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- **In Vivo Imaging of Receptor Occupancy: A Critical Tool for Targeted Therapy Development**
- **Optimized Approaches to a Successful Preclinical Formulation Development**
- **Getting Clearance for Takeoff: Options to Assess Drug CL Before FHD**
- **Rules of Thumb for Optimizing and Selecting Non-Orally Delivered Drug Candidates**

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2016 Drug Design and Delivery Symposium

	I - Time: The Fourth Dimension in Drug Discovery
January 28	The Importance of Drug-Target Kinetics in Drug Design Robert Copeland - Epizyme, Inc Dan Erlanson - Carmot Therapeutics
February 25	Long-Acting Injectable Medications: Strategies and Mechanistic Considerations Jules Remenar - Alkermes Annette Bak - Merck
March 31	Modified Release Formulations for Solubility Starved Compounds Mengwei Hu - Merck John Morrison - BMS
April 28	*The Medicinal Chemist of Tomorrow (Special Topic)* Joel Barrish - Achillion Ravi Nargund - Merck Molly Schmid - Tech Coast Angels
	II - Beyond Traditional Small Molecules
May 19	Design of Deliverable Macrocycles Scott Lokey - UC Santa Cruz Nicholas Meanwell - BMS
June 23	Dreaming Big and Thinking Small: Applying Medicinal Chemistry Strategy to Antibody-Drug-Conjugates L. Nathan Tumey - Pfizer Peter Senter - Seattle Genetics
July 28	Nucleic Acids Therapeutics: Making Sense of Antisense Oligonucleotides Punit Seth - Ionis Richard Olson - BMS
August 18	Crystallography as a Drug Design and Delivery Tool (Special Topic) Robert Wenslow - Crystal Pharmatech Vincent Stoll - Abbvie Andrew Brunskill - Merck
	III - Pharmacology Revisited
September 29	Dealing with Reactive Drug Metabolites in Drug Discovery: Can We Predict Toxicities of Drug Candidates that form Reactive Metabolites? Deepak Dalvie - Pfizer Frederick Peter Guengerich - Vanderbilt University
October 27	Rational Design of Small Molecules Targeting RNA Matt Disney - Scripps Rl Florida Amanda Garner - University of Michigan

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Meet the Organizers


Nicholas Meanwell
BMS


John Morrison
BMS

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12

Upcoming ACS Webinars
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Thursday, October 6, 2016

Treating Cancer with Nanoparticles Powered by the Sound of Light

Justin Harris, Lead Research Scientist, NanoHybrids

Mark Jones, Executive External Strategy and Communications Fellow, Dow Chemical

Thursday, October 13, 2016

Failure: Why Science Is So Successful

Stuart Firestein, Author and Professor of Neuroscience, Columbia University

Darren Griffin, Professor of Genetics, University of Kent

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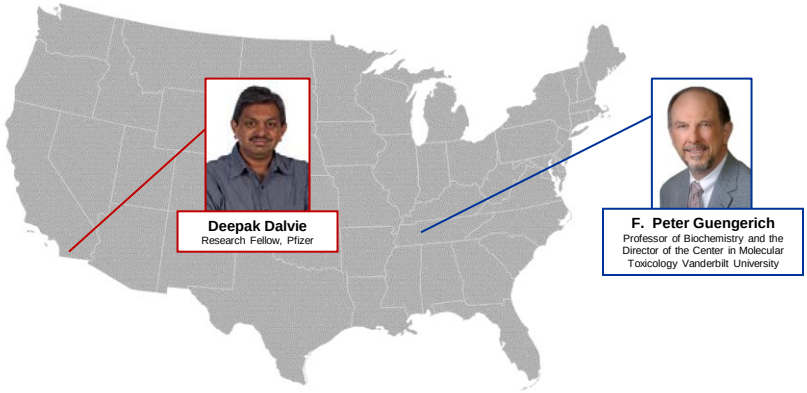
13

2016 Drug Design and Delivery Symposium

"Dealing with Reactive Drug Metabolites in Drug Discovery"



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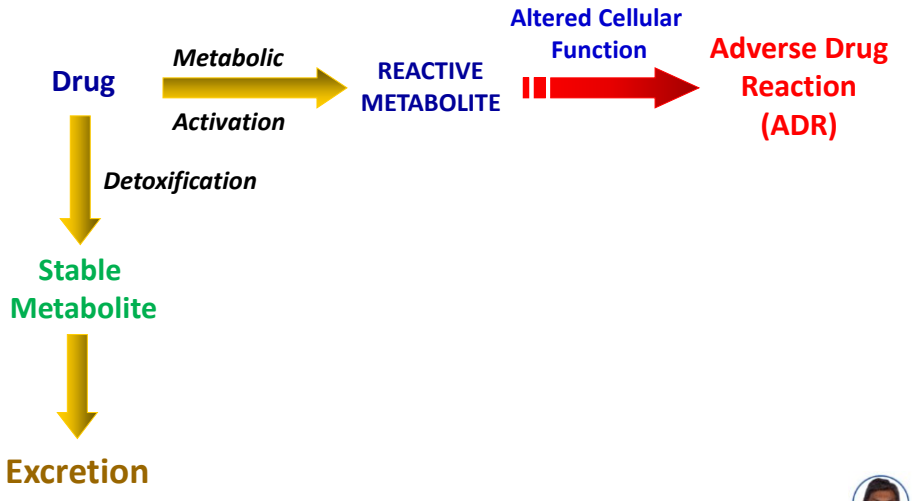
Dealing with Reactive Metabolites in Drug Discovery:

Can we Predict Toxicities of Drug Candidates that form Reactive Metabolites?

Deepak Dalvie
Pharmacokinetics, Dynamics and Metabolism Department
Pfizer

Addressing Reactive Metabolites in drug discovery has become regular routine

Circumstantial evidence links reactive metabolites to adverse drug reactions



Predicting Toxicity of Reactive Metabolites can be Challenging

Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

Which of these groups are considered to be "structural alerts" in medicinal chemistry?

A) 

B) 

- A
- B
- Both
- Neither

Agenda

★ **Background**

- Idiosyncratic Adverse Drug Reactions (IADRs)
- Reactive metabolites
- Reactive Metabolite Assays

★ **Discuss why predicting toxicity of RM-positive compounds can be challenging?**

★ **Approaches to deal with reactive metabolites from a DM-PK perspective**

Adverse Drug Reactions

A Leading Cause of Candidate Attrition and Drug Recalls

Idiosyncratic Drug Reactions (IADRs)
A Major Problem

★ **Unpredictable**

★ **Not easy to study**

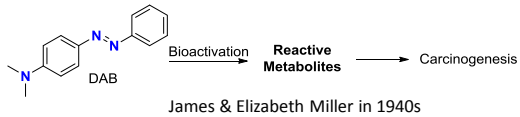
★ **The underlying mechanism is not clear**

★ **Believed to be immune mediated!**

Reactive Metabolites A Major Risk Factor in Toxicity

★ **Carcinogenic**

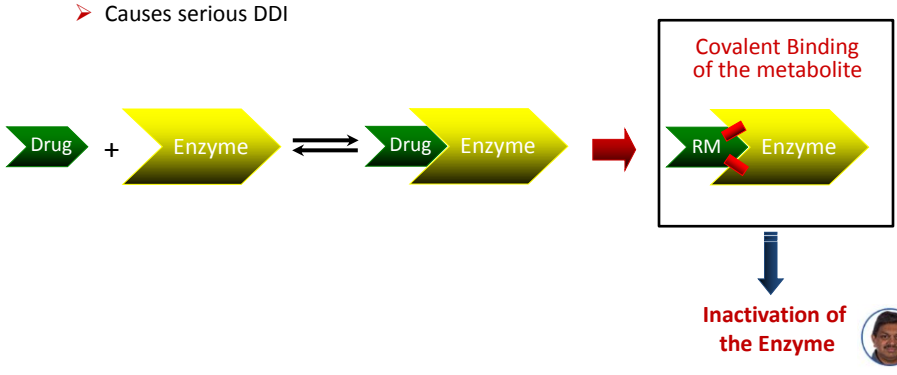
- Modify DNA
  - Genotoxicity



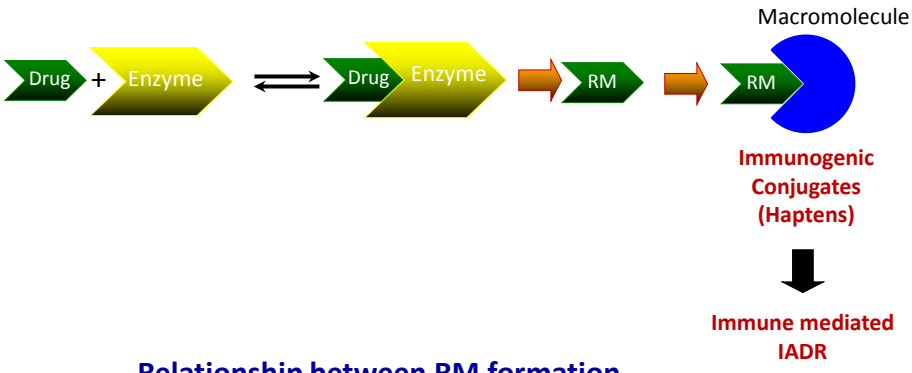
James & Elizabeth Miller in 1940s

★ **RM also associated with inhibition of enzymes – mainly P450**

- Covalently bind to the heme or apoprotein
- Results in inactivation of enzyme
- Causes serious DDI



Reactive Metabolites & Idiosyncratic Adverse Drug Reactions (IADR)



Relationship between RM formation and IADR is not well understood

★ **Basic Premise:**

Molecules <u>that do not produce</u> Reactive Metabolites will <u>not</u> cause IADRs

Basic Tactic Used By Pharma Industry
"Avoid Reactive Metabolites"

★ **Two approaches frequently adopted:**

★ **Exclude chemical functionalities undergoing metabolic activation**

- So called "Structural Alerts" or *known toxicophores*

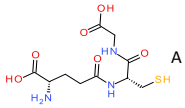
★ **Screen for Reactive Metabolite Formation (RM Assays)**

Commonly Used Methods

Covalent Binding Method

- ★ **Uses Radiolabel**
- ★ **Most definitive**

Trapping Electrophilic Species With Nucleophilic Reagents



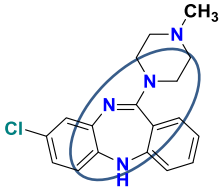
Glutathione
A Commonly Used Nucleophile

- ✦ **Most popular in a discovery setting**
- ✦ **Acts as a surrogate of covalent binding**

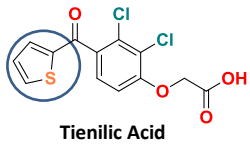
Evans D. C. et. al. *Chem. Res. Toxicol*. 2004

★ **Ultimate Goal: Eliminate the liability of RM formation**

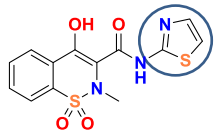
Drugs that Possess a Structural Alert can be Toxic!



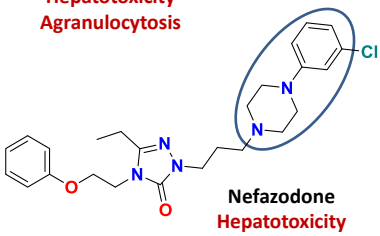
Clozapine
Hepatotoxicity
Agranulocytosis



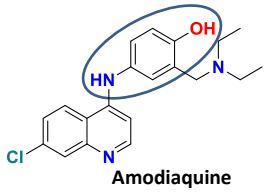
Tienilic Acid
Immune-mediated Hepatotoxicity



Sudoxicam
Hepatotoxicity



Nefazodone
Hepatotoxicity

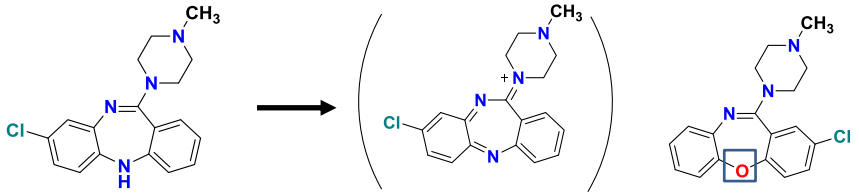


Amodiaquine
Hepatotoxicity

~ 80 % of the drugs associated with IADR
contain structural alerts and form reactive metabolites

Stefan, A. et. al. 2011 *Chem. Res. Toxicol.* 24:1345-1410

Absence of Structural Alerts Improves Safety Profile of Drugs

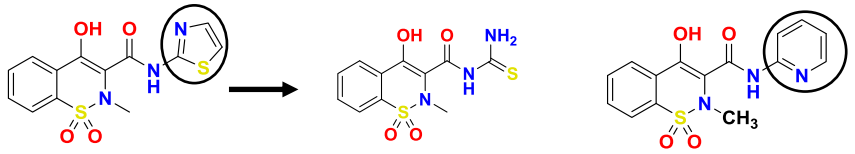


Clozapine
Toxic!

Reactive Iminium
Intermediate

Loxapine
Safer

Uetrecht et. al. 1997, Chem-Bio Interactions 104:117



Sudoxicam
Hepatotoxic

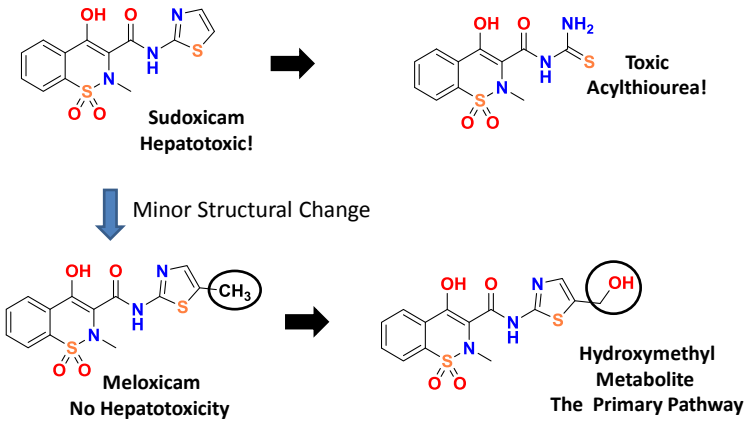
Toxic
Acylthiourea!

Piroxicam – Safer

Definitely a good idea to replace motifs that form reactive metabolites!

Not that Straightforward

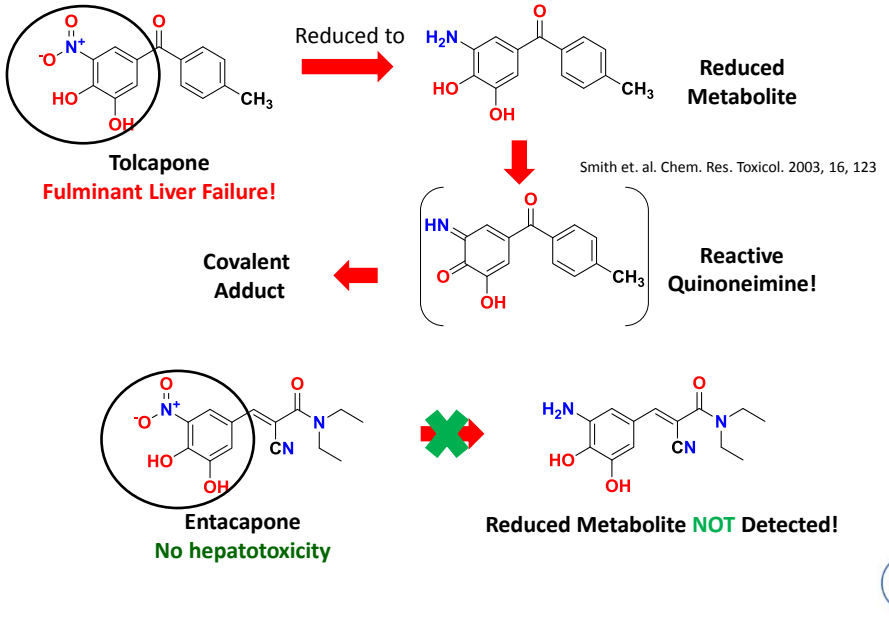
All Molecules with Structural Alerts Are Not Bioactivated!



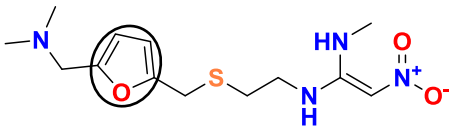
Introduction of methyl group dramatically alters the metabolic profile

Obach et.al. CRT 2008 21:1890

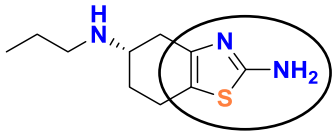
Tolcapone versus Entacapone



Some Molecules Contain A Structural Alert BUT the Clearance Mechanism is Different



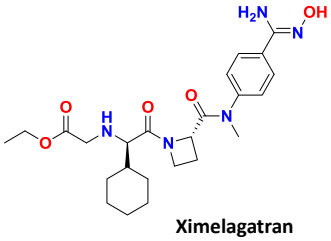
Ranitidine
SA - Furan



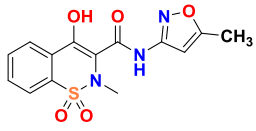
Pramipexole
SA - Aminothiazole

Both drugs are renally excreted!

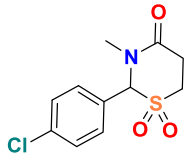
Some Molecules Devoid of SA Are Toxic



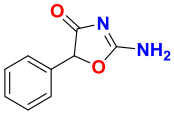
Ximelagatran



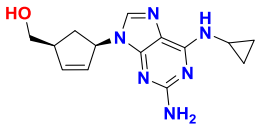
Isoxicam



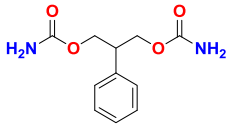
Chlormezanone



Pemoline

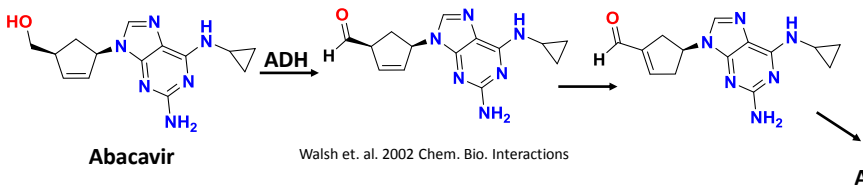


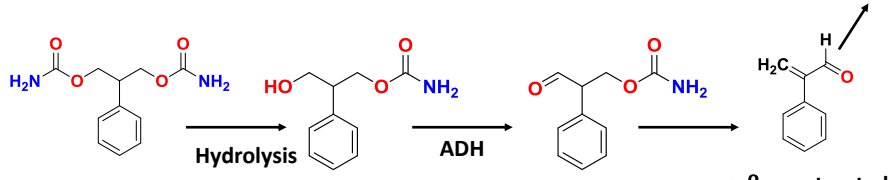
Abacavir



Felbamate

Felbamate and Abacavir – No Typical SA But Bioactivated!



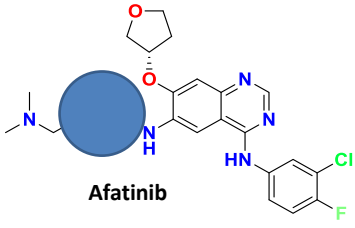


Relying on structural alert database alone may not be the right thing!

As new motifs are introduced – new structural alerts may surface

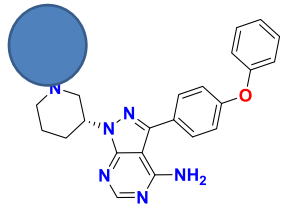
Structural Alerts Are Back!!

Recently Approved Irreversible Inhibitors



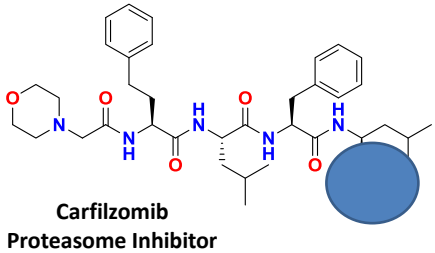
Afatinib

Covalent Modifier of EGFR



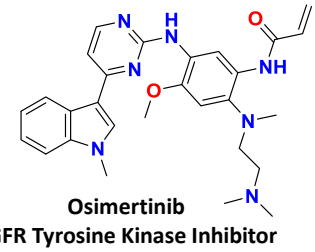
Ibrutinib

Bruton Tyrosine Kinase inhibitor



Carfilzomib

Proteasome Inhibitor

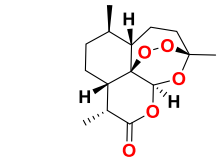


Osimertinib

EGFR Tyrosine Kinase Inhibitor

Received accelarated approval in 2015

Some Compounds Need Bioactivation to Exert Action

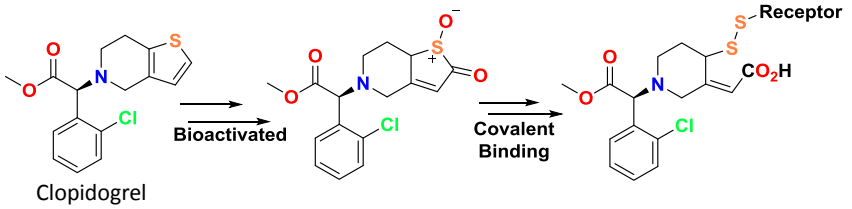


Artemisinin

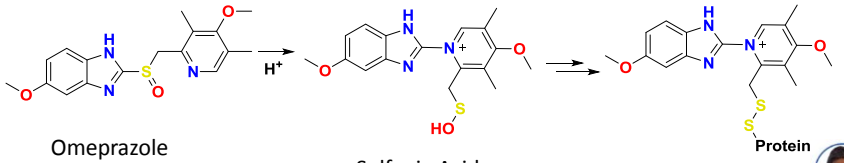
Bioactivated By Heme and Fe(II) oxide

Free Radicals

Antimalarial Activity



Clopidogrel



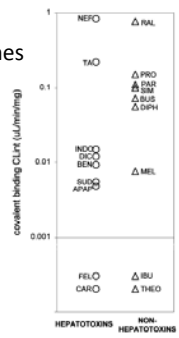
Omeprazole

Sulfenic Acid

What About Reactive Metabolite Assays?

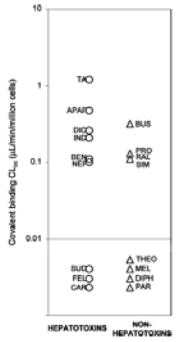
Data Generated By Obach and his Colleagues

Microsomes



Some Differentiation

With Hepatocytes



Hepatocytes

★ **Overlap observed between hepatotoxins and non-hepatotoxins**

Bauman *Chem. Res. Toxicol*. 2009, 22, 332-340
Obach *Chem. Res. Toxicol*. 2008, 21, 1814-1822

Covalent Binding or GSH adduct formation only suggests the presence of an electrophilic intermediate!

★ **Lack of Covalent Binding or GSH Adduct formation does not guarantee safety**

What Does This Tell Us?

★ **Avoiding SA and eliminating RM formation– makes sense**

- Avoids Risks

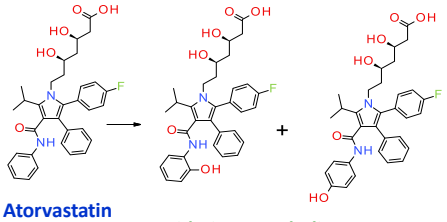
★ **Reactive Metabolite formation does not predict toxicity**

- No guarantee that elimination of reactive metabolites will make a safe drug
- Presence of a **structural alert** will **not** make the molecule **toxic**!

★ **Several widely prescribed drugs form reactive metabolites**

- Stefan, A. et. al. 2011 *Chem. Res. Toxicol*. 24:1345-1410

Even a #1 Blockbuster drug
contains SA
and can form potential RMs



Atorvastatin

Oxidative Metabolites

Bottomline

NO ASSAY OR KNOWLEDGE BASED SYSTEMS
CAN PREDICT THE POTENTIAL OF A DRUG TO CAUSE IADR !

More to it than just bioactivation!

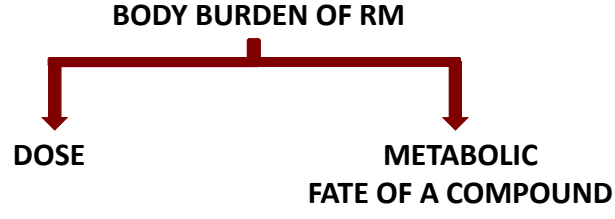
Why is there a Disconnect?

- ★ Several factors influence toxicity
  - ➢ Bioactivation is a part of it
- ★ Some other factors that may result in IADRs
  - ➢ Direct association with Human Leukocyte Antigen (HLA)
    - ❑ Examples:
      - ❖ Abacavir
      - ❖ Allopurinol
      - ❖ Carbamazepine
      - ❖ Flucloxacillin
  - ➢ Transporters – Inhibition of the Bile Salt Export Pump (BSEP)
    - ❑ Examples
      - ❖ Bosentan
      - ❖ Imitanib
  - ➢ It is multi-factorial most of the times
    - ❑ Troglitazone

What is the Path Forward?

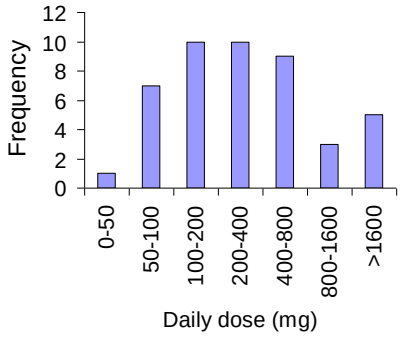
★ Body Burden of a Reactive Metabolite can influence IADR

★ One Strategy - Reduce the body burden of the electrophilic intermediate



Impact of DOSE – Now a Well Known Concept!

★ **Low dose reduces the risk of IADRs**



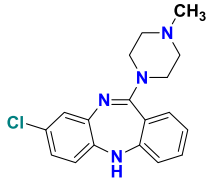
Lower the Dose
↓
Lesser reactive metabolite formed
↓
Lower the Risk

Kalgutkar, A. et. al.2005, *Curr. Drug Metab.* 6:161:225

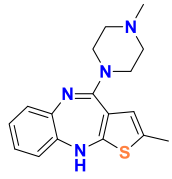
Uetrecht, J. 1999 *Chem. Res. Toxicol.* 12:387-95
Lammert, C. et. al. 2008, *Hepatology* 47:2003-2009
Stefan, A. et. al. 2011 *Chem. Res. Toxicol.* 24:1345-1410

Impact of Dose on Toxicity

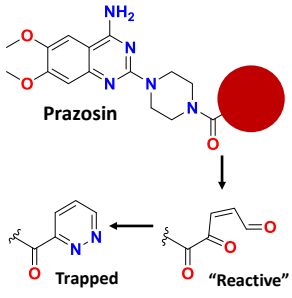
Mol Pharmacol 1998 53:999



Clozapine
Dose: 300-900 mg



Olanzapine
Dose: 10 mg



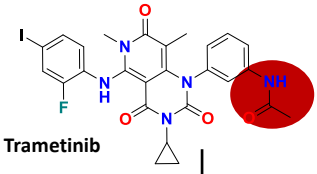
Prazosin

Dose: ~1 mg

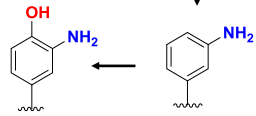
Trapped **"Reactive"**

Erve et. al. DMD 2007 35:908

Dose: ~ 2 mg



Trametinib

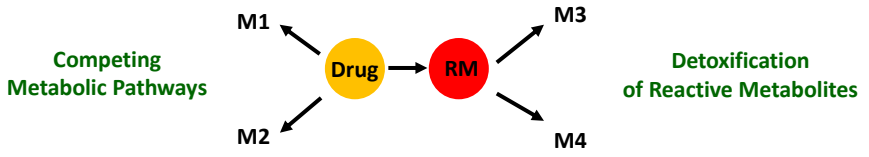


Both Are Structural Alerts!

Other Driver of RM Body Burden – Metabolic Fate

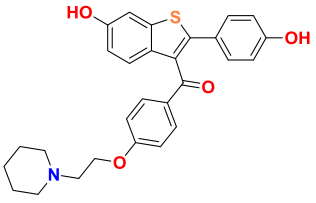
★ **Amount of a Reactive Metabolite Formed depends on:**

- Contribution of competing metabolic routes
- Contribution of pathways that detoxify the reactive metabolite

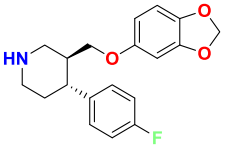


More the pathways ⇨ lesser the amount of RM

Raloxifene & Paroxetine



Raloxifene

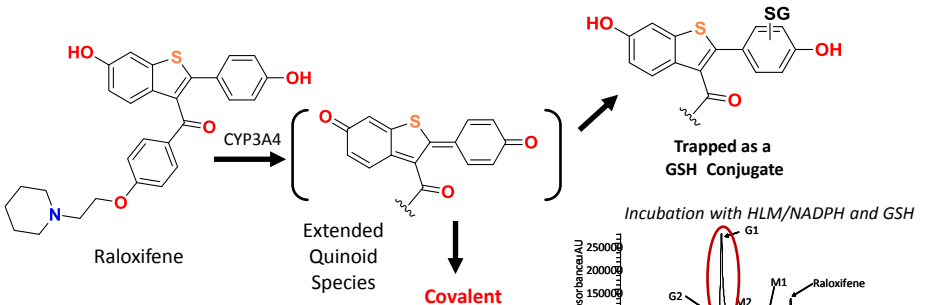


Paroxetine

Two cases that illustrate influence of metabolic pathways on RM body burden

The Raloxifene Case

- ★ **Raloxifene - A selective estrogen receptor modulator (SERM)**
- ★ **Bioactivated by CYP3A4 to quinoid intermediates**
- ★ **A mechanism-based inhibitor of CYP3A4 –** *covalently binds to Cys residue in CYP3A4 (CRT 2007)*
- ★ **No IADRs or DDIs reported**



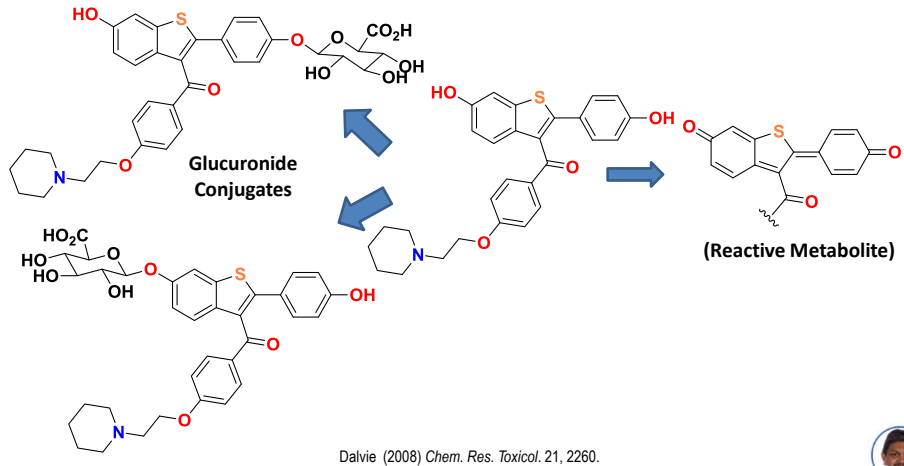
Yu, L. et. al. Chem. Res. Toxicol. 2004 17, 879

Covalent Binding to Human liver Microsomes

Dalvie et. al. *Chem.Res.Toxicol.* 2008, 21:2260-2271

Why is Raloxifene is Devoid of any IADRs or DDI ?

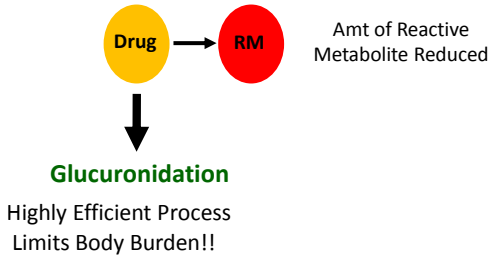
- ★ **Primary route of raloxifene metabolism – Glucuronidation**
- ★ **Highly efficient glucuronidation pathway competes with oxidation**



Dalvie (2008) *Chem. Res. Toxicol.* 21, 2260.

Competing Pathways Make A Difference

- **Glucuronidation limits the amount of raloxifene undergoing bioactivation**

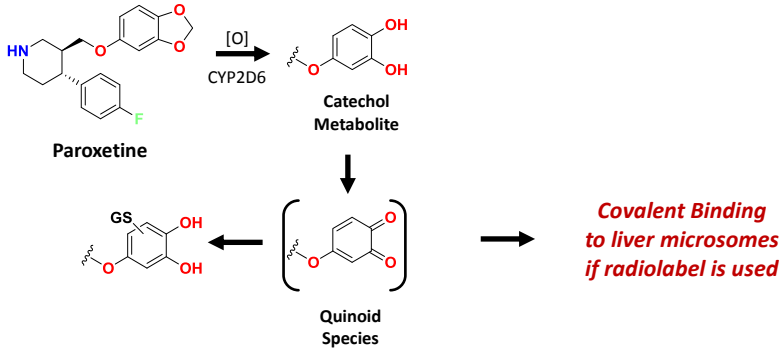


Dalvie et.al., (2008) *Chem. Res. Toxicol.* 21, 2260.

- **Dose may also influence (Dose of raloxifene – 60 mg QD)**

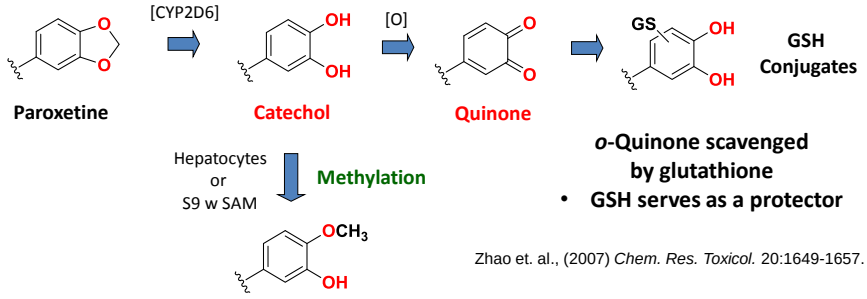
Paroxetine Case

- ★ **Paroxetine – a widely used antidepressant**
- ★ **Undergoes metabolic activation**
- ★ **But Paroxetine is not a hepatotoxin!**



Zhao et. al., (2007) *Chem. Res. Toxicol.* 20:1649-1657.

Detoxification of Reactive Metabolite and its Precursor Influences its Body Burden



***o*-Quinone scavenged by glutathione**

- **GSH serves as a protector**

Zhao et. al., (2007) *Chem. Res. Toxicol.* 20:1649-1657.

★ **Methylation limits the amount of catechol being oxidized**

★ **Dose may contribute:**

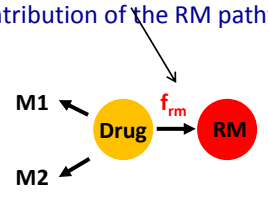
- Paroxetine - Low daily dose (20 mg QD)
- Reactive metabolite burden is readily handled by endogenous glutathione pool

What did we learn from this ?

★ **It is all about Body Burden!**

★ **RM Body Burden is dependent on**

- Dose
- Contribution of the RM pathway



★ **Knowledge of the complete metabolite profile is important**

- Important to use integrated tissue systems
- **Liver S9 w co-factors or hepatocytes** rather than liver microsomes

Take Home Message!

★ **Predicting toxicity of RM-Positive compounds is challenging**

★ **Good to avoid Structural Alerts**

- Replace if a bioisotere is available
  - If there is no loss of pharmacological activity
- Avoids unnecessary risk assessment

★ **If the Structural Alert is necessary for activity**

- Demonstrate if the SA is prone to RM using met ID

★ **RM Assays are good "Gate Keepers"**

- A "flag" to trigger additional studies
- GSH adducts provide a mechanistic understanding of bioactivation
- **Results** need to be put in right context

Take Home Message!

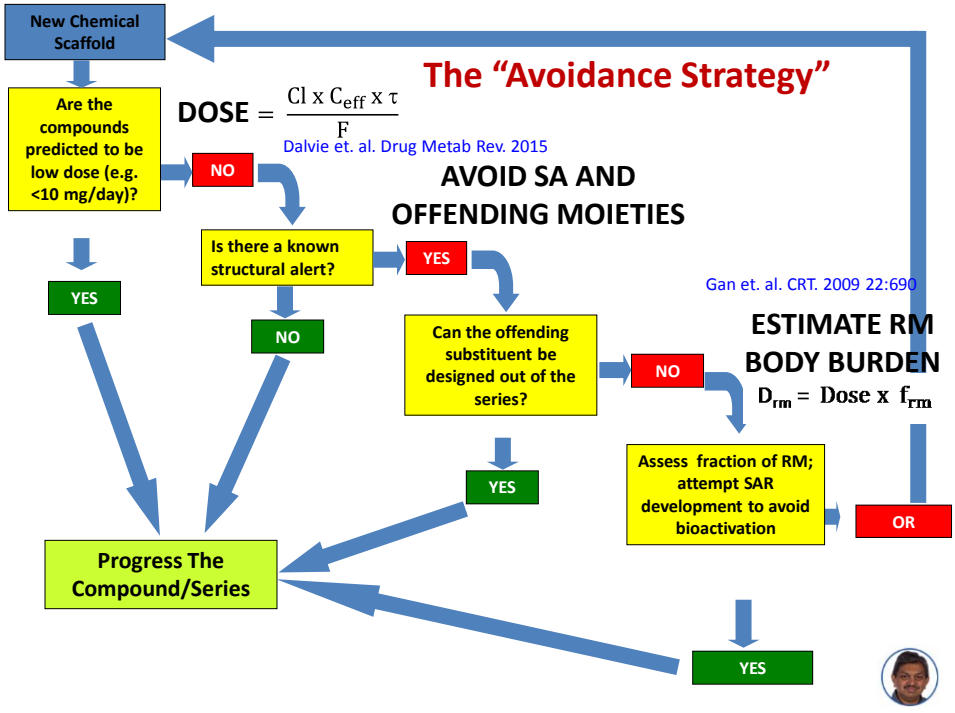
★ **Keeping the dose low is important**

- Exposure to RM is tolerated

★ **Estimation of RM Body Burden could be useful**

- A more positive step towards prediction of IADR Risks

B.K. Park et. al. *Nature Drug Discovery* 10, 292-309 (2011) – A seminal paper!



Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

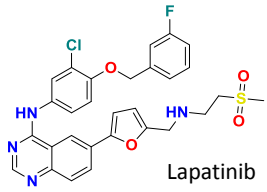
What other factors can you not forget when thinking about level of risk? (multiple correct answers)

- Indication
- Route of Administration
- Target Population
- pH
- Medical need

Never Forget Other Factors!

★ **Level of Risk also depends on factors such as**

- Indication
- Medical need
- Target population …



Lapatinib

High Dose – 1250 mg
Black box warning
Metabolites – contain SA
Indication - ONCOLOGY

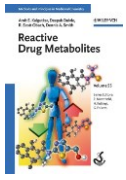
★ **These factors will influence the avoidance strategy**

Acknowledgements

Amit Kalgutkar – Cambridge Site Scott Obach – Groton Site

Kalgutkar AS. and **Dalvie DK.** ***Annu. Rev. Pharmacol. Toxicol*****. 2015. 55:35–54**

Dalvie D, Kalgutkar AS. and Chen W. ***Drug Metab Rev*****. 2015. 47:56-70.**



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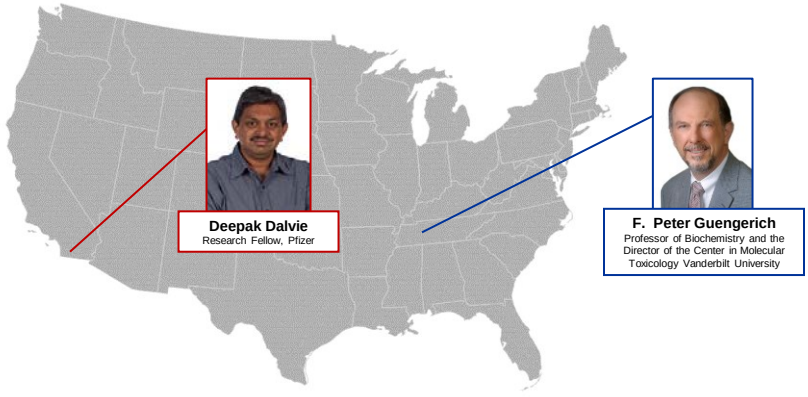

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Stuart Firestein, Author and Professor of Neuroscience, Columbia University

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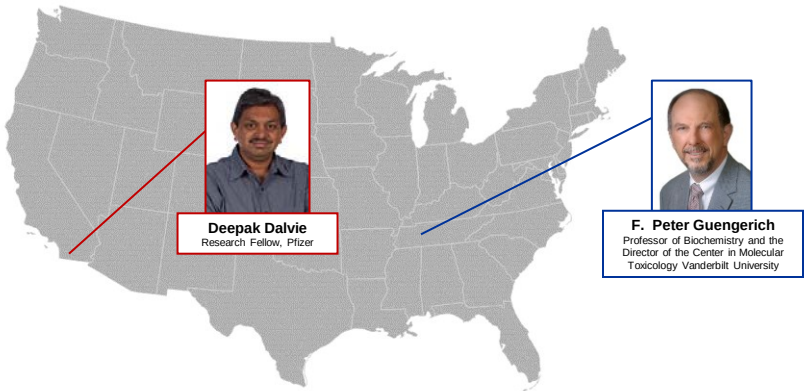

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