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Discovery, Design and Synthesis of the First Reported Potent and Selective Sphingosine-1-Phosphate 4 (S1P₄) Receptor Antagonists

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Abstract

Selective S1P₄ receptor antagonists could be novel therapeutic agents for the treatment of influenza infection in addition to serving as a useful tool for understanding S1P₄ receptor biological functions. 5-(2,5-dichlorophenyl)-*N*-(2,6-dimethylphenyl)furan-2-carboxamide was identified from screening the Molecular Libraries-Small Molecule Repository (MLSMR) collection and selected as a promising S1P₄ antagonist hit with moderate *in vitro* potency and high selectivity against the other family receptor subtypes (S1P_{1–3,5}). Rational chemical modifications of the hit allowed the disclosure of the first reported highly selective S1P₄ antagonists with low nanomolar activity and adequate physicochemical properties suitable for further lead-optimization studies.

Keywords

S1P₄ receptor antagonists; S1P_{1–3,5} receptor family; 5-aryl furan-2-arylcarboxamide

Sphingosine-1-phosphate (S1P) is a pleiotropic lysophospholipid that is formed in various cells including platelets, mast cells and macrophages in response to various stimuli such as growth factors, cytokines and antigens. It serves as an important biological mediator that influences endothelial and lung epithelial integrity^{1,2} as well as lymphocyte recirculation.^{3–6} S1P exerts its biological effects via extracellular signaling through five G-protein coupled receptor subtypes, namely S1P₁ through S1P₅.⁷ While S1P_{1–3} receptors are expressed ubiquitously, S1P₄ expression is highly restricted to hematopoietic and lymphatic tissue and S1P₅ is expressed in the central nervous system.⁸ S1P₄ is coupled to Gα_i and Gα_o proteins and activates the ERK, MAPK, PLC cascades.⁹ Local modulation of S1P receptors in the

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lungs has been shown to alter dendritic cell activation and accumulation in the mediastinal lymph nodes, resulting in blunted T cell responses and control of immunopathological features of influenza virus infection, without involvement of the S1P₁ immunosuppressive activities.¹⁰ Reports showing that in dendritic cells S1P₅ expression is very low whereas S1P₄ is highly expressed,¹¹ suggest that chemical activation of the S1P₄ subtype in the airways may be effective at controlling the immunopathological response to viral infections. It has been reported that S1P₄ is upregulated during megakaryocytes differentiation suggesting that S1P₄ antagonists are a suitable target for reactive thrombocytosis.¹² However, aspects of the biological role of S1P₄ remain unclear partly due to the lack of ligands, neither agonists nor antagonists, with high selectivity against the S1P_{1-3,5} subtypes. Herein we report on the discovery, synthesis and structure-activity relationships (SAR) of novel S1P₄ selective antagonists as a valuable tool to elucidate the biological and pharmacological profile of the target receptor.

In house high-throughput screening (HTS) of the Molecular Libraries-Small Molecule Repository (MLSMR) collection identified a 5-aryl furan-2-arylcarboxamide derivative **1** (Figure 1) as a S1P₄ antagonist hit with acceptable *in vitro* potency/selectivity profile. With the aim to validate the identified hit, **1** was re-synthesized (Scheme 1) confirming IC₅₀'s of 78 nM at S1P₄, 14 μM at S1P₅ and no activity at concentrations up to 25 μM towards S1P₁₋₃ subtypes. However, inadequate values of logarithm of partition coefficient and total polar surface area (CLogP = 4.8, tPSA = 38.5) were calculated for **1**. We sought to increase potency and reduce lipophilicity (ClogP and tPSA optimal values ranges of 2.5–3.5 and 60–90 respectively)¹³ through rational chemical modifications of the hit structure, thereby altering the predicted physicochemical properties and S1P₄ binding affinity. **1** was formally fragmented into 2 regions to investigate its SAR (Figure 1): (A) aryl ring C-linked to furan, (B) arylamide.

The preparation of various analogs with modified substituents on the phenyl ring of region B was easily achieved as presented in Scheme 1. Coupling of commercially available acylchloride **2** with a variety of anilines provided, in good yields, a structurally and electronically diverse set of analogs **4a–z**. The obtained compounds were submitted to S1P₄ functional assay (Table 1).¹⁴

Potency and lipophilicity were not significantly affected by attaching small alkylic groups on positions 2 and 6 (**4b**, **4c**, **4f**) compared to the hit. When polar substituents, hydrogen donors or acceptors, were attached in the same positions the potency decreased more substantially (**4d**, **4e**, **4h**, **4i**). Similar activity to the hit was observed for the 2,4,6-trimethyl derivative **4q**. Incorporating the amidic nitrogen into a 5-member ring suppressed completely the activity (**4j**). Two major reasons were formulated to explain this phenomenon: (a) the constriction of the C–N bond rotation, (b) the loss of hydrogen bond donor capability. To evaluate the contribution of the N–H hydrogen bond donor, a *N*-methylated derivative **4r** was synthesized. **4r** was approximately 128-fold less potent than the homolog **4q**; therefore the hydrogen-bond donor capability of the amide group was hypothesized to be essential for the potency. Noteworthy, position 4 of the phenyl ring tolerated lipophilic substituents (**4q**, **4y**) as well as polar and ionizable groups as observed for **4n**, **4p**, **4w**, **4x**, **4y** and **4o**, **4s–4v**, **4z**, the latter showing enhancement of both potency and physicochemical properties. Particularly, the amine **4v**(CYM50358) [CLogP = 3.8, tPSA = 64.4] and alcohols **4t** [CLogP = 3.8, tPSA = 58.6], **4z** [CLogP = 4.0, tPSA = 67.8] were more potent than the hit compound **1** with more desirable physicochemical properties.

Next, SAR studies of the aryl ring A were performed while maintaining 2,6-dialkylanilines in the pendant moiety B. The general synthetic route to afford **9a–q** is outlined in Scheme 2.

Amide coupling of 2-furoic acid **5** with appropriate aniline **6** followed by Suzuki cross coupling under standard conditions¹⁵ with a wide range of aryl boronic acids afforded **9a–q**.

S1P₄ activity of **9a–q** is reported in Table 2. The 2,5-dimethyl analogue **9i** showed similar potency to the hit compound. Deletion of 2-chlorine (**9b**) had a higher impact on the loss of activity than the removal of 5-chlorine (**9c**) (6- vs 3-fold). Notably, the 2,4-dichlorophenyl regioisomer **9a** was 25- and 7-fold less potent than the hit and the mono-chlorinated **9c** respectively; thus indicating that substitution at position 4 was detrimental for the potency. 2,6-dimethylated derivative **9j** was found to be inactive probably due to the anti-coplanar orientation of the phenyl ring. In an attempt to reduce lipophilicity, polar substitutions at positions 2 and 5 were installed, but were found detrimental for the activity (**9e**, **9f**, **9g**, **9h**), suggesting that region A binds to a lipophilic pocket. Successively, a new set of analogs was prepared by replacing the phenyl ring. Interestingly, thiophene and furan rings were found to be good bioisosteres. The 3-thienyl **9k** and 2-thienyl **9m** analogs were slightly more potent than the phenyl derivative **9d**. As the presence of either chlorine or methyl groups in positions 2 and 5 of the phenyl ring were found to be essential for the activity, methylated and chlorinated thienyl derivatives (**9l**, **9n** and **9o**) were synthesized. Interestingly, 4-methyl-3-thienyl **9l** was 1.5-fold more potent than **9k** (similar trend was observed in the phenyl series, **9c** vs **9d**) whereas 3-methyl-2-thienyl **9o** was equipotent to **9m**. Moreover, equipotency was found for 3-chlorophenyl **9b** and 2-furanyl **9p** compared to 5-chloro-2-thienyl **9n** and thienyl **9m** respectively. Interestingly, thienyl and furanyl derivatives showed more suitable physicochemical properties ($3.3 \leq \text{CLogP} \leq 4.3$) within the hit class.

To merge SAR studies of region A and B, hybrid molecules **15** and **16** (**CYM50374**) were synthesized (Scheme 3). 5-bromofuran **10** underwent Suzuki cross coupling with thiophene boronic acid **11** followed by ester hydrolysis to afford carboxylic acid **12** in good yields. Amide coupling of **12** with the opportune anilines **13** and **14** yielded the final compounds in moderated yields.

Indeed, **15** ($\text{CLogP} = 3.0$, $\text{tPSA} = 58.6$) and **16** ($\text{CLogP} = 2.7$, $\text{tPSA} = 58.6$) were potent S1P₄ antagonists ($\text{IC}_{50} = 46$ and 34 nM respectively), with lower lipophilicity compared to the hit compound.

A set of the most active compounds was selected for functional assays at S1P_{1–3,5} subtypes (Table 3). Notably, all the selected compounds displayed an exquisite selectivity for the S1P₄ receptor *versus* the other receptor subtypes; among them **4v** (**CYM50358**) and **16** (**CYM50374**) showing the most suitable physicochemical properties were selected as lead compounds to initiate a lead-optimization program.

In summary, we conducted a systematic SAR analysis of novel selective S1P₄ antagonists based on 5-aryl furan-2-arylcarboxamide **1** scaffold, identified by our HTS efforts. Physicochemical properties (CLogP and tPSA) were calculated for a preliminary evaluation of drug-like properties. Notably, introduction of different ionizable and polar groups at position 4 of region B led to the identification of molecules of particular interest (**4v** and **16**) with attractive *in vitro* biological profile and adequate physicochemical properties. As the first disclosure of S1P₄ antagonists with low nanomolar potency and high selectivity against S1P_{1–3,5} receptor subtypes, the class of molecules herein reported represents a significant milestone that may allow experiments aimed to gain more insights into the biological functions of S1P₄ in fundamental immunological processes. Details of more in-depth ongoing SAR for lead-optimization of the identified lead molecules will be communicated in due course.

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Figure 1.

Scheme 1.

Synthesis of 5-(2,5-dichlorophenyl)-2-arylcarboxamides **1**, **4a–z**.

Reagents and conditions: i.- **2** (1 equiv), **3** (2 equiv), DIPEA (2 equiv), CH₂Cl₂, rt, 2–4 h, 60–95%.

Scheme 2.

Synthesis of 5-Aryl-N-(2,6-dialkylphenyl)furan-2-carboxamides **9a-q**.

Reagents and conditions: (i) **5** (1 equiv), **6** (1.2 equiv), EDCI (1.2 equiv), HOBT (1.2 equiv), DMF, rt overnight, 90%; (ii) **7** (1 equiv), **8** (1.5 equiv), Pd(PPh₃)₄ (0.1 equiv), 2M aq NaCO₃ (2 equiv), 1,4-dioxane, 80 °C, 4–6 h, 20–85%.

Scheme 3.

Synthesis of molecules **15**, **16**.

Reagents and conditions: (i) **10** (1 equiv), **11** (1.5 equiv), Pd(PPh₃)₄ (0.1 equiv), 2M aq Na₂CO₃ (2 equiv), 1,4-dioxane, 80 °C, overnight; (ii) LiOH (1.6 equiv), THF/MeOH/H₂O (2: 2:1), rt, 3 h, 65% (over 2 steps); (iii) **12** (1 equiv), **13** or **14** (1.5 equiv), EDCl (1.5 equiv), HOBT (1.5 equiv), DMF, overnight, 60–70%.

Table 1

S1P₄ antagonists (IC₅₀ nM)^a

cpd	R	Ar	ClogP ^β	tPSA ^β	IC ₅₀
4a	H		5.5	38.3	165
4b	H		5.2	38.3	41
4c	H		5.5	38.3	89
4d	H		4.0	56.8	417

cpd	R	Ar	ClogP ^β	tPSA ^β	IC ₅₀
4e	H		3.3	58.6	573
4f	H		6.2	38.3	105
4g	H		3.8	72.5	302
4h	H		5.1	38.3	169

cpd	R	Ar	ClogP ^β	tPSA ^β	IC ₅₀
4i	H		4.5	47.6	163
4j	-CH ₂ -CH ₂ -		5.6	29.5	NA
4k	H		4.5	38.3	6400
4l	H		6.2	38.3	253

cpd	R	Ar	ClogP ^β	tPSA ^β	IC ₅₀
4m	H		5.0	47.6	931
4n	H		4.0	47.6	589
4o	H		4.2	58.6	58
4p	H		4.9	47.6	80
4q	H		5.4	38.3	63
4r	Me		5.5	29.5	8100
4s	H		3.4	90.7	52

cpd	R	Ar	ClogP ^β	tPSA ^β	IC ₅₀
4t	H		3.8	58.6	21
4u	H		4.7	64.6	34
4v	H		3.8	64.3	25
4w	H		3.7	67.4	94
4x	H		4.7	41.6	348
4y	H		6.7	38.3	115
4z	H		4.0	67.8	44

^α Data are reported as mean for $n = 3$ determinations. NA = no active at concentrations up to 25 μ M.

^β ClogP and tPSA values are obtained from ChemDraw 12.0 V.

Table 2

S1P₄ antagonists (IC₅₀ nM)^a

cpd	R	Ar	ClogP ^β	tPSA ^β	IC ₅₀
9a	H		4.9	38.3	2000
9b	H		4.4	38.3	497
9c	Me		4.4	38.3	269
9d	H		3.6	38.3	575

cpd	R	Ar	ClogP $^{\beta}$	tPSA $^{\beta}$	IC $_{50}$
9e	H		4.0	38.3	308
9f	H		5.5	38.3	NA
9g	Me		2.4	78.8	NA
9h	Me		3.4	56.8	513

cpd	R	Ar	ClogP $^{\beta}$	tPSA $^{\beta}$	IC $_{50}$
9i	H		4.3	38.3	64
9j	H		4.0	38.3	NA
9k	H		3.3	38.3	448
9l	H		3.5	38.3	286
9m	H		3.5	38.3	118

cpd	R	Ar	ClogP ^{β}	tPSA ^{β}	IC ₅₀
9n	H		4.3	38.3	515
9o	H		3.7	38.3	93
9p	Me		3.3	47.6	130

^a Data are reported as mean for $n = 3$ determinations. NA = no active at concentrations up to 25 μ M.

^{β} ClogP and tPSA values are obtained from ChemDraw 12.0 V.

Table 3

S1P selectivity counter screen (IC₅₀ nM, percentage of inhibition)^{a,b,c}

cpd	SIP ₄ IC ₅₀	SIP ₁ IC ₅₀	SIP ₂ IC ₅₀	SIP ₃ IC ₅₀	SIP ₅ IC ₅₀
4b	41	NA	50% ^c	55% ^c	50% ^c
4c	89	NA	NA	NA	NA
4o	58	5300	2800 (90%) ^b	5400	3000
4p	80	20% ^c	2800 (70%) ^b	30% ^c	40% ^c
4t	36	45% ^c	3000 (85%) ^b	60%	40%
4v	25	6400	3900 (90%) ^b	95%	5500 (90%) ^b
4z	44	35% ^c	2400 (90%) ^b	70% ^c	60% ^c
9o	93	95% ^c	2600	20% ^c	40% ^c
15	46	75% ^c	80% ^c	10% ^c	NA
16	34	80% ^c	90% ^c	NA	NA

^a Data are reported as mean for *n* = 3 determinations.

^b Percentage of inhibition.

^c Percentage of inhibition at 25μM. NA = no active at concentrations up to 25 μM.