A High-Throughput Screening Assay to Detect Thyroperoxidase Inhibitors and Discover Structural Alerts

Steven O. Simmons

[1]National Center for Computational Toxicology;
ORD, U.S. EPA, RTP, NC

In support of the Endocrine Disruption Screening Program (EDSP21), the US EPA ToxCast program is developing assays to enable screening for chemicals that may disrupt thyroid hormone synthesis. Thyroperoxidase (TPO) is critical for TH synthesis and is a known target of thyroid-disrupting chemicals that adversely impact neurodevelopment. The AUR-TPO assay was recently developed to screen >1,900 ToxCast chemicals for potential TPO inhibition activity. Parallel assays were used to determine which AUR-TPO actives were more selective for TPO inhibition. Additionally, the TPO inhibition activities of 150 chemicals were compared between the AUR-TPO assay and an orthogonal peroxidase oxidation assay using guaiacol as substrate to confirm putative TPO inhibition profiles. Bioactivity results from the AUR-TPO assay were used to identify chemical substructures associated with in vitro TPO inhibition. Substructure profiles were generated for each chemical in the ToxCast test set using the publicly-available ToxPrint 2.0 chemotypes. Chemotypes enriched among the putative TPO inhibitors were identified using a cumulative hypergeometric probability ($p < 0.01$). Of the total 729 chemotypes evaluated, 44 were overrepresented among TPO inhibitors. Another 24 chemotypes were found to be significantly underrepresented among AUR-TPO actives. Examination of these chemotypes revealed four basic pharmacophores that accounted for 70% of the ToxCast chemicals active in the AUR-TPO assay: aromatic alcohols, aromatic amines, thiocarbonyls and phosphothioates. Chemico-structural analysis of AUR-TPO screening results enabled the identification of chemical features that likely drive TPO inhibition in the AUR-TPO assay, highlighting the potential to identify thyroid-disrupting chemicals in silico using structural alerts identified by chemotype analysis and confirmed by in vitro testing. The pharmacophores identified using this approach also offer key insights into mechanisms of TPO inhibition, which should strengthen the development of predictive tools. *This abstract does not necessarily reflect the policy of the US EPA.*