Uncommon Biotransformation Pathways: Challenges and Strategies

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Delaware Drug metabolism Discussion Group Symposium, June 13, 2017

Known and Unknowns?

As we know,
There are known knowns.
There are things we know we know.

We also know
There are known unknowns.
That is to say
We know there are some things
We do not know.

But there are also unknown unknowns,
The ones we don't know
We don't know.

—D.H. Rumsfeld
Feb. 12, 2002, Department of Defense news briefing

Presentation Outlines

- Introduction/Background
  - Need for metabolite identification
- Issues and Tools for Metabolite ID
  - Discovery
  - Development
- Challenges for metabolite ID of Unknowns
- Opportunities and tools
  - Examples
- Summary and Conclusions

Why Focus on Metabolite ?

- Metabolites facilitate the excretion of drugs (chemical) from the body- Detoxification process-This is good.
- The metabolites modulate the efficacy of drugs in the treatment of disease
- Metabolites represent new chemicals to which a human is exposed; thus, risk assessment is prudent
  - Coverage of human metabolites in tox species (MIST)
- Metabolites may contribute to
  - Pharmacologic activity (efficacy)
  - Toxicity
  - Drug-drug Interactions
- Pharmaceutical industries are mandated by regulatory agencies to identify metabolites of drug candidates

Questions Related to Metabolites

- U.S. FDA Office of Clinical Pharmacology question based review (QBR) template
  - 2.2.3 Are the active moieties in the plasma or other biological fluid appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?
  - 2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for *efficacy?*
  - 2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for *safety*
  - 2.2.5 What are the PK characteristics of the drug and its major metabolite?
  - 2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?
  - 2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?
  - 2.2.5.6 What are the characteristics of drug metabolism?
  - 2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?
  - 2.4.2.3 Is the drug or a major metabolite an inhibitor and/or an inducer of CYP enzymes and/or transporters?
  - 2.6.2 Which metabolites have been selected for analysis and why?

Metabolite Identification in Discovery

- Issues to Address
  - Identify “soft spots” (Guide synthesis of stable analogs)
  - Explain species differences in Clint
  - Minimizing bioactivation potential (screen reactive metabolites)
  - Characterizing the PK/PD Disconnect
- Tools to Use
  - *In Cerebro* – biotransformation scientist, knowledge based
  - In silico (Metasite, Meteor, CYP Score)
  - Liver microsomes (predominantly rat, dog and human)
  - Hepatocytes (rat and human)
  - Plasma/urine samples from *in vivo* PK studies in animals
- Techniques to Use
  - High throughput/LC-MS
  - Non-radiolabeled compound
  - Metabolite ID softwares

Metabolite ID beyond Discovery

- Issue to Address
  - Species comparison (qualitative and quantitative)
  - Pharmacological activity
  - Reactive intermediates
  - Comparison of human metabolic pathways with those of tox species
  - Define clearance mechanism
  - Guide DDI clinical studies
  - Regulatory submission quality
- Tools to Use
  - Radioactive tracer/ β–PAM (quantitative)
  - Urine, bile, fecal samples from radiolabeled non-clinical ADME studies
  - Urine, plasma from Clinical (SD/MD) studies
  - Urine, plasma and fecal samples from radiolabled human AME studies

Known Knowns Metabolite Detection

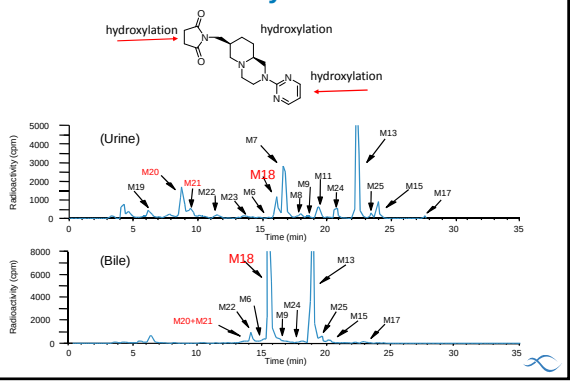
- Common Phase I routes of metabolism
  - hydroxylation, dihydroxylation, demethylation, dehalogenation, etc
- Phase II conjugates of parent & metabolites
  - Sulphate, glucuronide, acetylation
- Generate appropriate accurate mass XIC's
  - (+14, +16, +32, -14, +2, +80, +176, etc)
  - Easy to automate through Excel worksheet
- Comparison vs control samples

Metabolite ID of Unknowns: Techniques

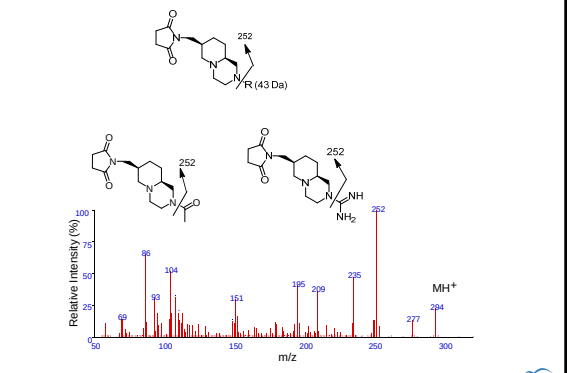
- LC-MS/MS
  - Accurate Mass measurements
  - High/Low energy MS (MS^E)
- Derivatization
- Hydrolysis (enzymatic, acid, base)
- Stable Isotope techniques
  - (H/D exchange, ^{18}O)
  - Isotope cluster
- LC-NMR (Continuous flow or stopped flow)
- CYP mimetic chemical models
- Microorganisms

Example 1: N-Heterocyclic Ring Scission

HPLC-Radiochromatograms of Sunepitron Metabolites in Monkey Urine and Bile

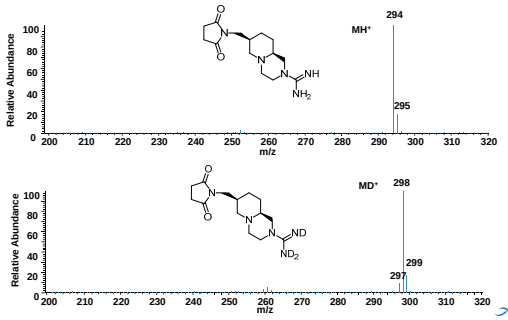


CID product ion spectrum of M18

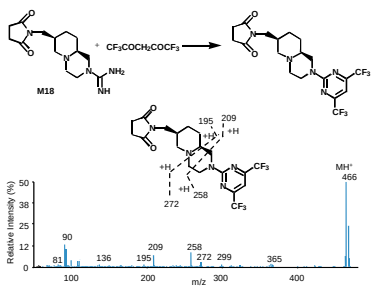


Determine exchangeable protons

Mass Spectra of M18 Before and After Deuterium Exchange

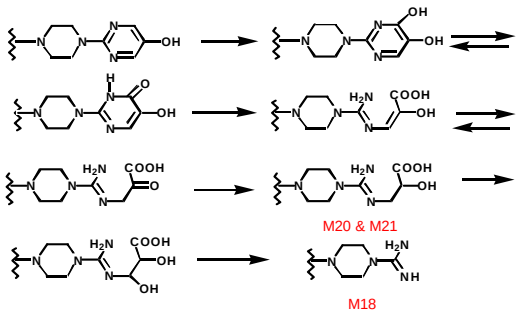


CID Product Ion Spectrum of M18 After Treatment With HFAA

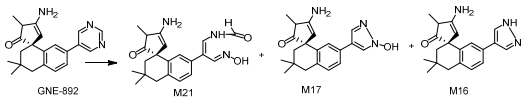


- Reduced polarity
- Diagnostic mass increase

Possible Mechanism for the Formation of M18

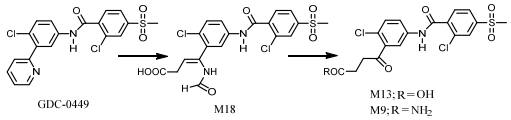


Scission of pyrimidine ring to pyrazole



Takahashi et al. DMD 2014;42:890-898

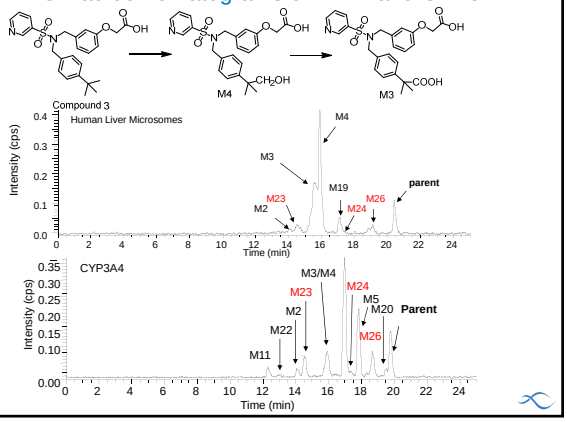
Scission of pyridine ring



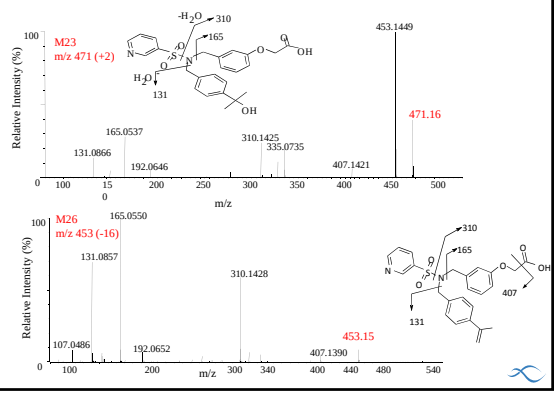
Khojasteh et al. DMD 2014;42:343-351

Example 2: C-C bond cleavage
C-Demethylation

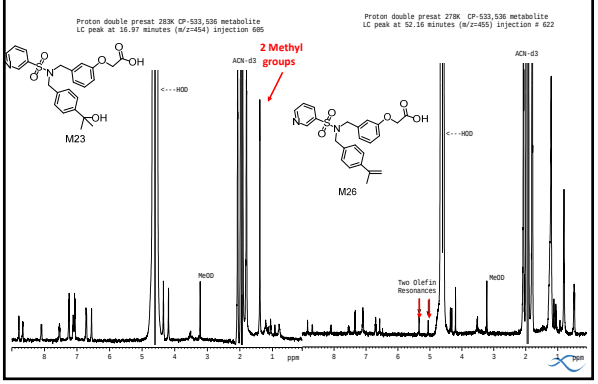
HPLC Radiochromatograms on HLM and CYP3A4



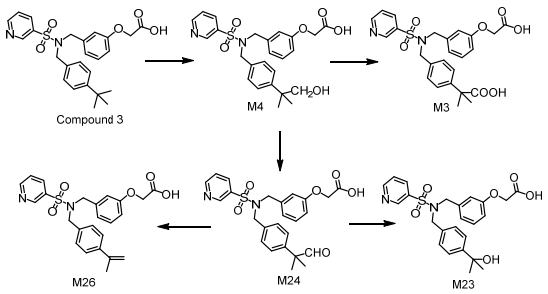
CID product ion mass spectra of M23 & M26



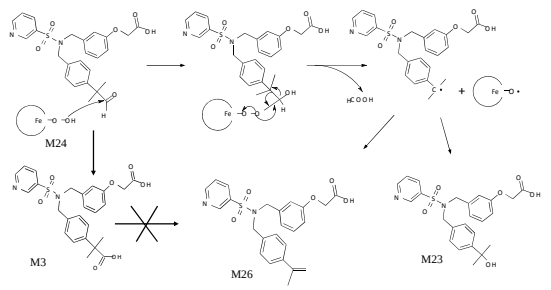
1H NMR spectra of M23 and M26



Metabolism of Compound 3

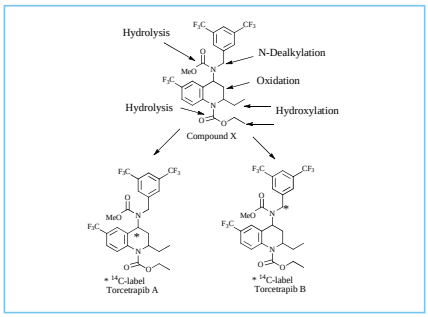


Mechanism for the formation of M23 & M26



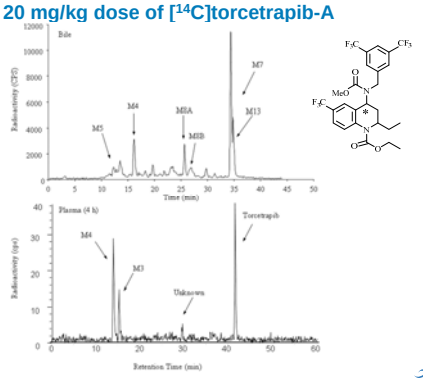
Example 3: Multiple Oxidations, Aromatization

Metabolism of Compound 4

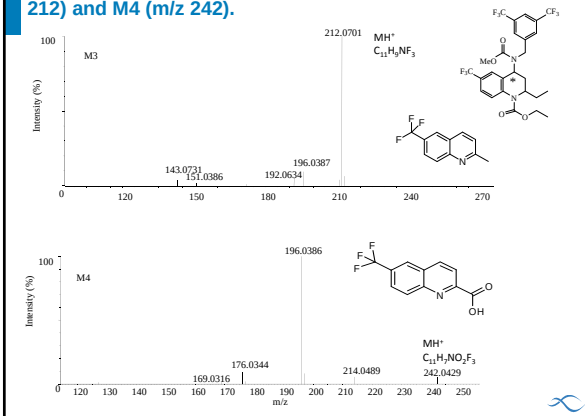


Prakash et al , DMD, 2008

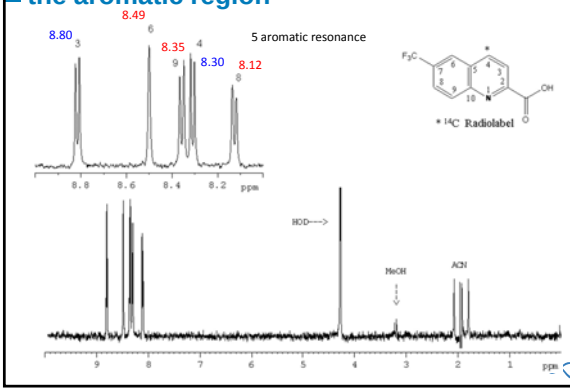
HPLC-radiochromatograms of torcetrapib metabolites in bile and plasma (4 h) of rats after oral administration of a single 20 mg/kg dose of [^{14}C]torcetrapib-A



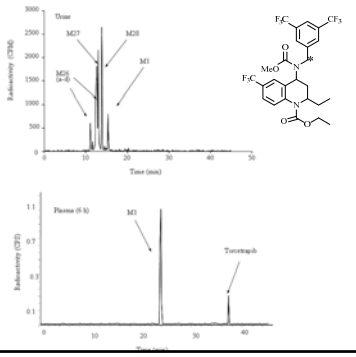
CID product ion mass spectra of metabolites M3 (m/z 212) and M4 (m/z 242).



1H NMR spectrum of M4 with expansion of the aromatic region

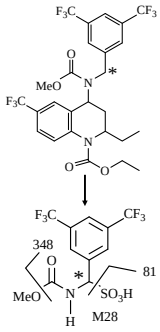


HPLC-radiochromatograms of torcetrapib metabolites in urine and plasma of rats after oral administration of a single 20 mg/kg dose of [^{14}C]torcetrapib-B

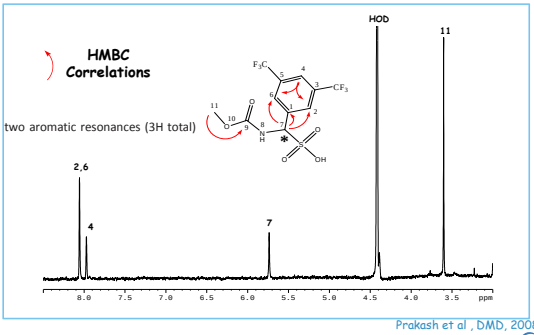


Characteristics of M28

- Metabolite M28 was detected in the urine of rats dosed with C-14 labeled at the benzylic position
- M28 displayed a deprotonated molecule, $[M-H]^-$, of *m/z* 380 in the negative ion mode.
- Accurate mass of M28 suggested the empirical formula of $C_{11}H_9F_6NO_5S$
- The product ion mass spectrum of *m/z* 380 gave a major fragment ion at *m/z* 81, which was attributed to the sulfonate moiety and minor fragment ions at *m/z* 348 and 240.
- The fragment ion at *m/z* 348 indicated a loss of the methanol moiety from *m/z* 380, and the ion at *m/z* 240 suggested the loss of the methylcarbamoyloxy and sulfonate moieties from *m/z* 380.
- These data suggested that the metabolite was a sulfite adduct of methyl carbamate.

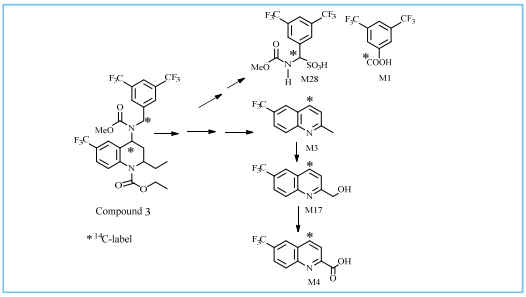


^{1}H NMR Spectrum of M28



Prakash et al , DMD, 2008

Proposed uncommon metabolites of Compound 3



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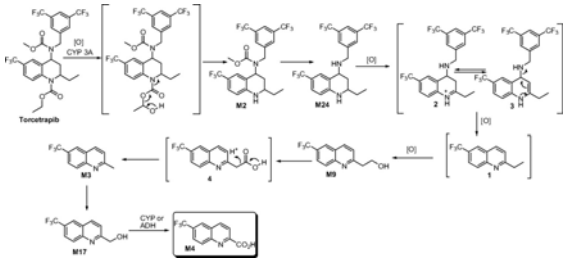
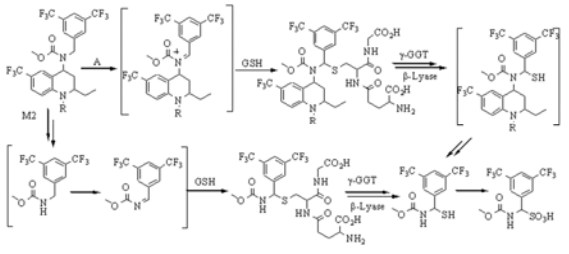
Proposed mechanism for the formation of M3 and M4



Proposed mechanism for the formation of M28



Summary and conclusions

- Understanding the metabolic fate of drug candidates is a critical component of drug discovery and development
  - Impacts PK prediction
  - Impacts safety
  - Impacts efficacy
- Many tools are available to characterise metabolism… and more are emerging
  - In silico approaches
  - Novel detection and identification technologies
- Strategic considerations must be fit-for-purpose
  - Early information is important for compound selection
  - Rational assessment of safety implications is key
- Identified several novel and unusual metabolites of several structurally different drugs
- Combination of LC/MS/MS with other analytical approaches (LC-NMR, H/D exchange, derivatization) is a powerful tool for solving difficult problems encountered in the analysis of drug metabolites.

An Important Reminder

- What you do every day changes people's lives
- Every job is critical to the success of our mission to develop human therapeutics
- Our belief in what we do as "essential to our success and ability to help patients" is critical
- You make a difference in the lives of people you will never meet.

Acknowledgements

- Pfizer Colleagues
- Biogen Colleagues
- Agios Colleagues

Questions?