Original citation:

Permanent WRAP url:
http://wrap.warwick.ac.uk/64235

Copyright and reuse:
The Warwick Research Archive Portal (WRAP) makes this work of researchers of the University of Warwick available open access under the following conditions.

This article is made available under the Creative Commons Attribution 4.0 International license (CC BY 4.0) and may be reused according to the conditions of the license. For more details see: http://creativecommons.org/licenses/by/4.0/

A note on versions:
The version presented in WRAP is the published version, or, version of record, and may be cited as it appears here.

For more information, please contact the WRAP Team at: publications@warwick.ac.uk
The Potent Oxidant Anticancer Activity of Organoiridium Catalysts**

Zhe Liu, Isolda Romero-Canelón, Bushra Qamar, Jessica M. Hearn, Abraha Habtemariam, Nicolas P. E. Barry, Ana M. Pizarro, Guy J. Clarkson, and Peter J. Sadler*

Abstract: Platinum complexes are the most widely used anticancer drugs; however, new generations of agents are needed. The organoiridium(III) complex [(η5-Cp*bxiph)Ir(phpy)(Cl)] (1-Cl), which contains π-bonded bipyridyltetramethylcyclopentadienyl (Cp*bxiph) and C,N-chelated phenylpyridine (phpy) ligands, undergoes rapid hydrolysis of the chlorido ligand. In contrast, the pyridine complex [(η5-Cp*bxiph)Ir(phpy)(py)]+ (1-py) aquates slowly, and is more potent (in nanomolar amounts) than both 1-Cl and cisplatin towards a wide range of cancer cells. The pyridine ligand protects 1-py from rapid reaction with intracellular glutathione. The high potency of 1-py correlates with its ability to increase substantially the level of reactive oxygen species (ROS) in cancer cells. The unprecedented ability of these iridium complexes to generate \( \ce{H2O2} \) by catalytic hydride transfer from the coenzyme NADH to oxygen is demonstrated. Such organoiridium complexes are promising as a new generation of anticancer drugs for effective oxidant therapy.

Three platinum-based anticancer drugs, cisplatin, carboplatin, and oxaliplatin (OXA), are involved in nearly 50% of all anticancer therapies worldwide; however, problems of platinum resistance and undesirable side effects are limiting their future use. This highlights the need to develop anticancer agents with new mechanisms of action (MoAs). In contrast to the DNA-targeting platinum drugs, some organoiridium complexes are promising as a new generation of inert and labile Ir(III) complexes as anticancer agents. The activity of half-sandwich cyclopentadienyl anticancer complexes [(η5-Cp)Ir(p(η2)(X)(Y))Cl]Cl, where Cp* can be a pentamethylcyclopentadienyl (Cp*), phenyltetramethylcyclopentadienyl (Cp*bxiph), or biphenyltetramethylcyclopentadienyl (Cp*bxiph) moiety, and X=Y is a chelating ligand, is highly dependent both on the Cp* substituents and on the X=Y ligand. These chelido complexes all hydrolyze rapidly (within minutes at 310 K), including [(η5-Cp*bxiph)Ir(phpy)Cl] (1-Cl; php = 2-phenylpyridine), which is one of the most potent complexes.

Herein, we show that the monodentate ligand can have a major influence on both chemical reactivity and anticancer potency. We compare the aquation of the chlorido complex 1-Cl with that of the pyridine (py) complex [(η5-Cp*bxiph)Ir(phpy)py]+ (1-py). We investigated their activity towards a wide range of cancer cells and their selectivity for cancer cells over normal cells and used COMPARE analysis to explore the potential MoAs. We related cellular accumulation of iridium and production of ROS in cells to the redox chemistry of the complexes. In particular, we asked whether the ability of the cyclopentadienyl Ir(III) complexes to accept a hydride from the coenzyme NADH can be linked to ROS production. We demonstrate that organoiridium iridium complexes can be used as highly effective, even catalytic, oxidants for the treatment of cancer.

The novel compound 1-py PF6, was synthesized from the chlorido analogue 1-Cl, isolated as the PF6 salt (Figure 1a), and fully characterized by \(^1\)H and \(^{13}\)C NMR spectroscopy, ESI-MS, CHN elemental analysis, HPLC (Figure S1), and X-ray crystallography (Figure 1b; for details see the Supporting Information, Tables S1 and S2).
First, we assessed the anticancer activity of 1-py in comparison with that of 1-Cl (and cisplatin) and probed their MoAs. We then studied chemical reactions of 1-py that might play a key role in determining its biological activity, especially novel pathways for the production of ROS.

Complex 1-py showed high potency with an IC_{50} value (the concentration at which 50% of cell growth is inhibited) of 120 nm towards A2780 human ovarian cancer cells, which renders it six times more active than 1-Cl,[6c] and approximately ten times more active than cisplatin (Figure 2a and Table S3). Moreover, 1-py is thirteen times less toxic towards normal cells (MRC-5 human lung fibroblast cells) than towards A2780 cancer cells, whereas 1-Cl has a much lower selectivity factor of four (Figure 2a). Interestingly, the antiproliferative activity of 1-py towards A2780 cells after exposure for four hours is the same as that after 24 hours, which implies that the onset of cell death is a relatively rapid process (Figure S2 and Table S4).

The antiproliferative activities of 1-Cl[7] and 1-py were further evaluated by the National Cancer Institute NCI-60 human cancer cell screen,[8] which consists of nine tumor subtypes and approximately 60 cell lines (Figure 2c and Figure S3). Three endpoints were determined: the GI_{50} (the concentration that causes 50% cell growth inhibition), TGI (concentration that causes 100% cell growth inhibition), and LC_{50} values (the concentration that decreases the original cell count by 50%). Complex 1-py is six (GI_{50}) to thirteen (LC_{50}) times more potent than CDDP and approximately three times...
more potent than 1-Cl (Figure 2b). Complex 1-py shows high potency towards a wide range of cancer cell lines (Figure S3), with particular selectivity towards colon, melanoma, and non-small-cell lung cancer (NSCLC). Complex 1-py displayed its highest potency towards the MDA-MB-468 breast cell line with a GI₅₀ value of 132 nM. The high potency of 1-py contrasts with the loss of activity when a chloride in a Ruᴵᴵ arene anticancer complex [(η⁶-hexamethylenbenzene)Ru(en)Cl]⁺ is substituted by pyridine.⁹

The heat map highlights the distinct differences between the iridium compounds and the platinum drugs (Figure 2c). Strikingly, 1-py is more active in almost all of the cell lines, and the pattern of selectivity is very different for the iridium and platinum complexes, suggesting different MoAs. We used the NCI COMPARE algorithm, which quantitatively compares the selectivity in the NCI-60 screen of a seed compound with a database of compounds, to produce a Pearson’s correlation coefficient between -1 (negative correlation) and +1 (positive correlation), as a measure of similarity.⁷,⁸ COMPARE analysis of 1-py showed no correlation to any platinum compounds when we assessed the top 100 correlations with the DTP/NIH synthetic compound database, which hosts more than 40000 pure, natural and synthetic compounds. This result quantitatively suggests that the MoA of 1-py is different from that of cisplatin and other platinum compounds. In contrast, COMPARE analysis gave a correlation coefficient of 0.744 for 1-py and 1-Cl across the NCI-60 panel, suggesting that they have similar MoAs.

We also investigated the accumulation of complexes 1-Cl and 1-py in A2780 cells. After 24 hours of drug exposure, the amount of iridium that had accumulated in the cells was 20 times larger when complex 1-py was used instead of 1-Cl (8.3 ± 0.3 ng Ir and 0.39 ± 0.05 ng Ir per 10⁶ cells, respectively; Table S3). Uptake of 1-py by A2780 cells was concentration- and time-dependent, rising in the first 30 minutes, and slowly increasing during the four-hour study (Figure S4).

Some anticancer metal complexes disturb cellular redox homeostasis by increasing the level of oxidative stress.¹⁰ To assess whether redox chemistry is involved in the MoA, we co-administered 1-py and l-buthionine sulfoximine (l-BSO) to A2780 cells. The tripeptide glutathione (GSH, γ-L-Glu-L-Cys-Gly) is an important antioxidant in cells and a scavenger of ROS. l-BSO, an inhibitor of γ-glutamylcysteine synthetase, is often used to deplete the level of cellular GSH. A two-fold decrease in the IC₅₀ value (60 ± 3 nM) was observed upon co-incubation of 1-py with a non-toxic dose of l-BSO (5 μM; Figure S5 and Table S3). These data are consistent with a MoA for 1-py that involves redox processes, as cells are exposed to higher levels of ROS on co-incubation with l-BSO.

To detect changes in general oxidative stress, we determined the levels of ROS in A2780 cells that are induced by 1-py at concentrations of one third of the IC₅₀ value, the IC₅₀ value, and three times the IC₅₀ value by flow cytometry (Figure 3). This allowed the determination of the total level of oxidative stress (combined levels of H₂O₂, peroxy and hydroxyl radicals, peroxynitrite, and NO in the FL1 channel), whilst also monitoring superoxide production (in the FL2 channel). All flow-cytometry experiments were conducted with a drug exposure of just one hour, during which 1-py achieved 78% of its maximum antiproliferative activity (Table S4). We observed a substantial increase in the total ROS (×1230) and superoxide (×700) levels in cells treated with 1-py compared to untreated cells (Figure S6). No significant changes in the ROS level were observed with increased concentrations of 1-py, which suggests that low doses of 1-py (1/3 of the IC₅₀) are sufficient to maximize ROS generation. Similar experiments were carried out using 1-Cl and revealed that although 1-Cl also generated ROS, the level of superoxide induction is significantly lower than for 1-py (Figure 3B). Therefore, the level of ROS induced by the complexes 1-py and 1-Cl correlates with their anticancer activity.

This appears to be the first report of an organometallic iridium anticancer complex that is able to generate significant ROS levels in cancer cells. The highly amplified ROS levels that are induced by 1-py and 1-Cl are likely to play an important role in their activity. Non-enzymatic production of...
superoxides by xenobiotics has previously been related to the MoAs of organic anticancer drugs such as doxorubicin. [11]

Interestingly, we found that even in the presence of a thiol, such as the ROS scavenger N-acetyl-L-cysteine (NAC), the iridium complex is able to cause an increase in the level of superoxide in A2780 cancer cells (Figure 3b; see also the Supporting Information).

Cancer cells display a redox metabolism that is distinctly different from that of healthy cells. [3a] Normal cells are able to support a hydride to aqua IrIII cyclopentadienyl complexes and provide a pathway to an oxidant MoA. Previously, we have shown that NADH can produce ROS and thus provide a potential MoA and interaction with possible oxidant MoAs (e.g., for cancer therapy). [3]

Next, we investigated the aqueous chemistry of 1-py and, in particular, possible reactions that could produce ROS. First, we studied the hydrolysis (aquation) of 1-py, as this may provide a potential MoA and interaction with possible biological targets. The 1H NMR data reveal that the hydrolysis equilibrium was established after four hours at 310 K (63.3 % hydrolyzed, $t_1/2 = 77.8$ min; Figure S7). Aquation was reversed when pyridine was added (Figure S8). We previously reported that 1-Cl undergoes rapid hydrolysis; this process reached equilibrium within minutes even at 278 K. [15] Thus, the introduction of pyridine significantly slows down the hydrolysis rate, which leads to an activation time that is more compatible with transport to biological target sites.

Given the high chloride concentration in the body, we investigated the stability of 1-py in the presence of NaCl (104, 23, and 4 mm), mimicking the Cl− concentration in blood plasma, cell cytoplasm, and cell nucleus, respectively. [15] After one hour, 7−18 % of 1-py had reacted with chloride to give 1-Cl (Figure S9).

Coenzyme NADH plays a key role in numerous biocatalyzed processes. Previously, we have shown that NADH can donate a hydride to aqua IrIII cyclopentadienyl complexes and induce the reduction of protons to H2 and that of quinones to semiquinones. [15] Now, we have investigated whether reactions of 1-py and 1-Cl with NADH can produce ROS and thus provide a pathway to an oxidant MoA.

When NADH (3.5 mol equiv) was added to a 0.25 mM solution of 1-Cl, a sharp singlet at $-14.7$ ppm was observed in the 1H NMR spectrum within ten minutes; this resonance corresponds to the Ir III hydrido complex ([(η-Cp)H]Ir(=CpH)(H)] (1-H; Figure 4a). The large upfield shift of this peak compared to that for [(η-Cp)Ir(CO)H]+ (ca. $-11.1$ ppm) [14] is notable. NADH was converted into its oxidized form NAD+ (new peaks at 8.98, 9.35, and 9.58 ppm assignable to the hydrogen atoms at the C4, C6, and C2 positions of the nicotinamide ring of NAD+). These data suggest that 1-Cl can accept a hydride from NADH. Similar results were obtained for the reaction of NADH with 1-py (Figure S10), but the reaction was much slower (a few hours), perhaps because of the difference in hydrolysis rates of the two iridium complexes. Strikingly, data from UV/Vis spectrosocopy suggested that 1-Cl and 1-py can act as catalysts for hydride transfer from NADH with turnover numbers (TONs) of 8.2 and 7.6, respectively; the concentration of reacted NADH is calculated by measuring the absorption difference at 339 nm (Figure 4b). Importantly, the ROS hydrogen peroxide (H2O2) was detected by the appearance of a blue color on an H2O2 test stick in a solution of 1-py (1 mM) with NADH (3 mol equiv) in MeOH/H2O (3:7; Figure 4c), revealing that H2O2 was present in a concentration of approximately 0.22 mM, the level probably being limited by the solubility of oxygen (ca. 0.23 mM at 298 K). [15] No H2O2 was detected in the presence of added catalase or when the reaction was carried out under a nitrogen atmosphere.

To the best of our knowledge, hydride transfer from NADH to O2 has not been reported previously, although Noyori-type transfer hydrogenation catalysts, such as [(η-Cp)Ir(TsDPEN)(H)] (TsDPEN = H2NCHPhCHPhN(SO2CH2CH2)2), can undergo oxidative addition of O2 to give hydroperoxide intermediates and H2O as a product in MeCN and CH2Cl2. [16] The production of the ROS H2O2 by electron transfer from NADH to O2 might therefore be involved in the activity of 1-py (and 1-Cl) in cancer cells.

Electrochemical studies ruled out the possibility that an iridium-centered redox process is related to the ROS production (Figure S11).
GSH is abundant (at millimolar concentrations) in cells and participates in the detoxification of many anticancer drugs. Therefore, we investigated whether the reactions of 1-py and 1-CI with GSH might be involved in the difference in their anticancer activities. 1H NMR spectra showed that 95% of 1-py had reacted with GSH after 12 hours to yield complex [(η^2-Cp^biph)Ir(phpy)(SG)]^- (1-SG; Figure S12). The four CH3 groups in the Cp^biph ring of 1-py give rise to three singlets with an intensity ratio of 1:1:2, but split into six peaks with an intensity ratio of 1:1:2:1:1:2 for the glutathione adduct 1-SG (Figure S12). Complex 1-py, which contains an unsymmetric chelating ligand, is chiral; therefore, two diastereomeric glutathione adducts are expected. Other 1H NMR peaks for the generation of the ROS hydrogen peroxide. In cells, cancer cells, which is followed by the reaction with NADH and subsequent deactivation processes. Indeed, decreasing the cellular level of GSH after treatment with 1-CI results in a larger increase in the level of ROS in ovarian cancer cells within one hour and is the first reported organometallic iridium compound to do so. This new strategy for the rational design of oxidant catalytic organo-iridium drugs may be highly effective for treating platinum-resistant cancers.

Keywords: anticancer drugs · biocatalysts · hydride transfer · iridium · reactive oxygen species

Received: December 23, 2013
Published online: March 11, 2014