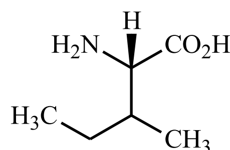
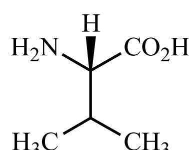
Answers to end-of-chapter questions

1) The structures of isoleucine and valine are as follows.



Isoleucine



Valine

Isoleucine has a larger side chain than valine, and so there is less space available in that region of the active site for COX-1 than there is in the corresponding region in COX-2. Drugs can be designed that take advantage of this difference such that they fit into the active site of COX-2 but not the active site of COX-1.

2) The reaction catalysed by acetylcholinesterase on acetylcholine is the hydrolysis of the ester to produce acetic acid and choline. With neostigmine, the corresponding reaction would be the following:

Neostigmine

In fact, this does not happen and a stable complex with the enzyme is formed. Following the mechanism through, serine acts as a nucleophile and a nucleophilic substitution reaction takes place on the urethane with loss of the phenol group. However, once this has occurred, serine is 'capped' with a stable urethane group that is resistant to hydrolysis.

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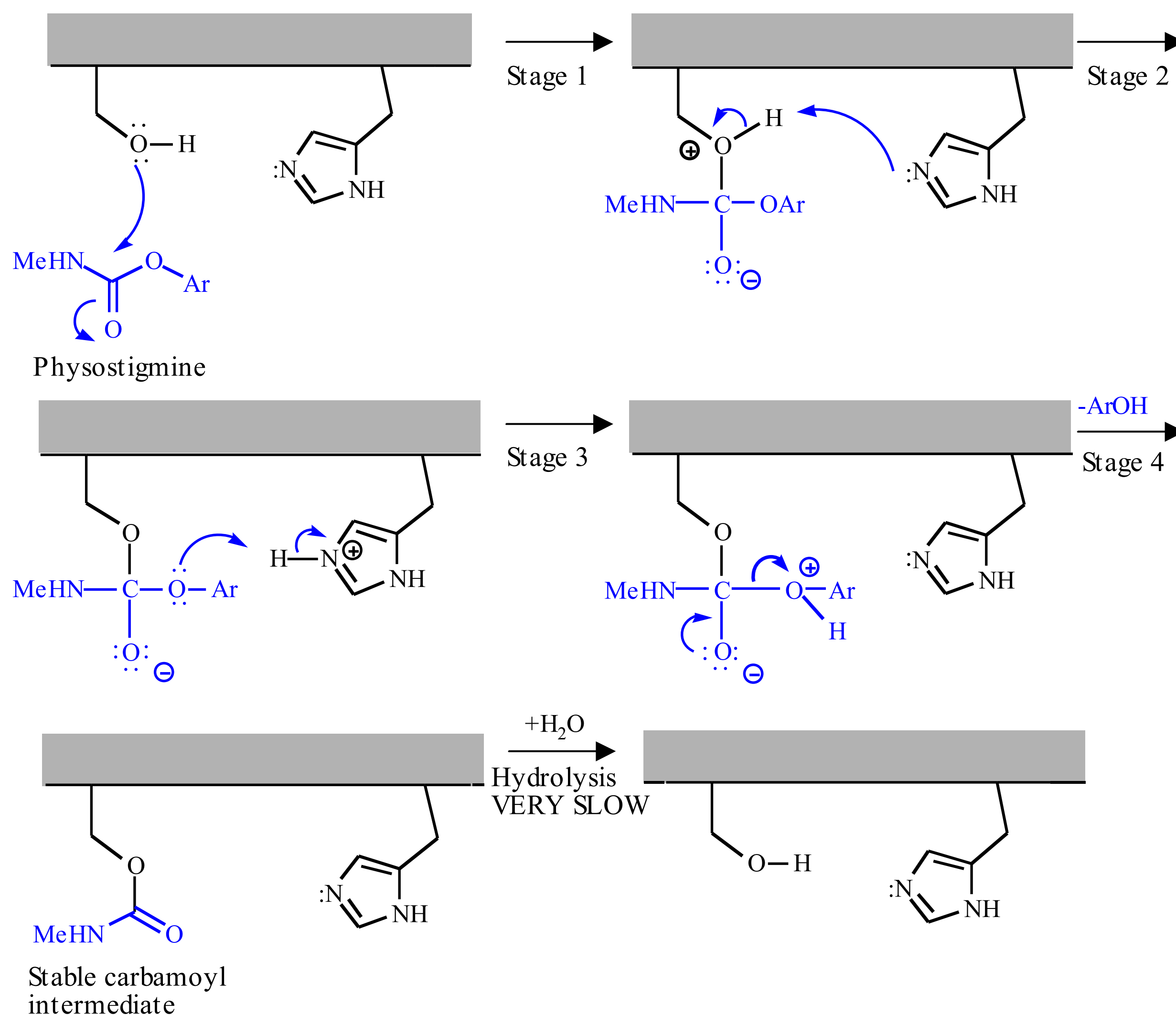

Full details of this process are described in section 22.13.1.1.

3) A transition-state inhibitor is a drug which mimics the transition state for an enzyme-catalysed reaction. Such a drug should bind more strongly than either the substrate or the product, and be a strong inhibitor as a result.

The enzyme-catalysed hydrolysis of the peptide bond between Phe and Pro should involve the enzyme using a nucleophilic group to form a bond to the carbonyl group, resulting in the intermediate shown.

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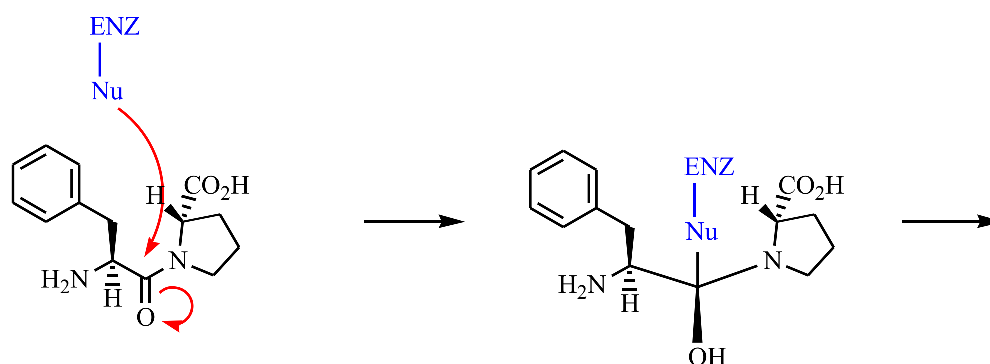
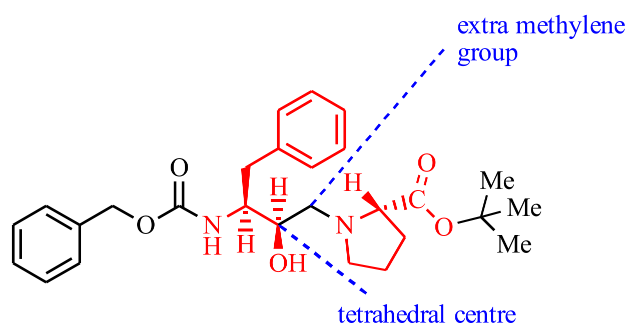


The transition state for this stage should resemble the intermediate more than the starting material and so drugs mimicking the transition state should have characteristics also seen in the intermediate. The similarities in structure I to the intermediate are highlighted in colour below.



Note the presence of the tetrahedral centre bearing the alcohol group - also present in the intermediate. Note also that there is an extra methylene group between this tetrahedral centre and proline. This is necessary if the inhibitor is to be stable to the enzyme-catalysed reaction. Further details regarding the design of this inhibitor can be found in sections 20.7.4.2 and 20.7.4.3.
An IC_{50} 6500 nM means that the concentration of inhibitor required to inhibit the enzyme by 50% is 6500 nanomolar.

4) It is proposed that the binding interactions between an enzyme and a substrate are optimal during the transition state of the enzyme-catalysed reaction. This is a reasonable proposition since the speed and effectiveness of a catalysed reaction is crucially dependent on how much the catalyst stablises the transition state. The more stable the transition state, the lower the activation energy. This is in turn results in a faster rate of reaction, which explains why binding interactions play an important role in an enzyme's role as a catalyst. Therefore, it is more important that enzymes form their strongest interactions with 'guest molecules' at the transition state of the reaction, rather than to the substrate at the beginning of the process, or the product at the end. Indeed, strong interactions with substrate or product are likely to be detrimental since it could result in a slow 'turnover' with substrate and product spending too much time in the active site.

5) In both cases, the enzyme-catalysed process is stalled. Both 5-azacytidine and 5-fluoro-2'-deoxycytidine can react with the enzyme to form structures I and II

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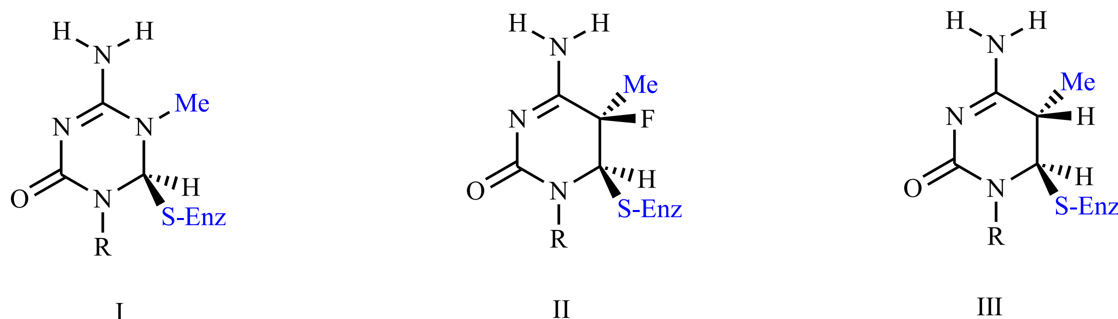
respectively. These are the equivalent structures to the normal intermediate III. In the normal enzyme mechanism, the final stage involves loss of a hydrogen from structure III to form a double bond within the ring, resulting in cleavage of the C-S bond linking the structure to the enzyme. This cannot occur in either structures I or II. In structure II, this mechanism would require the loss of a highly unstable F^+. In structure I, one could draw a mechanism involving the lone pair of the nitrogen, but this would involve the nitrogen gaining a positive charge which is not favoured.



5) The following table shows the values necessary for both the Michaelis Menton and Lineweaver-Burk plots in the absence of inhibitor.

Substrate concentration [S] (mol dm^{-3})	Initial rate (mol dm^{-3} s^{-1})	1/(substrate concentration) 1/[S] (mol^{-1} dm^3)	1/Initial rate (mol^{-1} dm^3 s)
0.05	28.6	20	0.035
0.10	51.5	10	0.0194
0.25	101	4	0.0099
0.50	141	2	0.0071
1	172	1	0.0058

The Lineweaver-Burke plot has 1/initial rate on the y-axis and 1/substrate concentration on the x-axis. This should give a straight line having an intercept of 0.00400 on the y-axis and a slope of 0.00155
Therefore, the maximum rate of reaction = 1/intercept = 250.2 mol dm^{-3} s^{-1}
K_M = slope x (maximum rate of reaction) = 0.387 mol dm^{-3}
The maximum rate of reaction is achieved at high substrate concentration.
K_M is the Michaelis constant and corresponds to the substrate concentration at which the initial rate of reaction is half of J_{max}.

A Michaelis Menton plot of the maximum rate of reaction versus substrate concentration shows a curve where the line is approaching a maximum plateau, but has not yet reached it. Therefore, any attempt to define the maximum rate of reaction from this kind of plot can only be an estimate. Since we need to know the maximum rate of reaction in order to define K_M, the value of K_M will also be inaccurate.
The Lineweaver-Burke plot gives a straight line and allows us define the maximum rate of reaction and K_M more accurately.

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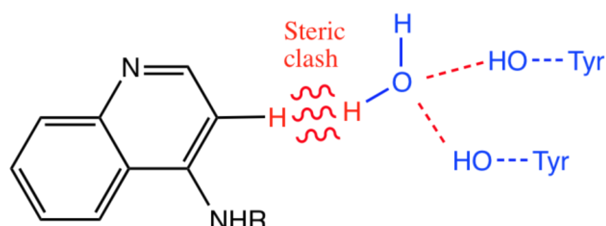
The table for the experiment carried out in presence of 4nM inhibitor is as follows

Substrate concentration [S] (mol dm^{-3})	Initial rate J (mol dm^{-3} s^{-1})	1/(substrate concentration) 1/[S] (mol^{-1} dm^3)	1/Initial rate 1/J (mol^{-1} dm^3 s)
0.05	10	20	0.1
0.10	20	10	0.05
0.20	35.1	5	0.0285
0.40	62.5	2.5	0.0160
0.833	100	1.2	0.01

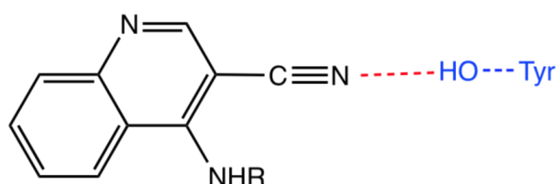
The Lineweaver-Burk plot in the presence of the inhibitor should give a straight line plot crossing the y-axis at the same point as the Lineweaver-Burk plot in the absence of inhibitor.
As the lines cross the y-axis at the same point, this indicates competitive inhibition.
The slope of the line in the presence of inhibitor is steeper than the line in the absence of inhibitor.
Slope in the presence of inhibitor = 0.00477
Maximum rate of reaction = 250.3
K_M(app)=1.19

alpha = K_M app/K_M = 1.19/0.387 =3.075
Therefore, Ki = [I] / (alpha-1) = 1.93

7) Analogue I is less active than the original quinazoline as there is no nitrogen to participate in the hydrogen bonding network involving water and two tyrosine residues. Moreover, the additional hydrogen atom would clash with the water molecule and displace it. Therefore, there are fewer binding interactions for analogue II, making it a weaker inhibitor



Analogue II has a nitrile substituent which will also displace the water molecule. However, the nitrile group can form a hydrogen bonding interaction directly to one of the tyrosine residues. This binding interaction must be stronger than the original hydrogen bonding network involving the quinazoline structure.



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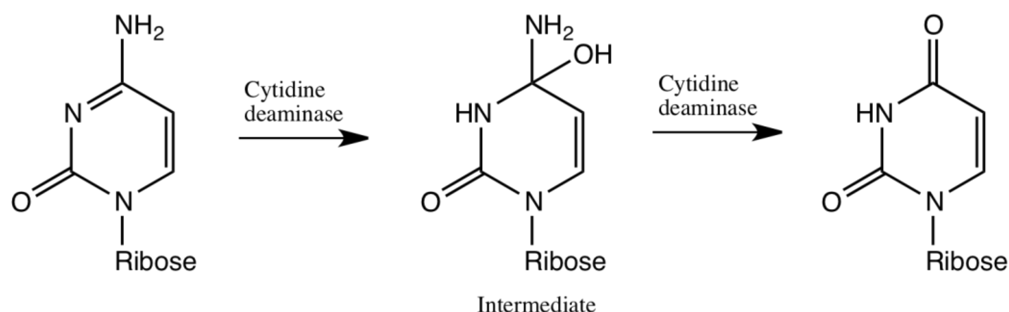
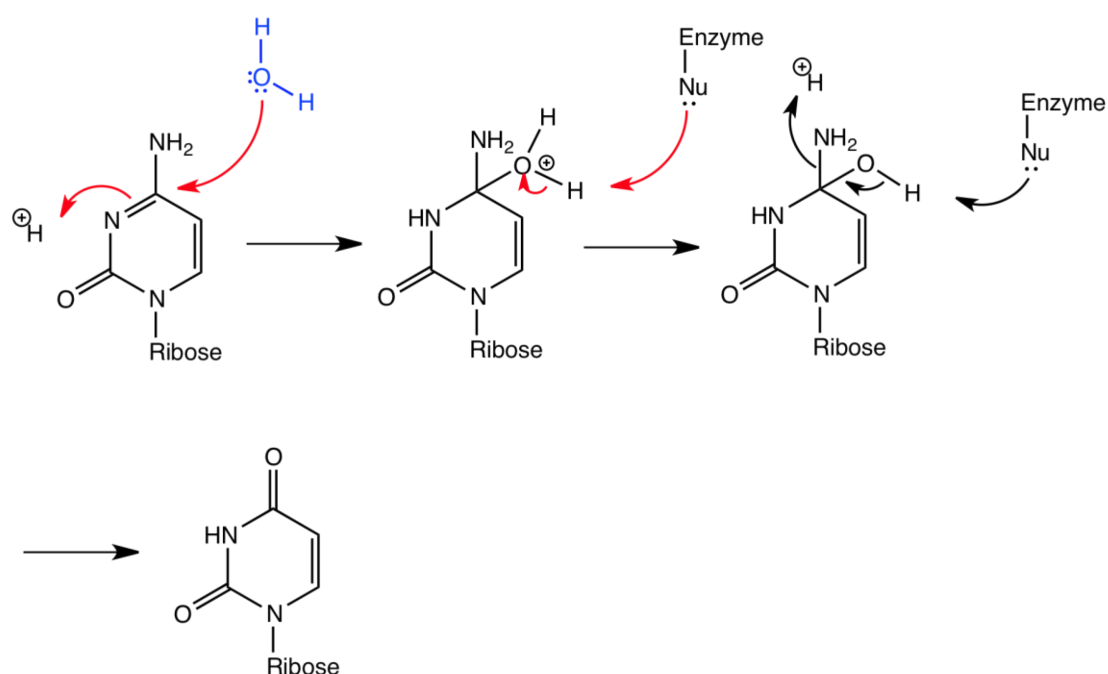
8) The reaction catalysed by cytidine deaminase is as follows and includes an intermediate where an OH group has been added to form a tetrahedral centre. The OH group is provided by the conserved water molecule present in the active site



A possible mechanism is as follows



The proton provided in the mechanism is likely to be provided by an amino acid residue such as histidine.

The enzyme-catalysed reaction mechanism carried out on zebularine will create the structure below which serves as a transition-state analogue and enzyme inhibitor. A transition state analogue is an effective inhibitor as it is believed that the transition state of an enzyme-catalysed reaction mechanism is bound more strongly than either the substrate or the product. Although a transition state is a highly unstable species, it is believed that it bears more of a similarity to the reaction intermediate than to the substrate or product. Therefore, a transition state analogue should contain a tetrahedral centre and relevant binding groups. In this case, the OH group can form crucial hydrogen-bonding interactions and is orientated in a similar manner to the normal reaction intermediate. Unlike the usual reaction intermediate, however, the transition

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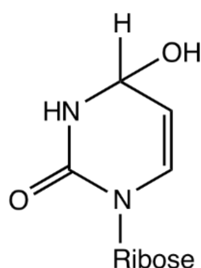
state analogue is stable as there is no leaving group available to allow the formation of the carbonyl group.



The transition state analogue formed from zebularine

3,4-Dihydrozebularine lacks the C=N bond within the heterocyclic ring, which means that an enzyme-catalysed reaction is not possible with the conserved water molecule. Therefore, the structure binds without any alteration and no transition-state analogue is formed. The structure binds more weakly as there is no OH group present to form an additional hydrogen-bonding interaction.

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