

F1-1975

AU-FQ antibacterial compounds: activity against Gram+ organisms and development of an emulsion formulation.

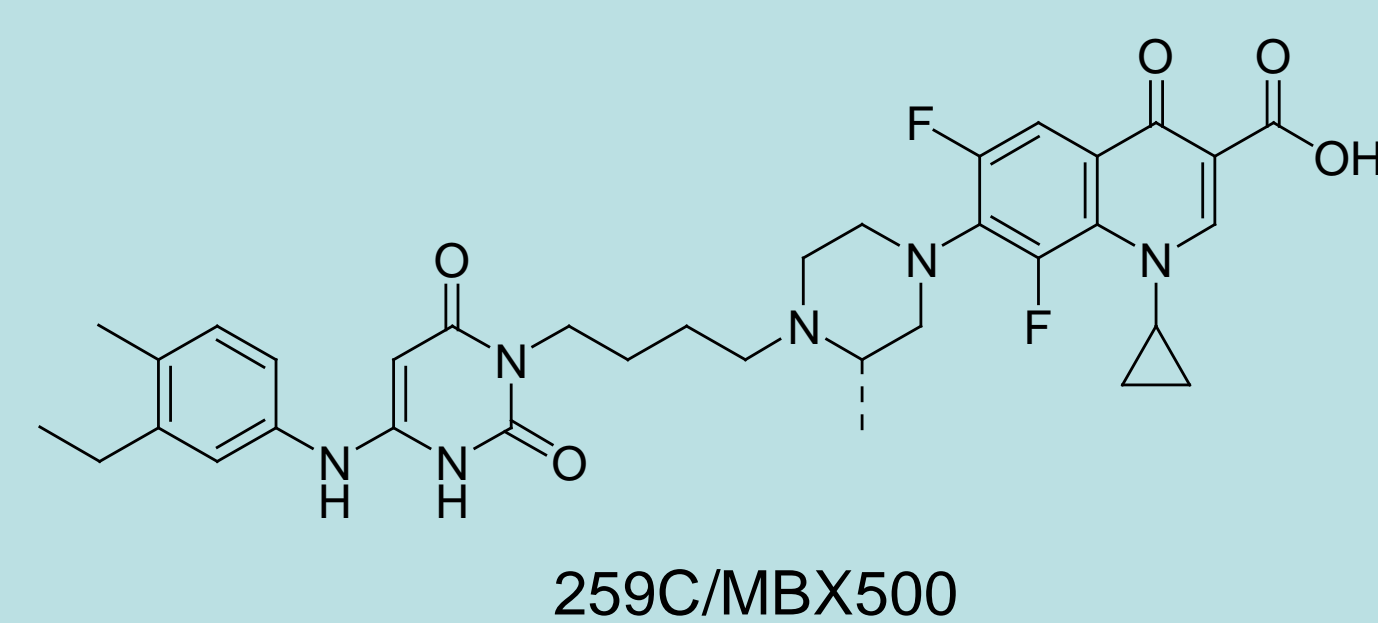
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ABSTRACT

- Background:** "AU-FQ" hybrid antibacterials - 6-anilinoauracils connected via the 3 position to the 7-piperazinyl group of fluoroquinolones - are potent antibacterials *in vitro* and *in vivo*, and show promise as parenteral treatments for multiantibiotic resistant Gram+ infections. The preclinical candidate 259C, aka MBX500 (see structure), has posed delivery challenges because of its high dose and its pH-dependent solution stability. The product profile requires a dosage form that is easy to use parenterally and stable under standard storage conditions. These challenges have been addressed by the development of F-20, a stable, pH-neutral, detergent-free, and lyophilized formulation.
- Methods:** 259C was synthesized by a proprietary method. Screening against Gram+ clinical isolates was done by Micromyx LLC, Kalamazoo, MI. Animal studies employed Swiss-Webster mice and Fischer rats, and analyses of parent compound and metabolite in plasma were done by LC-MS. Intravenous emulsions were rationally developed using a unique Nano-E™ emulsion technology, and analyzed by laser light scattering, microscopic evaluation, pH measurements, and HPLC.
- Results:** 259C was an effective antibacterial with MIC₉₀ values of 0.12-4 µg/mL against multiple clinical isolates of 18 Gram+ strains. Experimental emulsions of 259C up to 8 mg/mL were prepared and analyzed for particle size and stability. The best emulsion "F-20" was stable for at least 6 months in liquid form at 2-8 °C and under accelerated conditions as lyophilized powder (30°C/70% RH). Analysis of plasma of IV-dosed rats revealed complex elimination of the parent compound and conversion to a single major metabolite, likely the carboxyl glucuronide.
- Conclusions:** Successful development of the F-20 emulsion formulation for 259C has enabled GLP preclinical toxicity studies in rats to be initiated. The emulsion does not adversely affect efficacy, pharmacokinetics or metabolism of 259C in the mouse or rat. The liquid emulsions were stable for at least 6 months at 2-8°C, and the lyophilized versions, after reconstitution, were stable up to 6 months.

BACKGROUND



259C/MBX500 is the lead preclinical candidate from a group of hybrid antibacterials, consisting of a DNA polymerase III inhibitory "EMAU" moiety (left hand) covalently bound to a "fluoroquinolone, FQ" antibacterial moiety via the 7-cyclic amine function (right hand). Members of this family of antibacterials are potent inhibitors of DNA polymerase III from Gram+ bacteria, and weak inhibitors of topoisomerase/gyrase. It is believed that the compounds owe their potent antibacterial activity to facilitation of uptake in sensitive organisms by the FQ moiety, rather than to dual target activity – for example, the hybrids are equally active against cipro-resistant and sensitive organisms (Table 1).

Antibacterial activity

Gram-positive spectrum: 259C/MBX500 is a potent and selective inhibitor of Gram-positive aerobic bacteria. Table 1 summarizes the MIC₅₀ and MIC₉₀ values of the compound against 306 clinical isolates, and the effect of comparators vancomycin, linezolid, daptomycin, ciprofloxacin and oxacillin against the same organisms. MIC₅₀ values of 259C varied from 0.06-2 µg/mL and MIC₉₀ values varied from 0.12-4 µg/mL. Strains that were resistant to comparators were uniformly sensitive to 259C.

Organism	Phenotype	# isolates	MIC ₅₀ , MIC ₉₀ (µg/ml)					Oxacillin
			259C	Vanco	Linezolid	Dapto	Cipro	
<i>S. aureus</i>	MSSA	25	0.5,1	1,1	4,4	1,1	0.5,1	0.2,0.5
	MRSA	25	2,4	1,1	2,4	1,1	>32,>32	64,>64
	CA-MRSA*	25	0.5,2	1,1	2,2	0.5,1	0.5,>32	32,>64
<i>S. epidermidis</i>	MSSE	25	1,2	2,2	1,2	0.5,1	0.25,>32	≤0.06,0.12
	MRSE	25	2,4	2,2	1,2	0.5,1	>32,>32	64,>64
<i>E. faecalis</i>	VSE	15	2,4	1,2	1,2	2,4	>32,>32	>64,>64
	VRE	10	2,2	>64,>64	1,1	4,4	>32,>32	>64,>64
<i>E. faecium</i>	VRE	15	2,4	1,1	2,2	8,8	>32,>32	>64,>64
	VRE	10	2,4	>64,>64	2,2	4,8	>32,>32	>64,>64
<i>E. faecalis/faecium</i>	LRE†	11	2,4	>64,>64	16,32		>32,>32	
<i>S. pneumoniae</i>	PSPP	10	1,2	0.5,0.5	0.5,1	0.25,0.5	1,2	≤0.06,0.25
	PIPP	10	0.5,1	0.25,0.25	0.5,1	0.12,0.25	0.5,1	2,4
"	PRSP	10	0.5,1	0.25,0.5	0.5,1	0.12,0.25	0.5,2	8,8
		25	0.06,0.12	0.5,0.5	1,1	0.06,0.12	0.12,2	≤0.06,≤0.06
<i>S. pyogenes</i>		25	1,1	0.5,0.5	1,1	0.25,0.5	1,1	0.5,0.5
<i>S. agalactiae</i>		25	1,1	>64,>64	8,16			≤0.008,0.015
<i>H. influenzae</i>		20	1,1	>64,>64	8,16			≤0.03,≤0.03
<i>M. catarrhalis</i>		20	0.5,1	64,>64	4,4			

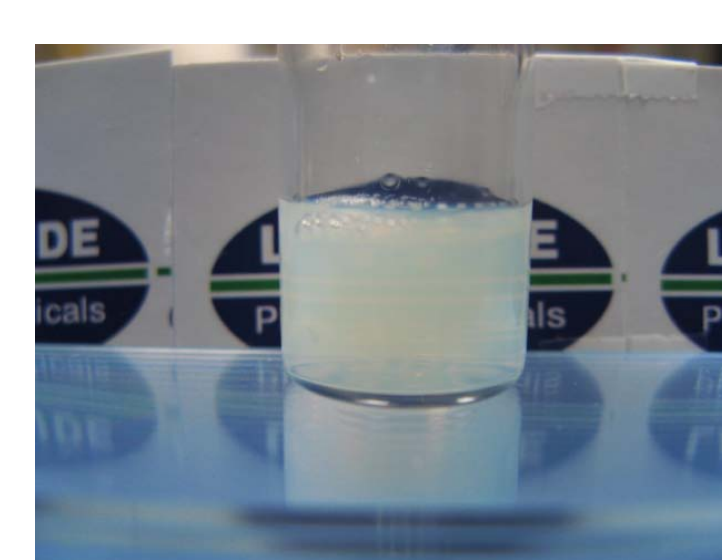
*community acquired MRSA. †linezolid resistant Enterococci.

Emulsion development

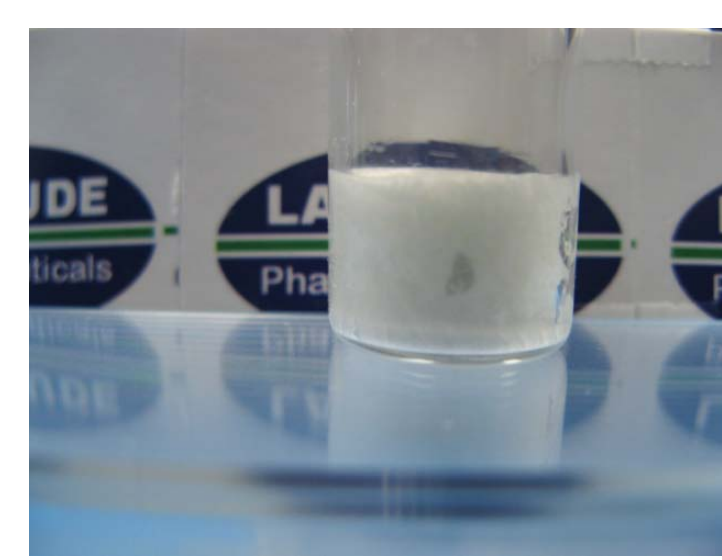
A stable intravenous nanoemulsion formulation for 259C (F-20, Table 2) was developed using proprietary Nano-E™ technology, which is especially suitable for formulating highly insoluble small molecules, peptides and proteins. All components of the Nano-E™ system are GRAS listed and of compendial grade. A Nano-E™ emulsion is a pH neutral, semi-transparent and lyophilized emulsion with an average droplet size below 200 nm allowing for sterilization by filtration.

Components	Vehicle	6 mg/g	8 mg/g
	% (w/w)	% (w/w)	% (w/w)
259C	0	0.6	0.8
Soybean oil, super-refined	7	7	7
Miglyol 812 (medium chain triglycerides)	7	7	7
Phospholipon 90G (soy lecithin)	7	7	7
Sodium oleate*	0.3	0.3	0.3
Sucrose	15	15	15
NaOH and HCl (for pH adjustments)	8.0-8.2	8.0-8.2	8.0-8.2
Water-for-Injection	QS	QS	QS
Total	100	100	100

F-20 has achieved a high drug load to about 8 mg/mL for the highly insoluble 259C. Both liquid and lyophilized forms have been prepared (see Figures). The lyophilized form can be reconstituted with water for injection.



Liquid emulsion



Lyophilized emulsion

Emulsion stability

F-20 stability parameters include appearance (visual and microscopic), pH, droplet size (see Table 3), assay (see Table 4) and chromatographic purity. No changes were observed in appearance and pH after 6 months of the liquid emulsion at 2-8 °C or of the lyophilized emulsion up to 25 °C.

Table 3. Average droplet size by laser light scattering (RH, relative humidity).

Sample	Storage condition	Initial	1 week	2 weeks	3 months	6 months
Liquid F-20	2-8°C ambient RH	71.3	73.2	93.5	76.9	77.1
	25°C 60% RH		79.2	83.3	90.8	105
	2-8°C ambient RH		168	127	120	138
25°C 60% RH	125	121		141	163	

Table 4. 259C concentration by HPLC.

Sample	Storage condition	Initial	1 week	2 weeks	3 months	6 months
Liquid F-20	2-8°C ambient RH	8.0	7.8	8.0	7.8	7.9
	25°C 60% RH		7.7	7.7	7.2	7.1
Lyophilized F-20	2-8°C ambient RH	7.8	8.2	8.2	7.9	7.9
	25°C 60% RH		8.0	8.1	7.9	7.6

The Nano-E™ technology has proven to be an excellent delivery method for the insoluble 259C. The F-20 emulsion is free of any toxic, irritating, allergenic or non-approvable ingredient, and the F-20 vehicle has shown a superior local and systemic safety profile with no adverse toxicity observed in animal studies. F-20 demonstrated good physical and chemical stability at 2-8 °C, and it can be freely diluted with 5% dextrose solution to allow for dose and infusion rate adjustment. The manufacturing process for F-20 has been scaled to kilogram quantity.

Efficacy and toxicity in mice

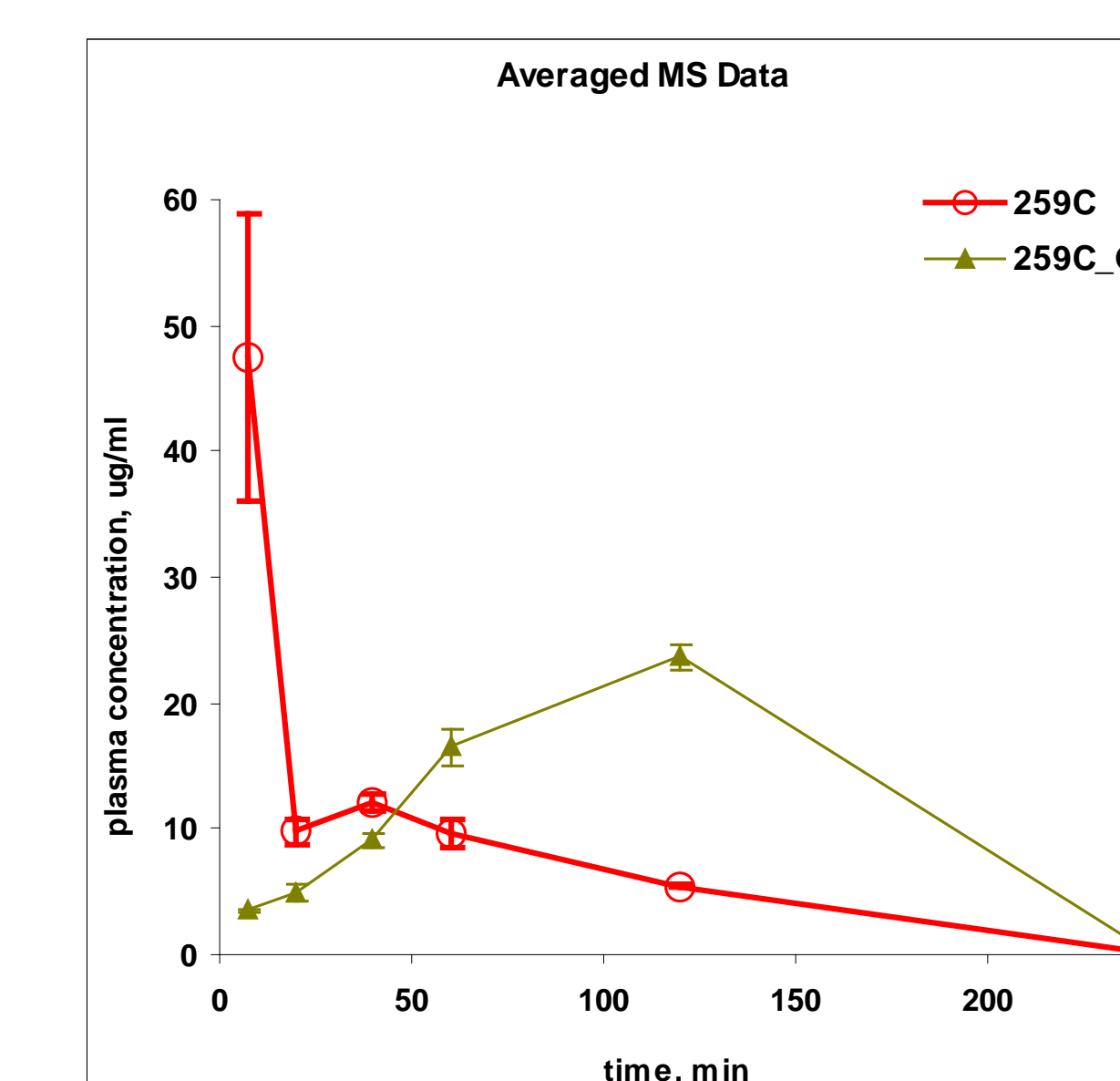
The F-20 emulsion formulation enabled delivery of 259C via the tail vein to Swiss-Webster mice to determine antibacterial efficacy against IP infection with *S. aureus* (Smith) and to determine acute toxicity. Efficacy and toxicity were similar to those obtained with the artificial vehicle "DCP" (Table 5), but without the severe thrombophlebitis observed at the site of injection with the latter vehicle.

Vehicle	ED ₅₀ † mg/kg	LD ₅₀ mg/kg
DCP	16	220
F-20	18	200

* via tail vein. † Sa(Smith) IP infection. DCP is 10% dimethylacetamide, 10% Cremophor in PBS, pH ca. 9.5.

Pharmacokinetics and metabolism in rats

The 259C emulsion was given as a bolus injection of 40 mg/kg in the tail vein of Sprague-Dawley rats. Blood samples were removed at the indicated times from groups of 6 rats. The plot shows the average plasma concentration of 259C obtained from LCMS analysis vs. time. A major metabolite, the glucuronide 259C_G, independently confirmed by MS, is seen with peak plasma concentration at ca. 120 min.



PK parameters were derived from WinNonLin using a one compartment model (Table 6). Results were similar to those from dosing of 259C in DCP or in 5% DMSO in saline, pH 10.2, via jugular vein catheters (not shown).

Parameter	Value	StdError	CV%
t _{1/2 β}	40.9 min	21.2	52
Cl	0.0273 L/min	0.0096	35.3
V _{ss}	1.61 L/kg	0.53	33.4
PK parameters for 259C_G			
C _{max}	25.0 µM	2.49	10.0
T _{max}	120 min		

Conclusions

- 259C is a broad, Gram+ spectrum antibacterial with no cross-resistance to current antibiotics.
- The F-20 emulsion formulation enables IV dosing of 259C without irritation at the SOI.
- The F-20 emulsion does not adversely affect the efficacy of 259C in mice.
- The F-20 emulsion does not alter the PK properties of 259C in the rat.

GLP preclinical tox/path and safety pharmacology studies are underway with 259C in F-20 emulsion at 8 mg/ml.

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