NOVEL GPR39 AGONISTS: CORRELATION OF BINDING AFFINITY USING LABEL-FREE BACK-SCATTERING INTERFEROMETRY WITH POTENCY IN FUNCTIONAL ASSAYS

Daniel Brown[1], Niklas Larsson[2], Ola Fjellström[3], Anders Johansson[3], Sara Lundqvist[2], Johan Brengdahl[2], and Richard J. Isaacs[1]

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[1]Molecular Sensing, Inc., Nashville, Tennessee, USA
[2]AstraZeneca Discovery Sciences, Mölndal, Sweden
[3]AstraZeneca CVMD iMED, Mölndal, Sweden

AstraZeneca

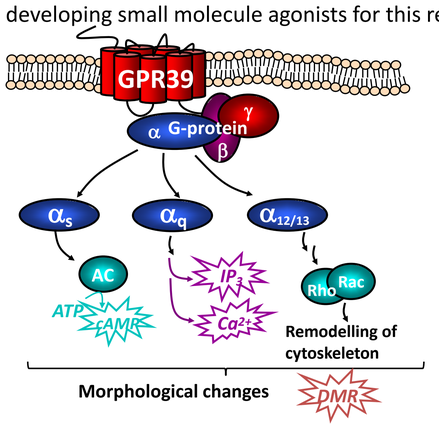
Abstract

Back-scattering interferometry (BSI) is an emerging label-free, conformation-sensitive detection technology for quantitative mass- and matrix-independent biophysical characterization of small molecule interaction with complex drug target proteins under native-like conditions (1). Integral membrane proteins such as GPCRs are critical targets for drug discovery but present a host of challenges to the investigation of their biophysical properties. Of paramount interest to drug discovery efforts is the characterization of the interaction of GPCRs with small molecule compounds as a component of library screening, mechanism of action (MOA) determination, drug candidate profiling, and other aspects of intermolecular binding that inform pharmacology and medicinal chemistry. The difficulty associated with obtaining small molecule affinity data for functionally intact GPCRs effectively restricts the range of assay techniques suited to quantifying these interactions *in vitro*.

Herein, we describe the application of BSI to the characterization of small molecule ligand binding to human GPR39 overexpressed in crude membrane fractions in free solution. GPR39 is a Zn^{2+}-responsive GPCR under investigation as a therapeutic target for type-2 diabetes (T2D) (2). The ability to measure the affinity of small molecule agonists such as $Zn^{2+}$is especially novel, given the unfavorable mass ratio and fast off rate that complicates the use of more established binding assays. Results from screening representatives from multiple novel GPR39 agonist series is presented, including how BSI-derived affinity and functional assay-derived potency correlate for compounds of varying scaffolds.

Background

GPR39 is a GPCR expressed in metabolic tissues including pancreatic β-cells and has been proposed as a target for treatment of T2D. Zn^{2+} has been shown to be a stimulator of GPR39 activity via the $G\alpha_q$, $G\alpha_s$ and $G\alpha_{12/13}$ pathways and is considered an important physiological regulator of the receptor. Several *in vivo* studies of GPR39 function suggest a role of this receptor in β-cell function. Overall, available studies support the hypothesis that GPR39 agonists increase glucose stimulated insulin secretion and may be beneficial as glucose lowering agents in T2D, thus demonstrating the value of developing small molecule agonists for this receptor.



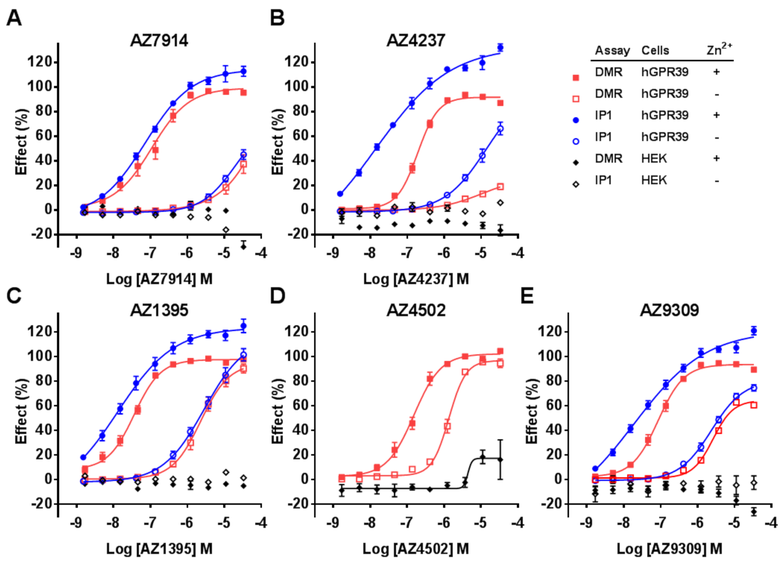
In this study, we have identified and characterized GPR39 agonists *in vitro*. Cell-based functional assays are vital for screening and characterization of drug candidates such as GPR39 agonists. However, it is critical to further verify that a pharmaceutical compound engages with a defined target as well as determining the affinity of the interaction. Thus, a minimal *in vitro* inter-molecular binding assay platform such as BSI provides critical information that cannot be supplied by functional assays. The combination of potency data from cell-based assays with affinity data from BSI assays provides 'on-target' characterization of drug function, adding value during drug development.

References

1. Bornhop DJ, Latham JC, Kussrow A, Markov DA, Jones RD, Sorensen HS. Free-solution, label-free molecular interactions studied by back-scattering interferometry. Science. 2007 Sep 21;317(5845):1732-6.

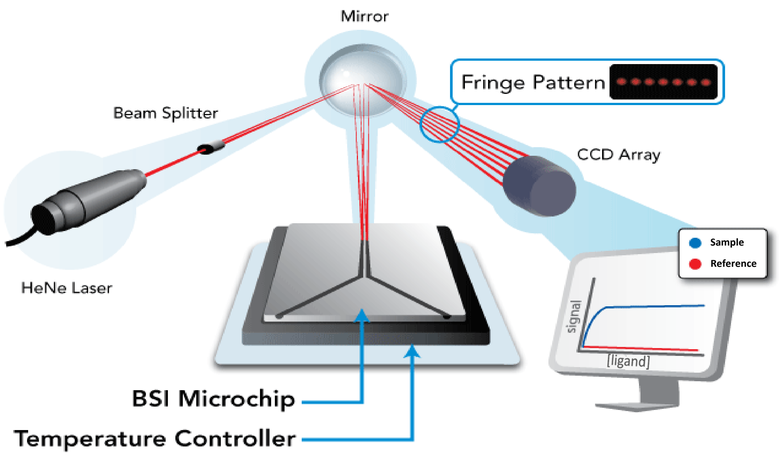
2. Popovics P, Stewart AJ. GPR39: A Zn^{2+}-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. Cell Mol Life Sci. 2011 Jan;68(1):85-95.

GPR39 Agonists in Functional Assays



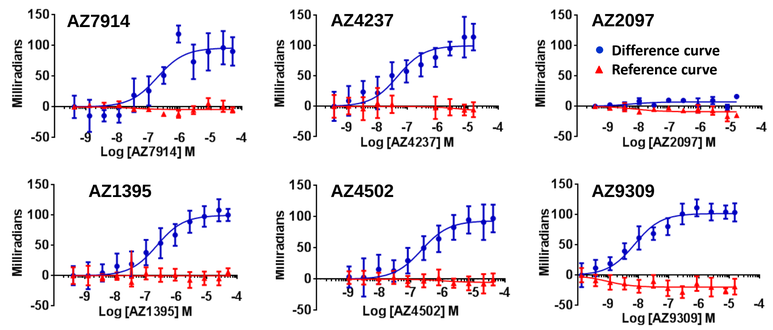
Characterization of GPR39 agonists in functional assays. Multiple Zn^{2+} modulated GPR39 agonist series were identified by HTS. AZ7914 (*A*), AZ4237 (*B*), AZ1395 (*C*), AZ4502 (*D*) and AZ9309 *(E)* represent one chemical series each. Here, concentration response curves in functional GPR39 assays are shown. Responses were measured with DMR (Corning Epic) and IP_1 (Cis-Bio) assays in the absence (-) or presence of 5 μM Zn^{2+} (+) employing HEK293s-hGPR39 (hGPR39) or untransfected HEK293s (HEK) cells. The DMR assay measures the integrated cell response and is therefore agnostic of signaling pathways whereas the IP_1 assay measures signaling via the $G\alpha_q$ / Ca^{2+} release pathway. Values are means ± SEM of two to eighteen experiments.

The BSI Platform



The Back-Scattering Interferometer employs a simple optical train comprised of a coherent light source (low-power He-Ne laser), a microfluidic interferometric channel and a CCD camera. The interaction of the laser beam with the fluid-filled channel results in a high-contrast interference pattern and phase shift detection. The fringe shifts in a predictable manner with molecular binding events within the optical channel. When molecular complexes form with constitutive changes in conformation, the fringe shift is detected as a change in RI with a resolution of 50 nanometers. The output of the system is a concentration dependent plot of fringe position.

GPR39 Agonists in BSI Assays



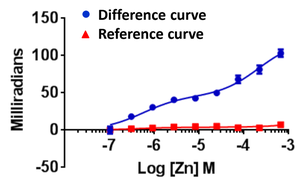
Quantification of GPR39 agonist affinity by BSI. GPR39 was expressed in HEK293s cells and membranes were prepared by standard methods. Membranes (both GPR39 and non-expressing reference membranes) were diluted in binding buffer containing 5 μM $ZnCl_2$ and mixed with serially-diluted test compounds, incubated 2 h at room temperature, and analyzed with the BSI device. Reference membrane signal was subtracted from target signal at each concentration point to generate difference curves (blue). Reference curves (red) are included to demonstrate lack of high background signal. One-site binding models were used to calculate the K_D values summarized in Table 1. AZ2097 is a close analog to AZ9309, but inactive in functional assays, and was included as a negative control. As an additional control, the GPR40 agonist TAK-875 was also analyzed. No specific binding was detected for TAK-875 (data not shown).

Table 1. Summary of functional (DMR, IP_1) and BSI data for GPR39 agonists

	DMR	IP_1	BSI
	EC_{50} (μM)	EC_{50} (μM)	K_D (μM)
AZ7914	0.10	0.074	0.16
AZ4237	0.19	0.034	0.048
AZ1395	0.036	0.019	0.233
AZ4502	0.15	-	0.22
AZ9309	0.079	0.044	0.007
AZ2097	ND	-	ND

Zn^{2+} Binding to GPR39

BSI enables binding quantification of very low molecular weight ligands such as Zn^{2+} to integral membrane proteins such as GPR39 under conditions where other binding assay technologies fail. The data fits best to a two-site binding model with a high-affinity K_D of 0.54 ± 0.06 μM and low-affinity K_D of 215 ± 34 μM.



Summary

- We have identified multiple novel Zn^{2+}-modulated GPR39 agonist series
- BSI has successfully been applied to measure affinity between GPR39 and small molecule agonists in the presence of Zn^{2+}
- GPR39 agonist potencies measured in functional assays are in the same range as binding affinities measured by BSI
- Our GPR39 agonist series provide tools to test the hypothesis that GPR39 agonism would improve glucose stimulated insulin secretion *in vivo*