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Abstract Nuclear translocation is an important step in glucocorticoid receptor (GR) signaling and assays that measure this process allow the identification of nuclear receptor ligands independent of subsequent functional effects. To facilitate the identification of GR-translocation agonists, an enzyme fragment complementation (EFC) cell-based assay was scaled to a 1536-well plate format to evaluate 9,920 compounds using a quantitative high throughput screening (qHTS) strategy where compounds are assayed at multiple concentrations. In contrast to conventional assays of nuclear translocation the qHTS assay described here was enabled on a standard luminescence microplate reader precluding the requirement for imaging methods. The assay uses beta-galactosidase alpha complementation to indirectly detect GR-translocation in CHO-K1 cells [Fung, P., et al. *Assay Drug Devel. Technol*. 2006, 4(3): 263–272]. 1536-well assay miniaturization included the elimination of a media aspiration step, and the optimized assay displayed a Z′ of 0.55. qHTS yielded EC_{50} values for all 9,920 compounds and allowed us to retrospectively examine the dataset as a single concentration-based screen to estimate the number of false positives and negatives at typical activity thresholds. For example, at a 9 μM screening concentration the assay showed an accuracy that is comparable to typical cell-based assays as judged by the occurrence of false positives that we determined to be 1.3% or 0.3%, for a 3σ or 6σ threshold, respectively. This corresponds to a confirmation rate of ~30% or ~50%, respectively. The assay was consistent with glucocorticoid pharmacology as scaffolds with close similarity to dexamethasone were identified as active, while, for example, steroids that act as ligands to other nuclear receptors such as the estrogen receptor were found to be inactive.

Keywords:
qHTS, HTS, EFC, PubChem, glucocorticoid receptor, nuclear translocation, suspension cells
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