# Web article 7

**The design of indinavir (CrixivanR)**

Indinavir arose from work originally carried out on a series of compounds containing a hydroxyethylene transition state isostere. The Merck company screened a collection of renin inhibitors which they had previously synthesized, and identified L364505 as a potent inhibitor of HIV protease (Fig. 1). However, antiviral activity was low due to the poor pharmacokinetics of the molecule (i.e. vulnerability to degradative enzymes, rapid biliary clearance and poor oral absorption). Thus, a range of analogues was made in order to find a smaller molecule which retained potent activity. It was discovered that the left hand (P) half of the molecule could be truncated by removing the two phenylalanines to give L682679 without loss of activity. Moreover, this compound lacked any activity against renin. Attention now turned to varying the right hand (P') half of the molecule, and in particular to remove the Leu-Phe dipeptide such that the peptide character of the molecule would be reduced. Replacing the dipeptide with a simple benzylamino group resulted in a drop in activity (structure II), but the fact that activity *was* still present encouraged the research team to look at further amines which were conformationally restrained, arguing that this might constrain the group into a favourable binding pocket. A fivefold increase in activity was observed when an indane ring system was incorporated (structure III). Adding substituents to the indane ring led to the discovery that a *cis* hydroxyl group gave a 70 fold increase in activity resulting in L685434. This suggests that the hydroxyl group is binding to a hydrogen bonding region in the S2' subsite. This compound also had antiviral activity, but since it showed poor oral bioavailability it was not taken forward to clinical trials. The compound also has poor water solubility and so it was reasoned that the introduction of an amine nitrogen into the P half of the molecule might improve both the solubility and bioavailability of the compound. A molecular modelling exercise was carried out where L-685,434 was docked into a model of the active site. This revealed that the aromatic rings at P1 and P1' were pointing towards the surface of the enzyme and that it should be possible to add substituents at the *para* position which could increase water solubility, but which would not interfere with binding interactions with the active site. Indeed such groups should be capable of retaining hydrogen bonding interactions with water at the surface of the enzyme. This led to structure L689502 where a morpholine group (known to be good for increasing water solubility) was linked to the *para* position through a hydroxyethyl 'spacer'. Although the activity against the isolated enzyme dropped slightly, antiviral activity increased as a result of improved cell penetration. Oral bioavailability also improved to 5%. Unfortunately, the compound had liver toxicity in animal studies. Nevertheless, the results demonstrated that oral bioavailability could be improved by improving water solubility.

At this stage a radically new approach was taken. Since the active site is symmetrical, the S and S' subsites are equivalent which means that either half of an inhibitor should fit the S or the S' subsites equally well. Taking this argument further, it should be possible to combine half of one PI with half of another to give a structurally distinct hybrid inhibitor. A modelling study was carried out to check the hypothesis and the Merck team decided to combine the P' half of their own hydroxyethylene transition state inhibitor with the P' half of the hydroxyethylamine transition state inhibitor saquinavir. The P' moiety of saquinavir was chosen for its solubility enhancing potential and the P' moiety of L685434 is attractive for its lack of peptide character. The resulting hybrid structure (L704,486) was less active as an inhibitor but was still potent. Moreover, the presence of the decahydroisoquinoline ring system resulted in better water solubility and oral bioavailability (15%) as intended. However, antiviral activity proved relatively weak.

Hydroxethyelene

Ph isostere Ph

O O

BocPhePheHN

OH

LeuPheNH2

Ph

BocHN

OH

LeuPheNH2

Ph

L364,505 (IC50=1nM)

L682,679 (IC50=0.6nM)

Ph

BocHN

OH

O

NHCH2Ph

Ph

Ph

O

BocHN N

H

OH

Ph

Indan ring system

Conformationally restrained bond

1. (IC50=111nM)
2. (IC50=21nM)

O

O N

N H H H

H

H N

N N

H H H

HO O

Ph

O O

O N N

H H

OH OH

H H2NOC

P' P

Saquinavir (Ro 31-8959/003)

P

L-685,434 R=H

P'

R

IC50 0.3nM

O

N

L-689,502 R=

O

IC50 0.45nM

O NH

O

N N

L-704,486 H H

IC50 7.6nM HO OH

H

**Fig. 1** Development of L704,486 (Boc=Me3C-O-(CO)-).

It was now decided to replace the decahydroisoquinoline ring with a piperazine ring for two reasons, First it would be possible to functionalise the nitrogen at the 4-position allowing the possibility of adding a ligand capable of binding to the S3 subsite and which could be varied in its hydrophilic/hydrophobic character. Second, the additional amine should improve aqueous solubility and oral bioavailability. The resulting piperazine structure L732,747 (Fig. 2) had improved activity both on the isolated enzyme and in the antiviral cell based assay, and was co-crystallised with HIV protease for X-ray crystallographic studies. These revealed that the binding pockets S2 to S2' were all filled and that the benzyloxycarbonyl group on piperazine fitted the lipophilic S3 binding pocket.

One of the critical aspects of the cell based assay used in these studies is that the drug should cross a cell membrane and so high activity versus the isolated enzyme does not necessarily correlate with antiviral activity. A variety of substituents was placed at the 4-position of piperazine and it was found that a benzyl group increased activity by 2-3 fold in the cell based assay (structure IV). However, water solubility decreased. This was solved by replacing the aromatic ring with a pyridine ring

which was lipophilic enough to retain the hydrophobic interactions with the S3 pocket but included a weakly basic nitrogen to improve water solubility and oral bioavailability. This resulted in indinavir which is a potent inhibitor of both HIV-1 and HIV-2 proteases, showing negligible inhibition of mammalian proteases. Indinavir reached the market in 1996. It has better oral bioavailability than saquinavir and is less highly bound to plasma proteins (60%).

S2 S2'

L-704,486 IC50 7.6nM

O NH

S3 O

N N

H

O N HO OH

O

S1 S1'

L732,747 IC50 0.5nM

O NH

O

N

N HO

O NH

O

N N N

H H

OH N N HO OH

(IV) IC 50 0.3nM

Indinavir (MK-639) (L-735,524)

Ki 0.34 nM

IC50 0.56nM

**Fig. 2** Development of indinavir.

EC95 0.10M