Antitumor Antibiotics Drug Design. Part I. Synthesis of a 6-Chloro-2-methoxy-9-triethylenetetraaminoacridine Iron Complex which Binds to DNA and Causes Strand Scissioning *In Vitro*

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Abstract

6-Chloro-2-methoxy-9-triethylenetetraaminoacridine iron complex has been synthesized and was found to bind to duplex DNA and to cause strand scissioning in the presence of a reducing agent *in vitro*. The advantage of this compound is its high water solubility.

Introduction

The acridine moiety is a well-known efficient intercalating agent [1]. Recently, the incorporation of the acridine moiety in the design of new antitumor antibiotics has been widely employed. Lown *et al.* [2] have successfully used acridine as an intercalative portion for their design on bleomycin models. According to Lown [2], the acridine moiety is responsible for bringing other reactive centers of the antibiotics in close proximity to the DNA in order to exert its antitumor activities.

The design, synthesis and biological testing *in vitro* of new antitumor antibiotics based on an analysis of the structural requirement of the natural antibiotic, such as bleomycin [3], for metabolic reductive activation and DNA binding has been described. The 6-chloro-2-methoxy-9-triethylenetetraaminoacridine is found to intercalate strongly with duplex DNA via the acridine moiety and, in the presence of a reducing agent, to effect strand scissioning of close circular DNA (PM-2) via the triethylenetetraamino iron complex. Therefore, this new compound is a potential antitumor agent.

Experimental

Materials

PM-2 covalently closed circular (ccc) DNA was prepared as described previously by Morgan *et al.* [4]. Ethidium bromide and calf thymus (CT) DNA were from Sigma; and triethylenetetraamine and dithiothreitol (DTT) were from Aldrich. 6,9-dichloro-2-methoxyacridine was prepared starting with 2,4-dichloro-benzoic acid and *p*-anisidine [5] (it is also commercially available from Aldrich). Reaction of the above with a large excess (100 fold) of triethylenetetraamine gave the title compound, 6-chloro-2-methoxy-9-triethylenetetraaminoacridine [6].

6-Chloro-2-methoxy-9-triethylenetetraaminoacridine Iron Complex

Dimethylfornamide (6 ml) was brought to reflux under nitrogen and 6-chloro-2-methoxy-9-triethylenetetraaminoacridine (100 mg) was added, letting it reflux for a further 15 min. After this time, anhydrous ferrous chloride (40 mg, 1.1 equivalent) was added to the reaction mixture, which was then refluxed for 30 min. The solvent was removed under vacuum and the remaining solid dissolved in methanol (filtered, if necessary). Ether was then added to the methanol solution, giving a brownish solid precipitate. The solid was then successively purified using methanol/ether to afford the pure product (95 mg, 82%). $UV_{(methanol)}$ 265 nm (uncomplexed, 278 nm). $IR_{(nujol)}$, V_{max} 1385, 1377 cm^{-1}. FAB mass spectrum m/e 444 ($M^+ - 2\,Cl^-$, 0.3%).

Biological Studies

A. G. K. Turner and Associate Model 430 spectrofluorometer was used (excitation wavelength = 525 nm, emission wavelength = 600 nm).

(a) Binding constant

Calf Thymus (CT) DNA (1 μl, 12.8 O.D.) was added to an ethidium assay solution of pH 8.0 [7a] and the fluorescent intensity was adjusted to an arbitrary unit (ranging from 70–85). The drug (8.5×10^{-5} M solution in H_2O) was added in 1–3 μl aliquot portions and the fluorescent values measured until more than 50% loss of the initial fluorescent occurred.

(b) Scissioning studies

The ethidium assay method of Morgan *et al.* [7b] was used. The conversion of PM2-ccc-DNA to PM2

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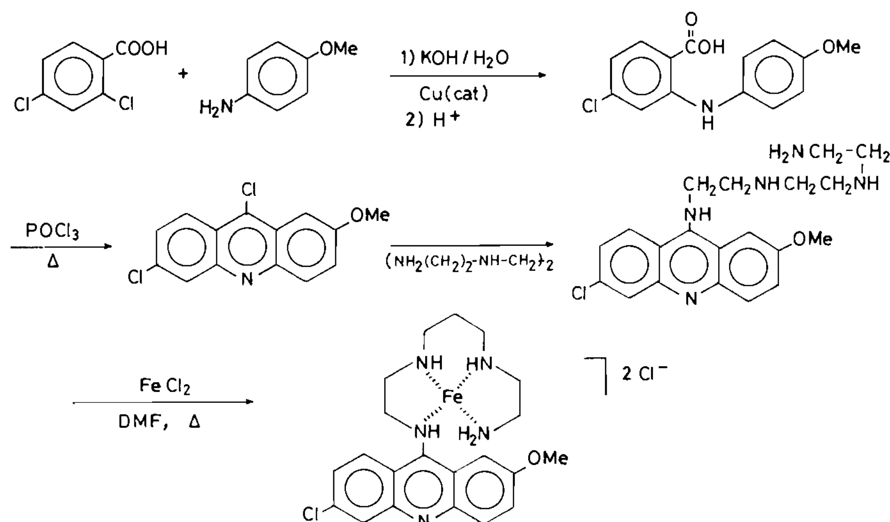

Scheme 1. Synthetic route of 6-chloro-2-methoxy-9-triethylenetetraaminoacridine iron complex.

open circular DNA by the drug resulted in an initial 33–35% increase in fluorescence (depending on the superhelical density of the sample) in the assay solution which contained 0.5 μg ml^{-1} of ethidium bromide and 0.2 mM EDTA in 20 mM potassium phosphate buffer pH 11.8; a 100% loss of fluorescence occurred after the heating and cooling cycle (96 °C for 4 min, then cooled in ice, 23 °C, for 5 min) because the strands separated. The reaction of 6-chloro-2-methoxy-9-triethylenetetraaminoacridine iron complex with DNA was performed at 37 °C in a volume of 100 μl containing 1.02 O.D units of PM2-ccc-DNA, 88% ccc) in a potassium phosphate buffer (pH 6.0) and 5×10^{-5} M of the drug in the presence of 20 mM dithiothreitol (DTT).

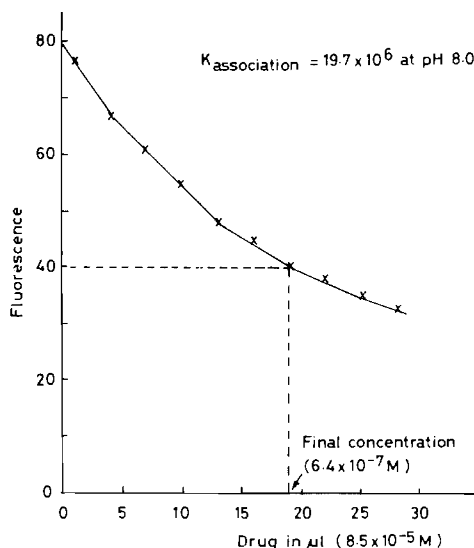

Fig. 1. Binding studies. Inhibition of ethidium binding to calf thymus DNA by the addition of 6-chloro-2-methoxy-9-triethylenetetraaminoacridine iron complex.

Results and Discussion

The synthetic route for the 6-chloro-2-methoxy-triethylenetetraaminoacridine iron complex is illustrated in Scheme 1. This compound not only dissolves in organic solvents (methanol, DMF or DMSO) but is also highly water soluble, which is a great advantage for a bioactive molecule.

It was found that the 6-chloro-2-methoxy-triethylenetetraamino iron complex binds to DNA readily by intercalation of the acridine ring, $K_{association}$ 19.6×10^6 mol^{-1}. The experimental result obtained is illustrated in Fig. 1. A 5×10^{-5} M solution of the above compound in the presence of 20 mM dithiothreitol (DTT) with free access to air causes 82% scissioning of PM2 supercoiled covalently closed circular DNA in 30 min at pH 6.0, comparable to the efficiency of bleomycin [8]. In the absence of the reducing agent (DTT), no scissioning is observed, as illustrated in Fig. 2. Dithiothreitol was choosen as the reducing agent in this study because a 20 mM concentration was found to cause minimal (2%) scissioning of ccc-DNA in a control. Dithiothreitol now replaces the frequently used mercaptoethanol.

The 6-chloro-2-methoxy-9-triethylenetetraamino-acridine iron complex was found to be as effective as the more complex, naturally occurring bleomycin or the haemin–acridine model of bleomycin [2] as reported in *in vitro* studies. In this case, the acridine intercalates to the DNA, bringing the triethylenetetra-amino iron complex moiety into close proximity to the DNA. Complexation of oxygen to the metallo-iron moiety probably results in the generation of a

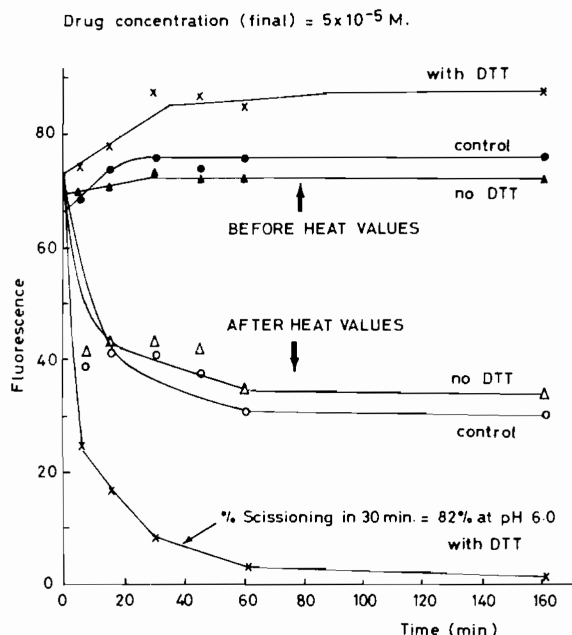

Fig. 2. Scissioning studies. Cleavage of PM2-ccc-DNA by 6-chloro-2-methoxy-9-triethylenetetraaminoacridine iron complex and its enhancement by reducing agent.

reactive species, such as the superoxo- or hydroxyl radical, which will then cause the DNA strand to break; this mechanism was proposed by Lown for his haeminacridine bleomycin model [9]. The reducing agent is required for the reduction of the oxidised Fe^{III} back to Fe^{II} for the continuous binding of oxygen; thus it is the reactive radical species for biological activity. The mechanism of the above synthesized drug for antitumor activity *in vitro* is found to be parallel to that of bleomycin.

Conclusion

Based on the results obtained, 6-chloro-2-methoxy-9-triethylenetetraaminoacridine iron complex showed antitumor activity *in vitro*. For a compound to be effective as a potential antitumor agent, it requires only an effective intercalating moiety and a metallo-iron center (or any center that will generate reactive radical species). The present new compound has the advantage of high water solubility, an important criterion for future clinical use. The other bleomycin models synthesized thus far have the problem of poor solubility [6].

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