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Effects of histidin-2-ylidene vs. imidazol-2-ylidene ligands on the anticancer and antivascular activity of complexes of ruthenium, iridium, platinum, and gold

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ABSTRACT

Couples of *N*-heterocyclic carbene complexes of ruthenium, iridium, platinum, and gold, each differing only in the carbene ligand being either 1,3-dimethylimidazol-2-ylidene (IM) or 1,3-dimethyl-*N*-boc-*O*-methylhistidin-2-ylidene (HIS), were assessed for their antiproliferative effect on seven cancer cell lines, their interaction with DNA, their cell cycle interference, and their vascular disrupting properties. In MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assays only the platinum complexes were cytotoxic at single-digit micromolar IC_{50} concentrations with the (HIS)Pt complex being on average twice as active as the (IM)Pt complex. The former was highly efficacious against cisplatin-resistant HT-29 colon carcinoma cells where the latter had no effect. Both Pt complexes were accumulated by cancer cells and bound to double-helical DNA equally well. Only the (HIS)Pt complex modified the electrophoretic mobility of circular DNA in vitro due to the HIS ligand causing greater morphological changes to the DNA. Both platinum complexes induced accumulation of 518A2 melanoma cells in G2/M and S phase of the cell cycle. A disruption of blood vessels in the chorioallantoic membrane of fertilized chicken eggs was observed for both platinum complexes and the (IM)gold complex. The (HIS)platinum complex was as active as cisplatin in tumor xenografted mice while being tolerated better. We found that the HIS ligand may augment the cytotoxicity of certain antitumoral metal fragments in two ways: by acting as a transmembrane carrier increasing the cellular accumulation of the complex, and by initiating a pronounced distortion and unwinding of DNA. We identified a new (HIS)platinum complex which was highly cytotoxic against cancer cells including cisplatin-resistant ones.

1. Introduction

N-Heterocyclic carbene (NHC) complexes have been used mainly as catalysts for a wide range of reactions [1–4]. More recently, further applications came to the fore, including functional materials and antibacterial or anticancer drugs [5–10]. Once important cellular and molecular targets of bioactive NHC complexes had been identified, such as mitochondrial function, cell cycle progression or DNA repair, a rational design of more specific, 'targeted', and pleiotropic derivatives became possible. The most convincing progress of this kind has been made for antimicrobial silver complexes [11,12], and for antitumoral complexes of gold [13–16] and platinum [17–19]. An intriguing approach to increase the bioavailability of such drugs is the attachment of carrier fragments for which dedicated transport and metabolisation pathways exist, such as sugars [20], steroids [21], or amino acids and peptides [22]. Herein, we report a focused study of four couples of metal complexes bearing either an ordinary 1,3-dimethylimidazol-2-ylidene or an analogous histidine-derived NHC ligand (Fig. 1). The aim was to check whether the histidine residue acts as an uptake shuttle and efficacy booster and how such effects are mediated by the nature of the central metal.

2. Experimental

2.1. General

Complexes **1a** [23], **2a** [17], **3a** [24], **4a** [25], and **2b** [26] were prepared according to literature. A sufficient purity of >95% was verified by elemental analysis (for **2a** and **4a**) or by HPLC analysis of their

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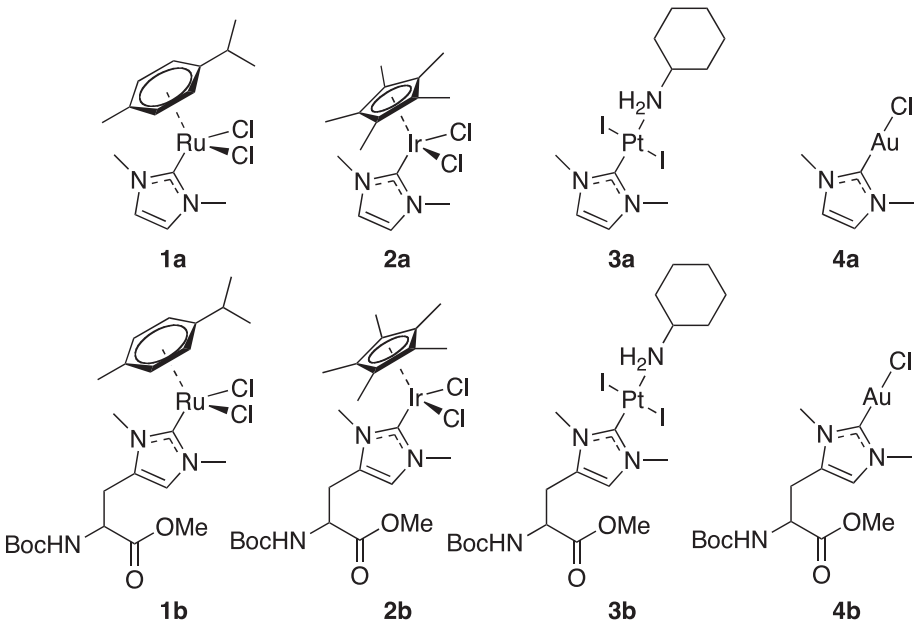

Fig. 1. Structures of carbene complexes of ruthenium **1**, iridium **2**, platinum **3**, and gold **4** with either 1,3-dimethylimidazol-2-ylidene ligands **a** or 1,3-dimethyl-*N*-boc-*O*-methylhistidin-2-ylidene ligands **b**.

stock solutions in DMF (for **1a**, **2b**, **3a**). Microanalysis of **2a**: Calcd: C, 36.43; H, 4.69; N, 5.67%; found: C, 36.58; H, 4.78; N, 5.78%. Microanalysis of **4a**: Calcd: C, 18.28; H, 2.45; N, 8.53%; found: C, 18.37; H, 2.70; N, 8.36%. Metalation reactions were carried out under strict exclusion of light and air using standard Schlenk technique under an atmosphere of dry nitrogen or argon. Dichloromethane was dried by passage through a solvent purification column. All other reagents were used as received unless otherwise stated. NMR spectra were recorded on Varian and Bruker spectrometers operating at 400 or 500 MHz. Chemical shifts (δ in ppm, J in Hz) were referenced to $SiMe_4$. Signal assignments are based on homo- and heteronuclear (multiple-bond) correlation spectroscopy. Elemental analysis: Exeter Analytical 440CE elemental analyzer. High-resolution mass spectrometry: Micromass/Waters HPLC TOF spectrometer with ESI source. Flameless atomic absorption spectroscopy (FAAS): Varian AA240Z Zeeman atomic absorption spectrometer equipped with a GTA 120 graphite tube atomizer.

2.2. Chemistry

2.2.1. 1,3-Dimethyl-N-boc-O-methylhistidinium iodide 5 [26]

N-Boc-L-histidine (1.16 g, 4.54 mmol), iodomethane (2.83 mL, 45.4 mmol) and potassium carbonate (1.89 g, 13.6 mmol) were stirred in acetonitrile (150 mL) for 20 h at 65 °C. The solvent was removed under reduced pressure and the residue was suspended with CH_2Cl_2 and filtered. The filtrate was concentrated and hexane (100 mL) was added to afford the product as a white solid (1.83 g, 95% yield) of m.p. 39 °C; 1H NMR (500 MHz, $CDCl_3$) δ 9.93 (s, 1H,NCHN), 7.19 (br., s, 1H, C = CHN), 5.62 (d, J = 6.4 Hz, 1H, HNCO), 4.57 (dd, J = 12.0, 6.4 Hz, 1H, CHCOO), 4.01 (s, 3H, NCH_3), 3.97 (s, 3H, NCH_3), 3.82 (s, 3H, OCH_3), 3.26–3.32 (m, 1H, CH^aH), 3.13–3.22 (m, 1H, CHH^b), 1.42 (s, 9H, $(CH_3)_3$); 13C NMR (125 MHz, $CDCl_3$) δ 170.6 (*C*OOMe), 155.3 (NCOO), 137.1 (NCN), 131.6 (*C* = CH), 121.6 (C = *C*H), 80.6 (*C*Me_3), 53.1 (OCH_3), 52.0 (*C*COO), 36.8 (NMe), 34.3 (NMe), 28.1 ($(CH_3)_3$), 26.4 (CH_2).

2.2.2. Dichlorido[(p-cymene)-(1,3-dimethyl-N-boc-O-methylhistidin-2-ylidene)]ruthenium(II) **1b**

1,3-Dimethyl-*N*-boc-*O*-methylhistidinium iodide **5** (0.225 g, 0.53 mmol) and Ag_2O (0.061 g, 0.26 mmol) were stirred in CH_2Cl_2 (10 mL) at room temperature for 1 h. $[Ru(p\text{-cymene})Cl_2]_2$ (0.162 g, 0.26 mmol) was added, the resulting mixture was stirred for a further hour and then filtered through celite which was eluted with CH_2Cl_2. The filtrate was concentrated to 1 mL and diethyl ether (50 mL) was added to afford complex **1b** as an orange solid (0.230 g, 72%) of m.p. > 120 °C (decomp.); $C_{24}H_{37}Cl_2N_3O_4Ru$ (603.54) requires: C, 47.76; H, 6.18; N, 6.96%. Found: C, 47.45; H, 6.17; N, 6.47%; 1H NMR (400 MHz, $CDCl_3$) δ 6.79 (s, 1H, C = CHN), 5.41–5.39 (m, 2H, CH^{cym}), 5.22–5.17 (m, 1H, CHCOO), 5.12 (d, J = 5.8 Hz, 2H, CH^{cym}), 4.61–4.57 (m, 1H, CH^aH), 3.93 (s, 3H, NCH_3), 3.91 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 3.17–3.11 (m, 1H, CHH^b), 2.99–2.91 (m, 1H, *CH*Me_2), 2.07 (s, 3H, $C^{cym}CH_3$), 1.44 (s, 9H, $(CH_3)_3$), 1.26 (d, J = 7.1 Hz, 6H, $C(CH_3)_2$); 13C NMR (101 MHz, $CDCl_3$) δ 174.1 (CRu), 171.6 (NHCO, 155.3 (COO), 130.7 (*N*C = CH), 122.2 (NC = *C*H), 109.3 (C^{cym}), 98.9 ($C^{cym}CH_3$), 85.1 ($CH^{cym}CCH$), 85.0 ($CH^{cym}CCH$), 82.8 ($CH^{cym}CCH_3$), 80.5 (*C*Me_3), 53.6 (*C*HCOO), 53.0 (OCH_3), 39.6 (NCH_3), 36.7 (NCH_3), 30.8 ($C^{cym}CH$), 28.4 ($(CH_3)_3$), 28.3 (CH_2), 22.6 ($CH(CH_3)_2$), 18.8 ($C^{cym}CH_3$); MS (EI): m/z 503 (11%, –Boc), 429 (16), 355 (21), 281 (13), 221 (19), 134 (24), 119 (100), 85 (30), 57 (93), 43 (62).

2.2.3. Diiodido[(cyclohexylamine)-(1,3-dimethyl-N-boc-O-methylhistidin-2-ylidene)]platinum(II) ***3b***

Diiodido[(pyridine)-(1,3-dimethyl-N-boc-O-methylhistidin-2-ylidene)]platinum(II) ***6***: 1,3-Dimethyl-*N*-boc-*O*-methylhistidinium iodide **5** (425 mg, 1.0 mmol), sodium iodide (1.50 g, 10 mmol), potassium carbonate (1.38 g, 10 mmol) and $[PtCl_2]_n$ (0.266 g, 1.0 mmol) were suspended in pyridine (20 mL). The mixture was sonicated for 45 min and then heated at 100 °C for 72 h. The solvent was removed under reduced pressure and the residue was diluted with CH_2Cl_2 (30 mL) and filtered. The filtrate was evacuated to yield a crude product which was purified by column chromatography (silica gel 60; eluent CH_2Cl_2/MeCN 2:1) to give 0.453 g (55%) of diiodido[(pyridine)-(1,3-dimethyl-*N*-boc-*O*-methylhistidin-2-ylidene)]platinum(II) as a yellow solid. An analytically pure sample was obtained after recrystallization from MeOH/Et_2O, m.p. 91 °C; $C_{19}H_{28}I_2N_4O_4Pt$ (841.38) × MeOH requires: C, 28.02; H, 3.76; N, 6.53%. Found: C, 28.29; H, 3.11; N, 6.28%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.05 (dt, J = 4.9, 1.5 Hz, 2H, $H^{py,ortho}$), 7.73 (tt, J = 7.6, 1.5 Hz, 1H, $H^{py,para}$), 7.29–7.39 (m, 2H, $H^{py,meta}$), 6.67 (s, 1H, C = CH), 5.17 (d, J = 7.0 Hz, 1H, NH), 4.53–4.64 (m, 1H, CHCOO), 3.91 (s, 3H, NMe), 3.90 (s, 3H, NMe), 3.77 (s, 3H, OMe), 2.99–3.13 (m, 2H, CH_2), 1.47 (s, 9H, $(CH_3)_3$); ^{13}C NMR (125 MHz,