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Discovery of heterocyclic sulfonamides as sphingosine 1-phosphate receptor 1 ($S1P_1$) antagonists

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ABSTRACT

We have discovered a novel class of heterocyclic sulfonamides that act as antagonists of the $S1P_1$ receptor. While members of this series identified from a high-throughput screen showed promising levels of potency in a cell-based assay measuring the inhibition of receptor internalization, most compounds were excessively lipophilic and contained an oxidation-prone thioether moiety. As a result, such compounds suffered from poor physical properties and metabolic stability, limiting their utility as in vivo probes. By removing the thioether group and systematically developing an understanding of structure–activity relationships and the effects of lipophilicity on potency within this series, we have been able to identify potent compounds with vastly improved physical properties. A representative enantiopure triazole sulfonamide (**33**) has measurable bioavailability following a low (3 mg/kg) oral dose in rat, highlighting an achievement of the early hit-to-lead efforts for this series.

Sphingosine 1-phosphate (S1P), a bioactive metabolite found widely in mammalian plasma, has been shown to be involved in signaling in a number of cellular processes. This phospholipid binds to and activates a family of five cell-surface G protein-coupled receptors known as the S1P receptors ($S1P_1$–$S1P_5$), and has demonstrated effects on cell proliferation and migration, cellular architecture, immune cell trafficking, vascular integrity, and angiogenesis.[1]

Given the breadth of biological activities in which S1P is involved, S1P receptor signaling has been implicated in a variety of disorders, such as inflammatory disease[2] and cancer.[3] Compounds that can affect S1P receptor signaling therefore have the potential to treat such diseases. For example, FTY720 (fingolimid), a sphingolipid mimetic approved for treatment of multiple sclerosis,[4] is a potent agonist of four of the five isoforms of S1P receptor ($S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$).[5,6] Following acute activation of $S1P_1$, fingolimid causes irreversible downregulation of the receptor by inducing internalization[7] and subsequent proteasomal degradation.[8,9] It is this 'functional antagonism' of $S1P_1$ that results in the inhibition of lymphocyte egress from secondary lymphoid organs, which is the mechanistic basis for the therapeutic benefit of this compound.

Our interest in S1P signaling stems from reports of the role of $S1P_1$ in angiogenesis and vascular development. For example, $S1P_1$ function is critical for vascular maturation during embryonic development in mice,[10] and $S1P_1$ siRNA gene silencing results in the suppression of vessel formation in vivo in Matrigel implants.[11] Importantly, the functional antagonist activity of fingolimid has been shown to result in the inhibition of angiogenesis in multiple in vivo models.[12] Given the promise of compounds that inhibit angiogenesis as cancer therapeutics, we initiated a program to develop an orally bioavailable, selective $S1P_1$ receptor modulator to assess the feasibility of such a compound as an antitumor agent.

While other $S1P_1$ agonists under clinical development[13] would be expected to have antiangiogenic effects similar to fingolimid due to the same functional antagonist activity, $S1P_1$ agonism is known to cause activation of G protein-coupled inwardly rectifying potassium ($GIRK/I_{KACh}$) channels in human cardiomyocytes, resulting in instances of transient bradycardia observed in clinical trials with such compounds.[14] Pharmacological modulation of the $S1P_3$ receptor has similarly been linked to adverse cardiovascular events in rodents.[15,16] We therefore desired to identify compounds that were $S1P_1$-selective antagonists, devoid of any agonist activity. At the time we initiated our program, only sparse reports such compounds were available.[17–19] As analogs of the phospholipid S1P, these compounds were useful tools but had limited utility as orally available agents. In the last few years, however, additional reports of orally bioavailable, small-molecule $S1P_1$ antagonists have

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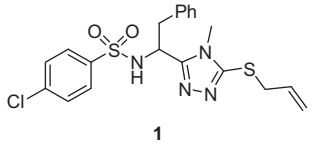
emerged in the literature.[20–22] In this manuscript, we describe the initial hit-to-lead efforts of our program resulting in the identification of $S1P_1$-selective antagonists with oral bioavailability.

In order to identify novel antagonists of the $S1P_1$ receptor, we conducted a high throughput screen of the AstraZeneca compound collection in a cellular assay measuring the ability of compounds to inhibit S1P-induced receptor internalization.[23] This assay format readily allowed us to assess the potency of individual compounds while also differentiating between compounds that act as receptor agonists and antagonists. Among the hits identified from this screening effort, a series of triazole thioether-based compounds containing an aryl sulfonamide moiety (such as compound **1**, Fig. 1) were prioritized for further exploration due to their promising potency in comparison with other hits. In addition, there was a strong correlation between the measured potency in this cell-based assay and an assay measuring binding affinity for compounds of this substructure (Supplementary information), which gave us confidence that we could utilize the cell-based receptor internalization assay to drive SAR development within this series. Lastly, the compounds in this series did not show any activity in a cellular assay measuring antagonism of the $S1P_3$ receptor, suggesting that $S1P_1$-selective compounds could be identified from this scaffold.

An important goal of our initial chemistry efforts was to identify potent compounds with properties suitable for oral administration in rodent in vivo studies in order to address key biological questions surrounding the feasibility of $S1P_1$ as a viable target for cancer therapy. Early in our exploration of this scaffold, however, it became clear that the relatively high lipophilicity of this class of compounds would be a liability that would hinder further progression of the series. Many of these compounds had poor physical properties, and were plagued by very limited stability in the presence of human and rat liver microsomes which contributed to rapid in vivo clearance (equal to or greater than the rate of hepatic blood flow) and a lack of oral bioavailability in rat pharmacokinetic studies.

We postulated that the metabolic instability of this series was largely due to the oxidation-prone sulfur atom of the thioether moiety, and that excision of this liability from the scaffold would result in improved in vivo exposure. Therefore, our initial chemistry strategy was to identify the critical elements of the pharmacophore, and to design thioether-free compounds with decreased lipophilicity in the hope that oral bioavailability could be achieved. A significant portion of our initial work to follow up the HTS hits exemplified by **1** established that only relatively modest changes to the 4-chlorophenylsulfonamide moiety are tolerated, with small substituents at the 3- and 4-positions affording compounds with measurable antagonist activity. While a more detailed exploration of this group was conducted later in the program in the context of other scaffolds, we chose to keep the 4-chlorophenylsulfonamide group constant in our initial campaign to allow for the direct comparison of newly prepared compounds to the initial hits.

A representative synthetic route to access compounds described in this Letter is depicted in Scheme 1. We intended to prepare the target compounds as racemates in order to quickly develop an understanding of the SAR for the scaffold, and then evaluate the individual enantiomers for compounds of interest at a later time.



1

Figure 1. Representative HTS hit from triazole sulfonamide series.

Thus, racemic phenylalanine was converted to intermediate **2** by installation of the aryl sulfonamide moiety followed by esterification. This compound was converted to hydrazide **3**, which was then acylated with the appropriate acid chloride (or formylated by heating in formic acid in the case of **4**). Dehydration using phosphorous oxychloride afforded the oxadiazole **5**, which could be converted to the target triazole **6** by heating in the presence of the desired primary amine under microwave irradiation.

We first prepared a series of matched pairs of the HTS hit **1** (EC_{50} = 1.03 μM), in which the *S*-allyl group was either removed altogether or replaced with small alkyl groups in order to establish SAR at this position of the triazole (Table 1, **6**–**10**). From this focused set of compounds, it became evident that small alkyl groups in place of the thioether moiety are preferred over leaving this position unsubstituted. In particular, the methyl- and ethyl-substituted analogs (**7** and **8**) provided the biggest boost in potency in the $S1P_1$ translocation assay (>10-fold and >50-fold improvements, respectively) relative to the unsubstituted compound **6**. Because of relatively nonpolar nature of the remainder of the scaffold, we were mindful of the impact of these changes on lipophilicity in addition to potency, and found the use of the lipophilic ligand efficiency (LLE) metric informative in the analysis of data for compounds in this series.[24] Thus, with the addition of an alkyl substituent in compounds **7** and **8**, the gain in potency outweighs the increase in lipophilicity, as indicated by an increased LLE relative to **6**. This observation implies that the enhancement in potency for these compounds results from improved interactions between the ligand and the receptor rather than simply an increase in desolvation energy upon binding.

A set of matched pairs of **7**, in which the triazole 4-substituent was modified, was similarly prepared (Table 1, **11**–**14**). A clear preference for small alkyl substitution at this position was observed, with groups larger than ethyl resulting in a loss in potency. Since an ethyl group appeared to be preferred at both positions, we then prepared compound **15** and were pleased to find that this compound resulted in further improvement in the inhibition of $S1P_1$ receptor internalization while maintaining the higher lipophilic ligand efficiency seen with **7**, **8**, and **11**.

Having established the preferred substitution patterns on the triazole ring, we next turned our attention to variation of the group appended to the position adjacent to the sulfonamide nitrogen. As part of extensive exploration of the structure–activity relationships at this position, we prepared a series of matched pairs of compound **11** (Table 2) using chemistry analogous to that detailed in Scheme 1. Relatively subtle changes to the benzyl ring of **11** had modest effects on potency in the $S1P_1$ receptor translocation assay (compounds **16** and **17**). Interestingly, we found that replacement of the aryl ring with a simple olefin (compound **18**) did not result in a significant loss in potency, suggesting truncation of this group was possible. However, saturation of this olefin (compound **19**) or incorporation of branching (compound **20**) resulted in less effective and somewhat more lipophilic antagonists. Ultimately, we found that the compound with a simple methyl group in place of the benzyl (**21**) had comparable potency, which coupled with a significant reduction in lipophilicity resulted in a promising lead compound with decent physical properties (380 μM solubility in pH 7.4 buffer) and encouraging metabolic stability in vitro (human microsomal CL_{int} < 5 μL/min/mg, human hepatocyte CL_{int} < 2 μL/min/10^6 cells).

Alongside our optimization of the 1,2,4-triazole scaffold, we explored the option of utilizing other appropriately substituted heterocycles as $S1P_1$ receptor antagonists (Table 3). 1,2,4-Oxadiazole **22**, which was a synthetic intermediate in the preparation of compounds **8** and **15** (Scheme 1), is considerably less potent, emphasizing the importance of the *N*-4 substituent in the triazole system. We also prepared two imidazole analogs of