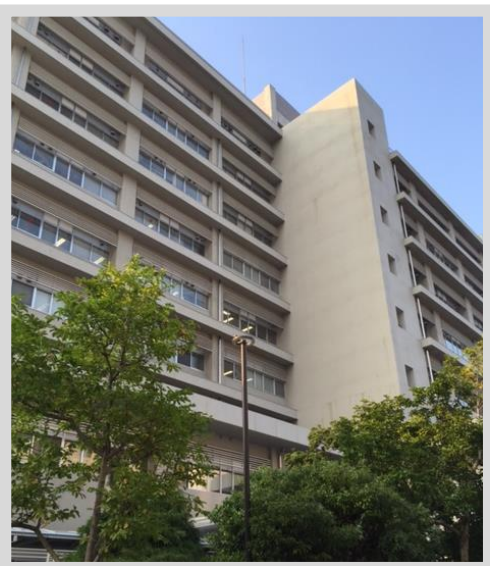


The studies on aldehyde oxidase-mediated metabolism
and its inhibition considering species differences

アルデヒド酸化酵素における代謝および阻害の種差に着目した薬物動態研究



Hiroshima University

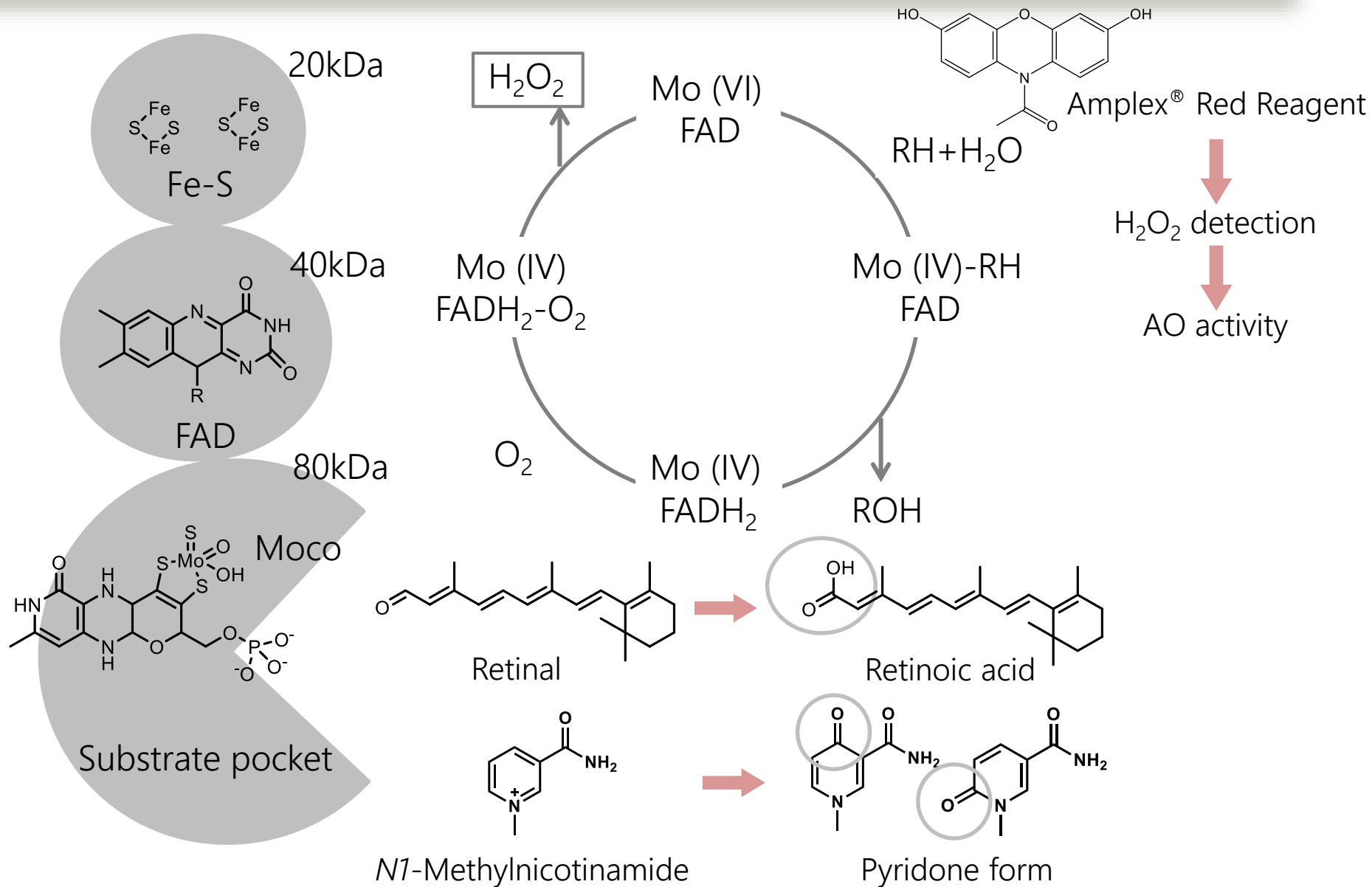
Seigo SANOH

佐能 正剛

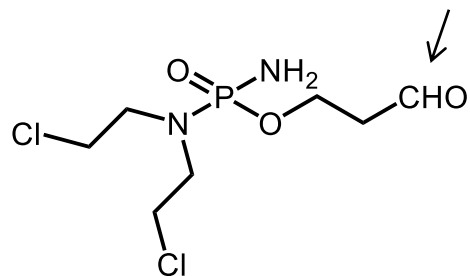
Graduate School of Biomedical and Health Sciences,
Hiroshima University

広島大学大学院医系科学研究科

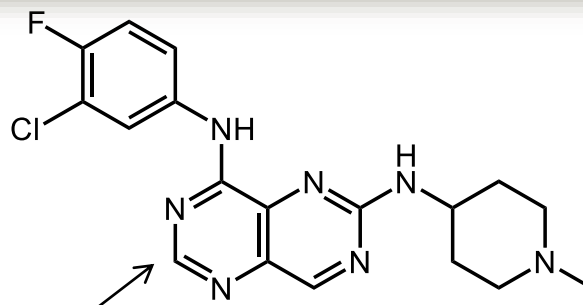
Metabolic mechanism of AO



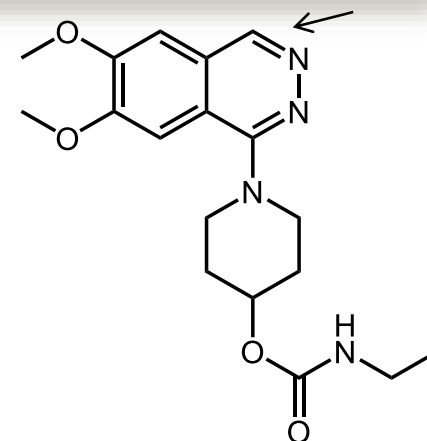
Pharmaceuticals and drug candidates metabolized by AO



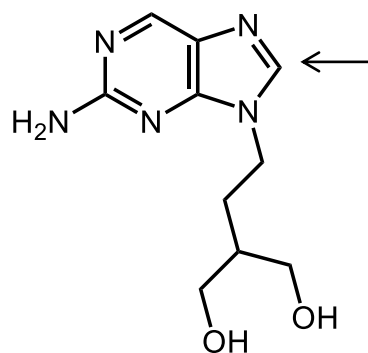
Aldophosphamide
(Cyclophosphamide metabolite)



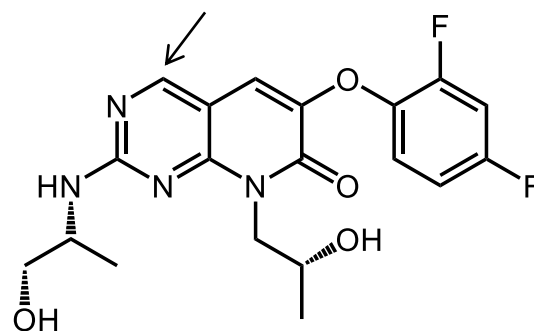
BIBX1382
Low
bioavailability



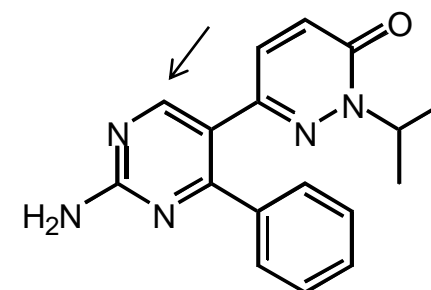
Carbazeran
Low
bioavailability



6-Deoxypenciclovir
(Famciclovir metabolite)



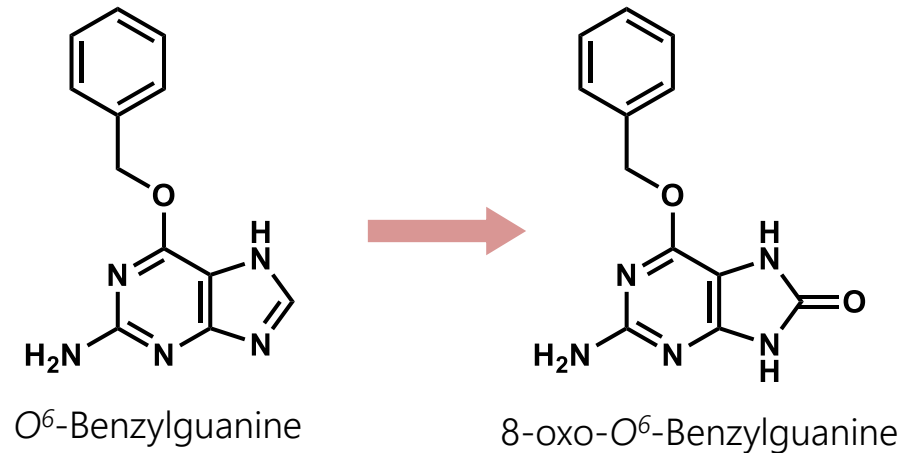
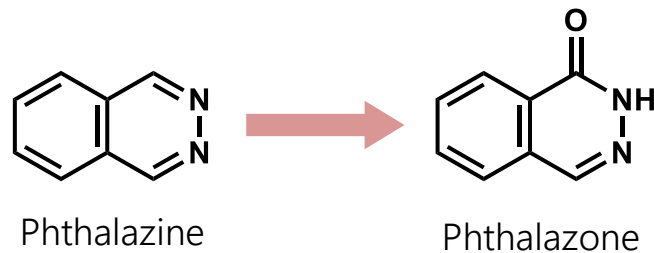
RO-1
Short half-life



FK3453
Low
bioavailability

Rat; $CL_t = 14.9 \text{ mL/min/kg}$, BA = 30.5%,
Dog; $CL_t = 5.0 \text{ mL/min/kg}$, BA = 87.1%

Substrate specificity of AO isoforms



Isoenzyme	Phthalazine		O^6 -Benzylguanine	
	K_m (μM)	V_{max} (nmol/min/mg)	K_m (μM)	V_{max} (pmol/min/mg)
human AOX1	60.2 \pm 13.3	5.91 \pm 0.56	175 \pm 48.6	75.8 \pm 8.98
mouse AOX1	123 \pm 32.3	2.19 \pm 0.30	126 \pm 27.8	6.13 \pm 0.51
mouse AOX3	7.18 \pm 2.15	1.61 \pm 0.12	ND ^a	ND ^a

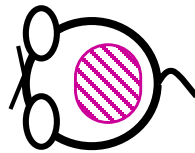
All values are expressed as mean \pm S.E.M. of 3. replicates.

a : kinetic parameters could not be determined because metabolite was not detected.

Chimeric mice transplanted with human/rat hepatocytes

Chimeric mice transplanted with **human** hepatocytes

Human hepatocytes



uPA/SCID mice

Rat hepatocytes

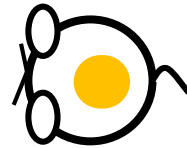
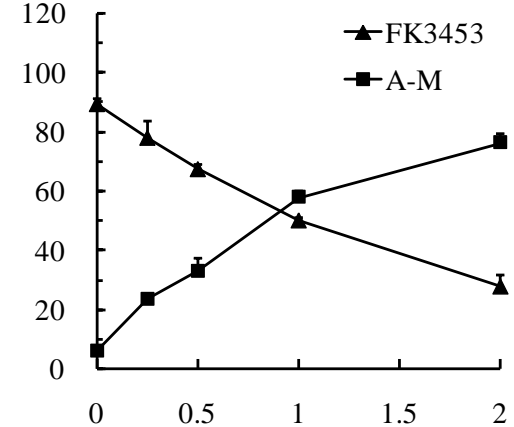
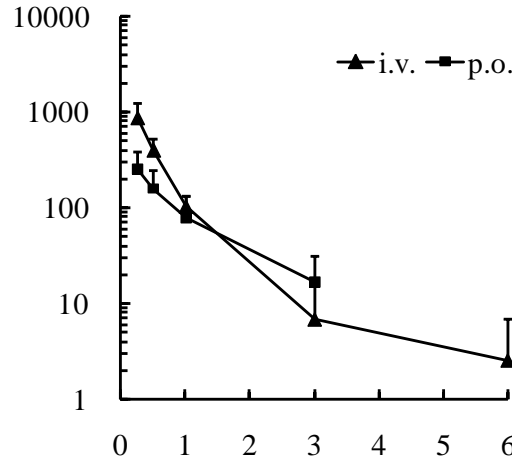
Chimeric mice transplanted with **rat** hepatocytes



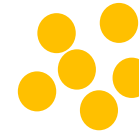
FK3453



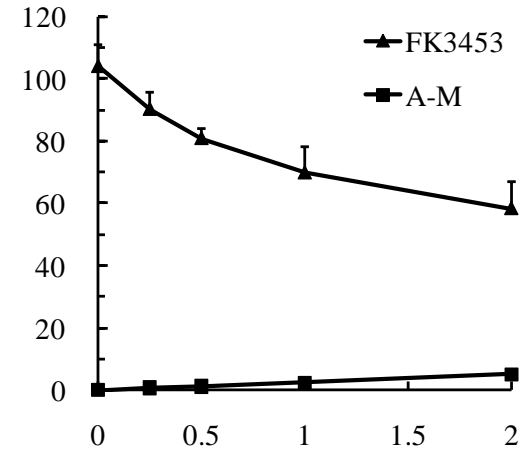
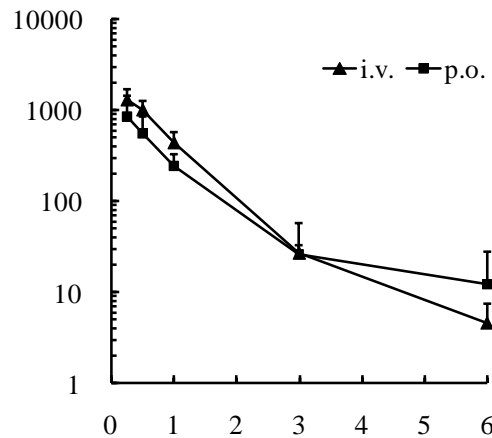
Human hepatocytes



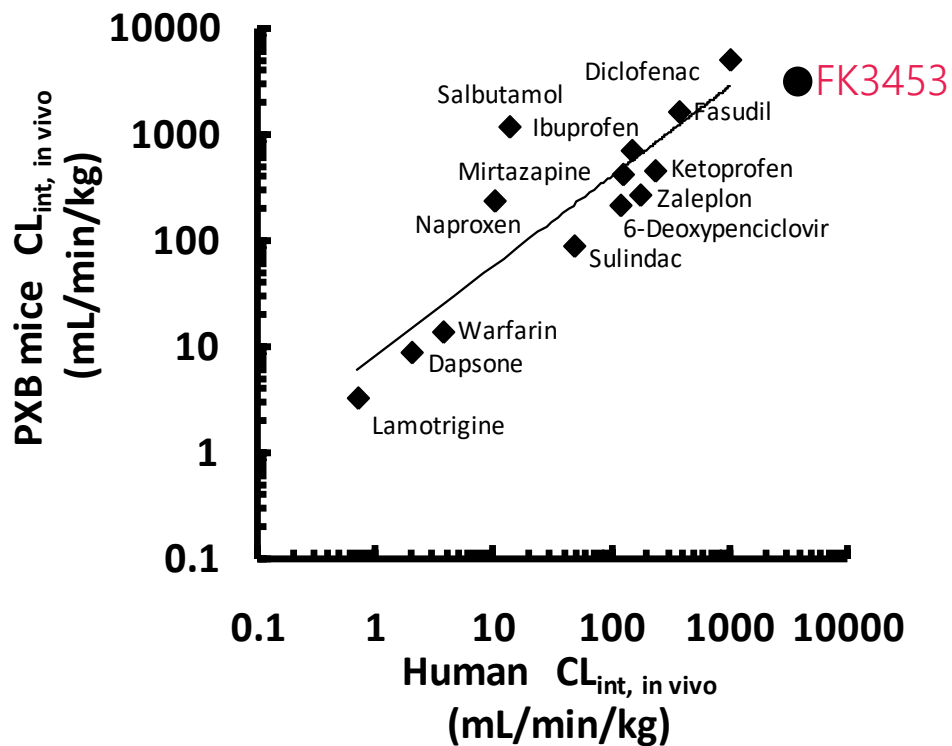
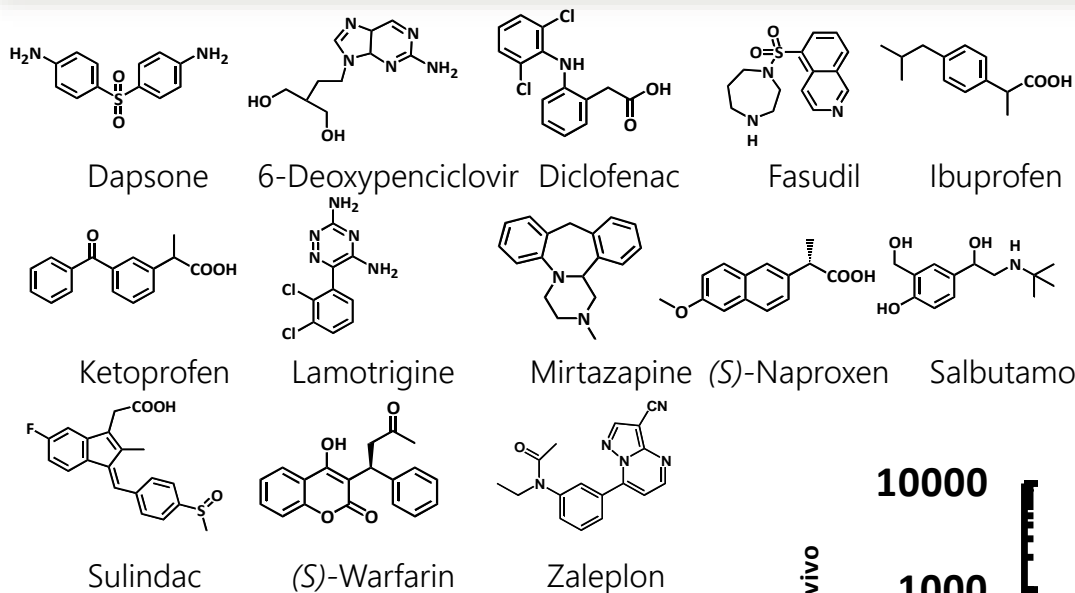
FK3453



Rat hepatocytes



Predictability of clearance using chimeric mice with humanized liver ⁶



<Single species allometric scaling>

$$\text{Predicted } CL_t = CL_{\text{PXB mice}} \times (\text{B.W.}_{\text{human}} / \text{B.W.}_{\text{PXB mice}})^a \text{ (eq.1)}$$

$$\text{Predicted } Vd_{ss} = Vd_{ss \text{ PXB mice}} \times (\text{B.W.}_{\text{human}} / \text{B.W.}_{\text{PXB mice}})^b \text{ (eq.2)}$$

<Complex Dedrick plot >

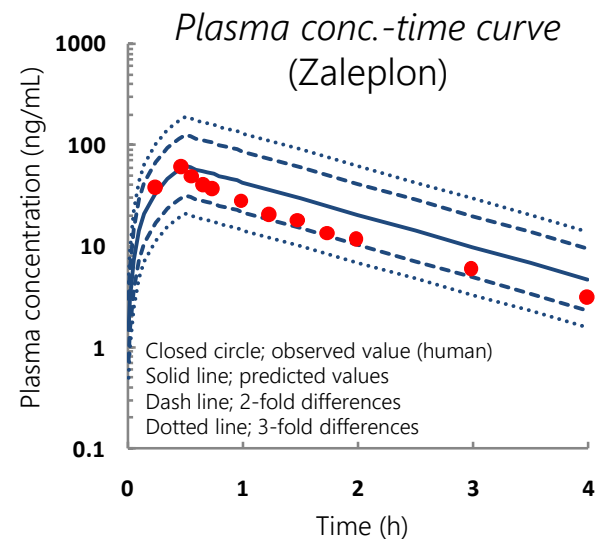
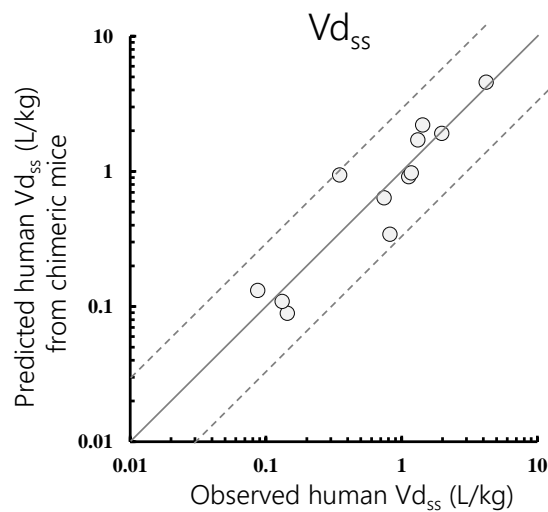
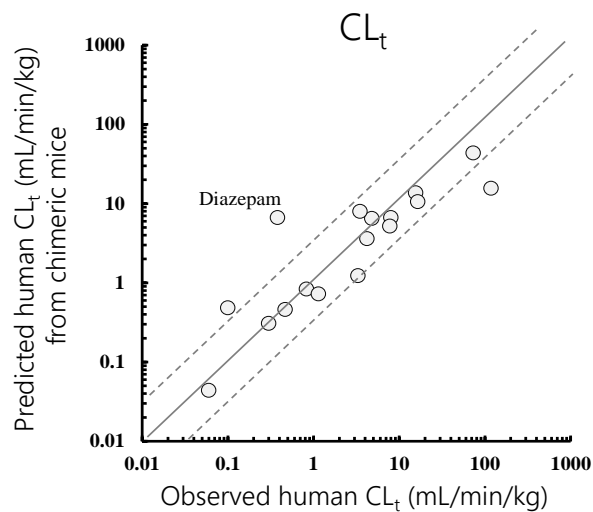
$$\text{Predicted time} = \text{Real time}_{\text{PXB mice}} \times [(\text{B.W.}_{\text{human}})^{b-a} / (\text{B.W.}_{\text{PXB mice}})^{b-a}] \text{ (eq.3)}$$

$$\text{Predicted plasma conc. (C}_p) = C_{p \text{ PXB mice}} \times [(\text{B.W.}_{\text{human}})^{1-b} / (\text{B.W.}_{\text{PXB mice}})^{1-b}] \text{ (eq.4)}$$

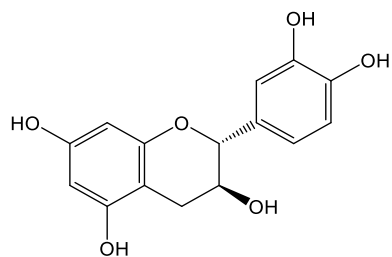
	CL _t		Vd _{ss}	
	Chimeric mice	Rats	Chimeric mice	Rats
Number of compounds	13		8	
Average of exponential values	0.783	0.700	0.894	0.799
AAFE	2.73	6.42	1.62	1.60
Within 3-fold error (%)	84.6	53.8	100	100

Predictability of PK using chimeric mice with humanized liver

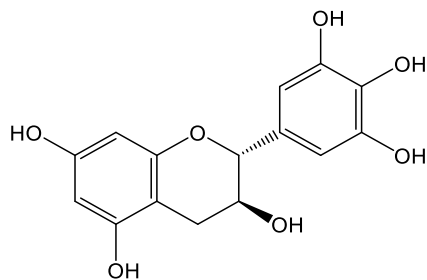
	CL_t	Vd_{ss}
Number of compounds	17	12
Average of exponential values	0.814	0.900
AAFE	2.97	1.50
Within 3-fold error (%)	82.4	100



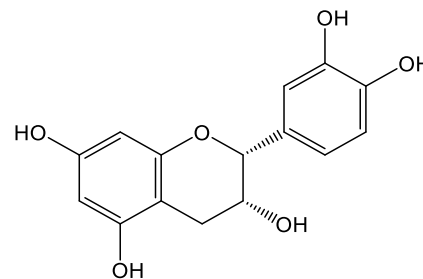
Comparison of inhibitory effects of drugs on AO isoforms



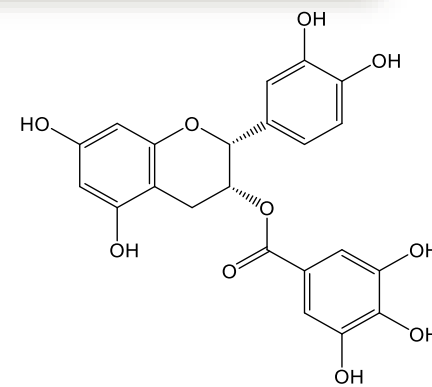
(+)-Catechin
(IC₅₀=21.3μM)



(-)-Epigallocatechin
(IC₅₀=67.3μM)



(-)-Epicatechin
(IC₅₀=20.6μM)

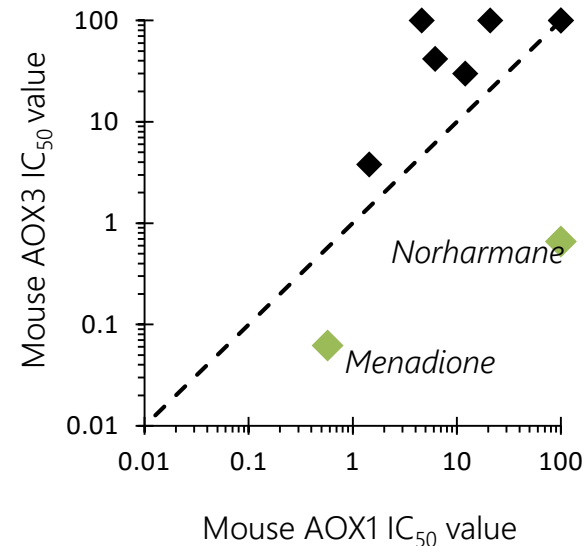
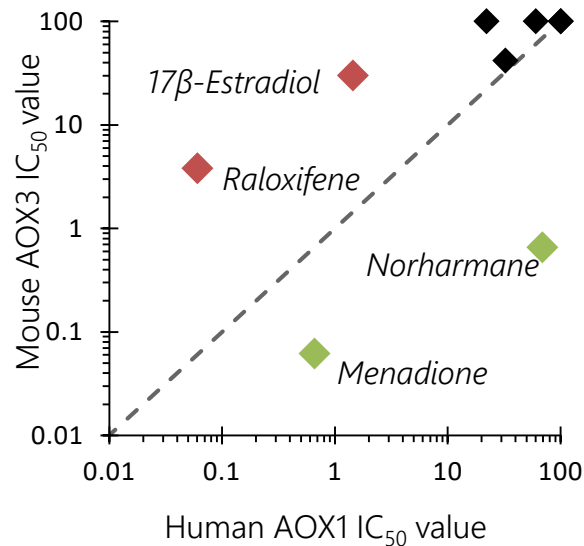
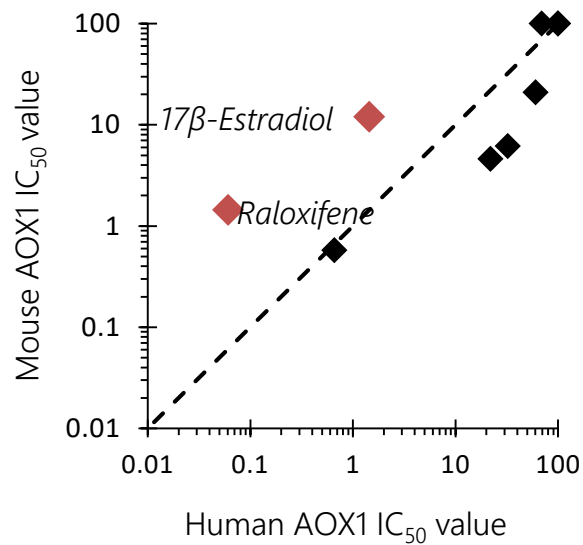


(-)-Epicatechin gallate
(IC₅₀=58.3μM)

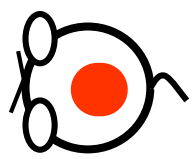
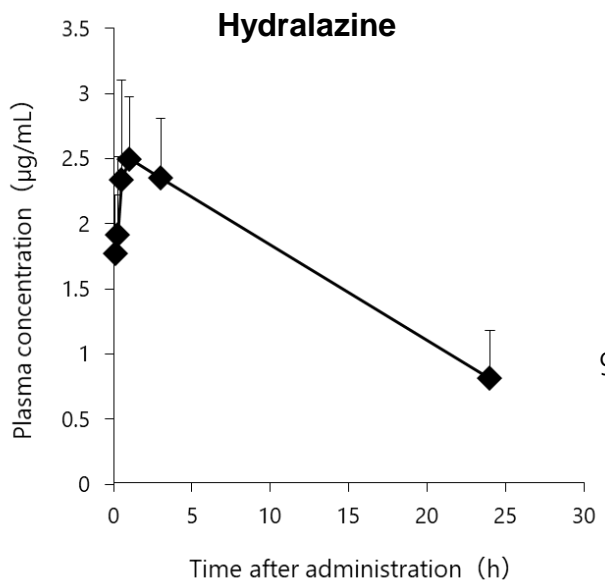
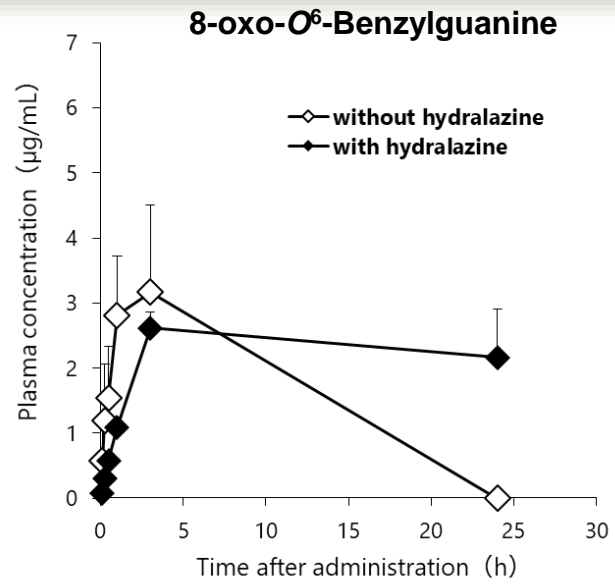
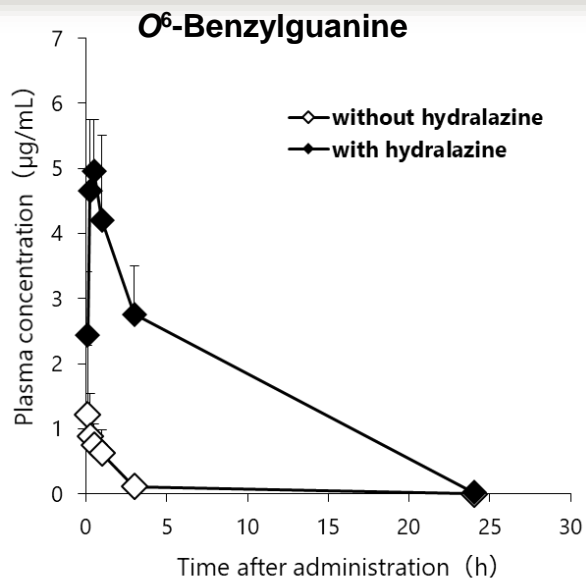
human AOX1 vs. mouse AOX1

human AOX1 vs. mouse AOX3

mouse AOX1 vs mouse AOX3



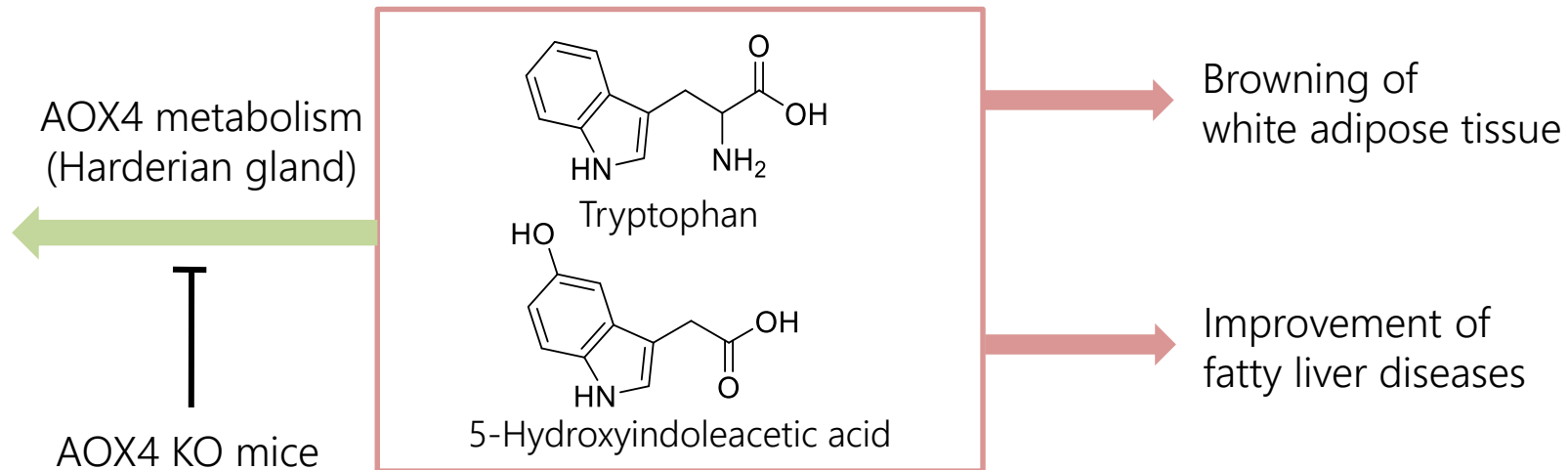
DDI study on AO metabolism using chimeric mice



Chimeric mice with 95.4 % humanized liver

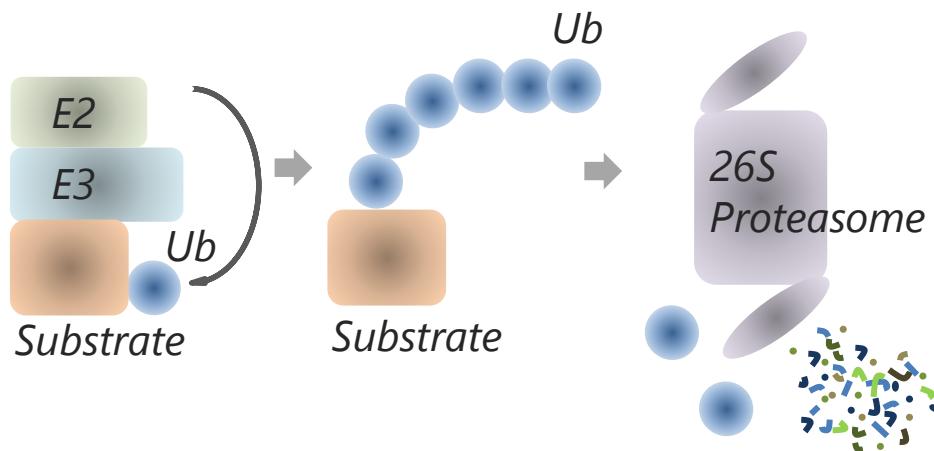
- Orally injection
- 10 mg/kg O⁶-benzylguanaine
- 30 mg/kg hydralazine (1h pre-treatment)

■ Physiological significance



■ Regulation of AOX expression

→ Contribution of ubiquitin proteasome system



Acknowledgement

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