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Identifying Thyroid-Active Chemicals Using High-Throughput Screening

Stephanie A. Eytcheson[1,2], Alexander D. Zosel[2,3], Michael W. Hornung[2], Sigmund J. Degitz[2]

[1]Oak Ridge Institute for Science and Education Postdoctoral Fellow, Oak Ridge, TN, USA

[2]U.S. Environmental Protection Agency, Office of Research and Development, Center for Computational Toxicology and Exposure, Great Lakes Toxicology and Ecology Division, Duluth, MN, USA

[3]Oak Ridge Associated Universities Student Services Contractor, Oak Ridge, TN, USA

Introduction

High-throughput screening (HTS) is used to rapidly assess chemicals for bioactivity at a specific molecular target. The goal of HTS is to reduce the cost of generating data to support assessing the risk of exposure to chemicals. HTS data can be used to identify potential molecular initiating events within adverse outcome pathways. 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 ligand binding to these carrier proteins may result in thyroid axis disruption.[1]

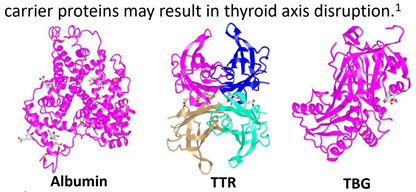


Image from Rabah et al. 2019[1]

Objectives

- Utilize a two-tiered approach to screen chemicals for binding to TTR and TBG
  - Tier 1: Single point screening at a high concentration to identify chemicals with the potential to inhibit TH binding to TTR or TBG
  - Tier 2: Concentration-response testing to establish IC50 for active chemicals

Methods

Chemicals

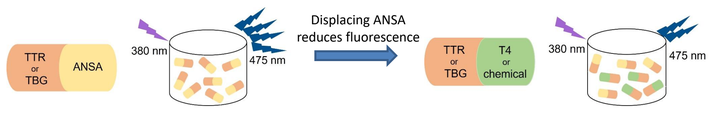
- ToxCast phase1_v2, phase 2, and e1k chemicals[2] were obtained in 96-well plates at target concentrations of 20 mM in dimethyl sulfoxide (DMSO).

Displacement assays

- TBG and TTR displacement assays closely followed Montaño et al.[3]
- 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.
- 8-Anilino-1-naphthalenesulfonic acid (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 4°C 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 maximum fluorescence (100% ANSA bound to TBG or TTR) and to the highest concentration of T4 as complete inhibition.

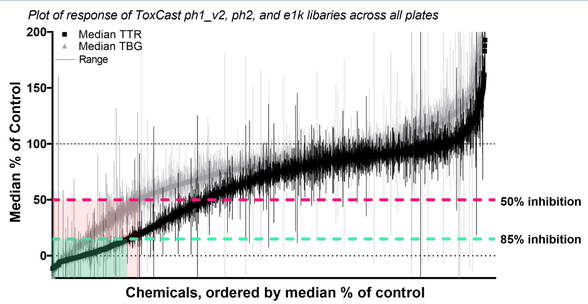
Strategy

- 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 activity greater than 50% in the TBG assay or 85% in the TTR assay moved on to concentration-response testing.



Stephanie A. Eytcheson | eytcheson.stephanie@epa.gov | 218-529-5127

Single Point Screening Results



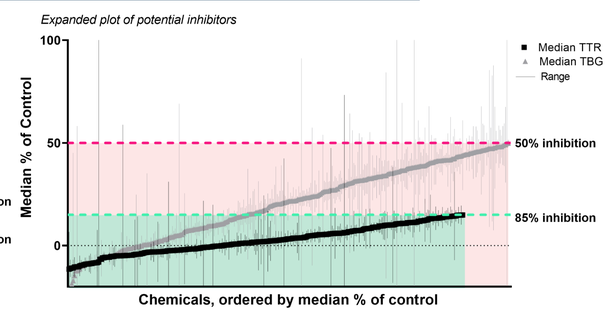


Table 1. Summary of single point screening

	TBG		TTR	
% inhibition	Total # Chemicals	%	Total # Chemicals	%
NA	118	7	146	8
<20%	918	51	698	38
≥20%	777	43	969	53
≥50%	328	18	591	32
≥85%	133	7	294	16
Total	1813		1813	

- Percent inhibition is calculated as 100 – median % of control.
- 328 and 294 chemicals were moved on to concentration response testing in the TBG and TTR assay, respectively.

Concentration-Response Results

Concentration-response data for chemicals screened in the TBG assay (A-J) and the TTR assay (K-T). 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.

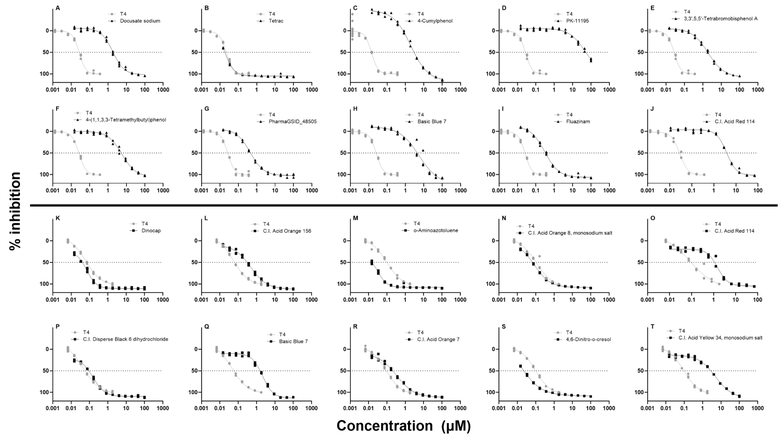


Table 2. Single point screening and concentration-response of example chemicals

			Rank		Median % inhibition		Conc-Resp.	
	Plot	Chemical Name	Single Point	Conc-Resp	Single Point at 100 µM	Conc-Resp at 100 µM	IC50 (µM)	Hill Slope
TBG	A	Docusate sodium	1	27	122.5	104.1	1.995	-1.63
	B	Tetrac	2	1	122.2	106.5	0.022	-3.59
	C	4-Cumylphenol	3	42	118.7	113.4	3.27	-1.83
	D	PK-11195	4	202	117.1	66.8	47.01	-1.43
	E	3,3',5,5'-Tetrabromobisphenol A	5	28	114.6	104.7	2.00	-1.16
	F	4-(1,1,3,3-Tetramethylbutyl)phenol	6	63	112.4	101.4	5.32	-1.18
	G	PharmaGSID_48505	7	10	112.2	101.2	0.495	-1.42
	H	Basic Blue 7	8	51	111.5	107.1	3.86	-1.25
	I	Fluazinam	9	8	111.3	106.9	0.382	-1.47
	J	C.I. Acid Red 114	10	49	110.1*	102.6*	3.80	-2.30
TTR	K	Dinocap	1	14	111.5	112	0.036	-1.19
	L	C.I. Acid Orange 156	2	52	111.1	110.8	0.29	-0.8
	M	O-Aminoazotoluene	3	2	111.0	109.4	0.018	-1.44
	N	C.I. Acid Orange 8, monosodium salt	4	22	110.9	109.1	0.072	-0.946
	O	C.I. Acid Red 114	5	77	110.5*	105.6*	0.73	-0.839
	P	C.I. Disperse Black 6 dihydrochloride	6	24	110.4	111.4	0.08	-0.891
	Q	Basic Blue 7	7	95	110.3	111.7	1.47	-1.20
	R	C.I. Acid Orange 7	8	44	110.3	111.4	0.2	-0.762
	S	4,6-Dinitro-o-cresol	9	3	109.9	109.5	0.02	-0.777
	T	C.I. Acid Yellow 34, monosodium salt	10	120	109.6	108.6	2.47	-0.592

*Maximum concentration = 70 µM

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. Alternatively, TTR has two binding sites and can bind retinol-binding protein as well as thyroid hormones, thus perhaps allowing for less specific binding.
- 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.

Acknowledgements

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