



Approaches to Minimize the Bioactivation Potential of 11- β -Hydroxysteroid Dehydrogenase-1 Inhibitors in Lead Optimization

Deepak Dalvie

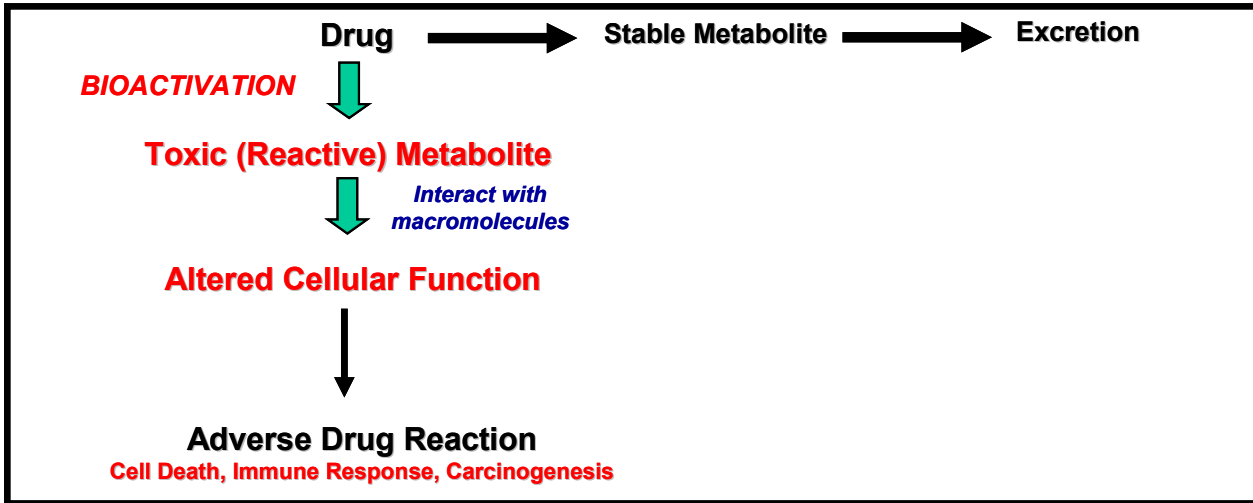
**Pharmacokinetics, Dynamics and Metabolism
Pfizer
San Diego**

- Drug Metabolism can play a key role in the lead optimization stage of drug discovery
 - **Identifying undesirable ADME characteristics**
 - Fixing metabolic liability
 - Identifying soft spots in a compound

Identification of pharmacologically active metabolites

- Understanding PK-PD & structure activity relationships
 - May lead to a discovery of new class of agents
 - **Provide IP protection**
-
- **Identifying factors that may impact drug safety**
 - **Drug Safety – a primary cause for high rate of attrition in development**
 - Identifying toxicophores – ‘Structural Alerts’
 - Especially the ones that undergo ‘**metabolic activation**’
 - Identifying metabolites with undesirable pharmacological & toxicological profile

Metabolic Activation and Idiosyncratic Adverse Drug Reactions (IADR)



- ◆ It is not easy to predict toxicological risks
 - Circumstantial evidence links **metabolic activation to IADR**
 - No animal models

- ◆ One approach adopted by most pharmaceutical industries
 - **Eliminate the candidate's potential to undergo metabolic activation**
 - Screen drugs for their ability to form reactive metabolites
 - **Avoid chemical functionalities that are susceptible to bioactivation**
 - No guarantee that this will make drugs safer but it is worth a try
 - **TALL ORDER BUT AVOIDS RISK!**
 - Saves \$\$
 - Saves time and effort

Methods to Assess Bioactivation Potential

Two Methods Commonly Used

Covalent Binding to Proteins

- ◆ Most definitive
- ◆ Useful in quantitation of the reactive metabolite
- ◆ Helps to detect all reactive metabolites
- ◆ Limited by availability of radiolabel

Trapping with nucleophiles

- ◆ Most popular in a discovery setting
- ◆ Nucleophiles used to trap are:
 - Glutathione
 - N-Acetylcysteine
 - Other nucleophiles - cyanide
- ◆ Acts as a surrogate marker of covalent binding

General Method Used



Drug (10 – 50 μ M) + Human Liver Microsomes + GSH (3 mM)

↓ NADPH regenerating system (+/-)

Incubation at 37° C for 1.0 hr

↓
Samples analyzed for GSH adducts using various LC-MS methods



Advantages and Disadvantages of the Bioactivation Assay

PROs

- ◆ Can be used rapidly & easily in a discovery setting
- ◆ Sensitive
- ◆ Has a generic end-point – GSH Adduct
- ◆ Amenable to a 96 well-format

CONs

- ◆ The presence of a GSH adduct in vitro may not translate to toxicity
- ◆ Only detects reactive metabolites that form GSH adducts
 - Other reactive metabolites such as aldehydes, imines etc. cannot be captured
 - Some GSH adducts are hydrolyzed readily
- ◆ May result in false adducts/positives
- ◆ May eliminate potentially good candidates
- ◆ Microsomal assays do not address all metabolic pathways for a compound

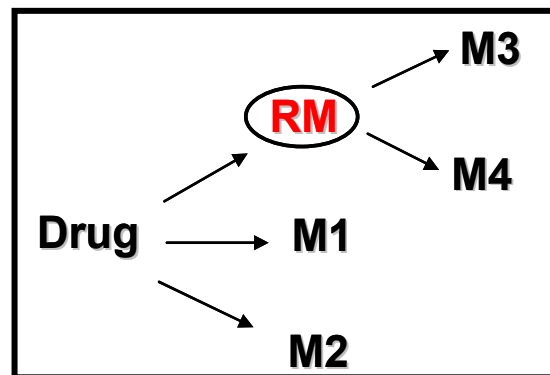


What does a Positive GSH Adduct Mean?

GSH adduct formation - Not a kill shot

- **Just one type of an alert**
- **A 'Flag' that may trigger additional studies**
 - **In vitro & in vivo**
- **GSH conjugation is a detoxification step**
- **Several other factors need to be weighed in**
 - **Is it a prototype or a back up?**
 - **Indication**
 - **Is the drug being developed for an unmet medical need?**
 - **First in class?**
 - **Dosing regimen**
 - **Is it a acute dosing or a multiple/chronic dosing drug?**
 - **Drugs that are used chronically are more prone to IADRs**
 - **Weigh the Benefits versus Risks**

- ◆ **Studies to consider to mitigate the risk in light of a positive signal**
 - **Assess contribution of other detoxification/metabolic pathways**
 - **Other studies:**
 - **Assess the formation of reactive metabolites in preclinical species**
 - **Assess the degree of covalent binding using radiolabel in the presence of other metabolic pathways**
 - **Predict the dose**
 - **Lower the dose**
↓
 - **Lesser amount of the reactive metabolite formed**
↓
 - **Lower Risk**
 - **“It is all about body burden of the RM”**



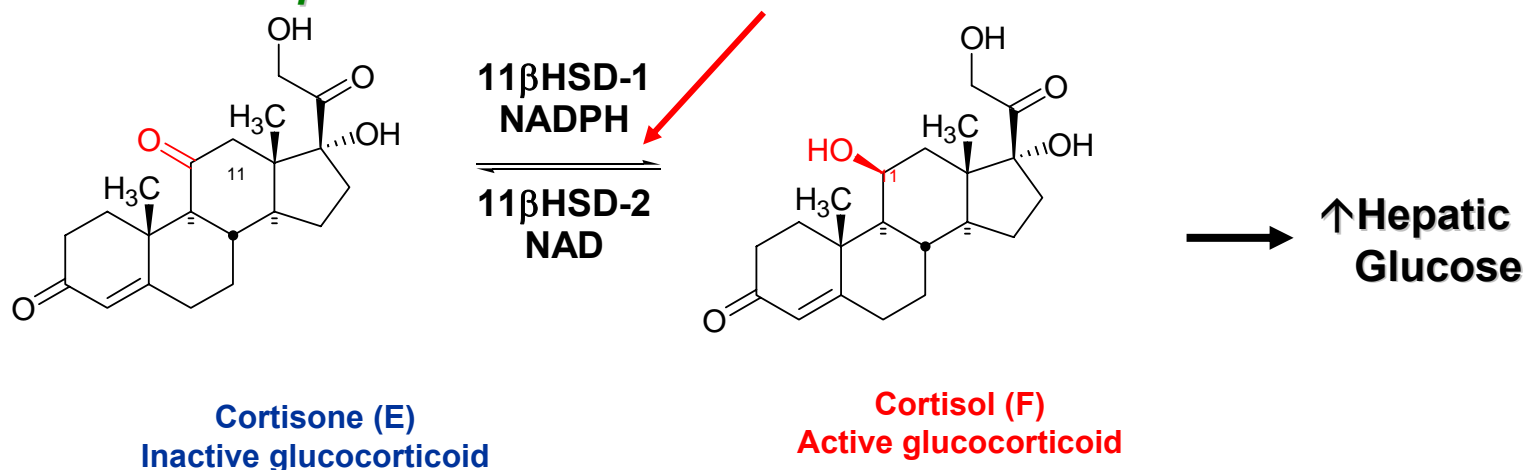
Minimizing the Bioactivation Potential of 11- β -HSD-1 Inhibitors

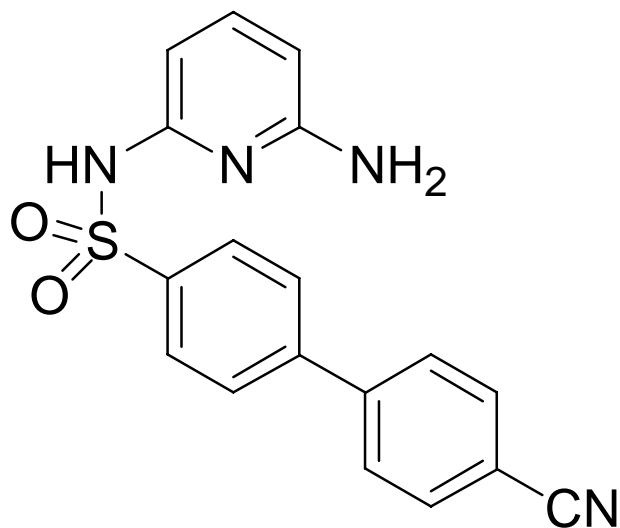
Project Goal

- ◆ Develop an **11 β HSD1** (11 β -**H**ydroxy**S**teroid **D**ehydrogenase type-**1**) inhibitor for the treatment of Type II diabetes

Background

11 β HSD1 inhibitor inhibits conversion of cortisone to cortisol



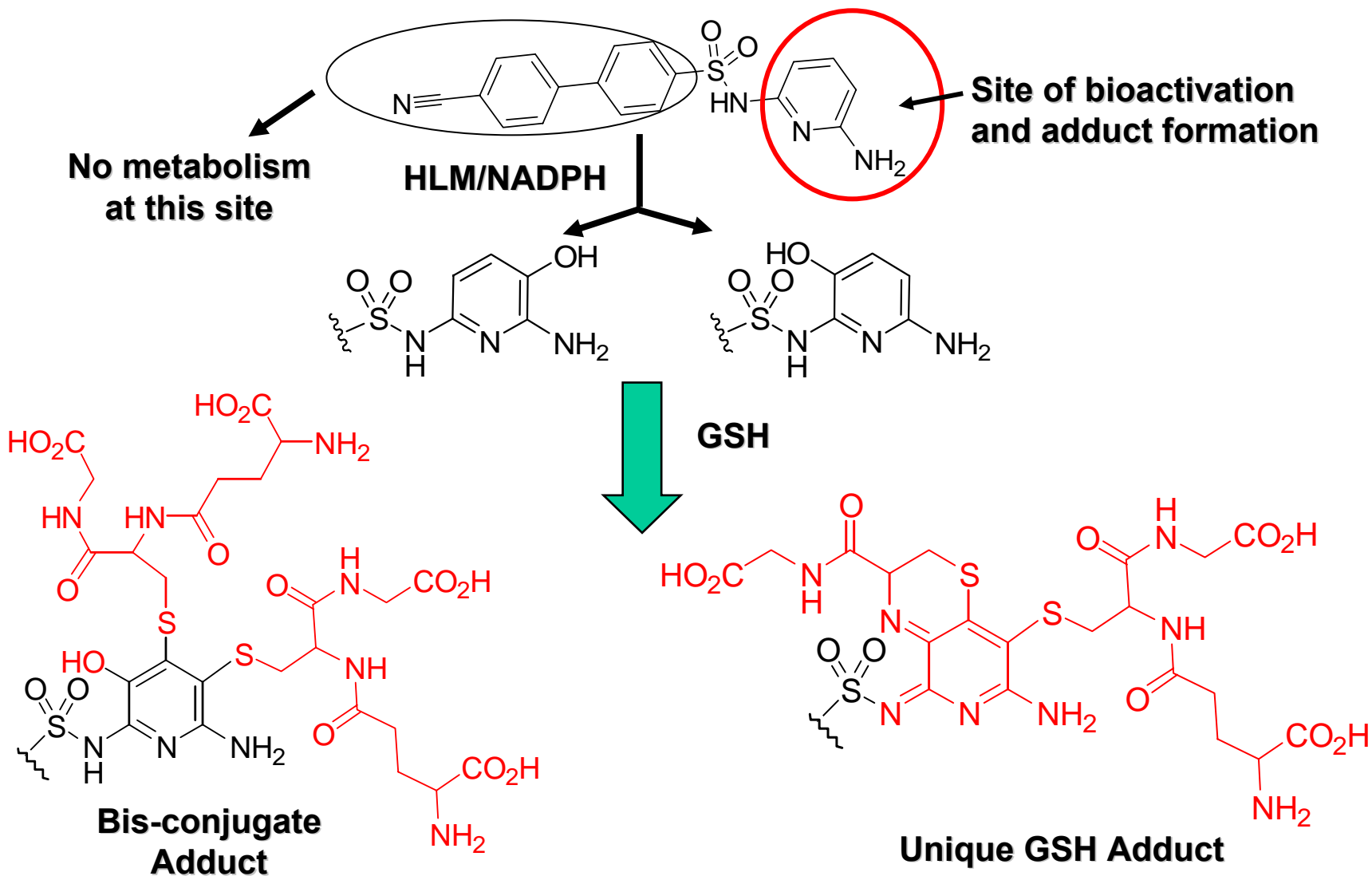


PF-915275

- ◆ Has great physicochemical properties
- ◆ Good potency
- ◆ Pharmacokinetic Attributes
 - Low Cl and Vdss in preclinical species
 - Long half-life –suitable for QD dosing
- ◆ Other Attributes
 - Not a PgP substrate
 - Clearance mechanism: **metabolism**
 - Primary route - **oxidative**
 - Glucuronidation (minor)
- ◆ Low DDI risk: $IC_{50} > 25 \mu M$ towards major CYPs

- ◆ Key Issues from a metabolism perspective
 - **Risk of bioactivation**
 - **No Structural Alerts – yet +ve signal in RM assay**

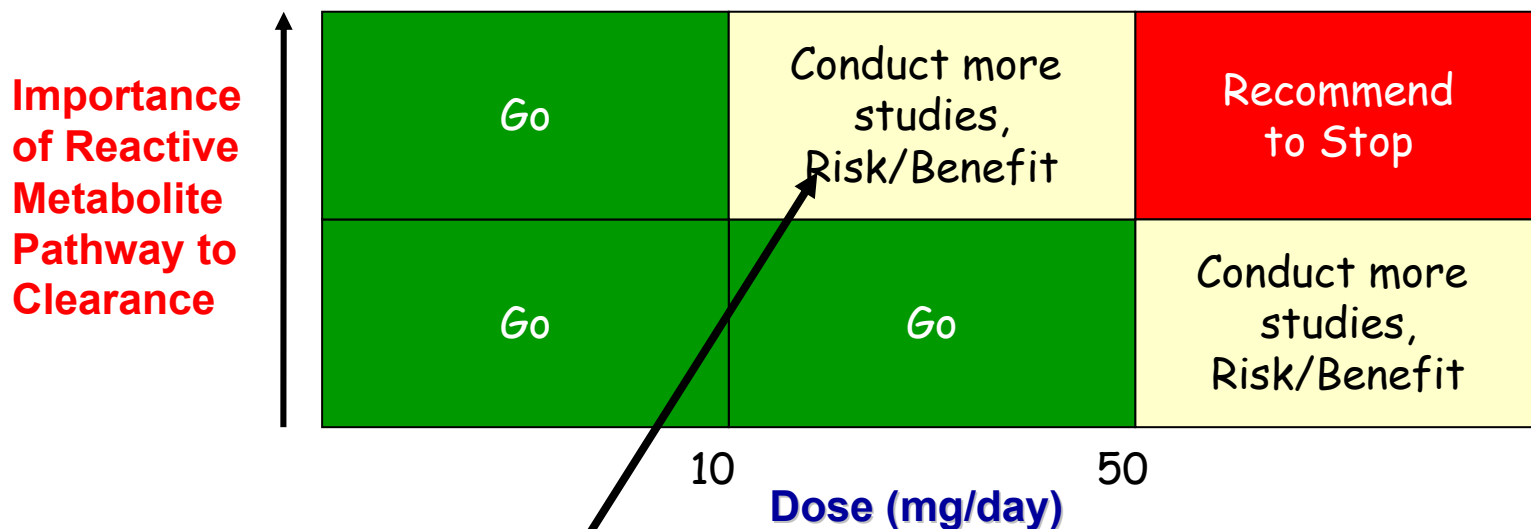
Metabolism of PF-915275



Reactive metabolites trapped as Novel GSH Adducts

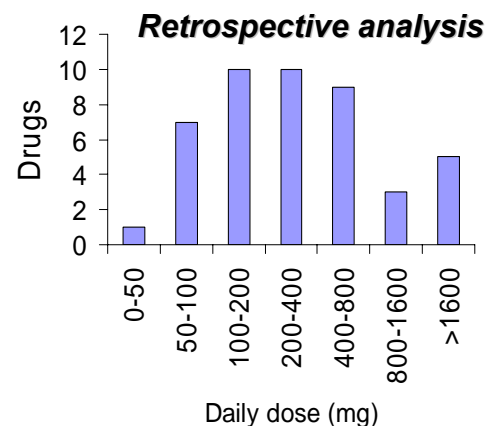
Risk Assessment for PF-915275

- ◆ Oxidation leading to reactive metabolites – a primary route
- ◆ Preliminary dose prediction 1-50 mg (predicted dose 30-50 mg)
 - uncertainties in clearance and C_{eff}

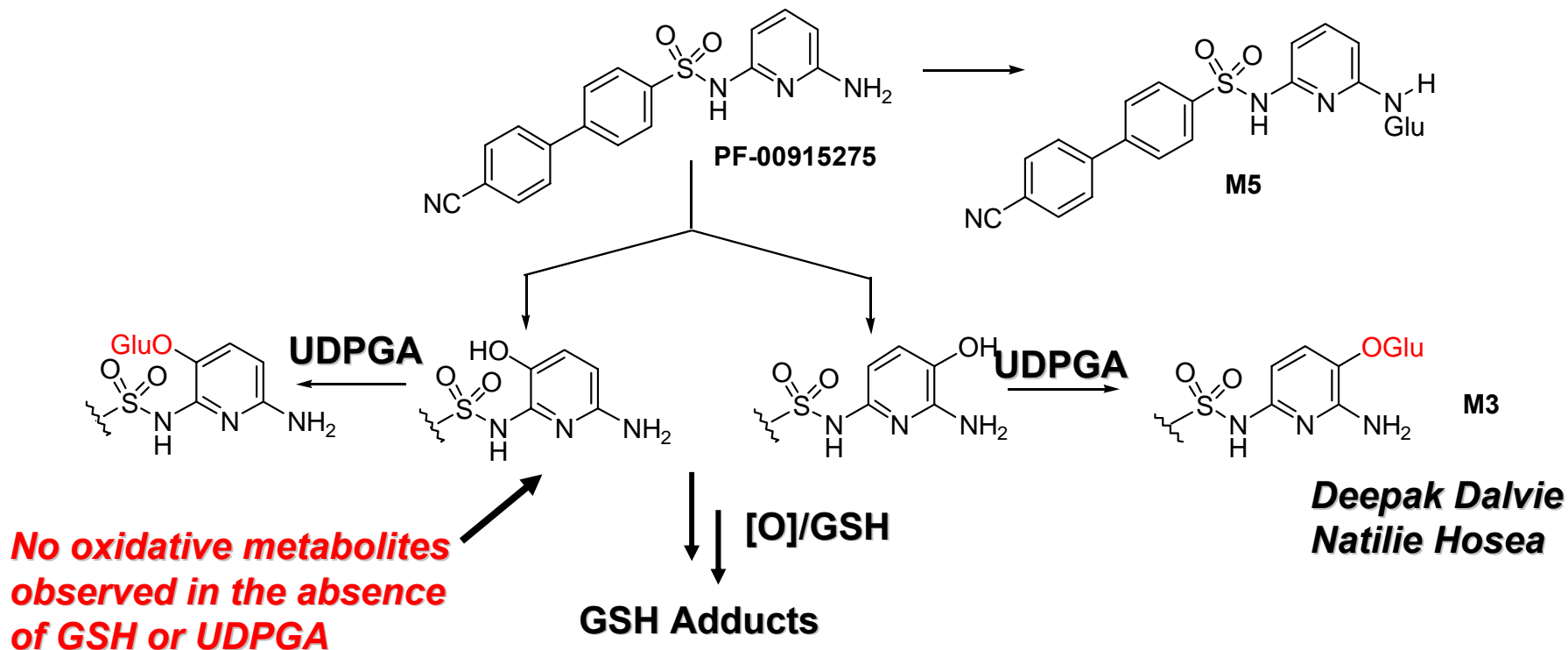


PF-915275

Pfizer's Risk Assessment Grid

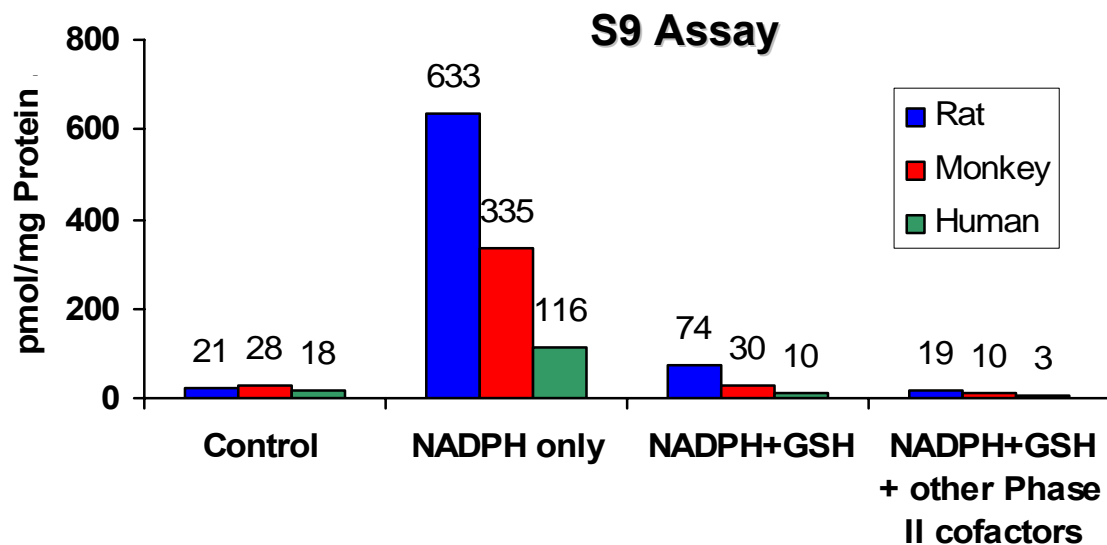
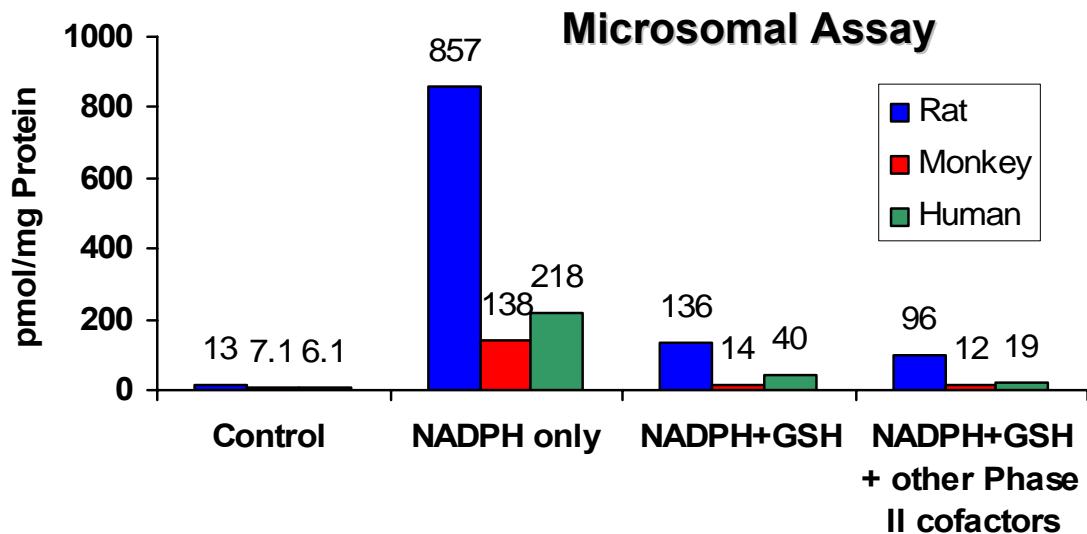


Preliminary Risk Assessment of Reactive Metabolite Formation in LM/NADPH/UDPGA

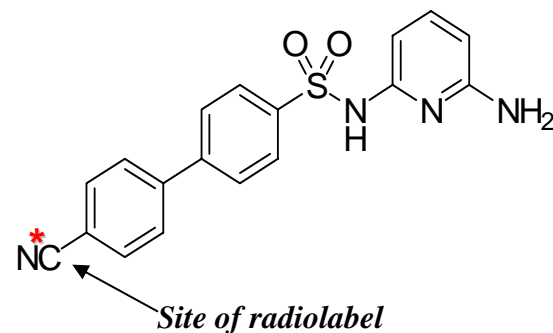
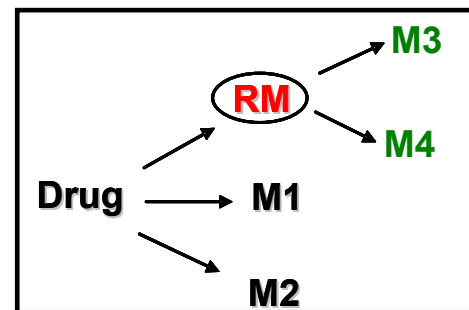


- ◆ **Assessment of role of other detoxification pathways**
- ◆ **Not a time-dependent inhibitor of CYPs**
- ◆ **[¹⁴C]-PF-915275 was synthesized**
 - **Assessment of covalent binding in vitro and in vivo was initiated**

Covalent Binding Studies in LM and S9 Using [14C]-PF-915275



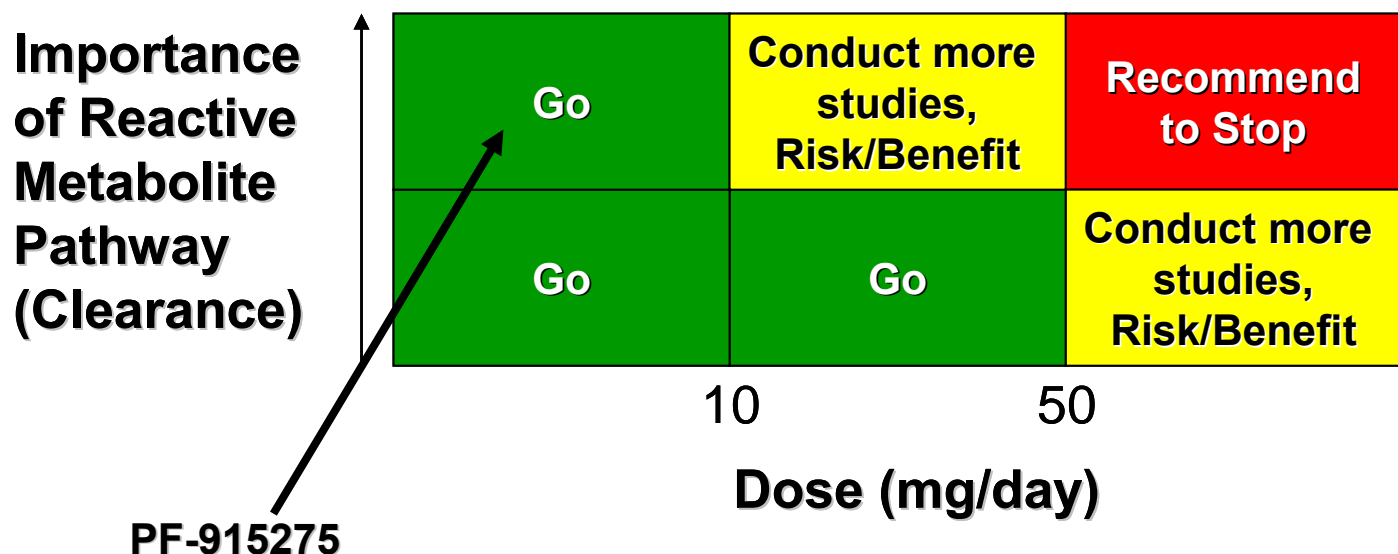
- ✦ Impact of other metabolic pathways on covalent binding
- ✦ Pathways considered
 - Glucuronidation
 - Sulfation



Ping Kang
Sue Zhou

De-risking Factors to Move PF-915275 into Development

- ◆ Phase II detoxification pathways observed in S9 and in vivo in rat
 - In vitro covalent binding reduced to near background
 - Very low binding to liver protein in vivo (**0.21 pmol/mg; <0.05% of dose**)
 - No GSH conjugates or related metabolites observed in vivo
- ◆ Refinement of dose prediction led to low predicted human dose
 - **Refined Dose = 0.3 to 3 mg using monkey PK/PD data**
- ◆ No toxicity observed in in-vivo tox models including a GSH depleted arm

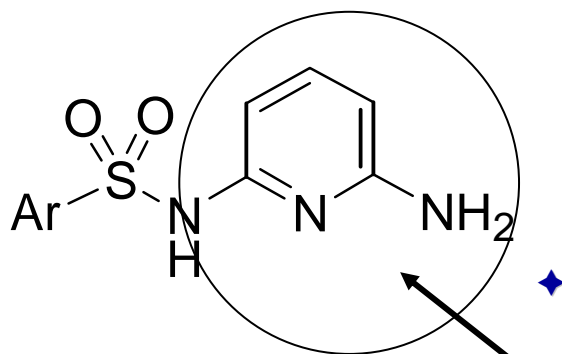
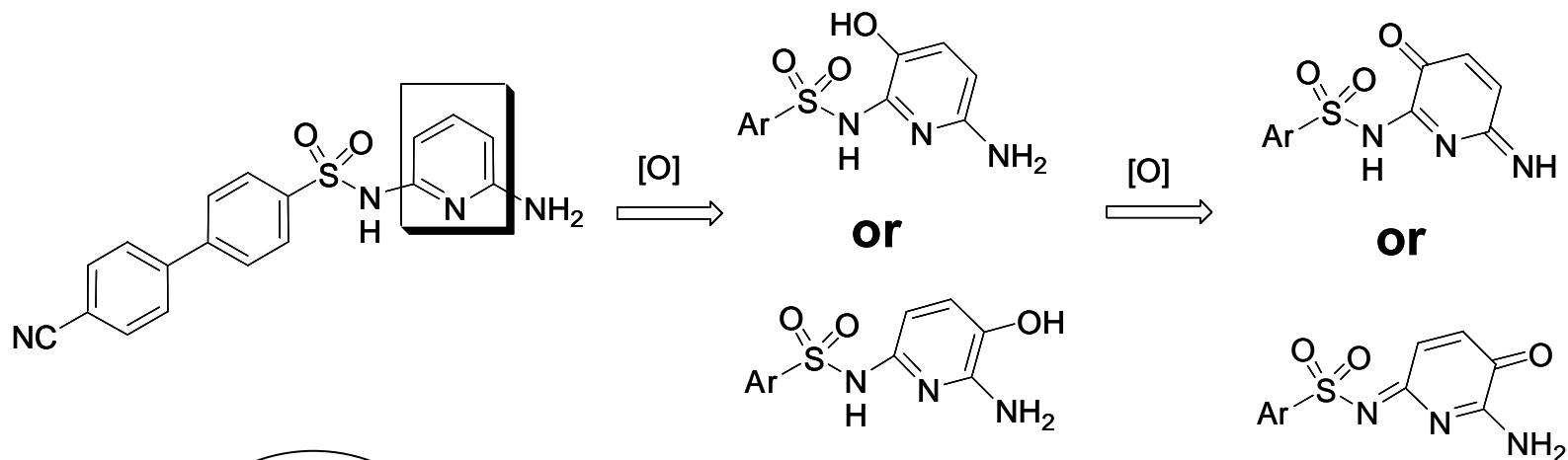




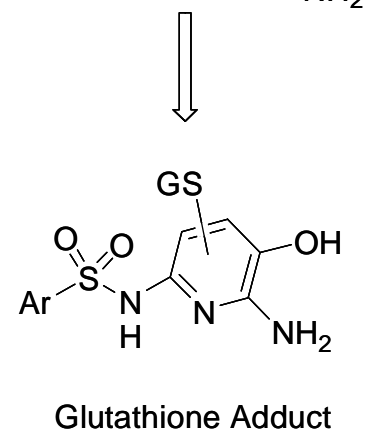
Biotransformation Profile for Back up to PF-915275

Back up Profile =
Attributes of the lead compound
+
Eliminate the Bioactivation Risk

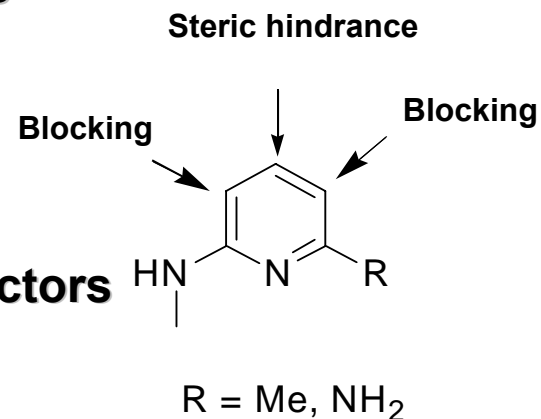
Proposed Mechanism of Bioactivation of PF-915275



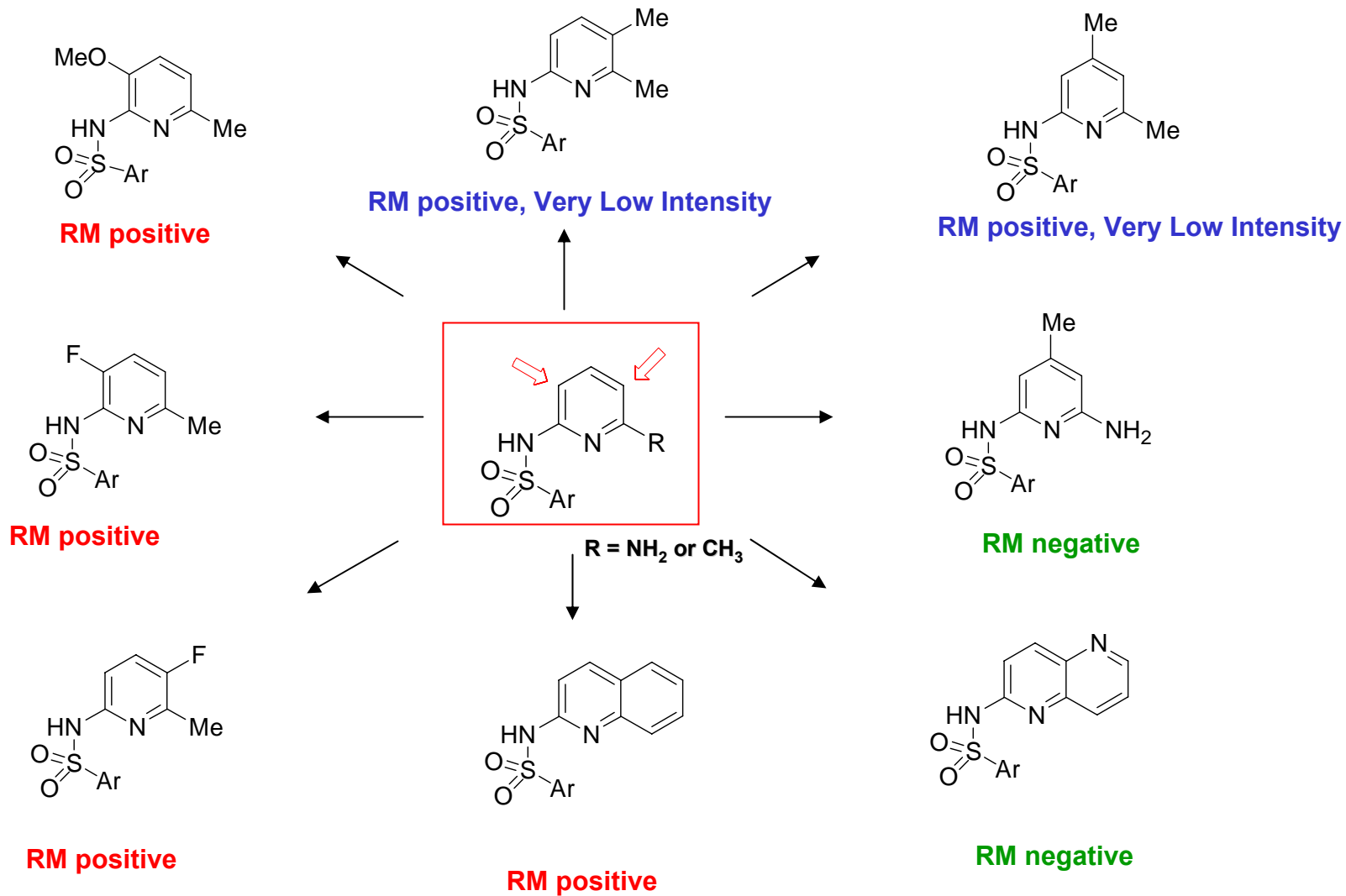
- ◆ **Electron rich moiety makes it susceptible to bioactivation.**
- ◆ **Electron rich moiety necessary for biological activity**



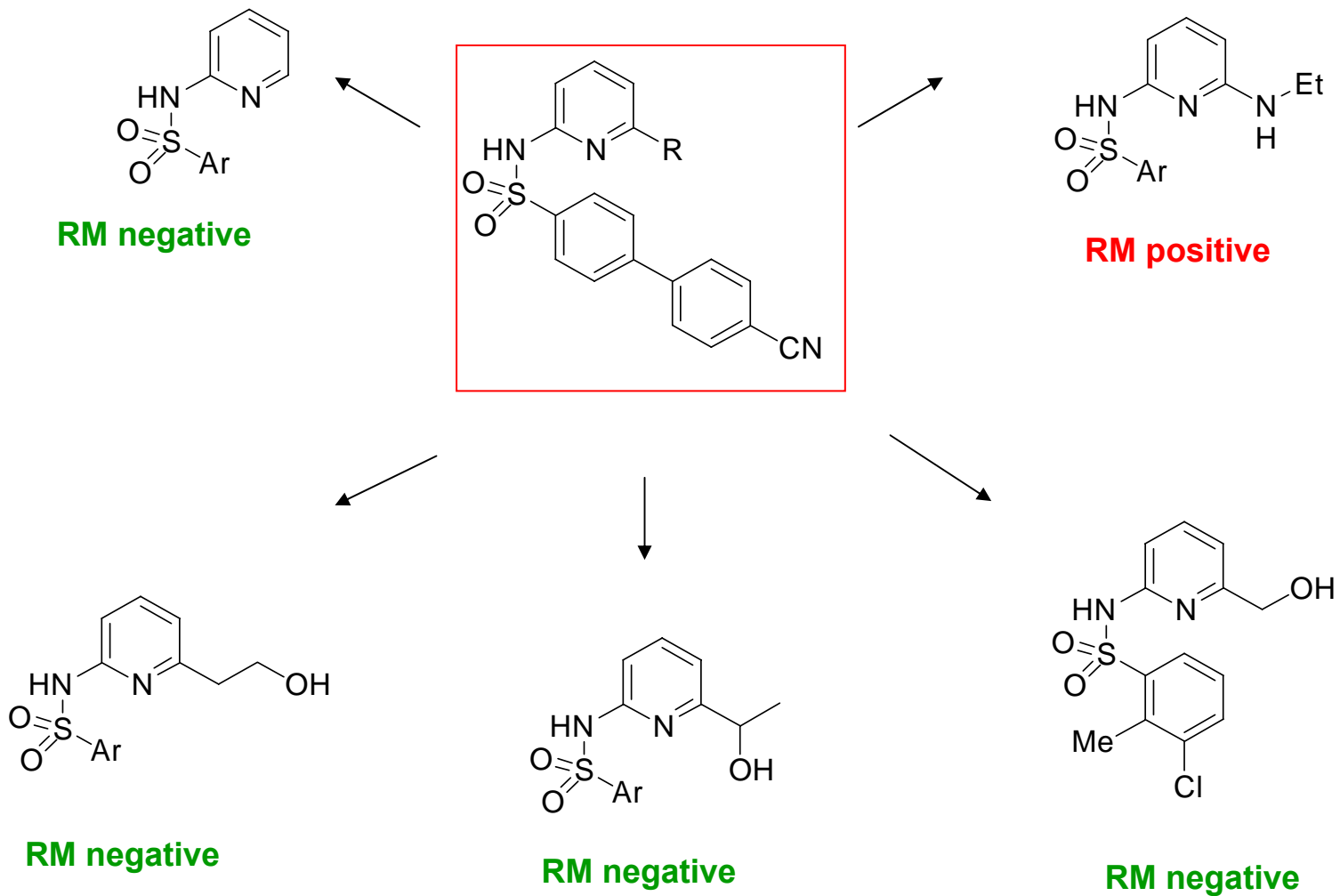
- ◆ **Chemical modifications to pyridine ring**
 - Blocking proposed sites of metabolism on pyridine ring
 - Steric hindrance
- ◆ **Metabolic Switching**
 - Introduce soft spots that could result in divergence from the bioactivation pathway
 - Long half-lives and low clearance provided an opportunity to introduce new metabolic soft spots
- ◆ **Reactive metabolite assay**
 - Evaluate “intrinsic” bioactivation in LM/NADPH
 - Evaluate other detoxification pathways in S9/cofactors



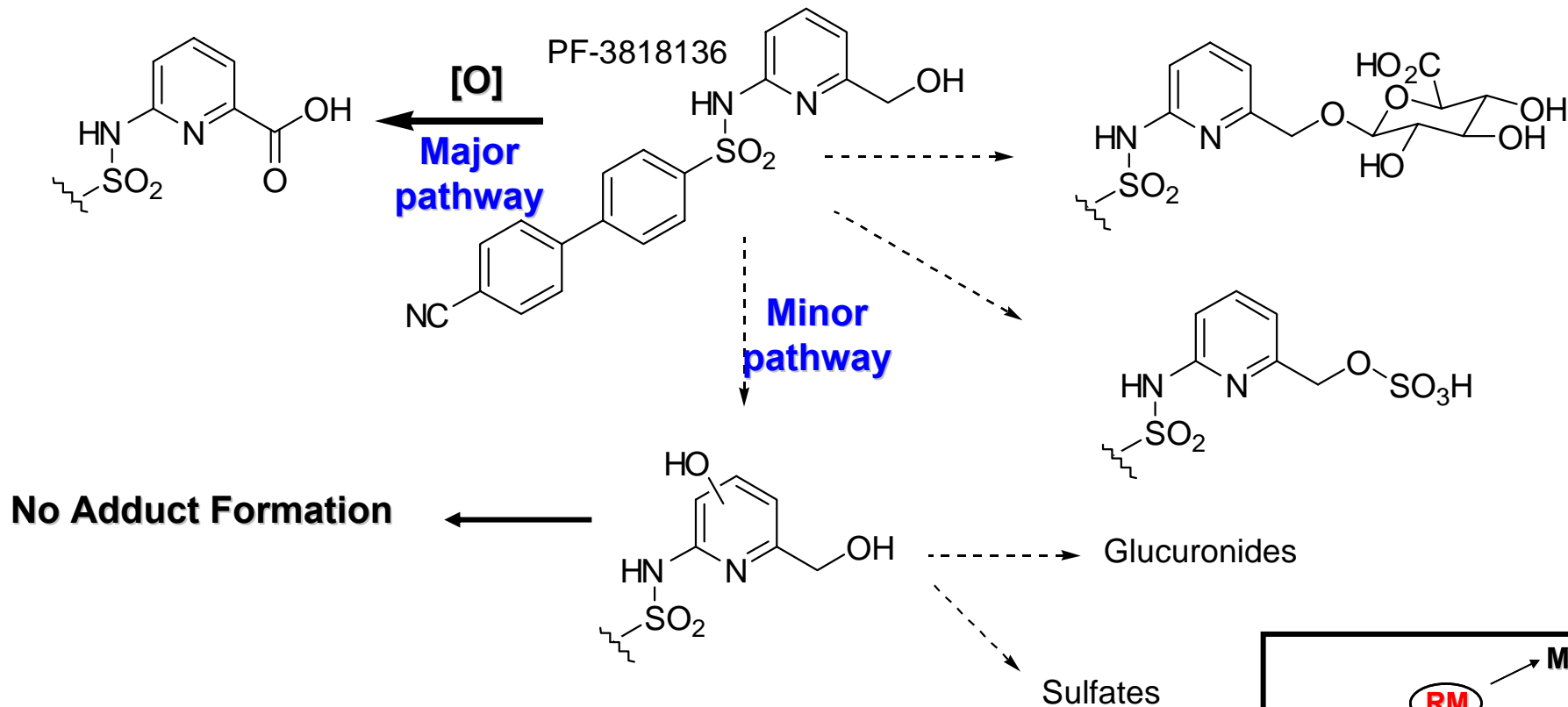
SAR: Blocking/Steric Hindrance



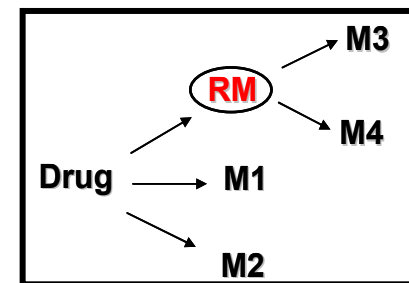
SAR: Metabolic Switching



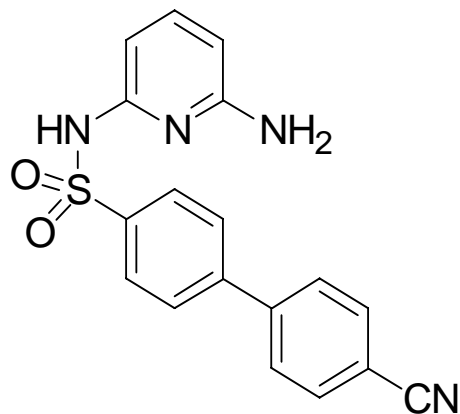
Impact of Metabolic Switching on Bioactivation



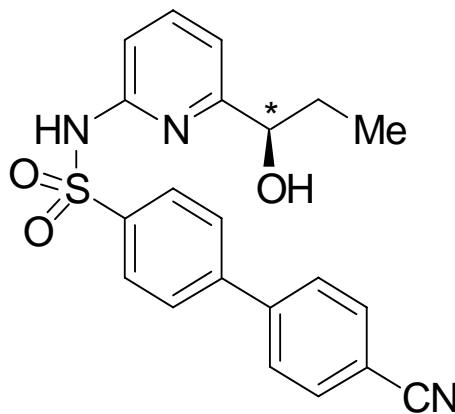
Metabolism of PF- 3818136
Assessment of Bioactivation Potential in LM and S9



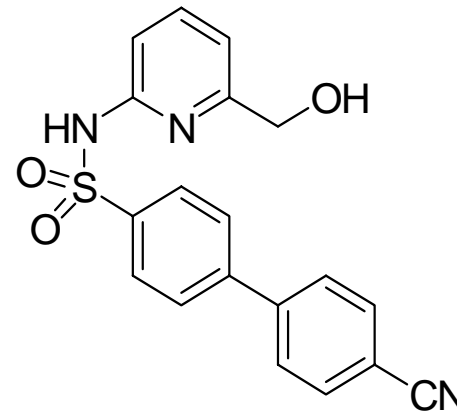
Candidate Nomination based on the Mitigation Strategies



LEAD
PF-915275



PF-3651639



PF-3818136

BACK UP

- ✦ *Both compounds possessed PK properties similar to PF-915275*
- ✦ *Did not undergo metabolic activation*



Some Important Points to Take Home

- ◆ **Addressing reactive metabolites early on**
 - Reduces attrition at late stages of development
 - Saves \$\$
 - Saves time & efforts

- ◆ **GSH adduct formation - Not a kill shot**
 - Should be used to flag in Lead Development
 - GSH conjugation is a detoxification step
 - Consider additional studies to de-risk if reactive metabolite is detected

- ◆ **Liver microsomal assays do not address all metabolic pathways for a compound**
 - Evaluate other metabolic pathways using suitable co-factors and liver S9
 - Early Dose Prediction may be useful

- ◆ **Other factors need to be considered**
 - Prototype
 - Indication
 - Dosing regimen
 - Disposition of the drug
 - Observations from toxicity studies

- ◆ **Active involvement of biotransformation experts helps in understanding and solving the reactive metabolite issue**



Acknowledgements

PDM

- ◆ **Natalie Hosea**
- ◆ **Ping Kang**
- ◆ **Bill Pool**
- ◆ **Amy La Paglia**
- ◆ **Catherine Lee**
- ◆ **Sue Zhou**

Chemistry

- ◆ **Sajiv Nair**
- ◆ **Chris Smith**
- ◆ **Michael Siu**
- ◆ **Yong Wang**
- ◆ **Ted O Johnson**
and several others



Back ups



Steps to Mitigate the Risk of PF-915275 in Light of a Positive Signal in the GSH Assay

GSH Adducts Detected in LM

**Assess role of other metabolic pathways
(S9 with cofactors, Hepatocytes)**

**Investigate GSH Adduct Formation
in Microsomes, or S9
of rat, dog & monkey**

*Helps to select
the appropriate
tox species*

**Work with SS
Conduct in vivo Toxicity studies**

**Predict the Correct Dose
(If Dose < 10 mg)**

All studies are clean

CAN

Prioritize Radiolabel



'Early Discovery Screening' Strategy For CAN2

Med Chem
strategies
to
eliminate
risk

