Utilizing High-Throughput Screening to Rank and Prioritize Thyroid-Active Chemicals

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Introduction

High-throughput screening (HTS) is used to rapidly assess chemicals for bioactivity at a specific molecular target. Data from these assays can be used to develop a framework to predict *in vivo* effects with the goal of reducing animal testing. One gap in thyroid-related HTS assays includes the thyroid hormone carrier proteins transthyretin (TTR) and thyroxine-binding globulin (TBG). TTR and TBG maintain levels of free versus bound thyroid hormone and serve as circulating hormone transport proteins to deliver thyroid hormone to target tissue. Inhibition of these carrier proteins may result in thyroid axis disruption.[1]

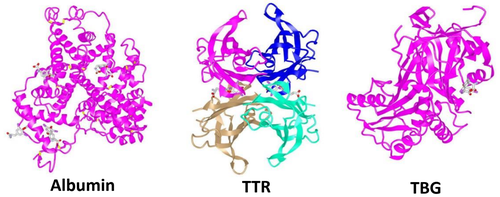


Image from Rabah et al. 2019[1]

Objectives

- Utilize a two-tiered approach to screen chemicals for inhibition of TTR and TBG
  - Tier 1: Single point screening at a high concentration
  - Tier 2: Concentration-response testing

Methods

Chemicals

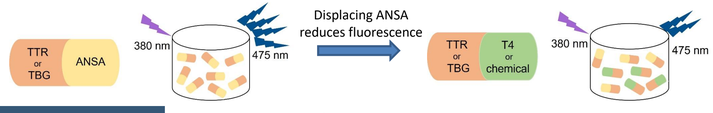
- ToxCast phase1_v2, phase 2, and e1k chemicals were obtained in 96-well plates at target concentrations of 20 mM in DMSO.

Inhibition assays

- The TBG and TTR inhibition assays closely followed Montaño et al.[2]
- 200 µL reactions were set up in 96-well plates with three replicate reaction plates per chemical source plate.
- 0.0625 µM TBG and 0.125 µM TTR were added to each reaction.
- ANSA fluoresces when bound to TBG or TTR. The concentration of ANSA in each reaction was 0.6 µM and 1.2 µM for TBG and TTR, respectively.
- Reactions were incubated at 4C for 2 hours before reading on a Biotech Synergy Neo2 plate reader (Winooski, VT). ANSA was excited with a 380 nm filter, and emission was measured with a 475 nm filter.
- T4, an endogenous ligand of TTR and TBG, displaces ANSA and reduces fluorescence. T4 was used to prepare a standard curve ranging from 0.0013 µM to 1.8 µM.
- Fluorescence was corrected by subtracting autofluorescence of TBG or TTR
- Fluorescence was normalized to DMSO controls as 100% activity and to the highest concentration of T4 as complete inhibition.

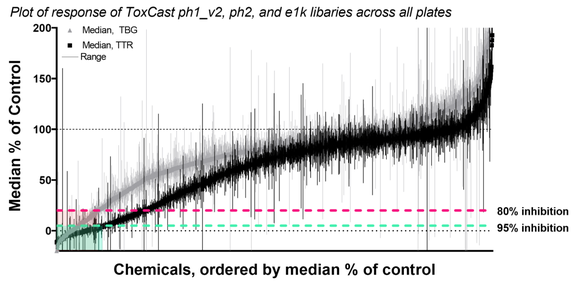
Strategy

- Initial single concentration screen at 100 µM. 20 mM plated stock chemical in DMSO used at 1:200 dilution in reactions with final DMSO at 0.5%
- Chemicals with inhibition greater than 80% in the TBG assay or 95% in the TTR assay moved on to concentration-response testing.



Single Point Screening Results

Plot of response of ToxCast ph1_v2, ph2, and e1k libaries across all plates



Expanded plot of potential inhibitors

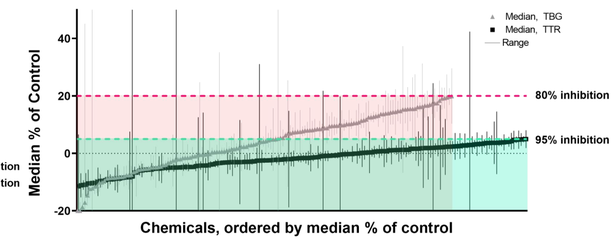


Table 1. Summary of single point screening

	TBG		TTR	
% inhibition	Total # Chemicals	%	Total # Chemicals	%
NA	118	7	146	8
<20%	918	51	698	38
≥20%	777	43	969	53
≥80%	154	8	338	18
≥95%	82	4.5	184	10
Total	1813		1813	

- Percent inhibition is calculated as 100 – median % of control.
- Chemicals with ≥80% inhibition in the TBG assay and ≥95% inhibition in the TTR assay move on to concentration response.

Concentration-Response Results

Concentration-response data for chemicals screened in the TBG assay (A-F) and the TTR assay (G-L). All plots include the T4 standard curve (gray circles) run in the same 96-well plate as the chemical [black triangles (TBG) or black squares (TTR)]. The T4 standard curves ranged from 0.0013-0.3645 µM in the TBG assay and 0.0067-1.8 µM in the TTR assay.

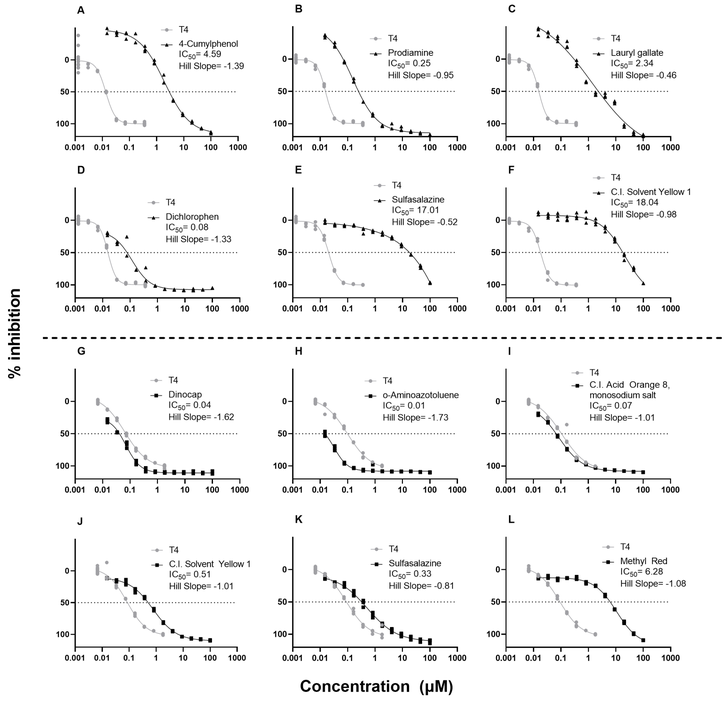


Table 2. Single point screening and concentration-response of example chemicals

	Plot	Chemical Name	Rank in Single Point	Median % inhibition: Single Point at 100 µM	Median % inhibition: Conc-Resp at 100 µM	Median % inhibition: Conc-Resp at 45 µM	Conc-Resp. IC50 (µM)	Conc-Resp. Hill Slope
TBG	A	4-Cumylphenol	3	118.68	113.35	106.9	4.59	-1.39
	B	Prodiamine	11	110.09	114.98	113.55	0.25	-0.95
	C	Lauryl gallate	14	108.54	117.55	115.34	2.34	-0.46
	D	Dichlorophen	16	108.44	104.36	107.01	0.08	-1.33
	E	Sulfasalazine	25	106.18	96.24	70.92	17.01	-0.52
	F	C.I. Solvent Yellow 1	34	104.73	97.55	74.4	18.04	-0.98
TTR	G	Dinocap	1	111.49	111.95	111.37	0.04	-1.62
	H	o-Aminoazotoluene	3	111	109.36	108.81	0.01	-1.73
	I	C.I. Acid Orange 8, monosodium salt	4	110.91	109.12	108.58	0.07	-1.01
	J	C.I. Solvent Yellow 1	11	109.59	109.61	107.99	0.51	-1.01
	K	Sulfasalazine	12	109.33	109.42	107.55	0.33	-0.81
	L	Methyl red	13	109.09	108.84	98.33	6.28	-1.08

Conclusions and Future Work

- A greater percentage of chemicals were active (*i.e.,* ≥ 20% inhibition) in the TTR assay than the TBG assay in single point screening. One possible reason for this could be that TTR is a homotetramer, and displacement of ANSA may be due to dissociation of the tetramer as opposed to the chemical at the binding site.
- Concentration-response screening is ongoing. The data will be processed through the ToxCast Analysis Pipeline to define the IC_{50} with the goal of ranking and prioritizing chemicals for further testing.
- Future work may include *in vivo* testing of the chemicals identified as inhibitors of TBG or TTR to determine whether disruption of the thyroid axis can be inferred from *in vitro* activity.

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References

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