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Antitumor Antibiotics Drug Design. Part II*. Synthesis of 4-Ethylamido[5,(2′-thienyl)-2-thiophene]imidazole iron(II) Complex, a New N_2S_2-Metallocycle with a 'Built In' Intercalating Moiety which Causes DNA Scissioning *In Vitro*

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Abstract

A new metal chelating moiety, 4-ethylamido[5,(2′-thienyl)-2-thiophene]imidazole iron(II) (**1**) was synthesized and showed antitumor activity *in vitro*. The bisthiophene moiety which sterically resembles the bithiazole units of bleomycin may allow us to probe further the mechanism of antitumor action by bleomycin. The cyclic voltammetry for the new compound **1** in DMSO showed a nearly reversible Fe^{3+}/Fe^{2+} transition. The electron spin resonance spectrum consisted of a fairly broad band resonance centered at $g = 2.00989$, similar to that of a bleomycin–Fe^{2+} complex. The new compound **1** causes cleavage of double helical DNA without the requirement of an extra intercalating group.

Introduction

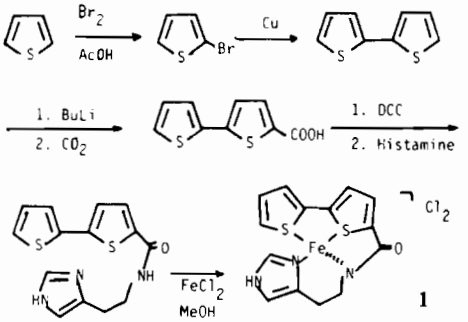
The glycopeptide antitumor antibiotics bleomycin and tallysomycin are clinically useful in treating many malignant diseases. They exhibit a unique mode of action by binding to DNA and cause an oxygen dependent single strand and double strand break. Hexacoordination of metals at the N-terminus [2] is generally thought to activate oxygen by the generation of reactive oxygen radicals which attack DNA, although until now there is only a limited amount of direct evidence. There are several recent reports containing data whose interpretation appears inconsistant with this view, these include the suggestion that the bithiazole unit is involved in metal chelation [3] and is responsible for some of the antitumor activity.

In this study, we have synthesized a model compound, 4-ethylamido[5,(2′-thienyl)-2-thiophene] imidazole iron(II) (**1**), containing a bisthiophene unit which may sterically resemble the bithiazole unit in bleomycin. Initial physico-chemical studies of compound **1** may help us probe further the role played by bithiazole metal complex in bleomycin. Another key feature of this newly synthesized compound **1** is that the intercalating group is 'built into' the molecule. Compound **1** has been shown to cleave double helical DNA at a relatively slow rate, and binds weakly with DNA. Other analogues of the chelating part of bleomycin such as iron–porphyrin used by Lown [4], ethylenediaminetetraacetic acid used by Dervan [5], and tetraphenylporphyrin derivatives [6], have been reported not to cause significant double helical DNA cleavage on their own, and require an intercalating group to bring them into close proximity with the DNA for biological activities.

Results

The new iron complex **1** was synthesized as illustrated in Scheme 1. The coupling reaction between 5-(2′-thienyl)-2-thienoic acid and histamine can be effectively carried out using dicyclohexylcarbodiimide. Iron was inserted into the N_2S_2-macrocycle by heating with ferrous chloride in methanol to afford the metallocycle in good yield.

The cyclic voltammetry (CV) of compound **1** was found to be nearly reversible as indicated by a peak separation (E) of 80 mV. Also, the other criteria for



Scheme 1. Synthesis of 4-ethylamido[5,(2′-thienyl)-2-thiophene]imidazole iron(II) complex **1**.

*Part I is ref. 1.

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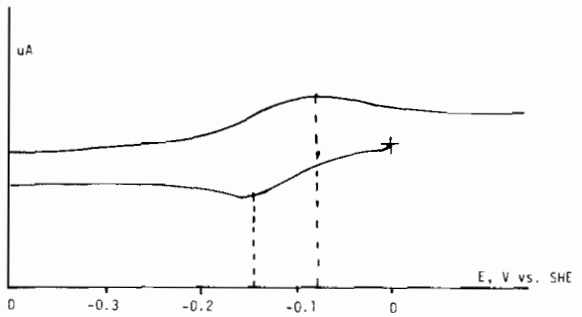

Fig. 1. Cyclic voltammogram of compound **1** in DMSO (1×10^{-3} M).

reversibility is fulfilled, that is $i_a/i_c = 1$ (see Fig. 1). The electron spin resonance of compound **1** was measured in dimethylformamide from α-diphenyl-β-picryhydrazyl (DPPH) and was found to consist of a fairly broad resonance, centered at $g = 2.00989$, which suggests that the equatorial ligands consist of N,N,S,S atoms (see Fig. 2).

The binding constant of compound **1** was measured using the ethidium fluorescence assay technique developed by Morgan [7a] with calf thymus DNA. **1** was found to have a rather low binding affinity with DNA. A solution of 5×10^{-5} M compound **1** in the presence of 20 mM dithiothreitol (DTT) with unlimited excess of air was found to cause 50% scissioning of PM2-supercoiled covalently closed circular (CCC) DNA in 4 h at pH 7.0 (Fig. 3). The control experiment carried out using the pure ligand of **1** showed no observable scissioning of the PM2-CCC DNA. Thus in comparison with bleomycin, compound **1** was found to have a lower efficiency for scissioning of PM2-CCC DNA.

Discussion

The data presented here show that the new metal binding moiety with a 'built in' intercalating group, **1**, has a nearly reversible Fe^{2+}/Fe^{3+} transition and an ESR resonance parameter of $g = 2.00989$, comparable to that of bleomycin–Fe^{2+} complex ($g = 2.0057$). **1** by analogy to haemaglobin [8] will be expected to bind reversibly with oxygen (the additional ligand in the sixth coordinating position may be solvent). The hexacoordinated iron(II) complex, **1**, must therefore be capable of activating oxygen to generate reactive oxygen radical species which then attack DNA, as observed by the scissioning of PM2-CCC DNA in the presence of reducing agent (DTT).

Our bisthiophene iron model **1** thus pinpoints an additional important role that might be played by the

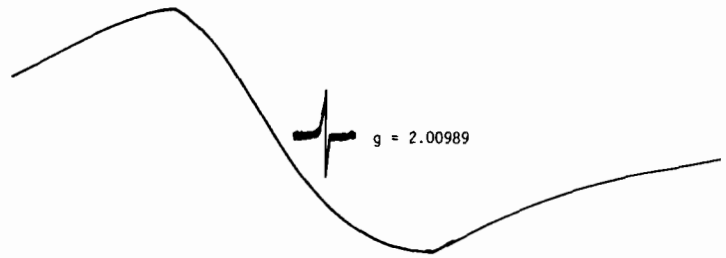

Fig. 2. Electron spin resonance spectrum of compound **1** in DMF.

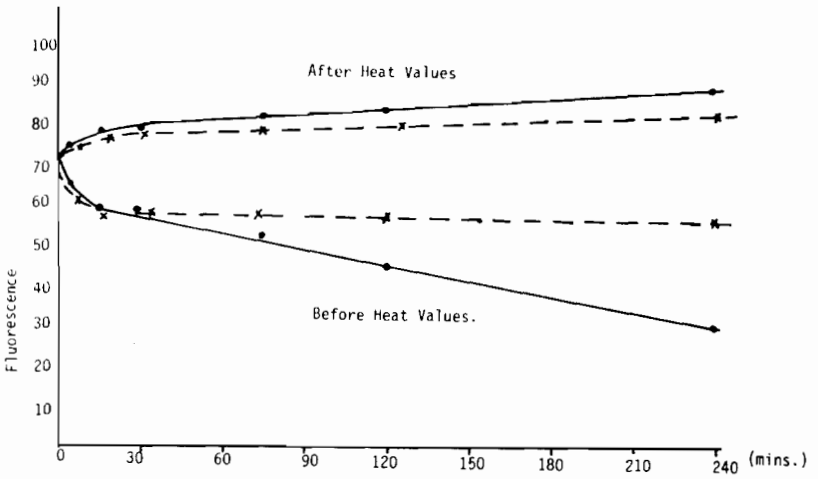

Fig. 3. Scissioning study of compound **1** with PM2-CCC DNA. ——, compound **1** with DDT; – – –, control with DDT and neutral ligand of **1** with DDT. % scissioning = 50% (2 h).

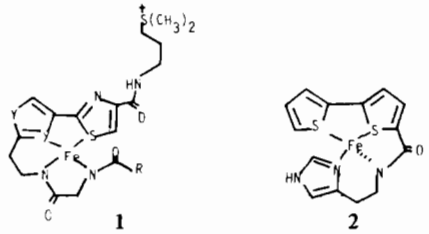

Fig. 4. A comparison of the bleomycin model and the synthetic model.

sterically resembling bithiazole region of the bleomycin in metal chelation, **2**, for some of its observed antitumor activities (Fig. 4). The major role of the bithiazole unit has been thought to be that of intercalation, although evidence exists for the presence of metal ions in close proximity to this terminus of the molecule [3]. Our initial studies not only provide additional evidence for the role of bithiazole metal complex in DNA scissioning, but also include the design of a new antitumor compound with a 'built in' intercalating group. The lower efficiency in the cleavage of DNA by compound **1** may be due to the lack of an electrostatic binding group that is present in bleomycin. An electrostatic binding group should further promote DNA scissioning by bringing the reactive hexacoordinated iron(II) complex **1** into closer proximity with the DNA for a longer than average period of time. Compound **1** and DNA should equilibrate fairly rapidly between the bound and unbound forms, thus lowering the efficiency of DNA cleavage as observed.

Conclusions

This simple synthetic model, 4-ethylamido[5,(2'-thienyl)-2-thiophene]imidazole iron(II) complex, could be an efficient tool to probe further the exact role of the bithiazole unit in bleomycin and in designing more efficient antitumor agents base on bleomycin model. The synthesis of more elaborate molecules is currently being carried out in this laboratory.

Experimental

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infra spectra were obtained on a Nicolet 7199FT spectrometer. Proton NMR measurement were made on either a Varian 100 MHz or Bruker WH-200 spectrometer. Mass spectra were measured with AEI MS-12 or MS-9. Elemental analysis were performed where possible.

In the biological studies, fluorescence was measured on a Turner 430 spectrofluorometer, equipped with a cooling fan to minimize fluctuations in the xenon lamp source and using 1 cm^3 cuvettes at ambient temperature. Binding and scissioning studies were carried out using calf thymus and PM2 supercoiled covalently closed circular DNA, respectively, by the methods of Morgan [7a, b].

2-Bromothiophene [9] and 2,2'-bithiophene [10] were prepared according to literature methods.

5-(2'-Thienyl)-2-thienoic acid

A solution of 2,2'-bithiophene (15 g) in dry ether (190 ml) under nitrogen was cooled to −60 °C, after which n-butyl lithium solution (57 ml, 1.6 M solution) was added dropwise with stirring. After the addition, the solution was left to warm up to room temperature, and then cooled again to −50 °C and dry carbon dioxide gas was passed through the reaction mixture for about an hour. The reaction mixture was again left to attain room temperature, quenching with cold water (350 ml) to dissolve the lithium salt. The ethereal layer was separated, and washed with water. The combined water layer was heated to 80 °C and acidified with concentrated hydrochloric acid, in which the crude product precipitated and was filtered and dried (yield 15 g). Recrystallization from chloroform and subsequently from carbon tetrachloride yielded the pure product (9.5 g, 50%); melting point (m.p.) 184–185 °C. IR (nujol) 1670(br) cm^{-1}; NMR (DMSO-d_6) δ 7.11(1H, dd, J = 6.0 Hz., 4'-H), 7.33(1H, d, J = 6.0 Hz., 4-H), 7.50(1H, dd, J = 1.5 and 6.0 Hz., 3'-H), 7.63(1H, dd, J = 1.5 and 6.0 Hz., 3-H), 7.68(1H, d, J = 6.0 Hz., 5'-H). *Anal.* Found: C, 51.31; H, 2.87; S, 30.40; O, 14.93. Calc. for $C_9H_6O_2S_2$: C, 51.43; H, 2.88; S, 30.47; O, 15.23%.

4-Ethylamido[5,(2'-thienyl)-2-thiophene]imidazole

A mixture of 5-(2'-thienyl)-2-thienoic acid (420 mg) and carbonyl diimidazole (350 mg) was dissolved in dimethyl formamide (10 ml) and stirred for 3 h at room temperature. After this time, the reaction mixture was cooled to 0 °C, and histamine (222 mg) added, after which it was left to attain room temperature overnight. The DMF was removed under high vacuum (temperature = 60 °C). To the solid obtained was added ether to remove unreacted starting material. The remaining solid was washed with 10% MeOH/$CHCl_3$ to remove further impurities. The remaining solid was filtered and dried to give product (570 mg, 85%); m.p. 216 °C (decomposed). IR (nujol) 3175(br), 1610, 1455 cm^{-1}; NMR (DMSO-d_6) δ 8.70(1H, m, imidazole-NH), 8.00–6.85(7H, m, 5 × thiophene-CH and 2 × imidazole-CH), 3.48(3H, m, $CONHCH_2$, amide disappear with D_2O shake), and 2.80(2H, t, CH_2). *m/e* 303.0508 (M^+, 28%).

4-Ethylamido[5,(2'-thienyl)-2-thiophene]imidazole Iron(II) Complex

To a suspension of 4-ethylamido[5,(2'-thienyl)-2-thiophene]imidazole (180 mg) in methanol (15 ml) a

solution of iron(II) chloride (120 mg) in methanol (2.5 ml) was added at room temperature under nitrogen and stirred for 60 h. After this time, most of the solid dissolved. The reaction mixtures was then filtered and the filtrate concentrated under high vacuum at room temperature to give a brownish solid. Column chromatography of the brownish solid on silica gel was found to afford the pure product (120 mg, 65%); m.p. 147–149 °C. IR (nujol) 3120, 1600, 1550, 1530 cm^{-1}. *m/e* 356 ($M^+ - (2H + Cl)$) and 92 (M^+ for FeCl).

The cyclic voltammograms were performed using a EG & E Princeton VA-scanner (Model 175) and a VA-detector (Model 173) equipped with a Princeton plotter RE-0089. The experiment was carried out with 1×10^{-3} M Fe^{2+}–complex solution in DMSO with 0.1 M TEAP at 25 °C. ESR spectra were obtained on a Varian E9 spectrometer with the Fe^{2+}–complex in DMF. For calibration DPPH was used as external standard (*g* was found to be 2.00989).

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References

1 C. W. Ong, *Inorg. Chim. Acta, 123,* 221 (1986).
2 S. M. Hecht (ed.), 'Bleomycin, Chemical, Biochemical and Biological Aspects', Springer, New York, 1979.
3 R. P. Sheriden and R. K. Gupta, *J. Biol. Chem., 256,* 1242 (1981).
4 J. W. Lown and A. V. Joshua, *J. Chem. Soc., Chem. Commun.,* 1298 (1982).
5 R. P. Hertzberg and P. B. Dervan, *J. Am. Chem. Soc., 104,* 313 (1982).
6 Y. Hashimoto, C. S. Lee, K. Shudo and T. Okamoto, *Tetrahedron Lett.,* 1523 (1983).
7 (a) A. R. Morgan, J. S. Lee, D. E. Pulleyblank, N. L. Murray and D. E. Evans, *Nucleic Acids Res., 7,* 547 (1979); (b) 594 (1979).
8 C. Walsh, 'Enzymatic Reaction Mechanism', Freeman, San Francisco, 1979, pp. 464–500.
9 F. E. Blicke and J. H. Bruckholter, *J. Am. Chem. Soc., 64,* 977 (1942).
10 H. Wynberg and A. Loqothetis, *J. Am. Chem. Soc., 78,* 1958 (1956).