Hit-to-lead (H2L) and Lead Optimization in Medicinal Chemistry

Drug Discovery: Lead Optimization

Lecture Overview

- **Ligand-protein interactions.**
- **Physico-chemical properties and drug design: attributes of a lead molecule.**
- **Introduction to medicinal chemistry and lead optimization.**
- **Best practices in medicinal chemistry.**
- **Case-studies (Alzheimer's disease):**
  - Fragment-based approaches in discovery of beta-secretase inhibitors
  - Identification of a PDE9 clinical candidate

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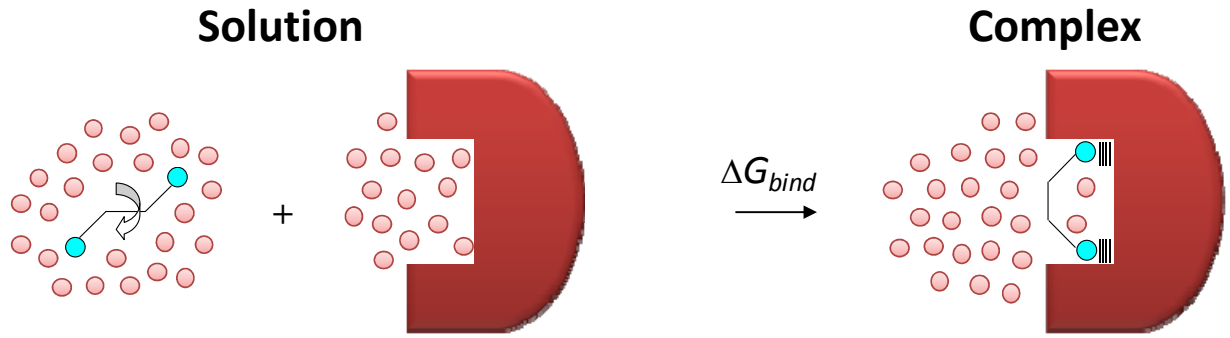
Ligand-protein binding event



$$K_a = e^{-\Delta G/RT} = \frac{1}{K_d}$$

$$\Delta G = \Delta H - T\Delta S$$

Other commonly used potency measures: IC_{50}, Ki

$$K_i \approx K_d$$

Something to remember:

–1.36 kcal/mol ~ 10-fold gain in affinity
–2.72 kcal/mol ~ 100-fold
–4.08 kcal/mol ~ 1000-fold

Some Factors Affecting ΔG_{bind}

Favor Binding	Oppose Binding
Entropy and enthalpy gain due to the "hydrophobic effect" – taking ligand out of water eliminates the penalty associated with the solvent cavity creation.	Ligand desolvation enthalpy – loss of interactions with the solvent.
Entropy and enthalpy gain due to the "hydrophobic effect" for ordered waters bound to protein moving to bulk solvent.	Binding pocket desolvation enthalpy – loss of interactions with the solvent.
Residual vibrational entropy in protein-ligand complex.	Translational and rotational entropy loss for ligand and protein upon binding (loss of 3 translational and 3 rotational degrees of freedom).
Protein-ligand intermolecular interactions .	Loss of conformational, torsional, and vibrational entropies for ligand and protein.
	Strain energy in protein-ligand complex.

Thermodynamics: basic concepts

Potency
10 fold = 1.4 kcal/mol
$\Delta G = \Delta H - T\Delta S$ $\Delta G = -1.4 \log Ki$
1 kcal = 4.184 kJ

Binding Energy		
Potency	–logKi	ΔG
1nM	9	-12.6
10nM	8	-11.2
100nM	7	-9.8
1μM	6	-8.4

Binding energy

- maximal affinity for non-covalent binders ~15 kcal/mol ($-\log K_i \sim 11$)
- enthalpy/entropy compensation
  - *J. Phys. Chem.* **1994**, *98*, 1515.
  - *Biochem. Pharmacol.* **2000**, *60*, 1549.
  - *Chem. Biol.* **1995**, *2*, 709.

Enthalpy

- fundamentally, ΔH reflects strength of ligand interaction with target relative to solvent
- all types of favorable interactions contribute to enthalpy of binding
- accounting for desolvation penalty is an integral part of enthalpy optimization
- ligand internal strain upon binding is often underappreciated

Entropy

- unfavorable contributions
  - loss of conformational degrees of ligand upon binding
  - loss of conformational degrees of protein upon binding
- favorable contributions
  - release of water into bulk solvent

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"A medicinal chemist's guide to molecular interactions"
J. Med. Chem. **2010**, *53*, 5061-5084.

Guiding principles for binding enthalpy optimization

Why bother?

- entropy is usually easier to fix – try to have good enthalpy from the outset
- will likely result in lower lipophilicity
- can result in higher ligand efficiency
- maybe a better starting point for potency improvement

How?

- make *good* H-bonds
  - distance, angle
  - interact with multiple partners on the protein
- group polar functionalities together in your ligand to minimize desolvation penalty
- appreciate desolvation penalty
- assess ligand strain in the bound conformation
- enthalpy improvement does not have to come from polar groups only
- target "unstable" waters

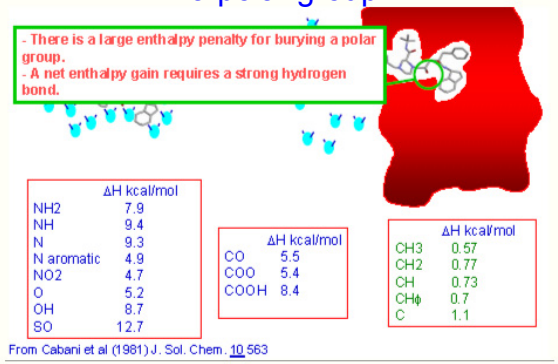
Distances for productive interactions

Hydrogen bond *e.g* C=O···H–O	2.7 - 3.0
π stack C···C	3.3 - 4.3
Edge to face C···C	3.7 - 4.7

Possible energy gain

Energy Estimates	* potency change	
Group	ΔG kcal/mole	x fold*
H-bond	–1.4	10
Salt bridge	–3.4	300
–Me from lipophilicity	–0.7	3
–Cl from lipophilicity	–1.0	5
–Ph from lipophilicity	–2.5	60
–Me buried	–1.4	10
–Ph buried	–3.4	300
Single bond rot	0.7	3
Entropy loss (300 MWt)	+7.0	100,000
Ethane rot barrier	3.0	140
C-C bond energy	80.0	10^{57}

Desolvation penalty for fully burying a polar group



Proton donor and acceptor scales: *e.g.,* M. H. Abraham, *J. Chem. Soc., Perkin II*, **1989**, 1355.

Guiding principles for binding entropy optimization

Why bother?

- the fastest way to improve affinity
- if you want a certain binding profile, adjust the entropy contribution to get there fast
- if you have unfavorable entropy, you can probably deduce why – and fix it!

How?

- lipophilic interactions
- target water clusters
- “prepay” conformational penalty by rigidifying your ligand into a bound conformation
- decrease conformational mobility of your ligand

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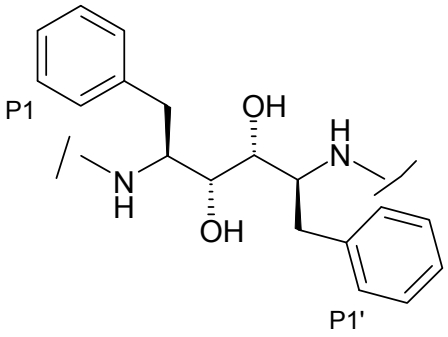
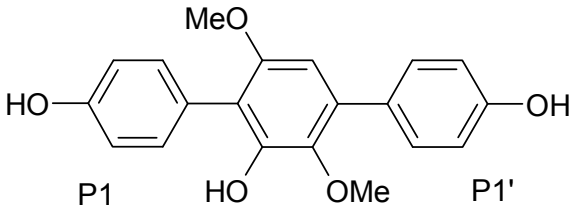
Design of HIV protease inhibitor

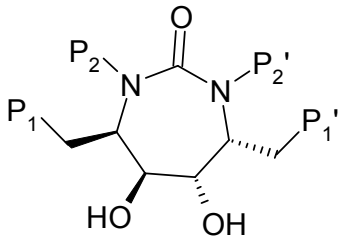
2 OH bind through bound water to enzyme

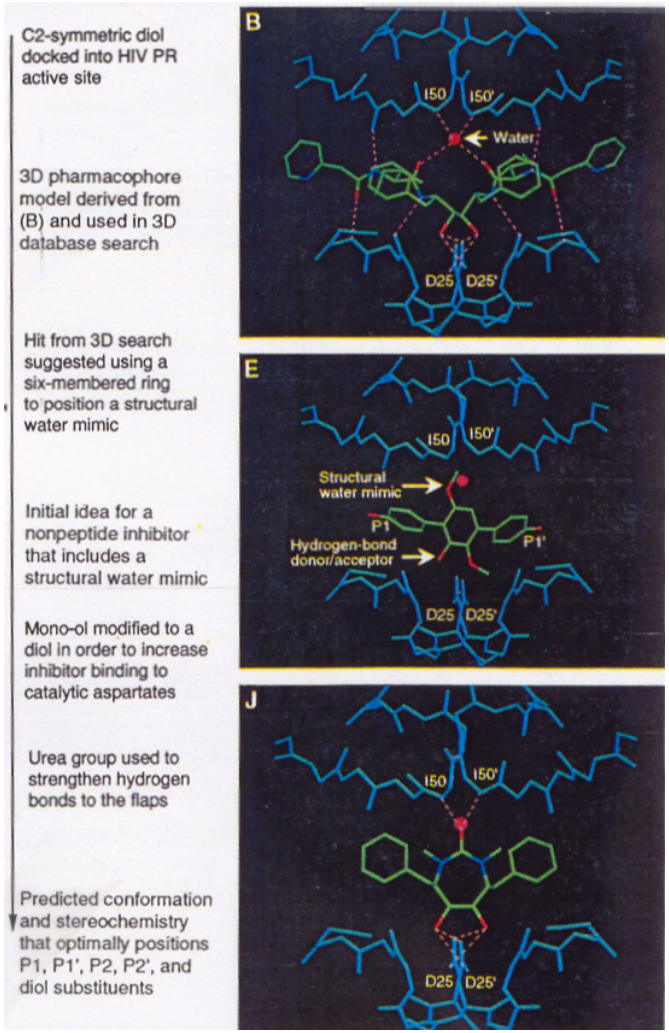


MeO displaces bound water



Ketone displaces water
P1, P2, P1’ and P2’ optimised





Science **1994**, 380-382.

Kinetics: basic concepts and examples

$K_i = \frac{k_{off}}{k_{on}}$

k_{on}	Circumstance
10^9 $M^{-1}s^{-1}$	rare: diffusion control
10^7 $M^{-1}s^{-1}$	common: fast association
10^5 $M^{-1}s^{-1}$	rare: rearrangement

Potency	Occupancy $t_{1/2}$ if $k_{on} = 10^7$ $M^{-1}s^{-1}$
10 nM	7 sec
1 nM	70 sec
0.1 nM	7 min
10 pM	1.9 h

half-life = $0.693/k_{off}$

Target	Drug or drug candidate	Dissociative half-life
Escherichia coli dihydrofolate reductase	Trimethoprim	8 minutes
HMG-CoA reductase	Compactin	15 minutes
Chicken dihydrofolate reductase	Methotrexate	35 minutes
Xanthine oxidase	Allopurinol	5 hours
Adenosine deaminase	Deoxyconformycin	40 hours
HSP90	Geldanamycin	4.6 hours
Human PNP	DADMe-Imm-H	20 minutes
Human PNP	DADMe-Imm-G	2 hours
COX2	Rofecoxib (Vioxx)	9 hours
Viral neuroaminidase	Oseltamivir (Tamiflu)	47 minutes
ERBB2/EGFR	Lapatinib	5 hours
Human angiotensin II type 1 receptor	Candesartan	1–3 hours

Why focus on the off-rate?

Drugs exert their effects when they are bound

- exceptions: hysteresis, posttranslational modifications
- residence time is determined by the *off*-rate only

Even from the potency perspective, why not focus on *on*-rate?

- ultimately limited by diffusion
- *on*-rate affected by diffusion, desolvation, molecular orbital reorientation…
    - difficult to impact by design
    - SAR would be entirely empirically driven
- *on*-rate may not be a limiting factor of the P-L complex formation *in vivo*

More interpretable SAR: consider only ligand-protein complex

Selectivity is time-dependent and is thus a function of the *off*-rate

Effects on *in vivo* activity

- proximity effect
    - high local concentration
    - nonspecific binding
    - rebinding is second order
- as a consequence, long *off*-rate can result in extended duration of action
- it has been demonstrated that a very long *off*-rate can significantly affect efficacy and dosing regimen

"Drug-target residence time and its implications for lead optimization" *Nature Rev. Drug Disc.* **2006**, *5*, 730-739.

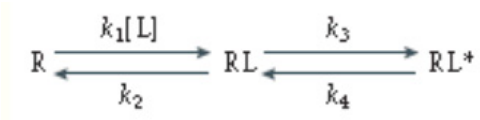
Can we design for longer *off*-rate?

Easier said than done

***Off*-rate is a measure of P-L complex stability – focus on that**

- optimize van der Waals interactions (attractive forces work on very short distances unlike electrostatic interactions)
- do not incur entropic penalty – there is at least anecdotal evidence that entropy and *off*-rate may correlate (evolving science)
- minimize ligand strain

L-P complex isomerization can lead to really long *off*-rates



$k_{off} = k_2k_4/(k_2 + k_3 + k_4)$

If possible, monitor continuously and follow the SAR

- decoupled from the *on*-rate, SAR should be easier to interpret (provided there is enough data points)

Mitigating factors for using very slowly dissociating ligands

Rate of target resynthesis (especially for antibacterial targets)

Possible immune response for cell-surface targets in systemic circulation
- altered receptor conformation can be recognized as foreign
- roxifiban (DuPont; antagonist of platelet-surface receptor glycoprotein IIb/IIIa)

Safety window for mechanism-related toxicity
- toxicity and desired pharmacological effect may have different temporal profiles
- was suggested for D2 receptor blockers

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Lipophilicity is your friend

Why is Lipophilicity important?

Lipophilicity is a key physical property for predicting biological activity of drugs. Why?

The body is made up of fatty compartments (cell membranes) and aqueous compartments (inter- and intra-cellular fluid)

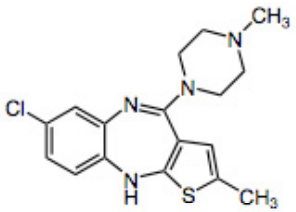
Drugs need to pass between these different compartments to get to their site of action

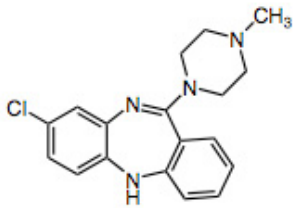
A suitable balance between lipophilicity and hydrophilicity is essential for the drug to have the right transport properties to be effective

Lipophilicity can wreck everything

Lipophilicity and bioavailability

Olanzapine ®
Brand name ZYPREXA: a medication approved for the treatment of schizophrenia and acute mania





	Olanzapine	Chlozapine
Lipophilicity	Log P = 2.94 Log $D_{7.4}$ = 2.21	Log P = 3.72 Log $D_{7.4}$ = 3.32
Usual Dose/ Dose Frequency	5 – 10 mg (once a day)	300 mg (twice a day)
Human Bioavailability	~100%	55%

"Lipophilicity in drug discovery" *Expert Opin. Drug Disc.* **2010**, *5*, 235-248.

The Rule of 5 - Origins

- Lipinski et al. (Pfizer, Groton) *Advanced Drug Delivery Reviews* **1997,** *23*, 3-25.

- Considered factors which could affect SOLUBILITY and PERMEABILITY of compounds.

- Looked at > 50,000 compounds.

- 'Rule of 5' emerged. *As a guideline.*

- What does it predict? What rules should we consider today?

The Rule of 5 - Definition

Poor absorption/permeation and solubility are <u>likely</u> when:

Number of H-bond donors (NH, OH) > 5
Number of H-bond acceptors > 10
MW > 500
clogP > 5

~ 90% of oral drugs adhere to this rule.

Why is this image here?

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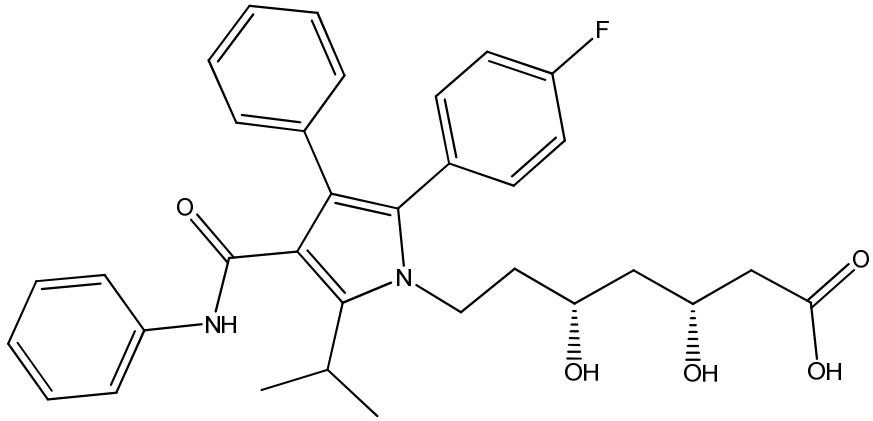
So how about this compound?

no. of H-bond donors (NH, OH) > 5

no. of H-bond acceptors > 10

MW > 500

clogP > 5



Computed Properties of the Parent Compound	
Andrews Binding Energy	18.60
Calculated Exact (Non-isotopic)	558.25
Calculated log Partition Coefficient (ClogP)	4.46
Heavy Atom Count	41
Hydrogen Bond Acceptor Count	5
Hydrogen Bond Donor Count	4
LogD @ pH = 6.5	1.54
LogD @ pH = 7.4	0.74
NH/OH Count	4
Nitrogen/Oxygen Count	7

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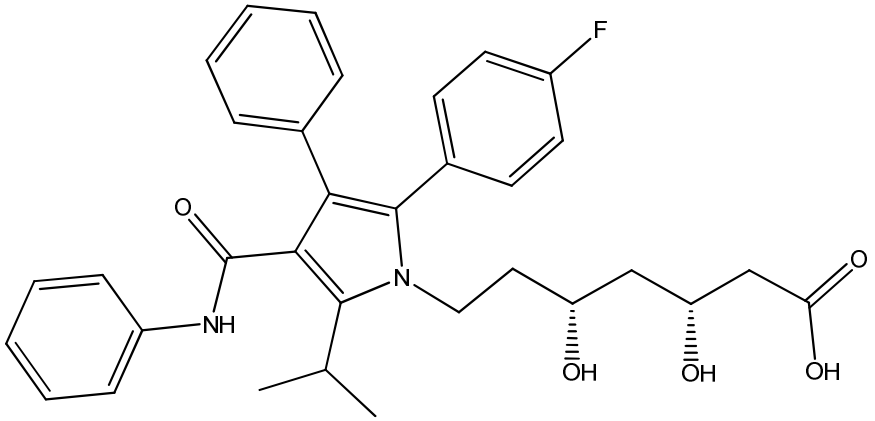

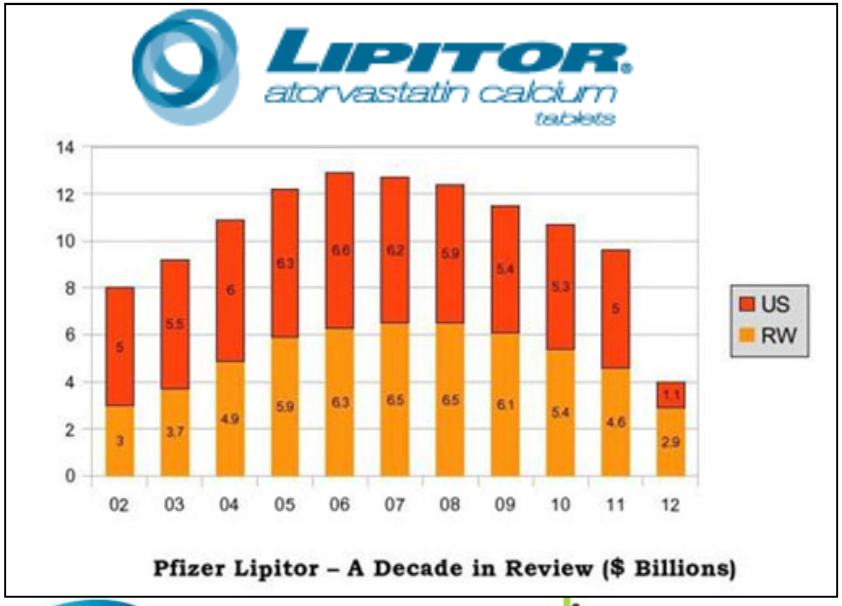

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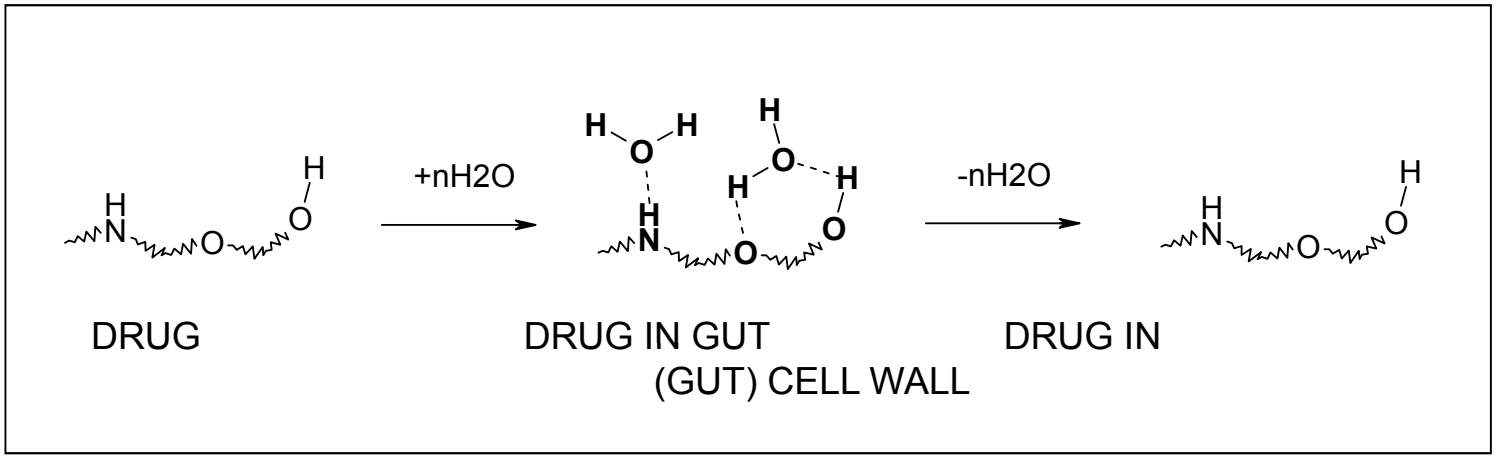
The Rule of 5 – How does it work?

(1) H-bond Donors and Acceptors



- Too many H-bond donors/ acceptors make desolvation too difficult, preventing absorption across the gut wall.
- H-bond donors (NH, OH) are 2-3x worse than acceptors (O, N).

The Rule of 5 – How does it work?

(2) clogP > 5

- lipophilic compounds have poor aqueous solubility => poor absorption
- CYPs metabolise lipophilic compounds => poor bioavailability
- Keep clogP < 5

The Rule of 5 – How does it work?

(3) MW > 500

for MW >500 there is ~ no middle ground

Few NH/OH/N/O =>

TOO LIPOPHILIC

Lots of NH/OH/N/O =>

CAN'T DESOLVATE

ALSO: as MW ↑ Sites of metabolism ↑

AND: as MW ↑ Membrane penetration ↓

Basicity, Neutrality and Acidity

Neutral Compounds

- not ionised/ protonated at physiologically relevant pH (7.4)
  - *ca*. 1/3 of all drugs

Basic Compounds

- protonated at physiological pHs
- *ca*. 1/3 of all drugs

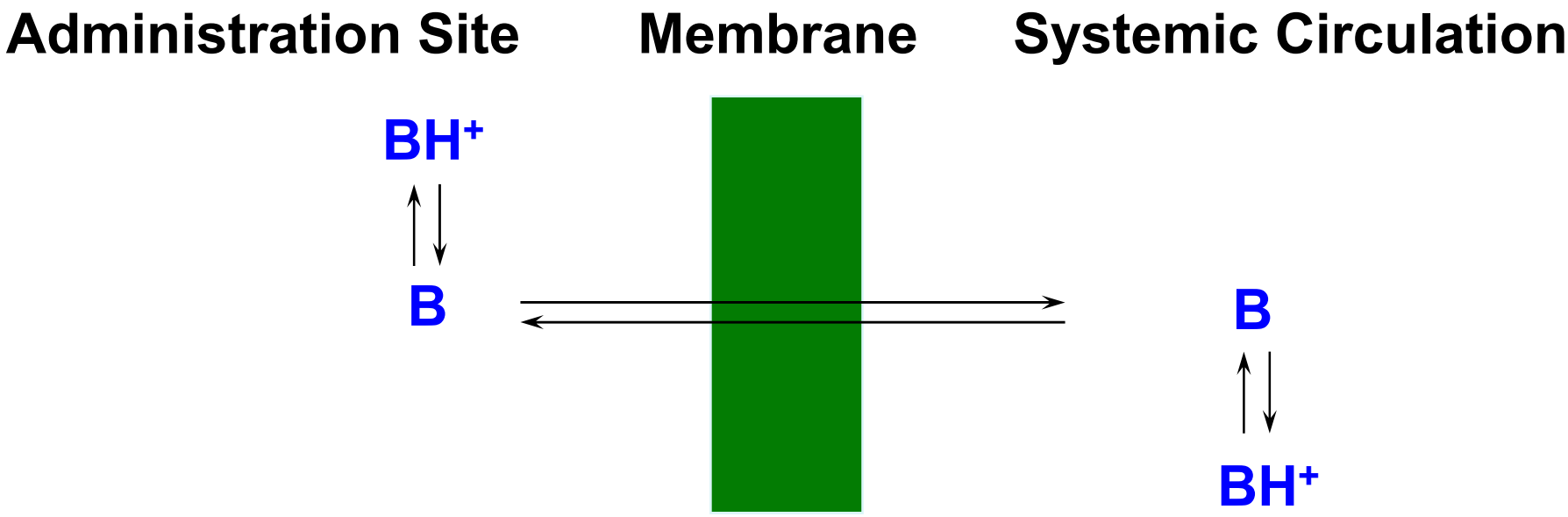
Acids

- deprotonated/ionised at physiological pH
- *ca*. 1/3 of all drugs

pKa can/will effect

- potency
- logD
- solubility
- salt forms/ crystallinity
- membrane permeability
- plasma protein binding
- volume of distribution
- metabolism…

Ionizable Compounds: Membrane Partitioning



- Only unionised drug can cross the membrane.
- Ionised drug must first lose charge.
- Dependent upon pKa and permeation rate of unionised form.

Metabolism as a function of pK_a

- CYP metabolism increases as lipophilicity goes up.

- Neutral compounds, acids and bases are typically metabolised by different CYPs, *e.g.:*

CYP2C9 - acids
CYP2D6 - bases
CYP3A - acids, bases, neutrals

Physico-chemical Properties: Summary

- We can predict / calculate / measure pKa, logD and logP.
- Typical drug profile:

  clogP < 5 (ideally <3)

  0 < logD < 2.5

  MW <500

  Low H-bond Donor/ acceptor count (<5, 10 respectively)

  “Ro5 compliant”

- We aim to work well within these limits!

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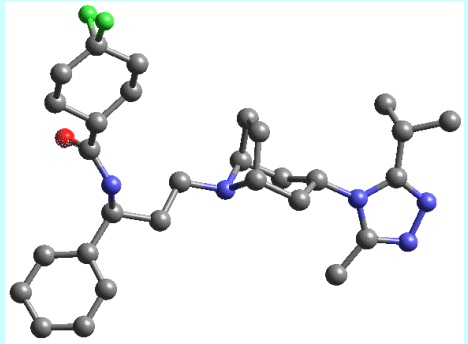
What is a Drug?



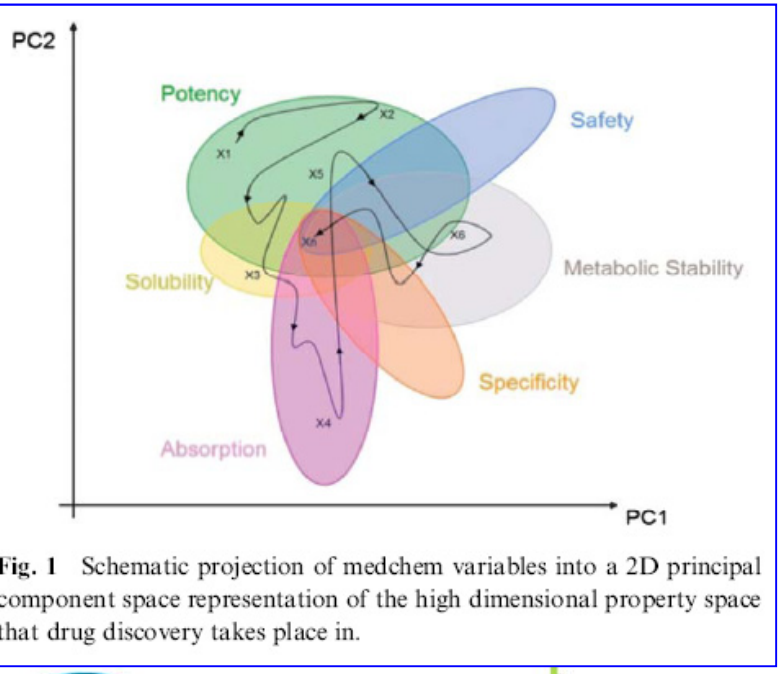


Fig. 1 Schematic projection of medchem variables into a 2D principal component space representation of the high dimensional property space that drug discovery takes place in.

Not the most potent
Nor the most stable
Nor the best absorbed
Nor the least active against cardiac ion channels

BUT – best balance

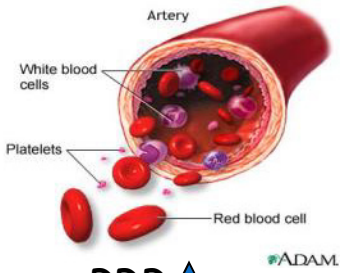
"Molecular obesity, potency and other addictions in drug discovery" *Med. Chem Commun.* **2011**, *2*, 349-355.

Lead Optimization – a Balancing Act

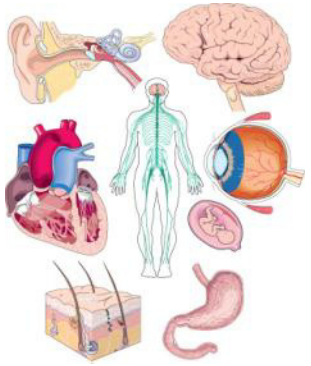
• An oral drugs journey from the gut to target includes interactions with water, membranes and proteins. All are very different environments!

• These differing environments mean we spend a lot of time optimising molecular properties and balancing these with potency/ selectivity.

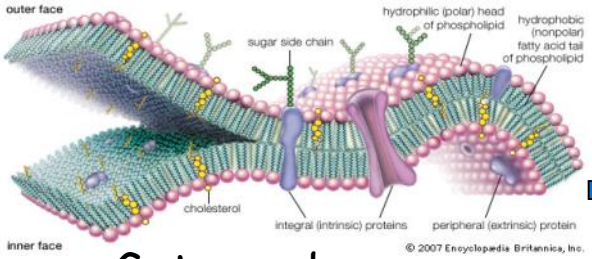
Solubility



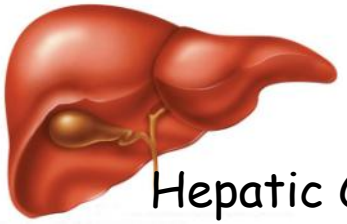
PPB



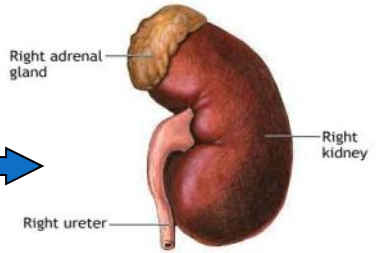
Tissue distribution



Gut membrane permeation



Hepatic Cl

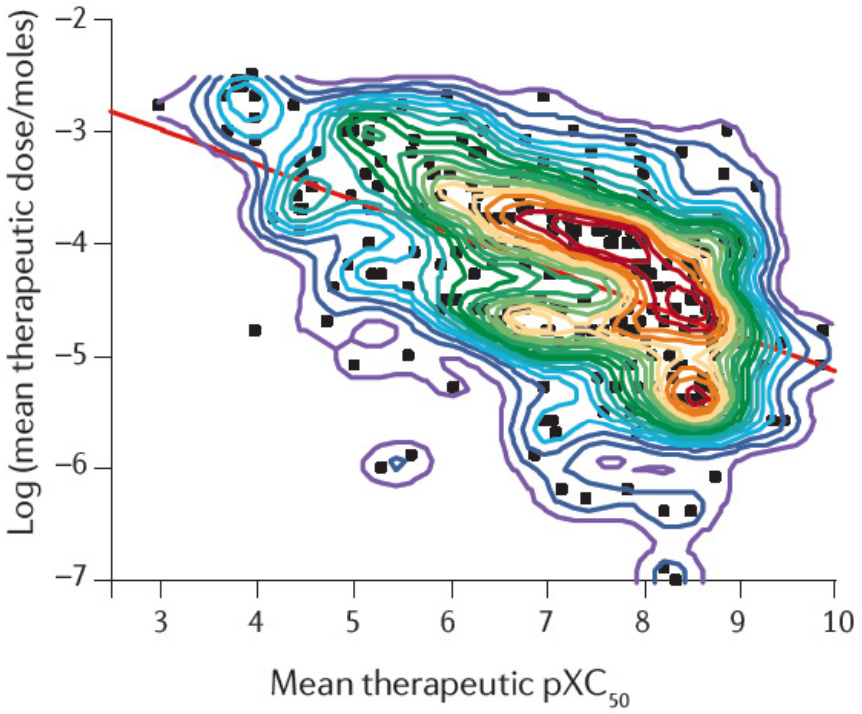


Renal Cl

It is not just potency!

$$\text{Dose} = \frac{C_{eff,free} \cdot CL_{int} \cdot \tau}{f_a}$$

- Distribution
- Affinity
- Intrinsic activity
- Intrinsic stability
- Solubility
- Permeability



"Probing the links between in vitro potency, ADMET and physicochemical parameters" *Nature Rev. Drug Disc.* **2011**, *10*, 197-208.

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Lead Optimization

Refining the chemical structure of a confirmed hit to improve its drug characteristics.

- Synthesis of analog series.
- Testing the series to correlate changes in chemical structure to biological and pharmacological data to establish structure-activity relationships (SAR):
  - Potency
  - Bioavailability
  - Stability
  - Selectivity
- Optimization cycle is repeated until the candidate molecule is selected.

A Concept of Ligand Efficiency

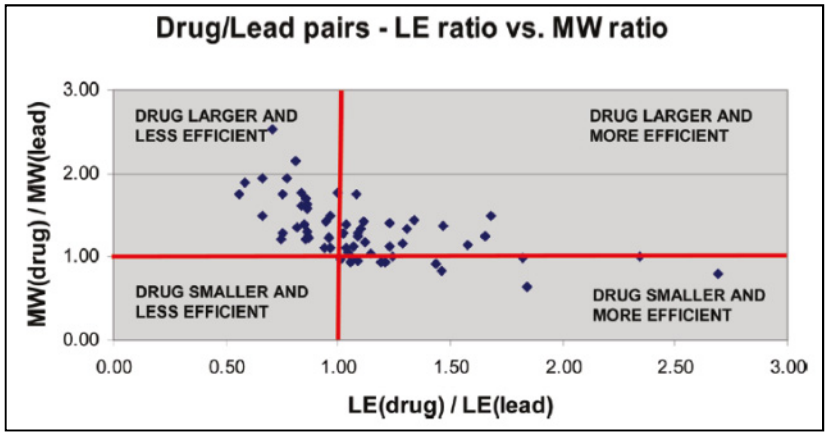
- Kuntz: free energy of binding per atom

  $\Delta g = \Delta G / N_{\text{non-H atoms}}$

  $\Delta g = 1.4 \log(IC_{50}) / N_{\text{non-H atoms}}$

- LE is related to potency and number of atoms.
- LE is critical in assessment of hit quality and should be closely monitored during lead optimization.

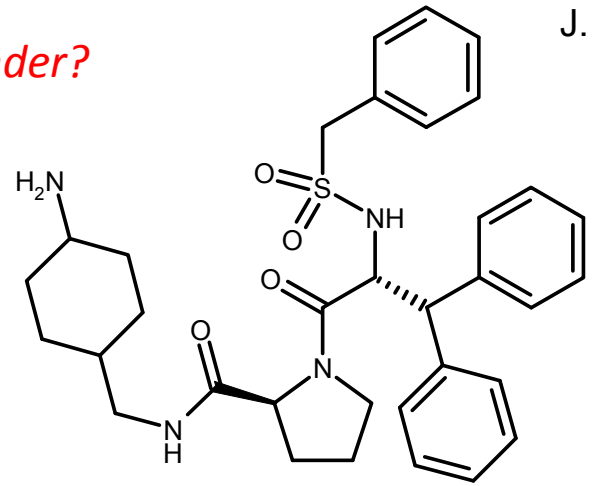
"An analysis of the binding efficiencies of drugs and their leads in successful drug discovery programs" *J. Med. Chem.* **2010**, *53*, 2986-2997.

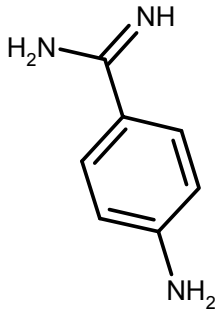


Ligand Efficiency – Small is Beautiful

Which compound is a better binder?

J. Med. Chem.**1997**, *40*, 830-832.



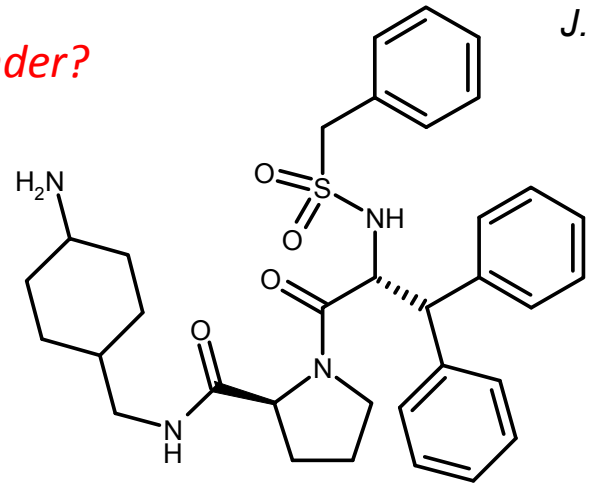


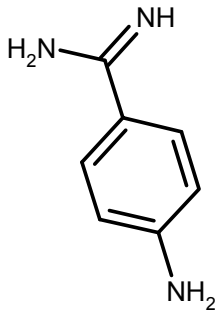
K_i (nM)	0.0025	30000

Ligand Efficiency – Small is Beautiful

Which compound is a better binder?

*J. Med. Chem.***1997**, *40*, 830-832.





K_i (nM)	0.0025	30000
Energy (kcal/mol)	15.8	6.2
Atoms	43	10
MW	604	136
LE (kcal/mol/atom)	0.37	**0.62**

Micromolar ligand twice as efficient binder as picomolar ligand!

Lead Optimization – Top 10 Tactics

1. Start with a good lead
   - Low MW and logP, potent, selective, novel and functionally active!
2. Look before you leap
   - 'Why waste 2 hours in the library when you could spend 2 weeks in the lab'
3. Chemistry should allow rapid diversification
   - Multiple sites of variation and chemistry suitable for parallel follow-up
4. Optimise Lipophilic Interactions
   - LogP/Potency plots & Ligand Efficiency– spot outliers
5. Optimise Polar interactions
   - Look for specific H-bonds and meaningful loss (or gains) in potency

Lead Optimization – Top 10 Tactics

6. Hetero-atom Insertion
   - Aryl/heterocycle switch or CH_2/O/N switch
7. Bioisosteres
   - Amide reversal
   - Isoelectronic and/or isosteric replacement
8. Optimise Dipole
   - F or CF_3 substitution
   - N/C-F switch
9. Conformational control
   - If you see a ring break it. If you don't then make it.
   - Preorganisation can be very beneficial to potency (If you get it right!)
10. Challenge your own hypotheses & invest in alternative templates/series
    - Get out of the box!

Lipophilicity and promiscuity

Lipophilicity will likely buy you potency…

- … but not just for your target.
- hydrophobic interactions are mostly not directional and, thus, are much less specific than polar interactions.
- lipophilic amines are particularly bad in this regard (red line in the plot below).

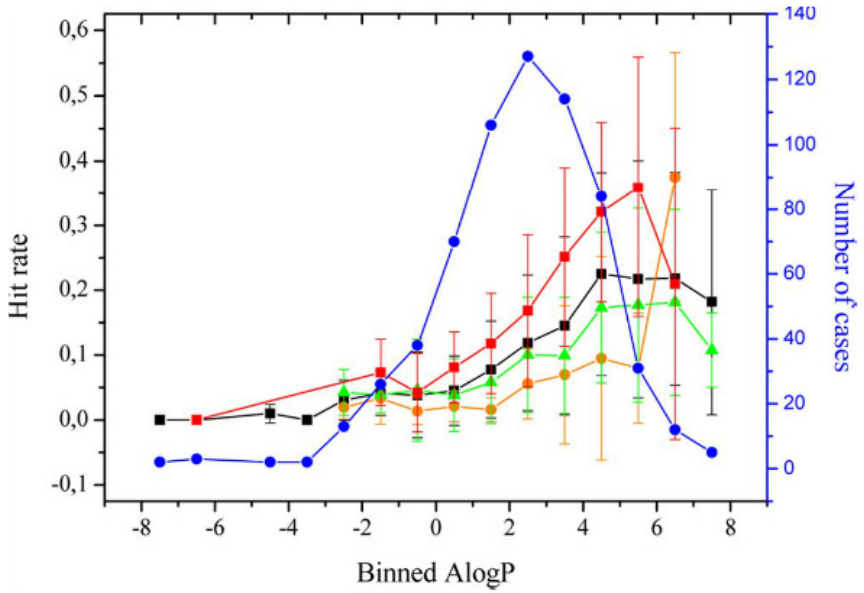


Figure 1. Relationship between AlogP and hit rate on off-targets for 638 marketed drugs investigated on 40–73 targets.[33] Left *Y*-axis shows the hit rate. Black, green, orange, and red lines and symbols represents all, neutral, acidic, and basic compounds, respectively. Right *Y*-axis and blue line with symbols represent all compounds regardless of the ionization state (corresponds to black squares of left axis).

"Contributions of molecular properties to drug promiscuity" *J. Med. Chem.* **2013**, *56*, 1789-1795.

"The influence of drug-like concepts on decision-making in medicinal chemistry" *Nature Rev. Drug Disc.* **2007**, *6*, 881-890.

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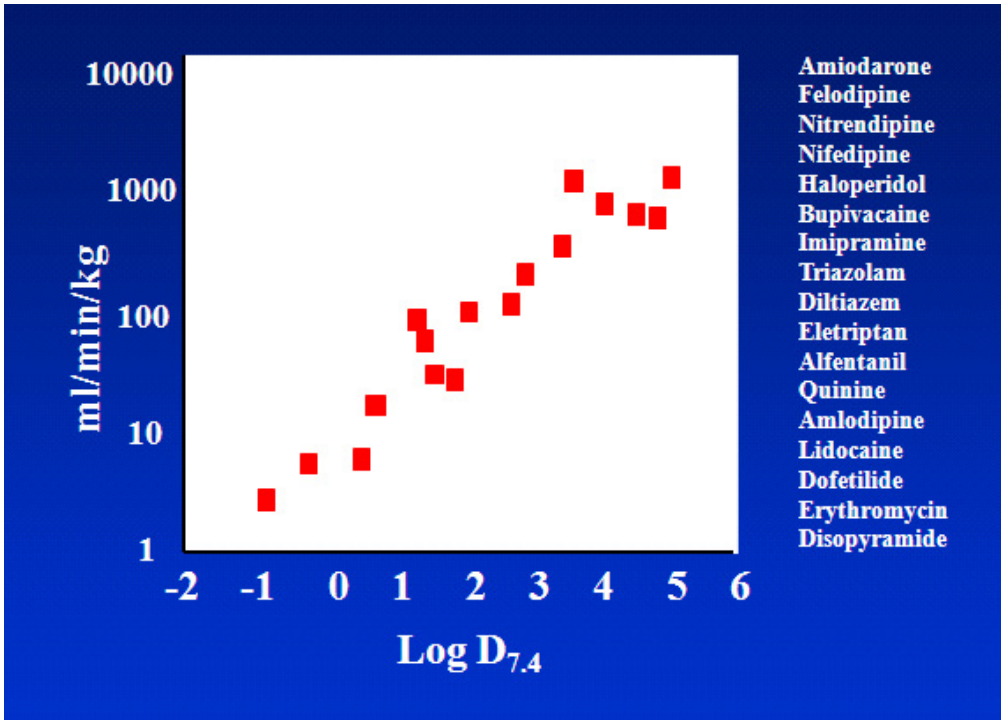
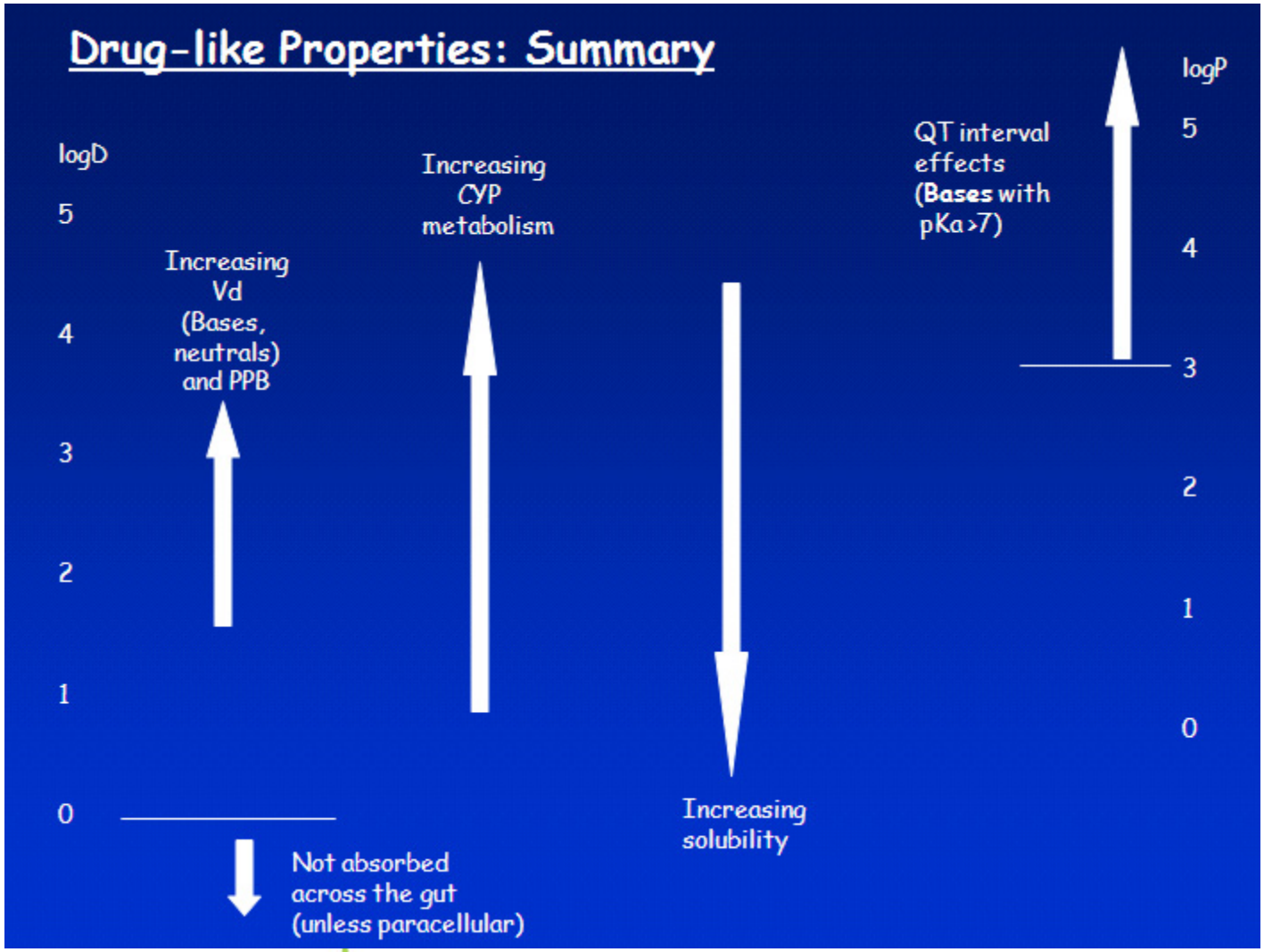
Lipophilicity and clearance

CYP3A4 substrates as a test case:

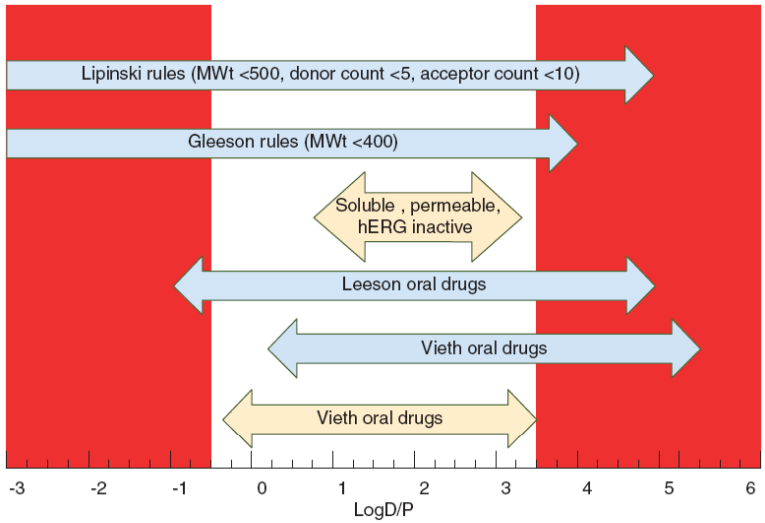


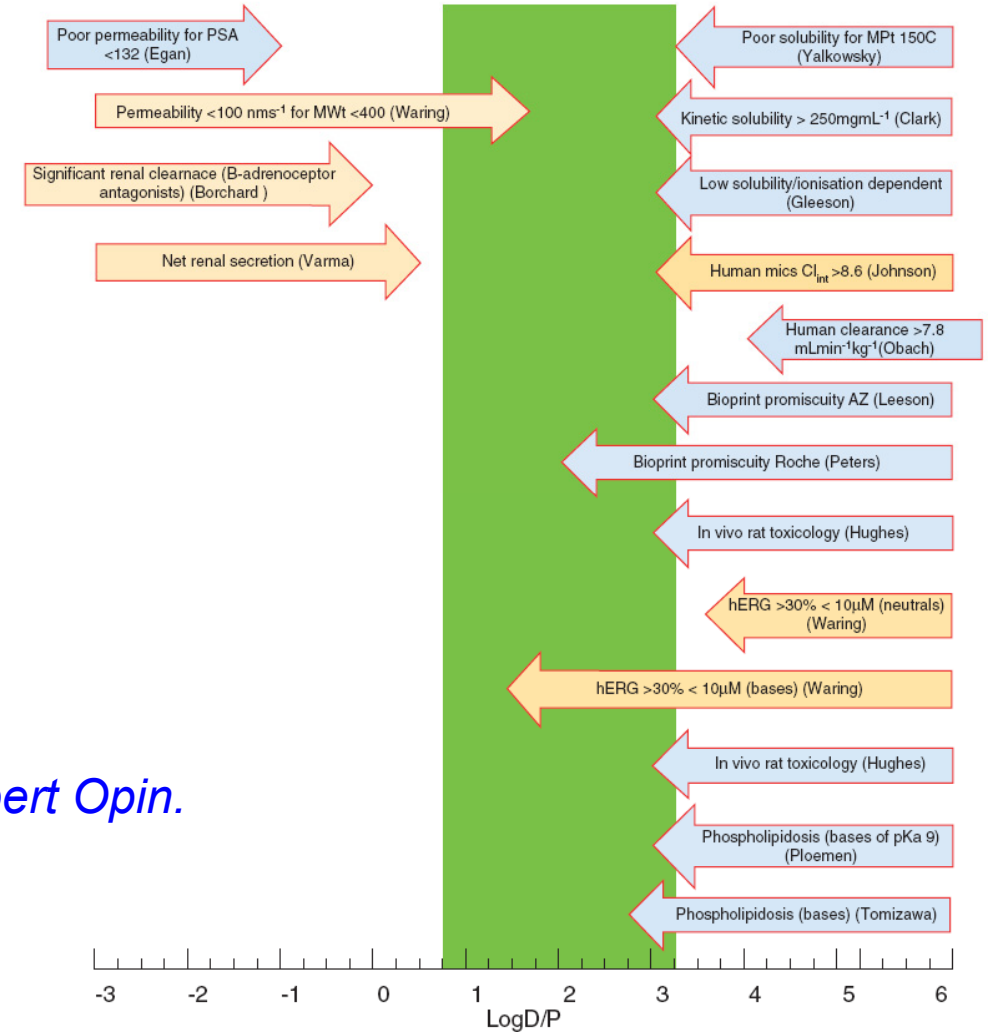
This trend is rather general.

Opposing factors in lead optimization



Is there a preferred lipophilicity range for oral drugs?





"Lipophilicity in drug discovery" *Expert Opin. Drug Disc.* **2010**, *5*, 235-248.

ADME predictions based on phys-chem properties

neutral molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	average	lower
permeability*	higher	average/higher
bioavailability	average	lower
volume of Dist.**	average	average
plasma protein binding	average	higher
CNS penetration***	higher/average	average/lower
brain tissue binding	lower	higher
P-gp efflux	average	higher/average
in-vivo clearance	average	average
hERG Inhibition	lower	lower
P450 inhibition****	lower 2C9, 2C19, 2D6 & 3A4 inhibition	higher 2C9, 2C19 & 3A4 inhibition
P450 inhibition****	higher 1A2 inhibition	lower 1A2 inhibition
P450 inhibition****		average 2D6 inhibition

basic molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	higher/average	lower/average
permeability*	higher/average	average
bioavailability	average	lower
volume of Dist.**	higher/average	higher
plasma protein binding	lower	average
CNS penetration***	higher/average	average/lower
brain tissue binding	lower	higher
P-gp efflux	average	higher/average
in-vivo clearance	average	higher/average
hERG Inhibition	average/higher	higher
P450 inhibition****	lower 1A2, 2C9, & 2C19 inhibition	lower 1A2 inhibition
P450 inhibition****	average 2D6 & 3A4 inhibition	average 2C9, 2C19 inhibition
P450 inhibition****		higher 2D6 & 3A4 inhibition

acidic molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	higher	average/higher
permeability*	lower	average/lower
bioavailability	average	average
volume of Dist.**	lower	lower
plasma protein binding	average/higher	higher
CNS penetration***	lower	lower
brain tissue binding	lower	higher
P-gp efflux	lower	lower
in-vivo clearance	lower/average	average
hERG Inhibition	lower	lower
P450 inhibition****	lower 1A2, 2C9, 2C19, 2D6 & 3A4 inhibition	lower 1A2, 2C19, 2D6 & 3A4 inhibition
P450 inhibition****		higher 2C9 inhibition

(d) zwitterionic molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	higher	average/higher
permeability*	lower	lower/average
bioavailability	lower	lower
volume of Dist.**	lower	average/lower
plasma protein binding	average/lower	higher
CNS penetration***	average/lower	lower
brain tissue binding	lower	higher
P-gp efflux	average	average
in-vivo clearance	average	average
hERG Inhibition	lower	average/lower
P450 inhibition****	lower 1A2, 2C9, 2C19, 2D6 & 3A4 inhibition	lower 1A2, 2C19 & 3A4 inhibition
P450 inhibition****		average 2C9, 2D6 inhibition

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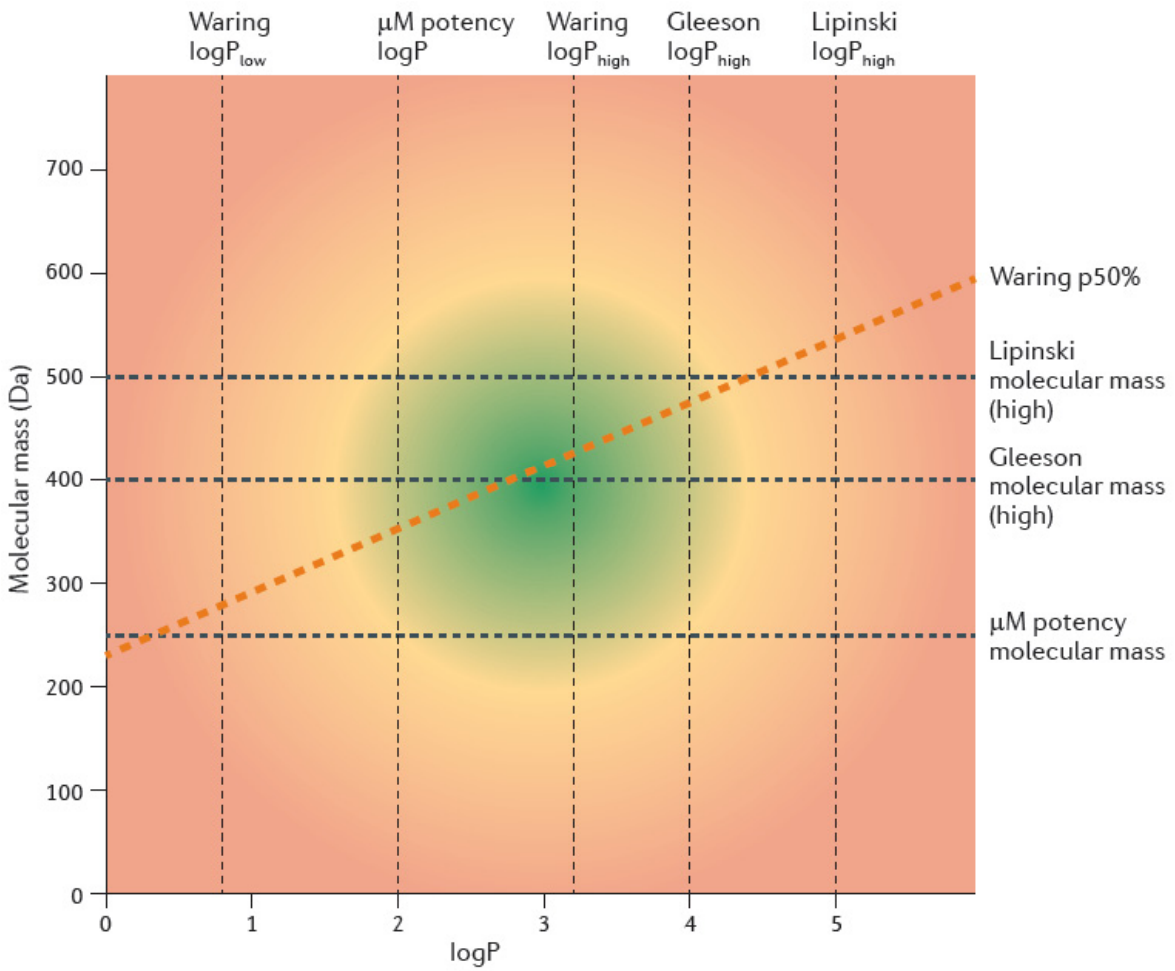
"Generation of a set of simple, interpretable ADMET rules of thumb" *J. Med. Chem.* **208**, *51*, 817-834.

ADME predictions based on phys-chem properties

"Generation of a set of simple, interpretable ADMET rules of thumb" *J. Med. Chem.* **208**, *51*, 817-834.

How about this?



"Finding the sweet spot: the role of nature and nurture in medicinal chemistry" *Nature Rev. Drug Disc.* **2012**, *11*, 355-365.

Guiding principles for lead optimization

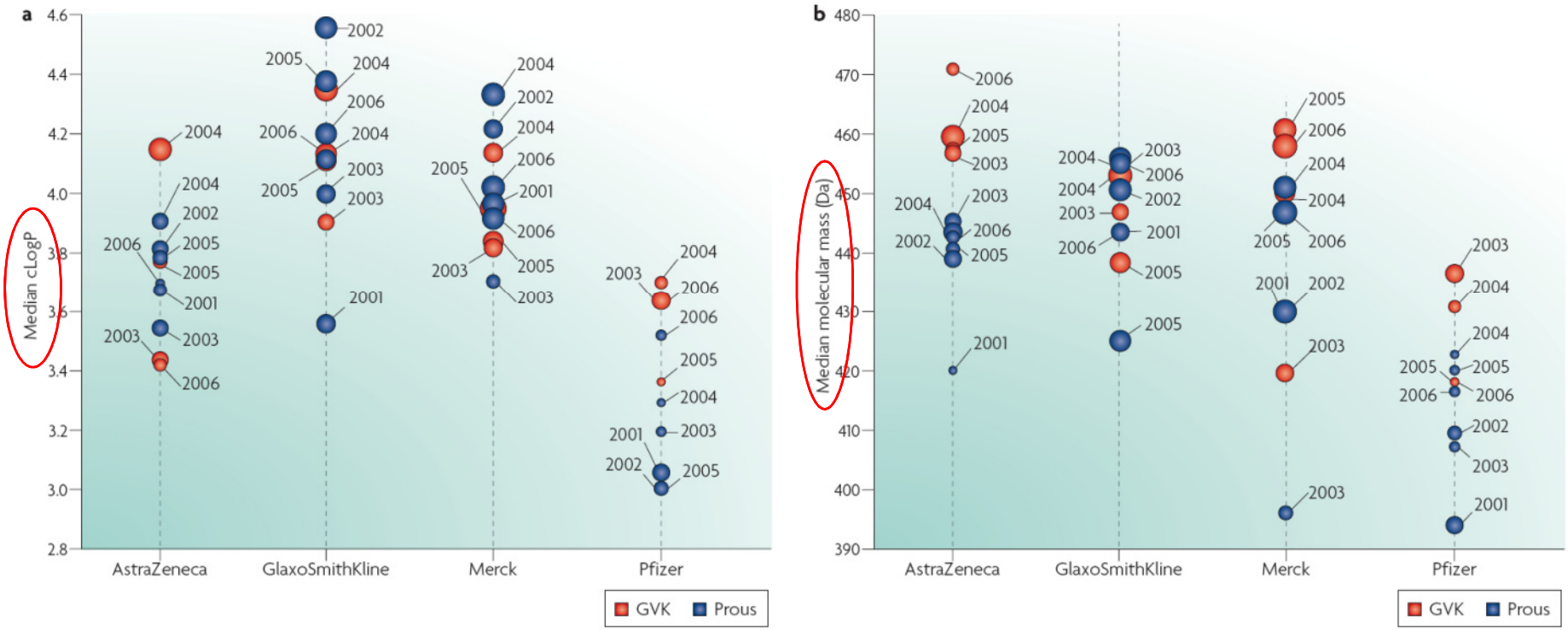
Box 2 | **Proposed medicinal chemistry guidelines**

- Consider the chemical tractability (ligandability) of the target, and if it is poor then investigate different mechanisms of action or different pathways
- Select multiple, low-complexity polar starting points with high binding enthalpy, and optimize enthalpically towards the lead compound
- Select appropriate metrics for multidimensional optimization; use ligand efficiency and lipophilic efficiency metrics in hit-to-lead optimization and change to more complex metrics emphasizing dosage to support lead optimization
- Evaluate available chemistries when entering extensive optimization; prepare what you designed and really want rather than what you can readily synthesize; design, synthesize and use proprietary building blocks rather than depend on chemistry catalogues
- Do not be afraid to revert to a series of lower potency if it has better physicochemical properties. Extensive optimization of a scaffold that is not amenable to achieving a desirable balance of potency and ADME (absorption, distribution, metabolism and excretion) properties is likely to be a waste of time and resources
- Stay focused on the 'sweet spot' and committed to deliver high-quality compounds, but remain open-minded to the many ways this can be achieved
- Resist timelines that compromise compound quality

"Finding the sweet spot: the role of nature and nurture in medicinal chemistry" *Nature Rev. Drug Disc.* **2012**, *11*, 355-365.

Drug likeness and med-chem culture

- **Attention to the concepts of drug-likeness, both on an individual and an institutional level, will have tangible consequences.**



"The influence of drug-like concepts on decision-making in medicinal chemistry " *Nature Rev. Drug Disc.* **2007**, *6*, 881-890.

"Molecular obesity, potency and other addictions in drug discovery" *Med. Chem Commun.* **2011**, *2*, 349-355.

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Two MedChem Worlds

We don't know how good a compound is until we make it.

MedChem is a voyage of discovery.

We can predict enough data to ensure we make better compounds and succeed sooner.

We know so much already!

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Lecture Overview

- Ligand-protein interactions.
- Physico-chemical properties and drug design: attributes of a lead molecule.
- Introduction to medicinal chemistry and lead optimization.
- **Best practices in medicinal chemistry.**
- Case-studies (Alzheimer's disease):
  - Fragment-based approaches in discovery of beta-secretase inhibitors
  - Identification of a PDE9 clinical candidate

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“What Good Medicinal Chemists Do (and Don’t Do)?”

From a survey of 33 current and past Pfizer medicinal chemists on the topic set out above.

Three General Themes

Technical expertise

Знание научных дисциплин

Strategic thinking and judgment

Стратегическое мышление

Individual behaviors in a collaborative environment

Навыки работы в коллективе

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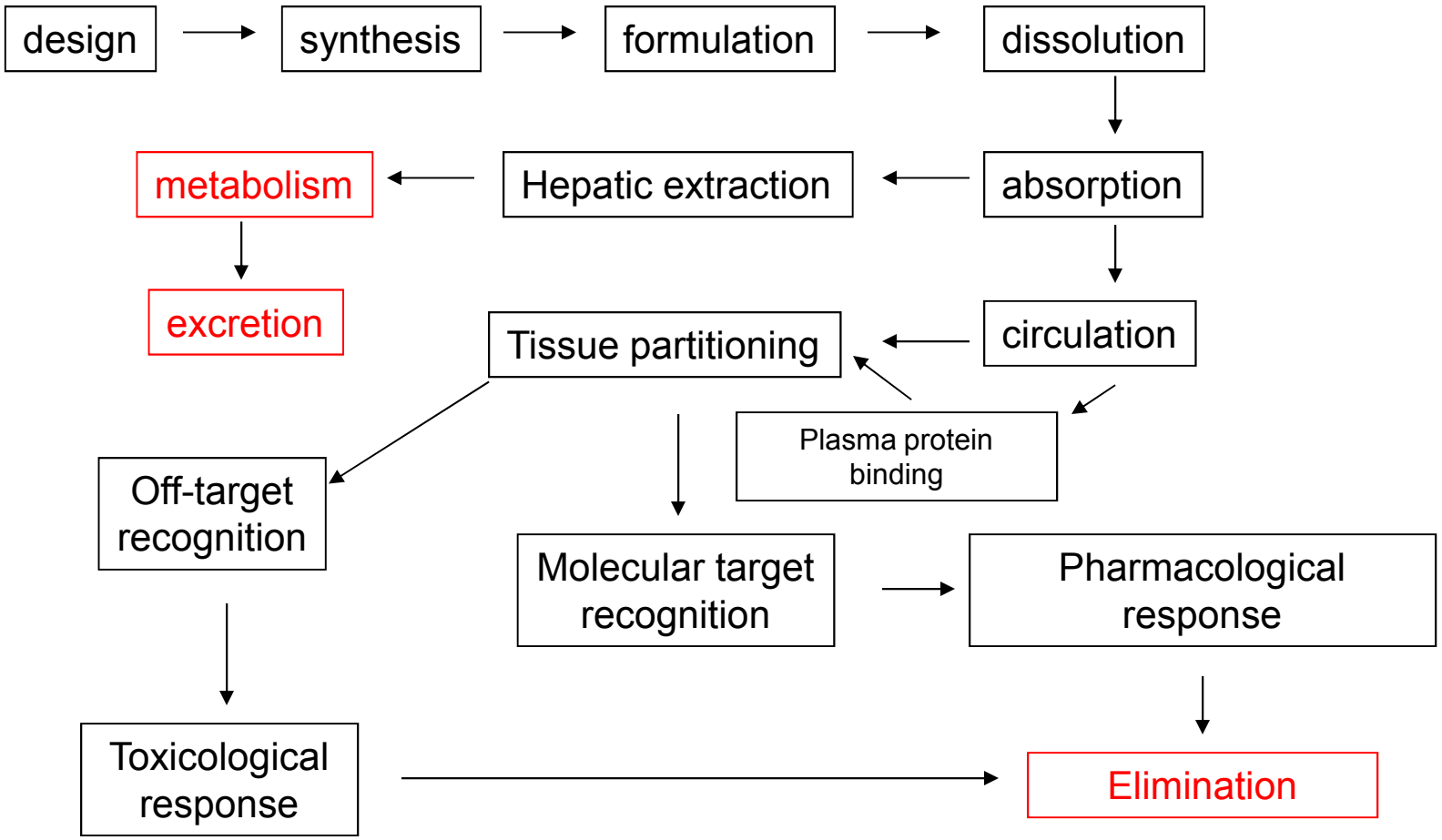
Topic A

Technical Expertise of Good Medicinal Chemists

1. It's All Chemistry



2. Understand Thermodynamics

Respect the physical laws of nature

Уважай законы физики!

"For me, the first essential is to understand thermodynamics. If your project requires a suspension of those laws anywhere it is a dead dog."

Enthalpy and entropy

Solid state

Dissolution and solvation

Desolvation and partitioning

Ligand-macromolecule interaction

3. Understand Protein Function and Mechanism

Learn kinetics and what they mean.

Не забывай о кинетике.

“Understand how enzymes catalyze reactions - it is really very cool.”

4. Think in 3D and Dynamically

Consider the conformational dynamics of ligand and protein.

Рассматривай динамику формирования комплекса Л-П.

Learn to treasure structural insights, but don't be misled/seduced.

3D-информация может быть очень полезной, а может и сбить с правильного пути.

Stereochemistry can be your friend.

Хиральность приносит сложности, но при этом может и помочь.

Understand steric and electronic influences on conformational dynamics.

Думая о конформационном анализе, рассматривай и стерические, и электронные эффекты.

5. Become and Expert in Ionization Chemistry

95% of drugs bear ionizable functionality.

Drug properties change incrementally with degree of ionization.

Dissolution, solubility, partitioning, ligand association, tissue distribution are all *pH dependent.*

Local (including intramolecular) influences on ionization potential must be understood.

6. Become an Expert in Non-Covalent Interactions

Hydrogen bonds (most important interactions in ligand-macromolecular binding)

π-cation

π–π

Ion pairing

Van der Waals/hydrophobic

Understand the impact of suboptimal angles and distances on the strength of the bonding interaction.

Не надо забывать, что неоптимальные углы и расстояния очень сильно влияют на силу (водородных) связей.

7. Understand the Chemistry of Drug Metabolism

Learn to recognize potential metabophores.

Know how CYPs work.

Know about other metabolic pathways and transformations.

Understand why knowledge of CYP P450 interactions are important.

8. Become an Expert in Drug Design

Good medicinal chemists cherish each and every analog as the embodiment of a *hypothesis.*

Хорошие мед. химики рассматривают каждое синтезированное соединение как тест отдельной гипотезы.

Good medicinal chemists focus on the end, not the means.

Хорошие мед. химики фокусируются на цели, а не на средствах (когда формулируют гипотезу).

Good medicinal chemists recognize bad signs:

Хорошие мед. химики умеют распознавать "признаки беды":

- Potency tracking with increased lipophilicity
- Flat SAR (“the wall”)
- Nonsensical results

9. Understand the Principles of Pharmaceutics and PK

Pharmaceutics is all about understanding the relationships of compound properties to drug behaviors.

Фармацевтика, в принципе, наука о том, как свойства соединения влияют на его поведение в организме.

Understand the origins of the rule of five and what they really mean.

Усвой откуда произошло "правило пяти" и что оно означает на самом деле.

Read: “Time related differences in physical property profiles of oral drugs” *J. Med. Chem.* **2004**, *47*, 6338-6448.

10. Understand How Technologies Can Help

Get close with your computational chemist, your protein chemist, your structural biologists, your analytical specialists, your purification team.

Установи крепкие профессиональные отношения с коллегами в других дисциплинах.

Learn enough about what they do to present them with well defined questions.

Знай достаточно об их работе, чтобы задавать хорошие вопросы.

Don’t expect miracles!

Не жди чуда!

11. Understand and Leverage Data

Even simple biochemical assays are extremely complex at the molecular level:

Даже относительно простые скрины(?) являются сложными на молекулярном уровне:

- Every assay is variable in outcome
- Identical assays are not the same

Complexity of an assay (or organism) inversely correlates with data precision.

Сложность скрина/организма обратно пропорциональна точности данных.

Quality of experimental design dictates the quality of the data.

Качество эксперимента определяет качество данных.

Wouldn't hurt to understand a little bit of statistics.

Знание статистики полезно.

Use the tools (such as Spotfire) to analyze data.

"Because biological data generates real numbers such as IC_{50}s, it's tempting to assign more precision to the numbers than is merited."

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Topic B

Strategic Thinking and Judgment of a Good Medicinal Chemist

1. Never Assume You Understand – Get Proof

Be critical of good news - you will be surprised how many times it can't be repeated.

Скептически относись к очень хорошим новостям/данным.

Be critical of bad news that doesn't make sense.

Скептически относись к плохим данным, которые трудно объяснить.

Don't assume that a result derives from just one thing.

Не думай, что у результата (плохого или хорошего) есть только одна причина.

Too little knowledge is both dangerous and delusional.

"I have seen many a chemist accept blindly (and miss-interpret) meaningless data. Once you understand something about the biology, be open-minded about possible never-seen-before observations, but keep in mind that, if the data make no sense, there could be a fairly mundane explanation (like poor solubility) rather than some ground-breaking new biological event."

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2. Think Small

Recognize that optimization of a lead almost always requires increased molecular size and complexity.

Оптимизация лида практически всегда ведет к возрастанию сложности и молекулярного размера соединений.

Understand that certain therapeutic target classes have their own rules: *e.g.,* CNS drugs tend to be smaller, with lower polar surface area.

Различные терапевтические классы соединений требуют различных параметров.

Every route of administration requires a unique properties profile.

Различные пути администрации лекарств (oral, IV, topical, inhaled...) обычно требуют особенных, и часто очень различных, свойств.

"Medicinal chemists should understand the principle of ligand efficiency and the beauty of increasing potency by improving the fit of their molecule to its target rather than just by increasing lipophilicity."

3. The Difference Between Must Have and Nice to...

Perfection is unattainable. Near-perfect is rare. Decide in advance what compromises may be acceptable, because you will be making some.

Идеал не доступен. Почти идеальный профиль тоже бывает редко. Заранее реши, какие компромиссы возможны, потому что их придется делать.

Also decide on those attributes where compromise is not an option.

Также определи заранее, какие параметры критичны и компромиссу не подлежат.

"So the answer is setting the product profile early...what are the must haves...not negotiables but the must haves, and getting to key milestones which help you to decide if you are succeeding or failing....if you can not get to key milestones...DROP IT!!!"

4. Know When Hold ‘Em or Fold ‘Em

Avoid emotional ties to a project - it is not in your best interest or the company’s.Passion does not change the data no matter how many stars you wish upon.

Не привязывайся эмоционально к проекту - данных это не изменит.

Respect the data.

Develop an intuitive sense for trouble, and a talent for defining the “killer experiment”- then, respect and stand by the results.

Уважай данные.

Разработай ключевой эксперимент, но потом уже не придумывай оправданий, если данные не нравятся.

5. Don't Succumb to Dogma and Cast Iron Principles

Every pharma company has its own biases based more on singular anomalies than on a systematic establishment of principle. Challenging institutional knowledge requires some diplomacy, but good medicinal chemists will recognize when dogma stands in their way.

У каждой компании свои догмы. Обходить их не легко, но хорошие специалисты понимают, когда игра стоит свеч.

"Don't blindly follow "rules" like the rule of 5 or the "structural alerts" known so well at Pfizer. They are good guidelines, but in my opinion good scientists should follow the science and sometimes that means actually doing the experiment."

"Be data-driven and aggressively challenge perceptions and dogma. These are innovation killers."

Topic C

Individual behaviors in a collaborative environment

6. Respect the Expertise of Your Team Mates

They know more about their particular discipline than you do - don't try to out-think them.

Твои коллеги лучше разбираются в своей области, чем ты. Уважай их знания.

Talk to others, ask questions, learn from them. You won't be sorry and these critically important relationships will grow (more on this later).

Общайся с коллегами, задавай вопросы, учись. В жизни пригодится.

Don't create problems for others down the road with fixes to problems at hand.

Не создавай будущих проблем для коллег, чтобы решить свои сиюминутные проблемы.

2. Become a “Gatekeeper’

Gatekeepers are:

- Curious
- Collaborative
- Approachable
- Science first
- Quality controllers
- And above all, expert networkers

Ключевые специалисты:

- Любознательны
- Хорошо работают с другими
- Ставят науку перед эмоциями/амбициями
- Фокусируются на качестве
- Имеют обширные профессиональные знакомства и/или умеют их устанавливать

3. Be a Self-Motivated Drug Hunter

“Always remember what the goal is: to make someone's life a little bit better by creating a molecule that is both safe and effective.”

“Никогда не забывай о конечной цели: помочь людям, создав лекарство, которое и эффективно, и безопасно.”

Organizational de-motivators:

- Unclear expectations
- Poor communication
- Under-resourcing of projects
- Failure to enable and reward good decisions
- Risk-averse cultural environment
- Confusing tactics with strategy

Демотивирующие факторы:

- Расплывчатые планы
- Плохое общение (horizontal and vertical)
- Недостаток ресурсов
- Игнорирование качественно сделанных решений
-Боязнь риска в компании
- Путаница между тактикой и стратегией

Lecture Overview

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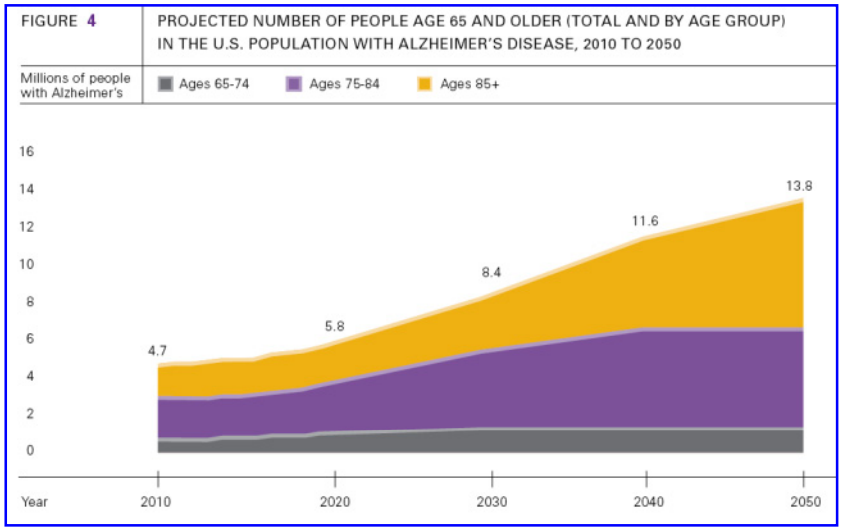
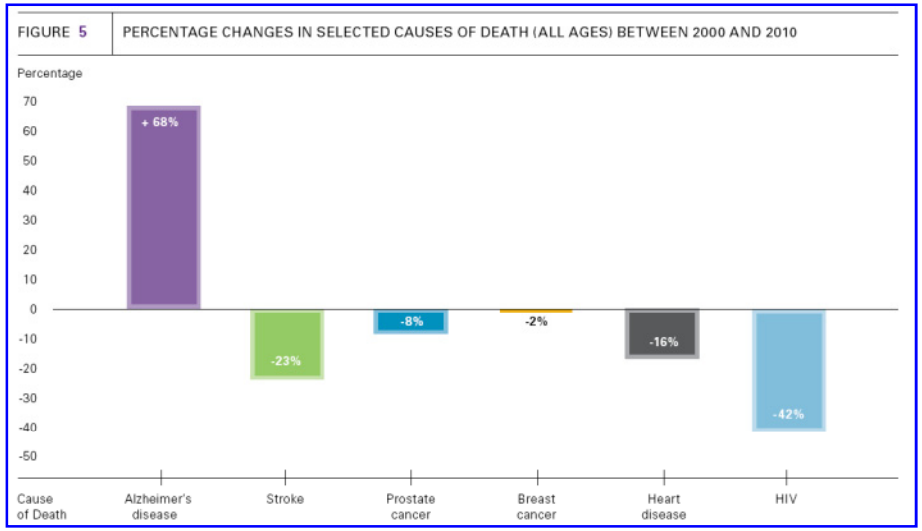
Alzheimer's Disease – Medical need





1 IN 3 SENIORS DIES WITH ALZHEIMER'S OR ANOTHER DEMENTIA.

ALZHEIMER'S DISEASE IS THE SIXTH-LEADING CAUSE OF DEATH.

From "2013 Alzheimer's Disease Fact and Figures" report

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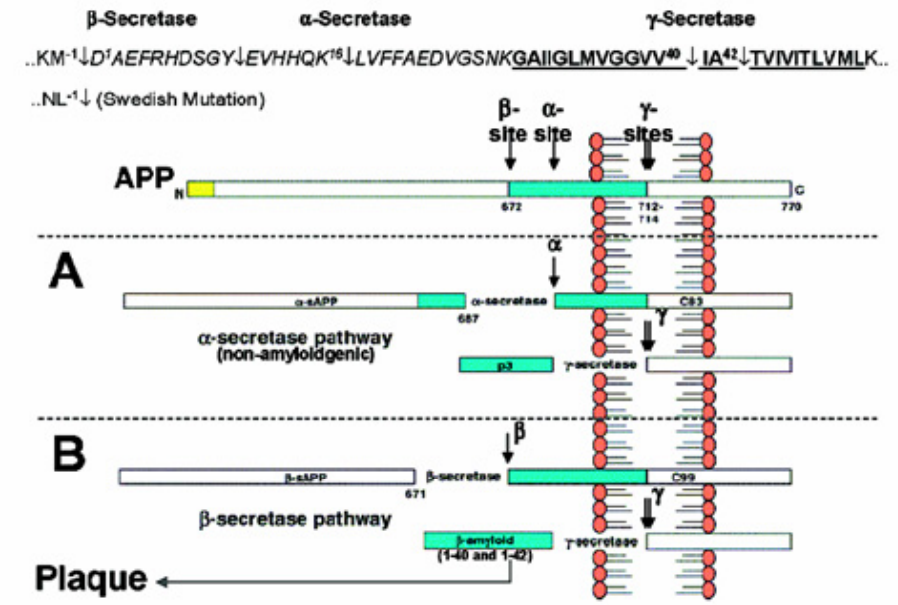
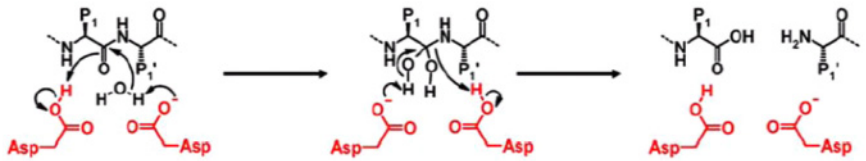
BACE (BACE1, β-secretase) as the target for AD

- Alzheimer's Disease is an enormous unmet medical need.
- Genetic mutation at BACE1 cleavage site in APP (SWE) tied to early-onset familial AD.
- Postmortem brain: BACE mRNA, enzymatic activity and protein expression elevated in the frontal cortex. Correlated with elevated brain Aβ.
- Clearance of Aβ40 and Aβ42 is decreased by 25 and 30%, respectively, in AD *vs.* control subjects, as revealed by stable isotope labeling of newly-synthesized Aβ protein levels in CSF (Bateman, 2010).
- APP A673T mutation protects the carriers from developing AD by minimizing amyloidogenic pathway processing of APP (2012).
- Preclinical evidence suggest that small (>25%) reductions in brain Aβ have profound effect on plaque deposition and behavioral (cognitive) measures in APP transgenic animals (McConlogue *et al.*, 2009).
- However, BACE is a tough target from the perspective of drugability.



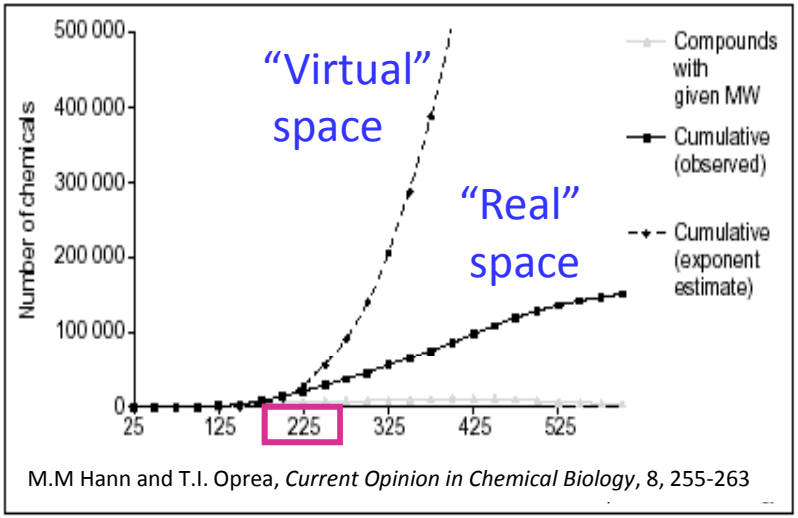
from J. Varghese *Current Topics Med. Chem.* 2006, *6*, 569-578



from J. Eder, *et al. Current Pharmaceutical Design* 2007, *13*, 271-285

Drivers for FBDD

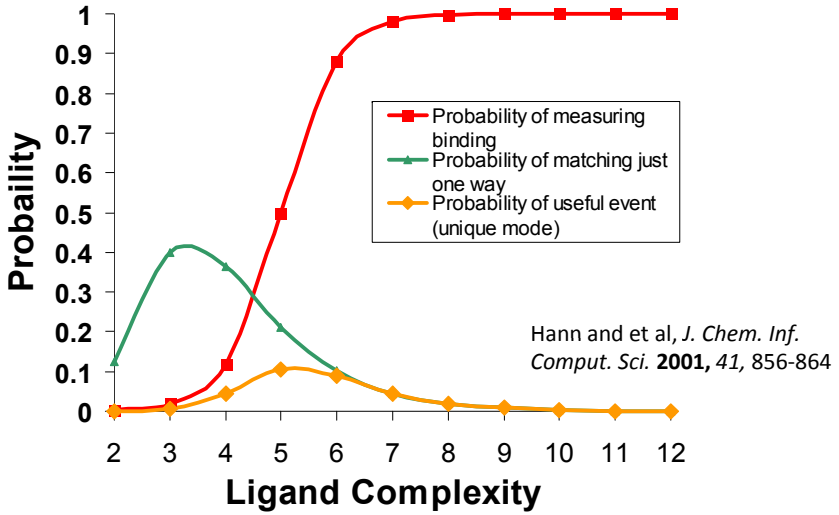
Efficient Sampling of Chemical Space



M.M Hann and T.I. Oprea, *Current Opinion in Chemical Biology*, 8, 255-263

Sampling of more diverse chemical space with fewer compounds (102-104) than HTS.

Probability of Detecting Interactions



Hann and et al, *J. Chem. Inf. Comput. Sci.* **2001,** *41,* 856-864

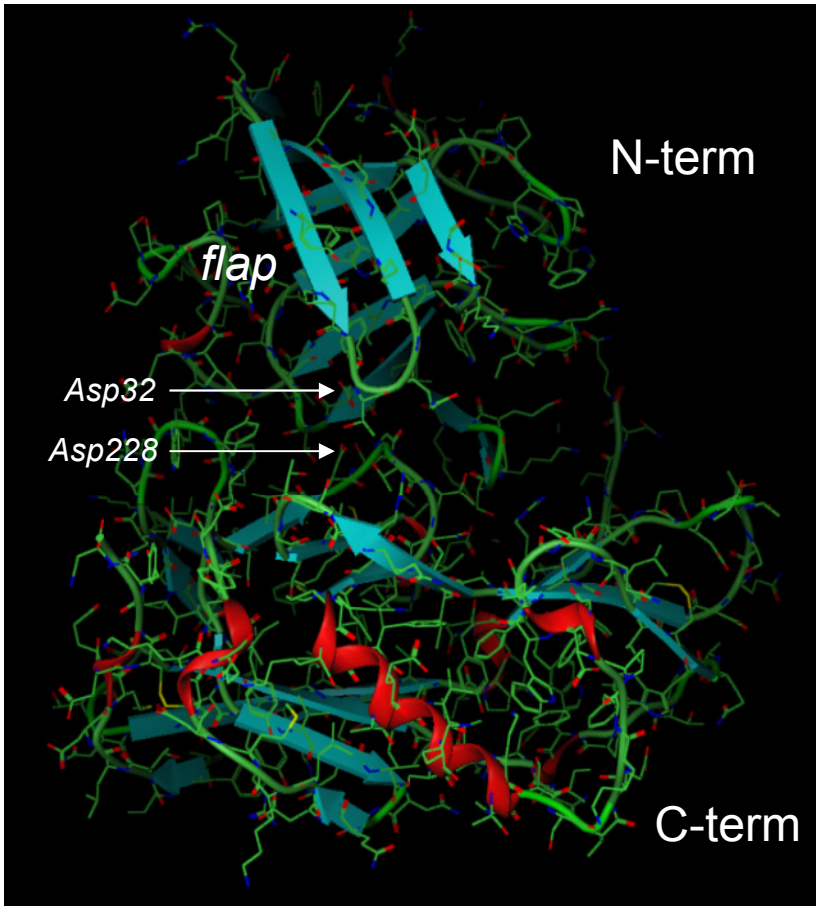
Probability of finding a good match between receptor and ligand decreases exponentially with increasing ligand complexity.

Screening at high concentration to identify weak (μM – mM) but ligand efficient fragments hit, which bind specifically to targets.

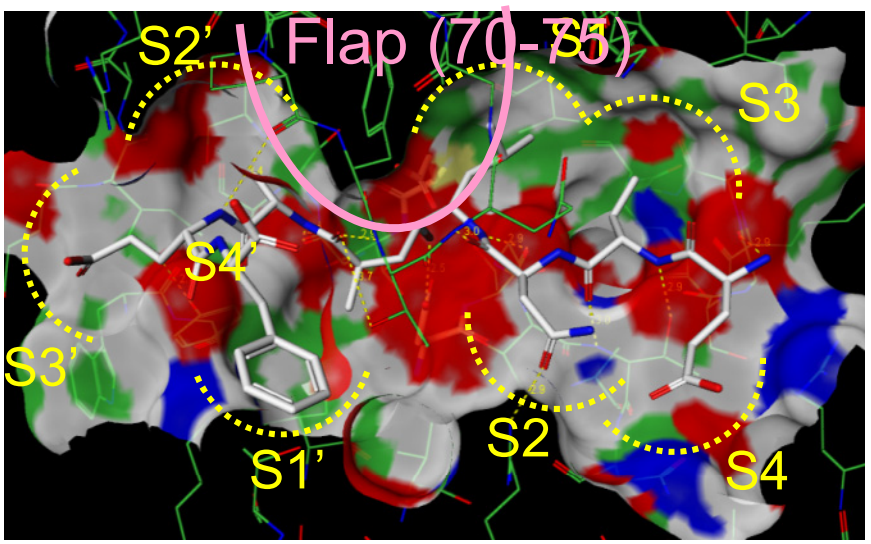
Characterization using structural and functional information to enable rapid and rational design in hit-to-lead.

By design hits reside in attractive physicochemical space from the start – ability to co-optimize potency and ADMET in parallel.

BACE is a 400 amino acid aspartyl protease



- BACE1 (β-secretase, memapsin-2) is a member of the aspartyl protease family.
- In same class as renin, cathepsin D/E, pepsin A/C, napsin A): share similar fold, catalytic mechanism.



- Anchored to a six-stranded β-sheet "platform“
- “Flap" is a highly mobile region of all Asp proteases
- Wide, long active site with 7 distinct subsites (S4-S3’)
- Largely hydrophobic interactions (S3, S1, S1’, S2’) with some hydrophilic character (S4, S2, S3’)
- *N*-term and *C*-term domains are highly twisted 8-stranded β-sheets

Benzimidazoles as BACE fragment hits

How weak is too weak?

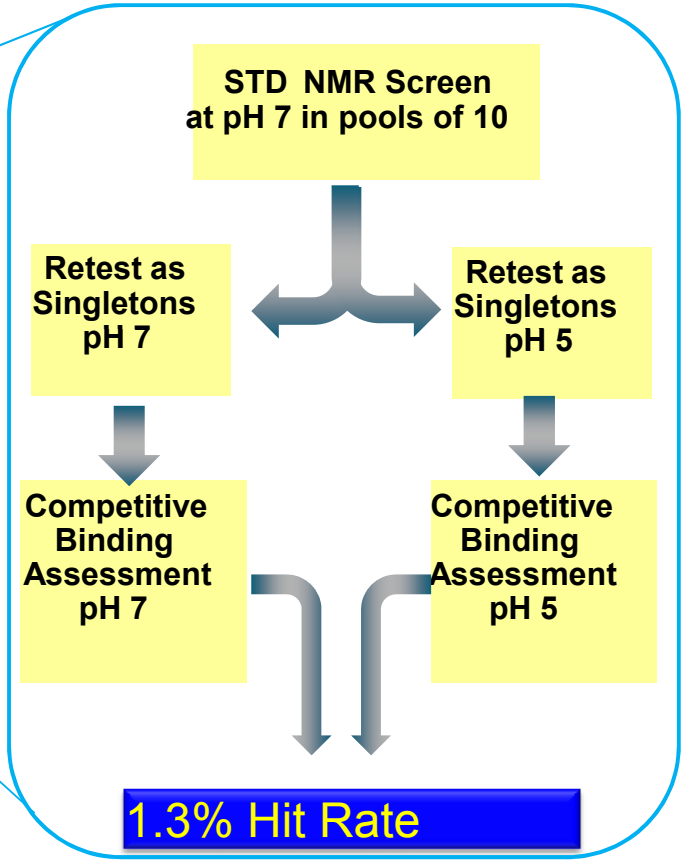
Primary screening by NMR – hit identification

2592-member proprietary Pfizer library
("GFI" library – Global Fragment Initiative)
W. F. Lau *et al. Design of a Multi-purpose Fragment Screening Library using Molecular Complexity and Orthogonal Diversity Metrics.* Journal of Computer-Aided Molecular Design 2011: p. 1-16.

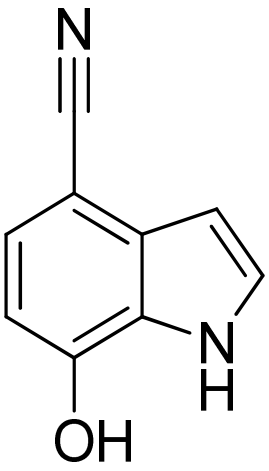
Hit identification
Primary STD-NMR Screen

Hit Validation
FUNCTIONAL NMR TROSY SPR X-RAY

Hit Optimization and Expansion
FUNCTIONAL NMR TROSY SPR X-RAY
FILE MINING DIRECTED SYNTHESIS



Indole hit identification and validation



Compound 1

Molecular properties

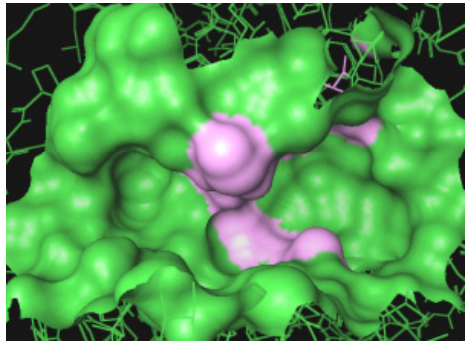
18 heavy atoms
MW = 158.16
CLogP = 1.7

Initial screening data

Soluble at pH 5.0
Soluble at pH 7.0
Medium-strong STD at pH 5.0
Strong STD at pH 7.0
Partially competed out at pH 5.0

Hit profiling

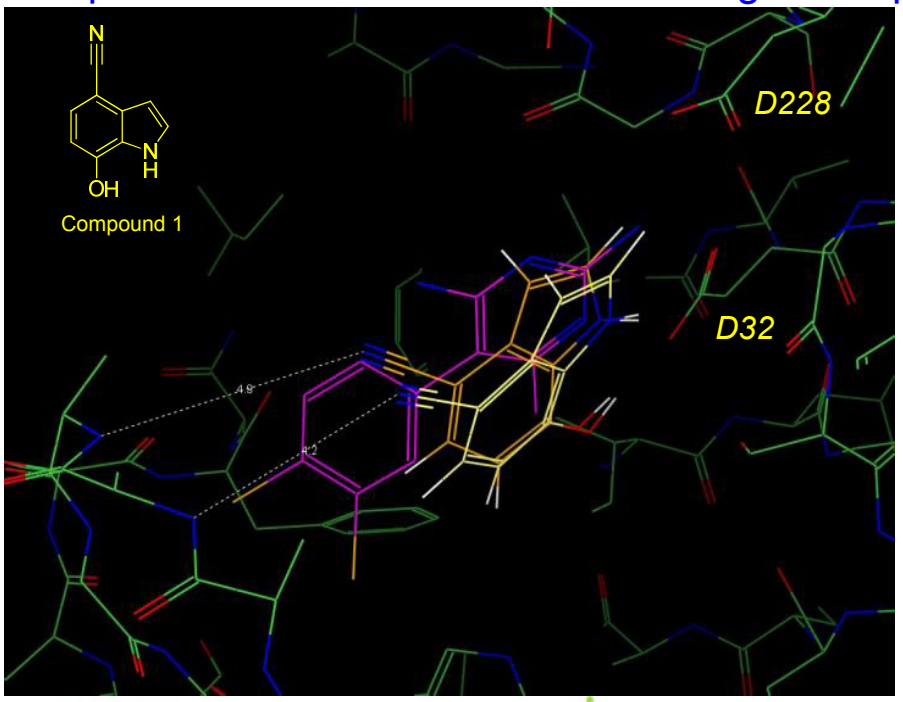
no density in X-ray
solubility issues above 1 mM at pH 5.0
no binding detected by OCTET
only weak TROSY signals – at the active site with the catalytic Asp and the flap



- Compound **1** was a single representative from one of the several chemotypes identified in the primary NMR screening**.**
- How weak is too weak?
- Need to prosecute multiple fragment hits in parallel.
- At the time, novel chemical matter for β-secretase was extremely valuable.
- **What would you do next?**

Computational chemistry to the rescue

- Potential binding modes of hits were assessed by docking and MCSS (Multiple Copy Simultaneous Search).
- Compound **1** was unique in a sense that these two orthogonal computational methods resulted in the nearly identical predicted binding orientations (yellow and orange in the picture below).
- These data increased our interest in the chemotype represented by compound **1** and provided an avenue for rational fragment optimization.



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Key observations

Interaction predicted between the indole N and catalytic aspartate 32

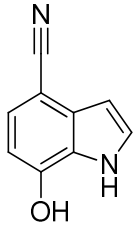
Catalytic Asp228 is too far to be engaged by compound 1

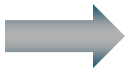
Good overlap predicted with a validated diaminopyrimidine fragment (X-ray structure shown in magenta; *vide infra*)

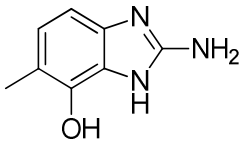
Hypothesis

Productively engage the second catalytic aspartate by installing an H-bond donor next to the ring nitrogen (with amino group being the most promising substituent)

Hit expansion





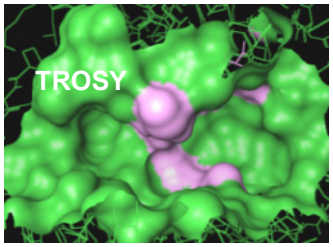


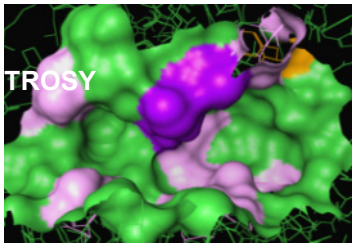
Compound 1

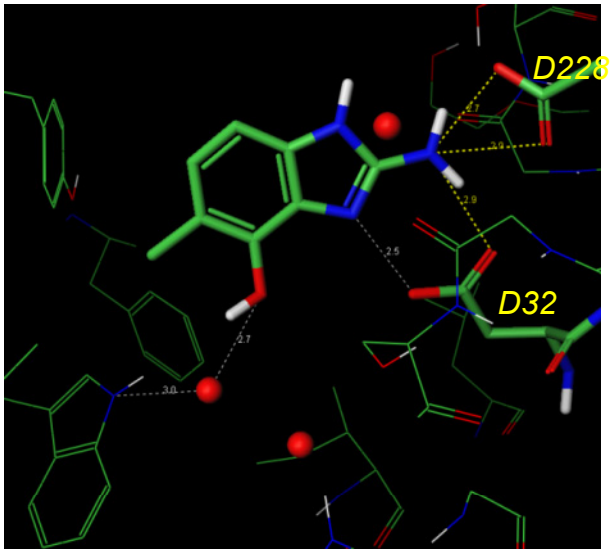
12 heavy atoms
MW = 158.16
CLogP = 1.7
strong STD
minor TROSY perturbations
no density in X-ray

Compound 2

12 heavy atoms
MW = 163.18
CLogP = 1.7
strong STD
significant TROSY perturbations
successful X-ray!







- File mining based on the similarity to the original hit and, especially, with the formulated hypothesis in mind has produced promising results:
  - direct interactions with Asp228 have been engineered in, just as predicted by modeling;
  - phenolic OH makes a water-mediated H-bond to Trp76.
- Still, no binding in Octet.
- **What is the next step?**

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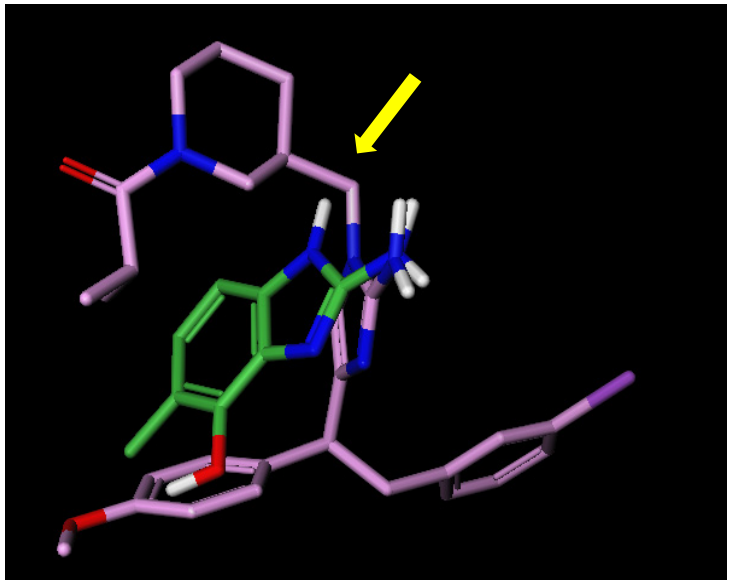
Overlap with aminoimidazoles

- Binding mode of **2** (green) was similar to other aminoheterocycles in the Pfizer portfolio of BACE inhibitors being pursued at the time**.**
- In particular, there was an obvious growth vector based on overlap with some members of the aminoimidazole series.
- A possibility of growth in this direction has been explored as the next step.

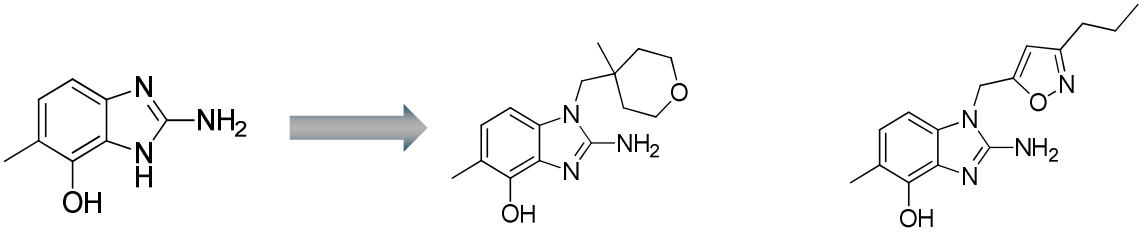
Overlap of X-ray structures of compound 2 and a BACE inhibitor from the aminoimidazole series (M. Brodney *et al. "Amino imidazoles as β-secretase inhibitors for treatment of Alzheimer's disease"* 240th ACS National Meeting, Boston, MA, United States, August 22-26, 2010: 1011614.)



Hit optimization

Parallel chemistry afforded compounds which:

- validated the proposed initial scaffold growth trajectory
- had potency detectable by the biochemical assay
- were functionally capable
- possessed a profile predictive of brain penetration
- however, no easy access to the traditional Schechter subsites



Compound 2

MW = 163.18
CLogP = 1.7
strong STD
significant TROSY perturbations
no detectable binding in Octet
successful X-ray

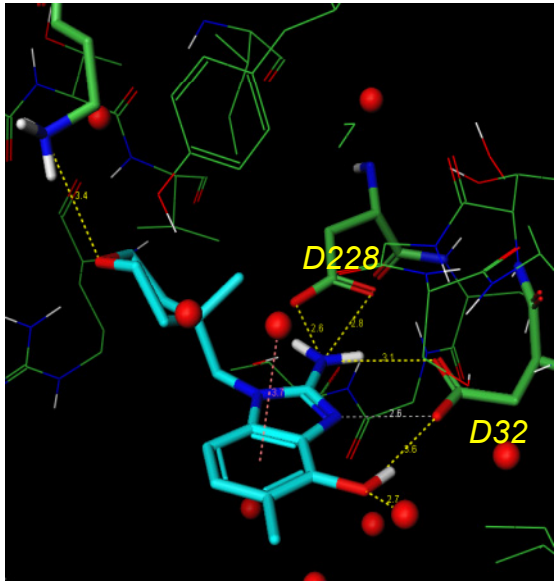
Compound 3

MW = 275.35
CLogP = 2.6
Octet KD = 70 μM
(LE = 0.29 kcal/mol/atom)
NMR IC50 = 150 μM
CFA IC50 = 296 μM
RRCK AB = 15 x 10^{-6} cm/s
MDR BA/AB = 3.5
HLM CLint = 12 μL/min/mg

Compound 4

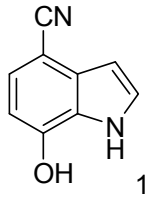
MW = 286.33
CLogP = 2.6

CFA IC50 = 223 μM
RRCK AB = 22 x 10^{-6} cm/s
MDR BA/AB = 2.2
HLM CLint = 24 μL/min/mg



X-ray of 3 confirmed the expected binding mode

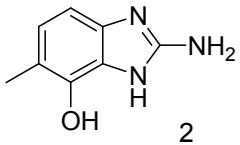
Summary - aminobenzimidazoles



1

GFI hit by STD NMR
Minimal competition
No KD by NMR

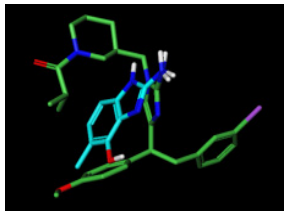
predicted binding mode



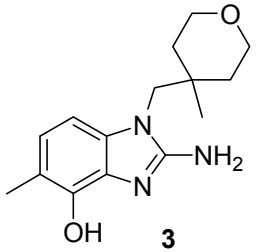
2

successful X-ray!

Weak STD @ pH 5
Sol OK

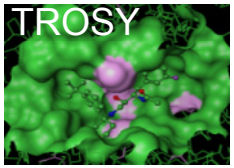


merge with aminoimidazole SAR



3

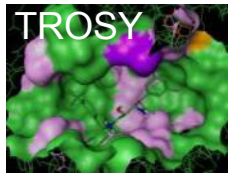
Strong STD @ pH 5
Sol OK
~ 100% competition



Weak TROSY but key interactions with catalytic ASP

Octet no binding up to 4 mM

No density in X-Ray



Octet no binding up to 4 mM

X-Ray Solved
Confirms modeling
Interaction with cat. ASPs

X-Ray Solved
Good density
Binding as predicted

Octet KD = 70 µM

NMR IC50 = 150 µM
CFA IC50 = 296 µM

- **Hypothesis-driven design led to fast identification of a novel (at the time) chemical series starting from a very weak fragment hit.**
- While the series has been deprioritized in favor of other, more promising, series in the Pfizer BACE portfolio, the series progression and evaluation were achieved very expediently.
- Multidisciplinary approach was the key in assessment and optimization of this chemotype.

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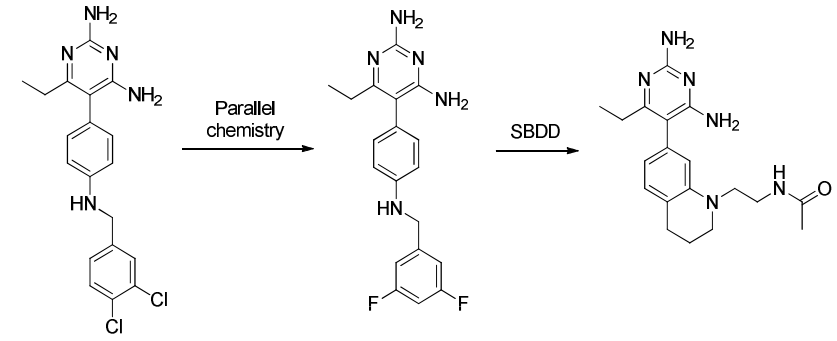
Example of a “target hopping” using a fragment-based approach

Renin as a possible starting point

- Renin is an aspartic protease closely related to BACE.
- Nonpeptidic drug-like chemical equity has been successfully optimized for this target:



Renin IC_{50} = 27000 nM
MW = 388
cLogP = 4.80

Renin IC_{50} = 4600 nM
MW = 355
cLogP = 3.78

Renin IC_{50} = 178 nM
MW = 354
cLogP = 2.36

- An opportunity to utilize renin chemical matter as a jump board for the BACE program seemed attractive.
- However:
  - screening of nonpeptidic renin inhibitors did not produce usable BACE hits;
  - attempts to modify renin leads based on docking in BACE have not been successful.
- **Should we try something smaller??**

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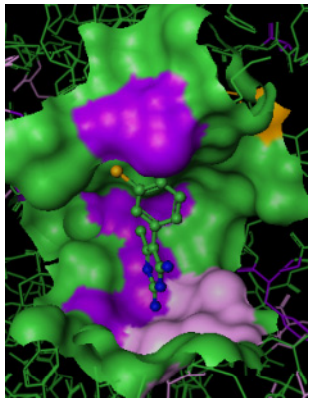
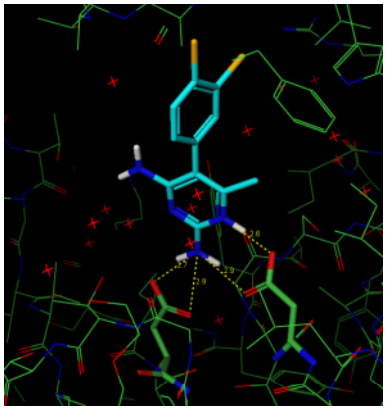
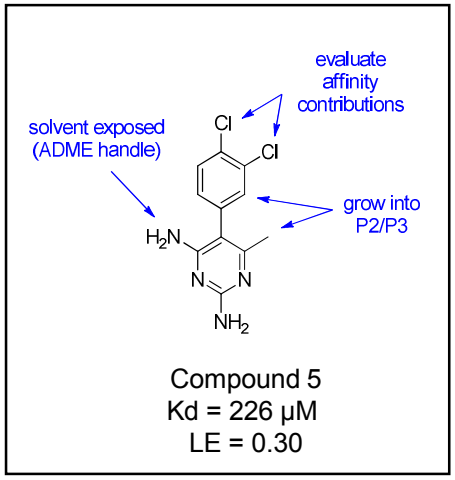
“Target hopping” – renin-based BACE inhibitors

6 fragments selected for NMR screening – a rudimentary representation of renin equity



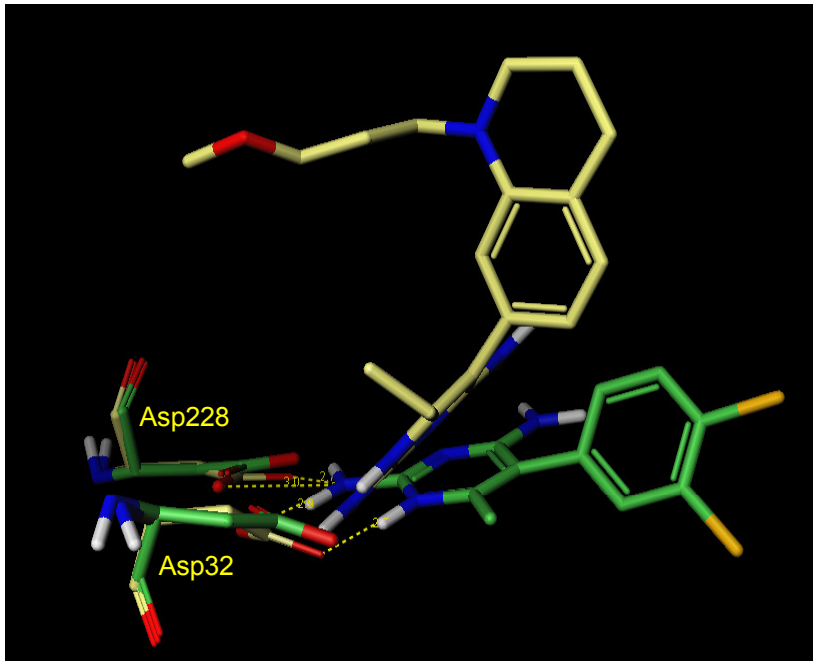




Compound 5
Kd = 226 μM
LE = 0.30

- A small number of scaffolds was selected to represent the renin chemical equity.
- NMR screening detected robust binding.
- A successful X-ray structure was obtained as a follow-up and led to:
  - formulation of an initial SAR strategy;
  - understanding of why larger renin leads were not suitable starting point (next slide).

Impact from the X-ray structure



Yellow – Diaminopyrimidine-containing renin inhibitor.

Green – BACE fragment hit.

Catalytic aspartates of BACE and renin are shown (BACE numbering of the residues)

- Despite similarities in the protein structures and the same ligand binding motif, the binding mode is significantly different - which explained difficulties in the approach of using renin leads as chemical matter for BACE.
- The difference in the dihedral angle of interest turned out to be consistent between multiple renin structures and diverse aminopyrimidine BACE binders developed on the basis of the initial hit.

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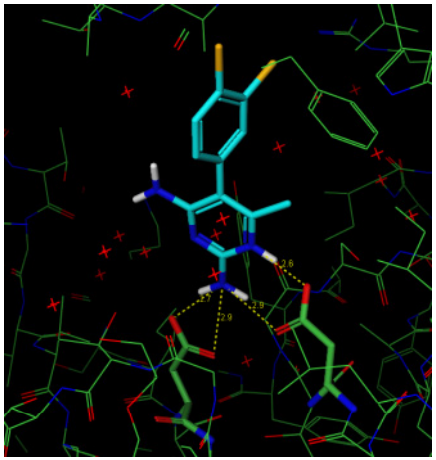
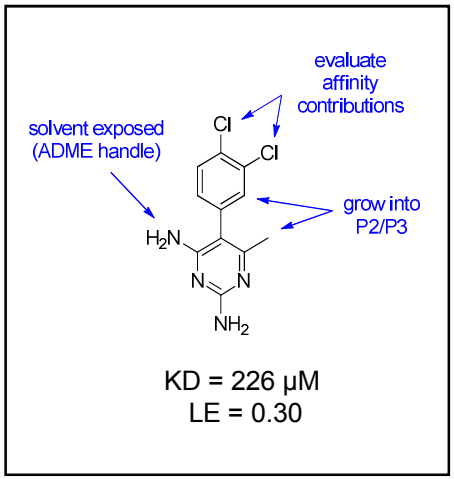
Summary - diaminopyrimidines





4 new aminoheterocyclic scaffolds were quickly identified in the course of the hit expansion efforts

- Fragment-based approach was successful where mining/modification of leads for a related target was not. It is an important takeaway which could be relevant for other target classes.
- Structural information was the key to understanding the target differences and outlining an SAR expansion strategy.
- Four new chemotypes of BACE inhibitors were expediently identified as the consequence of this finding.

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Spirocyclic pyrrolidines

millimolar to micromolar –
optimization of an X-ray hit

Efremov, Ivan V. *et al.* “Discovery and optimization of a novel spiropyrrolidine inhibitor of β-secretase (BACE1) through fragment-based drug design”
J. Med. Chem. **2012**, *55*, 9069-9088.

X-ray screening of Pfizer GFI (Global Fragment Initiative) library

Rationale

- Broad approach to fragment screening for difficult targets – both NMR and X-ray fragment screening campaigns
- X-ray screening offers different solution conditions, concentrations and molecular dynamics in comparison to NMR and other methods

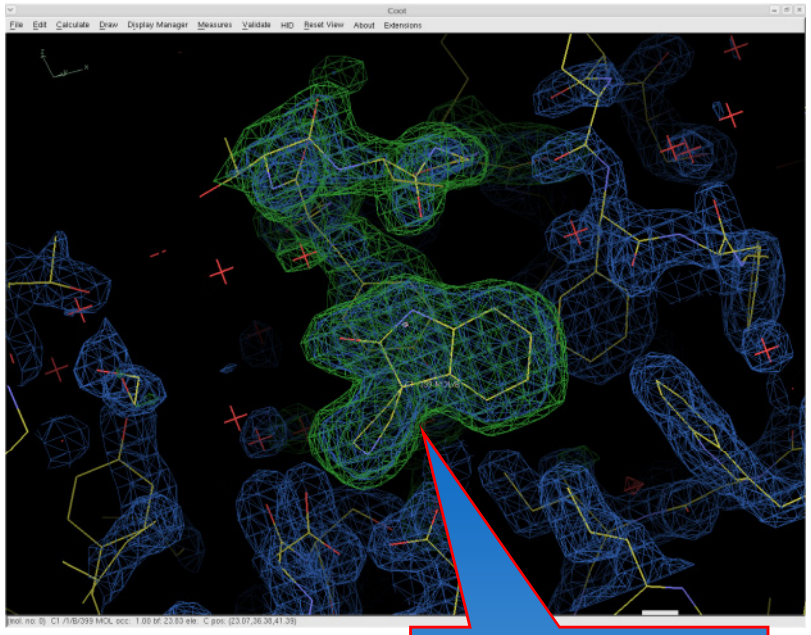
Library format

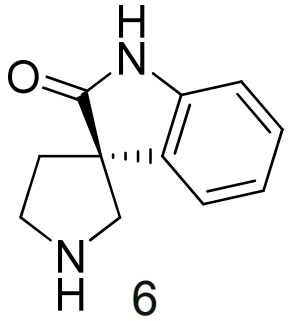
- 340 cps @ 4 cps/mix = 85 mixtures
- Final concentration 20 mM/cmpd in 10% DMSO

IMCA offered the most suitable location for X-ray fragment screening

- Estimated beam time needed - minimum of 72 hours assuming 30'/dataset
- Synchrotron radiation (higher flux than in-house => shorter data collection times)
- Sample handling robotics: ACTOR robot combined with remote data collection capabilities

Oxindole-containing spirocyclic pyrrolidine has been identified as a single fragment hit in this screening campaign





6

funct . NMR IC_{50} = 1.0 mM
LE = 0.30 kcal/mol/atom

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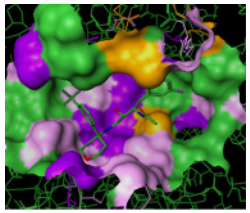
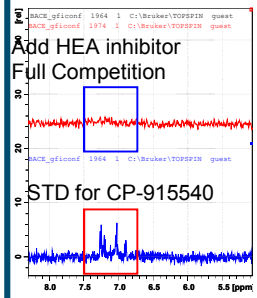
Fragment hit characterization and analysis

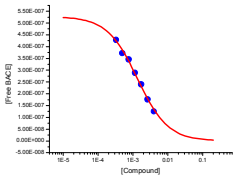
Hit profiling



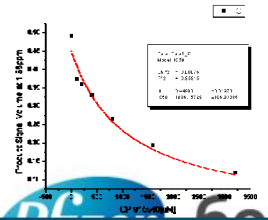
Binding:
- Significant TROSY perturbations in the active site



Binding:
- Strong STD
- 90% competition with known active site binder
- Solubility OK



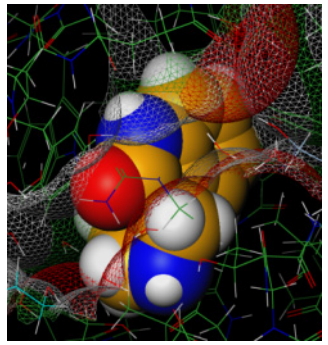
Affinity:
- Octet Kd = 1.4 mM



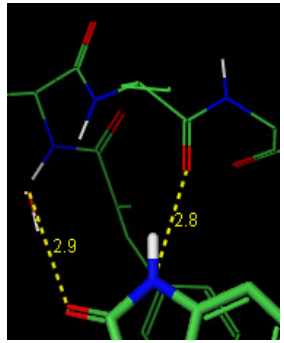
Potency:
- Functional NMR IC_{50} = 1.09 mM

Binding mode analysis

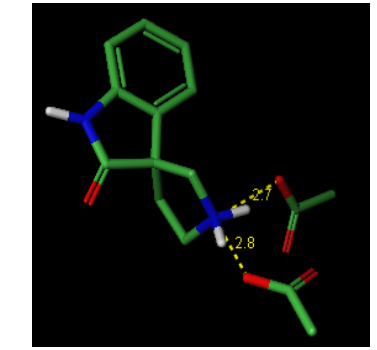
- Notice a perfect fit in the binding site.
- Nicely sequestered from solvent.
- Not many vectors are open for elaboration.
- No obvious access to S3 pocket.



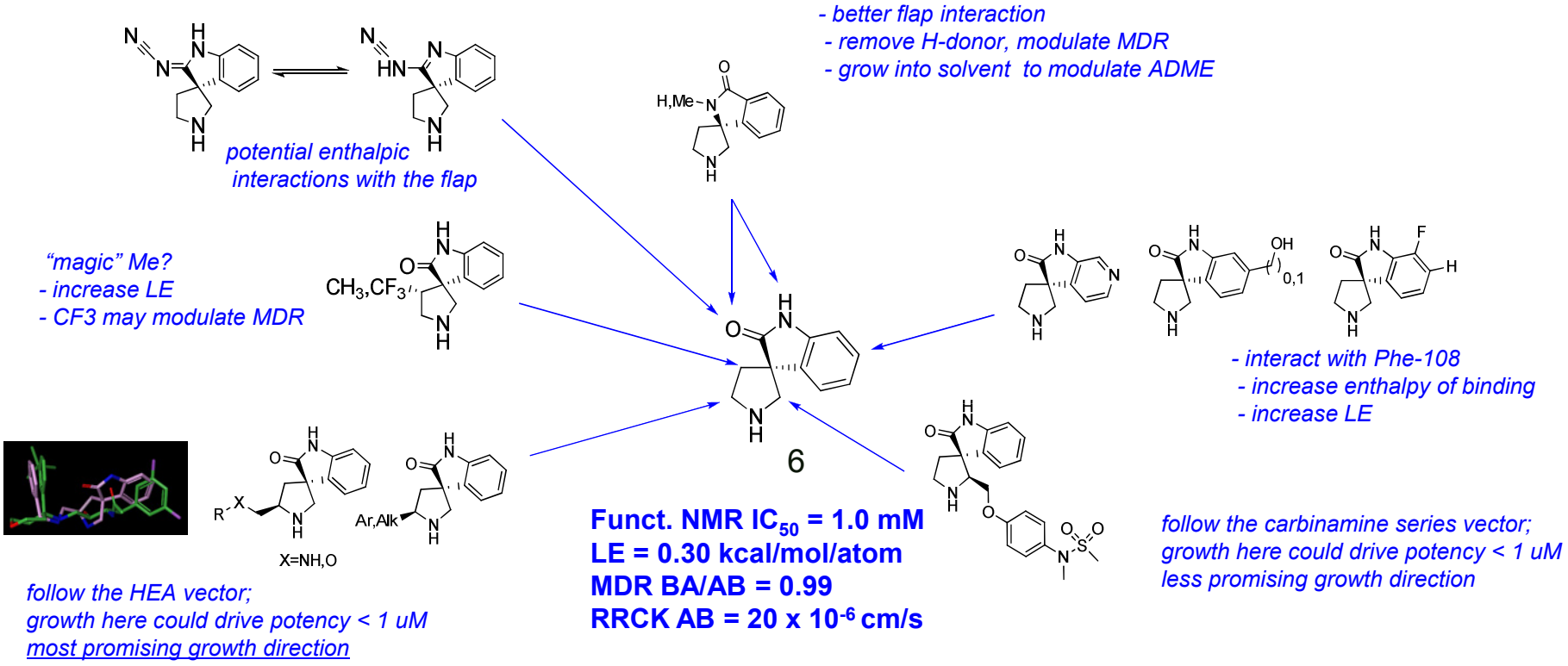
- NH of the lactam interacts with a carbonyl on the flap. In fact, this flap residue is more commonly rotated and the NH instead of CO forms an H-bond with the ligands.
- An opportunity to remove H-bond donor from our inhibitors.



- The catalytic aspartates are almost orthogonal to each other.

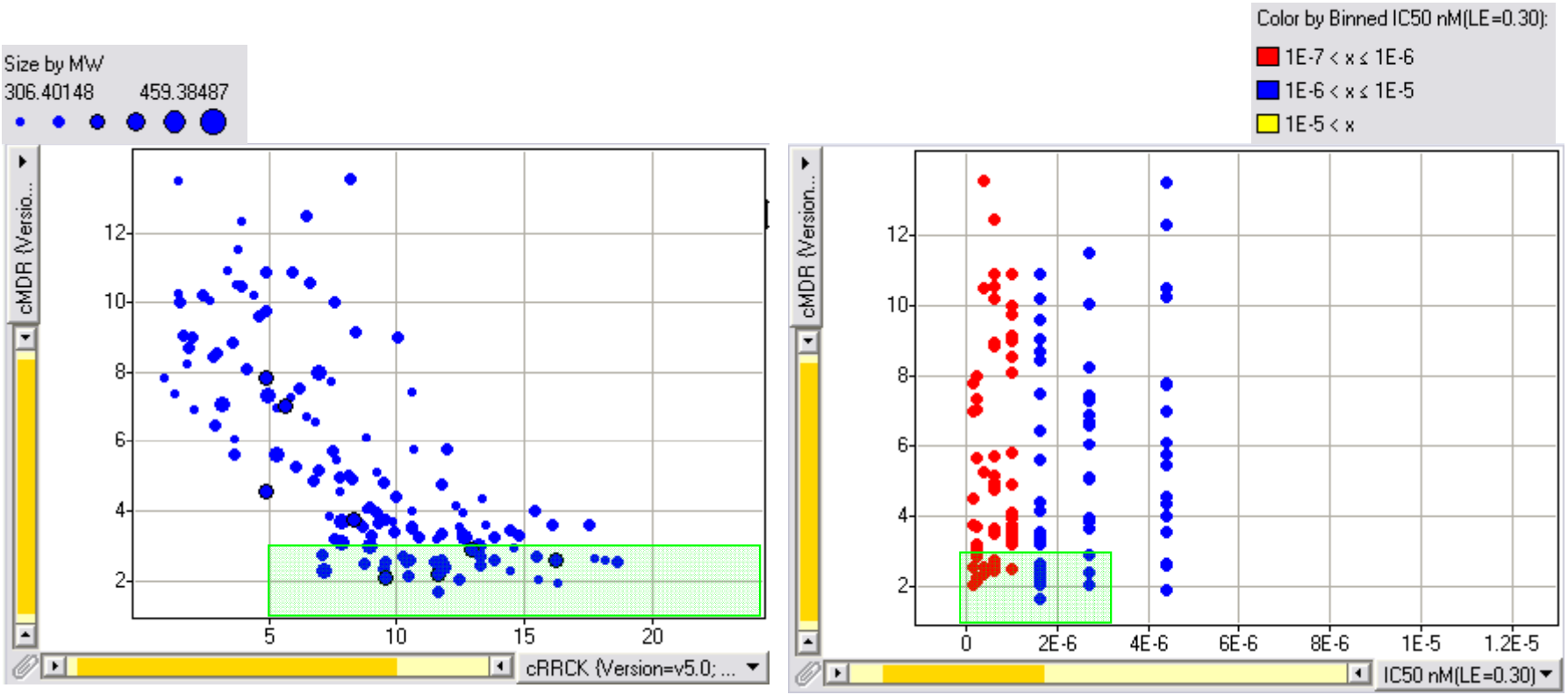


Initial chemistry strategy based on analysis of the binding mode



- **Comparison to other pyrrolidine fragments is favorable in terms of MDR and permeability.**
- **Good ligand efficiency (for BACE) – an important consideration.**
- **Not many avenues for growth – important to identify these to reach the desired potency level.**
- **Key consideration for decision making is identification of the productive growth vector.**

Is there room for growth?



- A significant fraction of the enumerated compounds (along the most promising vectors – illustrated here with P2') was predicted to reside in good chemical space, including heavier compounds as well.

- Assuming LE = 0.3 (and even 0.28), it was realistic to expect submicromolar compounds with good MDR profile.

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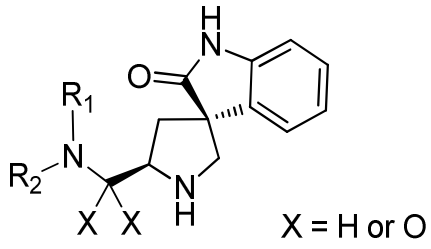
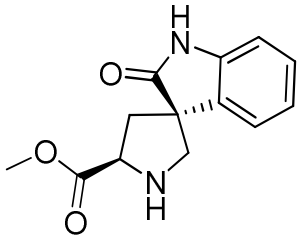
Initial exploration of the S2'-S3' "HEA vector"

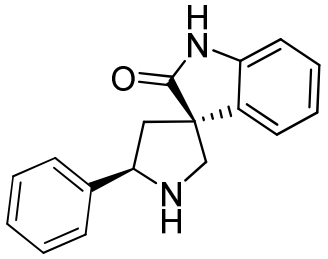


Low affinity or LE



7

NMR IC50 = 242 μM
(LE = 0.28 kcal/mol/atom)
OCTET Kd = 570 μM
CFA: 34% at 300 μM
RRCK AB = 35 x10^{-6} cm/s
MDR BA/AB = 1.13



8

CFA: 64% at 300 μM
RRCK AB = 30 x10^{-6} cm/s
MDR BA/AB = 0.98
HLM CLint, app = 17 uL/min/kg

- **Initially examined amines/amides turned out to be poor binders.**
- **Methyl ester 7 was found to be a robust binder in spite of the fact that the Me group has nto yet reach any Schechter subsites.**
- **BACE inhibitory activity of the phenyl analog 8 demonstrated that the isosteric replacement of the ester functionality was possible.**
- **Use of the ester group was a facile way to mine out SAR of this growth vector.**

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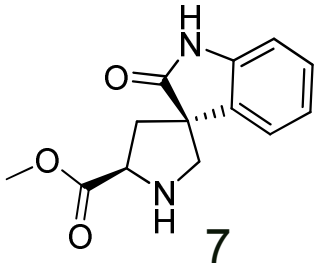
Initial mapping of S1'/S2' pockets using esters



7

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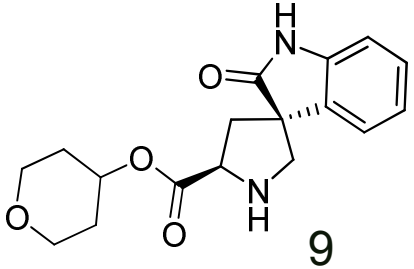
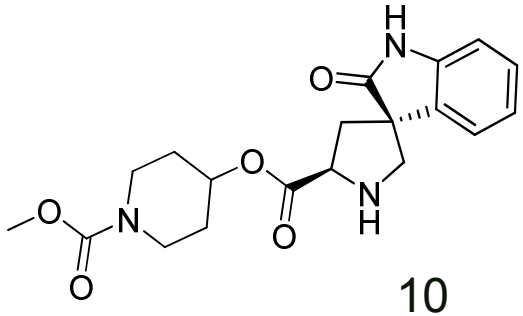


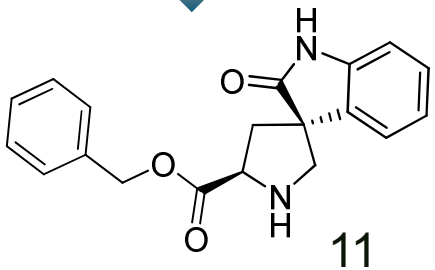
9

NMR IC50 = 32 μM (LE = 0.27)
OCTET Kd = 40 μM
CFA IC50 = 114 μM
RRCK = 25 x 10^{-6} cm/s
MDR BA/AB = 1.02
dofetilide: 4% at 10 μM



10

NMR IC50 = 14 μM (LE = 0.25)
ELISA IC50 = 84 μM
RRCK = 21 x 10^{-6} cm/s
MDR BA/AB = 1.91

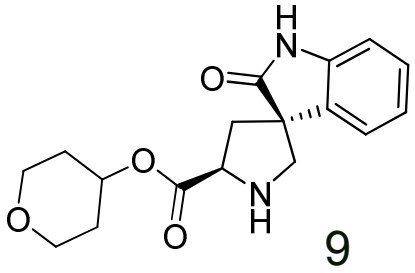


11

NMR IC50 = 33 μM (LE = 0.26)
CFA IC50 = 86 μM
RRCK = 30 x 10^{-6} cm/s
MDR BA/AB = 0.97
no CYP inhibition

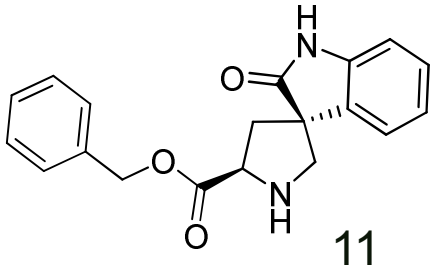
- **Responsive SAR has been observed by elaboration of the ester moiety.**
- **Ease of chemistry allowed to quickly explore this direction thus confirming the initial assumption about viability of this growth vector.**
- **Importantly, good permeability and low MDR efflux were observed for bigger analogs – as projected.**

SAR using structural information: esters in the spiropyrrolidine series



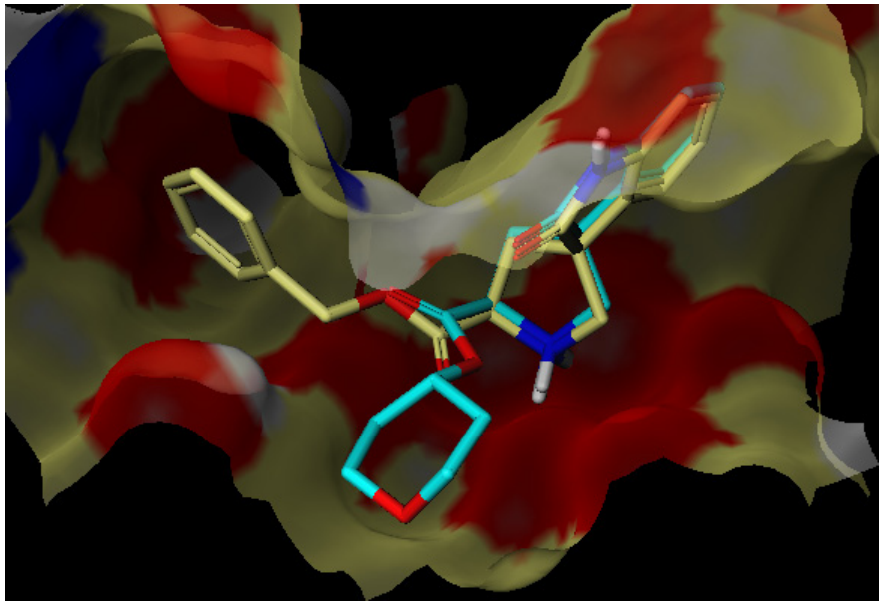
9

NMR IC50 = 32 μM (LE = 0.27)
CFA IC50 = 114 μM



11

NMR IC50 = 33 μM (LE = 0.26)
CFA IC50 = 86 μM



- **Without structural info it could be a hindrance due to the confusing SAR.**
- **With structural information it became a benefit – we used the same chemistry to map out SAR in 2 different pockets.**
- **This example highlights importance of obtaining structural information for key compounds – especially for targets with large binding sites.**

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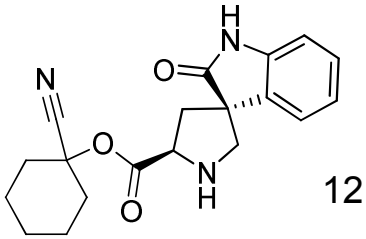
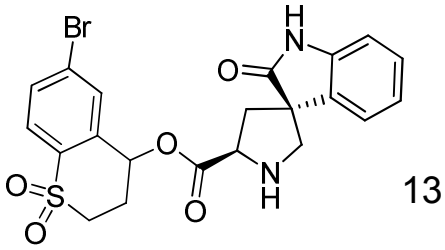
Examples of a productive flap engagement



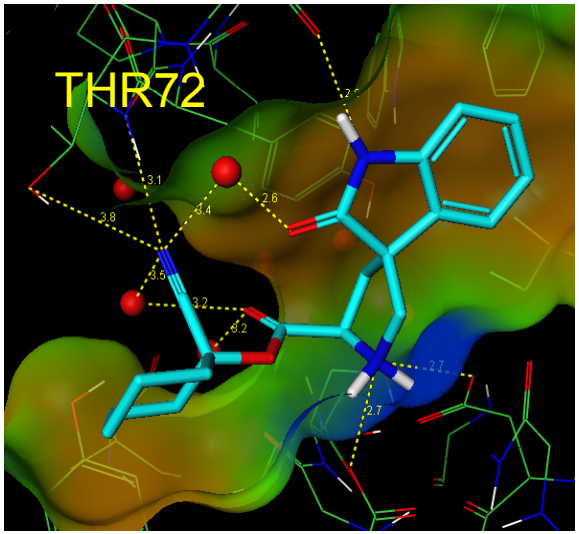
12

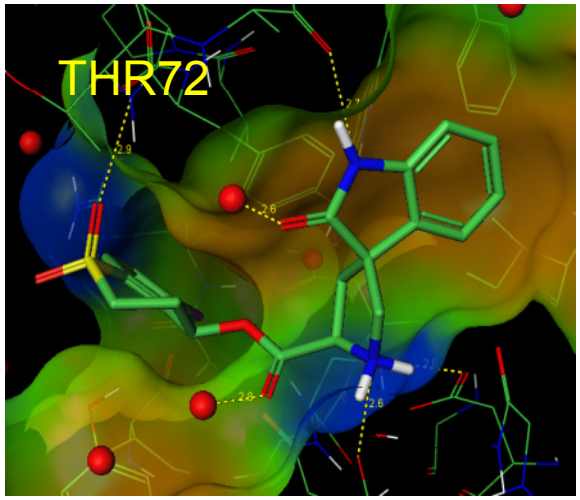
NMR IC50 = 11 μM (LE = 0.29)
CFA IC50 = 25 μM
RRCK = 23 x 10^{-6} cm/s



13

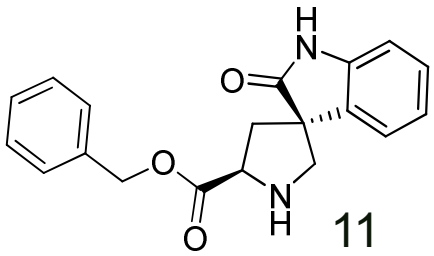
NMR IC50 = 1.0 μM (LE = 0.28)
CFA IC50 = 4.0 μM
RRCK = 49 x 10^{-6} cm/s
MDR BA/AB = 6.85





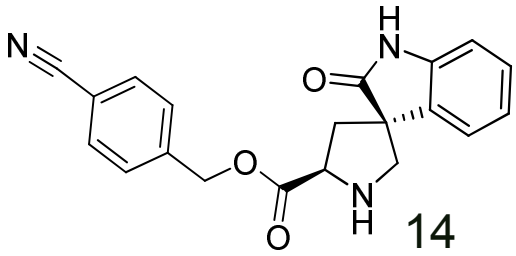
In addition to the potency increases due to occupancy of the S1' and S2' subsites, productive interactions with the flap residues have been engineered in.

Optimization of P2’ substituents



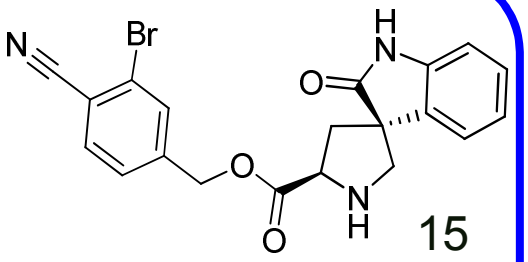
11

NMR IC50 = 33 μM (LE = 0.26)
CFA IC50 = 114 μM
RRCK = 30 x 10^{-6} cm/s
MDR BA/AB = 0.97



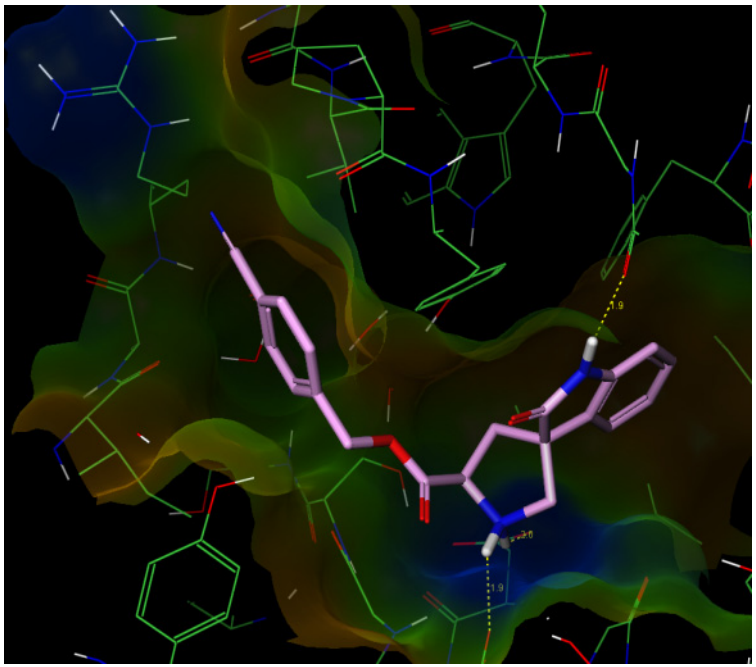
14

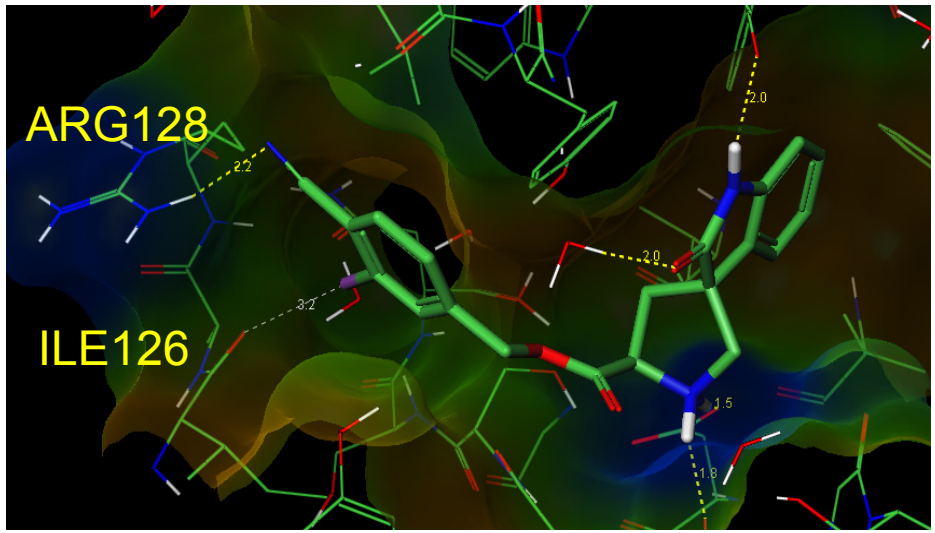
NMR IC50 = 7.0 μM (LE = 0.28)
CFA IC50 = 12 μM
RRCK = 18 x 10^{-6} cm/s
MDR BA/AB = 0.91



15

NMR IC50 = 2.0 μM (LE = 0.30)
CFA IC50 = 6.3 μM
RRCK = 10 x 10^{-6} cm/s
MDR BA/AB = 2.58

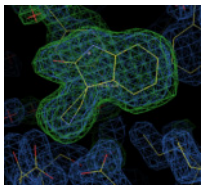


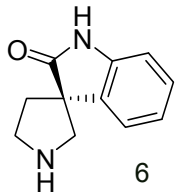


Progress in the spiropyrrolidine series

Decision to use FBDD approach in BACE project

Crystallography-based screening of the X-ray subset of the GFI collection

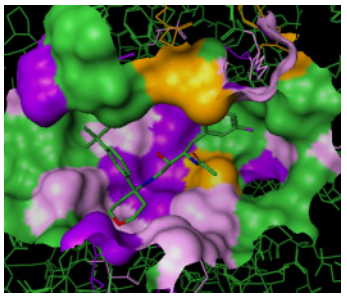




6

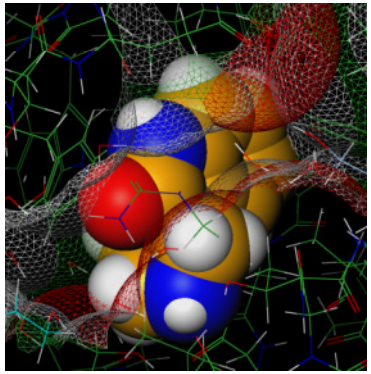
oxindole-containing spiropyrrolidine yields “spectacular” density

Hit validation and profiling

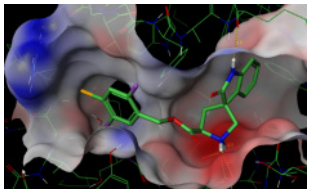


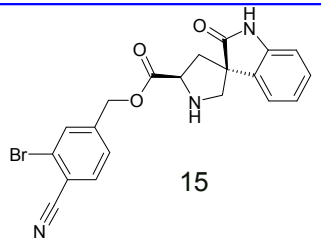
NMR IC50 = 1.09 mM (LE = 0.3)
OCTET Kd = 1.36 mM
full competition with HEA
significant TROSY perturbations
RRCK AB = 20 x 10^{-6} cm/s, MDR BA/AB = 0.99

Multidisciplinary analysis of the binding mode



Design cycles





15

CFA IC50 = 6.3 μM
NMR IC50 = 2 μM (LE = 0.30)
RRCK AB = 10 x 10^{-6} cm/s
MDR BA/AB = 2.58
(many analogs with better MDR)

- **Hypothesis generation and testing by a multidisciplinary team led to fast progress in identifying single-digit micromolar BACE inhibitors.**
- **Almost 3 orders of magnitude potency improvement has been achieved while maintaining favorable ligand efficiency and ADME profiles.**
- **Initial efforts in identifying ester isosteres have been successful.**

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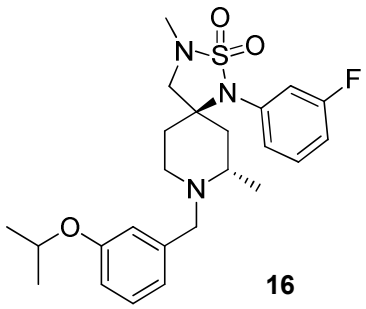
Summary - spiropyrrolidines

- X-ray based fragment screening was an effective way to identify a novel type of BACE binders.
- Multidisciplinary approach was the key to fragment hit profiling and optimization efforts.
- Spirocyclic pyrrolidine scaffold was shown to be a suitable platform for development of efficient BACE inhibitors with ADME parameters predictive of good brain penetration.
- With a few design loops, potency of the starting hit was improved 500-1000 fold while maintaining the favorable ligand efficiency and ADME profile.
- Next iteration of optimization work focused on additional scaffold optimization and isosteric replacements of the ester functionality.

Spiropiperidines

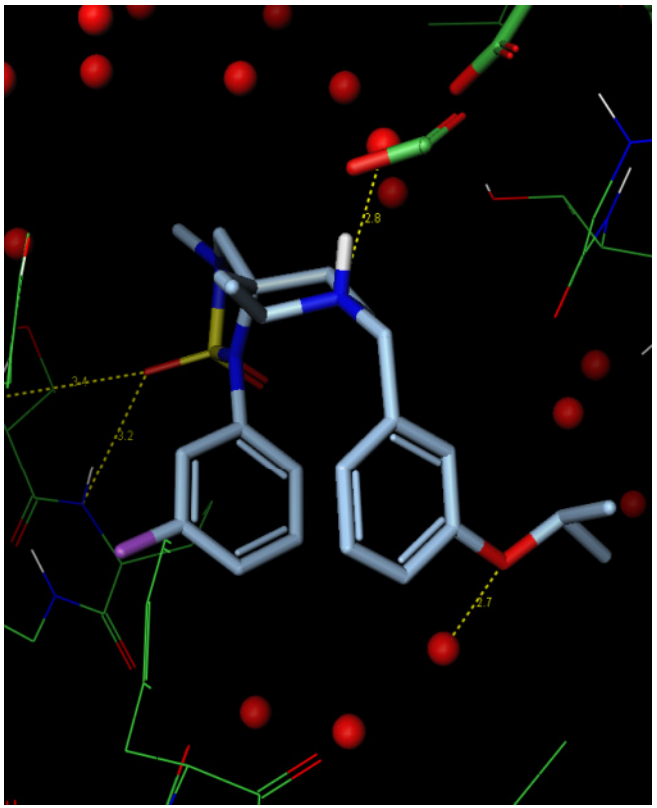
impact from fragment-based work on lead optimization

Spiropiperidine series – identification of a key H-bond interaction



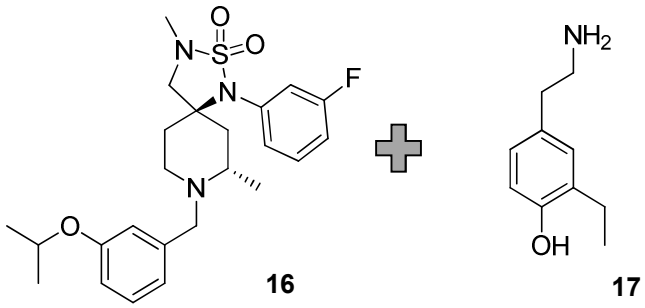
16

CFA IC50 = 2.37 μM
WCA IC50 = 1.80 μM



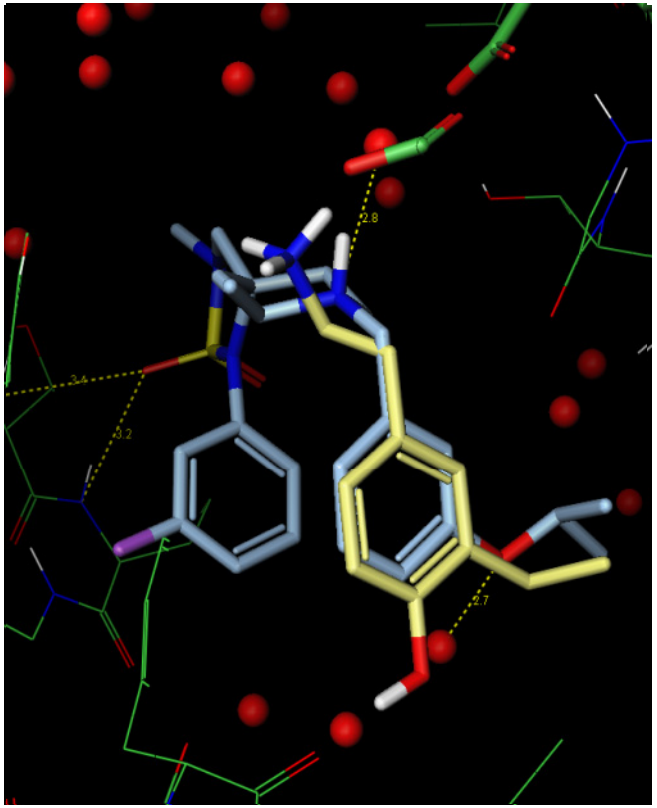
- Productive occupancy of the S1 subsite is a key feature of the spiropiperidine class of BACE inhibitors.
- X-ray structure of the tyramine derivative 17 recapitulated the binding pose of the P1 aryl substituent and indicated presence of the well-positioned H-bond to the carbonyl of Phe108.
- Transfer of this SAR observation to the spiropiperidine scaffold resulted in a significant potency improvement.
- It was one of the key observations for SAR advancement in this series.

Spiropiperidine series – identification of a key H-bond interaction



CFA IC50 = 2.37 µM
WCA IC50 = 1.80 µM

tyramine-based fragment hit*
OCTET KD ~ 6 mM



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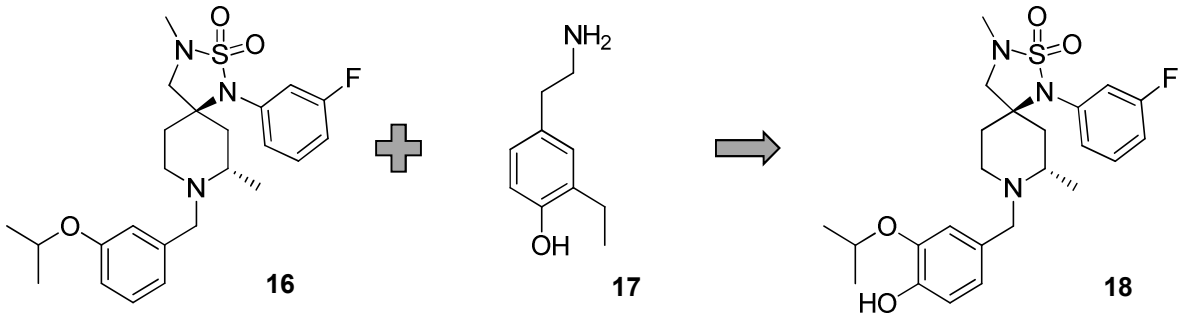
* Kuglstatter et al. *Bioorg. Med. Chem. Lett.* 2008, 18 (4), 1304-1307.

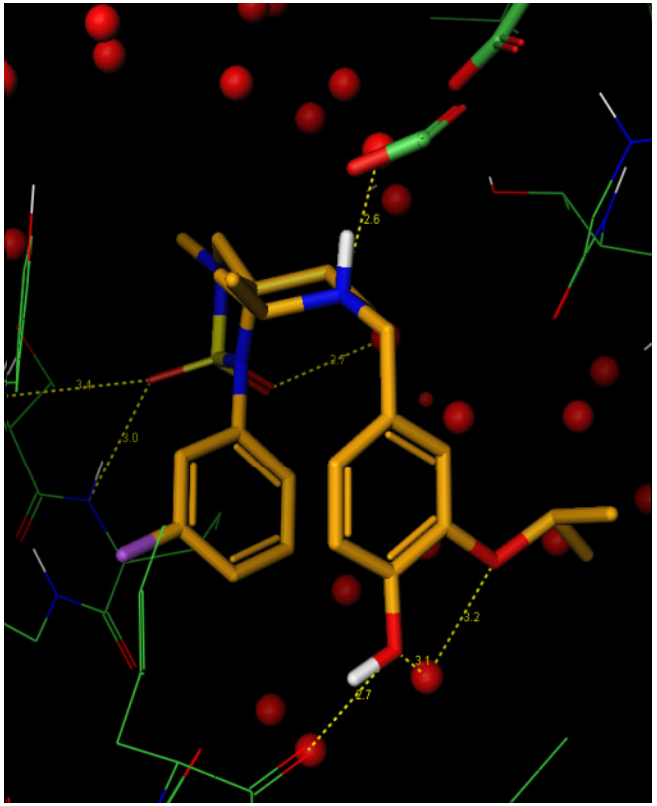
Spiropiperidine series – identification of a key H-bond interaction



CFA IC50 = 2.37 µM
WCA IC50 = 1.80 µM

tyramine-based fragment hit
OCTET KD ~ 6 mM

CFA IC50 = 0.106 µM
WCA IC50 = 0.100 µM



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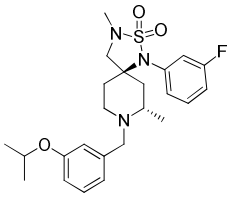
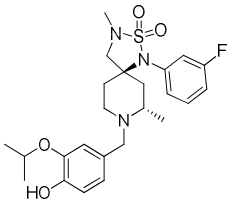
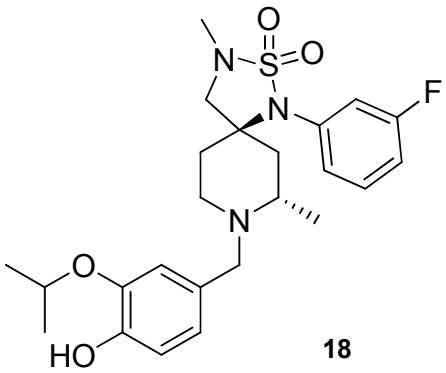
Effect of the installed H-bond interaction on the thermodynamic parameters of binding

Compound #	CFA IC_{50} (μM)	ITC K_D (μM)	ΔG (kcal/mol)	ΔH (kcal/mol)	TΔS (kcal/mol)
 **16**	2.37	1.21	-8.15	-10.3	-2.17
 **18**	0.106	0.174	-9.88	-11.6	-1.68

- Thermodynamic data aligned well with the biochemical assay.
- This data package illustrates that the well-positioned H-bond avoids enthalpy-entropy compensation phenomenon and leads to a significant affinity increase despite the higher desolvation penalty.
- The change in entropy/enthalpy balance results in an even more enthalpically driven binding in compound 18 compared to compound 16.

Profile of the spiropiperidine lead compound



18

clogP = 3.7
MW = 478
HBD = 1
PSA = 82

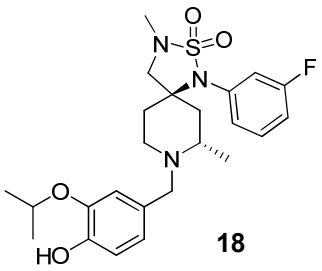
WT WCA (total Aβ) IC50 = 25 nM
WT WCA (sAPPβ) IC50 = 66 nM

CFA CatD IC50 **> 100 μM**
CFA BACE2 IC50 = 280 nM

HLM Clint = 15.4 mL/min/kg
RLM Clint = 59.7 mL/min/kg

CNS Penetration
MDCK AB = 17.6 x 10^{-6} cm/s
MDR Er = 3.3
B/P (mouse) = 0.35
fu,brain = 0.12
fu,plasma = 0.17
Cub/Cup = 0.27

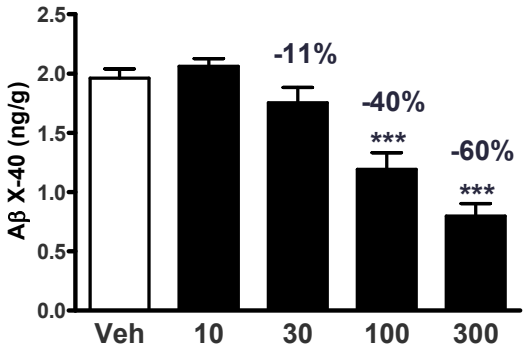
Acute Reduction of Central Aβ in Wild Type Mice @ 3h post dose (sc dosing)



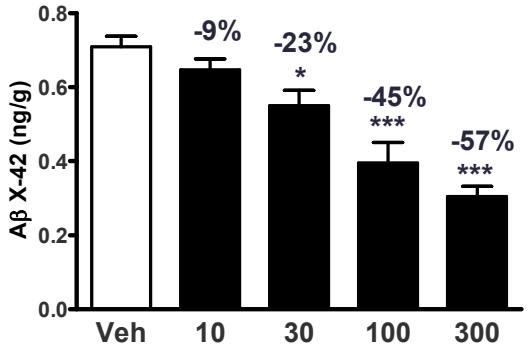
18

N=8/group
Statistical analysis: one-way ANOVA, post-hoc Dunnett's test,
* P<0.05
** P<0.01
*** P<0.001
vs. vehicle

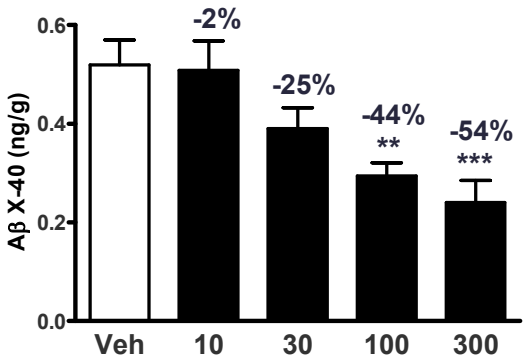
Brain Aβ40



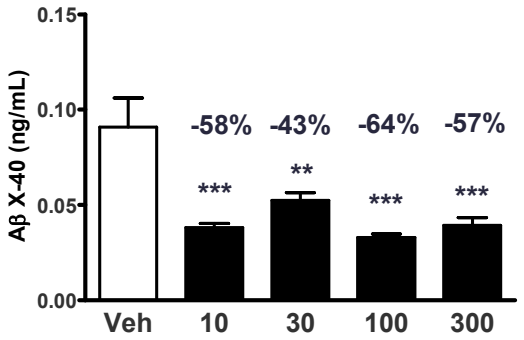
Brain Aβ42



CSF Aβ40



Plasma Aβ40



- **Significant reduction of plasma Ab was observed at all doses tested.**
- **Brain and CSF reached statistical significance at 100, 300 mg doses.**

Summary - spiropiperidines

- A very productive H-bond was engineered in a lead scaffold by translation of the structural information from a fragment compound.
- Even a very weak compound can provide a glimpse into a highly useful SAR direction – need to leverage such tactics outside of formal FBDD programs.
- Fragment info can come from a variety of sources. How broadly is this information being actively mined by medicinal chemists?

Acknowledgements

Chemistry
- Michael Brodney
- Tommy Chen
- Jason Dutra
- Karen Coffman
- Steven Goldstein
- Christopher Helal
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- Kevin Ogilvie
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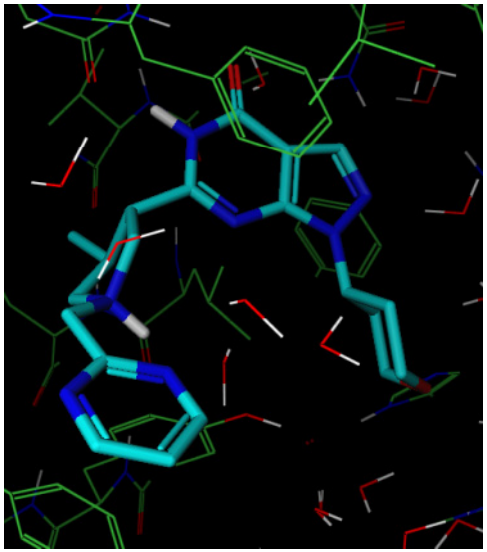
- Lorraine Lanyon
- JianHua Liu
- Stephen Noell
- Christine Oborski
- Charles Nolan

Lecture Overview

- Ligand-protein interactions.
- Physico-chemical properties and drug design: attributes of a lead molecule.
- Introduction to medicinal chemistry and lead optimization.
- Best practices in medicinal chemistry.
- **Case-studies (Alzheimer's disease):**
  - Fragment-based approaches in discovery of *beta*-secretase inhibitors
  - Identification of a PDE9 clinical candidate

Identification of a PDE9 Clinical Candidate for the Treatment of Alzheimer's Disease Utilizing Prospective Design and Novel Library Protocol Development



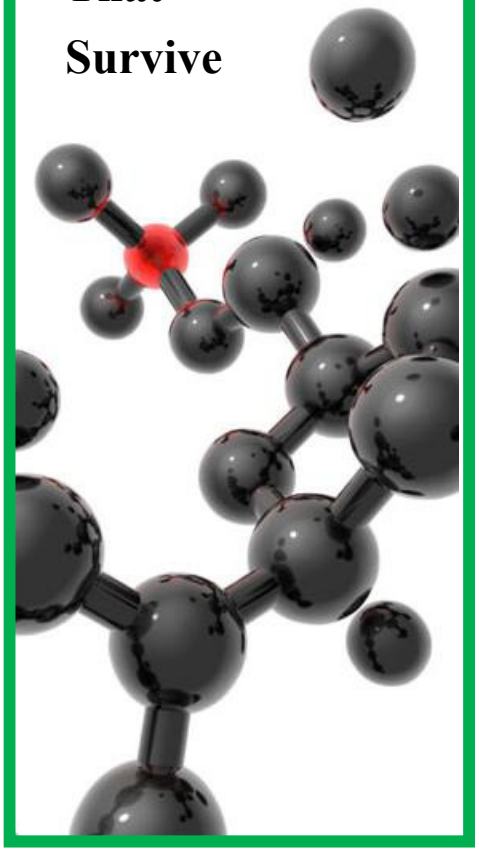
Verhoest, Patrick R. *et al.* "Design and Discovery of 6-[(3S,4S)-4-Methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (PF-04447943), a Selective Brain Penetrant PDE9A Inhibitor for the Treatment of Cognitive Disorders" *J. Med. Chem.* **2012**, *55*, 9045-9054.

Overall Strategy

Be Right
About
Targets
More Often

Select the
Right Patients

Design
Molecules
That
Survive

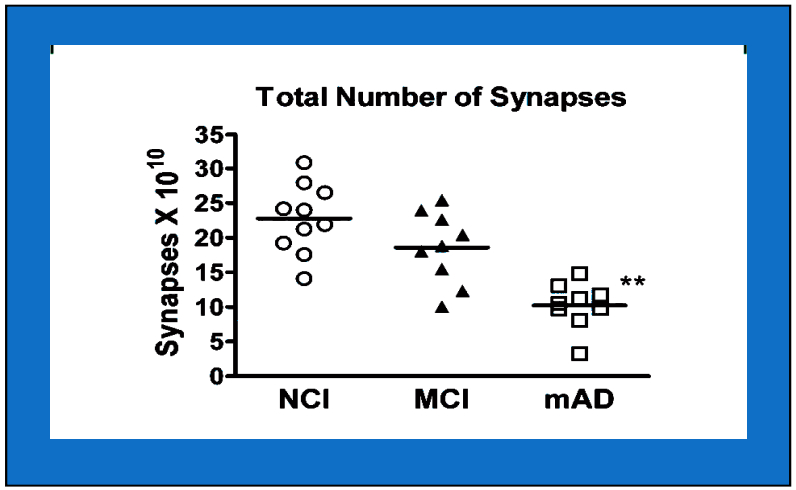


Speed

AD and Synaptic Dysfunction

Alzheimer's Disease is, fundamentally,a synaptic failure.
Selkoe (2002) *Science* **298**:789-791.

Synapse loss in AD



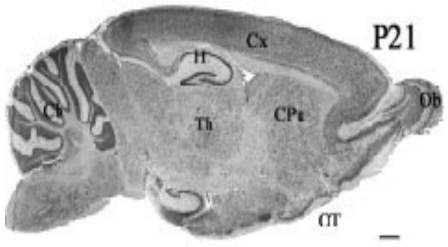
- **Occurs very early in the disease**
  - –Scheff et al., *Neurology* (2007)
- **Preceeds amyloid deposition**
  - –Masliah et al., *Neurosci. lett* (1994)
- **Observed as decreased synapse density and expression of synaptic proteins**
  - –Masliah et al., *Neurology* (2001)
- ***Correlates most closely with cognitive decline***
  - –Terry et al., *Ann. Neurol.* (1991)

➢ Stabilization of vulnerable synapses may restore cognitive function and slow progression of the disease.

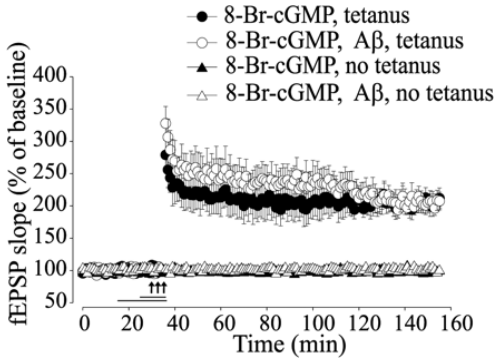
PDE9 Inhibition to Stabilize Synapses

Novel hypothesis (PDE9)

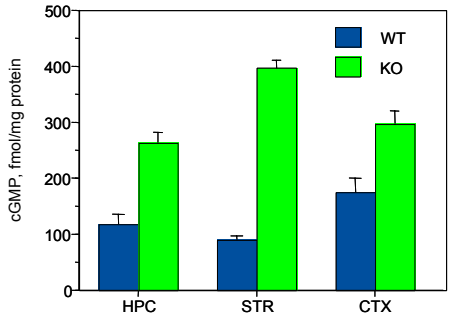
- cGMP reverses Aβ induced Long Term. Potentiation (LTP) deficits in hippocampal slices.
- PDE9 KO Mice have elevated LTP and cGMP.
- cGMP has shown to be active in models of cognition.
- PDE9 has widespread CNS distribution and has the highest affinity of PDEs for cGMP.



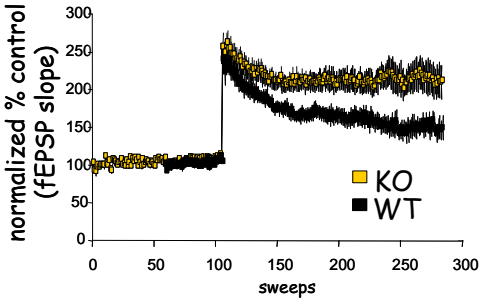
PDE9 mRNA in rat brain
Van Staveren et al., (2003) *J Comp Neurol,* **467**:566
Andreeva et al. (2001) *J Neurosci.* **21**:9068



PDE9 KOs have elevated cGMP levels

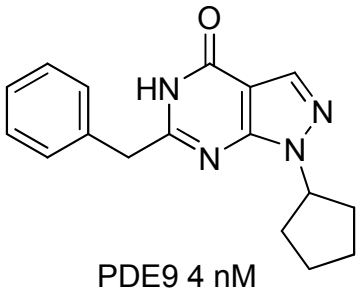


PDE9A KO mice

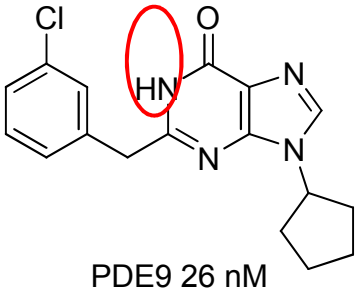


Choosing Chemical Matter to Pursue (CNS Penetration)

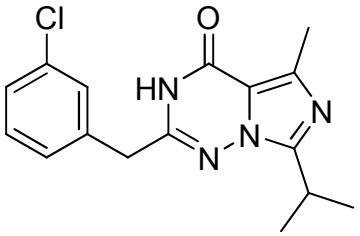
- HTS only yielded known Chemical Matter.
- Utilized known internal PDE inhibitor matter.
- All have good calculated properties: ClogP, MW, LE, LipE, B/P (0.5-0.8).



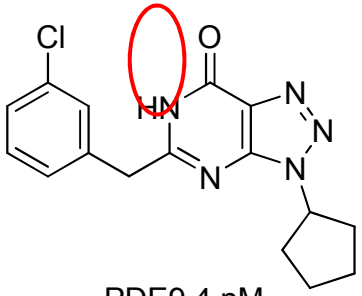
PDE9 4 nM
PDE1c 1 nM
MDR/MDCK=1
Mouse B/P=1.2



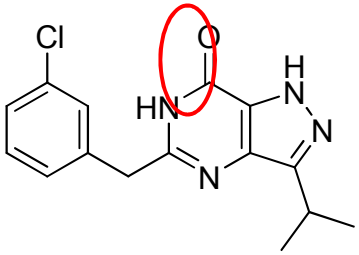
PDE9 26 nM
PDE1c 88 nM
MDR/MDCK=1.5
Mouse B/P=0.1



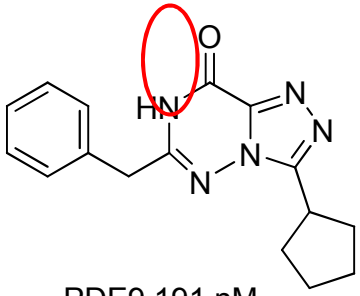
PDE9 47 nM
PDE1c 80 nM
MDR/MDCK=1.1
MouseB/P=0.8



PDE9 4 nM
PDE1c 1 nM
MDR/MDCK=1
Rat B/P=0.08



PDE9 35 nM
PDE1c 2 nM
MDR/MDCK=2.2
Mouse B/P=0.07



PDE9 191 nM
PDE1c 10 nM
MDR=1.1
Mouse B/P=0.05

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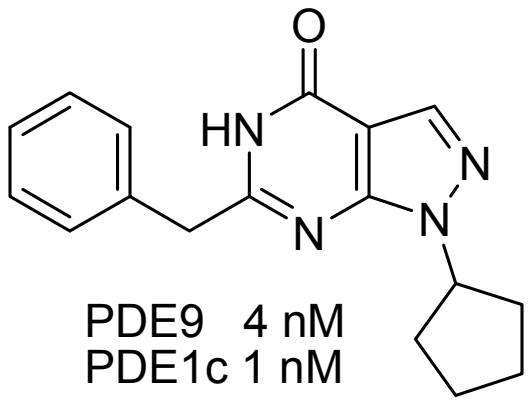
Prospective Design



PDE9 4 nM
PDE1c 1 nM

Prospectively Design Libraries To Address Multiple Issues

Potential/Known Issues

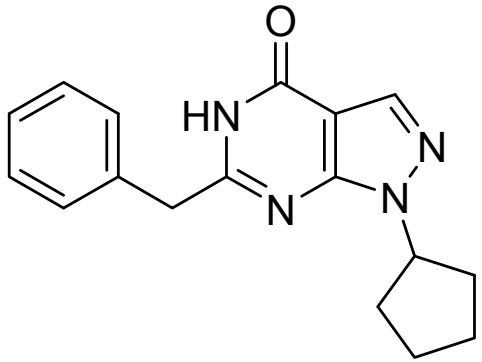
Selectivity
Brain Penetration/PgP
Solubility/exposure
Clearance
No Library Protocol

Engaged PDE9 through Library protocol development

Design Cycle 1

Goals: Enable the chemistry, improve selectivity and properties
Identify a CIR tool

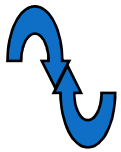
Ideal: Address all issues in one design



Potential/Known Issues

- Selectivity
- Brain Penetration/PgP
- Solubility/exposure
- Clearance
- No Library Protocol

Design 1



Selectivity
Solubility/exposure
No Library Protocol

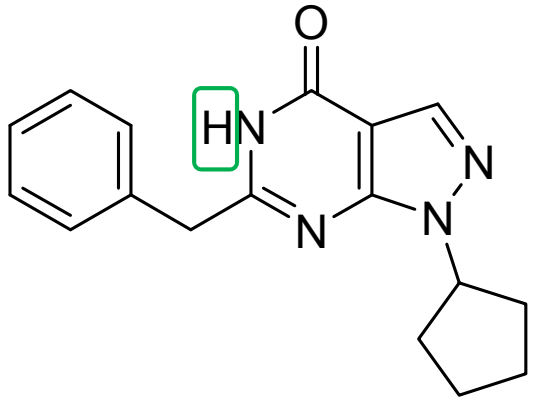
SBDD MoViT
Chemical Efficiencies

Library Design: Building Cores Provides More Diversity

Chemical Enablement – *In Situ* Synthesis of Core Template

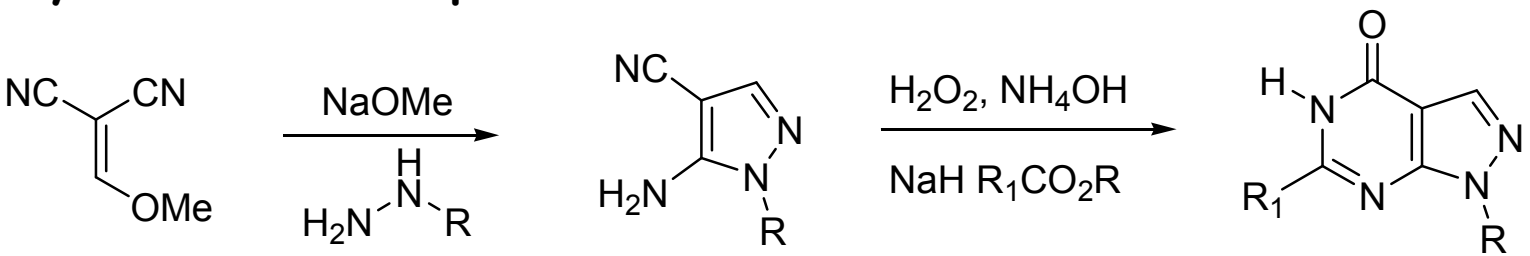
- Increases diversity
- Improves properties
- Form of Lead Hopping

Ester Monomer



Hydrazine Monomer

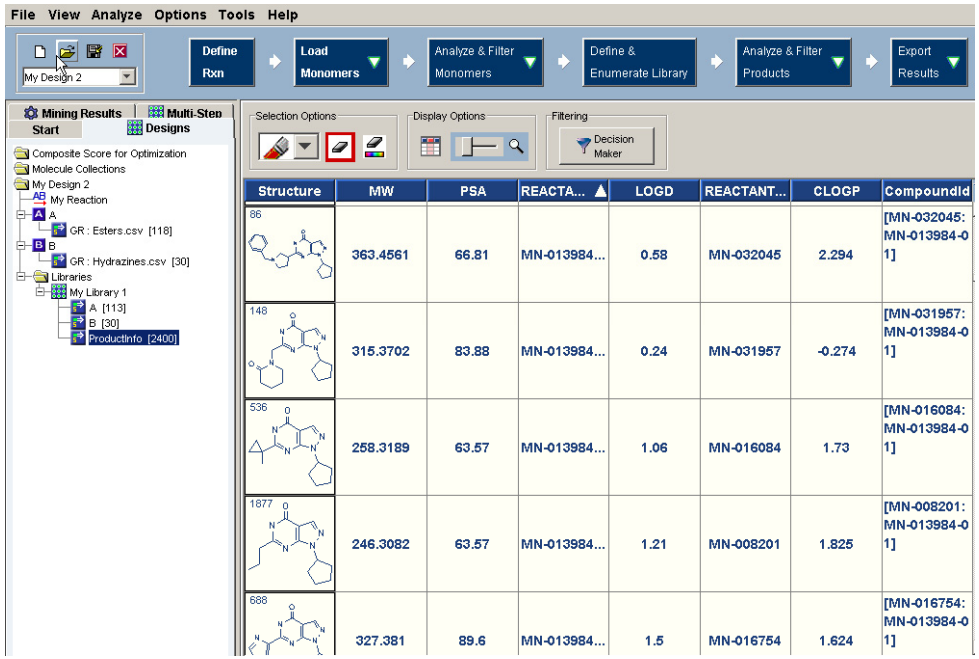
Library Protocol Development



Monomer Selection is Critical

89,000 compounds!

PGVL Design



Filtered Monomers

Mw<210

Cross reactivity

No Proton Donors

Hydrazines (poor properties)

Visually sorted monomers

Keep Enabled Monomers

(30x118)

For large libraries filtering monomers can be easier

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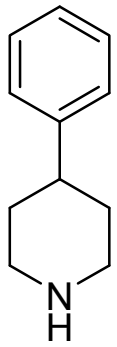
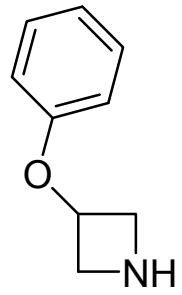
Enabled Monomers Increase Diversity

"**Enabled Monomers**" were given priority to allow for further library chemistry
Have higher value – Allow for further parallel chemistry

Difficult Library enablement

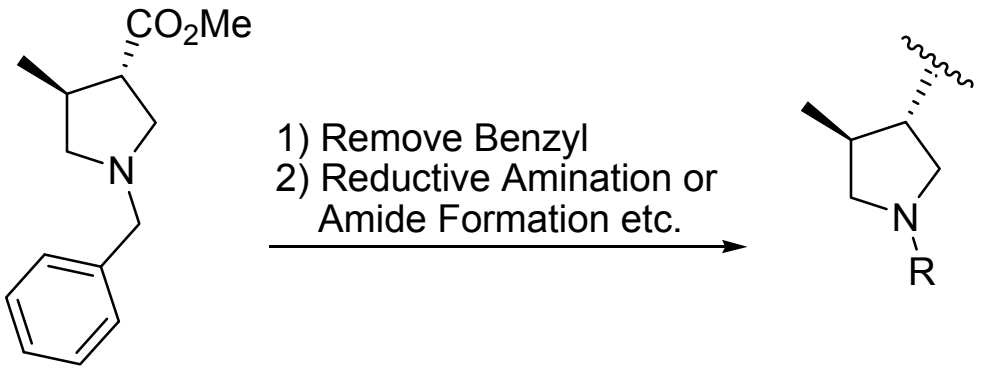


vs



Library enablement for Ar replacement

PDE9 Enabled Monomer:



CNS drug Space Filtering

3540 Compounds

↓

Enumerated

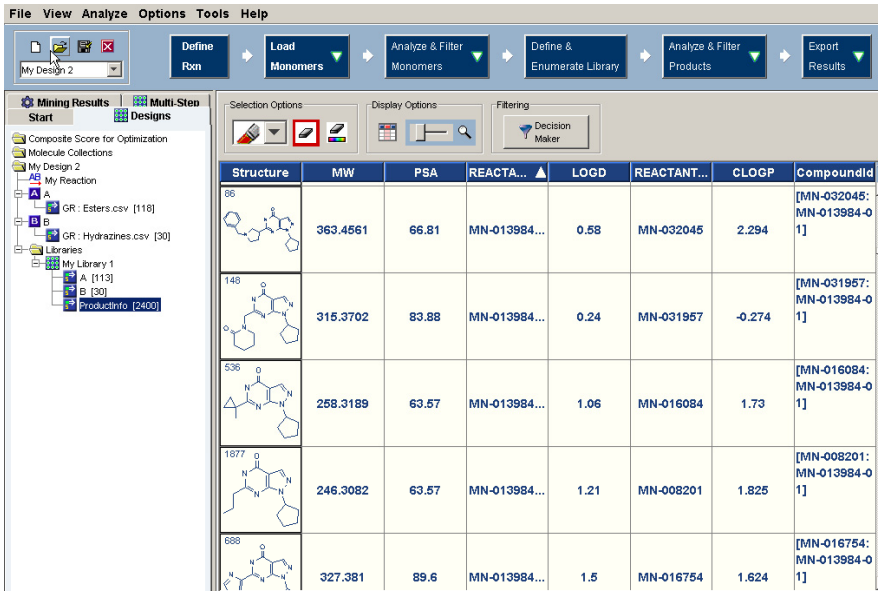
↓

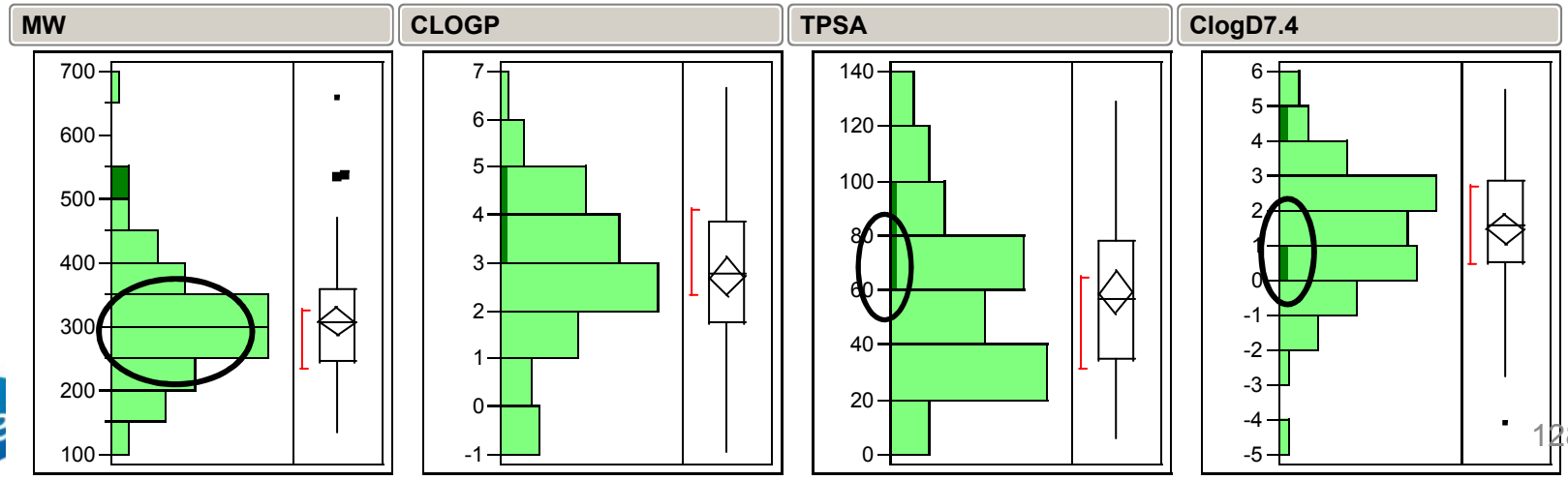
Filter CNS Drug Space

MW<420

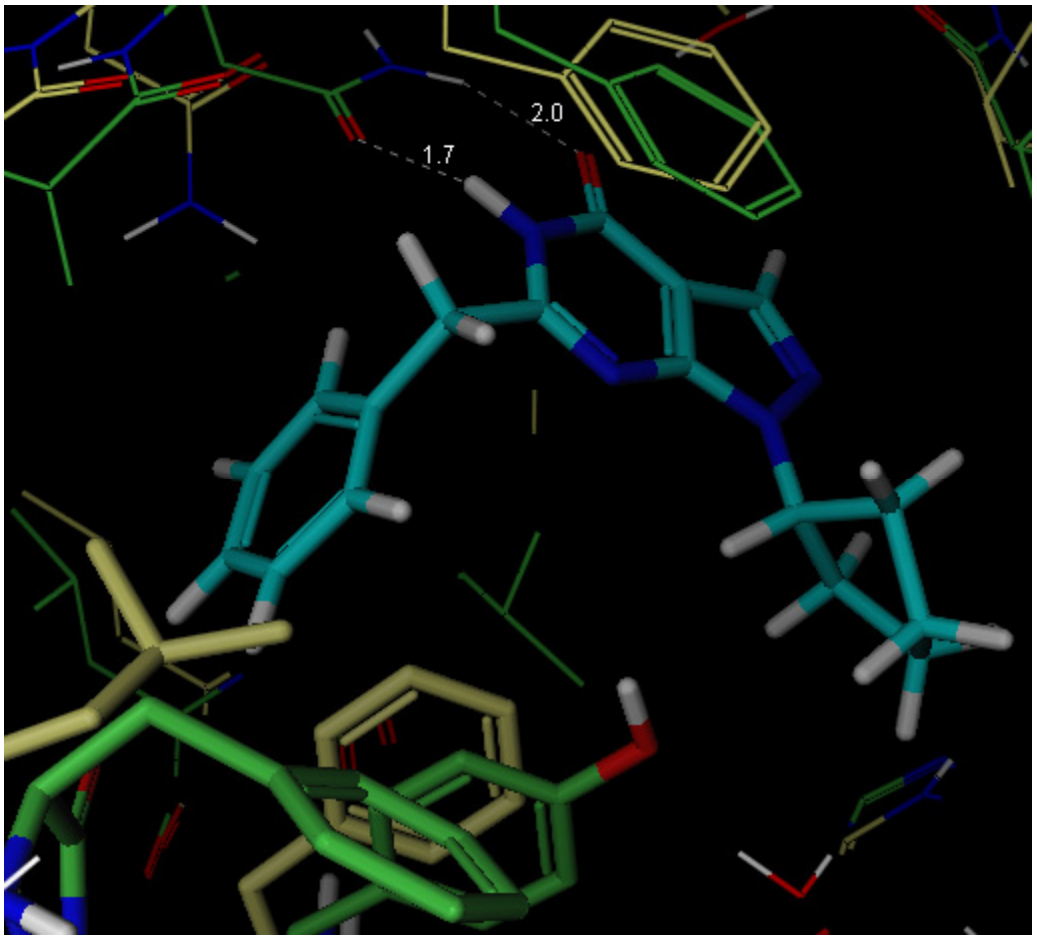
cLogD<3

TPSA<110





Docking in PDE9 Binding Site: Targeting Residue Differences



Green is PDE9: Yellow is PDE1C

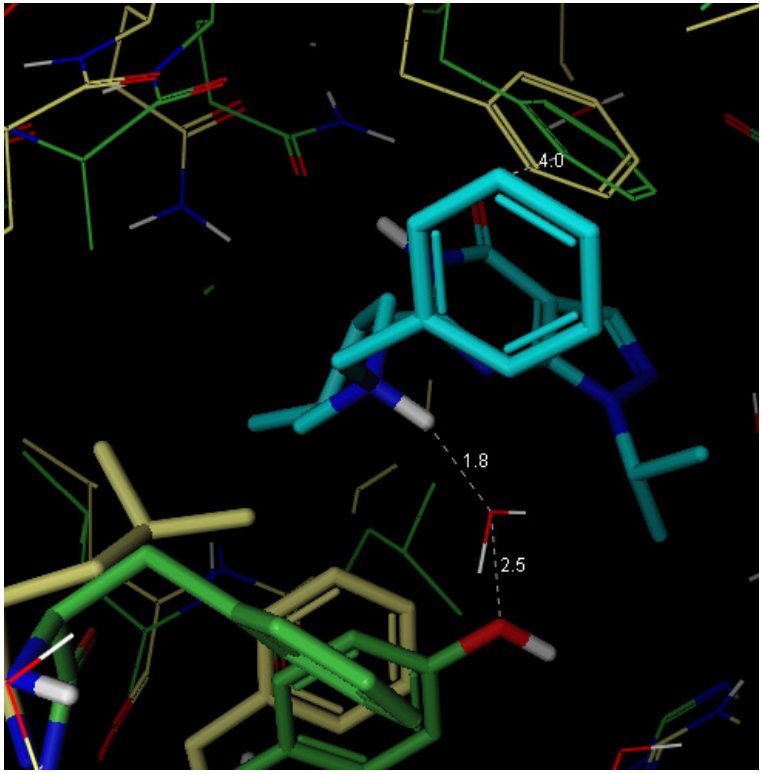
SBDD

Docked 2400 Compounds From Library

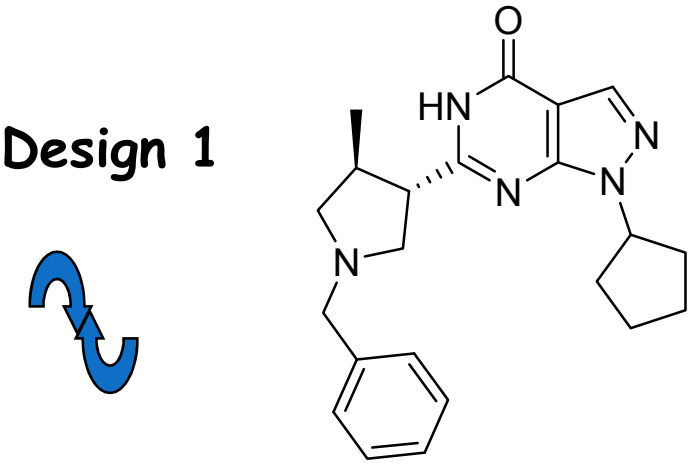
Selected monomers based On HT Scores and Visual Inspection 500 selected

"Rational approaches to improving selectivity in drug design" *J. Med. Chem.* **2012**, *55*, 1424-1444.

Hydrogen Bonding provides Selectivity



Design 1



Issues:
In-vivo Efficacy
Clearance

9 nM
270 nM PDE1c
B/P=4.8
HLM CLint 210

- The pyrrolidine N forms a hydrogen bonding network through a water to Tyr424.
- This provides a >100 fold shift in selectivity for PDE9.
- In addition the basic amine improves solubility and is an Enabled Monomer.

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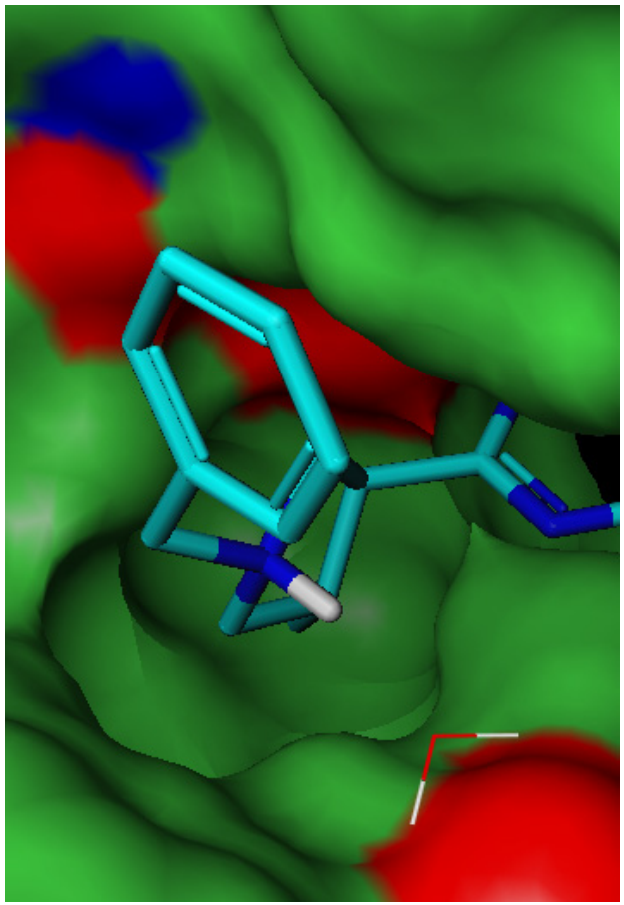
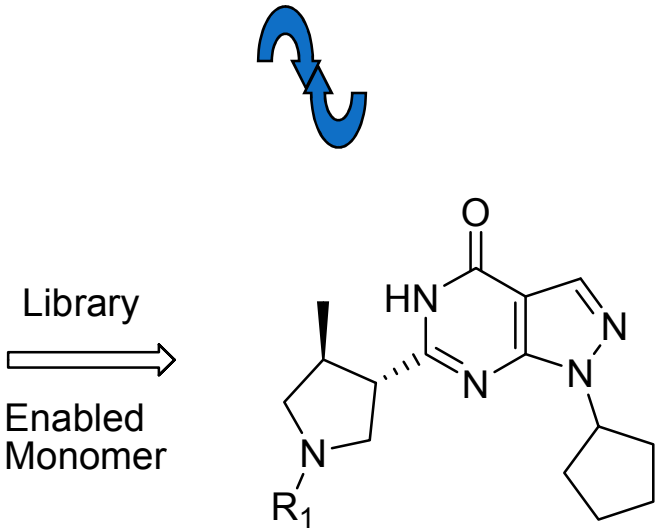
"Rational approaches to improving selectivity in drug design" *J. Med. Chem.* **2012**, *55*, 1424-1444.

SBDD: Lipophilic Face Provides Efficacy Opportunity

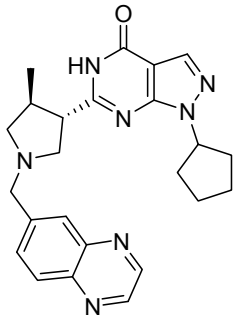
Design 2 and 3 Conducted in Parallel



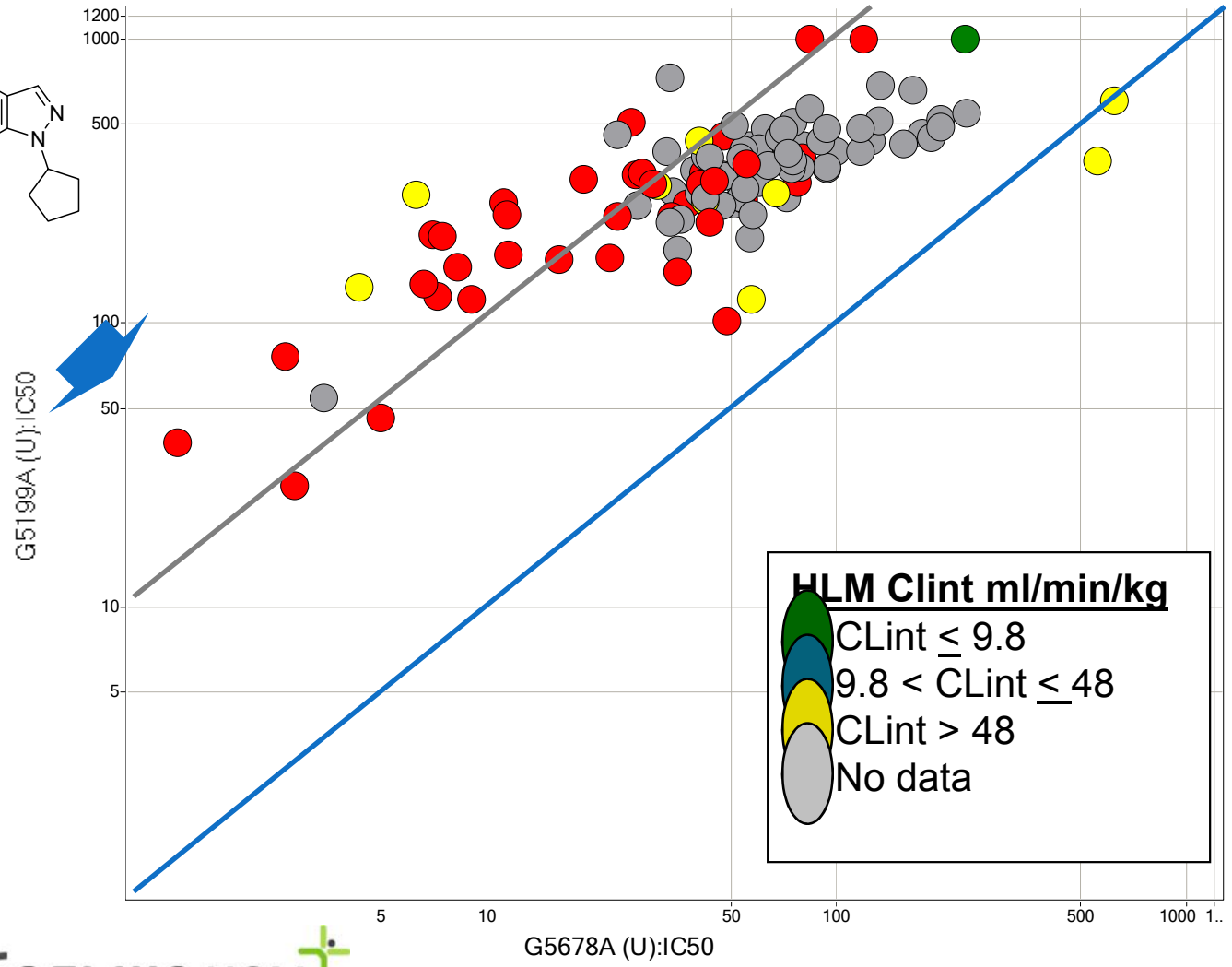
Design 2-Efficacy Enabled Monomer



Library Identified a CIR Tool Series-Trending for Selectivity



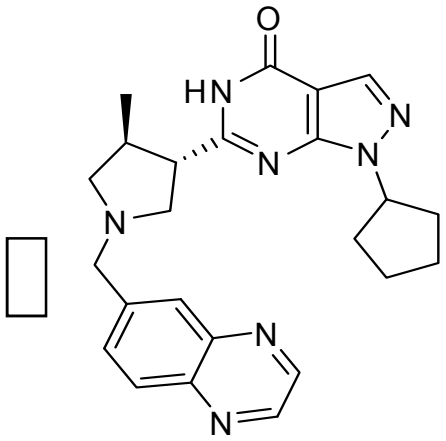
PDE1c



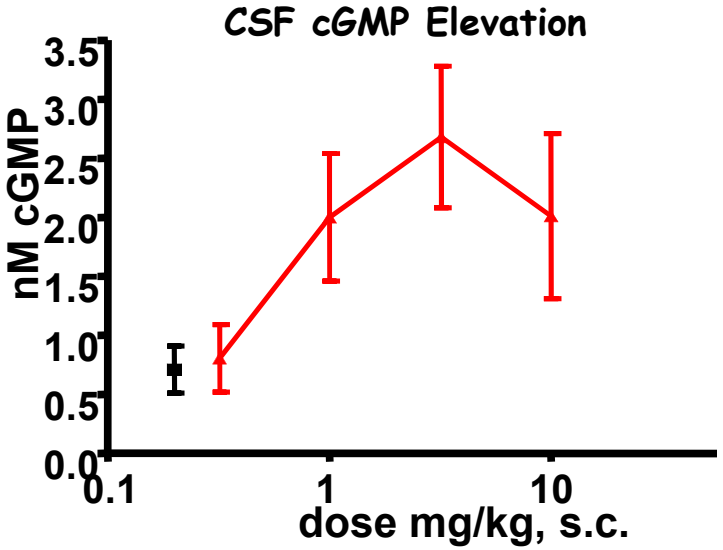
PDE9

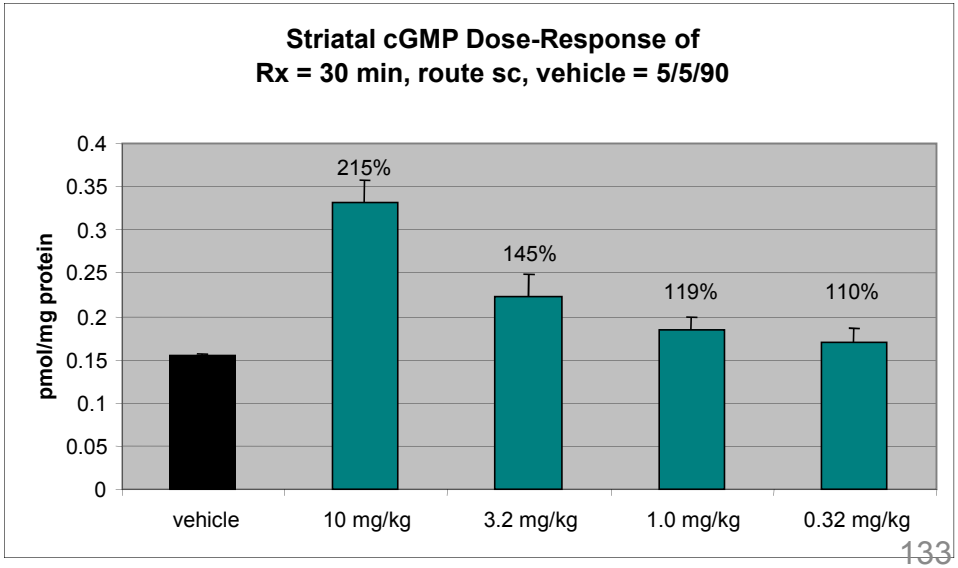
Design Cycle 2: Enabled Monomer Provides the in-vivo "Tool"

"In-vivo Tool"
Built CIR



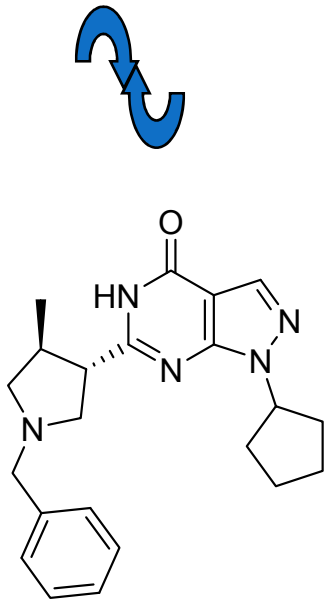
PDE9 1.2 nM
PDE1c 45 nM
240% cGMP@32
HLM CLint 149
High Permeability
Dof: 4% @ 10uM
Improved solubility
Dose Responsive Exposure
B/P=1.4
CSF=free plasma





Design Cycle 3: Improved Clearance

Design 1

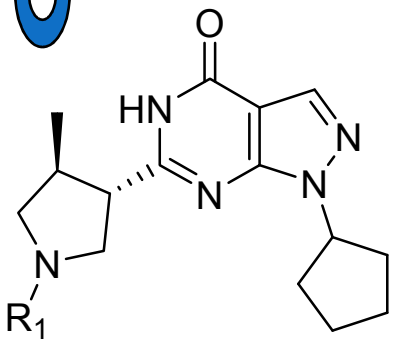


Issues:
In-vivo Efficacy
Clearance

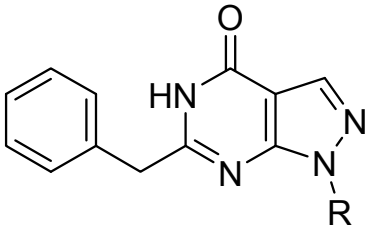
Design 2-Efficacy

Library

Enabled Monomer



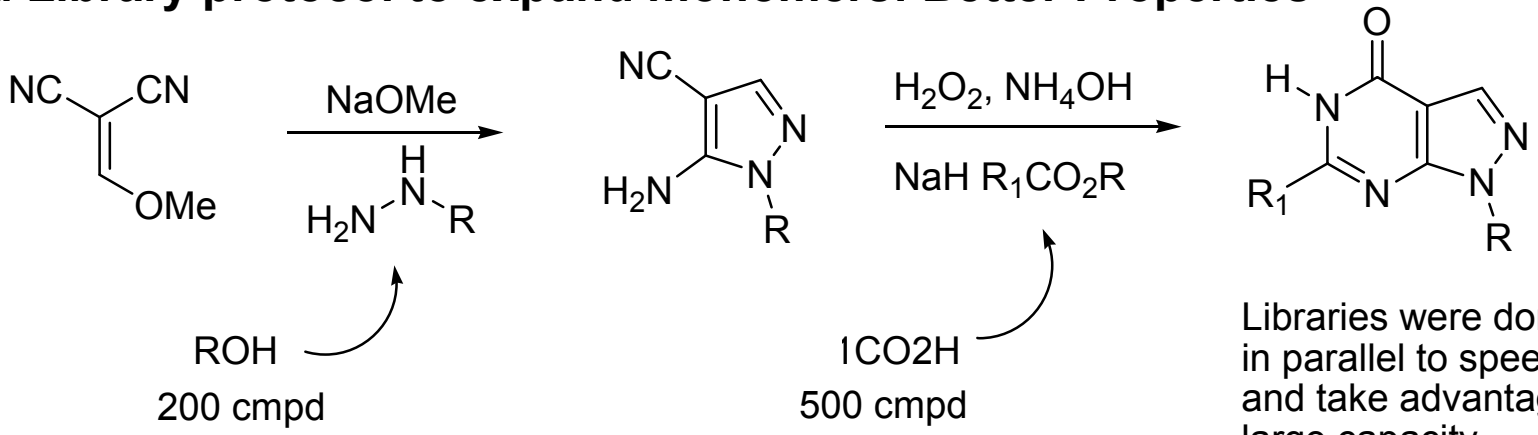
Design 3-Clearance



Design Cycle 3: Libraries Targeting Improved Clearance

Prospective Design: Can not address CLh with hydrazine monomer set

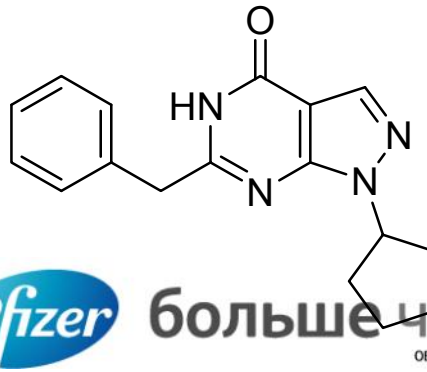
2nd Library protocol to expand monomers: Better Properties



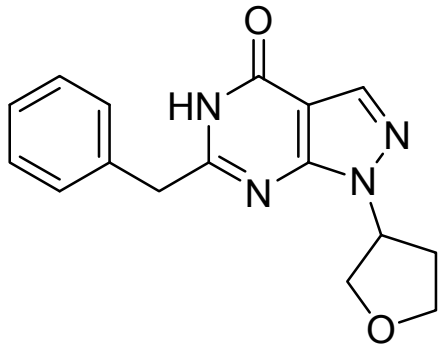
Libraries were done in parallel to speed process and take advantage of the large capacity

In-Situ Monomer Generation Allows For:

Improved Clearance Predictions and Calculated Properties

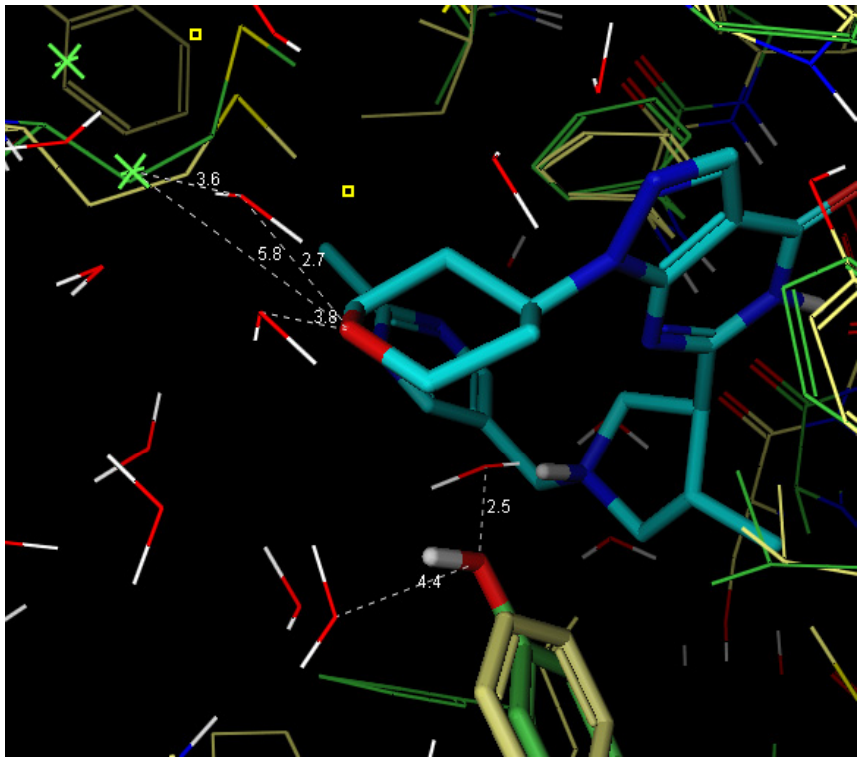


Clog D=2.2
Mic Pred Unstable
(Low Confidence)

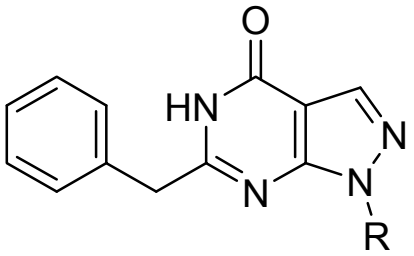


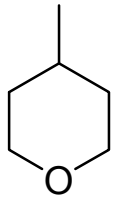
Clog D=1.1
Mic Pred Stable
(High Confidence)

Pyran Improves Selectivity and Clearance

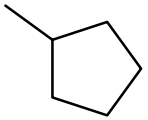


In-Situ Hydrazine Synthesis





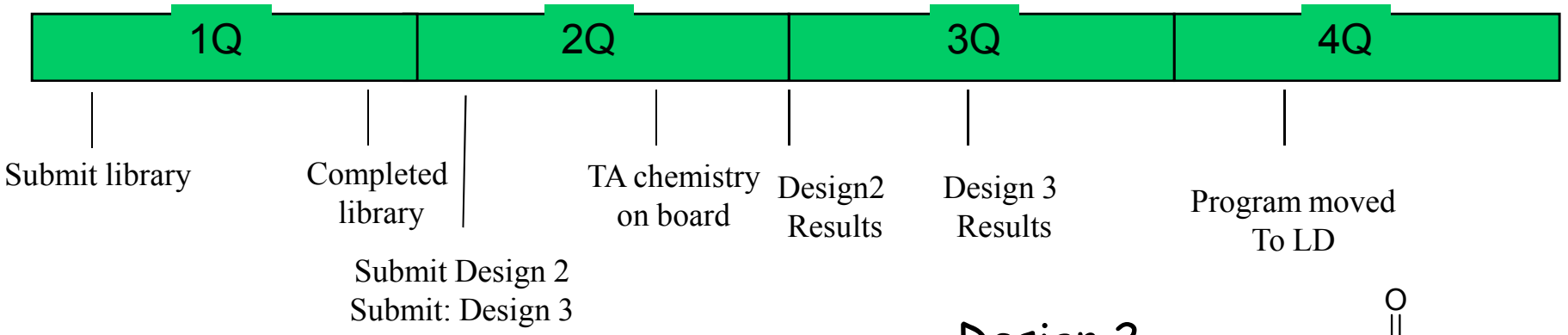
PDE9 2nM
PDE1c 12 nM
HLM Clint 14



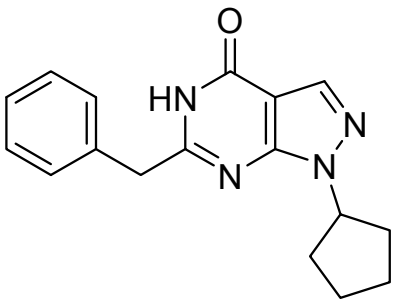
PDE9 4 nM
PDE1c 1 nM
HLM CLint 199

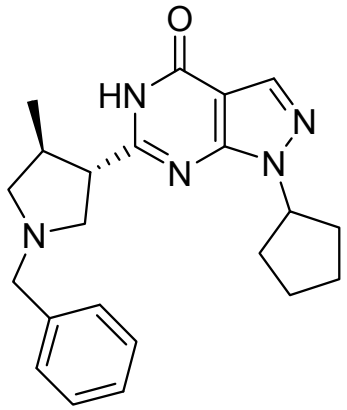
- PDE9 is a more polar environment than PDE1c and forms a better hydrogen bonding network through waters to the magnesium

Three Library Designs Moved the Program to Lead Development

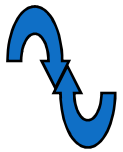


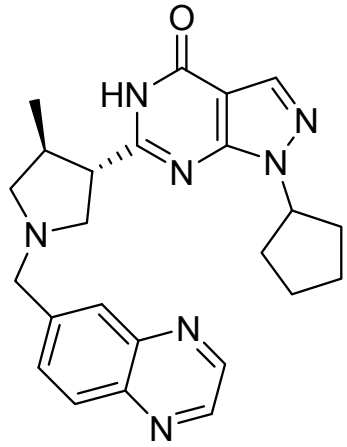
Design 1



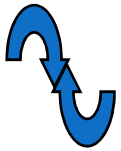


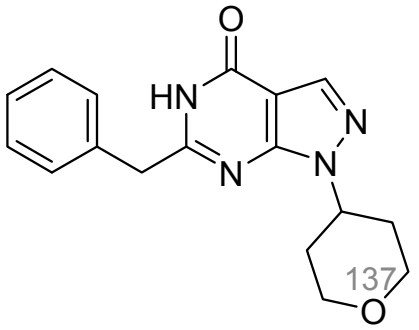
Design 2





Design 3



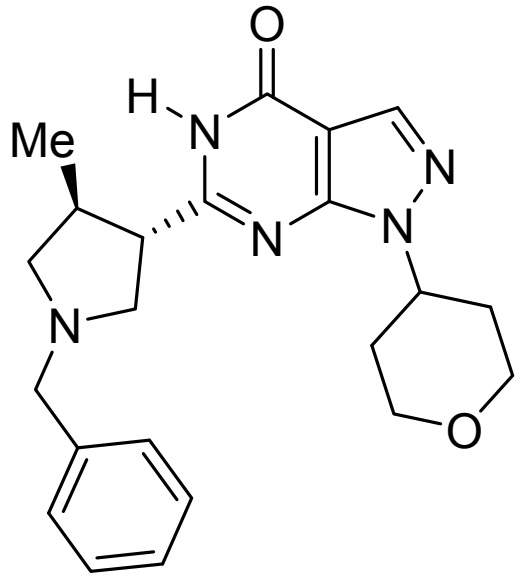


Utilizing Library Knowledge and SBDD to design Singletons

Increasing Me size
May improve potency

Benzyl Substitution
is important for
potency

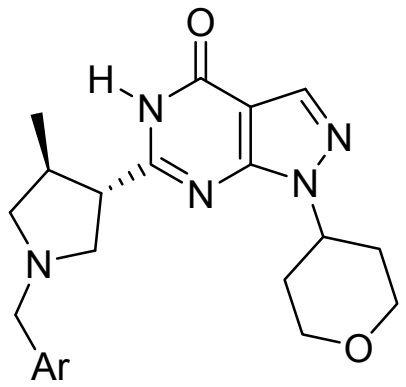
Heterocycles further
Improve clearance

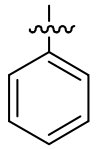
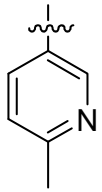
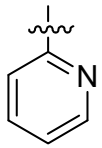
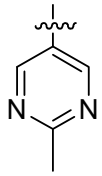
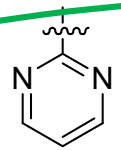


Maintain Pyran for
improved clearance
And selectivity

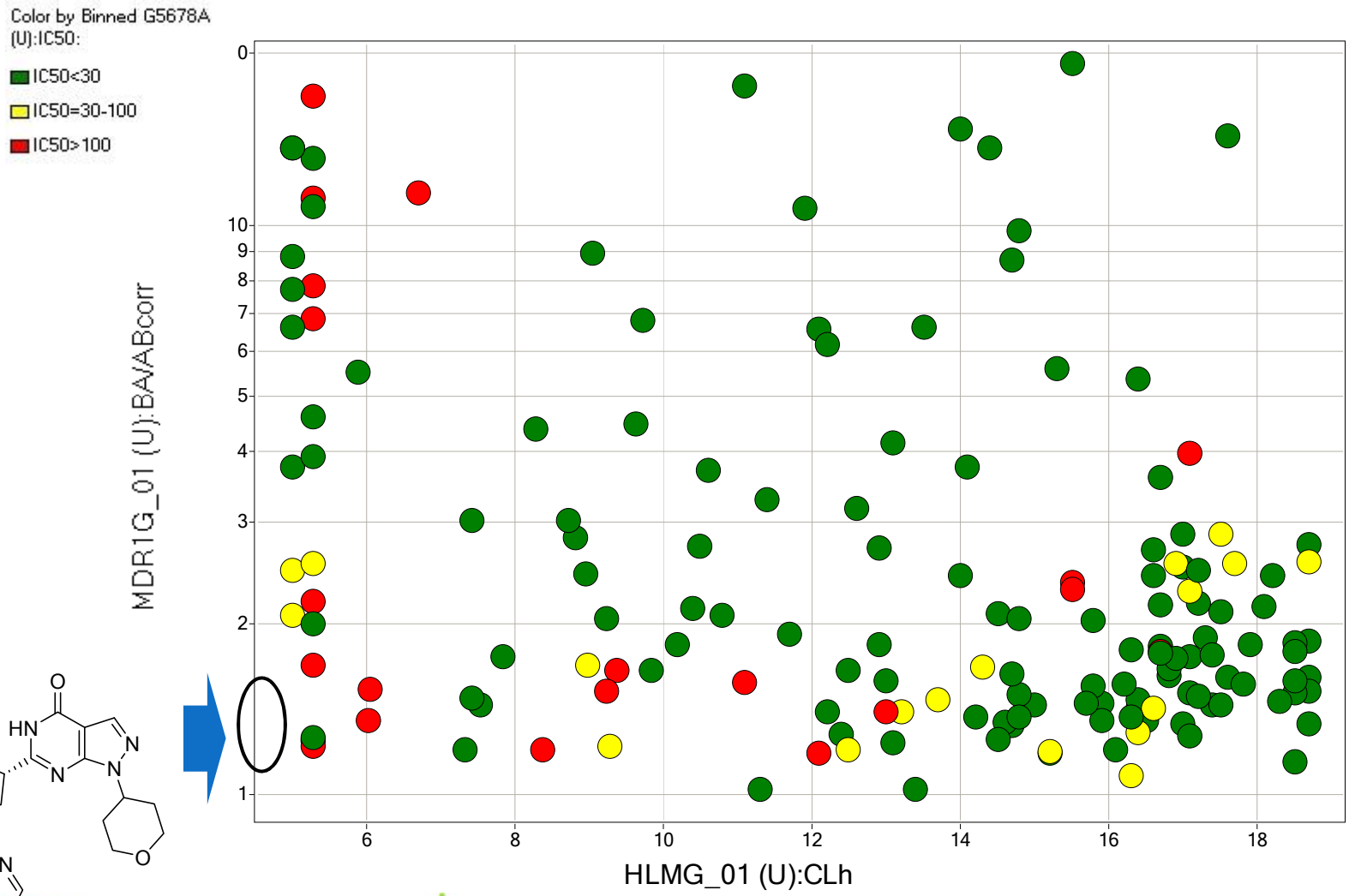
PDE9=7nM
PDE1C=>1uM
HLM Clint 39
MDR=1.4
Mw=393, ClogP=1

Heterocycles Effect Clearance and P-gp



Ar	Potency	HLM CLint	MDR BA/AB	Rat B/P
	7 nM	**39**	1.2	1.2
	4 nM	18	**4.5**	**0.09**
	6 nM	15	**1.4**	**0.4**
	4 nM	<7	10	0.02
	12 nM	<7	1.1	0.6

CNS-Difficult Balance for MDR and Clearance



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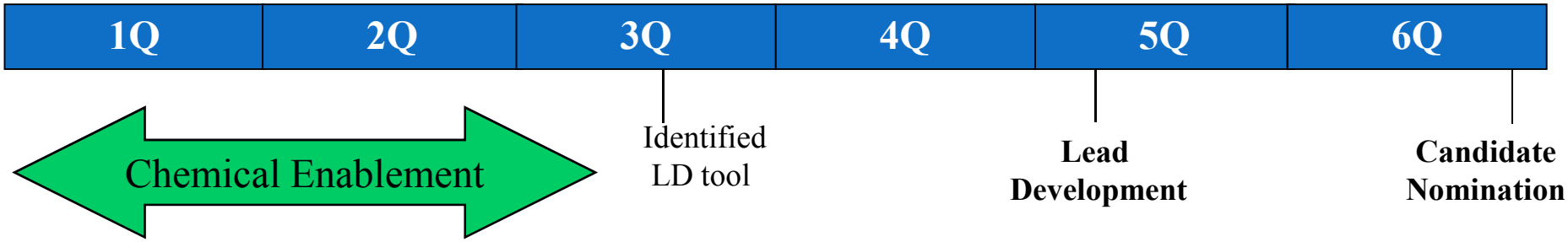
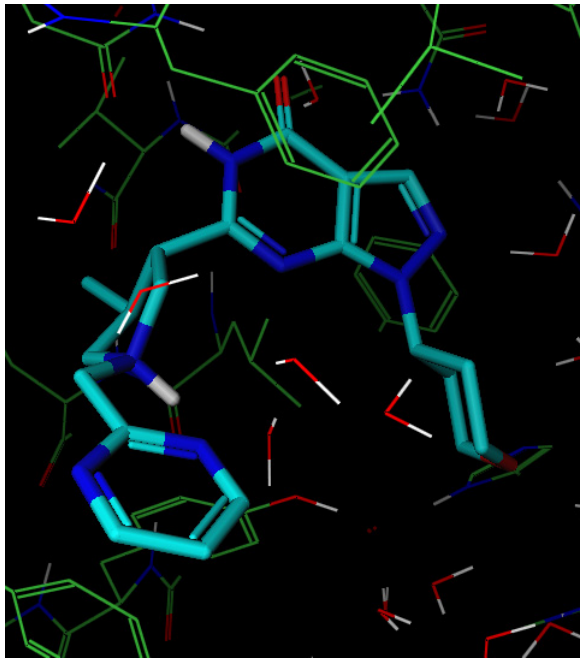
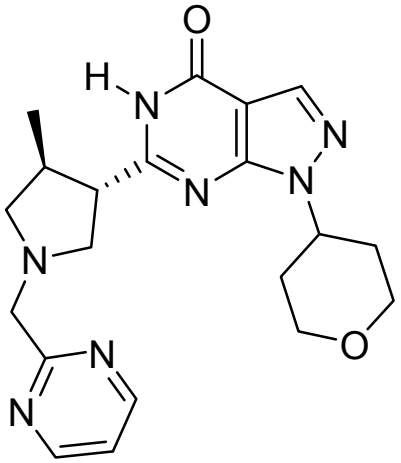
Library Design and SBDD Enhanced Speed







PF-04447943		
cLogP	-1.8	1.00
LogD7.4	-0.7	1.00
TPSA	102.0	0.61
MW	395.0	0.75
HBD	1	0.83
pKa	7.8	1.00
Desirability Score		5.19

PreClinical Candidate Profile

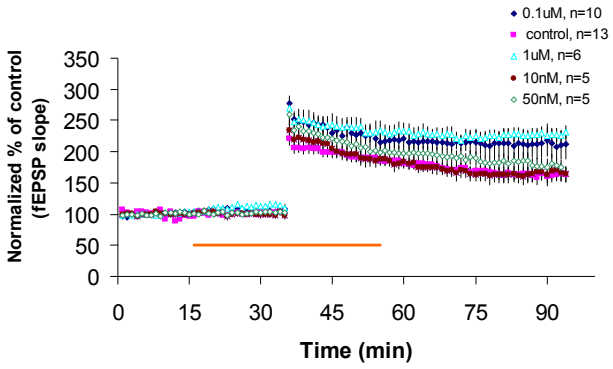
PDE9 = 12 nM (>100x)
CLogP = -1.0, MW = 395
LE = 0.37, LipE = 9.36
CNS MPO = 5.19
HLM Clint =<7 ml/min/Kg
Oral bioavaility >75%
High Solubility
C_{eff} = ~15 ng/mL
f_u= 93%
Dose Projection ~10mg

"Defining desirable central nervous system drug space through the alignment of molecular properties, in vitro ADME and safety attributes" *ACS Chem. Neurosci.* **2010**, *1*, 420-434.

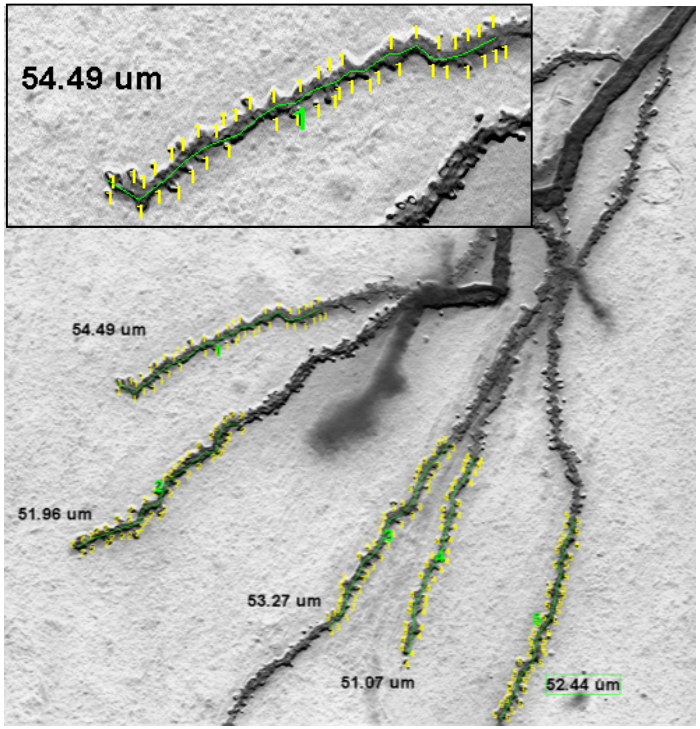
PF-4447943 PDE9i Enhances Synaptic Strength and Density

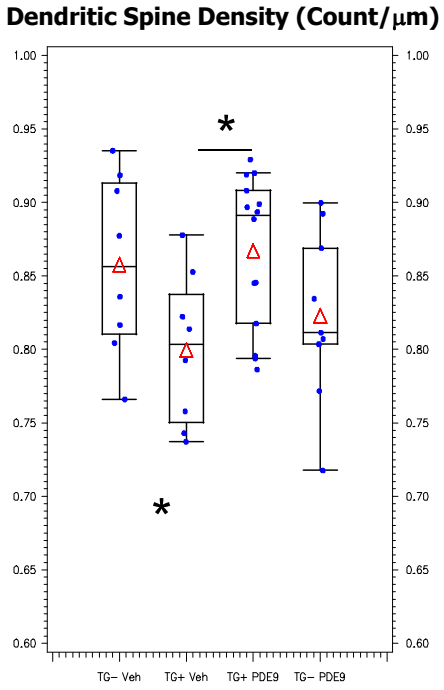
Hypothesis: PDE9 inhibitors will stabilize vulnerable synapses in the face of a AB insult by restoring synaptic plasticity mechanisms that provide activity-dependent stabilization.

LTP produces activity-dependent increases in synaptic strength



- cGMP plays a critical role in LTP
- PDE9 inhibition enhances LTP
- AB disrupts LTP
- cGMP reverses AB effects on LTP





- tg2576 mice exhibit elevations in AB and deficits in hippocampal synaptic spine density prior to plaque deposition.
- Chronic treatment with PF-04447943 via mini-pump for 30 days in 4 month old tg2576 mice prevented deficits in spine density in CA1 hippocampal dendritic fields.
- Exposure measured was in range of expected Ceff: CSF exposure was 38-100nM

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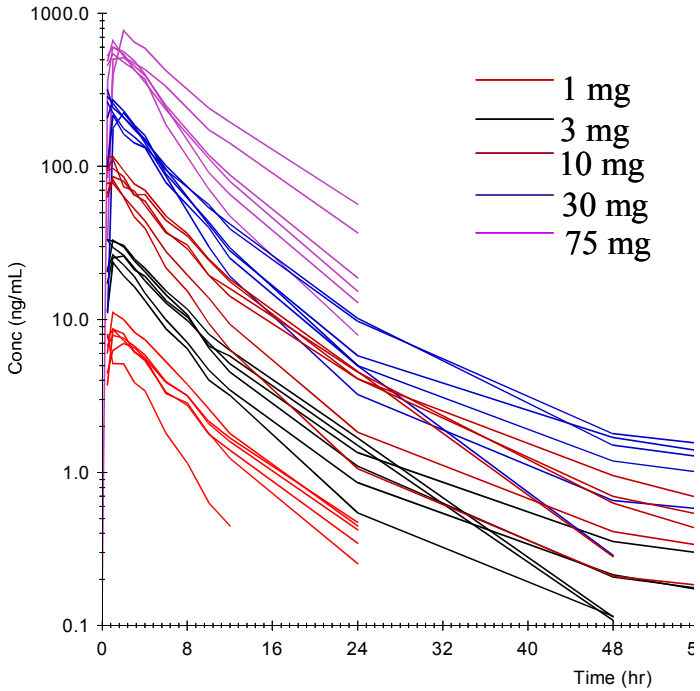
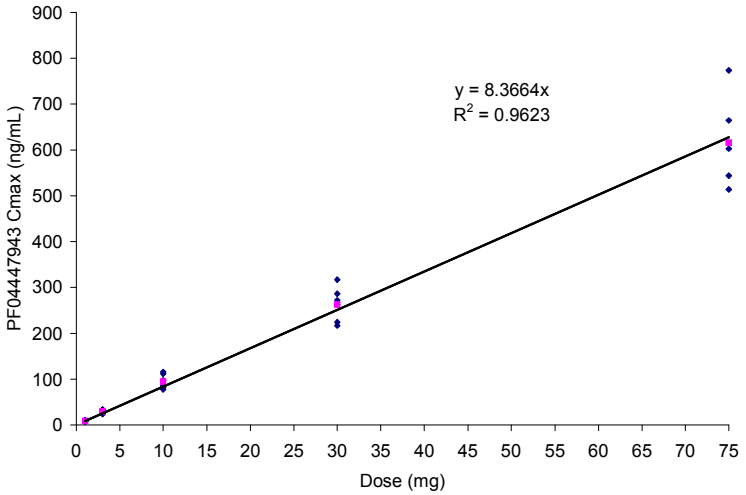
Single-Dose PK Results

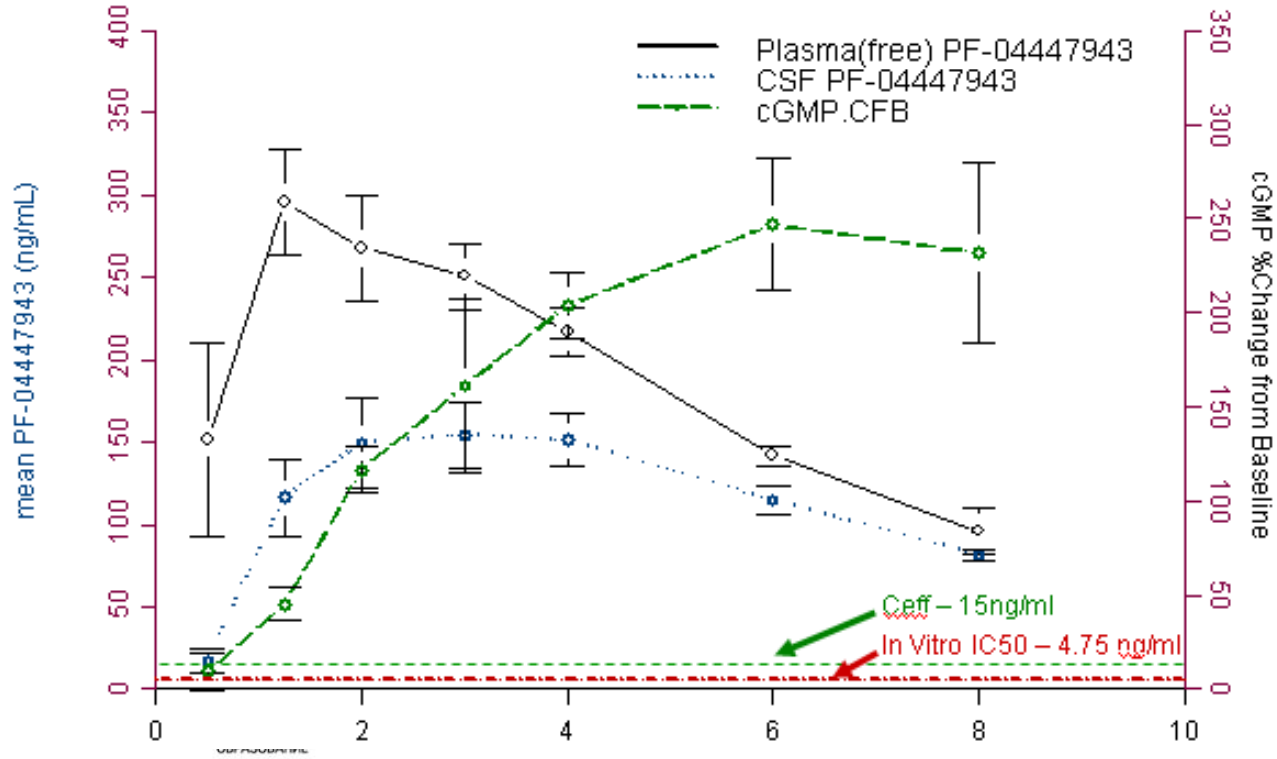
- **PF-4447943 is absorbed rapidly with median Tmax of 0.75 to 1 hr following a single oral dose of 1 to 75 mg PF-4447943**
- **Exposure of PF-4447943 (mean Cmax and AUC) following 1 to 75 mg single oral dose increased approximately proportionally with dose**
- **Human $T_{1/2}$ is 12+ hours**





PF-4447943 Clinical Phase 1 Results

- Well tolerated at all doses tested-no serious AEs
- Maximum dose tested in two week study was 35 mg BID
- Elevated CSF cGMP 250+% in humans (40 mg SD)
- Single dose CSF/Plasma AUC ratio is 0.63 (8 hours)
- PF-4447943 will enter a Phase 2 AD Trial



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