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**Inorganic Chemistry Communications** 



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# Mono- and dinuclear ( $\eta^6$ -arene) ruthenium(II) benzaldehyde thiosemicarbazone complexes: Synthesis, characterization and cytotoxicity

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### ARTICLE INFO

Article history: Received 30 November 2010 Accepted 16 March 2011 Available online 9 April 2011

Keywords: Bioorganometallic chemistry Arene ruthenium(II) Thiosemicarbazones Anticancer drugs

## ABSTRACT

A series of mono- and dinuclear ( $\eta^{6}$ -arene) ruthenium(II) complexes were prepared by reaction of thiosemicarbazone ligands derived from benzaldehyde and ruthenium(II) precursors of the general formula [Ru( $\eta^{6}$ -arene)( $\mu$ -Cl)Cl]<sub>2</sub>, where arene =  $p^{-i}$ PrC<sub>6</sub>H<sub>4</sub>Me or C<sub>6</sub>H<sub>5</sub>C<sub>3</sub>H<sub>6</sub>COOH. These complexes were characterized by NMR and IR spectroscopy, ESI-mass spectrometry and elemental analysis. The molecular structure of the mononuclear *p*-cymene complex was determined by X-ray diffraction analysis, revealing a pseudo-tetrahedral piano stool conformation and a bidentate *N*,*S* coordination mode of the thiosemicarbazone ligand. The complexes and ligands were evaluated for their *in vitro* cytotoxicity against the WHCO1 oesophageal cancer cell line.

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Metal-based therapeutic agents have been used as anticancer drugs in recent years [1]. Drugs specifically derived from platinum, have been used extensively as anticancer agents. The benchmark discovery of the anticancer properties of *cis*-diamminedichloroplatinum(II) (cisplatin) was made by Rosenberg and co-workers in the mid 1960's [2]. This discovery single-handedly propelled research based on using metalcontaining compounds as anticancer agents [2]. To date, cisplatin is considered to be one of the most effective and widely used anticancer drugs. Second generation platinum drugs including carboplatin and oxaliplatin have been developed for clinical application [3].

The efficacy of these drugs, including cisplatin, however, is reduced by increasing tumour resistance and in the case of cisplatin, high toxicity. This in turn affects the administration of the drugs [4–8]. These limitations have aroused interest towards the design and evaluation of transition metal complexes other than platinum-based derivatives for therapeutic use. In recent years, several ruthenium-based complexes have been investigated for potential antitumor activity. Two ruthenium (III) complexes namely [indH]*trans*-[Ru(*N*-ind)<sub>2</sub>Cl<sub>4</sub>] (KP1019) [9–11] and [imiH]*trans*-[Ru(*N*-imi)(*S*-dmso)Cl<sub>4</sub>] (NAMI-A) [12,13] (imi = imidazole and ind = indazole) have successfully completed phase I clinical trials.

Half-sandwich arene ruthenium(II) complexes have generated great interest as potential anticancer agents. Many mono- and polynuclear arene ruthenium(II) compounds have displayed *in vitro* and/or *in vivo* antiproliferative activity [14–18]. Complexes of the

general formula [Ru( $\eta^6$ -arene)(en)Cl]PF<sub>6</sub>, (where en = ethylenediamine and arene = benzene, *p*-cymene, tetrahydroanthracene, dihydroanthracene and biphenyl) have also exhibited anticancer activity. This included activity against cisplatin-resistant cells [19,20]. More recently, a series of arene ruthenium(II) complexes containing 1,3,5triaza-7-phosphaadamantane (PTA) derivatives were evaluated for their activity [21–25].

Thiosemicarbazones (TSCs) are versatile ligands as they possess a number of donor atoms which may coordinate in various ways. In addition to this, thiosemicarbazones possess a variety of biological properties including antiproliferative activity [26]. Studies have demonstrated that TSCs are potent inhibitors of the enzyme ribonucleotide reductase (RR) and are capable of interrupting DNA synthesis and repair [27]. Incorporation of metals onto these TSC ligands can result in alteration or enhancement of their biological activity [28]. In a recent study, half-sandwich ( $\eta^6$ -p-cymene) ruthenium(II) complexes derived from bidentate thiosemicarbazones were evaluated for their biological activity against breast and colorectal carcinoma cells. The complexes exhibited good in vitro cytotoxic activity against all cancer cell lines [29]. Our previous work regarding the anticancer activity of dithiosemicarbazone palladium (II) complexes has been problematic due to the poor solubility of these compounds [30]. Many arene ruthenium complexes have been known to possess good biological activity as well as solubility in aqueous media. This paper reports the synthesis, characterization and cytotoxicity of half-sandwich ( $\eta^6$ -arene) ruthenium(II) complexes of benzaldehyde derived mono- and dithiosemicarbazones.

The benzaldehyde monothiosemicarbazone (L1) was prepared by modification of the literature method [31]. Benzene-1,4-dithiosemicarbazide was prepared according to the reported procedure [32] and

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<sup>1387-7003/\$ –</sup> see front matter 0 2011 Elsevier B.V. All rights reserved. doi:10.1016/j.inoche.2011.03.041

subsequently refluxed with benzaldehyde in a 1:2 stoichiometric ratio in MeOH affording the desired dithiosemicarbazone ligand (**I2**) in 91% yield [33]. Complexes **1** and **2** were prepared by reaction of **L1** and  $[\text{Ru}(\eta^6-p-\text{cymene})(\mu-\text{Cl})\text{Cl}]_2$  [34] or  $[\text{Ru}(\eta^6-\text{C}_6\text{H}_5\text{C}_3\text{H}_6\text{COOH})(\mu-\text{Cl})\text{Cl}]_2$  [35] in a 2:1 molar ratio (Scheme 1). Complexes **3** and **4** were obtained by reaction of **L2** and  $[\text{Ru}(\eta^6-p-\text{cymene})(\mu-\text{Cl})\text{Cl}]_2$  or  $[\text{Ru}(\eta^6-\text{C}_6\text{H}_5\text{C}_3\text{H}_6\text{COOH})(\mu-\text{Cl})\text{Cl}]_2$  using a 1:1 molar ratio (Scheme 2). All reactions were carried out by stirring the contents at ambient temperature for 16–24 h. All complexes were obtained as air-stable red/orange solids in moderate to good yields (62–89%) [36]. Complexes **1** and **3** are readily soluble and stable in CH<sub>2</sub>Cl<sub>2</sub>, CHCl<sub>3</sub>, MeOH, EtOH and DMSO and sparingly soluble in water, while complexes **2** and **4** are less soluble in chlorinated solvents.

All compounds were characterized using NMR and IR spectroscopy, ESI-mass spectrometry and elemental analysis. The <sup>1</sup>H NMR spectrum of L2 displays a signal for the imine protons at 8.52 ppm and a signal for the hydrazinic protons at 12.06 ppm. The protons of the phenyl bridge appear as a singlet at 7.92 ppm attesting to the symmetrical nature of the molecule. The <sup>1</sup>H NMR spectra of the complexes (1-4) suggest a loss of the two-fold symmetry of the *p*-cymene and the carboxylic acid arene ligands upon coordination of the metal to the thiosemicarbazone ligands. In these complexes the ruthenium atoms are stereogenic due to the coordination of four different ligator atoms. The loss of symmetry is evidenced by the appearance of a set of four doublets in the region of 4.60 and 5.40 ppm accounting for the protons of the *p*-cymene rings of complexes 1 and 3. The methyl substituents of the isopropyl group are observed as two distinct doublets in the aliphatic region of the spectra, which further confirms the loss of symmetry as the two methyl groups are non-equivalent. These methyl protons are observed at 1.07 and 1.13 ppm for complex **1** and at 1.09 and 1.15 ppm for complex **3**. In the case of the carboxylic acid derivatives (2 and 4), three pseudo-triplets and two doublets are observed for the arene ring and are found in the region of 5.00-5.50 ppm. The loss of symmetry of the arene rings in all cases suggests that these complexes have similar modes of coordination. Singlets for the imine protons are observed at 9.05 (1), 8.81 (2), 9.04 (3), and 8.90 ppm (4). A downfield shift is observed for these signals compared to the free ligands upon coordination to ruthenium, suggesting coordination of the metal to the azomethine nitrogen atom as the signal becomes more deshielded in each case. This is also observed for similar arene ruthenium(II) thiosemicarbazone complexes [29,37].

The infrared spectrum of **L2** displays absorption bands at 1600 and 860 cm<sup>-1</sup> for the C=N and C=S stretching frequencies, respectively. The infrared spectra of the complexes suggest that the thiosemicarbazones act as neutral ligands upon coordination. The metals are coordinated to the ligands via the imine nitrogen and the thione sulfur atoms. Absorption bands for the azomethine stretching frequencies ( $\nu_{C=N}$ ) of complexes **1** and **3** are observed at approximately 1618 and 1614 cm<sup>-1</sup>, respectively. This particular band is found at approximately

1600 cm<sup>-1</sup> for complexes **2** and **4**. Bands for the thione stretching frequencies ( $\nu_{C=S}$ ) are observed in the region of 845–875 cm<sup>-1</sup>. The IR spectra of complexes **2** and **4** display absorption bands for the carbonyl stretching frequencies ( $\nu_{C=O}$ ) at 1698 and 1727 cm<sup>-1</sup>, respectively.

The ESI mass spectra display base peaks corresponding to the fragment [M-HCl-Cl]<sup>+</sup> for complexes **1** and **2**. A peak of 85% intensity corresponding to the fragment  $[M-4HCl-H+2Na]^+$  is observed for complex **3**, while a fragment corresponding to  $[M-2Cl-CO_2]^+$  is observed for complex **4**. Elemental analysis further confirms the composition of these compounds. The data obtained for these complexes are consistent with the proposed structures.

The mode of coordination of one of these compounds was confirmed by X-ray diffraction. Crystals suitable for structure determination were grown by slow diffusion of diethyl ether into a MeOH/CH<sub>2</sub>Cl<sub>2</sub> (80:20 v/v %) solution [38]. The compound crystallizes in the monoclinic space group C2/c as its chloride salt alongside one molecule of methanol. An ORTEP drawing [39] with the corresponding atom labelling scheme is presented in Fig. 1 together with the selected bond lengths and angles.

The molecular structure confirms coordination of the thiosemicarbazone ligand by the ruthenium metal through the imine nitrogen and thione sulfur donor atoms in a bidentate fashion forming a fivemembered chelate ring. This N.S-coordination mode is the most common for thiosemicarbazone ligands, however N,S-coordination in which a four-membered chelate ring is formed has been as well observed [40-44]. The complex adopts the commonly observed piano-stool geometry of many half-sandwich arene ruthenium(II) complexes [29,37,44–46]. In this case, the *p*-cymene ring forms the seat of the piano-stool, while the bidentate thiosemicarbazone (N,S)and the chlorido ligand form the three legs of the stool. Intermolecular distances of 2.969(8) Å between Cl(2) and N(1) as well as 3.146(5) Å between N(1) and O(1) of the solvated methanol molecule are indicative of strong hydrogen bonding (Fig. 2). The ruthenium center adopts a pseudo-tetrahedral geometry as the p-cymene ligand essentially occupies one coordination site. Bond angles of 81.82(12), 83.45(12) and 86.52(5)° are observed for N(3)-Ru(1)-S(1), N(3)-Ru (1)-Cl(1) and S(1)-Ru(1)-Cl(1), respectively. On closer examination of the bond lengths, it appears that the p-cymene ligand is asymmetrically coordinated to the metal center as the Ru-Carene bond lengths are slightly varied from 2.162(5) for Ru(1)-C(10) to 2.266(6) Å for Ru(1)-C(12). This is also observed for a similar anthraldehyde TSC ruthenium complex [29]. It is believed that this distortion may minimize steric interaction between the *p*-cymene moiety and the thiosemicarbazone ligand [29].

Complexes **1–4** along with their corresponding ligands (**L1** and **L2**) were evaluated for their cytotoxic potencies against WHCO1 oesophageal cancer cells [47]. This was carried out by means of a colorimetric MTT assay and the IC<sub>50</sub> values obtained are listed in



Scheme 1. Synthesis of mononuclear ( $\eta^6$ -arene) ruthenium(II) thiosemicarbazone complexes (1 and 2).



**Scheme 2.** Synthesis of dinuclear ( $\eta^6$ -arene) ruthenium(II) thiosemicarbazone complexes (**3** and **4**).

Table 1. Two complexes (1 and 4) displayed moderate cytotoxicity against this particular cell line. Complex 4 displayed the best activity ( $IC_{50} = 8.96 \mu$ M), however, in comparison to its free ligand, a decrease in potency is observed. The low activity may be attributed to inadequate accumulation of these compounds inside the cells. Low *in vitro* cytotoxic activity has often been observed for ruthenium compounds, including NAMI-A. In this case of NAMI-A, the compound displays low *in vitro* activity but increased activity against tumour metastases *in vivo*. Many other ruthenium compounds have been found to display enhanced cytotoxicity *in vivo* may reveal promising results.

In conclusion, two mono- and two dinuclear ( $\eta^6$ -arene) ruthenium (II) thiosemicarbazone complexes have been prepared and charac-



**Fig. 1.** Molecular structure of cation **1** at 50% probability level with chloride anion and methanol molecule omitted for clarity. Selected bond lengths (Å) and angles (°): Ru(1)-Cl(1) 2.4004(15), Ru(1)-S(1) 2.3579(15), Ru(1)-N(3) 2.131(4), C(1)-S(1) 1.695(6), C (1)-N(1) 1.314(7), C(1)-N(2) 1.338(6), N(2)-N(3) 1.400(6), C(2)-N(3) 1.285(6); Cl(1)-Ru(1)-S(1) 8652(5), Cl(1)-Ru(1)-N(3) 83.45(12), S(1)-Ru(1)-N(3) 81.82(12), N(1)-C (1)-S(1) 121.8(4), S(1)-C(1)-N(2) 120.7(5), N(1)-C(1)-N(2) 117.5(5), N(2)-N(3)-C(2) 113.6(4).

terized using standard spectroscopic and analytical techniques. Single crystal X-ray diffraction confirmed coordination of the thiosemicarbazone in a bidentate N–S manner to ruthenium, resulting in a pseudo tetrahedral geometry about the metal center. Two complexes displayed moderate activity against WHCO1 cells.

#### Acknowledgements

We gratefully thank the University of Cape Town, National Research Foundation (NRF) of South Africa, CANSA, Carnegie Corporation and the Swiss–South African Joint Research Programme for the financial support, the Anglo Platinum Corporation as well as the Johnson Matthey Research Centre for a generous loan of ruthenium chloride hydrate.

## Appendix A. Supplementary material

CCDC 797327 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc. cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].



Fig. 2. Hydrogen bonded network in [1]Cl · MeOH.

#### Table 1

 $IC_{50}$  values obtained for complexes (1-4) as well as their corresponding free ligands (L1 and L2) against WHCO1 cells.

Compound	$IC_{50}~(\mu M)~^a$	95% Confidence Interval
L1	>200	not applicable
L2	0.21	0.11-0.38
1	81.13	69.87-94.21
2	>200	not applicable
3	>200	not applicable
4	8.96	1.26-63.78

<sup>a</sup> Drug concentration required for 50% inhibition of cell viability.

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°C. FT-IR (KBr, cm<sup>-1</sup>): 1600 (C=N), 860 (C=S). <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) = 7.76 (m, 6H, CH (benzaldehyde)), 7.92 (s, 4H, Ph), 8.24 (m, 4H, benzaldehyde), 8.52 (s, 2H, CH=N), 10.38 (s, 2H, -CNHC-); 12.06 (2H, s, -NHN=). <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  (ppm) 125.92, 128.05-134.43 (benzaldehyde), 136.62, 143.30 (C=N), 176.43 (C=S). Elemental analysis (%): Calc. for C<sub>22H20</sub>N<sub>6</sub>S<sub>2</sub>: C, 61.08; H, 4.66; N, 19.43; S, 14.38. Found: C, 60.73; H, 5.17; N, 19.67; S, 14.28. ESI-MS: m/z 455 (100%, [M+Na]<sup>+</sup>).

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- [36] General Procedure for the synthesis of 1-4: Complexes were prepared by reaction of  $[Ru(\eta^6\text{-}arene)(\mu\text{-}Cl)Cl]_2$  and L1 (1:2) or L2 (1:1) in  $CH_2Cl_2$  or MeOH. All reactions were carried out by stirring the contents at ambient temperature for 16-24 hours. Soluble products were precipitated using diethyl ether after solvent reduction. The solids were obtained as red or orange solids. Complex 1: Yield = 0.155 g (62%). M.p.: 177–181 °C. FT-IR (KBr, cm<sup>-1</sup>) 1618 (C=N), 875 (C=S). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 1.07 (d, 3H, CH<sub>3</sub> (<sup>i</sup>Pr), <sup>3</sup>J<sub>H-H</sub> = 6.81), 1.13 (d, 3H, CH<sub>3</sub> (<sup>†</sup>Pr), <sup>3</sup>*J*<sub>H-H</sub> = 6.94), 2.05 (s, 3H, CH<sub>3</sub>), 2.61 (m, 1H, CH (methine)), 4.64 (d, 1H, CH (*p*-cymene), <sup>3</sup>*J*<sub>H-H</sub> = 5.72), 4.80 (d, 1H, CH (*p*-cymene), <sup>3</sup>*J*<sub>H-H</sub> = 5.82), 4.88 (d, 1H, CH (*p*-cymene), <sup>3</sup>*J*<sub>H-H</sub> = 5.79), 5.41 (d, 1H, CH (*p*-cymene), <sup>3</sup>*J*<sub>H-H</sub> = 5.79), (d, m, Gr (*p*-cynterle),  $y_{H-H} = 5.79$ , 5.47 (d, m, Gr (*p*-cynterle),  $y_{H-H} = 5.79$ , 7.57 (m, 4H, CH (benzaldehyde), NNH), 8.15 (d, 2H, CH (benzaldehyde),  $^{3}J_{H-H} = 6.99$ ), 9.05 (s, 1H, CH=N).  $^{13}C(^{1}H)$  NMR (100 MHz, CDCI<sub>3</sub>):  $\delta$  (ppm) = 18.57, 21.39, 23.05, 30.73, 81.35-88.78 (*p*-cymtene), 103.85, 127.84, 128.86-133.34 (benzaldehyde), 161.65 (C=N), 177.54 (C=S), Elemental analysis (%): Calc, for C<sub>18</sub>H<sub>23</sub>Cl<sub>2</sub>N<sub>3</sub>RuS MeOH: C, 44.10; H, 5.26; N, 8.12; S, 6.20. Found: C, 44.22; H, 4.82; N.7.92; S, 6.07. ESI-MS: *m*/z 414 (100%, [M-HCI-CI]<sup>+</sup>). Complex 2: Yield = 0.225 g (75%). M.p.: decomposes at 163 °C. FT-IR (KBr, cm<sup>-1</sup>): 1698 (C=O), 1601 (C=N), (75%). M.p.: decomposes at 163 °C. FT-IR (KBr, cm<sup>-1</sup>): 1698 (C=O), 1601 (C=N), 871 (C=S). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) = 1.81 (qn, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>, COOH), 2.37 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CHOOH,  ${}^{3}J_{H-H} = 7.14$ ), 2.44 (t, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>COOH, <sup>3</sup>J<sub>H+H</sub> = 7.09), 5.09 (d, 1H, CH (arene),  ${}^{3}J_{H-H} = 5.84$ ), 5.17 (d, 1H, CH (arene),  ${}^{3}J_{H-H} = 5.89$ ), 5.29 (t, 1H, CH (arene),  ${}^{3}J_{H-H} = 5.72$ ), 5.43 (t, 1H, CH (arene),  ${}^{3}J_{H-H} = 5.85$ ), 5.57 (t, 1H, CH (arene),  ${}^{3}J_{H-H} = 5.77$ ), 7.71 (m, 3H, CH (benzaldehyde)), 8.24 (d, 2H, CH (benzaldehyde),  ${}^{3}J_{H-H} = 5.86$ ), 8.81 (s, 1H, CH=N).  ${}^{13}C{}^{14}$ H NMR (100 MHz, CD<sub>3</sub>OD): δ (ppm) = 24.36, 32.13, 32.48, 81.25-106.25 (arene), 128.63-133.47 (benzaldehyde), 162.35 (C=N), 174.98 (C=O), 177.82 (C=S) Elemental analysis (%): Calc. for C<sub>18</sub>H<sub>2</sub>(Cl<sub>N</sub>N<sub>2</sub>O<sub>7</sub>RUS; C, 41.94; H. 177.82 (C=S). Elemental analysis (%): Calc. for C<sub>18</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>RuS: C, 41.94; H, 4.11; N, 8.15; S, 6.22. Found: C, 41.52; H, 4.13; N, 7.86; S, 5.91. ESI-MS: m/z 444 (100%, [M-HCl-Cl]<sup>+</sup>). Complex 3: Yield = 0.187 g (68%). M.p.: decomposes at 224 °C. FT-IR (KBR, cm<sup>-1</sup>): 1614 (C=N), 869 (C=S). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  $(ppm) = 1.09 (d, 6H, CH_3 ({}^{i}Pr), {}^{3}J_{H-H} = 6.74); 1.15 (d, 6H, CH_3 ({}^{i}Pr), {}^{3}J_{H-H} = 6.82),$ 2.07 (s, 6H, CH<sub>3</sub>), 2.64 (m, 2H, CH (methine)), 4.69 (m, 2H, CH (p-cymene)), 4.82 (d, 2H, CH (*p*-cymene),  ${}^{3}J_{H-H} = 5.39$ ), 4.92 (d, 2H, CH (*p*-cymene),  ${}^{3}J_{H-H} = 4.86$ ), 5.41 (d, 2H, CH (*p*-cymene),  ${}^{3}J_{H-H} = 5.67$ ), 7.58 (m, 12H, CH (benzene), CH (benzaldehyde), NNH), 8.19 (d, 4H, CH (benzaldehyde),  $^{3}_{H-H} = 6.44$ ), 9.04 (s, 2H, CH=N), 10.66 (s, 2H, CNHC-).  $^{13}C(^{1}H)$  NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 18.65, 21.40, 23.14, 30.76, 81.91-88.88 (p-cymene), 103.97, 124.35 (benzene), 124.48, 128.90-133.49 (benzaldehyde), 135.28, 161.45 (C=N), 175.97 (C=S). Elemental analysis (%): Calc. for C42H48Cl4N6Ru2S2·1/2CH2Cl2: C, 46.94; H, 4.54; N, 7.73; S, 5.90. Found: C, 46.54; H, 4.69; N, 7.44; S, 5.78. ESI-MS: m/z 945 (85%, [M-4HCl-H+ 2Na]<sup>+</sup>). Complex 4: Yield = 0.179 g (89%). M.p. decomposes at 158 °C. FT-IR (KBr, cm<sup>-1</sup>): 1727 (C=O), 1600 (C=N), 845 (C=S). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ (ppm) = 1.81 (qn, 4H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>COOH); 2.30 (m, 8H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>COOH); 4.99 (d, 2H, CH (arene),  ${}^{3}_{J_{H+H}}$  = 5.73), 5.04 (d, 2H, CH (arene),  ${}^{3}_{J_{H+H}}$  = 6.14), 5.13 (t, 2H, CH (arene),  ${}^{3}_{J_{H+H}}$  = 6.66), 5.40 (t, 2H, CH (arene)), 5.40 (t, (arene), <sup>3</sup>*J*<sub>H-H</sub> = 5.87), 7.42 (s, 4H, benzene), 7.57 (m, 6H, CH (benzaldehyde)), 8.14 (d, 4H, CH (benzaldehyde),  ${}^{3}J_{H-H} = 5.48$ ), 8.90 (s, 2H, CH=N).  ${}^{13}C{}^{1}H$  NMR  $(100 \text{ MHz}, \text{CD}_3\text{OD}): \delta \text{ (ppm)} = 25.35, 33.19, 33.60, 82.66-100.94 \text{ (arene)}, 125.10$ (benzene), 129.68-135.22 (benzaldehyde), 137. 00, 164.86 (C=N), 170.61 (C=O), 174.77 (C=S). Elemental analysis (%): Calc. for C<sub>42</sub>H<sub>44</sub>Cl<sub>4</sub>N<sub>6</sub>O<sub>4</sub>Ru<sub>2</sub>S<sub>2</sub>: C, 45.65; H, 4.01; N, 7.61; S, 5.80. Found: C, 45.60; H, 4.49; N, 7.54; S, 5.67. ESI-MŠ: m/z 495 (100%, [M-2Cl-CO<sub>2</sub>]<sup>2+</sup>).
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and kindly provided by Professor Rob Veale (University of Witwatersrand, South Africa). IC<sub>50</sub> determinations were carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 3000 cells were seeded per well in 96-well plates. Cells were incubated at 37 °C under 5% CO<sub>2</sub> (24 hours), after which aqueous DMSO solutions of each compound (10  $\mu$ l, with a constant

final DMSO concentration of 0.2%) were plated at various concentrations. After a 48 hour incubation period, MTT (10  $\mu$ ) solution was added to each well. After further 4 hour incubation, solubilisation solution (100  $\mu$ l) was added to each well, and plates were incubated overnight. Plates were read at 595 nm using a BioTek microplate reader.