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Organometallic complexes of (thio)allomaltol-based Mannich-products: Synthesis, stability and preliminary biological investigations

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ABSTRACT

Organometallic complexes with (thio)pyrone-based ligands have been shown to possess promising cytotoxic properties. To extend the class of potential metallodrugs, the (thio)pyrone backbone was modified *via* Mannich reaction with morpholine, *N*-methylpiperazine and piperidine as cyclic amine. The ligands and organometallic complexes were characterized by means of 1D and 2D NMR, ESI MS and also in one case by X-ray diffraction analysis. Due to the high aqueous solubility, the behavior and stability in aqueous solution of the synthesized complexes was studied by ^{1}H NMR spectroscopy. In addition, the influence of these modifications on cytotoxicity in human cancer cell lines was investigated by means of the MTT assay.

Introduction

Ruthenium complexes are promising agents for the treatment of cancer and the most prominent representatives of this class of metallodrugs, sodium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (NKP-1339 or IT-139) and imidazolium *trans*-[tetrachlorido(1*H*-imidazole)(dimethylsulfoxide-κS)ruthenate(III)] (NAMI-A), have been investigated in clinical trials (Fig. 1) [1–4]. NKP-1339 is administered intravenously and binds rapidly to blood proteins such as albumin and transferrin. The formed adducts prevent the complex from fast aquation and decomposition in aqueous solution and are then accumulated in the tumor tissue. There the metal center is reduced under hypoxic conditions (activation by reduction), yielding the corresponding highly reactive Ru(II) species, which are thought to interact with cellular targets [3,5].

Due to the supposed reduction of the clinically tested Ru(III) metallodrugs under hypoxic conditions, the class of Ru(II) complexes has been investigated for anticancer potential as well. Ru(II) is prone to oxidation reactions, but it can be easily stabilized by facial coordination of an arene moiety, yielding compounds with a piano-stool geometry of the general formula [(η^6-arene)Ru(X,Y,Z)], where X,Y are monodentates or a chelating moiety and Z is the leaving group, typically a halido ligand. With variation of the remaining coordination sites, chemical reactivity and anticancer activity can be fine-tuned, notably bidentate chelating ligands showed a high impact on the stability [6–8]. The hydrophobic arene may enhance the transport through the cell membrane and facilitate cellular uptake [9]. The two most investigated representatives of this class so far are the RAPTA compounds (*e.g.* RAPTA-C), developed by Dyson and coworkers, and 1,2-diaminoethane complexes established by Sadler's group (*e.g.* RM175). Both paved the way for further investigation of organometallic Ru(II)-arene compounds as potential anticancer agents and are in an advanced preclinical stage of drug development [6,10–13].

3-Hydroxypyrones are widely used as chelating agents due to their ability to form stable complexes with a broad range of different metal ions. Advantages include the easy accessibility, high biocompatibility and the high amount of possible modifications. Recently, metal complexes bearing 3-hydroxy(thio)pyrones as a chelating motif have been shown to possess promising cytotoxic activity *in vitro* and are currently being tested *in vivo* [14–16]. These neutral complexes undergo rapid hydrolysis within seconds, yielding the respective aqua species. It has been found that

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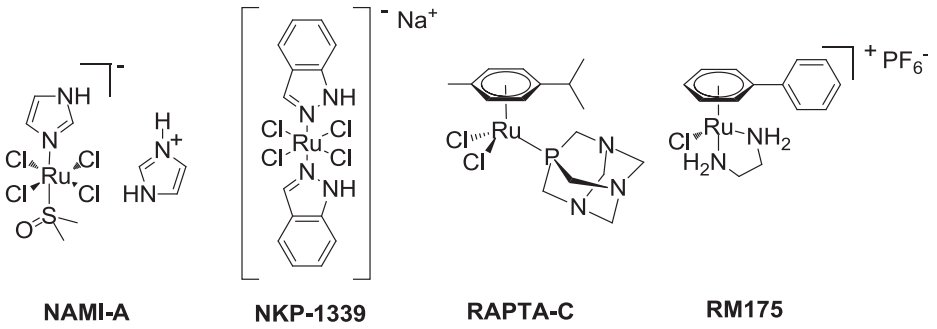

Fig. 1. Structures of ruthenium anticancer agents investigated in clinical trials (NAMI-A and NKP-1339) or in an advanced preclinical stage of drug development (RAPTA-C and RM175).

thiopyrone analogs are significantly more stable in the presence of biomolecules than the analogous pyrone derivatives, where ligand cleavage was observed over time [15,17]. The increase in stability is supposed to be the crucial factor for the observed high cytotoxic activity in the low micromolar range [15,17,18].

Due to the lower affinity of oxygen to ruthenium, *O*,*O*-bidentate ligands tend to be cleaved off after dissolving in water or in the presence of biomolecules. Kasser and coworkers have already reported a series of organometallic Mannich products of kojic acid. The utilization of kojic acid as precursor resulted in a high solubility; however, the stability under biological conditions was limited due to the formation of $[Ru_2(p\text{-cym})_2(OH)_3]^+$ after a short period of time [19]. Utilization of thiopyrones might circumvent this drawback, and the aim of this work was the extension of the (thio) pyrone library by modification of the heterocyclic backbone *via* Mannich reaction and subsequent conversion to corresponding Ru(II)-, Os(II)-arene and Rh(II)-Cp* complexes. The solubility and stability of the novel compounds in water was examined. The impact of the metal center variation and the introduction of hydrophilic groups on the anticancer activity was elucidated by the colorimetric MTT assay in different human cancer cell lines.

Experimental

General

All solvents were of analytical grade and used without further purification. Utilized chemicals were purchased from Sigma Aldrich or Acros and used as received. The dimeric metal precursors $[Ru(p\text{-cym})Cl_2]_2$, $[Os(p\text{-cym})Cl_2]_2$, and $[Rh(Cp^*)Cl_2]_2$ were prepared according to the literature [20,21]. Allomaltol was synthesized according to literature protocols. The synthesis of thioallomaltol was slightly modified, compared to the standard procedure [15,22–24]. ^{1}H NMR spectra were recorded at 25 °C and 500.10 MHz and ^{13}C{H} NMR spectra at 25 °C and 125.75 MHz using a Bruker FT-NMR spectrometer Avance III™ 500 MHz. D_2O, DMSO-d_6 and $CDCl_3$ were used as solvents for the NMR experiments and the concentrations of the samples were around 2–4 mM. Electrospray ionization mass spectra were recorded on a Bruker Esquire 3000 in positive and negative mode; the samples were dissolved in methanol. Elemental analyses were carried out with a Eurovector EA3000 Elemental Analyzer by the Microanalytical Laboratory of the University of Vienna. If not otherwise stated, the substances were synthesized and purified according to general procedures.

Synthesis

5-Hydroxy-2-methyl-thiopyran-4(1H)-one (**2**)

Allomaltol (1.00 g, 7.9 mmol) and Lawesson's reagent (1.61 g, 4.0 mmol) were suspended in 20 mL dry 1,4-dioxane and refluxed for 4 h under argon atmosphere. After cooling to room temperature the solvent was evaporated and the crude product was purified by column chromatography (*n*-hexane/ethyl acetate = 10:1, Rf = 0.15). Yield: 80%. ^{1}H NMR (DMSO-d_6, 500.10 MHz, 25 °C): δ = 2.32 (s, 3H, CH_3), 7.33 (s, 1H, H3), 8.25 (s, 1H, H6), 8.41 (s, 1H, −OH) ppm.

General procedure for Mannich reactions

A mixture of cyclic secondary amine (1.30 mmol, 0.8 eq.) and aqueous formaldehyde solution (35%, 0.127 mL, 1.59 mmol, 1 eq.) in methanol (6 mL) was heated to reflux temperature. Allomaltol (200 mg, 1.59 mmol, 1 eq.) or thioallomaltol (203 mg, 1.586 mmol, 1 eq.) was added and the mixture was refluxed for a further 5 min. After cooling to room temperature, the solution was concentrated under reduced pressure and the formed precipitate was separated by filtration, washed with petrol ether and dried *in vacuo*.

3-Hydroxy-6-methyl-2-[(morpholin-4-yl)methyl]-pyran-4(1H)-one (***3a***)

The reaction was performed according to the general procedure using morpholine (113 μL, 113 mg) and allomaltol (200 mg). Yield: 83%, colorless solid. ^{1}H NMR (DMSO-d_6, 500.10 MHz, 25 °C): δ = 2.25 (s, 3H, CH_3), 2.43 (t, $^3J_{H,H}$ = 5 Hz, 4H, H_{mor}), 3.48 (s, 2H, CH_2–N), 3.56 (t, $^3J_{H,H}$ = 5 Hz, 4H, H_{mor}), 6.22 (s, 1H, H5), 8.85 (br s, 1H, −OH) ppm. ^{13}C NMR (DMSO-d_6, 127.75 MHz, 25 °C): δ = 173.5 (C4), 164.7 (C6), 146.2 (C2), 143.4 (C3), 111.1 (C5), 66.1 (CH_{2mor}) 53.9 (CH_2–N), 53.0 (CH_{2mor}), 19.4 (C7) ppm. Elemental Anal. Calc for $C_{11}H_{15}NO_4$: C, 58.66; H, 6.71; N, 6.22; Found: C, 58.56; H, 6.51; N, 6.04%. ESI-MS (neg) *m*/*z*: 223.4 $[M - H]^-$. ESI-MS$^+$ *m*/*z*: 225.6 $[M + H]^+$, 247.5 $[M + Na]^+$, 472.4 $[2M + H]^+$.

3-Hydroxy-6-methyl-2-[(piperidin-1-yl)methyl]-pyran-4(1H)-one (***3b***)

The reaction was performed according to the general procedure using piperidine (128 μL, 110 mg) and allomaltol (200 mg). After standard work up, the product was obtained as highly viscous oil. Therefore, the oil was dissolved in dichloromethane and concentrated to dryness, yielding the compound as a colorless solid. Yield: 88%, colorless solid. ^{1}H NMR (DMSO-d_6, 500.10 MHz, 25 °C): δ = 1.33–1.38 (m, 2H, H_{pip}), 1.46–1.50 (m, 4H, H_{pip}) 2.25 (s, 3H, CH_3), 2.39–2.41 (m, 4H, H_{pip}), 3.45 (s, 2H, CH_2–N), 6.20 (s, 1H, H5), 8.88 (br s, 1H, −OH) ppm. ^{13}C NMR (DMSO-d_6, 127.75 MHz, 25 °C): δ = 173.4 (C4), 164.5 (C6), 146.8 (C2), 143.2 (C3), 111.1 (C5), 54.4 (CH_2–N), 53.7 (CH_{2pip}), 25.4 (CH_{2pip}), 23.6 (CH_{2pip}), 19.3 (C7) ppm. Elemental Anal. Calc for $C_{12}H_{17}NO_3 \cdot 0.1CH_2Cl_2$: C, 62.71; H, 7.48; N, 6.04; Found: C, 62.69; H, 7.27; N, 5.78%. ESI-MS$^-$ *m*/*z*: 221.4 $[M - H]^-$. ESI-MS$^+$ *m*/*z*: 223.7 $[M + H]^+$, 446.4 $[2M + H]^+$, 468.5 $[2M + Na]^+$.

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*3-Hydroxy-6-methyl-2-[(4-methylpiperazin-1-yl)methyl]-pyran-4(1H)-one (**3c**)*

The reaction was performed according to the general procedure using *N*-methylpiperazine (144 μL, 130 mg) and allomaltol (200 mg). Yield: 92%, colorless solid. ^{1}H NMR (DMSO-d_6, 500.10 MHz, 25 °C): δ = 2.15 (s, 1H, N–CH_3), 2.26 (s, 3H, CH_3), 2.31 (br s, 4H, H_{paz}), 2.45 (br s, 4H, H_{paz}) 3.49 (s, 2H, CH_2–N), 6.22 (s, 1H, H5), 8.89 (br s, 1H, –OH) ppm. . ^{13}C NMR (DMSO-d_6, 127.75 MHz, 25 °C): δ = 173.5 (C4), 164.6 (C6), 146.6 (C2), 143.2 (C3), 111.1 (C5), 54.4 (CH_{2pip}), 53.5 (CH_2–N), 52.3 (CH_{2pip}), 45.6 (N–CH_3), 19.4 (C7) ppm. Elemental Anal. Calc for $C_{12}H_{18}N_2O_3 \cdot 0.15CH_3OH$: C, 60.03; H, 7.71; N, 11.52; Found: C, 60.26; H, 7.37; N, 11.20%. ESI-MS$^-$ *m/z*: 236.5 [M – H]$^-$. ESI-MS$^+$ *m/z*: 238.7 [M + H]$^+$, 476.5 [2M + H]$^+$, 498.5 [2M + Na]$^+$.

*3-Hydroxy-6-methyl-2-[(morpholin-4-yl)methyl]-thiopyran-4(1H)-one (**3d**)*

The reaction was performed according to the general procedure using thioallomaltol (203 mg) and morpholine (113 μL, 113 mg). After removal of the solvent, the oily residue was dissolved in diethyl ether and stored overnight at 4 °C. The precipitate was collected, washed with diethyl ether and dried *in vacuo*. Yield: 73%, brown solid. ^{1}H NMR (DMSO-d_6, 500.10 MHz, 25 °C): δ = 2.34 (s, 3H, CH_3), 2.46 (t, $^3J_{H,H}$ = 4 Hz, 4H, H_{mor}), 3.57 (t, $^3J_{H,H}$ = 4 Hz, 4H, H_{mor}), 3.61 (s, 2H, CH_2–N), 7.32 (s, 1H, H5), 8.31 (br s, 1H, –OH) ppm. ^{13}C NMR (DMSO-d_6, 127.75 MHz, 25 °C): δ = 187.5 (C4), 159.5 (C6), 149.37 (C3), 143.2 (C2), 123.1 (C5), 66.1 (CH_{2mor}) 54.4 (CH_2–N), 53.0 (CH_{2mor}), 18.8 (C7) ppm. Elemental Anal. Calc for $C_{11}H_{15}NO_3S$: C, 54.75; H, 6.27; N, 5.80; S, 13.29 Found: C, 54.54; H, 6.23; N, 5.62; S, 13.29%. ESI-MS$^-$ *m/z*: 239.4 [M – H]$^-$. ESI-MS$^+$ m/z: 241.6 [M + H]$^+$, 263.6 [M + Na]$^+$

Synthesis of the ruthenium(II) and rhodium(III) complexes

General procedure for the synthesis of pyrone-based organometallic complexes. Ligand (1.1 eq) and sodium methoxide (1.1 eq) were dissolved in methanol (10 mL) and stirred for 15 min under argon atmosphere. The respective dimeric metal precursor (150 mg, 1 eq) was added to the solution and the mixture was stirred overnight. The solvent was removed under reduced pressure, dissolved in dichloromethane and filtered. The volume was reduced to ~2 mL, *n*-hexane was added and the suspension was stored overnight at 4 °C. The formed solid was separated by filtration, washed with *n*-hexane and dried *in vacuo*.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(morpholin-4-yl)methyl]pyran-4(1H)-onato-κO}(η^6-p-cymene)ruthenium(II) (**4a**).*

The reaction was performed analogous to the general complexation procedure, using **3a** (120 mg, 0.53 mmol), sodium methoxide (29 mg, 0.53 mmol) and [Ru(*p*-cym)Cl_2]$_2$ (150 mg, 0.25 mmol). Yield: 66%.

^{1}H NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 1.29 (d, $^3J_{H,H}$ = 7 Hz, 3H, $CH_{3cym\text{-}c}$), 1.33 (d, $^3J_{H,H}$ = 7 Hz, 3H, $CH_{3cym\text{-}c}$), 2.26 (s, 3H, CH_3), 2.30 (s, 3H, $CH_{3cym\text{-}a}$), 2.54–2.63 (m, 4H, H_{mor}), 2.90 (sept, $^3J_{H,H}$ = 7 Hz, 1H, $CH_{cym\text{-}b}$), 3.48 (d, $^2J_{H,H}$ = 13 Hz, 1H, CH_2–N), 3.71 (t, $^3J_{H,H}$ = 5 Hz, 4H, H_{mor}), 3.85 (d, $^2J_{H,H}$ = 13 Hz, 1H, CH_2–N), 5.24 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.28 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.48 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.50 (d, $^3J_{H,H}$ = 6 Hz, 1H, H $_{cym}$), 6.28 (s, 1H, H5) ppm. ^{13}C NMR ($CDCl_3$, 127.75 MHz, 25 °C): δ = 185.1 (C4), 164.5 (C6), 158.2 (C3), 149.4 (C2), 109.5 (C5), 99.5 ($C_{cym\text{-}4}$), 95.93 ($C_{cym\text{-}1}$), 80.2 ($C_{cym\text{-}3}$), 80.0 ($C_{cym\text{-}5}$), 78.2 ($C_{cym\text{-}2}$), 77.5 ($C_{cym\text{-}6}$), 67.0 (CH_{2mor}), 54.4 (CH_2–N), 53.5 (CH_{2mor}), 31.2 ($CH_{cym\text{-}b}$), 22.6 ($CH_{3cym\text{-}c}$), 22.4 ($CH_{3cym\text{-}c}$), 20.2 (C7), 18.7 ($CH_{3cym\text{-}a}$) ppm. Elemental Anal. Calc for $C_{21}H_{28}ClNO_4Ru$: C, 50.96; H, 5.70; N, 2.83; Found: C, 50.66; H, 5.36; N, 2.79%. ESI-MS$^+$ *m/z*: 459.5 [M – Cl]$^+$, 373.5 [M – Cl – M_{mor}]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(piperidin-1-yl)methyl]pyran-4(1H)-onato-κO}(η^6-p-cymene)ruthenium(II) (**4b**).*

The reaction was performed analogous to the general complexation procedure, using **3b** (119 mg, 0.53 mmol), sodium methoxide (29 mg, 0.53 mmol) and [Ru(*p*-cym)Cl_2]$_2$ (150 mg, 0.25 mmol). Yield: 73%.

^{1}H NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 1.28 (d, $^3J_{H,H}$ = 7 Hz, 3H, $CH_{3cym\text{-}c}$), 1.33 (d, $^3J_{H,H}$ = 7 Hz, 3H, $CH_{3cym\text{-}c}$), 1.43 (br s, 2H, CH_{2pip}), 1.59 (br s, 4H, CH_{2pip}), 2.25 (s, 3H, CH_3), 2.30 (s, 3H, $CH_{3cym\text{-}a}$), 2.50–2.56 (m, 4H, H_{pip}), 2.89 (sept, $^3J_{H,H}$ = 7 Hz, 1H, $CH_{cym\text{-}b}$), 3.45 (br s, 1H, CH_2–N), 3.89 (d, $^2J_{H,H}$ = 13 Hz, 1H, CH_2–N), 5.23 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.28 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.47 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.47 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 6.27 (s, 1H, H5) ppm. ^{13}C NMR ($CDCl_3$, 127.75 MHz, 25 °C): δ = 184.8 (C4), 164.3 (6), 109.5 (C5), 99.6 ($C_{cym\text{-}4}$), 96.1 ($C_{cym\text{-}1}$), 80.1 ($C_{cym\text{-}3}$), 79.9 ($C_{cym\text{-}5}$), 87.2 ($C_{cym\text{-}2,6}$), 54.6 (N–CH_3), 54.4 (CH_{2pip}), 31.2 ($CH_{cym\text{-}b}$), 26.0 (CH_{2pip}), 24.2 (CH_{2pip}), 22.7 ($CH_{3cym\text{-}c}$), 22.3 ($CH_{3cym\text{-}c}$), 20.2 (C7), 18.7 ($CH_{3cym\text{-}a}$) ppm. Elemental Anal. Calc for $C_{22}H_{30}ClNO_3Ru$: C, 53.60; H, 6.13; N, 2.84; Found: C, 53.40; H, 5.90; N, 2.74%. ESI-MS$^+$ *m/z*: 457.5 [M – Cl]$^+$, 373.5 [M – Cl – M_{pip}]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(4-methylpiperazin-1-yl)methyl]pyran-4(1H)-onato-κO}(η^6-p-cymene)ruthenium(II) (**4c**).*

The reaction was performed analogous to the general complexation procedure, using **3c** (128 mg, 0.53 mmol), sodium methoxide (29 mg, 0.53 mmol) and [Ru(*p*-cym)Cl_2]$_2$ (150 mg, 0.25 mmol). Yield: 66%

^{1}H NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 1.28 (d, $^3J_{H,H}$ = 7 Hz, 3H, $CH_{3cym\text{-}c}$), 1.33 (d, $^3J_{H,H}$ = 7 Hz, 3H, CH–$CH_{3cym\text{-}c}$), 2.25 (s, 3H, CH_3), 2.30 (s, 3H, N–CH_3), 2.30 (s, 3H, $CH_{3cym\text{-}a}$), 2.47 (br s, 4H, H_{paz}), 2.63 (br s, 4H, H_{paz}), 2.89 (sept, $^3J_{H,H}$ = 7 Hz, 1H, $CH_{cym\text{-}b}$), 3.46 (d, $^2J_{H,H}$ = 13 Hz, 1H, CH_2–N), 3.96 (d, $^2J_{H,H}$ = 13 Hz, 1H, CH_2–N), 5.24 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.28 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.47 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 5.49 (d, $^3J_{H,H}$ = 6 Hz, 1H, H_{cym}), 6.27 (s, 1H, H5) ppm. ^{13}C NMR ($CDCl_3$, 127.75 MHz, 25 °C): δ = 185.0 (C4), 164.4 (C6), 158.2 (C3), 149.9 (C2), 109.5 (C5), 99.6 ($C_{cym\text{-}4}$), 96.0($C_{cym\text{-}1}$), 80.2 ($C_{cym\text{-}3}$), 79.8 ($C_{cym\text{-}5}$), 87.4 ($C_{cym\text{-}2,6}$), 55.2 (CH_{2paz}), 53.8 (CH_{2paz}), 52.8 (CH_2–N), 46.1 (N–CH_3), 31.2 ($CH_{cym\text{-}b}$), 22.7 ($CH_{3cym\text{-}c}$), 22.4 ($CH_{3cym\text{-}c}$), 20.22 (C7), 18.74 ($CH_{3cym\text{-}a}$) ppm. Elemental Anal. Calc for $C_{22}H_{31}ClN_2O_3Ru \cdot 0.5H_2O$: C, 51.11; H, 6.24; N, 5.42; Found: C, 51.07; H, 5.91; N, 5.23%. ESI-MS$^+$ *m/z*: 472.5 [M – Cl]$^+$, 373.5 [M – Cl – M_{pip}]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(morpholin-4-yl)methyl]pyran-4(1H)-onato-κO}(η^5-1,2,3,4,5-pentamethylcyclopentadienyl)rhodium(III) (**6a**).*

The reaction was performed analogous to the general complexation procedure, using **3a** (120 mg, 0.53 mmol), sodium methoxide (29 mg, 0.53 mmol) and [Rh(Cp^*)Cl_2]$_2$ (150 mg, 0.24 mmol). Yield: 64%.

^{1}H NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 1.70 (s, 15H, Cp^*), 2.24 (s, 3H, CH_3), 2.62 (br s, 4H, H_{mor}), 3.39 (d, $^2J_{H,H}$ = 14 Hz, 1H, CH_2–N), 3.69–3.76 (m, 4H, H_{mor}), 4.03 (d, $^2J_{H,H}$ = 14 Hz, 1H, CH_2–N), 6.25 (s, 1H, H5) ppm. ^{13}C NMR ($CDCl_3$, 127.75 MHz, 25 °C): δ = 184.6 (C4), 163.7 (C6), 158.4 (C3), 149.2 (C2), 110.1 (C5), 91.2 (Cp^*), 67.2 (CH_{2mor}), 54.5 (CH_2–N), 53.5 (CH_{2mor}), 20.2 (C7), 9.02 (Cp^*) ppm. Elemental Anal. Calc for $C_{21}H_{29}ClNO_4Rh$: C, 50.67; H, 5.87; N, 2.81; Found: C, 50.76; H, 5.84; N, 2.62%. ESI-MS$^+$ *m/z*: 461.6 [M – Cl]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(piperidin-1-yl)methyl]pyran-4(1H)-onato-κO}(η^5-1,2,3,4,5-pentamethylcyclopentadienyl)rhodium(III) (**6b**).*

The reaction was performed analogous to the general complexation procedure, using **3b** (119 mg, 0.53 mmol), sodium

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methoxide (29 mg, 0.53 mmol) and $[Rh(Cp^*)Cl_2]_2$ (150 mg, 0.24 mmol). Yield: 50%.

^{1}H NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 1.40–1.45 (m, 2H, CH_{2pip}), 1.56–1.63 (m, 4H, CH_{2pip}), 1.70 (s, 15H, Cp*), 2.23 (s, 3H, CH_3), 2.55 (m, 4H, H_{pip}), 3.35 (d, $^2J_{H,H}$ = 14 Hz, 1H, CH_2–N), 4.03 (d, $^2J_{H,H}$ = 14 Hz, 1H, CH_2–N), 6.24 (s, 1H, H5) ppm. ^{13}C NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 184.4 (C4), 163.5 (C6), 158.1 (C3), 150.4 (C2), 110.0 (C5), 91.3 (Cp*), 54.8 (N–CH_3), 54.4 (CH_{2pip}), 26.1 (CH_{2pip}), 24.3 (CH_{2pip}), 20.2 (C7), 9.0 (Cp*) ppm. Elemental Anal. Calc for $C_{22}H_{31}ClNO_3Rh$: C, 53.29; H, 6.30; N, 2.82; Found: C, 53.46; H, 6.32; N, 2.67%. ESI-MS$^+$ *m*/*z*: 460.2 [M − Cl]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(4-methylpiperazin-1-yl)methyl] pyran-4(1H)-onato-κO}(η^5-1,2,3,4,5-pentamethylcyclopentadienyl) rhodium(III) (**6c**).*

The reaction was performed analogous to the general complexation procedure, using **3c** (127 mg, 0.53 mmol), sodium methoxide (29 mg, 0.53 mmol) and $[Rh(Cp^*)Cl_2]_2$ (150 mg, 0.24 mmol). Yield: 51%.

^{1}H NMR ($CDCl_3$, 500.10 MHz, 25 °C): δ = 1.70 (s, 15H, Cp*), 2.23 (s, 3H, CH_3), 2.29 (s, 3H, N–CH_3), 2.48 (br s, 4H, H_{paz}), 2.67 (br s, 4H, H_{paz}), 3.39 (d, $^2J_{H,H}$ = 14 Hz, 1H, CH_2–N), 4.12 (d, $^2J_{H,H}$ = 14 Hz, 1H, CH_2–N), 6.24 (s, 1H, H5) ppm. ^{13}C NMR ($CDCl_3$, 127.75 MHz, 25 °C): δ = 185.5 (C4), 163.6 (C6), 158.3 (C3), 149.6 (C2), 110.0 (C5), 91.3 (Cp*), 55.0 (CH_{2paz}), 53.9 (CH_{2paz}), 52.8 (CH_2–N), 46.2 (N–CH_{3paz}), 20.2 (C7), 9.02 (Cp*) ppm. Elemental Anal. Calc for $C_{22}H_{32}ClN_2O_3Rh \cdot 0.5H_2O$: C, 50.83; H, 6.40; N, 5.39; Found: C, 51.08; H, 6.05; N, 5.24%. ESI-MS$^+$ *m*/*z*: 474. [M–Cl]$^+$, 375.4 [M-Cl-M_{pip}]$^+$.

Synthesis of the thiopyrone-based organometallic complexes

General procedure for the synthesis of thiopyrone-based organometallic complexes.

Thiopyrone (1 eq) and sodium methoxide (1 eq) were dissolved in dry methanol (10 mL, dried over molecular sieves 3 Å) and stirred for 15 min under inert conditions. The solution was transferred into a Schlenk tube containing the corresponding metal dimer (50 mg, 1 eq.) and the mixture was stirred for 1.5 h at room temperature. The solvent was removed under reduced pressure, dissolved in dichloromethane and filtered. The volume was reduced to ~2 mL, the product precipitated upon addition of *n*-hexane and the resulting suspension was stored at 4 °C overnight. The formed precipitate was removed by filtration, washed with *n*-hexane and dried *in vacuo*.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(morpholin-4-yl)methyl]pyran-4(1H)-thionato-κS}(η^6-*p*-cymene)ruthenium(II) (**4d**).*

The reaction was performed analogous to the general complexation procedure, using **3d** (39 mg, 0.16 mmol), sodium methoxide (9 mg, 0.16 mmol) and $[Ru(p\text{-cym})Cl_2]_2$ (50 mg, 0.08 mmol). Yield: 75%.

^{1}H NMR (D_2O, 500.10 MHz, 25 °C): δ = 1.22 (d, $^3J_{H,H}$ = 7 Hz, 6H, $CH_{3cym\text{-}c}$), 2.21 (s, 3H, CH_3), 2.43 (s, 3H, $CH_{3cym\text{-}1}$), 2.71 (sept, $^3J_{H,H}$ = 7 Hz, 1H, $CH_{cym\text{-}b}$), 2.99 (br s, 4H, H_{mor}), 3.32–3.34 (m, 1H, CH_2–N), 3.87 (br s, 4H, H_{mor}), 3.98–4.00 (m, 1H, CH_2–N), 5.65 (d, $^3J_{H,H}$ = 7 Hz, 2H, H_{cym}), 5.84 (d, $^3J_{H,H}$ = 7 Hz, 2H, H_{cym}), 7.47 (s, 1H, H5) ppm. Elemental Anal. Calc for $C_{21}H_{28}ClNO_3RuS \cdot 0.7CH_2Cl_2$: C, 45.69; H, 5.19; N, 2.46; Found: C, 45.86; H, 5.43; N, 2.65%. ESI-MS$^+$ *m*/*z*: 476.3 [M − Cl]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(morpholin-4-yl)methyl]pyran-4(1H)-thionato-κS}(η^6-*p*-cymene)osmium(II) (**5d**).*

The reaction was performed analogous to the general complexation procedure, using **3d** (31 mg, 0.16 mmol), sodium methoxide (9 mg, 0.16 mmol) and $[Os(p\text{-cym})Cl_2]_2$ (50 mg, 0.06 mmol). Yield: 79%.

^{1}H NMR (D_2O, 500.10 MHz, 25 °C): δ = 1.22 (d, $^3J_{H,H}$ = 7 Hz, 6H, $CH_{3cym\text{-}c}$), 2.30 (s, 3H, CH_3), 2.42 (s, 3H, $CH_{3cym\text{-}a}$), 2.62 (sept, $^3J_{H,H}$ = 7 Hz, 1H, $CH_{cym\text{-}b}$), 2.97 (br s, 4H, H_{mor}), 3.32–3.34 (m, 1H, CH_2–N), 3.86 (br s, 4H, H_{mor}), 3.97–3.99 (m, 1H, CH_2–N), 5.97 (d, $^3J_{H,H}$ = 7 Hz, 2H, H_{cym}), 6.15 (d, $^3J_{H,H}$ = 7 Hz, 2H, H_{cym}), 7.54 (s, 1H, H5) ppm. Elemental Anal. Calc for $C_{21}H_{28}ClNO_3OsS \cdot 0.75CH_2Cl_2$: C, 39.35; H, 4.48; N, 2.10; Found: C, 39.16; H, 4.48; N, 2.39%. ESI-MS$^+$ *m*/*z*: 566.2 [M − Cl]$^+$.

*Chlorido{3-(oxo-κO)-6-methyl-2-[(morpholin-4-yl)methyl]pyran-4(1H)-thionato-κS}(η^5-1,2,3,4,5-pentamethylcyclopentadiene) rhodium(III) (**6d**).*

The reaction was performed analogous to the general complexation procedure, using **3d** (39 mg, 0.16 mmol), sodium methoxide (9 mg, 0.16 mmol) and $[Rh(Cp^*)Cl_2]_2$ (50 mg, 0.08 mmol). Yield: 55%.

^{1}H NMR (MeOD, 500.10 MHz, 25 °C): δ = 1.73 (s, 15H, Cp*), 2.40 (s, 3H, CH_3), 2.79 (br s, 4H, H_{mor}), 3.20–3.24 (m, 1H, CH_2–N), 3.74 (br s, 4H, H_{mor}), 3.85–3.88 (m, 1H, CH_2–N), 7.32 (s, 1H, H5) ppm. Elemental Anal. Calc for $C_{21}H_{29}ClNO_3RhS \cdot 0.5CH_2Cl_2$: C, 46.42; H, 5.44; N, 2.52; S, 5.76; Found: C, 46.49; H, 5.61; N, 2.40; S, 5.92%. ESI-MS$^+$ *m*/*z*: 467.3 [M − Cl]$^+$.

Single-crystal X-ray diffraction analysis

Single crystals of **4a**, suitable for X-ray diffraction analysis, were obtained by the slow diffusion method from dichloromethane/*n*-hexane and were measured on a X8 APEX2 diffractometer at 100 K. A single crystal was positioned 40 mm from the detector and 914 frames with a 90 s exposure time over 1° scan width were measured. SAINT was used to process the data and the structures were solved by direct methods and refined by full-matrix least-squares techniques [25]. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted at calculated positions and refined with a riding model. The following programs for structure solution, refinement, molecular diagrams and scattering factors were used: SHELXS-97 [26], SHELXL-2013 [26], OLEX2 [27] SHELXLE [28] and ORTEP-3 [29].

Cytotoxicity in human cancer cell lines

Cell lines and culture conditions

Human CH1 (ovarian carcinoma) cells were kindly provided by Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, UK) and SW480 (colon carcinoma) as well as A549 (non-small cell lung cancer) cells by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). Cells were grown in 75 cm^2 culture flasks (CytoOne, Starlab, Hamburg, Germany)) as adherent monolayer cultures in complete culture medium, i.e. minimal essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM L-glutamine and 1% v/v non-essential amino acids (from 100× ready-to-use stock solution) (all purchased from Sigma–Aldrich, Vienna, Austria). Cultures were maintained at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2.

Cytotoxicity tests in cancer cell lines

Cytotoxicity was determined by a colorimetric microculture assay (MTT assay). For this purpose, CH1, A549, and SW480 cells were harvested from culture flasks by trypsinization and seeded in complete culture medium into 96-well microculture plates (CytoOne, Starlab) in densities of 1×10^3 (CH1), 2×10^3 (SW480), 3×10^3 (A549) viable cells/well, respectively. After 24 h pre-incubation, cells were exposed to dilutions of the test compounds

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in 200 μL/well complete culture medium for 96 h. At the end of exposure, drug solutions were replaced with 100 μL/well of a 6:1 mixture of RPMI 1640 medium (supplemented with 10% heat-inactivated FBS and 4 mM L-glutamine) and MTT solution (MTT reagent in phosphate-buffered saline, 5 mg/mL). After incubation for 4 h, medium was removed and the formazan product formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities at 550 nm were measured with a microplate reader (Biotek ELx808), using a reference wavelength of 690 nm to correct for unspecific absorption. The percentage of viable cells was expressed relative to untreated controls (defined as 100%), and 50% inhibitory concentrations (IC_{50}) were calculated from concentration–effect curves by interpolation. Evaluation is based on at least three independent experiments (in case of IC_{50} > 200 μM on at least two experiments), each comprising triplicates for each concentration level.

Results and discussion

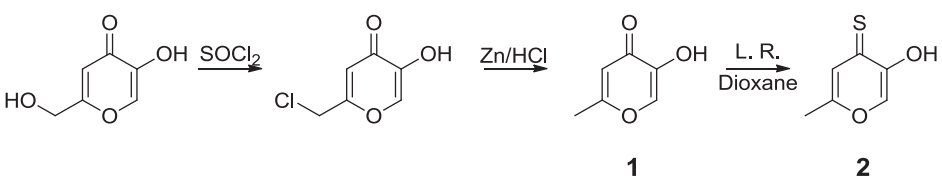
Thiopyrone-based organometallic complexes were found to be highly active in human cancer cell lines; however, their solubility under physiological conditions is significantly lower compared to their pyrone analogs. This drawback can be prevented by the insertion of highly hydrophilic groups such as morpholine, piperidine or *N*-methylpyrazine. The modification of the pyrone scaffold can be easily performed by Mannich reaction at position 6 of the pyrone ring and has already been reported by Aytemir et al. [30,31] Recently, Kasser *et al*. reported the synthesis of kojic acid-based Mannich products and the corresponding ruthenium(II)-arene complexes [19]. However, the organometallics were reported to be insufficiently stable under physiological conditions, due to rapid ligand cleavage. Direct thionation of the already reported ligands was unsuccessful. Allomaltol was utilized as precursor for this synthetic approach and was synthesized according to the protocol *via* two-step synthesis. First, kojic acid was converted with thionyl chloride to the respective chlorokojic acid and was subsequently reduced with Zn/HCl to allomaltol [15,22,23]. Thioallomaltol **2** was obtained by reaction of **1** with an equivalent amount of Lawesson's reagent (Scheme 1) [32]. The utilization of Lawesson's reagent compared to the reported synthesis with phosphorus pentasulfide improved the yield to 80%, and the purification of the compound can be easily performed *via* column chromatography [24,33].

The conversion to the respective Mannich products was achieved by the conversion of allomaltol **1** or thioallomaltol **2**, with equimolar amounts of 35% aqueous formaldehyde and the respective cyclic secondary amine (morpholine, piperidine or *N*-methylpiperazine) and refluxing the reaction mixture for 5 min. After work up, the products were obtained in high yields (83–88%), in the same range as the kojic acid analogs [19]. However, under these reaction conditions, the yield of the already reported ligand **3b** could be significantly increased from 30 to 88% [30].

The metal complexes were obtained according to established literature procedures by deprotonation of the ligand with sodium methoxide and reaction with the respective dimeric metal precursor [20,34]. After separation of byproducts by extraction and subsequent filtration, the products were obtained in moderate to good yields by precipitation from dichloromethane/*n*-hexane (Scheme 2, Table 1). We have recently shown that thiopyrone-based organometallics provide an increased stability under physiological conditions and promising anticancer potential *in vitro*. The high affinity of sulfur to the ruthenium center prevents ligand cleavage and inactivation of the complexes [18]. Therefore, **3d** was converted with the respective organometallic precursor yielding **4d**, **5d** and **6d**. Small traces of water in the reaction solvent led to decomposition of the formed complexes and formation of side products during the conversion. It was essential to work strictly under inert conditions. In addition, utilization of equimolar ratios of the reactants reduces the amount of formed side products and facilitates the work up procedure. Overall, the complexes were isolated in moderate to good yields (55–79%) and in high purity. All synthesized compounds were characterized by 1D and 2D NMR spectroscopy (Figs. S1–10), electrospray ionization mass spectrometry, elemental analysis and **4a** by X-ray diffraction analysis. In the case of the pyrone derivatives **3a**–**c** the shift of the H5 proton was between 6.20 and 6.22 ppm, and the broad singlet for the hydroxyl proton was at around 8.85 ppm. Formation of the Mannich products was confirmed by the disappearance of the H2 proton and the formation of new signals, assigned to the methylene group between 3.45 and 3.49 ppm and the pendant cyclic amines [30,31]. A downfield shift was observed for the H5 of the thionated ligand **3d** by around 1 ppm, which is typical for the thiopyrone backbone [35]. The signal for the methyl group and the amino moiety were found in the same area as for the *O*,*O*-analog.

The formation of the desired complexes was confirmed by disappearance of the –OH group at position 3 and the shift of the respective arene protons upon coordination. Spectra of all *O*,*O*-chelating complexes were recorded in $CDCl_3$, due to the observed increased stability of the compounds in this solvent. The protons of the arene ligand were observed as four doublets, and the two methyl groups also split in two doublets, which can be explained by the hindered inversion at the metal center in aprotic solvents. Coordination of an organometallic fragment to the (thio)pyrone ligand induced a diastereotopic splitting into two doublets with a geminal coupling constant of 14 Hz. These signals were unambiguously assigned to the methylene group in position 2 by HSQC and HMBC experiments. A similar behavior was observed for organometallic complexes bearing *N*-substituted 2-pyridiones as chelating ligands [34]. This diastereotopic splitting of the methylene group was observed for all synthesized metal complexes. For the rhodium compounds **6a**–**d** the characteristic singlet for the η^5-1,2,3,4,5-pentamethylcyclopentadienyl ligand (Cp^*) was found around 1.75 ppm.

The ^1H NMR spectra of the thionated compounds of the **d**-series were recorded in D_2O, and methanol-d_4 due to their solubility, better resolution and insufficient stability in either $CDCl_3$ or DMSO-d_6. Broadening of the aromatic signals of the *p*-cymene, which was induced by coordination to the metal center, is obviously apparent in the spectra of the osmium complex **5d**. Compared to the arene signals of the ruthenium analog **4d** with 5.65 and 5.84 ppm there is a downshift to 5.97 and 6.15 ppm. However, recording of ^{13}C NMR spectra was not possible for this series due to the surprising



Scheme 1. Synthesis of the (thio)pyrone precursors **1** and **2**.

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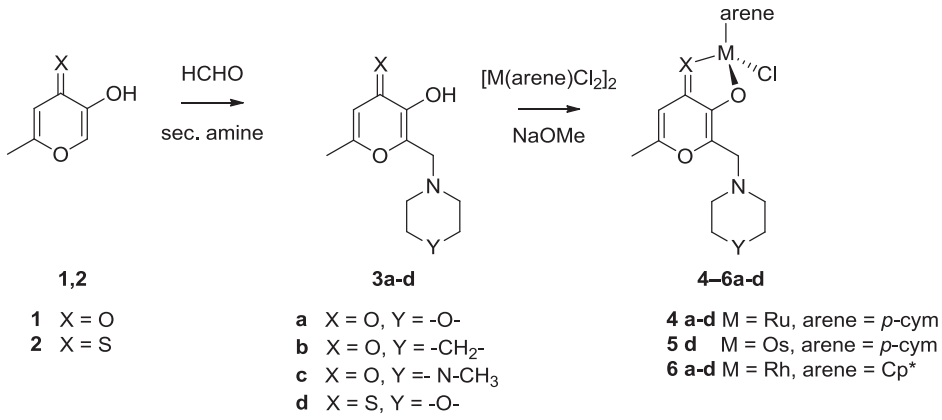

Scheme 2. Synthesis of the *O,O*- and *O,S*-chelates and organometallic Ru(II) (**4a**–**d**), Os(II) (**5d**) and Rh(III) (**6a**–**d**) complexes.

insufficient stability and decomposition of the thiopyrone complexes over the experiment time.

Poor solubility can seriously affect the suitability as a drug [36]. For example, the maximum tolerated dose of KP1019 could not be reached in the clinical setting due to solubility problems necessitating large infusion volumes [37]. Overall, the attachment of cyclic secondary amines such as piperazine, morpholine or piperidine enhanced the solubility of the organometallic complexes tremendously. The morpholine series showed the highest solubility of 240 mM for the ruthenium complex **4a** and the rhodium analog **6a**. The solubility of **4b** and **4c** were found around 200 mM. The rhodium complexes were soluble in the range of 60 mM for the rhodium(III)-*N*-methylpiperazine derivative (**6c**). The complexes of the **d** series were substantially less soluble, but even the osmium analog (**5d**) showed 20 mM solubility.

X-ray diffraction analysis

Single crystals of **4a**, suitable for X-ray diffraction experiments, were obtained by the slow diffusion method from dichloromethane/*n*-hexane. The complex crystallized in the triclinic space group *P-1* (Fig. 2). Details on the crystallographic measurements and the crystal parameters are provided in Table 2. The complex adopts the typical "piano-stool" configuration, where the deprotonated pyrone acts as an anionic *O,O*-chelate forming a five-membered ring with an envelope conformation. The Ru–O bond lengths 2.0819(19) Å (Ru–O3) and 2.0877(20) Å (Ru–O2) were found to be in the same range, which is in contrast to related structures [15,34,38,39]. This behavior was only observed for the maltol-based complex described by Peacock *et al.*, where two solvent molecules formed hydrogen bonds to the *O,O*-donor atoms [12]; ,however, no co-crystallized solvent molecules were found in the case of **4a**. The Ru–Cl distance of 2.4162(8) Å is comparable to those of already published Ru(II)-arene complexes [15]. The asymmetric coordination sphere leads to a chiral center at the ruthenium atom and both enantiomers were found in the unit cell in a 1:1 ratio.

Table 1
Overview of synthesized complexes with corresponding yields.

	Metal	Pyrone	Sec. amine	Yield
4a	Ru	Allomaltol	Morpholine	66%
4b	Ru	Allomaltol	Piperidine	73%
4c	Ru	Allomaltol	*N*-Methylpiperazine	66%
4d	Ru	Thioallomaltol	Morpholine	75%
5d	Os	Thioallomaltol	Morpholine	79%
6a	Rh	Allomaltol	Morpholine	64%
6b	Rh	Allomaltol	Piperidine	50%
6c	Rh	Allomaltol	*N*-Methylpiperazine	51%
6d	Rh	Thioallomaltol	Morpholine	55%

Stability of ruthenium(II) complexes in aqueous solution

Most of the clinically applied anticancer agents are administered intravenously, and therefore the stability in aqueous solution is a crucial parameter for drug development. Those compounds should act as prodrugs, which are activated by hydrolysis yielding the highly reactive aqua-species [40,41]. The stability in water was investigated by ^{1}H NMR spectroscopy in D_2O due to the high aqueous solubility of the complexes. Samples were dissolved in D_2O and spectra were recorded over a period of 72 h. All synthesized organometallics undergo hydrolysis to the corresponding aqua-complexes within seconds. However, the thiopyrone-based complexes were surprisingly very unstable, and even minor traces of water induced decomposition, which was rather unexpected given to the high stability reported for thiopyrone-based organo-ruthenium complexes [15]. Even the osmium analogueanalog,

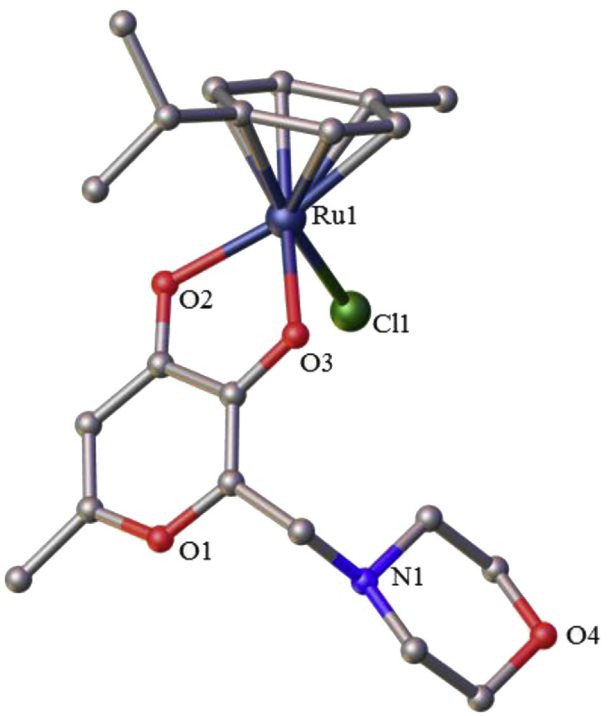

Fig. 2. Solid-state structure of complex **4a**. Hydrogen atoms were omitted for clarity.

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Table 2
Crystallographic data, data collection parameter und structure refinement details for **4a**.

	4a
Empirical formula	$C_{21}H_{28}ClNO_4Ru$
Formula weight [g/mol]	494.97
Temperature/K	100(2)
Wavelength/Å	0.71073
Crystal size/mm	0.08 × 0.08 × 0.1
Crystal system	triclinic
Space group	*P-1*
a [Å]	9.2542(4)
b [Å]	10.3922(4)
c [Å]	12.7027(5)
α [°]	67.543(2)
β [°]	70.582(3)
γ [°]	68.259(2)
Volume [Å^3]	1022.41(7)
Z	2
Calculated density/mg/m^3	1.608
Absorption coefficient/mm^{-1}	0.925
F(000)	508
Θ range for data collection	1.78–27.49°
Index ranges	$-12 \le h \le 11$ $-13 \le k \le 13$ $-16 \le l \le 16$
Reflections collected/unique	17,207/4609
Data/restraints/parameters	4609/15/222
R(Int)	0.0300
Final *R* indices [$I > 2\sigma(I)$]	
R_1[a]	0.0343
wR_2[b]	0.0853
GOF[c]	1.022

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.
[b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2}$.
[c] GOF $= \{\Sigma[w(F_o^2 - F_c^2)^2]/(n-p)\}^{1/2}$, where *n* is the number of reflections and *p* is the total number of parameters refined.

which should be the most stable of this series according to the HSAB principle, was far too unstable under the applied conditions (Figs. S12 and 13).

Interestingly, only complex **4a** of the ruthenium series showed sufficient stability attributed to the crucial influence of the *N*-heterocycle of the synthesized organometallic compounds. In Fig. 3, the aromatic region of **4a** is illustrated, and the formation of dimeric species, $[Ru(p\text{-cym})_2(OH)_3]^+$, can be observed over time (see Fig. S11 for complete spectra). After 3 days less than 10% of complex **4a** reacted to the dimeric species; however, complex **4c** was significantly less stable, where the same amount of dimer was formed after 6 h. With piperidine as secondary amine, after 5 min around 7% and after 6 h over 20% of the ruthenium complex **4b** was converted to the dimeric species. NMR experiments confirmed that the rhodium complexes **6a**–**c** were stable in aqueous solution for over 72 h without any traces of ligand cleavage or decomposition and are therefore suitable for biological testing.

Due to the obtained stability data, **4b**–**d**, **5d** and **6d** were not further investigated in biological experiments, and only compounds **4a** and the Rh(III) complexes **6a**–**c** were tested for their cytotoxicity.

Cytotoxicity

The investigated complexes **4a** and **6a**–**c** were tested for their cytotoxic activity in three human cancer cell lines, namely CH1 (ovarian carcinoma), SW480 (colon carcinoma), and A549 (non-small cell lung cancer), by means of the colorimetric MTT assay (Table 3, Fig. S14). In the rather chemoresistant cell line A549, IC_{50} values greater than 200 μM were observed for all complexes. This also holds true for SW480 cells, with the only exception being the Rh-piperidine derivative **6b**, which yielded an IC_{50} of about 100 μM.

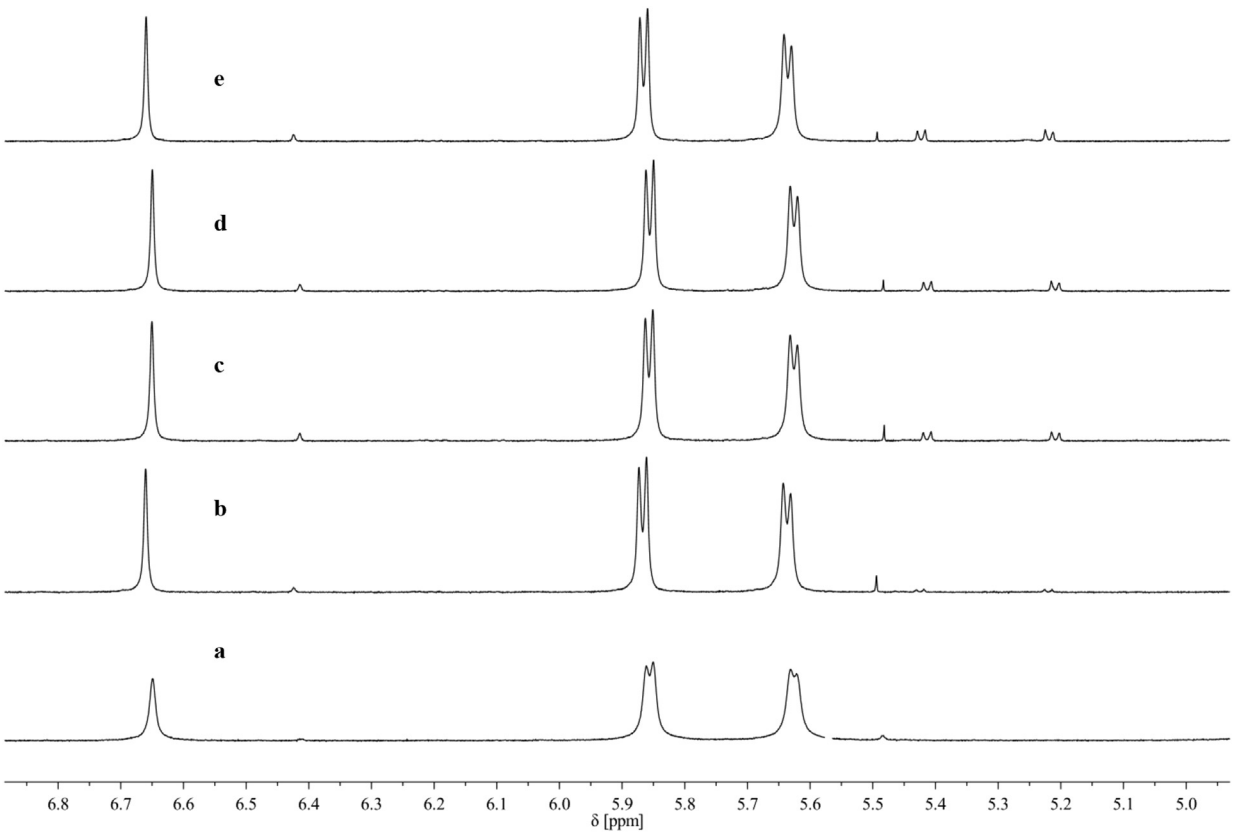

Fig. 3. ^{1}H NMR spectra of **4a** in D_2O after a) 5 min, b) 3 h, c) 24 h, d) 48 h and e) 72 h showing the formation of $[Ru_2(p\text{-cym})_2(OH)_3]^+$ at 5.1 and 5.4 ppm, respectively.

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Table 3
Solubility and cytotoxicity of complexes (**4a**, **6a**–**c**) in three human cancer cell lines in comparison with the Rh-allomaltol complex and NKP-1339 [42,43].

Compound	Solubility [mM]	IC_{50} [μM][a]		
		A549	CH1	SW480
4a	240	>200	56 ± 5	>200
6a	240	>200	199 ± 42	>200
6b	100	>200	136 ± 27	109 ± 29
6c	70	>200	201 ± 29	>200
Rh-allomaltol	35	>200	104 ± 20	145 ± 5
NKP-1339	18	156 ± 11	62 ± 9	88 ± 19

[a] 50% inhibitory concentrations in A549, CH1 and SW480 cells in the MTT assay (96 h exposure). Values are means ± standard deviations obtained from two (if > 200) or three independent experiments.

In the cell line CH1, as the most chemosensitive one, all complexes showed activity in the tested concentration range. In this cell line, the Ru complex **4a** exhibited an IC_{50} value of about 60 μM, which is more potent than the Rh analog **6a** and in the range of cytotoxicity of NKP-1339. For the Rh complexes, the side chain moiety exerts a slight influence on activity, with cytotoxicity decreasing in the following order: piperidine > morpholine ≈ methylpiperazine. However, the IC_{50} values of the rhodium series **6a**–**b** in CH1 cells are higher than that of the unsubstituted Rh-allomaltol complex, which might be explained by the high hydrophilicity of the compounds.

Conclusions

In this study, the synthesis and characterization of organometallic Ru(II), Os(II)-arene and Rh(III)-Cp* complexes bearing novel (thio)pyrones as chelates is discussed. The (thio)pyrone scaffold was modified *via* Mannich reaction using formaldehyde and a cyclic secondary amine (amine = morpholine, piperidine and *N*-methylpiperazine). The organometallics were characterized by standard analytical methods, and the crystal structure of **4a** confirmed the proposed structures of the products. The introduction of the pendant *N*-heterocycles increased the solubility of the complexes dramatically. Coordination of the thionated analogs to organometallic moieties yielded surprisingly highly unstable compounds, which rapidly decomposed in aqueous solution. Overall, three Rh(III) and one Ru(II) complexes were sufficiently stable for further biological experiments. The cytotoxicity of the organometallics was determined in three human cancer cell lines, and only the Ru compound **4a** exhibited moderate activity in CH1 cells.

Overall, the high solubility of the complexes facilitates not only their biological evaluation, but also enlarges the window of bioavailability for possible pharmaceutical applications. However, further investigations and an extended panel of compounds are required to gain a better understanding of the mode of action and structure–activity relationships of Rh-Cp* in comparison to Ru-arene complexes.

Acknowledgments

We thank the University of Vienna for financial support.

Appendix A. Supplementary material

CCDC 1007969 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Appendix B. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jorganchem.2014.10.044.

References

[1] C.S. Allardyce, P.J. Dyson, Platin. Met. Rev. 45 (2001) 62.
[2] C.G. Hartinger, M.A. Jakupec, S. Zorbas-Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P.J. Dyson, B.K. Keppler, Chem. Biodivers. 5 (2008) 2140.
[3] A. Bergamo, G. Sava, Dalton Trans. 40 (2011) 7817.
[4] R. Trondl, P. Heffeter, C.R. Kowol, M.A. Jakupec, W. Berger, B.K. Keppler, Chem. Sci. (2014).
[5] M.J. Clarke, S. Bitler, D. Rennert, M. Buchbinder, A.D. Kelman, J. Inorg. Biochem. 12 (1980) 79.
[6] A.F.A. Peacock, P.J. Sadler, Chem. Asian J. 3 (2008) 1890.
[7] A.M. Pizarro, P.J. Sadler, Biochimie 91 (2009) 1198.
[8] J. Reedijk, Platin. Met. Rev. 52 (2008) 2.
[9] M.J. Clarke, Coord. Chem. Rev. 236 (2003) 209.
[10] W. Han Ang, P.J. Dyson, Eur. J. Inorg. Chem. 2006 (2006) 4003.
[11] C.G. Hartinger, P.J. Dyson, Chem. Soc. Rev. 38 (2009) 391.
[12] A.F.A. Peacock, M. Melchart, R.J. Deeth, A. Habtemariam, S. Parsons, P.J. Sadler, Chem. Eur. J. 13 (2007) 2601.
[13] I. Romero-Canelón, P.J. Sadler, Inorg. Chem. 52 (2013) 12276.
[14] B.S.P. Michael, A. Jakupec, B.S.P. Bernhard, K. Keppler, Curr. Top. Med. Chem. 4 (2004) 1575.
[15] W. Kandioller, C.G. Hartinger, A.A. Nazarov, C. Bartel, M. Skocic, M.A. Jakupec, V.B. Arion, B.K. Keppler, Chem. Eur. J. 15 (2009) 12283.
[16] W. Kandioller, A. Kurzwernhart, M. Hanif, S.M. Meier, H. Henke, B.K. Keppler, C.G. Hartinger, J. Organomet. Chem. 696 (2011) 999.
[17] S.M. Meier, M. Hanif, W. Kandioller, B.K. Keppler, C.G. Hartinger, J. Inorg. Biochem. 108 (2012) 91.
[18] W. Kandioller, C.G. Hartinger, A.A. Nazarov, M.L. Kuznetsov, R.O. John, C. Bartel, M.A. Jakupec, V.B. Arion, B.K. Keppler, Organometallics 28 (2009) 4249.
[19] J.H. Kasser, W. Kandioller, C.G. Hartinger, A.A. Nazarov, V.B. Arion, P.J. Dyson, B.K. Keppler, J. Organomet. Chem. 695 (2010) 875.
[20] M.A. Bennett, A.K. Smith, J. Chem. Soc. Dalton Trans. (1974) 233.
[21] B.L. Booth, R.N. Haszeldine, M. Hill, J. Chem. Soc. Inorg. Phys. Theor. (1969) 1299.
[22] Z.D. Liu, H.H. Khodr, D.Y. Liu, S.L. Lu, R.C. Hider, J. Med. Chem. 42 (1999) 4814.
[23] Y. Ma, W. Luo, P.J. Quinn, Z. Liu, R.C. Hider, J. Med. Chem. 47 (2004) 6349.
[24] J.A. Lewis, D.T. Puerta, S.M. Cohen, Inorg. Chem. 42 (2003) 7455.
[25] M.R. Pressprich, J. Chambers, SAINT + Integration Engine, Program for Crystal Structure Integration, Bruker Analytical X-ray systems, Madison, 2004.
[26] G.M. Sheldrick, Acta Crystallogr. A 64 (2007) 112.
[27] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, J. Appl. Crystallogr. 42 (2009) 339.
[28] C.B. Hübschle, G.M. Sheldrick, B. Dittrich, J. Appl, Crystallogr 44 (2011) 1281.
[29] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565.
[30] M.D. Aytemir, U. Calis, Hacet. Univ. J. Fac. Pharm. 27 (2007) 1.
[31] M.D. Aytemir, B. Özçelik, Eur. J. Med. Chem. 45 (2010) 4089.
[32] S. Chaves, M. Gil, S. Canário, R. Jelic, M.J. Romão, J. Trincão, E. Herdtweck, J. Sousa, C. Diniz, P. Fresco, M.A. Santos, Dalton Trans. (2008) 1773.
[33] J.A. Lewis, S.M. Cohen, Inorg. Chem. 43 (2004) 6534.
[34] M. Hanif, H. Henke, S.M. Meier, S. Martic, M. Labib, W. Kandioller, M.A. Jakupec, V.B. Arion, H.-B. Kraatz, B.K. Keppler, C.G. Hartinger, Inorg. Chem. 49 (2010) 7953.
[35] D. Brayton, F.E. Jacobsen, S.M. Cohen, P.J. Farmer, Chem. Commun. (2006) 206.
[36] M.J. Clarke, F. Zhu, D.R. Frasca, Chem. Rev. 99 (1999) 2511.
[37] E.S. Antonarakis, A. Emadi, Cancer Chemother. Pharmacol. 66 (2010) 1.
[38] M.-G. Mendoza-Ferri, C.G. Hartinger, R.E. Eichinger, N. Stolyarova, K. Severin, M.A. Jakupec, A.A. Nazarov, B.K. Keppler, Organometallics 27 (2008) 2405.
[39] S.M. Meier, M. Novak, W. Kandioller, M.A. Jakupec, V.B. Arion, N. Metzler-Nolte, B.K. Keppler, C.G. Hartinger, Chem. Eur. J. 19 (2013) 9297.
[40] A.S. Kearney, Adv. Drug Deliv. Rev. 19 (1996) 225.
[41] A.K. Sinhababu, D.R. Thakker, Adv. Drug Deliv. Rev. 19 (1996) 241.
[42] O. Dömötör, S. Aicher, M. Schmidlehner, M.S. Novak, A. Roller, M.A. Jakupec, W. Kandioller, C.G. Hartinger, B.K. Keppler, É.A. Enyedy, J. Inorg. Biochem. 134 (2014) 57.
[43] P.-S. Kuhn, V. Pichler, A. Roller, M.A. Jakupec, W. Kandioller, B. Keppler, submitted for publication.