Identification of Androgen Receptor Antagonists using Tox21 qHTS Data and the MARCoNI Nuclear Receptor Co-regulator Recruitment Assay

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Androgen receptor (AR) antagonism is a mechanism of endocrine disruption by environmental chemicals that is associated with a number of adverse effects including reproductive organ cancers and abnormalities. To identify chemicals that may act as AR antagonists, quantitative high-throughput screening efforts were undertaken by the Tox21 federal agency collaboration. 8307 chemicals were screened in an AR luciferase-based reporter gene assay using MDA-kb2 cells (human breast cell line) (LUC) stimulated with 10 nM R1881 to identify antagonists. 7523 of these chemicals were also tested at a lower agonist concentration (0.5 nM, LUC2) in order to detect whether a shift in agonist concentration also resulted in a shift in the effective concentration needed to produce antagonism, indicating a true, competitive inhibitor effect. 875 (11%) and 1206 (16%) of the chemicals were active in the LUC and LUC2 assays, respectively. 706 chemicals were active in both. To identify structural similarities amongst active chemicals, we clustered chemicals in a self-organizing map using ToxPrint chemotypes. We calculated the median ΔAC50 values between the two assays for each cluster, assuming a higher median ΔAC50 would correlate to a higher confidence in true antagonism. The clusters ~~that had~~with the highest values included chlorinated bisphenols, estrogens, and hydroxyfluorenes. We selected 318 chemicals for testing in the Microarray Assay for Real-time Coregulator-Nuclear receptor Interaction (MARCoNI) platform using the AR and 154 co-regulator peptides. We identified two recruitment patterns that distinguished between the reference antagonists hydroxyflutamide and bicalutamide, as well as patterns that correlated to dyes and detergents that are likely false positive. Using these assay technologies, we identified novel AR antagonists and structural features that are related to AR antagonism as well as increased our confidence in sorting true antagonists from false positives for this important target of endocrine disruption. This abstract does not reflect federal agency policy.