



Synthesis and characterization of (Ru(II), Co(III)) heterobimetallic complexes formed with a 1,10-phenanthroline based hydroxamic acid conjugate

Imre Nagy^a, Etelka Farkas^{a, **}, Jana Kasparkova^{b, c}, Hana Kostrhunova^c, Viktor Brabec^{b, c}, Péter Buglyó^{a, *}

^a Department of Inorganic and Analytical Chemistry, University of Debrecen, Egyetem tér 1, 4032, Debrecen, Hungary

^b Department of Biophysics, Palacky University, Slechtitelu 27, 78371, Olomouc, Czech Republic

^c Czech Academy of Sciences, Institute of Biophysics, Kralovopolska 135, CZ-61265 Brno, Czech Republic

ARTICLE INFO

Article history:

Received 30 January 2020

Received in revised form

25 March 2020

Accepted 28 March 2020

Available online 2 April 2020

Keywords:

SAHA analogue

Hydroxamate

Bimetallic

Anticancer

Enzyme inhibition

Cobalt

Ruthenium

ABSTRACT

A novel ambidentate type hydroxamic acid derivative (phenhaH, **1**) containing an (O,O) and a 1,10-phenanthroline (phen) based (N,N) donoratom set together with its heterobimetallic complexes incorporating an octahedral [Co(4 N)]³⁺ (4 N = tris(2-aminoethyl)amine (tren) or tris(2-methylpyridyl)amine (tpa)) and a half-sandwich type [(η⁶-p-cym)Ru]²⁺ entity have been synthesized and characterized using various analytical techniques. Reaction of [Co(4 N)Cl]₂ with **1** proved the exclusive (O,O) coordination of the ligand to the [Co(4 N)]³⁺ core to yield [Co(tpa)(phenha)](ClO₄)₂ (**2**). Subsequent treatment of **1** with [Ru(η⁶-p-cym)Cl]₂ and [Co(4 N)Cl]₂ in a one-pot reaction resulted in the formation of [(η⁶-p-cym)Ru(Cl)(phenha)Co(tren)]Cl(PF₆)₂ (**3**) and [(η⁶-p-cym)Ru(Cl)(phenha)Co(tpa)](PF₆)₃ (**4**) 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 **4** could 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 **4**. Complexes **2–4** 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, **4** being the most active. Complex **4** displayed better activity against MDA-MB-231 cells than cisplatin.

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Interest in designing molecules capable of binding metal ions at the heart of metalloenzymes represents an active area in drug discovery nowadays. Within this subject, during the past ca. two decades, numerous histone deacetylase (HDAC) enzyme inhibitors have received much attention as anticancer drug candidates [1–3]. Among them, a large number of hydroxamate-based compounds have been developed and tested [4] and suberoylanilide hydroxamic acid (SAHA, see Fig. 1) was the first FDA-approved drug for clinical use against cutaneous T-cell lymphoma in 2006 [4,5]. Fig. 1

also shows the function of the different parts of the molecule.

The mechanism of action of SAHA has not been clarified in the full details yet, but as it is shown in Fig. 1, SAHA can bind directly to the Zn(II) ion in the active site of the HDAC enzyme through O,O-hydroxamate chelation. The aliphatic linker chain is thought to fit into the protein's narrow channel, while the phenyl ring is a recognition domain [4,5]. It is also known, however, that several sub-families belong to the HDAC protein family [6]. Therefore, the requirement for specific and selective inhibition of HDACs to achieve therapeutic efficacy remains topics of debate, and considerable efforts are ongoing nowadays to develop new HDAC inhibitors with the capability of selectively targeting sub-family or specific enzymes.

Numerous examples illustrate that combining two or more entities with some desired functionality into one drug molecule offers advantages over the single agents. This is illustrated, e.g. by a very recent innovative trial based on a DNA-targeted metallo-prodrug

* Corresponding author.

** Corresponding author.

E-mail addresses: efarkas@science.unideb.hu (E. Farkas), buglyo@science.unideb.hu (P. Buglyó).

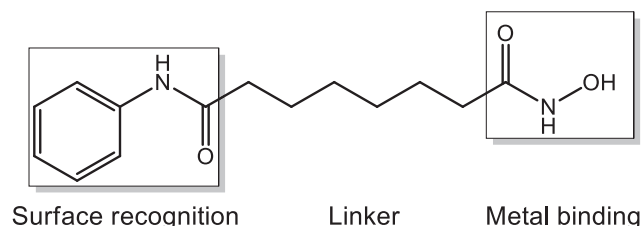


Fig. 1. Formula of SAHA showing the different parts of the molecule with their function.

strategy, in which HDAC inhibition with oxidative stress has been combined and promising results have been collected. In the synthesized prodrug complexes, two of the four equatorial coordination sites of the Cu(II) ion are occupied by the hydroxamate-O,O chelate of the HDAC inhibitor SAHA, while the other two by a phenanthrene-type N,N-binding ligand as DNA intercalative component [7]. Within this subject, the development of various new metallated SAHA-based drug candidates has also received a significant interest during the past few years [8–14]. As it is known, numerous characters of organic drug molecules can be modified via the incorporation of a metal ion into them. There are examples, where metal ions act as key structural scaffolds supporting this way e.g. adequate orientation of the organic molecules [11], but on the other hand, the metal ions on their own right can also show cytotoxic effect like, for example, the platinum-based conjugates prepared as dual type drug candidates (containing two biofunctional moieties) [9]. Developing drugs that incorporate metals other than platinum has also been actively investigated and over the past few years, numerous HDAC inhibitors have been synthesized in which the phenyl head group has been functionalized with a metal complex. Among others, potential inhibitor molecules incorporating rhenium [11,12], ruthenium [13,14] or rhodium [14] have been synthesized and characterized. In some cases, improved cytotoxicity relative to SAHA was observed with these functionalized derivatives. Comparable activity to the clinically approved inhibitor SAHA has been observed with the recently developed Ru(II) and Rh(III) piano stool complexes of phen-derivatized SAHA, but it is an advantage that the 3-dimensional structure of the metal complex can be easily modified, what might lead to development of drug candidates with increased effectivity and selectivity [14].

The complexes developed in this cited work [14] leave the hydroxamic moiety of the SAHA derivative free for coordination to the Zn(II) at the active site of HDACs. However, as it is well-known from many previous results, a hydroxamate function is an effective chelator moiety towards numerous metal ions and prefers some of them more or even much more over Zn(II) [15,16]. Consequently, metal complexation, what might occur during transportation of the above cited cytotoxic complexes to the target, decreases their effectivity and selectivity and can cause side effects. To overcome the problem, transportation of a drug molecule to the site of action in an inactive form and activation of it only at the site is one of the known approaches [17,18]. If the drug molecule is a potential chelating ligand, like the SAHA or its derivative, inactivation may be achieved via its chelation to the metal centre of a kinetically inert pro-drug complex. The very large difference in kinetic lability between the complexes of cobalt(III) and cobalt(II) (the former complexes are very inert, the latter are fairly labile ones) makes this metal suitable to construct hypoxia selective pro-drug complexes [18]. Moreover, as it was observed in previous work the Co(II) ions, which can be formed upon reduction of Co(III) at the hypoxic target, caused mitochondrial DNA damage in rat neuronal PC12 cells. This means that the Co(II) ions themselves may also have a cytotoxic effect

on their own right [19].

Consequently, it might be possible that greater target specificity is achieved if Co(III)-SAHA/derivative complex is used instead of the pure organic drug molecule or its metallated derivative, in which the hydroxamate function is left free during the transportation. However, according to the best of our knowledge, any previous result in this subject has not been published. For this reason, based on our knowledge and skills in synthesis and characterization of metal complexes of hydroxamate-based ligands [20–22], one new phen-derivatized SAHA analogue compound, incorporating a somewhat shorter aliphatic chain compared to the parent molecule SAHA (Fig. 2. Structure 1) has been designed and prepared. Using this ligand the monometallic ternary Co(III) complex (Structure 2) and two bis-metallic Co(III)-Ru(II) complexes (Structures 3 and 4) were also synthesized in the present study.

In the synthesized complexes a tripodal 4N-donor ligand, either tris(2-methylpyridyl)amine (tpa) (complexes 2 and 4) or tris(2-aminoethyl)amine (tren) (complex 3) occupies four coordination sites and stabilizes +3 oxidation state of the central Co ion, while the coordination sphere is completed via the hydroxamate oxygens of the phen-conjugated SAHA derivative (complex 2) or its Ru(II) piano stool complex (complexes 3 and 4).

Results obtained during the chemical characterization and in preliminary biological tests of these new complexes are summarized in the present paper.

2. Experimental

2.1. Materials and reagents

Methyl-4-(chloroformyl)butyrate, 1,10-phenanthroline, 5-amino-1,10-phenanthroline, DMAP, $\text{NH}_2\text{OH}\cdot\text{HCl}$, NH_4PF_6 , NaClO_4 and various solvents were commercial products from Toronto Research Chemicals, Merck, SigmaAldrich, VWR, Reanal and used as received. $[\text{Ru}(\eta^6\text{-p-cym})\text{Cl}_2]_2$, $[\text{Co}(\text{tren})\text{Cl}_2]\text{Cl}$, $[\text{Co}(\text{tpa})\text{Cl}_2]\text{Cl}$ were synthesized and purified according to literature procedures [20,21]. Cisplatin was obtained from Sigma-Aldrich (Prague, Czech Republic).

2.2. NMR, IR and ESI-MS measurements

The NMR measurements were carried out using a Bruker DRX 400 NMR spectrometer at room temperature on samples prepared in $\text{DMSO-}d_6$. Calibration was performed using the signals of the solvents: 2.50 ppm for DMSO and 4.79 ppm for D_2O . IR spectra as KBr pellets were recorded on PerkinElmer FTIR Paragon 1000 PC instrument at the Department of Organic Chemistry, University of Debrecen. ESI-TOF MS measurements in the positive mode were carried out on Bruker micrOTOF-Q type Qq-TOF-MS instrument, Bruker Daltonik, Bremen Germany, at the Department of Inorganic and Analytical Chemistry, University of Debrecen. The concentration of the samples was 10 $\mu\text{g/mL}$, and the solvent was methanol. The instrument was equipped with an electrospray ion source, where the voltage was 4 kV. The drying gas was N_2 . The flow rate was 4.0 mL/min and the drying temperature was 200 $^\circ\text{C}$. The spectra were recorded by means of a digitizer at a sampling rate of 2 GHz. The mass spectra were calibrated externally, using the exact masses of clusters $[(\text{HCOONa})_n + \text{Na}]^+$ generated from the electrosprayed solution of sodium-formate (HCOONa). The spectra were evaluated with DataAnalysis 4.4 software from Bruker. The samples were introduced directly into the ESI with a syringe pump.

2.3. Synthesis of the ligand and its complexes

Methyl-5-((1,10-phenanthroline-5-yl)amino)-5-oxopentanoate.

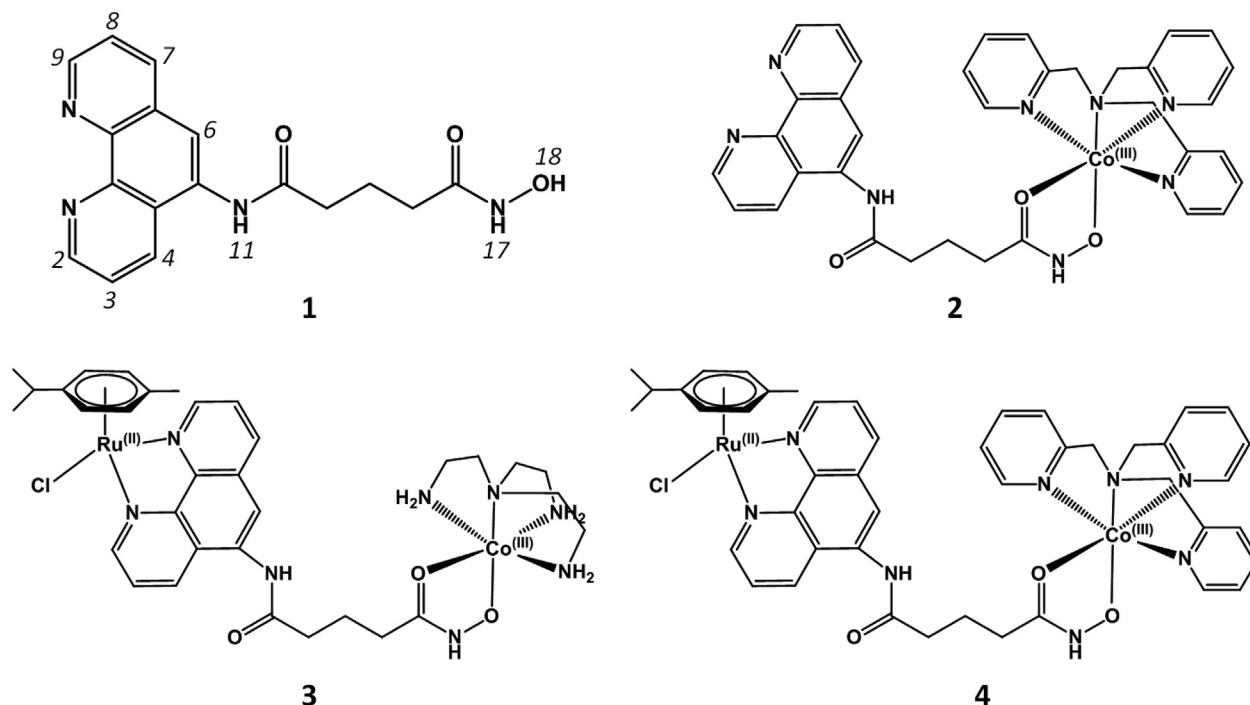


Fig. 2. Structure of the novel phenanthroline-based hydroxamic acid conjugate, phenhaH (1), its monometallic ternary complex with $[\text{Co}(\text{tpa})]^{3+}$ (2) and its bis-metallic Ru(II)/Co(III) complexes, in which the 4N-donor ligand is either tren (3) or tpa (4).

The compound was prepared by the slight modification of the appropriate literature method [23]. 1.93 g (10.0 μmol) of 5-amino-1,10-phenanthroline and 122 mg (100 μmol) of DMAP were dissolved in 70 mL dry DMF under nitrogen atmosphere at room temperature. The mixture of 1.52 mL (10.0 μmol) of methyl-4-(chloroformyl)butyrate, and 30 mL dry DMF was added dropwise to the reaction in 1 h. The mixture was stirred for 8 h and then evaporated to 10 mL. 100 mL dry Et_2O was added to the mixture and cooling at 4 $^\circ\text{C}$ overnight afforded a beige precipitate. The solid was filtered and dried in vacuo. Yield: 3.233 g, 99%. ^1H NMR: (400 MHz, D_2O): δ/ppm = 9.20 (d, 1H, Ar-H); 9.13 (d, 1H, Ar-H); 8.92 (d, 1H, Ar-H); 8.75 (d, 1H, Ar-H); 8.19 (s, 1H, Ar-H); 8.16 (m, 1H, Ar-H); 8.09 (m, 1H, Ar-H); 3.74 (s, 3H, CH_3); 2.75 (t, 2H, CH_2); 2.61 (t, 2H, CH_2); 2.14 (m, 2H, CH_2).

N^{17} -Hydroxy- N^{11} -(1,10-phenanthrolin-5-yl)pentanediamide, phenhaH (1).

1.25 g (3.84 mmol) of methyl-5-((1,10-phenanthrolin-5-yl)amino)-5-oxopentanoate was dissolved in dry MeOH (10 mL) under nitrogen, and chilled in an ice-bath to 0 $^\circ\text{C}$. The pH of the mixture was changed to 9 with 0.3 M KOH/MeOH solution. 0.483 g (6.91 mmol) of $\text{NH}_2\text{OH}\cdot\text{HCl}$, and 0.542 g (9.63 mmol) of KOH were dissolved in 5 mL MeOH each in separated flasks and chilled to 0 $^\circ\text{C}$. The solution of $\text{NH}_2\text{OH}\cdot\text{HCl}$ and KOH were mixed and filtered into the flask of ester. The mixture was stirred for 12 h. The solution was neutralized with 1 M HCl. The resulting beige solid was filtered and dried in vacuo. Yield: 60.0 mg (48%). ^1H NMR: (400 MHz, D_2O): δ/ppm = 9.07 (d, 1H, Ar-H); 9.00 (d, 1H, Ar-H); 8.72 (d, 1H, Ar-H); 8.59 (d, 1H, Ar-H); 8.03–7.93 (m, 3H, Ar-H); 2.64 (t, 2H, CH_2); 2.30 (t, 2H, CH_2); 2.04 (t, 2H, CH_2). ^{13}C NMR: ($\text{DMSO}-d_6$) δ/ppm = 172.3 (C^{12}); 165.4 (C^{16}); 149.0 (C^2); 146.0 (C^9); 141.0 (C^4); 140.0 (C^1); 135.1 (C^5); 133.3 (C^{10}); 129.2 (C^6); 125.1 (C^4); 125.0 (C^7); 118.4 (C^8); 106.9 (C^3); 99.3 (C^6); 35.1 (C^{13}); 33.0 (C^{15}); 20.5 (C^{14}). HR MS (ESI, positive ion): m/z : 325.1267 ($\text{C}_{17}\text{H}_{17}\text{N}_4\text{O}_3$: 325.1295; $[\text{phenhaH} + \text{H}]^+$), 347.1086 ($\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}_3\text{Na}$: 347.1115; $[\text{phenhaH} + \text{Na}]^+$), 363.0826 ($\text{C}_{17}\text{H}_{16}\text{N}_4\text{O}_3\text{K}$: 363.0854; $[\text{phenhaH} + \text{K}]^+$), 369.2374

($\text{C}_{17}\text{H}_{15}\text{N}_4\text{O}_3\text{Na}_2$: 369.0934; $[\text{phenha} + 2\text{Na}]^+$).

$[\text{Co}(\text{tpa})(\text{phenha})](\text{ClO}_4)_2$ (2).

110 mg (339 μmol) of phenhaH and 167 mg (339 μmol) of $[\text{Co}(\text{tpa})\text{Cl}_2]\text{Cl}\cdot\text{H}_2\text{O}$ were dissolved in 20 mL MeOH:H $_2\text{O}$ ratio 1:1. 13.6 mg (339 μmol) of NaOH was also dissolved in MeOH:H $_2\text{O}$ and the NaOH solution was added to the mixture. The purple solution was stirred at 60 $^\circ\text{C}$ for 3 h. After it cooled to room temperature 95.0 mg (678 μmol) NaClO_4 was added. The volume of the solution was reduced to 5 mL. On standing at room temperature purple crystals were obtained. Yield: 210 mg (63%) ^1H NMR (400 MHz, DMSO): 13.20 (s, 1H, NH); 9.17 (m, 3H, Ar-H); 8.59 (m, 4H, Ar-H); 8.15 (m, 4H, Ar-H); 7.80 (m, 4H, Ar-H); 7.71 (m, 4H, Ar-H); 7.40 (m, 1H, Ar-H); 5.64 (m, 3H, CH_2); 5.22 (m, 3H, CH_2); 2.22 (t, 2H, CH_2). IR (KBr)/ cm^{-1} : 3445.21, 3079.76, 2933.20, 1610.27, 1094.40, 771.39, 622.90. Anal. required for $\text{C}_{35}\text{H}_{33}\text{Cl}_2\text{CoN}_8\text{O}_{11}\cdot 0.5\text{CH}_3\text{OH}\cdot 1.5\text{H}_2\text{O}\cdot \text{NaCl}$: C, 43.82, H, 3.94, N, 11.52%. Found: C, 43.76, H, 3.80, N, 11.36%. MS (ESI, positive ion): m/z : 671.192 ($\text{C}_{35}\text{H}_{33}\text{CoN}_8\text{O}_3$: 671.190; $[\text{Co}(\text{tpa})(\text{phenha})-\text{H}]^+$), 771.148 ($\text{C}_{35}\text{H}_{33}\text{CoN}_8\text{O}_3\text{ClO}_4$: 771.163; $[\text{Co}(\text{tpa})(\text{phenha})(\text{ClO}_4)]^+$).

$[(\eta^6\text{-p-cym})\text{Ru}(\text{Cl})(\text{phenha})\text{Co}(\text{tren})]\text{Cl}(\text{PF}_6)_2$ (3).

100 mg (163 μmol) of $[\text{Ru}(\eta^6\text{-p-cym})\text{Cl}_2]_2$ and 106 mg (327 μmol) phenhaH were dissolved in 5 mL MeOH and stirred for 2 h at room temperature. To this solution 1 mL aqueous solution of 108 mg (327 μmol) of $[\text{Co}(\text{tren})\text{Cl}_2]\text{Cl}$ and 5 mL MeOH solution of 18.0 mg (327 μmol) KOH were added. The mixture was stirred at 60 $^\circ\text{C}$ for 4 h. After cooled down 5 mL MeOH solution of 160 mg (981 μmol) of NH_4PF_6 were added. Slow evaporation of the mixture gave a green solid. The solid was filtered off, washed with water and recrystallized from MeOH:acetonitrile 1:1. The green solid appeared was filtered off and dried in vacuo. Yield: 220 mg (55%). ^1H NMR (400 MHz, DMSO): 11.96 (d, 1H, NH); 10.60 (m, 1H, NH); 9.96 (d, 1H, Ar-H); 9.83 (d, 1H, Ar-H); 9.11 (m, 1H, Ar-H); 8.86 (d, 1H, Ar-H); 8.51 (d, 1H, Ar-H); 8.21 (t, 1H, Ar-H); 8.11 (t, 1H, Ar-H); 6.34 (d, 2H, Ar-H); 6.11 (d, 2H, Ar-H); 5.40 (m, 2H, CH_2); 4.88 (m, 3H, CH_2); 3.62 (m, 1H, CH_2); 3.16 (m, 3H, CH_2); 2.99 (m, 4H, CH_2);

2.68 (m, 6H, -CH₂); 2.32 (m, 2H, -CH₂); 2.17 (s, 3H, -CH₃); 1.94 (m, 3H, -CH₂); 0.91 (m, 7H, -CH, -CH₃). IR (KBr)/cm⁻¹: 3407.6, 3233.07, 1631.48, 1533.13, 1159.01, 842.74, 558.29. Anal. required for C₃₃H₄₇CoN₈O₃RuCl₂P₂F₁₂·4H₂O: C, 33.12, H, 4.63, N, 9.36%. Found: C, 33.16, H, 4.22, N, 9.37%. MS (ESI, positive ion): *m/z*: 399.087 (C₃₃H₄₆CoN₈O₃RuCl: 399.088; [(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tren)-H]²⁺), 943.130 (C₃₃H₄₆CoN₈O₃RuClPF₆: 943.140; [(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tren)(PF₆)-H]⁺), 1089.111 (C₃₃H₄₇CoN₈O₃RuClP₂F₁₂: 1089.112; [(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tren)(PF₆)₂]⁺).

[(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tpa)](PF₆)₃ (**4**).

129 mg (211 μmol) of [Ru(η⁶-*p*-cym)Cl₂]₂ and 137 mg (422 μmol) phenhaH were dissolved in 10 mL MeOH and stirred for 2 h at room temperature. To this solution 208 mg (422 μmol) of [Co(tpa)Cl₂]Cl and 5 mL MeOH solution of 24.0 mg (422 μmol) KOH were added. The mixture was stirred at 60 °C for 4 h. After cooled down 5 mL MeOH solution of 206 mg (1.27 mmol) of NH₄PF₆ was added. The solid was filtered and recrystallized from MeOH:acetonitrile 1:1. Slow evaporation of the mixture gave a green solid that was filtered off and dried in vacuo. Yield: 305 mg (52%). 12.86 (s, 1H, NH); 10.55–10.31 (m, 1H, NH); 9.96 (m, 1H, Ar-H); 9.83 (m, 1H, Ar-H); 9.17 (m, 1H, Ar-H); 8.90–8.61 (m, 2H, Ar-H); 8.61–8.40 (m, 2H, Ar-H); 8.33 (s, 1H, Ar-H); 8.12 (m, 4H, Ar-H); 7.98 (q, 1H, Ar-H); 7.78 (t, 2H, Ar-H); 7.69 (m, 2H, Ar-H); 7.61 (m, 1H, Ar-H); 7.40 (m, 1H, Ar-H); 6.34 (m, 2H, Ar-H); 6.10 (m, 2H, Ar-H); 5.59–4.75 (m, 6H, Ar-H); 2.70 (m, 2H, -CH₂); 2.61 (m, 1H, -CH₂); 2.33 (m, 1H, -CH₂); 2.17 (m, 3H, -CH₃); 1.94 (m, 1H, -CH₂); 1.55 (m, 1H, -CH₂); 0.91 (m, 7H, -CH, -CH₃). IR (KBr)/cm⁻¹: 3444.24, 1639.20, 1464.67, 1315.21, 846.60, 558.29. Anal. required for C₄₅H₄₇ClCoN₈O₃RuP₃F₁₈·2H₂O: C, 38.22, H, 3.63, N, 7.92%. Found: C, 38.40, H, 3.70, N, 7.97%. MS (ESI, positive ion): *m/z*: 471.090 (C₄₅H₄₆ClCoN₈O₃Ru: 471.088; [(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tpa)-H]²⁺), 1087.140 (C₄₅H₄₆ClCoN₈O₃RuPF₆: 1087.140; [(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tpa)(PF₆)-H]⁺), 1233.112 (C₄₅H₄₇ClCoN₈O₃RuP₂F₁₂: 1233.112; [(η⁶-*p*-cym)Ru(Cl)(phenha)Co(tpa)(PF₆)₂]⁺).

2.4. Cyclic voltammetric studies

Cyclic voltammetric experiments were performed within the voltage range 200 to -1400 mV, at room temperature in a H₂O:MeOH 1:1 mixture, using a BASi Epsilon Eclipse equipped with a three-electrode system, which consists of an Ag/AgCl/3 M KNO₃ reference electrode (*E*_{1/2} = 209 mV vs. NHE), a platinum wire auxiliary electrode (ALS Co. Japan), and a glassy carbon (CHI104) working electrode. The samples were measured after degassing with argon. The concentration of the complexes was 1 mM, the sweep rates were varied within the range 10–500 mV/s during the determination of the redox potentials. In all cases, KNO₃ (0.20 M) was used as a supporting electrolyte. The aqueous solution of K₃[Fe(CN)₆] was used to calibrate the system, as published in an earlier paper [21].

2.5. Antiproliferative activity testing

Commercial reagent grade chemicals, solvents, and reagents for the preparation of solutions for biological work were used as received from commercial suppliers without further purification. Complexes **2**, **3**, and **4** were freshly dissolved in water at the concentration of 3 mM. The stock solution of cisplatin was freshly made 2 mM in water. For the experiments, the stock solutions were diluted by the cell culture media to the respective concentrations. The concentrations of Ru, Co or Pt in the stock solutions, as well as in the culture media, were verified by ICP-MS (Agilent, Japan).

The human cervical carcinoma HeLa cells, human breast cancer MCF-7 cells, and human colorectal carcinoma cells HCT116 were kindly supplied by Professor B. Keppler, University of Vienna

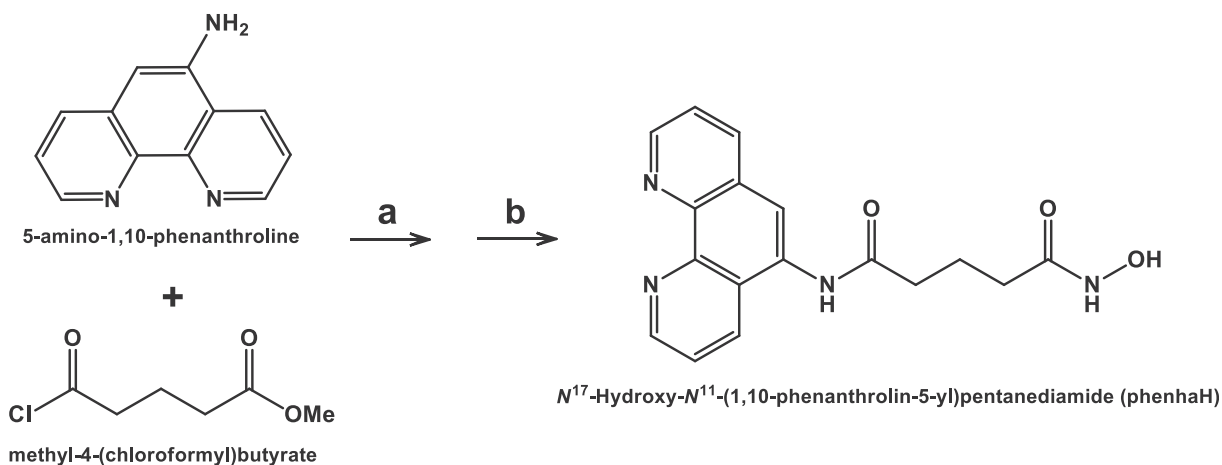
(Austria). Highly invasive breast carcinoma MDA-MB-231 cells were purchased from the European collection of authenticated cell cultures (ECACC) (Salisbury, UK). The cells were grown in DMEM medium (high glucose 4.5 g/L, PAA, Pasching, Austria) supplemented with gentamycin (50 μg/mL, Serva) and 10% heat inactivated fetal bovine serum (PAA). The media for MDA-MB-231 were also supplemented with 1% non-essential amino acids (Sigma-Aldrich, Prague, Czech Republic). The cells were cultured in a humidified incubator at 37 °C in a 5% CO₂ atmosphere and subcultured 2–3 times a week with an appropriate plating density. Antiproliferative activity of complexes **2–4** and, for comparative purposes, also cisplatin was assayed by commonly used MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test as already described in detail [10,22]. Briefly, the cells were seeded in 96-well tissue culture plates at a density of 3000 (MCF-7) or 5000 (HeLa, MDA-MB-23, HCT116) cells/well overnight. Then, the cells were treated with various concentrations of the compounds tested in this work diluted in DMEM medium. After 72 h of treatment, the MTT solution was added to the cells, and the plates were incubated for additional 4 h. The medium was subsequently removed, and the formazan crystals produced by the cell metabolism were dissolved in DMSO. The cell viability was evaluated by measurements of the absorbance at 570 nm (reference 620 nm), using an Absorbance Reader (SUNRISE TECAN, SCHOELLER). The reading values were converted to the percentage of control (% cell survival), and the IC₅₀ values were calculated from the curves constructed by plotting the cell survival (%) versus drug concentration (μM) (IC₅₀ = concentration of the agent inhibiting cell proliferation by 50%).

3. Results and discussion

3.1. Synthesis and characterization of ligand **1** and complex **2**

The synthesis of the novel ligand is outlined in Scheme 1. Compared to the previous result [23] in the new compound the chain length was shortened, and the formation of the hydroxamic acid group was also modified (cf. chapter 2.3 of Experimental in this paper and the corresponding part in ref. 23). In the present work, methyl-4-(chlorophormyl)butyrate was reacted with 5-amino-phenanthroline in dry DMF and the obtained ester was further converted to the target hydroxamic acid using hydroxyl-amine in methanol. The ligand was obtained as a beige solid. Both the NMR and the HR-MS spectra indicated the identity and purity of the ligand shown in Fig. 2, Structure 1.

As the novel ligand with ambidentate chelating character is capable of binding in principle both metal cores at both ends of the molecule, we have tested the donor atom preference of the [Co(4 N)]³⁺ and the [(η⁶-*p*-cym)Ru(H₂O)₃]²⁺ units by reacting them separately with **1** in 1:1 metal ion to ligand ratio. It was found that if the [Co(tpa)]³⁺ was tried to be reacted with **1** in the absence of any base, no reaction occurred. By adding base to the system, a purple coloured solid **2** could be isolated upon cooling in the presence of ClO₄⁻ ions. Although the NMR spectrum of the Co complex (**2**) did not provide unambiguous proof for the coordination site of the metal core because of the significant overlapping of the phen moiety and tpa signals in the aromatic region both MS and CHN data clearly supported the [Co(tpa)(phenha)](ClO₄)₂ stoichiometry of **2**. To obtain information on the coordination preference of the [Co(4 N)]³⁺ core instead of **1** its simple N,N model, 1,10-phenanthroline (phen) was also reacted with [Co(tren)Cl₂]Cl but no reaction was found in MeOH at 60 °C either in the absence or in the presence of base. This indirectly but clearly provides support for the coordination of the [Co(4 N)]³⁺ to the hydroxamate unit in **2**. The lack of coordination detected with the model phen is



Scheme 1. Synthetic route for phenhaH, **a** dry DMF/DMAP **b** MeOH/ $\text{NH}_2\text{OH} \cdot \text{HCl}$ /KOH.

originated most probably due to the significant sterical hindrance occurring between the $[\text{Co}(4\text{ N})]^{3+}$ unit and the phen ligand or the phen part of the **1**. Recently various stable and inert complexes with the $[\text{Co}(4\text{ N})(\text{HA})]^{2+}$ general composition (HA = monohydroxamate) with the O,O coordination of the hydroxamate were reported in the literature [21].

Regarding the $[(\eta^6\text{-}p\text{-cym})\text{Ru}]^{2+}$ core, some previous results discuss the conditions, under which the coordination either to a phen-moiety [14] or a hydroxamate/hydroxymate function can occur [20]. In the present study, when the dimeric complex $[\text{Ru}(\eta^6\text{-}p\text{-cym})\text{Cl}_2]_2$ was reacted in methanol with **1** in a 1:2 ratio on standing a small amount of burgundy coloured solid was isolated. The overlay of the NMR spectra of this isolated complex and that of the free ligand (Fig. 3) indicated changes at the signals of the phen moiety, but not in the aliphatic region supporting the N,N coordination of ligand **1** to the Ru containing core. As Fig. 3 shows, the highest difference (ca. 0.8 ppm) is observed for the shifts of the

corresponding H2 and H9 hydrogens of **1**, located nearest to the coordinating N donors, in the free and the coordinating ligand. Due to the electron withdrawing effect of the half-sandwich cation, the higher chemical shifts belong to the hydrogens situating at the aromatic part of the coordinating phenhaH ligand. This finding on the donor atom preference of the $[(\eta^6\text{-}p\text{-cym})\text{Ru}]^{2+}$ core towards **1** is in agreement with the previous results with N^{20} -hydroxy- N^{11} -(1,10-phenanthrolin-5-yl)octanediamide [14].

3.2. Synthesis and characterization of the heterobimetallic complexes, **3** and **4**

For the synthesis of the complexes **3** and **4** one-pot reactions have been carried out during which first $[(\eta^6\text{-}p\text{-cym})\text{RuCl}_2]_2$ and ligand **1** were reacted then, without isolation of the Ru-complex, the $[\text{Co}(4\text{ N})]^{3+}$ unit was added and upon treatment with base, the desired heterobimetallic complexes containing tren (**3**) or tpa

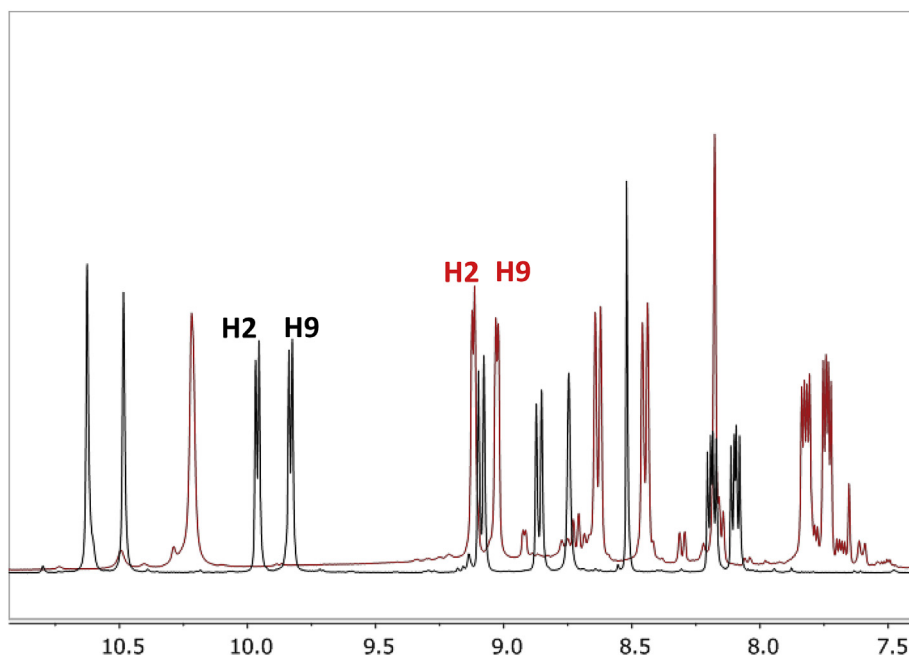


Fig. 3. Overlay of the ^1H NMR spectra registered in the range 11.5–7.0 ppm for the ligand **1**, phenhaH (red), and for $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (black) isolated from the reaction of $[\text{Ru}(\eta^6\text{-}p\text{-cym})\text{Cl}_2]_2$ and **1**. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

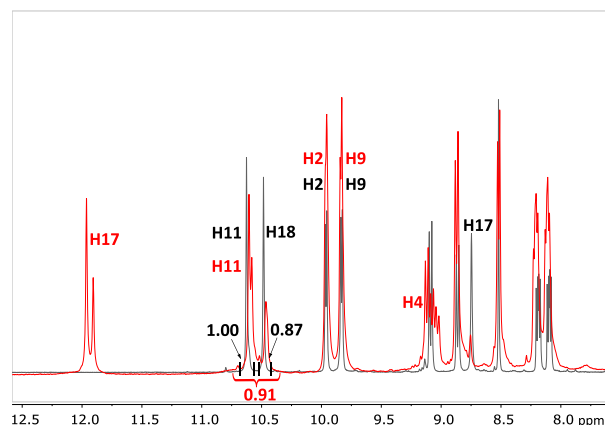


Fig. 4. Overlay of the ^1H NMR spectra in the range 7.5–12.5 ppm for $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (grey) and $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenha})\text{Co}(\text{tren})]\text{Cl}(\text{PF}_6)_2$ (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

(4) were isolated in the form of the PF_6^- salts as described in Experimental section. The identity and purity of the novel complexes were checked using various analytical techniques. Among them CHN and ESI-MS in the positive mode provided clear proof for the purity and composition of complexes. In particular, in the MS spectra, the appropriate fragment ions containing both of the metal ions and in some cases other smaller fragments could be identified. Interestingly, in agreement with earlier results on $[\text{Co}(\text{4 N})(\text{HA})]^{2+}$ (HA = monohydroxamate) type complexes, under MS conditions, extra deprotonation of **1** can be detected with the most likely proton loss of the hydroxamate unit [21].

The complexity of the NMR spectra of these complexes hindered the complete assignment of all signals. The reason for the presence of a large amount of resonances in the spectra is the formation of different isomers for **3** and **4**. If the 4 N donor tripodal amines (tren, tpa) coordinate to the octahedral Co(III), hydrogens on one arm being in the symmetry plane become magnetically different from the others at the two other arms resulting in the doubling of the NMR signals. Coordination of an asymmetric hydroxamate function to the remaining two sites of this Co(III) core yields two geometrical isomers thus in principle already four signals in the NMR spectra can be assumed. If the half-sandwich Ru(II) unit also binds to the (N,N) donor set of the phen moiety, due to asymmetry of the N,N donor ligand the half-sandwich Ru(II) also becomes a chirality centre resulting in the theoretical formation of two enantiomers.

Despite the above issues in order to shed light on the chelating environment of the two metal ions in the heterobimetallic complexes **3** and **4**, Fig. 4 displays the overlay of the aromatic region of the NMR spectra of $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (already shown in Fig. 3) with that of **3** since (unlike tpa) the presence of the tren ligand in **3** does not complicate the aromatic region of the spectrum. As Fig. 4 reveals the signals of the H2 and H9 protons located next to the N,N donors of the phen ring (see the red spectrum in Fig. 4.) remained unchanged in **3** compared to $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (with N,N coordination of the Ru core, grey spectrum) indicating identical binding mode for this metal in the two complexes. Furthermore, while for $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ two resonances with an integral of one each can be seen in the range 10.7–10.4 ppm most likely belonging to H11 and H18 [14], for **3** in this ppm range the total integral of all the signals very close to one. This strongly suggests the disappearance of the signal of H18 due to deprotonation of the hydroxamate function upon coordination to the $[\text{Co}(\text{4 N})]^{3+}$ core and the formation of various isomers as detailed above causing the appearance of more

signals for H11. Interestingly, the signal of H4 situated in the vicinity of H11 also displays several resonances in the spectrum of complex **3** but not in $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$.

To support the above assumption i.e. the disappearance of the H18 (hydroxamic OH) signal of **3** upon coordination to Co(III) the COSY spectra of $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ and **3** were also acquired. Overlay of the aromatic region of these spectra of $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (grey) and $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenha})\text{Co}(\text{tren})]\text{Cl}(\text{PF}_6)_2$, **3**, (red) in Fig. 5 clearly reveals that a cross-peak between H17 and H18 for $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (grey) can be seen indicating intact hydroxamic function, furthermore, no coupling is observable for H11 at 10.63 ppm. On the contrary, for **3** (see the red plots in Fig. 5.), the above cross-peak disappeared, indicating the proton loss of the hydroxamic function due to the coordination to the Co(III) core. Furthermore, a new signal can also be seen in the spectrum of **3** (red) at 11.91 ppm without any cross-peak, most likely belonging to H17, the NH proton of the coordinating hydroxamate function. The H11 proton that gives a signal without cross-peak at 10.63 ppm for $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ shows an upfield shift and two resonances in **3** at 10.61 and 10.46 ppm (red spectrum, in Fig. 5.) with a total integral of 0.91 due to the presence of isomers for the dimetallic complex.

Although similar metal ion binding preference of **3** and **4** can be expected for **4** the presence of the tpa signals in the aromatic region hindered a similar comparison of the NMR spectra as for **3** (*vide supra*). For **4**, therefore, a comparative ESI-TOF-MS study was carried out where **4** and a sample in which to **2** $[\text{Ru}(\eta^6\text{-}p\text{-cym})\text{Cl}_2]_2$ in methanol was added and after 2 h of stirring the solutions were analysed. The preparation of the latter solution is expected to result in the formation of **4** too, but with the reversed order of adding the metal ions i.e. first the $[\text{Co}(\text{tpa})]^{3+}$, then the $[(\eta^6\text{-}p\text{-cym})\text{Ru}]^{2+}$ core was used. The MS results in Fig. 6 clearly reveal the presence of different species in which the $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenha})\text{Co}(\text{tpa})]^{3+}$ cation can be detected with various amount of PF_6^- (for **4**) or ClO_4^- (for the other) counter ions in both cases with the appropriate m/z values. These results indicate that the order of adding the two metal ion containing units does not influence the binding architecture of the heterobimetallic complexes.

In order to explore the redox properties of the ligand and the novel complexes, CV measurements were also carried out. Out of the two chelating, phen and hydroxamate, functions in our novel ligand **1**, some results have been collected for the redox behaviour of the latter one in our previous work. These previous results did not show any redox activity of monohydroxamate functions in the investigated voltage range (the same range and conditions were also used in the present work) [21]. Electrochemical behaviour of phen and its various derivatives have been intensively studied in some labs during the past few years and the high complexity of the systems have been found [24–26]. For example, reduction waves arising from adsorption of phen on the surface of GCE was found [26]. As a consequence, in the first CV experiment of the present work, a voltammogram of ligand **1** was registered under the conditions detailed in the Experimental part. Any redox activity within the investigated voltage region on a freshly prepared sample was not detected. If, however, the cycles were repeated on the same sample several times, the irreversible reduction wave started to appear on the voltammogram. Because, investigation of the redox processes of the species adsorbed and activated on the electrode surface was beyond the scope of this work, voltammograms registered always and strictly on freshly prepared samples. Based on these results, for **2**, which is a ternary complex with Co(III) and contains the phen-part of the ligand **1** in uncomplexed form, an irreversible peak at cca. -200 mV was detected, which is attributable to the irreversible reduction of the Co(III) centre based on our previous results [21].

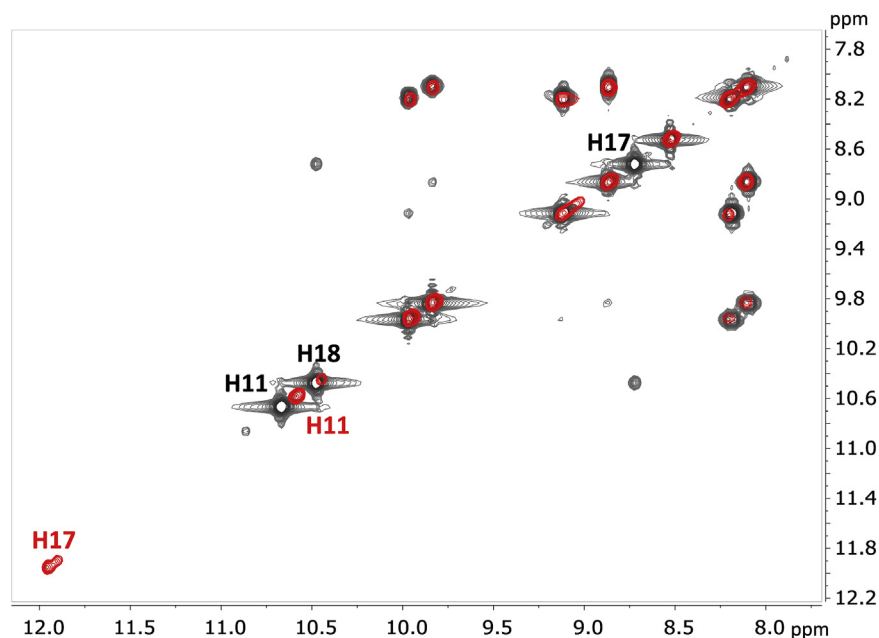


Fig. 5. Overlay of the aromatic region of ^1H - ^1H COSY NMR spectra of $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenhaH})]\text{Cl}$ (grey) and $[(\eta^6\text{-}p\text{-cym})\text{Ru}(\text{Cl})(\text{phenha})\text{Co}(\text{tren})]\text{Cl}(\text{PF}_6)_2$ (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Out of the collected voltammograms, those registered for the complexes **3–4** are shown in Fig. 7.

As Fig. 7 shows, the reduction wave, which is most probably attributable to the Co(III)/Co(II) reduction, is significantly more negative for the tren-containing complex (**3**) than that for the tpa-containing one (**4**) (those are -736 mV and -593 mV, respectively). This finding is in complete agreement with the previous results and comes from the electronic effect of the π -back-bonding interaction of the three pyridyl rings of tpa with the cobalt centre, which results in decreased electron density in the complex **4** at the central metal ion, compared to that in **3** [21]. Moreover, the above-mentioned π -back-bonding interaction of the three pyridyl rings results not only in the increased reducibility of the Co(III) , but also in the decreased lability of a ligand coordinating to a Co(III) -tpa core, compared to Co(III) -tren [22]. This difference results in measurable reversibility of the Co(III)/Co(II) process in the case of the tpa-containing complex **4** (the anodic peak potential appears at 413 mV), while in the case of the tren-containing complex, **3**, the process seems irreversible.

3.3. Antiproliferative activity

The effect of compounds **2–4** on human cancer cells was tested using the MTT assay. Human cervical carcinoma HeLa cells, human breast adenocarcinoma MCF-7 cells, breast carcinoma MDA-MB-231 cells, and human colorectal carcinoma cells HCT116 were incubated with complexes **2–4**, and the viability of cells was evaluated as described in the Experimental section. The IC_{50} values determined for 72 h of the treatment are reported in Table 1 and compared with those found for cisplatin. As indicated, compounds **2–4** displayed a moderate antiproliferative activity with IC_{50} values in double-digit micromolar concentrations. The bis-metallic Ru/Co complex **4** was the most active among all three tested complexes; it showed activity comparable or better than clinically used cisplatin, particularly in breast cancer cells. Interestingly, complex **4** is more active than cisplatin in highly aggressive triple-negative breast carcinoma cells MDA-MB-231.

4. Conclusion

As a part of our project, in which development of new metal based potential drug candidates is the final goal, in the present work, a novel phenanthroline-conjugated ligand (**1**) analogous to the well-known, clinically used drug, suberoylanilide hydroxamic acid (SAHA), has been synthesized and characterized together with its monometallic ternary complex with Co(III) (**2**) and two bimetallic Co(III) - Ru(II) ternary complexes (**3** and **4**).

The novel phen-based ambidentate ligand, **1**, has a somewhat shorter aliphatic chain compared to the parent molecule, SAHA, and, in addition to the metal binding O,O-donor hydroxamate, it also incorporates another chelating site, the N,N-donor phen moiety. Regarding the complexation of **1** with the studied two metals, all the results showed that, most probably, due to steric hindrance, the phen moiety was unable to bind to the two available coordination sites of the $[\text{Co(III)}(4\text{N})]^{3+}$ core. In complex **2**, therefore, the exclusive hydroxamate type coordination **1** was found leaving the phen-containing part free in the complexes **3** and **4** and capable of binding the $[(\eta^6\text{-}p\text{-cym})\text{Ru}]^{2+}$ unit with proven antiproliferative activity.

Out of the two selected metal ions, $[(\eta^6\text{-}p\text{-cym})\text{Ru}]^{2+}$ is a well-known entity of complexes with cytotoxic activity. Co(III) is known to form kinetically inert complexes, but upon its reduction to $2+$ cation, a kinetically labile complex is resulted. The idea was that, if transportation of this new potentially bioactive chelating ligand to the target can be achieved in a kinetically inert Co(III) complex and if the reduction as well as dissociation of the complex occurs only at the target, unwanted complexation reactions of the ligand during its transportation might be eliminated, so increased effectivity and selectivity might be obtained. Moreover, some previous results have indicated that Co(II) ions themselves can also have a cytotoxic effect [19]. In all three synthesized ternary complexes, the $3+$ oxidation state of the Co(III) ion was stabilized by a 4N-donor ligand, by tpa in the complexes **2** and **4**, while by tren in the complex **3**.

Beside characterization of the complexes by elementary analysis, NMR, and ESI-MS techniques, their redox activity by CV

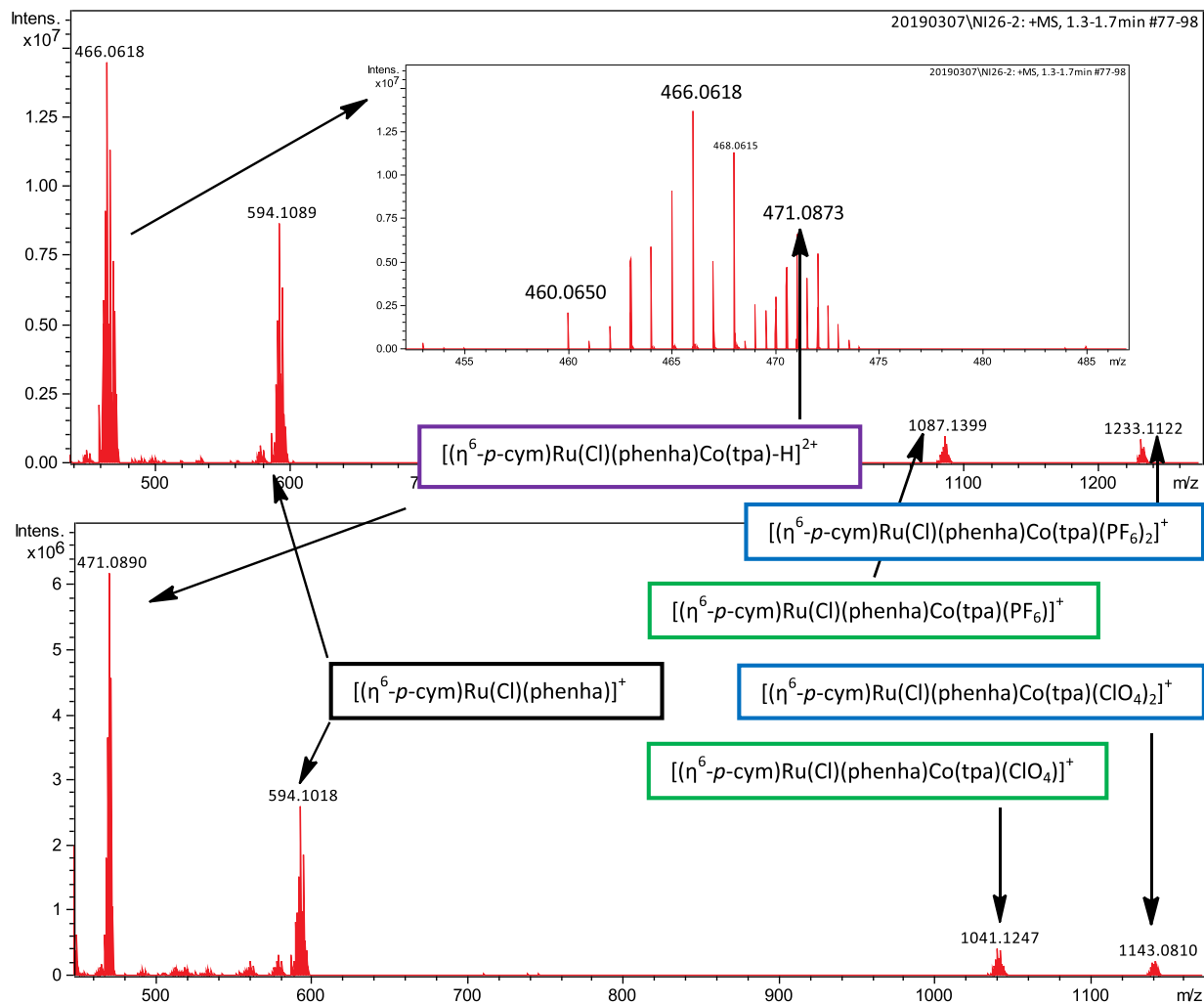


Fig. 6. ESI-MS spectrum of **4** (top) and that of a solution obtained from **2** by adding one equivalent of $[(\eta^6\text{-}p\text{-cym})\text{Ru}]^{2+}$ (bottom) in the positive mode showing the ions formed.

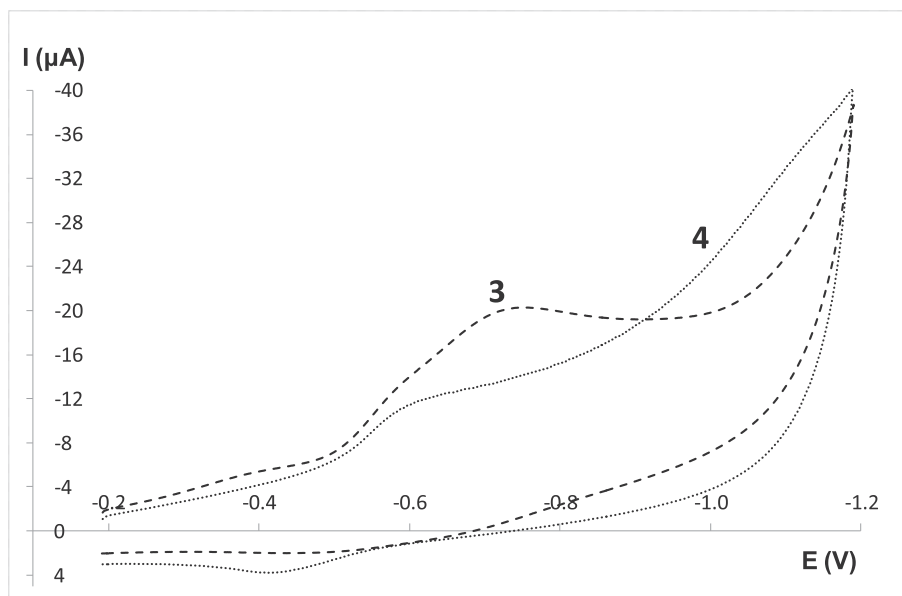


Fig. 7. CV curves registered for **3** (broken line) and **4** (dotted line) in water:MeOH 1:1, referenced to Ag/AgCl electrode at a potential sweep rate of 50 mV/s for **3** and 100 mV/s for **4** and $c_M = 1$ mM.

Table 1

The IC₅₀ mean values^a (μM) obtained for **2**, **3**, **4**, and cisplatin (after 72 h of incubation) by MTT assay.

IC ₅₀ (μM)	HeLa	MCF-7	MDA-MB-231	HCT116
2	29 ± 4	26 ± 3	24 ± 4	51 ± 7
3	61 ± 7	40 ± 5	30 ± 5	109 ± 9
4	30 ± 5	16 ± 3	18 ± 2	30 ± 3
cisplatin	14.8 ± 0.1	17 ± 1	42 ± 5	6.7 ± 0.1

^a Data represent a mean ± SD from at least three independent experiments, each of them made in triplicate.

method was investigated. Because of the complexity of the electrochemical behaviour of the phen containing compound and its metal complexes, several questions have remained unanswered in this respect. However, the corresponding results clearly show that, in agreement with previous findings on Co(III)-tpa-monohydroxamate ternary complexes, complex **2** also exhibits an irreversible reduction peak on the voltammogram at ca. 200 mV. It is also in agreement with the expectations that for the bimetallic complex **3**, in which the 4N-donor ligand is tren, the Co(III)/Co(II) reduction occurs at a significantly more negative potential (below the potential range maintained by bioreductants) than that in the case of the tpa-containing one, **4**. This is interpreted by the electronic effect of the π -back-bonding interaction of the three pyridyl rings of tpa with the cobalt centre resulting in decreased electron density in the complex **4** at the central metal ion, compared to that in **3** [21]. Moreover, this π -back-bonding interaction also results in the decreased lability of the ligand and, in parallel, in the measurable reversibility of the Co(III)/Co(II) process for the tpa-containing **4**. Further investigations are necessary to figure out why the reducibility of the Co(III) is significantly decreased in the bimetallic complex **4**, compared to the monometallic complex **2** (both having tpa as the 4 N donor).

The antiproliferative activity of compounds **2–4** in various cancer cells (human cervical carcinoma HeLa cells, human breast adenocarcinoma MCF-7 cells, breast carcinoma MDA-MB-231 cells, and human colorectal carcinoma cells HCT116) was tested using the MTT assay. The bimetallic complex **4** was found to be the most active among the tested complexes; it showed activity comparable or better than clinically used cisplatin. Interestingly, **4** is more active than cisplatin against the highly aggressive triple-negative breast carcinoma cells, MDA-MB-231. Consequently, additional studies on **4** seem to be worth carrying out.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The research was supported by the EU and co-financed by the European Regional Development Fund under the project GINOP-2.3.2-15-2016-00008 and the Hungarian Scientific Research Fund (OTKA K112317). The work of JK, HK, and VB was supported by the Czech Science Foundation (Grant No. 18-09502S).

Abbreviations

HDAC	histone deacetylase
SAHA	suberoylanilide hydroxamic acid
phen	1,10-phenanthroline

phenhaH	N ¹⁷ -hydroxy-N ¹¹ -(1,10-phenanthroline-5-yl)pentanediamide
tren	tris(2-aminoethyl)amine
tpa	tris(2-methylpyridyl)amine
DMAP	N,N-dimethylaminopyridine
DMF	N,N-dimethylformamide
CV	cyclic voltammetry
GCE	glassy carbon electrode
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
DMSO	dimethylsulfoxide

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jorgchem.2020.121265>.

References

- [1] W.K. Kelly, P.A. Marks, Nat. Clin. Pract. Oncol. 2 (2005) 150–157. <http://doi:10.1038/nclonc0106>.
- [2] A.C. West, R.W. Johnstone, J. Clin. Invest. 124 (2014) 30–39. <http://doi:10.1172/JCI69738>.
- [3] M. Manal, M.J.N. Chandrasekar, G.P. Jeyapal, M.J. Nanjan, Bioorg. Chem. 67 (2016) 18–42. <https://doi.org/10.1016/j.bioorg.2016.05.005>.
- [4] C.J. Marmion, J.P. Parker, K.B. Nolan, Hydroxamic acids: an important class of metalloenzyme inhibitors, in: Comprehensive Inorganic Chemistry, vol. 3, Elsevier, 2013, pp. 683–708 (chapter 3).23.
- [5] H. Rajak, A. Singh, K. Raghuwanshi, R. Kumar, P.K. Dewangan, R. Veerasamy, P.C. Sharma, A. Dixit, P. Mishra, Curr. Med. Chem. 21 (23) (2014) 2642–2664. <https://doi.org/10.2174/09298673113209990191>.
- [6] W.S. Xu, R.B. Parmigiani, P.A. Marks, Oncogene 26 (2007) 5541–5552. <http://doi:10.1038/sj.onc.1210620>.
- [7] T.J.P. McGiverna, C. Slatorb, A. Kellett, C.J. Marmion, Mol. Pharm. 15 (2018) 5058–5071. <http://doi:10.1021/acs.molpharmaceut.8b00652>.
- [8] R.G. Kenny, C.J. Marmion, Chem. Rev. 119 (2019) 1058–1137. <http://doi:10.1021/acs.chemrev.8b00271>.
- [9] D. Griffith, M.P. Morgan, C.J. Marmion, Chem. Commun. (2009) 6735–6737. <http://doi:10.1039/b916715c>.
- [10] V. Brabec, D.M. Griffith, A. Kisova, H. Kostrhunova, L. Zerzankova, C.J. Marmion, J. Kasparkova, Mol. Pharm. 9 (2012) 1990–1999. <https://doi.org/10.1021/mp300038f>.
- [11] K.J. Kilpin, P.J. Dyson, Chem. Sci. (2013). <https://doi.org/10.1039/c3sc22349c>.
- [12] D. Can, H.W. Peindy, N. Dongo, B. Spingler, P. Schmutz, P. Raposinho, I. Santos, R. Alberto, Chem. Biodivers. 9 (2012) 1849–1866. <https://doi.org/10.1002/cbdv.201200076>.
- [13] E. Meggers, Curr. Opin. Chem. Biol. 11 (2007) 287–292. <https://doi.org/10.1002/cbdv.201200076>.
- [14] J.M. Cross, T.R. Blower, N. Gallagher, J.H. Gill, K.L. Rockley, J.W. Walton, ChemPlusChem 81 (2016) 1276–1280. <https://doi.org/10.1002/cplu.201600413>.
- [15] R. Codd, Coord. Chem. Rev. 252 (2008) 1387–1408. <http://doi:10.1016/j.ccr.2007.08.001>.
- [16] D.M. Griffith, B. Szöcs, T. Keogh, K. Yu Suponitsky, E. Farkas, J. Inorg. Biochem. 105 (2011) 763–769. <http://doi:10.1016/j.jinorgbio.2011.03.003>.
- [17] P.D. Bonnichsa, B.J. Kim, R. Hocking, J.K. Clegg, P. Turner, S.M. Neville, T.W. Hambley, Dalton Trans. 41 (2012) 11293–11304. <http://doi:10.1039/c2dt30727h>.
- [18] D.C. Ware, P.J. Brothers, G.R. Clark, W.A. Denny, B.D. Palmer, W.R. Wilson, J. Chem. Soc., Dalton Trans. (2000) 925–932.
- [19] G. Wang, T.K. Hazra, S. Mitra, H.-M. Lee, E.W. Englander, Nucleic Acids Res. 28 (10) (2000) 2135–2140. <http://doi:10.1093/nar/28.10.2135>.
- [20] P. Buglyó, E. Farkas, Dalton Trans. 38 (2009) 8063–8070. <http://doi:10.1039/b908173a>.
- [21] P. Buglyó, I. Kacsir, M. Kozsup, I. Nagy, S. Nagy, A.Cs. Bényei, É. Kováts, E. Farkas, Inorg. Chim. Acta. 472 (2018) 234–242. <https://doi.org/10.1016/j.ica.2017.07.026>.
- [22] M. Kozsup, E. Farkas, A.Cs. Bényei, J. Kasparkova, H. Crlíkova, V. Brabec, P. Buglyó, J. Inorg. Biochem. 193 (2019) 94–105. <https://doi.org/10.1016/j.jinorgbio.2019.01.005>.
- [23] R.-R. Ye, Z.-F. Ke, C.-P. Tan, L. He, L.-N. Ji, Z.-W. Mao, Chem. Eur. J. 19 (2013) 10160–10169. <https://doi.org/10.1002/chem.201300814>.
- [24] P. Gayathri, A.S. Kumar, Langmuir, Langmuir 30 (34) (2014) 10513–10521. <https://doi.org/10.1021/la502651w>.
- [25] G. Shul, M. Weissmann, D. Bélange, Langmuir 30 (22) (2014) 6612–6621. <https://doi.org/10.1021/la500349t>.
- [26] G. Shul, M. Weissmann, D. Bélange, Electrochim. Acta 162 (2015) 146–155. <https://doi.org/10.1016/j.electacta.2014.12.116>.