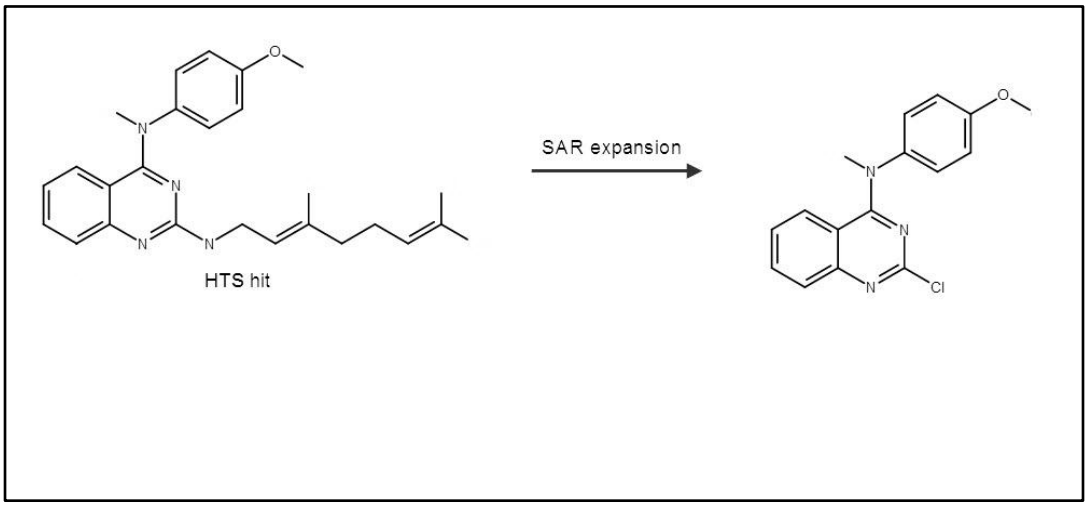
What are the Known Activities of my Chemotype on Non Primary Target Classes?

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).

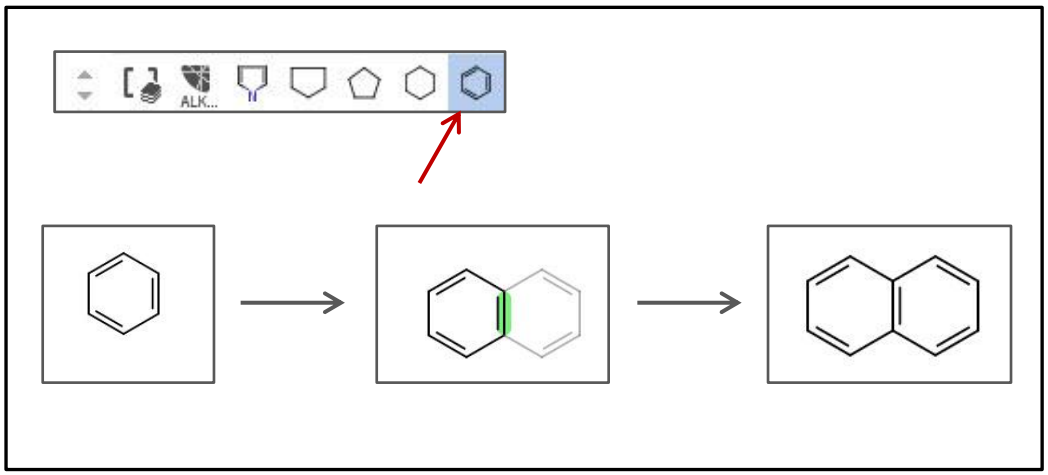


What is known about this chemotype/template (Compound 2) in Reaxys Medicinal Chemistry?

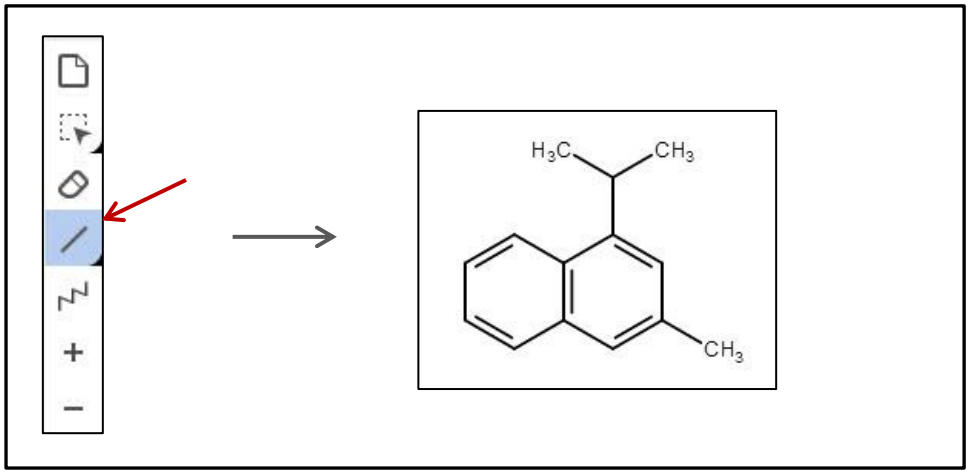
❖ Define Search Query

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

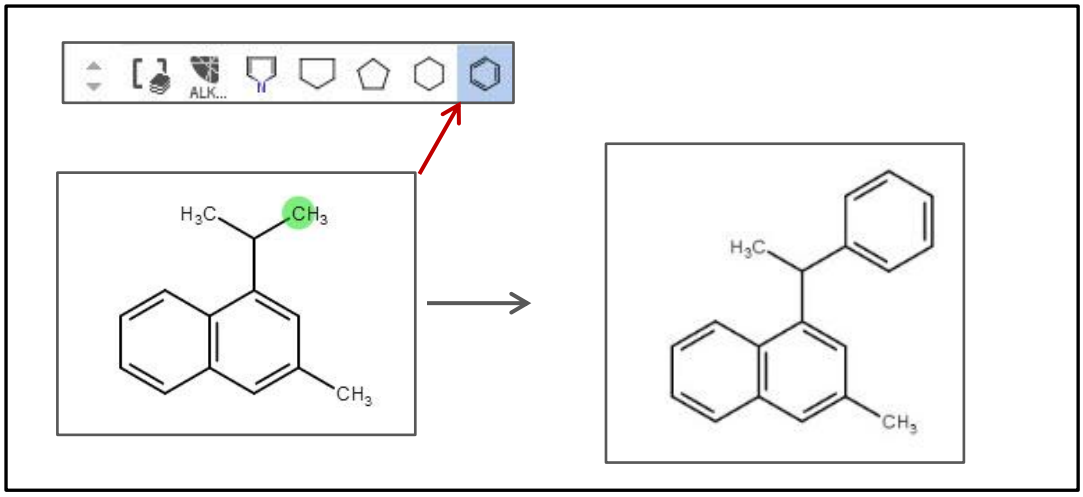
2. Select the ***Benzene*** tool in the Template Toolbar along the bottom
   a. Click to place a benzene ring as shown
   b. Click the right vertical bond of the existing ring to add a second benzene ring



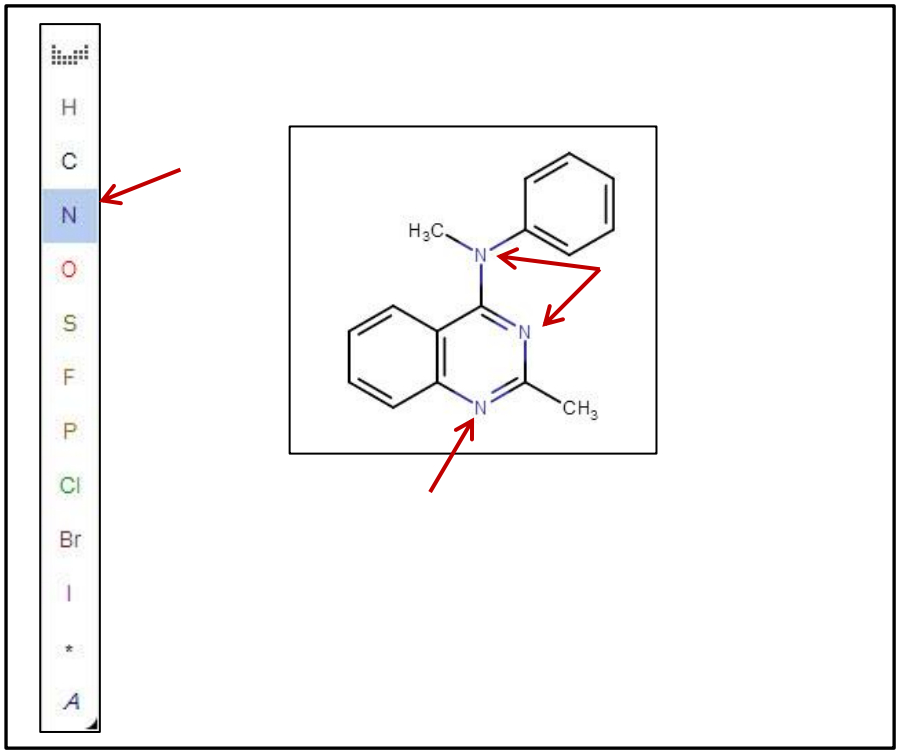
3. Select the ***Single bond*** tool from the Tools Toolbar on the left
    a. Add four single bonds as shown



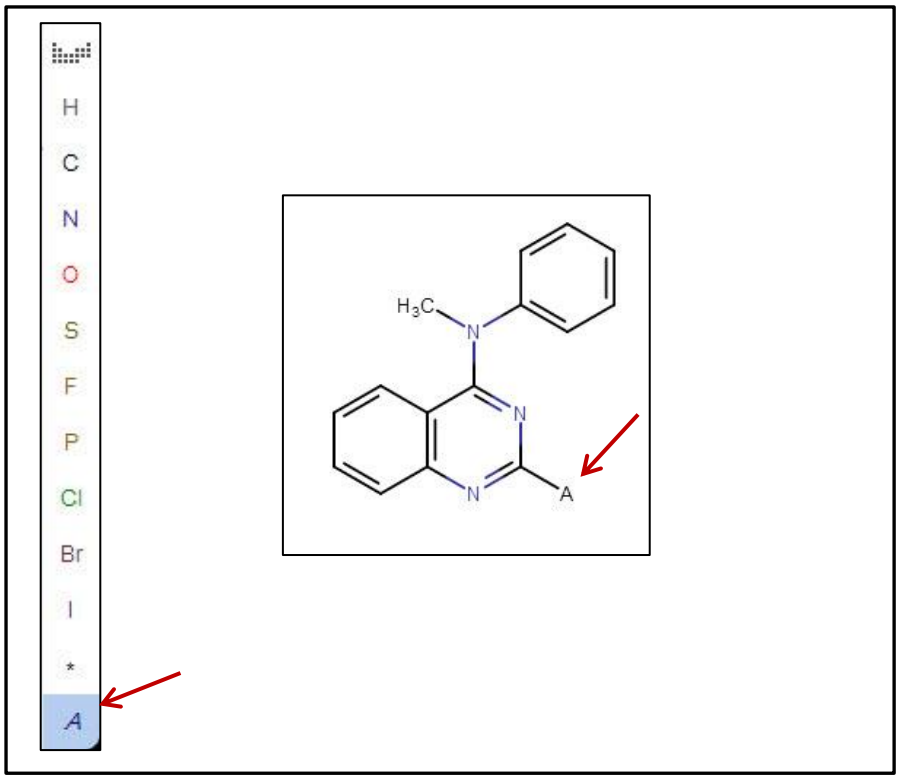
4. Select the ***Benzene*** tool
    a. Click to place a benzene ring as shown



5. Change atoms as necessary:
   a. Click '***N'*** in the atom toolbar, define the three Nitrogen atoms shown:

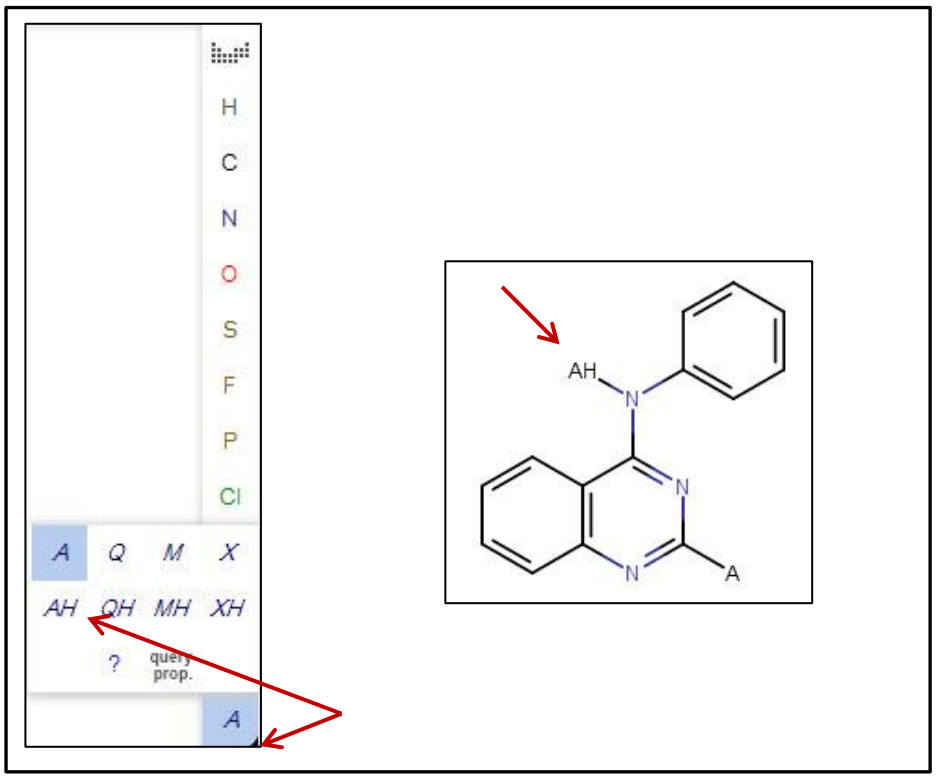


   b. Click '***A'*** in the atom toolbar (for *any atom except hydrogen)*
   c. Define the atom shown:

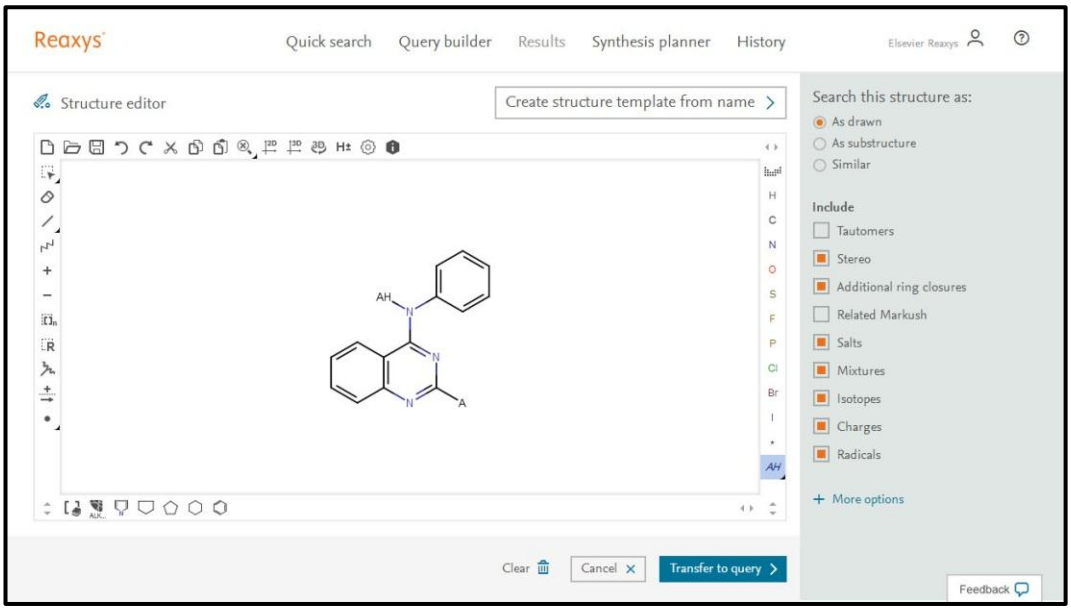


d. Click '***AH'*** in the atom toolbar (for *any atom including hydrogen)*

e. Define the atom shown:

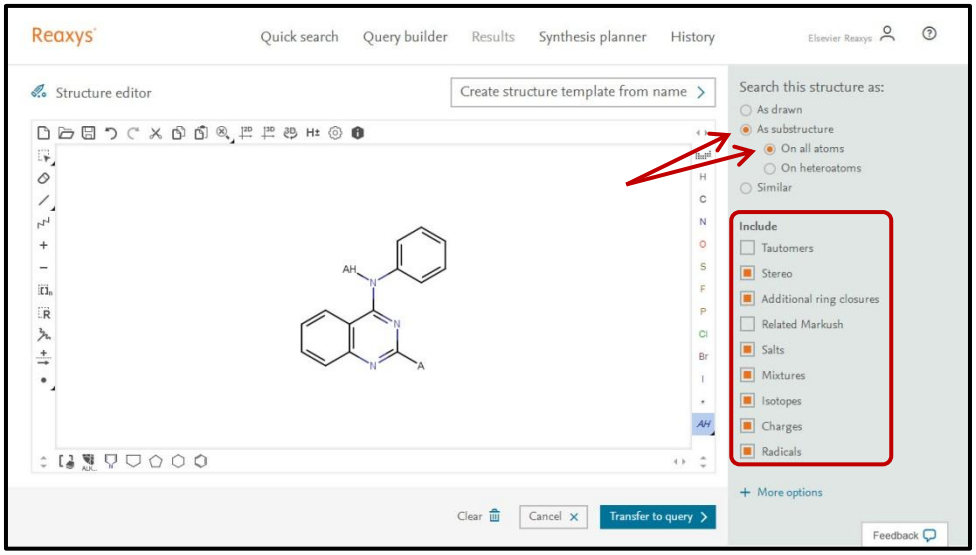


The query looks like this:

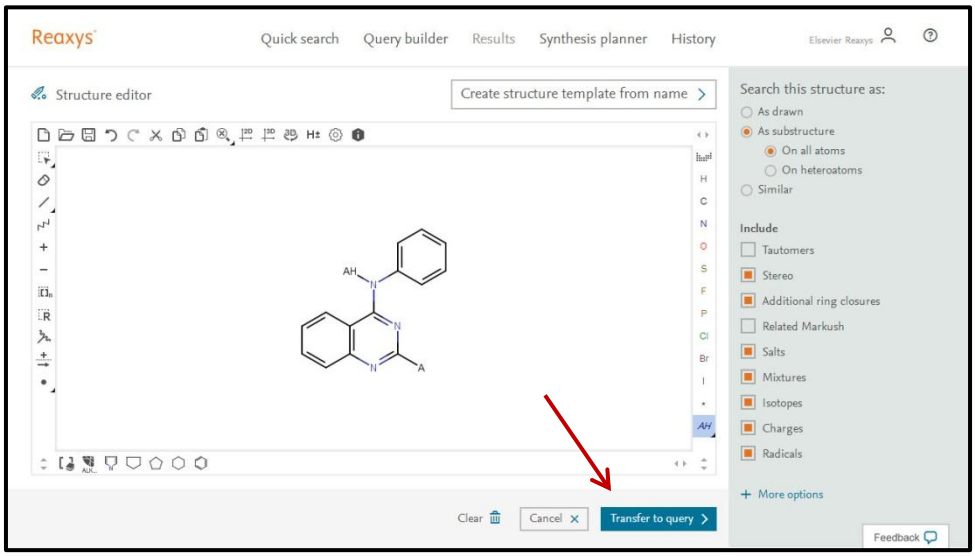


6. In the **Search this structure as**: panel, there are three options.
    - **As drawn**: Reaxys will find results for the query as drawn
    - **As substructure**: Reaxys offers two sub-options:
        - **On all atoms** will substitute any explicit or implicit hydrogen with any other atom or group
        - **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*



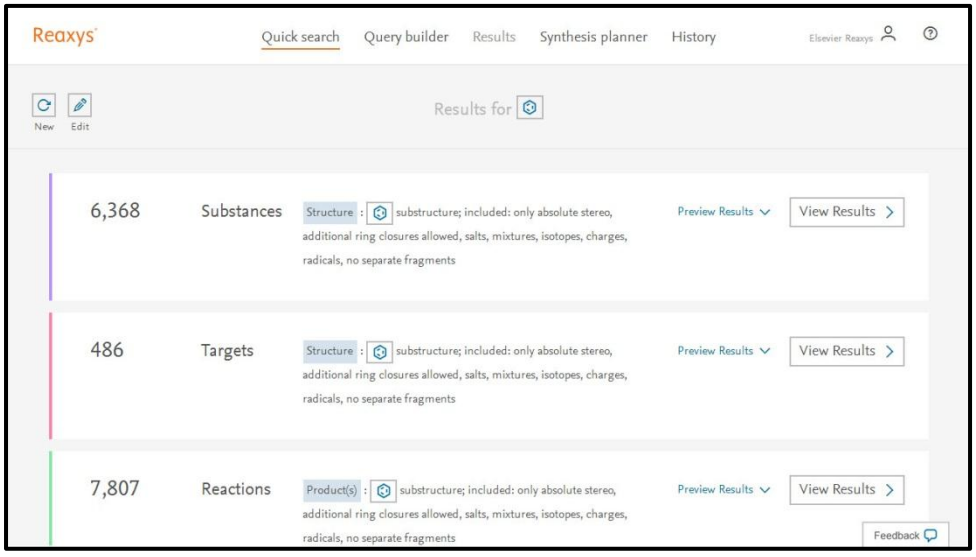
7. Click ***Transfer to query***



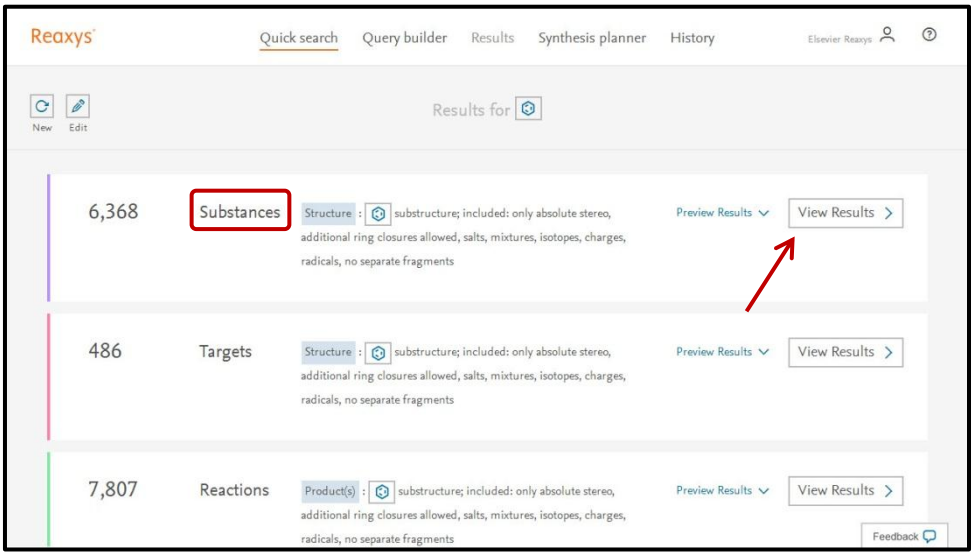
8. Click ***Find***.

The Results Preview is displayed.

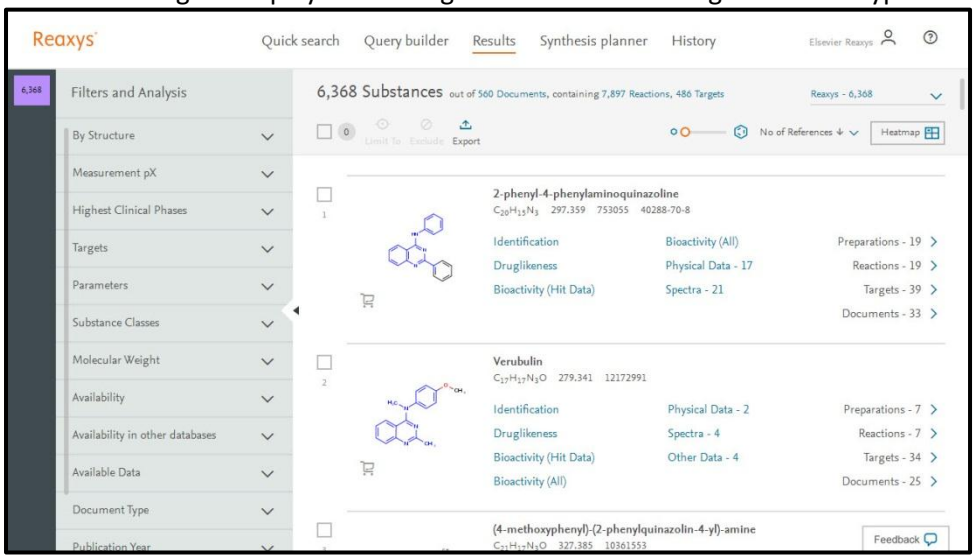
- Reaxys Medicinal Chemistry will present a Results Preview showing different variations of the entered query to provide you with options, which you may not have thought of at query formulation time:
  - Substances having the drawn chemotype.
  - Targets on which the drawn chemotype was test on.
  -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



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

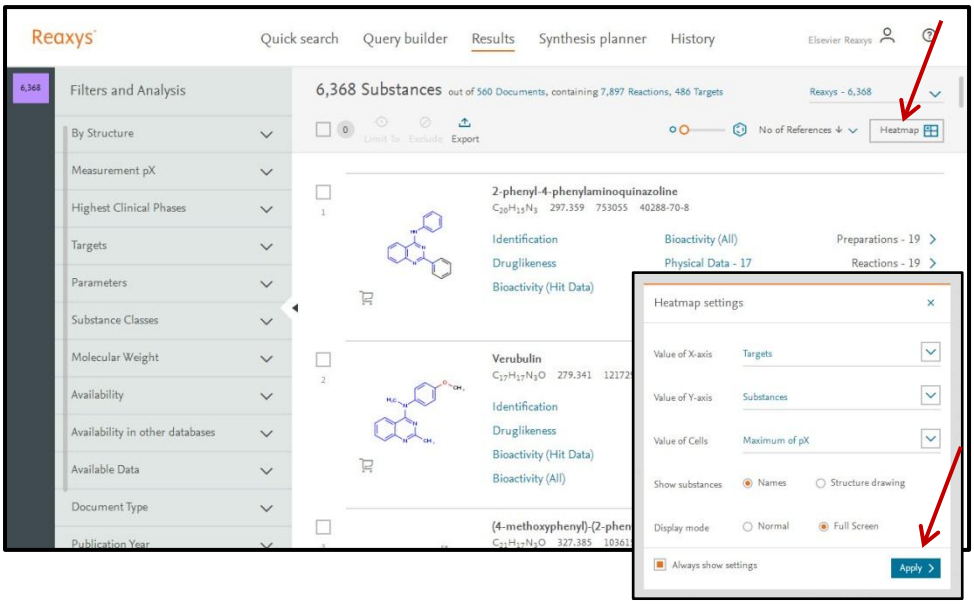


The Results Page is displayed showing the substances having this chemotype.



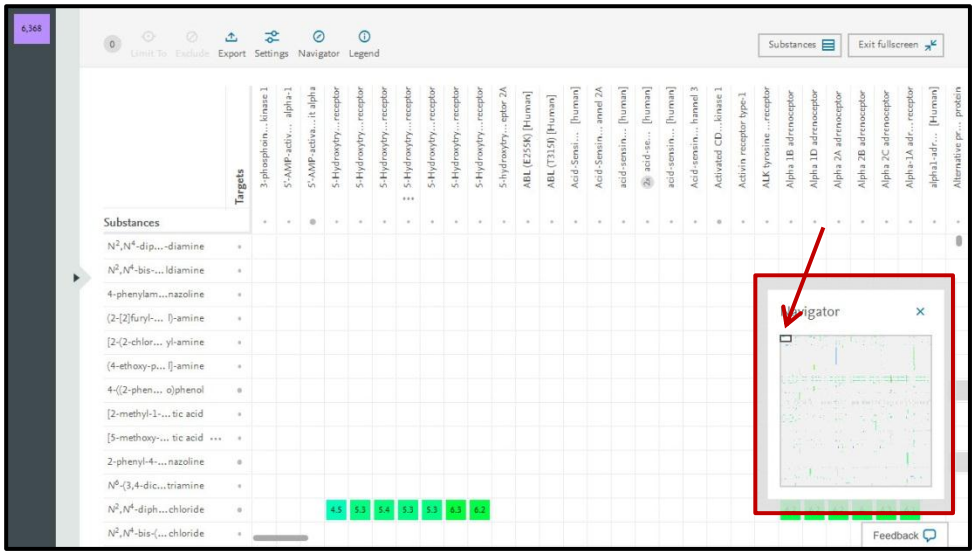
❖ View the Heatmap

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

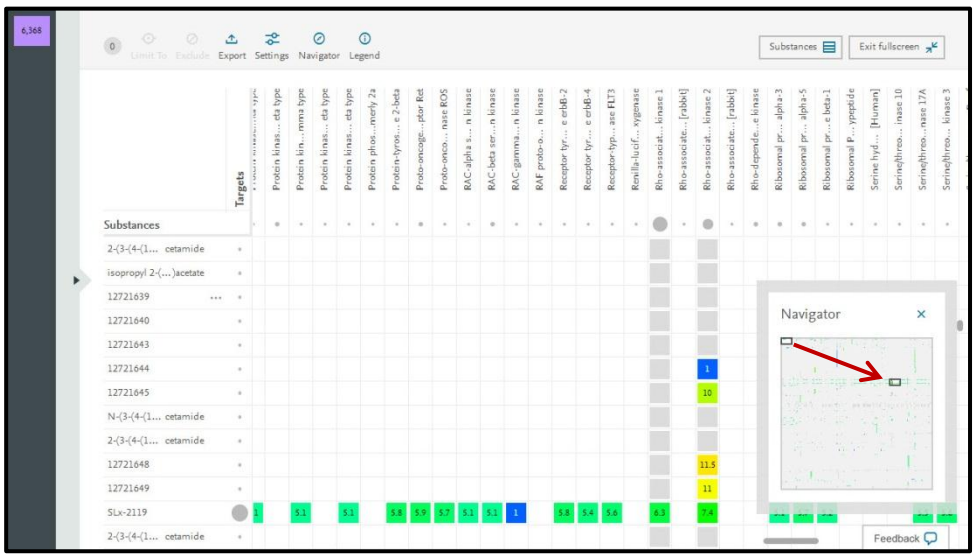


The Heatmap is displayed providing an overview of the Structure activity relationship.

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.



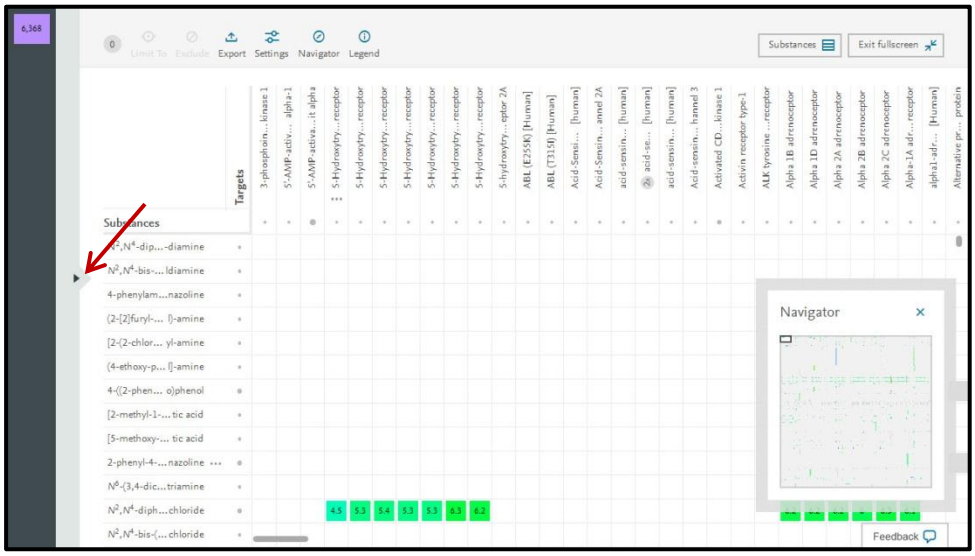
2. By **dragging and dropping** this rectangle to another location within the Navigator, users are able to explore the entire heatmap.



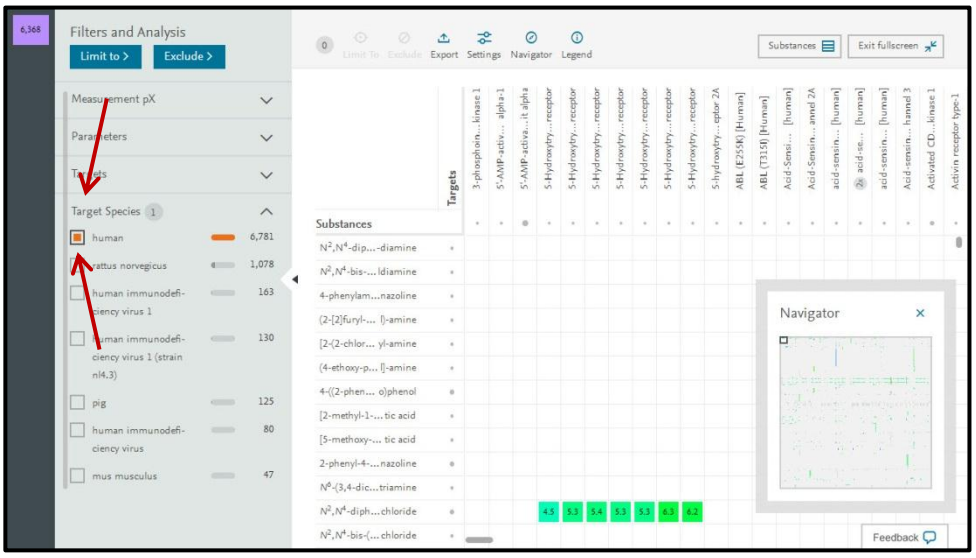
a. **Return the Navigator rectangle** to the upper left.

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

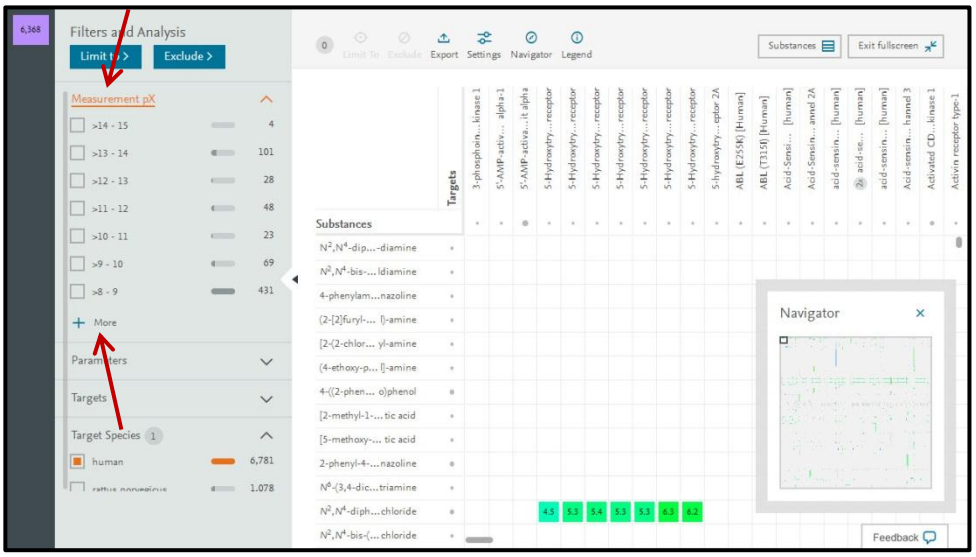
3. Click to display the ***Filters and Analysis*** panel.



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

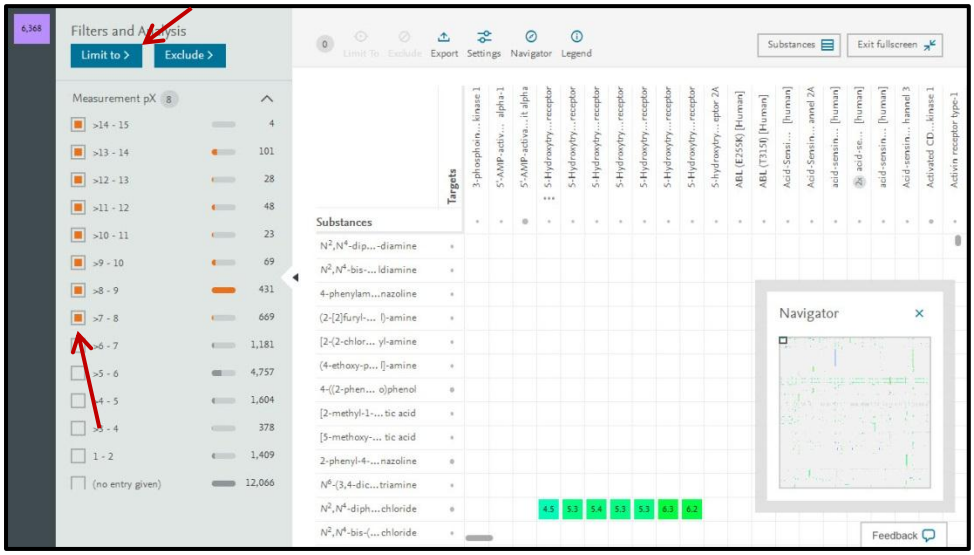


5. Expand ***Measurement pX.***
    a. Click ***More***



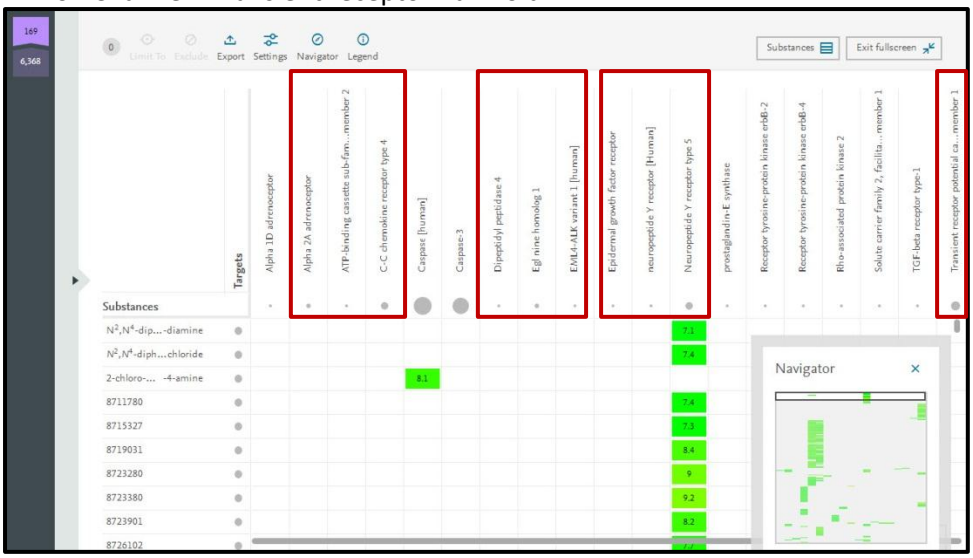
b. Check ***pX values over 7*** (affinity below 0.1µM) to select active compounds.

c. Click ***Limit to***



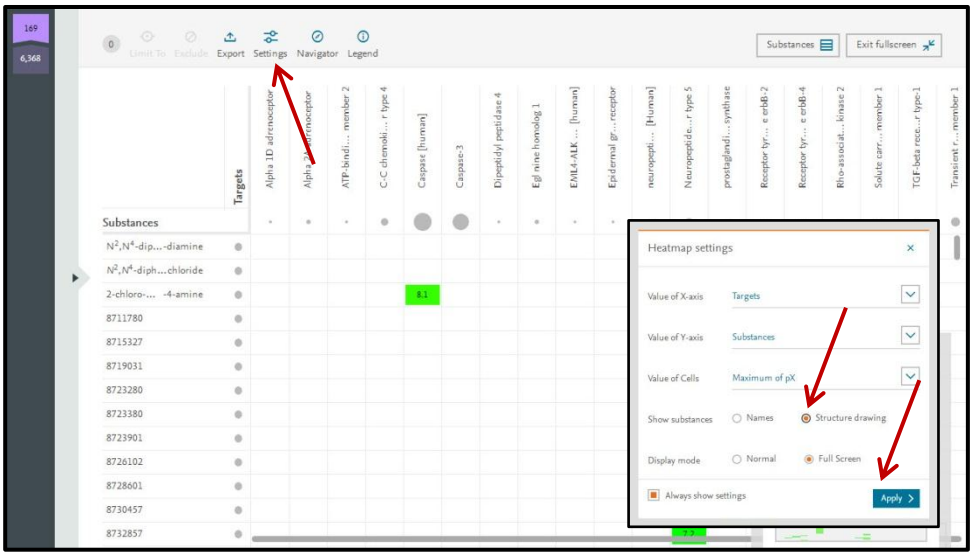
The chemotype potentially bind on the following targets:

- GPCR : Alpha2a Adrenergic, Chemokine CCR4, Neuropeptide Y5
- Kinase : EGFR, Rock2, tgfbr1
- Histone : Histone deacetylase
- Peptidase : Dipeptidyl peptidase 4
- Ion Channel : Transient receptor Vanilloid 1



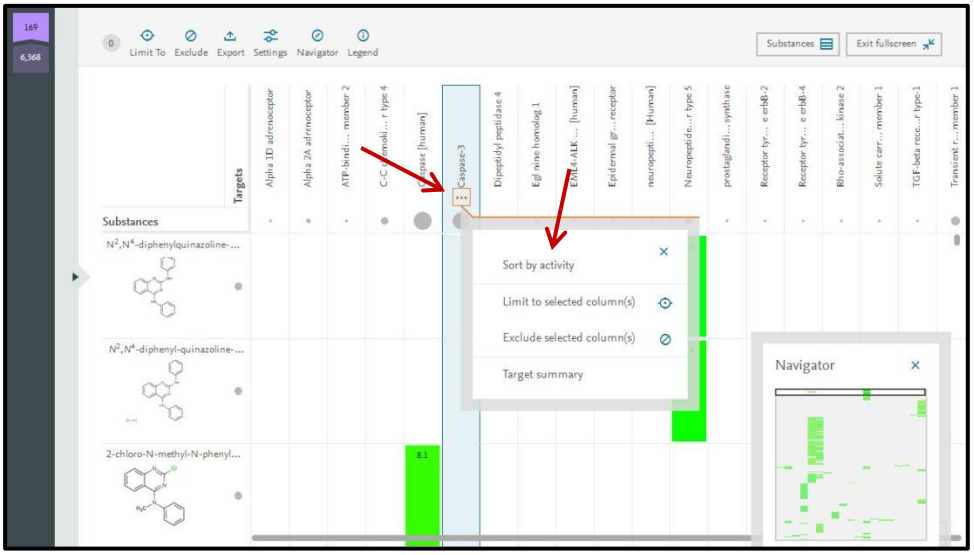
Heatmap settings allow you to display the *chemical structure* in the heatmap instead of names (the default display).

6. Click ***Settings***
    a. Turn on ***Structure drawing*** and click ***Apply***



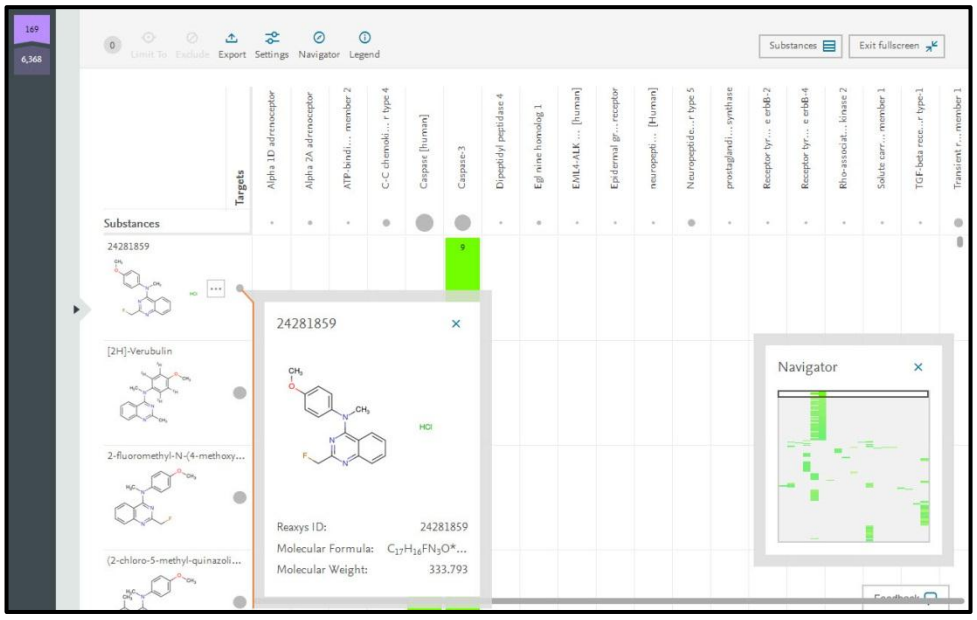
Sort compounds by descending bioactivities on Caspase 3:

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

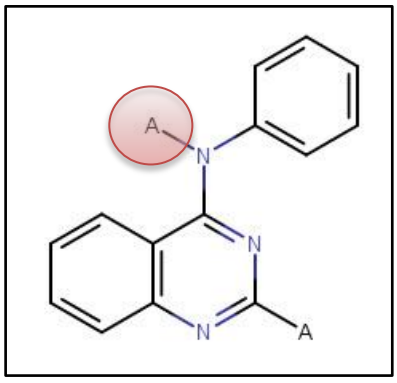


Looking at the chemical compounds carefully, you see that compounds active on caspase have a methyl group on the aniline nitrogen, whereas all the other active compounds on non-caspase target have no methyl substituent in the same position.

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

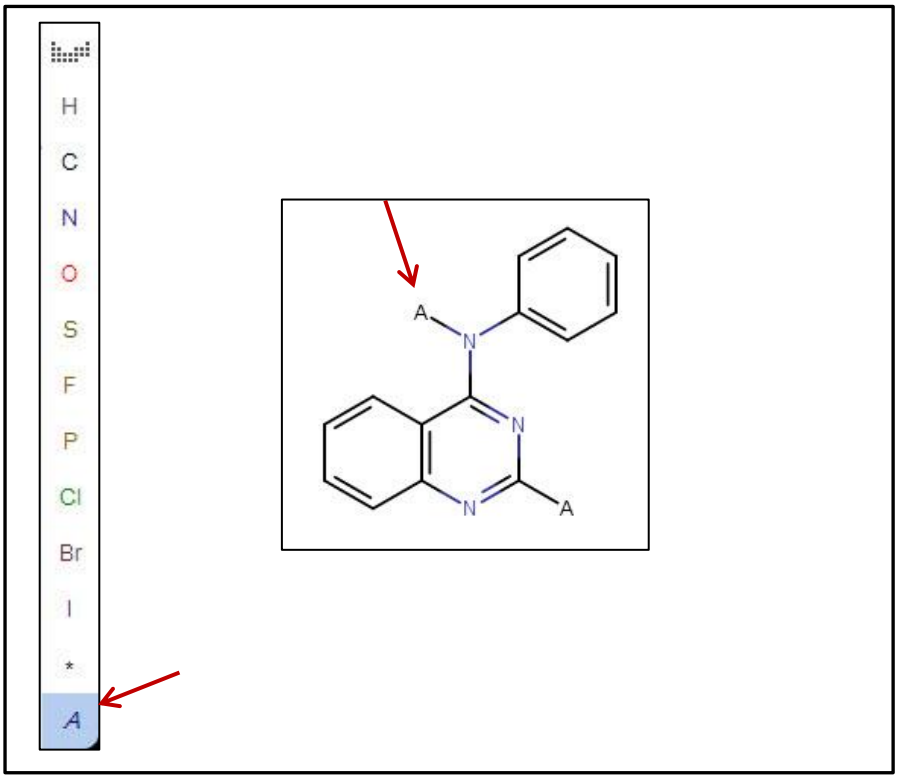


How do we check this? Let's perform a new search by changing the substructure search to:

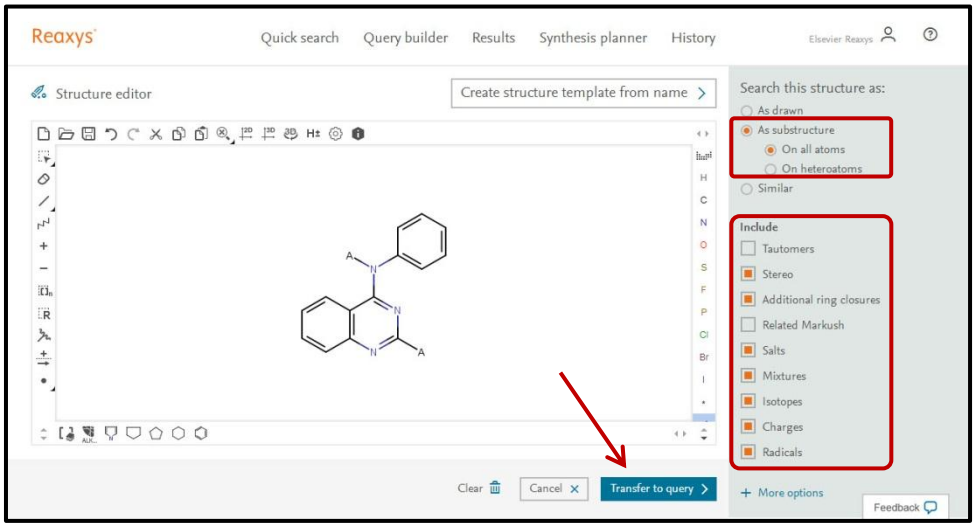


❖ Revise Search Query

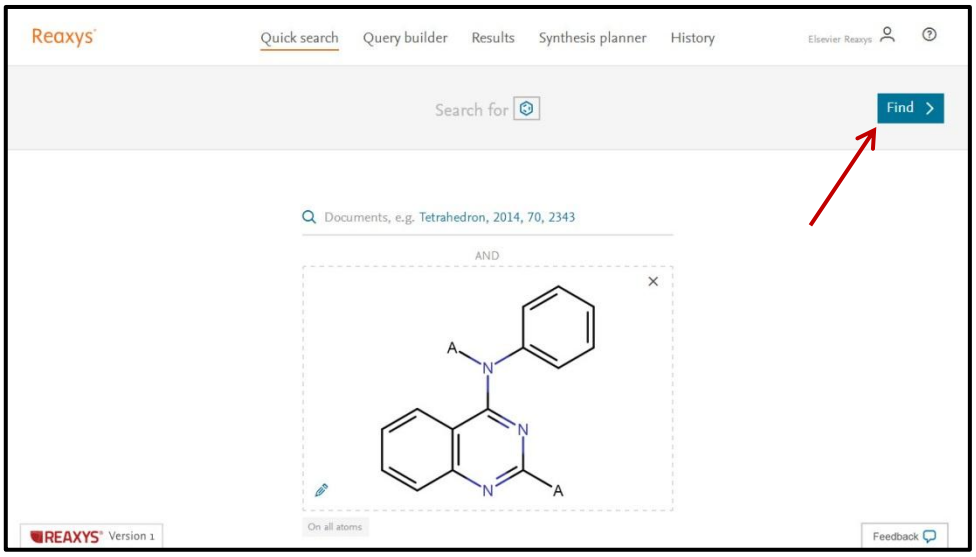
1. Click ***Exit fullscreen***
   a. Click ***Quick search***
   b. On the Quick search page, click **the structure**
2. Click '***A'*** in the atom toolbar (for *any atom except hydrogen)*
   a. Define the atom shown:



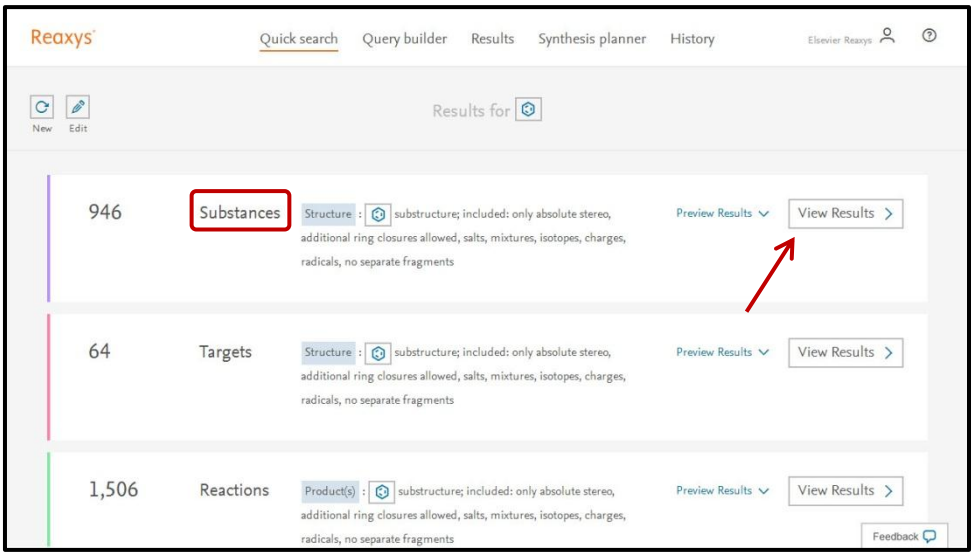
3. If necessary, click ***As substructure*** and ***On all atoms***
   a. Click ***Transfer to query***



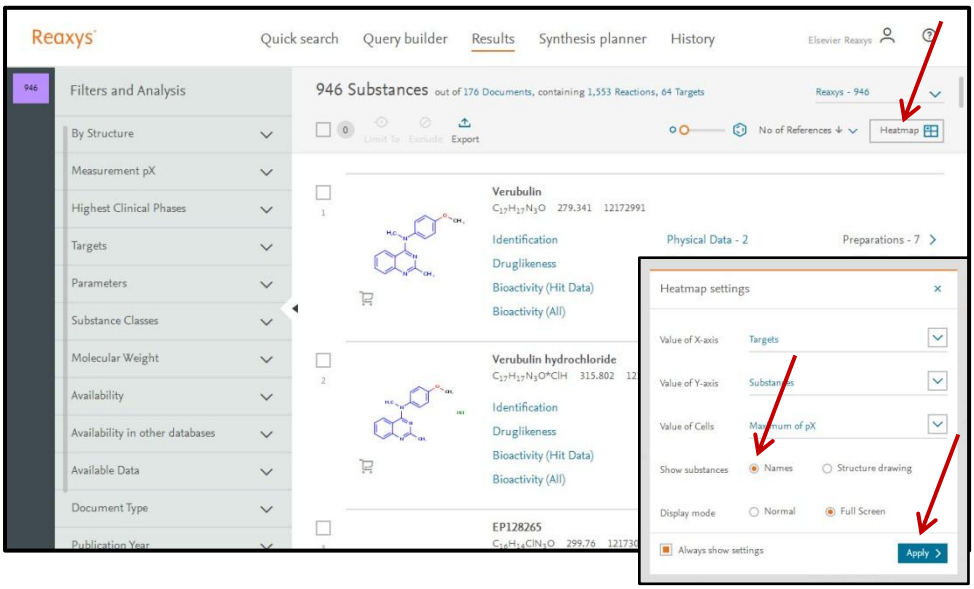
4. Click ***Find***.



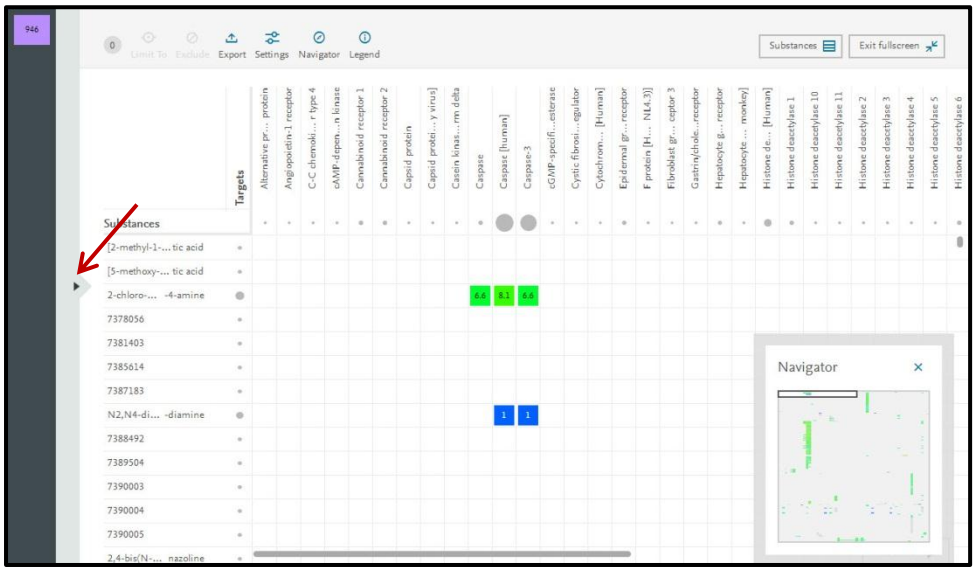
5. Click ***View Results*** for the first result set (Substances).



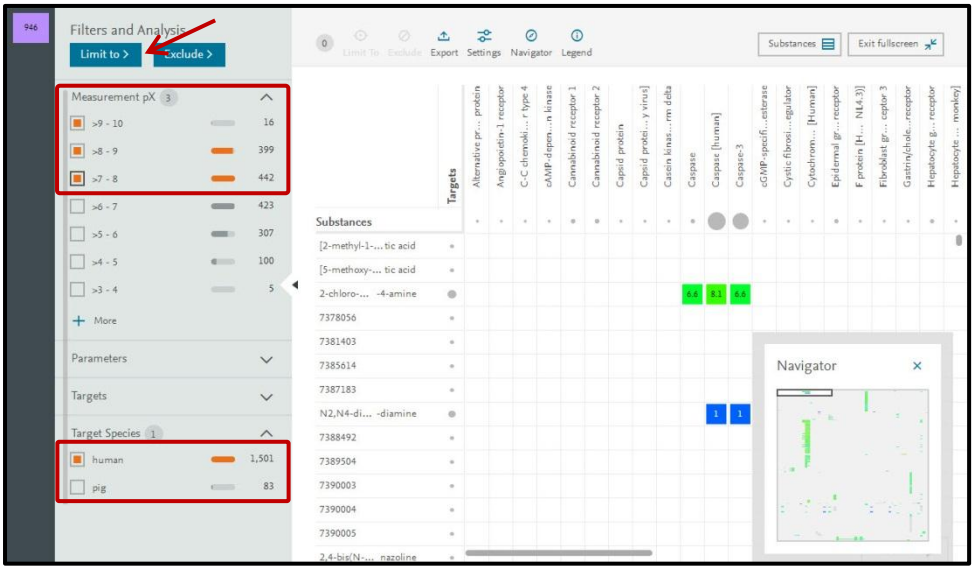
6. On the Results Page, click ***Heatmap***.
    a. If necessary, turn on ***Names*** and click ***Apply***.



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.