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JSCS: Inorganic Chemistry Sub Editor

**Manuscript title: " New mixed-ligand Ni (II) and Zn (II) macrocyclic complexes with bridged bicyclo-[2,2,1]-hept-5-en-*endo*-2,3-*cis*-dicarboxylate: synthesis, characterization, antimicrobial and cytotoxic activity”**

Dear Prof. Miloš Đuran,

Thank you for useful comments and suggestions on the structure of our manuscript. We thank you for the opportunity to re-review our manuscript in spite of the big corrections too.

Accordingly, we revised the manuscript and the detailed corrections are listed below at the point. Corrected sentences are highlighted in the text on the manuscript.

Responsee to the referees` comments and demands

**Inorganic Chemistry Sub Editor**

Comment The sentence “Many of them have been examined because of their potential as dyestuffs or pigments,3 various industrial applications, involved in a number of biological processes such as photosynthesis and dioxygen transport4 catalytic properties, potential applications as metal extractants, radiotherapeutic and high potential in antitumor therapy.5” is hard to understood and data are scrambled presented. I suggest to be separated into two sentences with aspects concerning industrial and biological applications with corresponding examples.

Response: The sentence is replaced with the following: Many of them have been examined because of their potential as dyestuffs or pigments.3 Some of them are involved in a number of biological processes such as photosynthesis and dioxygen transport 4, and also have high potential in antitumor therapy.5

Comment . The sentence “Nickel Schiff base complexes have a strong role in bioinorganic chemistry and redox enzyme systems.7” has no connection with the paper topic. Instead the authors should present the Ni(II) role for biological systems similar with Zn(II).

Response: Nickel(II) complexes of macrocyclic ligands are well known to be biologically important and interesting because of their anticarcinogenic, antibacterial and antifungal properties. Also, they have been screened for their medicinal properties because they possess some degree of cytotoxic activity. 11b

11 b) M. Tyagi, S. Chandra, *J Saudi Chem Soc***18** (2014)53(DOI: <http://dx>. doi:10.1016/j.jscs.2011.05.013)

Comment. The mL instead of ml must be homogenized in whole paper.

Response: The mL instead of ml were corrected in whole paper.

Comment. Electronic spectra must be presented in solid state also having in view that in solvents with coordinative properties (such acetonitrile) the coordination sphere could be modified in solution.

Response Diffusion-reflection spectra were recorded. Results and interpretations are given in the chapter Spectral properties, UV-Vis spectra.

Comment. The absorption maxima of the d-d bands for Ni(II) must be provided instead a range. The measure unit must be provided at the end of enumeration. The assignments for the d-d bands must be modified as: 3A2g→3T1g(P), 3A2g→3T1g(F) and 3A2g→3T2gconsidering the measure units (nm) presented.

Response: The section:

**The electronic spectrum of the Ni(II) complex shows an absorption bands in the regions 497-525 nm, 606 nm and 702-888 nm. These are assigned to the spin-allowed transitions 3A2g(F)→3T2g(F), 3A2g(F)→3T1g(F), and 3A2g(F)→3T1g(P) respectively, consistent with their well-defined octahedral configuration. A characteristic feature of the spectra of octahedral nickel(II) complexes is that the molar absorbances are low, at the Ni(II) complex are in the range from 35-74 dm3 mol−1 cm−1. The positions of the bands and their intensities are characteristic of octahedral geometry. The bands in the range from 221 to 260 nm (ε =4400-4800 dm3 mol−1 cm−1) was assigned to charge transfer bands.**

is replaced with:

The electronic spectrum of the Ni(II) complex in CH3CN shows an absorption bands at the 402, 606 and 877 nm attributed to d-d transitions. These are assigned to the spin-allowed transitions 3A2g(F)→3T1g(P), 3A2g(F)→3T1g(F), and 3A2g(F)→3T2g(F) and respectively, consistent with their well-defined octahedral configuration. The reflectance spectrum in the visible region shows bands at 410, 610, and 870. Reflectance and solution spectra of the Ni(II) complex corresponded to five- or six-coordinated nickel (II) and its magnetic moments (3.4 μB/Ni(II)) also suggests a high-spin nickel state. A characteristic feature of the spectra of octahedral nickel(II) complexes is that the molar absorbance are low, at the Ni(II) complex are in the range from 35-74 dm3mol−1cm−1. In UV part the several sharp unresolved bands in the range from 221 to 260 nm (*ε*=4400-4800 dm3 mol−1cm−1) was assigned to intraligand transitions bands. Intraligand transitions were found in the spectra of ligands in the range 220-280 nm.

Comment. The oxidation state two is very stable for both nickel and zinc and as result the electronic spectra do not usually present charge transfer bands. The bands that appear in UV in my opinion correspond to π→π\*. As result the complexes spectra most be presented in comparison with that of macrocycle ligand. Also for Zn(II) the absorption maxima must be presented.

Response: Ligands spectra were recorded and compared with the electronic spectrum of the complex.

For Zn(II) the sentence:The transitions of very strong intensity at 349–335 nm and 245– 208 nm have been attributed to the charge transfer from the ligands to the metal centers.25**was replaced with**: The Zn(II) complex is white and electronic spectra show only the intraligand transitions, very strong intensity at 207, 214 and 245 nm (*ε*=4265-4652 dm3 mol−1cm−1). 25 The intraligand transitions in both complexes to be slightly shifted during complexation.

Comment. The band at 1100 cm-1 arise from to the ν3(ClO4) while that at at ~620 cm-1 from ν4(ClO4). A Table with the most important IR bands must be provided, especially with that discussed in the text, in comparison with that corresponding for ligands.

Response: The most important FTIR bands of complexes and ligands were presented in Table I. Figure 2 was replaced with a new table (Table I) so that now the total number of figures is 2 and tables is 4.

Comment .The Δν is not a splitting but a difference and must be corrected.

Response: In manuscript instead of splitting, Δν was corrected difference, Δν.

Comment .The sentence “The control of many of the organisms mentioned above is key to health sustainability as many are noted on the recent World Health Organization (WHO) priority pathogens list of new antibiotics are urgently needed.30 ???” is hard to understood and must be revised.

Response: For this reasons, discovery of new compounds with antimicrobial activity is an exclusively important objective 30

Comment . The sentence “This is due to increased cellular permeability.” is not correct having in view that the complexes do not modify the cellular permeability but can penetrate this membrane when is lipophilic. As result this must be revised

Response: The sentence “This is due to increased cellular permeability.” was deleted.

Comment.

Some expression must be corrected:

|  |  |  |
| --- | --- | --- |
| Expression/word used | Correct expression/word | Page/row |
| mixed-ligand | mixed-ligands | 1 - title |
| are prepared | were prepared | 1-abstract |
| bonded for the metal ions | bonded to metal ions | 1-abstract |
| Other ways of dicarboxylate bridge bonding cannot be excluded cmpletely. | - | 1-abstract |
| coordination numbers, different geometries | coordination numbers and different geometries | 2 |
| Zn complexes | Zinc complexes | 2 |
| reports on the synthesis | reports the synthesis | 2 |
| For tetrahedral nickel(II) large orbital contribution is expected. The observed values of magnetic moment range between 3.5 and 4.2 μB/Ni(II). | For tetrahedral nickel(II) a large orbital contribution is expected and as result the observed values of magnetic moment range between 3.5 and 4.2 μB/Ni(II). | 7 |
| The magnetic measurements showed two unpaired electrons per Ni(II) ion suggesting also an octahedral or tetrahedral/square pyramidal geometry for the Ni(II) complexes.23 This data is in the range 2.8-3.4 μB/Ni(II) and suggesting octahedral environment around Ni(II).  | The magnetic measurements showed two unpaired electrons per Ni(II) ion suggesting also an octahedral geometry for the Ni(II) complexes.23  | 7 |
| studied complex | complex | 9 |
| analysed complex | complex | 9 |
| which induces the coordination | which indicates the coordination | 10 |
| variety of other biological activities (antiviral, antitumor, or anticanceractivity)(antitumor and anticancer represent the same activity) | a variety of other biological activities such as antiviral or antitumor. | 11 |
| The lipid membrane surroundingthe cell favors the passage of lipid soluble materials only and it is known that liposolubility isan important factor that controls antimicrobial activity that metal chelates have higher antibacterial activity than the free ligand. | The lipid membrane of the cellfavors the passage of lipid soluble materials only and it is known that liposolubility isan important factor that controls antimicrobial activity. reference | 12 |
| Synthesized new | The new synthesized  | 13 conclusion |
| 4 N | four nitrogen atoms | 13 |
| 2O / 1O | two oxygen atoms / one oxygen atom | 13 |

Response: Comments from the table are corrected.

**Reviewer A:**

Comment: Biological activity is insufficiently tested. Antimicrobiology is done to four strains, and cytotoxicity to two cell lines. The basic question is where does it stimulate biological activity. Complexes were tested, not ligand tpmc and bicyclic dicarboxylate ligand. It can not be said that complexes are biologically active, not comparable to ligands. The ligands themselves could exhibit biological activity. For antimicrobial study there is no antibiotic or mycotic, so for a given strains could not talk about their more or less activity. It is not clear that the activity is not solely due to free perchlorates, as well.

Response: In the experimental part, testing of ligands ((tpmc and K2C9H8O4·H2O) and salts (Zn(ClO4)2∙6H2O, Ni(ClO4)2∙6H2O) were added. The results obtained were commented on in Chapter 3. Results and discussion, *In vitro antimicrobial activity a*nd the sentence reads: We tested the starting compounds, but they did not show antimicrobial effect (data not shown).

In Table III, the results of Ampicillin and Nystatin were inserted.

Comment: Figure 2 may be omitted and the data be included in Table 1. The quality of Figure 3 is too low to read any symbols.

Response: The Figure 2 is replaced withTable I.

**Reviewer C:**

Comment: Instead of ”bonded for the metal ions” I suggest using ”coordinated for the metal ions.” This should be replaced through the all text.

Response:The ”bonded” was replaced with ”coordinated”.

Comment: Instead of UV/Vis it should be UV-Vis. This should be followed through all the text.

Response: The UV-V is instead UV/Vis was corrected.

Comment: In Fig. 1 it is not clear what (a) and (b) are. Also, this should be clarified in Fig. 3.

Response: In Figures 1 and 3 (now Figure 2) are marked parts a) b) ...

**Reviewer D**

Comment:What is a positive control for antimicrobial assay, as was reported in the antiproliferative assay? It must be included.

Response: The same as comment 2 and response for rew A.

Comment:Why authors did not test antimicrobial and anticancer effect of Zn and Ni salts and respective ligands? How we could know whether the tested complexes were more active than salts and ligands?

Response: The same as comment 2 and response for rew A.

Comment :Molecular weight of both compounds have to be written exactly - 1133.27 instead of 1133, and so on.

Response: It was corected

We hope that our paper is now sutable for publication in Journal of the Serbian ChemicalSociety.

March 5th, 2019. Sincerely yours,

Dr Branka Dražić