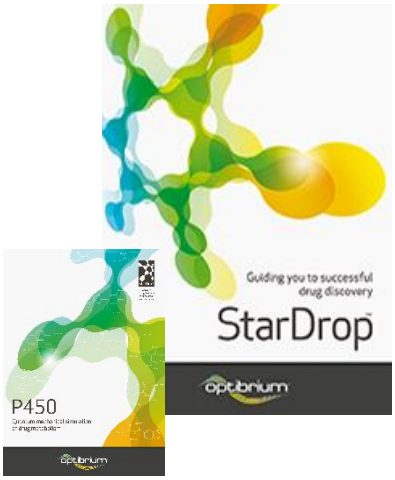
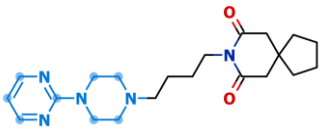


Worked Example:

Fast Follower: Optimising P450 Metabolic Stability

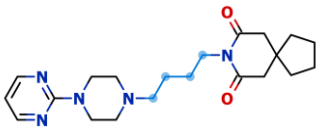
In this example, we will explore the feasibility of pursuing a fast-follower for Buspirone, a 5-HT_{1A} ligand used as an anti-anxiolytic therapeutic. Buspirone has a known liability due to rapid metabolism by CYP3A4, leading to low oral bioavailability and a short half-life in man. The project wished to efficiently identify analogues of Buspirone with an *in vitro* CYP3A4 half-life 3-times longer than Buspirone and a minimum loss of receptor affinity.

The structure of Buspirone can be broken down into three regions:



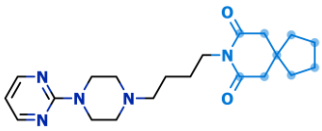
Arylpiperazine

- Protonatable recognition element, receptor affinity
- Metabolism: Hydroxylation at pyrimidine C5



Tetramethylene linker

- Metabolism: N-dealkylation α to piperazine N4



Piperidinedione moiety

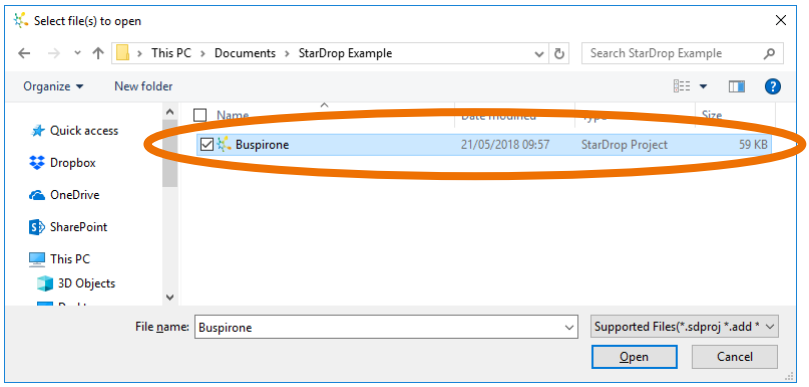
- Metabolism: oxidation of spirocyclopentane ring

Our objective is to identify structural modifications that reduce the vulnerability of key sites of metabolism, as indicated by decreasing the **site lability,** and identify molecules that are likely to meet the project goal of increased half-life with respect to metabolism by CYP3A4 by reducing the **composite site lability** (CSL).

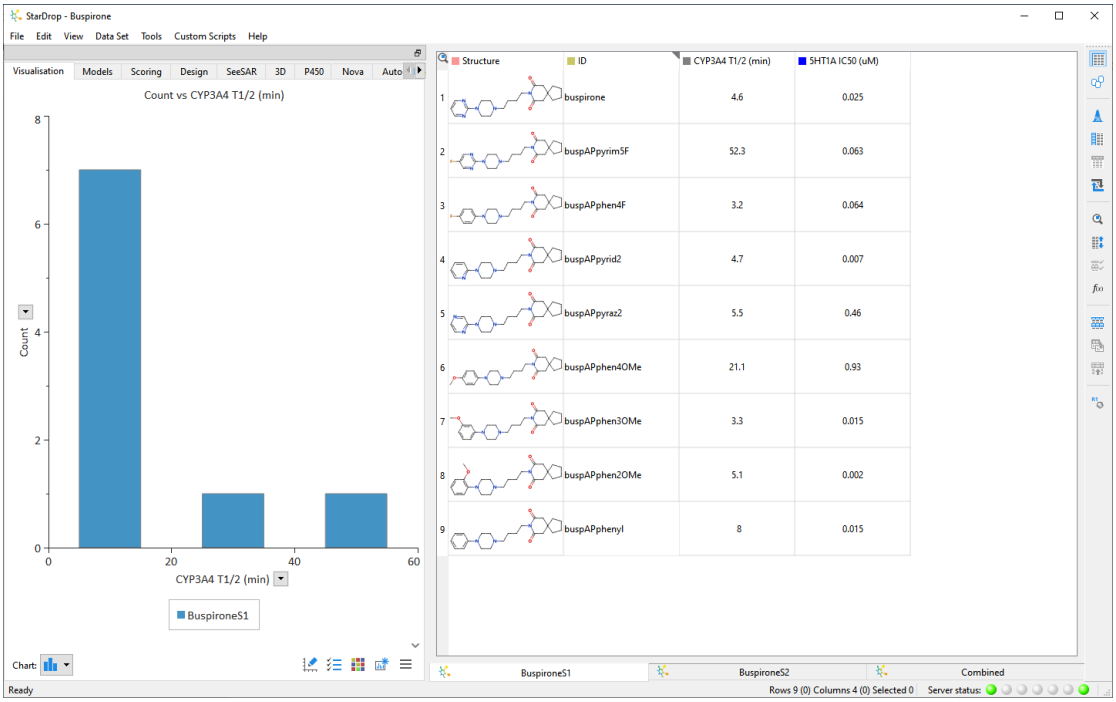
We will investigate modifications to the different regions of Buspirone identified above using two different series: Series 1 will explore alternative aryl substitutions on the piperazine; Series 2 will explore modifications to the tetramethylene linker and piperidinedione moiety. Finally, we will explore the impact of combinations of the most promising modifications.

Exercise

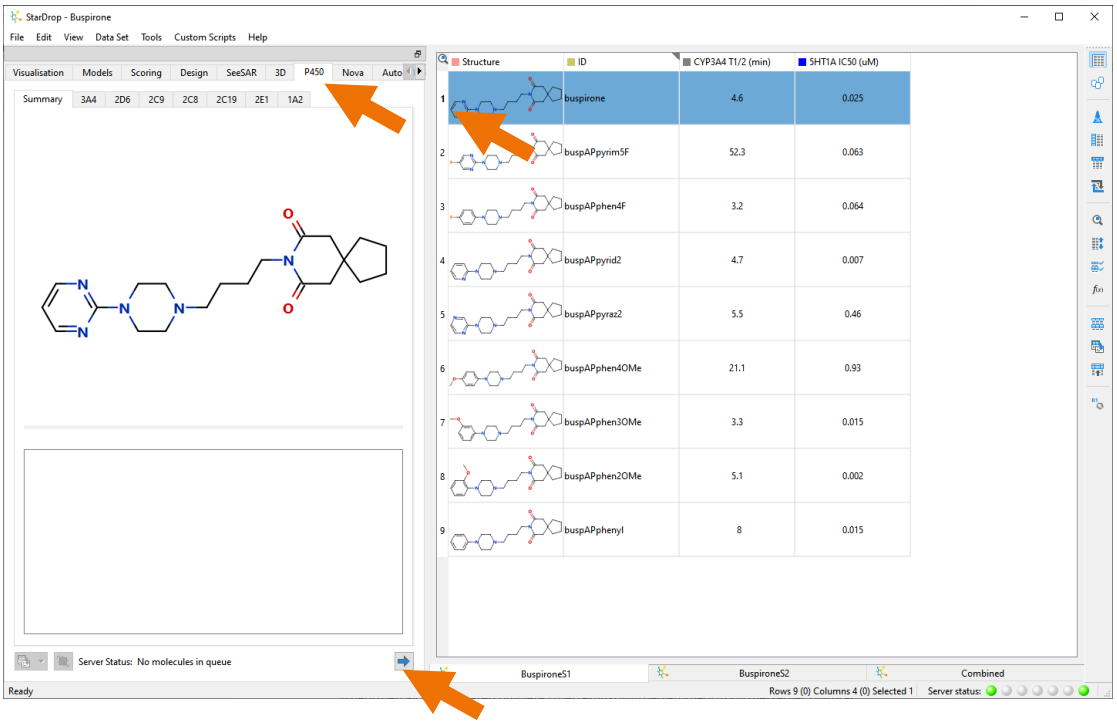
- In StarDrop, open the StarDrop project file **Buspirone.sdproj** by selecting **Open** from **File menu**.



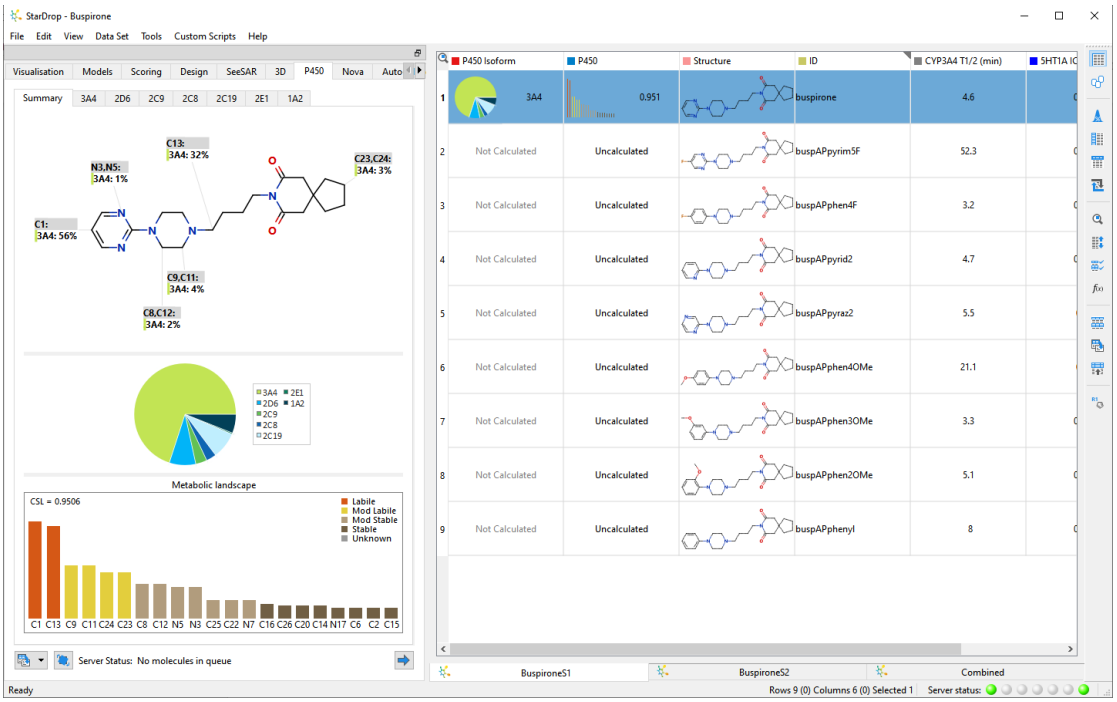
- The first data set displayed, **BuspironeS1**, contains 9 compounds, the first of which is Buspirone. For each compound, its structure, identifier, and experimental measurements of half-life with respect to metabolism by CYP3A4 and IC_{50} against $5HT_{1A}$ are shown.



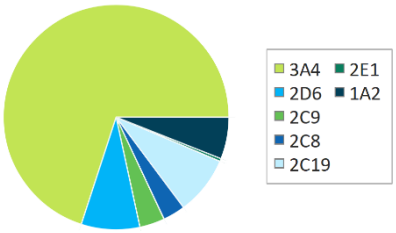
- Change to the **P450** area, select the first compound in the table, Buspirone, and submit this compound to the P450 models by clicking on the  button.



When the calculations are complete, the results will be returned from the server, with a summary displayed in the table and in the P450 area (**Hint:** the regions of the P450 area can be resized to enlarge the regioselectivity, WhichP450™ and site lability views). The calculation will take roughly 1-2 minutes for a compound; however, if the results for a compound have previously been calculated on your server, the results will be returned instantly.

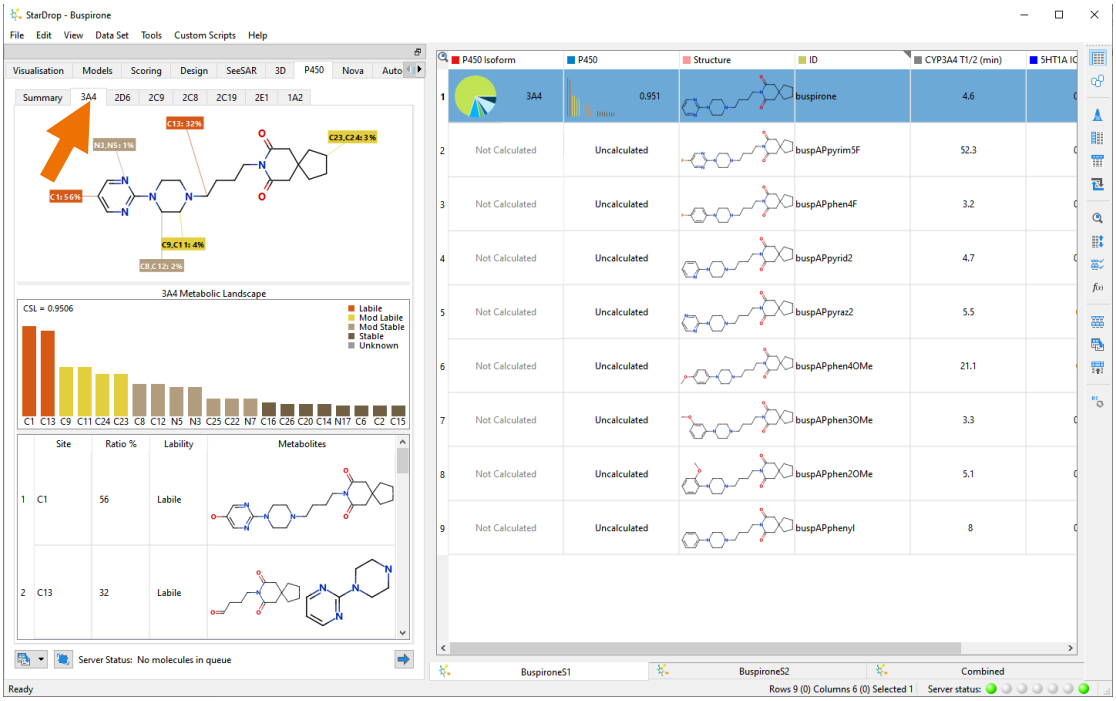


The result from the WhichP450 model, shown by the pie chart, indicates that Buspirone is most likely to be metabolised by CYP3A4, in agreement with its known primary route of metabolism.



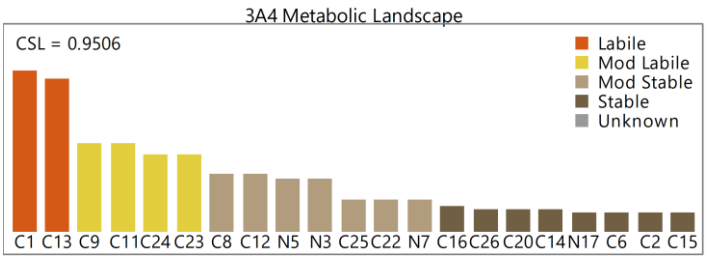
Above this is a summary of the predicted regioselectivity of metabolism by the major isoforms predicted to contribute significantly, in this case only CYP3A4.

- Click on the **3A4** tab within the P450 area to focus on the predicted metabolism by this isoform.



Here we can see good agreement between the predictions from the model and the known metabolism of Buspirone by CYP3A4. The predominant sites of metabolism are predicted to be at the para position of the pyrimidine (C1) and the carbon in the tetramethylene linker α to the piperazine nitrogen (C13).

The **Metabolic Landscape** indicates the lability of each site with respect to metabolism by CYP3A4 in absolute terms to guide the optimisation of compounds with improved metabolic stability.



This indicates that, although the proportion of metabolism on the spirocyclopentane moiety (C23, C24) is predicted to be small, this is predicted to be a moderately labile site, again in agreement with the observation of metabolism at this position. C9 and C11 on the piperazine are also predicted to be moderately labile, although these are not observed experimentally.

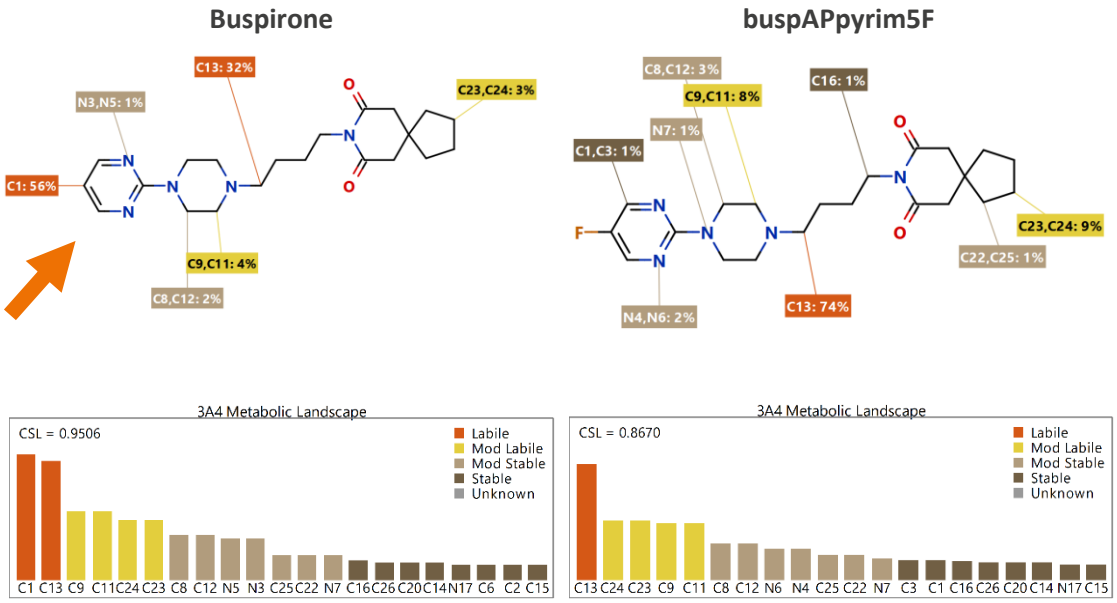
The **CSL** (composite site lability) is shown in the top-left of the metabolic landscape, as well as in the **P450** column in the data set. This is a measure of the efficiency of the product formation step in the catalytic cycle of CYP3A4. Thus, a lower CSL value indicates a greater likelihood of improved stability.

We will now explore the impact of potential modifications on the predicted lability at each observed site of metabolism. The **BuspironeS1** data set explores alternative aryl substitutions on the piperazine.

- Click on the top-left corner of the table below the button to select all the rows in the data set and submit these to the P450 models by clicking on the button.

- Selecting a row in the spreadsheet will display the detailed results in the P450 area.
- Examine each of the compounds, in turn, to identify modifications to the aryl group that improve the vulnerability of this region of the molecule to metabolism by CYP3A4. This will be indicated by lower **site lability** bars shown for the corresponding

sites in the **Metabolic Landscape** view. An example of such a modification is shown here:

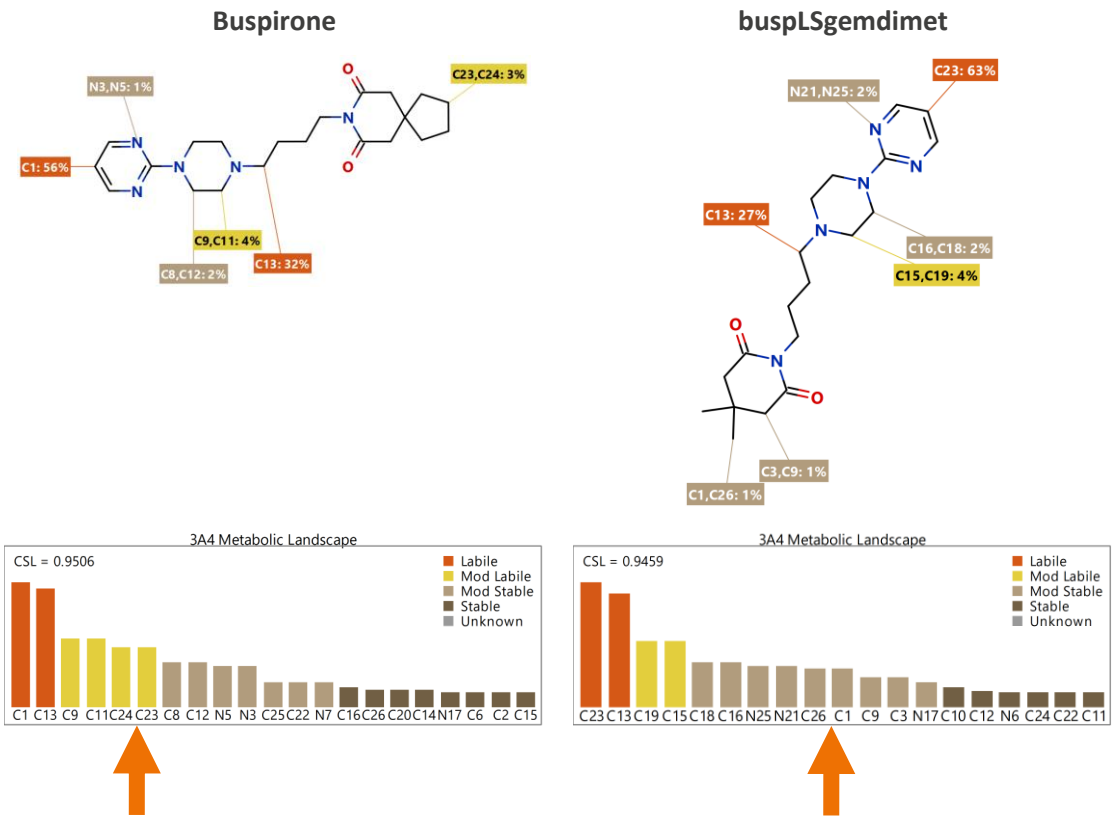


The experimental data indicate that this change alone results in an improvement in half-life by a factor of 10 while maintaining an IC_{50} of approximately 60 nM.

The CSL for many of the compounds remains high because, in this case, we are modifying only one region of the molecule, but other labile sites remain; therefore, there may be only a small change in the overall CSL, even for a beneficial modification. Also, other factors influence the overall rate of metabolism (in particular logP and pK_a); therefore, we do not necessarily expect a direct correlation between the small changes to CSL and the CYP3A4 half-life at this stage.

- Change to the **BuspironeS2** data set by clicking on the corresponding tab at the bottom of the data set. This contains the compounds in Series 2 that explore modifications to the tetramethylene linker and piperidinedione moiety.
- Run the P450 calculations for Series 2, as described above for Series 1, and explore the results, paying particular attention to changes that reduce the site labilities on the tetramethylene linker and piperidinedione moiety.

An example result is shown below:

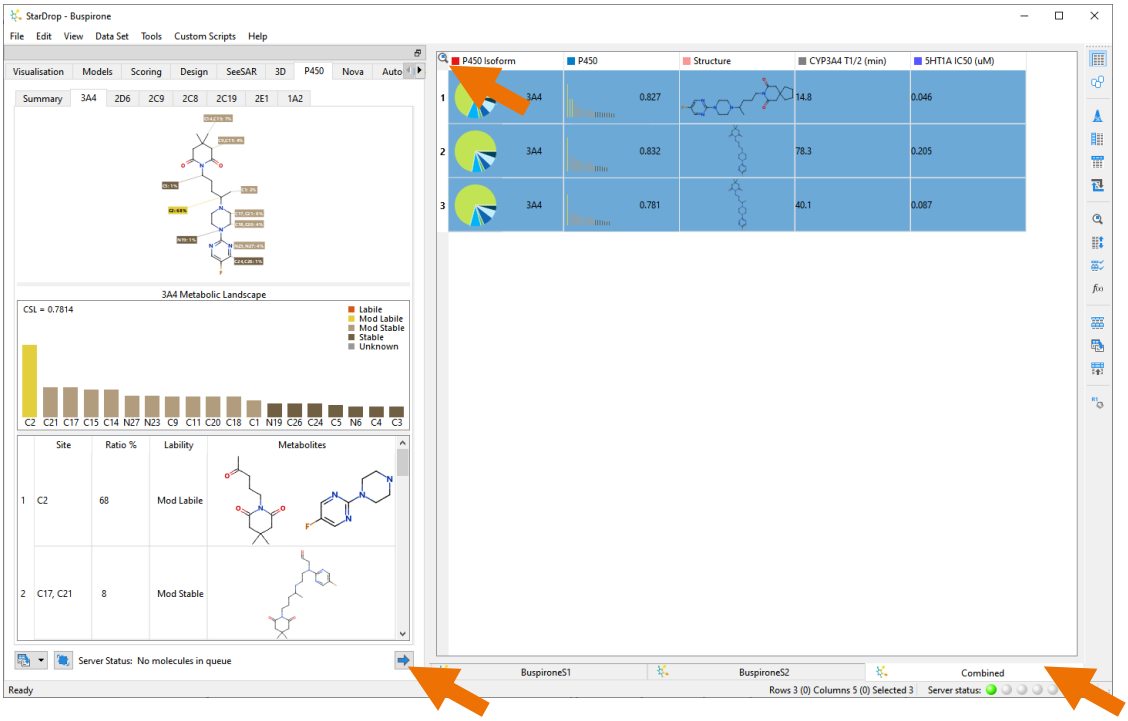


This indicates that replacing the spiropentane with a dimethyl substitution removes two moderately labile sites. Perhaps surprisingly, given the remaining presence of two highly labile

sites on this compound, this modification is sufficient to improve the half-life from 4.6 minutes to 30.5 minutes.

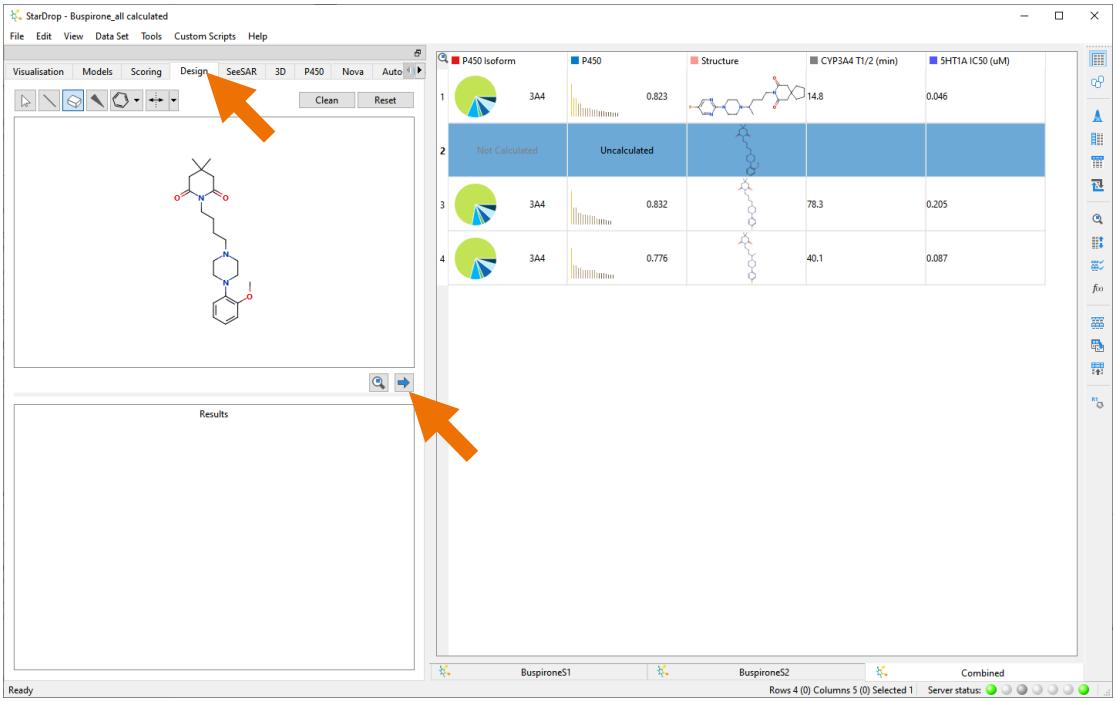
Finally, we would like to explore combinations of the modifications we have identified to find compounds with improved overall stability while avoiding those changes that caused a large decrease in potency.

- Change to the **Combined** data set to run the P450 models on these three examples, as described previously.



Note that all of these compounds have significantly better (lower) CSL values than Buspirone and meet the objective of greater than 3-times the half-life of Buspirone. Furthermore, in two cases, IC_{50} values against 5-HT_{1A} of less than 0.1 μM have been retained.

- You can explore further modifications by drawing new molecules in the **Design** area. Add these to the dataset using the  button before switching to the **P450** area and submitting the molecules to the P450 models.



Further details of the chemistry, assays and results in this study can be found in Tandon et al. The design and preparation of metabolically protected new aryl-piperazine 5-HT1A ligands. Bioorg. Med. Chem. Lett. 2004 14(7) pp. 1709-12. If you have any questions, please feel free to contact stardrop-support@optibrium.com.