Evaluation of FXR-active Chemicals Identified from Tox21 Screening

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Nuclear receptors play a key role in physiological functions. Assessing how chemicals interact with this superfamily of proteins can provide mechanistic data that supports the construction of toxicity pathways related to human disease. Farnesoid X receptor alpha (FXRα, NR1H4) is a member of the nuclear receptor superfamily with demonstrated importance in bile acid homeostasis, glucose metabolism, lipid homeostasis, and hepatic regeneration. In this study, we evaluated a select set of compounds previously identified in Tox21 qHTS *in vitro* screens as FXRα agonists and antagonists using four experimental approaches. Transactivation studies were conducted to validate potency and efficacy of putative human FXR agonists and antagonists and determine interactions with medaka FXRα. Functional analyses of ligand-induced receptor:coregulator interactions were conducted to gain mechanistic insights beyond receptor transactivation studies. 3D molecular docking studies evaluated the respective binding modes of putative agonists and antagonists in the FXR active site. Finally, a larval medaka assay was used to evaluate gene expression changes induced by FXR ligands *in vivo*. Transactivation reported in the Tox21 FXR-*bla* assay was generally confirmed in our transactivation studies, although we found diuron, which was labeled inactive in Tox21, to be a potent antagonist with both human and medaka FXR. FXR agonists identified as “active” displayed significantly diverse and complex ligand-induced protein:protein interactions with FXR and selected NR coregulators. Docking experiments indicated that a number of these chemicals have favorable interactions within the binding pocket of the FXR crystal structure. Expression of Fxr hepatic gene targets (BSEP, CYP7a, SHP) following compound exposures in medaka larvae demonstrated *in vivo* activities of both FXR agonists and FXR antagonists. In summary, the current study generally confirmed qHTS *in vitro* results, provided orthogonal data on protein:protein interactions and receptor docking, and translated those results to an *in vivo* system. *This project was funded in whole or in part with federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.*

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