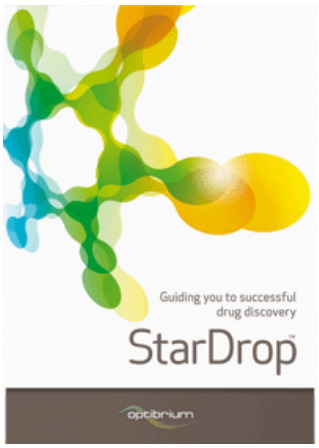
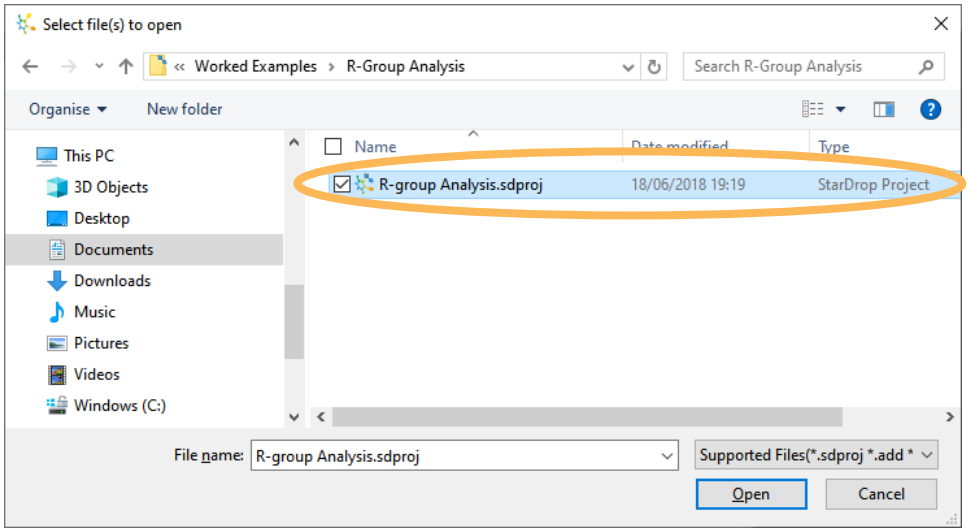


Worked Example:

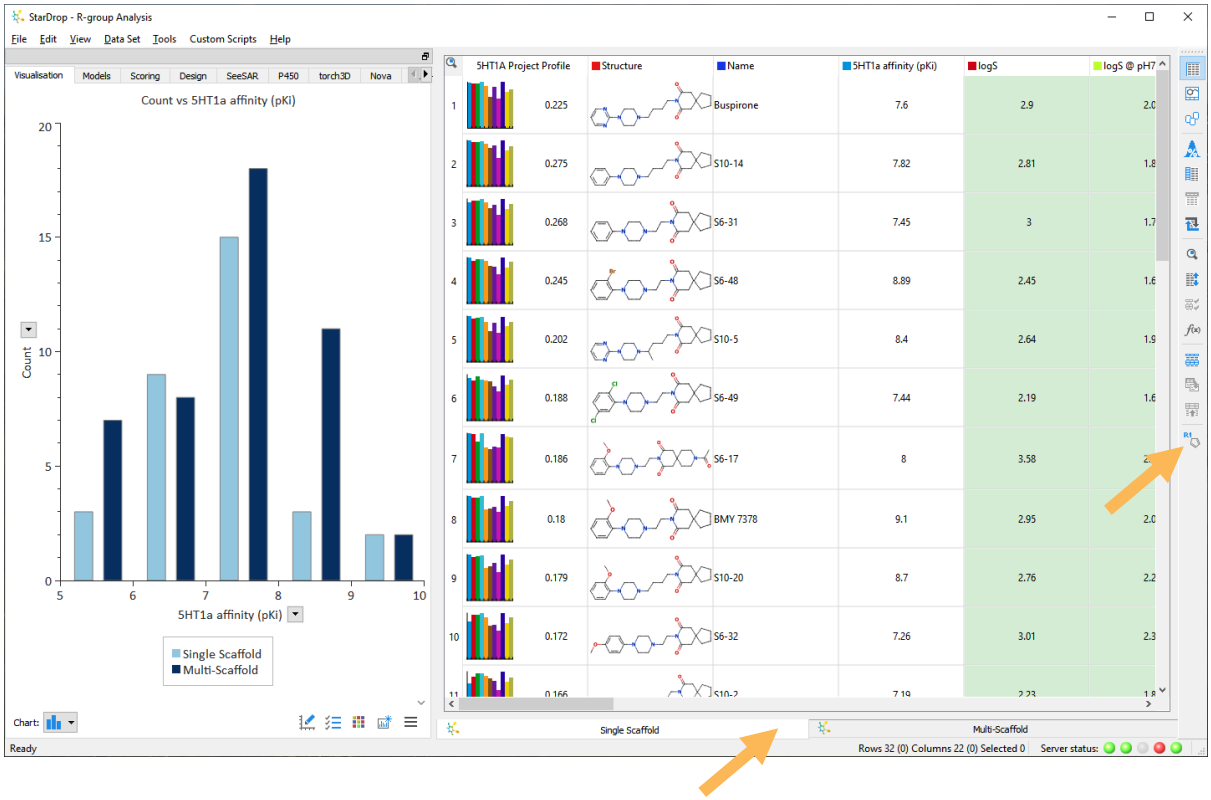
R-group Analysis

In this example we are going to take a look at an R-group analysis of two data sets to identify functionalities which are influencing potency. The first data set contains Buspirone and some close analogues based on a common scaffold. In the second data set there are different series, based around multiple scaffolds, where the substitution points are equivalent because they share a similar binding pose.

- In StarDrop™, open the file **R-Group Analysis.sdproj** by selecting **Open** from the **File** menu.



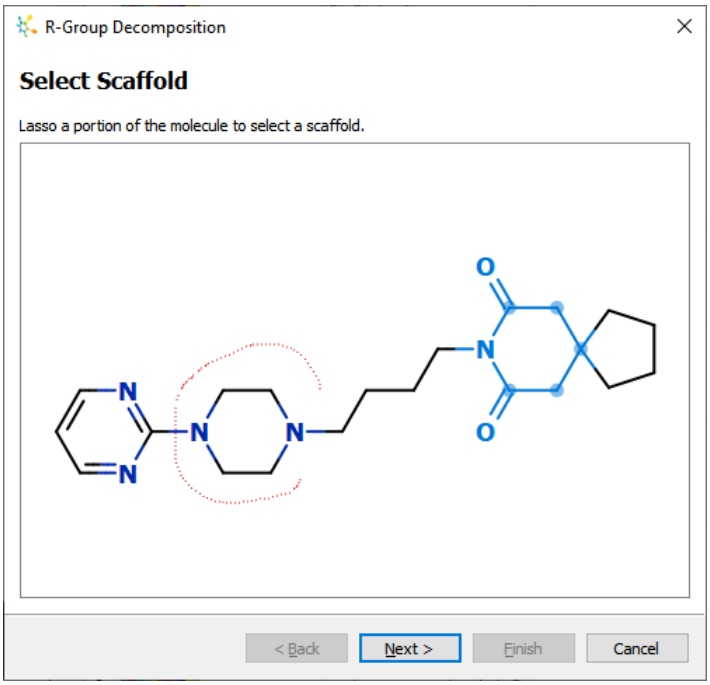
The project contains the two data sets which you can view by clicking on the tabs at the bottom. It also shows a histogram giving a quick overview of the distribution of potency data in each set.



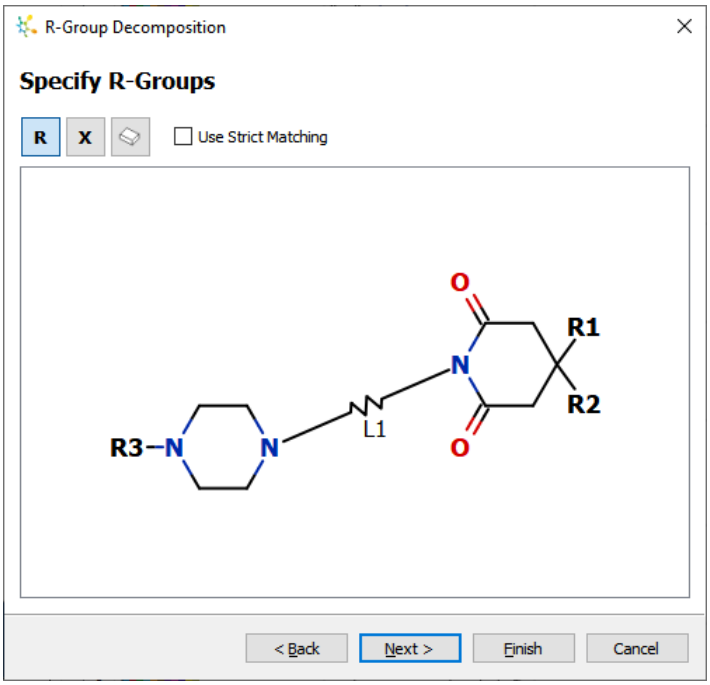
- Select the first row (Buspirone) in the first dataset (Single Scaffold) and start the R-group decomposition wizard by clicking the **R-group Decomposition button**  on the right-hand toolbar (it is also available from the **Tools** menu).

The selected molecule is displayed in the wizard so that you can now choose which regions of the molecule make up the scaffold.

- Select the piperazine and piperidinedione rings (as shown in the screenshot to the right) by drawing around them with the mouse, holding down the **CTRL** key to select a second region.

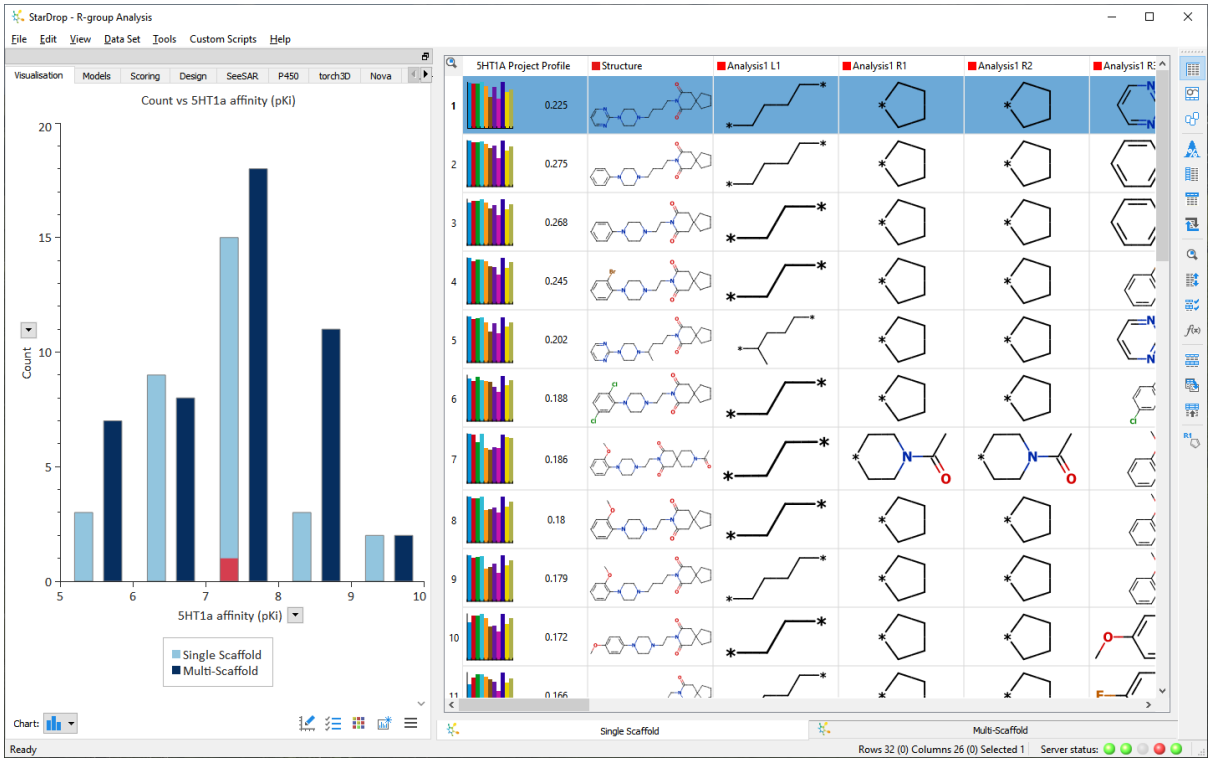


- Click **Next** to confirm the R-groups that will be analysed. In this example, because we selected two separate regions, the connecting functionalities will be considered to be variations in linkers.



Please note that you can add R-groups by clicking the **R-group button** R and then clicking at the point on the scaffold where they should be analysed. If you wish to specify variable atoms or fragments, click the **Variable Atom button** X and then select atoms on the scaffold which may vary between the compounds being analysed. In this case, no additional R-groups, variable atoms or fragments are required.

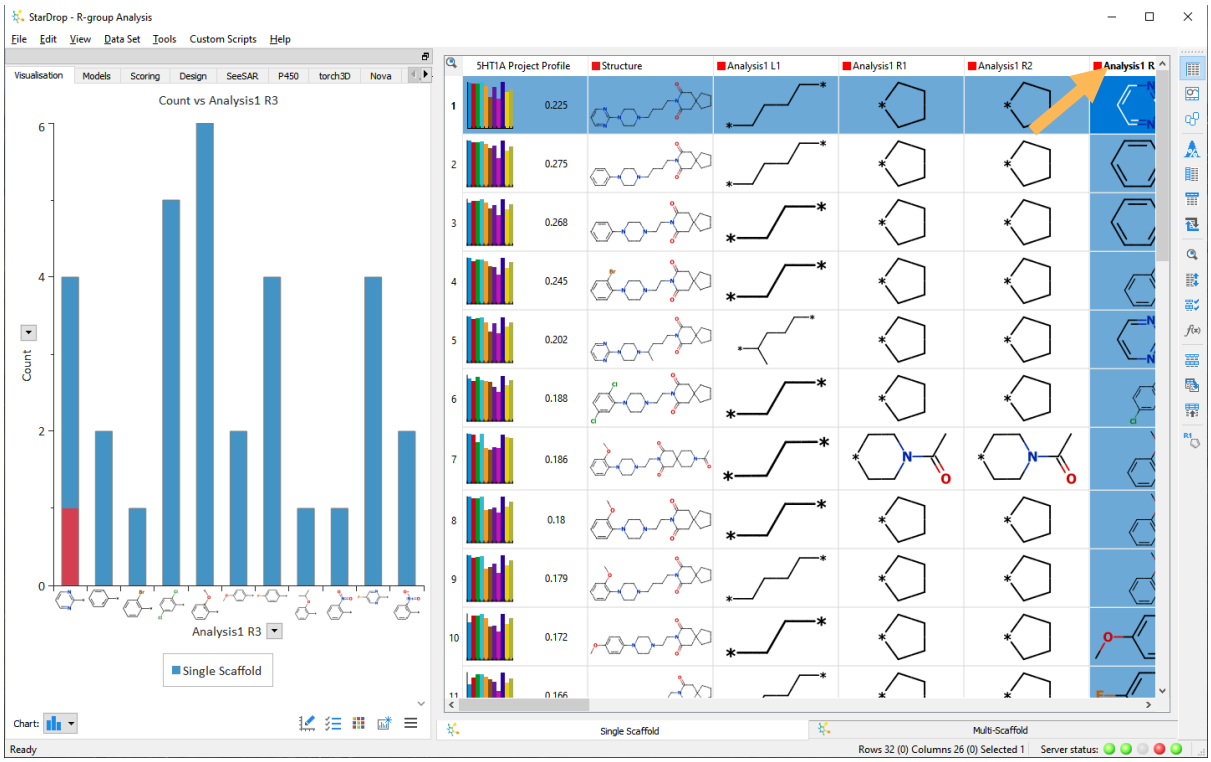
- Click the **Finish button** and new columns will be added to the data set indicating the R-groups and linkers that have been found for each compound.



- Hover over any of the R-groups to see a pop-up of the scaffold. To keep a scaffold window open, right-click on the R-group column header and choose **View Scaffold**. This window can be docked above the main StarDrop tabs and if you have more than one analysis in your data set the displayed scaffold will change to show the scaffold associated with the selected R-group column at any given time.

In the **Visualisation area** you can now create charts to analyse the SAR in the series.

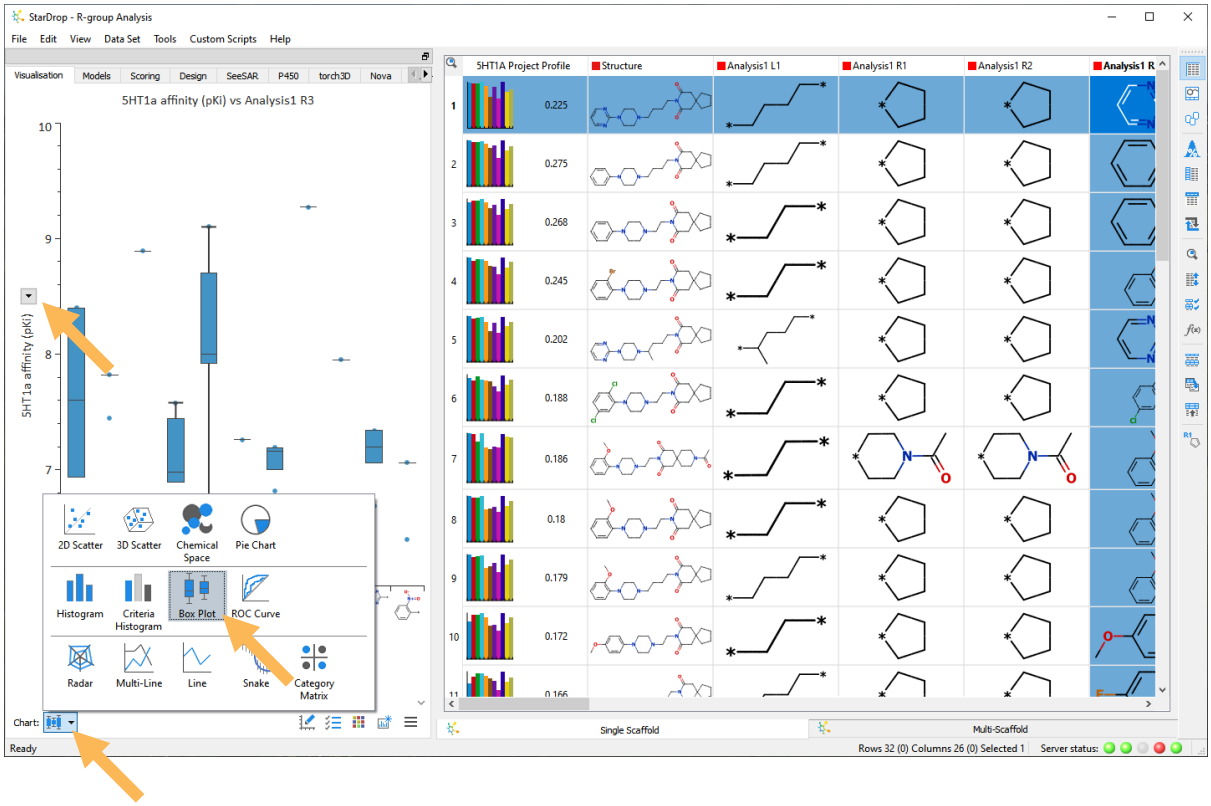
- Click on the header of the column **Analysis1 R3** in the data set to select it.



A histogram will be displayed showing the number of compounds with each group at position R3. Pointing at the picture of an R-group on the axis will pop-up a larger version.

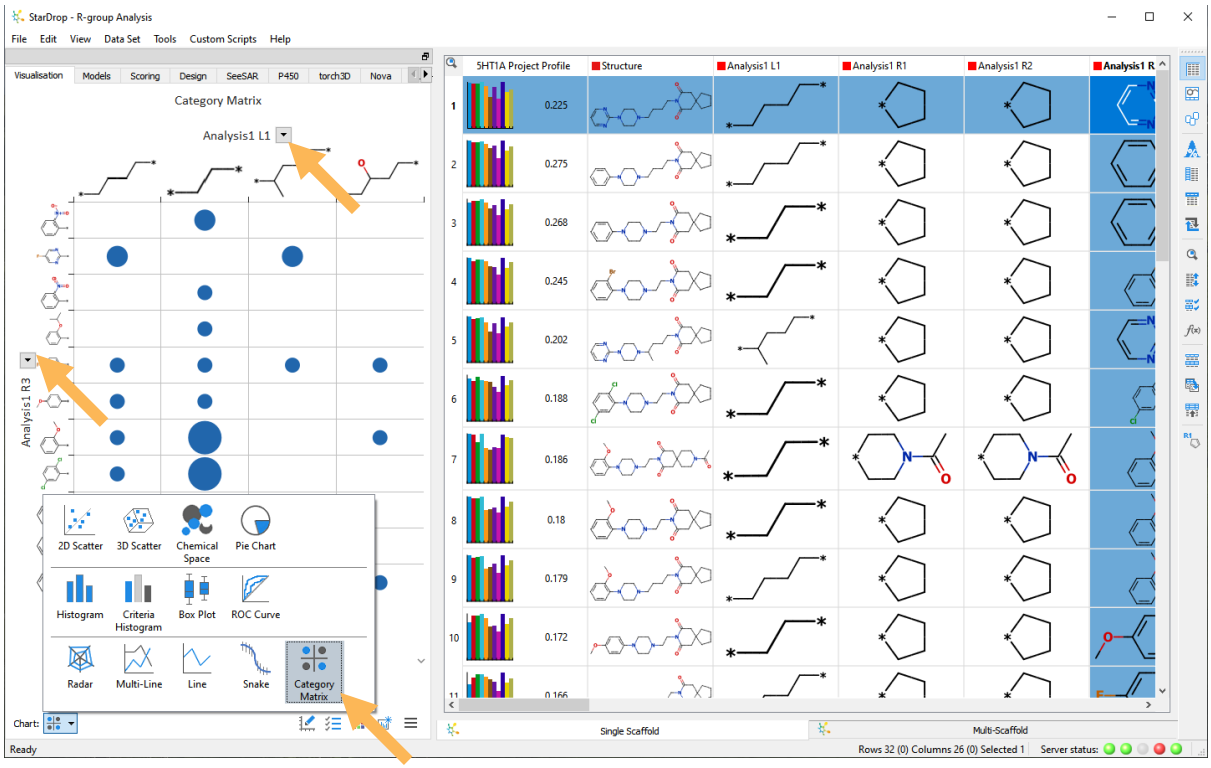
Other data can be plotted on the y-axis and other chart types can be used to see more detailed information.

- From the **Chart Menu** at the bottom of the Visualisation area, select **Box Plot**.
- On the y-axis, use the drop-down menu to change the property to **5HT affinity (pKi)**.



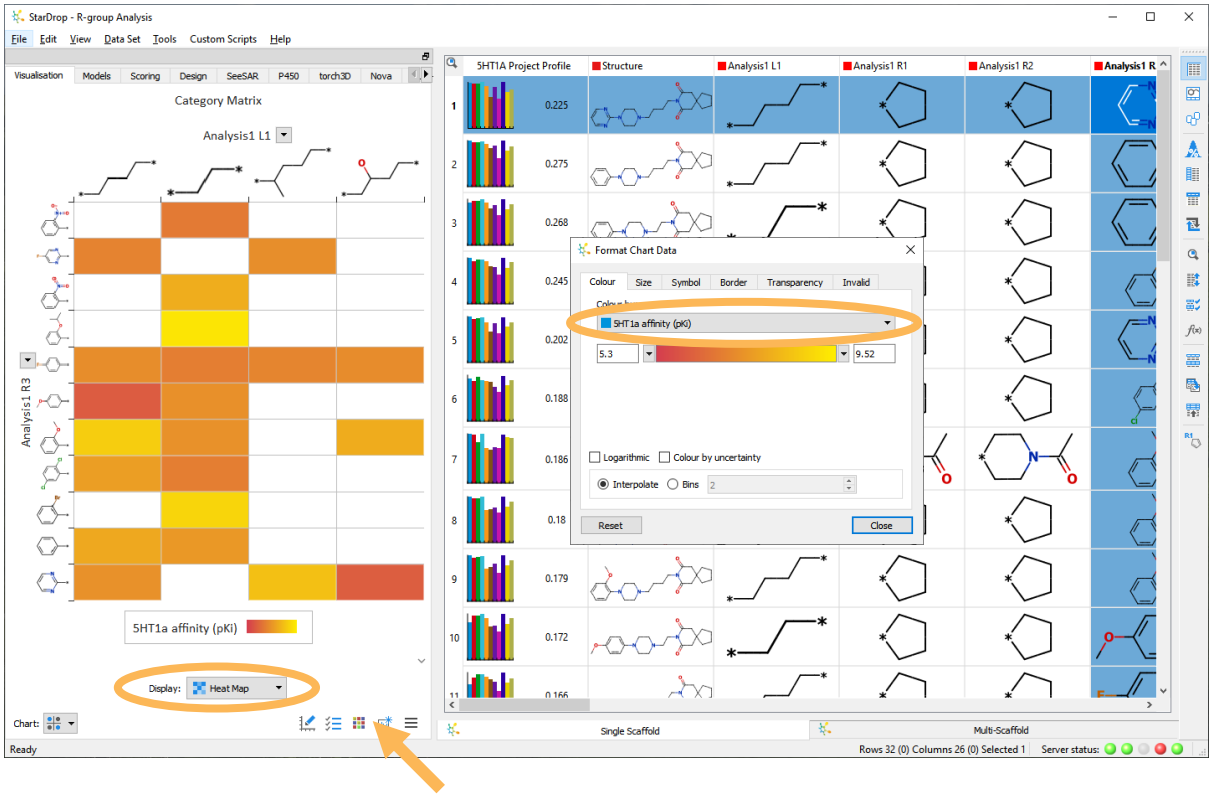
This enables us to see the distribution of potency data associated with each R-group. To analyse the properties associated with combinations of R-groups we can create an SAR table.

- From the **Chart Menu** again, select **Category Matrix** and choose **Analysis1 R3** for the y-axis and **Analysis1 L1** for the x-axis.



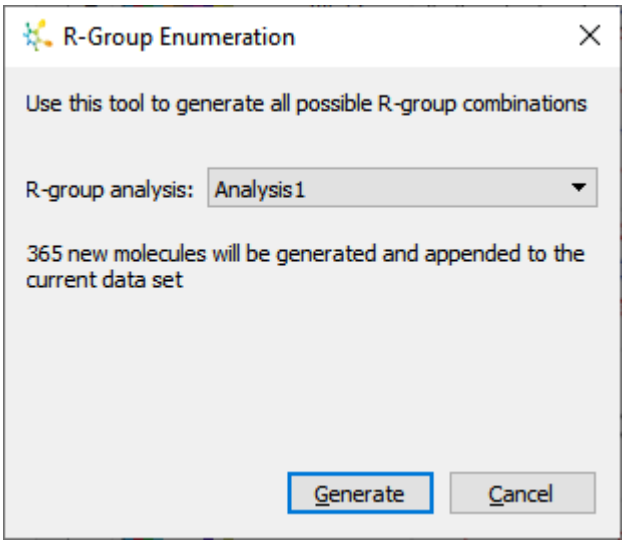
By default, this will show pie charts where the size indicates the number of compounds represented, but we can change this to show a heatmap using the menu below the chart.

Click the Format button at the bottom of the Visualisation area and choose to **Colour by** the property **5HT1a Affinity (pKi)**.



If you use the default colours, then the compounds with the highest pKis will be highlighted yellow, the lowest red. The different types of charts that you show in each cell enable addition formatting options such as using size, symbols, borders, etc. to represent additional properties.

Finally, StarDrop can 'fill in' the missing combinations of R-groups and linkers that are not present in the series, helping to explore other, potentially interesting, compounds.

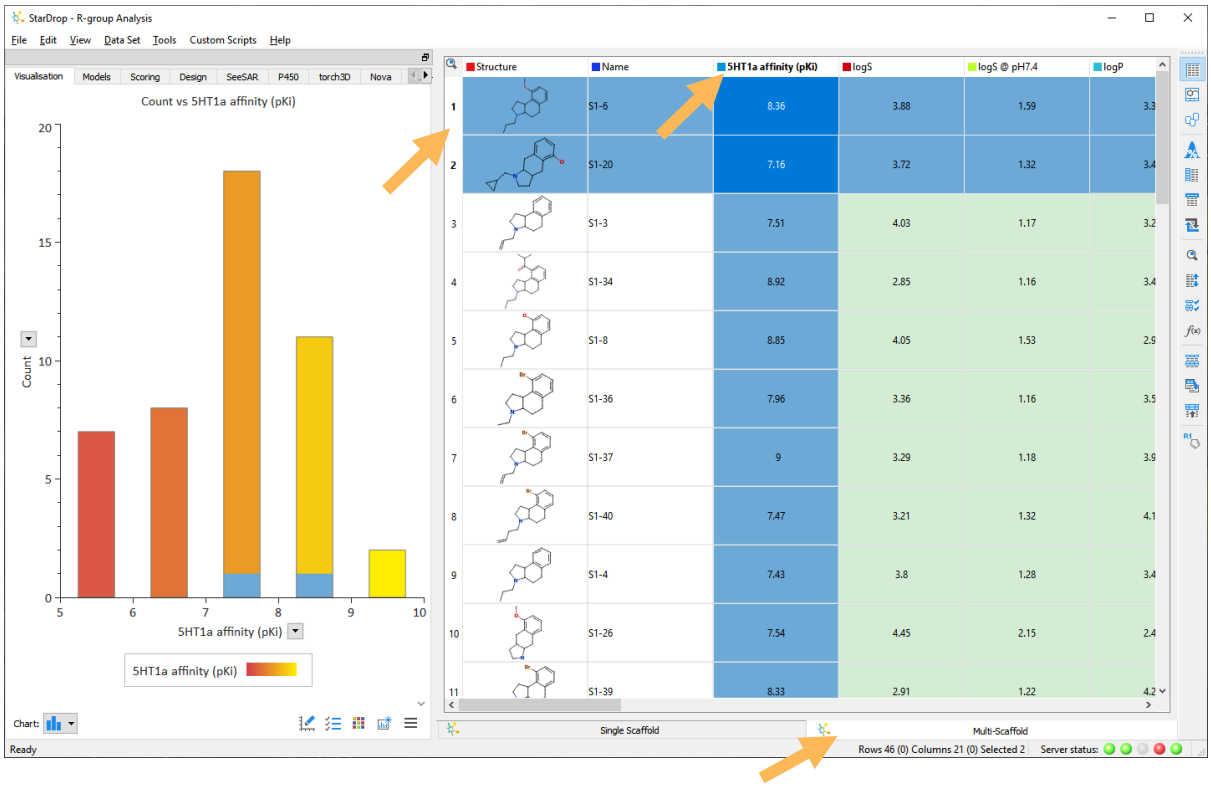


- Right-click on one of the R-group column headers and choose **R-Group Enumeration** from the menu.
- Click **Generate** and the new compounds will be added to the data set and any visualisations you have created, with their predicted properties automatically calculated.

Multi-scaffold R-group Analysis

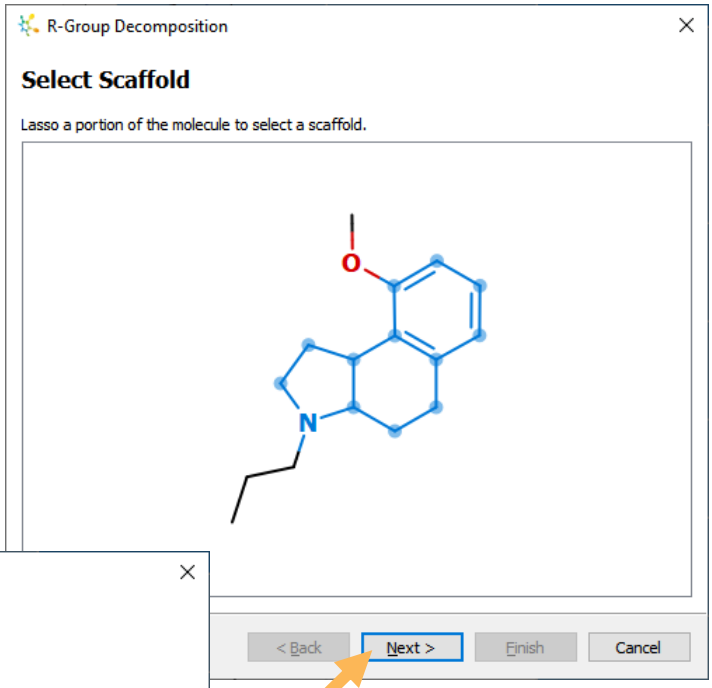
In the second data set in this project, called **Multi-Scaffold**, we have different series, based around multiple scaffolds, where the substitution points are equivalent because they share a similar binding pose. We would like to identify common patterns for substitutions at equivalent positions or look for the effect of the core replacements on compound properties. In this example, we will show how multi-scaffold R-group analysis can be performed in StarDrop.

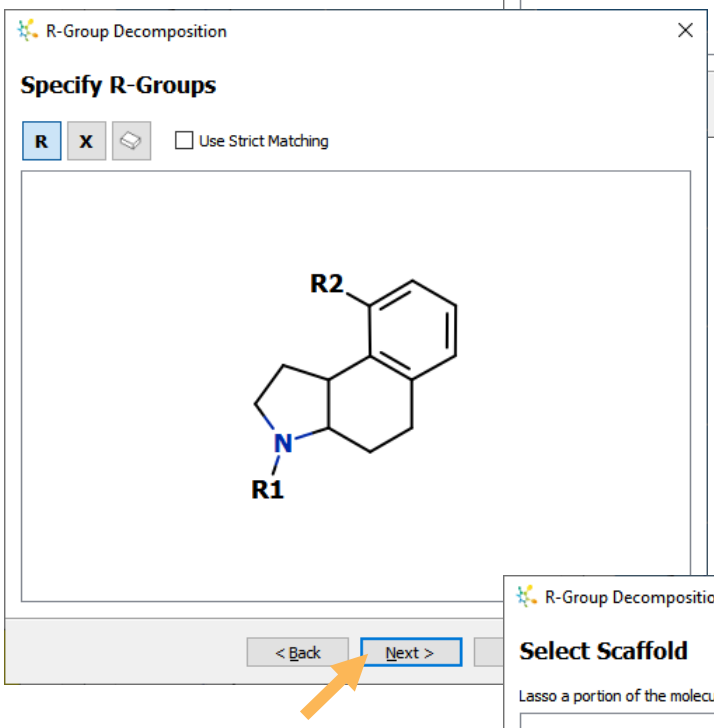
- Change to the Multi-Scaffold data set by clicking on the tab at the bottom of the data sets and select the column called 5HT1a affinity (pKi).
- Select the first two rows (holding down the **CTRL** key to select the second) and note that they are based on similar scaffolds with equivalent substitution points.



- As before, click the clicking the **R-group Decomposition button**  on the right-hand toolbar to start the **R-Group Decomposition wizard**.

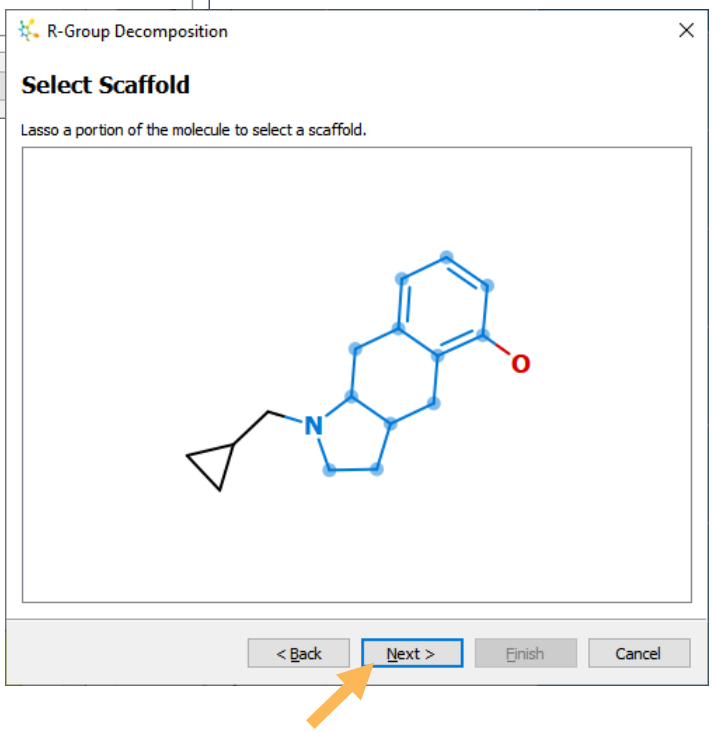
- The first compound will be shown on the **Select Scaffold** page of the wizard. Draw around the fused ring system to define this as the first scaffold (which will be highlighted in blue, as shown right) and click **Next**.



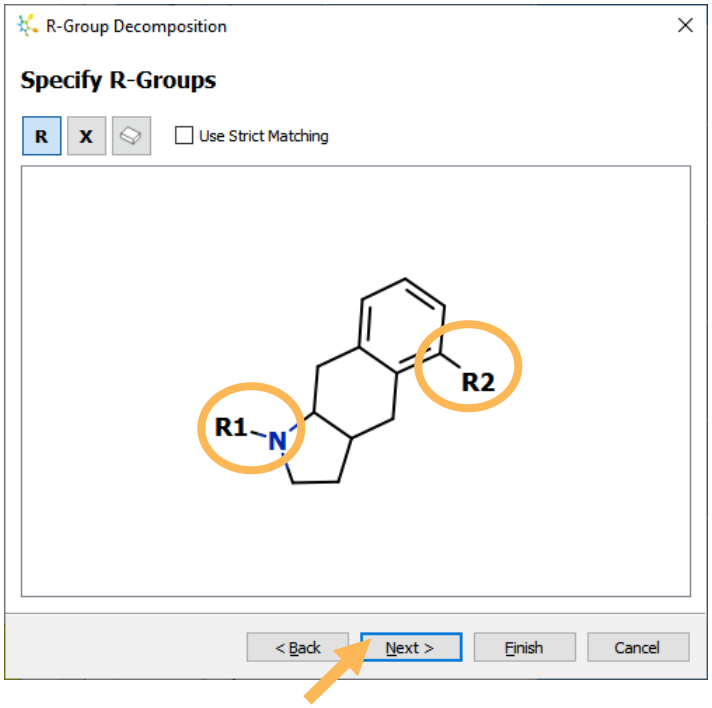


- The **Specify R-Groups** page will automatically show groups R1 and R2 at the substitution points for the selected compounds, as shown right. We do not need to add any other points of variation, so click **Next** again.

- The **Select Scaffold** page will now be shown again, this time displaying the second compound. Again, draw around the fused ring system to define the second scaffold as highlighted in blue to the right. Click **Next** to define the R-groups for this scaffold.

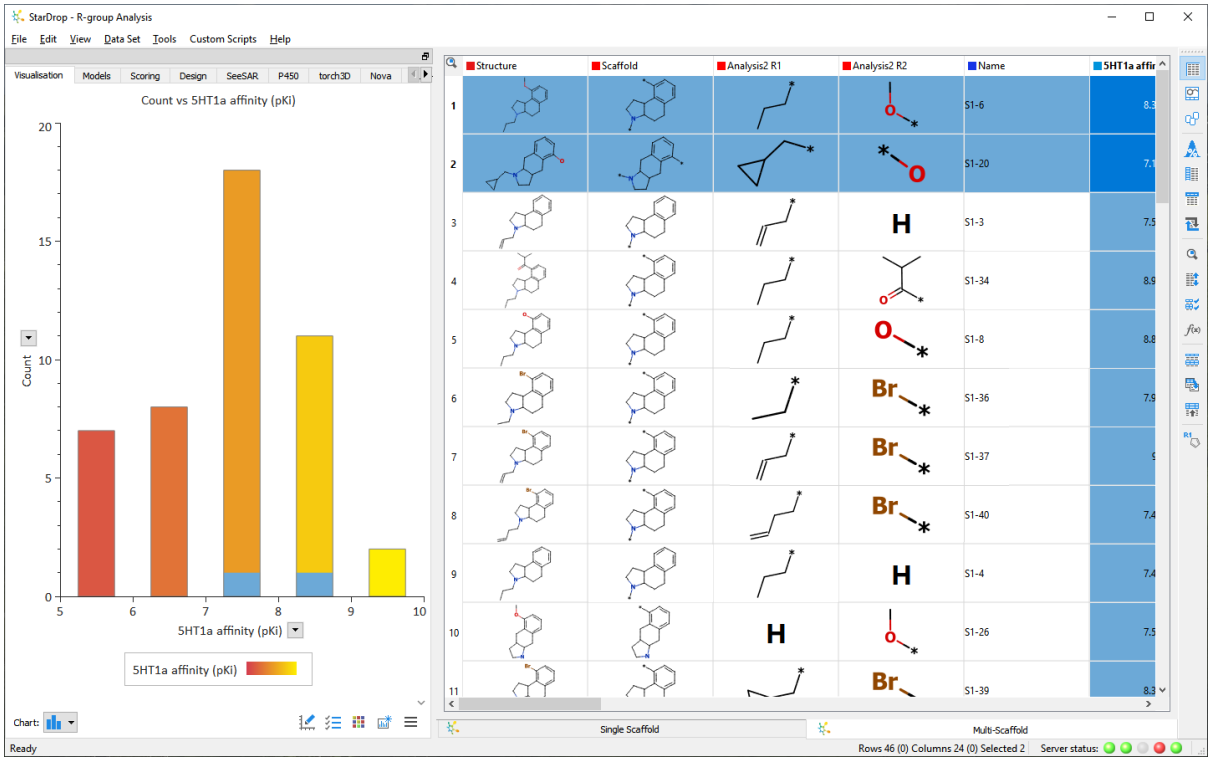


- The **Specify R-groups** page will be shown again for this scaffold. In this case, it is important to ensure that the groups at equivalent positions in each scaffold have the same label (R1 and R2). If it is necessary to change the defaults, you can edit them simply by clicking on the label (using the **R** button). Please ensure that the R1 and R2 labels are in the positions shown to the right. Again, click **Next**.

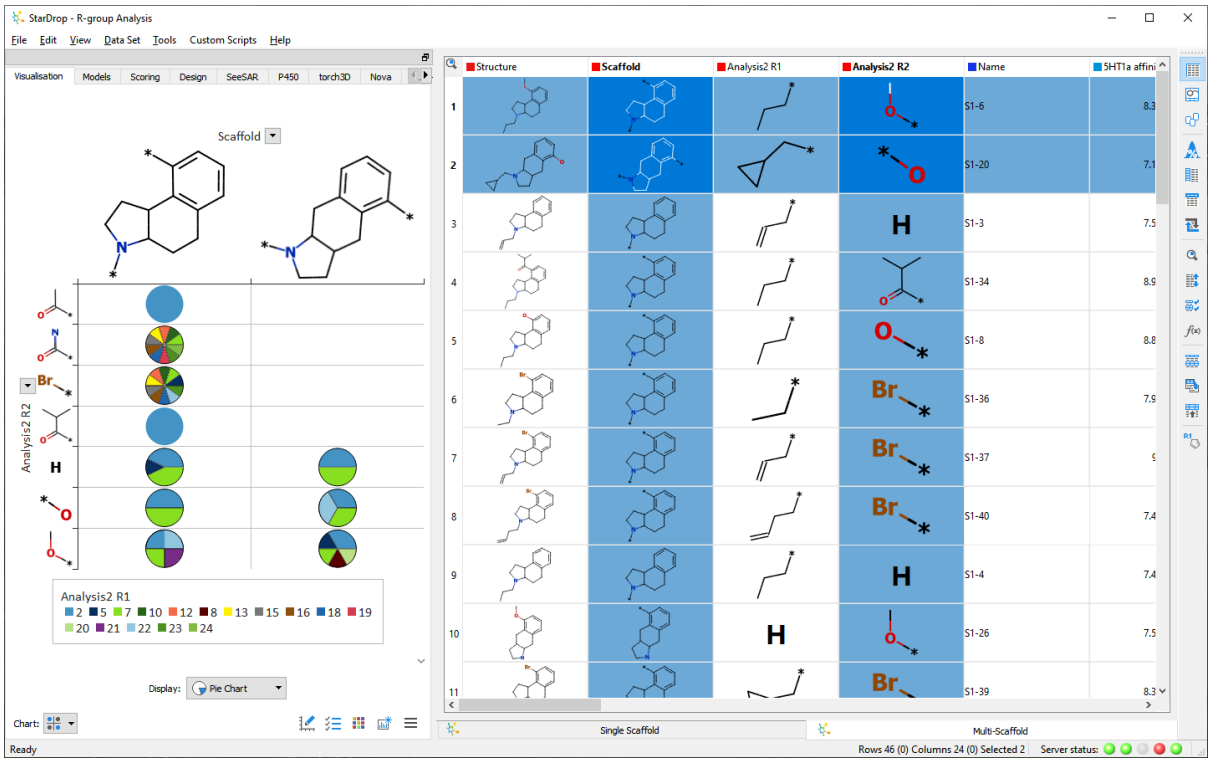


- If you wish to give the analysis a name, click **Next**, otherwise click **Finish** to run the R-group decomposition.

Three new columns will be added to the data set, showing the scaffold, R1 and R2 groups for each compound.



As with R-groups, the scaffold can be used in visualisations, by selecting the corresponding column or choosing the scaffold from the axis drop-downs in the **Visualisation area**.



In this example, the pie-charts have been coloured by R1 to show which R1 group has been tried with each scaffold and R2.