**Web article 19 Tipranavir**

Tipranavir (Fig. 1) is an example of a PI which was designed from a non peptide lead compound. High throughput screening of 5000 structurally diverse compounds from a compound bank led to the discovery that the anticoagulant **warfarin** (Fig. 1) was a weak PI with antiviral activity. This was followed up by testing various warfarin analogues leading to the discovery that the simplified structure **phenprocoumon** was a more potent competitive enzyme inhibitor with weak antiviral activity. Both these structures are used therapeutically for other purposes and have high oral bioavailability. Therefore they serve as promising lead compounds for non peptide like antiviral agents with good oral bioavailability.

A crystal structure of the enzyme inhibitor complex was determined showing that the 4-OH group of the coumarin ring was located within hydrogen bonding range of the catalytic aspartate residues, while the two lactone oxygens were hydrogen bonding

directly to the NH amides of the isoleucine groups (Ile 50 & Ile 50') in the enzyme flaps (Fig. 17.34). Unlike all the previous PIs, there was no bridging water involved in this interaction. Therefore, these compounds represented a new class of inhibitors with a novel pharmacophore of hydrogen bonding interactions. The crystal structure also showed that the ethyl and phenyl groups approximately fitted the S1 and S2 subsites respectively while the benzene ring of the coumarin ring system fitted the S1 subsite. However, the way this ring is positioned in the S1' subsite is such that substituents would not be directed to the S2' subsite.

Ile50 Ile50'

O O

O OH

O O

P2

4

P1 OH

P1'

Phenprocoumon

Ki 1M; ED50 100-300M

Warfarin

Me IC50 30M

Me

Asp 25 Asp 25'

**Fig. 1** Warfarin and phenprocoumon.

Therefore, it was decided that a study of 4-hydroxypyrones might be worth considering (Fig. 2). In these structures, the aromatic moiety (P1') binding to S1' is removed, but placing suitable substituents at position 6 might allow the introduction of groups which could bind to both the S1' and S2' subsites. Structure PNU 95929 was synthesized to test this hypothesis and was co- crystallised with the enzyme to study its binding interactions. It was shown that the ethyl and phenyl groups at the 3a position lie approximately in the S1 and S2 subsites respectively, while the phenethyl substituent lies close to the S2' subsite. It was then reasoned that an ethyl substituent placed at the 6a-position might fit subsite S1' and move the phenethyl group into the S2' subsite. PNU 96988 was obtained as a mixture of four stereoisomers due to the presence of two chiral centres. It can be viewed as being pseudosymmetrical in terms of the substituents present at positions 3 and 6 of the pyrone ring. The compound had an improved binding affinity and Ki for the enzyme. It also had good oral bioavailability and so clinical trials were started in 1993. However, the compound was withdrawn due to disappointing potency.

The company then moved to structures bearing two substituents at position 6 of the dihydropyrone ring (Fig. 17.35). Structure I had similar activity to PNU96988 but a crystal structure showed that it was binding differently with the hydroxyl group interacting with one of the catalytic aspartate groups rather than both. Attempts to use identical substituents at position 6 in order to remove a chiral centre were unsuccessful. Modification of the aromatic ring occupying the S2 subsite was now carried out. Substituents were added to access the S3 subsite leading to structure II which had good activity in cell assays. This was a mixture of four diastereoisomers and so the mixture was co-crystallised with the protease enzyme to identify the active diastereomer. The enzyme-inhibitor complex which was isolated was analysed by X-ray crystallography and shown to be the 3aR,6R diastereomer. The hydroxy group interacted almost symmetrically with both catalytic aspartates by hydrogen bonding, while the carbonyl oxygen displaced the normal bridging water and interacted with both isoleucine groups in the flap region of the enzyme. The

subsites S3-S2' were occupied as shown in figure 17.35, and the sulfonamide group formed strong hydrogen bonds to residues in the active site.

Further work led to PNU109112, but the sulfonamide group proved metabolically labile to an enzyme called glutathione *S*- transferase, resulting in cleavage of the bond between the sulfonamide group and the pyridine ring (Fig. 3). This is an unusual metabolic reaction for sulphonamides and studies showed that the reaction involved the enzyme carrying out a nucleophilic attack on the pyridine carbon bearing the sulphonamide group. The bond between the pyridine and the sulphonamide group was then cleaved with loss of sulfur dioxide and the amine half of the molecule. This reaction was found to take place if the pyridine carbon concerned was electrophilic, and since the cyano group at the *para* position of the pyridine ring is a strong electron withdrawing group, it activates this carbon towards this reaction. Replacing the cyano group with a group which was less electron withdrawing (i.e. CF3) led to tipranavir which had increased metabolic stability. X-Ray crystallographic analysis showed

that tipranavir bound to the active site in a similar fashion to structure II. The compound entered phase II clinical trials in 2000.

P1'

R

O O 6 \*

6

P2 3



\*

P1 OH

Ph

P2'

O O \* P2

\*

P1 OH

P2' Ph

P1'

Me

Me Me

PNU-95929 (R=H) Ki 0.5M; ED50 1mM I

PNU-96988 (R=Et)Ki 38nM; ED50 3M Ki 35nM; ED50 3M

S3

H N

SO2HN N

Me

S2

S1

II

O O 6

\*

3

\*

OH

Me

Me

S2'

S1'

NC

N

SO2HN

O O Ph

OH Me

Me

PNU-109112

Ki=1nM; IC50 0.5 M

Mixture of diastereomers

Ki 7pM IC50 40nM

F3C

N

SO2HN

O O Ph

OH

\* Chiral centre

Tipranavir (PNU-140690) Ki 8pM; IC50 30nM

**Fig. 2** Development of tipranavir (\* chiral centre).

Enzyme

N

 N Enzyme N

N C

SO2HN

R

HN C

SO2HN

R

NC

SO2

Enzyme

+ H2NR

**Fig. 3** Metabolic cleavage of PNU-109112.