

Antineoplastic Activity of a Series of Ferrocene-containing Alcohols

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Abstract. *Background:* Often potentially good chemotherapeutic drugs find limited clinical use due to the many negative medical and physical side-effects they may exhibit. To combat these negative side-effects, new antineoplastic materials are continuously being synthesised and evaluated. Ferrocene-containing compounds under certain conditions may show appreciable anticancer activity. Some of the factors that determine this activity have been investigated. *Materials and Methods:* Ferrocene-containing alcohols were tested for cytotoxicity against the HeLa cancer cell line. Cell survival was measured by means of the colorimetric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide assay. *Results:* The 50% lethal dosage of 4-ferrocenylbutanol was 5.72 $\mu\text{mol. dm}^{-3}$ and for 2-ferrocenylethanol and 3-ferrocenylpropanol it was 35.0 and 17 $\mu\text{mol. dm}^{-3}$ respectively while for ferrocenylmethanol IC_{50} was $>100 \mu\text{mol. dm}^{-3}$. *Conclusion:* A drug activity-structural relationship exists in that ferrocenyl drugs with longer side chains are more cytotoxic. Compounds with lower ferrocenyl group formal reduction potential are also more cytotoxic.

Due to the success of cisplatin, $(\text{PtCl}_2(\text{NH}_3)_2)$, as an anticancer drug (1), interest in the use of transition metal complexes in medicine and other biological areas has grown rapidly over recent years (2). The antineoplastic activity of some ferricenium salts against Ehrlich ascites tumour cell lines has been reported (3). It has been shown that some of these ferricenium salts have more favourable 50% lethal dosage values than cisplatin (4) and that the lack of aqueous solubility of ferrocene itself is probably the reason it is inactive as an antineoplastic agent. Kansawa and co-workers (5) showed that at relatively high concentrations ($100 \mu\text{g. cm}^{-3}$), ferrocenyl acetic acid induced good to excellent inhibition rates against

human adenocarcinoma, squamous cell carcinoma and large cell carcinoma of the lung in *in vitro* human tumour clonogenic assays. Ferrocenoic acid does not display any tumour inhibiting activity even though it is water-soluble as the carboxylate (6), but in its oxidised form, *i.e.* as the ferrocenium salt, it exhibits appreciable inhibitory activity (3, 6). The antitumour effect of the ferrocenyl group may thus also be related to the oxidation state of the central iron atom. The 3+ oxidation state of the iron in ferrocenium cations appears to be an active form of ferrocene-containing drugs. Thus, from the work by Osella and co-workers (6), it is known that the mechanism of action of the ferrocenyl group in chemotherapy involves as a first step oxidation of the Fe^{II} -containing ferrocenyl group of the drug to the Fe^{III} -containing ferricenium species by redox active enzymes in a particular body compartment. The ferricenium species then interacts with water and oxygen to generate the hydroxyl radical (OH^\bullet). The hydroxyl radical cleaves the DNA strands which results in cell death. The question arises as to whether ferrocene-containing drugs would be more active in cancer treatment if the reduction potential of the ferrocenyl group could be systematically lowered. The electrochemistry of ferrocene is very much ideal in that it represents an electrochemically reversible one electron transfer process according to the equation $\text{Cp}_2\text{Fe}^{\text{III}} + e^- \rightleftharpoons \text{Cp}_2\text{Fe}^{\text{II}}$, where Cp = the cyclopentadienyl anion, C_5H_5^- .

Towards the goal of improved performances of drugs against malignant cells, the results of *in vitro* cytotoxicity tests of a series of ferrocene-containing alcohols 1-4 against HeLa cells is reported here. The results have also been correlated with the formal reduction potentials of the ferrocenyl moiety of all the investigated compounds.

Materials and Methods

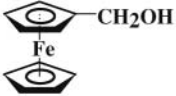
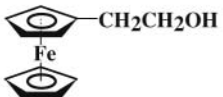
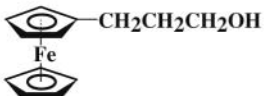
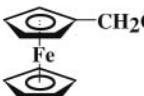
Compounds. The ferrocene-containing alcohols [1-4] (Table I) were synthesised as described elsewhere (7).

Sample preparation. Ferrocene-containing alcohols [1-4] were dissolved in DMSO to give stock concentrations of $10 \mu\text{mol. dm}^{-3}$ and diluted in growth medium supplemented with foetal

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Key Words: Ferrocenyl derivatives, alcohols, HeLa, electrochemistry, cytotoxicity.

Table I. Chemosensitivity of drugs expressed as IC_{50} ($\mu\text{mol. dm}^{-3}$) values^a after 7 days of incubation against HeLa cell lines. Formal reduction potentials, E° , of 1-4 are measured in volts (V) versus an Ag/Ag^+ reference electrode in acetonitrile.

Compound	E°/V^b	$IC_{50}/\mu\text{M}$
1  CH_2OH	0.111	>100
2  $\text{CH}_2\text{CH}_2\text{OH}$	0.029	35.0
3  $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$	0.029	17.0
4  $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$	0.026	5.7

^aData from three experiments are expressed as the mean drug concentration ($\mu\text{mol. dm}^{-3}$) causing 50% cell death. ^bData from reference (7).

calf serum (FCS) to give final DMSO concentrations not exceeding 0.5% and drug concentrations of 1-2000 $\mu\text{mol. dm}^{-3}$ in cell experiments.

Cell cultures. The human cervix epitheloid cancer cell line, HeLa (ATCC CCL-2), was grown as monolayer culture in MEM. The growth medium was fortified with 10% FCS, and 1% penicillin and streptomycin. Incubation of the cultures was at 37°C under 5% CO_2 . The cells were seeded at 2,000 cells/well for 24 h incubation experiments and 400 cells/well for 7-day incubation experiments in 96-well microtiter plates in a final volume of 200 μl of growth medium in the presence or absence of different concentrations of experimental drugs [1-4]. Appropriate solvent control systems were included. Wells without cells and with cells but without drugs were included as controls. After incubation at 37°C for 7 days, cell survival was measured as a percentage of living cells in relation to a control that was not exposed to the drug by means of the colorimetric 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazodium bromide (MTT) assay (8, 9).

Results and Discussion

The cytotoxicity of ferrocene-containing alcohols [1-4], were determined by observing their effects *in vitro* on cultured HeLa cell lines for 7 days of continuous drug incubation. Survival curves (Figure 1) indicating cell survival as a percentage of living cells in relation to a control that was not exposed to the drug were plotted as a function of drug

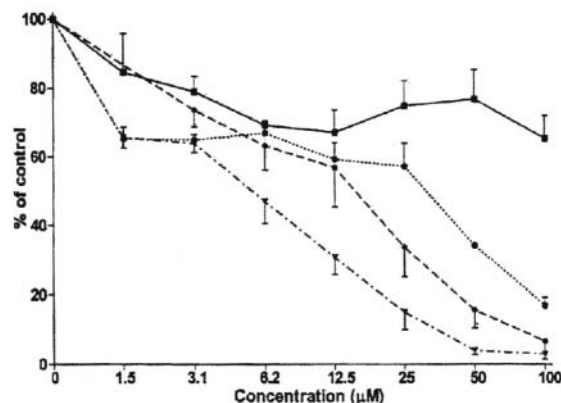


Figure 1. Percentage cell survival of HeLa cancer cells, relative to the control, vs. concentration of ferrocenyl alcohols 1 (■), 2 (●), 3 (*) and 4 (▼) after 7 days of incubation.

dose. From these survival curves, IC_{50} values (drug dose required for 50% cell death) were estimated by extrapolation and are summarised in Table I.

A clear activity-structure correlation was observed. The activity of the alcohols [1-4] increased with the increase in the number of methylene (CH_2) spacers that separate the ferrocenyl group from the alcohol group. From the IC_{50} , it can be seen that ferrocenylbutanol [4], was the most effective of the alcohol series compounds in killing HeLa cells ($IC_{50}=5.2 \mu\text{M}$). Ferrocenylbutanol [4], was more than 20 times more effective than the short chain alcohol [1], almost 8 times more effective than alcohol [2] which has a CH_2CH_2 spacer between the ferrocenyl and OH groups, and three times more effective than alcohol [3] in which the ferrocenyl and OH moieties are separated by three methylene spacers. Figure 2 (left) shows the relationship between the IC_{50} and the number of C-atoms (CH_2 groups) in the alcohol side chain.

An activity-ferrocenyl formal reduction potential (E°) correlation was also observed. The alcohols with more negative E° values were more active, while those with more positive E° values (Table I) were less active. This is consistent with a mechanism of action that first involves the oxidation of the ferrocenyl group by biological oxidising agents to the active ferrocenium species. The ferrocenium species could then be involved in electron transfer reactions to the cell DNA (6), which ultimately lead to cell death. Figure 2 (right) shows the relation between IC_{50} and the formal reduction potentials, E° , for 1-4.

The present series of ferrocene-containing alcohols are not as active as cisplatin. Experiments performed under identical conditions showed that $IC_{50}=1.3 \mu\text{M}$ for cisplatin. This implies that cisplatin is four times more active than [4], the most active ferrocene-containing alcohol studied here.

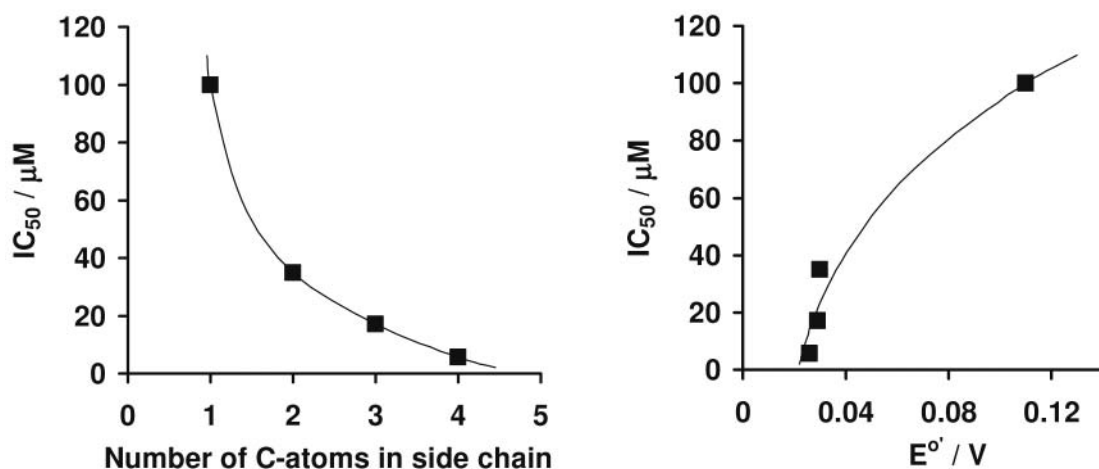


Figure 2. Relationship between the number of C-atoms in the alcohol side chain and the IC_{50} (left) and between $E^{\circ'}$ values and the IC_{50} (right) for the ferrocenyl alcohols [1-4].

Conclusion

It was convincingly demonstrated in this study that a definite structure-activity relationship exists in ferrocene-containing alcohols. The compounds having longer alkyl chains (*i.e.* more methylene spacers) between the ferrocenyl antineoplastic moiety and the hydroxy group were more active. These compounds also exhibited the smallest ferrocenyl formal reduction potential. Compounds with the smallest ferrocenyl reduction potential are more prone to enzymatic oxidation in a particular body compartment (6), generating the active ferrocenium drug species.

Acknowledgements

The authors acknowledge financial support from the Department of Trade and Industry Technology and Human Resources for Industry Programmes, the Cancer Association of South Africa and the Central Research Fund of the University of the Free State.

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Received April 2, 2007

Revised July 13, 2007

Accepted July 24, 2007