Quantum Mechanical Models of P450 Metabolism to Guide Optimization of Metabolic Stability

Optibrium Webinar 2015, June 17 2015

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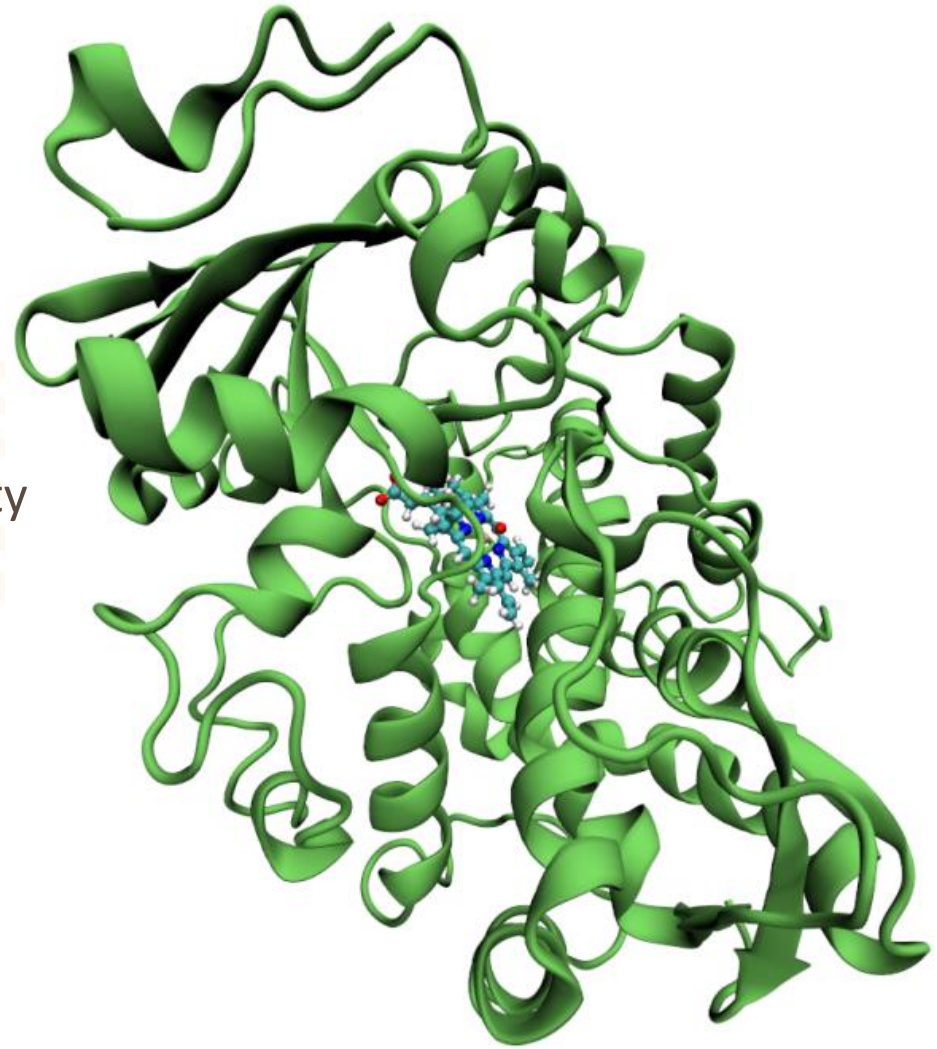
Overview

- Cytochrome P450
- Predicting P450 Metabolism
  - Reactivity - ‘electronic’ contributions
  - Accessibility - steric and orientation
- Site Lability
- Example Application 1
  - Finding stable and potent analogues of Buspirone
- Example Application 2
  - Fast follower: Focused library design for metabolic stability
- Conclusions

Cytochrome P450s

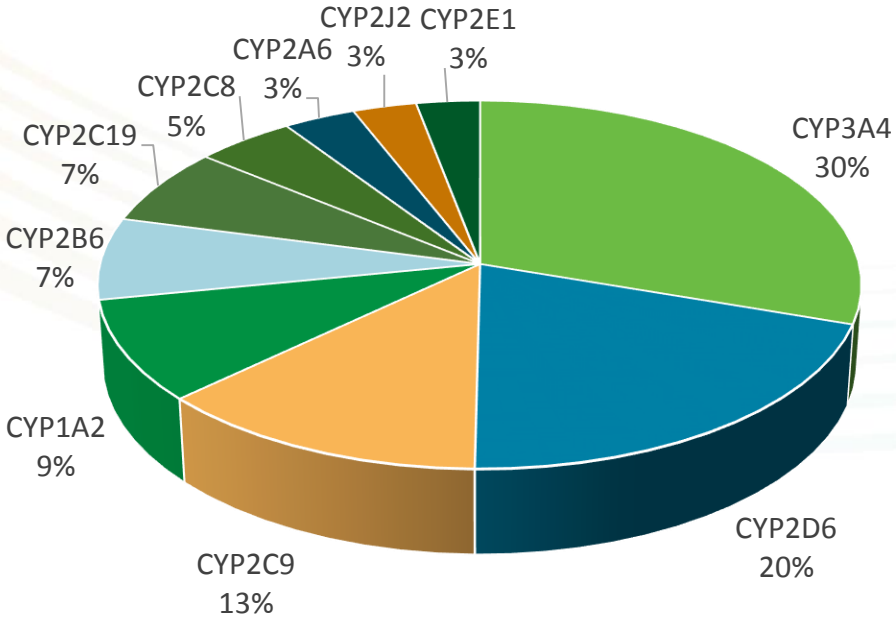
- Ubiquitous superfamily of haem-containing monoxygenase enzymes



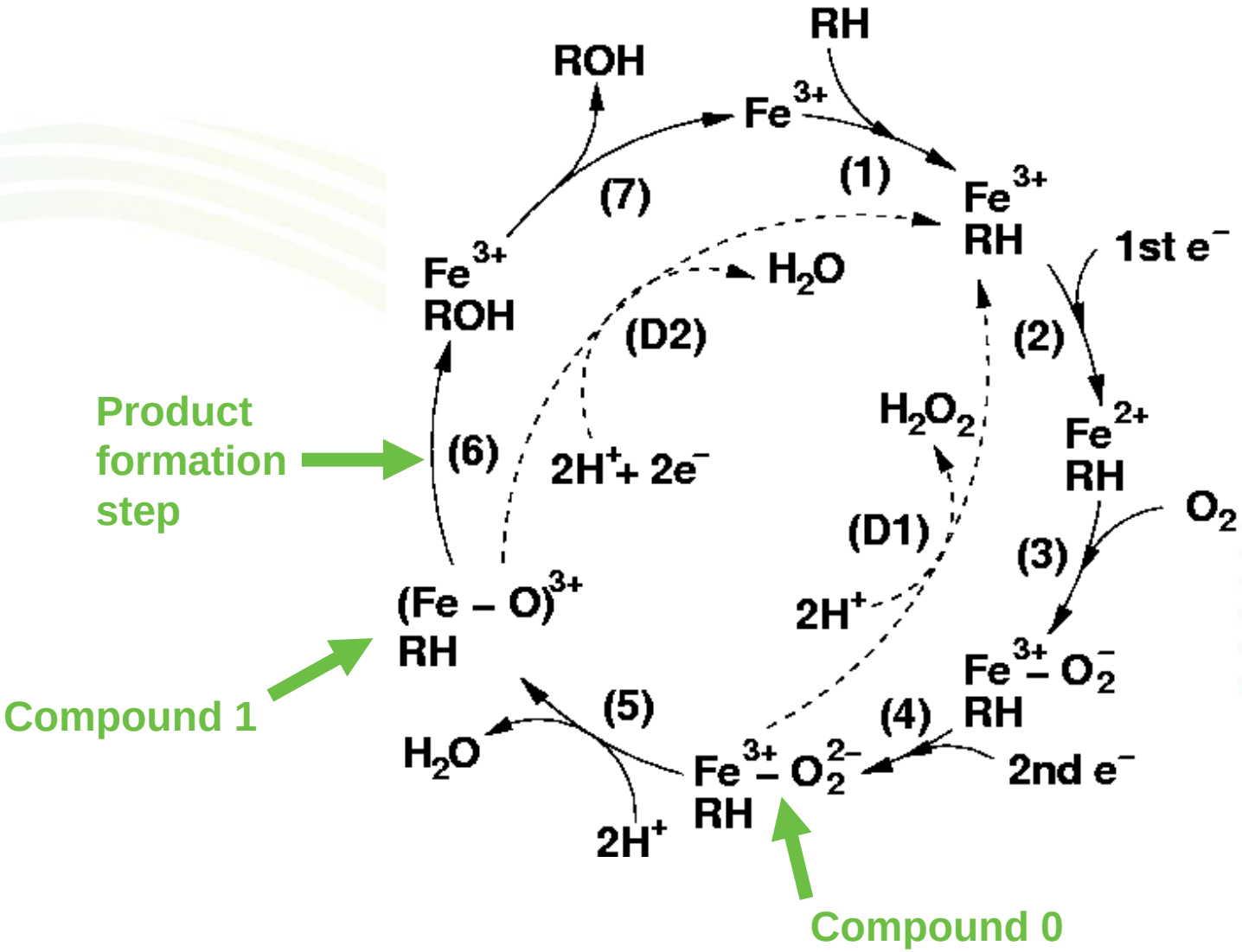
- Responsible for ~70-80% of drug metabolism, leading to:
  - Rapid clearance or low bioavailability
  - Potential for drug-drug interaction
  - Impact of P450 polymorphism
  - Bioactivation to form reactive/toxic metabolites

Cytochrome P450s

- Primary isoforms responsible for drug metabolism in human
- Insertion of oxygen into substrate
  - increase hydrophilicity
  - facilitate secondary metabolism
  - facilitate excretion



P450 Catalytic Cycle



Shaik et al, Chemical Reviews, 110, 949-1017 (2010)

Predicting P450 Metabolism

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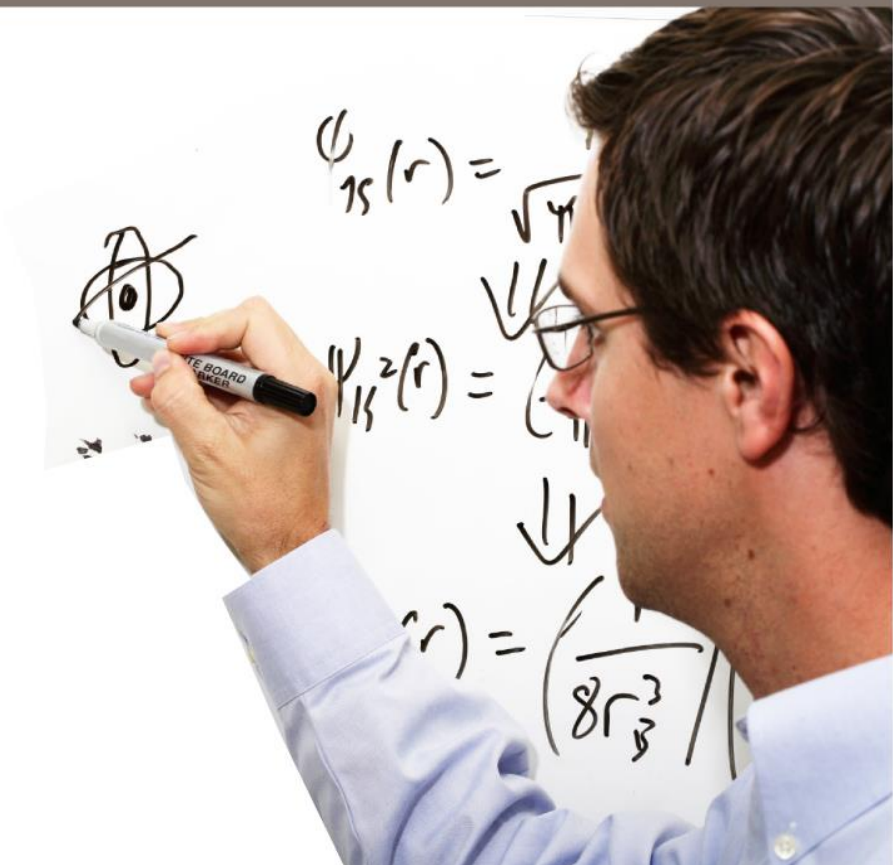


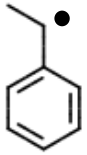
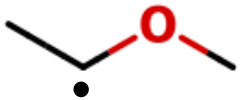
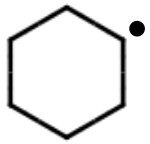
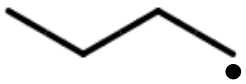
Methods

Two primary factors determine the site of metabolism:

- Electronic properties of substrate
  - H abstraction – aliphatic oxidation, N-dealkylation, O-dealkylation
  - Direct oxidation – aromatic oxidation, epoxidation, N oxidation, S oxidation
  - Activation barrier to abstraction of H and direct oxidation
  - Independent of isoform
- Orientation of substrate in active site
  - Dependent on isoform and substrate
  - Freedom to move
  - Electrostatic interactions with between protein and substrate
  - Steric accessibility

Electronic Effects

Trends in Metabolism Correlate with Radical Stability

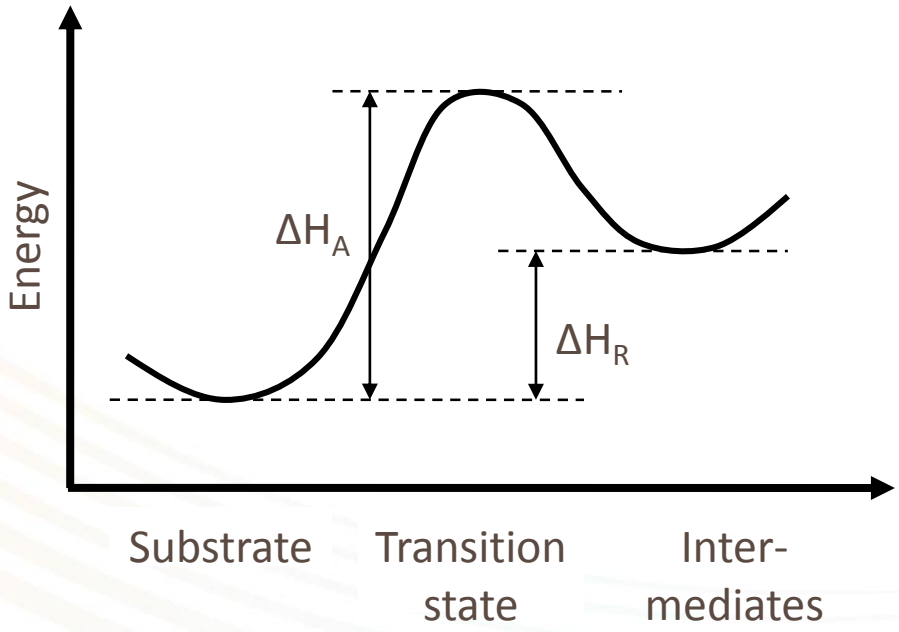
Radical	$\delta\Delta H_f$ (kcal/mol)	Reaction Type
	17.3	N-dealkylation
	19.6	benzylic hydroxylation
	26.6	O-dealkylation
	27.7	aliphatic hydroxylation
	28.6	aliphatic hydroxylation
	33	ω-hydroxylation

Increasing occurrence of metabolism

Korzekwa, Jones and Gillette, J. Am. Chem. Soc., **112**, p. 7042 (1990)

Electronic Models for CYP Reactivity

- Semi-empirical QM methods used to calculate energies of substrate and reaction intermediates
- Brönsted relationship to generate activation energy
- Site considered in context of molecular environment
  - Not considered as a discrete uniform entity
  - Subtle longer range effects can be captured
  - Important when developing a lead series



$$\Delta H_A \propto \Delta H_R$$

Korzekwa, Jones and Gillette, J. Am. Chem. Soc., **112**, p. 7042 (1990)

Electronic Models for CYP Reactivity

- Energy relationships have been developed to predict activation energies for oxidation reactions
  - Hydrogen atom abstraction
  - Aromatic oxidation
  - S-oxidation
  - N-oxidation
  - Epoxidation
  - ….
- Models have been parameterized with:
  - Experimental data*
  - *Ab initio* calculations†

*Jones, Mysinger & Korzekwa, Drug Metab. Dispos., **30**(1) p. 7 (2002)

†Rydberg *et al.* ACS Med. Chem. Lett. **1** p. 96 (2010)

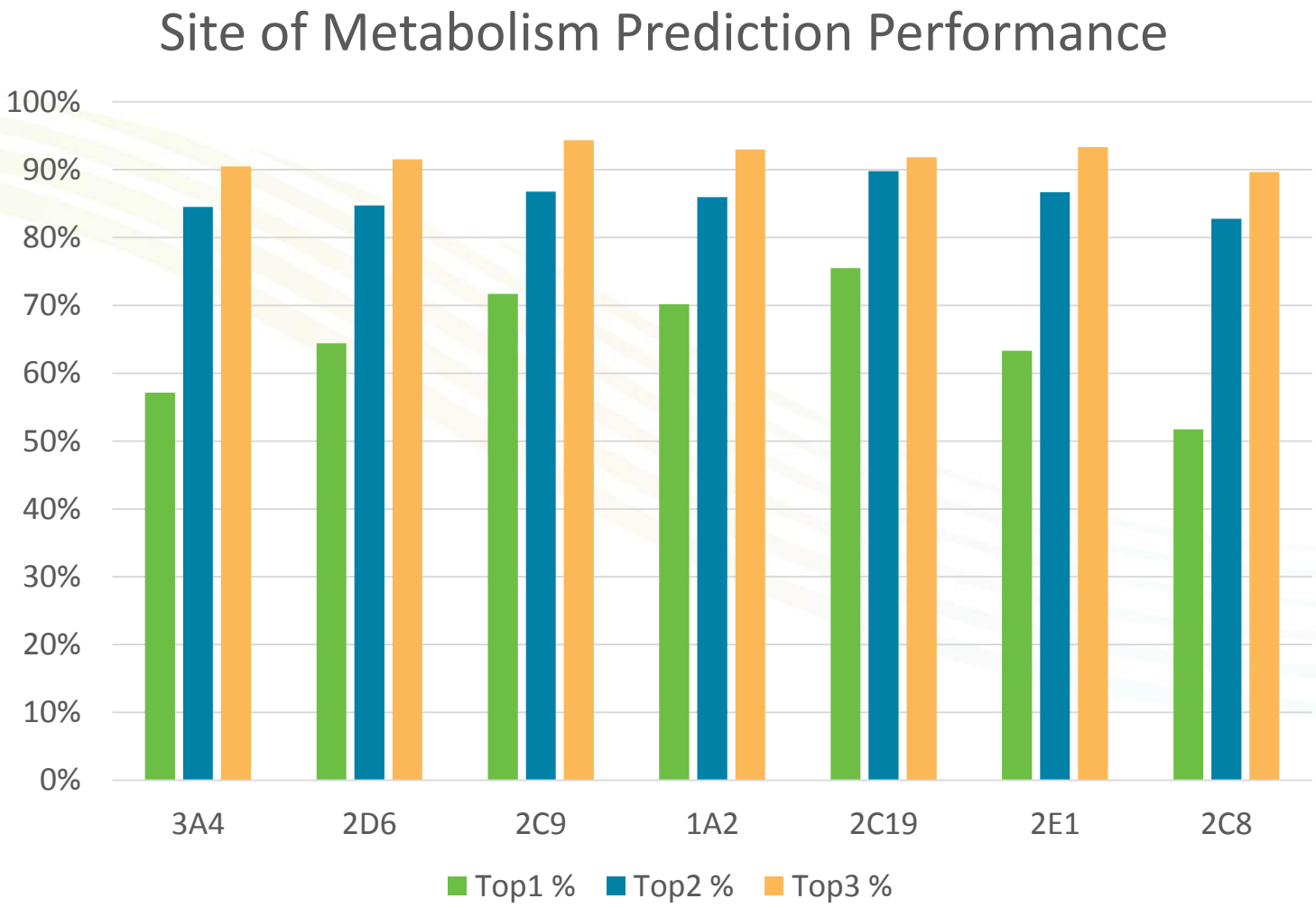
Steric and Orientation Effects

- Binding within active site restricts the accessibility of sites to the active oxy-haem species
- Structure of substrate introduces steric hindrance
- Corrections to activation energy estimated with statistically trained model using 2D descriptors, including
  - Distances to charged functionalities, H-bond acceptors/donors, lipophilic groups
  - Distances to rings, flexible linkers, 'bulky' groups
- Trained and tested using high-quality regioselectivity data sets carefully curated from the literature

Isoform	Number of Molecules
3A4	305
2D6	202
2C9	193
1A2	201
2C19	184
2E1	105
2C8	106

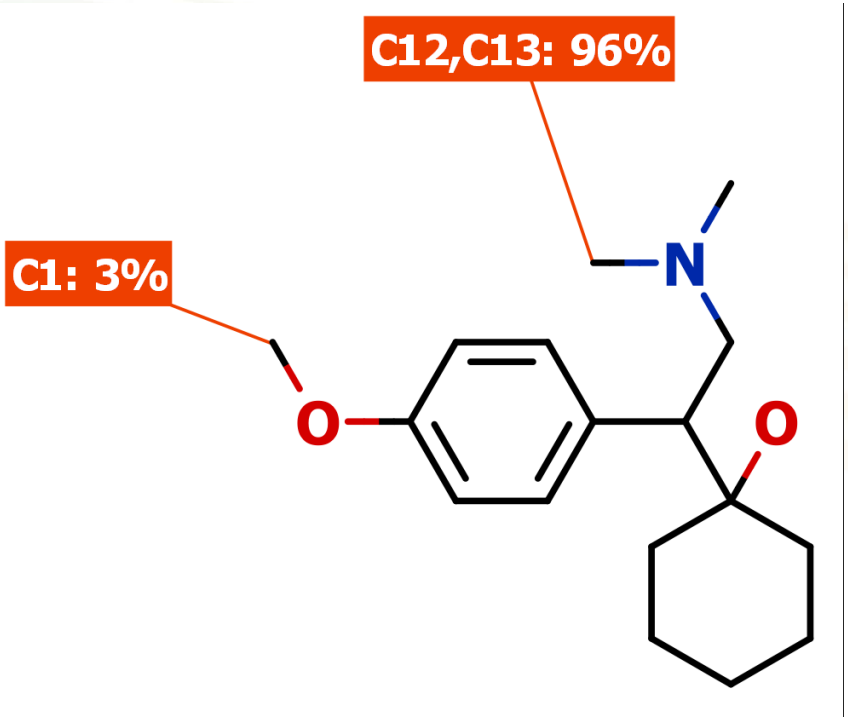
Validation

Independent test sets of 30% of data

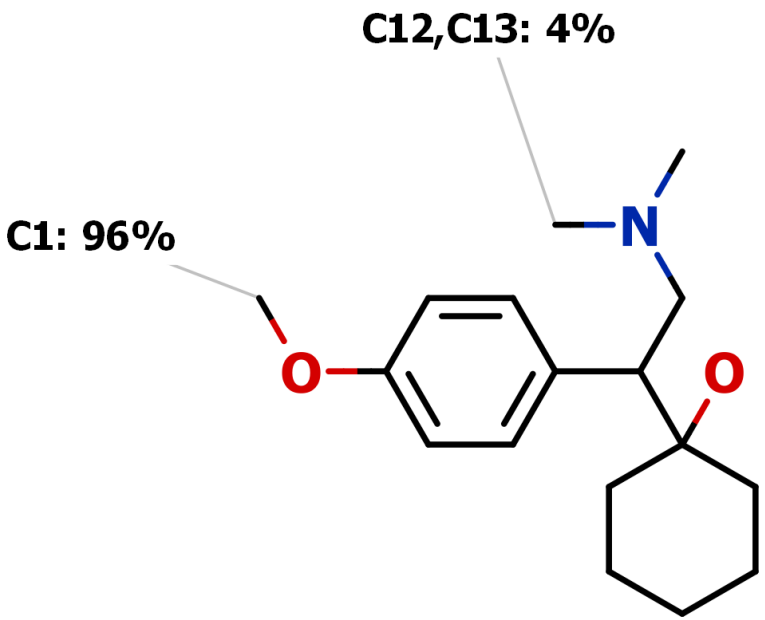


Example Regioselectivity Prediction
Venlafaxine

CYP3A4



CYP2D6



Site Lability

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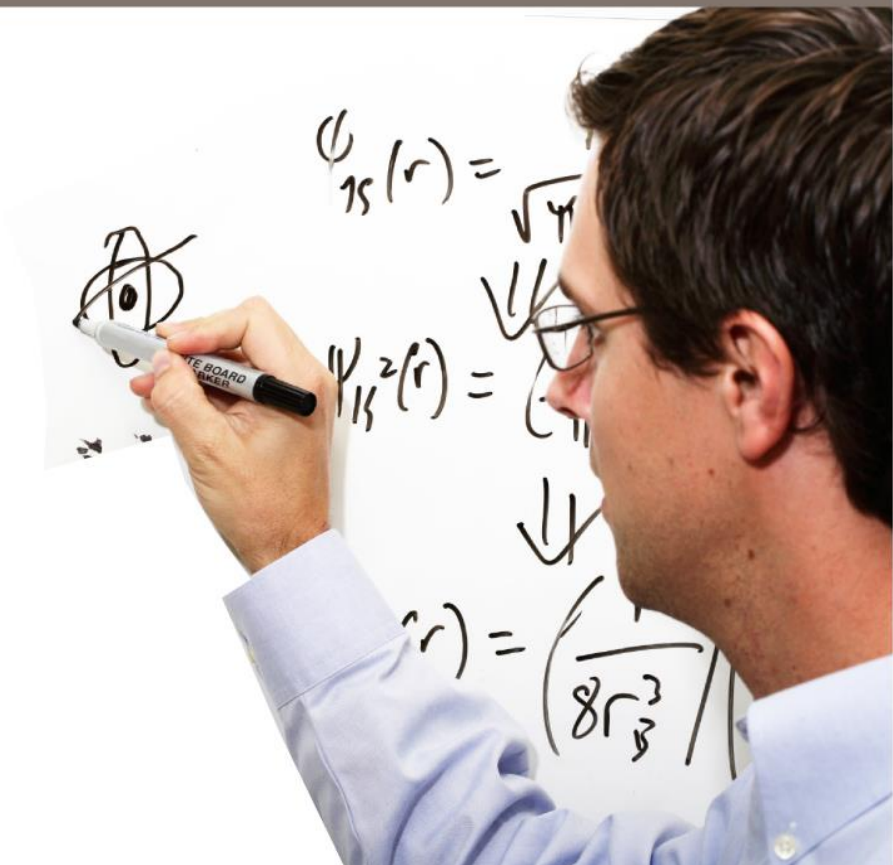
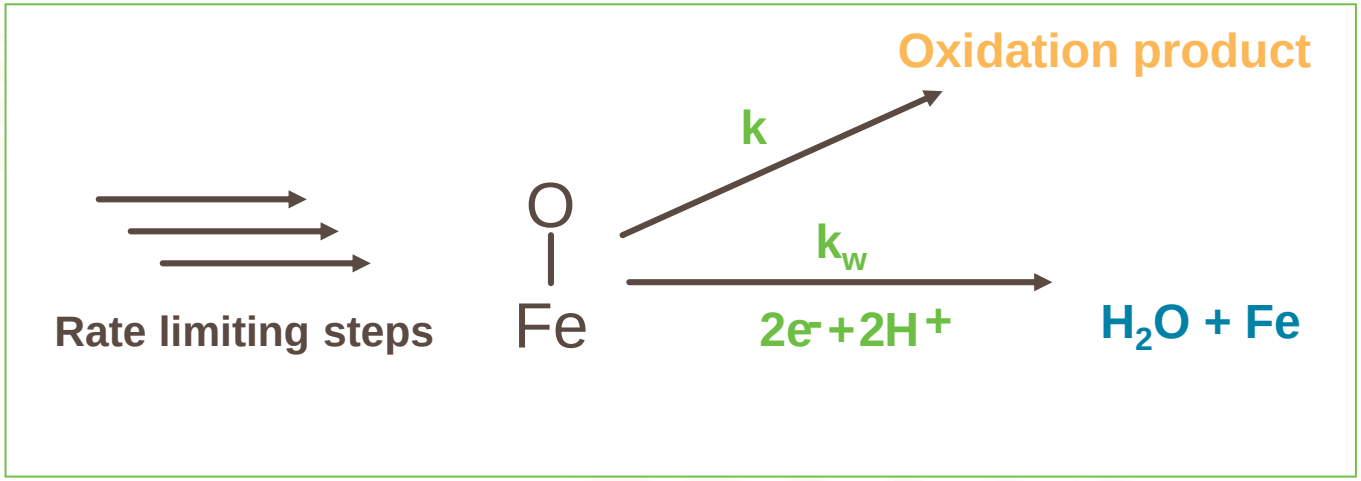


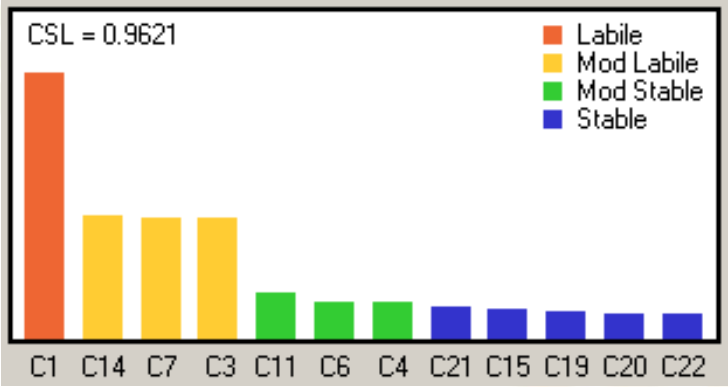
P450 Metabolic Lability



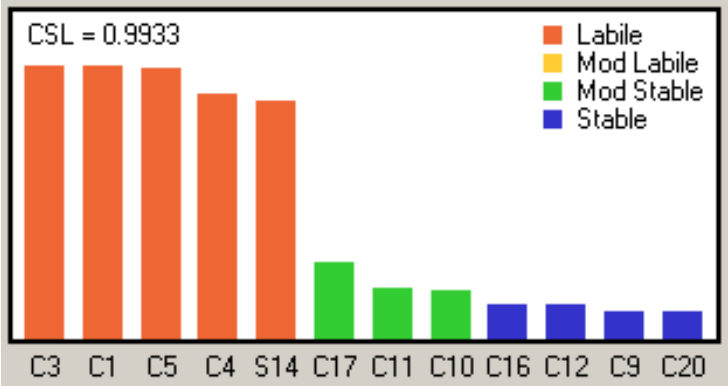
- Oxidation of a site on the molecule is in competition with water formation (and deactivation of the P450 active site).
- Site lability is a measure of how easily a site is oxidised compared to water formation, governing the efficiency of product formation.

P450 Metabolic Landscape

A



B



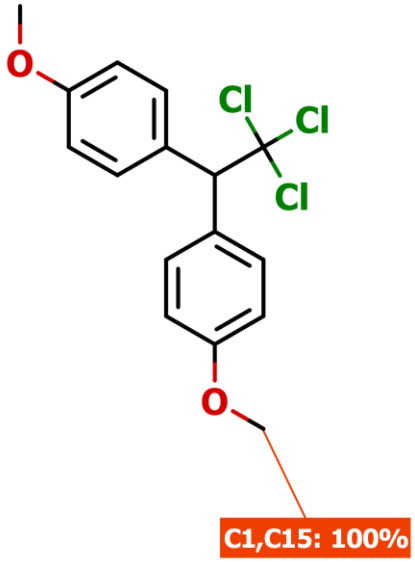
- This output indicates how **vulnerable** a molecule is to metabolism by CYP3A4, **if** it binds as a substrate
- Which compound is a better opportunity for optimisation?

Composite Site Lability (CSL)

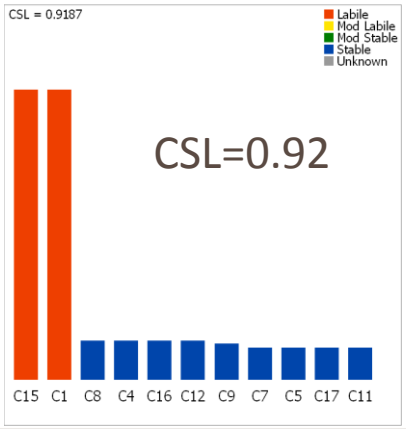
- The **Composite Site Lability** is a measure of the efficiency of metabolism of a molecule by CYP3A4
- CSL varies between 0.0 and 1.0
  - Lower values imply greater metabolic stability
- A **labile site** on a molecule may need modification to improve its stability
- Site lability is an important factor affecting rate of metabolism, but other factors are important
  - E.g. binding affinity, reduction rates (type I and type II binding)

Example
Methoxychlor

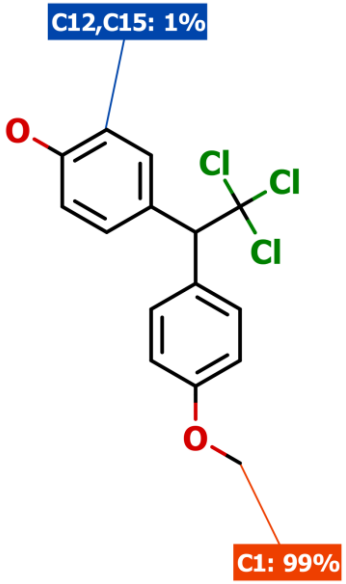
Methoxychlor



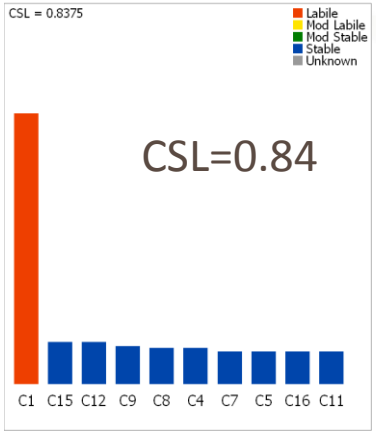
3A4 Metabolic Landscape



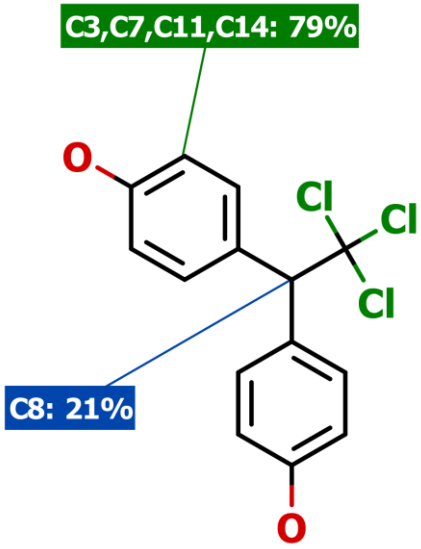
Mono-hydroxy methoxychlor



3A4 Metabolic Landscape



Bis-hydroxy methoxychlor



3A4 Metabolic Landscape

Example Application

Finding stable, potent analogues of Buspirone

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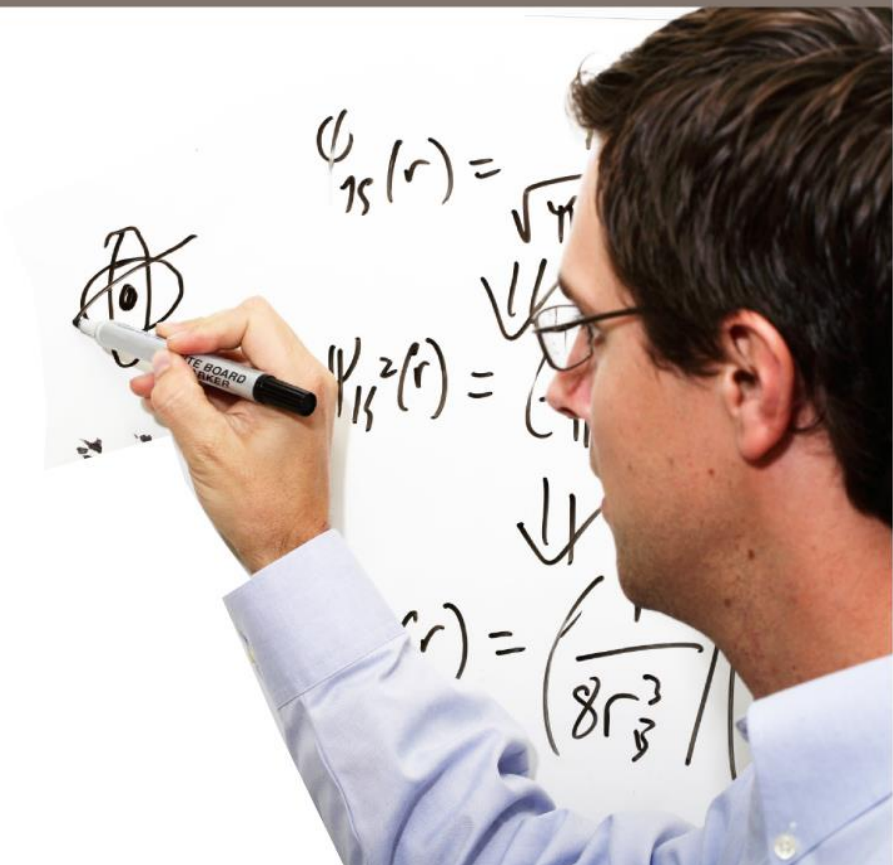
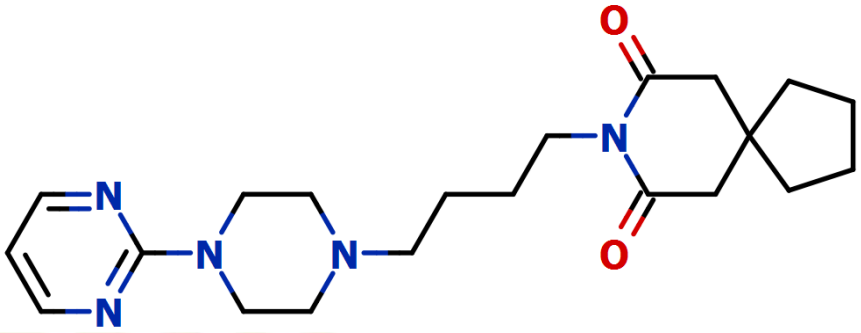


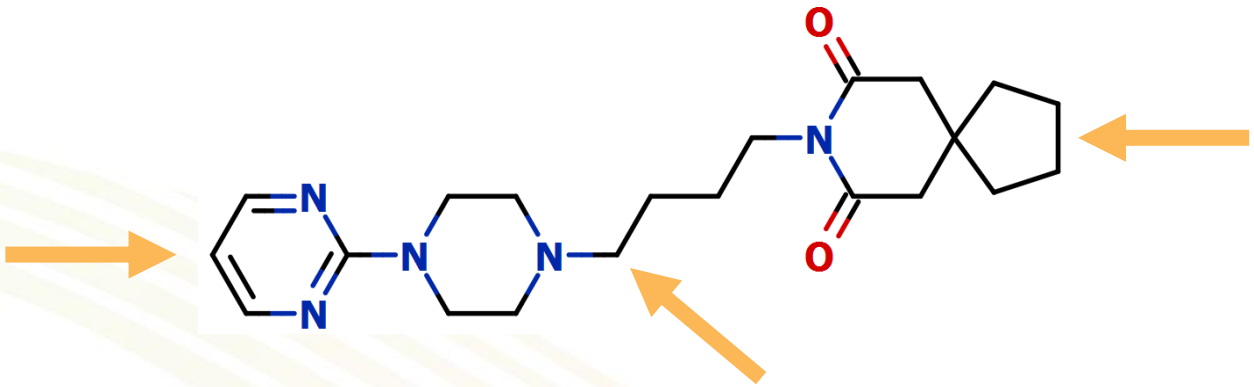
Buspirone



- Anti-anxiolytic drug, 5-HT_{1A} ligand
  - Receptor affinity: IC_{50} = 25 nM (pIC_{50} = 7.6)
- Poor oral bioavailability (4%)
  - Due to metabolism by P450 CYP3A4
  - *In vitro* CYP3A4 stability: $t_{½}$ = 4.6 minutes
- Goal: Identify buspirone analogue with
  - Improve *in vitro* CYP3A4 stability >3-fold: $t_{½}$ = 15 minutes
  - Retain receptor affinity IC_{50} < 250 nM (pIC_{50} > 6.6)

 M. Tandon et al. Bioorg. Med. Chem. Lett. **14** p. 1709 (2004)

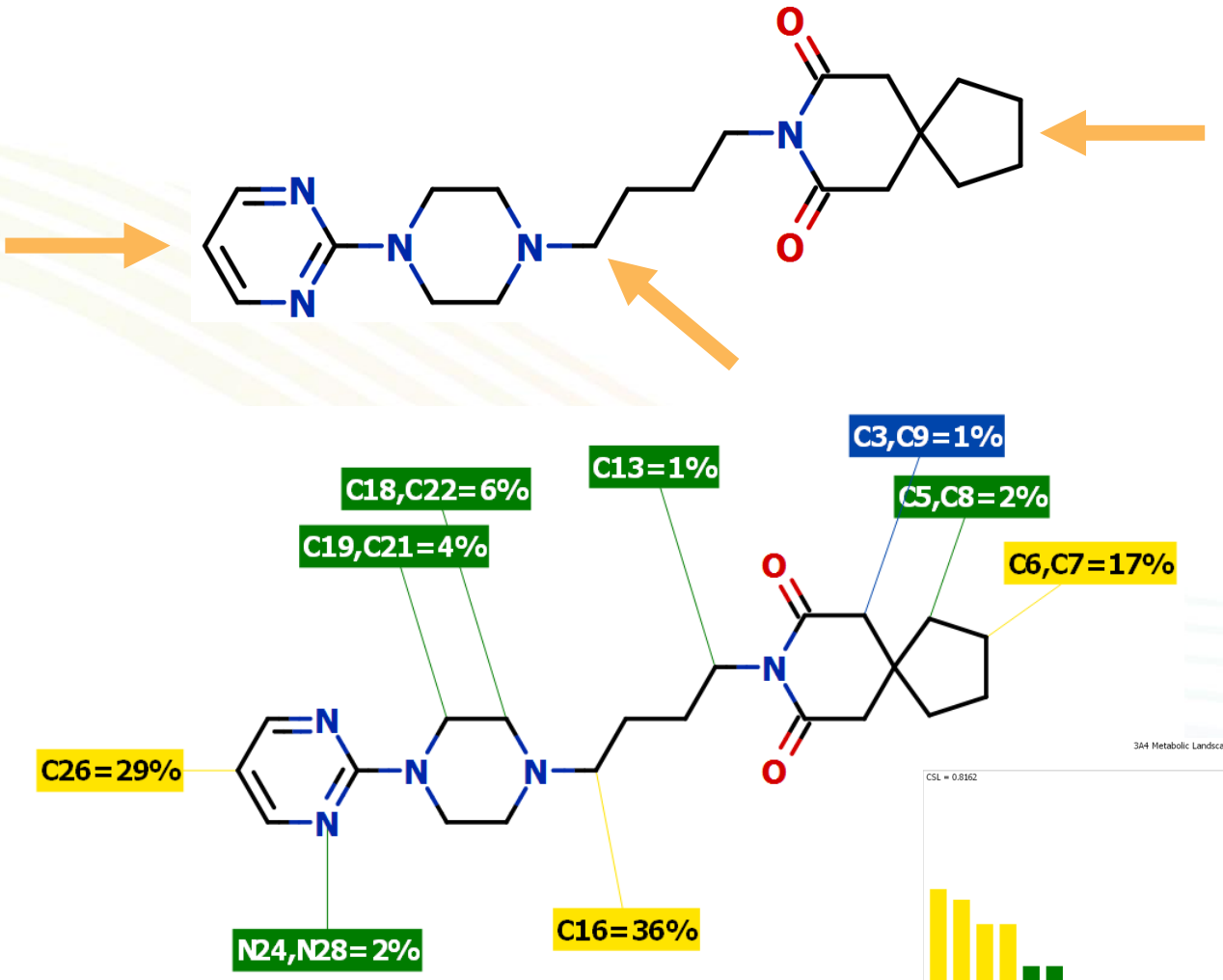
Buspirone Metabolism



- Hydroxylation at pyrimidine C_5
- N-dealkylation α to piperazine N_4
- Oxidation of spirocyclopentane ring

 M. Tandon et al. Bioorg. Med. Chem. Lett. **14** p. 1709 (2004)

Buspirone Metabolism



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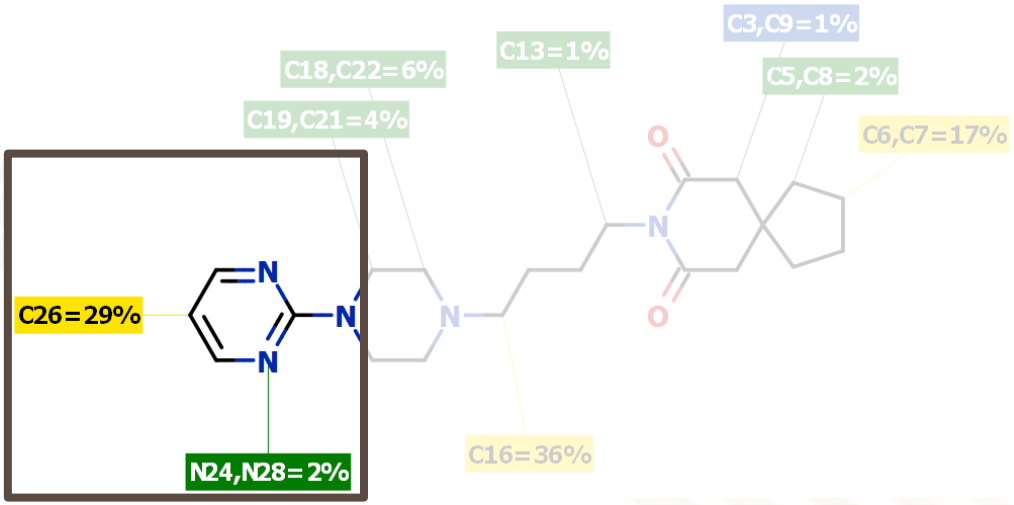
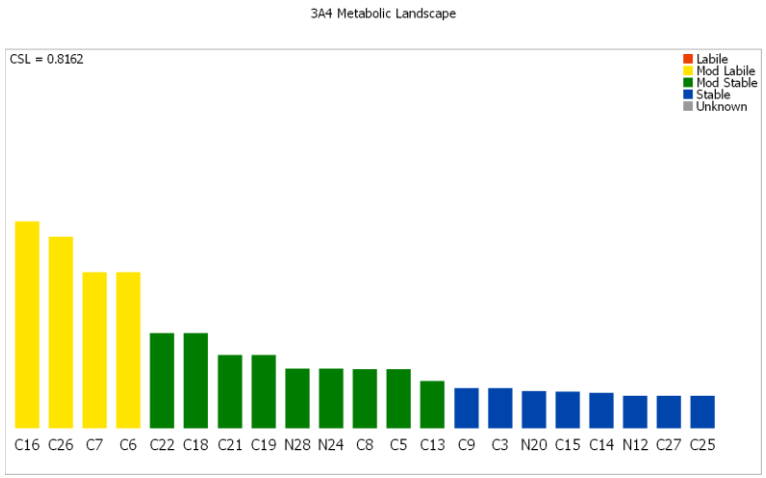
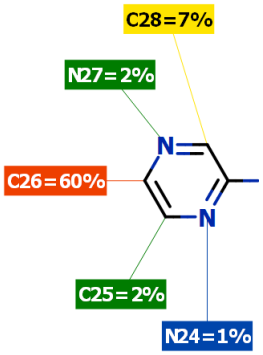
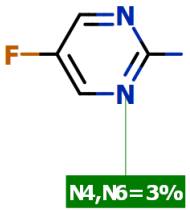
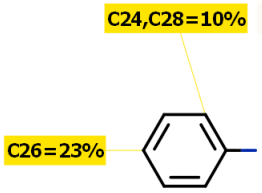
Strategies for Optimising Stability
Arylpiperazine moiety

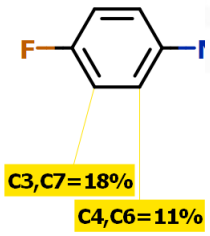


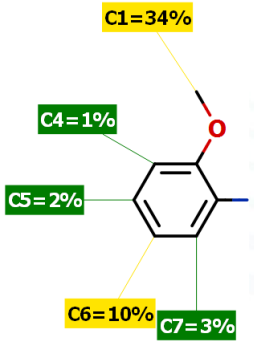


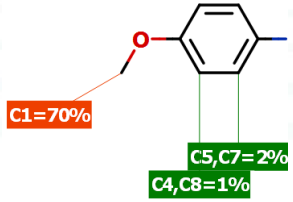


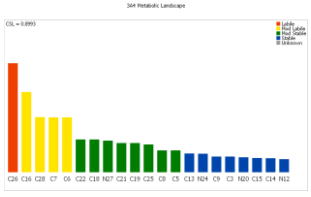


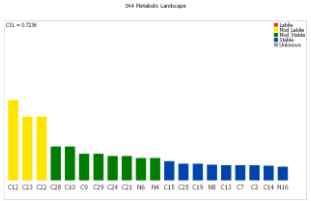


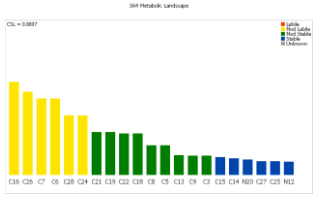


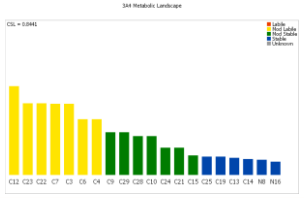


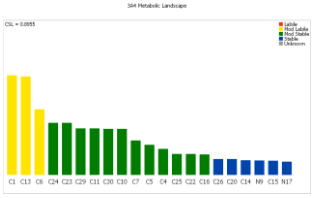


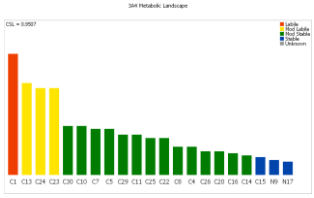






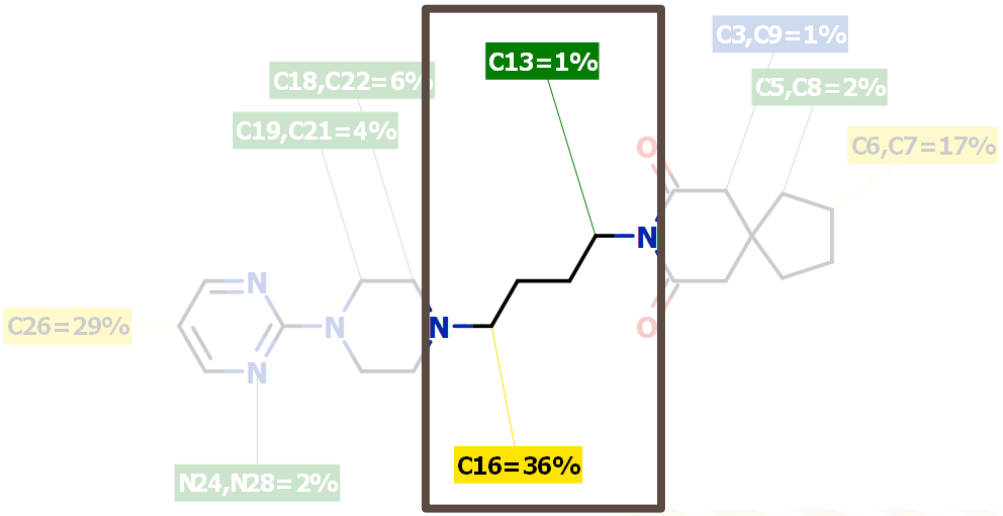


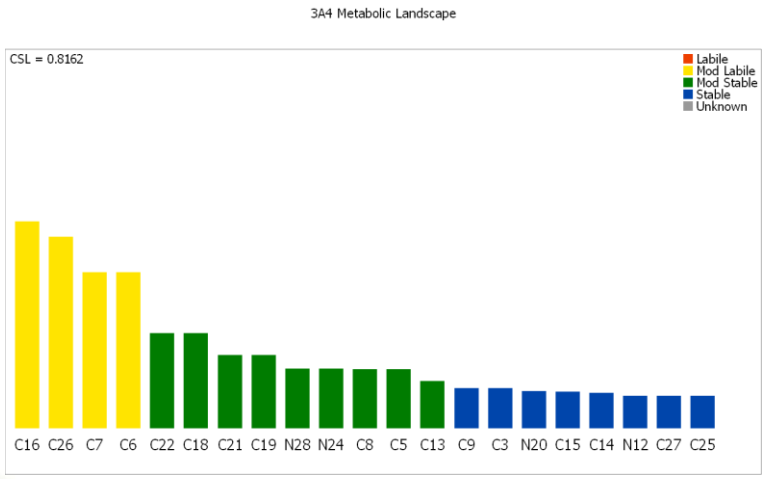


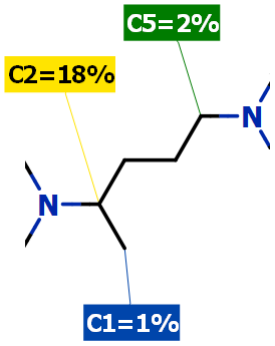


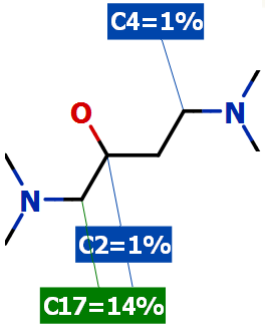
M. Tandon et al. Bioorg. Med. Chem. Lett. **14** p. 1709 (2004)

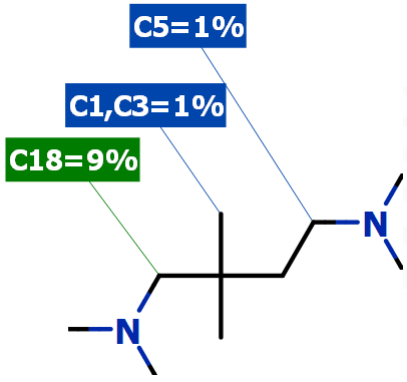
Strategies for Optimising Stability
Tetramethyline linker

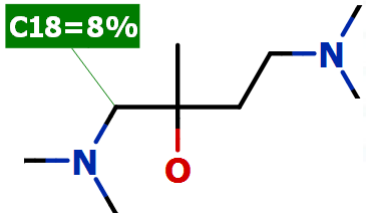


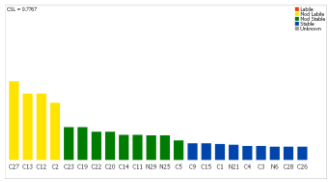


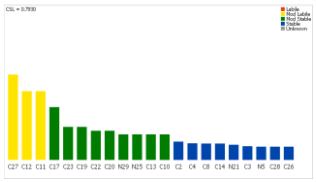


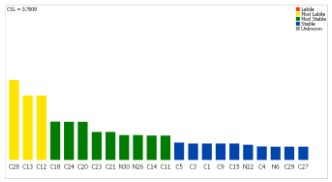


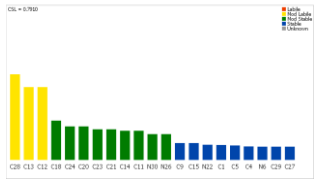












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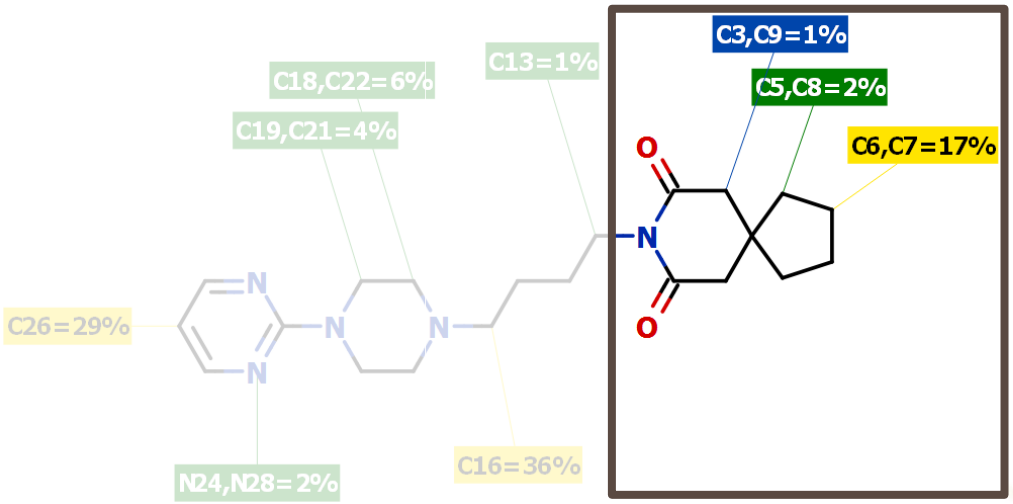
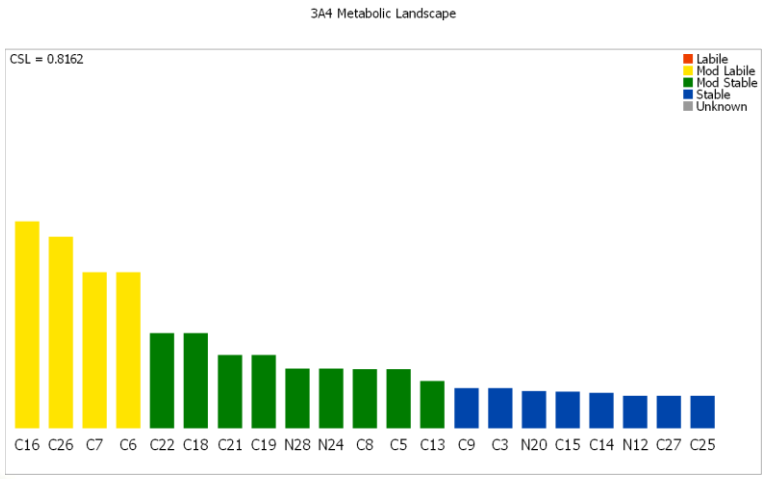
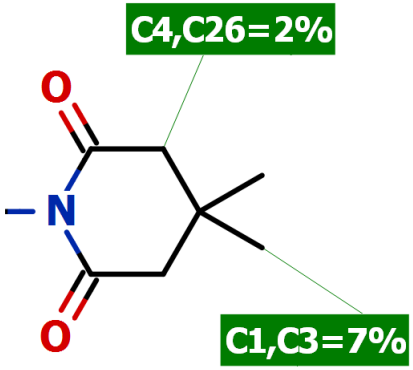
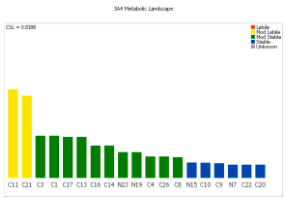
Strategies for Optimising Stability
Piperidinedione moiety









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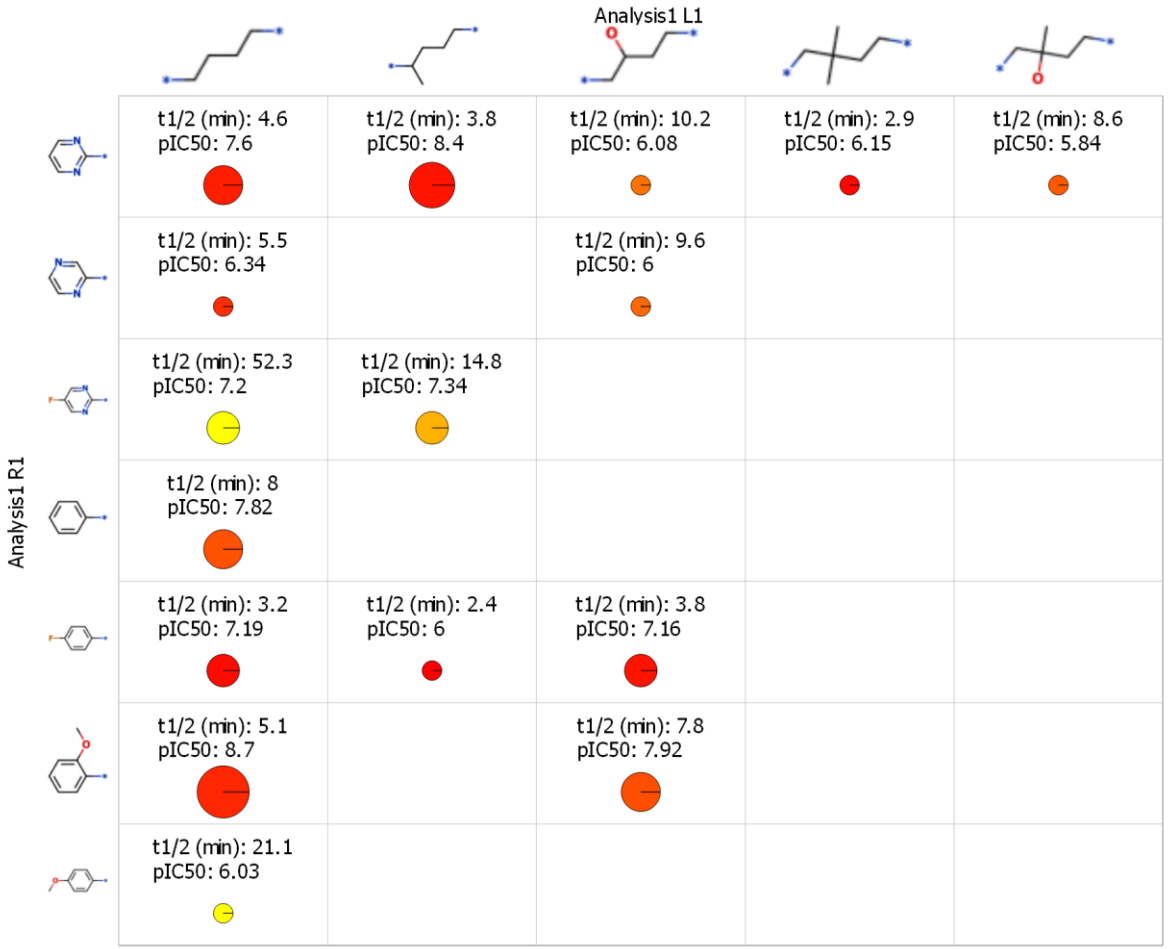
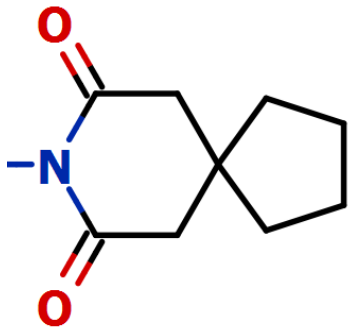
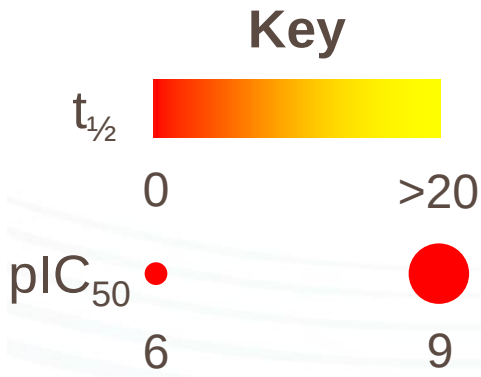
SAR

3,3-tetramethyleneglutarimide







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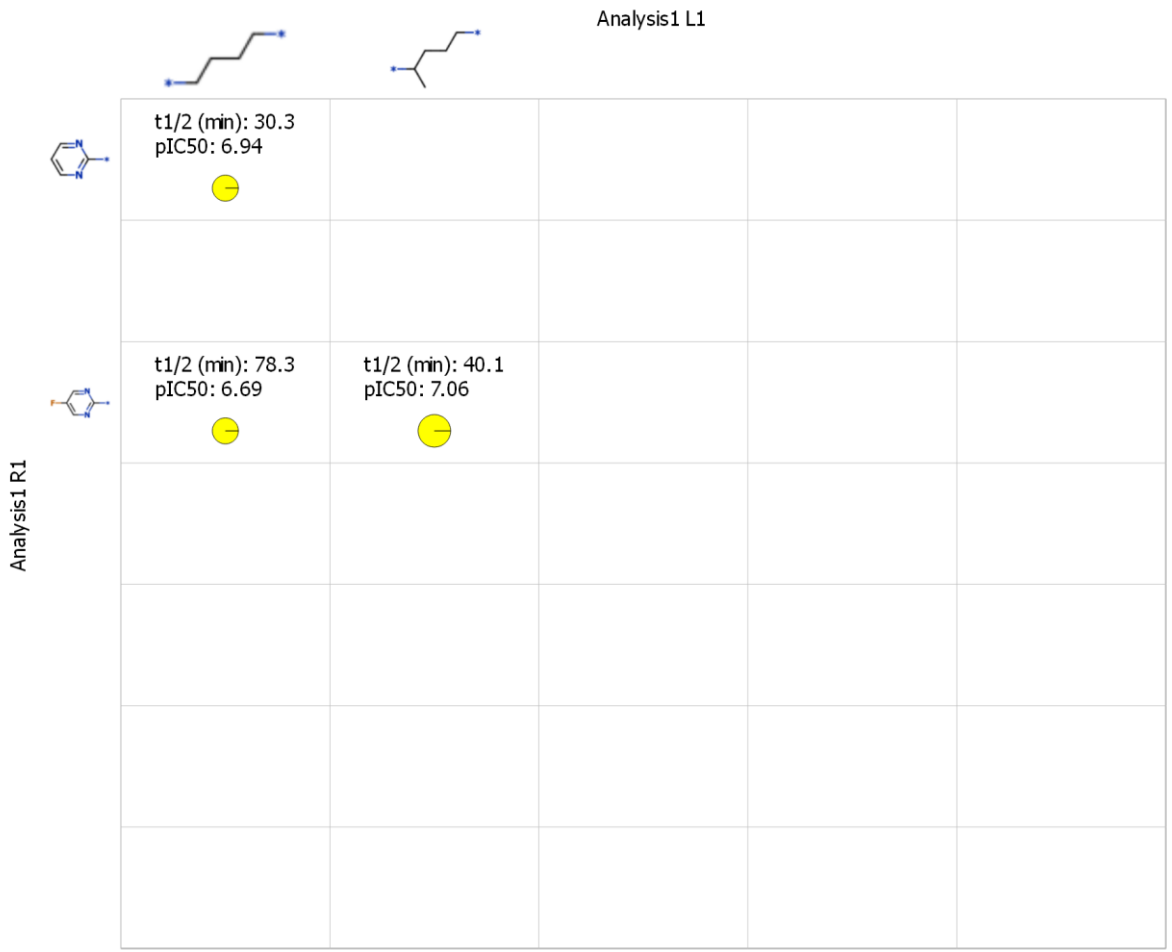
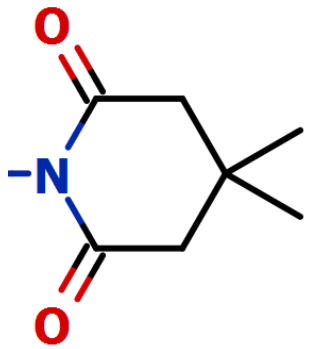
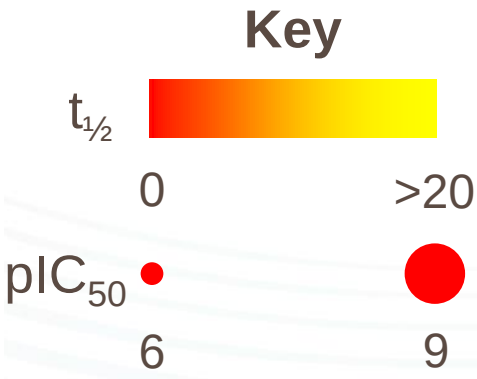
SAR
4,4-dimethylpiperidine-2,6-dione







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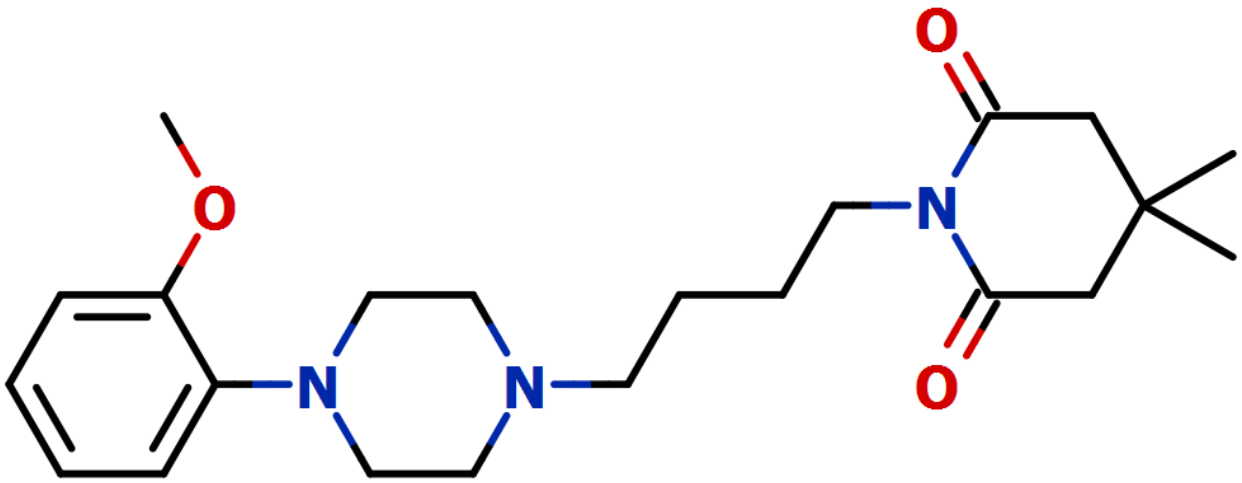
Outcome
'Best' compound



- Stability improved by ~10×
  - $t_{½}$ = 43 min
- Potency improved by ~10×
  - IC_{50} = 2 nM

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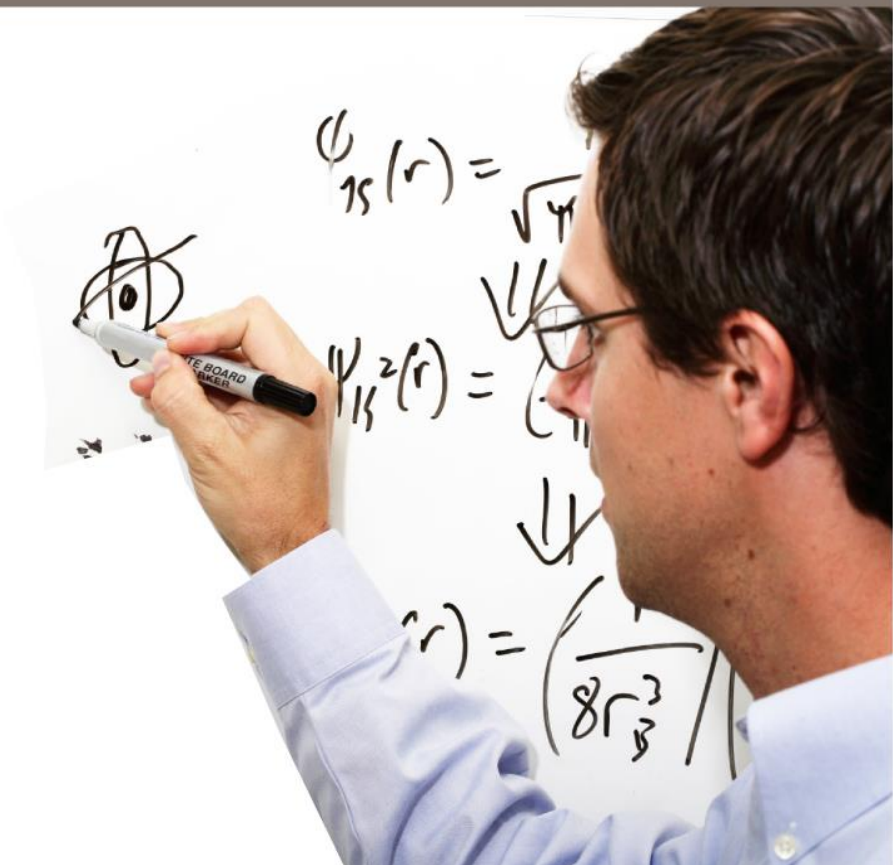
Example Application

Fast follower: Focused library design for metabolic stability

optibrium



Project Overview

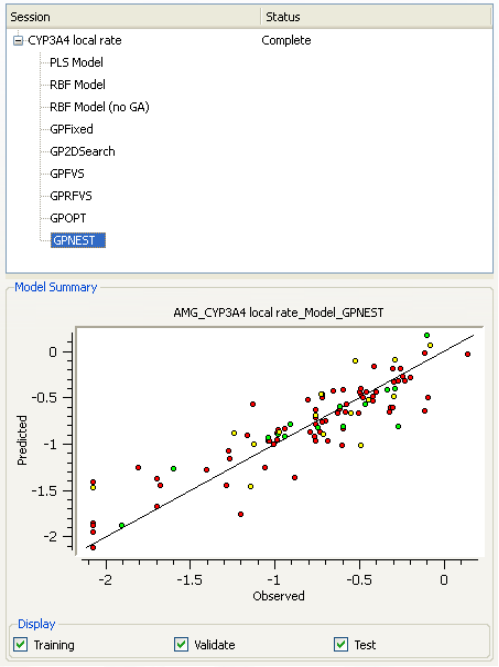
- Fast follower for existing drug with issues:
  - Poor oral bioavailability
  - Short and variable half-life
- Issues caused by rapid metabolism by CYP3A4
- Project Goal: Identify lead series for fast follower with
  - >2× half-life with respect to CYP3A4 metabolism *in vitro*
  - Maintain potency <200 nM against primary target
- Project strategy
  - Initial virtual library of 13,000 compounds exploring core replacements
  - Phase I: Exploration of library and model building
  - Phase II: Application of models to focus on high quality leads

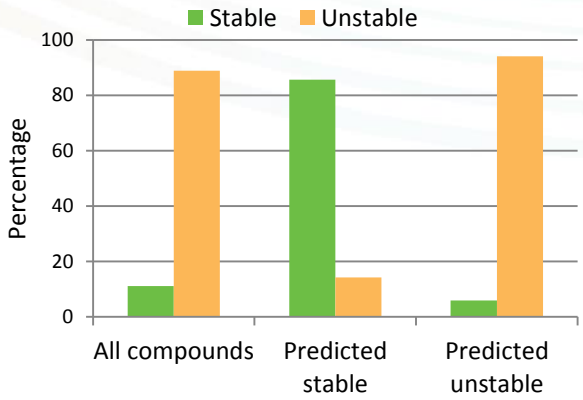
Phase I

- Selected 100 compounds with range of properties
  - Synthesized and tested for *in vitro* potency and CYP3A4 stability
- Results
  - 70% met criterion for potency
  - 11% met criterion for CYP3A4 stability
  - Only 3% met both criteria
- Used data to build models of CYP3A4 stability and potency

Modelling of CYP3A4 Stability

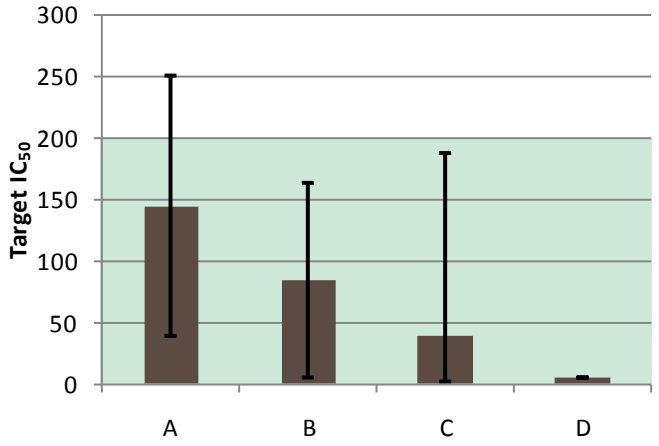
- StarDrop Auto-Modeller used to build model of CYP3A4 rate (log k)
- Descriptors used:
  - Composite site lability (CSL) from P450 models
  - logP
  - log of neutral fraction at pH7.4
- Best model:
  - $R^2 = 0.66$ ($r^2_{corr} = 0.72$)
  - High specificity for stable compounds

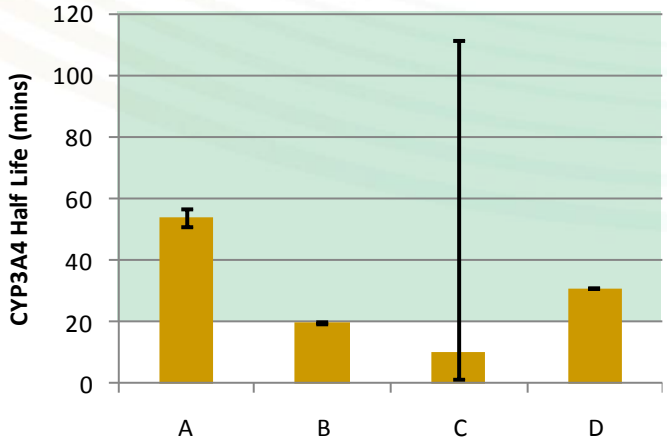




Phase II

- Applied models to full library of 13,000 compounds
- 40 compounds predicted to be both stable and potent
- Synthesized and tested *in vitro* for potency and stability
- Result: 4 lead series





Outcome

- 140 compounds synthesized in two iterations
- Identified four high quality lead series
  - Potent
  - Increased stability with respect to CYP3A4 metabolism
  - All predicted to be soluble, absorbable and pass blood-brain-barrier

Conclusions

- Prediction of P450 metabolism can help to guide the design of compounds with improved metabolic stability
- Predicting sites of metabolism is useful but not sufficient
- QM approaches can be used to estimate lability on an absolute scale
  - With corrections for steric accessibility and orientation
  - Sites considered in their molecular environment
- Acknowledgement
  - This research has received funding from the Union Seventh Framework Programme 2013 under the grant agreement no602156

HECATOS