Integrating *in silico* and *in vitro* data to identify putative thyrotropin-releasing hormone receptor ligands

ACS 2020 Fall Meeting

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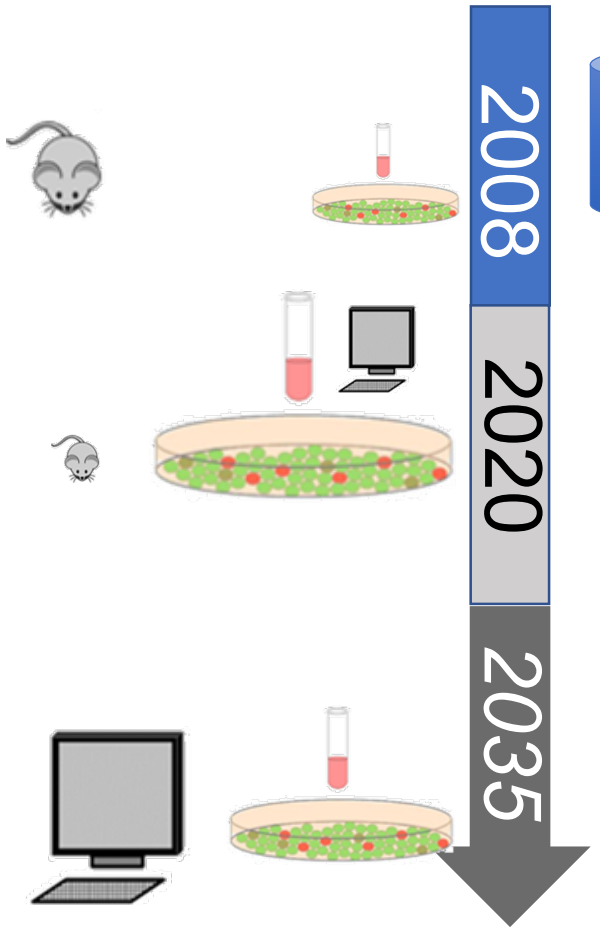
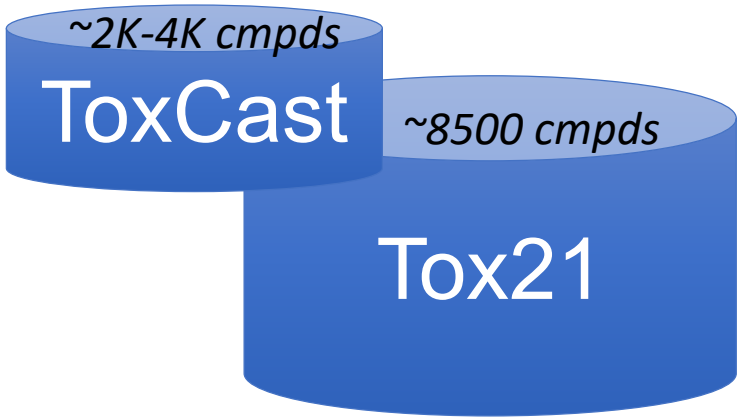
High-throughput screening (HTS) in risk assessment



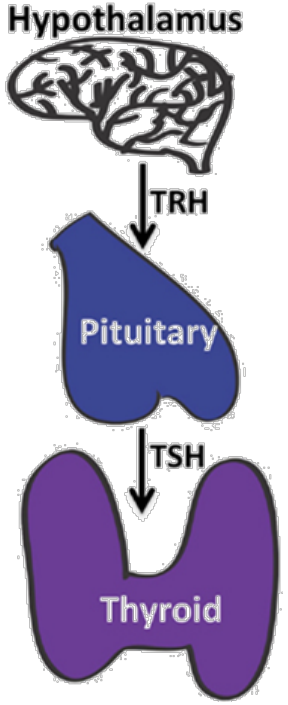


Data

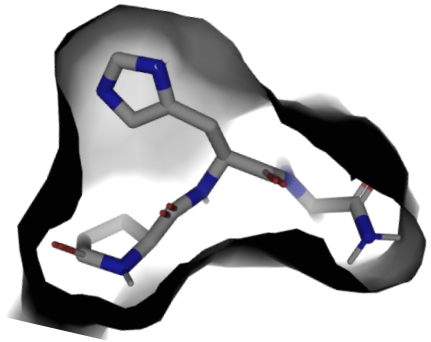
Models

How many environmentally-relevant chemicals interact with the thyrotropin-releasing hormone receptor (TRHR)?

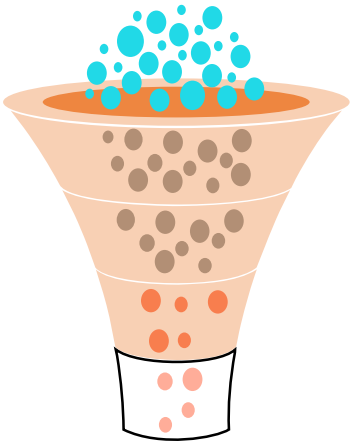
Molecular initiating event (MIE)



If a chemical interacts with TRHR, it may disrupt thyroid hormone production.



Experimental approach



Are all hits reliable?
Are we missing important hits?
How can we increase confidence in results?

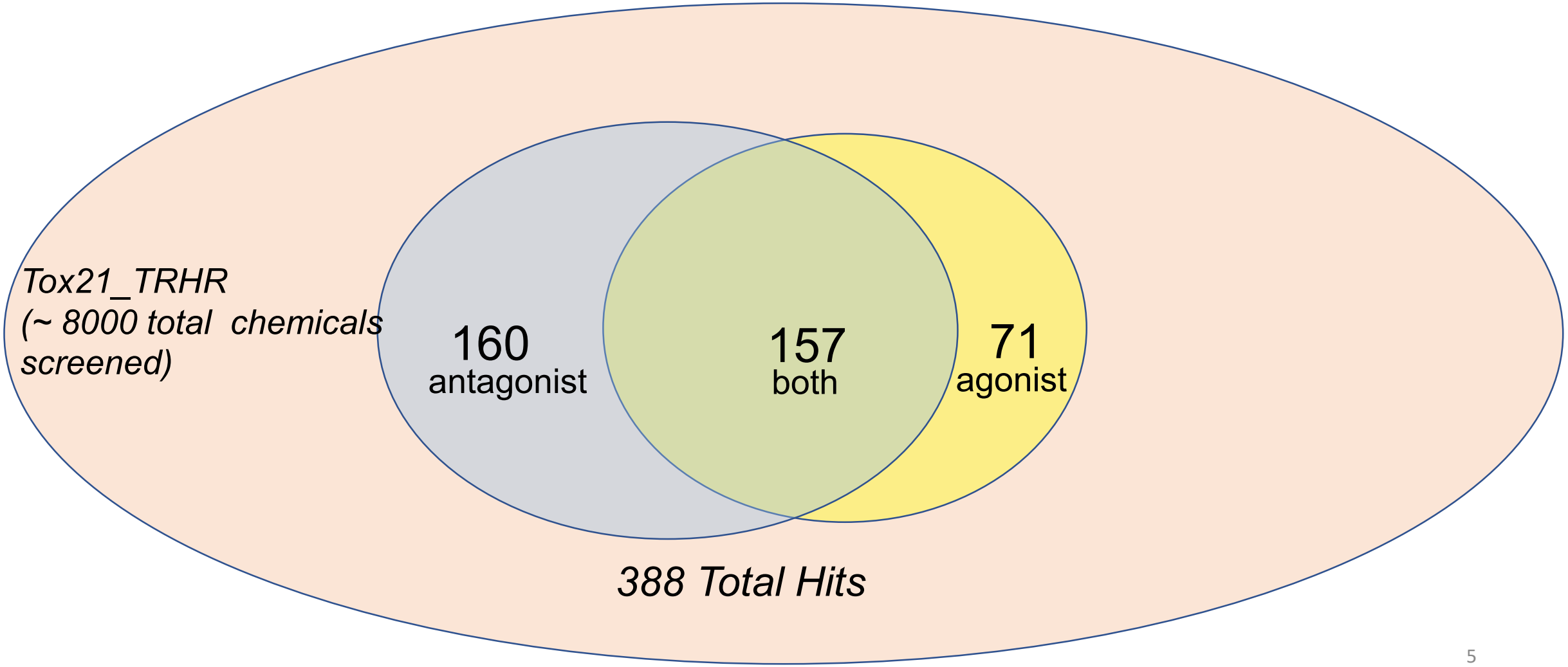
Tox21_TRHR Assay Design

Thyrotropin-releasing hormone receptor (TRHR) assay

<u>Hypothesis:</u> Tox21_TRHR assay measurement indicates changes in the receptor (TRHR) response to its specific ligand (TRH).

Steps I-IV can influence hit interpretation

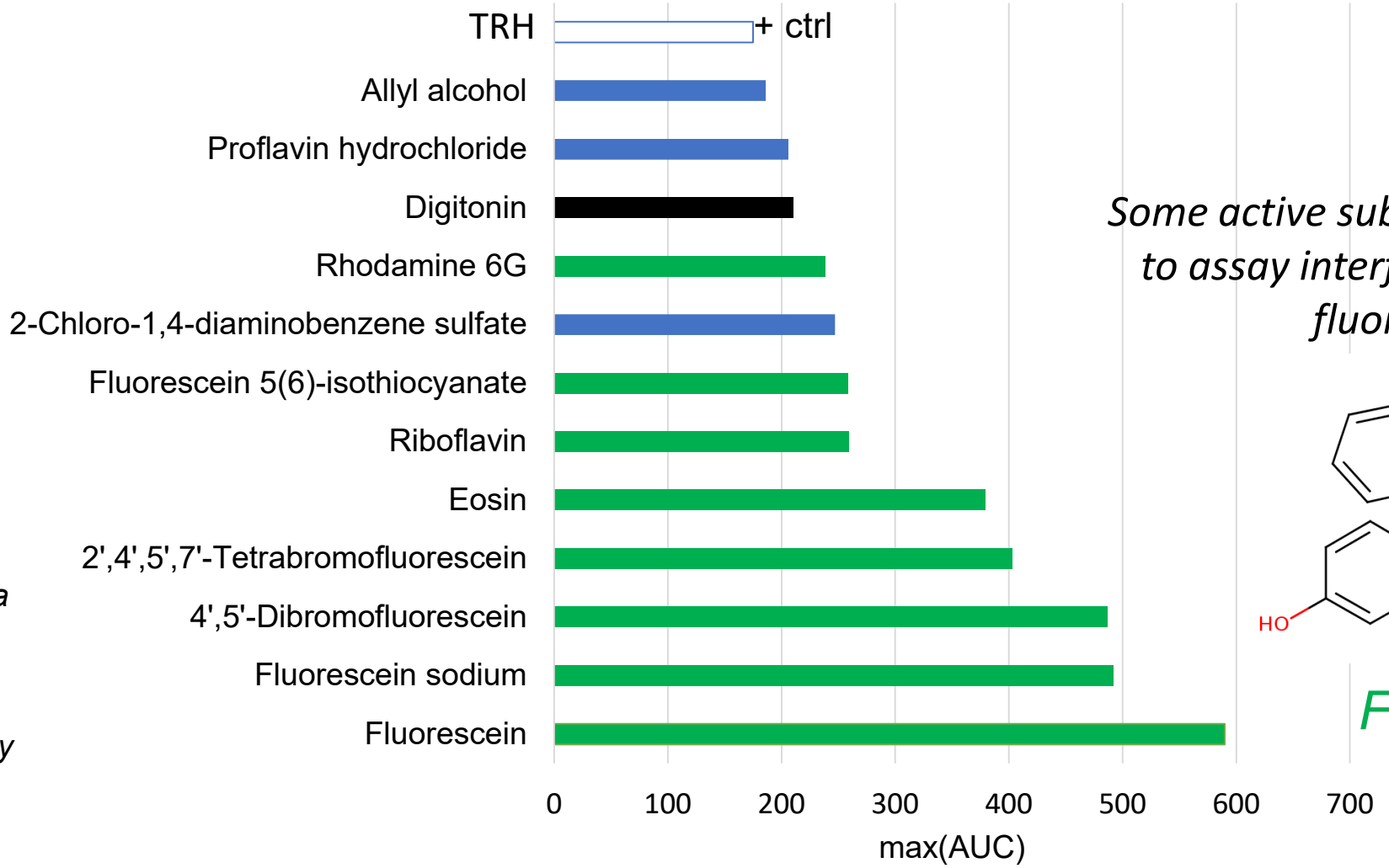
Tox21_TRHR Agonist & Antagonist Actives



Problem Statement & Approach

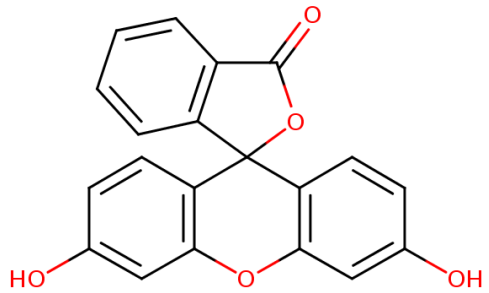
- Tox21_TRHR assay provides an indirect measure of potential TRHR activity
- Large number of diverse environmental chemicals screened, yet **false negatives and positives** are expected
- Goal is to identify subset of likeliest true actives from the full set of assay results
- Approach is to prioritize subset of actives (true hits) and inactives (potential false negatives) for follow-up testing using:
  - *domain knowledge*
  - *chemotype enrichments*
  - *in silico computational chemistry models*

Examples of most active Tox21_TRHR actives



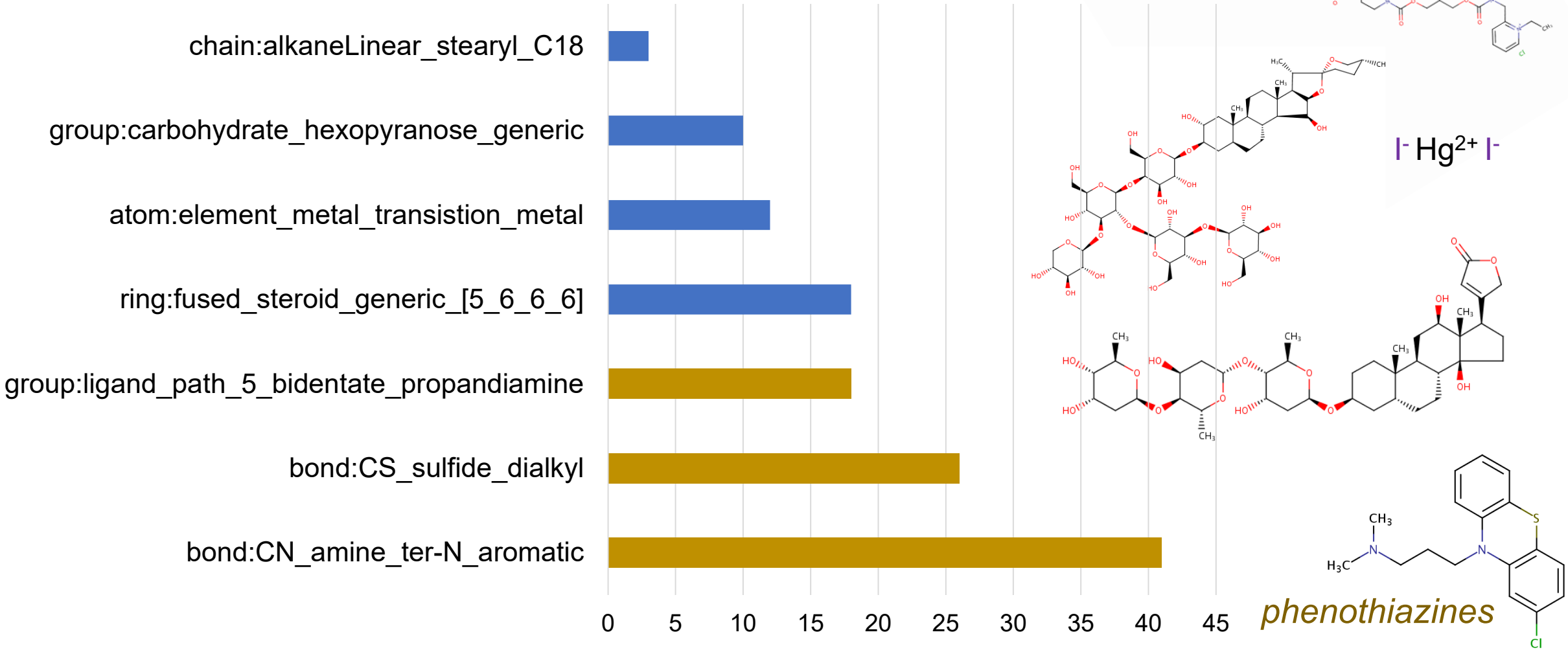
Maximum AUC by chemical is the area under the curve-fit from the ToxCast Pipeline, and compresses potency and efficacy into a single metric.

Some active substances may be due to assay interference from auto-fluorescence.

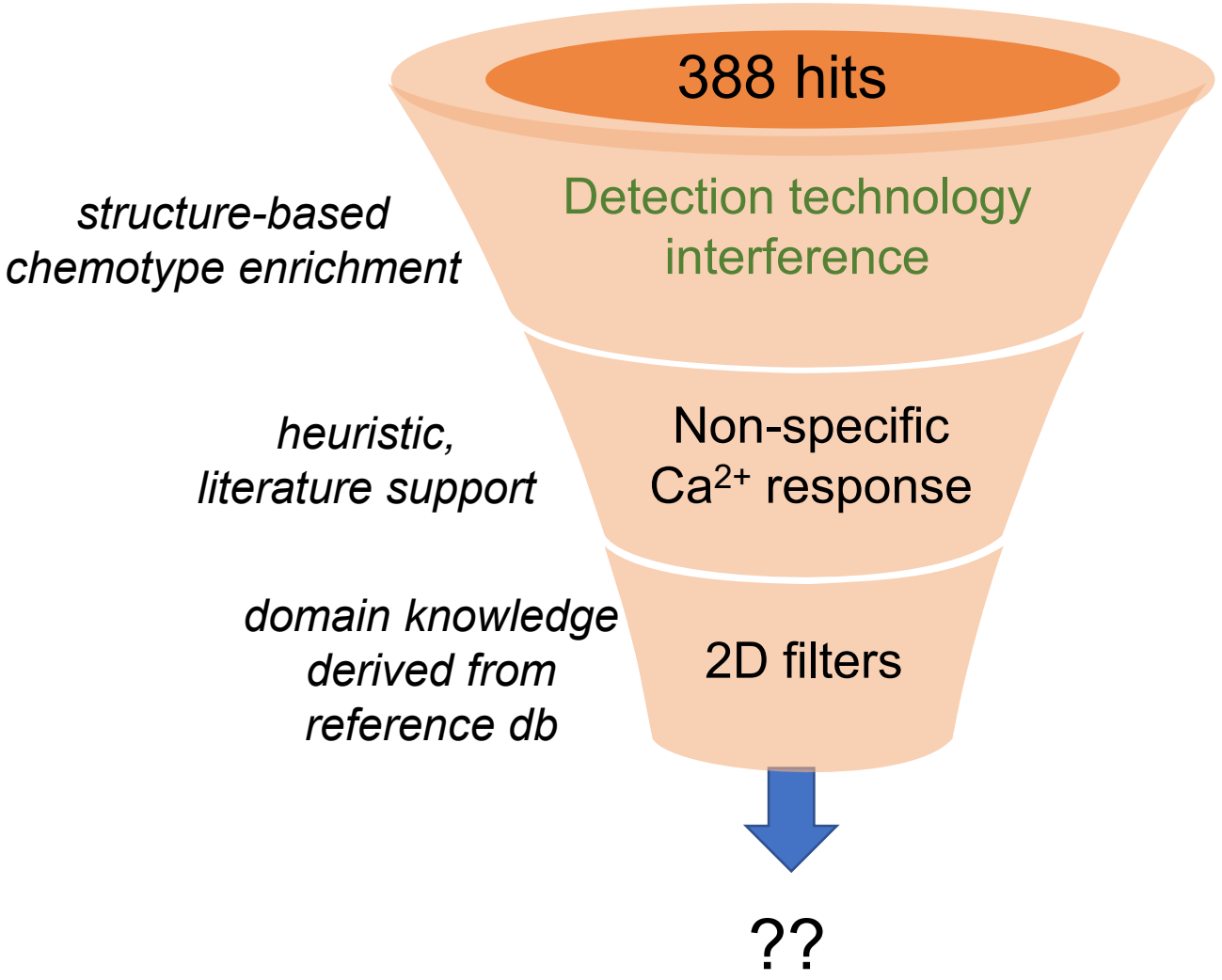


Fluorescent

Privileged scaffolds from chemotype enrichment analysis



Filtering potential sources of false positive hits



Binding reference dataset and modeling

10 Journal articles
Patents
BindingDB

Data curation

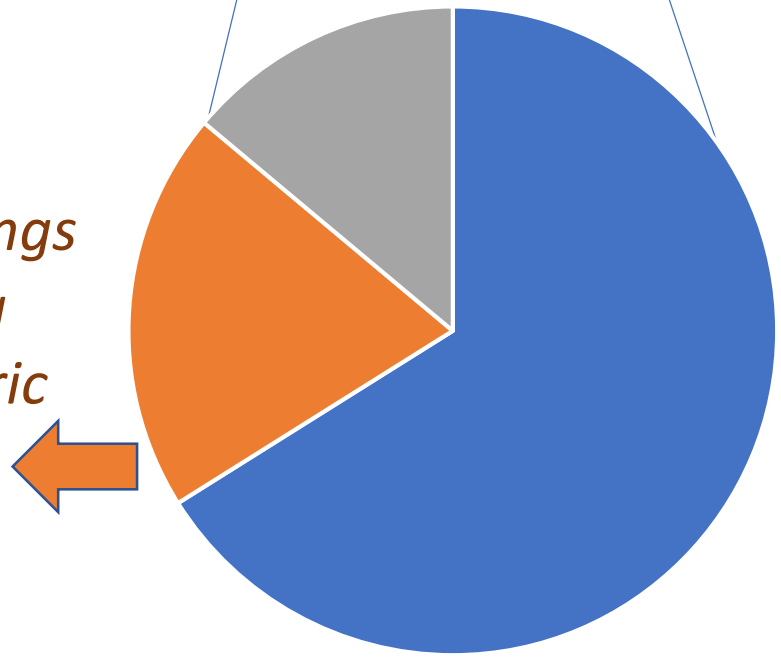
115 unique structures & activity values

Data mining
3D modeling

association rules
pharmacophore models

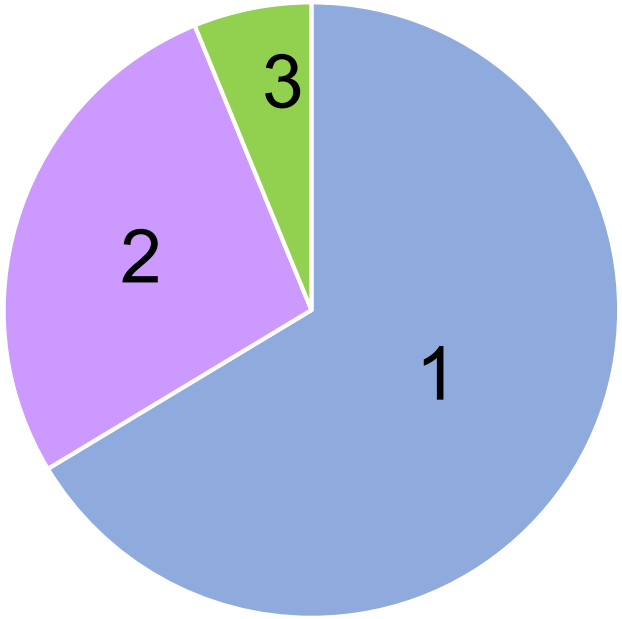
Reference dataset of binding data to:

- *Eliminate atypical binders with < 3 rings*
- *Train models using 3D pharmacophoric features*



(76) Binders rings with ≥ 3 rings
(16) Non-binders with ≥ 3 rings
(23) Non-binders with < 3 rings
(0) Binders with < 3 rings)

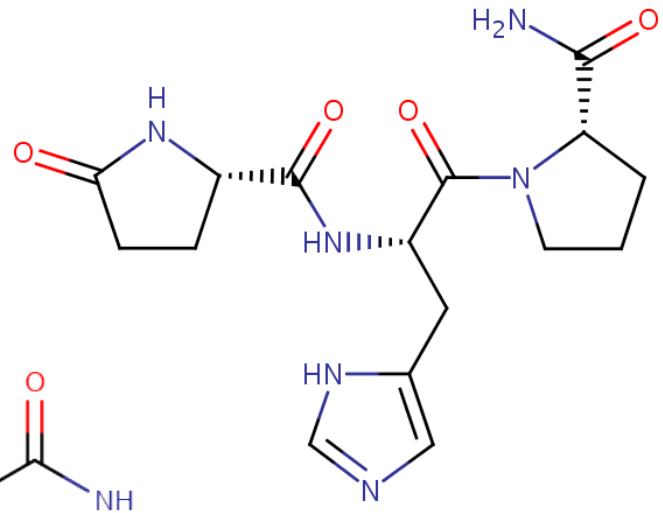
Binding reference dataset: structural diversity



K_i or IC_{50}
0.01 – 300 uM

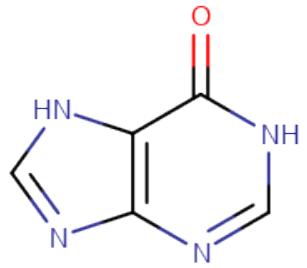
1. Analogs to the natural TRHR ligand

bond:C(=O)N_carboxamide_generic (**75**)



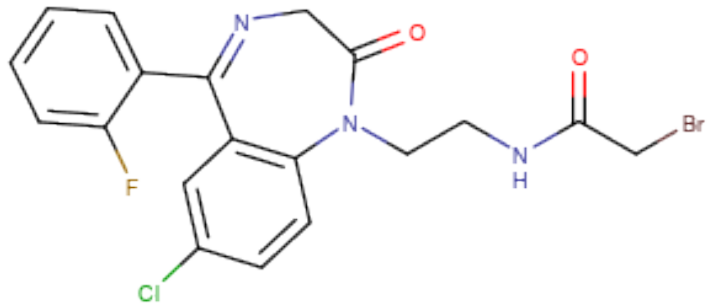
2. Heterocyclic compounds

ring:hetero_[6]_Z_generic (**31**)

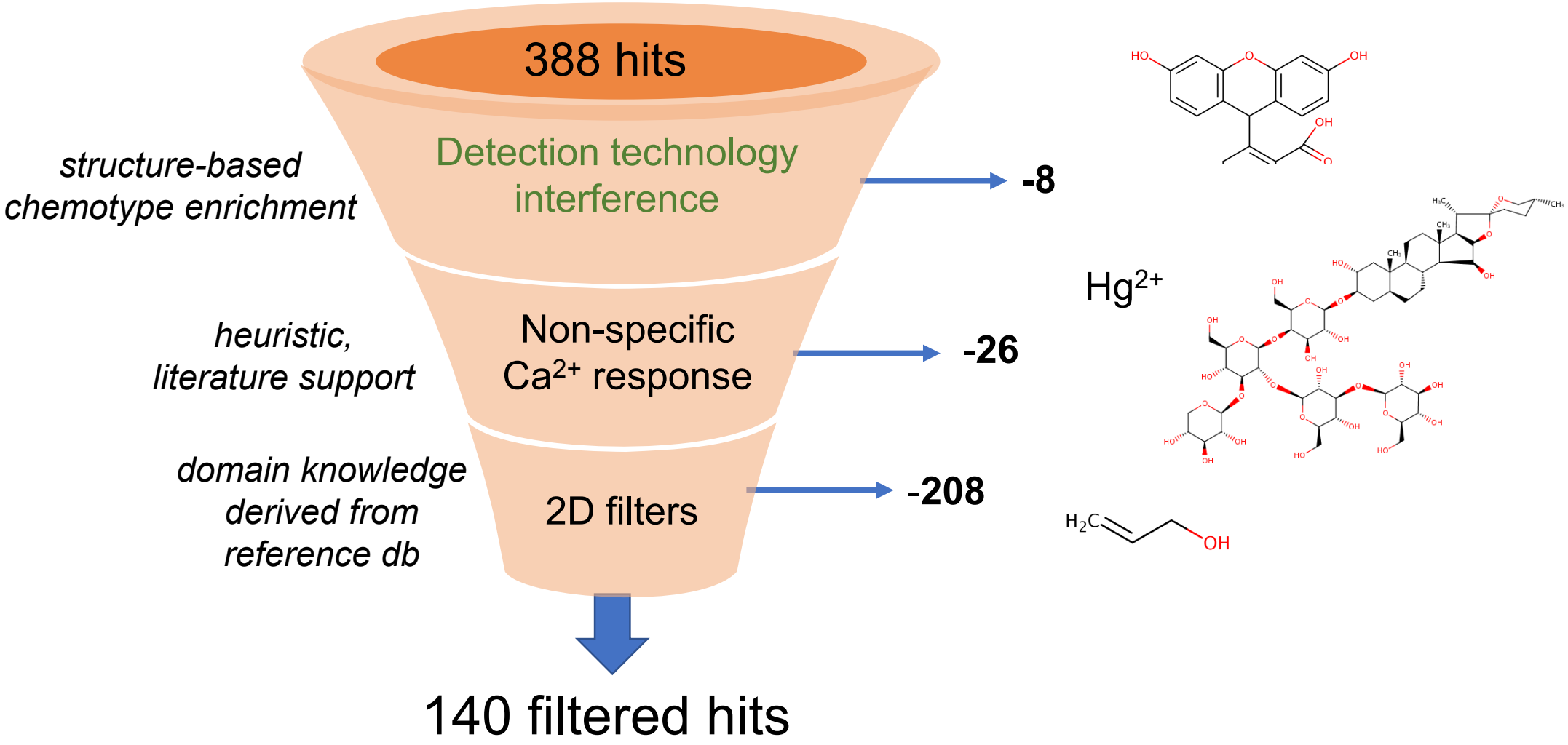


3. Benzodiazepine-like structures

ring:hetero_[6_7]_N_benzodiazepine_(1_4-) (**7**)



Filtering potential sources of false positive hits



Pharmacophore modeling

Analysis based on reference dataset

Generation of 3D conformations

Molecular alignment of binders

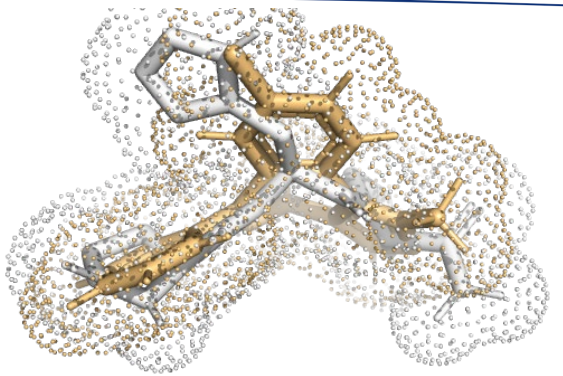
Generation & validation of pharmacophore models

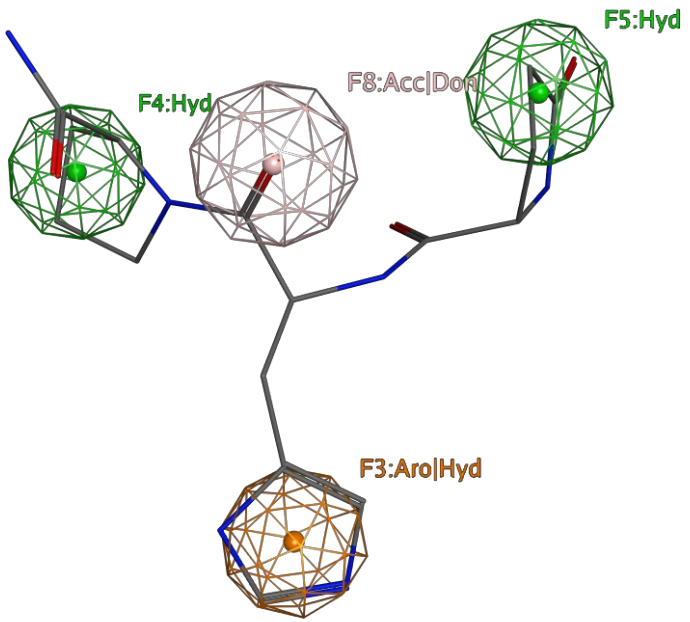
Apply to all tested chemicals

Querying structure database

Analysis of model results

MOE





mol	rmsd
	0.4799
	0.2784
	0.2636
	0.5092
	0.2334
	0.3147
	0.4857
	0.2842
	0.3047
	0.3550
	0.2474
	0.3806
	0.3041
	0.2422
	0.2404
	0.2885
	0.4383
	0.5112
	0.2485
	0.3482

Prioritizing active hits

388 hits

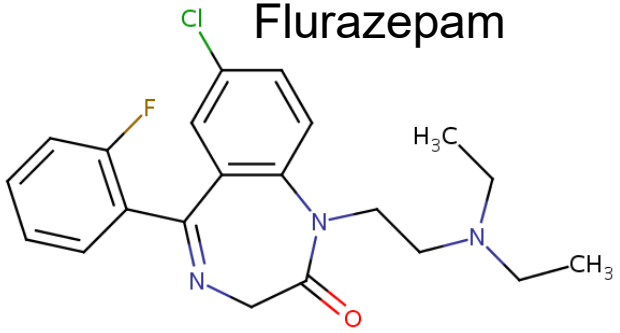
140 filtered hits

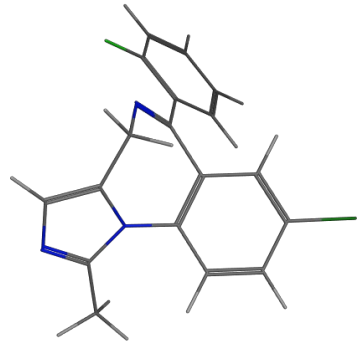
HIGH

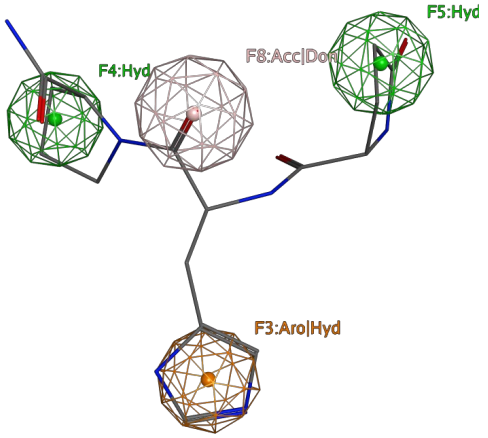
MODERATE

LOW

Flurazepam







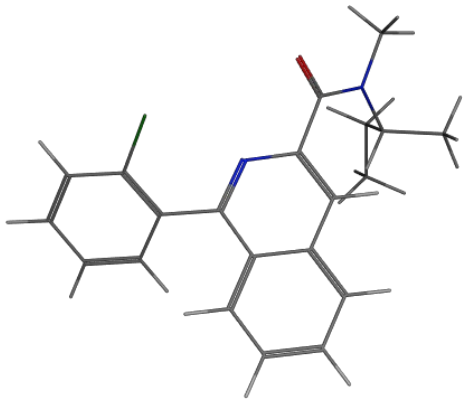
[TRH model, benzo model 1, benzo model 2, mixed model 1 , mixed model 2 ….]

[0, **1**, 0, **1**, 0, **1**, **1**, 0, **1**, 0, 0, 0, 0, 0, **1**]

Concordance:
Models &
Assay &
Expert

MODERATE:
at least one model + quality check

Predicted TRHR inhibitor: PK-11195 (Moderate binder)



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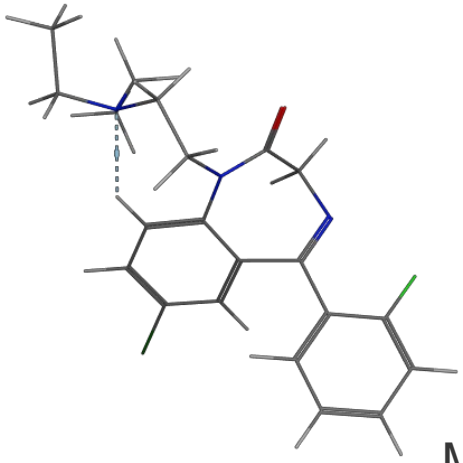
3D Modeling yields plausible results in the drug realm

isoquinoline carboxamide binds selectively to the peripheral benzodiazepine receptor (PBR)



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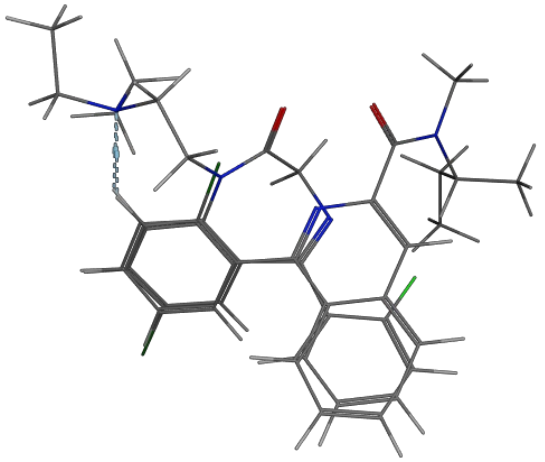
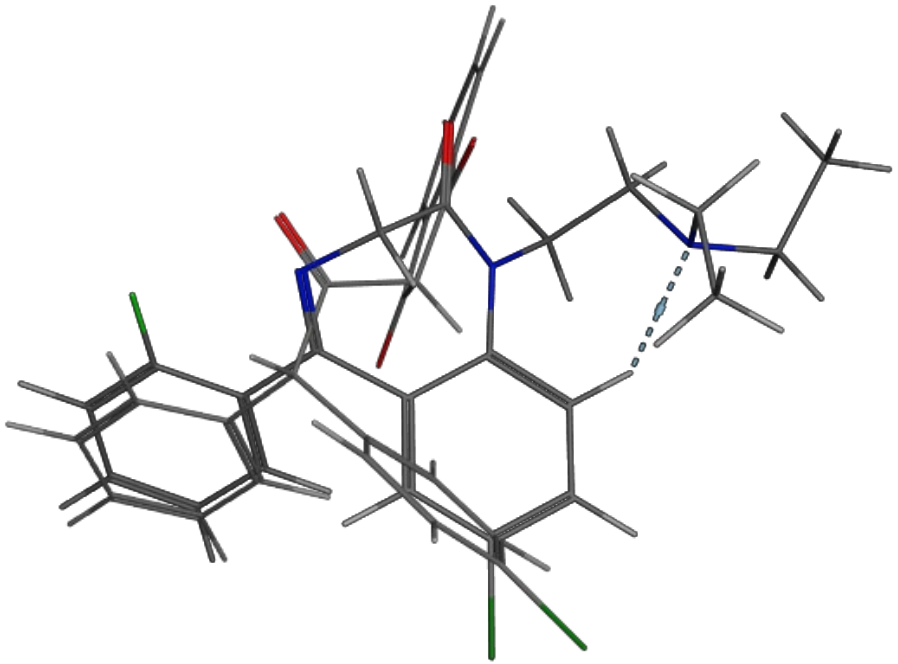
Midazolam, known TRHR binder

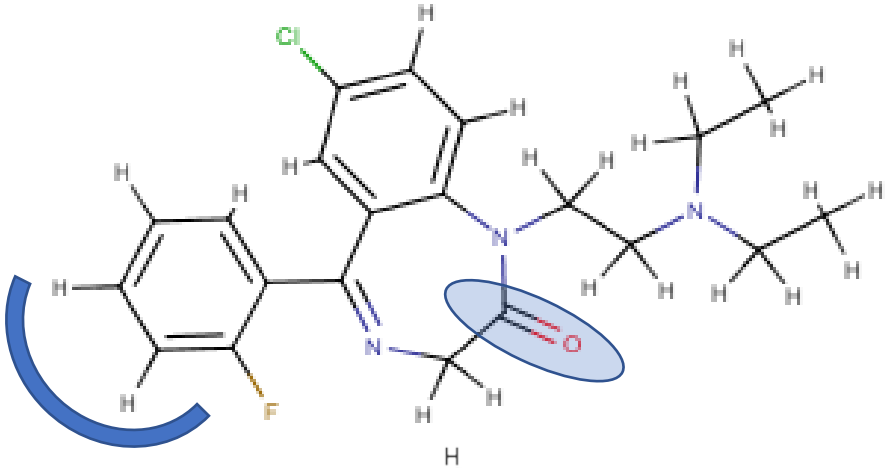


PK-11195 aligned to Midazolam

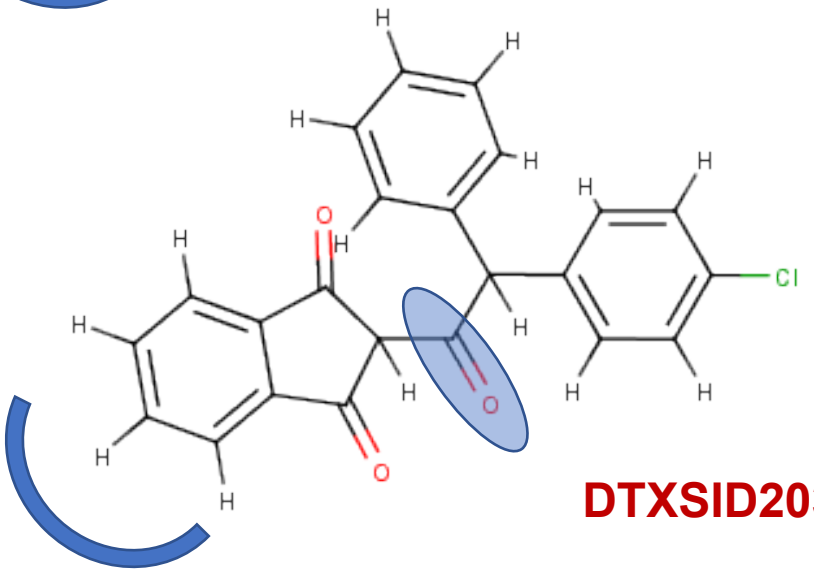
Moderate-plausibility assay active: Chlorophacinone



Chlorophacinone has structural features associated with Benzodiazepine inhibition of TRHR [hit in **5** models]



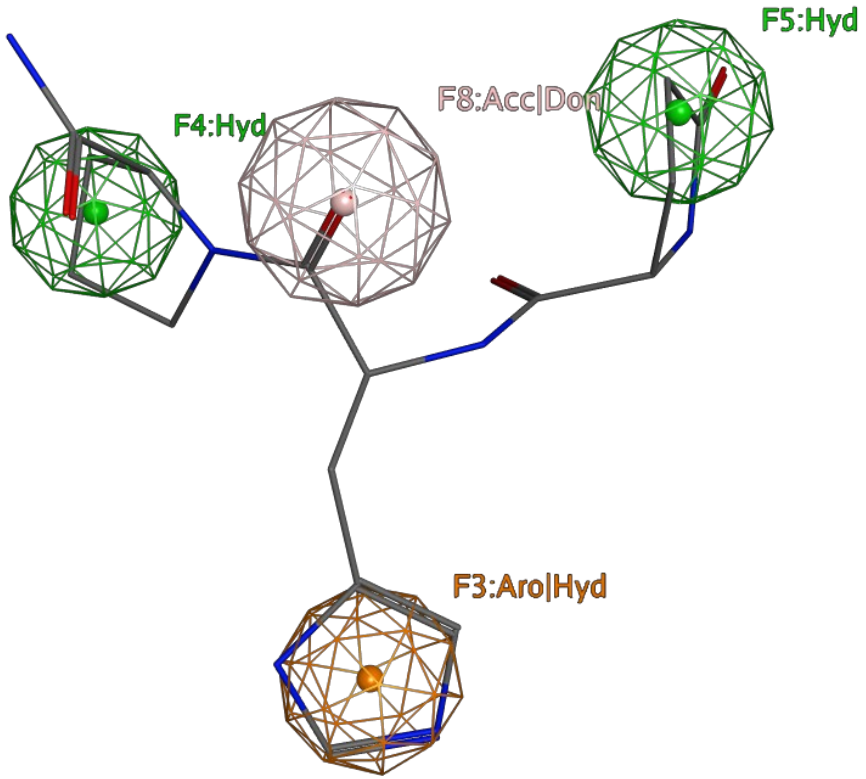
TRHR antagonist
DTXSID1023071



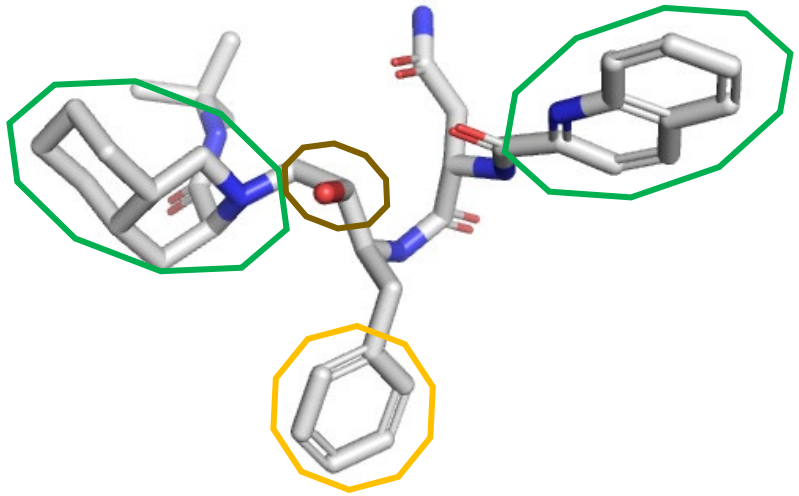
DTXSID2032348

High-plausibility assay active: Saquinavir mesylate

TRH



Saquinavir mesylate



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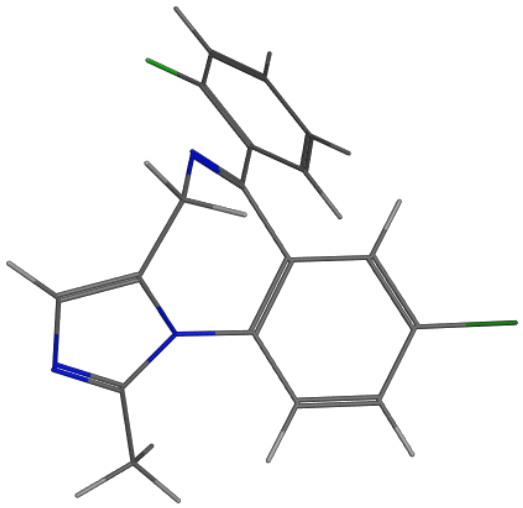
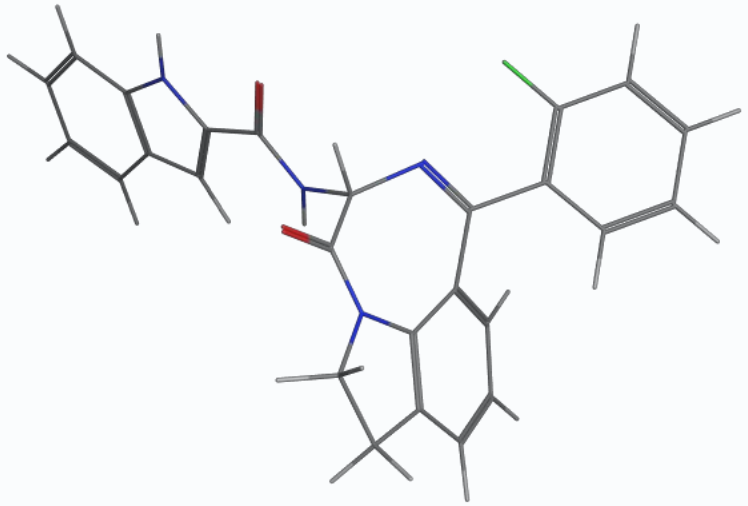
Newly identified TRHR candidate modulators from actives

Benzodiazepine



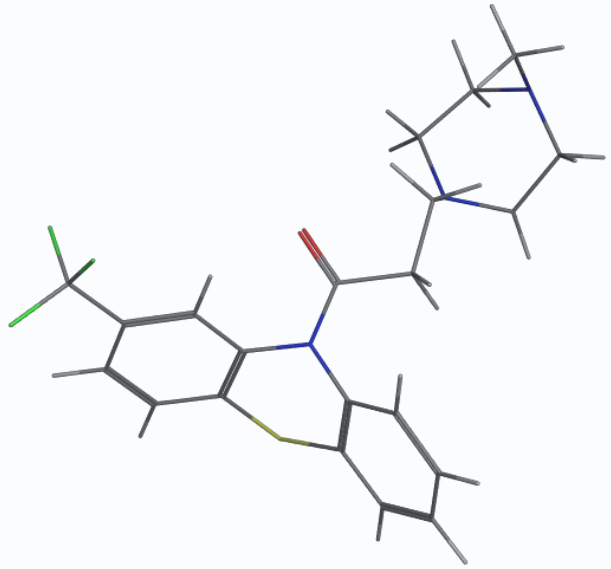
Known TRHR competitive inhibitor (midazolam), assay active

New atypical Benzodiazepine



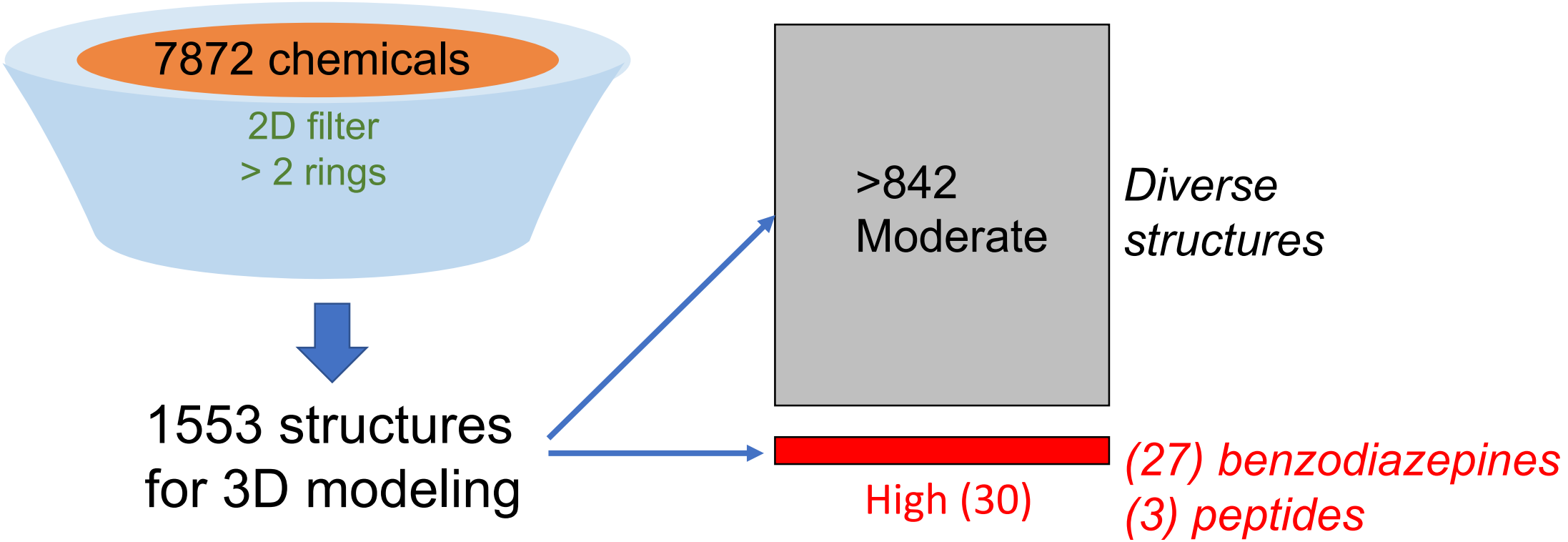
Does not bind GABA receptor, inhibits peptide receptor (CCK)

Opioid antagonist

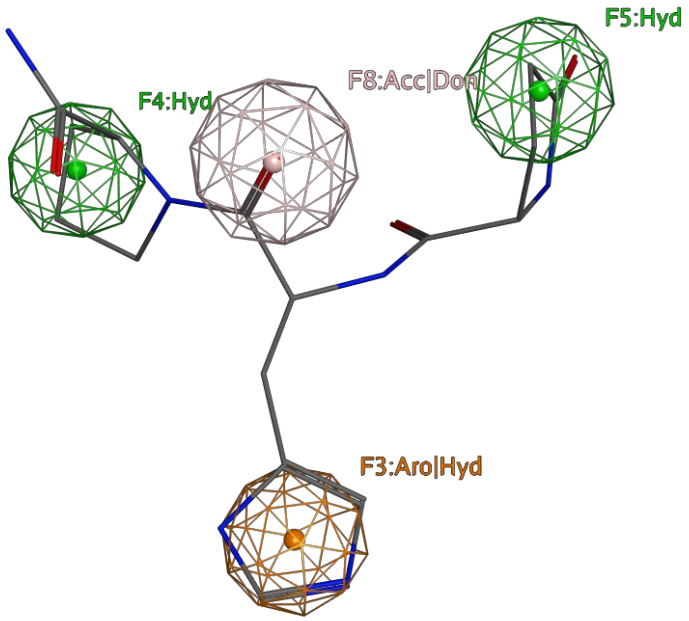


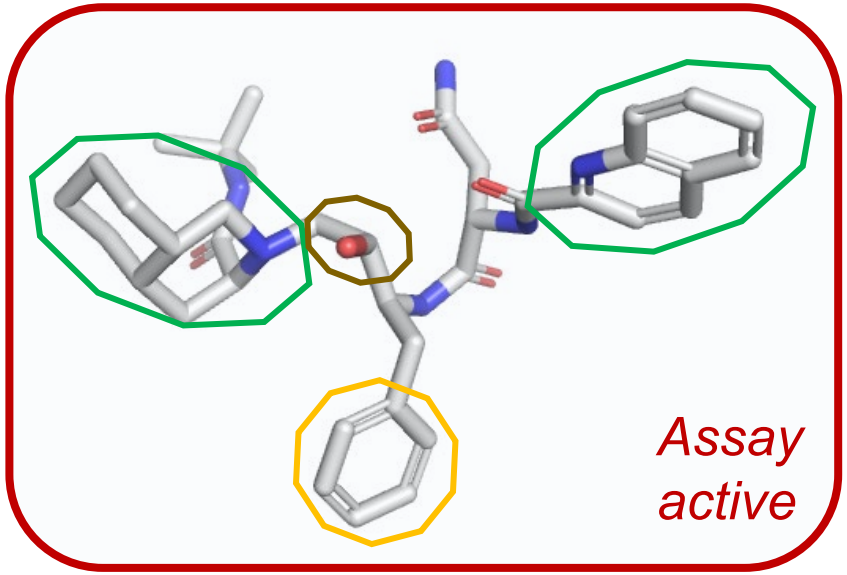
Unclear, warrants further inquiry

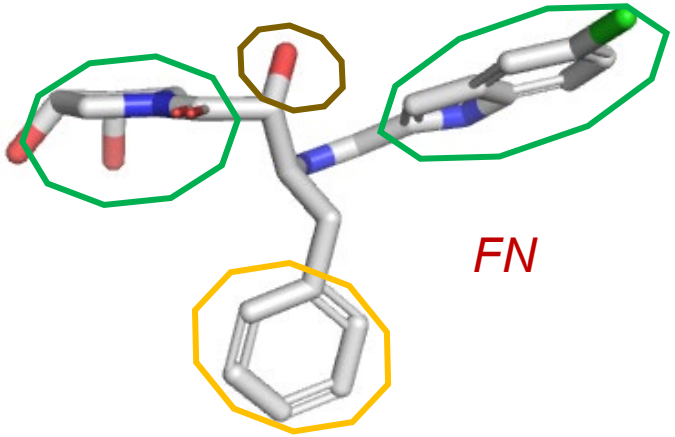
Identifying false negatives



Prioritized peptide-like structures







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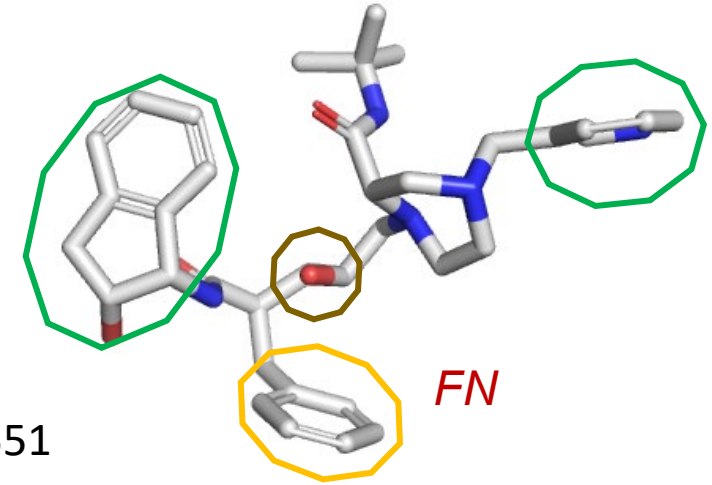

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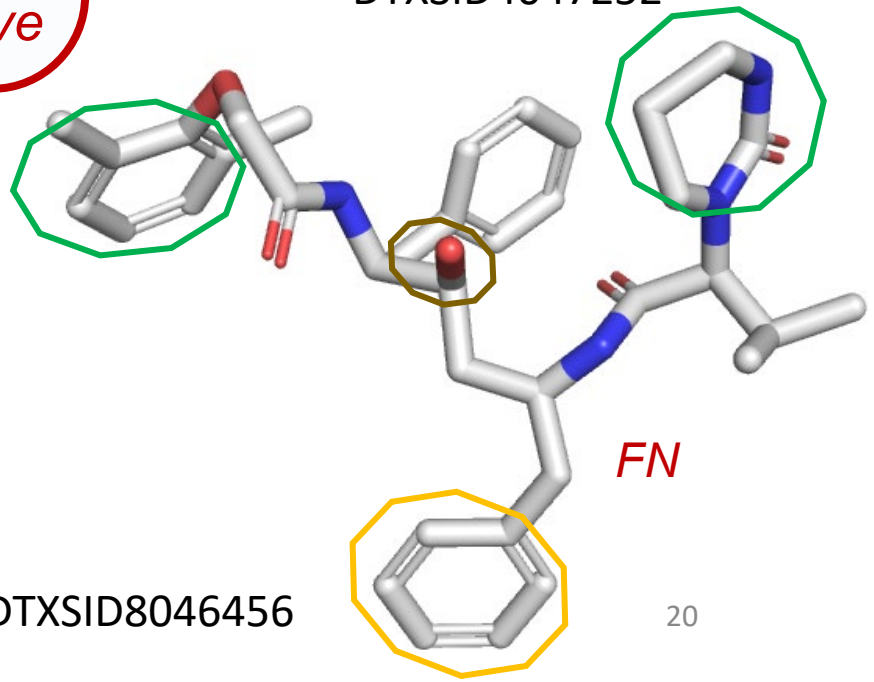

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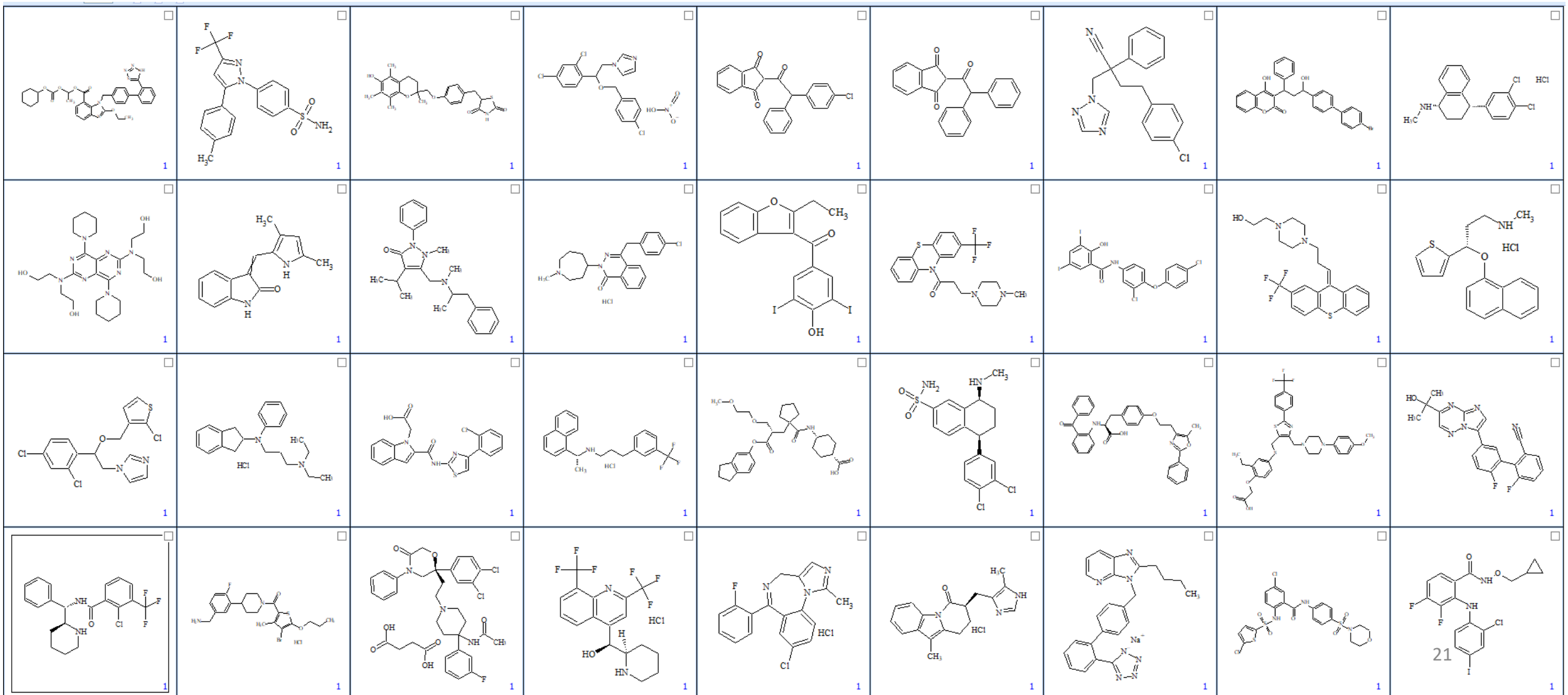
Predicted Moderate Binders (*in assay hits*)

- Structurally diverse
- Multi-ring structures
- Primarily drugs

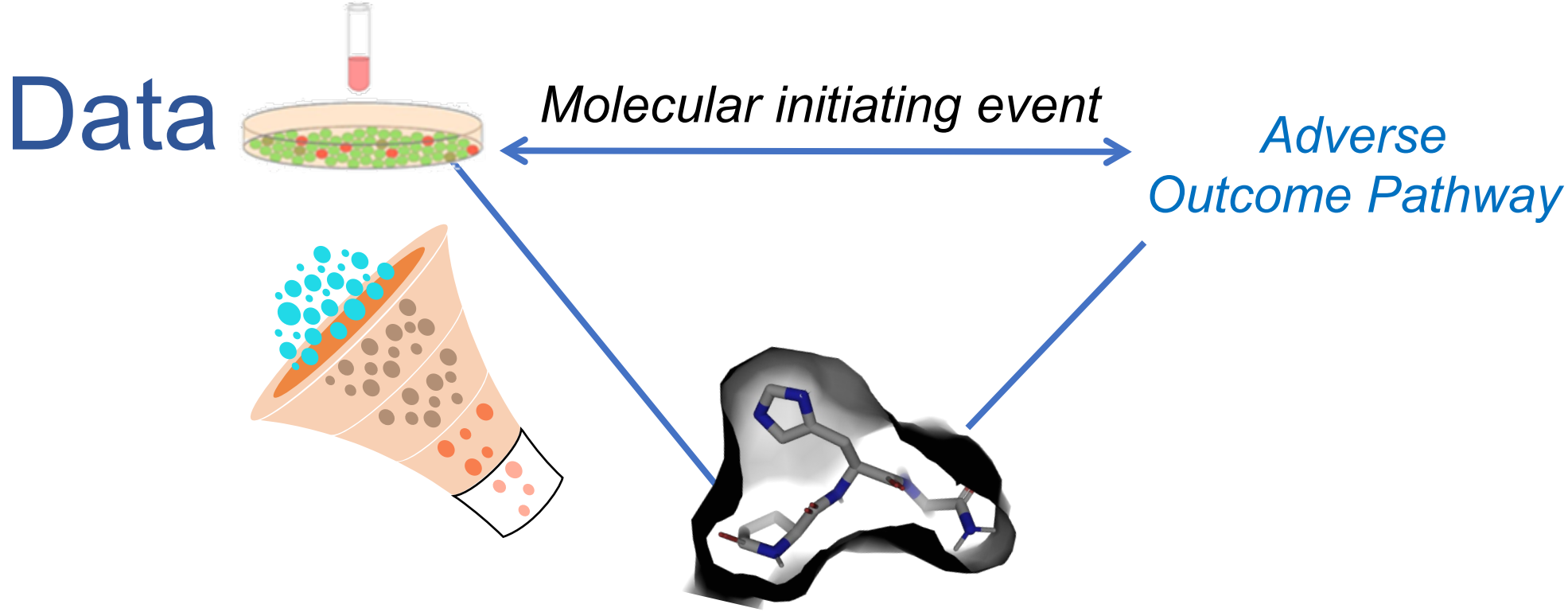


Summary & Conclusions

- >50% assay false positive; enriched features associated with artifacts
- Multistep prioritization workflow combines
  - *existing domain knowledge*
  - *in vitro results*
  - *in silico computational chemistry*
- Integrated approach points to small number of true active candidates in both the hits and negatives, including a novel benzodiazepine-type structure
- *A limitation of this work is that the 3D modeling assumes the TRHR binding pocket in the native conformation.*
- 3D models predict larger set of structurally diverse moderate binders among hits that are of potential environmental significance, warranting follow-up evaluation

Summary & Conclusions

Multistep workflow is generalizable and can be applied to other high-throughput assay results to improve ability to filter out false positives and identify potential true actives for follow-up screening.



Acknowledgements

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- Tox21 & ToxCast assay collaborative group