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A general strategy to add diversity to ruthenium arene complexes with bioactive organic compounds *via* a coordinated (4-hydroxyphenyl) diphenylphosphine ligand†

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Esterification of (4-hydroxyphenyl)diphenylphosphine, coordinated to the [Ru(η^6-*p*-cymene)Cl_2] fragment, allows a series of bioactive carboxylic acids to be introduced directly into the organometallic molecule. Evaluation of the compounds on human ovarian cancer cells reveals synergistic enhancements in their antiproliferative activity relative to their bioactive organic and organometallic precursors.

Ruthenium compounds are promising candidates as anticancer drugs that potentially overcome the limitations exhibited by the platinum-based chemotherapics currently used in the clinic.[1] For example, [imidazolium][*trans*-Ru(*N*-imidazole)(*S*-dmso)Cl_4], NAMI-A, and [indazolium]*trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)], KP1019, have undergone clinical evaluation.[2] In addition to these Ru(III) coordination complexes, organometallic Ru(II) complexes based on the Ru(η^6-arene) scaffold have attracted considerable interest.[3] In this context, ruthenium arene complexes comprising 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane (PTA, affording RAPTA complexes)[4] or ethylene-1,2-diamine[5] as ligands have emerged as among the most interesting species with relevant antitumor properties.[6] It should be noted that several RAPTA analogues in which the PTA is replaced with triphenylphosphine-derived ligands have been studied.[7,8] Although the lipophilicity of these phosphines result in a decrease in water solubility of the complex, the more hydrophobic phosphines enhance cellular uptake and cytotoxicity.[7a,b]

With the aim of modulating the biological activity of Ru(η^6-arene)-type complexes, a number of molecules with a known biological/pharmacological function have been tethered to this unit.[9] Such compounds have been usually realized by inclusion of prior-functionalized arene species,[10] or by binding appropriately modified *O*,*O*,[11] *N*,*O*(*S*)[12] and *N*,*N*[13] chelate, or monodentate P[8] and N ligands[8,14] to the ruthenium(II). Direct modification of a coordinated arene ligand has also been realized using suitable protecting groups in order to suppress undesired reactions at the ruthenium centre during the coupling process.[15]

Herein we describe an alternative strategy,[16] in which a generic Ru(η^6-arene)Cl_2 compound with a reactive phosphine ligand can be modified, to introduce diverse bioactive organic components (Chart 1). The selected carboxylic acids are known to exert a biological function and some of them were previously incorporated within anticancer metal compounds.[17–22]

The esterification reactions were conveniently carried out directly on complex **1**, which includes a (4-hydroxyphenyl) diphenylphosphine ligand,[23] with the complex tolerating the reaction conditions negating the need of protecting strategies (Scheme 1).

The synthetic approach (Scheme 1) avoids manipulation and purification procedures of non coordinated (4-hydroxy-

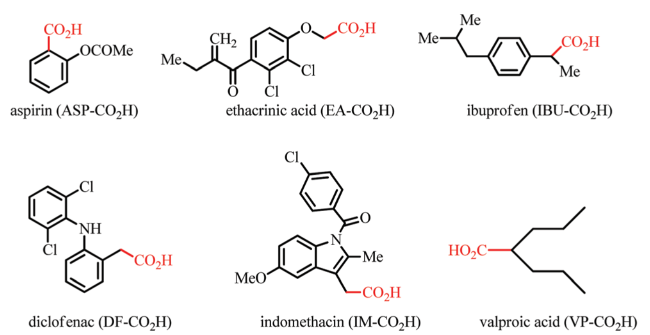

Chart 1 Bioactive carboxylic acids used in this work.

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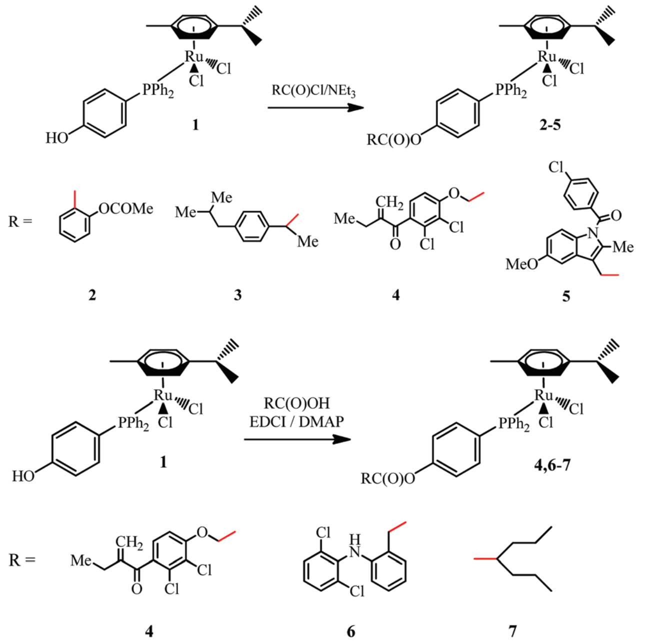

Scheme 1 Preparation of ruthenium compounds with bioactive organic fragments **2–7** from the starting complex **1**.

phenyl)diphenylphosphine and of its ester derivatives, which are air sensitive (see ESI† for details).

Complexes **2–7** were isolated in *ca.* 60–80% yield after purification using either chromatographic separation or extraction from CH_2Cl_2/H_2O. Complexes **2** and **3** are functionalized with aspirin and ibuprofen, respectively, which have been conjugated to cisplatin analogues[17b,19a] and other metals,[17a,19b] leading to more efficient anticancer behaviour.

Complexes **2–7** were characterized by analytical and spectroscopic techniques (see ESI† for full details). They all exhibit an IR absorption in the region 1740–1780 cm^{-1} due to the ester group. The ^{31}P NMR spectra contain a unique resonance around 24 ppm. The salient feature in the ^{13}C NMR spectra corresponds to the resonance emanating from the ester carbon, being at *ca.* 9 ppm higher frequency with respect to that observed for the uncoordinated carboxylic acids. The molecular structures of **1** and **2** were determined by single crystal X-ray diffraction and are shown in Fig. 1 and 2, together with relevant bonding parameters.

Compounds **1** and **2** comprise the expected three-leg piano-stool geometry typical of other ruthenium(II)-arene compounds.[24] The bonding parameters around the Ru(II) centres are similar to those reported for $[RuCl_2(p\text{-cymene})(PAr_3)]$ structures.[8,25] The bonding parameters of the acetylsalicylic acid ester in **2** are not significantly different to those in other reported structures.[26]

The cytotoxicity of **1–7** and the bioactive precursors was assessed in human ovarian A2780 and A2780CisR cancer cells, the latter having acquired resistance to cisplatin, and human embryonic kidney HEK-293 cells using the MTT assay (Table 1).

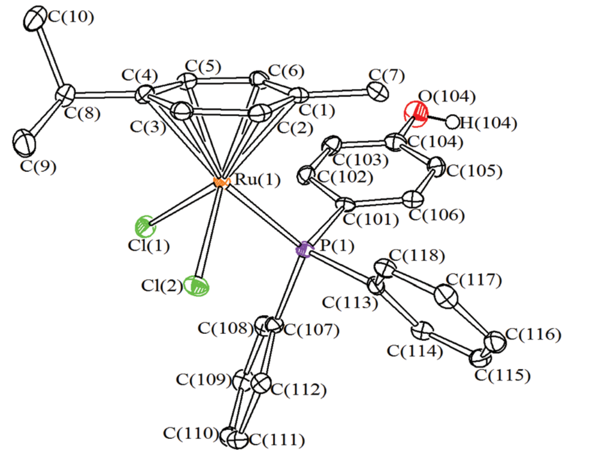

Fig. 1 Structure of **1** with key atoms labelled. Displacement ellipsoids are at the 50% probability level. C–H hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (°): Ru(1)–(η^6-*p*-cymene)$_{av}$ 2.217(10), Ru(1)–Cl(1) 2.4193(9), Ru(1)–Cl(2) 2.4106(9), Ru(1)–P(1) 2.3584(9), C(104)–O(104) 1.349(5), Cl(1)–Ru(1)–Cl(2) 87.69(3).

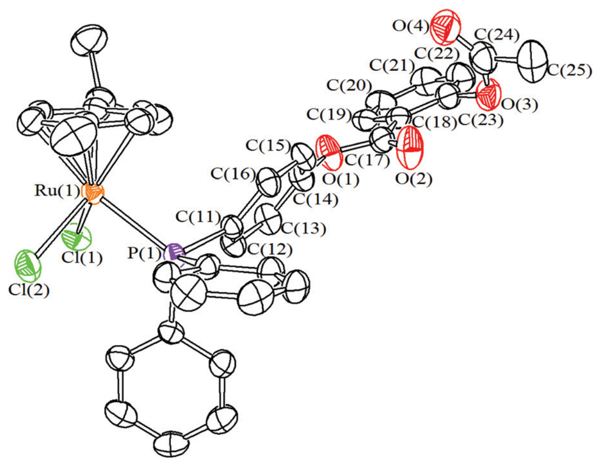

Fig. 2 Structure of **2** with key atoms labelled. Hydrogen atoms are omitted for clarity. Displacement ellipsoids are at the 50% probability level. Selected bond lengths (Å) and angles (°): Ru(1)–(η^6-*p*-cymene)$_{av}$ 2.215(10), Ru(1)–Cl(1) 2.4038(11), Ru(1)–Cl(2) 2.4223(11), Ru(1)–P(1) 2.3741(11), C(14)–O(1) 1.391(5), O(1)–C(17) 1.359(5), C(17)–O(2) 1.179(5), C(23)–O(3) 1.399(6), O(3)–C(24) 1.354(6), C(24)–O(4) 1.189(6), C(24)–C(25) 1.496(8), Cl(1)–Ru(1)–Cl(2) 89.33(4), C(14)–O(1)–C(17) 121.3(4), sum at C(17) 360.0(7), C(23)–O(3)–C(24) 117.6(4), sum at C(24) 360.0(9).

Compounds **2–4** and **6–7** are more cytotoxic to the A2780 cell line relative to the parent ruthenium compound **1** and the corresponding bioactive carboxylic acids. In particular, **3** and **7**, derivatised with ibuprofen or valproic acid, show cytotoxicity in the low micromolar range against the A2780 cell line, with approximately 5 fold lower IC_{50} values compared to **1**. Compounds **2**, **3** and **7** show a marked increase in activity compared to ASP-CO_2H, IBU-CO_2H and VP-CO_2H, respectively. Compound **4** is 2-fold more cytotoxic than EA-CO_2H, and **6** is 5-fold more active than DF-CO_2H against the A2780 cell line. In contrast, a *ca.* 6-fold loss in activity is observed for **5**

Table 1 IC_{50} values (μM) determined for **1–7** and other relevant control compounds on human ovarian carcinoma (A2780), human ovarian carcinoma cisplatin resistant (A2780CisR) and human embryonic kidney (HEK-293) cell lines after 72 h exposure. Values are given as the mean ± SD

Compound	A2780	A2780CisR	HEK-293
1	50 ± 2	67.9 ± 0.2	69 ± 1
2	21 ± 1	32 ± 2	24 ± 1
3	11.6 ± 0.3	14 ± 2	7.9 ± 1.1
4	19.1 ± 0.1	38 ± 1	3.8 ± 0.4
5	173 ± 2	>200	>200
6	41 ± 3	74 ± 8	61 ± 5
7	9 ± 1	10.4 ± 1.3	7.4 ± 1.2
L2═O	>200	>200	>200
ASP-CO$_2$H	>200	>200	>200
IBU-CO$_2$H	>200	>200	>200
EA-CO$_2$H[8b]	40 ± 3	53 ± 5	—
IM-CO$_2$H[8a]	27 ± 2	112 ± 1	67 ± 1
DF-CO$_2$H[8a]	202 ± 16	84 ± 3	186 ± 14
VP-CO$_2$H[27]	>100	>100	63 ± 38
RAPTA-C[28]	230	270	>1000
Cisplatin	1.9 ± 0.7	23 ± 3	9 ± 1

compared to IM-CO$_2$H against the A2780 cell line. Complexes **1–7** are all less cytotoxic to the cisplatin resistant A2780CisR cells, and do not display appreciable cancer cell selectivity, *i.e.* the IC_{50} values in the tumorigenic and non-tumorigenic HEK-293 cell lines are similar.

Spectroscopic and conductivity measurements (see ESI†) indicated partial release of the phosphine ligand over 72 hours, when **1–7** were maintained in dmso/water solutions at 37 °C. Thus, the antiproliferative activity of the complexes is mainly due to phosphine-bound Ru species (note that **L2═O** was inactive against the cell lines). The cleavage of the ester bond linking the bioactive group to the phosphine moiety was not observed in all the compounds, however, once inside a cell esterases could cleave the ester bond to separate the bioactive fragment from the ligand/complex.[29] In summary, a versatile method that allows bioactive organic compounds to be directly incorporated into a ruthenium(II)-arene structure has been developed. Remarkably, the chloride ligands in the complex are not affected by the reaction facilitating the direct transformation and negating the need of protecting groups employed elsewhere.[15] The complexes are more cytotoxic than the ruthenium(II)-arene precursor and the bioactive organic compounds. It seems likely that the present approach could be extended to many other metal-based systems, allowing the rapid synthesis of bioactive organometallic and metal–organic compounds with structural diversity.

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