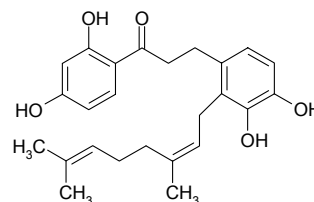


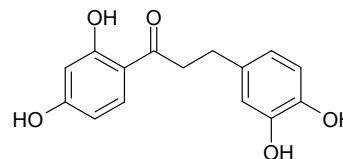
Enzyme Inhibition and Inactivation

1. 5-Lipoxygenase is an enzyme that catalyses the conversion of arachidonic acid (AA) into leukotrienes. The physiological role of leukotrienes is to mediate inflammation and hypersensitivity reactions. AA-induced ear edema in mice is a useful disease model for inflammation.

a) New selective inhibitor of 5-lipoxygenase from Indonesian plant *Artocarpus communis* was reported to suppress AA-induced ear edema with IC₅₀ value of 50 nM. The compound binds to the same form of enzyme that arachidonic acid binds. Sketch the Michaelis-Menten plot and the double reciprocal plot in the presence of 0, 5, and 50 nM concentration of the inhibitor when the concentration of arachidonic acid ($K_m = 10 \mu\text{M}$) is varied from 0 to 200 μM . Make sure to label your axes and lines completely. Only qualitative analysis is required.



b) Assume that the compound shown on the left forms a ternary complex with the enzyme and the substrate, and also binds to the free enzyme. Sketch the double reciprocal plot in the presence of 0, 5, and 50 nM concentrations of the inhibitor. Make sure to label your axes and lines completely. Only qualitative analysis is required.



c) Which compound is likely to be stronger inhibitor of 5-lipoxygenase? Justify your answer.

2. Several drugs once thought to be “silver bullets” have lost their efficiency because of the emergence of drug resistant pathogens. Discuss reasons and molecular mechanisms behind emergence of drug resistance for:

- AZT as HIV drug
- Vancomycin as a drug of last defense against *Streptococcus aureus*
- Penicillin as bacteriocidal drug

3. In order for cells replicate, sufficient quantities of nucleotides, such as deoxythymidylate (dTTP), are needed. dTTP is made from deoxyuridylate (dUTP) by thymidylate synthase. Enzymes responsible for synthesis of dUTP from uridine are ribose-1-phosphate uridine phosphorylase, uridine phosphorylase, UMP kinase, ribonucleotide reductase, orotate phosphoribosyltransferase, thymidine kinase, and dUTP diphosphohydrolase. Significant decrease in thymidylate synthase activity is seen after administration of drug 5-fluorouracil. However, this compound does not significantly reduce the activity of the enzymes that are involved in conversion of uracil to dUTP.

- Explain why 5-fluorouracil does not inhibit enzymes that convert uracil to dUTP.
- Explain why administration of 5-fluorouracil leads to reduced thymidylate synthase activity.

Project development.

You will be submitting a drug design proposal at the end of this course. In this stage, you are expected to come up with a plan how to assay your potential drug candidates. Please discuss what the rationale behind using such an assay is and identify any potential limitations. Your assay can be either molecular-biology based (e.g. quantification of particular mRNA using molecular beacons), biochemical (e.g. measuring ligand binding to the receptor), histological (analysis of tissue appearance), clinical (determination of viral load), or end-point assay (improvement in host health). You do not need to hunt down the original research in which the assay was developed but you need to provide evidence that a particular assay or animal model is appropriate to your target and disease. Keep in mind that we are not talking about human clinical trials here yet but at most animal models of the disease.