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

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Abstract and Background

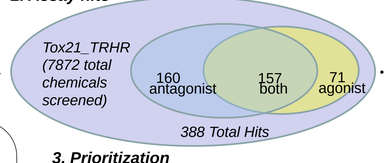
Despite progress in applying high-throughput screening (HTS) technologies to toxicology, exemplified by the Tox21 and ToxCast programs, the challenge of relating biochemical outputs to molecular initiating events (MIEs) and adverse outcome pathways (AOPs) remains challenging, particularly when the relationship between the biochemical output and MIE is indirect. Thyrotropin-Release Hormone Receptor (TRHR) activation is an MIE in the Thyroid-Hormone (TH) AOP, with unknown risk by environmentally-relevant chemicals. A Tox21 HTS biochemical TRHR assay is available but currently without orthogonal or confirmatory Tox21 or ToxCast assays to help confidently differentiate TRHR positive and negative responses. To determine if environmentally relevant chemicals can directly interact with TRHR, we developed an in-silico cheminformatic workflow to amplify biological signals and improve confidence and specificity in the TRHR assay results. The tiered approach: 1) identifies structure-activity patterns using chemotype-enrichment analysis; 2) filters noise from cytotoxicity or assay interference; and 3) prioritizes potential TRHR modulators by likelihood of binding. To build a training set, we created a curated reference dataset from literature studies of competitive binding that reported chemical concentration required to displace binding of radiolabeled TRH. The dataset is balanced between binders and non-binders and mainly contains TRH derivatives and psychoactive drugs. Using pharmacophore modeling, 3D descriptors discriminated between binders and non-binders. Preliminary results suggest that less than 11% of actives in the Tox21 assay contain TRH-like binding features, due to the latter reflecting conservation of the TRHR ligand binding site and structural similarity to known TRHR modulators, benzodiazepines and neuropeptides. The presented tiered workflow increases the value of *in vitro* data for chemical prioritization as it is grounded by the physical determinants directly related to relevant signals in experimental results. Our findings suggest that combining structure-based methods and data enrichment analysis can increase confidence in HTS results and define a scope of prediction for risk

Background: In vitro screening of Tox21 library for TRHR activity

1. Tox21_TRHR assay design

- TSHR 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.

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.

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.

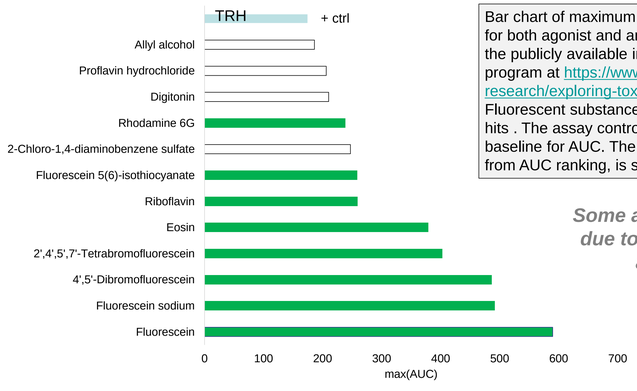
3. Prioritization

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*

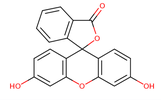
Cheminformatic analysis of assay data

Activity ranking from dose-response modeling



Bar chart of maximum AUC values per chemical computed for both agonist and antagonist endpoint data obtained from the publicly available invitrodb database in the ToxCast program at https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data. Fluorescent substances (Green) are within the top-ranking hits . The assay control TRH (Blue) sample indicates the baseline for AUC. The structure of Fluorescein, the top hit from AUC ranking, is shown in the bottom right.

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



Data gathering and analysis to create a reference dataset of TRHR binders

Literature review and data mining

10 Journal articles
Patents
BindingDB

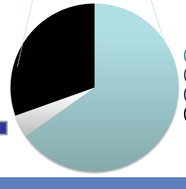
Data curation

115 unique structures & activity values

Data mining 3D modeling

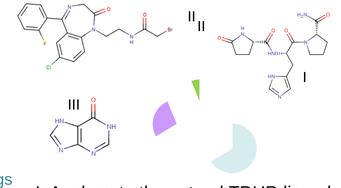
association rules
pharmacophore models

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

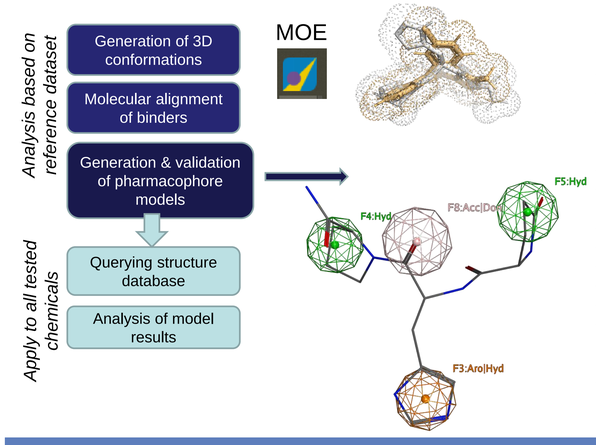


I. Analogs to the natural TRHR ligand
II. Heterocyclic compounds
III. Benzodiazepine-like structures

Methods

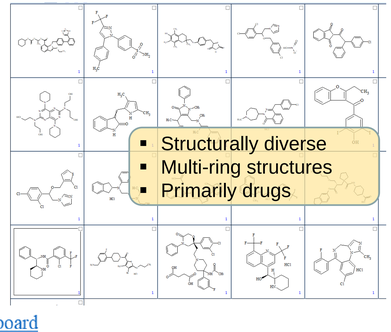
- Cheminformatics analysis of *in vitro* assay data [**Tox21_TRHR**[1]]
- Building reference dataset [**research articles**[2]]
- 2D Filters/3D modeling [**MOE**[3]]
  - chemotype enrichments & heuristics

In silico modeling and prioritization workflow

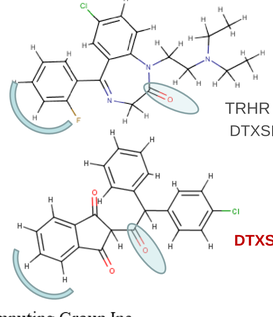


mol	rmsd
	0.4799
	0.2784
	0.2636
	0.5092
	0.2334
	0.3147
	0.4857
	0.2842
	0.3047
	0.3550
	0.2474
	0.3806
	0.3041
	0.2422
	0.2404
	0.2885
	0.4383
	0.5112
	0.2485
	0.3482

Recommendations for future testing



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



1. https://comptox.epa.gov/dashboard
2. PMID: 8648595, 17035026, 16162016
3. MOE (The Molecular Operating Environment), software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A 2R7 (http://www.chemcomp.com

U.S. Environmental Protection Agency
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