Synthesis and Antibacterial Activity of 2,3-Dehydroofloxacin

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The 2,3-dehydro analog **2** of the potent quinolone antibacterial agent ofloxacin (**1**) was synthesized by an efficient six step route beginning with ethyl 2,3,4,5-tetrafluorobenzoylacetate. Formation of the oxazine ring of **2** was accomplished by ozonolysis of 1-(1-buten-3-yl)quinolone **5** to the corresponding aldehyde, which cyclized upon treatment with base *via* intramolecular displacement of the C-8 fluorine to afford tricyclic ester **6**. The antibacterial activities of 2,3-dehydroofloxacin (**2**) and ofloxacin (**1**) are compared.

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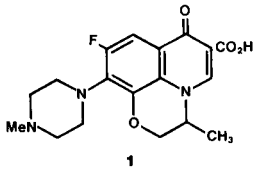
Ofloxacin (**1**) [1] belongs to a new class of antibacterial agents known as quinolones [2], which exert their activity by inhibition of the target enzyme DNA gyrase [3]. Quinolones typically contain the 1,4-dihydro-4-oxoquinoline-3-carboxylic acid (or 1,8-naphthyridine-3-carboxylic acid) heterocyclic nucleus, with ofloxacin possessing an additional oxazine ring appended to the 1- and 8-positions. A recent US patent [4] describing the synthesis of 2,3-dehydroofloxacin (**2**) prompts us to disclose our synthesis of this interesting quinolone analog possessing the 7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxacine heterocyclic nucleus. Our interest in 2,3-dehydroofloxacin (**2**) stems from the fact that the electron donating ability of the N-1 substituent (*e.g.*, *p*-fluorophenyl, cyclopropyl and methoxy) of the 4-oxoquinolone nucleus strongly influences antibacterial potency [2]. Thus, the additional double bond in 2,3-dehydroofloxacin (**2**) would be expected to have an interesting effect on antibacterial activity relative to ofloxacin (**1**) because it creates a resonance interaction between the oxygen and carbon-3 of the benzoxazine ring, thereby adding electron density to the quinolone nitrogen.



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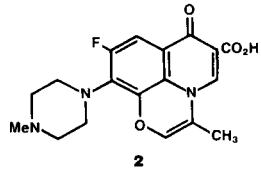

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Our approach to the synthesis of the 3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid nucleus of 2,3-dehydroofloxacin (**2**) was based on formation of the oxazine ring by the intramolecular displacement reaction of enolate **5A**, depicted in Scheme 1. Related intramolecular displacement reactions of the C-8-fluorine in the quinolone series by alkoxide [5], phenoxide [6] and thiophenoxide [7] nucleophiles have been previously reported. It was further expected that quinolone **5**, possessing an olefinic side chain at N-1, could serve as the precursor to enolate **5a** by ozonolysis followed by treatment of the resulting aldehyde with base.

As shown in Scheme 1, 1-(1-buten-3-yl)quinolone **5** was prepared in a straightforward manner from ethyl 2,3,4,5-tetrafluorobenzoylacetate (**3**), based on a procedure used in synthesis of the corresponding 1-(1-hydroxyprop-2-yl) derivative, an intermediate in the synthesis of ofloxacin [5]. Thus, treatment of **3** with triethyl orthoformate and acetic anhydride gave the oily ethoxymethylene derivative, which upon treatment with 3-amino-1-butene afforded vinylogous amide **4** in 68% yield. Cyclization of **4** to quinolone **5** occurred in 95% yield upon exposure to sodium hydride in glyme. The use of glyme as solvent in this reaction was critical.

Conversion of **5** to pyrido[1,2,3-*de*][1,4]benzoxazine **6** was accomplished in essentially a one-pot procedure. A methanolic solution of **5** was ozonolyzed at −78° and the ozonide was quenched with dimethylsulfide. The solvent was removed and the residue was redissolved in DMF and was added to a 0° suspension of sodium hydride in DMF. Upon warming to room temperature, the desired cyclization reaction rapidly ensued (as monitored by tlc) to produce **6** in 74% yield. Since only the (*Z*)-enolate of **5A** is capable of cyclizing, it was assumed that equilibration of the (**E**)- and (**Z**)-enolates occurred under the reaction conditions.

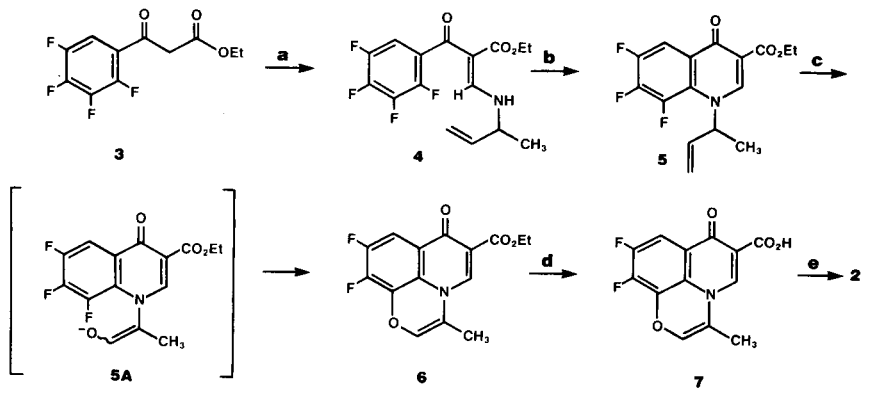
2,3-Dehydroofloxacin (**2**) then was prepared by acid hydrolysis of ester **6** to acid **7** (80% yield), followed by displacement of the 9-fluorine with *N*-methylpiperazine (79% yield).

A comparison of the *in vitro* antibacterial activity of 2,3-dehydroofloxacin (**2**) and ofloxacin (**1**) against three organism is shown in Table 1. Interestingly, introduction of the Δ^2-unsaturation into the ofloxacin nucleus led to a dramatic reduction in antibacterial potency, as shown by the higher MIC values for 2,3-dehydroofloxacin (**2**). Studies are currently underway to determine whether the loss of activity is due to weak inhibition against the target enzyme DNA gyrase or to poor penetration across the cellular membrane.

Table 1
In Vitro Antibacterial Activity (Minimum Inhibitory Concentration, μg/ml)

Compoud No.	*S. aureus* 005	*E. coli* 125	*Ps. aeruginosa* 104
1	0.39	<0.25	0.25
2	12.5	0.39	12.5

Scheme 1



a) i: $HC(OEt)_3$, Ac_2O, Δ; ii: 3-amino-1-butene, 95% EtOH; b) NaH, glyme, 0°C;
c) i: O_3, MeOH, -78°C; ii: Me_2S; iii: NaH, DMF, 0° → 25°C; d) 2N HCl, THF, Δ;
e) N-methylpiperazine, DMSO, 70°C.

EXPERIMENTAL

The ^1H nmr spectra were determined with a Varian XL 300 spectrometer. Chemical shifts are expressed in ppm relative to deuteriochloroform. Significant ^1H nmr data are tabulated in order (number of protons, multiplicity, coupling constant (Hertz)). Mass spectra were determined with a Finnigan-4510 GSMS mass spectrometer. Infrared spectra were determined with a Perkin-Elmer Model 283B infrared spectrophotometer. Elemental analyses were performed by the Pfizer Analytical Chemistry Department. Melting points are uncorrected and were obtained in open capillaries on a Thomas-Hoover melting point apparatus. Ozonolyses were performed on a Welsbach ozonator. Solvents were removed with a rotary evaporator.

Anhydrous, Gold Label *N,N*-dimethylformamide and ethylene glycol dimethyl ether (glyme) were purchased from Aldrich Chemical Co and were used without purification.

Flash chromatography was performed using 32-63 μm Silica gel (Woelm®) according to the method described by Still *et al.* [8]. Analytical thin-layer chromatography (tlc) was performed on 250 micron, 2.5 x 10 cm silica gel plates (Analtech) using ultraviolet light or potassium permanganate spray for visualization.

Ethyl 3-(1-Buten-3-ylamino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (**4**).

A mixture of 5.00 g (18.9 mmoles) of ethyl 2,3,4,5-tetrafluorobenzoylacetate (**3**) [9], 4.20 g (28.4 mmoles) of triethyl orthoformate, and 4.83 g (47.3 mmoles) of acetic anhydride was heated at 145° for 2 hours. The volatiles were removed by high vacuum distillation at 145°, leaving behind a viscous red oil (R_f 0.24, 3:1 hexane:ethyl acetate). To a solution of this crude enol ether in 80 ml of 95% ethanol was added dropwise at 0° a solution of 1.47 g (20.7 mmoles) of 3-amino-1-butene [10] in 12 ml of 95% ethanol. After stirring the mixture for 2 hours at 0°, the solvent was removed and the residual oil was purified by flash chromatography (12 cm height, 6.5 cm diameter), using 3:1 hexane:ethyl acetate as eluant, to afford 4.38 g (68%) of ethyl 3-(1-buten-3-ylamino)-2-(2,3,4,5-tetrafluorobenzoyl)acrylate (**4**) as a white solid following trituration with pentane, mp 62-63°; ^1H nmr (deuteriochloroform): 300 MHz, δ 0.96 and 1.09 (3H, two t, J = 7), 1.43 and 1.45 (3H, two d, J = 7), 3.96-4.12 (3H, m), 5.20-5.28 (2H, m), 5.77 (1H, m), 6.90-7.04 (1H, m), 8.09 and 8.10 (2H, two d, J = 13); ir (potassium bromide): 1688, 1622, 1483 cm^{-1}.

Anal. Calcd. for $C_{16}H_{15}F_4NO$: C, 55.66; H, 4.38; N, 4.06. Found: C, 55.71; H, 4.45; N, 4.04.

Ethyl 1-(1-Buten-3-yl)-1,4-dihydro-6,7,8-trifluoro-4-oxoquinoline-3-carboxylate (**5**).

Into a 200 ml three-necked flask was placed 0.64 g (13.3 mmoles) of 50% sodium hydride in mineral oil; this was washed with three 10 ml portions of pentane, mixed with 100 ml of anhydrous glyme (dimethoxyethane), and chilled to 0°. A solution of 4.18 g (12.1 mmoles) of ethyl 3-(1-buten-3-ylamino)-2-(2,3,4,5-tetrafluorobenzoyl) acrylate (**4**) in 15 ml of glyme was then added dropwise. The mixture was stirred for 1 hour at 0° and was quenched by the dropwise addition of excess (3 ml) acetic acid. Most of the solvent was removed by evaporation and the residual slurry was poured onto 100 ml of crushed ice and water. The resulting white suspension was stirred for 30 minutes; the precipitate was collected by filtration and washed with pentane to yield 3.75 g (95%) of ethyl 1-(1-buten-3-yl)-1,4-dihydro-6,7,8-trifluoro-4-oxoquinoline-3-carboxylate (**5**) (R_f 0.22, 1:1 hexane:ethyl acetate), mp 113-115°; ^1H nmr (deuteriochloroform): 300 MHz, δ 1.44 (3H, t, J = 7), 1.72 (3H, t, J = 7), 4.42 (2H, q, J = 7), 5.41 (1H, d, J = 15), 5.51 (1H, d, J = 10), 5.66 (1H, broad q), 6.04-6.16 (1H, m), 8.17-8.25 (1H, m), 8.58 (1H, s); ms (m/e), 325 (M^+), 55 (base); ir (potassium bromide): 1730, 1695, 1648, 1612 cm^{-1}.

Anal. Calcd. for $C_{16}H_{14}F_3NO_3$: C, 59.08; H, 4.34; N, 4.31. Found: C, 58.80; H, 4.20; N, 4.29.

Ethyl 9,10-Difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (**6**).

A stream of ozone was bubbled into a mixture of 2.90 g (8.9 mmoles) of ethyl 1-(1-buten-3-yl)-1,4-dihydro-6,7,8-trifluoro-4-oxoquinoline-3-carboxylate (**5**), 80 ml of methanol, and 20 ml of methylene chloride for 1 hour at −78°. The disappearance of the starting olefin was monitored by tlc (1:1 ethyl acetate:hexane) and by the appearance of a blue color. The reaction mixture was purged with nitrogen and excess dimethylsulfide (7 ml) was add-

ed dropwise at −78°. The mixture was allowed to warm slowly to room temperature, the solvent was removed and the residual oil was dried under high vacuum. Meanwhile, in a 200 ml three-necked flask was prepared a 0° suspension of 0.96 g (19.9 moles) of 50% sodium hydride in mineral oil (washed with pentane, 3 x 10 ml) in 100 ml of anhydrous *N,N*-dimethylformamide; to it was added dropwise a solution of the crude aldehyde in 20 ml of *N,N*-dimethylformamide. When the addition was complete, the ice bath was removed and a mild exotherm ensued (30°). After stabilizing to room temperature, the mixture was quenched carefully with excess acetic acid and the solvent was removed. The residue was dissolved in 30 ml of chloroform, washed with water (4 x 30 ml), dried (magnesium sulfate), and evaporated to a brown solid. Purification of the solid by flash chromatography (13 cm height, 6.5 cm diameter) using 97.5:2.5 chloroform:methanol as eluant afforded 2.11 g (74%) of ethyl 9,10-difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylate (**6**) as a pale yellow solid (R_f 0.44, 5:95 methanol:chloroform) after trituration with ether, mp 259-261°; ^{1}H nmr (deuteriochloroform): 300 MHz, δ 1.40 (3H, t, J = 7), 2.09 (3H, s), 4.39 (2H, q, J = 7), 6.27 (1H, s), 7.68-7.75 (1H, m), 8.28 (1H, s); ms: (m/e) 307 (M^+), 262, 235 (base), 206, 179, 151, 132: ir (potassium bromide): 1721, 1606, 1566, 1504 cm^{-1}. The analytical sample was prepared by recrystallization form isopropanol, mp 266-267°.

Anal. Calcd. for $C_{15}H_{11}F_2NO_4$: C, 58.64; H, 3.61; N, 4.56. Found: C, 58.37; H, 3.31; N, 4.40.

9,10-Difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (**7**).

A mixture of 1.00 g (3.60 mmoles) of ethyl 9,10-difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (**6**), 50 ml of aqueous 2*N* hydrochloric acid solution, and 20 ml of tetrahydrofuran was heated to reflux for 5 hours. The disappearance of the ester was monitored by tlc (5:95 methanol:chloroform). The solvent was removed and the solid residue was triturated with ether and pentane to yield 0.80 g (80%) of 9,10-difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (**7**) as a pale yellow solid: ^{1}H nmr (deuteriochloroform): 300 MHz, δ 2.14 (3H, s), 6.41 (1H, s), 7.70-7.77 (1H, m), 8.47 (1H, s,); ms: (m/e) 279 (M^+), 235 (base), 206, 179, 151, 138. The analytical sample was prepared by recrystallization from 2-propanol, mp 267-269°.

Anal. Calcd. for $C_{13}H_{17}F_2NO_4$: C, 55.93; H, 2.53; N, 5.02. Found: C, 55.67; H, 2.32; N, 4.99.

9-Fluoro-3-methyl-10-(4-methyl-1-piperizinyl)-7-oxo-7*H*-pyrido-[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (2,3-Dehydroofloxacin) (**2**).

A mixture of 0.100 g (0.36 mmole) of 9,10-difluoro-3-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (**7**), 0.240 g (2.20 mmoles) of *N*-methylpiperazine, and 3 ml of dimethyl sulfoxide was heated to 70° for 16 hours. The solvent and excess *N*-methylpiperazine were removed by vacuum distillation and the remaining solid was triturated with ethyl acetate to yield 102 mg (79%) of 9-fluoro-3-methyl-10-(4-methyl-1-piperizinyl)-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid (2,3-dehydroofloxacin) (**2**) as a yellow solid, mp 264-266° dec; ^{1}H nmr (deuteriochloroform): 300 MHz, δ 2.08 (3H, s), 2.37 (3H, s), 2.50-2.62 (4H, m), 3.20-3.37 (4H, m), 6.36 (1H, s), 6.55 (1H, d, J = 13), 8.32 (1H, s); ms: (m/e) 359 (M^+), 315. An analytical sample was prepared by recrystallization from ethyl acetate, mp 264-266°.

Anal. Calcd. for $C_{18}H_{18}O_4N_3F$: C, 60.16; H, 5.05; N, 11.69. Found: C, 59.90; H, 4.67; N, 11.31.

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