Predicting Regioselectivity and Lability of Cytochrome P450 Metabolism using Quantum Mechanical Simulations

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Overview

• Cytochrome P450

• Predicting P450 Metabolism
  – ‘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
- Primary isoforms responsible for drug metabolism in human

Zanger and Schwab, Pharmacol. & Therapeut. 138(1) p. 103 (2013)
P450 Catalytic Cycle

Product formation step

1. Fe$^{3+}$ to Fe$^{2+}$
2. Fe$^{2+}$ to Fe$^{3+}$
3. Fe$^{3+}$ to Fe$^{2+}$
4. Fe$^{2+}$ to Fe$^{3+}$
5. Fe$^{3+}$ to Fe$^{2+}$
6. 2H$^+$ + 2e$^-$ to H$_2$O
7. ROH to RH
Predicting P450 Metabolism
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
  - Electrostatic interactions with between protein and substrate
  - Freedom to move
  - Steric accessibility
# Electronic Effects

## Trends in Metabolism Correlate with Radical Stability

<table>
<thead>
<tr>
<th>Radical</th>
<th>$\delta\Delta H_f$ (kcal/mol)</th>
<th>Reaction Type</th>
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<tr>
<td>![N radical]</td>
<td>17.3</td>
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<tr>
<td>![benzylic radical]</td>
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<td>benzylic hydroxylation</td>
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<tr>
<td>![O radical]</td>
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<tr>
<td>![aliphatic radical]</td>
<td>27.7</td>
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<td>![aliphatic radical]</td>
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<tr>
<td>![ω-hydroxylation radical]</td>
<td>33</td>
<td>ω-hydroxylation</td>
</tr>
</tbody>
</table>

Increasing occurrence of metabolism

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Electronic Models for CYP Reactivity

- Semi-empirical QM methods used to calculate energies of substrate and reaction intermediates
- Brönsted relationship to generate activation energy
- Fragment 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

\[ \Delta H_A \propto \Delta H_R \]
Electronic Models for CYP Reactivity

- Free 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)
Steric and Orientation Effects

• Binding within active site restricts the accessibility of sites to the active oxy-haem species

• Structure of ligand 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
Validation
Independent test sets of 30% of data

Site of Metabolism Prediction Performance

[Bar chart showing the prediction performance for different sites with top 1%, top 2%, and top 3% accuracy rates for sites 3A4, 2D6, 2C9, 1A2, 2C19, 2E1, and 2C8.]
Metabolite Structure Generation

- SMIRKS patterns used to generate metabolite structures, e.g.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>SMIRKS</th>
</tr>
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<tbody>
<tr>
<td>Aliphatic hydroxylation</td>
<td>[C;X4:1][H:2]&gt;&gt;[C:1][O][H:2]</td>
</tr>
<tr>
<td>Aromatic hydroxylation</td>
<td>[c:1][H:2]&gt;&gt;[c:1][O][H:2]</td>
</tr>
<tr>
<td>N dealkylation</td>
<td>[#7:1][C:2][H])&gt;&gt;[#7:1][H].[C:2]=[O]</td>
</tr>
<tr>
<td>S oxidation</td>
<td>[#16:1] &gt;&gt; <a href="=%5BO%5D">#16:1</a></td>
</tr>
</tbody>
</table>

- Metabolites can be exported into a new data set for further analysis, e.g. activity, physicochemical properties, etc.
  - Output exact mass to aid metabolite ID experiments
Example Regioselectivity Prediction
Venlafaxine

CYP3A4

C1=7%

C12,C13=91%

CYP2D6

C1=88%

C17,C19=1%

C18=3%
Site 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.
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
Clozapine vs Amoxapine

CSL = 0.97

CSL = 0.84
Example Application
Finding stable, potent analogues of Buspirone
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$_{1/2} = 4.6$ minutes

- Goal: Identify buspirone analogue with
  - Improve CYP3A4 stability >3-fold: t$_{1/2} = 15$ minutes
  - Retain receptor affinity IC$_{50} < 250$ nM (pIC$_{50} > 6.6$)

• Hydroxylation at pyrimidine C₅
• N-dealkylation α to piperazine N₄
• Oxidation of spirocyclopentane ring
Buspirone Metabolism

Strategies for Optimising Potency

Arylpiperazine moiety

Strategies for Optimising Potency

Tetramethyline linker

Strategies for Optimising Potency
Piperidinedione moiety

### SAR

**3,3-tetramethyleneglutaramide**

<table>
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<th>Analysis R1</th>
<th>t1/2 (min)</th>
<th>pIC50</th>
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<td></td>
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<table>
<thead>
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<th>Analysis L1</th>
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<th>pIC50</th>
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</table>

**Key**

- **t1/2**
  - 0
  - >20
- **pIC50**
  - 6
  - 9

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SAR

4,4-dimethylpiperidine-2,6-dione

Key

Outcome

‘Best’ compound

- Stability improved by $\sim 10\times$
  - $t_{1/2} = 43$ min

- Potency improved by $\sim 10\times$
  - $IC_{50} = 2$ nM

Conclusions

• Models of P450 metabolism can accurately predict site of metabolism and metabolites

• Predicting sites of metabolism is useful but not sufficient for compound design

• QM approaches can be used to estimate lability on an absolute scale
  – With corrections for steric accessibility and orientation
  – Fragments considered in their molecular environment

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