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Increasing Anti-Cancer Activity with Longer Tether Lengths of Group 9 Cp* Complexes

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Here in, we report the cytotoxicity of both rhodium and iridium functionalised Cp* analogues of the [Cp*MCl₂]₂ dimers. The functionalised dimers contain a hydroxy tethered arm of differing carbon length. These show promising IC₅₀ values when tested against HT-29, A2780 and cisplatin-resistant A2780cis human cancer cell lines, with the cytotoxicity improving proportionally with an increase in carbon tether length of the Cp* ring. The most promising results are seen for the 14-carbon Cp* tethered rhodium (**2d**) and iridium (**3b**) complexes, which show up to a 24-fold increase in IC₅₀ compared to the unfunctionalised [Cp*MCl₂]₂ dimer. All complexes were potent inhibitors of purified thioredoxin reductase suggesting that disruption of cellular anti-oxidant function is one potential mechanism of action.

In a search for less toxic and more potent alternatives to cisplatin, organometallic complexes have shown promising activity as anti-cancer agents.¹⁻⁴ There are relatively few reported studies on the anti-cancer activity of rhodium and iridium Cp* complexes.⁵⁻¹⁷ Sadler *et al.* reported that for complexes with the general structure [Cp[†]Ir(XY)Cl]^{0/+}, where Cp[†] is either Cp* or arene-functionalised Cp* ligands. Their potency towards A2780 human ovarian cancer cells increases with an increase in aryl substitution on the cyclopentadienyl ligand.¹⁷ Recently we demonstrated the use of various iridium Cp* piano stool complexes with promising activity for HT-29 and MCF-7 cell lines, where [Cp*IrCl₂]₂ was inactive.¹⁶ Therefore, breaking up the rhodium and iridium dihalide Cp* dimers with different bidentate ligands led to the development of complexes which show high *in vitro* cytotoxic activity against tumour cell lines.⁵⁻¹⁹

Where tested, studies have reported negligible activities of the starting dimers, i.e. [Cp*MCl₂]₂ (M = Rh or Ir)¹⁶ and there

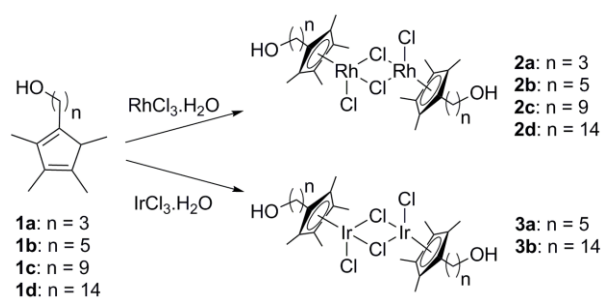
have been no previously reported examples of group 9 dihalide functionalised Cp* dimers demonstrating cytotoxic behaviour. We are interested in the potential of converting these inactive dimers [Cp*MCl₂]₂ (M = Rh or Ir) to active cytotoxic agents through modifications of the Cp* ligand. Herein, we report the synthesis of ten complexes and a discussion of five new crystal structures of the type, [Cp[†]MCl₂]₂ (M = Rh or Ir). These complexes have also been evaluated for their cytotoxic activity *in vitro* against three tumour cell lines. Previous data has shown that the monomers were cytotoxic and therefore these dimers were tested in the hope to deliver two metal centres to the cancerous cells, potentially increasing the potency of the complex by up to 2-fold. To allow comparisons to be made between monomer and dimer, we have explored the effects of adding monodentate pyridine ligands and bidentate picolinamide ligands to both of the functionalised Cp[†] iridium and rhodium dimers.

Complexes **1b**, **1d**, **2b**, **2d**, **3a** and **3b** have been previously reported for their catalytic potential.²⁰⁻²² We investigated the immobilisation of these complexes onto Wang resin for use as recyclable transfer hydrogenation catalysts.²¹ In the hope to maximise the amount of metal that can reach the cancerous cells and to increase the drugs potency, the library of dimers has been tested against human colorectal adenocarcinoma (HT-29), human ovarian carcinoma (A2780) and cisplatin-resistant human ovarian carcinoma (A2780cis). Due to the ability of the cyclopentadienyl hydroxyl-substituent to bind well to solid supports and with the previous evidence we have of the Cp* ligand binding to a metal centre,²³ it is proposed that this alcohol could provide stability to the metal centre if the dimer breaks apart during its interaction with cells. The preparation of the dimeric complexes **2a-2d**, **3a** and **3b** are shown in **Scheme 1**. The addition of monodentate pyridine and bidentate picolinamide ligands to the dimers, gave complexes **4a**, **4b**, **5a** and **5b**. These were synthesised by a reaction of **3a** or **3b** with the corresponding ligand, as shown in **Scheme 2**. All of the complexes were characterised by ¹H and ¹³C{¹H} NMR spectroscopy, mass spectrometry and the novel complexes by elemental analysis.

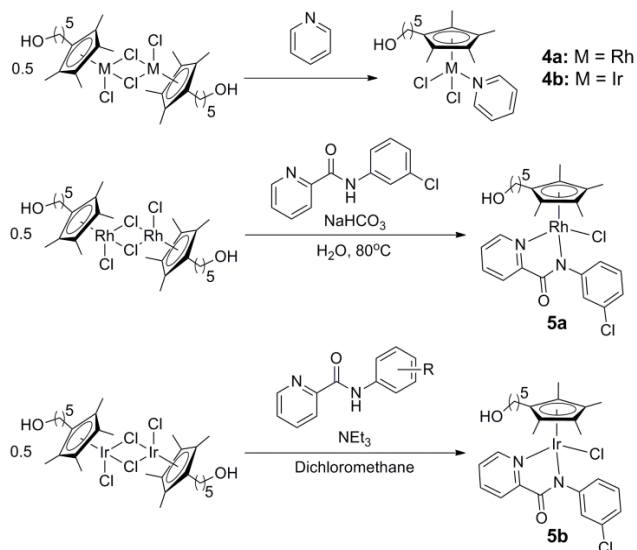
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[†] Electronic Supplementary Information (ESI) available: Experimental procedures for complexes, chemosensitivity studies, Trx-R enzyme assay and crystal structure determination details. The cifs for complexes **2a**, **2c**, **4a**, **5a** and **5b** were deposited to the CCDC with codes 927162-927163 and 947905-947907 respectively. See DOI: 10.1039/b000000x/



Scheme 1 Formation of alcohol functionalised Cp⁺ rhodium dimers **2a-2d** and iridium dimers **3a-b**.



Scheme 2 Formation of alcohol functionalised Cp⁺ rhodium and iridium pyridine and picolinamide complexes **4a**, **4b**, **5a** and **5b**.

Single crystals of **2a** and **2c** were obtained by layer diffusion of hexane into a dichloromethane solution and vapour diffusion from dichloromethane/pentane solvent system respectively. The alcohol chain in **2c** showed disorder, with four of the carbons equally occupied over two positions each (Figure S2). The rhodium and iridium pyridine complexes, **4a** and **4b**, were recrystallised from vapour diffusion of dichloromethane/diisopropylether and chloroform/pentane solvent systems respectively. Complex **4b** could not be solved to a publishable quality, however, it clearly shows the connectivity of iridium to a pyridine ligand, two chloride ligands and a 5 carbon functionalised Cp⁺ ligand. The picolinamide complexes, **5a** and **5b**, were crystallised using slow diffusion from a methanolic solution and layer diffusion from a dichloromethane/hexane solvent system respectively.

The complexes were obtained as orange-red single crystals and solutions were performed in either monoclinic (**2a**, **2c**, **5a**), triclinic (**4a**), or orthorhombic (**5b**) space groups. Selected bond lengths (Å) are presented in Table 1 and are in the range expected for these structures. All of the angles around the metal centre show the geometry expected for *pseudo* octahedral complexes which is common for these “piano-stool” structures (Tables S1-2, Supplementary Information). The angles between the metal and bidentate ligands are in the range 77-91°, with the remaining three coordination sites

Bond Lengths (Å)	M(1)-Cl(1)	M(1)-X	M(1)-Y	M(1)-Cg
2a (X=Cl(2), Y=Cl(2'))	2.4341(7)	2.4857(6)	2.4775(6)	1.774
2c (X=Cl(2), Y=N(1))	2.4353(8)	2.4693(8)	2.4776(9)	1.769
4a (X=Cl(2), Y=N)	2.4502(5)	2.4393(5)	2.150(1)	1.789
5a (X=N(1), Y=N(2))	2.4416(8)/ 2.4452(8)	2.121(3)/ 2.127(3)	2.114(2)/ 2.111(2)	1.8091/ 1.812
5b (X=N(1), Y=N(2))	2.451(2)	2.136(5)	2.125(4)	1.823

Table 1 Selected bond lengths (Å) for complexes **2a**, **2c**, **4a**, **5a** and **5b**

occupied by the functionalised Cp⁺ ligand and the angles observed for their centroids (Cg) to the chloride or ligands ranges between 125-134°. Molecular structures for complexes **2a**, **2c**, **4a**, **5a** and **5b** are shown in Figure 1.

The cytotoxic activities of complexes **2b-2d**, **3a** and **3b** along with [Cp⁺RhCl₂]₂, [Cp⁺IrCl₂]₂ and cisplatin, were tested against HT-29, A2780 and A2780cis cell lines. The IC₅₀ values (μM) ± Standard Deviation are presented in Table 2. The potency of the monomeric pyridine complexes, **4a** and **4b**, were assessed against HT-29 cells and the picolinamide complexes, **5a** and **5b**, were assessed against A2780 cells. The pyridine complexes were less active than their dimeric precursors, **2b** and **3a**, with a 3-fold decrease in activity observed against HT-29 between **2b** (30 ± 1 μM) and **4b** (92 ± 1 μM). Against A2780, the iridium picolinamide complex, **5b** (52.5 ± 0.8 μM), was also less active than its starting dimer, **3a** (23.2 ± 0.8 μM) with a 2.2-fold decrease in activity observed. The dimeric species, except for the anomalous result of **2b** against A2780cis, upon replacing a Cp⁺ methyl group with a longer aliphatic chain alcohol substituent, an increase in cytotoxicity is observed against all cell lines. The cytotoxicity improves upon increasing the functionalisation of the Cp⁺ ligand, i.e. a longer alcohol chain decreases the complex's IC₅₀ value and is therefore more potent. These results give the indication that the chain length of the Cp⁺ could be a feature required for increased potency.

Both the rhodium and iridium complex **2d** or **3b** are the most potent of the dimers, with values comparable to cisplatin. Complex **2d** is the first example of a cytotoxic functionalised Cp⁺ rhodium complex. Previous rhodium Cp⁺ complexes with reported cytotoxic activity have contained bidentate ligands that were designed to intercalate with DNA.^{11, 18} This rhodium 14-carbon tether dimer **2d** (3.9 ± 0.1 μM), shows a 24-fold increase in IC₅₀ against A2780 when compared to the unsubstituted dimer, [Cp⁺RhCl₂]₂ (95.0 ± 2.0 μM). The iridium complex **3a**, with a 5 carbon chain, is significantly more active than its rhodium analogue **2b**, most noticeably against the cisplatin-resistant A2780cis line with an IC₅₀ of 28.4 ± 0.6 μM compared to 127.0 ± 6.0 μM. Against the A2780cis line, complexes **2d** (4.9 ± 0.2 μM) and **3b** (5.3 ± 0.1 μM) have a 2-fold increase in active when compared to cisplatin (11.1 ± 0.6 μM), showing these complexes potential to circumvent cisplatin-resistance in cells.

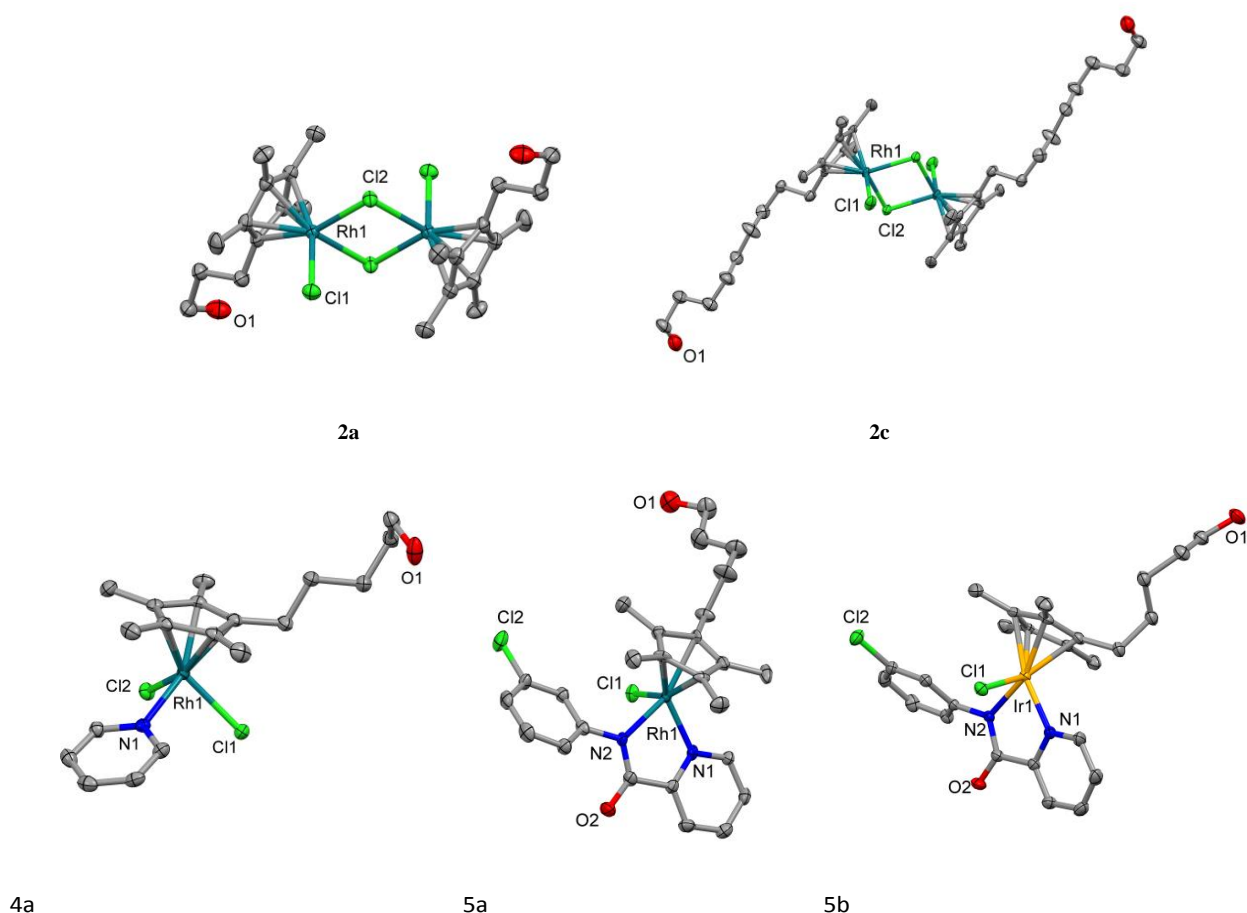


Figure 1 Molecular structures of **2a**, **2c**, **4a**, **5a** and **5b**. Hydrogen atoms are omitted for clarity and displacement ellipsoids are at the 50% probability level.

Complex	IC ₅₀ (μM) ± SD		
	HT-29	A2780	A2780cis
cisplatin	2.5 ± 0.1	0.95 ± 0.05	11.1 ± 0.6
[RhCp*Cl ₂] ₂	141.0 ± 2.0	95.0 ± 2.0	90.0 ± 2.0
[IrCp*Cl ₂] ₂	92.0 ± 4.0	30.9 ± 0.4	43.0 ± 3.0
2b	123.0 ± 2.0	85.0 ± 7.0	127.0 ± 6.0
2c	13.0 ± 0.2	6.2 ± 0.3	10.8 ± 0.7
2d	12.7 ± 0.4	3.9 ± 0.1	4.9 ± 0.2
3a	30.0 ± 1.0	23.2 ± 0.8	28.4 ± 0.6
3b	10.6 ± 0.8	5.2 ± 0.2	5.3 ± 0.1
4a	132.0 ± 2.0	-	-
4b	92.0 ± 1.0	-	-
5a	-	85.0 ± 4	-
5b	-	52.5 ± 0.8	-

Table 1 IC₅₀ (mM) values ± SD (standard deviation) from a 5 day MTT assay for complexes 2ab-d, 3a-b, 4a-b and 5a-b along with cisplatin, [IrCp*Cl₂]₂ and [RhCp*Cl₂]₂ for reference. (-) indicates no data is available

The *in vitro* MTT assay for determining chemosensitivity is a useful indication of drugs cytotoxicity but it provides little information as to individual drugs' mode or modes of action. To gain a preliminary insight into this, the effects of the complexes on thioredoxin reductase activity were assessed. Thioredoxin reductase (Trx-R) is one of the key anti-oxidant enzymes in the cell, is commonly over expressed in tumours and can promote tumour growth and progression.²⁴ We have

previously reported a range of iridium picolinamide complexes that inhibit Trx-R, with IC₅₀ values in the nanomolar range.^{19, 25} This prompted us to investigate whether these novel complexes **2b**, **2c**, **2d**, **3a** and **3b** reported here are also able to inhibit Trx-R activity. Significantly, all the active rhodium and iridium functionalised Cp*⁺ dimers were found to be potent Trx-R inhibitors with IC₅₀ values in the nanomolar or low micromolar range (**Table 3** and **Figure S1**).

Complex	IC ₅₀ (nM) ± SD
2b	510 ± 59
2c	398 ± 71
2d	552 ± 34
3a	217 ± 50
3b	2098 ± 410

Table 3. Thioredoxin reductase 1 (Trx-R) IC₅₀ values (nM) ± SD for complexes **2b-d** and **3a-b**.

Complexes **2b** and **2c** are the most potent when assessing the chemosensitivity studies, however, they are not the most potent inhibitors of Trx-R. In fact, the least chemosensitive complex **3a** is the most potent Trx-R inhibitor, with an IC₅₀ value of 217 ± 50 nM. These results show that Trx-R inhibition may contribute to the mode of action and the anti-cancer activity of some or all of our novel complexes; although it is likely that additional mechanisms may also be important and future studies will aim to explore this further.

Conclusions

We have extended our library of complexes of the type [Cp[†]MCl₂]₂ where M = Rh/Ir and Cp[†] = C₅(CH₃)₄(CH₂)_nOH, and have evaluated the complexes' *in vitro* activities against HT-29, A2780 and A2780cis cell lines. In general, the anti-cancer activity improves upon increasing the chain length between the Cp ring and the OH, with the most promising IC₅₀ values seen for the 14-carbon length tethered dimers. The most significant result was that of the rhodium 14-carbon tether dimer **2d** (3.9 ± 0.1 μM), in which a 24-fold increase in IC₅₀ was observed when compared to the unsubstituted dimer, [Cp[†]RhCl₂]₂ (95.0 ± 2.0 μM). This is the first example of a cytotoxic functionalised Cp[†] rhodium complex. The mechanism of action of organometallic complexes is generally complex but here we demonstrate that one potential mechanism involves inhibition of, thioredoxin reductase (Trx-R) activity. In a cell free assay, complexes **2b-d** and **3a** inhibited Trx-R activity with IC₅₀ values in the nanomolar range. Whilst the active complexes **2b-d** and **3a-b** all show potent inhibitory activity against Trx-R. There was no strict correlation with the differential cytotoxicity of the complexes suggesting that additional mechanisms might also contribute to the mode of action of these complexes.

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