Bleomycin Models. Haemin–Acridines which bind to DNA and cause Oxygen-dependent Scission

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Haemin–acridines intercalate into duplex DNA *via* the acridine moiety and, in the presence of reducing agents, cause oxygen dependent scission and show antileukemic properties analogous to the action of the glycopeptide antibiotic bleomycin.

The glycopeptide antibiotic bleomycin is active clinically against a range of malignant diseases,[1] the principal cell target probably being DNA.[1] The DNA is degraded in an oxygen-dependent reaction mediated by iron[2,3] which is hexaco-ordinated by the glycopeptide and which gives rise to reactive oxygen species.

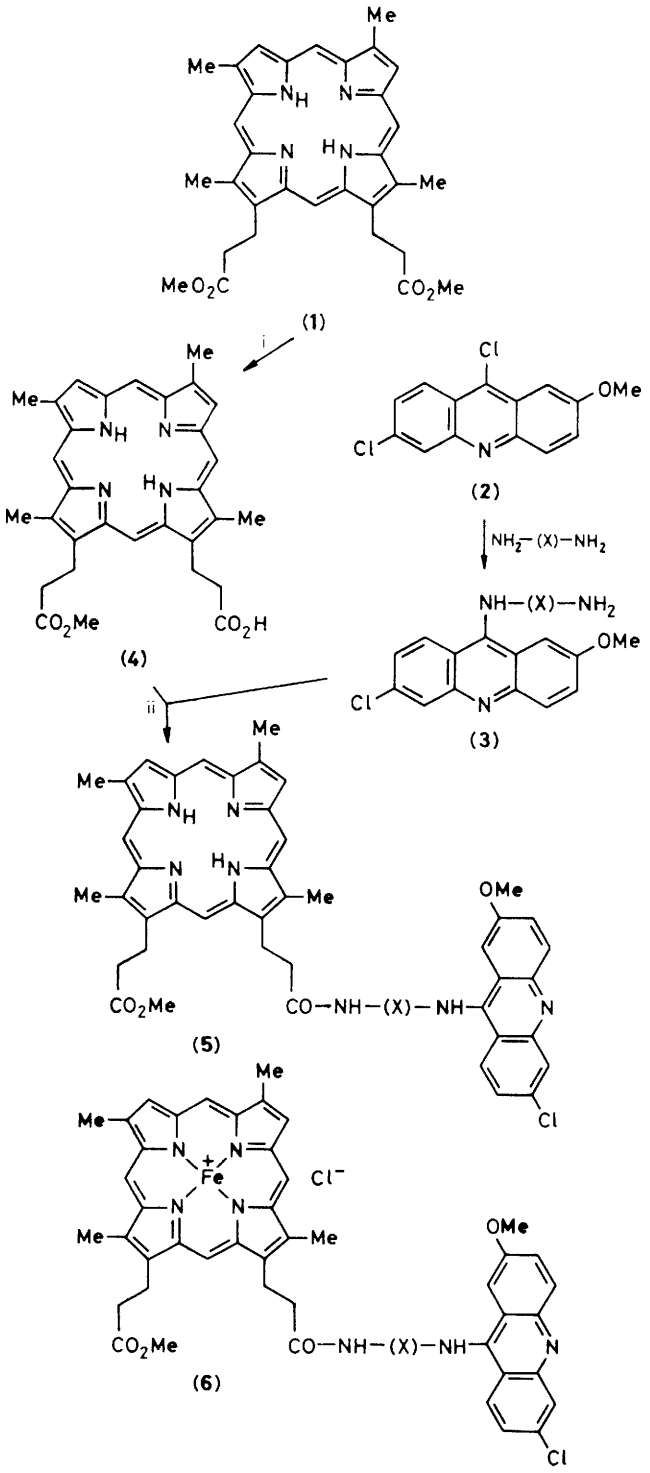
We report the synthesis of certain porphyrin–acridines to which iron may be bound and which reproduce the essential features of bleomycin action on DNA and its anticancer properties at comparable concentrations.

Controlled hydrolysis of deuteroporphyrin IX dimethyl ester[4] (**1**) with 4 M hydrochloric acid gave a mixture of di- and mono-carboxylic acids (**4**) from which the latter could be separated by chromatography. Heating of the 9-chloroacridine (**2**) with a large excess of the linking diamine or triamine at 80 °C for 2 h gave derivatives (**3**)† which were purified as their hydrochlorides.† Coupling of the aminoacridines (**3**) to the regioisomeric esters (**4**) was carried out as shown in Scheme 1[5] and the products were purified by repeated chromatography. The linked products (**5a**—**c**) were obtained in 50—60% yields† while (**5d**, **e**) were obtained in 40—50% yield. Iron was inserted into (**5a**—**c**) by refluxing with anhydrous ferrous chloride in *N*,*N*-dimethylformamide (DMF)[6] to afford (**6a**—**c**).†

The porphyrin–acridines (**5**), the haemin–acridines (**6**), and the linking diamine substituted acridines (**3**) bind to duplex DNA readily in a pH-dependent manner (lower pH favours binding). Compound, $K_{assoc}/10^6$ l mol^{-1};‡ (**3a**) 12.6; (**3b**) 27.4; (**3c**) 31.5; (**3d**) 30.0; (**3e**) 57.3; (**5a**) 1.9; (**5b**) 1.6; (**5c**) 1.4; (**5d**) 2.3; (**5e**) 3.0; (**6a**) 3.2; (**6b**) 3.9; (**6c**) 2.7; (**6d**) 6.3; and (**6e**) 9.2. Since in contrast neither porphyrin (**4**) nor the

† All new compounds described gave spectral (n.m.r., m.s.) and analytical data in accord with their assigned structures.

‡ Determined by the ethidium bromide binding assay described previously (A. R. Morgan, J. S. Lee, D. E. Pulleyblank, N. L. Murray, and D. H. Evans, *Nucleic Acids Res.*, 1979, **7**, 547).



a; X = $[CH_2]_2$
b; X = $[CH_2]_3$
c; X = $[CH_2]_4$
d; X = $[CH_2]_2NH[CH_2]_2$
e; X = $[CH_2]_3NH[CH_2]_3$

Scheme 1. Preparation of deuteroporphyrin–acridines (**5**) and haemin–acridines (**6**). *Reagents:* i, 4 M HCl, ii, di-imidazol-1-yl ketone, *N*-ethylmorpholine, dimethyl sulphoxide.

corresponding haemin bind to DNA it is concluded that (**3**), (**5**), and (**6**) bind *via* the acridine intercalating group. Among compounds (**5**) and (**6**) the strongest binding is found for the (**d**) and (**e**) derivatives suggesting that in each case additional

Table 1. Scission of PM2-CCC-DNA[a] by 5 × 10^{-5} M deutero-haemin–acridines at 37 °C, pH 7.0, in the presence of 20 mM 2-mercaptoethanol.

Compound	% DNA scission in 20 min		
	pH 6.0	pH 7.1	pH 8.0
(**6a**)	70	67	60
(**6b**)	75	67	51
(**6c**)	79	69	53
(**6d**)	84	70	45
(**6e**)	90[b]	88[b]	85

[a] CCC = covalently closed circular. [b] 2.5 × 10^{-5} M.

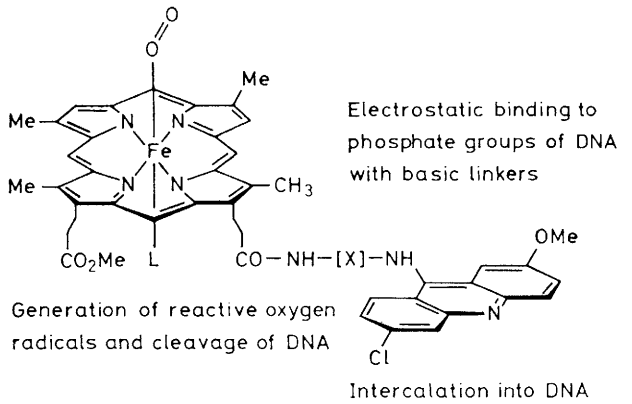


Figure 1. Depiction of the molecular mechanism of action of the oxygen complex of haemin–acridines involved in intercalative binding to DNA and subsequent oxidative scission.

binding is afforded by the basic amine-substituted link as is found in the native antibiotic.[1]

Solutions of 2.5 × 10^{-5} M (**6e**) in the presence of 20 mM 2-mercaptoethanol with free access to air caused 88% scission of PM2-supercoiled covalently closed circular (CCC)DNA in 20 min‡ at pH 7.1 comparable to efficiencies found with bleomycin.[3] The presence of both a reducing agent and oxygen are essential since in the absence of 2-mercapto-ethanol no DNA scission is observed while in its presence careful degassing with argon reduced the extent of DNA scission by 5 × 10^{-5} M (**6a**) at pH 7.1 to 38% from 67%. Greater efficiency in oxygen-dependent DNA scission is observed for those haemin–acridines (**6d**) and (**6e**) which bind more strongly to the DNA (Table 1). For a given agent, comparatively more DNA scission is observed as the pH is lowered. The lack of reactivity of the zinc complex [formed from the porphyrin–acridine (**5a**) by the $ZnCl_2$–DMF method][5] towards DNA either in the presence or in the absence of reducing agent demonstrates the need for a reducible metal, as in the case of bleomycin.

By analogy with the reversible binding of oxygen to haemoglobin[7] one expects an initial iron–oxygen complex in the haemin group, Figure 1. The additional ligand L in the sixth co-ordinating position may be solvent or, in the case of the unbound compound, this may be accommodated by intramolecular participation of a nitrogen ligand.[7] It was found that 5 × 10^{-3} M benzyl isonitrile caused complete inhibition of the DNA scission by (**6e**) in the presence of 2-mercaptoethanol. Precisely similar selective and competitive inhibition of bleomycin action is obtained with ethyl iso-nitrile and is caused by preferential binding of the latter to the sequestered iron.[8]

Dilute solutions (5 × 10^{-5} M) of the haemin corresponding to (**4**) (*i.e.* incapable of binding to DNA) when reduced with 2-mercaptoethanol in the presence of DNA caused no scission

of the latter. This indicates that close proximity to the target is essential as expected if indiscriminately reactive species such as hydroxyl are being generated. Hydroxyl radicals have been suggested to be among the ultimate species generated by bleomycin which cleave DNA.[9]

Compounds (**5a**—**e**) and (**6a**—**e**) all exhibit growth inhibitory activity ($ID_{50} < 1.0\ \mu g\ ml^{-1}$) against murine L1210 leukemia cells in culture while (**6e**) gave a 22% increase in life span against P388 leukemia in mice.§ Compound (**6d**) strongly inhibited (at 40 $\mu g\ ml^{-1}$) *E. coli* growing in aerobic conditions which is a typical feature of bleomycin.§

After this work was completed it was reported that a (methidium-propyl)iron(II) ethylenediaminetetra-acetic acid complex binds to DNA and causes oxygen-dependent scisson.[10]

This work was supported by the Natural Sciences and Engineering Research Council of Canada, the National Cancer Institute of Canada, and the National Foundation for Cancer Research.

Received, 19th July 1982; Com. 838

§ We are indebted to Dr. J. P. McGovren and Dr. L. J. Hanka of the Upjohn Co., Kalamazoo, for these data.

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