Novel C-7 Carbon Substituted Fourth Generation Fluoroquinolones Targeting *N. Gonorrhoeae* Infections

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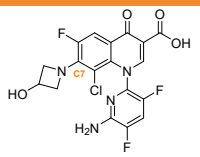
INTRODUCTION

Fluoroquinolones (FQ) are a first-line antibacterial therapy, used to treat both Gram-negative and Gram-positive bacterial infections. FQ efficacy is achieved by disrupting the bacterial enzyme's DNA gyrase and topoisomerase IV. There is extensive SAR analysis of this class of antibiotics - both in terms of microbiological activity[1-3] and target related toxicity. Delafloxacin, a fourth generation FQ has broad spectrum activity and reduced FQ related toxicity[4-5]. However, it has recently failed a Phase 3 clinical trial for treatment of *N. gonorrhoeae* infections. This was due to lack of sufficient efficacy at the dose administered[6].



Delafloxacin

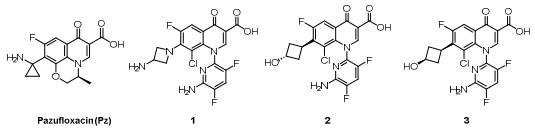
HYPOTHESIS AND DISCLOSURE

► The PK/PD of the FQ class is established and efficacy is known to be driven by the fAUC/MIC[7]. Despite a low MIC_{90} values vs *N. gonorrhoeae* of 0.125 µg/mL[8] , Delafloxacin has a low fAUC/MIC ratio relative to other FQ's. This is in part due to its PPB profile and its low oral bioavailability[9].

► Increased FSP[3] and p*Ka* attenuation are known strategies for modulating solubility and PK profiles[10-11]. Given the extensive literature precedence of fluoroquinolone SAR, new investigative FQ's are dominated by contemporary cyclic amines at the C-7 position. A less exploited strategy is to replace the C-7 nitrogen for a carbon linker. Of which, there is only one marketed FQ, Pazufloxacin (Pz, see image below). Hitherto there are no **direct** comparisons between Nitrogen-for-Carbon linked FQ's. A Nitrogen-for-Carbon swap with Delafloxacin is anticipated to change the physicochemical properties of the molecule by attenuating the conjugation throughout the quinolone core, specifically reducing the relative electron distribution around the β-keto acid moiety and thus potentially modulating the acid p*Ka*, which could allow for differentiated bioavailability and salt formulations.

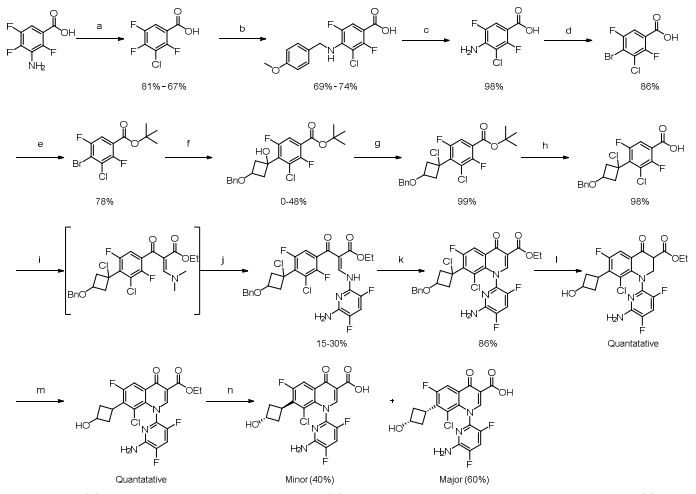
► We disclose the first **direct** Nitrogen-for-Carbon swap at the C7 position of a known FQ, Delafloxacin (see image below) with biological and ADMET profiles. The N-for-C swap in Delafloxacin gives rise to two stereoisomers (**2** and **3**, see below), with distinct electrostatic vectors - which can impact physchem properties and target engagement.

► In addition, an amino-azetidine analogue **1** was synthesised and profiled. As anticipated, compound **1** (zwitterionic) proved to have significant physchem differences from Delafloxacin (anionic). Compound **1** does not feature in any peer-reviewed articles, and is reported in only one patent[12] .



Pazufloxacin (Pz) **1** **2** **3**

SYNTHESIS



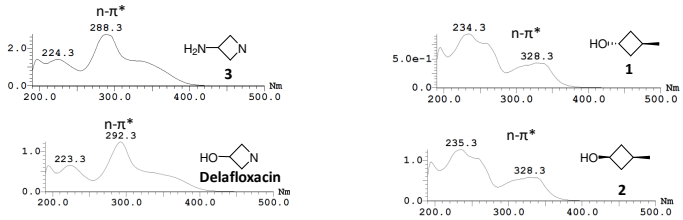
Reagents and conditions: (a) *tert*-Butyl nitrite, CuCl, MeCN, 60°C, 1 hr; (b) para-methoxybenzyl amine, NEt_3, DMSO, 90°C, 18 hrs; (c) TFA, 60°C, 1 hr; (d) *tert*-butyl nitrite, $CuBr_2$, MeCN, 60°C, 30 mins; (e) $(BOC)_2O$, DMAP, *t*-butanol, 60°C, 2 hrs; (f) ^{i}PrMgBr, 3-benzoxycyclobutanone, 2-MeTHF, -20°C – 0°C, 5 min; (g) $SOCl_2$, DCM, 40°C, 2hrs; (h) 4M HCl in dioxane, 70°C, 6 hr; (i) Oxalyl chloride, DCM, DMF, RT, 1 hr then Ethyl 3-(N,N-dimethylamino)acrylate, DIPEA, toluene, 70°C, 1 hr; (j) 2,6-Diamino-3,5-difluoropyridine, 80°C, 2 hr; (k) NaH, THF, 0°C - RT, 2 hrs; (l) H_2 (9 bar), 10% Pd/C, toluene, MeOH, 1.5 hr; (m) DDQ, dioxane, reflux, 30 min; (n) NaOH, EtOH, RT. Separation of isomers achieved by preparative HPLC.

► 14 step total synthesis (4.72% overall yield) involving a diverse range of chemistry including:
► Controlled Grignard step with a poly-halogenated aryl ring directs alkylation and prevents benzyne formation (step f).
► Tertiary alcohol cannot be removed by hydrogenation, TES/TFA, Barton deoxygenation conditions or mesylate activation/displacement.
► Tertiary alcohol is only displaced with $SOCl_2$ upon heating (step g), which is subsequently removed via hydrogenation (step l).
► Desired isomeric targets (**2** and **3**) were separated by preparative HPLC and each isomer characterised by ^{1}H NMR/^{13}C NMR, NOSEY and UPLC.

REFERENCES AND ACKNOWLEDGEMENTS

1) D. T. Chu et al., R. V. Chari *et al.*, *Antimicrobial Agents and Chemotherapy,* 1989, **33(2)**: 131–135. 2) V T. Andriole *Clinical Infectious Diseases*, 2005, **41(2)**: S113–S119. 3) S. Balasubramaniyan *et al., RSC Adv.*, 2018, **8**, 23629-23647. 4) T. Lodise *et al., Open Forum Infect. Dis.* 2018; **5(10)**. 5) S. R. Dawe et al., *Photochem Photobiol Sci.* 2018; **17(6)**:773-780. 6) *Sex Transm. Dis.* 2019 Jan 19. doi: 10.1097/OLQ.0000000000000971. 7) I. Elisabet et al., *Antimicrobial Agents and Chemotherapy,* 2011, **55(10)**: 4619–4630. 8) O. Olusegun *et al.*, *Antimicrobial Agents and Chemotherapy,* 2016, **60(5)**: 3106-3111. 9) S. Jorgensen *et al.*, *Infect. Dis. Ther.,* 2018, **7(2)**: 197–217. 10) F. Lovering *et al., J. Med. Chem.*, 2009, **52(21)**, 6752-6756. 11) S. D. Fried *et al., Annu. Rev. Biochem.*, 2017 **20(86)**: 387–415. 12) Y. Akira *et al.*, Patent number EP0911327, 1999, Wakunaga Pharma Co Ltd. 13) R. Aura *et al., Acta Medica Marisiensis*, 2015, **61(4)**:328-336. Our appreciation goes to John Moat from the University of Warwick for microbiological profiling, Michael Morton from Apconix for ion channel assays and to Andrew Kennedy from Pion for coordinating p*Ka* measurements.

UV PROFILING



► Compounds **2** and **3** UV in acidic media are differentiated from traditional fluoroquinolones with a blue shift for the n-π* transition of the β-keto moiety[13] – indicative of a lower electron density around this region. As a result, the p*Ka*'s of compounds **2** and **3** are anticipated to be lower than Delafloxacin.

MICROBIOLOGICAL PROFILING

		Microbiological activity (µg/mL)†			
	Strain\Compound	**1**	**2**	**3**	**Delafloxacin**
Gram -ve	*N.gonorrhoea* ATCC 49226	0.003	0.01	0.06	0.06
	N.gonorrhoea (WHO L)	0.06	0.12	0.12	0.03
	A.baumannii ATCC 19606	2	64	16	8
	P.aeruginosa PA01	0.12	2	4	1
	K.pneumoniae ATCC 700603	0.25	8	4	2
	E.coli ATCC 25922	0.001	0.12	0.12	0.01
Gram +ve	*S.aureus* ATCC 29213	0.003	0.03	0.03	0.003
	E. faecalis ATCC 29212	0.12	0.5	0.25	0.03

† All minimum inhibitory concentrations (MICs) performed with a biological repeat to Clinical Laboratories Standards Institute (M7-A9). Media used for MIC was cation-adjusted Muller Hinton broth.

► Compounds **2** and **3** have similar microbiological profiles within experimental error across all strains.
► Compound **2** and **3** have a similar microbiological profile to Delafloxacin across all strains.
► Compound **1** has the lowest MICS across all strains, including the clinically relevant MDR *N.gonorrhoea* (WHO L) strain.

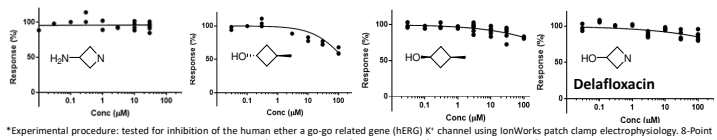
PHYSCHEM AND ADME PROFILING

Compound ID	R1	$logD_{7.4}$	KSOL	HPPB	HLM	HHeps	MPPB	MLM	CYP450 inhibition**	P*Ka*
			µM	%Fu	Clint (µL/min/mg)	Clint (µL/min/10 6 cells)	%Fu	Clint (µL/min/mg)	µM	
1		-0.3	3.6	29.7	5	3	7.0	8.2	>50, >50, 12.7, >50, 13.6	5.33, 7.72
2		0.3	59.3	10.5	5	6.8	1.3	16.3	>50, >50, 41.7, >50,>50	5.04
3		0.3	44.8	12.4	5	9.2	0.5	5	>50, >50, >50, >50, 24.8	4.96
Delafloxacin		0.7	58.1	14.9	5	3	0.9	23.7	>50, >50, >50, >50,>50	5.47

**CYP inhibition values in order as follows: 1A2, 2C19, 2C9, 2D6, 3A4

► $LogD_{7.4}$ measurement of **2** and **3** show a 0.4 log unit drop compared to Delafloxacin - indicating that introducing a heteroatom for a non-polar atom results in a compound that is counterintuitively less lipophilic. P*Ka* measurements confirm this observation, with p*Ka* values of 5.33 (7.72), 5.04, 4.96 and 5.47 for compounds **1**, **2**, **3** and Delafloxacin respectively.
►Compound **1** shows poor kinetic solubility and inhibits CYP2C9 and CYP3A4 at 12.7 and 13.6 µM respectively but has a differentiated PPB profile.
► Cyclobutanol isomers **2** and **3** display different CYP inhibition profiles.

CARDIOTOX LIABILITY PROFILING (hERG IC_{50})



*Experimental procedure: tested for inhibition of the human ether a go-go related gene (hERG) K+ channel using IonWorks patch clamp electrophysiology. 8-Point concentration-response curves were generated using three-fold serial dilutions from a maximum final test concentration of 100mM.

► Compound **1** does not confer any hERG liability at 100 µM. Cyclobutanol isomers **2** and **3** display different hERG inhibition profiles at the concentrations tested.

SUMMARY

Physchem: Compounds **2** and **3** show a 0.4 log unit drop in $LogD_{7.4}$ compared to Delafloxacin, which may be explained by their reduced electron density in the quinolone ring. This hypothesis was confirmed by UV profiling and p*Ka* measurements and may aid future formulations of analogous molecules.
Microbiology: Compounds **2** and **3**: Replacing the C-7 linked azetidinol for a cyclobutanol is well tolerated in terms of microbiological activity. Compound **1** is more active against gram-negative strains compared to Delafloxacin due to it's zwitterionic physchem properties.
ADME: Replacing the C-7 linked azetidinol for a cyclobutanol is well tolerated in terms of *in-vitro* ADME profiling. Although there is a difference in CYP450 inhibition profiles.
Cardiotox: The hERG inhibition profiles need to be assessed at higher concentrations although there is qualitative difference between the isomers.