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Anti-tumour activity in non-small cell lung cancer models and toxicity profiles for novel ruthenium(II) based organo-metallic compounds

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ARTICLE INFO

Article history:
Received 29 July 2005
Accepted 18 October 2005

Keywords:
Ruthenium(II) organo-metallic complex
Lung cancer
Hepatotoxicity

ABSTRACT

Novel ruthenium(II) organo-metallic compounds are active in ovarian cancer models [Aird RE, Cummings J, Ritchie AA, Muir M, Morris RE, Chen H, et al. In vitro and in vivo activity and cross resistance profiles of novel ruthenium(II) organometallic arene complexes in human ovarian cancer. Br J Cancer 2002;86(10):1652–7]. $[(\eta^6\text{-}C_6H_5C_6H_5)Ru(en)Cl]^+$ (as a PF_6 salt, where en = ethylenediamine (RM175)) has been evaluated in a 13-cell line panel. Particular sensitivity (~10-fold lower than mean IC_{50}) was noted in breast cancer and non-small cell lung cancer cell lines. In addition, IC_{50} in the A549 was 2 μM and RM175 (25 mg kg^{-1}, days 1 and 5, i.p.) caused a significant ($p = 0.004$) growth delay in a xenograft model. HC11 [$(\eta^6$-tetrahydroanthracene)Ru(en)Cl]PF_6 was more potent in the A549 cell line (IC_{50} 0.5 μM). HC11 (25 mg kg^{-1}, days 1, 8 and 15, i.p.) was also active in vivo. Following RM175 25 mg kg^{-1}, days 1 and 5, and 15 mg kg^{-1}, days 1–5, HC11 25 and 40 mg kg^{-1}, day 1, elevated alanine transaminase levels were detected, suggesting hepatotoxicity. No changes were observed in kidney or haematological parameters. In liver sections, multi-focal hepatic necrosis was seen, becoming confluent at high doses of HC11. In vitro studies confirmed that HC11 was more toxic than RM175 to fresh human hepatocytes and equitoxic to mithramycin. Liver toxicity may be related to the arene ligand and modification may reduce the potential for hepatic toxicity, while maintaining the anti-tumour activity seen.

1. Introduction

The metal ruthenium (Ru) possesses several favourable chemical properties that indicate it may be a strong candidate to form a basis for rational anticancer drug design [2,3]. Ru^{II} complexes demonstrate similar ligand exchange kinetics to those of platinum (Pt^{II} and Pt^{IV}) while displaying only low toxicity. Ruthenium(III) has the ability to mimic iron in binding to plasma proteins including transferrin and albumin [3]. Transport and sequestration of Ru into tumour cells may be mediated via protein transport and receptor mediated uptake [4,5]. Due to differing ligand geometry between their

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doi:10.1016/j.bcp.2005.10.053

complexes, some Ru^{III} compounds (octahedral) bind to DNA forming predominately inter-strand crosslinks as opposed to the intra-strand crosslinks favoured by square-planar Pt^{II} in cisplatin [6,7]. In addition, non-nuclear targets, such as the mitochondrion and the cell surface, have also been implicated in the antineoplastic activity of Ru complexes, particularly in the case of the clinically investigated Ru^{III} antimetastatic drug *trans*-[$RuCl_4$(DMSO)(Im)](ImH) (NAMI-A) [8]. NAMI-A has completed clinical phase I evaluation, and cutaneous toxicity was dose-limiting. Nausea and vomiting was also noted. Mild reversible nephrotoxicity was noted and a pre- and post-hydration regimen was utilised. Neurotoxicity and hepatic toxicities were not identified. Stable disease was identified in a patient with non-small cell lung cancer.

Previous investigators have focused on Ru^{III} complexes as potential anti-tumour agents [9–11]. These are thought to be activated by reduction to Ru^{II} in the body (Clarke et al. [2]). In contrast, we have developed a series of novel organo-metallic Ru^{II} arene complexes [12] including [(η^6-$C_6H_5C_6H_5$)Ru(en)Cl]$^+$ (RM175) and [(η^6-tetrahydroanthracene)Ru(en)Cl]$^+$ (HC11). The presence of the arene ligand stabilises the Ru^{II} oxidation state [13]. These Ru^{II} complexes have been evaluated for activity in both in vitro and in vivo models of human ovarian cancer, and cross-resistance profiles established in cisplatin and multi-drug resistant (MDR) variants [1]. Issues relevant to the cytotoxicity of these compounds are their selectivity for guanine on DNA, leading to the formation of an N7–Ru bond together with the formation of a strong hydrogen bond between the C6 carbonyl group of guanine and an NH proton of the ethylenediamine ligand.

In this paper we report results of studies to assess the anti-tumour activity in a range of tumour types using an automated screen approach and subsequent studies focusing on non-small cell lung cancer models. We also report preliminary toxicity data, to illustrate the likely therapeutic index of these compounds.

2. Materials and methods

RM175 and HC11 (Fig. 1) were synthesised and characterised using the methods reported previously [12,14]. A549 and H520 cell lines were obtained from Cancer Research UK Cell Services. Fresh human hepatocytes were obtained from the UK Human Tissue Bank after ethical approval from NHS Lothian.

2.1. *In vitro cytotoxicity using propidium iodide fluorescence assay*

Human tumour cells (see Table 1) were plated in 96-well flat-bottomed microtitre plates (50 μL cell suspension, 1×10^5 or 5×10^4 cells/mL) and additional 50 μL of culture medium was added. After a 24 h recovery, 50 μL of culture medium containing 50 μg/mL gentamycin was added into the six control wells or medium containing the test drug was added to the wells. Each drug concentration was plated in triplicate. Following 3–6 days of incubation, depending on cell doubling time, a modified propidium iodide (PI) assay [15] was performed. Culture medium was replaced by fresh medium and 50 μL of an aqueous propidium iodide solution (25 μg/mL) was added to each well. PI does not cross intact cell membranes and enters only the nucleus of dead cells by intercalation into DNA and RNA. The fluorescence signal correlates with the number of dead cells. Fluorescence (FU_1) was measured using a Millipore Cytofluor 2350 microplate reader (excitation 530 nm, emission 620 nm). Microplates were then kept at −18 °C for 24 h, yielding in a total cell kill. After thawing of the plates and a second fluorescence measurement (FU_2) the amount of viable cells was calculated by subtraction of FU_2 from FU_1.

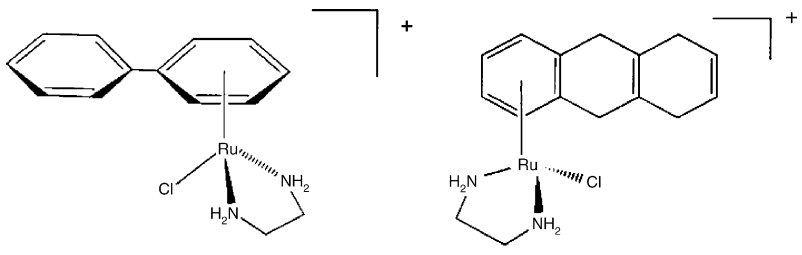
Growth inhibition was expressed as treated/control × 100 (%T/C); inhibiting concentrations (IC) were determined by plotting compound concentration versus cell viability. Mean IC_{50}, IC_{70} and IC_{90} values were calculated for each individual cell line. From all IC_{70} values the mean IC_{70} was calculated according to the formula:

$$\text{Mean } IC_{50,70} = 10^{\frac{\left(\sum_1^n \log(IC_{50,70})_x\right)}{n}}$$

where x is the value of specific tumour cell line and n is the total number of tumour cell lines studied. If an IC_{50} or IC_{70} could not be determined within the examined dose range, the lowest or highest concentration studied was used for the calculation.

2.2. *In vitro cytotoxicity in A549 and H520 using sulforhodamine B assay*

The cytotoxic activity of the test compounds was assessed using a cell death assay based on detection of cells by sulforhodamine B (SRB), a stain specific for proteins, used for this purpose by the United States National Cancer Institute [16]. Briefly, A549 and H520 were plated out at densities,



RM175 HC11

Fig. 1 – Chemical structures of HC11 and RM175.

Table 1 – In vitro growth inhibition in lung cancer cell lines

	A549		H520	
	IC50 (μM)	Range	IC50 (μM)	Range
RM175	3.0	2.9–3.3	3.5	3–3.8
HC11	0.50	0.49–0.51	0.53	0.53–0.54
CDDP	2.6	2.9–4.2	9.5	9.3–9.8

determined by growth curve analysis (data not shown), that gave the best range of log phase growth after 2–3 days culture in 96-well plates. After this time the media were removed from each well by aspiration and fresh media containing drug at known concentrations were added to each well. Cells were incubated a further 24 h and the media removed by aspiration. Wells were washed in warmed PBS (200 μL) and then layered with fresh culture media. Cells were then incubated 72 h, at 37 °C/5% CO_2. To each well was then added trichloroacetic acid (TCA), 10% final concentration and the plates were incubated 1 h at 4 °C in order to fix the cells. Wells were washed gently under running water and allowed to air dry. SRB, 50 μL, was then added to each well and the plates incubated 30 min at room temperature. The excess SRB was then washed off with four washes of 1% acetic acid and the plates allowed to completely air dry. To each well was then added Tris–HCl, pH 10.5, 150 μL/well and the plates were incubated 1 h at room temperature and then very gently mixed by shaking. Absorbance was read at 540 nm on a 96-well microplate reader.

2.3. *In vitro cytotoxicity in fresh human hepatocytes using the MTT assay*

Fresh human hepatocytes (20,000 viable cells) were plated in collagen-I pre-coated 96-well plates (BD Biosciences, Oxford, UK), in William's E medium (WEM) (Cambrex, Wokingham, UK) supplemented with 10% FCS, L-glutamine, penicillin (100 U/mL), streptomycin (100 μg/mL), 10 nM insulin and 30 nM dexamethasone and allowed to adhere for 12 h at 37 °C/5% CO_2 in a humidified atmosphere. Stock solutions of HC11 and RM175 were prepared with 5% (v/v) dimethylsulfoxide (DMSO) so that the final concentration of DMSO the hepatocytes were exposed to was never greater then 0.5%. A stock solution of mithramycin (Sigma) was prepared using sterile water. Cells were treated with 50 μL of RM175 (1–50 μM), HC11 (0.07–13.5 μM) or mithramycin (0.1–10 μM) for 24 h. At the end of drug exposure, the media were aspirated and the cells washed with 50 μL PBS before adding 200 μL/well of fresh complete WEM. Cytotoxicity studies were performed using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay 24 h after the end of drug treatment. Cells were incubated in 200 μL fresh 37 °C WEM containing 0.4 mg/mL MTT (Sigma, Gillingham, UK) for 3 h in the dark at 37 °C. Medium was then removed, and formazan crystals were solubilised in 200 μL DMSO. The optical density was measured at 570 nm using a Biohit BP800 microplate reader. A comparison between the MTT and SRB assays was also undertaken (data not shown) to ensure relevant and valid comparison of IC_{50} values obtained using both assays.

2.4. *In vivo toxicity of RM175 and HC11*

These studies were performed in C57/Bl 6 mice 6-weeks old. Treatment schedules for RM175 administration were: RM175 schedule 1—25 mg kg^{-1} administered i.p. days 1 and 5 (50 mg kg^{-1} total); RM175 schedule 2—15 mg kg^{-1} administered i.p. days 1–4 (60 mg kg^{-1} total). For HC11, the following schedules were used: HC11 schedule 1—25 mg kg^{-1} administered i.p. on day 1; HC11 schedule 2—40 mg kg^{-1} administered i.p. on day 1. The following parameters were measured in plasma: haematology (RBC, WBC and platelets) and biochemistry (ALT, ALP, total bilirubin, creatinine and urea) and the liver, spleen, lungs, kidneys and peritoneum were collected for subsequent evaluation. Three animals were to be culled at each of the following time-points: RM175 schedule 1—days 3, 5, 8 and 10; RM175 schedule 2—days 2–5; HC11 schedules 1 and 2—days 2–5.

2.5. *Pathology studies*

Liver and spleen from experimental and control animals were removed immediately after sacrifice and fixed by immersion in 10% buffered formalin. Liver lobes were embedded in paraffin wax and sectioned at 5 μm for histopathological assessment. All sections were stained with haematoxylin and eosin (H&E) using standard techniques. Sections were scanned and assessed for significant changes on two occasions. Selected liver sections were examined for fatty change using oil-red-O stain.

2.6. *In vivo efficacy of RM175 in LXFL xenografts*

RM175 was tested for in vivo activity in the large cell lung cancer model LXFL 529 growing subcutanously in serial passage in NMRI-nude mice. LXFL 529 was derived from a patient with untreated large cell lung cancer. Master stocks were frozen down at passage 7 in between liquid nitrogen and these experiments were performed in passage 24. The characteristics of the models have been described previously [17].

Nu/nu athymic mice of NMRI background from an in-house breeding facility were used for all experiments. Tumours were implanted s.c. in both flanks of 6-week-old nude mice. Treatment commenced when tumours reached diameters of 5–6 mm. Animals were randomly assigned into treatment groups. Food and water were provided ad libitum. Tumour growth was followed by serial two-dimensional caliper measurements and body weight documented concomitantly twice a week. Tumour volumes were calculated according to the formula (length × $width^2$)/2 and mean as well as median relative tumour volume (vol. day x/day 0 × 100) were used for analyses. Data were presented as mean relative tumour volumes ± S.E., optimal T/C values of the test divided by the control relative tumour volumes were calculated.

2.7. *In vivo efficacy of RM175 and HC11 in A549 xenografts*

Xenografts were established from the A549 cell lines by subcutaneous implantation of 10^7 cells in serum free media in the flank of animals. All animal experiments were carried out

according to UKCCCR guidelines [18]. Female nu/nu mice were implanted on both flanks with 2–3 mm^3 fragments of viable tumour and randomised when tumour volumes reached 30–100 mm^3. Animals were randomised into control and drug treated groups (5 animals/group). Ru^{II} complexes were administered as 10% DMSO solutions in sterile saline at a volume of 0.1 mL/10 g of body weight i.p. HC11 was administered at a dose of 25 mg kg^{-1} on days 1, 8 and 15. RM175 was administered at a dose of 25 mg kg^{-1} on days 1 and 5. Tumour xenografts were measured three times a week in perpendicular diameters and tumour volume calculated as (length × $width^2$)/2.

3. Results

3.1. *In vitro testing of RM175 in the 14 cell line panel*

RM175, $[(\eta^6\text{-}C_6H_5C_6H_5)Ru(en)Cl]^+$ (as a PF_6 salt), was shown to have a mean IC_{70} of 3.9 μg/mL (~8 μM) and the IC_{50} value was 3 μM, confirming the level of activity we have reported previously (Aird et al. [1]) and demonstrating it has a broad spectrum of activity (Fig. 2). Particular sensitivity (~10-fold lower than mean IC_{50}) was noted in a breast cancer cell line (401NL) and a non-small cell lung cancer (NSCLC) cell line (LXFL 529L). Comparative analyses were performed to ascertain whether the pattern of sensitivity correlated with other known agents. The strongest correlation was with mitoxantrone (0.61), but this was not thought to be significant, considering the number of cell lines tested (13).

The cytotoxicity of both compounds was also determined in two other non-small cell lung cancer cell lines, H520 and A549 (Table 1). HC11 $[(\eta^6\text{-tetrahydroanthracene})Ru(en)Cl]PF_6$ was highly cytotoxic in both cell lines with IC_{50}'s of 0.53 and 0.5 μM, respectively. RM175 was less active than HC11 with IC_{50}'s of 3 and 3.5 μM in A549 and H520, respectively, but similar to cisplatin (IC_{50} = 2.6 and 9.5 μM for A549 and H520, respectively).

3.2. *In vivo toxicity of RM175 and HC11*

In order to evaluate the activity of both compounds, the determination of a non-toxic dose was necessary. For RM175, the previously determined active dose of 25 mg kg^{-1} on days 1 and 5 and a more dose intense schedule of 15 mg kg^{-1} day^{-1} × 5 were evaluated. Two dose levels, 25 and 40 mg kg^{-1}, 1, were tested for HC11, administered on day 1 only.

Weight loss of 10% was observed after administration of 15 mg kg^{-1} day^{-1} of RM175 on day 5 but this did not have a significant impact on the animals. In contrast, HC11 administered at a dose of 40 mg kg^{-1} induced significant weight loss with all animals losing 10% body weight by day 3 (range 5–16%). Animals were unwell and were culled earlier than the proposed days 5 and 8 time-points.

The evaluation of blood biochemistry parameters showed a rise in alanine transaminase (ALT), noted following the administration of HC11, in particular (Fig. 3). Following a single dose of 25 mg kg^{-1}, i.p., the elevation of ALT was maximal at 48 h (mean 418 U L^{-1} in the treated group, mean 97 U L^{-1} in the control groups) and had resolved by day 8. Following

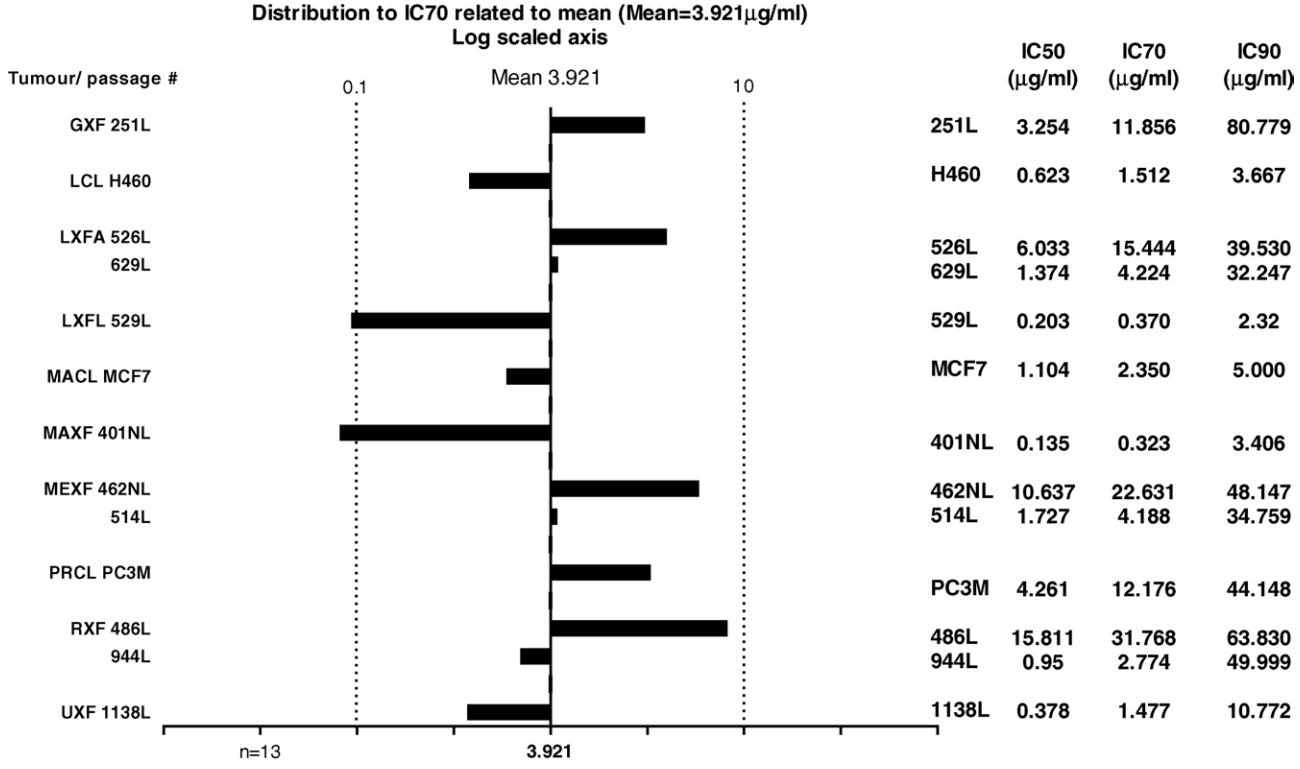


Fig. 2 – In vitro data from the 13 cell lines in the Freiburg screen. IC_{70} data (μg/mL) are expressed relative to the mean IC_{70} (3.921 μg/mL).

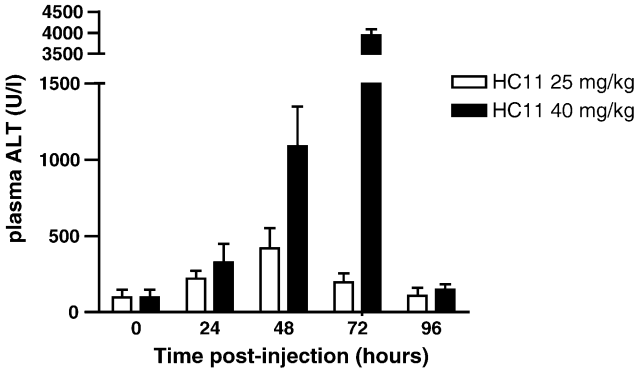


Fig. 3 – Changes in alanine transaminase (ALT) plasma levels in animals following administration of HC11 at the dose of 25 mg kg^{-1} (white bars) or 40 mg kg^{-1} (black bars) as a single injection i.p. on day 1. Results are mean ± S.D. of three animals at each time-point.

40 mg kg^{-1}, there was a marked elevation of ALT by 72 h (mean 3907 U L^{-1}) compared to untreated animals (mean 97 U L^{-1}). These changes were associated with histopathological changes (Fig. 4): following treatment with HC11 (25 mg kg^{-1}), on day 2, no major differences were identified between treated and control animals, although there was a low level of fatty-like vacuolar change around large blood vessels (portal tracts) in the treated group. However, on days 3 and 5 the occurrence of multiple small and larger foci of necrosis of hepatocellular parenchyma accompanied by infiltrations of polymorphs (neutrophils) and macrophages was noted. In the animals sacrificed on day 8, a similar lesion was identified in 1/3 animals and showed features suggestive of scarring. At all time-points, in animals receiving 40 mg kg^{-1}, there was a variable but often severe focal or more diffuse/lobar necrotising reaction with polymorph and macrophage presence. These infiltrations were either parenchymal or, more often, perivascular. Individual apoptotic hepatocytes in the adjacent hepatocellular parenchyma were also seen in many of these livers. Where lobar lesions were present the adjacent lobes were usually unaffected, however. The lesions were more marked in the animals culled on days 4 and 5.

Following administration of RM175, elevation of ALT was seen in the group receiving 15 mg kg^{-1} on days 1–5 after 96 h (mean 355 U L^{-1}) compared to control animals (62 U L^{-1}). No elevation of ALT was seen in the group receiving 25 mg kg^{-1} on days 1 and 5. The histopathology showed only minor changes such as small polymorph and macrophage inflammatory foci adjacent to the hilar region in 2/3 animals on day 4. The significance of this is unclear, as low-level inflammatory cells were present in the extrahepatic omental hilar tissue in many of the sections in the series, but were not seen in control animals. A solitary peripherally located small necrotic focus with a few associated polymorphs was seen in 1/3 animals on day 5. The cause of this was not apparent, but it is similar, but less severe than the changes associated with HC11.

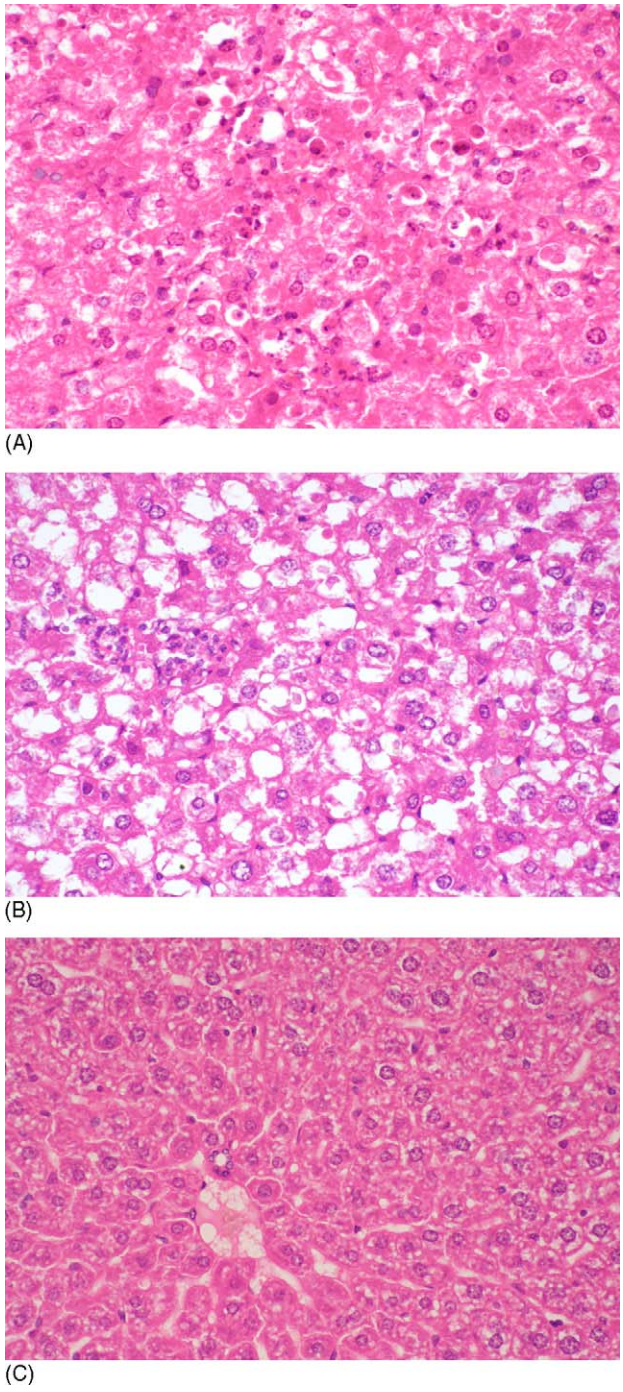


Fig. 4 – Microscopic evidence of liver toxicity after HC11 administration. Animals were treated with HC11 i.p. on day 1. Livers were collected on day 3 and pathology examination was carried out after H&E staining. (A) HC11 40 mg kg^{-1} showing extensive necrosis; (B) HC11 25 mg kg^{-1} showing vacuolar change, consistent with fatty degeneration; (C) control.

No changes were noted in urea or creatinine over the course of the experiment. A single animal in the high dose (40 mg kg^{-1}) HC11 study was noted to be thrombocytopenic, but this occurred on day 5 when the animal was profoundly unwell.

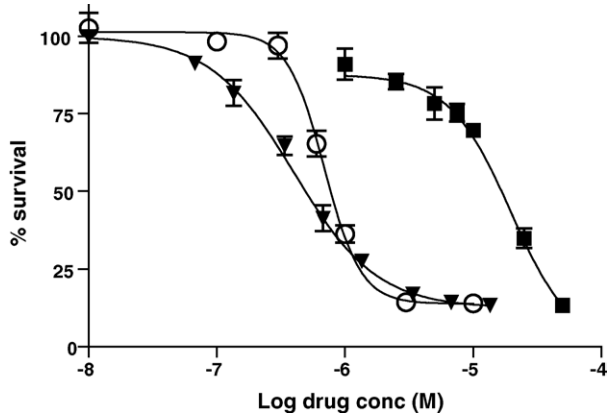


Fig. 5 – In vitro cytotoxicity of RM175 (■), HC11 (▼) and mithramycin (○) in fresh human hepatocytes. Cells were treated over a range of concentrations with RM175, HC11 or mithramycin for 24 h. Twenty-four hours after the end of drug treatment, the MTT assay was performed as described. Results are expressed as percentage of survival as compared to untreated cells and are the mean ± S.E.M of triplicate samples.

3.3. *In vitro cytotoxicity of HC11 and RM 175 in fresh human hepatocytes*

To ascertain whether the changes noted in the in vivo experiments in rodents might be relevant in patients, we evaluated the cytotoxicity of RM175 and HC11 in vitro in fresh human hepatocytes, using the known hepatotoxic drug, mithramycin, as a positive control. Both ruthenium compounds induced a rapid cytotoxic effect (Fig. 5), detectable at the end of drug exposure (24 h). IC_{50} values for the two ruthenium compounds were markedly different, with HC11 being 40-fold more toxic (RM175 IC_{50} 24 μM, HC11 IC_{50} 0.6 μM). In comparison, the IC_{50} for mithramycin was 0.7 μM.

3.4. *In vivo anti-tumour activity of RM175 and HC11*

The anti-tumour activity of RM175 and HC11 was evaluated using 25 mg kg^{-1}, days 1 and 5 of RM175 and 25 mg kg^{-1} of HC11 on days 1, 8 and 15. RM175 was also evaluated at two dose levels in the LXFL 529L xenografts, in Freiburg. Both RM175 and HC11 were evaluated in the A549 model. Significant growth delay was noted in the A549 model (Fig. 6A) on day 46 for both RM175 ($V_t/V_0 = 5.3 \pm 1.9$, $p = 0.004$) and HC11 ($V_t/V_0 = 4.7 \pm 0.7$, $p = 0.001$) compared to controls (9.8 ± 1.1). However, anti-tumour activity was not seen in the LXFL 529L model (Fig. 6B), although this study was performed in the NMRI mouse strain and only 4% body weight loss was noted (data not shown), so possibly below the maximally tolerated dose.

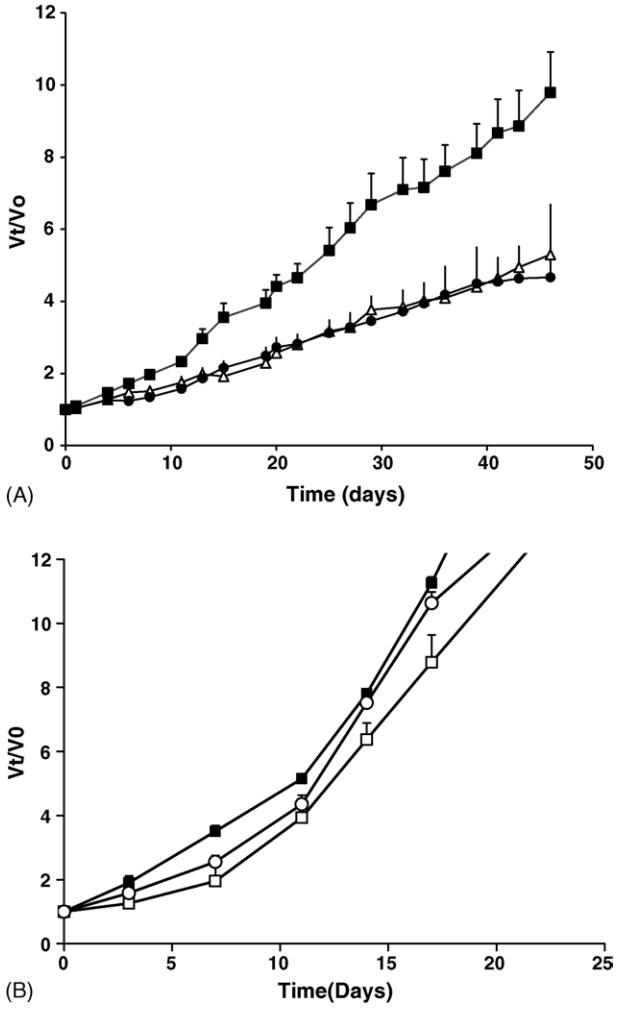


Fig. 6 – In vivo efficacy of RM175 and HC11 on A549 (A) and LXFL lung cancer xenografts (B). (A) Animals bearing A549 xenografts were treated with RM175 25 mg kg^{-1}, i.p., on days 1 and 5 (●), HC11 25 mg kg^{-1} on days 1, 8 and 15 (△) or vehicle (■). (B) Animals bearing LXFL xenografts received vehicle (■) or RM175 i.p. on days 1, 5 and 13 at the dose of 15 mg kg^{-1} (○) or 25 mg kg^{-1} (□). Results are expressed as the average growth rate (V_t/V_0) of each group ± S.E.

4. Discussion

This paper presents the anti-tumour activity and safety profiles of two Ru^{II} arene complexes, RM175 and HC11, in vitro and in vivo. In vitro assessment of RM175, $[(\eta^6\text{-}C_6H_5C_6H_5)Ru(en)Cl]^+$ (as a PF_6 salt) using the Freiburg screen, demonstrated a mean IC_{50} of 3 μM, confirming the level of activity we have reported previously and demonstrating RM175 has a broad spectrum of activity. Particular sensitivity (~10-fold lower than mean IC_{50}) was noted in breast cancer (401NL) and non-small cell lung cancer (529L) cell lines. Using the data from the Freiburg screen, it was not possible to identify compounds with a similar spectrum of activity, or to identify a particular mechanism of action, i.e. RM175 was ''compare-negative'' in this screen.

The particular activity (RM175 $IC_{50} < 0.5$ μM) noted in the large cell lung cancer model, in vitro, in the Freiburg screen, led us to undertake further in vitro studies in two additional non-small cell lung cancer cell lines, A549 and H520. The

IC_{50} values were similar to those obtained in the Freiburg screen for RM175 which confirmed the activity seen in lung cell lines. Moreover, HC11 was more active than RM175 and cisplatin in these two cell lines. HC11 has been shown previously to be 10-fold more active than RM175 in the ovarian cancer cell line, A2780 [1]. These results are in contrast with the lack of cytotoxicity of NAMI-A observed in vitro against breast and colon cancer cell lines [19] and suggest a different cellular target for these two classes of compounds. These Ru^{II} arene compounds have been shown to interact strongly with guanine bases in DNA [14].

To provide a preliminary assessment of the potential therapeutic index for these agents, we evaluated myelosuppression, liver and renal toxicity, as possible sequelae of the use of a heavy metal containing anti-proliferative complex. Previous pharmacokinetic studies using NAMI-A reported high concentrations of ruthenium in the kidneys after a single i.v. administration [20]. When NAMI-A was administered daily i.p., the highest concentrations of ruthenium were observed in the liver and kidneys [21]. In this study, no clear evidence of either bone marrow or kidney toxicity was identified. However, liver toxicity was observed with both RM175 and HC11. It was mild and reversible in animals receiving RM175 and HC11 at low doses (25 mg kg^{-1}) but became severe in animals treated with HC11 at a dose of 40 mg kg^{-1}, suggesting a low therapeutic index for this particular compound.

Fresh hepatocyte models have been used previously to investigate liver toxicity and are considered to be one of the best in vitro models for this purpose [22]. Mithramycin is a known hepatotoxic anti-cancer drug [23] and has been included as a positive control in this study with fresh hepatocytes. All three compounds (HC11, RM175 and mithramycin) caused hepatocyte toxicity after drug treatment. However, HC11 was 40-fold more cytotoxic (IC_{50} 0.6 μM) than RM175 (IC_{50} 24 μM) and equitoxic to the known hepatotoxin, mithramycin (IC_{50} 0.7 μM). Moreover, the IC_{50} for RM175 was approximately 10-fold higher in fresh hepatocytes, when compared to the IC_{50} observed for A549 and H520 (see Table 1), suggesting a log difference between drug concentrations active in tumour cells and concentrations toxic for normal cells. However, HC11 displayed similar IC_{50} values in fresh hepatocytes and in NSCLC cell lines, suggesting a low (~1) therapeutic index. It is unlikely that the cytotoxic effect observed in vitro is due to the opening of the arene ring in the tissue culture medium since previous studies have shown the stability of these ruthenium complexes in presence of amino acids, peptides, proteins and nucleotides [24–26].

The anti-tumour activity of both RM175 and HC11 was evaluated in animals bearing A549 xenograft and RM175 was also evaluated in Freiburg in the LXFL lung model. Both RM175 and HC11 were active in the A549 model, but RM175 did not show activity in the LXFL model. Pharmacokinetic studies of NAMI-A have reported an excellent distribution in the lungs [21]. This was associated with an antimetastatic effect of this compound against Lewis lung carcinoma in mice. A preferential uptake of ruthenium by lung cells may also be suggested considering the anti-tumour activity of NAMI-A against Lewis lung carcinoma grown as xenografts [21].

Hepatotoxicity is not a recognised side effect following the administration of platinum containing drugs, at standard dosages, and was not a feature of the NAMI-A phase I trial. In a study of the administration of high-dose carboplatin, reversible biochemical hepatotoxicity was identified, but doses six times higher than the standard dosage regimen were being used [27]. Titanocene dichloride is also an organo-metallic compound containing cyclic aromatic hydrocarbons (cyclopentadienyl). It was associated with reversible liver and GI tract damage, observed for 4–8 days after administration at 40–60 mg kg^{-1} in pre-clinical studies [28]. In subsequent phase I trials, renal toxicity was the major dose-limiting event, although hepatotoxicity was dose-limiting in one patient [29,30]. It is known that cyclic aromatic hydrocarbons can be hydroxylated and conjugated in the liver leading to the generation of potentially toxic species. Therefore, studies to evaluate the role of cytochrome P450s in induction of liver toxicity are being performed and these will be incorporated into screening protocols, if appropriate. The present study suggests that fresh human hepatocytes can be used to evaluate the potential hepatotoxicity of this class of agents and that indeed HC11 may be potentially hepatotoxic. However, further chemical synthesis is ongoing to ''design out'' this toxicity and improve the therapeutic index of this series of compounds.

Acknowledgements

We thank the Edinburgh Technology Fund and Oncosense Ltd. for their support for this work and Drs. Robert Morris, Haimai Chen and Abraha Habtemariam for the synthesis of compounds.

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