Bulgarian Journal of Agricultural Science, 18 (No 3) 2012, 446-450
Agricultural Academy

ANTIMICROBIAL ACTIVITY *IN VITRO* OF FOUR NICKEL COMPLEXES

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Abstract

POPOVA, T. P., R. I. ALEXANDROVA, R. TUDOSE, E.-M. MOSOARCA and O. COSTISOR, 2012. Antimicrobial activity *in vitro* of four nickel complexes. *Bulg. J. Agric. Sci.,* 18: 446-450

The *in vitro* effect of four nickel complexes with ligands containing the antipyrine moiety N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) against 33 pathogenic bacterial strains, as well as control ones, was examined. The antifungal activity of the complexes against four *Candida* spp. was established too. Two routine methods were used: of Bauer-Kirby and the determination of minimum inhibitory concentrations. The compounds $Ni(TAMEN)(NCS)_2$ and $Ni(TAMEN)(ClO_4)_2$ manifested good antibacterial activity *in vitro* against the tested bacteria, especially towards the Gram-positive strains, as well as against *K. pneumoniae* and *P. aeruginosa*. Among all tested microorganisms, the sensitivity of the strains of *E. coli* to the examined complexes proved most weak. *Candida* spp. showed high sensitivity to $Ni(TAMEN)(NCS)_2$ and $Ni(TAMEN)(ClO_4)_2$ too and weaker to $Ni_2(BAMP)(Cl)_4$ and $Ni_2(BAMP)(Ac)_4$.

Key words: nickel complexes, antibacterial activity
Abbreviations: MIC - minimum inhibitory concentrations; BAMP - N,N'-bis(4-antipyrylmethyl)-piperazine; TAMEN - N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane

Introduction

Nickel is one of the metals with antimicrobial properties. Its ions can penetrate in microbial cells and kill them mainly by inactivating their enzymes (Chohan, 2000). Many investigations show that different nickel (II) complexes manifest antimicrobial action (Rao and Reddy, 1990; Thirumalaikumar et al., 1999; Chohan et al., 2002; Singh et al., 2003; Patel et al., 2004). Dependence of the inhibitory activity of the metal ions from the chelate geometry and thermal stability of the metal complexes has been established (Kamalakannan and Venkappayya, 2002). Appreciable antimicrobial activity in comparison with the ligands has been proved in some nickel compounds (Naik et al., 2002; Shivankar and Takkar, 2003). Blasco et al. (1996) established that in complexation with sulfanilamide derivatives, nickel (II) and other metal ions enhance their inhibitory activity *in vitro* against such important relative pathogens as *E. coli* and *S. aureus*.

It is interesting to study the effect of nickel complexes on *Candida spp*. The investigations in this direction are insufficient up now (Sharma and Varshney, 1991; Shivankar and Takkar, 2003).

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In this study we directed our efforts to investigate the antibacterial and antifungal activity *in vitro* of five nickel compounds.

Material and Methods

In the experiments were used pure cultures of 33 pathogenic bacterial strains, isolated from animals, as well as control strains. Half of them were Gram-positive (6 strains of *Staphylococcus aureus*, 4 of *Bacillus subtilis* and 7 of *Streptococcus pyogenes*) and the rest - Gram-negative (5 strains of *Escherichia coli*, 2- of *Klebsiella pneumoniae*, 6-*Pseudomonas aeruginosa* and 3-*Pseudomonas fluorescens*). The strains were received from patients subject to continuous treatment with different antibacterial means and showed higher drug resistance *in vitro*. In the experiments were included pure cultures of 3 pathogenic strains of *Candida albicans* and 1 of *Candida tropicalis*.

In the experiments were used four nickel complexes with ligands containing the antipyrine moiety N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) (Figure 1) andN,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) (Figure 2): $Ni_2(BAMP)(Cl)_4$, $Ni_2(BAMP)(Ac)_4$, $Ni(TAMEN)(NCS)_2$ and $Ni(TAMEN)(ClO_4)_2$. The compounds were obtained according to the methods described in previous works (Costisor et al., 1981; Costisor et al., 1994 a, b).

The dilutions of the compounds and the controls were prepared in sterile phosphate buffered saline (PBS) with pH 7.2.

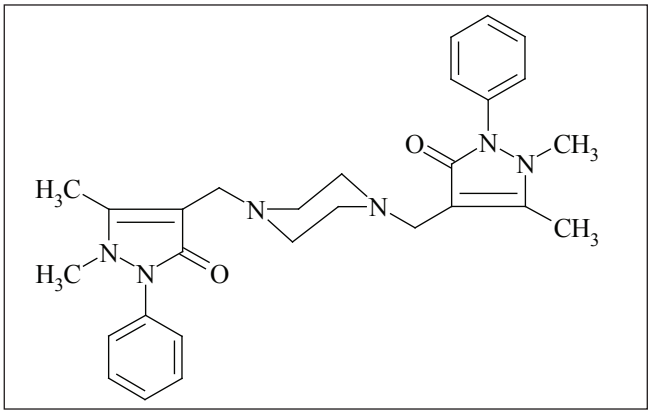

Fig. 1. N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP)

The antibiotic thiamphenicol was used as a control for comparison at the determination of the antibacterial activity of the salts.

Studies were carried out by the classic agar-diffusion method of Bauer, Kirby et al. (1966). Bacterial suspensions were inoculated at a dose of 2.10^6 cells/ml on Mueller-Hinton's agar with pH 7.2 – 7.4 and 4 mm layer thickness in Petri dishes with diameter 9 mm. The compounds and controls were applied in PBS solutions by dropping of 0.1 ml in 9-mm holes in the agar at doses 100 μg/well for nickel complexes and ligands and 30 μg/well for the antibiotic. Results were recorded by measuring the diameters of clear zones around the holes in mm, including the whole diameter. Inhibitory effect of the salts was established at zones > 12 mm and >17mm for the antibiotic.

The determination of the minimum inhibitory concentrations (MICs) was performed by the method of twofold serial dilutions on Mueller-Hinton's agar as per Ericsson and Sherris (1971). $MICs_{50}$ were calculated mathematically depending on the number of inhibited colonies of the medium with the respective nickel compound, ligand or antibiotic dilution compared to the control medium colonies without drugs. According to Rodríguez-Argüelles et al. (2005) absence of antimicrobial activity was recorded at MIC>100 μg/ml and antimicrobial effect – at MIC≤100 μg/ml. The activity of the metal compounds tested was defined as high in MIC≤50 μg/ml.

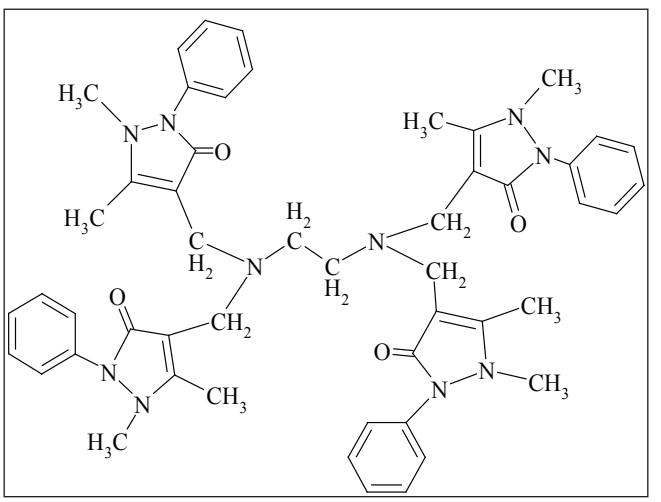

Fig. 2. N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN)

Statistical analysis was made using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

Results and Discussion

The results from the studies performed by the agar-gel diffusion method are presented in Table 1. From the data it is seen that the compounds Ni(TAMEN)$(ClO_4)_2$ and Ni(TAMEN)$(NCS)_2$ manifest good antibacterial activity against the tested bacteria. Largest mean diameters of inhibitory zones were measured in compound Ni(TAMEN)$(NCS)_2$ (20.8±0.9) and Ni(TAMEN)$(ClO_4)_2$ (18.9±0.9), very close with these in thiamphenicol (22.5±0.9) and considerably exceeded the zones in the other examined compounds ($P<0.01$) and the ligands TAMEN (10.9±0.2, $P<0.001$) and BAMP (10.9±0.2, $P<0.001$). Vastly lesser were the sterile zones in compounds $Ni_2(BAMP)(Ac)_4$ (11.9±0.3) and $Ni_2(BAMP)(Cl)_4$ (11.8±0.3) but they reliable exceeded these in the controls TAMEN and BAMP ($P<0.01$). The differences between the mean diameters of the zones of all examined complexes with thiamphenicol were reliable ($P<0.01$). Higher sensitivity to Ni(TAMEN)$(ClO_4)_2$ and Ni(TAMEN)$(NCS)_2$ showed *B. subtilis*, *K. pneumoniae, P. fluorescens* and *C. albicans*. The sensitivity of the tested strains of *E. coli* to the compounds proved most weak. The diameters of the measured zones of inhibition were small.

These results correspond to the data of other authors, which established an enhanced antibacterial activity of Ni (II) complexes in comparison with the ligands in the

Table 1
Inhibitory effect of nickel compounds on pathogenic bacteria and fungi in the agar-diffusion method

Microorganisms	No of strains	Inhibitory zones in mm						
		Ni 1	Ni 2	Ni 3	Ni 4	TAMEN	BAMP	Th
S. aureus	6	15.9±2.4	19.4±2.9	11.3±0.5	10.8±0.3	10.5±0.5	10.5±0.3	22.7±2.2
S. pyogenes	7	18.9±2.6	20.2±1.5	11.8±0.2	11.5±0.4	10.3±0.3	10.5±0.5	26.7±1.5
B. subtilis	4	24.9±0.7	25.1±1.0	no data	11.0±0.4	no data	no data	29.2±1.3
E. coli	5	14.1±2.1	15.6±2.2	11.5±0.5	11.7±0.7	11.5±0.5	11.3±0.5	18.4±1.7
K. pneumoniae	2	23.0±0.3	26.8±1.9	no data	15.8±1.6	no data	no data	22.7±1.0
P. aeruginosa	6	18.0±2.7	18.8±2.5	13.0±0.7	11.5±0.4	11.5±0.3	11.5±0.5	19.8±2.2
P. fluorescens	3	21.5±2.0	24.8±2.3	no data	14.5±0.9	no data	no data	26.1±2.3
Candida spp.	4	20.3±2.2	23.4±2.9	no data	12.0±0.9	no data	no data	13.8±1.0
All strains	37	18.9±0.9	20.8±0.9	11.9±0.3	11.8±0.3	10.9±0.3	10.9±0.3	22.5±0.9

Ni 1 - Ni(TAMEN)$(ClO_4)_2$; Ni 2 - Ni(TAMEN)$(NCS)_2$; Ni 3 - $Ni_2(BAMP)(Ac)_4$; Ni 4 - $Ni_2(BAMP)(Cl)_4$

Table 2
Minimum inhibitory concentrations of nickel compounds and their ligands against pathogenic Gram-positive and Gram-negative microorganisms

Microorganisms	No of strains	MIC_{50}						
		Ni 1	Ni 2	Ni 3	Ni 4	TAMEN	BAMP	Th
S. aureus	6	44.0±18.6	19.3±9.1	144.0±40.3	122.7±31.1	112.0±16.0	160.0±32.0	10.4±4.8
S. pyogenes	7	22.6±8.2	5.6±1.9	128.0±0.0	68.6±10.9	80.0±16.0	106.0±56.32	4.4±2.0
B. subtilis	4	10.5±0.9	5.8±0.3	no data	84.0±12.0	no data	no data	3.0±0.0
E. coli	5	65.6±18.9	103.6±24.4	160.0±32.0	121.6±6.4	104.0±24.0	82.0±28.9	2.0±0.8
K. pneumoniae	2	6.0±1.2	14.0±5.8	no data	40.0±4.6	no data	no data	13.7±1.2
P. aeruginosa	6	51.0±18.4	49.5±19.1	160.0±32.0	133.3±26.7	112.0±16.0	130.0±50.6	15.5±4.5
P .fluorescens	3	10.7±2.9	12.7±5.9	no data	80.0±9.2	no data	no data	7.0±1.2
Candida spp.	4	21.0±14.4	19.3±14.9	no data	21.8±4.0	no data	no data	56.0±24.0
All strains	37	33.1±5.9	30.7±7.1	148.0±13.9	96.8±8.4	102.0±7.6	119.5±16.7	12.6±3.6

MIC_{50} - 50% inhibition; Ni 1 - Ni(TAMEN)$(ClO_4)_2$; Ni 2 - Ni(TAMEN)$(NCS)_2$; Ni 3 - $Ni_2(BAMP)(Ac)_4$; Ni 4 - $Ni_2(BAMP)(Cl)_4$

agar-gel diffusion method (Chohan et al., 2001; Shivankar and Takkar, 2003).

Similar results were received at the establishment of the MICs. The data are presented in Table 2. In this method all tested bacteria were most sensitive to Ni(TAMEN)$(NCS)_2$ (x=30.7±7.1). The difference with thiamphenicol (x=12.6±3.6) was not significant (P>0.05), but the differences towards the mean values of MICs in compounds $Ni_2(BAMP)(Ac)_4$ (148.0±13.9) and $Ni_2(BAMP)(Cl)_4$ (96.8±8.4), as well as in the controls TAMEN (102.0±7.6) and BAMP (119.5±16.7), were significant (P<0.001). The established MICs were low and in Ni(TAMEN)$(ClO_4)_2$ (x=33.1±5.9) too, in spite that the differences with the antibiotic were reliable (P<0.001). The established MICs in $Ni_2(BAMP)(Ac)_4$ and $Ni_2(BAMP)(Cl)_4$ were vastly higher, significantly exceeding these in Ni(TAMEN)$(NCS)_2$, Ni(TAMEN)$(ClO_4)_2$ and thiamphenicol. In compound $Ni_2(BAMP)(Ac)_4$ have received higher mean values of the MICs in comparison with these of the ligands TAMEN and BAMP (P<0.001). In compound $Ni_2(BAMP)(Cl)_4$ the mean MIC's values were higher than these of TAMEN (P<0,001) and BAMP (P>0,05) too. MIC's values were lowest in thiamphenicol, like they significantly differ from these in the rest compounds and controls tested (P<0,001), except of Ni(TAMEN)$(NCS)_2$.

As it is seen from the results, received by the two methods, the Gram-positive bacteria tested were most sensitive to Ni(TAMEN)$(NCS)_2$. The received results were closed to these in thiamphenicol (P>0.05). Very good inhibitory effect against the Gram-positive bacteria showed Ni(TAMEN)$(ClO_4)_2$ too, mostly against *Bacillus* spp. $Ni_2(BAMP)(Ac)_4$ seemed to be less active against the Gram-positive bacteria. Similarly, Kasuga et al. (2001) established selective antibacterial activity of other nickel compounds against some Gram-positive bacteria too.

The established MICs for the Gram-negative microorganisms tested were higher then these of the Gram-positive ones. The examined Gram-negative bacteria were most sensitive to Ni(TAMEN)$(ClO_4)_2$ and Ni(TAMEN)$(NCS)_2$, mainly *K. pneumoniae* and *Pseudomonas* spp., in which the received MICs were commensurable with these in the control antibiotic (P>0.05). More over, the sterile zones of the strains of *K. pneumoniae* were wider then these of thiamphenicol.

In the two methods used, *Candida* spp. showed high sensitivity to Ni(TAMEN)$(NCS)_2$ and Ni(TAMEN)$(ClO_4)_2$ too and weaker to $Ni_2(BAMP)(Cl)_4$. The differences with thiamphenicol in the agar-gel diffusion method were significant (P<0.05). Naturally, the tested strains of genus *Candida* showed low sensitivity to the antibiotic. Their results in $Ni_2(BAMP)(Cl)_4$ were similar – the MICs were a little bit higher then these in thiamphenicol (P>0.05).

The metal complexes, examined by us, were more active against the tested bacteria and fungi than the parent compounds. From the results is seen dependence of antibacterial activity of the tested complexes on their structure, what corresponds with the findings of other authors (Kasuga et al., 2001; Chohan et al., 2002).

The examined nickel compounds were found to be biologically active. The complexes showed more significant antimicrobial activity against one or more bacterial and fungal species in comparison to uncomplexed ligands. Some authors established higher antibacterial and antifungal activity of some nickel complexes than the ligands too (Kamalakannan and Venkappayya, 2002). Other explorers founded different antibacterial and antifungal action of different nickel compounds, with better activity against Gram-positive bacteria. Like us, Kasuga et al. (2001) established that some of the examined by them nickel complexes have not inhibited the ground of the tested bacteria and fungi.

Conclusion

Nickel complexes, examined by us, manifest biologically activity. They showed good antimicrobial effect, especially these with TAMEN - Ni(TAMEN)$(NCS)_2$ (mean inhibitory zones 20.8±0.9 mm and mean MIC_{50} 30.7±7.1) and Ni(TAMEN)$(ClO_4)_2$ (mean inhibitory zones 18.9±0.9 mm and mean MIC_{50} 33.1±5.9). High sensitivity to these compounds showed the Gram-positive bacteria, *Candida* spp., as well as *K. pneumoniae* and *P. aeruginosa*. The sensitivity of the strains of *E. coli* to the examined complexes proved most weak.

The complexes had higher activity against the tested bacteria and fungi than the parent compounds TAMEN (mean inhibitory zones 10.9±0.2 mm and mean MIC_{50}102.0±7.6) and BAMP (mean inhibitory zones 10.9±0.2 mm and mean MIC_{50}119.5±16.7). These re-

sults disclosed dependence of antibacterial activity of the tested complexes on their structure.

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Received August, 23, 2011; accepted for printing February, 2, 2012.