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Supplemental Material

Limited Chemical Structural Diversity Found to Modulate Thyroid Hormone Receptor in the Tox21 Chemical Library

Katie Paul-Friedman, Matt Martin, Kevin M. Crofton, Chia-Wen Hsu, Srilatha Sakamuru, Jinghua Zhao, Menghang Xia, Ruili Huang, Diana A. Stavreva, Vikas Soni, Lyuba Varticovski, Razi Raziuddin, Gordon L. Hager, and Keith A. Houck

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Figure S1. Quantitative high-throughput screening for TR modulators and follow-up workflow. The Tox21 chemical library was screened at 15 concentrations in three independent runs in agonist and antagonist modes. A cell viability assay was run in parallel. Data were fit to concentration-response curves using the tcpl package and active calls determined. Manual review of the curves based on fitting warning flags resulted in fewer actives. The ratio of TR antagonist AC50 ($AC50^{TR}$) to the viability assay ($AC50^{Via}$) was calculated to help rule out cytotoxic compounds. All 28 agonist candidates along with additional compounds that had some evidence of activity in the qHTS were characterized in multiple characterization assays to confirm activity as direct TR agonists or indirect ones acting through RXR. The best candidate antagonists were characterized in additional assays.

Figure S2. Structures assigned as TR activators. Highest confidence agonist chemicals for TR working either directly through TR (A) or indirectly through RXR (B). Also shown are likely false positive results from the qHTS (C) Note that cresol is represented by a Markush structure which represents a group of related chemical structures having variation in the shaded region.

Figure S3. Structures assigned as TR antagonists/inhibitors. Highest confidence agonist chemicals for TR working either directly through TR (A) or indirectly or potentially artefactual mechanisms (B).

Figure S4. Promiscuity and cytotoxicity of bisphenol A. The EPA's invitrodbv3 database was queried for bioactivity of bisphenol A using the Chemistry Dashboard (<http://comptox-dev.epa.gov/dashboard/dsstoxdb/results?search=DTXSID7020182#bioactivity>). The numbers of active assay hit calls are displayed in a histogram using AC₅₀ values as intervals (blue bars) with the cumulative frequency shown by the orange line. The AC50 for the TR_LUC_GH3_Antagonist assay is indicated by the blue arrow and the Cytotoxicity Limit from the Dashboard by the red arrow.

Additional File- Excel Document