Etrasimod, a Novel Sphingosine-1-Phosphate (S1P) Receptor Modulator, Lacks Functional Activity at S1P2 Receptors

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Introduction

The S1PR receptor modulator FTY720 (Fingolimod), a prodrug that is phosphorylated in vivo, possesses potent immunomodulatory activities and has been approved for the treatment of multiple sclerosis. Numerous next-generation S1PR modulators, including etrasimod (APD334), are currently at different stages of clinical development for the treatment of various autoimmune diseases. Selectivity of S1PR modulators is an important issue, both from standpoint of engagement of additional receptor driven signaling pathways beneficial for clinical efficacy, as well as for mitigation of potential safety concerns. FTY720-phosphate (FTY720(P)) possesses functional activity at S1P1, 3, 4, and 5 receptor subtypes but, until recently, was thought to lack activity at the S1PR2 subtype. Similar lack of activity at S1PR2 was attributed to numerous additional S1P receptor modulators, based mostly on the absence of activity in β-arrestin recruitment assays. S1PR2 is a negative regulator of cell migration. Here, we employed various signaling pathway specific assays in addition to pathway agnostic approaches to detect functional activity of FTY720(P) and other S1PR modulators at S1PR2.

Methods

The pharmacology of selected S1PR modulating drugs at S1PR2 was characterized in the following panel of functional assays.

β-Arrestin recruitment: Performed in a clonal S1PR1 and S1PR2/HEK293 cell line using the PathHunter technology from DiscoveRx.

Dynamic Mass Redistribution assays: Compounds were evaluated in label-free dynamic mass redistribution assays (Corning Epic®) using the same S1PR2 expressing HEK293 cells, to capture a more holistic view of functional responses.

Receptor internalization: Performed using HEK293 cells stably expressing N-terminally HA-tagged S1PR2. Cells were exposed to test compounds at 37C and then fixed. Cell surface receptor was quantified using a fluorescently labelled anti-HA antibody by flow cytometry.

Chemotaxis assays: Migration of S1P2R expressing HEK293 cells was studied in a Neuroprobe 96-well chemotaxis system. Cells were placed in the upper chamber of the device and chemotaxis to fibronectin in the lower chamber evaluated. S1PR modulators were evaluated for their ability to block this chemotaxis, when placed in the lower chemoattractant-containing chamber, in a similar manner to S1P. Cells pre-treated with S1PR modulators were then evaluated to determine whether S1P-mediated blockade of chemotaxis was affected.

Results

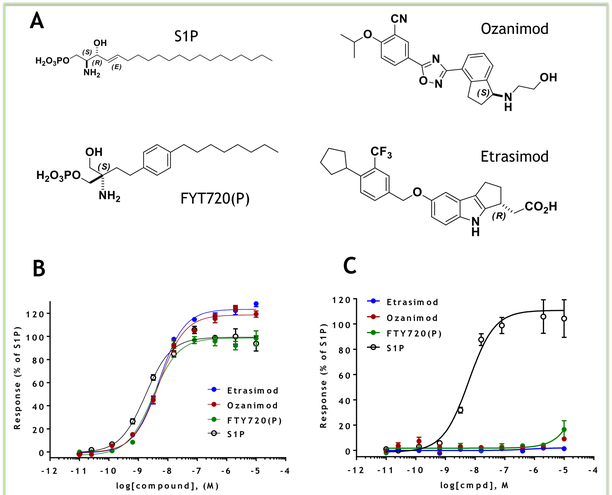

Figure 1. *Pharmacology of S1PR modulators at S1PR1 and S1PR2 in β-arrestin recruitment assays. **(A)** Chemical structures of S1PR modulators. **(B)** Dose-responses of compounds at S1PR1. **(C)** Dose-responses of compounds at S1PR2.*

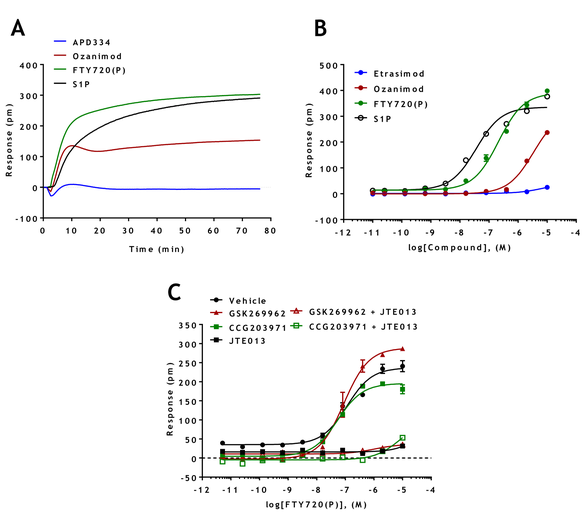

Figure 2. *S1PR modulator activity in label-free Dynamic Mass Redistribution (DMR) assays. **(A)** Drug induced kinetic DMR response traces (2 μM). **(B)** Dose-response effects of S1PR modulators. **(C)** Effects of S1PR2 antagonist JTE013 and Rho pathway inhibitors GSK269962 and CCG203971 on FTY720(P) induced DMR responses.*

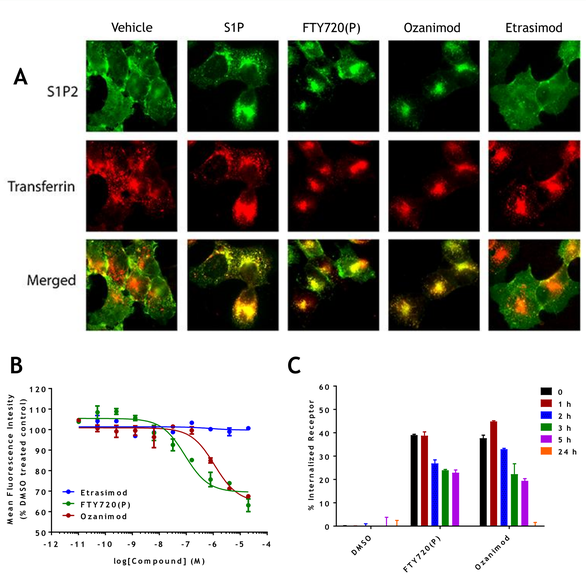

Figure 3. *S1PR modulator induced S1P2R internalization and kinetics of recycling. **(A)** Treatment with ozanimod and FTY720(P), but not etrasimod induced S1P2R internalization, as determined by fluorescence microscopy. **(B)** Concentration-response curves for S1P2R internalization by S1PR modulators determined by flow cytometry. **(C)** Kinetics of reappearance of S1PR2 on the cell surface following drug washout.*

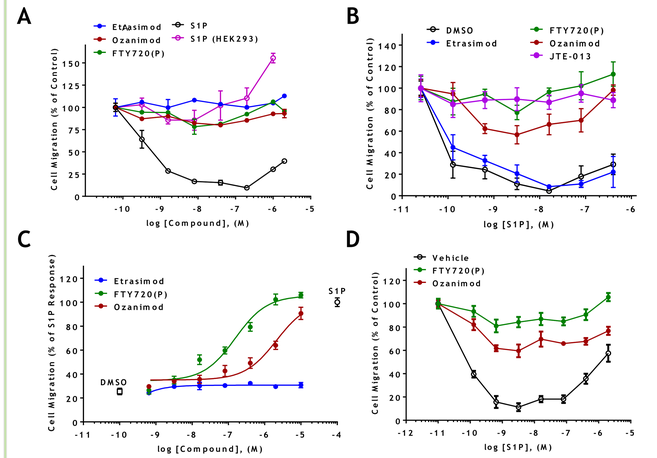

Figure 4.

***(A)** S1P potently inhibits migration of S1PR2 expressing cells toward fibronectin, while S1PR modulators were inactive. Test compounds were included in the lower, chemoattractant containing chamber.*

***(B)** With the exception of etrasimod, when cells were exposed to S1PR modulators (10 μM) for 1h prior to evaluation, S1P (5 nM placed in the lower chamber) no longer inhibited their migration to fibronectin.*

***(C)** Dose-response of S1PR modulators in reversing the inhibitory action of S1P on cell migration.*

***(D)** Persistence of S1PR modulator effects on S1PR2 function. Cells were incubated with indicated S1PR modulators (10 μM) for 1h then washed extensively, and equilibrated for 2h before setting up the chemotaxis assay. S1PR modulator exposed cells remained unresponsive to S1P.*

Conclusions

- S1P induces S1PR2-mediated β-arrestin recruitment and G-protein activation (GTPγS), while all of the S1PR modulators were inactive
- FTY720(P) and ozanimod modulate S1PR2 receptor signaling, as revealed by label-free DMR assays
- FTY720(P) and ozanimod induce persistent internalization of S1PR2 through arrestin-independent pathways
- S1PR modulator DMR responses are not affected by inhibition of Rho, suggesting additional S1PR2-mediated signaling activities by these modulators
- Unlike S1P, S1PR modulators do not inhibit cell migration
- FTY720(P) and ozanimod functionally antagonize S1P-S1PR2 mediated inhibition of cell migration in a persistent fashion
- Etrasimod displays no detectable activity at S1PR2

Table 1. Pharmacology of S1P1R modulators on S1PR2 receptors

Indicated values are pEC_{50} +/- StDev, in parenthesis - EC_{50} in nM. NR - no response, NA- not applicable, ND - not determined

Compound	GTPγS	β-Arrestin Recruitment	Dynamic Mass Redistribution	Internalization	Reversal of S1P Inhibition of Chemotaxis
S1P	6.23 +/- 0.01 (587)	7.90 +/- 0.44 (12.7)	7.32 +/- 0.11 (48)	6.69 +/- 0.46 (297)	NA
Etrasimod	NR	NR	NR	NR	NR
FTY720(P)	NR	NR	6.86 +/- 0.41 (176)	6.88 +/- 0.19 (142)	6.68 +/- 0.20 (228)
Ozanimod	NR	NR	5.58 +/- 0.10 (2660)	5.73 +/- 0.32 (2271)	5.60 +/- 0.16 (2633)

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