Short thesis for the degree of doctor of philosophy (PhD)

Redox properties of mixed ligand Co(III) complexes and synthesis of their heterobimetallic derivatives

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I. INTRODUCTION AND OBJECTIVES

In our society, tumorous diseases have a serious impact on our everyday live. To be able to fight these types of illnesses effectively, new drugs should be developed that could act in a more selective way without damaging the healthy cells and as a result, they would have fewer side effects.

One way of increasing compound selectivity is taking advantage of the differences existing between tumour cells and normal ones. One of these differences is tumour hypoxia that means a lack of oxygen in tumour cells therefore, a more reductive environment is present in them. Hypoxia-activated prodrugs can be good candidates for this purpose.

Hypoxia-activated prodrugs can be developed from cobalt(III) complexes. The inactive Co(III) compound cannot release the biologically active molecule in the body but only in a suitable, more reductive environment, where the selective reduction of the metal ion (Co(III)/Co(II)) may occur. From the more labile Co(II) complex that may be formed under hypoxic conditions, the targeted release of the biomolecule can happen. According to previously published results, it would be beneficial to apply Co(III) molecules whose coordination spheres consist of 4N and 2O donor atoms. In most of the cases, the applied 4N donors are different kinds of tripodal amines while the 2O donors are molecules that have antitumour or antibacterial activities. However, in order to make the reduction of the complexes selective, the systematic study and fine-tuning of their redox properties are crucial.

Another approach applied in our research group is the combination of a hypoxia-activated complex with an anticancer platinum-based drug into one molecule that could also increase the efficiency of the drugs already in use.

Based on the results previously mentioned in my thesis, one goal of my doctoral work was to explore in detail the effects of the variation of the 4N and 2O donors in terms of the redox properties of the Co(III) complexes with the aid of using model hydroxamic acids. We also wanted to explore the effect of the replacement of the 4N donor ligands

by 2x2N ones on the redox and kinetic properties of the Co(III) complexes. For this reason several model $[Co(2N)_2(2O)]^{x+}$ type compounds were synthesized, in which maltol and its derivatives were used as 2O donors. The maltolato ligands were also served as models and building blocks of novel chelating, ambidentate ligands to be developed in the second part of my work and mentioned below.

In the continuation of the work we aimed to synthesize, characterize and study the $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ and $[(\eta^5\text{-Cp*})Rh(H_2O)_3]^{2+}$ binding capabilities of novel ambidentate ligands that are capable of coordinating both the hard type cobalt(III) entity via their 2O donor atoms and a soft platinum group metal ion (PGM) with proven anticancer potential via their 2N donors.

With the aid of the results of the solution equilibrium studies we intended to synthesize and characterize mononuclear and hetero dinuclear Co(III)/PGM metal complexes.

Our goal was also to explore the cytotoxic activity of the newly obtained compounds via screening of selected human derived cancer cell lines in the framework domestic and international collaborations.

II. EXPERIMENTAL METHODS

The ligands and complexes were characterized using NMR, IR, ESI-MS and elemental analysis techniques while for the equilibrium studies pH-potentiometric, ESI-MS and ^{1}H NMR measurements were carried out. By using pH-potentiometric experimental data dissociation constants (pK) of the ligands and overall stability constants (lgβ) of the complexes in the $[(\eta^6\text{-}p\text{-cym})Ru]^{2+}$ and $[(\eta^5\text{-Cp*})Rh]^{2+}$ containing systems were determined.

Titrations were performed with the help of Mettler Toledo DL50, T50 or T5 titrators equipped with Mettler Toledo DGi 115-SC electrode at an ionic strength of 0.20 M KCl and 25.0±0.1 °C temperature. SUPERQUAD and PSEQUAD softwares were used during the calculations.

NMR measurements were carried out on a Bruker Avance I 400 MHz spectrometer. The chemical shifts in the acquired spectra were given from the signal of the solvent (2.50 ppm) in d^6-DMSO or internal standard (TSP, 0.00 ppm) in D_2O. Samples also contained 0.20 M KNO_3 or KCl ionic strength during titrations. To set up the detected pH* values DNO_3 and NaOD solutions were used.

ESI-MS measurements were carried out on a Bruker MaXis II. uhr ESI-TOF MS instrument at the Department of Inorganic and Analytical Chemistry, University of Debrecen by Dr. Attila Gáspár and co-workers. For the evaluation of the spectra Data Analysis 3.0 software was used. The concentration of the complexes were ~ 0.01 mg/ml while during the equilibrium studies metal ion concentration was ~ 0.03 mM.

Elemental analysis was performed at the Department of Organic Chemistry, University of Debrecen by Dr. Attila Kiss on an Elementar Vario MICRO CUBE instrument while infrared spectra were acquired on a Perkin Elmer FTIR Paragon 1000 PC equipment using KBr pellets at the same department by Anita Kónya-Ábrahám.

Redox properties of the studied complexes were explored by cyclic voltammetry and the measurements were carried out on a Metrohm 746

VA Trace Analyzer or a BASi Epsilon EClipse instrument with the aid of glassy carbon working electrode (CHI104), Ag/AgCl/3M NaCl reference electrode (E^0 = +209 mV) and platinum wire auxiliary electrode (ALS Co. Japan). Every sample solution was degassed with argon for five minutes before measurements and the scan rate was 200 mV/s. For the calibration $K_3[Fe(CN)_6]$ solution was used (E^0= +458 mV, I = 0.5 M KCl) while the concentration of each samples were ~ 1.0 mM also maintaining 0.20 M (KNO_3) ionic strength.

Single crystal analysis (XRD) was carried out on a Bruker-D8 Venture diffractometer, on Mo Kα (λ = 0.71073 Å) or Cu Kα (λ = 1.54178 Å) characteristic radiation at the Department of Physical Chemistry, University of Debrecen by Dr. Attila Bényei.

Hydrophilic and lipophilic character (lg*D*) of the complexes was determined in water/n-octanol (1/10 ratio) using a classical shake flask method in approximately 1 mM solutions in the 400-800 nm wavelength range.

MTT assay was used to determine the antiproliferative activity of the selected complexes against HeLa cell line. HeLa cells were treated with various concentrations of the complexes for 72 h. After the incubation 10 µl of a 5 mg/ml solution of MTT in PBS was added to each well and the plates were incubated for another 1.5 h. The medium was removed and the formazan crystals formed were dissolved in 100 µl DMSO. The absorbance was measured at 540 nm using a Thermo Scientific Multiscan Go spectrophotometer. IC_{50} values were calculated from the raw data by GraphPad Prism 8.0.1 software.

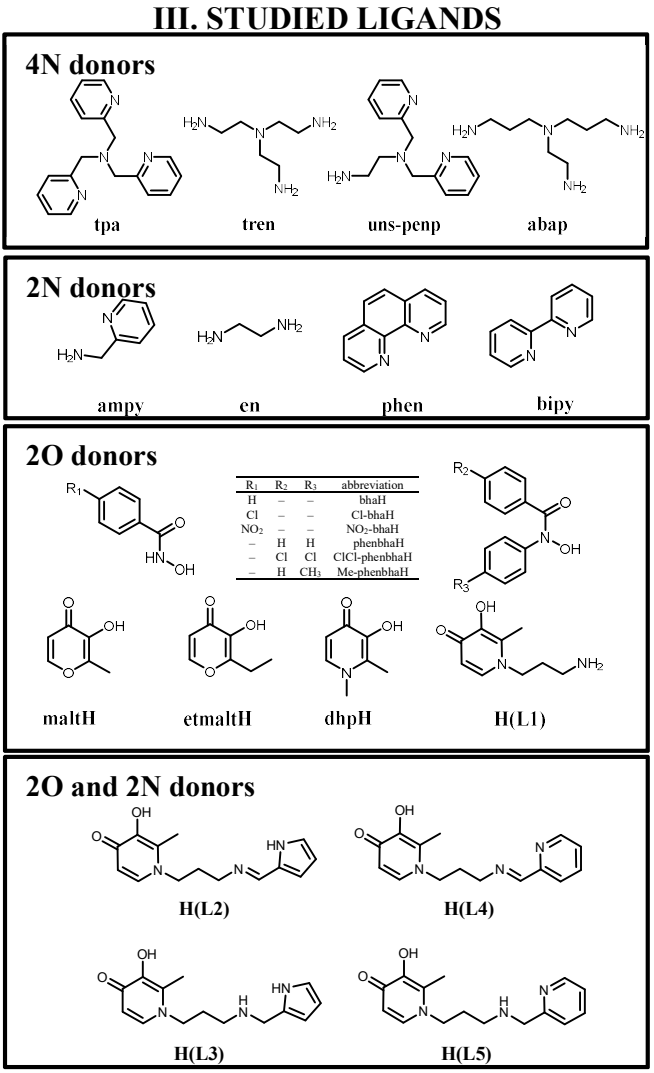


Figure 1. Structure of the studied ligands with the used abbreviations

IV. NEW SCIENTIFIC RESULTS

4.1 Novel $[Co(2N)_2(2O)]^{2+}$ type cobalt(III) complexes were synthesized and characterized.

4.1.1 The targeted compounds were obtained from $[Co(2N)_2Cl_2]Cl$ type complexes (where 2N = phen, bipy, en, ampy) in the presence of base and a suitable 2O donor (2O = bhaH, maltH, etmaltH, dhpH).
4.1.2 The entity and purity of the complexes were proven by NMR, IR, ESI-MS and elemental analysis data.
4.1.3 Single crystal analysis supported the expected octahedral geometry of the novel Co(III) compounds and the absolute configurations of the crystals were also described.

4.2 Redox properties of 4N and 2x2N type Co(III) complexes were explored.

4.2.1 In the case of the studied 4N and 2x2N donor containing complexes the stability difference ($\Delta\lg\beta$) of the Co(III) and the appropriate Co(II) species formed after cathodic reduction was found to increase in the direction of aliphatic amins (Fig. 2). This trend can be explained with the back donation capability of the aromatic-N donors that can stabilize the Co(II) form more than the aliphatic N donors.
4.2.2 The cobalt(III) complexes that bind tripodal ligands (tren, tpa; capable of forming fused chelates), had higher $\Delta\lg\beta$ values than those containing 2N donors.
4.2.3 The redox potential did not change significantly when bhaH was functionalized in para position but the presence of the phenyl ring (phenbha) caused a considerable positive shift (~ 200 mV).
4.2.4 By varying the 2O donors in a given 4N or 2N containing complex, $\Delta\lg\beta$ increased in line with the pK_a values of the 2O donor ligands: malt ~ etmalt << dhp.

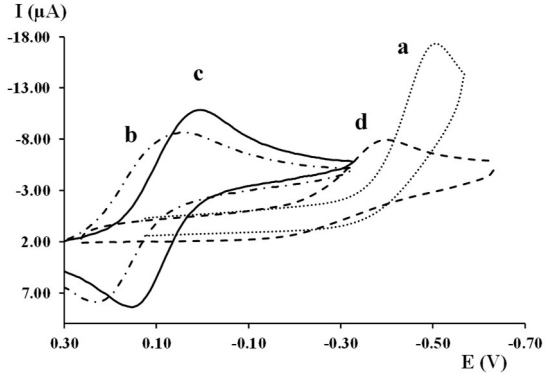


Figure 2. Cyclic voltammograms of $[Co(en)_2(malt)](ClO_4)_2$ (a), $[Co(phen)_2(malt)](ClO_4)_2$ (b), $[Co(bipy)_2(malt)](ClO_4)_2$ (c) and $[Co(ampy)_2(malt)](ClO_4)_2$ (d), registered in water. $c_M \sim 0.01$ M, I = 0.20 M (KNO_3), v = 200 mV/s).

4.3 The anticancer activity of $[Co(2N)_2(2O)]^{2+}$ and $[Co(4N)(2O)]^{2+}$ type complexes was explored against HeLa cell line.

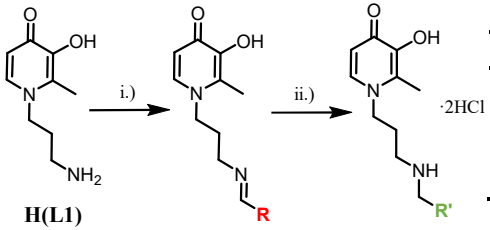
4.3.1 Before the biological tests the aqueous stability measurements proved the very inert character of the studied Co(III) complexes.

4.3.2 According to the lipophilicity measurements that were carried out in n-octanol/water mixture, the highly hydrophilic character of the complexes was found.

4.3.3 The 2N complexes turned to be active and $[Co(phen)_2(malt)](ClO_4)_2$, $[Co(phen)_2(dhp)](ClO_4)_2$ and $[Co(bipy)_2(dhp)](ClO_4)_2$ inhibited the growth of the HeLa tumour cells better than cisplatin or carboplatin. However, the studied 4N donor derivatives did not show any cytotoxic activity against the cell line.

4.4 Novel ambidentate, chelating pyridinone derivatives (H(L3) and H(L5)) capable of binding two metal ions simultaneously were synthesized and their interactions with $[(\eta^6\text{-}p\text{-cym})Ru]^{2+}$ and $[(\eta^5\text{-Cp*})Rh]^{2+}$ were explored in detail.

4.4.1 Two new Schiff-bases and their reduced derivatives were synthesized, characterized (Fig. 3) using ^{1}H NMR, IR, ESI-MS, elemental analysis, XRD and their proton dissociation processes explored with the aid of pH-potentiometry.



	R	R'
H(L2)	pyrrole	–
H(L3)	–	pyrrole
H(L4)	pyridine	–
H(L5)	–	pyridine

Figure 3. Synthesis of the ambidentate ligands. Reagents and conditions: i) abs. EtOH, pyrrole-2-carboxaldehyde/pyridine-2- carboxaldehyde, 85 °C, 2 h. ii) MeOH, $NaBH_4$, 0 °C (~2h), RT overnight, 6M HCl, abs. EtOH.

4.4.2 H(L3) was found to coordinate the organoruthenium ion via its oxygen donors while H(L5) can bind both of the metal ions in (N,N) chelate.

4.4.3 The solution studies revealed that for H(L3) the complexation starts with both organometallic cations via (O,O) coordination. While with $[(\eta^6\text{-}p\text{-cym})Ru]^{2+}$ only the pyridinone unit binds the metal ion, resulting in the exclusive formation of 1:1 complexes, with $[(\eta^5\text{-Cp*})Rh]^{2+}$, at a 2:1 metal ion ligand ratio, dinuclear species could also be detected.

4.4.4 H(L5) was capable of binding an excess of the studied organorhodium and organoruthenium cations via forming 2:1 type complexes.

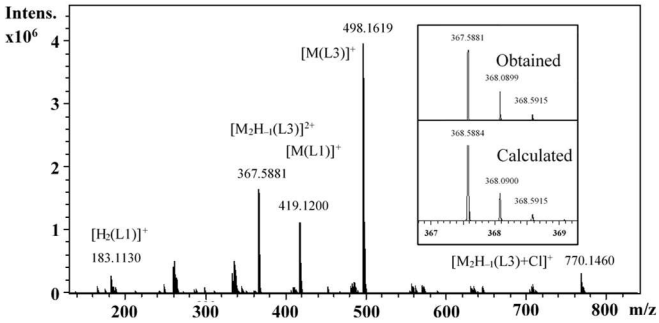


Figure 4. ESI-MS spectrum registered in the $[(\eta^5\text{-Cp*})Rh]^{2+}$–L3 system at 1:1 metal-ion-to-ligand ratio, pH = 7.0. Inlet shows the observed and the calculated isotope pattern for $[M_2H_{-1}L]^{2+}$.

4.5 Novel $[Co(4N)H(L5)]^{3+}$, $[Co(2N)_2H(L3/L5)]^{3+}$ type as well as heterobimetallic Co/PGM complexes incorporating both cobalt(III) for hypoxia activation and a platinum group metal ion (Ru or Rh) with anticancer potential were synthesized and characterized.

4.5.1 In the reaction of H(L5) and $[Co(tren)Cl_2]Cl$ or $[Co(tpa)Cl_2]Cl$ novel $[Co(4N)H(L5)]^{3+}$ type complexes were synthesized and fully characterized (NMR, IR, ESI-MS, elemental analysis). Based on the results (O,O) coordination of the ligand was proven.

4.5.2 $[Co(2N)_2H(L3/L5)]^{3+}$ type complexes were also successfully synthesized and the coordination of the O-donors of the pyridinone ring of the ligand was proven by the molecular structure of $[Co(bipy)_2H(L5)](BF_4)_3{\cdot}2H_2O$ (Fig. 5).

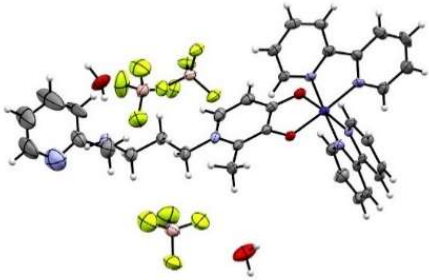


Figure 5. Molecular structure of $[Co(bipy)_2H(L5)](BF_4)_3 \cdot 2H_2O$.

4.5.3 $[Co(tpa)(L5)(\eta^5\text{-}Cp^*)RhCl](ClO_4)_3$ and $[Co(tpa)(L5)(\eta^6\text{-}p\text{-}cym)RuCl](ClO_4)_3$ were obtained in the reaction of half equivalent $[(\eta^5\text{-}Cp^*)RhCl_2]_2$ or $[(\eta^6\text{-}p\text{-}cym)RuCl_2]_2$ and the aqueous solution of $[Co(tpa)H(L5)]^{3+}$. Analytical characterization suggests the successful synthesis and the formation of four isomers (Fig. 6).

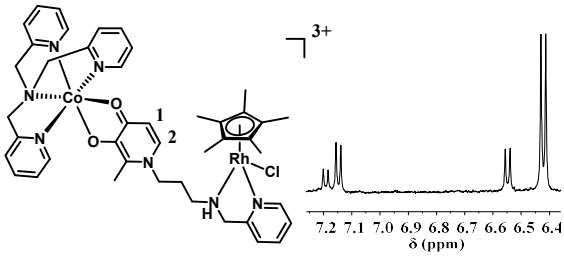


Figure 6. The signals of „*1"* proton in the ^1H NMR spectrum of $[Co(tpa)(L5)(\eta^5\text{-}Cp^*)RhCl](ClO_4)_3$ (d^6-DMSO).

V. POSSIBLE APPLICATIONS OF THE RESULTS

During this research 47 cobalt(III) complexes were studied, out of which I synthesized 31 as new. For the novel complexes the suggested coordination mode, structure and purity were also proven with the aid of different analytical techniques. Redox properties of the complexes were explored using cyclic voltammetry. Our results provided a detailed view on these processes and on the options of the fine tuning of the cobalt(III)/cobalt(II) redox processes. Based on our findings, it may become possible to create more selective hypoxia-activated prodrugs via tailoring their redox properties.

In addition, new, chelating, ambidentate ligands were designed, synthesized and characterized capable of binding Co(III) and platinum group metals simultaneously. Detailed solution and synthetic studies with the new ligands revealed that in Co(III)/PGM heterodinuclear complexes (N,N)-chelated PGM and (O,O)-chelated Co(III) metal cores are present reflecting the donor atom preference of these metal ions. Based on the results new type of platinum complexes with dual action can be obtained having a hypoxia-activatable Co(III) core beside the platinum group ion with anticancer potential and that may result in the development of drug candidates with less side effects and higher selectivity.

PUBLICATIONS

Articles related to the dissertation (3)

1. Péter Buglyó, István Kacsir, Máté Kozsup, Imre Nagy, *Sándor Nagy*, Attila Bényei, Éva Kováts, Etelka Farkas
   Tuning the redox potentials of ternary Co(III) complexes containing various hydroxamates
   Inorg. Chim. Acta **472** (2018) 234-242
   IF: 2.433
2. *Sándor Nagy*, Emese Tóth, István Kacsir, Attila Makai, Attila Csaba Bényei, Péter Buglyó
   Effect of the replacement of tripodal 4 N donors by two 2 N chelators on the redox and cytotoxic activity of maltolato and deferipronato containing Co(III) complexes
   J. Inorg. Biochem. **220** (2021) 111372
   IF: 4.155 (2020)
3. *Sándor Nagy*, András Ozsváth, Attila Csaba Bényei, Etelka Farkas, Péter Buglyó
   Donor atom preference of organoruthenium and organorhodium cations on the interaction with novel ambidentate (N,N) and (O,O) chelating ligands in aqueous solution
   Molecules **26** (2021) 3586
   IF: 4.411 (2020)

Articles not related to the dissertation (5)

1. Daniele Sanna, Valeria Ugone, Péter Buglyó, *Sándor Nagy*, István Kacsir, Eugenio Garribba
   Speciation of potential anticancer agent $[V^{IV}O(oda)(H_2O)_2]$ and its interaction with low and high molecular mass blood bioligands
   Inorg. Chim. Acta **472** (2018) 127–138
   IF: 2.433

2. Daniele Sanna, Jessica Palomba, Giuseppe Lubinu, Péter Buglyó, *Sándor Nagy*, Franc Predih, Eugenio Garribba
   Role of Ligands in the Uptake and Reduction of V(V) Complexes in Red Blood Cells
   J. Med. Chem. **62** (2019) 654-664
   IF: 6.205
3. Máté Kozsup, Orsolya Dömötör, *Sándor Nagy*, Etelka Farkas, Éva Anna Enyedy, Péter Buglyó
   Synthesis, characterization and albumin binding capabilities of quinizarin containing ternary cobalt(III) complexes
   J. Inorg. Biochem. **204** (2020) 110963
   IF: 4.155
4. Hana Crlikova, Hana Kostrhunova, Jitka Pracharova, Máté Kozsup, *Sándor Nagy*, Péter Buglyó, Viktor Brabec, Jana Kasparkova
   Antiproliferative, DNA binding and cleavage properties of dinuclear Co(III) complexes containing the bioactive quinizarin ligand
   J. Biol. Inorg. Chem. **25** (2020) 339-350
   IF: 3.358
5. Daniele Sanna, Péter Buglyó, *Sándor Nagy*, Franc Perdih, Jessica Palomba, Valeria Ugone, Eugenio Garribba
   Interaction of V(V) complexes formed by picolinic acid and pyrazinecarboxylic acid derivatives with red blood cells
   Polyhedron, (2021) 115590
   IF: 3.052 (2020)

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Subject: PhD Publication List

Candidate: Sándor Nagy
Doctoral School: Doctoral School of Chemistry
MTMT ID: 10068222

List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

1. **Nagy, S.**, Ozsváth, A., Bényei, A., Farkas, E., Buglyó, P.: Donor Atom Preference of Organoruthenium and Organorhodium Cations on the Interaction with Novel Ambidentate (N,N) and (O,O) Chelating Ligands in Aqueous Solution.
   Molecules. 26 (12), 1-23, 2021. ISSN: 1420-3049.
   DOI: http://dx.doi.org/10.3390/molecules26123586
   IF: 4.411 (2020)

2. **Nagy, S.**, Tóth, E., Kacsir, I., Makai, A., Bényei, A., Buglyó, P.: Effect of the replacement of tripodal 4N donors by two 2N chelators on the redox and cytotoxic activity of maltolato and deferipronato containing Co(III) complexes.
   J. Inorg. Biochem. 220, 1-10, 2021. ISSN: 0162-0134.
   DOI: http://dx.doi.org/10.1016/j.jinorgbio.2021.111372
   IF: 4.155 (2020)

3. Buglyó, P., Kacsir, I., Kozsup, M., Nagy, I., **Nagy, S.**, Bényei, A., Kováts, É., Farkas, E.: Tuning the redox potentials of ternary cobalt(III) complexes containing various hydroxamates.
   Inorg. Chim. Acta. 472, 234-242, 2018. ISSN: 0020-1693.
   DOI: http://dx.doi.org/10.1016/j.ica.2017.07.026
   IF: 2.433

List of other publications

Foreign language scientific articles in international journals (5)

4. Sanna, D., Buglyó, P., **Nagy, S.**, Perdih, F., Palomba, J., Ugone, V., Garribba, E.: Interaction of V(V) complexes formed by picolinic and pyrazinecarboxylic acid derivatives with red blood cells.
   Polyhedron. 212, 1-7, 2022. ISSN: 0277-5387.
   DOI: http://dx.doi.org/10.1016/j.poly.2021.115590
   IF: 3.052 (2020)

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5. Crlikova, H., Kostrhunova, H., Pracharova, J., Kozsup, M., **Nagy, S.**, Buglyó, P., Brabec, V., Kasparkova, J.: Antiproliferative, DNA binding, and cleavage properties of dinuclear Co(III) complexes containing the bioactive quinizarin ligand.
J. Biol. Inorg. Chem. 25 (2), 339-350, 2020. ISSN: 0949-8257.
DOI: http://dx.doi.org/10.1007/s00775-020-01765-4
IF: 3.358

6. Kozsup, M., Dömötör, O., **Nagy, S.**, Farkas, E., Enyedy, É. A., Buglyó, P.: Synthesis, characterization and albumin binding capabilities of quinizarin containing ternary cobalt(III) complexes.
J. Inorg. Biochem. 204, 1-9, 2020. ISSN: 0162-0134.
DOI: http://dx.doi.org/10.1016/j.jinorgbio.2019.110963
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7. Sanna, D., Palomba, J., Lubinu, G., Buglyó, P., **Nagy, S.**, Perdih, F., Garribba, E.: Role of Ligands in the Uptake and Reduction of V(V) Complexes in Red Blood Cells.
J. Med. Chem. 62 (2), 654-664, 2018. ISSN: 0022-2623.
DOI: http://dx.doi.org/10.1021/acs.jmedchem.8b01330
IF: 6.054

8. Sanna, D., Ugone, V., Buglyó, P., **Nagy, S.**, Kacsir, I., Garribba, E.: Speciation in aqueous solution and interaction with low and high molecular mass blood bioligands of [V IV O(oda)(H 2 O) 2], a V compound with in vitro anticancer activity.
Inorg. Chim. Acta. 472, 127-138, 2018. ISSN: 0020-1693.
DOI: http://dx.doi.org/10.1016/j.ica.2017.07.064
IF: 2.433

Total IF of journals (all publications): 30,051
Total IF of journals (publications related to the dissertation): 10,999

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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