

Addressing Toxicity Issues Due to Drug Metabolites During Discovery and Early Development

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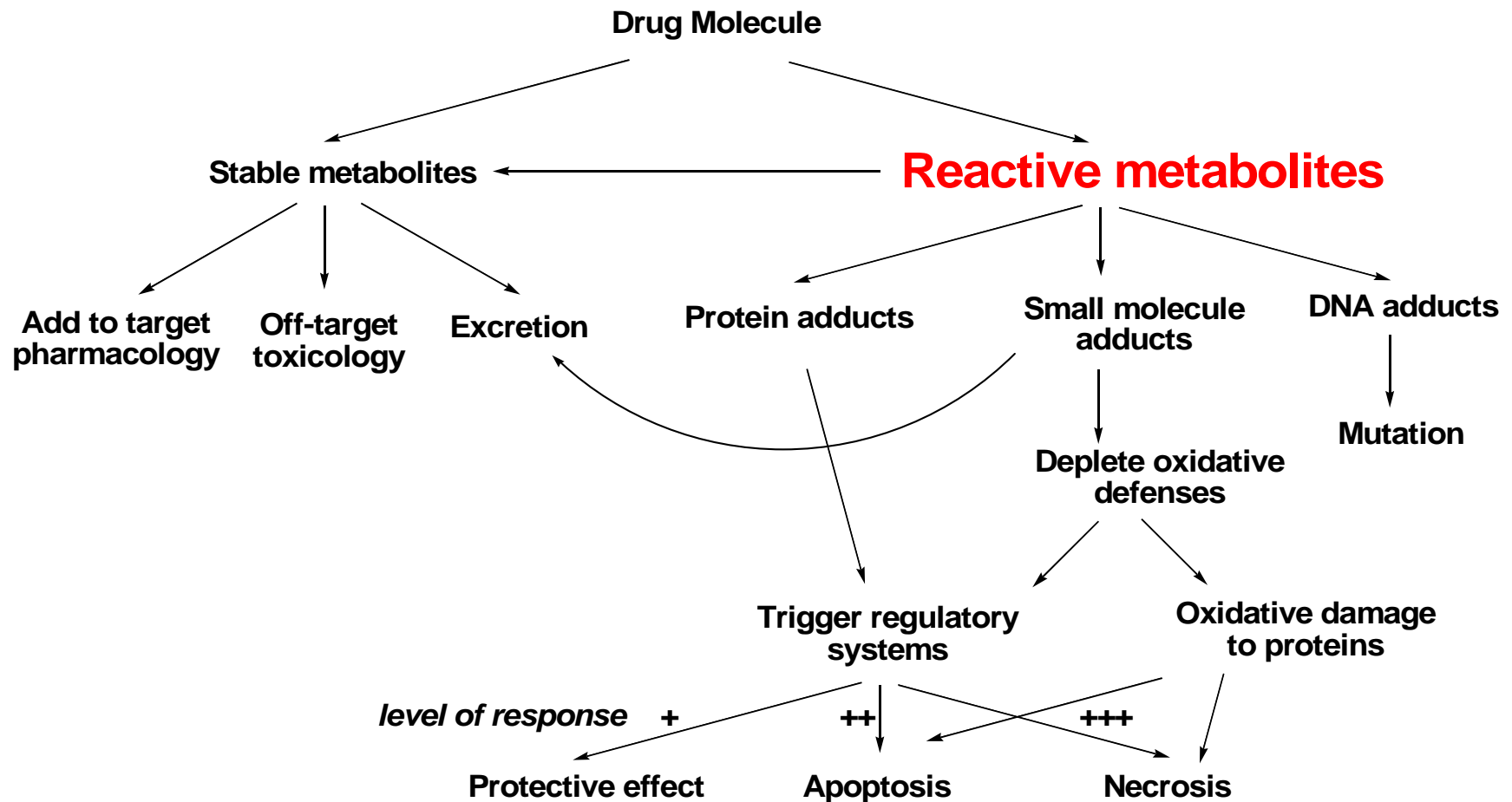
Safety Testing of Drug Metabolites

- Goal: Proper testing of the safety of a new drug requires that the metabolites that humans are exposed to are also tested in the species used for toxicological evaluation.
 - Obligation to patient safety during clinical trials and post-approval
 - Regulatory requirements

Safety Testing of Drug Metabolites

- History
 - Prior guidance from FDA
 - ICH S3A, March 1995
 - Carcinogenicity Study Protocol Submission, since 1997
 - PhRMA MIST task force, 1999-2002 (culminated in publication - Baillie et al, TAP, 182, 188-106, 2002)
 - PhRMA/FDA Joint workshop, Nov 2000
 - FDA Draft Guidance, June 2005
 - PhRMA/FDA/DruSafe Joint workshop, Nov 2005
 - FDA Guidance Issued, Feb 2008

Safety Testing of Drug Metabolites



Adapted from Liebler and Guengerich, Nature Reviews, 4, 410-420.

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What are we most concerned with regarding metabolites

- Most of the cases of metabolite mediated toxicity have to do with the formation of reactive intermediates
- All of the examples cited in the FDA draft guidance arguing for the need to closely monitor metabolites deal with cases where reactive metabolites are generated
- *Reactive Metabolites:*
 - *Need to define and minimize pathways leading to the formation of reactive metabolites (IDRs)*
 - *Need to understand the role that these pathways may play in observed toxicology*

Reactive Metabolic Intermediates

When good reactions go bad

- Chemically reactive, electrophilic metabolites formed during normal biotransformation reactions (able to leave the enzyme active site)
- Catalyzed by the same enzymes responsible for detoxification
- Determinant of good vs. bad is chemical nature of metabolized molecule

Consequences of reactive metabolite formation

- covalent binding: proteins and DNA
 - electrophilic metabolites will react with nucleophilic molecules
 - protective mechanisms exist, but can be overwhelmed
- oxidative stress
 - disruption of reduced environment and formation of ROS
 - protective mechanisms also exist, but can also be overwhelmed

The Problem

- The formation of reactive metabolic intermediates can often be demonstrated to be associated with molecules that cause acute and delayed idiosyncratic toxicities
- Very difficult to predict which reactive intermediates will cause problems
- Fundamental lack of understanding about what happens between covalent binding and cell death
- 52% of acute liver failures in U.S. are drug induced (Kaplowitz, DMR, 36, 301)
- 10% of all drugs registered between 1975 and 2000 have been withdrawn or have a “black box” warning of adverse drug reactions (ADRs). *JAMA* (2002) 287: 2215
- Recent examples such as troglitazone (diabetes), tolcapone (extrapyramidal disorders), trovafloxacin (antibiotic), felbamate (anticonvulsant), zalcitabine (antiviral)

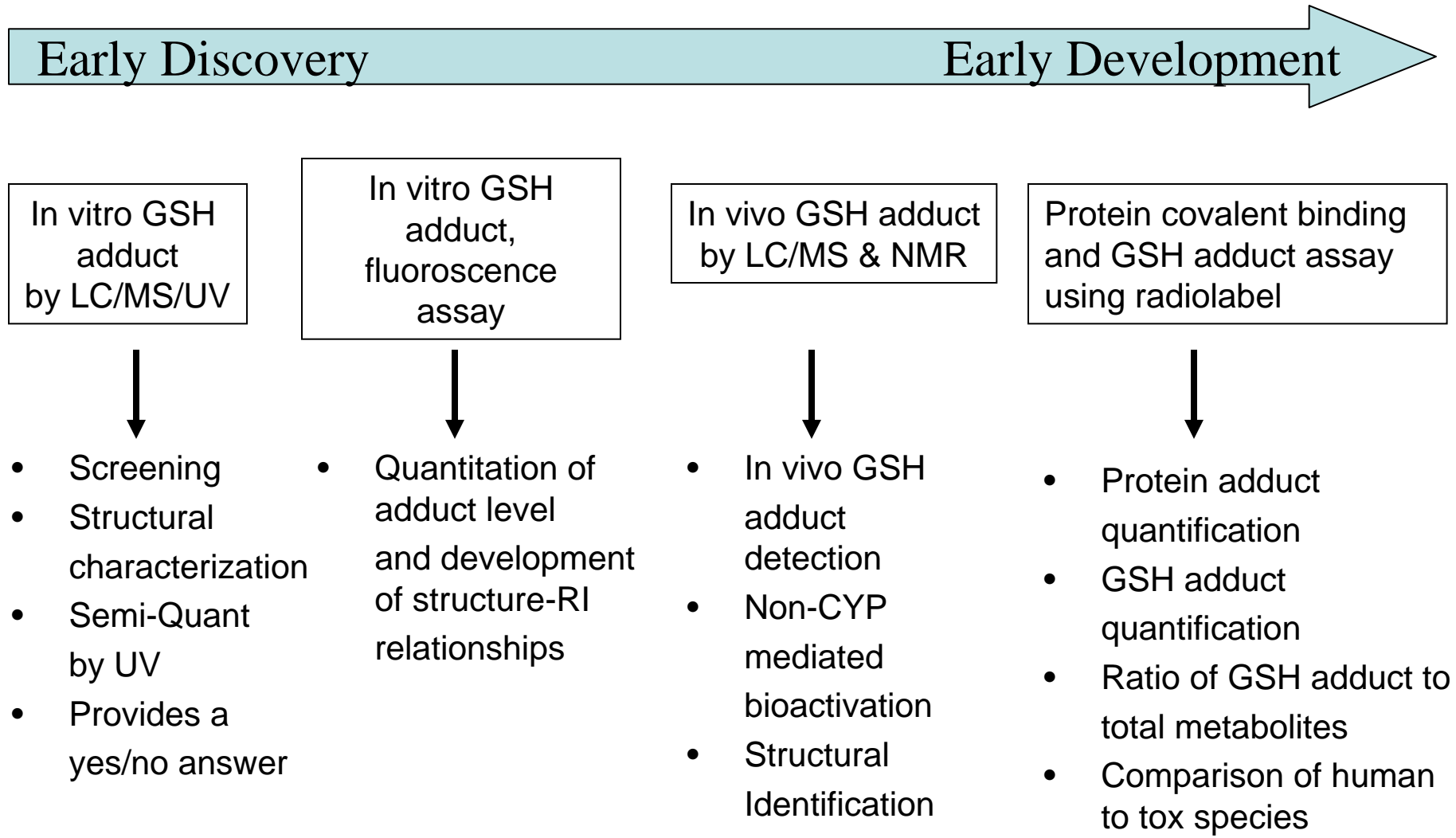
Idiosyncratic Toxicities – Association with Reactive intermediates

- There is a very large body of literature that demonstrates that many drugs that produces IDRs also form reactive intermediates that covalently modify intracellular targets
- However, the association is almost completely built around observations made with drugs that produce IDRs and not extended to try to address questions such as:
 - Is the reactive intermediate profile significantly different between drugs that cause IDRs and drugs that have superior safety, *i.e.* the “safe profile” (Obach, APISSEX poster)

Choices

- Ignore the problem
 - rely on toxicity testing to catch acute toxins
 - hope that the relatively low frequency of IDRs doesn't haunt us
- Elucidate mechanisms of IDRs in order to devise screens
 - entire scientific careers have been spent attempting this
 - No predictive animal models for immunologically-based idiosyncratic hepatotoxicity
- Develop strategy to detect and minimize reactive intermediate formation and covalent binding

Reactive Intermediate Detection



Large Molecule Covalent Binding Detection

- Assay can be used to characterize both the: 1) absolute risk to humans based on findings in human tissue fractions and 2) the relative risk assessment for human vs toxicology species, ie., does the assay predict a higher level of covalent adducts in the tox species relative to humans
- How to take into account candidates that demonstrate slow rates of in vitro turnover yet are predominately cleared through metabolism?
- Complete assessment of the absolute covalent binding number in this assay should take into account other factors regarding the clearance of the compound:
 - *For example*, Total burden of adduct = $\text{Dose} \times F_a \times F_m \times F_{\text{adduct}}$
 - F_{adduct} is the ratio of covalent adduct/total metabolite

How to put these findings into perspective?

- Dose
 - Low predicted human dose (≤ 10 mg) mitigates much of the risk of reactive intermediates (Utrecht, *Chem Res. Toxicol.* (1999) 12: 387)
- Duration of treatment
- Therapeutic target
 - unmet medical needs
 - life threatening indications

Method to investigate role of metabolism in observed toxicology

- The most commonly used method for modulation of metabolic pathways is through co-administration with a fairly non-toxic metabolic inhibitor, such as ABT (for CYP enzymes) or BNPP (for esterases)
- Other approaches include: enzyme induction, gender comparisons, species comparisons, k/o animals, transgenic animals, castration, etc.

Method to investigate role of metabolism in observed toxicology

- Why investigate observed toxicology to determine if it is metabolism related?
 - Maybe able to demonstrate species-specific toxicology (eg. Sustiva)
 - Need to separate compound-dependent from target-dependent toxicity

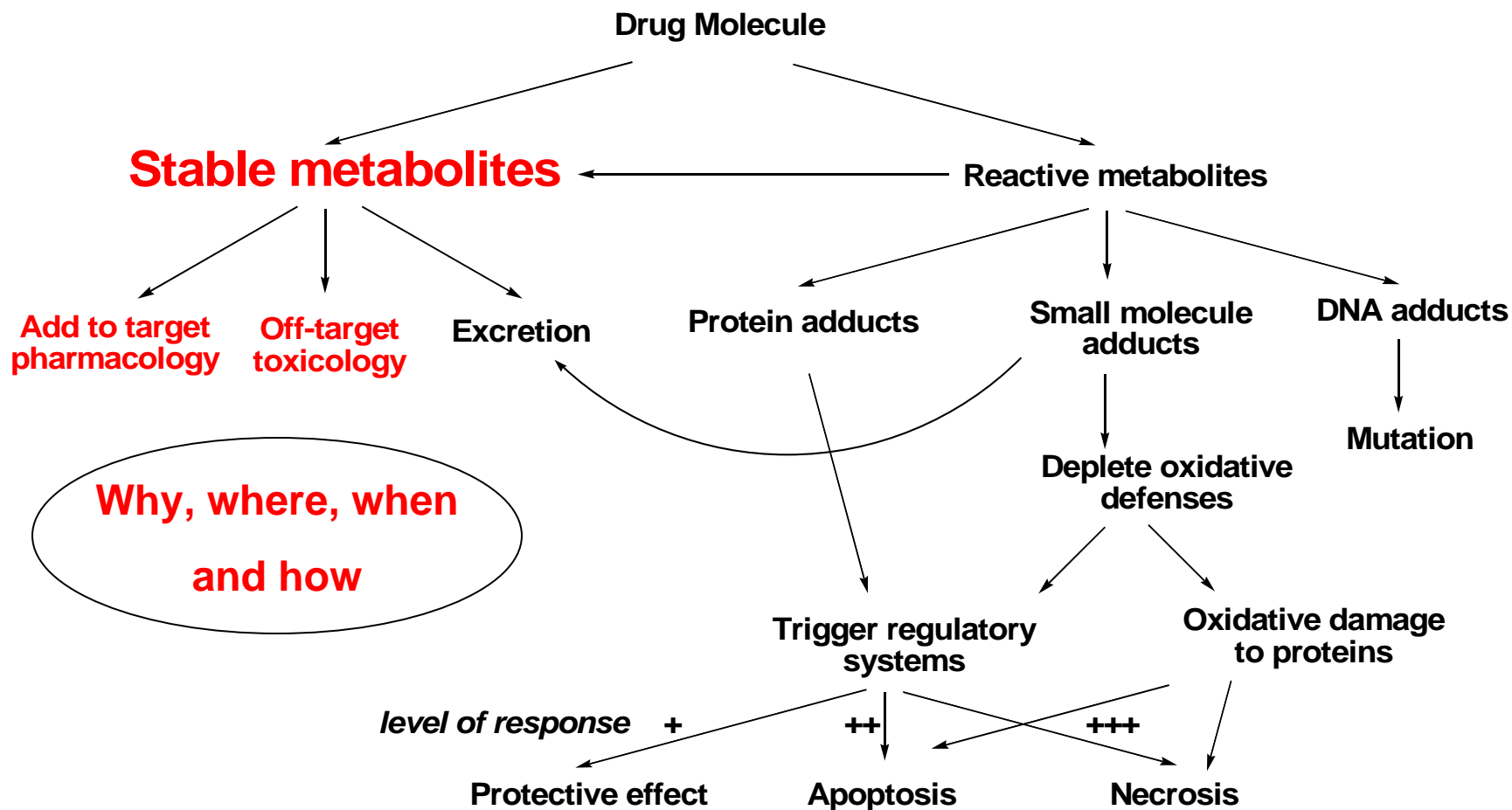
Role of metabolism in observed toxicology - example

- **Background:** A kinase inhibitor induced atrophy of the adrenal cortex in mouse, rat, dog and monkey.
- Radiolabeled drug accumulated in the rat adrenal gland to levels 30-fold greater than in liver.
- Binding to adrenal proteins and adrenal toxicity were abrogated by co-administration of aminobenzotriazole (ABT), a mechanism-based inhibitor of multiple CYP enzymes, to rats

Role of metabolism in observed toxicology - example

- Further mechanistic studies were performed in cultures of mouse Y-1 and human H295R adrenocortical cell lines.
- Cells were exposed to increasing concentrations of the kinase inhibitor in the presence or absence of general CYP inhibitors (ABT, keto), a specific inhibitor of CYP11A1 (aminoglutethimide) or CYP11B1/2 (metyrapone), or CYP inducers (ACTH, forskolin).
- Cells were also treated following > 90% inhibition of each steroidogenesis step by CYP-selective siRNAs. Endpoints included cell protein, ATP, and caspase 3/7 activity.
- **Conclusion:** Metabolism of the kinase inhibitor by CYP11A1 induced observed adrenal toxicity in rats (presumed to be similar mechanism in other species). Furthermore, apoptosis in mouse, but not in human adrenal-derived cells, indicated a species difference in metabolism (*O. Flint, et al., SOT, Seattle, 2008*).

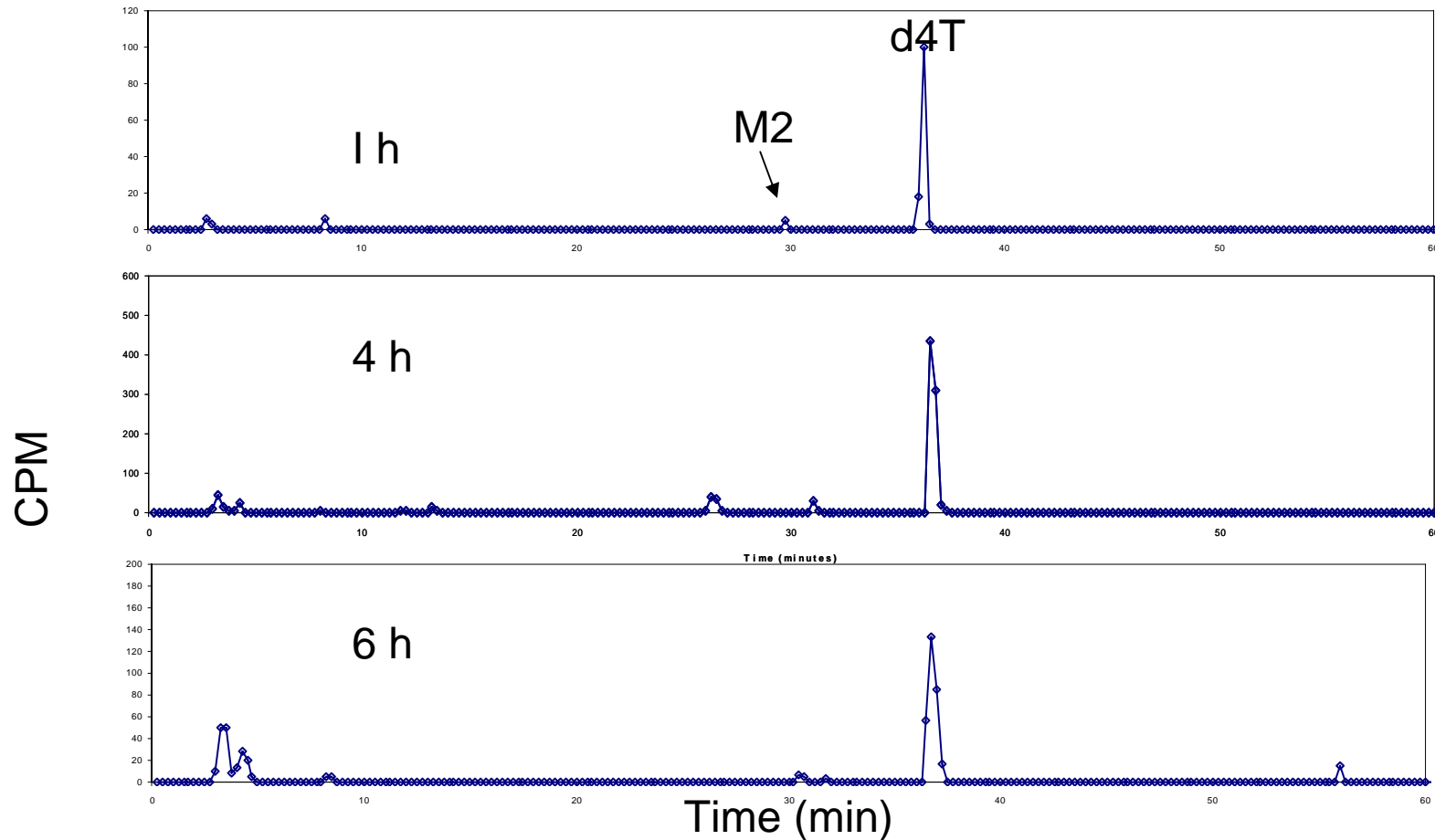
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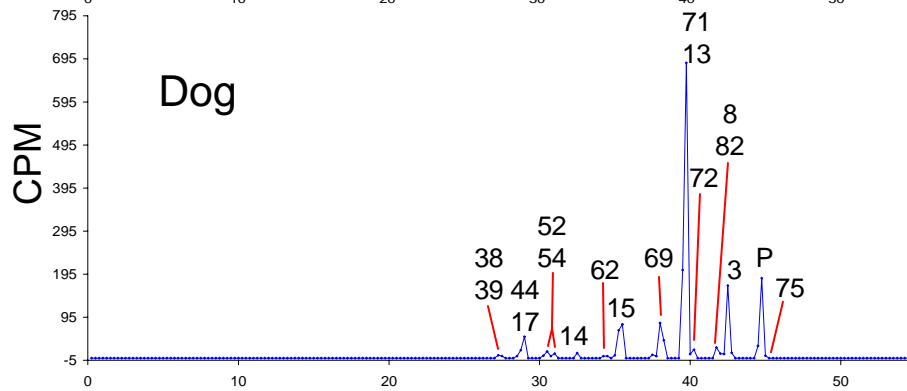
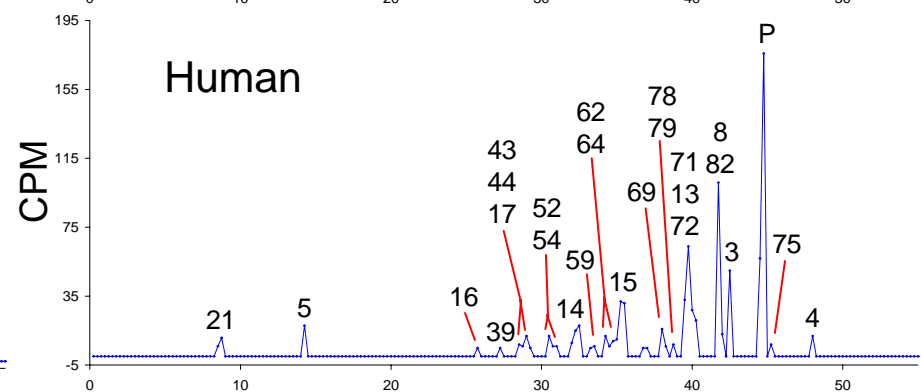
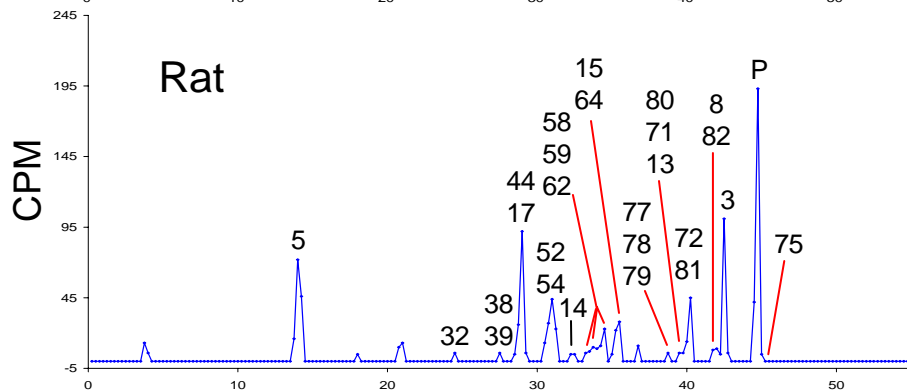
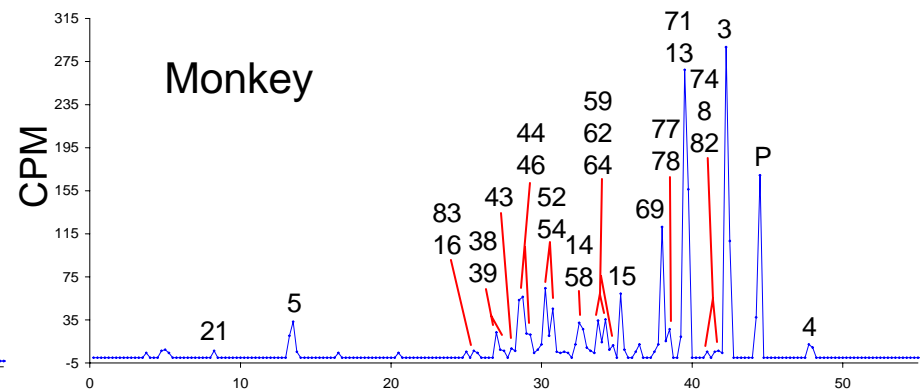
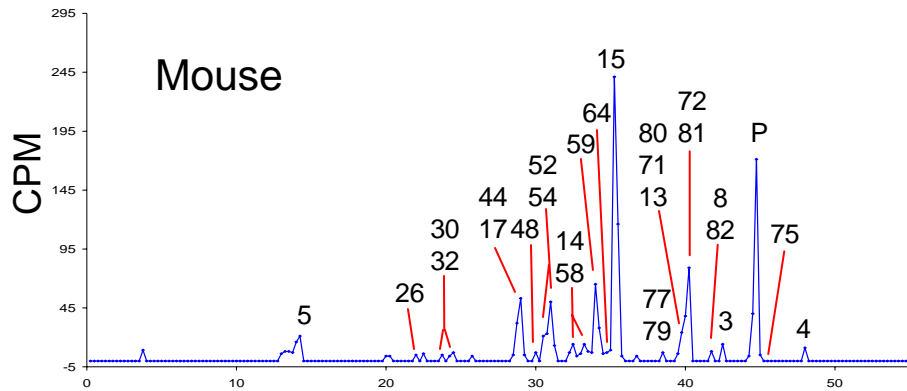
Safety Testing of Drug Metabolites



- Sometimes the goal of ensuring adequate exposure to human metabolites can be fairly simple

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Safety Testing of Drug Metabolites



• Sometimes the goal of ensuring adequate exposure to the complex mixture of human metabolites can be very challenging!

What are we most concerned with regarding metabolites

- Most of the cases of metabolite mediated toxicity have to do with the formation of reactive intermediates
- All of the examples cited in the FDA draft guidance arguing for the need to closely monitor metabolites deal with cases where reactive metabolites are generated
- *What about the more “typical” metabolic process giving rise to chemically stable metabolites?*

What biological activity will a metabolite likely possess?

- Typical metabolites are very closely related structurally to the parent, thus would be expected to have some degree of activity against the target receptor, ie, it is not surprising that they would follow the same SAR as parent (*Humphreys and Unger, CRT, 19, 1564-1569*)
- Conversely, these closely related species would not necessarily be expected to gain new activities not seen with parent
 - Does this hypothesis hold true? Seems to be from the literature as there are few examples of where a stable metabolite produced toxicity through a pathway not impacted as well by the parent.

What biological activity will a metabolite likely possess?

- How often do parent-metabolite pairs display significantly different off-target pharmacology?
 - Prodrugs are obviously a special case
 - Pairs such as terfenadine-fexofenadine are examples of parent-metabolite with significant off-target effects (HERG binding), however, the off-target activity resides with the parent
 - Metabolites can have significantly different physicochemical properties which may make them prone to physical deposition during elimination leading to toxicity (eg., guaifenesin induced urolithiasis)

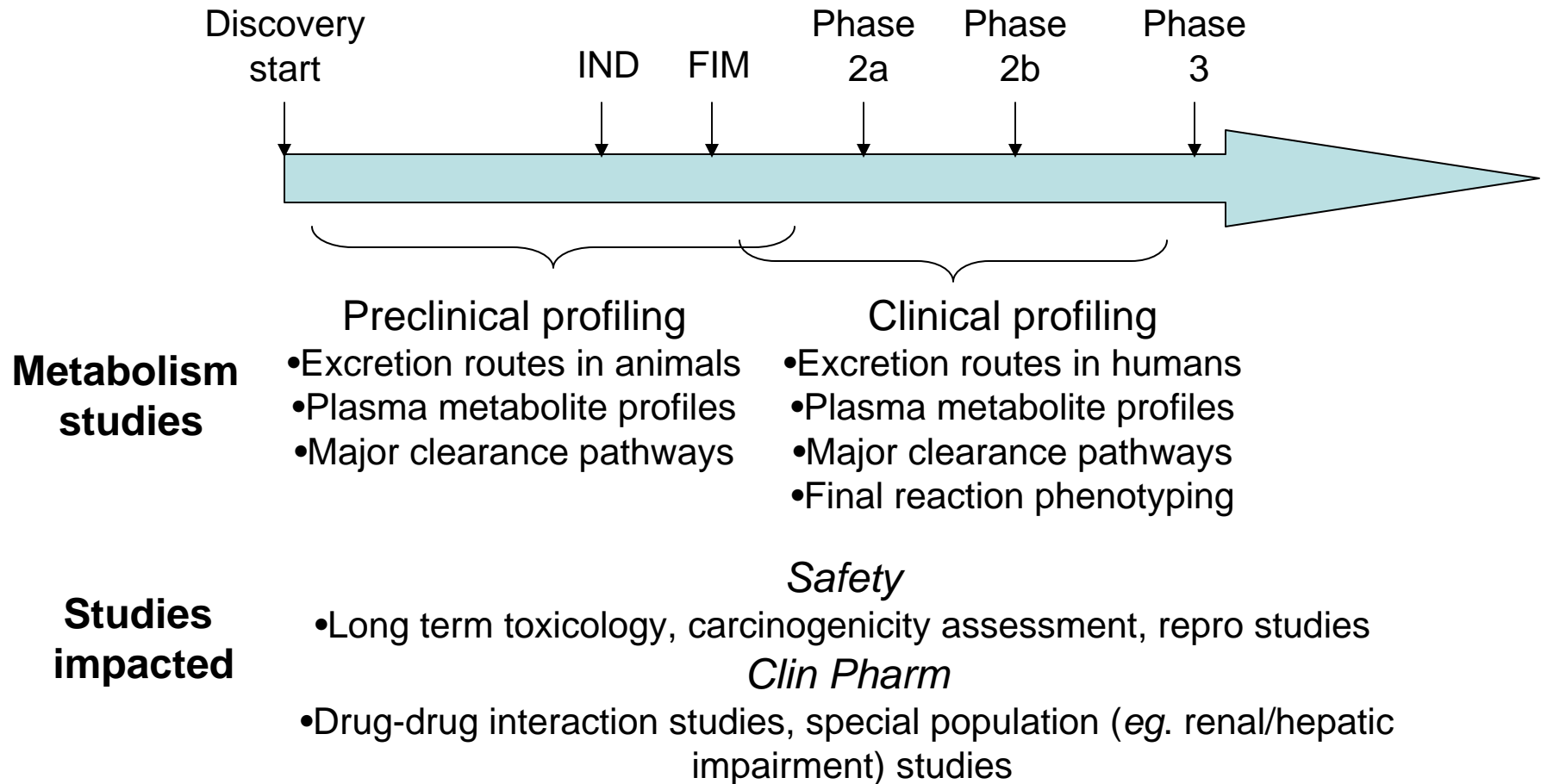
What biological activity will a metabolite likely possess?

- The probability of metabolites having new pharmacology would be expected to go up as the number of potential targets closely related to the intended target goes up, *eg.* ATP-binding site directed kinase targets
- This effect is seen in some cases with metabolites of antidepressants where the metabolites can have differential potencies towards serotonin, norepinephrine or dopamine reuptake or binding

So why characterize stable metabolites?

- There is a reasonable chance they will contribute to the pharmacological activity, and may even drive a significant portion of the observed efficacy
- Points regarding stable metabolites and off-target toxicity are completely based on empirical observation and only makes the relationship less likely
 - This is especially true for high dose drugs where metabolites could be in concentration ranges more likely to cause off-target effects
 - Example cited in FDA guidance refers to a case study where metabolite had significant cv liability

When to generate metabolite data



Generation of metabolite data

Types of metabolite data that are most important for:

Safety Program

- Human plasma metabolite profiles
- Cross-species comparative profiles, quantitative metabolite levels at doses used for toxicology studies relative to metabolite levels at the projected efficacious dose
- Human or animal data on metabolite concentrations that may relate to a signal in a toxicology study, especially a species selective signal

Clinical Pharmacology Program

- Human plasma metabolite profiles (active metabolites)
- Major routes of excretion
- Metabolic pathways of excretion (mass balance?)

Triggers for generation of complete metabolite information in early development

- Need for information to guide preparation of a carcinogenicity protocol
- Metabolite information as a potential go/no go decision point
- Early information on active metabolite concentrations
- Early information on coverage of predicted metabolites in tox species vs humans
- Useful to guide key DDI studies

When to generate definitive ADME data

- Best when it can be used to prospectively plan for safety and clinical pharmacology studies. Also, this allows questions regarding subject safety in early clinical trials to be addressed.
 - Early ADME studies also means significant data will be generated for compounds that will fail
- Late generation of data may lead to missed opportunities for metabolite data to be utilized to plan long term toxicity studies and drug-drug interaction studies
- Strategy should be a balance of two competing priorities
- *Is there any way to get an early look at the human metabolite plasma profile?*

Methods of obtaining human plasma metabolite profiles prior to radiolabel study

- Specific LC-MS assays of suspected metabolites using authentic standards
- AMS of plasma samples from early clinical study
- Use of radiolabeled “standards” generated from animal studies to generate MS response factors that are then applied to LC-MS analysis of human samples (*Yu, et al, Rapid Comm in Mass Spec, 21, 497-502; D. Zhang, et al., Drug Metab Lett, 1(4), 293*)
- Newer LC-MS techniques, such as high resolution mass spectrometry with mass defect filtering and other data mining techniques (*M. Zhu, et al., Drug Metab Dispos., 34(10), 1722-33; K. Bateman, et al. Rapid Comm in Mass Spec, 21, 1485-96, H. Zhang, et al., J. of Mass Spec. Epub, Feb 26.*)

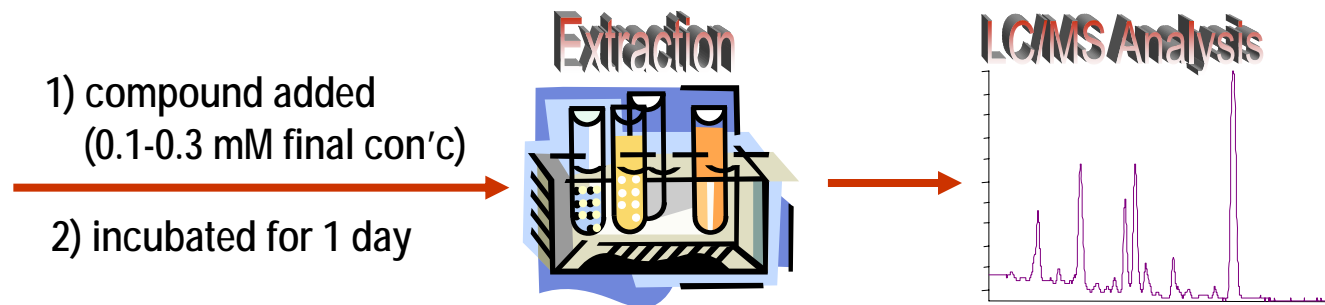
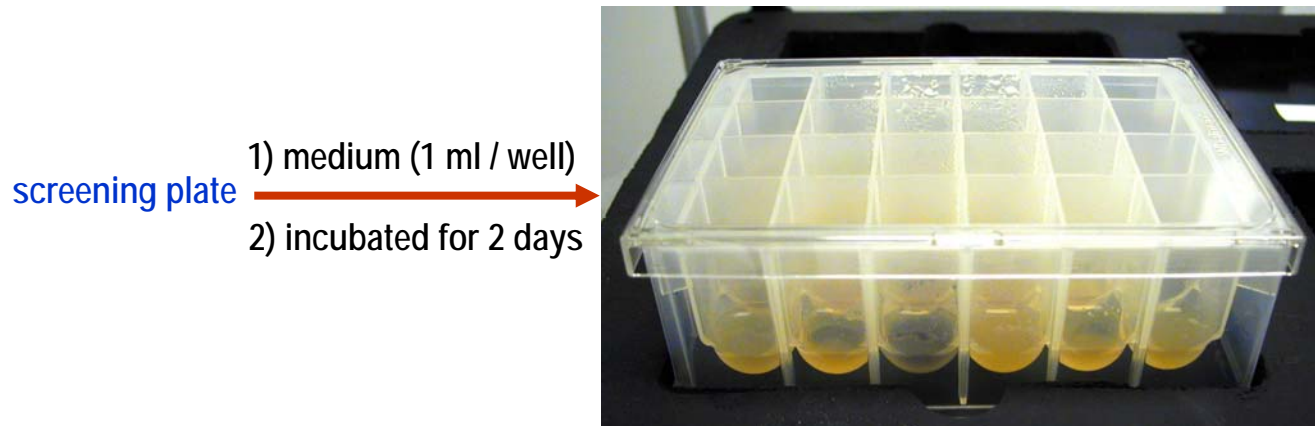
What are the typical steps of metabolite characterization

- Identification from in vitro or in vivo sample
 - Full structural ID (may involve chemical synthesis or NMR of isolated material)
- Synthesis of small batch of metabolite (1-50 mg)
 - Allows for testing of pharmacological activity
 - Limited liability testing
 - Use as authentic standard for early LC-MS assays
- Synthesis of large batch of metabolite (50 mg-several grams)
 - Allow for fully validated analytical assay development
 - Further liability testing

Synthesis of metabolites for early characterization

- Biosynthesis with microbial bioreactors
 - Compared with other metabolite synthesis methods, microbial biotransformation offers several advantages:
 - suitable for rapid screen
 - low scaling-up cost
 - high tolerance toward organic solvent.
 - However, screening with the conventional shake flask method is a time and labor consuming process.

Microbial biosynthesis

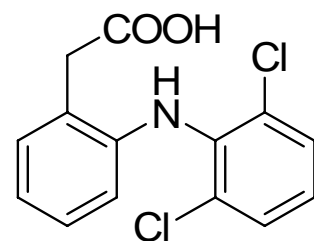
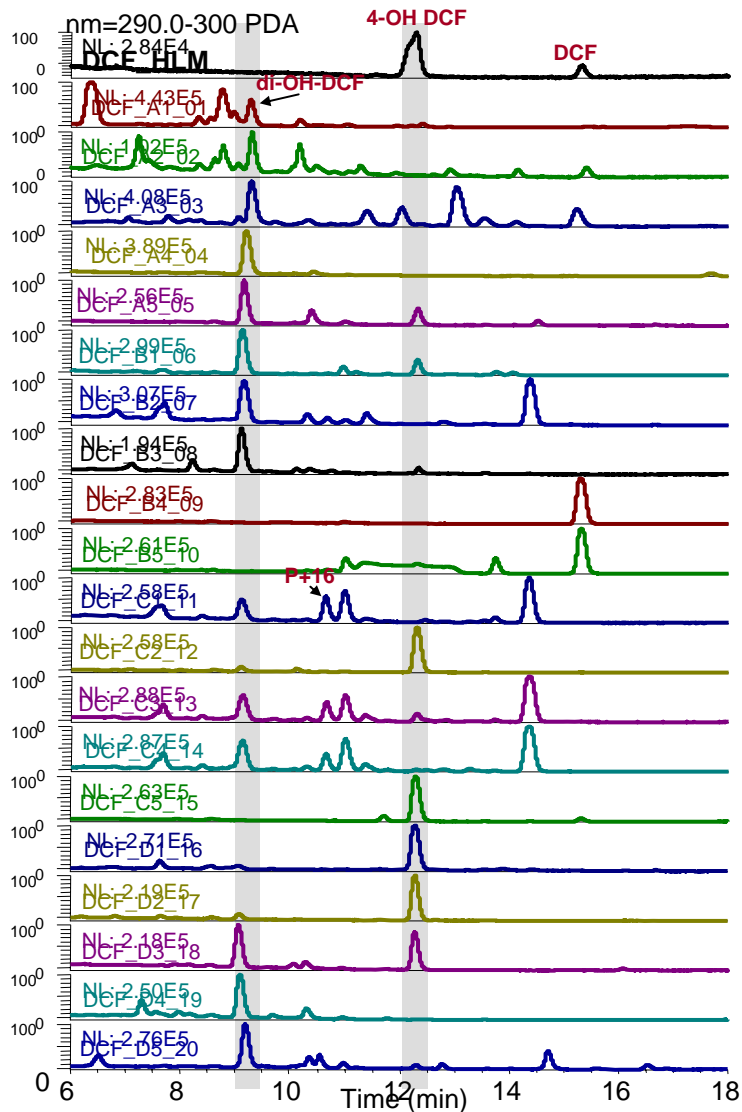


W Li, DMR, 37(2), 196

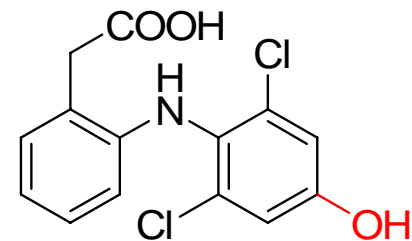
- Screening plates (24 well) prepared containing 20 strains of Actinomycetes bacteria
- Rapid procedure (all strains use the same media and growth conditions) to go from frozen plates to incubations results in 4 days

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Microbial biosynthesis



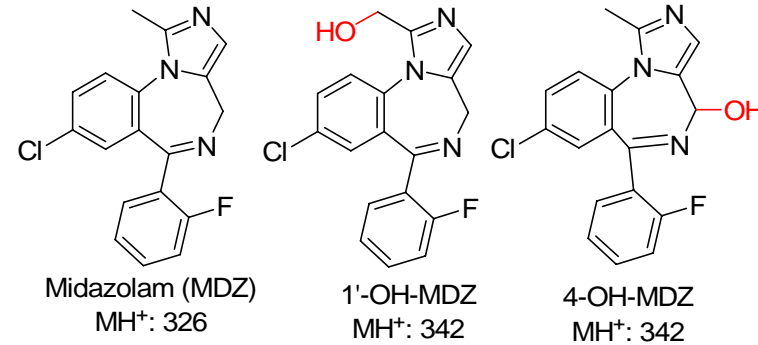
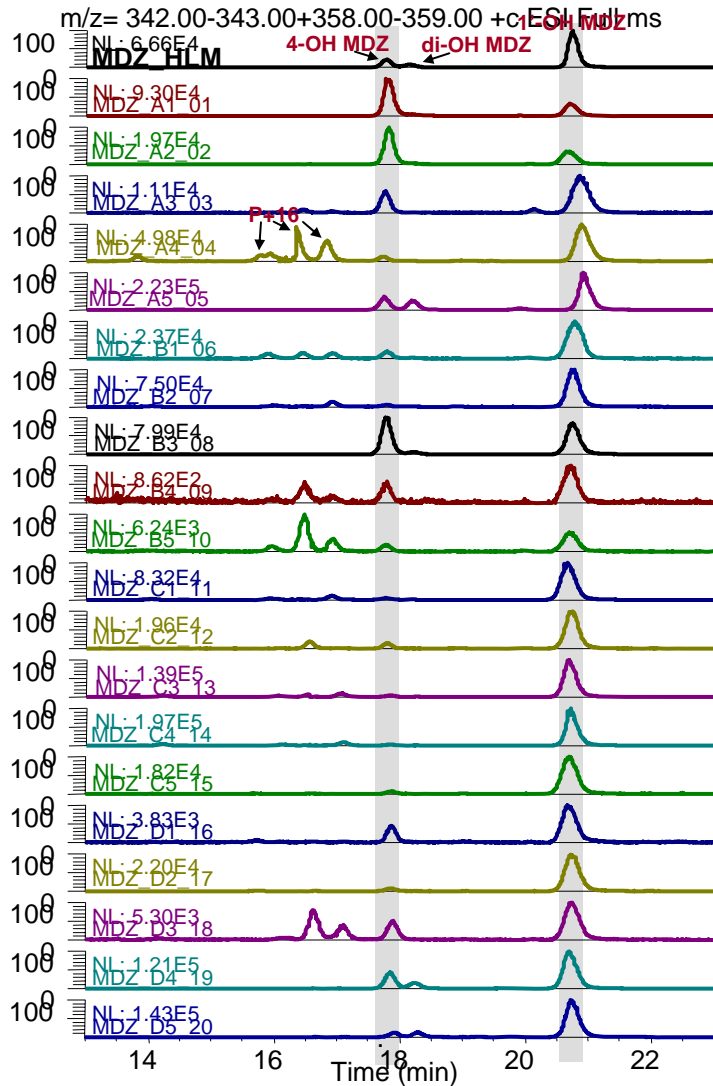
Diclofenac (DCF)
MH⁺: 296



4-OH-DCF, MH⁺: 312

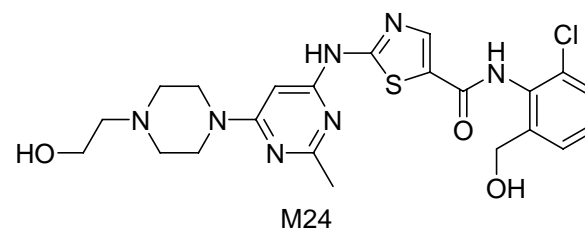
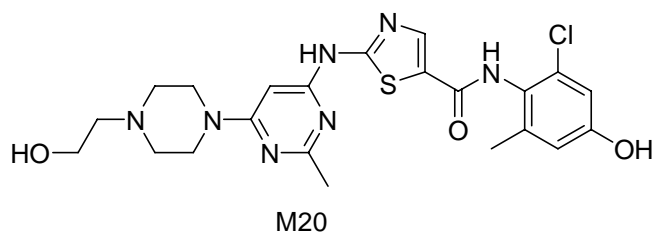
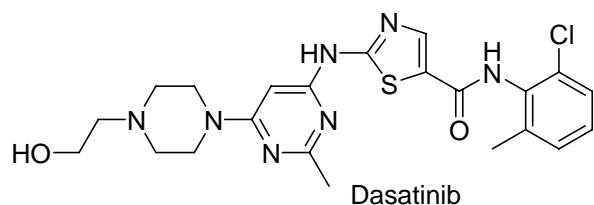
W Li, DMR, 37(2), 196

Microbial biosynthesis



W Li, DMR, **37(2)**, 196

Microbial biosynthesis



Metabolites of dasatinib (Sprycel) prepared via microbial method.
W. Li, et al., DMD, 36(4), 721-30.

*Synthesis of glucuronide conjugates, W. Li, APIS SX poster,
Shanghai, May 2008*

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Acknowledgements

BMS Biotransformation

Mingshe Zhu

Donglu Zhang

Li Ma

Weiping Zhao

Jonathan Josephs

Hong Su

Yue Zhong Shu

Rama Iyer

Wenying Li

Jinping Gan

Haiying Zhang

Lian Zhou

Lisa Christopher

Steve Unger

Oliver Flint