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# Mannich products of kojic acid and *N*-heterocycles and their Ru(II)–arene complexes: Synthesis, characterization and stability

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ABSTRACT

Ru(II)–$\eta^6$-*p*-cymene compounds bearing pyrone-derived ligands, which were obtained by Mannich reaction with piperidine and related analogues, have been synthesized. The compounds were characterized by NMR spectroscopy, mass spectrometry, thermogravimetric analysis and in the case of 2-(2,6-dimethyl-morpholin-4-ylmethyl)-3-hydroxy-6-hydroxymethyl-pyran-4-one by X-ray diffraction analysis. The chlorido complexes are prone to aquation in aqueous solution which results in the formation of dimers. Dimer formation can be inhibited by in situ replacement of the chlorido ligand by imidazole yielding compounds which are significantly more stable in water, as demonstrated by $^1$H NMR spectroscopy.



## 1. Introduction

Severe side effects, high toxicity and limited activity of platinum anticancer agents have led to the development of new metal-containing chemotherapeutics [1]. Ruthenium compounds are considered to be promising alternatives with complementary activity, i.e., in tumors that do not respond to platinum drugs, and moreover, exhibit a lower general toxicity.

Indazolium trans-[tetrachloridobis(1H-indazole)ruthenate(III)] (KP1019) and imidazolium trans-[tetrachlorido(S-dimethyl sulfoxide)(1H-imidazole)ruthenate(III)] (NAMI-A) entered clinical trials in recent years [2–4]. Protein binding after intravenous administration and activation by reduction in the tumor cell appear to be essential steps in the mode of action of these and related Ru(III) complexes [5–7].

More recently, organometallic Ru(II)–arene compounds (see Fig. 1 for some examples of mono- and polynuclear compounds) were identified as promising anticancer agents [8–10]. They exhibit activity in different tumor models compared to the established platinum-based anticancer agents and some are even active in platinum resistant cell lines [11–16] and against metastatic tumors in vivo [17,18]. The most widely developed compounds are cationic ethylenediamine (en) and RAPTA compounds [8–10,19]. The replacement of the chelating en moiety by *O*,*S*-containing bidentate ligands results in compounds which are significantly less active, at least in human tumor cell lines [20]. The lower potency to inhibit cell proliferation of, for example, maltolato complexes was related to the formation of stable dinuclear $[Ru_2(cym)_2(OH)_3]^+$ species (cym = $\eta^6$-*p*-cymene) in aqueous solution, as demonstrated by electrospray ionization mass spectrometry (ESI-MS) [20]. Replacement of pyronato by thiopyronato and pyridinonato ligands stabilizes the compounds and cytotoxicities in the low μM range were obtained [21–24]. However, more recently pyrone compounds were reported that do not undergo the described dimer formation but also do not exhibit antiproliferative activity [25]. This behavior was related to the decomposition of the compounds by reaction with biological nucleophiles, e.g., those present in the medium used for cultivating human tumor cell lines [25].

Kojic acid, or 5-hydroxy-2-hydroxymethyl-4H-pyran-4-one, is a bioactive γ-pyrone derivative produced by many species of *Aspergillus* and *Penicillium* [26]. The chelating properties of such pyrones to transition metal ions, e.g., Ru(II/III), Fe(III), Cu(II), [27–31] make them interesting ligands. Complexes of pyrones with vanadium and zinc have already been developed as insulin mimics and

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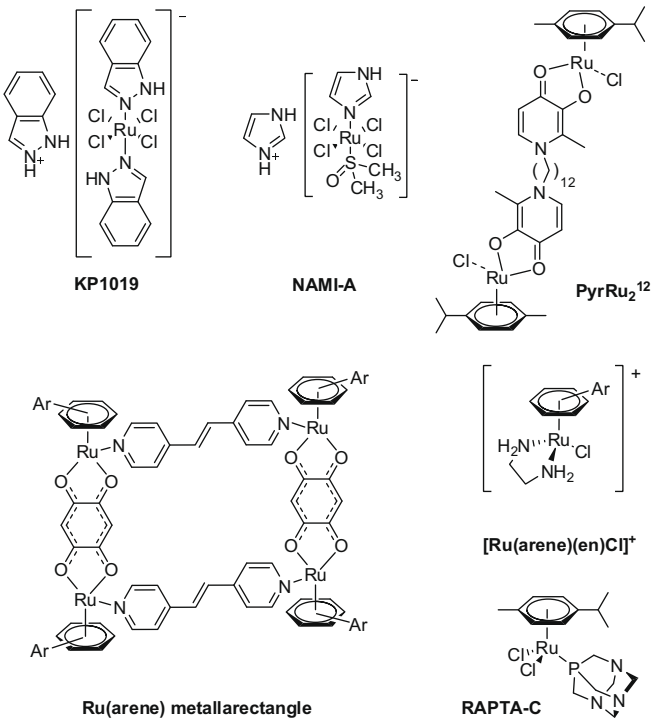



**Fig. 1.** Structures of ruthenium anticancer drug candidates.

vanadyl coordination compounds with maltol and ethylmaltol recently entered clinical trials [32–36]. Kojic acid has antibacterial and antifungal properties. Furthermore, kojic acid was shown to inhibit different enzymes relevant to the undesirable melanosis of agricultural products, which is related to its coordination ability to, e.g., copper, in the active site of tyrosinase [37–39].

In this study, the kojic acid scaffold was modified by a Mannich reaction with piperidine derivatives with the aim to link it to Ru(II)–arene fragments and to obtain compounds with anticancer activity. The synthesized complexes were characterized by standard methods and their behavior in aqueous solution and their reactivity towards imidazole was studied.

## 2. Experimental section

### 2.1. General

Analytical grade materials were obtained from commercial suppliers and used without further purification. Bis[dichlorido($\eta^6$-*p*-cymene)ruthenium(II)] was synthesized as described elsewhere [40]. Melting points were determined with a Büchi B-540 apparatus and are uncorrected. NMR spectra were recorded at 25 °C in DMSO-$d_6$ on a Bruker FT-NMR spectrometer Avance III™ 500 MHz at 500.10 ($^1$H) and 125.75 MHz ($^{13}$C{$^1$H}). Electrospray ionization mass spectra were recorded on a Bruker esquire$_{3000}$ in negative and positive ion modes. Elemental analyses were performed by the Laboratory for Elemental Analysis of the Faculty of Chemistry, University of Vienna, with a Perkin Elmer 2400 CHN Elemental Analyzer. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) measurements were carried out simultaneously with a Mettler-Toledo TGA/SDTA851e. The thermograms were recorded in the temperature range 25–900 °C with a heating rate of 5 °C per minute and nitrogen as purge gas with a flow rate of 50 mL/min. The IR spectra were recorded on a Bruker Vertex 70 FT-IR-spectrometer equipped with an ATR unit in the range of 4000–500 cm$^{-1}$.

### 2.2. General procedure for the reaction of kojic acid with secondary amines and formaldehyde – synthesis of **1a–1f**

The secondary amine (4.5 mmol) and 35% aqueous formaldehyde solution (0.44 mL, 5.5 mmol) were dissolved in MeOH (30 mL) and heated to 65 °C. Kojic acid (0.78 g, 5.5 mmol) was added to the refluxing solution and the reaction mixture was cooled under stirring to room temperature. If no precipitation occurred, the reaction mixture was concentrated under reduced pressure and stored at 4 °C overnight. In any case the resulting precipitate was filtered, washed with $Et_2O$ and dried in vacuo.

#### 2.2.1. 3-Hydroxy-6-hydroxymethyl-2-[(piperidin-1-yl)methyl]-pyran-4(1H)-one **1a**

The reaction was performed according to the general procedure, using piperidine (0.44 mL, 4.5 mmol). Yield: 1.01 g (94%) colorless solid. M.p. 167 °C; $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 1.32–1.41 (m, 2H, $H_{pip}$), 1.44–1.53 (m, 4H, $H_{pip}$), 2.36–2.44 (m, 4H, $H_{pip}$), 3.47 (s, 2H, $CH_2$–N), 4.30 (s, 2H, $CH_2$), 5.67 (br s, 1H, OH), 6.31 (s, 1H, H5) ppm; $^{13}$C NMR (DMSO-$d_6$, 125.75 MHz, 25 °C): $\delta$ = 24.1 ($C_{pip}$), 25.9 ($C_{pip}$), 54.2 ($C_{pip}$), 55.0 ($CH_2$–N), 60.1 ($CH_2$), 109.4 (C5), 144.1 (C6), 147.3 (C2), 168.0 (C3), 174.0 (C4) ppm. Elemental Anal. Calc. for $C_{12}H_{17}NO_4$: C, 60.24; H, 7.16; N, 5.85. Found: C, 60.23; H, 7.23; N, 5.90%. IR (ATR, cm$^{-1}$, selected bands): 3262, 2944, 2816, 1645, 1607, 1578, 1459, 1207. ESI-MS (neg): *m/z* 238.0 [M–H]$^-$. ESI-MS (pos): *m/z* 501.3 [2M+Na]$^+$, 240.1 [M+H]$^+$.

#### 2.2.2. 3-Hydroxy-6-hydroxymethyl-2-[(4-methylpiperidin-1-yl)-methyl]pyran-4(1H)-one **1b**

The reaction was performed according to the general procedure, using 4-methylpiperidine (0.53 mL, 4.5 mmol). Yield: 0.98 g (86%) colorless solid. M.p. 168 °C; $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 0.87 (d, 3H, $^3J_{H,H}$ = 7 Hz, –$CH_3$), 1.05–1.18 (m, 2H, $H_{pip}$), 1.24–1.36 (m, 1H, $H_{pip}$), 1.51–1.60 (m, 2H, $H_{pip}$), 1.98–2.09 (m, 2H, $H_{pip}$), 2.75– 2.83 (m, 2H, $H_{pip}$), 3.48 (s, 2H, $CH_2$–N), 4.29 (s, 2H, $CH_2$) 5.69 (br s, 1H, OH), 6.31 (s, 1H, H5) ppm; $^{13}$C NMR (DMSO-$d_6$, 125.75 MHz, 25 °C): $\delta$ = 22.2 ($C_{pip}$), 30.4 ($C_{pip}$), 34.3 ($C_{pip}$), 53.6 ($C_{pip}$), 54.6 ($CH_2$–N), 60.1 ($CH_2$), 109.4 (C5), 144.1 (C6), 147.3 (C2), 168.0 (C3), 174.0 (C4) ppm. IR (ATR, cm$^{-1}$, selected bands): 3269, 2926, 2817, 1645, 1607, 1576, 1459, 1203. Elemental Anal. Calc. for $C_{13}H_{19}NO_4$: C, 61.64 H, 7.56; N, 5.53. Found: C, 61.55; H, 7.65; N, 5.60%. ESI-MS (neg): *m/z* 252.1 [M–H]$^-$, 505.2 [2M–H]$^-$. ESI-MS (pos): *m/z* 254.2 [M+H]$^+$, 529.3 [2M+Na]$^+$.

#### 2.2.3. 3-Hydroxy-6-hydroxymethyl-2-[(morpholin-4-yl)-methyl]pyran-4(1H)-one **1c**

The reaction was performed according to the general procedure, using morpholine (0.39 mL, 4.5 mmol). Yield: 0.93 g (85%) colorless solid. M.p. 172 °C; $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 2.43 (t, $^3J_{H,H}$ = 4 Hz, 4H, $H_{morph}$), 3.50 (s, 2H, $CH_2$–N), 3.56 (t, $^3J_{H,H}$ = 4 Hz, 4H, $H_{morph}$), 4.31 (d, $^3J_{H,H}$ = 4 Hz, 2H, $CH_2$), 5.68 (t, $^3J_{H,H}$ = 6 Hz, 1H, OH), 6.32 (s, 1H, H5), 9.01 (br s, 1H, $OH_{pyrone}$) ppm; $^{13}$C NMR (DMSO-$d_6$, 125.75 MHz, 25 °C): $\delta$ = 53.40 ($C_{morph}$), 54.4 (C8), 60.1 (C7), 66.6 ($C_{morph}$), 109.4 (C5), 144.2 (C6), 146.7 (C2), 168.1 (C3), 174.1 (C4) ppm. IR (ATR, cm$^{-1}$, selected bands): 3254, 2946, 2826, 1648, 1608, 1575, 1451, 1198. Elemental Anal. Calc. for $C_{11}H_{15}NO_5$: C, 54.77; H, 6.27; N, 5.81. Found: C, 54.84; H, 6.33; N, 5.81%. ESI-MS (neg): *m/z* 240.2 [M–H]$^-$, 480.9 [2M–H]$^-$. ESI-MS (pos): *m/z* 505.0 [2M+Na]$^+$, 264.0 [M+Na]$^+$.

#### 2.2.4. 3-Hydroxy-6-hydroxymethyl-2-[(3-methylpiperidin-1-yl)-methyl]pyran-4(1H)-one **1d**

The reaction was performed according to the general procedure, using 3-methylpiperidine (0.53 mL, 4.5 mmol). Yield: 0.65 g (57%) colorless solid. M.p. 162 °C; $^1$H NMR (DMSO-$d_6$, 500.10 MHz,

25 °C): $\delta$ = 0.79–0.84 (m, 4H, $H_{pip}$), 1.38–1.49 (m, 1H, $H_{pip}$), 1.51–1.71 (m, 4H, $H_{pip}$), 1.92–2.00 (m, 1H, $H_{pip}$), 2.69–2.80 (m, 2H, $H_{pip}$), 3.48 (s, 2H, $CH_2$–N), 4.29 (s, 2H, $CH_2$), 5.68 (br s, 1H, OH), 6.31 (s, 1H, H5) 8.98 (br s, 1H, $OH_{pyrone}$) ppm; $^{13}$C NMR (DMSO-$d_6$, 125.75 MHz, 25 °C): $\delta$ = 20.0 ($C_{pip}$), 25.5 ($C_{pip}$), 31.1 ($C_{pip}$), 32.7 ($C_{pip}$), 53.7 ($C_{pip}$), 54.7 ($CH_2$–N), 60.1 ($CH_2$), 61.5 ($C_{pip}$), 109.4 (C5), 144.1 (C6), 147.3 (C2), 168.0. (C3), 174.0 (C4) ppm. IR (ATR, $cm^{-1}$, selected bands): 3266, 2928, 2803, 1649, 1609, 1578, 1460, 1202. Elemental Anal. Calc. for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.59; H, 7.64; N, 5.57%. ESI-MS (neg): *m/z* 252.5 $[M–H]^-$, 504.9 $[2M–H]^-$. ESI-MS (pos): *m/z* 254.5 $[M+H]^+$.

#### 2.2.5. 2-[(3,5-Dimethylpiperidin-1-yl)methyl]-3-hydroxy-6-hydroxymethylpyran-4(1H)-one **1e**

The reaction was performed according to the general procedure, using 3,5-dimethylpiperidine (0.60 mL, 4.5 mmol). Yield: 0.30 g (25%) colorless solid. M.p. 163 °C; $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 0.41–0.53 (m, 1H, $H_{pip}$), 0.76–0.83 (d, $^3J_{H,H}$ = 6 Hz, 6H, $H_{pip}$), 1.54–1.65 (m, 5H, $H_{pip}$ 2.73–2.81 (d, $^3J_{H,H}$ = 6 Hz, 2H, $H_{pip}$), 3.49 (s, 2H, $CH_2$–N), 4.29 (s, 2H, $CH_2$), 5.68 (br s, 1H, OH), 6.31 (s, 1H, H5), 8.98 (br s, 1H, $OH_{pyrone}$) ppm; $^{13}$C NMR (DMSO-$d_6$, 125.75 MHz, 25 °C): $\delta$ = 19.9 ($CH_3$), 31.1 ($C_{pip}$), 42.0 ($C_{pip}$), 54.4 ($CH_2$–N), 60.1 ($CH_2$), 61.2 ($C_{pip}$), 109.4 (C5), 144.1 (C6), 147.3 (C2), 168.0 (C3), 174.0 (C4) ppm. IR (ATR, $cm^{-1}$, selected bands): 3269, 2951, 2837, 1653, 1607, 1578, 1462, 1200. Elemental Anal. Calc. for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.78; H, 8.03; N, 5.37%. ESI-MS (neg): *m/z* 266.6 $[M–H]^-$. ESI-MS (pos): *m/z* 268.5 $[M+H]^+$.

#### 2.2.6. 2-[(2,6-Dimethylmorpholin-4-yl)methyl]-3-hydroxy-6-hydroxymethylpyran-4(1H)-one **1f**

The reaction was performed according to the general procedure, using 2,6-dimethylmorpholine (0.55 mL, 4.5 mmol). Yield: 0.69 g (57%) colorless solid. M.p. 168 °C; $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 1.03 (d, 6H, $^3J_{H,H}$ = 6 Hz, $CH_3$), 1.76 (t, 2H, $^3J_{H,H}$ = 10 Hz, $H_{morph}$), 2.70 (d, 2H, $^3J_{H,H}$ 10 Hz, $H_{morph}$), 3.49 (s, 2H, $CH_2$–N), 3.50–3.58 (m, 2H, $H_{morph}$), 4.30 (d, $^3J_{H,H}$ = 6 Hz, 2H, $CH_2$), 5.68 (t, $^3J_{H,H}$ = 6 Hz, 1H, OH), 6.32 (s, 1H, H5), 8.98 (br s, 1H, $OH_{pyrone}$) ppm; $^{13}$C NMR (DMSO-$d_6$, 125.75 MHz, 25 °C): $\delta$ = 19.4 ($CH_3$), 54.0 (C8), 59.12 ($C_{morph}$), 60.1 (7), 71.4 ($C_{morph}$), 109.4 (5), 144.2 (C6), 146.7 (C2), 168.1 (C3), 174.1 (C4) ppm. IR (ATR, $cm^{-1}$, selected bands): 3264, 2975, 2860, 1648, 1609, 1577, 1459, 1200. Elemental Anal. Calc. for $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11; N, 5.20. Found: C, 57.91; H, 7.18; N, 5.22%. ESI-MS (neg): *m/z* 268.5 $[M–H]^-$, 536.8 $[2M–H]^-$. ESI-MS (pos): *m/z* 560.9 $[2M+Na]^+$, 270.1 $[M+H]^+$.

### 2.3. General procedure for synthesis of the Ru(II) complexes **2a–2f**

The ligand (0.66 mmol) and sodium methoxide (42 mg, 0.77 mmol) were suspended in MeOH (30 mL) under an argon atmosphere. Bis[dichlorido($\eta^6$-*p*-cymene)ruthenium(II)] (200 mg, 0.33 mmol) in $CH_2Cl_2$ (20 mL) was added dropwise and the solution was stirred for 18 h at room temperature, filtered and concentrated under reduced pressure. The obtained residue was extracted with $CH_2Cl_2$ (3 × 15 mL), the organic phase was filtered and the volume was reduced to 5 mL, and the product precipitated with hexane (if necessary). The mixture was stored at 4 °C overnight and the resulting solid was removed by filtration and dried in vacuo.

#### 2.3.1. Chlorido{3-(hydroxy-κO)-6-hydroxymethyl-2-[(piperidin-1-yl)methyl]pyran-4(1H)-onato-κO}($\eta^6$-*p*-cymene)ruthenium(II) **2a**

The reaction was performed according to the general procedure for the synthesis of the Ru(II) complexes, using 3-hydroxy-6-hydroxymethyl-2-[(piperidin-1-yl)methyl]pyran-4-one (158 mg, 0.66 mmol). Yield: 291 mg (84%) red solid. IR (ATR, $cm^{-1}$, selected bands): 3380, 3058, 2958, 2868, 1601, 1567, 1513, 1475, 1209. Elemental Anal. Calc. for $C_{22}H_{30}ClNO_4Ru{\cdot}H_2O$: C, 50.13; H, 6.12; N, 2.66. Found: C, 50.33; H, 5.99; N, 2.66%. ESI-MS (pos): *m/z* 474.5 $[M–Cl]^+$, 390.4 $[M–Cl–piperidine]^+$.

#### 2.3.2. Chlorido{3-(hydroxy-κO)-6-hydroxymethyl-2-[(4-methylpiperidin-1-yl)methyl]pyran-4(1H)-onato-κO}($\eta^6$-*p*-cymene)ruthenium(II) **2b**

The reaction was performed according to the general procedure for the synthesis of the Ru(II) complexes, using 3-hydroxy-6-hydroxymethyl-2-[(4-methylpiperidin-1-yl)methyl]pyran-4-one (167 mg, 0.66 mmol). Yield: 288 mg (81%) red solid. IR (ATR, $cm^{-1}$, selected bands): 3268, 2920, 2870, 1603, 1563, 1505, 1474, 1201, 1051. Elemental Anal. Calc. for $C_{23}H_{32}ClNO_4Ru{\cdot}H_2O$: C, 51.05; H, 6.33; N, 2.59. Found: C, 51.20; H, 6.29; N, 2.53%. ESI-MS (pos): *m/z* 488.4 $[M–Cl]^+$, 390.4 $[M–Cl–4\text{-methylpiperidine}]^+$.

#### 2.3.3. Chlorido{3-(hydroxy-κO)-6-hydroxymethyl-2-[(morpholin-4-yl)methyl]pyran-4(1H)-onato-κO}($\eta^6$-*p*-cymene)ruthenium(II) **2c**

The reaction was performed according to the general procedure for the synthesis of the Ru(II) complexes, using 3-hydroxy-6-hydroxymethyl-2-[(morpholin-4-yl)methyl]pyran-4-one (159 mg, 0.66 mmol). Yield: 339 mg (98%) red solid. IR (ATR, $cm^{-1}$, selected bands): 3380, 3061, 2962, 2868, 1602, 1565, 1503, 1478, 1199, 1088. Elemental Anal. Calc. for $C_{21}H_{28}ClNO_5Ru{\cdot}0.5H_2O$: C, 48.51; H, 5.62; N, 2.69. Found: C, 48.43; H, 5.57; N, 2.77%. ESI-MS (pos): *m/z* 476.4 $[M–Cl]^+$.

#### 2.3.4. Chlorido{3-(hydroxy-κO)-6-hydroxymethyl-2-[(3-methylpiperidin-1-yl)methyl]pyran-4(1H)-onato-κO}($\eta^6$-*p*-cymene)ruthenium(II) **2d**

The reaction was performed according to the general procedure for the synthesis of the Ru(II) complexes, using 3-hydroxy-6-hydroxymethyl-2-[(3-methylpiperidin-1-yl)methyl]pyran-4-one (167 mg, 0.66 mmol). Yield: 308 mg (88%) red solid. IR (ATR, $cm^{-1}$, selected bands): 3334, 2927, 2871, 1602, 1564, 1476, 1276, 1201 Elemental Anal. Calc. for $C_{23}H_{32}ClNO_4Ru{\cdot}0.5H_2O$: C, 51.92; H, 6.25; N, 2.63. Found: C, 51.86; H, 6.22; N, 2.62%. ESI-MS (pos): *m/z* 488.5 $[M–Cl]^+$, 390.5 $[M–Cl–3\text{-methylpiperidine}]^+$.

#### 2.3.5. Chlorido{2-[(3,5-dimethylpiperidin-1-yl)methyl]-3-(hydroxy-κO)-6-hydroxymethyl-pyran-4(1H)-onato-κO}($\eta^6$-*p*-cymene)ruthenium(II) **2e**

The reaction was performed according to the general procedure for the synthesis of the Ru(II) complexes, using 2-[(3,5-dimethylpiperidin-1-yl)methyl]-3-hydroxy-6-hydroxymethyl-pyran-4-one (176 mg, 0.66 mmol). Yield: 324 mg (89%) red solid. IR (ATR, $cm^{-1}$, selected bands): 3318, 3043, 2950, 2871, 1597, 1564, 1491, 1276, 1035. Elemental Anal. Calc. for $C_{24}H_{34}ClNO_4Ru{\cdot}0.75H_2O$: C, 52.36; H, 6.50; N, 2.54. Found: C, 52.35; H, 6.33; N, 2.53%. ESI-MS (pos): *m/z* 502.5 $[M–Cl]^+$, 390.4 $[M–Cl–3,5\text{-dimethylpiperidine}]^+$.

#### 2.3.6. Chlorido{2-[(2,6-dimethylmorpholin-4-yl)methyl]-3-(hydroxy-κO)-6-hydroxymethyl-pyran-4(1H)-onato-κO}($\eta^6$-*p*-cymene)ruthenium(II) **2f**

The reaction was performed according to the general procedure for the synthesis of the Ru(II) complexes, using 2-[(2,6-dimethylmorpholin-4-yl)methyl]-3-hydroxy-6-hydroxymethyl-pyran-4-one (178 mg, 0.66 mmol). Yield: 297 mg (82%) red solid. IR (ATR, $cm^{-1}$, selected bands): 3349, 2970, 2871, 1603, 1563, 1501, 1477, 1199, 1082. Elemental Anal. Calc. for $C_{23}H_{32}ClNO_5Ru{\cdot}0.5H_2O$: C, 50.40; H, 6.07; N, 2.55. Found: C, 50.13; H, 5.93; N, 2.38%. ESI-MS (pos): *m/z* 504.3 $[M–Cl]^+$.

**Table 1**
Crystallographic data of **1f**.

| | |
|---|---|
| Chemical formula | $C_{13}H_{19}NO_5$ |
| $M_r$ (g mol$^{-1}$) | 269.29 |
| T (K) | 100(2) |
| Crystal size (mm$^3$) | 0.20 × 0.15 × 0.10 |
| Crystal color, shape | Colorless, block |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| $a$ (Å) | 17.9436(12) |
| $b$ (Å) | 7.4072(5) |
| $c$ (Å) | 10.3390(6) |
| $\beta$ (°) | 100.018(3) |
| $V$ (Å$^3$) | 1353.22(15) |
| Z | 4 |
| $D_{calc}$ (g cm$^{-3}$) | 1.322 |
| $\mu$ (mm$^{-1}$) | 0.102 |
| $F$(0 0 0) | 576 |
| $\theta$ range for data collection (°) | 2.98–30.11 |
| $h$ range | −25/25 |
| $k$ range | −10/10 |
| $l$ range | −14/1 |
| Reflections collected | 3983 |
| No. parameters | 174 |
| Independent reflections ($R_{int}$) | 0.0554 |
| $R_1$[a] | 0.0405 |
| w$R_2$[b] | 0.1130 |
| Goodness-of-fit (GOF) on $F^2$[c] | 1.009 |

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

### 2.4. X-ray diffraction analysis

X-ray diffraction measurements were performed with a single crystal of **1f** on a Bruker X8 APEXII CCD diffractometer at 100 K. The single crystal was positioned at 35 mm from the detector and 3472 frames were measured each for 30 s over 1° scan width. The data were processed using the SAINT software package [41]. Crystal data, data collection parameters, and structure refinement details are given in Table 1.

The structure was solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms were inserted at calculated positions and refined with a riding model. The following computer, software and tables were used: structure solution, SHELXS-97 [42]; refinement, SHELXL-97 [43]; molecular diagrams, ORTEP-3 [44]; computer, Pentium IV; scattering factors [45].

### 2.5. Thermogravimetry/differential thermal analysis

The compounds were characterized by TG/DTA under nitrogen atmosphere (flow rate 50 mL min$^{-1}$). The temperature diagram was set to a heat rate of 5 °C min$^{-1}$ from 25 to 900 °C followed by 120 min at 900 °C.

## 3. Results and discussion

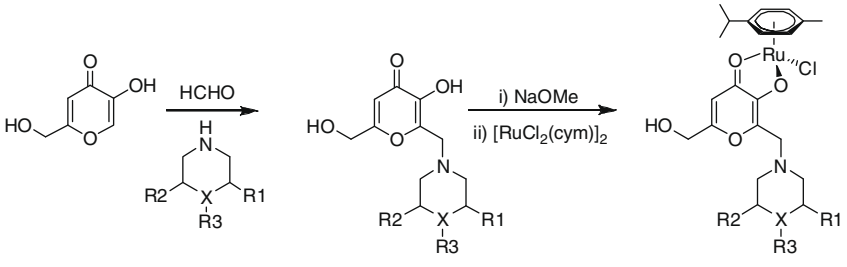
Mannich reactions between kojic acid, a close analogue to allomaltol, and cyclic secondary amines resulted in the ligands **1a–f** (Scheme 1) with the yield (25–94%) strongly depending on the steric bulk of the N-containing heterocycle. Alkyl substitution in the para-position was favorable to meta substitution whereas ortho derivatization did not show any conversion to the desired product. Reactivity at the 2-position in the pyrone ring appears to be significantly lower for kojic acid compared to allomaltol, for which a series of Mannich products were reported [46]. Accordingly, the procedure used to derivatize allomaltol, i.e., an aldol reaction with aldehydes at room temperature [47], was not suitable. However, the coordination of the Ru to the ligand was performed using a standard procedure, i.e., following deprotonation of the ligand with sodium methoxide in methanol. In the next step bis[dichlorido($\eta^6$-*p*-cymene)ruthenium(II)] was added and the complexes **2a–f** were obtained in high yield (82–98%).

The compounds were characterized by 1D and 2D NMR spectroscopy (**1a–f**), IR spectroscopy, thermogravimetry/differential thermal analysis (TG/DTA), electrospray ionization mass spectrometry (ESI-MS) and elemental analysis. Single crystals of **1f** suitable for X-ray diffraction analysis were obtained from methanol.

The chemical shifts of the H5 proton of the pyrone ring and of the aliphatic hydroxymethyl protons are very similar for all ligands in DMSO-$d_6$. The complexes **2a–f** react rapidly with solvents such as $CDCl_3$, DMSO-$d_6$, MeOH-$d_4$ and $D_2O$ forming several species, as evidenced by NMR spectroscopy. In order to reduce the rate of the hydrolysis reaction in $D_2O$, NaCl was added to the reaction mixture. However, in contrast to recent studies with other Ru(II)–arene compounds [13], addition of NaCl did not shift the equilibrium to the chlorido complexes. A similar behavior was observed for structurally related (thio)pyrone complexes [22,25,47].

The IR spectra of the ligands show sharp bands at around 3260 cm$^{-1}$, assignable to the hydroxy functionality of the pyrone ($\nu$O–H), and bands at ca. 1645, 1605 and 1575 cm$^{-1}$ corresponding to the $\delta$O–H and $\nu$C=C, $\nu$C=O as well as $\nu$C=C vibrations [48]. After complex formation the band assigned to the OH group is significantly weakened and becomes much broader. The signals between 1645 and 1570 cm$^{-1}$ are shifted to lower energies and appear at approximately 1600 ($\nu$C=C), 1560 ($\nu$C=O) and 1500 cm$^{-1}$ ($\nu$C=C), as reported for other pyrone complexes [48].

The X-ray structure of **1f** shows similar features to other pyrones (Fig. 2) [49,50]. The pyrone ring in **1f** is not perfectly planar with an O1–C2–C3–C4 torsion angle of 5.95(16)°, compared to 2.0(4) in allomaltol and 3.00/−2.11° in two polymorphic forms of




**a** R1 = R2 = R3 = H, X = C
**b** R1 = R2 = H, R3 = $CH_3$, X = C
**c** R1 = R2 = H, X = O
**d** R1 = R3 = H, R2 = $CH_3$, X = C
**e** R1 = R2 = $CH_3$, R3 = H, X = C
**f** R1 = R2 = $CH_3$, X = O

**Scheme 1.** Synthesis of the ligands and of the corresponding Ru(II)–$\eta^6$-*p*-cymene complexes.

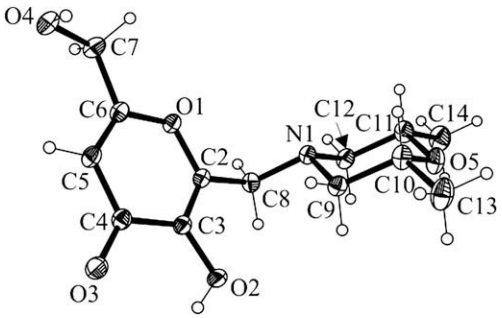
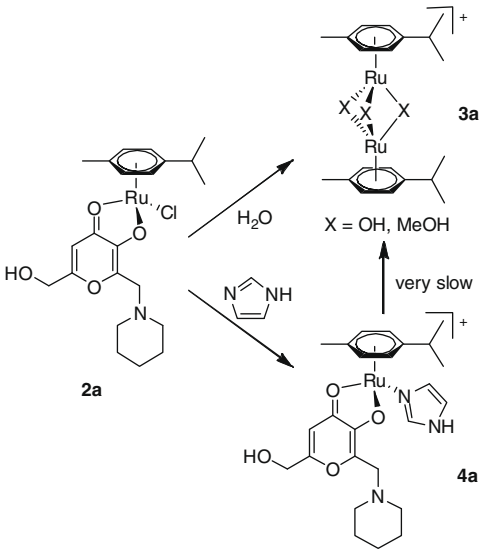



**Fig. 2.** The molecular structure of **1f**. The displacement ellipsoids are drawn at 50% probability level. Selected bond lengths (Å) and angles (°): O2–C3 1.3435(13), O3–C4 1.2502(14), O4–C7 1.4062(14), O1–C2 1.3702(13), O1–C6 1.3420(13), C2–C3 1.3585(16), C5–C6 1.3485(16); C3–C4–O3 121.06(10), C4–C3–O2 120.81(10), C5–C4–O3 124.25(11).

maltol. The bond lengths and angles are similar to those observed for maltol [49] and allomaltol [50]. It appears that the pyrone C2(X)=C3 bonds are generally slightly longer when X is an aliphatic substituent (1.36 vs. 1.32 for X = H), probably due to the electron donating effect. The morpholine ring adopts a chair conformation and the two methyl groups are in equatorial positions and cis to each other, although the starting material for the synthesis contains a mixture of isomers. **1f** is involved in a network of intermolecular hydrogen bonding interactions in the solid state (Fig. S1). The O2 partakes in a bifurcated hydrogen bond to O3 and N1$^{i}$ (see (Fig. S1). Another short hydrogen bonding interaction is evident between the O4 as proton donor and O3$^{ii}$ as proton acceptor.

The synthesized compounds were characterized by TG/DTA under a nitrogen atmosphere. The TG curves of the ligands were very similar and melting points between 160 and 180 °C were observed in all cases (see Section 2). As soon as **1a**–**f** are heated to temperatures greater than the melting point, decomposition occurs with an initial loss of the $CH_2OH$ group. For compounds **2a**–**f** several degradation steps were observed (Fig. 3 for **2b**), but no clear-cut melting points were determined by DTA. The first step in the TG curve is assignable to the release of water (ca. 0.50–0.92 eq.), followed by the pyrone ligand in a first order reaction (e.g., for **2b** 47.7% [theor. 48.2%]) and by decomposition of the residue in higher




**Scheme 2.** Reaction of **2a** with $H_2O$ and imidazole to yield **3a** and **4a**.

order reactions under release of the *p*-cymene and chlorido ligands (e.g., for **2b** 31.3% [theor. 32.4%]). However, no characteristic caloric effects were observed which are necessary to exactly determine the decomposition steps. The final residue for all complexes is assumed to be elemental Ru after heating up to 900 °C (e.g., for **2b** 18.5% [or 19.0% considering the drying effect in step 1; theor. 19.5%]), which is at slightly lower temperature than reported in literature (above 1000 °C) [51].

ESI-MS studies of the ligands were performed in positive and negative ion modes and peaks assignable to protonated, sodiated and deprotonated species were observed. For the complexes analyzed in positive ion mode immediately after dissolution the most abundant peaks may be assigned to $[M–Cl]^+$ ions. However, as mentioned above, these compounds are unstable and their MS change after prolonged incubation. For example, **2a** was dissolved in water and analyzed by ESI-MS after 60 h (sample was mixed with MeOH in order to facilitate the spraying process) and the most abundant peaks at *m/z* 519.4, 551.2 and 565.1 were assigned to $[(cym)Ru(\mu\text{-}CH_3OH)(\mu\text{-}OH)Ru(cym)–2H]^+$, $[(cym)Ru(\mu\text{-}CH_3OH)_2$-

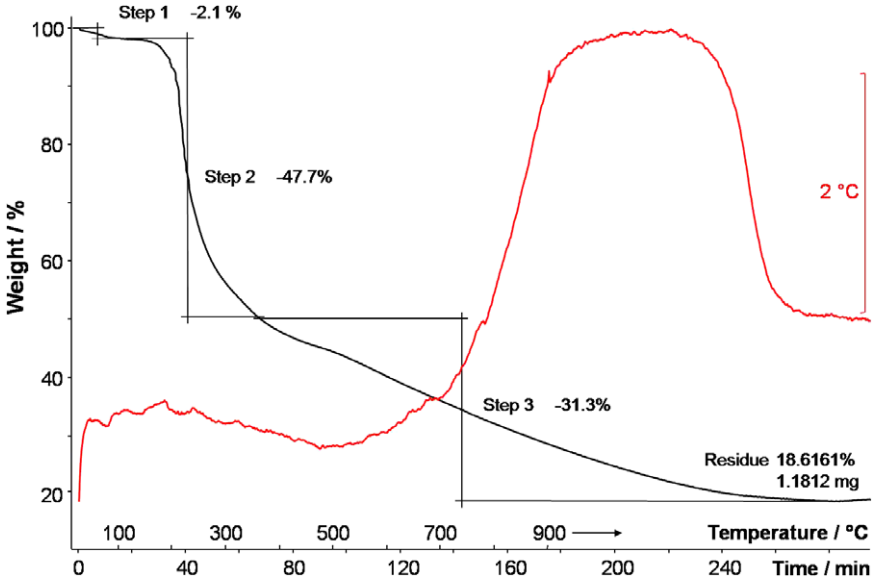



**Fig. 3.** Thermogravimetric and differential thermo analysis curves of **2b**, determined in a temperature range between 25 and 900 °C. The measurement parameters are given in Section 2.

($\mu$-OH)Ru(cym)–2H]$^+$ and [(cym)Ru($\mu$-$CH_3OH)_3$Ru(cym)–3H]$^+$, respectively. In addition, a signal of low abundance at *m/z* 474.6 corresponding to [**2a**–Cl]$^+$ was observed. Mass spectra of the complexes revealed in aqueous solution the presence of dimeric species, such as **3a** (Scheme 2), which are formed by cleavage of the chelating pyrone ligand. All the identified signals contained the characteristic isotope pattern of either mono- or dinuclear ruthenium compounds (mass spectrum of a freshly prepared solution of **2f**, Fig. 4). Similar observations were reported recently for structurally related compounds [20,22,25].

In an attempt to stabilize the Ru complex and prevent dimer formation, the chlorido complex **2a** was reacted with an equimolar amount of imidazole in $D_2O$ to the respective cationic compound **4a** (Fig. 5, Scheme 2). In keeping with other Ru(II)–arene imidazole compounds [52], **4a** showed a much lower rate of hydrolysis to the dimeric compound **3a** in $D_2O$ than the parent complex **2a**. After 18 h incubation both the imidazole complex and decomposition products were present in the reaction mixture, and the ratio of **4a**:**3a** was much higher than the ratio of **2a**:**3a** observed under similar conditions.

The low stability of complexes **2** in aqueous solution limits the compounds for further development as anticancer agents, and makes them unsuitable for biological testing. However, coordination of nucleophiles to the Ru center to replace the chlorido ligands might be an option to obtain compounds with sufficient stability for further studies.

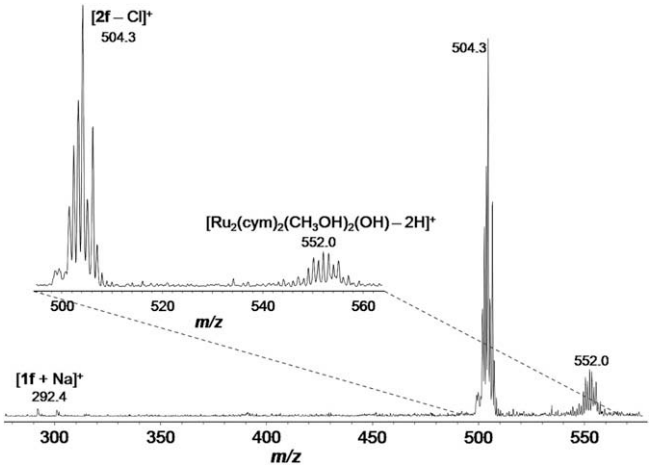



**Fig. 4.** Mass spectrum recorded for **2f** in $H_2O$/MeOH.

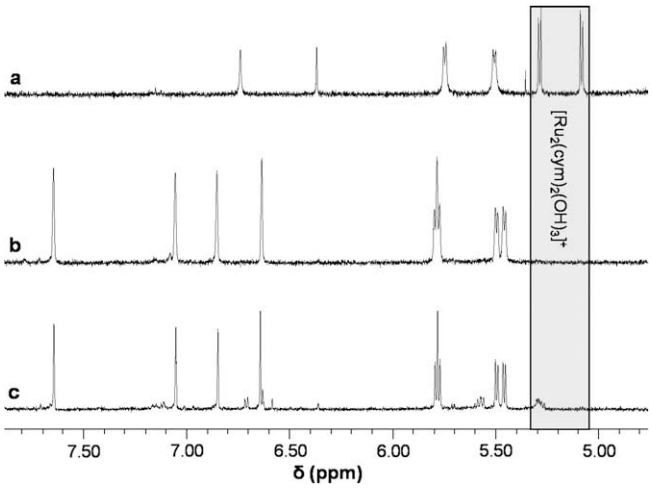



**Fig. 5.** $^1$H NMR spectra from the reaction of **2a** with an equimolar amount of imidazole. (a) **2a** after incubation in $D_2O$ for 18 h, (b) 5 min after addition of imidazole and (c) after a further 18 h.

## 4. Conclusions

Organometallic Ru(II)–arene compounds with chelating ligands have demonstrated potential as anticancer agents. However, one major requirement is stability in aqueous solution in order to administer such drugs formulated for intravenous infusion. Complexes bearing pyrone ligands have been shown to form often stable species suitable for biological applications. In the present study, a series of new pyrone-derived ligands was synthesized from kojic acid in a Mannich reaction with aliphatic *N*-heterocycles and the respective organometallic Ru(II)–arene compounds were obtained. In contrast to related compounds, the complexes were not sufficiently stable to characterize them in solution by NMR spectroscopy (formation of dimeric Ru species) and therefore further biological development appears to be limited. However, the compounds were characterized by IR, TG/DTA, ESI-MS and elemental analysis confirming the proposed structures. In an attempt to stabilize the compounds they were reacted with imidazole to replace the labile chlorido ligand. This approach yields stable species which do not undergo hydrolysis and subsequent reaction to form dimeric Ru(II) compounds. This reaction opens the route to obtain cationic compounds with different properties in comparison to the well established chlorido complexes.

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## Appendix A. Supplementary material

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 743776. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2010.01.007.

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