MEDCH 527 1/23/2017

HYDROLASES: Carboxylesterases and Epoxide Hydrolases

References

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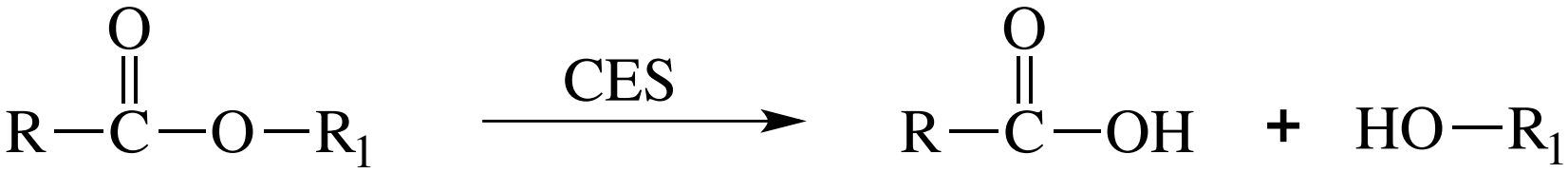
Hydrolases:

Carboxylesterases (CES) [Cholinesterases, Paraoxonase, AADAC]
Epoxide Hydrolases (EPHX)



- CES and EPHX belong to the α/β-Hydrolase-fold structural class of proteins

- CES and EPHX possess active site catalytic triads.

- CES - Ser (Cys) proteases
- EPHX - Asp proteases

Hemmert et al., *Mol Pharmacol*. (2010).

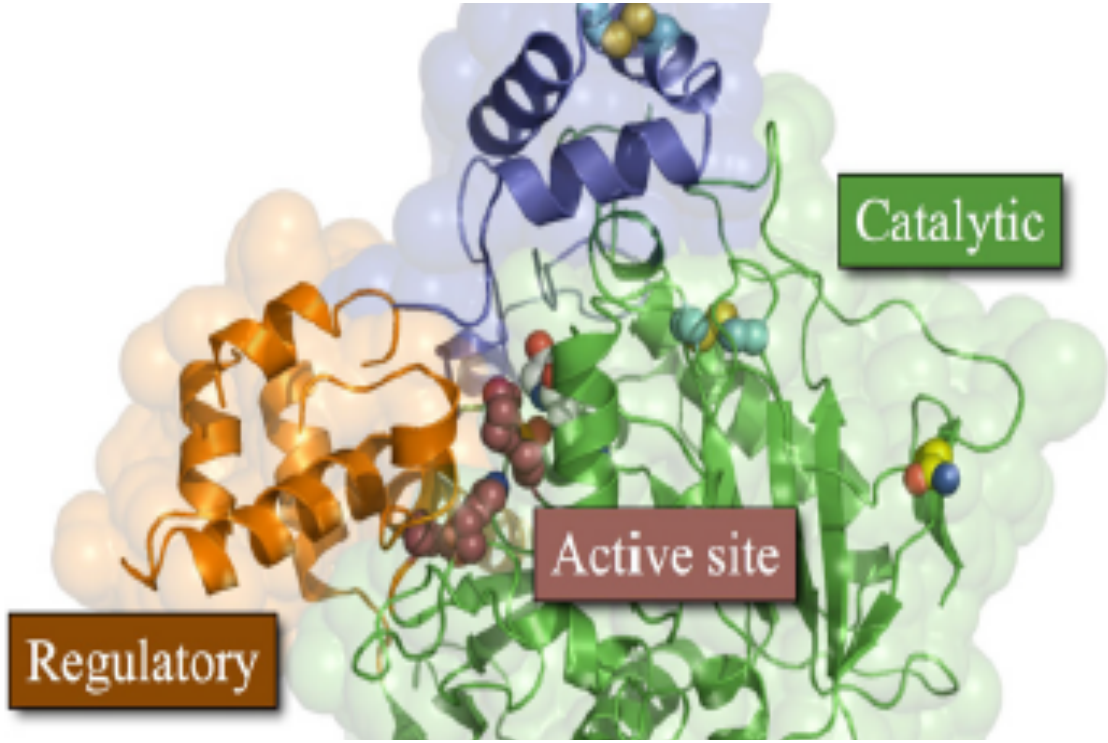


Fig. 2. hCE1 monomer. Each protein monomer has three domains; the α/β domain (blue) forms the trimer interface and caps the active site; the regulatory region (orange) contains the secondary surface binding site (Z-site) and Glu354 of the catalytic triad; and the catalytic domain (green) contains the central β-sheet conserved in serine hydrolases and the two catalytic residues His468 and Ser221. There are two disulfide bonds (cyan), one in the α/β domain and the other in the catalytic domain, and one glycosylation site, at Asn79 (yellow).

Esterases

- A large and diverse group of enzymes that can **hydrolyze peptides, amides and halides, in addition to carboxylesters, thioesters and phosphate esters**.
- **Carboxylesterases** (CES) are important from a clinical viewpoint because ester derivatives of therapeutic agents are widely used as **prodrugs to improve absorption, bioavailability, taste, stability and to prolong duration of action**.
- Some (AChE) have clearly defined endogenous functions, whereas the function of others are emerging (PON1) or remain obscure (BChE and CES).

Esterases: Comparative Tissue Localization and Catalytic Mechanism (simplified)

Intestine →	**Liver** →	**Blood** →	**Brain**	**(Mechanism)**
CES2 (AADAC)	CES1/2 (AADAC)	BChE	AChE	(catalytic triads)
		PON1		(dyad?)
		(Albumin)		(non-specific?)

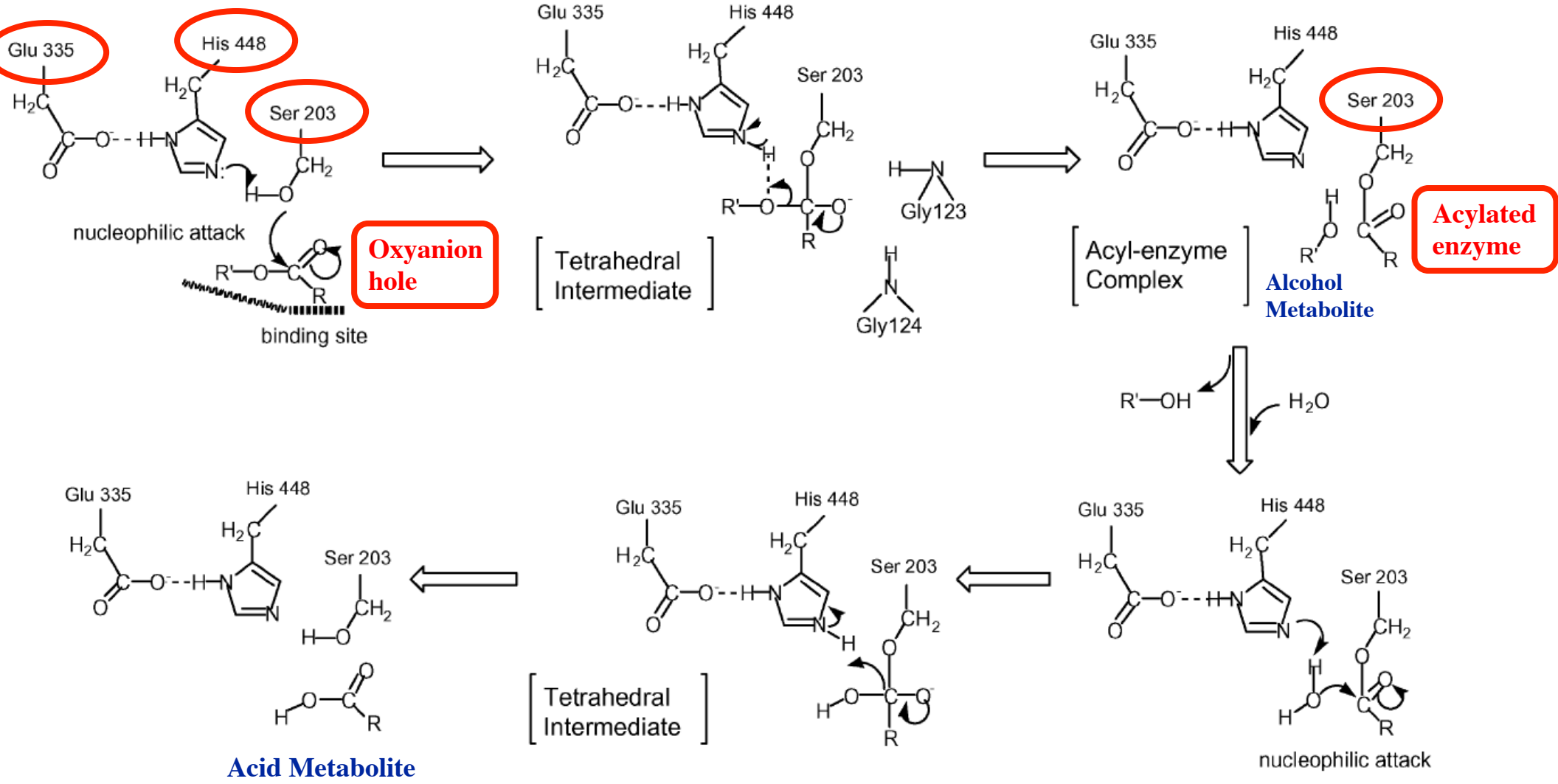
Carboxylesterase (CES) Characteristics

- At least five human genes, CES1, 2, 3, 4A, 5A
- CES1/CES2/AADAC most important for hepatic drug metabolism, share ~50% amino acid identity
- All possess a **catalytic triad** (Ser, Glu/Asp, His) and an oxyanion hole

	Oxyanion Hole Loop	Serine	Catalytic Triad Glu/Asp	Histidine	Serine Codon
AADAC	RGLFYIHGGGWCVG	IGISGDSAGGNLAAAV	ITCQYDLLRDDG	VEDGFHGAFSFLG	AGT
AADACL-1	RSVVYIHGGGWALA	ICISGDSAGGNLAAAL	LTCEHDVLRDDG	FEDGFHGCMIFTS	AGT
AADACL-3	PGIVYYHGGGGVMG	VVVCGDSFGGAIAAVV	VSCEYDALRDNS	MEDGFHGVLRTID	AGT
AADACL-4	RGIIFYHGGATVFG	VVVCGESVGGAAVAAI	VSCENDILRDDS	LYDGFHGSIIFFD	AGC
AChE-E	PVLVWIYGGGFYSG	VTLFGESAGAASVGMH	GVVKDEGSYFLV	WMGVPHGYEIEFI	AGC
AChE-S	PVLVWIYGGGFYSG	VTLFGESAGAASVGMH	GVVKDEGSYFLV	WMGVPHGYEIEFI	AGC
AChE-R	PVLVWIYGGGFYSG	VTLFGESAGAASVGMH	GVVKDEGSYFLV	WMGVPHGYEIEFI	AGC
BChE	-VLIWIYGGGFQTG	VTLFGESAGAASVSLH	GVNKDEGTAFLV	WMGVMHGYEIEFV	AGT
CE1	PVMVWIHGGGLMVG	VTIFGESAGGESVSVL	GINKQE-FGWLI	TVIGDHGDELFSV	TCA
CE2	PVMVWIHGGALVFG	VTIFGESAGGTSVSSL	GVNNNE-FGWLI	HMKADHGDELPFV	TCT
CE3	PVMVWVHGGALITG	VTVFGGSAGGSIISGL	GVNNHE-FSWLI	WVKADHGAEGAFV	TCT
CE4	PVLVWFPGGAFKTG	VTIFGESAGAISVSSL	GVNNHE-CGFLL	FVKADHADEVRFV	TCC
CE5	PVMVWFPGGAFIVG	VTLFGQSAGAMSISGL	GVNNLE-FNWLL	TDGADHGDEMYFL	TCG
CEL-1	PVMIWIYGGAFLMG	ITLFGESAGGASVSLQ	GTNNMDGHIFAS	WVGADHADDIQYV	TCT
CEL-2	PVMIWIYGGAFLMG	ITLFGESAGGASVSLQ	GTNNMDGHIFAS	----------	TCT

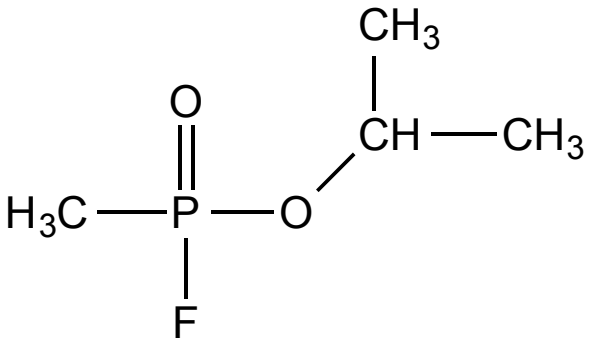
Fig. 5. Alignment of amino acid residues surrounding the oxyanion hole loop and the catalytic triad for arylacetamide deacetylase (AADAC), arylacetamide deacetylase like (AADACL), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), carboxylesterase (CE), and carboxyl ester lipase (CEL). Also included is the nucleotide sequence of the serine codon. All sequences are of the human forms.

Carboxylesterase Catalytic Cycle

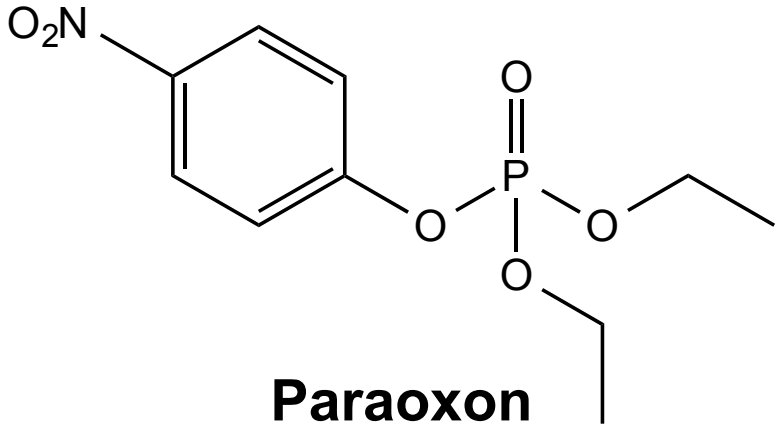


Satoh and Hosokawa, 2006

Esterase Classification by OP Interactions (Aldridge, 1953)



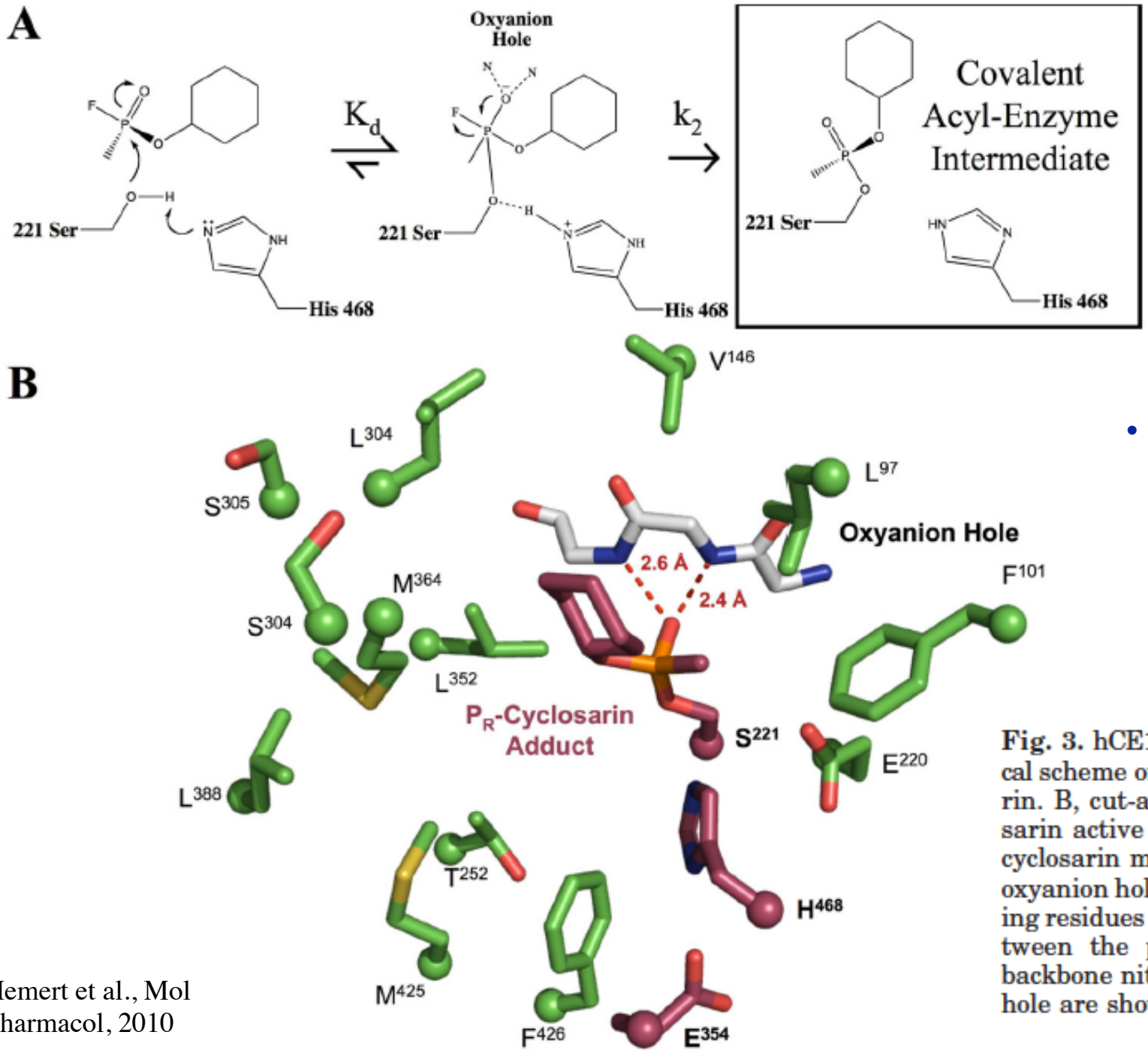
Sarin



Paraoxon

- A-esterases: e.g. paraoxonase (PON1) hydrolyzes organophosphates (OPs)

- B-esterases: e.g. carboxylesterases (CES) and cholinesterases BChE/AChE) are irreversibly inhibited by OPs



- **In A, during k2, instead of alcohol formation, F^- is displaced.**

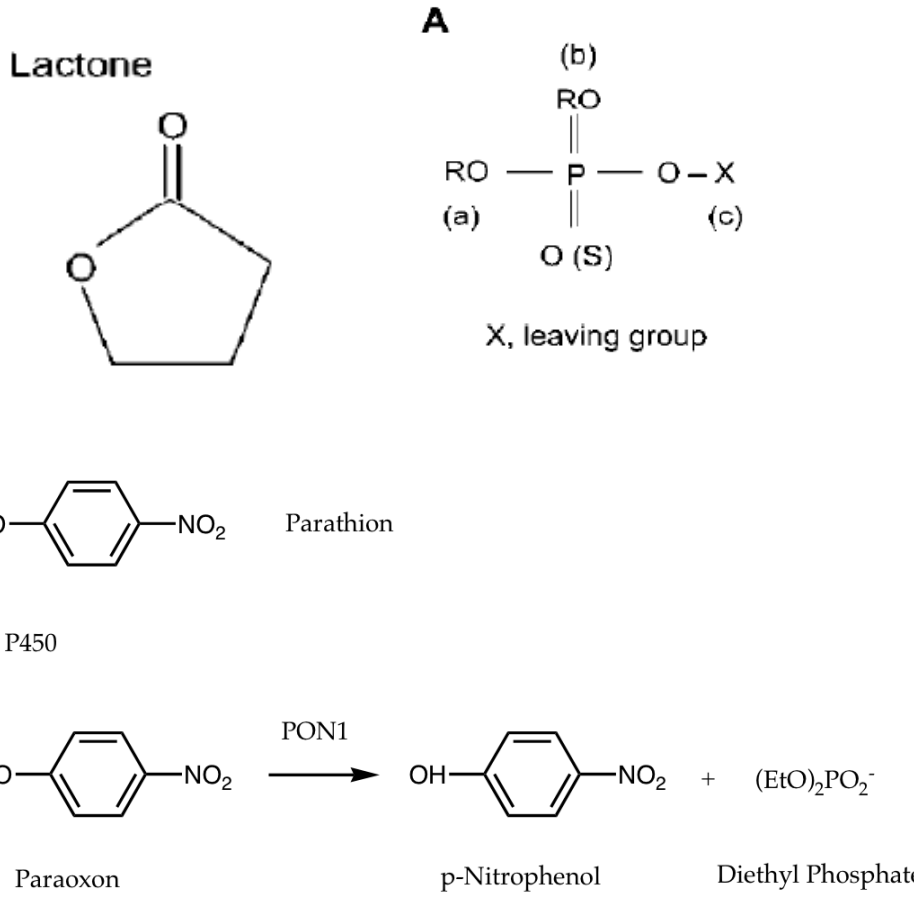
Fig. 3. hCE1-cyclosarin complex. A, chemical scheme of hCE1 reacting with cyclosarin. B, cut-away view of the hCE1-cyclosarin active site. The catalytic triad and cyclosarin molecule are shown in purple, oxyanion hole in white, and the surrounding residues in green. Hydrogen bonds between the phosphoryl oxygen and the backbone nitrogen atoms in the oxyanion hole are shown in red.

Hemert et al., Mol Pharmacol, 2010

Paraoxonase (PON1)

• PON1 native activity is that of a lactonase, but also (fortuitously) metabolizes phosphoric acid triesters (A), many of which are toxic organophosphates (OP).

• OPs are widely used as pesticides applied as relatively non-toxic sulfur derivatives which are bioactivated by P450 to the oxon forms.

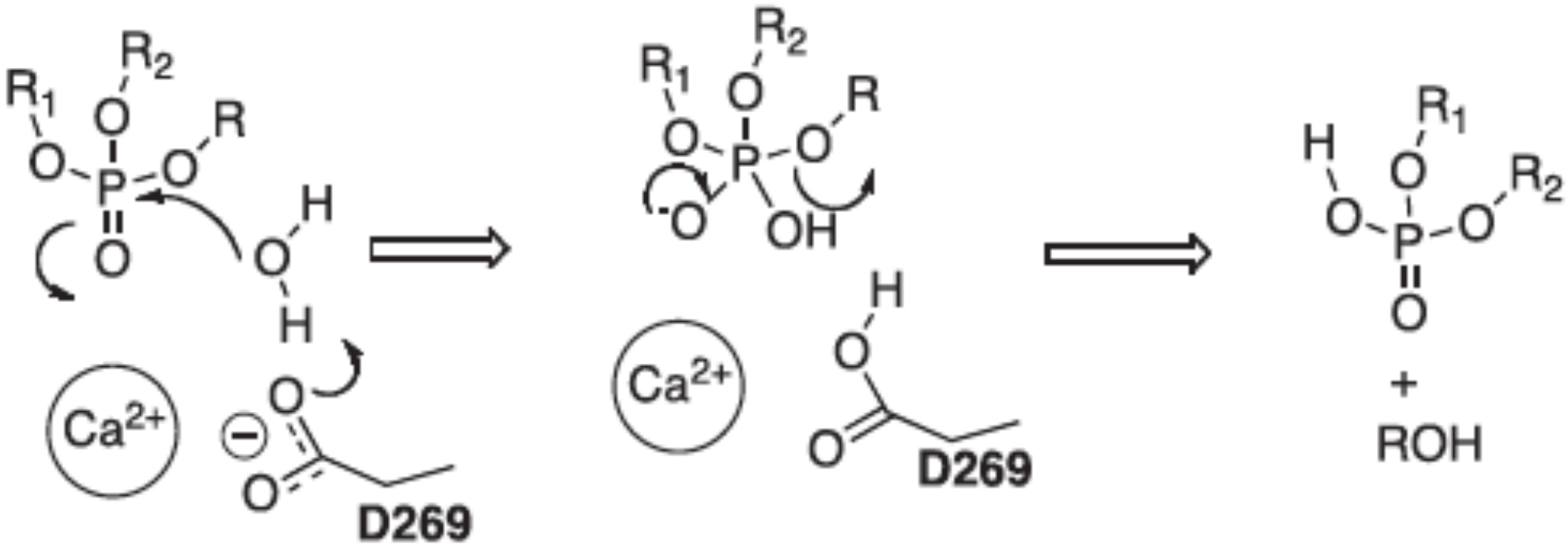


• OP pesticides are usually applied as the non-toxic sulfur derivatives, which are bioactivated by P450 to the oxon forms. The oxon binds irreversibly to acetylcholinesterase causing neurotoxicity.

• PON1 neutralizes this effect by hydrolyzing the oxon.

PON Mechanism (aspartyl protease)

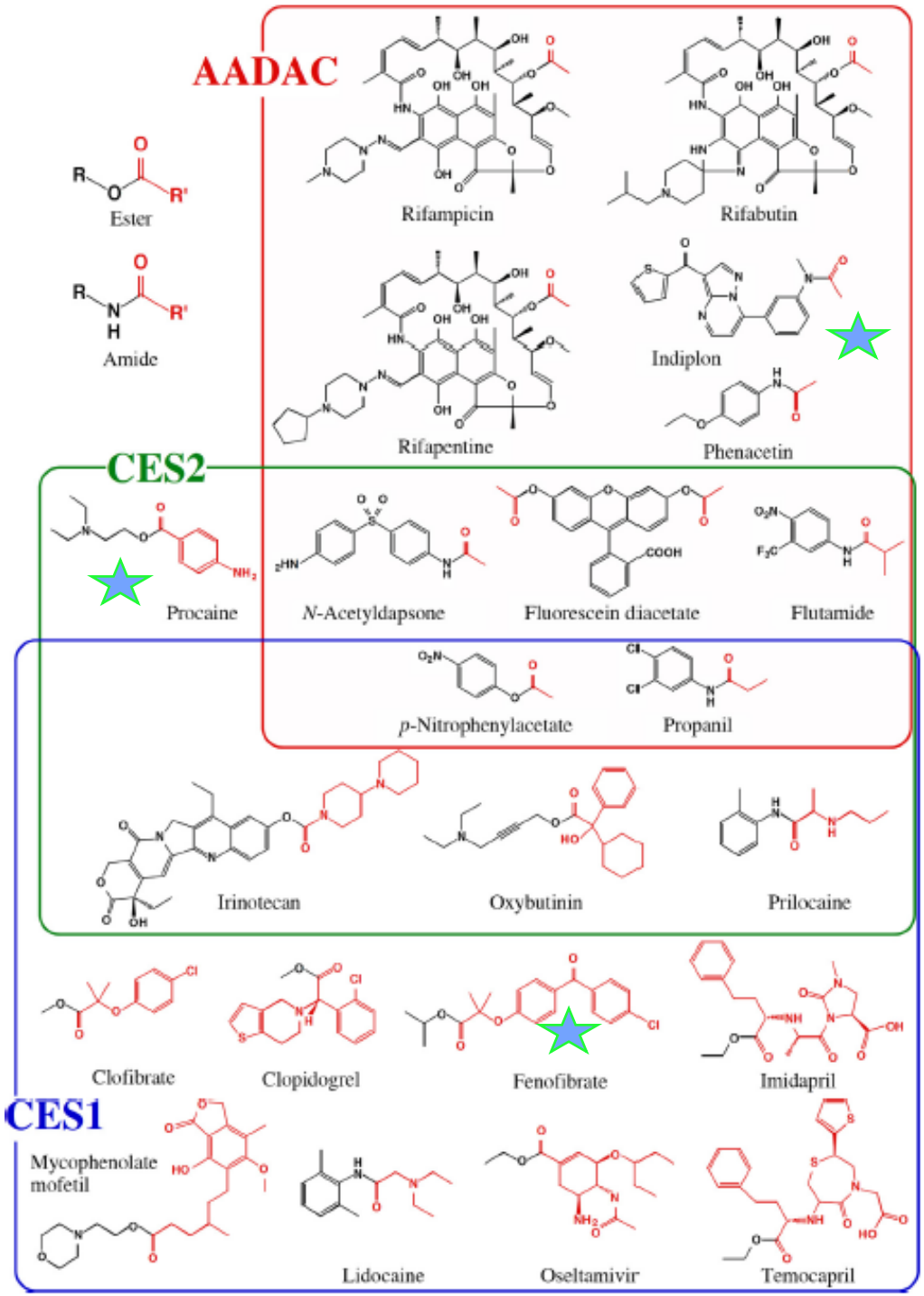
Muthukrishnan et al., J. Org. Phys. Chem. (2012)



- Phosphotriester hydrolysis by activated water molecule
- No active site Ser to attack substrate, no acylated PON intermediate
- Asp269 and possibly another His residue appear to be important catalytic residues
- Calcium ions required!

Other Biochemical Features of CES

- Found ubiquitously, but concentrated in the **small intestine, liver,** and lung
- Mainly microsomal localization (no cofactors)
- ~60 kDa mass; glycosylation necessary for activity – facilitates trimer formation.
- Large hydrophobic substrate binding pocket (15 A° deep)
- Proper orientation of carbonyl carbon into oxyanion hole leads to structural restrictions on substrates:
  - CES1 prefers esters with small alcohol group
  - CES2 prefers esters with large alcohol and small acyl groups
  - AADAC (closer to CES2)

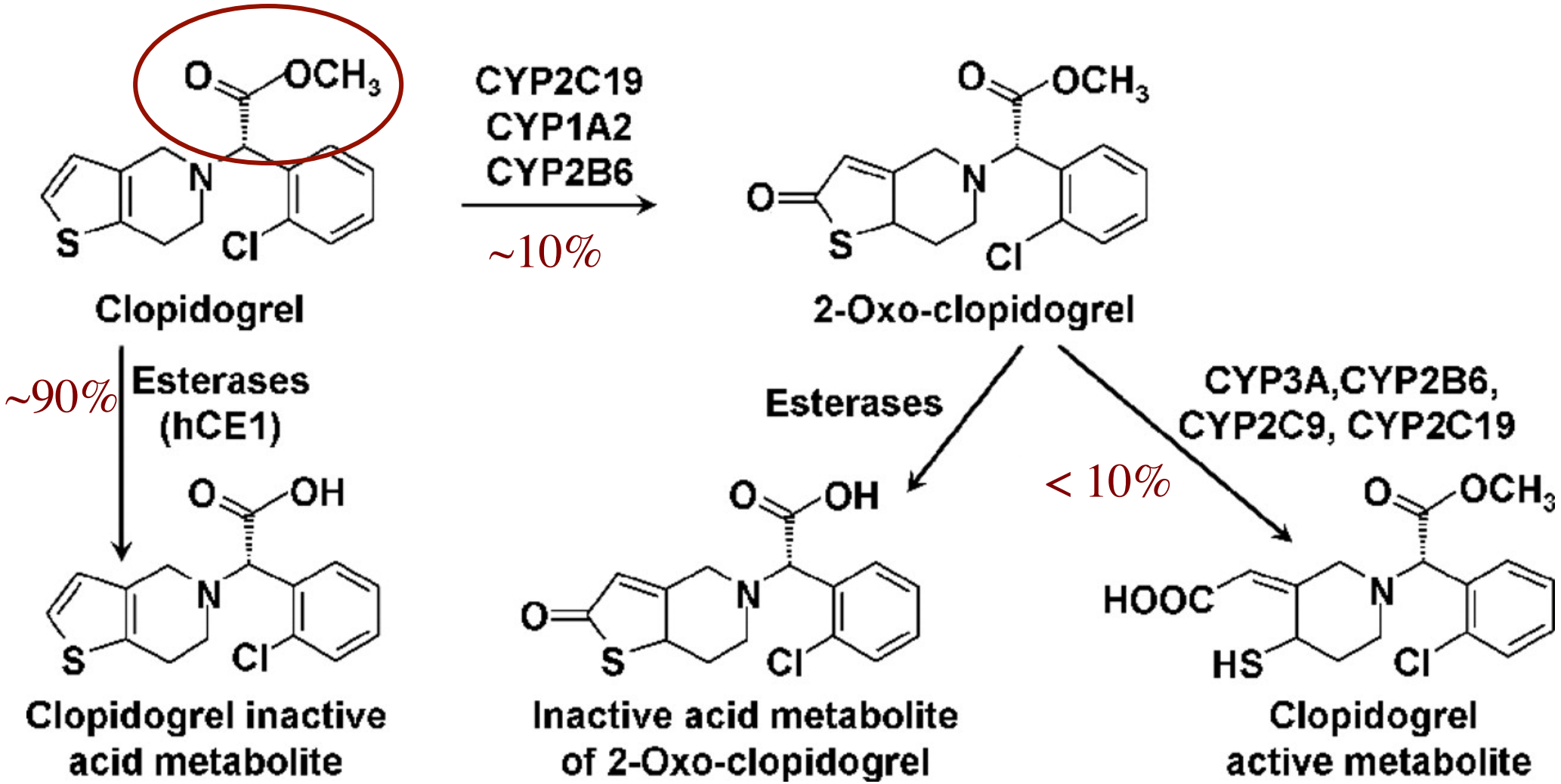


- CES1, CES2 and AADAC demonstrate overlapping substrate selectivities.
- Fenofibrate and several other substrates demonstrate high selectivity for CES1.
- Procaine appears to be a selective substrate for CES2.
- Indiplon, and a few other substrates appear to be selective for AADAC.

CES Catalyze Both Activation and Inactivation Rxns

	CES1	CES2
Hydrolysis to active metabolite	Oseltamivir	Prasugrel[a]
	Benazepril	Irinotecan
	Quinapril	Lovastatin
	Imidapril	Simvastatin
Hydrolysis to inactive metabolite	Clopidogrel	Cocaine
	Methylphenidate	Aspirin
	Meperidine	

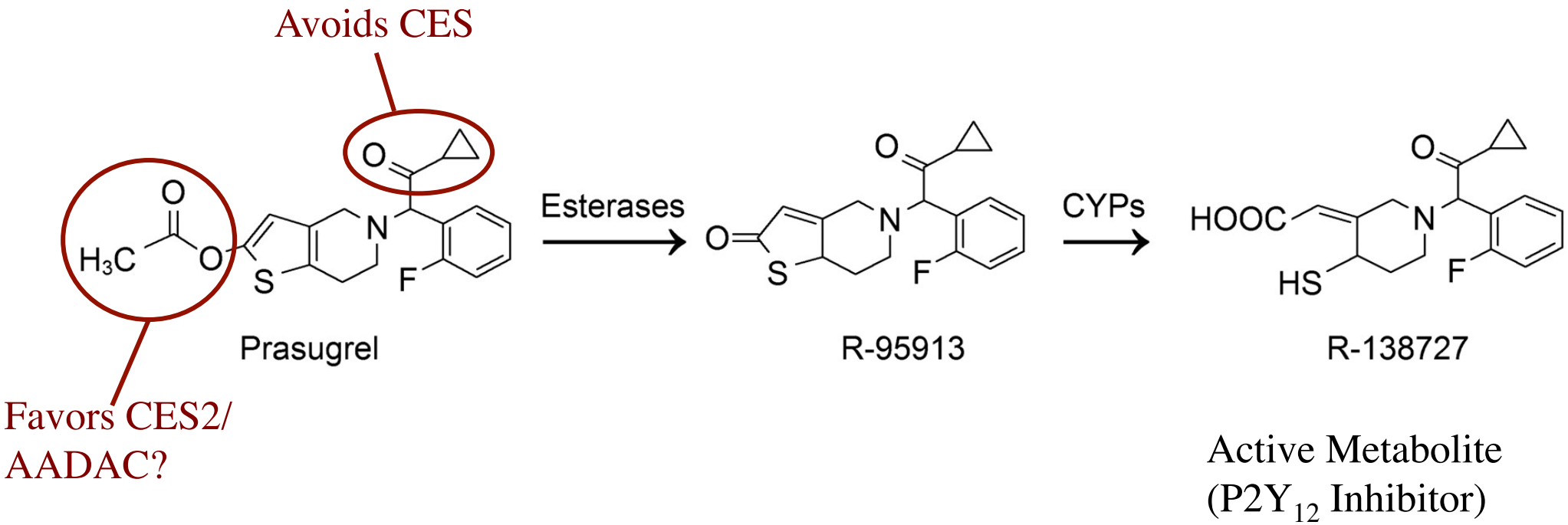
[a] Prasugrel is hydrolyzed to an inactive metabolite that is the precursor of the active moiety.



- Clopidogrel, a thienopyridine prodrug ($P2Y_{12}$ inhibitor), is well-absorbed; metabolism occurs in the liver and the "inactivation pathway dominates.

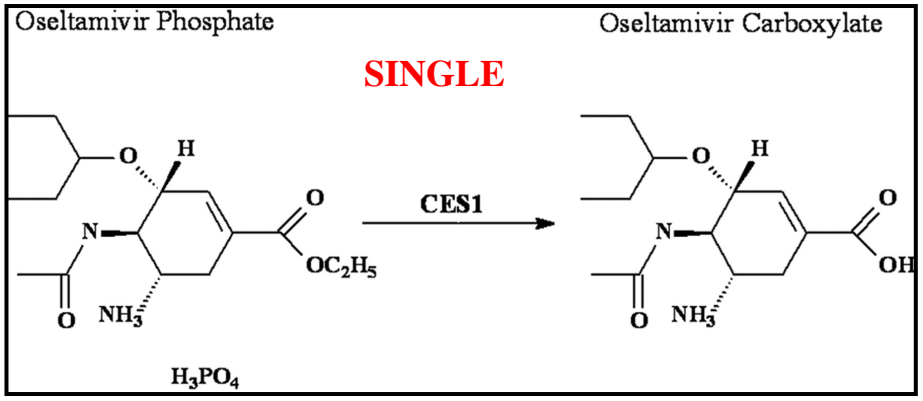
Farid et al, J Clin Pharmacol, 2010

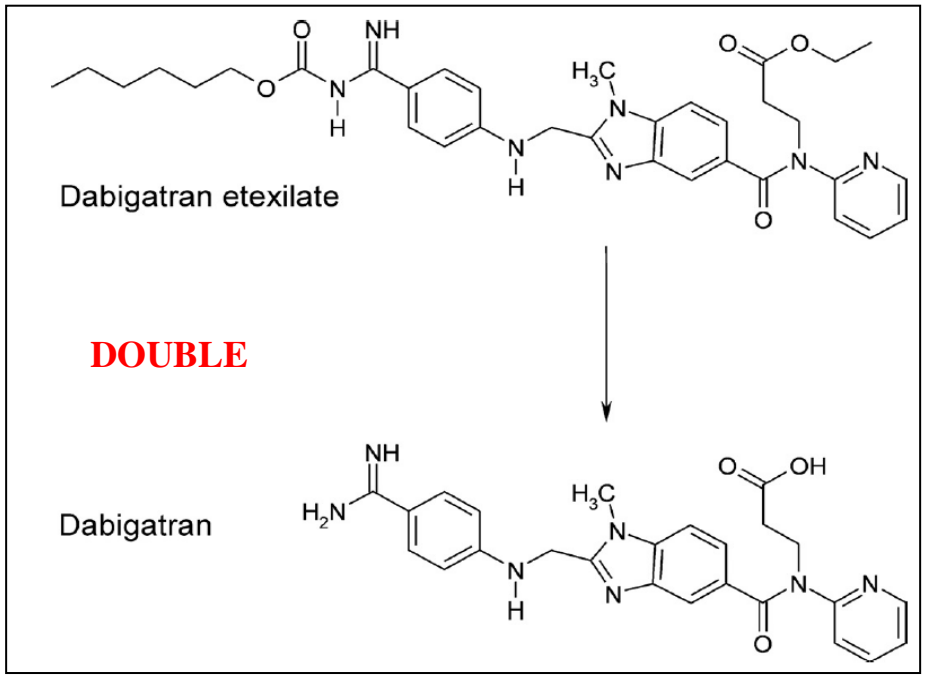
Metabolism of Prasugrel to Active Metabolite

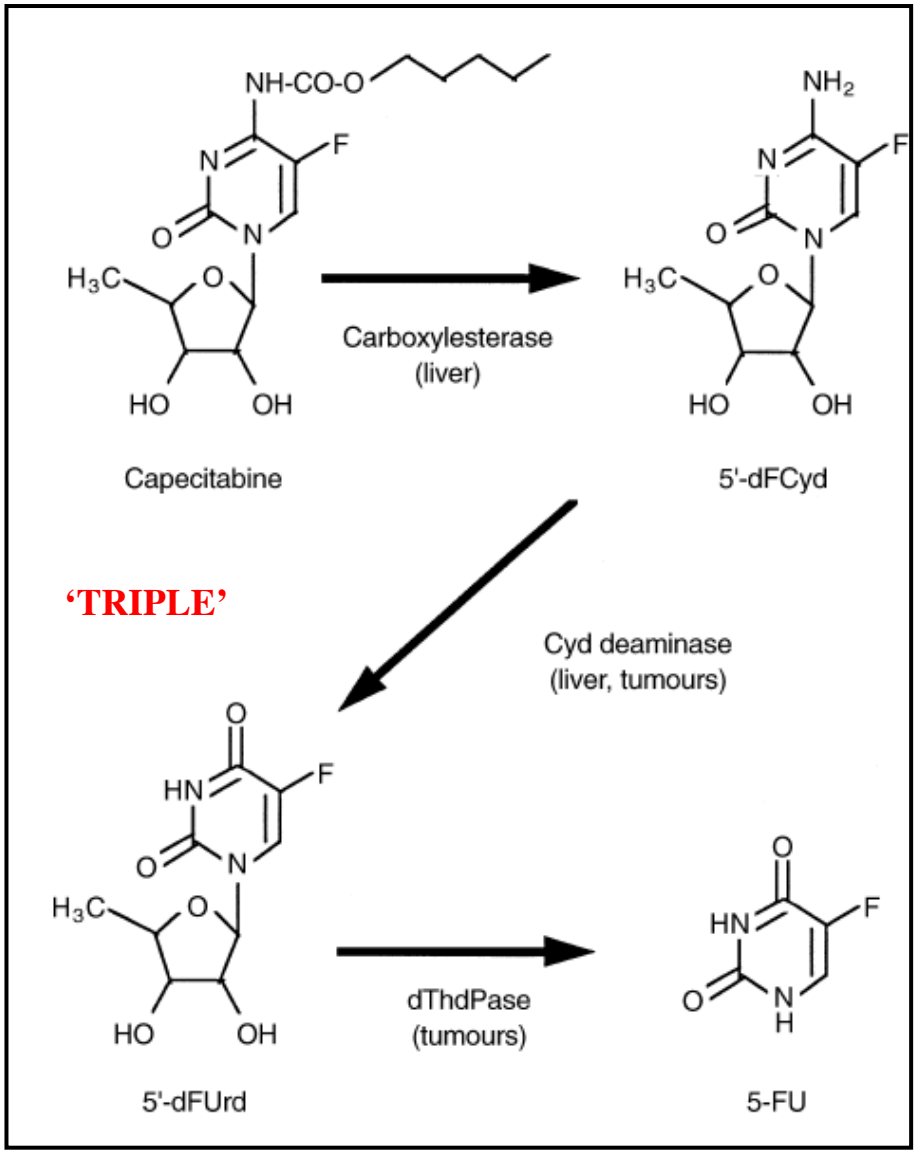


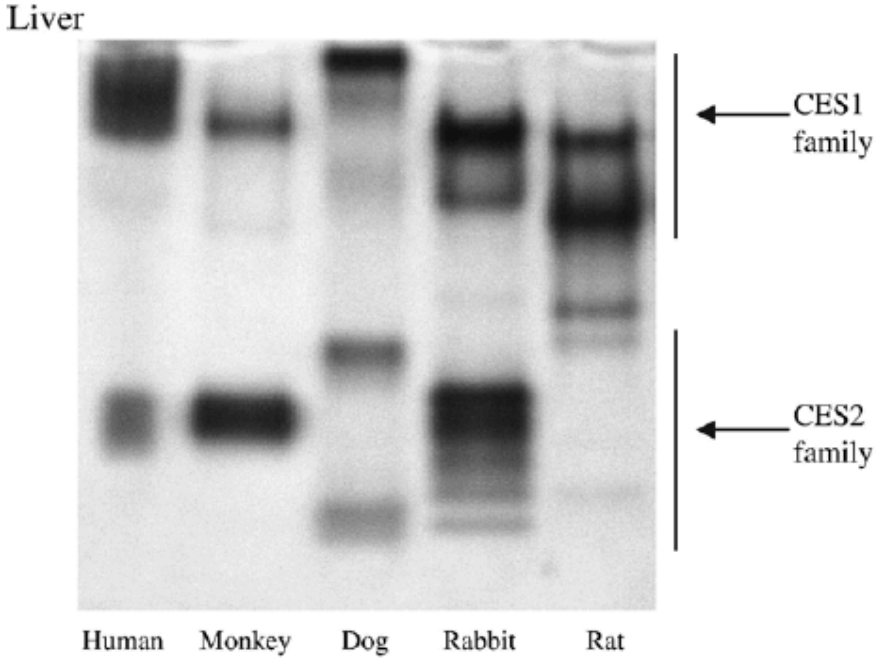
- Daiichi/Lilly developed prasugrel as an alternative to clopidogrel, avoiding sequential P450 oxidations to reach the active metabolite.

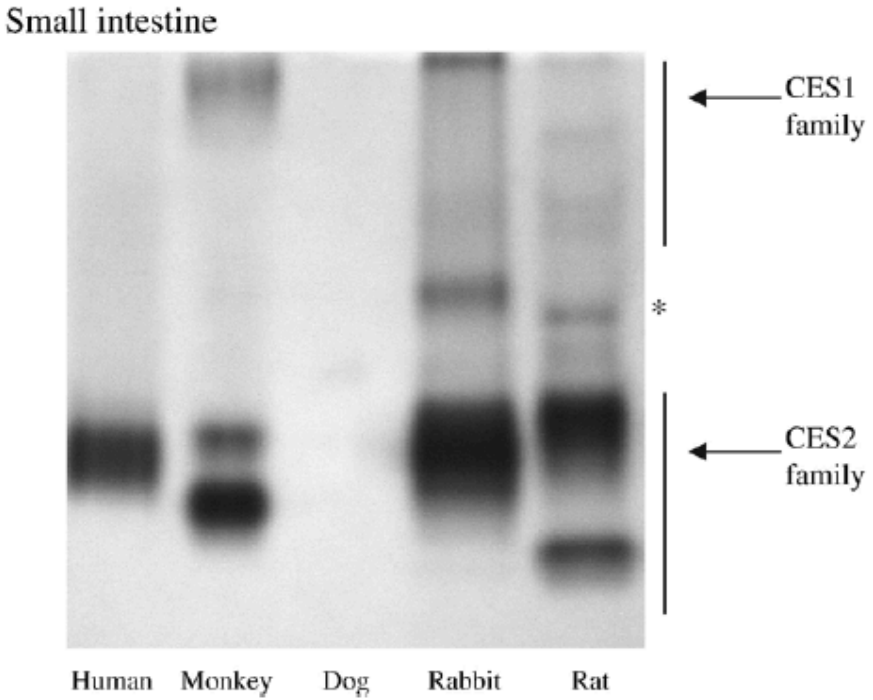
Other Examples of Prodrug Bioactivation by CES









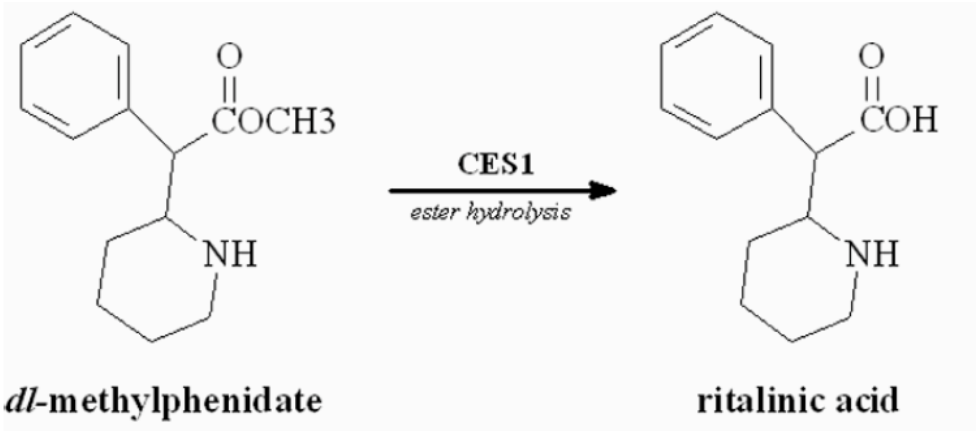


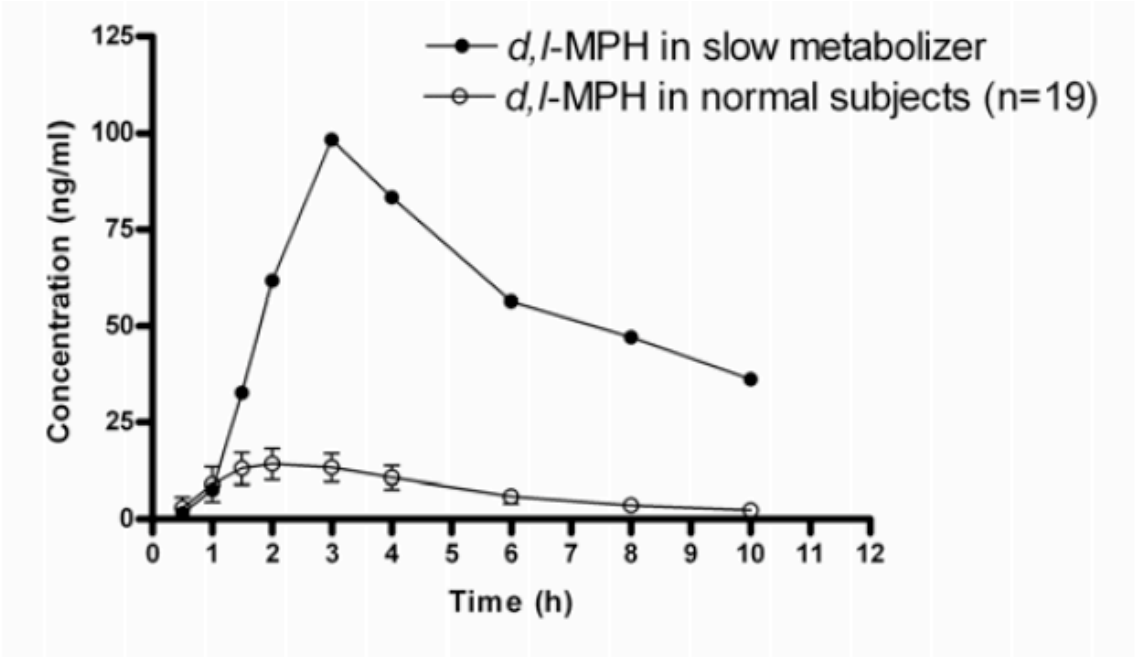
Tissue and Species Comparisons

- CES1 and CES2 present in liver, but CES1 dominates
- CES2 in the intestine
- CES1 and CES2 proteins found in all toxicity testing species, except notably the beagle dog, which lacks CES2 in the intestine.

Taketani et al, 2007

Genetic Variation: CES1 and Methylphenidate

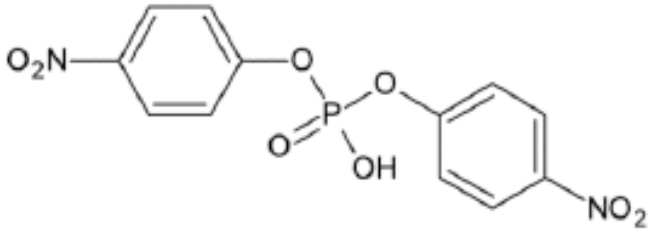




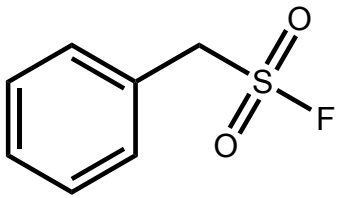
Zhu et al., Am. J Hum Genet. 2008

- Methylphenidate used for ADD
- Atypical pharmacokinetic profile following racemic methylphenidate dosed to healthy volunteers led to the discovery of two coding SNPs affecting CES1 function: **Gly143Glu**; Asp260fs.
- Low allele frequencies; 0.01-4%, but potential profound effects
- **Gly143 is a residue in the oxyanion hole**
- No sig. CES2/AADAC genetic variation known

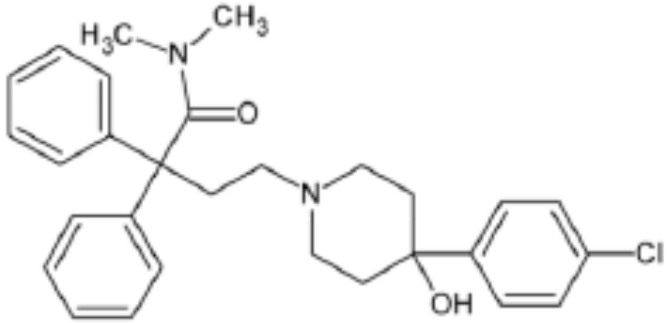
Diagnostic CES Inhibitors



Bis(4-nitrophenyl) phosphate (BNPP)



PMSF

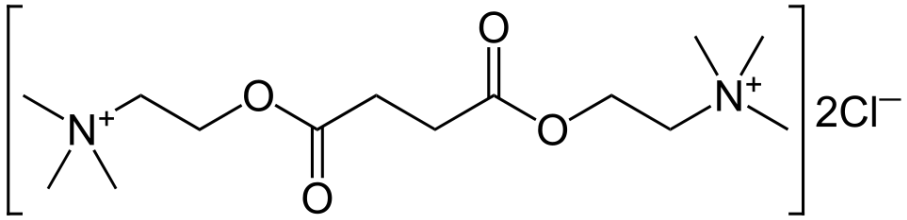


Loperamide

- BNPP (bis 4-nitrophenylphosphate) used at 0.1 mM is a general, non-selective, CES1, CES2 and AADAC inhibitor
- PMSF is a general serine protease inhibitor, but does not inhibit AADAC at 0.1 mM.
- Loperamide is a selective CES 2 inhibitor (Ki = 1.5 uM).

Hatfield and Potter, Exp. Op. Ther. Pat. (2011)

BChE Polymorphisms and Drug Response



Succinylcholine (muscle paralysis)

- Era of pharmacogenetics ushered in by the discovery that patients with *BChE* gene mutations could not metabolize succinylcholine, and so could not breathe on their own for several hours following a dose intended to paralyze for only a few minutes.
- Observed in ~ 1/1800 patients.

BChE: Butyrylcholinesterase

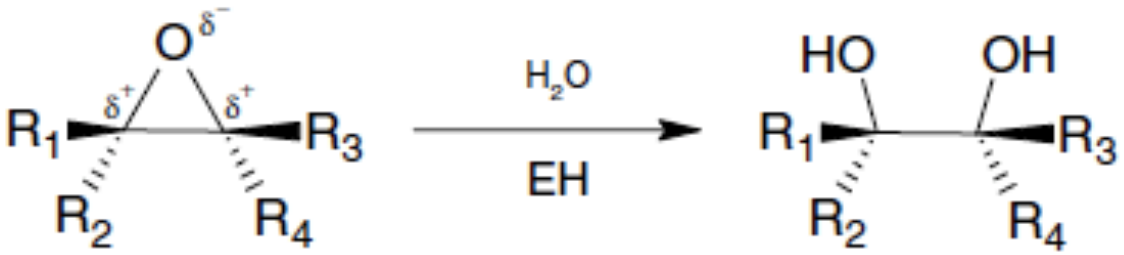
- 4-Carbon acyl chain optimum, contrasts with AChE (2-carbon)
- BChE tolerates fairly sizeable and lengthy alcohol chains, e.g. benzoyl and naphthyl esters (methylprednisolone, festolol)
- Dominant drug metabolizing esterase in the eye
- Ubiquitous distribution, but a key site is the blood (plasma cholinesterase)

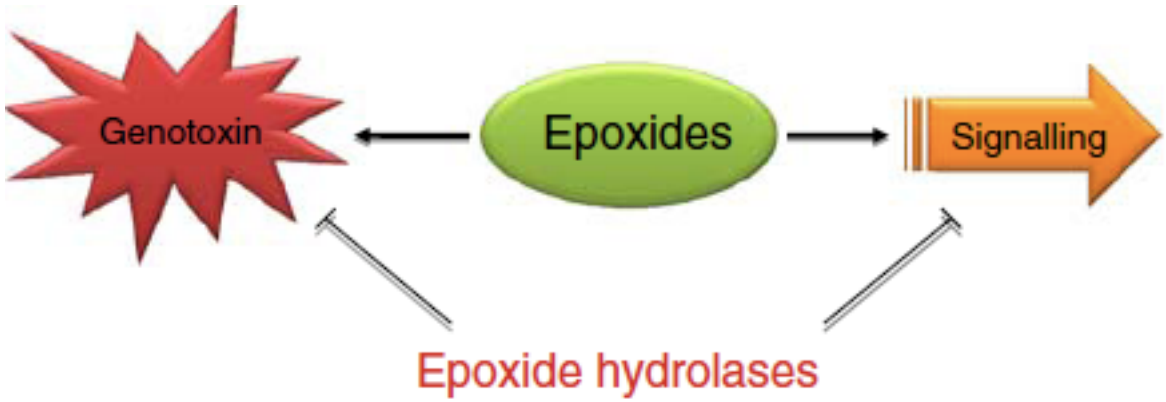
Plasma Esterases

Table 1
Clinically relevant compounds hydrolyzed by human plasma esterases

Drug action	BChE	PON1	Albumin
Analgesic	Aspirin		Aspirin
Analgesic	Isosorbide diaspirinate		
Anti-asthma	Bambuterol		
Local anesthetic, vasoconstrictor	(−)-Cocaine		
Anti-cancer	Irinotecan (CPT-11)		
Beta-blocker	Flestolol		Flestolol
Alpha-blocker, erectile dysfunction	Moxisylyte		
Stimulates eating	*n*-Octanoyl ghrelin		
Analgesic	Heroin		
Local anesthetic	Procaine		
Muscle relaxant	Succinylcholine		
Muscle relaxant	Mivacurium		
Anti-inflammatory	Methyl prednisolone acetate		
Local anesthetic	Chloroprocaine		
Local anesthetic	Tetracaine		
Diuretic		Spironolactone	
Lower cholesterol		Lovastatin, mevastatin, simvastatin	
Pesticide		Paraoxon	Paraoxon
Pesticide		Diazoxon	
Nerve agent		Sarin	
Antibacterial		Prulifloxacin	
Anti-asthma		Glucocorticoid-lactones	
Anticholinesterase		Mono(diethylphosphoryl) obidoxime	
Antabuse			Disulfiram
Anti-cancer			Cyclophosphamide
Anti-inflammatory			Ketoprofen glucuronide
Topical analgesic			Nicotinate esters
Pesticide			Carbaryl
Insecticide			*O*-Hexyl *O*-2,5-dichlorphenyl phosphoramidate

EPOXIDE HYDROLASE: Roles in Xenobiotic Metabolism and Cell (Lipid) Signaling





Epoxide hydrolases (EHs) typically catalyze formation of **vicinal** diols.

Microsomal EH (EPHX1) both detoxifies (reactive) xenobiotic epoxides, but also has a role in roxicity

Soluble EH (EPHX2) terminates the **signaling action** of endogenous (lipid) epoxides.

Mammalian Epoxide Hydrolases

Gene description present	Recommended	Protein description recommended
EPHX1 (HYL1)	EPHX1	Microsomal epoxide hydrolase, mEH
EPHX2 (HYL2)	EPHX2	Soluble epoxide hydrolase, sEH
ABHD9	EPHX3	Epoxide hydrolase 3, EH3
ABHD7	EPHX4	Epoxide hydrolase 4, EH4
Peg1/MEST	(EPHX5, possibly)	MEST

Peg1/MEST should only be termed EPHX5 when the MEST protein has confirmed epoxide hydrolase activity. We do not recommend involving non-α/β hydrolase fold EHs, e.g. Leukotriene A_4 hydrolase (LTA_4H), or other mammalian EHs like Hepoxilin A_3 hydrolase and Cholesterol epoxide hydrolase (ChEH) in the respective nomenclature, unless sequence information is available

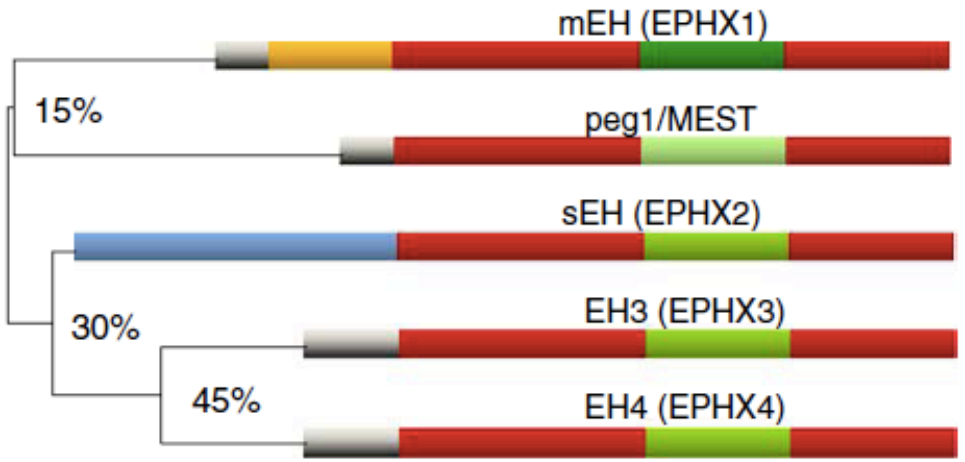


Fig. 2 Phylogenetic tree of mammalian epoxide hydrolases. Protein sequence comparison of human epoxide hydrolases sEH, mEH, EH3, EH4 as well as MEST in their α/β hydrolase fold domains (displayed in *red*). The percent sequence identity is indicated at the branches. The lid domains are coloured in *green*. All proteins contain variable N-terminal extension, such as the phosphatase domain (*blue*) in case of the soluble epoxide hydrolase, membrane anchors (*grey*) or the N-terminal meander of microsomal epoxide hydrolase (*yellow*)

<u>Others:</u>

LTA4 hydrolase

Cholesterol epoxidase

Hepoxilin and trioxilin epoxidases

Decker et al, Arch. Toxicol. 2009

Microsomal Epoxide Hydrolase (mEH, EPHX1)

- mEH is widely distributed across tissues with the highest levels found in the liver. The enzyme is encoded by a single gene, and cross-species amino acid homologies are about 90%.

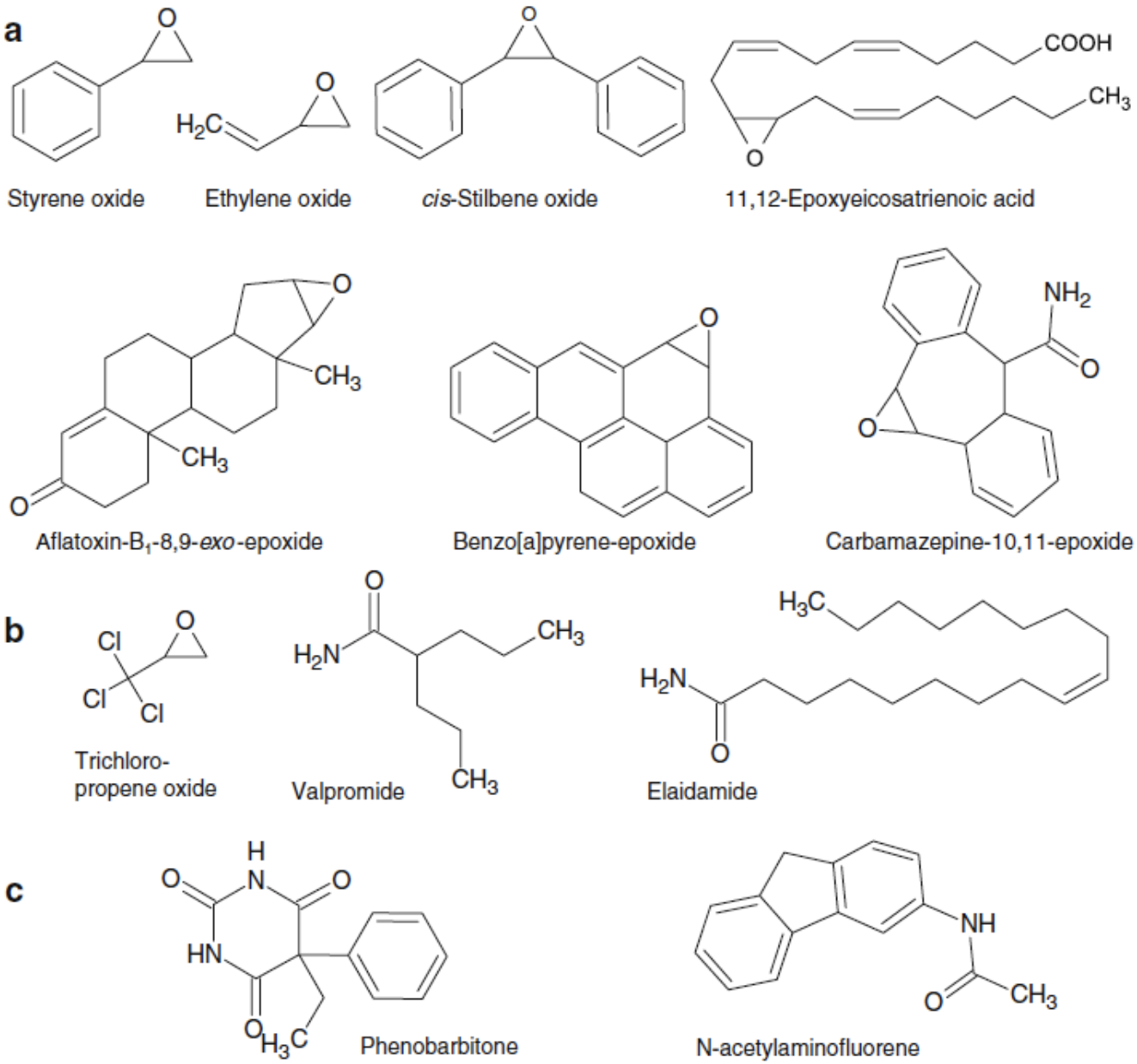
- mEH bears practically no primary structural similarity to sEH and has yet to be crystallized.

- mEH is a remarkably stable enzyme which hydrates a variety of alkene and arene oxides. In most instances mEH acts as a detoxifying enzyme by converting electrophilic epoxides to vicinal trans-dihydrodiol metabolites which are much less reactive. mEH exhibits a broad substrate specificity with a preference for hydrophobic epoxides without extensive substitution.

- Styrene oxide and cis-stilbene oxide are common substrate probes the *in vitro* determination of mEH activity.

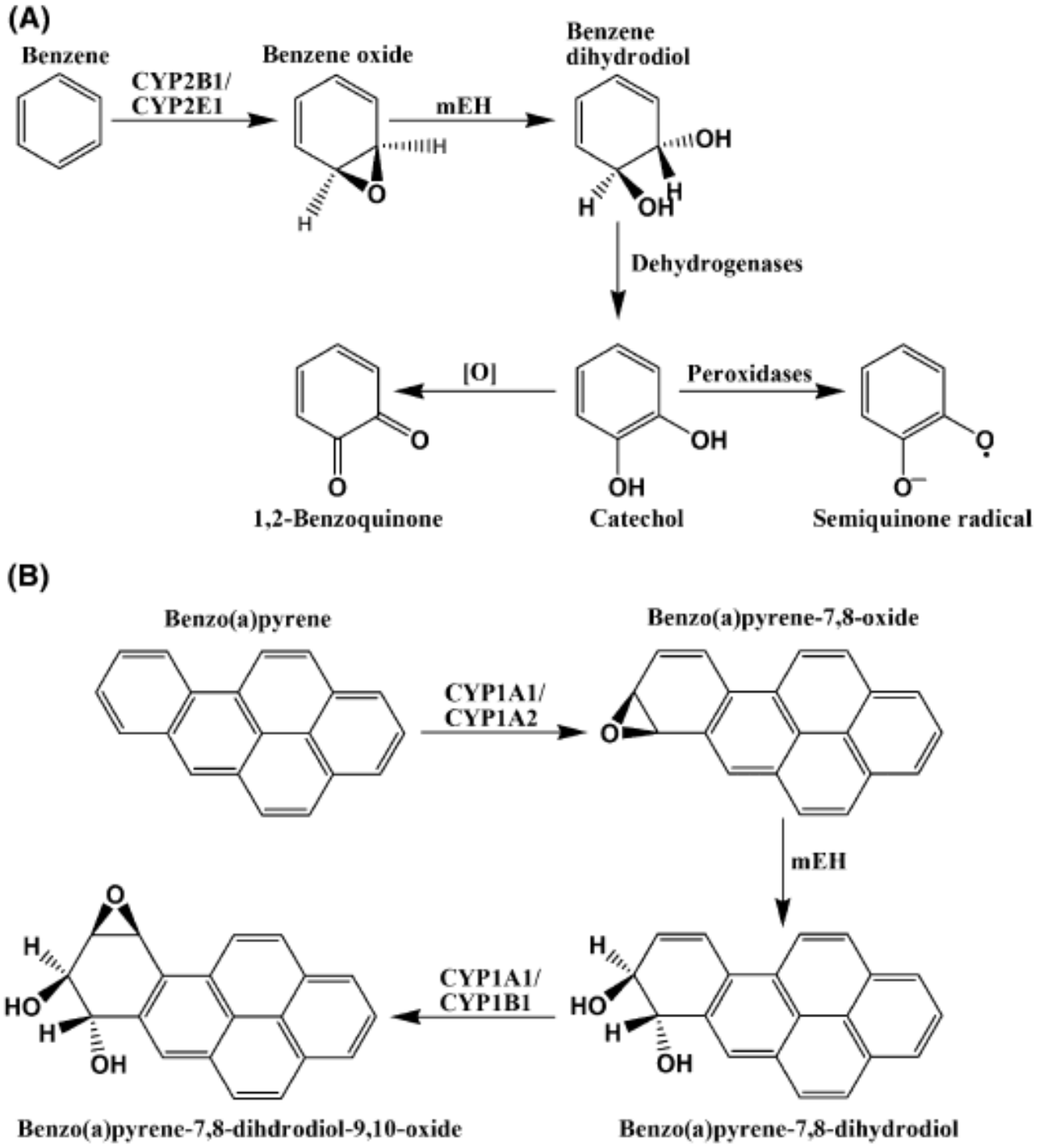
mEH Substrates (a) Inhibitors (b) Inducers (c)

The enzyme prefers to metabolize unhindered epoxides



Role of mEH in benzene and polycyclic hydrocarbon toxicities

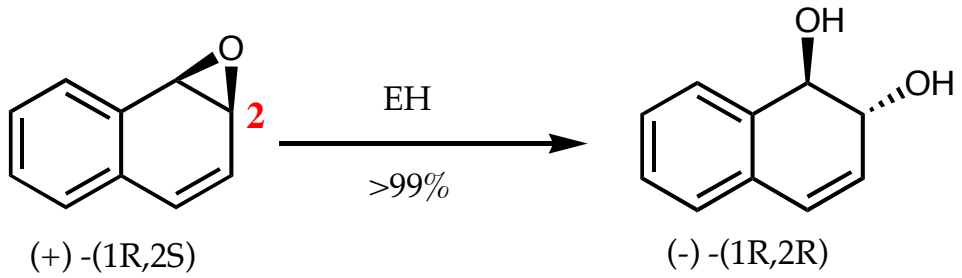
mEH plays a pivotal role in formation of catechol and dihydrodiol epoxides



Chemical mechanism of epoxide hydrolysis by mEH

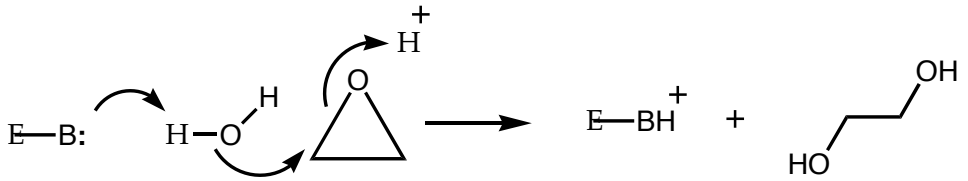
- Nucleophile attack occurs:

1) at the less hindered carbon (or the carbon with (S) absolute stereochemistry.
2) Backside attack with inversion of stereochemistry.



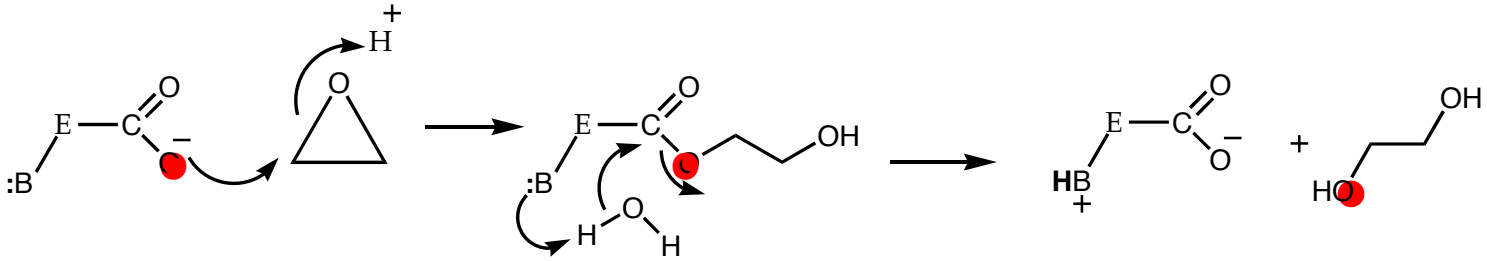
• A "classic" labeling experiment provided the earliest experimental evidence for the involvement of an ester intermediate in the catalytic mechanism of microsomal EH.

• Isotopic composition data shows that, under "single-turnover" conditions it was possible to label both the diol metabolite and mEH with an 18O atom from water, and that in a second "single-turnover" experiment using re-isolated enzyme from the first experiment and 16O water, that an 18O atom was transferred to the product.



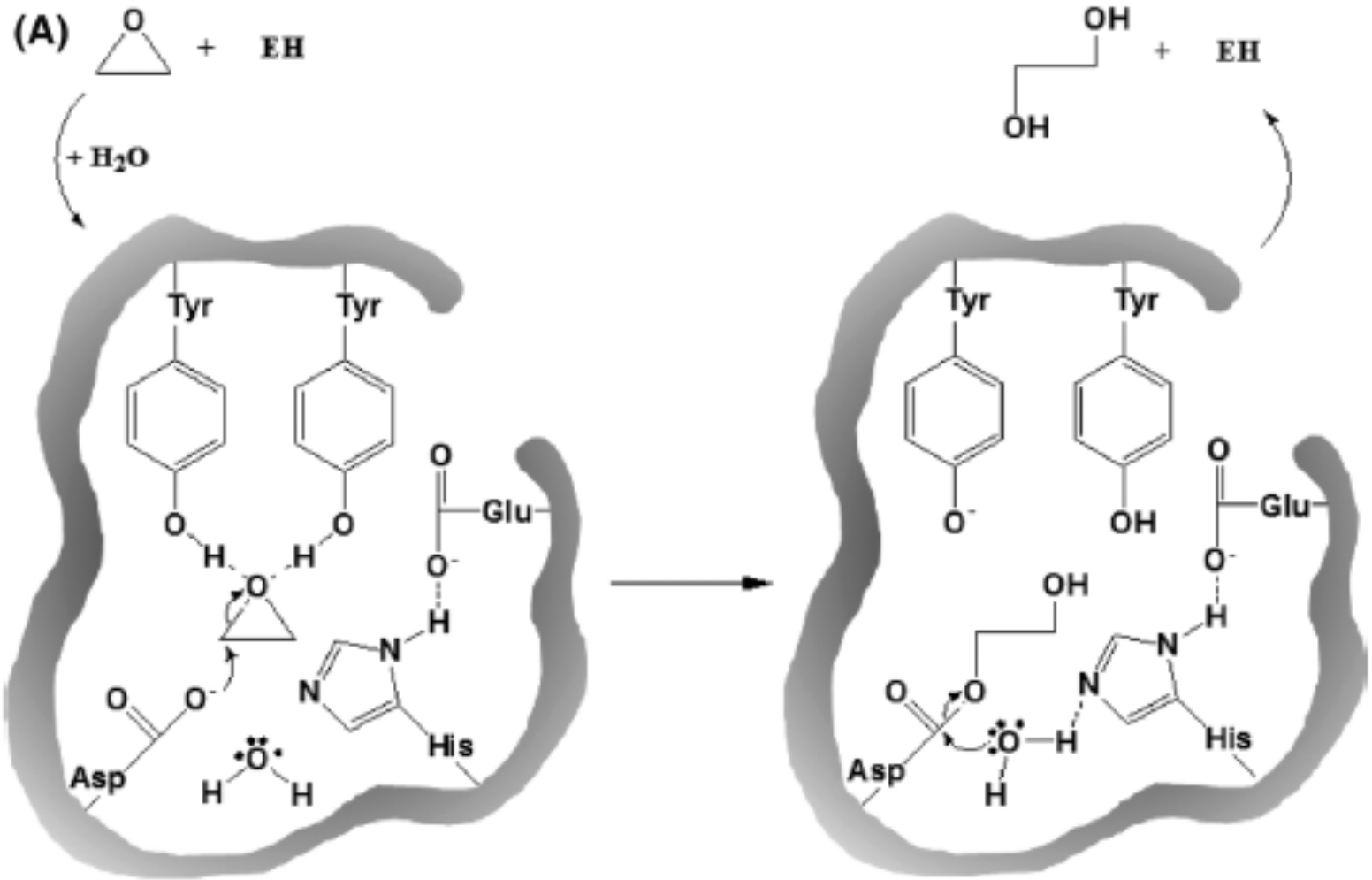
Lacourciere and Armstrong,
JACS 115:10466, 1993

Awesome expt!!



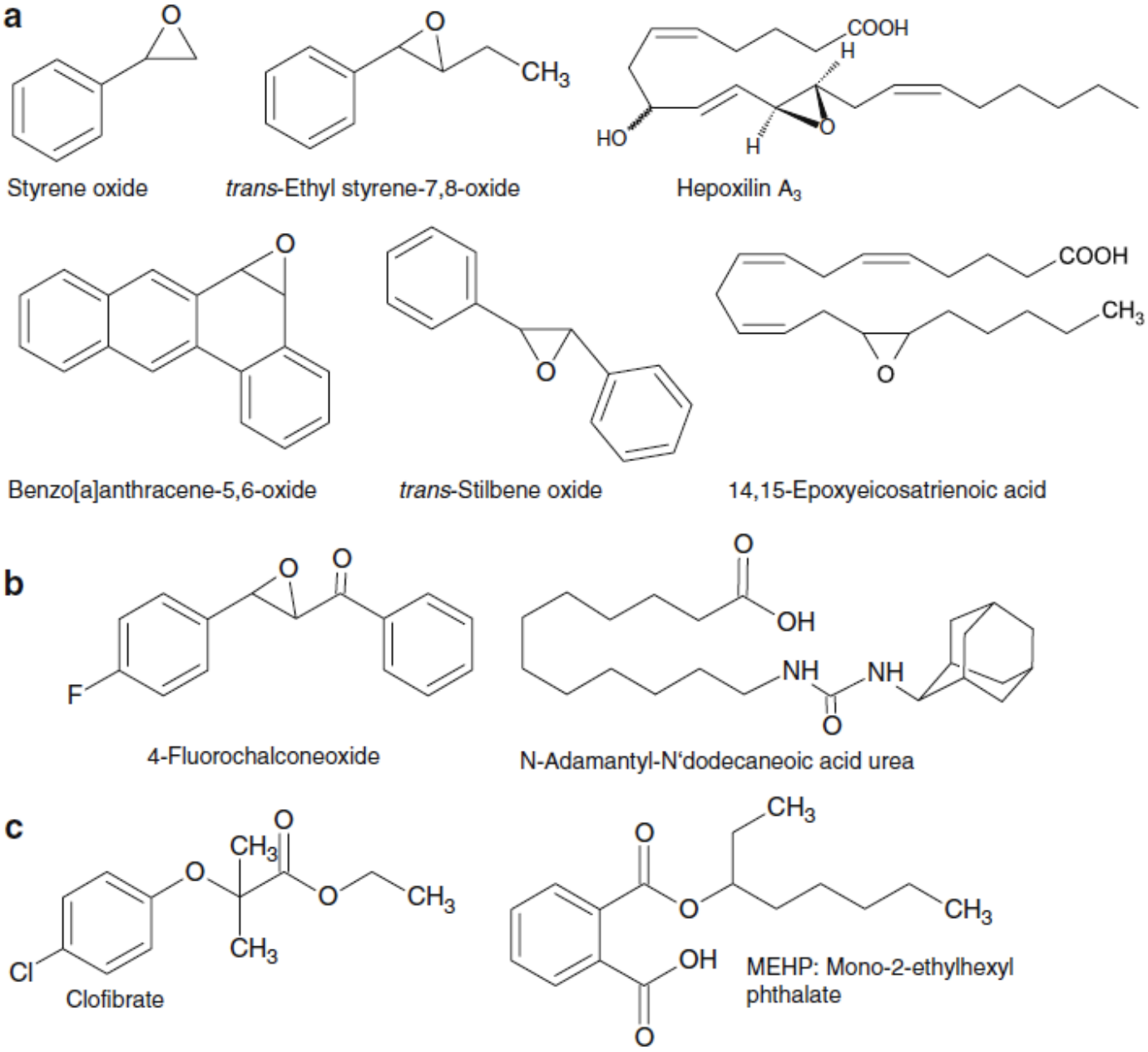
Catalytic Mechanism

- A nucleophilic residue - **Asp**– covalently binds to substrate to form an ester intermediate.
- **His** and an acidic residue (**Glu/Asp**) cooperatively activate a water molecule which hydrolyzes the acyl intermediate.
- Active site **Tyr residues** stabilize developing charge on the epoxide oxygen.



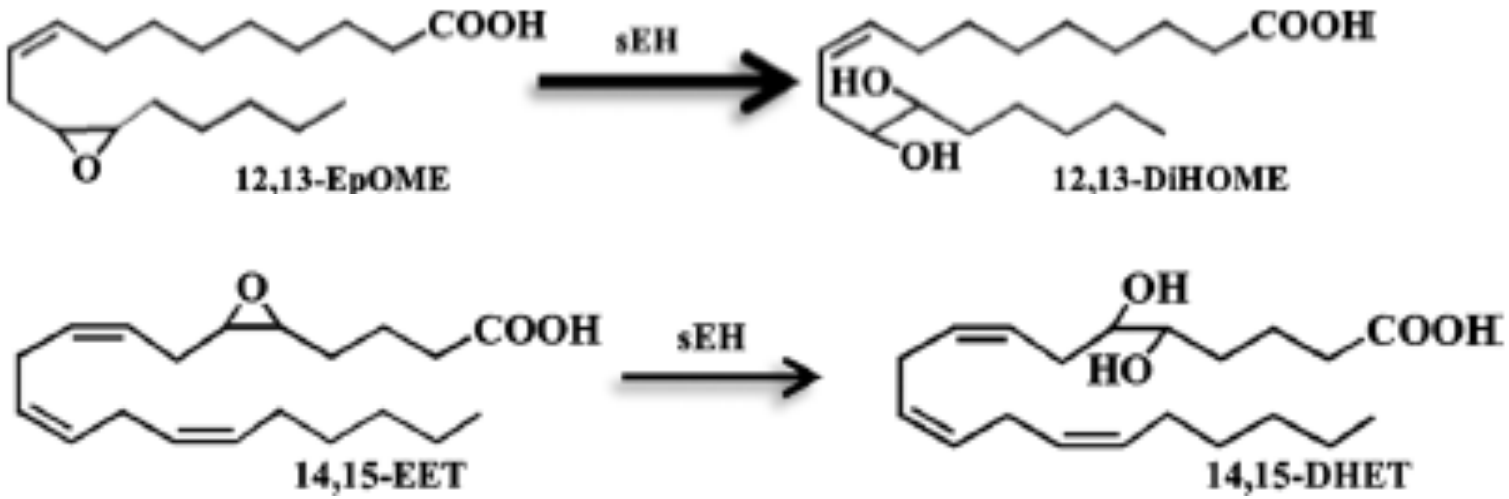
sEH
Substrates (a)
Inhibitors (b)
Inducers (c)

The enzyme readily metabolizes <u>hindered</u> epoxides



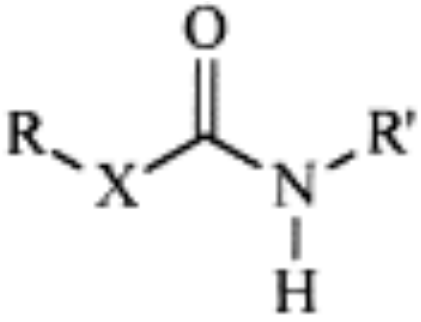
Physiological Roles for sEH

- sEH is involved in the metabolism of arachidonic, linoleic, and other fatty acid epoxides, endogenous chemical mediators that play an important role in blood pressure regulation and inflammation.
- Epoxyeicosatrienoic acids (EETs) have antihypertensive and anti-inflammatory properties.
- Deletion of the sEH gene in male mice lowered systolic blood pressure and altered arachidonic acid metabolism.



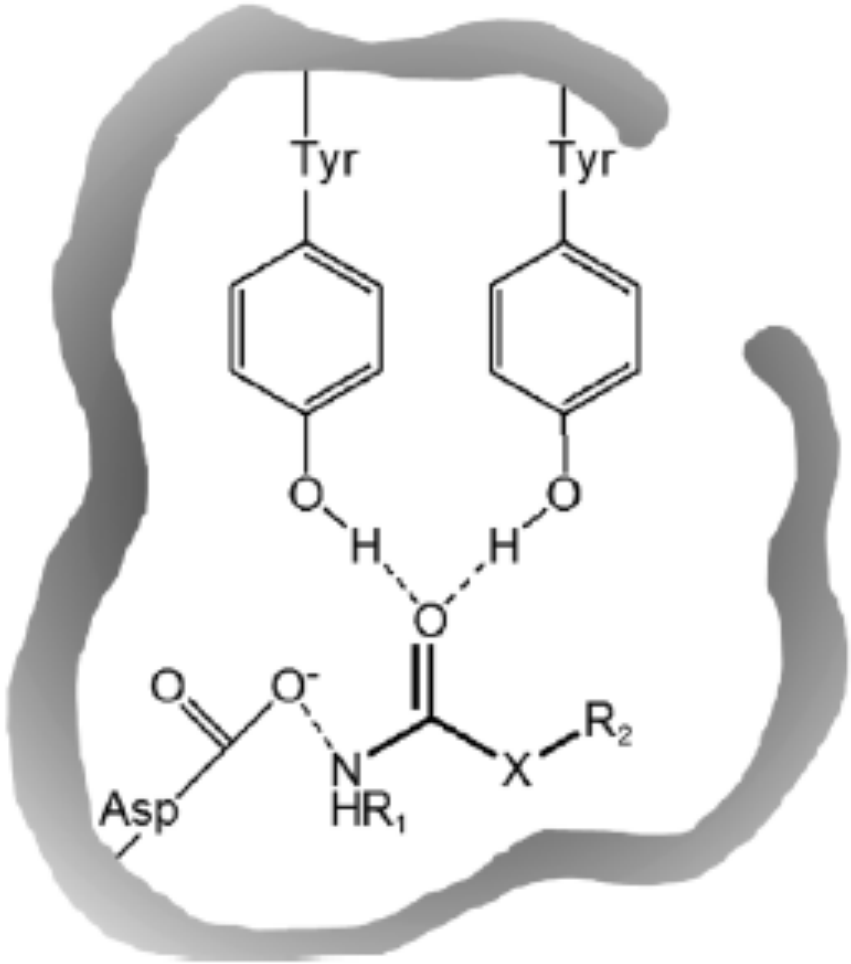
sEH Inhibitors as Therapeutic Agents

• Bruce Hammock's group have designed a variety of potent and selective amide, carbamate and urea-based inhibitors of she. Early example shown below is N,N-dicyclohexylurea.



X: NH, O, or CH_2.

R and R': alkyl or aryl groups.



mEH and the Carbamazepine-Valproic acid Drug Interaction