

# **ADME/TOX in Drug Discovery: Assay Design and Case Studies**

**Li Di**

**Wyeth Research**

**Princeton, NJ**

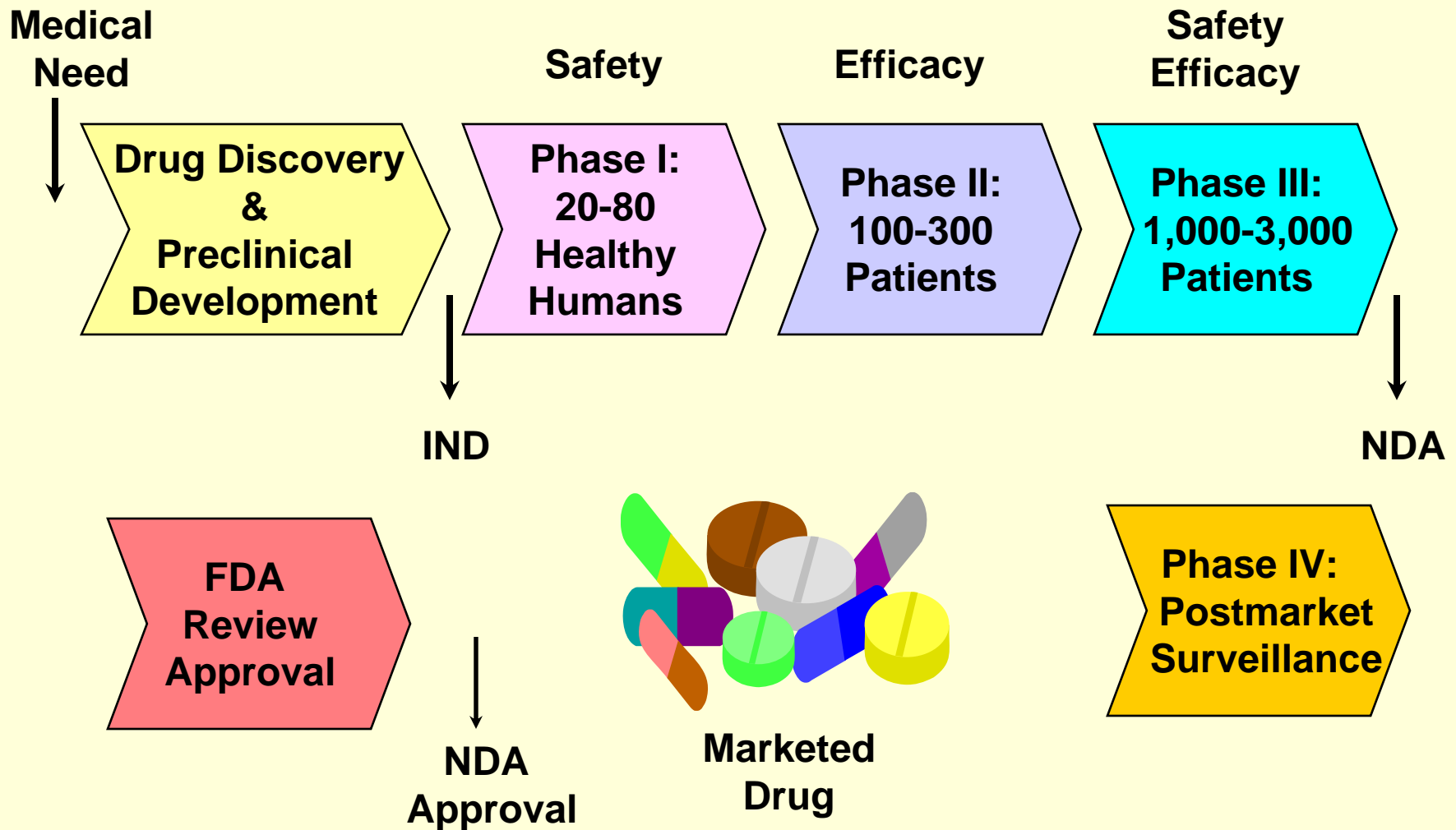
**LRIG**

**Somerset, NJ**

**September 20, 2007**

**Wyeth**  
Research

# Stages of Drug Discovery and Development Process



# Drug Discovery and Development Cascade

<b>High Throughput Screening</b>	<b>1,000,000</b>
<b>HTS Hits</b>	<b>2,000</b>
<b>HTS Actives</b>	<b>1,000</b>
<b>Discovery Program</b>	<b>50</b>
<b>Drug Candidate</b>	<b>10</b>
<b>Drug</b>	<b>1</b>

**12 years, \$800 million, 10% Success Rate**

M.M. Hann & T. I. Oprea, Curr Opin Chem Bio, 2004, 8(3), 255-263

M. Dickson, J. P. Gagnon, Nature Rev Drug Disc. 2004, 3, 417 - 429

**Wyeth**  
Research

# Importance of Pharmaceutical Profiling

---

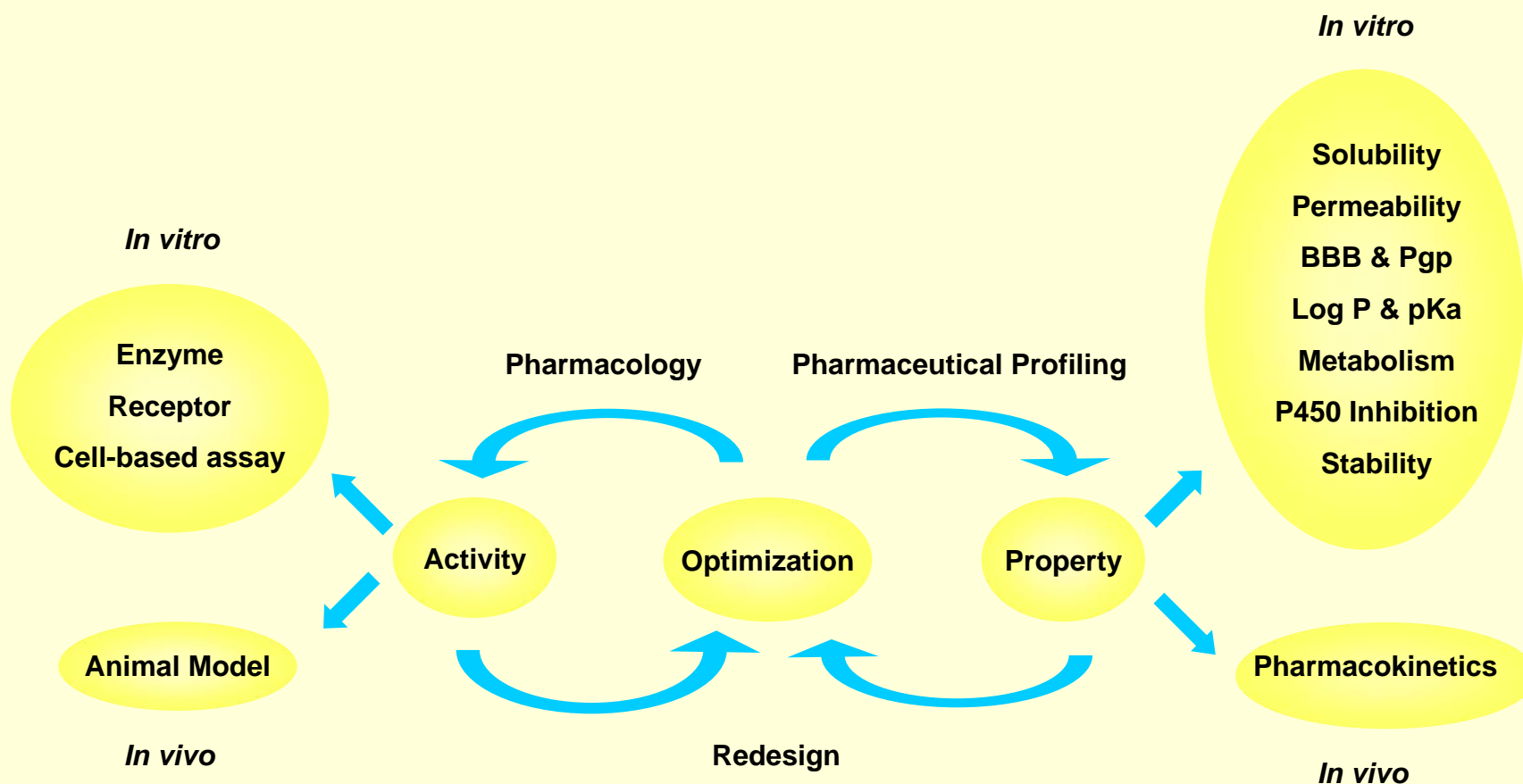
<u>Reason for Failure</u>	<u>Percentage</u>
Toxicity	22 %
Lack of Efficacy	31 %
Market Reasons	6 %
<b>Poor Biopharmaceutical Property</b>	<b>41%</b>

# ADME-TOX Properties

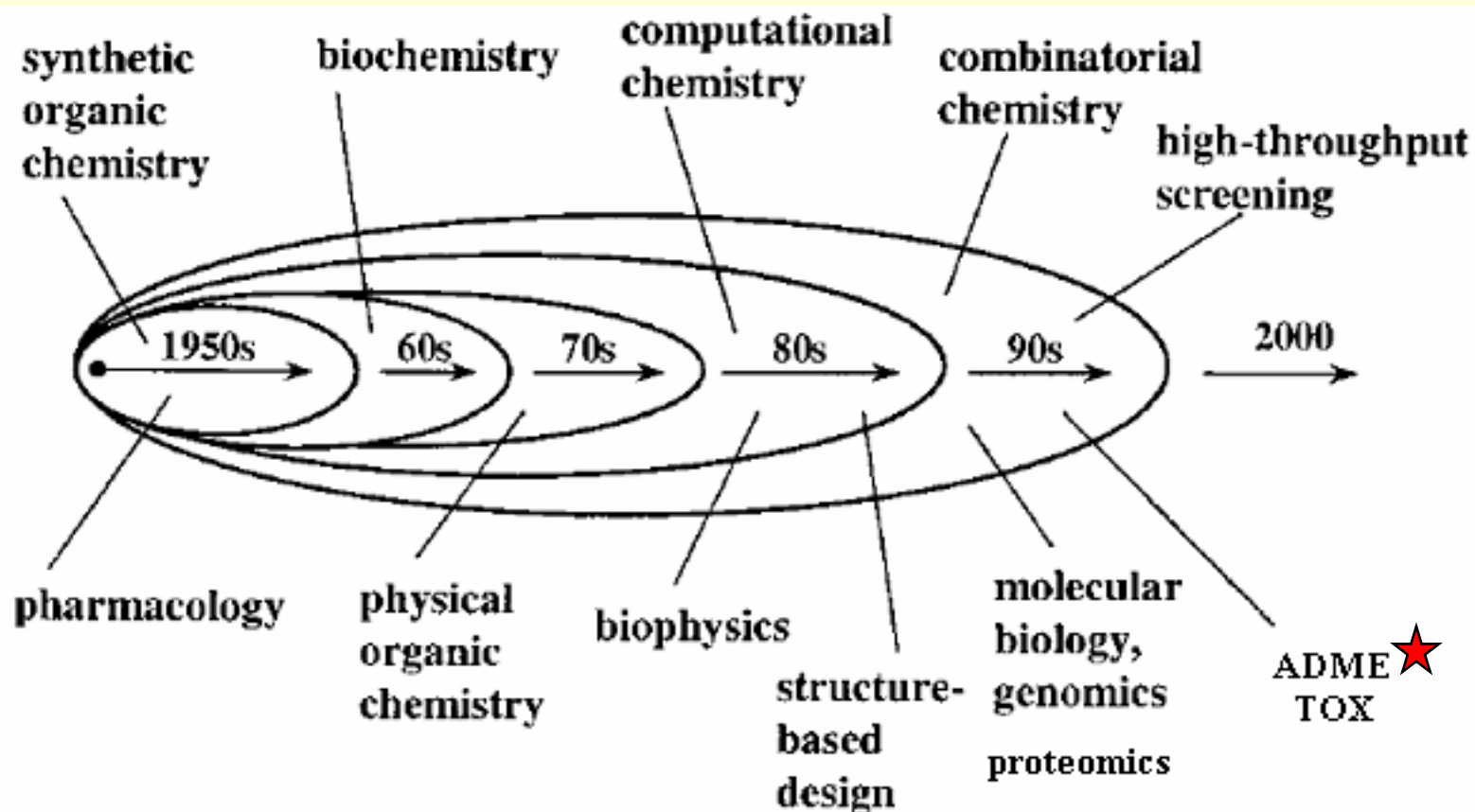
---

- **A**bsorption
- **D**istribution
- **M**etabolism
- **E**xcretion
- **T**oxicity

# Successful Drug = Potency + Properties

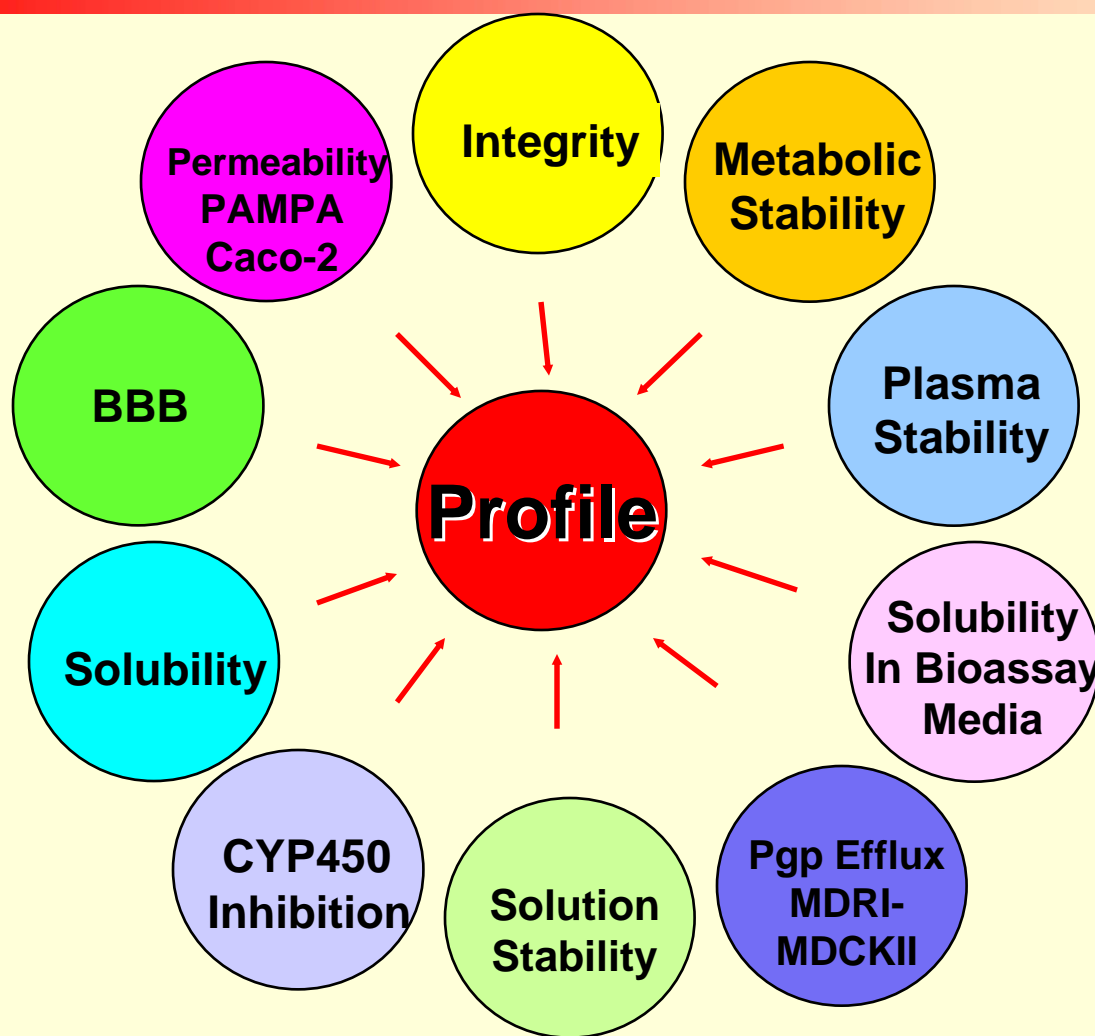


# ADME/TOX Screening: New Tool for Drug Discovery



Modified from Han van de Waterbeemd, et al, *J. Med. Chem.*, 1313-1333, 44(9), 2001

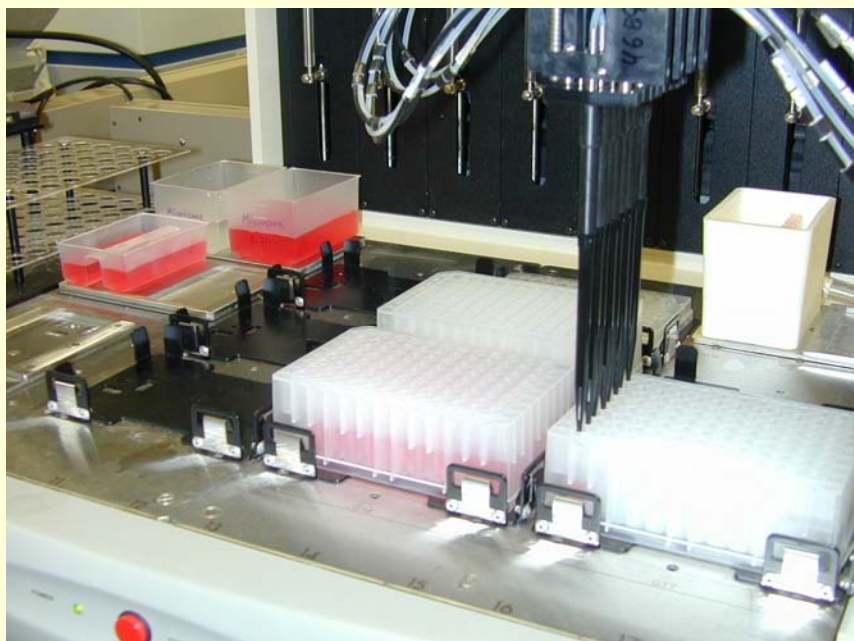
# Wyeth Pharmaceutical Profiling Assays



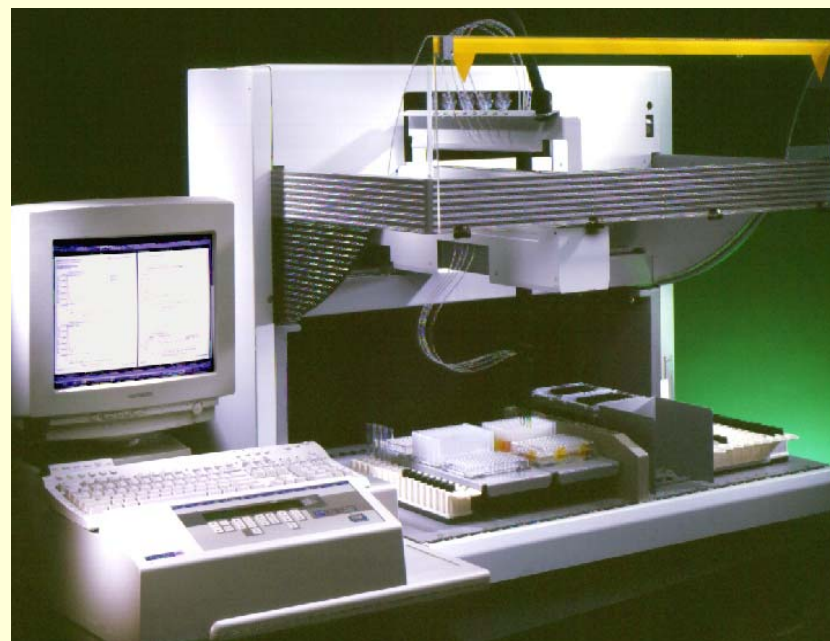
- High Throughput
- 96-Well Format
- ~ 3 mg Material
- Timely: 1- 2 Weeks

Modified from Edward H. Kerns, J. Pharm. Sci., 2000, 90(11), 1838-1858

# Robotic Sample Preparation



**Packard Robot**  
**Microsomal Stability**  
**CYP450 Inhibition**



**Tecan Robot**  
**PAMPA, BBB**  
**Solubility**

# High Throughput Sample Analysis



**LC-MS-MS**

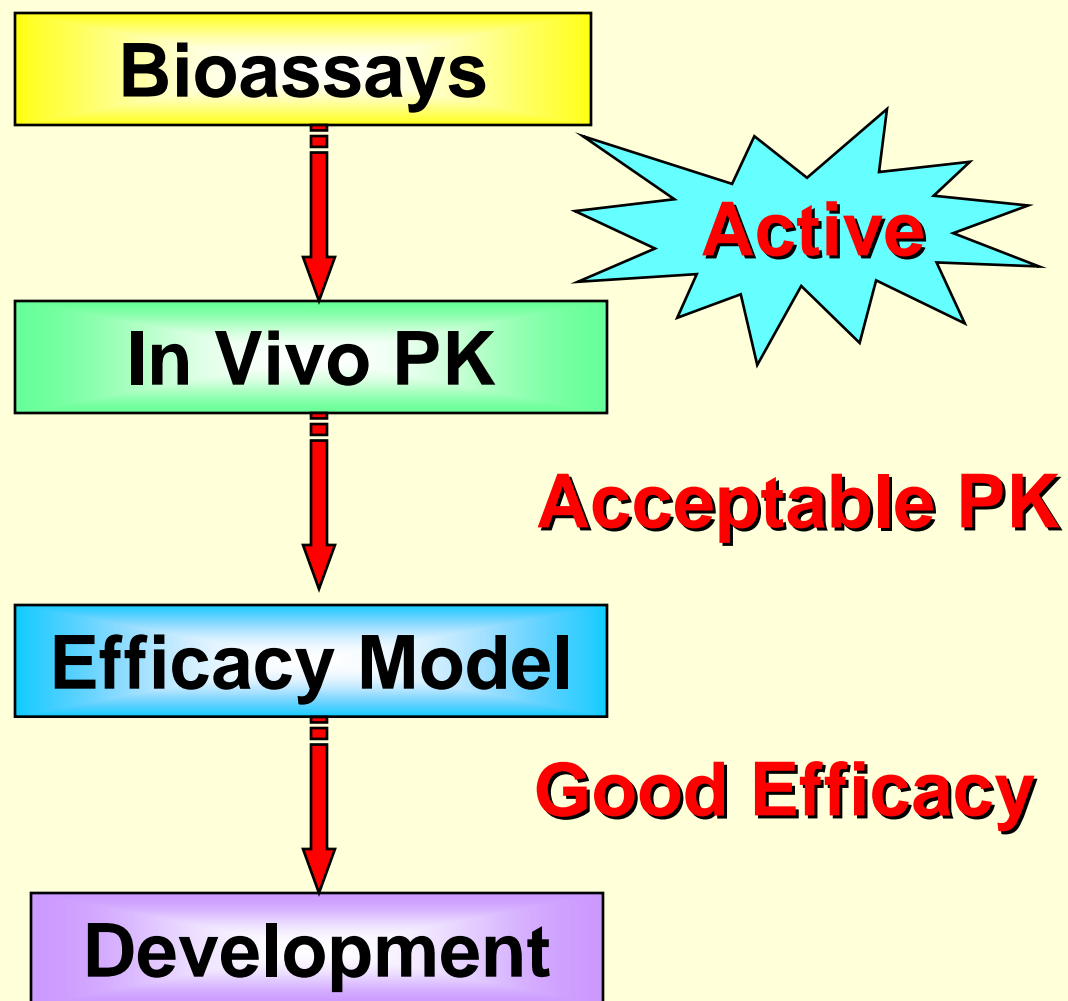


**Fluorescent Plate Reader**



**UV/Vis Plate Reader**

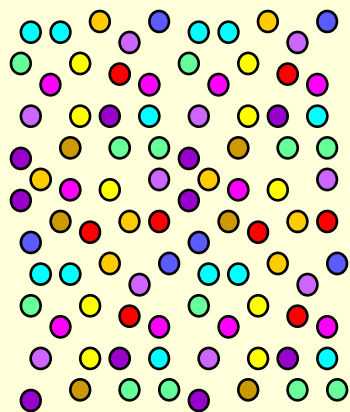
# Screening Paradigm in the Past



**Activity was the sole driver for discovery programs**

# Past: Activity-Driven Screening

Compounds



Bioassays



Very Active

$K_i = 7 \text{ nM} !$

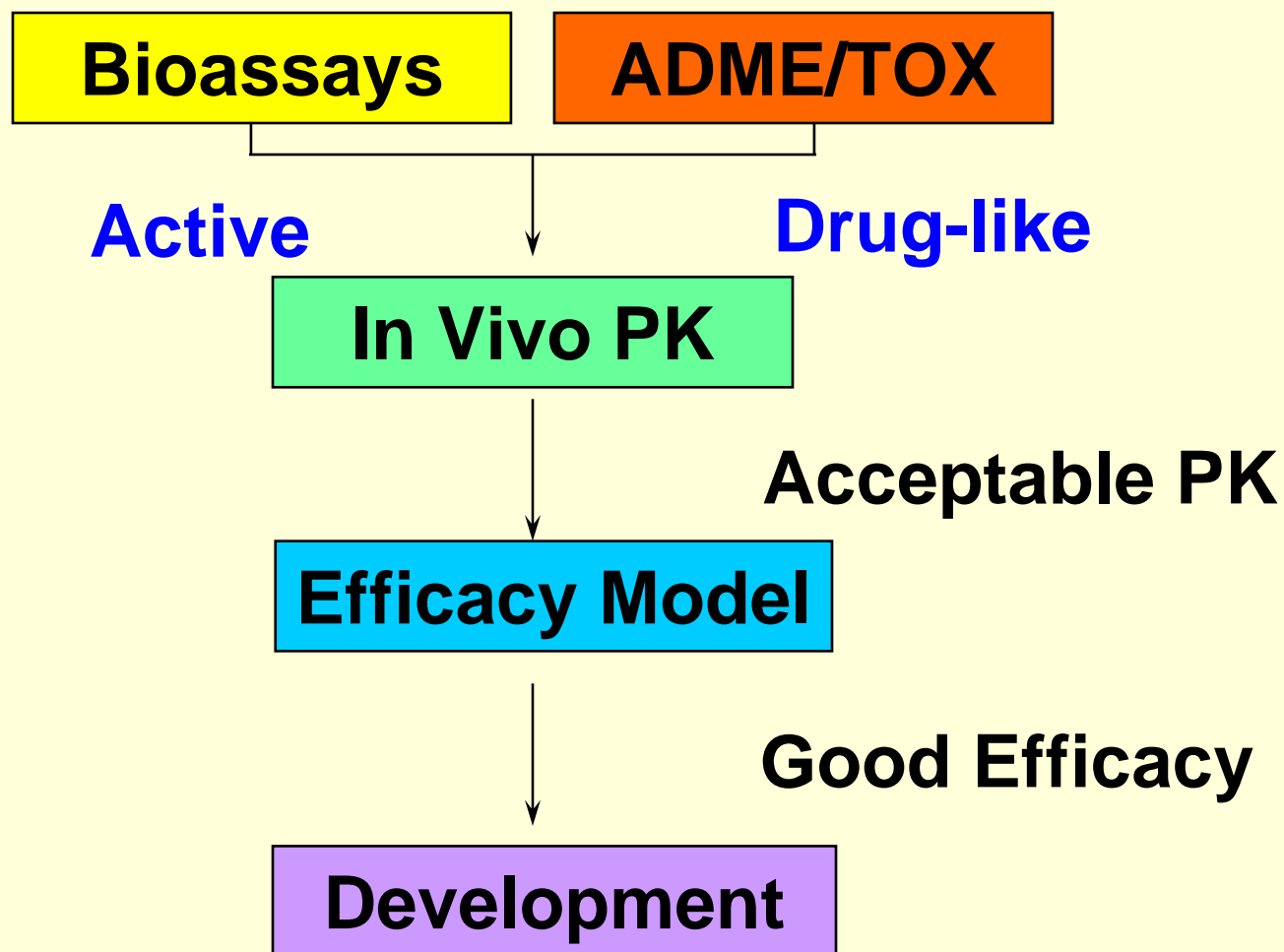


$F\% = 0$

Inactive  
Terminated

Long Cycle Time  
High Cost  
Low Success Rate

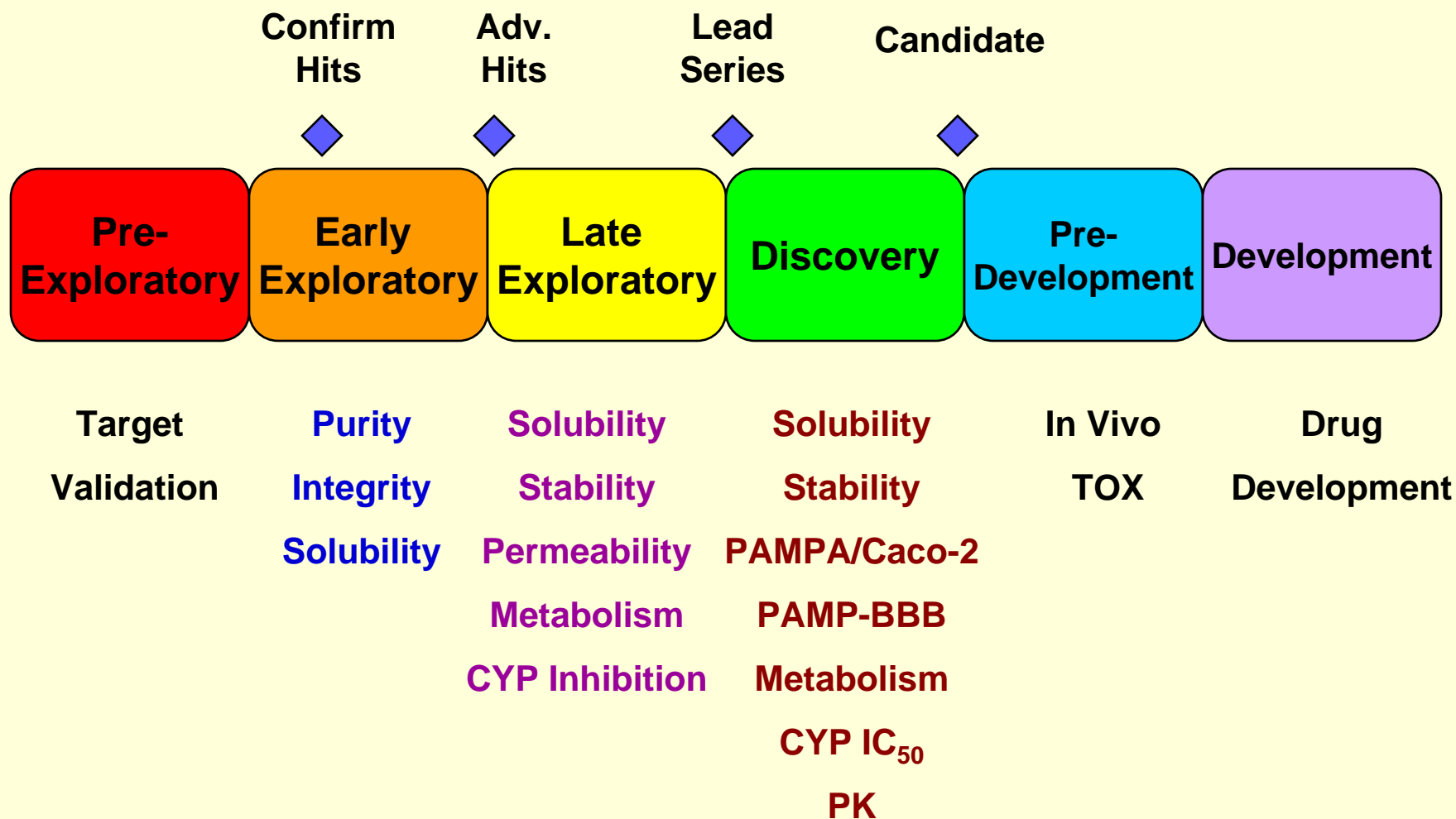
# Present Drug Discovery Screening Paradigm



**ADME/TOX: Advancement Criteria**

**Wyeth**  
Research

# ADME/TOX Screening in Drug Discovery



**Ensure Quality of Development Candidates**

# Industry Development ADME/TOX Criteria

---

- Solubility > 100  $\mu\text{g/mL}$
- Oral Bioavailability > 20%
- CYP  $\text{IC}_{50}$  > 10  $\mu\text{M}$  or  $\text{C}_{\text{max}} / \text{K}_i < 0.1$
- hERG  $\text{IC}_{50} / \text{C}_{\text{max}} > 30$

---

---

**“You can’t manage it, if you can’t measure it. ”**

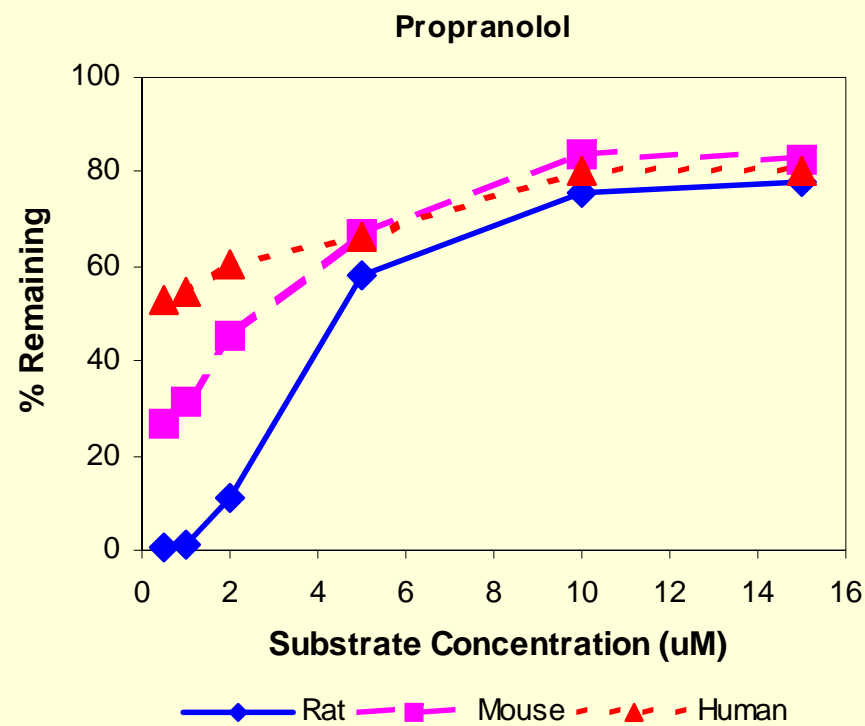
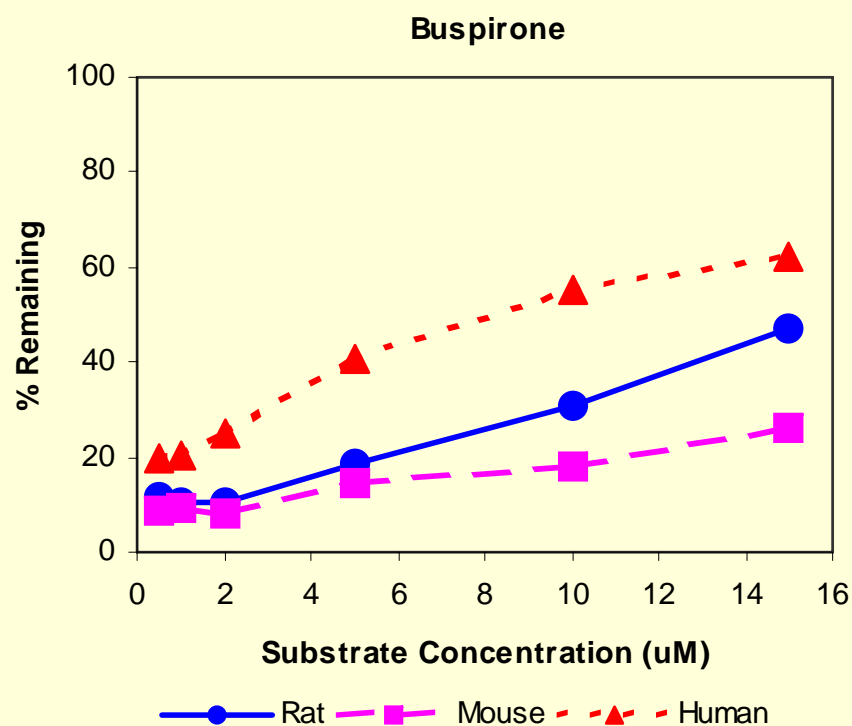
**Robert R. Ruffolo**  
**Wyeth R&D President**

# Critical Factors for Successful ADME/TOX Profiling

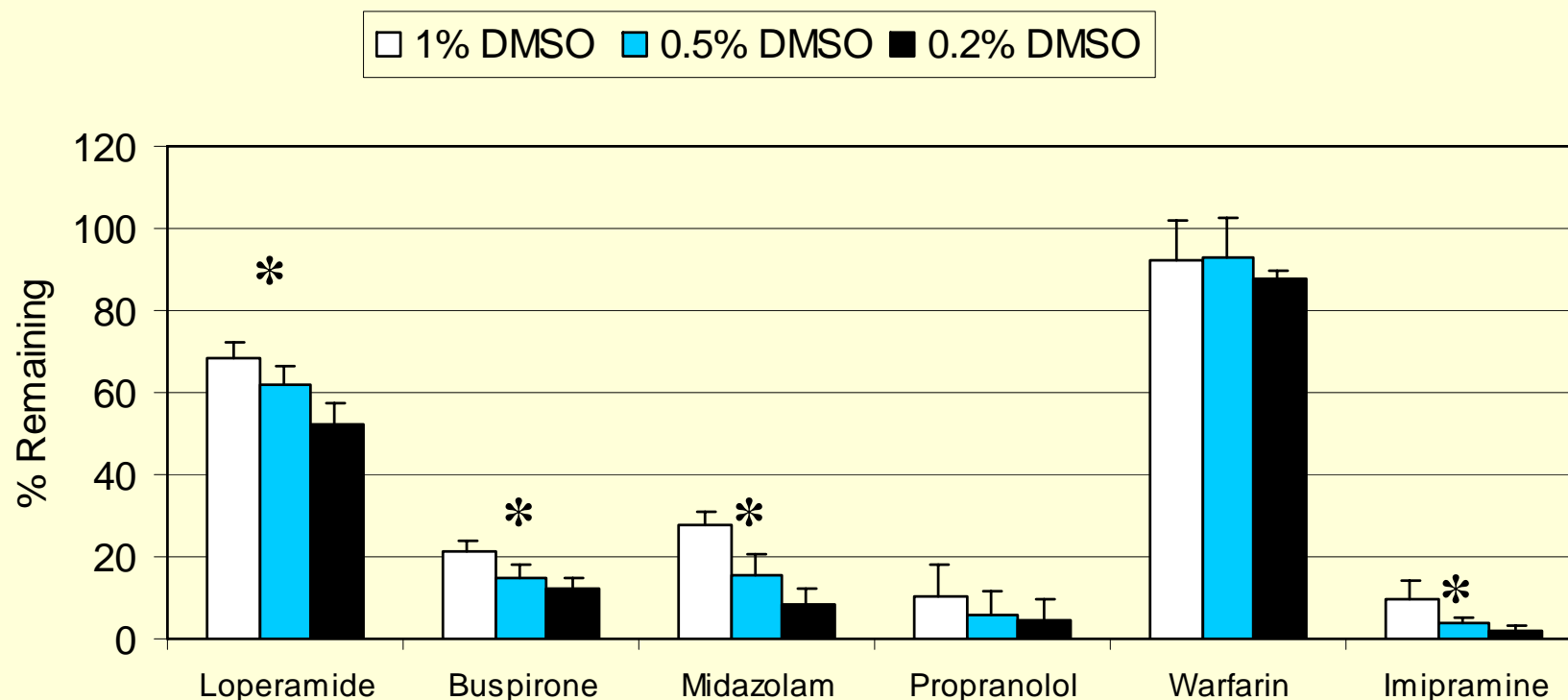
---

- **Assay Design**
- **Throughput**
- **Speed**
- **Timing**

# Effect of Substrate Concentration on Microsomal Stability

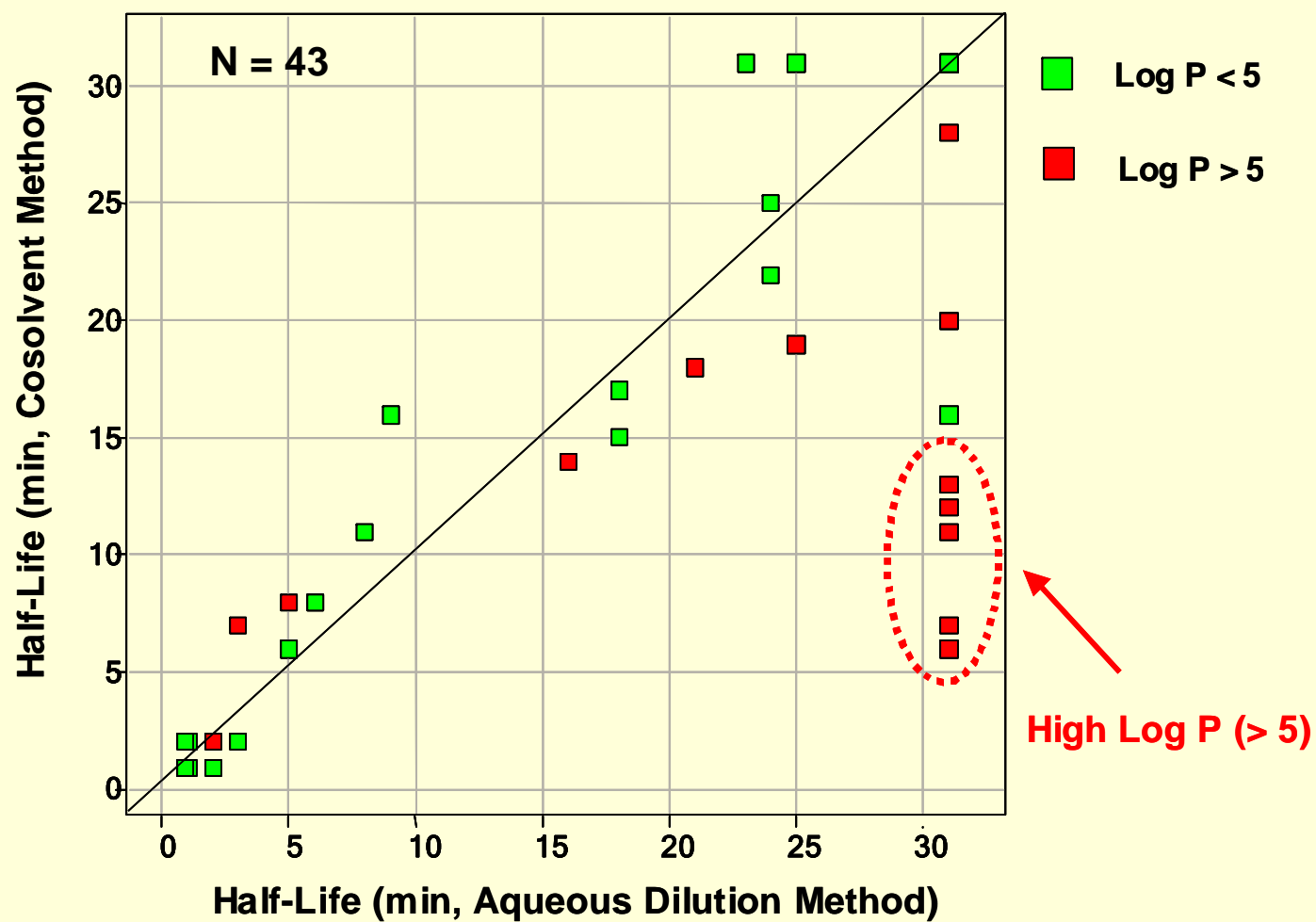


# Effect of DMSO on Microsomal Stability Assay



Assay is sensitive to DMSO. Compounds (\*) show statistically significant difference between 1% and 0.2% using LSD test at P-value < 0.05.

# Avoid Aqueous Dilution



## Microsomal Stability: Single Time Point vs. Multiple Time Point

Validation Data	t1/2 (mins)	t1/2 (mins)
Compounds	Wyeth	Literature*
Midazolam	3	4
Verapamil	6	10
Diltiazem	15	21
Zolpidem	> 30	44
Tenoxicam	> 30	38

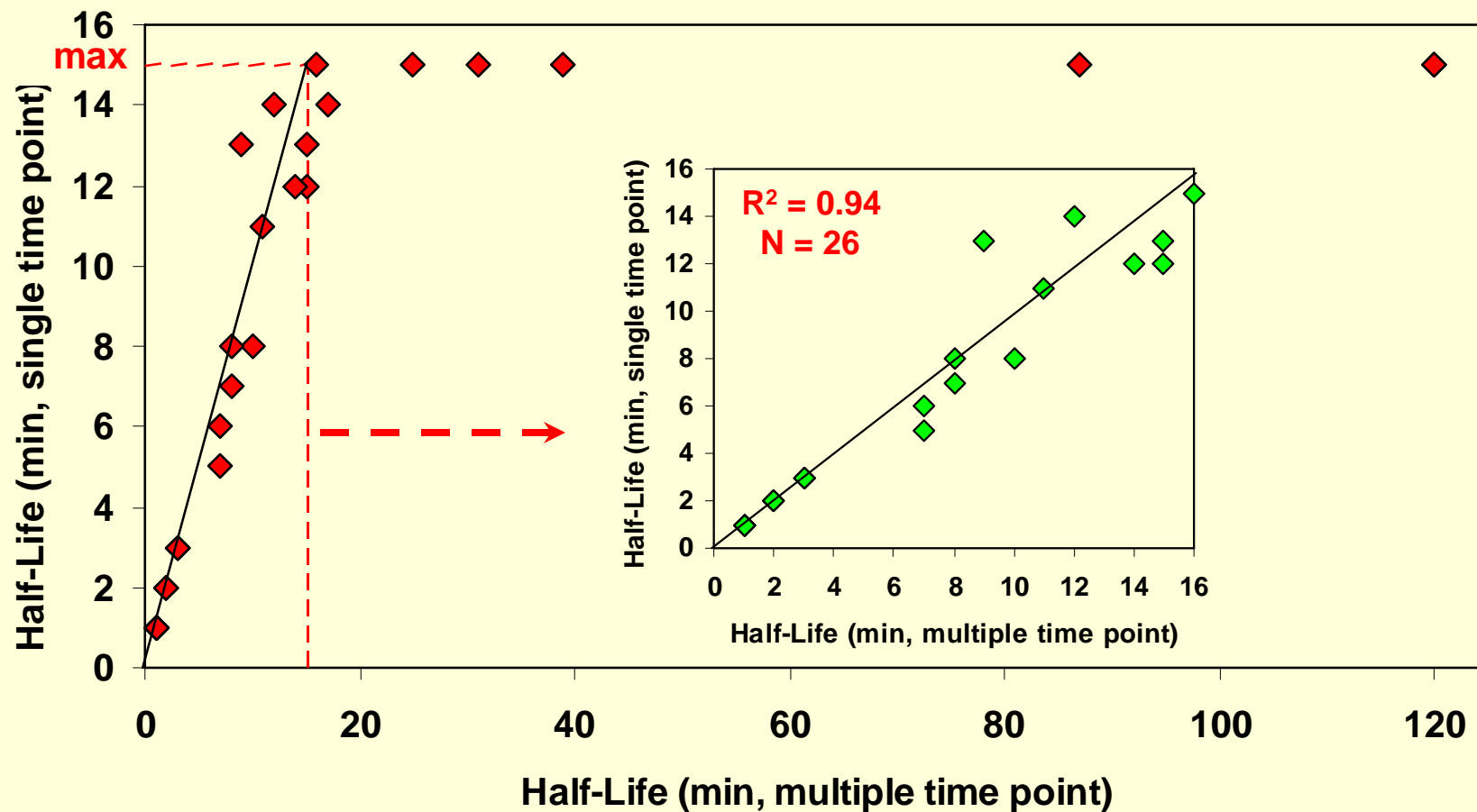
\* Scott Obach, Drug Met. Disp., 1999, 27(11), 1350-1359

**Good correlation between single time point and multiple time point**

Li Di, Ed Kerns, et al, J. Pharm. Sci. 2004, 93, 1537-1544.

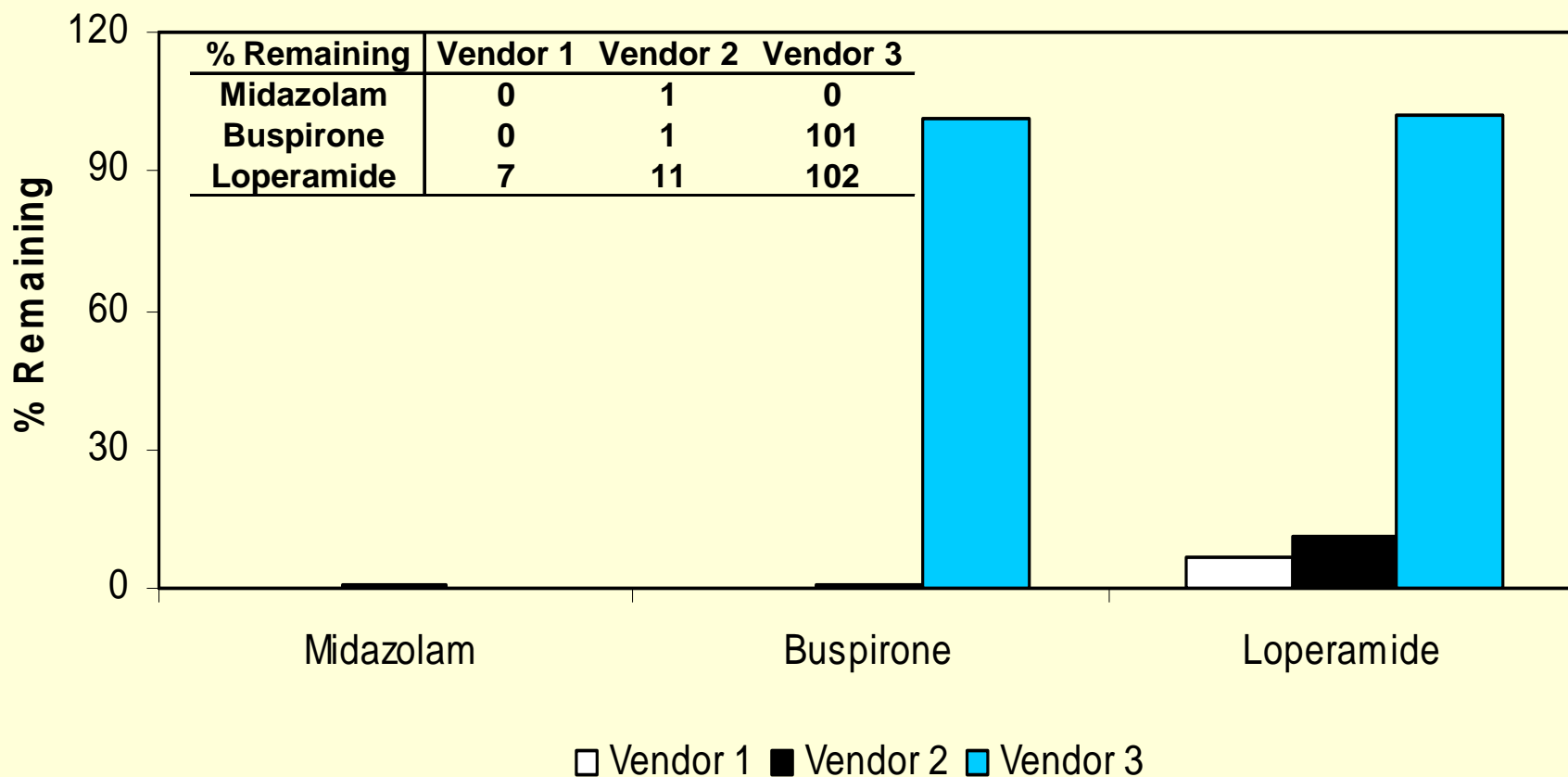
**Wyeth**  
Research

# Correlation of Single Time Point and Multiple Time Point for Wyeth Compounds (5 min incubation)



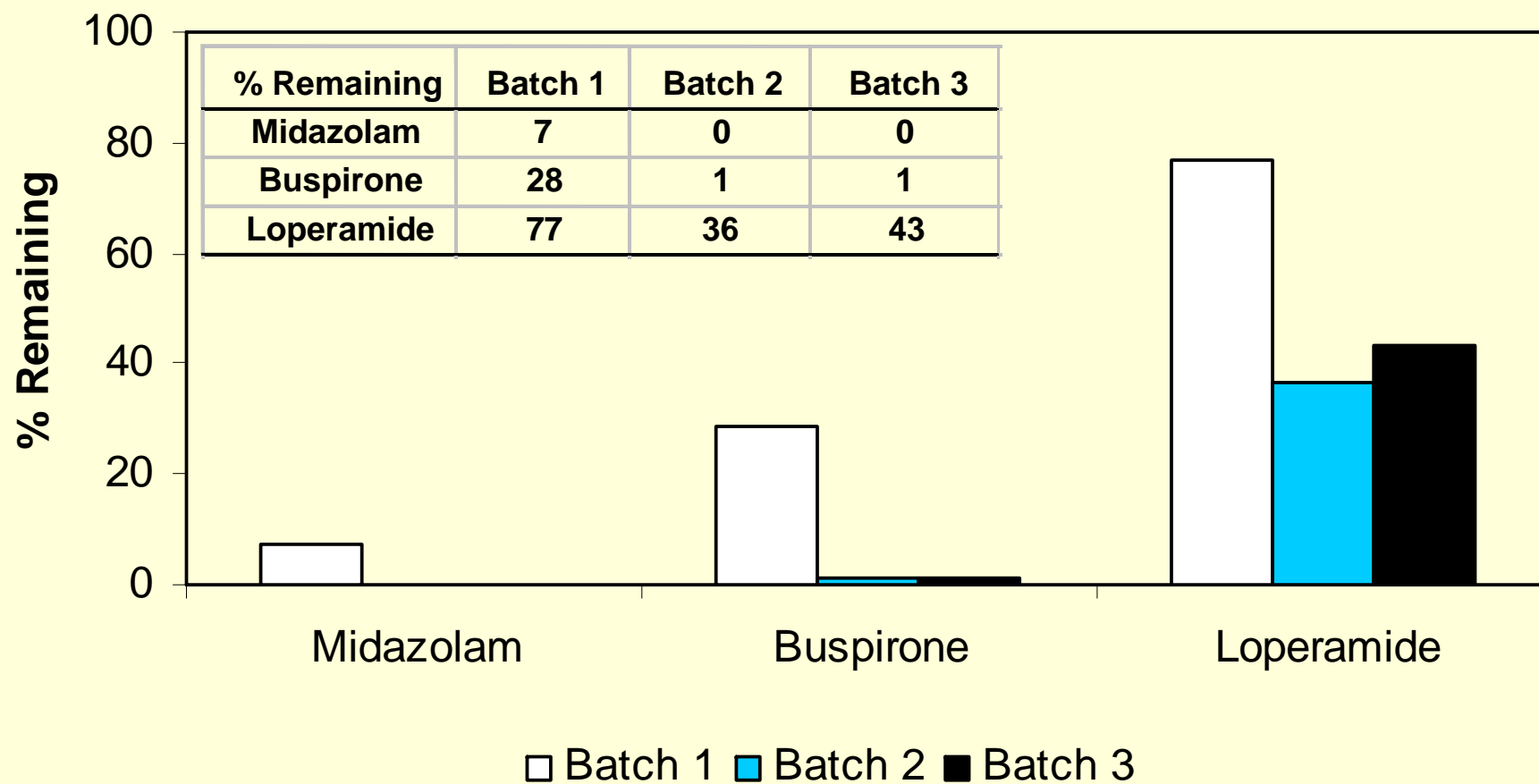
Li Di, Ed Kerns, et al, J. Pharm. Sci. 2004, 93, 1537-1544.

## Vendor-to-Vendor Variation of Rat Liver Microsomes



**Large variation from vendor-to-vendor**

# Batch-to-Batch Variation of Rat Liver Microsomes



# Final Microsomal Assay Conditions

---

- **Compound concentration: 1  $\mu$ M**
- **Protein concentration: 0.5 mg/mL**
- **Cosolvents: 0.2% DMSO, 0.8% ACN**
- **No aqueous dilution**
- **Incubation time: 15 min**

Li Di, Ed Kerns, et al, Int. J. Pharm. 2006, 317, 54-60

Li Di, Ed Kerns, et al, J. Pharm. Sci. 2004, 93, 1537-1544.

Li Di, Ed Kerns, et al, J. Biomol. Screening, 2003, 8, 453-462.

# Past and Present Assay Design

---

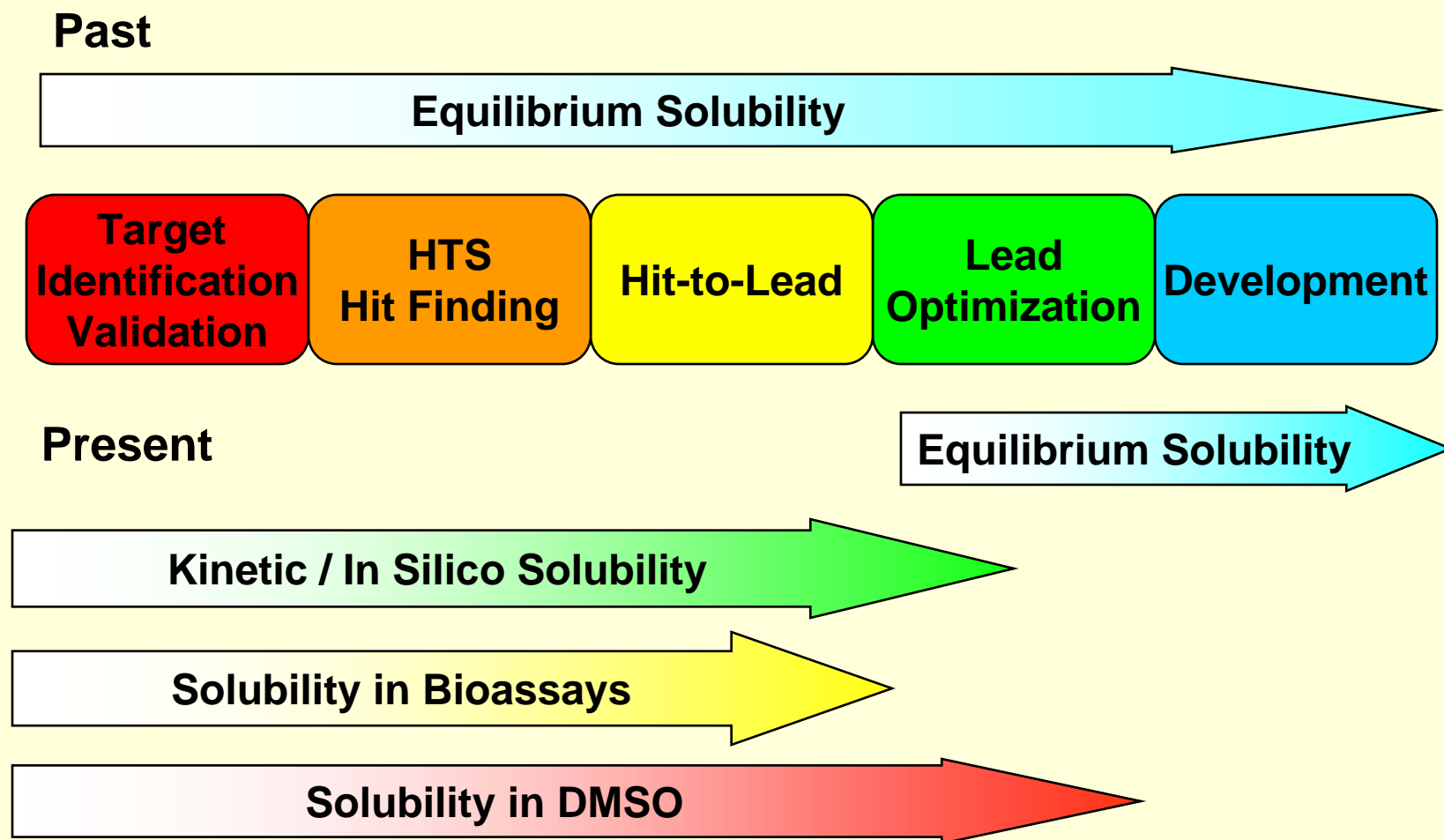
- **Past**

- ▶ One size fits all
- ▶ Multiple time points
- ▶ Multiple concentrations ( $IC_{50}$ )

- **Present**

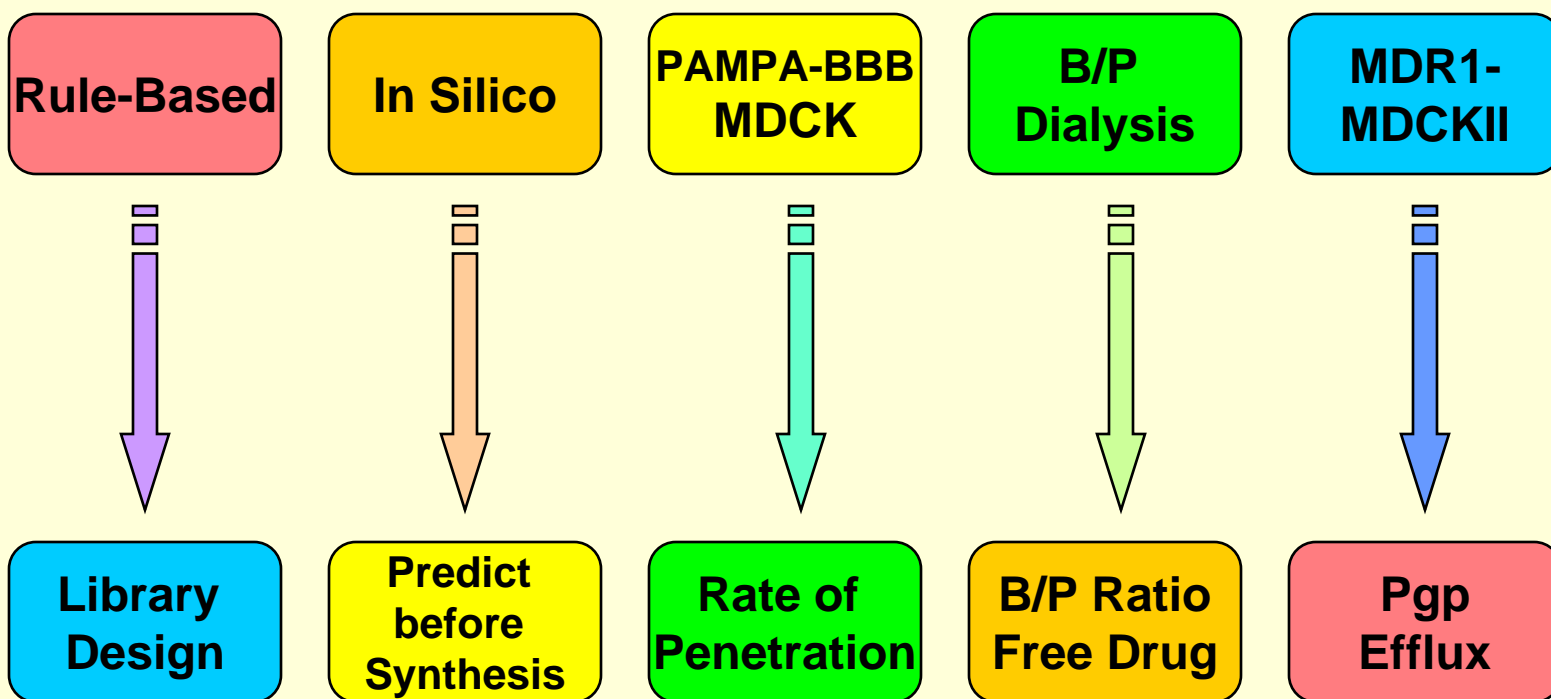
- ▶ Tier approach
- ▶ Custom assays

# Solubility Assays in Drug Discovery



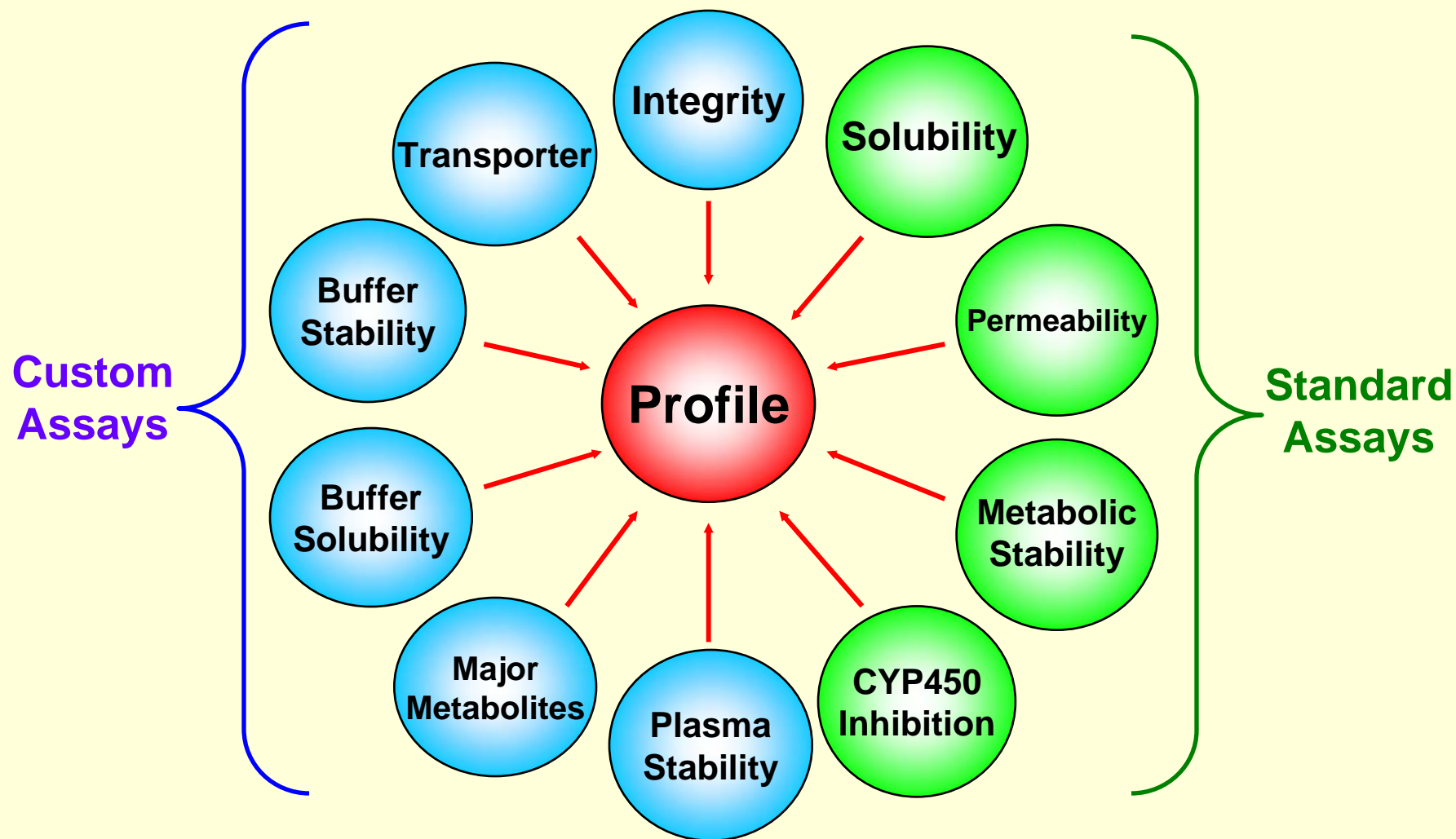
**Different Assays at Different Stages for Various Purposes** **Wyeth**  
Research

# Tier Approach to Blood-Brain Barrier



**Determine Underlying Mechanisms**

# Standard and Custom Assays



**Informed Decisions**

**Wyeth**  
Research

## Throughput: Past - Low; Present - High

### Past

- 500 / year
- Limited information
- Not enough for SPR
- Not for selection
- Profile only actives

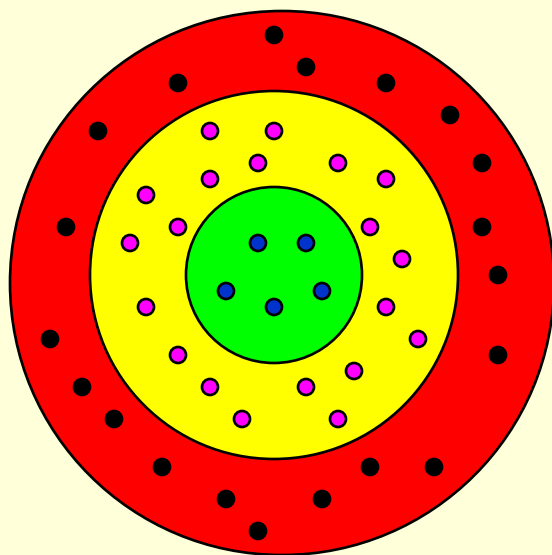
### Present

- 50,000 / year
- Rich information
- Guide SPR
- Selection criteria
- Profile all

# Past: Long Turnaround Time

## Present: Short Turnaround Time

Turnaround Time = 1 month



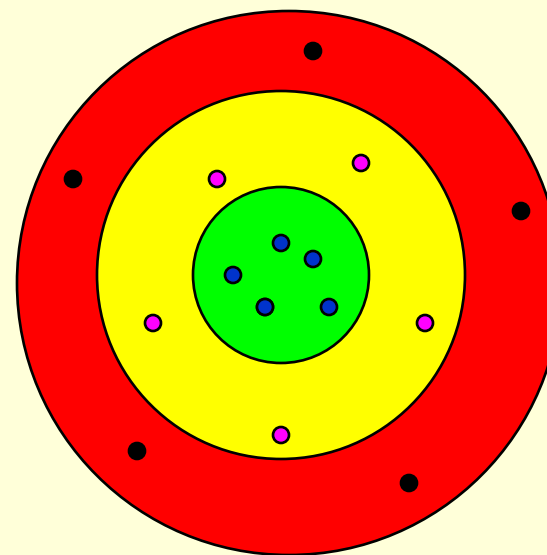
**40** Compounds  
**2 months**

■ unstable

■ moderate

■ stable

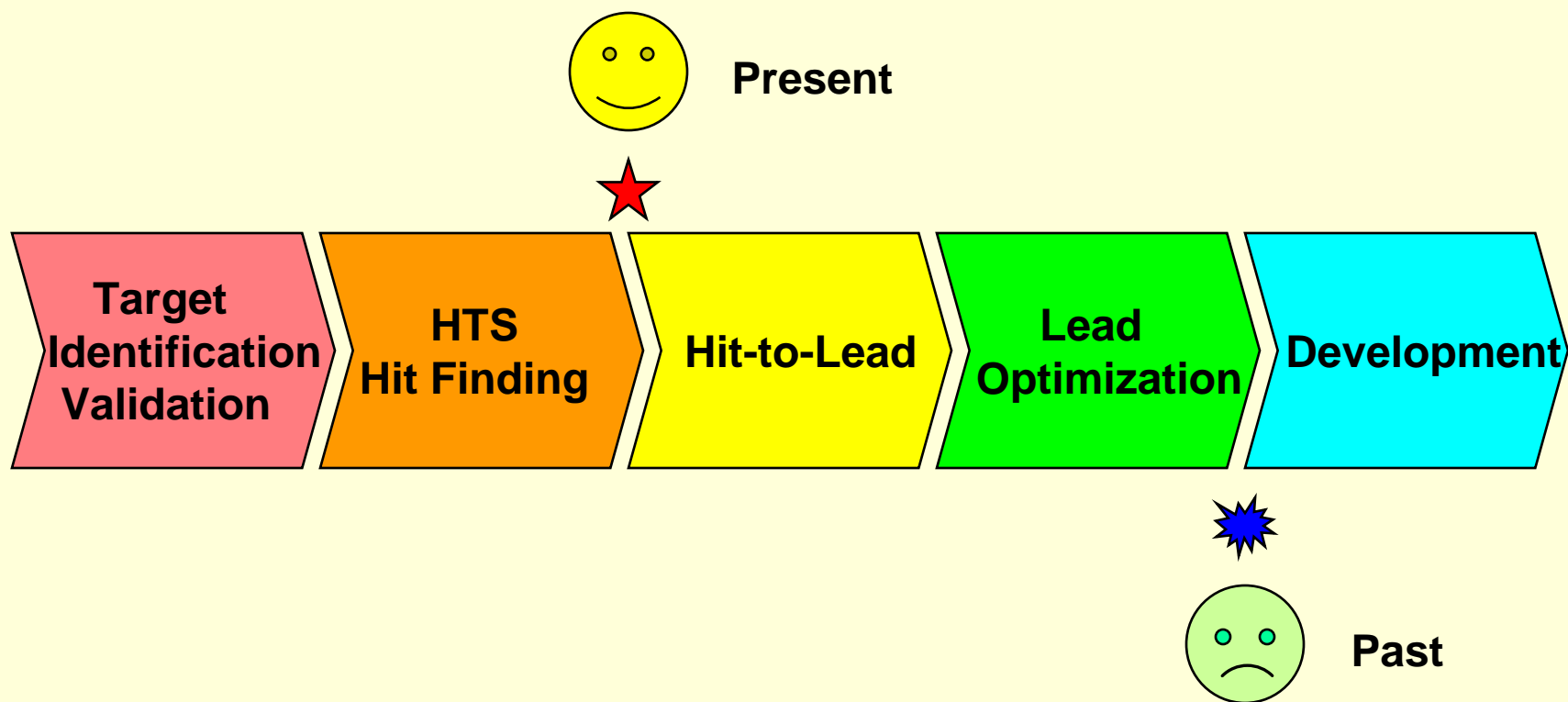
Turnaround Time = 1 week



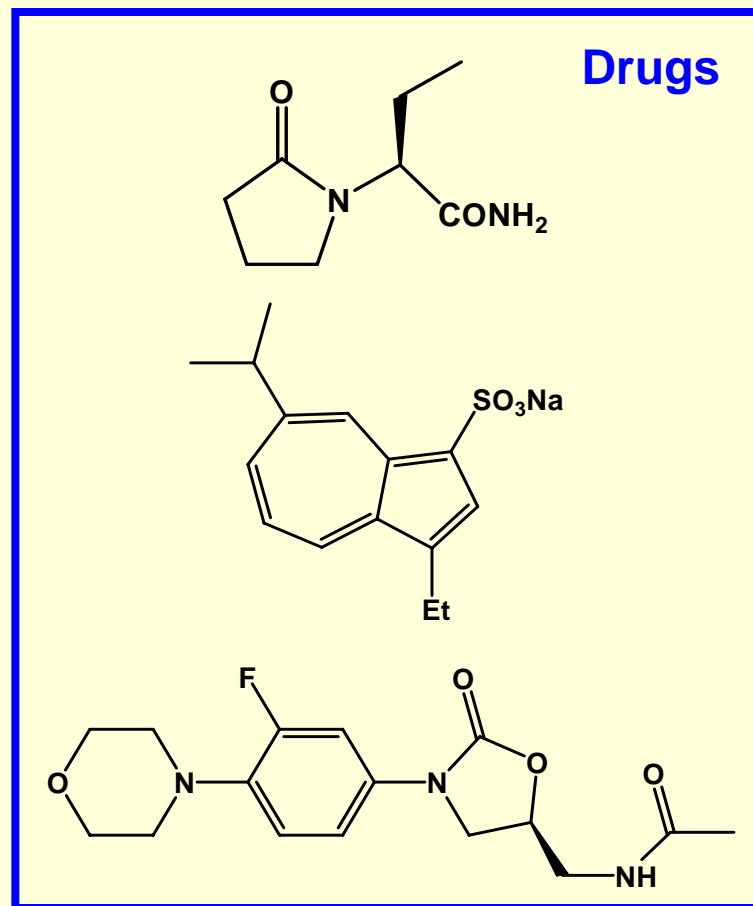
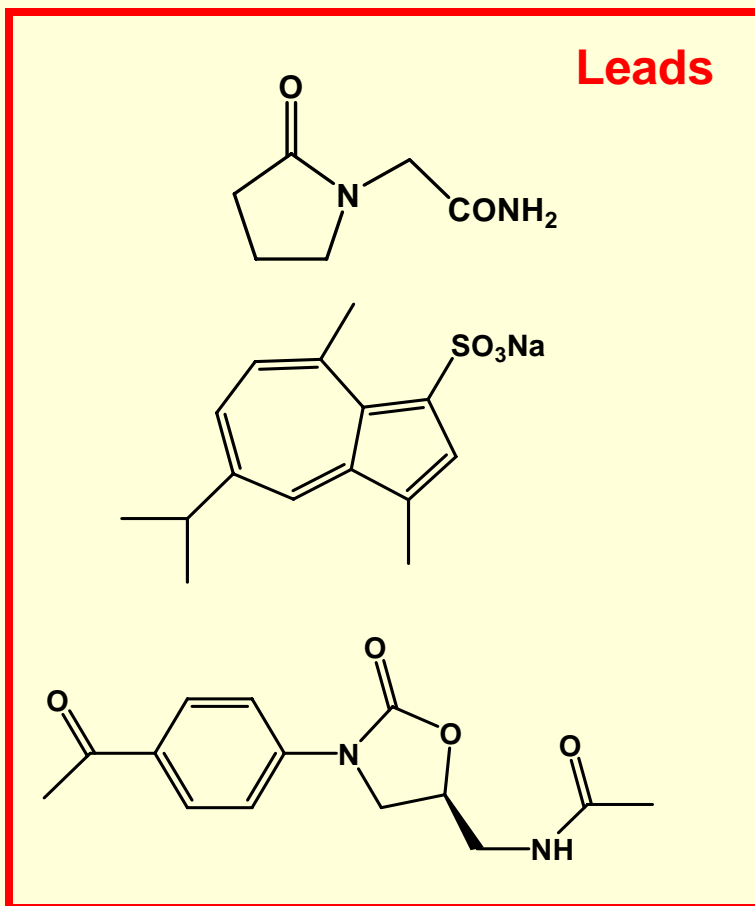
**10** Compounds  
**2 weeks**

Assumes Discovery Team makes 5 compounds / week

# Timing: Past-Too Late; Present-Early



# Drug Structure Similar to Lead Structure



Great opportunity to improve the properties is at exploratory stage

# Hit-to-Lead: Lead Profiles

Project X	Hit	Lead 1	Lead 2	Desirable Profile
Enzyme IC 50 (nM)	542	161	198	< 1000
Cell Assay ( nM)	30000	19047	4823	< 10000
Selectivity	> 100	> 100	> 100	> 10
MW	344	316	350	< 450
clog P	3.9	3.2	4.6	< 4.5
TPSA	62	75	62	< 80
Aq Sol. (ug/mL)	14	28	6	> 60
PAMPA( $10^{-6}$ cm/s)	12	2.6	4.7	> 1.0
CYP3A4	-4	2	24	< 15
CYP2D6	-6	0	6	< 15
CYP2C9	9	13	8	< 15
RLM $t_{1/2}$ (min)	< 1	3	> 30	>15
MLM $t_{1/2}$ (min)	> 30	14	> 30	>15
HLM $t_{1/2}$ (min)	12	2	> 30	>15
hERG (%)	25	39	22	< 50%
Definable Series	Yes	Yes	Yes	Yes
Definable SAR	Yes	Yes	Yes	Yes
Novelty	Yes	Yes	Yes	Yes

Potency & Selectivity

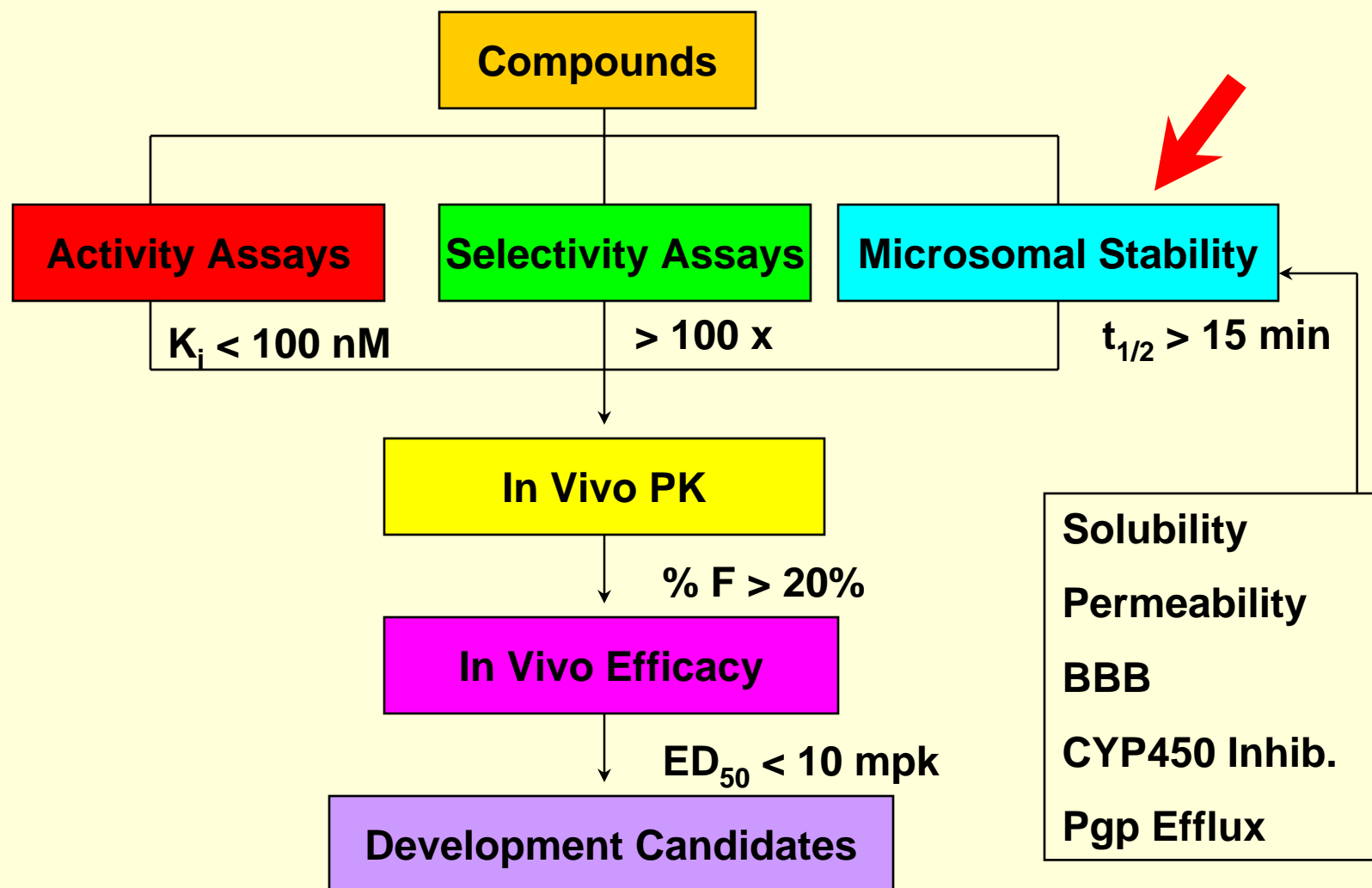
Calculated Properties

ADME/TOX  
Properties

Chemical Series  
Novelty

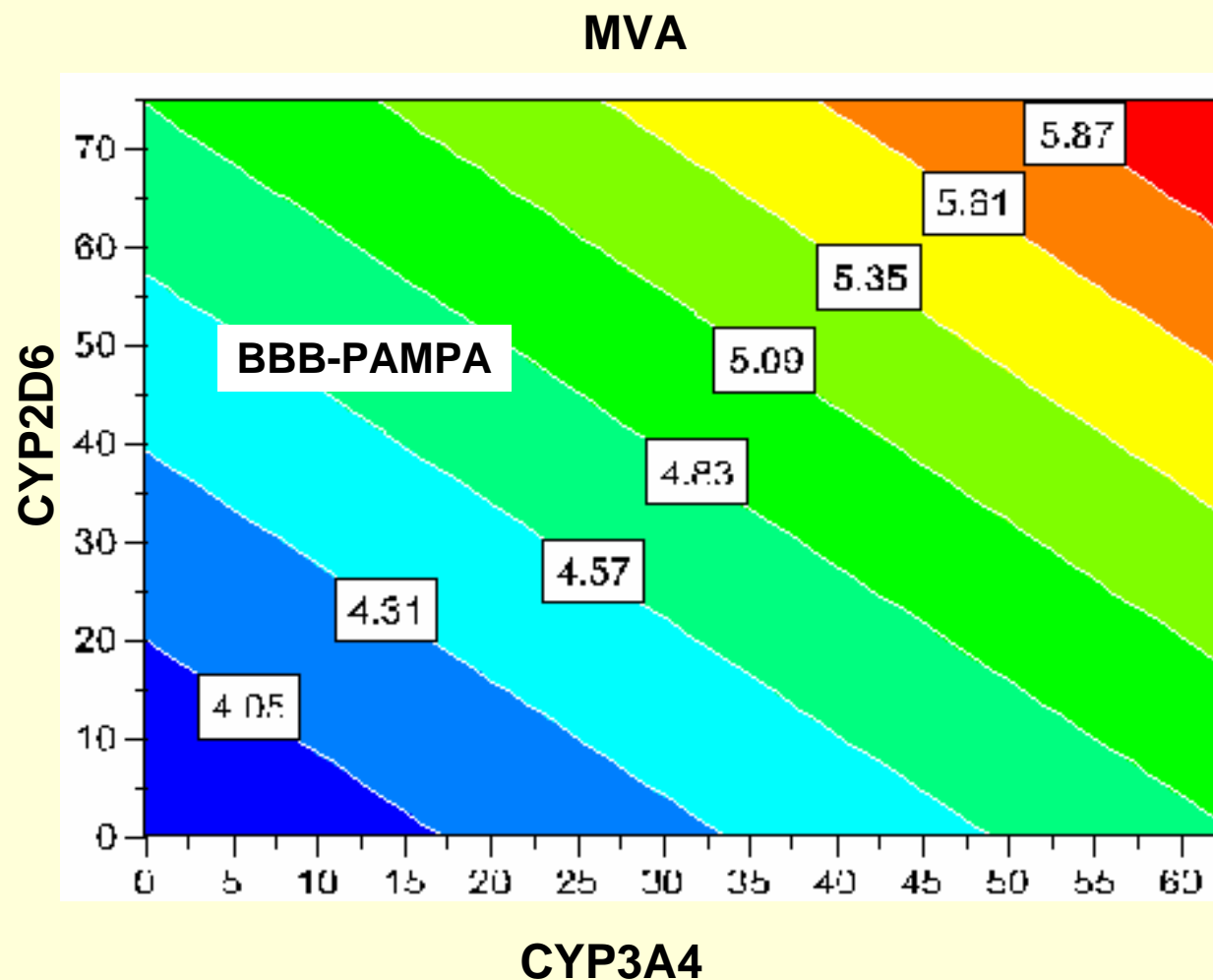
Decision Making

# Property Screening is Incorporated into Compound Selection



Selection criteria: Active, Selective and Stable

# Identify Series with High Potential for Optimization of BBB and CYP



- Reverse amide series
- BBB-PAMPA values > 4 and acceptable CYP 3A4/CYP 2D6 inhibition can be obtained

*Courtesy of Adam Gilbert*

# Guide Structural Modification

## Screen solubility of 500 compounds

### Lead

- ▶ Solubility = 0  $\mu\text{g/mL}$
- ▶ % F = 0 (Tw/MC)
- ▶ Efficacy: Corn Oil

### Candidate

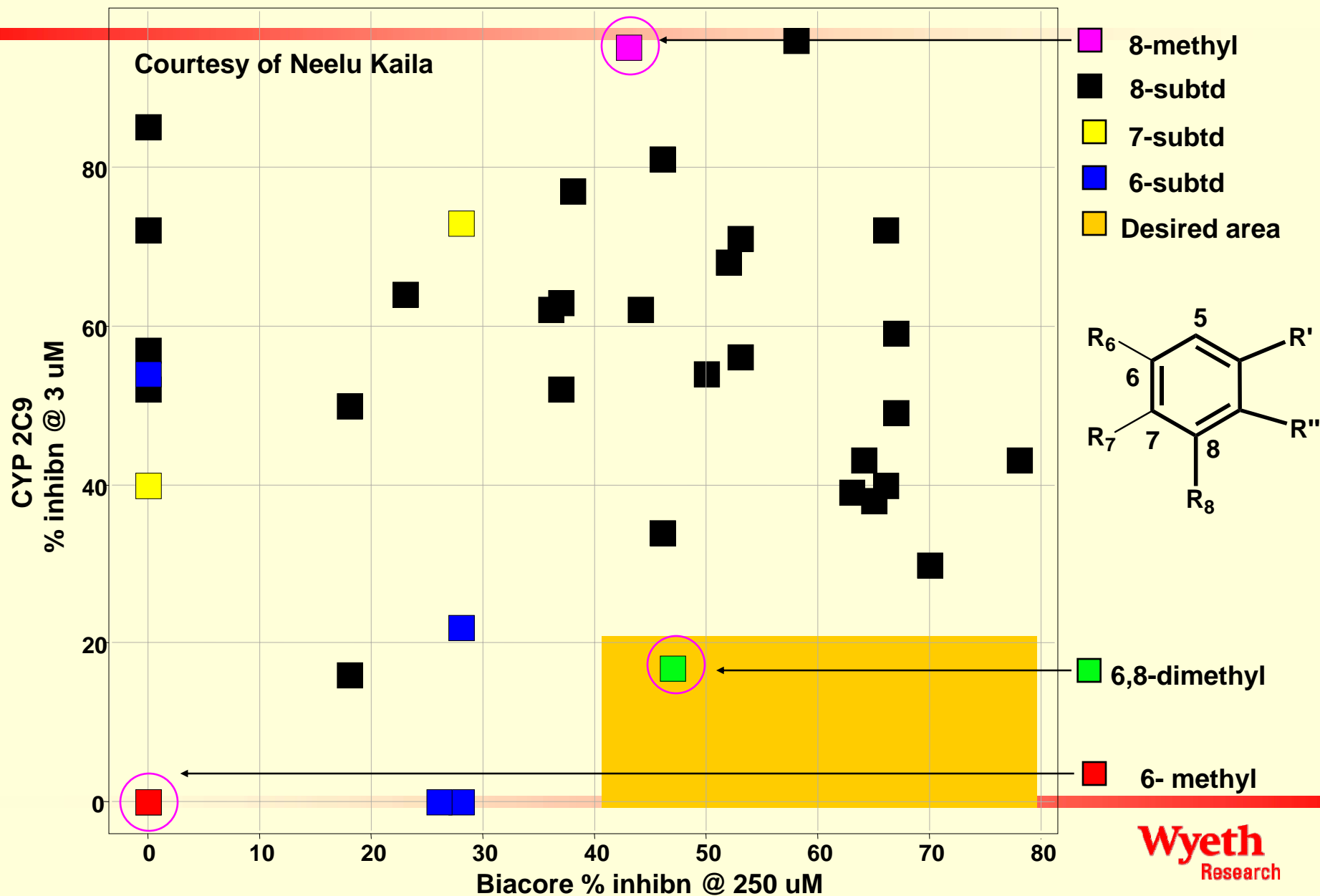
- ▶ Solubility > 6 mg/mL
- ▶ % F > 20 % (Tw/MC)
- ▶ Efficacy: Tw/MC

**Quality Development Candidate**

**Wyeth**  
Research

# Parallel Optimization of Potency and CYP Inhibition

6,8 Disubstituted analogs may help get away from CYP inhibition

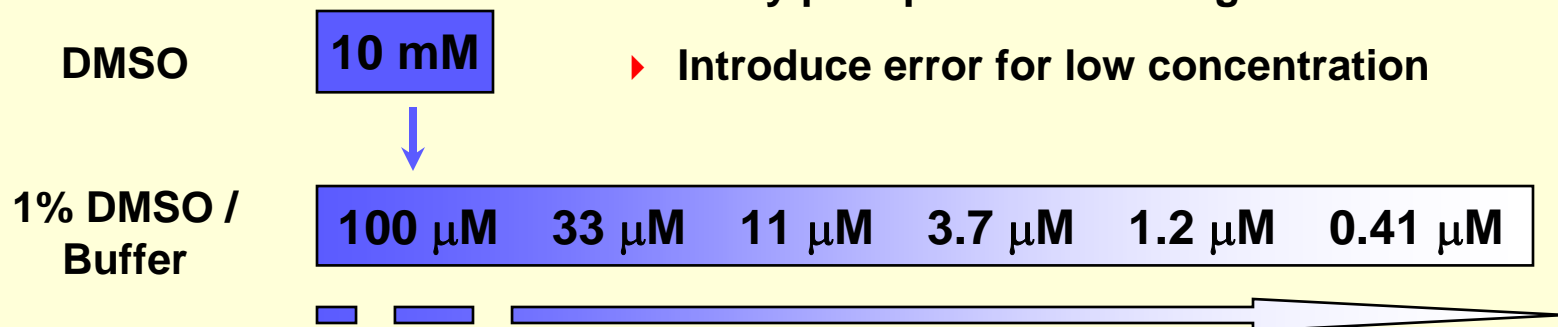


# Solubility in Bioassay Medium Guide Assay Optimization

Conditions	Old Protocol	New Protocol
		15 $\mu$ M Dose
Stock DMSO Concentration	20 mM	1.5 mM
1st Dilution to RPMI Buffer	150 $\mu$ M, 0.75% DMSO	30 $\mu$ M, 2 % DMSO
Final % DMSO	0.075% DMSO	1% DMSO

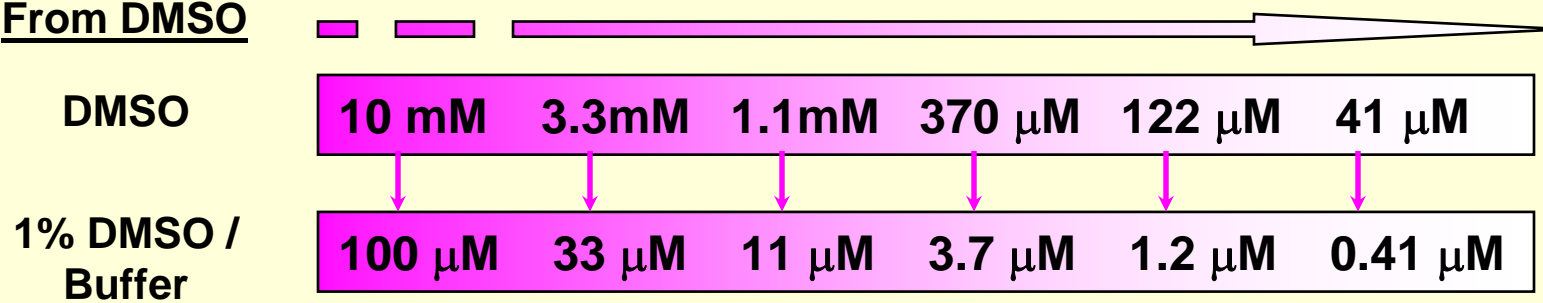
# Strategies for Serial Dilution: Dilute in DMSO

## From Aqueous



- ▶ Carry precipitation from high concentration
- ▶ Introduce error for low concentration

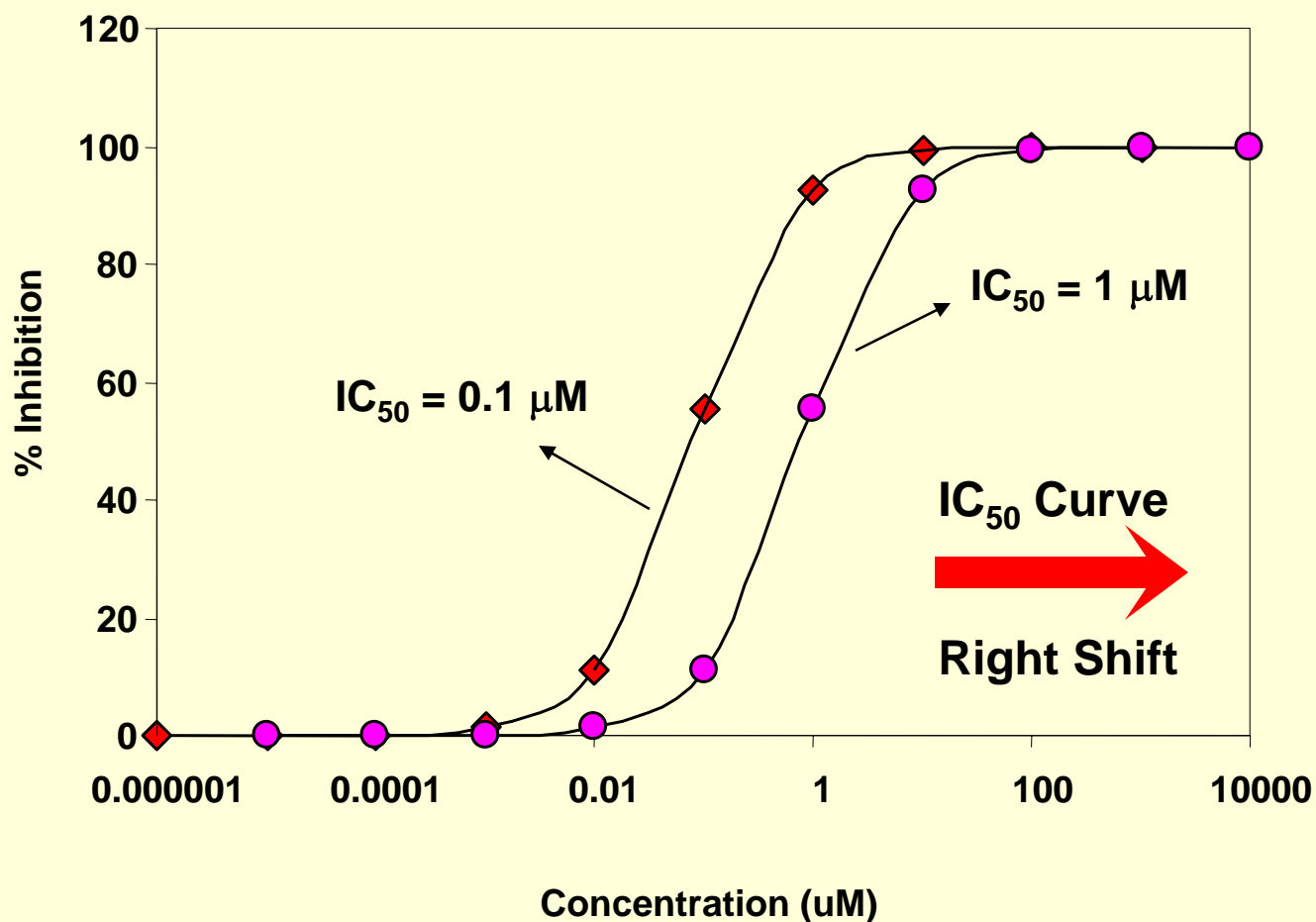
## From DMSO



- ▶ High concentration might still precipitate, but will not affect low concentration

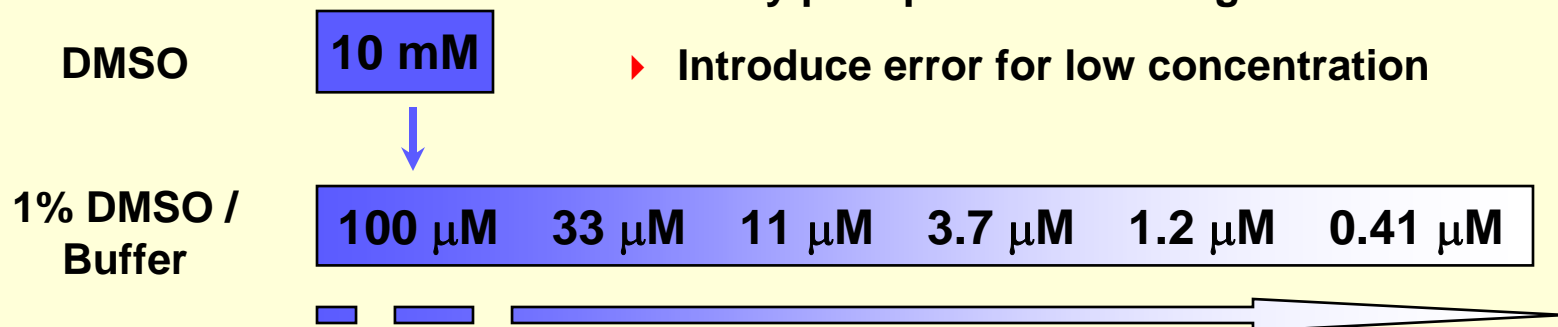
# Right Shift of $IC_{50}$ due to Low Solubility

When all the concentrations in assay buffers are lower:



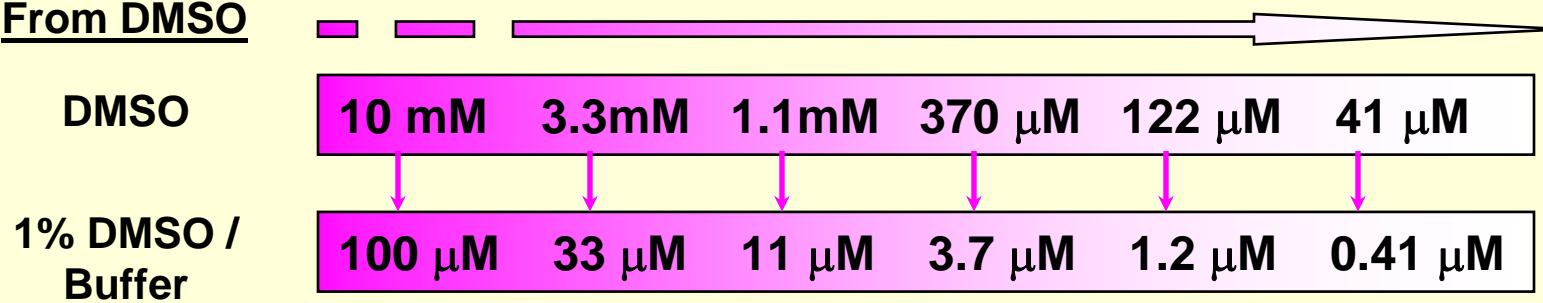
# Strategies for Serial Dilution: Dilute in DMSO

## From Aqueous



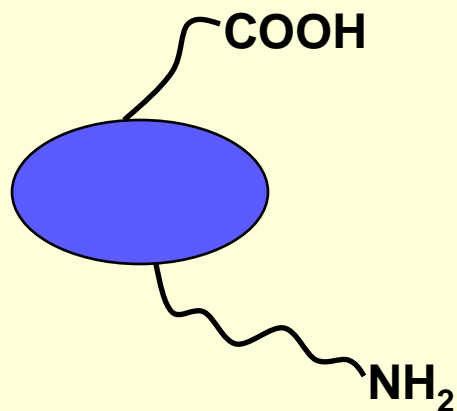
- ▶ Carry precipitation from high concentration
- ▶ Introduce error for low concentration

## From DMSO



- ▶ High concentration might still precipitate, but will not affect low concentration

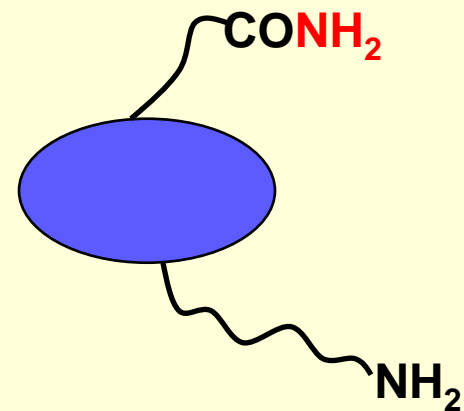
# Stability Affects Assay Results



**Low oral bioavailability**

**Poor Permeability**

**IV Administration**

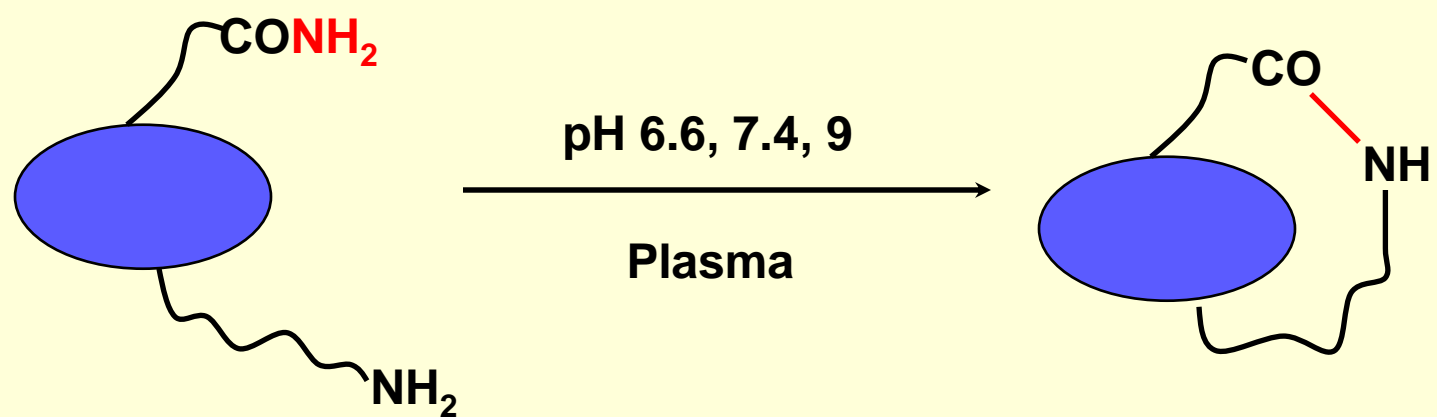


**Very active in vitro**

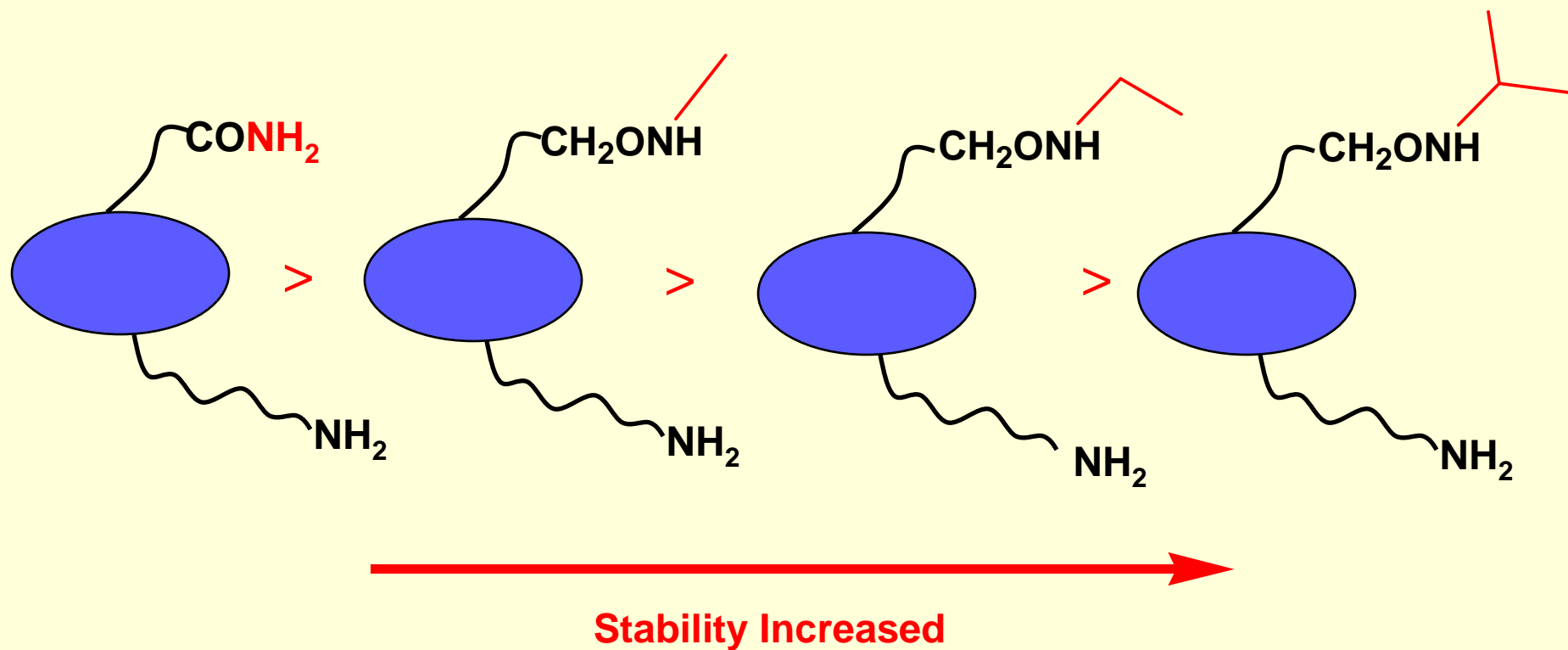
**Very active in vivo**

**But.....**

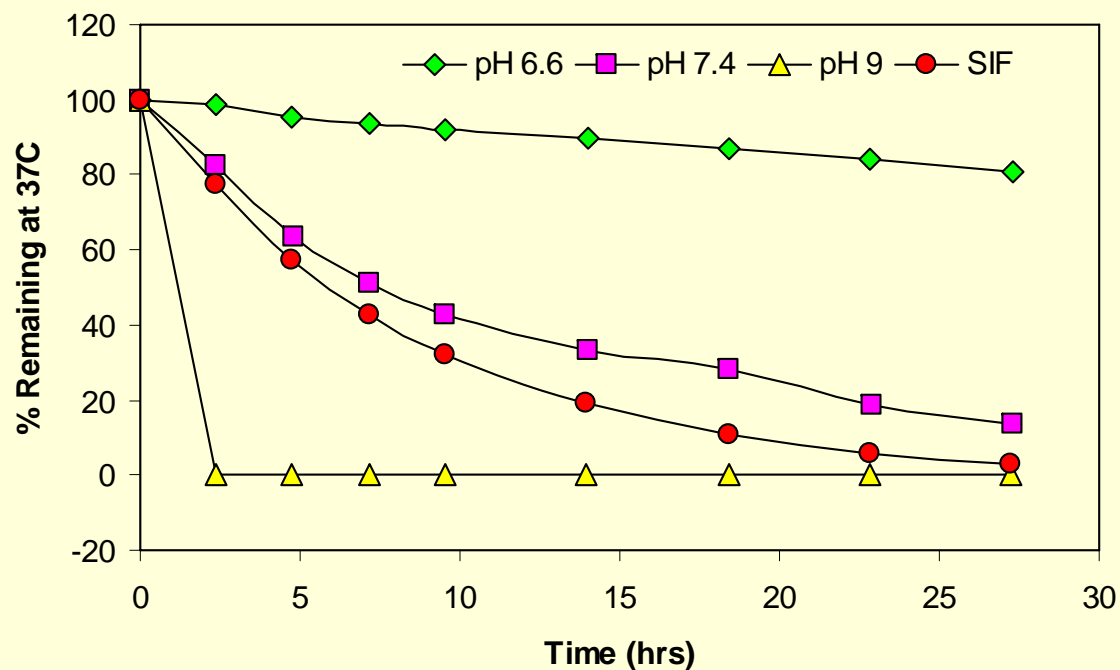
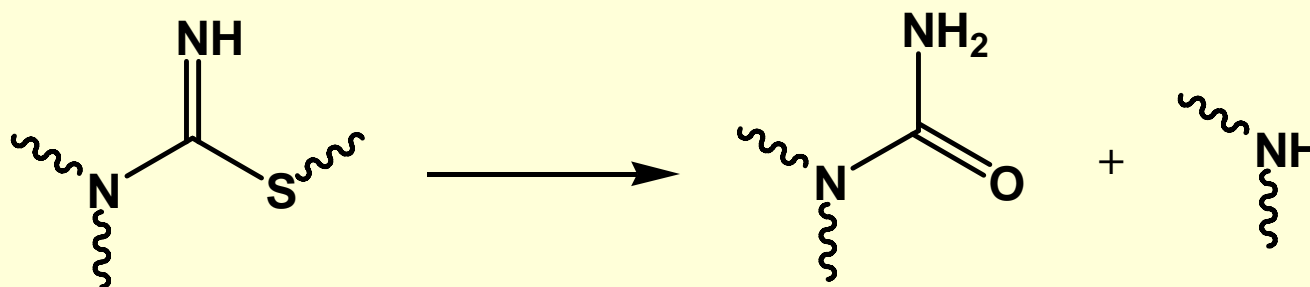
# Compound Rapidly Cyclized



# Guide Structural Modification



# Isothiourea Rapidly Hydrolyzed in Buffer



Project was terminated

# Guide Structural Modification

Screen ~1000 metabolic stability and ~100 Pgp

## Lead

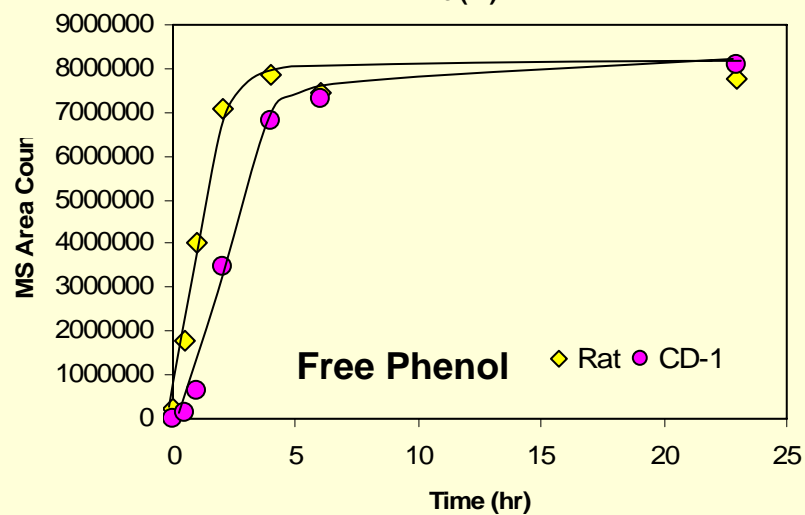
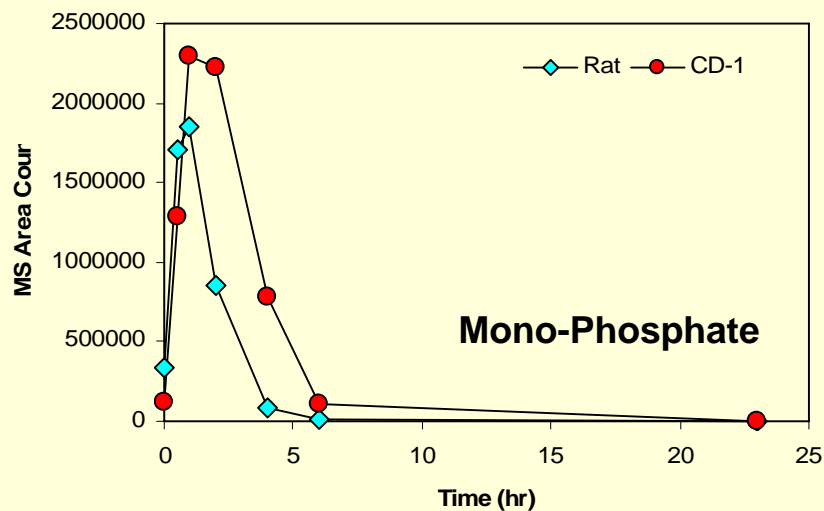
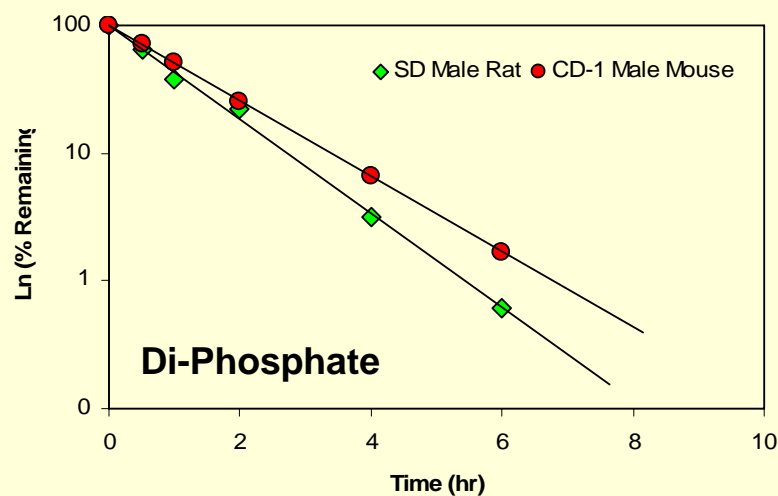
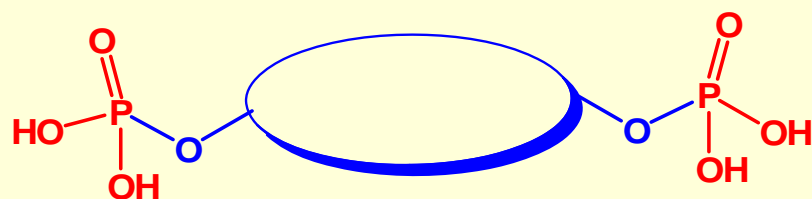
- ▶ Met.  $t_{1/2} < 5$  min
- ▶ Pgp efflux  $> 10$
- ▶ B/P ratio  $< 0.02$
- ▶ No Efficacy

## Candidate

- ▶ Met.  $t_{1/2} > 30$  min
- ▶ Pgp efflux  $< 2.5$
- ▶ B/P ratio  $> 0.60$
- ▶ Efficacious

**Enhanced Success**

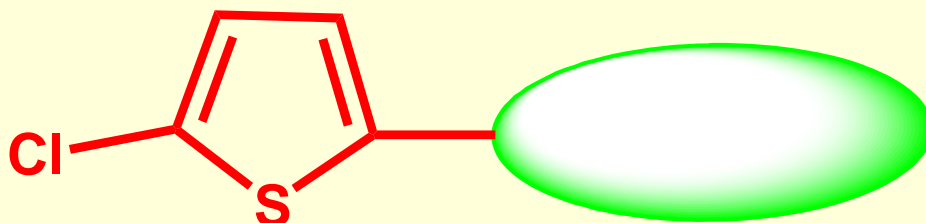
# Screening of Prodrugs



Identify prodrug with desirable properties **Wyeth**  
Research

# Develop Structure-Property Relationships

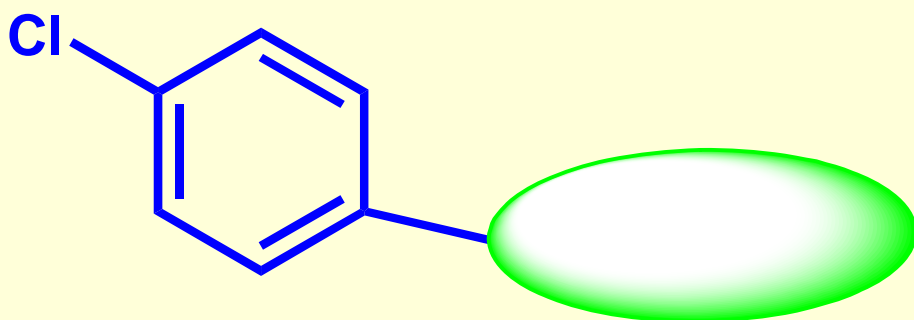
2-Cl Thiophene



ED <sub>50</sub>	5-13 nM
Rat Stab.	< 1 min
Mouse Stab.	< 1 min
Human Stab.	4 min

**Inactive in vivo**

p-Cl Benzene



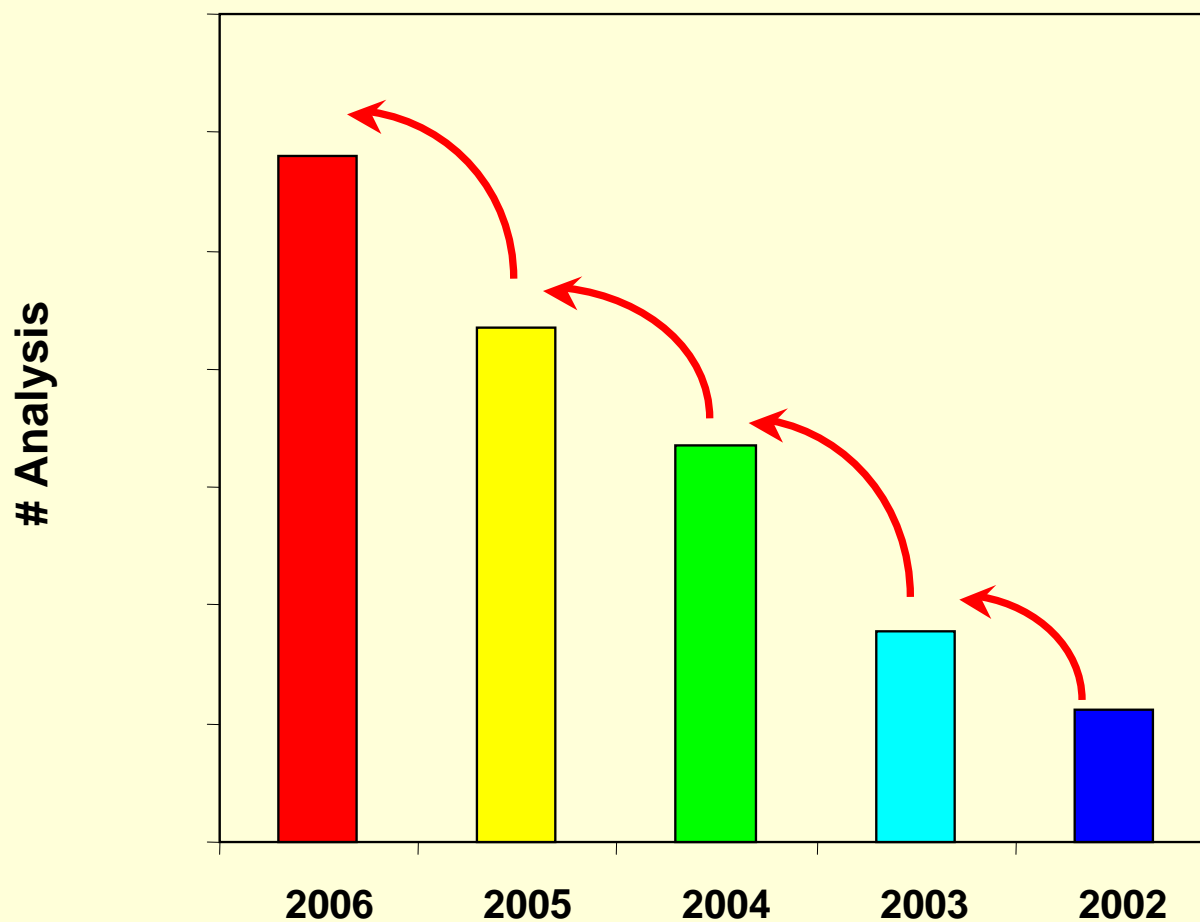
ED <sub>50</sub>	12 nM
Rat Stab.	21 min
Mouse Stab.	13 min
Human Stab.	11 min

**Active in vivo**

**Improved Stability and In Vivo Performance**

**Wyeth**  
Research

# Microsomal Stability: >30% Increase Per Year



**Success Breeds Success**

**Wyeth**  
Research

# Future Prospective

---

- **More predictive software**
- **Miniaturization**
- **Predictive toxicity**
- **Earlier profiling**

# Enhance Predictability and Intelligence of Software

---

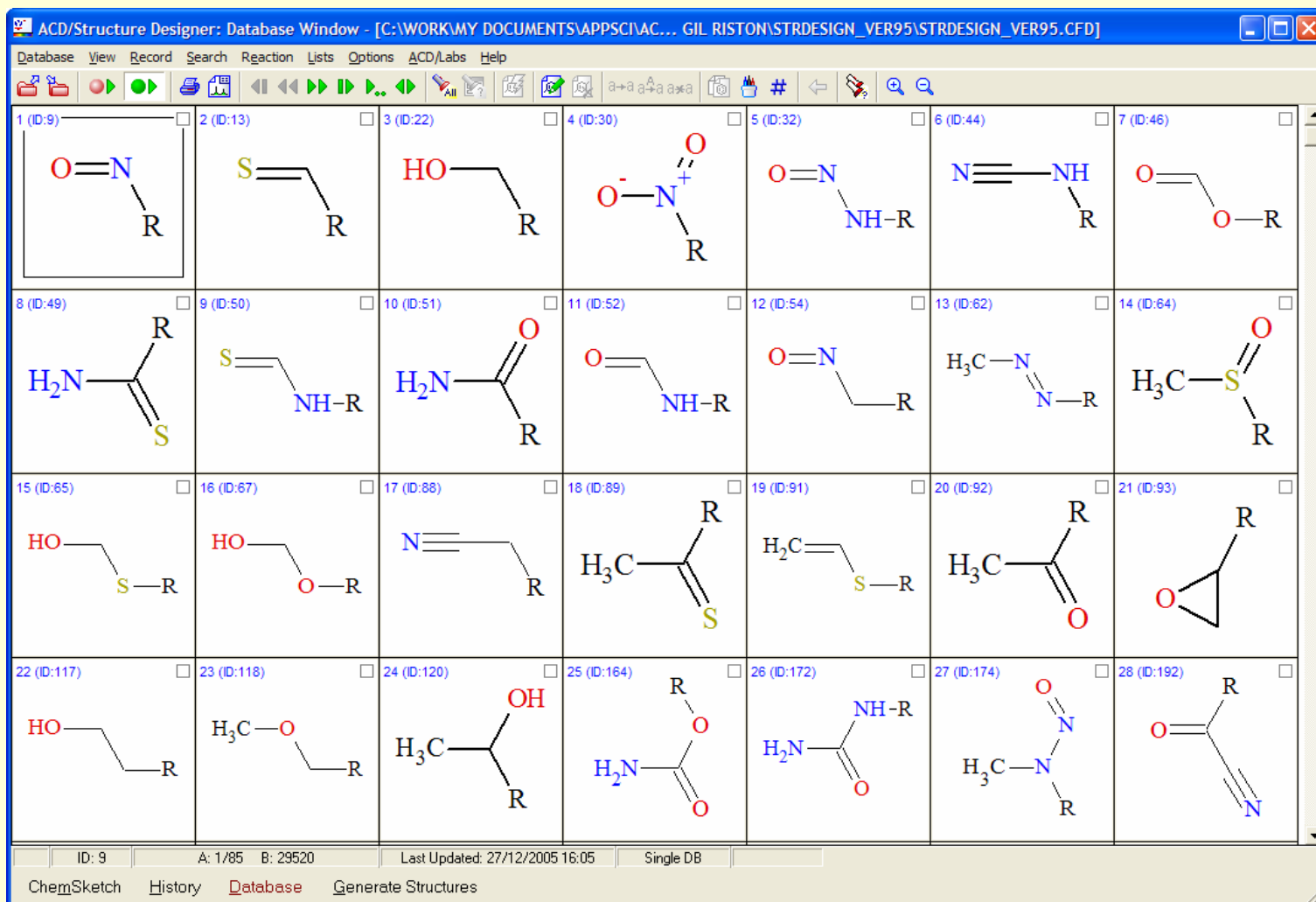
- **Predictability**

- ▶ Accuracy
- ▶ Coverage
- ▶ Custom models

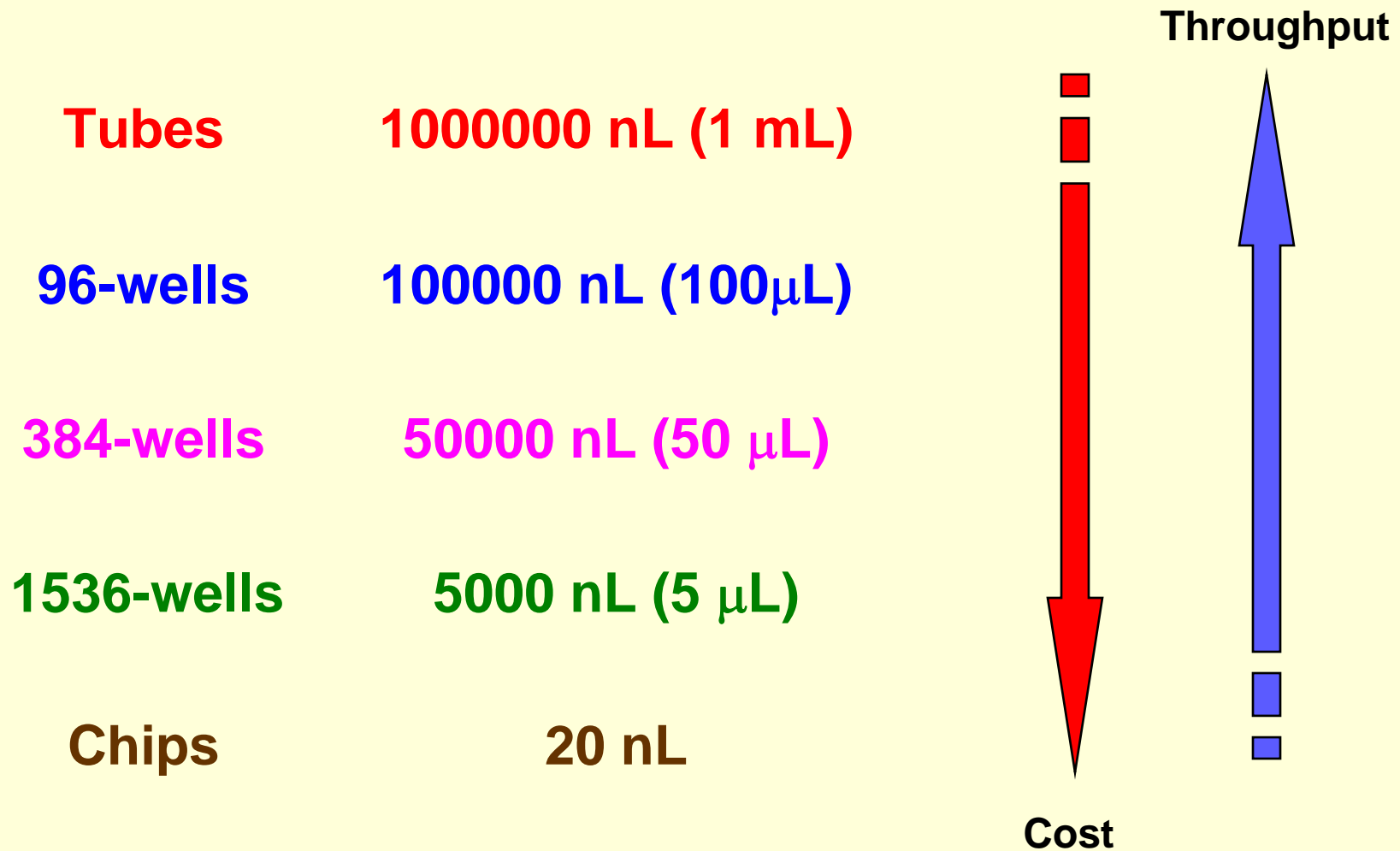
- **Intelligence**

- ▶ Propose substituents
- ▶ Design new compounds

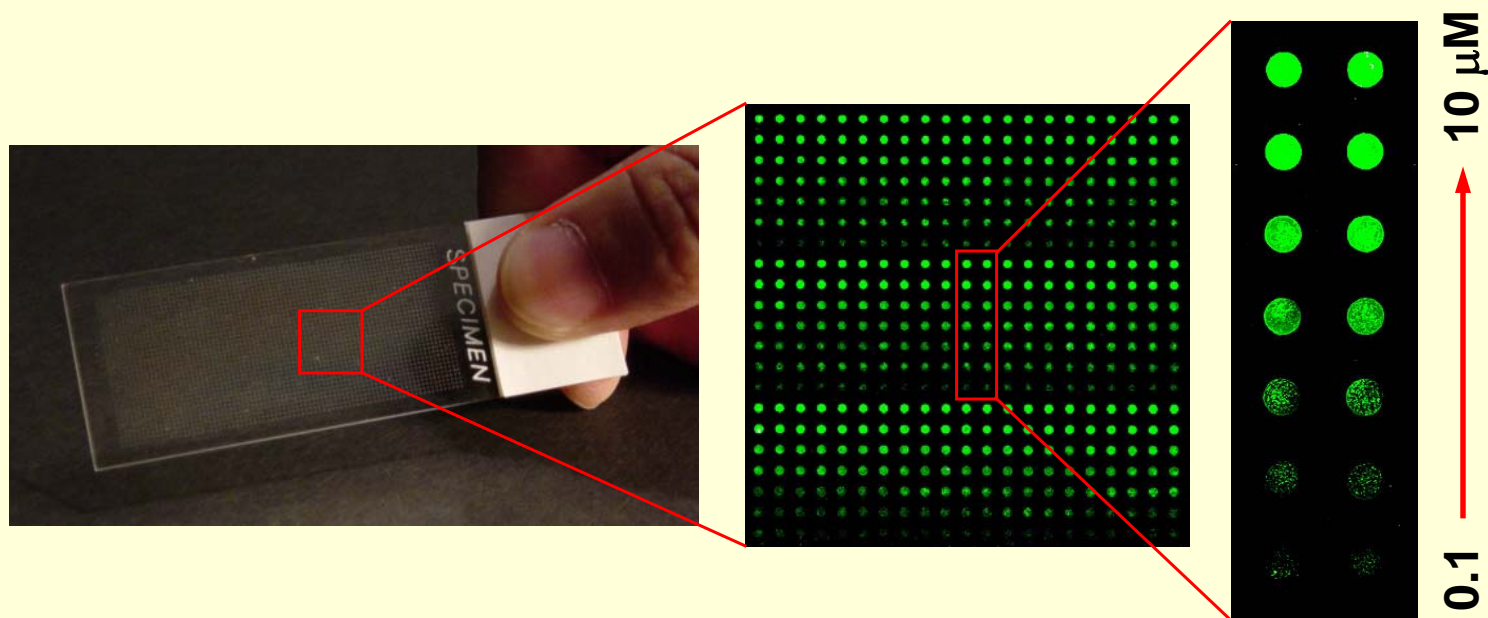
# ACD Structure Designer: Propose Substituents



# Future: Miniaturization

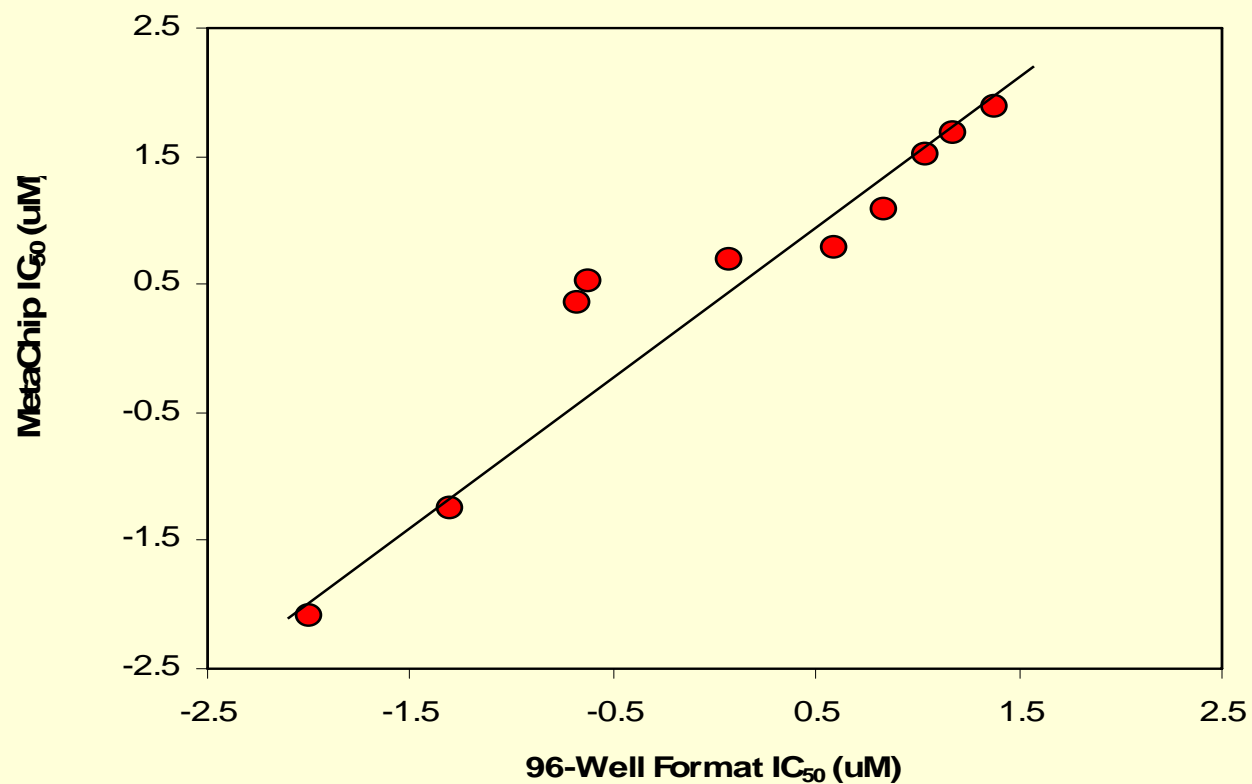


# Future Miniaturization: Metachip from Solidus

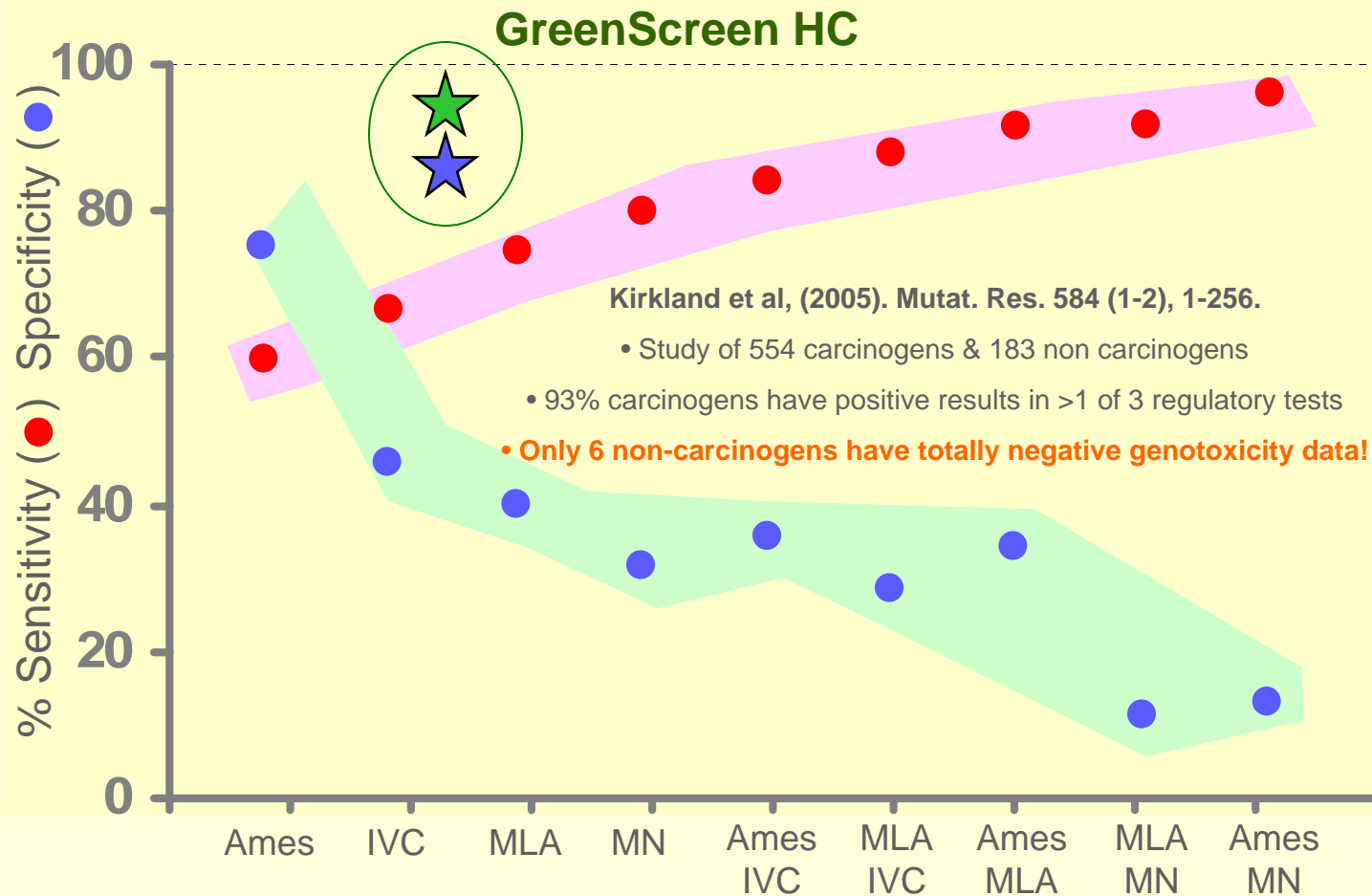


- **> 1000x volume reduction (20 nL): savings**
- **11,200 spots / slide: throughput and speed**

# Correlation Between MetaChip and 96-Well Format for CYP3A4 Inhibition



# More Predictive TOX Assays



Peter McCulloch, Gentronix

# Impact of Pharmaceutical Profiling on Attrition

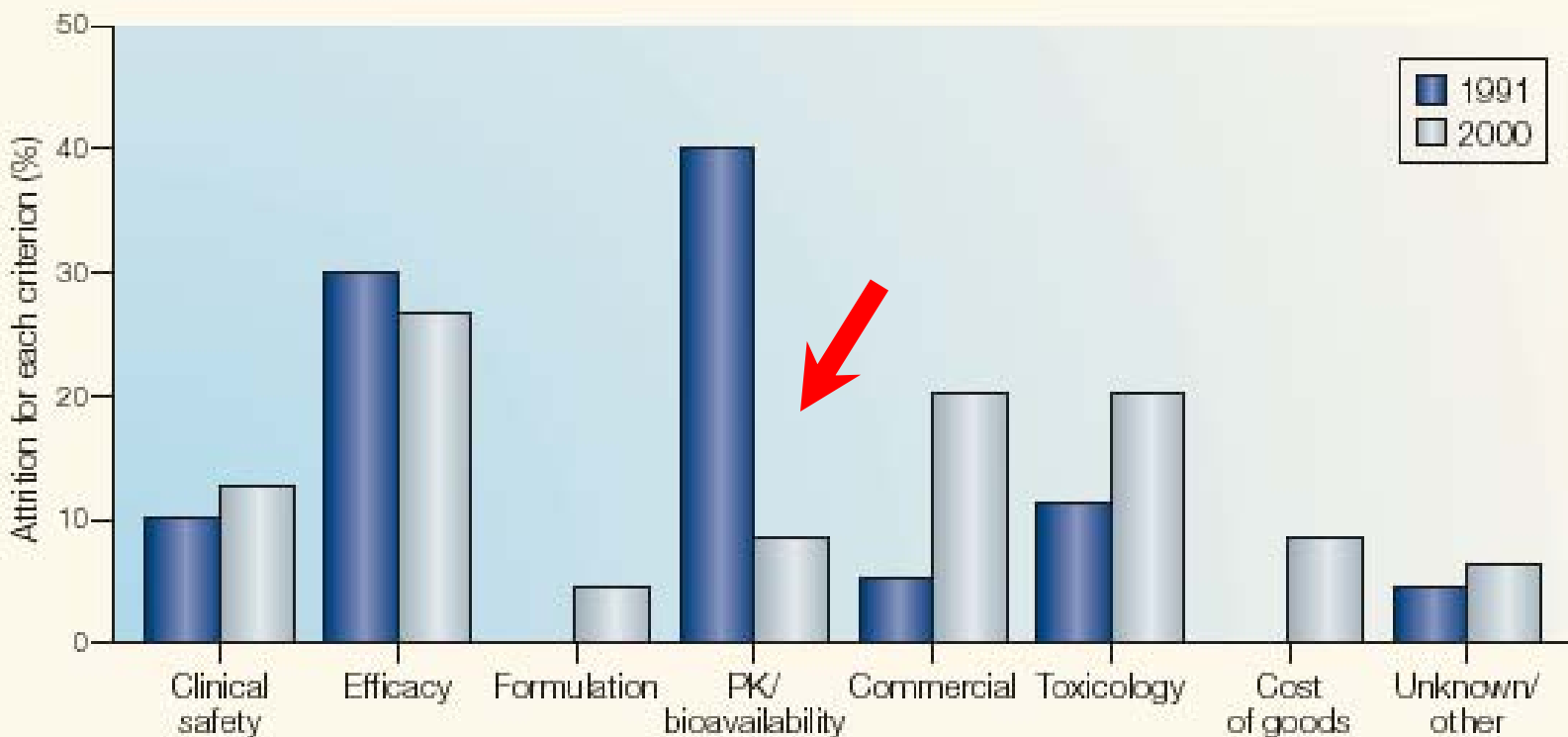
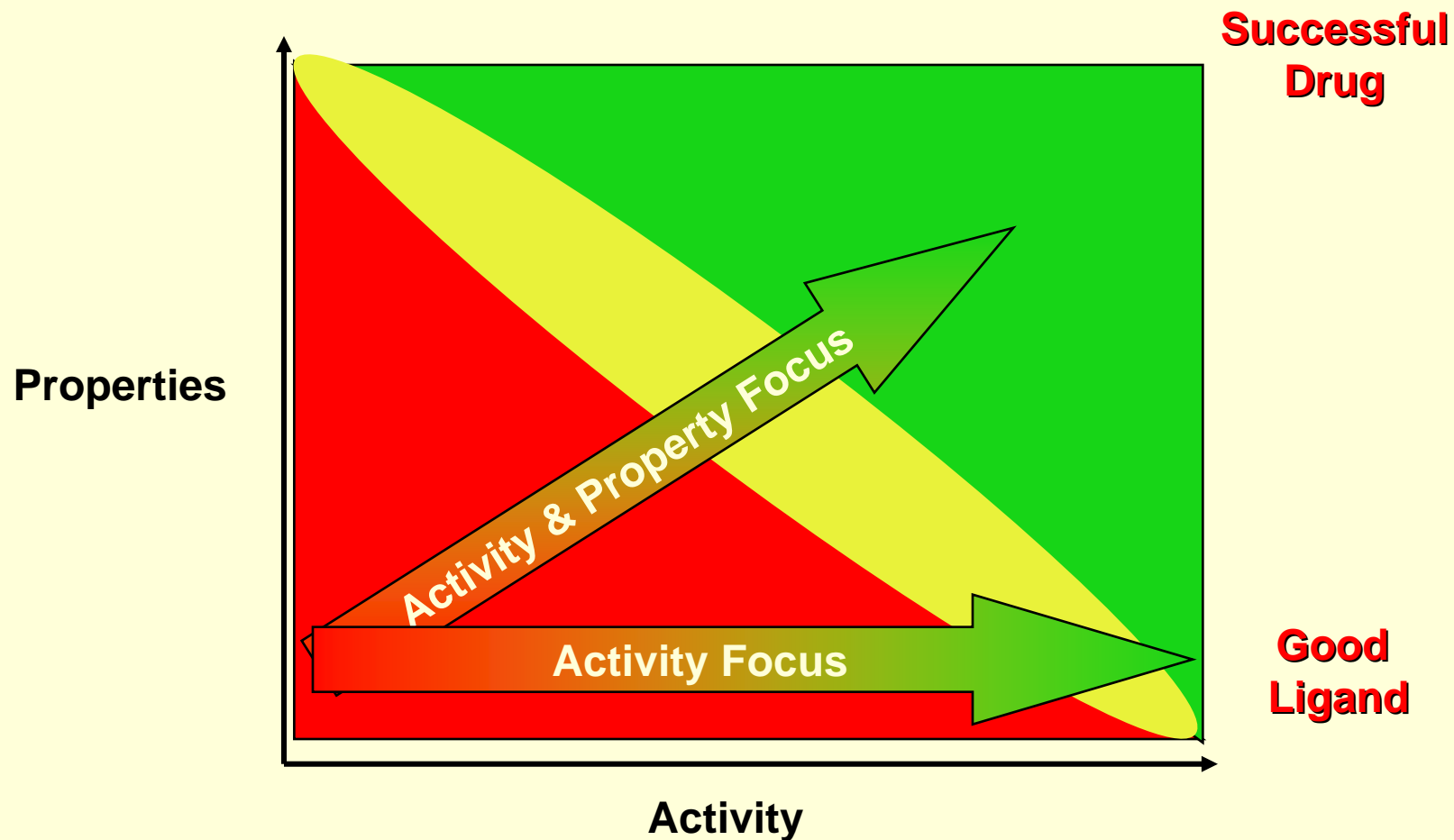


Figure 3 | Reasons for attrition (1991–2000). PK, pharmacokinetics.

# Conclusions



# Acknowledgments

---

- **Princeton:** Edward Kerns, Susan Petusky, Susan Li, Zhen Lin, Hong Jin, Ian Bezar
- **Pearl River:** Yelena Pyatski, Deanna DeGrandi, Adam Pitkin, Joe Marini, Barry Press
- **Chem./Bio.:** Neelu Kaila, Adam Gilbert, Baihua Hu, Jay Wroble, Jeremy Levin, Jeff Pelletier, Jonathon Gross, Martin DeGrandi, Lee Jennings, Mike Malamas, Paul Dollings, Tim Lock, Paige Mahaney, Bill Moore, Andy Fensome, Yuren Wang, Derek Cole, Al Robichaud, John Butera, Boyd Harrison, John Ellingboe
- **Leadership:** Oliver McConnell, Guy Carter, Magid Abou-Gharbia

# Further Information

- Contact: [DIL@WYETH.COM](mailto:DIL@WYETH.COM)
- ACS Short Course on “Drug-like properties”
- Book: available Jan. 25, 2008

