Organometallic rhodium(III) and iridium(III) cyclopentadienyl complexes with curcumin and bisdemethoxycurcumin co-ligands†

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A series of half-sandwich cyclopentadienyl rhodium(III) and iridium(III) complexes of the type \([\text{Cp}^*\text{M(curc/bdcurc)Cl]}\) and \([\text{Cp}^*\text{M(curc/bdcurc)(PTA)]SO}_3\text{CF}_3}\), in which \(\text{Cp}^*\) = pentamethylcyclopentadienyl, curcH = curcumin and bdcurcH = bisdemethoxycurcumin as O^O-chelating ligands, and PTA = 1,3,5-triaza-7-phosphaadamantane, is described. The X-ray crystal structures of three of the complexes, i.e. \([\text{Cp}^*\text{Rh(curc)(PTA)]SO}_3\text{CF}_3}\) (5), \([\text{Cp}^*\text{Rh(bdcurc)(PTA)]SO}_3\text{CF}_3}\) (6) and \([\text{Cp}^*\text{Ir(bdcurc)(PTA)]SO}_3\text{CF}_3}\) (8), confirm the expected “piano-stool” geometry. With the exception of 5, the complexes are stable under pseudo-physiological conditions and are moderately cytotoxic to human ovarian carcinoma (A2780 and A2780cisR) cells and also to non-tumorigenic human embryonic kidney (HEK293) cells, but lack the cancer cell selectivity observed for related arene ruthenium(II) complexes.

Introduction

Curcumin is a naturally occurring polyphenol derived from the rhizomes of turmeric (Curcuma longa). Curcumin (curcH) is the major component of three curcuminoids that give turmeric its characteristic yellow color. The minor curcuminoid components are demethoxycurcumin (dcurcH) and bisdemethoxycurcumin (bdcurcH) in which one or both –OMe functionalities at the outer phenol rings are removed.

Curcumin displays potent activity in vitro and in animal studies, acting as an anti-proliferative, anti-metastatic and antiangiogenic agent. Numerous studies have also explored the antioxidant, anti-hepatotoxic, anti-hyperlipidemic, antiviral, and anti-Alzheimer’s disease effects. Despite the promising biological effects of curcumin at the preclinical and clinical levels its therapeutic applications are, however, restricted by poor solubility and rapid metabolism resulting in very low bioavailability following oral administration. One strategy used to improve the biological (drug-like) properties of curcumin, which avoids covalently modifying the compound, is to coordinate it to metal complexes.

Ruthenium compounds have emerged, in recent years, as promising alternatives to platinum drugs by displaying specific activities against different cancers and favourable toxicity and clearance properties. Previously, we have demonstrated that half-sandwich ruthenium-arene complexes with curcumin and bisdemethoxycurcumin and an ancillary chloride ligand show promising activity as anticancer agents. We have also shown that the replacement of chloride with the 1,3,5-triaza-7-phosphaadamantane ligand (PTA) led to the formation of RAPTA-type complexes with superior solubility properties and also superior cytotoxicities. It was found that the presence or absence of peripheral methoxy groups in curcumin and the different arene rings do not strongly influence the biological activity whereas the PTA ligand appears to significantly improve the pharmacological properties of the curcumin-modified arene ruthenium(II) complexes.

Rhodium and iridium complexes have been explored for their anticancer properties with initial efforts being focused on Rh(i) and Ir(i) compounds with a square-planar geometry similar to that of cisplatin. Recently, more stable cyclopenta-dienyl Rh(II) and Ir(II) complexes, with higher structural diversity and a higher coordination number (6 versus 4), have been shown to have highly potent anticancer activity. Based on the highly promising pharmacological properties of the arene ruthenium(II) complexes incorporating curcumin and bisdemethoxycurcumin co-ligands, combined with the exciting developments on the anticancer properties of Rh(II)
and Ir(m) organometallic compounds, we decided to integrate these two areas and prepare and evaluate some pentamethycyclopentadienyl Rh(m) and Ir(m) compounds containing curcum-based ligands and, in some cases, PTA. It should be noted that two curcum complexes of Rh(m) and Ir(m) have been reported, but structural data and biological studies were not described.

Results and discussion

Synthesis

The rhodium(m) and iridium(m) pentamethycyclopentadienyl complexes (1–4) were prepared in high yield (78–93%) from the reaction of the appropriate dimer, [Cp*MCl2]2 (M = Rh or Ir, Cp* = η5-pentamethycyclopentadienyl) with the appropriate pro-ligand and KOH in methanol (Scheme 1). Analytical and spectral data for 1 and 3 are in accordance with literature data. Complexes 1–4 are air stable and soluble in acetone, acetonitrile and DMSO and slightly soluble in alcohols. Additionally, the curcum complexes, 1 and 3, are also soluble in chlorinated solvents. Such different solubilities appear to be due to the different R substituents of curcum ligands (Scheme 1). The IR spectra of 1–4 show the typical ν(C=O, C=C) bands of curc and bdcure at lower wavenumbers than the corresponding bands in the free ligands as a consequence of coordination through both the carbonyl arms to the metal. In the far-IR region, several absorptions were observed in the range 482–445 and 252–242 cm⁻¹. These may be assigned to ν(M–O) and ν(M–Cl) stretches, respectively.

The NMR spectra of 1–4 corroborate the expected structures containing the bidentate curcum and bisdemethoxy-curcum ligands. For example, a doublet is observed in the 1H NMR spectra of rhodium derivatives 1 and 4 corresponding to the species [Cp*M(curc/bdcurc)]⁺, in which the chlorid ligand has dissociated.

The chloride ligand in 1–4 is readily replaced by the watersoluble phosphine 1,3,5-triaza-7-phosphaadamantane (PTA), by treatment of the complexes with AgSO2CF3 in methanol containing PTA, affording [Cp*M(curc/bdcurc)(PTA)] [SO2CF3] (M = Rh 5 and 6 and M = Ir 7 and 8), as depicted in Scheme 2. The substitution of the chlorine ligand by PTA and the formation of an ionic compound were confirmed by the disappearance of the ν(M–Cl) band in the IR spectra of 5–8. Moreover, a characteristic absorption pattern in the region 1000–1200 cm⁻¹, indicative of a non-coordinated O3SCF3⁻ anion, has also been observed. The 1H NMR spectra of 5–8 in CD3CN display the expected signals due to the coordinated Cp*, curc or bdcurc and PTA ligands. The resonances due to the PTA are observed at a lower field with respect to those of uncoordinated PTA, thus confirming coordination to the metal center.

The 31P NMR spectra of rhodium complexes 5 and 6 in CD3CN display a doublet centered at ca. −34 ppm with a 1JPr-Rh value of ca. 151 Hz, and the iridium derivatives 7 and 8 comprise a single resonance at −56 ppm, in a range typical of related compounds.

The ESI mass spectra of 5–8 in acetonitrile show two main peak envelopes, that of highest relative intensity corresponding to the fragment [Cp*M(curc/bdcurc)]⁺, upon dissociation of PTA, the other corresponding to the intact species [Cp*M(curc/bdcurc)(PTA)]⁺.

Complex 5 in water–DMSO (80–20%) solution is stable, its 31P NMR spectra remaining unchanged after 96 h. The stability of 5 was also determined under pseudo-pharmacological conditions in 5 mM NaCl solution (being a model for the low intracellular chloride concentration in cells) and in 100 mM NaCl solution (approximating to the higher chloride levels in blood). Solutions of the complexes (c = 2.0 mM) in aqueous NaCl (c = 5 mM or 100 mM in D2O containing 20% of [D6]-DMSO) were prepared and maintained at 37 °C for 7 days. The decomposition of the complexes was monitored by 1H and 31P NMR spectroscopy.

The 31P [1H] spectrum of 5 displays a doublet at −36.0 ppm (JPrH = 155 Hz), corresponding to the starting species, [Cp*Rh(curc)(PTA)]⁺ (RhA) (Scheme 3 and Fig. 1).
After 1 day, signals indicative of two new species are observed in both 5 and 100 mM aqueous NaCl solutions. The first doublet in the range $-35.7/ -36.0$ ppm ($J_{PRh} = 147$ Hz) corresponds to the $[\text{Cp*Rh(PTA)Cl}]^+$ (RhB) species, previously reported by Macchioni and co-workers, arising from the release of the curc ligand and its replacement with a chloride and a second PTA from another $[\text{Cp*Rh(curc)(PTA)}]^+$ species, which in turn likely affords a third neutral species of the formula $[\text{Cp*Rh(curc)Cl}]$ (RhC). The second doublet in the range $-37.3/-37.4$ ppm ($J_{PRh} = 134$ Hz) corresponds to the $[\text{Cp*Rh(PTA)Cl}_2]$ (RhD) species.48

After 96 h an excess of PTA was added and, immediately, the resonance of $[\text{Cp*Rh(PTA)Cl}_2]$ (RhD) disappeared. Whereas, the intensity of $[\text{Cp*Rh(PTA)Cl}]^+$ (RhB) increased and the resonances of $[\text{Cp*Rh(curc)(PTA)}]^+$ (RhA) reappeared, together with a new signal due to $[\text{Cp*Rh(PTA)}]^{2+}$ ($J_{PRh} = 120$ Hz).48

The $^{31}$P NMR spectrum of the iridium compound 7 in water–DMSO (80–20%) solution showed immediately the formation of the hydrolysis products $[\text{Cp*IrCl}_2(\text{PTA})]$ (IrC: $-63.8$ ppm) and $[\text{Cp*IrCl(OD}_2)(\text{PTA})]^+$ (IrB: $-65.7$ ppm), in equilibrium with the starting species $[\text{Cp*Ir(curc)(PTA)}]^+$ (IrA: $-54.0$ ppm). The nature of $[\text{Cp*IrCl}_2(\text{PTA})]$ has been confirmed by adding an excess of NaCl (Fig. 2b), whereas the $[\text{Cp*IrCl(OD}_2)(\text{PTA})]^+$ species has been hypothesized on the basis of a previous work by Peruzzini.47

The difference in stability of iridium complexes with respect to rhodium analogues has been previously observed for complexes with other ligands such as poly(pyrazolyl)borates.49,50

The $^{31}$P NMR spectra of 5 in D$_2$O containing 20% DMSO were recorded at different pD values to determine the pK$_a$ value of the coordinated PTA ligand. The chemical shift was plotted against pD (Fig. 3) and the curve fitted using the Henderson–Hasselbalch equation to give a pK$_a$ of 3.36 ± 0.02 (0.44 is subtracted to account for the difference between the pH and pD).51

The solid-state structures of 5, 6 and 8 were established by X-ray crystallography (see the Experimental section). Their structures are shown in Fig. 4 and relevant bond distances and angles are given in the caption. The three metal complexes...
display the standard piano-stool geometry. Ligands show an almost planar geometry (the angles between the aromatic rings being 1.3°, 4.6° and 4.0° for compounds 5, 6 and 8, respectively), with a small twist around the central backbone (the calculated dihedral angles for the -C=C(=O)-C= moiety varies from 1.7° in 5 to 8° in 8 to 8.8° in 6). The bond distances and angles around the metal centers (see the caption to Fig. 4) fall in the range of values found in the literature for similar compounds.\textsuperscript{19} Strong intermolecular hydrogen bonds are observed between the ligands and the anions (CF\textsubscript{3}SO\textsubscript{3}⁻).

Fig. 4 Molecular structures of 5 (top), 6 (middle) and 8 (bottom). The counter-anion has been omitted for clarity. Bond distances (Å) and angles (°): [5] Rh1–O1, 2.107(1); Rh1–O2, 2.091(1); Rh1–P1, 2.314(1); Rh1–η\textsuperscript{5}(centroid), 1.802(1). [6] Rh1–O1, 2.109(2); Rh1–O2, 2.089(2); Rh1–P1, 2.313(1); Rh1–η\textsuperscript{5}(centroid), 1.808(2). [8] Ir1–O1, 2.117(4); Ir1–O2, 2.101(4); Ir1–P1, 2.299(2), Ir1–η\textsuperscript{5}(centroid), 1.810(3).

Cytotoxicity data for the A2780 cell line are comparable with the IC\textsubscript{50} value obtained for [(η\textsuperscript{5}-Cp*)Ru(curc)Cl\textsuperscript{2+}] containing N\textsuperscript{3}-N-(phenanthroline, 2,2\textsuperscript{′}-bipyridine, ethylenediamine) and N\textsuperscript{2}-O-(picolinate) chelating ligands, are inactive on A2780 cells with IC\textsubscript{50} values >100 μM.\textsuperscript{31,33} Even with modified Cp\textsuperscript{∗}-type ligands\textsuperscript{31,56}. However, varying the chelating ligand\textsuperscript{12,40,57–59} can lead to compounds with IC\textsubscript{50} values considerably higher than cisplatin.

Cytotoxicity data for the A2780 cell line are comparable with the IC\textsubscript{50} value obtained for [(η\textsuperscript{5}-Cp*)Ir-(L\textsuperscript{−}L\textsuperscript{′}Cl)]\textsuperscript{2+} containing N\textsuperscript{3}-N-(phenanthroline, 2,2\textsuperscript{′}-bipyridine, ethylenediamine) and N\textsuperscript{2}-O-(picolinate) chelating ligands, are inactive on A2780 cells with IC\textsubscript{50} values >100 μM.\textsuperscript{31,33} Even with modified Cp\textsuperscript{∗}-type ligands\textsuperscript{31,56}.

The rhodium(III) and iridium(III) complexes with chloride or allomaltol as O\textsuperscript{2+}-chelating ligands were all inactive toward A2780 cells with IC\textsubscript{50} values >100 μM.\textsuperscript{54} The antiproliferative effects of 1–8 were investigated in two human ovarian cancer cell lines, A2780 and the cisplatin-resistant A2780cisR cell line, as well as in non-tumorigenic human embryonic kidney (HEK293) cells. IC\textsubscript{50} values for compounds 1–8 were determined after a 72 hour exposure period using the MTT assay (Table 1 and the Experimental section).

<table>
<thead>
<tr>
<th>Compound</th>
<th>A2780 (IC\textsubscript{50}, μM)</th>
<th>A2780cisR (IC\textsubscript{50}, μM)</th>
<th>HEK293 (IC\textsubscript{50}, μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>curcH</td>
<td>4.0 ± 0.1</td>
<td>3.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>bdcurcH</td>
<td>4.6 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>2.2 ± 0.1</td>
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<tr>
<td>1</td>
<td>14.9 ± 2.1</td>
<td>12.3 ± 0.3</td>
<td>13.7 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>20.0 ± 2.0</td>
<td>18.1 ± 0.2</td>
<td>31.8 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>23.2 ± 0.8</td>
<td>14.3 ± 2.5</td>
<td>16.3 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>20.7 ± 5.6</td>
<td>23.6 ± 2.3</td>
<td>26.1 ± 2.9</td>
</tr>
<tr>
<td>5</td>
<td>12.5 ± 0.5</td>
<td>16.0 ± 3.0</td>
<td>17.2 ± 4.8</td>
</tr>
<tr>
<td>6</td>
<td>14.7 ± 2.2</td>
<td>17.7 ± 0.2</td>
<td>19.0 ± 4.0</td>
</tr>
<tr>
<td>7</td>
<td>23.2 ± 2.3</td>
<td>39.4 ± 9.4</td>
<td>29.4 ± 3.9</td>
</tr>
<tr>
<td>8</td>
<td>21.0 ± 1.0</td>
<td>33.3 ± 3.6</td>
<td>15.6 ± 1.4</td>
</tr>
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</table>

All the complexes were cytotoxic at moderate micromolar concentrations against both cancer cell lines with IC\textsubscript{50} values in the 12–23 μM range for A2780 cells and in the 12–40 μM range for A2780cisR cells, indicating a lack of cross-resistance. In HEK293 cells comparable IC\textsubscript{50} values were obtained (14–32 μM) suggesting a lack of cancer cell selectivity.

Previously reported Rh(III) Cp\textsuperscript{∗} complexes containing maltol or allomaltol as O\textsuperscript{2+}-chelating ligands were all inactive toward A549, CH1 and SW480 cell lines,\textsuperscript{52} whereas the lapachol complex shows good activity and selectivity toward the CH1 ovarian carcinoma cell line.\textsuperscript{53} A cyclometalated rhodium(III) complex shows lower or comparable IC\textsubscript{50} values toward A2780 cells.\textsuperscript{39,55} Ir(III) Cp\textsuperscript{∗} complexes of the formula [(η\textsuperscript{5}-Cp*)Ir-(L\textsuperscript{−}L\textsuperscript{′}Cl)]\textsuperscript{2+} containing N\textsuperscript{3}-N-(phenanthroline, 2,2\textsuperscript{′}-bipyridine, ethylenediamine) and N\textsuperscript{2}-O-(picolinate) chelating ligands, were investigated in two ovarian carcinoma cell line.\textsuperscript{53} A cyclometalated rhodium(III) complex shows lower or comparable IC\textsubscript{50} values toward A2780 cells.\textsuperscript{39,55} However, varying the chelating ligand\textsuperscript{12,40,57–59} can lead to compounds with IC\textsubscript{50} values considerably higher than cisplatin.

Table 1 Cytotoxicity of 1–8 following incubation for 72 h on human ovarian carcinoma A2780 and A2780R (cisplatin-resistant) and non-tumorigenic human embryonic kidney HEK293 cell lines

The antiproliferative effects of 1–8 were investigated in two human ovarian cancer cell lines, A2780 and the cisplatin-resistant A2780cisR cell line, as well as in non-tumorigenic human embryonic kidney (HEK293) cells. IC\textsubscript{50} values for compounds 1–8 were determined after a 72 hour exposure period using the MTT assay (Table 1 and the Experimental section).

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This behaviour contrasts to that of the related ruthenium(n) complexes, in which the activity/selectivity of the PTA-containing complexes was superior to the chloride-based complexes. However, in keeping with the ruthenium(n) complexes, the nature of the curcuminoind ligand employed, i.e. curcumin and bisdemethoxycurcumin, plays little discernible role. It is not unreasonable to assume that following dissociation of the curcuminoind ligand the rhodium(m)/iridium(m) complexes interact with different biological targets compared to the arene ruthenium(n) derivatives, implying a different mechanism of action.

Conclusions

Compared to platinum- or ruthenium-based anticancer agents, the study of the medicinal properties of rhodium and iridium complexes is poorly developed. Here, we described a series of novel pentamethylcyclopentadienyl rhodium(m) and iridium(m) complexes with curcumin or bisdemethoxycurcumin co-ligands and a chloride or PTA ligand, these complexes being closely related to the previously studied arene ruthenium(n) complexes. Unlike related ruthenium(n) complexes with a PTA ligand, which display 100 times higher cytotoxicity towards cancer cells versus healthy cells, the compounds reported here are moderately cytotoxic to both human ovarian cancer cells and non-tumorigenic human embryonic kidney cells. Since it has been shown that all these complexes are able to release their curcuminoind ligands under physiological-like conditions it would suggest that the differences in activity between the pentamethylcyclopentadienyl M(III) (M = Rh/Ir) and arene ruthenium(n) compounds originate from the different metal fragments, which are known to have different binding preferences for certain biological targets.

Experimental section

General procedures

The dimers [Cp*MCl2], (M = Rh or Ir) were purchased from Aldrich. Curcumin and bisdemethoxycurcumin were purchased from TCI Europe and were used as received. All other materials were obtained from commercial sources and were used as received. IR spectra were recorded from 4000 to 30 cm\(^{-1}\) on a Perkin-Elmer Frontier Spectrometer FT-IR/FIR instrument. \(^1\)H and \(^13\)C NMR spectra were recorded on a 400 Mercury Plus Varian instrument operating at room temperature (400 MHz for \(^1\)H and 100 MHz for \(^13\)C) relative to TMS. Positive and negative ion electrospray ionization mass spectra (ESI-MS) were obtained on a Series 1100 MSI detector HP spectrometer using methanol as the mobile phase. Solutions (3 mg mL\(^{-1}\)) for analysis were prepared using reagent-grade methanol. Masses and intensities were compared to those calculated using IsoPro Isotopic Abundance Simulator, version 2.1.28. Melting points are uncorrected and were recorded on a STMP3 Stuart scientific instrument and on a capillary apparatus. Samples for microanalysis were dried in vacuo to constant weight (20 °C, ca. 0.1 Torr) and analysed on a Fisons Instruments 1108 CHNS-O elemental analyzer.

Synthesis of complexes 1–8

[Cp*Rh(curc)Cl] (1). Curcumin (curc, 184.2 mg, 0.5 mmol) was dissolved in methanol (20 mL) and KOH (28.05 mg, 0.5 mmol) was added. The mixture was stirred for 1 h at room temperature and then [Cp*RhCl2] (154.52 mg, 0.25 mmol) was added. The mixture was stirred for 24 h at room temperature and an orange precipitate formed which was filtered off and washed with 5 mL of ethyl ether (278.82 mg, 0.43 mmol, yield 87%). The residue was concentrated to ca. 2 mL and stored at 4 °C. Red crystals were slowly afforded within 2 days.

1 is soluble in acetonitrile, acetone, nitromethane, DMSO and slightly soluble in alcohols. M.p. 264–265 °C. Anal. Calcd for C\(_{31}\)H\(_{34}\)ClO\(_6\)Rh: C, 58.09; H, 5.35. Found: C, 58.00; H, 5.16. IR (cm\(^{-1}\)): 2917 m, 1620 m, 1590 m, 1450 m, 1380 m, 1112 m, 1026 m, 930 m. ESI-MS (+) CH\(_3\)OH (m/z, relative intensity %): 605 [100] [Cp*Rh(curc)Cl].

[Cp*Rh(bdcurc)Cl] (2). Compound 2 was prepared following a procedure similar to that reported for 1 by using bdcurc (270.12 mg, 0.46 mmol, yield 93%). 2 is soluble in acetonitrile, acetone, nitromethane, DMSO and slightly soluble in alcohols. M.p. 236–237 °C. Anal. Calcd for C\(_{29}\)H\(_{30}\)ClO\(_4\)Rh: C, 59.96; H, 5.21. Found: C, 59.84; H, 5.16. IR (cm\(^{-1}\)): 2918 m, 1621 m, 1587 m, 1505 s, 472 m, 454 w, 242 s. H NMR (CD\(_3\)CN, 293 K): \(\delta\) 1.69 (s, 15H, C(H3Cp*), 95.5 (d, CCp*, trans = 16 Hz). 13C NMR (CD\(_3\)CN, 293 K): \(\delta\) 8.8 (s, CH\(_{3}\)Cp*), 56.1 (s, O of curc). ESI-MS (+) CH\(_3\)CN (m/z, relative intensity %): 545 [100] [Cp*Rh(bdcurc)Cl].
(s, C(6, 6'H, OH of curc), 7.20 (s, 6H, C(7, 7'H) of curc), 7.55 (d, 2H, C(4, 4'H) of curc, Jtrans = 16 Hz)).

**[Cp*Ir(bdcurc)Cl]** (4). Compound 4 was prepared following a procedure similar to that reported for 2 by using [Cp*IrCl2](298.25 mg, 0.44 mmol, yield 89%). 4 is soluble in alcohols, acetone, acetonitrile, DMSO and slightly soluble in chlorinated solvents. M.p. 230–232 °C. Anal. Caled for C30H35ClIrO3: C, 51.97; H, 4.51. Found: C, 51.84; H, 4.48. IR (cm^-1): 3214 w, 1621 sh, 1598 m ν(C=C), 1493 s, 1269 s, 1158 s, 482 m, 445 w, 252 s (υ(C=C)).

**[Cp*Rh(bdcurc)(PTA)][SO3CF3]** (5). Ag2SO3CF3 (64 mg, 0.25 mmol) was added to a solution of 1 (160 mg, 0.25 mmol) in acetonitrile. The reaction mixture was stirred for 1 h and filtered to remove AgCl. PTA was added and the reaction was carried out for 24 h at room temperature. The solvent was removed under reduced pressure and methanol and diethyl ether (20 mL) was added. The mixture was left at 4 °C until an orange precipitate formed. The orange crystalline powder was recovered by filtration and air-dried (154.9 mg, 0.169 mmol, yield 68%) and it was identified as 5. It is soluble in alcohols, acetone, acetonitrile, DMSO and slightly soluble in chlorinated solvents and water. M.p. 253–254 °C. Anal. Caled for C136H159Ir2N2O4P6Rh2S: C, 50.06; H, 5.09; N, 4.61; Found: C, 50.00; H, 5.01; N, 4.55. IR (cm^-1): 3258 w, 2925 w, 1621 sh, 1601 m, 1583 m ν(C=C), 1496 s, 1270 s, 1148 s, 1029 s ν(SO3CF3), 570 m ν(Rh-P), 472 m, 454 w, 421 w ν(Rh-P).

**[Cp*Ir(curc)(PTA)][SO3CF3]** (7). Compound 7 was prepared following a procedure similar to that reported for 5 by using precursor 3 (157.66 mg, 0.15 mmol, yield 63%). 7 is soluble in alcohols, acetone, acetonitrile, DMSO and slightly soluble in chlorinated solvents and water. M.p. 227–229 °C. Anal. Caled for C147H165Ir3N3O6P6Rh3[SO3CF3]: C, 53.50; H, 4.30; N, 4.14. IR (cm^-1): 2924 br, 1613 sh, 1599 m ν(C=C), 1493 s, 1270 s, 1149 s, 1028 s ν(SO3CF3), 575 m ν(υ Ir-P), 472 m, 453 w, 390 w ν(υ Ir-P). 1H NMR (CD3CN, 293 K): δ = 8.05 (d, 2H, C(3, 3')H of curc, Jtrans = 16 Hz), 6.98 (d, 2H, C(9, 9')H of curc, Jtrans = 16 Hz), 6.91 (sbr, 2H, OH of curc, 7.16 (dd, 2H, C(10, 10')H of curc, Jtrans = 16 Hz), 7.28 (s, 2H, C(6, 6')H of curc), 7.61 (d, 2H, C(4, 4')H of curc, Jtrans = 16 Hz).

**[Cp*Ir(curc)(PTA)][SO3CF3]** (6). Compound 6 was prepared following a procedure similar to that reported for 5 by using precursor 2 (129.8 mg, 0.15 mmol, yield 61%). 6 is soluble in alcohols, acetone, acetonitrile, DMSO and slightly soluble in water. M.p. 237–239 °C. Anal. Caled for C136H159Ir2N2O4P6Rh2S: C, 50.77; H, 4.97; N, 4.93. Found: C, 50.68; H, 4.86; N, 4.82. IR (cm^-1): 3157 br, 1617 sh, 1602 m, 1588 m ν(C=C), 1493 s, 1277 s, 1160 s, 1025 s ν(SO3CF3), 580 m, 571 m ν(Rh-P), 476 s, 450 w, 346 w ν(Rh-P).

1H NMR (CD3CN, 293 K): δ = 1.67 (d, 15H, CH2Cp, JCH2Cp = 3.4 Hz), 4.16 (s, 6H, CH2N, PTA), 4.48 (s, 6H, PCH2N, PTA), 5.65 (s, 1H, C(1)H of bdcure, Jtrans = 16 Hz), 6.88 (m, 4H, C(7, 7'H) and C(9, 9'H) of bdcure), 7.40 (s, 2H, OH of bdcure), 7.55 (m, 4H, C(6, 6')H and C(10, 10')H of bdcure, Jtrans = 16 Hz). 13C NMR (CD3CN, 293 K): δ = 8.5 (s, CH2Cp), 48.8 (d, NCH2P, PTA, JCP = 9.9 Hz), 72.5 (d, NCH2N, PTA, JCP = 11.1 Hz), 91.6 (s, CCH2, J(CH2Cp) = 7.0 Hz), 104.0 (s, C(1) of bdcure), 116.1 (s, C(7, 7') and C(9, 9') of bdcure), 121.4 (s, C(6, 6') and C(10, 10') of bdcure, 126.5 (s, C(5, 5') of bdcure), 131.0 (s, C(4, 4') of bdcure), 141.0 (s, C(3, 3') of bdcure), 160.5 (s, C(8, 8') of bdcure), 183.8 (s, C(2, 2')=O of bdcure).

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ESI-MS (+) CH$_3$CN (prepared as DMSO solutions and then dissolved in the culture in a medium supplemented with 10% FBS. Compounds were approximately 5000 cells per well and pre-incubated for 24 h), and pre-incubated for 24 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 590 nm using a multwell plate reader, and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from at least two independent experiments, each comprising triplicates per concentration level.

**Determination of pK$_a$ values**

The pH values of NMR samples in D$_2$O were measured at 298 K, directly in the NMR tube, using a 713 pH meter (Metrohm) equipped with an electrode calibrated with buffer solutions at pH values of 4, 7, and 9. The pH values were adjusted with dilute HNO$_3$ and NaOH. The pH titration curves were fitted to the Henderson–Hasselbalch equation using the program Matlab (MathWorks Software) with the assumption that the observed chemical shifts are weighted averages according to the populations of the protonated and deprotonated species. The resonance frequencies change smoothly with pH between the chemical shifts of the charged form HA$^-$, stable in acidic solution, and those of the neutral, deprotonated form A, which is present at a high pH. At any pH, the observed chemical shift is a weighted average ($\delta_{av}$) of the two extreme values $\delta$(HA$^-$) and $\delta$(A):

$$\delta_{av} = \frac{\delta$(HA$^-$)$[HA^+] + \delta$(A)$[A]}{[HA^+] + [A]}$$

The midpoint of the titration occurs when the concentrations of the acid and its conjugate base are equal: [HA$^-$] = [A], that is, when the pH equals the pK$_a$ of the compound. The pH at the midpoint of the curve is corrected by subtracting 0.44 to the pH values since the measurements were made in D$_2$O.

**Acknowledgements**

This work was financially supported by the University of Camerino (Fondo di Ateneo per la Ricerca 2011–2012), by MIUR (PRIN 2010-2011; 2010BNZ3F2) and by COST (CM 1302, European Network on Smart Inorganic Polymers).

**Notes and references**
