**Web article 18 Nelfinavir (ViraceptR)**

The Lilly company began work on the development of nelfinavir, starting with the hydroxyethylene

transition state inhibitor LY289612 (Fig. 1) as their lead compound. This structure has the same groups P1-P3 as those used in saquinavir, while the original proline at P1' has been replaced with an *ortho* substituted benzamide. This latter group was introduced since it was anticipated that the amide side chain would be forced out of the plane of the aromatic ring and allow the carbonyl oxygen to take part in hydrogen bonding to the water molecule located in the flap region of the enzyme. LY289612 is a potent inhibitor of HIV-1 protease and also has good antiviral activity in whole cell assays. However, the compound has poor oral bioavailability. Therefore, the company set out to improve enzyme inhibition,

antiviral activity and oral bioavailability. To achieve this an iterative process was carried out whereby the crystal structure of the enzyme-inhibitor complex was studied to identify what modifications might be beneficial to the inhibitor. Once synthesized, the new inhibitor was co-crystallised with the enzyme to identify whether binding interactions were as predicted and then further modifications were carried out.

Thus, a crystal structure of the inhibitor(LY289612)-enzyme complex was studied and revealed that the binding subsites S3-S2' were occupied as shown in figure 17.30. It was also shown that the benzamide carbonyl had rotated out of the plane to allow the expected interaction with the water molecule in the flap region. It was further noted that the P1 phenyl and P3 quinoline rings were relatively close (3.2Å) and could actually interact with each other. This suggested that it might be possible to modify and expand the P1 substituent such that it could bind to both the S1 and S3 subsites, allowing the P3 substituent to be truncated or removed. Computer modelling studies suggested that replacing the phenyl group with *S*-phenyl or 2-*S*-Naphthyl would achieve this goal. A variety of structures was synthesized and this led to the discovery of LY297135 with slightly improved activity despite the replacement of the quinoline group (P3) with a methyl group. LY297135 was co-crystallised with the enzyme to study the actual binding interactions and this revealed the expected binding with the S-naphthyl group spanning the S1 and S3 subsites. The sulfur atom was found to occupy the S1 subsite providing good hydrophobic interactions, while the aromatic ring occupied the S3 subsite. Unfortunately, the antiviral activity of LY297135 in the whole cells assay proved less impressive (ED50=23nM) than its inhibition of the enzyme.

S3 ca. 3.2A S1

Ph O

O

H N

N N

H

O OH

CONH2

S2

LY289612 (IC50 1.5 nM)

S2'

H N

S1'

H

S O N

O

H N

N H

O OH

CONH2

LY297135 (IC50 1.1 nM)

(ED50 23 nM)

**Fig. 1** Development of a substituent spanning the S1 and S3 subsites.

Attention now turned to the P2 substituent. The presence of arginine in both LY289612 and LY297135 gives these structures a peptide character which may account for their poor oral bioavailability, and so an alternative P2 substituent was sought. It was decided that a bicyclic tetrahydroquinoline ring system would be capable of filling the S2 subsite and this led to AG1204 (Fig. 2). Studies of the crystal structure of the inhibitor-enzyme complex revealed that the inhibitor was binding as expected, with the bicyclic tetrahydroquinoline ring system positioned out of plane with the carbonyl group. These studies then led to the conclusion that the bicyclic tetrahydroquinoline system could be simplified to an aromatic ring containing an amino group at position 3 and a methyl group at position 2 (AG1232). The amino substituent matches the nitrogen present in tetrahydroquinoline while the methyl substituent acts as a conformational blocker and forces the aromatic ring out of plane with the carbonyl group. AG1232 had comparable activity to AG1204, and X-ray crystallography was carried out on the enzyme inhibitor complex. Unexpectedly, this revealed a different mode of binding where the methyl substituent was buried deep in the S2 pocket and the amine group was able to form a hydrogen bond to the side chain carboxylate group of Asp30. To achieve this, the aromatic ring had flipped 180o and there was an edge to face interaction with the *S*-naphthyl ring. The amino group was replaced with a phenol since the latter is a better hydrogen bond donor, and this provided AG1254 which was 10 times more active in the enzyme assay and 5 times more active in the whole cell antiviral assay. Thus, a structure had been designed with comparable enzyme activity to LY289612 but with decreased molecular weight. Unfortunately, antiviral activity was still less than optimal and the compound had poor aqueous solubility

It was now decided to switch direction and see what effect the newly designed P2 substituent would have if it was incorporated into saquinavir in place of the P2/P3 moieties. A molecular modelling experiment was first carried out to check whether this was feasible by superimposing the enzyme conformations of AG1254 and saquinavir. This proved favourable and so AG1310 was synthesized and found to have slightly weaker enzyme inhibitory activity but greatly increased antiviral activity, due possibly to better cell penetration. Oral bioavailability was also greatly increased.

The next step was to see whether the same strategy used in designing LY297135 could be used (i.e. extending the P1 substituent such that it interacted with both the S1 and S3 subsites). This was achieved using an *S*-phenyl group in place of phenyl to give nelfinavir with a 10 fold improvement in enzyme inhibition. A crystal structure of nelfinavir bound to the enzyme was studied which showed that the molecule is bound in an extended conformation where the binding interactions involving the molecular backbone are similar to saquinavir. A tightly bound water molecule serves as a hydrogen bonding bridge between the two amide carbonyls of the inhibitor and the flap region of the enzyme in a similar manner to other inhibitor-enzyme complexes. The crystal structure also showed that *S*-phenyl group resides mainly in the S1 site and partially extends into the S3 site. The substituted benzamide occupies the S2 pocket with the methyl substituent interacting with valine and isoleucine through van der Waals interactions, and the phenol interacting with Asp 30 through hydrogen bonding.

T etrahydro-

quinoline S O H

N

O

HN

N H

OH

H2N

H

S O N

Me O

N H

OH

AG1204 Ki 24nm

AG1232 Ki 49nm

H HO

H

Ph O N

Me O

S O

Me O

HO

N H

OH

N N N

H H

OH

H

AG1254

Ki 3nm

LY309391/AG1310

Ki 21nm

Ile 84

S3 S1

Val 32

H

S O N

S2'

Asp 30

HO

S2

Me O

N H

H

N H

OH

H

S1'

Nelfinavir (AG 1343)

Ki 2.0 nM EC50 0.008-0.02 M

Asp25 Asp25'

**Fig. 17.31** Development of nelfinavir.

**Fig. 2** Development of nelfinavir.

Nelfinavir mesylate was marketed in 1997 and is used as part of a four drug combination therapy. Like indinavir and ritonavir, nelfinavir is more potent than saquinavir due to its better pharmacokinetic profile. Compared to saquinavir, it has a lower molecular weight and logP, and an enhanced aqueous solubility, resulting in enhanced oral bioavailability. It can inhibit the metabolic enzyme CYP3A4 and

thus affects the plasma levels of other drugs metabolized by this enzyme. It is 98% bound to plasma proteins.

Surprisingly, the analogue containing the original *S*-naphthyl group proved to be a weaker enzyme inhibitor than AG1310. It was not clear why this was the case.