Fenarimols And Nitros: Potential Drug Candidate Series

ERIC CHATELAIN, HEAD OF DRUG DISCOVERY

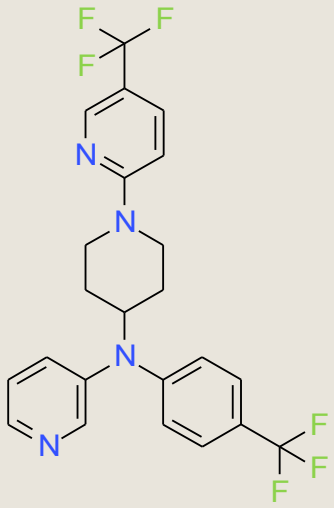
DNDi
Drugs for Neglected Diseases *initiative*

ICTMM,
September 2012, Rio de Janeiro

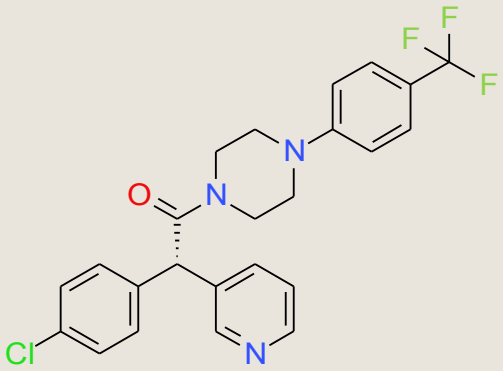
Fenarimol Series

From a single Hit to 2 potential Candidates that fulfill the TPP Criteria

- EPL-BS0967



- EPL-BS1246



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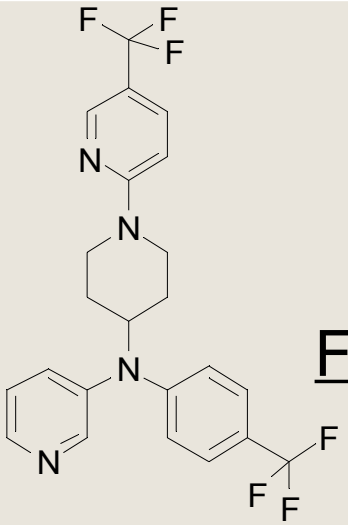
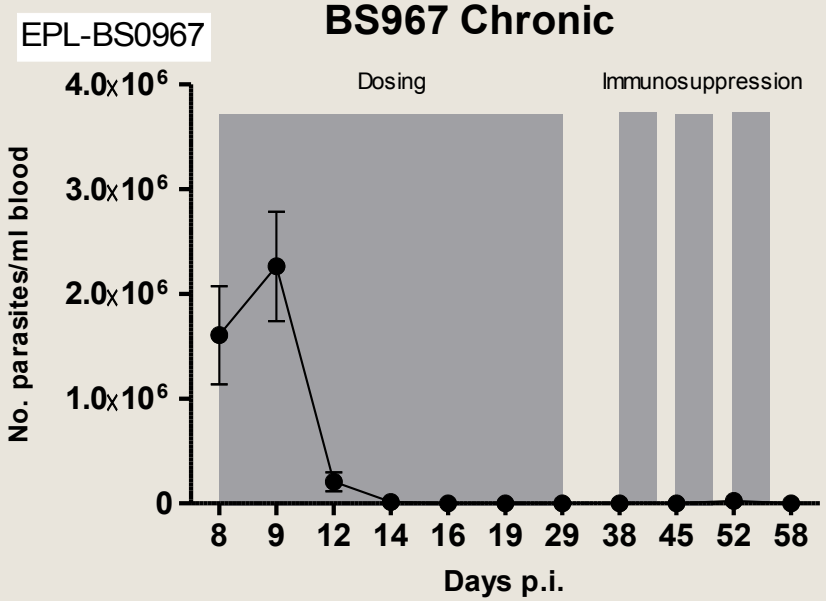
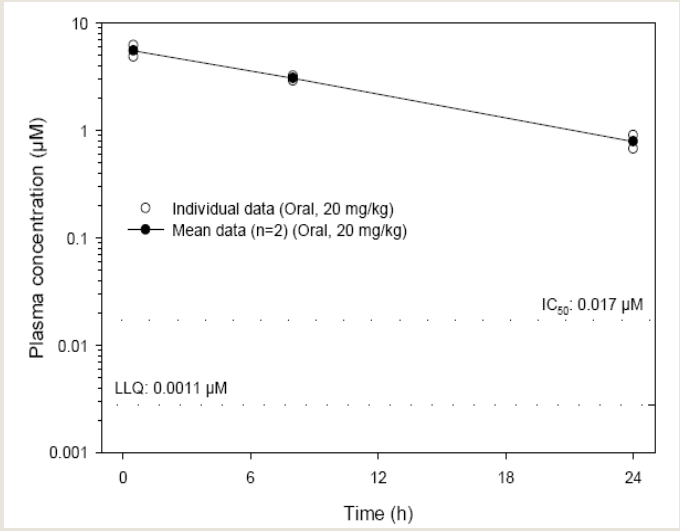
EPL-BS0967

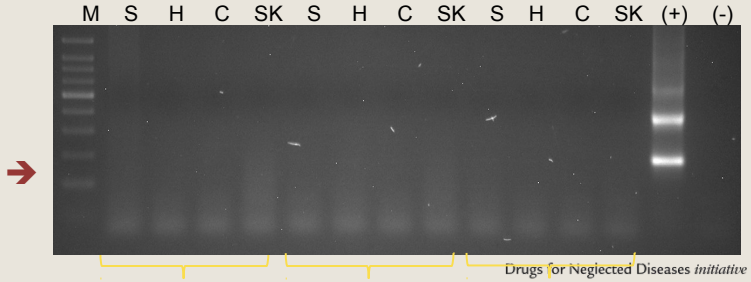


T. cruzi IC_{50} (Tc VI)	Selectivity Index	SAR	Chemical tractability	CYP3A4 IC_{50}	hERG IC_{50}
0.014-0.017µM	>3500	> 60 compounds with broad activity	4 steps No chiral center	>20 µM	>30 µM

Fulfills the TPP Criteria







EPL-BS0967: In Vitro DMPK Properties

- High plasma protein binding in all species (>99%)
- Good agreement between predicted plasma clearance based on in vitro studies, and measured in vivo clearance
- Low CYP inhibition compared to posaconazole

	EPL-BS0967 IC50 (μM)	**Posaconazole IC50 (μM)**
CYP1A2	>20	>30
CYP2C9	8.1	9.5
CYP2C19	9.8	20.9
CYP2D6	>20	>30
CYP3A4/5 Testosterone	>20	<0.25

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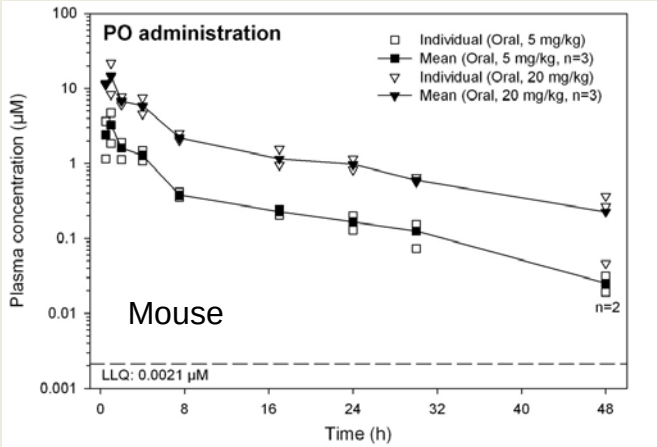
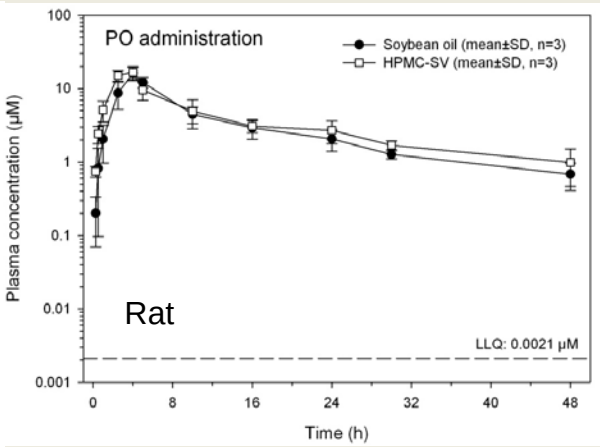
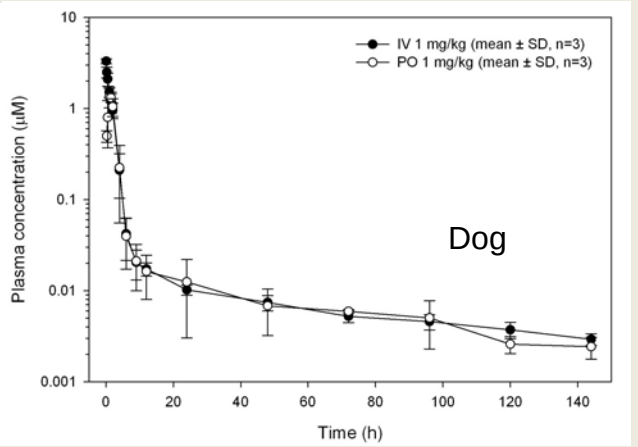
EPL-BS0967: Summary of PK Properties

- Low clearance, high volume of distribution and long half life
- High bioavailability in all species
- High volume of distribution and likely accumulation with repeat dosing







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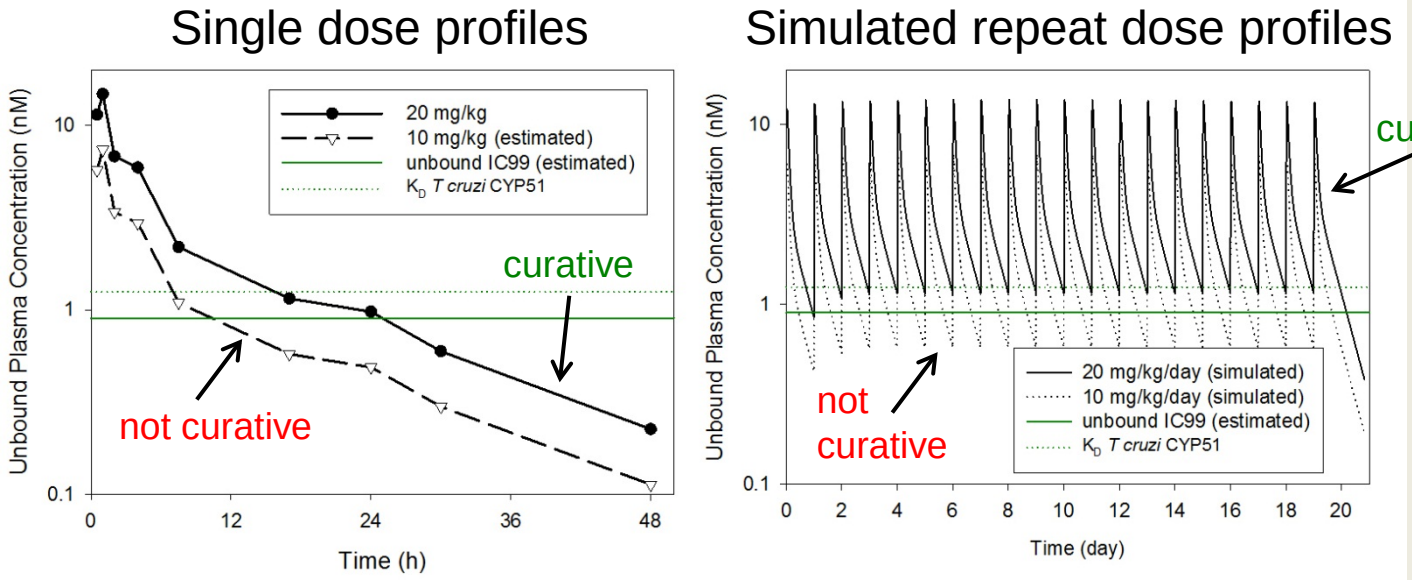
EPL-BS0967: PK/PD Relationships

- Cures obtained in mice with 20 mg/kg/day for 20 days, but not with 10 mg/kg/day
- Data suggests that unbound plasma concentrations need to be maintained above the unbound IC_{99} over the dosing period to achieve cures



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EPL-BS0967
In vitro and *in vivo* Toxicity

- *In vitro*
  - Cytotoxicity assessed in L-6 cells. CC_{50} = 59 µM ; SI >3500
  - hERG IC_{50} >30 µM (patch clamp)
  - Not genetoxic (Ames negative with and w/o S9 activation)
  - No signals in enzyme assays at 10 µM
  - Receptor binding assays: Some signals identified at 10 µM

- *In vivo*
  - 20-Day Efficacy Studies in Mice: No signs of toxicity at daily doses of 20 mg/kg
  - 14-Day Oral Exploratory Toxicity Studies in Rats: Estimate of safety margin based on C_{max} on D14 at 20 mg/kg (8-12 µM) and expected C_{av} needed for efficacy (3.5 µM)

  Safety margin based on available data ~ 2-3

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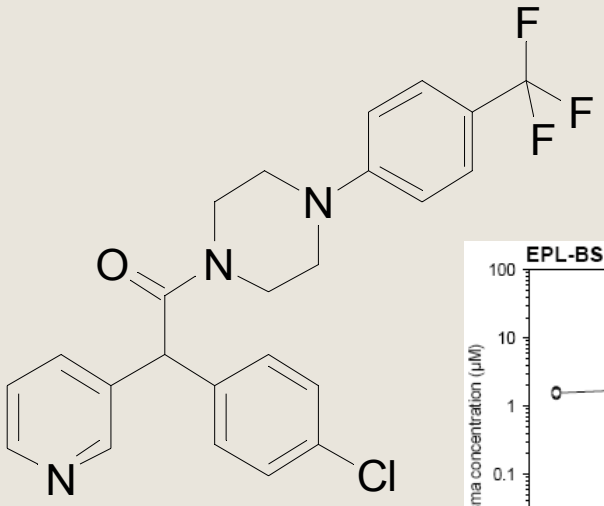
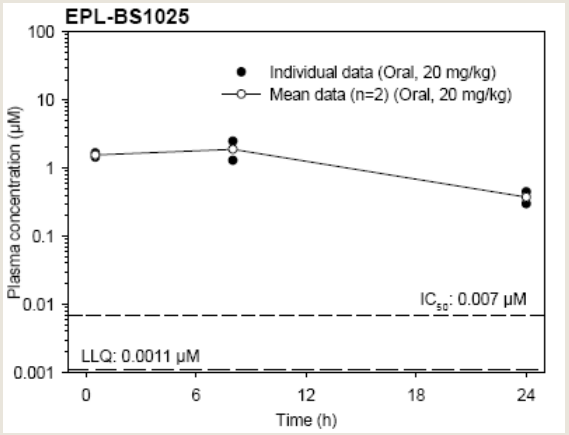
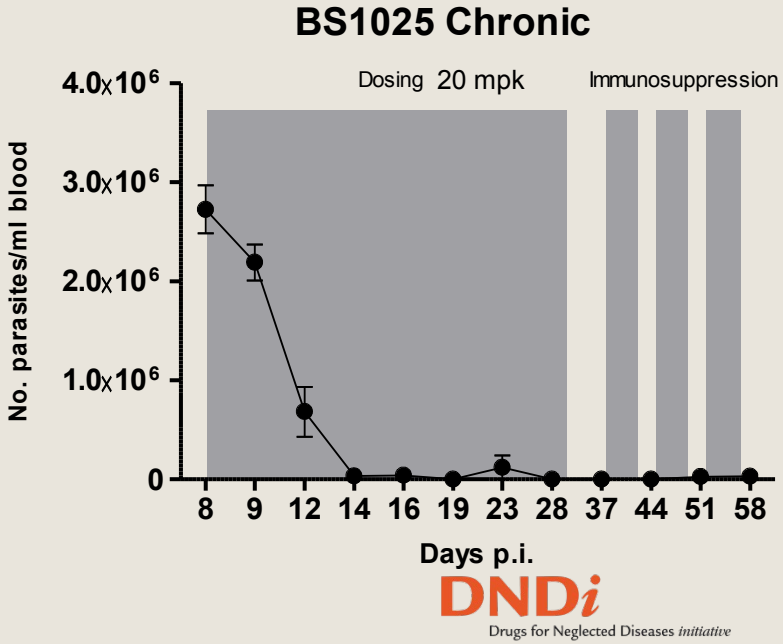
EPL-BS1246

	T. cruzi IC_{50} (Tc VI)		Chemical tractability	CYP3A4 IC_{50}	hERG IC_{50}
BS1025	6-7nM	Racemate	6 steps ,1 chiral center	16 µM	12 µM
BS1245	192 nM	R	ND	20 µM	8 µM
BS1246	7.5 nM	S	ND	17 µM	18 µM



EPL-BS1025





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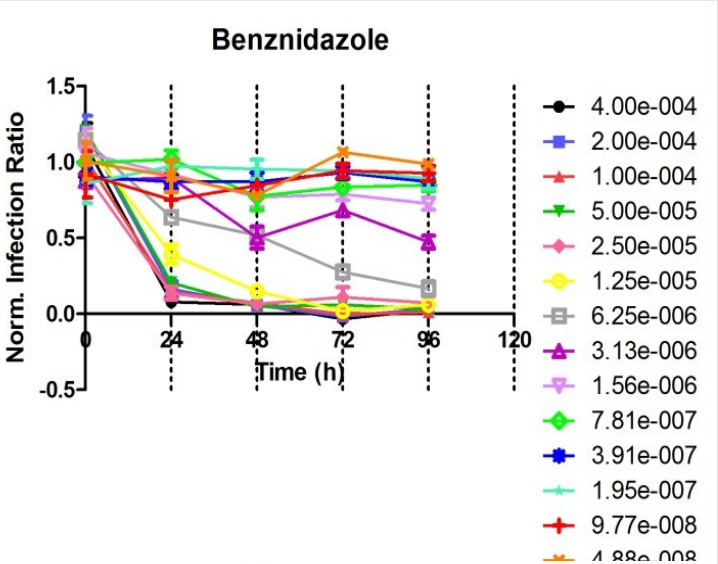
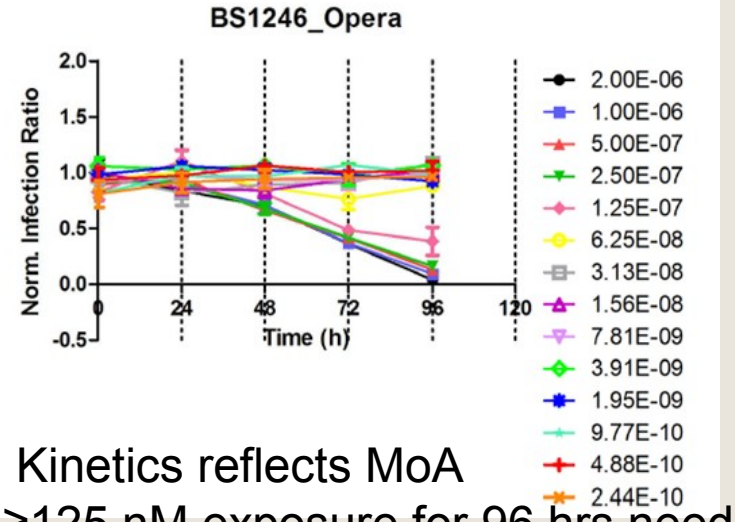
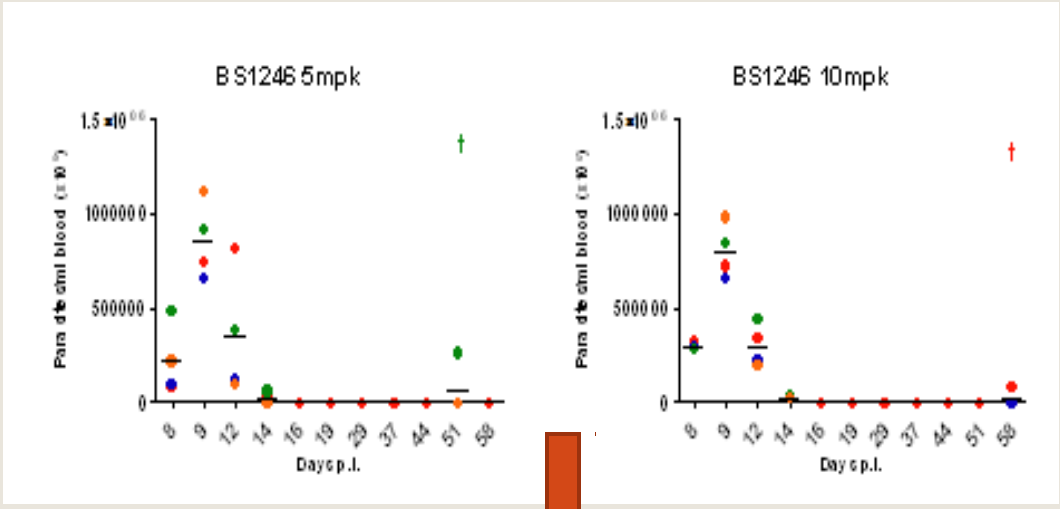
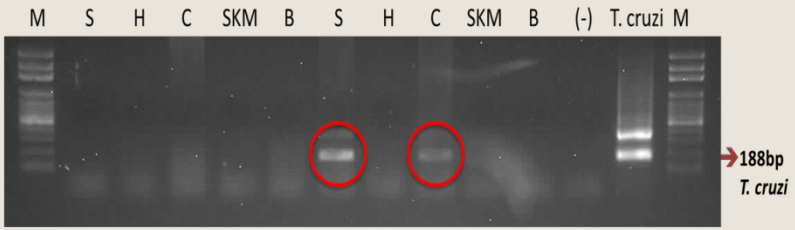
EPL-BS1246
Time-Kill *in vitro* and efficacy *in vivo*





Kinetics reflects MoA
≥125 nM exposure for 96 hrs needed





M: Ladder DNA marker
S: spleen
H: heart
C: colon
SKM: skeletal muscle
B: blood
(-): PCR negative control

PCR-confirmed cure in 50% of mice following treatment at 10 mg/kg p.o.

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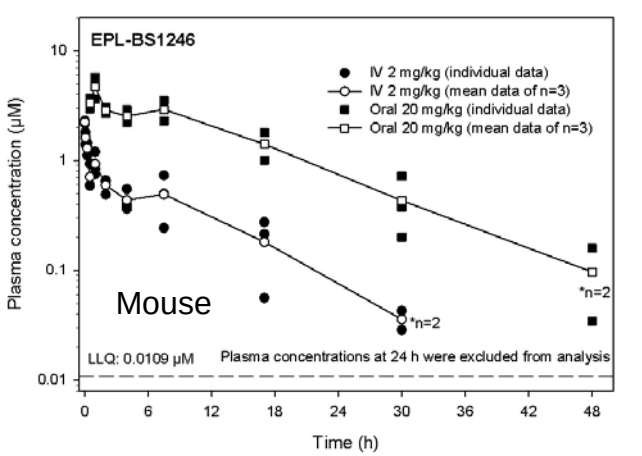
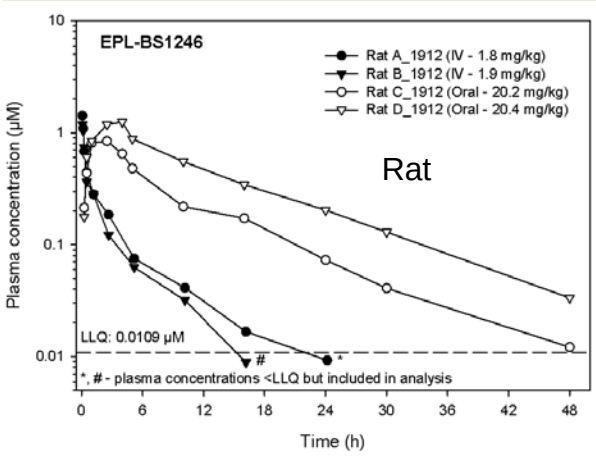
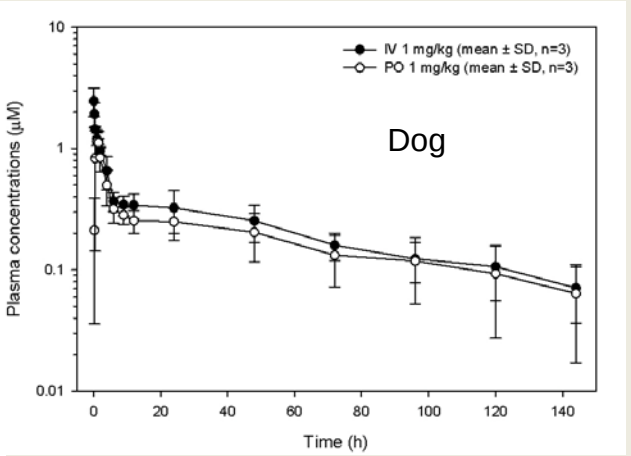
EPL-BS1246: Summary of PK Properties

- Low clearance, high volume of distribution and long half life
- High bioavailability in all species
- High volume of distribution and likely accumulation with repeat dosing







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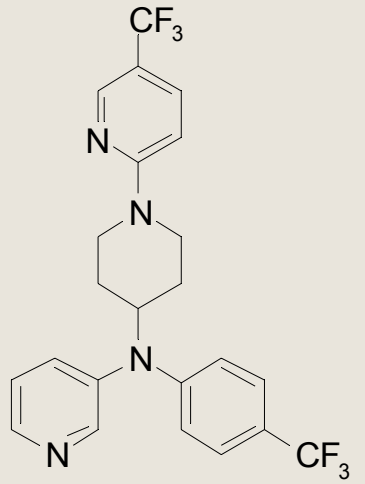
EPL-BS1246
In vitro and *in vivo* Toxicity

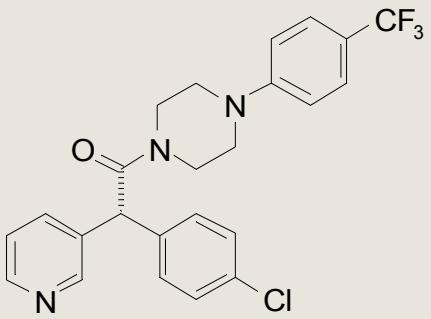
- *In vitro*
  - Cytotoxicity assessed in L-6 cells. CC_{50} = 38 µM ; SI >3700
  - hERG IC_{50} 18 µM (patch clamp)
  - Genetoxicity study ongoing (Ames test)
  - No signals in enzyme assays at 10 µM
  - Receptor binding assays: Some signals identified at 10 µM
- *In vivo*
  - 20-Day Efficacy Studies in Mice: No signs of toxicity at daily doses of 20 mg/kg
  - 14-Day Oral Exploratory Toxicity Studies in Rats: Estimate of safety margin based on C_{max} pending TK data

Fenarimol Series Summary

- Review of these 2 potential Candidates took place
  - Both compounds very efficacious in the Chagas model
  - Low risk for DDI
  - Potential for low CoG
  - Concern for low safety window with EPL-BS0967
- 14-day Explo Toxicity study in rats with EPL-BS1246 predicts better safety margin
  - Wait for definitive TK data before moving forward with EPL-BS1246
- Additional studies ongoing related to MoA (TcCYP51 inhibition, co-crystalization, ergosterol synthesis inhibition)



EPL-BS0967



EPL-BS1246

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Nitros: An Old Class with Potential but also Major Limitations

- Nitros are a validated compound class for their potential for Chagas Disease
  - Current Drugs used for treatment belong to this class
  - “Nitros” (-furanes, -imidazoles, -triazoles) from various sources are efficacious in murine model e.g ENH-5, Ro-XXX compounds, RJ compounds, Fexinidazole, albeit at high dose (300 mg/kg/day)
  - Cidal compounds

- General Liabilities include
  - Toxicity (Genotoxicity, hERG, other)
  - Safety margin: in general not very potent compounds (µM range)

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Rationale for a new Nitro

Considering this data for either Benznidazole or Nifurtimox, there is room for improvement

A “Nitro” with:

- Higher potency
- Better PK profile
- Better safety
- Better compliance

A Solution?

Our better understanding of PK/PD for Chagas could be applied to develop a better and safer Nitro

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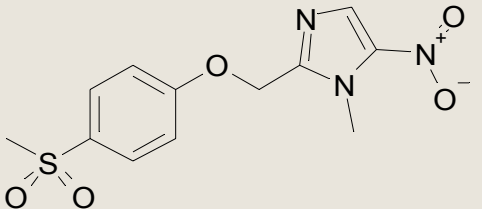
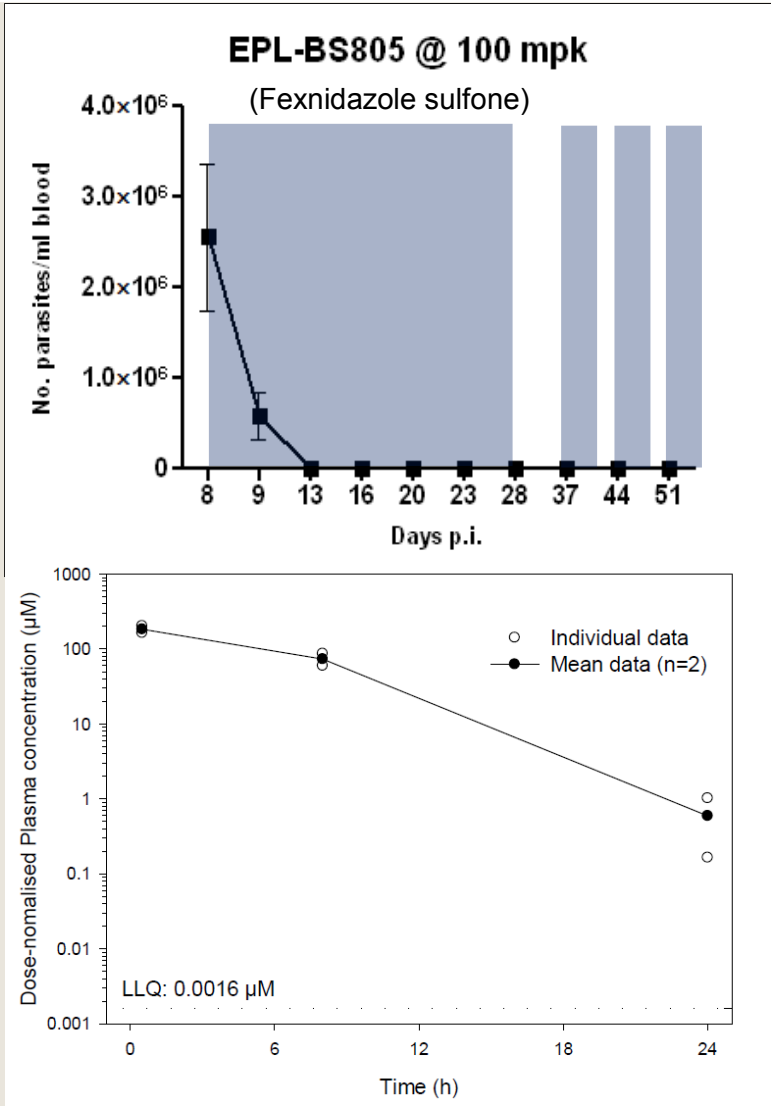
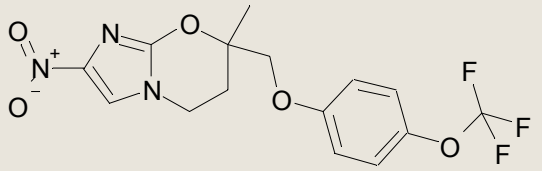
FEXINIDAZOLE SULFONE



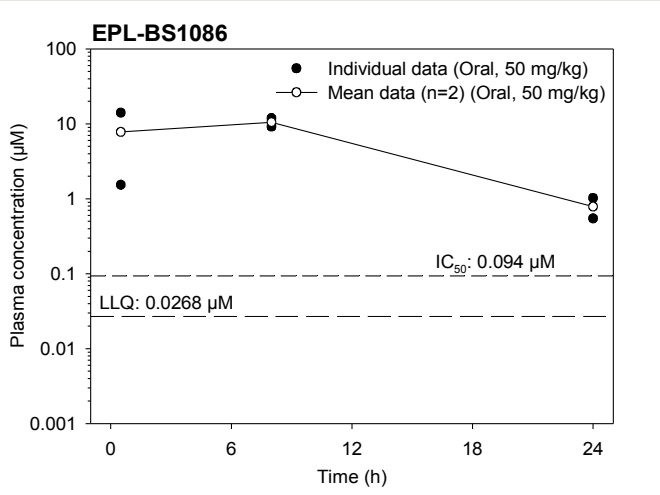
- Fexinidazole M2 metabolite
- 100% cure in mice (negative PCR) at 100 mg/kg with two *T. cruzi* strains
  - 50 mg/kg < ED_{50} < 100 mg/kg
- Good DMPK Properties
- Issues
  - QT prolongation observed in Phase I for Fexi (Fexi, M1, M2)
  - Safety margin?
- Next steps
  - Review data, Go/NoGo decision

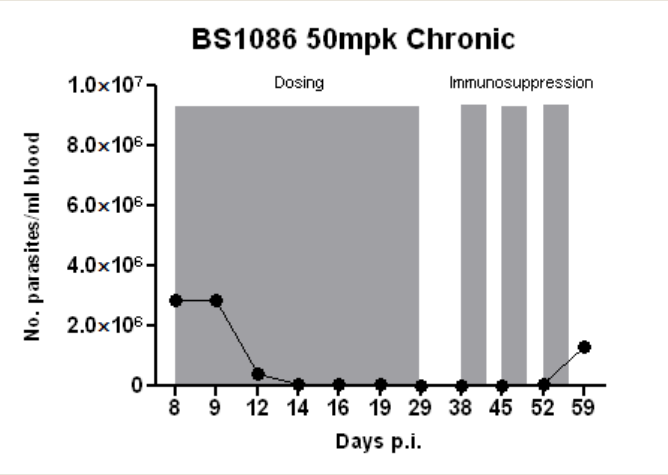


NITROIMIDAZO-OXAZINES



- EPL-BS1086: Proof of Concept in murine Chagas immunosuppressive model
  - E_H < 0.28
  - LogD 3.5, Kin. Sol. 1.6-3.1 µg/ml
  - hERG IC_{50} 3.8 µM
- Series generally characterised by:
  - Low solubility & moderately high LogD values
  - Minimal CYP3A4/5 inhibition (IC_{50} values all >20 µM)
  - Oral exposure (in mice) correlates well with predicted E_H values (in HLM)





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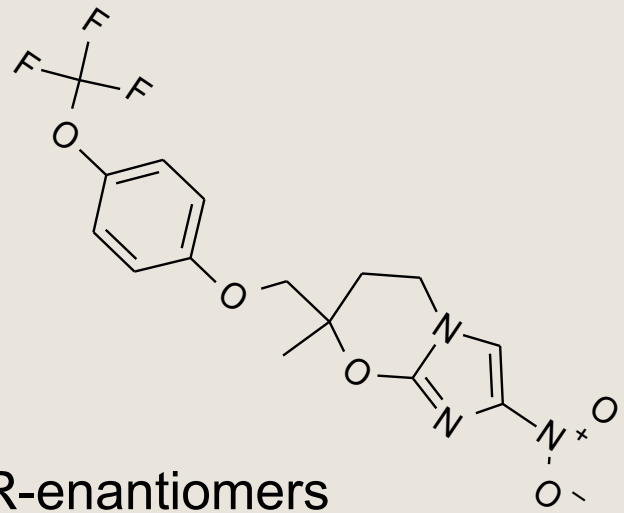
NITROIMIDAZO-OXAZINES (2)

- Next steps
  - 7-substituted-oxazines series
    - Profile enantiomers of EPL-BS1086
  - Several new starting points identified
    - Nitrotriazolooxazines series
    - PA824 class: Greater potency of the R-enantiomers
    - 6-substituted-oxazines analogues



- Issues / Points to consider
  - hERG and AMES as flags in that series
  - No cure yet observed with that series in the murine immunosuppressive model

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Conclusions/Critical issues

- Different liabilities from current leads/candidates identified may preclude their development as drug candidates
  - QTc prolongation observed with Fexinidazole in Phase 1 → Risk/Benefit for Chagas Disease?
  - Clinical efficacy of Posaconazole in Chagas patients → Impact for the Fenarimols (EPL-BS1246) and other EBIs in general

→ Need for more chemical diversity

- Better understanding of the PK/PD relationships for Chagas disease and relevance of animal models and *T. cruzi* strains

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Acknowledgments

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DMPK

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Susan Charman

DNDi

Ivan Scandale

Delphine Launay

Stephanie Braillard

Tom von Geldern

THANK YOU

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Back-up Slides

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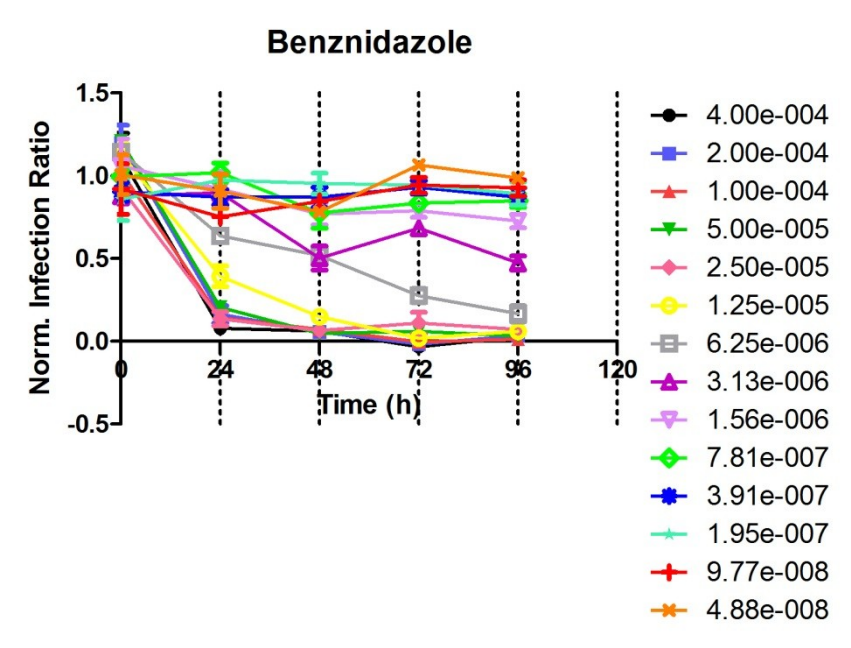
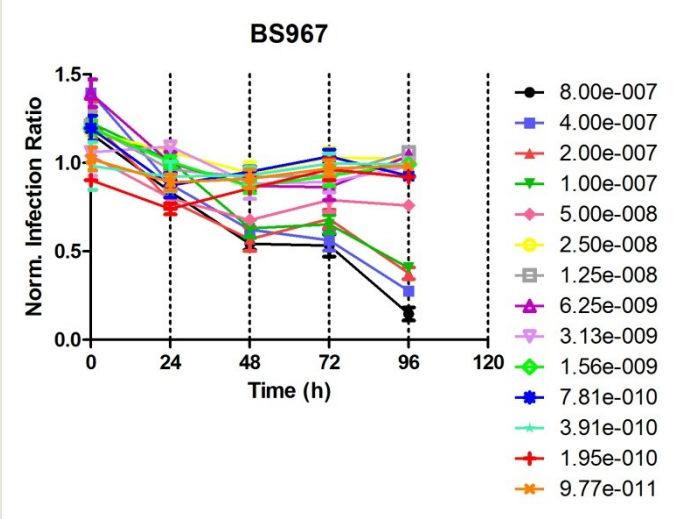
CHAGAS PK/PD: A few preliminary examples (1)

- Assays for one representative of each Tc Group (I to VI) in place → Relevance?
- *In vitro* Time Kill





100 nM

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Kinetics of intracellular *T. cruzi (Y strain, TcII)* Killing *in vitro*

Seed U2OS cells into 384-w plates

Incubate 24 h 37˚C, 5% CO_2

Seed *T. cruzi* parasites (Y strain)

Incubate 24 h 37˚C, 5% CO_2

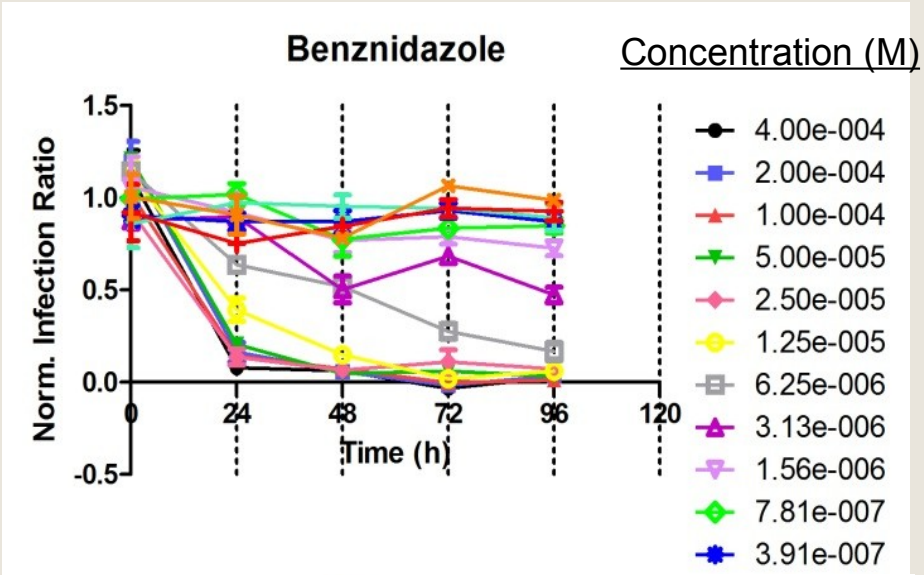
Add compounds in DRCs

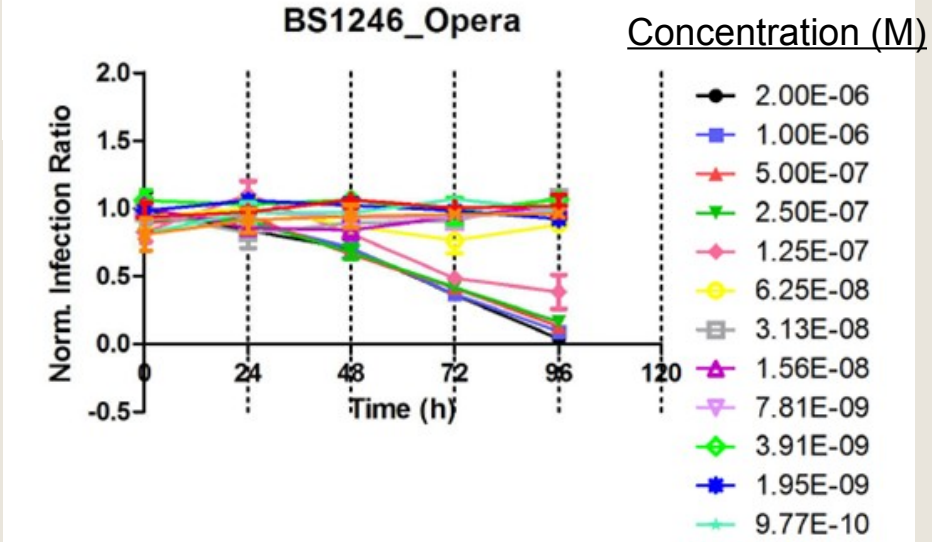
Culture at 37˚C, 5% CO_2 for the following exposure times:
0h – 24h – 48h – 72h – 96h

Fix with 4% PFA at each time-point

Stain with 5 µM DRAQ5

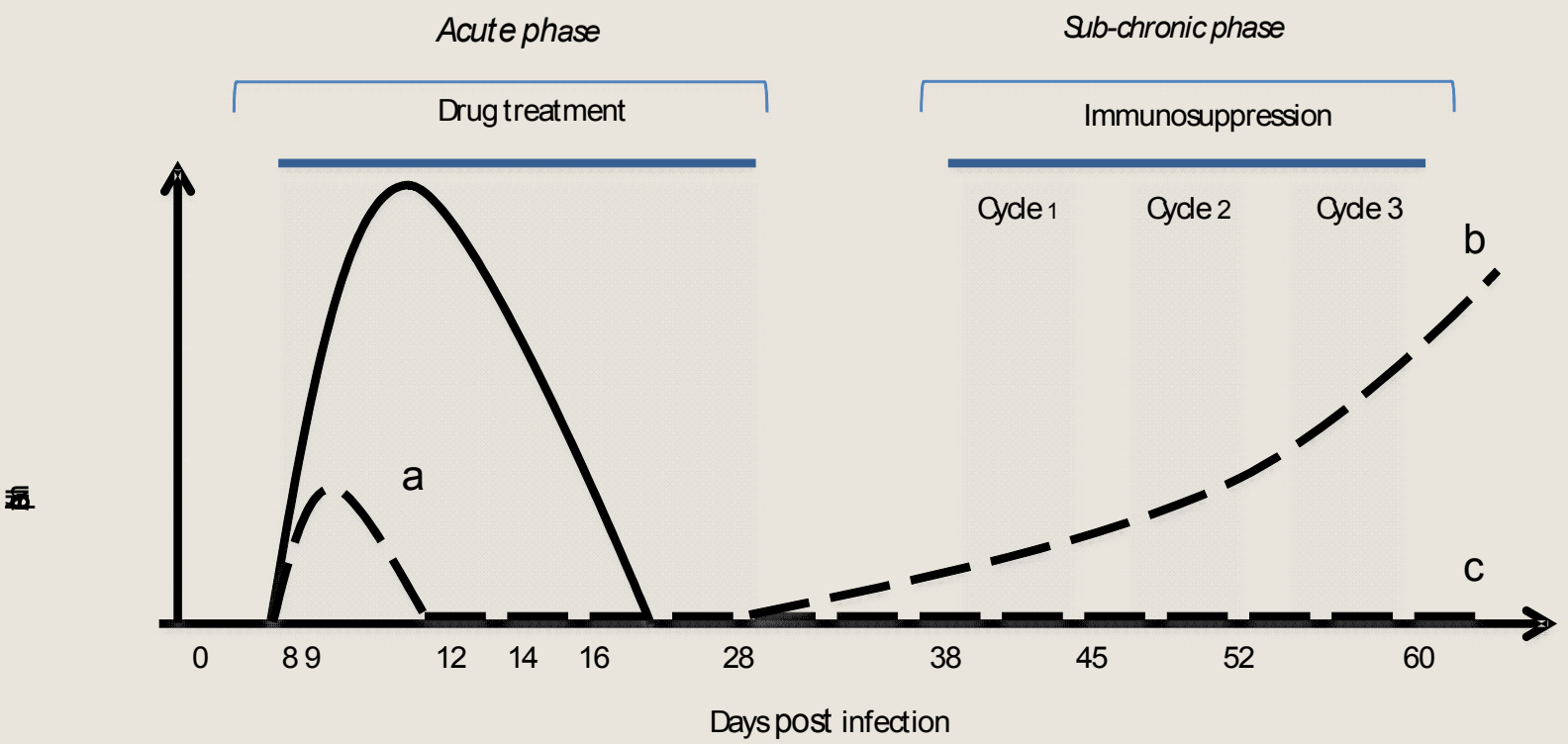
Read in OPERA





- Kinetics reflects MoA
- ≥125 nM exposure for 96 hrs needed

Chagas mouse model for *in vivo* efficacy testing compatible with Lead Optimization



Illustrates normal course of infection
(parasites eventually enter tissues/organs and are no longer detected in blood)

Indicates possible positive outcomes following drug treatment, i.e.,

a. Significant reduction in parasitemia ➔ if 100% efficacy is observed, animals are

b. Parasite rebound after immunosuppression ➔ test at higher dose or longer treatment

c. No parasite rebound after immunosuppression ➔ confirm cure with PCR

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Figure 1: Plasma exposure and heart concentrations (at 24 h) of EPL-BS0803 and EPL-BS0805 following oral administration of EPL-BS0803 to mice at a nominal dose of 100 mg/kg.

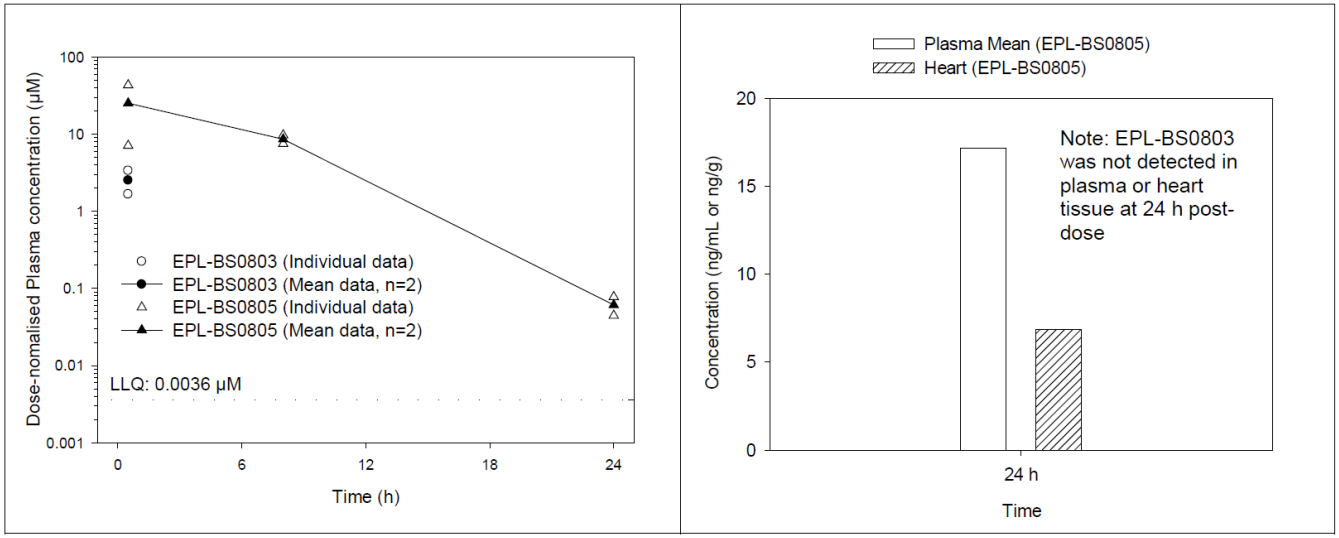
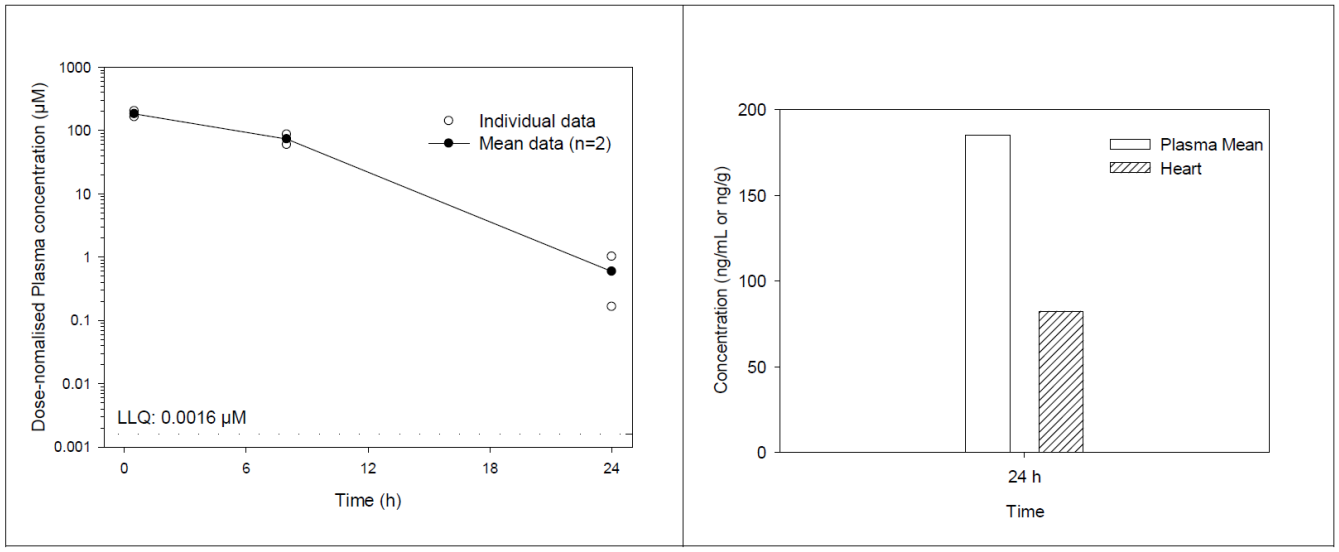


Figure 2: Plasma exposure and heart concentrations (at 24 h) of EPL-BS0805 following oral administration to mice at a nominal dose of 100 mg/kg.



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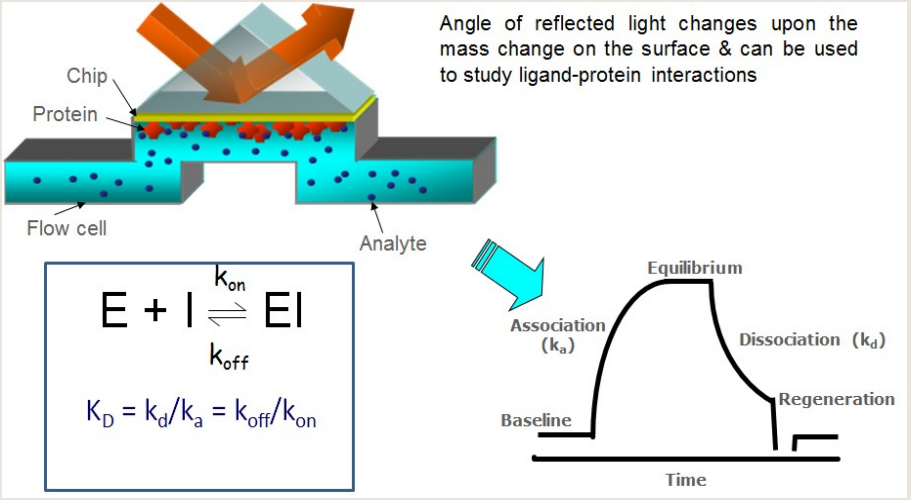
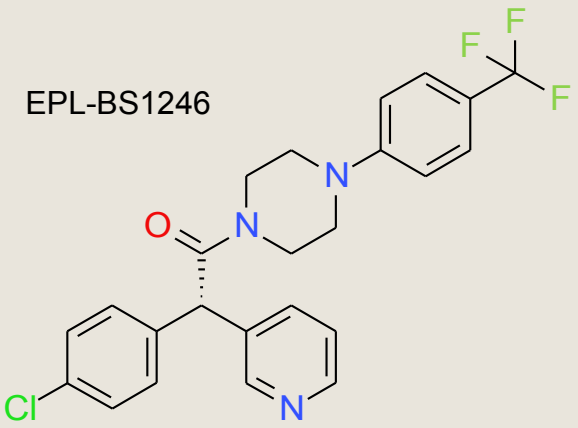
EPL-BS1246 binds *T. cruzi* CYP51





	k_a	k_d	K_D
EPL-BS1246	9.054e5	9.426e-4	1.04 nM
posaconazole	5.97e5	5e-5	0.084 nM
Fluconazole	9010	0.05594	6.21 µM

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EPL-BS1246 is potent and selective inhibitor of *T. cruzi*

- Tulahuen LacZ strain (TcVI): IC_{50} = 7.5 ± 2.0 nM

- *T. b. rhodesiense* IC_{50} > 10 µM
- L-6 cells: CC_{50} ≈ 38-50 µM
- CC_{50}/IC_{50} ratio: SI > 3700

  - IC_{50} benznidazole = 2.0 ± 0.5 µM
  - IC_{50} Posaconazole = 0.7 ± 0.2 nM

- *T. cruzi* strains

	Group	EPL-BS1246	Benzn.
Dm28c	TcI	217.0 nM	2.3 µM
Y	TcII	45.9 nM	4.4 µM
ARMA13	TcIII	t.b.d.	5.5 µM*
ERA	TcIV	39.4 nM	1.4 µM
92-80	TcV	t.b.d.	0.6 µM
Tulahuen WT	TcVI	t.b.d.	4.3 µM*
CL Brener	TcVI	t.b.d.	4.4 µM

DNDi
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