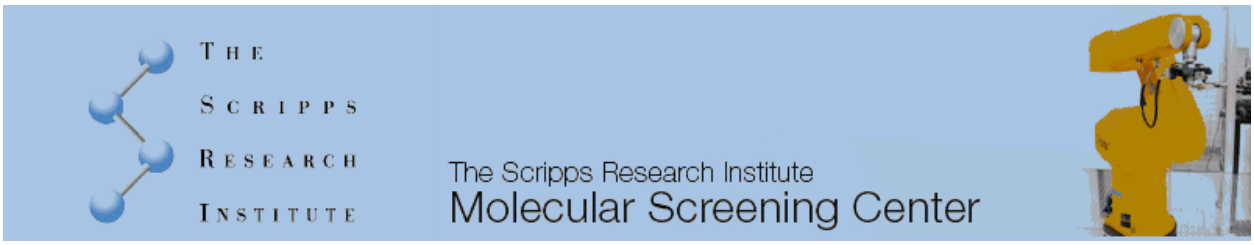


Probe Report

Project: S1P1 Antagonist

Grant Number: 1R03MH076534-01

Screening Center: The Scripps Research Institute Molecular Screening Center

Principal Investigator of Screening Center: Hugh Rosen, MD PhD

Assay Submitter & Institution: Germana Sanna, The Scripps Research Institute

PubChem Bioassay Identifer (AID):

Assay or Pathway Target: S1P1

Assay Target: S1P1

Probe PubChem Substance Identifier (SID): 24257742

Specific Aim: Identify compounds which provide insight into the molecular mechanism of S1P biological function.

Significance: S1P1 agonists are currently in late-stage clinical trials and recent results suggest that agonists have differing effects upon receptor activation and desensitization. Antagonists of the S1P1 receptor will provide useful probes into the biology of S1P receptors and in vivo mechanism of S1P1 angonists.

Rational: In light of the proposed chronic administration of agonists and probable importance of maintaining S1P1 receptor activity or tone, understanding the balance between activation and desensitization is important.

Assay Results
Evaluation of rationally designed compounds identified SID 24257742 as an antagonist of the S1P1 receptor.
S1P activation of the S1P1 receptor is inhibited by SID 24257742 (2a in Figure 1) and a chiral enantiomer(2b in Figure 1) with estimated Ki of 77 nM and 4.6 uM, respectively.[1] In vivo efficacy is demonstrated in Figure 2. The antagonist blocks in vivo effects of co-administered S1P1 agonist, SEW2871 (3 in Figure 2).

Prior Probes:

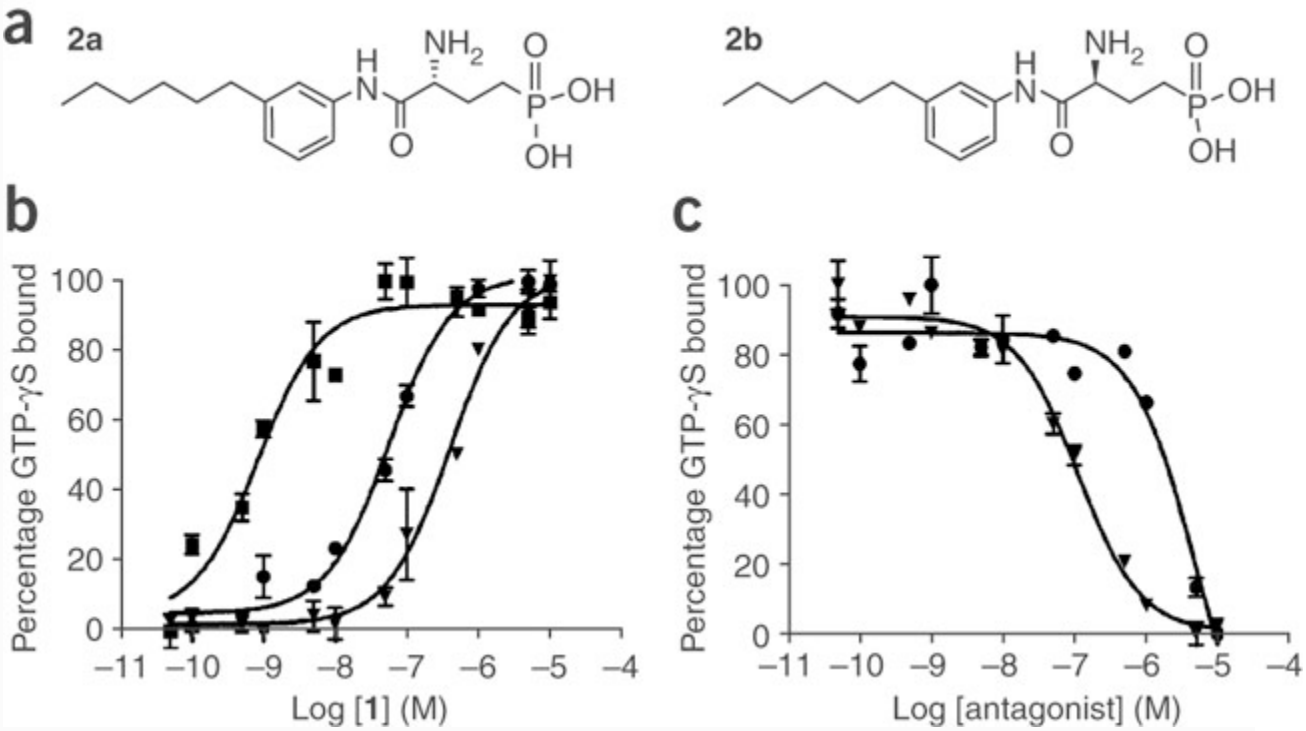
VPC23019 and VPC44116, previously reported S1P1 antagonists, have poor solubility, stability and lack in vivo efficacy (VPC44116 the phosphonate analog of VPC-23019 and is reported to have similar a S1P receptor selectivity profile as VPC23019)[2, 3].

Probe Summary

SID 24257742 and the S-enantiomer are commercially available from Avant Polar Lipids (cat numbers 857390 and 857391).

FIGURE 1. Compound 2a is a selective antagonist for human S1P1 (hS1P1) receptor activation by the physiological agonist S1P.

From the following article
Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral $S1P_1$ antagonist *in vivo*
M Germana Sanna, Sheng-Kai Wang, Pedro J Gonzalez-Cabrera, Anthony Don, David Marsolais, Melanie P Matheu, Sindy H Wei, Ian Parker, Euijung Jo, Wei-Chieh Cheng, Michael D Cahalan, Chi-Huey Wong and Hugh Rosen
Nature Chemical Biology **2**, 434-441 (2006)
doi:10.1038/nchembio804



(**a**) Structures of **2a** and **2b**. (**b**) Shift in EC_{50} for S1P activation of $hS1P_1$ receptor by **2a** and **2b**. We tested CHO cell membranes expressing stably transfected $hS1P_1$ receptors for antagonism in a GTP-γS binding assay. Activation of $hS1P_1$ receptors by **1** alone (closed squares) or in the presence of 10 μM **2a** (inverted triangles) and **2b** (circles) is expressed normalized to percentage GTP-γS binding induced at maximal S1P concentration. The EC_{50} value was 0.8 nM for **1** alone; it was 398 nM and 50 nM in the presence of **2a** and **2b**, respectively. (**c**) We derived K_i values for S1P activation of the $hS1P_1$ receptor from antagonist competition curves; they were 77 nM for **2a** (inverted triangles) and 4.63 μM for **2b** (circles).

FIGURE 2. Systemic S1P1 antagonism reverses agonist-induced sequestration of blood lymphocytes and medullary thymocyte phenotypic maturation.

From the following article
Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral $S1P_1$ antagonist *in vivo*
M Germana Sanna, Sheng-Kai Wang, Pedro J Gonzalez-Cabrera, Anthony Don, David Marsolais, Melanie P Matheu, Sindy H Wei, Ian Parker, Euijung Jo, Wei-Chieh Cheng, Michael D Cahalan, Chi-Huey Wong and Hugh Rosen
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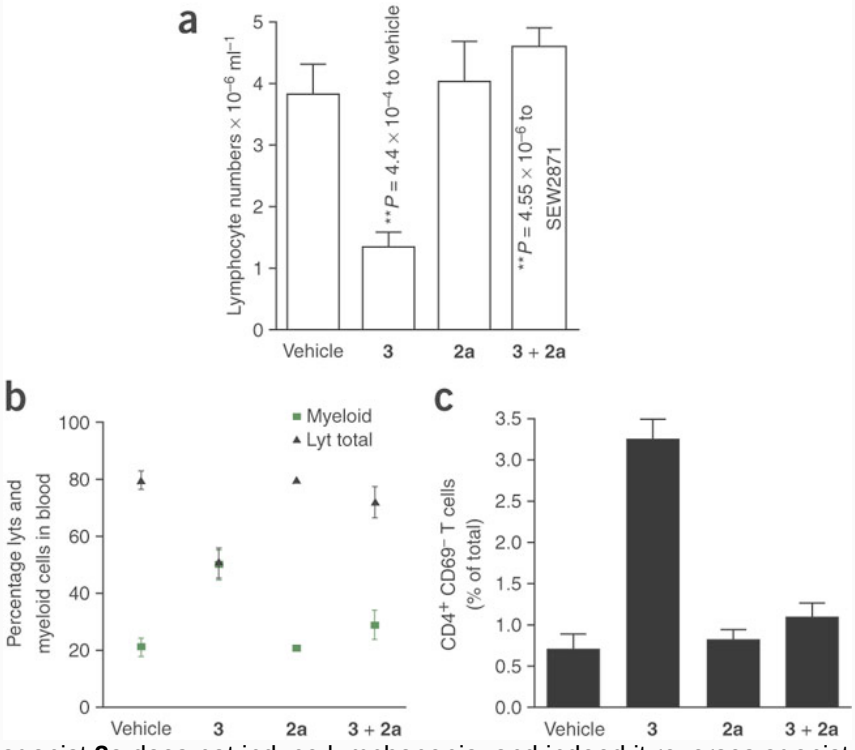

(**a**) The $S1P_1$ antagonist **2a** does not induce lymphopenia, and indeed it reverses agonist-induced lymphopenia. We administered **2a** (10 mg kg^{-1}) i.p. in C57BL6 mice at the time of oral dosing of **3** (20 mg kg^{-1}) or vehicle. We measured blood lymphocyte numbers as described[2] at 5 h. The data shown are pooled from four independent experiments with a total $n = 12$ mice per group. Mean ±s.d. and P values from Student's t-test are shown. (**b**) $S1P_1$ antagonist reverses agonist-induced alterations in differential leukocyte count (namely the percentage of lymphocytes, monocytes and neutrophils in the volume of blood counted) under the conditions and methods described in Figure 5a. Representative data ($n = 3$) from one of four similar experiments is shown. Because S1P agonists sequester lymphocytes (lyts) passing through specific lymphoid microenvironments, neutrophils and monocytes (myeloid cells) that do not traverse the same barriers are not sequestered. The number of myeloid cells remains constant while lymphocytes disappear, and therefore the percentage of myeloid cells rises. (**c**) $S1P_1$ antagonist reversal of agonist-induced loss of CD69 in late medullary thymocytes. We quantified CD69 expression on single-positive $CD4^+$ thymocytes by FACS at 5 h after administration of 20 mg kg^{-1} of **3** in the presence or absence of 10 mg kg^{-1} **2a**. Mean ±s.d., $n = 3$, data from one of two similar experiments is shown.

References

1. Sanna, M.G., et al., *Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral S1P1 antagonist in vivo.* Nat Chem Biol, 2006. **2**(8): p. 434-41.
2. Davis, M.D., et al., *Sphingosine 1-phosphate analogs as receptor antagonists.* J Biol Chem, 2005. **280**(11): p. 9833-41.
3. Awad, A.S., et al., *Selective sphingosine 1-phosphate 1 receptor activation reduces ischemia-reperfusion injury in mouse kidney.* Am J Physiol Renal Physiol, 2006. **290**(6): p. F1516-24.