Bioorganic & Medicinal Chemistry Letters, Vol.2, No.9, pp. 929-932, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00
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A FERROCENE-INTERCALATOR CONJUGATE WITH A POTENT CYTOTOXICITY

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(*Received* 20 *March* 1992)

Abstract : Ferrocenium radical cation is known to possess cytotoxic properties. It was of interest to bring the biooxidizable ferrocenyl group into close proximity with DNA using an intercalator. The new ferroceneacridine conjugate was synthesized and found to possess potent cytotoxic activities against four cell lines.

Organometallic compounds of the metallocene type have a wide array of biological properties, and recent attention has been focused on their antitumor activities.[1] These metallocenes can be divided into two main types : $[(\eta^5\text{-}C_5H_5)_2MX_2]$ where M = Ti, V, Zr, Nb, Mo,[2] and $[(\eta^5\text{-}C_5H_5)_2M]^+X^-$ where M = Fe.[3] Their antitumor activity is strongly dependent upon the central metal atom and the halide ligands. There exist some indirect indications that their antitumor activity is due to interaction with DNA. In the case of ferrocene, the ferrocenium radical cation formed during oxidation by the glucose oxidase β-D-glucose system in the presence of peroxidase is a relatively stable species which can interact with the nucleophilic DNA for the expression of cytotoxic activity.[4] In this paper, we report the incorporation of the ferrocenyl group to DNA binding compounds for bringing the reactive group into close proximity to DNA with the hope of enhancing the cytotoxic effect. The need to bring reactive species into close proximity to DNA for the expression of antitumor activity is best seen in the case of bleomycin and its models.[5]

(Hydroxymethyl)ferrocene and (2-aminoethyl)ferrocene[6] were chosen to covalently link with DNA interacting compounds through the respective ether and amine bonds. The moieties employed were phenyl, biphenyl and acridine. [2-(Benzylamino)ethyl]ferrocene (**1**) and N-(2-ferrocenylethyl)-6-chloro-2-methoxyacridin-9-amine (**2**) were obtained by treatment of (2-

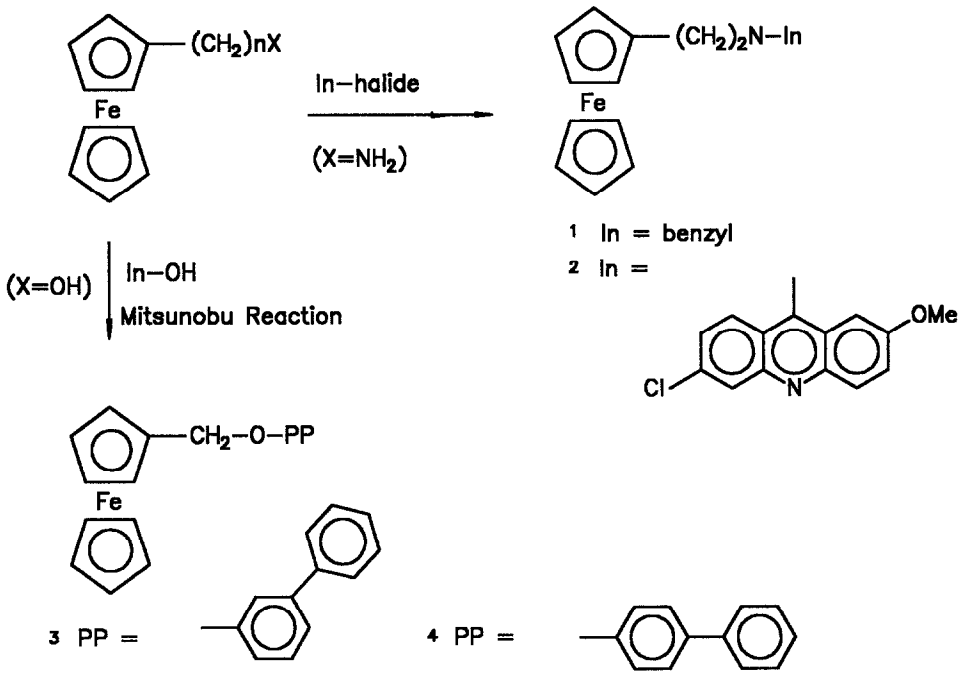

Table 1

In Vitro Cytotoxicity of Compounds 1-4, Ferrocene and 692-Acr

Compound	IC_{50} (μg/mL)			
	KB	Hep.	Hela	Colo--205
1	-	-	-	-
2	1.0	2.0	1.0	1.0
3	-	18.5	14.9	-
4	-	14.0	12.0	-
Ferrocene[a]	-	-	-	-
692-Acr[a,b]	-	-	-	-

a. Inhibition of 50% cell growth cannot be obtained with drug dosage of 40 μg/ml during MTT assay[10].

b. 692-Acr : 6,9-dichloro-2-methoxyacridine

aminoethyl)ferrocene with benzyl bromide and 6,9-dichloro-2-methoxy-acridine,[7] respectively. The ether linked [(3-phenylphenoxy)methyl]ferrocene (**3**) and [(4-phenylphenoxy)-methyl]ferrocene[8] (**4**) were prepared by the Mitsunobu coupling[9] of (hydroxymethyl)ferrocene with the appropriate phenylphenols.

The *in vitro* cytotoxicity of the synthesized compounds **1-4** against a series of tumor cell lines is shown in Table 1. The data showed that compound **2**, the combination of the ferrocenyl group with the DNA intercalator (acridine), is highly cytotoxic against all the cell lines tested. The ferrocene derivatives **3** and **4**, the biphenyl moieties of which have been reported to bind DNA sluggishly, ($K_{assoc.}$ 3 x $10^3 M^{-1}$)[11] were found to give low cytotoxicity. The inability of the phenyl derivative **1** to bind DNA was in good agreement with the lack of cytotoxicity.

The results presented here show that the substitution of a ferrocenyl group to a strong DNA intercalator allows for strong interactions with the key cellular target. The ferrocenium cation which is formed by the oxidation with glucose oxidase β-glucose system in the presence of peroxidase can now be specifically targeted towards DNA. By the appropriate choice of DNA binding compounds, it may be possible to prepare derivatives having varying affinities and sequence specific interaction and degrading DNA.

Acknowledgement : The authors wish to thank the Triservice Hospital for the facilities to perform the *in vitro* antitumor assay and the NSC of Taiwan for financial support.

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7. **1** : ^{1}H NMR ($CDCl_3$, 90 MHz.): δ 7.65 (m, 5H, arom.), 5.30 (s, 2H, N-CH_2), 4.15 (br. s, 9H, ferrocene), 3.72 (br, 1H, NH), 3.50 (t, 2H, CH_2), 2.68 (t, 2H, CH_2).
2 : Mp 144^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz.): δ 8.00-7.30 (m, 7H, acridinyl-H and NH), 4.25 and 4.15 (2s, 9H, ferrocene), 4.10 (m, 2H, CH_2), 3.95 (s, 3H, OMe), 2.90 (t, 2H, CH_2).

8. **3** : Mp 108^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz.) s 7.70-6.90 (m, 9H, phenylphenoxy), 4.80 (s, 2H, s, CH_2O), 4.35 (s, 2H, ferrocene), 4.20 (s, 7H, ferrocene).
4 : Mp 116^{0}C; ^{1}H NMR ($CDCl_3$, 300 MHz.) : δ 7.60-7.00 (m, 9H, phenylphenoxy), 4.80 (s, 2H, CH_2), 4.30 (s, 2H, ferrocene), 4.10 (s, 7H, ferrocene).
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10. Cells were taken from exponential phase cultures and were allowed to grow in a plate containing 96 wells using RPMI-1640 medium supplemented with 5% fetal bovine serum, 1 mM glutamine and antibiotics (penicillin and streptomycin) at 37^{0}C in a CO_2 incubator. Cell suspensions were trysinized and disaggregated and approx. 3 x 10^3 cells were inoculated into each well in 0.18 mL of medium, to which 0.02 mL of drug was added. After 4 days of culture, 0.1 mg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added to each well and the plate incubated for 4 h. The medium was then removed, 0.2 mL DMSO added to each well and the plate agitated for 10 min. The optical density of each well was measured at 545 nm test wavelength and a 690 nm reference wavelength using a Titertek Multiskan plate reader. Absorbance levels from drug tested cells were corrected against untreated control values.
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