Necroptosis-Inducing Rhenium(V) Oxo Complexes

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CANCER AND CISPLATIN

Estimated 8.2 million cancer related deaths in 2012

Increased from 2008 by 600,000

- Cisplatin has been used to treat a large number of cancers:
  - Approved: Bladder, ovarian, and testicular
  - Also used for: head and neck, mesothelioma, cervical, lung, esophageal, brain, and neuroblastomas

- Discovered in 1968, due to broad spectrum of activity, became and still is a widely used cancer chemotherapeutic
  - Over 50% of cancer treatment regimens involve cisplatin and its derivatives


CISPLATIN

- Disadvantages (*common* side effects)
  - Kidney damage
  - Decreased blood levels of magnesium, potassium, and calcium
  - Nausea/vomiting
  - Low white blood cell, red blood cell, and platelet counts
  - Taste changes, including metallic taste of foods
  - Sensation of pins and needles or numbness in hands and/or feet caused by irritation of nerves
  - Swelling in hands, feet, or legs
  - Fetal changes if pregnant during treatment

- A number of other platinum based compounds have been pursued
  - Carboplatin, approved 1989 (improved safety)
  - Oxaliplatin, FDA approved 2002 (broader spectrum)
  - Satraplatin, not approved (orally bioavailable)
  - Picoplatin, phase III results unsatisfactory (active in some Cisplatin resistant cancers)


PLATINUM ALTERNATIVES

- Due to high cross resistance of platinum drugs, other transition metal based compounds have been sought as a replacement
- Some classes of transition metal containing complexes:
  - Iridium, titanium, iron, ruthenium, osmium, gold, silver, molybdenum, gallium, rhenium
  - Various mechanisms of action from DNA binding, apoptosis induction, nucleobase binding, to induction of ROS production
- Several have begun phase I and phase II trials
- Difficulties:
  - Aqueous solubility
  - Hydrolytic stability
  - Toxicity

RHENIUM

- Re agents have been used as in vitro and in vivo imaging agents
- Also $^{186}$Re and $^{188}$Re have been used in radiotherapy
  - However, its antiproliferative activities have not been studied as well
- Appealing for catalytic potential and lipophilicity
- Re(I) compounds have proven to be some of the most active Re antiproliferative compounds reported (acting via covalent interaction with DNA or protein side chains)
  - IC$_{50}$ as low as 700 nM
- In 2010, Mitsopoulou and co workers showed that several oxo Re(V) complexes were able to intercalate into DNA and upon irradiation cause DNA strand breaks suggesting potential use as cancer chemotherapeutic
COMPOUNDS 1 AND 2

KReO₄ $\xrightarrow{\text{PPh₃, HCl, EtOH, reflux}}$ Re$^{2+}$Cl₂$\cdot$PPh₃

$\xrightarrow{\text{MeOH, 24 h, 50°C}}$ Re$^{2+}$Cl₂$\cdot$OMe

1

2
**IN VITRO ACTIVITY**

**IC\textsubscript{50} Values (nM) in Various Cancerous and Healthy Cell Lines after 72 h Exposure**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cancer type</th>
<th>1</th>
<th>2</th>
<th>Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>Lung carcinoma</td>
<td>207 ± 4</td>
<td>157 ± 15</td>
<td>3230 ± 467</td>
</tr>
<tr>
<td>HeLa</td>
<td>Cervical adenocarcinoma</td>
<td>445 ± 4</td>
<td>695 ± 21</td>
<td>4100 ± 113</td>
</tr>
<tr>
<td>U2OS</td>
<td>Bone osteosarcoma</td>
<td>274 ± 6</td>
<td>209 ± 31</td>
<td>4600 ± 600\textsuperscript{a}</td>
</tr>
<tr>
<td>NTERA-2</td>
<td>Testis carcinoma</td>
<td>230 ± 28</td>
<td>255 ± 35</td>
<td>385 ± 49</td>
</tr>
<tr>
<td>A2780</td>
<td>Ovarian carcinoma</td>
<td>670 ± 40</td>
<td>150 ± 10</td>
<td>700 ± 200\textsuperscript{a}</td>
</tr>
<tr>
<td>A2780CP70</td>
<td>Ovarian carcinoma</td>
<td>42 ± 15</td>
<td>56 ± 2</td>
<td>8415 ± 205</td>
</tr>
<tr>
<td>MRC-5</td>
<td>Lung fibroblast</td>
<td>1351 ± 228</td>
<td>709 ± 76</td>
<td>530 ± 600\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Values from literature
**ACTIVITY IN CISPLATIN RESISTANT CELL LINES**

**IC$_{50}$ Values (nM) a Panel of Cisplatin-Resistant Cell Lines after 72 h Exposure**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cancer type</th>
<th>1</th>
<th>2</th>
<th>Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-29</td>
<td>Colorectal adenocarcinoma</td>
<td>85 ± 11</td>
<td>95 ± 20</td>
<td>29640 ± 1329</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast adenocarcinoma</td>
<td>475 ± 161</td>
<td>1735 ± 275</td>
<td>43600 ± 7071</td>
</tr>
<tr>
<td>MCF-7</td>
<td>Breast adenocarcinoma</td>
<td>285 ± 35</td>
<td>805 ± 21</td>
<td>9740 ± 537</td>
</tr>
<tr>
<td>PC-3</td>
<td>Prostate adenocarcinoma</td>
<td>270 ± 14</td>
<td>780 ± 10</td>
<td>10250 ± 919</td>
</tr>
<tr>
<td>DU 145</td>
<td>Prostate carcinoma</td>
<td>2840 ± 38</td>
<td>1370 ± 84</td>
<td>&gt;100000</td>
</tr>
</tbody>
</table>
CLASSIFICATION OF 1 AND 2 MECHANISM OF ACTION

RNAi signature of Eμ-Mycp19arf−/− lymphoma cells treated with 1 μM (LD80-90) 1 (A) or 2 (B) after 72 h.

- Neither 1 nor 2 act via a mechanism of action similar to that of drugs in the reference set
- 1 and 2 are a novel class (mechanistically) of cancer drug compound
MOA: RELATED TO CASPASE-MEDIATED APOPTOSIS?

1 and 2 do not induce caspase-mediated apoptosis
MOA: RELATED TO APOPTOSIS?

1 and 2 do not induce apoptosis via caspase cascade nor ripoptosome formation
MOA: RELATED TO NECROPTOSIS?

- Effect seen in various cell lines
- 1 and 2 induce cell death via necroptosis but not unregulated necrosis
MOA: RELATED TO NECROPTOSIS

1 and 2 act via the RIP1-RIP3 necrosome rather than upregulation of either individual protein.
NECROPTOSIS

HOW DO 1 AND 2 INDUCE NECROPTOSIS?

- Necrostatin-1 blocks 1 and 2 induced necroptosis
- Necrosome formation is important in 1 and 2 mechanism of action
HOW DO 1 AND 2 INDUCE NECROPTOSIS?

Pretreatment with a ROS inhibitor (N-acetylcysteine)
- 1 and 2 cause increase in ROS in order to cause necroptic cell death

Red = PI stained cells
Blue = cells treated with 1 (A) or 2 (B)
Orange = cells pretreated with N-acetylcysteine (1 h) followed by 1 (A) or 2 (B)
HOW DO 1 AND 2 INDUCE NECROPTOSIS?

Red = ROS levels in untreated cells

Blue = in cells treated with 1 (A) or 2 (B)

Orange = in cells treated with 1 and necrostatin-1

C. ROS levels after treatment with H2O2 (1 mM)

D. ROS levels after treatment with shikonin (necroptosis inducer)

- 1 and 2 induce necroptosis in a necrosome-dependent manner via elevated ROS levels
HOW DO 1 AND 2 INDUCE NECROPTOSIS?

- Supported by immunoblotting which showed no increase in expression of markers of DNA damage
- 1 and 2 mechanism of action is independent of PARP-1
HOW DO 1 AND 2 INDUCE NECROPTOSIS?

- Supported by RNAi data which show little to no correlation between 1 and 2 activity and p53 status
- 1 and 2 mechanism of action is independent of p53
EFFECTS OF 1 AND 2 ON THE CELL CYCLE

Treated cells become stalled in G1 and then a large amount of cellular debris is seen indicating cell death (pattern characteristic of necroptosis).
IN VIVO TOXICITY AND STABILITY

- Mice injected with up to 36 mg/kg (single dose, IP) and monitored 6 days post injection
- No significant acute toxicity
- 30 mg/kg Cisplatin causes acute nephrotoxicity
- $t_{1/2}$ in whole human blood = 29.1 min
- $t_{1/2}$ for cisplatin = 21.6 min
CONCLUSION / FUTURE WORK

- Compounds 1 and 2 selectively killed cancer cells (including cisplatin resistant cell lines) over normal cells
  - With greater potency over cisplatin (up to nearly 350x improved potency in some cases)
- 1 and 2 appear to induce cell death via a novel mechanism of action – necroptosis
  - Via necrosome activity, increased ROS generation, G1 cell cycle arrest, and cell membrane disruption
- Potentially very useful due to no cross resistance between apoptosis inducing agents and 1 and 2 for the treatment of chemoresistant cancers
- What is the cellular target of compounds 1 and 2?
- In vivo activity?