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https://www.acs.org/content/acs/en/acs-webinars/drug-discovery.html
Service Dogs in Your Chemistry Lab

Date: Wednesday, September 22, 2021 @ 2-3pm ET
Speakers: Patricia Bediako, Saint Peter's University; Joey Ruppi, Empower Ability Consulting, LLC; Ashley Seybert, Independence Science
Moderator: Pantha Baez, Indiana University-Purdue University Indianapolis

What You Will Learn:
- What does the Americans with Disabilities Act cover regarding access rights for service dogs
- How to hire a service dog specific to your job duties, and what type of training is required
- What types of service dogs exist and what is the process to obtain one

Co-produced with: Chemists with Disabilities (CWD) Committee, ACS Department of Diversity Programs, and ACS Diversity, Inclusion & Respect Advisory Board

Register for Free

Molecules to Manufacturing to Marketplace: 3D Printing of Sulfonated Polystyrenes for Controlled Release

Date: Thursday, September 23, 2021 @ 2-3:15pm ET
Speakers: Timothy Long, Arizona State University; and Michael, Burton, Virginia Tech
Moderator: Bryan Twedt, American Chemical Society

What You Will Learn:
- What is the impact of polystyrene and its microstructure on printability and performance of 3D-printed structures?
- How to leverage rheology for predictive additive manufacturing system design and materials screening
- A snapshot of the topologies and concepts captured in the ACS Polymer Chemistry: Principles and Practice short course held at Virginia Tech

Co-produced with: ACS Professional Education

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Designing Around Structural Alerts in Drug Discovery

FREE Webinar | TODAY at 2pm ET

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Designing around Structural Alerts in Drug Discovery

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This ACS Webinar is co-produced with the ACS Division of Medicinal Chemistry, American Association of Pharmaceutical Scientists, and ACS Publications.

Outline

- The problem:
  - adverse drug reactions and manifestations of toxicity
  - drug withdrawals, BBWs and rejections due to liver toxicity
- Drug-induced liver disease – DILI
  - underlying mechanisms
- Metabolic activation of drugs and toxicity
  - background studies that attempt to provided perspective
  - assessing reactive metabolite formation and covalent binding to proteins
- A synopsis of structural alerts
  - problematic functionality and the underlying mechanistic organic chemistry
- Approaches to mitigating reactive metabolite problems
  - strategies and tactics
- Conclusion

A. Kalgutkar, J. Med. Chem. 2020, 63, 6276-6302
**ADVERSE DRUG REACTIONS AND WITHDRAWALS**

*The Role of Metabolic Activation*

Adverse Drug Reactions (ADRs)

- ADRs were estimated to be the 4th leading cause of death in the US in 1994
  - deaths estimated at 106,900 (95% CI 76,000-137,000)
  - ADR death rates increased between 1999 and 2006
  - over 2 million serious ADRs per year: $136 billion yearly cost
- ADRs have been divided into 5 categories
  - Type A accounts for 80%
  - Type B has an underlying chemical basis

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Underlying Effect</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Augmented Reactions</td>
<td>Dose-related extension of pharmacology</td>
<td>Excessive hypotension with antihypertensive agents; rhabdomyolysis with statins</td>
</tr>
<tr>
<td>B</td>
<td>Bizarre Reactions</td>
<td>Idiosyncratic – immune or non-immune mediated Rare: 1 in 10,000-50,000</td>
<td>Troglitazone and tienvil acid hepatotoxicity</td>
</tr>
<tr>
<td>C</td>
<td>Chemical Reactions</td>
<td>Dose-related; molecular understanding</td>
<td>Acetaminophen, isoniazid hepatotoxicity</td>
</tr>
<tr>
<td>D</td>
<td>Delayed Reactions</td>
<td>Occur after many years of drug ingestion</td>
<td>Teratogenicity after drug intake during pregnancy - thalidomide</td>
</tr>
<tr>
<td>E</td>
<td>End-of-treatment Reactions</td>
<td>Adverse reactions on drug withdrawal</td>
<td>Withdrawal seizures after stopping phenytoin</td>
</tr>
</tbody>
</table>


Withdrawals of Prescription Drugs 1960-1999

121 Drugs withdrawn from world markets 1960-1999 for safety reasons

- NSAIDs most common category associated with drug withdrawal
- Many of the antidepressants withdrawn are MAO inhibitors
- Hepatotoxicity is the leading cause of drug withdrawal

“Hepatotoxicity is the most common adverse effect causing major drug problems including withdrawals and refusal to approve”
Dr Robert Temple (FDA): Drug-Induced Liver Injury: A National and Global Problem,
Feb. 12-13th, 2001, Westfields Conference Center, Chantilly, VA

Drugs With Liver Toxicity Problems

- Structurally disparate and mechanistically diverse
- Reactive metabolites suspected and examined in several cases
  - iproniazid, tienilic acid, troglitazone
  - sitaxsentan and trovafloxacin ultimately withdrawn by Pfizer
- Sitaxsentan and trovafloxacin ultimately withdrawn by Pfizer after warning labels added
- Difficult to establish definitive cause and effect relationship
  - no evidence that ximelagran is associated with reactive metabolites
  - immune mediated: human leukocyte antigen (HLA) - HLA-DRB1*07
Drug-Induced Liver Injury (DILI)

- Most instances of DILI are idiosyncratic in nature
  - no reliable biomarkers
  - focus on reactive metabolites: retrospective studies

- Mitochondrial toxicity is an uncommon but distinctive form of liver toxicity
  - tetracycline, amiodarone, valproic acid
  - problem with HIV-1, HBV nucleoside analogues: inhibition of host DNA polymerase

- Cholestatic DILI - transporter involvement
  - bile salt export pump (BSEP, ABCB11): cyclosporin, rifampicin
  - multi-drug resistance-associated protein 2 (MRP2, ABCC2)
  - multi-drug resistance protein 3 (MDR3)
    - these transporters are genetically polymorphic proteins

- Immune mechanisms of DILI
  - antibodies to liver proteins: hapten hypothesis - tienilic acid
  - human leukocyte antigen (HLA) allele binding

#### CAUSES OF ALF WORLDWIDE

![Graph showing causes of ALF worldwide]

- USA 1998-2000
- USA 1994-1996
- Denmark 1973-1990
- India 1987-1993
- France 1972-1990
- UK 1993-1994

- Other
- Acetaminophen
- Drug
- HBV
- HAV

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**METABOLIC BIOACTIVATION**

*In Vitro Techniques and Metabolic Pathways*
Metabolic Activation and Drug Toxicity

Chemically reactive species from Phase 1 or 2 metabolism - or a combination
- Highly reactive species bind to heme and inactivate CYP
- Moderately reactive products may react with CYP protein
  - dissociate from CYP, decorate other proteins
  - glutathione may be protective
  - most problematic species
- Low reactivity metabolites captured by GSH or H₂O

Decoration may inactivate a protein or produce a hapten
- DNA decoration can be mutagenic
  - guanine particularly susceptible to modification
  - C-8 the most reactive site

Protein or DNA Decoration

Uricosuric diuretic agent introduced in Europe in 1976,
- US FDA approval followed in 1979
- Withdrawn in the US in 1980
  - severe hepatotoxicity in <1% of patients: 10% fatality rate
- Drug-induced immunoallergic hepatitis
  - anti-LKM₂ antibodies detected (liver-kidney microsome)
- Most compelling example of haptenization hypothesis
  - thienyl peroxide, thiolactone potential electrophiles
- Iso-tienilic acid an impurity in early lots of tienilic acid
  - toxicity profiles of the 2 compounds differ
  - tienilic acid induces immune-mediated hepatitis in humans, not rats
  - iso-tienilic acid directly causes hepatitis in rats
- In vitro metabolic studies comparing tienilic acid & iso-tienilic acid
  - some illumination of the chemistry underlying the observed toxicity


Tienilic Acid (Ticrynafen)

Assessing Reactive Metabolites

- Incubate compound with human liver microsomes (HLM)
- Analyze for protein covalent binding (PCB) – radio-labeled drug
- Evaluate in the presence and absence of glutathione (GSH) or derivative
  - GSH is a natural protective mechanism
- Protein binding measured as pmol eq./mg protein
  - 50 pmol eq./mg protein in vitro and in vivo suggested as a standard
  - differentiate between propensity to be toxic/non-toxic
- Analyze for PCB in presence and absence of GSH to assess potential for protection in vivo
- Analyze for (GSH) adducts
  - can be done with cold drug
  - GSH: soft nucleophile for soft electrophiles
- Trap with Na\textsuperscript{14}CN
  - CN\textsuperscript{-} is a hard nucleophile
  - used to trap hard electrophiles like iminium ions

Protein Covalent Binding and Toxicity

- Bioactivation/PCB and toxicity correlation not absolute
  - meta isomer of acetaminophen not liver toxic in mice
  - comparable levels of PCB
  - PCB is measure of bioactivation not toxicity
- PCB in vitro in HLM or in vivo shows poor correlation for clinically toxic drugs
  - problematic drugs exhibited higher PCB than safe drugs
  - 1 study separated safe drugs based on dose
- Necessitates caution in extrapolating PCB to clinical or pre-clinical toxicity
- Drugs may be metabolized in vivo by different pathways to in vitro
  - losartan forms GSH adducts via the imidazole moiety in vitro
  - metabolism in vivo: oxidation of CH\textsubscript{2}OH; tetrazole glucuronidation
- Follow RM assessment in LM with studies in S9 and hepatocytes
  - understand clearance pathways in vivo
  - develop an integrated view of metabolism
- Clinical indication, drug dose are additional factors that provide context
  - low dose drugs less likely to cause idiosyncratic toxicity

Drug Clearance Pathways in Humans

- Clearance mechanisms for the top 200 US drugs in 2002
- CYP-mediated metabolism dominates and CYP 3A4 is the major catalyst

CYP 450s dominate (~90%)
1A2, 2C9, 2C19, 2D6 & 3A4 account for 75%

4192 reactions, 860 drugs

4058 reactions, 860 drugs

The CYP 450 Catalytic Cycle

- Oxidation
  - CYPs are powerful oxidants
  - Fe=O(O=O) the most powerful species
  - rebound mechanism most common pathway for C-H
  - Fe=O=O-H can oxidize soft atoms

- Reduction
  - CYPs can act as reducing agents
  - occurs with some substrates when binding of O2 is slow

Oxidation cycle is initiated by substrate binding
- Fe3+ (ferric) is reduced to Fe2+ (ferrous) by e transfer from NADPH
- Fe2+ species binds O2

Fe2+-O2 complex is a reducing agent whilst waiting for an electron
- the single electron can be transferred to the substrate
- facilitated by some substrates blocking O2 binding
- can reduce N-O, N-N bonds, C-halogen bonds

Fe2+-O=O-H is an oxidant and a nucleophile (Cpd 0)
- sulfur, amines (CYP 1A2), soft atoms
- may hydrolyze nitriles (Fe2+-O-O)

Fe3+ (ferrous) is readily oxidized to Fe3+ (ferric)
Fe=O2+ (can be written as Fe=O) is the major oxidizing species (Cpd I)
- capable of a broad repertoire of reactions
A SYNOPSIS OF STRUCTURAL ALERTS

And The Underlying
Mechanistic Organic Chemistry

Structural Alerts

- Phenyl rings feature prominently
  - may reflect ubiquity in drug design
  - most common ring in marketed drugs

- Common functionalities can be problematic
  - carboxylic acids
  - olefins
Structural Alerts – a Survey of Toxicophores

Anilines & Masked Anilines
Darunavir, Dapsone, Procainamide

Nitrobenzenes
Nifedipine, Dantrolene, Tolcapone, Flutamide

Marketed drugs

- Anilines
- Nitrobenzenes

- 3 factors identified as contributing to aniline mutagenicity:
  F1: facility of the aniline binding to CYP 1A2 active site
  F2: ease of proton abstraction from ArNH₂
  F3: susceptibility of ArNH-OH bond to H⁺-mediated heterolysis
- The 3 factors operate strictly in a sequential fashion
  - order of importance: F1>F2>F3
  - disruption of 1 factor will make the subsequent steps irrelevant
- Applies to some heterocycles
  - food-derived mutagens PhIP
  - amino pyrazoles,
  - amino triazoles,
  - amino thiazoles
- Amino pyridazines OK

Structural Alerts – a Survey of Toxicophores

Cyclopropylamines
Ciprofloxacin, Nevirapine, Tranzylopromine, Abacavir

Allylic Amines
Terbinafine

1,2,3,6-Tetrahydopyridines
Haloperidol

2-Halo- and 2-Cyano Pyridines, Pyrimidines
DUP453

Haloaikanes
Chloramphenicol, Halothane

- May extend to other leaving groups – e.g. RSO₂, acidic heterocycles
- CCl₄ undergoes reductive activation to CCl₃ radical – can react with DNA
### Structural Alerts – a Survey of Toxicophores

#### Alkenes
- Alclofenac, Zolpidem

#### Acetylenes
- Efavirenz, Erlotinib, Terbinafine, Selegiline, Rasagiline

#### Methylene dioxy Aromatics
- Paroxetine, Tadalafil

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#### Thioureas, Thioamides
- Ethionamide, Methimizole, Quazepam, Epalrestat, Enzalutamide, PF-06282999

#### 1,4-Hetero-Substituted Aromatics
- Acetaminophen, Amodiaquine extends to thiophenes

#### 5-OH, OMe or Amino Indoles
- Umifenovir, Delavirdine
### Structural Alerts – a Survey of Toxicophores

<table>
<thead>
<tr>
<th>Phenols, Hydroquinones</th>
<th>Raloxifene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benzene, Bromo- &amp; Iodo benzenes</strong></td>
<td>Cobimetinib</td>
</tr>
<tr>
<td><strong>Substituted Toluenes</strong></td>
<td>Salbutamol</td>
</tr>
<tr>
<td><strong>Thiophenes</strong></td>
<td>Duloxetine, Olanzapine, Tiotropium; Rivaroxaban, Clopidogrel</td>
</tr>
<tr>
<td><strong>Furans, Oxazoles, Thiazoles</strong></td>
<td>Ranitidine, Prazosin, Furosemide, Dantrolene, Mometasone, Ritonavir</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Benzylamines</th>
<th>Sertraline, Imatinib, Cetilizine, Donepezil, Cinacalcet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carboxylic Acids</strong></td>
<td>Valproic Acid, Atorvastatin, Pregabalin, Dalcetrapib, Fluticasone (thioesters)</td>
</tr>
</tbody>
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Bristol-Myers Squibb
Amines: Some Special Cases with Concern

- Cyclic amines
  - α-hydroxylation & H₂O elimination:
  - can lead to cyclic iminium species
  - react with hard electrophiles (CN⁻)
  - aldehyde dissociation with acyclic amines
- Nomifensine withdrawn due
  - hemolytic anemia and hepatotoxicity
  - iminium reacts readily with CN⁻ but not GSH
- Fluorinated amines
  - elimination of HF after α-OH’ation
- Fluoroacetic acid release
  - naturally occurring toxin
  - Krebs cycle: inhibits aconitase
  - lethal doses (mpk):
    - dog: 0.05; rat 0.1-5
    - humans: 2-5
- Cyclopropyl amines undergo ring opening
  - tranylcypromine metabolized to cinnamaldehyde
- Trovafoxicin had BBWs for liver toxicity
  - ultimately withdrawn due to hepatotoxicity

STRATEGIES FOR MITIGATING REACTIVE METABOLITES
Strategies for Reducing Potential Problems

- Maximize potency, minimize dose
  - reduces reactive metabolite burden

- Structural modification
  - remove or modify problematic structural elements

- Introduce steric effects
  - steric shielding of metabolic sites to slow bioactivation
  - reactive metabolites will also likely be subject to steric hindrance

- Electronic effects
  - metabolic modification will be kinetically slower, reduced throughput
  - BUT…… metabolic activation produces highly reactive species
  - potential source of problems

- Introduce a metabolic soft spot
  - redirects metabolism away from problematic elements

- Intramolecular capture
  - proximal nucleophile can capture reactive intermediates

Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Which of these strategies for reducing potential problems are you familiar with? (Select all that apply)

- Structural Modification
- Introduce Steric Effects
- Electronic Effects
- Introduce a Metabolic Soft Spot
- Intramolecular Capture
REACTIVE METABOLITE MITIGATING STRATEGIES

Structural Modification of Problematic Elements

Quinonediimines in Bradykinin Antagonists

- Diamino pyridine moiety susceptible to oxidation in bradykinin antagonists
- Solution - isostere of phenylene diamine moiety
  - reduce pyridine moiety to ethylene diamine; add C=O to mimic N
  - dimethyl provides conformational bias - Thorpe-Ingold effect
- Cyclopropyl optimal – improved topology
  - electronic overlap with C=O confers additional conformational bias

Clinical utility of felbamate limited by aplastic anemia and hepatotoxicity

Atropaldehyde is potently electrophilic and toxic to fibroblasts
- thiol adducts found in rat and human urine

Strategic deployment of F based on detailed understanding of metabolism

F atom of fluorofelbamate prevents elimination of carbamate
- atropaldehyde not formed
REACTIVE METABOLITE MITIGATING STRATEGIES

*Introduce Steric Effects*

**Melanocortin-4-Receptor Antagonist & Pim Kinase**

- MCR4: time-dependent CYP 3A inhibitor in HLM
  - cyanide adduct identified in HLM containing KCN
- With rCYP 3A4, saw $I_{max}$ at 450 nM – MI complex
  - consistent with amine oxidation to nitroso derivative
- PO administration to rats increased indinavir plasma levels 3x
  - suggested potential for DDIs in humans
  - development terminated
- $\alpha$-CH$_3$ derivative did not inhibit CYP
  - steric shielding of amine
  - also blocks metabolic path

**PIM kinase**

- CYP 3A TDI in HLM
  - met. ID studies implicated azepine
- Fluorine played critical role in TDI
  - saw GSH adducts
  - negated nitroso pathway
- Consistent with Michael acceptor formation
  - $\alpha$-hydroxylation to afford C=O
  - elimination of F
  - blocked by $\alpha$-CH$_3$

Data consistent with this pathway

CYP 3A4 $IC_{50}$ <0.1 $\mu$M


Bristol-Myers Squibb
Problems with a Fluorinated Pyrimidine

- **BACE-1 inhibitors**
  - saw time-dependent CYP 450 inhibition
- **Structure-inhibition studies implicated F-pyrimidine**
  - Met ID studies identified minor metabolite of F-pyrimidine +OH, -F
  - with GSH: +OH, -F, +GSH, +2H
- **Dimethylated pyrimidine reduced CYP 3A4 TDI: retained BACE-1 inhibition**

Enhancing Acyl Glucuronide Stability

- **Potent DGAT-1 antagonist**
  - blocks triglyceride synthesis, storage
- **Acyl glucuronide the 1º metabolite**
- **Added bulk to cyclohexane**
  - increases stability of acyl glucuronide
  - $t_{1/2}$ for hydrolysis = 64 h in buffer
  - <15% rearrangement over 80 h

- **Steric bulk increases AG stability**
- $t_{1/2}$ of 21 AGs of marketed & withdrawn drugs
- **Zone classification for predicting toxicity of AGs**
- $t_{1/2}$: safe drugs ≥7.2 h; unsafe drugs ≤1.7 h
- **Regression analysis**
  - gave a $t_{1/2}$ of 3.6 h dividing point

<table>
<thead>
<tr>
<th></th>
<th>Unsafe</th>
<th>Safe</th>
<th>Dividing Point</th>
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<tr>
<td>$t_{1/2}$</td>
<td>≤1.7 h</td>
<td>≥7.2 h</td>
<td>3.6 h</td>
</tr>
</tbody>
</table>

Introduce sterically demanding proximal substituents
**REACTIVE METABOLITE MITIGATING STRATEGIES**

*Modulate Electronic Properties*

**Avoiding Quinone-Type Metabolites**

- Short-acting Ca²⁺-sensing receptor antagonists for osteoporosis
- Lead candidate underwent sequential NADPH-dependent oxidation
  - gave catechol and ortho-quinone in HLM based on GSH trapping
- Modifying the phenol ring to a pyridine reduced propensity for oxidation
  - calculations indicated higher oxidation potential
  - 2 F atoms also introduced to the distal phenyl ring
- 56-fold lower GSH adducts with modified molecule
- Challenge: maintaining high clearance rate to minimize off-target activities

- HIV-1 attachment inhibitors
  - demethylation/oxidation to quinone
  - 6-aza would metabolize to amide

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REACTIVE METABOLITE MITIGATING STRATEGIES

Introduce a Metabolic Soft Spot or Redirect Metabolism

Olefins in Benzodiazepine Receptor Ligands

- GABA-chloride channel ligands – ω₁ benzodiazepine receptor
- Alpidem – anxiolytic marketed in 1991, withdrawn in 1995 due to liver toxicity
  - peripheral ω₁ – partial agonist – binds to mitochondrial receptor
  - dose: 50 mg TID
  - forms GSH adducts in vivo and depletes GSH in hepatocytes
- Zolpidem – structurally related hypnotic (Ambien®)
  - 10 mg QD dose
  - central ω₁ – full agonist; no peripheral activity
  - no GSH adducts in vivo or in hepatocytes
  - metabolic pathways involve oxidation of the two CH₃ moieties
- Structurally similar but markedly different pharmacology and toxicology
REACTIVE METABOLITE
MITIGATING STRATEGIES

Combination Approaches

Avoiding Iminoquinone Metabolites in CRF$_1$

- Lead identified within a series of potent CRF$_1$ receptor inhibitors
  - 60% of dose identified as oxidized metabolites in bile
  - 25% of dose excreted as GSH adducts of phenyl ring
- Phenyl ring modification focused on pyridine analogue
  - a survey of pyridyl analogues indicated substantially reduced levels of bioactivation
  - incorporated into molecule selected for further development
- Pyrazinone ring also subject to bioactivation
  - required further structural modification to electron deficient CN moiety
- Major metabolic pathway – O-demethylation of alkyl ether introduced as a soft spot

Conclusion

- Several functionalities have been associated with problems
  - in drug discovery & development; post-marketing
  - frequent association with bioactivation
- Establishing cause-effect toxicity has been difficult in many cases
  - retrospective search for an understanding of the problem
- Effect of a particular structural alert can be contextual
  - many examples of successful drugs that contain potential toxicophores
  - ~50% of small molecule drugs in the top 200 contain structural alerts
- Metabolism-based toxicity can sometimes be difficult to predict
  - idiosyncratic toxicity produces low frequency events
  - not always observe in preclinical species
  - utility of drug will depend on severity and availability of alternate therapy
- Establishing cause-effect toxicity has been difficult in many cases
  - tienilic acid is the most compelling example
- Would appear to be prudent to minimize metabolic activation
  - low dose drugs less frequently associated with problems
  - % metabolized by a particular pathway,
  - alternative pathways of metabolism in vivo
  - context of disease for therapy
Designing around Structural Alerts in Drug Discovery

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