Metabolic Activation and Idiosyncratic Drug Toxicity: By Avoiding Structural Alerts, Do We Mitigate Risks?

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Cause(s) of Attrition in Drug Discovery

- In the early 90’s, major cause of attrition was poor pharmacokinetics\(^1,2\)
  - Largely resolved via involvement of DM/PK groups at early stages of drug discovery (Exploratory/Lead development/candidate-seeking)

- Of late: lack of efficacy (achieving POM for novel targets) and **drug safety** are the leading causes of candidate attrition
  - Pharmacology tactics to counterbalance attrition
    - Better understanding of pharmacological targets
    - Incorporation of translational pharmacology (PK/PD, disease biomarkers, etc)
    - Probe concept (exploratory INDs, etc)
  - Tactics to counterbalance safety-related attrition arising from IADRs
    - ?

Safety-Related Attrition: Adverse Drug Reactions (ADRs)

• ADRs Contribute to patient morbidity and mortality
  – One of the most common causes for drug recalls or black box warning labels
    • Of a total of 548 drugs approved in the period from 1975-1999, 45 drugs (8.2%) acquired 1 or more black box warnings, 16 (2.9%) were withdrawn from the market

• ADR Classification
  – Type A ADRs: ~ 80% of ADRs fall in this category
    • Type A ADRs can be predicted from known drug pharmacology (e.g. Hemorrhage with anticoagulants)
    • Dose dependent – can be reversed with dose reduction
    • Generally identified in preclinical species (animal models of pharmacology)
  – Type B (Bizarre) or Idiosyncratic ADRs (e.g., hepatotoxicity, skin rashes, aplastic anaemia, agranulocytosis) – e.g., black box warning for “sulfonamides”
    • Unrelated to primary pharmacology
    • Dose independent (with the exception of some drugs) – can occur at any dose within the therapeutic range
    • Temporal relationship - Symptoms subsides after cessation of treatment; rapid onset upon re-challenge
      • Can be severe - maybe fatal - most common cause for drug withdrawal
      • Cannot be predicted from traditional toxicological studies in animals
      • Rare - frequency of occurrence - 1 in 10,000 to 1 in 100,000
        – Normally not observed until phase III or post launch
Associating a Functional Group with Adverse Drug Reactions

– Sulfonamides have achieved notoriety with respect to hypersensitivity (e.g., skin rashes)

**BLACK BOX WARNING**

FATALITIES ASSOCIATED WITH THE ADMINISTRATION OF SULFONAMIDES, ALTHOUGH RARE, HAVE OCCURRED DUE TO SEVERE REACTIONS, INCLUDING STEVENS-JOHNSON SYNDROME, TOXIC EPIDERMAL NECROLYSIS, FULMINANT HEPATIC NECROSIS, AGRANULOCYTOSIS, APLASTIC ANEMIA, AND OTHER BLOOD DYSCRASIAS. SULFONAMIDES, INCLUDING SULFONAMIDE CONTAINING PRODUCTS SUCH AS TRIMETHOPRIM/SULFAMETHOXAZOLE, SHOULD BE DISCONTINUED AT THE FIRST APPEARANCE OF SKIN RASH OR ANY SIGN OF ADVERSE REACTION. In rare instances, a skin rash may be followed by a more severe reaction, such as Stevens-Johnson syndrome, toxic epidermal necrolysis, hepatic necrosis, and serious blood disorder (see PRECAUTIONS). Clinical signs, such as rash, sore throat, fever, arthralgia, pallor, purpura, or jaundice may be early indications of serious reactions.

![Chemical structures of Sulfamethoxazole and Trimethoprim](image)
The Concept of Xenobiotic Bioactivation to Reactive Metabolites (RMs)

Origins in the field of chemical carcinogenicity

Ames Test for genotoxicity has *S-9/NADPH-dependent bioactivation* arm; required for FDA submissions

- **RM “covalently adducts” to DNA resulting in genotoxic response**
  - Fungal mycotoxin aflatoxin B$_1$ (AFB$_1$) – established hepatocarcinogen†
    - *Exposure occurs primarily through ingestion of mold-contaminated foods (e.g., corn and peanuts)*
  
- **Rate-limiting step is P450-catalyzed RM formation**

\[ \text{AFB}_1 + \text{P450} \rightarrow \text{Furan epoxide (A reactive metabolite)} \]


RM “covalently adducts” to metabolizing enzymes (e.g., cytochrome P450) responsible for its formation

- Leads to enzyme inactivation and:
  - Non-linear PK if P450 enzyme is also primarily responsible for clearance
  - Drug-drug interactions (DDIs) (Atorvastatin/Grapefruit juice)
    - Furanocoumarins, bergamottin and 6’,7’-dihydroxybergamottin, the abundant constituents of GFJ, are mechanism-based inactivators of P4503A4

\[ R = H_2C - \]

Toxic Drug Metabolites – Acetaminophen as an Example

- Brodie et al. (National Institutes of Health) first to demonstrate:
  - Bioactivation of acetaminophen and covalent binding to liver tissue
- Nelson et al. elucidated the mechanism of acetaminophen bioactivation (involving a 2 electron oxidation to a reactive quinone-imine intermediate)
  - “Gold standard” of human and animal hepatotoxicity assessments

Dose-dependent (> 1 gm/day) hepatotoxin
- Depletes GSH upon toxic overdose
- Covalent binding to > 30 hepatic proteins
- Liver toxicity can be observed in animals
- N-Acetylcysteine as antidote

\[
\text{Acetaminophen} \quad \xrightarrow{\text{P450}} \quad \text{NAPQI} \quad \xrightarrow{\text{GSH}} \quad \text{GSH = glutathione – endogenous antioxidant (~ 10 mM concn. in mammals)}
\]

\[ \text{GSH} \]

\[ \text{HN} \quad \text{O} \quad \text{CH}_3 \]

\[ \text{O} \quad \text{HN} \quad \text{O} \quad \text{CH}_3 \]

\[ \text{N} \quad \text{O} \quad \text{CH}_3 \]

\[ \text{O} \quad \text{HN} \quad \text{O} \quad \text{CH}_3 \]

\[ \text{S} \quad \text{NH} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{COOH} \quad \text{O} \quad \text{NH}_2 \quad \text{COOH} \]

\[ \text{14C-APAP covalent binding to microsomes prevented by GSH; confirms the protective role of the thiol} \]
## Drugs Associated With IADRs

### Drugs Withdrawn

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Drug Name</th>
<th>Adverse Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiinflammatory</td>
<td>Aclclofenac</td>
<td>Hepatitis, rash</td>
</tr>
<tr>
<td>Anxiolytic</td>
<td>Alpidem</td>
<td>Hepatitis, agranulocytosis</td>
</tr>
<tr>
<td>Antituberculosis</td>
<td>Isoniazid</td>
<td>Hepatitis (can be fatal)</td>
</tr>
<tr>
<td>Antimalarial</td>
<td>Amodiaquine</td>
<td>Hepatitis, agranulocytosis</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Amineptine</td>
<td>Hepatitis, cutaneous ADRs</td>
</tr>
<tr>
<td>Antirheumatic</td>
<td>Benoxaprofen</td>
<td>Hepatitis, cutaneous ADRs</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Carbutamide</td>
<td>Bone marrow toxicity</td>
</tr>
<tr>
<td>Analgesic</td>
<td>Aminopyrine</td>
<td>Bone marrow toxicity</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>Captopril</td>
<td>Cutaneous ADRs, agranulocytosis</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Carbamazepine</td>
<td>Hepatitis, agranulocytosis</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Clozapine</td>
<td>Agranulocytosis, agranulocytosis</td>
</tr>
<tr>
<td>Antifungal</td>
<td>Dapsone</td>
<td>Agranulocytosis, cutaneous ADRs, aplastic anaemia</td>
</tr>
<tr>
<td>Antiinflammatory</td>
<td>Diclofenac</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Antituberculosis</td>
<td>Felbamate</td>
<td>Hepatitis (fatal), aplastic anaemia, severe restriction in use</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Tolcapone</td>
<td>Hepatitis (fatal)</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Furosemide</td>
<td>Agranulocytosis, cutaneous ADRs, aplastic anaemia</td>
</tr>
<tr>
<td>Antithrombotic</td>
<td>Ticlopidine</td>
<td>Hepatitis (fatal), aplastic anaemia</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Trazodone</td>
<td>Valproic acid (anticonvulsant)</td>
</tr>
<tr>
<td>Antifungal</td>
<td>Thalidomide</td>
<td>Teratogenicity</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Valproic acid</td>
<td>Thalidomide (immunomodulator)</td>
</tr>
</tbody>
</table>

### Temp. Withdrawn or Withdrawn in other Countries

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Drug Name</th>
<th>Adverse Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesic</td>
<td>Aminopyrine</td>
<td>Hepatitis (&gt; 200 deaths)</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Nefazodone</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Antibacterial</td>
<td>Trovan</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Antibacterial</td>
<td>Zileuton</td>
<td>Hepatitis</td>
</tr>
</tbody>
</table>

### Marketed Drugs

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Drug Name</th>
<th>Adverse Effect(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antituberculosis</td>
<td>Abacavir</td>
<td>Cutaneous ADRs</td>
</tr>
<tr>
<td>Analgesic</td>
<td>Acetaminophen</td>
<td>Hepatitis (fatal)</td>
</tr>
<tr>
<td>Antihypertensive</td>
<td>Captopril</td>
<td>Cutaneous ADRs, agranulocytosis</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Carbamazepine</td>
<td>Hepatitis, agranulocytosis</td>
</tr>
<tr>
<td>Antipsychotic</td>
<td>Clozapine</td>
<td>Agranulocytosis, cutaneous ADRs</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Diclofenac</td>
<td>Hepatitis</td>
</tr>
<tr>
<td>Antituberculosis</td>
<td>Felbamate</td>
<td>Hepatitis (fatal), aplastic anaemia</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Tolcapone</td>
<td>Hepatitis (fatal)</td>
</tr>
<tr>
<td>Antidepressant</td>
<td>Trazodone</td>
<td>Hepatitis (fatal)</td>
</tr>
<tr>
<td>Anticonvulsant</td>
<td>Trimethoprim</td>
<td>Agranulocytosis, cutaneous ADRs</td>
</tr>
<tr>
<td>Antifungal</td>
<td>Triamterene</td>
<td>Hepatitis (fatal), teratogenicity</td>
</tr>
</tbody>
</table>

For many drugs associated with IADRs, circumstantial evidence suggests a link with RM formation. Structure-toxicity relationships – evident and present a compelling case against RM positives.
Structure-Toxicity Relationships – Example 1

Enol-carboxamide-containing NSAIDs

**Sudoxicam**
- Hepatotoxic (acute liver failure)
- Withdrawn from Phase III trials

**Meloxicam**
- “Clean” drug

**Piroxicam**
- “Clean” drug
Rationalizing the Differences in Toxicological Profile Through Differences in Metabolism

Thioureas are toxic substances – Can oxidize proteins, glutathione, etc

Principal metabolism in humans is hydroxylation on methyl
Very minimal thiazole ring opening

Structure-Toxicity Relationships – Example 2

Antipsychotic agents


**Clozapine**

Agranulocytosis /Hepatotoxicity

*(Black box warning – requires intensive monitoring)*

**Quetiapine (Seroquel®)**

Commercial blockbuster

**Loxapine**

“Clean” drug
Rationalizing the Differences in Toxicological Profile Through Differences in Metabolism


Bioactivation of clozapine catalyzed by peroxidases in neutrophils
Reactive metabolite responsible for covalent binding to neutrophils

Quetiapine and loxapine cannot form electrophilic iminium like clozapine does
RM Detection – Electrophile Trapping

- Reactive metabolites (with the exception of acyl glucuronides) are unstable
  - Need derivatization techniques for indirect characterization
- RM trapping with exogenous nucleophiles
  - Can be used with diverse metabolism vectors
    - Liver microsomes, S-9, hepatocytes, etc
    - Glutathione, N-acetylcysteine (soft nucleophiles)
      - Traps soft electrophiles (e.g., Michael acceptors — quinones)
    - Methoxylamine, semicarbazide, cyanide (hard nucleophiles)
      - Traps hard electrophiles (e.g., aldehydes, iminium ion)
  - LC-MS/MS and/or NMR methodology for structure elucidation of conjugate
RM Detection – Covalent Binding

- Limited to availability of radiolabeled drug candidate
  - May not be suitable in early discovery

- **In Vitro** covalent binding can be assessed with diverse metabolism vectors
  - Effect of competing/detoxicating drug metabolizing enzymes on covalent binding can also be examined

- **In vivo** covalent binding can be assessed in preclinical species

- Covalent binding data is quantitative
  - No information on nature of proteins modified
Utility of RM Detection Tools in Drug Discovery - Identifying the Metabolic Basis for Mutagenicity

CP-809,101

Selective and potent 5-HT$_{2c}$ Agonist
- Excellent in vivo pharmacology for weight reduction
- Excellent predicted human pharmacokinetics
- Potential as an anti-obesity agent

- Mutagenic in Salmonella Ames assay
  - Requires Ariclor Rat S-9/NADPH
    - Suggests DNA-Reactive Metabolites Formed
  - Compound dropped from development

No toxicophore / Structural alert present; clean in DEREK assessment

GOAL

Need to elucidate mutagenic mechanism(s) for design of follow-on candidates

Available tools: [${}^{14}$C]-CP-809,101, RM traps (GSH, CH$_3$ONH$_2$, etc)

### TABLE 1

S-9/NADPH-dependent covalent binding of [\(^{14}\!)\text{-CP-809,10 to calf-thymus DNA}]

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Test I</th>
<th>Test II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean DPM(^a)/20 (\mu) g DNA</td>
<td>Mean DPM(^a)/20 (\mu) g DNA</td>
</tr>
<tr>
<td>Vehicle (DMSO)</td>
<td>35 (34, 35)</td>
<td>23 (24, 24, 22)</td>
</tr>
<tr>
<td><strong>CP-809101</strong> (0.5 (\mu)M) - S9</td>
<td>51 (54, 47)</td>
<td>52 (48, 48, 59)</td>
</tr>
<tr>
<td><strong>CP-809101</strong> (0.5 (\mu)M) + incomplete S-9</td>
<td>40 (39,41)</td>
<td>42 (42, 41, 43)</td>
</tr>
<tr>
<td><strong>CP-809101</strong> (0.5 (\mu)M) + complete S-9</td>
<td>105 (104, 106)</td>
<td>124 (126, 121)</td>
</tr>
<tr>
<td><strong>CP-809101</strong> (5.0 (\mu)M) + complete S-9</td>
<td>495 (490,501)</td>
<td>462 (472, 451)</td>
</tr>
</tbody>
</table>

- S-9, without metabolic activation; + incomplete S-9 (-NADPH); complete S-9 (+ NADPH) NADPH was used in test I and NADPH regenerating system was used in test II.

\(^a\) mean DPM represents average from two to three separate experiments.

NADPH-dependent covalent binding to DNA suggests P450-mediated bioactivation to DNA-reactive metabolite(s)
Deciphering CP-809101 Bioactivation Pathways

Covalent binding to DNA significantly attenuated in the presence of CH$_3$ONH$_2$ and GSH
Rational Chemical Modifications to Circumvent Mutagenicity

Metabolic soft spots (Minimal ring opening)

Non-mutagenic in Ames Assay

Primary Pharmacology Maintained

PK Attributes Maintained

Cannot form quinone-methide

Compound 1 identified as meeting desired criteria for primary in vitro pharmacology and progressed for further profiling (e.g., in vitro ADME, metabolism studies, etc) as part of lead optimization efforts.
Glutathione Trapping Studies on 1

A. HLM + NADPH
B. HLM + NADPH + GSH
C. HLM – NADPH + GSH
D. GSH in buffer
E. Cytosol + GSH
F. GST + GSH

Kalgutkar AS, Sharma R, Walker GS et al. Unpublished data
Mass Spectra of M4-1 and M5-1

M4-1 (Exact mass + H⁺ = 638.2238)

M5-1 (Exact mass + H⁺ = 654.2182)

(- H₂O)
Additional Confirmation of Adduct Structure using NMR

<table>
<thead>
<tr>
<th>Position</th>
<th>Group</th>
<th>δ (¹H) ppm</th>
<th>δ (¹³C) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>C? O</td>
<td>3.30</td>
<td>170.4</td>
</tr>
<tr>
<td>b</td>
<td>CH</td>
<td>1.88</td>
<td>26.8</td>
</tr>
<tr>
<td>c</td>
<td>CH₂</td>
<td>2.34</td>
<td>31.5</td>
</tr>
<tr>
<td>d</td>
<td>C? O</td>
<td></td>
<td>171.8</td>
</tr>
<tr>
<td>e</td>
<td>NH</td>
<td>8.71</td>
<td>51.6</td>
</tr>
<tr>
<td>f</td>
<td>CH</td>
<td>4.61</td>
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<td>g</td>
<td>C? O</td>
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<td>3.52</td>
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<td>C</td>
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<td>r</td>
<td>CH</td>
<td>5.52</td>
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<tr>
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<td>t</td>
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<td>u</td>
<td>CH</td>
<td>3.22,3.33</td>
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<td>CH</td>
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<td>y</td>
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<tr>
<td>z</td>
<td>CH</td>
<td>3.22,3.33</td>
<td>42.0</td>
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</tbody>
</table>

* Proton chemical shifts were measured relative to DMSO-"d₆" signal at 2.50 ppm. Carbon chemical shifts were obtained from the g-HSQC and g-HMBC spectra and measured relative to the DMSO-"d₆" signal at 39.5 ppm.
The Cyanide Group in 1 is Essential for Nucleophilic Displacement by GSH

Cyanide substituent required for nucleophilic displacement by glutathione

Conclusions –
GSH adduct formation does not require “bioactivation”
4-Aryloxy-5-cyanopyrimidines can function as potential affinity labels (protein alkylation) or cause GSH depletion

Cyano replacements in the current scaffold avoided
Eliminating Toxicity Risks in Drug Discovery Setting

• Structure-toxicity analyses teaches us that avoiding RM formation with drug candidates represents one potential solution to preventing drug toxicity

• Avoid chemical functionalities known to be susceptible to reactive metabolites
  – Tall order but avoids risk
Examples of Functional Groups Susceptible to RM Formation

- Anilines (masked anilines)
- p-Aminophenols
- Nitrobenzenes
- Hydrazines (phenylhydrazines)
- Benzylamines
- Catechols
- Cyclopropylamines
- 1,2,3,6-Tetrahydopyridines
- 2-Halopyridines and pyrimidines
- Haloalkanes
- Unsubstituted alkenes
- Acetylenes
- Imides
- Formamides
- Sulfonylureas
- Thioureas
- Methylenedioxy groups
- Reduced aromatic thiols
- 5-Hydroxy(or methoxy) indoles
- 3-Methylindoles
- Unsubstitued furans
- Unsubstitued thiophenes
- Unsubstitued thiazoles
- Unsubstitued oxazoles
- Thiazolidinediones
- Fatty acids (medium to long chain)
- Carboxylic acids
- Hydroxylamines
- Hydroxamic acids
- Michael Acceptors
- Hydroquinones
- Bromobenzene
- BENZENE !!!!
And What about the False Negatives?

I really don’t see a “ugly” looking structure here

I checked for glutathione conjugates in HLM and human hepatocytes and saw none

I assessed covalent binding to HLM and human hepatocytes and saw nothing of significance


But this is the anti-convulsant felbamate (Daily Dose > 3000 mg)

Within a year of its release in 1993

- 34 cases of aplastic anemia resulting in 13 deaths (Incidence rate 1:4800 – 1:37000)
- 23 cases of hepatotoxicity resulting in 5 deaths (Incidence rate 1:18000 – 1:25000

Black box warning (severe restriction in use)

- ~ 12,000 patients estimated to be on drug
In Vivo Observations on Felbamate Conversion to RMs in Humans

metabolites in human urine

R = CH$_2$OH or CO$_2$H

A heavy duty electrophile

Felbamate


And What about the False Negatives?

No obvious structural alert
No evidence of glutathione conjugate formation \textit{in vitro or in vivo}

Primary metabolic pathways in humans – ester hydrolysis, reduction to active drug “Melagatran”

Ximelagatran (Exanta®), the first orally active thrombin inhibitor (anticoagulant) was withdrawn due to several cases of hepatotoxicity

- Daily dose 20 – 60 mg BID
- Short term use (< 12 days) in humans did not indicate hepatotoxic potential
- Long term use (> 35 days) in human showed elevated hepatic enzyme levels in 0.5% of patients
  - Withdrawal triggered from severe liver damage in a patient
  - Immune component demonstrated upon pharmacogenomic analysis

Furthermore, How do we Handle the False Positives?

Adding insult to injury, some are commercial blockbusters

**Paroxetine (Paxil®)**
- RM = Catechol /quinone

**Olanzapine (Zyprexa®)**
- RM = iminium

**Clopidogrel (Plavix®)**
- RM = Thiophene ring opening

**Prazosin (Minipress®)**
- RM = Furan Ring Opening

**Raloxifene (Evista®)**
- RM = quinone

**Aripiprazole (Abilify®)**
- RM = quinone imine

GSH conjugate/covalent binding demonstrated for all compounds
RM Detoxication as a Mitigating Factor for IADRs

The case of paroxetine

Detoxication

\[
\text{CYP2D6} \rightarrow \text{COMT} \rightarrow \text{Detoxication} \rightarrow \text{GSH} \rightarrow \text{Covalent Binding to Hepatic Tissue}
\]

The case of Raloxifene

Bioactivation

\[
\text{P4503A4} \rightarrow \text{Detoxication} \rightarrow \text{GSH} \rightarrow \text{Detoxication} \rightarrow \text{Bioactivation}
\]

Raloxifene
Dose Size as a Mitigating Factor for IADR Potential of New Drug Candidates

There are many examples of two structurally related drugs that possess a common structural alert prone to bioactivation, but the one administered at the lower dose is much safer than the one given at a higher dose.

Atypical anti-schizophrenia agents

Clozapine

*Agranulocytosis in 2% of patients*

Daily Dose = 300 mg

Olanzapine

*Safe and Successful Drug*

Sales > US $ 2 billion

Forms GSH conjugates via the iminium ion in a manner similar to clozapine

Covalent binding to proteins

Only 3 cases of agranulocytosis

Higher than recommended dose

Daily Dose = 10 mg
Bioactivation Data Needs to be Placed in Proper Context — Risk/Benefit Assessments (Qualifying Considerations)

• **Nature of the medical need**
  – Life-threatening disease / unmet medical need
  – First in class

• **Target population**
  – Underlying disease state (immune-compromised patients)

• **Is the drug candidate intended to provide proof of a novel mechanism?**

• **What % of clearance mechanism involves bioactivation**
  – Existence of detoxication pathways; renal excretion, etc

• **What is the daily dose of the drug?**
  – IADRs are rare for drugs dosed below 20 mg QD
For an account of a discovery strategy for dealing with RM positive compound(s) as a drug candidate, please see:


N\_N\_N\_CF_3\_O\_Me\_Ph

1

CaSR IC$_{50}$ = 41 nM
HLM $CL_b$ = 12 mL/min/kg
RM positive
(GSH-EE conjugate peak area = 166810)

Predicted Human Dose = 45 mg QD

11

CaSR IC$_{50}$ = 8 nM
HLM $CL_b$ = 7.4 mL/min/kg
RM positive
(GSH-EE conjugate peak area = 3000)

Predicted Human Dose = 10 mg QD

13

CaSR IC$_{50}$ = 64 nM
HLM $CL_b$ = 6.0 mL/min/kg
RM negative

Reactive metabolite (RM) formation pathway

\[ \text{X = CH, N} \]

\[ \text{HO} \]

\[ \text{HO} \]

\[ \text{HO}_2\text{C} \]

\[ \text{NH}_2 \]

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{OH} \]

\[ \text{NH} \]

\[ \text{COOR} \]

\[ \text{GSH: R = H} \]

\[ \text{GSH-EE: R = CH}_2\text{CH}_3 \]