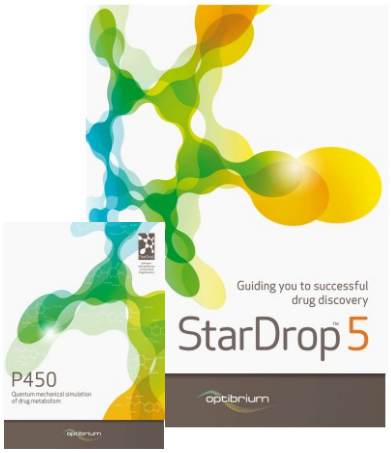
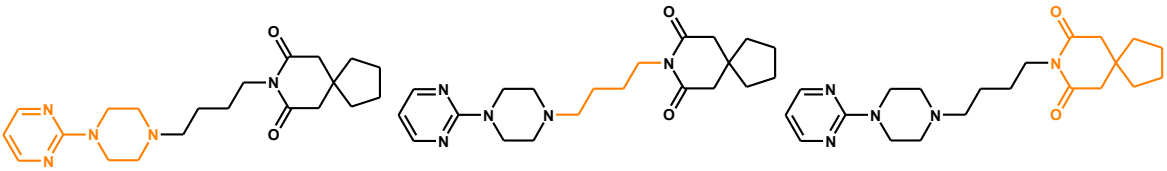


Worked Example:

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

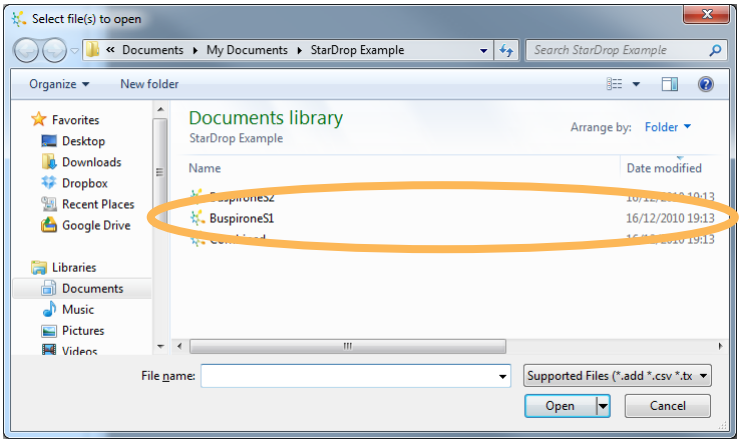
This example illustrates the use of the P450 metabolism models to explore structural modifications in each of these regions in order to identify those most likely to significantly improve the stability with respect to CYP3A4 metabolism.

Exercise

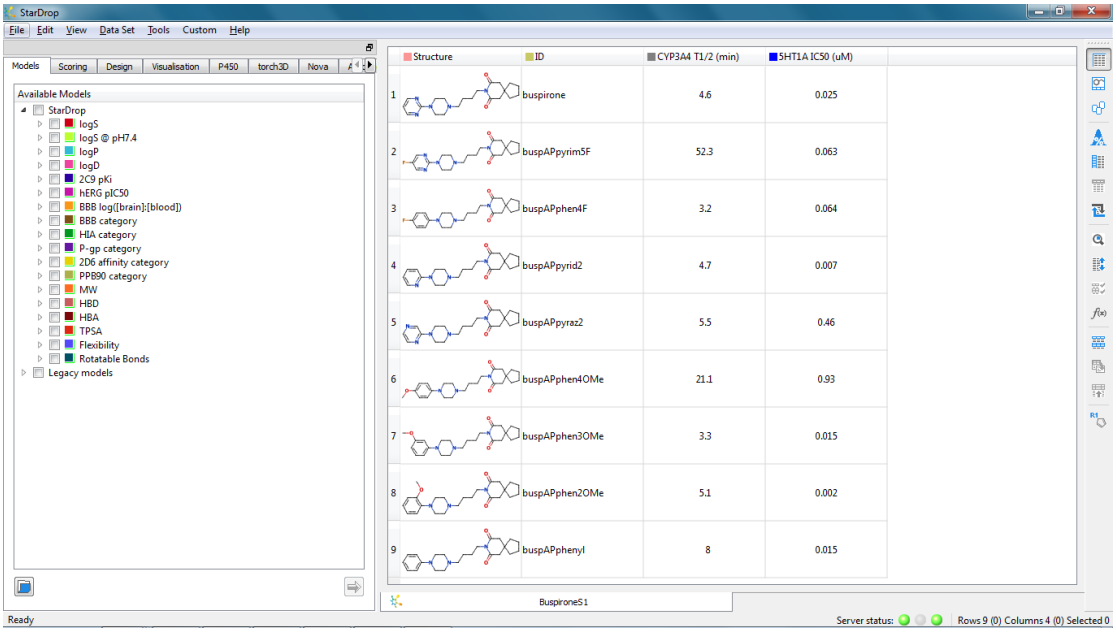
Our objective is to identify structural modifications that reduce the vulnerability of key sites of metabolism, as indicated by decreasing the **site lability** and, ultimately, identify molecules that are likely to meet the project goal of increased half-life with respect to metabolism by CYP3A4 by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

We will explore 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. These series were designed to maintain potency against 5-HT_{1A} as well as improve metabolic stability.

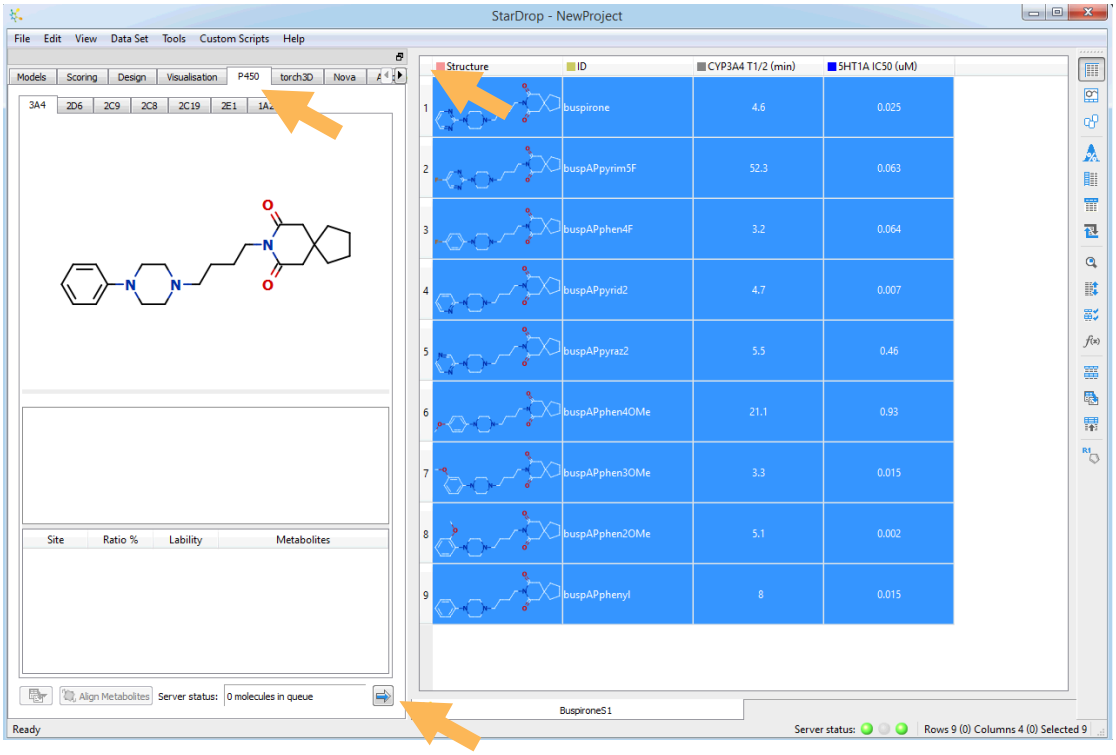
- Start StarDrop from the Start menu.
- Open the file **BusironeS1.add** by using the **File -> Open** menu option. This contains the compounds in Series 1.



- You will see a spreadsheet containing structures, identifiers and their measured half-life with respect to metabolism by CYP3A4. The spreadsheet contains 9 compounds, the first of which is Buspirone.

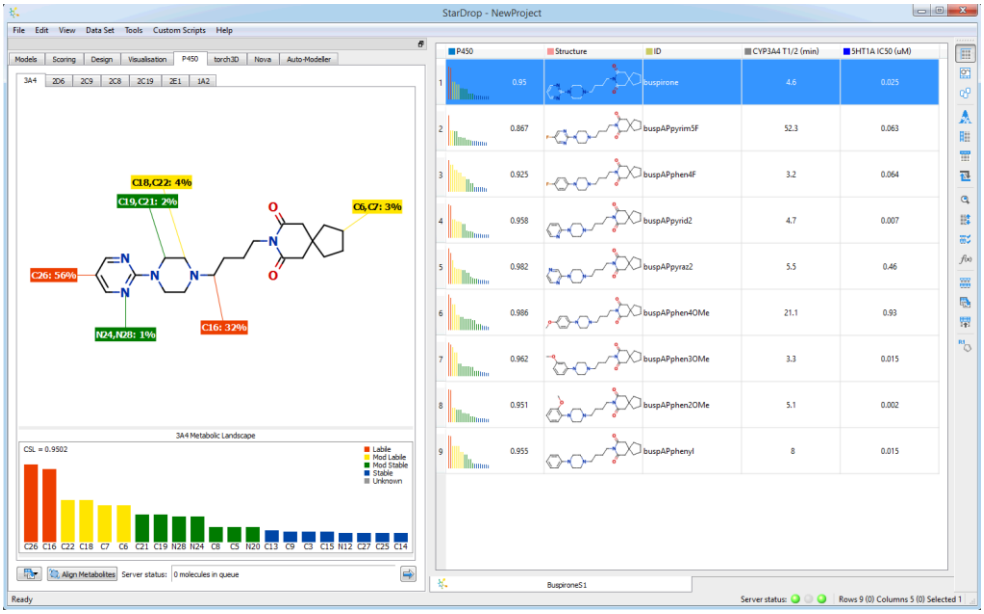


- Change to the P450 tab, select all of the compounds in the data set by clicking in the top left corner of the spreadsheet and submit these to the P450 models by clicking on the  button.

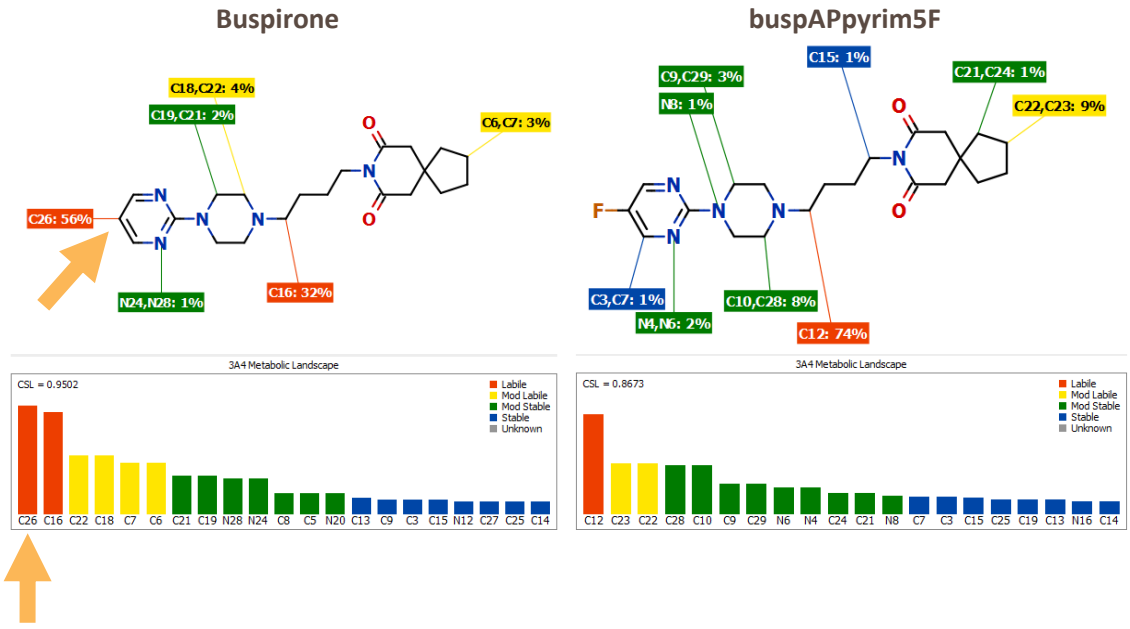


When the calculations are complete, the results will be returned from the server and a summary will be displayed in the spreadsheet. Each molecule will take roughly 2-3 minutes to calculate; however, if the results for a molecule have previously been calculated on your server, the results will be returned instantly.

- Selecting a row in the spreadsheet will display the detailed results for a single molecule in the P450 tab (**Hint:** the regions of the P450 tab can be resized to enlarge the regioselectivity and site lability views). The results for Buspirone are shown below:



- 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 below:

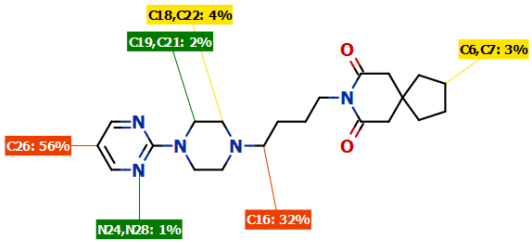


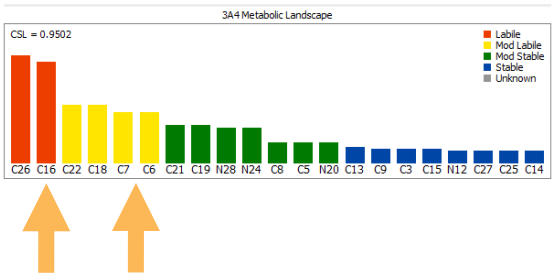
- The **composite site lability** (CSL) for a molecule is a measure of the efficiency of the product formation step in the catalytic cycle of CYP3A4. Thus, a lower CSL value

indicates greater stability. In this case, as we are modifying only one region of the molecule, other moderately labile sites remain, so 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 pKa) therefore we do not necessarily expect a direct correlation between the small changes to CSL and the CYP3A4 half-life at this stage.

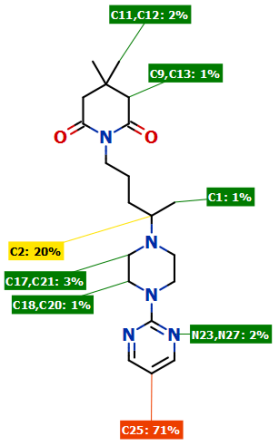
- Open the file **BuspironeS2.add** by using the **File -> Open** menu option. This contains the compounds in Series 2.
- Run the P450 calculations for Series 2, as described above for Series 1, and explore the resulting Metabolic Landscapes, paying particular attention to changes that reduce the site labilities on the tetramethylene linker and piperidinedione moiety. An example is shown below:

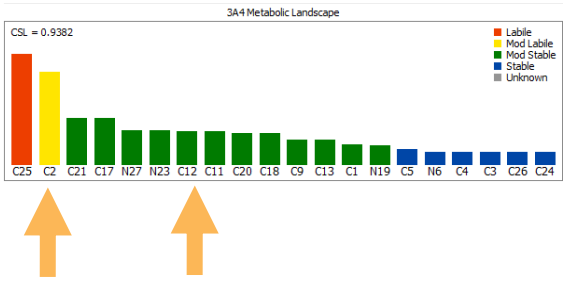
Buspirone



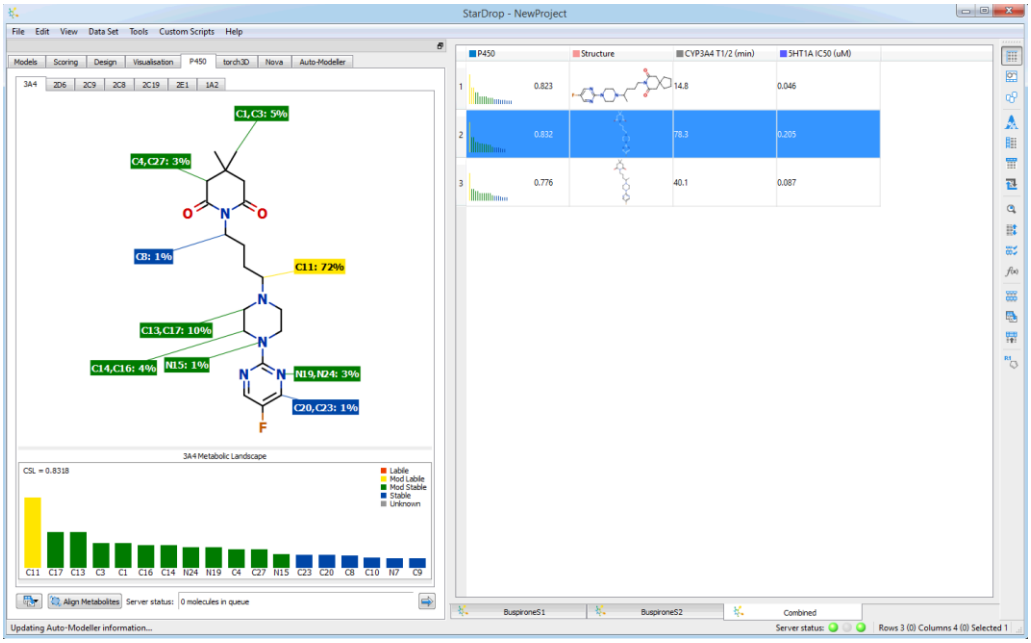


buspLSamegemdimet



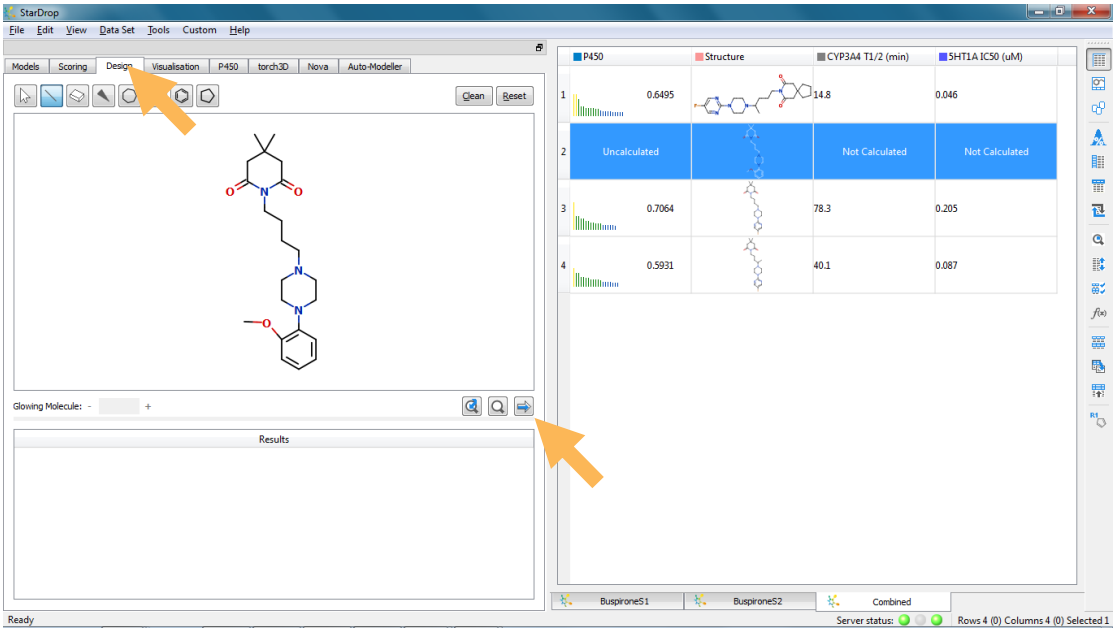


- Finally, we would like to explore combinations of the modifications we have identified to find compounds with improved overall stability, while avoiding those changes that caused a large decrease in potency. Open the file **Combined.add** to load three such examples and run the P450 models as described above.



- 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.
- Further modifications can be explored by drawing new molecules in the **Design** tab.

  Add these to the dataset using the  button before switching to the **P450** tab 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 arylpiperazine 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.