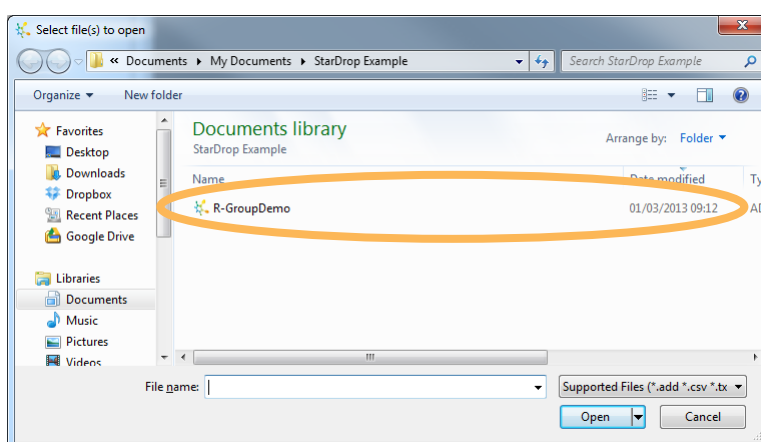



## Worked Example:

### R-group Analysis

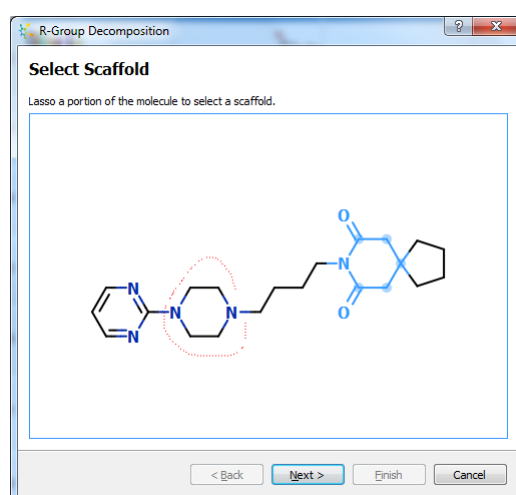
In this example we are going to take a look at an R-group analysis of two data sets to identify functionalities which are influencing potency. The first data set contains Buspirone and some close analogues based on a common scaffold.

- Start StarDrop from the **Start** menu.
- Open the file **R-GroupDemo** by using the **File -> Open** menu option.



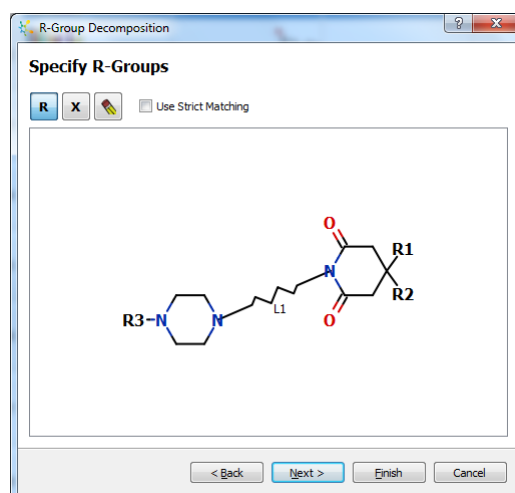
Select the first row (Buspirone) and start the R-group decomposition wizard by clicking the  button on the toolbar (it is also available from the **Tools** menu).


- 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 two ring functionalities indicated in the screenshot to the right by drawing around them to lasso the area, holding down the **SHIFT** or **CTRL** button to select a second region.




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- 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.



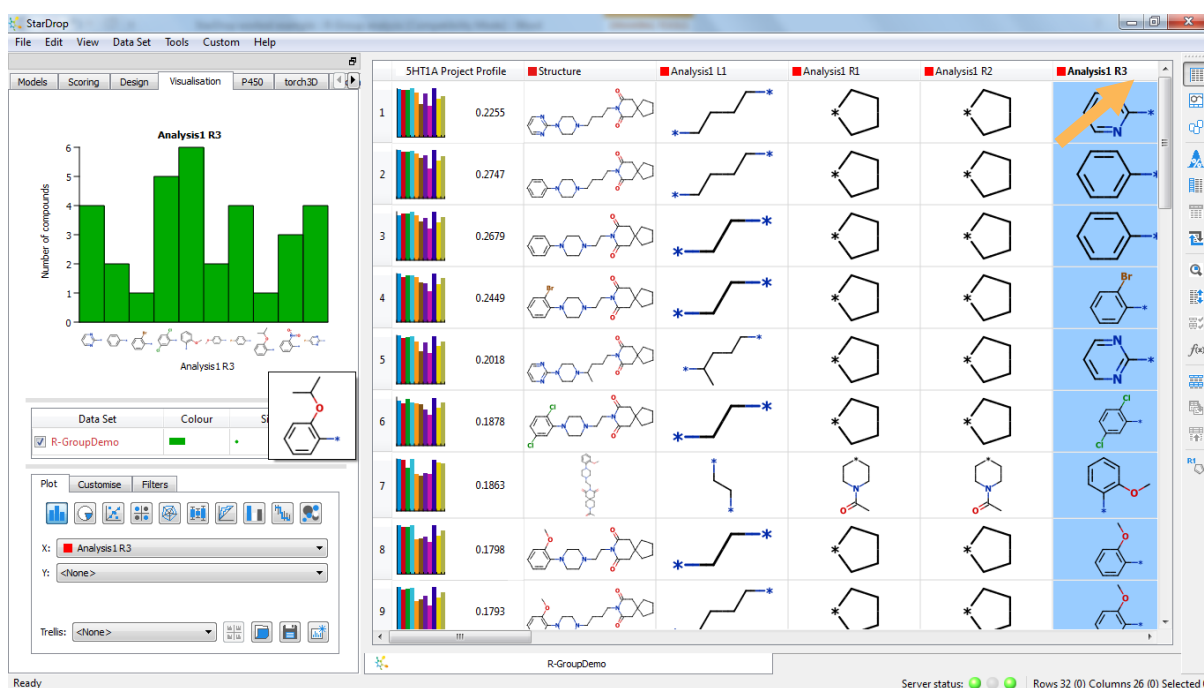
Please note that you can add R-groups by clicking the  button and then clicking at the point on the scaffold where they should be analysed. If you wish to specify variable atoms or


fragments, click the  button 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.

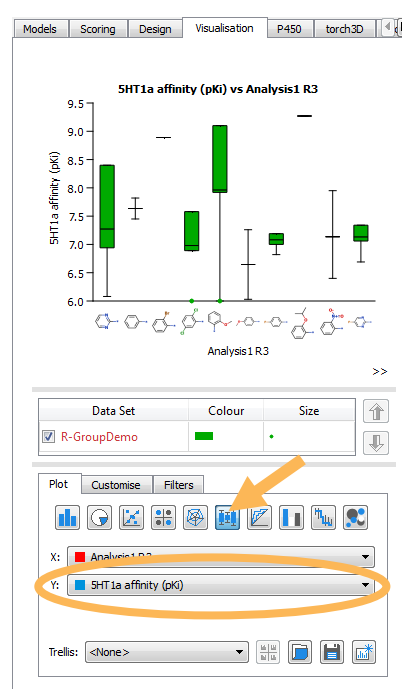
- Click the **Finish** button and new columns will be added to the data set indicating the R-group and linkers that have been found for each compound.


Row	Score	Structure	Analysis1 L1	Analysis1 R1	Analysis1 R2	Analysis1 R3
1	0.2255					
2	0.2747					
3	0.2679					
4	0.2449					
5	0.2018					
6	0.1878					
7	0.1863					
8	0.1798					
9	0.1793					

- 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 in the tabbed area 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.
- You can visualise the SAR in the series using the visualisation tools in StarDrop. Change to the **Visualisation** tab, and click on the header of the column **Analysis1 R3** in the data set. 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 plot types can be used to see more detailed information. For example, click the  button on the **Visualisation** tab and select **5HT1a affinity (pK<sub>i</sub>)** from the Y: drop-down to display a box plot showing the distribution of potency for compounds with each group at position R3.



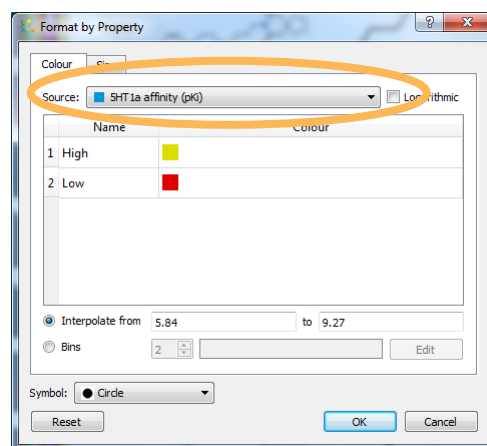
- Create an SAR plot by clicking the  button on the **Visualisation** tab and choose **Analysis1 R3** from the **X:** drop-down menu and **Analysis1 L1** from the **Y:** drop-down menu. This will display an SAR table of R3 against L1 as shown right:

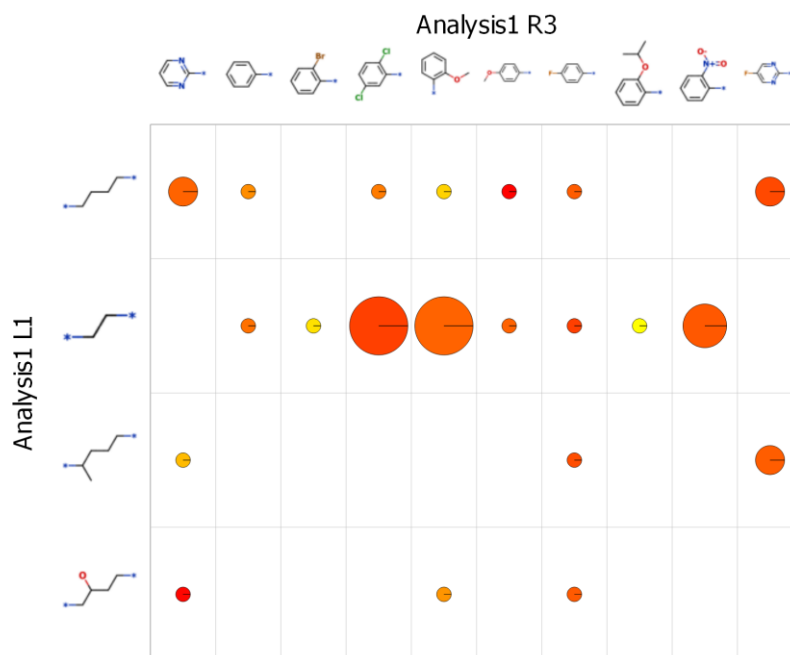


- You can colour the SAR plot by different properties. For example, to colour the plot by the **5HT1a affinity (pK<sub>i</sub>)** values in the data set, click on the block of colour next to 'R-GroupDemo' in the key, as shown below, to bring up the **Format by Property** dialogue.

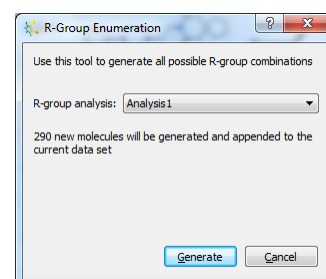
Data Set	Colour	Size
<input checked="" type="checkbox"/> R-GroupDemo		

- In the resulting dialogue, change the **Source** for the colour to **5HT1a affinity (pK<sub>i</sub>)** and click **OK**.
- The compounds with the highest pK<sub>i</sub>s will be highlighted yellow, the lowest red. The size of the circles indicates the number of compounds which contain those groups.





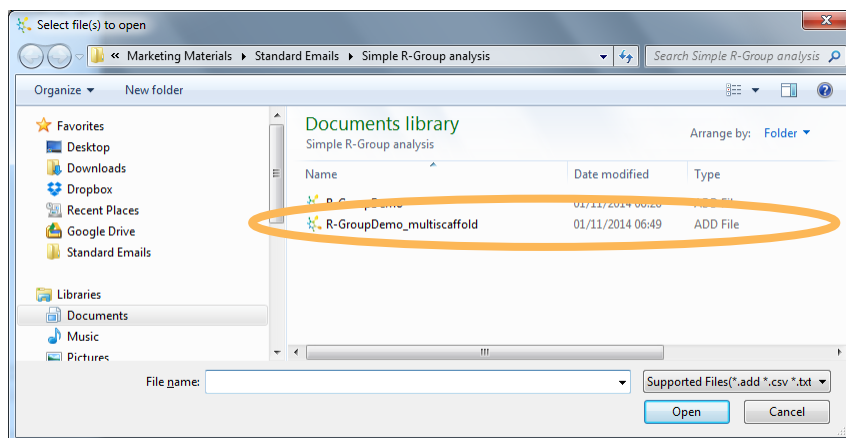
- You can also change the size of the circles according to another property by clicking on the circle under **Size** in the key and there are many other ways to view your data, for example by plotting pie charts, histograms or radar plots in the SAR table. Click on the **Customise** tab to see some of these options.
- Other visualisations can be plotted by selecting an R-group column on its own or in combination with a property. Feel free to explore!
- 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. To do this, right-click on one of the R-group column headers to choose **R-Group Enumeration...** from the menu. This will estimate the number of new compounds that will be generated
- Click **Generate** and the new compounds will be added to the data set with their predicted properties automatically calculated.



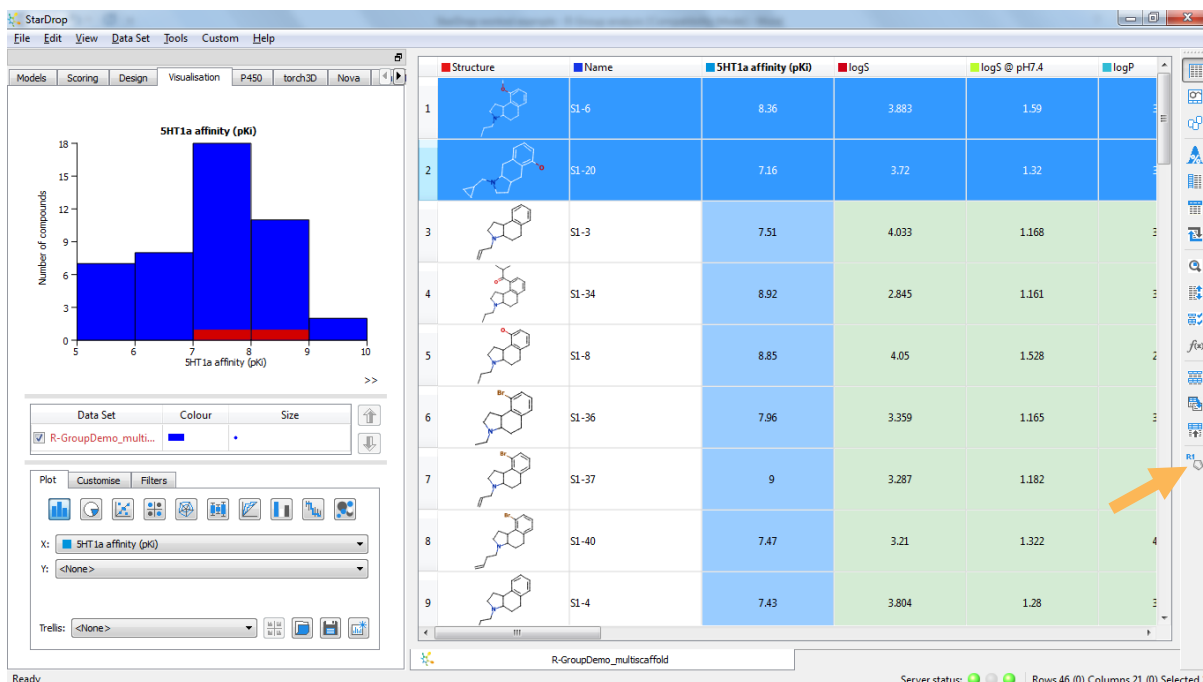
## Multi-scaffold R-group Analysis

We may have different series, based around multiple scaffolds, where the substitution points are equivalent because they share a similar binding pose. In these cases, 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.

- Open the file **R-GroupDemo\_multiscaffold** by using the **File -> Open** menu option.

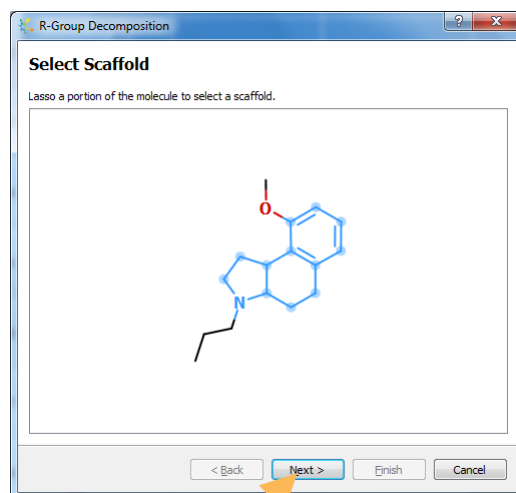


- In the resulting data set, you will note that there are two, similar scaffolds with equivalent substitution points. Examples of each are shown in rows 1 and 2, so select both of these rows (holding down the **CTRL** key to select the second row).

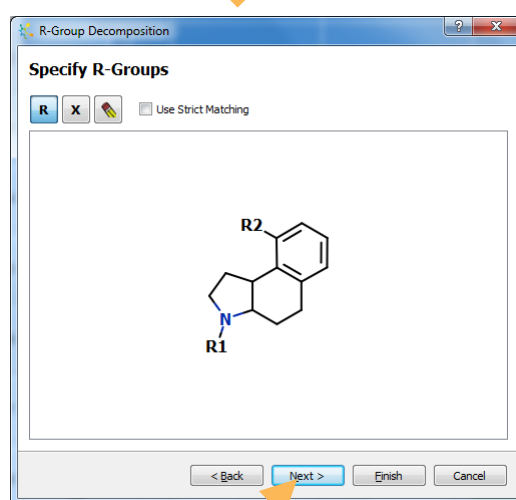


- Click the  button on the toolbar to open the **R-Group Decomposition** wizard.

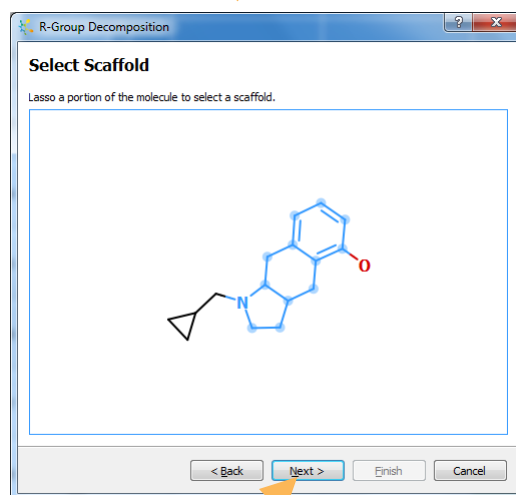
- The first compound will be shown in the **Select Scaffold** tool. Draw around the fused ring system to define this as the first scaffold (which will be highlighted in blue, as shown right) and click **Next**.



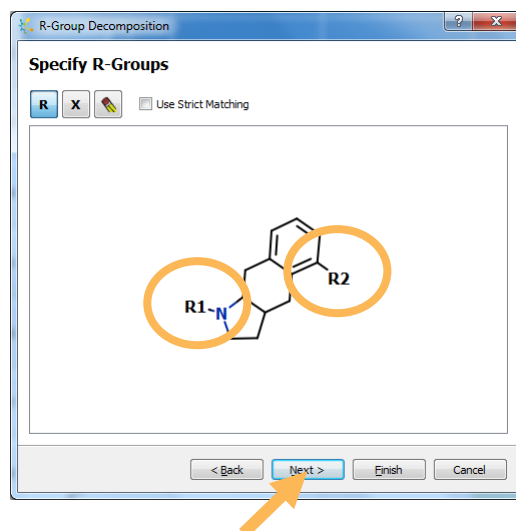
- The **Specify R-Groups** tool will automatically show groups R1 and R2 at the substitution points for the selected compounds, as shown right. We do not need to add any other points of variation, so click **Next** again.



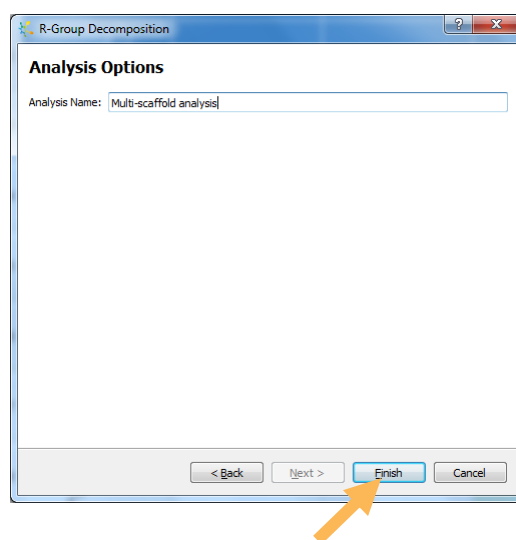
- The **Select Scaffold** tool will now be shown again, this time displaying the second compound. Again, draw around the fused ring system to define the second scaffold as highlighted in blue to the right. Click **Next** to define the R-groups for this scaffold.



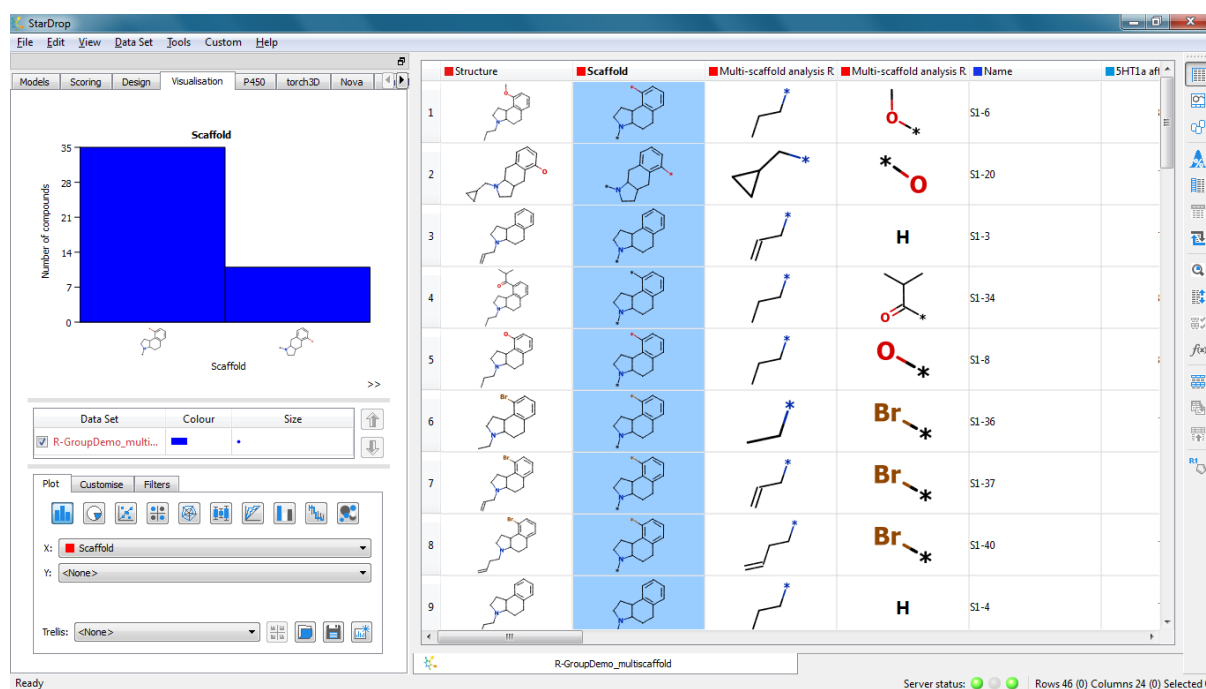
- The **Specify R-groups** tool will be shown again for this scaffold. In this case, it is important to ensure that the groups at equivalent positions in each scaffold have the same label (R1 and R2). If it is necessary to change the defaults, you can edit them simply by clicking on the label. Please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



- On the final page of the tool, you can give the analysis a name if you wish. Click **Finish** to run the R-group decomposition.

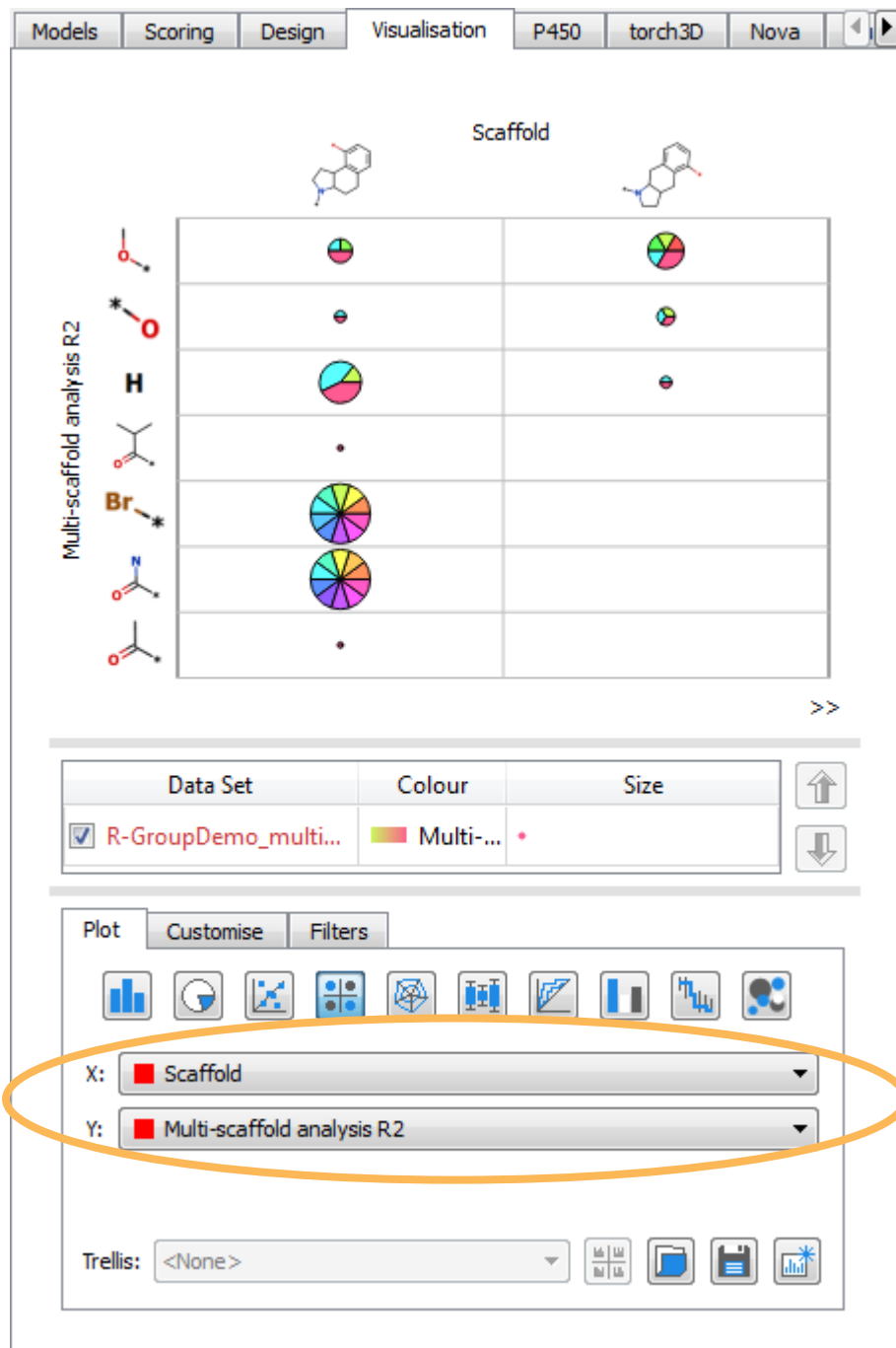


Three new columns will be added to the data set, showing the scaffold, R1 and R2 groups for each compound:





As with R-groups, the scaffold can be used in visualisations, including SAR plots, by selecting the corresponding column or choosing the scaffold from the **X:** or **Y:** menu in the **Visualisation** tab.



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