


Medicinal Chemistry
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Anti-Viral Agents Part – 2

So welcome to today's lecture in this lecture we will cover various aspects of anti-viral agents as we have discussed quite a bit about, how the HIV and related viruses can infect mammalian cell and how they are able to use the host machinery to produce new virions which then can go ahead and infect newer host cell and so on, so in today's lecture we will discuss at some strategies to counter this virus.

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Protease inhibitors

- In the mid 1990s, the use of X-ray crystallography and molecular modelling led to the structure-based design of a series of inhibitors which act on the viral enzyme HIV protease
- Unlike the reverse transcriptase inhibitors, the PIs are not prodrugs and do not need to be activated.



Patrick, G. L.

So the first major class of inhibitors of we want to look at today are this protease inhibitors, so somewhere in the 90's X-ray crystallography become quite prevalent in drug discovery and so you could crystalize proteins along with ligand and therefore you could understand and how the major interaction occur and also parallelly molecular modeling had measure to some extent as a field and so it was being used to design new structure, so using the synergy of X-ray crystallography and molecular modeling a lot of structure design of inhibitors was possible.

So these enzyme called as viral enzyme HIV protease was important enzyme to inhibit, so we have already previously looked at the reverse transcriptase inhibitors and since reverse transcriptase is an enzyme that is present a primarily in a viruses is not a humans this was

something that we could exploit, so but unless unlike those reverse transcriptase inhibitor which need a phosphor relation to be activated these protease inhibitor is not pro-drug and so they need not be activated they can they are active in their actual administered form.

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The HIV protease enzyme

- The HIV protease enzyme is an example of an enzyme family called the **aspartyl proteases** —enzymes which catalyse the cleavage of peptide bonds and which contain an aspartic acid in the active site that is crucial to the catalytic mechanism.



So look at the HIV protease enzyme, so this is an enzyme which is belong to the family of aspartyl proteases and we have already looked at this previously several lectures back or several weeks back actually about how aspartyl proteases is work will look at it briefly once again but these enzyme catalyze the cleavage of peptide bonds and they contain aspartic acid in the active site and that is crucial for the catalytic mechanism, so here is the active site of this enzyme and there is something interesting called as flaps which we are look at later.

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The HIV protease enzyme

- The enzyme can be obtained by synthesis or cloned and purified...
- The enzyme is crystallized with or without an inhibitor bound to the active site, making it an ideal target for structure-based drug design where X-ray crystallographic studies of enzyme-inhibitor complexes allow the design of novel inhibitors.



Patrick, G. L.

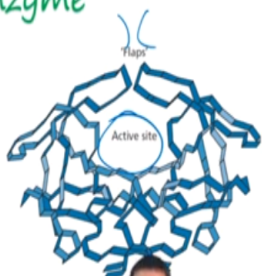
So this enzyme can be obtained by synthesis or it can be cloned and purified and so we can have access to large amount enzyme pretty much, so then it is crystallized with or without an inhibitor bound to be active site, so what happens is that when you crystallized it and it is may to form then you get a structure which probably resembles the form in which it is going to bind inhibitor and once the inhibitor is bound that would represent the induced fit that happens.

So now based on this we can have a good idea about what happens during the binding process and after it is bound so this kind of information provides an ideal mechanism or ideal vehicle for us to understand or to be able to carry out structure based design so using this we would be able design new inhibitors.

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The HIV protease enzyme

- The HIV protease enzyme is a symmetrical dimer made up of two identical protein units, each consisting of 99 amino acids.
- The active site is at the interface between the protein units and is also symmetrical with twofold rotational (C_2) symmetry.



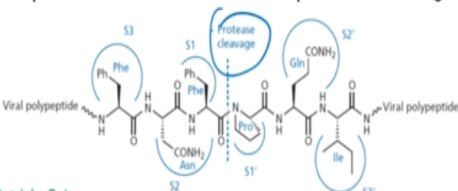
Patrick, G. L.

So the HIV protease is a symmetrical dimer made up of two identical units, so it is shown here this is one unit and this is second unit and it contains about 99 amino acids the active site is at the interface between the protein units and is also symmetrical with twofold rotational symmetry, so here is the active site which is also has C_2 symmetry in it.

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The HIV protease enzyme

- The enzyme has a broad substrate specificity and can cleave a variety of peptide bonds in viral polypeptides, but, crucially, it can cleave bonds between a proline residue and an aromatic residue (phenylalanine or tyrosine)
- The cleavage of a peptide bond next to proline is unusual and does not occur with mammalian proteases such as renin, pepsin, or cathepsin D, and so the chances of achieving selectivity against HIV protease over mammalian proteases are good



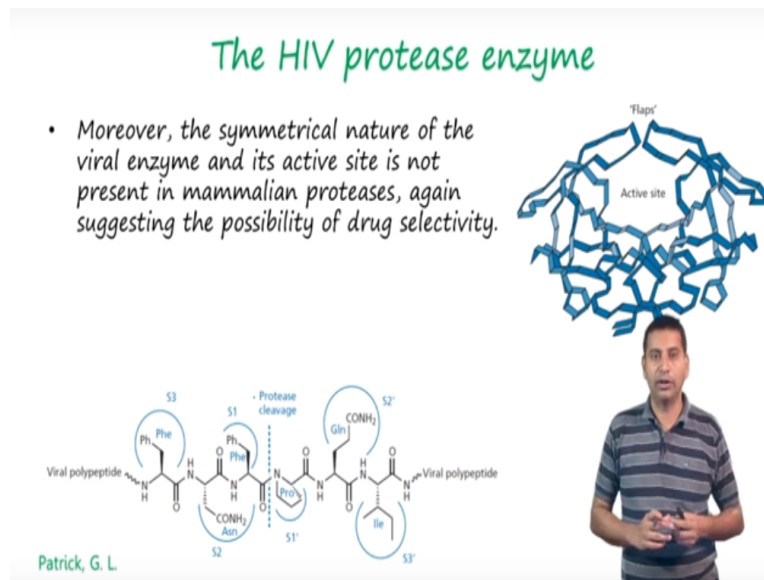
Patrick, G. L.

So the enzyme actually has broad substrate specificity and it can cleave a variety of peptide bond in viral polypeptides but crucially it can cleave the bond between a pro-line residue and I shown here pro-line residue and an aromatic amino acid residue such as phenylalanine or tyrosine, so

the cleavage of the peptide bond next to the pro-line is unusual and does not occur in mammalian proteases which we are already familiar with such renin, pepsin or cathepsin D, so the chances of achieving selectivity against HIV protease is actually quite good, so this is another aspect of drug design that we have discussed previously.

So when we are using or when we are identifying an enzyme as a target we need to keep in mind that the enzyme may or may not be present in the mammalian cell or the host cell as well as there is anything unique about the one code, so that one code achieves selectivity so here this enzyme satisfies both the criteria that is it is present in mammalian cells or human but it is not going to cleave that particular bond and so therefore one could use it for drug development.

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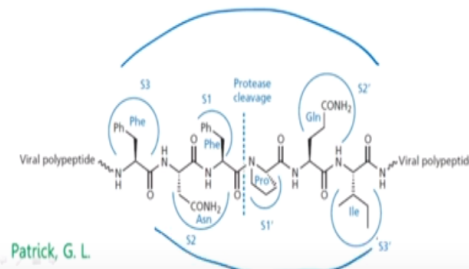


So the symmetrical nature of the viral enzyme and its active site is not present in mammalian proteases, so there is a significant difference in the activity of the structure of the mammalian version and the viral version.

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The HIV protease enzyme

- There are eight binding subsites in the enzyme—four on each protein unit, located on either side of the catalytic region
- These subsites accept the amino acid side chains of the substrate and are numbered **S1–S4** on one side and **S1'–S4'** on the other side

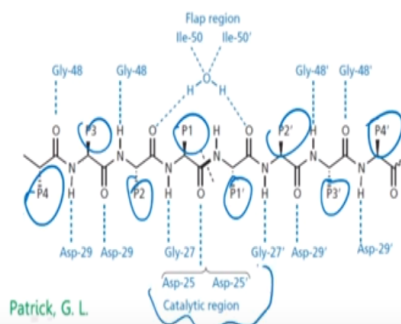


So in this enzyme there are binding sub-sites in the enzyme four on each of the protein unit and located on either side of the catalytic region and so these are numbered S and S4 and or S1 prime to S4 prime on the other side so you can see here this is these are the sub-units.

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The HIV protease enzyme

- The relevant side chains on the substrate are numbered **P1–P4** and **P1'–P4'**
- Peptide bonds in the substrate are also involved in hydrogen bonding interactions with the active site



Now the relevant side chains on the substrate are numbered as P1 to P4 and P1 prime to P4 prime so here is P1 P2 P and P4 and the corresponding other sides so the peptide bond in the substrate is also involved in hydrogen bonding interaction with the active site and here is the catalytic region.

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The HIV protease enzyme

- A water molecule is present in the active site which acts as a hydrogen bonding bridge to two isoleucine NH groups on the enzyme flaps.
- This hydrogen bonding network has the effect of closing the flaps over the active site once the substrate is bound.

The diagram illustrates the active site of the HIV protease enzyme. A water molecule (H₂O) is positioned in the center, acting as a hydrogen bonding bridge. It forms hydrogen bonds with the NH groups of two isoleucine residues (Ile-50 and Ile-50') located on the enzyme flaps. The flaps are shown closing over the active site. Other residues shown include Gly-48, Gly-48', Gly-27, Gly-27', Asp-29, Asp-29', and Asp-25, Asp-25' in the catalytic region. The substrate is shown as a polypeptide chain with residues P1, P1', P2, P2', P3, P3', P4, and P4'.

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Now an interesting thing about this protease enzyme is that there is a water molecule present in the active site which acts as a hydrogen bond bridge, so here is the water molecule and this is the hydrogen bridge that we are talking about and what happens is that this bridges to isoleucine NH groups on the enzyme flaps, so we have already at those two flaps in the enzyme and so the hydrogen bonding network has the effect of closing the flaps over the active sites once the substrate is bound, so it is almost like how the flaps are open and after the substrate is bound the flaps closed.

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The HIV protease enzyme

- The aspartic acids Asp-25 and Asp-25' on the floor of the active site are involved in the catalytic mechanism.
- Each of these residues is contributed by one of the protein subunits, and the carboxylate side chains interact with a bridging water molecule during the hydrolysis mechanism

The diagram illustrates the active site of the HIV protease enzyme, focusing on the catalytic region. A water molecule (H₂O) is positioned in the center, acting as a hydrogen bonding bridge. It forms hydrogen bonds with the NH groups of two isoleucine residues (Ile-50 and Ile-50') located on the enzyme flaps. The flaps are shown closing over the active site. Other residues shown include Gly-48, Gly-48', Gly-27, Gly-27', Asp-29, Asp-29', and Asp-25, Asp-25' in the catalytic region. The substrate is shown as a polypeptide chain with residues P1, P1', P2, P2', P3, P3', P4, and P4'.

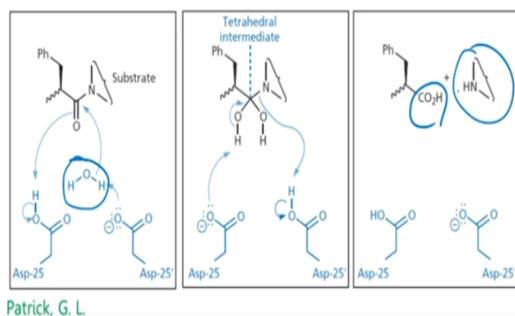
Patrick, G. L.

So the aspartic acids unit over shown here ASP-25 and 25 prime on the floor of the active site are involved in the catalytic mechanism, each of this residue is contributed by one of the protein subunits and the carboxylate side chains interact with a bridging water molecule during the hydrolysis mechanism this is again some thin that we looked at previously.

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Design of HIV protease inhibitors (PIs)

- A mechanism similar to the one that we have previously discussed is operational (renin)

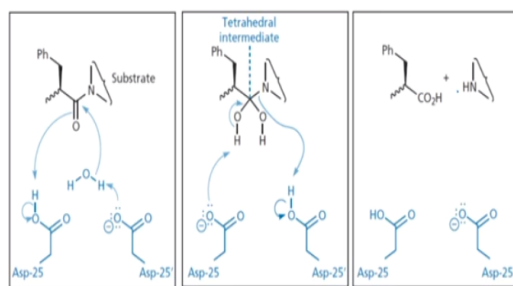


So here is the mechanism that we discuss previously for a renin so here is the bridging water molecule and this water molecule is actually activated by the aspartate residue here and by assistance from the aspartate these water molecule attacks the might bond and forms the tetrahedral intermediate and subsequently under goes in which to produce an are main and a carboxylic acid and therefore this mechanism is what is operating in renin a very similar mechanism is operational in HIV protease.

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Design of HIV protease inhibitors (PIs)

- Transition-state inhibitors are suggested... many of the strategies resulting from the development of renin inhibitors were adapted to the design of HIV PIs.



Patrick, G. L.



So based on this mechanism one could suggest that transition state inhibitors can be used and now there are many strategies there have been used which have previously used for inhibitors and this can be adapted for the design of HIV protease inhibitors.

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Transition State Inhibitors

- Transition-state inhibitors are designed to mimic the transition state of an enzyme-catalysed reaction.
- The transition state is likely to be bound to the active site more strongly than either the substrate or product.
- Therefore, inhibitors resembling the transition state are also likely to be bound more strongly.

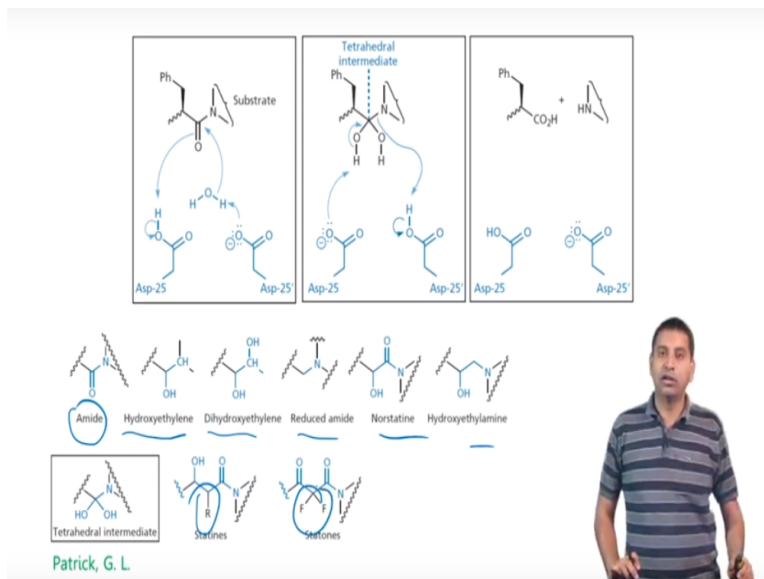
Patrick, G. L.



So just a sort of recap transition state inhibitors are designed to mimic the transition state of an enzyme catalyzed reaction so here the logic is that if the inhibitor resembling the transition state then the enzyme binds to the transition state significantly stronger compared to the substrate or the pro-drug and, so once this happens then it results in a situation where it is competitive

natural substrate, so therefore inhibitors resembling the transition state are quite actively sort after.

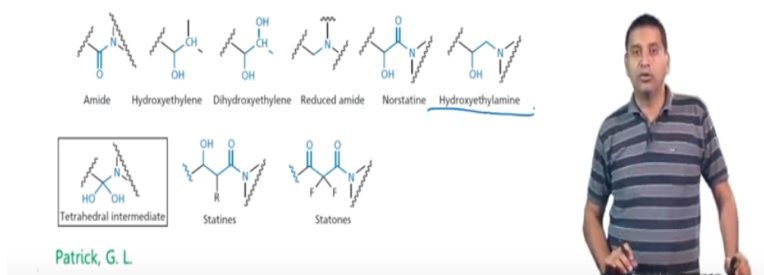
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So based on the chemistry that had already been developed on from rinine inhibitors so there are number of sort of inhibitors that one could proposed which have all these various functional groups like dihydroxyethylene, hydroxyethylene, reduced amide, hydroxyethylamine and so on and so forth and of course we could also use a you know statines and statones as potential inhibitors.

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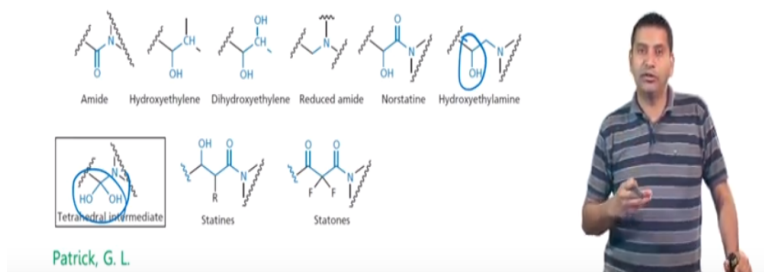
- A large number of structures were synthesized incorporating these isosteres...
- The hydroxyethylamine isostere was found to be the most effective



So large numbers of this structure were synthesis which incorporated these isosteares and what was found the hydroxyethylamine was found to be the most effective.

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- This isostere has a hydroxyl group which mimics one of the hydroxyl groups of the tetrahedral intermediate and binds to the aspartate residues in the active site.



So this isosteare has a hydroxyl group which mimics the of the hydroxyl in the tetrahedral intermediate so here is the tetrahedral intermediate and here is the hydroxyl group and therefore it can bind to the aspartate residues in the active site.

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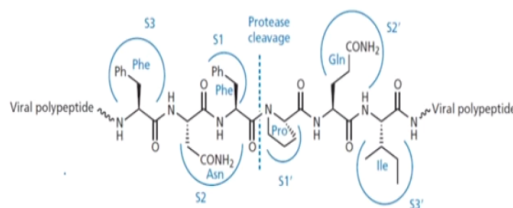
- Having identified suitable transition-state isosteres, inhibitors were designed based on the enzyme's natural peptide substrates, as these contain amino acid residues which fit the eight subsites and allow a good binding interaction between the substrate and the enzyme.

Patrick, G. L.

So having identified this suitable transition state isosteres, inhibitors were then designed based on the enzymes natural peptide substrates, so that it can go and bind to the HIV protease and because it is an inhibitor therefore does not undergo trend over it is there is no amide bond to cleave therefore it would bind to the enzyme and prevent for the substrate from acting on it.

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- There are eight binding subsites in the enzyme—four on each protein unit, located on either side of the catalytic region
- These subsites accept the amino acid side chains of the substrate and are numbered S1–S4 on one side and S1'–S4' on the other side.

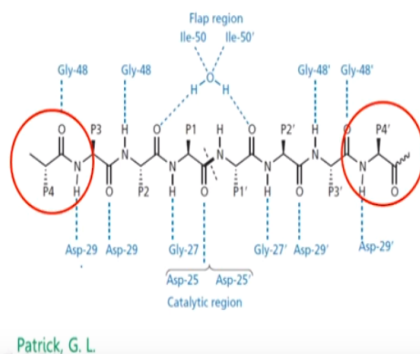


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So there are eight binding sub-sites in the enzyme as we discuss previously and these are numbered are S1 to S4 on one side and S1 prime to S4 prime the other side.

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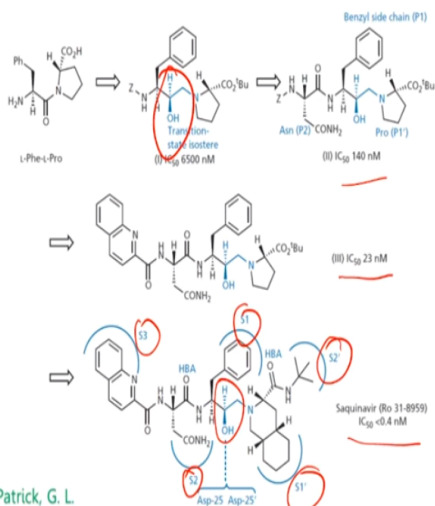
- The relevant side chains on the substrate are numbered P1–P4 and P1'–P4'



Now the relevant side chains on the substrate are again numbered labeled as P1 to P4 and P1 prime to P4 prime.

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- The molecular weight would be quite large! Poor oral bioavailability...

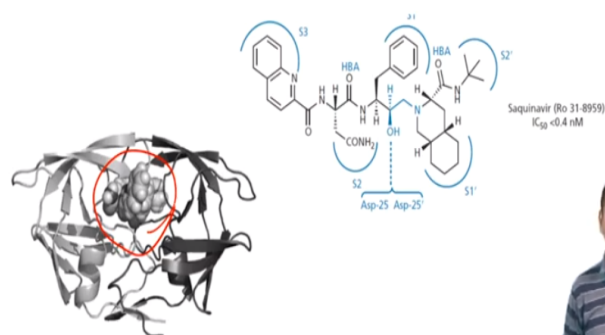


So using this concept there are been number of inhibitors that have been developed here is transition state isostere which has a IC 50 of about 6500 Nano molar and subsequently this is been further optimized to reduce the CI 50 to 140 Nano molar and finally further modification relate to an improved IC 50 of 20 Nano molar, so we are getting really closed to where we want to get to and finally based on the interaction of the S3 S1 S2 sub-units and keeping in the

transition state in mind as well as S2 prime and S1 prime this molecule which is quinavir was identified this has an IC 50 of less than 0.4 Nano molar and that is fantastic for use as an HIV protease inhibitor.

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- Optimizing this gave the final structure Saquinavir



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So finally this structure Saquinavir was developed which has the following structure and if you cocrystallize this molecule in with HIV protease you find a rate fits very beautifully into the active sites with the following binding interactions.