Abstract

High-throughput screening (HTPS) assays to detect inhibitors of thyroperoxidase (TPO), the enzymatic catalyst for thyroid hormone (TH) synthesis, are not currently available. Herein we describe the development of a HTPS TPO inhibition assay. Rat thyroid microsomes and a fluorescent peroxidase substrate, Amplex UltraRed (AUR, LifeTechnologies), were employed in an endpoint assay for comparison to the existing kinetic guaiacol (GUA) oxidation assay. Following optimization of assay metrics including Z', dynamic range, and activity using methimazole (MMI), the assay was tested with a 21-chemical training set. The potency of MMI-induced TPO inhibition was greater with AUR compared to GUA. The dynamic range and Z' score with MMI were as follows: 127-fold and 0.62 for the GUA assay, 18-fold and 0.86 for the 96-well AUR assay, and 11.5-fold and 0.93 for the 384-well AUR assay. The 384-well AUR assay drastically reduced animal use, requiring one-tenth of the rat thyroid microsomal protein needed for the GUA 96-well format assay. Fourteen chemicals inhibited TPO, with a relative potency ranking of MMI > ethylene thiourea > 6-propylthiouracil > 2,2',4,4'-tetrahydroxy-benzophenone > 2-mercaptobenzothiazole > 3-amino-1,2,4-triazole > genistein > 4-propoxyphenol > sulfamethazine > daidzein > 4-nonylphenol > triclosan > iopanoic acid > resorcinol. These data demonstrate the capacity of this assay to detect diverse TPO inhibitors. Seven chemicals acted as negatives: 2-hydroxy-4-methoxybenzophenone, dibutylphthalate, diethylhexylphthalate, diethylphthalate, 3,5-dimethylpyrazole-1-methanol, methyl 2-methyl-benzoate, and sodium perchlorate. This assay could be used to screen large numbers of chemicals as an integral component of a tiered TH-disruptor screening approach.