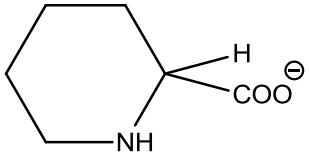
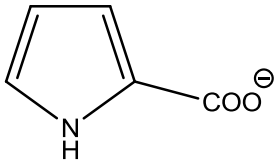
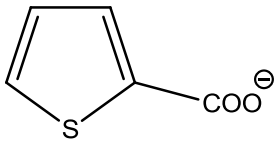
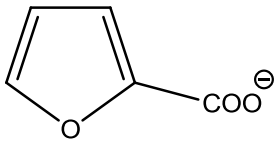
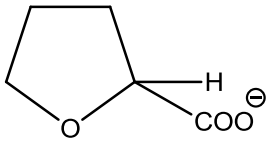
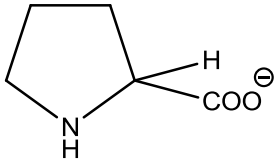
Inhibition of Proline Racemase

Proline racemase, a bacterial enzyme, catalyzes the interconversion of D and L-proline. The K_m values for D and L-proline are 2.3 mM and 3.8 mM respectively. The maximal velocity is 8×10^{-3} mol/mg per min for L-proline. Various compounds have been tested as inhibitors of the enzymes. Their structure and extent of inhibition are indicated below.

Inhibitor	Concentration [M]	Percent Inhibition*
 Pipecolate	1.1×10^{-1}	18
 Pyrrole-2-carboxylate	5.7×10^{-2} 3.6×10^{-4}	98 50
 2-thiophenecarboxylate	5.7×10^{-2}	73
 2-furoate	5.7×10^{-2}	11
 tetrahydrofuroate	1.1×10^{-1}	10
 Proline	5.7×10^{-2} M Present in all of the inhibitor reactions	0

1. Write the reaction catalyzed by proline racemase. What is the equilibrium constant for this reaction? What would be a reasonable structure for the transition state?

2. Using graph paper draw a Lineweaver-Burk Plot (1/v vs. l/[S]) for the uninhibited enzyme. Label the axes appropriately. On the same sheet draw the plot expected when 3.6×10^{-4} M pyrrole-2-carboxylate is present as a competitive inhibitor.

3. Calculate the turnover number for proline racemase. The enzyme is composed of two identical subunits each with a molecular weight of 38,000 daltons.

4. Rank the five compounds listed above in order of their inhibitory action. Explain why the best inhibitor has a K_1 approximately 160 times lower than the K_m values for proline.

Based on Cardinale & Abeles, Biochemistry 7:3970 (1968)
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