# Synthesis and Antibacterial Evaluation of Certain Quinolone Derivatives

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A number of 7-substituted quinolone derivatives were synthesized and evaluated for antibacterial and cytotoxic activities. Preliminary results indicated that most compounds tested in this study demonstrated better activity against methicillin-resistant *Staphylococcus aureus* than norfloxacin. Among them, 1-(4-amino-2-fluorophenyl)-6-fluoro-1,4-dihydro-7-{4-[2-(4-methoxyphenyl)-2-hydroxyiminoethyl]-1-piperazinyl}-4-oxo-3-quinolinecarboxylic acid (**11d**) and its ketone precursor **10d** exhibited significant activities against *Klebsiella pneumoniae*, methicillin-resistant *S. aureus*, erythromycin- and ampicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus faecalis*. Due to strong cytotoxicities of **11d** (a mean log $GI_{50}$ of −5.40), compound **10d**, with good antibacterial activities and low cytotoxicities (a mean log $GI_{50}$ of −4.67), is a more potential drug candidate.

## Introduction

Although the structure−activity relationships of antibacterial fluoroquinolones have been extensively investigated, the optimum substituent at the C-7 position which has a great impact on potency, spectrum, solubility, and pharmacokinetics has not been precisely defined.[1−5] Extensively investigated substituents are piperazin-1-yl and its 4-substituted derivatives. For example, pefloxacin, the 4′-methylnorfloxacin, and other 4-substituted piperazin-1-yl prodrugs of norfloxacin were prepared to improve the bioavailability of the parent.[6−8] A number of fluoroquinolones with an oxime or a substituted oxime attached to the pyrrolidine or piperazine ring at C-7 position were also synthesized and evaluated for antibacterial activities.[9−14] In our previous report, we described the preparation and evaluation of certain norfloxacin derivatives with an additional functional moiety such as 4-hydroxyaminoalkyl on the C-7 piperazin-1-yl group. Most of these compounds demonstrated better activity against methicillin-resistant *Staphylococcus aureus* than norfloxacin and proved to possess selective cytotoxicity against renal cancer cell lines.[15] In continuing our efforts to establish the structure−activity relationships of antibacterial fluoroquinolones, further derivatization of preceding oximes as well as their 8-fluoro and 1-aryl derivatives were synthesized and evaluated.

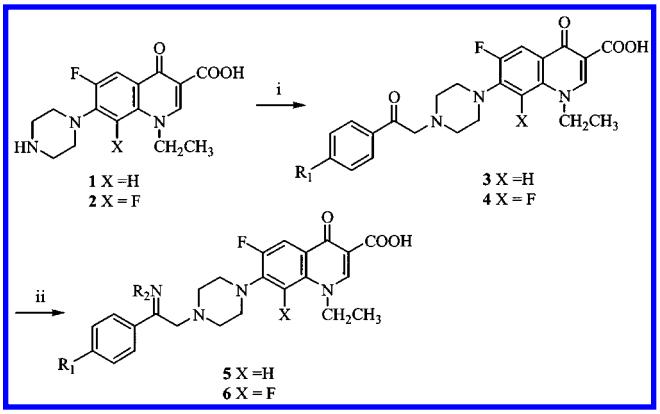
## Chemistry

The general procedure for the preparation of 6-fluoro-1,4-dihydro-7-[4-(2-substituted-iminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **5a**−**i** (see Chart 1) and their 6,8-difluoro analogues **6b**−**h** are described in Scheme 1. Norfloxacin (**1**) was treated with $NaHCO_3$ and a bromomethyl ketone to give its *N*-(2-oxo-2-(4-substituted phenyl)ethyl derivatives **3a**−**i**. Reaction of **3a**−**i** with hydroxyamine, methoxyamine, hydrazine, semicarbazide, and thiosemicarbazide, respectively, in EtOH gave the corresponding **5a**−**i** in 29−56% overall yield. Among them, the sole *Z*-form isomer of **5b**, **5c**, and **5e** was isolated while both *E*- and *Z*-form isomers of **5a**, **5d**, and **5f**−**i** were obtained with *Z*-isomers predominating.[15,16] Accordingly, 6,8-difluoro-1,4-dihydro-7-[4-(2-

**Chart 1.** Side Chains Employed as the $R_1$ and $R_2$ Substitutents

| | $R_1$ | $R_2$ |
|---|---|---|
| **a** | H | OH |
| **b** | H | $NHC(=O)NH_2$ |
| **c** | H | $NHC(=S)NH_2$ |
| **d** | F | OH |
| **e** | F | $NH_2$ |
| **f** | Cl | OH |
| **g** | Cl | Ome |
| **h** | OMe | OH |
| **i** | OMe | OMe |

**Scheme 1**[a]




[a] Reagents: (i) $NaHCO_3$, $R_1PhCOCH_2Br$ in DMF; (ii) $R_2NH_2$ in EtOH.

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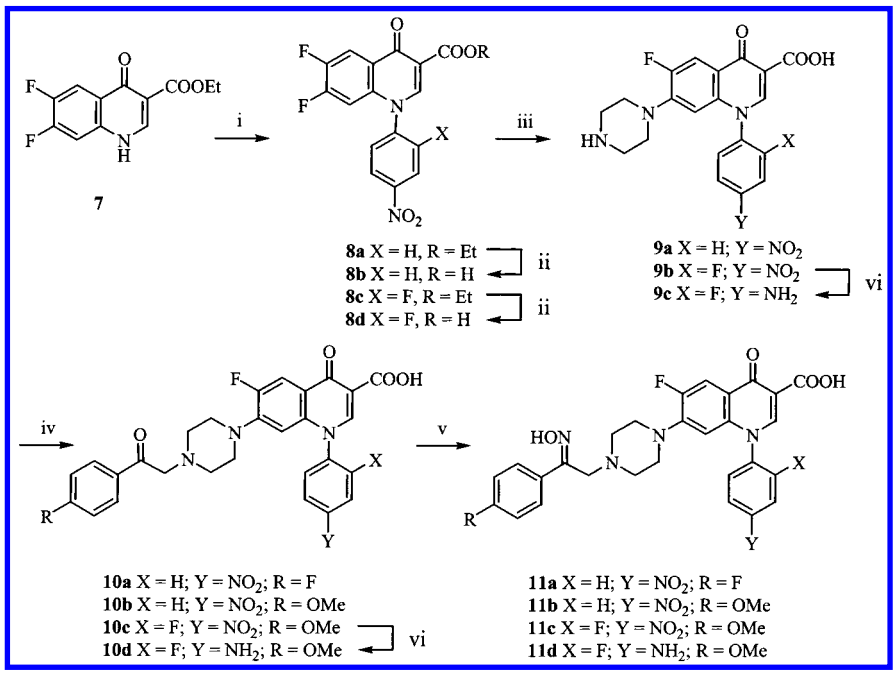
**Scheme 2**[a]




[a] Reagents: (i) $K_2CO_3$, 4-fluoronitrobenzene or 3,4-difluoronitrobenzene in DMF; (ii) HCl, AcOH; (iii) piperazine in $CH_3CN$; (iv) $K_2CO_3$, $R_1PhCOCH_2Br$ in DMF; (v) $NH_2OH$ HCl in EtOH; (vi) $H_2$, Pd/C in $CH_2Cl_2$.

substituted-iminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **6b**−**d** and **6f**−**h** were obtained from 8-fluoronorfloxacin (**2**) in 52−70% overall yield.

The preparation of 1-aryl-6-fluoro-1,4-dihydro-7-[4-(2-hydroxyiminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **11a**−**d** is described in Scheme 2. Reaction of ethyl 6,7-difluoro-4-oxoquinoline-3-carboxylate (**7**) with 3,4-difluoronitrobenzene and $K_2CO_3$ in dry DMF at 80 °C gave ethyl 6,7-difluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (**8c**) in 67% yield.[17,18] Compound **8c** was hydrolyzed with HCl in acetic acid, and the crude intermediate without purification was treated with piperazine in $CH_3CN$ to afford 6-fluoro-1-(2-fluoro-4-nitrophenyl)-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinoline-3-carboxylic acid (**9b**) in 78% yield. Compound **9b** was reduced by catalytic hydrogenation on Pd/C to its 1-(4-amino-2-fluorophenyl) counterpart **9c** in 85% yield. Alkylation of **9a** and **9b** with $K_2CO_3$ and a 4′-substituted 2-bromoacetophenone gave their respective *N*-[2-oxo-2-(4-substituted-phenyl)-ethyl] derivatives **10a**−**c** which were then reacted with hydroxylamine to give 1-aryl-6-fluoro-1,4-dihydro-7-[4-(2-hydroxyiminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **11a**−**c** in 69−81% overall yield.[19,20] Compound **11d** was obtained by hydroxylamination of **10d**, which was synthesized via hydrogenation of **10c** with catalytic Pd/C in $CH_2Cl_2$.

## Results and Discussion

A number of quinolone derivatives prepared for this study were tested in vitro against five susceptible strains and three resistant strains. The minimum inhibitory concentrations (MIC) are presented in Table 1. In general, 6-fluoro-1,4-dihydro-7-[4-(2-hydroxyiminoethyl)piperazin-1-yl]-4-oxoquinoline-3-carboxylic acids **5a**−**i** are less active than norfloxacin with the exception of the inhibitory activity against methicillin-resistant *S. aureus* (*S. aureus* M-R). With an ethyl substituent at N-1 and a phenyl (or a substituted phenyl) at the C-7 piperazinyl side chain, the imino nitrogen substituted with a hydroxy group (**5a**, $R_2$ = OH) exhibited the most potent inhibitory activity on G(−) bacteria and *S. aureus* M-R while a thiourea (**5c**, $R_2 = NH_2CSNH_2$) is most favorable for the inhibition of erythromycin- and ampicillin-resistant *Streptococcus pneumoniae* (*S. pneumoniae* E-R and A-R) and vancomycin-resistant *Enterococcus faecalis* (*E. faecalis* V-R). An amino substituent (**5e**, $R_2 = NH_2$) is more active than its hydroxy counterpart (**5d**, $R_2$ = OH) which in turn is more potent than a methoxy substituent (**5h** vs **5i**). Introduction of an 8-fluoro substituent enhanced antibacterial activities (**3a** vs **4a**, **3h** vs **4h**, **5b** vs **6b**, **5h** vs **6h**). For the N-1 substituent, 4-nitrophenyl is comparable to its ethyl counterpart (**11a** vs **5d**; **11b** vs **5h**) while 2,4-disubstituted phenyl (**11c**, **11d**) is most active. In fact, even though the ketone precursors **3a**−**h** and **10a**−**b** are inactive against the growth of resistant strains, the parent 2,4-disubstituted phenyl quinolones **9b** and **9c**, and their ketone precursors **10c** and **10d**, exhibited significant activities. Due to strong cytotoxicities of **11c** (a mean log $GI_{50}$ of −5.34) and **11d** (a mean log $GI_{50}$ of −5.40), the ketone precursor, **10d**, with good antibacterial activities and low cytotoxicities (a mean log $GI_{50}$ of −4.67), is a more potential drug candidate.[21]

## Conclusion

A number of 7-substituted quinolone derivatives were synthesized and evaluated for antibacterial and cytotoxic activities. Preliminary results are as follows: (1) For 1-ethyl quinolones, 7-[4-(2-oxoethyl)piperazin-1-yl]-derivatives **3**, with exception of **3f**, are narrow-spectrum and only active against methicillin-resistant *S. aureus* while their 2-iminoethyl counterparts **5** are more active and broad-spectrum antibacterial agents. Besides, 8-fluoro derivatives are more active antibacterial agents than their respective unsubstituted counterparts. (2) For

**Table 1.** In Vitro Antibacterial Activity of 4-Oxoquinoline-3-carboxylic Acids [MIC, $\mu$M ($\mu$g/mL)][a]

| compd | *S. aureus* M-R | *S. pneumoniae* E-R, A-R | *E. faecalis* V-R |
|---|---|---|---|
| **2** | 2.31 (0.78) | 18.5 (6.25) | 18.5 (6.25) |
| **3a** | 7.15 (3.13) | >200 (>100) | >200 (>100) |
| **3d** | 6.87 (3.13) | >200 (>100) | >200 (>100) |
| **3f** | >200 (>100) | >200 (>100) | >200 (>100) |
| **3h** | 6.70 (3.13) | >200 (>100) | >200 (>100) |
| **4a** | 1.71 (0.78) | 13.7 (6.25) | 13.7 (6.25) |
| **4h** | 0.62 (0.30) | >200 (>100) | >200 (>100) |
| **5a** | 0.44 (0.20) | >200 (>100) | >200 (>100) |
| **5b** | 3.15 (1.56) | 25.3 (12.5) | 25.3 (12.5) |
| **5c** | 1.53 (0.78) | 24.5 (12.5) | 12.2 (6.25) |
| **5d** | 2.13 (1.0) | >200 (>100) | >200 (>100) |
| **5e** | 1.66 (0.78) | 26.6 (12.5) | 26.6 (12.5) |
| **5f** | >200 (>100) | >200 (>100) | >200 (>100) |
| **5g** | nd[b] | >200 (>100) | >200 (>100) |
| **5h** | 0.41 (0.20) | 13.0 (6.25) | 3.23 (1.56) |
| **5i** | 1.57 (0.78) | 12.6 (6.25) | 12.6 (6.25) |
| **6b** | 0.39 (0.20) | 6.11 (3.13) | 6.11 (3.13) |
| **6c** | 0.74 (0.39) | >200 (>100) | >200 (>100) |
| **6d** | 0.61 (0.30) | 6.14 (3.0) | 2.05 (1.0) |
| **6f** | 0.59 (0.30) | 19.8 (10) | 19.8 (10) |
| **6g** | >200 (>100) | >200 (>100) | >200 (>100) |
| **6h** | 0.20 (0.10) | 6.25 (3.13) | 3.12 (1.56) |
| **9b** | 7.27 (3.13) | 0.46 (0.20) | 29.0 (12.5) |
| **9c** | 0.97 (0.39) | 3.90 (1.56) | 15.6 (6.25) |
| **10a** | >200 (>100) | >200 (>100) | >200 (>100) |
| **10b** | 5.58 (3.13) | >200 (>100) | >200 (>100) |
| **10c** | 2.70 (1.56) | 5.41 (3.13) | 43.2 (25) |
| **10d** | 0.55 (0.30) | 1.82 (1.0) | 5.47 (3.0) |
| **11a** | >200 (>100) | >200 (>100) | >200 (>100) |
| **11b** | 1.36 (0.78) | 10.9 (6.25) | 21.7 (12.5) |
| **11c** | 0.34 (0.20) | 1.31 (0.78) | 5.27 (3.13) |
| **11d** | 0.53 (0.30) | 1.77 (1.0) | 5.32(3.0) |
| Nf[c] | 4.89 (1.56) | 19.6 (6.25) | 9.80 (3.13) |

[a] Organisms selected are as follows: *S. aureus* M-R, *Staphylococcus aureus*, methicillin-resistant; *S. pneumoniae* E-R and A-R, *Staphylococcus pneumoniae*, erythromycin- and ampicillin-resistant, clinical isolates; *E. faecalis*, V-R, *Enterococcus faecalis*, vancomycin-resistant, clinical isolates. [b] nd, not determined. [c] Nf, norfloxacin.

1-aryl quinolones, 7-[4-(2-oxoethyl)piperazin-1-yl]-derivatives **10a** and **10b** are inactive while **10c** and **10d** are active antibacterial agents with no significant cytotoxicity. The 2-iminoethyl derivative **11a** is inactive whereas **11b**−**d** are active against all three resistant strains. However, due to strong cytotoxicities of **11c** and **11d**, the ketone precursor, **10d**, with good antibacterial activities and low cytotoxicities, is a more potential drug candidate.

## Experimental Section

**In Vitro Antibacterial Assay. Determination of MIC:** 2 mg of each test compound was dissolved in an appropriate solvent (100% DMSO) and serially diluted with DMSO into the desired testing concentration ranges. Each series of testing solution (0.01 mL) was added into the 48-well plate with 0.99 mL of media broth containing 1−5 × $10^5$ CFU/mL testing microorganism. Thus the final maximal concentration of DMSO was 1%, and the initial concentration of testing solution was 300 $\mu$M. Media used were as follows: nutrient broth (NB, DIFCO) for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*; Mueller-Hinton broth (DIFCO) for *S. aureus*, methicillin-resistant (MRSA), and *Proteus vulgaris*; brain heart infusion broth (BHI, DIFCO) for *Mycobacterium ranae*; and tryptic soy broth (DIFCO) containing 7% of calf serum for *S. pneumoniae* (EM & AM Res. Clinical Isolates) and *E. faecalis* (VRE, Clinical Isolates). The plates were incubated for 20−72 h at 37 °C, and then the MIC was determined by a visual turbidity readout or by microscope observation of microorganism growth. Vehicle and reference agents were used in every test as the negative and positive controls, and the assays were performed in duplicate.

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**Supporting Information Available:** Experimental procedures, characterization, and elemental analyses for compounds **4a**, **4d**, **4f**, **4h**, **5b**, **5c**, **5e**, **5g**, **5i**, **6b**−**d**, **6f**−**h**, **8c**, **8d**, **9c**, **9d**, **10a**−**d**, and **11a**−**d**; tables containing data for antibacterial activities against *E. coli*, *M. ranae*, *P. aeruginosa*, *K. pneumoniae*, and *P. vulgaris* and the inhibitory activities on renal cancer cell lines for **3a**, **3f**, **3h**, **4a**, **4h**, **5a**−**i**, **6c**, **6d**, **10a**−**d**, and **11a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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