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Discovery of thiadiazole amides as potent, $S1P_3$-sparing agonists of sphingosine-1-phosphate 1 ($S1P_1$) receptor

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ABSTRACT

High-throughput screening of GSK compound collection led to the discovery of a novel series of thiadiazole amides as potent and $S1P_3$-sparing sphingosine-1-phosphate 1 ($S1P_1$) receptor agonists. Synthesis, structure and activity relationship, selectivity, and some developability properties are described.

The sphingosine-1-phosphate (S1P) receptors have been receiving greater attention as a therapeutic target with the recent FDA approval of FTY720 for oral treatment of remitting–relapsing multiple sclerosis (RRMS).[1] S1P is a bioactive lysolipid with pleiotropic functions that mediates a large variety of biological effects via extracellular signaling through five distinct G-protein coupled receptors, numbered $S1P_1$ through $S1P_5$.[2] Agonism of S1P receptors prevents the migration of autoimmune lymphocytes from lymph nodes and other secondary lymphoid organs into the periphery including the central nervous system.[1] This interruption of lymphocyte migration, ostensibly without affecting cell types of the innate immune system (e.g., neutrophils or macrophages) as well as affecting cellular reactivity of lymphocytes to antigen challenge, promises a new immunomodulatory therapeutic principle for a variety of autoimmune diseases.

FTY720 is a prodrug and its phosphorylated form FTY720-P exerts in vivo pharmacological effects.[3] FTY720-P is poorly selective and proves to be a potent agonist of four of the five S1P subtypes ($S1P_1$, $S1P_{3-5}$).[4] $S1P_1$ receptor agonism has been shown to correlate with lymphocyte recirculation, while $S1P_3$ receptor agonism has been linked to a number of side effects in preclinical development and clinical trials.[5–7] To attenuate the potential risk of adverse effects associated with activation of $S1P_3$ receptor and therefore to improve the safety profile, more selective compounds for $S1P_1$ are needed. Herein, we describe the discovery of a novel series of thiadiazole amides as potent and selective $S1P_1$ receptor agonists.

A high throughput screening (HTS) using a GTPγS assay[8] was employed to search the GSK compound collection for $S1P_1$ receptor agonists. The thiadiazole amides such as **3** and **4** were identified as one of the most interesting hit series with EC_{50}s of ~1 μM (Fig. 1). The hit structures feature a tertiary amide directly linked to a thiadiazole core and represent a novel chemistry starting point. To follow up the hit series with aim of improving the potency as well as other drug-like properties, the structure–activity relationship (SAR) was explored based on the core template of thiadiazole amides.

The general synthetic methods employed to prepare thiadiazole amides are summarized in Scheme 1. Thiadiazole **6** was prepared by reaction of acid chlorides with amino thiourea, followed by cyclization under acid conditions. Thiadiazole **6** was then alkylated with different alkyl aldehydes using standard reductive animation procedures to give amines **7**, which was coupled with various acid chlorides to afford the targeted thiadiazole amides **8**. One interesting finding during the course of the synthesis is that if amides were formed first, alkylation reaction took place at the nitrogen on the thiadiazole ring rather than amide nitrogen as illustrated in Scheme 2. Therefore, alkylated thiadiazole **10** was formed as the major product with a trace amount of **8**. To the best of our knowledge, very few examples were reported in the literature demonstrating alkylation reactions for thiadiazole rings.[9–12]

With the robust synthesis route developed, we first explored the SAR of the right-hand side (RHS) moiety by preparing analogs

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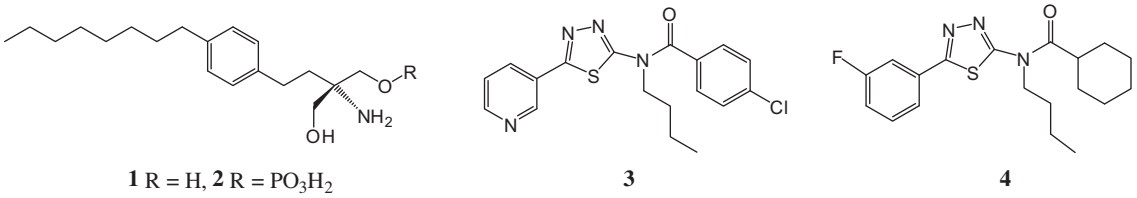
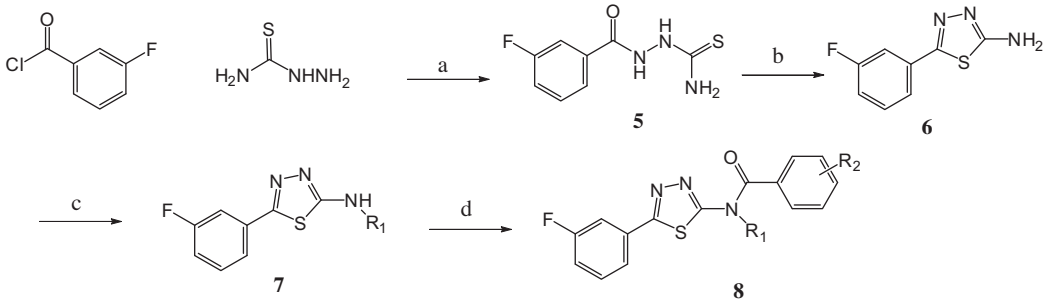
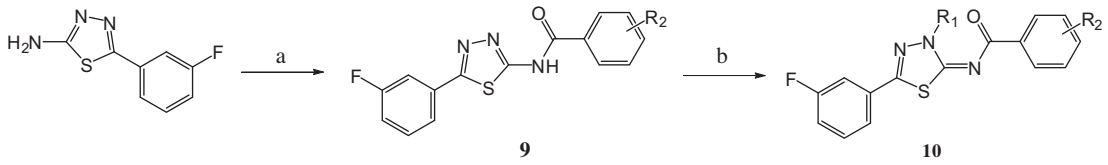


Figure 1. Structures of FTY720 (**1**), its metabolite FTY720-phosphate (**2**) and two HTS hits (**3** and **4**).

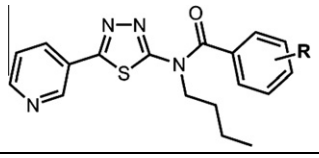


Scheme 1. Reagents and conditions: (a) pyridine, RT, overnight; (b) H_2SO_4, 40 °C, 30 min, 30% from two steps; (c) $NaBH(OAc)_3$, aldehydes, THF, 50 °C, overnight, 35–45%; (d) acid chlorides, Et_3N, microwave, 120 °C, 2 h, ~50%.



Scheme 2. Reagents and conditions: (a) acid chlorides, pyridine, RT, 4 h, 35–45%; (b) alkyl bromides, K_2CO_3, DMF, ~30%.

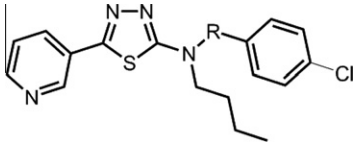
Table 1
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds **3** and **11a–l**

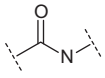


Compd	R	$S1P_1$[a] pEC_{50}	$S1P_3$[a] pEC_{50}
11a	H	6.0	<5.0
11b	2-Cl	6.3	<5.0
11c	2-F	6.5	<5.0
11d	2-OMe	6.2	<5.0
11e	2-Me	6.2	<5.0
11f	3-Cl	6.1	<5.0
11g	3-F	5.9	<5.0
11h	3-OMe	5.9	<5.0
11i	3-Me	6.2	<5.0
3	4-Cl	6.6	<5.0
11j	4-F	5.9	<5.0
11k	4-OMe	5.9	<5.0
11l	4-Me	6.7	<5.0

[a] $S1P_1$ and $S1P_3$ assays were performed as described in Ref. 13.

Table 2
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds **3** and **12a–c**



Compd	R	$S1P_1$[a] pEC_{50}	$S1P_3$[a] pEC_{50}
3		6.6	<5.0
12a		<5.0	<5.0
12b		<5.0	<5.0
12c		5.2	<5.0

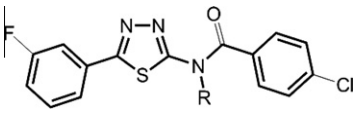
[a] $S1P_1$ and $S1P_3$ assays were performed as described in Ref. 13.

containing different small substituents on the phenyl ring. As shown in Table 1, various groups were tolerated in the RHS ($S1P_1$ pEC_{50}s: 5.5–6.7). All compounds listed in Table 1 showed good selectivity for $S1P_1$ over $S1P_3$ (10-fold at least) and 100-fold selectivity could be achieved with some compounds.

In order to evaluate the importance of the amide function, various replacements were then synthesized. As shown in Table 2, sulfonamide, and amine moieties yielded much less active or completely inactive compounds. This amide moiety seems to be part of the pharmacophore and remains in future compound design.

Subsequently, the efforts were focused on the alkyl moiety on the amide nitrogen. As shown in Table 3, the size of the alkyl moiety is important for $S1P_1$ potency. $S1P_1$ potency (pEC_{50}) were all less than 5.5 with H, Me and Et and started to increase with the length of the alkyl moieties from *n*-Pr to *n*-Bu, while potency

Table 3
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds **13a–g**



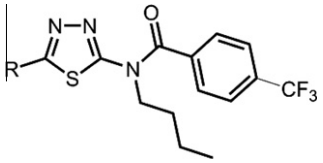
Compd	R	$S1P_1$[a] pEC_{50}	$S1P_3$[a] pEC_{50}
13a	H	<5.0	<5.0
13b	Me	<5.0	<5.0
13c	Et	<5.5	<5.0
13d	*n*-Pr	6.2	<5.0
13e	*n*-Bu	6.9	<5.0
13f	Cyclopropyl-CH_2-	6.6	<5.0
13g	*n*-Pentyl	6.2	<5.5

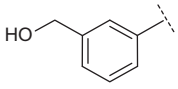
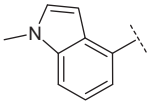
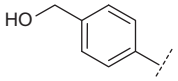
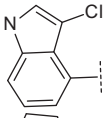
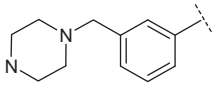
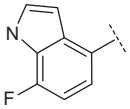
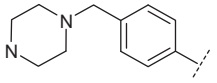
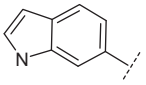
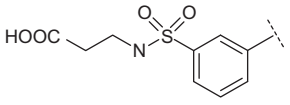
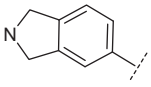
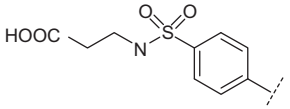
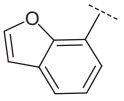
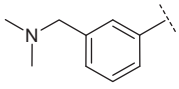
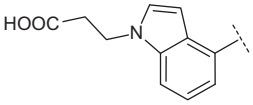
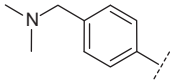
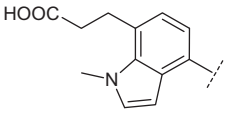
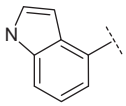
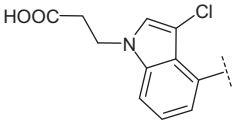
[a] $S1P_1$ and $S1P_3$ assays were performed as described in Ref. 13.

dropped after bigger/longer *n*-Pentyl group was introduced (H ≈ Me ≈ Et <*n*-Pr <*n*-Bu >*n*-Pentyl, **13a–13g**). We hypothesize this might be due to the optimal hydrophobic interactions with $S1P_1$ receptors since other secondary forces could not be reasonably projected for these pure alkyl groups.

Based on the structural features of FTY720 as well as other published S1P receptor agonists,[9,14–17] hydrophilic portions (e.g., acid, amine, alcohol) are typically needed in the molecule to gain the potency. With this in mind, we then focused our efforts in exploring the left-hand side (LHS) moiety by introducing polar moieties. As shown in Table 4, analogs with different hydrophilic groups in the *para* position of the phenyl ring (see **14b**, **14d** and **14h**) provided a significant increase in $S1P_1$ potency to EC_{50} 10–100 nM. Particularly, EC_{50} values for analogs (**14f** and **14p–r**) bearing a carboxylic acid group fell into nanomolar range. However, having polar groups at the *meta* position of the phenyl ring did not enhance potency. Besides substituted phenyls, indole as an example of bicyclic ring was found to be a good moiety for $S1P_1$ potency enhancement. Further derivatization of the indole nitrogen or introduction of halogen substitutions also yielded potent compounds (see **14j–l**). Notably, when thiadiazole portion was shifted from 5 to 7-position of the indole ring, potency was dramatically decreased by

Table 4
$S1P_1$ and $S1P_3$ pEC_{50} values for compounds **14a–r**



Compd	R	$S1P_1$[a] pEC_{50}	$S1P_3$[a] pEC_{50}	Compd	R	$S1P_1$[a] pEC_{50}	$S1P_3$[a] pEC_{50}
14a		7.2	<5.0	**14j**		8.1	<5.5
14b		7.9	<5.0	**14k**		7.5	<5.0
14c		5.4	<5.0	**14l**		8.2	<5.0
14d		7.3	<5.0	**14m**		5.6	<5.0
14e		6.6	5.6	**14n**		7.1	<5.5
14f		8.9	6.3	**14o**		6.6	<5.0
14g		6.2	<5.0	**14p**		10.4	5.8
14h		7.5	<5.0	**14q**		9.4	<5.0
14i		8.9	<5.5	**14r**		8.8	<5.0

[a] $S1P_1$ and $S1P_3$ assays were performed as described in Ref. 13.

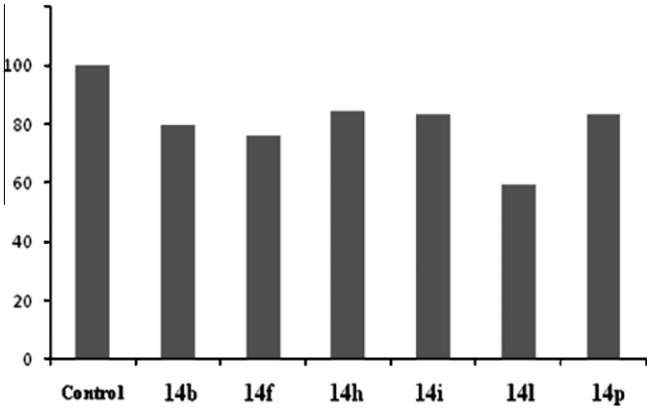


Figure 2. Reduction of blood lymphocyte counts by IP dose of selected compounds. Compounds were evaluated for its ability to induce peripheral lymphocyte reduction following a single administration. Naïve mice were given compounds through ip (2 mg/kg) and blood samples were taken at 4 h after dosing. Lymphocytes in mice were counted by FACS within the lymphocyte gate. Percentages indicate cell counts relative those of the control.

Table 5
Intrinsic clearance with liver microsomes of selected compounds[a]

Compd	Mouse (μL/min × mg protein)	Rat (μL/min × mg protein)	Dog (μL/min × mg protein)	Human (μL/min × mg protein)
14b	712.4	278.6	370.2	61.2
14f	137.4	99.6	595.8	13.8
14h	478.4	539.0	1151.0	109.4
14i	63.0	43.2	823.4	77.2
14l	59.4	43.0	464.2	48.6
14p	91.2	80.6	625.2	118.8

[a]Intrinsic clearance assays were performed as described in Ref. 18.

~1000-fold (comparing **14m** with **14i**). Electronic deficient ring seems to provide less potent compound, which was exemplified by **14l** compared to **14i** with a half log decrease of potency. Dihedral angle between thiadiazole and indole moieties can further negatively impact $S1P_1$ activity. This was supported by the evidence that analogs with Cl substituent on the 3-position of the indole were found to be more than 10-fold less potent than those without this substituent due to the combination of electronic and steric effects (comparing **14k**, **14r** with **14i**, **14p**, respectively).

Compounds **14b**, **14f**, **14h**, **14i**, **14l**, **14p** were further evaluated in in vivo lymphopenia model. Hence, the number of lymphocytes circulating in blood was measured at 4 h after intraperitoneal (ip) administration. Given the variability of the lymphocyte count, the reduction was only considered to be very mild (**14b**, **14f** and **14l**) or none (**14h**, **14i** and **14p)** (Fig. 2). The lack of pharmacodynamic effect (i.e., lymphopenia) may be attributed to their poor pharmacokinetic profiles. A generally high in vitro intrinsic clearance in liver microsomes was observed in these molecules (Table 5). Compound **14l**, with the lowest in vitro intrinsic clearance in mouse, gave the most significant lymphopenia. The screening pharmacokinetic (PK) model (ip, 3 time points) also demonstrated low blood exposures within time of period of 0–4 h for **14i** and **14p** (133 and 114 h ng/mL for AUC_{0-4} of **14i** and **14p**, respectively). These results confirmed our hypothesis that the mild lymphopenia was due to poor drug exposure. More efforts are being focused on improving the PK profile of the thiadiazole amides, and progress will be reported in due course.

In summary, a series of thiadiazole amides as novel and selective $S1P_1$ receptor agonists were identified from the high throughput screening. Based on the template, an initial SAR was explored, leading to the identification of the thiadiazole amide series with nanomolar potency. Although the series has high clearance resulting in poor pharmacokinetic profiles, the series appears to be an acceptable starting point for further lead optimization. Ongoing work is to design and synthesize cyclic amides as well as other heterocyclics as replacements or bioisosteres of the tertiary amide moiety to increase their metabolic stability while retaining $S1P_1$ potency.

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