Dynamic Mass Redistribution phenotypic assay for identifying ligands active at GPR35 receptors.

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Introduction

GPR35 is an orphan receptor reported to be involved in inflammatory disorders[1], CNS disfunction[2, 3], pain[4], diabetes[5] and immunologycal diseases such as asthma[6, 7]. Kinurenic acid[1], 2-oleil lisophosphatidic acid[8] and recently chemokine CXCL17[8] have been suggested as potential endogenous ligands but without confirmation; showing differences in efficacy between human and rat orthologues[7]. Phenotypic assays based on Dynamic Mass Redistribution (DMR) revealed as powerful tool screening libraries in orphan receptors which signalling pathways and biology remain unknown. We aimed to develop a miniaturized phenotypic assay based on DMR label-free technology[9] that enables the detection of new ligands for human and rat GPR35 receptors.

Material & Methods

Cell Seeding

HT-29 (ATCC)
Culture medium: McCoy´s 5A (ATCC 30-2007)
+ FBS-dyaliced (Sigma F0392) 10% + Penicillin-Streptomycin (Sigma P781) + Hepes 25mM pH=7.4.

IEC-6 (ATCC)
Culture medium: DMEM (ATCC 30-2002)
+ FBS-dyaliced (Sigma F0392) 10% + Insulin (Sigma I1882) 0.1 u/mL Penicillin-Streptomycin (Sigma P781) + Hepes 25mM pH=7.4.

10000-30000 cell/well. Volume: 50µL in LFC-384 well microplates (PerkinElmer 6057408).
O/N 37ºC CO_2 5%.

Buffer exchange

HT-29 (ATCC)
Assay Buffer: McCoy´s 5A (ATCC 30-2007) or HBSS (Sigma H6648)
+ Hepes 20mM pH=7.4 + DMSO 0.01%.

IEC-6 (ATCC)
Assay Buffer: HBSS (Sigma H6648) or DMEM (ATCC 30-2002)
+ Hepes 20mM pH=7.4 + DMSO 0.01%.

JANUS® Liquid handler
Wash cycles = 3
Wash volume = 25µL
Asp/disp speed = 10µL/sec
Asp/disp height = 2mm
Final soack volume = 50µL

DMR measurement

Thermal Equilibration: 90´Inside EnSpire reader

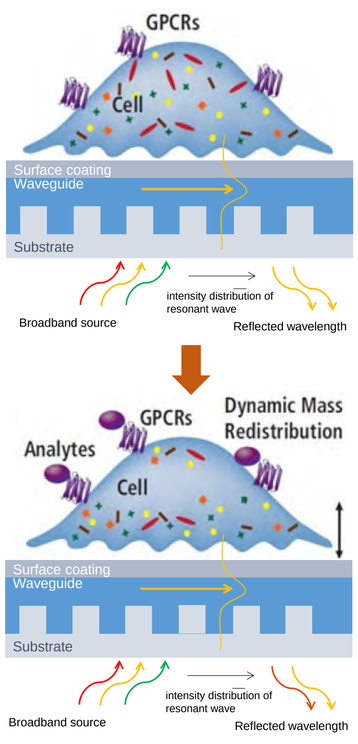
Baseline measurement: 15 repeats

Compound addition:
Volume: 10µL
Dispense speed = 10µL/sec
Dispense height = 2mm

Mix after addition:
Cycles: 3
Volume: 20µL
Dispense speed = 20µL/sec
Dispense height = 2mm

Final measurement:
60 repeats (1 per minute)

DMR Measurement principle



Results

Optimization of cell number and assay buffer at HT-29(hGPR35) and IEC-6 (rGPR35) cell line.

HT-29

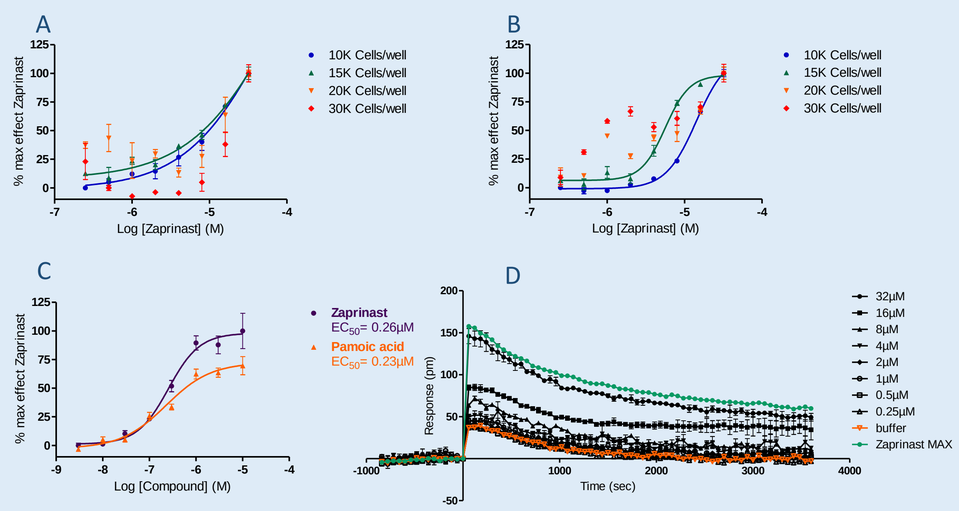


Fig.1. Effect of Zaprinast at HT-29 cells.
A: Sigmoidal concentration-response curve for Zaprinast effect using HBSS as assay buffer. B: Sigmoidal concentration-response curve for Zaprinast effect using McCoy´s 5A medium as assay buffer. C: Sigmoidal concentration-response curve for Zaprinast and Pamoic acid effect at 15000 cells/well using McCoy´s 5A medium as assay buffer. D: Kinetic response plot of Zaprinast at 15000 cells/well using McCoy´s 5A medium as assay buffer. The mean ± SEM (vertical bars) of each measure determined in triplicate of the percentage of inhibition is shown.

IEC-6

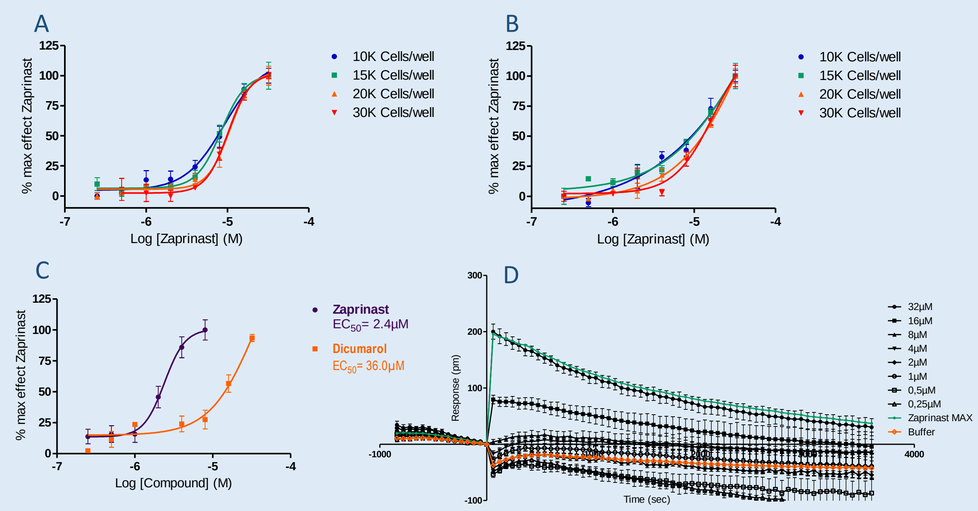


Fig.2. Effect of Zaprinast at IEC-6 cells.
A: Sigmoidal concentration-response curve for Zaprinast effect using HBSS as assay buffer. B: Sigmoidal concentration-response curve for Zaprinast effect using DMEM medium as assay buffer. C: Sigmoidal concentration-response curve for Zaprinast and Dicumarol effect at 15000 cells/well using HBSS as assay buffer. D: Kinetic response plot of Zaprinast at 15000 cells/well using HBSS as assay buffer. The mean ± SEM (vertical bars) of each measure determined in triplicate of the percentage of inhibition is shown.

Conclusions

The optimal conditions for activity measurement at HT-29 (hGPR35) cell line: 15000 cells/well and McCoy´s 5A (ATCC 30-2007) medium as assay buffer.
The optimal conditions for activity measurement at IEC-6 (rGPR35) cell line: 15000 cells/well and HBSS (Sigma H6648) as assay buffer.
We have developed miniaturized phenotypic assays based on DMR label-free technology to measure the agonist or antagonist activity of compounds in human and rat cell lines expressing the orphan GPR35 receptor.

References

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