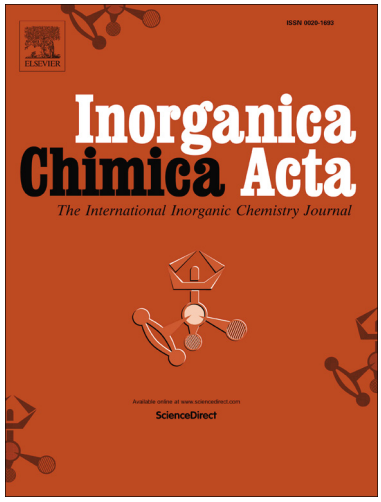

Ruthenium(II) trithiacyclononane complexes of 7,3',4'-trihydroxyflavone, chrysin and tectochrysin: synthesis, characterisation, and cytotoxic evaluation

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Ruthenium(II) trithiacyclononane complexes of 7,3',4'-trihydroxyflavone, chrysin and tectochrysin: synthesis, characterisation, and cytotoxic evaluation

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Competing Interests

The authors have no potential conflict of interest to report.

Ruthenium(II) trithiacyclononane complexes of 7,3',4'-trihydroxyflavone, chrysin and tectochrysin: synthesis, characterisation, and cytotoxic evaluation

This study describes a simple, two-step method for obtaining ruthenium(II) trithiacyclononane ([9]aneS_3) flavonate complexes in yields of 23 to 43%. With 7,3',4'-trihydroxyflavone (thflv), a neutral complex, [Ru(II)([9]aneS_3)(DMSO)(thflv)] (**1**), is formed by coordination at the catechol group. With chrysin (chrys) and tectochrysin (tchrys), Ru(II) binds to the chromenone fragment to form cationic complexes that are isolated as [Ru(II)([9]aneS_3)(chrys)(DMSO)]Cl (**2**) and [Ru(II)([9]aneS_3)(DMSO)(tchrys)]Cl (**3**). The structure of the complexes is characterised by FT-IR, NMR and ESI^+-MS. Furthermore, the flavones and the corresponding complexes, **1**-**3**, were investigated regarding their *in vitro* cytotoxic activity towards four different human tumour cell lines, PC-3 (prostate), MG-63 (osteosarcoma), MCF-7 and MDA-MB-231 (both breast adenocarcinoma).

Keywords: flavones • ruthenium(II) complexes • trithiacyclononane • cytotoxicity • cancer cell lines

1. Introduction

Flavonoids are polyphenolic natural compounds occurring in a wide variety of edible plants and known to benefit human health due to their antioxidant and, in a few cases, anti-inflammatory action [1]. Flavonoids were recently associated with other interesting medicinal effects, namely protection against fatty liver [2], cardiovascular disease [3] and with chemopreventive and chemotherapeutic actions. The vast potential of application of flavonoids has made them subject of intense scientific research. In the year 2017 alone, the number of publications on the subject was around half a thousand. In the present work, focus is given to three natural flavonoids: 7,3',4'-trihydroxyflavone (thflv), 5,7-dihydoxyflavone or

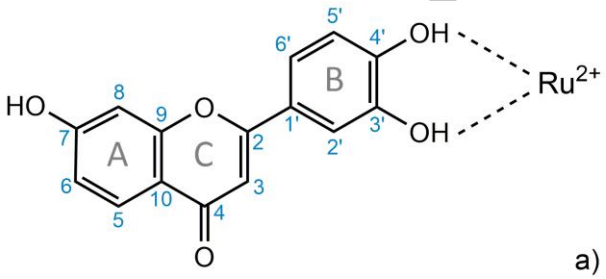
chrysin (chrys), and 5-hydroxy-7-methoxyflavone or tectochrysin (tchrys). 7,3',4'-trihydroxyflavone is also named 5-deoxyluteolin and it can be found in white clover, *Trifolium repens* [4] and in alfalfa, *Medicago sativa* [5]. It also occurs in *Butea monosperma*, a Thailandese fabaceae tree commonly known as 'flame of the forest' for its attractive, large, bright orange-reddish flowers [6]. Its application as an anti-inflammatory compound is under evaluation [7]. Chrysin occurs naturally in propolis [8], honey [9] and several species of the *Passiflora* genus [10], and it exhibits cytotoxic activity against human adenocarcinoma [11] and prostate cancer cell lines [12, 13]. Methoxyflavones and polymethoxyflavones are also in the spotlight for their recently discovered growth-inhibitory properties on human colon cancer cell lines [14, 15]. Tectochrysin (tchrys) is isolated from *Kaemperia parviflora*, or Thailand's black ginger, a herbaceous plant of the Zingiberacea family native to Thailand [16]. It was shown to induce cell death of colorectal cancer cells by the ROS-mediated mitochondrial apoptosis pathway [14].

Flavonoids have well-known metal chelating abilities, namely with iron, copper, and zinc, which may cause their depletion *in vivo* but may also be used to design new metal drugs active towards a variety of diseases related to the biological role of metals [17]. For instance, several aminoflavone complexes with cytotoxic activity are described in the literature, namely *cis*-dichlorobis(3-aminoflavone)platinum [18], *cis*-dichlorobis(3-imino-2-*R*-*O*-flavanone)ruthenium(II) (where R = CH_3 or CH_2CH_3) [19], *cis*-dichloro(3-nitrosoflavone)(3-hydroxyiminoflavanone)ruthenium(II) [20], and dichloro(*p*cym)(6- or 7-amino-flavone)ruthenium(II), where *p*cym is η^6-*p*-cymene [21]. Ruthenium complexes with *O,O'*-donor ligands are less common due to the lower affinity of this metal ion to bind oxygen, which makes their preparation more challenging. A few known examples are *trans*-*bis*(DMSO)*bis*(4'-*R*-flavone)ruthenium(II), with R = Cl, NO_2, OCH_3 or $N(CH_3)_2$ [22], *bis*(2,2'-bipyridine)(flavonolate)ruthenium(II) [23], and two families of organometallic

complexes, (*p*cym)(chlorido)(4'-*R*-3-hydroxyflavonate)ruthenium(II), with R = H, Cl, F [24], and (*p*cym)(halogeno)(4'-*R*-3-hydroxyflavonate)ruthenium(II), with R = H, Cl and *halogen* = Br, I [25]. Ruthenium complexes are interesting alternatives to platin compounds for antitumoral chemotherapy. This is associated with their more versatile ligand-exchange abilities, in tandem with the octahedral geometry (instead of planar four-coordinated Pt) and the possibility of existing in two redox states in vivo, (+2 and +3 states) [26]. The complex indazolium [*trans*-Ru(III)Cl_4bisindazole], commonly known as KP1019 and first described in the late 1990s [27], attracted interest for its excellent *in vitro* apoptotic activity against colorectal cancer [28]. To date, it remains a leading example of transition to the clinic, having successfully overcome phase I clinical trials and being presently under phase II studies [29]. Within Ru(II) complexes, emerging families include complexes with facial arene ligands, namely the RAPTA family (from *Ruthenium Arene PTA*), based on complex RAPTA-C, [Ru(II)Cl_2(1,3,5-triaza7-phosphatricyclo-[3.3.1.1]decanephosphine)(*p*cym)] [30]. A few RAPTA complexes have presently completed preclinical trials [31-33]. With the demonstration that the facial arene ligand is not essential to cytotoxicity and it can be replaced by other ligands such as the face-capping trithiacyclononane ([9]aneS_3) [34], a new family of cytotoxic Ru(II) complexes emerged [35, 36]. We have developed and tested several Ru(II)([9]aneS_3) complexes, both with aza ligands [37, 38] and with natural ligands such as glycine [39] and curcumin [40]. Within those with aza-ligands, highlight goes to [Ru(II)([9]aneS_3)Cl(phpz)], where phpz = 5-(2-hydroxyphenyl)-3-[(4-methoxystyryl)-pyrazole), that has a good cytotoxicity against prostate cancer (PC-3) and breast cancer (MDA-MB-231) cells [37].

The present paper reports the two-step syntheses of three new Ru(II) trithiacyclononane ([9]aneS_3) complexes with three naturally occurring flavones. In the particular case of 7,3',4'-trihydroxyflavone, having a catechol in the B-ring position,

coordination occurs *via* the catechol moiety to form a five-membered ring. This is, to the best of our knowledge, the first report of ruthenium coordination *via* the catechol group in large polyphenolic ligands. The other two complexes feature *O,O'*-coordination *via* the chromen-4-one moiety to Ru(II), thus yielding a six-membered heterocyclic ring containing the Ru(II) atom. The *in vitro* cytotoxic activities of these flavonoids are largely unexplored, and for this reason they were evaluated against four human cancer cell lines, namely prostate cancer (PC-3), breast cancer (both hormone-dependent (MCF-7) and triple-negative (MDA-MB-63)) and osteosarcoma (MG-63). The cytotoxicity of the Ru(II) complexes was also assessed, revealing that metal coordination causes a reduction of the antitumor activity.



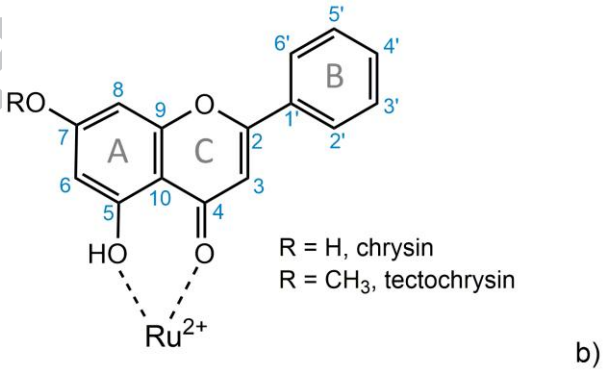


Figure 1. Structure and atom labelling of the ligands used in the present work. The available sites for coordination to Ru(II) are highlighted with dashed lines: a) 7,3',4'-trihydroxyflavone binds ruthenium *via* the catechol; b) chrysin and tectochrysin coordinate in the positions 4 and 5.

2. Experimental

2.1. Chemicals

All the chemicals were used as received from the distributors. 5,7-dihydroxyflavone, also known as chrysin, was acquired from Alfa Aesar (Karlsruhe, Germany) and 7,3',4'-trihydroxyflavone was purchased from the Indofine Chemical Company (Hillsborough, USA). 5-hydroxy-7-methoxyflavone, with the common name tectochrysin, is not commercially available, therefore it was prepared as described in 2.3. Tetrabutylammonium hydroxide (TBAOH) methanol solution (1M) was acquired from Sigma Aldrich (Sintra, Portugal). [Ru(II)([9]aneS_3)Cl_2(*S*-DMSO)] was prepared according to the procedures described in our previous work [37–42]. Our procedure is adapted from the method described in 1994 by Landgrafe and Sheldrick [43], replacing the solvent $CHCl_3$ with a non-toxic solvent, ethanol, and increasing the reaction time to 4h [42].

Solvents were at least of analytical grade and used without further purification.

The cell lines – MCF-7 (estrogen receptor-positive human epithelial breast adenocarcinoma), MDA-MB-231 (human estrogen receptor-negative epithelial breast adenocarcinoma), PC-3 (castration-resistant prostate cancer) and MG-63 (osteosarcoma) – were purchased from the European Collection of Authenticated Cell Cultures (ECACC, https://www.phe-culturecollections.org.uk/collections/ecacc.aspx)

2.2. Instrumentation

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 300 spectrometer at 300.13 MHz and 75.47 MHz, respectively, at room temperature. ^{13}C assignments were made based on 2D heteronuclear single quantum coherence spectroscopy (HSQC, ^{1}H,^{13}C), and heteronuclear multiple bond correlation (HMBC, delay for long-range $J_{C/H}$ couplings were optimised for 7 Hz) experiments. The residual protic solvent signal

(DMSO-d_6: ^{1}H δ 2.50 ppm and ^{13}C δ 39.5 ppm) was used as internal reference. Chemical shifts are quoted in parts per million (ppm) and the coupling constants (*J*) in Hertz (Hz).

Fourier-transform infrared (FT-IR) spectra, in the 4000-380 cm^{-1} range, were collected as KBr pellets using a Unicam Mattson Mod 7000 FTIR spectrophotometer, by averaging 64 scans at a maximum resolution of 2 cm^{-1}. In a typical preparation, 2 mg of sample were mixed in a mortar with 200 mg of KBr (Sigma-Aldrich, ≥ 99%).

Mass spectra were recorded by C. Barros on a Micromass® Q-TOF 2 mass spectrometer with electrospray ionisation (ESI^{+}-MS), using methanol as the solvent. The *m/z* ratios presented in the characterisation data of the sample are monoisotopic, calculated using the mass of the most abundant natural isotope of each element (^{1}H, ^{12}C, ^{14}N, ^{16}O, ^{32}S, ^{35}Cl and ^{102}Ru).

Elemental analysis of the newly synthesised complexes was performed by M. Marques in a TruSpec 630-200-200 CHNS Analyser.

The cell optical density results of the MTT assay were measured using a BioTek μQuant MQX200 UV-Visible spectrophotometer equipped with the Gen5 *software*.

2.3. Synthesis of 5-hydroxy-7-methoxyflavone (tchrys)

A solution of chrysin (101.7 mg, 0.40 mmol) and potassium carbonate (165.9 mg, 1.2 mmol) in refluxing acetone (5 mL) was treated with dimethyl sulphate (41.6 μL, 0.44 mmol). The mixture was refluxed for one hour to form a yellow precipitate, which was filtered and subject to column chromatography in silica-gel using as eluent a mixture of 3% methanol in dichloromethane. Further purification was carried out by recrystallisation in dichloromethane with 5% methanol over three days. Crystals were washed with ethanol (10 mL) and dried. Yield: 85 mg (79%).

FT-IR selected bands ν(tilde) = 3340 m (ν_{O-H}), 3088 m, 3071 m, 3058 m, 3014 m, 2980 m, 2951 m, 2924 m, 2900 m, 2843 m, 1669 vs ($\nu_{C=O}$), 1626 s, 1609 vs ($\nu_{C2=C3}$), 1588 vs, 1567 m

($\nu_{C2=C3–C4}$), 1495 s, 1453 s, 1436 s, 1423 m, 1352 vs, 1309 m, 1247 m, 1202 vs, 1170 s, 1161 vs, 1121 s, 1103 m, 1041 s, 906 m, 866 s, 850 s, 828 m, 808 s, 721 s, 695 s, 646 s, 566 m, 500 m, 469 m, 390 m.

^{1}H NMR (300.13 MHz, DMSO-d_6): δ(ppm) = 12.82 (1H, s, O*H*-5), 8.13-8.09 (2H, m, H-2',6'), 7.66-7.55 (3H, m, H-3',4',5'), 7.05 (1H, s, H-3), 6.83 (1H, d, *J* 2.3 Hz, H-8), 6.41 (1H, d, *J* 2.3 Hz, H-6), 3.88 (3H, m, 7-OC*H*$_3$). Data is in good agrement with literature values [44].

^{13}C NMR (75.47 MHz, DMSO-d_6): δ (ppm) = 182.2 (C-4), 165.4 (C-7), 163.5 (C-2), 161.2 (C-5), 157.4 (C-9), 132.3 (C-4'), 130.6 (C-1'), 129.2 (C-3',5'), 126.5 (C-2',6'), 105.4 (C-3), 105.0 (C-10), 98.2 (C-6), 92.9 (C-8), 56.2 (7-O*C*H$_3$).

2.4. Synthesis of [Ru(II)([9]aneS$_3$)(S-DMSO)(7,3',4'-trihydroxyflavone)] (1)

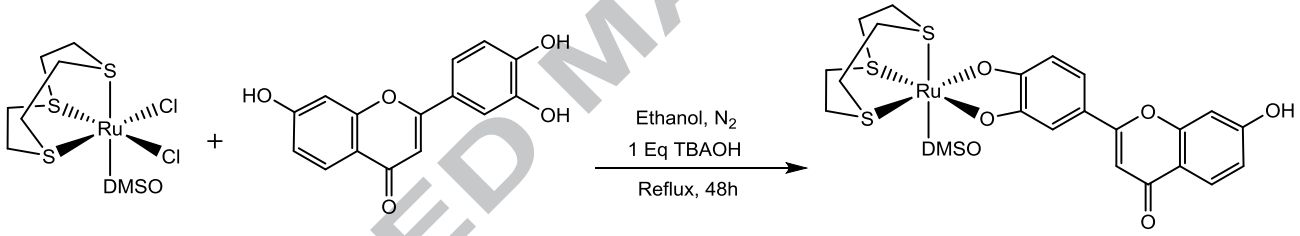
A solution of thflv (94.5 mg, 0.35 mmol) in refluxing ethanol (15 mL) was treated with one equivalent of tetrabutylammonium hydroxide (350 μL, 0.35 mmol). The colour of the solution changed from light yellow to orange. After 20 minutes, the complex [Ru([9]aneS$_3$)Cl$_2$(DMSO)] (150 mg, 0.35 mmol) was added and the reaction mixture was left under reflux for 48 h (Scheme 1). During this time the solution became dark orange and a dark green precipitate was formed. The hot solution was cannula-filtered and the remaining solid was washed with ethanol (20 mL), diethyl ether (20 mL) and dried (90 mg, 38% yield). Elemental analysis for [Ru($C_6H_{12}S_3$)($C_{15}H_8O_5$)(C_2H_6SO)]·3(H_2O) (Mr = 681,8): C, 40.52; H, 4,73 %. Found: C, 40.07; H, 4.28 %.

FT-IR selected bands ν(tilde) = 3443 m (νO–H), 2985 m, 2949 m, 2914 m (all three νC–H), 1624 vs (νC···O + νC···C $_{(thflv)}$), 1595 m, 1560 s (both νC···C $_{(thflv)}$), 1490 vs (non-assigned), 1449 m, 1413 m (both δC–H$_{([9]aneS3)}$), 1305 s, 1257 s, 1160 m (all three δC–H $_{(DMSO)}$), 1090 s (νS=O), 721 w (νC–S $_{(DMSO)}$), 680 w, 650 vw (both νC–S $_{([9]aneS3)}$), 490 w, 461 vw (both νRu–S $_{([9]aneS3)}$), 426 w (νRu–S $_{(DMSO)}$), 397 vw (νRu–O).

ESI$^+$-MS (MeOH) *m/z* (relative intensity %): 522 ([Ru([9]aneS_3–CH_2CH_2)(thflv)]$^+$, 100); 628 ([Ru([9]aneS_3)(*S*-DMSO)(thflv)]$^+$, 95).

^{1}H NMR (300.13 MHz, DMSO-d_6): δ(ppm) = 10.58 (1H, br s, OH-7), 7.78 (1H, d, *J* 8.7 Hz, H-5), 6.90 (1H, dd, *J* 2.0, 8.4 Hz, H-6'), 6.85 (2H, d, *J* 2.0 Hz, H-2',8), 6.81 (1H, dd, *J* 2.0, 8.7 Hz, H-6), 6.35 (1H, d, *J* 8.4 Hz, H-5'), 6.30 (1H, s, H-3), 3.19 (6H, s, $SO(CH_3)_2$), 2.87–2.58 (12H, m, CH_2 of trithiacyclononane).

^{13}C NMR (75.47 MHz, DMSO-d_6): δ (ppm) = 176.0 (C-4), 170.1 (C-4'), 165.0 (C-2), 163.5 (C-3'), 162.4 (C-9), 157.5 (C-7), 125.9 (C-5), 116.8 (C-10), 115.7 (C-1'), 114.7 (C-5',6'), 113.9 (C-6), 110.5 (C-2'), 102.1 (C-8), 100.8 (C-3), 43.6 ($SO(CH_3)_2$), 34.1, 31.9 and 30.7 (CH_2 of trithiacyclononane).



Scheme 1. Synthetic procedure used in the preparation of the complex **1**.

2.5. Synthesis of [Ru(II)([9]aneS_3)(chrysin)(S-DMSO)]Cl (2)

A solution of 5,7-dihydroxyflavone or chrysin (63.5 mg, 0.25 mmol) in refluxing methanol (20 mL) was treated with half equivalent of tetrabutylammonium hydroxide (125 μL, 0.125 mmol). The solution turned from light to dark yellow. After 20 minutes, the complex precursor [Ru([9]aneS_3)Cl_2(*S*-DMSO)] (107.5 mg, 0.25 mmol) was added affording a light orange solution, which was allowed to reflux for 24 h (Scheme 2). The volume of the solution was reduced to roughly one half and a yellow solid was formed; this solid was identified as unreacted precursor [Ru([9]aneS_3)Cl_2(*S*-DMSO)]. The supernatant was filtered through a cannula, collected and added with 60 mL of diethyl ether. After storage at 4ºC for one week, the product [Ru(II)([9]aneS_3)(chrys)(*S*-DMSO)]Cl (**2**) was isolated as a dark

orange precipitate. The product was filtered, washed with cold ethanol (5 mL), diethyl ether (20 mL) and dried (40 mg, 23% yield).

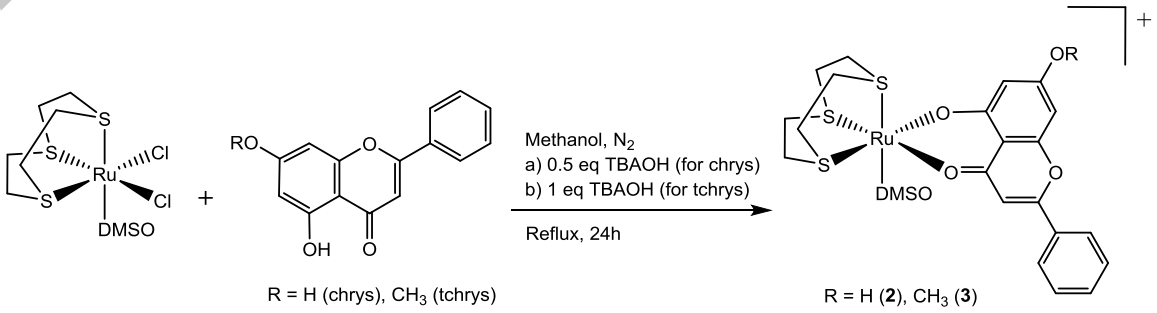
Elemental analysis for $[Ru(C_6H_{12}S_3)(C_{15}H_9O_4)(C_2H_6SO)]Cl\cdot 1.6H_2O$ (Mr = 675.0): C, 40.80; H, 4,50 %. Found: C, 41.00; H, 4.63 %.

FT-IR selected bands ν(tilde) = 3147 m (νO–H), 2992 sh, 2975 m, 2924 m (all three νC–H), 1631 vs (νC···O + νC···C $_{(chrys)}$), 1598 s, 1577 s, 1528 vs (all three νC···C $_{(chrys)}$), 1450 m, 1413 m (both δC–H $_{([9]aneS3)}$), 1297 w, 1266 m, 1165 s (all three δC–H $_{(DMSO)}$), 1099 m, 1082 s (both νS=O), 716 vw (νC–S $_{(DMSO)}$), 678 w, 653 w (both νC–S $_{([9]aneS3)}$), 492 vw, 457 vw (both νRu–S $_{([9]aneS3)}$), 424 m ($\nu_{Ru–S\ (DMSO)}$), 400 vw (νRu–O).

ESI^+-MS (MeOH) *m/z* (relative intensity %): 507 ($[Ru([9]aneS_3–CH_2CH_2)(chrys)]^+$, 100); 613 ($[Ru([9]aneS_3)(chrys)(S\text{-DMSO})]^+$, 49).

^1H NMR (300.13 MHz, DMSO-d_6): δ (ppm) = 10.54 (1H, s, OH-7), 8.07-8.02 (2H, m, H-2',6'), 7.64-7.52 (3H, m, H-3',4',5'), 7.05 (1H, s, H-3), 6.24 (1H, d, *J* = 2.3 Hz, H-8), 6.11 (1H, d, *J* = 2.3 Hz, H-6), 2.96 (3H, s, $SO(CH_3)_2$), 2.95 (3H, s, $SO(CH_3)_2$), 2.93-2.58 (12H, m, CH_2 of trithiacyclononane).

^{13}C NMR (75.47 MHz, DMSO-d_6): δ (ppm) = 178.3 (C-4), 168.2 (C-5), 164.3 (C-7), 159.4 (C-2), 158.9 (C-9), 132.0 (C-4'), 130.4 (C-1'), 129.3 (C-3',5'), 126.2 (C-2',6'), 106.7 (C-10), 105.2 (C-3), 103.3 (C-6), 91.1 (C-8), 43.1, 42.4 ($SO(CH_3)_2$), 34.6, 34.1, 32.5, 32.4, 29.9 and 29.8 (CH_2 of trithiacyclononane).



Scheme 2. Synthetic procedure used in the preparation of complexes **2** and **3** (herein represented in their cationic form).

2.6. Synthesis of [Ru(II)([9]aneS$_3$)(S-DMSO)(tectochrysin)]Cl (3)

A solution of tchrys (18.8 mg, 0.07 mmol) in refluxing methanol (13 mL) was treated with one equivalent of tetrabutylammonium hydroxide (70 μL, 0.07 mmol). The solution colour changed from light yellow to fluorescent yellow. After 20 min, the complex [Ru([9]aneS$_3$)Cl$_2$(*S*-DMSO)] (30 mg, 0.07 mmol) was added causing the solution to turn orange. The reaction mixture was left under reflux for 24 h (Scheme 2). After cooling to room temperature, 20 mL of diethyl ether and 20 mL of dichloromethane were added to the reaction mixture, but rather than isolating a solid product, an oily product, with dark orange colour, was obtained. This product was isolated from the solution and recrystallised over one week in dichloromethane. This allowed isolating a powdered solid that was filtered, washed with diethyl ether (10 mL) and vacuum-dried (20 mg, 43% yield).

Elemental analysis was not done due to low amount of sample.

FT-IR selected bands ν(tilde) = 3405 m (νO–H), 2988 m, 2961 m, 2932 m, 2910 m (all νC–H), 1636 vs (νC$^{\cdots}$O + νC$^{\cdots}$C $_{(tcrys)}$), 1599 s, 1578 m, 1549 s (all three νC$^{\cdots}$C $_{(tcrys)}$), 1451 m, 1431 m (both δC–H $_{([9]aneS3)}$), 1160 m (δC–H $_{(DMSO)}$), 1099 sh, 1087 m ($\nu_{S=O}$), 719 w (νC–S $_{(DMSO)}$), 681 w, 657 w (both νC–S $_{([9]aneS3)}$), 491 w, 457 vw (νRu–S $_{([9]aneS3)}$), 424 w (νRu–S $_{(DMSO)}$), 398 vw (νRu–O).

ESI$^+$-MS (MeOH) *m/z* (relative intensity %): 521 ([Ru([9]aneS$_3$–CH$_2$CH$_2$)(tchrys)]$^+$, 100); 627 ([Ru([9]aneS$_3$)(*S*-DMSO)(tchrys)]$^+$, 57).

^{1}H NMR (300.13 MHz, DMSO-d$_6$): δ (ppm) = 8.08 (2H, m, H-2',6'), 7.65-7.54 (3H, m, H-3',4',5'), 7.11 (1H, s, H-3), 6.44 (1H, d, *J* 2.5 Hz, H-8), 6.26 (1H, d, *J* 2.5 Hz, H-6), 3.80 (3H, s, 7-OCH$_3$), 2.97 (6H, s, SO(CH$_3$)$_2$), 2.94-2.66 (12H, m, CH$_2$ of trithiacyclononane).

^{13}C NMR (75.47 MHz, DMSO-d$_6$): δ (ppm) = 178.8 (C-4), 168.4 (C-5), 165.1 (C-7), 159.8 (C-2), 158.7 (C-9), 132.1 (C-4'), 130.2 (C-1'), 129.3 (C-3',5'), 126.3 (C-2',6'), 107.4 (C-10),

105.3 (C-3), 101.3 (C-6), 90.1 (C-8), 55.6 (7-O*C*H_3), 43.1, 42.5 (SO(CH_3)$_2$), 34.7, 34.1, 32.6, 32.3, 30.0 and 29.8 (CH_2 of trithiacyclononane).

2.7. Cytotoxicity assays

2.7.1. Preparation of the solutions

All stock solutions were freshly prepared (details described in Table 1). Water soluble compounds were prepared in phosphate buffered saline (PBS) solution (140.0×10^{-3} M NaCl, 2.7×10^{-3} M KCl, 1.5×10^{-3} M KH_2PO_4, 8.1×10^{-3} M Na_2HPO_4 in Mili-Q water, pH 7.4) and sterilised by filtration. Poorly water soluble agents were prepared in 50% DMSO in PBS or in 100% DMSO. Further dilutions of these stock solutions were made with the corresponding percentages of each solvent, in order to obtain the final concentrations required for the biological assays (Table 1). Note that the maximal concentration tested was 200 μM for the compounds prepared in DMSO/PBS (50/50), and 100 μM for those prepared in 100% DMSO, thus assuring that, in all experiments and for both control and treated cells, the highest concentrations of DMSO in each well was 0.5% (*v/v*). Previous studies (which are not presented) showed that cell viability is not affected upon exposure to 0.5% DMSO, for all four cell lines studied. Complex **1** was tested to the limit of its solubility in DMSO (18.5 μM).

Table 1. Composition and concentration of the stock solutions and of the highest concentrations tested for the flavones and complexes **1**-**3**.

Compound	Stock Solution	Highest concentration
7,3',4'-thflv	40 mM, DMSO	100 μM
Complex **1**	3.7 mM, DMSO	18.5 μM
Chrysin	20 mM, DMSO	100 μM
Complex **2**	20 mM, DMSO/PBS	200 μM
Tectochrysin	20 mM, DMSO	100 μM
Complex **3**	20 mM, DMSO/PBS	200 μM

2.7.2. Cell culture

Four human cancer cell lines were tested in the present work, representing three types of cancer: osteosarcoma (MG-63), prostate (PC-3) and breast – both hormone-dependent (MCF-7) and triple-negative, highly metastatic (MDA-MB-231). The cell lines were cultured as monolayers and maintained at 37°C, in a humidified incubator under 5%, and sub-cultured twice a week. Cells were harvested upon addition of trypsin/EDTA (0.05% trypsin/EDTA solution).

MG-63 cells were grown and maintained in MEM medium supplemented with 10% inactivated FBS, non-essential amino acids (1 mM), 1% penicillin-streptomycin and sodium pyruvate (1 mM). PC-3 cells were grown and maintained in RPMI 1640 medium supplemented with 1,5 g of $NaHCO_3$, 10% inactivated FBS and 1% penicillin-streptomycin. The MCF-7 cells were maintained in DMEM medium supplemented with 1.5 g of $NaHCO_3$, 10% inactivated FBS and 1% penicillin-streptomycin. The MDA-MB-231 cells were grown and maintained in RPMI 1640 medium, supplemented with 1.5 g of $NaHCO_3$, 10% inactivated FBS, non-essential amino acids (1 mM), 1% penicillin-streptomycin and sodium pyruvate (1 mM).

2.7.3. Toxicity and cell viability assays

Cell viability was assessed by the mitochondrial dehydrogenase activity colorimetric assay (MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) [45]. Three independent experiments with triplicates for each drug concentration were performed. Cells were seeded (MG-63 at 2.5×10^4 cells/cm^2, PC-3 at 1.5×10^4 cells/cm^2, MCF-7 at 3.0×10^4 cells/cm^2 and MDA-MB-231 at 1.5×10^4 cells/cm^2) in 48-well plates (500 µl/well) and incubated at 37 ºC. After allowing cells to adhere for 24 hours, different concentrations of the test compounds were added. In the control group the corresponding percentages of solvent, PBS, DMSO or 50% DMSO in PBS were added. Cisplatin was used as a positive control.

The plates were incubated for 72 hours, followed by the addition of MTT solution (5 mg/mL in PBS) to each well (final concentration 0.5 mg/mL) and incubated for 2 hours. The MTT containing medium was removed and 200 µl of DMSO was added to each well in order to dissolve the purple formazan crystals formed by MTT reduction in live viable cells. The plates were gently homogenised using circular motion at room temperature, to dissolve the precipitate. The optical density (OD) was then measured at 570 nm.

2.7.4. Statistical analysis

The software *GraphPad PRISM 5.00* for *Windows* (*GraphPad Software*, San Diego, California, USA, www.graphpad.com) was used for the analysis of the data from the cell growth inhibition assays. Results are expressed as a mean and standard deviation obtained from three independent experiments (n=3), each comprising three replicate measurements performed for each concentration of each compound tested plus the untreated control. Statistical analysis was performed by one-way ANOVA followed by the Dunnett's *post hoc* test for statistical comparison between the experimental data, *p*-values less than 0.05 having been considered as significant. The IC_{50} values were determined for the compounds which presented a significant statistical difference relative to the control. The results for each compound were fitted using nonlinear regression analysis, in sigmoidal dose-response curves (variable slope).

3. Results and Discussion

3.1. Synthesis of tectochrysin or 5-hydroxy-7-methoxyflavone (tchrys)

Tectochrysin occurs naturally in a variety of plants but it is still not available as a commercial flavone. This way, for it to be used as a ligand in the ruthenium complexes herein described,

it had to be prepared by methylation of chrysin. The reaction is easily achieved using dimethyl sulphate ($(CH_3O)_2SO_2$) as the alkylating agent. The product, tectochrysin, was isolated and purified by crystallisation and its structure was verified by NMR, which is in good agreement with literature data [44].

3.2. Synthesis of the complexes

The complexes are prepared by a two-step procedure. The flavonol ligand was dissolved in either methanol or ethanol according to its solubility and treated with one equivalent (or half equivalent, in the case of chrysin) of base, tetrabutylammonium hydroxide (TBAOH), to afford the corresponding flavonolate. To the same solution is then added [Ru([9]aneS_3)Cl_2(DMSO)] in equimolar amounts. Noteworthy, 7,3',4'-trihydroxyflavone (thflv) affords a neutral complex, [Ru(II)([9]aneS_3)(DMSO)(thflv)] (**1**), with a yield of 38%. The same procedure, using two equivalents of TBAOH (instead of one) also leads to the formation of the complex **1** with similar yield (37%). Chrysin (chrys) and tectochrysin (tchrys) afford two monocationic complexes, [Ru(II)([9]aneS_3)(chrys)(DMSO)]Cl (**2**) and [Ru(II)([9]aneS_3)(DMSO)(tchrys)]Cl (**3**), in moderate yields: 23% for **2** and 43% for **3**. For comparison, the yield reported for [Ru(II)Br(*p*cym)(3-hydroxyflavonate)] was 47% [25] and that of [Ru(II)Cl(*p*cym)(3-hydroxyflavonate)] was 71% [24].

3.3. Infrared spectroscopy

The most relevant FT-IR band frequencies of the powdered complexes, in comparison with those of the flavones, are presented in the Table 2. Complexation affected mostly the bands of the flavonoids, in particular those attributed to stretching modes of the ketone group and the aromatic rings. The assignment of vibrational frequencies of chrysin was carried out by Sundaraganesan et al., by combining experimental methods and quantum mechanics [46]. The authors explain that the C=O bond in the pyrone ring has a large dipole moment and a

strong degree of conjugation, giving rise to two bands at 1612 and 1655 cm^{-1}. Note also that these bands have a mixed character, comprising ν(C=C) and ν(C=O) contributions [46]. The same observations apply to the bands at 1626 and 1669 cm^{-1} of tectochrysin. In the corresponding complexes, **2** and **3**, the carbonyl stretch appears as a single band at 1631 and 1636 cm^{-1}, respectively, which is associated with changes in polarisation brought about by the formation of a six-membered ring with ruthenium (in combination with the deprotonated C5-O^- of ring A). Other reported complexes with *O,O'*-coordination, including a bis-flavone ruthenium complex [47] and a curcuminate ruthenium complex [40], exhibited a red shift of the carbonyl upon metal coordination.

In 7,3',4'-trihydroxyflavone, a single band is observed for the carbonyl [48], which appears very slightly blushifted (by only 2 cm^{-1}) in the corresponding ruthenium complex, **1**. This is likely associated with the distinct geometry of coordination, which occurs via the catechol group and thus has a lower influence on the chromenone group frequencies.

Regarding the backbone of the complexes **1-3**, three bands are worth mentioning. These are the Ru–S stretching band, around 424 cm^{-1}, which helps confirm the presence of coordinated trithiacyclononane, a band around 397–400 cm^{-1} that can be tentatively assigned to the Ru–O stretch, which is expected to occur in the 460–400 cm^{-1} region [49], and the S=O stretch of coordinated DMSO that confirms this ligand is retained upon coordination with the flavones. Note also that the S=O stretching band in complexes **1-3** is found between 1082 and 1099 cm^{-1} (Table 2), that is, blushifted in regard to free DMSO (at 1055 cm^{-1}), thus indicating *S*-coordination; in the case of O-coordination the band would be expected to appear redshifted to the 862 - 997 cm^{-1} interval [50].

Table 2. Selected FT-IR vibrational frequencies (in cm^{-1}) for 7,3',4'-trihydoxyflavone (thflv), chrysin (chrys), tectochrysin (tchrys) and the complexes **1**-**3**.

thflv [a]	**1**	chrys [b]	**2**	tchrys	**3**	Description
1622	1624	1655, 1612	1631	1669, 1626	1636	ν(C=O) + ν(C···C)
1598, 1568	1595, 1560	1595, 1577, 1500	1598, 1577, 1528	1609, 1588, 1567	1599, 1578, 1549	ν(C···C)
—	1413	—	1413	—	1410	δ(CH), [9]aneS$_3$
—	1090	—	1082	—	1099, 1087	ν(S=O)
—	426	—	424	—	424	ν(Ru–S), DMSO
—	397	—	400	—	398	ν(Ru–O) [c]

[a] 7,3',4'-trihydroxyflavone group frequencies are assigned according to the report of De Carvalho et al. [48].
[b] Chrysin group frequencies are assigned according to the work of Sundaraganesan et al. [46].
[c] The Ru-O stretch is assigned with base on the report of M. Ashok et al. [49].

3.4. Mass spectrometry

The ESI^+-MS spectra of the complexes were collected in methanol. The spectrum of the complex **1** shows two major peaks at *m/z* 522 and 628, which correspond respectively, to a fragment and the molecular cation, $[Ru([9]aneS_3)(DMSO)(thflv)]^+$. The fragment is generated by loss of one ethylene unit of the macrocycle along with the coordinated DMSO, $[Ru([9]aneS_3–CH_2CH_2)(thflv)]^+$. Such type of fragmentation pattern is typical of ruthenium(II) trithiacyclononane complexes, as demonstrated by the studies developed by the group of Santana-Marques [51, 52]. The same fragmentation pattern is observed for the complexes **2** and **3**. Besides the peaks corresponding to the molecular cation, found at *m/z* 613 for **2** and 627 for **3**, another intense peak in each spectrum is found respectively at *m/z* 507 and 521, and attributed to the fragment $[Ru([9]aneS_3–CH_2CH_2)(flavonate)]^+$ (flavonate = chrys, tchrys) generated by loss of one ethylene of the macrocycle and the labile ligand DMSO. For all the complexes under study, the isotopic distribution for each set of peaks is characteristic of ruthenium-bearing species, with ratios of *c.a.* 5% for ^{96}Ru, 14% for ^{99}Ru, 16% for ^{100}Ru, 30% for ^{102}Ru and 15% for ^{104}Ru. This distribution tallies well with the theoretical models for the empirical formula of each species, as well as with the reported experimental data for Ru stable isotopes [53].

3.5. NMR

Solution phase NMR determined the structure and geometry of coordination of the complexes **1-3** and helped confirm the findings resulting from FT-IR data that show the presence of DMSO in the first coordination sphere (^{1}H and ^{13}C spectra are presented in the *Electronic Supplementary Information*).

Complex **1** presents small shifts to lower frequencies for several proton signals of thflv when compared to the free ligand (Figure 2 and Table 3). Indeed, H-5, H-6 and H-8 shift *ca.* 0.1 ppm, H-3 shifts 0.3 ppm and H-2', H-5' and H-6' shift *ca.* 0.5 ppm (refer to Figure 1 for labeling). The shielding effect is stronger in the protons of ring B due to the increase of the electronic density in the 3' and 4' positions as a result of the coordination to Ru(II). In turn, the O*H*-7 is shifted upfield by 0.19 ppm. The aliphatic region of the spectrum presents almost no shifts in comparison with the Ru(II) precursor. The methyl groups of the *S*-coordinated DMSO appear as a single signal at δ 3.19 ppm and the macrocyclic protons as a multiplet in the δ 2.87-2.58 ppm range.

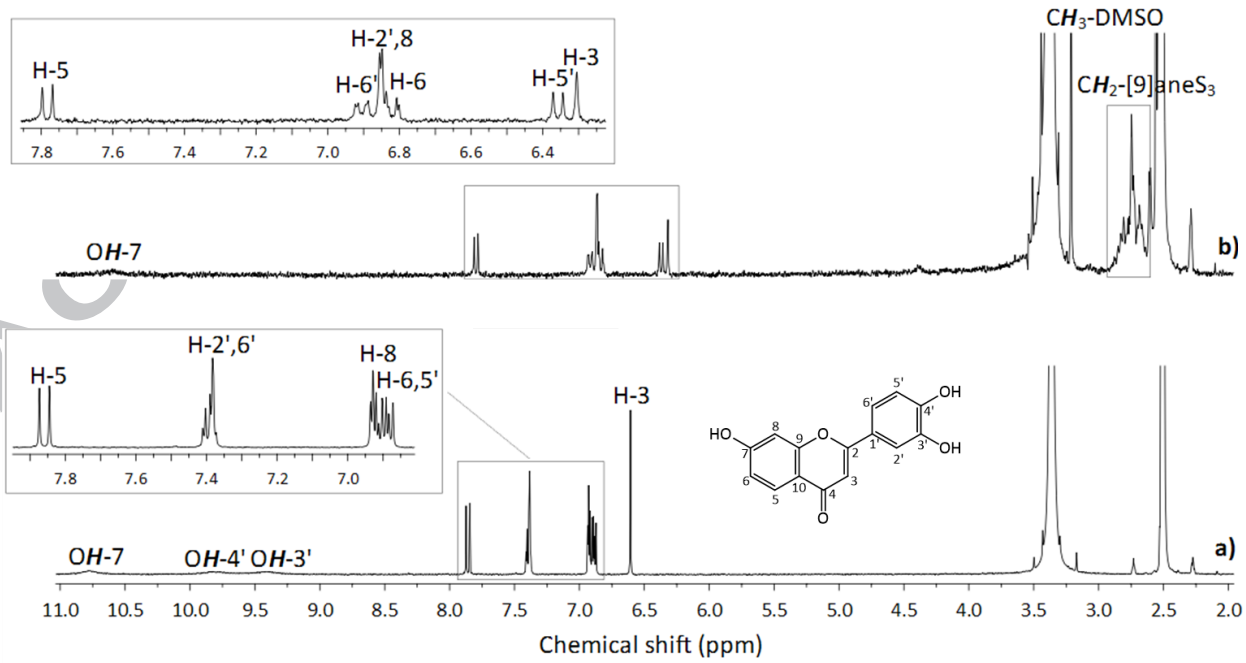


Figure 2. ^{1}H NMR spectra in DMSO-d_6 of a) thflv and b) complex **1**.

Table 3. ^{1}H NMR chemical shifts (in ppm) of complex **1** in DMSO-d_6, in comparison with those of the pure flavone ligand.

^{1}H	7,3',4'-thflv	Complex **1**
H-2'	7.45 – 7.30 (m)	6.85 (d)
O***H***-3'	9.40 (s)	n.o.
O***H***-4'	9.83 (s)	n.o.
H-5'	6.91 – 6.87 (m)	6.35 (d)
H-6'	7.45 – 7.30 (m)	6.90 (dd)
H-3	6.61 (s)	6.30 (s)
H-5	7.86 (d)	7.78 (d)
H-6	6.91 – 6.87 (m)	6.81 (dd)
O***H***-7	10.77 (s)	10.58 (br s)
H-8	6.93 (d)	6.85 (d)
C***H***$_3$-DMSO	—	3.19 (s)
C***H***$_2$-[9]aneS_3	—	2.87 – 2.58 (m)

The DMSO-d_6 signal (2.50 ppm) was used as the internal reference.

n.o. – not observed

The low solubility of the complex **1**, even in DMSO, has hampered the detection of aromatic carbons and for this reason the data from 2D NMR experiments (HSQC and HMBC spectra, presented in the *Electronic Supplementary Information*) was vital towards identification and attribution of attribution of the carbon resonances. $^{2/3}J_{C\text{-}H}$ HMBC correlations allowed identifying the signals of C-2 (H-6', H-3 → C-2), C-4 (H-5, H-3 → C-4), C-7 (H-5, H-6 → C-7), C-9 (H-5 → C-9), C10 (H-8, H-3 → C-10), and C-1' (H-2', H-5' → C-1'). As expected, many ^{13}C resonances of coordinated thflv are shifted in comparison to the signals of free thflv. The full list of values, for the complexes and the free flavones, is indicated in the Table 5. We highlight the strong shift observed for the C-3' and C-4' resonances (ring B), with values of *c.a.* 20 ppm that result from the direct coordination of ring B to Ru(II).

Complex **2** features significant shifts in the ^{1}H NMR signals of the rings A and C of chrysin, as listed in the Table 4 (atom and ring labelling in Figure 1; spectrum in the Figure S3 of the *Electronic Supplementary Information*). The formation of a 6-membered ring between Ru(II), the deprotonated OH-5 and the 4-carbonyl restores the electronic density of the C_2=C_3 unsaturated bond, inducing an anisotropic shift of the H-3 resonance to higher

frequencies. The H-6 signal appears deshielded, which evidences the decrease in the electronic density due to the ligand-to-metal sigma (σ) donation. Such effect is known to attenuate with distance [54] and thus it affects the other protons to a lesser extent. The aliphatic region of the spectrum presents two singlets at δ 2.95 and 2.96 ppm, attributed to two non-equivalent methyl groups of an *S*-coordinated DMSO and a multiplet in the δ 2.93-2.58 ppm range for the methylene protons of the macrocycle. The shifts of the two DMSO methyl groups are different from those of the precursor, [Ru(II)([9]aneS_3)Cl_2(*S*-DMSO)], and of those observed for the complex **1**, consistent with the different coordination environment in **2** and the rotational constrains for the DMSO ligands (that result from the presence of the bulkier flavonate ligand in the coordination sphere). Regarding ^{13}C resonances (Figure S4 and Table 3), chrysin signals were assigned by $^{2/3}J_{C\text{-}H}$ HMBC correlations, namely C-2 (H-6', H-2', H-3 → C-2), C-4 (H-3 → C-4), C-5 (H-6 → C-5), C7 (H-6, H-8→ C-7) C-9 (H-8 → C-9), and C-10 (H-8, H-3, H-6 → C-10). In complex **2**, Ru(II) is coordinated with rings A and C of chrysin, which is acting, as mentioned, as an electron donor ligand and features significant changes in its carbon chemical shifts: C-5, C-6 and C-10 appear shifted downfield by *c.a.* 6.7, 4.2, and 2.7 ppm, respectively, and C-8 is shifted upfield by 3.1 ppm (regarding pure chrysin).

Table 4. ^{1}H NMR chemical shifts (in ppm) of complexes **2** and **3** in DMSO-d_6, in comparison with those of the pure flavone ligands.

^{1}H	Chrysin	Complex **2**	Tectochrysin	Complex **3**
H-2'	8.09 - 8.04 (m)	8.07 – 8.02 (m)	8.13 – 8.09 (m)	8.08 (dd)
H-3'	7.64 – 7.53 (m)	7.64 – 7.52 (m)	7.66 – 7.55 (m)	7.65 – 7.54 (m)
H-4'	7.64 – 7.53 (m)	7.64 – 7.52 (m)	7.66 – 7.55 (m)	7.65 – 7.54 (m)
H-5'	7.64 – 7.53 (m)	7.64 – 7.52 (m)	7.66 – 7.55 (m)	7.65 – 7.54 (m)
H-6'	8.09 - 8.04 (m)	8.07 – 8.02 (m)	8.13 – 8.09 (m)	8.08 (dd)
H-3	6.97 (s)	7.05 (s)	7.05 (s)	7.11 (s)
O***H***-5	12.83 (s)	n.o.	12.82 (s)	n.o.
H-6	6.22 (d)	6.11 (d)	6.41 (d)	6.26 (d)
O***H***-7 or 7-OC***H***$_3$	10.94 (s)	10.54 (s)	3.88 (s)	3.80 (s)
H-8	6.52 (d)	6.24 (d)	6.83 (d)	6.44 (d)
C***H***$_3$-DMSO	—	2.95 (s), 2.96 (s)	—	2.97 (br s)
C***H***$_2$-[9]aneS_3	—	2.93 – 2.58 (m)	—	2.94 – 2.66 (m)

The DMSO-d_6 signal (2.50 ppm) was used as the internal reference.

n.o. – not observed

The ^{1}H NMR shifts of complex **3** in DMSO are also presented in the Table 4 (for the ^{1}H spectrum, refer to Figure S7). Given that this complex bears tchrys, a ligand with strong structural similarities to chrys, its protons present, as expected, chemical shift values similar those of the complex **2** These confirm coordination of Ru(II) to the deprotonated C5–O^{-} and the 4-carbonyl of the chromenone. The aliphatic region is also similar to that of **2**, with the twelve methylene protons of [9]aneS_3 appearing as a multiplet at δ 2.94-2.66 ppm and two non-equivalent methyl groups of *S*-coordinated DMSO appearing collapsed in a single broad resonance centred at δ 2.97 ppm. Note that non-equivalency is best observed in the ^{13}C spectrum (Figure S8, Table 5), in which two carbon resonances are observed in the region corresponding to the methyl carbons of coordinated DMSO. The ^{13}C resonances of C-5 and C-6 appear shifted downfield by 2.3 and 2.4 ppm, respectively, in regard to free tchrys, as a result of ligand electron-donating effect. Also noteworthy is the fact that, in **3**, the coordination of Ru(II) to the ketone (C-4) is accompanied by an electronic metal-to-ligand π-backdonation, as evidenced by the shift of the C-4 and C-2 resonances to lower frequencies.

Table 5. ^{13}C NMR chemical shifts (in ppm) of complexes **1**-**3** in DMSO-d_6, in comparison with those of the pure flavone ligands.

^{13}C	7,3',4'-thflv	Complex **1**	Chrysin	Complex **2**	Tectochrysin	Complex **3**
C-1'	122.2	115.7 [a]	130.8	130.4	130.6	130.2
C-2'	113.2	110.5 [b]	126.5	126.2	126.5	126.3
C-3'	145.7	163.5 [a]	129.2	129.3	129.2	129.3
C-4'	149.2	170.1 [a]	132.1	132.0	132.3	132.1
C-5'	116.0	114.7 [b]	129.2	129.3	129.2	129.3
C-6'	118.6	114.7 [b]	126.5	126.2	126.5	126.3
C-2	162.6	165.0 [a]	163.2	159.4	163.5	159.8
C-3	104.5	100.8 [a]	105.2	105.2	105.4	105.3
C-4	176.3	176.0 [a]	182.0	178.3	182.2	178.8
C-5	126.5	125.9 [b]	161.5	168.2	161.2	168.4
C-6	114.8	113.9 [b]	99.1	103.3	98.2	101.3
C-7	162.6	157.5 [a]	164.5	164.3	165.4	165.1
C-8	102.4	102.1 [b]	94.2	91.1	92.9	90.1
C-9	157.4	162.4 [a]	157.5	158.9	157.4	158.7
C-10	116.2	116.8 [a]	104.0	106.7	105.0	107.4
O-CH_3	—	—	—	—	56.2	55.6
CH_3 (DMSO)	—	43.6	—	43.1, 42.4	—	43.1, 42.5
CH_2 ([9]aneS_3)	—	34.1, 31.9, 30.7	—	34.6, 34.1, 32.5, 32.4, 29.9, 29.8	—	34.7, 34.1, 32.6, 32.3, 30.0, 29.8

The DMSO-d_6 signal (39.5 ppm) was used as the internal reference.
[a] Projected from HMBC
[b] Projected from HSQC

3.6. Cytotoxicity assays

The effect of complexes **1** to **3** on the viability of several human cancer cells – prostate cancer (PC-3), breast cancer (MCF-7 and MDA-MB-231) and osteosarcoma (MG-63) – was assessed upon a 72 h incubation time. The results were compared with the free ligands – thflv, chrys and tchrys (Table 6) –, and cisplatin was used as the reference drug.

Table 6. IC_{50} (μM) values for 7,3',4'-trihydoxyflavone (thflv), chrysin (chrys), tectochrysin (tchrys), cisplatin and the complexes **1**-**3** against four human cancer cell lines.

Compound	MG-63	PC-3	MCF-7	MDA-MB-231
thflv	38.1	23.2	36.9	24.8
complex **1**	> 18.5 [a]	> 18.5 [a]	> 18.5 [a]	> 18.5 [a]
chrys	41.0	15.7	29.5	17.1
complex **2**	> 200 [b]	146.2	> 200 [b]	180.6
tchrys	67.9	32.8	113.5	44.8
complex **3**	> 200 [b]	> 200 [b]	> 200 [b]	> 200 [b]
cisplatin	4.5	6.6	13.8	9.5

[a] 18.5 was the highest concentration tested for this compound due to low solubility in aqueous medium
[b] growth inhibition at 200 μM, the highest concentration tested, was lower than 50%.

The results presently obtained confirm the cytotoxic activity of the pure flavonoids, thflv and chrys having the most interesting IC_{50} values. Noteworthy is the lower activity observed for tchrys, a 5-methoxylated derivative of chrys, which demonstrates the relevance of free hydroxyl groups for the antitumoral activity of flavones. Furthermore, it is worth highlighting that all the tested flavonoids displayed antineoplastic activity against osteosarcoma, a kind of cancer with a high incidence in children and teenagers and for which the currently available chemotherapeutic strategies are still very limited. The compounds´ antitumor efficacy towards the MG-63 cell line, albeit lower than that of the reference drug

cisplatin, is within the 38-68 μM range. Overmore, the side effects are expected to be milder since flavonoids are natural compounds with reported good tolerability. A chrysin glucoside perfusion was reported to be safely used on mice as a natural hypotensive agent [55] and intravenous chrysin nanoparticles were shown to control tumour growth in xenograph mice [56].

Regarding the complexes, **1** could only be tested at a maximal concentration of 18.5 μM, due to poor water solubility (see details in the Experimental section, Table 1). At this concentration, no inhibition was observed for any of the tested cell lines, and it can then be considered as inactive. Complexes **2** and **3**, in turn, displayed high IC_{50} values against the tested cell lines (> 146 μM), revealing a loss of activity as compared to the free ligands. These results are somewhat unexpected, given the known cytotoxic activity of complexes of the Ru(II)(trithiacyclononane) family on several of the herein used human tumoral cells, (MG-63, PC-3 and MDA-MB-231) [37, 38], as well as on a murine cell line (TS/A) [57]. It may, though, be a phenomenon associated with *O*,*O*'–Ru(II) coordination, which was previously observed for some Ru(II) complexes with curcumin (curc), namely [Ru(II)Cl(curc)(*p*cym)]) [58], [Ru(II)Cl(curc)(hexamethylbenzene)]) [59], and [Ru(II)([9]aneS_3)(curc)(DMSO)]Cl) [40].

4. Conclusions

The work herein reported describes the first catechol-coordinated ruthenium flavone complex, [Ru([9]aneS_3)(DMSO)(thflv)] (**1**), as well as two novel complexes [Ru([9]aneS_3)(chrys)(DMSO)]Cl (**2**) and [Ru([9]aneS_3)(DMSO)(tchrys)]Cl (**3**), with a six-membered ring formed between Ru and the oxygen atoms at positions 4 and 5 of the chromenone moiety of each flavone. The complexes **1**-**3** are obtained in moderate yields of 38, 23 and 43% respectively, using a one-pot generic synthetic procedure that involves an initial step of deprotonation of the flavone followed by treatment with the ruthenium

precursor, [Ru([9]aneS_3)Cl_2(DMSO)]. The unequivocal confirmation of the geometry of complexes **1-3** was achieved by use of both solution and solid-state characterisation techniques, namely FT-IR, 1-D and 2-D NMR and ESI^+-MS.

The cytotoxic activity against several human cancer cells was studied for the flavones as well as their complexes. The free ligands showed good inhibitory action on the various tested cell lines (prostate cancer, breast cancer, both hormone-dependent and triple-negative) and, more importantly, osteosarcoma (a strongly chemoresistant, low prognosis type of cancer). Anti-osteosarcoma action has been reported for other flavones and their derivatives, in particular for baicalin [60], nobiletin [61], luteolin [62], and wogonin [63], but it is, to the best of our knowledge, unprecedented for chrysin, tectochrysin and 7,3',4'-trihydroxyflavone (also called 5-deoxyluteolin). In turn, complexes **1** to **3** displayed no antitumor activity against the MG-63 osteosarcoma cell line. Particularly for complexes **2** and **3**, tested at concentrations up to 200 μM, a very reduced activity was obtained. Complex **2**, comprising chrys as the ligand, prompts a decreased viability of PC-3 prostate cancer and MDA-MB-231 breast cancer cells, but with high IC_{50} values (146.2 and 180.6 μM, respectively). As for complex **3**, with tchrys as the ligand, an $IC_{50} > 200$ μM was determined, which is in line with the higher IC_{50} values observed for free tchrys in these cell lines. Hence, the low activity of the metal complexes may be somewhat associated with the properties of the ligand, and partly caused by solubility issues that do not allow the complexes to achieve their cellular targets in suitable amounts. Future directions towards the improvement of these structures should thus contemplate an improved cellular uptake and increased water solubility, either by incorporating a solubilising group as PTA (1,3,5-triaza-7-phosphaadamantane) in their coordination sphere or by loading them into adequate carriers such as cyclodextrins and liposomes.

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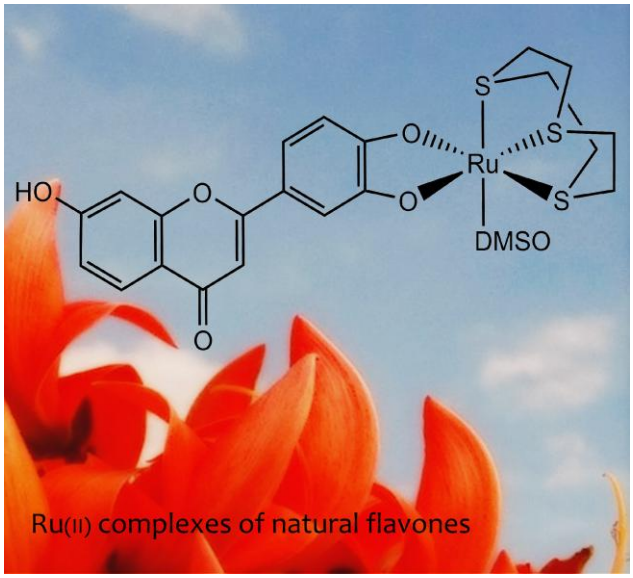
Graphical abstract contents



Ruthenium(II) trithiacyclononane complexes of three naturally occurring flavones are obtained in yields of 23 to 43%. One of these, [Ru(II)([9]aneS_3)(thflv)(DMSO)], (thflv = 7,3',4'-trihydroxyflavone) exhibits coordination at the catechol group, a rare feature in Ru(II)-O,O' complexes. The *in vitro* cytotoxic activity of the flavones and their corresponding Ru(II) complexes is presented

Highlights

- 7,3',4'-trihydroxyflavone forms a neutral Ru(II) complex via catechol O,O'-coordination
- Chrysin and techtochrysin form cationic complexes with Ru(II) via bidentate chromenone binding
- Chrysin may be a natural alternative to cisplatin in osteosarcoma treatment