MLSCN Probe Summary: S1P1 Agonist

The Scripps Research Institute Molecular Libraries Screening Center (SRIMSC)

Grant Number: 1R03MH076534-01
Principal Investigator: Germana Sanna

CRISP Project Title: MLSCN HTS Assays R03 - S1P1

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. More potent agonists of the S1P1 receptor will provide useful probes into the biology of S1P receptors.

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.

PubChem Bioassay Identifier (AID): 449

PubChem Bioassay Name: Primary HTS and Confirmation Assays for S1P1 Agonists and Agonism Potentiators

Assay Description
A cell line containing the human S1P1 receptor as well as the beta-lactamase (BLA) reporter-gene under control of the cyclic AMP response element (CRE) promoter was used to measure S1P1 activation. Since the S1P1 receptor is a member of Gi/o protein coupled receptor family, agonism was measured by adding test compounds in the presence of a forskolin challenge. Through stimulation of adenylate cyclase, forskolin increases the production of cAMP and therefore the transcription of the CRE-BLA reporter gene. S1P1 agonists would abrogate this effect; similarly agonism potentiators would increase the potency of an S1P1 agonist. Therefore the amount of BLA activity was inversely proportional to the concentration of agonist or agonism potentiators in the

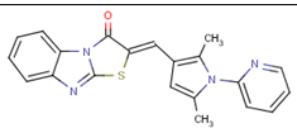
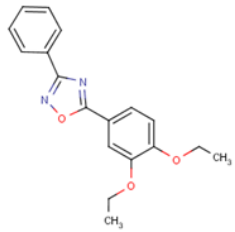
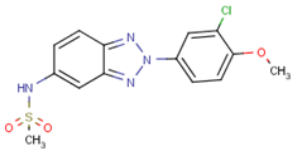
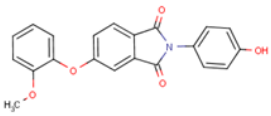
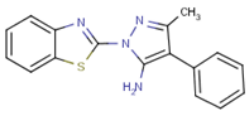
presence of an S1P1 agonist. BLA activity was measured with a fluorescent BLA substrate.

The primary HTS assay was conducted in 1536-well format. All compounds were tested once at a 10 micromolar final concentration.

Center Summary of Screen and Followup:
Screening, Dose Response and Parental Cell line Counterscreen: We screened 55,727 compounds and 315 were identified as actives. Compounds with activity greater than 50% of control were selected for followup, cherrypicked, and dose response plates prepared. Dose response results were clustered by structural similarity.

Compound Optimization
PK 917 appears to tissue distribute and has very high clearance
Confirmation: by internalization
Receptor Tone

Figure 1 Cluster

Structure	SID	PUR.	Cluster ID	Cluster Num	S1P1 AG EC50 uM	S1P1 PTR EC50 uM	S1P1 Parental EC50 uM	S1P1 Inhib Prim %	S1P3 AG Inhib Prim %	S1P3 ANT Inhib Prim %
	860541	100	273	1	0.132	0.111	>95.1	101.3	-1.4	N/A
	7969091	100	23	17	0.147	0.107	>95.1	107.5	14.2	-13.4
	4256514	100	110	1	0.148	0.126	>95.1	102.9	-0.7	6.4
	860064	100	105	2	0.217	0.151	>95.1	111.5	-0.7	14.9
	4256120	100	60	2	0.415	0.385	>95.1	95.3	-0.7	10.3

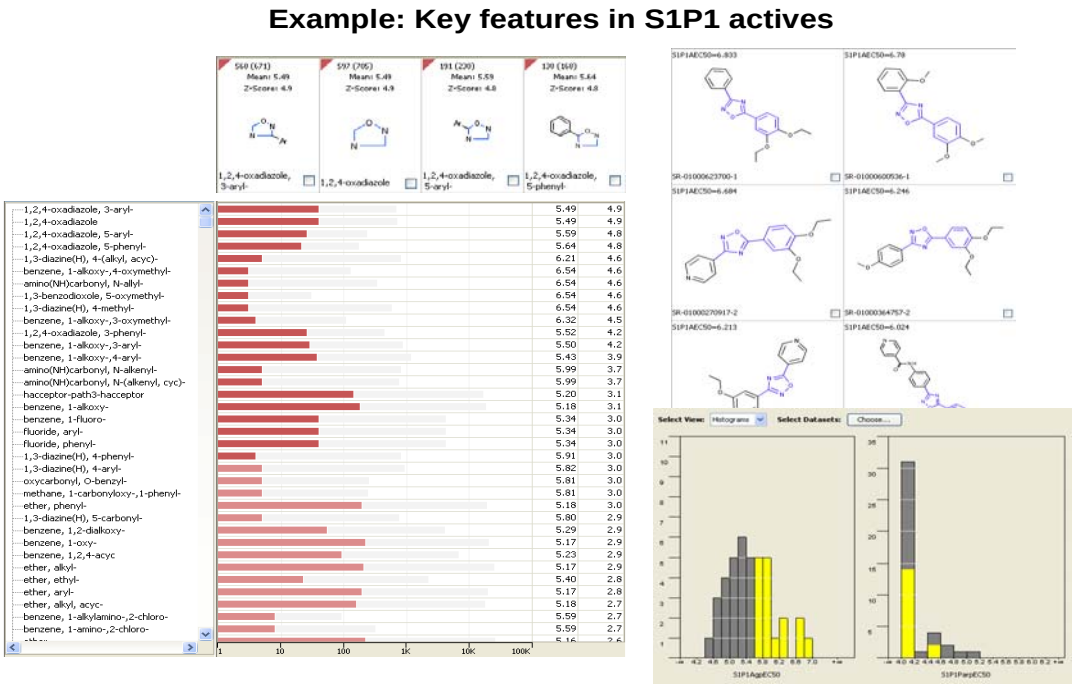


Figure 2. Key chemical features for S1P1 agonist activity immediately lead to aryl oxadiazole derivatives. Some examples are shown. The histograms illustrate the selectivity against the parental cell line.

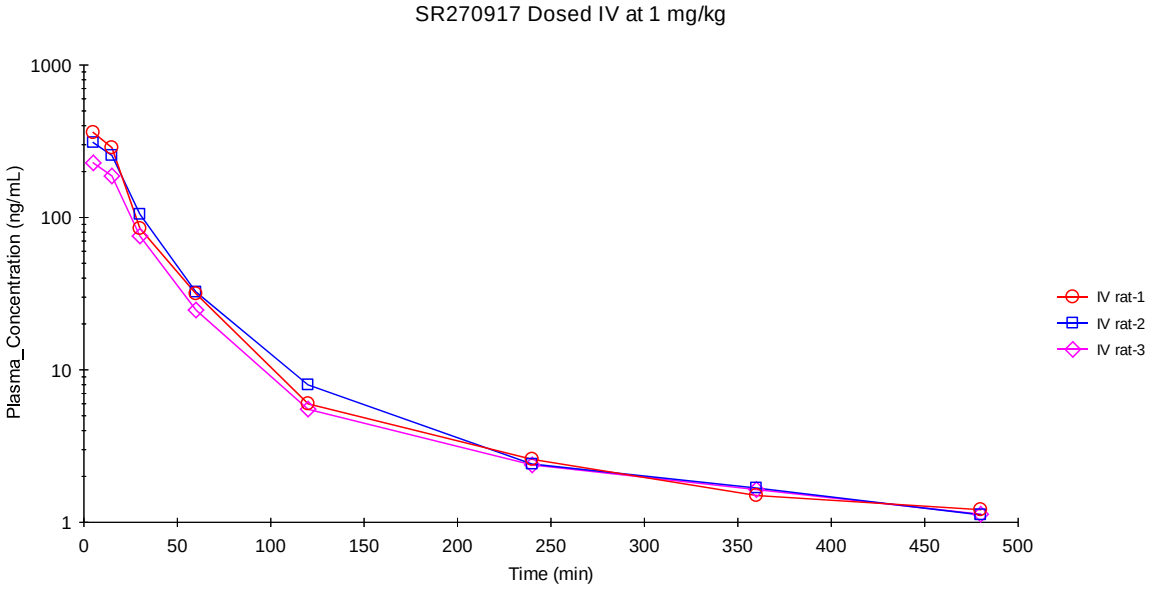
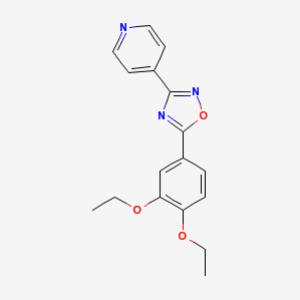


Figure 3. SR-917 was formulated in 10%DMSO,10% Tween80, 80%water and dosed at 1mg/kg.

- CID 976135



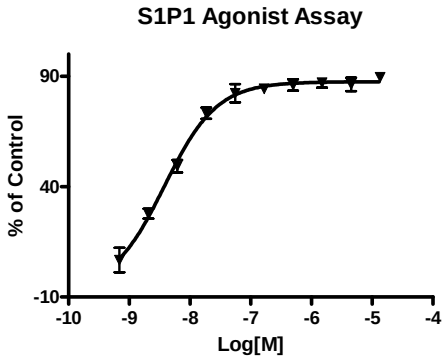


Figure 4. Identification of an active compound in the screen. A) Structure of compound 976135[CID]. B) Dose response curve of 976135[CID].

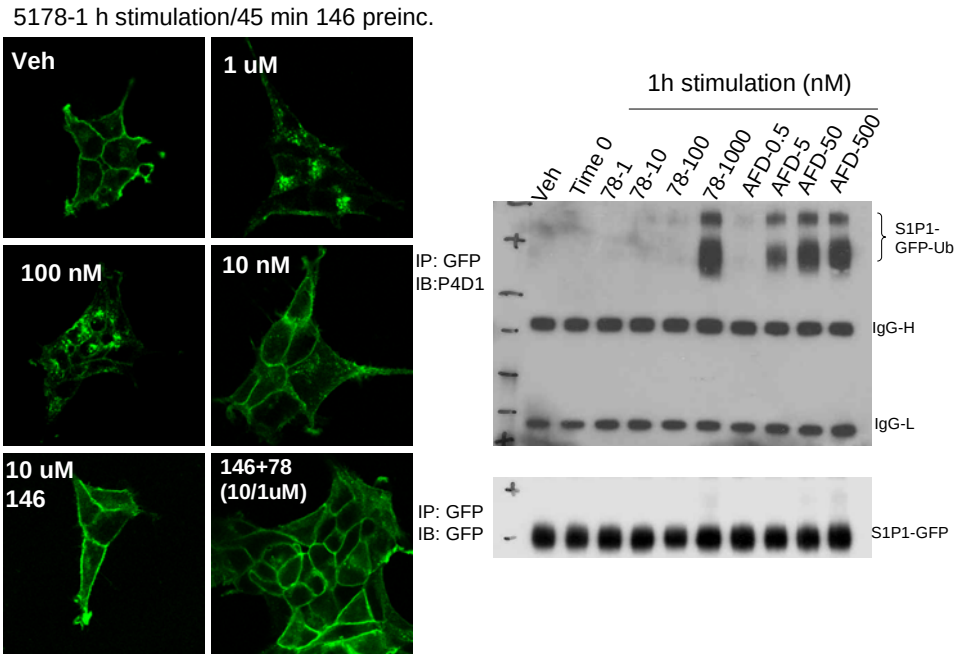


Figure 5.