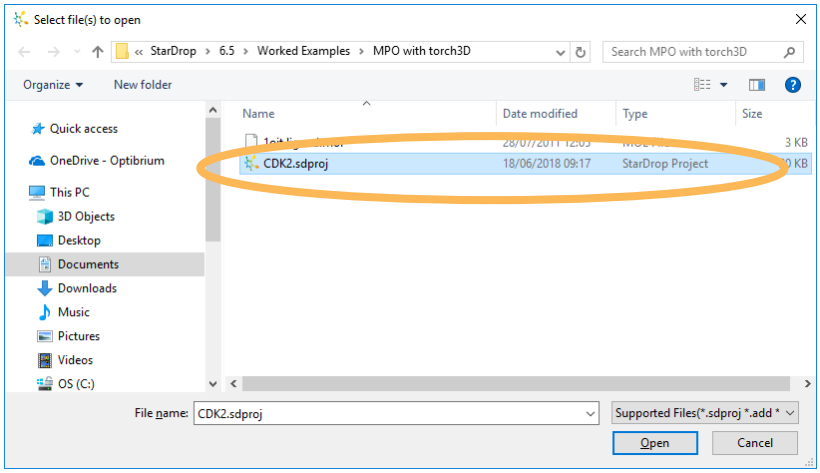
Worked Example:

Multi-Parameter Optimisation of 3D SAR

In this example, we will explore the multi-parameter optimisation of a series of CDK2 inhibitors, combining a 3D insight into the structure-activity-relationship (SAR) gained from StarDrop's torch3D™ module and predictions of ADME and physicochemical properties, using StarDrop's unique Probabilistic Scoring approach.

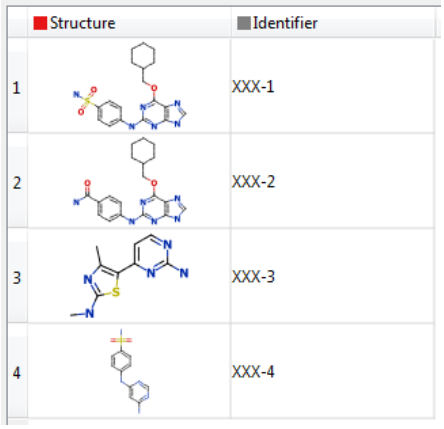
Exercise

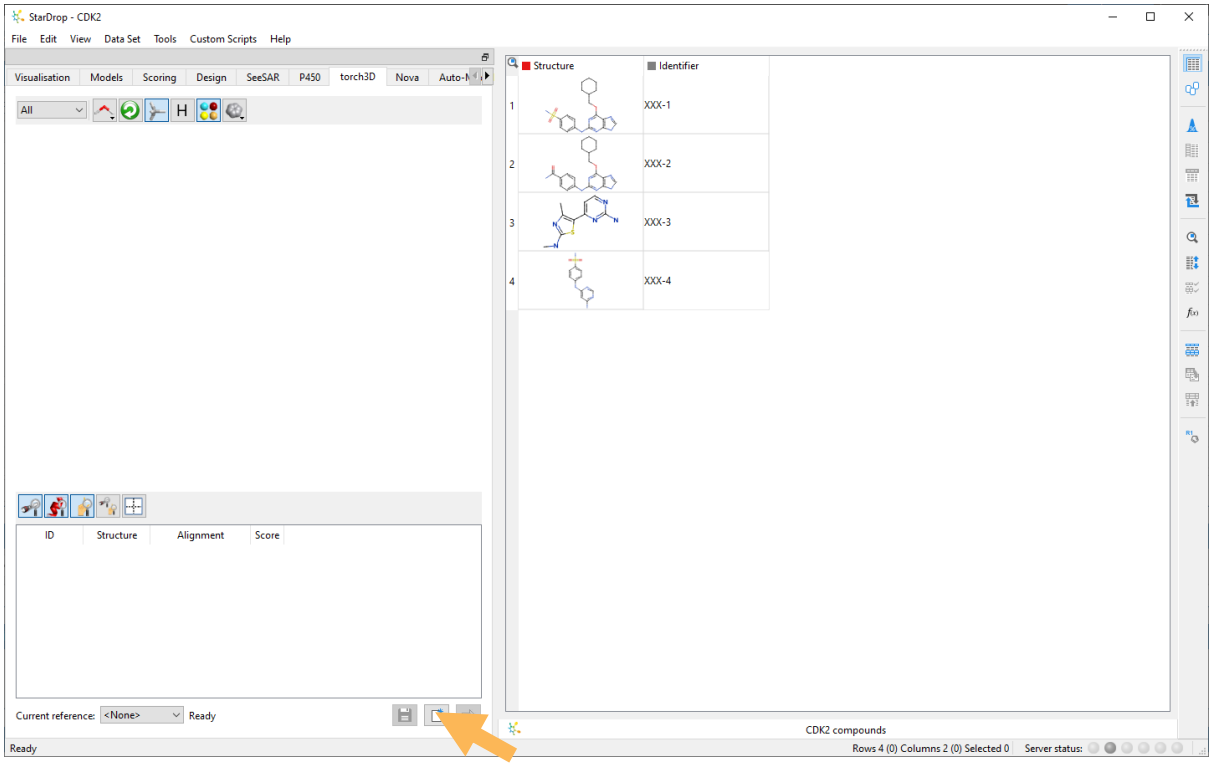
- In StarDrop, open the project **CDK2.sdproj** by selecting **Open** from the **File** menu.



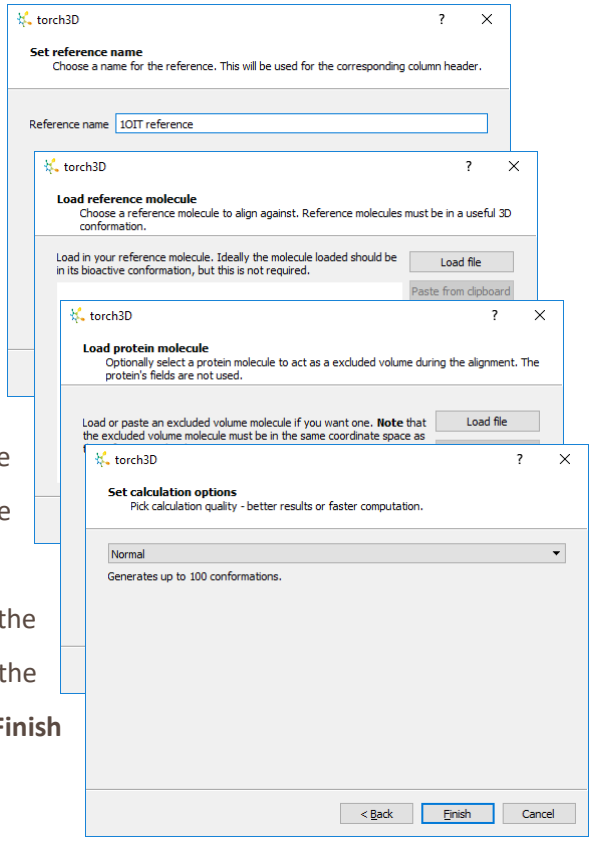
This will open a small data set containing four compounds which are active against the CDK2 target.

We're going to explore the 3D SAR of these compounds by comparing them to a reference compound with a known bioactive conformation in CDK2, using the torch3D™ module developed in collaboration with Cresset.

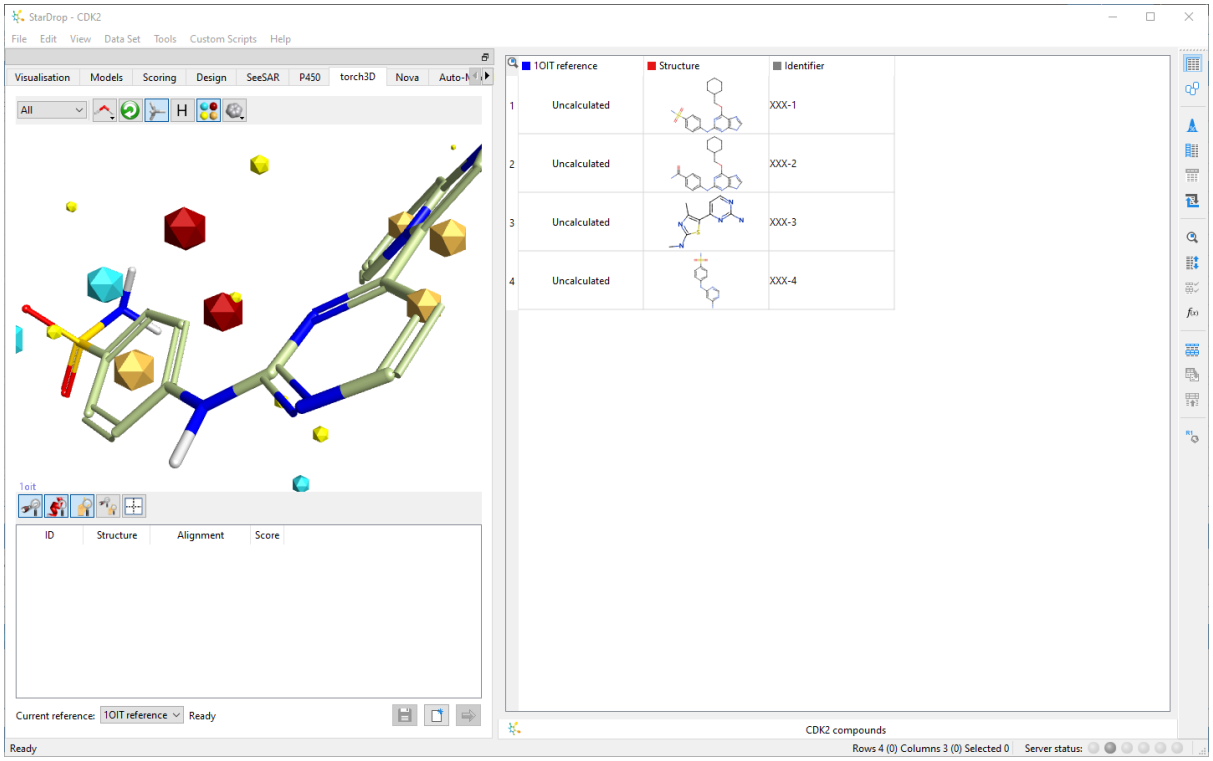




- At the bottom of the torch3D area, click the **New reference** button  to start the torch3D wizard and define a reference against which new compounds will be compared.
- On the first page of the wizard, enter the name "**1OIT reference**" (Please use *exactly* this name because it will be used later).
- Click the **Next** button and then load the reference molecule by clicking the **Load file** button and selecting the file **1oit ligand.mol**.
- Click the **Next** button to (optionally) load a protein structure that can be used to define an excluded volume - in this example, we shall omit this step.
- Click **Next** to specify the speed of the calculations – in this case, we will use the default **Normal** setting and then click the **Finish** button.

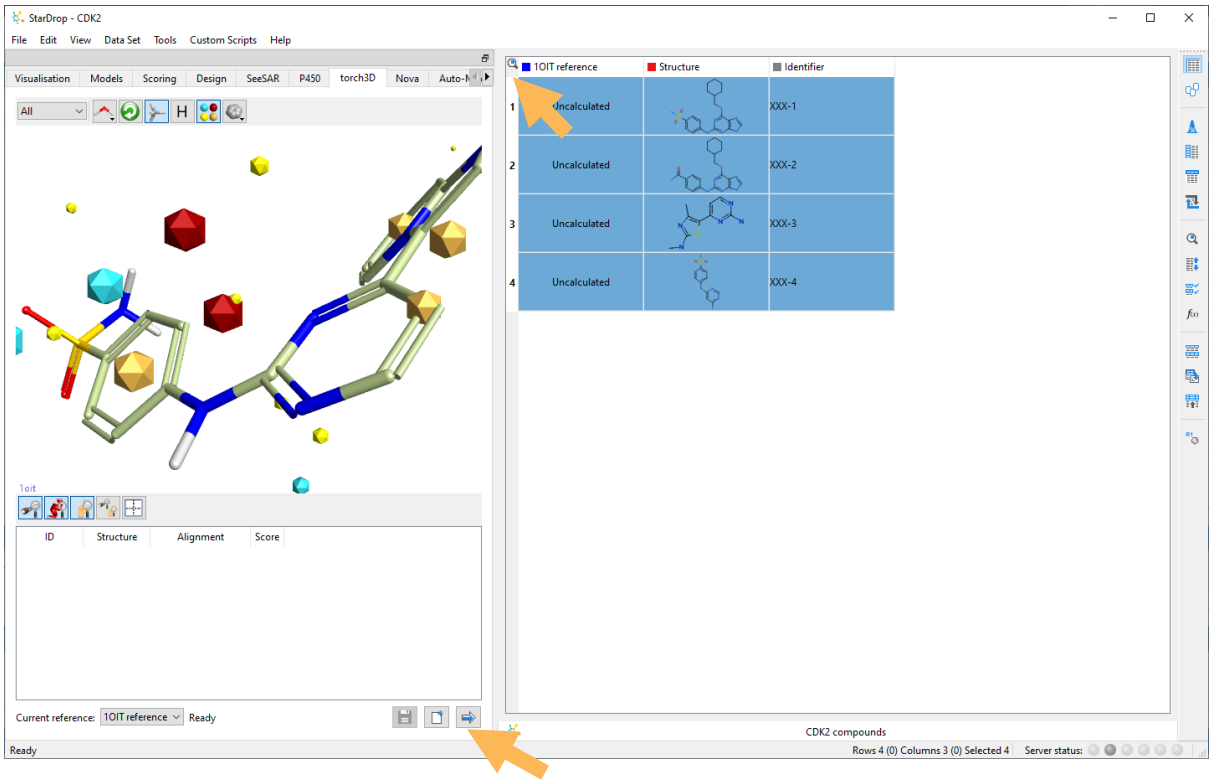


The reference molecule will be displayed, and a new column will be added to the data set.



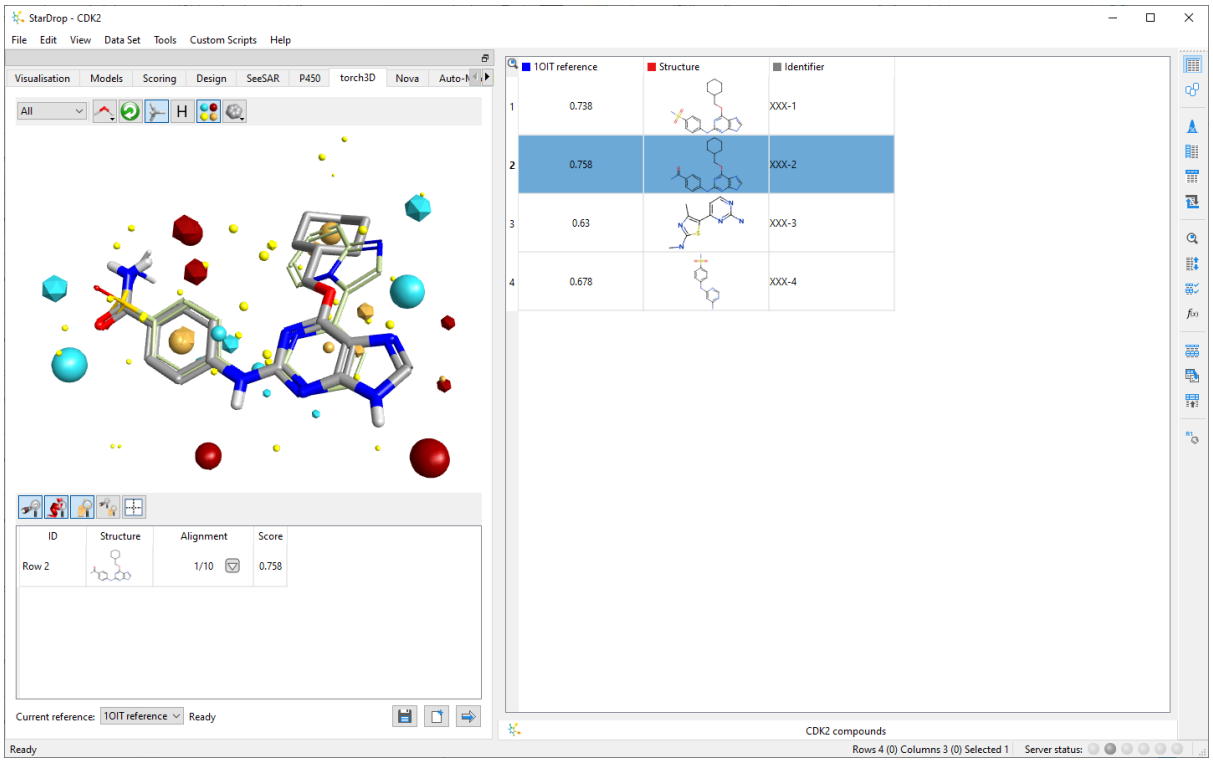
Now the reference has been set up, we can compare the compounds in the data set with this, based on the pattern of fields generated by the structures as well as the shape.

- Select all the rows in the data set (**Hint**: click the top-left corner of the data set, just below the button) and then click the  button to start the calculations.



- Once the FieldScores have been calculated for all the compounds, select row 2.

- Use the mouse to zoom into and rotate the 3D molecules to see how their fields compare.



In torch3D larger field points represent stronger points of potential interaction and the field points are coloured as follows:

- Blue: Negative field points (like to interact with positives/H-bond donors on a protein)
- Red: Positive field points (like to interact with negatives/H-bond acceptors on a protein)
- Yellow: van der Waals surface field points (describing possible surface/vdW interactions)
- Gold/Orange: Hydrophobic field points (describe regions with high polarisability/hydrophobicity)

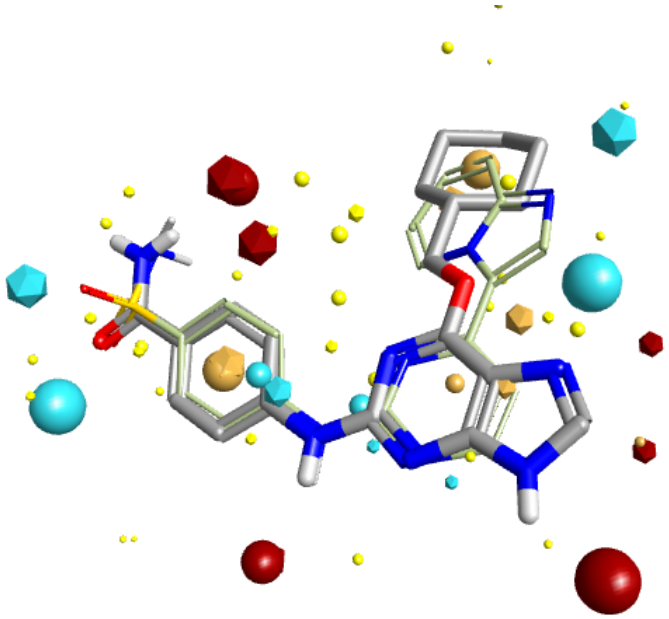
For further details about the interpretation of the field points, please see Section 12.3 of the StarDrop Reference Guide, which can be accessed from the **Help** menu in StarDrop.

The FieldScore (a value between 0 and 1, where scores closest to 1 are the best match) is calculated by considering how similar the fields around the aligned conformation are to those of the reference compound. The best score is shown, but the scores for the next best conformations (10 in total) are available, enabling you to view other possibilities. Using the up and down arrows in the table below the 3D window, you can choose which conformation is displayed.

Note: The score is an important factor in deciding the validity and potential activity of particular alignments and molecules. However, it is not the only factor to be considered before embarking on the synthesis of a compound designed in torch3D. The top-scoring result is the one that is the most

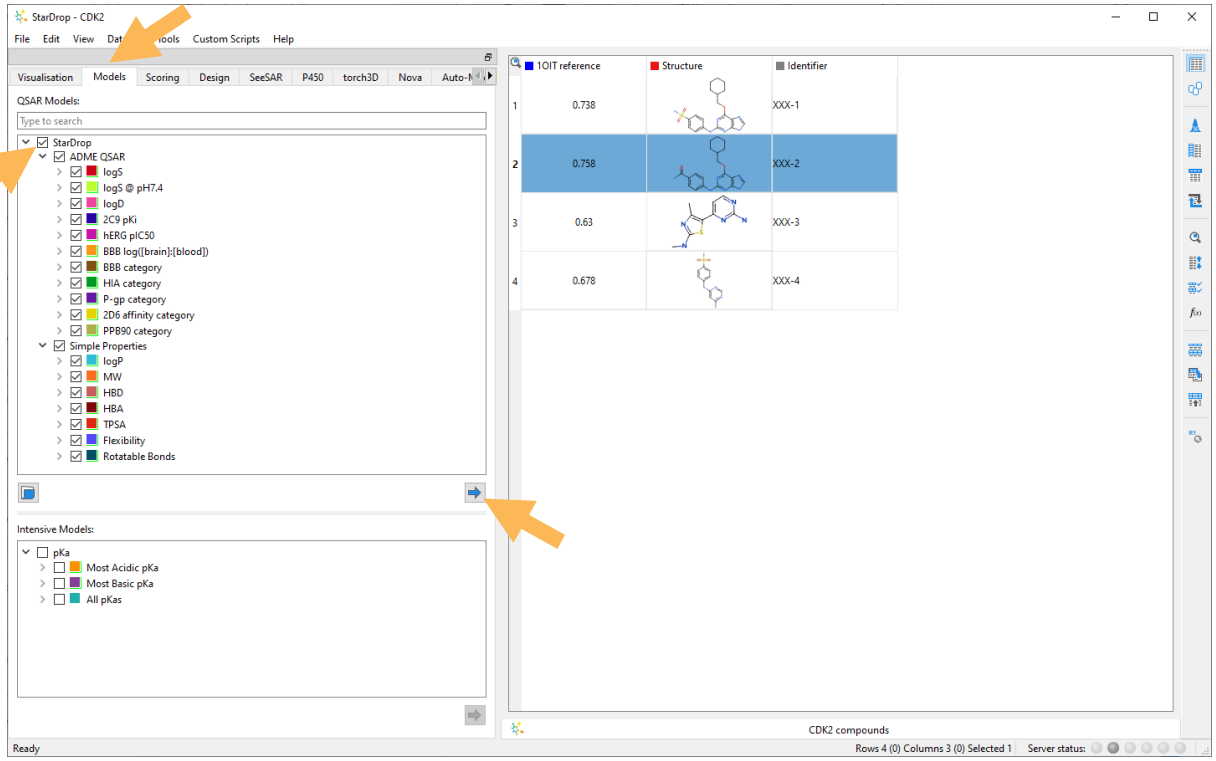
similar to the target molecule in terms of fields and shape. That doesn't necessarily mean that it is the most likely to be active or that it's the one you should make first.

Explore the different compounds in the data set and note that, despite the difference in the chemical series, there is a strong similarity in the alignment of their fields and their shape, explaining their similar bioactivity. See, for example, compound **XXX-2** below, overlaid on the reference.



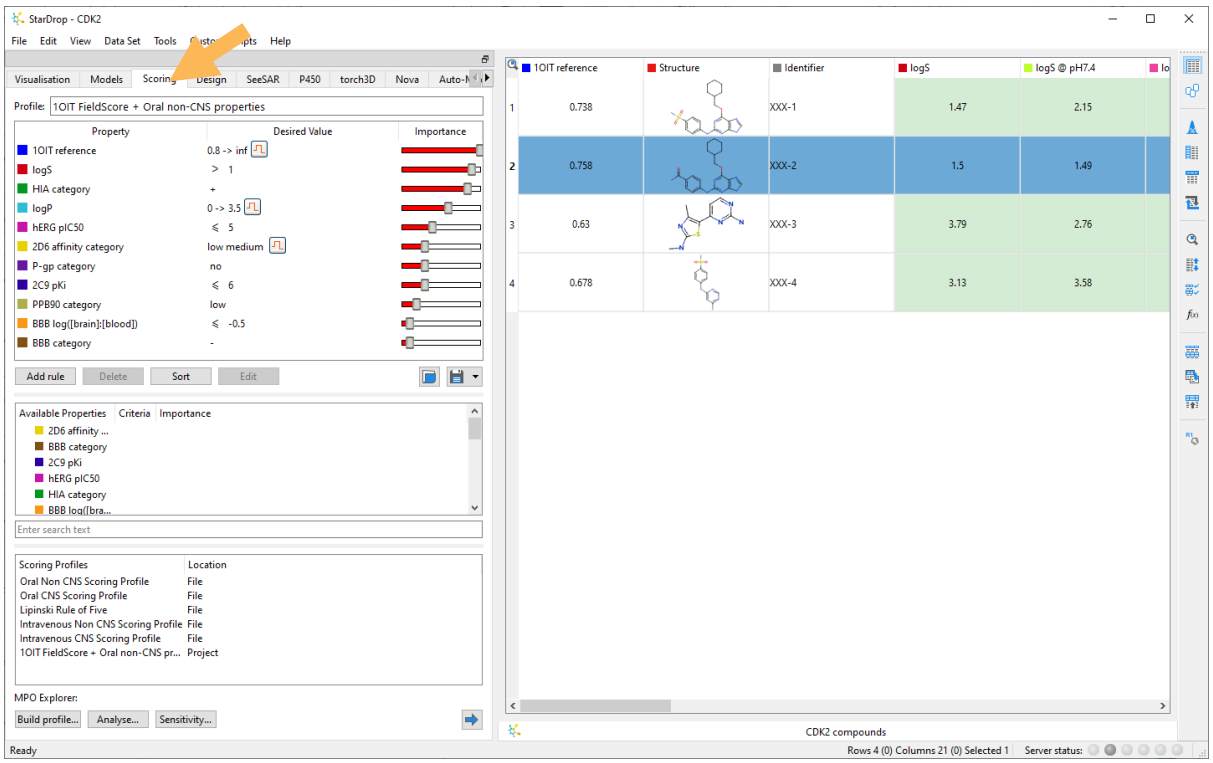
This can provide us with valuable information to guide the design of novel compounds with improved potency. However, potency is not the only factor to consider when optimising compounds, so to help us to design compounds with an optimal *balance* of properties, we will also make predictions of relevant ADME and physicochemical properties using StarDrop's ADME QSAR module.

- Change to the **Models** area.
- In the list of **QSAR Models**, check the box next to **StarDrop** to select all of StarDrop's ADME QSAR models and click the  button to calculate the selected properties for all of the compounds in the data set.



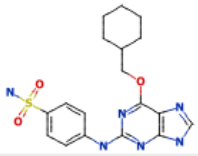
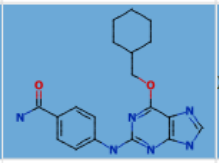
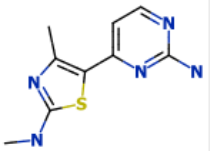
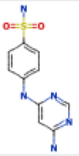
Now we have a lot of data for each of the compounds; too much to easily keep track of all of the properties while designing new compounds. Therefore, we'll use StarDrop's Probabilistic Scoring approach to multi-parameter optimisation to assess each compound's properties against the overall profile required by the project. We can then use this score to track our progress as we attempt to design compounds with an improved *balance* of properties.

- Change to the **Scoring** area.



This project already contains a scoring profile of property criteria that have been defined by the project team; in this case, the team would like a compound with a high FieldScore and appropriate properties for an orally dosed compound against a CNS target. The importance of each criterion has also been specified, enabling acceptable compromises to be defined.

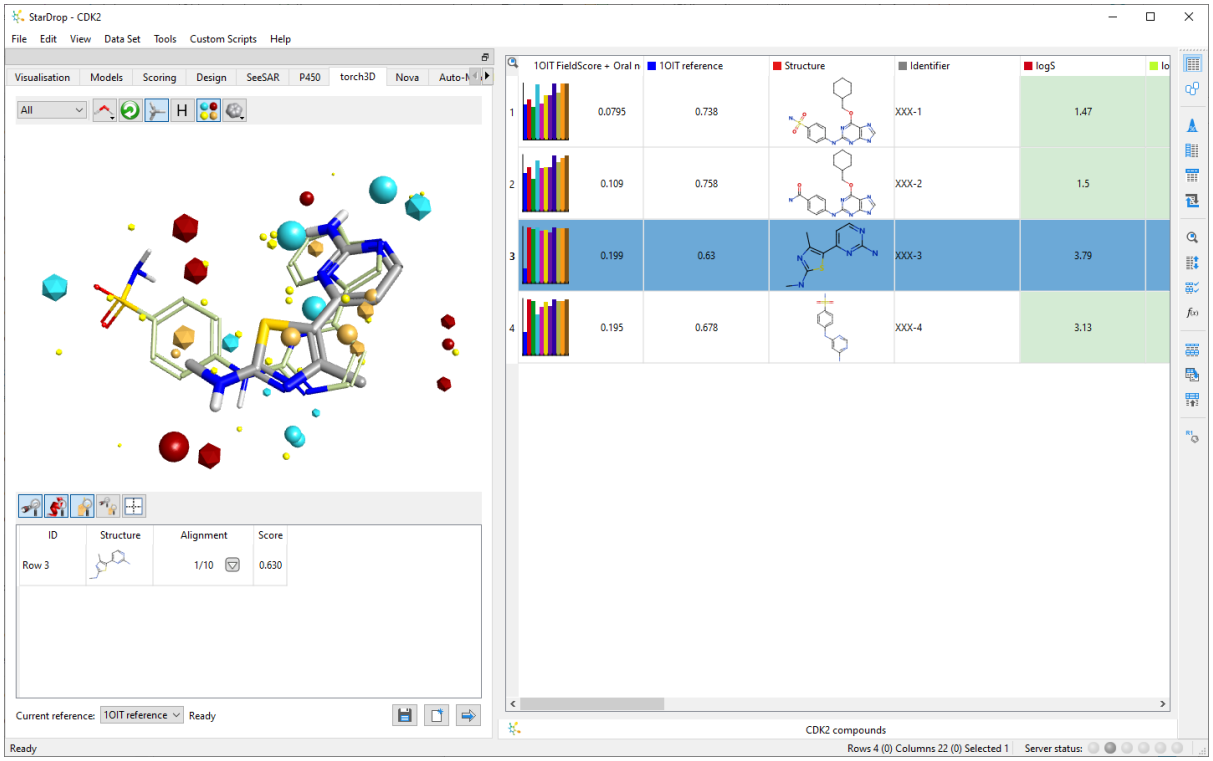
- Click the  button to generate a score for each compound against this profile.

	1OIT FieldScore + Oral non-C.	1OIT reference	Structure	Identifier
1	0.0795	0.738		XXX-1
2	0.109	0.758		XXX-2
3	0.199	0.63		XXX-3
4	0.195	0.678		XXX-4

The score is in the range of 0 to 1 (the higher, the better) and represents the likelihood of success of each compound against the overall profile of property requirements, taking into account not only the property values but also the uncertainty in each prediction or measurement. The histogram shows the impact of each individual property on the overall score; the colours correspond to the key in the scoring profile.

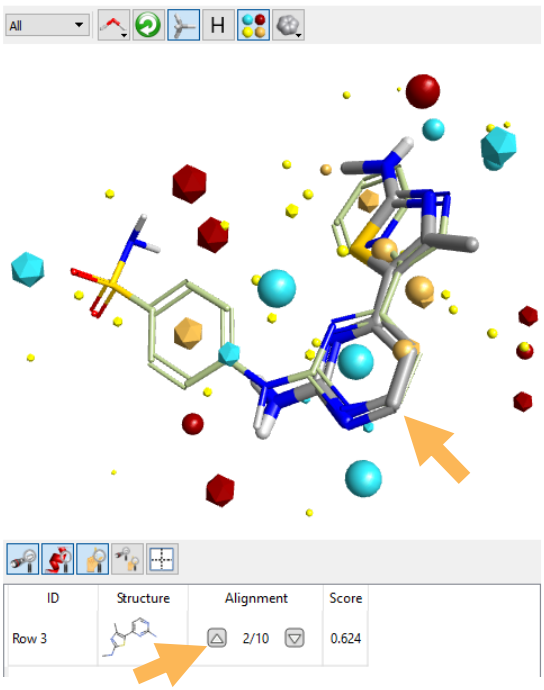
From the scores, we can see that the highest-scoring compounds achieve a score of approximately 0.2. Given the uncertainty in the predicted data, this is quite good, but ideally, we would like to try to find even better compounds to synthesise and test, so let's explore the multi-dimensional optimisation of these compounds.

- Return to the **torch3D** area in StarDrop and select row three in the data set which contains the compound **XXX-3**



Despite its small size, this fragment achieves a reasonably good FieldScore. The best alignment identified by torch3D is shown by default, but torch3D compares many, energy-minimised conformations with the reference and different alignments with good FieldScores may suggest possible alternative binding modes and indicate opportunities for further optimisation. As an example, we will explore alternative alignments of compound **XXX-3**.

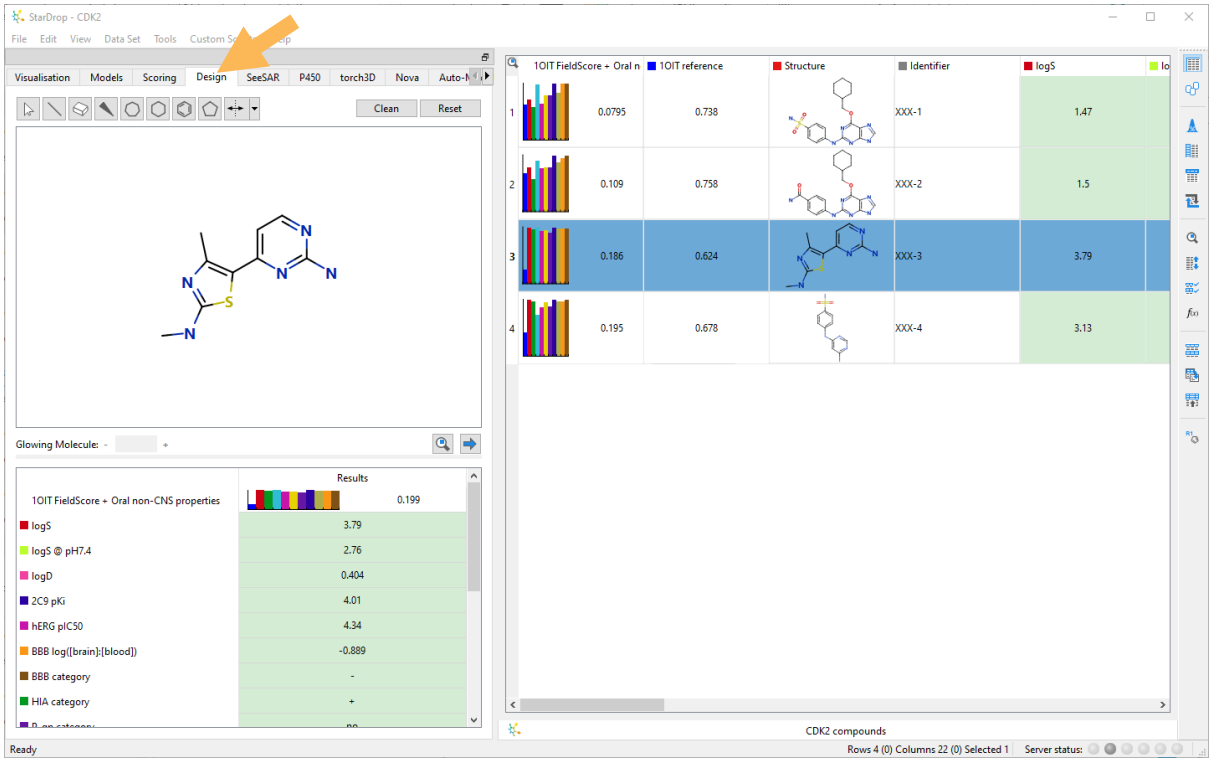
- Examine the alternative alignments by clicking the ▽ or △ buttons in the **Alignment** column in the table under the 3D visualisation.



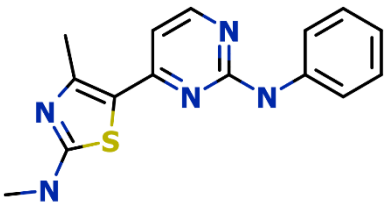
It is notable that in some alternative conformations generated by torch3D, there is a good match between the piperidine rings. In the second conformation, such an alignment is observed, and the fragment has a similar FieldScore to the best alignment. Comparing this with the bioactive reference compound, it would appear that there is potential to extend this fragment and form additional interactions.

To explore the effect of further variations, we're going to use StarDrop's interactive designer.

- Change to the **Design** area. The selected compound **XXX-3** will appear in the designer, ready to modify.



- Modify the structure to be the one shown below using the chemical structure editor.

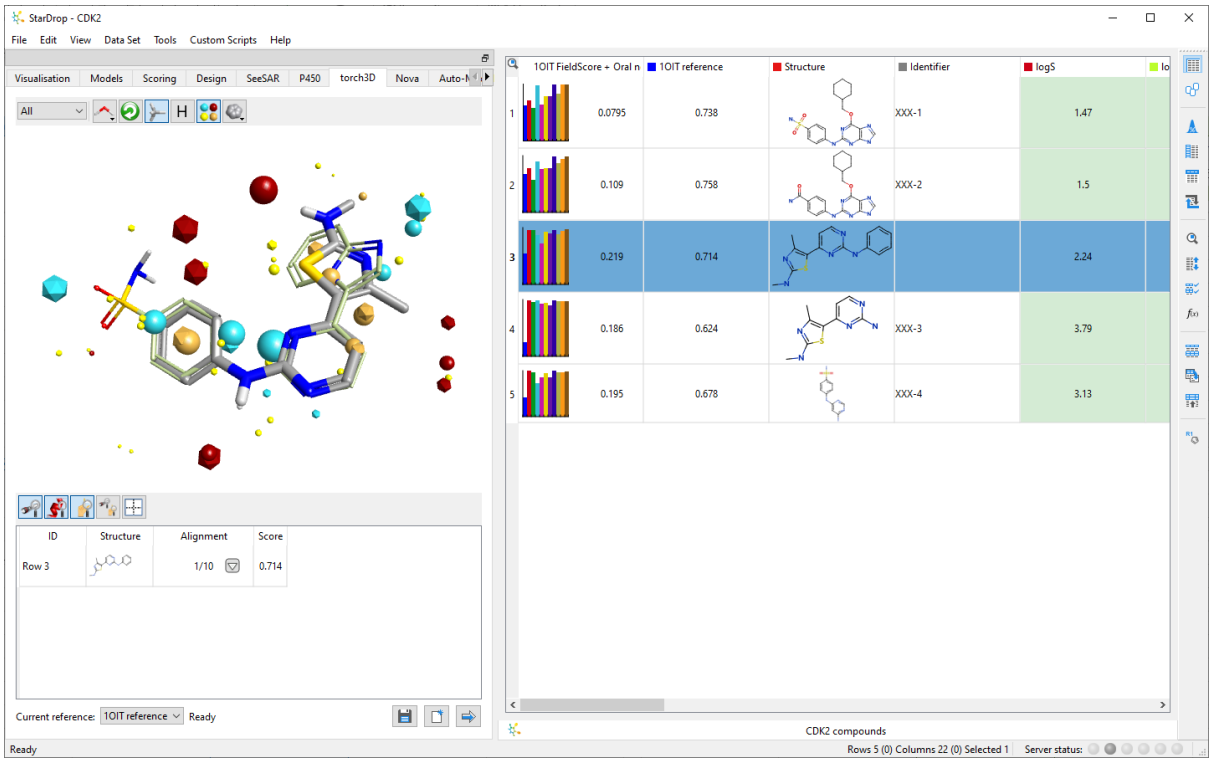


Hints:

- The **Bond** tool  enables you to add atoms and bonds.
- To change the element of an atom, simply point at the atom and type the symbol. To change from one heteroatom to another, select the atom, delete the symbol and type the new symbol.
- The Template tool  enables you to add a benzene ring. To add a ring, position the ring over an atom and click to fuse it onto the molecule (a blue circle will appear).

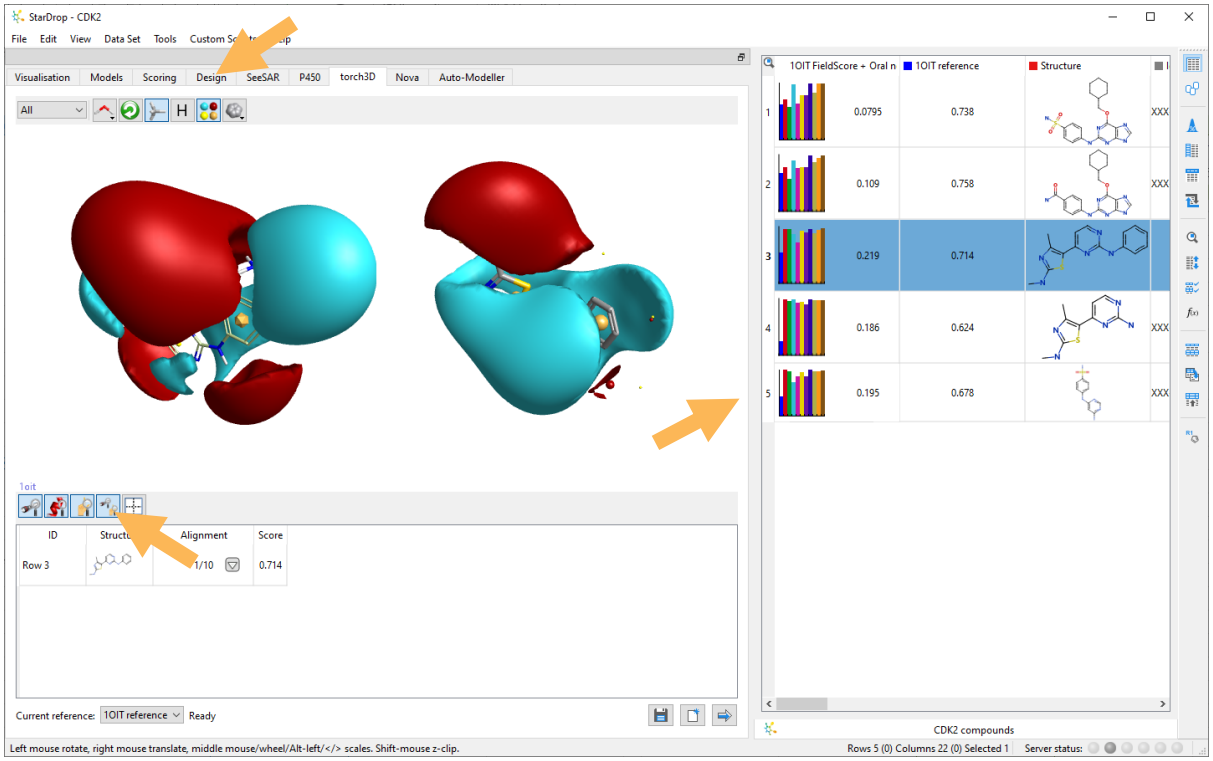
You will notice that the results for the QSAR models below the editor update as you edit the structure. However, the FieldScore calculation takes too long to provide an instant update and so isn't displayed in the list. To calculate the FieldScore, we'll add the new compound to the data set.

- Click the  button in the **Design** area to add the structure to the data set.
- Return to the **torch3D** area and click the  button to calculate the FieldScore for this new compound.



This has resulted in an increase in the FieldScore and the overall score, however, the introduction of the phenyl group has resulted in increased logP and hERG affinity.

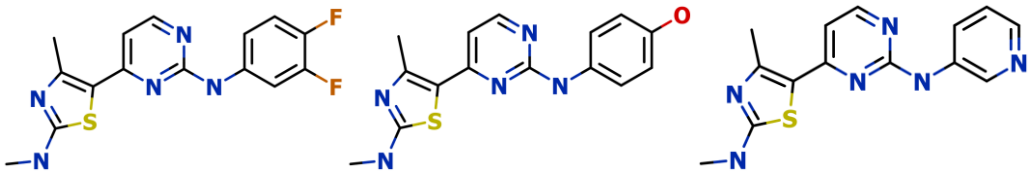
- In the **torch3D** area, click the  button to separate the reference from the aligned compound and click the  button and turn on both the positive and negative fields.



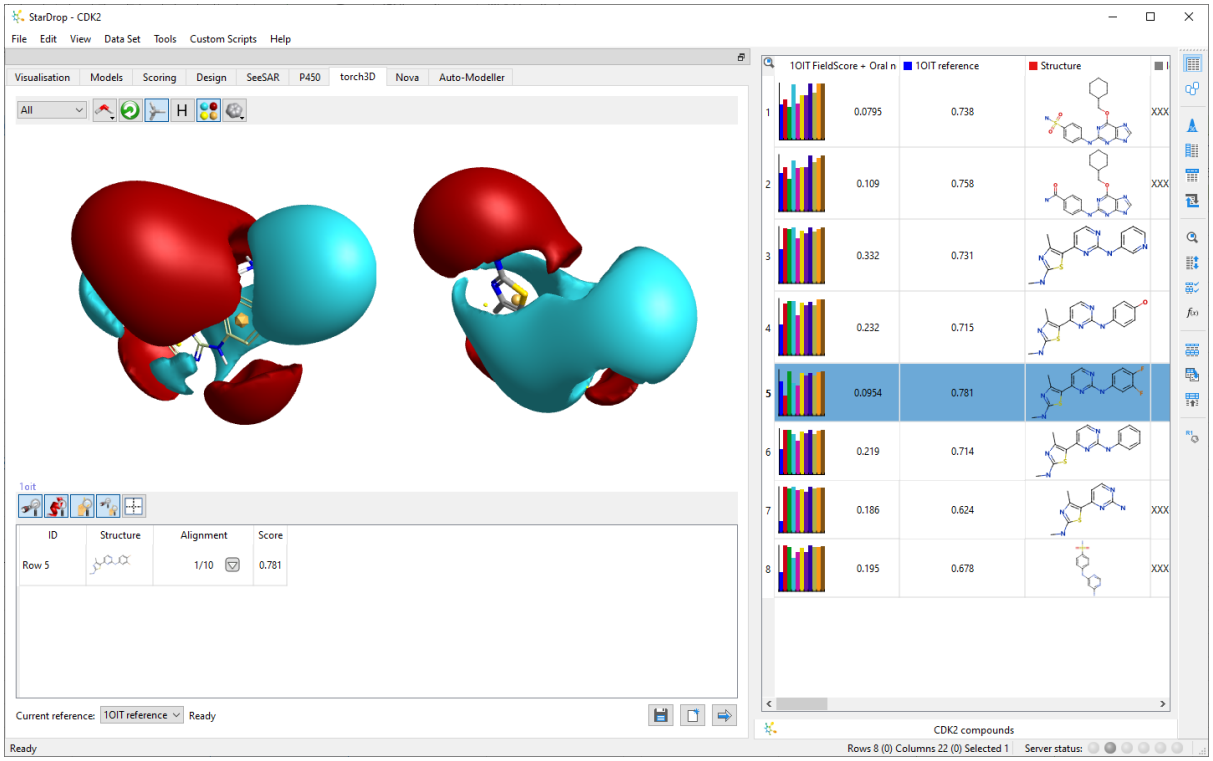
Note: you can drag the spacer between the data set and the torch3D area to make more space.

There may be an opportunity for further optimisation; note the strong fields around the sulphonamide on the reference where the phenyl group has no strong pattern at all. One approach to reproducing this field pattern would be by substituting a difluorobenzene, phenol or pyridine, so we are going to explore these different substitutions to try and improve the overall score:

- Return to the **Design** area and add these structures below to the data set.



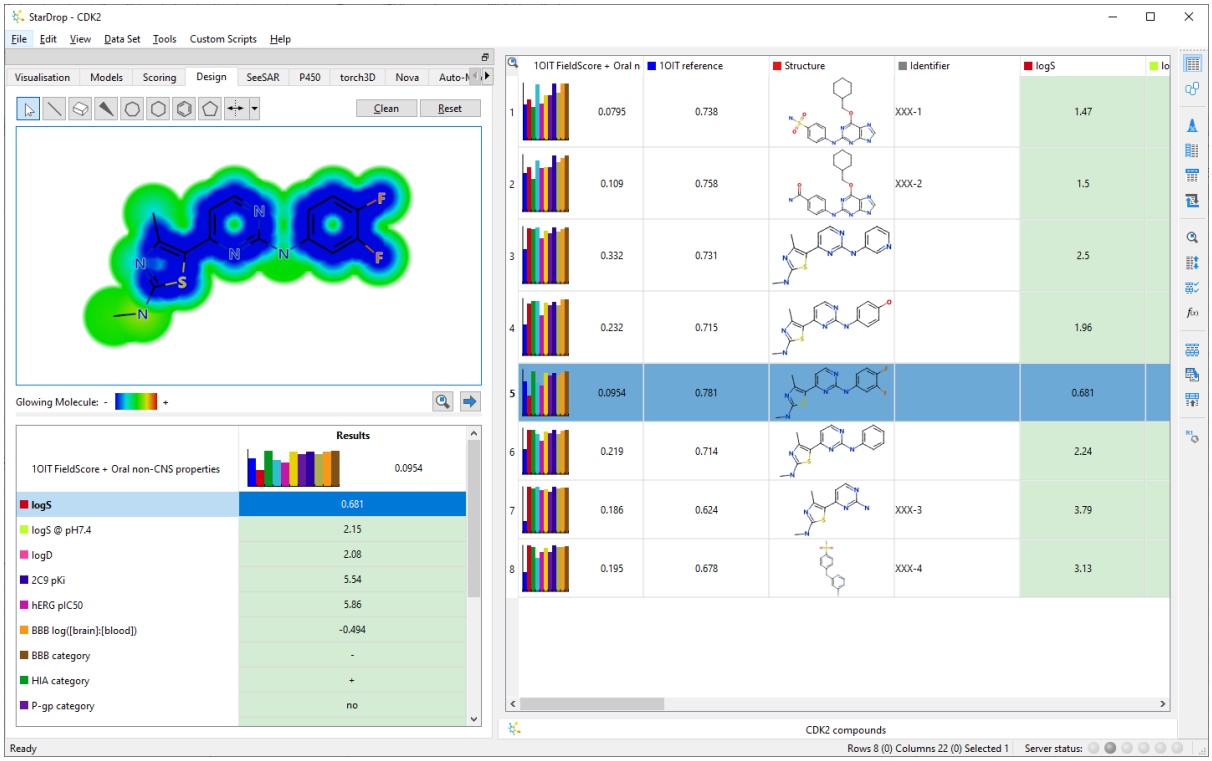
- In the **torch3D** area, calculate the FieldScore for these compounds, and the overall scores will be updated.



Here you can see that the substitution has had the desired effect on the FieldScore. The image above shows the fields for the difluorobenzene and clicking on the pyridine and phenol derivatives you can see similar patterns. For the pyridine the overall effect is very positive because a number of important properties, such as solubility and logP have also improved; however, for the difuorobenzene the overall score is now much lower. Despite having a very positive effect on the FieldScore, this has been at the cost of significantly reducing the solubility, suggesting that this is unlikely to yield a high-quality compound.

You can see the impact of the Fluorine substitutions on the predicted solubility using StarDrop's Glowing Molecule™.

- Change to the **Design** area, select the row containing the difluorobenzene derivative and click on the logS value below the editor to see the Glowing Molecule for this compound.



The blue colour around the Fluorines indicates their significant negative effect on the predicted solubility.

- Using torch3D, the ADME QSAR modules and probabilistic scoring, explore different substitutions to see how high a score you can achieve by simultaneously improving the FieldScore, ADME and physicochemical properties. Looking at the other compounds in the data set may provide some useful suggestions.

This example has used some of StarDrop's capabilities to explore the multi-parameter optimisation of a series of compounds; in particular probabilistic scoring, interactive design and Glowing Molecule along with the optional torch3D and ADME QSAR modules.