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Predictions of *In Vivo* Heterotropic Activation of Cytochrome P450 Enzymatic Activity from *In Vitro* Systems

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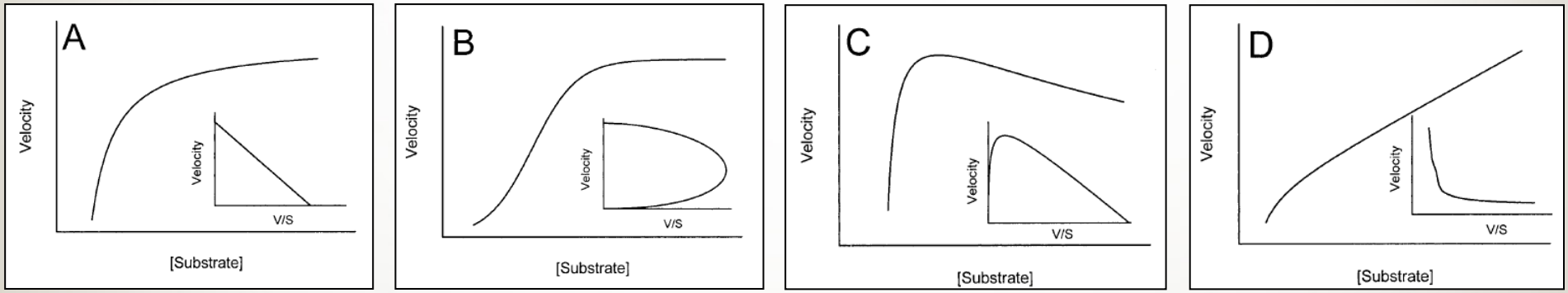
Atypical Kinetic Profiles for P450: Heterotropic Effects

- Atypical (non-MM) kinetic profiles for P450:
  - Activation, autoactivation, substrate inhibition, partial inhibition, biphasic metabolism

- Notion of homotropic vs. heterotropic cooperativity with P450
  - Heterotropic effects: activation and inhibition
  - Activation mostly noted with P450s 3A4 and 2C9
  - α-naphthoflavone activates hydroxylation of benzo[*a*]pyrene, others.

- Two binding site hypothesis (vs. remote allosteric site)
  - X-ray crystal structures of P450 3A4: unliganded, inhibitors, substrates
  - Kinetic studies, mutagenesis, modeling



Michaelis-Menten | Autoactivation | Substrate Inhibition | Biphasic

Hutzler and Tracy (2002) DMD: 30: 355.

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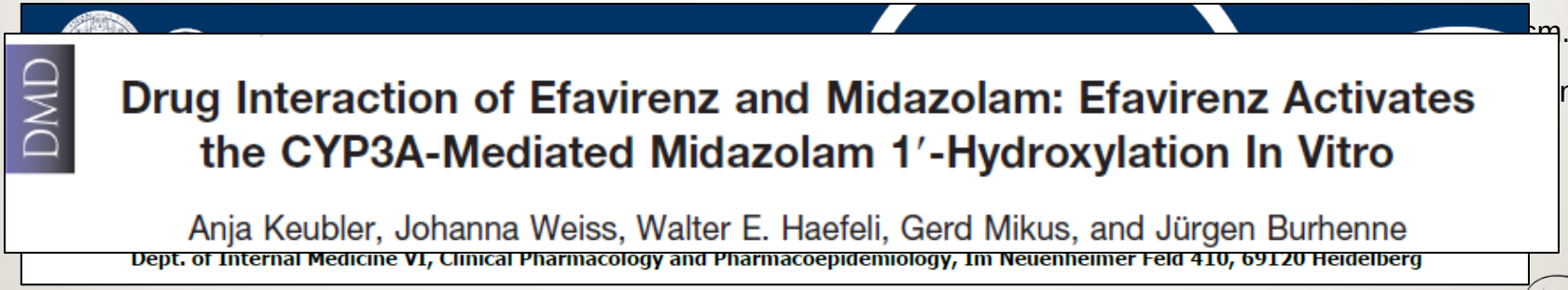
- Two binding site hypothesis (vs. remote allosteric site)
  - X-ray crystal structures of P450 3A4: unliganded, inhibitors, substrates
  - Kinetic studies, mutagenesis, modeling

- Typically hear about TDI/induction and DDI potential – is there a concern with activation?

- Limited *in vivo* observations:


DMD

Drug Interaction of Efavirenz and Midazolam: Efavirenz Activates the CYP3A-Mediated Midazolam 1′-Hydroxylation In Vitro

Anja Keubler, Johanna Weiss, Walter E. Haefeli, Gerd Mikus, and Jürgen Burhenne

Dept. of Internal Medicine VI, Clinical Pharmacology and Pharmacoepidemiology, Im Neuenheimer Feld 410, 69120 Heidelberg

In Vitro Tools for Predicting Heterotropic Effects *In Vivo*

- Recombinant systems
  - Changes in K_m, V_{max}
  - Typically human P450 isoforms (translation to rodent or non-human primate?)
  - Artificial system with co-expressed or added partner proteins, co-factors
- Hepatic microsomal systems
  - Methods to monitor disappearance of substrate and accumulation of metabolite
  - More complex system with multiple enzymatic pathways present
  - Translation of human metabolism to other species (substrate dependence, correct homolog?)
  - Does not account for non-microsomal sources of metabolism (AO); permeability
- Hepatocytes
  - More physiological system with relevant levels and proximity of co-factors/partner proteins
  - Allows for other sources of metabolism (non-P450, phase II)
  - Large donor pools (human) can mix gender, polymorphs, race, ethnicity
  - Translation to other species? Role of non-hepatic metabolism?

Heterotropic Effects on P450 Enzymes: An Emerging Trend

ACTIVATION
ANA
J. MATTHEW H

servations

In Vivo Drug I
Midazolam: He
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Hiroshi Yamaza
Masato Nakamu

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WEI TANG, RALPH A.
NATHAN X. YU, DENNI

Heterotropic Effects on Drug-Metabolizing Enzyme Activities: *In Vitro* Curiosity Emerges as a Clinically Meaningful Phenomenon (Perhaps?)

RS Obach[1]

Alterations in cytochrome P4503A4 are the most frequent underlying cause of drug–drug interactions (DDIs). This enzyme exhibits some unusual behaviors; for example, it has been observed that certain inhibitors can affect some CYP3A4-catalyzed reactions more than others, even for the same substrate. This has been proposed to be due to the simultaneous binding of more than one ligand to the enzyme. This behavior has been frequently observed *in vitro*, but seldom are analogous effects evident *in vivo*.

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Drug Metab Dispos. 2013 Sep 3. [Epub ahead of print]

Heterotropic Activation of the Midazolam Hydroxylase Activity of CYP3A by a Positive Allosteric Modulator of mGlu5: In Vitro to In Vivo Translation and Potential Impact on Clinically Relevant Drug-Drug Interactions.

Blobaum AL, Bridges TM, Byers FW, Turlington M, Mattmann M, Morrison RD, Mackie C, Lavreysen H, Bartolome JM, Macdonald GJ, Steckler T, Jones CK, Niswender CM, Conn PJ, Lindsley CW, Stauffer SR, Daniels JS.

Vanderbilt University;

Abstract

Allosteric modulation of G-protein coupled receptors (GPCRs) has gained considerable attention in the drug discovery arena as it opens avenues to achieve greater selectivity over orthosteric ligands. We recently identified a series of positive allosteric modulators (PAMs) of metabotropic glutamate receptor 5 ($mGlu_5$) for the treatment of schizophrenia that exhibited robust heterotropic activation of CYP3A4 enzymatic activity. The prototypical compound from this series, VU0448187, was found to activate CYP3A4 to > 100 % of its baseline intrinsic midazolam (MDZ) hydroxylase activity in vitro; activation was CYP3A substrate-specific and $mGlu_5$ PAM-dependent. Additional studies revealed the concentration-dependence of CYP3A activation by VU0448187 in multi-species hepatic and intestinal microsomes and hepatocytes, as well as diminished effect observed in the presence of ketoconazole. Kinetic analyses of the effect of VU0448187 on MDZ metabolism in recombinant CYPs or human liver microsomes resulted in a significant increase in V_{max} (minimal change in K_m) and required the presence of cytochrome b5. The atypical kinetics translated to in vivo, as rats receiving an intraperitoneal administration of VU0448187 prior to MDZ treatment demonstrated a significant increase in circulating 1- and 4-hydroxy midazolam (1-OH-MDZ, 4-OH-MDZ) levels compared to rats administered MDZ alone. The discovery of a potent, substrate-selective activator of rodent CYP3A with an in vitro to in vivo translation, serves to illuminate the impact of increasing intrinsic enzymatic activity of hepatic and extrahepatic CYP3A in rodents, and presents the basis to build models capable of framing the clinical relevance of substrate-dependent heterotropic activation.

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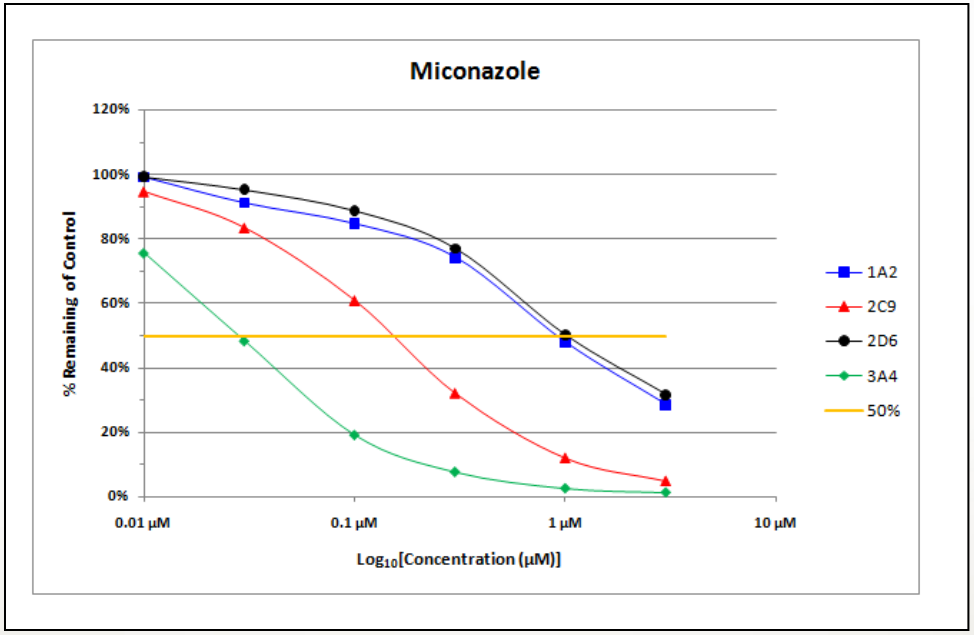
Blobaum, et al (2013) DMD, In press

Discovery of Heterotropic Activation of P450 3A

• A cocktail inhibition approach with human liver microsomes is used in our laboratory to monitor the ability of various compounds to inhibit 4 of the major P450 enzymes.

• A specific substrate/metabolite combination is utilized for each enzyme (midazolam/1-OH-midazolam – P450 3A4) with levels of metabolites being detected by specific MRMs via LC-MS/MS approaches.

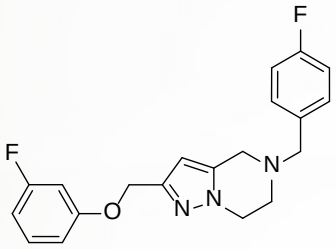
• A pan-P450 inhibitor (miconazole) is included as a positive control.



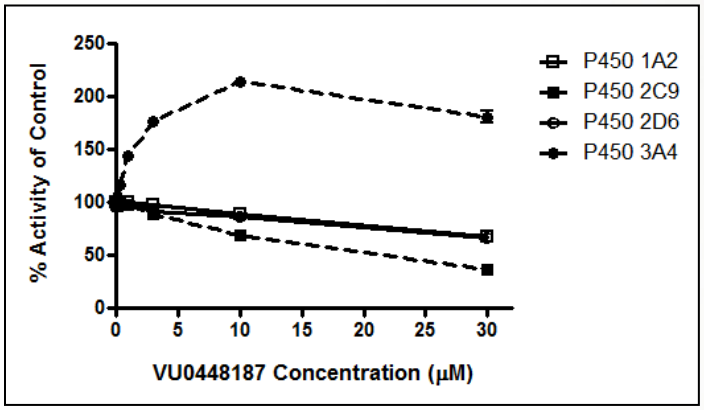
Discovery of Heterotropic Activation of P450 3A

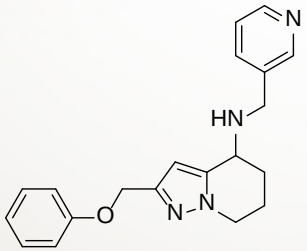
• We discovered a series of mGlu5 positive allosteric modulators that exhibited activation of P450 3A4 midazolam 1-hydroxylase activity in human liver microsomes.

• The prototype compound, designated VU0448187, was chosen for further exploration of this phenomenon *in vitro* and *in vivo*.

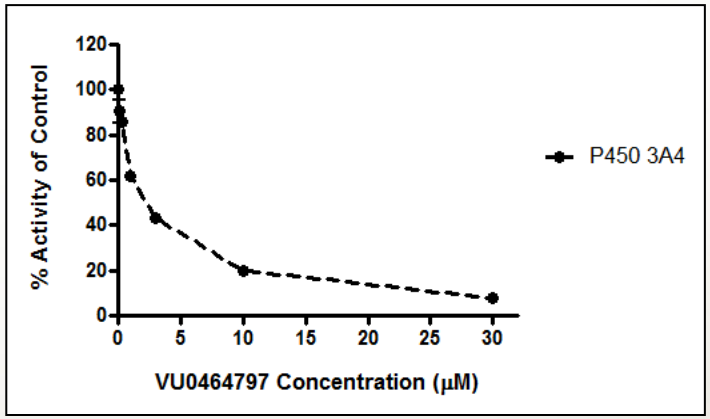


VU0448187
1900 nM EC_{50} hmGlu5





VU0464797
620 nM EC_{50} hmGlu5



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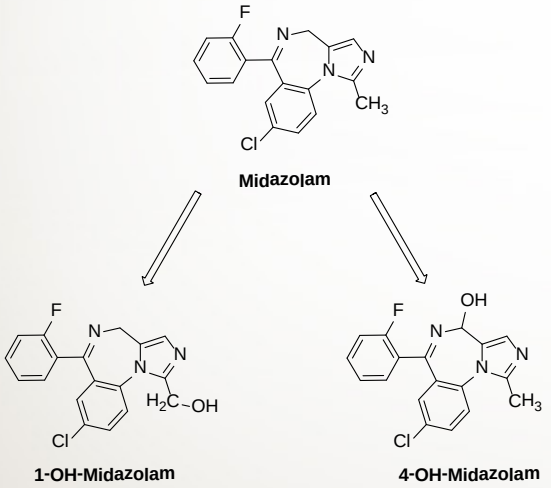
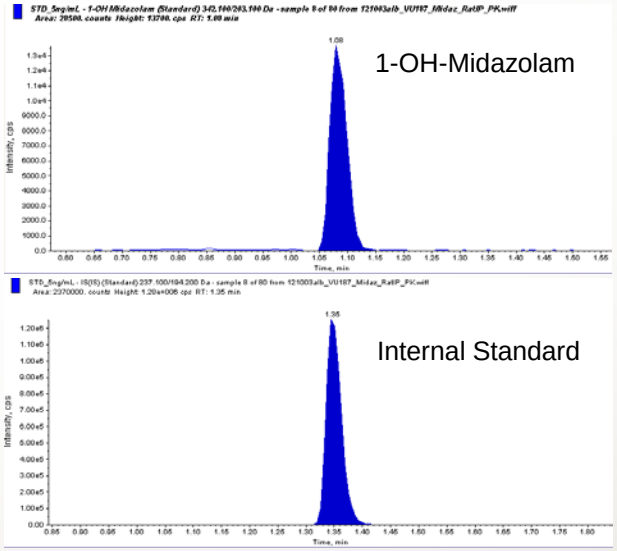
Assay Development: MS-Based Paradigm for Analysis

• LC-MS/MS methodology was developed to monitor the levels of VU0448187, midazolam, 1-OH-midazolam, and 4-OH-midazolam simultaneously from multiple sample matrices (rP450s, microsomes, hepatocytes, rodent plasma and tissue homogenates).

• The activation of P450 3A enzymatic activity was specific for the conversion of midazolam to its hydroxylated metabolites.

• Levels of analyte were quantified against a 12-point standard curve. Based on the study design, data were reported as % of control, product formed per unit time per concentration of P450/protein, or ng/mL (*n* = 2 or more).





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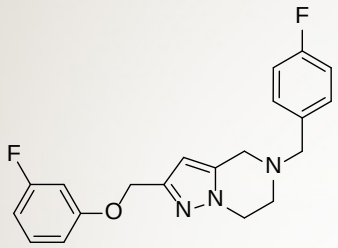
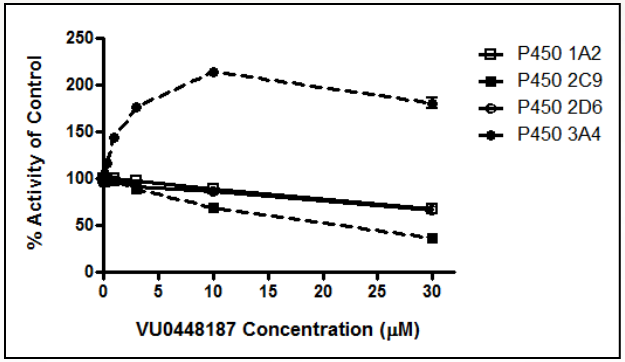
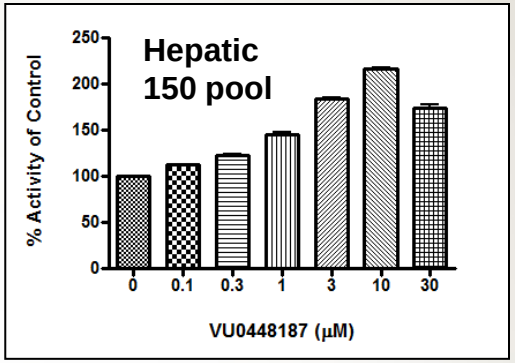
Observations in Multi-Species Liver and Intestinal LMs



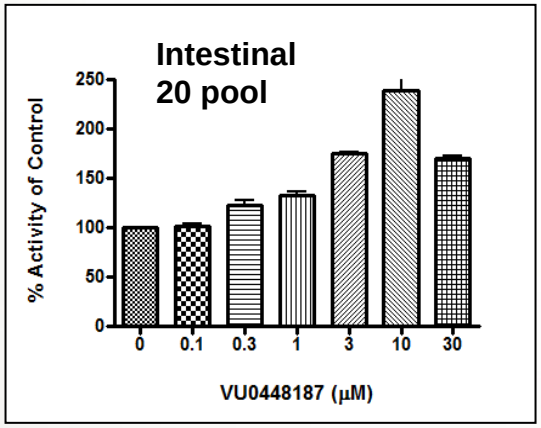
VU0448187



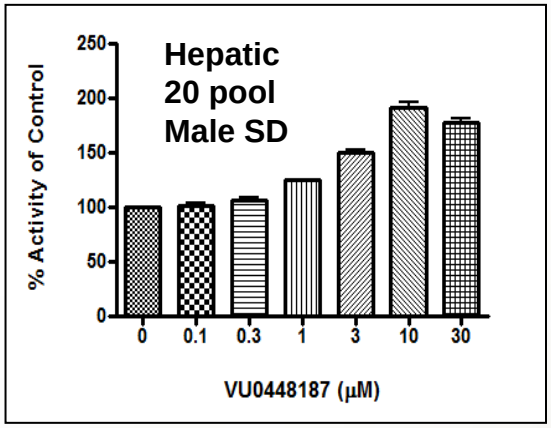
Results of P450 Cocktail in HLM



HLM – 1-OH-MDZ Formation

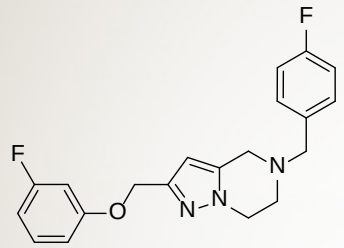


HIM – 1-OH-MDZ Formation

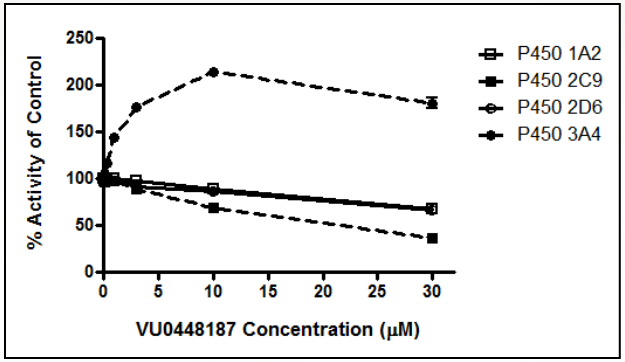


Rat LM – 1-OH-MDZ Formation

Effects of 3A Inhibitors and Alternative Substrates in HLM



VU0448187



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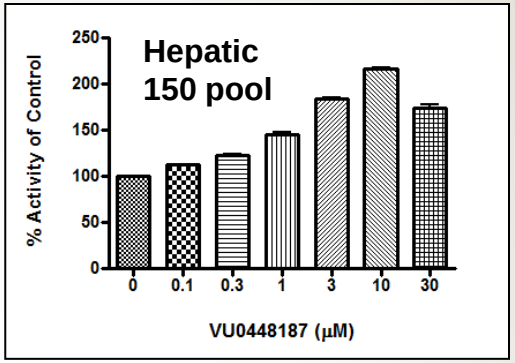
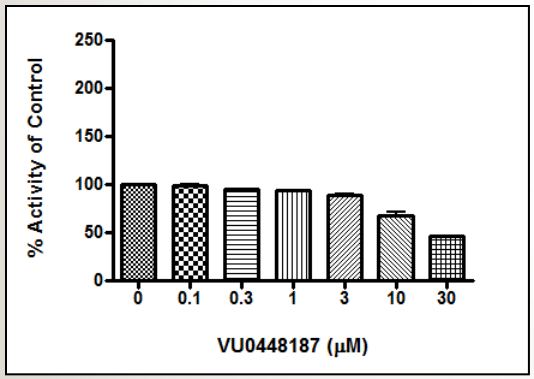


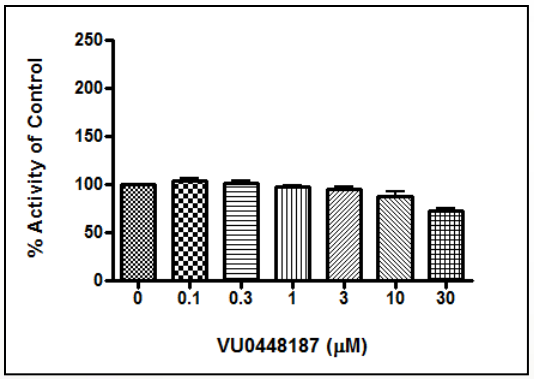
HLM – 1-OH-MDZ Formation

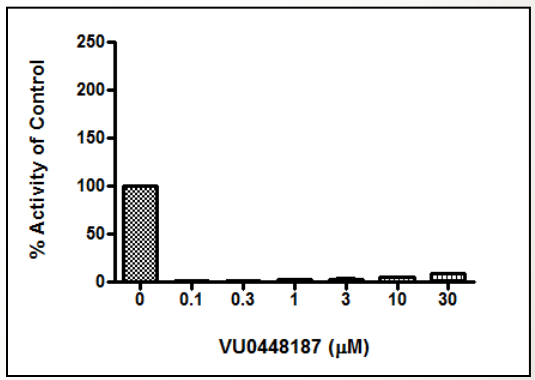
+ Ketoconazole (1 μM)

HLM – Testosterone Hydroxylation



HLM – Progesterone Hydroxylation





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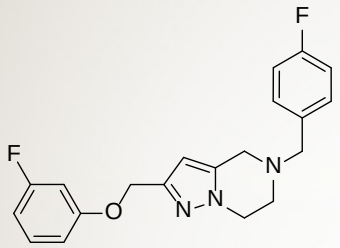
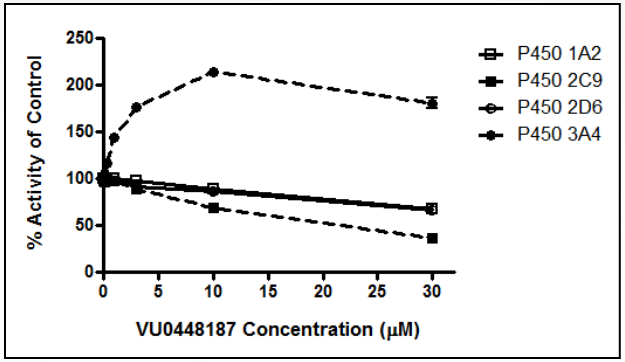
Kinetics of 3A4 Activation in Human LM and rCYPs



VU0448187



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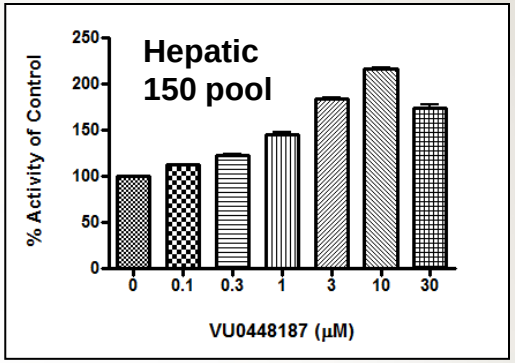
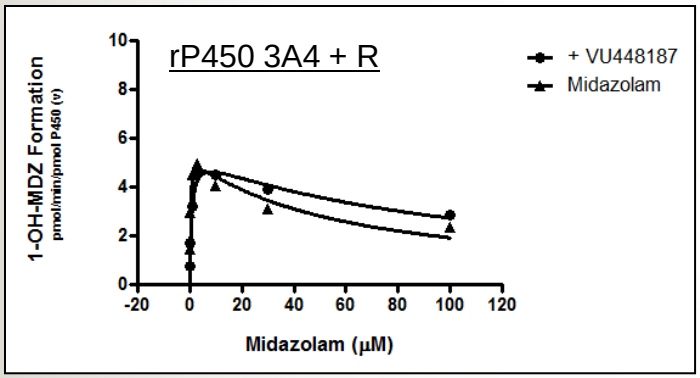
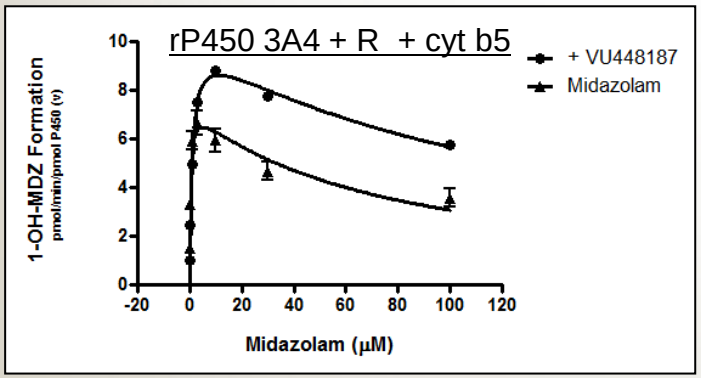


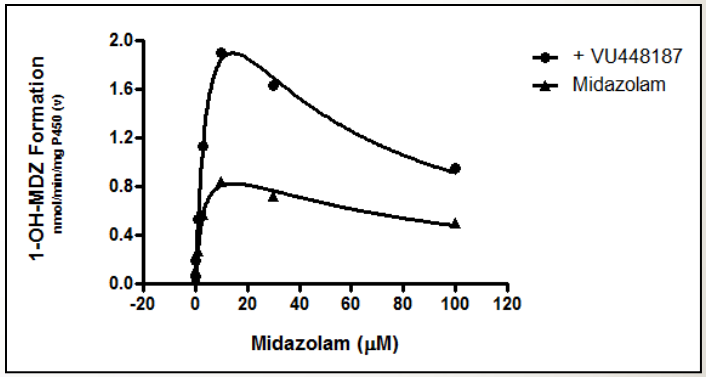
HLM – 1-OH-MDZ Formation

V_{max} driven



Recombinant CYPs





Determination of kinetic parameters in HLM

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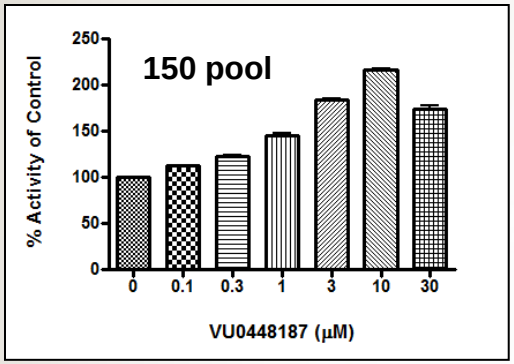
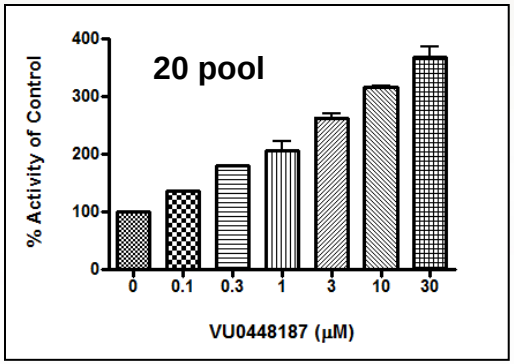
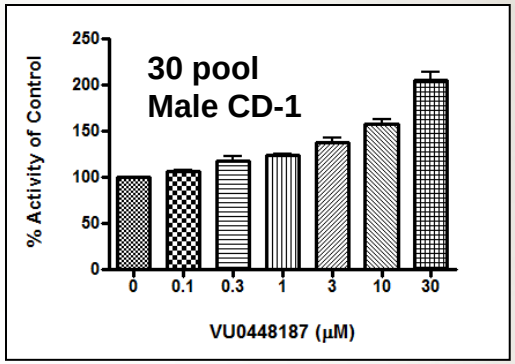
Are Hepatocytes a Better System for *In Vivo* Predictions?



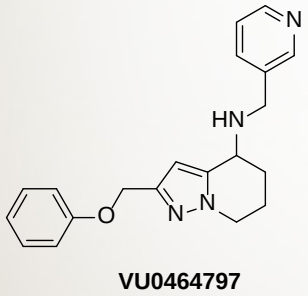
HLM – 1-OH-MDZ Formation



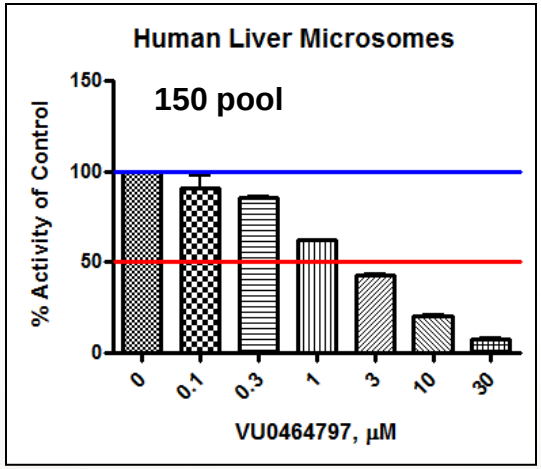
Human Hepatocytes
LiverPool

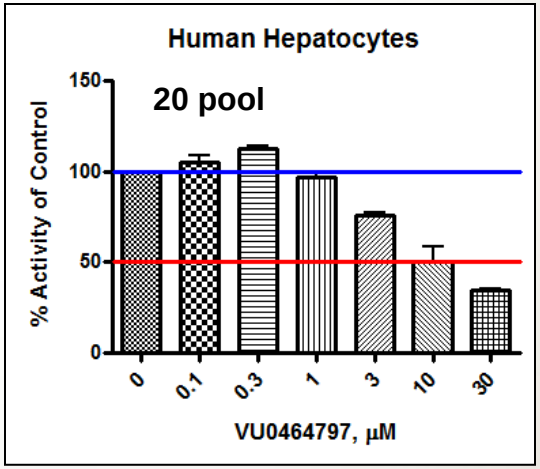


Murine Hepatocytes

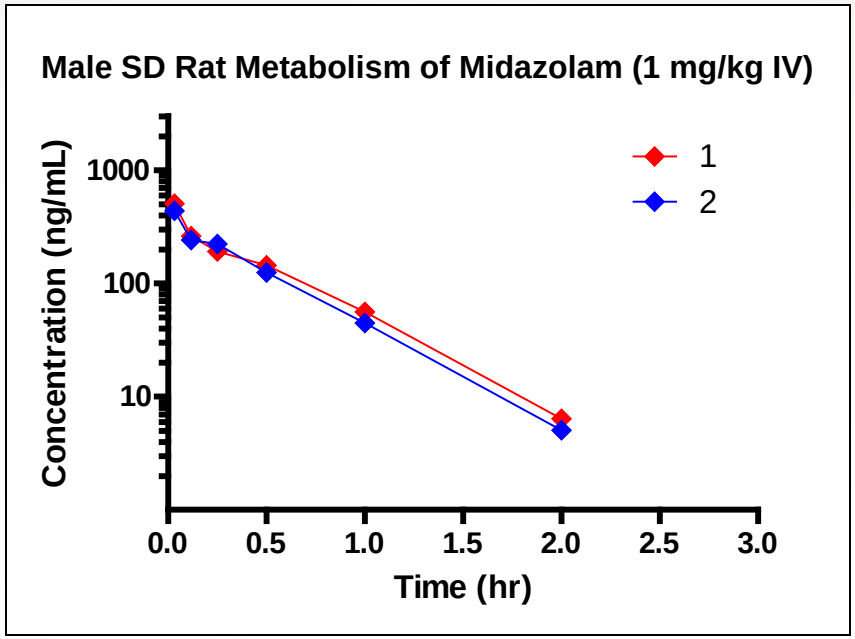


VU0464797





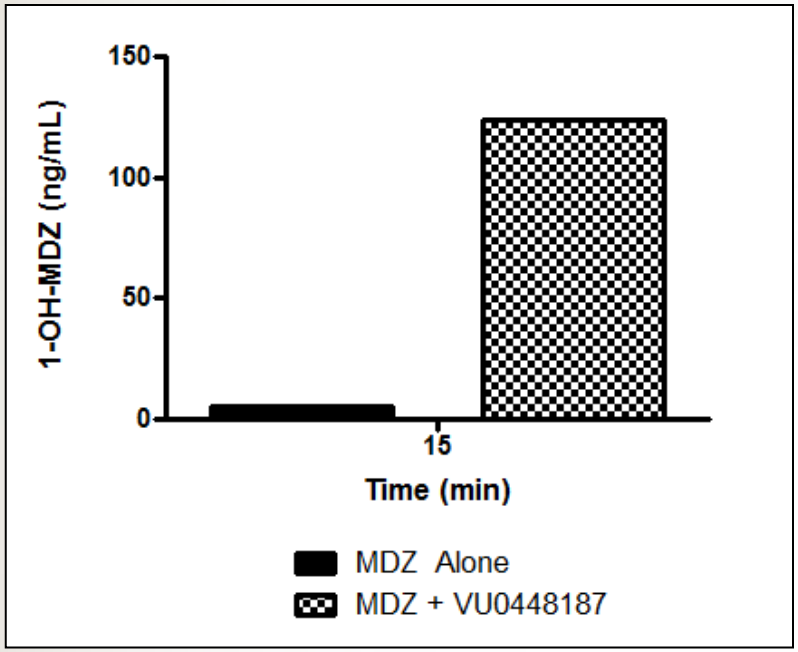
Heterotropic Activation: *In Vivo* PK Studies in SD Rats

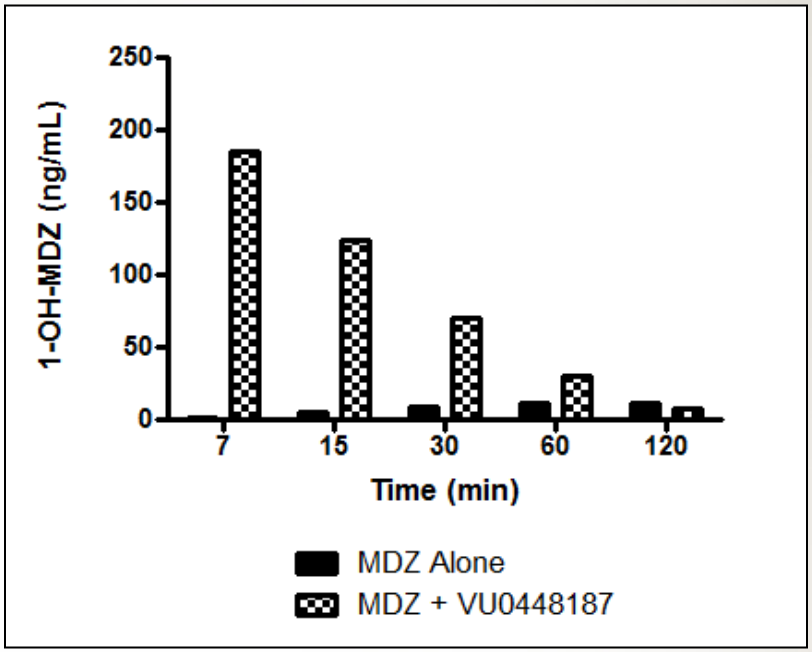


PK Parameter		1	2
CLp	mL/min/kg	80.0	88.1

- Clearance of midazolam from plasma was suprahepatic (> 70 mL/min/kg) in SD rats.
- It was difficult to detect 1-OH-midazolam and 4-OH-midazolam from a 1 mg/kg IV midazolam dose.
- Higher dose of midazolam would be required *in vivo*; change in route of administration (IP).

Heterotropic Activation: *In Vivo* Observations in SD Rats

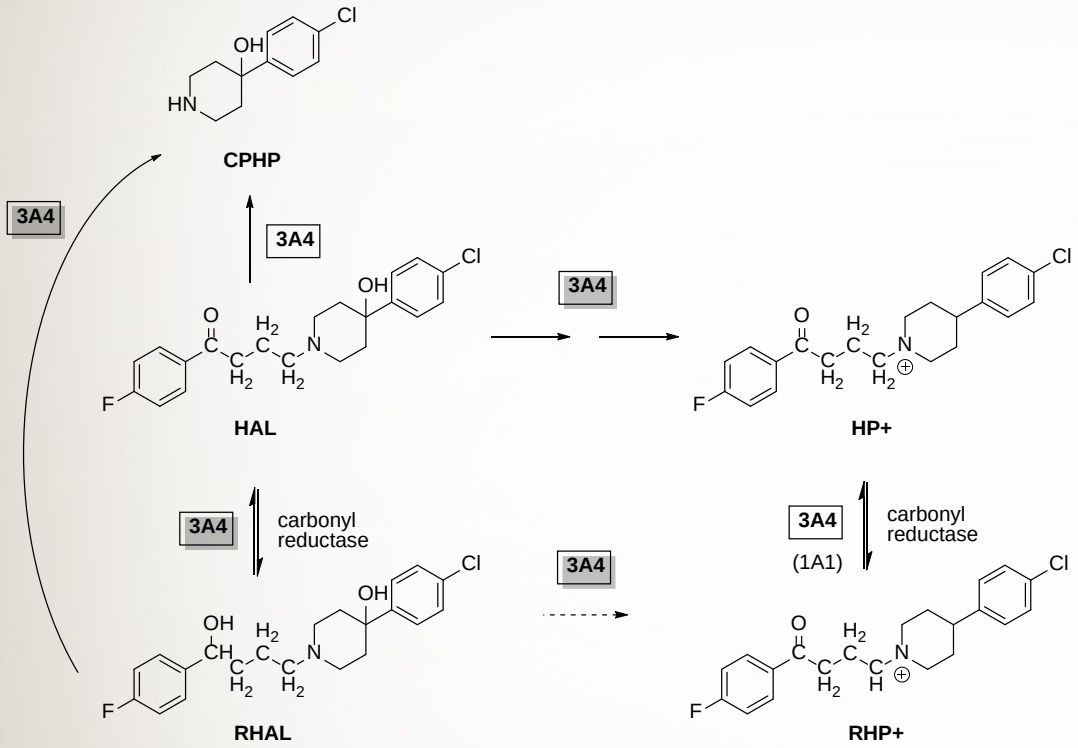




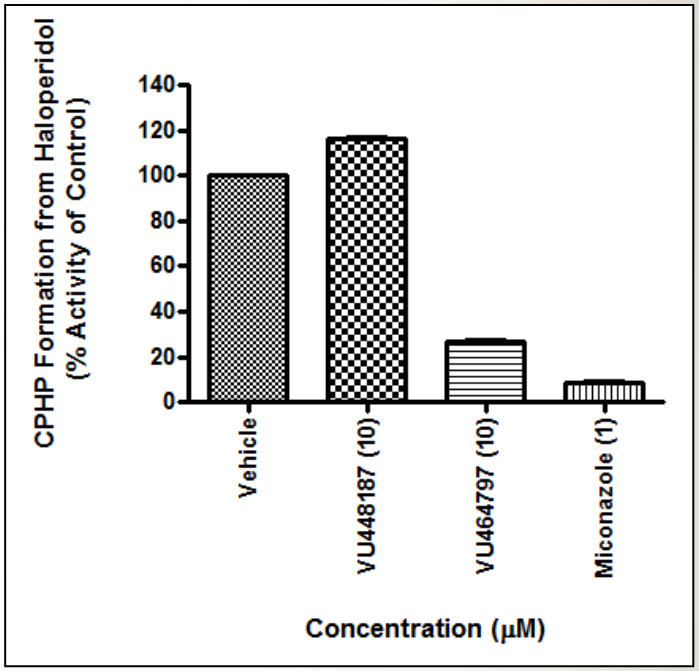
• Male Sprague-Dawley rats (*n* = 2) pre-treated for 1 hour with VU0448187 (IP, 10 mg/kg) followed by midazolam (IP, 10 mg/kg) showed a significant increase in plasma 1-OH midazolam levels.

• 4-OH midazolam levels were also monitored and showed an increase in the presence of VU0448187.

Heterotropic Activation of 3A by VU0448187: Haloperidol



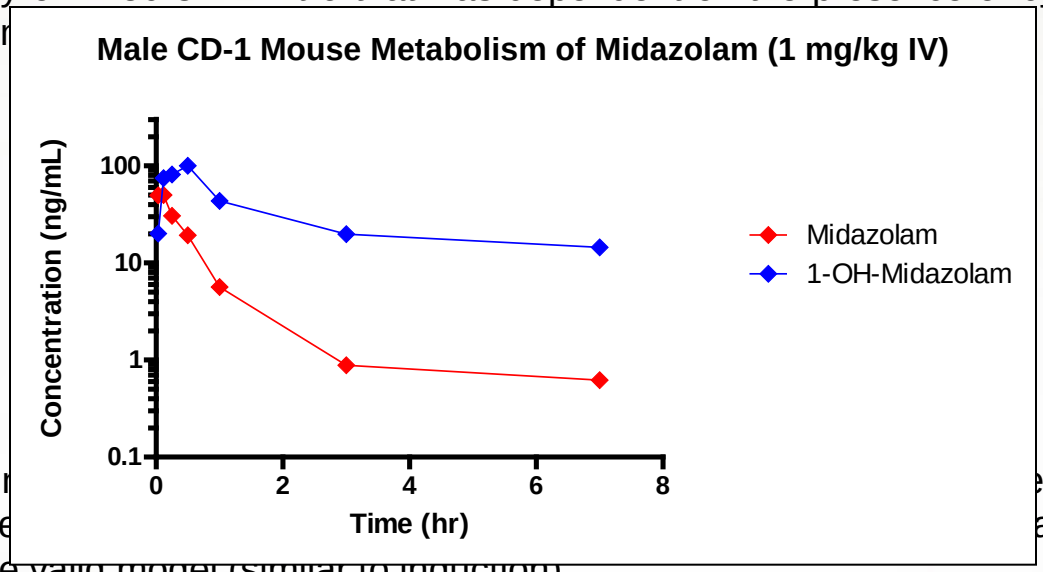
CPHP Levels in HLM



Fang, et al. (2001) DMD: 12: 1638.

Heterotropic Activation of 3A4 by VU0448187: Summary

• VU0448187 demonstrated a multi-species and substrate-dependent increase in the (midazolam) hydroxylase activity of P450 3A *in vitro* that was dependent on the presence of cyt b5, resulted in a change in V_{max} (mir



• For this compoun ere more robust in hepatocytes, sugge activation effects *in vivo* may be a more valid model (similar to induction).

PK Parameter	1	2
CLp	mL/min/kg	62.9	40.8

• Multi-species paradigms exist for modeling heterotropic activation of 3A *in vivo*. In male SD rats, VU0448187 showed robust activation of MDZ hydroxylase activity that resulted in an increase in both 1-OH and 4-OH-MDZ metabolites.

• Preliminary pharmacokinetic studies in mice indicate a potential *in vivo* model to monitor changes in MDZ clearance, as well as metabolite formation in the presence of VU0448187.

The Path Forward – Future Studies

- **Future *in vitro* studies with focus on:**
  - Alternative substrates; clinical relevance (i.e. Haloperidol)
  - Collaboration goals: studies with mutants, modeling, crystallography
- **Future *in vivo* studies in rat will focus on:**
  - The ability of ketoconazole to block the effects of VU0448187 on MDZ metabolism
  - The substrate-dependence of 3A activation by VU0448187 (i.e. no observed increase in OH-testosterone levels)
  - The scaffold dependence of 3A activation (allosteric modulators from a different structural class do not show the same effect *in vivo*)
- **Future *in vivo* studies in mice with focus on:**
  - Determining the best dosing route and time course for observing heterotropic activation of 3A midazolam hydroxylase activity by VU0448187
  - Follow up studies in humanized mice

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