ANTIVIRAL DRUGS: HIV-1 PROTEASE INHIBITORS

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2016-2017
The aspartic protease of human immunodeficiency virus (HIV-1 PR) is responsible for the cleavage of the viral Gag and Gag-Pol polyprotein precursors into mature, functional viral enzymes and structural proteins. This process, called viral maturation, which leads to the final morphological rearrangements, is indispensable for production of infectious viral particles.

If HIV-PR is inhibited, the nascent virions cannot go on to attack other cells and the spreading of HIV is therefore stopped.

Introduction of HIV protease inhibitors (PIs) in 1995 and the application of highly active anti-retroviral therapy (HAART).
**Antiviral Drugs: HIV-1 Protease Inhibitors**

- Symmetrical protein made up of two identical protein subunits (99-residue monomers assembled into a $C_2$-symmetric structure)
- Active site is symmetrical unlike human proteases
- Selective inhibition might be possible
- Flaps help to seal the substrate and inhibitors into the active site
- Member of the aspartyl proteases
**Antiviral Drugs: HIV-1 Protease Inhibitors**

**NOTES**

- 8 binding subsites available for amino acid side chains (S4-S4’)
- HIV-Protease can cleave between Phe and Pro
- No similar reaction in mammalian proteases
- Possibility of selective inhibitors
**Substrate Binding and Cleavage**

**Antiviral Drugs: HIV-1 Protease Inhibitors**

**FLAP REGION**

Ile-50  Ile-50'

Gly-48   Gly-48

**CATALYTIC REGION**

Asp-29  Asp-29

Gly-27

Asp-29'  Asp-29'  Gly-27'  Gly-27'

Asp-25  Asp-25'

**NOTES**

- Hydrogen bonding interactions involving peptide backbone
- Binding of amino acid side chains to binding subsites
- Bridging water molecule to flap region
**Reaction Mechanism**

**Antiviral Drugs: HIV-1 Protease Inhibitors**

**Reaction mechanism**

**NOTES**

- Asp-25 and Asp-25’ are crucial to the mechanism
- Bridging water is involved
1. HIV-1 Protease Inhibitors (PIs)

- Saquinavir (Fortovase and Invirase) - first protease inhibitor used clinically (1995)
- Ritonavir (Norvir) (1996)
- Indinavir (Crixivan) (1996)
- Nelfinavir (Viracept) (1997)
- Amprenavir (Agenerase) (1999)
- Atazanavir (2003)
- Lopinavir with ritonavir (Kaletra).
- Lopinavir active against ritonavir-resistant strains of HIV
- Resistance occurs if protease inhibitors used on their own
- Used in combination with reverse transcriptase inhibitors for HIV
2. Design of HIV Protease Inhibitors

- Similar strategy to renin inhibitors
- Designed as transition-state inhibitors
- Mimic the transition state of the reaction mechanism
- Stable to the enzyme-catalysed reaction
- Introduce a stable tetrahedral feature to mimic the tetrahedral stereochemistry of the transition state: transition-state isostere
- Hydroxyethylamine isostere proven to be effective
Antiviral Drugs: HIV-1 Protease Inhibitors

Protease Inhibitors (PIs)

- Saquinavir, 1995
  SQV, Invirase® & Fortovase®
  X1

- Indinavir, 1996
  IDV, Crixivan®
  X2

- Ritonavir, 1996
  RTV, Norvir®
  X3

- Nelfinavir, 1997
  NFV, Viracept®
  X4

- Amprenavir, 1999
  APV, Agenerase®
  X5

- Lopinavir, 2000
  LPV
  Kaletra® & Aluvia®
  (LPV+RTV)
  X6

- Atazanavir, 2003
  ATV, Reyataz®
  X7

- Fosamprenavir, 2003
  FPV
  Lexiva® & Telzir®
  X8

- Tipranavir, 2005
  TPV, Aptivus®
  X9

- Darunavir, 2006
  DRV, Prezista®
  X10
3. Saquinavir

**Lead compound**

- Pol is a viral polypeptide substrate for HIV protease
- Includes a pentapeptide sequence containing the susceptible Phe-Pro linkage

![Chemical structure of Saquinavir](image)
3. Saquinavir

**Lead compound**

![Saquinavir structure](image)

- **Benzylloxycarbonyl (Z) protecting group**
- **NH4Bu protecting group**
- **Hydroxyethylamine transition-state isostere**
3. Saquinavir

Lead compound

Benzylloxycarbonyl (Z) protecting group

NOTES
• Stable to enzyme-catalysed reaction
• 5 side chains fit subsites S3-S2’
• Weak inhibitor ($IC_{50}$ 750 nM)

DISADVANTAGES
• High molecular weight
• High peptide character
• Poor oral bioavailability
3. Saquinavir

Drug Design

- Simplification: start from a dipeptide rather than a pentapeptide
- Aims: to lower MW and peptide character

NOTES

- Hydroxyethylamine transition-state isostere
- Stable to enzyme-catalysed reaction
- Protecting groups present on N- and C-termini
- Side chains (P1 and P1’) occupy enzyme subsites (S1 and S1’)
- Weak inhibitor ($IC_{50}$ 6500 nM)
3. Saquinavir

Drug Design

- Extension strategy: add an extra amino acid
- Aim: to increase binding interactions

**NOTES**

- Side chain of Asn (P2) occupies subsite (S2)
- Results in increased binding
- 40 fold increase in inhibition (**IC₅₀ 140 nM**)
3. Saquinavir

Drug Design

NOTES

- Asn is the optimum amino acid for P2
- Benzyl group is the optimum side chain for P1
3. Saquinavir

Drug Design / X-Ray Crystallography

NOTES

- Protease crystallised with above ligand
- Structure determined by X-ray crystallography
- Tertiary butyl protecting group occupies subsite S2’
- Protecting group (Z) occupies large hydrophobic subsite S3
- Modify Z to increase binding interactions to S3
3. Saquinavir

**Drug Design**

- Modify Z to increase binding interactions to S3

**NEXT:**
- Six fold increase in inhibition ($IC_{50} 23$ nM)
- Modify the proline side chain (P1')
- Modify the tertiary butyl ester (P2')

Quinoline
3. Saquinavir

Drug Design

- R-Stereochemistry is essential for the transition-state isostere
- 60 fold increase in activity (IC$_{50}$ 0.4 nM)
- Saquinavir was the first protease inhibitor to reach the clinic

**Protease Inhibitors (PIs): Saquinavir**

**Antiviral Drugs: HIV-1 Protease Inhibitors**
3. Saquinavir

Binding interactions

NOTES

• Studied by X-ray crystallography
• Five subsites are occupied (S3-S2’)
• The S3’ subsite is inaccessible
• The transition state isostere interacts with the catalytic aspartates
• Carbonyls act as HBAs to the bridging water molecule in the flap region
3. Saquinavir

**Binding interactions**

**Disadvantages**

- Poor oral bioavailability
- Susceptible to drug resistance
- Subsequent research on more modern protease inhibitors aims to reduce MW and peptide character
4. Ritonavir and Lopinavir

- Developed by Abbott Pharmaceuticals
- Designed to take advantage of the symmetrical nature of the active site
- Symmetrical inhibitors should be capable of binding left to right or right to left
- Symmetrical inhibitors likely to show greater selectivity over mammalian proteases
- Symmetrical inhibitors likely to be more resistant to hydrolytic breakdown by peptidases
- Lead compound designed to have C2 symmetry
4. Ritonavir and Lopinavir

*De novo* design of a symmetrical lead compound

- **C2** symmetry of active site
- **P₁**  \( \rightarrow \)  **P₁'**

**NOTES**

- *De novo* design is based on the enzyme-catalysed reaction intermediate
- Benzyl group is retained - strong binding group to S1
- Symmetrical reaction intermediate contains two benzyl groups
- Remove one alcohol group to stabilise the molecule
- Molecular modelling confirms that molecule should bind to the active site
- The target alcohol is synthesised and tested
4. Ritonavir and Lopinavir

Drug Design

- Target alcohol (I) acts as a weak enzyme inhibitor
- Inactive *in vitro*
- But still represents the successful *de novo* design of a lead compound

\[
\text{Target alcohol (I)}
\]

\[
\text{IC}_{50} > 10,000 \text{ nM}
\]

\[
\text{Target alcohol (II)}
\]

\[
\text{IC}_{50} 590 \text{ nM}
\]

Activity increases with the addition of valines
4. Ritonavir and Lopinavir

Drug Design

Target alcohol (I)
$IC_{50} > 10,000 \text{ nM}$

(II)
$IC_{50} 590 \text{ nM}$

A74704
$IC_{50} 3 \text{ nM}$

- Activity increases with the addition of protecting groups
- Resistant to proteolytic breakdown
4. Ritonavir and Lopinavir

Binding interactions for A74704

**NOTES**
- Symmetrical binding pattern
- Binding interactions to Gly-27 and Gly-27’ are not optimum
- Increasing the distance between relevant NH groups may be beneficial
4. Ritonavir and Lopinavir

*De novo* design of a symmetrical lead compound II

**NOTES**

- *De novo* design is based on the enzyme-catalysed reaction intermediate
- Axis of symmetry is designed to go through the centre of a bond rather than the reaction centre
- Allows the introduction of an extra atom
- Increases the separation of the NH groups
4. Ritonavir and Lopinavir

Comparison of A74704 and its diol equivalent

A74704
IC$_{50}$ 3 nM

Diol equivalent
IC$_{50}$ 0.22 nM
4. Ritonavir and Lopinavir

Properties of the diol equivalent of A74704

- Diol equivalent of A74704 shows 10-fold greater activity
- Poor water solubility
- Terminal portions are exposed to solvent (crystal structure)
- Possible to add more polar groups to increase solubility
4. Ritonavir and Lopinavir

**Drug design**

![Chemical structure of Ritonavir and Lopinavir]

**NOTES**

- Pyridines and N-Methylureas increase polarity and water solubility
- Poor oral bioavailability
- Entered clinical trials as an intravenous agent
- EC\textsubscript{50} 0.2 mM; K\textsubscript{i} 140 pM
- Does not bind as predicted by molecular modelling
- Asymmetric binding is observed in the crystal structure
- R-OH forms two hydrogen bonds to both catalytic aspartates
- S-OH forms only one hydrogen bond
- Remove S-OH to avoid energy penalty involved in desolvation
4. Ritonavir and Lopinavir

Drug design

Removal S-OH

NOTES

- Improved activity ($K_i$ 17 pM)
- Similar binding mode to A 77003
4. Ritonavir and Lopinavir

Drug design

![Chemical structure of A80987]

**NOTES**

- Modifications aimed at varying molecular weight, aqueous solubility and hydrogen bonding
- Fine tuning modifications
- Urethanes good for plasma half life and potency
- Urea replaced by urethane, valine removed from right hand segment
- Molecule no longer symmetrical, but smaller
- Retained activity ($EC_{50} 0.13$ mM) and improved oral bioavailability
- Relatively short plasma lifetime
- Binds strongly to plasma proteins
4. Ritonavir and Lopinavir

Drug design

- Pyridine rings susceptible to metabolism (N-oxidation)
- Steric shields and electron-withdrawing substituents fail to block metabolism
- Replace pyridine rings with alternative heterocycles (bio-isosteres)
4. Ritonavir and Lopinavir

**Drug design**

- Thiazolyl ring used as a bio-isostere for the pyridine ring (P3)
- Thiazolyl ring is bad for water solubility
- Replacing a urethane group with N-methylurea restores water solubility
- Activity improved by having an alkyl substituent on the thiazolyl ring and by shifting the OH group
- A83962 shows 8-fold increase in activity relative to A80987
4. Ritonavir and Lopinavir

Drug design

**NOTES**

- Thiazolyl ring is used as a bio-isostere for the pyridine ring (P2’)
- Improved activity (EC$_{50}$ 30 nM) and better oral bioavailability
- Thiazolyl nitrogen forms a hydrogen bond to Asp-30
- 20 x more stable to metabolism than A80987
- Therapeutic levels last 24 hours following oral administration
4. Ritonavir and Lopinavir

Drug design

NOTES

• Drug resistance arises when ritonavir is used alone

• Due to mutation of Val-82 to Ala, Thr or Phe

• Disrupts an important hydrophobic interaction between Val-82 and the isopropyl group
4. Ritonavir and Lopinavir

**Drug design**

NOTES

- P3 thiazolyl group removed and replaced with a cyclic urea
- Permits enhanced hydrogen bonding interactions with the S2 subsite
- Compensates for the loss of bonding interactions due to the thiazolyl group
- No interaction with Val-82
- Active against ritonavir-resistant strains
5. Indinavir

- Designed by Merck using a hybridisation strategy
- Takes advantage of the symmetrical nature of the active site
- Link one half of one inhibitor with one half of another
- Hybrid formed from P’ halves of L685,434 and Saquinavir

- Contains hydroxyethylene transition-state isostere
- Potent inhibitor (IC\textsubscript{50} 0.3 nM)
- Poor bioavailability
- Liver toxicity
5. Indinavir

Drug design

L685,434
IC\textsubscript{50} 0.3 nM

Saquinavir
IC\textsubscript{50} 0.4 nM

L704,486
IC\textsubscript{50} 7.6 nM
5. Indinavir
Drug design

- P’ half of Saquinavir is good for water solubility
- P’ half of L685,434 lacks peptide character
- L 704,486 is less active but still potent
- Oral bioavailability 15%

IC\textsubscript{50} 7.6 nM

L704,486
5. Indinavir
Drug design

NOTES
- P half of L704,486 is modified
- Decahydroisoquinoline ring is replaced with a piperazine ring
- Better water solubility and oral bioavailability
- Allows further substitution (benzyloxy carbonyl group)

L704,486
IC<sub>50</sub> 7.6 nM

L732,747
IC<sub>50</sub> 0.5 nM
5. Indinavir

Binding interactions for L732477

NOTES

- Crystal structure of L732,747 bound to HIV-protease determined
- High activity vs HIV protease
- Good enzyme inhibition does not necessarily mean high antiviral activity
- Drug has to cross the cell membrane
- Variety of substituents tried at the 4-position of piperazine
- Benzyloxycarbonyl group accesses the S3 subsite - extra interaction

L732,747
IC\textsubscript{50} 0.5nM
5. Indinavir
Drug Design

Benzyl group increases activity two-fold

Water solubility is decreased

Structure IV
IC$\text{50}$ 0.3 nM
Antiviral Drugs: HIV-1 Protease Inhibitors

6. Indinavir

Drug Design

NOTES

• Aromatic ring is replaced with a pyridine ring
• Pyridine ring is lipophilic and interacts with the lipophilic subsite S3
• Pyridine ring contains nitrogen
• Improves water solubility and oral bioavailability
• Potent inhibitor with negligible activity against mammalian proteases
• Less highly bound to plasma proteins compared to saquinavir
• Reached market in 1996

Indinavir

IC$_{50}$ 0.56 nM
K$_i$ 0.34 nM
EC$_{95}$ 0.10 mM
5. Indinavir

Binding interactions for Indinavir

Indinavir

IC₅₀ 0.56 nM
Kᵢ 0.34 nM
EC₉₅ 0.10 mM