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The synthesis and biological evaluation of novel series of nitrile-containing fluoroquinolones as antibacterial agents

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Abstract—Several novel series of nitrile-containing fluoroquinolones with weakly basic amines are reported which have reduced potential for hERG (human ether-a-go-go gene) channel inhibition as measured by the dofetilide assay. The new fluoroquinolones are potent against both Gram-positive and fastidious Gram-negative strains, including Methicillin resistant *Staphylococcus aureus* and fluoroquinolone-resistant *Streptococcus pneumoniae*. Several analogs also showed low potential for human genotoxicity as measured by the clonogenicity test. Compounds **22** and **37** (designated PF-00951966 and PF-02298732, respectively), which had good in vitro activity and in vitro safety profiles, also showed good pharmacokinetic properties in rats.

Antibacterial resistance is a growing problem[1,2] which necessitates the discovery of new antibiotics with activity against resistant strains. The fluoroquinolone class of antibiotics is an important weapon against bacterial infections, in particular Ciprofloxacin and Levofloxacin (see Fig. 1), but resistance is increasing in this class as well.[3] Some more recently approved fluoroquinolones have had concerns with cardiovascular safety, in particular the possibility of QT_c prolongation which can lead to potentially fatal *torsades de pointes*.[4,5] These concerns led to Sparfloxacin and Grepafloxacin being removed from the market and bolded warnings being added to the package inserts for Moxifloxacin and Gatifloxacin.

Due to the serious consequences of inducing QT_c prolongation, much work has been done to understand the effect and to develop various methods to predict it.[6] Typical in vitro assays include a hERG (human ether-a-go-go related gene) functional assay and a dofetilide binding assay which measures the ability of a compound to displace dofetilide—a potent inhibitor in the functional assay—from the hERG channel. The dofetilide assay has a higher throughput than the hERG functional assay and is thus more useful at the discovery stage.[6] Several pharmacophore models have been developed for hERG activity, and the general findings indicate that a basic nitrogen and two or three aromatic components at appropriate distances from the amine confer increased hERG activity.[6] We set out to decrease the hERG activity by decreasing the basicity of the amine with the electron-withdrawing nitrile group, while at the same time maintaining activity against resistant organisms.

Keywords: Fluoroquinolones; Antibacterial; Resistance; Nitriles; Dofetilide; hERG; pK_a; PF-00951966; PF- 02298732; PF-02789402.
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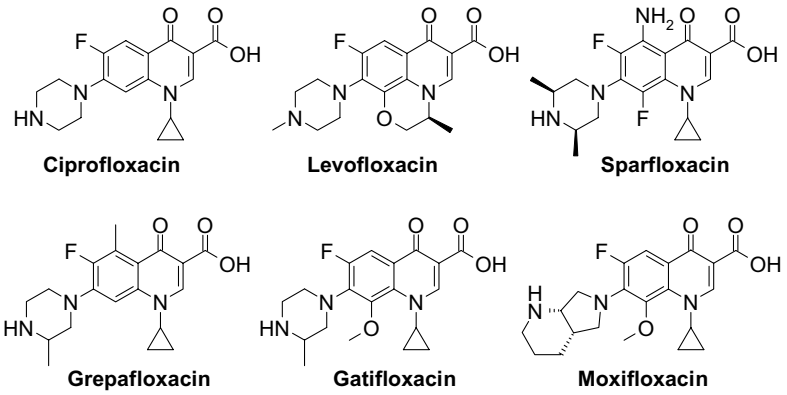


Figure 1. Chemical structures of some approved fluoroquinolones.

Our initial series was derived from the C_7-pyrrolidine side chains (see Table 1 for fluoroquinolone numbering) developed by Domagala et al.[7] The previous series had excellent activity against resistant strains but showed significant displacement of dofetilide in the dofetilide binding assay. The pyrrolidine side chain series also showed various levels of mammalian genotoxicity, so we also monitored the compounds in a clonogenicity assay which has been shown to be predictive in the fluoroquinolone class.[8]

We have found that incorporating a nitrile group either onto the carbon framework of the pyrrolidine side chain (C-substituted series, R = CH_2CN in Table 1, e.g., **20**) or as a substituent on the nitrogen (N-substituted series, R′ = CH_2CH_2CN, e.g., **17**) significantly increased the K_i in the dofetilide assay. Further exploration of nitrile-containing fluoroquinolones led to the discovery of compounds with either pyrrolidine or azetidine side chains which have activity against key Gram-positive and Gram-negative bacteria along with improved in vitro safety measures.

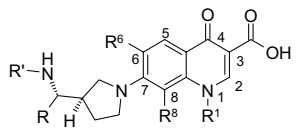
The fluoroquinolones with pyrrolidine side chains were prepared as described previously for the non-nitrile-containing analogs,[7,9] an example of which is shown in Scheme 1. The 8-Me fluoroquinolone analog (Table 1, **28**) could be prepared in higher yield by using DMSO instead of acetonitrile and Hunig's base rather than triethylamine. In the case of the analogs containing azetidine side chains (**34** and **36**), the final deprotection (removal of trifluoro-acetate, see Scheme 4) was accomplished with sodium carbonate in methanol/water at 70 °C. To prepare the analogs where the nitrogen was substituted with the ethylcyano group (Table 1, R′ = CH_2CH_2CN, e.g., **17**), the unsubstituted fluoroquinolone (e.g., **16**) was heated with excess acrylonitrile (ca. 5 equiv) and triethylamine in methanol at 50 °C.

The synthesis of the nitrile-containing pyrrolidine side chains **6a** and **6b** is shown in Scheme 2. The ester **2**, prepared by the method of Yoshiyasu et al.[10] was converted by standard procedures into the Cbz-protected aldehyde **3**, which was then transformed into the nitrile olefin **4** as a mixture of isomers by a Horner–Emmons–Wadsworth olefination. After our initial attempts to introduce an amine surrogate (azide, phthalimide, carbamic acid *tert*-butyl ester) to **4** by a Michael addition were unsuccessful, we found that either ammonia or methyl amine could be added directly to **4** with excess amine (5–10 equiv) in ethanol at 80 °C in a sealed tube. After Boc protection of the amine, the diastereomers were separated by silica gel chromatography, and then the enantiomers of the slower eluting diastereomer were separated by preparative chiral HPLC to give the desired stereoisomer (vide infra).[11] After Cbz-deprotection of the pyrrolidine, the side chain was coupled to various fluoroquinolone cores to give **20**, **22**, **27**, **32**, and **33** (Table 1).

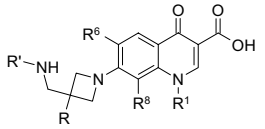
The absolute stereochemistry of the desired stereoisomer of **5** ((*S*)-3-amino-3-(*R*)-pyrrolidin-3-yl-propionitrile) was confirmed to be the same as in the des-nitrile case[7] by an independent, stereoselective synthesis (Scheme 3). The aldehyde **7**, synthesized by the method of Baldwin et al.[12] was converted to amine **8** by reductive amination. Removal of the TBS protecting group from **8** followed by heating at reflux in THF induced a translactamization from the β-lactam to the γ-lactam, which formed the pyrrolidine ring with the desired stereochemistry. After global reduction and Boc protection of the amine, the *N*-benzyl group was replaced with a Cbz protecting group, the swap being necessary for later removal in the presence of a nitrile. The nitrile was then introduced via the mesylate to give side chain **5a**, the stereochemistry of which was confirmed to be that of the desired stereoisomer by chiral HPLC. Methylation of **5a** led to **5b** which was also confirmed by chiral HPLC to be the desired stereoisomer in the *N*-methyl analogs.

The azetidine side chains were synthesized as shown in Scheme 4 following the route developed by Frigola et al.[13] starting from the known tertiary alcohols **12a**[13] (R = Me) and **12b**[14] (R = Et). After reaction with methanesulfonyl chloride, the resulting mesylate of the alcohol **12** could be efficiently transformed into the nitrile with sodium cyanide, provided the solvent was a mixture of DMF and water—running the displacement in anhydrous DMF gave inconsistent and generally poor yields. The nitrile **13** was then reduced to the amine, which was protected as the trifluoroacetate. Finally, hydrogenolysis of the benzhydryl group freed the azeti-

Table 1. Structure–activity relationships in nitrile-containing fluoroquinolones

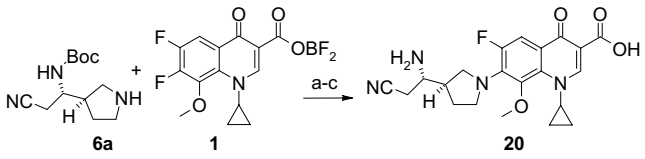


Compound	R	R′	R^6	R^8	R^{1a}	*Staphylococcus aureus* MIC[b] (μg/mL)	*Staphylococcus aureus*^{R} MIC[c] (μg/mL)	*Streptococcus pneumoniae* MIC[d] (μg/mL)	*Streptococcus pneumoniae*^{R} MIC[e] (μg/mL)	*Haemophilus influenzae* MIC[f] (μg/mL)	*Moraxella catarrhalis* MIC[g] (μg/mL)	Dofet.[h] KI (μM)	Clono[i] IC_{50} (μg/mL)
Ciprofloxacin						0.125	1	0.5	16	0.008	0.03	>100	500
Levofloxacin						0.125	0.5	0.5	16	0.015	0.06	>150 (10)	>500
16	H	H	F	OMe	cp	0.015	0.06	0.06	1	0.015	0.125	24	56
17	H	CH_2CH_2CN	F	OMe	cp	0.03	0.06	0.125	2	0.125	0.5	106	252
18	Me	H	F	OMe	cp	0.002	0.008	0.004	0.03	0.004	0.015	85	30
19	Me	CH_2CH_2CN	F	OMe	cp	0.004	0.008	0.015	0.125	0.06	0.06	>150 (2)	48
20	CH_2CN	H	F	OMe	cp	0.001	0.002	0.008	0.125	0.008	0.03	>150 (29)	24
21	Me	Me	F	OMe	cp	0.015	0.06	0.008	0.125	0.015	0.06	>150 (40)	>125
22	CH_2CN	Me	F	OMe	cp	0.008	0.06	0.06	0.5	0.03	0.06	>150 (9)	139
23	H	H	H	OMe	cp	0.03	0.25	0.125	4	0.06	0.5	64	331
24	H	CH_2CH_2CN	H	OMe	cp	0.03	0.25	0.125	2	0.25	1	>100	326
25	Me	H	H	OMe	cp	0.008	0.03	0.015	0.125	0.125	0.06	62	314
26	Me	CH_2CH_2CN	H	OMe	cp	0.03	0.125	0.25	2	0.5	1	>100	38
27	CH_2CN	H	H	OMe	cp	0.008	0.06	0.03	0.5	0.06	0.125	>150 (30)	465
28	Me	H	F	Me	cp	0.002	0.03	0.002	0.03	0.004	0.015	51	22
29	Me	CH_2CH_2CN	F	Me	cp	0.002	0.008	0.008	0.06	0.03	0.015	72	27
30	Me	H	F	OCH_2CHMe		0.015	0.125	0.03	0.25	0.008	0.06	>100 (35)	143
31	Me	CH_2CH_2CN	F	OCH_2CHMe		0.015	0.03	0.06	0.25	0.06	0.125	>150 (10)	210
32	CH_2CN	H	F	OCH_2CHMe		0.008	0.06	0.015	0.125	0.008	0.125	>150 (22)	159
33	CH_2CN	Me	F	OCH_2CHMe		0.06	0.125	0.125	2	0.03	0.25	>150 (19)	279

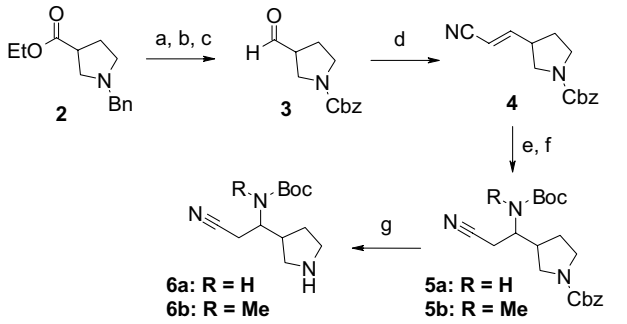


34	Me	H	F	Me	cp	0.06	0.06	0.06	2	0.015	0.06	112	112
35	Me	CH_2CH_2CN	F	Me	cp	0.03	0.03	0.125	2	0.06	0.125	>150 (18)	268
36	Et	H	F	Me	cp	0.015	0.03	0.06	1	0.015	0.125	45	50
37	Et	CH_2CH_2CN	F	Me	cp	0.03	0.03	0.125	2	0.06	0.25	>150 (25)	253

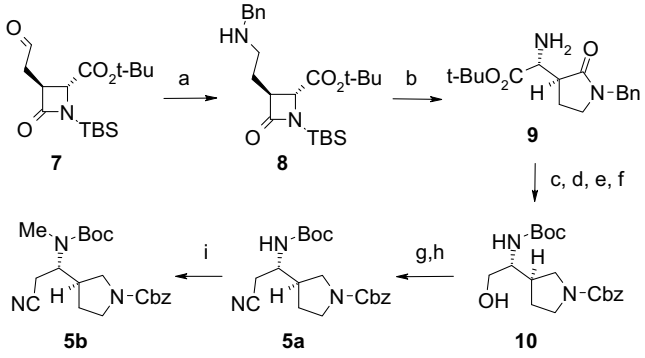
[a] cp = cyclopropyl, OCH_2CHMe between R^1 and R^8 is the (*S*)-stereoisomer as in Levofloxacin.
[b] *Staphylococcus aureus* UC-76.
[c] Methicillin resistant *Staphylococcus aureus* SA-2552.
[d] *Streptococcus pneumoniae* SV-1.
[e] Fluoroquinolone resistant *Streptococcus pneumoniae* SP-3765.
[f] *Haemophilus influenzae* HI-3542.
[g] *Moraxella catarrhalis* BC-3531.
[h] See Ref. 17 for assay conditions, number in parentheses is % inhibition at 300 μM.
[i] Clonogenicity, see Ref. 8 for assay conditions.



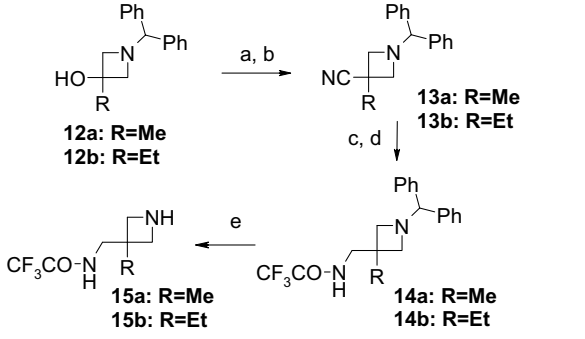
Scheme 1. Example coupling of side chain **6a** with fluoroquinolone core **1**; Reagents and conditions: (a) triethylamine, acetonitrile, 80 °C; (b) triethylamine, ethanol, 80 °C (79%, 2 steps); (c) HCl, dioxane, CH_2Cl_2 (84%).



Scheme 2. Reagents and conditions: (a) CbzCl, $CHCl_3$ (85%); (b) $NaBH_4$ (56%); (c) TEMPO, NaOCl (78%); (d) $(EtO)_2POCH_2CN$, Cs_2CO_3 (74%); (e) RNH_2, EtOH, sealed tube, 80 °C; (f) Boc_2O (83–85%, 2 steps); (g) Pd/C, H_2, THF (quant).



Scheme 3. Reagents and conditions: (a) $BnNH_2$, AcOH; then $NaBH_3CN$; (b) NaF, MeOH; then reflux in THF/MeOH, (40%, 2 steps); (c) $LiAlH_4$, (d) Boc_2O (60%, 2 steps); (e) Pd/C, H_2; (f) CbzCl (50%, 2 steps); (g) MsCl; (h) NaCN (95%, 2 steps); (i) NaH, DMF; then MeI (72%).



Scheme 4. Reagents and conditions: (a) MsCl, triethylamine (R = Me: 96%, R = Et: 98%); (b) NaCN, 4:1 DMF/H_2O, 60 °C (R = Me: 75%, R = Et: 86%); (c) $LiAlH_4$, THF, reflux (R = Me: 95%, R = Et: 92%); (d) $(CF_3CO)_2O$ (R = Me: 98%, R = Et: 53%); (e) Pd/C, H_2, MeOH, HCl.

dine nitrogen for subsequent coupling to the 8-Me fluoroquinolone core to give **34** and **36** (Table 1).

The new fluoroquinolones were tested for activity against key pathogens for both community and hospital infections (Table 1), including Methicillin-resistant *Staphylococcus aureus*, Ciprofloxacin-resistant *Streptococcus pneumoniae*, and the fastidious Gram-negative species *Haemophilus influenzae* and *Moraxella catarrhalis*.[15] In the SAR portion of this paper, we will focus on the resistant *S. pneumoniae* species since the MIC values for the other species were generally less than or equal to our target value of 0.5 μg/mL.

The SAR of various pyrrolidine side chains has been previously discussed by Domagala et al.[7] The addition of a nitrile in the C-substituted series decreased the activity in *S. pneumoniae*^{R} by 4-fold (**20** vs **18**, **22** vs **21**, and **27** vs **25**) except in the case of the levofloxacin core where it showed no decrease in activity (**32** vs **30**). The N-substituted pyrrolidine series generally showed a 2-fold decrease in activity for the R^6 = F cores (e.g., **16** vs **17**, **28** vs **29**), whereas the R^6 des-fluoro core showed a 16-fold decrease in activity with R = Me (**25** vs **26**) but no significant change with R = H (**23** vs **24**).

The SAR of the azetidine side chains has been examined by Frigola et al.[13] in which the side chain of **34** was tested on the R^8 = H and R^8 = F cores. In that study, the addition of the R = Me group versus R = H led to equivalent activity with R^8 = H and about a 4-fold decrease in activity with R^8 = F. In our series, we were able to increase the activity by switching to the R^8 = Me core and using R = Et on the side chain to give sufficient activity against the *S. pneumoniae*^{R} strain (1 μg/mL).[16] As with the N-substituted pyrrolidine series, the N-substituted azetidine compounds also showed only a small decrease in activity compared to the unsubstituted analogs (**34** vs **35**, **36** vs **37**).

In every case where a nitrile was added to the side chain, the new compound showed less displacement of dofetilide in the dofetilide binding assay[17] compared to the des-nitrile case (e.g., **16** vs **17**, **18** vs **20**). For the R^8 = Me core, the increase in K_i is only modest (**28** vs **29**). Since the R^8 = Me core is the most lipophilic core (analog **29** clog *D* @7.0 is 0.7 higher than for the R^8 = OMe core analog **19**), the greater affinity for the hERG channel[18] may be influenced more by lipophilicity than amine basicity.[19] In some cases, the des-nitrile and nitrile-containing analogs both showed K_i > 150 μM, and therefore the %inhibition at 300 μM was used for comparison (**21** vs **22**, **30** vs **31**–**33**). Three of the new fluoroquinolones (**19**, **22**, and **31**) showed inhibition in the dofetilide assay at 300 μM as low as Levofloxacin (10%), a marketed fluoroquinolone with low risk for QT_c prolongation.[4] Two of these (**22** and **31**, designated PF-00951966 and PF-02789402) also met the criteria for clonogenicity—vida infra.

The increase of the K_i in the dofetilide assay was predicted from the reduced basicity of the amines in the nitrile-containing compounds. The reduced basicity was measured [20]

Table 2. Rat PK on selected nitrile-containing fluoroquinolones[a]

Compound	AUC, iv (μg h/mL)	CL, iv (mL/min/kg)	V_d, iv (L/kg)	$t_{1/2}$, iv (h)	C_{max}, po (μg/mL)	%*F*, po
19	3.5	24	2.5	2.7	1.2	70
22	4.4	19	1.8	2.4	1.4	53
27	5.2	16	0.5	1.8	1.4	22
34	2.5	33	2.5	1.3	0.3	18
37	6.5	13	1.0	1.4	2.6	92

[a] Rats were dosed at 5 mg/kg orally (po) or 1 mg/kg intravenously (iv). All values are an average of at least $n = 3$.

in the C-substituted series ($pK_a = 9.5$ vs 6.7 for **21** vs **22**), in the N-substituted pyrrolidine series ($pK_a = 9.9$ vs 6.9 for **18** vs **19**), and in the N-substituted azetidine series ($pK_a = 8.6$ vs 6.5 for **34** vs **35**).

Clonogenicity has been shown to be a useful predictor of human genotoxicity in the fluoroquinolone class.[21] Suto et al.[8] have shown that the clonogenicity in the fluoroquinolone class is affected by the nature of the core as well as the substituent R′ on the amine (see Table 1). We sought to control the clonogenicity using similar changes to obtain a compound with an IC_{50} of >100 μg/mL.

Compared to the R^8 = OMe core, the R^8 = Me core analogs were more genotoxic in the clonogenicity assay (**19** vs **29**), as was observed in the previous study.[8] In contrast, changing to the R^6 = H (des-F) core or the R^8–R^1 = (*S*)-OCH_2CHMe (Levofloxacin) core generally improved the clonogenicity (e.g., **20** vs **27** and **32**), and in many cases the target criterion of >100 μg/mL was satisfied.

Substitution on the nitrogen of the side chain also brought about improvements in the clonogenicity. N-Methylation showed a dramatic increase in clonogenicity IC_{50} from 24 μg/mL up to 139 μg/ml in **20** vs **22**, respectively, although the antibacterial activity did show a modest decrease. In the azetidine series, the N-substitution of the ethylcyano group showed a pronounced increase in clonogenicity IC_{50} (e.g., **34** vs **35** and **36** vs **37**), although little or no effect was seen by the same substitution in the pyrrolidine series (e.g., **23** vs **24** and **28** vs **29**).

Selected compounds which met the criteria for antibacterial activity, dofetilide, and/or clonogenicity were tested in vivo in rats to assess their pharmacokinetic performance, and the results are shown in Table 2. The nitrile-containing fluoroquinolones showed good overall pharmacokinetics. In the C-substituted series, the pyrrolidine analog **22** (designated PF-00951966) showed both good bioavailability and AUC. The azetidine analog **37** (designated PF-02298732) showed excellent bioavailability and a good AUC. On the other hand, the R^6 des-fluoro analog (**27**) showed a sub-optimal volume of distribution and low bioavailability. The non-nitrile-containing fluoroquinolone, **34**, shown for comparison, showed higher clearance and lower bioavailability compared to the other compounds tested.

In summary, we have synthesized a set of nitrile-containing fluoroquinolones with activity against resistant strains of *S. aureus* and *S. pneuomoniae* and low displacement of dofetilide in the dofetilide binding assay. By modifying the fluoroquinolone core and the side chain nitrogen substituents, we were able to attenuate the genotoxicity and discover several compounds which had both potency and good safety profiles in our in vitro assays. Further work on the activity of these compounds in an expanded panel of organisms and in vivo efficacy models will be reported in due course.

Acknowledgments

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11. *S. pneumo.*[R] MICs for stereoisomers of **22**: diastereomer A (faster eluting) = 8 μg/mL, diastereomer B (slower eluting) = 1 μg/mL, and enantiomer B1 (faster eluting) = 8 μg/mL, enantiomer B2 (slower eluting, desired) = 0.5 μg/mL. Diastereomers were separated on silica gel eluted with hexanes/ethyl acetate. Enantiomers were separated by chiral HPLC on a ChiralPak AD column eluted with methanol/ethanol.
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organisms. For example, the MIC_{50} for compound 22 against *Escherichia coli* strains and ESBL producing strains of *Klebsiella pneumoniae* is 32 μg/ml.
16. Compounds in the azetidine series with R = *n*-propyl, isopropyl, and cyclopropropyl were also active, but did not offer any advantages over R = ethyl (**37**).
17. A filter binding assay with [^{3}H]-dofetilide was used as described in: Greengrass, P. M.; Stewart, M.; Wood, C. M. World Patent WO2003021271, 2003.
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