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New Fluoroquinolone Finafloxacin HCl (FIN): Route of Synthesis, Physicochemical Characteristics and Activity under Neutral and Acid Conditions

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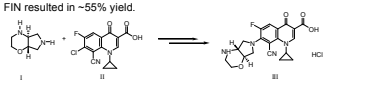
Revised Abstract

Background: FIN, a novel fluoroquinolone (FQ), is a representative of a new 8-cyano subclass. FIN exhibits optimal efficacy at slightly acidic pH under which other FQs lose activity. FIN is therefore intended for therapeutic use against bacterial infections in acidic environments, e.g. *H. pylori* eradication, UTI.

Methods: I and II were synthesized and combined to III (FIN). Physicochemical characterization was performed by; NMR, X-ray, HPLC (solubility), titration (ionisation constants). MICs were determined using CLSI methodology for broth microdilution at different pH.

Results: I and II were synthesized in 7 steps at ~25% and ~30% yield, respectively. Coupling of I and II and subsequent crystallization into FIN resulted in ~55% yield.



Characterization of FIN included elucidation of the chemical and crystal structure, determination of solubility (mg/mL; 5.5 (pH 7), 1.9 (pH 4.5)) and ionisation constants (pK_{a1}=5.6, pK_{a2}=7.8). FIN MICs (mg/L) against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were 0.06 and 0.25 (pH 7.2) and 0.008 and 0.06 (pH 5.8), respectively.

Conclusions: FIN displays exceptional antibacterial activity at low pH, unlike other FQs, making it a prime candidate to treat infections in acidic environments, such as the gastrointestinal or urogenital tract.

Introduction

Finafloxacin (FIN, Figure 1) is a novel, broad spectrum fluoroquinolone (FQ) that belongs to a new 8-cyano subclass. FIN contains a novel chiral base component which confers improved antibacterial activity at slightly acidic pH (pH 5.0 – 6.0) under which other marketed FQs exhibit significantly reduced activity [1].

FIN exhibited superior activity to comparator FQs against adherent bacteria *in vitro* [2] and in a wide range of rodent infection models [3,4]. Additionally, FIN displayed an excellent safety profile in a wide range of predictive, *in vitro*, toxicity assays [5] and was well tolerated in healthy human volunteers [6]. These attributes suggest that FIN warrants clinical investigation for bacterial infections that are associated with low pH such as urinary tract infection and *Helicobacter pylori* eradication.

The present study was performed to determine the physiochemical characteristics of FIN and the effect of pH on its basic antibacterial activity.

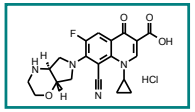


Figure 1. Finafloxacin hydrochloride (FIN).

Methods

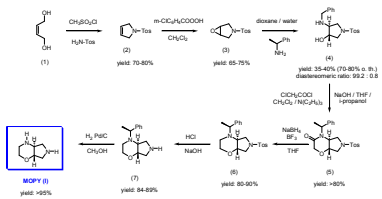
Chemistry: Finafloxacin HCl (FIN, III) was synthesized by combining MOPY,-(1*S*,6*S*)-Morpholinopyrrolidine (I), with Cyano-FQA, 7-chloro-8-cyano-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (II), followed by crystallization to the hydrochloride in two steps with a ~55% overall yield. The two components, MOPY (I) and Cyano-FQA (II) were prepared in 7-step syntheses each, with ~25% yield and 30% yield, respectively. The synthesis of MOPY (I) started from 2-butendiol (1) and p-toluenesulfonamide to form 1-tosylpyrroline (2), which was converted into epoxide (3) by 3-chloro-perbenzoic acid. Chirality was introduced by opening the epoxide ring with (*S*)-1-phenylethylamine and retrieval of the desired diastereomere (4) by crystallization. Oxo-morpholine (5) was synthesized by acylation of (4) with chloro-acetylchloride and subsequent cyclization. (5) was reduced to (6) with a sodium borhydride bortrifluoride-THF-complex prior to de-tosylation to (7) and final hydrogenation to MOPY (I). The Cyano-FQA (II) synthesis started with fluoro-*m*-xylene (8) reacting to (9), which was chlorinated to form hepta-chloro-xylene (10) under UV-irradiation. Starting from (10) formylbenzoic acid (11) and the corresponding cyanobenzoic acid (12) were subsequently formed before cyano-benzoyl-chloride (13) resulted from reacting with thionylchloride. Esterification of (13) with β-ethyl-3-dimethylaminoacrylate (β-DAASE), reaction with cyclopropyl-amine followed by cyclization lead to (14), which after acidic ester-hydrolysis yielded Cyano-FQA (II).

Physicochemical characterisation: Chemical and physical properties of FIN (III) were determined by 1- and 2D-NMR as well as X-ray crystal analysis, using standard methods. Polymorphism studies comprised spectroscopical (IR, Raman, X-ray powder diffraction), thermal (differential scanning cloriometry, thermomicroscopy, thermogravimetry) and hygroscopical (dynamic vapor sorption) analysis of various crystallization experiments (phase equilibration, evaporation, vapor diffusion, precipitation, drying, desolvation).

***In vitro* activity:** All minimum inhibitory concentration (MICs) were performed using CLSI methodology for broth microdilution with the pH being adjusted to 7.2 or 5.8 with 1M NaOH or 1M HCl.

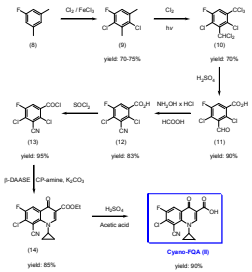
Results and Discussion

Scheme 1. Synthesis of the MOPY (I) building block.



Results and Discussion

Scheme 2. Synthesis of the Cyano-FQA (II) building block.



Physicochemical characteristics of FIN (III).

Description
white to yellowish substance

Average molecular weight
434.8545 g/mol - $C_{20}H_{19}FN_4O_4$ x HCl

Optical purity
α^{20}_D: -129° (based on dried substance)

Water content (25°C, 60% rh; 40°C, 75% rh; in PE bags)
~ 7.7% or ~2x H_2O per molecule FIN (III)

Table 1. *Solubility* (at 25±5 °C)

Solvent	mg/mL
Water	5.5
Water, pH 4.5	1.9

Table 2. *Partition coefficients (P)*

System	log P
Octanol/Water	-1.5
Octanol/pH1	-1.7
Octanol/pH7	-0.6

Table 3. *Ionisation constants* (by potentiometric titration)

	pK_a values
pK_{a1} (carboxylate function)	5.6
pK_{a2} (nitrogen at C7 substitute)	7.8

Polymorphism

FIN (III) crystallized in at least 9 modifications (2 non solvated and 7 solvated forms) with the dihydrate and an anhydrate form as the most stable and preferred variants under ambient conditions.

Scheme 3. Synthesis of FIN (III).

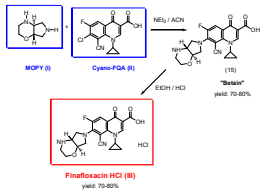
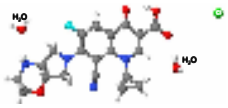
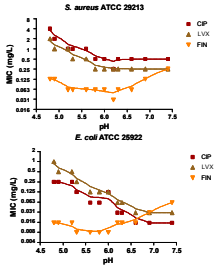


Figure 2. X-ray crystal structure of FIN (III).



Antibacterial properties of FIN (III).

Figures 3 and 4. pH dependant MIC.



pH dependent MIC: MICs of FIN (III), ciprofloxacin (CIP) and levofloxacin (LVX) were determined over a range of pH. The antibacterial activity of FIN was optimal between pH 5 – pH 6, under which the activities of CIP and LVX were significantly lower.

Table 5. Antibacterial activity of FIN (III) compared to ciprofloxacin (CIP) and levofloxacin (LVX) against a panel of pathogenic bacteria.

Organism	Strain	MIC (mg/L)					
		FIN		CIP		LVX	
		pH 7.2	pH 5.8	pH 7.2	pH 5.8	pH 7.2	pH 5.8
E. coli[1]	ATCC 25922	0.06	0.0078	0.015	0.06	0.03	0.125
E. coli[1]	ATCC 700928	0.125	0.015	0.015	0.125	0.03	0.25
E. coli[1]	ATCC 10536	0.125	0.0078	0.0078	0.03	0.03	0.125
K. pneumonia[2]	52145	0.125	0.03	0.03	0.25	0.06	0.5
P. aeruginosa[3]	ATCC 27853	8	1	0.25	1	1	2
P. aeruginosa[3]	PA01	4	0.5	0.125	0.25	0.5	1
P. mirabilis[4]	ATCC 14153	1	0.125	0.03	0.125	0.125	0.25
S. aureus[5]	ATCC 29213	0.25	0.06	0.5	0.5	0.25	0.25
S. aureus[5]	ATCC 33591	0.25	0.06	0.5	0.5	0.25	0.5
S. saprophyticus[5]	ATCC 15305	0.5	0.125	1	0.5	1	0.5
E. faecalis[6]	ATCC 29212	1	0.25	1	2	1	2

[1]*Escherichia coli*, [2]*Klebsiella pneumoniae*, [3]*Pseudomonas aeruginosa*, [4]*Proteus mirabilis*, [5]*Staphylococcus aureus*, [5]*Staphylococcus saprophyticus*, [6]*Enterococcus faecalis*.

MIC: FIN (III) showed good activity compared to CIP and LVX at pH 7.2. Overall, under acidic pH conditions, FIN (III) was superior to both competitors against a range of pathogens.

Conclusions

- A scalable synthesis of the two building blocks and the novel fluoroquinolone finafloxacin hydrochloride (FIN, III) was established.
- The chemical structure of FIN (III) and important physiochemical characteristics were determined.
- The basic antibacterial activity of FIN (III) against Gram-negative and Gram-positive pathogens was determined.
- FIN (III) exhibited excellent and overall superior antibacterial activity at low pH, demonstrating an exceptional potential to treat infections in acidic environments, such as in the gastrointestinal or urogenital tract, in abscesses, intra-abdominal infections, TB, CF and others.
- In acidic environment FIN (III) outperformed relevant reference quinolones, most likely due to its lower intrinsic basic capacity enabling a more efficient uptake into the cell at lower pH.

Literature

[1] Kresken *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2037.
[2] Goh *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2042.
[3] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No.F1-2044.
[4] Endermann *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2045.
[5] Schmuck *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2047.
[6] Patel *et al.*, 48th ICAAC, Washington DC 2008, Poster No. F1-2048.