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

Design, synthesis and characterization of new, multifunctional, bioactive compounds based on metal complexes

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I. INTRODUCTION AND OBJECTIVES

Helping to identify cancers as quickly as possible and cure them as effectively as possible is a constant task of science. Nowadays, conditions that make difficult to cure, such as resistance and mutation, are particularly challenging to reduce.

In drug therapy, some metal complexes have become prominently important. Although only Pt(II)-containing complexes are commonly used in the clinic, a number of other metal ions and their very large number of complexes have recently become the focus of interest in the development of potential anti-cancer drugs. In addition to metal ions, it has become essentially a common consideration in the selection of ligands or in the development of new ones that they have biological activity on their own. Thus, the development of more effective, more selective drug molecules that attack cancer cells at multiple points is the goal of much research today.

The aim of my research was to synthesize and characterize new metal complexes that contain metal ions of different character, charge and hydrolytic properties with known anti-cancer effects. The selection of ligands was also determined by their ability to have biological activity on their own.

Accordingly, we aimed to prepare complexes of some potentially anti-cancer metal ions. The metal ions Co(III), Ru(II), Ru(III), Os(VI) and Ga(III) were chosen for the synthesis of the complexes. In the case of the complexes, we wanted to increase the efficiency and selectivity of the target delivery by developing prodrug complexes in which the central metal ion of the kinetically inert Co(III)tren/tpa complex can be reduced to Co(II) only in a hypoxic medium, followed by dissociation of the labile complex and may result in the components becoming active. The second metal ion is a potentially anti-cancer metal ion, Ru(II), Ru(III), Os(VI) and Ga(III),

which are used to form the multi-targeted bimetallic complexes. The coupling of the two units required the development of ambidentate ligands capable of coordinating to Co(III) with their O,O donor atoms and the listed metal ions with their N,N donor atoms.

In the first stage of our research, two ambidentate maltol derivatives were synthesized to link the Co(III)tren/tpa unit, however, the preparation of bimetallic complexes was failed by a side reaction.

Therefore, the above challenging ambident ligand was replaced with a SAHA derivative, which was successfully prepared and used to obtain the desired bimetallic complexes. This ligand was phenhaH, which coordinates with the O,O donor atoms to the Co(III)tren/tpa unit and with the N,N donor atoms to the $[(\eta^6\text{-}p\text{-cym})Ru]^{2+}$ complex. In addition to analytical studies, the new compounds were also tested on cancer cell lines and the redox properties of the complexes were determined.

For deferasirox, (H_3L_B), in addition to the anti-cancer activity of the complexed metal ion, the strong ability of the ligand to bind iron(III) is also advantageous for the synthesis of multi-targeted complexes. In the first stage of the research, the bis complex of Co(III) with decarboxy-deferasirox, the bis complex of deferasirox with Co(III), Ru(III) and Ga(III) furthermore the mono complex of Os(VI) were prepared. The water-soluble sulfonated deferasirox derivatives were used to explore the solution equilibria with Ga(III) in aqueous medium.

The deferasirox conjugate formed with a naphthyl or a phenanthrolyl unit that also function as fluorophores may also be potentially suitable for the development of multi-targeted complexes. The bis complex of Co(III) with deferasirox phenanthroline amide was found to be able to coordinate to two of $[(\eta^6\text{-}p\text{-cym})Ru]^{2+}$ cations with its N,N donor atoms.

Analytical characterization of the prepared complexes and ligands (Figure 1) was performed by NMR, elemental analysis, IR, UV-Vis and single crystal X-ray diffraction methods, and we also planned some biological assays on selected complexes. With the help of solution equilibrium studies we wanted to obtain information about the stoichiometry and stability of the complexes formed in aqueous medium.

II. EXPERIMENTAL METHODS

Characterization of ligands and complexes was performed using various analytical methods.

NMR spectroscopic analyzes were performed on Bruker WP 360 SY and Bruker Avance 400 instruments using d^6-DMSO and D_2O deuterated solvents, and the spectra obtained were evaluated using the MestReNova program.

Equilibrium studies of the complex formation processes in solution were carried out using **pH potentiometric** techniques. SUPERQUAD and PSEQUAD softwares were used to evaluate the titration curves. Measurements were performed with Mettler Toledo DL50, T50 or T5 titrators equipped with a Mettler Toledo DGi 115-SC combined glass electrode at constant temperature (25.0 ± 0.1 ° C) and ionic strength (I = 0.20 M KCl).

Infrared spectroscopic (IR) studies were performed at the Department of Organic Chemistry, University of Debrecen with a Perkin Elmer FTIR Paragon 1000 PC instrument, KBr pellet method, and **elemental analysis** measurements were carried out with an Elementar Vario MICRO CUBE instrument.

Mass spectrometry (ESI-MS) measurements were performed at the Department of Inorganic and Analytical Chemistry, University of Debrecen with a Bruker MaXis ESI-TOF MS instrument. Measurements were taken in positive and negative modes using water or methanol as a solvent. Evaluation of the spectra was performed with the DataAnalysis 3.4. software.

The structure of the prepared single crystals was determined by **single crystal X-ray diffraction** analysis at the Department of Physical Chemistry, University of Debrecen, using a Bruker-D8 Venture type diffractometer. During the measurements, the characteristic K_a radiation of Cu or Mo (Cu Kα = 1.54184 A, Mo Kα =

0.71073 A) was used. Mercury 3.8 software was used to construct the structural image of the complexes and to determine the bond lengths and bond angles.

The redox properties of the ligands and their complexes were examined with a Metrohm 746 VA Trace Analyzer or a BASi Epsilon EClipse **cyclic voltammeter** (CV). The working electrode was glassy carbon (CHI104), the auxiliary electrode was platinum and the reference electrode was Ag/AgCl. The concentration of the samples was 1 mM, and a 1:1 mixture of water and MeOH was used as the solvent. An ionic strength of 0.20 M KNO_3 was used during the measurements. The instrument was calibrated with an aqueous solution of $K_3[Fe(CN)_6]$. The samples were deoxygenated with argon gas before measurements. The potential sweep rates were varied within the range of 10-500 mV/s.

The *in vitro* anti-cancer activity of selected metal complexes involved in the **biological studies** was determined by Prof. Jana Kasparkova and co-workers using the MTT assay. The efficacy of the complexes was tested on HeLa, MCF-7, MDA-MB-231 and HCT116 cancer cell lines, and the results were compared with those of cisplatin under the same conditions. Solutions of different concentrations of the tested complexes were incubated with the model cancer cells in a 96-well plate for 72 h and then the MTT solution was added. The cell culture medium was then removed and the amount of dye taken up was measured photometrically and compared to that taken by the cisplatin-treated cells.

III. STUDIED LIGANDS

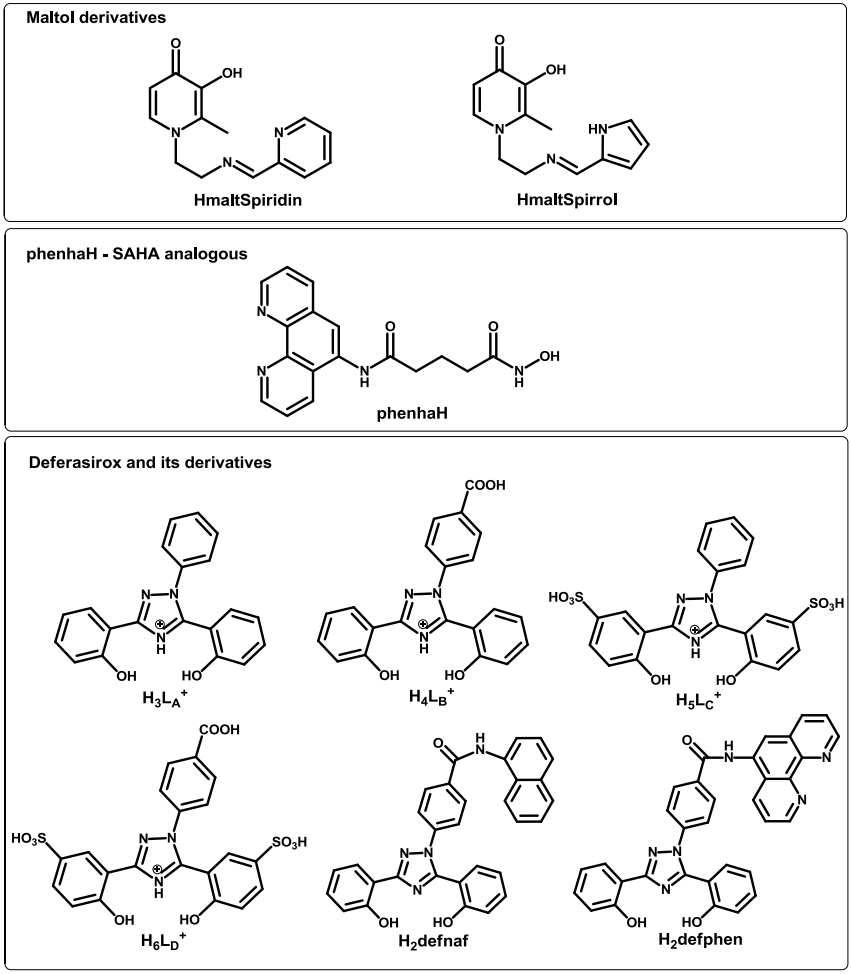

Figure 1. Ligands discussed in the PhD dissertation.

IV. NEW SCIENTIFIC RESULTS

4.1 Novel ambidentate, chelating maltol derivatives were prepared and characterized, and experiments were performed to prepare their bimetallic complexes of Co(III) and Ru(II).

4.1.1 The starting material HmaltEtN was reacted with pyridine carbaldehyde and pyrrole carbaldehyde in the presence of NaOMe base. Two new ambidentate ligands, HmaltSpiridine and HmaltSpirrol, were formed in the two reactions, both containing O,O and N,N donor atoms.
4.1.2 The structure, identity and purity of the prepared compounds were also confirmed by NMR methods.
4.1.3 During the experiments on the preparation of the Co(III) complex, an undesired redox reaction was detected. In the mass spectrum, only a partially formed complex containing the Co unit and ligand could be identified according to the m/z values.

4.2 phenhaH and its metal complexes were prepared and characterized.

4.2.1 A new ligand, the ambidentate phenhaH was prepared, in which the hydroxamic acid group provides complexing O,O donor atoms while the phenanthroline unit has N,N donor atoms. The ligand was characterized by NMR and MS methods.
4.2.2 A novel mononuclear complex containing Co(III)tpa was prepared, as well as dinuclear complexes consisting a $[(\eta^6\text{-}p\text{-cym})Ru]^{2+}$ unit and either Co(III)tren, or Co(III)tpa entities (Figure 2). The products were characterized by NMR, elemental analysis, IR, MS and CV confirming that the Co(4N) unit is bound by the ligand O,O, while the organoruthenium cation is bound by the N,N chelate.
4.2.3 Based on the CV analysis of the dinuclear complexes, the voltammogram of both tren and tpa complexes shows a reduction

peak, while the voltammogram of the tpa-containing complex also shows reversible processes. The difference between the reduction potentials of the two complexes is ~150 mV, suggesting a correlation between the redox potential and the structure. This is important in the tunability of the selective reducibility of complexes.

4.2.4 Biological assay of selected metal complexes on four cancer cell lines was performed with the framework of international collaboration. The bimetallic tpa complex showed the highest cytotoxicity compared to the reference cisplatin. The cisplatin was outperformed the activity of the studied complexes against the HCT116 cell line. Complexes containing tpa showed greater cytotoxicity than those containing tren. For the aggressive, triple-negative breast cancer MDA-MB-231 cell line, all of our tested complexes were found to be more active than cisplatin.

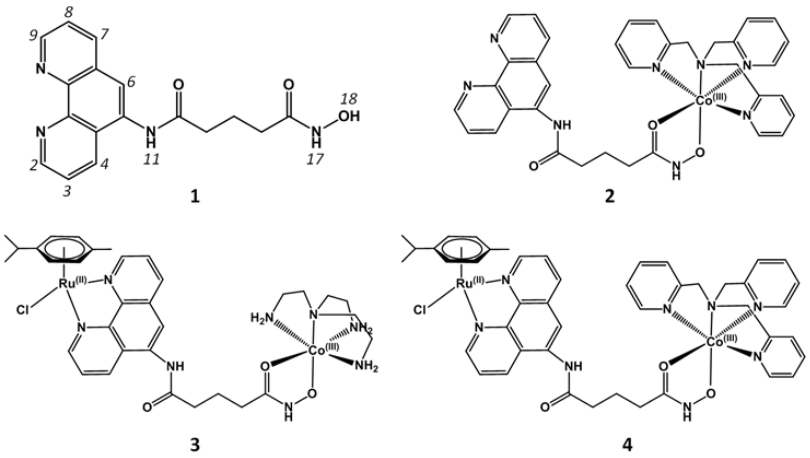

Figure 2. The structure of the new phen-based hydroxamic acid conjugate, phenhaH (1), its mononuclear complex with $[Co(tpa)]^{3+}$ (**2**), the dinuclear Ru(II)/Co(III) complexes, where 4N ligands are tren (**3**) or tpa (**4**)

4.3 New derivatives and metal complexes of deferasirox were prepared and characterized.

4.3.1 To increase the water solubility of deferasirox and decarboxy-deferasirox, new disulfonated derivatives of the two ligands, H_4L_C and H_5L_D, were prepared. The two new ligands were also characterized by NMR and single crystal X-ray diffraction.

4.3.2 Novel bis complexes of deferasirox with Ru(III), Co(III), and Ga(III) were prepared and characterized using NMR (Figure 3.), IR, MS and elemental analysis.

4.3.3 A new deferasirox complex with the molecular formula $K_2[(Os^{VI}O_2)L_BOH]$ was prepared, in which the starting osmium (IV) formed a dioxo cation due to an unplanned redox reaction, thus forming a mono complex with deferasirox. Furthermore, the bis complex of Co(III) decarboxy deferasirox was synthesized, the molecular structure of which was also determined by single crystal X-ray diffraction.

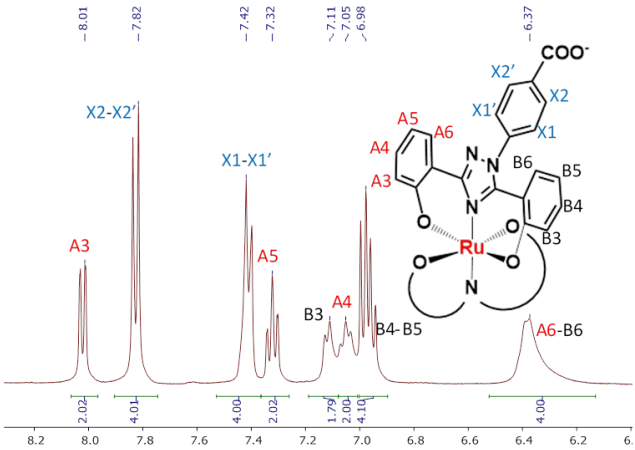

Figure 3. The ^{1}H NMR spectrum of $K_3[Ru(L_B)_2]$ in DMSO supplemented with proton assignment.

4.3.4 The stoichiometry of the formed complexes and their stability constant values were determined by studying the interaction between the prepared H_4L_C and H_5L_D ligands and Ga(III). Based on the calculated concentration distribution curves, the complex formation starts around pH 2, and at physiological pH the bis complex is the major species.

4.4 Carbonyl-substituted conjugates of deferasirox were prepared and characterized.

4.4.1 A new method was developed for the synthesis of the acide chloride derivative of deferasirox allowing the preparation of further new conjugates.
4.4.2 The H_2defnaf conjugate was prepared in which the naphthyl unit as a fluorophore allows the tracking of the ligand or its metal complexes in the cell. The new product was characterized by NMR analysis.
4.4.3 A novel ambidentate ligand, H_2defphen was prepared, in which the phenanthroline unit is capable of coordinating via the N,N chelating set to a second metal ion, thus forming a multinuclear complex. The new product was characterized by NMR analysis.
4.4.4 The bis complexes of H_2defnaf and H_2defphen with Co(III) were prepared. The products were characterized by NMR (Figure 4).
4.4.5. ESI-MS showed that the Co(defphen)$_2$ complex is able to bind two organoruthenium ions using the N,N chelates of free phen units.

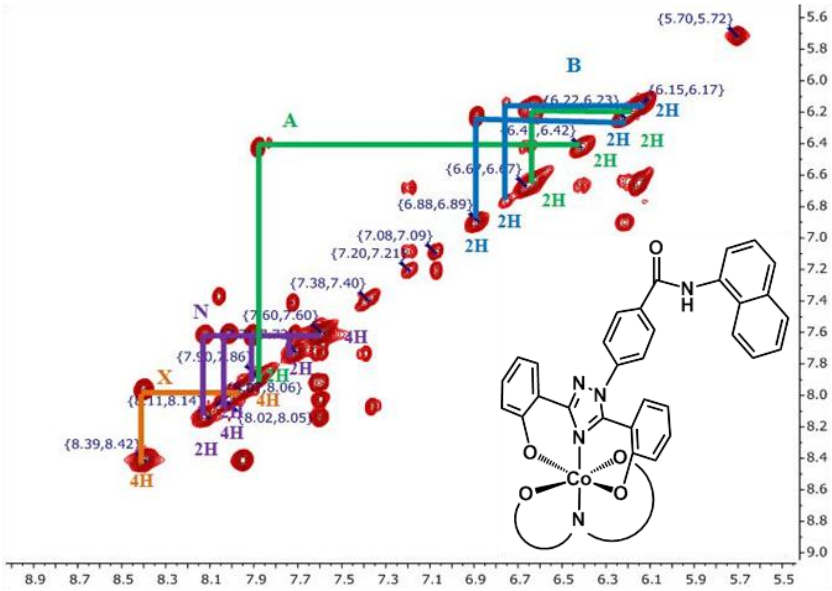

Figure 4. Aromatic range of the 2D COSY spectrum of the $K[Co(defnaf)_2]$ complex.

V. POSSIBLE APPLICATIONS OF THE RESULTS

In my research, I have carried out mainly synthetic work, during which the aim was to obtain and characterize new metal complexes with likely anti-cancer potential in which the constituent components have an anti-cancer effect on their own resulting thus the formation of multi-targeted complexes.

The eight new maltol, SAHA and deferasirox derivatives in total and their Co(III), Ru(II)/(III), Os(VI) and Ga(III) complexes were prepared and characterized (nine complexes, two of them are dinuclear, containing two metal ions) and we hope to contribute to the further drug development work that may result in more efficient and selective drug candidate molecules. Importantly, during my PhD work, synthetic pathways and procedures were developed that can successfully be used to produce new, hopefully effective drug candidate molecules.

PUBLICATIONS

Articles related to the dissertation (2)

1. *I. Nagy*, E. Farkas, J. Kasparkova, H. Kostrunova, V. Brabec, P. Buglyó
   Synthesis and characterization of (Ru(II), Co(III)) heterobimetallic complexes formed with a 1,10-phenanthroline based hydroxamic acid conjugate
   Journal of Organometallic Chemistry, **2020**, *916*, 121265.
   IF: 2.369

2. *I. Nagy*, G. Fereczik, L. Bíró, E. Farkas, A. Cs. Bényei, P. Buglyó
   Metal complexes of deferasirox derivatives: A solid state and equilibrium study
   Polyhedron, **2020**, *190*, 114780.
   IF: 3.052

Articles not related to the dissertation (2)

1. P. Buglyó, L. Bíró, *I. Nagy*, B. Szőcs, E. Farkas
   Hydroxypyronate, Thiohydroxypyronate and Hydroxypyridinonate derivatives as potential Pb^{2+} sequestering agents
   Polyhedron, **2015**, *92*, 7-11.
   IF: 2.108

2. P. Buglyó, I. Kacsir, M. Kozsup, *I. Nagy*, S. Nagy, A. C. Bényei, É. Kovács, E. Farkas
   Tuning of the redox potentials of ternary cobalt(III) complexes containing various hydroxamates
   Inorganica Chimica Acta, **2018**, *472*, 234-242.
   IF: 2.433

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Doctoral School: Doctoral School of Chemistry
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List of publications related to the dissertation

Foreign language scientific articles in international journals (2)

1. **Nagy, I.**, Ferenczik, G., Bíró, L., Farkas, E., Bényei, A., Buglyó, P.: Metal complexation of deferasirox derivatives: A solid state and equilibrium study.
   Polyhedron. 190, 1-13, 2020. ISSN: 0277-5387.
   DOI: http://dx.doi.org/10.1016/j.poly.2020.114780
   IF: 3.052

2. **Nagy, I.**, Farkas, E., Kasparkova, J., Kostrhunova, H., Brabec, V., Buglyó, P.: Synthesis and characterization of (Ru(II), Co(III)) heterobimetallic complexes formed with a 1,10-phenanthroline based hydroxamic acid conjugate.
   J. Organomet. Chem. 916, 1-9, 2020. ISSN: 0022-328X.
   DOI: http://dx.doi.org/10.1016/j.jorganchem.2020.121265
   IF: 2.369

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List of other publications

Foreign language scientific articles in international journals (2)

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

4. Buglyó, P., Bíró, L., **Nagy, I.**, Szőcs, B., Farkas, E.: Hydroxypyronate, thiohydroxypyronate and hydroxypyridinonate derivatives as potential Pb2+ sequestering agents. *Polyhedron. 92*, 7-11, 2015. ISSN: 0277-5387.
DOI: http://dx.doi.org/10.1016/j.poly.2015.03.006
IF: 2.108

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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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