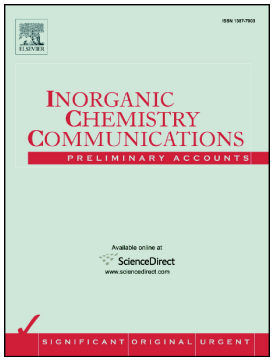

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David C. Kennedy, Brian R. James

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Synthesis and antiproliferatory activity of ruthenium complexes containing N-heterocyclic carboxylates

David C. Kennedy, Brian R. James

Department of Chemistry, The University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1

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Abstract

Solvates of the complexes $Ru_2Cl(pic)_4(EtOH)$(**2**), $Ru(Im\text{-}CO_2)_3$ (**3**), and $Ru(Im\text{-}CO_2)(Im\text{-}CO_2H)Cl_2$ (**4**), were synthesized from reaction of $RuCl_3{\cdot}3H_2O$ or $K_3[RuCl_6]$ with the N-heterocyclic carboxylic acids pyridine-2-carboxylic acid (picolinic acid, Hpic), and imidazole-4-carboxylic acid ($Im\text{-}CO_2H$). Crystals of **2**-**4** could not be grown and hence characterization was done by elemental analysis, NMR, IR, and conductivity data; **2** and **4** were tested for antiproliferatory activity *in vitro* against a human breast cancer cell line, but were less active than, for example, Ru complexes containing bis-imidazole or 4,4'-biimidazole that we studied previously [see Can. J. Chem. 89 (2011) 948]. Preliminary work with a third potential ligand, 3-nitro-1,2,4-triazole-5-carboxylic acid (abbreviated HCANT), and other nitro heterocyclic compounds is also presented.

Keywords: Ruthenium; N-heterocyclics; Carboxylates; Antiproliferatory activity

Our group first published work on the use of ruthenium complexes as radiosensitizers in 1986, reporting on a fluorinated nitroimidazole ligand system [1], and have since continued interest in Ru-imidazole and -4,4'-biimidazole complexes [2] and their 'biological properties', particularly antiproliferatory activity against cancer cells. Ruthenium species we have studied include co-ligands such as sulfoxides [3], and acac and the related maltolato and pyridonate anions were also studied as ligands or co-ligands [4]. Meantime, we have naturally followed the great interest in development through clinical trials of the anticancer agents [HIm][*trans*-$Ru^{III}Cl_4(Im)$(*S*-DMSO)] (Im = imidazole) and [HInd][*trans*-$Ru^{III}Cl_4(Ind)_2$] (Ind = indole), labelled NAMI and KP1019, respectively [5]. We reported, in 2011, the exponential general interest in 'bioinorganic' Ru-systems [4d], and noted that 'many synthetic-type coordination and organometallic researchers in the Ru chemistry area are now testing their new complexes for antiproliferatory activity!' This trend, including studies of other biological properties, has continued and, for example, around 400 publications are listed in *SciFind* for 2014, 2015 and the first 6 months of 2016; as well as ref. 5(a-c), 13 other arbitrary 2016 references are listed [6(a-m)].

We decided to study as ligands imidazoles containing a carboxylic acid substituent because this would likely allow for any derived Ru complexes to be soluble in aqueous media, depending on pH – an important property for biological tests. This paper reports the syntheses and characterization work of the complexes, as well as some preliminary data on their antiproliferatory properties. A further N-heterocyclic carboxylic acid, pyridine-2-carboxylic acid (Hpic), was also studied because the tris(η^2-*N*,*O*-picolinate) complex *mer*-$Ru^{III}(pic)_3$ was known [7], and its characterization data were thought to be of possible value for our studies. Because of the ability of nitroimidazoles to function as radiosensitizers and hypoxic markers [1, 8], studies were also initiated on carboxylic acid derivatives of nitro-substituted N-heterocyclic compounds: examples include synthesis of 3-nitro-1,2,4-triazole-5-carboxylic acid and its reactivity with Ru-precursors, and attempts to synthesize 2-nitroimidazole-4-dicarboxylic acid and the reported 2-nitroimidazole-4,5-dicarboxylic acid [9].

Some ligand syntheses required the following precursors that were purchased from Aldrich: imidazole-4-carboxylic acid (Im-CO_2H), imidazole-4,5-dicarboxylic acid, 4-(hydroxymethyl)-imidazole, and 3-amino-1,2,4-triazole-5-carboxylic acid; Hpic, trityl chloride, and other common chemicals were also Aldrich products. $RuCl_3 \cdot 3H_2O$ (with 39% Ru) was donated by Colonial Metals Inc., while $K_3[RuCl_6]$ [10] and $Ru_2Cl(\mu\text{-}O_2CMe)_4$ [11] were made by literature methods. Some solvents (Fisher products) were dried and distilled prior to use: MeOH and EtOH (distilled from Mg), THF (Na); other solvents were used as received. The deuterated solvents D_2O, CD_3OD, $CDCl_3$, and DMSO-d_6 (Cambridge Isotope Labs products) were also used as received. Medical grade N_2 was used as received from Praxair.

^1H NMR spectra (s = singlet, t = triplet, q = quartet, br = broad, and m = multiplet) were recorded at room temperature (~20 °C) on a Bruker AV300 instrument, and referenced using residual protonated solvent signals: 7.24 ($CDCl_3$), 4.65 (D_2O), 3.30 (CD_3OD), and 2.49 (DMSO-d_6). IR spectra (KBr pellets, s = strong, m = medium) were recorded as cm^{-1} on a Bomem-Michelson MV-100 FT-IR spectrometer. Mass spectral data were measured on a Bruker Esquire electrospray (ESI ion-trap) spectrometer and a Micromass LCT electrospray time of flight (ESI TOF); M^+ is defined as the positive molecular ion of a given species and does not include the mass of solvated molecules. Conductivity measurements, carried out on a RCM151B Serfass conductivity bridge (A.H. Thomas Co.) with a 3403 cell (Yellow Springs Instrument Co.), were calibrated using 0.0100 M aq. KCl solution (Λ_M = 141.3 Ω^{-1} cm^2 mol^{-1} at 25 °C, cell constant =

1.016 cm^{-1}), and data are given in Λ_M units. Elemental analyses were done on a Carlo Erba, EA 1106 analyzer in this department.

All reagents, cell handling techniques, and details for a so-called MTT colourimetric biological assay, which examines antiproliferatory activity of compounds over large concentration ranges [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium], have been described in detail in earlier publication [3, 4c]. The MTT assay gives IC_{50} values (the drug concentration at which 50% of the cells are viable relative to a control) that are determined from plots of % cell viability vs. drug concentration (logarithmic scale). The Ru complexes were examined on human breast cancer cells (MDA-MB-435S), purchased from ATCC (American Type Culture Collection).

Modified literature methods [7] were used to synthesize mer-Ru(pic)$_3$·H_2O (**1**). (a) Hpic (0.14 g, 1.2 mmol) was added to a red solution of $K_3[RuCl_6]$ (0.17 g, 0.39 mmol) in 5 mL H_2O, and the solution then heated at 60 ^oC for 5 h. The yellow precipitate that formed was filtered off, washed with MeOH (2 x 5 mL) and H_2O (1 x 5 mL), and then dried *in vacuo* at ~80 ^oC for 48 h; yield: 0.11 g (58 %). (b) Hpic (0.096 g, 0.78 mmol) was added to a brown solution of $Ru_2Cl(\mu$-$O_2CMe)_4$ (0.062 g, 0.13 mmol) in water /MeOH, 1:1 (10 mL), and the mixture was refluxed for 8 h to give the yellow precipitate that was isolated by filtration, washed with H_2O (2 x 5 mL), and dried as above. Yield: 0.049 g (77 %). IR: 3443 (O-H, s), 1661 ($C=O_{asym}$, s), 1565 (m), 1315 ($C=O_{sym}$, m), 1280 (s), 1057(m), in agreement with literature data [7]. ESI-MS: 468 (M^+). Anal. Calcd. for $C_{18}H_{12}N_3O_6Ru·H_2O$: C, 44.54; H, 2.91; N, 8.66. Found: C, 44.5; H, 2.7; N, 8.3.

For synthesis of $Ru_2Cl(pic)_4(EtOH)•H_2O$ (**2**), Hpic (0.072 g, 0.59 mmol) was added to a brown solution of $RuCl_3·3H_2O$ (0.051 g, 0.19 mmol) in 10 mL EtOH, and the solution was then refluxed for 6 h, with the colour becoming orange; after evaporation to a volume of ~1 mL, addition of Et_2O (10 mL) gave an orange precipitate that was collected, washed with Et_2O (2 x 5 mL), and then dried *in vacuo* at ~80 ^oC for 24 h. Yield: 0.066 g (86 %). ^1H NMR (D_2O): δ 8.76-7.92 (m, pic), 4.53 (br q, coordinated $HOC\mathit{H}_2CH_3$), 3.55 (q, free $OHC\mathit{H}_2CH_3$), 1.45 (br t, coordinated $HOCH_2C\mathit{H}_3$), 1.12 (free $HOCH_2C\mathit{H}_3$). IR: 3468 (O-H, s), 3124 (N-H, m), 1672 ($C=O_{asym}$, s), 1639 ($C=O_{asym}$, s), 1600 (m), 1456 (m), 1396 ($C=O_{sym,}$ m), 1316 ($C=O_{sym,}$ m), 1282 (m), 1147 (m), 856 (m), 759 (s), 691 (m). ESI-MS: 727 (M^+–EtOH), 382 ($Ru(pic)_2Cl^+$), 346 ($Ru(pic)_2^+$). Λ_M (MeOH) = 112. Anal. Calcd. for $C_{26}H_{22}N_4O_9ClRu_2·H_2O$: C, 39.52; H, 3.06; N, 7.09. Found: C, 39.4; H, 2.9; N, 6.9.

$Ru(Im\text{-}CO_2)_3{\cdot}2H_2O$ (**3**) was synthesized by two methods: (a) $Im\text{-}CO_2H$) (0.094 g, 0.84 mmol) was added to a solution of $K_3[RuCl_6]$ (0.12 g, 0.28 mmol) in H_2O (10 mL). Refluxing for 6 h afforded a yellow precipitate that was collected at r.t., washed with MeOH (2 x 5 mL), and dried *in vacuo* at 78 ^oC for 48 h; yield: 0.074 g (57 %). (b) $Im\text{-}CO_2H$ (0.092 g, 0.82 mmol) was added to a solution of $Ru_2Cl(\mu\text{-}O_2CMe)_4$ (0.065 g, 0.14 mmol) in 1:1water/MeOH (10 mL) that was then refluxed for 8 h to give the yellow precipitate, isolated as in (a). Yield: 0.043 g (67 %). ^1H NMR (DMSO-d_6): δ 0.31, 0.09, –0.09 (s, H_5-Im), –2.67, –13.55, –17.87 (s, H_2-Im). IR: 3423 (O-H, s), 3109 (N-H, s), 1642 ($C{=}O_{asym}$, s), 1324 ($C{=}O_{sym,}$ s), 1201 (m), 1090 (m), 1024 (m), 930 (m), 829 (m). ESI-MS: 435 (M^+). Anal. Calcd. for $C_{12}H_9N_6O_6Ru{\cdot}2H_2O$: C, 30.64; H, 2.79; N, 17.87. Found: C, 30.9; H, 2.6; N, 17.5.

For synthesis of $RuCl_2(Im\text{-}CO_2)(Im\text{-}CO_2H)_2{\cdot}EtOH{\cdot}H_2O$ (**4**), $Im\text{-}CO_2H$ (0.084 g, 0.75 mmol) was added to a solution of $RuCl_3{\cdot}3H_2O$ (0.065 g, 0.25 mmol) in EtOH (10 mL); on being refluxed for 4 h, the solution became yellow. The volume was then reduced to ~2 mL, when addition of Et_2O (15 mL) afforded a yellow precipitate that was collected, washed with Et_2O (3 x 5 mL), and dried as for **3**. Yield: 0.11 g (76 %). ^1H NMR (CD_3OD): δ 3.45 (q, $HOC\mathit{H}_2CH_3$), 1.12 (t, $HOCH_2C\mathit{H}_3$), –4.49, –7.20, –10.16 (s, H_5-Im), –14.83, –16.75, –23.92 (br s, H_2-Im). IR: 3467 (O-H, s), 3217 (O-H, m), 3149 (N-H, m), 1718 ($C{=}O_{asym}$, s), 1706 ($C{=}O_{asym}$, s), 1570 ($C{=}O_{asym,}$ s), 1384 ($C{=}O_{sym,}$ m), 1350 ($C{=}O_{sym,}$ m), 1313 ($C{=}O_{sym,}$ m), 1188 (m), 1089 (m). ESI-MS: 508 (M^+), 472 (M^+–Cl), 396 (M^+–Im–CO_2H), 360 (M^+–Im–CO_2H–Cl). Λ_M (MeOH) = 19. Anal. Calcd. for $C_{12}H_{11}N_6O_6Cl_2Ru{\cdot}EtOH{\cdot}H_2O$: C, 29.43; H, 3.35; N, 14.71. Found: C, 29.4; H, 3.2; N, 14.4.

3-Nitro-1,2,4-triazole-5-carboxylic acid (HCANT) was made by a modified literature method for conversion of amino-triazoles to the nitro derivative [12]. After addition of $NaNO_2$ (1.10 g, 15.9 mmol) to 7 mL of H_2SO_4 at –5 ^oC, glacial CH_3CO_2H (15 mL) and finely ground 3-amino-1,2,4-triazole-5-carboxylic acid (2.00 g, 15.6 mmol) were added. The mixture was then stirred for 10 min to dissolve most of the triazole; H_2O (25 mL) was then added at a temperature of around zero. The resulting yellow solution was then added dropwise to a nitrite solution (200 g $NaNO_2$ in 200 mL H_2O) at 50 ^oC (a rapid addition generates a hot foam of diazonium salts, with contents erupting from the flask). The green product solution on heating for 2 h at 50 ^oC becomes colourless, and is then extracted with EtOAc (4 x 50 mL); evaporation of the extracts gives

HCANT (1.41 g, 57 %). IR: 3416 (N-H, s), 3257 (O-H, m), 1710 (C=O, s), 1574 (NO_2, m), 1383 (NO_2, m), 1268 (m), 720 (m). ESI-MS: 159 (M^+). Anal. Calcd. for $C_3H_2N_4O_4$: C, 22.78; H, 1.28; N, 35.44. Found: C, 22.5; H, 1.4; N, 35.4.

For synthesis of methyl imidazole-4-carboxylate (**5**), conc. H_2SO_4 (1.5 mL) was added to a suspension of Im-CO_2H (1.00 g, 8.92 mmol) in MeOH (20 mL), and the mixture was refluxed for 24 h to give a solution that was then cooled to 0 ^oC and neutralized to pH 8 using 5 M NaOH. Evaporation *in vacuo* left a white residue that was re-dissolved in a minimal volume of boiling water; cooling the solution deposited white crystals of the ester that were collected, washed with cold H_2O (1 x 5 mL), and dried *in vacuo* at r.t. for 24 h. Yield: 0.87 g (77 %). ^1H NMR (DMSO-d_6): δ 7.74 (s, 1H, H_5-Im), 7.65 (s, 1H, H_2-Im), 2.51 (s, 3H, CH_3-Im). IR: 3105 (N-H, s), 2976, 2846 (C-H, m), 1619 (C=O, m), 1363 (s), 1156 (m), 864 (m). ESI-MS: 127 (M^+). Anal. Calcd. for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.5; H, 4.85; N, 21.7.

For methyl 1-tritylimidazole-4-carboxylate (**6**), trityl chloride (1.77 g, 6.35 mmol) was first stirred into a solution of **5** (0.80 g, 6.3 mmol) in DMF (20 mL) under N_2. NEt_3 (0.98 mL, 7.0 mmol) was then added, and the mixture stirred for 16 h at r.t. before being poured over ice; the cold mixture was then filtered and the isolated solid was washed with H_2O (2 x 5 mL), and then dried *in vacuo* at r.t. for 24 h. Yield: 2.01 g (86 %). ^1H NMR ($CDCl_3$): δ 7.65 (s, 1H, H_5-Im), 7.52 (s, 1H, H_2-Im), 7.03-7.36 (m, 15H, Ph_3C), 2.42 (s, 3H, CH_3-Im). ESI-MS: 369 (M^+), 243 (CPh_3). Anal. Calcd. for $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60. Found: C, 78.35; H, 5.6; N, 7.4.

4-Hydroxymethyl-1-tritylimidazole (**7**) was made by two methods: (a) a modified, reported method was first used [9]. A hexanes solution (5.5 mL) of 1.0 M diisobutylaluminum hydride (Dibal-H) was added to a THF solution (15 mL) of **6** (1.02 g, 2.72 mmol) under N_2, and the mixture was stirred for 1 h, and then cooled to 0 ^oC. The following were then slowly added in sequence: H_2O (0.8 mL), 15 % aq. NaOH (1.0 mL), and H_2O (0.8 mL). The mixture was then filtered, and the precipitate washed with THF (3 x 10 mL), the combined filtrate and washings then being evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 and the solution, after being washed with H_2O to remove any remaining inorganic salts, was evaporated *in vacuo* to give a white product that was further dried for 24 h at r.t. *in vacuo*. Yield: 0.41 g (44 %). (b) To a stirred solution of 4-(hydroxymethyl)imidazole (0.52 g, 5.3 mmol) in DMF (10 mL) under N_2 was added trityl chloride (1.48 g, 5.30 mmol), and after ~10 min NEt_3 (0.90 mL, 6.4 mmol) was

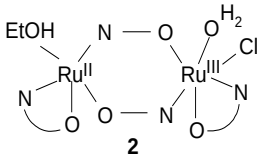
added. This mixture was then stirred for 16 h, and poured onto ice; the resulting precipitate was collected, washed with H_2O (2 x 10 mL), and dried *in vacuo* for 24 h. Yield: 1.53 g (85 %). ^{1}H NMR ($CDCl_3$): δ 7.39 (s, 1H, *H*$_2$-Im), 7.27-6.97 (m, 15H, CPh_3), 6.80 (s, 1H, *H*$_5$-Im), 4.62 (s, 2H, C*H*$_2$OH). ESI-MS: 341 (M^+), 243 (CPh_3). Anal. Calcd. for $C_{23}H_{20}N_2O$: C, 81.15; H, 5.92; N, 8.23. Found: C, 81.2; H, 5.9; N, 7.9. The ^{1}H NMR data agree with reported values [9].

4,5-Dihydroxymethyl-1-tritylimidazole was made by a modified, reported method [13]. A hexanes solution (10.5 mL) of 1.0 M Dibal-H solution was added to a THF solution (20 mL) of dimethyl 1-tritylimidazole-4,5-dicarboxylate (1.03 g, 2.41 mmol) (see below) under N_2, and the mixture was stirred for 4 h, and then cooled to 0 ^{o}C; the following were then added: H_2O (1.5 mL), 15 % aq. NaOH (2 mL), and H_2O (1.5 mL). The mixture was then filtered, and the precipitate washed with warm THF (3 x 10 mL), the combined filtrate and washings then being evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2, the solution filtered through Celite (~10 g), and the filtrate was evaporated. The resulting white solid was dried *in vacuo* at r.t. for 24 h. Yield: 0.42 g (44 %). ^{1}H NMR ($CDCl_3$) δ 7.67 (s, 1H, *H*$_2$-Im), 7.41-7.19 (m, 15H, CPh_3), 4.83 (s, 2H, C*H*$_2$OH), 3.89 (s, 2H, C*H*$_2$OH). ESI-MS: 371 (M^+), 243 (CPh_3). Anal. Calcd. for $C_{24}H_{22}N_2O_4$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.7; H, 5.9; N, 7.4.

The following compounds were made by the indicated literature methods: 4-hydroxymethyl-2-nitroimidazole (**8**)**,** but in only 3% yield from 4-hydroxymethyl-1-tritylimidazole [9]; dimethyl imidazole-4,5-dicarboxylate, in 85% yield, from imidazole-4,5-dicarboxylic acid [13]; and dimethyl 1-tritylimidazole-4,5-dicarboxylate, in 71% yield, from dimethyl imidazole-4,5-dicarboxylate [13].

First, it is worth noting that formation of Ru^{III} or Ru^{II} complexes via the mixed-valence $Ru_2Cl(\mu\text{-}O_2CMe)_4$ is common [7b], but details of the redox chemistry remain unclear. The structurally characterized Ru^{III}-picolate yellow complex, *mer*-$Ru(pic)_3\cdot H_2O$ (**1**), was synthesized from reaction of Hpic with $K_3[RuCl_6]$ and with $Ru_2Cl(\mu\text{-}O_2CMe)_4$ by modified literature methods [7] in 58 and 77% yields, respectively; however, **1** was too insoluble in the medium required for the IC_{50} measurements [3, 4c]. A reported reaction [14] giving **1** in 62% yield using $RuCl_3\cdot 3H_2O$, and Na_2CO_3 to deprotonate the Hpic, was also tested but generated only a black solid; it should be noted that commercially available '$RuCl_3\cdot 3H_2O$' contains variable amounts of Ru^{IV} impurities [15], and irreproducibility of some Ru^{III} chemistry using the trichloride precursor

is not surprising. Fortunately, when the Na_2CO_3 was omitted, a bright orange complex formulated as $Ru_2Cl(pic)_4(EtOH)\bullet H_2O$ (**2**) was obtained in 86% yield, and was readily water-soluble. Elemental analysis (EA) strongly supports this formulation, and the MS data (in MeOH) show a Ru_2 splitting pattern with a parent peak for $[M^+–EtOH]$ at 727 (Fig. S1); this pattern is distinctly different from that of a mononuclear species (Fig. S2). The IR data, specifically the difference in the asymmetric and symmetric carboxylate stretching frequencies [16], which is very similar to that of **1** that contains solely N,O-bonded picolinate (ν_{asym} = 1661; ν_{sym} = 1315 cm^{-1}), implies the absence of a bridging carboxylate; for μ-carboxylates, the difference is typically < 150 cm^{-1} for a Ru_2 species [16]. The band at 3468 cm^{-1} is assigned to ν_{OH} of the H_2O, the value being close to one of 3460 cm^{-1} in **1**, in which the H_2O is H-bonded to carboxylate O-atoms, this resulting in chains within the crystal [7b]. Coordinated EtOH is identified by ^1H NMR resonances (in D_2O) at δ 1.45 (the Me-triplet) and δ 4.53 (the CH_2 quartet) that are downfield from those seen also for free EtOH (δ 1.12 and 3.55, respectively); the relative intensities of the two sets of signals (Fig. S3) imply ~40% of **2** is converted to a species with coordinated D_2O. Conductivity for **2** in MeOH is in the 1:1 electrolyte range [17], consistent with dissociation of a terminal Cl^-; loss of a bridged Cl^- would likely decompose the bimetallic unit, but the ESI-MS data in MeOH show this remains intact. In their synthesis of **1**, Barral et al. [7b] isolated also a co-product $Ru_2(pic)_4$, with IR data for the carboxylate being again consistent with coordination via N- and O-atoms; possible coordination of the keto group of a carboxylate at an axial position of a further $Ru_2(pic)_4$ unit to give a polymeric structure was also suggested to rationalize low solubility of the complex. An 'imaginative' non-polymeric Ru^{II}-Ru^{III} solid state structure for **2** containing chelated bridging and non-bridging *N,O*-pic ligands is shown above; an alternative structure with four bridging pic ligands is also feasible. The ^1H NMR spectrum (Fig. S3) supports only qualitatively this type of structure, because the partial loss of coordinated EtOH and likely loss of chloride means that several species are present; meaningful integration of the ^1H signals was not possible. The related mixed-valence complex, $Ru_2Cl(hp)_4(Hhp)$ where Hhp is 2-hydroxypyridine, similarly contains bridging and non-bridging *N*,O-ligands [18].

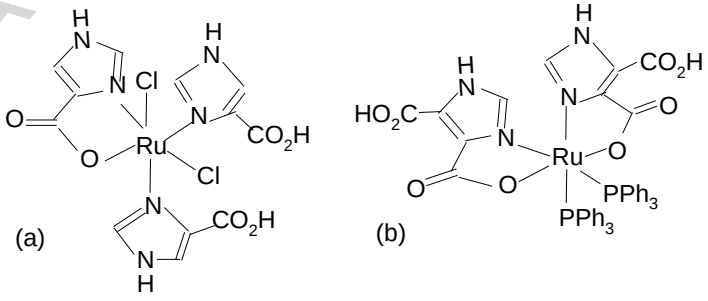


2

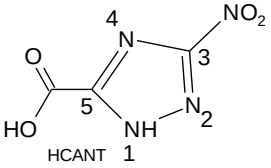
The yellow complex $Ru^{III}(Im\text{-}CO_2)_3\cdot 2H_2O$ (**3**), like **1**, was synthesized in good yield using reaction of either $K_3[RuCl_6]$ or $Ru_2Cl(\mu\text{-}O_2CMe)_4$ with Im-CO_2H in H_2O or MeOH, respectively,

and characterized by methods used for **1**. The EA is consistent with a dihydrate, and the presence of H_2O is shown by ν_{OH} at 3423 cm^{-1}, and an increase in intensity of the residual H_2O signal in the ^1H NMR spectrum (in DMSO-d_6) compared with that seen in the absence of the complex; **3** is only sparingly soluble in DMSO (and insoluble in other solvents) and the NMR spectrum recorded over 6 h (~20,000 scans) shows 6 weak signals for the bound imidazoles at δ 0.31, 0.09, and –0.09 for H(5) and δ –2.67, –13.55, and –17.87 for H(2), the assignments being based on those observed earlier for monodentate imidazole ligands at Ru^{III} [4d]. The $\nu_{asym} - \nu_{sym}$ value of 318 cm^{-1} for the carboxylates in the IR spectrum is consistent with a chelated structure with binding via the imidazole-N(3) and one carboxylate-O atom as in **1**.

Unlike the reaction with $RuCl_3 \cdot 3H_2O$ with Hpic, reaction with Im-CO_2H does not form a bimetallic complex. A reflux reaction in EtOH yields a product of formulation $Ru^{III}Cl_2$(Im-CO_2)(Im-$CO_2H)_2 \cdot$EtOH$\cdot H_2O$ (**4**) that satisfies the EA; one possible isomer consistent with the data is shown below in (a). The IR data show two types of carboxylates with ν_{asym} = 1718, 1706, and 1570 cm^{-1}. The reported complex $Ru^{II}(PPh_3)_2$(L-H)$_2$, where L-H_2 = imidazole-4,5-dicarboxylic acid, has two coordinated carboxylates and two non-coordinated carboxylic acid groups [see (b)] with respective ν_{asym} values of 1627 and 1720 cm^{-1} [19]. This suggests that for **4**, the stretches at 1570 and 1718/1706 cm^{-1} are for the bound $–CO_2^-$ and $–CO_2H$ groups, respectively; a strong 3467 cm^{-1} band likely results from the H_2O solvate. ESI-MS data show a parent M^+ peak at 508 (Fig S4) with the same isotope splitting pattern as a calculated spectrum (Fig. S2). In MeOH, the conductivity of **3**, consistent with a neutral species, remained unchanged over 3h. The ^1H NMR spectrum in CD_3OD shows three signals for both the H(2) and H(5) protons of the Im-CO_2H and ImCO_2^- ligands, consistent with structure (a), where the two Im-CO_2H ligands are in different environments; resonances for the free solvate EtOH are also seen.



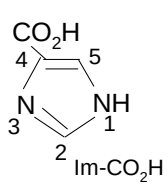
To our knowledge, there are no previous reports on Ru complexes containing Im-CO_2H or $ImCO_2^-$. Indeed, complexes with the imidazole-4-carboxylate ligand are surprisingly rare; one type is $M(CO)_3(H_2O)$(Im-CO_2), where M = Re, Tc [20]. There is a $RuH(CO)$(MeIm-CO_2)$(PPh_3)_2$ complex, but this contains an N-methylated imidazole -2-carboxylate ligand [21].

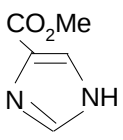


HCANT

HCANT was synthesized by modifying a reported Sandmeyer reaction with the 3-amino precursor that was stated to be soluble in acetic acid [12]. However, this is incorrect, and we used a higher H_2SO_4:$MeCO_2H$ ratio to solubilize the amino-triazole; addition of the diazonium salt solution to the $NaNO_2$ solution needs to be dropwise with rapid stirring and gentle heating to prevent foam erupting from the flask. The isolated solid was well characterized. Attempts to form Ru complexes from HCANT via methods analogous to those described with Hpic and $ImCO_2H$ in were unsuccessful. Reaction of $K_3[RuCl_6]$ with 3 equiv. of HCANT in H_2O gave a purple solution from which a purple solid was isolated, the MS showing a parent peak consistent with the formulation $Ru^{III}Cl_2(CANT)(HCANT)_2$ analogous to **4**. There are IR bands at 1677, 1665, 1652 cm^{-1} (ν_{asym} of C=O), and 1542 cm^{-1} (ν_{NO2}), but an intense 1912 cm^{-1} band may be due to a coordinated CO or NO (see below); also, for the above formulation, EA data were variable but always low in C (e.g. 11.5 *vs*. 16.74) and N (21.6 *vs*. 26.05), and high in H (3.0 *vs*. 0.78) suggesting the presence of considerable water (consistent with an observed, intense broad IR band at ~3400 cm^{-1}). A purple solid was also isolated with use of $Ru_2Cl(\mu\text{-}O_2CMe)_4$ as a precursor. The IR data are very similar to those noted above, except that the 1912 cm^{-1} band is even more intense. ESI-MS (negative, MeOH) showed a single peak at 444 with a Ru isotope splitting factor consistent with the formulation $Ru(CANT)_2(NO)$, and the 1912 cm^{-1} band is in the range for a $Ru^{II}(NO)$ species [22]; a variable EA was again always low in C and N, and high in H for this formulation. Further studies are needed to characterize these purple solids.

Synthesis of 2-nitroimidazole-4-carboxylic acid was attempted using intermediates **5**–**8**. Treatment of Im-CO_2H with MeOH in conc. H_2SO_4 generated the methyl ester (**5**), the procedure





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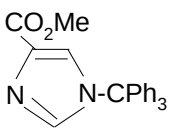

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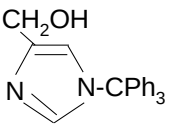

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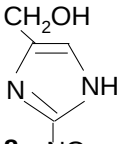

8

following that reported for an analogous ethyl ester [9]. Then the trityl group was used to form **6** with a protected imidazole-N(1) position, as this group is easily removed later with dilute acid, as shown in reported syntheses of 4,4'-biimidazole derivatives [23], and used by us previously [2a]. The protected ester **6** was then reduced at r.t. using Dibal-H to give 4-hydroxymethyl-1-tritylimidazole (**7**) in 44% yield (an 86% yield was reported for $LiAlH_4$ reduction of the ethyl ester [9], but attempts to use this for the methyl ester were unsuccessful); **7** was also made by treatment of 4-(hydroxymethyl)imidazole with trityl chloride in 85% yield. Nitration of **7** using $^{n}PrNO_3$, on the planned route to the 2-nitro derivative (**8**), gave only a 3% yield, despite a reported 29% [9]. We found that the same nitrate procedure to make 2-nitroimidazole from 1-tritylimidazole gave a 39% yield (*vs*. the reported 35-50% [9]). The low yield of 4-hydroxymethyl-2-nitroimidazole prevented attempts at oxidation to the 4-carboxylic acid derivative.

In imidazole-4,5-dicarboxylic acid, there is only the C(2) position available for substitution in the ring, and direct nitration using $^{n}PrNO_3$, HNO_3 or HNO_3/H_2SO_4 seemed plausible; that nitration of 4-nitroimidazole to form 2,4-dintroimidazole has been reported [24] shows that nitration at C(2) is possible. However, neither imidazole-4,5-dicarboxylic acid nor its dimethyl ester was reactive. Treatment of 4,5-bis(hydroxymethyl)-1-tritylimidazole with $^{n}PrNO_3$ also gave no nitrated products.

IC_{50} values ($\pm$10%) against MDA-MB-435S cells for complexes **2** and **4** are 100 and 400 μM, respectively, with the values for the free associated ligands Hpic and Im-CO_2H being 950 and 4000 μM; an example of a cell viability *vs*. concentration plot is shown in Fig. S5 (for complex **2**). The IC_{50} values for some Ru-imidazole/biimidazole complexes (and cisplatin) are an order of magnitude lower [2a, 4d]. Thus, the introduction of carboxylic acid groups into the ligand N-heterocyclic rings would appear from limited data to decrease antiproliferatory activity of these types of Ru complexes.

Conclusions

A mixed-valence, dinuclear Ru_2 complex, and two Ru^{III} complexes containing N-heterocyclic carboxylate ligands, have been synthesized, and tested via an MTT assay for antiproliferatory activity *in vitro* against a human breast cancer cell line. Limited findings, compared with literature data, suggest that inclusion of a carboxylic acid group into an imidazole ligand

decreases such activity. Efforts to make nitro-substituted derivatives of carboxylated imidazoles, and their Ru complexes, of potential interest as radiosensitizers, are also described.

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Supplementary material

Supplementary data for this article (ESI-MS for **2** and **4**, ^{1}H NMR spectrum for **2**, and cell viability *vs*. [**2**] for IC_{50} measurment) can be found online at ……

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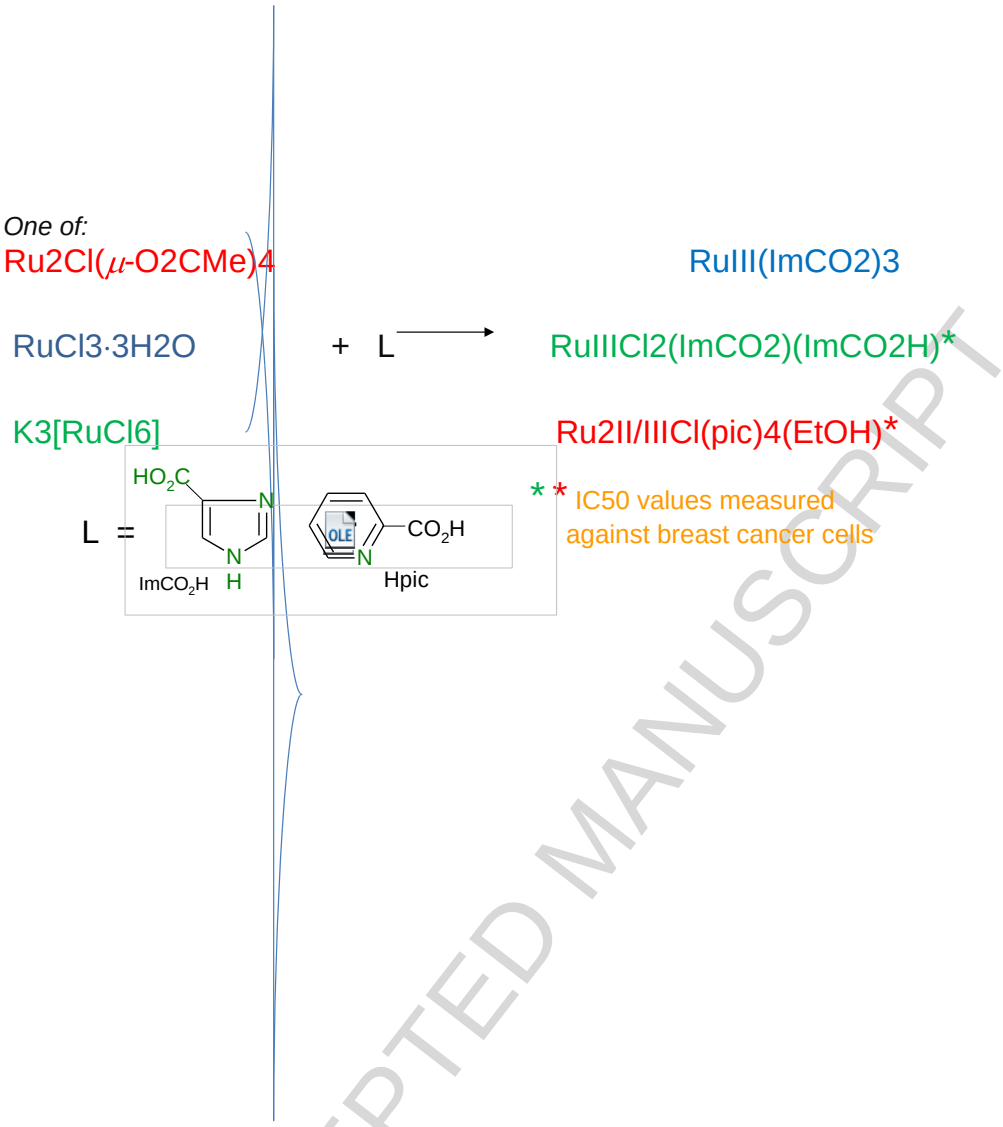

Graphical abstract

Highlights

- Ru complexes containing carboxylated imidazole ligands
- Antiproliferatory activity (IC_{50} values)