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SYNTHESIS, BIOACTIVITY, AND DNA-CLEAVING ABILITY OF DESFERRIOXAMINE B-NALIDIXIC ACID AND ANTHRAQUINONE CARBOXYLIC ACID CONJUGATES

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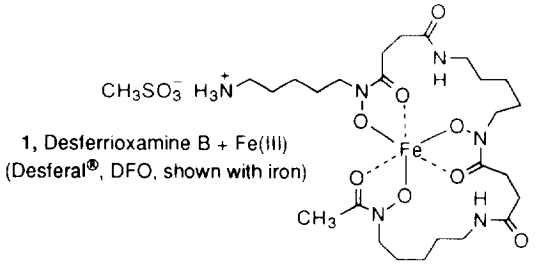
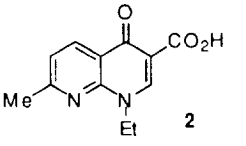
Abstract: Synthesis and *in vitro* activity of two novel conjugates (**4** and **5**) of desferrioxamine B, with nalidixic acid (**2**) and anthraquinone carboxylic acid (**3**), are described.

The need to acquire extremely insoluble extracellular ferric iron for use in physiological redox chemistry has led to the microbial evolution of specific iron-sequestering agents called siderophores.[1] Desferrioxamine B (**1**, DFO, Desferal®), a representative trihydroxamate-containing siderophore, is the current drug of choice for the treatment of transfusional iron overload in β-thalassemic patients,[2] and also has found recent application as an effective antimalarial agent.[3] However, a major concern associated with siderophore therapy is that continuous use often induces severe septicemia, especially in immunocompromised patients, since siderophores are potent microbial growth promoters. Our focus has been on the synthesis of conjugates of siderophores and antimicrobial agents with the anticipation that such conjugates could be used to treat iron overload while preventing septicemia. In addition, siderophore-drug conjugates may be more broadly useful as species selective, active drug transport agents. We recently reported the synthesis and activity of a number of trihydroxamic acid and catechol-based siderophore-β-lactam conjugates.[4] Thus, while our early studies have focused on the use of siderophores as drug delivery agents, we also realized that the iron itself may serve as the toxic agent. For example, if the siderophore-iron complex could be localized in or near the minor groove of DNA, redox chemistry might induce DNA damage by the Fenton reaction. As a preliminary test of the feasibility of this concept, we describe in this article the syntheses and initial biological evaluation of two conjugates (**4** and **5**) obtained by conjugating DFO (**1**) to nalidixic acid (**2**) and anthraquinone carboxylic acid (**3**).



1, Desferrioxamine B + Fe(III)
(Desferal®, DFO, shown with iron)



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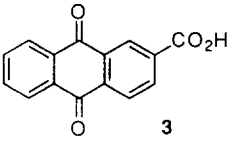
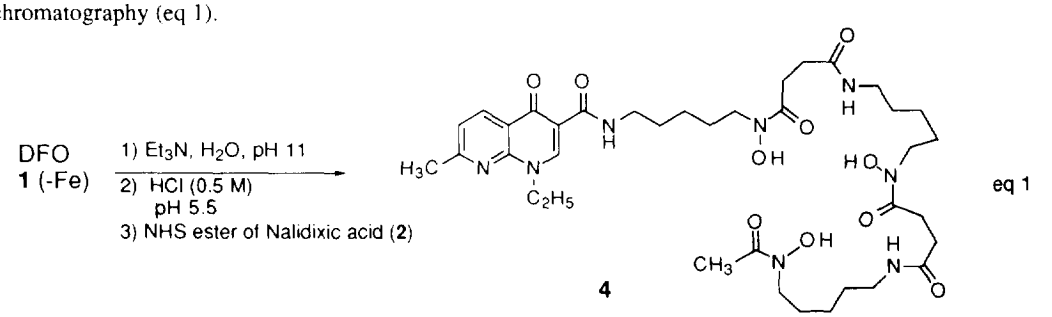


3

Nalidixic acid is a quinolone antibiotic[5] and it is known that the free carboxylic acid is necessary for its antibacterial activity. However, as amide conjugates with DFO we intended that the nalidixic and

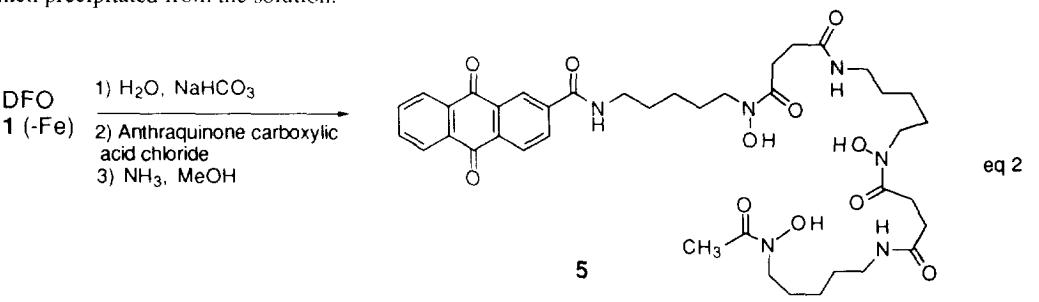
anthraquinone carboxyclic acids would serve as DNA intercalators,[6] thereby anchoring the iron complex close to DNA and then potentially allow the iron to induce redox damage to DNA.[7] Site selective DNA cleavage would not necessarily be anticipated, nor required, since randomly induced DNA damage by an actively transported and microbially selective conjugate might prove to be therapeutically valuable. Furthermore, determining the general antibiotic and antifungal properties of these unique conjugates was of interest.

Synthetic Aspects: The syntheses of conjugates **4** and **5** involved direct coupling of the carboxyl group of **2** and **3** with the amine terminus of DFO. This simple approach was anticipated to minimize disruption of the required molecular recognition of the siderophore component by keeping intact the essential trihydroxamate iron chelating component of DFO while attaching the drug as far as possible from the metal chelator site. Indeed, it has been shown that amino-terminal derivatization does not affect the iron binding capacity of DFO,[8] and in fact, some *N*-acylated derivatives exert improved antimalarial activity relative to DFO itself. Despite the anticipated ease of forming direct amide linkages between nalidixic acid or anthraquinone carboxylic acid and DFO, many conventional techniques proved to be ineffective, perhaps because of competing nucleophilicity of the hydroxamate components of DFO. Interestingly, however, increasing the amount of base (Et_3N) from one to four equivalents during generation of the free amine of **1**, followed by treatment of the *N*-hydroxysuccinimide (NHS) ester of **2** (obtained from overnight reaction of **2** with DCC and NHS in THF at 70 °C), afforded an improved yield of the acylated product **4**[9] (~55%) after reverse phase chromatography (eq 1).



eq 1

Acylation of DFO with four equivalents of the acid chloride of anthraquinone carboxylic acid (obtained by refluxing **3** with $SOCl_2$ for 2h) under Schotten-Baumann conditions provided the tetraacylated product.[10] A methanol/CH_2Cl_2 solution of the tetraacylated product was saturated with ammonia to give **5**[9] which precipitated from the solution.



eq 2

DNA cleavage experiment: The ability of conjugates **4** and **5** to interact with and cleave DNA by Fenton-type[11] chemistry was probed in the presence and absence of Fe(II), H_2O_2 and ascorbate (Figure 1). The experiments were performed using a 29 bp DNA fragment radiolabeled at one end with [^{32}P]. Each reaction contained approximately 30,000 cpm of labeled substrate with 0.05 μg/μL of *E. coli* *t*RNA as carrier. The cleavage reactions were carried out at room temperature for 2 min in 100 mM Tris-HCl pH 8.0 buffer (total volume of 20 μL), and were quenched by the addition of 0.1 M thiourea (2 μL). The DNA was ethanol precipitated and washed with 70% EtOH. The dried samples were resuspended in formamide loading dye and were resolved by electrophoresis on a 15% polyacrylamide gel containing 7 M urea. Separate control experiments were performed in the presence of each reactive component (data not shown). DNA cleavage occurred only when both the siderophore and the reducing agents were present. Another structurally similar, hydroxamate-containing siderophore (iron chelator) N^5-acetyl-N^5-hydroxy-L-ornithine, and EDTA, which do not contain DNA intercalators also induced cleavage under identical experimental conditions, indicating that the Fenton-type chemistry was responsible for DNA damage. Fe[EDTA]$^{2-}$, which is a solvent-based generator of hydroxyl radicals,[12] cuts DNA uniformly. However, the cleavage patterns for conjugates **4**, and especially **5**, containing the potential DNA intercalators, are not random. Presumably, this is due to proximity effects during the Fenton chemistry and provides evidence that these molecules do bind directly to DNA with some site selectivity.

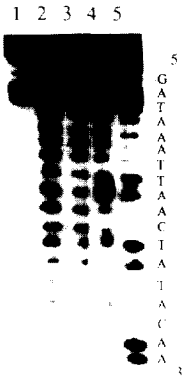


Figure 1. Autoradiograph showing cleavage patterns produced by DFO-nalidixic acid conjugate (**4**) and DFO-anthraquinone carboxylic acid conjugate (**5**) with 3'-^{32}P end-labeled DNA: **Lane 1**, 10 μM Fe(II), 0.03% H_2O_2; **Lane 2**, 20 μM EDTA, 10 μM Fe(II), 9 mM ascorbate, 0.03% H_2O_2; **Lane 3**, 20 μM N^5-acetyl-N^5-hydroxy-L-ornithine, 10 μM Fe(II), 9 mM ascorbate, 0.03% H_2O_2; **Lane 4**, 35 μM DFO-nalidixic acid conjugate (**4**), 8.8 μM Fe(II), 8 mM ascorbate, 0.03% H_2O_2; **Lane 5**, 35 μM DFO-anthraquinone carboxylic acid conjugate (**5**), 8.8 μM Fe(II), 8 mM ascorbate, 0.03% H_2O_2.

Antimalarial activity: Conjugate **4** displayed considerable antimalarial activity against *P. falciparum* D6 and *P. falciparum* W2 (IC_{50} ~0.6 μg/mL for both). Since these two strains (D6 and W2) are resistant to mefloquine and a combination of chloroquine and pyrimethamine, respectively, use of two drugs with different modes of action often is required for treatment. Thus, the identical inhibitory concentrations of **4** against both strains of *P. falciparum* is especially intriguing. Interestingly, conjugate **5** did not display significant antimalarial activity. Further biological evaluation of **4** and **5**, and related conjugates, are under way and will be reported in due course.

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9. Characterization data: for compound **4**; mp 143-144°C; ^{1}H NMR (DMSO-d$_6$) δ 1.0-1.5 (m, 21H), 1.90 (s, 3H), 2.20 (br t, 4H), 2.40-2.55 (m, 7H), 2.85-3.02 (m, 6H), 3.20-3.50 (m, 6H), 4.50 (q, *J*=7.0 Hz, 2H) , 7.41 (d, *J*=8.2 Hz, 1H), 7.76 (t, *J*=4.8 Hz, 2H), 8.49 (d, *J*=8.1 Hz, 1H), 8.90 (br s, 1H), 9.55-9.72 (m, 3H), 9.78 (t, *J*=5.6 Hz, 1H); ^{13}C NMR (DMSO-d$_6$) δ 175.87, 172.61, 172.05, 171.95, 171.41, 170.67, 170.15, 163.63, 162.96, 158.12, 149.83, 148.07, 147.68, 139.02, 135.86, 124.82, 121.24, 119.62, 112.16, 47.07, 46.78, 45.95, 45.52, 38.67, 38.42, 30.37, 30.00, 28.99, 27.61, 25.99, 25.19, 24.82, 23.61, 23.48, 20.29, 14.98, 8.55; HRMS (FAB) calcd. for MH$^+$ $C_{37}H_{59}O_{10}N_8$: 775.4354, found: 775.4341; for compound **35** mp 188-190°C; ^{1}H NMR (DMSO-d$_6$) δ 1.10-1.65 (m, 18H), 1.90 (s, 3H), 2.30 (br t, 4H), 2.50-2.65 (m, 4H), 3.00. (br t, 4H), 3.20-3.60 (m, 8H), 7.60-7.80 (m, 2H), 7.93-7.98 (m, 2H), 8.22-8.45 (m, 7H), 8.66-8.69 (m, 2H); HRMS (FAB) calcd. for MH$^+$ $C_{40}H_{55}O_{11}N_6$: 795.3929. Found: 795.3928.
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