



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



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- 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:

Structure editor	Create structure templ	late from name > Search this structure as:
日		As substructure Similar Had G Tautomers N Stereo Additional ring closures Related Markush P Salts Gl Mixtures Br Isotopes I G Charges Related A
13970000		+ More options



- 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.

Reaxys	Quick search Query builder Results Synthesis planner History	Elsevier Reaxys 🔍 💿
	Search for 🔕	Find >
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	AH	
REAXYS * Version 1	On all atoms	Feedback 🖵

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.

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C New Edit		Results for 🙆			
6,368	Substances	Structure : Substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results 🗸	View Results >	
486	Targets	Structure : Substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results 🗸	View Results >	
7,807	Reactions	Product(s) : 🕝 substructure; included: only absolute stereo, additional ring closures allowed; salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results 🗸	View Results >	2



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



The Results Page is displayed showing the substances having this chemo	oty	pe.
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axys	Quick	search Query build	ler <u>Results</u> Synthesis plan	ner History	Elsevier Reaxys	?
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Measurement pX	~					
Highest Clinical Phases	~		2-phenyl-4-phenylaminoqu C ₂₀ H ₁₅ N ₃ 297.359 753055	u inazoline 40288-70-8		
Targets	~		Identification	Bioactivity (All)	Preparations - 19	
		bl.d	Druglikeness	Physical Data - 17	Reactions - 19	
Parameters	~	מ	Bioactivity (Hit Data)	Spectra - 21	Targets - 39	
Substance Classes	~	Ľ			Documents - 33	
Molecular Weight	~		Verubulin			
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			Identification	Physical Data - 2	Preparations - 7	
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D. H. W. W.			(4-methoxyphenyl)-(2-pher	ylquinazolin-4-yl)-amine	Feedback (ς



View the Heatmap

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

Filters and Analysis	6,	368 Substances out o	f 560 Documents, containing 7,897 Rea	ections, 486 Targets	Reaxys	- 6,368
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Available Data	~	p:	Bioactivity (All)	Show substances	Names O St	tructure drawing
Document Type	\sim					
5.11 J. N			(4-methoxyphenyl)-(2-phen	Display mode	 Normal F 	full Screen

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.

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N ² N ⁴ -bis-(chloride																												Food	haak	ā



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.

- £ \$ Ø 0 0 Substances 📄 🛛 Exit fullscreen it alpha dinase. -endle 5'-AMP-activ.. gets Navigator × ov-... tic acid 2-phenyl-4-...nazoline Nº-/3 4-dic triamine N², N⁴-diph...chloride 4.5 5.3 5.4 5.3 5.3 6.3 6.2 N², N⁴-bis-(... chlo Feedback 💭
- 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*.

6,368	Filters and Analysis Limit to > Exclud	le >		C O O Limit To Exclude	1 Export	Setti	ngs	Ø Navig	ator	() Legend							5	iubsta	nces		Exit	t fulls	creen	7 ⁴
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				N ² , N ⁴ -bis-(chloride		_																Feed	back	0

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

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- b. Check *pX values over 7* (affinity below 0.1μ M) to select active compounds.
- c. Click *Limit to*

6,368	Filters and Alexysis Limit to > Exclu	de >			0 O O Limit To Exclude	1 Export	Setti	ngs	(Navig	ator	Leger	d							S	ubsta	nces		Exi	t fulls	creen	7 ^K]
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					N ² , N ⁴ -bis-(chloride		_																	Feed	iback	P	1

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

Gmit To Exclude	Export	Settings	Naviga	tor Lege	end										Sub	stances [Exit fulls	creen
	Targets	Alpha 1D adrenoceptor	Alpha 2Asurenoceptor	ATP-bindi member 2	C-C chemoli r type 4	Caspase [human]	Caspase-3	Dipeptidyl peptidase 4	Egl nine homolog 1	EML4-ALK [human]	Epidermal gr receptor	neuropepti [Human]	Neuropeptider type 5	prostaglandisynthase	Receptor tyr e erbB-2	Receptor tyr e erbB-4	Rho-associat kinase 2	Solute carr member 1	TGE, hoto rece e ture.1
Substances			-		.0	۲	۲	- *			- 20								_
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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

Structure editor	Create structure templa	te from name > Search this structure as:
で 「」 「」 「」 「」 「」 「」 「」 「」 「」 「」 「」 「」 「」	H± 🛞 🚯	A substructure On all atoms On heteroatoms Similar
ζ, τ τ, τ - Ω.		N Include C Tautomers S Stereo F Tautomers
R A + -		Additional ring closures Related Markush Salts Mictures
		Isotopes G Charges



4. Click Find.



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

Reaxys	Quic	k search Query builder Results Synthesis planner	History	Elsevier Reaxys 🝳 💿
New Edit		Results for 🙆		
946	Substances	Structure : (substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results 🗸	View Results >
64	Targets	Structure : Substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results 🗸	View Results >
1,506	Reactions	Product(s) : 💽 substructure; included: only absolute stereo, additional ring closures allowed, salts, mixtures, isotopes, charges, radicals, no separate fragments	Preview Results 🗸	View Results >



- 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.

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	Targets	Alternative pr protein	Angiopoietin-1 receptor	C-C chemoki r type 4	cAMP-depenn länase	Cannabinoid receptor 1	Cannabinoid receptor 2	Capsid protein	Capsid protei y virus]	Casein kinas rm delta	Caspase	Caspase [human]	Caspase-3	cGMP-specifiesterase	Cystic fibrosiegulator	Cytochrom [Human]	Epidermal grreceptor	F protein [H NL4.3)]	Fibroblast gr ceptor 3	Gastrin/cholereceptor	Hepatocyte g receptor	Hepatocyte monkey]	Histone de [Human]	Histone deacetylase 1	Histone deacetylase 10	Histone deacetylase 11	Histone deaoetylase 2	Histone deacetylase 3	Histone deacetylase 4
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- 8. In the *Filters and Analysis* panel:
 - a. Filter by Target Species = human
 - b. Filter by Measurement pX >7
 - c. Click *Limit to*

946	Filters and Analysis Limit to >		0 O O Limit To Exclude	1 Export	Settin	ngs	Ø Navig	ator	() Legend	3						S	ubstar	nces		Exit	t fullso	creen	я ^к		
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Compounds are mainly active on Caspase 3. Consequently methyl groups on the aniline nitrogen are mandatory for caspase activity and selectivity.

82 946	O O C	ort Settings Navigator Legend	Substances 🗮 🛛 Exit fullscreen 🧏
		Caspase [human]	Сакране-З
	Substances	•	•
	2-chloro4-amine	81	
	N-(4-meth4-amine	8.4	8.5
- P	Verubulin	87	8.7
	2-chloro4-amine	81	7.5
	2-chloro4-amine	7.8	
	2-chloro4-amine	7.8	Navigator ×
	N4-(4-metdiamine	8.1	
	EP128265	8.7	
	2-chloro4-amine	8	
	N-(4-azido4-amine	83	
	2-chloro4-amine	7.9	
	2-ethyl-N4-amine	82	
	N-(4-ethyl4-amine	82	
	2-methoxy4-amine	83	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1