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Synthesis and evaluation of 1-cyclopropyl-2-thioalkyl-8-methoxy fluoroquinolones

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ABSTRACT

Novel fluoroquinolone derivatives substituted with a 2-thioalkyl moiety, with and without a concomitant 3-carboxylate group, were synthesized to evaluate the effect of C-2 thioalkyl substituents on gyrase binding and inhibition. The presence of a 2-thioalkyl group universally decreased activity as compared to parent fluoroquinolones. However, with derivatives of moxifloxacin the presence of either a 2-thioalkyl group or a 3-carboxylate moiety increased activity over the 2,3-unsubstituted derivative. Energy minimization of structures provides an explanation for relative activities of fluoroquinolones having a C-2 thio moiety.

Fluoroquinolones are broad-spectrum antimicrobials[1,2] that inhibit DNA gyrase or topoisomerase IV (TopoIV) in bacteria.[3–6] Of primary concern in the clinical use of fluoroquinolone drugs is the emergence of fluoroquinolone-resistant strains of bacteria.[7–9] The quinolone class of antibacterial agents has undergone many structural changes in efforts to gain potency against resistant strains.[10] These antibiotics are generally based on the structure of the naphthyridone nalidixic acid (Fig. 1), the first active agent in the class. Subsequent generation quinolones consist of fluoroquinolones such as norfloxacin and ciprofloxacin, which contain a C-6 fluorine. These were followed by the 8-methoxy fluoroquinolones (e.g., gatifloxacin and moxifloxacin). The addition of an 8-methoxy substituent generally affords more equipotent inhibition of DNA gyrase and TopoIV, and it has been shown to facilitate more rapid killing and chromosome fragmentation.[1] Continued exploration of structural diversity in substituents at the N-1, C-2, C-7 and C-8 positions has led more recently to a variety of unique tricyclic structures that include the C-2-thio derivative ulifloxacin and the thiazoloquinolones (Fig. 1).[3,11-13]

Recently, several crystal structures with fluoroquinolone bound to DNA–TopoIV or DNA–DNA gyrase have been reported.[14–17] Even though none of these structures were obtained using a C-2 thioquinolone, analysis of the structures revealed possible binding contacts for a C-2 sulfur atom, and it suggested that a C-2 thioalkyl group may form additional binding interactions with the enzyme. This evidence is supported by studies in which we modeled ulifloxacin into the putative binding site of TopoIV (unpublished).

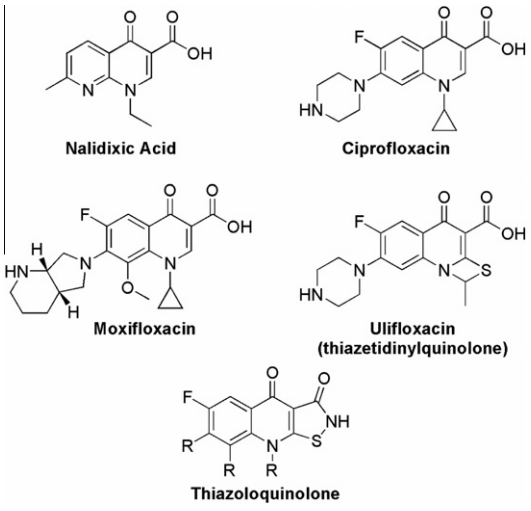

Figure 1. Representative structures of quinolone-class antibiotics and fluoroquinolones used or referred to in this work.

Literature examples of 2-thioquinolone derivatives where the C-2 sulfur atom is not incorporated into a fused ring are limited[18]; only ring-fused structures such as the thiazoloquinolones and

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thiazetidinylquinolones (Fig. 1) and are reported to have potent antibacterial activity.[19–21] Despite extensive modification of the fluoroquinolone core, little precedent exists for removing the 3-carboxylate due to its assumed requirement for activity.[13] However, quinazoline-2,4-diones and 1,3-diones have recently been shown to possess potent, broad-spectrum antibacterial properties despite the lack of a 3-carboxylate substituent,[22–30] thus suggesting the possibility that further modification to the C-2 and C-3 positions of quinolones will provide novel structures that maintain antibacterial activity.

Described here are studies initiated to evaluate the effect of a C-2 thioalkyl group on fluoroquinolone inhibition of DNA gyrase and antibacterial activity. Because the presence of a 3-carboxylate was anticipated to effect gyrase binding of C-2 *S*-alkyl derivatives, C-2 *S*-alkyl fluoroquinolone derivatives were synthesized with and without an adjacent C-3 carboxylate group. Synthesis of the 2-thioalkyl fluoroquinolone derivatives was achieved by modification of reported procedures for creating tricyclic C-2 sulfur-containing quinolones (Scheme 1).[31] Decarboxylation of parent fluoroquinolones and the 2-thioalkyl derivatives was anticipated to proceed using an established method for cyanide-mediated decarboxylation of fluoroquinolones.[32] All compounds were evaluated for bacteriostatic activity (MIC) with wild-type *Escherichia coli*, *E. coli tolC* knockout (an efflux pump knock out), and *Mycobacterium smegmatis*.

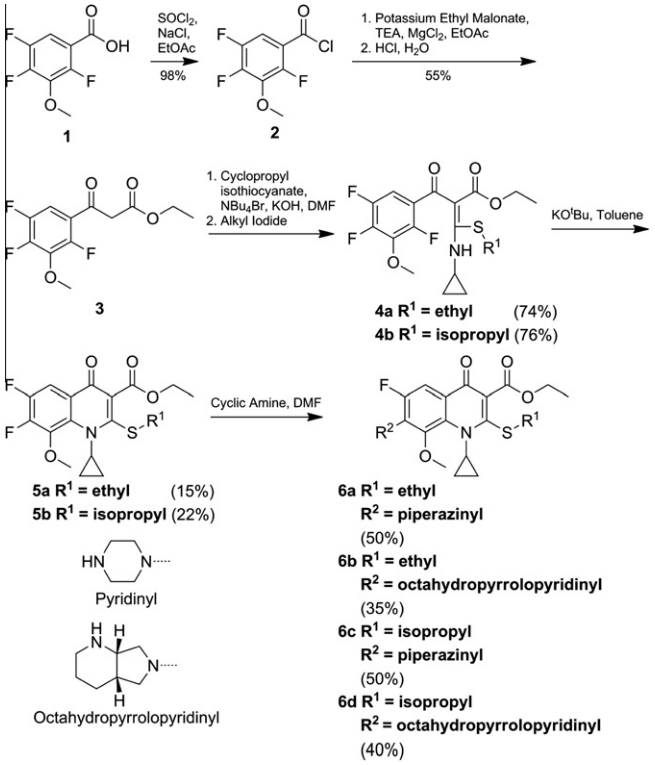
Commercially available 2,4,5-trifluoro-3-methoxy benzoic acid (**1**) was stirred at reflux in ethyl acetate with thionyl chloride to form the acid chloride (**2**) in near quantitative yield. Malonate ester **3** was then generated in a two-step process by reacting acid chloride **2** with potassium ethyl malonate in the presence of anhydrous magnesium chloride followed by decarboxylation with 6 N hydrochloric acid and crystallization of product to give the aryl propionate ethyl ester **3**.

Deprotonation of **3** with potassium hydroxide followed by coupling with cyclopropyl isothiocyanate and trapping the thiolate intermediate with ethyl iodide or isopropyl iodide in situ afforded ethyl esters **4a** and **b**, respectively, in high yield. Potassium *tert*-butoxide catalyzed intramolecular nucleophilic aromatic substitution yielded the target fluoroquinolone core structures **5a** and **b**. Nucleophilic aromatic substitution of the C-7 fluorine with the piperazine and octahydropyrrolopyridine rings was carried out in DMF at 60 °C to yield moxifloxacin-like and ciprofloxacin-like fluoroquinolone ethyl esters **6a–d** (Scheme 1).[33] The choice of piperazine and octahydropyrrolopyridine as the C-7 substituents is derived from their inclusion at the same position of ulifloxacin and ciprofloxacin, and moxifloxacin, respectively (Fig. 1).

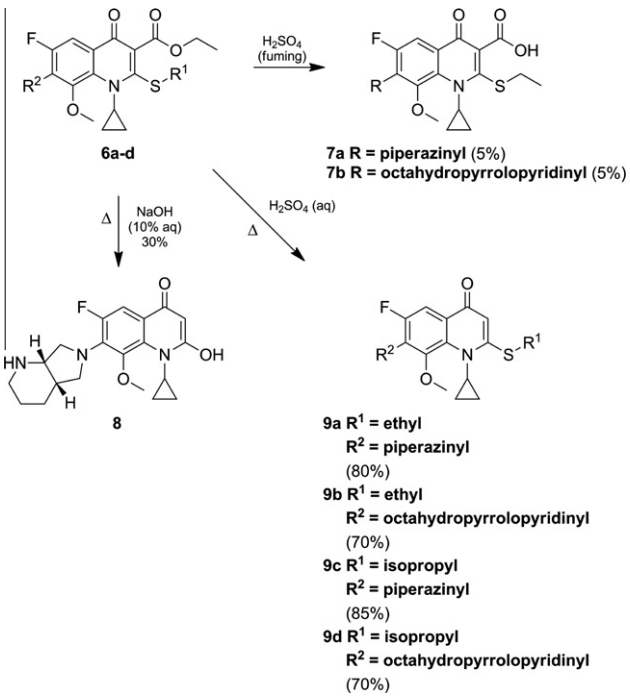
To our surprise, hydrolysis of ethyl esters **6a–d**[34–36] to give the C-2 thioalkyl substituted fluoroquinolones was highly problematic. Previously described conditions for the hydrolysis of many other fluoroquinolone esters and modifications to these procedures were generally unsuccessful with the C-2 thioalkyl compounds here.[37–42] We found that no reaction occurred with **6a–d** under aqueous hydrolytic conditions without elevated temperature. However, at the elevated temperatures under both acidic and basic pH decarboxylation products rather than hydrolysis products were observed (Scheme 2).

Formation of the 2-hydroxyl compound (**8**) during hydrolysis of **6b** and **d** likely follows an addition/elimination mechanism previously described for cyanide-mediated decarboxylation of fluoroquinolones.[32] In this reported mechanism for fluoroquinolone decarboxylation, the 1,4-addition of cyanide to the C-2 position delocalizes electrons to the alpha carbon at C-3, which promotes decarboxylation. Upon decarboxylation the double bond is reformed by ejecting cyanide as the leaving group. We propose a similar mechanism for the loss of C-2 thioalkyl groups upon base-mediated hydrolysis. Hydroxide anion first attacks the C-2 position of a C-2 thioalkyl substituted quinolone, and upon decarboxylation the thioalkyl group serves as a better leaving group than hydroxide. The thioalkyl group is ejected, and decarboxylated 2-hydroxy fluoroquinolone is obtained.

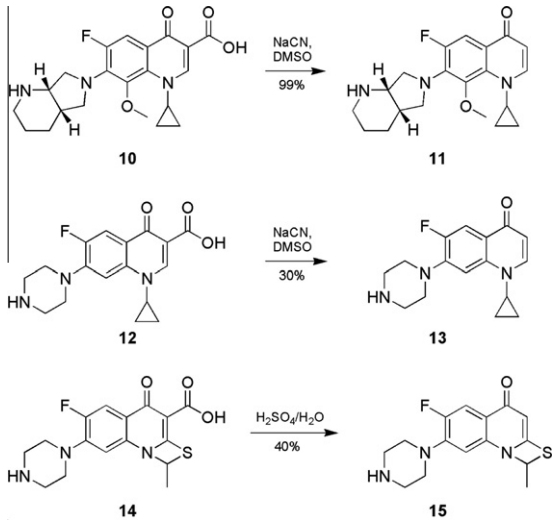
Hydrolysis of esters **6a–d** under acid conditions also afforded unexpected results. Following established literature procedures,[38,43] the heating of esters **6a–d** in 1–10% sulfuric acid



Scheme 1. Synthesis of C-2 S-alkyl fluoroquinolone ethyl esters.



Scheme 2. Ester hydrolysis and decarboxylation reactions.



Scheme 3. Decarboxylation of control fluoroquinolones.

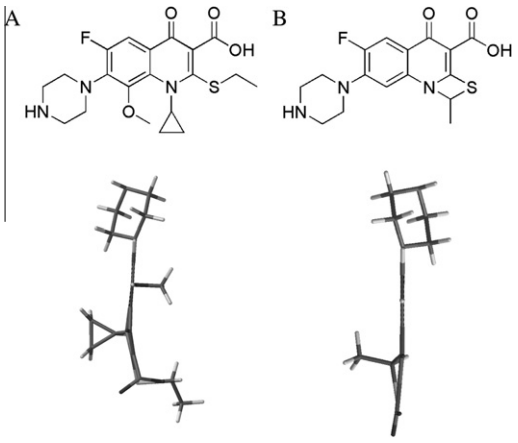


Figure 2. Comparison between **7a** (A) and ulifloxacin (B) showing loss of planarity when C-2 thioether is not constrained in the thiazetidine ring system. Minimizations performed by MM2 (shown) and HF 6-31G** were similar.

solution provided the decarboxylated C-2 thioalkyl products in good yield (Scheme 2). Ultimately, hydrolysis of the ethyl esters using fuming sulfuric acid at room temperature[42,44] gave isolatable C-2 thioethyl products **7a** and **b** in poor yield. Having these compounds characterized and in hand, analytical HPLC studies of **7a** and **b** in water at various pH revealed the compounds to be stable at neutral pH but unstable under both acidic and basic conditions. We were then able to use HPLC to observe hydrolysis of the C-2-*S*-isopropyl derivatives **6c** and **d** to give desired 3-carboxylate-2-thioisopropyl analogs, but these compounds proved to be unstable, readily undergoing decarboxylation. Thus their isolation was abandoned.

Decarboxylated control compounds were prepared from parent fluoroquinolones. Moxifloxacin (**10**) and ciprofloxacin (**12**) were decarboxylated with cyanide to give **11** and **13** (Scheme 3).[32] This procedure was ineffective when applied to ulifloxacin (**14**), further enforcing the detrimental effect of a C-2 thio moiety on reactions of the 3-carboxylate that are routinely performed with other fluoroquinolones. However, guided by experimental observations, treatment of ulifloxacin (**14**) with 10% sulfuric acid for 6 days at 100 °C gave descarboxyulifloxacin **15** in good isolated yield after preparative HPLC (Scheme 3).

Bacteriostatic activity of the parent 3-carboxy fluoroquinolones was compared with activity of the C-2-thioalkyl and decarboxylated derivates (Table 1). As expected the decarboxylated analogs of ciprofloxacin, moxifloxacin and ulifloxacin were 60-fold to over 25,000-fold less active than the parent agents against each strain tested (Table 1). Interestingly, both the parent and the decarboxylated compound showed lower MIC with the *tolC* knockout of *E. coli* as compared to wild type, demonstrating the 3-carboxylate group is not important for recognition and efflux by the TolC transport system.

MIC values for all C-2-thioalkyl derivatives are high in comparison to parent fluoroquinolones. In contrast, comparison of MICs for descarboxy moxifloxacin with the decarboxylated C-2-thioethyl and C-2-thioisopropyl derivatives shows that, in the absence of a C-3 carboxylate group, a C-2-thioalkyl group can impart lower MIC when in place of the C-2H. For example, incorporating a C2-*S*-isopropyl group into descarboxy moxifloxacin (compound **9d**) gives a lower MIC than for descarboxy moxifloxacin with each strain tested. These data demonstrate that either a 2-thioalkyl group or a 3-carboxylate moiety can afford increased activity over corresponding 2,3-unsubstituted derivatives, with the 3-carboxylate imparting a much lower MIC than the 2-thioalkyl group.

Unlike ulifloxacin, where the 3-carboxyl group and the thiazetidine ring combined to afford a significant increase in potency (lower MIC), compounds **7a** and **b**, which have both the 3-carboxylate and a 2-thioethyl group, have high MICs. In fact, the elevated MICs of the C-2-*S*-alkyl compounds overall is in contrast to typically low

Table 1
MIC of C-2 and C-3 modified quinolones in *E. coli* and *M. smegmatis*

Compound	C-2	C-3	C-7	Bacterial Strain (MIC μg/mL)		
				E. coli[a]	*E. coli* tolC[b]	*M. smegmatis*[c]
Ciprofloxacin	H	CO_2H	Piperazinyl	0.04	0.008	0.156
13	H	H	Piperazinyl	50	1.56	6.25
Ulifloxacin	Thiazetidine	CO_2H	Piperazinyl	0.062	0.016	3.13
15	Thiazetidine	H	Piperazinyl	>200	200	200
Moxifloxacin	H	CO_2H	Octahydropyrrolopyridinyl	0.156	0.004	0.08
11	H	H	Octahydropyrrolopyridinyl	>200	100	>200
7a	*S*-ethyl	CO_2H	Piperazinyl	200	12.5	>200
7b	*S*-ethyl	CO_2H	Octahydropyrrolopyridinyl	>50	>50	50
9a	*S*-ethyl	H	Piperazinyl	>50	25	>50
9b	*S*-ethyl	H	Octahydropyrrolopyridinyl	>200	100	>200
9c	*S*-isopropyl	H	Piperazinyl	>50	>50	>50
9d	*S*-isopropyl	H	Octahydropyrrolopyridinyl	>50	50	50

[a] KD65.
[b] KD1397.
[c] KD1163.

MICs reported in the literature for ulifloxacin and for other C-2-*S* substituted quinolones in which the C-2 sulfur is incorporated into a fused ring system such as isothiazolidinone (isothiazoquinolones) and thiazetidine (ulifloxacin) rings (Fig. 1).[19–21] Similar results were found when direct inhibition and poisoning of purified gyrase was characterized: the C-2-thioalkyl compounds were orders of magnitude less active than the fused-ring congeners (data not shown).

A likely reason for the improved activity of isothiazole- and thiazetidine-containing fluoroquinolones is binding interactions with gyrase involving the C-2 sulfur atom. However, addition of thioalkyl groups to position 2 of quinolones, as described in this work, while modestly enhancing activity over 2-unsubstituted 3-descarboxy fluoroquinolone, does not enhance antibacterial activity. Molecular modeling studies provide a likely explanation for this dramatic difference in activity for C-2-thioalkyl versus C-2-*S*-fused ring compounds. As shown (Fig. 2, panel A), steric conflict between a C-2-thioalkyl group and the 3-carboxylate moiety significantly distorts orientation of the 2-thioalky group and carboxylate out of planarity with the quinolone core. In contrast, when the C-2 sulfur atom is incorporated into a fused ring system, the 3-carboxylate and fused ring system remains co-planar with the quinolone ring system (exemplified by ulifloxacin in Fig. 2, panel B).

In conclusion, we have identified structural features of 2-thio derivatives of fluoroquinolones that contribute to both increased and decreased antibacterial activity. Synthetic methods employed in this work have revealed additional types of 1,4-addition reactions that can be exploited to functionalize position 2 on the fluoroquinolone core. Thus guided by these results and the recently reported quinolone–topoisomerase–DNA crystal structures, we are now working to characterize the potential binding interactions of a C-2-sulfur in the drug–topoisomerase complex and to synthesize new type II topoisomerase inhibitors that will be active against mutants resistant to current agents.

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33. Compound **5a** was prepared by stirring 182.7 mg (0.45 mmol) **4a** and 53.0 mg (0.47 mmol) KO^{t}Bu in toluene while heating to reflux for 19 h. Product **5a** was purified by flash column eluted with 3:1 hexanes. ^{1}H NMR ($CDCl_3$) δ 0.69 (br s, 2H), 1.17 (br s, 2H), 1.34 (m, 6H), 3.06 (q, J = 7.4 Hz, 2H), 3.70 (m, 1H), 4.06 (d, J = 2.3 Hz, 3H), 4.38 (q, J = 7.1 Hz, 2H), 7.75 (dd, 1H). ^{19}F NMR ($CDCl_3$) δ −146.03 (m, 1F), −137.12 (m, 1F). MS, ESI, calcd (M+H$^+$) 384.10, found 384.02. Compound **5b** was prepared similarly.
34. Compound **6a** was prepared by stirring 37.8 mg (0.10 mmol) **5a** with 44.3 mg (0.5 mmol) piperazine in 2 mL DMF at 130 °C for 26 h. Product was purified by semi-preparative HPLC. Compounds **6b–d** were prepared similarly. Compound **6b** ^{1}H NMR (CD_3CN) δ 0.62 (br s, 2H), 0.99 (br s, 2H), 1.31 (m, 6H), 1.83 (m, 4H), 2.73 (m, 1H), 3.06 (m, 4H), 3.35 (m, 1H), 3.55 (s, 3H), 3.74 (m, 1H), 3.89 (br s, 1H), 4.04 (t, J = 1.1 Hz, 1H), 4.29 (dq, J = 7.1, 1.6 Hz, 2H), 7.32 (d, $J_{H\text{-}F}$ = 12.8 Hz, 1H), 9.70 (br s, 1H). MS, ESI, calcd (M+H$^+$) 490.21, found 490.18.
35. Compound **7a** was prepared by stirring 71 mg **6a** in 1 mL fuming sulfuric acid for 2 h. Product was purified by semi-preparative HPLC and confirmed by ESI MS, calcd (M+H$^+$) 422.15, found 422.03. Compound **7b** was prepared similarly. MS, ESI, calcd (M+H$^+$) 462.18, found 462.11.
36. Compound **9a** was prepared by stirring **6a** in 0.1 M H_2SO_4 solution at reflux for 24 h. Pure **9a** was recovered by semi-preparative HPLC. **9b–d** and **15** were prepared similarly. Compound **9b** ^{1}H NMR (DMSO-d_6) δ 0.68 (br d, 2H), 1.13 (br d, 2H), 1.33 (t, 3H), 1.73 (m, 4H), 2.64 (m, 1H), 3.03 (m, 4H), 3.23 (d, 1H), 3.49 (s, 3H), 3.58 (m, 2H), 3.69 (m, 1H), 3.88 (m, 1H), 4.03 (m, 1H), 6.08 (s, 1H), 7.40 (d, 1H), 8.59 (br s, 1H), 9.26 (br d, 1H). MS, ESI, calcd (M+H$^+$) 418.19, found 418.22.
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