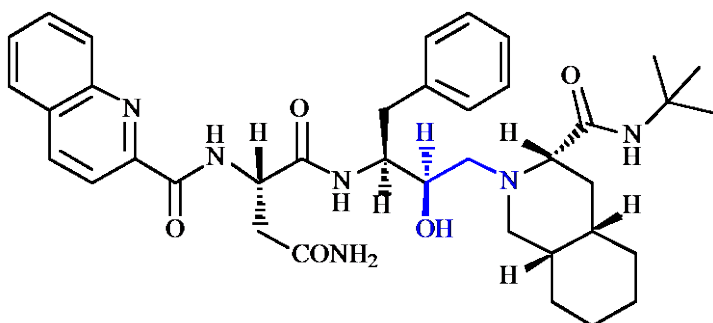**MOLECULAR MODELLING EXERCISES**

**EXERCISE 20.4   Comparison of Protease inhibitors**

INTRODUCTION

Saquinavir was the first HIV-protease inhibitor to enter the market as a treatment for HIV infection. Since then, a number of other protease inhibitors have also entered the clinic such as ritonavir, indinavir and nelfinavir (Fig. 1) (section 20.7.4 in the textbook). In this exercise, we will compare the crystal structures of HIV-protease with these inhibitors, and identify the active conformations of the four inhibitors.
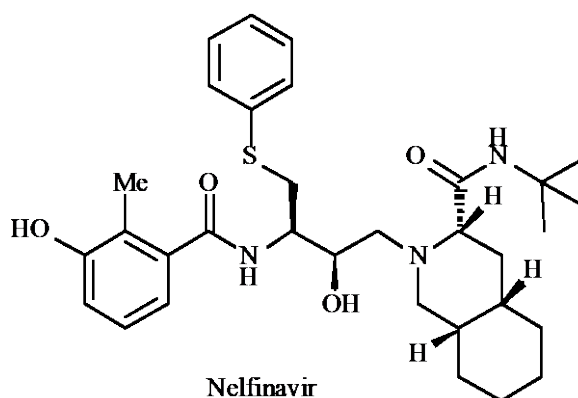
Saquinavir
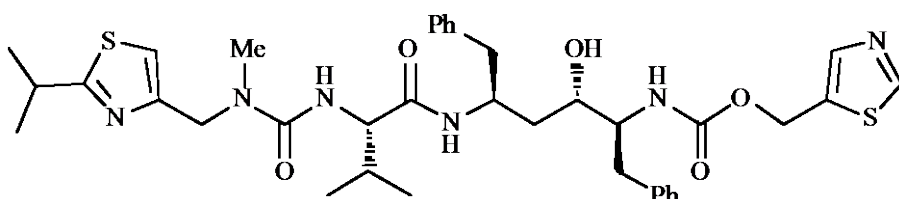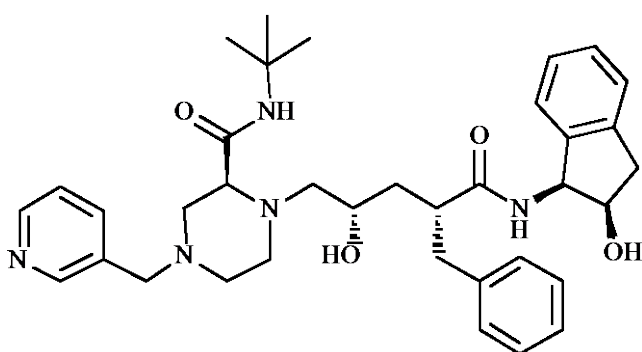
Ritonavir

Figure 1 Structures of protease inhibitors.

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.

*Download the crystal structures of the HIV-protease enzyme with the four bound protease inhibitors saquinavir, ritonavir, indinavir, and nelfinavir. The pdb codes for these crystal structures are 1hxb, 1hxw, 1hsg, and 1ohr respectively.
*Compare the shapes of the enzyme-ligand structures.
*Extract the protease inhibitors from the crystal structures and compare their structures, identifying the orientation of the side chains that occupy binding subsites.

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.

1.Retrieve the crystal structure of saquinavir bound to HIV-protease from the protein data bank.
You will need to be connected to the internet in order to do this.
*Open ChemBio3D or **Chem3D**.
*From the **Online** menu, *choose* **Find Structure from PDB ID**. *Enter* the PDB code for the crystal structure of saquinavir bound to the protease enzyme (**1HXB**).
Click on **Get File** and the protein will appear as a ribbon diagram, with the ligand presented as a ball and stick model.
 The protein is represented as a ribbon structure with a couple of short light purple sections corresponding to α-helices, and blue sections corresponding to beta sheets. The thin pink strands are regions of the polypeptide chain that do not have a secondary structure.
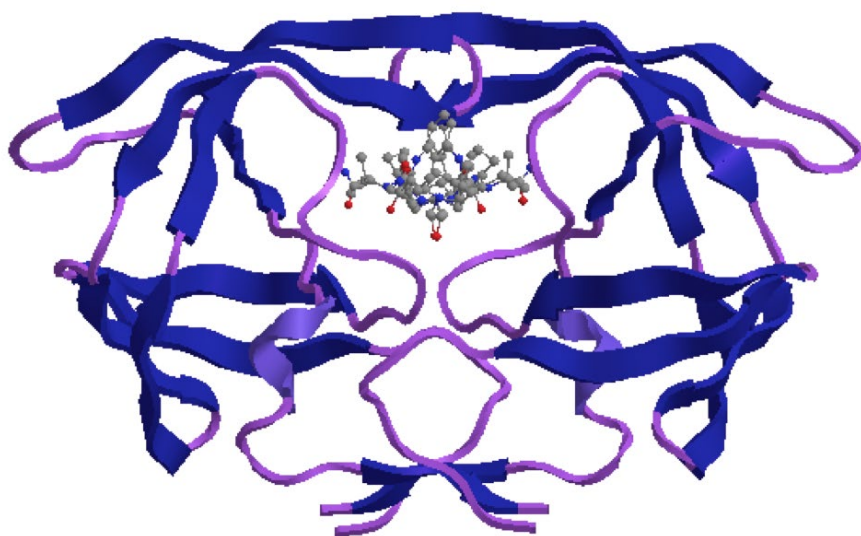

Figure 2 Crystal complex of saquinavir and the protease enzyme.

2. Open the crystal structures (1hxw; 1hsg; 1ohr).
Repeat the procedures described in section 1 above to open the relevant crystal structures and rotate them such that they are viewed in the same way (Fig. 3).
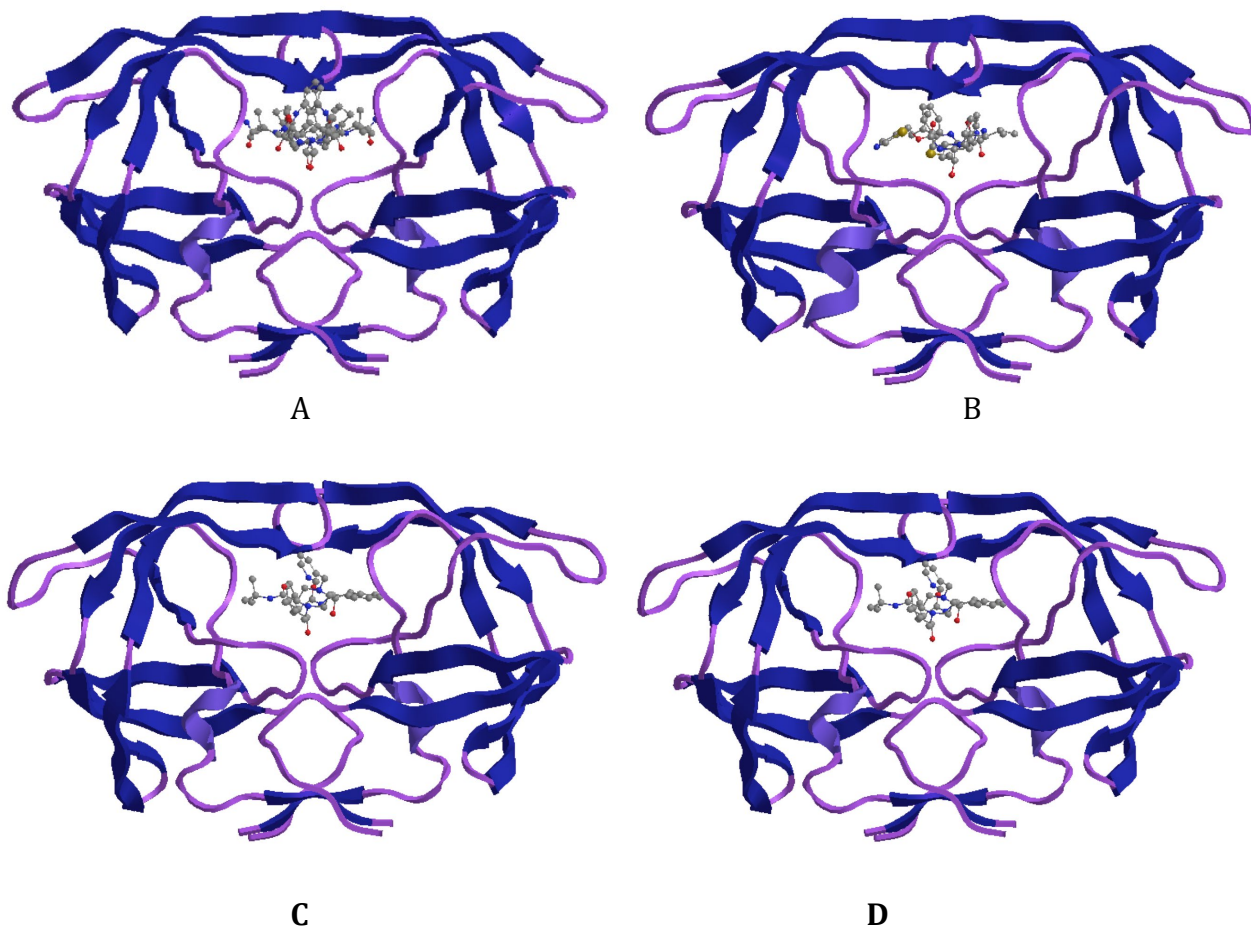

Figure 3 Crystal structures of the protease enzyme with A) saquinavir (1hxb), B) ritonavir (1hxw), C) indinavir (1hsg) and D) nelfinavir (1ohr).

3. Extract the protease inhibitors from the active sites
*From the **File** menu, *choose* **New** to open a new window.

*For each crystal structure, *choose* the **Select** tool then *double click* on any part of the ligand to select the whole molecule. Alternatively, *select* the ligand from the **Model Explorer** table.

Ritonavir is identified as **Ligand-2** once you expand the entry for **Chain B**.
Indinavir is identified as **Ligand-2** once you expand the entry for **Chain B**.
Nelfinavir is identified as **Ligand-2** once you expand the entry for **Chain A**.
The situation with saquinavir is more complex since two molecules of saquinavir are shown in the binding site for pdb 1hxb. This is an experimental anomaly and does not represent the true situation. Only one molecule of saquinavir can occupy the binding site at any one time. To select one of the saquinavir molecules present, carry out the following;
Expand **Chain A**, then *expand* **Ligand-2**.
Expand **ROC-100**, then *select* **ROC-66**. This represents a single saquinavir ligand which can be copied.

*For each selected inhibitor, *choose* **Copy** from the **Edit** menu, then *paste* the structure into the new window (Fig. 4). Do not attempt to energy minimise any of the structures or they will move away from the active conformation.

It is also 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 the extracted ligands.
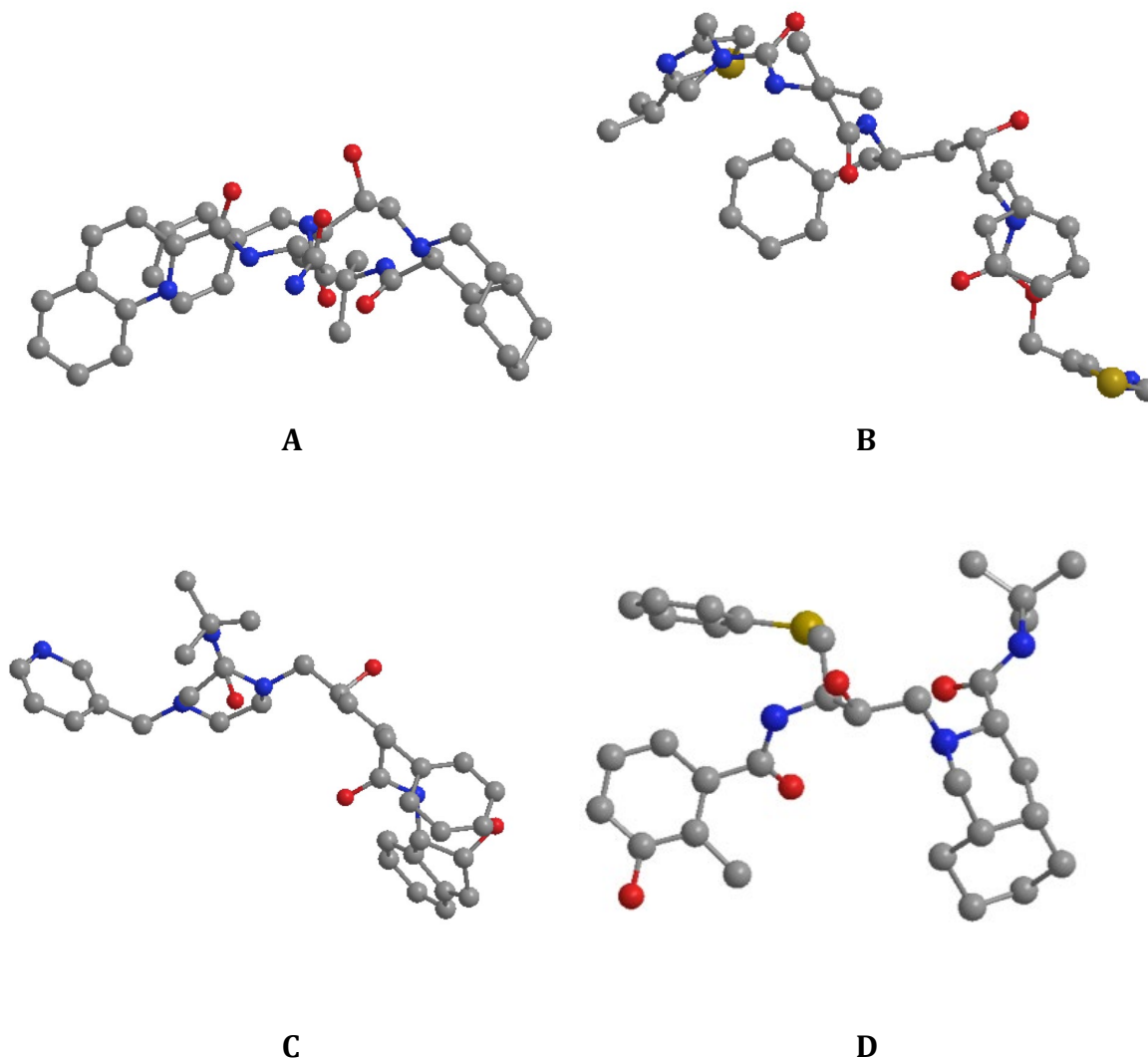

A **B**

C **D**

Figure 4 Active conformations for A) saquinavir, B) ritonavir, C) indinavir, and D) nelfinavir.

4. Identify common features in the ligands and use these features as a guide in aligning them.

The following figure (Fig. 5) shows saquinavir with orange-coloured carbon atoms, ritonavir with green-coloured carbon atoms, indinavir with purple-coloured carbon atoms, and nelfinavir in its original colour scheme.
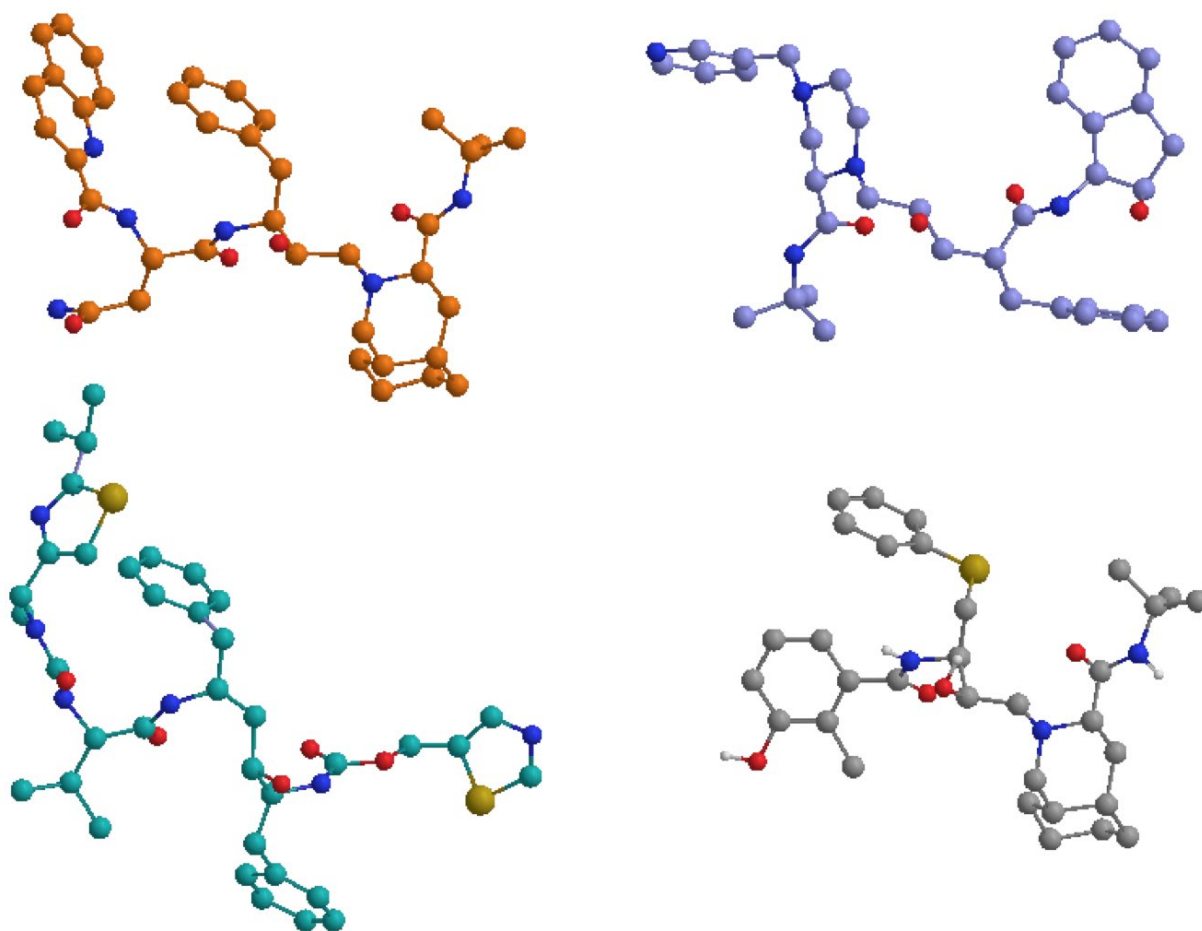
Figure 5 Comparison of the four protease inhibitors saquinavir (orange), ritonavir (green), indinavir (purple) and nelfinavir (grey).

5. Compare the structures and identify the relative positions of the binding subsites occupied by the substituents of the inhibitor structures.

All four structures have extended conformations with substituents pointing alternately up and down with respect to the main chain. For example, saquinavir and ritonavir each have five substituents that occupy the five subsites (S3 – S1 and S1'-S2') (see also fig 6). Indinavir has 4 substituents occupying subsites S3, S2, S1' and S2' (Fig. 7). The piperazine ring that is present as part of the main chain binds to the S1 subsite. Nelfinavir has an extended substituent that can bind to both the S1 and S3 subsites. These subsites are next to each other in the active site.
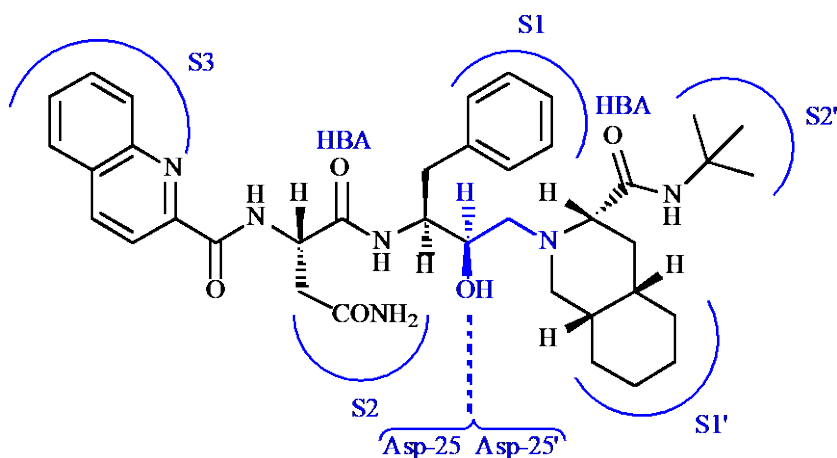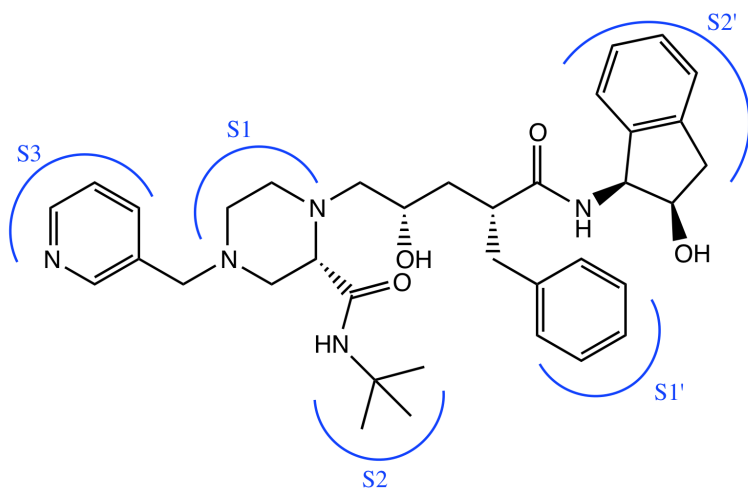
Figure 6 Binding interactions for saquinavir.
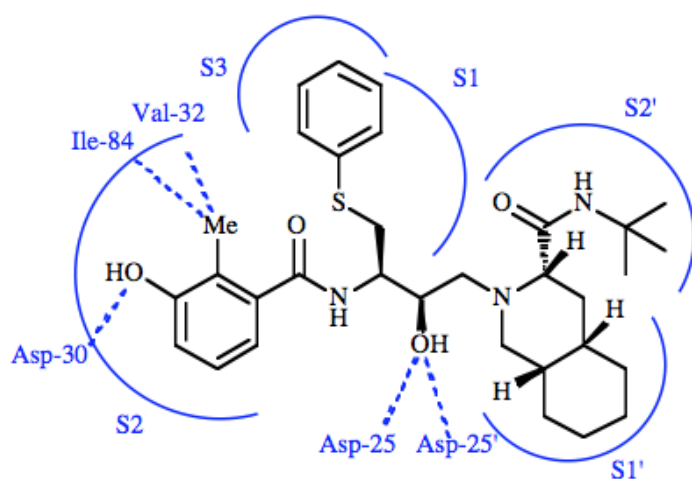

Figure 7 Binding interactions for indinavir.

Figure 8 Binding interactions for nelfinavir.