

Deuteration of Drugs for Pharmacokinetic Enhancement: Considerations Essential for Success



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Collaborators:

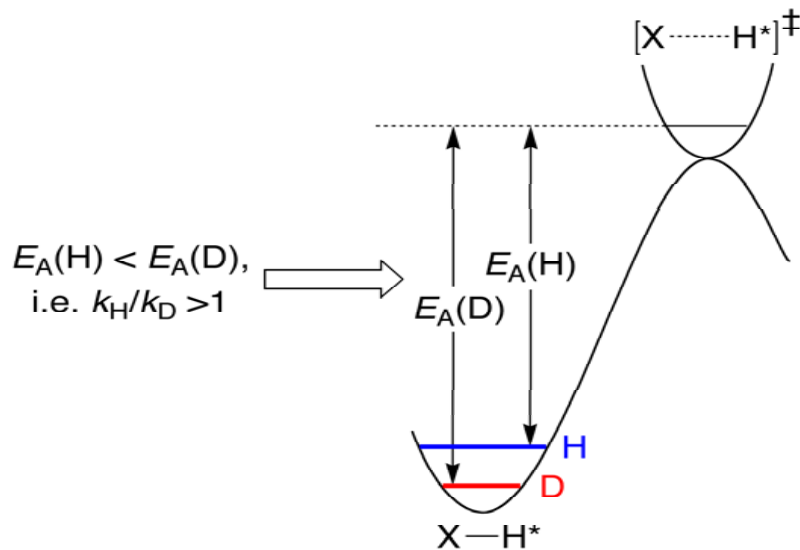
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Uses for Deuterated Drugs

- As an internal standard in quantitative analysis
 - Three or more non-exchangeable deuterium atoms incorporated to mass-differentiate the internal standard and analyte
- Alter pharmacokinetic or toxic properties
 - Deuterium substitution for hydrogen does not appreciably change physicochemical properties such as polar surface area, molecular volume, hydrogen bonding, is naturally abundant to 0.09%, and is NOT radioactive.
 - One or more deuterium atoms substituted at specific sites can slow metabolic clearance and result in:
 - Increases in C_{\max} , drug exposure (AUC), and systemic half-life ($T_{1/2}$)
 - Decreased metabolically generated toxic metabolites

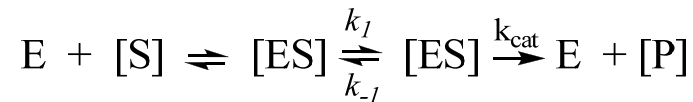
Origin of the Kinetic Deuterium Isotope Effect (KDIE)

- Difference in mass between deuterium and hydrogen results in a zero-point energy difference between C-H and C-D bonds, consequently an increase in energy required to break a C-D bond



If the transition state involves a symmetrical breaking of a C-H bond, substitution of hydrogen by deuterium slows down the reaction rate by a factor of 5 – 9

$$\text{KDIE} = k_{\text{H}}/k_{\text{D}} \sim 5 - 9$$



$$\text{Enzyme intrinsic isotope effect} = {}^{\text{D}}k_1/{}^{\text{H}}k_1$$

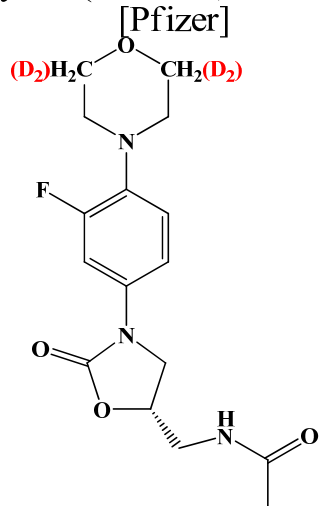
The ‘intrinsic isotope effect’ reflects the enzyme’s “commitment to catalysis”

In pharmacokinetics the ‘intrinsic clearance isotope effect’ reflects the kinetic isotope effect on the first order rate constant for disappearance of substrate or V_{max}/K_m

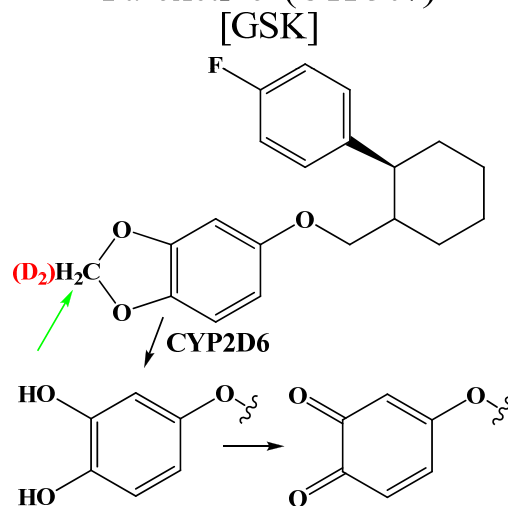
Press releases by Concert Pharmaceuticals

- 43% increase in preclinical (monkey) half life (4.5 to 6.3 hrs) for deuterated Linezolid Oct 27th 2008
- Phase 1 start for deuterated Paroxetine Sept. 25th, 2008; announced protection against CYP2D6 inactivation Sept. 29th, 2009
- Phase 1b multiple dose escalation study for deuterated Atazanavir Nov 9th, 2009
Expectation: QD dosing without Ritonavir co-administration

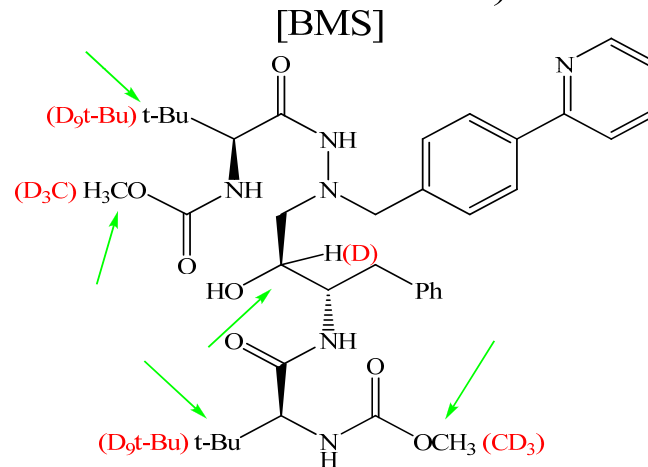
Zyvox (linezolid; C-20081)



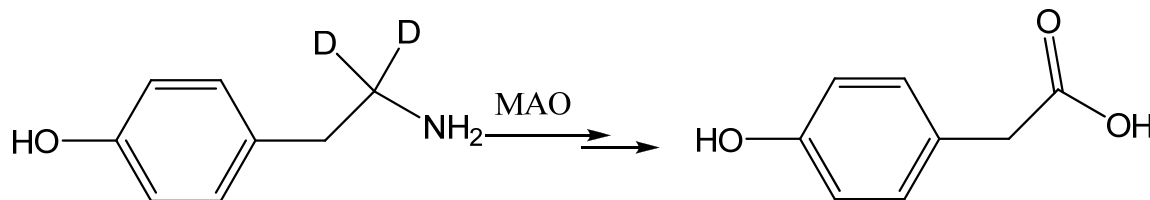
Paroxetine (CTP307)



Atazanavir CTP-518



Effect of deuterium substitution on sympathomimetic amines on adrenergic responses.
Belleau, B.; Burba, J.; Pindell, M.; Reiffenstein, J. *Science* (1961), 133 102-4.



Systemic Clearance Mechanism and KDIEs on Pharmacokinetics

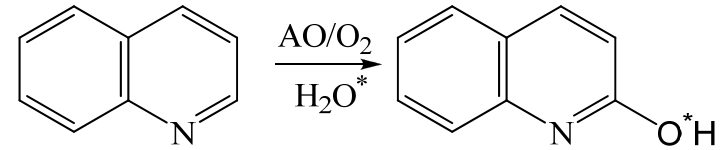
$$CL_{\text{systemic}} = CL_H + CL_{\text{extrahepatic}} + CL_{\text{urine}} + CL_{\text{other}}$$

$$CL_H = CL_{\text{metabolic}} + CL_{\text{bile}}$$

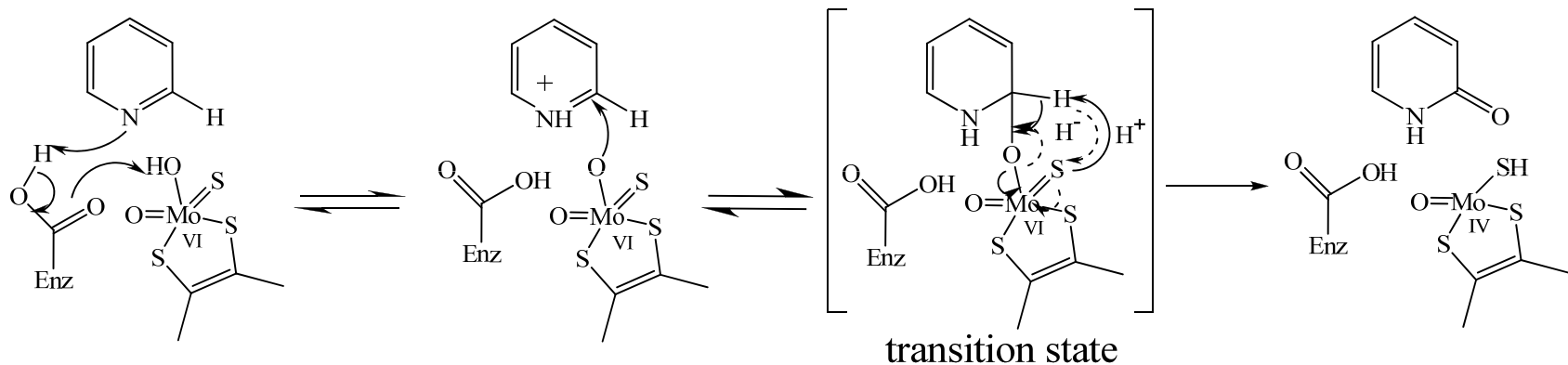
- Clearance enzymes where KDIEs apply
 - Aldehyde Oxidase 4 – 6
 - Monoamine Oxidases 2 – 9
 - Cytochromes P450 1 – 9
 - Alcohol/aldehyde dehydrogenases 6 – 8
- Clearance enzymes where KDIEs do not apply
 - Flavin monooxygenases
 - Glucuronyl transferases
 - Sulfotransferases
 - Glutathione-S-transferases
 - N-acetyl transferases
- For pharmacokinetic enhancement:
 - Metabolic clearance must determine systemic clearance
 - The enzyme(s) involved must have KDIEs on their intrinsic clearance

Why KDIEs with aldehyde oxidase?

Aldehyde Oxidase (AO) is a cytosolic molybdopterin class oxidase, hydroxylates nitrogen heterocycles α - to the nitrogen



- Experience with human PK failure due to high clearance by AO
- Interspecies variability that results in failure of allometric scaling
- Lack of direct *in vitro* to *in vivo* correlation in clearance scaling possibly due to wide tissue distribution
- Proposed reaction mechanism involves a rate-limiting hydride/proton abstraction

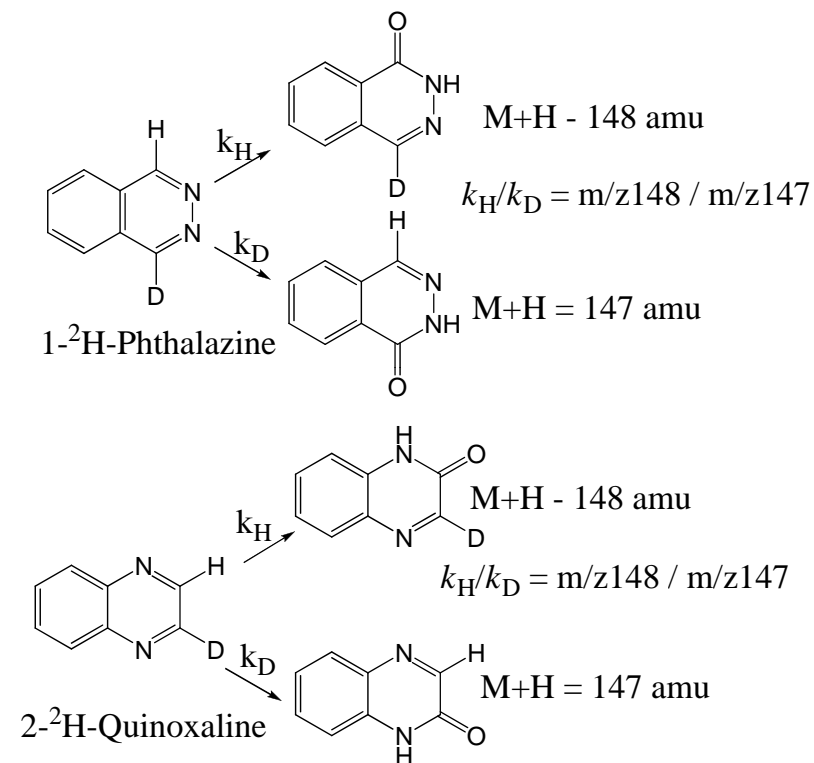
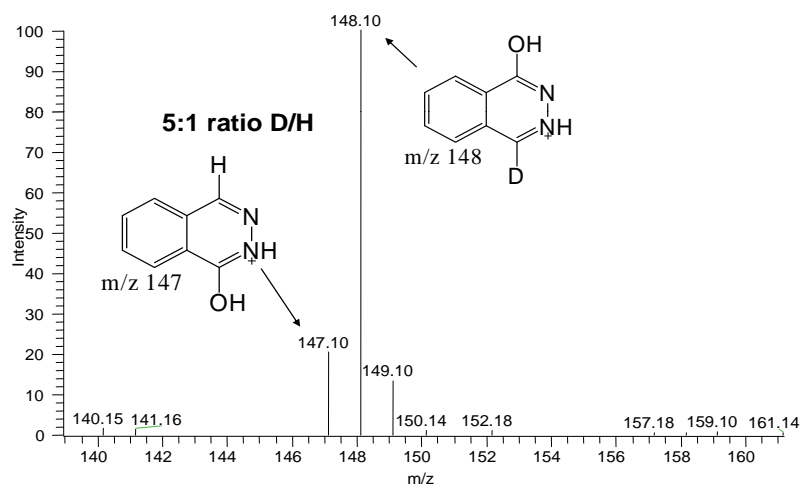


Potential to alter PK by use of KDIE

KDIEs to establish interspecies commonality in the AO reaction mechanism

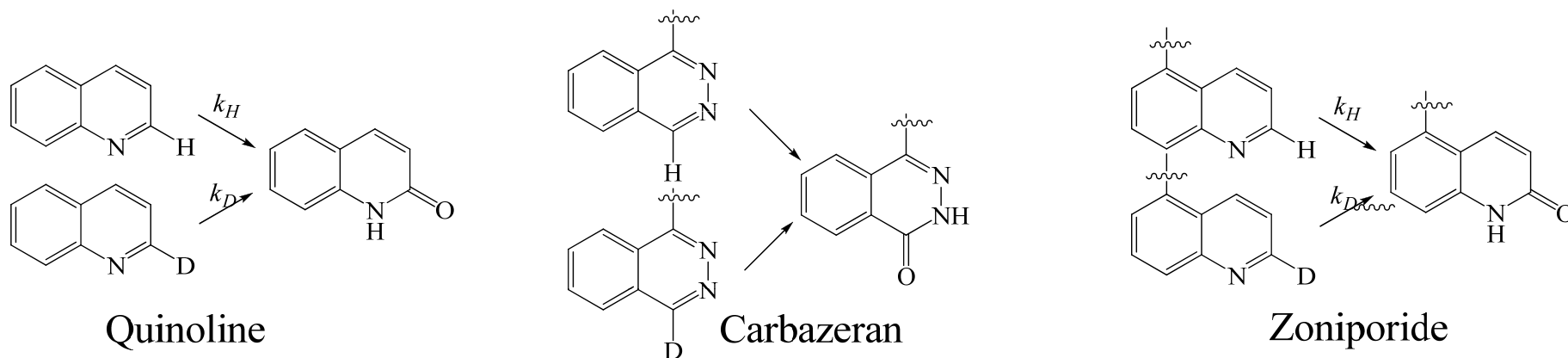
Intra-molecular isotope effect (intrinsic)

Spectra of hydroxylated metabolite of phthalazine after 30 minute incubation in guinea pig/ human cytosol



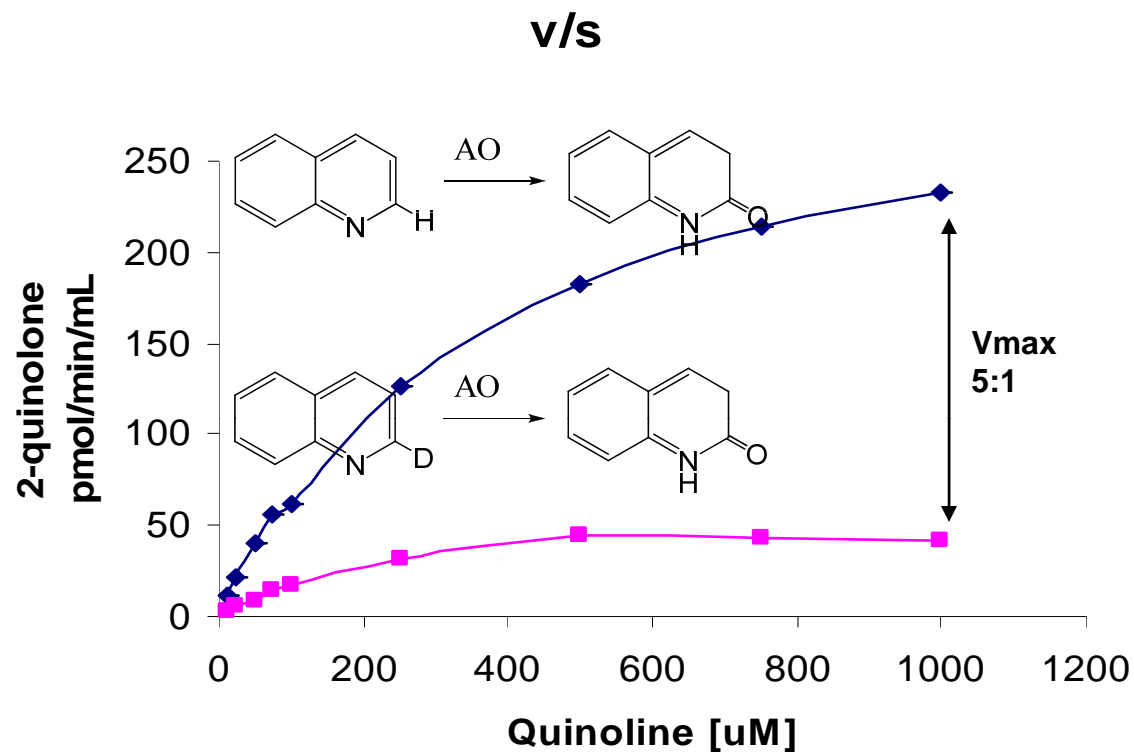
Substrate	$^Hk / ^Dk$ with cytosolic AO from		
	Guinea pig	Rat	human
2- ² H-Quinoxaline	4.7	5.1	5.0
2- ² H-Phthalazine	4.9	5.0	5.1

Inter-molecular KDIE on competitive first order elimination rate constants



Substrate	k_H / k_D with cytosolic AO from		
	human	rat	guinea pig
Quinoline	5.5	6.1	6.0
Carbazeran	4.8	5.0	6.0
Zoniporide	5.8	3.6	4.8

Inter-molecular KDIE on steady state kinetic constants



	Quinoline	2- ² H-Quinoline
K_m (mM)	212	193
V_{max} (pmol/min)	246	47
V_{max} / K_m	1.2	0.2

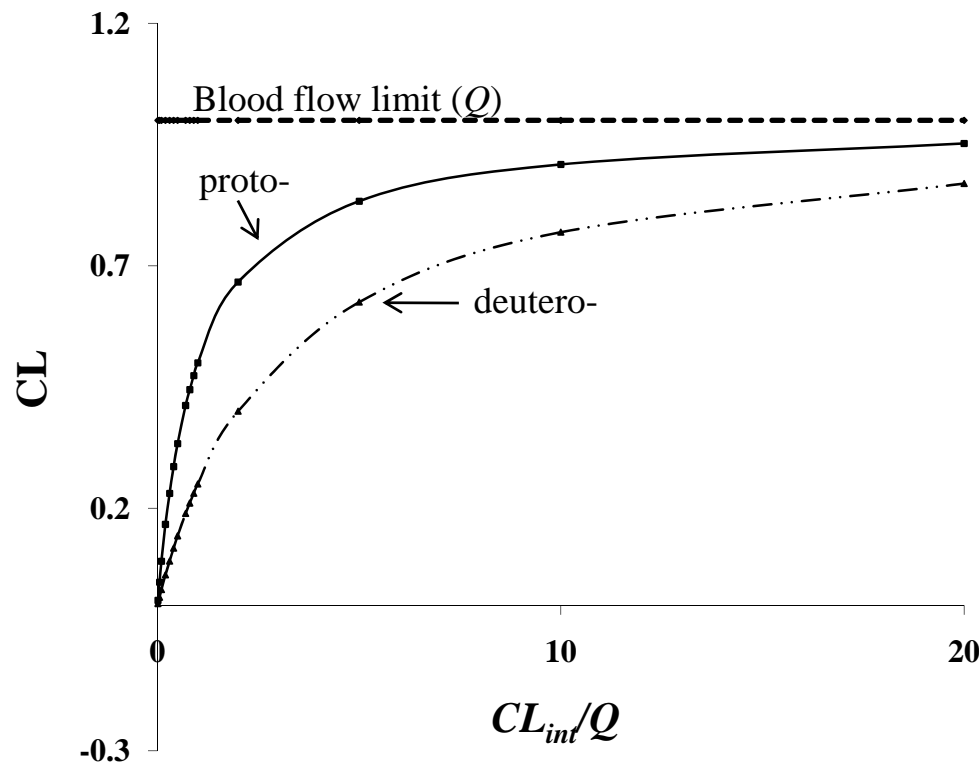
KDIE on $Cl_{int} = 6.0$

Conclusions from *in-vitro* KDIEs for AO-catalyzed reactions

- Across species the rate-limiting step in AO-catalyzed reactions is proton/hydride abstraction
- The KDIE for AO is fully expressed on the intrinsic clearance (V_{\max}/K_m)
- If systemic clearance of a drug is metabolically driven by AO, pharmacokinetics could be altered

Theoretical relationship between clearance (CL) and intrinsic clearance (CL_{int}) for a KDIE of 7.0

$$CL = \frac{Q \times CL_{int}}{Q + CL_{int}}$$



If $CL_{int} \gg Q$; $CL \cong Q$

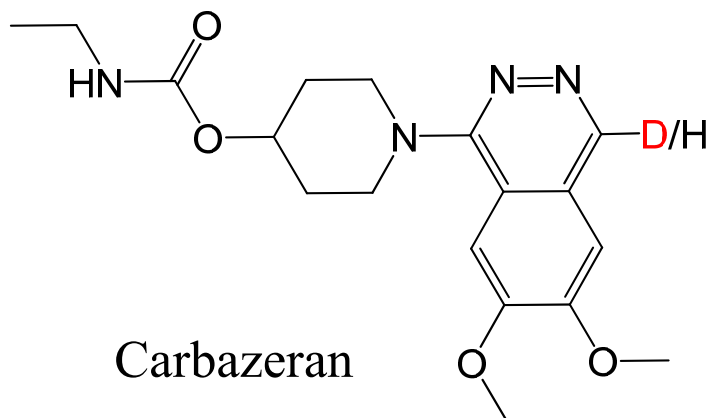
➤ Systemic half-life not expected to change for an IV or orally dosed drug.

➤ AUC and C_{max} may reflect the KDIE on the extra-hepatic contributions to overall clearance

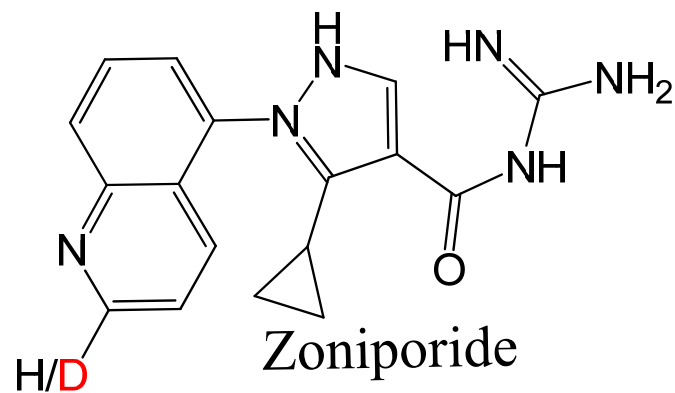
If $CL_{int} \ll Q$; $CL \cong CL_{int}$

➤ Systemic half-life, AUC and C_{max} may reflect the KDIE on the CL_{int}

Pfizer drugs examined *in-vitro* and *in-vivo*

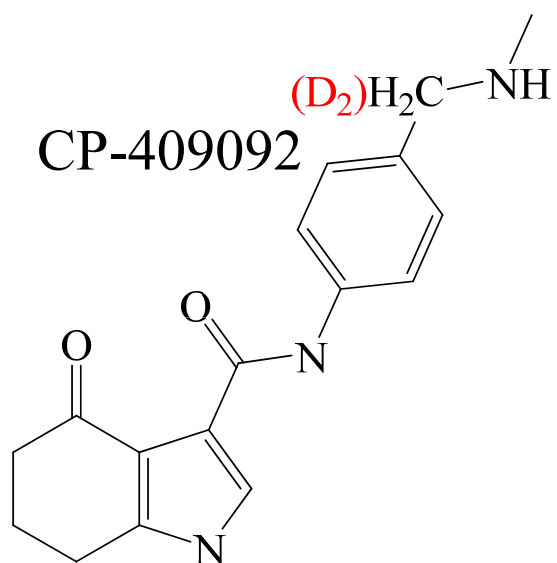


Carbazeran

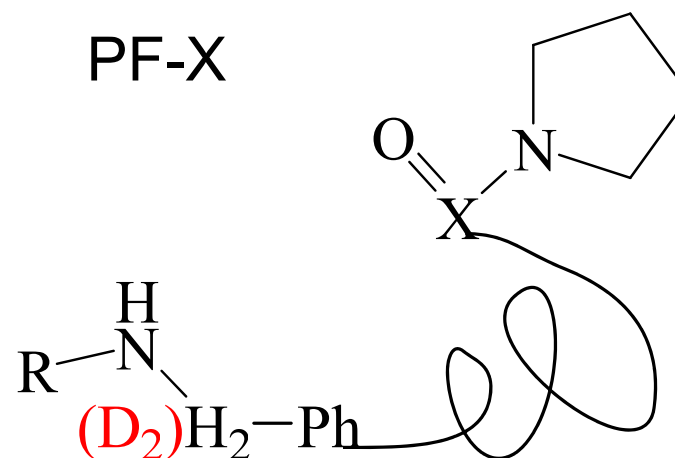


Zoniporide

Aldehyde Oxidase component to metabolism



CP-409092



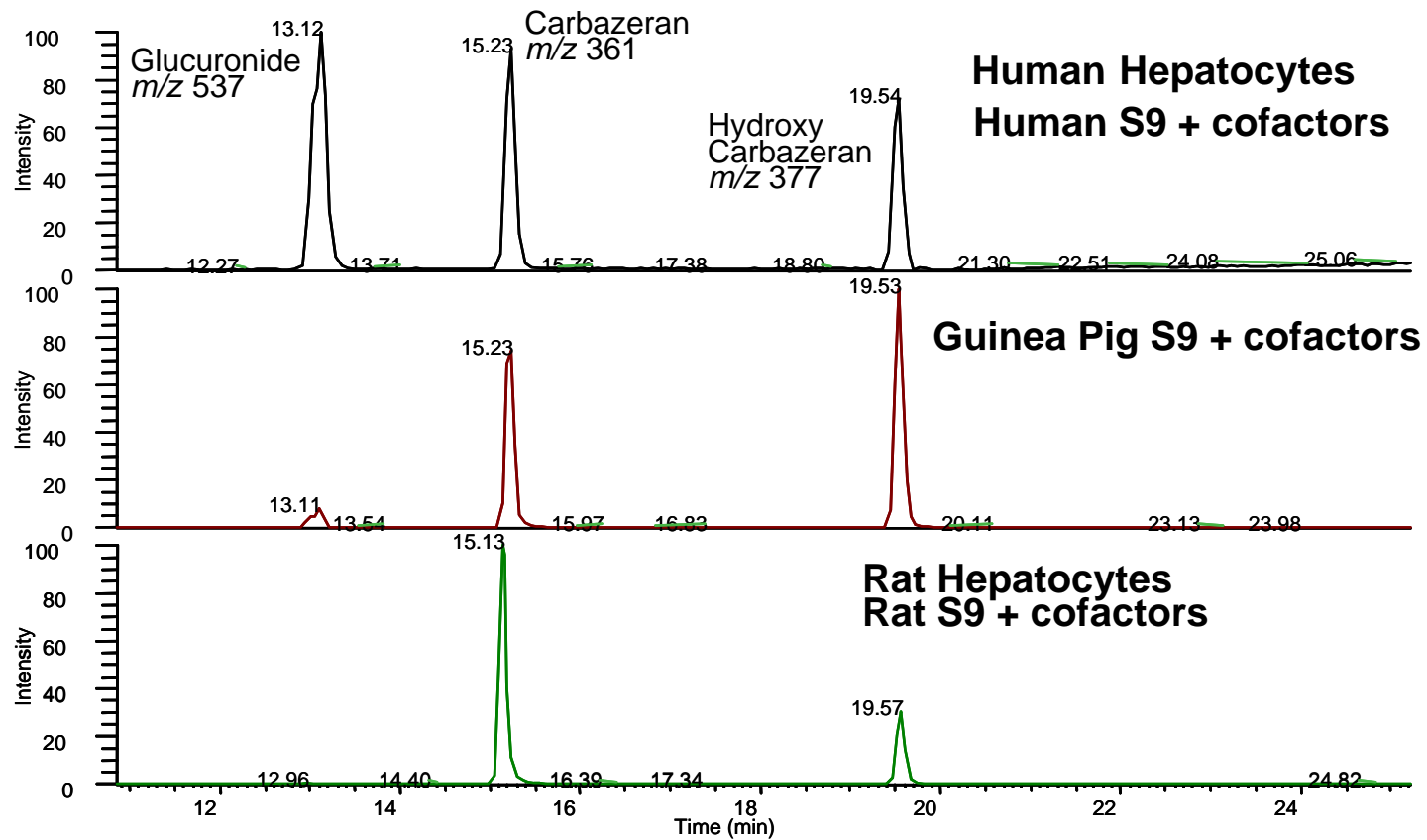
PF-X

Monoamine Oxidase component to metabolism

Intrinsic clearance KDIE for Carbazeran

Substrate	Human		Rat		Guinea Pig	
	Cytosol	Hepatocytes	Cytosol	Hepatocytes	Cytosol	S-9
Carbazeran	4.8	1.5	5.0	4.6	6.0	5.0

Metabolite profile for carbazeran



Prediction of Carbazeran pharmacokinetic outcome from *in-vitro* assays

- In human:

- No PK enhancement

- In guinea pig and rat:

- No effect on systemic half-life (blood flow limited clearance)

- Possible increases in C_{max} and AUC due to KDIE on intrinsic clearance (extrahepatic AO contribution)

KDIE on PK parameters for Carbazeran

IV-dosed

(Guinea pigs) KDIE (D/H)		
	AUC	T1/2
Mean	4.6	0.8
Std. Dev	0.7	0.1

(Rats) KDIE (D/H)		
	AUC	T1/2
Mean	2.0	1.1
Std. Dev	0.5	0.2

Orally-dosed

(Guinea pigs) KDIE (D/H)			
	AUC	T1/2	Cmax
Mean	21.9	0.5	22.5
Std. Dev	2.6	0.1	7.7

(Rats) KDIE (D/H)			
	AUC	T1/2	Cmax
Mean	2.3	1.27	1.5
Std. Dev.	0.2	0.1	0.0

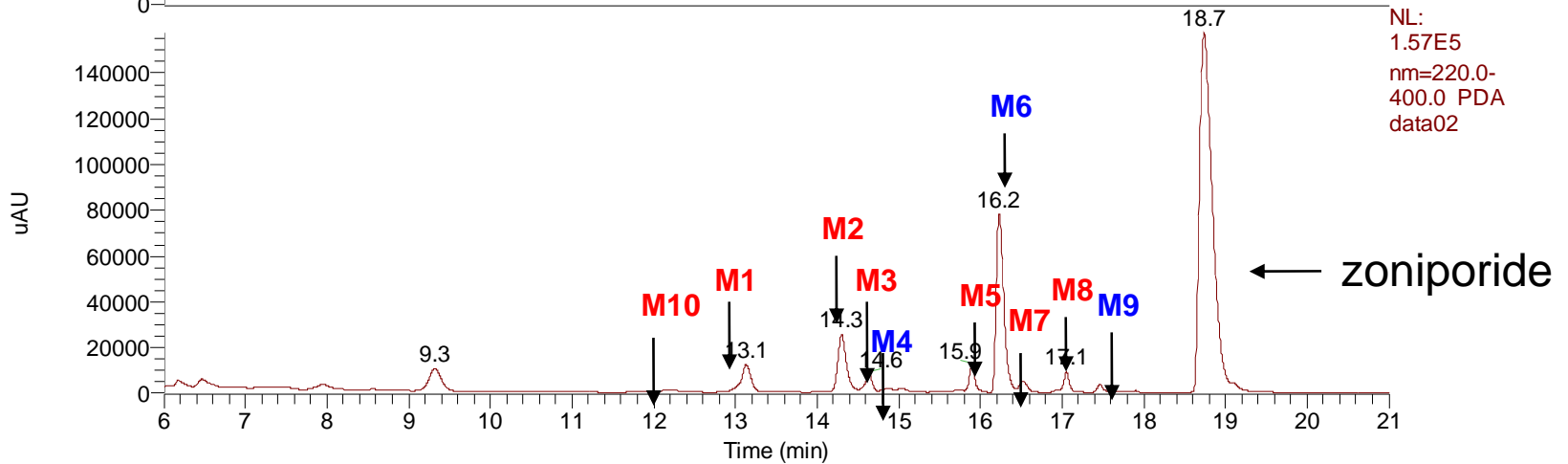
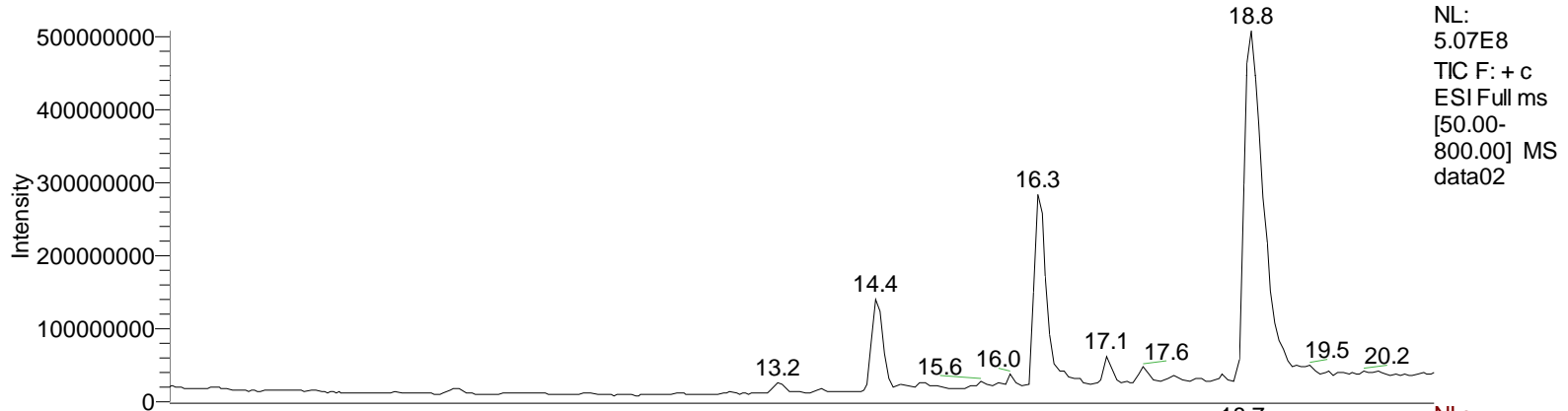
➤ Despite a common metabolic pathway, the guinea pig and rat differ in the outcome of KDIEs on the pharmacokinetic parameters, suggesting a species difference in their systemic clearance mechanisms

Intrinsic clearance KDIE for Zoniporide

Substrate	Human		Rat		Guinea Pig	
	Cytosol	Hepatocytes	Cytosol	Hepatocytes	Cytosol	S-9 supplemented
Zoniporide	5.8	1.9	3.6	2.7	4.8	1.5

Metabolite profile for Zoniporide in rat hepatocytes

RT: 6.0 - 21.0



KDIE on PK parameters for Zoniporide

IV-dosed

(Guinea pig) KDIE (D/H)			
	AUC	T1/2	
Mean	1.1	1.2	
Std. Dev	0.5	0.3	

(Rat) KDIE (D/H)			
	AUC	T1/2	
Mean	0.9	1.4	
Std. Dev	0.1	0.3	

Orally-dosed

(Guinea pig) KDIE (D/H)			
	AUC	T1/2	Cmax
Mean	1.13	1.2	0.9
Std. Dev	0.1	0.2	0.1

(Rat) KDIE (D/H)			
	AUC	T1/2	Cmax
Mean	0.6	1.12	0.7
Std. Dev	0.2	0.0	0.1

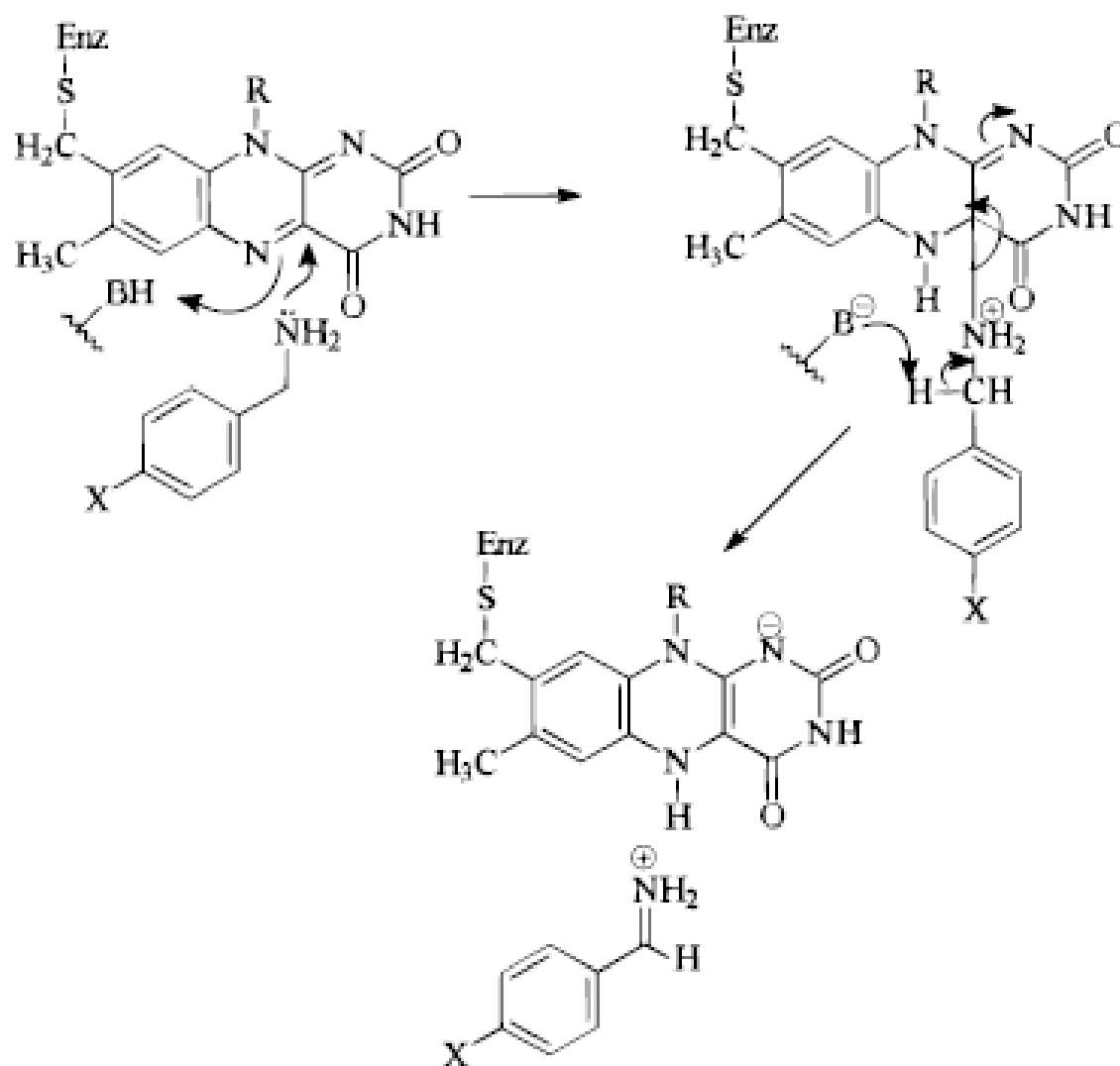
As predicted from *in-vitro* hepatocytes experiments, essentially no effect is observed on the pharmacokinetics of Zoniporide in guinea pig and rat

KDIEs on the steady-state kinetic parameters for oxidation of para-substituted phenethylamines by Monoamine Oxidase -A

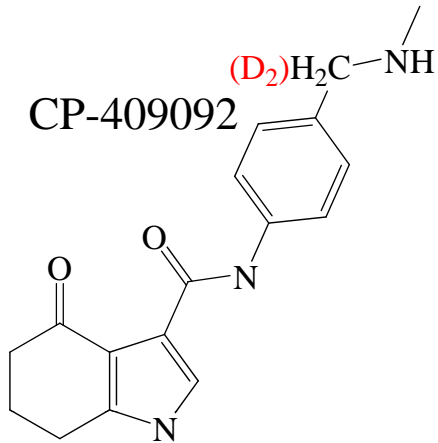
substituent	proteo		deutero		isotope effects	
	<i>k</i> _{cat}	<i>K</i> _m (<i>min</i> -1)	<i>k</i> _{cat} (μ M)	<i>K</i> _m (<i>min</i> -1)	^D <i>k</i> _{cat} (μ M)	^D (<i>k</i> _{cat} / <i>K</i> _m) ^D <i>CL</i> _{int}
H	64	1250	7.55	1410	8.5	9.6
OH	83.7	423	35.8	633	2.3	3.5
CF ₃	4.5	755	0.71	1190	6.3	9.9
F	111.5	1060	21.1	2050	5.3	10.2
Cl	26.6	320	3.45	380	7.7	9.2
Br	17.1	226	2.06	260	8.3	9.6
Me	18.6	150	2.05	152	9.1	9.2
NO ₂	200	4820	38.3	8270	5.2	9.0

Taken from: Nandigama and Edmondson, *Biochemistry* (2000), 39(49), 15258-15265

Proposed Mechanism of MAO-catalyzed deaminations

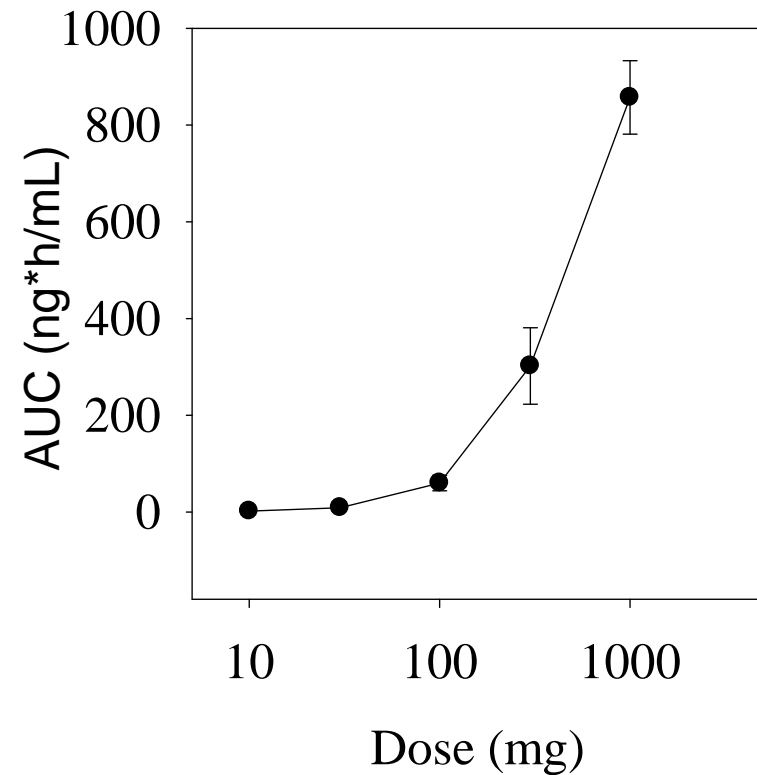
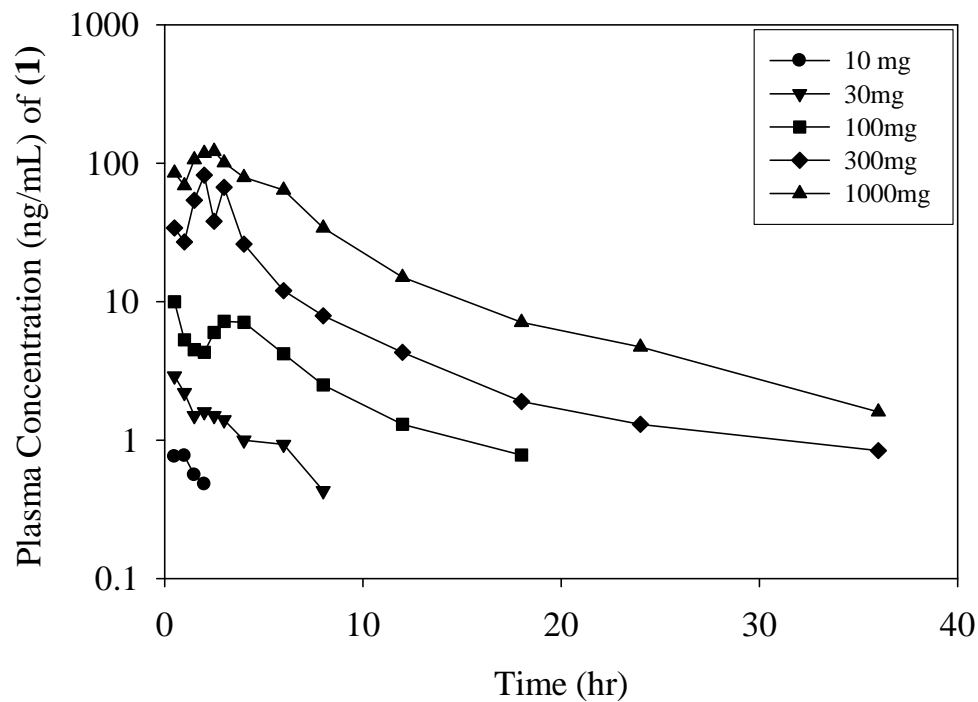


Properties and clinical results for CP409092



Known attributes:

- MAO-A substrate
- Un-desirable clinical PK
- characterized by $Cl_p/F > 200 \text{ mL/min/kg}$;
- Plasma elimination $t_{1/2} : \sim 8.5 \text{ h}$
- Large variability in PK
- Non-linear PK



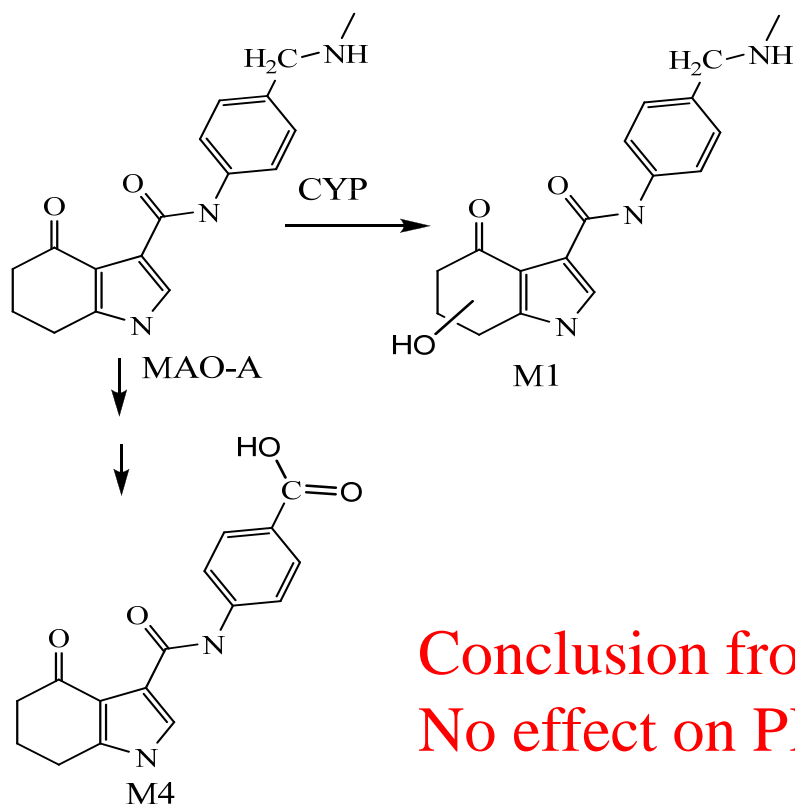
Low contribution of MAO to CP-409092 clearance in the rat

Intrinsic clearance KDIE for CP409092

System	H_k/D_k
Microsomes - NADPH	2.5
Microsomes + NADPH	1.1
Hepatocytes	1.1

Radiolabel Disposition of CP-409092 in Rat

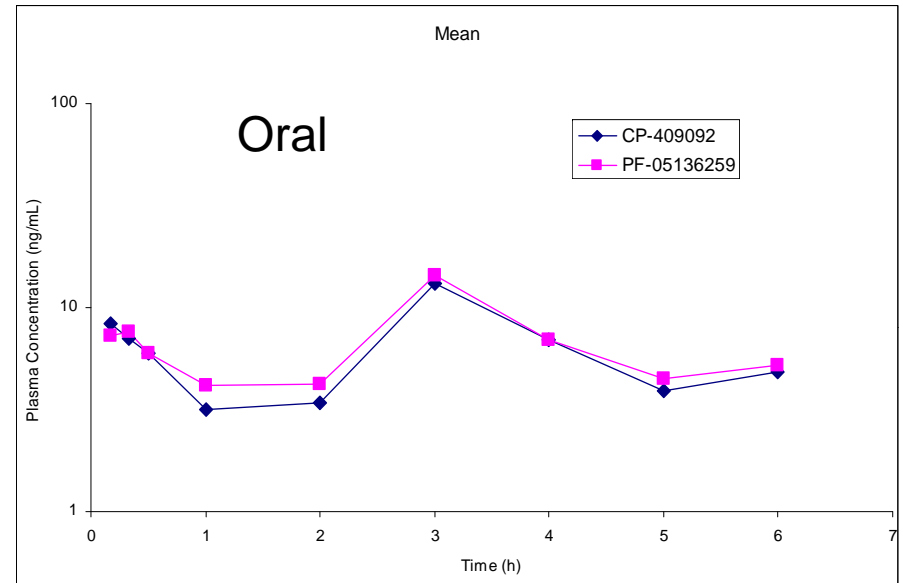
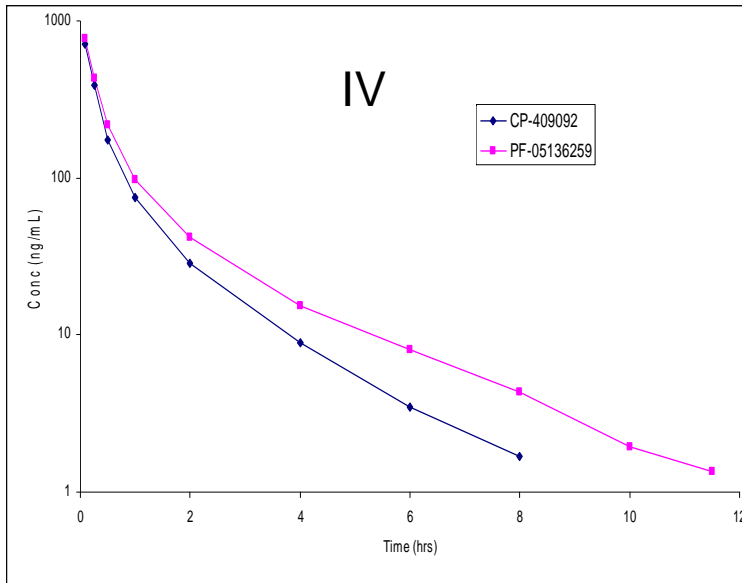
Metabolites	Urine (U)	Feces (F)	U+F
M1A	0.06	ND	0.06
M1	0.74	6.47	7.21
M1B	0.04	0.62	0.66
M2	0.88	3.5	4.39
M2A	0.08	ND	0.08
M4 (MAO-pathway)	0.62	3.24	3.86
M5	0.02	0.22	0.24
(unchanged drug)	1.01	72.4	73.41
Total	3.45	66.5	89.9



Conclusion from in-vitro KDIE
No effect on PK

Solubility: > 5 mg/mL;
 Caco₂: Papp AB : 1.2x
 10⁻⁶; Papp: **BA/AB: 7**

IV and oral pharmacokinetic isotope effect for CP-409092 in the rat



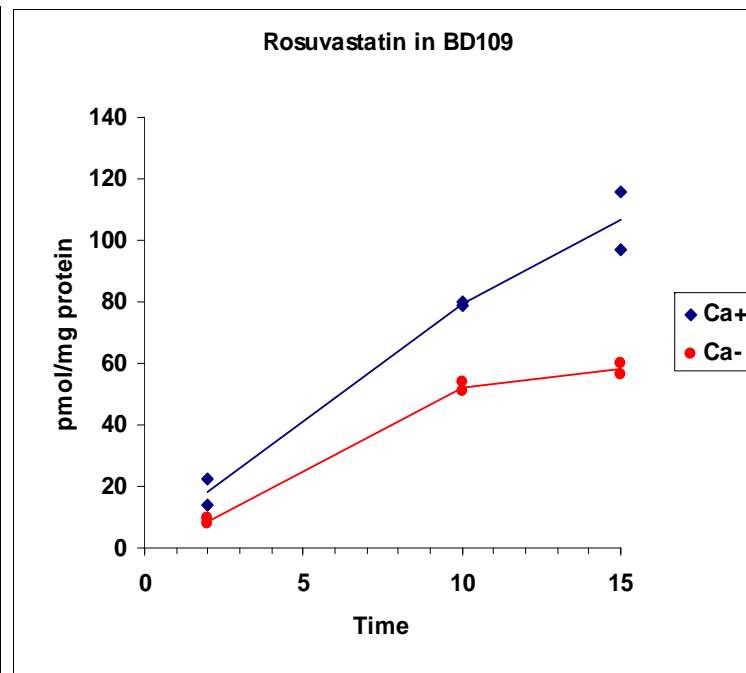
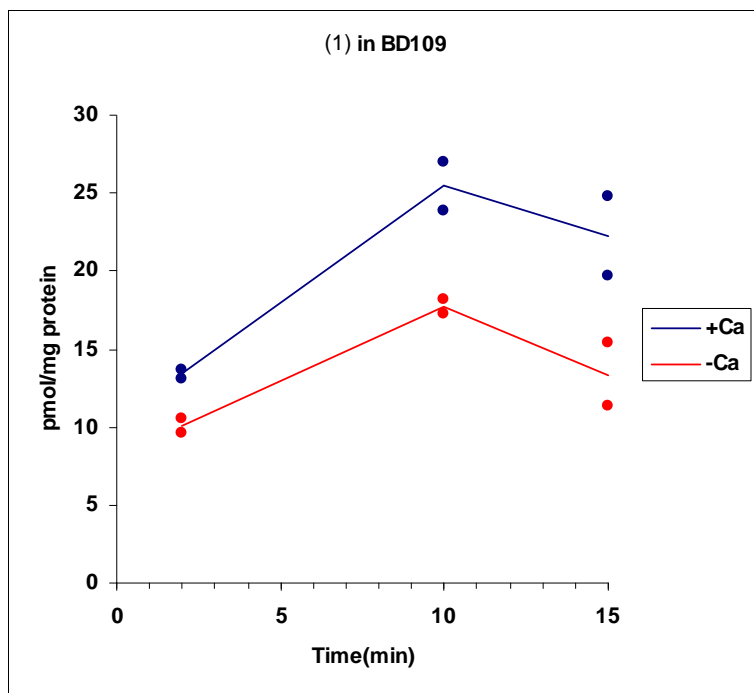
	KDIE (IV)	
Rat#	AUC	T1/2
Mean	1.2	1.2
Std. Dev.	0.1	0.1

KDIE in human in vitro systems for CP409092

System	H_k/D_k
	Human
Microsomes - NADPH	4.3
Microsomes + NADPH	3.8
Hepatocytes	5.7
rMAO-A	3.8

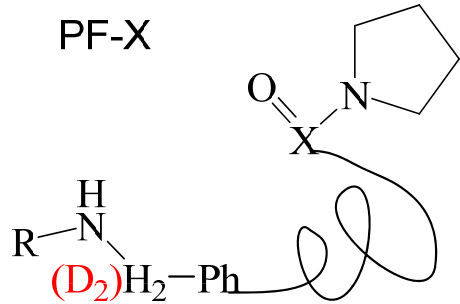
- Large KDIE in human in vitro systems suggest possible PK enhancement
- The presence of other clearance routes (biliary/absorption) may not favor overall enhancement of pharmacokinetic parameters

Sandwich cultured hepatocyte biliary excretion model



	Uptake, app (pmol/min/ mg)	Cl_b (uL/min/mg protein)	BEI (%)
Rosuvastatin¹	7.7	2.7	34
CP-409092	1.5	0.77	30

Studies with PF-X

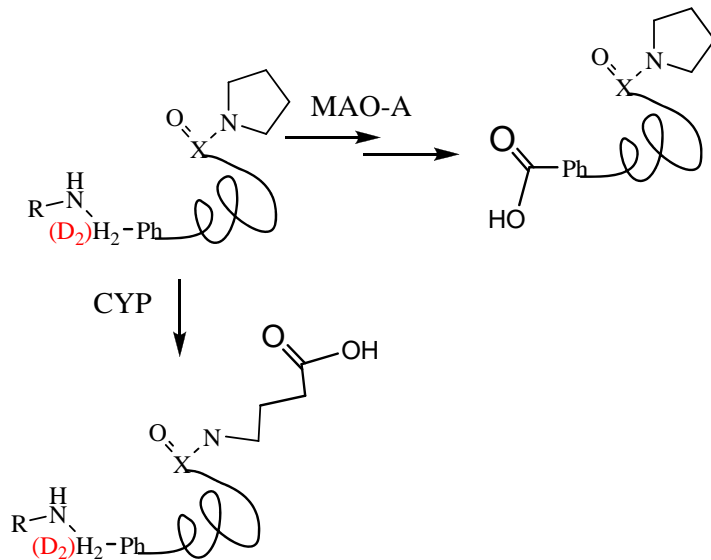


In Development
5X higher clearance at FIH than predicted
MAO contributes to metabolism

KDIE in rat and human in vitro systems

System	H_k/D_k
rMAO-A	2.8
RLM + NADPH	1.09
Rat hepatocytes	1.06
Human hepatocytes	1.4

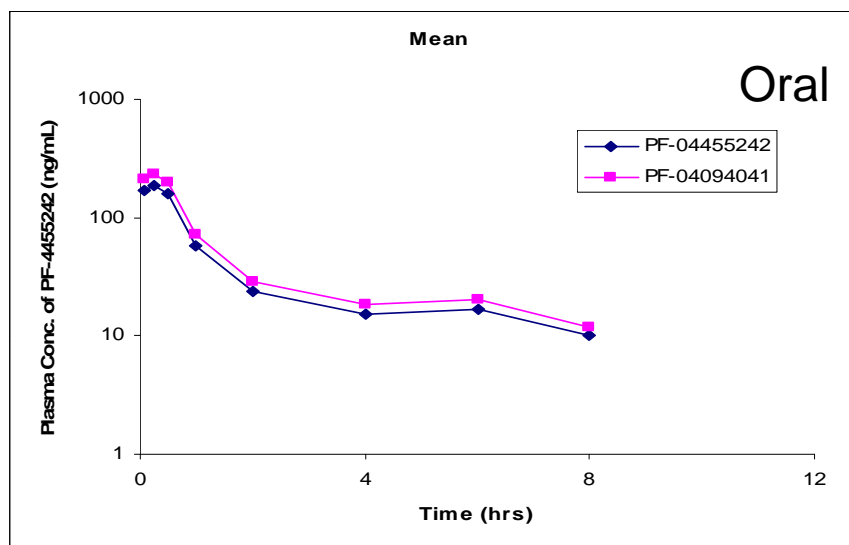
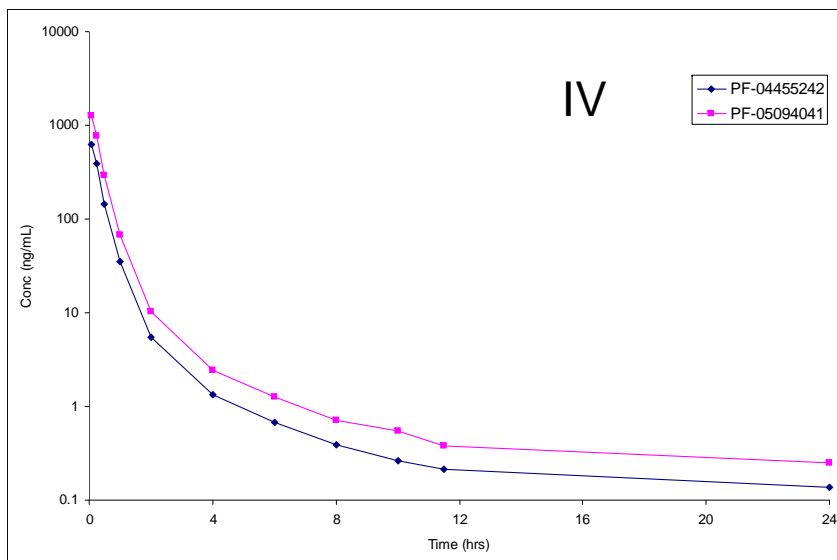
Known metabolic pathways



Conclusion:

No KDIE on the intrinsic clearance in rat hepatocytes and a small KDIE in human hepatocytes suggests deuteration will not enhance pharmacokinetics in either rat or humans

Rat IV and oral PK profiles for PF-X



Essentially no KDIE on IV or oral PK parameters for PF-X

Considerations for deuteration as a PK enhancement strategy

- The identity of enzymes involved in the metabolic clearance
- Knowledge of their reaction mechanisms
- The extent of their contribution to the overall metabolic clearance
- Magnitude of the intrinsic clearance isotope effect in hepatocytes (or equivalent) for multiple species
- Knowledge of other non-metabolic clearance mechanisms and the extent of their contribution to systemic clearance