Reactive Metabolites and Drug Safety

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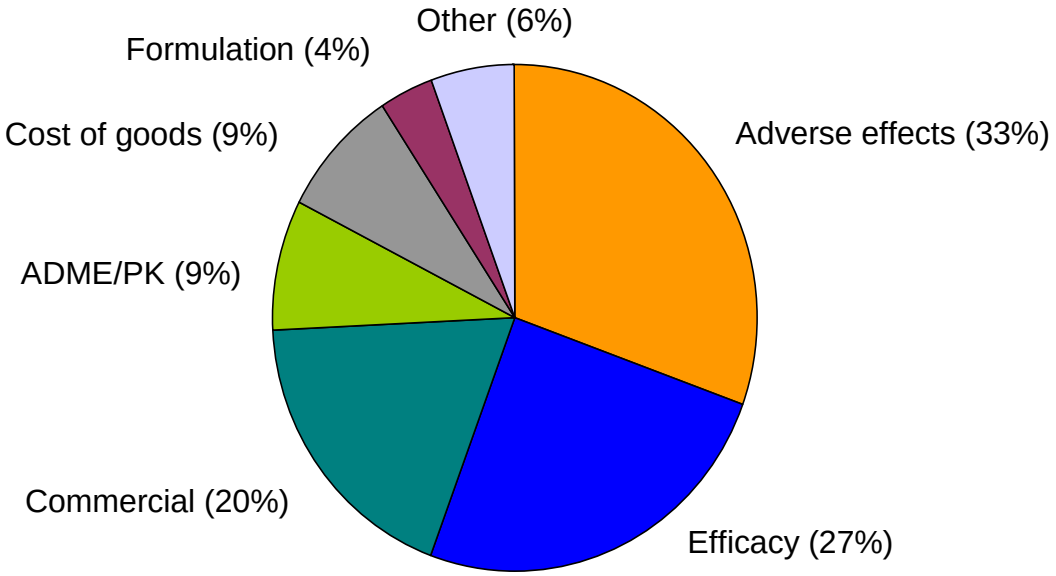
Reactive Metabolites and Drug Safety

- Adverse Drug Reactions (ADRs) and Drug Attrition
- Bioactivation/reactive metabolite formation
- Link between ADRs and Bioactivation
- Structural alerts
- Experimental techniques to detect reactive metabolites
- Strategies to avoid reactive metabolites
- Placing metabolic activation in proper context
- Summary

Adverse Drug Reactions (ADRs) and Drug Attrition

- Contribute to patient morbidity and mortality.
- Cause significant financial burden on the healthcare system.
- Main reason of black box warnings and drug withdrawals for marketed drugs.
- Actual leading cause of Drug Attrition for drugs in development.



Drug Attrition Rate-2000. Pharmaceutical R&D Benchmarking Forum, General Metrics 2001

Almirall

ADR Classification

Type A or Predictable Reactions (80% of ADRs):

- Identified in preclinical toxicological assays.
- Dose-dependent, can be reversed by dose reduction.
- Mechanism-related.

Hypotension with antihypertensives.

Gastrointestinal side effects with classical NSAIDS.

Type B, Unpredictable or Idiosyncratic Reactions (IADRs):

- Can occur at any dose.
- Low-rare frequency (1/10000 o 1/100000).
- Independent of pharmacology.

Hepatotoxicity. Hepatitis.

Cutaneous ADRs.

Agranulocytosis. Blood discrasia.

Examples of drugs associated to type B ADRs

Marketed drugs

Drug	ADRs
Abacavir	rash, fever
Acetaminophen	acute and chronic hepatitis
Amiodarone	hepatitis
Captopril	agranulocytosis, epidermal necrosis
Carbamazepine	hepatitis, agranulocytosis, rash
Chloramphenicol**	aplastic anaemia, cutaneous ADRs
Clozapine	**agranulocytosis, hepatotoxicity**
Cyclophosphamide	agranulocytosis, cutaneous ADRs
Dapsone	agranulocytosis, cutaneous ADRs
Diclofenac	hepatotoxicity
Dihydralazine	autoimmune hepatitis
Felbamate**	liver failure, aplastic anaemia
Flutamide	hepatitis, cutaneous ADRs
Furosemide	agranulocytosis, cutaneous ADRs, aplastic anaemia
Halothane	autoimmune hepatitis
Imipramine	hepatitis, agranulocytosis
Indomethacin	hepatitis
Isoniazid	severe and fatal hepatitis
Lamotrigine	rash
Methimazole	hepatitis, agranulocytosis, aplastic anaemia
Metroindazole	neutropenia, rash
Mianserin	agranulocytosis, neutropenia
Minocycline	autoimmune hepatitis, neutropenia, trombocytopenia
Phenytoin	agranulocytosis, neutropenia, cutaneous ADRs
Procainamide	hepatitis, agranulocytosis
Tacrine	hepatitis
Terbinafine	cutaneous ADRs
Thalidomide	teratogenicity
Ticlopidine	agranulocytosis, aplastic anaemia
Timetrophim	cutaneous ADRs, agranulocytosis, aplastic ananemia
Tolbutamide	teratogenicity
Tolcapone**	severe hepatotoxicity
Tranylcypromine	hepatitis, agranulocytosis
Trazodone	hepatotoxicity
Valproic acid	hepatotoxicity, teratogenicity
Zileuton	transaminase elevations

** limited use

Withdrawn drugs

Drug	ADRs
Aclofenac	rash, hepatitis
Alpidem	severe and fatal hepatitis
Aminetine	hepatitis, cutaneous ADRs
Aminopyirine	agranulocytosis
Amodiaquine	hepatitis, agranulocytosis
Benoxaprofen	hepatitis, cutaneous ADRs
Bromfenac	hepatotoxicity
Carbutamide	bone marrow toxicity
Ibufenac	severe and fatal hepatitis
Iproniazid	severe and fatal hepatitis
Metiamide	bone marrow toxicity
Nefazodone	acute liver failure
Nomifensine	fatal hepatitis, anaemia
Practolol	cutaneous ADRs
Remoxipride	aplastic anaemia
Sudoxicam	**hepatotoxicity**
Tienilic acid	autoimmune hepatotoxicity
Tolrestat	severe hepatotoxicity
Troglitazone	**hepatotoxicity**
Zimelidine	transaminase elevations
Zomepirac	hepatitis, cutaneous ARDs

All these drugs are susceptible to metabolic activation

Metabolic activation/Bioactivation

- The **biotransformation reactions** of a xenobiotic can be classified as:
  - **Phase I reactions**: oxidation, reduction, hydrolysis.
    Introduction of a functional group to increase aqueous solubility.
  - **Phase II reactions**: conjugation.
    Formation of O or N glucuronides, sulfates, acetates….to increase hydrophilicity.

- Usually, after a biotransformation reaction, the drug is biologically deactivated. **Metabolism** is regarded as a mechanism of **detoxification**.

- However, the metabolic events can generate **chemically reactive** and toxic **metabolites**: **BIOACTIVATION**.

IS BIOACTIVATION RESPONSIBLE FOR TYPE B ADRs?

Why is it difficult to predict type B ADRs?

There is not *in vivo* proof that reactive metabolites are ultimately responsible for type B ADRs.

- No direct 1:1 correlation has been established for reactive metabolism and toxicity.
- Precise mechanism of toxicity unknown.
- Lack of availability of animal models to evaluate type B ADRs in preclinical development.

The evidence linking *in vitro* drug bioactivation to type B ADRs is circumstantial.

- Genetic polymorphism/modulation of drug metabolizing enzymes as risk factors.
- Reactive metabolites.
- *Hapten hypothesis*: covalent binding to targe tissues.
- *Danger hypothesis*: drug-macromolecules conjugates lead to cell damage.
- Structure-toxicity relationships: chemical features that could lead to toxic side effects ⇢ **STRUCTURAL ALERTS**

Structural Alerts: Chemical functionalities known to be susceptible to form reactive metabolites

Structural Alert	Reactive metabolite
Aliphatic amines	Iminium ion
Alkylhalides	Acylhalides
Alkynes	Ketene, oxirene
Anilines/anilides	Quinone-imine, nitroso metabolite
Arenes, bromoarenes	Arene oxide
Benzylamines	Nitroso, oxime
Carboxylic acids	Acyl glucoronides
Cyclic secondary amines	*N*-Hydroxy or nitroxide metabolite
Cyclopropylamines	α,β-Unsaturated carbonyl metabolite
Dibenzazepines	Nitrenum ion
Formamides	Isocyanate
Furans	α,β-Unsaturated dicarbonyl
Hydantoins	Free radical

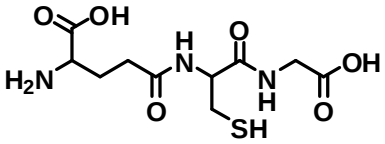
Structural Alert	Reactive metabolite
Hydrazine, hydrazide	Diazene or diazonium ion
Hydroquinones	*p*-Benzoquinone
Methylenedioxyphenyl	*o*-Quinone
3-Methyleneindoles	Imine-methide
Michael acceptors	Intrinsic electrophilicity
Nitrobenzenes	Nitroso derivative
o- or *p*-Alkylphenols	*o*- or *p*-Quinone methide
Sulfonylureas	Isocyanate
Thiazoles	Thioamide, glyoxal
Thiazolidinediones	α-Ketoisocyanate
Thiols	Disulfides
Thiophenes	α,β-Unsaturated dicarbonyl
Thioureas	*S*-oxide, isocyanate

A.S.Kalgutkar and J.R. Soglia, *Exper.Opin.Drug.Metab.Toxicol*. **2005**, *1(1)*, 91-142
A.S.Kalgutkar *et al*., *Curr.Drug.Metab*. **2005**, *6*, 161-225

In vitro strategies to detect reactive metabolites

Technical difficulty: - → +

- **Trapping electrophilic reactive metabolites *in situ***
  - Incubation of the drug with liver microsomes or hepatocytes in the presence of nucleophiles such as glutathione (GSH), N-acetylcysteine, lysine, histidine or cyanide.



glutathione (GSH)

- **Metabolite identification**
- **Covalent binding of reactive intermediates to biological macromolecules**
  - The extent of protein modification is based on the amount of radiolabel covalently bound to a protein following *in vitro* incubation to microsomes or hepatocytes.
  - Need radiolabeled compound in a stable (chemically and metabolically) position of the molecule.
- **Enzyme inactivation studies**
  - The drug is inactivating p450 enzymes: mechanism-based inactivators or suicide substrates.
- **Immunochemical approaches for identifying and characterizing protein targets of reactive metabolites**
  - Synthesis of an antibody that is able to identify haptenized proteins.

Strategies to avoid metabolic activation

- Direct replacement of the potential structural alert
  - Case study I: D_2/5-HT_2 antagonist family
  - Case study II: Taranabant

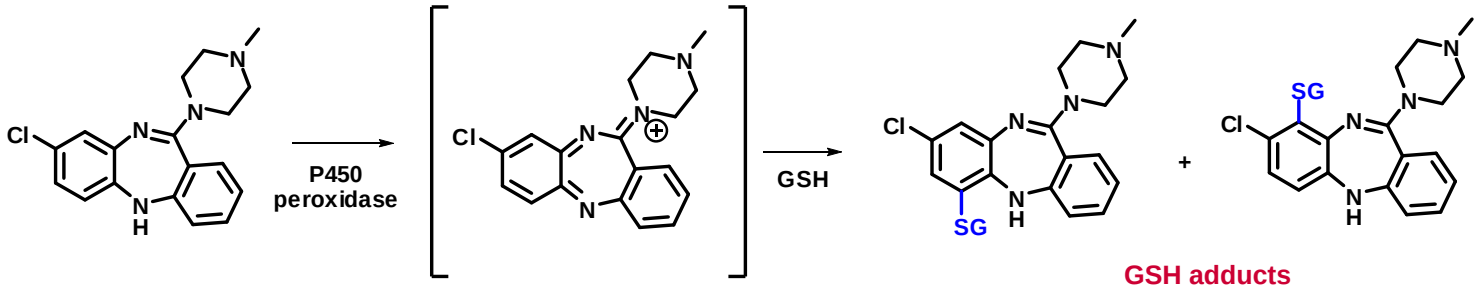
- Block site of bioactivation
  - Case study III: glucokinase (GK) activators

- Introduction of metabolic soft spots
  - Case study IV: Sudoxicam *vs* Meloxicam

Direct replacement of the structural alert

- Clozapine is an anti-psychotic agent (D_2/5-HT_2 antagonist) that has black box warnings associated with agranulocytosis and hepatoxicity.

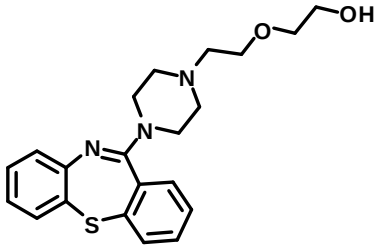
- Contains a **dibenzazepine structural alert**.



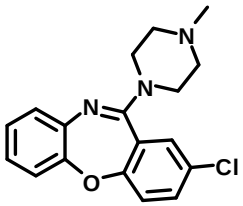
Reactive iminium cation: metabolite responsible for **covalent binding** in neutrophils

GSH adducts

- **Replace NH → Avoid iminium formation → NO AGRANULOCYTOSIS**



Quetiapine, Seroquel
One of the top 20 best-selling drugs

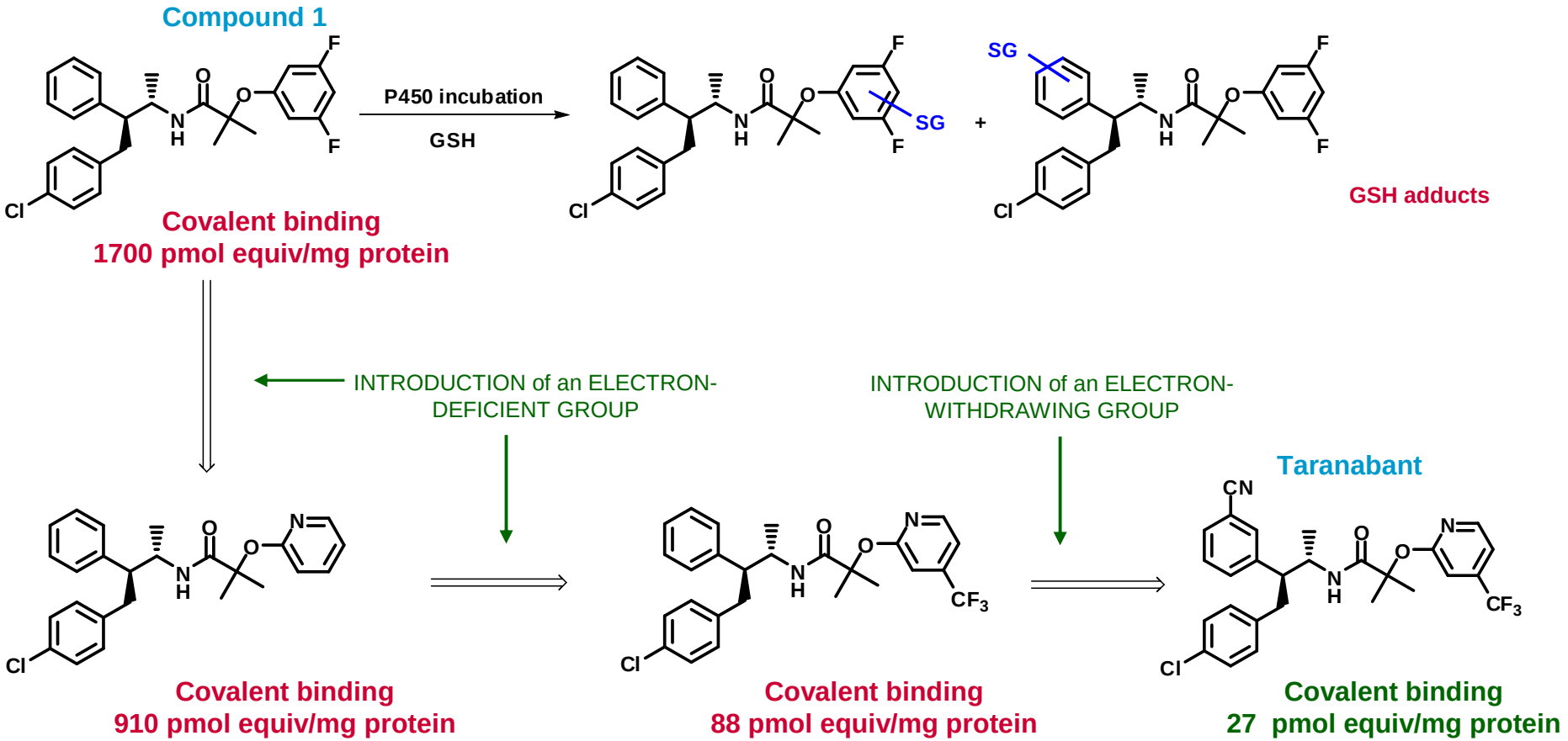


Loxapine

J.P. Uetrecht. *Chem.Res.Toxicol.* **1999**, *12(5)*, 387-395
A.S.Kalgutkar and J.R. Soglia, *Exper.Opin.Drug.Metab.Toxicol*. **2005**, *1(1),* 91-142

Direct replacement of the structural alert

Discovery of cannabinoid 1 (CB1) inverse agonists for obesity at Merck. From advanced lead compound **1** to development compound Taranabant.



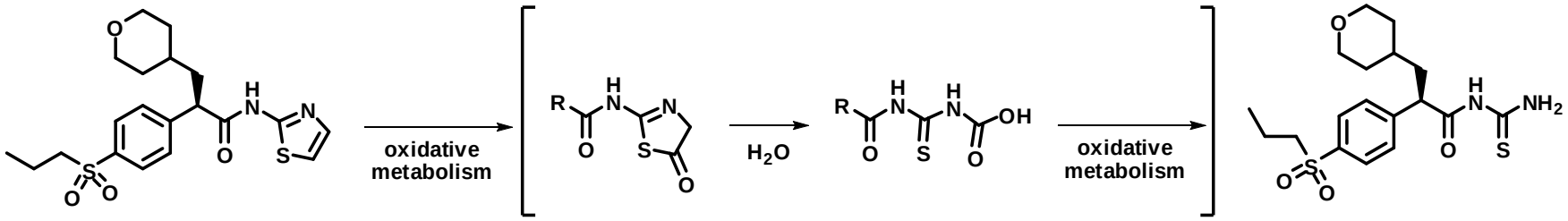
Merck cut off: 50 pmol equiv/mg protein

W.K. Hagmann *et al. J.Med.Chem.* **2006**, *49*, 7584-7587
S.Kumar *et al. J.Mass.Spectrom.* **2003**, *38,* 211-221

Block site of bioactivation

Discovery of glucokinase (GK) activators for type 2 diabetes at OSI Pharmaceuticals.

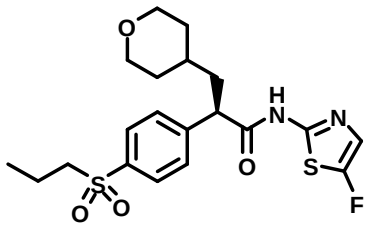
- Starting lead compounds with a **thiazole** ring showed **toxicity in rats**.



Toxic compound: rat death at 100 mg/Kg p.o.

Toxic metabolite detected *in vivo* in Sprague-Dawley rats

- Blocking oxidative metabolism by **introducing** a **fluorine** led to an active and **safe compound**.



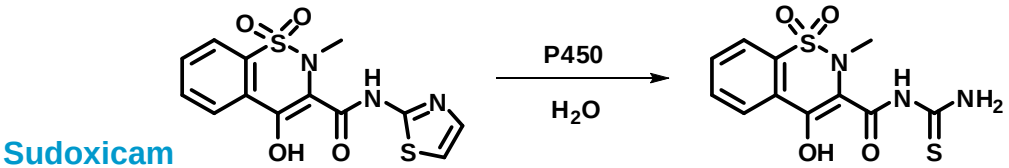
No evidence of toxicity in rats up to 250 mg/Kg
Thiourea not detected in rat plasma samples

L.S.Bertram *et al. J.Med.Chem.* **2008**, *51*, 4340-4345

Introduction of metabolic soft spots

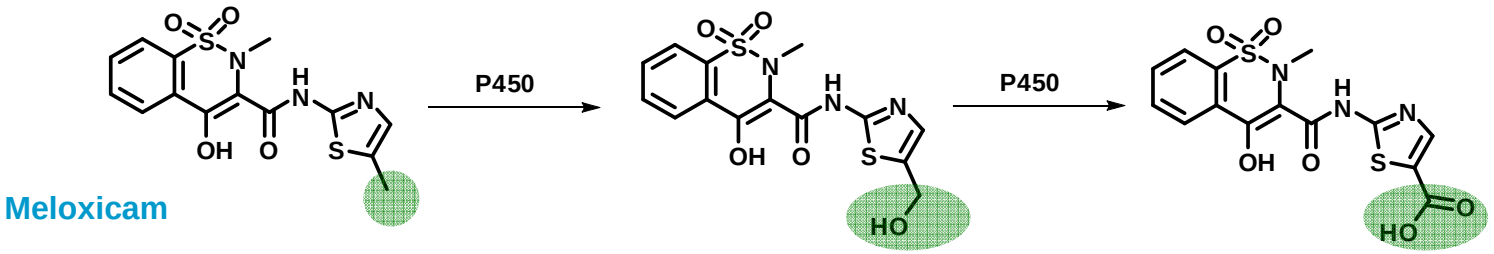
Enol-carboxamide-containing COX inhibitors for inflammation.

- Sudoxicam produced acute liver failure and it was withdrawn from Phase III clinical trials.
- Contains the **thiazole structural alert**.



Thiazole ring opening main metabolic route

- Meloxicam reached the market in 1996. Hepatic side effects are rare.



Oxidation of the methyl group is the main metabolic route
Methyl group: METABOLIC SOFT SPOT

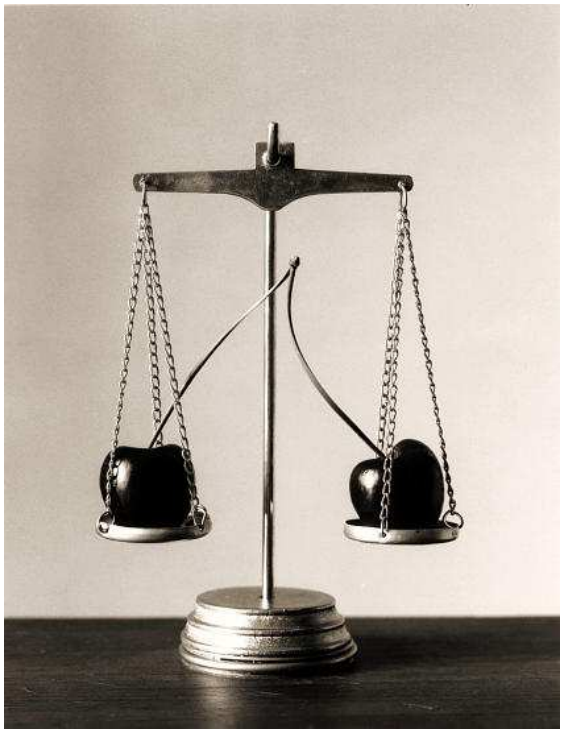
A.S.Kalgutkar *et al*., *Curr.Drug.Metab*. **2005**, *6*, 161-225

Placing metabolic activation in context

What is the nature of the medical need?

Target population Children? Immuno-compromised patients?

Is the drug intended for acute or chronic treatment?



Are *in vitro* techniques reliable predictors of type B ADRs?

Novel drug target lacking proof of principle

Chemical tractability of structural series

Projected clinical dose

RISK BENEFIT ASSESSMENT

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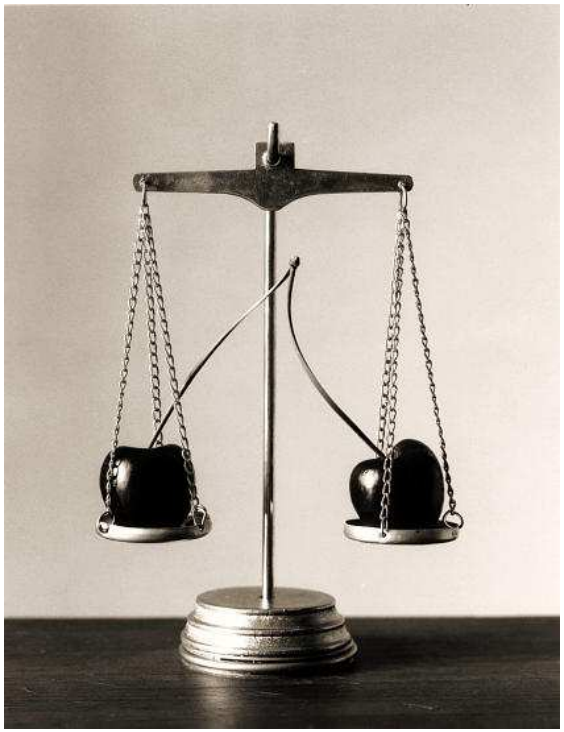

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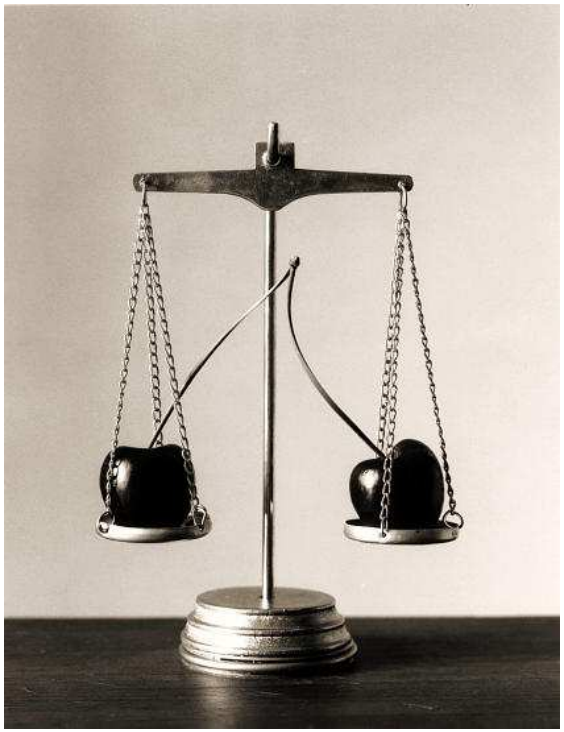

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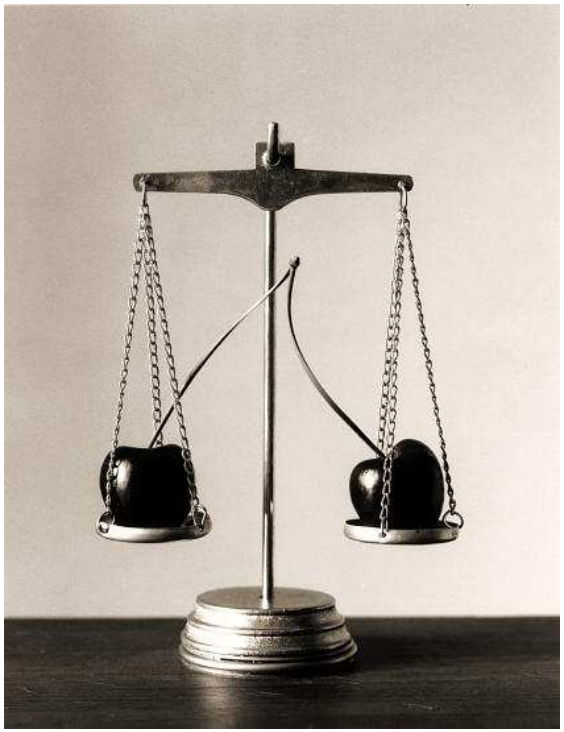

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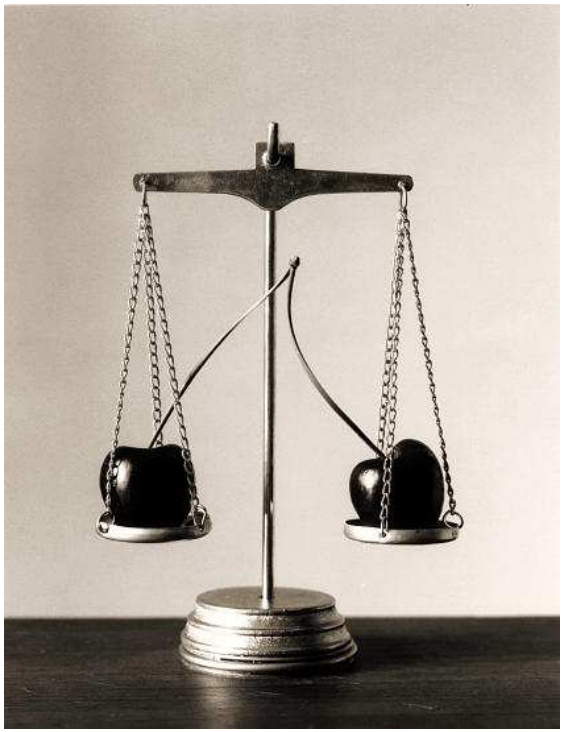

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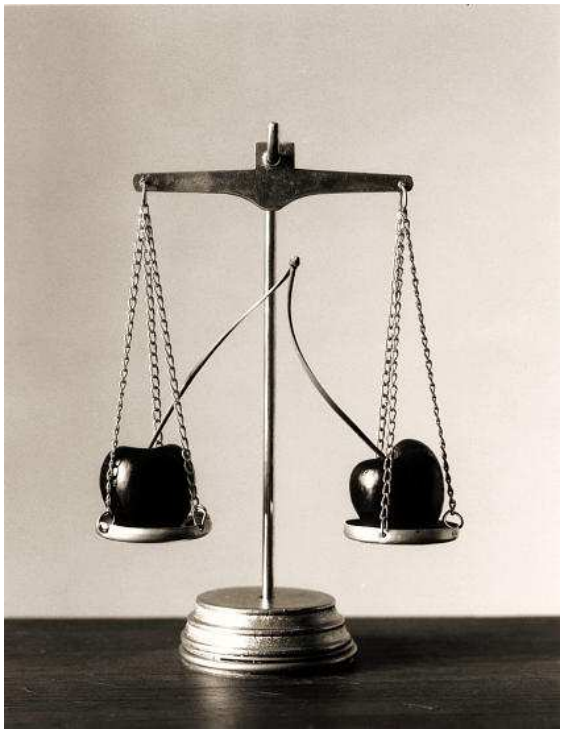

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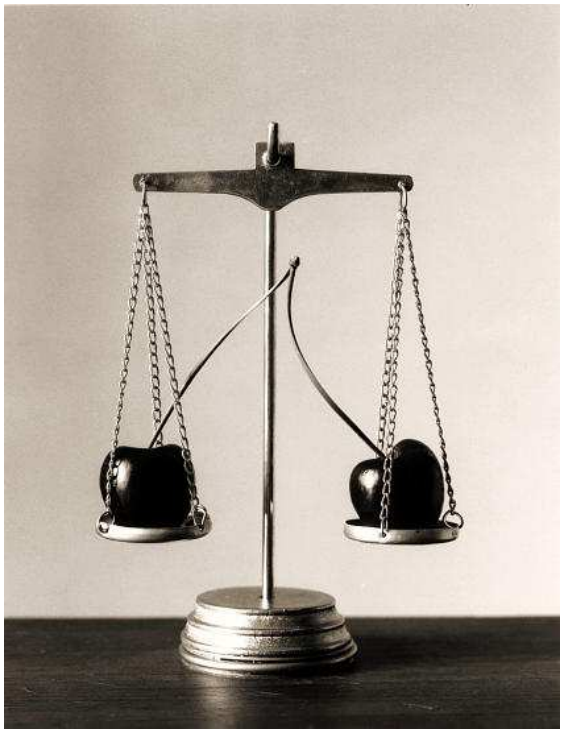


Are *in vitro* techniques reliable predictors of type B ADRs?

Case study V: Paroxetine

Novel drug target lacking proof of principle

Chemical tractability of structural series

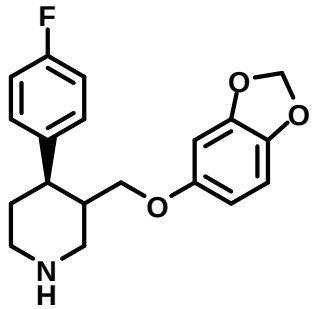
Projected clinical dose

RISK BENEFIT ASSESSMENT

Detoxication of reactive metabolites of Paroxetine

Studies on the bioactivation of Paroxetine, a serotonin reuptake inhibitor possessing anti-depressant activity.

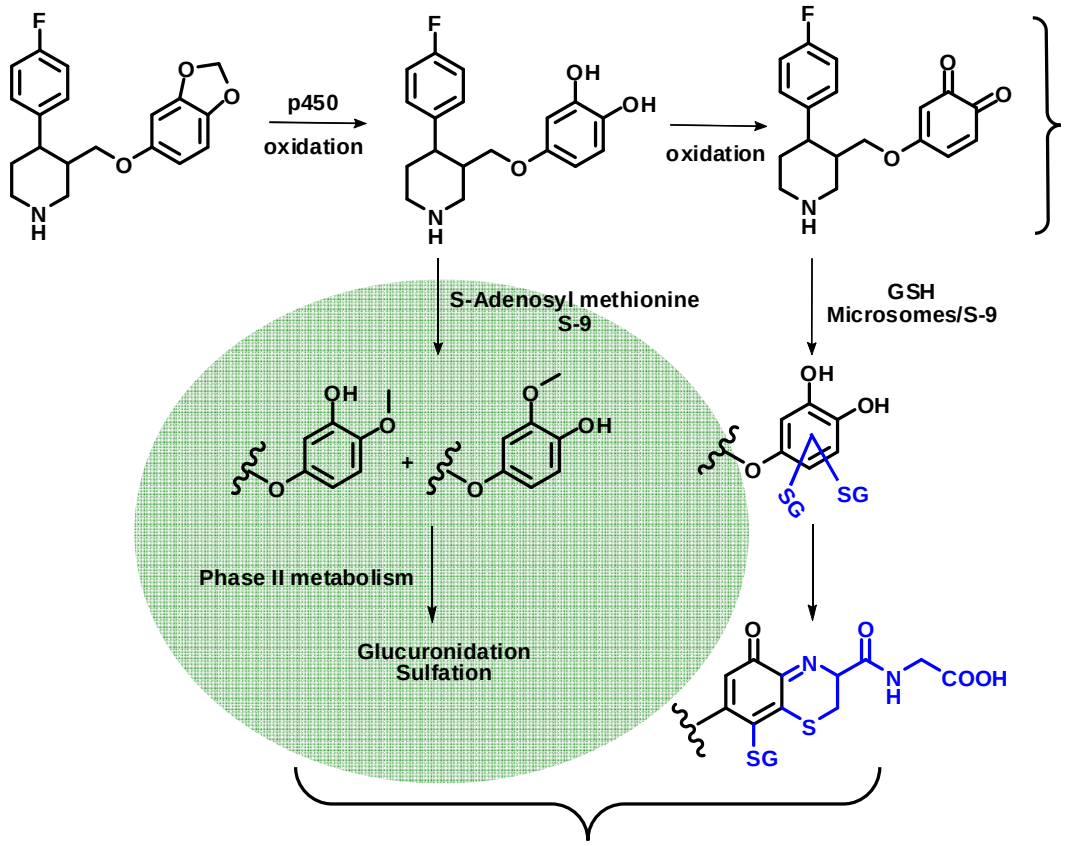
- Contains the methylenedioxyphenyl structural alert.
- Type B ADRs (especially hepatotoxicity) are extremely rare.



Paroxetine

Detoxication of reactive metabolites of Paroxetine

Overall summary of Paroxetine bioactivation/detoxication:



BIOACTIVATION
Covalent binding to microsomes/S-9

Major paroxetine metabolites in human are the guaiacol iomers and phase II conjugates thereof

Decreased covalent binding to microsomes/S-9
DETOXICATION

A.S.Kalgutkar *et al*., *Expert.Rev.Clin.Pharmacol.* **2008**, *1(4)*, 515-531
M.Segura *et al*., *Bioorg.Chem.* **2003**, *31*, 248-258

Placing metabolic activation in context

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Is the drug intended for acute or chronic treatment?

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Chemical tractability of structural series

Projected clinical dose

The same offending moiety can led to toxic or clean compounds, depending on the daily dose.

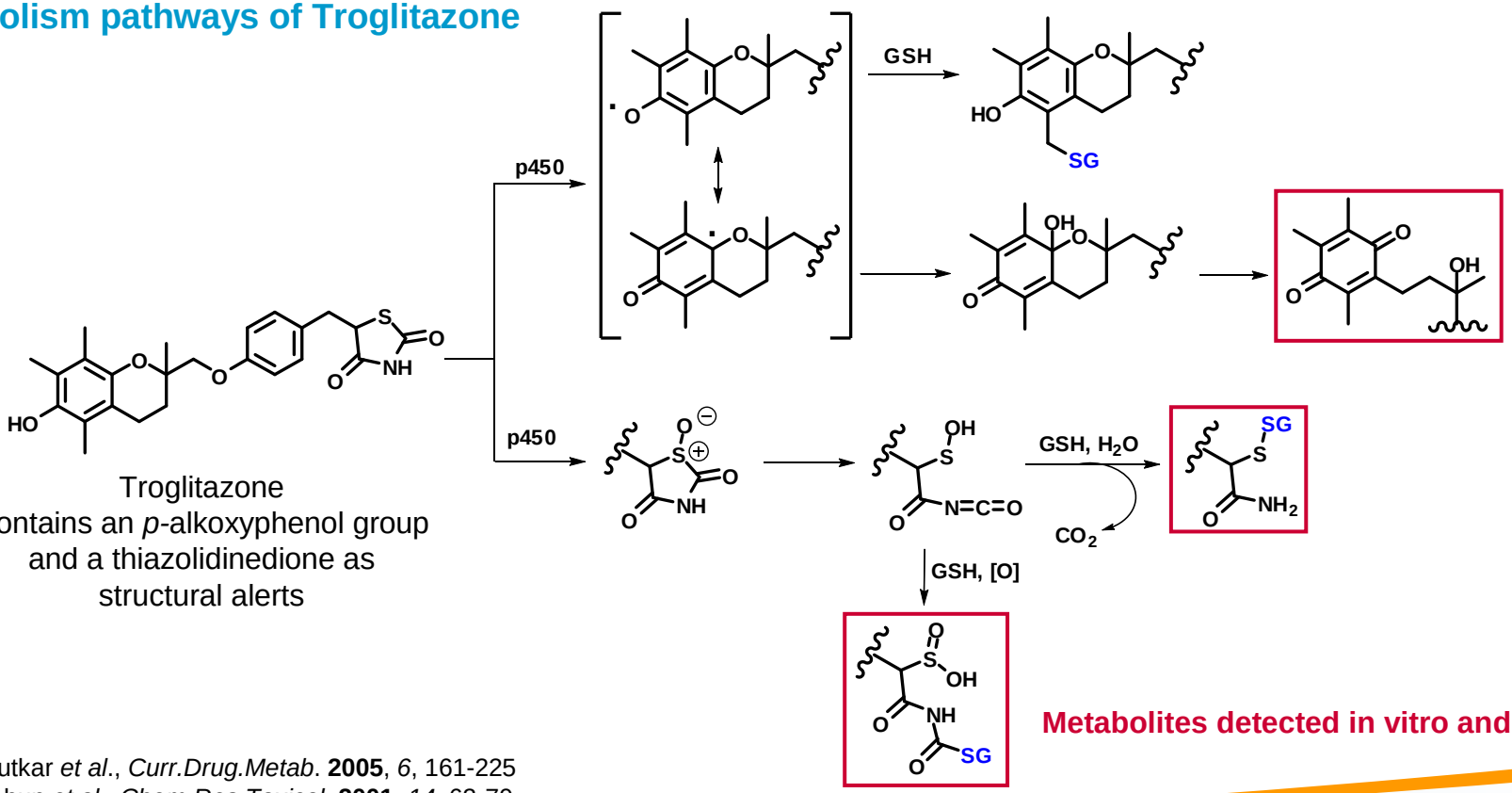
Case study VI: PPARγ family

RISK BENEFIT ASSESSMENT

The Glitazone-PPARγ story

- Troglitazone, a PPARγ agonist for the treatment of type 2 diabetes, was launched in 1997.
- Daily doses were 200-400 mg.
- After being on the market for 17 months, FDA received 560 reports of hepatotoxicity and 24 cases of acute liver failure. **Troglitazone was withdrawn from the market** in 2000.

Metabolism pathways of Troglitazone



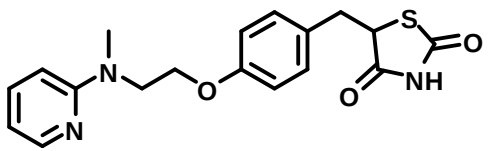
Troglitazone
Contains an *p*-alkoxyphenol group and a thiazolidinedione as structural alerts

Metabolites detected in vitro and in vivo

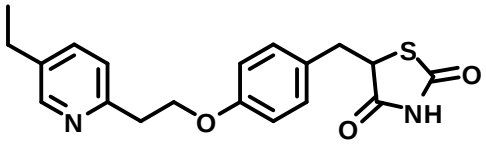
A.S.Kalgutkar *et al*., *Curr.Drug.Metab*. **2005**, *6*, 161-225
K. Kassahun *et al*., *Chem.Res.Toxicol.* **2001**, *14*, 62-70

The Glitazone-PPARγ story

Other successful members of the Glitazone-PPARγ family.



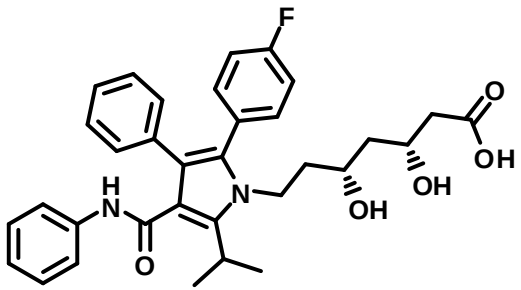
Rosiglitazone, Avandia
Daily dose: 4-8 mg



Pioglitazone, Actos
Daily dose: 15-45 mg

- Both compounds undergo thiazolidinedione ring scission mediated by p450, resulting in reactive metabolites trapped by GSH, but…
- Daily doses are < 50 mg.
- No evidence of drug-induced hepatotoxicity.

Things are not always what they seem…



ATORVASTATIN, LIPITOR®
……with 2006 sales of US$12.9 billion,
it is the largest selling drug in the world

When you see a giant, the first thing to do is to determine the position of the sun–and check to see if it's not actually the shadow of a pygmy

Novalis (Friedrich von Hardenberg), german poet

Summary

- It is still not possible to accurately predict the potential for toxicity of a compound that undergoes metabolic activation.

- In an effort to lessen the chances for type B ADRs, a strategy for minimizing reactive metabolite formation by iterative medicinal chemistry interventions should be implemented (especially in those cases when the predicted dosing regime is >50 mg).

- Reactive metabolite detection or covalent binding formation should be viewed as only one criterion in deciding whether to advance a compound into development.

- Appropriate consideration needs to be given to drug candidates for unmet and urgent medical needs.

Bibliography. Recent reviews

Toxicophores, reactive metabolites and drug safety: when is it a concern? A.S.Kalgutkar, G.Fate, M.T.Didiuk, J.Bauman, Expert.Review in Clinical Pharmacology (2008), 1(4), 515-531

Minimizing metabolic activation during pharmaceutical lead optimization: progress, knowledge gaps and future directions. S.Kumar, K.Kassahun, R.A.Tschirret-Guth, K.Mitra, T.A.Baillie. Current Opinion in Drug Discovery & Development (2008), 11(1), 43-52

Idiosyncratic Drug Reactions: Past, Present, and Future. J.Uetrecht. Chemical Research in Toxicology (2008), 21(1), 84-92.

Idiosyncratic drug reactions: current understanding. J.Uetrecht. Annual Review of Pharmacology and Toxicology (2007), 47 513-539.

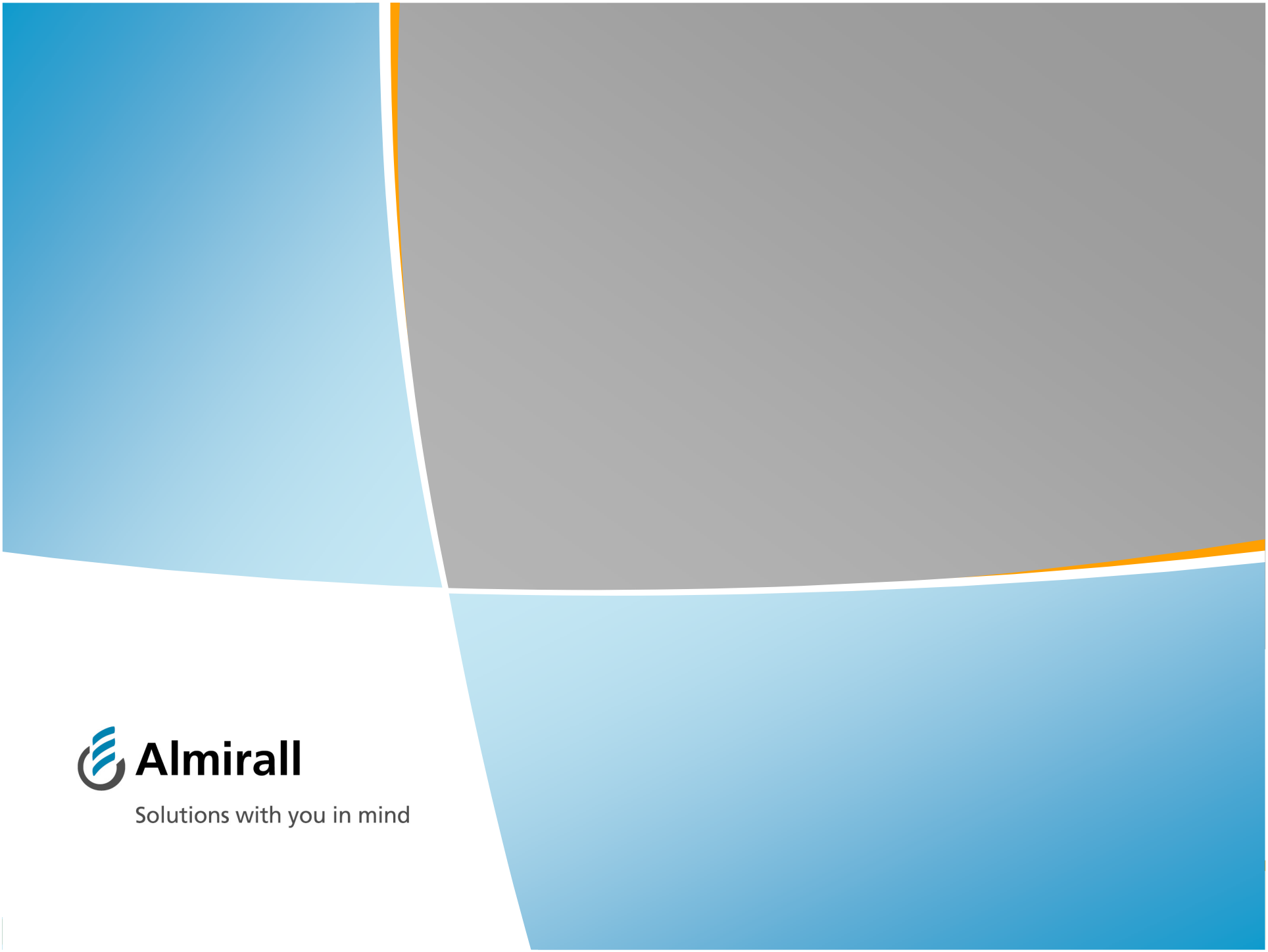
Applying mechanisms of chemical toxicity to predict drug safety. F.P.Guengerich, J.S.MacDonald. Chemical Research in Toxicology (2007), 20, 344-369.

Evaluation of which reactive metabolite, if any, is responsible for a specific idiosyncratic reaction. J. Uetrecht. Drug Metabolism Reviews (2006), 38(4), 745-753.

Minimising the potential for metabolic activation in drug discovery. A.S.Kalgutkar, J.R.Soglia. Expert Opinion on Drug Metabolism & Toxicology (2005), 1(1), 91-142.

A comprehensive listing of bioactivation pathways of organic functional groups. A.S.Kalgutkar, I.Gardner, S.R.Obach, C.L.Shaffer, E.Callegari, K.R.Henne, A.E.Mutlib, D.K.Dalvie, J.S.Lee, Y.Nakai, J.P.O'Donnell, J.Boer, S.P.Harriman. Current Drug Metabolism (2005), 6(3), 161-225.

Idiosyncratic toxicity: Mechanistic insights gained from analysis of prior compounds. J.F.Waring, M.G.Anderson. Current Opinion in Drug Discovery & Development (2005), 8(1), 59-65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