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In Vitro and *In Vivo* Characterization of Non-Hydroxamate LpxC Inhibitor FG-630

Min Teng*, Konstantin Taganov*, Baskar Nammalwar*, Xiaoming Li*, David Corbett#, Alastair Parkes#, Beth Williamson#, Lloyd Payne#, Swapna Vaddi #, Peter Warn# & David Puerta*

*Forge Therapeutics Inc., San Diego, CA; #Evotec (UK), Manchester, United Kingdom

Abstract

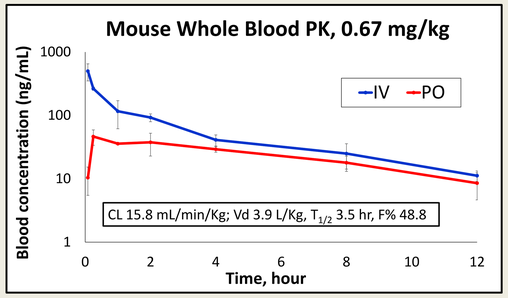
- **Background:** In an effort to identify a novel class of antibiotic for the treatment of infection by Gram-negative bacteria, the antimicrobial activity, pharmacokinetic profile and *in vivo* efficacy in murine models of *E. coli* infection were evaluated for the non-hydroxamic acid LpxC inhibitor FG-630.
- **Results:** FG-630 demonstrated an MIC against *E. coli* (ATCC 25922) of 1.0 µg/mL (4.0 µg/mL with 50% of FBS, 2.0 µg/mL with 20% rat urine). FG-630 showed activity against *K. pneumoniae* and other *Enterobacteriaceae,* including clinical isolates harboring plasmids containing the resistance genes mcr-1, ESBL, KPC, and NDM. FG-630 showed MIC of >128 µg/mL against *S. aureus*.
- FG-630 displayed favorable PK parameters in mice with CL of 15.8 mL/min/Kg; Vd of 3.9 L/Kg, $T_{1/2}$ of 3.5 hr and oral bioavailability of 49% when dosed at 0.67 mg/kg. When dosed at 60 mg/kg, a sustained urine exposure was evident, with a total of 3.8% of dose for IV and 2.1% of dose for PO over 12 hr, rendering a 55% oral bioavailability in urine.
- In an murine IP sepsis model, comparing with vehicle control 9 hr post infection, FG-630 (60 mg/kg, IV) and Tigecycline (20 mg/kg, IV), dosed at 1 hr and 5 hr post infection, reduced Log_{10} CFU/mL counts of MDR *E. coli* (BAA-2469) in the abdomen by 5.55 and 5.88, respectively.
- In a 5-day murine UTI infection model, FG-630 (60 mg/kg, q12h BID) and Ciprofloxacin (10 mg/kg, q12h BID) reduced susceptible *E. coli* (UTI98) Log_{10} CFU/mL counts in the kidneys by 3.91 and 3.01, respectively, the bladder by 3.18 and 2.45, respectively and the urine by 1.44 and 3.92, respectively. It is worth noting that at 15 mg/kg, FG-630 reduced bladder Log_{10} CFU/mL counts by 2.38.
- **Conclusion:** FG-630 demonstrated *in vitro* antimicrobial activity against WT and MDR *Enterobacteriaceae* and favorable PK properties in mice. FG-630 significantly reduced CFU burden in the abdomens, kidneys, bladders and urine of mice in models of MDR IP sepsis and UTI.

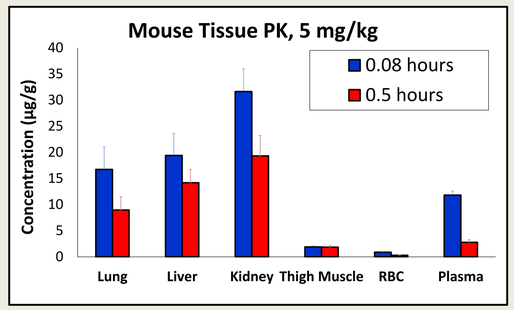
Introduction

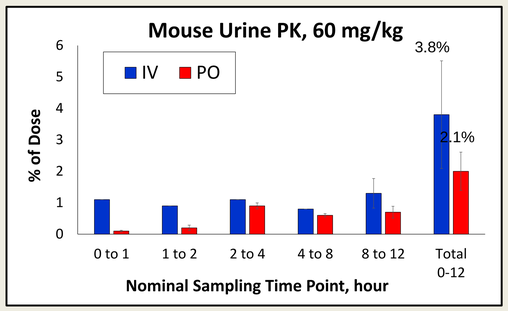
- The rise of drug-resistant Gram-negative (GN) infections caused by the ESKAPE pathogens *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species is an increasing danger to worldwide health.
- UTIs are one of the most common infections worldwide. *Enterobacteriaceae* are the main organisms associated with UTIs with *Escherichia coli* responsible for 60-80% of said infections.
- Fluoroquinolones (FQs) have been used as a mainstay for UTI treatment due to their oral bioavailability and deposition in the urinary tract tissues. However, there is a clear unmet need for novel oral antimicrobial agents due to the increasing levels of resistance to FQs and other antibiotics used to treat UTIs.
- LpxC (UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase) is a Zn(II) containing metalloenzyme that catalyzes the first committed step of lipid A biosynthesis required for virtually all GN bacteria. LpxC is an attractive target as it is found in nearly all GN bacteria, does not have a human homologue and its inhibition is cidal to most GN bacteria.
- The vast majority of LpxC inhibitors reported over the past 20 years utilize a hydroxamic acid metal binding pharmacophore which is a less than optimal therapeutic moiety due to known liabilities, including poor metabolic stability and/or limited oral bioavailability. Using our proprietary metalloenzyme platform, we have identified non-hydroxamate small molecule inhibitors of LpxC with antimicrobial activity against a broad panel of GN bacteria, including MDR strains.
- The non-hydroxamate LpxC inhibitor FG-630 demonstrated *in vivo* stability, sustained urine presence, rapid and broad organ distributions, and good oral bioavailability.
- Presented herein are the *in vitro* and *in vivo* characterization of FG-630, including results from whole blood, tissue and urine PK, mouse IP sepsis and UTI efficacy models.

DMPK/ADME Profile of FG-630

Properties	
MW	490
IC_{50} *E. coli*	45 nM
ΔT *E. coli* [a]	11
eLogD	1.96
mPPB fu (%)	0.93
CACO-2[b] P_{app} A-B/B-A	1.6/5.7
m/hHep[c]	19/4.1







FG-630 *in vitro* Antimicrobial Activity

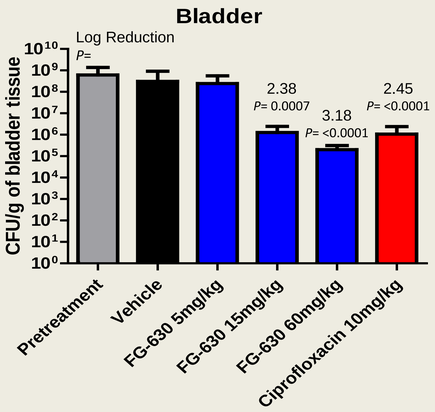
MIC (µg/mL)	
E. coli ATCC 25922 (50% FBS)	1 (4)
E. coli BAA-2469	1
K. pneumoniae ATCC 13883	8
E. cloacae ATCC 13047	2
P. aeruginosa PAO1	128
P. mirabilis ATCC 21100	>128
S. aureus ATCCC 29213	> 128

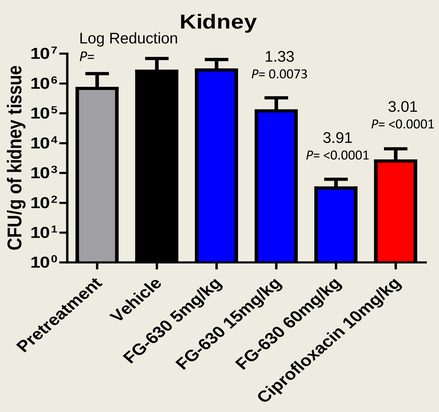
Activity FG-630 against a panel of Gram-negative and Gram-positive bacteria;
FBS = fetal bovine serum; MICs for levofloxacin are from *Antimicrobial Agents and Chemo.*, **2012**, 5103–12; *J Clin. Micro.*, **2010**, 2601–04. Additional MICs for comparator antibiotics were provided by ATCC or the CDC.
a, Thermal shift with *E. coli* LpxC, ºC;
b: 10^{-6} cm/s;
c: uL/min/million-cells

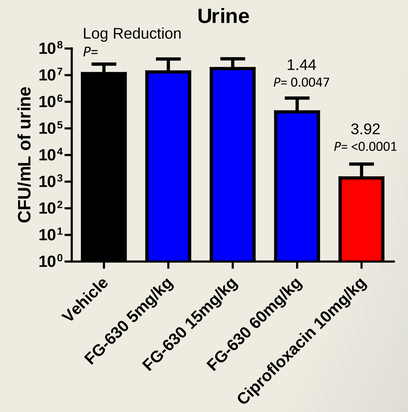
MIC (µg/mL)	BANK #	Resistance*	FG-630	LVF*
Shigella sonnei	30	KPC-, NDM-	4	<0.25
Salmonella typhimurium	31	KPC-, NDM-	4	<0.25
Klebsiella Oxytoca	71	CRE (-)	16	0.25
Citrobacter freundii	116	KPC-2	4	> 8
Serratia marcescens	122	SME	2	<0.25
Kluyvera ascorbata	144	KPC	4	4
Enterobacter cloacae	163	KPC, CTX-M-1	32	> 8
E. coli UTI89 (with 20% rat urine)	-	None	1 (2)	0.03 (0.125)

MIC in µg/mL	*E. coli* CDC AR Bank strains (41)			*E. coli* UTI isolates (100)		
	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
FG-630	1	2	0.5-2	2	4	1-8
Levofloxacin*	>8	>8	≤0.12 - >8	> 8	> 8	≤0.12 - >8

FG-630 in Murine UTI Study







Activity FG-630 in a mouse UTI model due to *E. coli* UTI89.

Bar chart indicate the CFU/mL tissue homogenate or mL urine following 3 days treatment.

The numbers and *p* value are the Log10 CFU/mL reduction in burden compared to vehicle treated mice and the statistical comparison compared to vehicle

Methods and Materials

MICs: Determined by CLSI M7-A10

Strains: A wide range of clinical and culture collection isolates recovered from worldwide sources including MDR strains expressing mcr-1, ESBL, BLA_{KPC}, and $BLA_{NDM-1,}$ resistance to aminoglycosides and fluoroquinolones.

UTI model:

Preconditioning: 5 days 5% glucose in drinking water

Mouse Strain: C3H/HeNR female 20-25g (8 mice per group)

Infection: Transurethral infection with ~$3.9x10^8$ CFU/mouse, *E. coli* UTI89

Treatment: Initiated 24h post infection. 5, 15, or 60mg/kg/dose IV FG-630 q12h BD for 3 days. Ciprofloxacin 10mg/kg/dose q12h IV BD for 3 days

Endpoints: Urine, bladder and kidney were harvested at 24h (pretreatment group) and 96h post infection and quantitatively cultured

IP sepsis:

Mouse Strain: ICR (CDI) male 25-30g (6 mice per group).

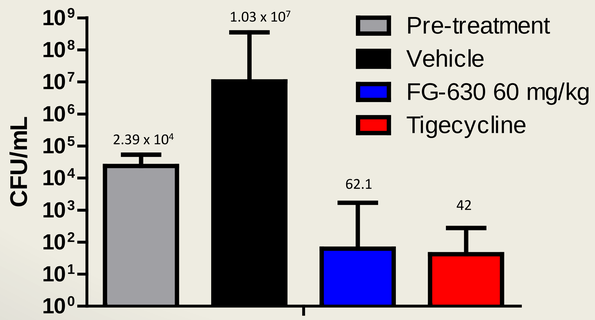
Infection: Bacterial suspension administered IP in 5% hog mucin ~$3.7x10^4$ CFU/mouse, *E. coli* ATCC BAA 2469

Pain Relief: Buprenorphine 0.03mg/kg SC

Treatment: Administered IV 1h and 5h post infection, vehicle, FG-630 60mg/kg/dose, or Tigecycline 20mg/kg/dose

Endpoints: IP wash at 1h (pretreatment group) and 9h post infection quantitatively cultured

IP Sepsis study



Bar chart of the geometric mean CFU/mL recovered from IP wash 9h post challenge with *E. coli* ATCC BAA 2469 compared to pre-treatment burden
Treatment with FG-630 60mg/kg/dose resulted in highly significant reductions in burden compared to vehicle (p=0.0001) and was as effective as Tigecycline 20mg/kg/dose

Summary and Conclusions

- FG-630 demonstrated *in vitro* activity and spectrum against a variety of Gram-negative bacteria, including clinical isolates harboring plasmids containing the resistance genes mcr-1, ESBL, KPC, and NDM.
- FG-630 was rapidly distributed into lung, liver and kidney upon dosed at 5 mg/kg, and displayed sustained level of exposure into urine upon dosed at 60 mg/kg to mice. Upon dosed orally, FG-630 showed 49% and 55% bioavailability in whole blood and urine, respectively.
- In the mouse IP sepsis model, FG-630 reduced MDR *E coli* Log_{10} CFU/mL counts in the abdomen by 5.55 when dosed at 60 mg/kg BID, which was comparable to 5.88 of Tigecycline (20 mg/kg, BID).
- In mouse UTI model, FG-630 reduced Log_{10} CFU/mL count in kidney and bladder by 3.91 and 3.18 when dosed at 60 mg/kg BID, which surpassed Ciprofloxacin at 10 mg/kg. The bacterial burden in urine was reduced to 1.44 log unit.
- We continue to optimize FG-630 for antibacterial spectrum and physiochemical properties with the aim of developing the first new class of Gram-negative antibiotic in decades.

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