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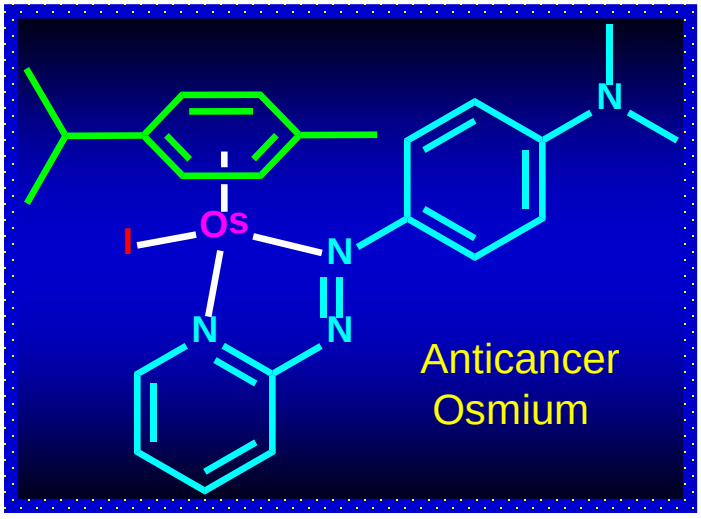
Anti-colorectal Cancer Activity of Organometallic Osmium Arene Azopyridine Complex

Steve D. Shnyder,*‡ Ying Fu, † Abraha Habtemariam,† Sabine H. van Rijt,† Patricia A. Cooper,‡ Paul M. Loadman,‡ and Peter J. Sadler*†

† Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, U.K, ‡ Institute of Cancer Therapeutics, University of Bradford, Richmond Road, Bradford, BD7 1DP, U.K.

Abbreviations List: L-BSO, L-buthionine sulfoximine; GSH, glutathione; ROS, reactive oxygen species

Graphic



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ABSTRACT We report that a single iv dose of the half-sandwich organometallic iodido osmium(II) arene complex [Os(η^6-*p*-cym)(4-(2-pyridylazo)-*N*,*N*-dimethylaniline)I]PF_6 (**1**, FY026) significantly ($p<0.01$) delays the growth of HCT116 human colon cancer xenografts in mice, with negligible toxicity. Compound plasma and tissue distribution studies carried out over 4 hours showed that detectable levels of osmium are evident in the tumor for the duration of the analysis. The anticancer activity of **1** appears to involve redox mechanisms. Combination treatment of A2780 ovarian cancer cells with L-buthionine-sulfoximine (L-BSO), a specific inhibitor of γ-glutamyl-cysteine synthetase, increased intracellular production of ROS and significantly increased the potency of **1**. This first report of in vivo antitumor activity for an organometallic osmium arene complex confirms the potential of this class of compounds as antitumor agents with a novel mechanism of action.

KEYWORDS Anticancer activity, organometallic complex, osmium, redox

Introduction

There is much current interest in exploring the anticancer activity of compounds containing metal-carbon bonds (organometallic compounds), especially those containing π-bonded aromatic cyclopentadienyl (C_5 binding, five-fold hapticity, η^5) and arene (C_6 six-fold hapticity, η^6) ligands. These include sandwich complexes of Fe^{II}, and Ti^{IV} containing two π-bonded rings,[1] and half-sandwich complexes of Ru^{II},1 and, more recently, Os^{II},[2] which contain just one π-bonded ring. The half-sandwich 'piano-stool' complexes have a particularly attractive scaffold for drug design since variations in the arene can modulate cell uptake and DNA intercalation, and ligands which constitute the 'legs' of the 'piano-stool' can control stability and reactivity. Initial cancer cell cytotoxicity data for ruthenium complexes of the type [(η^6-arene)Ru(XY)Z] where, for example, the arene = biphenyl, XY = an N,N-chelated diamine such as ethylenediamine, and Z a leaving group such as Cl, were encouraging, showing activity against a range of types of cancer cells at low micromolar doses and no cross-resistance with cisplatin. Moreover there was activity for these Ru complexes in vivo against human A2780 ovarian and A549 lung cancers.[3,4] These complexes with one reactive Ru-Cl bond are thought to target DNA.[5] However the biphenyl/ethylenediamine/Cl osmium analogue was subsequently found to be inactive in vivo in a mammary carcinoma model.[6] The introduction of XY = azopyridine, a strong π-acceptor, as the N,N-chelating ligand has a remarkable effect on the reactivity, especially when Z = iodide.[7] Both the Ru^{II} and the Os^{II} azopyridine complexes are relatively unreactive; for example, they are inert towards aquation.

. Recently we reported that the Os^{II} azopyridine complex [Os(η^6-*p*-cym)(azpy-NMe_2)I]PF_6, complex **1**, is active in human A2780 ovarian and cisplatin-resistant A2780, A549 lung, HCT-116 colon, MCF-7 breast, PC-3 prostate and RT-112 bladder cancer cell lines at sub-micromolar concentrations (IC_{50} values 180-620 nM).[8] We have now investigated the activity of this complex in vivo versus HCT116 human colon cancer xenografts and the distribution of osmium in plasma and tissues. Further insight into the mechanism of action of **1** was obtained from studies of its redox potential, its ability to

generate Reactive Oxygen Species (ROS) in cells, and the ability of L-buthionine-sulfoximine (L-BSO), a specific inhibitor of γ-glutamyl-cysteine synthetase known to reduce intracellular thiol levels,[9] to enhance the cytotoxicity of the complex.

Results and discussion

First we studied the anticancer efficacy of complex **1** *in vivo* versus the subcutaneously implanted HCT-116 xenograft model, when administered as a single intravenous injection at its maximum soluble dose of 40 mg/kg. The agent had negligible toxicity, with an observed maximum weight loss well within the normal limits. Complex **1** was seen to induce a statistically significant tumour growth delay in the HCT-116 model compared to the untreated control ($p<0.01$), and positive control compound, the standard agent cisplatin ($p<0.05$) (Fig. 1 and Table 1). The lack of toxicity seen, combined with the favourable tumor distribution reported below, would suggest that there is significant scope to administer this compound on a repeat-dose schedule to enhance its therapeutic ability.

Next we investigated the plasma and tissue distribution of osmium after administration of **1** *in vivo,* in HCT116 xenograft-bearing mice.The distribution of **1** was analysed in the liver, kidneys, tumour, lungs and plasma, 5, 60 and 240 min after administration. The results are shown in Table 2 and Figure S1. Osmium was detected in the tumour and all tissues over the time period of analysis, whilst amounts of osmium in the plasma were surprisingly low after just 5 min, suggesting a large volume of distribution or high level of tissue distribution.

Complex **1** is relatively inert. For example, it does not readily undergo hydrolysis in aqueous solution or bind to DNA bases, unlike chlorido diamine Os^{II} arene complexes.[10] Our initial studies on iodido azopyridine Os^{II} arene complexes8 suggested that their cytotoxic activity might involve redox mechanisms. Here we have investigated the ability of complex **1** to generate Reactive Oxygen Species (ROS) in cancer cells. ROS play important roles in regulating cell proliferation, death, and senescence. The redox system is also a significant

target for anticancer treatment.[11] Targeting the redox system can induce selective cell death in malignant cells and spare normal cells due to the higher baseline level of ROS in cancer cells.

We determined the level of ROS induced in A2780 human ovarian cancer cells by complex **1** using the probe 2′,7′-dichlorodihydrofluorescein-diacetate (DCFH-DA). This is taken up by live cells, hydrolyzed to 2′,7′-dichlorodihydrofluorescein (DCFH), and in turn oxidized to 2′,7′-dichlorofluorescein (DCF) in the presence of ROS and exhibits a green fluorescence.[12] Using this probe, we determined the level of general oxidative stress induced in cells[13] by **1**. We also investigated the effect of combined exposure to **1** and L-BSO. L-BSO, a specific inhibitor of γ-glutamyl-cysteine synthetase, depletes intracellular glutathione (GSH) Gutathione plays a central role in a wide range of cellular functions, including protection, detoxification, transport, and metabolism. A2780 cells were pre-incubated for 20 min with 10 μM DCFH-DA. The relative increase in DCF fluorescence was then detected over a period of 4 h after treatment of the cells with 1 μM **1**, or 1 μM **1** plus 50 μM L-BSO, or 50 μM H_2O_2 (Figure S2). On treatment with **1** alone, the ROS level increased by 21% compared to the control. This level increased further to 50% in the presence of L-BSO in addition to **1**. The mode of interference by **1** in the redox balance in cells and the production of ROS is not yet clear. Complex **1** underwent an electrochemical reduction in dimethylformamide -0.64 V (vs. Ag/AgCl). This reduction can be associated with addition of an electron into the π* orbital centered on the azo group of the phenylazopyridine ligand to form the azo anion radical, a process previously detected for the Ru^{II} analog, although occurring more readily in the latter case at a potential of -0.40 V. Unlike the Ru complex, **1** does not react catalytically with GSH.

These data suggested that L-BSO might enhance the cytotoxicity of complex **1** towards cancer cells. As can be seen from Figure 2, L-BSO alone at a dose of 50 μM had no significant effect of the growth of either A2780 human ovarian cells or A549 human lung cancer cells, but greatly enhanced the cytotoxicity of complex **1** at doses of 0.1 and 1 μM for A2780 cells, and 1 and 5 μM for A549 cells. These results imply that combination treatment with **1** and L-BSO may have potential as a therapeutic strategy. Clinical trials of L-BSO are currently are in progress on combinations with melphalan in patients with persistent or recurrent stage III malignant melanoma.[14]

Conclusion

In conclusion, we have demonstrated that the organometallic osmium arene azopyridine complex **1**, which has nanomolar activity in vitro in a panel of human cancer cell lines,[8] exhibits acivity *in vivo* against HCT116 human colon cancer xenografts in mice, with negligible toxicity. This appears to be the first demonstration of significant anticancer activity in vivo for organometallic half-sandwich osmium complexes. Studies on the plasma, tumor and normal tissue distribution of **1** suggest that there is scope to optimize the therapeutic activity using multiple-dose schedules without the risk of off-target toxicity.

SUPPORTING INFORMATION AVAILABLE Experimental procedures, Figures S1 and S2. This material is available free of charge via the Internet.

AUTHOR INFORMATION

Corresponding author: *To whom correspondence should be addressed. (S.D.S) Fax: (+44) 01274-233234. E-mail: S.D.Shnyder@Bradford.ac.uk. (P.J.S.) Fax: (+44) 024 76523819; E-mail: P.J.Sadler@warwick.ac.uk

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Chart 1. The structure of complex **1** (FY026)

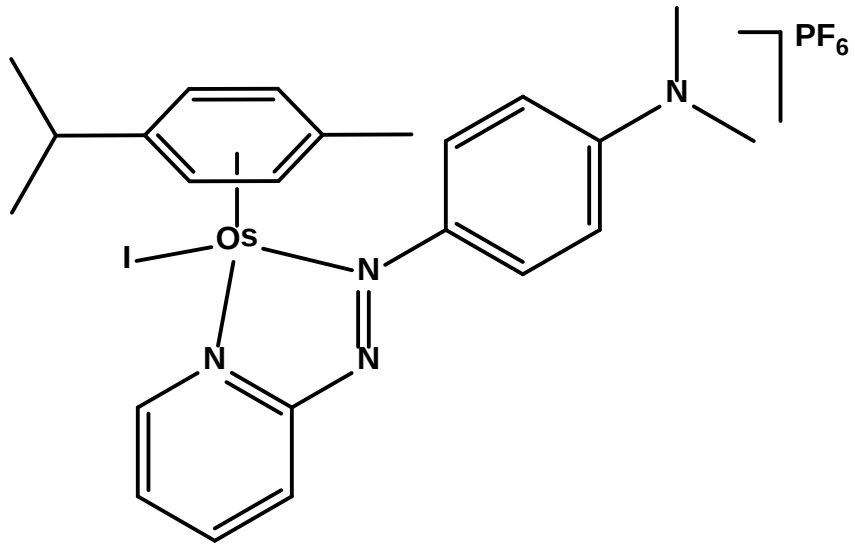


Table 1. Evaluation of the in vivo efficacy of **1** in the HCT-116 colon adenocarcinoma s.c. xenograft model

Compound (dose in mg kg^{-1})	Median tumour doubling time (days)	Significance	Maximum % weight loss (day)
Untreated controls	3.9		8 (8)
Complex **1** (40.0)	6.2	$p<0.01$	8 (1)
Cisplatin (8.0)	6.4	$p=0.05$	8 (6)

Table 2. Tumor, normal tissue and plasma distribution of osmium from **1** following i.v. administration of a single dose of 10 mg kg^{-1}.

Time/ min	μg Os / g tissue				
	Kidney	Lungs	Liver	Plasma	Tumor
5	1259.7(± 66.6)	5.81 (± 4.18)	88.6 (± 57.9)	19.6 (± 23.3)	11.9 (± 2.22)
60	513.5 (± 208.5)	40.3 (± 15.0)	136.4 (± 4.96)	21.8 (± 2.54)	8.80 (± 1.24)
240	418.6 (± 103.3)	28.3 (± 3.49)	77.2 (±10.5)	19.0 (± 10.6)	6.68 (± 2.77)

Figure captions

Figure 1. Evaluation of the in vivo efficacy of complex **1** when administered as a single intravenous injection at its maximum soluble dose of 40 mg kg^{-1} in the subcutaneously implanted HCT-116 human colon adenocarcinoma model. Complex **1** shows a greater efficacy than the standard agent cisplatin administered at its maximum tolerated dose in this model. Points represent mean ± S.D. (n=8).

Figure 2. Percentage cell survival after 24 h exposure to osmium complex **1** (FY026) with or without 50 μM L-BSO. (A) A2780 ovarian cancer cells; (B) A549 lung cancer cells. Previously determined[8] IC_{50} values are 0.2 μM for A2780 cells (24 h exposure to **1** followed by 72 h recovery period), and 0.4 μM for A549 cells (24 h exposure, 96 h recovery period).

Figure 1.

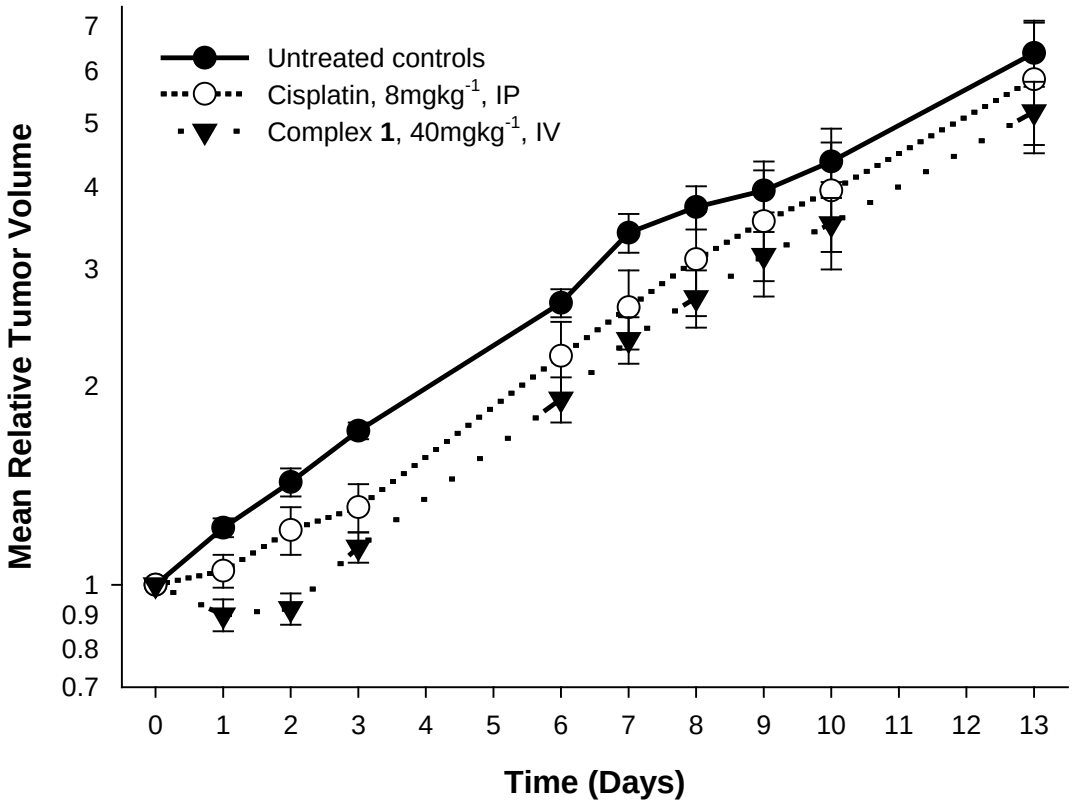
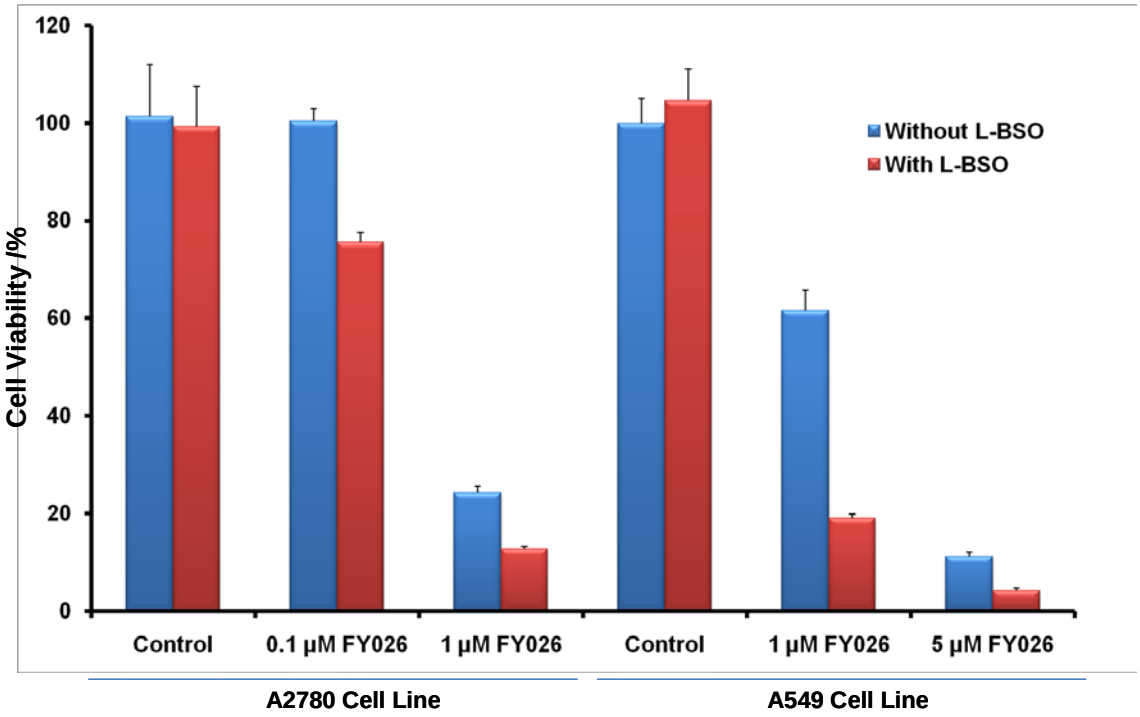

Untreated controls
Cisplatin, 8mgkg-1, IP
Complex 1, 40mgkg-1, IV
Mean Relative Tumor Volume
Time (Days)
7
6
5
4
3
2
1
0.9
0.8
0.7
0
1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 2.


120
100
80
60
40
20
0
Cell Viability /%
Without L-BSO
With L-BSO
Control
0.1 μM FY026
1 μM FY026
Control
1 μM FY026
5 μM FY026
A2780 Cell Line
A549 Cell Line