# Worked Example:

Binding Affinity and Torsion Angle Analysis of Virtual Libraries

This worked example explores ways to assess the binding affinity of docked compounds. The compounds in this example were generated as part of a virtual library 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-tumour activity. The beta resorcinol group participates in a hydrogen-bonding 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 based on an amide coupling reaction with a beta resorcylic acid core and commercially available secondary amines.
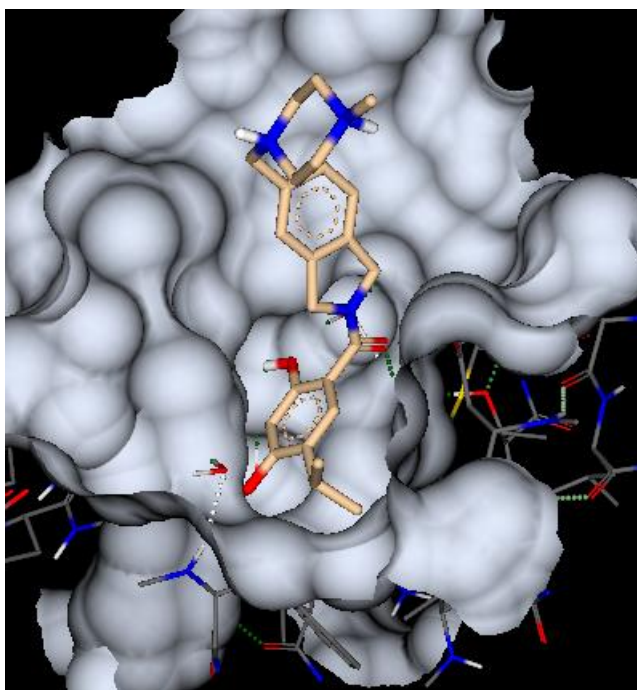
This example demonstrates how StarDrop's SeeSAR Affinity module can be used to consider binding affinity and torsion angle analysis for 3D poses generated by any docking software. If you have just

completed the SeeSAR Pose worked example, then you can carry on directly without reloading the initial file.

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.

Exercise

If you have completed the worked example "3D Analysis of Virtual Libraries", you can skip to page 5.

- In StarDrop, open the file **SeeSAR Affinity.sdproj** by selecting **Open** from the **File** menu.
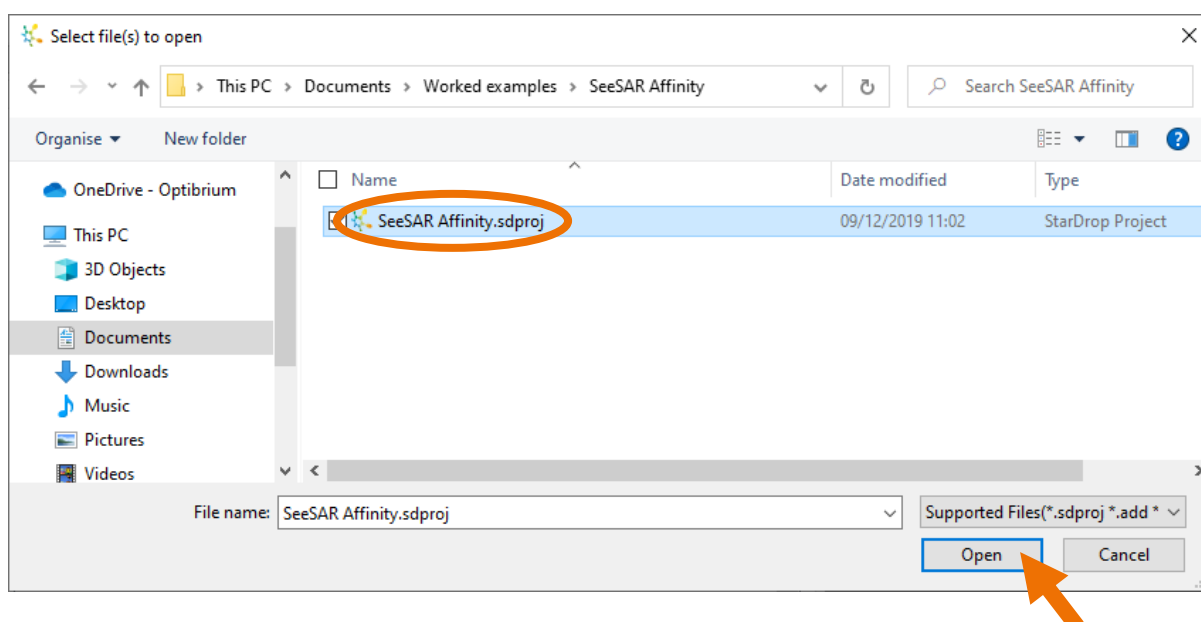
On the left, in the SeeSAR area, the protein HSP90 is displayed with its secondary structure and the co-crystallised ligand, Onalespib. The data set on the right contains ten compounds from the library. The compound in row 4 is also selected and displayed in the 3D viewer.
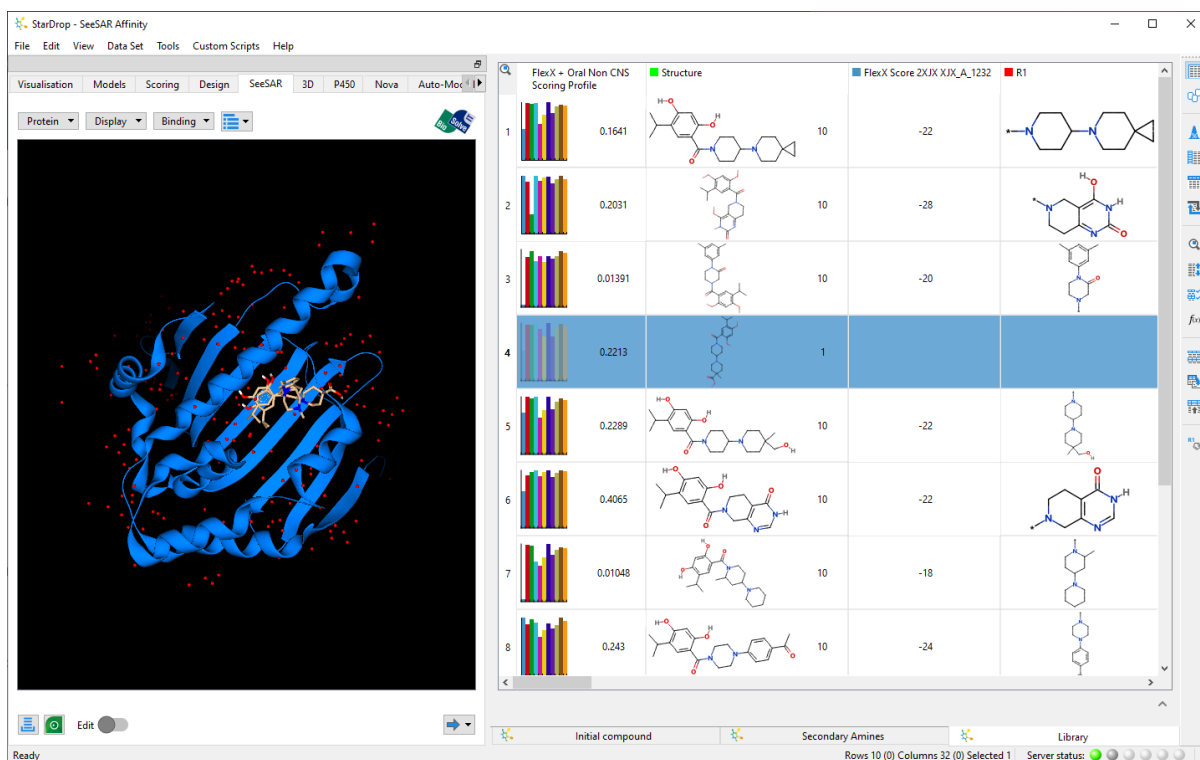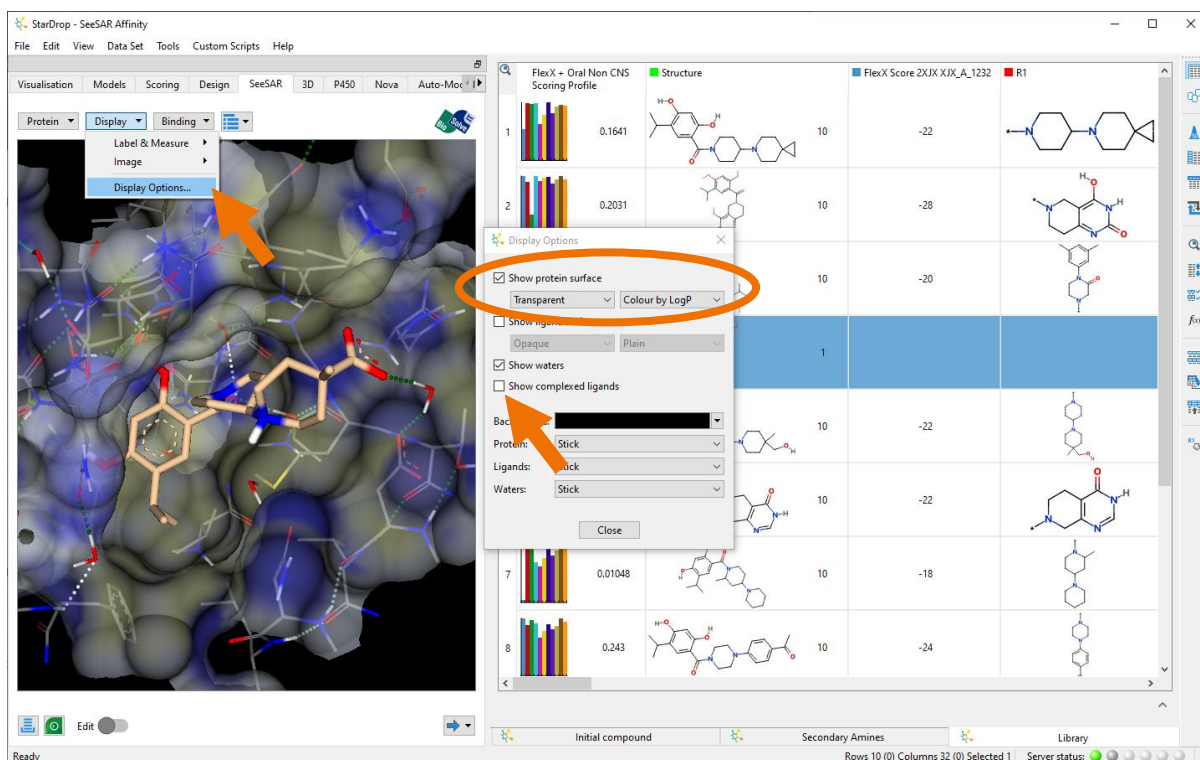
- From the **Binding** menu, select **Show Binding** to focus on the binding site.
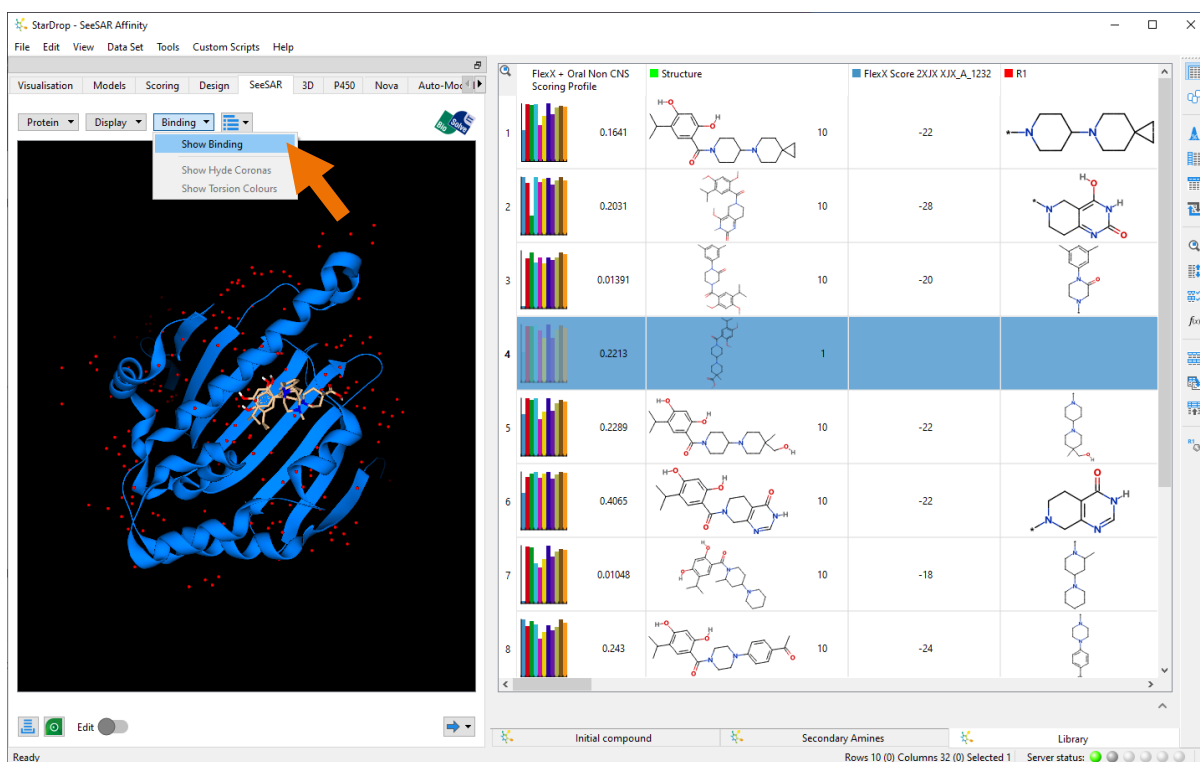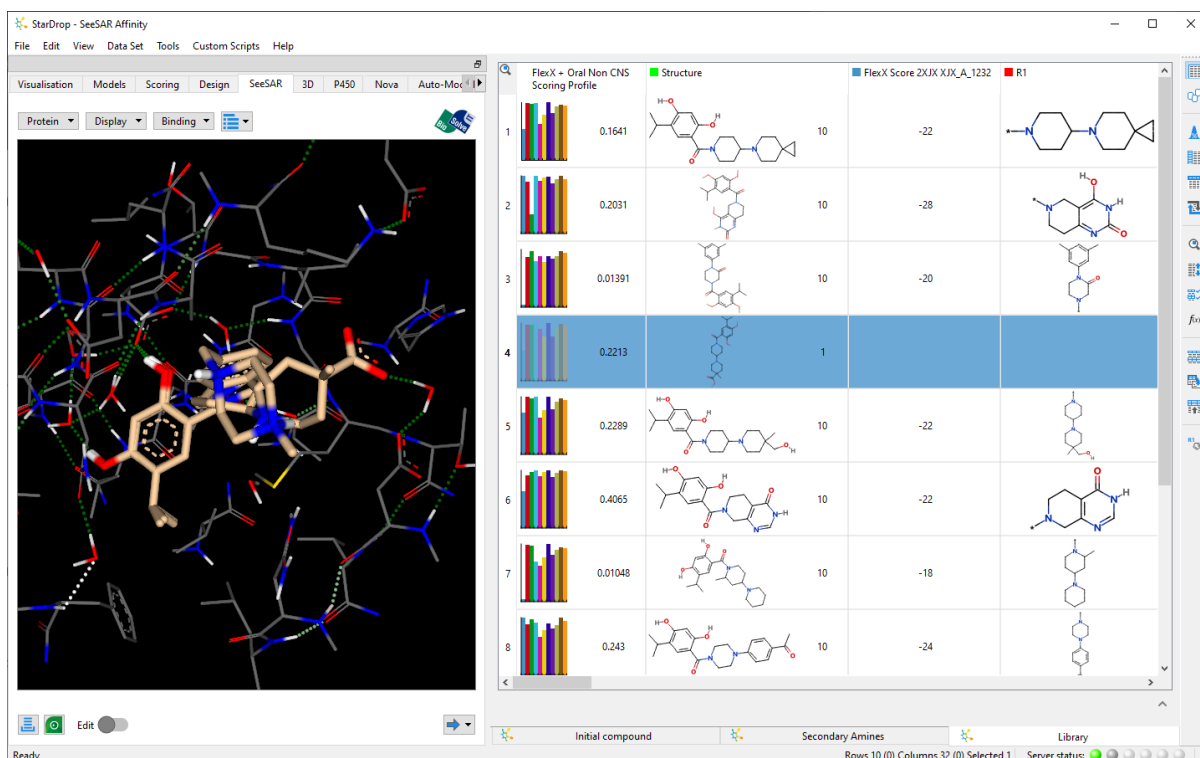
- From the **Display** menu, choose **Display Options**.

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

- In the **Display Options** dialogue, untick the **Show complexed ligands** option to hide Onalespib.

- Close the **Display Option** dialogue.

This data set already contains a column showing docking scores; however, these values don't necessarily provide a good indication as to the binding affinity we might expect. The SeeSAR Affinity module enables us to use BioSolve IT's HYDE scoring that provides a consistent approach for describing hydrogen bonding, the hydrophobic effect, and desolvation. It relies on HYdration and DEsolvation terms which are calibrated using the octanol/water partition coefficients of small molecules. The calibration does not employ measured affinity data and therefore, HYDE is generally applicable to all protein targets. HYDE reflects the Gibbs free energy of binding while only considering the essential interactions of protein-ligand complexes.

During our analysis, we will consider differences between the estimated binding affinities for the compounds in this library and their docking scores.

- Select the whole data set by clicking the button in the top-left corner of the data set (just below the search icon).

- From the menu at the bottom of the SeeSAR area, choose **Estimate binding affinity**. **Note:** This will generate poses for the entire data set. To generate poses for individual compounds, select these rows in the data set first.

You will see a warning telling you that you are about to generate estimated binding affinities for all the poses in the data set.

- Click the **OK** button.
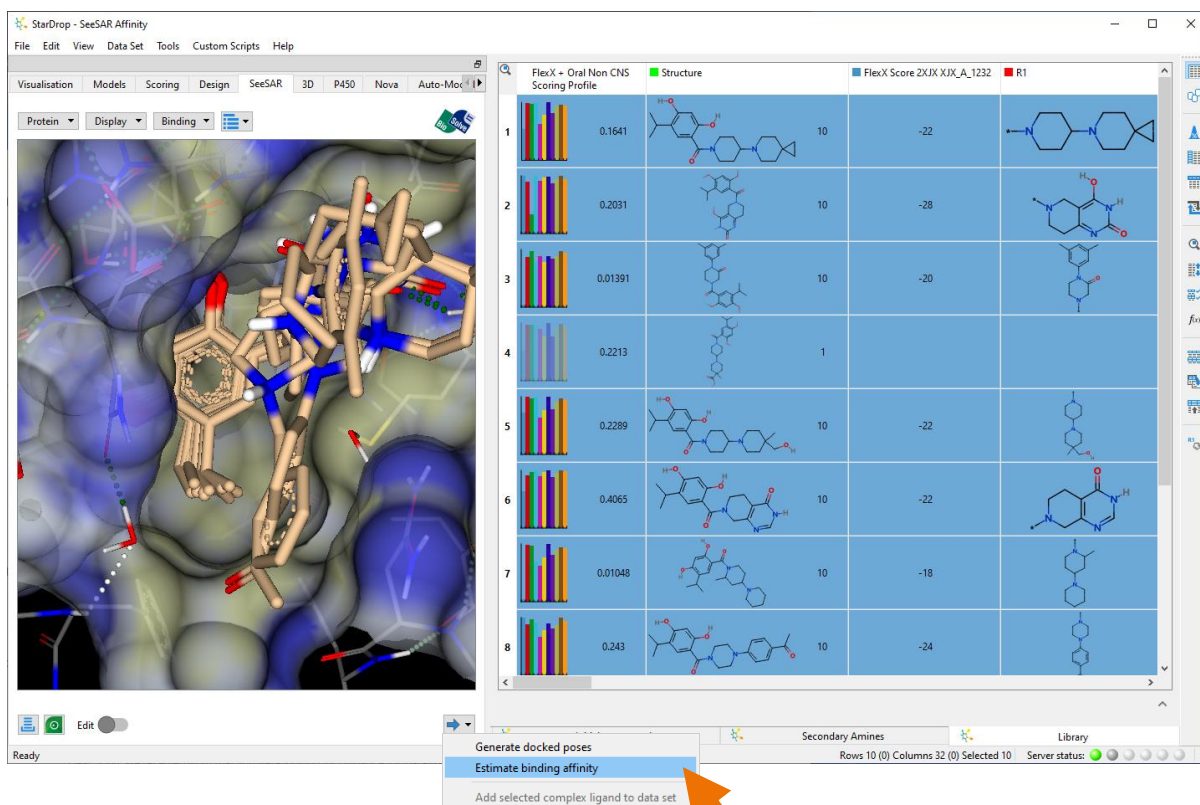
When estimating binding affinities, we need to define a binding site. A binding site can be defined either by selecting a column of docking results (for which a binding site has already been specified) or by choosing an individual ligand. In this case, we will use a ligand, specifically the co-crystallised ligand, Onalespib.
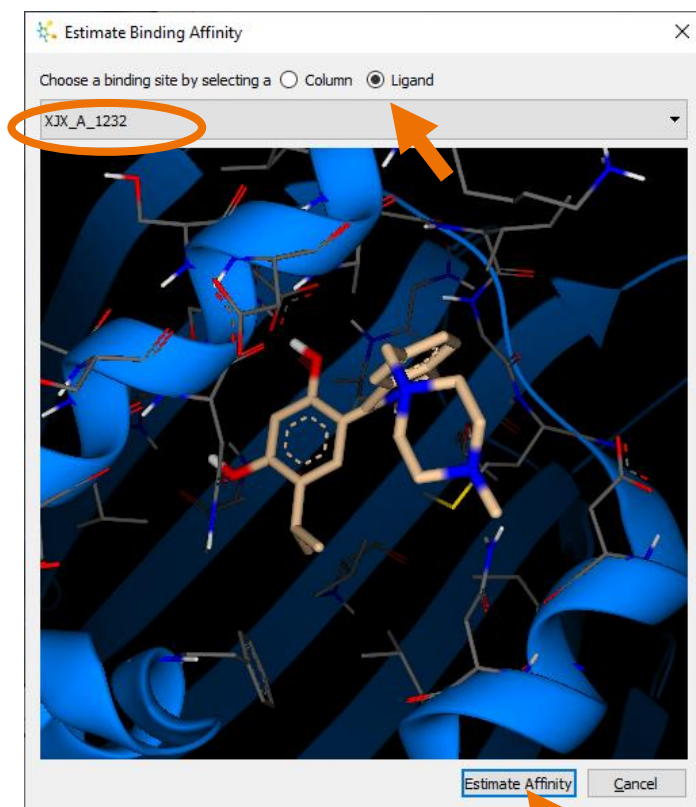

- Select the **Ligand** option and choose **XJX_A_1232** from the list.

- Click the **Estimate Affinity** button to start the process.

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You will see that a new column is added to the data set in which the HYDE score for the compound will be displayed once the calculations have been completed, providing estimates of the binding affinity for the selected compounds.

- When all of the calculations are completed, select the compound in **row 3**.
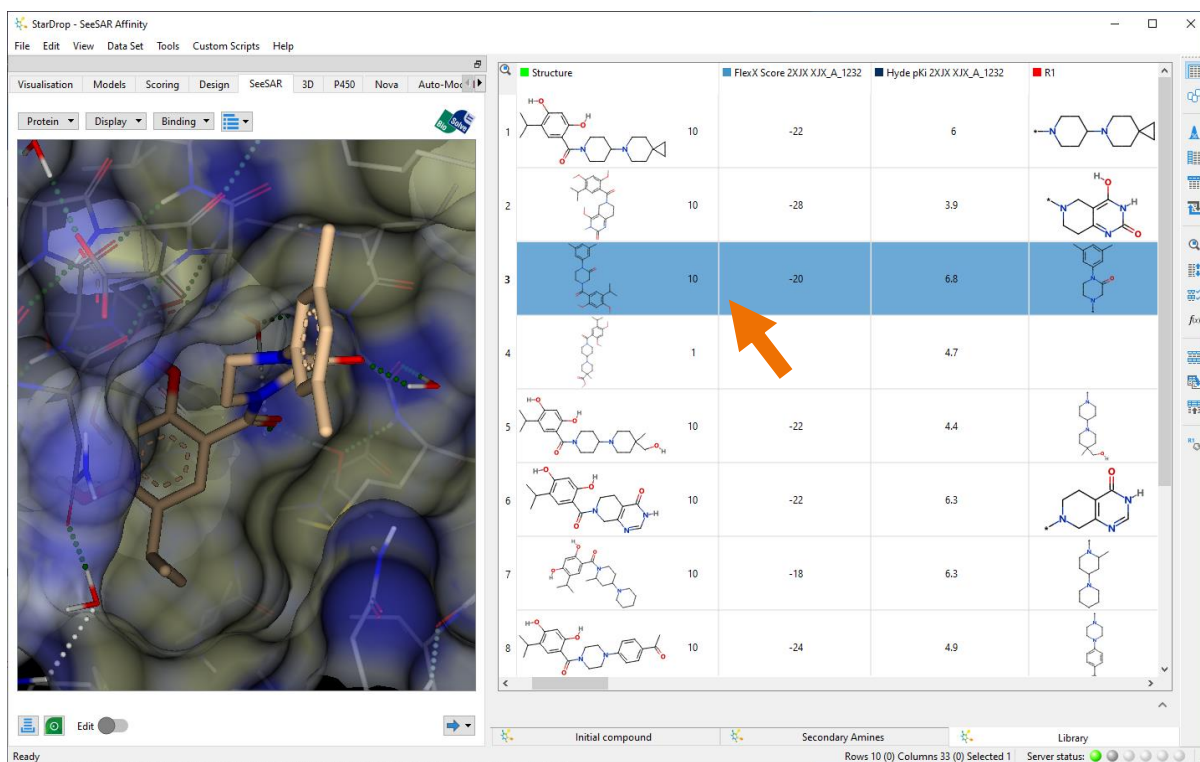
Note: Selecting a row in the data set enables you to view that compound, and its poses, in the binding site within the 3D viewer window. Using the mouse, you can *rotate*, *zoom* and *pan* the view.

The selected compound has an estimated binding affinity (pK_i) of 6.8, but one of the properties we may wish to analyse when exploring our docking results are torsion angles.

- From the **Binding** menu, select **Show Torsion Colours**
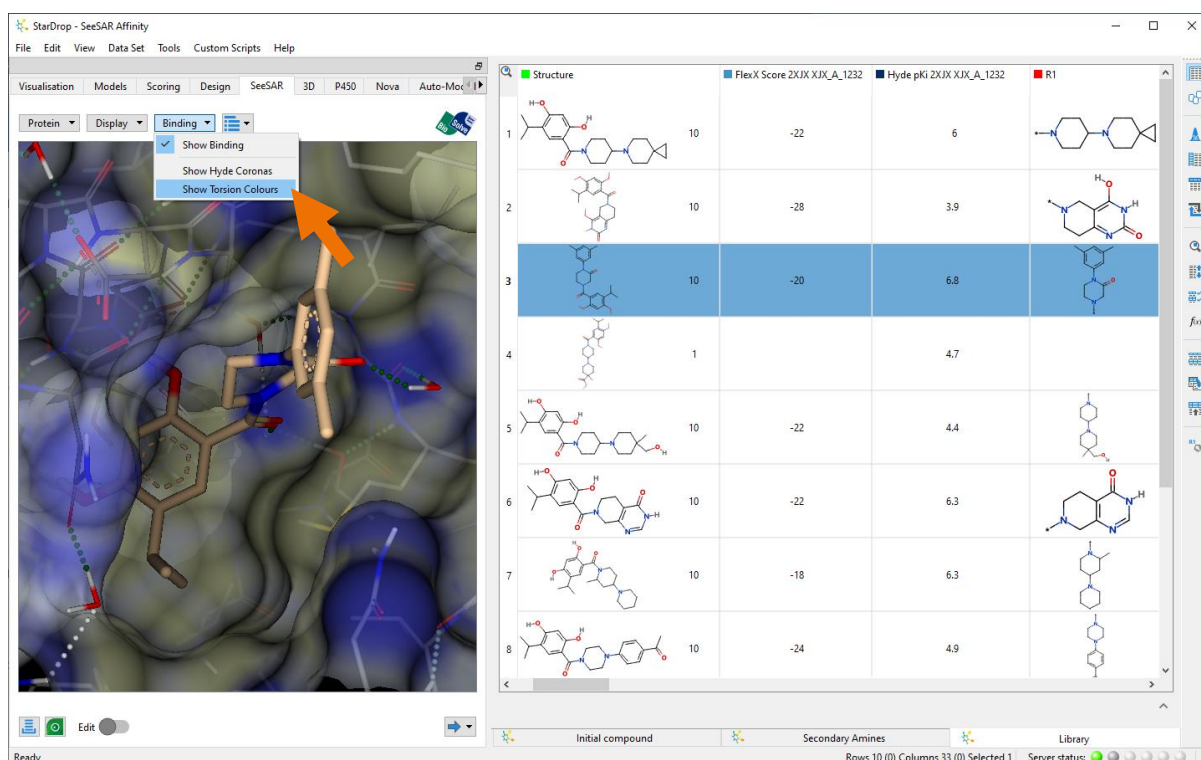
This analysis is based upon a comparison with the Torsion Library, developed jointly by F. Hoffmann-La Roche and the University of Hamburg that contain hundreds of rules for small molecule conformations derived from the Cambridge Structural Database (CSD). Those that lie within the first tolerance interval (i.e., those seen frequently) are coloured green. Those angles observed that lie outside the first tolerance interval (i.e., those seen less frequently) are coloured yellow. Any bonds coloured red reflects highly uncommon torsion angles, which are therefore questionable.
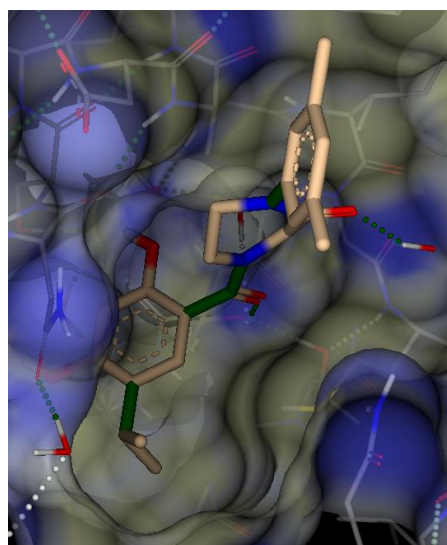

We can see from the analysis of the conformation that the torsion angles are coloured in green, indicating that they fall within the first interval of conformations as compared to compounds in the CSD. We can then explore some of the other docked poses generated to determine if any of the poses may possess greater binding affinity.

The individual poses can be accessed by clicking on the arrow [10] next to the structure in the data set and alongside each is its docking score and estimated binding affinity.

- Click on the [10] arrow and then select **pose 2**.
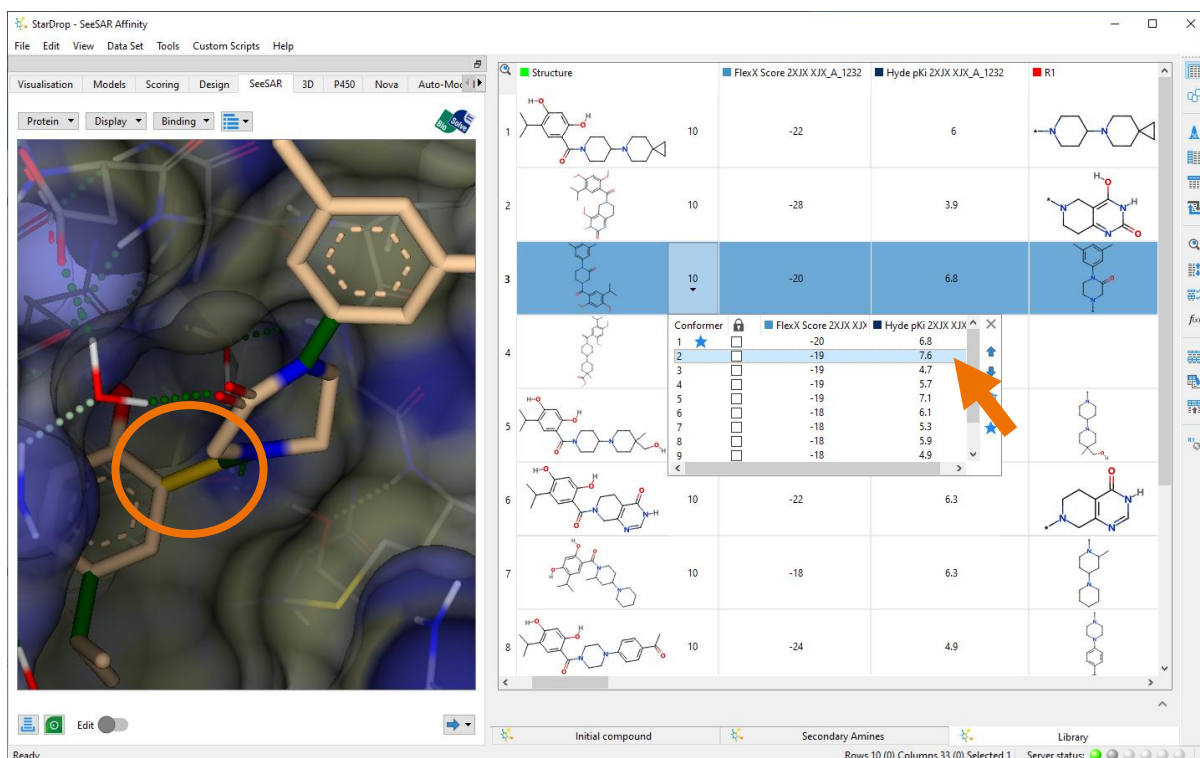
While the two conformations have similar docking scores, comparatively, the second pose shows a Hyde pKi affinity of almost a log unit higher. However, in the second pose, it can be observed that one of the bonds around the amide is coloured yellow. This may suggest that this conformation may have some associated strain. This is something we should be aware of, because if accurate, then it will likely have an impact upon the true binding affinity.

- From the **Binding** menu, deselect **Show Torsion Colours**

It is also important to note that for some compounds, we can see that, despite having a good docking score, the estimated binding affinity is very low (e.g., row 2 in the data set, which has the best docking score).

- Select the compound in **row 2**.
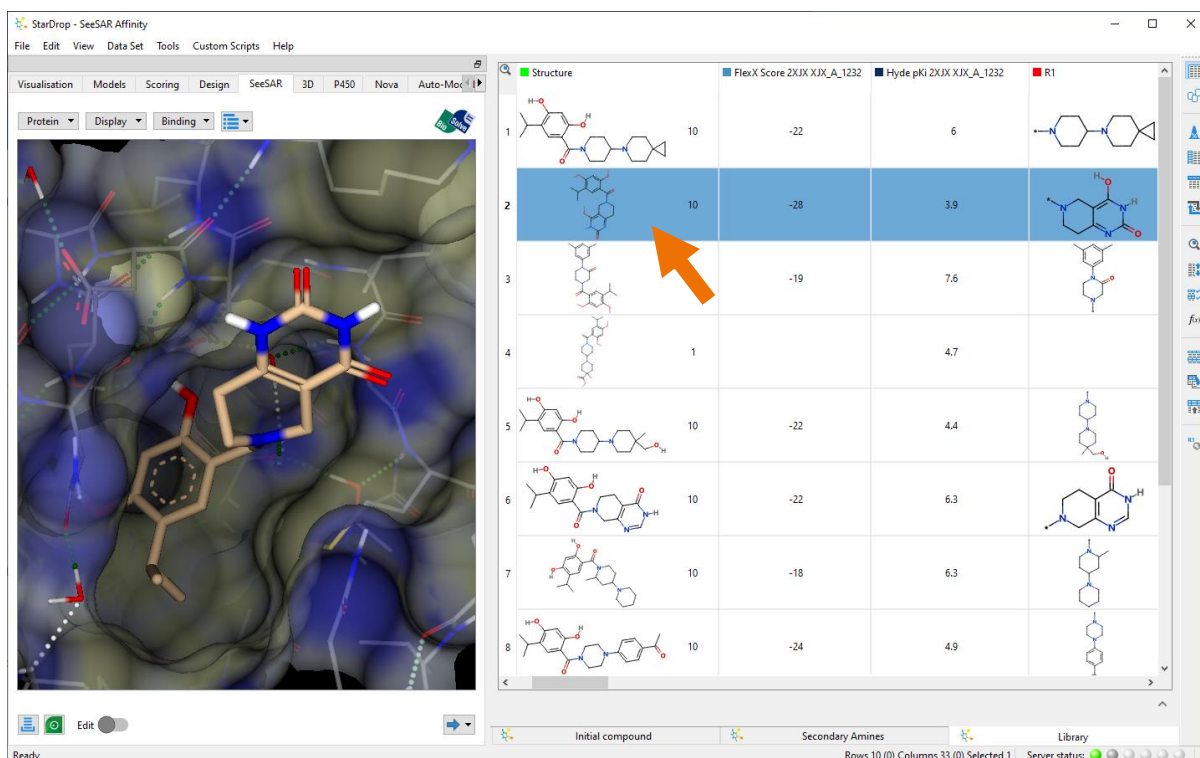
For this compound, we can see that the pose represents an alternative tautomer (compared with the original 2D structure). Some of the other poses for this compound do have better estimated binding affinities (although they are all still quite low).

- Select **pose 4**.

This pose does not have a good docking score but has a better estimated binding affinity (5.4) as compared to the first. If you scroll back to **pose 1**, you will see that it starred ⭐ to indicate that it is the primary pose (corresponding to the value for this pose which is displayed in the data set). We can change the primary pose to be **pose 4** based upon its better estimated binding affinity.
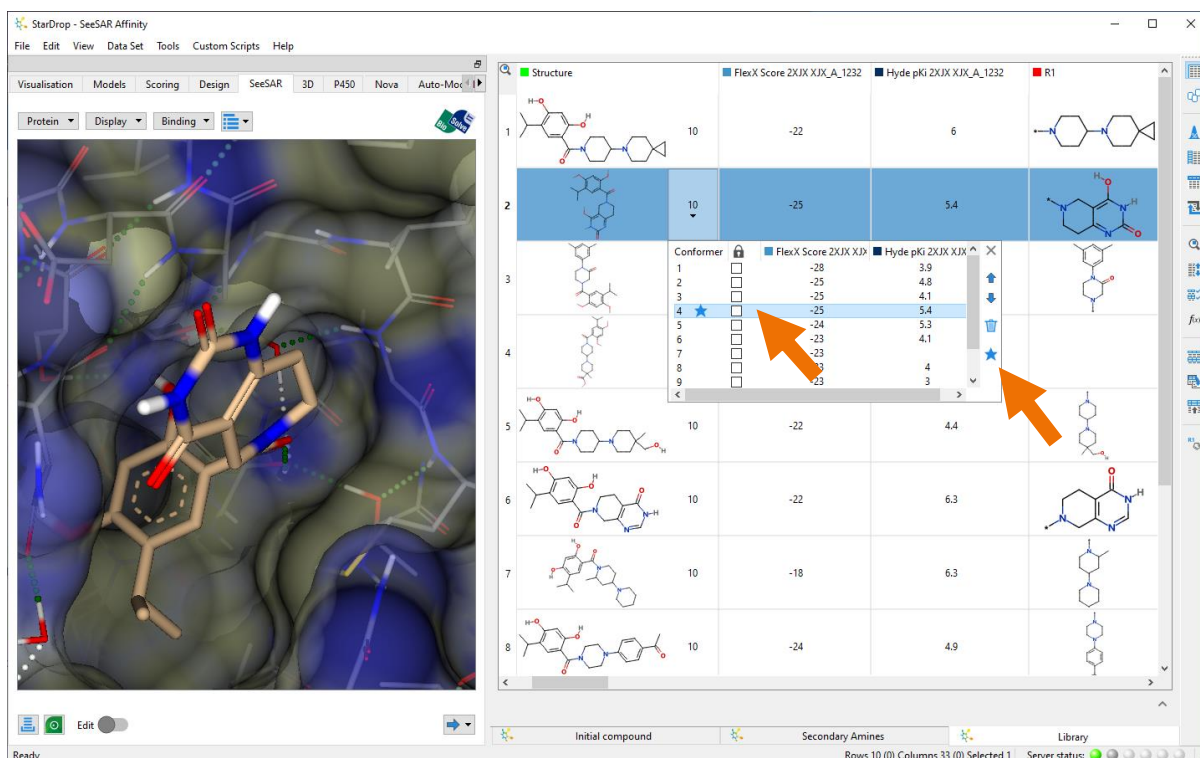
- Click on the ⭐ in the list of conformers to set **pose 4** as the primary pose.

- To understand better why there is a discrepancy between the docking scores and estimated affinities, we can use HYDE coronas to provide an atom-based overview of the affinity.

- From the **Binding** menu, select **Show Hyde Coronas**.
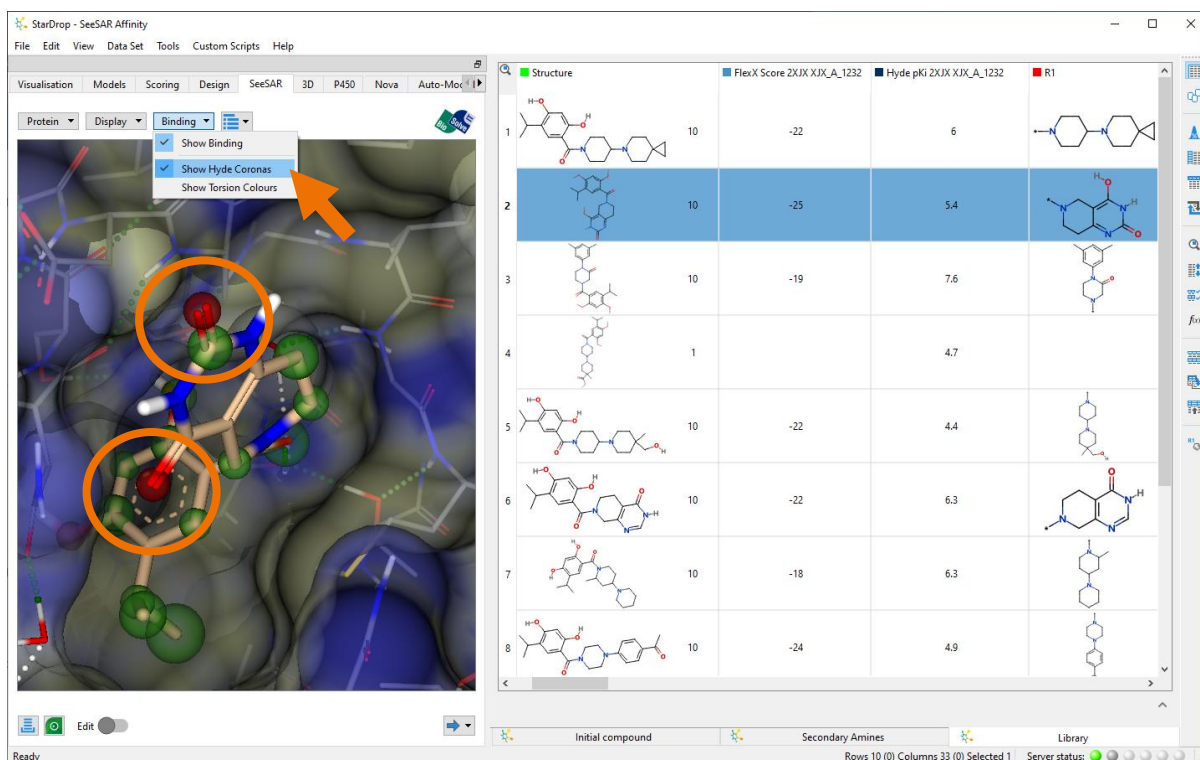
The coronas enable direct visualisation of the HYDE score where:

- A green corona indicates that the atom is making a favourable overall contribution to the HYDE score (estimated binding affinity)

- A red corona indicates an unfavourable contribution

- The magnitude of the corona sphere is proportional to the contribution to the score

- The absence of a corona indicates that the atom is not estimated to have a significant impact on the binding affinity

For this ligand, most of the coronas are green, but the red coronas around the carbonyls particularly stand out. This is because the carbonyls would normally act as hydrogen bond acceptors in solution, but here in this bound conformation, they are near a hydrophobic pocket and show unsatisfied hydrogen bonding. As such, this has a negative impact on the binding affinity of this conformation. We can perform this same analysis for any conformations loaded into StarDrop as a data set. At this stage, the next step we might try would be to modify one or both carbonyls to reduce their negative influence on the binding affinity. Another option would be to look more deeply at compounds that docked with scores comparable to the compound in row 2. Please note, the following steps are optional if you do not have the SeeSAR Pose module available; alternatively, if you have configured the Pose Generation Interface, you could try the same modifications using your own docking software.
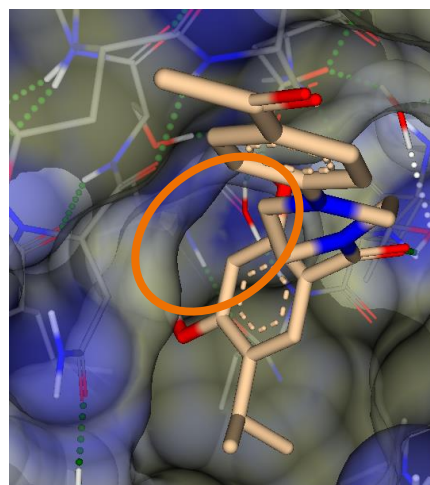
- Select the compound in **row 8**

This compound docked well but has a low HYDE pKi affinity value. From inspection of the HYDE coronas, it can be seen that the ketone oxygen is highlighted with a large red corona, signifying a negative contribution to the binding. However, if other docked poses are examined, a pose can be identified with an pKi affinity of 7.1. It also can be seen that all the coronas are green.

- Click on the arrow [10] next to the compound and then select conformer **pose 6**.

- Click on the ⭐ in the list of conformers to set **pose 6** as the primary pose.
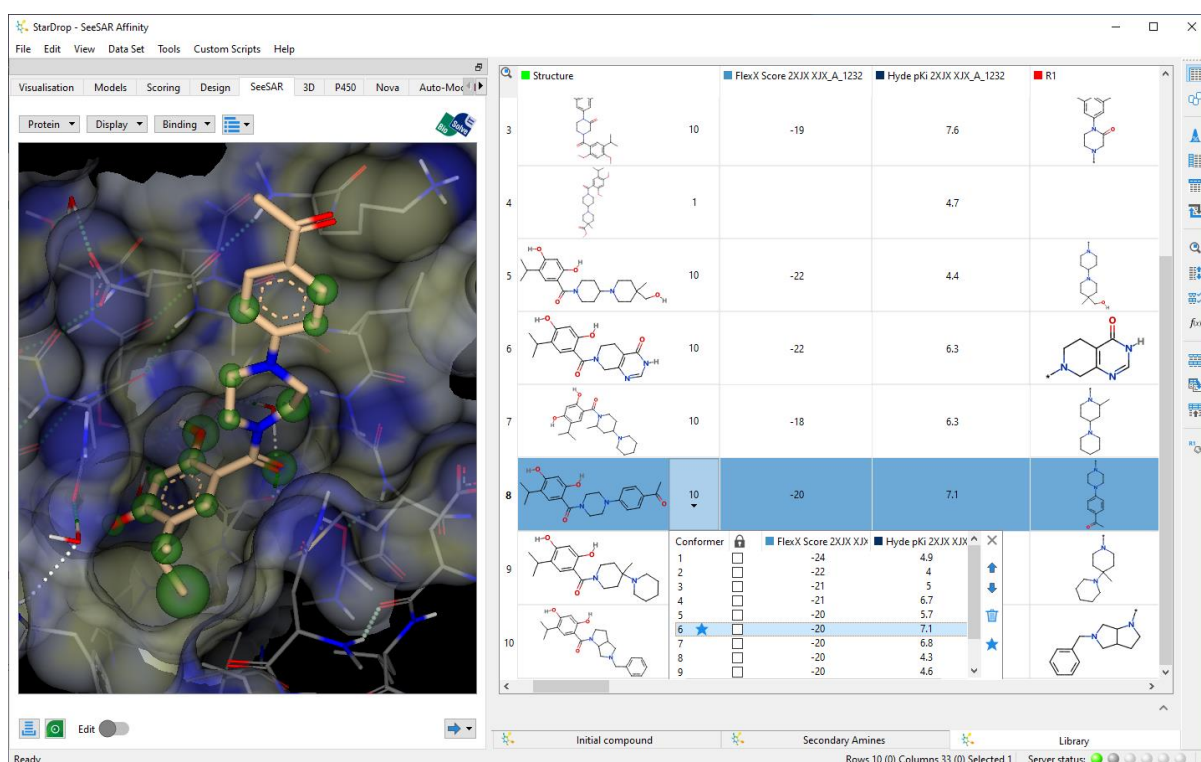
The ketone functional group is not a major contributor to the binding score, so further modifications of this moiety may allow for improvements in binding. Another possibility is to try and exploit a deep hydrophobic pocket that is occupied with a water molecule.

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

- In the sketch area, use the **Bond tool** and add an ethyl group to the resorcinol hydroxyl group that is circled, as shown below.

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. The new compound shows an improved binding affinity of 7.9 as compared to the original docked molecule. To keep this idea for further consideration, we can add it to our data set.

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

- Then close the **Edit** window.

If you are using alternative docking software, then this library can also be evaluated by docking in the HSP90 binding site using StarDrop's Pose Generation Interface to provide seamless integration with docking models from third-party platforms. Whilst this is beyond the scope of this exercise, if you would like to learn more about the Pose Generation Interface, please visit the following link in our online community videos: https://www.optibrium.com/videos/pose-generation-interface/