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Screening the Tox21 10K library for thyroid stimulating hormone receptor (TSHR) agonist and antagonist activity

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Thyroid-stimulating hormone (TSH) regulates thyroid hormone (TH) production via binding to its receptor (TSHR). The roles of TSHR in human pathologies including hyper/hypothyroidism, Grave's disease, and thyroid cancer are known, but it is currently unknown whether TSHR is an important screening target to identify chemicals that may perturb THs. The Tox21 collaboration enabled testing of the null hypothesis that TSHR is not targeted by environmentally-relevant chemicals. The TSHR is a G protein-coupled receptor that, when agonist-bound, activates adenylate cyclase with a resultant increase in cyclic adenosine 3',5' monophosphate (cAMP) that upregulates TH production. Commercially-available HEK293-TSHR cells were used in a 1536-well assay format to demonstrate agonism or antagonism of the TSHR, using cAMP as a marker of TSHR activation. A wildtype control was used to identify confounders, e.g. nonspecific modulators of cAMP. Homogeneous time-resolved fluorescence technology was used to quantify cAMP using a competitive immunoassay between native cAMP and dye-labeled cAMP. In agonist and antagonist mode optimization, TSH demonstrated signal-to-background ratios of 3.8 and 2.6, Z-factors of 0.75 and 0.53, and CVs of 3.6 and 5.7%, respectively, and an EC50 = 0.28 ng/mL. The Tox21 library (9667 chemicals) was tested in concentration-response in triplicate, and data were normalized by plate and plotted as %control, with the greater of a 20% change or 6*baseline median absolute deviation as the activity cutoff. Candidate agonists (493) and antagonists (340) were identified, with >70 chemical hits flagged as potentially nonspecific based on activity in wildtype cells, including cytotoxic organometallic compounds, autofluorescent or dye compounds, and cAMP upregulators active through other receptors or mechanisms. Potency (EC50/IC50) values ranged from 1e-5 to 150 µM for agonists and 1e-3 to 170 µM for antagonists. These chemicals have been prioritized for further screening as TSHR modulators using orthogonal assay technologies. *This abstract does not necessarily reflect the policy of the US EPA.*