**MOLECULAR MODELLING**

## EXERCISE 20.6 PLECONARIL

### INTRODUCTION

Pleconaril (Fig 1) is an antiviral agent that has undergone clinical trials for the treatment of the common cold. It binds into a hydrophobic pocket situated in the four proteins (VP1-VP4) making up the capsid coat of human rhinoviruses (see also section 20.9 in the textbook). In this exercise, you will download a crystal structure of the proteins VP1-VP4 with bound pleconaril, then extract the ligand to identify the active conformation. You will also identify hydrogen bonding interactions between pleconaril and the binding site

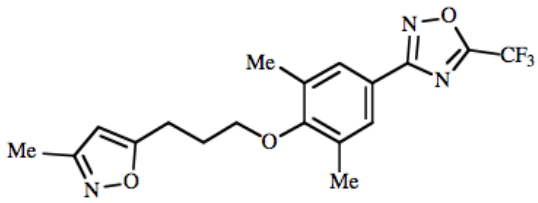



**Figure 1** Pleconaril.

### INSTRUCTIONS

It is suggested that you attempt to carry out the following instructions yourself before following the more detailed **Procedures** that follow. You may find the file entitled **Common Operations for ChemBio3D/Chem3D** a useful guide on how to carry out various operations. The ChemDraw file for pleconaril is available in the ChemDraw folder.

**PART A**

*Create an energy-minimised 3D-structure for pleconaril from its ChemDraw file.
*Identify the number of rotatable bonds.
*Identify different conformations for pleconaril using molecular dynamics.
*Identify different conformations using MMFF94 stochastic conformational sampling.

**PART B**

*Download the crystal structure of the HRV-14 protein with bound pleconaril (pdb file 1NA1).
*Identify the proteins that make up the complex.

**PART C**

*Locate pleconaril and the binding site.
*Extract pleconaril from the binding site.
*Compare the active conformation of pleconaril with the energy-minimised structure created in part A.
*Modify the energy-minimised conformation to form a similar conformation to the active conformation.

**PART D**

*Identify the expected binding interactions from the PoseVew image available from the pdb website.

**PART E**

*Create a model binding site with the amino acid residues labeled.

**PART F**

*Identify interactions between pleconaril and the amino acid residues Tyr-197, Val-191, Tyr-152, Val-188, and Phe-186.
*Identify the position of the isoxazole ring in the binding site.

**PROCEDURES**

There are various approaches that you can use to tackle these molecular modelling exercises. The following procedures illustrate how you might tackle this particular exercise, but they are not meant to be prescriptive. Note also that the results obtained may vary depending on the computer and the version of ChemBio3D/Chem3D used. For example, the specific conformations obtained from energy minimisation may differ, as may quantitative results such as steric energies. The ChemDraw file for pleconaril is available in the ChemDraw folder.

**PART A Pleconaril**

**1. Create an energy-minimised structure for pleconaril.**

**Open* **ChemBio3D** or **Chem3D**.

*From the **File** menu, *choose* **Open**, then *select* the ChemDraw file for pleconaril. *Click* **Open**.

*Energy minimise the structure MM2 →.

You may get something like the extended conformation shown in figure 2. The steric energy for this conformation was given in the bottom window as 41.6 kcal/mol.

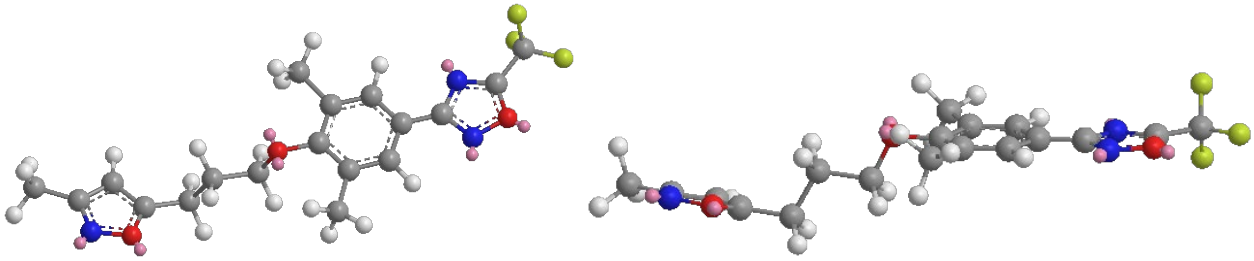


**Figure 2** Energy-minimised structure of pleconaril from two perspectives.

**2. Identify the number of rotatable bonds.**

*From the **Calculations** menu *choose* **Compute Properties**.

**Expand* **Molecular Topology** and *select* **Num Rotatable Bonds.** *Click* **OK**.

Note that rotatable bonds in this context are defined as those that result in distinct differences in conformation. Rotatable bonds that only alter the relative positions of hydrogen atoms (e.g. C-$CH_3$ etc) are not included in the total.

The number of rotatable bonds is given in the bottom window as 7. This presumably includes the bond to $CF_3$. In fact only 6 bonds are likely to generate significantly diffferent conformations (Fig. 3). A very large number of conformations are possible with those 6 rotatable bonds and so it is no simple task to identify the most stable conformation.

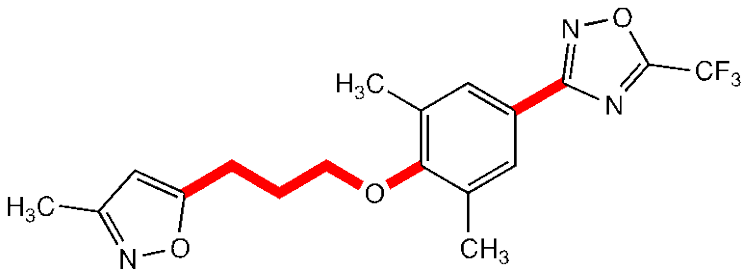



**Figure 3** Rotatable bonds in pleconaril that result in significant conformational changes.

**3. Search for conformations using molecular dynamics**

**Copy* and *paste* the energy-minimised conformation into a new window.

*From the **Calculations** menu, *choose* **MM2**, then **Molecular Dynamics**.

*A table will come up showing default entries that include a **Target Temperature** of **300K**.
*Keep the default values and *click* **Run**. You will see the molecule vibrating and adopting various conformations.
*If you wish to curtail the molecular dynamics process to look more closely at a specific conformation, *click* on **Stop Calculation** on the menu bar  , then energy minimise the conformation of interest.
The conformations shown in figure 4 were generated randomly by stopping the molecular dynamics programme at various stages, then energy minimising the resulting structures. During the molecular dynamics operation, it was observed that the right-hand region of the molecule stayed relatively static, whereas rotation took place within the chain in the left-hand region. The molecule eventually adopts different conformations where the overall shape of the molecule is L-shaped (Fig. 4).

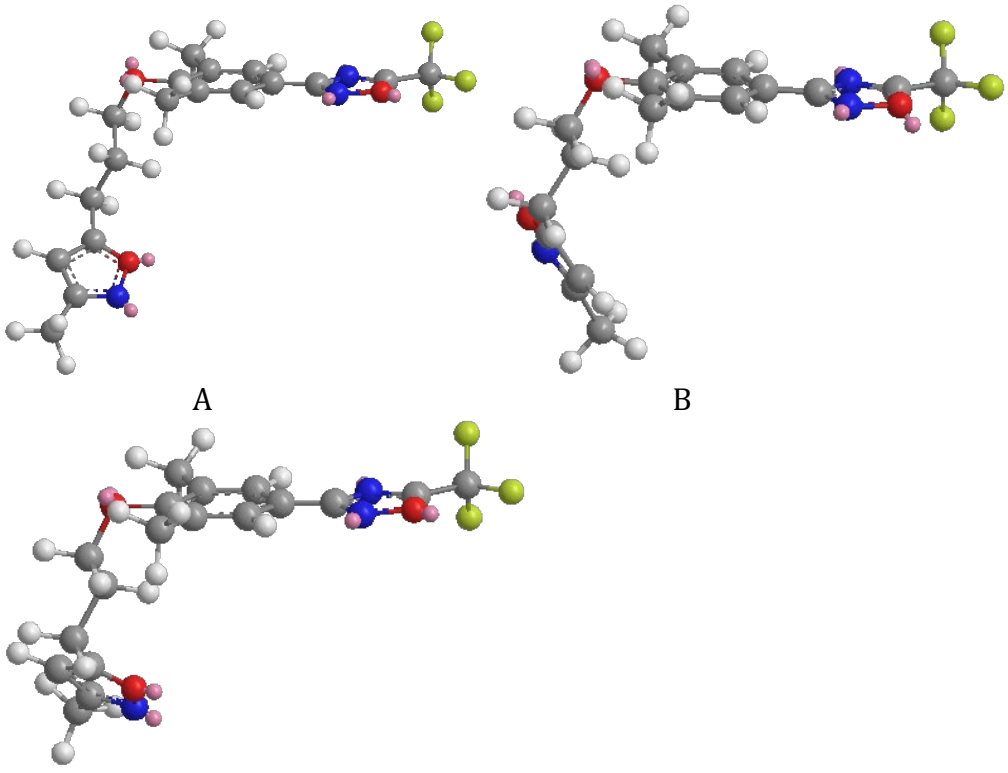



**Figure 4** Alternative energy-minimised conformations of pleconaril. The steric energies for A), B) and C) were 42.3, 42.5 and 42.3 kcal/mol respectively.

**4. Use MMFF94 stochastic conformational sampling to generate different conformations.**
The molecular dynamics program used in section 3 tends to get trapped in a specific region of conformational space. Stochastic sampling can generate random conformations that sample a wider range of conformational space.
**Copy* and *paste* the original energy-minimised structure into a new window.
*From the **Calculations** menu, *choose* **MMFF94**.
**Choose* **MMFF94 Stochastic Conformational Sampling**.
*In the resulting table, *set* **Maximum random offset for 10**.
**Choose* the **Number of conformations** as 20.
**Make* the **Maximum minimisation steps** for each structure generated as 2000.
**Click* **Run**.

*Once the operation is completed, open the **Structure Browser** to the left of the main window. This provides a list of conformations and their minimized energy. For the run carried out here, the energy varied only slightly between 84.4-86.2 kcal/mol.
The most stable of the conformations generated was copied and pasted into a new window, then energy minimised to give a conformation with a steric energy of 41.1 kcal/mol (Fig. 5). This is more stable than previous conformations. However, it cannot be stated whether this is the *most* stable conformation. There is also no way of telling what the active conformation might be as this does not necessarily correspond with the most stable conformation. Note also that steric energies reported in the Structure Browser are not for fully energy-minimised structures, and so it cannot be assumed that the order of energies observed will be the same once a full energy minimisation has been carried out.

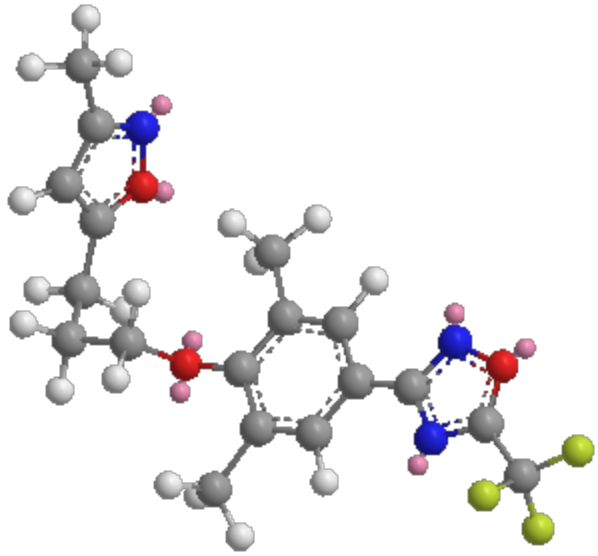


**Figure 5** An energy-minimised conformation obtained from stochastic sampling.

**PART B Crystal structure of pleconaril bound to its target**

**1. Download the crystal structure of the HRV-14 protein with bound pleconaril.**
You will need to be connected to the internet in order to do this.
*From the **Online** menu, *choose* **Find Structure from PDB ID**.
**Enter* the PDB code (**1NA1**) into the text box that appears.
**Click* on **Get File.**
The protein will appear as a ribbon diagram (Fig. 6). The ribbon represents the polypeptide backbone of the protein with light purple sections corresponding to α-helices, and blue sections corresponding to β sheets. The thin pink regions are connecting regions that do not have a secondary structure. The pleconaril ligand is represented in a ball and stick format.

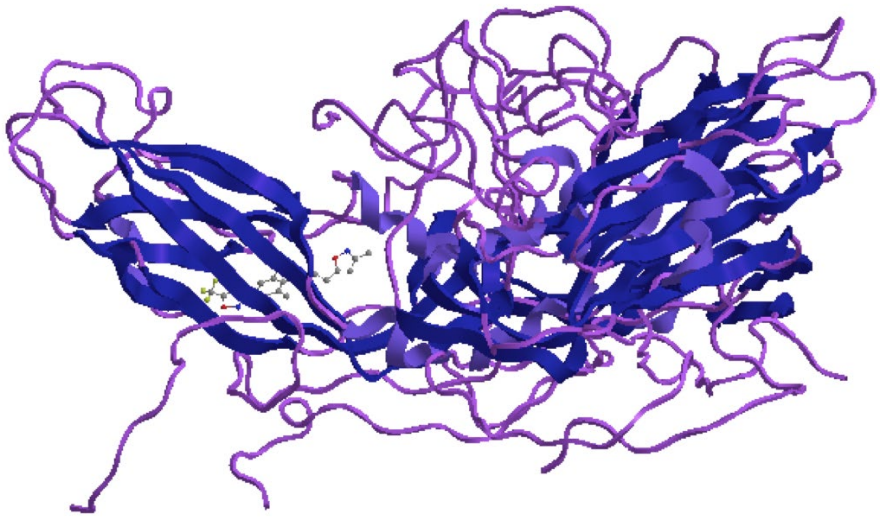


**Figure 6** Crystal structure of pleconaril bound to its target protein.

**2.Modify the colours to show the secondary structure more clearly.**
*From the **File** menu, *choose* **Model Settings**.
**Click* on the tab for **Colors and Fonts**.
*Under the section on **Model Colors**, *modify* the colours used for the Alpha helix, Beta sheet and Coil.
**Click* on **Apply** to see the effect in the main window. If satisfied, *click* **OK.**
The following structure (Fig 7) shows the helices in red, the sheets in blue and the coils in green. This colouring system makes it easier to identify the number of alpha helices that are present. You should be able to identify 9 helices, 5 of which are clustered together in the centre of the protein, and four of which are at the periphery.

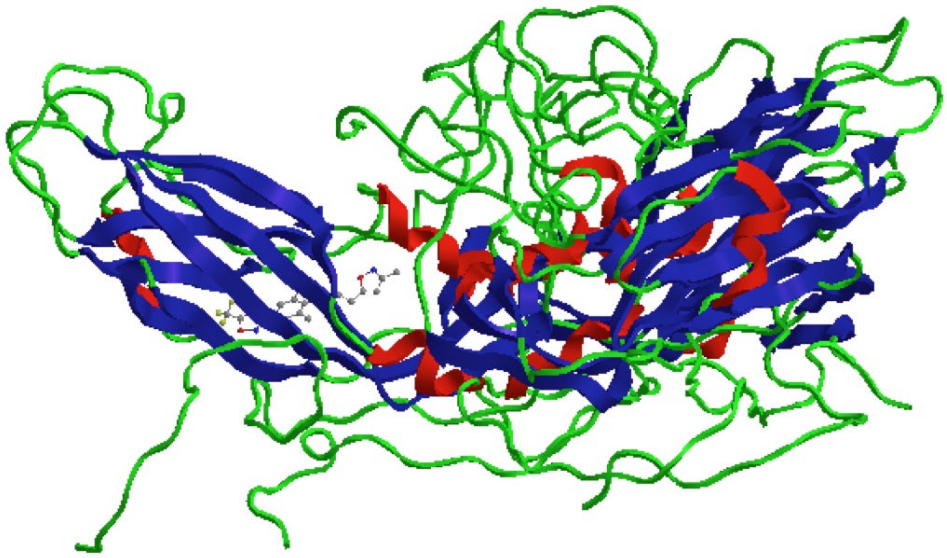


**Figure 7** Recoloured crystal structure.

**3. Identify the different features of the crystal structure.**
*Open the Model Explorer Table by *clicking* on the tab if present, or by *selecting* it from the **View** menu. The table should contain entries for four chains, Solvent and Backbone (Fig. 8A).
**Select* each of the entries for the four chains to highlight the four protein subunits in the main window (Fig. 9). Note that selecting Chain A also selects pleconaril.
**Expand* the entry for Chain A by clicking on the + sign to the left of the entry. This reveals **Fragment-17-289** and **Ligand-18** (Fig. 8B).
**Select* **Fragment-17-289** and the protein subunit is highlighted in the main window without highlighting pleconaril (Fig. 10A).
**Select* **Ligand-18** and pleconaril is highlighted (Fig. 10B).

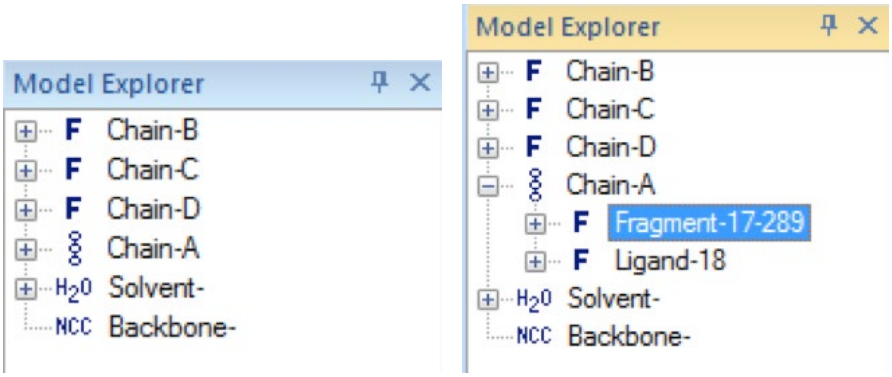



**Figure 8** A) Model Explorer table. B) Model Explorer table after expansion of Chain A.

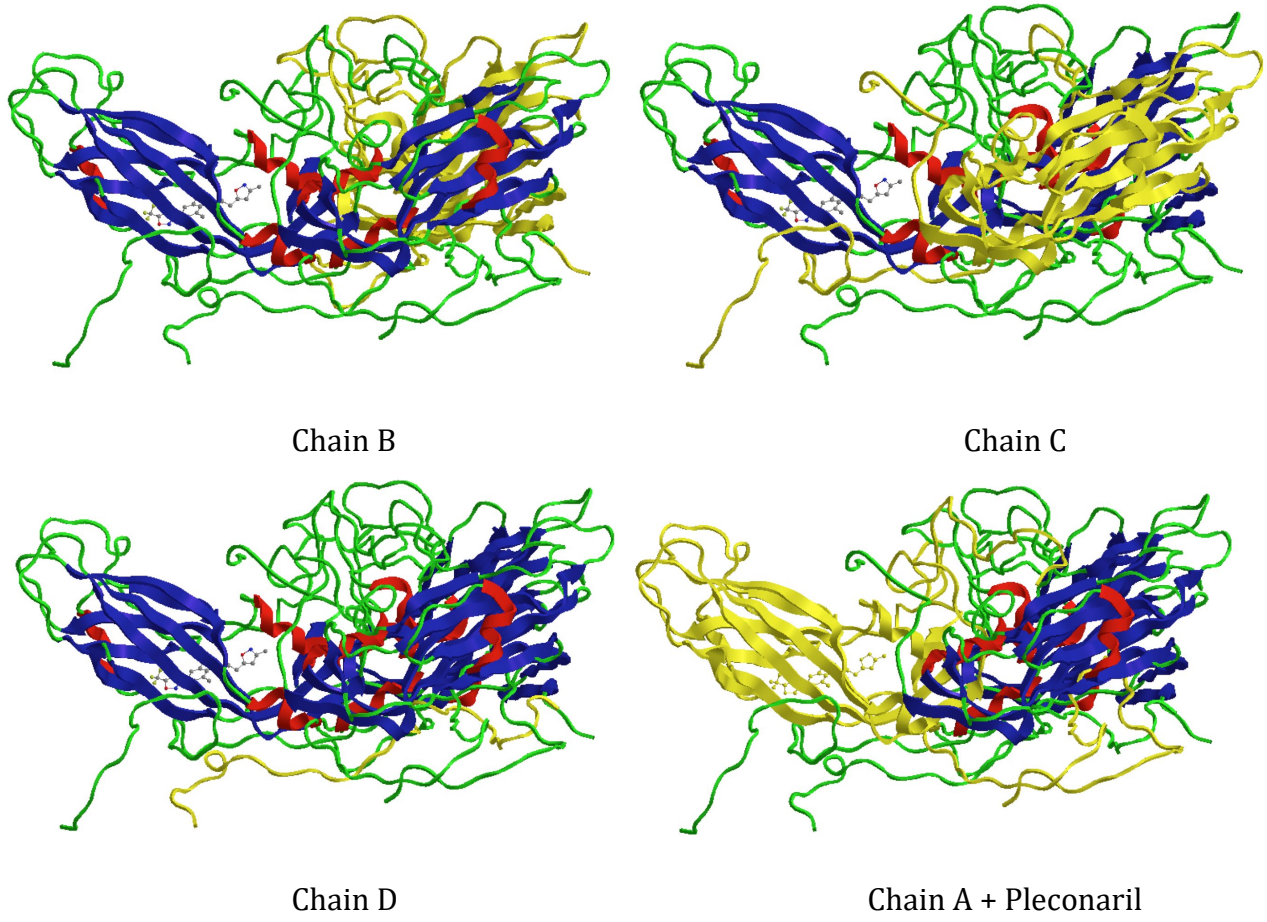



**Figure 9** Identification of the four protein subunits

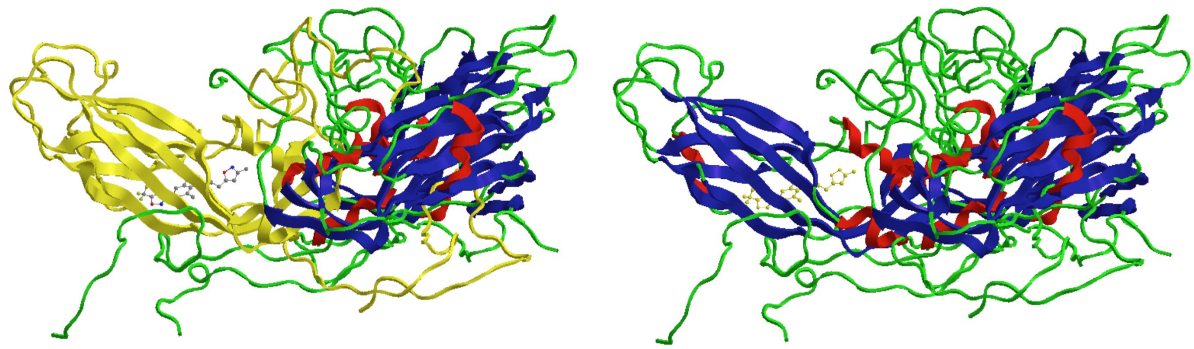



**Figure 10** A) Fragment-17-289 and B) Pleconaril.

**PART C Active conformation of pleconaril.**

**1. Extract pleconaril from the active site.**

*Zoom* in on the ligand by using the scroll on the mouse or the **zoom** tool (Fig. 11).

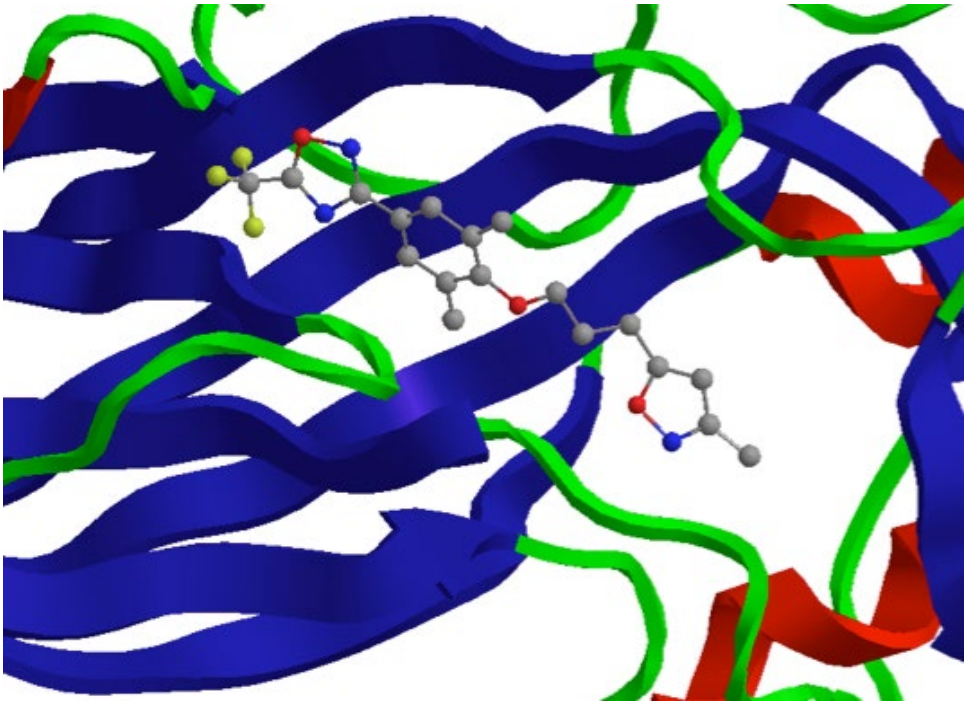


**Figure 11** Ligand in the binding site.

*Double click* on any part of the ligand such that the whole molecule is highlighted in yellow.
*From the **Edit** menu *select* **Copy**.
*From the **File** menu, *choose* **New** to open a new window.
*From the **Edit** menu, *choose* **Paste**. The conformation of pleconaril now appears in the new window (Fig. 12). Do not energy minimize the structure. The hybridizations of the atoms involved are not defined and an energy minimisation would consider them all to be $sp^3$ hybridised. Moreover, energy minimization would alter the conformation from the active conformation.

Note also: It is important to appreciate that there is a certain level of error involved in determining structures by X-ray crystallography, and that the bond angles and bond lengths are not at their optimal value in crystal structures.

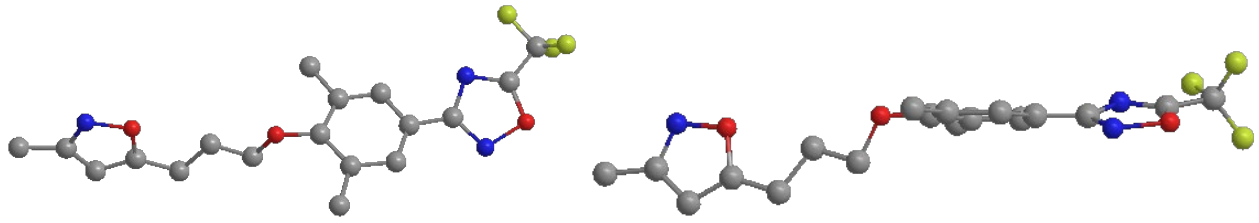


**Figure 12** Active conformation of pleconaril.

**2. Compare the active conformation of pleconaril with the energy-minimised conformations produced in part A.**

It is clear that the active conformation is an extended conformation rather than the L-shaped conformations generated in Part A. There is more of a similarity between the active conformation and the original energy-minimised extended conformation of pleconaril (Fig. 13). However, there is a significant difference in the orientation of the isoxazole ring at the 'left-hand' side of the structure (Fig. 13). In the active conformation, this ring is orthogonal to the other rings present, whereas it is coplanar in the energy-minimised structure.

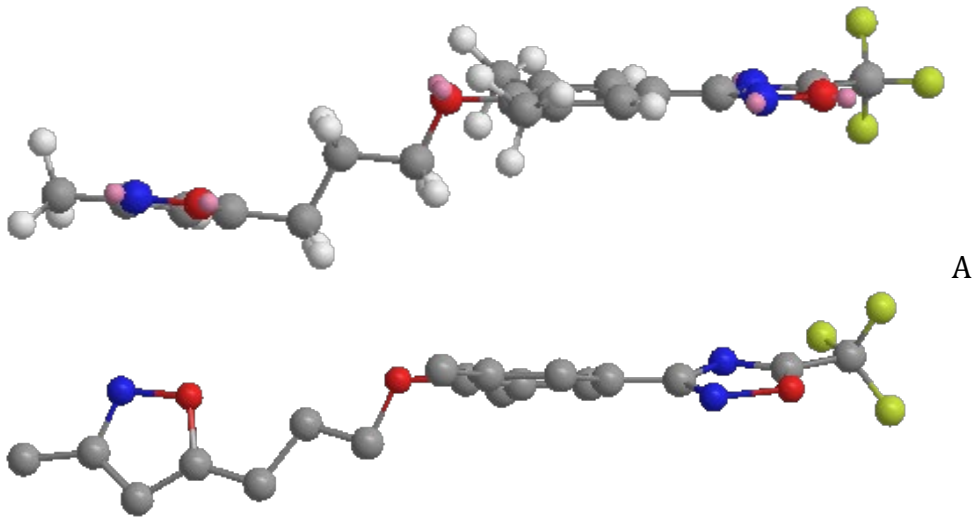



**Figure 13** Comparison of A) the original energy–minimised conformation from part A and B) the active conformation extracted from the crystal structure.

**3. Modify the energy-minimised conformation to resemble the active conformation.**

The isoxazole ring in the original energy-minimised conformation can be made orthogonal in the original energy-minimised conformation by a 90° bond rotation.

**Copy* and *paste* the original energy-minimised conformation into a new window.

**Select* the bond highlighted in figure 14.

**Open* the rotation dial and *select* the SW-pointing arrow at the bottom right.

***Enter*** 90 into the type box and *press* return on the keyboard.

**Energy minimise* the conformation.

Rotating the bond generates a conformation that is much closer to the active conformation, but energy minimisation causes the ring to revert back to its original coplanar position. This suggests that binding interactions in the binding site may be responsible for maintaining the isoxazole ring in the orthogonal orientation.

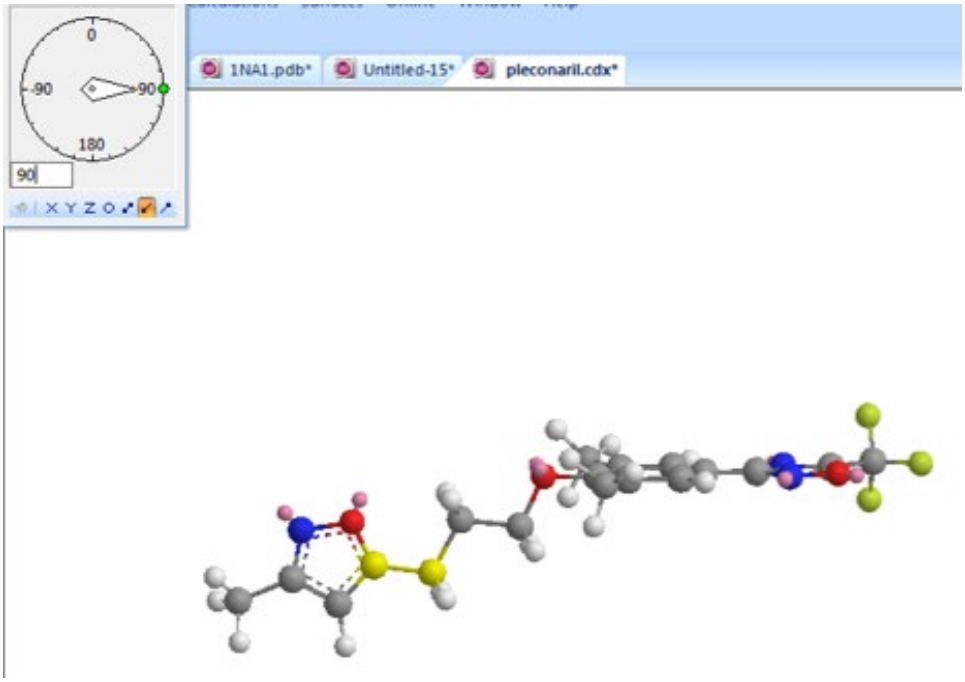



**Figure 14** Modification of the energy-minimised conformation.

**4. Identify a stable conformation where the isoxazole ring is in a similar orientation to the active conformation.**

Simply rotating the bond in figure 14 to reorientate the isoxzole failed to give a stable conformation that was not altered by energy minimisation. However, that does not mean that no such conformation exists. A more thorough assessment can be made using the dihedral driver to create a series of conformations generated by automatically rotating the bond by small amounts.

**Select* the same bond highlighted in figure 14.

*From the **File** menu, *choose* **Preferences**, then *click* on the **Dihedral Driver** tab to open a dialogue window.

*Use the slider bar that is present to set the resolution. This determines the number of degrees the bond will be rotated at each step of the process. Make it 5 degrees, then *click* on **Apply** and **OK**.

*From the **Calculations** menu, *choose* **Dihedral Driver**, then **Single Angle Plot**. You will see rotation taking place round the rotatable bond.

*On completion, a chart should appear showing how the steric energy has altered with rotation round the chosen bond. If the chart is not visible, *click* on the tab for the **Dihedral Driver Chart**, which is just above the main window. The chart shows two obvious energy minima (Fig. 15), which correspond to the energy-minimised structure of pleconaril plus a similar conformation where the isoxazole ring has been rotated 180°. The x-axis measures the dihedral angle formed by the atoms C(16)-C(17)-C(18)-O(22), which have been highlighted in the structure under the plot.

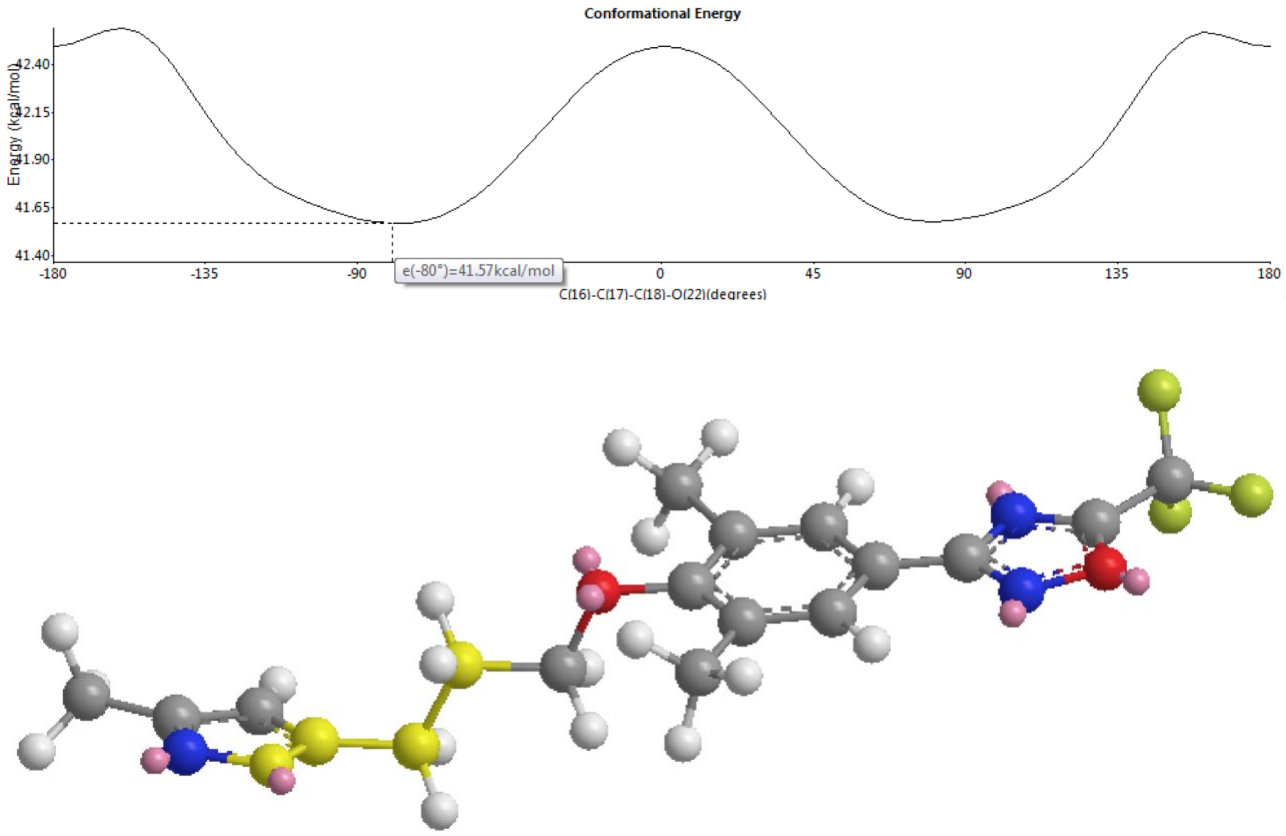



**Figure 15** Single Angle Dihedral Chart. The atoms involved in the dihedral angle have been highlighted in yellow.

We will now simplify the molecule to make comparisons of the different conformations easier.
*From the **View** menu, *select* **Model Display**, then **Show Hydrogen Atoms**. *Choose* **Hide**.
*From the **View** menu, *select* **Model Display**, then **Show Lone Pairs**. *Choose* **Hide**.
*To identify specific conformations, *click* on the plot line. The molecule in the main window will be converted to the relevant conformation, and a pop up window will show the energy and the dihedral angle measurement for that conformation.
**Click* on the two minimum points in the plot. The conformations relating to these points are shown in figure 16.

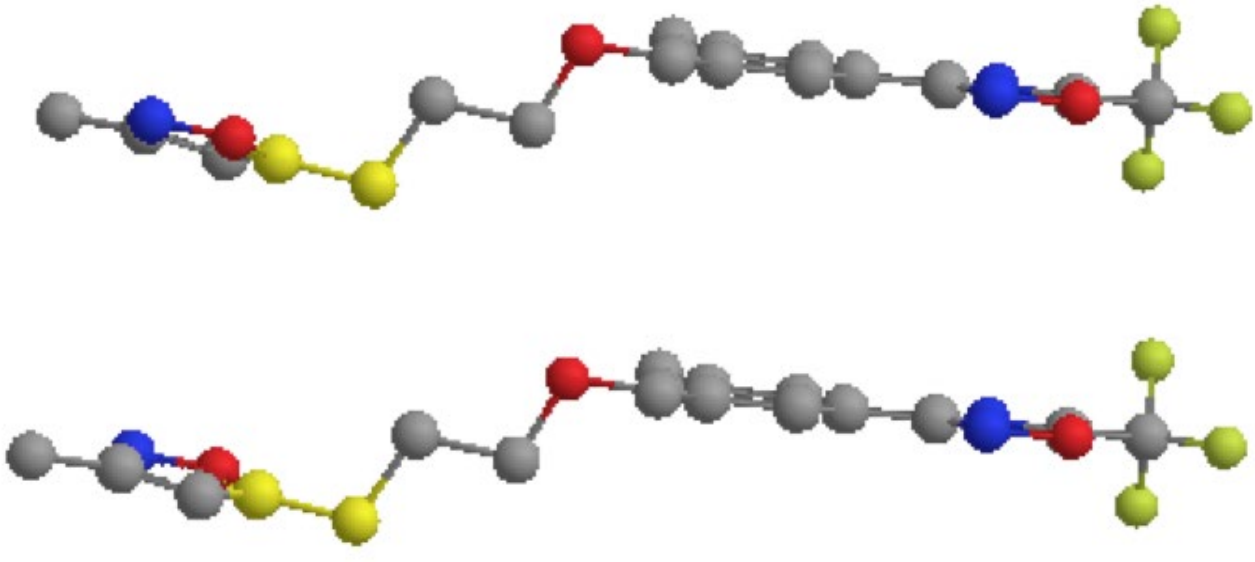


**Figure 16** Conformations corresponding to the two main energy minima (dihedral angles -80° and +80°).

Neither of these conformations have the isoxazole ring orthogonal to the central aromatic ring. However, closer inspection of the plot shows that there is another energy minimum at a dihedral angle 180°.
*_Click_ on the point of the plot corresponding to a dihedral angle of 180°.
This identifies the conformation shown in figure 17 where the isoxazole ring is orthogonal to the central aromatic ring.
*_Copy_ and _paste_ the conformation into another window and energy minimise it. In this case, the isoxazole ring remains in the orthogonal orientation when energy minimisation is carried out. A steric energy of 42.2kcal/mol was obtained which is very close to the steric energy of the original energy-minimised conformation (41.6 kcal/mol). However, although the isoxazole ring is orientated orthogonal to the central aromatic ring, this conformation does not match the active conformation. The isoxazole ring is orientated the opposite way with the methyl group 'up' and the heteroatoms 'down'. The conformation that most closely resembles the active conformation is at a dihedral angle of 0°, which corresponds to an energy peak in the middle of the chart (Fig. 18).

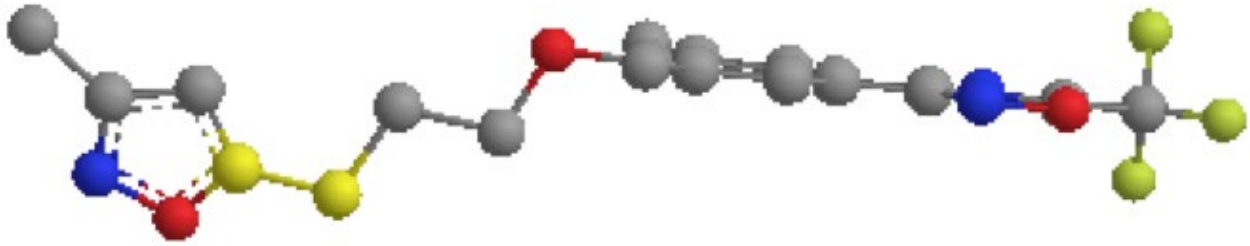


**Figure 17** Stable orthogonal conformation (dihedral angle 180°).

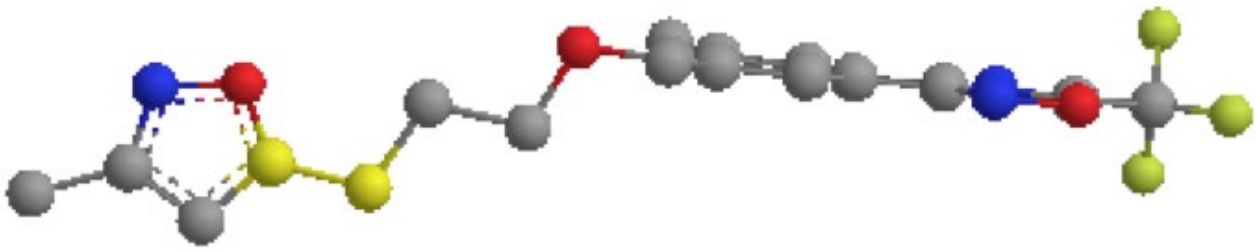


**Figure 18** Conformation that most closely resembles the active conformation (dihedral angle 0°).

Therefore, it is clear that binding interactions are helping to stablise the orientation of the isoxazole ring observed in the active conformation.

**PART D Identifying the PoseView analysis of binding interactions**

Useful information can be obtained about how a ligand binds to a binding site from the web site for the protein data bank.

*_Open_ the protein data bank website at www.rcsb.org.

*In the search box provided on the home page, _enter_ the PDB code (**1NA1**), then _click_ on **Go**. You will now be taken to the front page of that file.

*_Scroll_ down the page to a section headed **Small Molecules,** which refers to any ligands bound to the protein.

*Under the section on ligands, there are two diagrams shown under **2D Diagram and Interactions**. The left-hand structure shows the structure of the ligand. The right-hand diagram shows intermolecular binding interactions between the ligand and key amino acids within the binding site.

*_Click_ on the diagram to get a window showing an expanded view of the Poseview Image of the binding site (Fig. 19). There are no hydrogen bonds displayed in the image, but pi-pi interactions are shown by dashed green lines. Hydrophobic pockets are shown by green lines with the amino acids lining the pockets identified in green.

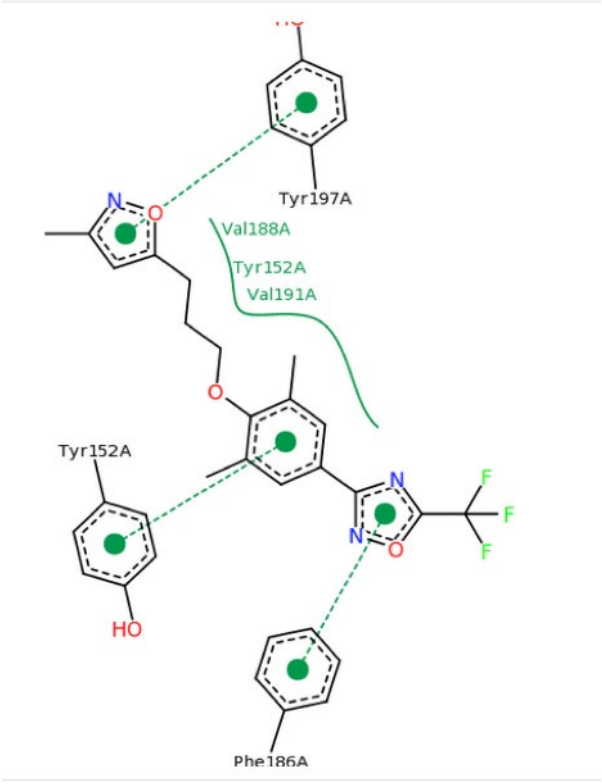



**Figure 19** PoseView image showing proposed binding interactions for pleconaril.

**Part E Create a model binding site.**

**1. Select the ligand and the amino acid residues closest to it.**
**Return* to the window with the crystal structure.
**Zoom* into the binding site such that pleconaril is clearly visible.
**Choose* the **Select** tool  and d*ouble click* on any of the ligand atoms to select the complete molecule.
**Hover* the mouse cursor over the selected area, then r*ight click* the mouse to open a menu.
**Choose* **Select**, then **Select groups within Distance of Selection**. *Choose* **4 Angstrom** (Fig. 20).

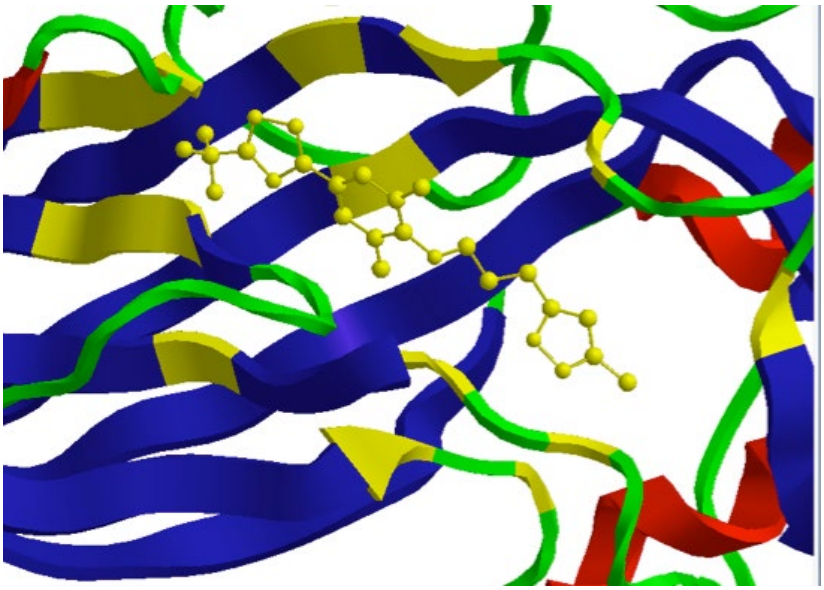


**Figure 20** Selected ligand and closest amino acid residues**.**

**2. Copy the selection and paste it into a new window.**
**From the **Edit** menu, *choose* **Copy.**
**From the **File** menu, *choose* **New** to open a new window.
**From the **Edit** menu, *choose* **Paste** to put the selection into the new window (Fig. 21).

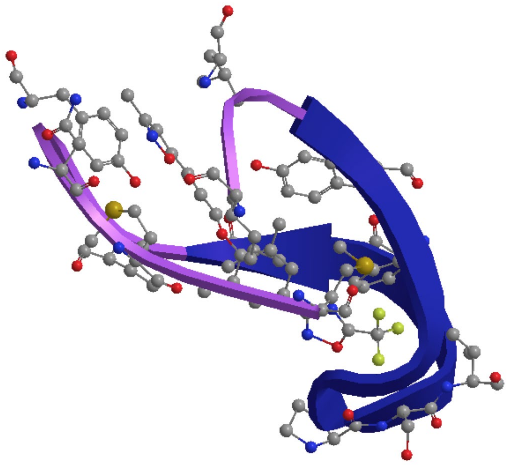


**Figure 21** Initial image of binding site.

**3. Display the labelled amino acids as sticks, and the ligand as a ball and stick model.**
*From the **View** menu, *choose* **Model Display**, then **Display Mode.** *Choose* **Sticks**.

To alter the ligand back to the ball and stick format, carry out the following procedure;
*Go to the **Model Explorer** table.
**Expand* the entry for **Chain A**, then *click* on **Ligand-18**.
*Keep the mouse cursor over the label and r*ight click* the mouse to produce a menu.
*From the menu, *choose* **Display Mode**, then **Ball and stick**.
*In the **Model Explorer** table, *click* on **Solvent**, then *right click* the mouse to reveal a menu.
**Choose* **Cut**. (This removes water molecules and allows the structure to be more easily rotated).
*From the **View** menu, *choose* **Model Display,** then *select* **Show Residue Labels.**
The model binding should now look like figure 22.

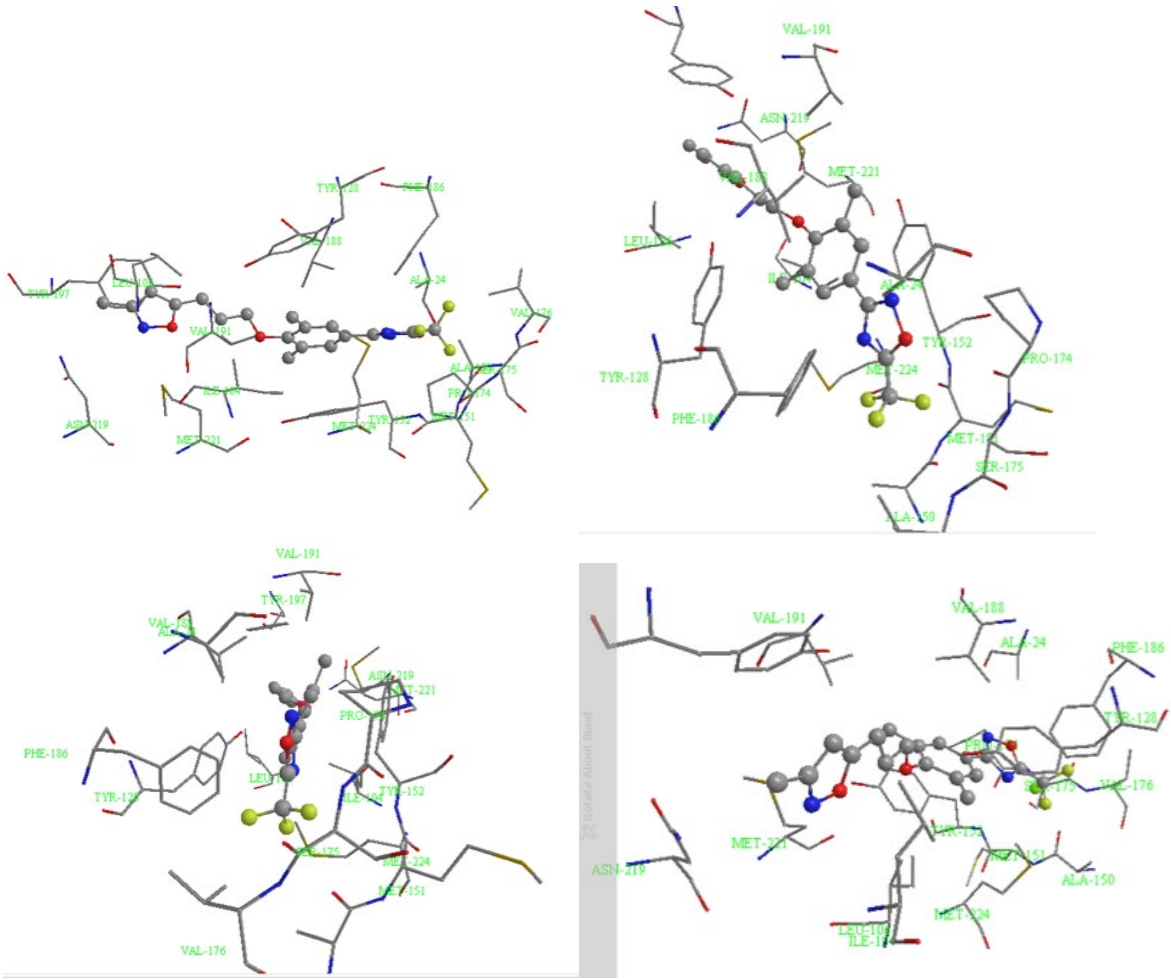



**Figure 22** The model binding site containing pleconaril and closest amino acids viewed from different perspectives.

**PART F Identification of interactions**

In this part, you can use the PoseView image to help you identify proposed interactions between the ligand and the binding site, then identify the interactions in the model binding site.

**1. Identify the interaction of the ligand with Tyr-197 and Val-191.**

*From the **View** menu, *choose* **Model Display**, then *select* **Perspective**.

*Manipulate the model binding site such that you can view the ligand and the two amino acids involved (Fig. 23). Both amino acids are on the same side of the ligand.

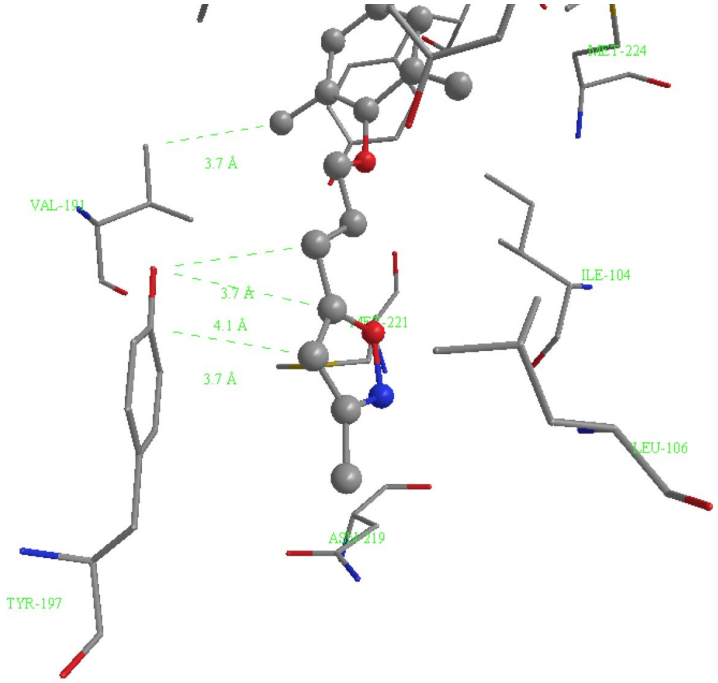



**Figure 23** Interaction of pleconaril with Tyr-197 and Val-191.

**2. Identify the interaction of the ligand with Tyr-152.**

The aromatic ring of Tyr-197 forms a face to face interaction with the aromatic ring of the ligand where the rings are separated by 3.4-3.6Å (Fig. 24). There is also a tyrosine residue (Tyr-128) on the opposite side of the ligand, which is 4.5Å away.

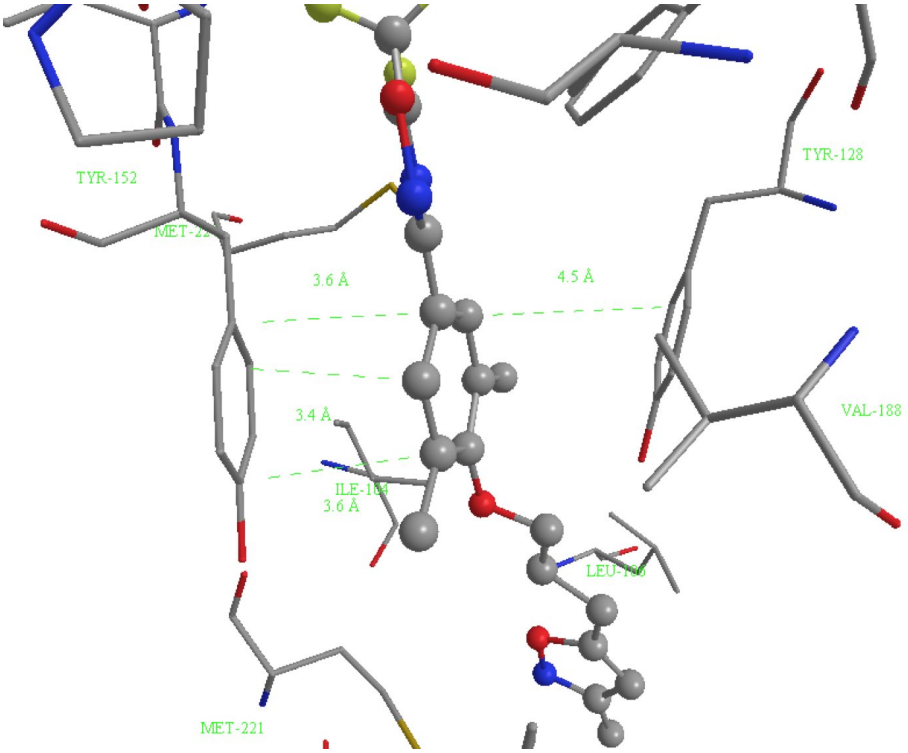
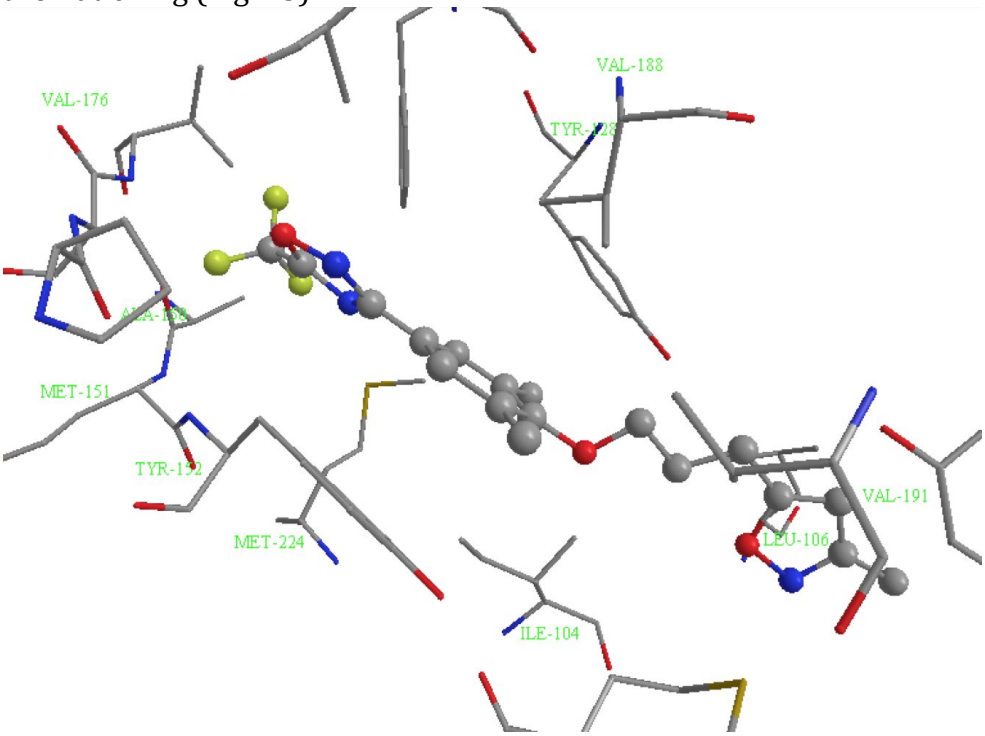



**Figure 24** Interaction of pleconaril with Tyr-152.

### 3. Identify the interaction of the ligand with Val-188 and Val-191.

Both these amino acid residues are on the same side of the ligand and can interact with the aromatic ring (Fig. 25).



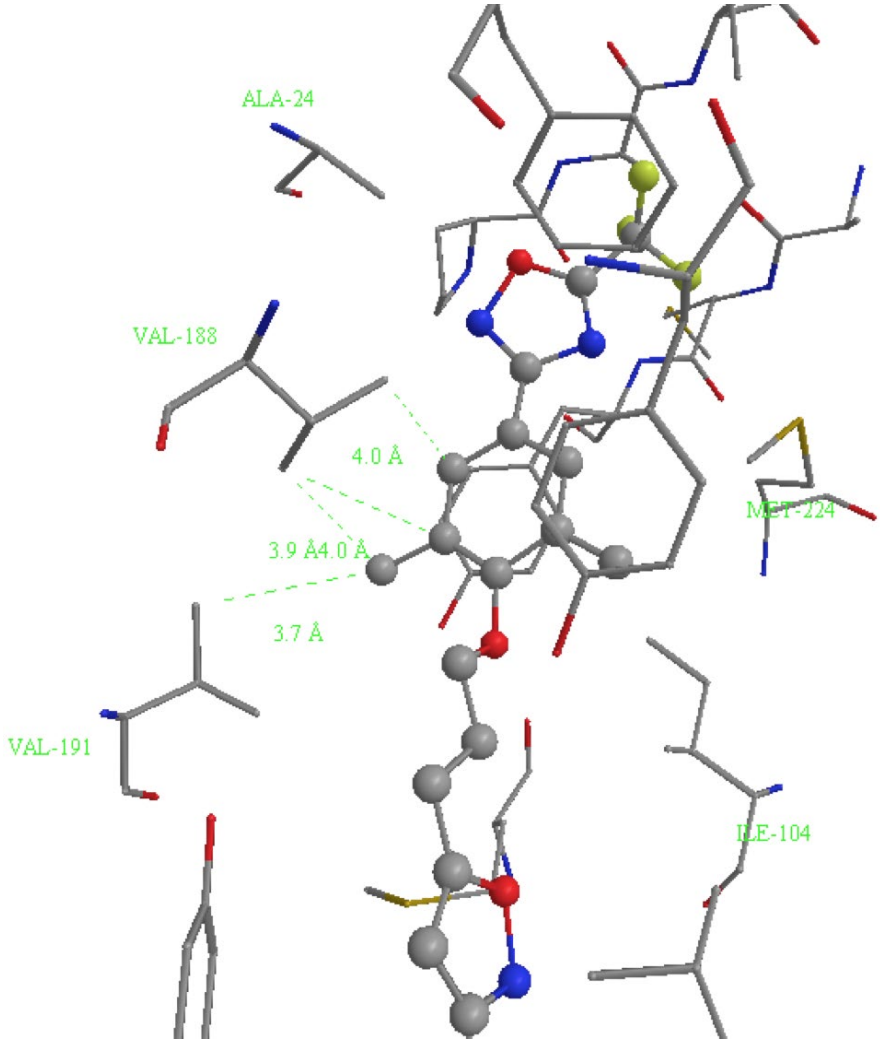



**Figure 25** Interaction of pleconaril with Val-188 and Val-191.

**4. Identify the interaction of the ligand with Phe-186.**
The aromatic ring of Phe-186 appears to be forming a pi-pi interaction with the oxadiazole heterocycle of the ligand (Fig. 26). Two of the heterocyclic atoms (C and N) are 3.1 Å and 3.2 Å from two of the aromatic carbon atoms.

A)

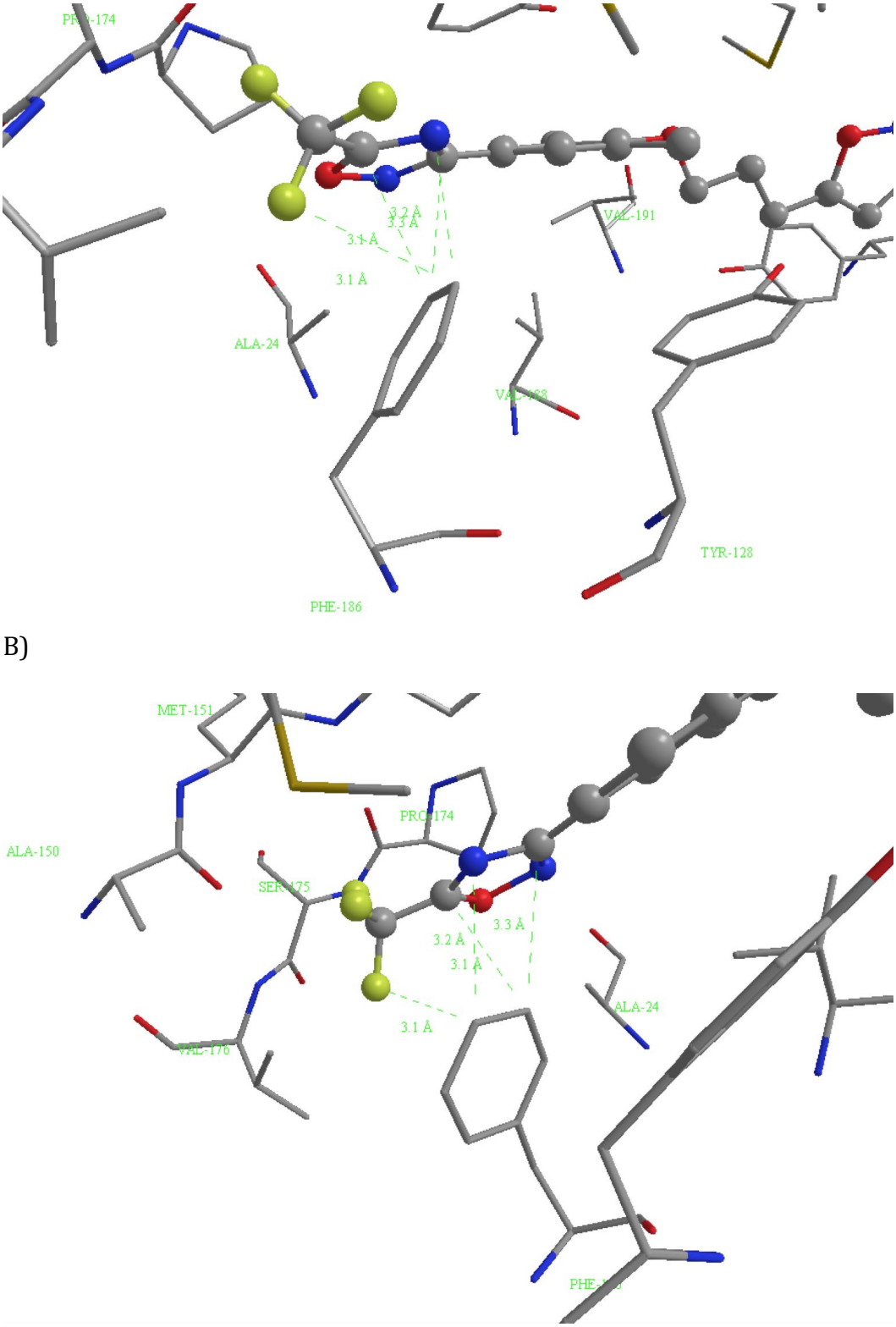



**Figure 26** Interaction of pleconaril with Phe-186 from two different perspectives.

**5. Identify the position of the isoxazole ring in the binding site**

As described earlier, the isoxazole ring of pleconaril is orthogonal to the central aromatic ring. The isoxazole ring is located in a pocket lined by several amino acid residues such as Tyr-122, Leu-106, Tyr-197, Asn-219, Val-191 and Met-221 (Fig. 27).

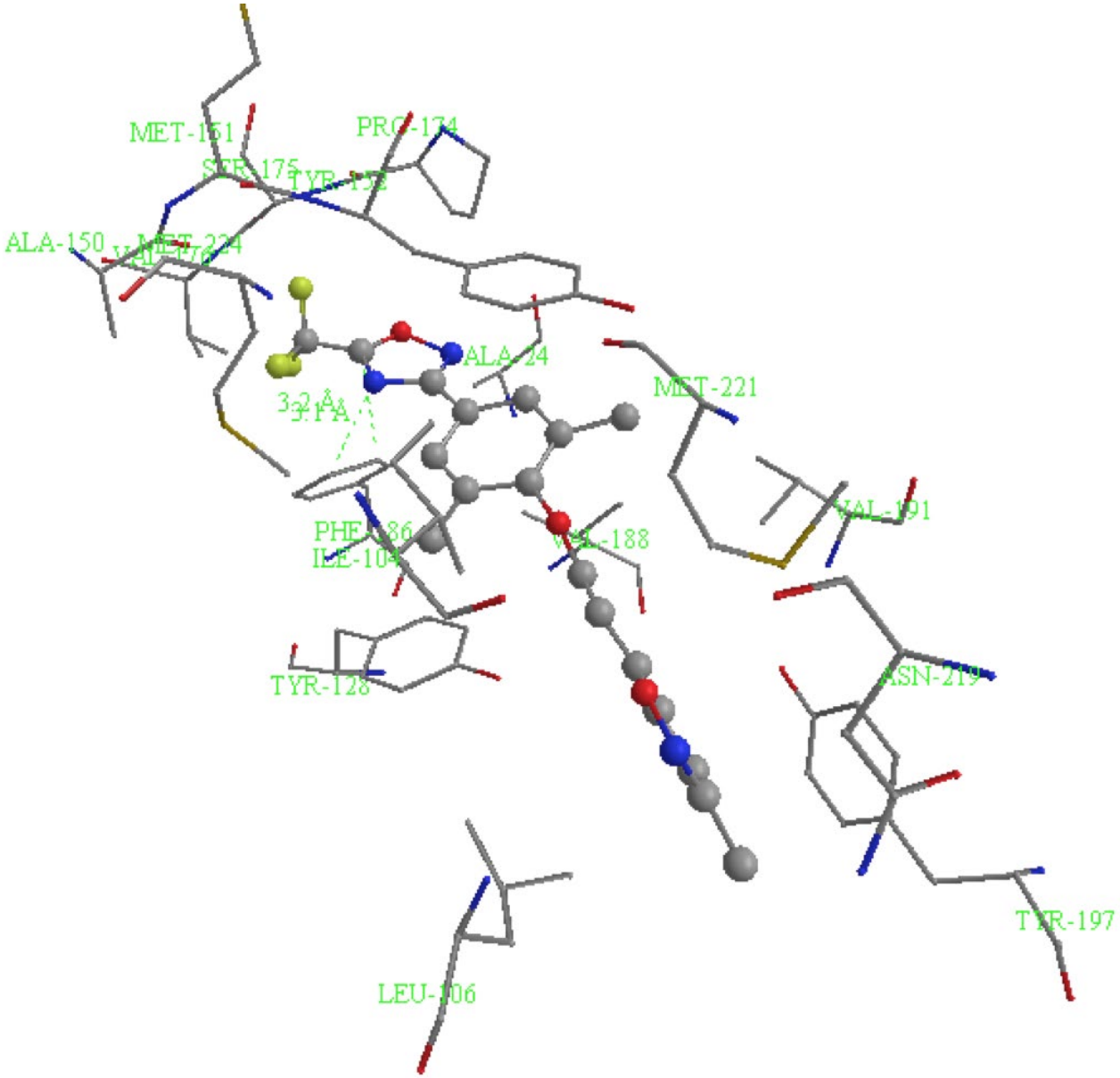



**Figure 27** Pocket accommodating the isoxazole ring (at the bottom).

A closer look at this pocket indicates a possible edge to face interaction with Tyr-197 (Fig. 28). The closest atoms in the two rings are 2.7Å apart.

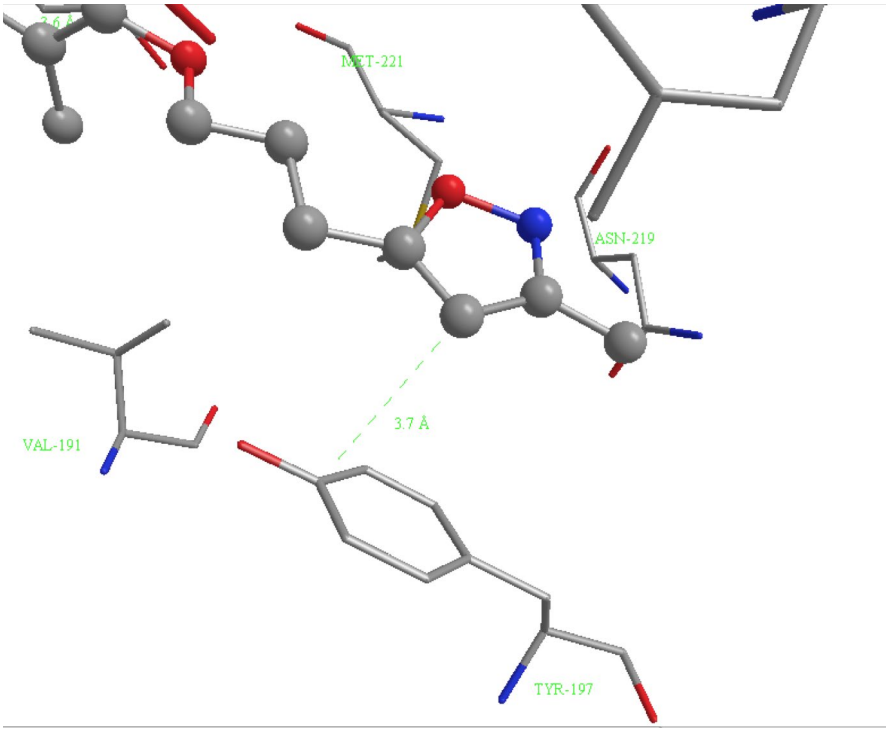



**Figure 28** Possible interaction with Tyr-197.