Impedance-based Secondary Screening of OX1 Receptor Antagonists using the 384-format RTCA HT Instrument

Impedance-based technologies have established themselves as powerful tools in cell biology. Impedance allows a label-free and sensitive kinetic readout of changes in cell morphology induced by a multitude of different stimuli, thus it is used in many different cell biological contexts. In drug discovery, impedance technologies are entering the High-Throughput (HTS) domain, especially in the field of GPCR (G-protein-coupled receptor) signaling assays this technology is regarded as a valuable complementation of already existing classical GPCR assays.

In this study, the recently developed 384-well impedance device RTCA HT (Roche Applied Science) was used as a secondary screening tool in a HTS for orexin type 1 (Ox1) receptor antagonists. Primary hits obtained by the classical calcium flux (FLIPR) technology using CHO-Ox1 cells were validated in newly developed fully-automated RTCA HT assays using the same cells. Impedance data were analyzed for antagonistic activities of the compounds, specificity of activity as well as robustness of these results (intra-assay and inter-assay variability). Finally, the antagonist hit confirmation rate compared to FLIPR data was determined. In conclusion, we find that with the novel 384-well RTCA HT technology assay robustness is comparable to classical GPCR assay technologies, hit confirmation rates were between 60-70 % with most of the non-confirmed hits being non-specific blockers of calcium signaling.

Introduction

Impedance technology allows a label-free, real-time readout of cellular morphological changes and is ideally suited to measure GPCR activation or inhibition independent of the coupling pathway in recombinant or non-recombinant cellular systems. As shown in Figure 1A all major G-protein pathways (i.e. Gs, Gq, Gi, G12/13) lead to changes in cellular morphology, thus the electrical impedance of an adherent monolayer can be measured when cells are grown on electrode surfaces. The newly developed 384-well RTCA HT instrument (Roche Applied Science) allows the analysis of such cellular responses to compounds in an HTS mode.

In this study we describe the use of the Roche RTCA HT instrument in a secondary screening within a HTS campaign searching for antagonists of the orexin type 1 receptor (Ox1). As seen in Figure 1B, 7160 compounds were evaluated in HTS using cell line assays (FLIPR) with CHO-Ox1 cells. 234 compounds thereof could be confirmed in the same assay. These 324 Ox1 antagonist hits were then subjected to FLIPR specificity assays using limited CHO-OX1 cells, and then analyzed in 2 fully-automated RTCA HT screenings on CHO-OX1 and CHO-S1P3 cells, respectively.

Methods

xCELLigence Technology

Integration of RTCA HT Stations into the Agilent Automation System

RTCA HT Screen Optimization

Results

Quality Metrics

Conclusions

We have integrated 2 xCELLigence RTCA HT Instruments (Roche Applied Science) into our double BioCel1200 Automation Platform from Agilent. Integration, performance and stability were assessed in automated screening protocols, by performing a secondary assay on hits that were previously identified in a calcium flux FLIPR assay to check the GPCR agonist screening.

The 2 RTCA HT Instruments could easily be integrated into the Agilent Automation System

Conclusion:

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