In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* high-throughput screening (HTS) results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

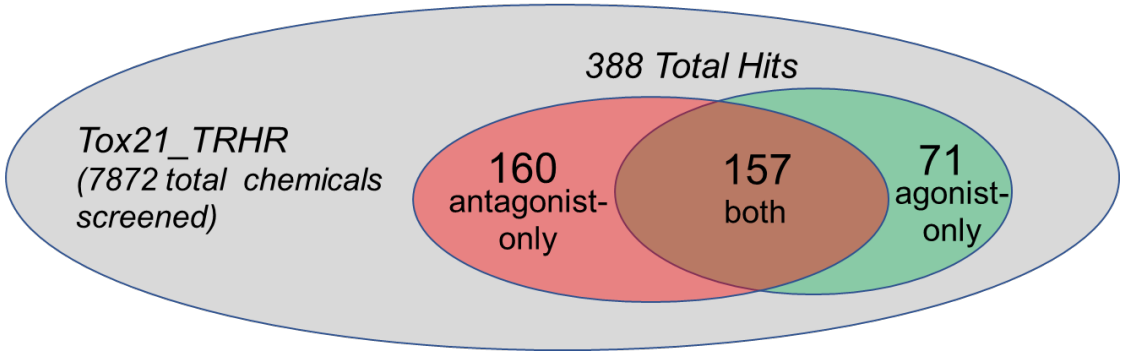
Abstract #252

Mahmoud Shobair[1*], Dan Chang[1], Ryan Lougee[3], Katie Paul Friedman[1], Chris Grulke[1], Jinghua Zhao[3], Ruili Huang[3], Menghang Xia[3], Ann Richard[1]
[1]Ctr. for Comput. Toxicol. & Exposure, ORD, U.S. EPA; [2]ORISE, Oak Ridge, TN, [3]Nat. Ctr. Adv. Translational Sciences, NIH, Bethesda, MD, *Currently P&G, Cincinnati, OH

Background

- Relating biochemical outputs to molecular initiating events (MIEs) and adverse outcome pathways (AOPs) is challenging, particularly when the relationship between the biochemical output and MIE is indirect, as is the case here
- Tox21 thyrotropin-releasing hormone receptor (TRHR) is a potential target in the thyroid hormone AOP, but is lacking confirmatory or orthogonal assays (*K. Paul Friedman, 2019*)

- TRHR is a GPCR (G protein-coupled receptor) with few known agonists or antagonists.
- Assay measures agonism or antagonism for TRHR through the Gq-Ca2+ pathway.



Objectives

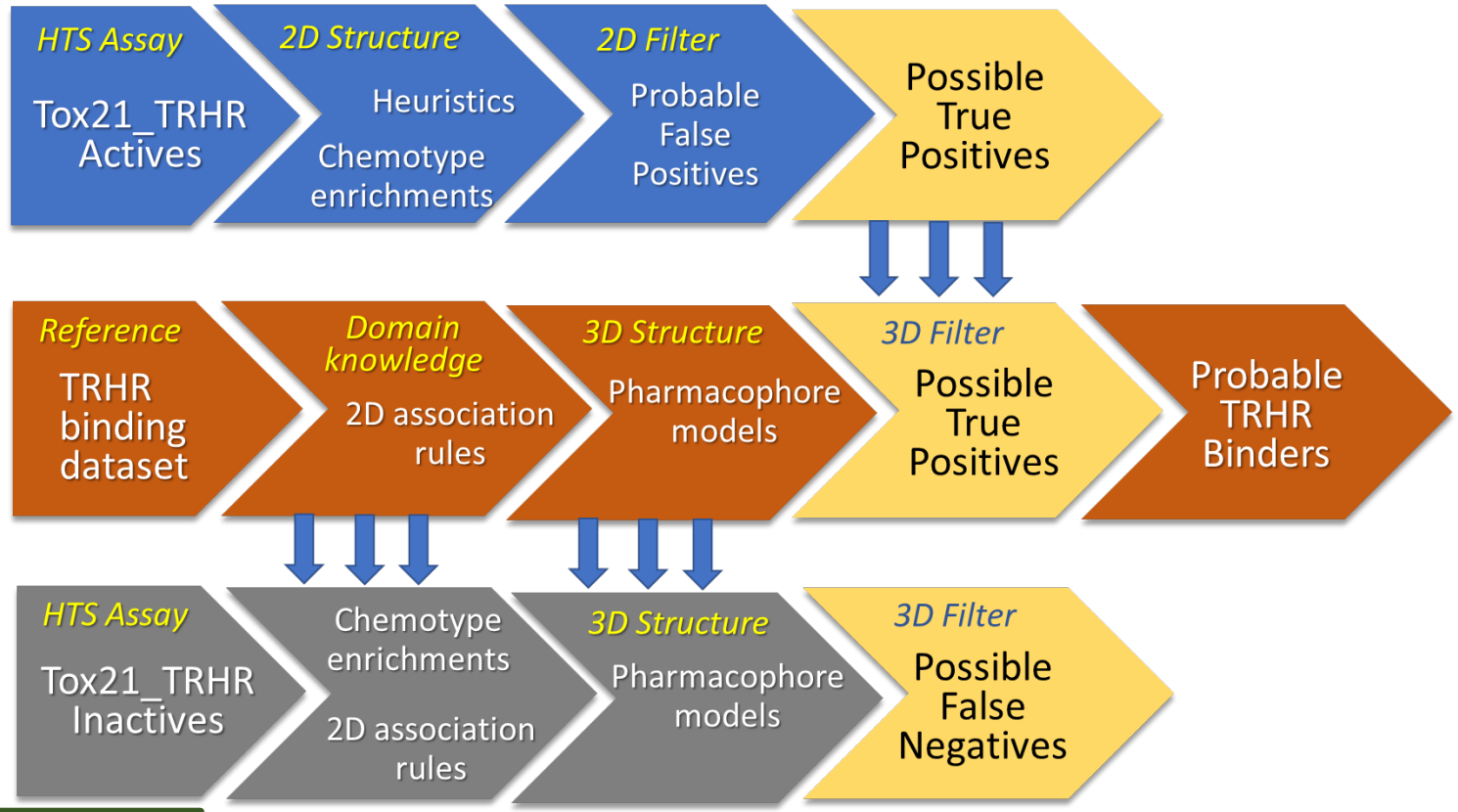
➢ ***Challenge:*** *prioritize Tox21_TRHR assay actives for hazard evaluation based on likelihood of binding to the receptor*

Develop and apply a structure-based data enrichment and knowledge contextualization workflow to identify likely TRHR false positives and discriminate true receptor binders

- ✓ identify structure-activity patterns using chemotype-enrichment analysis (*Wang et al., 2019*)
- ✓ filter noise from cytotoxicity or assay interference (*Borrel et al., 2020*)
- ✓ use 3D pharmacophore modeling to prioritize chemicals capable of binding to TRHR as well as

In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* HTS results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

Approach



Prioritize subset of actives (true hits) and inactives (potential false negatives) for follow-up testing using:

- *domain knowledge*
- *2D association rules*
- *chemotype enrichments*
- *QSAR models*
- *3D pharmacophore models*

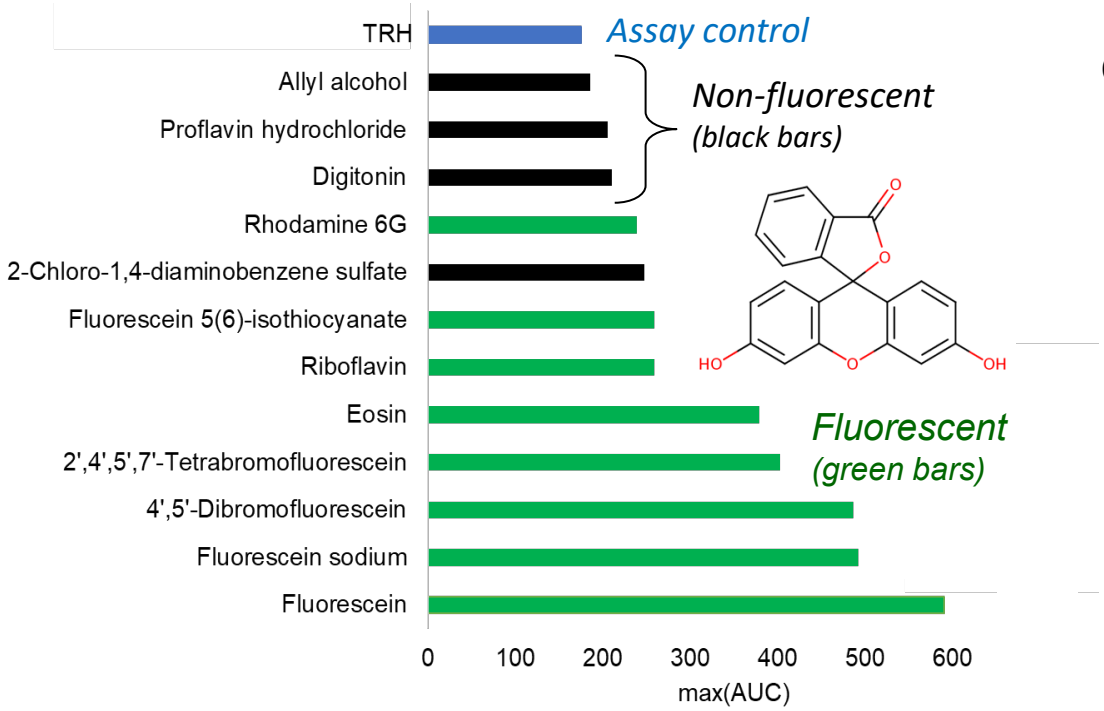
Methods

- Structures and Tox21_TRHR assay data downloaded from EPA dashboard https://comptox.epa.gov/dashboard
- Statistical analysis code at https://github.com/mshobair/cheminf; structures visualized https://chemotyper.org/
- Chemotype enrichment workflow (CTEW) and statistics described in *Wang et al., 2019;* ToxPrints at https://toxprint.org/
- Fluorescence prediction using InterPred web service for Machine Learning https://sandbox.ntp.niehs.nih.gov/interferences
- Pharmacophore modeling using Molecular Operating Environment (MOE) https://www.chemcomp.com/Products.htm

In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* HTS results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

Results: Cheminformatics Analysis

Dose-response curve analysis

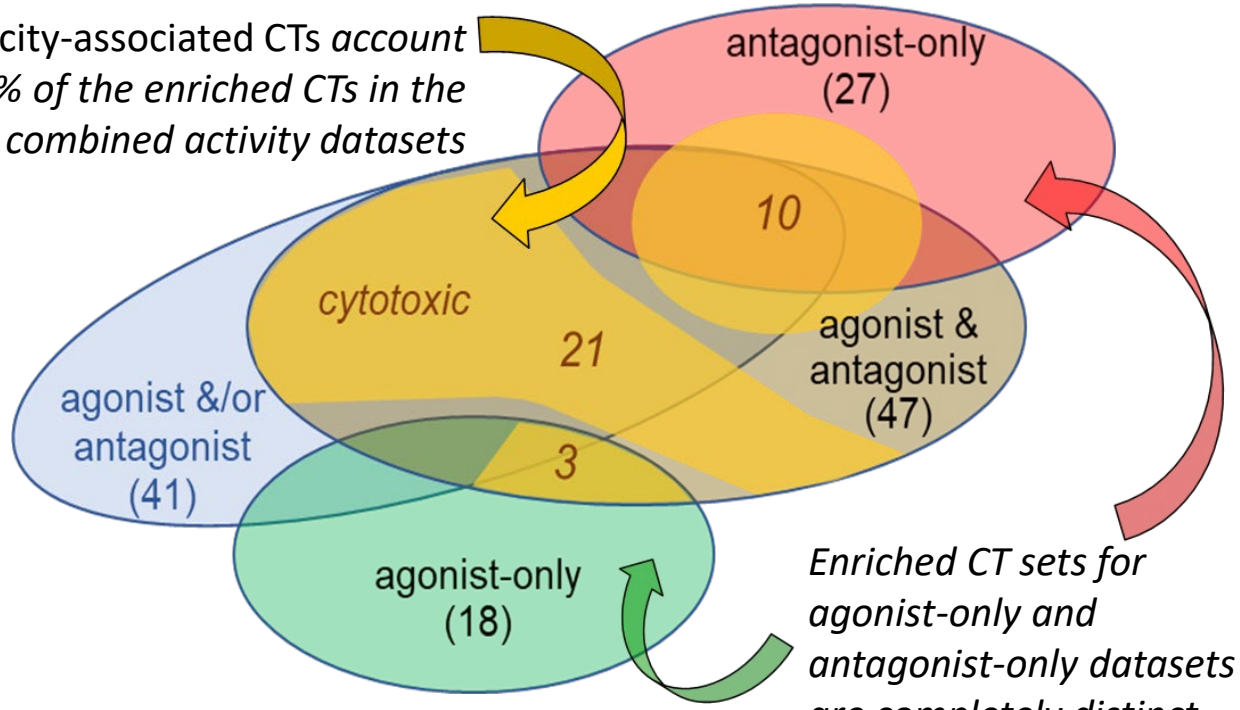


Maximum Area-Under-the-Curve (AUC) values per chemical computed for both agonist and antagonist endpoint data.

- *Majority of top potent/efficacious hits enriched in autofluorescent molecules, indicating source of false positive (FPos) activity.*

Chemotype-enrichment (CTE) analysis

Cytotoxicity-associated CTs account for >50% of the enriched CTs in the combined activity datasets



Enriched CT sets for agonist-only and antagonist-only datasets are completely distinct

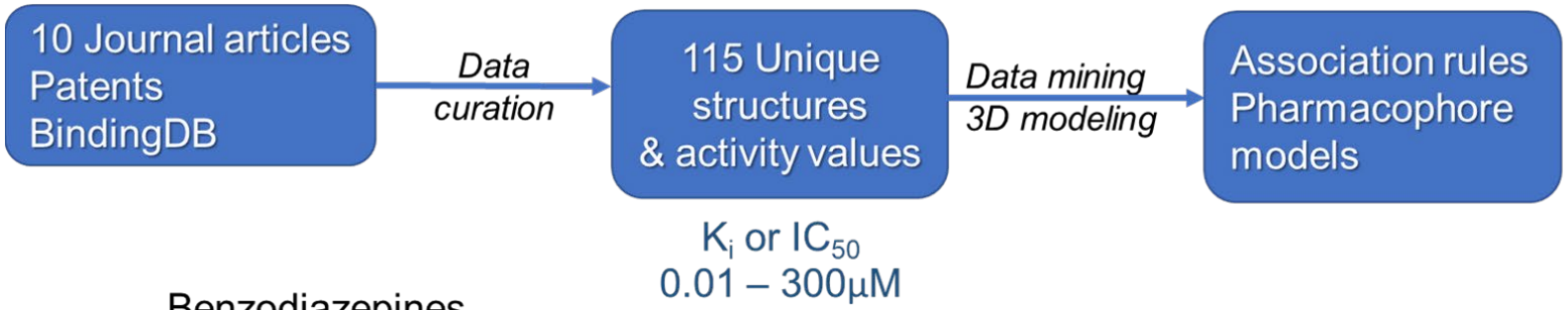
Number of ToxPrint chemotypes (CTs) identified from CTE analysis for 4 separate assay activity-datasets are shown.

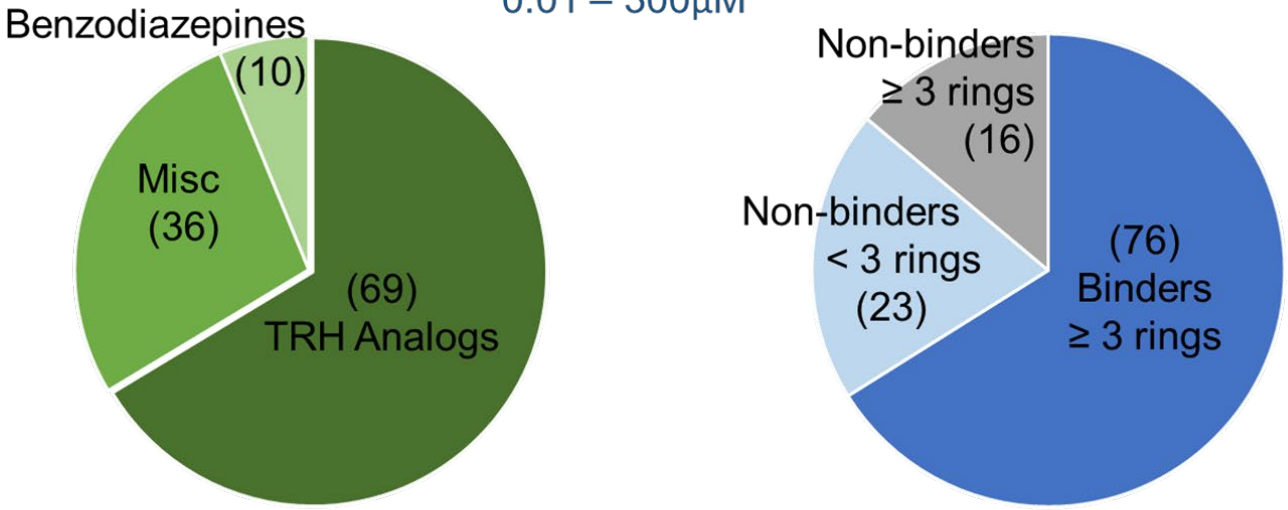
CTs included in the "cytotoxic" regions are significantly enriched in >10/42 Tox21 cytotoxicity-labeled assays

In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* HTS results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

Results: TRHR Binding Studies

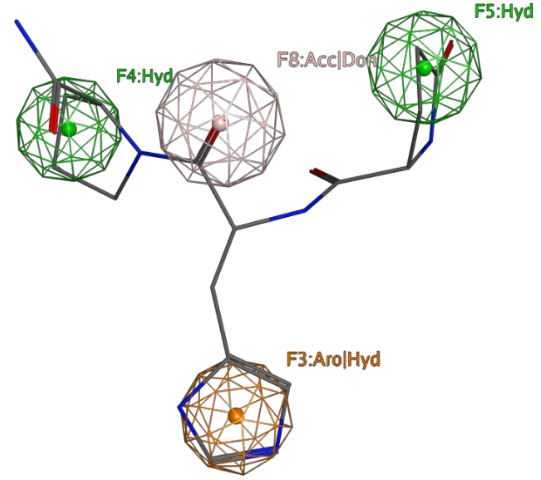
TRHR Binding Literature Survey



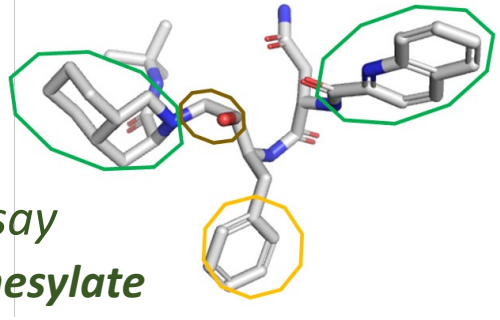


- Limited structural diversity of known TRHR binders
- Majority are TRH analogs containing 3 or more rings
- Benzodiazepines are known drug class of TRHR binders

TRHR Pharmacophore Modeling



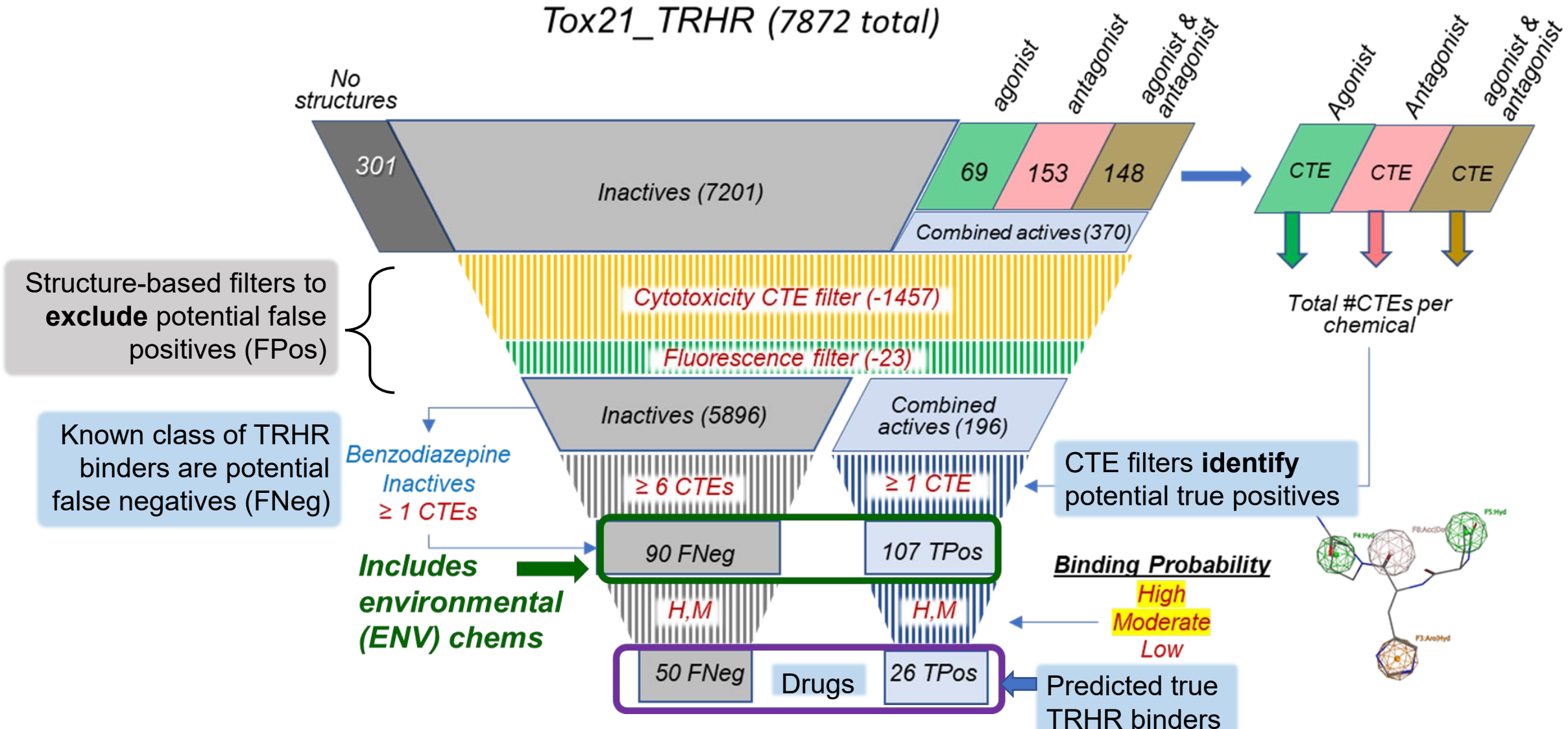
High probability binders limited to TRH analogs (mostly drugs)



Sample TRH-like assay active: ***Saquinavir mesylate***

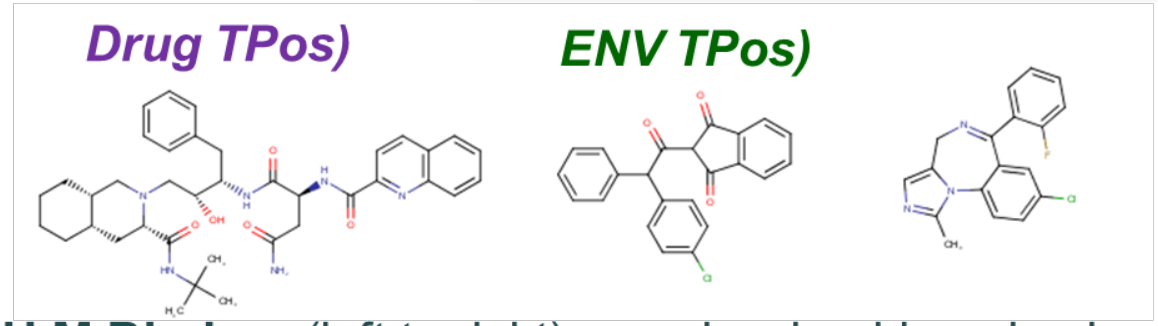
In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* HTS results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

Results: Structure-based prioritization workflow

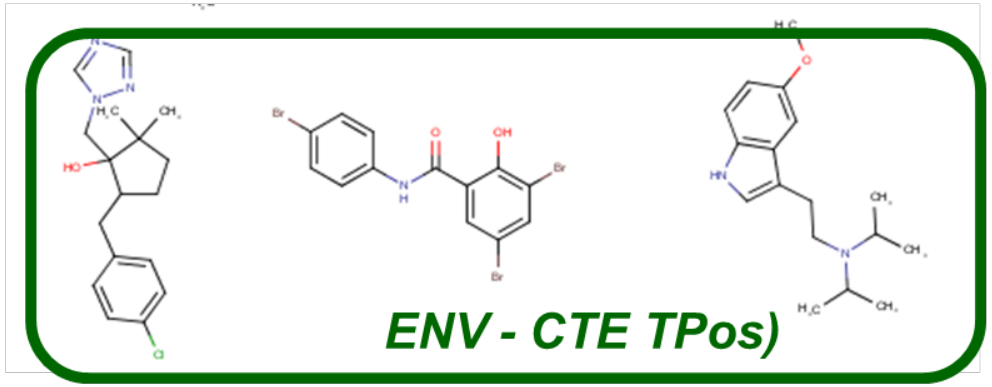


In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* HTS results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

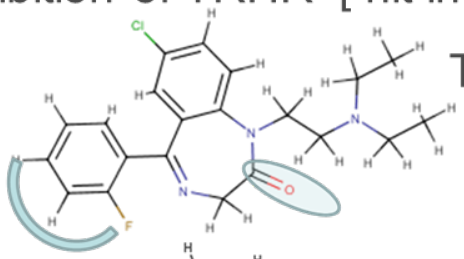
Recommendations for future testing



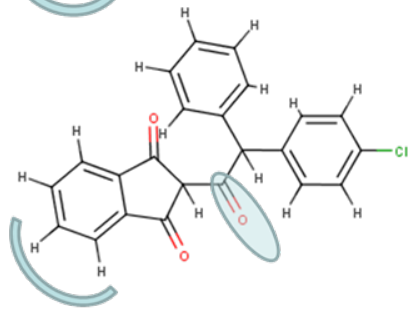
H,M Binders (left to right): saquinavir, chlorophacinone and midazolam



Chlorophacinone (no CTE) has structural features associated with Benzodiazepine inhibition of TRHR [hit in **5** models]



TRHR antagonist
DTXSID1023071



DTXSID2032348

- ***Chlorophacinone*** *predicted to be High binder but did not contain enriched CT for agonist or antagonist model;*
- *After filters and CTE analysis 3 environmental chemicals (left to right) –* ***Metconazole, Tribromsalan, 5-Methoxy-N,N-diisopropyltryptamine*** *– are predicted to be TPos;*
- *Recommend follow-up testing of the limited set of ENV TPos to substantiate TRHR or TR-related activity predictions.*

In silico and cheminformatics enrichment analysis to increase confidence in *in vitro* HTS results: Application to Tox21 thyrotropin-releasing hormone receptor (TRHR) assay

Results Summary

- 3D pharmacophore modeling and domain knowledge predict small set of mostly drugs to be high probability binders to TRHR (not all are active in Tox21_TRHR assay)
- Structure-based tiered workflow led to a 4-fold reduction in the number of predicted true positives for Tox21_TRHR assay, which included some environmental chemicals of potential concern for follow-up testing
- Environmental chemicals in this set are predicted to be low probability or weak receptor binders but with potential to impact TRHR activity

Conclusions

- In the absence of orthogonal or confirmatory assays, structure-based filtering and domain knowledge can help to filter out artifacts and identify potential true positives and false negatives in Tox21/ToxCast HTS assay results
- CTE approaches can leverage information from other Tox21 assays (e.g., cytotoxicity) and can help to isolate and amplify structure-activity signals
- A structure-based tiered workflow, such as presented here, can inform interpretation of HTS target-based assay results and can be used to prioritize chemicals for further testing

References

1. Paul-Friedman, K., Martin, M. et al. (**2019**). Limited chemical structural diversity found to modulate thyroid hormone receptor in the Tox21 chemical library. *Environ. Health Perspect.*, 127(9), 097009. https://doi.org/10.1289/EHP5314
2. Wang, J. Hallinger, D. et al. (**2019**) High-throughput screening and chemotype-enrichment analysis of ToxCast Phase II chemicals evaluated for human sodium-iodide symporter (NIS) inhibition. Environ. Int., 126:377-386. https://doi.org/10.1016/j.envint.2019.02.024
3. Borre,A., Huang, R. et al. (2020)High-Throughput Screening to Predict Chemical-Assay Interference. Sci Rep 10, 3986 (**2020**). https://doi.org/10.1038/s41598-020-60747-3
4. Kaur, N., Lu, X. et al. (**2005**). Thyrotropin-releasing hormone (TRH) analogues that exhibit selectivity to TRH receptor subtype 2. J. Med. Chem., 48(19), 6162–6165. https://doi.org/10.1021/jm0505462