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Research paper

Reactivity and biological activity of *N,N,S*-Schiff-base rhodium pentamethylcyclopentadienyl complexes

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ABSTRACT

Neutral piano-stool complexes of the general formula $[(\eta^5\text{-}C_5Me_5)Rh(L\text{-}OR)]$ (R = Me, **1**; R = Et, **2**; R = Pr^i, **3**) have been prepared in alcohols (methanol, **1**; ethanol, **2**; isopropanol, **3**) from the Schiff-base 5-methyl-4-{(pyridin-2-ylmethylene)amino}-4H-1,2,4-triazole-3-thiol (L-H) and the dinuclear precursor $[(\eta^5\text{-}C_5Me_5)RhCl_2]_2$. Concomitant with the coordination of the Schiff-base ligand, an alkoxilation occurs on the imine carbon atom of the ligand, thus forming the corresponding L-OR compounds. In these complexes, the L-OR ligand is *N,N,S*-coordinated, introducing chirality at the metal center. The antiproliferative activity of the piano-stool complexes **1**–**3** was evaluated on cancerous (A2780 and A2780cisR) and non-cancerous (HEK293) cell lines, showing no significant activity *in vitro* (IC_{50} > 200 μM), except for the ethanolate derivative **2**, which shows an IC_{50} of 21 μM on the ovarian cancer cell line A2780.

1. Introduction

The biological potential of piano-stool complexes incorporating multidentate ligands is well-established. The *in vitro* and *in vivo* anticancer activity [1], as well as the antimicrobial activity [2] of piano-stool complexes with symmetrical and unsymmetrical chelating ligands have been demonstrated. The use of chelating ligands reduces significantly the probability of ligand exchange [3], and modulate the electronic effects at the metal [4], two factors that play a major role in the biological activity of piano-stool complexes.

Among chelating ligands, Schiff-base derivatives have been widely explored as these multidentate ligands are relatively easy to synthesize, and they can be functionalized to generate chelating ligands with tailored properties [5]. They are generally obtained under mild reaction conditions, in high yields, by the direct condensation of aldehydes with aliphatic or aromatic primary amines [6]. Despite showing high stability as isolated organic molecules, some aromatic Schiff-bases are prone to hydrolyze, to regenerate the starting materials [7]. However, hydrolysis can be avoided by electronic and or steric constraints. Most studies dealing with piano-stool complexes incorporating multidentate Schiff-bases show the ligand in a bidentate mode [8], and only a few examples show Schiff-base ligands in a tridentate mode [9].

Remarkably, some Schiff-base compounds exhibit interesting behavior when coordinated to metal centers, with the carbon atom of the azomethine group becoming susceptible to nucleophilic attack. Alcohols [10], water [11] and 2,4-pentadione [12] can react with the azomethine carbon atom to afford the corresponding alkoxylate, hydroxylate, and acetylacetonate derivatives. In these systems, the nitrogen atom of the azomethine group is always coordinated to the metal ion, and is involved in five- or six-membered metallacycles. Therefore, this particular behavior can be used to introduce additional functionality onto Schiff-based complexes, and accordingly to modulate the biological activity of piano-stool complexes.

Therefore, to design new organometallic compounds with multidentate ligands and to confirm the Schiff-base carbon atom activation upon coordination to metal ions, a series of rhodium-based piano-stool complexes of the general formula $[(\eta^5\text{-}C_5Me_5)Rh(L\text{-}OR)]$ (R = Me, **1**; R = Et, **2**; R = Pr^i, **3**) has been prepared in alcohols (methanol, ethanol or isopropanol) from the tridentate Schiff-base ligand, 5-methyl-4-{(pyridin-2-ylmethylene)amino}-4H-1,2,4-triazole-3-thiol (L-H), and the dinuclear precursor $[(\eta^5\text{-}C_5Me_5)RhCl_2]_2$. The antiproliferative activity of all complexes was evaluated on various cell lines (A2780, A2780cisR, HEK293), and the results were compared to those obtained for cisplatin, $[(\eta^6\text{-}C_{10}H_{14})Ru(1,3,5\text{-triaza-7-phosphaadamantane})Cl_2]$ (RAPTA-C) and the arene ruthenium analogues $[(\eta^6\text{-}C_{10}H_{14})Ru(L\text{-}OR)]$ (R = Me, **4**; R = Et, **5**; R = Pr^i, **6**) [13].

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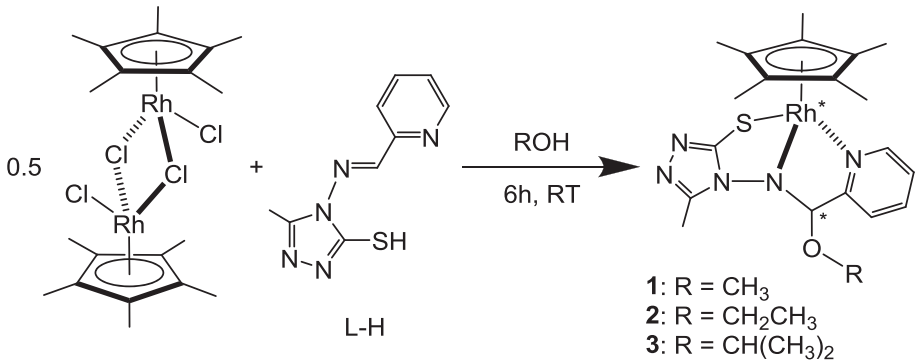


Scheme 1. Synthesis of the rhodium-based piano-stool complexes **1–3**, with the chiral centers indicated with the symbol * on the complexes.

2. Results and discussion

The dinuclear complex $[(\eta^5\text{-}C_5Me_5)RhCl_2]_2$ reacts at room temperature with 5-methyl-4-{(pyridin-2-ylmethylene)amino}-4H-1,2,4-triazole-3-thiol (L-H) to give a series of neutral complexes of the general formula $[(\eta^5\text{-}C_5Me_5)Rh(L\text{-}OR)]$ (R = Me, **1**; R = Et, **2**; R = Pr^i, **3**) (see Scheme 1). The reactions are conducted in alcohols (methanol, ethanol or isopropanol), and in parallel to the coordination of L-H to rhodium, an alkoxylation occurs on the imine carbon atom of L to generate, in accordance with the alcohol used, the L-OR ligands. The coordination of L-OR in a tridentated fashion and the insertion of the alkoxy group generate two chiral centers in these mononuclear complexes, one at the metal and the other on the imine carbon atom.

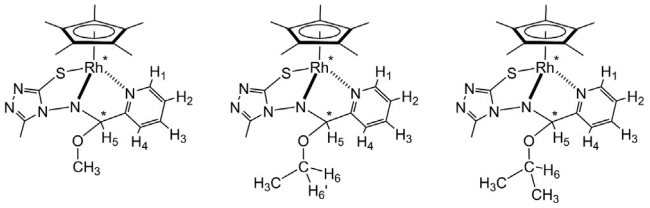
The addition of alkoxy groups on the Schiff-base ligand was confirmed by mass spectrometry. The electrospray ionization mass spectra (methanol, positive mode) of complexes **1–3** show $[M + H]^+$ peaks corresponding to mononuclear rhodium-based complexes (see Experimental section). The presence of rhodium in these compounds was evidenced from the isotopic pattern of the cationic peaks, which correlates exactly with the calculated theoretical isotopic distributions of rhodium species.

The exact position of the alkoxy group on the L-OR ligand was established by ^1H and ^{13}C NMR spectroscopy. Indeed, the azomethine proton, initially observed at 8.7 ppm in the pre-ligand (L-H) [13,14], is upfield-shifted and appears in the complexes around 5–6 ppm (see Table 1). A similar movement in chemical shift is observed for the corresponding carbon atom, which shifts from 164 ppm in L-H to ca. 106 ppm in the L-OR rhodium-based complexes. The insertion of an alkoxy group on the imine carbon atom generates an asymmetric carbon atom on L-OR (see Scheme 1). Therefore, in complexes **2** and **3**, the O-CH_2 moiety of the alkoxy group becomes diastereotopic. In complex **2**, two multiplets integrating for one proton each are observed at 4.2 and 3.9 ppm, respectively. The poor resolution of these signals, as well as others, confirms the existence of diastereoisomers in solution, which are due to the presence of two chiral centers, one at the metal, and one on the ligand. Integration of the signals and 2D NMR experiments confirm the presence of two species in solution with, in both cases, in a 2:3 molar ratio.

The cytotoxicity of complexes **1–3** was evaluated in human ovarian cancer cells, A2780 and A2780cisR (the latter having acquired resistance to cisplatin), as well as non-tumorigenic human embryonic kidney (HEK293) cells as a gauge of cancer cell selectivity. The cytotoxicity of complexes **1–3** were also compared to arene ruthenium analogues bearing the same L-OH ligands, i.e. $[(\eta^6\text{-}C_{10}H_{14})Ru(L\text{-}OR)]$ (R = Me, **4**; R = Et, **5**; R = Pr^i, **6**) [13], and benchmarked against cisplatin [15] and RAPTA-C [16] (see Table 2). Cisplatin is a widely used, cytotoxic alkylating agent and, as can be seen from Table 2, is cytotoxic to the A2780 cancer cells (IC_{50} = 2.3 ± 0.6 μM), but displays limited cancer cell selectivity when compared to the IC_{50} value of 8.4 ± 0.9 μM in the HEK293 cell line. In comparison, complex **2** is ca. 10 fold less cytotoxic to the A2780 cancer cells exhibitng an IC_{50} value of 21 ± 2 μM, but advantageously shows no discernable cytotoxicity towards the non-tumorigenic HEK293 cells at the maximum dose tested, i.e. 200 μM.

Remarkably, complex **2** is the only complex displaying any appreciable cytotoxicity below 200 μM. It should be noted, however, that

Table 1
^1H NMR data of complexes **1–3** (including multiplicities and integrations).



	1	2	3
H_1	8.55 (d, 1H)	8.54 (d, 0.6H), 8.52 (d, 0.4H)	8.53 (m, 1H)
H_2	7.91 (dd, 1H)	7.89 (dd, 1H)	7.89 (dd, 1H)
H_3	7.48 (m, 1H)	7.48 (m, 1H)	7.46 (m, 1H)
H_4	7.53 (d, 1H)	7.52 (d, 1H)	7.52 (d, 1H)
H_5	5.26 (s, 1H)	5.31 (s, 0.6H), 5.26 (s, 0.4H)	5.34 (s, 0.4H), 5.26 (s, 0.6H)
H_6	–	4.18 (m, 1H), 3.91 (m, 1H)	4.43 (m, 0.6H), 3.50 (m, 0.4H)
CH_3 (alkoxyl)	3.72 (s, 3H)	1.30 (dd, 3H)	1.30 (m, 6H)
CH_3 (triazole)	2.44 (s, 3H)	2.44 (s, 1.2H), 2.43 (s, 1.8H)	2.44 (s, 1.8H), 2.42 (s, 1.2H)
CH_3 (Cp*)	1.70 (s, 15H)	1.71 (s, 9H), 1.70 (s, 6H)	1.72 (s, 6H), 1.70 (s, 9H)

Table 2
IC_{50} values of complexes **1–6**, RAPTA-C and cisplatin in cancerous (A2780, A2780cisR) and non-cancerous cell lines (HEK293).

compound	A2780 (μM)	A2780cisR (μM)	HEK293 (μM)
cisplatin	2.3 ± 0.6	31 ± 3	8.4 ± 0.9
RAPTA-C	> 200	> 200	> 200
1	> 200	> 200	> 200
2	21 ± 2	> 200	> 200
3	> 200	> 200	> 200
4	> 200	> 200	> 200
5	> 200	> 200	> 200
6	> 200	> 200	> 200

ruthenium complexes which are weakly cytotoxic to cancer cells have progressed to clinical trials [17]. The half-sandwich complex RAPTA-C is also not cytotoxic to the cell lines studied, and unlike cisplatin, operates via an epigenetic mechanism of action [18]. Nonetheless, RAPTA-C show high efficacy *in vivo* against primary and metastatic tumors when administered at high doses [19], and when used in combination with other agents [20], it is effective at low doses, even against chemo-resistant cancers [21]. Consequently, although complexes **1–6** are not cytotoxic, with the exception of **2** in A2780 cells, they should not be excluded from further biological screening for putative anticancer activity.

3. Conclusions

Reactions between $[(\eta^5\text{-}C_5Me_5)RhCl_2]_2$ and the Schiff-base 5-methyl-4-{(pyridin-2-ylmethylene)amino}-4H-1,2,4-triazole-3-thiol in different primary and secondary alcohols lead to alkoxylation of the imine carbon atom of the ligand. Coordination of the Schiff-base in a *S,N,N*-tridentate fashion coupled to the nucleophilic attack on the imine carbon atom generate two chiral centers in the complexes. The complexes are not endowed with antiproliferative activity, except for the ethanolate derivative **2**, who possesses an IC_{50} of 21 μM on the ovarian cancer cell line A2780 and a remarkable degree of cancer cell selectivity.

4. Experimental section

4.1. Materials and methods

All chemicals were purchased from commercial sources and used as received unless specified otherwise. The starting materials $[(\eta^5\text{-}C_5Me_5)RhCl_2]_2$ [22] and 5-methyl-4-{(pyridin-2-ylmethylene)amino}-4H-1,2,4-triazole-3-thiol (L-H) [14] were prepared according to published methods. The ^{1}H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker Avance II 400 spectrometer using the residual protonated solvent as internal standard. Infrared spectra were recorded on a Thermo Scientific iS5 ATR/FTIR spectrometer. Electrospray ionization mass spectra were obtained in positive ion mode on a Bruker FTMS 4.7 T BioAPEX II mass spectrometer, University of Fribourg (Switzerland). UV–visible absorption spectra were recorded in methanol on a Perkin Elmer UV/Vis spectrophotometer at 10^{-5} M concentrations. The elemental analyzes were carried out by the Mikroelementaranalytisches Laboratorium, ETH Zürich (Switzerland).

4.2. General synthesis of the complexes $[(\eta^5\text{-}C_5Me_5)Rh(L\text{-}OR)$ (1–3).

The metal precursor $[(\eta^5\text{-}C_5Me_5)RhCl_2]_2$ (50.0 mg; 0.08 mmol) and 2 equivalents of LH (35.08 mg; 0.16 mmol) were dissolved in dry alcohol (methanol for **1**, ethanol for **2** and isopropanol for **3**) (15 mL), before being stirred at room temperature for 6 h. After removal of the solvent on a rotary evaporator, the orange precipitate was purified by column chromatography on silica gel, using a mixture of methanol/ethyl acetate/hexane as eluent (**1**:4/2/3; **2**:2/3/4; **3**:2/2/3). Then the isolated solid was dried under vacuum.

1: Yield: 25 mg, 0.051 mmol (64.8%). ^{1}H NMR (400 MHz, MeOD) δ (ppm): 8.55 (d, 1H, J = 5.2 Hz, C**H**$_{pyr}$), 7.91 (dd, 1H, J = 7.7 Hz, C**H**$_{pyr}$), 7.53 (d, 1H, J = 8.2 Hz C**H**$_{pyr}$), 7.48 (m, 1H, C**H**$_{pyr}$), 5.26 (s, 1H, NC**H**), 3.72 (s, 3H, OC**H**$_3$), 2.44 (s, 3H, C**H**$_3$), 1.70 (s, 15H, $C_5(CH_3)_5$). $^{13}C\{^1H\}$ NMR (101 MHz, MeOD) δ (ppm): 166.9 (S**C** = N), 165.7 (C**C** = N), 152.8 (**C**H$_{pyr}$), 149.1 (OCH**C**), 140.1 (**C**H$_{pyr}$), 127.1 (**C**H$_{pyr}$), 124.9 (**C**H$_{pyr}$), 107.4 (N**C**H), 97.52 (d, $J_{Rh\text{-}C}$ = 71 Hz, **C**$_5$(CH$_3$)$_5$), 28.0 (O**C**H$_3$), 11.1 (**C**H$_3$), 9.0 (C_5(**C**H$_3$)$_5$). IR: υ (cm^{-1}): 2913.1 (w, CH), 1417.4 (s, C═C), 1085.3 (m, C—O). UV–visible: (1.0×10^{-5} M, MeOH, 298 K): λ_{max} 322 nm (ε = 48070 M^{-1} · cm^{-1}), 454 nm (ε = 11330 M^{-1} · cm^{-1}), 756 nm (ε = 4990 M^{-1} · cm^{-1}). ESI-MS (MeOH): m/z = 488.1 [M + H]$^+$. Anal. (%): Calcd for $C_{20}H_{26}N_5ORhS{\cdot}H_2O$: C, 47.53; H, 5.58; N, 13.86: Found: C, 48.03; H, 5.38; N, 13.76.

2: Yield: 30 mg, 0.059 mmol (74.8%). ^{1}H NMR (400 MHz, MeOD) δ (ppm): Diastereoisomer A (60%): 8.54 (d, 1H, J = 4.2 Hz, C**H**$_{pyr}$), 7.89 (dd, 1H, J = 7.6 Hz, C**H**$_{pyr}$), 7.52 (d, 1H, J = 8.3 Hz, C**H**$_{pyr}$), 7.48 (m, 1H, C**H**$_{pyr}$), 5.31 (s, 1H, NC**H**), 4.18 (m, 1H, OC**H**$_2$CH$_3$), 3.91 (m, 1H, OC**H**$_2$CH$_3$), 2.43 (s, 3H, C**H**$_3$), 1.71 (s, 15H, C_5(C**H**$_3$)$_5$), 1.30 (dd, 3H, J = 7.0 Hz, OCH$_2$C**H**$_3$). Diastereoisomer B (40%): 8.52 (d, 1H, J = 4.2 Hz, C**H**$_{pyr}$), 7.89 (dd, 1H, J = 7.6 Hz, C**H**$_{pyr}$), 7.52 (d, 1H, J = 8.3 Hz, C**H**$_{pyr}$), 7.48 (m, 1H, C**H**$_{pyr}$), 5.26 (s, 1H, NC**H**), 4.18 (m, 1H, OC**H**$_2$CH$_3$), 3.91 (m, 1H, OC**H**$_2$CH$_3$), 2.44 (s, 3H, C**H**$_3$), 1.70 (s, 15H, C_5(C**H**$_3$)$_5$), 1.30 (dd, 3H, J = 7.0 Hz, OCH$_2$C**H**$_3$). $^{13}C\{^1H\}$ NMR (101 MHz, MeOD) δ (ppm). Diastereoisomer A (60%): 167.1 (S**C** = N), 165.6 (C**C** = N), 152.5 (**C**H$_{pyr}$), 149.0 (OCH**C**), 139.9 (**C**H$_{pyr}$), 126.9 (**C**H$_{pyr}$), 124.7 (**C**H$_{pyr}$), 106.5 (N**C**H), 97.33 (d, $J_{Rh\text{-}C}$ = 68 Hz, **C**$_5$(CH$_3$)$_5$), 64.6 (O**C**H$_2$CH$_3$), 15.6 (OCH$_2$**C**H$_3$), 10.9 (**C**H$_3$), 8.9 (C_5(**C**H$_3$)$_5$). $^{13}C\{^1H\}$ NMR (101 MHz, MeOD) δ (ppm). Diastereoisomer B (40%): 166.7 (S**C** = N), 165.5 (C**C** = N), 152.7 (**C**H$_{pyr}$), 149.0 (OCH**C**), 139.9 (**C**H$_{pyr}$), 127.0 (**C**H$_{pyr}$), 124.8 (**C**H$_{pyr}$), 107.3 (N**C**H), 97.40 (d, $J_{Rh\text{-}C}$ = 66 Hz, **C**$_5$(CH$_3$)$_5$), 64.6 (O**C**H$_2$CH$_3$), 15.6 (OCH$_2$**C**H$_3$), 10.9 (**C**H$_3$), 8.9 (C_5(**C**H$_3$)$_5$). IR: υ (cm^{-1}): 2982.5 (w, CH), 1415.6 (s, C═C), 1088.3 (s, C—O). UV–visible: (1.0×10^{-5} M, MeOH, 298 K): λ_{max} 434 nm (ε = 10160 M^{-1} · cm^{-1}), 644 nm (ε = 5200 M^{-1} · cm^{-1}). ESI-MS (MeOH): m/z = 502.1 [M + H]$^+$. Anal. (%): Calcd for $C_{21}H_{28}N_5ORhS{\cdot}H_2O$: C, 48.56; H, 5.82; N, 13.48: Found: C, 49.17; H, 5.57; N, 13.77.

3: Yield: 25 mg, 0.048 mmol (60.7%). ^{1}H NMR (400 MHz, MeOD) δ (ppm): Diastereoisomer A (40%): 8.53 (m, 1H, C**H**$_{pyr}$), 7.89 (dd, 1H, J = 8.6 & 7.3 Hz, C**H**$_{pyr}$), 7.52 (m, 1H, C**H**$_{pyr}$), 7.46 (m, 1H, C**H**$_{pyr}$), 5.34 (s, 1H, NC**H**), 3.50 (m, 1H, C**H**(CH$_3$)$_2$), 2.42 (s, 3H, C**H**$_3$), 1.72 (s, 15H, C_5(C**H**$_3$)$_5$), 1.30 (m, 6H, CH(C**H**$_3$)$_2$). Diastereoisomer B (60%): 8.53 (m, 1H, C**H**$_{pyr}$), 7.89 (dd, 1H, J = 8.6 & 7.3 Hz, C**H**$_{pyr}$), 7.52 (m, 1H, C**H**$_{pyr}$), 7.46 (m, 1H, C**H**$_{pyr}$), 5.26 (s, 1H, NC**H**), 4.43 (m, 1H, C**H**(CH$_3$)$_2$), 2.44 (s, 3H, C**H**$_3$), 1.70 (s, 15H, C_5(C**H**$_3$)$_5$), 1.30 (m, 6H, CH(C**H**$_3$)$_2$). $^{13}C\{^1H\}$ NMR (101 MHz, MeOD) δ (ppm): Diastereoisomer A (40%): 168.1 (S**C** = N), 165.7 (C**C** = N), 152.8 (**C**H$_{pyr}$), 149.3 (OCH**C**), 140.2 (**C**H$_{pyr}$), 127.0 (**C**H$_{pyr}$), 124.7 (**C**H$_{pyr}$), 104.4 (N**C**H), 97.65 (d, $J_{Rh\text{-}C}$ = 71 Hz, **C**$_5$(CH$_3$)$_5$), 65.6 (**C**H(CH$_3$)$_2$), 24.0 (CH(**C**H$_3$)$_2$), 24.0 (CH(**C**H$_3$)$_2$), 11.1 (**C**H$_3$), 9.4 (C_5(**C**H$_3$)$_5$). Diastereoisomer B (60%): 167.1 (S**C** = N), 166.0 (C**C** = N), 152.9 (**C**H$_{pyr}$), 149.3 (OCH**C**), 140.3 (**C**H$_{pyr}$), 127.3 (**C**H$_{pyr}$), 125.2 (**C**H$_{pyr}$), 107.5 (N**C**H), 97.72 (d, $J_{Rh\text{-}C}$ = 70 Hz, **C**$_5$(CH$_3$)$_5$), 70.6 (**C**H(CH$_3$)$_2$), 24.0 (CH(**C**H$_3$)$_2$), 24.0 (CH(**C**H$_3$)$_2$), 11.3 (**C**H$_3$), 9.3 (C_5(**C**H$_3$)$_5$). IR: υ (cm^1): 2919.2 (w, CH), 1416.0 (s, C═C), 1026.3 (s, C—O). UV–visible: (1.0×10^{-5} M, MeOH, 298 K): λ_{max} 327 nm (ε = 40830 M^{-1} · cm^{-1}), 453 nm (ε = 9510 M^{-1} · cm^{-1}), 745 nm (ε = 5900 M^{-1} · cm^{-1}). ESI-MS (MeOH): m/z = 516.2 [M + H]$^+$. Anal. (%): Calcd for $C_{22}H_{30}N_5ORhS{\cdot}AcOET$: C, 51.74; H, 6.35, N, 11.60: Found: C, 52.42; H, 6.30; N, 12.38.

4.3. Cell culture and cytotoxicity studies

Human ovarian carcinoma (A2780 and A2780cisR) cell lines were obtained from the European Collection of Cell Cultures. The human

embryonic kidney (HEK-293) cell line was obtained from ATCC (Sigma, Buchs, Switzerland). Penicillin streptomycin, RPMI 1640 GlutaMAX (where RPMI = Roswell Park Memorial Institute), and DMEM GlutaMAX media (where DMEM = Dulbecco's modified Eagle medium) were obtained from Life Technologies, and fetal bovine serum (FBS) was obtained from Sigma. The cells were cultured in RPMI 1640 GlutaMAX (A2780 and A2780cisR) and DMEM GlutaMAX (HEK-293) media containing 10% heat-inactivated FBS and 1% penicillin streptomycin at 37 °C and CO_2 (5%). The A2780cisR cell line was routinely treated with cisplatin (2 μM) in the media to maintain cisplatin resistance. The cytotoxicity was determined using the 3-(4,5-dimethyl 2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay [23]. Cells were seeded in flat-bottomed 96-well plates as a suspension in a prepared medium (100 μL aliquots and approximately 4300 cells/ well) and pre-incubated for 24 h. Stock solutions of compounds were prepared in MilliQ water. The solutions were sequentially diluted to give a final compound concentration range (0–200 μM). Cisplatin and RAPTA-C were tested as a positive (0–100 μM) and negative (200 μM) controls respectively. The compounds were added to the pre-incubated 96-well plates in 100 μL aliquots, and the plates were incubated for a further 72 h. MTT (20 μL, 5 mg/mL in Dulbecco's phosphate buffered saline) was added to the cells, and the plates were incubated for a further 4 h. The culture medium was aspirated, and the purple formazan crystals, formed by the mitochondrial dehydrogenase activity of vital cells, were dissolved in DMSO (100 μL/well). The absorbance of the resulting solutions, directly proportional to the number of surviving cells, was quantified at 590 nm using a SpectroMax M5e multimode microplate reader (using SoftMax Pro software, version 6.2.2). The percentage of surviving cells was calculated from the absorbance of wells corresponding to the untreated control cells. The reported IC_{50} values are based on the means from two independent experiments, each comprising four tests per concentration level.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ica.2019.119265.

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