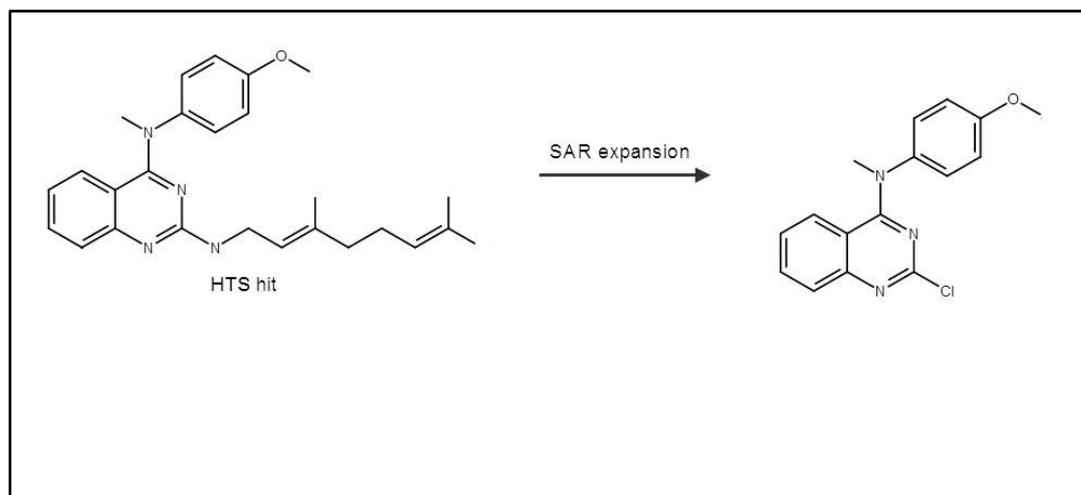


## What are the Known Activities of my Chemotype on Non Primary Target Classes?

An Apoptosis inducer 'chemotype' from a cell- and caspase-based apoptosis high-throughput screening was found (Compound 1). A structure activity relationship expansion lead to compound 2 (Schema1).



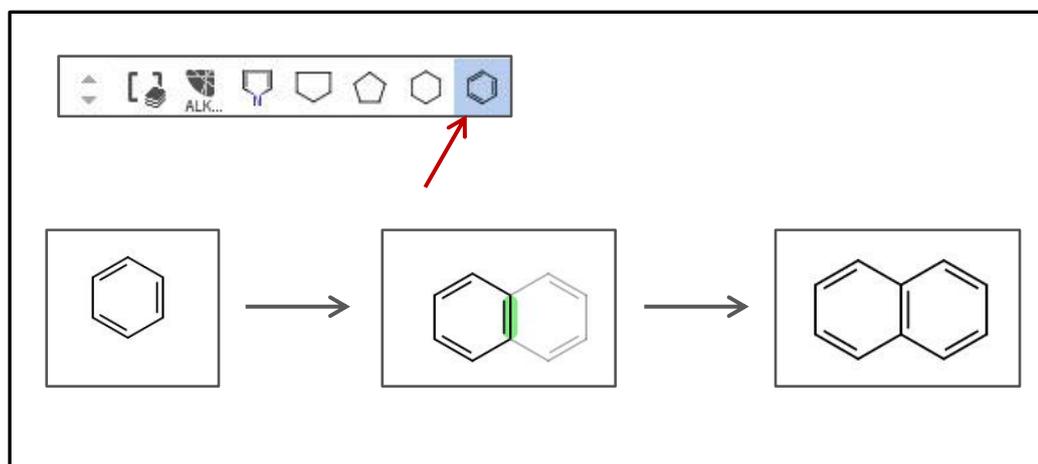
What is known about this chemotype/template (Compound 2) in Reaxys Medicinal Chemistry?

### ❖ Define Search Query

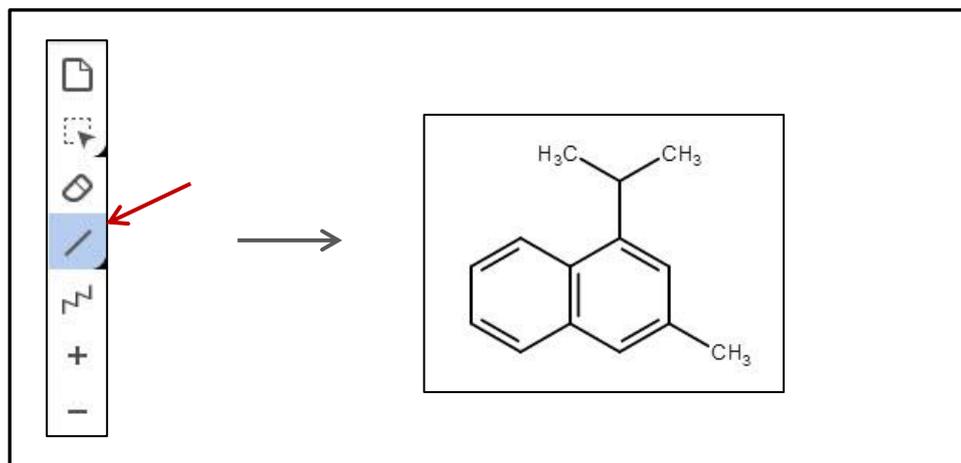
1. On the Reaxys home page click the **Create Structure or Reaction Drawing** box to open the structure editor (Marvin JS from ChemAxon).



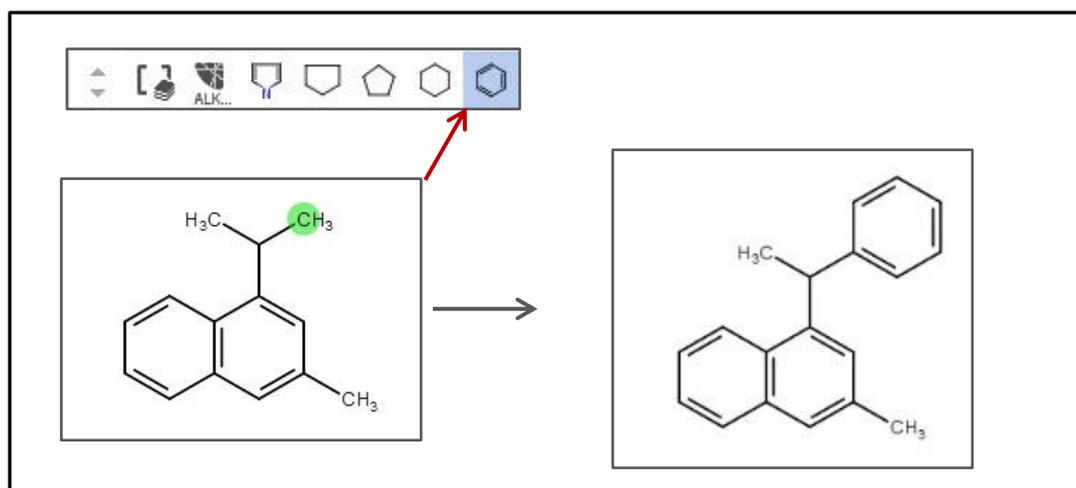
2. Select the **Benzene** tool in the Template Toolbar along the bottom
  - a. Click to place a benzene ring as shown
  - b. Click the right vertical bond of the existing ring to add a second benzene ring



3. Select the **Single bond** tool from the Tools Toolbar on the left
  - a. Add four single bonds as shown

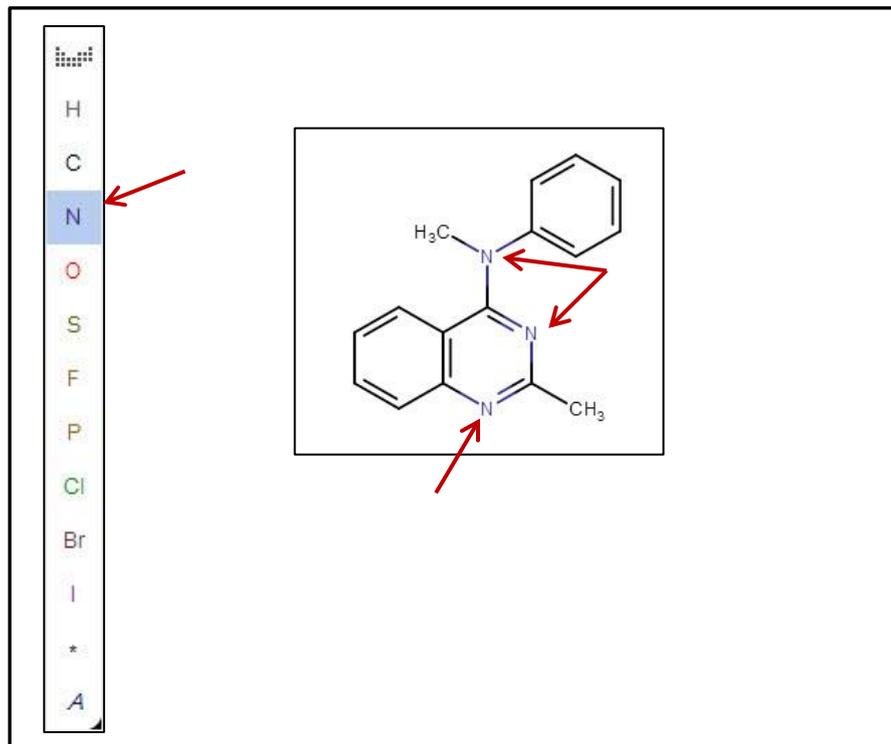


4. Select the **Benzene** tool
  - a. Click to place a benzene ring as shown



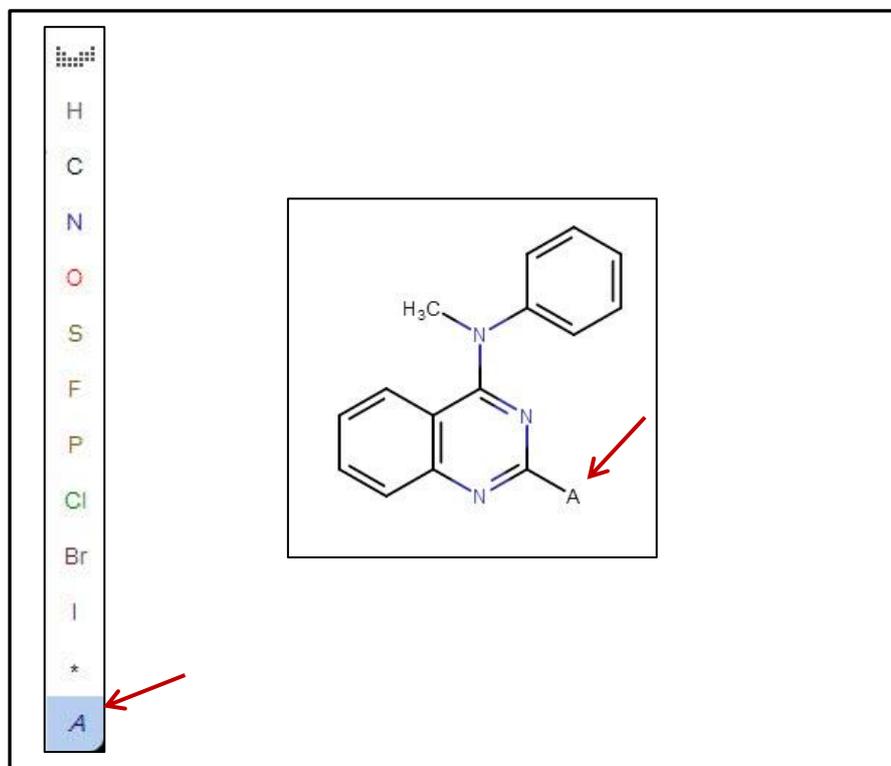
5. Change atoms as necessary:

a. Click 'N' in the atom toolbar, define the three Nitrogen atoms shown:

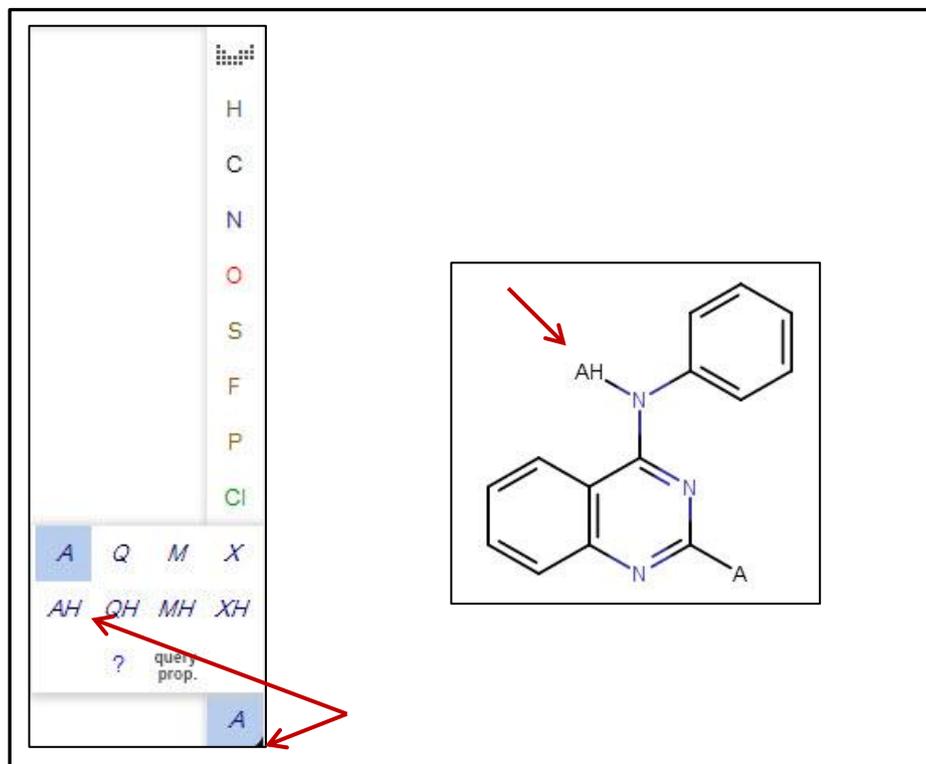


b. Click 'A' in the atom toolbar (for any atom except hydrogen)

c. Define the atom shown:

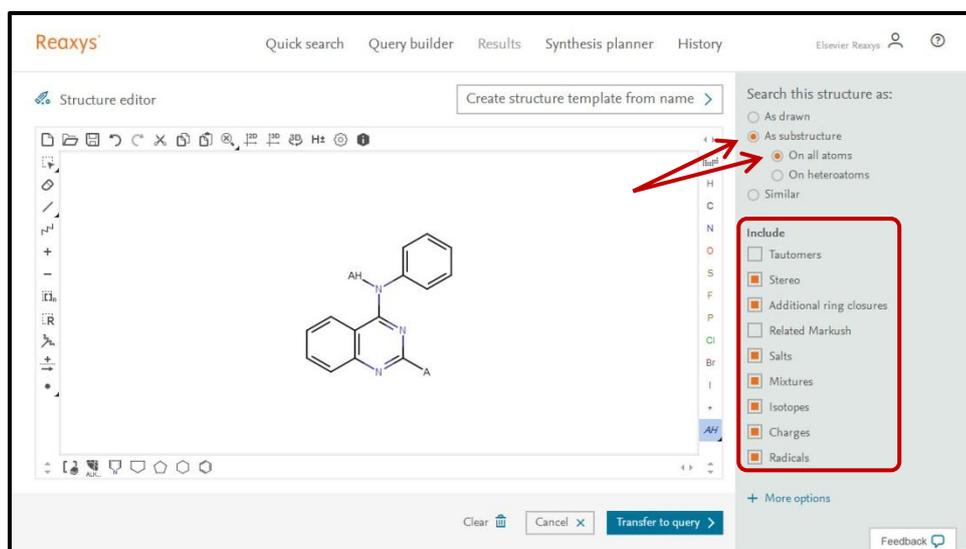


- d. Click 'AH' in the atom toolbar (for any atom including hydrogen)
- e. Define the atom shown:

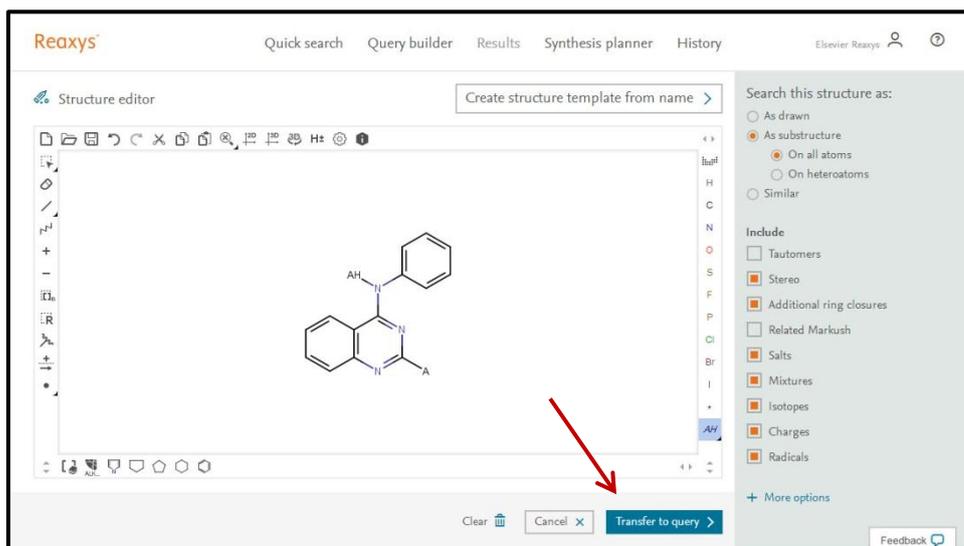


The query looks like this:

6. In the **Search this structure as:** panel, there are three options.
- **As drawn:** Reaxys will find results for the query as drawn
  - **As substructure:** Reaxys offers two sub-options:
    - **On all atoms** will substitute any explicit or implicit hydrogen with any other atom or group
    - **On heteroatoms** will do the same but only on heteroatoms
  - **Similar:** Reaxys will find results for a similarity search based on the drawn query
- a. Click **As substructure** and if necessary **On all atoms**
- b. Depending on the query and the type of answers you require you may wish to turn off some, or all, of the **Include** features. For this example, use the defaults which is all on except **Tautomers** and **Related Markush**



7. Click **Transfer to query**



## 8. Click **Find**.

The screenshot shows the Reaxys search interface. At the top, there are navigation tabs: Quick search, Query builder, Results, Synthesis planner, and History. A search bar is present with a 'Find >' button highlighted by a red arrow. Below the search bar, a chemical structure is displayed within a dashed box, labeled 'AND'. The structure is a benzimidazole ring system with a phenyl group attached to the nitrogen atom and an 'A' substituent on the benzimidazole ring. The interface also shows 'REAXYS Version 1' and 'On all atoms' options.

The Results Preview is displayed.

- Reaxys Medicinal Chemistry will present a Results Preview showing different variations of the entered query to provide you with options, which you may not have thought of at query formulation time:
  - Substances having the drawn chemotype.
  - Targets on which the drawn chemotype was test on.
  - Reactions performed to synthesized substances having the drawn chemotype.

The screenshot shows the Reaxys search results page. The search bar contains the chemical structure from the previous step. The results are displayed in three sections:
 

- Substances:** 6,368 results. Filter: Structure: substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments. View Results >
- Targets:** 486 results. Filter: Structure: substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments. View Results >
- Reactions:** 7,807 results. Filter: Product(s): substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments. View Results >

 The interface also shows 'New' and 'Edit' icons, and a 'Feedback' button at the bottom right.

9. Click **View Results** for the first result set (Substances).

The screenshot shows the Reaxys search results page. At the top, there are navigation tabs: Quick search, Query builder, Results, Synthesis planner, and History. Below the navigation, there are icons for 'New' and 'Edit'. The main content area displays three result sets:

- 6,368 Substances**: Structure: substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments. A red box highlights the 'Substances' label, and a red arrow points to the 'View Results' button.
- 486 Targets**: Structure: substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments.
- 7,807 Reactions**: Product(s): substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments.

Each result set has a 'Preview Results' dropdown menu and a 'View Results' button. A 'Feedback' button is located at the bottom right of the results area.

The Results Page is displayed showing the substances having this chemotype.

The screenshot shows the Reaxys Results page. The 'Results' tab is selected. The page displays a list of substances with their chemical structures and associated data. The 'Substances' result set is selected, and the page displays a list of substances with their chemical structures and associated data.

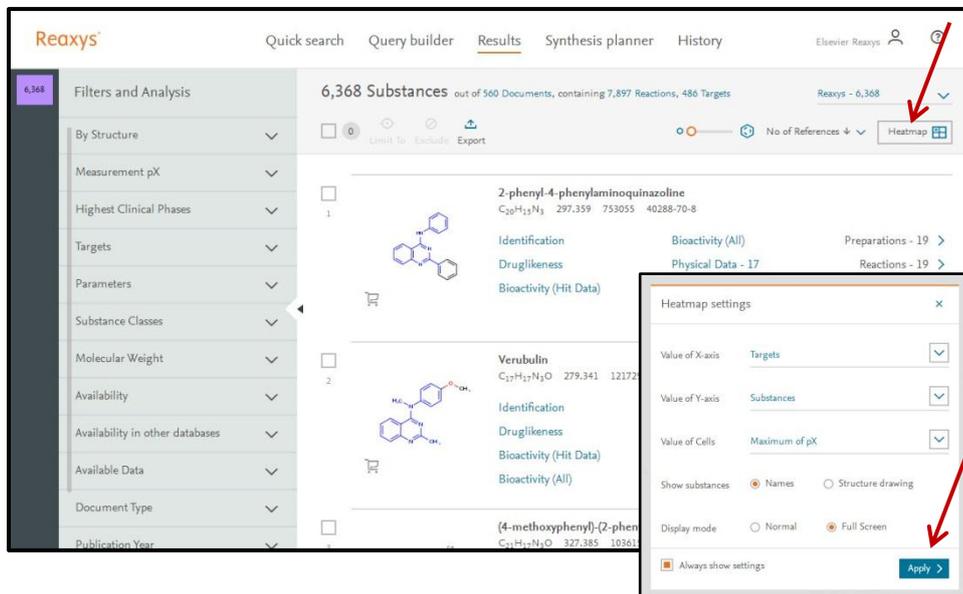
The left sidebar shows a 'Filters and Analysis' panel with various filters such as 'By Structure', 'Measurement pX', 'Highest Clinical Phases', 'Targets', 'Parameters', 'Substance Classes', 'Molecular Weight', 'Availability', 'Availability in other databases', 'Available Data', 'Document Type', and 'Publication Year'.

The main content area shows a list of substances with their chemical structures and associated data. The first substance is 2-phenyl-4-phenylaminoquinazoline (C<sub>20</sub>H<sub>13</sub>N<sub>3</sub>, 297.359, 753055, 40288-70-8). The second substance is Verubulin (C<sub>27</sub>H<sub>17</sub>N<sub>3</sub>O, 279.341, 12172991). The third substance is (4-methoxyphenyl)-(2-phenylquinazolin-4-yl)-amine (C<sub>21</sub>H<sub>17</sub>N<sub>3</sub>O, 327.385, 10361553).

Each substance entry includes a chemical structure, a name, a molecular formula, and a list of associated data points such as 'Identification', 'Bioactivity (All)', 'Physical Data', 'Spectra', 'Preparations', 'Reactions', 'Targets', and 'Documents'.

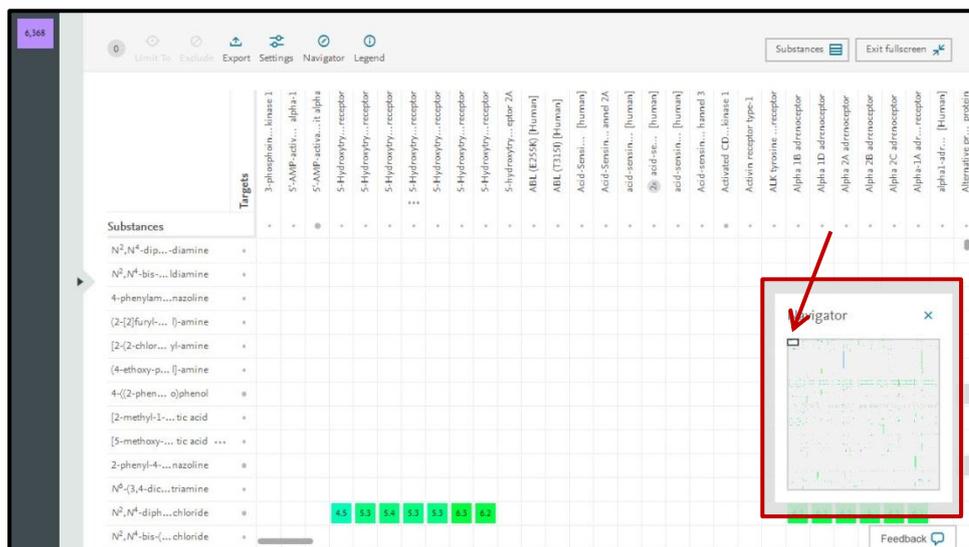
## ❖ View the Heatmap

1. Click **Heatmap**.
  - a. Review the Settings and click **Apply**.



The Heatmap is displayed providing an overview of the Structure activity relationship.

As there are many compounds and targets retrieved by the search, the screen displays only a subset of the whole heatmap. The whole heatmap is shown in the Navigator and what is seen on the screen is outlined by a rectangle.



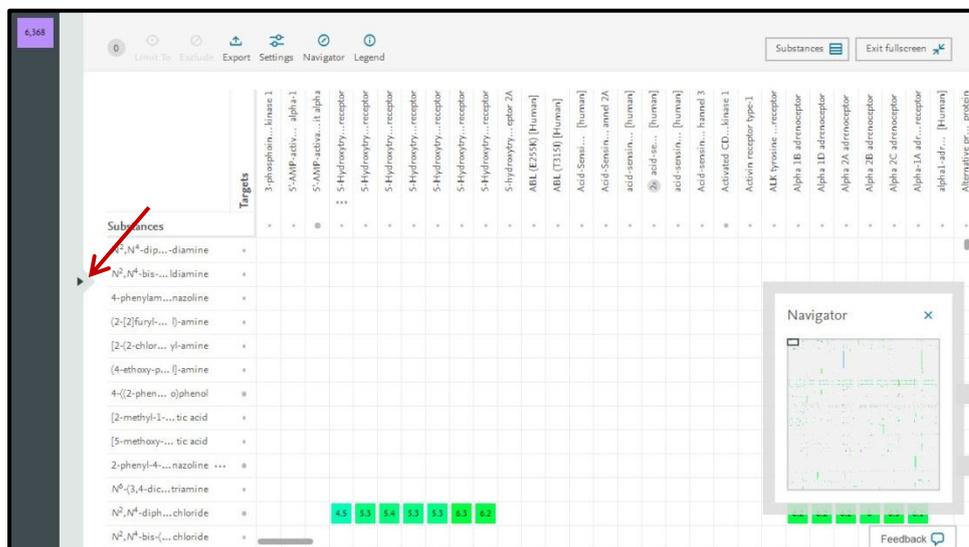
- By **dragging and dropping** this rectangle to another location within the Navigator, users are able to explore the entire heatmap.



- Return the **Navigator rectangle** to the upper left.

Nevertheless, in order to focus on the most interesting compounds, Filters on the left side are available.

- Click to display the **Filters and Analysis** panel.



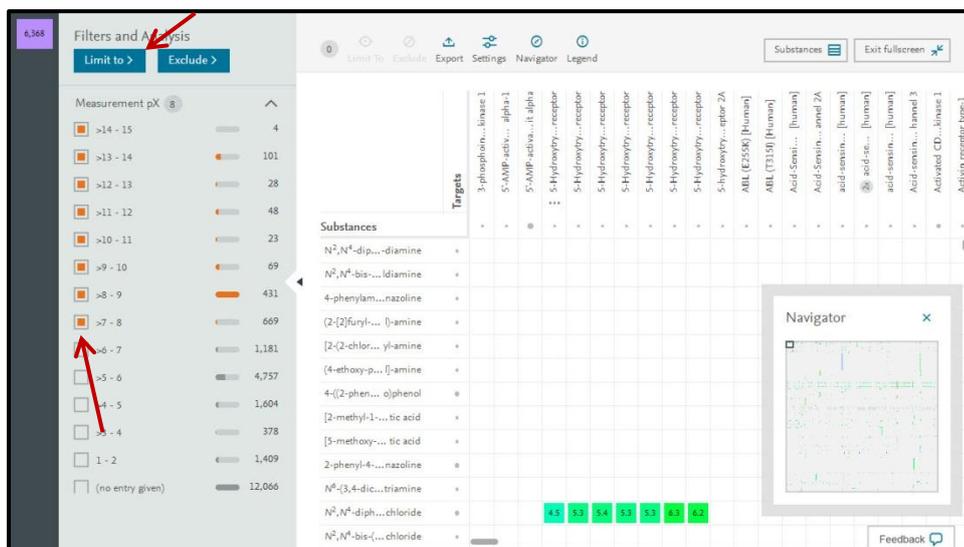
4. In the *Filters and Analysis* panel, expand **Target Species**.
  - a. Check the box for **human**.

The screenshot shows the 'Filters and Analysis' panel on the left. Under 'Target Species', the 'human' checkbox is checked, and the count is 6,781. Other species listed include *rattus norvegicus* (1,078), human immunodeficiency virus 1 (163), human immunodeficiency virus 1 (strain nl4.3) (130), pig (125), human immunodeficiency virus (80), and *mus musculus* (47). The main table displays a list of targets and substances. The 'Targets' column lists various receptors and enzymes, and the 'Substances' column lists their corresponding chemical names. A 'Navigator' window is open in the bottom right, showing a chemical structure.

5. Expand **Measurement pX**.
  - a. Click **More**

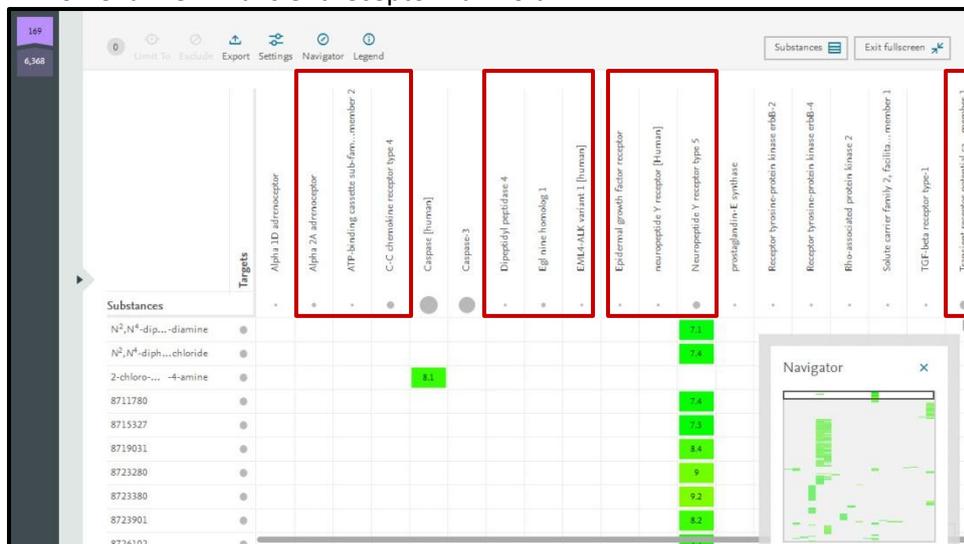
The screenshot shows the 'Filters and Analysis' panel on the left. Under 'Measurement pX', the 'More' button is highlighted with a red arrow. The 'Target Species' section is still visible, with 'human' selected. The main table displays a list of targets and substances. The 'Targets' column lists various receptors and enzymes, and the 'Substances' column lists their corresponding chemical names. A 'Navigator' window is open in the bottom right, showing a chemical structure.

- Check ***pX values over 7*** (affinity below 0.1 $\mu$ M) to select active compounds.
- Click **Limit to**



The chemotype potentially bind on the following targets:

- GPCR : Alpha2a Adrenergic, Chemokine CCR4, Neuropeptide Y5
- Kinase : EGFR, Rock2, tgfb1
- Histone : Histone deacetylase
- Peptidase : Dipeptidyl peptidase 4
- Ion Channel : Transient receptor Vanilloid 1



Heatmap settings allow you to display the *chemical structure* in the heatmap instead of names (the default display).

## 6. Click **Settings**

- a. Turn on **Structure drawing** and click **Apply**

The screenshot shows the Reaxys interface with a heatmap. The 'Settings' dialog box is open, showing options for 'Value of X-axis' (Targets), 'Value of Y-axis' (Substances), and 'Value of Cells' (Maximum of pK). Under 'Show substances', the 'Structure drawing' radio button is selected. The 'Apply' button is highlighted with a red arrow.

Sort compounds by descending bioactivities on Caspase 3:

## 7. Click the **Caspase 3 three dots** and **Sort by activity**.

The screenshot shows the Reaxys interface with the heatmap sorted by activity on the 'Caspase 3' column. A context menu is open over the 'Caspase 3' column header, with 'Sort by activity' selected. Red arrows point to the three-dot menu icon and the 'Sort by activity' option. A 'Navigator' window is also visible in the bottom right.

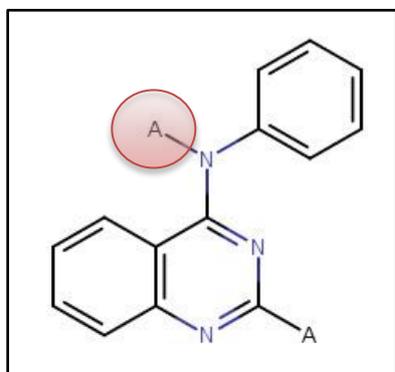
Looking at the chemical compounds carefully, you see that compounds active on caspase have a methyl group on the aniline nitrogen, whereas all the other active compounds on non-caspase target have no methyl substituent in the same position.

It appears that this methyl group is very important for the selectivity on caspase versus other targets.

The screenshot shows the Reaxys interface with a list of targets on the right and a list of substances on the left. The 'Caspase [human]' target is highlighted in green. A detail window for substance 24281859 is open, showing its chemical structure and properties:

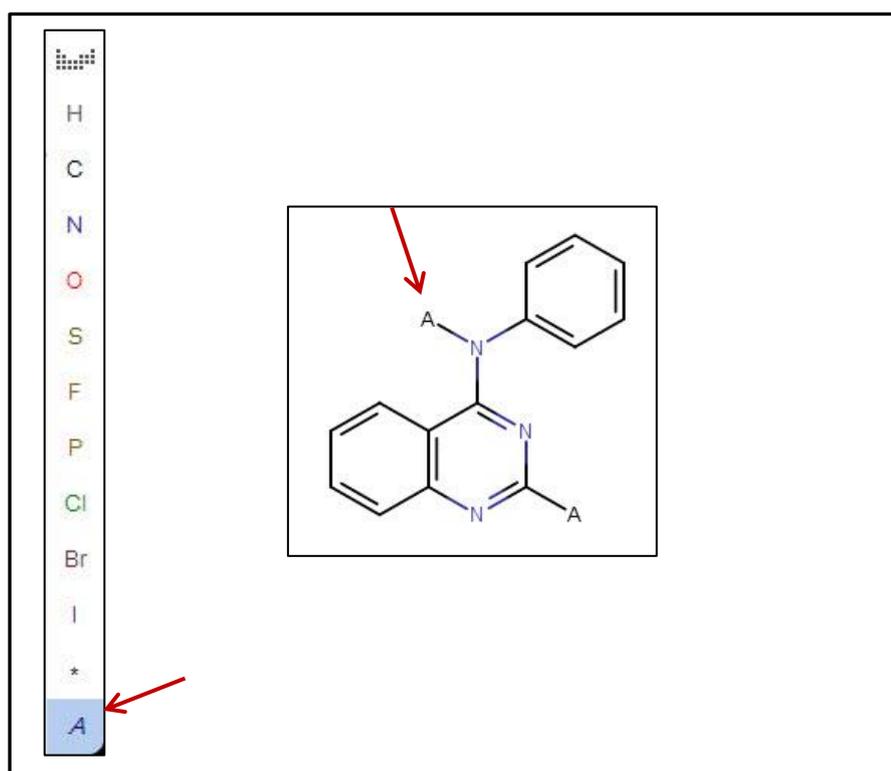
- Reaxys ID: 24281859
- Molecular Formula:  $C_{17}H_{16}FN_3O^+$ ...
- Molecular Weight: 333.793

How do we check this? Let's perform a new search by changing the substructure search to:



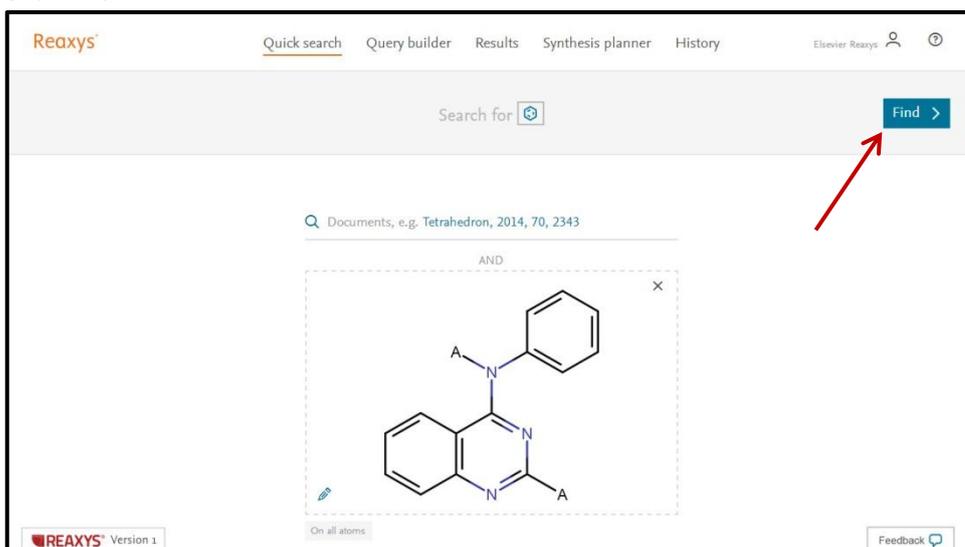
## ❖ Revise Search Query

1. Click **Exit fullscreen**
  - a. Click **Quick search**
  - b. On the Quick search page, click **the structure**
2. Click **'A'** in the atom toolbar (for *any atom except hydrogen*)
  - a. Define the atom shown:



3. If necessary, click **As substructure** and **On all atoms**
  - a. Click **Transfer to query**

4. Click **Find**.



Reaxys® Quick search Query builder Results Synthesis planner History Elsevier Reaxys

Search for  Find >

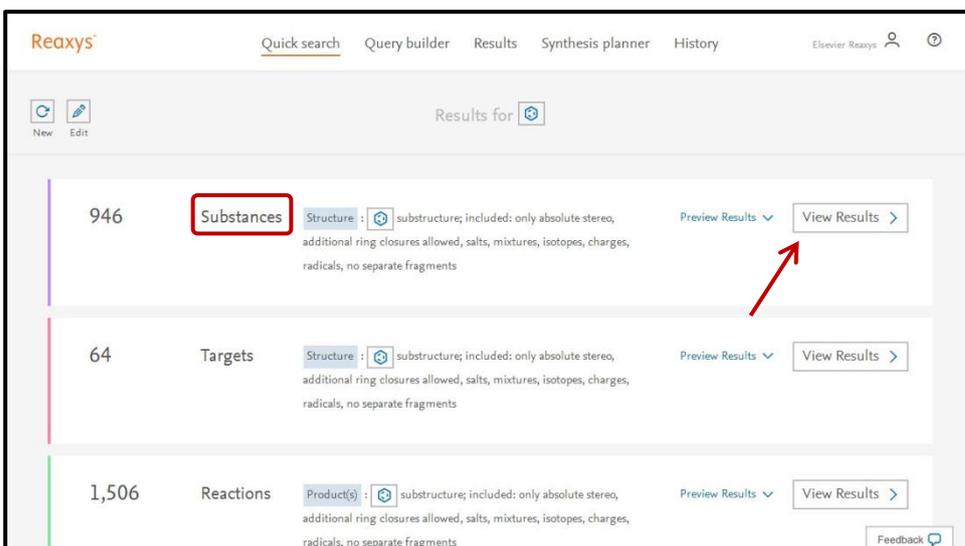
Q Documents, e.g. Tetrahedron, 2014, 70, 2343

AND

Chemical structure: A benzimidazole ring system with a phenyl group attached to the nitrogen atom and an 'A' substituent at the 2-position.

REAXYS® Version 1 On all atoms Feedback

5. Click **View Results** for the first result set (Substances).



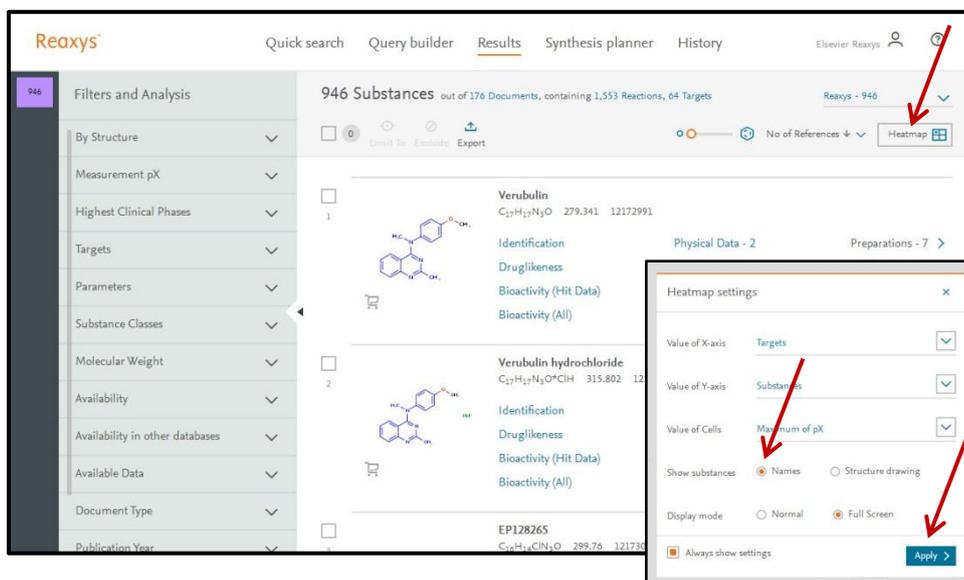
Reaxys® Quick search Query builder Results Synthesis planner History Elsevier Reaxys

New Edit Results for

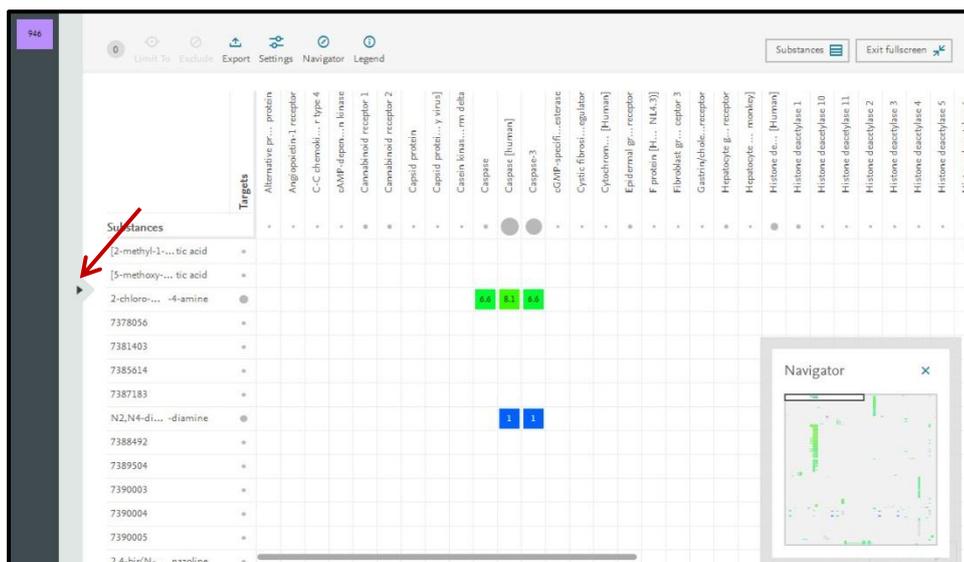
946	<b>Substances</b>	Structure: <input type="text"/> substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results <input type="text"/> View Results >
64	Targets	Structure: <input type="text"/> substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results <input type="text"/> View Results >
1,506	Reactions	Product(s): <input type="text"/> substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results <input type="text"/> View Results >

Feedback

6. On the Results Page, click **Heatmap**.
  - a. If necessary, turn on **Names** and click **Apply**.



7. Click to display the **Filters and Analysis** panel.



8. In the *Filters and Analysis* panel:
  - a. Filter by **Target Species = human**
  - b. Filter by **Measurement pX >7**
  - c. Click **Limit to**

Compounds are mainly active on Caspase 3. Consequently methyl groups on the aniline nitrogen are mandatory for caspase activity and selectivity.