Designing Around Problematic Functionalities in Drug Discovery

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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 survey of the chemistry of structural alerts
  - problematic functionality
  - mechanistic organic chemistry underlying bioactivation
- Strategies and tactics to mitigate reactive metabolite problems
  - understanding the underlying mechanism can inform of drug design approaches
- Conclusion

Adverse Drug Reactions and Withdrawals

The Role of Metabolic Activation

Adverse Drug Reactions (ADRs)

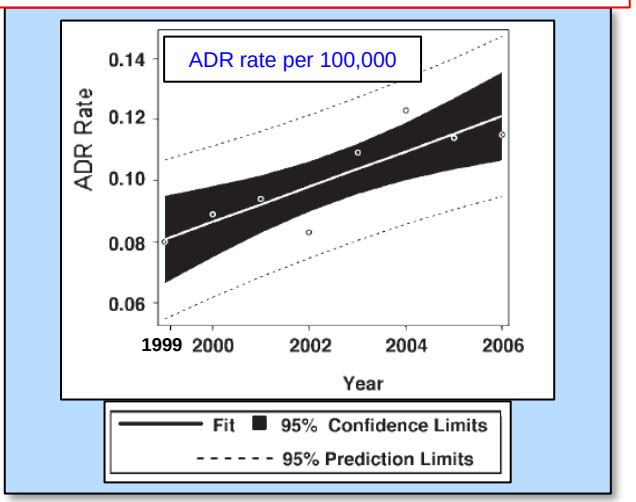
- ADRs were estimated to be the **4th leading cause of death** in the US in **1994**
  - deaths estimated at 106,900 (95% CI 76,000-137,000)
  - ADR death rates increased between 1999 and 2006
  - over 2 million serious ADRs per year: $136 billion yearly cost
- ADRs have been divided into 5 categories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

Type	Description	Underlying Effect	Examples
A (80%)	Augmented Reactions	Dose-related extension of pharmacology	Excessive hypotension with antihypertensive agents; rhabdomyolysis with statins
B	Bizarre Reactions	Idiosyncratic – immune or non-immune mediated Rare: 1 in 10-50,000	Troglitazone and tienilic acid hepatotoxicity
C	Chemical Reactions	Dose-related; molecular understanding	Acetaminophen, isoniazid hepatotoxicity
D	Delayed Reactions	Occur after many years of drug ingestion	Teratogenicity after drug intake during pregnancy - thalidomide
E	End-of-Treatment Reactions	Adverse reactions on drug withdrawal	Withdrawal seizures after stopping phenytoin

1994

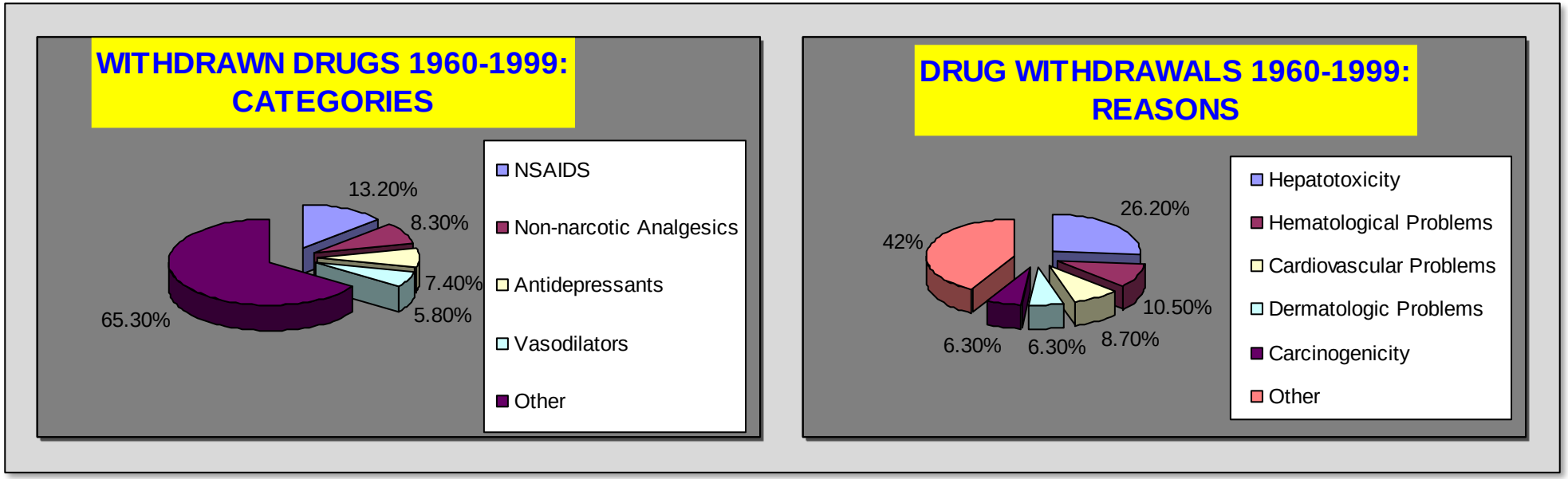
Disease	Per annum
Heart disease	743,460
Cancer	529,904
Stroke	150,108
ADRs	106,900
Pulmonary Disease	101,077
Accidents	90,523
Pneumonia	75,719
Diabetes	53,894

ADR death rates increased between 1999 and 2006



B. K. Park *et al*, *Chem. Res. Toxicol.*, 1998, **11**, 969-988; J. Lazarou *et al.*, *JAMA*, 1998, **279**, 1200-1205; G. Shepherd *et al.*, *Ann. Pharmacother*., 2012, **46**, 169-175
https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm

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

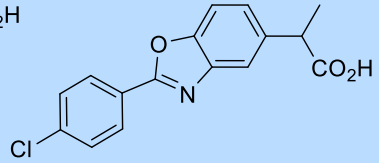
Man Fung *et al.*, *Drug Information Journal*, 2001, **35**, 293-317

Drugs With Liver Toxicity Problems

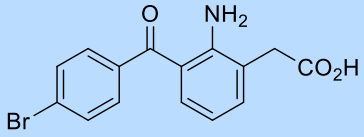
Withdrawn drugs

IPRONIAZID
ANTI-DEPRESSANT
1956

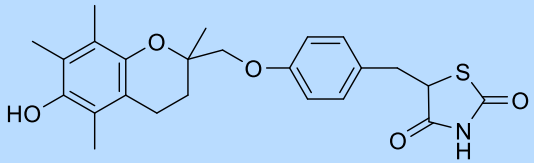
TIENILIC ACID (TICRYNAFEN)
DIURETIC
1979



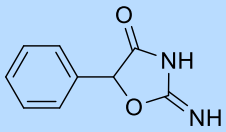
BENOXAPROFEN (ORAFLEX)
ANTI-INFLAMMATORY/ANALGESIC
1982



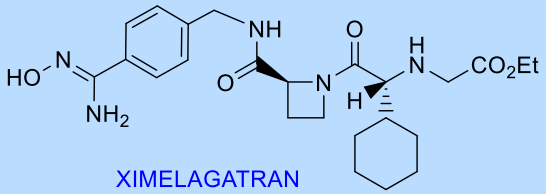
BROMFENAC
ANALGESIC
1998



TROGLITAZONE
ANTI-DIABETIC
2000



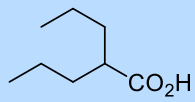
PEMOLINE
ADHD
2005



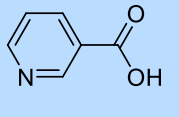
XIMELAGATRAN
THROMBIN INHIBITOR
2006

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

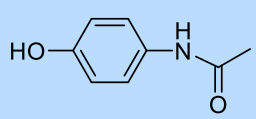
Drugs with warnings



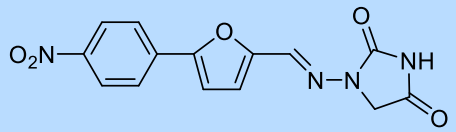
VALPROIC ACID
ANTI-CONVULSANT



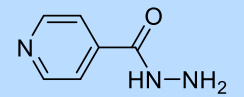
NIACIN
ANTI-HYPERLIPIDEMIC



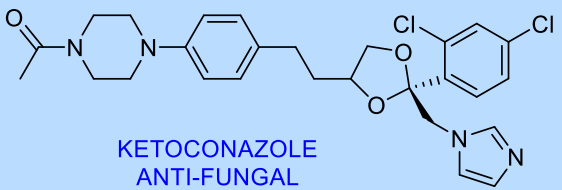
ACETAMINOPHEN
ANALGESIC/ANTI-PYRETIC



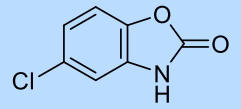
DANTROLENE
SKELETAL MUSCLE RELAXANT



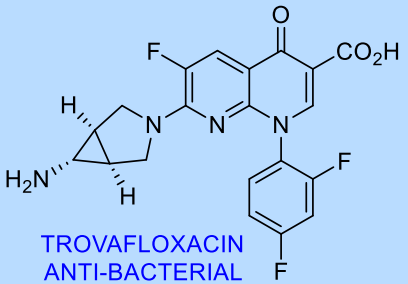
ISONIAZID
TUBERCULOSTATIC



KETOCONAZOLE
ANTI-FUNGAL

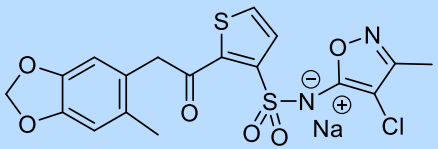


CHLORZOAZONONE
SKELETAL MUSCLE RELAXANT



TROVAFLOXACIN
ANTI-BACTERIAL

withdrawn worldwide 2001



SITAXSENTAN SODIUM
ENDOTHELIN ANTAGONIST
PULMONARY HYPERTENSION
CONGESTIVE HEART FAILURE

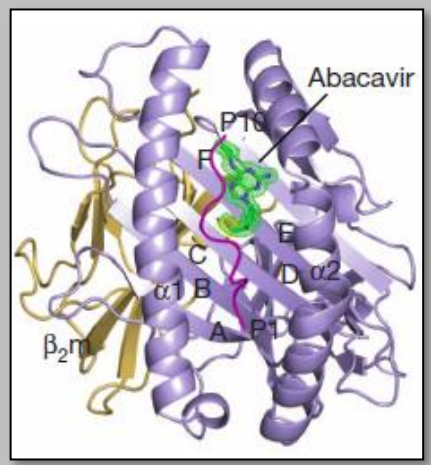
voluntarily withdrawn 2010

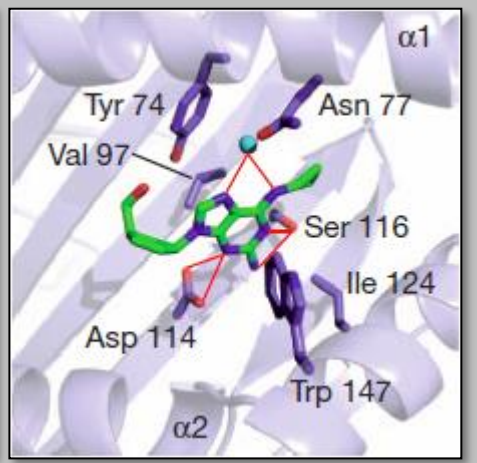
Drug-Induced Liver Injury (DILI)

- Most instances of DILI are idiosyncratic in nature
  - no reliable biomarkers
  - focus on reactive metabolites: retrospective studies
- Mitochondrial toxicity: an uncommon but distinctive form of liver toxicity
  - tetracycline, amiodarone, valproic acid
  - problem with HIV-1, HBV nucleoside analogues
    - inhibition of host DNA pol γ
- 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

- Hapten hypothesis:
  - drug-protein adducts create antigens
  - precipitates an immune response
- Danger hypothesis
  - concomitant background liver injury
  - due to inflammation or infection
  - LPS administration to animals sensitizes to hepatotoxic drugs
- Human leukocyte antigens (HLAs or MHCs) & DILI
  - some drugs bind to an HLA: **abacavir, carbamzepine**
    - alter peptide presentation to the immune system
  - genetic polymorphisms in HLA proteins
    - adds to the complexity

Drug	Allele	Comment
Flucloxacillin	*HLA-B*5701*	80-fold increased risk of DILI
Abacavir	*HLA-B*5701*	fever, rash, headache, nausea & vomiting
Amoxicillin-clavulanate	*HLA-DRB1*1501 also HLA-DQB1*06*	cholestatic liver injury
Lumiracoxib	*HLA-DRB*1501*	accounts for 6-8% incidence of DILI; reactive metabolites
Ximelagatran	*HLA-DRB1*07*	DILI – interferes with peptide binding to *HLA-DRB1*0701*

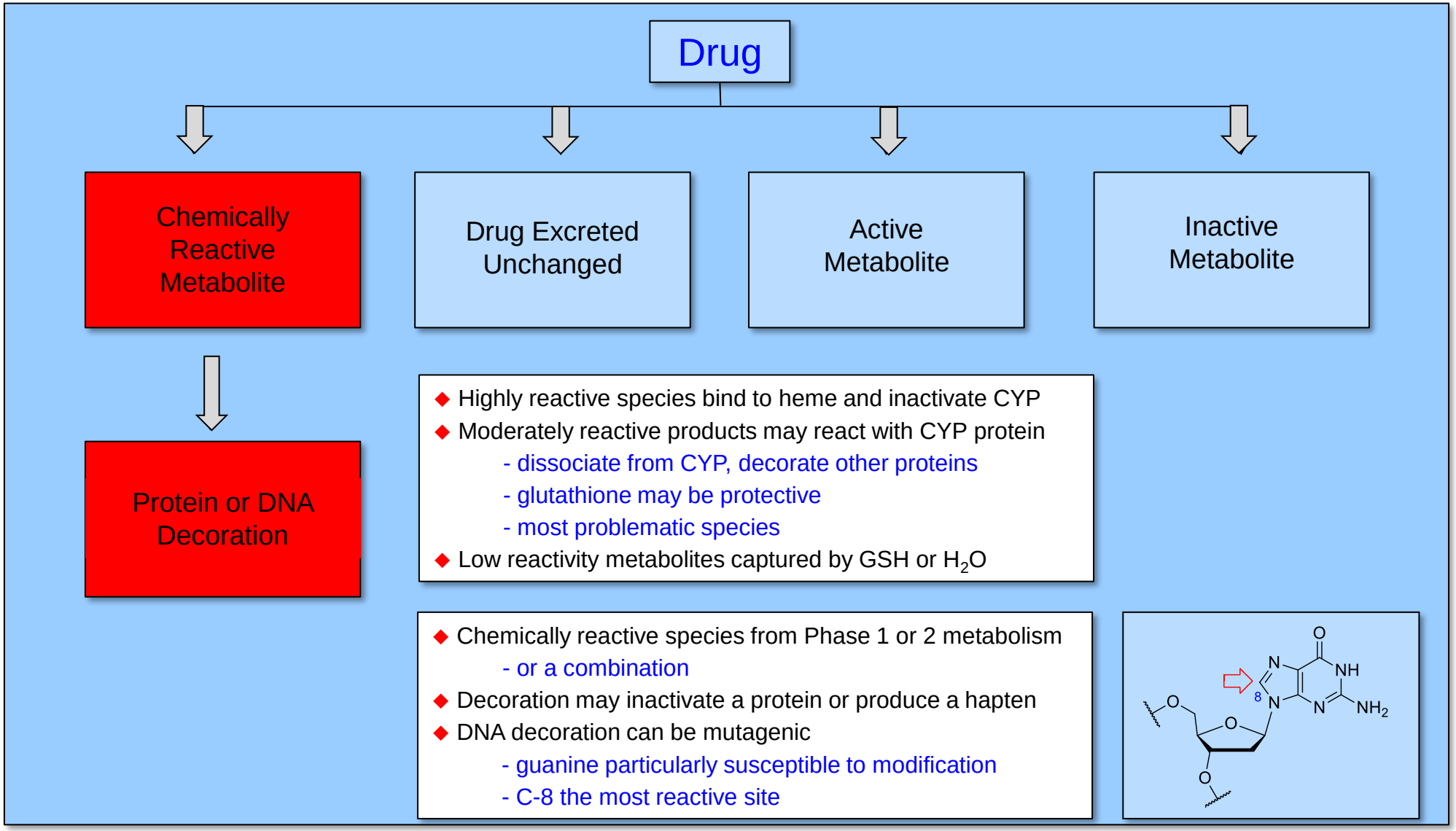




Abacavir bound to *HLA-B*5701*

S. Tujios and R.J. Fontana, *Nature Rev. Gastroenterol. Hepatol.*, 2011, **8**, 202-211; P.T. Illing *et al.*, *Nature,* 2012, **486**, 554-560; E.L. Reinherz, *Nature,* 2012, **486**, 479-481

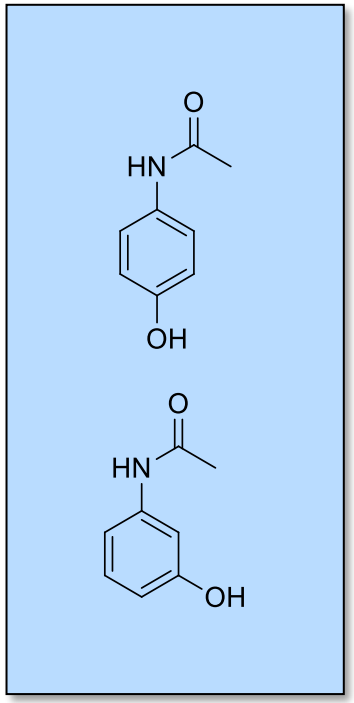
Metabolic Activation and Drug Toxicity



See P.M. Gannet *et al*., *Org. Biomol. Chem*., 2018, **16**, 2198-2209 for C-8 guanine modifications & role in cancer

Protein Covalent Binding and Toxicity

- Bioactivation/PCB & 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_2OH; 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



PCB = protein covalent binding

H. Takakusa *et al*., *Drug Metab. Disp*., 2008, **36**, 1770-1779; R.S. Obach *et al*., *Chem. Res. Toxicol*., 2008, **21**, 1814-1822; T. Usui *et al., Drug Metab. Disp*., 2009, **37**, 2383-2392

Dose and Incidence of Problems

- 53 of 68 withdrawn/black box label drugs associated with hepatic toxicity
  - rest due to blood dyscracia, cutaneous ADRs, anaphylaxis
- All ion classes included
  - 29 basic amines; 19 CO_2H; 20 neutral
- Broad range of physicochemical properties
  - MW: 137 - 808 Da
  - Log*P*: -0.67 to +6.35
  - TPSA: 27 - 224 Å^2
- No correlation between physicochemical properties & idiosyncratic toxicity
  - majority are high dose drugs: 100-2400 mg
- Analysis of 164 drugs with liver liability:
  - dose >100 mg & Log *P* >3 predicts DILI (Rule-of-2)
  - high % of false –ves (59%)
- Confirmed by ADRs reported in Sweden 1970-2004
  - drug doses of <50 mg less likely to be associated with DILI

Drug doses of >100 mg & drug Log *P* >3 predicts DILI (Rule-of-2)

Drug doses of <50 mg are less likely to be associated with DILI

Withdrawn/black box label drugs

Dose (mg)	Withdrawn	BBW
<10 mg	0%	0%
10-50 mg	6%	8%
50-100 mg	10%	11%
>100 mg	84%	81%

Drugs labeled for liver liabilities

164 drug set	% of DILI-concern drugs		
Log *P* → / Dose ↓	<1 (n = 49)	1 to ≤ 3 (n = 47)	>3 (n = 68)
<10 mg (n = 15)	60%	0%	20%
10 to <100 mg (n = 43)	60%	56%	41%
≥100 mg (n = 106)	65%	92%	96%

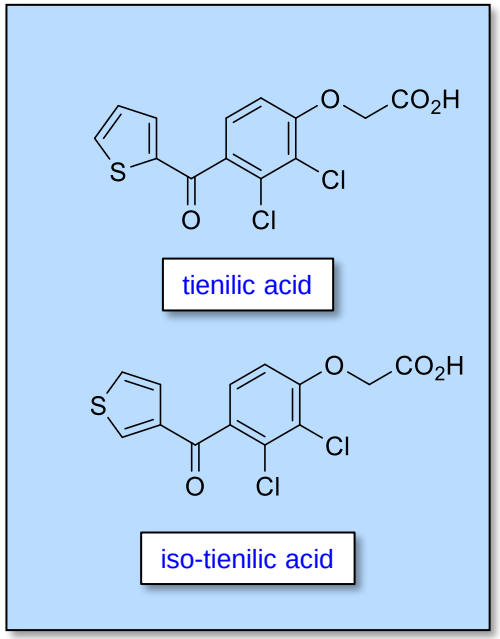
Daily Dose (n = 598)	≤10 mg	10-49 mg	≥50 mg
% Causing DILI	9	14.2	77
% fatal or liver transplant	2	9.4	13.2

Sweden 1970-2004

A.S. Kalgutkar *et al., Chem. Res. Toxicol.*, 2011, **24**, 1345-1410; C. Lammert *et al., Hepatology*, 2008, **47**, 2003-2009; W. Tong *et al., Hepatology*, 2013, **58**, 388-396

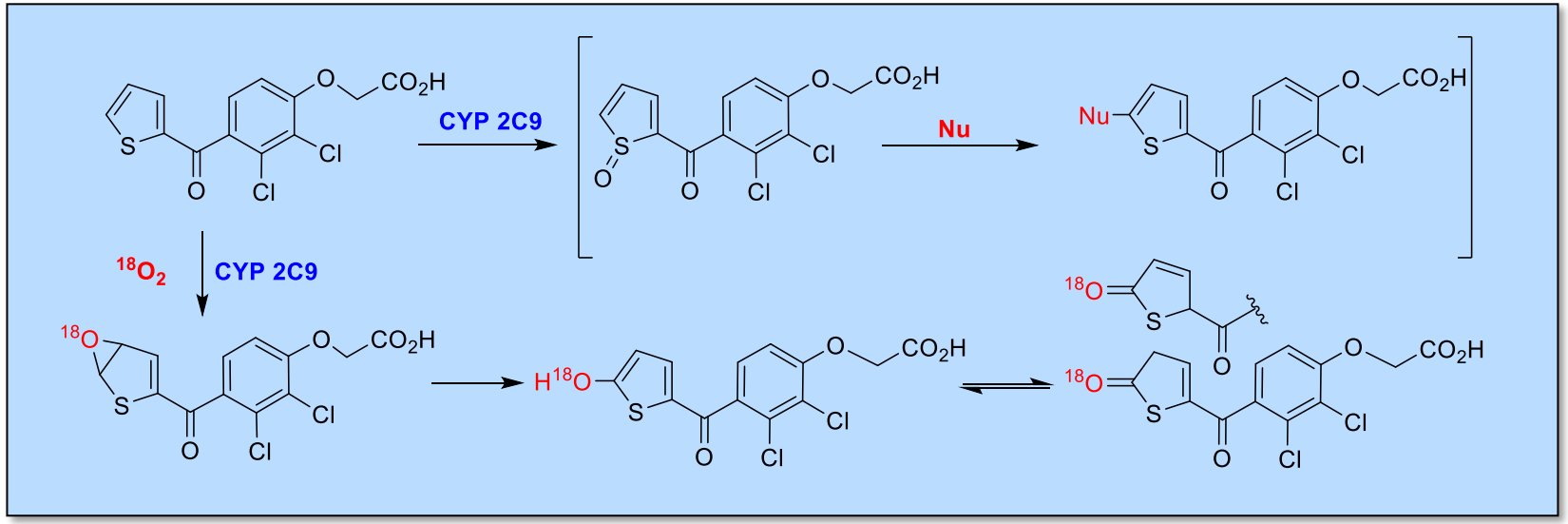
Tienilic Acid (Ticrynafen)

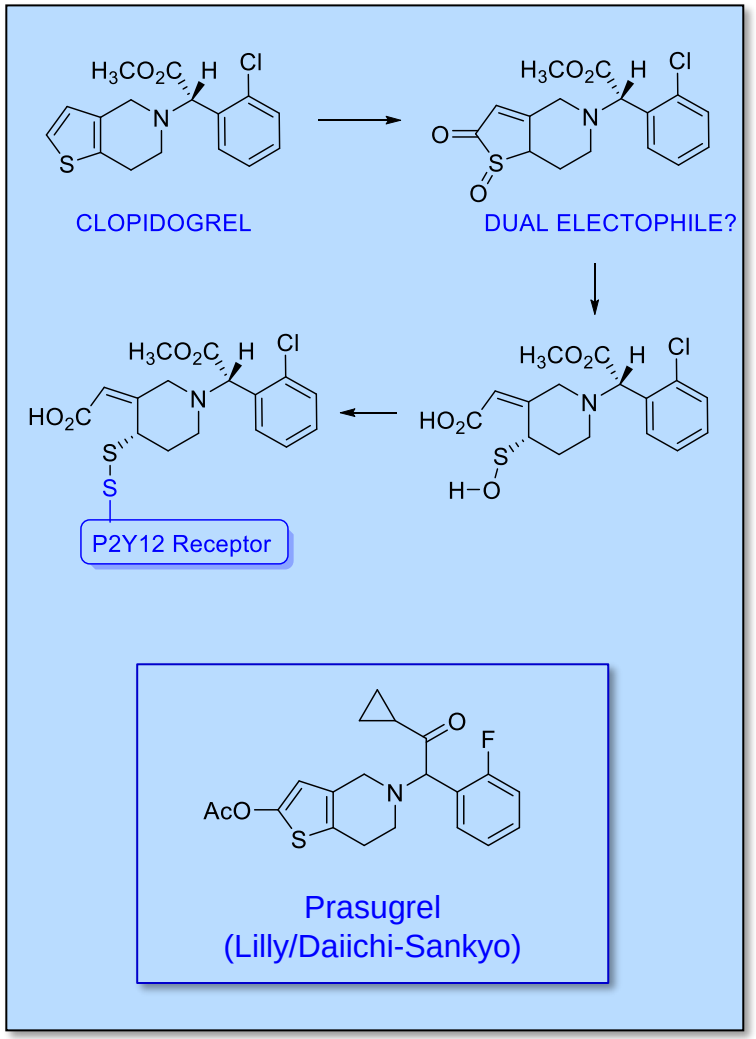
- Uricosuric diuretic agent introduced in Europe in 1976,
  - US FDA approval followed in 1979
- Withdrawn in the US in 1980
  - severe hepatotoxicity in <1% of patients
    - 10% fatality rate
- Drug-induced immunoallergic hepatitis
  - anti-LKM_2 antibodies detected (liver-kidney microsome)
- Anti-LKM_2 specifically recognizes CYP 450 2C9
  - tienilic acid is metabolized by CYP 450 2C9
  - covalently binds to a surface residue of 2C9
- Most compelling example of the haptenization hypothesis
- Iso-tienilic acid – topological isomer of 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



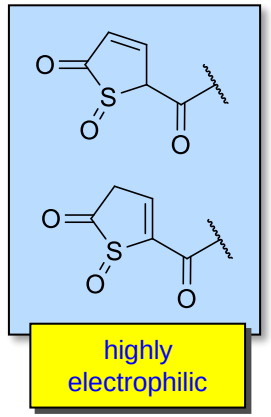
P. Beaune *et al., Proc. Natl. Acad. Sci. USA*, 1987, **84**, 551-555; *Mol. Pharmacol.,* 1996, **50**, 326-333

Metabolism of Tienilic Acid



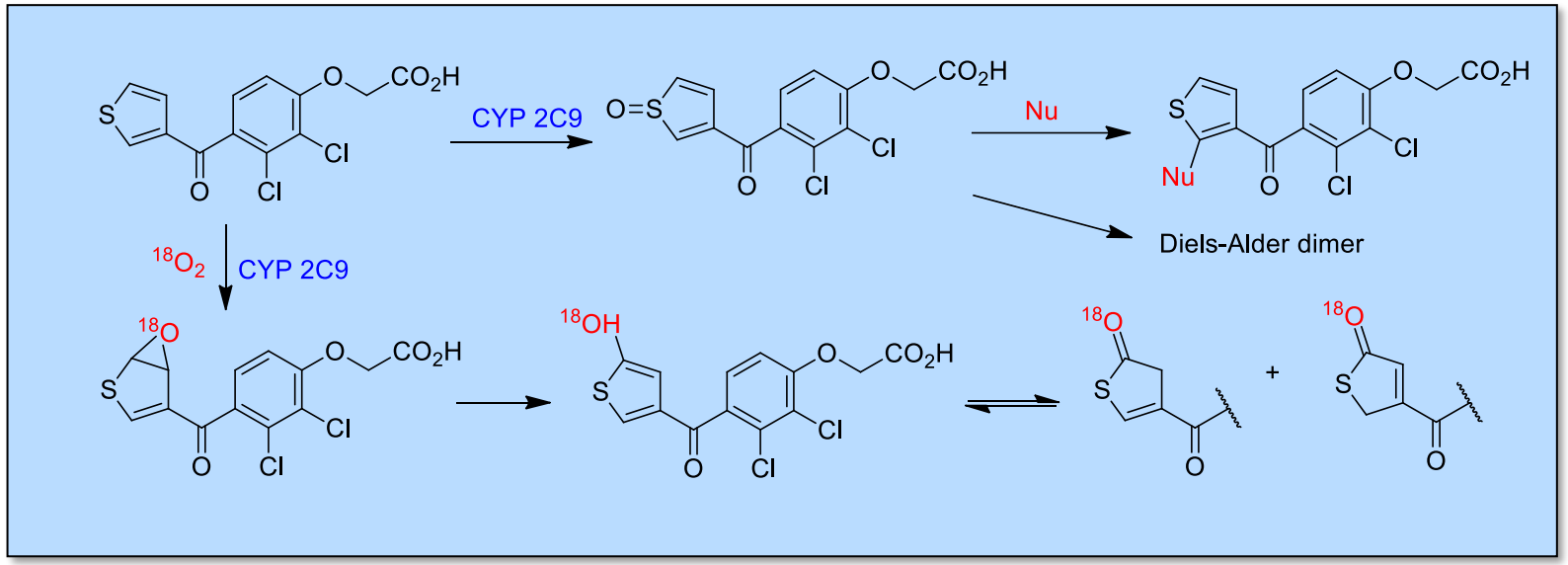


- CYP 2C9 adduct is +16 amu
  - O incorporated, labeling reduced by GSH
- 5-Hydroxy (thiolactone) derivative from S-oxide (Nu = H_2O) or epoxide
  - $^{18}O_2$ labeling studies suggest epoxide intermediate – 97% from $^{18}O_2$
  - no evidence of thiophene S-oxide detected in 2C9 supersomes
- Modeling studies suggest poor presentation of S to 2C9
  - C-4,C-5 bond exposed, readily oxidized
- Thiophene oxidation underlies the mode of action of clopidogrel
  - blood platelet ADP receptor antagonist
  - cyclic thioester oxide believed to be the reactive species



S.D. Nelson *et al*., *Chem. Res. Toxicol*., 2012, **25**, 895–903; P.M. Dansette *et al., Chem. Res. Toxicol.,* 2010, **23**, 1268-1274

Metabolism of iso-Tienilic Acid



- Iso-tienilic acid inactivates CYP 2C9 but not selectively
  - labels other microsomal proteins
- 2C9 metabolizes by the S-oxide and epoxide pathways
- S-oxide reacts with thiols at C-2
  - sterically more hindered than C-5 but more reactive
  - intercepted by GSH or dimerizes *via* a Diels-Alder reaction
  - may be the source of hepatotoxicity
- C4-C5 epoxide also formed
  - rearranges to electrophilic thiolactones
- 2C9 modeling studies suggest access of 2C9 to both S and olefin
  - S=O may be formed by Fe-O-O-H species due to remoteness from Fe

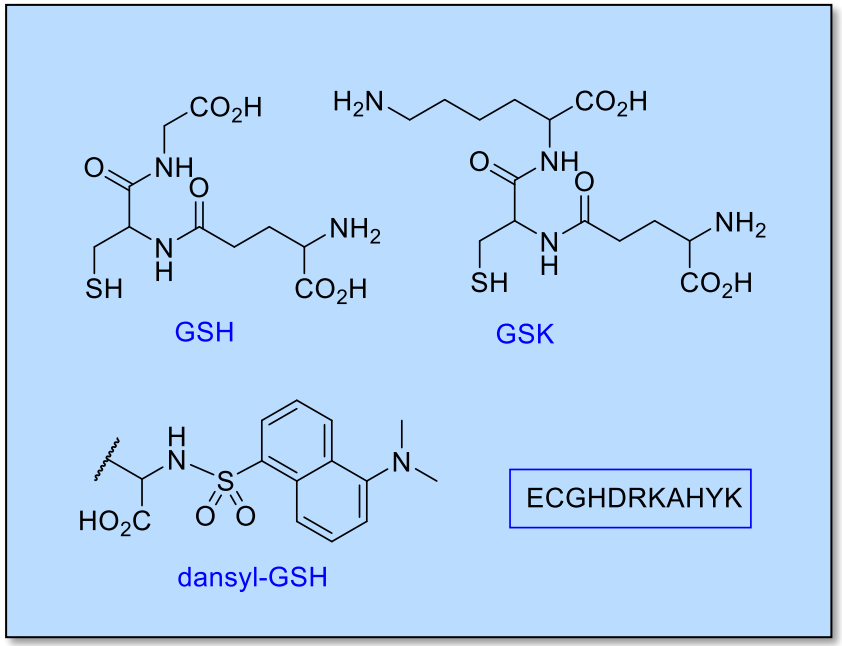
S.D. Nelson *et al*., *Chem. Res. Toxicol*., 2012, **25**, 895–903; P.M. Dansette *et al., Chem. Res. Toxicol.,* 2010, **23**, 1268-1274

Metabolic Bioactivation

In Vitro Techniques and Metabolic Pathways

Assessing Reactive Metabolites

- Incubate compound with human liver microsomes (HLM)
- Analyze for protein covalent binding (PCB)
  - use of radio-labeled drug optimal
- 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 $Na^{14}CN$
  - CN^- is a hard nucleophile
  - used to trap hard electrophiles like iminium ions



D. Evans *et al*., *Chem. Res. Toxicol*., 2004, **17**, 3-16; C. Prakash *et al., Curr. Drug Metab*., 2008, **9**, 952-964; M.P. Grillo, *Exp. Opin. Drug Metab. Toxicol*., 2015, **11**, 1281-1302.

Oxidizing and Conjugating Enzymes

- Cytochrome P450 family (CYP 450)
- Flavin-dependent monooxygenases (FMO)
- Monoamine oxidases A and B (MAO)
- Semi-carbazide-sensitive amine oxidase (SSAO)
- Esterases
- Cyclooxygenases and lipoxygenases
- Alcohol dehydrogenases
- Peroxidases

Phase 1 Processes

- UDP Glucuronosyl transferases
- Sulfotransferases
- Acetyl transferases
- Glutathione S-transferases

Phase 2 Conjugation Processes

- Processes can operate consecutively
  - elicit xenobiotic activation to reactive species
  - e.g. aniline metabolism

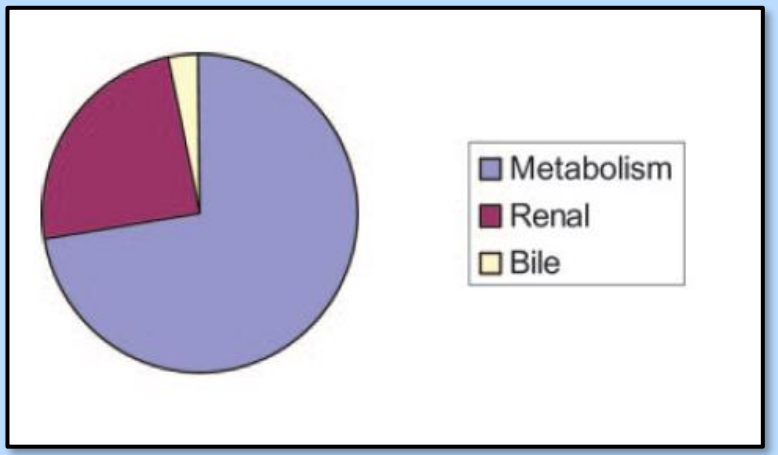
Phase 1/2 Conjugation Processes in Tandem

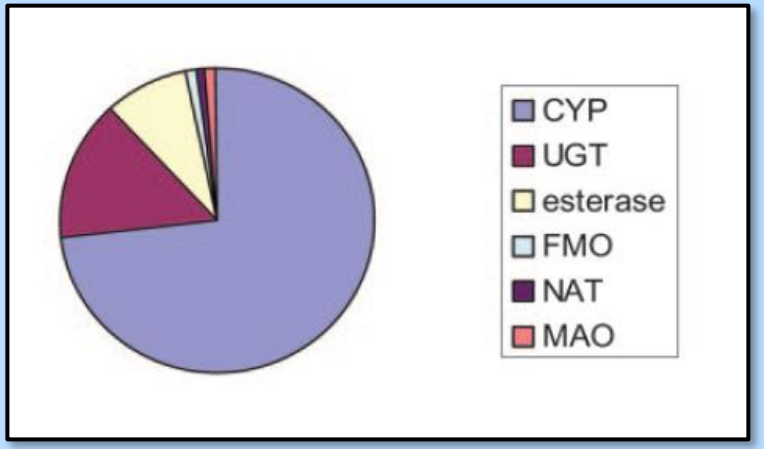
D.W. Nebert *et al.*, *Nature Rev. Cancer*, 2006, **6**, 947-960

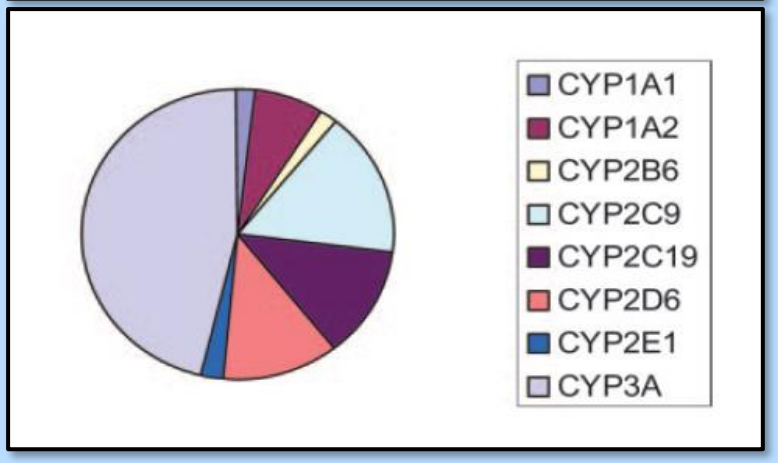
Drug Clearance Pathways in Humans

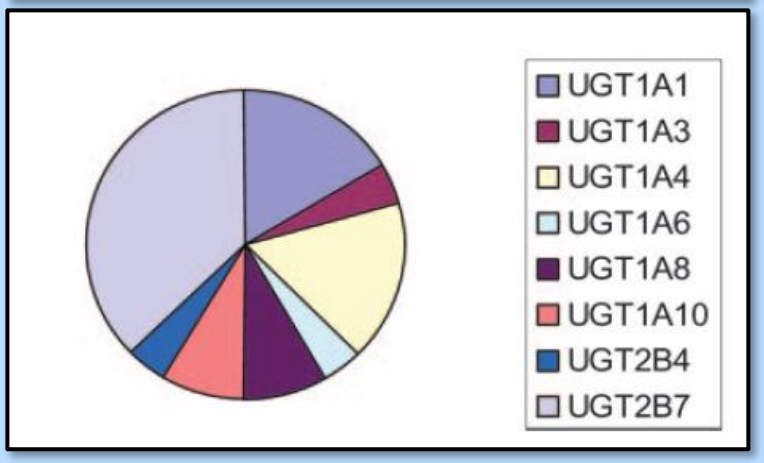
AKR: aldo-keto reductase
FMO: flavin monooxygenase
MAO: monoamine oxidase

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

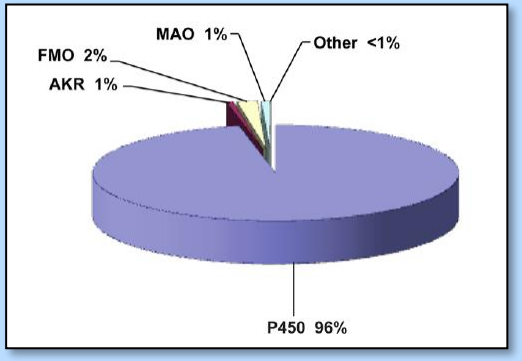






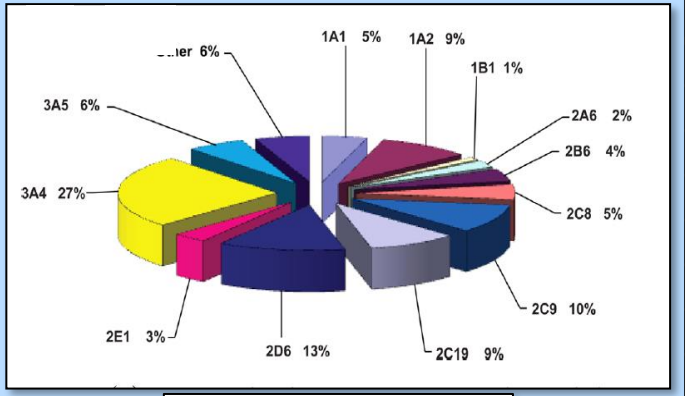


- CYP 450s dominate (>95%)
- 1A2, 2C9, 2C19, 2D6 & 3A4 account for 75%



P450	96%
FMO	25
AKR	1%
MAO	1%
Other	<1%

4192 reactions, 860 drugs

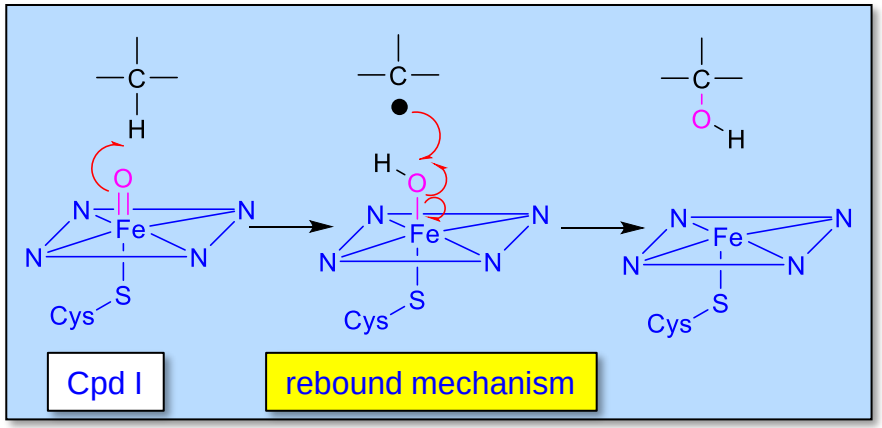


CYP	%
3A4	27
2D6	13
2C9	10
2C19	9
1A2	6
3A5	6
2C8	5
1A1	5

4058 reactions, 860 drugs

J.A. Williams *et al.*, *Drug Metab. Disp.,* 2004, **32**, 1201-1208; S. Rendic & F.P. Guengerich, *Chem. Res. Toxicol.,* 2015, **28**, 38-42

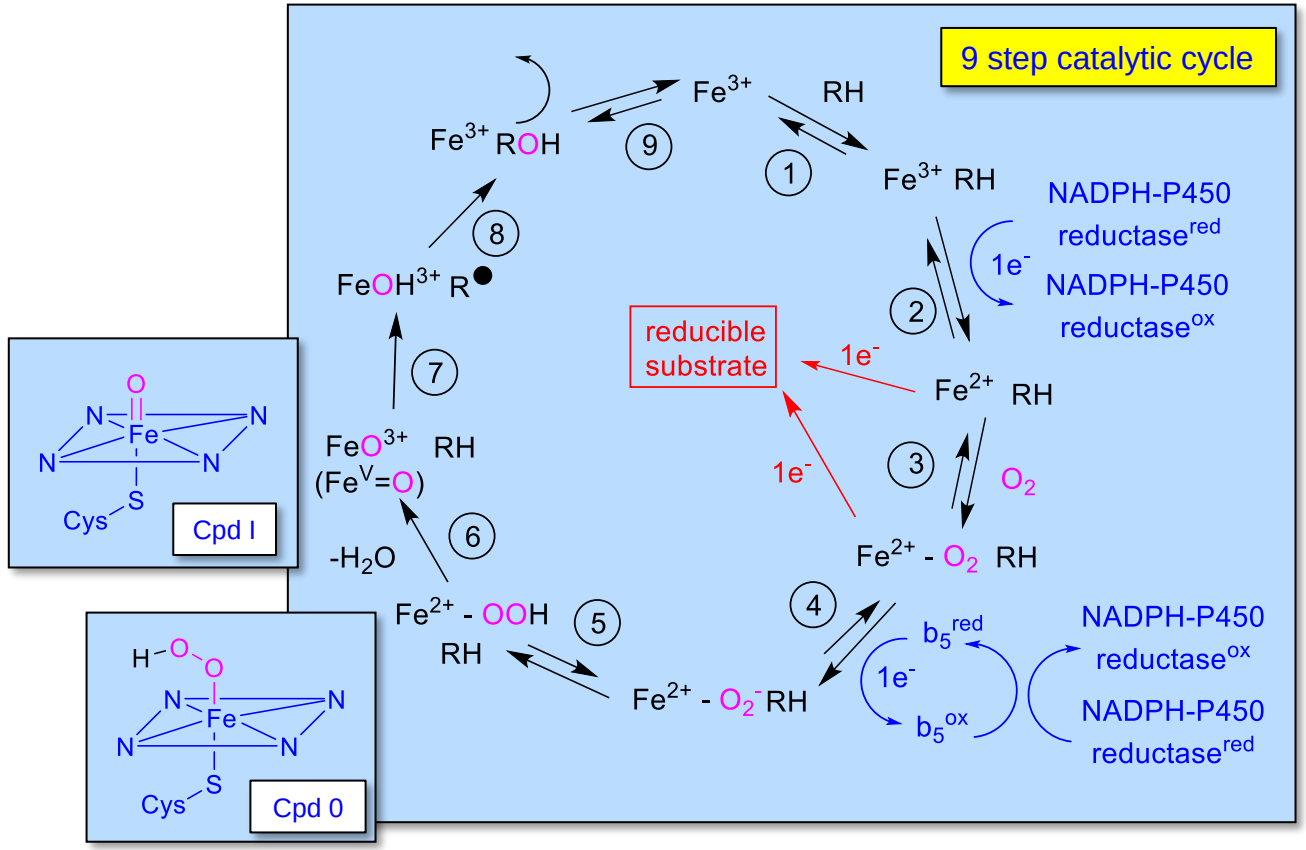
The CYP 450 Catalytic Cycle



- Oxidation
  - CYPs are powerful oxidants
  - $Fe{=}O^{3+}$ ($Fe^{V}{=}O$) the most powerful species
  - rebound mechanism most common pathway for C-H
  - Fe^{2+}-O-O-H can oxidize soft atoms

- Reduction
  - CYPs can act as reducing agents
  - occurs with some substrates when binding of O_2 is slow

- Oxidation cycle is initiated by substrate binding
  - Fe^{3+} (ferric) is reduced to Fe^{2+} (ferrous) by e^- transfer from NADPH
  - Fe^{2+} species binds O_2
- Fe^{2+}-O_2 complex is a reducing agent whilst waiting for an electron
  - the single electron can be transferred to the substrate
  - facilitated by some substrates blocking O_2 binding
  - can reduce N-O, N-N bonds, C-halogen bonds
- Fe^{2+}-O-O-H is an oxidant and a nucleophile (Cpd 0)
  - sulfur, anilines (CYP 1A2), soft atoms
  - may hydrolyze nitriles (Fe^{2+}-O-O^-)
- Fe^{2+} (ferrous) is readily oxidized to Fe^{3+} (ferric)
- $Fe{=}O^{3+}$ (can be written as $Fe^{V}{=}O$) is the major oxidizing species (Cpd I)
  - capable of a broad repertoire of reactions



F.P. Guengerich, *Chem. Res. Toxicol.,* 2001, **14**, 611-650; *J. Biol. Chem*., 2013, **288**, 17065-17073; *Trends Pharmacol. Sci*., 2016, **37**, 625-640
S. Shaik *et al., Acc. Chem. Res.,* 2019, **52**, 389-399

Structural Alerts

A Survey of Toxicophores
and the
Underlying Mechanistic Organic Chemistry

Structural Alerts – a Survey of Problematic Elements

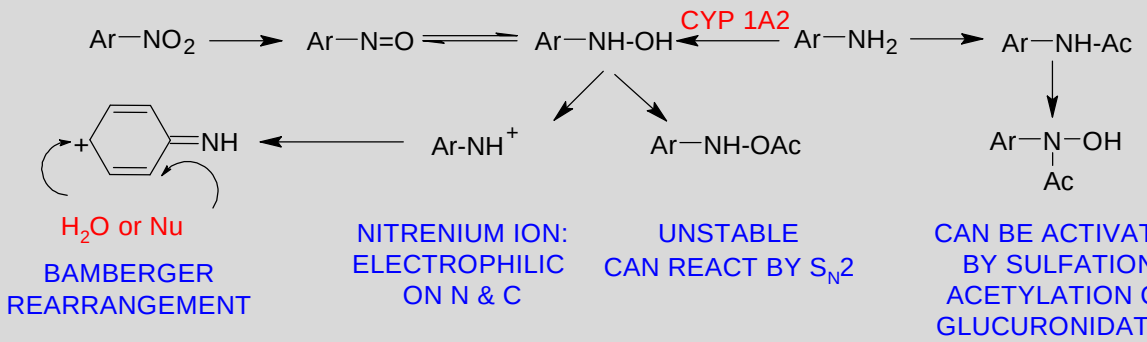
Anilines & Masked Anilines

Darunavir, Dapsone, Procainamide

Ar-NH-OH is the common problematic metabolite

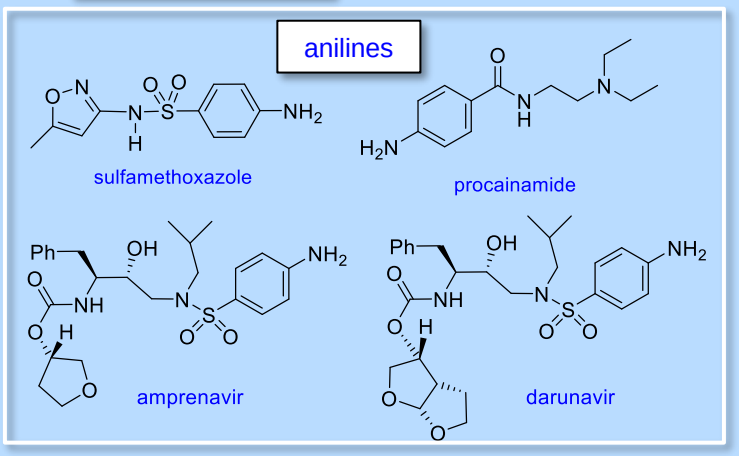
Nitrobenzenes

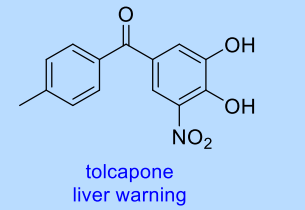
Nifedipine, Dantrolene, Tolcapone, Flutamide

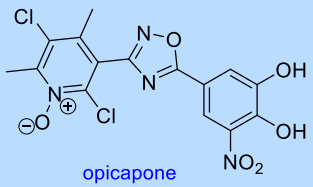


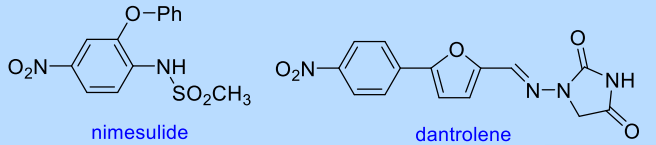
Marketed drugs

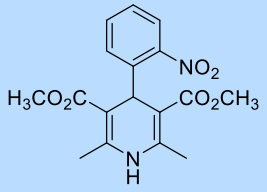
anilines









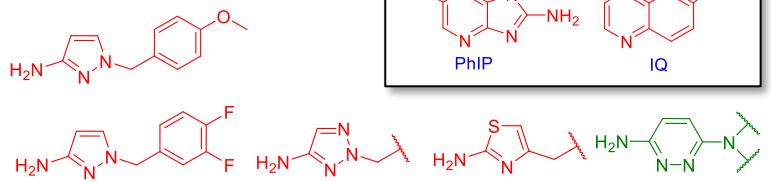


nitrobenzenes

- 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 $ArNH_2$: reacts with CYP-OOH
  - 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

CYP 1A2 believed to be main enzyme metabolizing anilines

- Applies to *some* heterocycles
  - food-derived mutagens PhIP
  - amino pyrazoles,
  - amino triazoles
  - amino thiazoles
- Amino pyridazines are OK



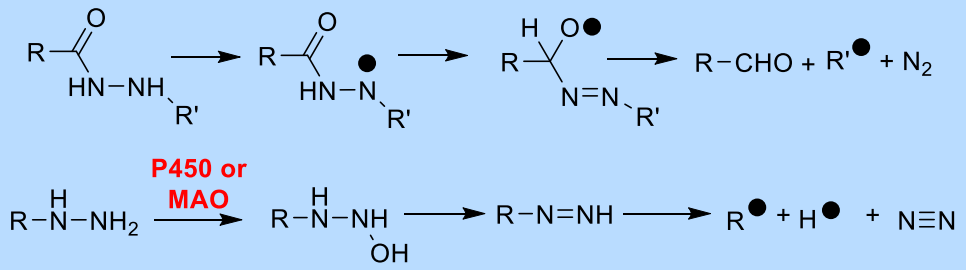
I. Shamovsky *et al., JACS,* 2011, **133**, 16168-16185; N.J. Gooderham *et al., Drug Metab. Disp*., 2001, **29**, 529-534
O. Bezençon *et al., J. Med. Chem*., 2017, **60**, 9769-9789; J.J. Crawford *et al*., *Chem. Res. Toxicol*., 2020, **33**, 1950-1959

Structural Alerts – a Survey of Problematic Elements

Hydrazines & Hydrazides

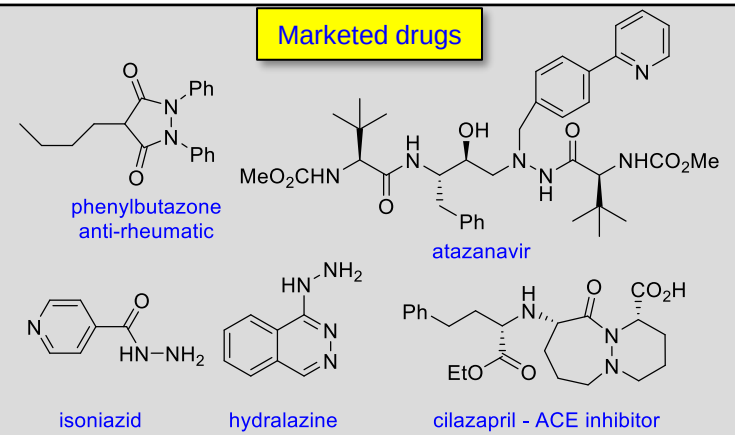
Isoniazid, Atazanavir, Phenylbutazone, Cliazapril, Hydralazine

isoniazid & hydralazine are problematic



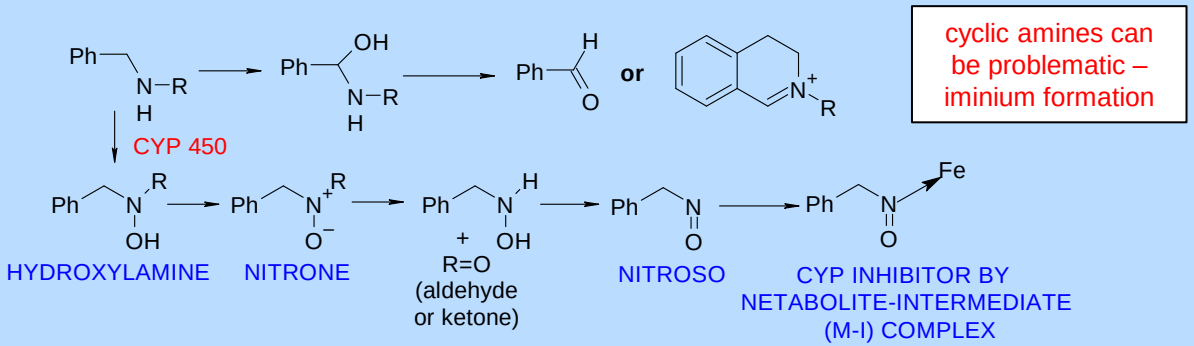
Less problematic hydrazines are heavily substituted

Marketed drugs

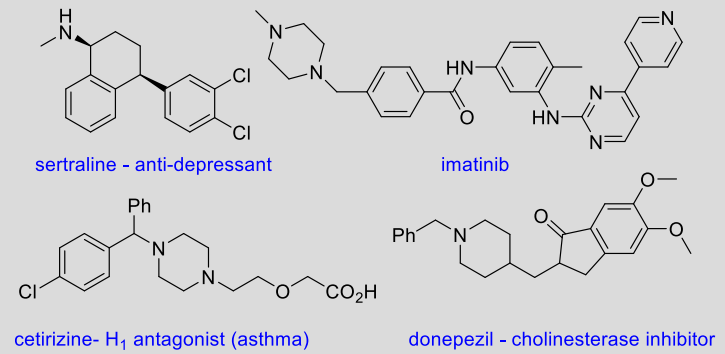


Benzylamines

Sertraline, Imatinib, Cetitizine, Donepezil, Cinacalcet

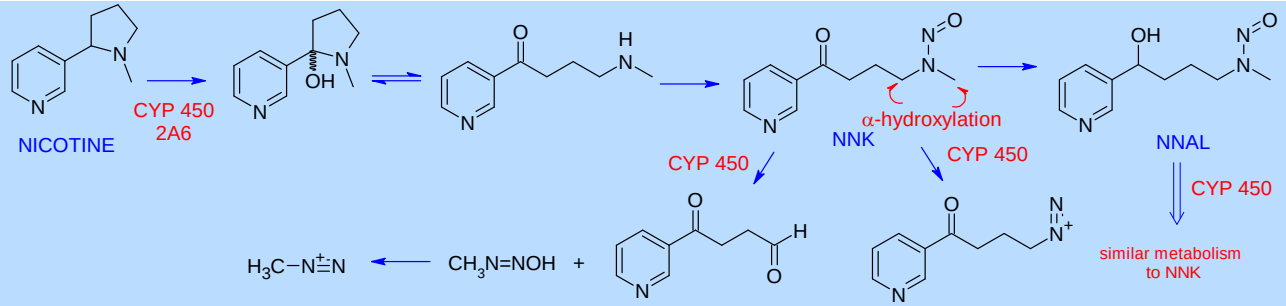


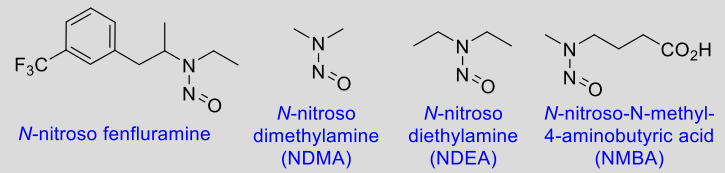
cyclic amines can be problematic – iminium formation



Nitrosamines

Amines & amides can be nitrosylated *in vivo*
- many drugs susceptible
Impurities in APIs

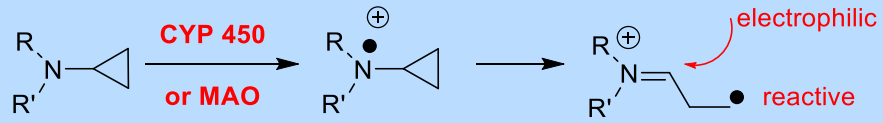
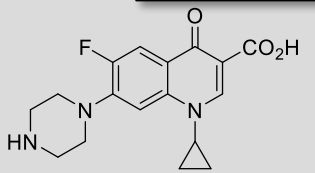
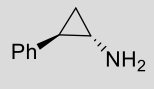
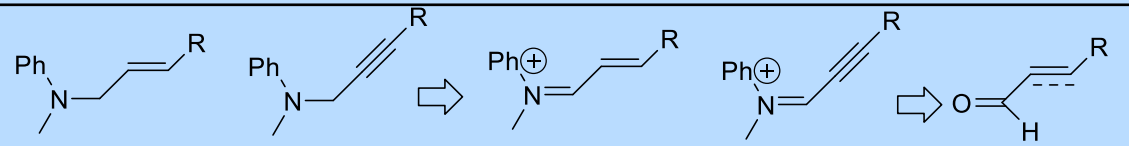
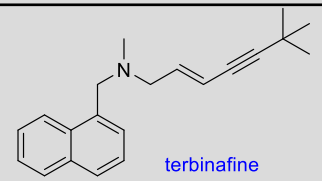
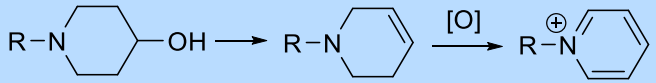
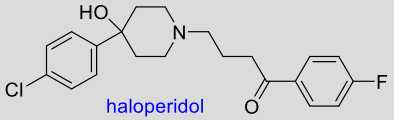
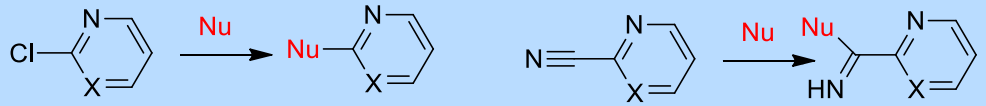
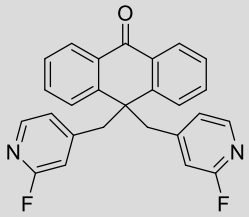
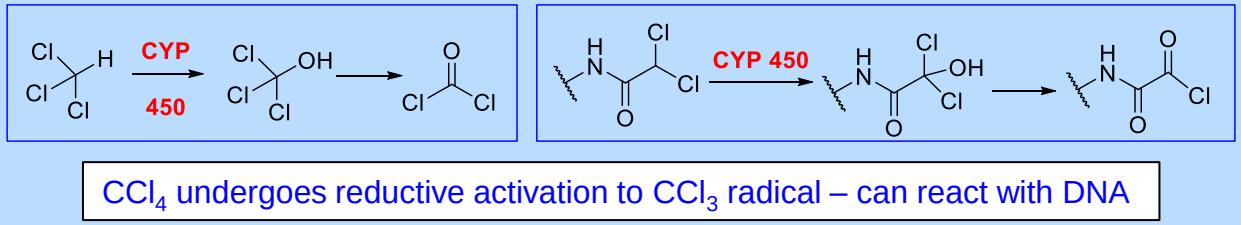
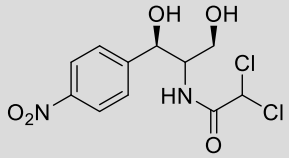
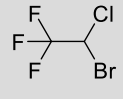




Nitroso fenfluramine - impurity - Europe 2000-2002 – 12 cases of acute liver toxicity
Nitrosamine impurities in drug products

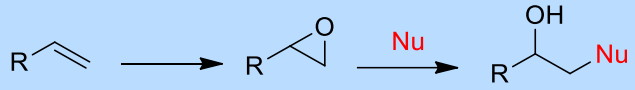
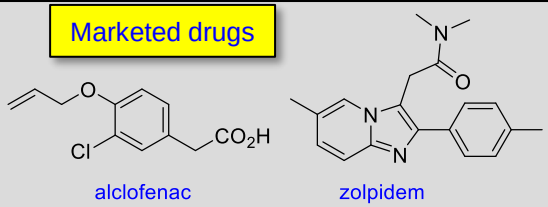
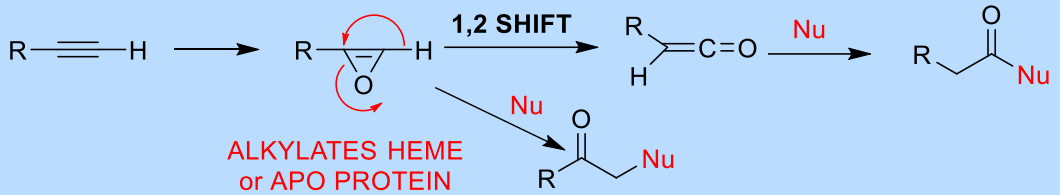
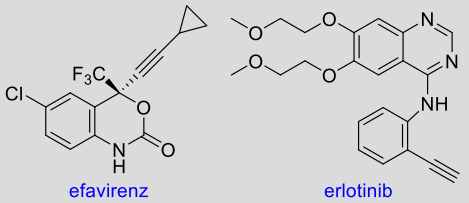
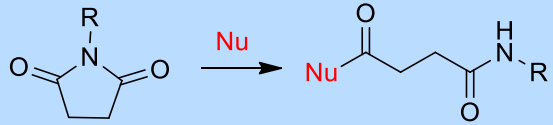
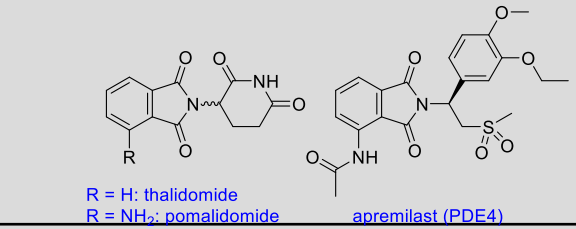
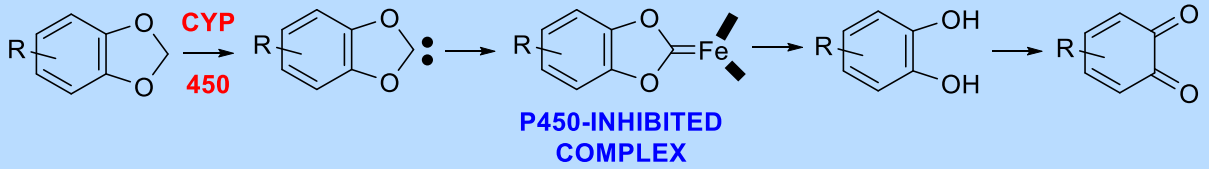
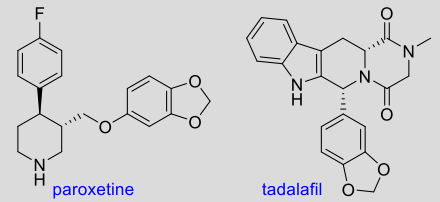
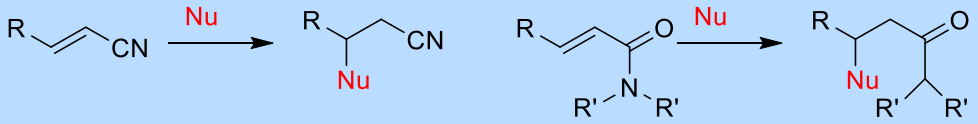
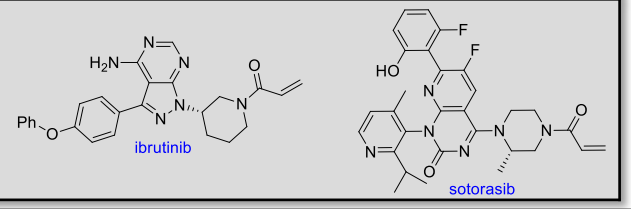
Hydrazines: B.K. Sinha *et al., J Drug Metab. Toxicol.*, 2014, **5**, 1000168; Benzylamines: A. Mutlib *et al., Chem. Res. Toxicol.,* 2002, **15**, 1190-1207
Nitrosamines: A.S. Kalgutkar *et al*., *J. Med. Chem*., 2022, **65**, 15584-15607; S.S. Bharate, *J. Med. Chem.,* 2021, **64**, 2923-2936

Structural Alerts – a Survey of Problematic Elements

		Marketed drugs
Cyclopropylamines Ciprofloxacin, Nevirapine, Tranylcypromine, Abacavir		 
Allylic Amines Terbinafine		
1,2,3,6-Tetrahydopyridines Haloperidol		
2-Halo- and 2-Cyano Pyridines, Pyrimidines DUP453		
Haloalkanes Chloramphenicol, Halothane		 

Cyclopropylamines : R.P. Hanzlik *et al., Arch. Biochem. Biophys.*, 2005, **436**, 265–275

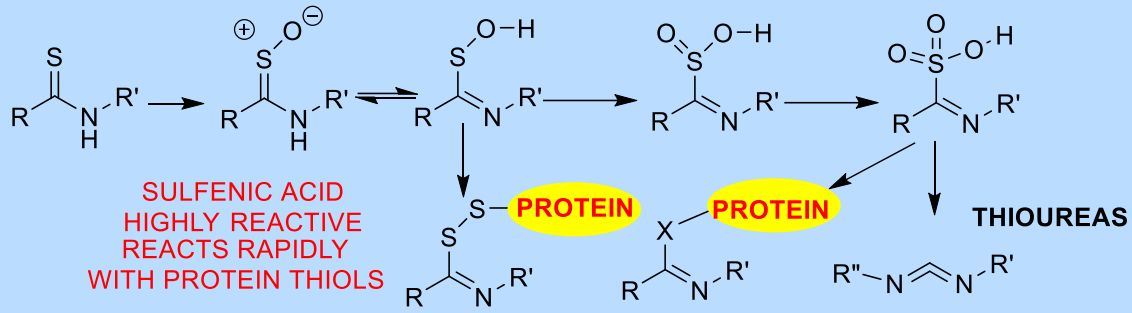
Structural Alerts – a Survey of Problematic Elements

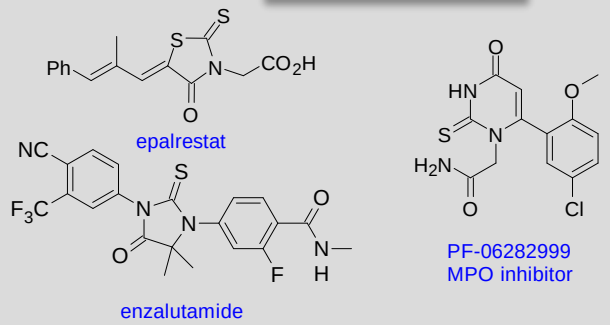
Alkenes Alclofenac, Zolpidem		Marketed drugs 
Acetylenes Efavirenz, Erlotinib, Terbinafine, Selegiline, Rasagiline		
Imides Thalidomide, Pomalidomide, Apremilast		
Methylenedioxy Aromatics Paroxetine, Tadalafil		
Michael Acceptors Ibrutinib, Dutasteride, Finasteride, Osimertinib		

Structural Alerts – a Survey of Problematic Elements

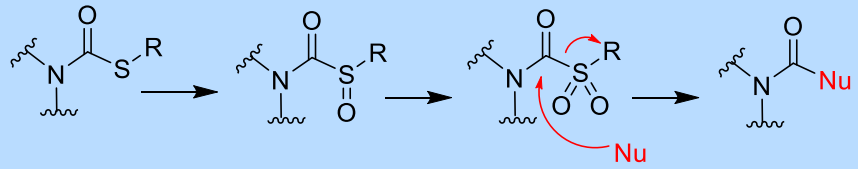
Marketed drugs

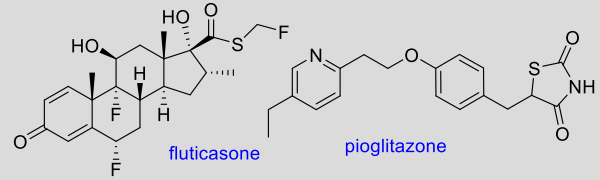
Thioureas, Thioamides
Ethionamide, Methimizole, Quazepam, Epalrestat, Enzalutamide, PF-06282999



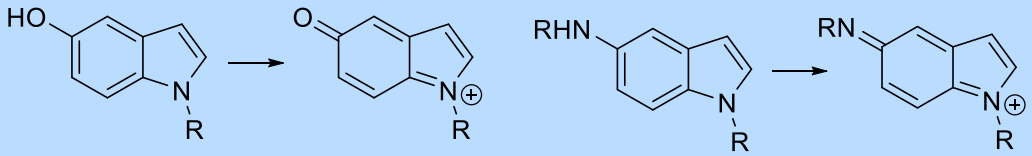


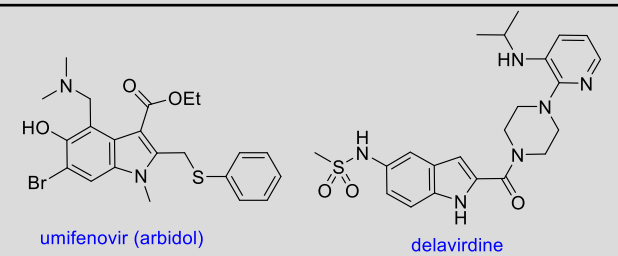
Thiocarbamates, Thioesters Thiazolidinediones
Fluticasone, Pioglitazone



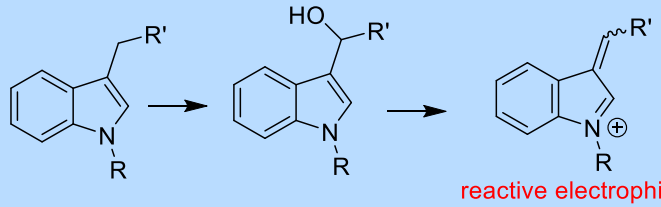


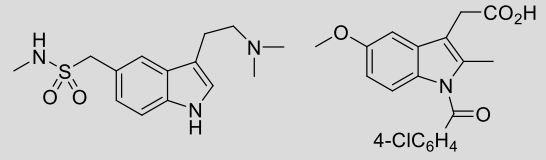
5-OH, OMe or Amino Indoles
Umifenovir, Delavirdine





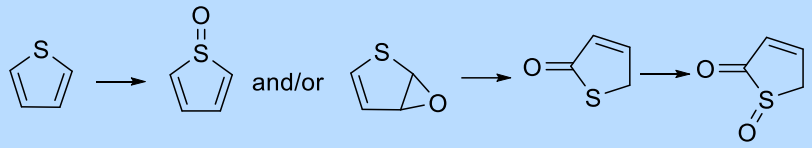
3-Alkylindoles
Sumatriptan, Eletriptan, Rizatriptan, Indomethacin



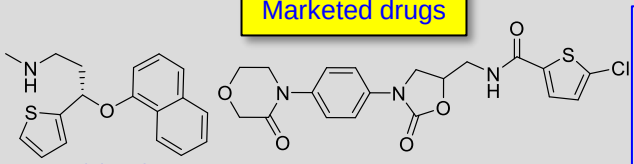


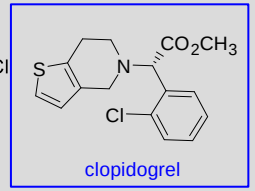
Structural Alerts – a Survey of Problematic Elements

Thiophenes
Duloxetine, Olanzapine, Tiotropium; Rivaroxaban, Clopidogrel

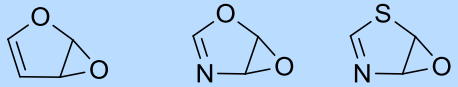


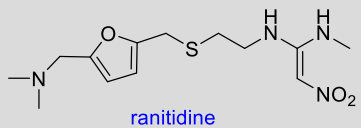
Marketed drugs

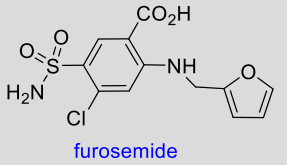




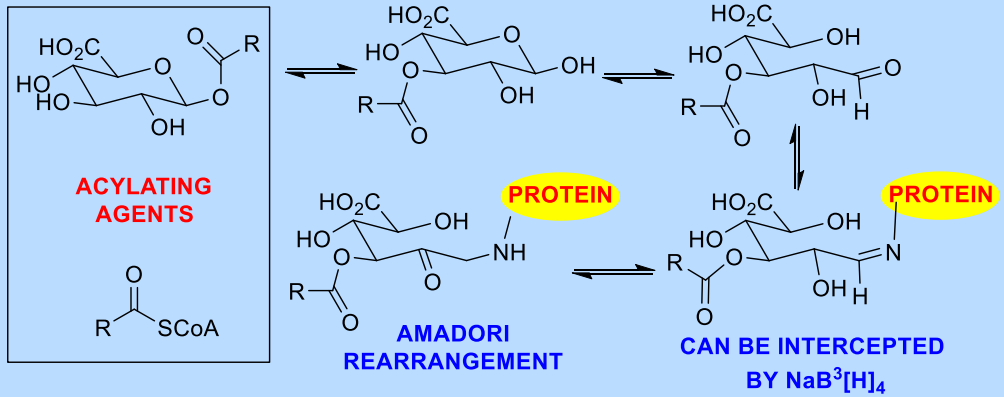
Furans, Oxazoles, Thiazoles
Ranitidine, Prazosin, Furosemide, Dantrolene, Mometasone, Ritonavir

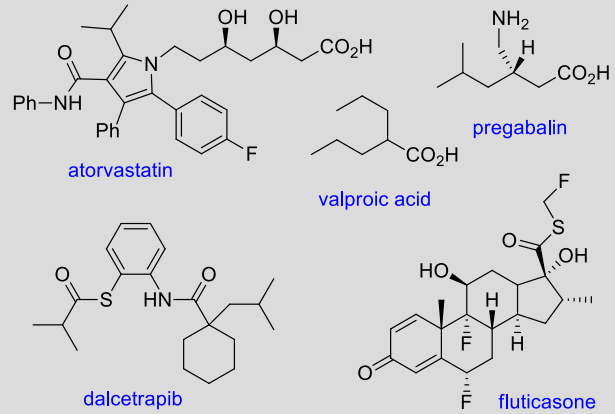




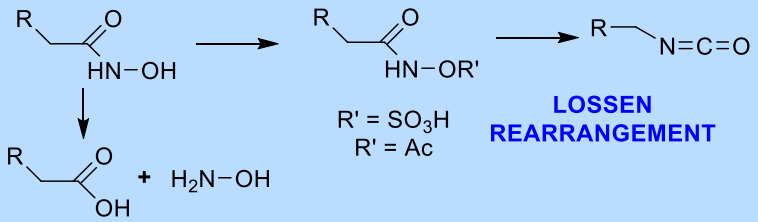


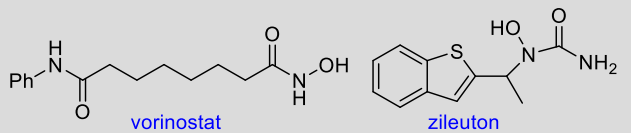
Carboxylic Acids
Valproic Acid, Atorvastatin Pregabalin, Dalcetrapib, Fluticasone (**thioesters**)





Hydroxamic Acids
Vorinostat, Zileuton

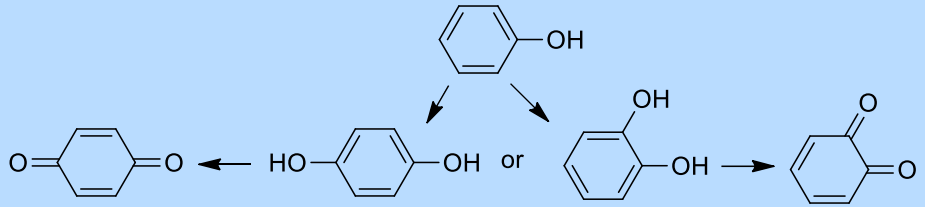


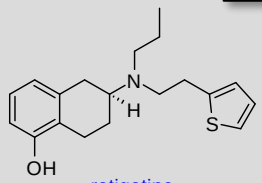


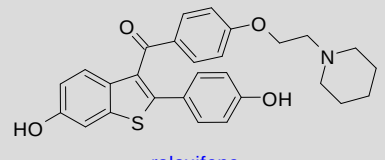
Structural Alerts – a Survey of Problematic Elements

Marketed drugs

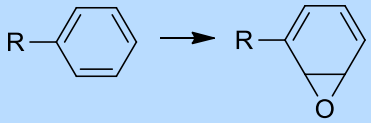
Phenols, Hydroquinones
Raloxifene

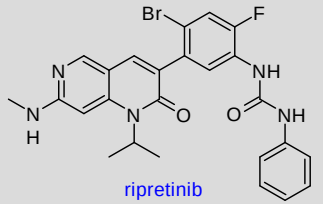


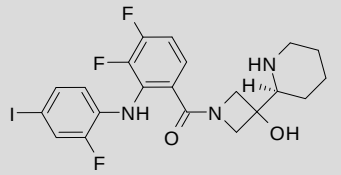

rotigotine


raloxifene

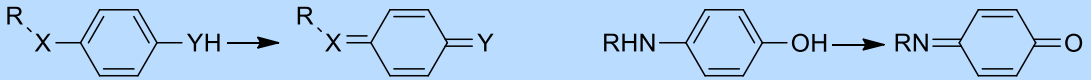
Benzene, Bromo- & Iodo benzenes
Cobimetinib

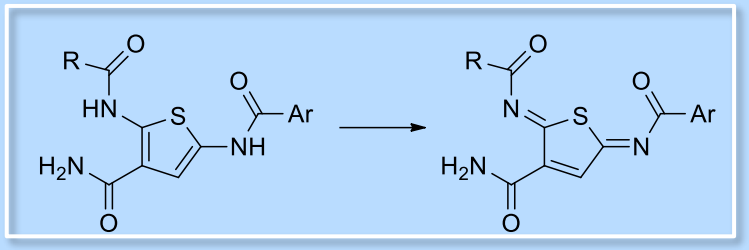


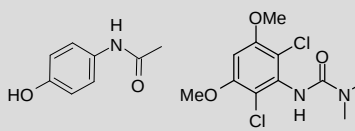

ripretinib


cobimetinib

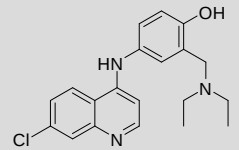
1,4-Hetero-Substituted Aromatics
Acetaminophen, Amodiaquine
Infigratinib
Retigabine & Flupirtine withdrawn
extends to thiophenes

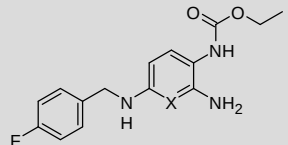




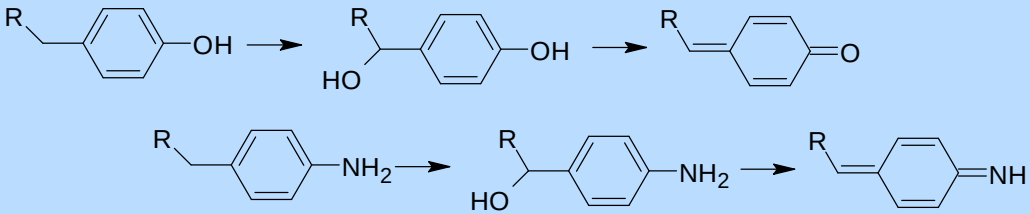

acetaminophen

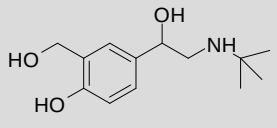
infigratinib

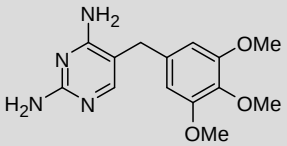

amodiaquine


X = CH: retigabine
X =N: flupirtine

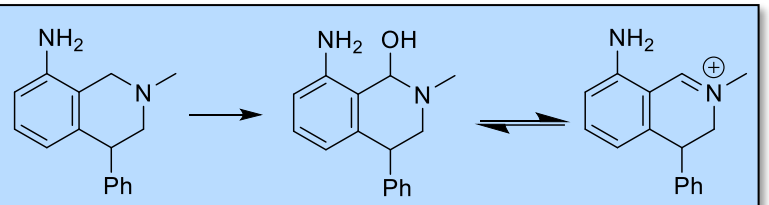
Substituted Toluenes
Salbutamol



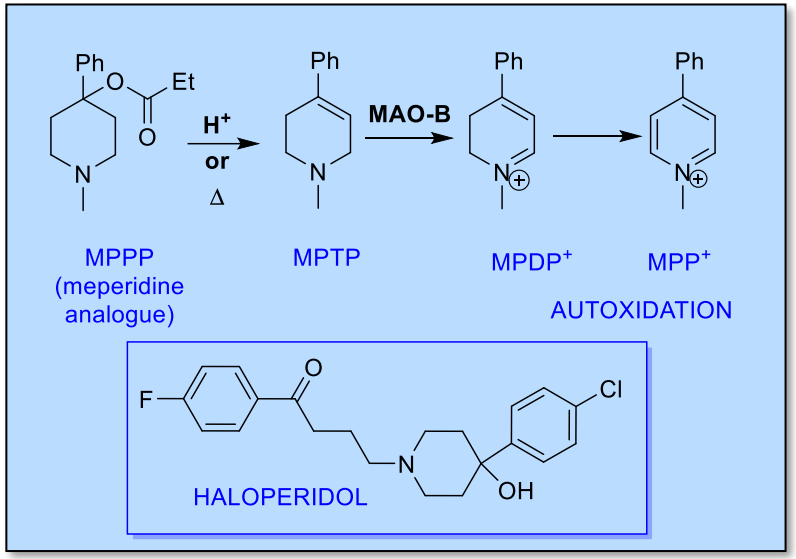

salbutamol


trimethoprim

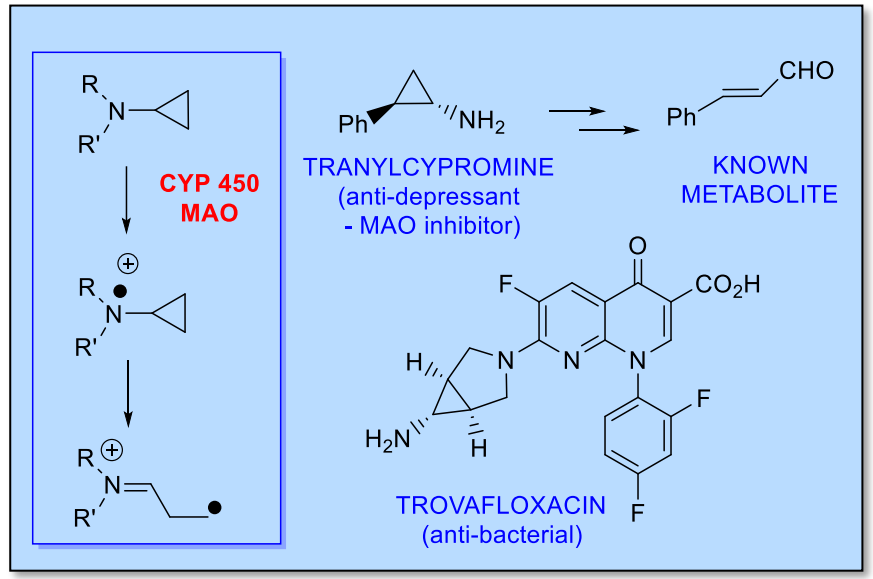
Amines: Some Special Cases with Concern



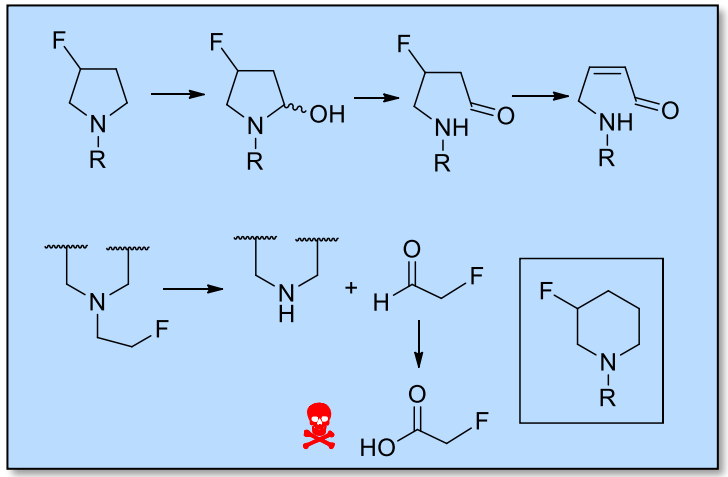
- Cyclic amines
  - α-hydroxylation & H_2O elimination
  - can lead to cyclic iminium species
  - react with hard electrophiles (CN^-)
  - aldehyde disassociation with acyclic amines
- Nomifensine withdrawn
  - hemolytic anemia and hepatotoxicity
  - iminium reacts readily with CN^- but not GSH



- MPPP causes neurotoxicity
  - haloperidol has similar metabolite



- Cyclopropyl amines undergo ring opening
  - tranylcypromine metabolized to cinnamaldehyde
- Trovafloxacin had BBWs for liver toxicity
  - ultimately withdrawn due to hepatotoxicity



- 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: 2-10

Strategies for Mitigating Reactive Metabolites

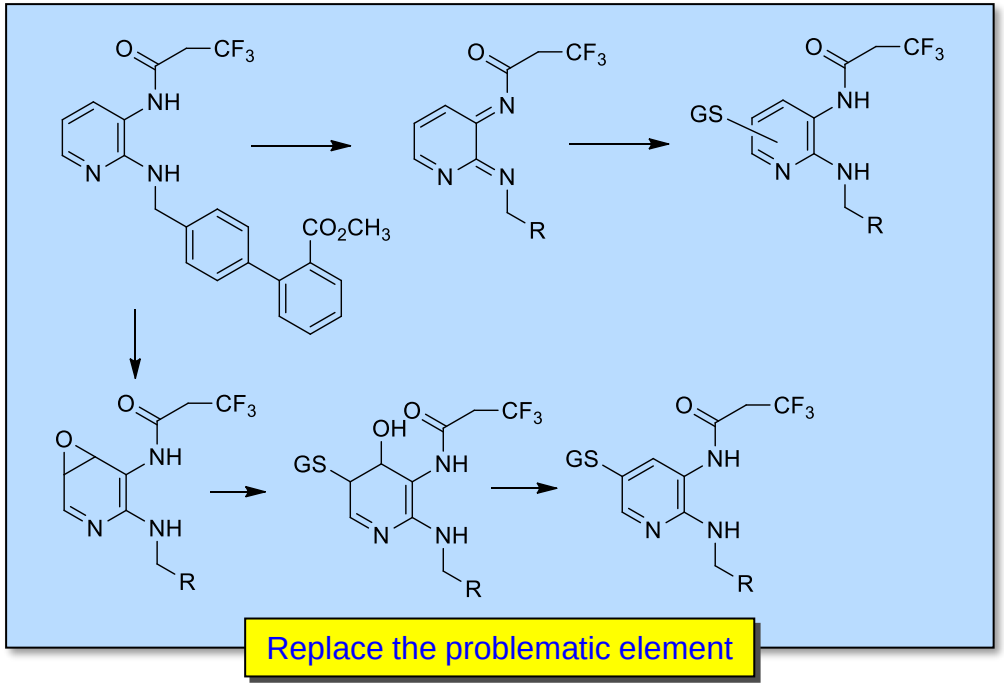
Strategies for Reducing Potential Problems

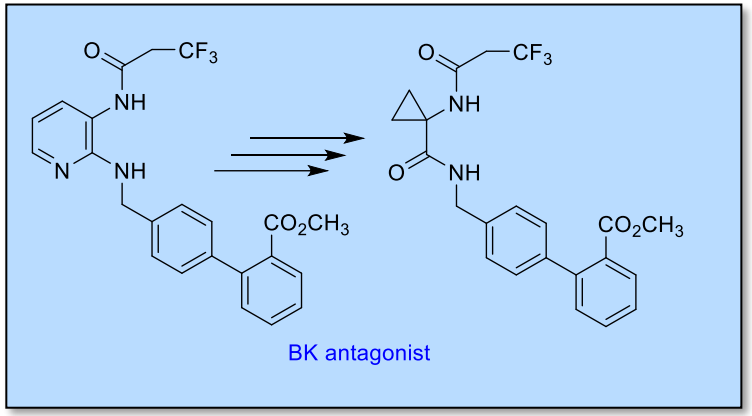
- Maximize potency, minimize dose
  - reduces reactive metabolite burden
- Structural modification
  - remove or modify problematic structural elements
- Introduce steric effects
  - steric shielding of metabolic sites to slow bioactivation
  - reactive metabolites will also likely be subject to steric 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

Reactive Metabolite Mitigating Strategies

Structural Modification of Problematic Elements

Quinonediimines in Bradykinin Antagonists

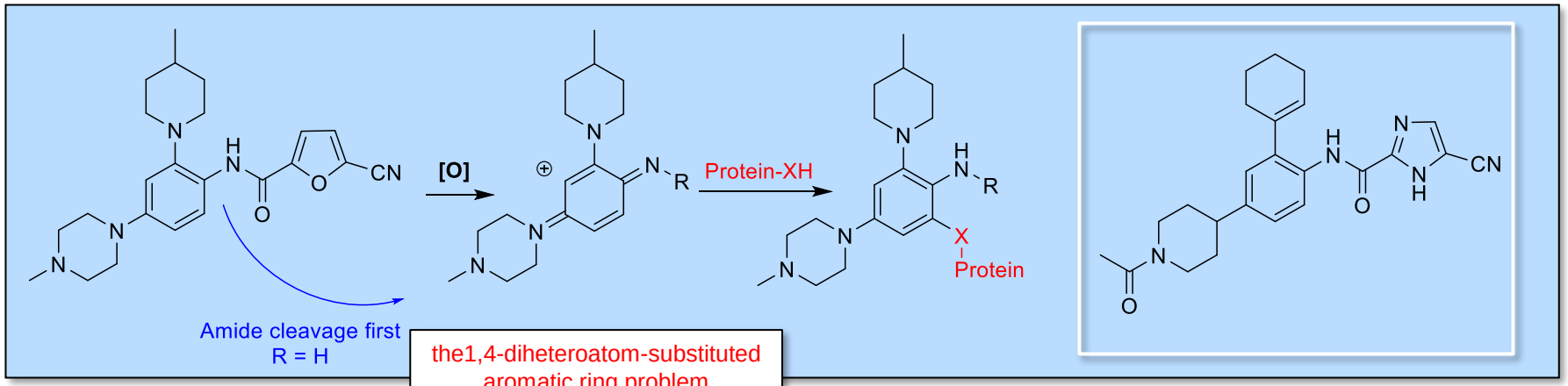




- Diamino pyridine moiety susceptible to oxidation in bradykinin antagonists
- Solution - isostere of phenylene diamine moiety
  - reduce pyridine moiety to ethylene diamine; add C=O to mimic N
  - dimethyl provides conformational bias - Thorpe-Ingold effect
- Cyclopropyl optimal: improved topology
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

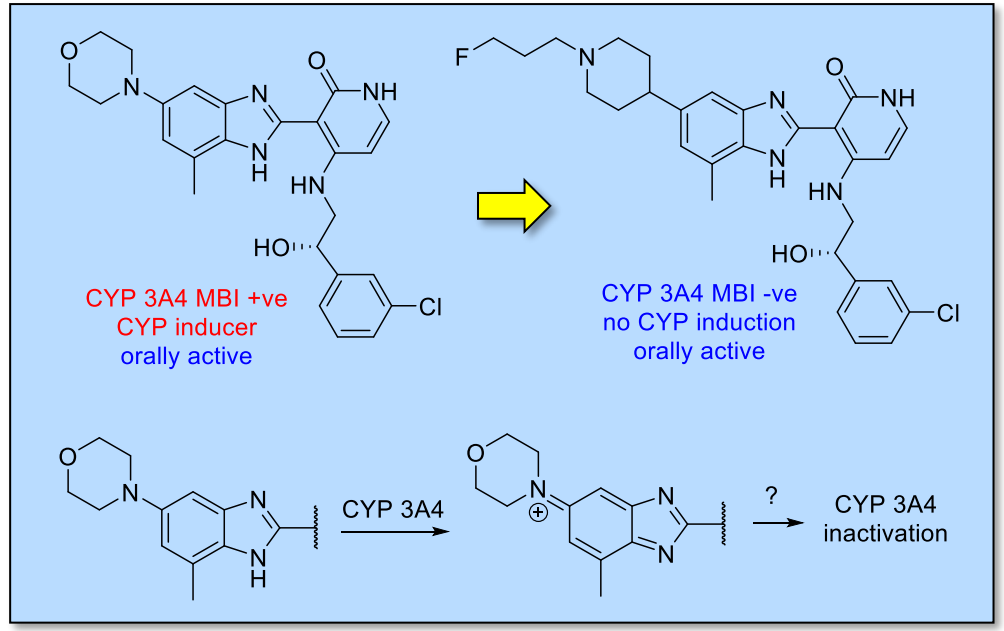
M.R. Wood *et al., J. Med. Chem.,* 2006, **49**, 1231-1234

Iminoquinones: FMS & IGFR



Replace the problematic element

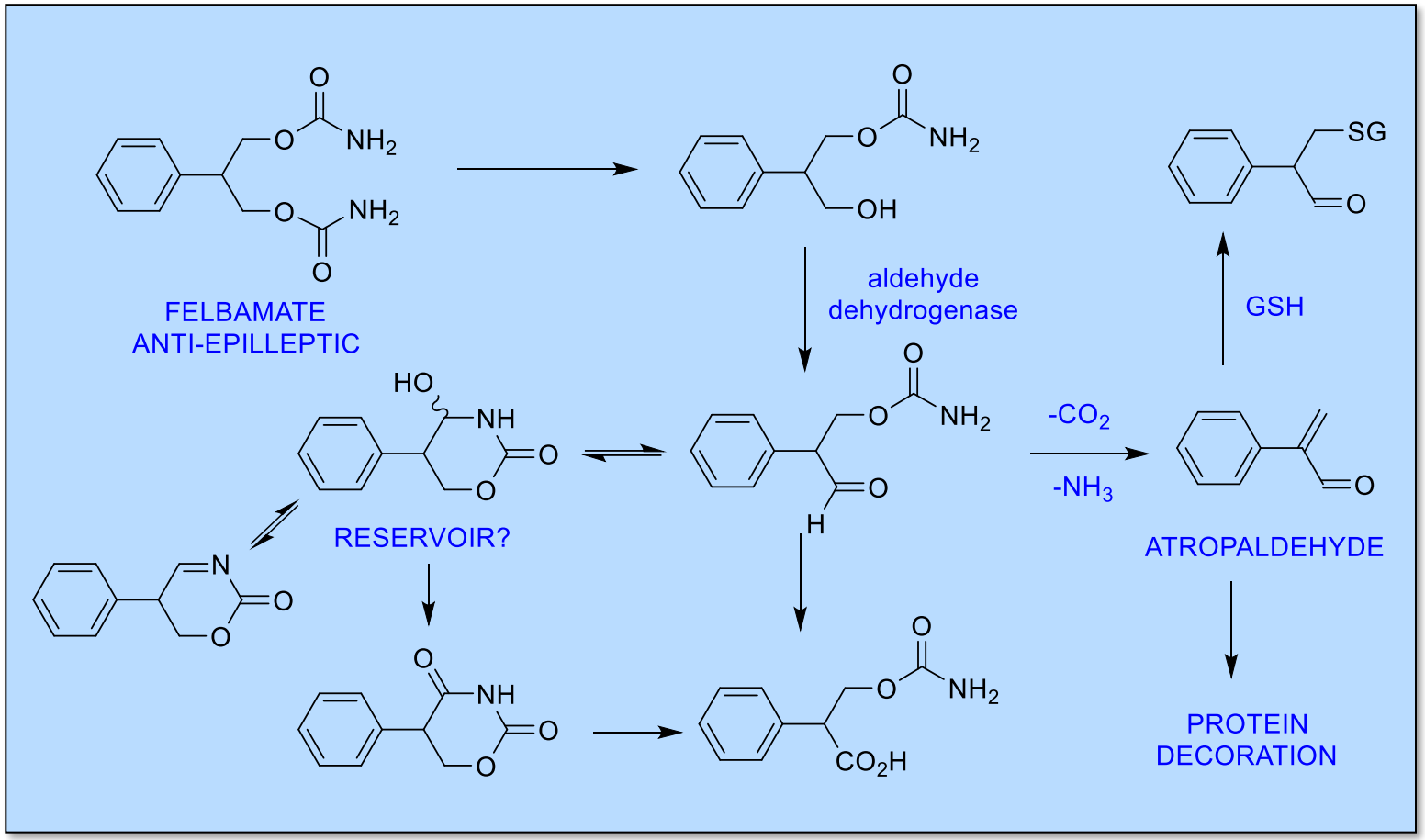
- FMS inhibitor: a type III receptor tyrosine kinase that recognizes CSF-1
  - an approach to treating arthritis and inflammation
- Electron-rich 1,2,4-phenylenetriamine core a cause for concern
  - the 1,4 heteroatom problem (also 1,2)
  - potential for release of an aniline
- GSH adducts of parent and aniline when incubated in mouse LM
- Solution: replace N atoms of piperazine and piperidine with C
  - furan also modified to imidazole



- Lead IGF1R inhibitor exhibited mechanism-based CYP 3A4 inhibition
  - replacing morpholine with piperidine, piperazine failed to address the problem
- C-linked piperidine provided a satisfactory solution
  - speculation of iminium quinone intermediate as source of MBI

S.K. Meegalla *et al., Bioorg. Med. Chem. Lett.*, 2008, **18**, 3632-3637; U. Velaparthi *et al., J. Med. Chem.*, 2008, **51**, 5897-5900

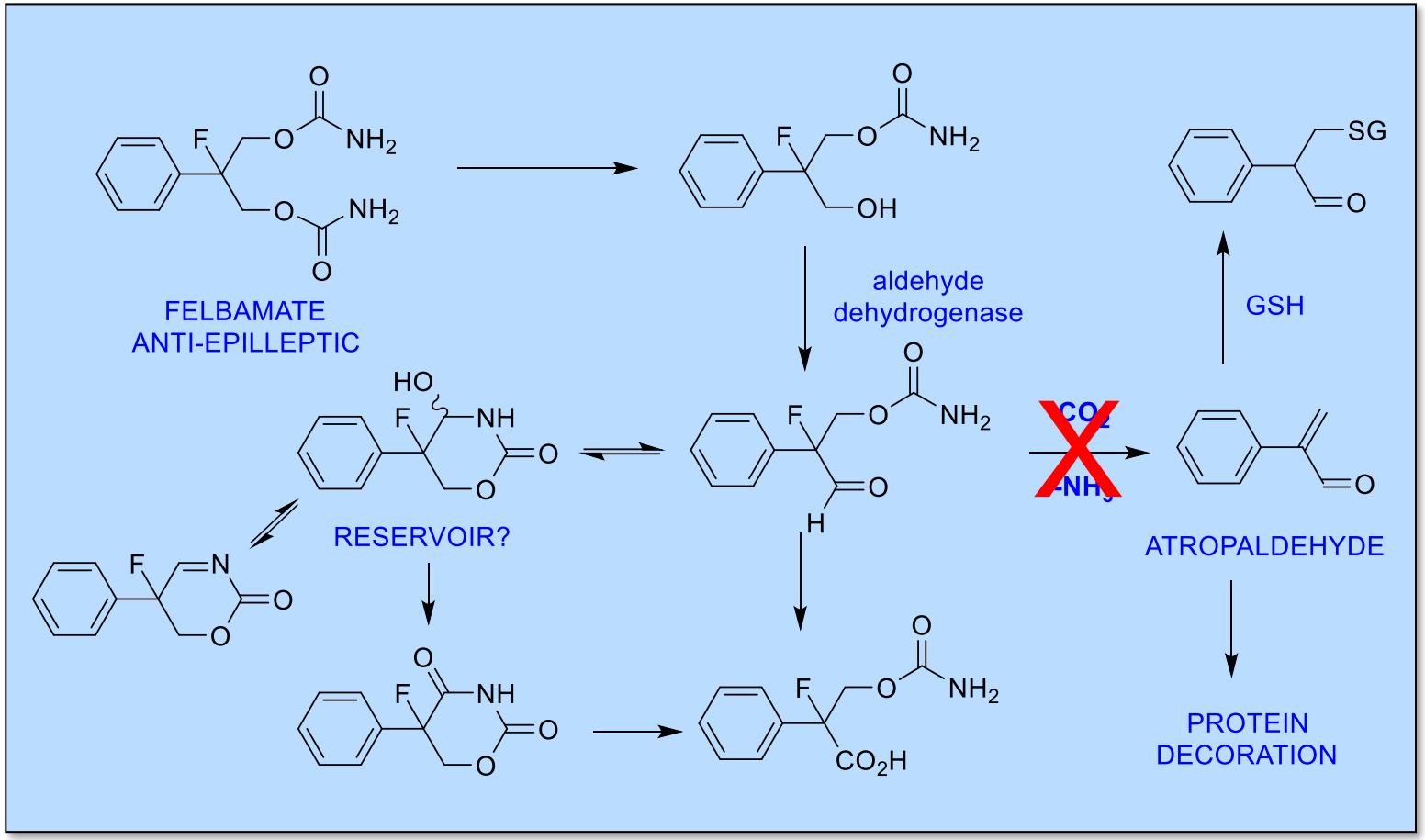
Felbamate Metabolism



- Clinical utility of felbamate limited by aplastic anemia & hepatotoxicity
- Atropaldehyde is potently electrophilic and toxic to fibroblasts
  - thiol adducts found in rat and human urine

C.M. Dieckhaus *et al., Chem. Biol. Interact*., 2002, **142**, 99-117; 119-134

F-Felbamate Mitigates Metabolic Activation

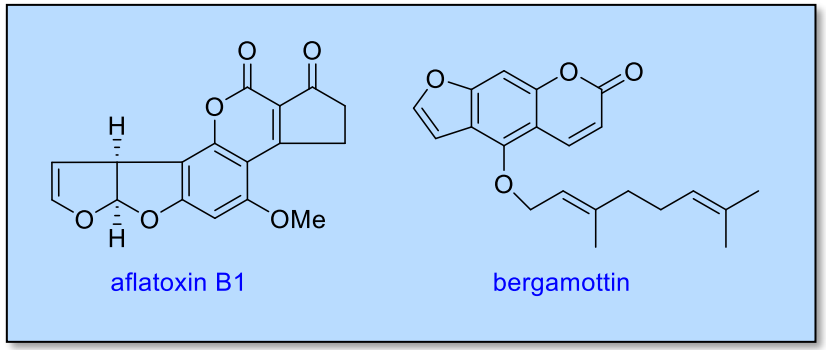


Block the metabolic pathway

- Strategic deployment of F based on detailed understanding of metabolism
- F atom of fluorofelbamate prevents elimination of carbamate
  - atropaldehyde not formed

R.J. Parker *et al., Chem. Res. Toxicol.,* 2005, **18**, 1842-1848

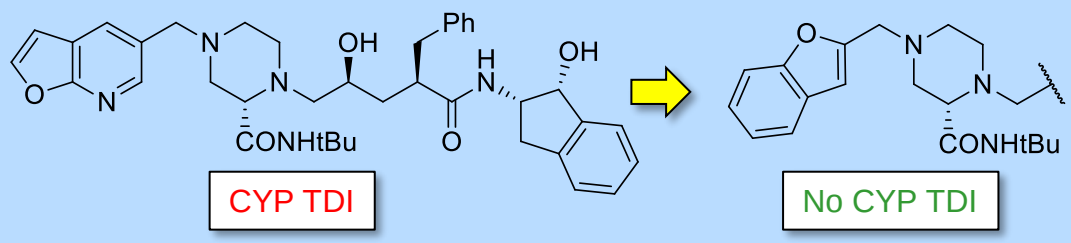
Avoiding Furan Metabolism



- Difuranocoumarins produced by common fungal molds
  - can contaminate human foodstuff and enter the diet
- Well-established link to hepatotoxicity & hepatocellular carcinoma
  - aflatoxin amongst the most carcinogenic substances known
- Metabolic activation has been established as the source of toxicity
  - dihydrofuran moiety activated *via* the epoxide

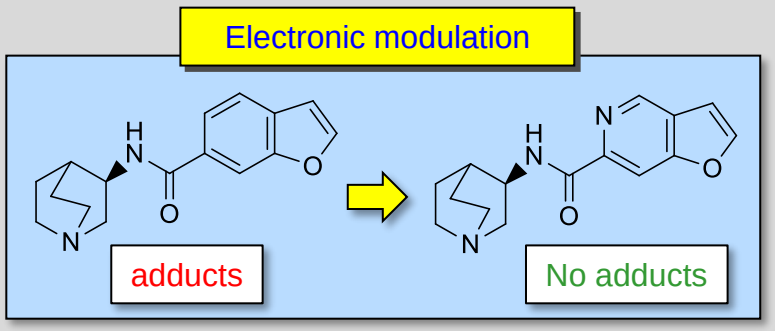
- Grapefruit juice found to increase felodipine bioavailability
  - 164-469% of that when dosed in H_2O; nifedipine F increased to 134%
  - due to inhibition of intestinal CYP enzymes
- Furanocoumarin derivatives have been implicated as the source of the effect
  - rapid, potent mechanism-based inhibitor of intestinal CYP 3A4
- Bergamottin & derivatives are mechanism-based CYP 3A4 inhibitors
  - bergamottin shows greater protein binding in LM – more hydrophobic
- Bergamottin also inhibits CYP 1A2, 2A6, 2C9, 2C19, 2D6, 2E1 & 3A4 in HLM

Rearrange topology to restrict access



- Merck: HIV-1 protease inhibitor
- Furopyridine is a mechanism-based CYP inhibitor
  - 2-linked benzofuran clean

Electronic modulation



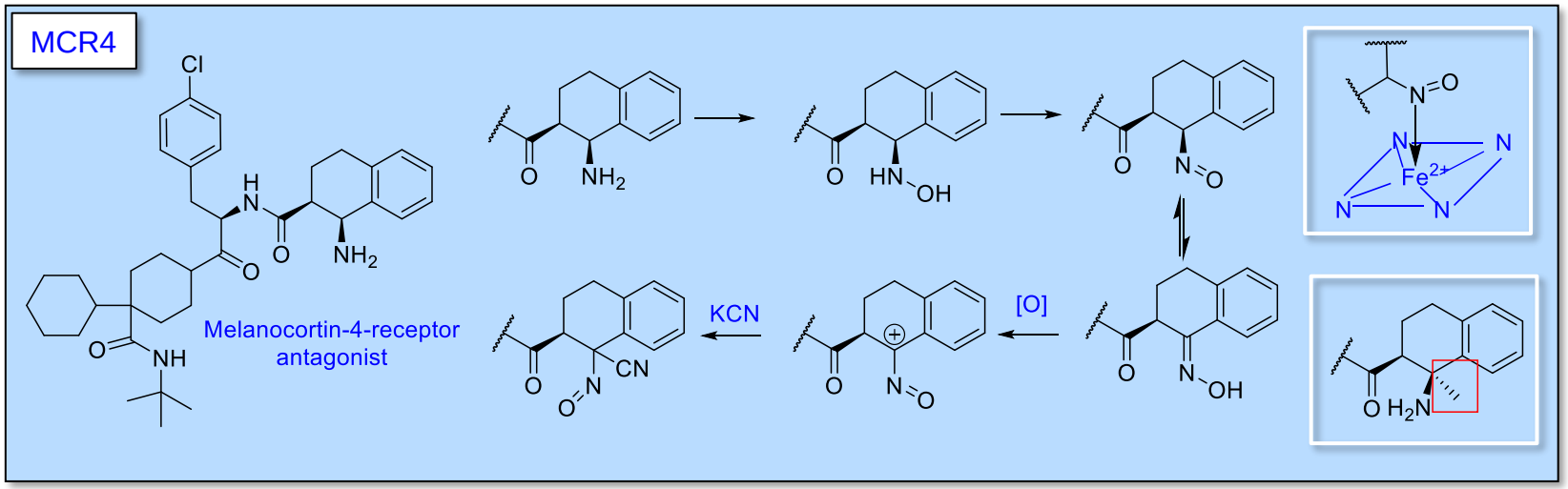
- Pfizer: α7-nicotinic acetylcholine receptor antagonists
- Benzofuran gave adducts in a CYP/GSE assay
- Furopyridine analogue was clean
  - advanced into clinical studies

D.G. Wishka *et al., J. Med. Chem.*, 2006, **49**, 4424-4436; B.D. Dorsey *et al., J. Med. Chem.*, 2000, **43**, 3386-3399

Reactive Metabolite Mitigating Strategies

Introduce Steric Effects

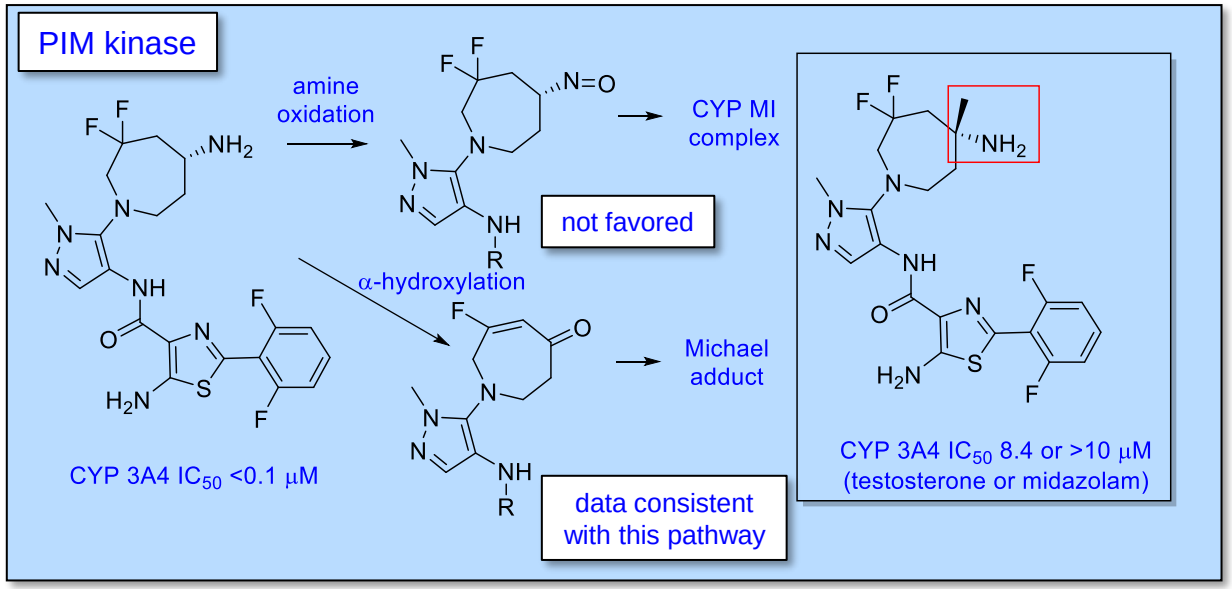
Melanocortin-4-Receptor Antagonist & Pim Kinase



- 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
  - α-hydroxylation to afford C=O
  - elimination of F
  - blocked by α-CH_3

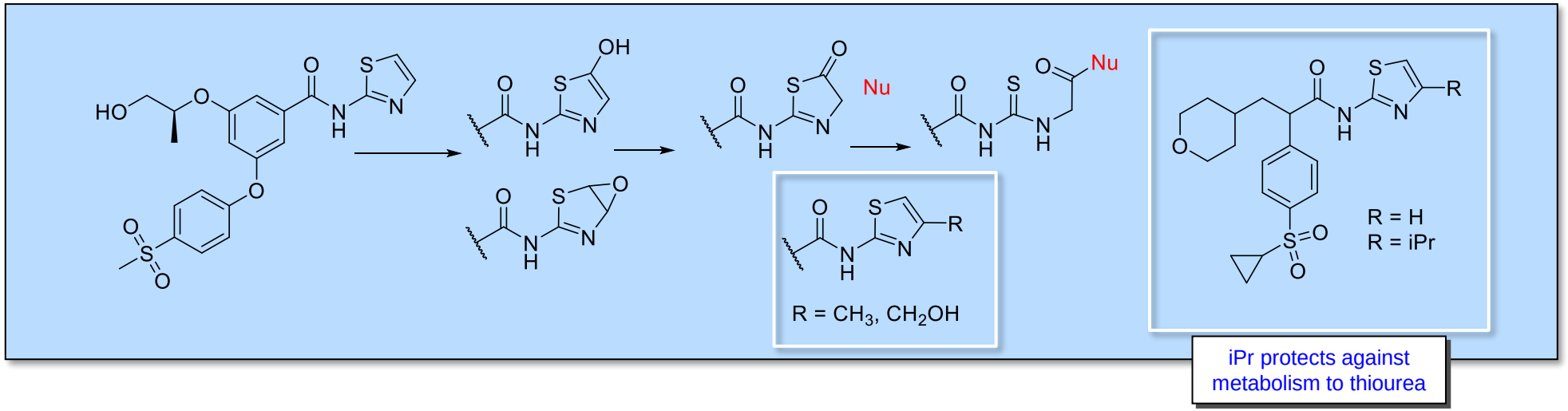
- MCR4: time-dependent CYP 3A inhibitor in HLM
  - cyanide adduct identified in HLM containing KCN
- With rCYP 3A4, saw I_{max} at 450 nM – MI complex
  - consistent with amine oxidation to nitroso derivative
- PO administration to rats increased indinavir plasma levels 3x
  - suggested potential for DDIs in humans
  - development terminated
- α-CH_3 derivative did not inhibit CYP
  - steric shielding of amine
  - also blocks the metabolic pathway

introduce steric/reactivity constraints



W. Tang *et al., Xenobiotica*, 2008, **38**, 1437-1451; X. Wang *et al., ACS Med. Chem. Lett.*, 2015, **6**, 925-929; B.M. Johnson *et al., J. Med. Chem.*, 2020, **63**, 6315-6386

Reducing Metabolic Activation of Thiazoles

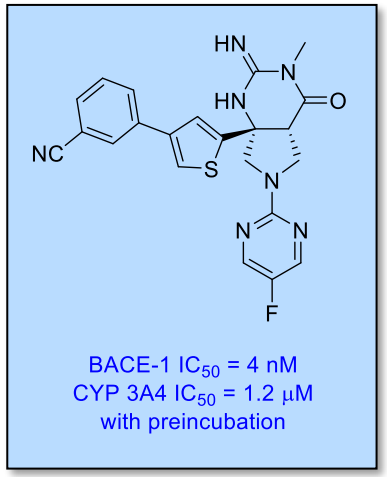


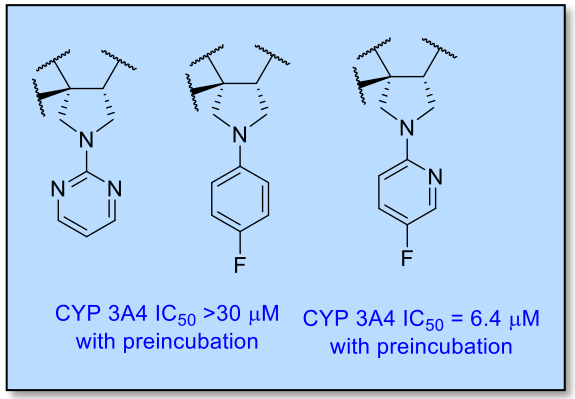
- Two series of glucokinase activators based on 2-amino thiazoles
- PCB when incubated with rat and human LM: thiazole moiety responsible
  - major metabolite in RLM was the thiourea acid, Nu = OH
  - trapped by GSH: adduct with Nu = GS
  - implicated the thiolactone as the key reactive intermediate
- Substitution of the thiazole with CH_2OH at C-4 or CH_3 at C-5 reduced PCB 2-5-fold
- Second series:
  - sterically demanding *i*-Pr substituent at C-4 of thiazole optimal for potency
  - no metabolism to the thiourea following oral dosing to rats at 50 mpk

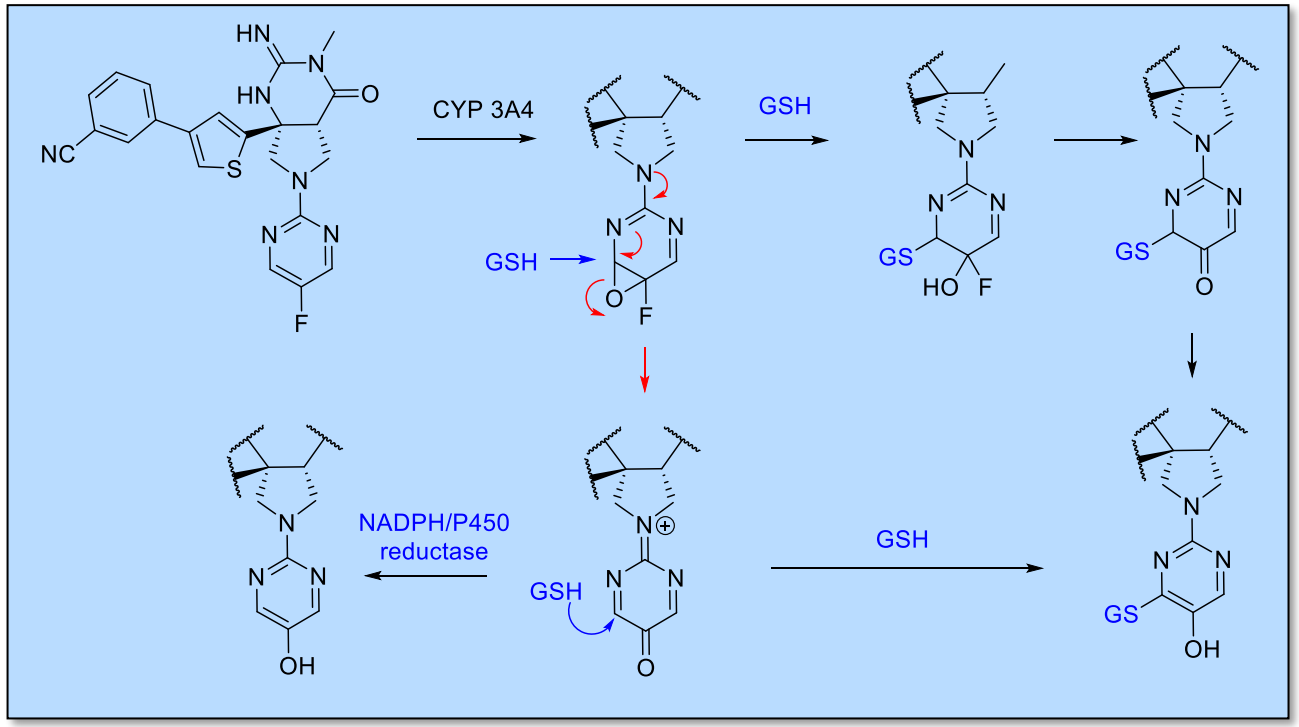
Introduce steric constraints

T. Lino *et al., Bioorg. Med. Chem. Lett.*, 2010, **20**, 1619-1622; F. Li *et al., Bioorg. Med. Chem.*, 2010, **18**, 3875-3884

Problems with a Fluorinated Pyrimidine

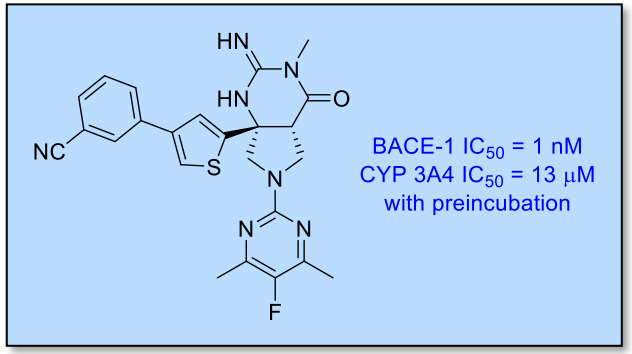






Introduce steric constraints

- 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



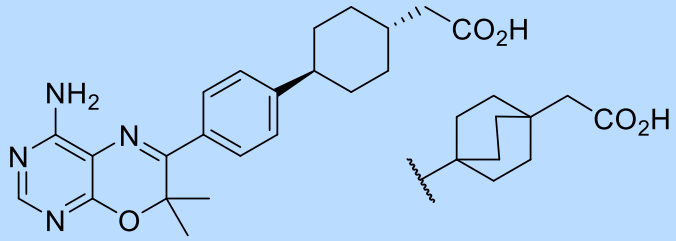
Enhancing Acyl Glucuronide Stability

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

Unsafe	Safe	Dividing Point
$t_{1/2}$ ≤1.7 h	$t_{1/2}$ ≥7.2 h	$t_{1/2}$ = 3.6 h

R, R'	*k* (h^{-1})
H, H	1.07
CH_3, H (*S*)	0.367
CH_3, H (*R*)	0.604
CH_3, CH_3	0.0302
Et, Et	0.00008

Introduce sterically demanding proximal substituents



hDGAT-1 IC_{50} = 15 nM hDGAT-1 IC_{50} = 15 nM

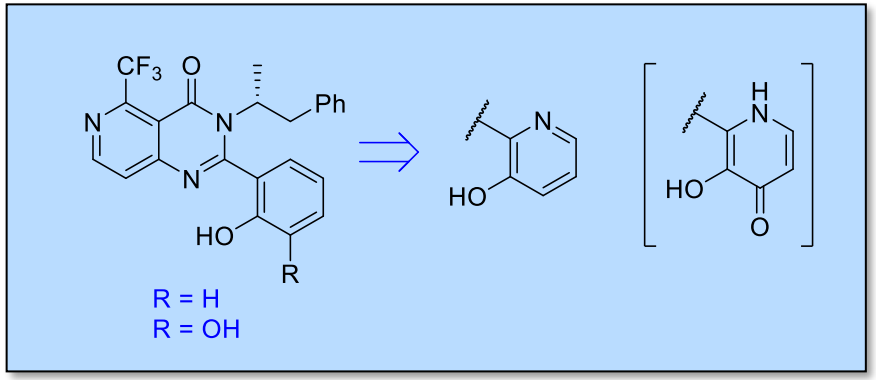
- Potent DGAT-1 antagonist
  - blocks triglyceride synthesis, storage
- Acyl glucuronide the 1° metabolite
- Added bulk to cyclohexane
  - increases stability of acyl glucuronide
  - $t_{1/2}$ for hydrolysis = 64 h in buffer
  - <15% rearrangement over 80 h

T. Yoshioka *et al., Chem. Res. Toxicol*., 2009, **22,** 158-172; 1559-1569; 1998-2008; A.M. Birch *et al*., *J. Med. Chem.,* 2009, **52**, 1558-1568
R. Sawamura *et al., Drug Metab. Disp*., 2010, **38**, 1857-1864; S.L. Regan *et al., Biopharm Drug Disp*., 2010, **31**, 367-395

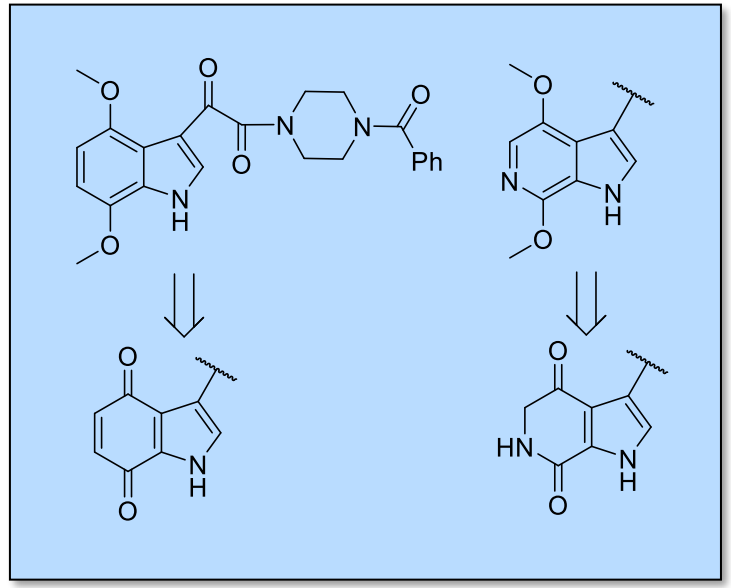
Reactive Metabolite Mitigating Strategies

Modulate Electronic Properties

Avoiding Quinone-Type Metabolites



Modulate electronic properties



- Short-acting Ca^{2+}-sensing receptor antagonists
  - potential therapy for osteoporosis
- Lead candidate underwent sequential NADPH-dependent oxidation
  - gave catechol & *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

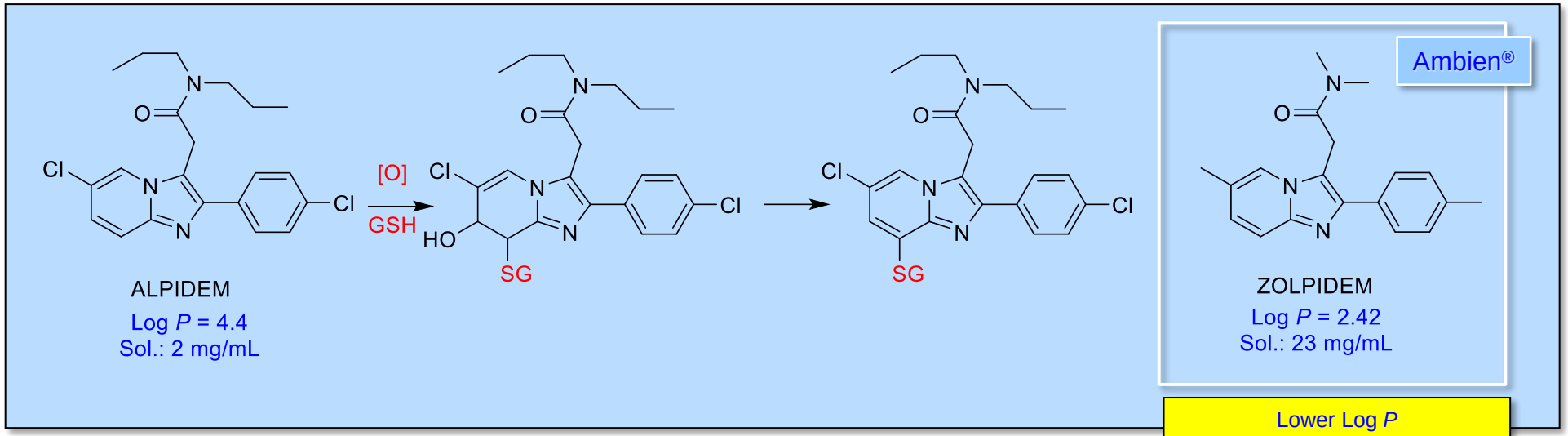
- HIV-1 attachment inhibitors
  - demethylation/oxidation to quinone
  - 6-aza would metabolize to amide

A. Kalgutkar *et al., Chem. Res. Toxicol.*, 2010, **23**, 1115-1126; T. Wang *et al., J. Med. Chem.*, 2009, **52**, 7778-7787

Reactive Metabolite Mitigating Strategies

Introduce a Metabolic Soft Spot or Redirect Metabolism

Olefins in Benzodiazepine Receptor Ligands



Lower Log *P*
Higher solubility
Lower dose
Alternate sites of metabolism

- GABA-chloride channel ligands – ω_1 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 CH_3 moieties
- Structurally similar but markedly different pharmacology and toxicology

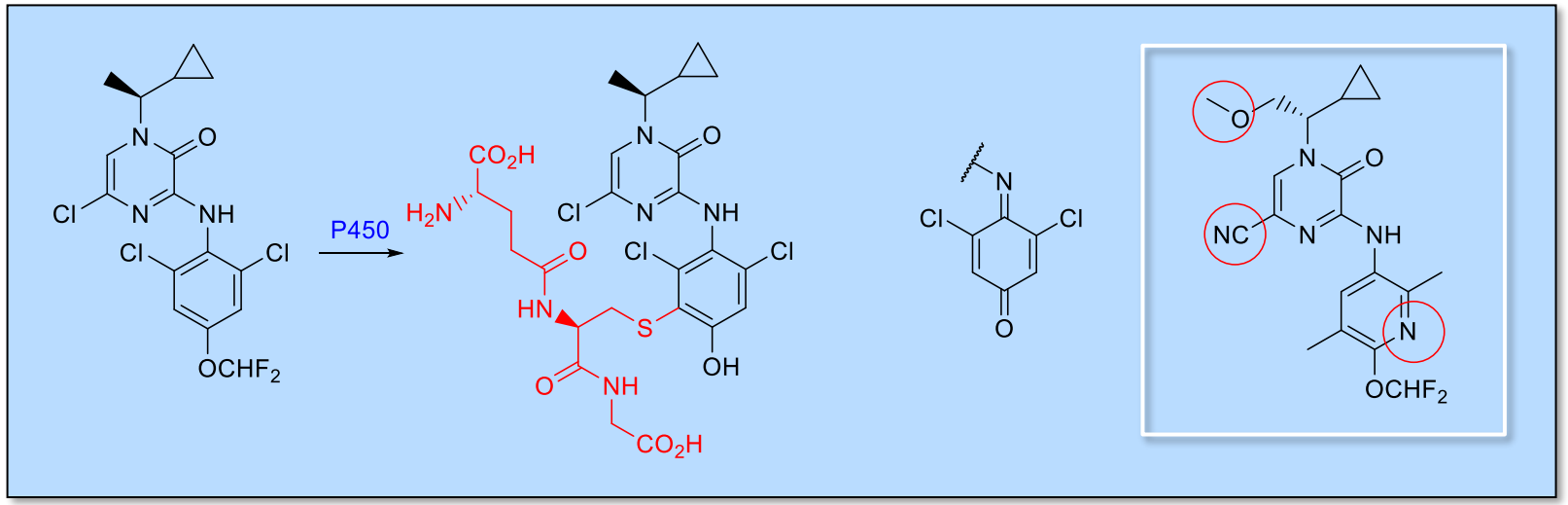
Redirect metabolism

A. Berson *et al., J. Pharmacol. Exp. Ther*., 2001, **299**, 793-800; A. Durand *et al., Drug Metabolism Rev*., 1992, **24**, 239-266
D. Garrigou-Gadenne *et al., Drug Metab. Disp*., 1991, **19**, 574-579, L. Pichard *et al., Drug Metab. Disp*., 1995, **223**, 1253-1262

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
  - epoxidation of the olefin
  - required further structural modification to electron deficient CN moiety
- Major metabolic pathway – *O*-demethylation of alkyl ether introduced as a soft spot

Combination of approaches: steric effects, redirect metabolism

R.A. Hartz *et al., J. Med. Chem.,* 2009, **52**, 7653-7658

Conclusion

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