Physicochemical Properties at Bayer HealthCare (Wuppertal) and Their Use in Medicinal Chemistry

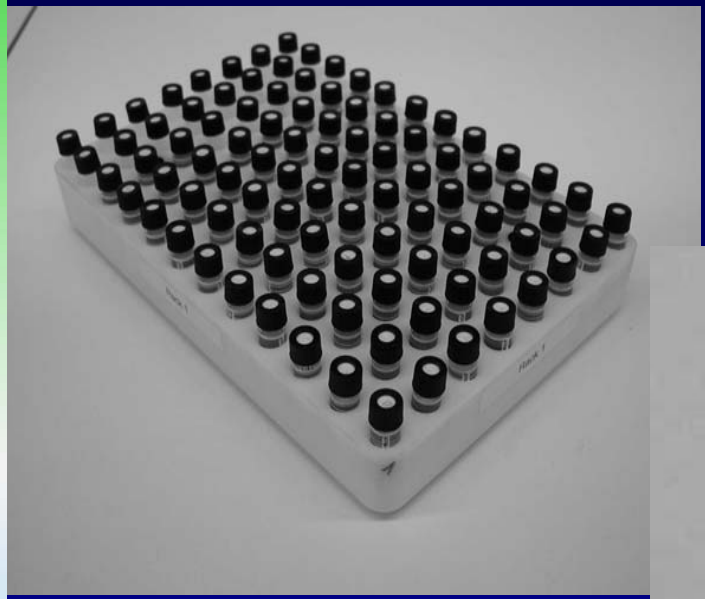
Contents

- Measured physico-chemical parameters
- Introduction of our laboratory
- Model systems for lipophilicity
- Solubility
- Use in medicinal chemistry

Physicochemical Properties Measured

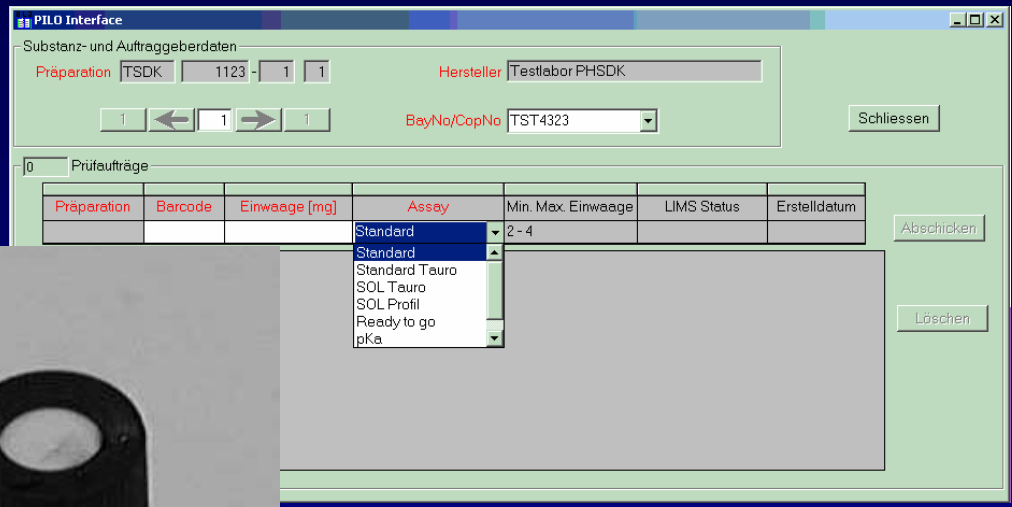
Lipophilicity	**Membrane Affinity**	**MA**
Plasma binding	**human serum albumin binding**	**HSA**
	rat serum albumin binding	**RSA**
pKa		**pKa**
Solubility	**screening in various buffers**	**SOL**
	equilibrium in buffer	
	equilibrium in galenic formulations	

Logistics for HT Physicochemistry



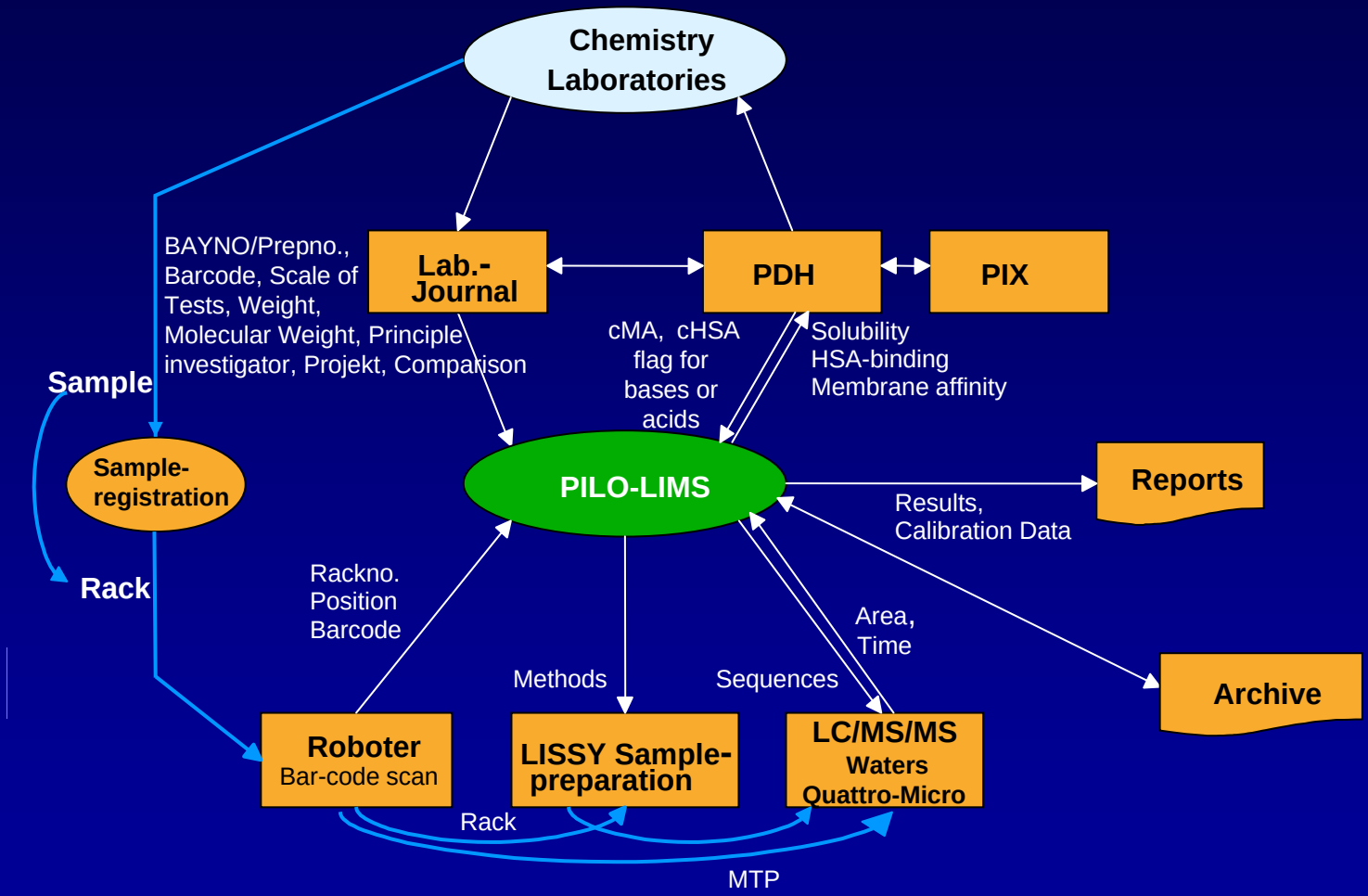
Vial collecting rack

Bar-coded vial for sample registration

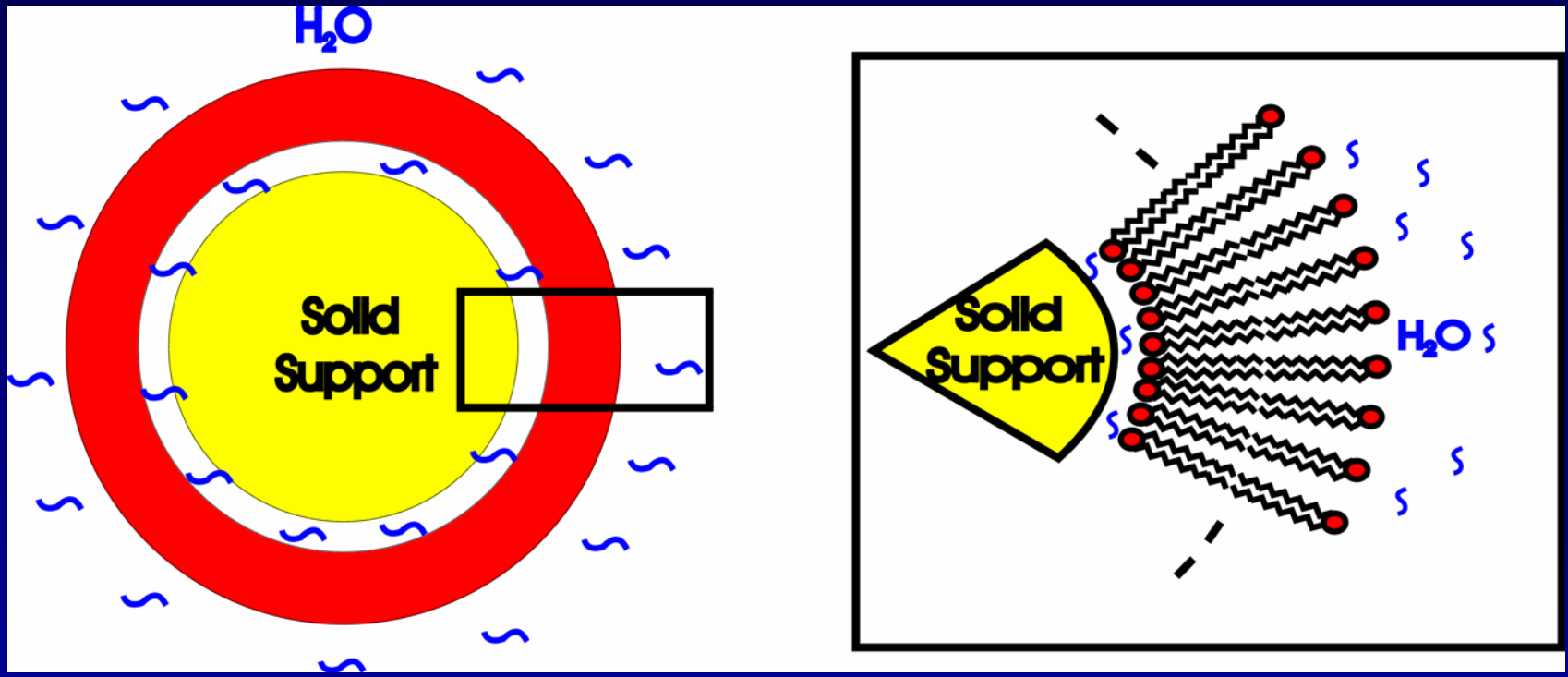


BLJ input for sample identification

Data Handling by Laboratory Information and Management System (LIMS)



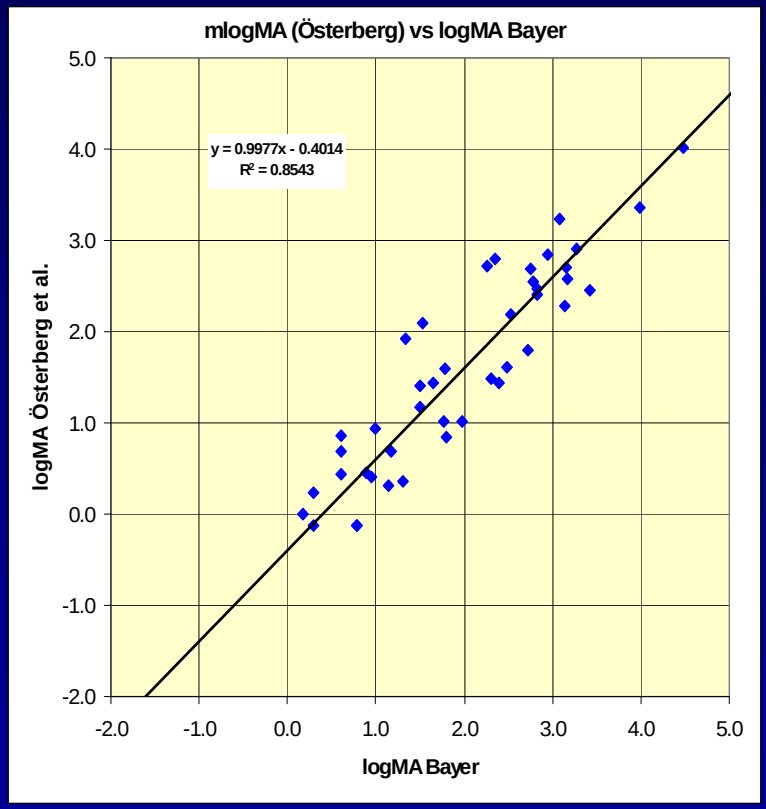
Our model system

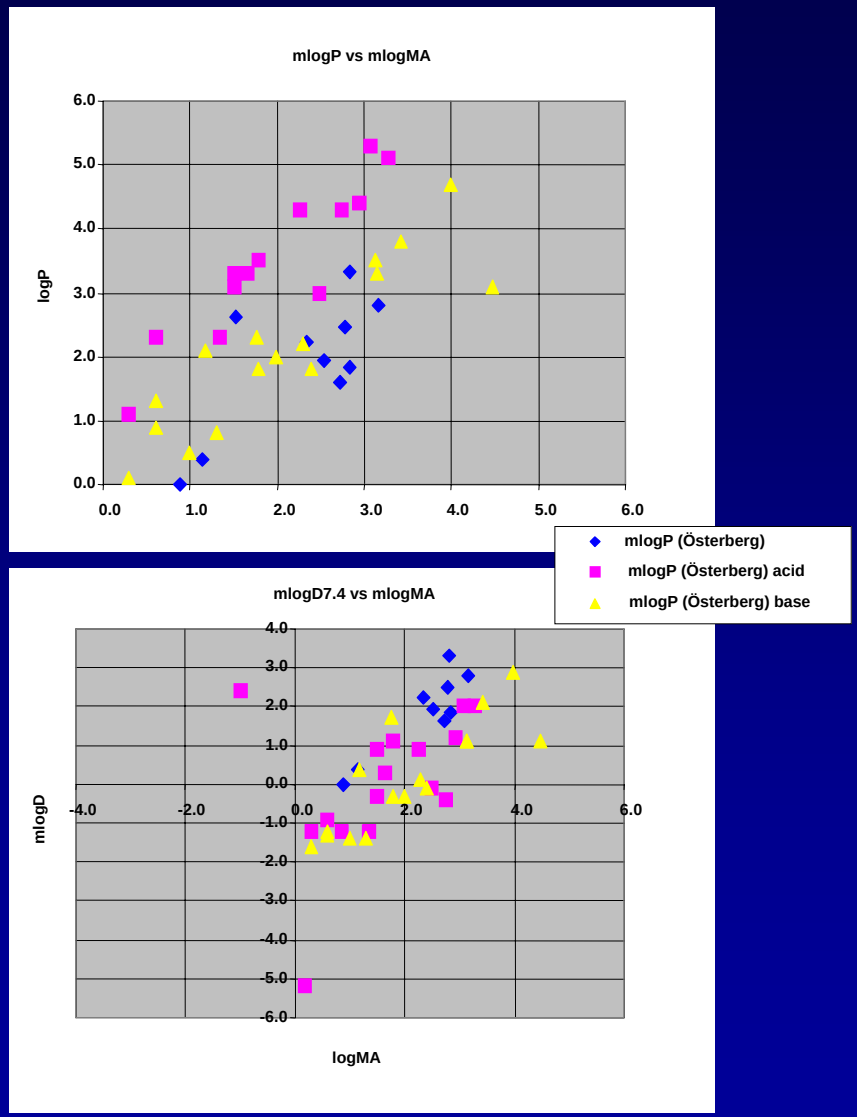


Solid-supported lipid membranes (TRANSIL®)

Why Use Membrane Affinity?

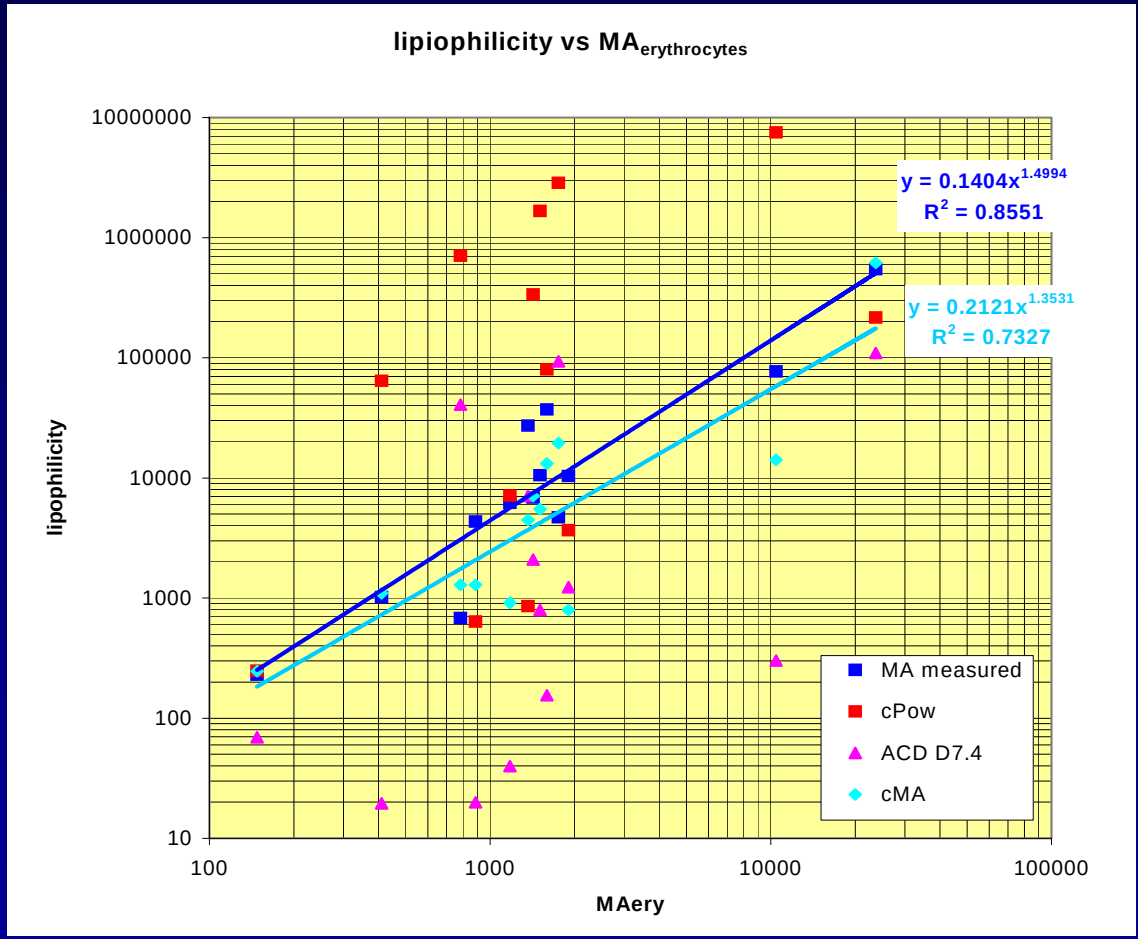
1. comparison with other lipophilicity descriptors





Why use membrane affinity?

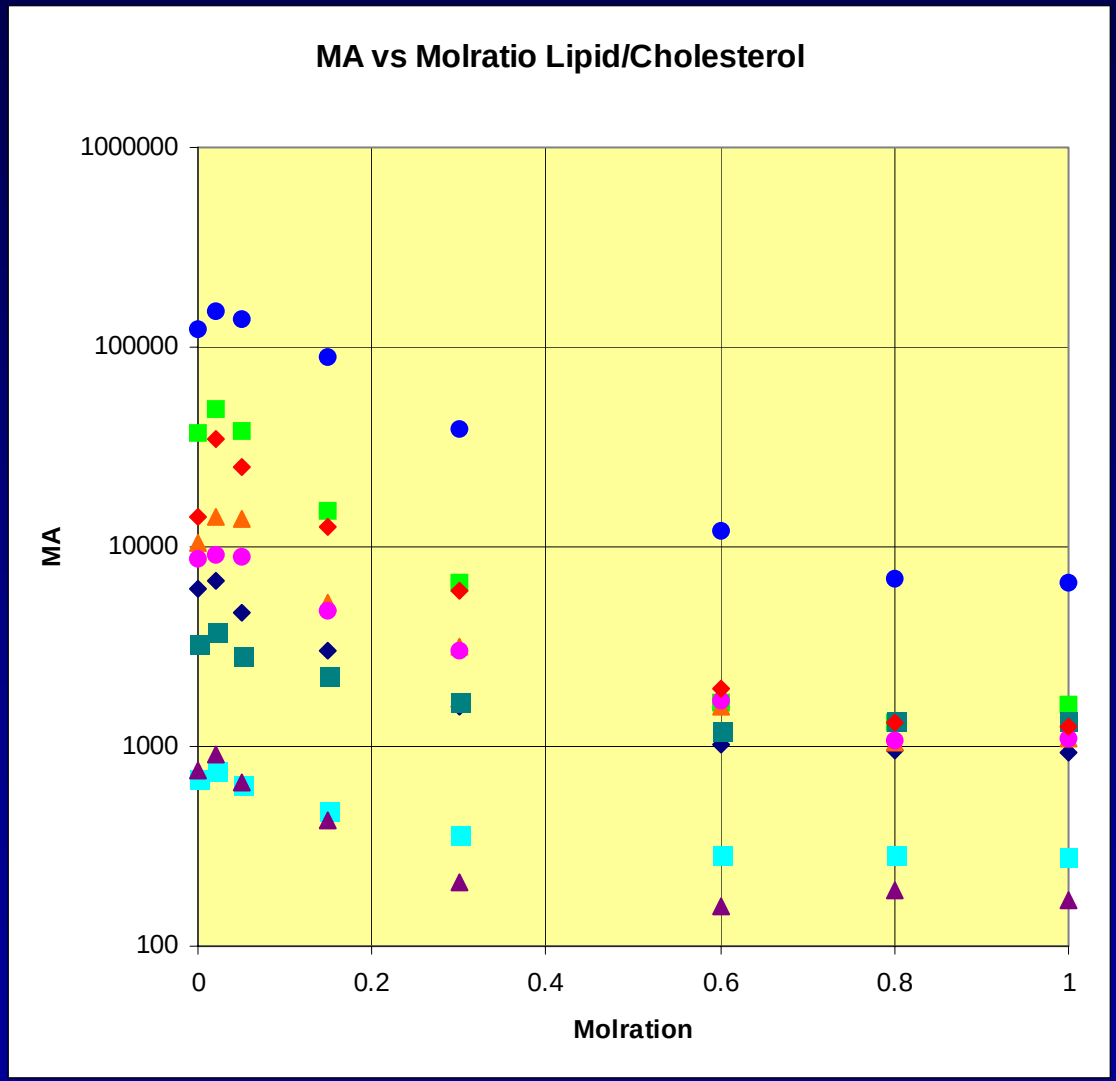
2. comparison with physiological membranes



Influence of cholesterol content has to be considered

Why use membrane affinity?

Influence of cholesterol



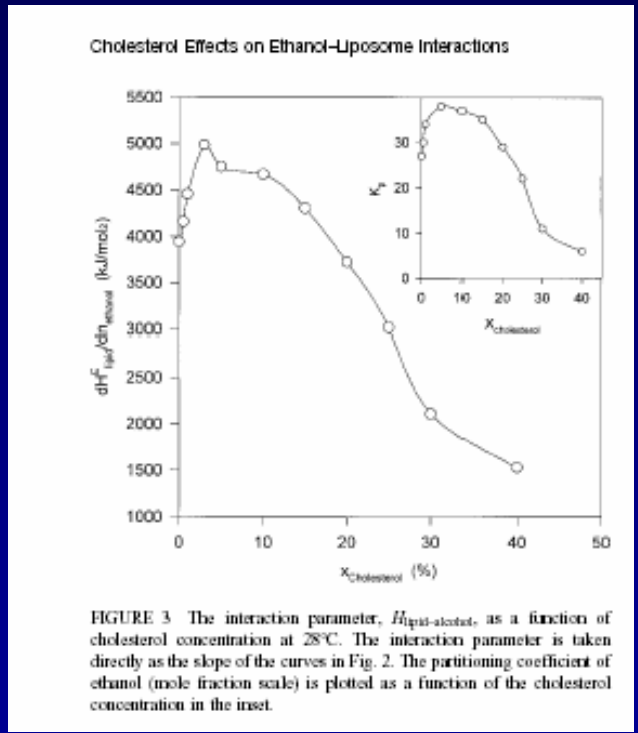
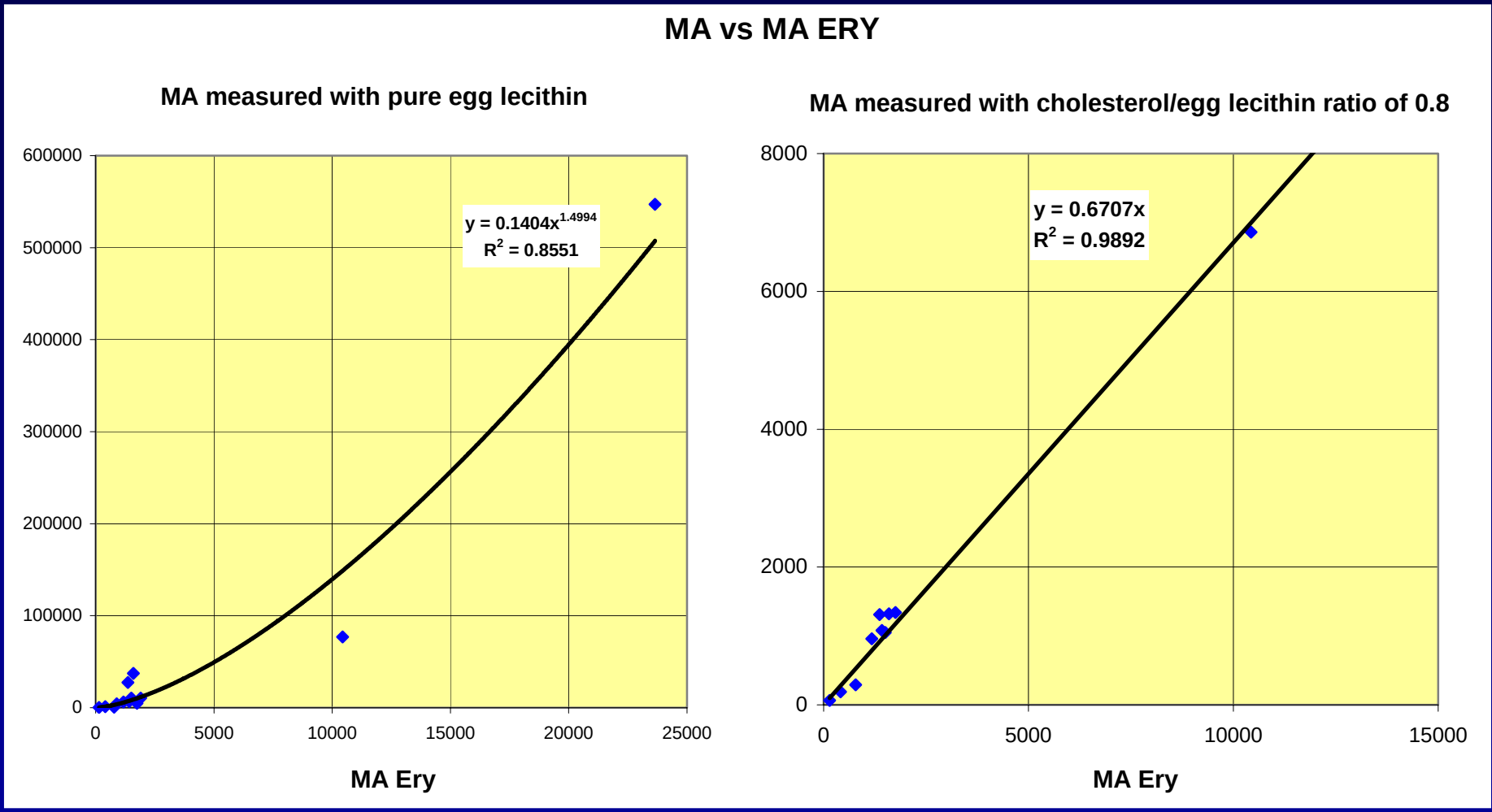


FIGURE 3 The interaction parameter, $H_{lipid\text{-}alcohol}$, as a function of cholesterol concentration at 28°C. The interaction parameter is taken directly as the slope of the curves in Fig. 2. The partitioning coefficient of ethanol (mole fraction scale) is plotted as a function of the cholesterol concentration in the inset.

C. Tradum et al.:Biophysical J. 78 2496-2492

Why use membrane affinity?

Influence of cholesterol



Why use membrane affinity?

Influence of cholesterol

- **Influence on passive permeation expected to be strong**
  - **flexibility of plasma membrane is strongly influence by cholesterol, content usually about 80 mol% of phospholipids content**
- **Influence on distribution expected to be low**
  - **80% of all membranes are intracellular with a cholesterol content about 4 mol% of phospholipids content**

Comparison of solubility methods

- **precipitation**
  - **all compounds**
  - **small amounts**
  - **fast analytics**
  - **compound dissolved in organic solvent**
  - **oversaturated solutions possible**
- **from powder**
  - **selected compounds**
  - **large amounts (two samples)**
  - **specific analytics**
  - **sensitive to morphology**
  - **sensitive to purity**
  - **sensitive to solvent impurities**

Comparison of solubility methods

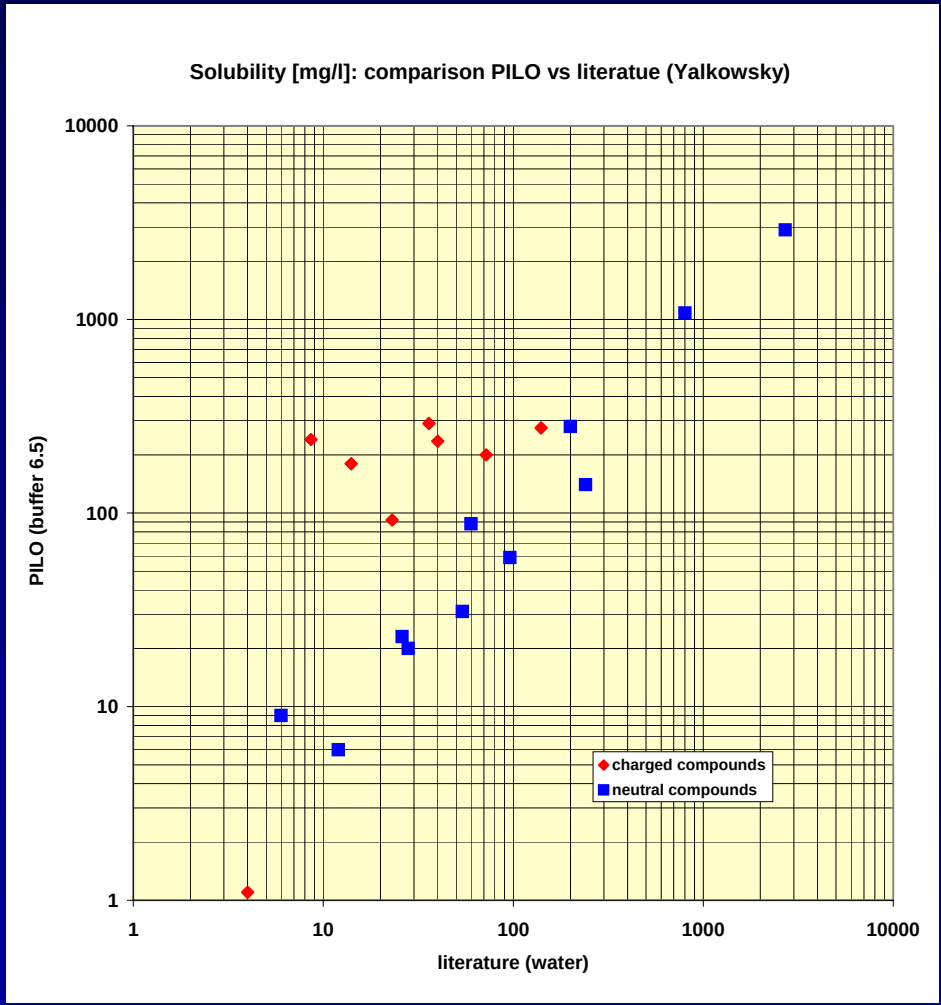
- **precipitation**
  - Dissolve compound in DMSO (2mg/40µl)
  - Add 10µl of this solution to 1000µl buffer (1% DMSO)
  - Shake for 24h at room temperature
  - Centrifuge to get supernatant
  - Establish LC/MS/MS method
  - Measure calibration standards and probe
- **from powder**
  - Weight an appropriate amount of compound as solid
  - Add 1000µl buffer
  - Shake for 24h at room temperature
  - Centrifuge to get supernatant
  - Establish LC/MS/MS method
  - Measure calibration standards and probe

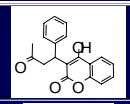
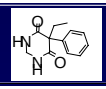
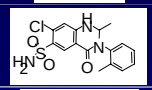
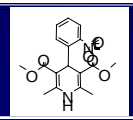
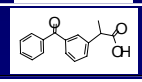
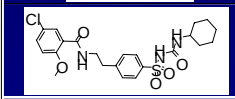
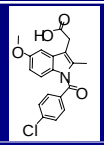
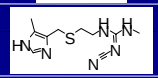
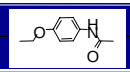
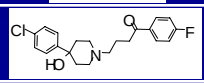
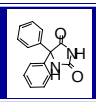
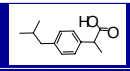
Comparison of solubility methods

EXAMPLES:

Compound		SOL (precipitation) [mg/l]	SOL (from powder) [mg/l]
	Cpd 1	0.5 ± 0,2	0.5 ± 0,3
	Cpd 2	7.9 ± 1,2	1.3 ± 0.2
	Cpd 3	8.8 ± 3,8	4.2 ± 1.1
	Cpd 4	3.9 ± 0.7	0.6 ± 0.1
Cpd 5	mod I	0.8 ± 0.08	0.4 ± 0.08
	mod II	1.5 ± 0.4	1.1 ± 0.08
Cpd 6	mod B	<0.1	<0.1
	amorphous	<0.1	<0.1
Cpd 7	mod I	350 ± 18	420 ± 17
	mod II	330 ± 26	380 ± 19

Comparison of solubility methods



Name	MOLSTRUCTURE	Yalkowsky (water)	charged compounds	neutral compounds
Warfarin		40	235	
Primidone		500		
Metolazone		60		88
Nifedipine		6		9
Ketoprofen		140	275	
Glyburide		4	1.1	
Indomethacin		8.6	240	
Cimetidine		11000		
Phenacetin		800		1080
Haloperidol		14	180	
Phenytoin		26		23
Ibuprofen		36	290	
Acetanilide		6300		

Lessons learned from solubility comparisons

- **method differences not really critical**
- **physical form very important**

  differences between research and development result from:

  - **morphology differences**
  - **impurities**
  - **solvent content**

- **counter-ions and buffers are important when compound is charged in solution**

Case Histories: The Use of Physicochemical Properties

Two different projects as examples:

1. Reducing lipophilicity and HSA binding to increase fraction unbound: erectile disfunction

2. Influence of solubility on in vivo efficacy: the HSV project

Reducing Lipophilicity and Protein Binding to Increase Fraction Unbound

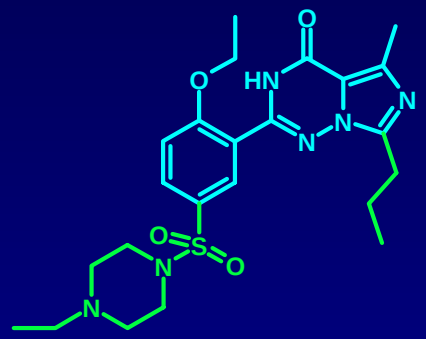
Starting point: initial compound
moderate effective IC50 PDE-5: 530nM

Insufficient physicochemical properties:

high membrane affinity: 16500
high protein binding: 1.7e-5 mol/l
solubility: below detection limit
fraction unbound: <1%

no in vivo efficacy

DP1 compound: Vardenafil
highly effective IC50 PDE-5: 2nM



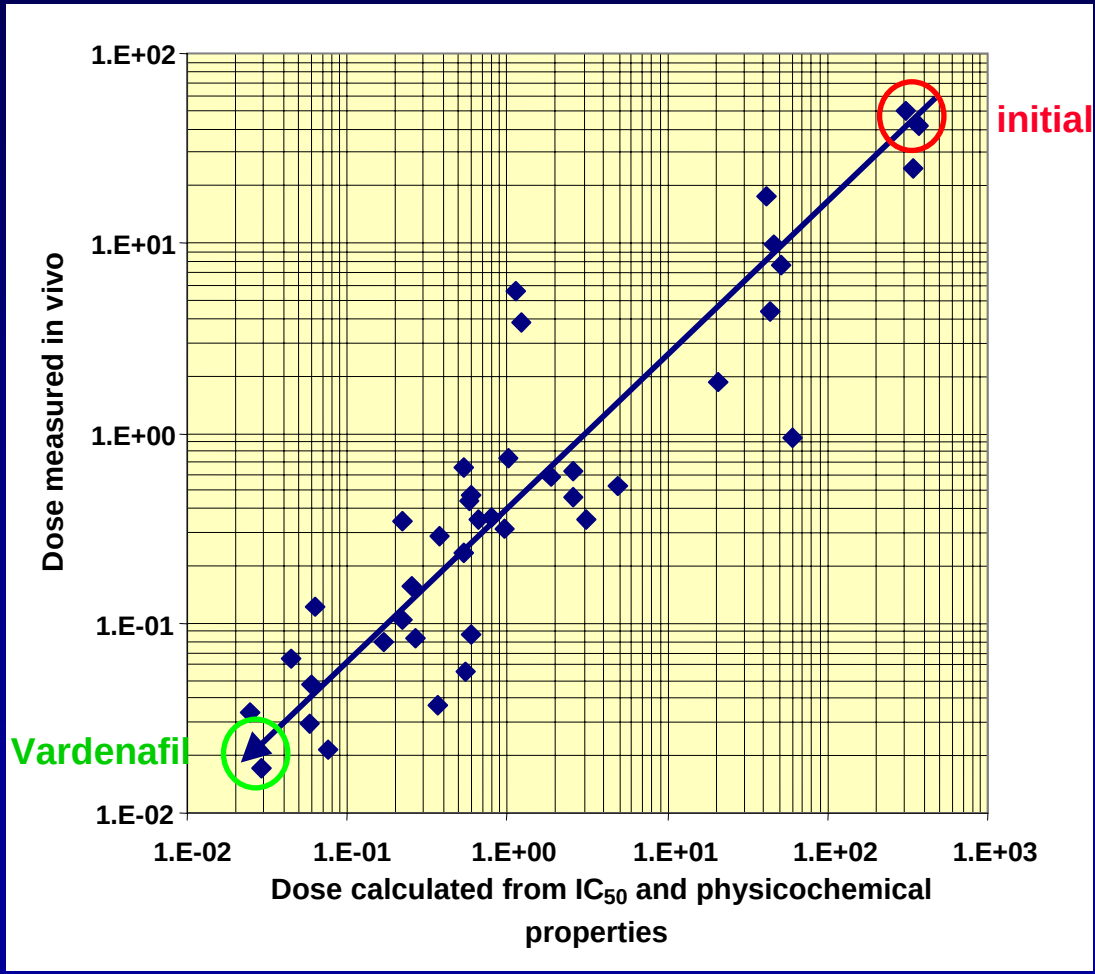
improved physicochemical properties:

reduced membrane affinity: 580
reduced protein binding: 1.2e-4 mol/l
solubility: 220 mg/l
fraction unbound: 14%

excellent in vivo efficacy

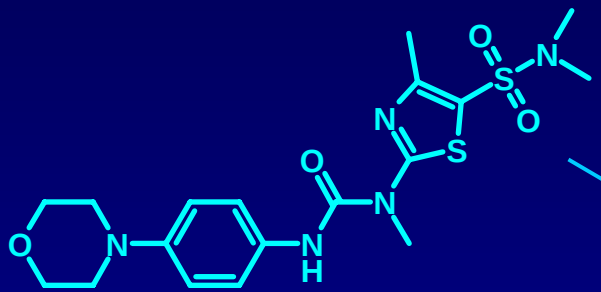
Reducing lipophilicity and protein binding to increase fraction unbound

Even the prediction of the in vivo effect from IC50 and fraction unbound (calculated from MA and HSA) was possible



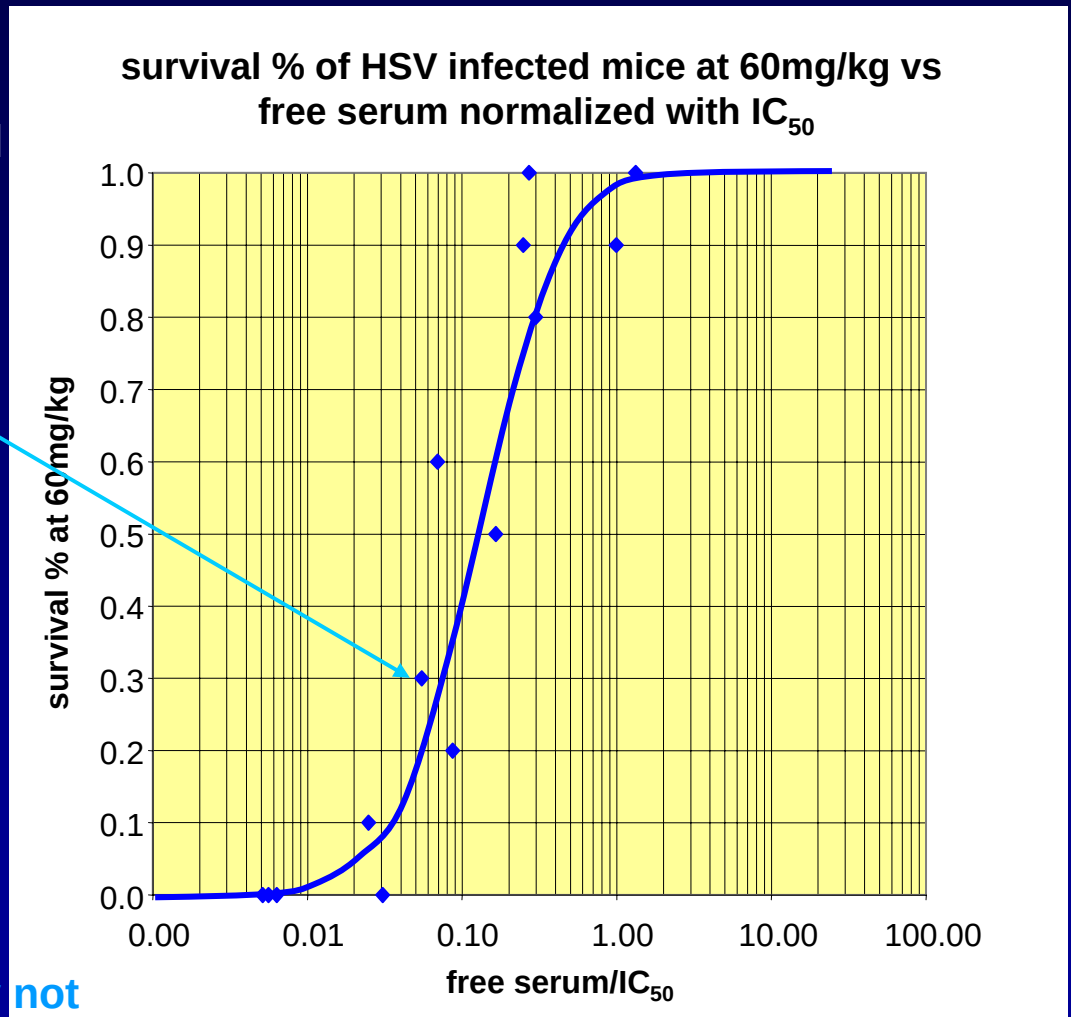
Influence of solubility on in vivo efficacy

Starting point: **Example 1**
moderate activity IC50: 750nM



Physicochemical properties:

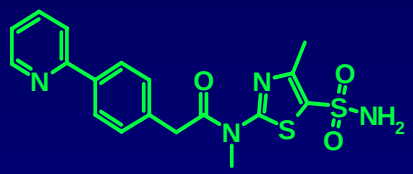
Membrane affinity: 1430
protein binding: 2e-4 mol/l
fraction unbound: 10%
Solubility: 17mg/l



optimization of physicochemistry not necessary, activity has to be improved

Influence of solubility on in vivo efficacy

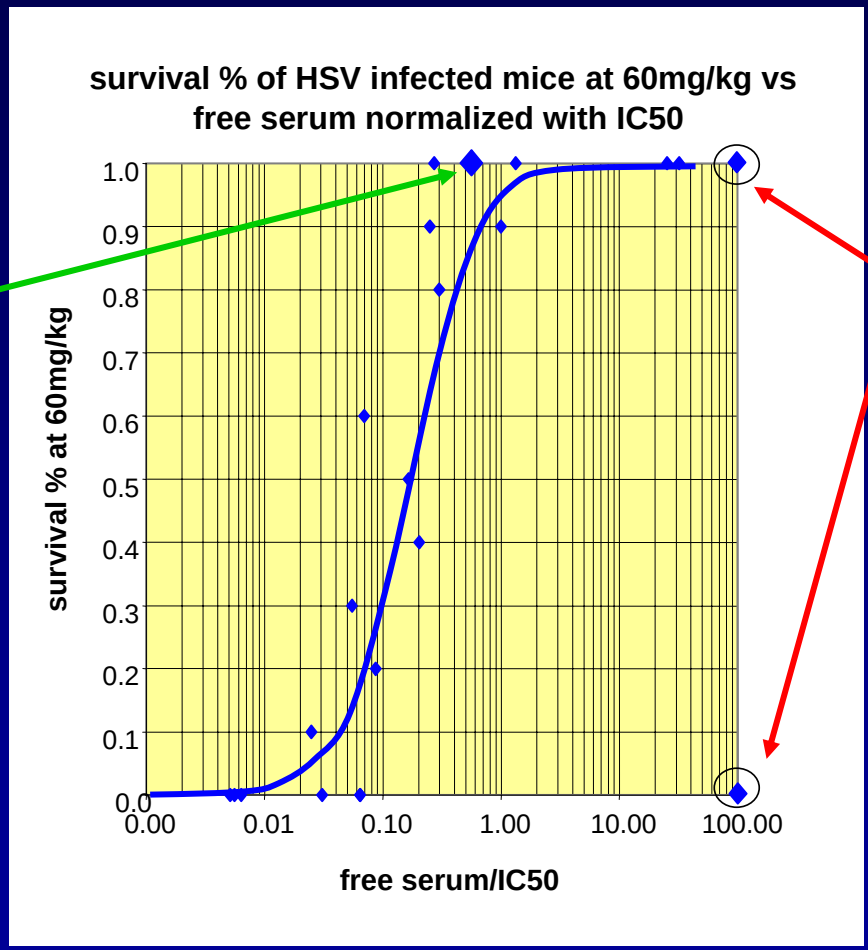
Development candidate
in vitro activity IC50: 20 nM



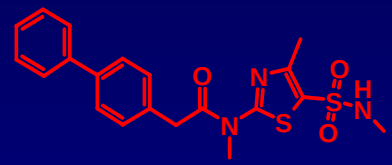
Physicochemical properties:

Membrane affinity: 1590
protein binding: 1e-5 mol/l
fraction unbound: 1%
Solubility: 2.7mg/l

good in vivo efficacy



Example 2: brilliant compound in vitro IC50: <1 nM



Physicochemical properties:

Solubility: <0.1 mg/l

excellent in vivo efficacy when administered as solution, no in vivo efficacy even as micronized powder

Conclusion

- **Impact of Physicochemistry Proven**
- **Physicochemistry/ADME Implemented in Medicinal Chemistry**
- **Properties Routinely Measured for Every Strategic Project**
- **Use in Lead Optimization and Exploratory Research Established**