Functional Activity of Etrasimod and a Diverse Panel of Sphingosine-1-Phosphate Receptor (S1PR) Modulators at S1P4 Receptors

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Introduction

The S1P receptor modulator FTY720 (Fingolimod) exerts numerous anti-inflammatory effects at least in part by producing sustained S1PR1 receptor internalization, resulting in loss of cellular responsiveness to S1P. FTY720 and a number of next-generation S1PR modulators have varying degrees of activity at the S1PR4 and 5 receptor subtypes.

Etrasimod is a next-generation small molecule S1PR modulator currently in clinical development for ulcerative colitis and additional indications. Like other S1PR1 modulators, etrasimod induces persistent internalization of S1PR1 on T-cells, preventing their egress from lymph nodes, producing lymphopenia and immunosuppression.

There is increasing awareness that, the S1PR4 receptor, which is expressed largely in immune cells (e.g. dendritic cells), may provide additional opportunities for immunomodulation. However, signaling via S1PR4 has so far been poorly characterized.

We therefore sought to characterize the functional activity of etrasimod and a panel of S1P receptor modulators in a range of S1PR4 functional assays designed to interrogate signaling pathways and receptor internalization.

Methods

S1P and a panel of S1P receptor modulators were evaluated in the following panel of functional assays:

β-Arrestin recruitment: Performed in a clonal S1PR4/HEK293 cell line using the PathHunter technology from DiscoveRx.

Receptor internalization: Performed using HEK293 cells stably expressing N-terminally HA-tagged S1PR4. 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.

GTPγS binding: G-protein activation was evaluated in GTPγS assays using membranes prepared from CHO cells stably expressing S1PR4 at high levels.

cAMP Modulation: Gαi-mediated reductions of forskolin-stimulated intracellular cAMP were measured in an S1PR4-CHO cell line (HTRF, Cisbio)

Dynamic Mass Redistribution assays: Compounds were evaluated in label-free dynamic mass redistribution assays (Corning Epic®) using the same S1PR4 expressing CHO cells, to capture a more holistic view of functional responses.

Results

All compounds produced robust arrestin recruitment with a range of efficacies. S1P was quite potent in this assay (4.5 nM) (**Fig. 1**).

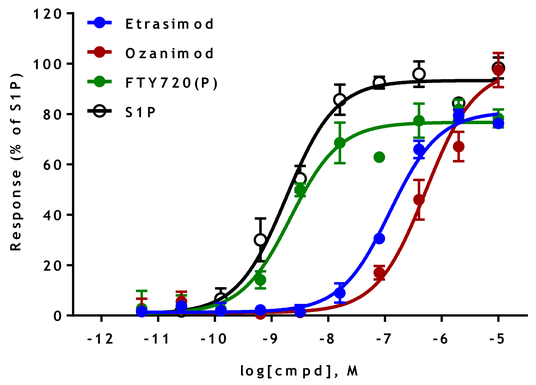

Fig. 1 *Dose-responses of S1PR modulator-induced recruitment of β-Arrestin to S1PR4.*

All of the compounds induced robust receptor internalization (**Fig. 2**). For most compounds, internalization potencies matched those observed in arrestin recruitment. The most notable exception was S1P, which was 100-fold less potent in producing internalization.

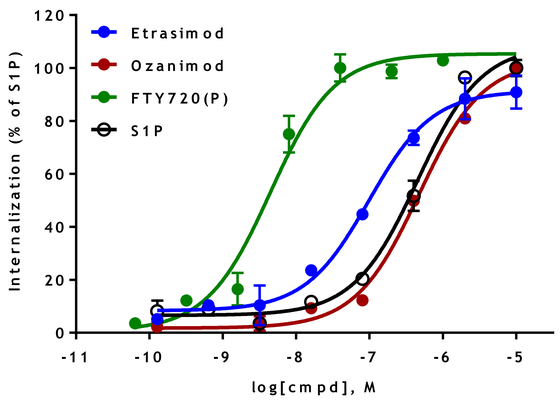

Figure 2 *Dose-responses of S1PR modulator-induced internalization of S1PR4.*

Receptor recycling was evaluated in a similar manner. Cells were exposed to S1PR modulators (10 μM) for 1.5h at 37C. Compounds were then removed and the cells thoroughly washed. Receptor expression at the cell surface was determined either immediately or after a 5h or 20h recovery period at 37C (**Fig. 3**).

Etrasimod, like FTY720(P), maintained robust receptor internalization for 20h. In contrast, normal cell surface S1PR4 expression was observed at the 5h timepoint for S1P, ozanimod and S1P.

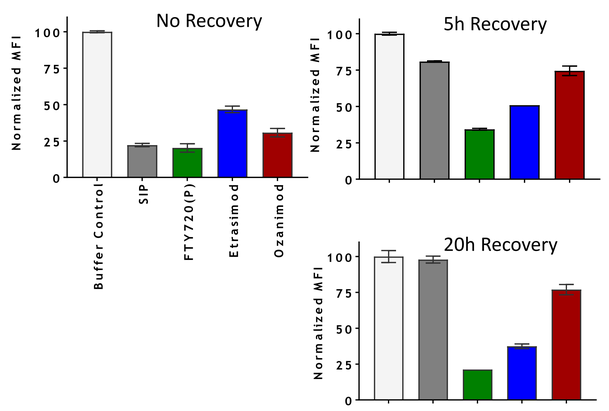

Figure 3 *S1PR4 internalization and receptor recovery at the cell surface 5h and 20h after compound wash-out.*

In GTPγS assays using CHO-S1PR4 membranes, the potency of S1P was 215 nM, consistent with a number of literature reports (**Fig. 4**). While most compounds produced G-protein activation, etrasimod was unique in exhibiting inverse agonist activity.

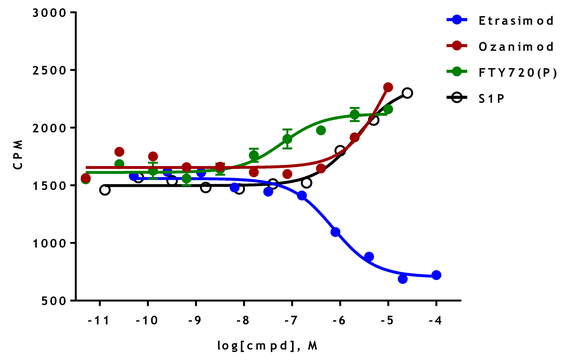

Figure 4 *S1PR4 mediated GTPγS responses.*

In cAMP assays using forskolin-stimulated S1PR4 CHO cells, S1P produced a low-potency response (**Fig. 5**). FTY720(P) elicited reductions in cAMP consistent with its activity in the GTPγS assay. Etrasimod and ozanimod were inactive in cAMP assays.

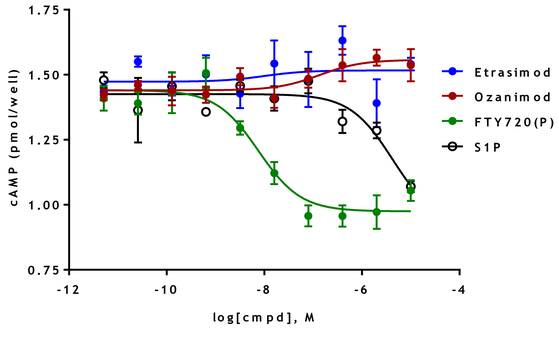

Figure 5. *S1PR4 mediated cAMP responses.*

Figure 6 shows label-free, dynamic mass redistribution responses obtained using the same S1PR4 expressing CHO cells.

With the exception of etrasimod, all compounds elicited DMR responses. The potencies of test compounds in all 5 functional assays are summarized in **Table 1**.

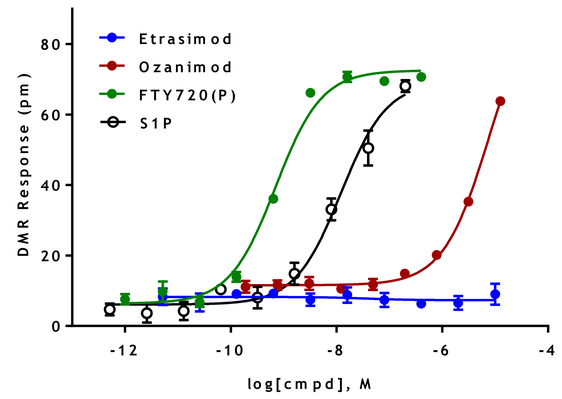

Figure 6. *S1PR4 mediated DMR responses.*

	Arrestin	Internaliz'n	DMR	GTPγS	cAMP
Etrasimod	147	206	NR	680*	NR
Ozanimod	482	549	5234	3310	NR
FTY720(P)	2.3	4.4	1.7	1.3	16
S1P	4.5	453	6.0	215	5397

Table 1 *Summary of potencies (nM) of S1PR modulators in all S1PR4 functional assays.*

Conclusions

- S1PR4 was confirmed to be Gi-coupled but high receptor expression was required to detect this activity in cAMP assays.
- The reported low potency of S1P induced G-protein activation at S1PR4 was confirmed. However, S1P-mediated β-arrestin recruitment was potent and robust, perhaps suggesting S1PR4 signals primarily through the arrestin pathway.
- Etrasimod exhibits clear, biased signaling at S1PR4, inducing robust arrestin recruitment and internalizing the receptor with no detectable G-protein signaling in cAMP or DMR assays, and clear inverse agonist activity in GTPγS assays.
- Etrasimod induces persistent internalization of S1PR4 that is maintained >20 h in recombinant systems.
- Etrasimod may act as a robust, functional antagonist at S1P4 receptors in the immune system, inducing prolonged receptor internalization without G-protein activation. Studies in primary immune cells are underway.

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