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J. Med. Chem., 2008, 51 (8), pp 2363–2371
DOI: 10.1021/jm701301m
Publication Date (Web): March 26, 2008

Abstract

Chromo/fluorophoric properties often accompany the heterocyclic scaffolds and impurities that comprise libraries used for high-throughput screening (HTS). These properties affect assay outputs obtained with optical detection, thus complicating analysis and leading to false positives and negatives. Here, we report the fluorescence profile of more than 70000 samples across spectral regions commonly utilized in HTS. The quantitative HTS paradigm was utilized to test each sample at seven or more concentrations over a 4-log range in 1536-well format. Raw fluorescence was compared with fluorophore standards to compute a normalized response as a function of concentration and spectral region. More than 5% of library members were brighter than the equivalent of 10 nM 4-methyl umbelliferone, a common UV-active probe. Red-shifting the spectral window by as little as 100 nm was accompanied by a dramatic decrease in autofluorescence. Native compound fluorescence, fluorescent impurities, novel fluorescent compounds, and the utilization of fluorescence profiling data are discussed.