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Discovery of Novel Dual-Target Topoisomerase Inhibitors Effective as Broad-Spectrum Agents: ADME and Anti-Microbial Efficacy

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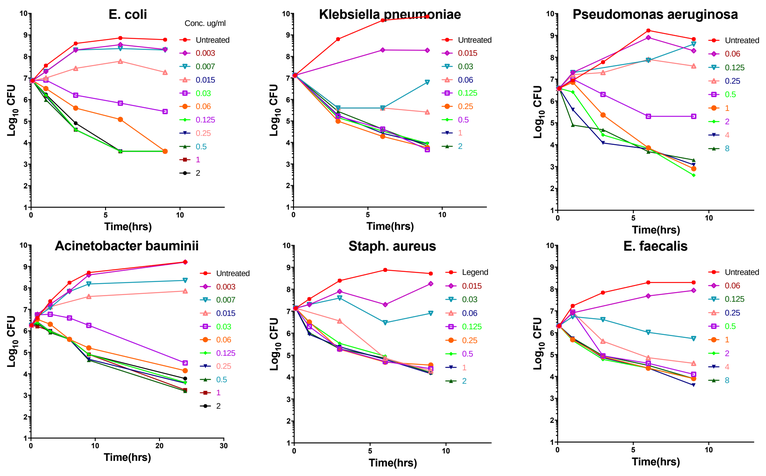
Background

GYROX is a novel potent broad spectrum antibacterial lead series. These compounds were assessed for their in vitro kill kinetics, in vitro Absorption, Distribution, Metabolism and Elimination, in vivo pharmacokinetics [PK] and efficacy in mouse models of infection.

Methods

In vitro kill kinetic assays were performed in microtitre plates over a period of 8 hours. LogD was measured using the Octanol:Buffer partitioning assay. Solubility was performed using the kinetic assay protocol. Plasma protein binding was done by the rapid equilibrium dialysis method. Intrinsic clearance in mouse, rat and human liver microsomes, and in rat and human hepatocytes, was estimated using the percent parent remaining method over a period of 30 minutes. Cytochrome P450 inhibition was estimated for human CYP1A2, 2D6, 3A4, 2C9, 2C19 in pooled liver microsomes using appropriate reference inhibitors. Intravenous and oral PK studies were performed in BALB/c mice and Wistar rats. Compound concentrations were quantified in plasma by LC/MS/MS. PK parameters were estimated using Noncompartmental methods in WinNonlin. Efficacy was evaluated in the mouse neutropenic thigh infection [MTI] and septicemia models [SM] with *E.coli* [ATCC25922].

In vitro Killing Kinetics



- Compound E, representative of the series, showed time dependent killing of ESKAPE bacteria
- Other compounds in the series (data not shown) yield similar time dependent killing kinetics

In vitro ADME properties

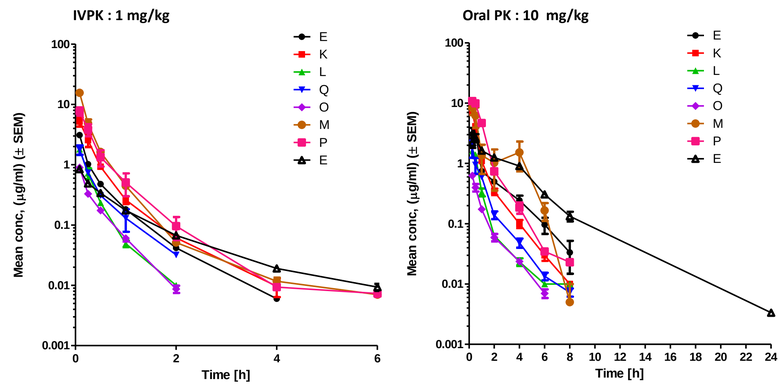
Cpd	elogD	Aq. solubility pH 7.4 (µM)	Human Hepatocyte CLint (µL/min/10^6 cells)	Rat Hepatocyte CLint (µL/min/10^6 cells)	Rat PPB [% free]	Human PPB [% free]
E	0.9	>500	7.3	4.2	ND	12.3
L	1.65	72	9	7.9	1.5	1.1
Q	1.7	174	14	ND	1.1	0.29
M	1.52	43	77	ND	2	1.6
O	1	177	8	ND	15.5	8.9
I	0.75	150	4.6	4.6	2.3	14.4
K	0.83	31	10.4	5.1	1.6	12.3

ND: Not Determined; CLint : Intrinsic clearance; PPB : Plasma protein binding

The in vitro ADME properties of GYROX was characterised by the following:

- low LogD (< 2)
- Solubility within range for acheiveing parenteral dosiing
- Low Intrinsic clearance in rat hepatocytes (< 8)
- Low to high Intrinsic clearance in human hepatocytes (8-40)
- Moderate plasma protein binding, ranging from 1 – 15% free in human plasma
- IC_{50}> 100 µM for the major isoforms: CY3A4, CYP2C9, CYP2C19, CYP2D6, CYP1A2
- Lack of inhibition of major Human CYP450 enzymes

Pharmacokinetics in Rats

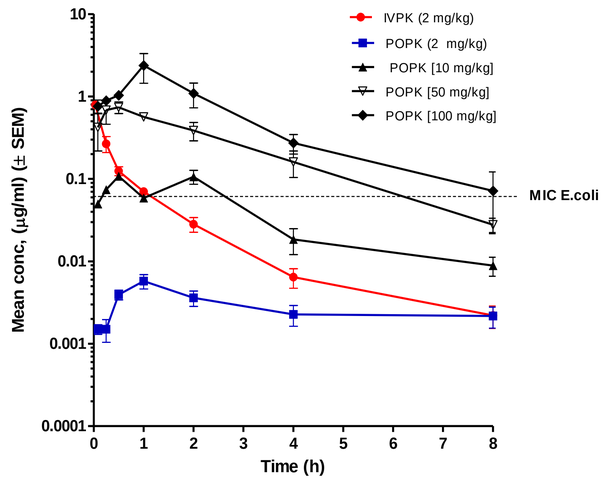


	IVPK 2 mg/kg							
Parameter	E	I	K	L	Q	O	M	P
$AUC_{0-\infty}$ (µg*h/mL)	686.5 ± 18.1	1111.9 ± 66.1	1989.3 ± 468.5	578.1 ± 56.6	688.9 ± 78.9	334.7 ± 23.7	4667.8 ± 346.6	3036.0 ± 1216.7
Clearance (L/h/Kg)	1.46 ± 0.04	1.8 ± 0.1	1.0 ± 0.3	3.5 ± 0.3	2.9 ± 0.3	6.0 ± 0.4	0.4 ± 0.0	0.7 ± 0.2
Vd_{SS} (L/Kg)	1.84 ± 0.2	0.8 ± 0.1	0.4 ± 0.1	1.0 ± 0.1	1.3 ± 0.4	2.2 ± 0.2	0.1 ± 0.0	0.3 ± 0.1
Half life (h)	1.86 ± 0.4	0.6 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.7 ± 0.1
	Oral PK: 10 mg/kg							
C_{max} (ng/mL)	2651.7± 704	3382.7 ± 1431.8	7071.6 ± 1055	1632.7 ± 244.7	1841.6 ± 1040.8	626.9 ± 34.4	10287.5	11008.3 ± 360.2
T_{max} (h)	0.5 ± 0.0	0.5 ± 0.0	0.25 ± 0.0	0.33 ± 0.1	0.25 ± 0.0	0.25 ± 0.0	0.25	0.33 ± 0.1
$AUC_{0-\infty}$ (ng*h/mL)	8305.4 ± 935	3861.3 ± 375.7	4593.3 ± 594.9	1251.1 ± 130.5	1509.2 ± 350.0	564.2 ± 48.0	12273.5	10611.0 ± 1431.1
Half life (h)	2.8 ± 0.2	1.3 ± 0.4	1.2 ± 0.3	1.9 ± 0.2	1.5 ± 0.3	1.4 ± 0.4	1.9	1.0 ± 0.2
Bioavailability (%F)	123 ± 13.6	69.5 ± 6.8	46.2 ± 6.0	43.3 ± 4.5	43.8 ± 10.2	33.7 ± 2.9	52.6	69.9 ± 9.4

IVPK:

- Multi-exponential disposition, low to high systemic clearance with respect to liver blood flow, low to high Volume of distribution at steady state
- Pharmacologically active exposures (C_{max} > MIC)
- **Oral PK:**
- Rapid absorption, $t_{1/2}$ range 2- 3 h, moderate to high oral bioavailability
- Pharmacologically active exposures (C_{max} >MIC)

Pharmacokinetics of Compound E in Mice



In vivo ADME properties

IV PK : 2 mg/kg

Parameter	Estimate
K_e (1/h)	0.403
$t_{1/2}$ (h)	1.72
$AUC_{0-\infty}$ (h*µg/ml)	0.352
CL (L/h/kg)	5.7
V_{ss} (L/kg)	13.3

Dose Escalation Oral PK

Parameter	Dose [mg/kg]			
	2	10	50	100
	Estimate			
K_e (1/h)	0.123	0.325	0.433	0.489
$t_{1/2}$ (h)	5.6	2.1	1.6	1.4
T_{max} (h)	1.0	0.50	0.50	1.0
C_{max} (µg/ml)	0.006	0.108	0.740	2.380
$AUC_{0-\infty}$ (h*µg/ml)	0.07	0.335	1.967	4.821
CL/F (l/h/kg)	27.6	29.8	25.4	20.7
F (%)	20.6	19.0	22.3	27.4
AUC/Dose	0.036	0.034	0.039	0.048
C_{max}/Dose	0.003	0.011	0.015	0.024

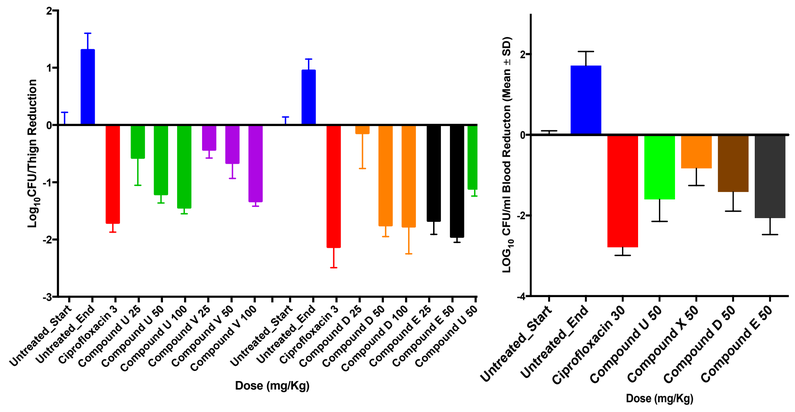
IVPK:

- High systemic clearance, very high Volume of distribution
- Exposures above MIC

Dose Escalation Oral PK:

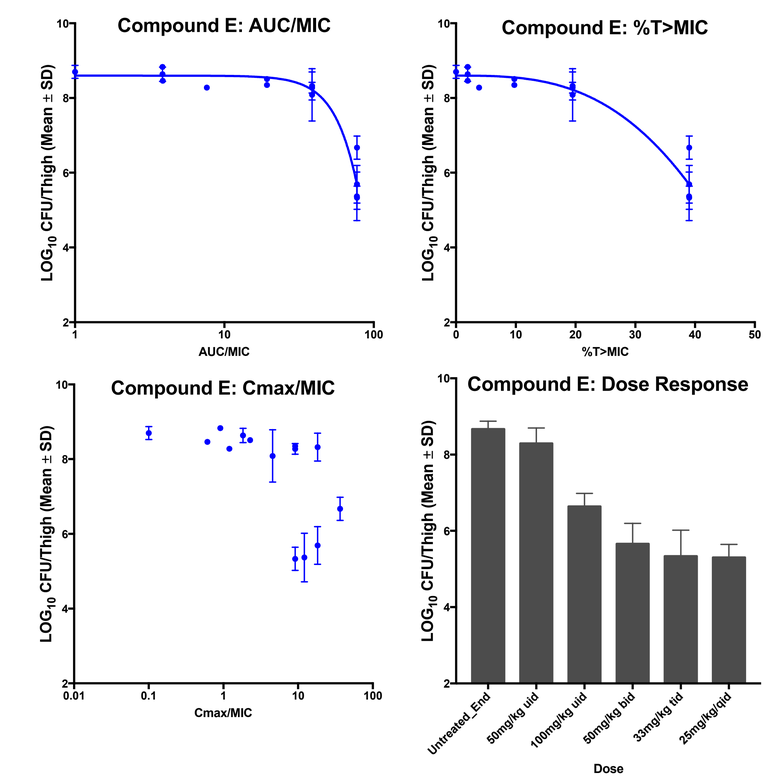
- Moderately Rapid absorption, moderate $t_{1/2}$, moderate oral bioavailability
- Linear PK
- Cmax was > MIC in a dose dependent manner
- Time above MIC increased in a dose dependent manner

Efficacy in Mouse Models



- The series has many members that show significant bactericidal efficacy (> 1 Log_{10}CFU/thigh) reduction in the thigh infection and and septicemia models in BALB/c mice, when challenged with E. coli ATCC 27922
- Efficacy was consistent with the PK for the respective compounds

Dose Fractionation in Mouse Thigh Infection Model



- Initial PK/PD studies reveal AUC/MIC & %Time>MIC as the drivers of efficacy

Conclusions

- The GYROX compounds exhibited promising in vitro ADME and in vivo PK properties which translated into significant efficacy in preclinical models of infection
- This series shows the potential for further development for antibacterial therapy

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