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# Synthesis and Biological Testing of Non-Fluorinated Analogues of Levofloxacin

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**Abstract**—Quinolones without the usual 6-fluorine substituent have been recently described as potent antibacterial agents. A series of non-fluorinated analogues of the antibacterial quinolone Levofloxacin were synthesized and tested.
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The quinolone antibacterials are a highly successful class of agents for the treatment serious infections. These drugs exert their effect by the inhibition of type II bacterial topoisomerases such as DNA gyrase and Topoisomerase IV. The first generation quinolones, such as Nalidixic Acid (Fig. 1), were characterized by activity against Gram-negative pathogens and were used primarily for the treatment urinary tract infections. The second generation quinolones were based on the finding of Koga that a fluorine at the C6 position of the quinolone combined with an amine containing group at C7 enhanced the spectrum and distribution of the drug.[1] Following this report, thousands of quinolones were synthesized that contained a C6 fluorine. Several very successful drugs were launched based on these results. Because of the prevalent C6 fluorine, the class is frequently referred to as fluoroquinolones antibacterials. Nearly ten years after Koga's disclosure, Ledoussal reported that potent quinolones with a C6 hydrogen could be made if the appropriate C7 substituent was chosen.[2] Since that time we[3] and others[4–11] have reported on the activity of non-fluorinated quinolones that have biological activity equivalent to the fluoroquinolones.

Levofloxacin,[12] a chiral version of the earlier drug Ofloxacin, is a successful fluoroquinolone antibacterial that departs from the typical quinolones substructure by having a fused ring connecting the N1 to C8 position.

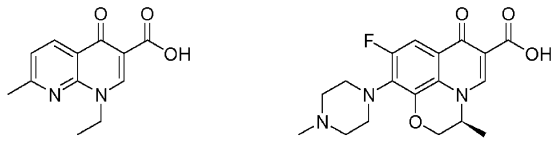


**Figure 1.** Structures of naladixic acid and levofloxacin.

This ring is connected with an ether moiety at C8. This type of substitution has the potential benefit of reducing any phototoxic problems seen with quinolones that have a halogen in the C8 position.[13] Several recently approved fluoroquinolones have a methoxy group at C8.

In the course of our exploration of non-fluorinated quinolones, we prepared a series of Levofloxacin analogues with a hydrogen at the C6 position. Interestingly, the original report on Ofloxacin disclosed several non-fluorinated analogues.[14] Limited microbiological data was presented on these racemic compounds and no topoisomerase inhibition values were available. Also, none of these analogues utilized the pyrrolidines and piperidines that were later found to increase the activity of many quinolones.

The synthetic route to the requisite quinolone nucleus followed an earlier report on the synthesis of the 6F quinolone (Scheme 1).[15] The keto-ester **1** was elaborated from the benzoyl chloride by standard methodology.[16] The ethoxy ethylene moiety was formed by treatment with acetic anhydride and triethylorthoformate and

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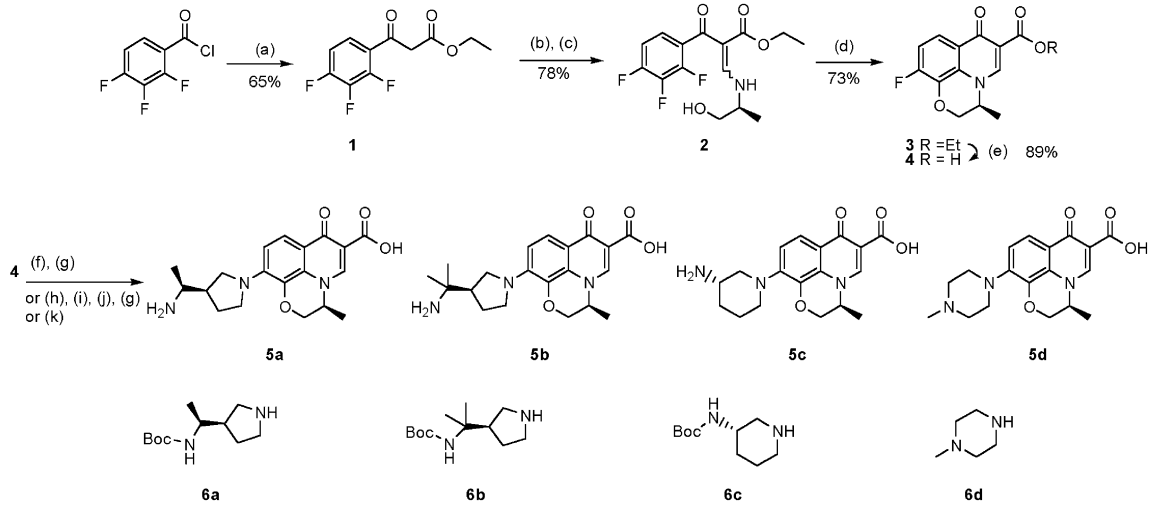


**Scheme 1.**[a] (a) Ethyl hydrogen malonate, 2 equiv *n*BuLi, THF, −78 °C to rt; (b) $(EtO)_3CH$, $Ac_2O$, 110 °C; (c) *S*-(+)-alaninol, $CH_2Cl_2$, 0 °C to rt; (d) 2 equiv NaH, THF, 0 °C to rt; (e) 10% KOH, THF, reflux. For **5a** and **5b** (f) **6a** or **6b**, $Et_3N$, NMP, 80 °C; (g) HCl, EtOH. For **5c** (h) $BF_3$ $Et_2O$, THF, reflux; (i) **6c**, $Et_3N$, DMF, 50 °C; (j) $Et_3N$, MeOH, reflux. For **5d** (k) pyridine, **6d**, 110 °C.
[a]**5a** and **5b** were synthesized in 19 and 30% yield, respectively, from **4**. New compounds gave satisfactory analytical data ($^1$H NMR, MS, HPLC, CHN analysis). **5c** and **5d** were synthesized in 10% and 20% yield, respectively, from **4**. New compounds gave satisfactory analytical data ($^1$H NMR, MS, HPLC, HRMS analysis).

used without isolation. Addition of commercially available *S*-(+)-alaninol afforded the enamine intermediate **2**. We found the closure of the N1 to C8 ring for benzoxazine formation was best effected by continued treatment with base in the quinolone ring formation step rather than with aqueous KOH in the ester hydrolysis step. The amines **6a**, **6b**, and **6c** were synthesized according to the reported protocols.[17–19] In the case of quinolones **5a**, **5b**, and **5d** coupling of the C7 side chain was performed on the quinolone carboxylic acid. For quinolone **5c** the boronate complex of the quinolone carboxylate was used to increase reactivity during coupling. The boronate was removed with triethylamine in refluxing methanol.

The biological evaluation of these compounds was carried out by both standard bacterial minimum inhibitory concentration (MIC) assays and DNA gyrase inhibition determinations. Our panel of organisms contains Gram-positive and Gram-negative bacteria (Table 1). Additionally, strains resistant to standard antibacterials were included. Topoisomerase inhibition values were also measured against wild-type, quinolone-resistant DNA gyrase (*E. coli*) and mammalian topoisomerase II.[20] The new pyrrolidine containing non-fluorinated quinolones **5a** and **5b** maintain good antibacterial potency relative to Levofloxacin. This result follows the general trends we have observed with the non-fluorinated quinolones. The direct 6-fluorinated congener of **5a** has been reported.[21] While direct comparisons cannot be made because of the different strains used in testing, the fluorinated version of this quinolone had few significant differences in activity using Levofloxacin as a reference. The aminopiperidine containing quinolone **5c** was dramatically reduced in potency compared to the pyrrolidine analogues. We have previously noted that side chains of this class are much more sensitive to substitution on other areas of the quinolone.[22] The non-fluorinated version of Levofloxacin (**5d**) was likewise quite poor in its antibacterial activity. This result was not unexpected

**Table 1.** Minimum inhibtory concentrations of new compounds

| Compd | | | **5a** | **5b** | **5c** | **5d** | Levofloxacin |
|---|---|---|---|---|---|---|---|
| Organism | Type[a] | Strain | MIC (μg/mL) | | | | |
| *S. aureus* | MSCS | MI246 | 0.031 | 0.063 | 2 | 4 | 0.12 |
| *S. aureus* | MSCS | MI273 | 0.125 | 0.125 | 4 | 8 | 0.12 |
| *S. aureus* | MSCR | MI345 | 1 | 2 | >32 | >32 | 2 |
| *S. aureus* | MRCS | MI300 | 0.063 | 0.063 | 2 | 4 | 0.12 |
| *S. aureus* | MRCR | MI339 | 2 | 2 | >32 | >32 | 2 |
| *S. saprophyticus* | | SS276 | 0.125 | 0.031 | 16 | 4 | 0.12 |
| *S. epidermidis* | MSCR | SE48 | 1 | 1 | >32 | >32 | 2 |
| *E. faecium* | VS | EF1 | 1 | 1 | >32 | >32 | 0.25 |
| *E. faecium* | VR | EF12 | 1 | 1 | NG | NG | 1 |
| *E. faecalis* | | STD44 | 0.031 | 0.125 | NG | NG | 0.25 |
| *S. pneumoniae* | PS | STP6301 | NG | 0.016 | >32 | 16 | 0.25 |
| *S. pneumoniae* | PS | STP64 | — | — | >32 | 16 | 0.25 |
| *S. pneumoniae* | PR | STP51 | 0.063 | 0.5 | >32 | 32 | 0.5 |
| *S. pyogenes* | | STA2 | 0.016 | 0.016 | >32 | 4 | 0.5 |
| *S. viridans* | | STV1 | 0.125 | 1 | >32 | 32 | 0.25 |
| *E. cloacae* | | AE63 | 0.125 | 0.25 | 0.25 | NG | 0.008 |
| *E. coli* | | ES142 | 0.5 | 0.5 | 1 | 8 | 0.016 |
| *E. coli* | | DC0[b] | 2 | 4 | 16 | >32 | 0.25 |
| *E. coli* | | DC2 | 0.063 | 0.125 | 2 | 16 | 0.25 |
| *M. catarrhalis* | | MC2 | 0.125 | 0.063 | 2 | 4 | 0.03 |
| *K. pneumoniae* | | KL21 | 0.016 | 0.031 | 0.25 | 1 | <0.004 |
| *K. pneumoniae* | CR | KL328 | >8 | >8 | >32 | >32 | 4 |
| *P. mirabilis* | | PR91 | 2 | 4 | 2 | 8 | 0.06 |
| *P. aeruginosa* | | PS96 | 1 | 4 | >32 | >32 | 0.5 |

[a]MSCS = Methicillin sensitive, ciprofloxacin sensitive; MRCR = Methicillin resistant, ciprofloxacin resistant; PS = Penicillin sensitive; PR = Penicillin resistant; VS = Vancomycin sensitive; VR = Vancomycin resistant.
[b]DC2 is a permeable mutant. DC0 is the parent strain. NG = No growth.

based on our previous work with piperazine derivatives with non-fluorinated quinolones and the previous report on the racemic version of this compound.[23] The topoisomerase inhibition values were in line with the MIC results (Table 2). The pyrrolidine containing compounds **5a** and **5b** were essentially the same as Levofloxacin against the wild-type DNA gyrase. They were less effective than similar non-fluorinated quinolones without the benzoxazine ring system against the quinolone resistant DNA gyrase. Quinolone **5a** was more potent than Levofloxacin against mammalian topoisomerase II, indicating potential detrimental activity.

**Table 2.** Inhibition values for *E. coli* DNA gyrase (wild type and fluoroquinolone resistant[a]) and mammalian topoisomerase II

| Compd | Wild type gyrase $IC_{50}$, μg/mL | Quinolone resistant gyrase $IC_{50}$, μg/mL | Topo II $IC_{50}$, μg/mL |
|---|---|---|---|
| **5a** | 6.4 | 51.2 | 38 |
| **5b** | 3.2 | >102 | 150 |
| **5c** | 63 | >500 | >500 |
| **5d** | 31 | >500 | 250 |
| Levofloxacin | 3.2 | 12.8 | >600 |

[a]Fluoroquinolone DNA gyrase was characterized as Ser83 to Trp mutant.

As seen in their antibacterial activity, analogues **5c** and **5d** were notably less active in inhibiting topoisomerases.

This non-fluorinated series of Levofloxacin analogues are equivalent or less potent than the parent molecule. In contrast to some non-fluorinated analogues where the N1 moiety is a cyclopropyl, there is a substantial decrease in activity versus known agents. The loss of freedom of movement at N1 and C8 appears to be responsible for these diminished results.

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