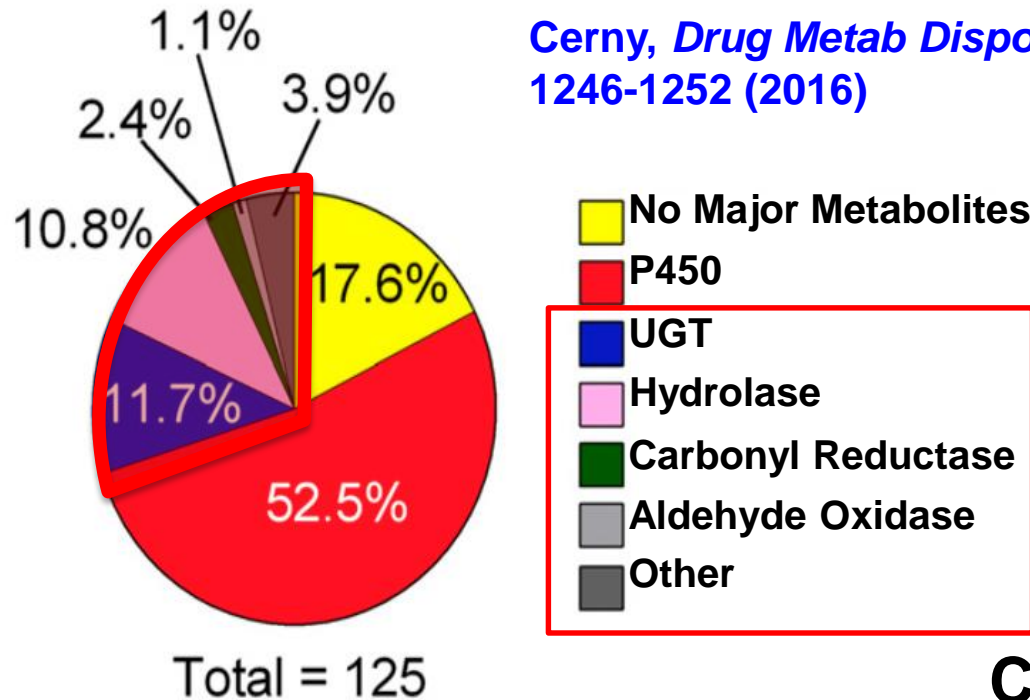


Elucidation of Novel Functions of Non-CYP Enzymes to Promote Understanding of Drug Toxicity and Pharmacokinetics

**医薬品毒性および動態理解を目指したnon-CYP
薬物代謝酵素の新規機能解明**

Tatsuki Fukami
Drug Metabolism and Toxicity
Kanazawa University

Contribution of Enzymes to Drug Metabolism



New chemical entities that are not metabolized by P450 are recently desired.



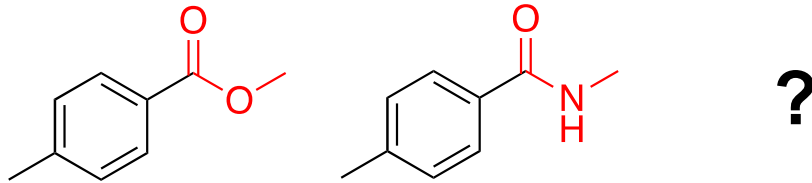
Contribution of non-P450 to drug metabolism tends to increase.



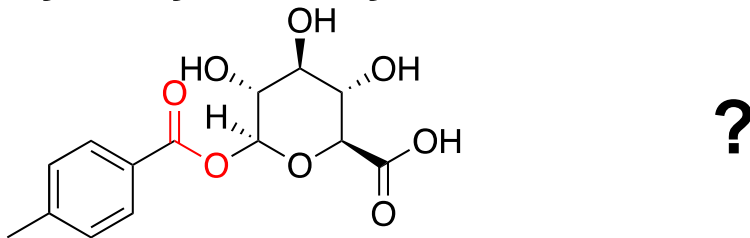
Non-P450 enzymes are receiving attention.

Unknown Non-CYP Enzymes

Many drugs containing ester or amide bonds are hydrolyzed by CES. However, some drugs were likely to be hydrolyzed by **enzyme(s)** other than CES in human liver.



Acyl-glucuronides possess ester bond. Because acyl-glucuronides are suggested to cause several toxicities, the **enzyme(s)** catalyzing their hydrolysis may attenuate their toxicities.

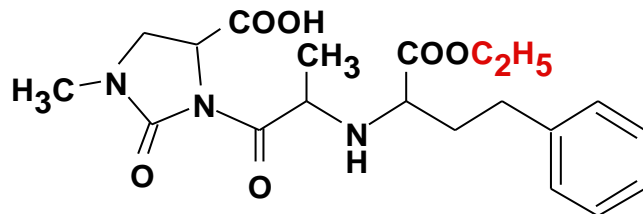


Some drugs containing nitro groups are metabolized to amino metabolites, but the responsible **enzyme(s)** had been unclear.

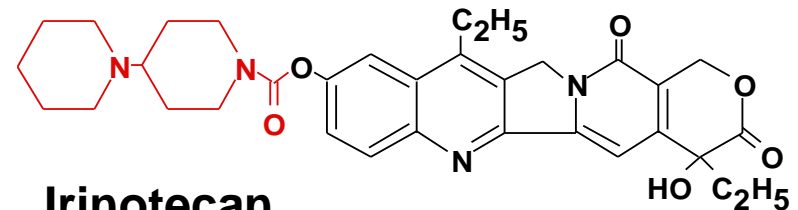


Hydrolase Activities of Various Drugs by Purified CES1 and CES2

Substrates	CES1			CES2		
	Km	Vmax	Vmax/Km	Km	Vmax	Vmax/Km
Ester-type drugs						
Camostat mesilate	0.707	0.958	1.356	2.700	0.090	0.015
Dilazep	0.154	0.122	0.727	0.089	0.119	1.326
Irinotecan	1.453	0.082	0.056	0.241	0.074	0.307
ONO-5046	1.060	0.132	0.125	2.080	0.476	0.229
Banazepril	0.734	0.886	1.207	0.785	0.330	0.420
Cilazapril	1.295	2.168	1.674	1.349	0.667	0.494
Quinapril	0.134	0.184	1.373	0.122	0.034	0.279
Temocapril	0.786	4.762	6.059	0.325	0.402	1.237
Delapril	1.502	1.569	1.045	-	-	-
Imidapril	0.287	0.195	1.679	-	-	-
Alacepril	-	-	-	-	-	-
Aspirin	-	-	-	2.270	0.244	0.107
Procaine	-	-	-	3.330	0.029	0.009
Oxybutinin	-	-	-	1.124	0.358	0.319
Diltiazem	-	-	-	-	-	-
Flavoxate	-	-	-	-	-	-
Propiverine	-	-	-	-	-	-



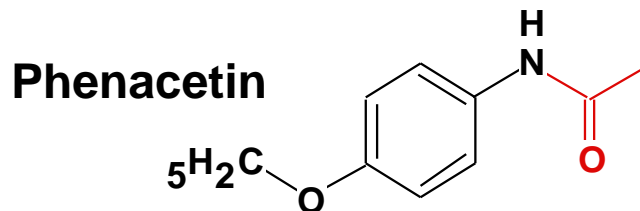
Imidapril



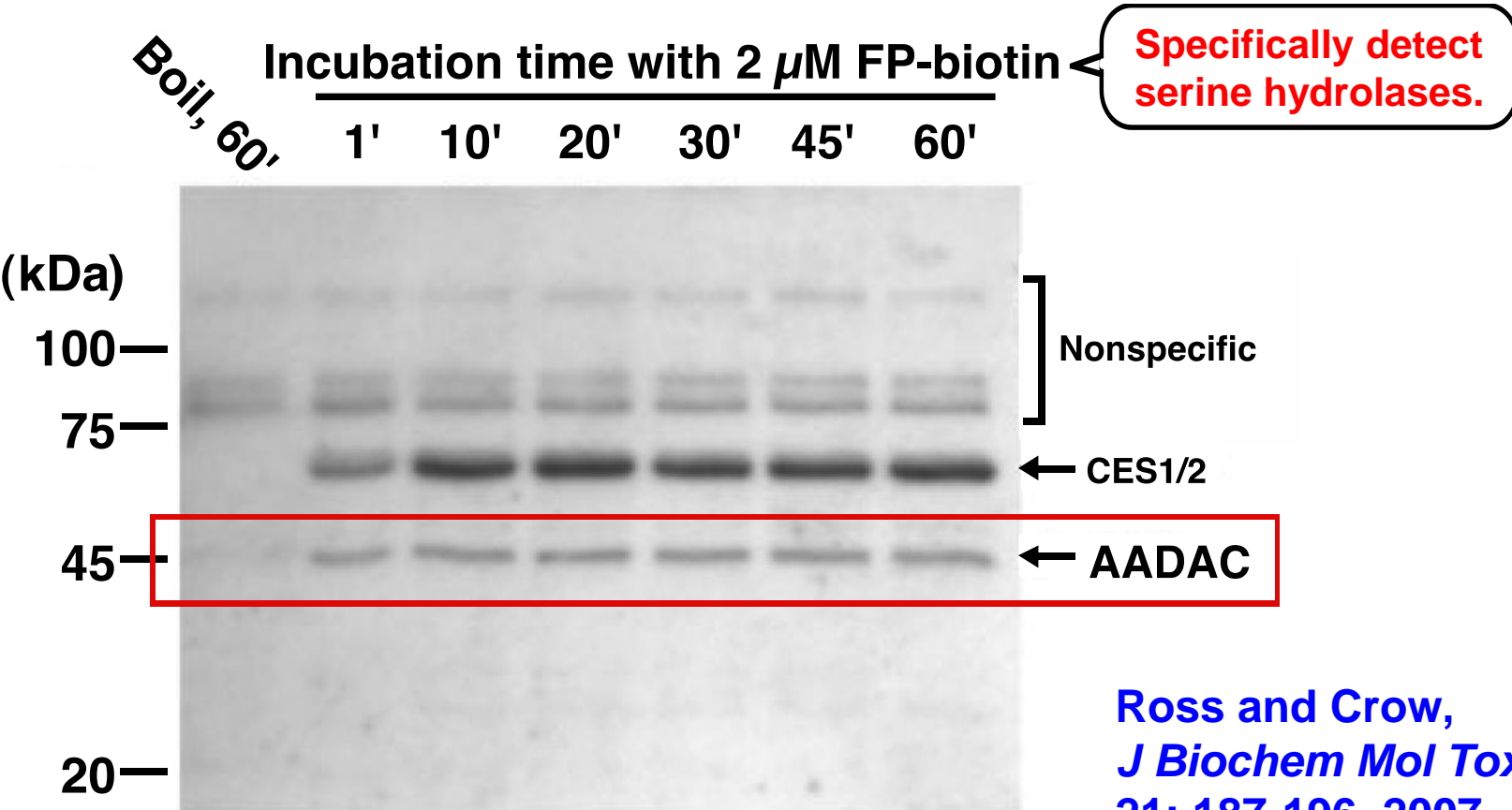
Irinotecan

Hydrolase Activities of Various Drugs by Purified CES1 and CES2

Substrates	CES1			CES2		
	Km	Vmax	Vmax/Km	Km	Vmax	Vmax/Km
Amide-type drugs						
Aniracetam	0.085	0.009	0.106	0.301	0.020	0.066
to anisic acid	0.095	0.007	0.074	0.412	0.349	0.847
to anisamidobutyric acid	-	-	-	-	-	-
Capsaicin	-	-	-	-	-	-
Captopril	-	-	-	-	-	-
Flutamide	-	-	-	-	-	-
Fominoben	-	-	-	-	-	-
Indomethacin	-	-	-	-	-	-
Lisinopril	-	-	-	-	-	-
Nefiracetam	-	-	-	-	-	-
Phenacetin	-	-	-	-	-	-
Phenobarbital	-	-	-	-	-	-
Pranlukast	-	-	-	-	-	-
Prazosin	-	-	-	-	-	-
Procainamide	-	-	-	-	-	-
Sultopride	-	-	-	-	-	-
Tiamamide	-	-	-	-	-	-
Endogenous substance Acetyl coenzyme A	-	-	-	-	-	-

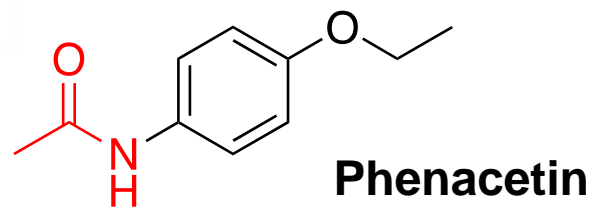
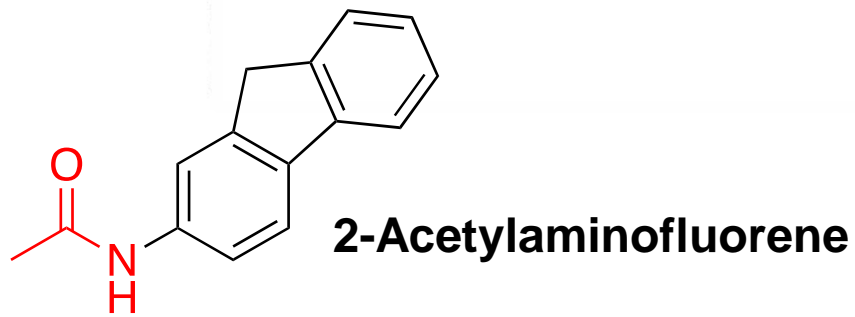


Serine Hydrolases Expressed in Human Liver Microsomes



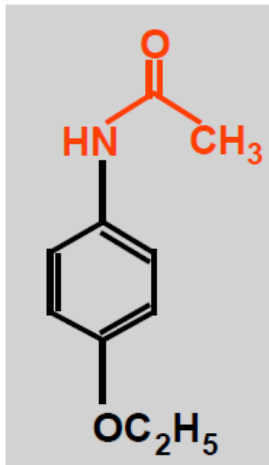
Ross and Crow,
J Biochem Mol Toxicol,
21: 187-196, 2007.

AADAC = Arylacetamide deacetylase



Metabolic Pathways of Phenacetin

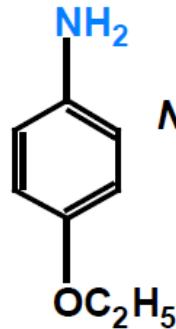
Prodrug



Phenacetin

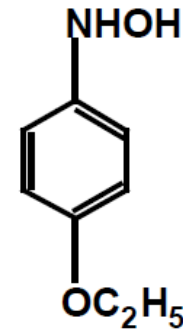
Hydrolysis

30%



p-Phenetidine

N-Hydroxylation



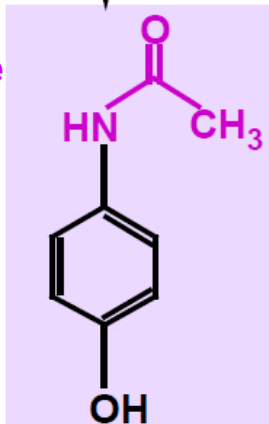
N-Hydroxyphenetidine



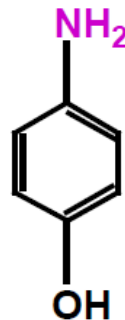
70%

CYP1A2

Active metabolite

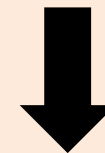


Acetaminophen (APAP)



p-Aminophenol

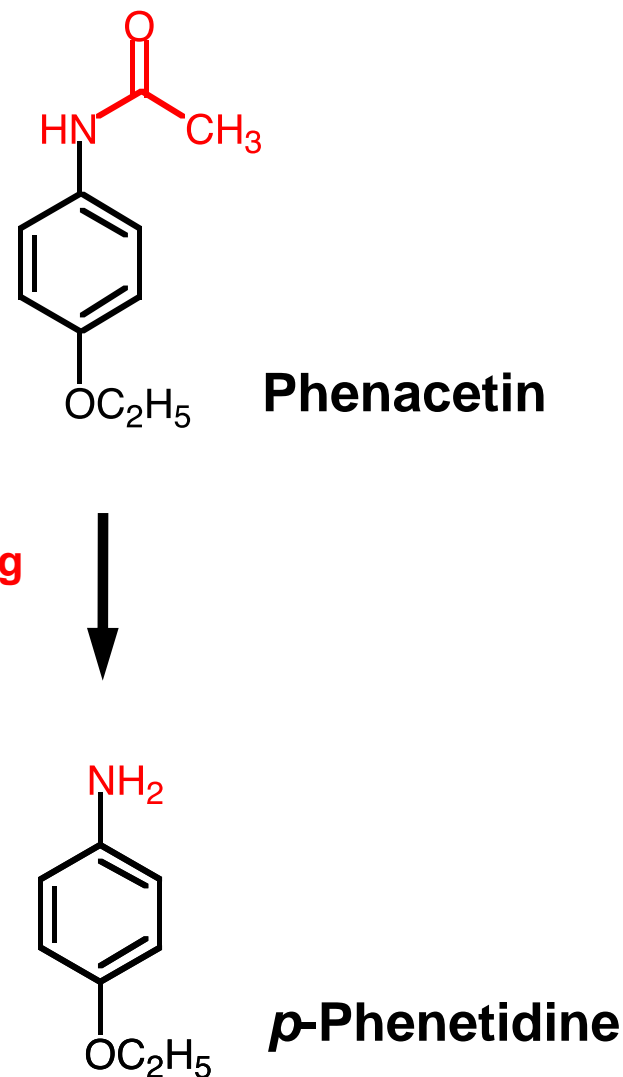
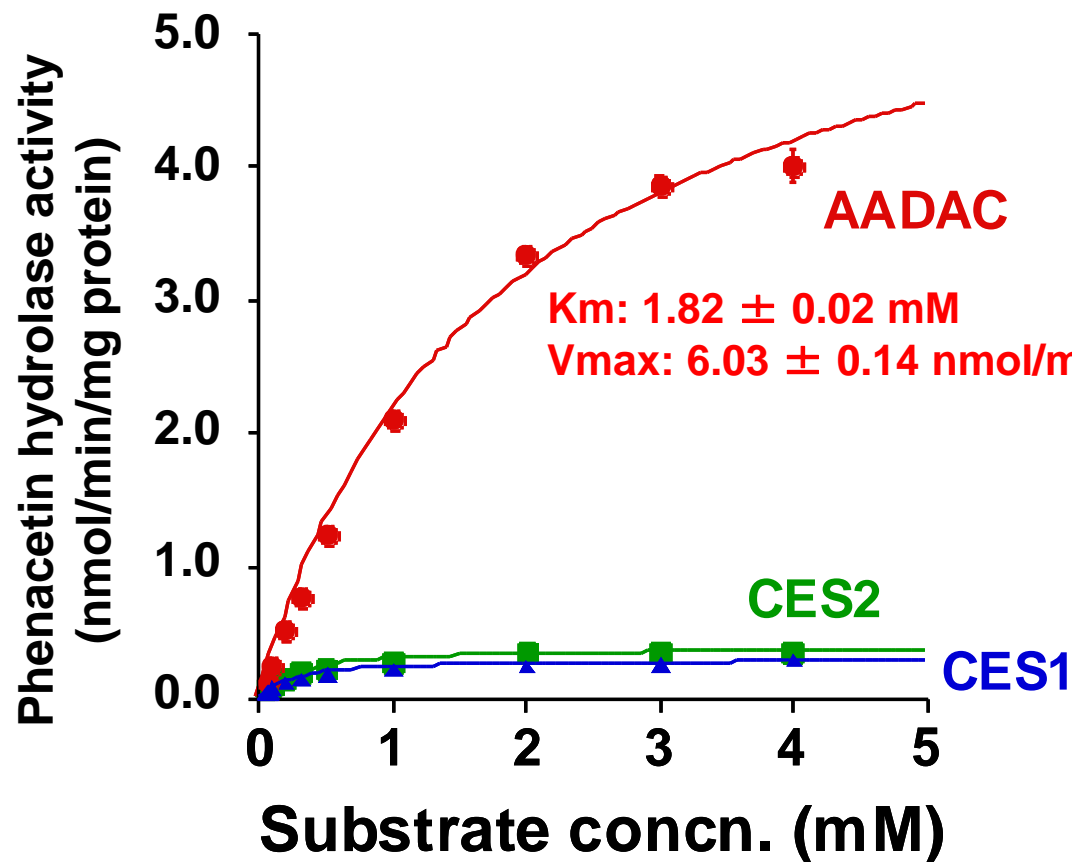
Methemoglobinemia
Pyelonephritis
Renal cell cancer



Withdrawn from the market (1991)

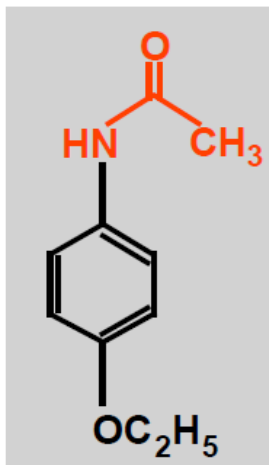
Phenacetin Hydrolase Activities by Recombinant AADAC, CES1, and CES2

Phenacetin

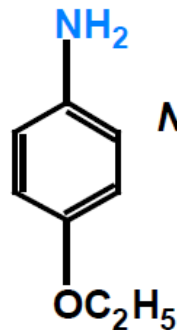
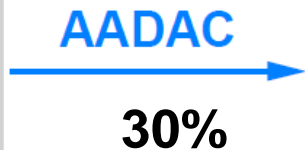


Metabolic Pathways of Phenacetin

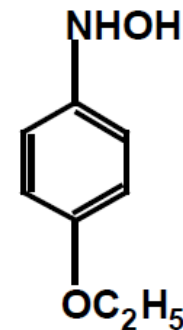
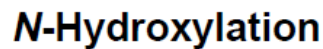
Prodrug



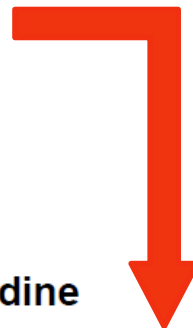
Phenacetin



p-Phenetidine

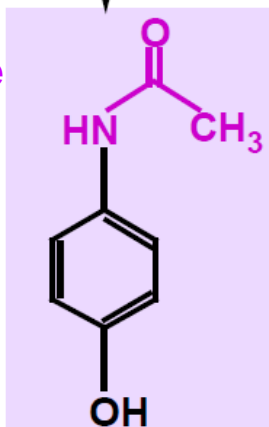


N-Hydroxyphenetidine



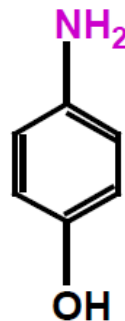
70%

CYP1A2



Acetaminophen (APAP)

Active metabolite



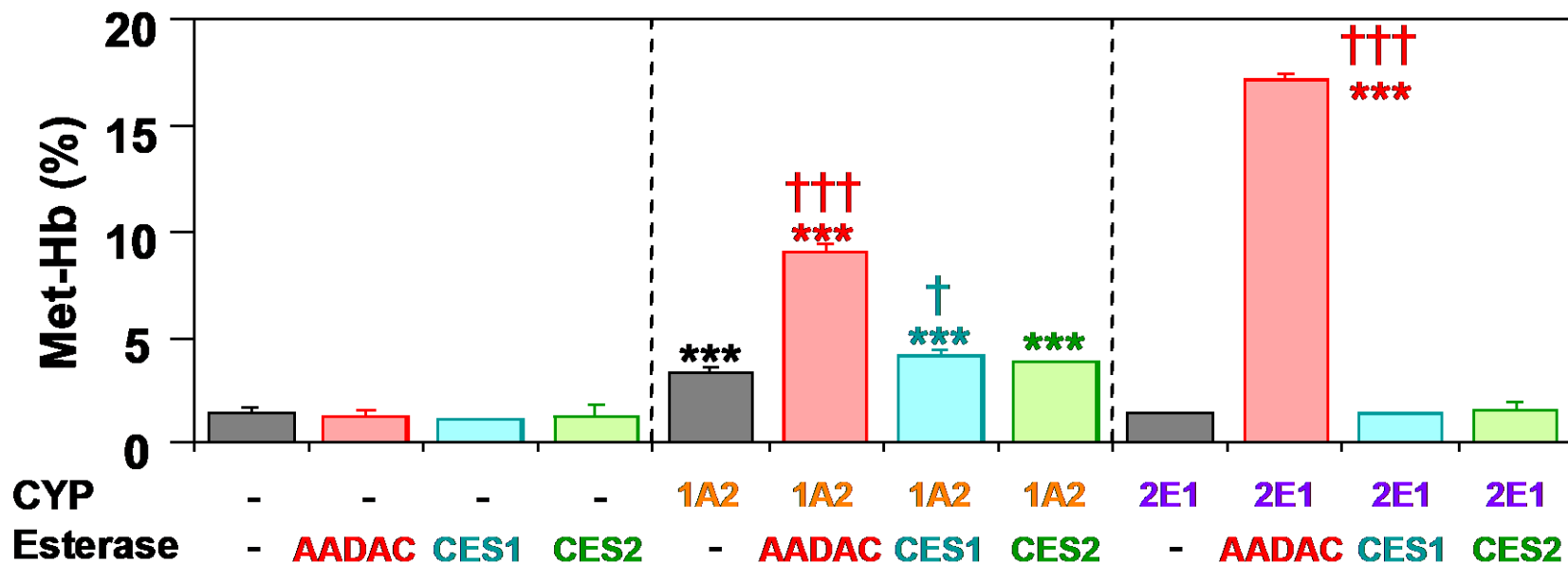
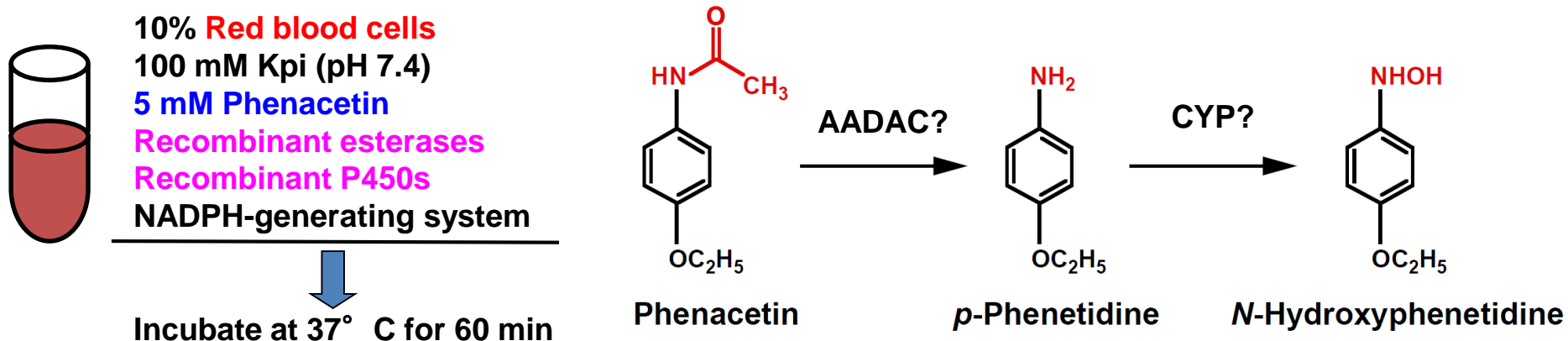
p-Aminophenol

Methemoglobinemia
Pyelonephritis
Renal cell cancer



Withdrawn from the market (1991)

Metabolism of Phenacetin by AADAC and CYP Triggers Methemoglobinemia

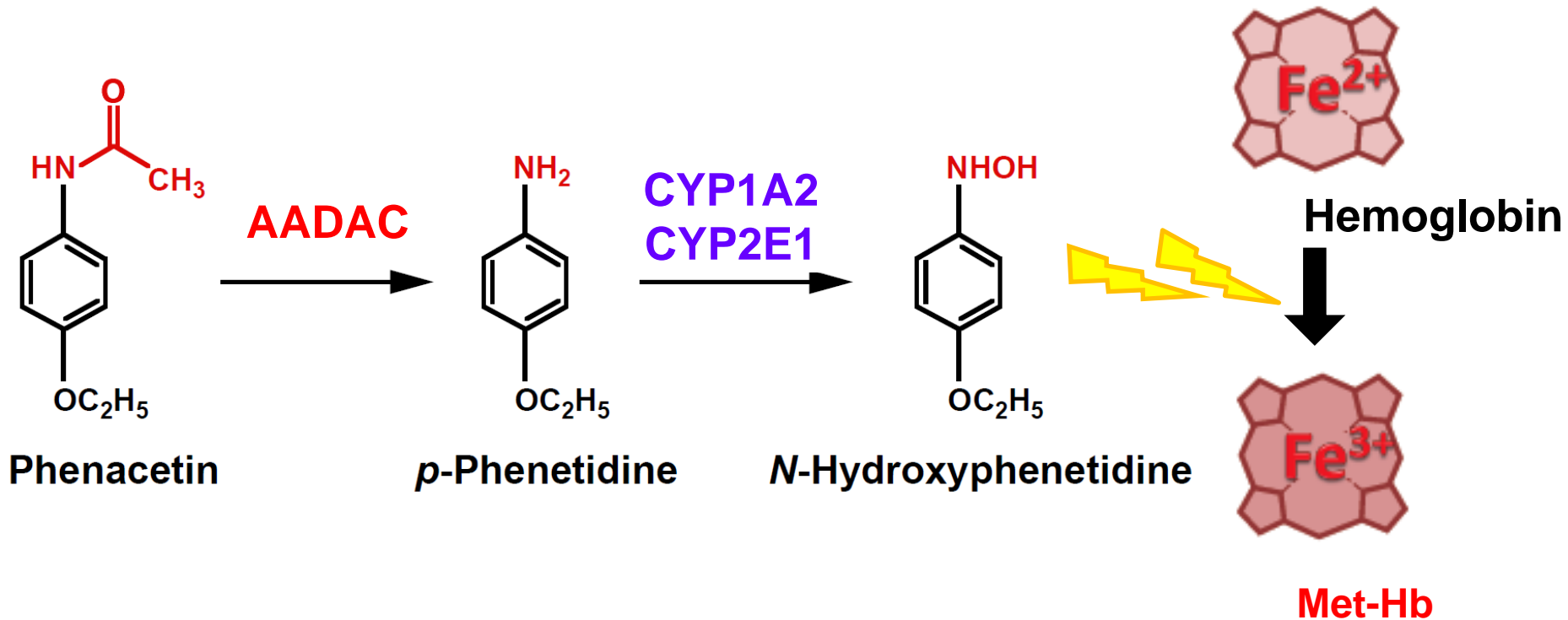


Data are mean \pm SD (n = 3).

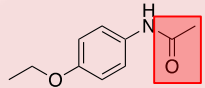
****P* < 0.001 compared with CYP (-) and esterase (-).

†*P* < 0.05 and †††*P* < 0.001 compared with CYP (+) and esterase (-).

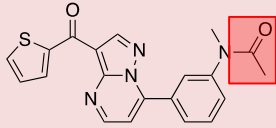
AADAC and CYPs are Involved in Methemoglobinemia Caused by Phenacetin



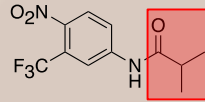
Substrate specificity of CES1, CES2, and AADAC



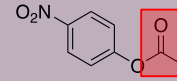
Phenacetin



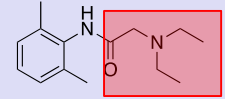
Indiplon



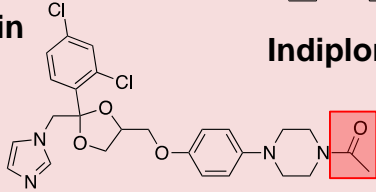
Flutamide



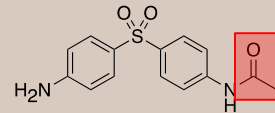
p-Nitrophenyl acetate



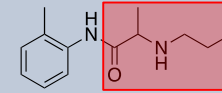
Lidocaine



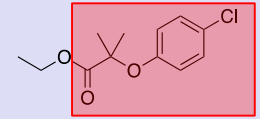
Ketoconazole



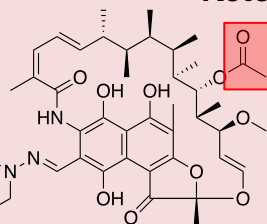
N-Acetyldapsone



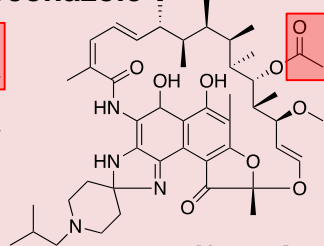
Prilocaine



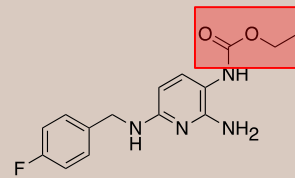
Clofibrate



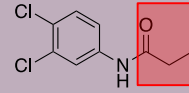
Rifampicin



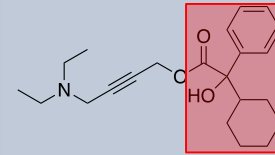
Rifabutin



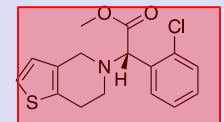
Flupirtine



Propanil



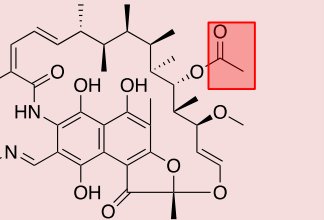
Oxybutynin



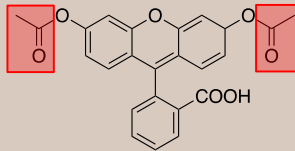
Clopidogrel



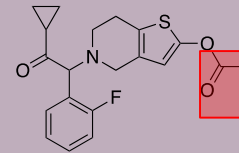
Rifapentine



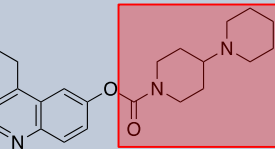
Rifabutin



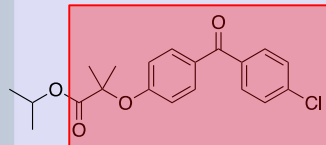
Fluorescein diacetate



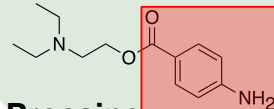
Prasugrel



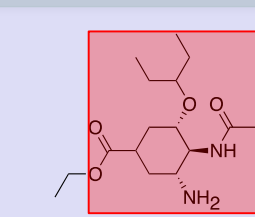
Irinotecan



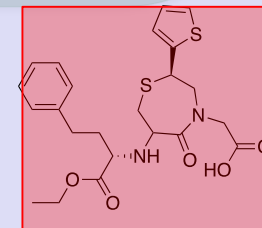
Fenofibrate



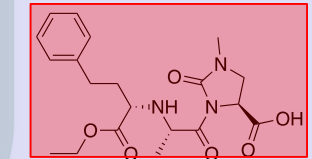
Procaine



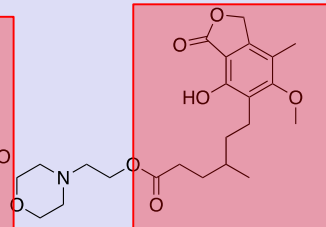
Oseltamivir



Temocapril



Imidapril

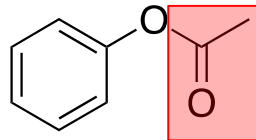


Mycophenolate mofetil

CES1

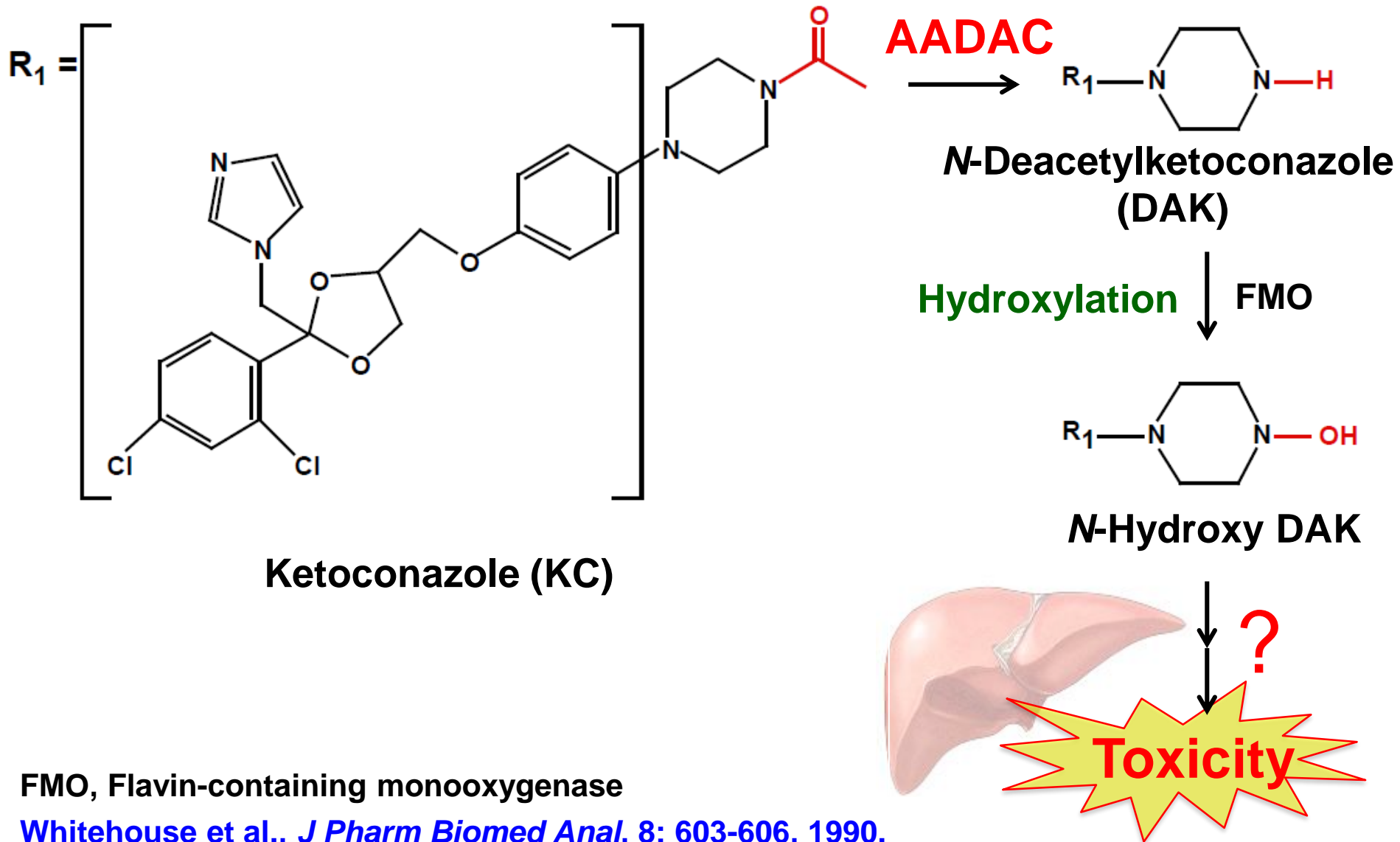
CES2

AADAC



Fukami et al.,
Eur J Pharm Sci,
78: 47-53, 2015.

Metabolic Pathway of KC

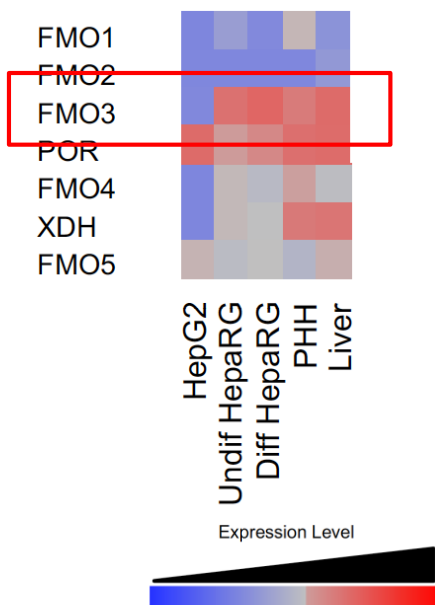
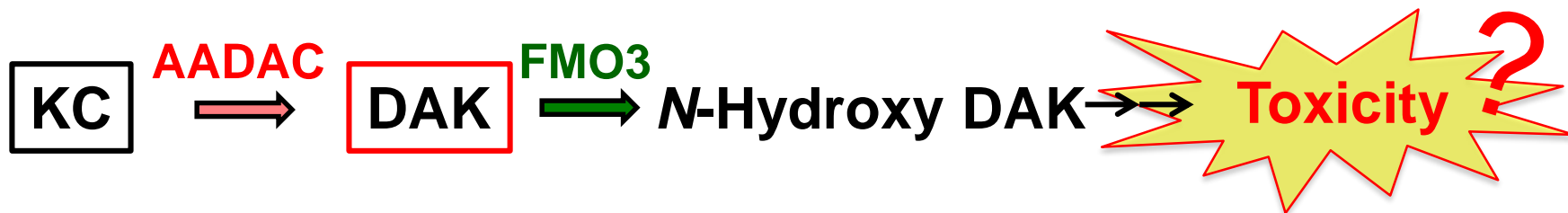


FMO, Flavin-containing monooxygenase

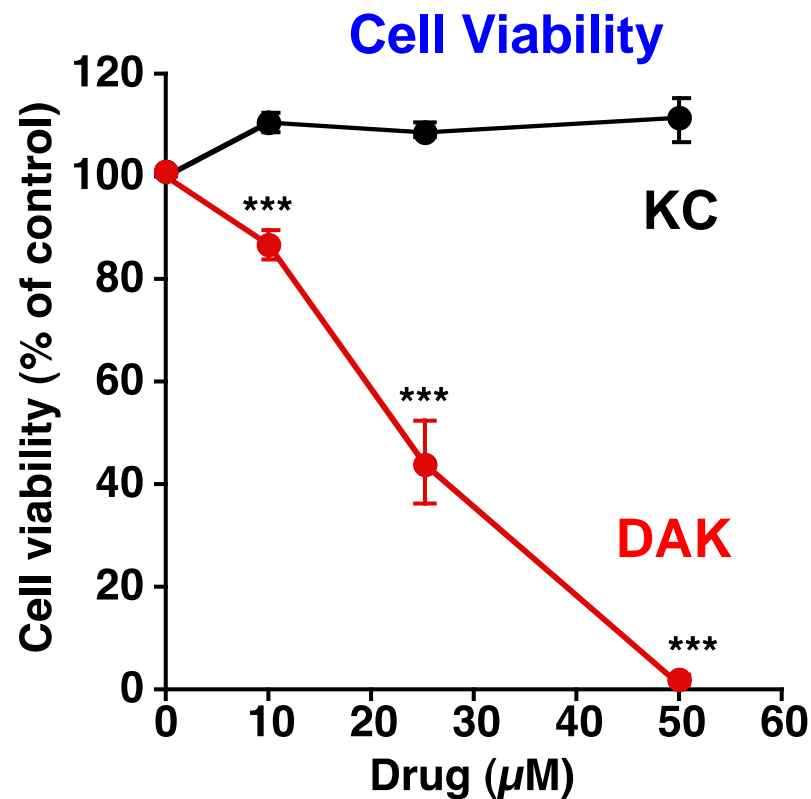
Whitehouse et al., *J Pharm Biomed Anal*, 8: 603-606, 1990.

Rodriguez and Miranda, *Drug Metab Dispos* 28: 1083-1086, 2000.

Cytotoxicity Induced by KC or DAK in HepaRG Cells

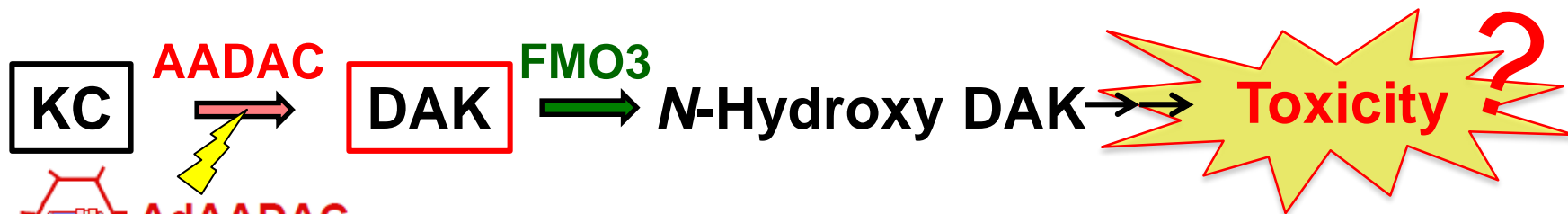


Hart et al., *Drug Metab Dispos*, 38: 988-994, 2010.

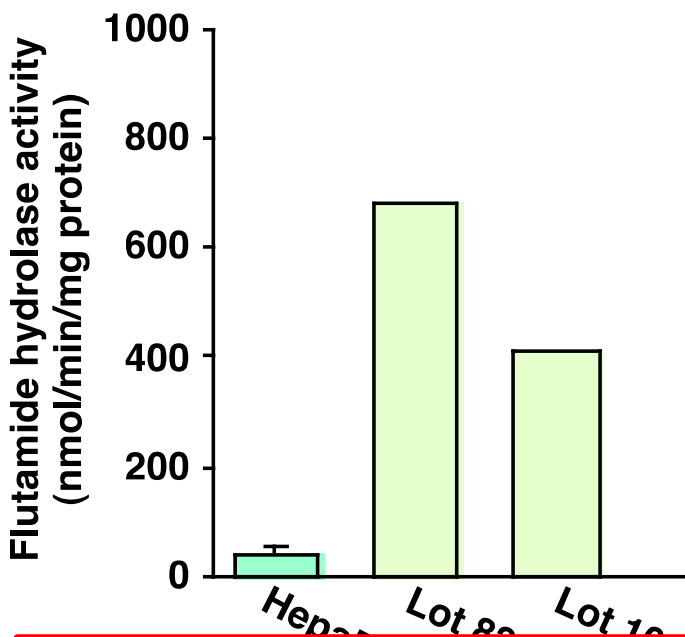


Fukami et al., *Biochem Pharmacol*, 116: 153-161, 2016.

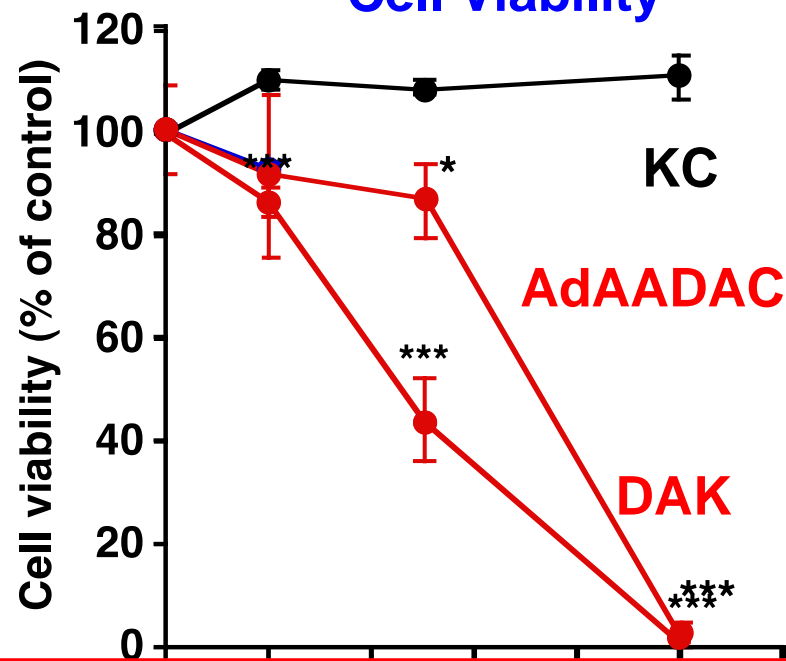
Cytotoxicity Induced by KC or DAK in HepaRG Cells



AADAC Activity



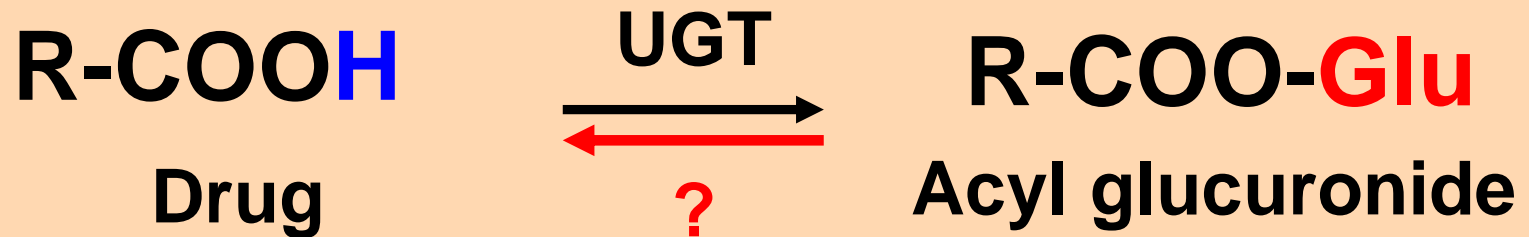
Cell Viability



AADAC exacerbates ketoconazole-induced toxicity.

hepatocytes

Acyl Glucuronide Formed from Drugs Containing A Carboxylic Acid Moiety



Toxicity of Drugs Containing A Carboxylic Acid Moiety

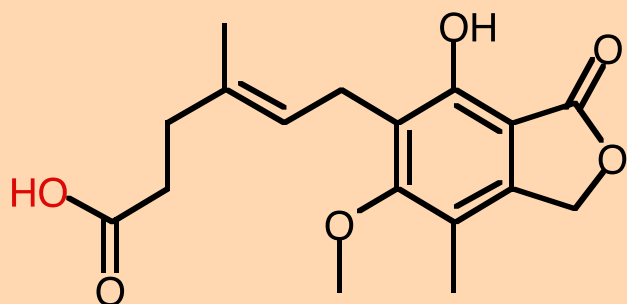
Drug	Toxicity
Diclofenac	Anaphylaxis, liver injury, SJS
Furosemide	Neutropenia, Thrombocytopenia
Ibuprofen	Anaphylaxis, SJS
Mefenamic acid	Anaphylaxis, SJS
Naproxen	Anaphylaxis, SJS
Probenecid	Anaphylaxis
Tolmetin	Anaphylaxis, liver injury, SJS

MPA-AG Formation and Hydrolysis in Human Liver Homogenates

K_m (μM)
173.7

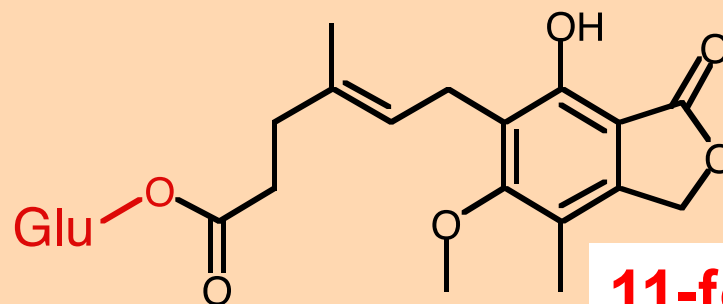
V_{max} (nmol/min/mg)
1.2

CL (μl/min/mg)
6.7



**Mycophenolic
acid (MPA)**

UGT2B7



**Mycophenolic acid
Acyl glucuronide
(MPA-AG)**

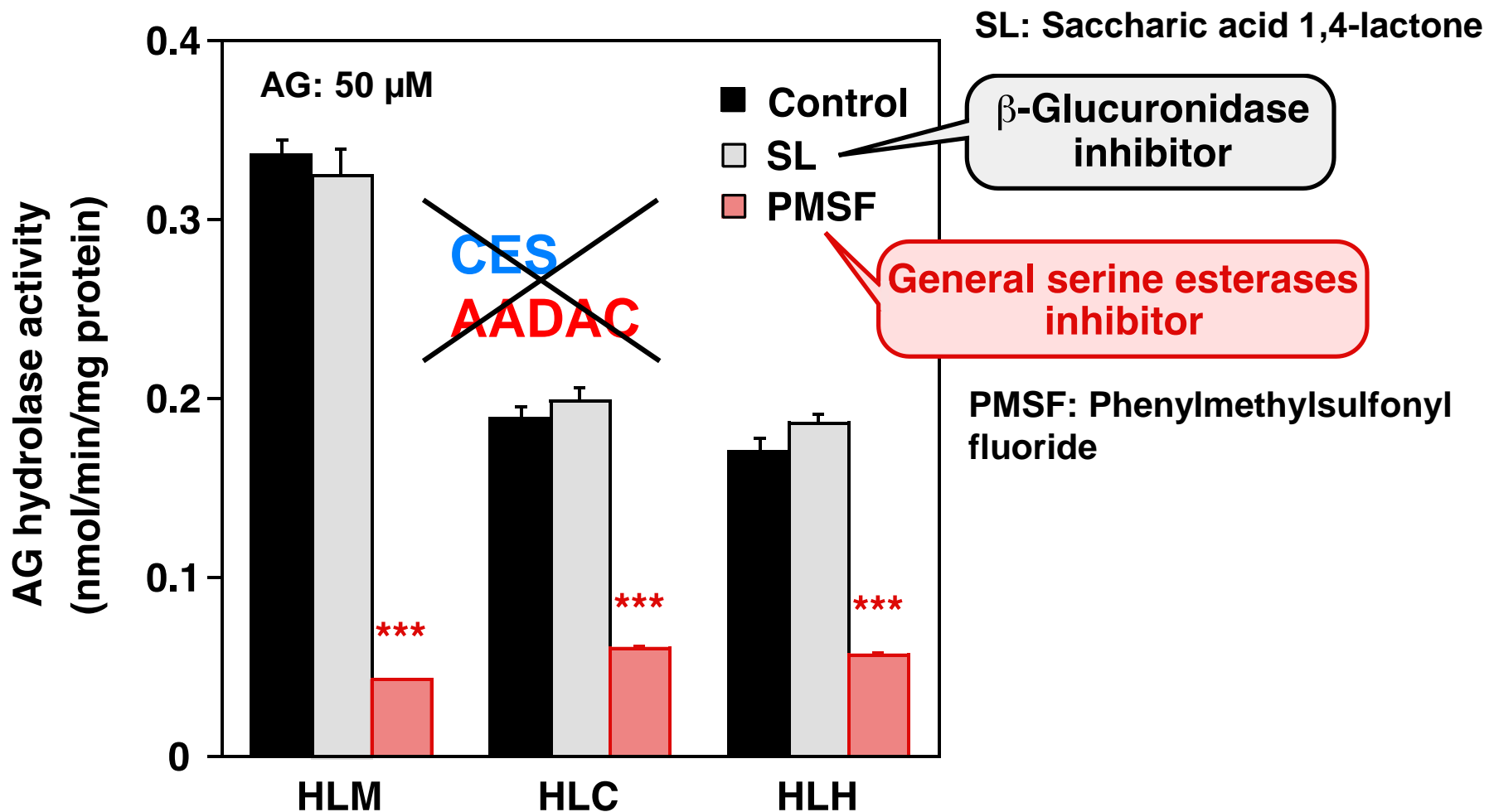
11-fold

K_m (μM)
623.0

V_{max} (nmol/min/mg)
0.4

CL (μl/min/mg)
0.6

MPA-AG Hydrolysis in Human Liver Preparations



Data are the mean \pm SD (n = 3).

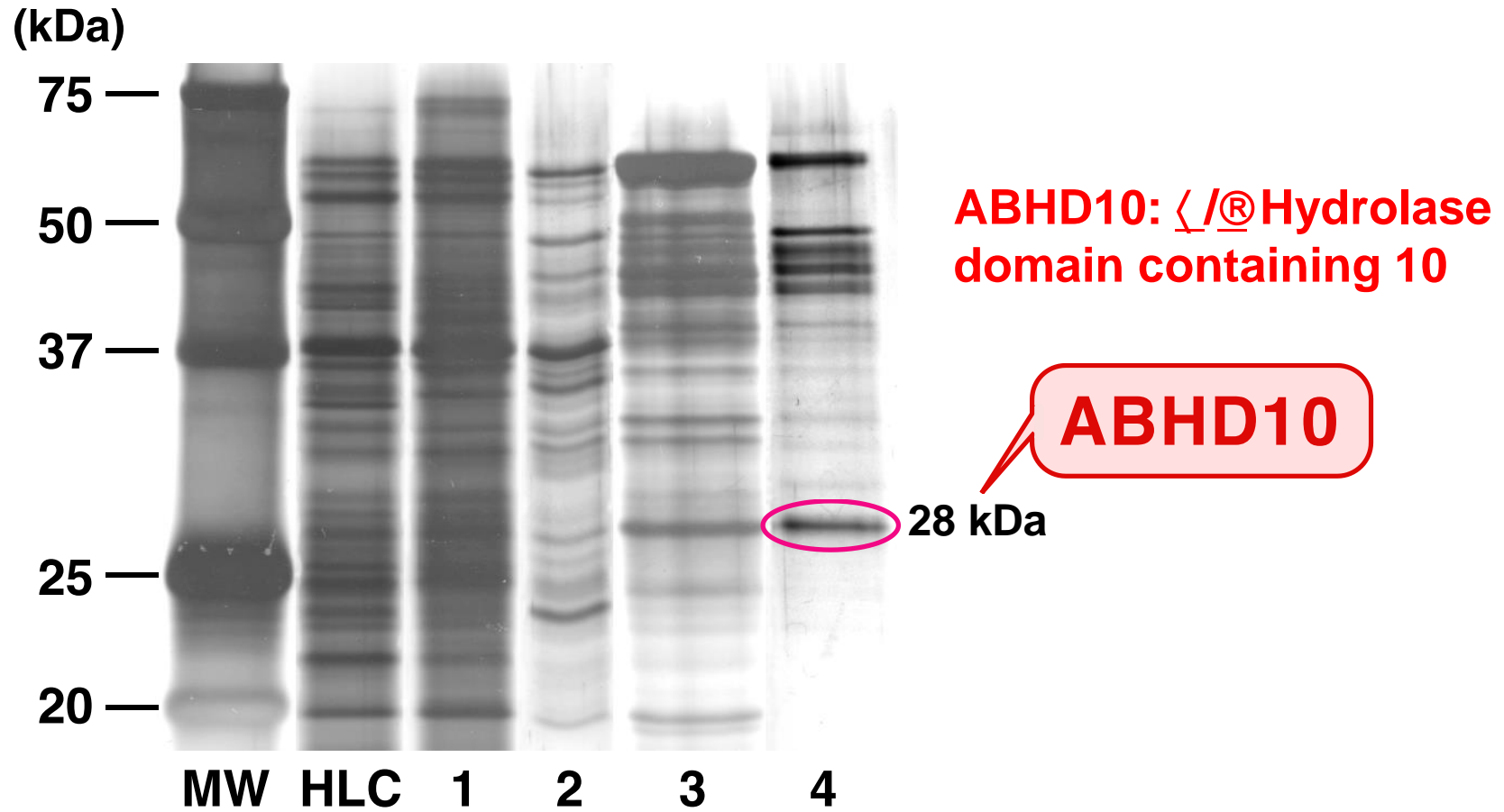
*** $P < 0.001$.

HLM: Human liver microsomes

HLC: Human liver cytosol

HLH: Human liver homogenates

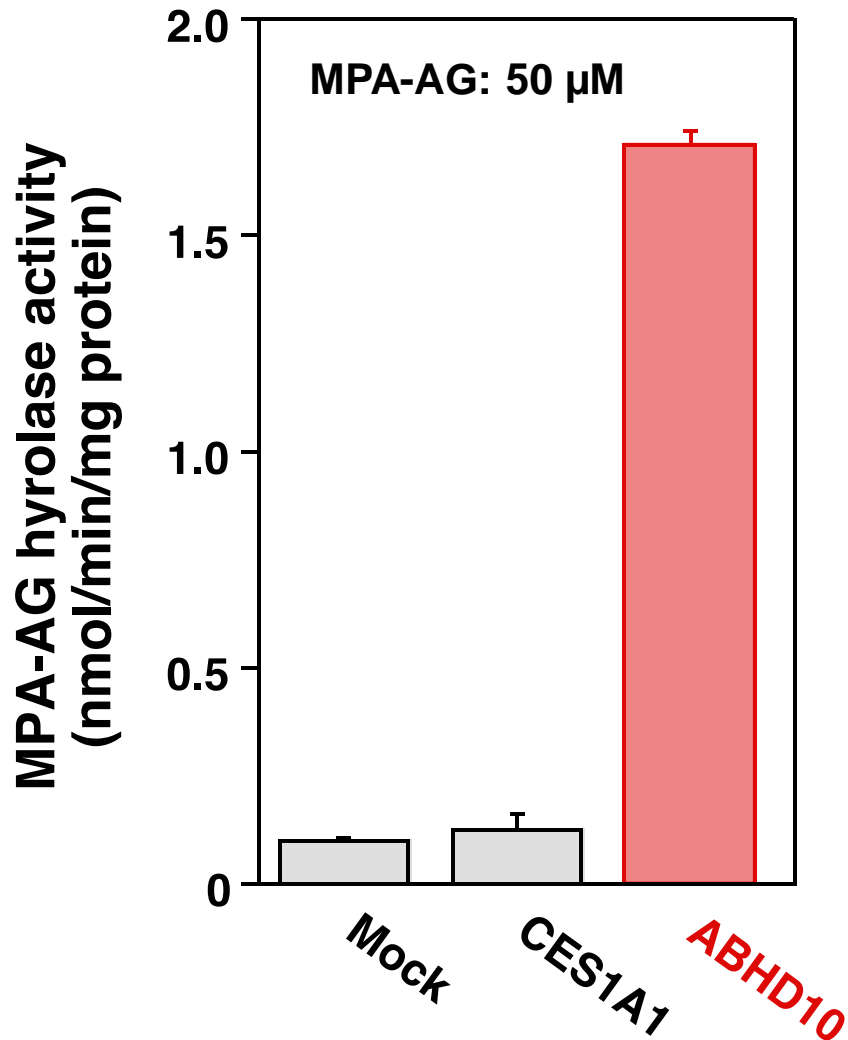
Purification of Enzyme(s) Responsible for MPA-AG Hydrolysis from Human Liver Cytosol



MW: Molecular weight standard
HLC: Human liver cytosol (10 µg)

1: Ammonium sulfate (50 - 70%)
precipitation fraction (10 µg)
2: CM Sepharose fraction (5.0 µg)
3: Mono P fraction (2.0 µg)
4: Superdex 200 fraction (1.0 µg)

MPA-AG Hydrolase Activity by Recombinant ABHD10

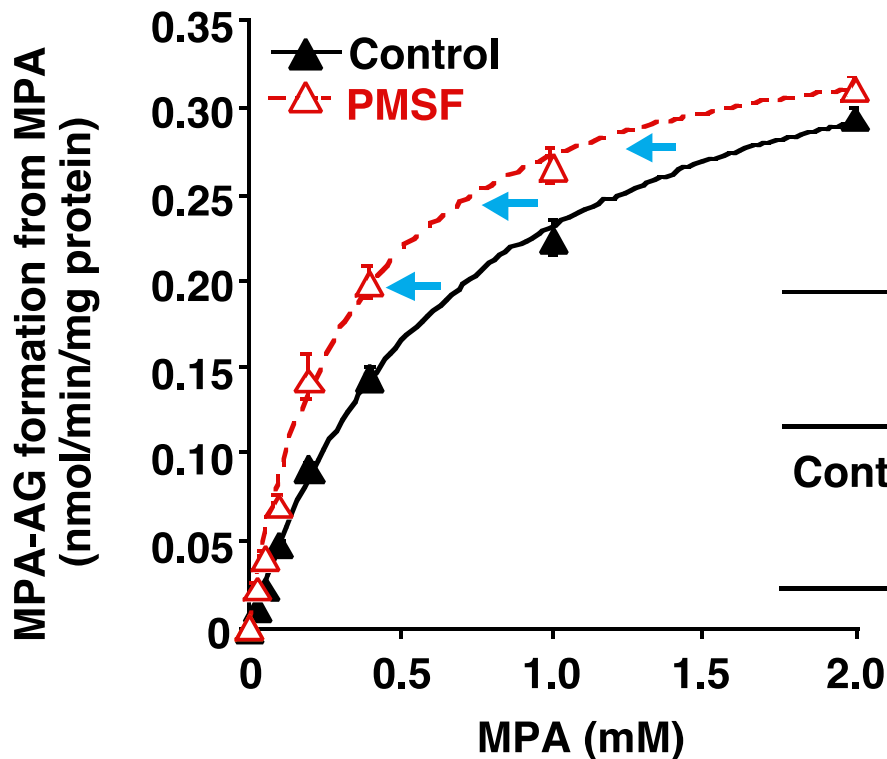
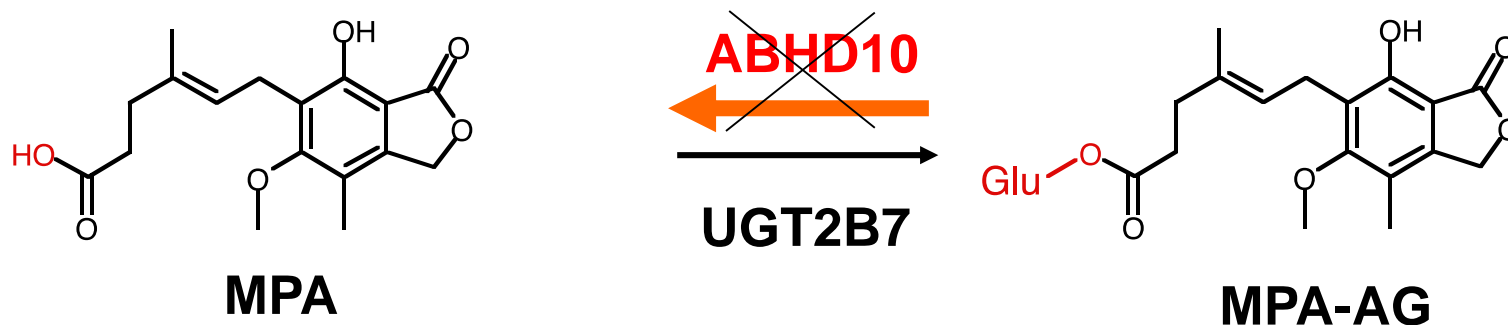


ABHD10 : α/β Hydrolase Domain Containing 10

- In human, 19 *ABHD* genes have been identified, but their **functions are largely unknown**.
- This is the **first report** for the role of ABHD enzyme in **drug metabolism**.

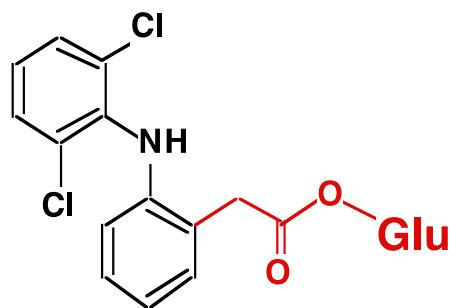
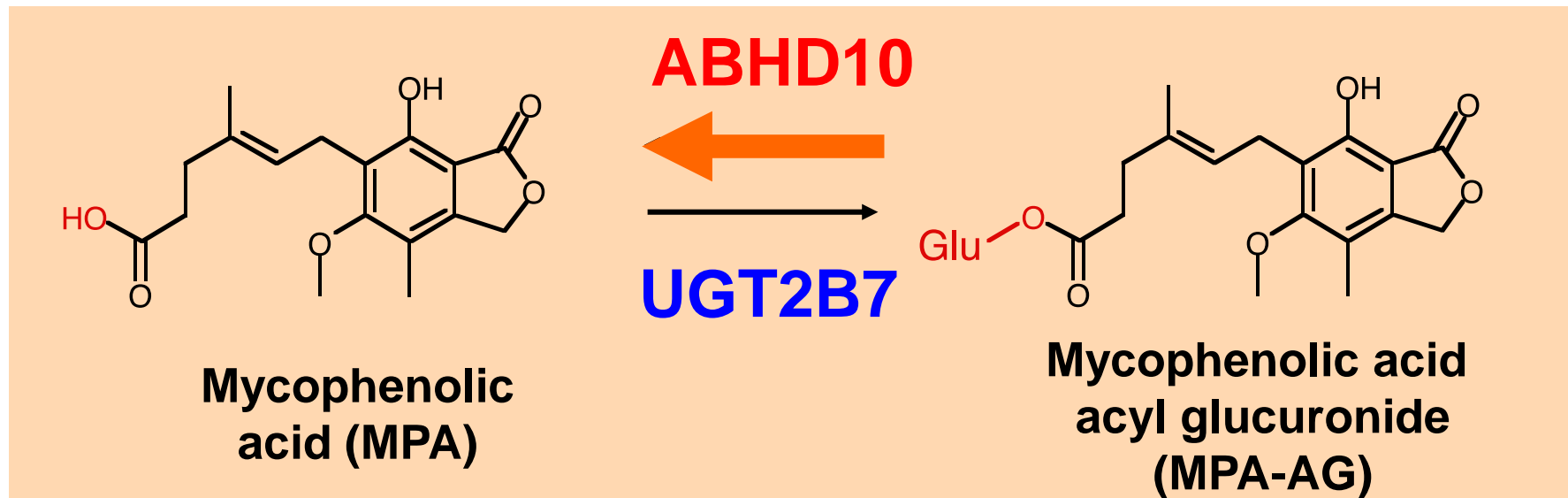
Data are the mean \pm SD (n = 3).

MPA Acyl Glucuronidation in Human Liver Homogenates is Affected by Inhibitor of ABHD10



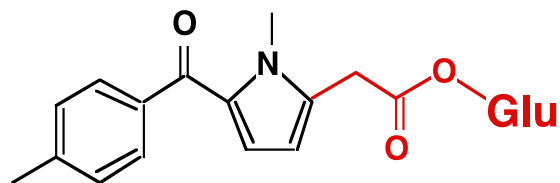
** $P < 0.01$ and *** $P < 0.001$.

ABHD10 Catalyzes Hydrolysis of Acyl Glucuronides in Human Liver

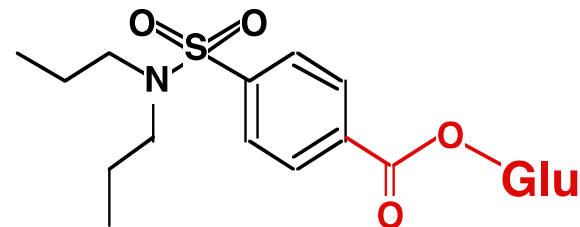


Diclofenac-AG

Iwamura et al., *J Biol Chem*, 287: 9240-9, 2012.



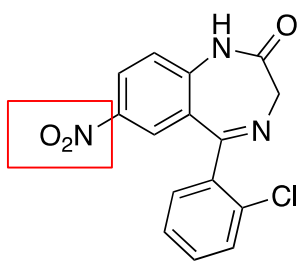
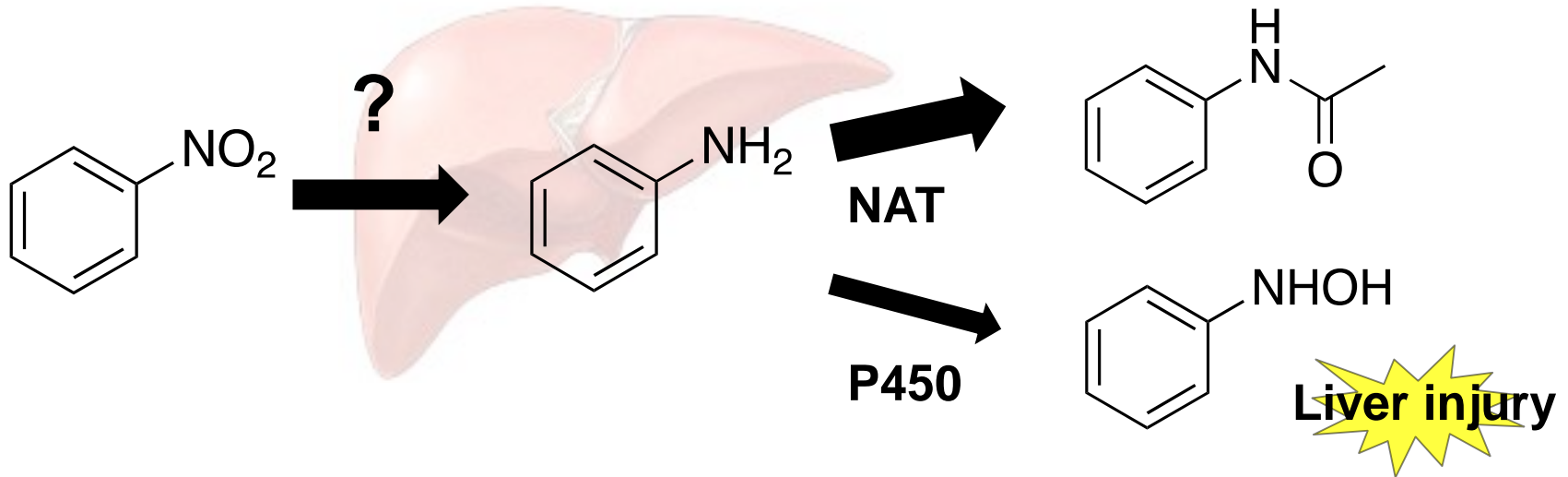
Tolmetin-AG



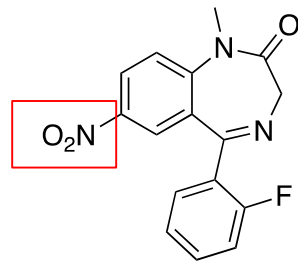
Probenecid-AG

Ito et al., *Drug Metab Dispos*, 42: 2109-16, 2014.

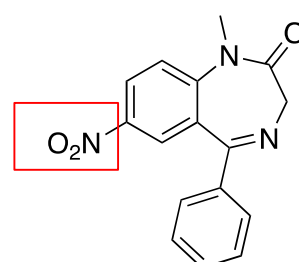
Reduction of Nitroaromatic Drugs



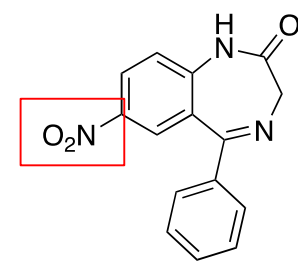
Clonazepam



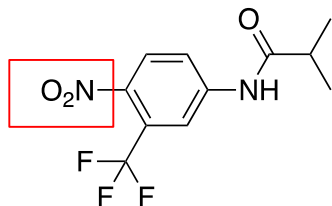
Flunitrazepam



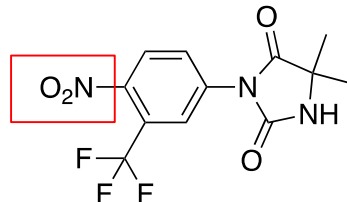
Nimetazepam



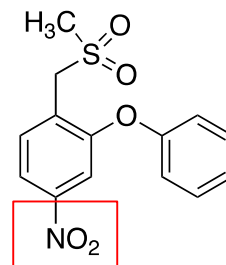
Nitrazepam



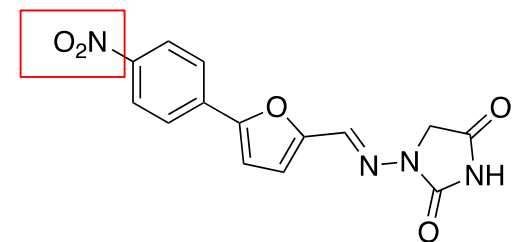
Flutamide



Nilutamide



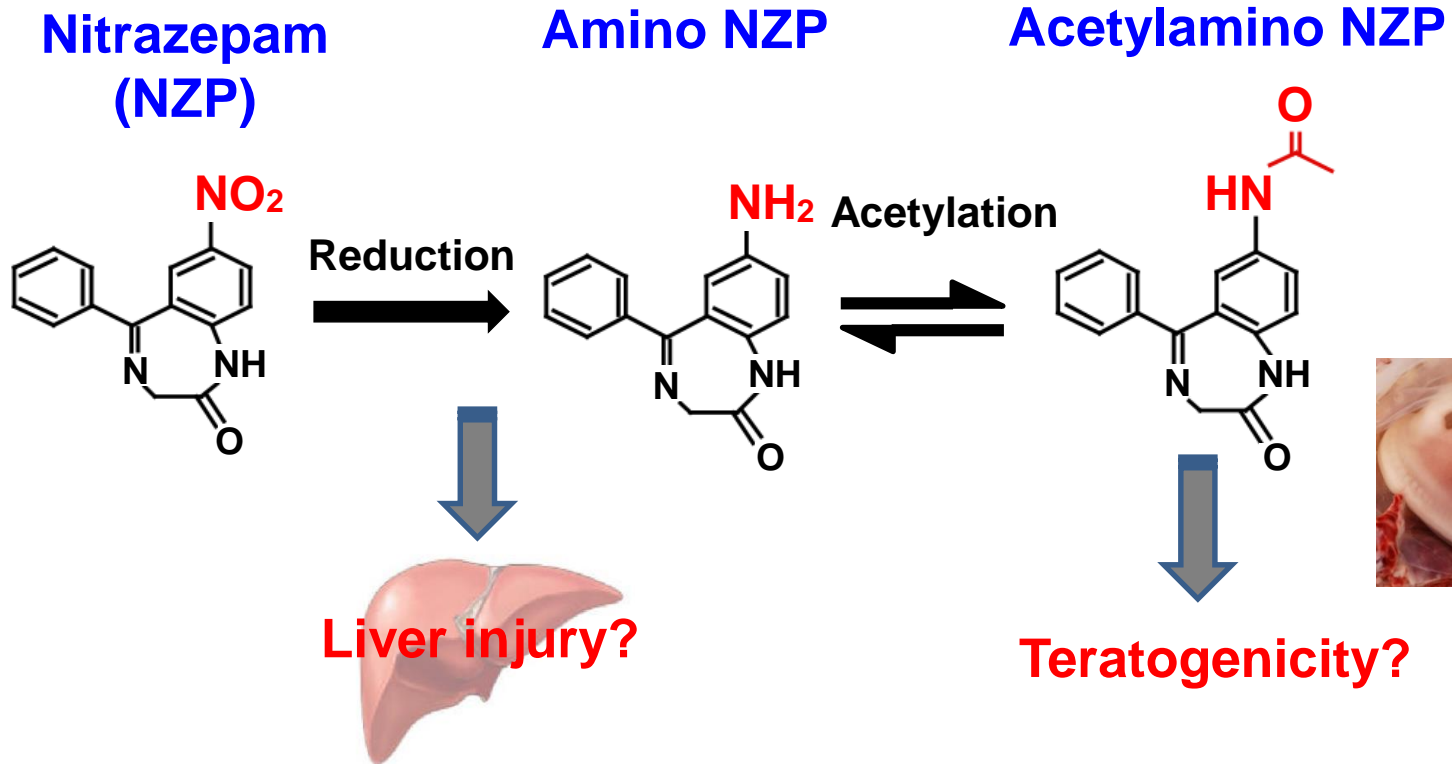
Nimesulide



Dantrolene

Nitrazepam

- ... is widely used as a hypnotic agent.
- ... rarely causes **liver injury** and **teratogenicity**.

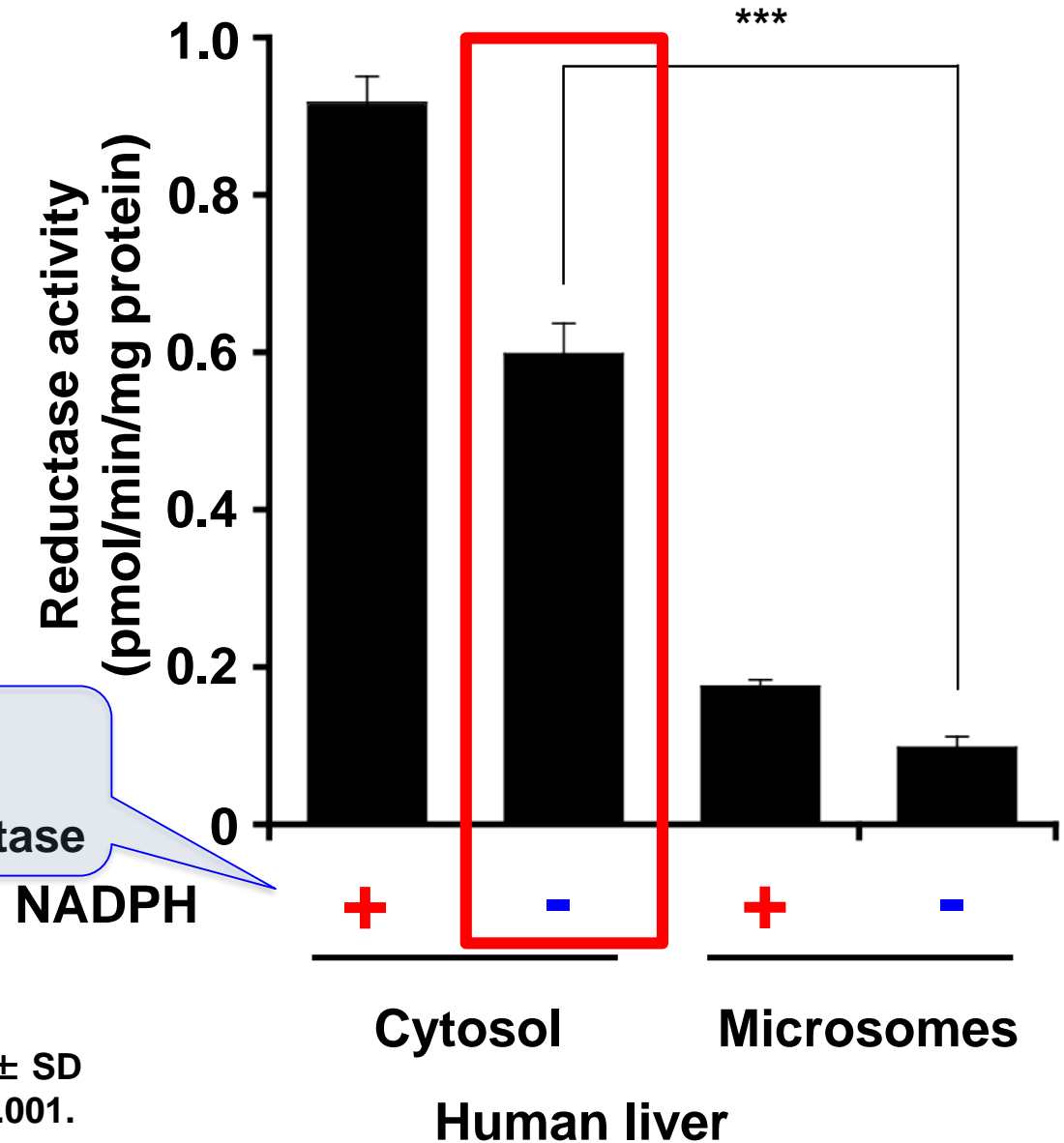


Takeo et al., *Toxicol Appl Pharmacol*, 1993.

Andrade et al., *Curr Drug Metab*, 2009.

Mizuno et al., *Drug Metab Dispos*, 2009.

NZP Reductase Activity in Human Liver

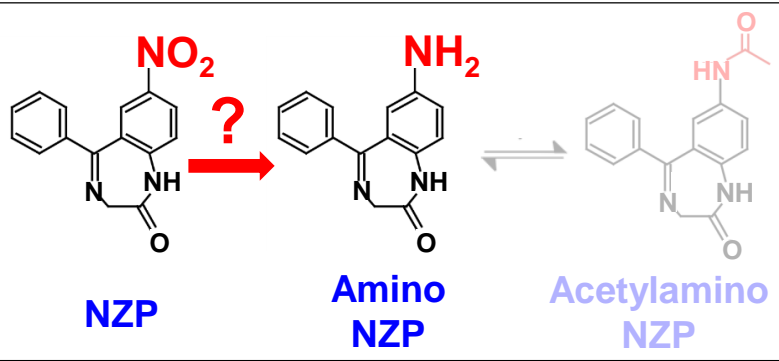


Aldo-keto reductase
Carbonyl reductase
NADPH-quinone oxidoreductase

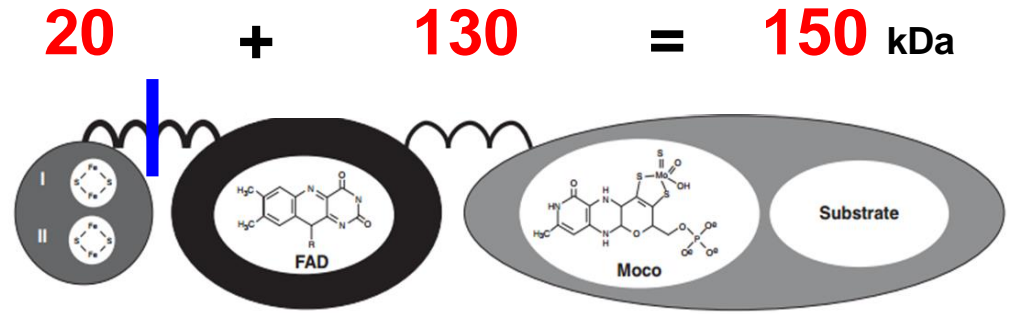
NADPH

Each column represents the mean \pm SD of triplicate determinations. *** $P < 0.001$.

Protein Purification for NZP Reductase

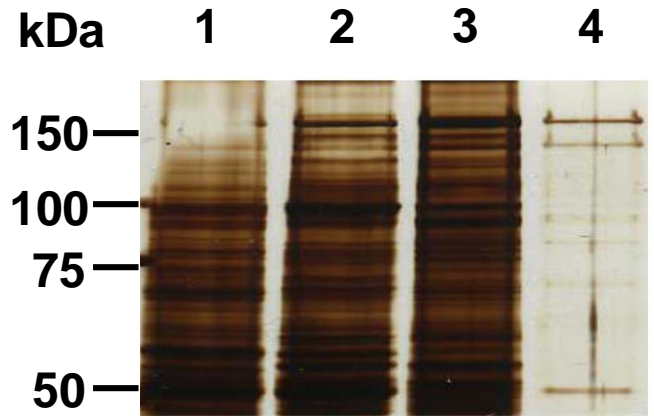


◆ Structure of AOX1



Garattini and Terao, *Expert Opin Drug Metab Toxicol*, 8: 487-503, 2012.

Silver staining

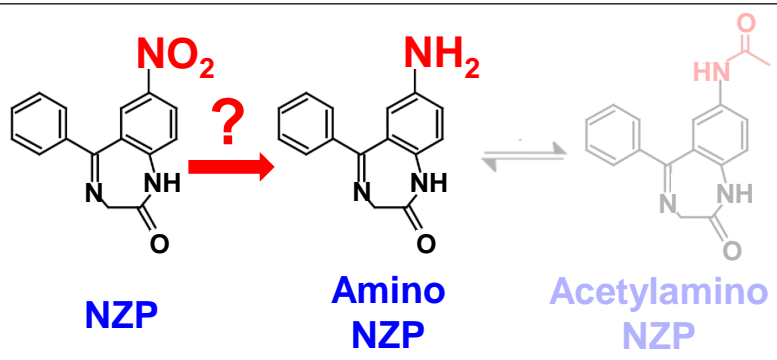


150 kDa
130 kDa \rightarrow **SDS-PAGE**

Aldehyde oxidase 1 (AOX1)
is cut in SDS-PAGE.

- Lane 1, HLC
- Lane 2, 40-60% Ammonium sulfate precipitated fraction
- Lane 3, CM Sepharose fraction
- Lane 4, DEAE Sephacel fraction

Western Blotting for AOX1

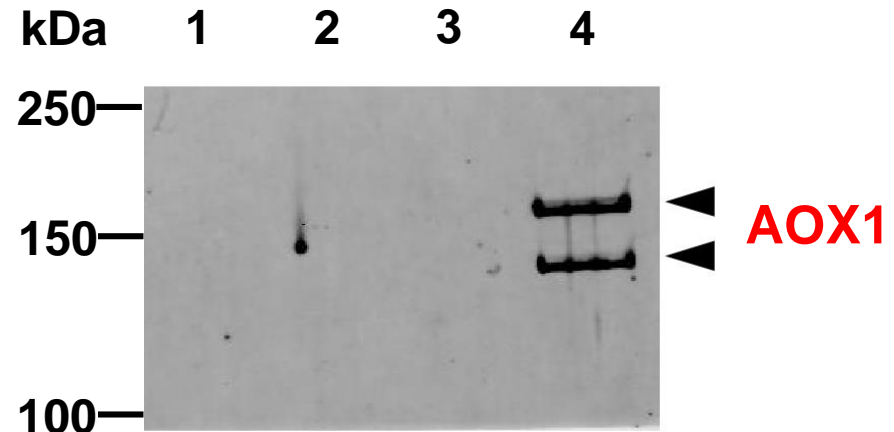
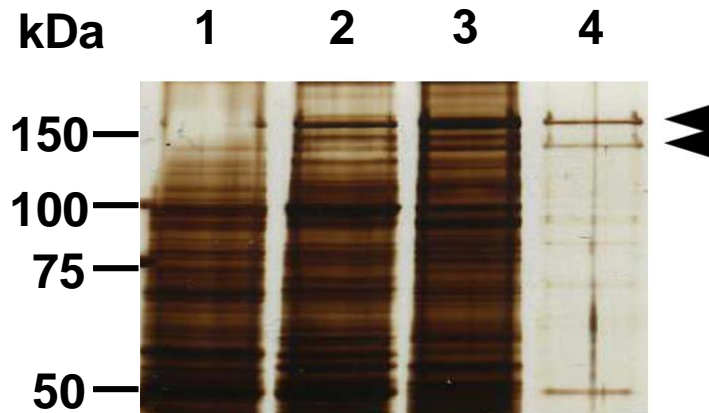


Methylnicotinamide (MNA) supplies electrons to AOX1 to increase activity.



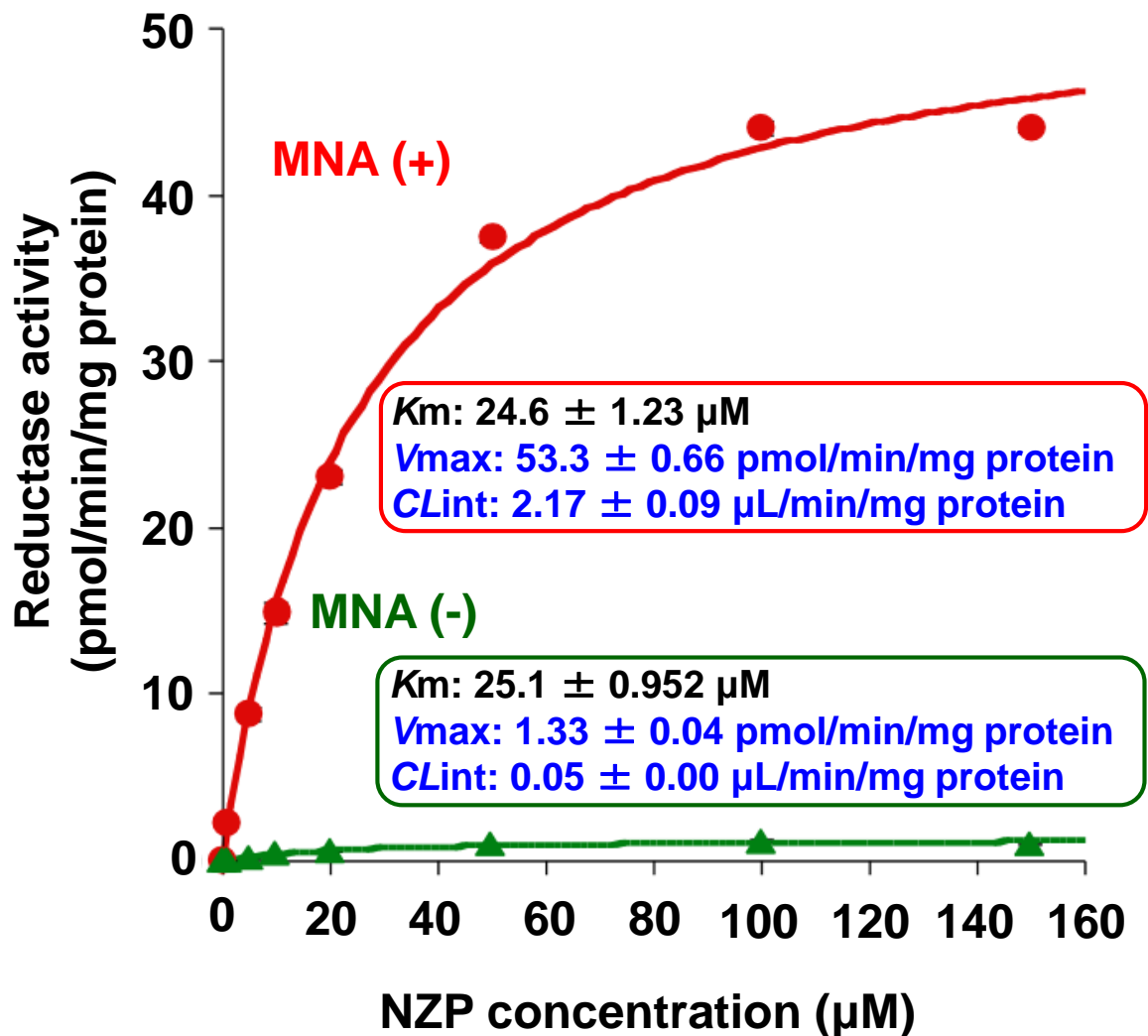
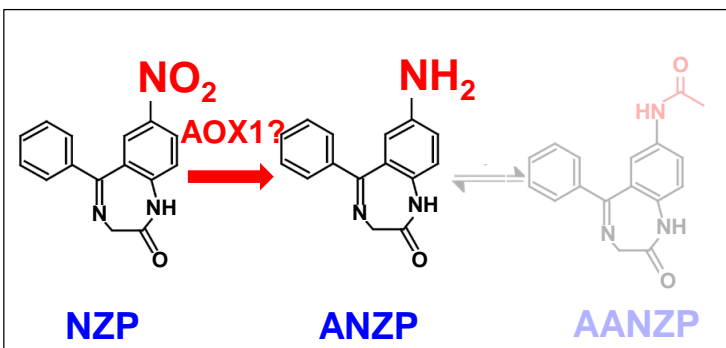
Silver staining

Western blotting



- Lane 1, HLC
- Lane 2, 40-60% Ammonium sulfate precipitated fraction
- Lane 3, CM Sepharose fraction
- Lane 4, DEAE Sephacel fraction

Kinetic Analyses of NZP Reduction by HLC in Presence of MNA

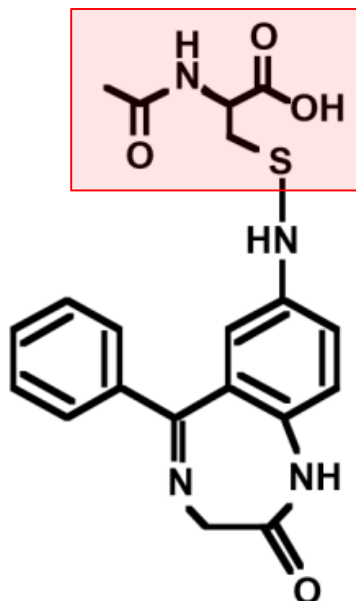
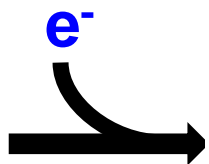


Each point represents the mean \pm SD of triplicate determinations.

Nitro Reduction via Hydroxylamine

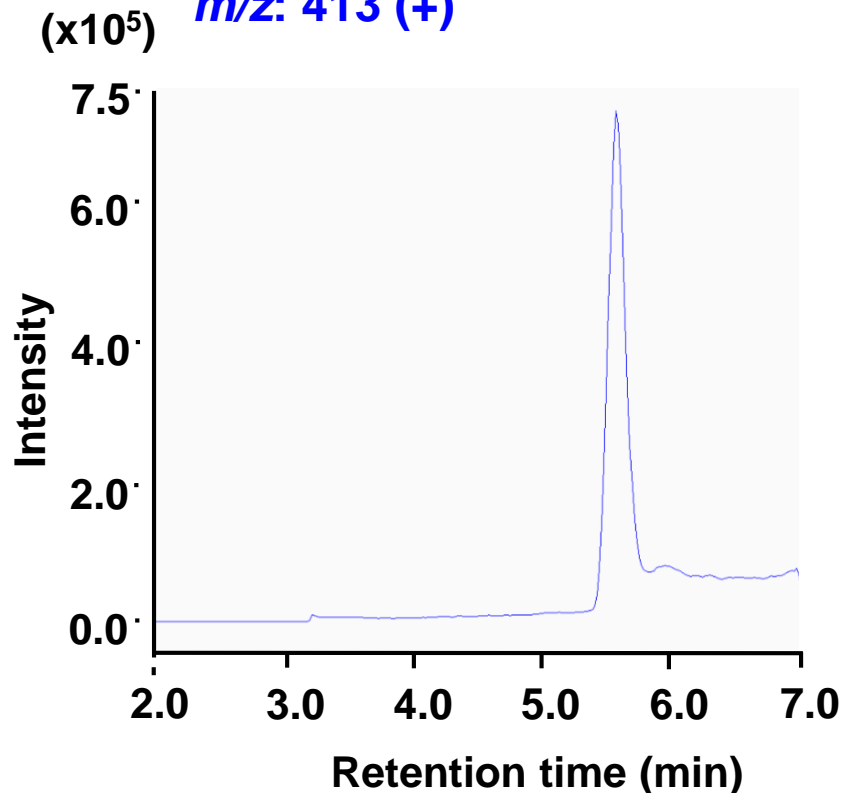
N-Acetyl-L-cysteine (NAC)

NZP

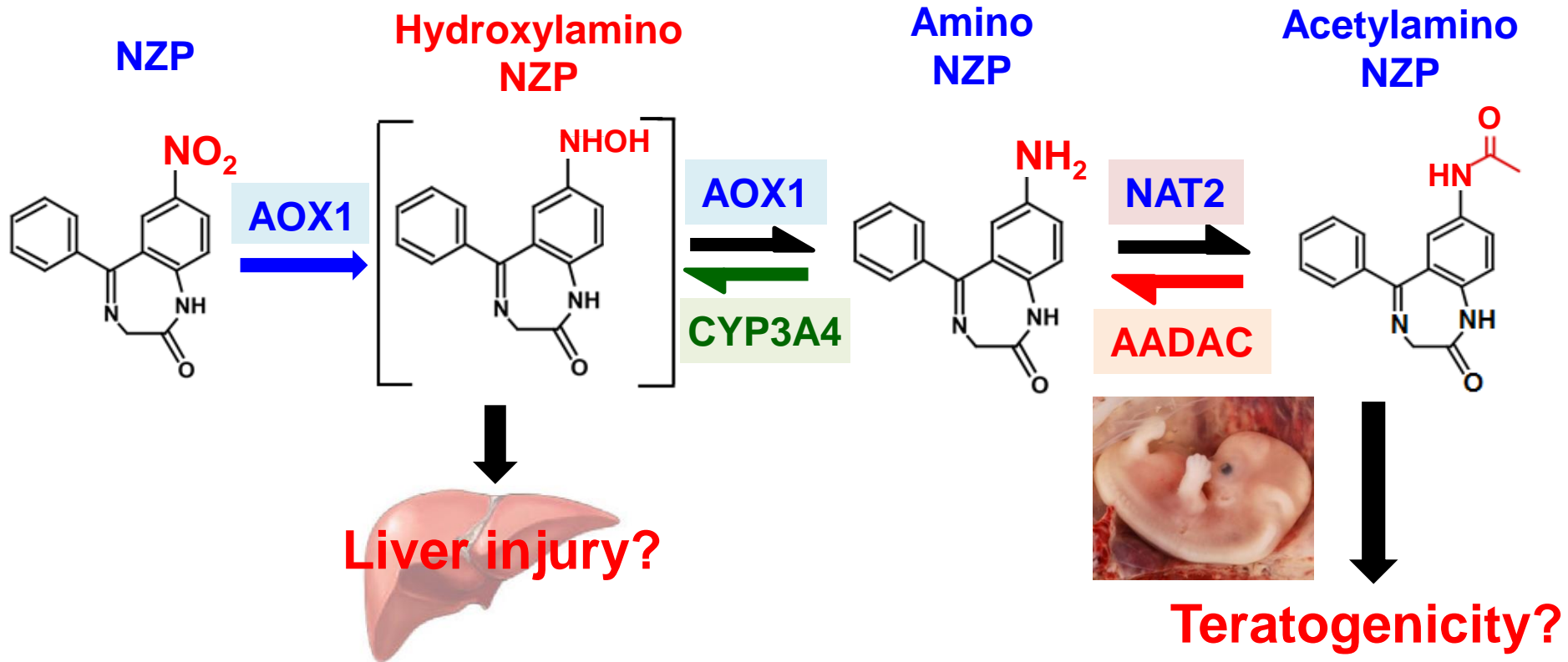


m/z : 413

Single ion monitoring
 m/z : 413 (+)



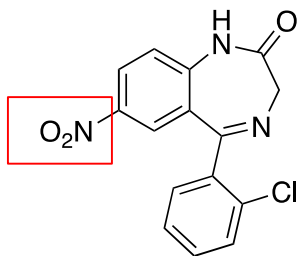
Metabolic Pathway of Nitrazepam in Human



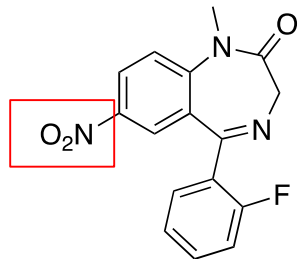
Konishi et al., *Biochem Pharmacol* , 140: 150-160, 2017.

The balance of metabolism would affect the sensitivity of the toxicities.

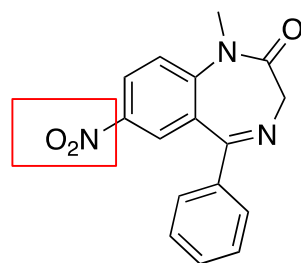
Reduction of Nitroaromatic Drugs



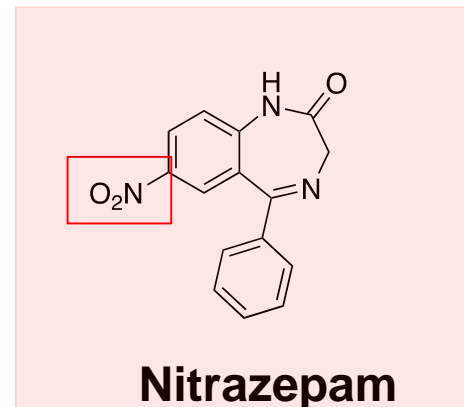
Clonazepam



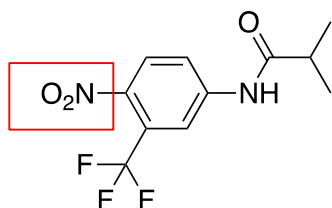
Flunitrazepam



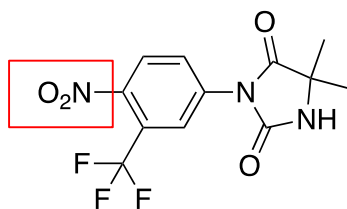
Nimetazepam



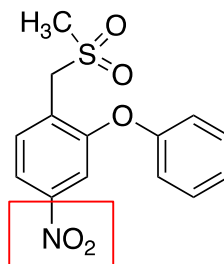
Nitrazepam



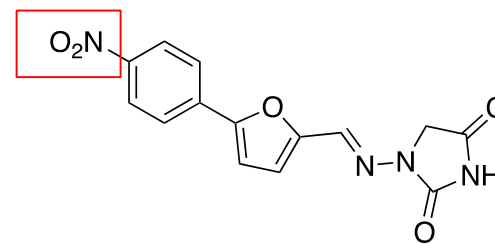
Flutamide



Nilutamide



Nimesulide

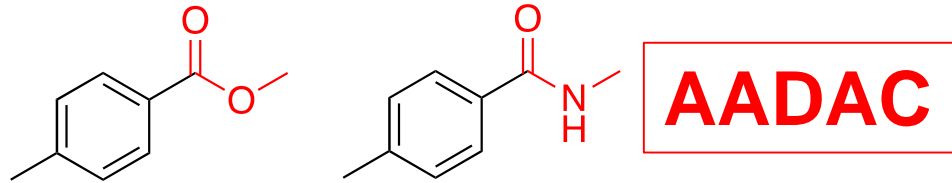


Dantrolene

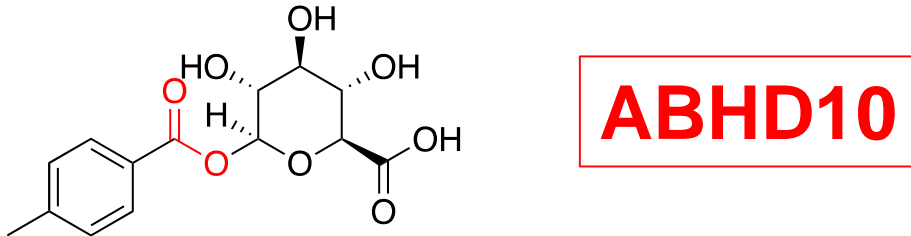
The reduction of nitroaromatic drugs by AOX1 would be of toxicological significance.

Unknown Non-CYP Enzymes

Many drug containing ester or amide bonds are hydrolyzed by CES. However, some drugs were likely to be hydrolyzed by **enzyme(s)** other than CES in human liver.



Acyl-glucuronides possess ester bond. Because acyl-glucuronides are suggested to cause several toxicities, the enzyme(s) catalyzing their hydrolysis may attenuate their toxicities.



Some drugs containing nitro groups are metabolized to amino metabolites, but the responsible enzyme(s) had been unclear.



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Keigo Konishi
Takuo Ogiso**

Fukami and Yokoi. The emerging role of human esterases. Drug Metab. Pharmacokinet., 27: 466-477, 2012.

Oda, Fukami, Yokoi, and Nakajima. A comprehensive review of UDP-glucuronosyltransferase and esterases for drug development. Drug Metab. Pharmacokinet., 30:30-51, 2015.