Detection of Species Selective Agonists

PathHunter™ β-Arrestin Assays: Determination of Pharmacology Differences Between Human and Ortholog GPCRs
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Abstract

Screening previously introduced a protein-protein interaction screening engine using enzyme fragment complementation that provides a robust, HTS-friendly format for maintaining the interaction of activated GPCRs and their receptors. To date, DiscoverRx has added this technology to generate over 170 pre-validated human cell lines. More recently, the coupling of mouse and/or GPCR in the brain imaging signaling pathway has been investigated. A systematic analysis of the chimeric receptor family and their respective mouse and human ligands revealed distinct efficacy and pharmacology differences in the ortholog receptors. Several ligands were found that provided cross-receptor selectivity. By contrast, non-human ligands were found to be non-selective and are thus not as superior agonists for the corresponding human receptor. Over 60 ortholog GPCR cell lines have now been developed using the PathHunter®-Arrestin platform to support our drug development programs and to explore the role of Arrestins screening in cross-species compound validation.

PathHunter™-Arrestin GPCR Assay System

Materials

- PathHunter™-Arrestin assay cell lines expressing the Arrestin β-Arrestin protein
- A human chimeric GPCR receptor isolated for the PathHunter®-Arrestin assay cell line

Methods

- Preparation of human and mouse cell lines expressing the Arrestin β-Arrestin protein
- A human chimeric GPCR receptor isolated for the PathHunter®-Arrestin assay cell line

Results

- Detection of Species Selective Agonists
  - Pharmacology is Independent of Receptor Expression Levels
  - Selectivity Compound Profiling
  - Analysis of Additional Chemokine Receptors

Discussion

- PathHunter™-Arrestin Assays: Determination of Pharmacology Differences Between Human and Ortholog GPCRs

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Notes

- m = mouse; c = canine; r = rat
- mRNA level
- Light
- RLU
- μL
- A & C
- B & D

Figure 1. PathHunter™-Arrestin GPCR Assay: isolation of the human chimeric GPCR receptor for the PathHunter®-Arrestin assay cell line

Figure 2. PathHunter™-Arrestin assay using human (A & C) and mouse (B & D) Chemokine (C-C motif) Receptor 3 (CCR3) expressing cell lines. Human CCL1 (µM) treated with CCR3 ligands: CCL2, CCL3 and CCL4. Human CCL1 (µM) treated with CCR3 ligands: CCL2, CCL3 and CCL4. Human CCL1 (µM) treated with CCR3 ligands: CCL2, CCL3 and CCL4. Human CCL1 (µM) treated with CCR3 ligands: CCL2, CCL3 and CCL4.

Figure 3. PathHunter™-Arrestin assay using human (A & C) and mouse (B & D) Chemokine (C-C motif) Receptor 6 (CCR6) expressing cell lines. Human CCL1 (µM) treated with CCR6 ligands: CCL2 and CCL4. Human CCL1 (µM) treated with CCR6 ligands: CCL2 and CCL4. Human CCL1 (µM) treated with CCR6 ligands: CCL2 and CCL4. Human CCL1 (µM) treated with CCR6 ligands: CCL2 and CCL4.

Figure 4. PathHunter™-Arrestin assay using human (A & C) and mouse (B & D) Chemokine (C-C motif) Receptor 2 (CCR2) expressing cell lines. Human CCL1 (µM) treated with CCR2 ligands: CCL2 and CCL4. Human CCL1 (µM) treated with CCR2 ligands: CCL2 and CCL4. Human CCL1 (µM) treated with CCR2 ligands: CCL2 and CCL4. Human CCL1 (µM) treated with CCR2 ligands: CCL2 and CCL4.