activity of this complex between Gram-positive and Gram-negative bacteria

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