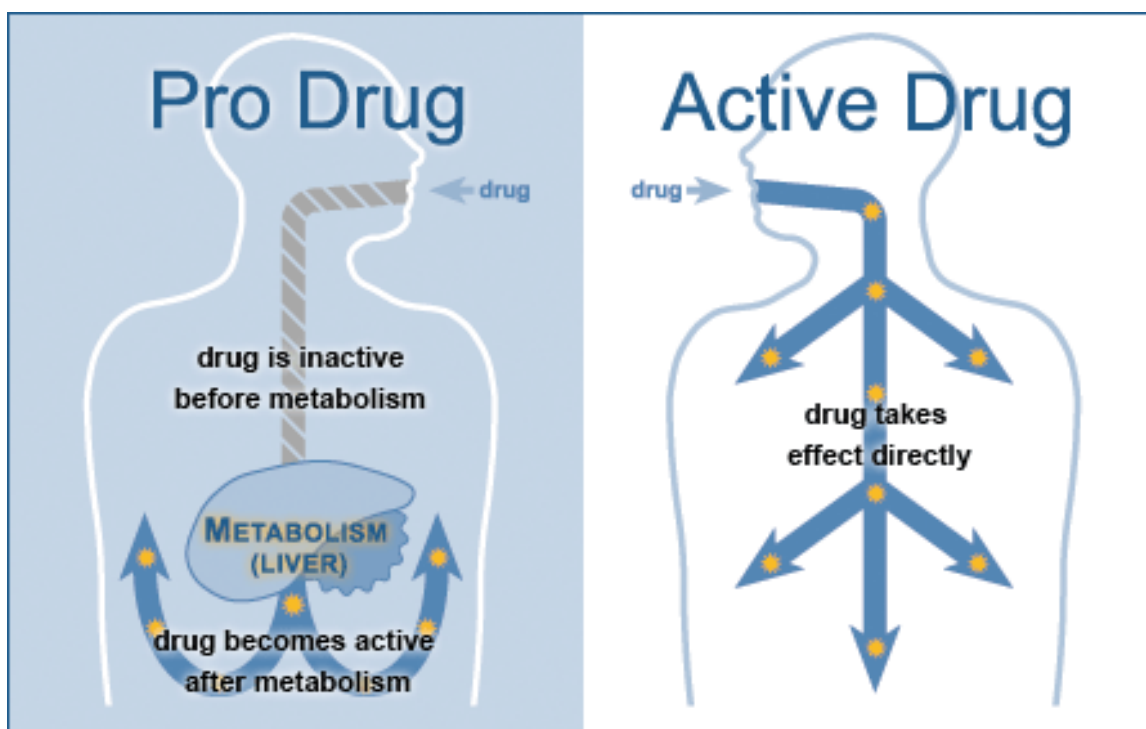


5. Prodrug Metabolism (2013)

Prodrug Definition:

Strict Definition: Prodrugs are inactive drugs that undergo a chemical or biochemical conversion to the active drug.



Biochemical or chemical process(es)

Prodrug
inactive



Drug
active

The definition of a prodrug is controversial in some circles.

1. Some consider various injectable salt forms to be prodrugs (Insulin).
2. Others argue that an inactive drug that is “activated” by a change in pH rather than an enzymatic event is also a prodrug (Omeprazole).
3. There are certainly gray areas. Are active drugs whose metabolites also circulate and are also active then prodrugs as well? Many examples of this are known (Prozac, Diltiazem). Since terfenadine did not achieve measurable levels in the blood in man was it just a toxic prodrug and the metabolite the real drug?

Approximately 7-10% of marketed drugs are considered to be prodrugs and the number is increasing. Two major reasons:

1. Because it works and allows us to circumvent problems delivering a new drug moiety to the site of action such as poor bioavailability and toxicity of the parent drug.
2. Improve the efficacy of older drugs, usually to improve absorption.

Where do the bioconversion occur? Major locations include:

1. Liver (major)
2. GI tract lumen and enterocytes
3. Blood
4. Target Cell (Intracellular and Cell Surface)
5. At the site of a depot injection.
6. Other

What enzymes catalyze the bioconversion reactions?

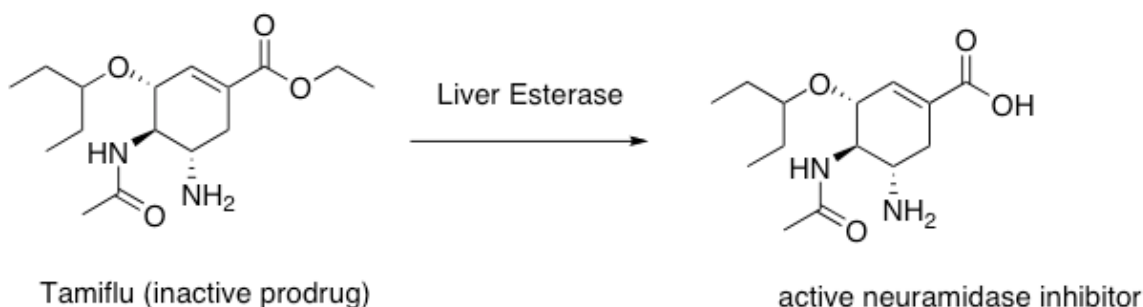
1. Carboxylesterases and phosphoesterases
2. Cytochrome P450 enzymes
3. Phosphoesterases.
4. Others (anticancer drugs)

What are some of the downsides to using enzymes to create active drugs from inactive prodrugs.

1. The enzyme may be subject to polymorphisms where the PM's are unable to get therapeutic benefit.
2. Polytherapy may lead to inhibition of the "activating" enzyme by other drugs (a type of DDI).
3. Other

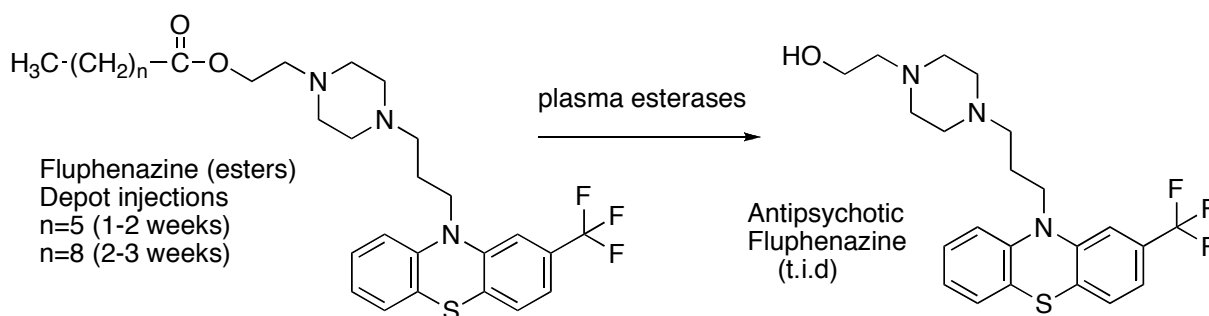
Carboxylesterases (Esterases)

A. Tamiflu (increased oral absorption/bioavailability; liver esterase)



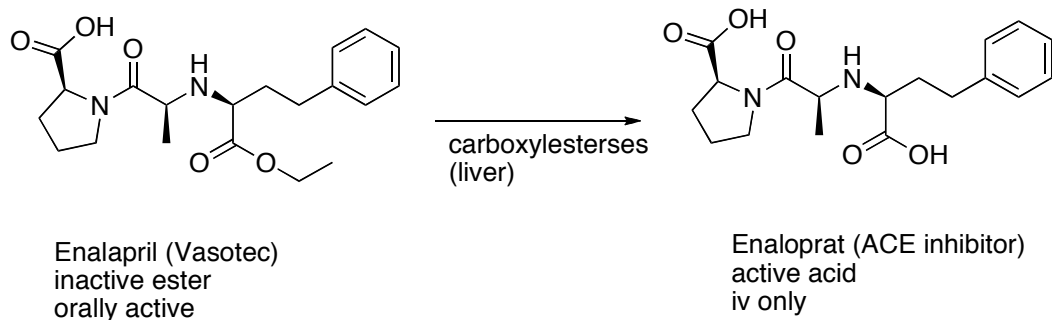
1. The presence of ionizable groups on a drug can hinder bioavailability since drugs that are ionized at physiological pH do not pass through membranes that line the GI tract.
2. Above we see that the active drug has two ionizable groups and is a zwitterion. Hence absorption/bioavailability is very poor.
3. The ethyl ester is reasonably well absorbed and undergoes rapid first pass hydrolysis by carboxylesterases that are present in the liver.
4. Here the carboxylic acid is retained in the active drug after cleavage of the ester bond.
5. Tamiflu was the first orally-active neuramidase inhibitor for use in flu.

B. Fluphenazine Esters (Depot Injection; Blood esterases)



1. Fluphenazine (right) is an antipsychotic agent used in the treatment of schizophrenia and other mental illnesses. Orally, this active drug is administered three times a day which can be a problem in compliance.
2. The fluphenazine esters ((n=5 is the heptanoate ester; n=8 is the decanoate ester) are the inactive prodrugs. Note the effect of chain length on the dosing interval.
3. They are very poorly soluble in water and are injected in a bolus IM. The esters slowly diffuse from the bolus site into the blood.
4. Bioactivation of the prodrug occurs in the blood by blood esterases.
5. Note that in this case the acid fragment that is produced by the esterase is “thrown away”. The active drug is the alcohol fragment and that can be administered orally.
6. This ester depot strategy is also used with some of the steroids.

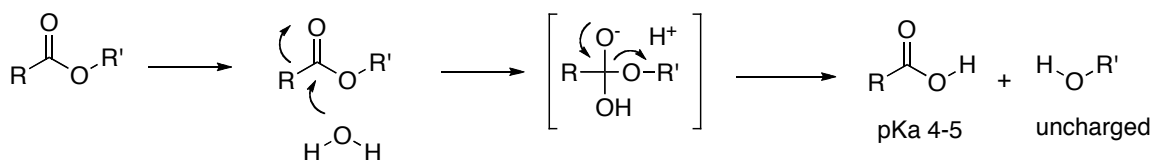
C. Enalapril (oral absorption; liver carboxylesterases)



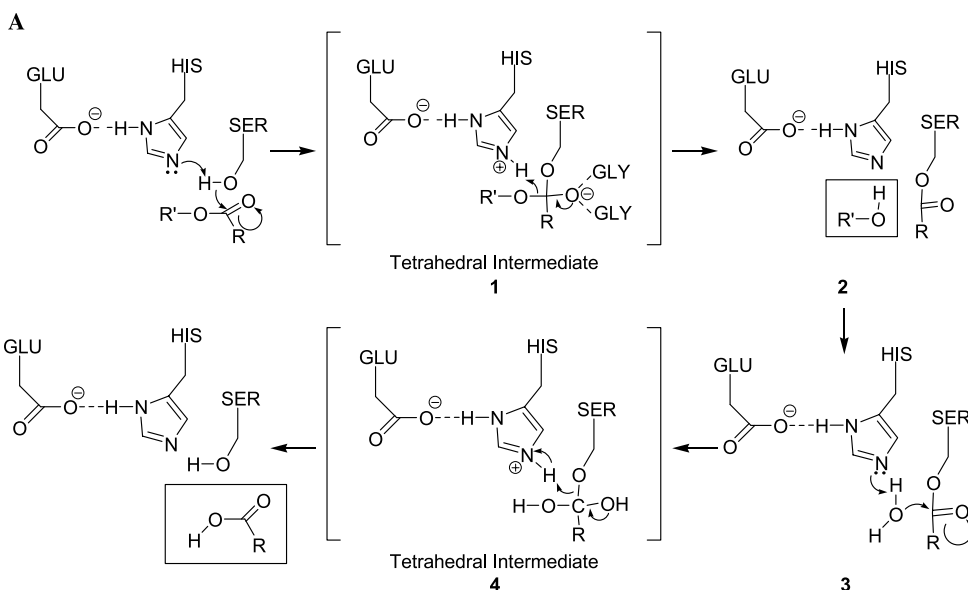
1. Enalaprat (right above) was developed as an angiotensin converting enzyme (ACE) inhibitor but was only effective IV (note it has two carboxylates and both are required for activity).
2. Merck made the inactive ethyl ester (Enalapril) which was absorbed and converted to the acid by carboxylesterases in the liver.
3. All but one of the ACE inhibitors on the market have the required two carboxylate groups of enalaprat where one of the carboxylates is the ethyl ester. Thus they are all prodrugs.

Aside but important on the mechanism of hydrolysis of an ester.

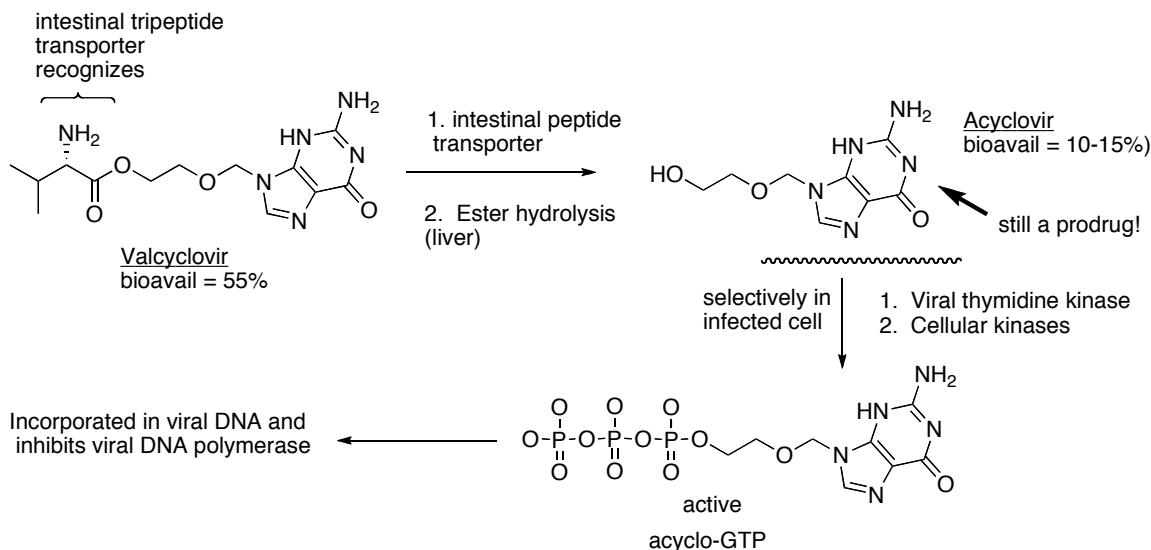
Many students on an exam when presented with an ester cannot show the products of ester hydrolysis correctly when presented with a real world drug. The following is an acceptable arrow pushing mechanism as a shorthand for the enzyme catalyzed mechanism. The key feature is that the carbonyl carbon is electrophilic and the tetrahedral intermediate collapses with cleavage of the indicate carbon oxygen bond. The other key thing to remember is that the acid product is mostly ionized at physiological pH and only the neutral acid will cross membranes.



The consensus mechanism for esterases is shown below so it is more complicated and not required for you to know.

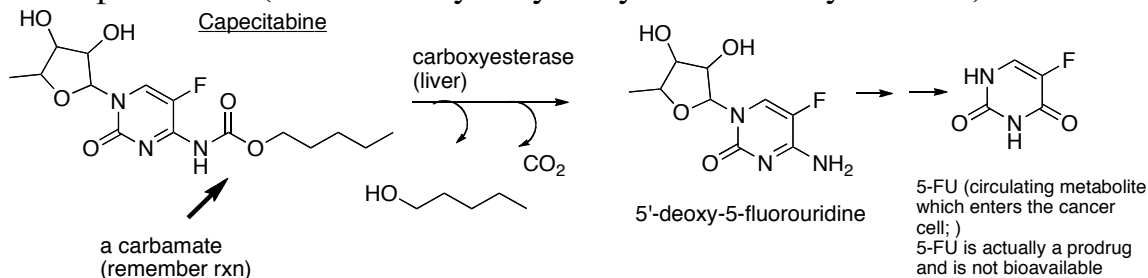


D. Valcyclovir (Valtrex) (oral absorption via tripeptide transporter; hydrolysis in the liver; final active agent made intracellularly in the target).



1. The tripeptide transporter is normally used to bring in small peptides from protein proteolysis in the GI tract.
2. Valtrex was made in order to increase the systemic exposure (AUC) to acyclovir and highlighted the tripeptide transporter as a potential way to increase bioavailability.
3. Ester hydrolysis in the liver provides the amino acid valine (not shown) and acyclovir. This is another case where the alcohol fragment is the important product
4. Acyclovir is a prodrug that is activated to the active agent intracellularly in the infected cells.
5. The pyrophosphorylated metabolite (acyclo-GTP) is formed much more rapidly by the viral thymidine kinase than mammalian thymidine kinase. This helps to protect uninfected cells.
6. The acyclo-GTP is trapped inside the cell but is eventually degraded.

E. Capecitabine (carbamate hydrolysis by liver carboxylesterase)



1. 5 Fluorouracil (5-FU; Efdudex) is a well known cancer drug that must be give iv and there are many serious side effects to the drug. As you will learn later 5-FU is actually a

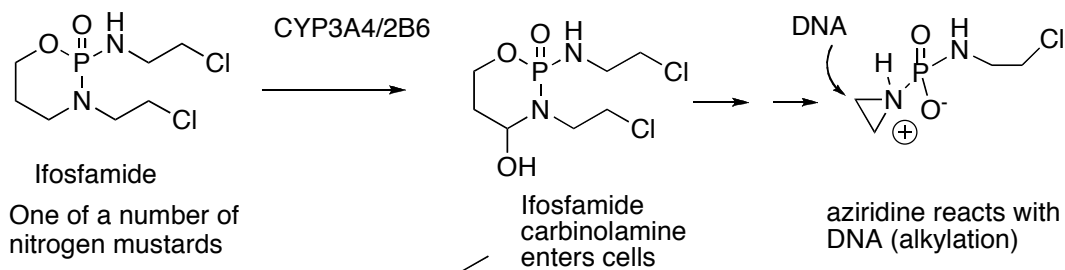
prodrug that is converted intracellularly to the active drug that inhibits thymidylate synthetase.

2. The prodrug Capecitabine has good oral bioavailability and peaks around 1.5 hours after dose. 5-FU and 5'-deoxy-5-fluoruridine levels peak around 2 hours and the latter accumulates with repeat dosing.
3. Capecitabine apparently inhibits CYP2C9 and causes excessive bleeding with warfarin (Black Box Warning).

Cytochrome P450 Enzymes

P450 enzymes can catalyze many reactions that activate pro-drugs to drugs.

F. Ifosfamide (P450 liver; further bioactivation in target cell to produce the reactive compound)

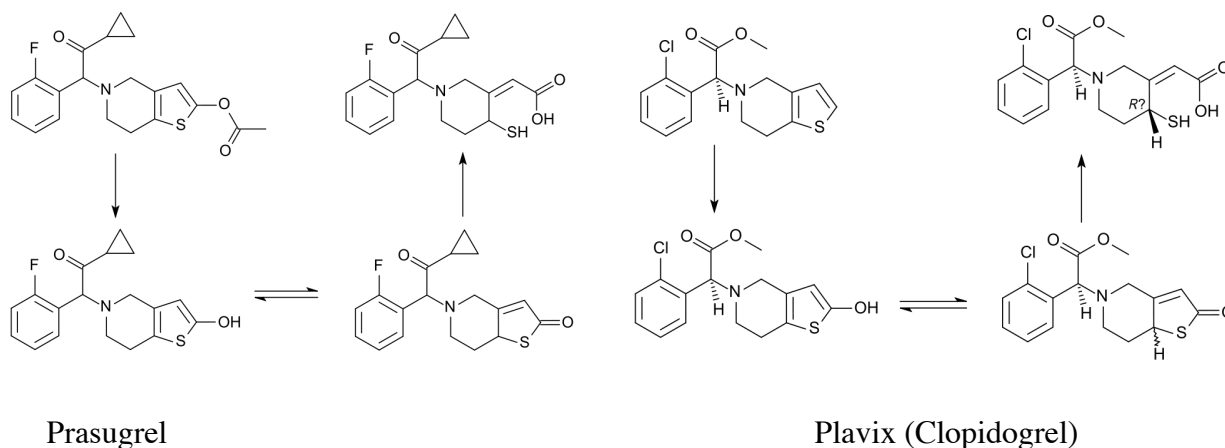
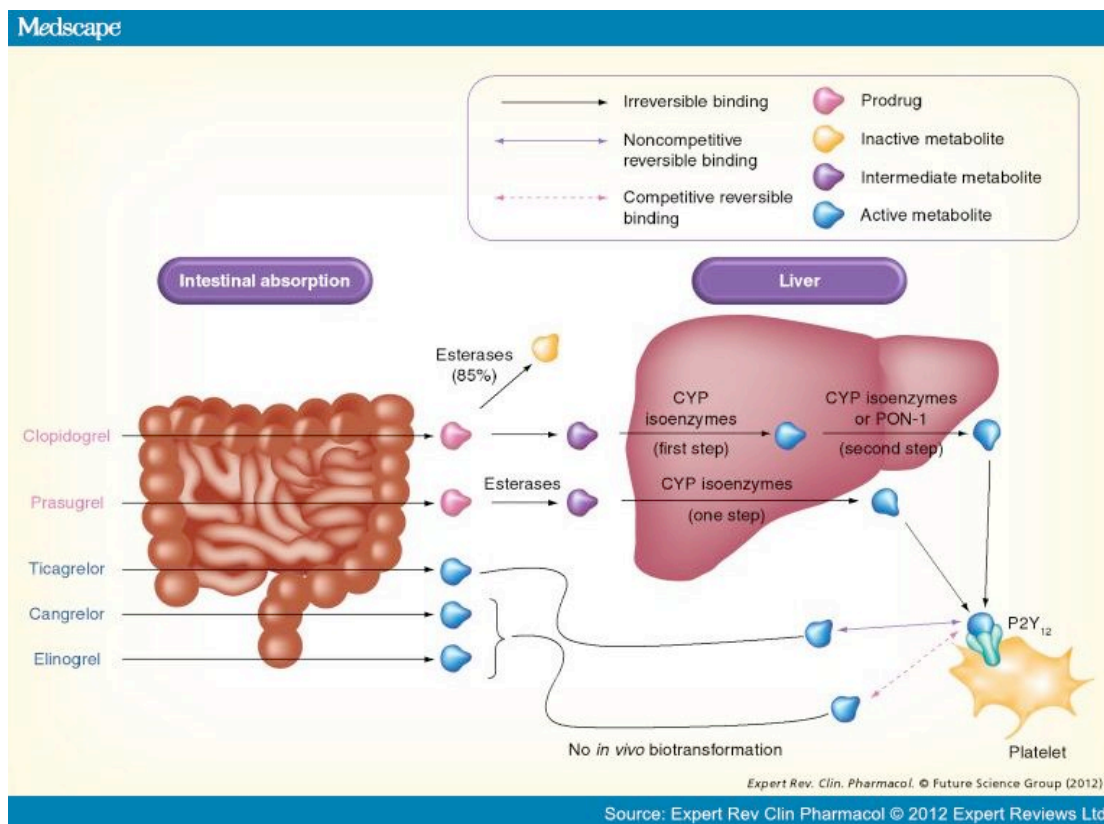


1. A number of anticancer drugs are classified as the nitrogen mustards. These drugs are for iv administration.
2. They are bioactivated via a series of steps that is initiated by cytochrome P450 catalyzed hydroxylation of a carbon adjacent a nitrogen in a rather unusual looking six membered ring to produce a carbinolamine which can exist in a ring opened form as well.
3. These metabolites enter the cell and undergo a number of reactions that eventually produce aziridine structures. (A veritable orgy of electron pushing to get there). This is probably the proximate alkylating agent that reacts covalently with DNA.
4. Here all of the intermediate compounds of the pathway are too short-lived to be formulated for administration.

G. Plavix and Prasugrel (P450 and carboxylesterases)

1. These drugs are administered orally. They inhibit platelet aggregation by irreversible binding of metabolites to the P2Y₁₂ receptor.
2. These drugs are used primarily before and after cardiac intervention procedures such a stent placemnet (percutaneous coronary intervention; PCI) to prevent clotting.
3. Plavix recently went generic and had peak sales of \$9.7 B. Prasugrel is more expensive and it's use is associated with a higher risk of bleeding (Black Box Warning).
4. There is a high interest in making better drugs.

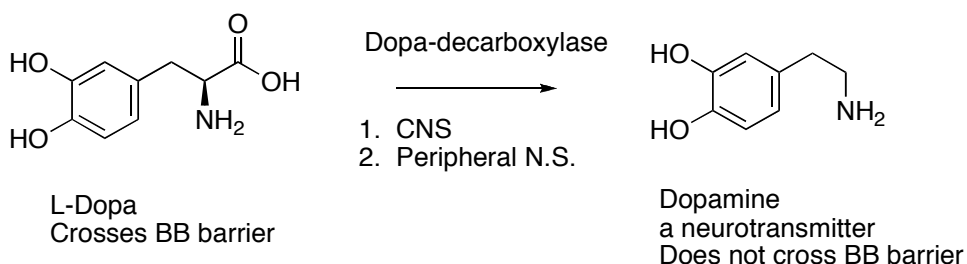
5. While both drugs are metabolized by esterases this reaction is used to clear plavix to inactive metabolites. By contrast the hydrolysis of the ester of prasugrel is required for the generation of the active compound.
6. P450 enzymes catalyze two steps in the bioactivation of plavix. The first of these is catalyzed by CYP2C19 to produce a hydroxylated thiene ring. A Black Box Warning has been issued due to the risk for CYP2C19 PMs. We would also be worried about DDI's with CYP2C19 inhibitors causing the same problem.
7. By contrast the prasugrel was designed so the hydroxythiene was generated by an esterase reaction. In both causes the thiolactone metabolism is multi enzyme.



There are a couple of points and/or errors in the colored diagram.

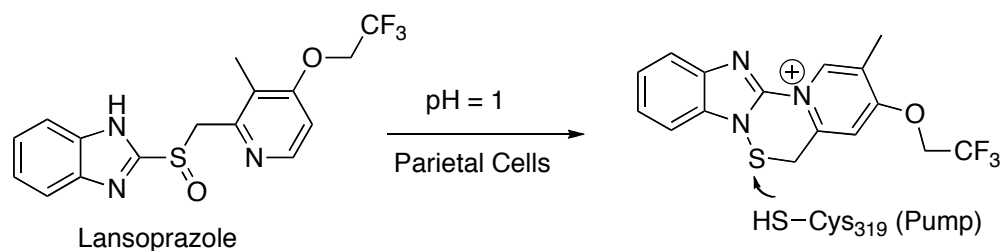
1. Prasugrel: The first esterase step actually occurs mostly in the enterocytes. While this drug is well absorbed (80% of dose found in urine) there is no parent prodrug in blood.
2. Prasugrel and Plavix: There are multiple P450 enzymes that carry out the final oxidative ring opening of the thiolactone in the liver so there is not a major issue with polymorphisms or drugs that produce DDI's. PON-1 is not important
3. Plavix: The arrow between intestine and the color of the product of the arrow seem to indicate an esterase step. That is not true, Plavix enters the hepatocytes unchanged.
4. Note that both released metabolites that are believed to be the real drug are irreversible inhibitors of P2Y₁₂. The other indicated drugs or drug candidates are reversible inhibitors of P2Y₁₂.

H. L-DOPA (blood brain barrier; CNS dopa-decarboxylase)



1. L-DOPA (levodopa) is administered orally for the treatment of Parkinsonism and is inactive. Parkinsonism is caused by the destruction of dopaminergic cells in the substantia nigra. The goal of treatment is to increase levels of the neurotransmitter dopamine in the central nervous system (CNS). L-DOPA bioavailability is 20-40%; note that it is an amino acid and is zwitterionic so it requires a transporter to cross membranes and be absorbed.
2. L-DOPA and the enzyme that catalyzes the conversion to dopamine participate in the normal pathway for the synthesis of dopamine in both the peripheral nervous system and the CNS. Thus L-DOPA levels are increased over normal levels when it is administered.
3. L-DOPA crosses the blood brain barrier (BB) while dopamine does not.
4. The increased conversion of the L-DOPA to dopamine in the peripheral nervous system causes unwanted side effects such as hypotension and nausea.
5. L-DOPA is usually given with a second drug, Carbidopa which inhibits dopa decarboxylase. The inhibitory effects of Carbidopa are confined to the peripheral nervous system as Carbidopa cannot cross the blood brain barrier.

I. Omeprazole, Lansoprazole (non-enzymatic bioactivation in a special compartment, the Parietal Cell)



1. Proton pump inhibitors are formulated to be released and absorbed in the intestine. They are acid labile and are destroyed by the acid pH of the stomach.
2. After absorption they travel through the blood and are taken up by the parietal cells of the stomach lining. These are the cells that secrete HCl into the lumen of the stomach.
3. The ATP-driven proton pump concentrates protons from the cytosol creates a local low pH and where they are converted to the active form by the acid pH of the pump itself. The active form, which many claim is the true active drug, reacts covalently with a critical cysteine residue and inactivates the pump.

