Cyclometalated Iridium(III) N-Heterocyclic Carbene Complexes as Potential Mitochondrial Anticancer and Photodynamic Agents

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Table S1 The lipophilicity and cellular uptake efficiency of complexes Ir1-Ir4.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Lipophilicity (log $P_{o/w}$)</th>
<th>Amount of iridium (nmol per cell)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ir1</td>
<td>0.94</td>
<td>0.63 ± 0.084</td>
</tr>
<tr>
<td>Ir2</td>
<td>0.77</td>
<td>0.49 ± 0.054</td>
</tr>
<tr>
<td>Ir3</td>
<td>1.14</td>
<td>0.82 ± 0.13</td>
</tr>
<tr>
<td>Ir4</td>
<td>0.82</td>
<td>0.56 ± 0.066</td>
</tr>
</tbody>
</table>

$^a$Data are presented as means ± standard deviation obtained in at least three independent experiments.

Table S2 IC$_{50}$ (µM) values of the tested complexes towards HeLa, U87 and LO2 cell lines at dark and 450 nm.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>HeLa Dark$^a$</th>
<th>Light$^b$</th>
<th>PI$^c$</th>
<th>U87 Dark$^a$</th>
<th>Light$^b$</th>
<th>PI$^c$</th>
<th>LO2 Dark$^a$</th>
<th>Light$^b$</th>
<th>PI$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ir1</td>
<td>1.3 ± 0.1</td>
<td>0.069 ± 0.013</td>
<td>19</td>
<td>1.6 ± 0.3</td>
<td>0.029 ± 0.008</td>
<td>55</td>
<td>1.2 ± 0.1</td>
<td>0.10 ± 0.03</td>
<td>12</td>
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<tr>
<td>Ir2</td>
<td>1.0 ± 0.1</td>
<td>0.26 ± 0.06</td>
<td>4</td>
<td>1.8 ± 0.3</td>
<td>0.046 ± 0.01</td>
<td>39</td>
<td>2.0 ± 0.3</td>
<td>0.81 ± 0.05</td>
<td>2.5</td>
</tr>
<tr>
<td>Ir3</td>
<td>1.6 ± 0.4</td>
<td>0.11 ± 0.04</td>
<td>15</td>
<td>1.4 ± 0.2</td>
<td>0.069 ± 0.015</td>
<td>20</td>
<td>1.1 ± 0.1</td>
<td>0.17 ± 0.07</td>
<td>6.5</td>
</tr>
<tr>
<td>Ir4</td>
<td>1.8 ± 0.2</td>
<td>0.11 ± 0.06</td>
<td>4</td>
<td>1.6 ± 0.2</td>
<td>0.040 ± 0.009</td>
<td>40</td>
<td>2.1 ± 0.3</td>
<td>0.50 ± 0.04</td>
<td>4.2</td>
</tr>
<tr>
<td>cisplatin</td>
<td>14.1 ± 1.1</td>
<td>13.0 ± 1.2</td>
<td>1.1</td>
<td>33.4 ± 2.3</td>
<td>33.1 ± 2.6</td>
<td>1.0</td>
<td>11.5 ± 0.5</td>
<td>10.9 ± 0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

$^a$IC$_{50}$ values are drug concentrations necessary for 50% inhibition of cell viability. Data are presented as means ± standard deviation obtained in at least three independent experiments. Cells are treated with complexes for 48 h.

$^b$Phototoxicity index is the ratio of the IC$_{50}$ value in dark to that obtained upon light irradiation. Cells were treated with the compounds for 12 h and then exposed to 450 nm LED light for 10 min.

$^c$PI (Phototoxicity index) is the ratio of the IC$_{50}$ value in the dark to that obtained upon light irradiation.
Fig. S1 A) UV/Vis spectra of **Ir1-Ir4** (20 μM) in CH₃CN at 298 K. B) UV/Vis spectra of **Ir1-Ir4** (20 μM) in CH₂Cl₂ at 298 K. C) Normalized emission spectra of **Ir1-Ir4** (20 μM) in CH₃CN at 298 K (λₑₓ = 405 nm). D) Normalized emission spectra of **Ir1-Ir4** (20 μM) in CH₂Cl₂ at 298 K (λₑₓ = 405 nm).
Fig. S2 Confocal images of A549 cells after incubation with Ir1 (10 μM) under different conditions. (A) Cells were incubated with Ir1 (10 μM) at 37 °C for 10 min. (B) Cells were incubated with Ir1 (10 μM) at 4 °C for 10 min. (C) Cells were pre-incubated with CCCP (10 μM) for 1 h at 37 °C and then incubated with Ir1 (10 μM) at 37 °C for 10 min. (D) Cells were pre-incubated with chloroquine (50 μM) for 1 h at 37 °C and then incubated with Ir1 (10 μM) at 37 °C for 10 min. Complex 7 was excited at 405 nm and emission was collected at 600 ± 20 nm. Scale bar: 10 μm.
Fig. S3 Determination of intercellular localization of complexes Ir1-Ir4 by confocal microscopy. A549 cells were incubated with LTDR (100 nM) for 20 min and then co-incubated with Ir1-Ir4 (10 μM) for another 10 min at 37 °C. The Ir(III) complexes were excited at 405 nm and the emission was collected at 600 ± 20 nm. LTDR was excited at 633 nm and the emission was collected at 660 ± 20 nm. Scale bar: 10 μm.
Fig. S4 Impact of complexes Ir1-Ir4 on MMP. The fluorescent intensity ration of A549 cells treated with Ir1-Ir4 at indicated concentrations for 6 h. Data shown are mean values ± standard deviations from three independent experiments. (*) P < 0.01, (**) P < 0.005, compared with the vehicle-treated cells.

Fig. S5 Activation of caspases-3/7 by Ir(III) treatment. A549 cells were exposed to cisplatin, Ir1 and Ir2 at the indicated concentrations for 12 h. Data shown are mean values ± standard deviations from three independent experiments. (**) P < 0.005, compared with the vehicle-treated cells.
Figure S6  ESI-MS spectrum of complexes Ir1. A) Ion isotopes spectrum of complexes Ir1. B) Ion isotopes spectrum of computer simulation using formula IrC₉H₃₂N₆, corresponding to [Ir1-Cl]⁺.

Figure S7  ESI-MS spectrum of complexes Ir2. A) Ion isotopes spectrum of complexes Ir2. B) Ion isotopes spectrum of computer simulation using formula IrC₃₅H₂₈N₆S₂, corresponding to [Ir2-Cl]⁺.
**Figure S8** ESI-MS spectrum of complexes Ir3. A) Ion isotopes spectrum of complexes Ir3. B) Ion isotopes spectrum of computer simulation using formula IrC45H44N6, corresponding to [Ir3-Cl]^+.

**Figure S9** ESI-MS spectrum of complexes Ir4. A) Ion isotopes spectrum of complexes Ir4. B) Ion isotopes spectrum of computer simulation using formula IrC41H40N6S2, corresponding to [Ir4-Cl]^+.
Figure S10 $^1$H NMR spectrum of complexes Ir1.

Figure S11 $^1$H NMR spectrum of complexes Ir2.
Figure S12 $^1$H NMR spectrum of complexes Ir3.

Figure S13 $^1$H NMR spectrum of complexes Ir4.