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Synthesis, characterization and cytotoxicity studies of Co (III)-flavonolato complexes

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ABSTRACT

Hypoxia activated Co(III) complexes as prodrugs may provide with a selective delivery of cytotoxic or antibacterial compounds. Whithin this field sixteen novel Co(III) ternary complexes with the general formula [Co(4N)(flav)]$(ClO_4)_2$, where 4N = tris(2-aminoethyl)amine (tren) or tris(2-pyridylmethyl)amine (tpa) and flav = deprotonated form of differently substituted flavonols have been synthesized, characterized, and their cytotoxicity assayed under both normoxic and hypoxic conditions. Molecular structures of two free flavonols and seven complexes are also reported. In all the complexes the bioligands exhibited the expected (O,O) coordination mode and the complexes showed a slightly distorted octahedral geometry. Cyclic voltammetric studies revealed that both the substituents of the flavonoles and the type of 4N donor ligands had an impact on the reduction potential of the complex. The ones containing tren demonstrated significantly higher stability than the tpa analogues, making these former compounds promising candidates for the development of hypoxia-activated prodrug complexes. Tpa complexes showed higher activity against both selected human cancer cell lines (A549, A431) than their free ligand flavonols, indicating that the anticancer activity of the bioligand can be enhanced upon complexation. However, slight hypoxia-selectivity was found only for a tren complex (**11**) with moderate cytotoxicity.

1. Introduction

Selective targeting is crucial in cancer therapy. The metal complexes currently used as anticancer agents are mostly Pt(II) compounds, having serious side effects due to the lack of selectivity. These problems are associated with the unwanted interaction of the drug molecules during their transportation to the cancerous cells [1]. One solution to protect the compounds from these interactions could be to chaperone them in an inactive form until they reach the site of action, where they should become activated [2–4]. The use of such prodrugs therefore is a strongly appealing option to increase selectivity. As a design principle, significant difference in kinetic lability between Co(II) and Co(III) complexes makes this metal suitable to construct prodrugs that can be activated under hypoxic conditions in the tumor tissues. While Co(III) complexes are kinetically inert and do not exchange ligands, upon reduction they become much more labile Co(II) complexes that can release a bioactive ligand in the tumor. Previously simple hydroxamates [3–5], an antimetastatic matrix metalloproteinase (MMP) inhibitor, marimastat [2], quinolones [6] were used among others to construct mixed ligand Co(III) complexes while light activation of Co(III)-curcumin prodrugs was also studied in an excellent paper by the Hambley group [7].

Flavonoids belong to the group of polyphenolic compounds that are mainly found in tea, apple, onion and fungi [8–10]. They have a wide range of biological activities, like antibacterial, antiviral, neuroprotective, cardioprotective, antioxidant and antitumor effects [9]. Anticancer activity of flavonoids is based on their ability to inhibit several enzymes, like protein kinases and topoisomerases that are necessary for the life cycles of cancer cells [11–16]. Furthermore, they inhibit the secreation of MMPs connected to tumor metastasis [17–19]. Having these properties flavonoid type compounds can inhibit cell growth and tumor cell invasion. Up to now four flavonoids, flavopiridol, silibinin, quercetin and a water-soluble glycine N-monosubstituted

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carbamate ester prodrug of quercetin, 3′-(*N*-carboxymethyl)carbamoyl-3,4′,5,7-tetrahydroxyflavone, (QC12), reached the phase of clinical investigations as anticancer agents, but none of them have been introduced into market. The main problem is their poor bioavailability resulting from susceptibility to glucourinidation and methylation by intestinal and liver metabolism [9]. Due to the presence of a hydroxyl group and an oxo group in chelating position in the structure of flavonols (a subgroup of flavonoids), they are potential chelating ligands by coordinating to metal ions via these two oxygen donor atoms. Complexation of flavonoids was shown to increase their pharmacokinetic and pharmacological properties [20,21].

In the present work the synthesis and characterization of redox-active cobalt(III) complexes of differently substituted flavonols are reported. Tris(2-aminoethyl)amine (tren) or tris(2-methylpyridyl)amine (tpa) tripodal amines were used as tetradentate ligands to synthesize the kinetically inert cobalt(III) complexes. A series of bioactive ligands with anticancer potential, i.e. 3-hydroxyflavone (flavH), 6-F-3′-NO_2-3-hydroxyflavone (NO_2FflavH), 3′-NO_2-3-hydroxyflavone (NO_2flavH), 4′-Me-3-hydroxyflavone (MeflavH), 6-Cl-4′-OMe-3-hydroxyflavone (ClOMeflavH), 6-Br-3-hydroxyflavone (BrflavH), 4′-iPr-3-hydroxyflavone (iPrflavH), chrysin (chrysH) and naringenin (narH), were used to complete the coordination sphere of the Co^{III} ion. The formulae and abbreviations of all ligands are summarized in Fig. 1 while numbering of the complexes in Table 1.

Table 1
Numbering of the Co^{III} complexes used in this study.

Complex	Abbreviation
[Co(tren)(flav)]$(ClO_4)_2$	**5**
[Co(tpa)(flav)]$(ClO_4)_2$	**6**
[Co(tren)(Brflav)]$(ClO_4)_2$	**7**
[Co(tpa)(Brflav)]$(ClO_4)_2$	**8**
[Co(tren)(Meflav)]$(ClO_4)_2$	**9**
[Co(tpa)(Meflav)]$(ClO_4)_2$	**10**
[Co(tren)(NO_2Fflav)]$(Cl)_2$	**11**
[Co(tpa)(NO_2Fflav)]$(ClO_4)_2$	**12**
[Co(tren)(NO_2flav)](Cl)(ClO_4)	**13**
[Co(tpa)(NO_2flav)]$(ClO_4)_2$	**14**
[Co(tren)(chrys)](Cl)(ClO_4)	**15**
[Co(tpa)(chrys)]$(ClO_4)_2$	**16**
[Co(tren)(ClOMeflav)]$(ClO_4)_2$	**17**
[Co(tren)(iPrflav)]$(ClO_4)_2$	**18**
[Co(tpa)(iPrflav)]$(ClO_4)_2$	**19**
[Co(tren)(nar)](Cl)(ClO_4)	**20**

2. Experimental

2.1. Materials and reagents

3-nitrobenzaldehyde, 2′-hydroxyacetophenone, 5′-F-2′-hydroxyacetophenone, flavH, $CoCl_2 \cdot 6H_2O$, $NaNO_2$, tren, chrysin, naringenin, $NaClO_4$, and methanol were commercial products from Merck, SigmaAldrich, TCI Chemicals, Acros Organics, Scharlau or Reanal and used as received. Tpa [22], [Co(tren)$(NO_2)_2$]Cl, [Co(tren)Cl_2]Cl, [Co(tpa)$(NO_2)_2$]Cl, [Co(tpa)Cl_2]Cl [23], MeflavH [24], ClOMeflavH [25], BrflavH [26], iPrflavH [27] were synthesized and purified according to literature procedures.

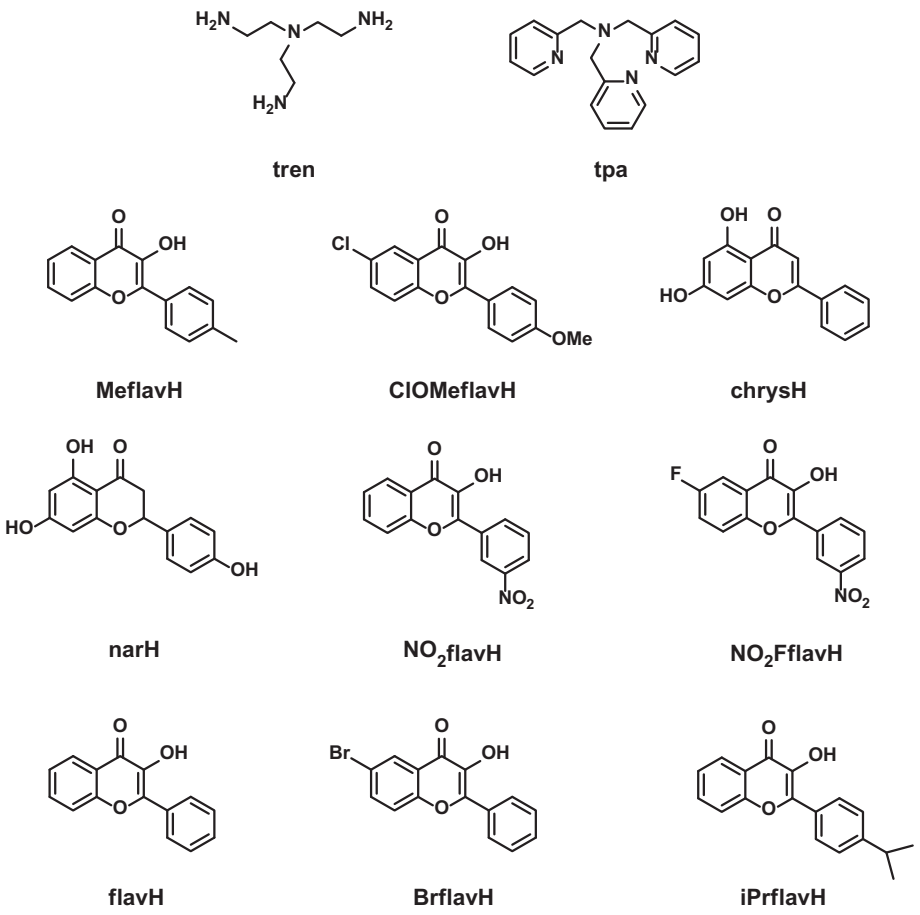


Fig. 1. The formulae and abbreviations of the ligands used in this study.

2.2. Syntheses

2.2.1. 5-NO_2-2′-hydroxychalcone (1)

3-Nitrobenzaldehyde (1.16 g, 7.0 mmol) was dissolved in 10 ml methanol and 2′-hydroxyacetophenone (0.83 g, 6.0 mmol) was added. Solution of 0.9 g NaOH in 0.9 ml water was added dropwise and the reaction mixture was stirred at room temperature overnight. The precipitated brown solid was treated with 20 ml 10% HCl and the mixture was filtered. The collected crude product was washed with water and recrystallized from methanol. The yellow crystalline solid was washed with water and dried in vacuo. Yield: 1.57 g (97%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 7.01–7.05 (m, 2H, Ar—H), 7.57–7.61 (m, 1H, Ar—H), 7.76 (t, 1H, Ar—H), 7.92–7.96 (d, 1H, -CH), 8.22–8.26 (d, 1H, -CH), 8.27–8.36 (m, 3H, Ar—H), 8.80 (m, 1H, Ar—H), 12.35 (s, 1H, -OH). IR (KBr)/cm^{-1}: 3432, 1646, 1590, 1525, 1359, 1100. Anal Required for $C_{15}H_{11}NO_4$: C, 66.91, H, 4.12, N, 5.20%. Found: C, 66.75, H, 4.04 N, 5.32%.

2.2.2. 5′-F-5-NO_2-2′-hydroxychalcone (2)

The synthesis was similar to that of **1**, using 5′-F-2′-hydroxyacetophenone (0.92 g, 6.0 mmol). The product was isolated as a yellow crystalline solid. Yield: 1.48 g (86%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 7.03–7.06 (m, 1H, Ar—H), 7.44–7.49 (m, 1H, Ar—H), 7.76 (t, 1H, Ar—H), 7.92–7.96 (d, 1H, -CH), 8.11–8.14 (m, 1H, Ar—H), 8.15–8.19 (d, 1H, -CH), 8.28–8.30 (m, 1H, Ar—H), 8.35 (d, 1H, Ar—H), 8.80 (m, 1H, Ar—H), 12.05 (s, 1H, -OH). IR (KBr)/cm^{-1}: 3434, 1649, 1593, 1525, 1353, 1174. Anal Required for $C_{15}H_{10}FNO_4$: C, 62.72, H, 3.51, N, 4.88%. Found: C, 62.76, H, 3.52 N, 5.02%.

2.2.3. 3′-NO_2-3-hydroxyflavone (3)

1 (1.00 g, 3.71 mmol) was dissolved in 21 ml methanol and 5 ml 8% NaOH. H_2O_2 (3.4 ml, 30%) was added dropwise to the well stirred reaction mixture for 15 min at 0 °C. The mixture was stirred for 1 h and poured into 80 g cracked ice. The pH of the mixture was adjusted to 1.0 using 20% HCl. The precipitated solid was filtered, washed with water and recrystallized from acetic acid. The pale brown crystalline solid was collected and dried in vacuo. Yield: 0.36 g (35%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 7.49 (m, 1H, Ar—H), 7.81–7.90 (m, 3H, Ar—H), 8.14 (d, 1H, Ar—H), 8.33 (d, 1H, Ar—H), 8.61 (d, 1H, Ar—H), 9.07 (m, 1H, Ar—H), 10.22 (s, 1H, -OH). IR (KBr)/cm^{-1}: 3250, 1608, 1570, 1527, 1347, 1137. Anal Required for $C_{15}H_9NO_5$: C, 63.61, H, 3.20, N, 4.95%. Found: C, 63.49, H, 3.24 N, 4.88%.

2.2.4. 6-F-3′-NO_2-3-hydroxyflavone (4)

The synthesis was similar to that of **3**, using **2** (1.20 g, 4.2 mmol). The product was isolated as a pale brown crystalline solid. Yield: 0.41 g (32%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 7.72–7.80 (m, 2H, Ar—H), 7.88 (t, 1H, Ar—H), 7.92–7.96 (m, 1H, Ar—H), 8.35 (m, 1H, Ar—H), 8.62 (d, 1H, Ar—H), 9.07 (m, 1H, Ar—H), 10.35 (s, 1H, -OH). IR (KBr)/cm^{-1}: 3343, 1626, 1581, 1522, 1349, 1083. Anal Required for $C_{15}H_8FNO_5 \cdot 0.6H_2O$: C, 57.74, H, 2.97, N, 4.49%. Found: C, 57.46, H, 2.89 N, 4.30%.

2.2.5. General procedure for the synthesis of the Co(III)-flavonolato/4N donor ligand ternary complexes

***CAUTION**: Although we never have experienced any problems, perchlorate salts are potentially explosive.*

The flavonol was dissolved in 15 ml methanol and NaOH (1 equiv.) was added. [Co(tren)Cl_2]Cl or [Co(*tpa*)Cl_2]Cl (1 equiv.) was added and the deep brownish reaction mixture was stirred at 60 °C overnight. After cooling, the mixture was filtered, using a cotton wool, and the solution was brought to dryness in rotavap. The residue was dissolved in 5 mL water and filtered. Solid $NaClO_4$ (2 equiv.) was added to the solution. On standing at room temperature crystalline solid appeared after few days. The solid was filtered, washed with water and dried in vacuo. In some cases the crude products were recrystallized from water or methanol.

2.2.5.1. [Co(tren)(flav)](ClO$_4$)$_2$ (5). Using flavH (76.43 mg, 0.32 mmol), NaOH (12.80 mg, 0.32 mmol) and [Co(tren)Cl_2]Cl (100.00 mg, 0.32 mmol). Brown crystalline solid, yield: 86.20 mg (42%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.78–2.90 (m, 3H, -CH_2 tren), 3.01 (m, 3H, -CH_2 tren), 3.10 (m, 2H, -CH_2 tren), 3.16 (m, 3H, -CH_2 tren), 3.52 (m, 0.6H, -CH_2 tren-isomer B) 3.66 (m, 1.4H, -CH_2 tren-isomer A), 5.08 (m, 1.4H, -NH_2 tren-isomer A) 5.22 (m, 0.6H, -NH_2 tren-isomer B), 5.39 (m, 4H, -NH_2 tren), 7.59 (m, 4H, Ar—H), 7.91–8.04 (m, 2H, Ar—H), 8.10 (d, 0.3H, Ar-H-isomer A) 8.40 (d, 0.7H, Ar-H-isomer B), 8.65 (m, 2H, Ar—H). IR (KBr)/cm^{-1}: 3447, 1491, 1099, 755, 625. Anal Required for $C_{21}H_{27}Cl_2CoN_4O_{11} \cdot 0.5H_2O$: C, 38.79, H, 4.34, N, 8.62%. Found: C, 38.85, H, 4.49 N, 8.55%. MS (ESI positive ion): *m/z*: 221.070 ([Co(tren)(flav)]$^{2+}$), 541.095 ([Co(tren)(flav)](ClO$_4$)$^+$).

2.2.5.2. [Co(tpa)(flav)](ClO$_4$)$_2$ (6). Using flavH (76.43 mg, 0.32 mmol), NaOH (12.80 mg, 0.32 mmol) and [Co(*tpa*)Cl_2]Cl (145.81 mg, 0.32 mmol). Pale brown solid, yield: 115.60 mg (46%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 5.22 (d, 1.3H, -CH_2 tpa-isomer A), 5.29 (d, 0.7H, -CH_2 tpa-isomer B) 5.32–5.34 (m, 2H, -CH_2 tpa), 5.58 (d, 1.3H, -CH_2 tpa-isomer A), 5.86 (d, 0.7H, -CH_2 tpa-isomer B) 7.44–7.50 (m, 1H, Ar—H), 7.57 (m, 4H, Ar—H), 7.79–7.89 (m, 5H, Ar—H), 7.97 (d, 0.3H, Ar-H-isomer B) 8.04–8.14 (m, 4H, Ar—H), 8.36–8.44 (m, 3H, Ar—H), 8.51 (d, 0.7H, Ar-H-isomer A), 9.03 (t, 1H, Ar—H), 9.17 (d, 0.3H, Ar-H-isomer B), 9.60 (m, 0.7H, Ar-H-isomer A). IR (KBr)/cm^{-1}: 3436, 1612, 1491, 1436, 1094, 766, 623. Anal Required for $C_{33}H_{27}Cl_2CoN_4O_{11} \cdot 0.5H_2O$: C, 49.89, H, 3.55, N, 7.05%. Found: C, 49.89, H, 3.53 N, 7.05%. MS (ESI positive ion): *m/z*: 293.073 ([Co(tpa)(flav)]$^{2+}$).

2.2.5.3. [Co(tren)(Brflav)](ClO$_4$)$_2$ (7). Using BrflavH (49.25 mg 0.16 mmol), NaOH (6.4 mg, 0.16 mmol) and [Co(tren)Cl_2]Cl (50.00 mg, 0.16 mmol). Brown solid, yield: 41.15 mg (36%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.74–2.86 (m, 3H, -CH_2 tren), 2.88–3.05 (m, 4H, -CH_2 tren), 3.08–3.19 (m, 3H, -CH_2 tren), 3.50 (m, 0.6H, -CH_2 tren-isomer B), 3.76 (m, 1.4H, -CH_2 tren-isomer A), 5.09 (m, 1.4H, -NH_2 tren-isomer A), 5.24 (m, 0.6H, -NH_2 tren-isomer B), 5.40 (m, 4H, -NH_2 tren), 7.54–7.68 (m, 3H, Ar—H), 7.98–8.17 (m, 2H, Ar—H), 8.63–8.67 (m, 3H, Ar—H). IR (KBr)/cm^{-1}: 3457, 1489, 1162, 769, 625. Anal Required for $C_{21}H_{26}BrCl_2CoN_4O_{11} \cdot 0.5H_2O$: C, 34.59, H, 3.73, N, 7.68%. Found: C, 34.52, H, 3.79 N, 7.64%. MS (ESI positive ion): *m/z*: 260.062 ([Co(tren)(Brflav)]$^{2+}$).

2.2.5.4. [Co(tpa)(Brflav)](ClO$_4$)$_2$ (8). Using BrflavH (45.50 mg, 0.14 mmol), NaOH (5.6 mg, 0.14 mmol) and [Co(*tpa*)Cl_2]Cl (65.51 mg, 0.14 mmol). Light brown solid, yield: 53.17 mg (44%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 5.19 (d, 0.7H, -CH_2 tpa-isomer B), 5.21 (d, 1.3H, -CH_2 tpa-isomer A), 5.32–5.33 (m, 2H, -CH_2 tpa), 5.59 (d, 0.7H, -CH_2 tpa-isomer B), 5.95 (d, 1.3H, -CH_2 tpa-isomer A), 7.45 (m, 1H, Ar—H), 7.54–7.60 (m, 3H, Ar—H), 7.72–7.89 (m, 5H, Ar—H), 7.95–8.19 (m, 5H, Ar—H), 8.43–8.49 (m, 3H, Ar—H), 8.65 (d, 0.7H, Ar-H-isomer A), 9.00 (m, 1H, Ar—H) 9.14 (d, 0.7H, Ar-H-isomer A) 9.25 (d, 0.3H, Ar-H-isomer B) 9.64 (d, 0.3H, Ar-H-isomer B). IR (KBr)/cm^{-1}: 3425, 1493, 1442, 1094, 769, 624. Anal Required for $C_{33}H_{26}BrCl_2CoN_4O_{11} \cdot 1.2H_2O$: C, 44.74, H, 3.23, N, 6.32%. Found: C, 44.79, H, 3.15 N, 6.24%. MS (ESI positive ion): *m/z*: 332.026 ([Co(tpa)(Brflav)]$^{2+}$).

2.2.5.5. [Co(tren)(Meflav)](ClO$_4$)$_2$ (9). Using MeflavH (50.00 mg 0.20 mmol), NaOH (8.0 mg, 0.20 mmol) and [Co(tren)Cl_2]Cl (61.78 mg, 0.20 mmol). Brown solid, yield: 64.32 mg (49%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.43 (m, 3H, -CH_3), 2.76–2.92 (m, 3H, -CH_2 tren), 2.99–3.02 (m, 3H, -CH_2 tren), 3.06–3.11 (m, 2H, -CH_2 tren), 3.14–3.18 (m, 2H, -CH_2 tren), 3.50 (m, 0.7H, -CH_2 tren-isomer B), 3.64 (m, 1.3H, -CH_2 tren-isomer A), 5.09 (m, 1.3H, -NH_2 tren-isomer A), 5.23 (m, 0.7H, -NH_2 tren-isomer B), 5.35–5.49 (m, 4H, -NH_2 tren), 7.40 (d, 1.3H, Ar-H-isomer A), 7.47 (d, 0.7H, Ar-H-isomer B), 7.60 (m, 1H, Ar—H), 7.92 (m, 1H, Ar—H), 7.98–8.03 (m, 1H, Ar—H), 8.10 (m, 0.3H, Ar-H-isomer B), 8.38

(m, 0.7H, Ar-H-isomer A), 8.56 (m, 2H, Ar—H). IR (KBr)/cm^{-1}: 3443, 1611, 1537, 1491, 1430, 1104, 753, 625. Anal Required for $C_{22}H_{29}Cl_2CoN_4O_{11}$: C, 40.26, H, 4.61, N, 8.54%. Found: C, 40.09, H, 4.53 N, 8.45%. MS (ESI positive ion): *m/z*: 228.579 ([Co(tren)(Meflav)]$^{2+}$).

*2.2.5.6. [Co(tpa)(Meflav)](ClO$_4$)$_2$ **(10)**.* Using MeflavH (43.26 mg, 0.17 mmol), NaOH (6.8 mg, 0.17 mmol) and [Co(*tpa*)Cl$_2$]Cl (78.06 mg, 0.17 mmol). Light brown solid, yield: 60.05 mg (44%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.39 (s, 1H, -CH_3-isomer B), 2.56 (s, 2H, -CH_3-isomer A), 5.20–5.34 (m, 4H, -CH_2), 5.57 (d, 0.6H, -CH_2 tpa-isomer B), 5.86 (d, 1.4H, -CH_2 tpa-isomer A), 7.39–7.60 (m, 4H, Ar—H), 7.68 (d, 1H, Ar—H), 7.79–7.90 (m, 4H, Ar—H), 7.98 (m, 0.7H, Ar-H-isomer A), 8.02–8.15 (m, 4H, Ar—H), 8.34 (d, 1H, Ar—H), 8.39 (d, 0.6H, Ar-H-isomer B), 8.48 (d, 1.4H, Ar-H-isomer A) 8.91 (d, 1H, Ar—H) 9.00 (m, 0.3H, Ar-H-isomer B), 9.15 (d, 0.7H, Ar-H-isomer A), 9.58 (d, 0.3H, Ar-H-isomer B). IR (KBr)/cm^{-1}: 3431, 1610, 1492, 1431, 1093, 770, 624. Anal Required for $C_{34}H_{29}Cl_2CoN_4O_{11}\cdot 0.8H_2O$: C, 50.11, H, 3.91, N, 6.88%. Found: C, 50.09, H, 3.72 N, 6.84%. MS (ESI positive ion): *m/z*: 300.079 ([Co(tpa)(Meflav)]$^{2+}$).

*2.2.5.7. [Co(tren)(NO$_2$Fflav)](Cl)$_2$ **(11)**.* Using NO_2FflavH (50.00 mg 0.17 mmol), NaOH (6.8 mg, 0.17 mmol) and [Co(tren)Cl$_2$]Cl (53.00 mg, 0.17 mmol). The solid was obtained without adding the perchlorate counter ion, isolated as a reddish crystalline solid. The pure product was obtained after recrystallization from MeOH. Yield: 37 mg (38%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.87 (m, 3H, -CH_2 tren), 2.94 (m, 2H, -CH_2 tren), 3.04–3.16 (m, 4H, -CH_2 tren), 3.56. (m, 3H, -CH_2 tren), 5.53 (m, 2H, -NH_2 tren), 6.05 (m, 2H, -NH_2 tren), 6.29 (m, 2H, -NH_2 tren), 7.81 (m, 1H, Ar—H), 7.89–7.98 (m, 2H, Ar—H), 8.19 (m, 1H, Ar—H), 8.38 (d, 1H, Ar—H), 8.86 (d, 1H, Ar—H), 9.76 (s, 1H, Ar—H). IR (KBr)/cm^{-1}: 3430, 1505, 1346, 1242, 797. Anal Required for $C_{21}H_{25}Cl_2CoFN_5O_5\cdot$MeOH: C, 43.44, H, 4.81, N, 11.51%. Found: C, 43.12, H, 4.73 N, 11.31%. MS (ESI positive ion): *m/z*: 252.559 ([Co(tren)(NO_2Fflav)]$^{2+}$), 504.110 ([Co(tren)(NO_2Fflav)-H]$^+$).

*2.2.5.8. [Co(tpa)(NO$_2$Fflav)](ClO$_4$)$_2$ **(12)**.* Using NO_2FflavH (50.00 mg, 0.17 mmol), NaOH (6.8 mg, 0.17 mmol) and [Co(*tpa*)Cl$_2$]Cl (75.64 mg, 0.17 mmol). Light brown solid, yield: 67.08 mg (46%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 5.38–5.43 (m, 4H, -CH_2 tpa), 5.53 (d, 0.7H, -CH_2 tpa-isomer B), 5.92 (d, 1.3H, -CH_2 tpa-isomer A), 7.47 (m, 1H, Ar—H), 7.57 (m, 2H, Ar—H), 7.77–7.90 (m, 4H, Ar—H), 8.01–8.29 (m, 6H, Ar—H), 8.36 (m, 0.4H, Ar-H-isomer B), 8.44 (m, 0.6H, Ar-H-isomer A), 8.54 (d, 1H, Ar—H), 8.59 (m, 0.6H, Ar-H-isomer A) 8.73 (d, 0.4H, Ar-H-isomer B), 8.88 (m, 0.4H, Ar-H-isomer B), 9.17 (d, 0.6H, Ar-H-isomer A), 9.28 (d, 0.6H, Ar-H-isomer A), 9.37 (s, 0.4H, Ar-H-isomer B), 9.65 (d, 0.4H, Ar-H-isomer B), 10.23 (s, 0.6H, Ar-H-isomer A). IR (KBr)/cm^{-1}: 3432, 1501, 1448, 1347, 1094, 624. Anal Required for $C_{33}H_{25}Cl_2CoFN_5O_{13}\cdot H_2O$: C, 45.75, H, 3.14, N, 8.08%. Found: C, 45.63, H, 3.12 N, 7.96%. MS (ESI positive ion): *m/z*: 680.134 ([Co(tpa)(NO_2Fflav) + OMe]$^+$).

*2.2.5.9. [Co(tren)(NO$_2$flav)](Cl)(ClO$_4$) **(13)**.* Using NO_2flavH (48.15 mg 0.17 mmol), NaOH (6.8 mg, 0.17 mmol) and [Co(tren)Cl$_2$]Cl (53.00 mg, 0.17 mmol). The pure product was obtained after recrystallization from MeOH. Orange solid, yield: 68.15 mg (52%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.85 (m, 3H, -CH_2 tren), 2.94 (m, 2H, -CH_2 tren), 3.08–3.15 (m, 4H, -CH_2 tren), 3.55. (m, 3H, -CH_2 tren), 5.49–5.57 (m, 4H, -NH_2 tren), 5.82 (m, 2H, -NH_2 tren), 7.63 (t, 1H, Ar—H), 7.94–8.01 (m, 2H, Ar—H), 8.08 (d, 1H, Ar—H), 8.13 (d, 1H, Ar—H), 8.38 (d, 1H, Ar—H), 8.84 (d, 1H, Ar—H), 9.77 (s, 1H, Ar—H). IR (KBr)/cm^{-1}: 3439, 1542, 1496, 1347, 1098, 624. Anal Required for $C_{21}H_{26}Cl_2CoN_5O_9\cdot$-MeOH: C, 40.38, H, 4.62, N, 10.70%. Found: C, 40.06, H, 4.47, N, 10.42%. MS (ESI positive ion): *m/z*: 243.564 ([Co(tren)(NO_2flav)]$^{2+}$).

*2.2.5.10. [Co(tpa)(NO$_2$flav)](ClO$_4$)$_2$ **(14)**.* Using NO_2flavH (48.15 mg 0.17 mmol), NaOH (6.8 mg, 0.17 mmol) and [Co(*tpa*)Cl$_2$]Cl (77.46 mg, 0.17 mmol). Brown solid, yield: 59.14 mg (42%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 5.27–5.39 (m, 4H, -CH_2 tpa), 5.54 (d, 0.6H, -CH_2 tpa-isomer B), 5.88 (d, 1.4H, -CH_2 tpa-isomer A), 7.47 (m, 1H, Ar—H), 7.54–7.59 (m, 2H, Ar—H), 7.78–7.97 (m, 5H, Ar—H), 8.05–8.15 (m, 5H, Ar—H), 8.35–8.44 (m, 2H, Ar), 8.55 (d, 1H, Ar—H), 8.59 (m, 0.6H, Ar-H-isomer A), 8.72 (m, 0.4H, Ar-H-isomer B) 9.06 (d, 0.4H, Ar-H-isomer B), 9.15 (d, 0.6H, Ar-H-isomer A), 9.29 (d, 0.6H, Ar-H-isomer A), 9.39 (s, 0.4H, Ar-H-isomer B), 9.59 (d, 0.4H, Ar-H-isomer B), 10.25 (s, 0.6H, Ar-H-isomer A). IR (KBr)/cm^{-1}: 3436, 1541, 1495, 1349, 1093, 768, 624. Anal Required for $C_{33}H_{26}Cl_2CoN_5O_{13}$: C, 47.73, H, 3.16, N, 8.43%. Found: C, 47.41, H, 3.05 N, 8.29%. MS (ESI positive ion): *m/z*: 315.564 ([Co(tpa)(NO_2flav)]$^{2+}$), 662.145 ([Co(tpa)(NO_2flav) + OMe]$^+$).

*2.2.5.11. [Co(tren)(chrys)](Cl)(ClO$_4$) **(15)**.* Using chrysH (81.36 mg 0.32 mmol), NaOH (12.8 mg, 0.32 mmol) and [Co(tren)Cl$_2$]Cl (100 mg, 0.32 mmol). Light brown solid, yield: 72.17 mg (38%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.75–2.77 (m, 4H, -CH_2 tren), 2.87 (m, 3H, -CH_2 tren), 3.18 (m, 3H, -CH_2 tren), 3.48 (m, 2H, -CH_2 tren), 5.31 (m, 2H, -NH_2 tren), 5.50 (m, 2H, -NH_2 tren), 5.70 (m, 2H, -NH_2 tren), 6.34 (m, 2H, Ar—H), 7.50 (s, 1H, -CH), 7.63 (m, 3H, Ar—H), 8.10 (m, 2H, Ar—H). IR (KBr)/cm^{-1}: 3420, 1634, 1531, 1430, 1169, 1107. Anal Required for $C_{21}H_{27}Cl_2CoN_4O_8\cdot 1H_2O$: C, 41.26, H, 4.78, N, 9.17%. Found: C, 41.13, H, 4.78 N, 9.22%. MS (ESI positive ion): *m/z*: 229.069 ([Co(tren)(chrys)]$^{2+}$), 457.129 ([Co(tren)(chrys)-H]$^+$).

*2.2.5.12. [Co(tpa)(chrys)](ClO$_4$)$_2$ **(16)**.* Using chrysH (81.36 mg 0.32 mmol), NaOH (12.8 mg, 0.32 mmol) and [Co(*tpa*)Cl$_2$]Cl (145.81 mg, 0.32 mmol). Brown solid, yield: 79.9 mg (28%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 4.97 (d, 1H, -CH_2 tpa-isomer A), 5.12 (d, 1H, -CH_2 tpa-isomer B), 5.30 (d, 2H, -CH_2 tpa), 5.59 (d, 1H, Ar-H-isomer A), 5.70 (d, 1H, Ar-H-isomer B), 6.43 (d, 1H, Ar—H), 6.56 (s, 0.5H, Ar-H-isomer A), 7.27 (s, 0.5H, Ar-H-isomer B), 7.41–7.46 (m, 1H, Ar—H), 7.51–7.62 (m, 3H, Ar—H), 7.72–7.84 (m, 4H, Ar—H), 7.92 (d, 1H, Ar—H), 8.00–8.06 (m, 2H, Ar—H), 8.11 (m, 2H, Ar—H), 8.23 (m, 2H, Ar—H), 8.28 (m, 2H, Ar—H), 9.26 (d, 0.5H, Ar-H-isomer A), 9.44 (d, 0.5H, Ar-H-isomer B), 11.05 (s, 0.5H, -OH-isomer A), 11.38 (s, 0.5H, -OH-isomer B). IR (KBr)/cm^{-1}: 3434, 1633, 1525, 1101, 771, 623. Anal Required for $C_{33}H_{27}Cl_2CoN_4O_{12}\cdot 1H_2O$: C, 48.37, H, 3.57, N, 6.84%. Found: C, 48.09, H, 3.61 N, 7.09%. MS (ESI positive ion): *m/z*: 301.069 ([Co(tpa)(chrys)]$^{2+}$), 601.129 ([Co(tpa)(chrys)-H]$^+$), 701.085 ([Co(tpa)(chrys) + ClO_4]$^+$).

*2.2.5.13. [Co(tren)(ClOMeflav)](ClO$_4$)$_2$ **(17)**.* Using ClOMeflavH (53.00 mg 0.17 mmol), NaOH (6.8 mg, 0.17 mmol) and [Co(tren)Cl$_2$]Cl (51.44 mg, 0.17 mmol). Brown solid, yield: 67.65 mg (56%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.76–2.85 (m, 2H, -CH_2 tren), 2.88–3.03 (m, 4H, -CH_2 tren), 3.07–3.12 (m, 2H, -CH_2 tren), 3.14–3.17 (m, 2H, -CH_2 tren), 3.51 (m, 0.7H, -CH_2 tren-isomer B), 3.73 (m, 1.3H, -CH_2 tren-isomer A), 3.89 (m, 3H, -CH_3), 5.07 (m, 1.3H, NH_2 tren-isomer A), 5.21 (m, 0.7H, NH_2 tren-isomer B), 5.36–5.43 (m, 4H, NH_2 tren), 7.14 (d, 1.3H, Ar-H-isomer A), 7.23 (d, 0.7H, Ar-H-isomer B), 7.91 (m, 1H, Ar—H), 7.99 (d, 0.4H, Ar-H-isomer B), 8.04 (d, 0.6H, Ar-H-isomer A), 8.10 (d, 0.4H, Ar-H-isomer B), 8.47 (d, 0.6H, Ar-H-isomer A), 8.64–8.67 (m, 2H, Ar—H). IR (KBr)/cm^{-1}: 3463, 3259, 1603, 1492, 1433, 1108, 626. Anal Required for $C_{22}H_{28}Cl_3CoN_4O_{12}\cdot H_2O$: C, 36.51, H, 4.18, N, 7.74%. Found: C, 36.28, H, 3.92, N, 7.52%. MS (ESI positive ion): *m/z*: 253.057 ([Co(tren)(ClOMeflav)]$^{2+}$), 505.107 ([Co(tren)(ClOMeflav)-H]$^+$), 605.063 ([Co(tren)(ClOMeflav) + ClO_4]$^+$).

*2.2.5.14. [Co(tren)(iPrflav)](ClO$_4$)$_2$ **(18)**.* Using iPrflavH (50.00 mg 0.18 mmol), NaOH (7.2 mg, 0.18 mmol) and [Co(tren)Cl$_2$]Cl (55.54 mg, 0.18 mmol). Brown solid, yield: 55.23 mg (45%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 1.27 (d, 6H, -CH_3), 2.78 (m, 3H, -CH_2 tren), 2.94–3.03

(m, 5H, -CH_2 tren, -CH), 3.16 (m, 3H, -CH_2 tren), 3.63 (m, 2H, -CH_2 tren), 5.39 (m, 2H, -NH_2 tren), 5.53 (m, 2H, -NH_2 tren), 5.89 (m, 2H, -NH_2 tren), 7.45 (d, 2H, Ar—H), 7.59 (t, 1H, Ar—H), 7.91 (t, 1H, Ar—H), 7.97 (d, 1H, Ar—H), 8.36 (d, 1H, Ar—H), 8.57 (d, 2H, Ar—H). IR (KBr)/cm^{-1}: 3512, 1615, 1603, 1493, 1090, 764, 623. Anal Required for $C_{24}H_{33}Cl_2CoN_4O_{11}\cdot 1.5H_2O$: C, 40.58, H, 5.11, N, 7.89%. Found: C, 40.69, H, 5.08 N, 8.03%. MS (ESI positive ion): *m/z*: 242.094 ([Co(tren)(iPrflav)]$^{2+}$), 483.180 ([Co(tren)(iPrflav)-H]$^{+}$).

2.2.5.15. [Co(tpa)(iPrflav)]$(ClO_4)_2$ ***(19)***. Using iPrflavH (50.00 mg 0.18 mmol), NaOH (7.2 mg, 0.18 mmol) and [Co(*tpa*)Cl_2]Cl (55.54 mg, 0.18 mmol). The pure product was obtained after recrystallization from MeOH. Brown solid, yield: 34.15 mg (22%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 1.23 (d, 2H, -CH_3-isomer B), 1.39 (d, 4H, -CH_3-isomer A), 2.96 (m, 0.3H, -CH-isomer B), 3.14 (m, 0.7H, -CH-isomer A), 5.20–5.29 (m, 2H, -CH_2), 5.32 (s, 0.6H, -CH_2-isomer B), 5.35 (s, 1.4H, -CH_2-isomer A), 5.58 (d, 0.6H, -CH_2-isomer B), 5.85 (d, 1.4H, -CH_2-isomer A), 7.79–7.89 (m, 4H, Ar—H), 7.96–7.98 (m, 1H, Ar—H), 8.04–8.08 (m, 2H, Ar—H), 8.12 (t, 2H, Ar—H), 8.34 (d, 0.7H, Ar-H-isomer A), 8.38–8.40 (m, 1H, Ar—H), 8.49 (d, 2H, Ar—H), 8.99 (d, 1H, Ar—H), 9.03 (d, 0.3H, Ar-H-isomer B), 9.18 (d, 0.7H, Ar-H-isomer A), 9.60 (d, 0.3H, Ar-H-isomer B). IR (KBr)/cm^{-1}: 3435, 1492, 1431, 1094, 765, 623. Anal Required for $C_{36}H_{33}Cl_2CoN_4O_{11}$: C, 52.25, H, 4.02, N, 6.77%. Found: C, 52.58, H, 4.15, N, 6.69%. MS (ESI positive ion): *m/z*: 314.096 ([Co(tpa)(iPrflav)]$^{2+}$).

2.2.5.16. [Co(tren)(nar)](Cl)(ClO_4) ***(20)***. Using narH (87.12 mg 0.32 mmol), NaOH (12.8 mg, 0.32 mmol) and [Co(tren)Cl_2]Cl (100.00 mg, 0.32 mmol). The pure product was obtained after recrystallization from MeOH. Brown solid, yield: 89.19 mg (46%). ^{1}H NMR (400 MHz, DMSO): δ/ppm = 2.70–2.89 (m, 4H, -CH_2), 2.91–2.99 (m, 2H, -CH_2), 3.00–3.15 (m, 3H, -CH_2), 3.18–3.37 (m, 5H, -CH_2) 2.78 (m, 3H, -CH_2 tren), 5.22–5.55 (m, 6.6H, -NH_2 tren, -CH-isomer A), 5.75 (d, 0.6H, Ar-H-isomer A), 5.78 (d, 0.4H, Ar-H-isomer B), 5.98 (m, 0.4H, -CH-isomer B), 6.05 (d, 0.6H, Ar-H-isomer A), 6.15 (d, 0.4H, Ar-H-isomer B), 6.88–6.95 (m, 2H, Ar—H), 7.37–7.42 (m, 2H, Ar—H). IR (KBr)/cm^{-1}: 3421, 1610, 1100, 836, 624. Anal Required for $C_{21}H_{29}Cl_2CoN_4O_9\cdot$-MeOH: C, 41.07, H, 5.17, N, 8.71%. Found: C, 40.81, H, 4.99, N, 8.54%. MS (ESI positive ion): *m/z*: 238.075 ([Co(tren)(nar)]$^{2+}$), 475.141 ([Co(tren)(nar)-H]$^{+}$), 575.098 ([Co(tren)(nar) + ClO_4]$^{+}$).

2.3. NMR, IR and Electrospray Ionization Mass Spectrometric (ESI-MS) measurements

NMR measurements were carried out using a Bruker Avance 400 NMR spectrometer at room temperature on samples prepared in d^6-DMSO. Calibration was performed using the residual solvent peak of DMSO (2.50 ppm). IR spectra as KBr pellets were recorded on a Perkin Elmer 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 a Bruker MaXis II. uhr ESI-TOF MS instrument at the Department of Inorganic and Analytical Chemistry, University of Debrecen. The concentration of the samples was 10 μg/ml and the solvent was methanol. The instrument was equipped with an electrospray ion source, where the voltage was 4.5 kV. The drying gas was N_2. The flow rate was 4 l/min and the drying temperature was 200 °C. Na-formate was injected after each separation enabling internal *m/z* calibration. The spectra were evaluated with the Bruker Compass Data Analysis 4.4. software.

2.4. Crystal structure analysis

X-ray data were collected on a Bruker D8 Venture type diffractometer with Mo (λ = 0.71073 Å) and Cu (λ = 1.54184 Å) radiation. Molecular structures of the compounds were solved using direct methods and refined on F^2 using SHELXL [28] program incorporated into WINGX [29] suite. Tables were extracted from the CIF files with PublCIF program [30] and PLATON [31] was used for the crystallographic calculations. Details of data collection and refinement with the data of the crystals were given in Supplementary Table S1. Deposition numbers for structures published in this paper are CCDC 2,041,489–2,041,497 for complexes **5**, **8**, **9**, **11**, **13**, **15**, **18** as well as for NO_2flavH **(3)** and MeflavH, respectively.

2.5. Cyclic voltammetric (CV) studies

Cyclic voltammetric measurements were performed within the voltage range 500 to −1200 mV, at room temperature in water:methanol = 1:1, using a BASi Epsilon Eclipse instrument equipped with a three-electrode system, that consists of Ag/AgCl/3 M KNO_3 reference electrode ($E_{1/2}$ = +209 mV vs. NHE), a platinum wire auxiliary electrode (ALS Co. Japan), and a glassy carbon (CHI104) working electrode. Aqueous solution of $K_3[Fe(CN)_6]$ was used to calibrate the system ($E_{1/2}$ = +458 mV vs. NHE in 0.50 M KCl) [32]. The samples were degassed before the measurements using argon gas. The concentration of the samples was 1 mM and the potentional sweep rates were 200 mVs^{-1} during the determination of the redox potentials. 0.20 M KNO_3 was used as supporting electrolyte.

2.6. Biological studies

2.6.1. Cytotoxicity studies

Cell Culturing. Cells were thawed and at least passaged twice under normoxia (21% O_2) before starting cytotoxicity experiments. Each cell line was cultured in Dulbecco's Modified Eagle Medium with phenol red, supplemented with 8.0% *v*/v fetal calf serum (FCS), 0.2% v/v penicillin/streptomycin (P/S), and 0.9% v/v Glutamine-S (GM) (DMEM complete). Cells were cultured in either 25 cm^2 or 75 cm^2 flasks and split at 70–80% confluence (three times per week for 25 cm^2 flasks, once per week for 75 cm^2 flasks). The normoxic cells were incubated in the dark at 21% O_2, 37 °C and 7.0% CO_2. The hypoxic cells were incubated in the dark using similar conditions except the O_2 concentration which was lower (1%, using a PHCbi O_2/CO_2 incubator, MCO-170M). The medium was refreshed three times a week. Cells used in all biological experiments were cultured for a maximum of eight weeks.

Cytotoxicity assay. Human cancer cell lines (A431, human epidermoid carcinoma; A549, human lung carcinoma) were distributed by the European Collection of Cell Cultures (ECACC), and purchased through Sigma Aldrich. Dulbecco's Minimal Essential Medium (DMEM, with and without phenol red, without glutamine), 200 mM Glutamine-S (GM), trichloroacetic acid (TCA), glacial acetic acid, sulforhodamine B (SRB), tris(hydroxylmethyl)aminomethane (tris base), 4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodo-fluorescein disodium salt (rose bengal), and cis-diamineplatinum(II) dichloride (cisplatin), were purchased from Sigma Aldrich. (2*R*,3*R*,4*R*,5*R*)-hexan-1,2,3,4,5,6-hexol (D-mannitol) was purchased from Santa Cruz Biotechnology via Bio-Connect. Fetal calf serum (FCS) was purchased from Hyclone. Penicillin and streptomycin were purchased from Duchefa and were diluted to a 100 mg/ml penicillin/streptomycin solution (P/S). Trypsin and Opti-MEM® (without phenol red) were purchased from Gibco® Life Technologies. Trypan blue (0.4% in 0.81% sodium chloride and 0.06% potassium phosphate dibasic solution) was purchased from BioRad. Plastic disposable flasks and 96-well plates were from Sarstedt. Cells were counted using a Bio-Rad TC10 automated cell counter with BioRad Cell Counting Slides. UV–vis measurements for analysis of 96-well plates were performed on a M1000 Tecan Reader. Cells were inspected with an Olympus IX81 microscope.

The cytotoxicity of the compounds to two human cancer cell lines (A431, A549) was assessed using an assay described by Hopkins et al. [33]. In short, cell cultures with a maximum confluence of 70–80% were trypsinized and centrifuged (1.5 min, 1.2 relative centrifugal force),

trypsin and DMEM complete were removed, and the cells were re-suspended using Opti-MEM supplemented with 2.4% *v*/v FCS, 0.2% v/v P/S, and 1.0% v/v GM, (hereafter called Opti- MEM complete). 10 μL of cell suspension and 10 μL of trypan blue were mixed and pipetted into a cell counting slide, and cells were counted using a BioRad TC10 automated cell counter. The cell suspension was diluted to the appropriate seeding density (A431, 8×10^3; A549, 5×10^3 cells/well) and seeded in the wells of a 96-well plate. The aqueous cisplatin positive control solution was prepared from a stock solution based on clinical formulation (3.3 mM cisplatin, 55 mM mannitol, 154 mM NaCl). Sterilized dimethylsulfoxide (DMSO) was used to dissolve the cobalt complexes or ligands, in such amounts that the maximum % (*v*/v) of DMSO per well did not exceed 0.5% (v/v).

Both for normoxic and hypoxic conditions the complete cytotoxicity experiment lasted 96 h: cells were seeded at $t = 0$ h, treated at $t = 24$ h, and the cells were fixed at $t = 96$ h. 96-well plates were seeded with the correct amount of cells in 100 μL Opti-MEM complete per well. Border wells B12-G12 were seeded with cells for qualitative positive controls such as cisplatin. In the remaining outer wells 200 μL of Opti-MEM complete was pipetted.

For the cytotoxicity assay under hypoxia, the following modification were applied following Lameijer et al. [34]: the cells were cultured at least for two weeks under hypoxia before starting the cytotoxic assay. For the assay itself, after seeding the cells in air, the plates were incubated under hypoxic conditions for 24 h. Treatment was realized in air at t = 24 h. The cells were put back in the hypoxic incubator and incubated for 72 h. The cells were finally fixed at $t = 96$ h for the end-point SRB assay.

Both for normoxic and hypoxic cytotoxicity assay, the end-point (t = 96 h) sulforhodamine B

(SRB) assay was performed following Vichai et al. In short, cells were fixed at t = 96 h by adding cold trichloroacetic acid (10% (*v*/v) 100 μL) in each well. The plates were stored at 4 °C for 14 h, then the TCA medium mixture was removed, the cells were rinsed with demineralized water three times, and air dried. Then, each well was stained with 100 μL SRB solution (0.6% *w/v* SRB in 1% v/v acetic acid) for 30 min, the SRB solution was removed, and washed with acetic acid (1% v/v) until no SRB came off, normally requiring 3–5 times. Once the plates were air-dried overnight, 200 μL of tris base (tromethamine, 10 mM) was pipetted to each well. To determine the cell viability the absorbance at 510 nm was measured using a M1000 Tecan Reader. To make sure all the SRB was dissolved, this measurement was performed at least 30 min after addition of tris base. The SRB absorbance data per compound per concentration were averaged over three identical wells (technical replicates, nt = 3) in Excel and made suitable for use in GraphPad Prism. Relative cell populations were derived from the average of the untreated controls ($n_t = 6$). The data from three independent biological replications were used to obtain the dose-response curves and EC_{50} values using non-linear regression of Hills-slope equation with a fixed Y maximum (100%) and minimum (0%) relative cell population values.

2.6.2. Lipophilicity measurements

Distribution coefficients ($D_{7.40}$) of compounds **5**, **6**, **9**, **10**, **15**, **16** were determined by shake-flask method in *n*-octanol/buffered aqueous solution at pH = 7.40 phosphate bufferered saline (PBS) at 25.0 °C. The complexes were dissolved in *n*-octanol pre-saturated buffered aqueous phase with the concentration of ~100 μM. Aqueous buffered phases and water pre-saturated *n*-octanolic phases were mixed in 1:3 volume ratios for 16 h. After phase separation, the UV–Vis spectrum of the complex in the aqueous phase was compared to that of the original stock solution and the corresponding $D_{7.40}$ values were calculated according to the following equation:

$$D_{7.40} = \left[\frac{\text{A (stock sol.)}}{\text{A (aqueous phase after separation)}} - 1 \right] \text{x} \frac{\text{V (aqueous phase)}}{\text{V } (n - \text{octanol phase})} \quad (1)$$

The UV–Vis spectra were recorded in the interval 200–500 nm using a Perkin Elmer Lambda 25 UV/Vis type spectrophotometer.

3. Results and discussion

3.1. Synthesis and characterization

The chalcone derivatives **1** and **2** were synthesized by Claisen condensation of the appropriately substituated acetophenone and 3-nitrobenzaldehyde in methanol (Scheme 1). Crude products were purified by recrystallization from methanol leading to the final compounds with high yield. The obtained chalcones were converted into the corresponding flavonols **3** and **4** in Algar-Flynn-Oyamada reaction using NaOH and 30% H_2O_2 (Scheme 1). The final products were obtained after recrystallization of the crude products from acetic acid [35–37].

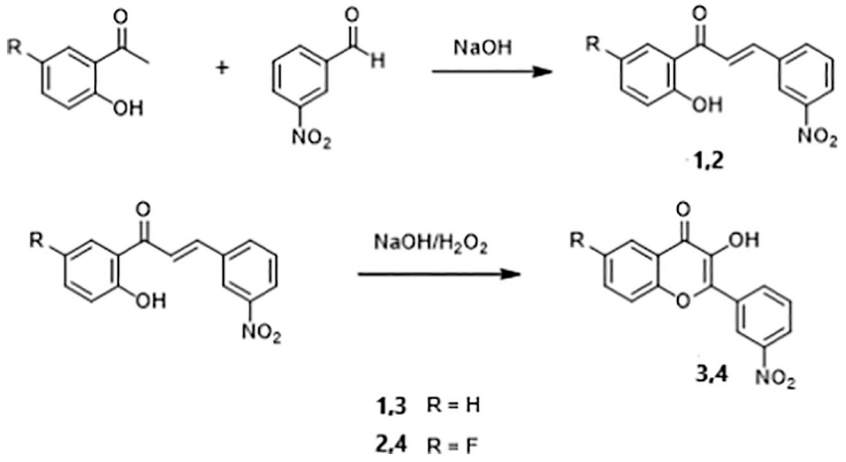
Stoichiometric amount of $[Co(4N)Cl_2]Cl$ (4N = tren or tpa) precursor and one of the different types of flavone/flavonol/flavanone ligands were reacted in MeOH in the presence of one equivalent base at 60 °C overnight. After the solvent was changed to water, mostly brownish coloured crystalline solids appeared in few days at room temperature by adding 2 eq. bulky ClO_4^- anion to the reaction mixture. For **11**, the complex was obtained in the absence of the ClO_4^- ion. For **3**, **5**, **8**, **9**, **11**, **13**, **15**, **18** and MeflavH slow evaporation of the solution at room temperature afforded single crystals of the compounds suitable for X-ray diffraction studies. All of the novel complexes are air stable crystalline solids soluble in polar solvents, (MeOH, DMSO) and, in low concentration, in water too.

^{1}H NMR spectra of the complexes showed the expected resonance signals and indicated the appearance of isomers in many cases. In the spectra of the tren complexes the signals of the $–CH_2$ groups of tren appear in the range of 3.6–2.6 ppm. Therefore, for the samples prepared in d^6-DMSO, signals of the $–NH_2$ groups also appeared at around 5.5 ppm. For these products the assignation of the signals of the different groups of the two ligands was simple, because of the aliphatic character of tren, unlike the flavH ligands with mostly aromatic protons. For **5**, **7**, **9**, **17**, **20** the ^{1}H NMR spectra of the compounds clearly indicated the presence of two geometric isomers. As it is illustrated in Fig. 2 these are likely the *cis*- and *trans*-isomers depending on the position of the coordinated deprotonated hydroxyl group to the tertiary amine of the tripodal tren or tpa.

The isomerisation was investigated on the signal of a $–CH_2$ group at around 3.5 ppm, the signals of the $–NH_2$ groups both belonging to the tren ligand and the signals of some aromatic or methyl protons of the bioligand, therefore these peaks were separated well enough to identify which isomer they belong to. Based on the integral values of these signals, the ratio of the two isomers was ~2:1 in all cases. Complete assignation of the aromatic peaks was achievable for **9** and **17**. As a representative example, the aromatic region of the spectrum of **9** with the assignation is presented in Fig. 3.

In Fig. 3 *3′* and *5′* protons have one signal in each isomers. The peak with higher intensity at 7.40 ppm belongs to the main A isomer while the lower intensity signal next to it, at 7.47 ppm relates to the same protons in isomer B. The corresponding signals of protons *2′* and *6′* of the isomers overlap at 8.5–8.6 ppm, but the difference between the intensity of the peaks of the two isomers is still detectable. For *6* (7.60 ppm) and *7* (7.92 ppm), the multiplicity of the peaks indicates the presence of the two isomers. Signals of *8* and *5* protons of the isomers are separated more in the spectrum. The highest difference in chemical shifts is observed between the peaks corresponding to *5* proton of the two isomers most likely due to the vicinity of this proton to the Co(4N) core. Assignation of the signals to the corresponding protons is further supported by COSY measurements (Supplementary Fig. S1). Notably, for **11**, **13**, **15**, **18** the isolated complexes contanied only one isomer. A representative aromatic region of spectrum of **18** is presented in Supplementary Fig. S2.

Assignation of the NMR signals was more difficult for the analogous tpa complexes due to the presence of the pyridyl signals making



Scheme 1. Synthesis of the chalcones and flavonols.

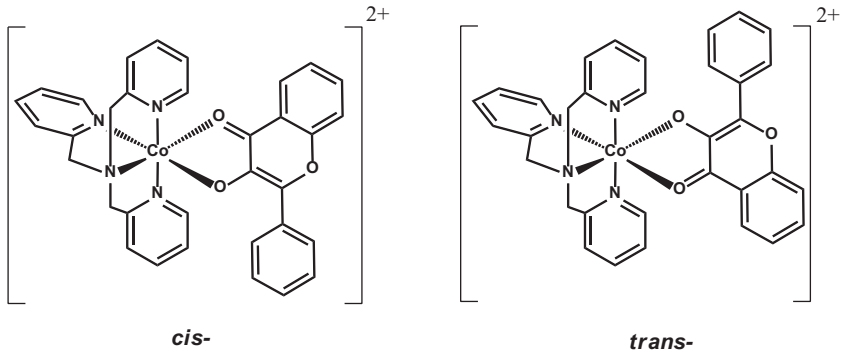


Fig. 2. The two isomers of the studied complexes using the example of $[Co(tpa)(flav)]^{2+}$ (**6**).

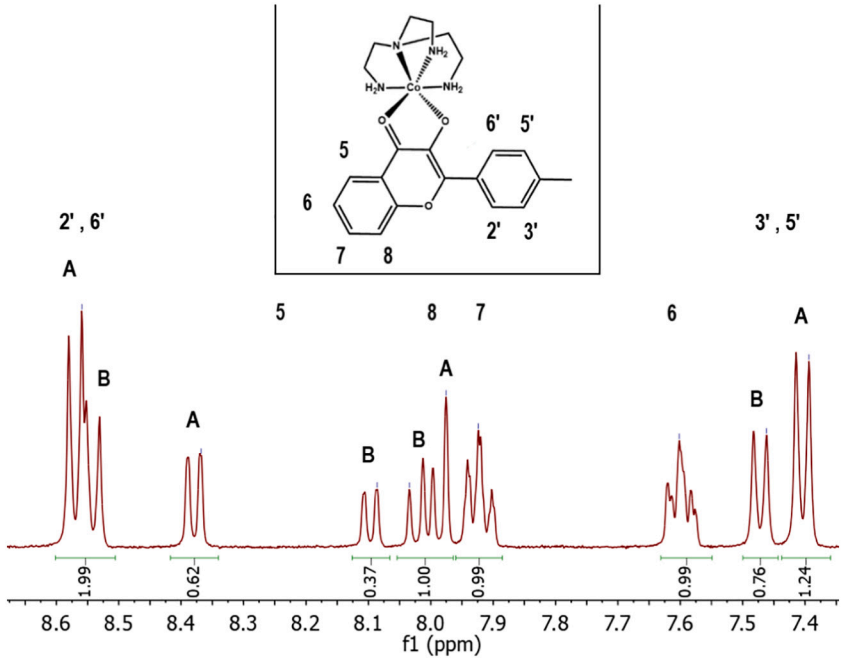


Fig. 3. Aromatic region of the ^{1}H NMR spectrum of **9** in d^{6}-DMSO with the assignation of the signals. A and B indicate the signals belonging to the two different isomers of the complex.

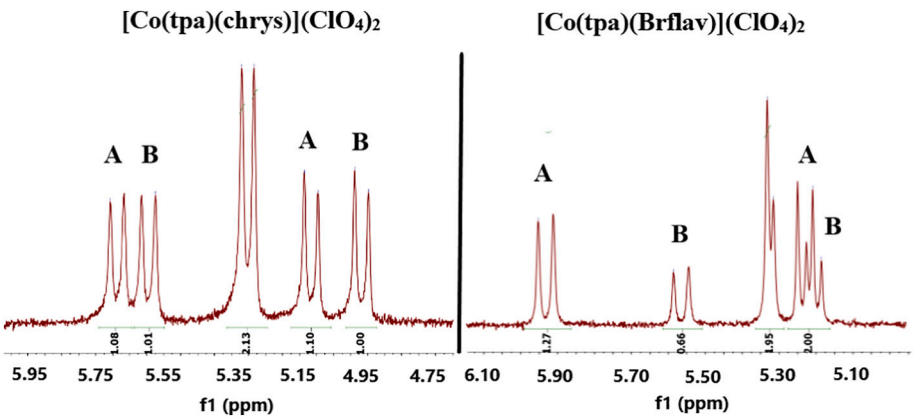


Fig. 4. Aliphatic region of the ^{1}H NMR spectrum of **16** and **8** in d^{6}-DMSO with the assignation of the signals of the isomers. For **16** the ratio of the two isomers is ~1:1 while for **8**– 2:1.

crowded the low field part of the spectra. As it is illustrated in Fig. 4 for **16** and **8**, in the aliphatic region the –CH_2 groups of coordinating tpa showed two sets of signals belonging to two isomers (A and B): two doublets and one singlet. This reveals that protons of two –CH_2 groups in axial position in the complexes are non-equivalent and coupling of them results in doublets in the spectrum. On the contrary, the singulet for the third methylene group supports the equivalence of its two protons due to equatorial position of this arm in the complexes [5,6]. For all these complexes (**6**, **8**, **10**, **12**, **14**, **16, 19**) ^{1}H NMR spectra showed the mixture of the two isomers. Based on the relative intensity of the –CH_2 tpa

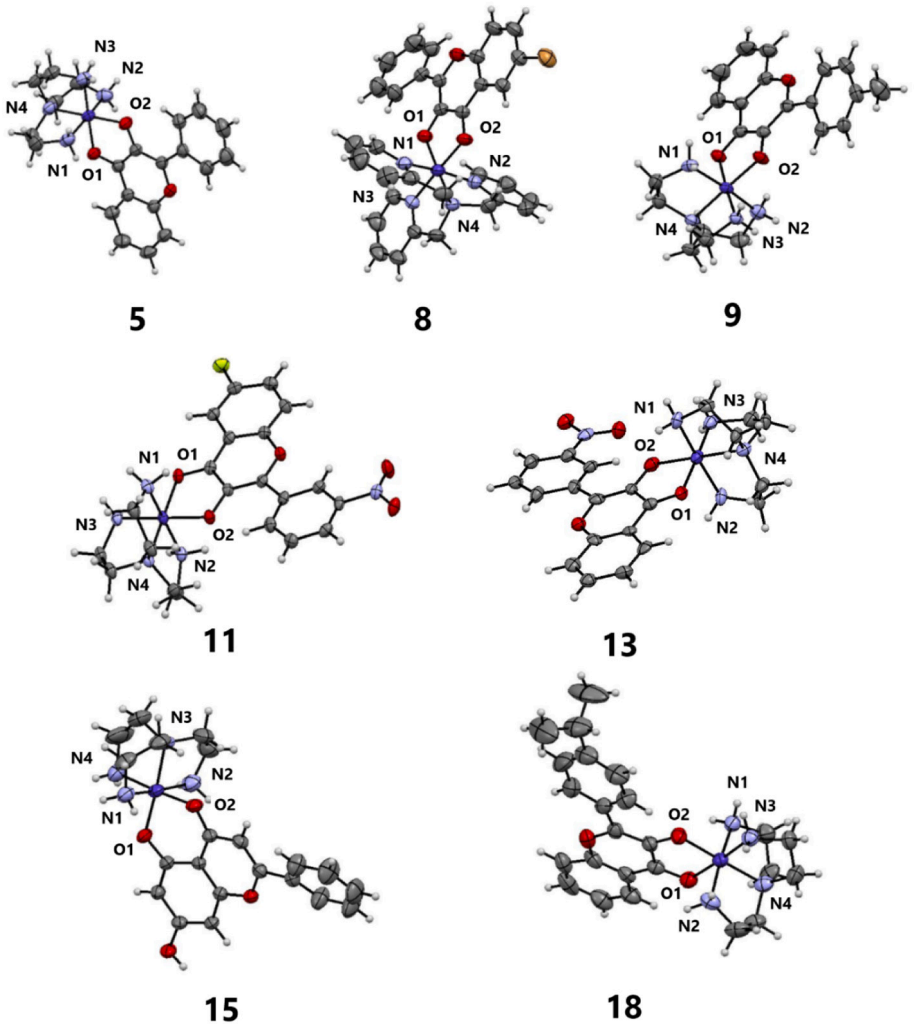


Fig. 5. Ortep view of [Co(tren)(flav)]$(ClO_4)_2 \cdot H_2O$ (**5**), [Co(tpa)(Brflav)]$(ClO_4)_2 \cdot 0.25H_2O$ (**8**), [Co(tren)(Meflav)]$(ClO_4)(Cl) \cdot H_2O$ (**9**), [Co(tren)(NO_2Fflav)]$(Cl)_2 \cdot 4$MeOH (**11**), [Co(tren)(NO_2flav)]$(ClO_4)_2 \cdot 2$MeOH (**13**), [Co(tren)(chrys)]$_2(ClO_4)_2(Cl)_2 \cdot 3H_2O$ (**15**) and [Co(tren)(iPrflav)]$(ClO_4)(Cl) \cdot$MeOH (**18**). Thermal ellipsoids shown at 40% probability level with partial numbering scheme. The counter ions and solvent molecules are omitted for clarity.

protons, the ratio of the isomers for **6**, **8, 10, 12, 14, 19** was found to be ~2:1, the same like in the case of tren complexes (vide supra). The only exception was **16**, where the intensity of the signals of the two isomers were almost identical indicating ~1:1 ratio of the isomers (Fig. 4).

ESI-MS analysis in the positive mode provided further proof for the identity of the complexes. In the mass spectra of all products, the peaks, belonging to the $[Co(4N)(flav)]^{2+}$ or $[Co(4N)(flav)\text{-}H]^{+}$ ions, appeared. For **5**, **16**, **17**, **20**, under ESI-MS conditions, the ClO_4^- counter ions did not dissociate completely either, in agreement with former literature findings [6]. All the mass spectra displayed the correct isotopic pattern (Supplementary Fig. S3).

For all the new ligands and complexes the microanalytical results also proved their integrity and purity as the experimental data are in good agreement with the calculated ones. For **13, 15** and **20** the CHN data showed that the complex crystallized with mixed counter ions, one ClO_4^- and one Cl^-. The results also indicated that most of the complexes also have water molecules in their crystal structure and this was also detected during the X-ray diffraction studies.

Molecular structure of the novel compounds **5**, **8**, **9**, **11**, **13**, **15**, **18** as well as **3** and MeflavH were investigated by single crystal X-ray structure determination. For the complexes the determined structures are shown in Fig. 5 while for **3** and MeflavH in Supplementary Fig. S4.

X-ray studies confirmed the expected structure and octahedral geometry of the complexes in all cases. The structures consist of one 4N donor ligand and one flavonolato ligand coordinating to the Co(III) centre. For all complexes the flavonols are bound to the metal ion via the carbonyl and the hydroxyl O donor atoms. Based on the obtained molecular structures complexes **3**, **5**, **8**, **9**, **13**, **15**, **18** were crystallized as the *trans*-isomer since the tertiary amine of the 4N ligand was observed to be *trans* to the deprotonated hydroxyl group of the flavonol ligand. Only complex **11** was found to be crystallized as the *cis*-isomer. It should be emphasized, however, that the crystals were selected by chance and in each case only one of them was analyzed. As the NMR pointed out some of the isolated solid complexes are mixture of isomers (vide supra). In the structure of **15** two cobalt complex molecules were found in the asymmetric unit with two Cl^- and two ClO_4^- counter ions proving the possibility that these type of complexes can be crystallized with mixed anions. The Co—O bond lengths were found to be shorter than the Co—N lengths, which is in accordance with the data of similar type of Co(III) complexes [38]. The length of the two different Co—O bonds were slightly different for all investigated complexes, that was obsereved in the case of previously published Ru(II) complexes of flavonols [36,39]. The key bond length and bond angle values are shown in Supplementary Table S2.

3.2. Electrochemical studies

Cyclic voltammetry was used to explore the redox properties and to determine the peak potential values of the synthesized Co(III) complexes. Electrochemical measurements were carried out in the +600 to −1000 mV voltage range under the conditions detailed in the Experimental part. All the determined cathodic (E_{pc}) or anodic (E_{pa}) peak potentials measured against Ag/AgCl were converted to NHE. The obtained E_{pc} and E_{pa} values of the complexes are summarized in Table 2.

Results of the electrochemical studies revealed major differences between the tren and tpa complexes in agreement with former literature findings [5,6,10,41]. In particular, the recorded cyclic voltammograms of the tren complexes showed irreversible reduction while those of the tpa analogues exhibited reversible redox processes of the complexes. Although typically the $\Delta E = E_{pa} - E_{pc}$ or the $E_{1/2} = (E_{pa} - E_{pc})/2$ values are used for the comparison of reversible redox processes inasmuch as this is not the case here for the tren complexes, Table 2 contains the E_{pa} and E_{pc} values instead. The difference in reversibility indicates that upon reduction the tren complexes dissociate practically completely due to the low stability of the corresponding Co(II) complex. On the contrary, the reversible reduction for the tpa complexes can be explained by the higher thermodynamic stability of the Co(II) complex formed and decreased rate of dissociation of the ligands, which are due the π-back-bonding capability of tpa. This feature of tpa also results in significantly more positive reduction peak potential values for the tpa complexes compared to the corresponding tren analogues; as a representative example this is demonstrated in Fig. 6 where voltammograms registered for **9** and **10** are presented. These results are in accordance with the findings on the redox properties of previously reported similar type Co complexes [6,40].

The reduction potential values of the complexes are also influenced by the different substituents of the flavonolato ligands. Voltammograms of 3′-NO_2-3-hydroxyflavone, **3**, and 6-F-3′-NO_2-3-hydroxyflavone **4**, complexes showed higher reduction potential values than the analogous complexes of the unsubstituted flavonol indicating that the strongly deactivating -NO_2 group with electron withdrawing character makes the complex more reducible by decreasing the electron density at the cobalt ion. Flavonolato complexes having activating substituents with electron donating property found to have more negative reduction potentials than the complexes of the unsubstituted flavonol. This can be explained by the increased electron density at the metal ion leading to a less reducible complex. Data in Table 2 also reveal that the chrysin and naringenin complexes exhibit the most negative E_{pc} values among the studied ones. This is most likely due to the size of the (O,O) chelate ring formed with the metal ion being six with these ligands. The low E_{pc} values indicate that these complexes with six membered chelate rings are harder to be reduced than the ones containing five membered chelate ring. Moreover, both chrysin and naringenin contain activating –OH substituent(s) that can also cause shift of the E_{pc} values of the complexes to the more negative region. Registered CV curves of all

Table 2
Cathodic (E_{pc}) and anodic (E_{pa}) peak potential values (mV vs. NHE) of the $[Co(4N)(flav)]^{2+}$ (4N = tren or tpa, see Table 1) complexes using glassy carbon electrode, in water: MeOH 1:1, I = 0.20 M KNO_3, at a sweep rate of 200 mVs^{-1}.

Flavonolate ligand in $[Co(4N)(flav)]^{2+}$	4N = tren		4N = tpa		
	#	E_{pc} (mV)	#	E_{pc} (mV)	E_{pa} (mV)
flav	**5**	−206	**6**	51	197
Brflav	**7**	−285	**8**	69	241
Meflav	**9**	−187	**10**	57	185
NO_2Fflav	**11**	−194	**12**	71	309
NO_2flav	**13**	−175	**14**	80	252
chrys	**15**	−331	**16**	−3	94
ClOMeflav	**17**	−264	**–**	–	–
iPrflav	**18**	−291	**19**	29	187
nar	**20**	−532	**–**	–	–

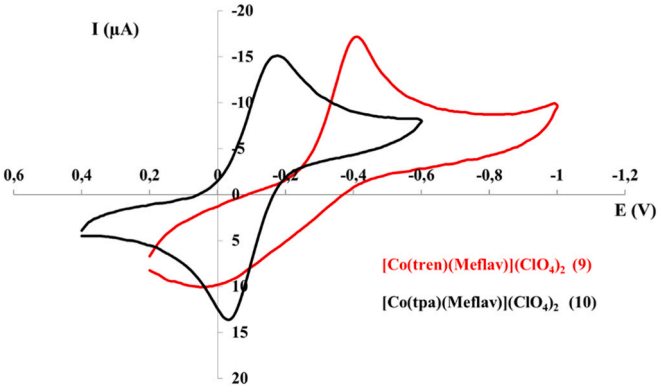


Fig. 6. Cyclic voltammetry curves registered for $[Co(tren)(MeFlav)](ClO_4)_2$ (**9**) and $[Co(tpa)(MeFlav)](ClO_4)_2$ (**10**) in H_2O:MeOH = 1:1, referenced to Ag/AgCl electrode at a potential sweep rate of 200 mV/s, I = 0.20 M KNO_3 and c = 1.0 mM.

complexes are presented in Supplementary Fig. S5.

Since the reduction potential range of cellular reductases is known to fall within the range of −200 to −400 mV vs. NHE [6,42–46], if the E_{pc} values of the complexes are considered in Table 2 the tren complexes might be suitable for selective reduction in the reductive environment of the tumor tissues. On the contrary, the E_{pc} values of the tpa analogues in general might be too high for the selective reduction of the complex in the cancer cells. In order to obtain more information regarding this assumption 10 fold excess of ascorbic acid was added to an aqueous solution of complex **7** and the UV–Vis spectrum in the range 350–800 nm was monitored for 48 h. No measureable change was observed revealing no reduction of the complex under these conditions.This indicates that the E_{pc} value alone is not enough to predict the mechanism of a reduction process occuring in a biological system.

3.3. Biological studies

Stability of the complexes in aqueous solution was monitored with the aid of ^{1}H NMR before the biological studies. The measurements were performed in a D_2O: DMSO = 5: 1 (*v*/v) solvent mixture. No changes were observed in the ^{1}H NMR spectra of the complexes after 48 h at room temperature, indicating that they are stable under these conditions and suitable for further biological studies. As an illustration ^{1}H NMR spectra of **9** recorded at 0 h and in 48 h are shown in Supplementary Fig. S6.

The cytotoxic activity of most of the complexes and of the free flavone ligands was tested against A549 (lung carcinoma) and A431 (skin epidermoid carcinoma) human cancer cell lines under both normoxic (21% O_2) and hypoxic (1% O_2) conditions. The determined cell growth inhibition effective concentrations (EC_{50} in μM) are indicated in Table 3.

In accordance with previous literature results none of the precursor $[Co(4N)Cl_2]Cl$ complexes were found to be active [6,40,41]. The A549 cell line was significantly more sensitive to the tested compounds than A431, with the exception of **9** for which the EC_{50} values were found lower for the A431 cells than for A549. Out of the free ligands MeflavH and chrysH showed no cytotoxicity against either cell types while flavH was slightly active against the A549 cell line under normoxic conditions only. Ligands NO_2flavH, NO_2FflavH and BrflavH were active only against A549 either but their cytotoxic effects were found to be significant in this cell line. These results show the remarkable effects of the different substituents of the biological flavonoid ligands on their cytotoxicity, in agreement with literature data [47].

Among the cobalt(III) compounds the tpa analogues were found to be significantly more active comparing to the corresponding tren complexes. This tendency was again in good agreement with the cytotoxicity results obtained for the tren and tpa cobalt complexes of the 1-methyl-3-(2-naphtyl)propane-1,3-dione ligand (naacH), investigated by the Hambley group [40]. Low IC_{50} values were found for complexes **6**, **8**, **12** and **14,** indicating their high antitumor activity against the A549 cell line, even under hypoxia. Since the corresponding free ligands showed lower cytotoxicity than their cobalt complexes, it appears that the obtained antitumor activity was clearly enhanced upon complexation. However, only compound **11** showed a statistically significantly lower EC_{50} value under hypoxia compared with normoxia; compound **7** showed a slightly lower EC_{50} value under hypoxia, but that was not

Table 3
Cytotoxicity of the compounds against A549 (lung carcinoma) and A431 (skin epidermoid carcinoma) human cancer cell lines under normoxic (21% O_2) and hypoxic (1% O_2) conditions determined after 72 h incubation by an end-point SRB assay. Cell-growing inhibition effective concentrations (EC_{50}) are reported in μM with 95% confidence interval (CI) in μM. Data is the mean over three independent experiments.

Compound	EC_{50}/μM							
	A431				A549			
	Normoxia	CI	Hypoxia	CI	Normoxia	CI	Hypoxia	CI
$[Co(tren)Cl_2]Cl$	>100		>100		>100		>100	
$[Co(tpa)Cl_2]Cl$	>100		>100		>100		>100	
chrysH	>100		>100		>100		>100	
flavH	>100		>100		36	+7 −4	>100	
MeflavH	>100		>100		>100		>100	
BrflavH	84	+29 −19	>100		7.0	+1.0 −1.0	12	+7 −4
NO_2flavH	64	+7 −6	>100		15	+3 −2	17	+3 −3
NO_2FflavH	>100		>100		16	+2 −2	15	+3 −2
$[Co(tren)(flav)](ClO_4)_2$ (**5**)	>100		>100		>100		>100	
$[Co(tpa)(flav)](ClO_4)_2$ (**6**)	45	+4 −3	47	+7 −6	8.3	+1.2 1.1	21	+3 −4
$[Co(tren)(Meflav)](ClO_4)_2$ (**9**)	44	+5 −4	88	+15 −12	>100		>100	
$[Co(tpa)(Meflav)](ClO_4)_2$ (**10**)	17	+3 −2	26	+4 −4	14	+1 −1	17	+11 −7
$[Co(tren)(Brflav)](ClO_4)_2$ (**7**)	55	+11 −9	91	+39 −23	89	+12 −12	73	+20 −16
$[Co(tpa)(Brflav)](ClO_4)_2$ (**8**)	13	+2 −2	17	+2 −2	6.8	+1.0 −0.8	8.9	+3.6 −2.9
$[Co(tren)(chrys)](ClO_4)_2$ (**15**)	>100		>100		>100		>100	
$[Co(tpa)(chrys)](ClO_4)_2$ (**16**)	68	+17 −13	64	+15 −12	34	+4 −3	33	+5 −4
$[Co(tren)(NO_2Fflav)](ClO_4)_2$ (**11**)	>100		>100		72	+15 −12	48	+8 −7
$[Co(tpa)(NO_2Fflav)](ClO_4)_2$ (**12**)	15	+3 −2	22	+4 −3	5.2	+0.7 −0.6	6.9	+1.2 −1.1
$[Co(tpa)(NO_2flav)](ClO_4)_2$ (**14**)	14	+4 −3	23	+5 −4	6.3	+1.0 −0.9	7.3	+1.3 −1.4
Cisplatin	3.2	+0.7 −0.6	5.6	+1.3 −1.1	1.8	+0.2 −0.2	2.4	+0.7 −0.6

statistically significantly different from the normoxia value, while all other complexes showed either identical or slightly lower toxicity under hypoxia, compared to normoxia.

While for the tren compounds only a mild toxicity or no toxicity at all was found, one should note that making cytotoxic agents that do not lose their cytotoxicity under hypoxia, is an interesting result. Indeed, many cytotoxic agents lose at least part of their toxicity due to increased resistance of hypoxic cells.

In order to explore the reason of the differences between the biological activity of tren and tpa complexes the partition coefficient log $D_{7.40}$ of a selection of six cobalt complexes were measured to assess on their lipophilicity and ability to cross biological membranes by passive diffusion. The log $D_{7.40}$ values of the studied complexes are shown in Table 4. In all cases the log $D_{7.40}$ values were higher for the tpa complexes, compared to the tren analogues, indicating the more lipophilic character of the former ones, while the latter ones were found all relatively water-soluble. This finding can be explained by the three aromatic pyridyl groups in the tpa ligand, which increase the lipopilicity of the complexes. These results also correlate with the cytotoxicity studies, as all complexes of the tpa series were found more cytotoxic than their tren analogue. Therefore, the higher lipophilic character of the tpa complexes is probably one reason for their higher anticancer activity.

Based on these results, we hypothesize that although the reduction potentials of the tren series of complexes are negative enough for them to be reduced in hypoxic cells, their anticancer activity was too low to measure significant activation under hypoxia because of their more hydrophilic character and low cellular uptake. Mild hypoxia activation was observed for the tren complex **11** which bears one of the most active cytotoxic flavonoid ligands of the series, i.e. NO_2Fflav. The reduction potential values of tpa complexes are probably too positive for triggering activation under hypoxia, although these complexes show significant cytotoxicity in A549 lung cancer cells due to their higher lipophilicity and probably better cellular uptake. Overall, an ideal hypoxia-activated cobalt(III) complex would be either a tpa derivative with a more negative E_{pc} value, or a more hydrophobic tren complex. It should also be noted that the high anticancer activity of most tpa-based cobalt(III) compound can probably not be related to the release of the active flavonol ligand, since their anticancer efficiency was found much higher than that of the corresponding free flavonol ligands. The activated cobalt(II) complex itself, reduced both under normoxia and hypoxia in the tpa series, might either have off-side cellular targeted that have not been identified yet, or generate reactive oxygen species for example via a Fenton-like reaction.

4. Conclusions

Nine substituted flavonols (flavH, MeflavH, BrflavH, ClOMeflavH, iPrflavH, NO_2flavH, NO_2FflavH, narH and chrysH) and two tetradentate nitrogen ligands (tren or tpa) were used to synthesize a series of sixteen $[Co(4N)(flav)]^{2+}$ complexes that were studied as potential hypoxia-activated anticancer agents. All analytical data, together with a series of X-ray crystal structures, are consistent with an (O,O) coordination mode of the flavonol ligand to the metal ion and with an octahedral geometry. Cyclic voltammetric studies of the complexes revealed that both the 4N ligand and the substituents on the flavonol bioligand influence the redox properties of the complexes. The tren-containing compounds were found to be reduced at significantly lower potential than their tpa analogues, while electron-donating substituents on the flavonol also shifted the reduction potential of the cobalt complex to the more negative region. All prepared tpa-containing complexes were found to have higher anticancer activity towards A549 and A431 human cancer cell lines than the corresponding free flavonol ligands, indicating that the anticancer efficiency of the bioligands can be enhanced upon complexation to the metal. However, no hypoxia activation was observed in this series of compounds because their reduction potentials are high enough to see activation already under normoxia. Although the tren cobalt complexes exhibited reduction in the good potential scale, they were not activated by hypoxia or very cytotoxic to the cancer cells, probably due to their too high hydrophilicity that is detrimental to cellular uptake. Complex **11** stands out as it showed a slight activation under hypoxia. Overall, the design of cancer-selective, hypoxia-activated cobalt complexes requires a careful balance among various parameters including the redox potential of the complex and its lipophilicity. Efforts are currently undergoing to obtain tren derivatives with higher lipophilicities and higher cellular uptake.

Table 4
Partition coefficient log $D_{7.40}$ values determined for a selection of cobalt(III) complexes at pH = 7.40 (PBS buffer), t = 25 °C.

Flavonolate ligand in $[Co(4N)(flav)]^{2+}$	4N = tren		4N = tpa	
	#	log $D_{7.40}$	#	log $D_{7.40}$
flav	**5**	−0.141	**6**	0.756
Meflav	**9**	−0.524	**10**	0.337
chrys	**15**	−0.349	**16**	−0.277

Author statement

Máté Kozsup: Synthesis, Investigation, Draft peraration; XueQuan Zhou: Investigation; Etelka Farkas: Conceptualization, Draft preparation; Attila Cs. Bényei: Investigation; Sylvestre Bonnet: Supervision, Methology, Draft preparation; Tamás Patonay (the late) and Krisztina Kónya; Providing some of the ligands; Péter Buglyó: Conceptualization, Methodology, Supervision, Writing and finalizing the manuscript.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinorgbio.2021.111382.

References

[1] L. Amable, Pharmacol. Res. 106 (2016) 27–36.
[2] T.W. Failes, C. Cullinane, C.I. Diakos, N. Yamamoto, J.G. Lyons, T.W. Hambley, Chem. Eur. J. 13 (2007) 2974–2982.
[3] T.W. Failes, T.W. Hambley, Dalton Trans. (2006) 1895–1901.
[4] P.J. Kim, R. Hocking, J.K. Clegg, P. Turner, S.M. Neville, T.W. Hambley, Dalton Trans. 41 (2012) 11293–11304.
[5] P. Buglyó, I. Kacsir, M. Kozsup, I. Nagy, S. Nagy, A.C. Bényei, É. Kovats, E. Farkas, Inorg. Chim. Acta 472 (2018) 234–242.
[6] M. Kozsup, E. Farkas, A.C. Bényei, J. Kasparkova, H. Crlikova, V. Brabec, P. Buglyó, J. Inorg. Biochem. 193 (2019) 94–105.
[7] A.K. Renfrew, N.S. Bryce, T.W. Hambley, Chem. Eur. J. 21 (2015) 15224–15234.
[8] M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, J. Agric, Food Chem. 40 (1992) 2379–2383.
[9] M. Khater, D. Ravishankar, F. Greco, H.M. Osborn, Future Med. Chem. 11 (21) (2019) 2845–2867.
[10] D. Raffa, B. Maggio, M.V. Raimondi, F. Plescia, G. Daidone, Eur. J. Med. Chem. 142 (2017) 213–228.
[11] C. Kanadaswami, L.T. Lee, P.H. Lee, J.J. Hwang, F.C. Ke, Y.T. Huang, M.T. Lee, In Vivo 19 (2005) 895–910.
[12] J.J. Champoux, Annu. Rev. Biochem. 70 (2001) 369–413.
[13] K. Chahar, N. Sharma, M.P. Dobhal, Y.C. Joshi, Pharmacogn. Rev. 5 (9) (2011).
[14] I.K. Wang, S.Y. Lin-Shian, J.K. Lin, Eur. J. Cancer 35 (1999) 1517–1525.
[15] C. Bailly, Curr. Med. Chem. 7 (2000) 39–58.

[16] Sukardiman, M. Tanjung, A. Darwanto, M.O. Darmadi, Clin. Hermorheol. Microcirc. 23 (2000) 185–190.
[17] L.M. Matrisian, Trends Genet. 6 (1990) 121–125.
[18] L.A. Liotta, Med. Sci. Am. 266 (1992) 54–59 (62–63).
[19] J.S. Rhee, L.M. Coussens, Trends Cell Biol. 12 (5) (1992) 209–211.
[20] M. Samsonowicz, E. Regulska, M. Kalinowska, Chem. Biol. Interact. 273 (2017) 245–256.
[21] M.M. Kasprzak, L. Szmigiero, E. Zyner, J. Ochocki, J. Inorg. Biochem. 105 (4) (2011) 518–528.
[22] Z. Tyeklár, R.R. Jacobson, N. Wei, N.N. Murthy, J. Zubieta, K.D. Karlin, J. Am. Chem. Soc. 115 (1993) 2611–2689.
[23] E. Kimura, S. Young, J.P. Collman, Inorg. Chem. 9 (5) (1970) 1183–1191.
[24] W. Adam, D. Golsch, L. Hadjiarapoglu, T. Patonay, Tetrahedron Lett. 32 (8) (1991) 1041–1044.
[25] R. Khanna, R. Kumar, A. Dalal, R.C. Kamboj, J. Fluoresc. 25 (2015) 1159–1163.
[26] D.A. Akrawi, T. Patonay, K. Kónya, P. Langer, Synlett. 24 (2013) 860–864.
[27] S. Das, I. Mitra, S. Batuta, M.N. Alam, K. Roy, N.A. Begum, Bioinorg. Med. Chem. Lett. 24 (21) (2014) 5050–5054.
[28] G.M. Seldrick, Acta Cryst A64 (2008) 112–122.
[29] L.G. Farrugia, J. Appl. Crystallogr. 32 (1999) 837–838.
[30] S.P. Westrip, J. Appl. Crystallogr. 43 (2010) 920–925.
[31] A.L. Spek, J. Appl. Crystallogr. 36 (2003) 7–13.
[32] E. Farkas, P. Buglyó, É.A. Enyedy, M.A. Santos, Inorg. Chim. Acta 357 (2004) 2451–2461.
[33] S.L. Hopkins, B. Siewert, S.H.C. Askes, P. Veldhuizen, R. Zwier, M. Heger, S. Bonnet, Photochem. Photobiol. Sci. 15 (2016) 644–653.
[34] L.N. Lameijer, D. Ernst, S.L. Hopkins, M.S. Meijer, S.H.C. Askes, S.E. Le Dévédec, S. Bonnet, Angew. Chem. Int. Ed. 56 (2017) 11549–11553.
[35] C.J. Mills, N.N. Mateeva, K.K. Redda, J. Heterocyclic Chem. 43 (2006) 59.
[36] A. Kurzwernhart, W. Kandioller, S. Bähler, C. Bartel, S. Martic, M. Buczkowska, G. Mühlgassner, M.A. Jakupec, H.B. Kraatz, P.J. Bednarski, V.B. Arion, D. Marko, B.K. Keppler, C.G. Hartinger, J. Med. Chem. 55 (2012) 10512–10522.
[37] C.X. Quin, X. Chen, R.A. Hughes, S.J. Williams, O.L. Woodman, J. Med. Chem. 51 (2008) 1874–1884.
[38] A.K. Renfrew, N.S. Bryce, T.W. Hambley, Chem. Sci. 4 (2013) 3731.
[39] A. Kurzwernhart, W. Kandioller, C. Bartel, S. Bähler, R. Trondl, G. Mühlgassner, M. A. Jakupec, V.B. Arion, D. Marko, B.K. Keppler, C.G. Hartinger, Chem. Commun. 48 (2012) 4839–4841.
[40] P.D. Bonnitcha, B.J. Kim, R.K. Hocking, J.K. Clegg, P. Turner, S.M. Neville, T. W. Hambley, Dalton Trans. 41 (2012) 11293.
[41] M. Kozsup, O. Dömötör, S. Nagy, E. Farkas, É.A. Enyedy, P. Buglyó, J. Inorg. Biochem. 204 (2020) 110963.
[42] B.P. Green, A.K. Renfrew, A. Glenister, P. Turner, T.W. Hambley, Dalton Trans. 46 (2017) 15897–15907.
[43] M.D. Hall, T.W. Failes, N. Yamamoto, T.W. Hambley, Dalton Trans. (2007) 3983–3990.
[44] V. Mallikarjun, D.J. Clarke, C.J. Cambell, Free Radic. Biol. Med. 53 (2012) 280–288.
[45] D.C. Ware, B.D. Palmer, W.R. Wilson, W.A. Denny, J. Med. Chem. 36 (1993) 1839–1846.
[46] J. Jiang, C. Auchinvole, K. Fischer, C.J. Cambell, Nanoscale 6 (2014) 12104–12110.
[47] A. Kurzwernhart, S. Mokesch, E. Klapproth, M.S. Adib-Ravazi, M.A. Jakupec, C. G. Hartinger, W. Kandioller, B.K. Keppler, Eur. J. Inorg. Chem. (2016) 240–246.