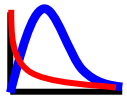
Pharmacodynamics

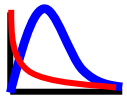
Chris Town
Research Pharmacokinetics



Definitions

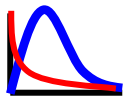
Pharmacodynamics:

the study of the biochemical and physiological effects of drugs and the mechanisms of their actions, including the correlation of action and effects of drugs with their chemical structure; also, the relationship between drug concentration and effect.



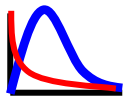
Pharmacodynamics

- **Principle: The Activity of all compounds is caused by interaction with receptors in the body**
  - **Receptors are biomolecules in the body**
    - **Enzymes**
    - **Cell Surface Receptors**
    - **Nuclear receptors**
    - **Etc.**



Drug Responses from Different Types of Interactions with Receptors

- Direct reversible effects
  - Ca Channel Blockers
  - Beta-Blocker
- Indirect reversible effects
  - Nuclear Hormone Receptors
  - Anti-coagulants
- Irreversible effects
  - Anti-Cancer drugs
  - Antibiotics



Pharmacodynamics

Processes driven by:

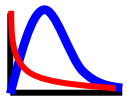
- **C_{max} (Maximum Concentration in Plasma)**
- **AUC (Overall Exposure)**
- **$C_{average}$ (Concentration during study)**

Examples of different processes in Antibiotics:

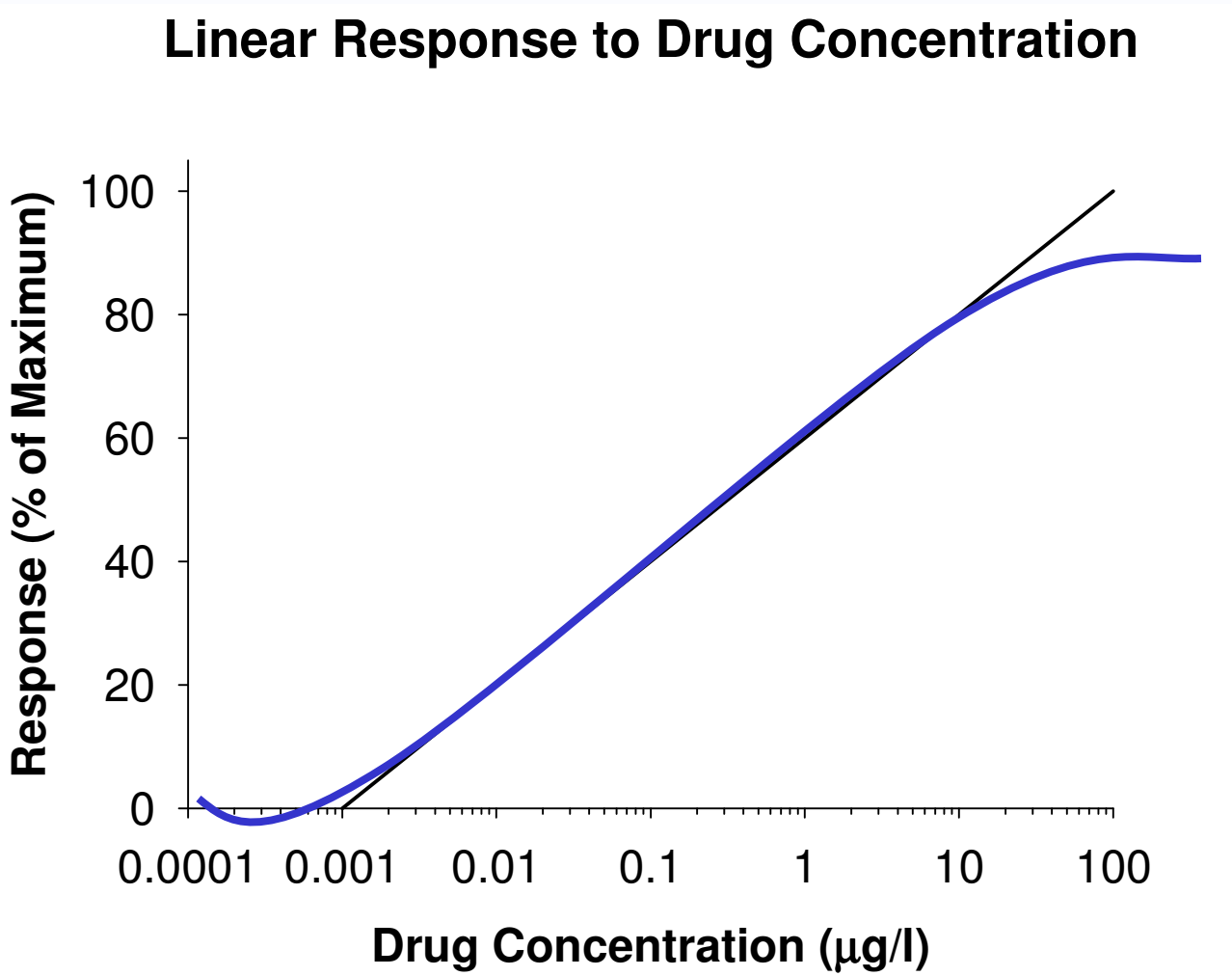
- **Beta-lactams (time dependent, no persistance)**
- **Tetracyclines (time-dependent, with persistance)**
- **Flouroquinloline (Concentration dependent, with persistance**

Examples in organs and tissues:

- **Nuclear Hormone Receptors**
- **Cell Surface Receptors**
- **Enzyme inhibitors**



Linear Pharmacodynamic Response



Receptor in Central Compartment

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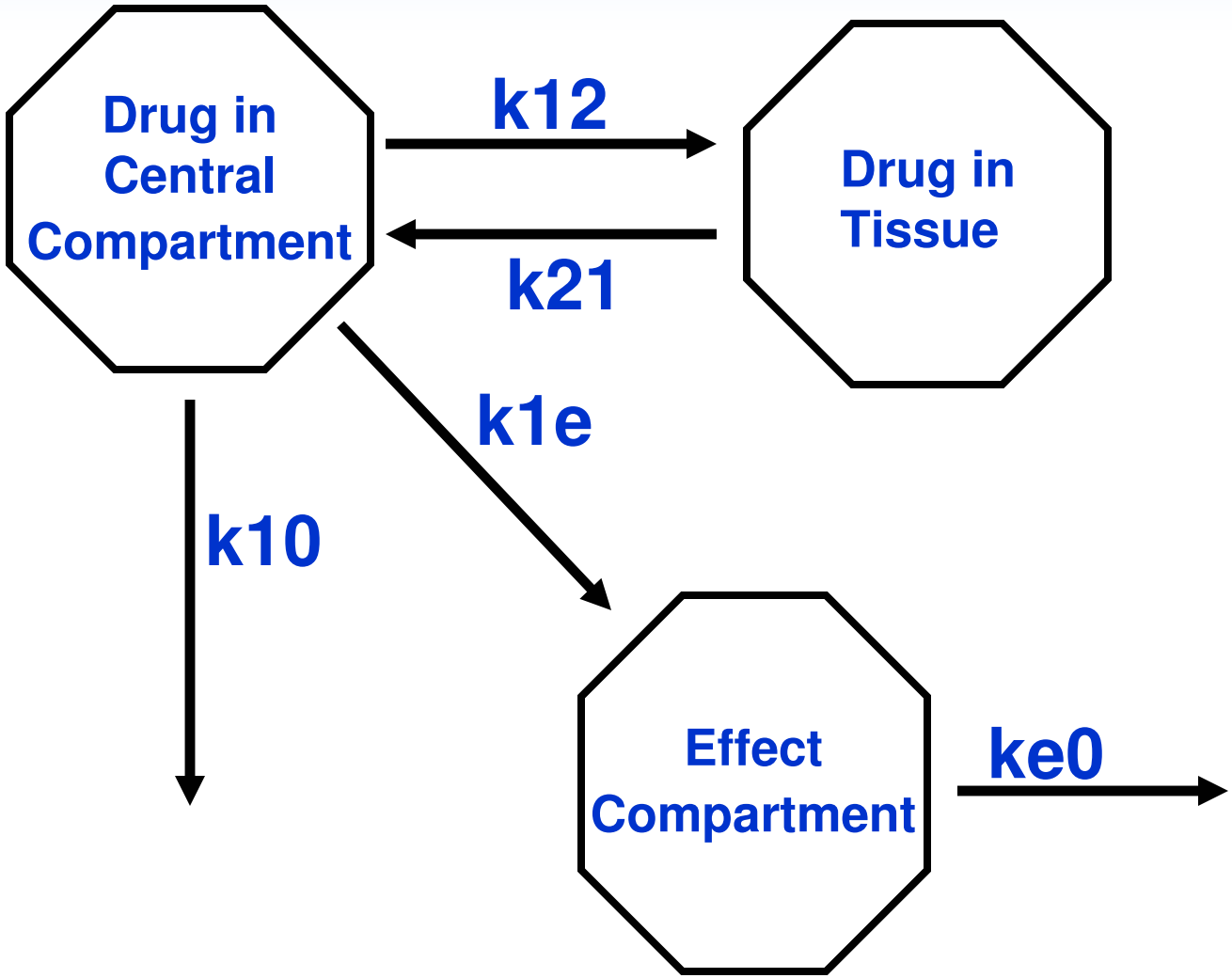
PK Model with Effect Compartment



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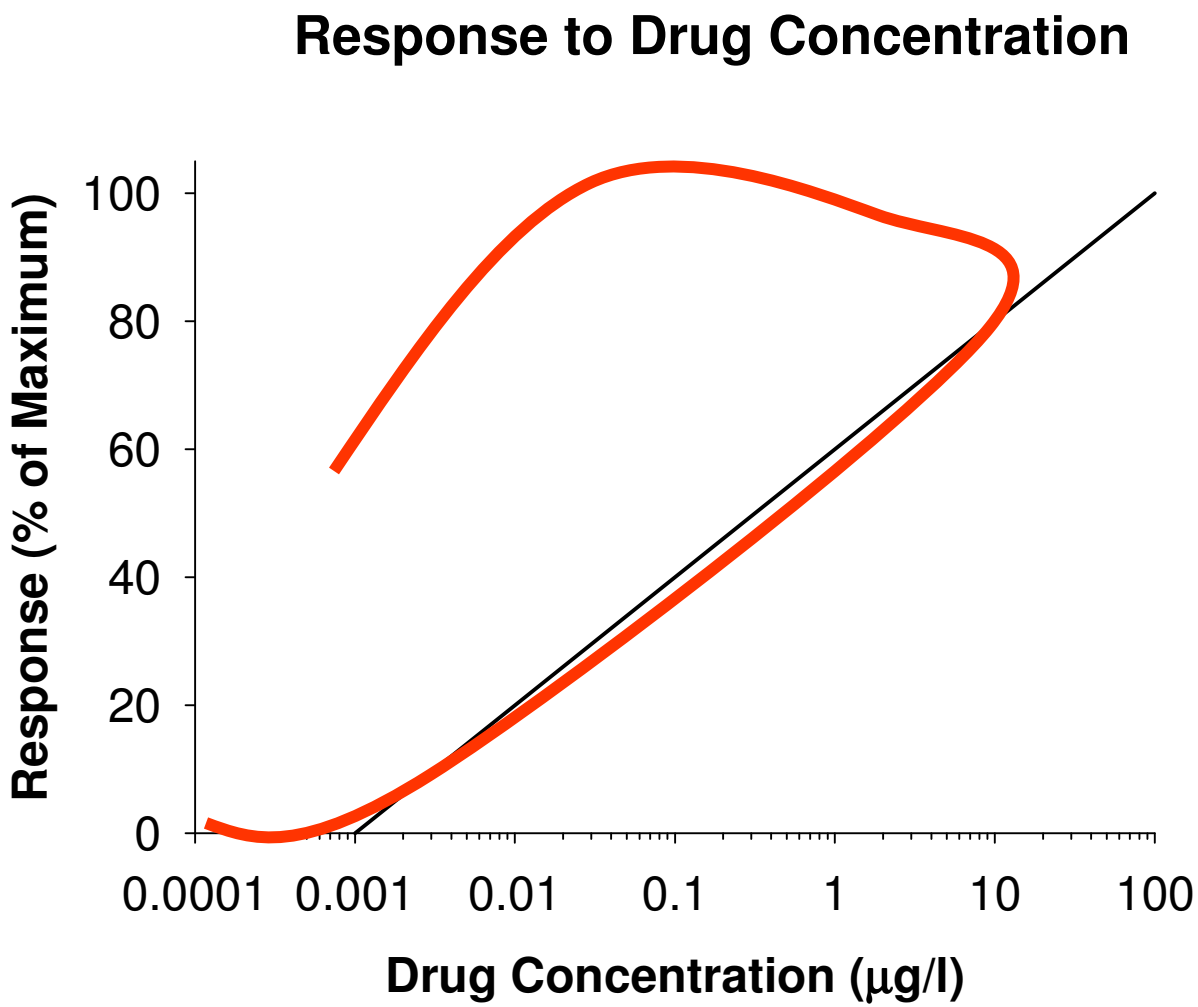
Hysteresis in Plot



Receptor Not in Central Compartment

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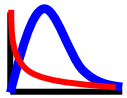
Pharmacodynamic Overview

- The interaction between different drugs and individual receptors may show different affinities and different potency and efficacy of response
- The reaction between drugs and receptors may have different response times
  - Different between t_{max} and Maximum response
- Modeling requires accurate bioanalytics and reasonable measurement of response with time



Exposure Screening

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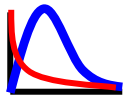

Typical Discovery Screening Cascade

In Vitro Enzyme Assay

Cell Based Assay

Selectivity

CYP Inhibition screening
Microsomal stability (high CL)
Exposure screening
Caco-2 permeability
IV/PO PK studies
solubility

Screening PK

Acute in vivo efficacy model

Expanded PK (definitive, TK, Formulations, etc…)

Chronic Efficacy model

DP1 Candidate

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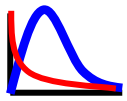
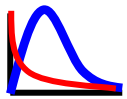


Defining the PK/DM Issues in a Project

How do we help define the issues?

- **Conduct a series of investigative studies on the Initial lead**
  - **IV/Oral PK study (in efficacy species)**
  - **Microsomal stability (efficacy & other species)**
    - **Metabolite ID work for very unstable compounds**
  - **Caco-2 permeability study (+/- Pgp inhibitor)**
  - **CYP inhibition (5 major isoforms)**
  - **Protein binding**
    - **(especially for compounds with major disparity between intrinsic and cell-based activity)**
  - **Solubility and Pharmaceutical Technology assessment (+solution/suspension comparison, as needed)**
  - **P450 induction**
  - **Target level analysis (e.g. tumor, brain, etc..)**
  - **In vitro plasma stability**
  - **Multi-cannulated rat model (as needed)**

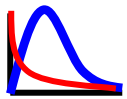


Screening of Initial Lead to aid in Chemical Plan

Design Screen to address Compound's shortcomings

- Metabolically Unstable (Microsomal Stability)
- Poor Absorption (CaCo-2 Cell Permeability)
- Rapid Elimination (IV administration in rats)
- CYP 450 Inhibitor (P450 Inhibition)

- Perform full test with random compounds to determine if problems with the chemical series have changed.



The Screening Cascade for HSL

HSL inhibition	<100 nM IC_{50}
Cellular activity assay	<100 nM EC_{50}
Selectivity	50x vs. ACPH, LPL, HL, PL, AChE
Fasted mouse model	Efficacy at 4h at ≤10 mg/kg p.o.
Toxicity Screening	100 / 300 mpk in rat
Microsome / blood stability	Mouse, rat, dog, human
PK plasma levels	Supports q.d. dosing
Diabetic rodent	> 50 mg/dL glucose decrease

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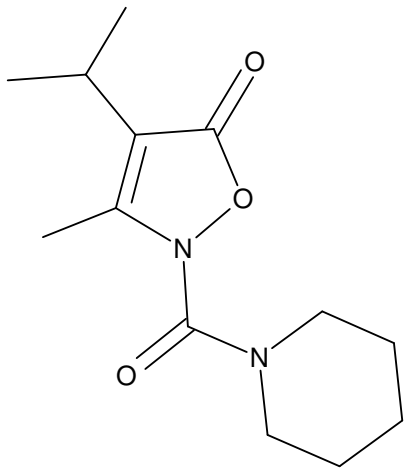
HSL Discovery Project

Lead Structure determined from HTS

- Active in vitro activity
- Low exposure in vivo
- Active in vivo
- PK issues may play a role



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Analysis of Compounds from In vitro Studies

- Concentrations determined using LC/MS/MS with a simple assay
  - No Internal Standard
    - Calculate Concentration from change in instrument response (Peak Area)
- Compare remaining drug concentration in presence and absence of NADPH (a necessary Cofactor for enzymatic breakdown) over time.

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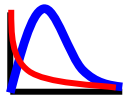
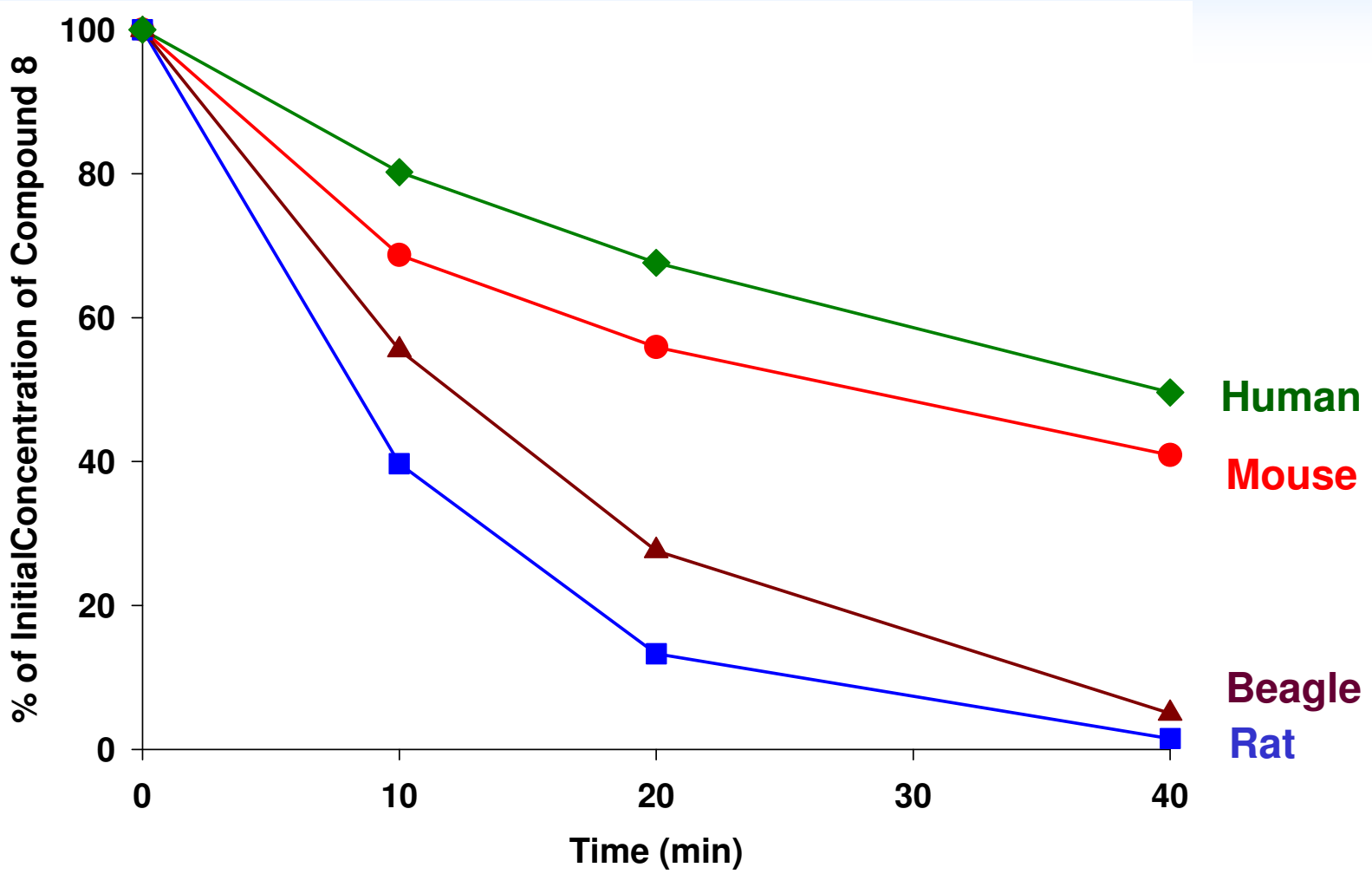


Compound 8 Stability in Liver Microsomes



Compound 8 most unstable in rat and dog liver microsomes more stable in human and mouse

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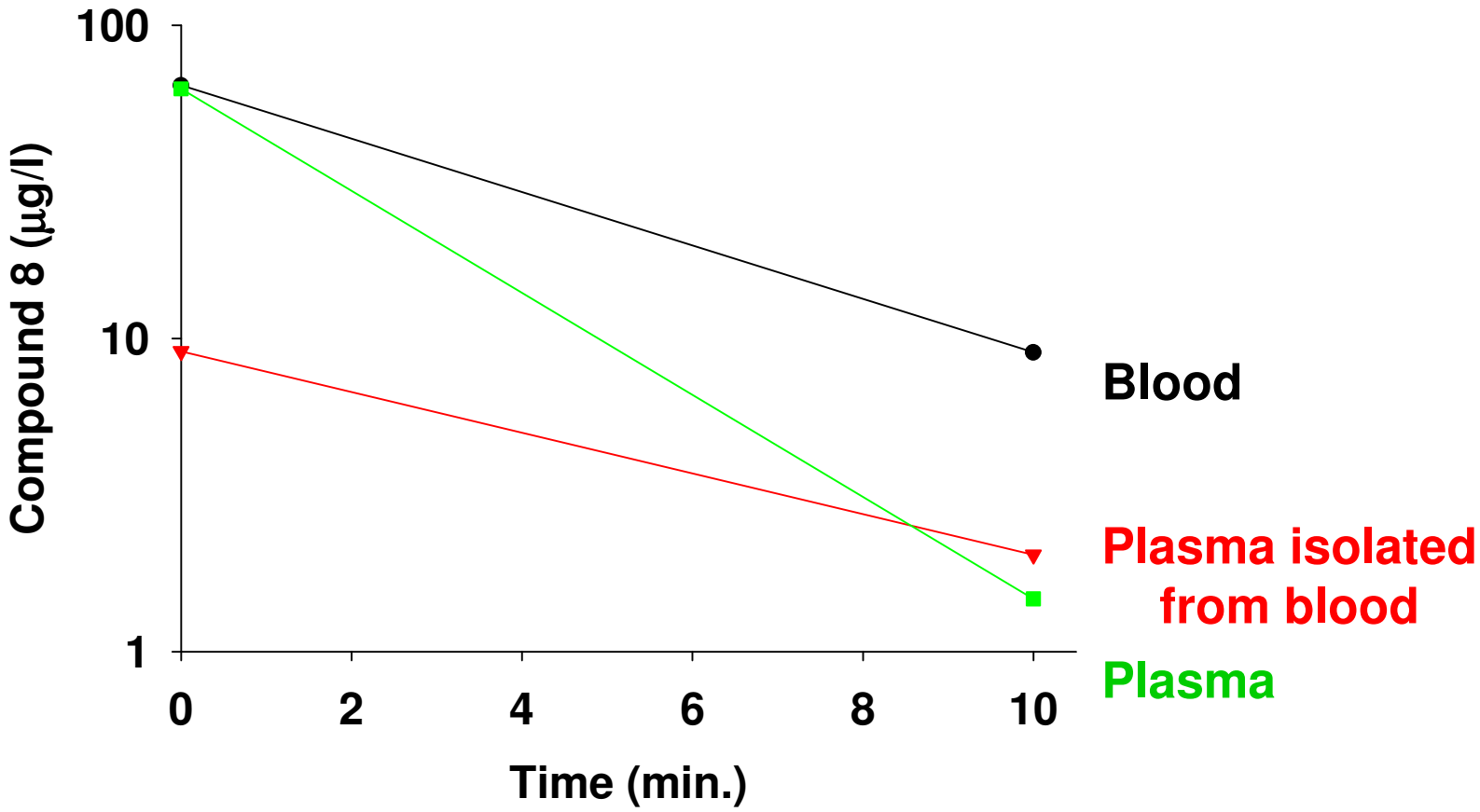
Stability in Rat Blood and Plasma

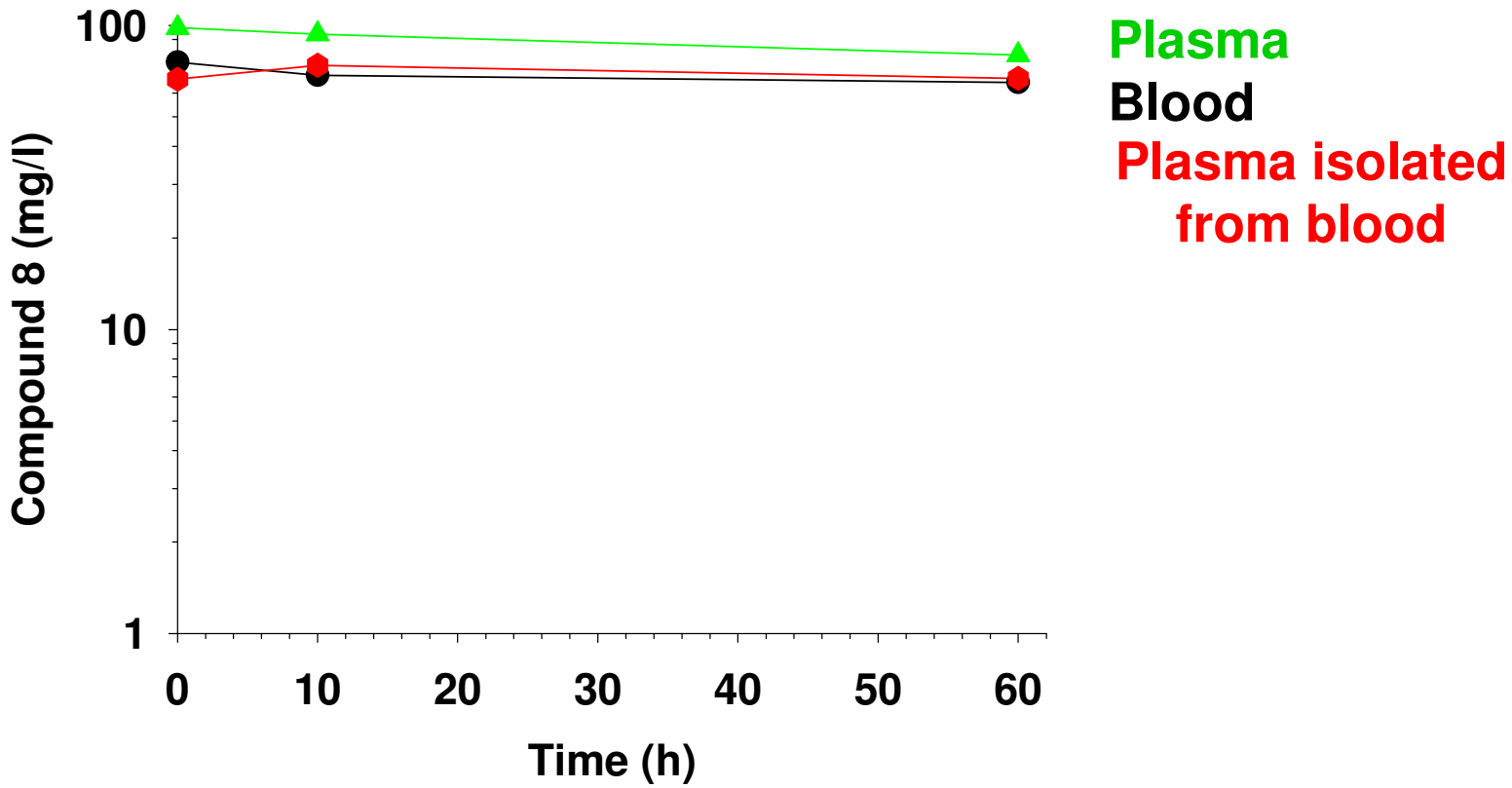
Disappearance of Compound 8 from rat blood and plasma



Compound 8 appears to break down in plasma and whole blood and apparently concentrates in Red Blood Cells

Stability in Human Blood and Plasma

Stability of Compound 8 in Human Blood and Plasma



Compound 8 is quite stable in human blood and plasma (similar findings with dog blood and plasma)

Analysis of Compounds from In vivo Studies

- Concentrations determined using LC/MS/MS
- Need Internal Standard
- Calibration curve made from 8 standards (5 – 5000 μg/l Calculate Concentration from change in instrument response (Peak Area)
- Quality Control samples added to sample run in duplicate or triplicate
- QC samples spread throughout sequence to make certain instrument is not changing over time
- Need a simple sample preparation procedure that works with most drugs
- Rugged LC column that can handles hundreds of samples
- LIMS system helps keep track of data.

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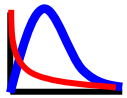

Reliable HPLC System

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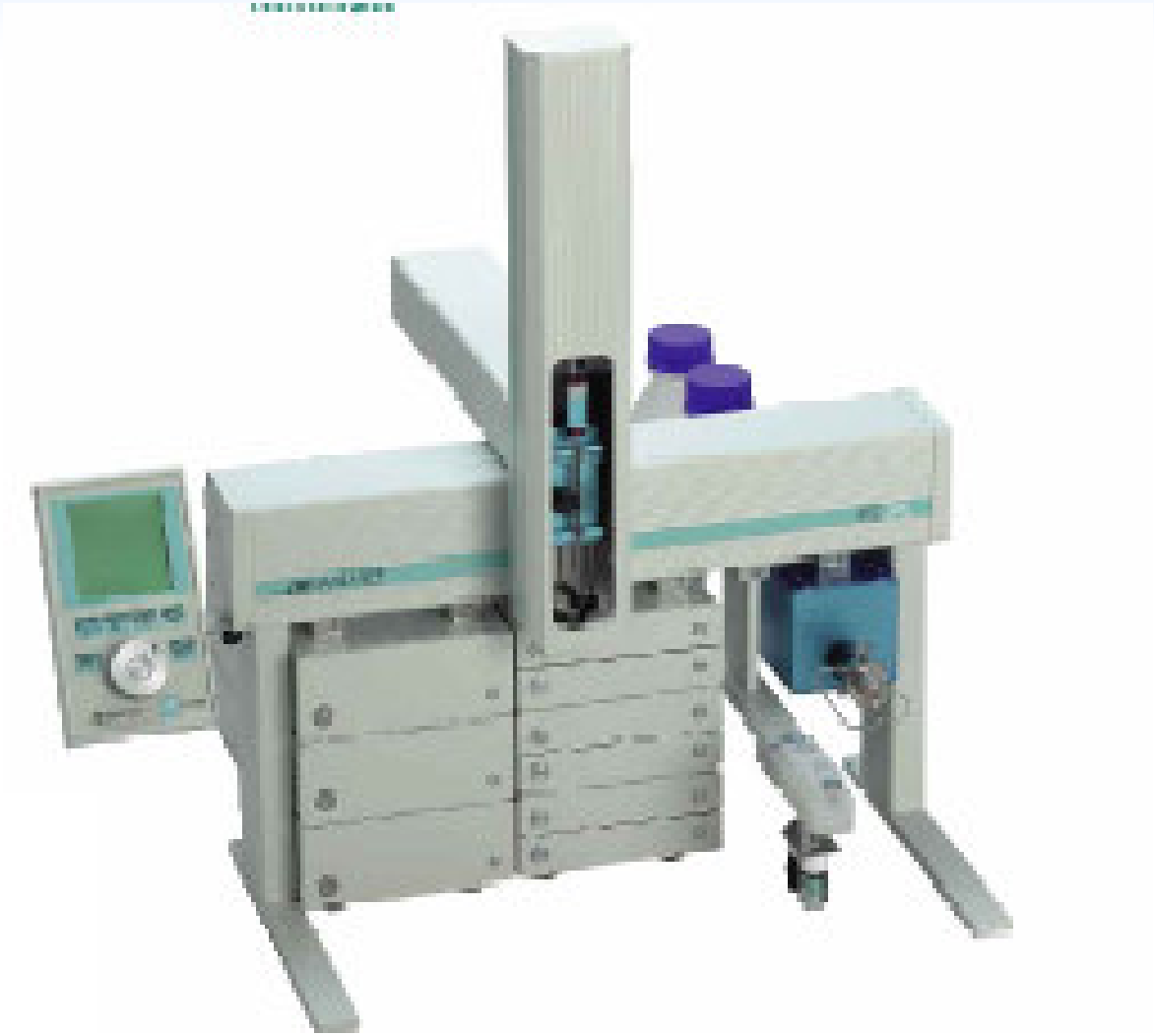
Rugged AutoInjector for LC System



Leap Technologies CTS Pal Autoinjector

March 22, 2005

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Versatile LC/MS/MS Instruments

Applied Biosystems API 4000

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Pharmacokinetics in Mice

Concentration of Compound 8 in mouse blood after oral dosing

Compound is measurable in whole blood when rapidly collected and inactivated. Efficacy appears to be C_{max} driven.

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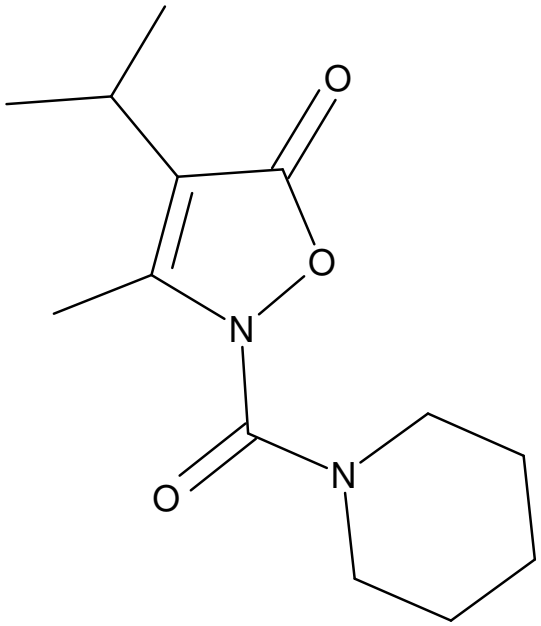
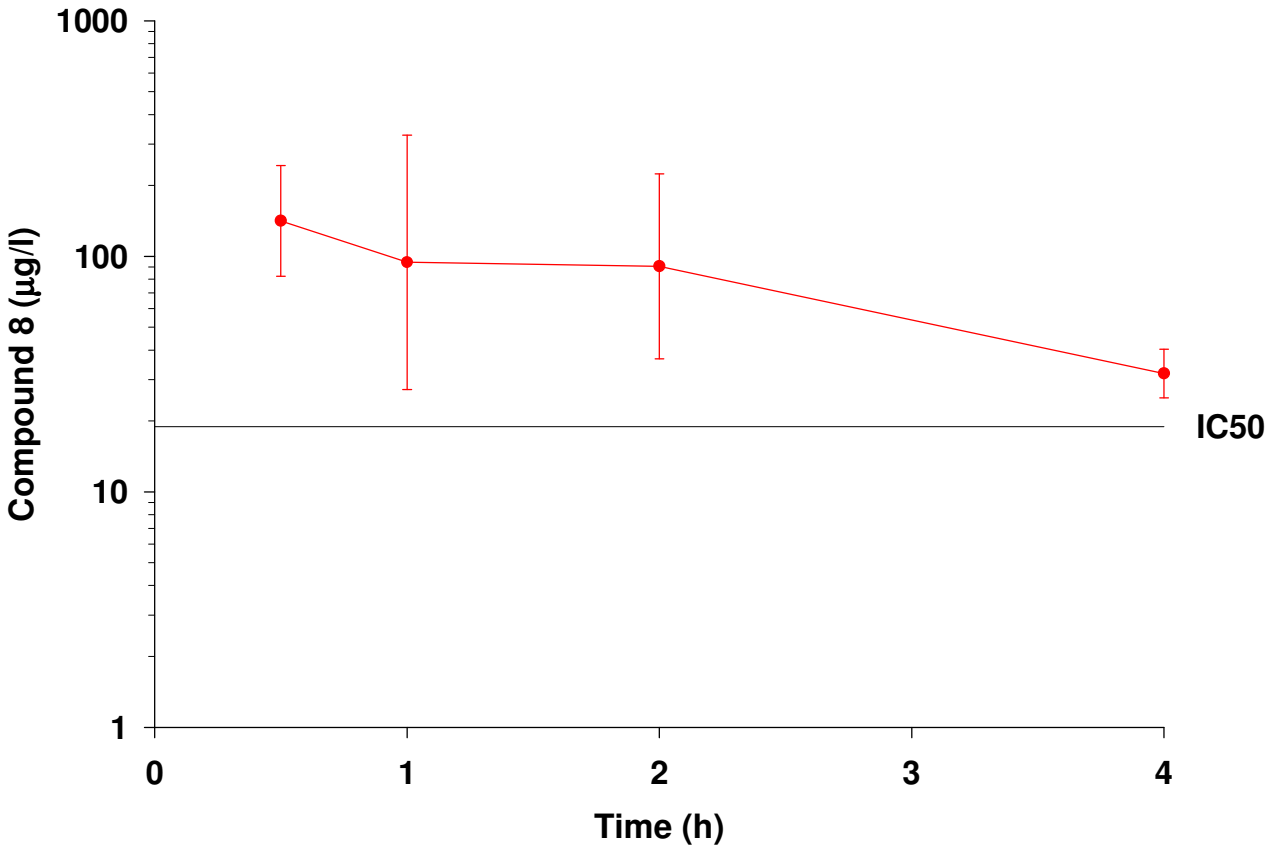
Compound 8 in Rats after Oral Administration

Rat Blood Concentrations after 100 mg/kg, PO





Compound 8 does not reach high concentrations in rats after high doses

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Compound 8 in rat blood and plasma

- Compound 8 breaks down in whole rat blood and plasma.
- Following isolation of plasma after spiking whole blood with Compound 8, the compound is found to concentrate more in red blood cells than in plasma.
- Compound 8 has about a 50-fold lower concentration than expected in serum when the compound has been spiked into whole blood prior to isolation of the serum.
- Analysis by LC/MS/MS allows the compound to be measured in whole blood or red blood cells without interference from the matrix.

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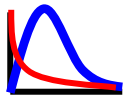
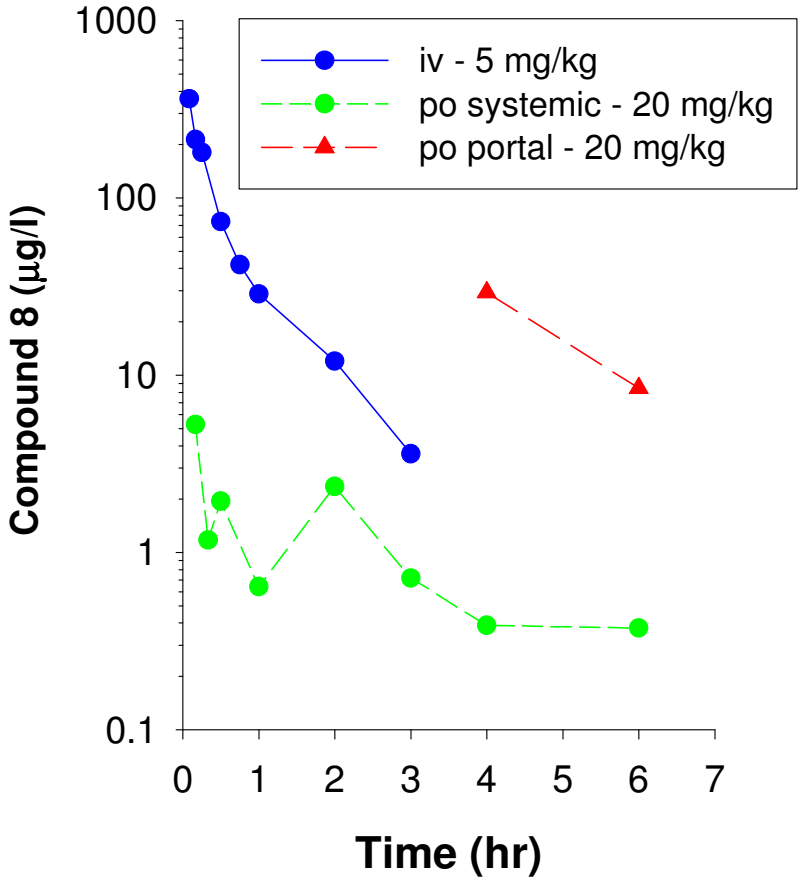


Compound 8 in Rat Blood



Comparison of portal vein concentrations and systemic concentrations suggests high first past

Microsomal Stability of Several Lead Compounds

% Compound Remaining at 10 Minutes							
Compound	Mouse (CD1)	Mouse (Balb/c)	Rat (SD)	Rat (Wistar)	Rat (STZ) (Wistar)	Dog (Beagle)	Human (pool)
Compound 8	68.7	43.5	39.7	58.9	61.2	55.5	80.2
Compound 30		31.7		18.0	43.4	34.4	82.2
Compound 57		52.9		51.9	61.3	63.4	81.8
Compound 65	17						69.7
Compound 72		44.8		78.0	77.1	81.6	72.2
Compound 76		51.8		72.2	74.8	91.7	83.1
Compound 79		29.4		59.4	62.5	90.6	77.9

The stability of all compounds in rodent is low
The extent of in vitro instability is not predictive of in vivo exposure

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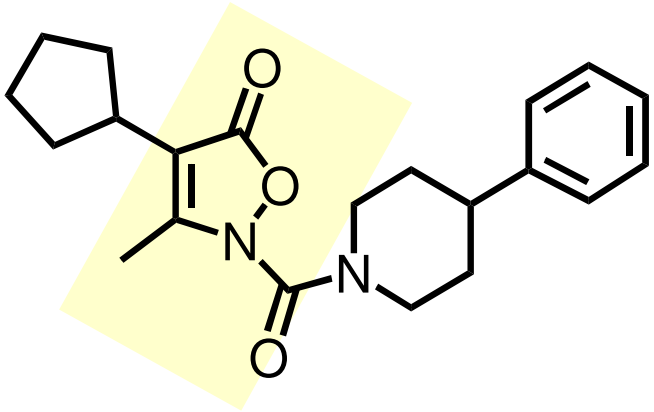
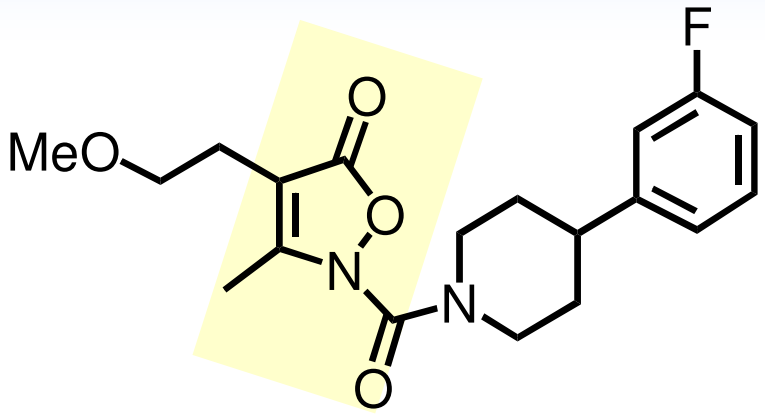
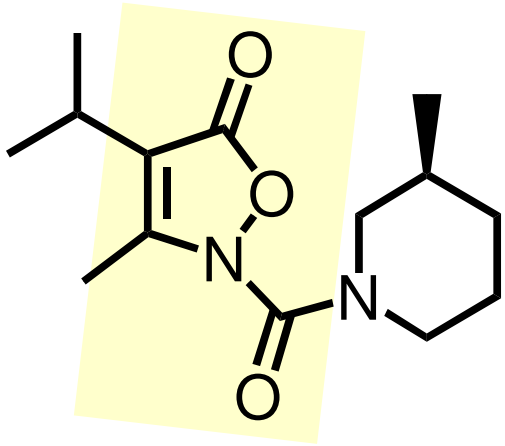
Lead Structures



Compound 72



Compound 76



Compound 57

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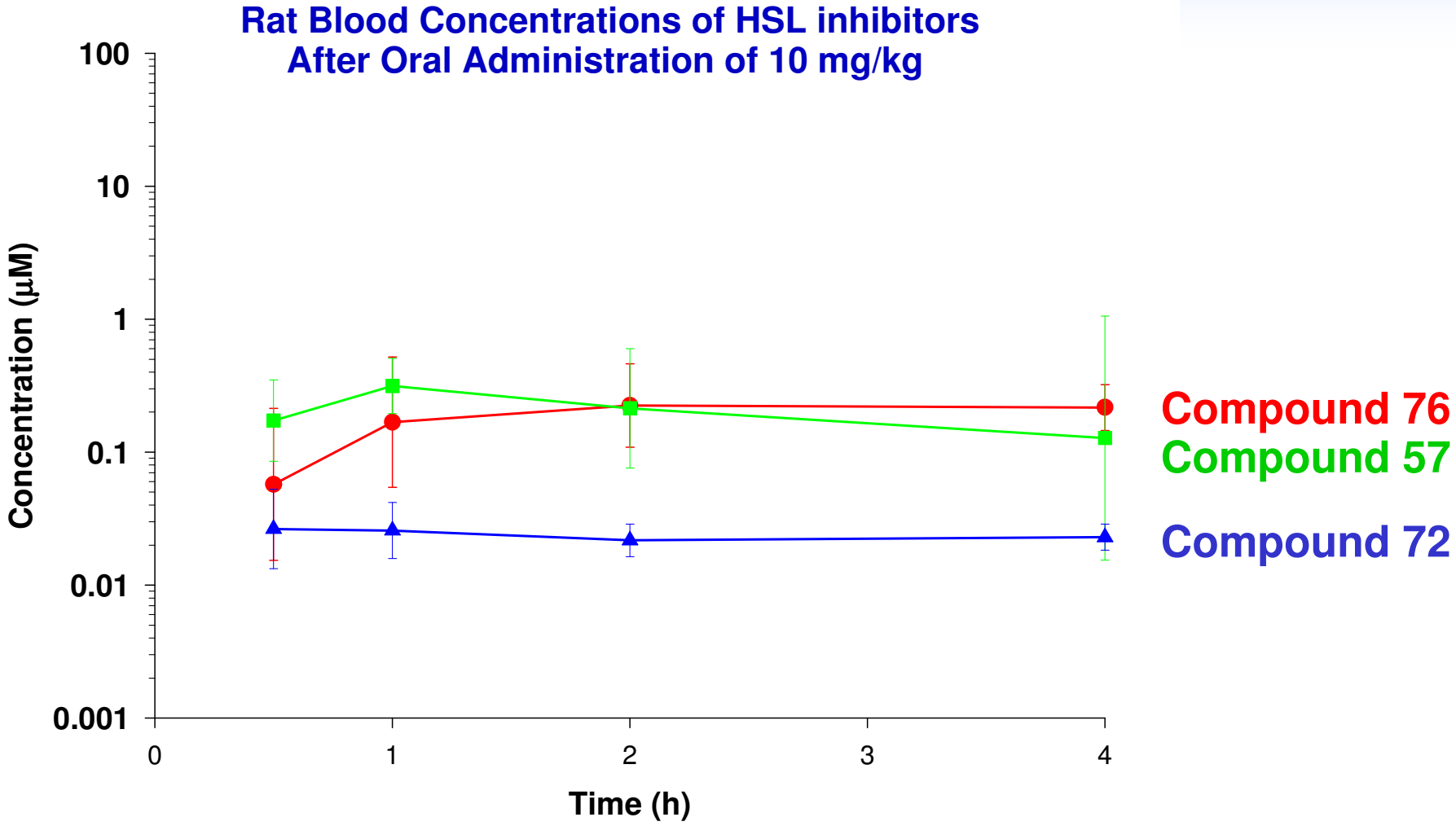
Exposure of 3 lead HSL Compounds in Rats



Compound 57 shows highest concentration at 1 h

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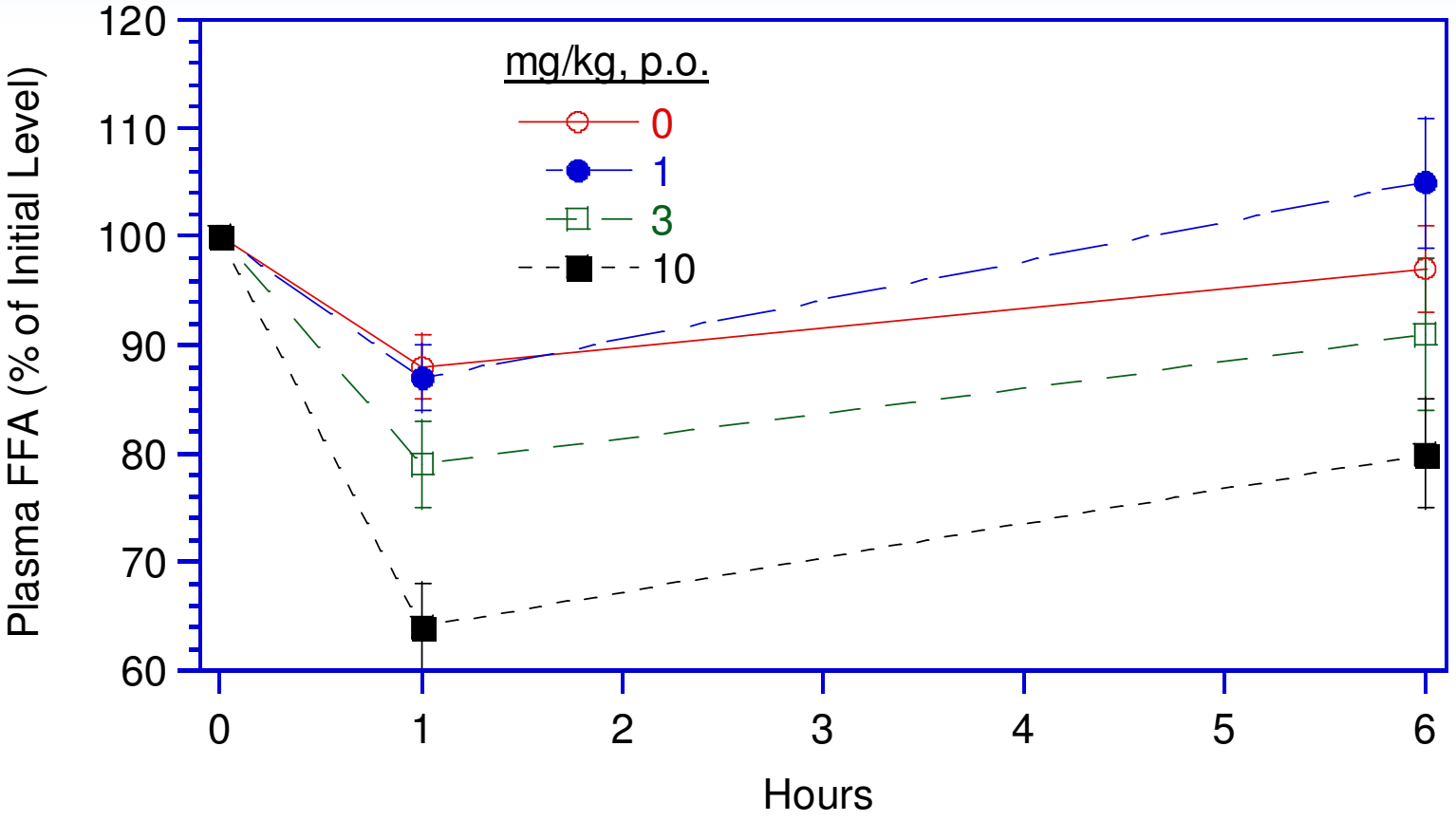
Efficacy of Compound 57 in Fasted Mice



Compound 57 shows dose-dependent efficacy in mice

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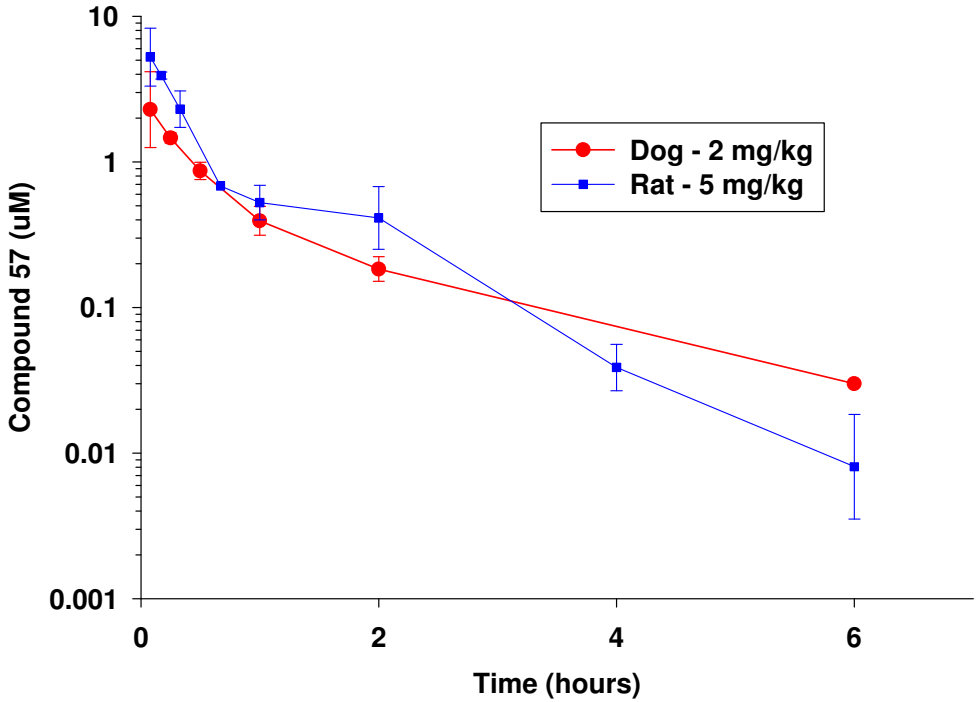
PK after Intravenous Administration

Concentrations of Compound 57 in blood after intravenous administration



Species	Dose	$AUC_{0\text{-}tn}$	C_0	$t_{1/2}$	Cl	Vd_{ss}
	mg/kg	(mg*h)/l	mg/l	h	l/h/kg	l/kg
Dog	2	235	846	1.14	4.25	4.64
Rat	5	161	2160	0.70	6.19	4.91

High clearance and volume of distribution in rat and dog

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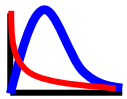
Identifying the best compound

HSL inhibition	> 800 (> 450 synthesized)
Cellular lipolysis assay	> 350 compounds
Selectivity	> 300 compounds
Fasted mouse model	50 compounds
Toxicity Screening	29 compounds
2-Week Rat Tox	4 compounds
Dog Tox	(1 compound)
DP1	

Pharmacokinetics did not play a major role in final compound choice

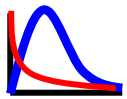
Compound 57 in vivo Properties

- shows good efficacy but low systemic exposure
- shows low stability in liver microsomes from all species tested, and rat blood and plasma
- high volume of distribution in both rat and dog
- showed toxicity in gut wall and testes
- might be concentrating in the tissues where it was causing toxicity
- radiolabeled material was made and a whole body autoradiography in rats was undertaken to check for accumulation

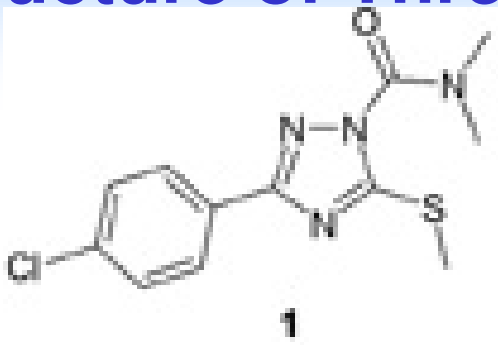


Conclusion of HSL Project

- Could not move forward into development because of issues of toxicity and inadequate PK
- Chemistry could not change core of molecule without losing activity
- The program has not moved forward into clinical studies



Structure of Three Competitor HSL Inhibitors



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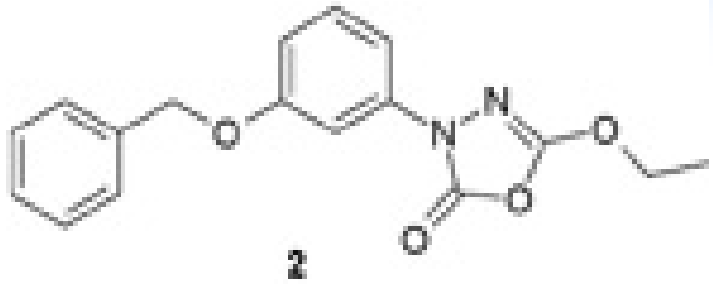

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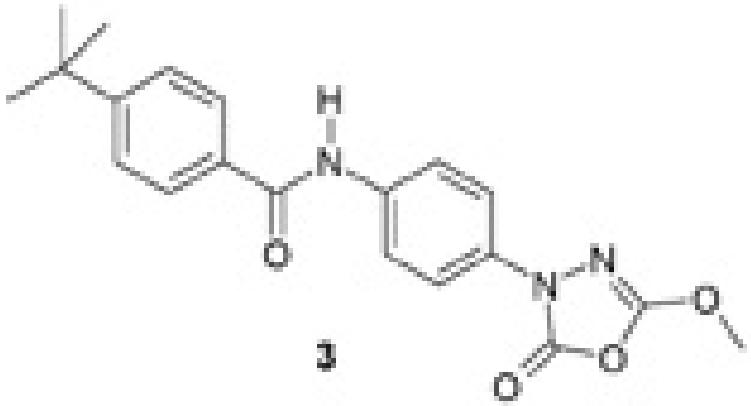

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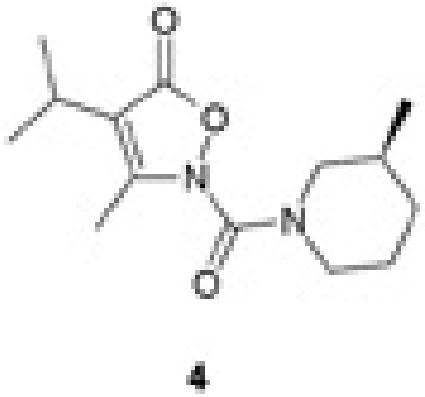


4

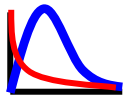
1. Novo Nordisk 2. Aventis 3. Aventis 4. Bayer

Ebdrup, S.; Sorensen, L. G.; Olsen, O. H.; Jacobsen, P. Synthesis and Structure-Activity Relationship for a Novel Class of Potent and Selective Carbamoyl-Triazole Based Inhibitors of Hormone Sensitive Lipase. J. Med. Chem. **2004; 47, (2), 400-410.**

HSL Knockout mice

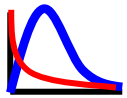
- HSL-deficient male mice are infertile
- Testes of male HSL-deficient mice are abnormal
- HSL appears to play a major role in spermatogenesis

Infertility and testicular defects in hormone-sensitive lipase-deficient mice. Chung S, Wang SP, Pan L, Mitchell G, Trasler J, Hermo L. Endocrinology. 2001 Oct;142(10):4272-81.



Lessons Learned

- It is very difficult to advance to human studies with efficacious compounds that have bad PK
- There is little that you can do with a chemical series if the core is metabolically unstable
- It is normally best to start a project with more than one chemical series
- Even the most promising methods of treatment can't produce a product unless a compound with good PK and tox profiles can be found



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