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Asymmetric transfer hydrogenation by synthetic catalysts in cancer cells

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Abstract

Catalytic anticancer metallodrugs active at low doses could minimise side-effects, introduce novel mechanisms of action which combat resistance, and widen the spectrum of anticancer drug activity. Here we use highly-stable chiral half-sandwich organometallic Os(II) arene sulfonyl diamine complexes, [Os(arene)(TsDPEN)] to achieve highly enantioselective reduction of pyruvate, a key intermediate in metabolic pathways, both in aqueous model systems and in human cancer cells, using non-toxic concentrations of sodium formate as a hydride source. The catalytic mechanism generates selectivity towards ovarian cancer cells versus non-cancerous fibroblasts (both ovarian and lung), which are commonly used as models of healthy proliferating cells. The formate precursor N-formylmethionine was explored as an alternative to formate in PC3 prostate cancer cells, which are known to over-express a deformylase enzyme. Transfer hydrogenation catalysts generating reductive stress in cancer cells offer a ground-breaking new approach to cancer therapy.

Introduction

Catalytic metallodrugs might be able to achieve high efficacy at low dosages and combat resistance through a multi-targeted mechanism of action. These are currently unmet clinical needs, and a problem for non-catalytic drugs, such as the platinum anticancer complex cisplatin [$PtCl_2(NH_3)_2$], yet platinum compounds are used in more than 50% of current chemotherapeutic treatments.[1,2] Over the last decade, catalytic complexes of ruthenium,[3-5] iridium,[6] and more recently osmium,[7] have attracted attention, allowing access to unique architectures and redox potentials, with the possibility of avoiding platinum-drug resistance, reducing side-effects, and widening the spectrum of activity.[8] The development of bio-compatible catalytic metallodrugs is however subject to hindrance by catalyst deactivation.[5] Unlike metalloenzymes which protect metal ions within cavities of the protein, metal centres in small-molecule catalysts are relatively exposed. Nevertheless, metallodrugs have been reported to catalyse a variety of bio-orthogonal transformations in cells including C-C bond formation and functional group modifications.[5] Complexes of ruthenium have been used in the deprotection of alloc/allyl-protected substrates,[9-11] and to carry out olefin metathesis in cells.[12] Metal-based catalysts have also been used to induce cell damage in the treatment of cancer. For example, organotelluride catalysts can generate hydrogen peroxide, inducing cancer cell death by oxidative stress.[13] Other complexes containing amino-terminal copper/nickel binding motifs can selectively cleave G-quadruplex telomeric DNA, inducing apoptosis in cancer cells.[14] We have recently reported the reduction of the cofactor nicotinamide adenine dinucleotide (NAD^+) to NADH inside cancer cells using non-chiral ruthenium[5] and rhodium[15] catalysts [M(arene)Cl(TsEn)], TsEn = N-tosyl-ethylenediamine. This new approach to killing cancer cells targets their inherent redox vulnerability, which results from dysfunctional mitochondria. Molecular mimics of NADH can be regenerated by iridium 'piano-stool' catalysts in aqueous media and, in tandem with a NADH-dependent enzymatic reaction, yield

chiral products with high conversion and enantiomeric excess.[16] Artificial transfer hydrogenation enzymes have been developed by Ward *et al* by introducing biotinylated iridium,[17] ruthenium[3] and rhodium[18] piano-stool complexes within streptavidin to achieve high enantioselectivity for the reduction of prochiral imines and ketones, but have not been used to reduce intracellular targets directly. We now show for the first time that asymmetric transfer hydrogenation of pyruvate to D-lactate can be achieved in both model aqueous systems, and in cancer cells, using a novel synthetic chiral organo-osmium catalyst and sodium formate as a hydride source.

Results

A successful catalyst for use in cells must be highly stable with respect to deactivation in the complex chemical environment of the cell. Transfer hydrogenation reactions are commonly achieved using bifunctional catalysts, such as the tosyl diamine complexes of ruthenium, rhodium or iridium reported by Noyori and co-workers.[19-22] Such catalysts can transfer hydrogen from a donor molecule (formic acid) to an acceptor (ketone) with high enantioselectivity. However, they require first the dissociation of a chloride ligand from the pre-catalyst. We recently reported chiral 16-electron osmium(II) complexes (Figure 1), analogous to the Noyori ruthenium(II) complexes, which are highly stable even in the catalytically-active 16-electron state, and have comparable efficiency to Ru(II) compounds.[23] As well as stability upon isolation and storage, we now find that these osmium complexes are particularly stable in aqueous media (phosphate-buffered saline) and also in DMSO, as studied by UV-visible and ^{1}H-NMR spectroscopy (Figure 2b and Supplementary Figure 4). This high stability suggested that it may be possible to deliver intact active organo-osmium catalysts inside cells and for them to remain functional in order to carry out catalytic reactions.

A series of sulfonyl-substituted *p*-cymene complexes (**3**-**6**) was synthesised with the view to optimise both catalytic and biological activities. The complexes retained the high stability of parent tosyl compound **2** in DMSO (Supplementary Figure 4) and biological media (Figure 2b), however catalytic efficiencies also remained very similar for the reduction of acetophenone, a widely-used test substrate in transfer hydrogenation (Table 1). Interestingly, all osmium complexes reduced acetophenone at higher rates (up to 3.5 × maximum turnover frequency, TOF_{max}) than the Noyori Ru catalyst (**9**) under identical conditions (Table 1). As an alternative approach to increase biological activity, the arene substituent (*p*-cymene) was exchanged for extended arenes (biphenyl and *m*-terphenyl) which increase the lipophilicity of piano-stool structures. Increased lipophilicity usually promotes increased uptake of metallodrugs by cells, hence increasing anticancer activity.[24] Single crystals of both enantiomers of biphenyl complex **7** suitable for X-ray diffraction were obtained as chloroform solvates (Figure 1b and Supplementary Figures 1-2). These confirmed the 16-electron nature of the catalysts, and are similar to those of **2**.[23]

The antiproliferative activities of complexes **2**-**8** against A2780 cancer cells ranged from moderate to high (IC_{50} = 4-30 μM) and were not statistically different between enantiomers (Table 1). The antiproliferative activity of **2** was re-determined after 24 h incubation in RPMI-1640 medium and did not differ significantly from the activity determined with freshly-prepared solutions, suggesting that *p*-cymene complex **2** remains highly stable in the cell culture medium. Total Os accumulation (ng Os × 10^6 cells) in A2780 cancer cells after 24 h drug exposure ranged from 4.8 to 31.9 ng Os × 10^6 cells (Supplementary Table 3). Further studies showed that the efflux of ***R,R*-2** occurred only slowly, and significant amounts of Os still remain in cells after 72 h (*ca*. 15 % of total Os; Supplementary Figure 5 and Supplementary Table 5). As a measure of hydrophobicity, partition coefficients (Log P) were also determined for complexes **2**-**8**, and range from 0.18 to 2.3 (Supplementary Table 3), and increase with

arene extension: *p*-cymene < biphenyl < *m*-terphenyl, as expected. In addition, there is an evident correlation between antiproliferative activities, or total metal accumulation, and partition coefficients (Pearson's r = -0.92 and r = 0.77 respectively) for the sulfonamide series **2**-**8** (Supplementary Figures 6 and 7).

Cell fractionation experiments using A2780 cancer cells treated with complex **2** provided evidence for the presence of Os in the cytoplasmic (47 ± 2 %) and membrane/organelle (48 ± 3 %) fractions, Supplementary Table 4). Cell cycle analysis of A2780 cancer cells (Supplementary Table 6), induction of apoptosis (Supplementary Table 7), and membrane integrity (Supplementary Table 8) were assessed after 24 h drug exposure to compound **2**. The complex caused G_1 arrest in A2780 cancer cells, but did not induce apoptosis, nor was the cellular membrane compromised by exposure to the osmium catalyst. The experiment was repeated to include 72 h of cell recovery time after drug exposure, but the results were not statistically different (Supplementary Tables 6-8).

The stability, high cellular metal accumulation (30 ± 2 ng Os × 10^6 cells) and relatively low cytotoxicity (15.5 ± 0.5 μM) made complex **2** a suitable candidate for *in-cell* catalysis studies, as a high cytoplasmic drug availability could be achieved (*ca*. 50 % of total accumulated Os, Supplementary Table 4) without causing cell death before initiation of catalysis. There appear to be no reports of the asymmetric reduction of the pro-chiral biomolecule pyruvate, a key metabolite in cells, by an external synthetic catalyst (Figure 2a). We first explored the reduction of pyruvate in formic acid using each enantiomer of complex **2**; confirming the formation of lactate by ^{1}H-NMR (Figure 2c). We then repeated the experiment in phosphate-buffered saline (PBS), using sodium formate as a hydride source to model our cell experiments. The rate (TOF / h^{-1}) of catalysis in aqueous medium was monitored by ^{1}H-NMR, and was found to be highly dependent on the concentration of formate; giving ca. 10× rate enhancement upon increasing formate concentration from 2 mol equiv. to 15 mol equiv. compared to pyruvate concentration

(Supplementary Figure 8, Supplementary Table 9). To determine enantiomeric excess, the concentrations of each enantiomer of lactate were determined using enantio-specific enzymatic assay kits. Remarkably, the catalysts retained asymmetric activity, and a large enantiomeric excess was generated using each catalyst (*e.e.* = 83 %). **(*R*,*R*)-2** formed D-lactate, and **(*S*,*S*)-2** formed L-lactate, respectively (Figure 2d, Supplementary Table 10).

After the successful catalysis by osmium of the asymmetric reduction of pyruvate in the model aqueous system with high enantioselectivity, we investigated whether the reduction could be detected in cancer cells. A2780 human ovarian cancer cells were exposed to equipotent concentrations of **2** or **7** (arene = *p*-cymene or biphenyl, respectively) together with a non-toxic concentration of sodium formate (Figure 3a, Supplementary Table 11) as a hydride source. Sodium formate significantly enhanced ($P<0.01$) the activities of the complexes in A2780 cancer cells by up to 13×. The decrease of cell proliferation with increase in formate concentration was similar for both enantiomers of *p*-cymene complex **2**, and unaffected for cells treated with sodium formate alone. Sodium formate did not significantly affect the degree of cellular accumulation of osmium in cells treated with either complex **2** or **7** ($P>0.77$; Figure 3a, Supplementary Table 13). We investigated selectivity in non-cancerous cells from the same tissue type (HOF human ovarian fibroblasts, Figure 3c) as well as normal cells from a different tissue (MRC5 lung fibroblasts, Figure 3d) using equimolar drug concentrations as in experiments with A2780 cells. Proliferation in both ovarian and lung primary fibroblasts was not significantly affected at the 95% confidence level, suggesting that such an anticancer treatment could selectively kill ovarian cancer cells with respect to healthy cells. Catalyst co-administration with sodium acetate instead of formate was also studied, but acetate had no effect on the activity of the complexes (Figure 3b, Supplementary Table 12). This is consistent with formate, but not acetate, being able to act as a hydride donor to the osmium catalyst.

Induction of apoptosis and membrane integrity in A2780 cancer cells were re-assessed upon co-administration of complex **2** with sodium formate (2 mM) and did not statistically differ from experiments conducted without formate (Supplementary Tables 7-8). Cells treated with osmium complex **2** and sodium formate exhibited slightly increased G_1 cell cycle arrest (Supplementary Table 6).

We also explored the possibility of substituting sodium formate by *N*-formylmethionine (fMet), a formate precursor and substrate for the enzyme peptide deformylase (PDF), which is overexpressed in some cancer cell lines, including PC3 human prostate cancer cells. Cleavage of fMet by peptide deformylase in cells would be expected to generate formate as hydride donor and co-catalyst. IC_{50} values for complex **2** and **7** in PC3 cells were found to be similar to those determined in A2780 cancer cells (Supplementary Table 3). Co-administration of osmium catalyst with *N*-formylmethionine to PC3 cells achieved a *ca.* 20% reduction in normalised proliferation relative to the normalised cells treated with only osmium complex **2**, without *N*-formylmethionine (Supplementary Table 14). Conversely, co-administration of *N*-acetyl-methionine (which cannot release formate) in place of formylmethionine, had no significant effect on cellular proliferation (Supplementary Table 14).

Potentiation of the antiproliferative activity of complexes **2** and **7** with non-toxic concentrations of sodium formate suggested that the drugs are likely to act as catalysts inside cells. Next we determined whether osmium-catalysed transfer hydrogenation inside cells occurs with high enantioselectivity, as observed in model experiments. In nature, pyruvate is selectively reduced to L-lactate by the enzyme lactate dehydrogenase in the cytosol. D-lactate is also present in cells, but only at low concentrations,[25] and therefore increased D-lactate levels are readily identifiable. A2780 cancer cells were exposed to each enantiomer of complex **2**, in the presence or absence of sodium formate, and intracellular D-lactate concentrations were determined using an enantioselective assay kit. The combination of ***R,R*-2** and sodium formate significantly

enhanced the concentration of D-lactate in cells relative to the Os-free controls ($P<0.02$) and ***R,R*-2** without formate ($P=0.0474$), whereas sodium formate alone did not increase the intracellular concentration ($P=0.7259$). Interestingly, ***R,R*-2** produced significantly higher levels of D-lactate than ***S,S*-2** ($P=0.0452$; Figure 4a, Supplementary Tables 15 and 16), suggesting that the enantioselectivity of lactate reduction observed in the aqueous model is maintained *in vitro*.

The extracellular D-lactate concentration was also determined (Supplementary Figure 8 and Supplementary Table 17), and was not influenced by either enantiomer of catalyst in the presence or absence of formate, or in the catalyst-free control ($P>0.1$; Supplementary Table 18 for full statistical analysis). These results are a clear indication that the reduction of pyruvate to D-lactate by catalyst ***R,R*-2** occurs intracellularly.

Discussion

Platinum-based therapies are typically DNA-targeting and provide little specific differentiation between cancer cells and healthy cells. New chemotherapies that target biochemical traits that are unique to cancer cells might have significant advantages. Our work recognizes two key vulnerabilities in cancer cells. Firstly, glycolysis is up-regulated in cancer cells (resulting in increased lactate accumulation, a phenomenon known as the Warburg Effect).[26] Lactate dehydrogenase (LDH) catalyses the inter-conversion of pyruvate and lactate,[27] and its overexpression in cancer cells has been linked to a high likelihood of metastases, and poor clinical prognosis.[28,29] Catalysing the conversion of pyruvate to lactate with an external synthetic catalyst and hydride source threatens the ability of cancer cells to harness energy and essential metabolites, such as NAD^+. Secondly, cancer cells are under redox stress due to the metabolic demands of rapid proliferation.[30] In contrast to cancer cells, healthy proliferating

cells have fully-functional mitochondria, and can recover from redox stress, and avoid damage.[31] Performing transfer hydrogenation reactions inside cancer cells can therefore perturb the delicate redox balance.

Complexes of the third-row transition low-spin $5d^6$ metal ion Os(II) are usually more kinetically inert than those of the second-row ion Ru(II); for example hydrolysis of the ethylenediamine (En) complex $[Os(biphenyl)(En)Cl]^+$ is *ca*. 40× slower than for Ru(II), despite both being isostructural.[32] Such behaviour and expectation of a less favorable catalytic performance may have previously deterred studies of osmium arene catalysts, but are likely to be favourable when considering the delivery of an active catalyst to cells, without deactivation. Osmium catalyst **2** displays properties which are compatible with biological use, including high stability both in aqueous solution and in the presence of DMSO, and the absence of degradation or inactivation by components of cell culture media over a 24 h period (310 K). Interestingly, osmium catalysis of acetophenone reduction occurs more rapidly than that of the Noyori-type ruthenium pre-catalysts, from which chloride must first dissociate to generate the active catalyst. Even after allowing the Ru(II) active catalyst to form *in situ*, the efficiency of the Os(II) complexes is higher than that of the ruthenium catalysts under identical conditions.

The established anticancer compound cisplatin, and traditional organo-metallic 'piano-stool' chlorido complexes, undergo activation by hydrolysis of a metal-halide bond as a crucial step in their mechanism of action,[33-35] but such an activation step is not required for activity of the 16-electron osmium catalysts used here. Cellular accumulation experiments demonstrate that complexes with higher hydrophobicity exhibit increased cellular osmium accumulation, as well as higher potency towards cancer cells (Supplementary Figures 6 and 7). The cellular distribution of Os studied for complex **2** showed that *ca*. 50% of the total osmium accumulated is present in the cytoplasm. However, the detection of osmium by ICP-MS does not give information regarding the speciation of the metal complex (i.e. cannot distinguish between

intact and poisoned catalyst). Interactions with cellular proteins and small biomolecules[36] are likely to cause catalyst deactivation and therefore the effective concentration of active catalyst in the cytosol is likely to be significantly lower.

Co-administration of an osmium complex (**2** or **7**) with increasing concentrations of sodium formate significantly decreased proliferation of A2780 cancer cells ($P<0.001$, Figure 3a). No significant change in cellular proliferation was observed in osmium-free experiments, indicating that the potency increase requires both osmium complex and formate. Experiments using acetate in place of formate showed no decrease in proliferation suggesting that hydride transfer from formate to osmium and catalytic hydrogenation is involved. Even at 0.5 mM, formate is in molar excess of the osmium complex, and therefore further increases in osmium potency at formate concentrations (Figure 3a) suggests that the mechanism of action is catalytic. The accumulation of osmium in cancer cells is not significantly affected by co-administration of sodium formate. Hence, decreased proliferation is not the result of increased osmium uptake (Figure 3a). Rather, increased potency can be attributed to a synergistic effect, in particular in-cell catalysis, involving both the osmium complex and formate. Importantly, we observed significant selectivity of antiproliferative activity towards ovarian cancer cells compared to healthy ovarian or lung cells. Modulation of antiproliferative activity by co-administration of the complexes with sodium formate was significantly more pronounced in A2780 cancer cells (*ca*. 85% decrease) than in MRC5 lung fibroblasts (*ca*. 15% decrease) and HOF ovarian fibroblasts (*ca.* 5% decrease).

The induction of apoptosis and membrane integrity in A2780 cancer cells treated with complex **2** did not differ in the presence of sodium formate (Supplementary Tables 7 and 8). Co-administration of formate increased the population of cells in G_1 cell cycle arrest (Supplementary Table 6). These data suggest that co-administration of formate does not change the mechanism of action, but instead enhances the effect. G_1 arrest has been associated as an

anti-apoptotic response to metabolic stress.[37] Together, these experiments suggest a highly cytostatic component of the mechanism of action (which is enhanced by the presence of formate). Existing platinum-based therapies induce apoptosis in cancer cells, which exhibit S/G_2M arrest resulting from DNA damage.[2,38] Considering the low nuclear accumulation of osmium and absence of S/G_2M cell cycle arrest, it seems likely that DNA damage is not involved in the mechanism of action of the sulfonamide complexes, which therefore differs significantly from that of Pt drugs.

This is, to the best of our knowledge, the first report of the use of a Noyori-type transfer hydrogenation catalyst to convert pyruvate to lactate, and importantly, the first demonstration of the enantioselective reduction of pyruvate by an external synthetic catalyst inside cells. D-lactate is produced in cytosol of cells by the glyoxylase pathway,[39] and is present only at low concentrations, compared to the major enantiomer, L-lactate. Additional D-lactate produced using the osmium complexes was therefore readily detected. ***R,R*-2** in combination with formate, produced significantly higher levels of D-lactate in cells than ***R,R*-2** without formate ($P=0.0474$; Figure 4a), showing that the enantioselectivity of reduction is maintained inside cells. In the earliest molecular modelling studies on the Noyori catalysts, a concerted transition state for hydrogen transfer was proposed, with enantioselectivity attributed to a favourable interaction between an aromatic ring in the substrate and the η^6-arene of the catalyst ('C-H---π' interaction), with additional contributions from dispersion and steric effects.[40,41] In more recent studies, including the specific involvement of solvent, the mechanism has been shown to be more likely stepwise in nature, with additional destabilisation of the disfavoured transition state by an unfavourable interaction with the SO_2 group on the ligand.[42-44] Other studies have revealed that multiple oxygen atoms in a substrate can engage in a process similar to the C-H---π interaction, i.e. where the electron-rich oxygen atoms take the place of the arene, in enantioselective reductions with Noyori catalysts.[45] Although we do not have direct evidence

for the proposed transition state, the absolute sense of reduction of pyruvate suggests that the carboxylate fulfils the same role as the electron-rich arene or multiple oxygen atoms in the substrate by engaging in a similar favourable interaction with the H atoms on the η^6-arene ligand (Figure 4b).

Whereas the intracellular D-lactate concentration was significantly modulated after treatment with ***R,R*-2** and formate, the extracellular D-lactate concentration was unaffected. Also osmium efflux from cells occurs only slowly (compared for example with some Ru complexes[11]), and therefore these data are indicative of *in-cell* reduction, as opposed to *on-cell* catalysis (which would involve efflux of pyruvate into the extracellular matrix containing the catalyst and formate, followed by uptake of D-lactate, since pyruvate is not present in the culture medium). Determination of the catalyst turnover number (TON) inside cells is complicated by several factors. The rate of transfer hydrogenation is limited by the available formate concentration, as shown in the model aqueous system (Supplementary Table 9). Also, the effective active concentration of catalysts is likely to be lowered by interactions with other biomolecules, including proteins. If for example a 5% availability of active catalyst is assumed, then the TON would be *ca*. 13.

Lactate production is unlikely to be the direct cause of cell death, since neither D- or L-lactate were not toxic to cells up to 2 mM (Supplementary Table 2). However, cells are dynamic systems, and therefore, perturbing local concentrations of either lactate enantiomer (or depletion of pyruvate) even at low levels might have a major effect on cell pathways, particularly if lactate can act as a strong inhibitor of other pathways (binding constant in micromolar or lower range).[46] Furthermore, it is possible that other unsaturated substrates may also be targeted by the Os catalyst, yielding a multi-targeted mechanism of action for the drug. Importantly, the experiments described here demonstrate that enantiomerically-selective

reduction of pyruvate to lactate can be achieved in cells, which now becomes a new cellular target worthy of further exploration.

Conclusion

We have shown that novel organo-osmium catalysts which are highly stable in aqueous media show promise not only as highly active transfer hydrogenation catalysts that out-perform ruthenium analogues under similar conditions, but also as cancer-cell-selective asymmetric catalysts with novel mechanisms of anticancer activity. Such new design concepts provide wide scope for further research into bio-compatible metallo-catalysts for use in the treatment of disease. Our work appears to provide the first example of both aqueous, and in-cell asymmetric transfer hydrogenation of pyruvate, with high enantioselectivity. Our studies demonstrate that an external catalyst may be used to produce a specific lactate enantiomer inside cells, depending on the chosen configuration of the catalyst chirality. Effective co-administration of catalyst and reducing agent might be achieved using nanoparticle encapsulation or polymeric micelles. Additionally, the use of formylmethionine as a hydride source is of great interest, as it can release formate when catabolised by the deformylase enzyme, which is overexpressed in certain cancer cells. Interactions of chiral biomolecules with enantioselective enzymes are crucial to cell metabolism and survival, and manipulation of enantiomeric ratios inside cells could provide a new strategy for fighting intractable diseases.

Experimental

Methods

The synthesis of sulfonamide ligands, osmium dimers and complexes, the instrumentation used, additional methods and numerical data are described in the Supplementary Information.

Catalytic reductions in cell-free aqueous media. Osmium complexes ***R*,*R*-2** or ***S*,*S*-2** were incubated with sodium pyruvate in PBS, in the presence / absence of sodium formate. Final concentrations: osmium complex = 15 μM (IC_{50} concentration determined in A2780); sodium pyruvate = 1 mM; sodium formate = 2 mM. After 24 h incubation at 310 K, concentrations of D and L-lactate were measured individually using enantio-specific detection assay kits (Cayman Chemical) as described in the manufacturer's instructions. Fluorescence (λ_{ex}: 530-540 nm, λ_{em}: 585-595 nm) was read using a Promega GloMax Multi+ microplate reader. Samples were measured in quadruplicate, and standard deviations calculated.

***In vitro* growth inhibition assay.** 5000 cells (A2780, HOF, MRC5 or PC3 cancer cells) were seeded per well (150 μL) in 96-well plates. The cells were pre-incubated in drug-free media at 310 K for 48 h before adding different concentrations of the compounds to be tested. Stock solutions (100 μM) of the osmium complexes were prepared in DMSO (5% v/v) and medium (95% v/v), and then further diluted in culture medium until working concentrations were achieved. Drug stock solutions were concentration-adjusted after ICP-OES analysis. The drug exposure period was 24 h. After this, supernatants were removed by suction and each well was washed with PBS. A further 72 h was allowed for the cells to recover in drug-free medium at 310 K. The SRB assay was used to determine cell viability.[47] Absorbance measurements of the solubilised dye (on a BioRad iMark microplate reader using a 470 nm filter) allowed the determination of viable treated cells compared to untreated controls. IC_{50} values

(concentrations which caused 50% of cell death), were determined as duplicates of triplicates in two independent sets of experiments and their standard deviations were calculated.

Co-administration of formate. Cell proliferation assays were carried out as described above with the following experimental modifications: A fixed (equipotent) concentration of each osmium complex was used, corresponding to ½ × IC_{50} concentration. Stock solutions of osmium complexes were prepared as described previously (Os concentration in drug stock solution was determined by ICP-OES before adminstration to cells, see SI). This stock solution was further diluted using the corresponding medium until working concentrations were achieved. Sodium formate was co-administered at three different concentrations (0.5, 1.0 and 2.0 mM). Both solutions were added to each well of cells independently, but within 5 min of each other. Cell viability was determined using the SRB assay as duplicates of triplicates. Cell viability modulation experiments were repeated using sodium acetate (0.5, 1.0, 2.0 mM) in place of sodium formate. *N*-formylmethionine modulation experiments were carried out similarly, using three concentrations (0.25, 0.5, 1.0 mM) in PC3 human prostate cancer cells, which are known to overexpress the peptide deformylase (PDF) enzyme.

Metal accumulation in cancer cells. The accumulation of osmium complexes in A2780 human ovarian cancer cells was studied. Briefly, 4 x 10^6 cells were seeded on a 6-well plate. After 24 h of pre-incubation time in drug-free medium at 310 K, the complexes were added to give final concentrations equal to IC_{50} / 3 (Os determined by ICP-OES before administration to cells) and a further 24 h of drug exposure was allowed, with no recovery time (with the exception of efflux experiments, in which cells were allowed to recover in drug-free medium for up to 72 h). After this time, cells were treated with trypsin, counted, and cell pellets were collected. Each pellet was digested overnight in concentrated nitric acid (72%) at 353 K; the

resulting solutions were diluted using doubly-distilled water containing thiourea (10 mM) and ascorbic acid (100 mg/L).[48] Concentrations were adjusted to give a final acid concentration of 3.6% v/v HNO_3 and the amount of Os taken up by digested cells was determined by ICP-MS in no-gas mode (see supplementary information). Experiments did not include recovery time in drug-free media; they were carried out in triplicate and the standard deviations were calculated. Statistical significances were determined using Welch's unpaired *t*-test.

Metal distribution in cancer cells. Cell pellets, obtained in triplicate (as described for metal accumulation studies) were fractioned using the Fraction PREP kit (BioVision). Samples were digested overnight in nitric acid (200 µL, 72% v/v) at 343 K, then diluted to achieve a final working acid concentration of 3.6% v/v (taking into account the volume of the sample: cytosolic and membrane fractions = 400 µL, nucleic fraction = 200 µL). Metal concentration in digested samples was determined by ICP-MS in no-gas mode.

Reduction of pyruvate to lactate in cells. The D-lactate assay detection kit (Cayman Chemical) was stored at 255 K before use. Complexes ***R*,*R*-2** and ***S*,*S*-2** were selected for screening. 30×10^6 A2780 human ovarian cancer cells were seeded in T75 flasks with 24 h pre-incubation. After this time, solutions of Os complexes and sodium formate were added independently, but within 5 min of each other (final working concentrations: IC_{50} concentration of the osmium complex, 2 mM sodium formate) with 24 h drug exposure. The supernatant was collected to determine the extracellular D-lactate concentration. The cells were then washed twice with PBS, detached using trypsin / EDTA, counted and centrifuged at 1000 g for 5 min to obtain cell pellets of 40×10^6 cells to determine the intracellular D-lactate concentration. Samples were processed per the manufacturer's instructions. Fluorescence (λ_{ex}: 530-540 nm, λ_{em}: 585-595 nm) was read using a Promega GloMax Multi+ microplate reader. Samples were

measured in triplicate, and standard deviations calculated. Statistics were calculated using Welch's *t*-test at the 95% confidence limit.

Data availability

Crystallographic data for complexes ***S,S*-7** and ***R,R*-7** can be found in the Cambridge Crystallographic Database (CCDC numbers 1507733 and 1507732, respectively). Datasets underpinning the research are included in the published manuscript and corresponding Supplementary Information. After the Open Access agreement has been established, underpinning datasets will be deposited in Warwick's Institutional Repository: Warwick Research Archive Portal (WRAP), according to the Open Access Agreement.

Associated content

Supplementary Information is available with the online version of this paper.

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Author Contributions

All authors were involved with the design and interpretation of experiments and with the writing of the script. Chemical and biological experiments were carried out by J.P.C.C., I.R.C.,

C.S.C. and A.H. G.J.C. carried out the x-ray crystallography. All authors have given approval to the final version of the manuscript.

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Abbreviations

ATH, asymmetric transfer hydrogenation; DPEN, 1,2-diphenylethylenediamine; dh-*m*-terp, 1,4-dihydro-*m*-terphenyl; En, ethane-1,2-diamine; fMet, *N*-formylmethionine; IC_{50}, half-maximal (50%) inhibitory concentration; NAD, nicotinamide adenine dinucleotide; *p*-cymene, 4-isopropyl-toluene; PDF, peptide deformylase; SRB, sulforhodamine B; TOF, turnover frequency; Ts, tosyl; *m*-terphenyl, 1,3-diphenylbenzene;

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Intracellular asymmetric transfer hydrogenation catalysis is demonstrated. Enantiomers of Os(II) arene catalysts can penetrate cell membranes, achieving reduction of pyruvate to D- or L-lactate using formate as a hydride source, with high enantioselectivity. The mechanism is selective for cancer versus normal cells and offers a new approach to cancer therapy.

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Figure Legends

Figure 1. Synthesis of osmium(II) arene sulfonamide catalysts **1**-**8**. (a) Dichlorido osmium pre-catalyst **1** (X = 4-methylphenyl) is formed *in situ* en route to 16-electron catalysts **2**-**8**.[23] (*R*,*R*)- and (*S*,*S*) configured complexes were synthesised from enantiomerically-pure ligands, to yield both enantiomers of the phenyl-substituted complexes (**2**, **4**-**8**), and methyl-substituted complex (**3**); (b) Mirror-image ORTEP diagrams of the x-ray crystal structures of [Os(biphenyl)(TsDPEN)] (**7**). ***R,R*-7 • 2CHCl$_3$** (left) and its enantiomer ***S,S*-7 • 2CHCl$_3$** (right), with thermal ellipsoids at 50% probability level. $CHCl_3$ molecules have been omitted for clarity.

Figure 2. Catalytic reduction of pyruvate to lactate in aqueous solution by [Os(*p*-cymene)(TsDPEN)] **2**, using formate as a hydride source. (a) Enantioselective reduction of pyruvate to lactate. (b) UV-visible spectrum of **2** in phosphate-buffered saline containing 5% DMSO does not change from 0 h (red) to 24 h (blue), 310 K, showing that it is highly stable. (c) Reduction of pyruvic acid by complexes ***S,S*-2** in formic acid : triethylamine azeotrope (5:2) at 310 K, monitored by ^{1}H NMR, S/C=200, 310 K (TOF_{max} = 296 ± 6 h^{-1}). (d) 24 h aqueous-phase reduction of pyruvate at 310 K in PBS by osmium catalysts ***R,R*-2** / ***S,S*-2**, in the presence of formate, with high enantioselectivity (final concentrations: Os complex = 15 μM; pyruvate

= 1 mM; formate = 2 mM). The major product using ***R,R*-2** is D-lactate (●) and for ***S,S*-2** is L-lactate (●). Error bars show ± one standard deviation from the mean. Statistics calculated using a two-tailed t-test with unequal variances (Welch's *t*-test).

Figure 3. Potentiation of the antiproliferative activity of osmium *p*-cymene complex ***S,S*-2** or biphenyl complex ***S,S*-7**. (a) Cell % survival (normalised) decreases upon co-administration of formate alongside Os catalyst in A2780 cancer cells (●) however cellular accumulation of Os is not affected by formate concentration (●). (b) Cell % survival (normalised) is not affected by co-administration of acetate in A2780 cells (●). (c) Cell % survival (normalised) is not significantly affected after co-administration of formate and Os catalyst in non-cancerous HOF ovarian fibroblasts (●). (d) Cell % survival (normalised) is not significantly affected in non-cancerous MRC5 fibroblasts after co-administration of formate and Os catalyst (●). Error bars show ± one standard deviation from the mean. Statistics using a two-tailed t-test with unequal variances (Welch's unpaired *t*-test). **p* < 0.05, ***p* < 0.01 ****p* < 0.001.

Figure 4. Enantioselectivity for the transfer hydrogenation (reduction) of pyruvate to selectively afford D-lactate is conserved in cells. (a) Intracellular D-lactate (μM) determined in A2780 cancer cells, after 24 h drug exposure at IC_{50} concentrations of complexes ***R,R*-2** or ***S,S*-2** (15 μM). Complex ***R,R*-2**, in contrast to ***S,S*-2**, shows significantly increased levels of D-lactate in the presence of formate (2 mM). * P<0.05 (Welch's unpaired *t*-test). Error bars show ± one standard deviation from the mean. See Supplementary Table 15 for full statistical analysis. (b) Proposed transition state for the reduction of pyruvate to D-lactate by osmium complex ***R,R*-2**.

Tables

Figure 1 table insert

	arene	X
2	*p*-cymene	4-methylphenyl
3	*p*-cymene	methyl
4	*p*-cymene	4-nitrophenyl
5	*p*-cymene	4-fluorophenyl
6	*p*-cymene	phenyl
7	biphenyl	4-methylphenyl
8	*m*-terphenyl	4-methylphenyl

Table 1. Catalytic data for acetophenone reduction and IC_{50} values (μM) against A2780 ovarian cancer cells for osmium complexes **2**-**8**, compared to established ruthenium transfer hydrogenation catalyst **9**.

	Acetophenone reduction [a]		IC_{50} in A2780 / μM [d]	
Catalyst	conv. (*e.e.*) [b]	TOF_{max} / h^{-1} [c]	(*R*,*R*)-isomer	(*S*,*S*)-isomer
2	99 (99)	63.9 ± 0.3	15.5 ± 0.5	15.2 ± 0.5
3	99 (96)	58 ± 2	30 ± 2	29.8 ± 0.5
4	99 (95)	61 ± 2	19.9 ± 0.5	19 ± 1
5	99 (96)	40 ± 2	17 ± 1	17 ± 1
6	92 (97)	58 ± 1	14 ± 1	15 ± 2
7	99 (95)	78 ± 1	6.5 ± 0.3	6.3 ± 0.1
8	95 (94)	43 ± 2	4.4 ± 0.3	4.5 ± 0.1
9	99 (99)	23 ± 1	8.2 ± 0.7	9 ± 1

[a] Reduction of acetophenone in formic acid / triethylamine (5:2) azeotrope (310 K, 0.5 mol% catalyst) using (*S*,*S*)-configured catalysts, giving the *S* enantiomer alcohol (final concentration: substrate ketone, 1.4M; catalyst, 6.9 mM). [b] Enantiomeric excess, determined by chiral GC-FID. [c] Maximum turnover frequency, determined by ^{1}H NMR. [d] Half-maximal inhibitory concentration (IC_{50} / μM) determined in A2780 human ovarian cancer cells (24 h drug exposure + 72 h recovery time, 310 K).