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

March 12-26, 2021
SOT Meeting Virtual

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Abstract

Despite progress in applying high-throughput screening (HTS) technologies to toxicology, exemplified by the Tox21 and ToxCast programs, the challenge of relating biochemical outputs to molecular initiating events (MIEs) and adverse outcome pathways (AOPs) remains challenging, particularly when the relationship between the biochemical output and MIE is indirect. To increase confidence in HTS results for hazard evaluation, a structure-based data enrichment and knowledge contextualization workflow was developed to identify likely false positives and discriminate true receptor binders. The tiered approach was applied to evaluate *in vitro* data for the Thyrotropin-Release Hormone Receptor (TRHR), an MIE in the thyroid hormone AOP. The tiered approach: 1) identifies structure-activity patterns using chemotype-enrichment analysis; 2) filters noise from cytotoxicity or assay interference; 3) uses 3D pharmacophore modeling to prioritize chemicals capable of binding to TRHR as well as modulators likely to interfere with binding. Preliminary results suggest that less than 11% of the Tox21 assay actives exhibit binding features of known TRHR receptor binders. These results presume conservation of the TRHR ligand binding site and structural similarity to known TRHR modulators, benzodiazepines and neuropeptides. The presented tiered workflow is grounded by physical determinants directly related to relevant signals in experimental results, thereby increasing the value of *in vitro* data for chemical prioritization. The workflow can be generalized to other *in vitro* biochemical assays and suggest that combining structure-based methods and data enrichment analysis can increase confidence in HTS results and define a scope of prediction for risk assessment.

Background: In vitro screening of Tox21 library for TRHR activity

1. Tox21_TRHR assay design

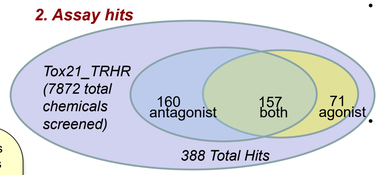
- TRHR is a GPCR with a few known agonists or antagonists. and antagonist modes.
- This assay measures agonism or antagonism for TRHR through the Gq-Ca2+ pathway.

The assay design includes potential sources of artefacts and non-specific interactions, as it indirectly measures TRHR activity leading to the sub-hypothesis:

The screening assay may not be very specific for TRHR agonists, and antagonists.

Objective: Create a workflow to identify & prioritize chemicals with structural features associated with binding to TRH binding site.

2. Assay hits



- 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

3. Prioritization

Approach is to prioritize subset of actives (true hits) and inactives (potential false negatives) for follow-up testing using:

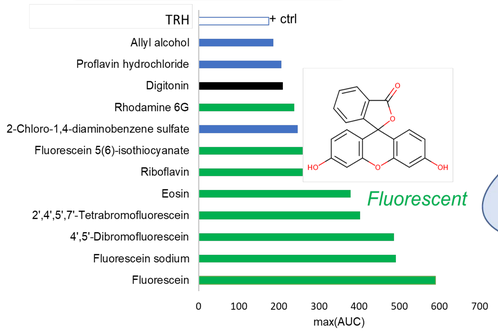
- *domain knowledge*
- *chemotype enrichments*
- *in silico computational chemistry models*

Methods

1. Structures and Tox21 assay data downloaded from EPA dashboard https://comptox.epa.gov/dashboard
2. Statistical analysis code at https://github.com/mshobair/cheminf. Structures visualized https://chemotyper.org/
3. InterPred web service for ML-based fluorescence prediction https://sandbox.ntp.niehs.nih.gov/interferences
4. Pharmacophore modeling using Molecular Operating Environment https://www.chemcomp.com/Products.htm

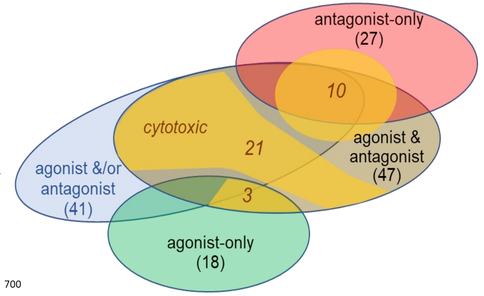
Cheminformatic analysis of assay data

Dose-response curve analysis



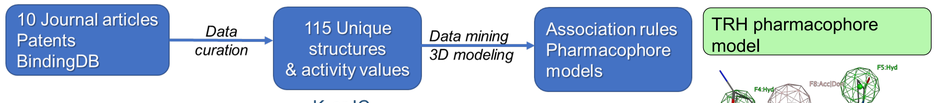
Bar chart of maximum Area-Under-the-Curve (AUC) values per chemical computed for both agonist and antagonist endpoint data, obtained from the publicly available invitrodb database in the ToxCast program. ***Majority of top 0.3% of potent/efficacious hits are enriched in autofluorescent molecules, indicating a source of false positive activity.***

Enriched chemical space partitioning by agonist and antagonist modes of action

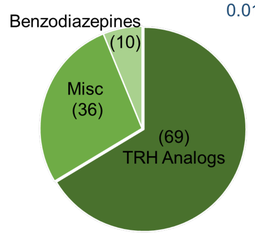


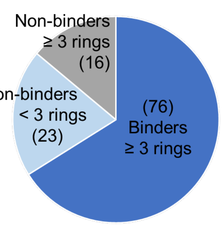
Venn diagram of ToxPrints identified from chemotype enrichment analysis per assay activity-bin: agonist-only, antagonist-only and agonist&antagonist. **Cytotoxicity-associated ToxPrints *overlap with >25% of the agonist&antagonist enriched chemical space. This warrants careful examination of the structure-activity signals when integrating multiple assay endpoint results.***

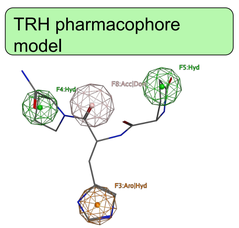
Data gathering and curation of TRHR binding reference dataset

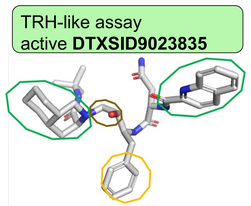


K_i or IC_{50}
0.01 – 300μM



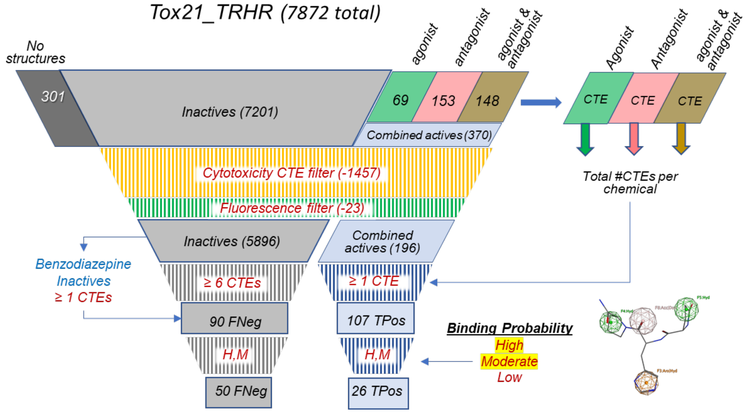






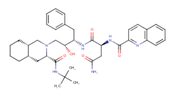
Integrating *in vitro* data & *in silico* modeling strengthens confidence in assay chemical space associated with the MIE. This allows for structure-based chemical prioritization based on known receptor mechanisms of action.

Structure-based chemical prioritization workflow

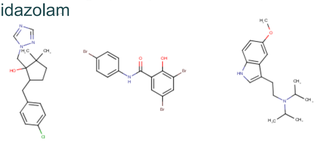


*Integrating chemotype enrichment analysis with binding models allows for **FN** & **TP** detection, and **FP** triage to maximize value of data for hazard evaluation and prioritization*

Recommendations for future testing



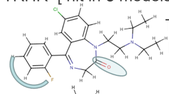
Binders (left to right): saquinavir, chlorophacinone and midazolam



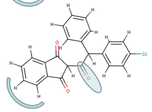
Environmental (CTE TPos)

***From CTE analysis** and likely weak binders (left to right)in the environmental chemicals: Metconazole, Tribromsalan and 5-Methoxy-N,N-diisopropyltryptamine*

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



TRHR antagonist **DTXSID1023071**



DTXSID2032348

- Structurally diverse
- Multi-ring structures

References

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