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Preparation and antibacterial evaluation of decarboxylated fluoroquinolones

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ABSTRACT

Decarboxylated ciprofloxacin (**3**) has been reported in the literature to have antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus subtilis*, *Enterobacter cloacae*, *Serratia marcescens* and especially potent activity against *Escherichia coli*. Herein, we report our syntheses of **3** and five additional decarboxylated fluoroquinolones (FQs). We have re-evaluated the antibacterial activity of these FQs. In contrast to previously reported data, none of these decarboxylated fluoroquinolones showed significant antibacterial activity in our assays using both the broth dilution and agar methods. Our study confirmed that the presence of a carboxylic acid group at the 3-position of the fluoroquinolone scaffold is essential for antibacterial activity.

Fluoroquinolones represent an important class of antibiotics, and are known for their activity against Gram-negative and -positive bacteria by inhibiting bacterial DNA gyrase.[1] Structure–activity relationship studies performed some years ago showed that modification of the 3-carboxylic acid group was detrimental to the antibacterial activities of quinolones unless the modified groups could be converted to the carboxylic acid functionality in vivo.[2] Substitution at the 3-position with other functionalities, such as sulfonic acid, sulfonamide, carboxymethyl, hydroxamic acid or phosphonic acid, led to much less active compounds.[3] However, several recent reports have disclosed that decarboxylated ciprofloxacin (**3**) displays antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Bacillus subtilis*, *Enterobacter cloacae*, *Serratia marcescens* and even more potent activity against *Escherichia coli* than ciprofloxacin.[4] Should this finding be true, the decarboxylated fluoroquinolones might be acting via a new mechanism. For example, Berge et al. reported that certain quinolones, which do not contain a carboxylic acid functionality at the 3-position, are methionyl *t*-RNA synthetase (MRS) inhibitors, and they were bactericidal.[5] After an extensive literature search, we found that decarboxylated norfloxacin (**7**) has also been synthesized. In contrast to **3**, compound **7** was reported to have no antibacterial activity.[6] Because the biological profiles of decarboxylated fluoroquinolones were limited and inconsistent, we decided to independently synthesize and examine the antibacterial activities of the decarboxylated derivatives of ciprofloxacin (**1**), norfloxacin (**5**), enrofloxacin (**8**) and 1-cyclopropyl-6,8-difluoro-7-(3-methylpiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (**10**).[7]

Following literature procedures, ciprofloxacin was treated with KCN in DMSO in an attempt to prepare **3**.[4a,8] However, in our hands this reaction gave a complex mixture containing the desired product and unidentified components, which were difficult to separate and isolate. However, when DMF was used as the solvent, a single product was obtained, which was not **3** but rather its *N*-formyl derivative **2**, which has not been previously reported (Scheme 1). Interestingly, in the absence of KCN, treatment of ciprofloxacin with DMF at elevated temperature provided **4** in good yield. To the best of our knowledge, this is a new method for the introduction of an *N*-formyl group to the piperazine ring of fluoroquinolones.[9] Methanolysis of **2** in the presence of K_2CO_3 conveniently provided compound **3**.

Similar procedures were applied to norfloxacin to prepare its decarboxylated derivatives **6** and **7**. For enrofloxacin, because the molecule does not contain a free NH group, its reaction with KCN in either DMSO or DMF provided the same decarboxylated product **9**. Interestingly, treatment of **10** with KCN in DMF provided the decarboxylated product **11** in good yield; no formylation product was observed. It is possible that steric hindrance of the methyl group in the piperazine ring prevented the transamidation process.

The NMR data for our compounds were consistent with the data reported by Park et al.[4a] and Koga et al.[6] for compounds **3** and **7**. However, the data reported by Al-Hajjar et al.[4b] for **3** differed significantly from ours. Upon inspecting the chemical shifts and coupling patterns, we determined that the structure of the compound prepared by Al-Hajjar et al.[4b] was misassigned. The ^{1}H NMR spectrum of compound **3** should have four distinctive doublets (and no singlet) in the aromatic region, two of which should share the same coupling constant attributable to the C(2) and C(3) protons. The data reported by Al-Hajjar lacked these two doublets and displayed

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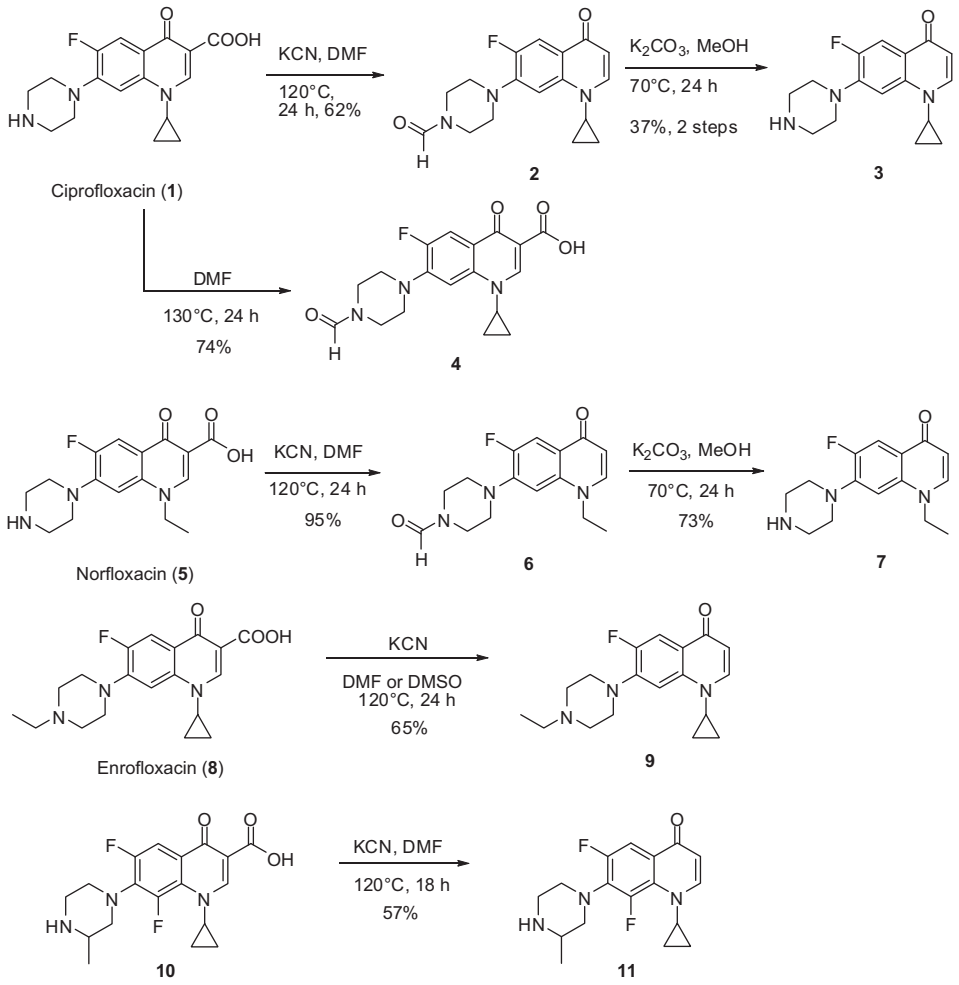

Scheme 1. Synthesis of decarboxylated fluoroquinolones.

a singlet at 8.7 ppm.[10] The singlet at low field is likely due to a C(2) proton adjacent to C(3), which still bears a carboxylic acid moiety.

Seven compounds that we prepared were tested in vitro against our panel of 16 bacterial strains using the standard broth dilution method. To our disappointment, none of the decarboxylated fluoroquinolones displayed any antibacterial activity.[11] To be consistent with the Park[4c] report and to rule out the possibility that the minimum inhibitory concentration (MIC) values might vary due to different culture media, we reran the MIC assay using the agar method and used the same strains of *E. coli*, *S. aureus* and *E. faecalis* that Park et al.[4a] used. Again, none of the decarboxylated compounds were active (Table 1). Among the seven compounds, only **4**, which still has the 3-carboxylic acid group, was active but less potent than ciprofloxacin.[12] Overall, our data consistently showed that decarboxylated fluoroquinolones displayed no significant antibacterial activity against a variety of bacterial strains.

In conclusion, we prepared six decarboxylated fluoroquinolones, four of which are new. Our assays showed that none of these compounds display significant antibacterial activity. It is possible that the biological data reported by Park et al.[4a], Al-Hajjar et al.[4b] and Marks et al.[4c] for compound **3** arose from contaminated samples,[13] incorrectly assigned samples or MIC values that were incorrectly recorded. Importantly, our study confirms that the 3-carboxylic acid group is essential for the antibacterial activity of fluoroquinolones.

Table 1
Minimum inhibitory concentration (MIC) assay data

Compound	MIC (μg/mL)					
	E. coli 29425		*S. aureus* 25923		*E. faecalis* 29212	
	Agar	Broth	Agar	Broth	Agar	Broth
2	>64	>80	>64	>80	>64	>80
3	>64	>80	>64	>80	>64	>80
4	0.5	0.31	0.5	0.31	1	0.63
6	>64	40	>64	>80	>64	>80
7	64	40	>64	>80	32	>80
9	>64	>80	>64	>80	>64	>80
11	>64	>80	>64	>80	>64	>80
Ciprofloxacin	<0.06	<0.02	0.5	0.31	0.5	1.25
Norfloxacin	0.01	0.12	2.0	0.63	2.0	2.5
Enrofloxacin	<0.06	0.05	0.13	0.16	0.13	0.63

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.060.

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11. See the Supplementary data for these data.
12. Ciprofloxacin, enrofloxacin and norfloxacin were included in the assays as internal standards.
13. If the samples were contaminated with small amounts of fluoroquinolones the MIC values could change significantly; see the Supplementary data for these data.