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Organometallic anticancer agents that interfere with cellular energy processes: a subtle approach to inducing cancer cell death.

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Two hybrid compounds comprising an antimetastatic ruthenium-arene fragment tethered to an indazole-3-carboxylic acid derivative that inhibits aerobic glycolysis in cancer cells have been prepared and evaluated in a variety of cancer cell lines, including highly relevant human glioblastoma cells, with an apparent synergistic action between the two components observed.

Metallocenes were evaluated for anticancer activity, following the introduction of cisplatin into the clinic,[1, 2] with titanocene dichloride being identified as a promising drug candidate.[3] It was subsequently evaluated in numerous in vitro and in vivo models and even in clinical trials on patients, but finally was not approved for use.[4] Jaouen later showed that derivatization of ferrocene with biologically active tamoxifens affords compounds that enhance the activity of the organic molecule leading to a strong cytotoxic effect in hormone-independent breast cancer cells (MDA-MBA231), where hydroxytamoxifen and ferrocene are inactive.[5, 6]

Following the early studies by Fish on the aqueous chemistry of transition metal-arene compounds and their reactivity with nucleobases, nucleosides and nucleotides,[7-9] we became interested in the application of ruthenium(II)-arene compounds in medicine. The complex Ru(η^6-*p*-cymene)(pta)Cl_2 (pta = 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane), termed RAPTA-C, is endowed with antitumour,[10] antimetastatic[11] and antiangiogenic[12] properties in vivo. These compounds exert their anticancer effect in a rather subtle manner; the compounds are not particularly cytotoxic, nor binding strongly to DNA, but rather interacting with protein targets with moderate binding constants leading to the interference of various cellular processes and pathways. A crystal structure of RAPTA-C bound to the nucleosome core particle reveals exclusive binding to the histone protein core via substitution of both chloride ligands, emphasizing the importance of the two labile chlorides in the RAPTA structure.[13]

The pta ligand may be replaced by other ligands including sugar-based phosphites that potentially target tumours[14-16] and imidazole ligands (Fig. 1).[17] The choice of the latter ligand was inspired by NAMI-A, one of the two ruthenium(III) coordination complexes currently undergoing clinical trials, which contains an imidazole ligand amongst others.[18, 19] Moreover, the cytotoxicity of ruthenium(II)-arene complexes can be increased by covalently linking organic inhibitors of proteins that are responsible for drug resistance in tumours via the imidazole group.[20, 21] This rather simple approach to ligand design, i.e. modifying an imidazole with a biologically active organic group, appears to be very effective provided appropriate bio-active compounds are selected. Related approaches have been reported by others.[22-27]

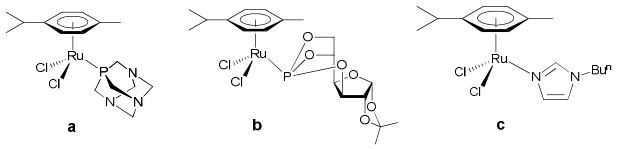
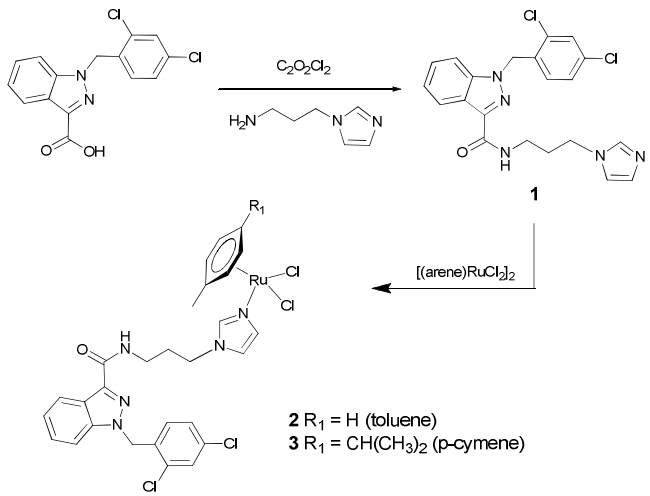


Fig. 1 The structures of RAPTA-C (**a**), an example of a sugar-based phosphite analogue (**b**) and an imidazole derivative, termed Ru-A (**c**).

In cancer chemotherapy drug combinations are almost always applied[28-30] and in this sense the organometallic and organic components can be viewed as a covalently linked drug combination. In this context, we hypothesized that it may be worthwhile combining the ruthenium(II)-arene dichloride unit with aerobic glycolysis inhibitors, and for this purpose lonidamine, [1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid], was selected. Lonidamine inhibits aerobic glycolysis in cancer cells while simultaneously enhancing aerobic glycolysis in normal cells,[31] thus providing a potentially highly selective ruthenium drug molecule. In this paper we describe the synthetic strategy adopted to afford the ruthenium(II)-lonidamine hybrid derivatives together with an account of their characterization and preliminary biological evaluation in relevant cancer cell lines.



Scheme 1 Synthesis of **1**, **2** and **3**.

Table 1 Cytotoxicity of lonidamine, **1** - **3**, RAPTA-C and cisplatin towards the ovarian cancer cell lines A2780 and A2780R and the human glioblastoma cell lines LN18, LN229, LNZ308, HEK and cortex neurons.

. Compound	Cell line, IC_{50} (72 h) /μM						
	A2780	A2780R	LN18	LN229	LNZ308	HEK	cortex neurons[a]
lonidamine	206.58±9.44	197.87±20.77	>50	>50	>50	350.76±39.60	89.4±6.0
1	10.72±1.59	11.56±2.41	9.4±2.8	6.4±0.9	12.5±3.5	20.63±5.81	21.9±5.4
2	20.81±6.16	24.12± 7.10				30.12±6.97	31.3±11.8
3	19.34±4.08	17.9±4.04	6.4±2.1	8.3±2.4	5.7±0.9	20.50±6.12	20.2±5.1
RAPTA-C	>250	>250					
Ru-A[21]	>100	>100					
cisplatin[32]	9.5 ± 2.4	31.5 ± 3.4					

[a] % from control after incubation for 72 h with 30.0 μM drug

The lonidamine-modified imidazole ligand, **1**, was prepared via reaction between the acid-chloride of lonidamine, prepared in situ, and N-(aminopropyl)imidazole (see Scheme 1 and Supporting Information for full details). Subsequent reaction of **1** with the ruthenium(II) arene dimers, [Ru(η^6-arene)Cl$_2$]$_2$ (arene = $C_6H_5CH_3$ toluene and *p*-$C_6H_4CH_3CH(CH_3)_2$ *p*-cymene), affords complexes **2** and **3**.

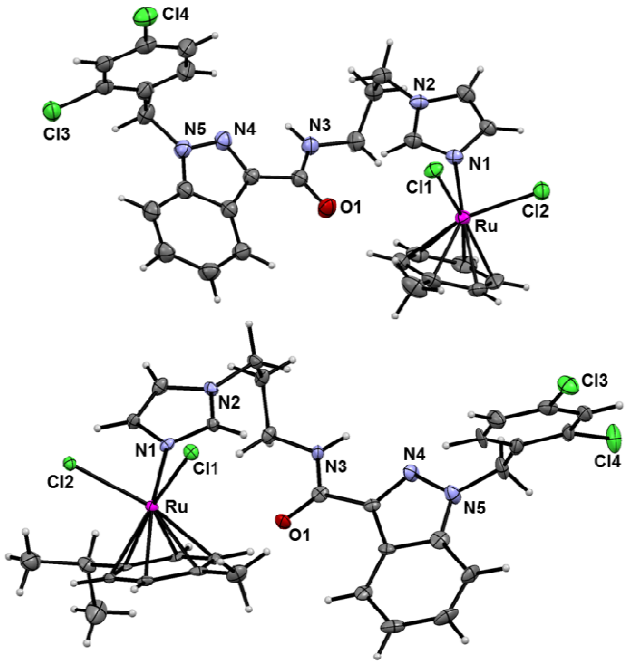


Fig. 2 Structures of (top) **2** and (bottom) **3** (one of 3 independent molecules in the asymmetric unit). Key bond lengths (Å) and angles (deg.) for **2**: Ru-arene centroid 1.658(4) , Ru-Cl1 2.4298(7), Ru-Cl2 2.4257(7), Ru-N1 2.108(2), Cl1-Ru-Cl 2 88.98(2), Cl1-Ru-N1 84.61(6), Cl2-Ru-N187.62(6) . For **3** (for structure on the figure): Ru-arene centroid 1.663(7), Ru-Cl1 2.4404(13), Ru-Cl2 2.4356(13), Ru-N1 2.118(4), Cl1-Ru-Cl2 89.24(4), Cl1-Ru-N1 85.05(12), Cl2-Ru-N1 85.21(11).

The ^{1}H and ^{13}C{^{1}H} NMR spectra and other data confirm the formation of the expected products. The structures of **2** and **3** were determined in the solid state on crystals grown from CH_2Cl_2/Et_2O mixtures. Crystallographic details are provided in the Supporting Information. The structures of **2** and **3** are combined with an organic inhibitor that has a very specific function.[20, 21]

To obtain more information on the cancer cell selectivity of **1** – **3** the compounds were evaluated on immortalised non-tumorigenic HEK cells and primary neuronal cultures of the cerebral cortex from Wistar rats (Table 1). A moderate preference towards the malignant cells, which is more pronounced for the relevant human glioblastoma cell lines, is observed.

shown in Fig. 2 and key bond parameters are listed in the caption. The geometry around the ruthenium(II) ion in both compounds are similar, with the expected 'piano-stool' structure composed from the arene ligand, lonidamine-derivatized imidazole and two chloride ligands. The structures show that the lonidamine moiety is attached to the imidazole group in the expected fashion, and moreover, the lonidamine structure is unperturbed relative to that of the free molecule.[33]

The cytotoxicity of lonidamine, **1-3** were evaluated against the A2780 and A2780cisR ovarian cancer cell lines, the latter having acquired resistance to cisplatin (Table 1). These two cell lines have been extensively used to evaluate the in vitro anticancer activity of ruthenium-based compounds and therefore allows comparisons to other classes of compounds to be made. Complexes **2** and **3** are markedly more cytotoxic than lonidamine and RAPTA-C or Ru-A ndicating that the two units operate together in a concerted fashion. Nevertheless, compared to cisplatin the complexes are less cytotoxic. However, their IC_{50} values in these cell lines are comparable to those of other promising ruthenium-based therapeutics.[34, 35]

Since the *p*-cymene derivative, **3**, is slightly more cytotoxic than **2**, and displays superior stability, this compound was evaluated further together with that of **1** in three human glioblastoma cell lines (Table 1) of direct relevance to lonidamine that is being clinically evaluated against brain cancers.[36]

Compared to lonidamine which showed essentially no activity (> 50 μM) towards the human glioblastoma cells **1** and **3** display excellent cytotoxicity, particularly for **3**. It is interesting to note that incorporation of the propyl-imidazole group to the lonidamine structure results in a superior cytotoxic effect, further enhanced in some cell lines by coordination to the ruthenium(II)-arene fragment. It has previously been shown the cytotoxicity of compounds can be enhanced when coordinated to the ruthenium(II)-arene unit as the broad therapeutic action of the organometallic unit, that can covalently (coordinate) bind to a range of biological targets operates more effectively when

Conclusions

The hybrid complex, **3**, based on lonidamine tethered to the ruthenium(II)-*p*-cymene unit via an imidazole group displays highly relevant cytotoxicites in human glioblastoma cell lines for which better chemotherapeutics are urgently required. This compound also exhibits a degree of selectivity towards these cells relative to primary neuronal cultures of the cerebral cortex. Lonidamine has been used in the treatment of brain tumours in combination with radiotherapy and temozolomide resulting in the inhibition of tumour growth, with lonidamine reducing the dose of temozolomide required for radiosensitization of brain tumours.[37] The hybrid system described herein represents another encouraging approach that will be studied further.

Notes and references

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† Electronic Supplementary Information (ESI) available: [materials and methods, synthetic procedures, NMR, X-ray diffraction data and cell culture and inhibition of cell growth]. See DOI: 10.1039/b000000x/
Crystallographic data for the structural analysis of **2** and **3** has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 897551 and 897552. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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