Synthesis of 4,12-Dihydro-4-oxoquino-[1,8a,8-*a,b*]quinoxaline-5-carboxylic Acid Derivatives

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The synthesis and antibacterial activity of 1-substituted amino-2-fluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acid derivatives are described. The synthetic route includes a carbon homologation and two intramolecular nucleophilic displacement cyclization reactions leading to the novel 4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acid heterocycle.

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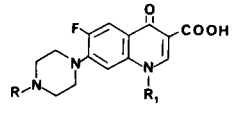
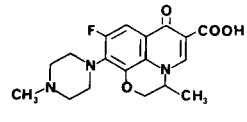
In recent years, the 4-pyridone-3-carboxylic acid class of orally active antibacterials known collectively as the quinolones has stimulated a great deal of interest in the synthesis of 1,4-dihydro-4-oxoquinoline-3-carboxylic acid derivatives. This class of antimicrobial agents includes Norfloxacin **1** [1], Ciprofloxacin **2**, Difloxacin **3** [3] and Ofloxacin **4** [4]. Compound **4** has an oxazine moiety. During our research on the synthesis of novel quinolone derivatives as antimicrobial agents, we synthesized a series of hybrid quinolone antibacterials containing the oxazine moiety. These compounds, 1-substituted amino-2-fluoro-4-oxo-4*H*-quino[8,8a-1-*b,c*][1,4]benzoxazine-5-carboxylic acid derivatives **5**, were found to possess antibacterial activity [5]. Since amino substitution in quinolone derivatives may improve the absorption or tissue distribution [6], compound **6**, which has an NH group in place of the ring oxygen in **5**, was chosen as a synthetic target.



1 R=H, R_1=C_2H_5
2 R=H, R_1=c-C_3H_5
3 R=CH_3, R_1=p-FC_6H_4



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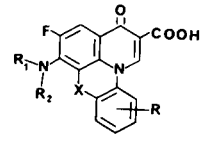
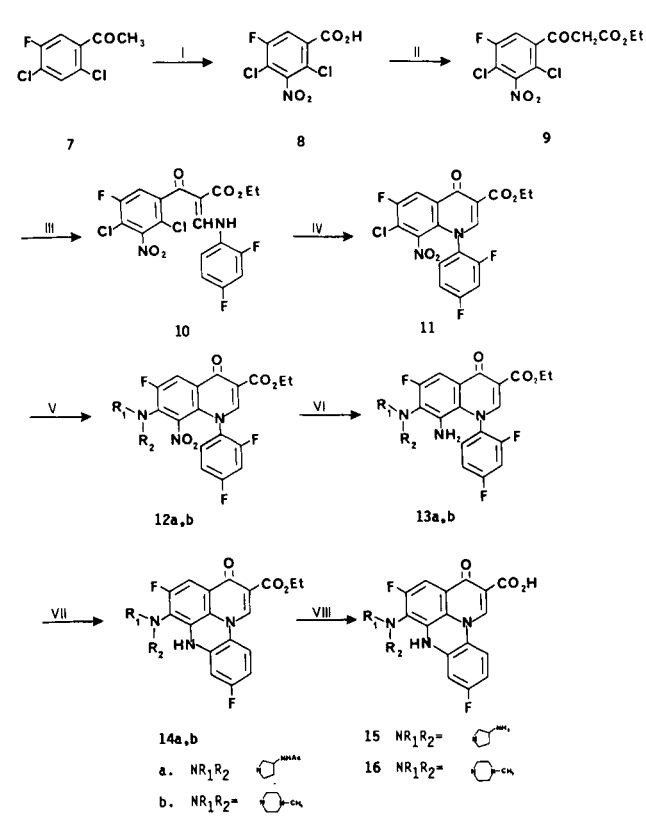


5 X=O
6 X=NH

The 4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acid (**6**) represents a novel heterocyclic ring system. In this paper, we would like to report the syntheses and antimicrobial evaluations of 1-(3-aminopyrrolidin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acid (**15**) and 1-(4-methylpiperazin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-a,b]quinoxaline-5-carboxylic acid (**16**). Our synthetic pathway to the desired ring skeleton required two intramolecular nucleophilic displacement cyclization reactions involving nitrogen as a nucleophile, a process that has been successfully applied to the syntheses of 1-aryquinolone [3], Amifloxacin [7] and benzoxazine derivatives [5]. Hence, compounds **15** and **16** were synthesized by a route illustrated in Scheme I.

Scheme I

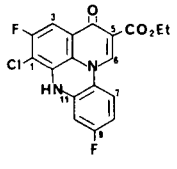


I) H_2SO_4/HNO_3 II) 1. $SOCl_2$, 2. $CH_2(COOEt)COOH/NBuli$ III) 1. $CH(OEt)_3/AC_2O$ 2. IV) NaH V) NR_1R_2 VI) $H_2/RaNi$ VII) pyridine Δ VIII) HCl

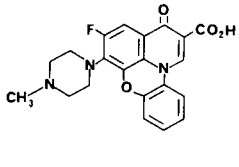
Oxidation and nitration of 2,4-dichloro-5-fluoroacetophenone (**7**) [8] was achieved in one reaction flask by reacting it with a mixture of concentrated sulfuric acid and nitric acid to yield the 2,4-dichloro-3-nitro-5-fluorobenzoic acid (**8**) (mp 188-191°, 82%). Treatment of this acid **8** with

thionyl chloride gave the corresponding acid chloride which, without purification, was reacted with the dilithiodianion of monoethyl malonate [9] to give ethyl 2,4-dichloro-3-nitro-5-fluorobenzoylacetate (**9**) as an oil (97%). It existed in both keto and enol forms. Treatment of this ester **9** with triethylorthoformate in acetic anhydride gave the one carbon homolog enol ether intermediate which was allowed to react with a slight excess of 2,4-difluoroaniline in methylene chloride at room temperature in give ethyl 3-(2,4-difluoroanilino)-2-(2,4-dichloro-3-nitro-5-fluorobenzoyl)acrylate (**10**). This enaminoketo ester exists in both *trans* and *cis* forms in solution with one of the isomers being predominant.

Regiospecific cyclization of the crude compound **10** with one molar equivalent of sodium hydride in tetrahydrofuran (THF) yielded ethyl 1-(2,4-difluorophenyl)-6-fluoro-7-chloro-8-nitro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**11**) (mp 189.5°, 79% overall from **9**). A desired synthetic pathway to a variety of amino derivatives would require a common intermediate having the basic ring skeleton with a leaving group at the 1-position such as compound **17**. Displacement of compound **17** at the 1-position with different amines would generate many amino derivatives. The compound **17** would, in theory, be derived from **11** by a reduction and cyclization synthetic sequence. The route described below, however, was chosen to take advantage of the activation of the chloro group by the α-nitro substituent, and thereby reduce the likelihood of displacement of the fluorine substituent. If the cyclization reaction were performed first to yield an intermediate such as **17**, the NH group would have had a deactivating effect on the chloro group; consequently, the displacement reaction was performed before cyclization.



17



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Displacement of **11** with an excess of 3-acetamidopyrrolidine or 4-methylpiperazine in THF yielded the ethyl 1-(2,4-difluorophenyl)-6-fluoro-7-substituted amino-8-nitro-1,4-dihydro-4-oxoquinoline-3-carboxylates **12a** (mp 117°, 83%) or **12b** (mp 204.5°, 73%). Reduction of **12a** or **12b** with Raney nickel yielded the ethyl 1-(2,4-difluorophenyl)-6-fluoro-7-substituted amino-8-amino-1,4-dihydro-4-oxoquinoline-3-carboxylates **13a** (mp 129-134°, 69.8%) or **13b** (mp 102-104°, 57%). Cyclization of **13a** and **13b** in refluxing pyridine yielded the 4,12-dihydro-4-oxoquino-[1,8a,8-*a,b*]quinoxaline derivatives **14a** (mp 233-234°, 73%) and **14b** (mp 240-242°, 73%). Hydrolysis of **14a** and **14b** with hydrochloric acid yielded 1-(3-aminopyrrolidin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acid hydrochloride (**15**) (mp >250°, 63%) and 1-(4-methylpiperazin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acid hydrochloride (**16**) (mp >250°, 94%).

Biological Results.

Table I summarizes the *in vitro* antibacterial activity of the 4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic acids against five gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P, *Staphylococcus aureus* CMX 686B, *Staphylococcus epidermidis* 3519, *Streptococcus faecium* ATCC 8043, and *Streptococcus pyogenes* 930) and six gram-negative organisms (*Escherichia coli* Juhl, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* 8045, *Pseudomonas aeruginosa* 5007, *Pseudomonas aeruginosa* K799/WT, and *Acinetobacter sp.* (MX669). The data for 1-(4-methylpiperazin-1-yl)-2-fluoro-4-oxo-4*H*-quino[8,8a,1-*a,b*][1,4]benzoxazine-5-carboxylic acid (**18**) is included for comparison. Inspection of Table I revealed that the two compounds, **15** and **16**, have moderate antibacterial activities against gram-positive bacteria. However, they are less potent than their oxobiostere analogs.

Table I

In Vitro Antibacterial Activity of 4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic Acids

	Minimal Inhibitory Concentration (MIC), μg/ml [a]										
	Organism										
Compound	Sa(A)	Sa	Se	Sf	Sp	Ec	Ea	Kp	Pa(5)	Pa(k)	A
15	6.2	12.5	6.2	12.5	6.2	100	>100	25	>100	>100	>100
16	6.2	6.2	3.1	3.1	0.78	12.5	50	6.2	>100	>100	12.5
18	1.56	1.56	1.56	1.56	3.1	3.1	6.2	1.56	12.5	12.5	3.1

[a] The MICs were detemined by the two-fold agar dilution on Brain-Heart Infusion agar. Organisms selected for inclusion in the table are: Sa(A), *Staphylococcus aureus* ATCC 6538P; Sa, *Staphylococcus aureus* CMX 686B; Se, *Staphylococcus epidermidis* 3519; Sf, *Streptococcus faecium* ATCC8043; Sp, *Streptococcus pyogenes* 930; Ec, *Escherichia coli* Juhl; Ea, *Enterobacter aerogenes* ATCC 13048; Kp, *Klebsiella pneumoniae* 8045; Pa(5), *Pseudomonas aeruginosa* 5007; Pa(k), *Pseudomonas aeruginosa* K799/WT; A, *Acinetobacter sp.* CMX 669.

EXPERIMENTAL

Melting points were taken in a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were obtained (unless otherwise specified) were within ±0.4% of the theoretical values. Microanalyses were performed by the Abbott analytical department. The nmr spectra were obtained on a Varian T-60 and HA-100 spectrometer using tetramethylsilane as an internal standard. The nmr peaks were designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Mass spectra were recorded on a Kratos MS-50 mass spectrometer. The ir spectra were recorded on a Perkin-Elmer Model 710 A infrared spectrometer. The ir, nmr, and ms data of all compounds were consistent with the assigned structures. Solutions were dried over magnesium sulphate.

2,4-Dichloro-3-nitro-5-fluorobenzoic acid (**8**).

2,4-Dichloro-5-fluoroacetophenone (**7**) [8] (47.4 g, 230 mmoles) was added slowly to concentrated sulfuric acid (500 ml) with stirring. The reaction temperature rose to 30° and a yellowish solution resulted. Ninety percent nitric acid (57.67 g, 920 mmoles) was added dropwise over a period of 3.5 hours. The rate of addition was controlled so that the reaction temperature was maintained at 55°. After the addition, the mixture was heated at 75° for 3 hours. The mixture was cooled to room temperature and poured into 4 liters of crushed ice. A solid appeared and was filtered and washed with water (5 x 200 ml). The solid was taken up in ether and extracted twice with dilute sodium hydroxide solution. The sodium hydroxide solution was acidified to *p*H 2 with concentrated hydrochloric acid. An oily precipitate appeared and was extracted with ether and dried. Evaporation of the ether under reduced pressure yielded 2,4-dichloro-3-nitro-5-fluorobenzoic acid (**8**) (47.9 g, 82%), mp 188-191°; ^{1}H nmr (deuteriochloroform): δ 7.95 (d, $J_{H\text{-}F}$ = 8 Hz, 1, aromatic H).

Anal. Calcd. for $C_7H_2Cl_2FNO_4$: C, 33.07; H, 0.79; F, 7.48; N, 5.51. Found: C, 33.04; H, 0.83; F, 7.38; N, 5.45.

Ethyl 2,4-dichloro-3-nitro-5-fluorobenzoylacetate (**9**).

One drop of dimethylformamide (DMF) was added to a solution of 2,4-dichloro-3-nitro-5-fluorobenzoic (29.46 g, 116 mmoles) in thionyl chloride (100 ml). After heating the mixture at 78° for 2 hours, the solvent was removed by evaporation under reduced pressure yielding a yellowish oil, 2,4-dichloro-3-nitro-5-fluorobenzoyl chloride. Monoethyl malonate (30.9 g, 234 mmoles) and 10 mg of biquinoline were dissolved in 500 ml dry tetrahydrofuran (THF) under nitrogen atmosphere and cooled to -50°. A solution of 2.5 M *n*-butyllithium in hexane was added until a pink color remained at -5° (200 ml). The reaction was then cooled to -78°. The acid chloride obtained as described above, dissolved in 200 ml of THF, was added dropwise. Upon addition, the dry ice bath was removed and the reaction was allowed to warm up to room temperature. The reaction was acidified with 300 ml of 1 *N* hydrochloric acid and was extracted with ether. The ether fraction was washed with saturated aqueous sodium bicarbonate solution and then water. The ether fraction was dried and evaporated to dryness yielding an oil **9** (36.44 g, 97%); ^{1}H nmr (deuteriochloroform): δ (2 set of signals) 1.27 (t, J = 7 Hz, 3, ethyl CH_3), 1.35 (t, J = 7 Hz, 3 ethyl CH_3), 4.02 (s, 1, CH_2), 4.21 (q, J = 7 Hz, 2, ethyl CH_2), 4.31 (q, J = 7 Hz, 2, ethyl CH_2), 5.62 (s, 1, vinyl H), 7.61 (d, $J_{H\text{-}F}$ = 10 Hz, 1 aromatic H), 7.62 (d, $J_{H\text{-}F}$ = 10 Hz, 1 aromatic H).

Anal. Calcd. for $C_{11}H_6Cl_2FNO_5$: C, 40.77; H, 2.49; N, 4.32. Found: C, 40.95; H, 2.27; N, 4.07.

Ethyl 1-(2,4-Difluorophenyl)-6-fluoro-7-chloro-8-nitro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**11**).

A solution of ethyl 2,4-dichloro-3-nitro-5-fluorobenzoylacetate (**9**) (12 g, 37 mmoles) in triethylorthoformate (9.1 ml, 59.2 mmoles) and acetic anhydride (24 ml, 167 mmoles) was heated at 125° for 3 hours with the removal of ethyl acetate formed during the reaction. The solution was evaporated under reduced pressure to a mobile oil which was then dissolved in methylene chloride (150 ml). 2,4-Difluoroaniline (4.18 ml, 40.7 mmoles) was added to the solution. After 15 minutes, the solution was evaporated to dryness yielding an oil **10** which was dissolved in dry tetrahydrofuran (100 ml). To this solution at ice bath was slowly added a 60% sodium hydride-in-oil suspension (1.69 g, 38.8 mmoles). The mixture was heated at 45° for 2 hours under nitrogen atmosphere and was cooled and evaporated under reduced pressure to approximately 10 ml. Ice cold water (500 ml) was added and the mixture was filtered. The yellowish solid was washed with water and ether and dried yielding 12.5 g of **11** (79%), mp 189.5°; ^{1}H nmr (deuteriochloroform): δ 1.41 (t, J = 7 Hz, 3, ethyl CH_3), 4.42 (q, J = 7 Hz, 2, ethyl CH_2), 7.05 (m, 2, aromatic H), 7.42 (m, 1, aromatic H), 8.27 (s, 1, vinyl H), 8.48 (d, $J_{H\text{-}F}$ = 8 Hz, 1, aromatic H).

Anal. Calcd. for $C_{18}H_{10}ClF_3N_2O_5$: C, 50.65; H, 2.34; N, 6.57. Found: C, 50.46; H, 2.34; N, 6.40.

Ethyl 1-(2,4-Difluorophenyl)-6-fluoro-7-(3-acetamidopyrrolidin-1-yl)-8-nitro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**12a**).

To a solution of the 1,4-dihydro-4-oxoquinoline-3-carboxylate **11** (1 g, 2.3 mmoles) in THF (20 ml) at room temperature was added in 3-acetamidopyrrolidine (986 mg, 6.9 mmoles). After stirring at room temperature under nitrogen atmosphere for 20 hours, the solution was evaporated to dryness under reduced pressure. The residue was treated with water (30 ml) and filtered and the residue was purified on silica gel column to yield 983 mg of **12a** (83%), mp 117°; ^{1}H nmr (deuteriochloroform): δ 1.40 (t, J = 7 Hz, 3, ethyl CH_3), 1.85 (m, 1, CH_2), 1.95 (s, 3, ethyl CH_3), 2.24 (m, 1, CH_2), 3.06 (m, 1, NCH_2), 3.31 (m, 2, NCH_2), 3.53 (m, 1, NCH_2), 4.39 (q, J = 7 Hz, 2, ethyl CH_2), 4.51 (m, 1 NCH), 5.62 (m, 1, NH), 7.01 (m, 2, aromatic H), 7.39 (m, 1, aromatic H), 8.18 (s, 1, vinyl H), 8.30 (d, $J_{H\text{-}F}$ = 12 Hz, 1 aromatic H).

Anal. Calcd. for $C_{24}H_{21}F_3N_4O_6 \cdot 2\frac{1}{2}\ H_2O$: C, 51.15; H, 4.62; N, 9.95. Found: C, 50.88; H, 4.50; N, 10.32.

Ethyl 1-(2,4-Difluorophenyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-8-nitro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**12b**).

Compound **12b** was prepared from **11** by using the same experimental procedure as the preparation of **12a** reacting **11** with *N*-methylpiperazine instead of 3-acetamidopyrrolidine, 73% yield, mp 204.5; ^{1}H nmr (deuteriochloroform): δ 1.23 (t, J = 7 Hz, 3, ethyl CH_3), 2.17 (s, 3, NCH_3), 2.30 (m, 4, NCH_2), 3.01 (m, 4, NCH_2), 4.17 (q, J = 7 Hz, 2, ethyl CH_2), 7.20 (m, 3, aromatic H), 8.18 (s, 1, vinyl H), 8.43 (d, $J_{H\text{-}F}$ = 12 Hz, 1, aromatic H).

Anal. Calcd. for $C_{25}H_{21}F_3N_4O_5$: C, 56.33; H, 4.29; N, 11.43. Found: C, 56.04; H, 4.27; N, 11.25.

Ethyl 1-(2,4-Difluorophenyl)-6-fluoro-7-(3-acetamidopyrrolidin-1-yl)-8-amino-1,4-dihydro-4-oxoquinoline-3-carboxylate (**13a**).

A solution of **12a** (750 mg, 1.44 mmoles) in ethyl acetate (50 ml) was hydrogenated under a nitrogen atmosphere in the presence of 1.4 g of Raney nickel. The mixture was then filtered and the solvent was evaporated to dryness to yield 409 mg **13a** (70%), mp 129-134°; ^{1}H nmr (deuteriochloroform): δ 1.40 (t, J = 7 Hz, 3, ethyl CH_3), 1.86 (m, 1, CH_2), 2.00 (s, 3, acetyl CH_3), 2.32 (m, 1, CH_2), 2.95 (m, 1, NCH_2), 3.19 (m, 1, NCH_2), 3.22 (m, 1, NCH_2), 3.49 (m, 1, NCH_2), 4.02 (bs, 2, NH_2), 4.39 (q, J = 7 Hz, 2, ethyl CH_2), 4.56 (m, 1, NCH), 5.73 (bs, 1, NH), 7.10 (m, 2, aromatic H), 7.55 (m, 1, aromatic H), 7.65 (d, $J_{H\text{-}F}$ = 12 Hz, 1 aromatic H), 8.28 (s, 1, vinyl H).

Anal. Calcd. for $C_{24}H_{23}F_3N_4O_4 \cdot 1\frac{1}{3}\ H_2O$: C, 56.20; H, 5.00; N, 10.92. Found: C, 56.23; H, 5.37; N, 10.79.

Ethyl 1-(2,4-Difluorophenyl)-6-fluoro-7-(4-methylpiperazin-1-yl)-8-amino-1,4-dihydro-4-oxoquinoline-3-carboxylate (**13b**).

Compound **13b** was prepared from **12b** using a similar experimental procedure as the preparation of **13a**, 57% yield, mp 102-104°; ^{1}H nmr (deuteriochloroform): δ 1.43 (t, J = 7 Hz, 3, ethyl CH_3), 2.39 (m, 4, NCH_2), 2.40 (s, 3, NCH_3), 2.87 (m, 4, NCH_2), 3.33 (bs, 2, NH_2), 4.40 (q, J = 7 Hz, 2, ethyl CH_2), 7.64 (m, 2, aromatic H), 8.23 (m, 1, aromatic H), 8.37 (d, $J_{H\text{-}F}$ = 12 Hz, 1, aromatic H), 8.73 (s, 1, vinyl H).

Anal. Calcd. for $C_{23}H_{23}F_3N_4O_3 \cdot H_2O$: C, 57.74; H, 5.23; N, 11.72. Found: C, 57.84; H, 5.08; N, 11.71.

Ethyl 1-(3-Acetamidopyrrolidin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carbonylate (**14a**).

A solution of **13a** (450 mg, 0.92 mmole) in pyridine (4 ml) was heated at 110° under nitrogen atmosphere for 36 hours. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in methylene chloride (50 ml) and was washed with sodium bicarbonate solution. Purification by column chromatography on silica gel gave the carboxylate **14a** (312 mg, 73%), mp 233-234, ^{1}H nmr (DMSO-d_6): δ 1.31 (t, J = 7 Hz, 3, ethyl CH_3), 1.81 (m, 1, CH_2), 1.86 (s, 3, acetyl CH_3), 2.27 (m, 1, CH_2), 2.90 (m, 1, NCH_2), 3.12 (m, 1, NCH_2), 3.20 (m, 1, NCH_2), 3.29 (m, 1, NCH_2), 4.23 (q, J = 7 Hz, 2, ethyl CH_2), 4.44 (m, 1, NCH), 6.72 (m, 1, CONH), 7.01 (m, 1, aromatic H), 7.05 (m, 1, aromatic H), 7.62 (m, 1, aromatic H), 8.18 (d, J_{H-F} = 10 Hz, 1, aromatic H), 8.79 (s, 1, vinyl H), 8.88 (s, 1, NH).

Anal. Calcd. for $C_{24}H_{22}F_2N_4O_4 \cdot ½\ H_2O$: C, 60.37; H, 4.86; N, 11.73. Found: C, 60.16; H, 4.62; N, 11.46.

Ethyl 1-(4-Methylpiperazin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino-[1,8a,8-*a,b*]quinoxaline-5-carboxylate (**14b**).

Compound **14b** was prepared from **13b** using similar experimental procedure as the preparation of **14a**, 73% yield, mp 240-242°; ^{1}H nmr (deuteriochloroform): δ 1.28 (t, J = 7 Hz, 3, ethyl CH_3), 2.29 (s, 3, NCH_3), 2.31 (m, 4, NCH_2), 2.77 (m, 4, NCH_2), 3.25 (bs, 1, NH), 4.35 (q, J = 7 Hz, 2, ethyl CH_2), 6.61 (m, 1, aromatic H), 7.26 (m, 1, aromatic H), 7.28 (d, J_{H-F} = 12 Hz, 1, aromatic H), 8.81 (s, 1, vinyl H).

Anal. Calcd. for $C_{23}H_{22}F_2N_4O_3 \cdot ½\ H_2O$: C, 61.47; H, 5.12; N, 12.47. Found: C, 61.41; H, 5.10; N, 12.40.

1-(3-Aminopyrrolidin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic Acid Hydrochloride (**15**).

A suspension of **14a** (250 mg, 0.533 mmole) in 6 *N* hydrochloric acid (2 ml) was heated at 110° for 6 hours. The solvent was removed under reduced pressure. The residue was refluxed in ethanol for 1 hour and was filtered yielding 158 mg of **15** (63%), mp >250°, ^{1}H nmr (DMSO-d_6): δ 2.12 (m, 1, CH_2), 2.39 (m, 1, CH_2), 3.00 (m, 1, NCH_2), 3.28 (m, 1, NCH_2), 3.47 (m, 2, NCH_2), 3.94 (m, 1, NCH), 6.79 (m, 1, aromatic), 7.16 (d, J_{H-F} = 12 Hz, 1, aromatic H), 8.36 (bs, 5, NH_3Cl, NH_2Cl), 9.02 (s, 1, vinyl H), 8.89 (bs, 1, COOH).

Anal. Calcd. for $C_{20}H_{18}Cl_2F_2N_4O_3$: C, 50.97; H, 3.85; N, 11.89. Found: C, 50.64; H, 3.79; N, 11.60.

1-(4-Methylpiperazin-1-yl)-2,9-difluoro-4,12-dihydro-4-oxoquino[1,8a,8-*a,b*]quinoxaline-5-carboxylic Acid Hydrochloride (**16**).

Hydrolysis of **14b** with 6 *N* hydrochloric acid under similar experimental condition as the preparation of **15** yielded compound **16**, 94% yield, mp >250°, ^{1}H nmr (deuteriotrifluoroacetic acid): δ 3.15 (m, 4, NCH_2), 3.75 (m, 7, NCH_2, NCH_3), 7.10 (m, 1, aromatic H), 7.76 (m, 1, aromatic H), 8.57 (m, 1, aromatic H), 9.43 (s, 1, vinyl H).

Anal. Calcd. for $C_{21}H_{18}F_2N_4O_3 \cdot HCl \cdot ½\ H_2O$: C, 55.08; H, 4.37; N, 12.24. Found: C, 54.82; H, 4.14; N, 12.53.

In Vitro Antibacterial Activity.

The *in vitro* antibacterial activity of the test compound was determined by conventional agar dilution procedures. The organisms were grown overnight in brain-heart infusion (BHI) broth (Difco 0037-01-6) at 36°. Two-fold dilutions of the stock solution (2,000 μg/ml) of test compound were made in BHI agar to obtain a test concentration ranging from 200 μg/ml-0.005 μg/ml. The plate was inoculated with approximately 10^4 organisms. It was then incubated at 36° for 18 hours. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compound that yielded no visible growth on the plate.

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