Proposition of Ph.D. thesis

Half- and full sandwich type Ru(II) complexes of histidine containing oligopeptides and their models: solution equilibrium studies and synthesis

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Interaction of $[(\eta^6\text{-arene})Ru(H_2O)_3]^{2+}$ organometallic cations with O-donor small molecules

I. INTRODUCTION AND THE AIM OF THE WORK

The square-planar cisplatin, $[(NH_3)_2PtCl_2]$, containing platinum(II) ion is used over 40 years in chemotherapy. It has, however, numerous drawbacks, among others serious side effects and unwanted cell resistance that can limit its use. Because of the mentioned disadvantages much effort is dedicated in the search of novel metal complexes that show the same activity, much better selectivity and low toxicity. Therefore the platinum metal group's ruthenium and its complexes are at the forefront of cancer research. Anticancer activity of ruthenium(II) and octahedral ruthenium(III) complexes (KP1019, KP1339, which is the sodium salt of KP1019, and also NAMI-A) have been demonstrated in the past decades. NAMI-A was the first ruthenium complex that entered into Phase I and later Phase II clinical trials. It was demonstrated in the literature that Ru(III) can be reduced to the more labile corresponding Ru(II) complex *in vivo* and that form is responsible for cytotoxicity. The +2 oxidation state of ruthenium can be stabilized in a half-sandwich "piano-stool" geometry with arenyl or arene ligands, in pentahapto or hexahapto binding mode, respectively. In these complexes there are three available coordination sites; two are very often occupied by a chelating (XY), for instance NN- or OO-donor ligand, while the remaining third position most commonly by a monodentate ligand. In the literature there are a lot of Ru(II) complexes that were characterized in solid phase, although practically there is no information about their solution behaviour.

Therefore, in the Bioinorganic Research Group of the Department of Inorganic and Analytical Chemistry, University of Debrecen solution equilibrium studies have carried out on half-sandwich type Ru(II) complexes that may provide important information to understand the biotransformation mechanism and ligand-exchange processes of Ru(II) complexes. Soft metal ions such Ru(II) shows preference toward S- and N-donor atoms, therefore for

instance, it will interact with peptides containing surface histidines and imidazole(s) in the side chain.

Beside the half-sandwich type compounds, the full sandwich type complexes have been the subject of several studies in recent years. There are only a few and promising result about the biological activity of full sandwich type ruthenium(II) complexes in the literature. These type of complexes can be synthesized using various synthetic routes, for instance by light irradiation with very good yield. Nowadays more research have published on the synthesis of peptide conjugates that contain a metal ion or a metal complex was conjugated to a bioactive peptide that can interact with the receptors of cancer cells. In this case the compound with anticancer potential can selectively accumulate in the target cells where it may exert its apoptotic effect. These type of bioconjugates contain a specific amino acid sequence, such as histidyl-alanyl-valinyl (His-Ala-Val, HAV) that can play an important role in biorecognition processes. These HAV-containing peptides have caused apoptosis of various cancer cells. Besides, they may have the potential application to modulate the intercellular junctions of the biological barrier to improve permeation of drugs. The interaction between radioactive gallium(III) and a peptide-based complex containing triaza chelator ligand may result in the formation of a heterobimetallic peptide conjugate complex that provides information on the biodistribution of the corresponding complex.

In the light of the above informations the aim of my Ph.D. work was to explore the interaction between half-sandwich type ruthenium(II) aquaion and imidazole containing ligands as simple models for the imidazole side chain of His-containing peptides. Our further aim was the synthesis, characterization and biological screening of a heterobimetallic, full sandwich type peptid conjugate complex.

II. EXPERIMENTAL METHODS

Oligopeptides containing histidines (*Figure 1:* **III.A-D**) and/or HAV sequence (*Figure 1:* **IV.A-B**) were synthesized using a microwave-assisted Liberty1™ **peptide synthesizer** (CEM, Matthews, NC) in our research group. The N-terminally free and also the acetylated oligopeptides were prepared using Fmoc/*t*Bu technique and Rink Amide AM resins as solid phase, containing amino group on their surfaces. The activation of carboxyl and amino groups was carried out with TBTU/HOBt/DIEA.

The purity of the ligands was checked by using **high-performance liquid chromatography (HPLC)**. The analytical studies were performed on a Vydac C18 reversed-phase column (250 mm × 4,6 mm; 300 Å pore size; 5 μm particle size) using a Jasco MD-2010 plus multiwavelength detector on 222 nm, 254 nm, 280 nm. For separation we used semi-preparative HPLC with Vydac C18 column (250 mm × 10 mm; 300 Å pore size; 5 μm particle size) and Jasco UV-2077 plus multiwavelength detector on 222 nm, 254 nm, 280 nm. The eluent A (water) and B (acetonitril) contained 0.1 % (V/V) TFA and flow rate of 1,0–2,0 mL/min was used.

pH-potentiometric titrations were used to check the purity of the oligopeptides and to determine the protonation constant of the ligands and the stability constants of half-sandwich type ruthenium(II) complexes. The measurements were carried out at 0.20 mol/dm^3 KNO_3 and in some cases at 0.20 mol/dm^3 KCl ionic strength at 298.0 K on Mettler Toledo instrument. Owing to the slow equilibrium processes individual samples containing the metal ion and ligand were also prepared. The samples were left to stand for 3–7 days and were checked by NMR. The overall stability constants of the complexes were calculated by means of the computational programs, PSEQUAD and SUPERQUAD. Based on the calculated stability constants the concentration distribution curves for the corresponding systems were constructed with the MEDUSA program.

In order to gain a deeper insight into the complex formation and to check the purity of the synthesized oligopeptides we used **NMR spectroscopy**. The ^{1}H, ^{13}C, ^{71}Ga and 2D NMR spectra were recorded on a Bruker AM 360 MHz FT-NMR or on a Bruker Avance DRX 400 MHz FT-NMR instrument. Analysis of the spectra were performed with 1D WIN-NMR and MestReNova softwares.

UV-Vis spectrophotometric measurements were carried out to study the complex formation. The spectra were recorded on a Perkin Elmer Lambda 25 double beam spectrophotometer using 1.000 cm cuvettes. For the analysis of the obtained spectra and determination of the thermodynamic stability constants of the complexes the PSEQUAD program was used.

Circular dichroism (CD) spectroscopy is a widely used technique for exploring the structure of optically active metal complexes. CD measurements were performed on a JASCO-810 spectropolarimeter. The CD spectra of individual Ru(II)-containing samples were recorded at different pH values in water and at room temperature using a 1.000 cm cell.

Electrospray ionization mass spectrometry (ESI-MS) was used to identify the synthesized ligands and their Ru(II) complexes. The measurements were performed in water and were carried out on a micrOTOF-Q ESI-TOF spectrometer in samples at 0.1–1.0 mmol/dm^3 ruthenium(II) concentration. DataAnalysis (version 3.4) was used for calculation.

In the framework of an international collaboration **Density Functional Theory (DFT)** calculations were carried out to optimize geometries of some Ru(II) complexes by Dr. Eugenio Garribba and Valeria Ugone (Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy) and Norbert Lihi (University of Debrecen). Geometries of the histidine (Ac-HAHH-NH_2; Ac-HAHAH-NH_2; H-AH-OH; H-HH-OH) containing Ru(II) complexes were fully optimized using the Gaussian 09 (revision C.01) software.

We also synthesized and fully characterized heterobimetallic (Ru(II) and Ga(III)) peptid conjugate complexes containing a histidyl-alanyl-valinyl (HAV) sequence. In the framework of an international collaboration with Dr. João D.G. Correia (Centro de Ciências e Tecnologias Nucleares (C^2TN), Instituto Superior Técnico, Universidade de Lisboa, Portugal) **cellular uptake, MTT viability assay** of the radioactive-compounds were studied in four human derived cancer cell lines.

III. STUDIED LIGANDS

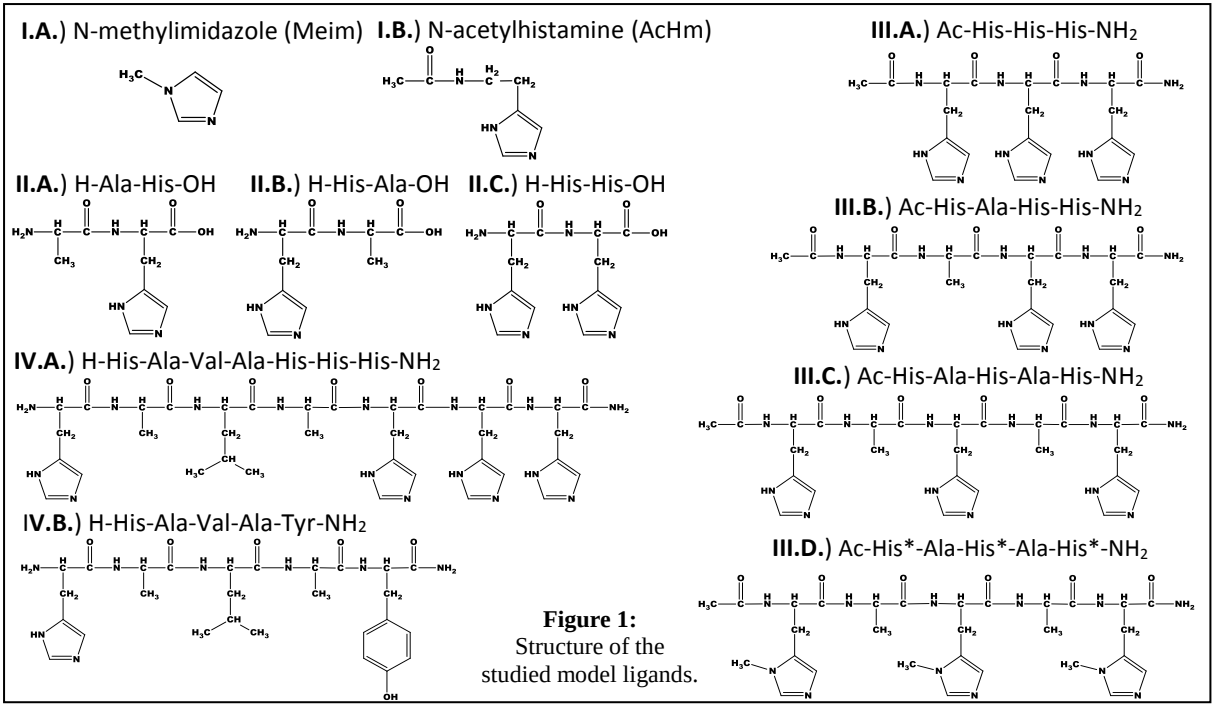

Figure 1: Structure of the studied model ligands.

IV. NEW SCIENTIFIC ACHIEVEMENTS

4.1. Synthesis and pH-potentiometric study of the novel ligands

4.1.1. We have synthesized novel, oligopeptide ligands (*Figure 1*: **III.A-IV.B**) using solid phase peptide synthesis. The identity and purity of the ligands were checked by HPLC, ESI-MS and NMR techniques and the deprotonation constants (except for *N3*-methylated Ac-H*AH*AH*-NH_2) were determined by pH-potentiometry at 0.20 mol/dm^3 KNO_3 ionic strength (*Table 1*).

Table 1: Deprotonation constants (p*K*) of the oligopeptides obtained by pH-potentiometry at 0.20 mol/dm^3 KNO_3, t = 25.0°C

	Ac-HHH-NH_2	Ac-HAHH-NH_2	Ac-HAHAH-NH_2	H-HAVAHHH-NH_2	H-HAVAY-NH_2
pK_1	5.61(1)	5.71(1)	5.80(1)	5.22(5)	5.44(1)
pK_2	6.32(1)	6.34(1)	6.33(1)	5.63(4)	7.27(2)
pK_3	6.95(1)	6.94(1)	7.02(1)	6.35(5)	9.52(3)
pK_4	-	-	-	6.83(3)	-
pK_5	-	-	-	7.91(4)	-
ΣpK	18.88	18.99	19.15	31.94	22.23

4.1.1. We have detected slow complex formation processes in the studied systems containing half-sandwich ruthenium(II) and the corresponding ligands (*Figure 1*: **I.A-IV.A**). Owing to the slow equilibrium processes individual samples containing Ru(II) and the

ligands in different ratios were prepared using 0.20 M KCl/KNO_3 ionic strength.

4.1.2. For Ac-HHH-NH_2 the formation of a yellow precipitate as low as pH 2.0 and a very limited solubility of the complex(es) even at elevated pH hindered subsequent solution equilibrium studies with this ligand.

4.2. Interaction between $[(\eta^6$-*p*-cym)$Ru(H_2O)_3]^{2+}$ and His-model ligands

We have studied in detail the complex formation between the half-sandwich type $[(\eta^6$-*p*-cym)$Ru(H_2O)_3]^{2+}$ and the small molecular weight, N-donor N-methylimidazole or N-acetylhistamine model ligands (*Figure 1*: **I.A-B**).

4.2.1. Steady pH readings were used to construct titration curves and to estimate the stability constants of the complexes present in solution. The calculated distribution curves for the $[(\eta^6$-*p*-cym)$Ru(H_2O)_3]^{2+}$–Meim system can be seen in *Figure 2*.

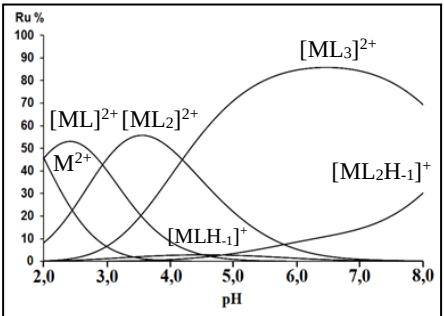

Figure 2: Calculated concentration distribution curves for the $[(\eta^6$-*p*-cym)$Ru(H_2O)_3]^{2+}$–Meim system at 1:3 ratio (c_{Meim} = 9.99 mM, 0.20 M KNO_3).

4.1.1. We have found the formation of two mixed hydroxido complexes ($[ML(OH)]^+$ and $[ML_2(OH)]^+$) at 0.20 M KNO_3, while mixed, chlorido containing complexes ($[MLCl_2]$ and $[ML_2Cl]^+$) were detected under biologically relevant conditions and also the hydrolysis was hindered. We have shown by ^{1}H NMR that Meim and AcHm (*Figure 1*: **I.A-B**) form stable complexes and prevent the metal ion from hydrolysis as low as 1:3 metal to ligand

ratio at pH 7.4. pH dependent ESI-MS measurements provided proof that the composition of the complexes are identical in the $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$–Meim and $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$–AcHm systems.

4.3. Interaction between $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ and histidyl containing oligopeptides

We have studied the complex formation between $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ and terminally not protected dipeptides containing one or two histidyl (*Figure 1:* **II.A-C**) units and those terminally protected oligopeptides (*Figure 1:* **III.A-D**) that contain three histidyl residues at different positions in the sequence.

4.3.1. Ruthenium(II) complexes of dipeptides containing one or two histidyl units

4.3.1.1. We have found using various NMR and MS measurements, that the complex formation with H-Ala-His-OH, H-His-Ala-OH and H-His-His-OH starts below pH 2 in the Ru(II) containing systems. Our NMR results revealed that chloride ions cannot coordinate to the metal ion even at 0.20 M KCl.

4.3.1.2. By the combined use of watergate NMR and MS measurements in the Ru(II)–H-Ala-His-OH system we have detected a $[ML]^+$ $(N^-_{amide}, \mathit{N3}_{im}, O^-_{carboxylate})NH_3^+$ binding isomer at acidic pH, which is in fast-exchange with $(N_{amino}, N^-_{amide}, \mathit{N3}_{im})O^-_{carboxylate}$ complex above pH 4.8. DFT calculations also support our result that the $\mathit{N1}_{im}$ coordination mode is preferred over the $\mathit{N3}_{im}$ one.

4.3.1.3. We have also identified a $(N_{amino}, \mathit{N3}_{im}, O^-_{carboxylate})NH_{amide}$ binding isomer, which is present over a wide pH range (1.7–7.0) in the Ru(II)–H-His-Ala-OH system.

4.3.1.4. Titration curves constructed using individual samples (*Figure 3*: c, d) indicated slow equilibrium processes in the Ru(II)–H-His-His-OH system and proved that no pH equilibrium is reached during the conventional pH-potentiometric titrations (*Figure 3*: b).

4.3.1.5. The almost identical shape of the titration curves of individual samples in the presence of KCl (c) or KNO_3 (d) demonstrated that chloride is not capable of coordinating. This result was also supported by ESI-MS and ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC NMR techniques.

4.3.1.6. With increasing the pH the ESI-MS results revealed the formation of $[ML]^{+}$ species formed from the $[MLH]^{2+}$ complex.

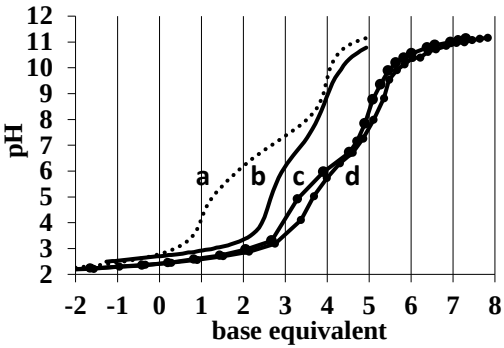

Figure 3: Representative base equivalent titration curves of H^{+}–H-His-His-OH (a) and $[(\eta^{6}$-*p*-cym)$Ru(H_2O)_3]^{2+}$–H-His-His-OH system at ratio 1:1 (b, c, d).

4.3.1.7. We have studied samples at 1:1, 1:2 and 2:1 ratio at pH 1.1 by ^{1}H NMR. We have demonstrated from the ratio of signals of complex, free ligand and free metal ion that the complex is formed at this pH is $[MLH]^{2+}$. Stability constant of $[MLH]^{2+}$ ($\log\beta_{[MLH]^{2+}}$ = 21.6(2)) was calculated using the known analytical concentrations of M, L, H^{+}, the protonation constants of ligand and the ratio of the signals belonging to the free metal ion and free ligand. We have also demonstrated from NMR spectra recorded at different pH values that the (N_{amino}, N_{im}, N_{im}) binding isomer of $[MLH]^{2+}$ is present in the system below pH 5. We have found that H-His-His-OH is capable of hindering the hydrolysis of the metal ion at pH 7.4.

4.3.1.8. DFT calculations were carried out to determine the most stable geometry of $[MLH]^{2+}$. The results, in accordance with the

equilibrium study, indicated that the (N_{amino}, N_{im}, N_{im}) coordination mode is more stable than the ($O^-_{carboxylate}$, N_{im}, N_{im}). We have shown that among the (N_{amino}, *N3*$_{im}$, *N3*$_{im}$) and (N_{amino}, *N3*$_{im}$, *N1*$_{im}$) coordination modes, the latter one is preferred, in which the "histamine type" coordination at the N-terminus with *N3* imidazole is formed, over the one when C-terminal histidine with *N1* nitrogen donoratom would coordinate to $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$.

4.3.1.9. CD spectra of the isomer with (N_{amino}, *N3*$_{im}$, *N1*$_{im}$) binding mode exhibited a large negative (420 nm) and a large positive (360 nm) Cotton-effect at pH 1.76, indicating the formation of a „histamine-type" (N_{amino}, *N3*$_{im}$) six-membered chelate. These results were also supported by DFT calculations providing further proof on the structure of binding isomer.

4.3.1.10. The $[M_2LH_{-1}]^{2+}$ species was detected by ESI-MS and the new complex formation processes above pH 5 (*Figure 3*: c, d) support the metal ion induced deprotonation and coordination of amide group.

4.3.2. Ruthenium(II) complexes of oligopeptides containing three histidyl units

4.3.2.1. We have demonstrated by the combined use of ^1H, ^{13}C, ^1H-^1H COSY, ^1H-^{13}C HSQC NMR and ESI-MS techniques that in the $[ML]^{2+}$ complex all the three imidazole nitrogens are coordinated to the metal ion in the Ru(II)–Ac-HAHH-NH_2 or –Ac-HAHAH-NH_2 systems at pH 7.4.

4.3.2.2. In the framework of an international collaboration DFT calculations were carried out to show that complex formation with *N1* nitrogen is preferred over *N3* (*Figure 4*).

4.3.2.3. We have also demonstrated by the comparison of the $\delta^{13}C$-imidazole chemical shifts (C2, C4, C5) of Ac-HAHH-NH_2, Ac-HAHAH-NH_2 ligands and those of $[ML]^{2+}$ complex, that *N1* coordination of the His units is likely to Ru(II).

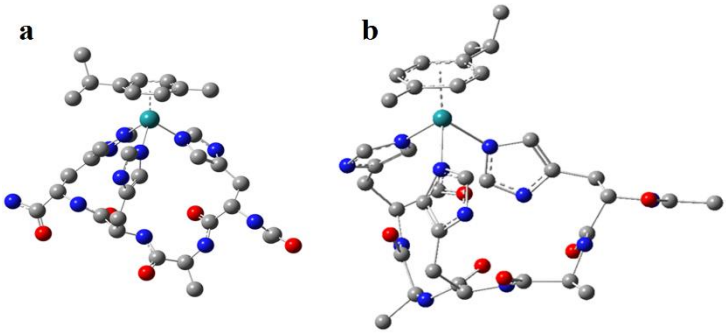

Figure 4: Optimized DFT structures of ML(*N1*) isomers, where L_1: Ac-HAHH-NH_2 (a) and L_2 = Ac-HAHAH-NH_2 (b). Hydrogen atoms have been ommited for clarity.

4.3.2.4. In order to obtain further proof on this binding mode a model pentapeptide, Ac-H*AH*AH*-NH_2 with three *N3*-methylated His units was also synthesized (*Figure 1*: **III.D**). We have demonstated by ESI-MS and NMR techniques that the three imidazoles are coordinated via *N1* about pH 8.

4.3.2.5. We have found by ^{1}H NMR that the hydrolysis of metal ion can be hindered below pH 9 in $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$–Ac-HAHH-$NH_2$ or –Ac-HAHAH-NH_2 systems at 1:1 ratio using 0.20 M KNO_3 ionic strength, while at 0.20 M KCl ionic strength that was hindered up to pH 11.5.

4.4. Ruthenium complexes of HAV containing peptides

The synthetic oligopeptides containing conserved histidyl-alanyl-valinyl (HAV) sequence with cell adhesion recognition capability exhibit antiproliferative effect on various cancer cells. These HAV-containing peptides may have potential application for the modulation of the intercellular junctions of the biological barrier to improve permeation of drugs. We have synthesized half- and full sandwich type complexes containing HAV sequence incorporating Ru(II) with anticancer potential. We used $[(\eta^6\text{-}p\text{-cym})Ru)]^{2+}$ and $[(\eta^5\text{-}$

Cp)Ru]$^{+}$ for the synthesis of the half-sandwich and for full sandwich type complex, respectively.

4.4.1. Half-sandwich type Ru(II) complexes containing HAV sequence

4.4.1.1. Although similarly to Ac-HHH-NH_2, in the sequence of H-HAVAHHH-NH_2 there are three histidyl residues next to each other, no formation of precipitate was detected at 1:1 metal ion to ligand ratio in contrast to the Ru(II)–tripeptide system. Using ESI-MS we have identified the formation of $[ML]^{2+}$ at pH 8.

4.4.1.2. We have also shown by ^{1}H, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC NMR and CD techniques, similarly to H-HA-OH and H-HH-OH, that the "histamine-type" (N_{amino}, $N3_{im}$) binding mode at the N-terminus of H-HAVAHHH-NH_2 is preferred in the range pH 2–8 (*Figure 5*) and the $[\{(\eta^6\text{-}p\text{-cym})Ru\}_2(OH)_3]^{+}$ hydroxido complex is formed only above pH 8.4.

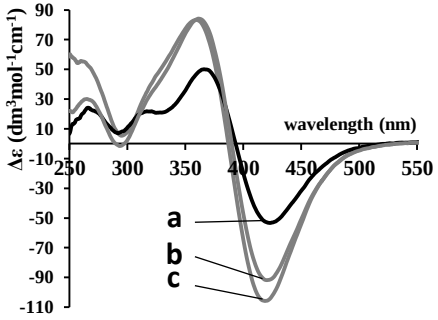

Figure 5: CD spectra of Ru(II)–H-HAVAHHH-NH_2 (pH = 2.65, a), Ru(II)–H-His-Ala-OH (pH = 2.15, b) and Ru(II)–H-His-His-OH (pH = 2.59, c) systems.

4.4.2. Synthesis, characterization and biological evaluation of a full sandwich type Ru(II) complex containing HAV sequence

4.4.2.1. We have synthesized and fully characterized by ^{1}H, ^{13}C, ^{71}Ga, ^{1}H-^{1}H COSY, ^{1}H-^{1}H ROESY, ^{1}H-^{13}C HSQC NMR and ESI-MS techniques the first heterobimetallic, full sandwich type peptid

conjugate complex containing Ru(II) and Ga(III) metal ions (*Figure 6*: f).

4.4.2.2. We have synthesized a full sandwich type (η^6-Tyr)RuCp)-HAVAY-NH_2 complex using visible-light irradiation of H-HAVAY-NH_2 in the presence of [(η^6-naphtalene)Ru(II)(η^5-Cp)]PF_6 precursor, in which the metal ion is bound to the Tyr (Y) unit of the peptide (*Figure 6*: b).

4.4.2.3. The coordinated Tyr residue in the pH-dependent NMR spectra of (η^6-Tyr-RuCp)-HAVAY-NH_2 showed unexpected multiplicity therefore we monitored by ^{1}H NMR whether light irradiation could also mediate the formation of full sandwich (η^6-Tyr-RuCp) complex with the model *L*-phenylalanine or *L*-tyrosine amino acids. We have demonstrated that the formation of the full sandwich type complex with a hexahapto bound phenyl group of the Tyr and the pentahapto Cp ring results in the change of the environment of protons of the phenyl ring and that can be rationalized with the change in hapticity from η^6 to η^5 if the pH is increased.

4.4.2.4. We have synthesized, purified and characterized the full sandwich peptide conjugate that is formed between H-HAVAY-NH_2 or (η^6-Tyr-RuCp)-HAVAY-NH_2 peptide complex and NODA-GA macrocycle (*Figure 6*: c, d). Reaction of the NODA containing compounds with ^{nat}Ga(III) or ^{67}Ga(III) afforded the corresponding gallium(III) complexes (*Figure 6*: e, f).

4.4.2.5. In the framework of an international collaboration we have screened the above mentioned compounds (*Figure 6*: a–f) on different human derived cancer cell lines. Although we have found significant cellular uptake of MDA-MB-231 cells, the complexes did not show inhibition of the cell proliferation on MCF-7, MDA-MB-231, A375 and PC-3 cell lines in the 0.1–200 μM concentration range.

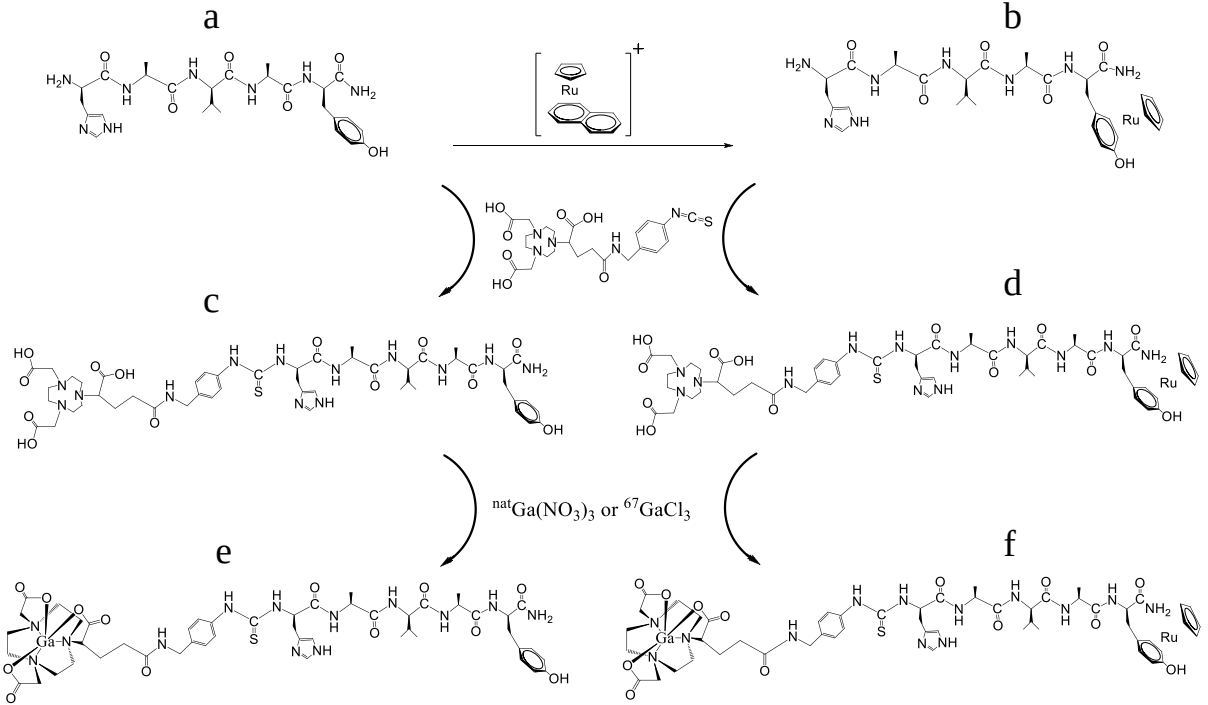

Figure 6: Synthetic routes of the Ga-NODA-GA-HAVAY-NH_2 (e) and Ga-NODA-GA-(η^6-Tyr-RuCp)-HAVAY-NH_2 (f) peptide complexes.

V. POSSIBLE APPLICATIONS OF THE RESULTS

The half-sandwich type $[(\eta^6\text{-}p\text{-cym})Ru(L)]^{n+/-}$ complexes may undergo various biotransformation reactions in blood serum with the formation of $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$, that can interact with the imidazolyl groups of side chain His residues of high molecular mass components in the biofluids.

In the light of the above information, the aim of our work was to study in detail the interaction between a half-sandwich type ruthenium(II) aqua complex and simple Im derivatives or imidazole side chain containing oligopeptides modeling surface accessible histidyl residues that contain the histidyl residues in various positions.

Although our investigations belong to the academic research, the obtained results allow to build up speciation models and help to characterize the complex formation between the half-sandwich type ruthenium(II) and bioligands containing imidazolyl N-donoratoms.

We have synthesized and fully characterized the first Ru(II)/Ga(III) heterobimetallic full-sandwich type peptidconjugate complex containing a histidyl-alanyl-valinyl (HAV) sequence for targeting, a full sandwich Ru(II) unit with potential anticancer activity and a ^{nat}Ga- or ^{67}Ga-NODA-GA moiety to monitor biodistribution. The use of our results and the modification of the structural units of the studied compounds may provide with information for the design and synthesis of novel metal complexes with improved antiproliferative potential.

VI. TUDOMÁNYOS PUBLIKÁCIÓK (PUBLICATIONS)

Az értekezés alapját képező közlemények (articles related to the dissertation)

Tudományos folyóiratban megjelent közlemények (3):

1. Zsolt Bihari, Valeria Ugone, Eugenio Garribba, Norbert Lihi, Péter Buglyó: Complex formation between $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ and oligopeptides containing three histidyl moieties.
   Journal of Organometallic Chemistry, 823, 116-125 (2016)
   IF: 2,336 (2015)
2. Zsolt Bihari, Filipe Vultos, Célia Fernandes, Lurdes Gano, Isabel Santos, João D. G. Correia, Péter Buglyó: Synthesis, characterization and biological evaluation of a ^{67}Ga-labeled (η^6-Tyr)Ru(η^5-Cp) complex with the HAV motif.
   Journal of Inorganic Biochemistry, 160, 189-197(2016)
   IF: 3,205 (2015)
3. Zsolt Bihari, Zoltán Nagy, Péter Buglyó: $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ binding capability of N-methylimidazole to model the interaction between the metal ion and surface histidine residues of peptides.
   Journal of Organometallic Chemistry, 782, 82-88 (2015)
   IF: 2,336 (2015); independent citation: 3

Konferencia kötetben megjelent tanulmány (1):

1. Bihari Zsolt, Bíró Linda, Buglyó Péter: A potenciálisan rákellenes hatású félszendvics típusú $[Ru(\eta^6\text{-}p\text{-cimol})(H_2O)_3]^{2+}$ kölcsönhatása hisztidintartalmú oligopeptidekkel és modelljeikkel, **XXXVI. Kémiai Előadói Napok, 2013, Szeged, Magyarország, p. 288, JATEPress, (ISBN 978-963-315-145-7).**

Tudományos kötetben megjelent fejezet (1):

1. Bihari Zsolt: A potenciálisan rákellenes hatású félszendvics típusú $[Ru(\eta^6\text{-}p\text{-cimol})(H_2O)_3]^{2+}$ kölcsönhatása hisztidintartalmú oligopeptidekkel és modelljeikkel. **Hatvani István Szakkollégium, Mi tendenciánk II. tanulmánykötet, 2013, Debrecen, Magyarország, p. 111-126, (ISNN 2063-6059).**

Az értekezés anyagából készült előadások (lectures related to the dissertation) (13):

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List of publications related to the dissertation

Foreign language scientific articles in international journals (3)

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List of other publications

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4. Bíró, L., Godó, A. J., **Bihari, Z.**, Garribba, E., Buglyó, P.: Tuning the Hydrolytic Properties of Half-Sandwich-Type Organometallic Cations in Aqueous Solution.
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