Cytotoxicity of Ferrocenyl–Ethynyl Phosphine Metal Complexes of Gold and Platinum

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Abstract. *Background: Ferrocene derivatives may possess antineoplastic activity. Those with low ferrocenyl reduction potentials often have the highest anticancer activity, as cell components have to oxidise them to the active ferrocenium species before cytotoxicity can be recorded. Some gold(I) complexes also possess anticancer activity. This study examined the cytotoxicity of ferrocenyl-ethynyl and ruthenocenyl-ethynyl complexes of gold and platinum. The results were related to the ease of iron oxidation in the ferrocenyl fragment and compared with the cytotoxicity of cisplatin, $[(H_3N)_2PtCl_2]$ and $[Au(PPh_2CH_2CH_2PPh_2)_2]Cl$. Materials and Methods: Ferrocene-containing gold and platinum complexes of the type Fc-C≡C-PPh_2, **1**, and Fc-C≡C-PPh_2→M with Fc=ferrocenyl ($Fe^{II}(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4)$), Ph=phenyl ($C_6H_5$) and M=Au-Cl, **2**, Au-C≡C-Fc, **3**, or Au-C≡C-Rc, **4** (Rc=ruthenocenyl, ($Ru^{II}(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4)$) and the complex $[(Fc\text{-}C{\equiv}C\text{-}PPh_2)_2PtCl_2]$, **5**, were investigated. Cytotoxicity tests were determined on the HeLa (human cervix epitheloid) cancer cell line, ATCC CCL-2. Cell survival was measured by means of the colorometric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide assay. Results: The IC_{50} values of compounds **1-4** from four experiments causing 50% cell growth inhibition, ranged between 4.6 and 27 μmol dm^{-3}. Drug activity was inversely proportional to the sum of all formal reduction potentials, $E^{o'}$, of the ferrocenyl groups of the Fc-C≡C-PPh_2 and Fc-C≡C- ligands coordinated to the gold centre. The Fc-C≡C-PPh_2→Au-Cl complex, compound **2**, was most cytotoxic with IC_{50}=4.6 μmol dm^{-3}, demonstrating the beneficial effect the Cl^- ion has on the cytotoxicity of these neutral gold complexes. The platinum complex $[(Fc\text{-}C{\equiv}C\text{-}PPh_2)_2PtCl_2]$, compound **5**, resembling the structure of cisplatin, in principle should exhibit good cytotoxicity, but was not tested due to its total insolubility in any biocompatible medium.*

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Potentially good chemotherapeutic drugs frequently find limited clinical use due to the many negative medical and physical side-effects they exhibit. For cisplatin, $[(H_3N)_2PtCl_2]$, such side-effects include *inter alia* poor aqueous solubility, a high excretion rate from the body, loss of appetite (anorexia), development of drug resistance after continued drug dosage, high toxicity especially to the kidneys and bone marrow, and, perhaps most important of all, the inability to distinguish between healthy and carcinomatous cells (1). To combat these negative side-effects, new antineoplastic materials are continuously being synthesised and evaluated (1), combination therapies are investigated in the hope of finding synergistic effects (2), new methods of delivering an active drug to a malignant growth are developed (3, 4) and new techniques of cancer treatment, such as photodynamic cancer therapy (5), are investigated.

In terms of new antineoplastic material, it has been shown that certain ferrocenium salts (6) have more favourable 50% lethal dosage values than cisplatin (7), while water-soluble ferrocenium-containing carboxylates (8) induce good-to-excellent cure rates against human adenocarcinoma, squamous cell carcinoma and large-cell carcinoma of the lung in *in vitro* human tumour clonogenic assays. It has also been shown that by anchoring the antineoplastic ferrocene derivative 3-ferrocenylbutanoic acid on a water-soluble polymeric drug carrier, an increase in drug activity of almost one order of magnitude is obtained (4).

The cytotoxicity of ferrocene-containing complexes is frequently dependent on the formal reduction potential of the ferrocenyl group. Related to ferrocene-containing alcohols, it was found that smaller $E^{o'}$ values lead to more

favourable (higher) cytotoxicity (9). In contrast, the free β-diketones $FcCOCH_2COR$ followed exactly the opposite trend (10). Two mechanisms by which the ferrocenyl group destroys antineoplastic growths were identified. The first was shown to involve homolytic action, *i.e.* radical-induced electron transfer processes between a ferrocenium group and water, *inter alia* to generate hydroxy radicals which cleave DNA strands (11). This implies that, after being administered to the body, a ferrocene-containing drug must first be oxidised by redox-active enzymes in the body to the ferrocenium species to show antineoplastic activity. Several redox-active enzymes and/or proteins are able to oxidise the iron(II) centre of the ferrocenyl group to iron(III) to liberate the positively charged radical ferrocenium species (12-16). It is, therefore, irrelevant whether the bioactive agent containing the ferrocenyl group is administered in the reduced ferrocenyl or oxidised ferrocenium state, provided that the formal reduction potential of the ferrocenyl group is low enough to allow ferrocenyl oxidation inside a cell. There are indications that the cut-off formal reduction potential of the ferrocenyl group where this cannot happen any more is 0.52 V *vs.* Fc/Fc^+ (9). $E^{o'}$ values of the ferrocenyl group of all four complexes **1-4** are less than 0.52 V (Table I), implying they may all have antineoplastic capability. In the second mechanism, the ferrocenyl group itself acts as a reducing agent when it reduces the tyrosyl radical of the R2 subunit of the enzyme ribonucleotide reductase (17). The active site of dimeric R2 consists of a tyrosil radical and two Fe(III) centers which are μ-oxo bridged (18, 19). This enzyme catalyses the reduction of ribonucleotides to deoxyribonucleotides, a key step in DNA syntheses (20) and its inactivation is, therefore, a goal in chemotherapy (21, 22).

Materials and Methods

Compounds. Complexes **1-5** (Figure 1) were synthesised according to published procedures (23).

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 cervix epitheloid cancer cell line, HeLa (ATCC CCL-2) (American Type Culture Collection, Manassas, VA, USA) was grown as monolayer cultures from MEM. The growth media were maintained at 37˚C under 5% CO_2 and fortified with 10% FCS and 1% penicillin and streptomycin.

Cancer cells were seeded at 500 cells/well in 96-well microtitre plates in a final volume of 200 μl of growth medium in the presence or absence of different concentrations of experimental drugs. Appropriate solvent control systems were included. After incubation at 37˚C for 7 days, cell survival was measured by means of the colourometric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) assay (24). 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.

Table I. *Chemosensitivity of complexes **1-7** expressed as IC_{50} (μmol dm-3) values[a] after 7 days of incubation with the HeLa cancer cell line. Formal reduction potentials, $E^{o'}$, of the ferrocenyl group are vs. a Fc/Fc^+ reference electrode.*

Compound	$E^{o'}$ (V)[b]	IC_{50} (μmol dm^{-3})
1 ($Fc\text{-}C{\equiv}C\text{-}PPh_2$)	0.300[c]	13+1
2 (**1**→AuCl)	0.350	4.6±0.7
3 (**1**→Au-C≡C-Fc)	0.000 (0.260)[d]	22±4
4 (**1**→Au-C≡C-Rc)	0.255 (0.270)[e]	27±4
5 [$(Fc\text{-}C{\equiv}C\text{-}PPh_2)2PtCl_2$]	[f]	[f]
6 Cisplatin [$(H_3N)_2PtCl_2$]	[f]	0.19±0.01
7 [$Au(PPh_2CH_2CH_2PPh_3)_2$]Cl	[f]	0.14±0.01

[a]Data are presented as mean drug concentration causing 50% inhibition of cell growth + standard error of the mean of four experiments. [b]Data from ref 23. [c]Unpublished data by E Fourie, JC Swarts and H Lang. [d]The first formal reduction potential at 0.000 V of this diferrocene-containing compound corresponds to the oxidation of the Fc group on the Au-C≡C-Fc molecular fragment. The second formal reduction potential is at 0.260 V and comes from the $Fc\text{-}C{\equiv}C\text{-}PPh_2$ molecular fragment. [e]The second $E^{o'}$ value is for the ruthenocenyl group, Rc. [f]Not determined due to lack of solubility, or the compound has no ferrocenyl group.

Results

Since it is known that ferrocene-based drugs often need an incubation period before they perform optimally in cell destruction, all the studies with complexes **1-4** were performed utilising seven days of drug exposure to the cells in accordance with previous studies (4, 9). The cell growth inhibitory properties of ferrocenyl alkynyl complexes **1-4** expressed as IC_{50} values are summarised in Table I and ranged between 4.6 and 27 μmol dm^{-3}. The lowest IC_{50} values correspond to the more active compounds. The most active drug was found to be the mono ferrocenyl, chloride-containing gold complex **2**. Complex **2**, Fc-C≡C-PPh2→Au-Cl with IC_{50}=4.6 μmol dm^{-3}, was three times more active than the free ligand $Fc\text{-}C{\equiv}C\text{-}PPh_2$, **1**, and clearly demonstrates the advantageous effect of introducing the gold(I) ion into anticancer drugs. Like the platinum(II) core of cisplatin [$(H_3N)_2PtCl_2$], the gold(I) centre also possesses a d^8 electronic configuration, although it exhibits a linear rather than square planar geometry. Drug activity was inversely proportional to the sum of the ferrocenyl formal reduction potentials, $E^{o'}$, of each compound.

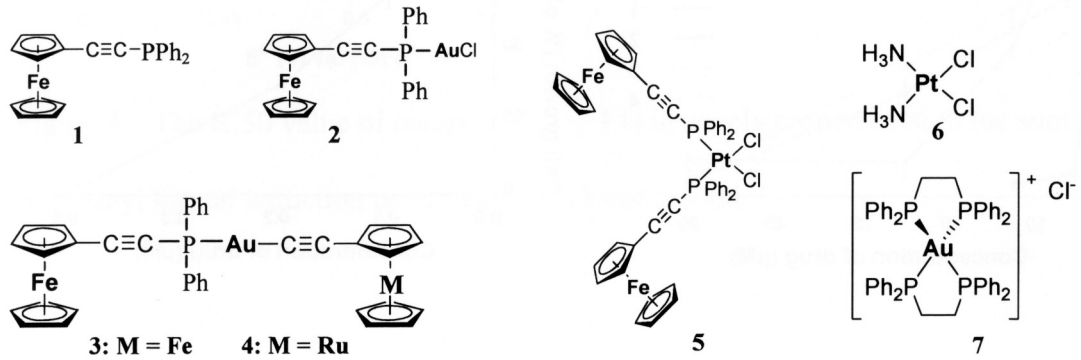

Figure 1. *Gold and platinum complexes. Ph, phenyl=C_6H_5.*

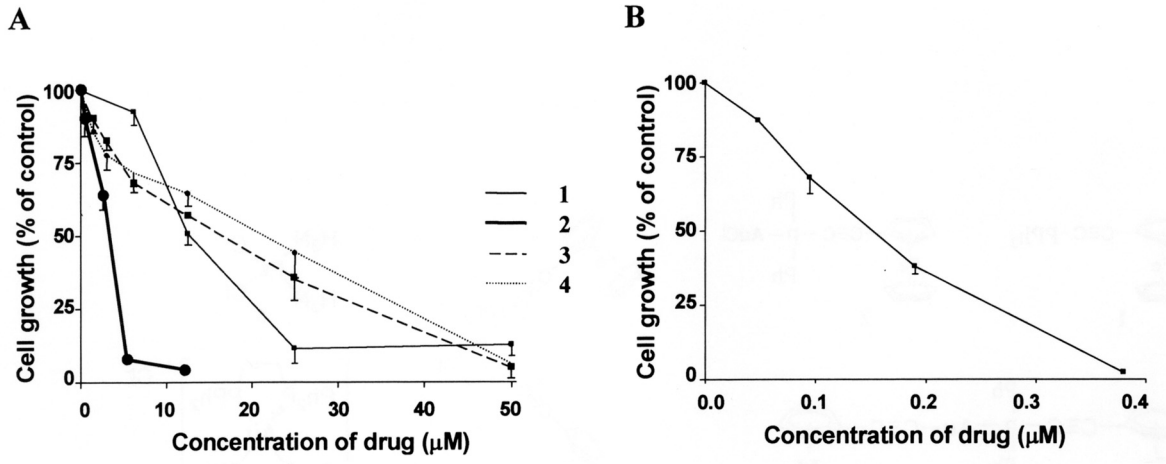

Figure 2. *Effect of concentration of compounds **1-4** (A) and compound **7**, [Au($PPh_2CH_2CH_2PPh_3$)$_2$]Cl, (B) on the survival of HeLa cancer cell line. Data are presented as the mean concentration±standard error of the mean of four experiments.*

Discussion

Surprisingly, replacement of the Cl^- ligand of the neutral complex 2 with another antineoplastic ferrocenyl group (Fc-C≡C- for complex **3**) or ruthenocenyl group (Rc-C≡C- for complex **4**) did not further enhance the activity of **2**. Compounds **3** and **4** were four and five times less reactive than compound **2**, respectively. This observation may be attributed to the greater reactivity of the Au-Cl moiety towards hydrolyses and disproportionation reactions in aqueous media. The alkynyl- and phosphine-gold bonds will not hydrolise as easily as an Au-Cl bond. The increased reactivity of the chloride species **2** was also mirrored by increased cytotoxicity of halogenated beta-diketonato complexes $FcCOCH_2COCF_3$ and $FcCOCH_2COCCl_3$ over the cytotoxicity of the halogen-free beta-diketonato complexes $FcCOCH_2COCH_3$ and the diferrocenylated complex $FcCOCH_2COFc$ (10).

Despite the promise that complex **5**, [(Fc-C≡C-PPh_2)$_2$$PtCl_2$], held as antineoplastic drug due to its similarity to cisplatin, [$(H_3N)_2PtCl_2$, complex **6**], and because it contains more than one antineoplastic active moiety (the –$PtCl_2$ and ferrocenyl centres), all attempts to determine the cytotoxicity of this complex met with failure due to its insolubility in water and all biologically compatible solvent systems. This clearly demonstrates that many promising anticancer drugs may never reach the clinical testing phase unless suitable ways can be found to make them water-soluble. Towards this goal, oligomeric ethylene glycol fragments and also water-soluble polymeric drug carriers have been developed as systems to take advantage of otherwise impossible-to-use ferrocene (4) and platinum (25) drugs in anticancer studies.

Another way of making complexes more water-soluble is to convert them to ionic compounds. Towards this end, the charged complex [Au($PPh_2CH_2CH_2PPh_2$)$_2$]$^+$[Cl^-], complex **7**,

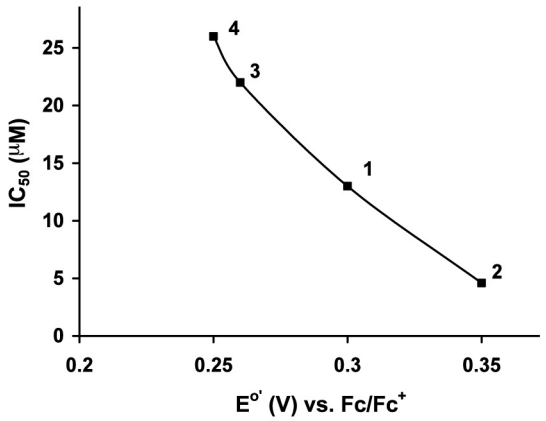

Figure 3. *The IC_{50} value of compounds **1-4** is inversely proportional to the sum of the ferrocenyl formal reduction potentials of each compound.*

was also investigated for cytotoxicity. This complex contained no antineoplastic ferrocenyl group but it still had the gold(I) ion, making comparisons with complexes **1-4** valuable. This ionic gold(I) complex with an IC_{50}=0.14 μmol dm^{-3} was even more cytotoxic than cisplatin (IC_{50}=0.19 μmol dm^{-3} under identical conditions) and at least one order of magnitude more cytotoxic than complex **2** (IC_{50}=4.6 μmol dm^{-3}). It is clear from this result that water solubility is a contributing parameter that determines the level of antineoplastic activity of compounds.

Previous studies indicated that ferrocene derivatives appear only to possess reasonable antineoplastic activity if the formal reduction potential, $E^{o'}$, of the ferrocenyl group is 0.52 V or less *vs*. Fc/Fc^+ (9). In this study, all ferrocene-containing complexes had $E^{o'}$ values of 0.35 V or less *vs*. Fc/Fc^+. The most active compound, complex **2**, had a formal reduction potential of 0.35 V *vs*. Fc/Fc^+ (Table I) while the less active complexes **1**, **4** and **5** had smaller formal reduction potentials, $E^{o'}$<0.280 V (Figure 3). This finding is in sharp contrast with previous research on ferrocene-containing polyamides (4) and alcohols (9), which indicated that compounds with lower reduction potentials should be more cytotoxic. Based on formal reduction potentials only, complex **3** would be expected to be the most active, not the least active, as it has the smallest ferrocene reduction potential of all the compounds investigated (Table I). It did, however, mirror the trend set by the beta-diketonato ferrocene-containing compounds $FcCOCH_2COR$ with R=CF_3, CCl_3, CH_3, Ph and Fc (10).

Evidently, the redox potential of the ferrocenyl group is not the only switch that determines the antineoplastic activity of a ferrocene compound. Structural features of ferrocene derivatives, such as substituent chain length (4, 9), have been shown to be important parameters in cytotoxicity. For the beta-diketone series $FcCOCH_2COR$, the relative acidity, expressed as pK_a values, was another important variable that determined the antineoplastic activity of ferrocene complexes. The most effective ferrocene-containing gold complex tested in this study, complex 2, may be subject to pH-controlled aqueous hydrolysis:

H_2O + Fc-C≡C-PPh_2-Au-Cl→Fc-C≡C-PPh_2-Au-OH + H^+ + Cl^-.

The phosphine ligands may interact with acid according to the reaction:

Fc-C≡C-PPh_2 + H_2O→Fc-C≡C-$P^+(H)Ph_2$ + ^-OH. Both reactions imply compound pK_a or ligand pK_b may also be an important parameter in the cytotoxic activity of the compounds of this study.

Conclusion

Of the five ferrocene-containing gold and platinum complexes that were tested for antineoplastic activity against the human HeLa cancer cell line, the Fc-C≡C-PPh_2-Au-Cl complex **2** was the most active, despite having the largest ferrocenyl formal reduction potential. Water solubility and charge, as demonstrated by complexes **5**, [(Fc-C≡C-$PPh_2)_2PtCl_2$] and **7** [$Au(PPh_2CH_2CH_2PPh_2)_2^+$][$Cl^-$], respectively, also play an important role in cytotoxicity. The higher activity of complex **2** is probably associated with its stronger acid strength, *i.e.* lower pK_a value.

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