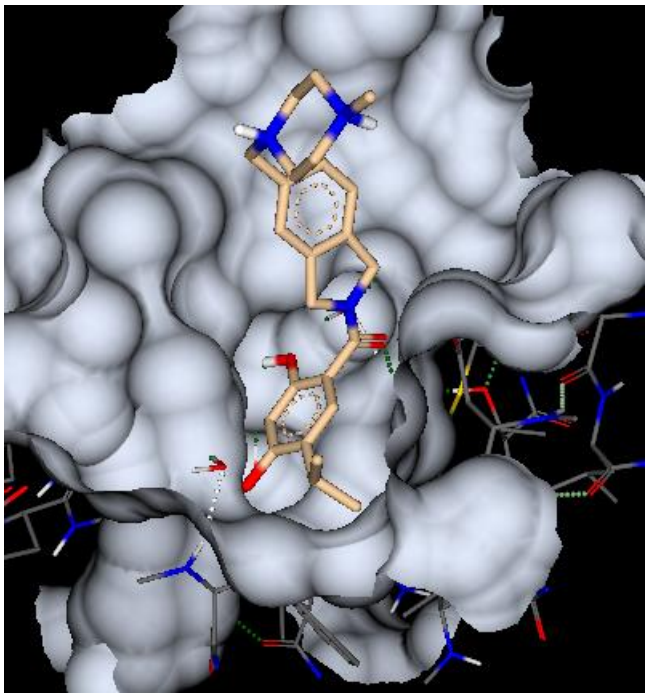
Worked Example:

3D Analysis of Virtual Libraries

This worked example explores ways to assess and design compounds in 3D using the SeeSAR Pose module. The compounds in this example have been generated as part of a virtual library (see the worked example “R-group Clipping of Reagents for Library Enumeration”) and are being considered for further optimisation.



The crystal structure on the right (PDB 2XJX) shows the binding site of Heat Shock Protein 90 (HSP90) with Onalespib as the co-crystallised ligand. Onalespib is a selective, potent HSP90 inhibitor that displays a long duration of anti-tumor activity. The beta resorcinol group forms a tight hydrogen bond network in the binding site, but the 5-(piperazin-1-ylmethyl)-isoindoline does not form any strong interactions with the protein.

The virtual compounds are all based on an amide coupling reaction with a beta resorcylic acid core and commercially available secondary amines.

Step-by-step instructions for all the features you will need to use in StarDrop are provided, along with screenshots and examples of the output you are likely to generate. If you have any questions, please feel free to contact stardrop-support@optibrium.com.

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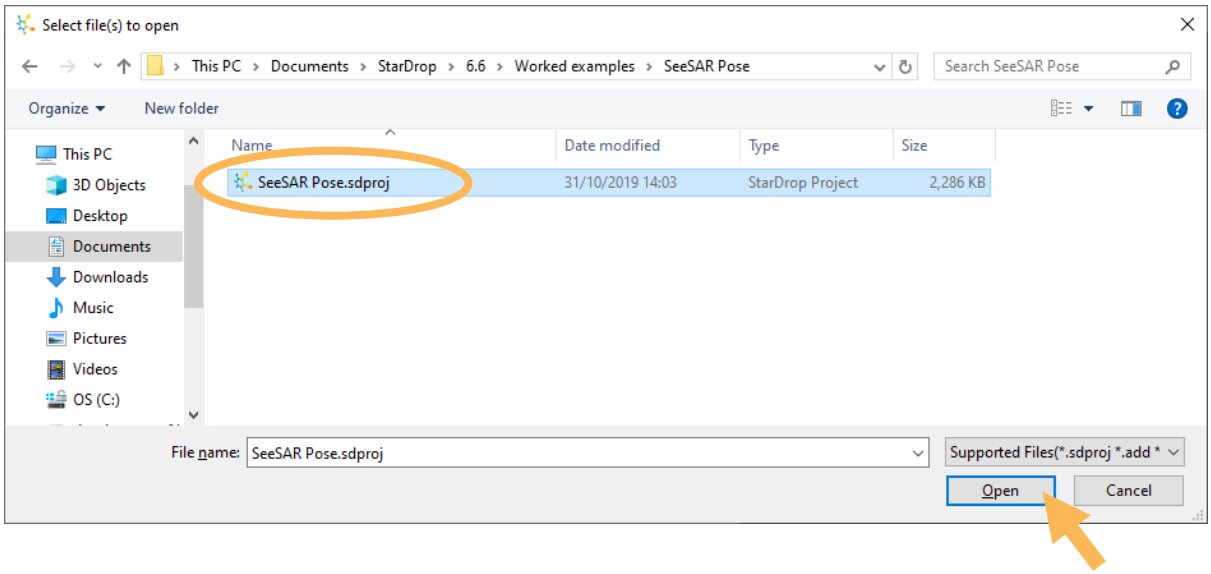
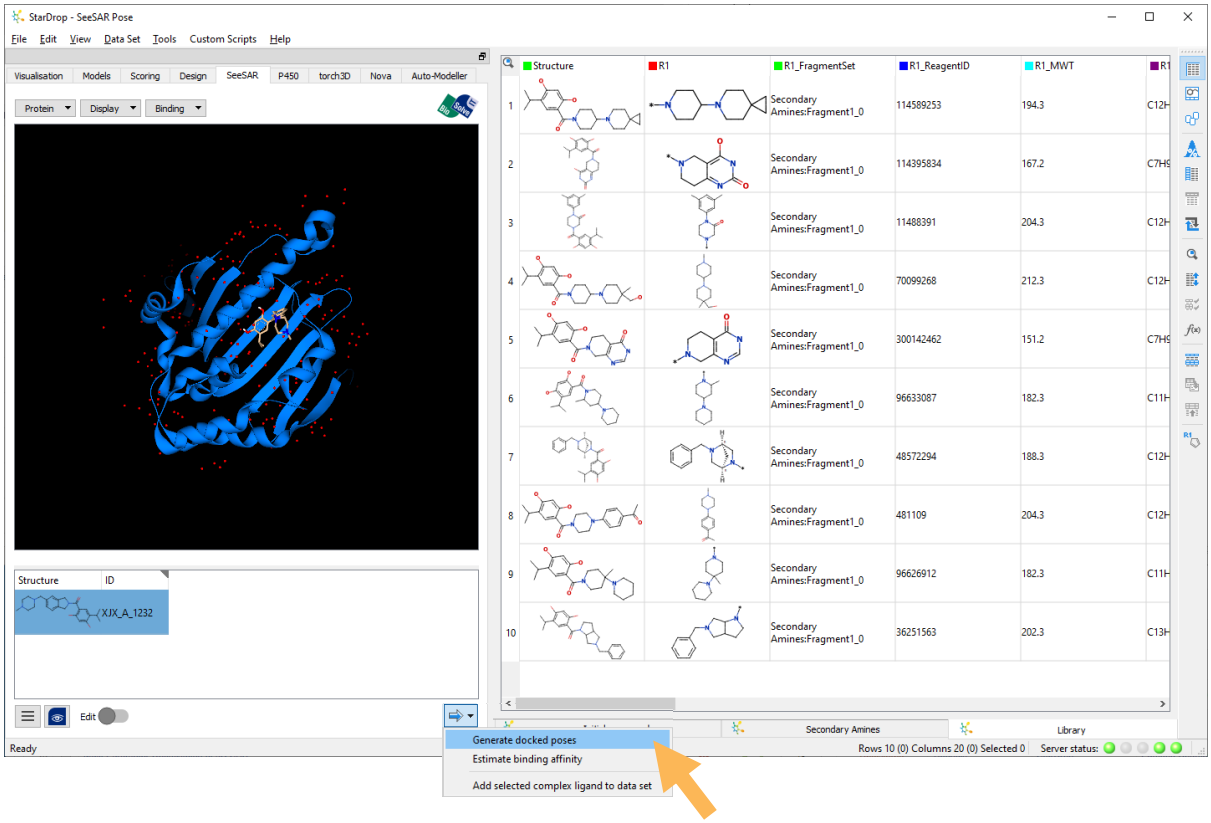
Exercise

- In StarDrop, open the file **SeeSAR Pose.sdproj** by selecting **Open** from the **File menu**.

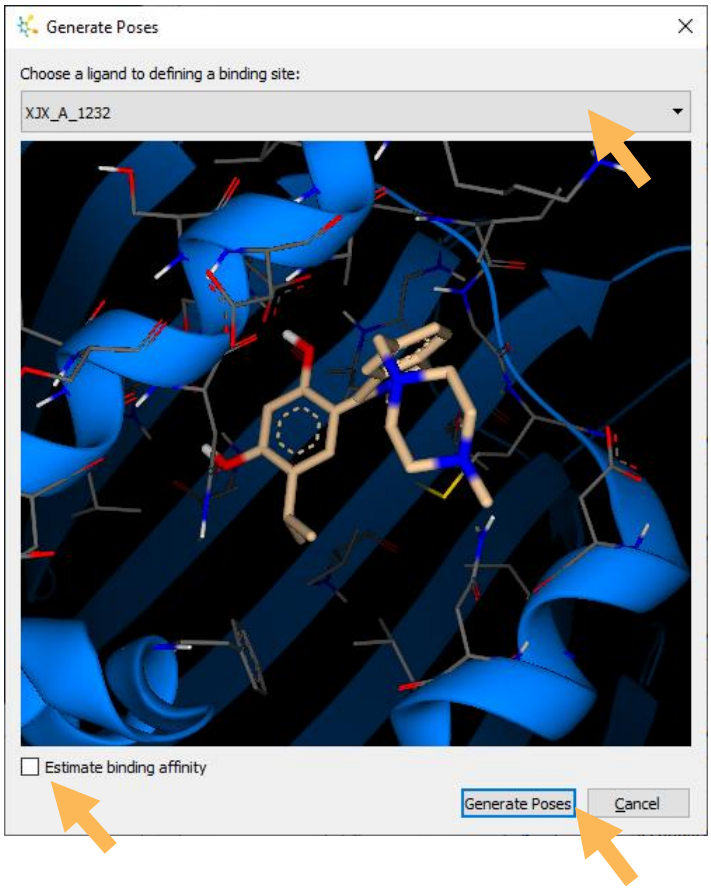


On the left, in the SeeSAR area, the protein HSP90 is displayed with its secondary structure and the co-crystallised ligand, Onalespib. The data set on the right contains 10 compounds from the library that we will analyse in 3D. To do this, we are going to generate a set of conformations using StarDrop's SeeSAR Pose module; however, if you wish to use 3rd-party docking software for such a task, then StarDrop's Pose Generation Interface can be used to connect seamlessly to your chosen platform and retrieve the docked conformations.



- From the  menu at the bottom of the SeeSAR area, choose **Generate docked poses**.

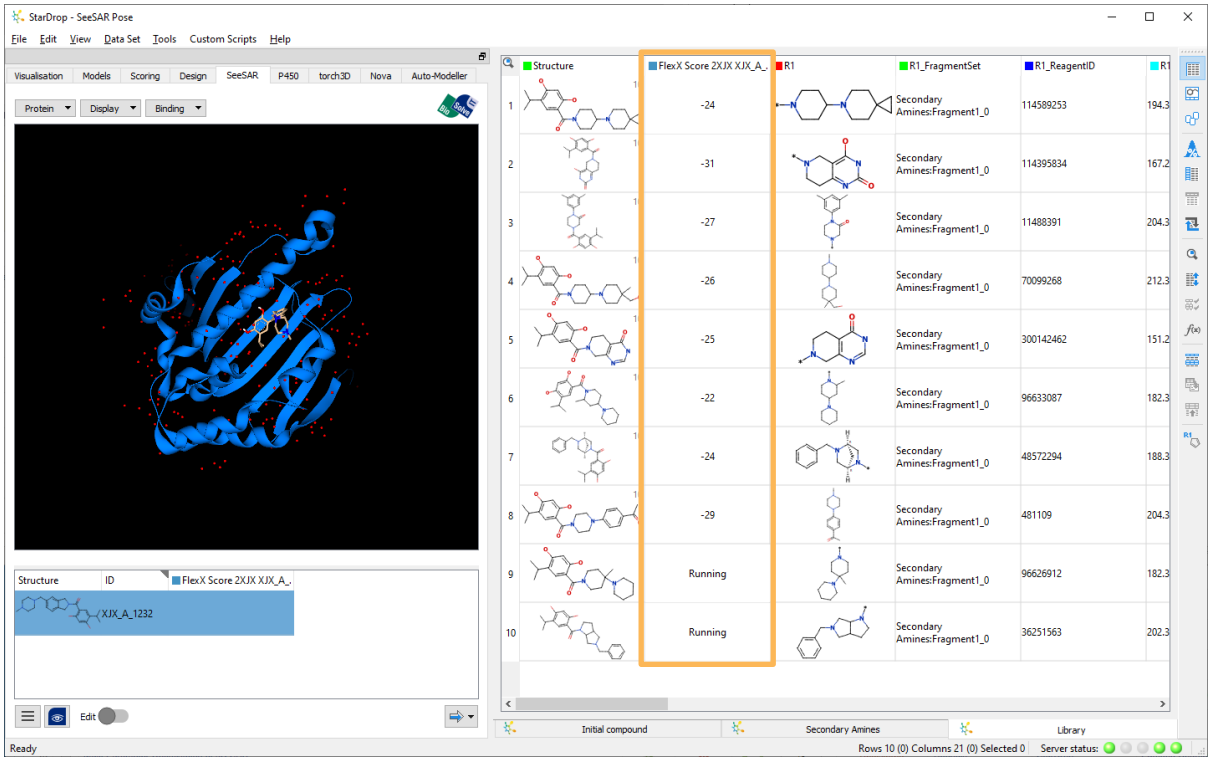
  Note: This will generate poses for the entire data set. To generate poses for individual compounds, select these rows in the data set first.



When generating poses, we need to define a binding site. By default, you will see that the co-crystallised ligand, Onalespib, is already selected and we will use this (**Note:** if we had already docked other ligands then we could use one of these to define the binding site instead). If you have the SeeSAR Affinity module then the **Estimate binding affinity option** is also checked, but we are not going to use this.

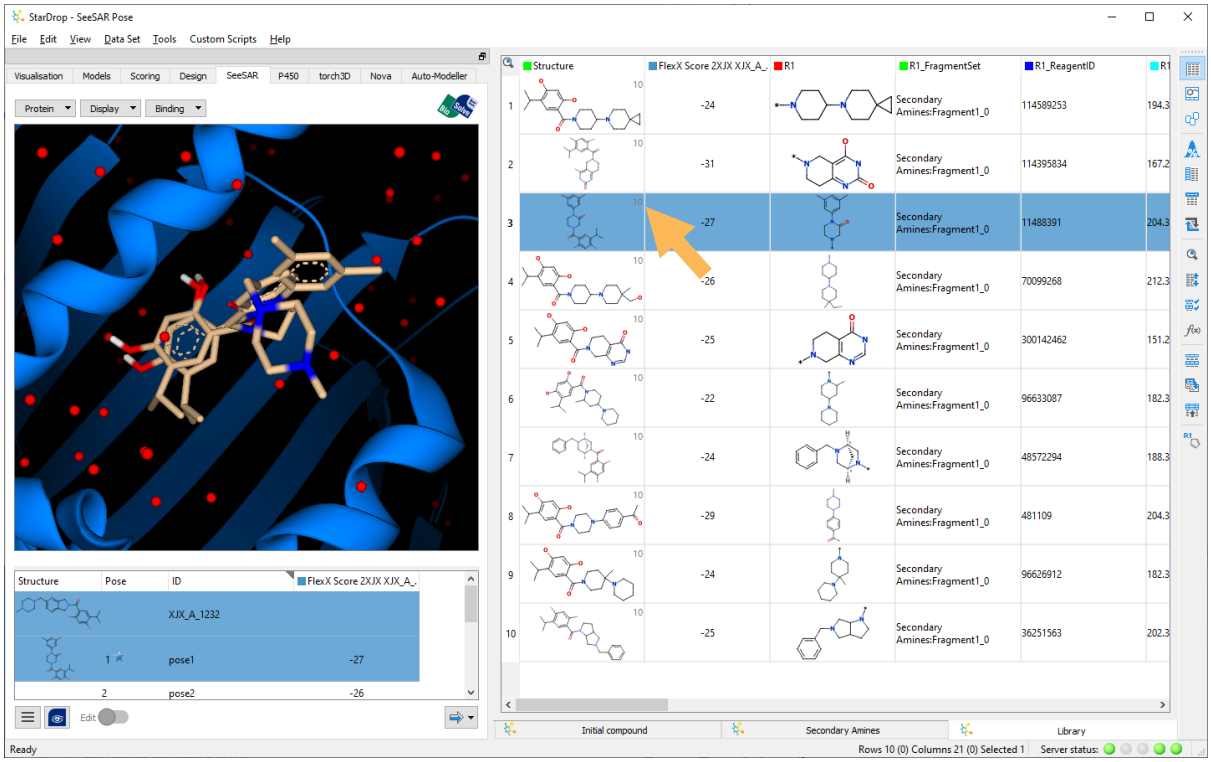
- Click the **Generate Poses button** to start the process.

You will see that a new column is added to the data set in which the FlexX Score for each compound will be displayed once the poses have been generated. While the calculations take place, the compounds will be listed as either being **Running** (the calculation is taking place) or **Queued** (the calculation will start when this compound reaches the front of the queue).



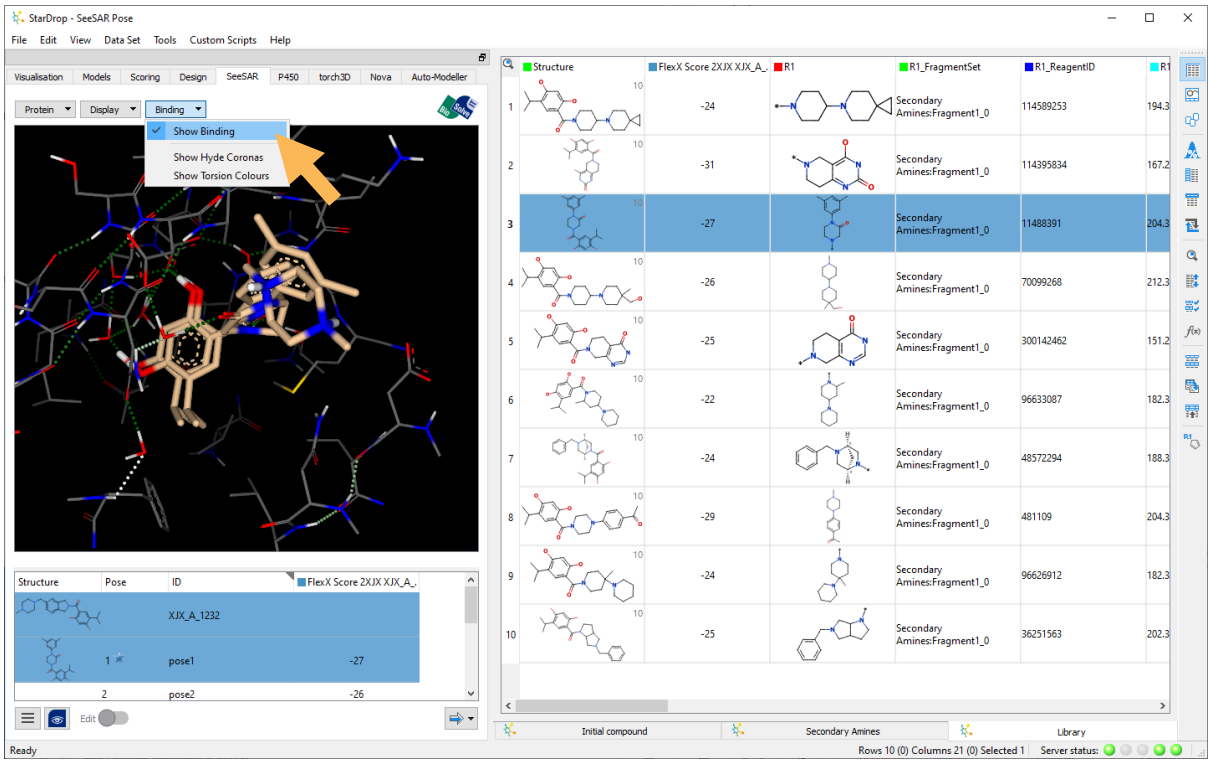
Once the calculations have completed, you will see that each compound has 10 poses generated (as shown to the top-right corner of the structure in each row).

- To view some of the poses that have been generated, select the compound in **row 3**.

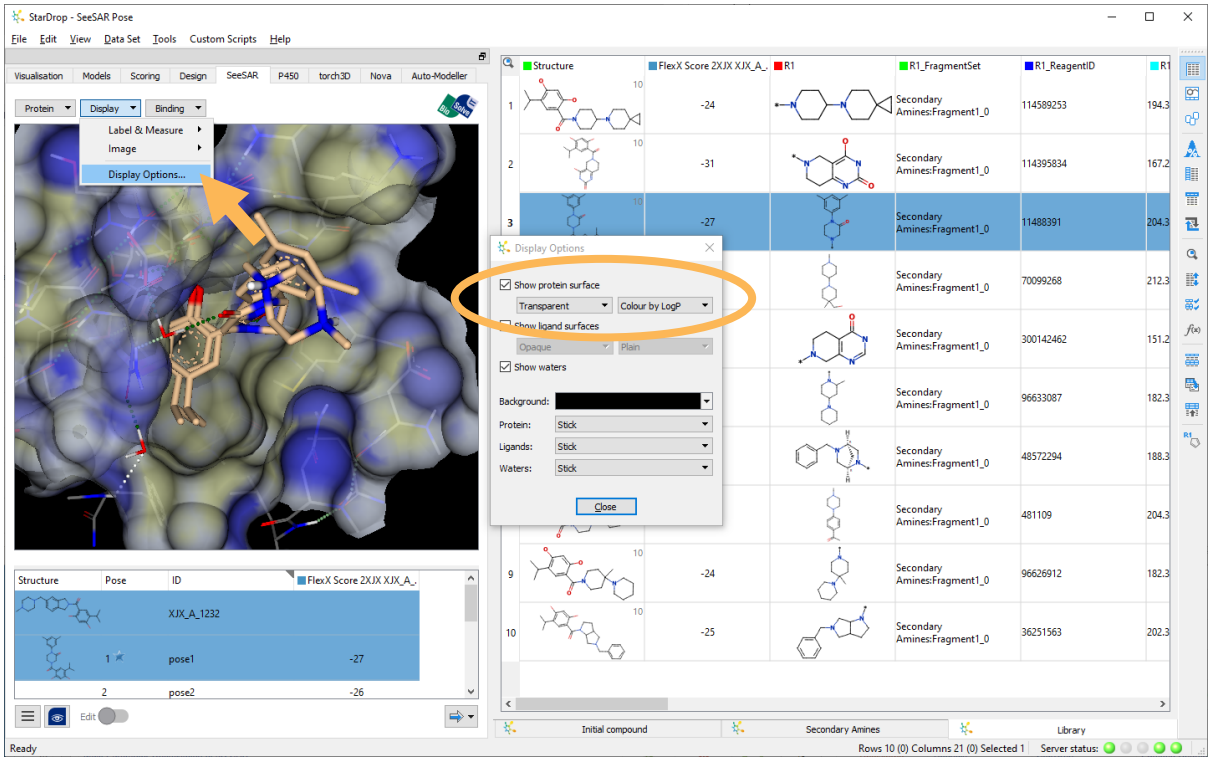


It is now loaded into the 3D viewer alongside the co-crystallised ligand.

- To view the binding site in more detail, select **Showing Binding** from the **Binding menu**.

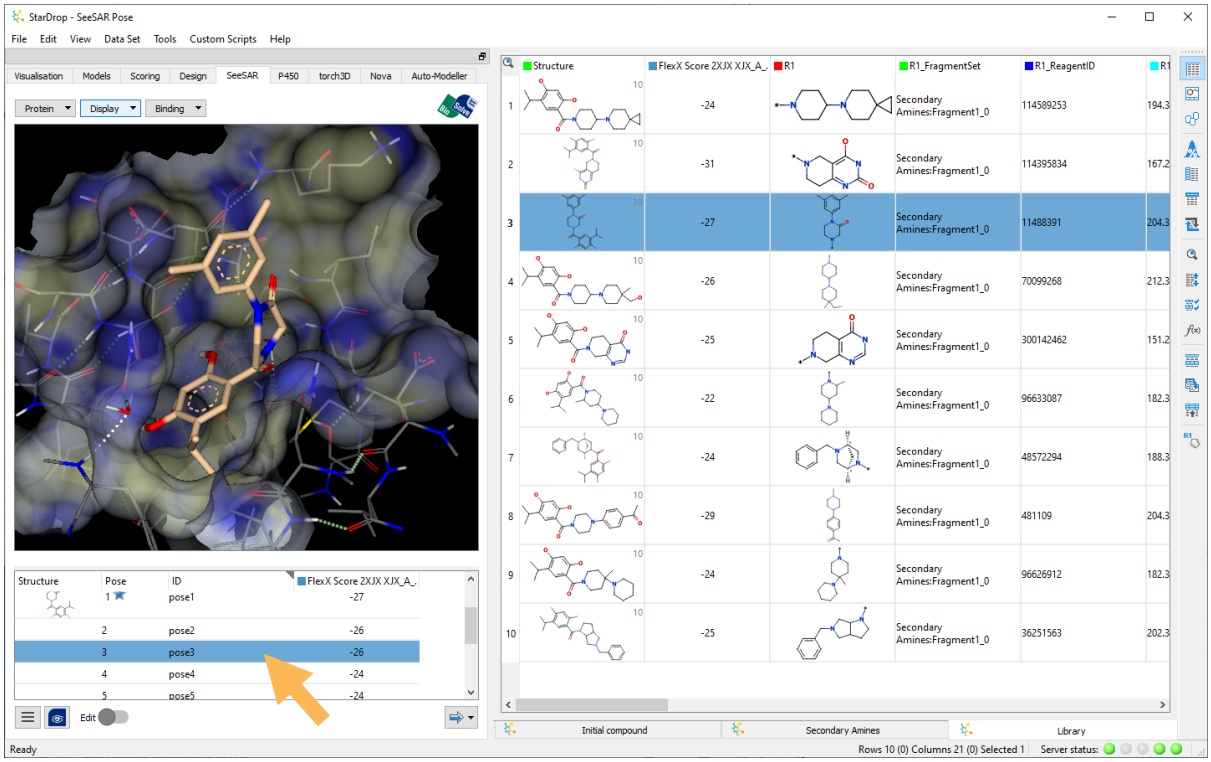


- From the **Display menu**, choose **Display Options**.
- In the **Display Options dialogue**, tick the **Show protein surface option** and choose **Transparent** and **Colour by LogP** from the menus.

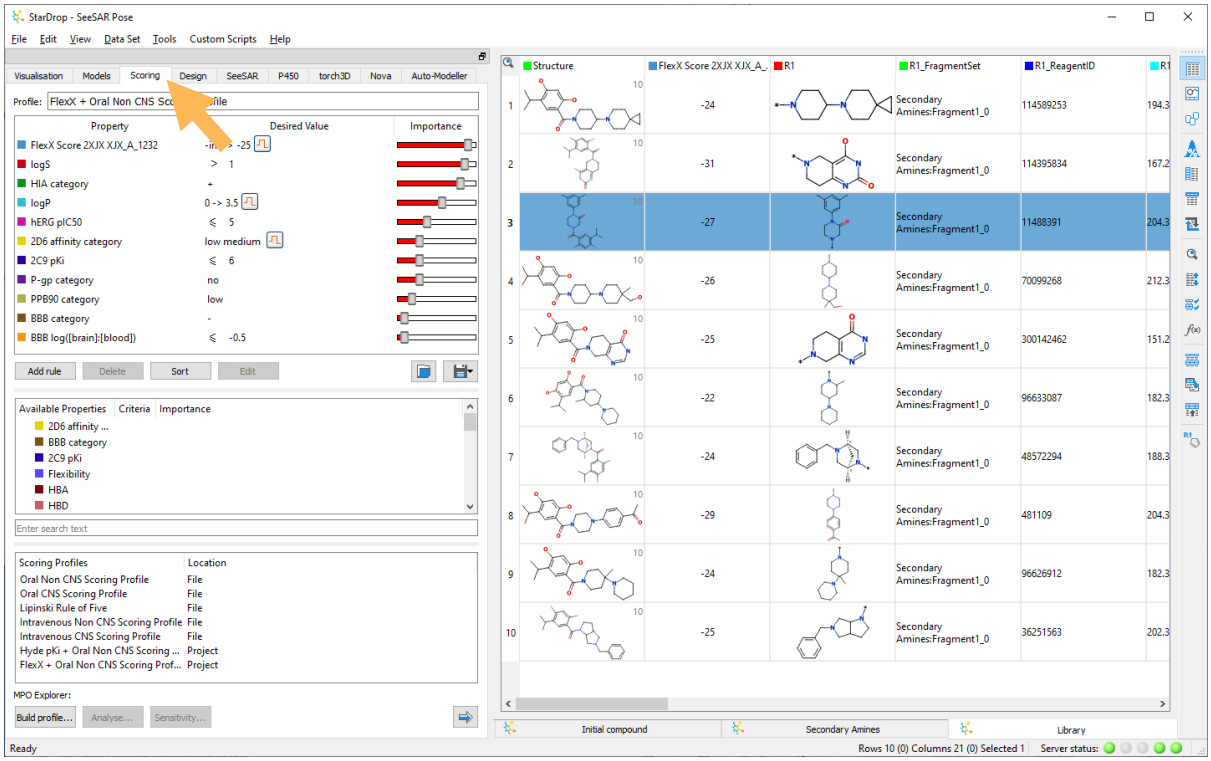


- Close the **Display Option dialogue**.

To select any pose in isolation, simply select it in the table below the 3D viewer. Indeed, although the top scoring pose for any selected compound is shown by default, we can select and view any of the others by selecting them in the table.



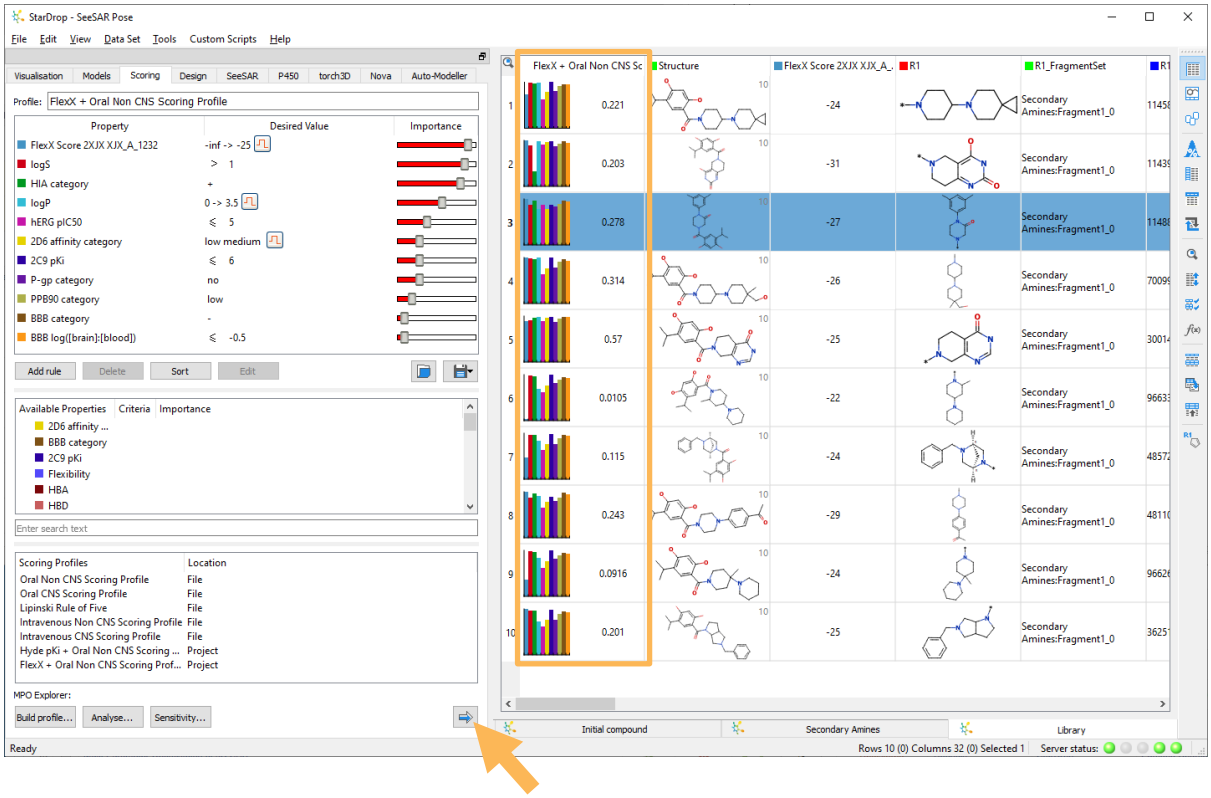
While the docking scores provide us with a qualitative idea of how we might rank-order the compounds, we'd like to combine this with more information about other compound properties.



- Select the **Scoring tab**.

In this example, we have created a multi-parameter scoring profile to reflect the range of properties we'd ideally like to see alongside the docking score for an oral non-CNS target.

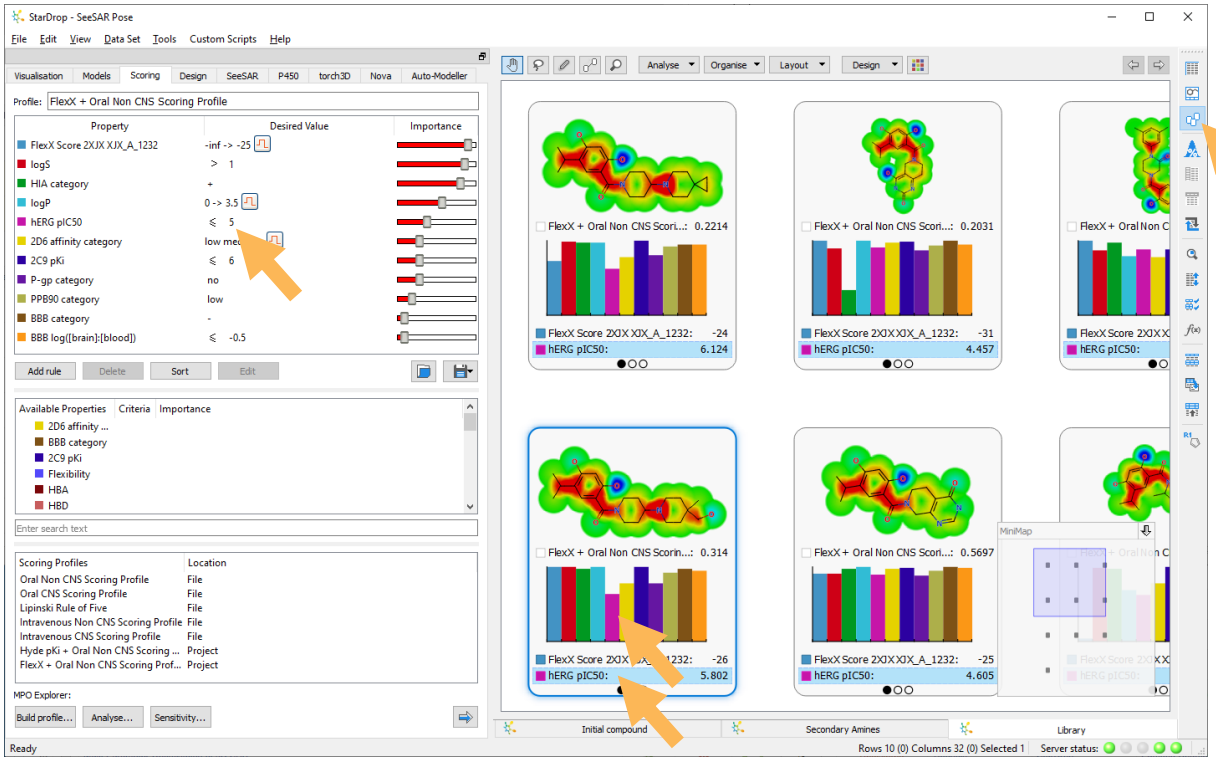
- Run the scoring profile by clicking the  button. (**Note:** you will see a message telling you that the docking score has no uncertainty associated with it. Ideally, all the data we score should have an appropriate reflection of the error and we know that the docking scores are not always accurate values, but in this case click **OK**.)



This generates a multi-parameter score for every compound and adds it to a new column. The score is a value between 0 and 1 and reflects the probability of the compound being successful given the criteria we have defined in the profile and the data available (a perfect score would be 1). Each score is accompanied by a histogram which gives a quick visual indicator as to how each of the compound properties contributed to the score. The lower the bar, the more of a problem that property may be for the compound.

We can examine this in more detail in Card View where we have already created a card design that shows the overall score, alongside the docking score and the hERG pIC_{50}.

- Click the **Card View button** near the top of the right-hand toolbar.

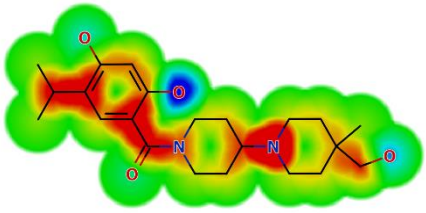


We can see in the second compound down on the left that the bar in the scoring histogram associated with hERG inhibition is shorter than the others, indicating a potential problem. From the property value shown at the bottom of the card we can see that the predicted value is 5.8 whereas in our scoring profile we indicated that an ideal compound would have a hERG pIC_{50} below 5.

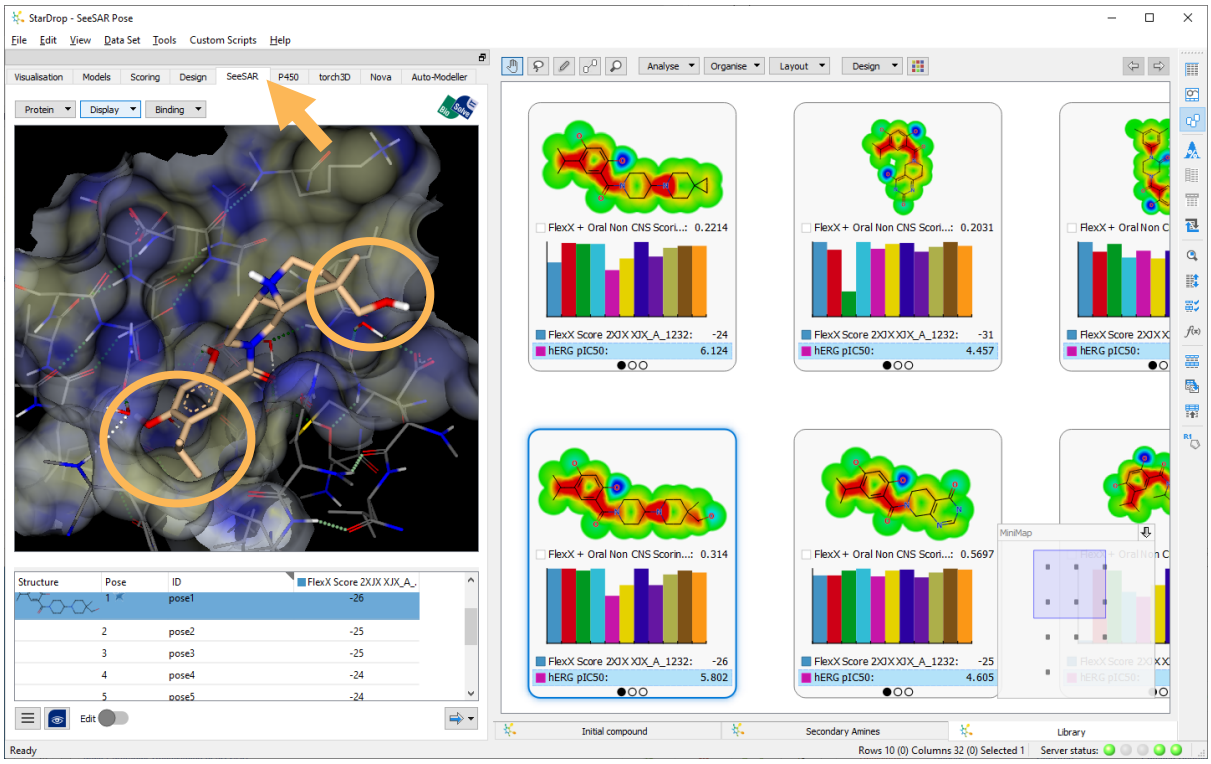
- On that card, click on the **hERG pIC50** property to display the Glowing Molecule for hERG pIC50.

The Glowing Molecule highlights regions of a compound that are having a significant impact upon its predicted properties. Regions of the molecule increasing the predicted value are coloured red, regions decreasing the predicted value are coloured blue, and regions having no overall influence are coloured green. The colours are interpolated between these extremes to reflect the influence of that region of the molecule on the property prediction.

We can see from the Glowing Molecule for our chosen compound that there are a number of regions glowing red, indicating that they are increasing the predicted hERG affinity; however, we should consider these alongside the 3D pose to consider their importance to the compound's ability to bind to the target protein.



- Click on the **SeeSAR tab** to return to the 3D viewer.



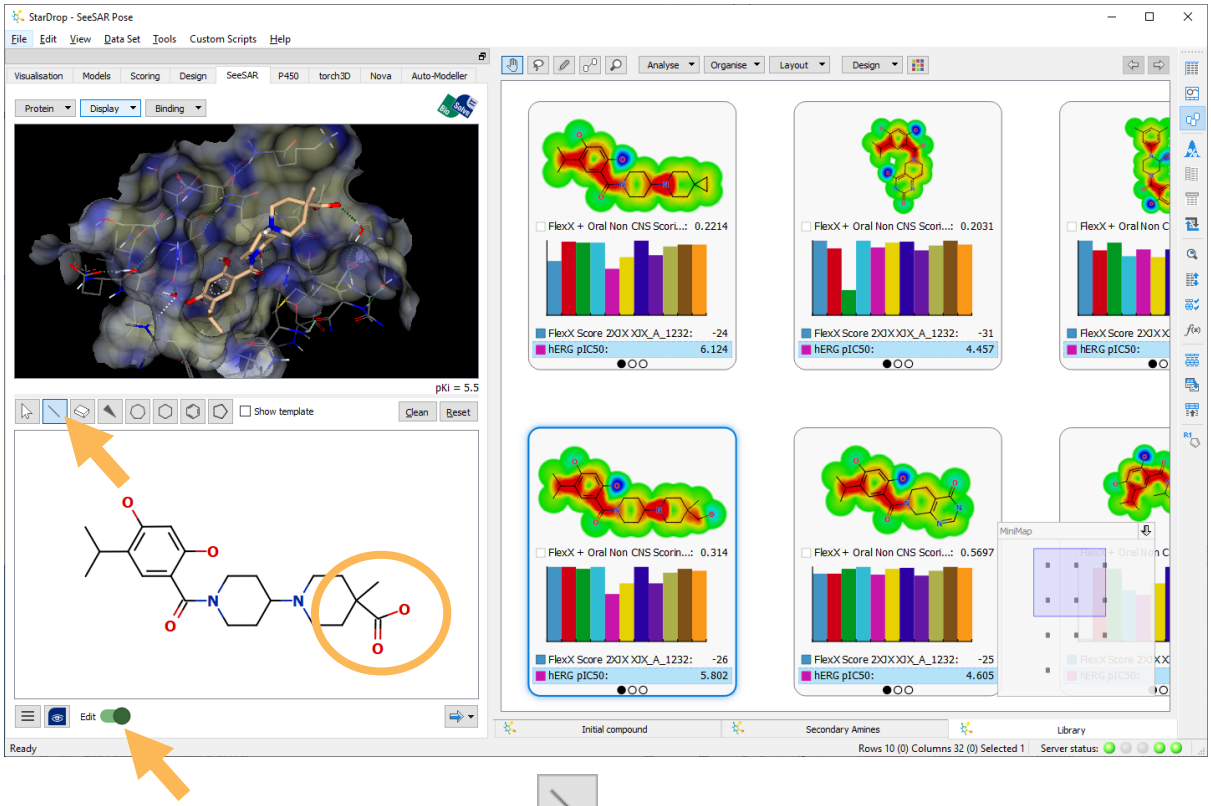
We can see from the Glowing Molecule that the resorcinol group is bright red and increasing the predicted hERG affinity, but we can see from the 3D viewer that this group is critical to the target binding affinity. In particular, we can see that the isopropyl group is very red, but this binds into a hydrophobic cleft and so we probably wouldn't want to change this because there is a significant risk that this would disrupt the binding.

On the other hand, the hydroxymethyl is also quite red and we can see that this is a lot more accessible. As such, making changes in this region may enable us to improve the hERG prediction without having an adverse effect upon the binding.

We can use the **Edit mode** in **SeeSAR Pose** to try out new ideas and get quick feedback about how the changes we have made may affect the binding.

- Click on the **Edit switch** at the bottom of the **SeeSAR area** to display the 2D editor.

Here we can use StarDrop's standard editing tools to make changes to the displayed structure. The selected structure will be used as a template for the newly edited structure to be docked into the same site.



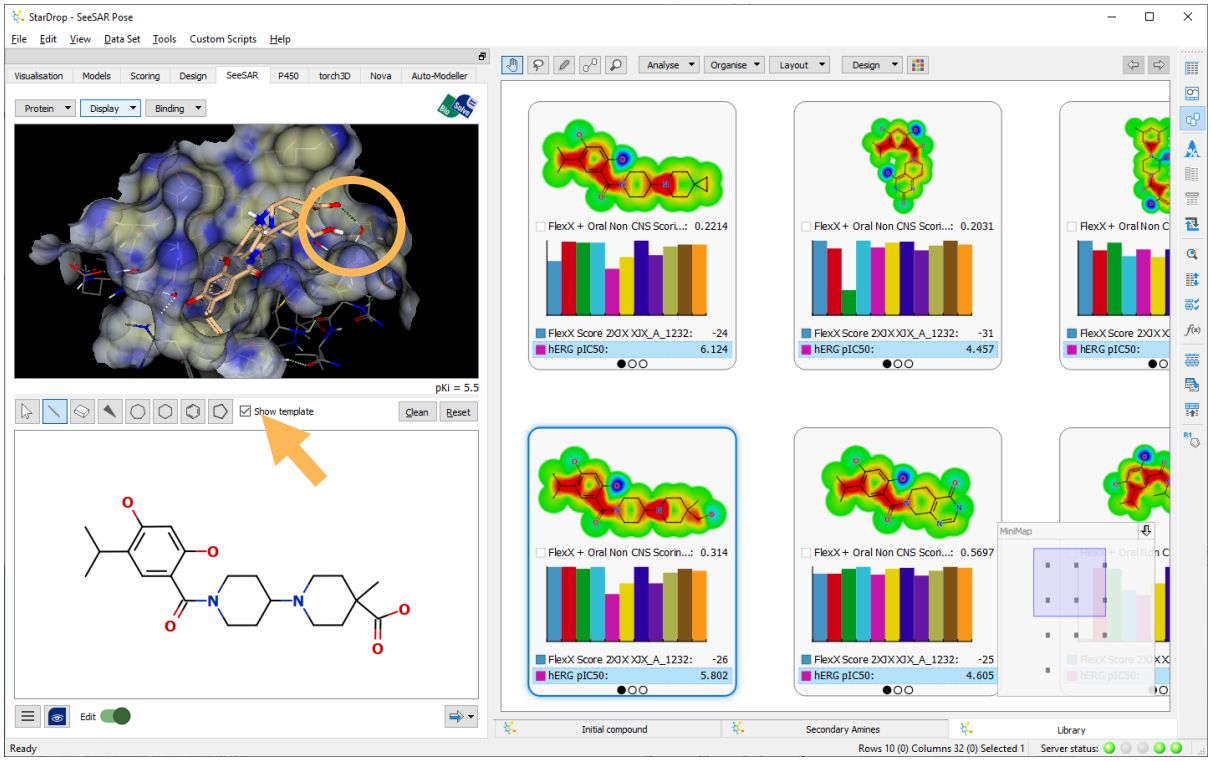
- In the sketch area, use the **Bond tool**  to change the hydroxymethyl into a carboxylic acid, as shown above.

Hint: To specify an element, hover over an atom and type the element symbol, in this case “O”. Bond types (single, double, triple) may be cycled by clicking on a specific bond.

As you edit the compound, you will see a message saying “Generating pose” below the 3D viewer and a new pose will be displayed within a few seconds.

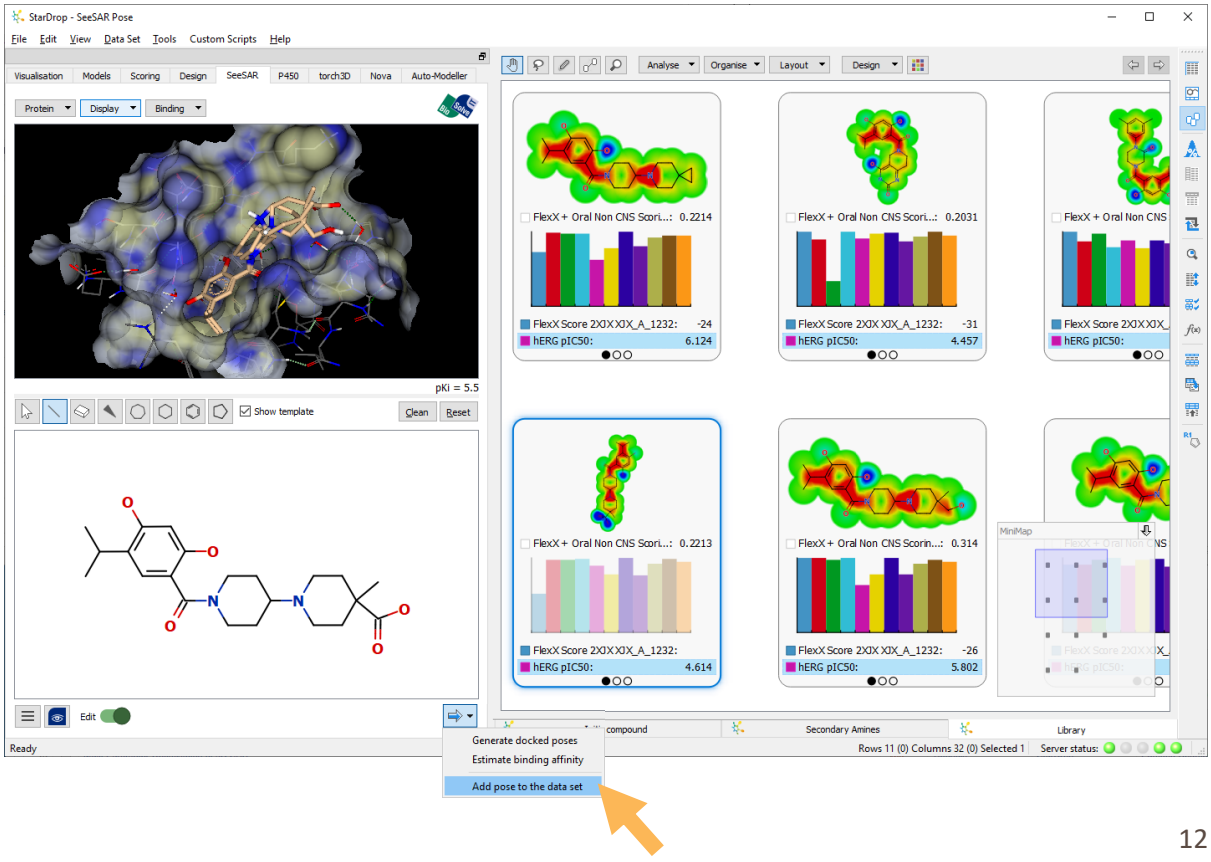
- To compare the new compound with the original, tick the **Show template option** below the 3D viewer.

Hint: You can change the relative sizes of the 3D viewer and the 2D sketch areas by dragging the slider in between.



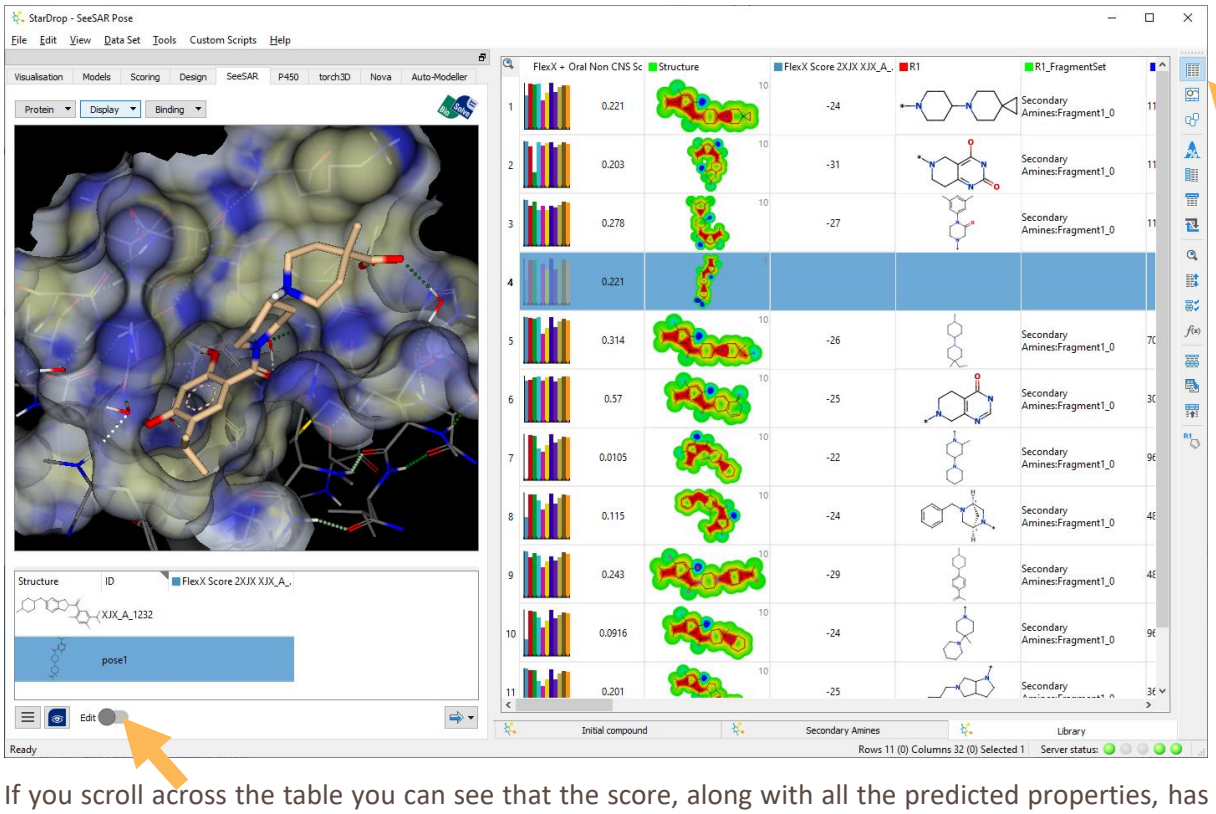
We can see that, in comparison to the original compound, the overall binding conformation has been maintained quite well and that the carboxylic acid forms an additional hydrogen bond with the target, which may assist the overall binding. As such, this may present us with a viable alternative, which we will add to our data set.

- From the  menu at the bottom of the SeeSAR area, choose **Add pose to data set**.



On the new card, you can see that the hERG pIC_{50} is now below 5, due to the addition of the carboxylic acid. This decrease is supported by the Glowing Molecule in which the carboxylic acid is coloured blue because it is decreasing the predicted hERG affinity.

- Click the **Edit switch** to come out of Edit mode.
- Click the **Table View button** to switch back into Table View.



If you scroll across the table you can see that the score, along with all the predicted properties, has been added to the data set along with the new compound.

The overall score is faded to indicate that there is a missing property, in this case, the docking score. These are not automatically calculated because they take a lot longer to generate, but it can be added easily by selecting this compound and running pose generation as we did earlier, selecting the co-crystallised ligand as the reference once again.

As further steps, we might wish to consider a more quantitative estimate of the binding affinity. This is something which you can do with the SeeSAR Affinity module, as demonstrated in the following worked example:
https://www.optibrium.com/community/tutorials/stardrop/475-seesar-affinity