Development, synthesis and characterization of bimetallic complexes as potential hypoxia-activated prodrugs with dual anticancer activity (K 112317)

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Final Report

Introduction and goals

Compared to healthy ones, cancer cells are characterized, among others, by altered cellular processes e.g. higher uptake of nutriments, lower pH, overexpression of matrix metalloproteinases (MMPs) or hypoxia (low cellular c_{O_2}). Hypoxia, therefore, also means a reductive environment which enhances e.g. the selective reduction of kinetically inert Co(III) complexes to the labile Co(II) ones. Previous studies suggested that Co(III)-hydroxamates can be used as bioreductively activated produgs under these conditions releasing free hydroxamate after the dissociation of the much less stable Co(II)-hydroxamate species. Other ligands with proven metalloenzime inhibitory effect and/or anticancer activity (sulphonamides, catecholates, flavonols, peptide-hydroxamates) could also be used in an identical manner and therefore, with the administration of these Co(III) prodrug complexes selective release of an anticancer agent in the cancer cells may be achieved. Linking a half-sandwich [M(η^6-*arene*)] (M) core to the rationally designed derivative of the above bioligands, beside the bioactive inhibitor, the metal core with potential anticancer activity may also be delivered to the cancer cell. To maintain the biological effect of these ligands careful design of the metal ion binding part is required. Furthermore, fine tuning of the kinetic inertness of the M-linker bond may result in the formation of free [M(η^6-*arene*)]$^{2+}$ too in the cell.

To achieve the goals of the outlined project the factors influencing the thermodynamic stability and kinetic inertness of the appropriate Co(II/III) or [(η^6-arene/η^5-arenyl)M]$^{2+}$ (M = Ru, Os, Rh, Ir) bonds in the suggested new, bimetallic complexes needed to be explored. Therefore, synthesis, characterization, redox studies of octahedral Co(III) model complexes with hydroxamates, hydroxamate derivatives of amino acids, simple peptides, flavonols and catecholates all incorporating an (O,O) binding site for the metal ion were planned. Another aspect of the research was the study of the [(η^6-arene/η^5-arenyl)M]$^{2+}$ binding of small mostly (N,N) chelator molecules and to highlight their kinetic and redox features. On the basis of the knowledge obtained rational derivatisation of the ligands with enzyme inhibitory effect was planned in order to modify them for binding the second metal ion too. In the remaining part of the project the synthesis, characterisation and biological evaluation of the novel complexes was planned.

Results

1. Ligands

For the synthesis of the planned complexes numerous, partly new tripodal amines, differently substituted monohydroxamic acids and flavonols (via the appropriate chalcones) as well as ambidentate ligands (incorporating phenantroline-, pyridyl or pyrrolyl and aliphatic N based chelating

N,N as well as hydroxamate or maltol-based chelating O,O donor atom sets) were synthesized and characterised using various (NMR, IR, CHN, ESI-TOF-MS) analytical techniques [1, 3, 6, 11, 15].

2. Complexation with platinum group metals

We have carried out solution equilibrium studies in the field of half-sandwich type platinum metal cation – (O,O) donor ligand systems. Regarding the interaction of the model $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ and various substituted flavonolates the formation of complexes with very limited aqueous solubility was detected hindering thus the determination of their stability constants, speciation and further studies in solution.

In order to gain information on the possibility to form hydroximato bridged bimetallic complexes, the interaction between $[Pd(en/pic)(H_2O)_2]^{2+}$ (en = ethylenediamine, pic = pyridine-2-methylamine) and primary hydroxamic acids (ahaH = acetohydroxamic, bhaH = benzohydroxamic acid) or secondary hydroxamic acids (meahaH = N-methyl-acetohydroxamic, pheahaH = N-phenylacetohydroxamic and phebhaH = N-phenylbenzohydroxamic acid) was studied in aqueous solution by pH-potentiometry, ^{1}H NMR and ESI-TOF-MS. Secondary hydroxamates were shown to form 1:1 species with the $[Pd(N,N)(H_2O)_2]^{2+}$ ions via the [O,O] chelating sets. Unexpectedly, in the primary ligands deprotonation and coordination of the hydroxamate-NH starts as low as pH <2, where the RCC(O)NO ion is capable of linking two $[Pd(diamine)]^{2+}$ units via the coordination through the [O,O] chelate to one unit and through the monodentate hydroxymato-N atom to the another one. As a consequence, primary ligands can bind an excess of metal ion too. A trinuclear complex predominates in a wide pH-range (5–10 for en, 3–10 for pic) and the hydroxide ion starts to compete with the hydroxymato ligand only above pH 10. In the trinuclear species two [O,O] chelated Pd(II) units are bridged via a third palladium core that binds to the hydroximato-N donors of the two ligands. This binding mode was also proved by MS studies in solution and by revealing the molecular structure of $[(Pd(en))_3(bhaH_{-1})_2](BF_4)_2{\cdot}2H_2O$, the first reported structure with a Pd(II)- hydroxymate-N monodentate coordination, characterized with single crystal X-ray diffraction in the solid state [18].

Complexation of the secondary N-methyl-acetohydroxamic acid (HMeaha) with $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$, $[(\eta^5\text{-Cp*})Rh(H_2O)_3]^{2+}$, $[(\eta^6\text{-}p\text{-cym})Os(H_2O)_3]^{2+}$ or $[(\eta^5\text{-Cp*})Ir(H_2O)_3]^{2+}$ cations and the primary acetohydroxamic acid (HAha) with the former two cations was studied in aqueous solution. $Meaha^-$ forms five-membered hydroxamate type chelated $[ML]^+$ and [M(OH)L], being the third coordination site occupied by a water molecule in the former complex and by a hydroxide ion in the latter one. While $[ML]^+$ remains predominant even under basic conditions in the Ru- and Rh-containing systems, it is measurable only in the slightly acidic region with the two 5*d* metal ions. Also, the tendency for hydrolysis of the aqua ligand in $[ML(H_2O)]^+$ complexes was detected to follow the trend of the cations for hydrolysis, Os > Ir > Ru > Rh. The X-ray result did not show any role of the H substituent of Aha^- in $[\{(\eta^5\text{-Cp*})Rh\}_2(\mu^2\text{-Aha})_2]^{2+}$, in solution, however, its significant role was supported especially with Ru. All the results are consistent with the formation of a stable trinuclear $[M_3H_{-2}L_3(H_2O)]^+$ species in which doubly deprotonated (hydroxymate type) ligands bridge two metal centres by coordinating via the two oxygens to the one cation and via the N-donor to the another one [10].

Complex forming capabilities of $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ with aminohydroxamates (2-amino-N-hydroxyacetamide (α-alahaH), 3-amino-N-hydroxypropanamide (β-alahaH) and 4-amino-

Nhydroxybutanamide (γ-abhaH)) having the primary amino group in different chelatable position to the hydroxamic function revealed the formation of stable [O,O] and mixed [O,O][N,N] chelated mono- and dinuclear species in partially slow with α-alahaH and β-alahaH or in fast processes with γ-abhaH and determined the formation constants of the complexes present. We have also synthesized and characterized novel dinuclear α-alaninehydroximato complexes containing the half-sandwich type Ru(II) core. The crystal and molecular structure of $[\{(\eta^6\text{-}p\text{-cym})Ru\}_2(\mu^2\text{-}\alpha\text{-alahaH}_{-1})(H_2O)Br]Br\cdot H_2O$ and $[\{(\eta^6\text{-}p\text{-cym})Ru\}_2(\mu^2\text{-}\alpha\text{-alahaH}_{-1})(H_2O)Cl]BF_4\cdot H_2O$ was determined by single crystal X-ray diffraction. This demonstates that in the complexes one half-sandwich core is coordinated by a hydroxamate [O,O] chelate while the other one by [N(amino),N(hydroxamate)] fashion of the bridging ligand. In both cases the remaining coordination sites of one of the Ru cores are taken by a halide ion whiles the other one by a water molecule. Reaction of them with 9-methylguanine indicates the N7 coordination of this simple DNA model. These complexes were also tested for their in vitro cytotoxicity using five human-derived cancer cell lines but showed no anti-proliferative activity in the micromolar concentration range [17].

Interaction of $[(\eta^5\text{-Cp*})Rh^{III}(H_2O)_3]^{2+}$ with aminohydroxamic acids (2-amino-N-hydroxyacetamide (α-alahaH), 3-amino-N-hydroxypropanamide (β-alahaH) and 4-amino-N-hydroxybutanamide (GABAha, γ-abhaH)) having the primary amino group in different chelatable position to the hydroxamic function revealed that the relative order of the pH-dependent conditional stability of the hydroxamate type (O,O) and (N_{amino},$N_{hydroxamato}$) chelates determine in high extent the coordination modes both in the mono- and various dinuclear species formed. While with α-alaha$^-$, the 5-membered (N,N) chelated mononuclear complex predominates, with β-alaha$^-$, in a wide pH-range, very stable dinuclear cluster ions exist. With γ-abha$^-$, in the most stable complexes, two ligands (in reverse variation) link two half-sandwich cations, coordinating each ligand via the hydroxamate chelate to one metal centre, while via the amino-N to the other one. This arrangement seems to be further stabilized by a hydrogen bond as DFT calculations support the extra stabilization effect of an internal H-bonding in $[\{(\eta^5\text{-Cp*})Rh^{III}\}_2H_{-1}(\gamma\text{-abha})_2]^+$. Synthesis, spectral (NMR, IR) and MS characterization of a novel complex with the iridium analogue, $[(\eta^5\text{-Cp*})Ir^{III}(\alpha\text{-alaha})Br]$ was also completed. This complex was tested for its *in vitro* cytotoxicity using human-derived cancer cell lines (A2780, HeLa, DU-145, A549, and MCF-7) and showed insignificant anti-proliferative activity in the micromolar concentration range [9].

To explore the suitability of a simple peptide backbone as (N,N) donor in this field, primary and secondary di- and tripeptide hydroxamic acids, Ala-Ala-NHOH, Ala-Ala-N(Me)OH, Ala-Gly-Gly-NHOH and Ala-Gly-Gly-N(Me)OH were synthesized and their interaction with Pd(II) (as a Pt(II) model but with faster ligand exchange reactions) was studied in aqueous solution in presence of Cl^- competitor ion by pH-potentiometric and ^1H NMR methods. We have demonstated that, except Ala-Gly-Gly-NHOH, the other three ligands act not only as coordination compounds, but also the hydrolysis of the coordinated ligands and formation of protonated hydroxylamine and Pd(II) complexes of the corresponding peptides under acidic conditions occured. The hydrolysis was rather slow with Ala-Gly-Gly-N(Me)OH slightly faster with Ala-Ala-NHOH, so speciation studies could also be performed successully on the systems containing one of the latter two ligands. This was, however, hindered for the Pd(II)-Ala-Ala-N(Me)OH system, where, in addition to the quite fast hydrolysis of the ligand, the reduction of Pd(II) to elementary metal by the N(Me)-hyroxylamine

formed was also observed. Speciation studies with Ala-Gly-Gly-NHOH revealed the predominance of a very stable 4N-donor complex, (NH_2,$2N_{amide}$, $N_{hydr.}$) over a wide pH-range. This is also capable of binding metal ion excess with the hydroxymate (O,O) set in dinuclear species. The formation of this latter type complex is hindered with the secondary analogue, Ala-Gly-Gly-N(Me)OH, where, in addition to the 3N donor atoms, the hydroxamate-O is also involved in the coordination of the most stable complex. However, the formation of mixed hydroxo species at high pH and a bis-complex in a rather slow process with $(NH_2,N_{amide})_2$ bonding mode in the presence of ligand excess was proven. Although the 3N coordination (NH_2,N_{amide}, N_{hydr}) results in a highly stable complex with the dipeptide derivative, Ala-Ala-NHOH, the fourth coordination site remains free for accepting an NH_2 moiety from excess ligand, or hydroxide ion at high pH. Likewise, the hydroxymate (O,O) set remains free to bind metal ion excess in a trinuclear species or a $[Co(4N)]^{3+}$ core in heterobimetallic complexes [6].

The $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ binding strength of the thioether ligands DL-methionine (H_2met^+) or S-methyl-Lcysteine (H_2mecys^+) was also studied by pH-potentiometry, NMR and MS in aqueous solution. Both ligands were found to form stable $[(\eta^6\text{-}p\text{-cym})RuA]^+$ complexes with [S, NH_2, COO^-] coordination of the amino acids over a wide pH-range. Comparison of the metal ion binding strength of the [O,O,O] (citrate), [O,N,O] (isoserine) and [S,N,O] (met) donor sets at pH = 7.4 revealed the exclusive formation of a [S,N,O] chelated metal complex. We have also synthesized and characterized $[(\eta^6\text{-}p\text{-cym})Ru(L)]X$, $[(\eta^6\text{-}p\text{-cym})Os(L)]Cl$ or $[(\eta^5\text{-Cp*})M(L)]Cl$ (L = mecys, DL-met, L-met; X = Cl, NO_3, CF_3SO_3; M = Rh, Ir) complexes and determined the molecular structures of the $[(\eta^6\text{-}p\text{-cym})Ru(mecys)]NO_3$ and $[(\eta^6\text{-}p\text{-cym})Ru(met)]NO_3$ by X-ray diffraction method. Both tridentate ligands form chiral-at-metal complexes in which the configuration of the metal centre is determined by the ligands containing a stereogenic centre (α_C) as well. Owing to the labile configuration of the thioether sulfurs epimerisation in both systems was detected and the ratio of the diastereomers was estimated [19].

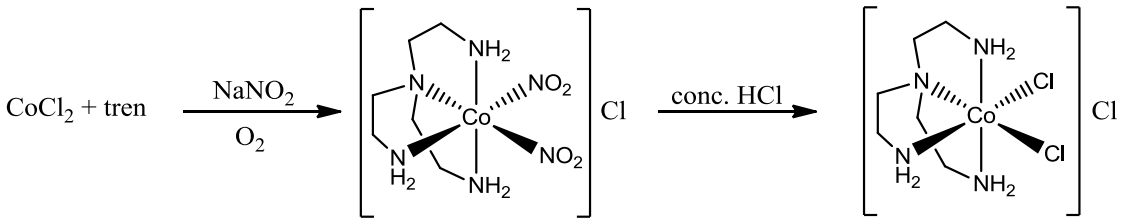
In order to model the biotransformation reactions of half-sandwich type platinum metal complexes with potential antiproliferative activity in the serum the interaction between $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ and N-methylimidazole was studied. The results indicate that up to three ligands can coordinate to the metal ion in rather slow processes resulting in mononuclear species. In the absence of chloride ions various mixed hydroxido complexes are also identified under basic conditions. Chloride was found to take the free coordination site(s) of the metal ion in the 1:1 and 1:2 species and to hinder effectively the hydrolytic processes. Our results indicate that even N-methylimidazole forms stable complexes with the metal ion under physiologically relevant conditions at 1:3 metal to ligand ratio and prevents it from complete hydrolysis at pH = 7.4 [20].

To model the $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ binding capabilities of high molecular mass components of blood the interaction between and terminally protected oligopeptides containing three histidyl moieties (Ac-HHH-NH_2, Ac-HAHH-NH_2, Ac-HAHAH-NH_2 and Ac-H*AH*AH*-NH_2, where A = L-alanyl, H = L-histidyl, H* = N^3-methyl-L-histidyl) were studied by potentiometric, MS, CD, NMR and DFT methods. Although for Ac-HHH-NH_2 the immediate formation of precipitation with $[(\eta^6\text{-}p\text{-cym})Ru(H_2O)_3]^{2+}$ hindered any further solution investigations results of the detailed NMR and MS studies revealed that the other three ligands coordinate to the metal ion in rather slow processes via the imidazole moieties forming $[(\eta^6\text{-}p\text{-cym})RuL]^{2+}$ (L = oligopeptide) type species in the slightly acidic, neutral pH-range. At pH ~7.5 identical binding mode of Ac-HAHH-NH_2 and Ac-

HAHAH-NH_2 in the $[(\eta^6\text{-}p\text{-cym})RuL]^{2+}$ via three imidazole nitrogens was found hindering completely the hydrolysis of the metal ion even at 1:1 metal ion to ligand ratio. At elevated pH MS evidences support the involvement of amide-N donor(s) in metal ion binding too beside partial hydrolysis. Both NMR and DFT results support the imidazole-N^1 over the N^3 coordination of the histidyl side chains of all these oligopeptides to the organometallic ruthenium(II) cation [8, 12].

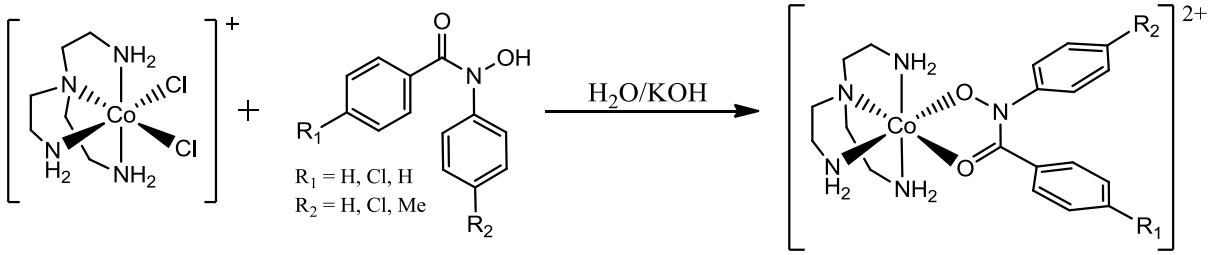
3. Synthesis and characterization of $[Co(4N)(2O)]^{n+}$ type complexes

We have synthesized tripodal [N,N,N,N] donor amines incorporating primary or pyridine based nitrogen donors, partly with the optimization of literature methods. Beside the symmetric ones (tpa; tren – commercially available) we have also explored the synthesis of asymmetric amines ($H_2NCH_2CH_2N(CH_2Py)_2$, $H_2NCH_2CH_2CH_2N(CH_2CH_2NH_2)_2$) for the systematic variation of the length and type of N donor atoms of the chelates in order to fine-tune the redox properties and inertness of the Co(III) complexes. Using the above 4N donor chelators the appropriate Co(III) precursors were also made as outlined in Scheme 1.



Scheme 1.

To check the effect on the redox properties of the cobalt complexes of the various substituents at phenyl rings various N-phenyl-benzohydroxamic acids as well as at flavonol derivatives these ligands were prepared. The synthesis of the mixed ligand compounds for the hydroxamates is outlined in Scheme 2 [11].



Scheme 2.

The novel Co(III) complexes bearing tripodal [4N] donor amines and (O,O) donor (hydroxamato, flavonolato, catecholato and quinolinato (floxacinato) type) bioligands with the potential being activated by hypoxia were characterised using NMR, IR, MS and microanalytical techniques. The crystal and molecular structures of $[Co(uns\text{-}penp)(H_2O)Cl]Cl_2\cdot H_2O$, $[Co(tren)(phebha)](ClO_4)_2$, $[Co(tpa)(bha)](ClO_4)_2\cdot C_2H_5OH\cdot H_2O$ and $[Co(tpa)(phebha)](ClO_4)_2$ have also been determined by single crystal X-ray diffraction method. To check, whether these new complexes show redox activity in a biologically relevant voltage-range, cyclic voltammetric (CV) study was performed. CV results indicated the irreversible reduction of Co(III) in all these complexes.

Out of the four studied tripodal amines, abap was found to decrease the Co(III/II) reduction potential far below the region of bioreductants. Decreasing of two of the chains by one CH_2 in tren compared to abap resulted in less negative reduction potential of the corresponding complex. Further positive shift was observed by introducing two (uns-penp), and especially three (tpa) π-back-bonding pyridyl rings into the chains of tetramines. In agreement with literature results, the 3+ oxidation state of the central cobalt ion was found to be extremely stabilized in the ternary complexes containing the doubly deprotonated benzohydroximate, but the metal ion is significantly more reducible in the ternary complexes with mono-deprotonated benzohydroxamate/derivative ligands. Measurable effect was not found on the redox potential via introduction of chloro or nitro substituents in para position into the phenyl moiety of bha^- (Cl-bha^- and NO_2-bha^-). Significant positive shift (ca. 200 mV) was obtained, however, when R_N = H was replaced by a phenyl ring in $phebha^-$ therefore complexes with this latter ligand can be likely candidates for the *in vitro* releasing of hydroxamates with proven biological activity [11].

Among the nine new mixed ligand Co(III) complexes incorporating floxacine ligands in $[Co(tren)(nor)](ClO_4)_2$, $[Co(tpa)(nal)](PF_6)_2$, $[Co(tpa)(nor)(Co(tpa)(norH)](PF_6)_3(Cl)_2 \cdot 5MeOH$ (nalH = nalidixic acid, norH = norfloxacin) the expected octahedral geometry of the complexes with one (O,O) donor floxacine and one 4N donor tren or tpa was detected by SCXD. CV studies revealed that the 4N donor ligands have much higher effect on the reduction potential of these ternary complexes than the quinolones. Due to the π-back-bonding interaction of the metal ion with the pyridyl-N's, the tpa containing compounds demonstrated lower stability and were easier to get reduced in a reversible manner. This character makes them unlikely candidates for the development of effective, highly selective hypoxia-activated pro-drug complexes, but this goal might be achieved by substitution of tpa by tren. $[Co(tren)(cip)](PF_6)_2$ (**1**) and $[Co(tpa)(cip)](PF_6)_2$ (**2**) (cipH = ciprofloxacin) showed slightly less antibacterial activity against *E. coli* than free ciprofloxacin and they found to have very low toxicity towards both selected cancer (HeLa, MCF 7, MDA-MB-239) and noncancerous (MRC5 pd30) cells. Interaction of **1** and **2** with ct DNA indicated the complexes to bind to DNA as intercalators [7].

We have also synthesized four Co(III) ternary complexes with the composition of $[(Co(4N))_2(quin)](ClO_4)_4$ or $[(Co(4N))_2(quinS)](ClO_4)_3$, where 4N = tren or tpa, $quinH_2$ = quinizarin (1,4-dihydroxy-9,10-anthraquinone), $quinSH_3$ = quinizarin-2-sulfonic acid (1,4-dihydroxy-9,10-anthraquinone-2-sulfonic acid) and their human serum albumin (HSA) binding capabilities were also tested. The complexes can be considered as likely chaperons of quinizarins which are structural models for anthracycline-based anticancer drugs like doxorubicin. All the Co(III) complexes are dinuclear and were isolated as mixture of isomers. Comparison of the cyclic voltammograms of the free ligands and the appropriate Co(III) complexes revealed that the new signals belonging to reversible processes in the range -400 – 0 mV (vs. Ag/AgCl) for the complexes can be attributed to the reversible reduction of the Co(III) centre. These potentials are in the range of typical (O,O) chelated Co(III) ternary complexes bearing 4N donor ligands and follow the order being more positive for the tpa containing complexes. Presence of the sulfonate group in the quinizarin resulted in slightly more negative reduction potential of the Co(III) complexes. HSA binding capabilities of the $quinH_2$ and $quinSH_3$ ligands as well as the appropriate complexes showed that $quinSH_3$ has higher affinity to the protein than $quinH_2$ while none of the complexes seem to bind to HSA. To gain a deeper insight into the antiproliferative activity of the quinizarine ternary complexes against human cancer cells their association with DNA in the cancer cells, DNA binding in cell-free media, and DNA cleavage

capability were investigated in detail. The results demonstrate that both complexes interact with DNA in tumor cells. However, their mechanism of antiproliferative action is different and this difference is mirrored by distinct antiproliferative activity. The cytostatic effect of the tren analogue is connected with its ability to intercalate into DNA and subsequently to inhibit activities of DNA processing enzymes. In contrast, the total antiproliferative efficiency of the tpa analogue, thanks to its redox properties, appears to be connected with its ability to form radicals and, consequently, with the ability of it to cleave DNA [2,4].

4. Synthesis, characterization and metal binding of novel ambidentate (NN)(OO) type ligands

Heterobimetallic complexes with the evolutionary, well-preserved, histidyl-alanyl-valinyl (HAV) sequence for cadherin targeting, an organometallic Ru core with anticancer activity and a radioactive moiety for imaging may hold potential as theranostic agents for cancer. To explore further this field, via visible-light irradiation of HAVAY-NH_2 pentapeptide in the presence of $[(\eta^5\text{-Cp})Ru(\eta^6\text{-naphthalene})]^+$ a full sandwich type complex, (η^6-Tyr-RuCp)-HAVAY-NH_2 was obtained in aqueous solution where the metal ion is connected to the Tyr (Y) unit of the peptide. Conjugation of this complex to 2,2′-(7-(1-carboxy-4-((4-isothiocyanatobenzyl)amino)-4-oxobutyl)-1,4,7-triazonane-1,4-diyl)diacetic acid (NODA-GA) and subsequent metalation of the resulting product with stable (^{nat}Ga) and radioactive (^{67}Ga) isotope yielded ^{nat}Ga/^{67}Ga-NODA-GA-[(η^6-Tyr-RuCp)-HAVAY-NH_2]. Cellular uptake and cytotoxicity of the radioactive and non-radioactive complexes, respectively, were evaluated in various human cancer cell lines characterized by different levels of N- or E-cadherins expression. Results indicate moderate cellular uptake of the radioactive complexes, however, the inhibition of the cell proliferation was not relevant [16].

During the project we have also developed, synthesized and characterized various novel ambidentate (NN)(OO) type ligands for the successful incorporation of the [Co(III)(4N)] and half-sandwich Ru/Rh entities to obtain heterobimetallic complexes. In the course of this project 1,10-phenantroline-, pyridyl- or pyrrolyl- and aliphatic N-based chelating (N,N) as well as hydroxamate- or maltolate-based chelating (O,O) donor atom sets were linked on the same molecule. In some cases a simple peptide backbone served as (N,N) donor part of the molecule [1].

Our results indicate that for a phen- and hydroxamate-based ligand, (phenhaH, **3)** the reaction with $[Co(4N)Cl]Cl_2$ proved the exclusive (O,O) coordination of the ligand to the $[Co(4N)]^{3+}$ core to yield $[Co(tpa)(phenha)](ClO_4)_2$, (**4**). Subsequent treatment of **3** with $[Ru(\eta^6\text{-}p\text{-cym})Cl_2]_2$ and $[Co(4N)Cl]Cl_2$ in a one-pot reaction resulted in the formation of $[(\eta^6\text{-}p\text{-cym})Ru(Cl)(phenha)Co(tren)]Cl(PF_6)_2$ (**5)** and $[(\eta^6\text{-}p\text{-cym})Ru(Cl)(phenha)Co(tpa)](PF_6)_3$ (**6)** in which the organometallic Ru core is coordinated by the phen part while the Co entity by the hydroxamate part of **1**. Cyclic voltammetry revealed that **6** can be reduced at a less negative potential and exhibits a reversible Co(III)/Co(II) redox process compared to **3** due to the π-back bonding interaction between the Co(III) centre and the pyridyl-N donors of tpa in **6**. Complexes **4–6** were tested for their *in vitro* cytotoxicity using human-derived cancer cell lines (HeLa, MCF-7, HCT116 and MDA-MB-231) and showed moderate anti-proliferative activity in the double digit micromolar concentration range **6** being the most active. **6** displayed better activity against MDA-MB-231 cells than cisplatin [1].

Personel, publications

During the whole time interval of the project 17 papers and 1 book chapter were published and 2 manuscripts submitted acknowledging the Fund. 8+32 lectures were presented in international and national conferences, respectively, and 13 posters were also shown in international conferences. Furthermore, 13 BSc, 10 MSc theses and 10 project works were also made in the framework of the research. Zsolt Bihari and Péter László Parajdi-Losonczi (supervisor: P. Buglyó) have succesfully defended their PhD theses while Máté Kozsup, Imre Nagy (supervisor: E. Farkas) and András Ozsváth (supervisor: P. Buglyó) are currently putting together their PhD theses based on the results obtained from the scientific results of this project.

Publications acknowledging the Fund in the framework of the projekt

1 I. Nagy, E. Farkas, J. Kasparkova, H. Kostrhunova, V. Brabec, P. Buglyó
Synthesis and characterization of (Ru(II), Co(III)) heterobimetallic complexes formed with a 1,10-phenanthroline based hydroxamic acid conjugate
J. Organomet. Chem., **2020**, *submitted*

2 H. Crlikova, H. Kostrhunova, J. Pracharova, M. Kozsup, S. Nagy, P. Buglyó, V. Brabec, J. Kasparkova
Antiproliferative, DNA binding and cleavage properties of dinuclear Co(III) complexes containing the bioactive quinizarin ligand
J. Biol. Inorg. Chem., **2020**, *under revision*

3 A. Ozsváth, L. Bíró, E. M. Nagy, P. Buglyó, D. Sanna, E. Farkas
Trends and exceptions in the interaction of hydroxamic acid derivatives of common di-and tripeptides with some 3d and 4d metal ions in aqueous solution
Molecules, **2019**, *24*, 3941

4 M. Kozsup, O. Dömötör, S. Nagy, E. Farkas, É. A. Enyedy, P. Buglyó
Synthesis, Characterization and Albumin Binding Capabilities of Quinizarin Containing Ternary Cobalt(III) Complexes
J. Inorg. Biochem., **2020**, *204*, 110963

5 A. Ryan, M-C. Fitzgerald, A. Ozsváth, B. Twamley, P. Buglyó, B. Murphy, D. Griffith
Ni(II), Pd(II) and Pt(II) Complexes of the Hedgehog Pathway Inhibitor, GANT61-D
Inorg. Chem., **2019**, *58*, 16075-16086

6 A. Ozsváth, E. Farkas, R. Diószegi, P. Buglyó
Versatility and trends in the interaction between Pd(II) and peptidehydroxamic acids
New J. Chem., **2019**, *43*, 8239-8249

7 M. Kozsup, E. Farkas, A. Cs. Bényei, J. Kasparkova, H. Crlikova, V. Brabec, P. Buglyó
Synthesis, characterization and biological evaluation of Co(III) complexes with quinolone drugs
J. Inorg. Biochem., **2019**, *193*, 94-105

8 B. D. Balogh, Zs. Bihari, P. Buglyó, G. Csire, Z. Kerekes, M. Lukács, I. Sóvágó, K. Várnagy
Metal binding selectivity of an N-terminally free multihistidine peptide, HAVAHHH-NH_2
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