Metabolic Activation and Covalent Binding: Significance in Lead Optimization and Relevance in Drug Development

MRC Centre for Drug Safety Science
Workshop on Chemically-Mediated Toxicity and Chemical Stress
Liverpool, UK, 13-14 April, 2010

Thomas A. Baillie

School of Pharmacy
University of Washington
Seattle, WA, USA

Mechanisms of Drug-Induced Toxicities

Type A - Normally reversible, involving a defined target leading to a predictable pharmacodynamic outcome (on- or off-target)

Type B - “Idiosyncratic” toxicities (not predictable, eg halothane)

Type C - Predictable, dose-dependent toxicities (eg acetaminophen)

Type D - Occur only after prolonged dosing (carcinogenicity, teratology)

- *Evidence suggests that reactive metabolites may play a causative role in several forms of drug-induced toxicity (Types B, C, and D)*
- *Type B (idiosyncratic) toxicities of greatest concern in drug development*

B. K. Park, M. Pirmohamed, and N. R. Ketteringham, *Chem. Res. Toxicol.,* **11**, 969-988 (1998)
D. A. Smith and R. S. Obach, *Chem. Res. Toxicol.,* **22**, 267-279 (2009)
J. Uetrecht, *Annu. Rev. Pharmacol. Toxicol.,* **47**, 513-539 (2007), and *Chem. Res. Toxicol.,* **21**, 84-92 (2008)

Chemically Reactive Drug Metabolites: Target Organs for Toxicity

The liver is especially vulnerable to damage by reactive metabolites since this organ possesses the highest level of drug metabolizing enzyme activity

Hypersensitivity reactions involving the skin, and blood dyscrasias, often are associated with exposure to reactive metabolites

Sometimes, more than one organ system is involved

Skin Reactions and Liver Failure with Intelence (Etravirine)

tibotec
THERAPEUTICS
DIVISION OF CENTOCOR ORTHO BIOTECH PRODUCTS, L.P.

Tibotec Therapeutics
430 U.S. Highway 22 East
Bridgewater, NJ 08807

www.tibotectherapeutics.com

IMPORTANT DRUG WARNING

August 2009

Dear Healthcare Professional:

Tibotec Therapeutics, in cooperation with the U.S. Food and Drug Administration, would like to inform you of an important safety update to the Severe Skin Reactions WARNINGS AND PRECAUTIONS section (5.1) of the INTELENCE™ (etravirine) tablets prescribing information.

Specifically, the existing Warning and Precaution regarding Severe Skin Reactions has been strengthened to reflect that there have been postmarketing reports of:

- fatality due to toxic epidermal necrolysis
- hypersensitivity reactions, sometimes accompanied by hepatic failure

Additionally, Guidance has been added that INTELENCE should be immediately discontinued when signs and symptoms of severe skin or hypersensitivity reactions develop. Given the clinical relevance of these adverse reactions, the following information regarding severe skin and hypersensitivity reactions has been included in the INTELENCE Prescribing Information:

Etravirine

Published on the web, 27 August, 2009

Bioactivation and Liver Toxicity

- A wide range of therapeutic agents have been withdrawn from use due to an unacceptably high incidence of hepatotoxicity:

  Aclofenac, alpidem, amadioquine, amineptine, benoxaprofen, bromfenac, ibufenac, iproniazid, nefazodone, nomifensine, sudoxicam, tienilic acid, tolresat, troglitazone, trovafloxacin, zileuton, zomepirac

- Many other marketed drugs have warnings for a risk of liver toxicity, or severe restrictions in their use

- For most of these agents, *bioactivation* to reactive metabolites has been demonstrated to occur either *in vitro* (human hepatic tissue) or *in vivo* (characterization of downstream stable metabolites)

- *High dose drugs* (>100mg/day) tend to be the ones which most frequently cause liver toxicity

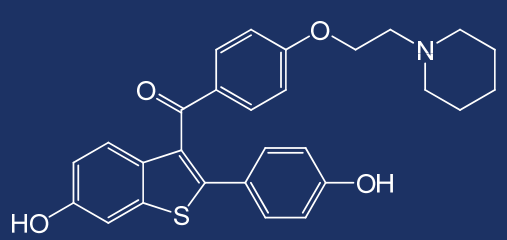
Chemically Reactive Drug Metabolites: The Problem for Drug Discovery & Development

- The weight of evidence (albeit mostly indirect) suggests that reactive metabolite formation represents a <u>risk factor</u> in drug development

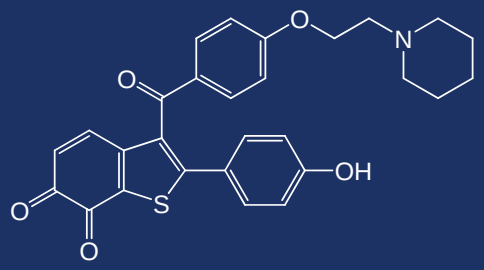
BUT

- Not all chemically reactive metabolites appear to be toxic, due to differences in:

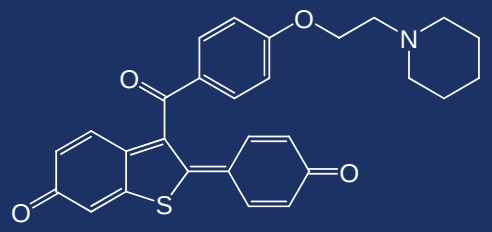
  electrophilicity, intracellular targets, stress signalling, detoxification pathways, reversibility in protein adduct formation, recognition of adducts by immune system, etc



Raloxifene

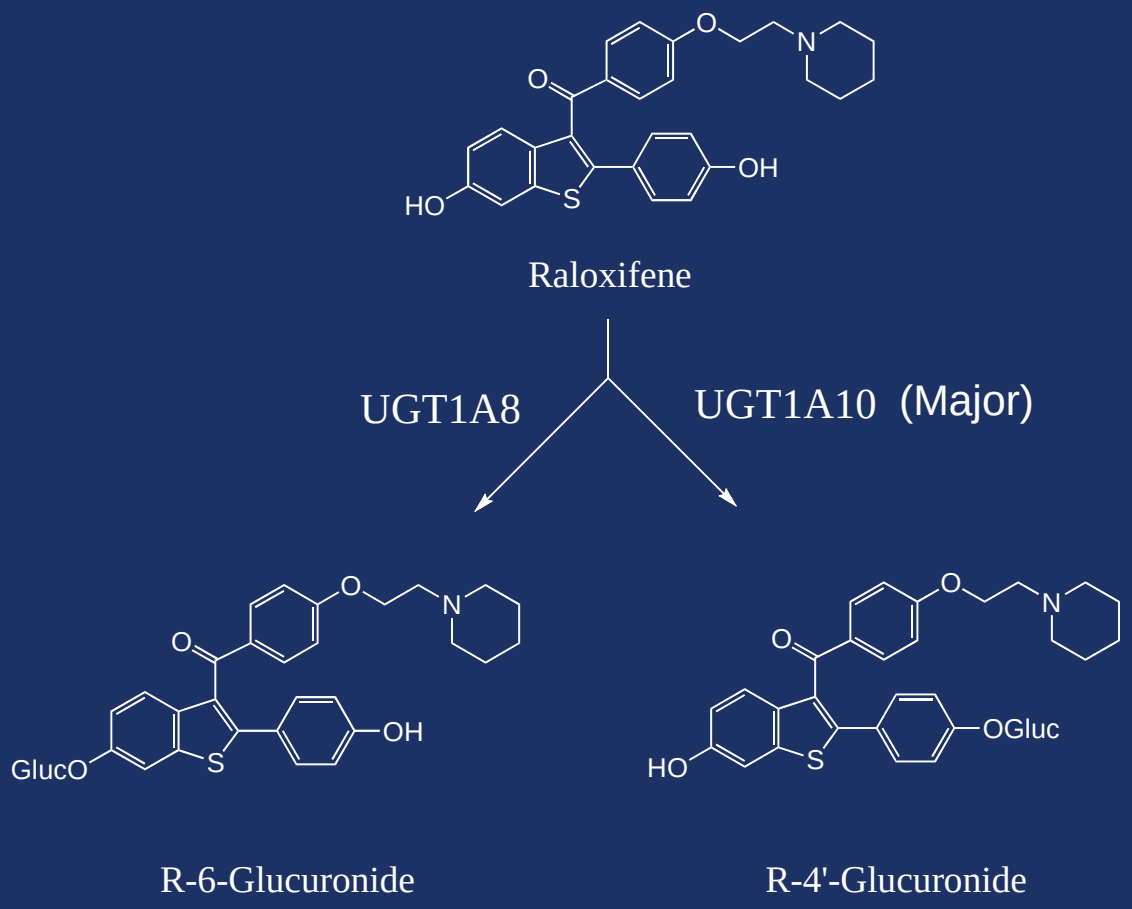


Raloxifene 6,7-*ortho*-Quinone $T_{1/2}$ = 69 min



Raloxifene Diquinone Methide $T_{1/2}$ < 1 sec

Intestinal Glucuronidation of Raloxifene in Humans



E. J. Jeong *et al., Drug Metab. Dispos.,* **33**, 785-794 (2005)

Chemically Reactive Drug Metabolites: The Problem for Drug Discovery & Development

- The interaction of electrophilic drug metabolites with biological systems is complex, multi-factorial, and poorly understood in terms of toxicological outcome:
  - Issues with *in vitro* to *in vivo* extrapolation
  - Relevance of animal toxicology data to human
  - Particular challenges with modeling the human immune system
- How to make decisions to advance drug candidates from discovery into development based on preliminary (ie very incomplete) data sets demonstrating metabolic activation?
- “Decision trees,” while subjective to a degree, are valuable in ensuring consistency in decision-making
- Pending new insights into mechanisms of reactive metabolite-mediated cellular injury, “avoidance strategies” represent the preferred option:
  - Reactive metabolite formation can be minimized, but rarely eliminated!

Assessing Formation of / Exposure to Reactive Drug Metabolites

(A) *In vitro* “trapping” experiments (eg with GSH, CN^-), or *in vivo* metabolic profiling studies:

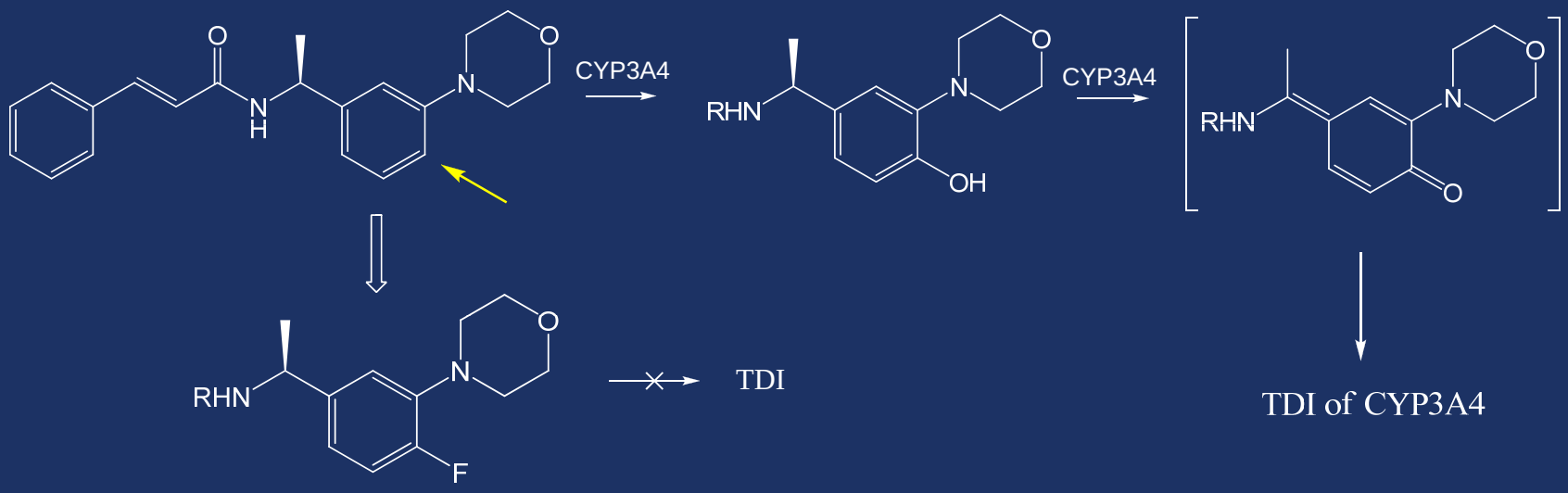
- Invaluable in enabling rational structural re-design

(B) Covalent binding studies:

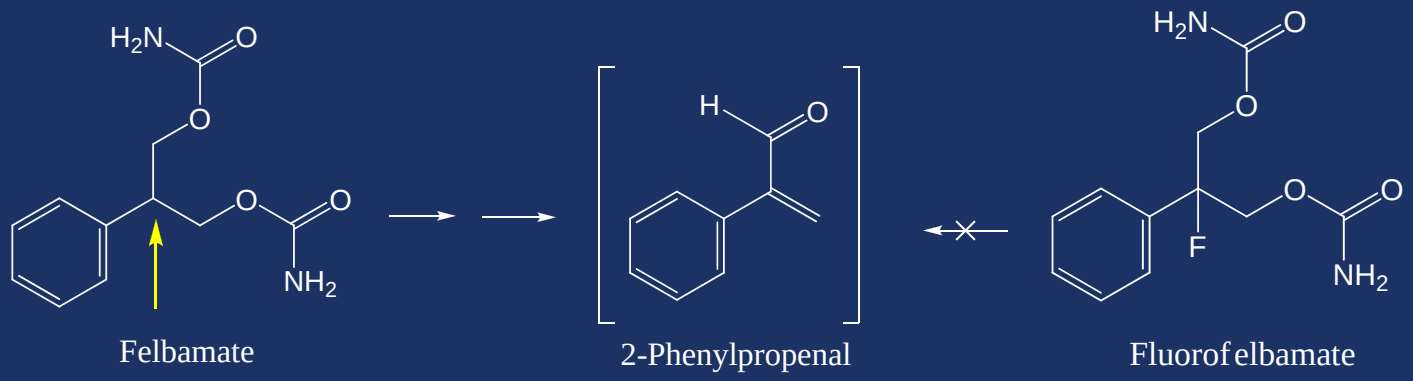
- Measures “total” burden of protein-bound drug residue
- Helpful complement to trapping studies

Nucleophilic trapping experiments and covalent binding studies employ different end-points and serve different purposes!

Minimizing Metabolic Activation: (1) Block Site of Metabolism

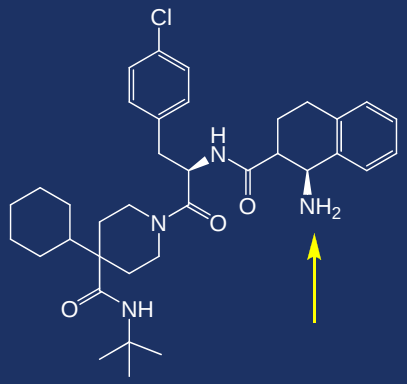


Y.-J. Wu *et al., J. Med. Chem.*, **46**, 3778-3781 (2003)

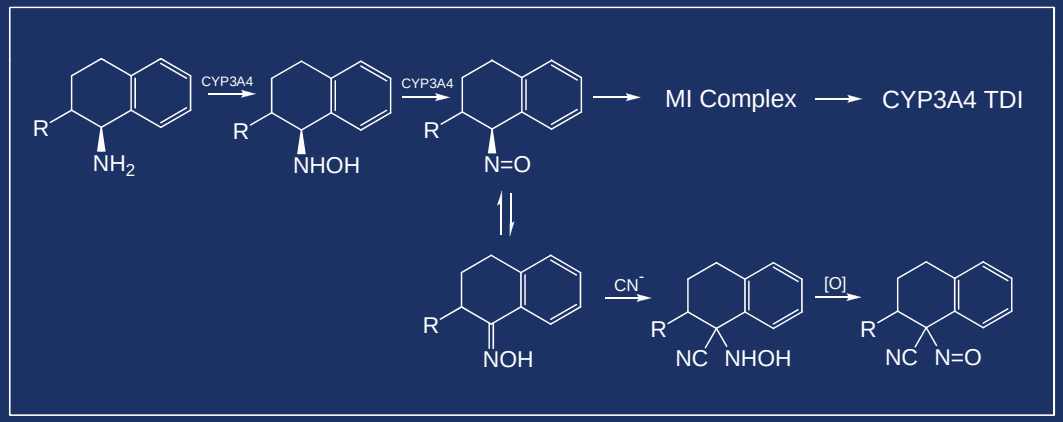


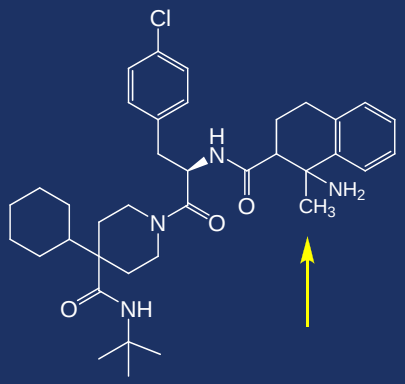
C. M. Diekhaus *et al., Chem.-Biol. Interact.*, **142**, 99-117 (2002)

Minimizing Metabolic Activation: (2) Introduce Steric Hindrance



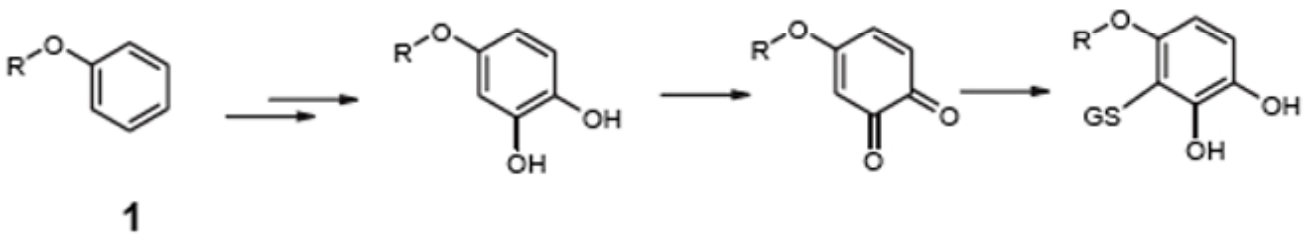
MC-4R Agonist Lead





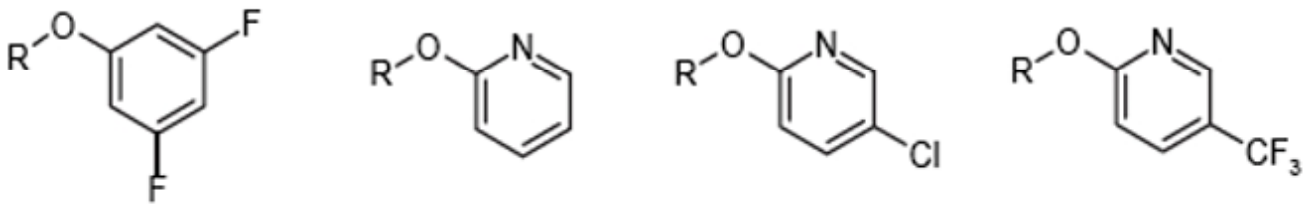
W. Tang *et al., Xenobiotica* **38**, 1437-1451 (2008)

Minimizing Metabolic Activation: (3) Introduce Electronic Changes



1

Figure 1 Metabolic activation of an aryloxy-substituted drug candidate (**1**).

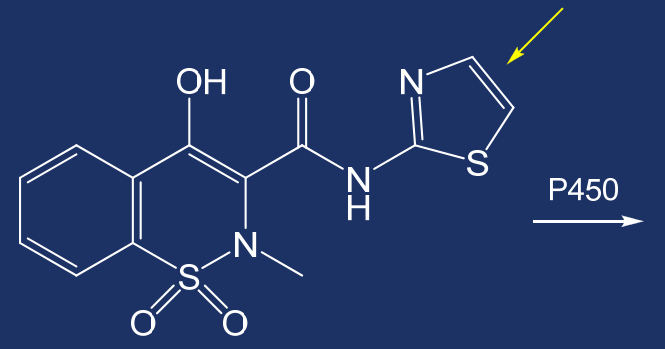


(1690) (911) (303) (88)

Figure 2 Improved analogs of the lead compound **1** showing reduced levels of covalent binding (pmol-equiv/mg protein, shown in parentheses).

G. A. Doss and T. A. Baillie, *Drug Metab. Rev.,* **38**, 641-649 (2006)
K. Samuel *et al., J. Mass Spectrom.,* **38**, 211-221 (2003)

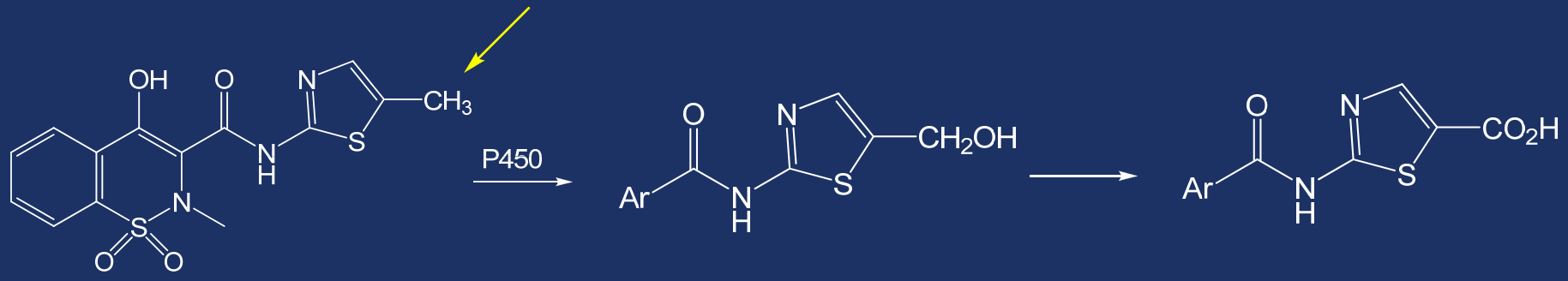
Minimizing Metabolic Activation: (4) Redirect Metabolism to "Soft Spot"



P450

Reactive metabolites of thiazole ring oxidation, thiourea formation

Sudoxicam
(Withdrawn during Phase III trials)



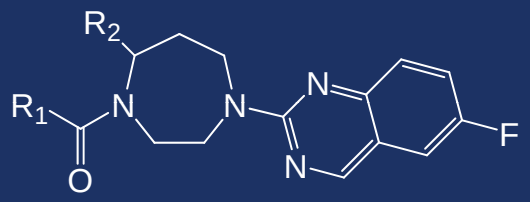
Meloxicam
(Non-hepatotoxic)

R. S. Obach *et al., Chem. Res. Toxicol.*, **21**, 1890-1899 (2008)

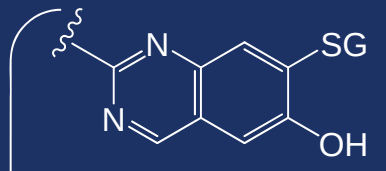
Minimizing Metabolic Activation: (5) Replacement of Structural Element

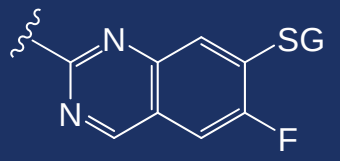
Orexin Receptor Antagonist Lead

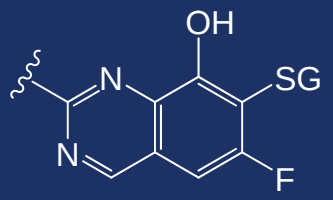
R_1 = Aryl
R_2 = Alkyl

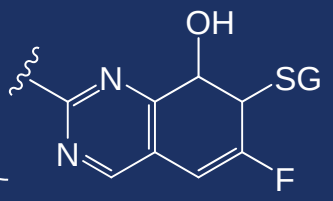


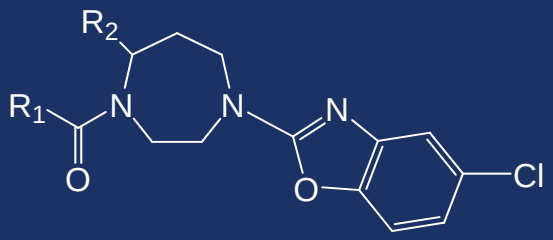
6-Fluoroquinazoline series











6-Chlorobenzoxazole series

No evidence of metabolic activation

Covalent Binding Studies

- In most cases, no simple relationship between covalent binding, GSH adduct formation, and toxicity *in vivo*
- Recent reports suggest that factoring-in estimates of "body burden" of reactive metabolites may improve our ability to predict hepatotoxic potential from covalent binding data (human hepatocytes)
- At present, true value of covalent binding data is that it serves as a tool to monitor net exposure of cells (or organs) to reactive metabolites of candidate drugs
- Tritium labeling is better suited to the discovery environment, carbon-14 to development
- Use of "microdosing" with ^{14}C and AMS to study covalent binding in man?

Chem. Res. Toxicol. **2010,** *23,* 152–158

Approach for *in Vivo* Protein Binding of 5-*n*-Butyl-pyrazolo[1,5-*a*]pyrimidine Bioactivated in Chimeric Mice with Humanized Liver by Two-Dimensional Electrophoresis with Accelerator Mass Spectrometry

Hiroshi Yamazaki,[*,†] Shunji Kuribayashi,[‡] Tae Inoue,[§,||] Chise Tateno,[§] Yasufumi Nishikura,[§] Ken Oofusa,[⊥] Daisuke Harada,[‡] Shinsaku Naito,[‡] Toru Horie,[§] and Shigeru Ohta[||]

Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan, Preclinical Assessment Department, Otsuka Pharmaceutical Factory, Inc., Naruto, Tokushima 772-8601, Japan, PhoenixBio, Co., Higashi-Hiroshima, Hiroshima 739-0046, Japan, Graduate School of Biomedical Sciences, Hiroshima University, Minami-ku, Hiroshima 734-8553, Japan, and Towa Environment Science Co., Suminoe-ku, Osaka 559-0034, Japan

Received September 9, 2009

Drug development of a potential analgesic agent 5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5-*a*]pyrimidine was withdrawn because of its limited hepatotoxic effects in humans that could not be predicted from regulatory animal or *in vitro* studies. *In vivo* formation of glutathione conjugates and covalent binding of a model compound 5-*n*-butyl-pyrazolo[1,5-*a*]pyrimidine were investigated in the present study after intravenous administration to chimeric mice with a human or rat liver because of an interesting capability of human cytochrome P450 1A2 in forming a covalently bound metabolite *in vitro*. Rapid distribution and elimination of radiolabeled 5-*n*-butyl-pyrazolo[1,5-*a*]pyrimidine in plasma or liver fractions were seen in chimeric mice after intravenous administration. However, similar covalent binding in liver was detected over 0.17−24 h after intravenous administration. Radio-LC analyses revealed that the chimeric mice with humanized liver preferentially gave the 3-hydroxylated metabolite and its glutathione conjugate in the plasma and liver. On the contrary, chimeric mice with a rat liver had some rat-specific metabolites *in vivo*. Analyses by electrophoresis with accelerator mass spectrometry of *in vivo* radiolabeled liver proteins in chimeric mice revealed that bioactivated 5-*n*-butyl-pyrazolo[1,5-*a*]-pyrimidine bound nonspecifically to a variety of microsomal proteins including human P450 1A2 as well as cytosolic proteins in the livers from chimeric mice with humanized liver. These results suggest that the hepatotoxic model compound 5-*n*-butyl-pyrazolo[1,5-*a*]pyrimidine was activated by human liver microsomal P450 1A2 to reactive intermediate(s) *in vivo* in humanized chimeric mice and could relatively nonspecifically bind to biomolecules such as P450 1A2 and other proteins.

Covalent Binding Studies in Humans: The Case of MaxiPost

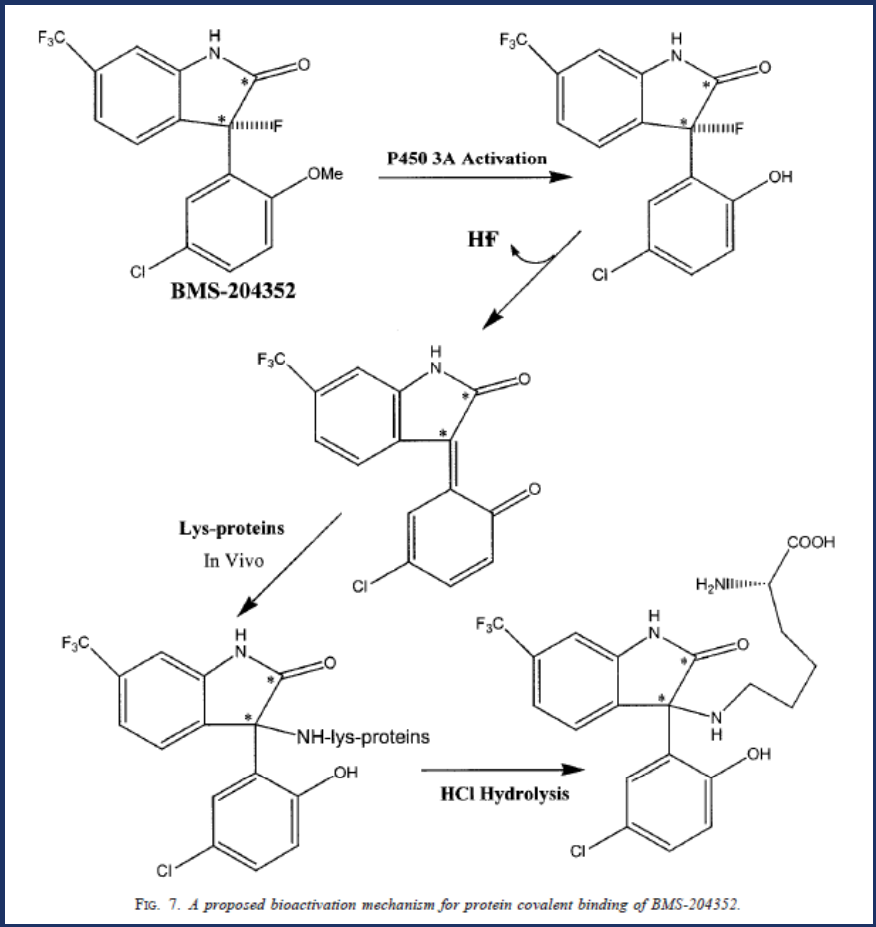


FIG. 7. *A proposed bioactivation mechanism for protein covalent binding of BMS-204352.*

D. Zhang *et al., Drug Metab. Dispos.*, **31**, 837-845 (2003)

MaxiPost – Potent and specific opener of maxi-K channels, targeted for the treatment of ischemic stroke

Preclinical studies demonstrated NADPH-dependent covalent binding of [^{14}C]MaxiPost *in vitro* (liver microsomes from rats, dogs, and humans), and time-dependent inactivation of CYP3A

Within 1 hr following IV dosing to rats, >85% of radioactivity in plasma was unextractable by organic solvents; appeared to be covalently bound to albumin

Acid hydrolysis of rat plasma afforded lysine adduct of *O*-desmethyl, desfluoro metabolite

Similar behavior in dogs

Preclinical tox studies in rats and dogs clean (10 mg/kg/day IV for 1 month)

Covalent Binding of Radioactivity to Human Plasma Proteins after Single 10mg IV Dose of [^{14}C]MaxiPost

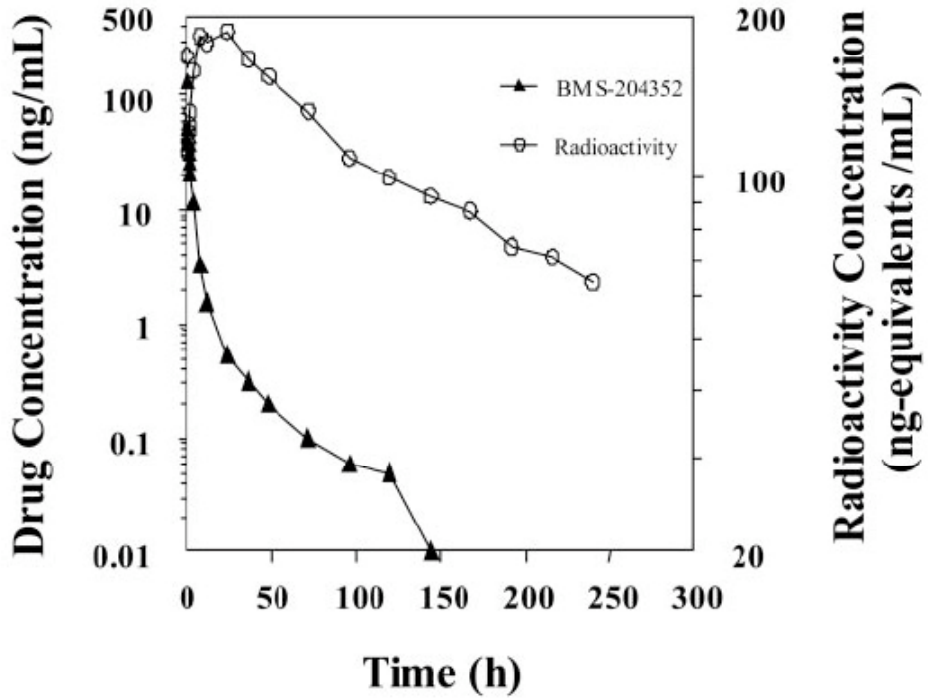


FIG. 1. Mean plasma concentration versus time profile of BMS-204352 and radioactivity following a 10-mg (50-μCi radioactivity) intravenous dose of [^{14}C]BMS-204352 (n = 8).

D. Zhang *et al., Drug Metab. Dispos.,* **33**, 83-93 (2005)

Fraction of unextractable radioactivity in plasma at 120 hr = 81%

$t_{½}$ for ^{14}C ~11 days (HSA $t_{½}$ ~19 days)

Acid hydrolysis gave same lysine adduct

Recovery of radioactivity (urine + feces) after 14 days = 97% (*N*-glucuronide of parent + unidentified metabolites)

Estimated fraction of dose covalently bound to plasma proteins ~0.5-5%

"Given the intended low clinical dose of <10mg/day, and the nature of the indication (stroke), protein covalent binding does not appear to adversely impact the development of BMS-204352"

Risk Assessment Considerations Based on Bioactivation Potential

- Chemical tractability of structural series?
  - What potential exists to modify structure?
  - Has metabolic activation been minimized relative to preceding compound?
- Availability of existing treatments for target disease?
- Is the prognosis disabling or life-threatening?
- Is the anticipated clinical dose <10mg?
- Are the metabolic clearance routes primarily non-Phase I?
  - Consider studies in hepatocytes
- Expected duration of therapy?
  - Will the drug be used chronically / prophylactically?
- What is the intended target population?
  - Is the drug intended for a pediatric indication?

D. C. Evans *et al., Chem. Res. Toxicol.,* **17**, 3-16 (2004)

Future Research on Biological Reactive Intermediates

Where is the field going?

- Key protein targets of reactive intermediates – what are they?
- Approaches to estimate “total body burden” of reactive metabolites
- Improved animal models of reactive metabolite-mediated toxicity (especially immune-mediated)
- Predictive toxicity biomarkers (based on genomics, proteomics, and metabolomics)
- Better understanding of cellular defense mechanisms (eg Keap1/Nrf2/ARE system)

What is needed?

- Basic research on mechanisms of foreign compound-mediated toxicity (this is not just a “pharmaceutical industry problem”)
- Industry-wide consortium and focused academic collaborations on “pre-competitive” aspects of drug-induced toxicity (eg biomarkers of exposure to reactive metabolites)
- Consensus on what constitutes a “Risk Assessment Plan” involving reactive metabolites (BRI VIII, Barcelona, July, 2010)

Reactive drug metabolites represent only one component of overall risk assessment!