Cytotoxicity of Ruthenocene-containing β-Diketones

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Abstract. *Background: Ferrocene-containing β-diketones and cisplatin, $[(NH_3)_2PtCl_2]$, possess strong antineoplastic activity. No information is available regarding the anticancer activity of the corresponding ruthenocene complexes. This study examined the cytotoxicity of stable ruthenocene-containing β-diketones. The results were related to the cytotoxicity of cisplatin and the ease of ruthenium electrochemical oxidation. Materials and Methods: The ruthenocene-containing β-diketones $RcCOCH_2COR$ where $Rc=Ru^{II}(C_5H_5)(C_5H_4)$ and $R=CF_3$ (**1**), CH_3 (**2**), $Ph=C_6H_5$ (**3**) and $Fc=Fe^{II}(C_5H_5)(C_5H_4)$ (**4**) were tested for cytotoxicity against HeLa (human cervix epithelioid) cancer, COR L23 (human large cell lung carcinoma) and the platinum-resistant CoLo 320DM (human colorectal) and COR L23/CPR cancer cell lines. Cell survival was measured by means of the colourimetric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) assay. Results: The 50% cell growth inhibition (IC_{50}) values of **1**-**4** towards the cells ranged between 8.2 and 84.6 μmol dm^{-3}, with **1** being the most cytotoxic complex. Drug activity was directly proportional to the electron density on the ruthenium centre as well as the oxidation potential of the ruthenium core but inversely proportional to the pK_a of the β-diketones. The strongest activity was observed against the COR L23 cell line, and the weakest activity against COR L23 CPR. Conclusion: A drug activity–structural relationship exists for ruthenocene-containing β-diketones in that drugs with the lowest electron density on the ruthenium centre are more cytotoxic. Compounds with larger ruthenium oxidation potentials and stronger acid strength (i.e. smaller pK_a values) are more cytotoxic.*

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High excretion rates from the body, the development of drug resistance after continued drug dosage, high toxicity, especially to the kidneys and bone marrow, and, perhaps most importantly of all, the inability to distinguish between healthy and carcinomatous cells, are some of the negative side-effects that cisplatin (1, 2) and many other chemotherapeutic drugs suffer from. As a consequence of this, new antineoplastic agents are continuously being synthesised and evaluated (3, 4), combinatorial therapies are being investigated in the hope of finding synergistic effects (5), new methods of delivering an active drug to a malignant growth are developed (6-8) and new techniques for cancer treatment, such as photodynamic cancer therapy (9), are being investigated.

It has been shown that free ferrocene-containing β-diketones (10) are more active than cisplatin against platinum resistant CoLo 320DM (human colorectal) and COR L23/CPR (human large lung cell) cancer cell lines. In contrast, carboxylato complex of ruthenium (11) were found to be two to four orders of magnitude less active than cisplatin.

In this study, we determined and compared the *in vitro* cytotoxicity of ruthenium-containing β-diketones **1**-**4** and diruthenium tetrakisacetate, **5**. The relationship between cytotoxicity and β-diketone acid strength expressed as pK_a values (12), ruthenium oxidation potentials, E_{pa} (12), and electron density on the ruthenium centre expressed as a function of group electronegativities of the R group, χ_R (13), are also reported.

Materials and Methods

Compounds. Complexes **1**-**4** (Figure 1) were synthesised according to published procedures (12). Cytotoxic results for **5** and cisplatin under the same conditions were described elsewhere (8, 11).

Sample preparation. The samples were dissolved in dimethyl sulphoxide (DMSO) giving stock concentrations of 20 mmol dm^{-3} and diluted in the appropriate growth medium supplemented with foetal calf serum (FCS) to give final DMSO concentrations not exceeding 0.5% and drug concentrations of 20-2,000 μmol dm^{-3} prior to the cell experiments.

Cell cultures. The human colorectal cell lines, CoLo 320DM (ATCC CCL-220) (American Type Culture Collection, Manassas, VA, USA),

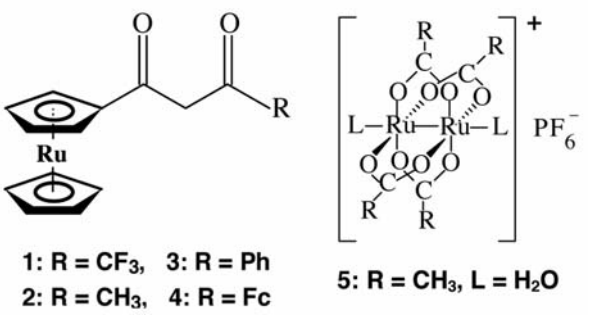

Figure 1. *Structure of ruthenocene-containing β-diketones **1**-**4** and the ruthenium carboxylate **5**. Ph=phenyl=C_6H_5, Fc=ferrocenyl=$Fe^{II}(C_5H_5)(C_5H_4)$.*

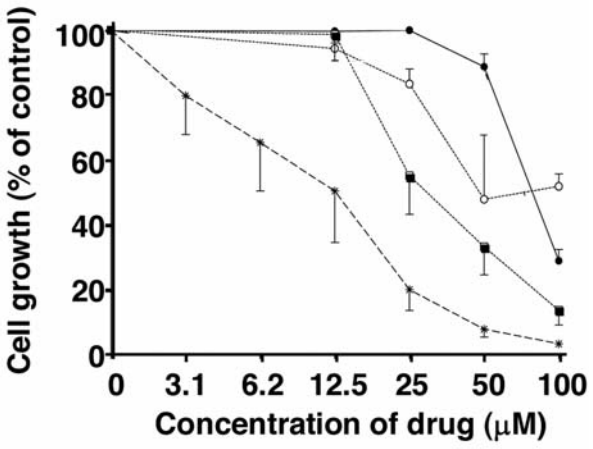

Figure 2. *Effect of concentration of **1** (*), **2** (■), **3** (●), and **4** (○) on the survival of COR L23 CPR cancer cells. Data are presented as the mean drug concentration±standard error of the mean of four experiments.*

was grown as a suspended culture in RPMI-1640. The human cervix epitheloid cancer cell line, HeLa (ATCC CCL-2) (American Type Culture Collection), human large cell lung carcinoma cell line, COR-L23 (ECACC 92031919) (European Collection of Animal Cell Cultures, Salisbury, Witshire, UK) and a cisplatin-resistant sub-line, COR-L23/CPR (ECACC96042336) (European Collection of Animal Cell Cultures), were grown as monolayer cultures using Eagle's minimum essential medium (MEM) in the case of HeLa cells and RPMI-1640 in the case of the other cell lines. The growth media were maintained at 37°C under 5% CO_2 and fortified with 10% FCS, and 1% penicillin and streptomycin. The cells were seeded at 400 cells/well in 96 well microtiter plates in a final volume of 200 μl of growth medium in the presence or absence of different concentrations of the experimental drugs. Appropriate solvent control systems were included.

In accordance with previous studies (10), incubation at 37˚C was allowed for 7 days before cell survival was measured by means of the colourimetric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazodium bromide (MTT) assay (14). The plates were read on a spectrophotometer at 570 nm with a reference wavelength of 630 nm. Wells without cells and with cells but without drugs were included as controls. Survival curves were plotted (Figure 2) as a function of drug dose and the drug concentration that caused 50% inhibition of cell growth (IC_{50}) was estimated by extrapolation.

Results

The cell growth-inhibitory properties of **1**-**5** are summarised in Table I. β-Diketone IC_{50} values ranged between 8.2 and 84.6 μmol dm^{-3}. Lower IC_{50} values correspond to more active compounds. The most active drug was found to be the fluorinated β-diketone **1**, while the COR L23/CPR cell line was the most resistant to the tested drugs. For this cell line, the cytotoxicity of complex **1** was the same as that of cisplatin, but for the other cell lines, cisplatin was slightly more cytotoxic than complex **1**. $RcCOCH_2COCH_3$, **3**, was up to 8 times less active than the CF_3 complex **1** and the least cytotoxic in the β-diketone series. All β-diketones were much more cytotoxic than the carboxylate salt $[Ru_2(CH_3COO)_4.2H_2O][PF_6]$, **5**. Complex **1** was two orders of maginitude (50-100 times) more cytotoxic than **5**.

Discussion

In this study, the electron density on the ruthenium centre of the Rc group of β-diketones **1**-**4** was manipulated by introducing electron-withdrawing or electron-donating R substituents to the β-diketone $RcCOCH_2COR$. It was previously shown that the electron density on the ruthenium core is a function of the electronegativity, χ_R, of each R group (12). Complex **1**, which contained the strongest electron-withdrawing group, CF_3 (χ_{CF3}=3.01), in its structure, is the complex with the electron-poorest ruthenium centre. This compound was also found to be the most cytotoxic (Table I). Because complex **4** with R of ferrocenyl has the strongest electron-donating R-group in its structure (χ_{Fc}=1.87), the ruthenium centre of complex **4** should be the most electron-rich and therefore it should possess the lowest cytotoxicity of the compound series **1**-**4**. However, the CH_3 derivative **3** (χ_{CH3}=2.34) was less cytotoxic than **4**, Table I. To account for this observation, the known high cytotoxicity of ferrocene-containing β-diketones is noted (10). It was concluded that a co-operative effect exists between the cytotoxic ferrocenyl and ruthenocenyl groups in **4** that overshadows any deactivation effect of the ruthenium centre that the electron-donating ferrocenyl group may induce. Since the CH_3 group does not possess similar cytotoxic behaviour to the ferrocenyl group, complex **3** was found to be the least cytotoxic in the present β-diketone series. The cytotoxicity of the phenyl derivative, **2**, was unexpectedly high and did not fit the cytotoxicity trend set by the other compounds.

The relationship between β-diketone acid strength, pKa, and cytotoxicity for HeLa cells is shown in Figure 3. From the observed trend, in the absence of any synergistic effects between the ferrocenyl and ruthenocenyl groups, the cytotoxicity of **4**

Table I. *Cytotoxicity of $RcCOCH_2COR$ complexes **1**-**4**, the ruthenium carboxylate **5** and cisplatin expressed as IC_{50} (μmol dm^{-3}) values[a] after 7 days of incubation with the indicated cancer cell lines.*

Compound	χ_R	pK_a	E_{pa} (Volts)	HeLa	COR L23	COR L23/CPR	CoLo 320DM
1 (R=CF_3)	3.01	7.36	0.609	9.0	9.4	12.6	8.2
2 (R=Ph)	2.21	11.31	0.451	17.1	16.7	26.7	21.2
3 (R=CH_3)	2.34	10.22	0.473	70.5	67.6	84.6	68.2
4 (R=Fc)	1.87	12.90	0.484	54.7	49.2	48.0	67.4
5 $[Ru2(OOCCH_3)_4]^+$	-	-	–0.317[b]	950.0	nd	nd	230.0
Cisplatin	-	-	-	5.6	6.3	12.3	3.0

[a]Data are presented as mean drug concentration causing 50% inhibition of cell growth±standard error of the mean of four experiments; [b]A reduction potential (15). Nd, Not determined; χ_R, Gordy scale group electronegativities of each R group, data are from (13); pK_a, acid strength of β-diketones, data are from (12); E_{pa}, oxidation potentials of the Ru centre *vs*. Fc/Fc^+, data are from (12) and (15).

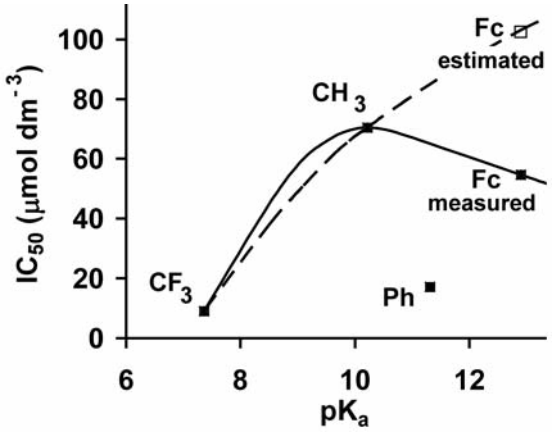

Figure 3. *pK_a-cytotoxicity relationship for β-diketones $RcCOCH_2COR$ with R=CF_3, Ph, CH_3 or Fc against HeLa cells. R-groups are noted next to each data point. The broken line estimates the IC_{50} value of **4** if there was no synergistic effect between the ferrocenyl and ruthenocenyl groups. Complex **2** with R=Ph was not fitted to the line.*

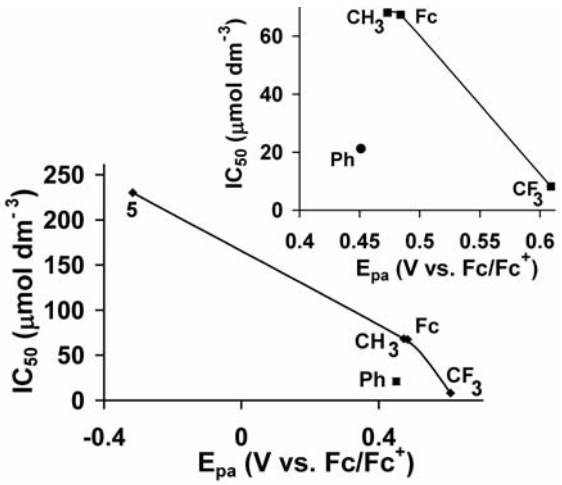

Figure 4. *Relationship between oxidation potential and cytotoxicity of $RcCOCH_2COR$ and $[Ru_2(OOCCH_3)_4.2H_2O][PF_6]$, **5**, against CoLo 320DM cells. Where relevant, R-groups are indicated next to each data point. Insert: β-diketone region only. Complex **2** with R=Ph was not fitted to the lines.*

would approach 100 μmol dm^{-3} or even higher rather than the observed 54.7 μmol dm^{-3}. Although the ferrocenyl group increased the expected cytotoxicity of **4** by probably at least two-fold, this cytotoxic activation was still not sufficient for **4** to become more cytotoxic than the CF_3 complex, **1**.

Figure 4 shows the relationship between ruthenium oxidation potentials, E_{pa}, and compound cytotoxicity for CoLo 320DM cancer cells. The highest cytotoxicity (lowest IC_{50} value) is associated with the largest oxidation potential. From this relationship the enhanced cytotoxicity of β-diketone ruthenium compounds over carboxylato ruthenium compounds **(11)** can be explained. The carboxylato ligands cause the ruthenium centre to have a very low redox potential compared to that of the β-diketones. This practically eliminates any useful cytotoxic properties the ruthenium centre may possess, probably because the ruthenium centres become too deactivated to become involved in electron transfer reactions with key biological molecules such as DNA.

Conclusion

The cytotoxicity of $RcCOCH_2COCF_3$ and cisplatin, $(H_3N)_2PtCl_2$, is comparable. Ruthenium-containing β-diketones are one to two orders of magnitude more cytotoxic than the corresponding ruthenium-containing acetate complex, **5**. Substituents that induce lower electron density on the ruthenium centre, such as CF_3, increase the cytotoxicity of the

$RcCOCH_2COR$ series of compounds substantially. A synergistic effect between the ruthenocenyl and ferrocenyl groups in $RcCOCH_2COFc$ cause this complex to be more cytotoxic than expected. The cytotoxicity of $RcCOCH_2COR$ complexes is directly proportional to the oxidation potential of the ruthenium centre of the ruthenocenyl group but inversely proportional to β-diketone acid strength (pK_a).

Acknowledgements

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