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Identification of benzoxazole analogs as novel, $S1P_3$ sparing $S1P_1$ agonists

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ABSTRACT

A novel series of benzoxazole-derived $S1P_1$ agonists were designed based on scaffold hopping molecular design strategy combined with computational approaches. Extensive SAR studies led to the discovery of compound **17d** as a selective $S1P_1$ agonist (over $S1P_3$) with high CNS penetration and favorable DMPK properties. **17d** also demonstrated in vivo pharmacological efficacy to reduce blood lymphocyte in mice after oral administration.

Modulation of biological actions of sphingosine-1-phosphate receptor-1 ($S1P_1$), a member of the G protein coupled receptor (GPCR) superfamily, has emerged as a new paradigm for the discovery of therapeutic agents in autoimmune diseases.[1] Recently, a novel $S1P_1$ agonist, FTY720 (Fig. 1), was approved as the first oral drug for the treatment of relapsing/remitting multiple sclerosis (RRMS).[2,3] The pharmacological efficacy of FTY720 was believed to be due to its agonistic activity at $S1P_1$ when phosphorylated in vivo. $S1P_1$ agonism could redirect the trafficking of peripheral T cells and B cells from systemic circulation into secondary lymphoid organs, thus reduce lymphocyte infiltration into CNS resulting in the observed efficacy in preclinical animal models for MS.[4] The primary adverse effect observed in clinical trials of FTY720 is transient and asymptomatic bradycardia. Its $S1P_3$ agonism (FTY720-P $S1P_3$ EC_{50} ~3 nM and $S1P_1$ EC_{50} ~0.3 nM)[2] was thought to be responsible for the hemodynamic and pulmonary side effects seen in clinical trials.[5–7] Hence $S1P_1$ agonists with selectivity over $S1P_3$ are preferred for development.

Various efforts have been followed as $S1P_1$ agonists with limited pharmacophores, including CS-0777,[8] ACT-128800,[9] AMG 369,[10] or PF-991[11] (Fig. 1). Herein, we reported the discovery of a novel benzoxazole series as selective $S1P_1$ agonists over $S1P_3$. The series demonstrated good overall pharmacokinetic properties, high CNS penetration property, and good in vivo efficacy in the mouse lymphopenia study.

In our previous study, we had discovered a series of indole-1,2,4-oxadiazole compounds as orally active $S1P_1$ agonists (exemplified by **1**, Fig. 2).[1,12] Compound **1** showed excellent $S1P_1$ potency (pEC_{50} = 11, Tango assay[13]) and good selectivity over $S1P_3$[14] (10^6-fold). The 3-chlorinated indole derivative **2** demonstrated similar potency and selectivity ($S1P_1$ pEC_{50} = 10.6; $S1P_3$ pEC_{50} <5), which suggests that small substitutions at the 3-position of the indole ring are tolerated. In order to further simplify the structure, we hypothesize the cyclization of indole with oxazole would maintain similar conformation and provide less unnecessary carbons (Fig. 3). Since alkyl amine in **4** is known to have very different physicochemical properties compared with the indole nitrogen in **3**, we decided to synthesize and examine the potency of the none-amine **5a** first which is also more synthetically feasible.

Our first task is to develop a convergent synthetic route targeting compound **5a**. As outlined in Scheme 1, starting from commercial available material **6**, acidic hydrolysis followed by selective iodination and reduction provided aniline **9**. Amide bond formation between aniline **9** and acid chloride **11** (prepared from acid **10**) gave amide **12**. Intermediate **12** was then subjected to the cyclization condition to give aza-benzoxazole **13**. Treatment of **13** with zinc reagent in the presence of suitable Pd catalyst resulted in the coupling product **14**. The final target molecules **5a**, **5c–f** were synthesized through ester hydrolysis. Compound **5b** was obtained from **13** via lithiation followed by quenching with dry ice.

With the synthetic approach successfully developed, we investigated the SAR on the carboxylic side chain. The first compound **5a** demonstrated reasonable potency (pEC_{50} 6.8) in the $S1P_1$ Tango assay[13] without $S1P_3$ activity (Table 1). Extension or reduction one carbon of the carboxylic acid side chain (**5e**, **5f**) maintained comparable $S1P_1$ potency and selectivity over $S1P_3$. However, if the side chain was further reduced (**5b**, **5c**, and **5d**) the enzymatic potency

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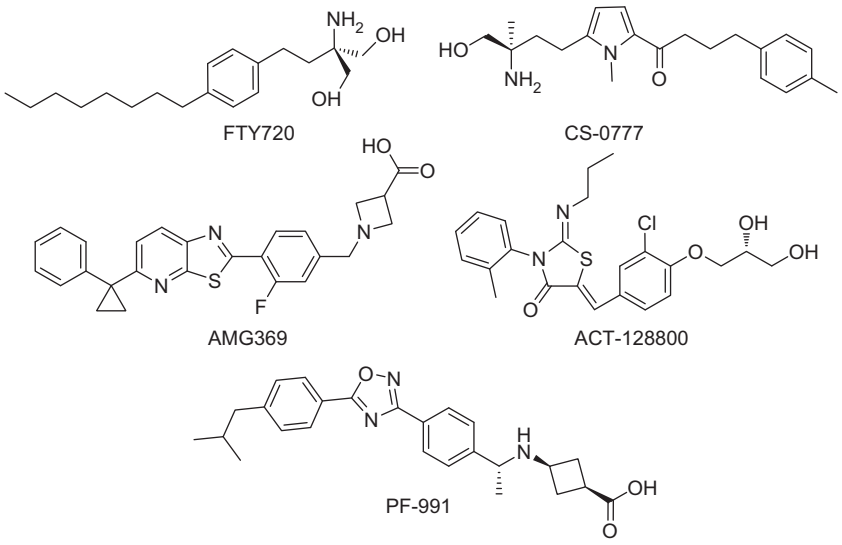


Figure 1. Structure of known $S1P_1$ agonists.

was decreased dramatically (pEC_{50} <5.5). Given the poor yield (<10%) leading to compound **5a** in the Negishi coupling step, compound **5f** was then selected as a starting point for further optimization due to its synthetic easiness.

Starting from **5f**, we turned our attention to optimize the left hand side of the molecule in order to improve $S1P_1$ potency (Table 2). The chloro-pyridine analogue (**15a**) proved to be least potent. Replacing the chloride with a more lipophilic trifluoromethyl group resulted in a much improved potency (**15b**). Its pyridine analogue demonstrated a slightly improved $S1P_1$ activity (**15c**). The most potent compound (**15d**) was obtained by the replacement of the isopropyl group with a phenyl group, with a subnanomolar $S1P_1$ agonistic activity. The potency decreased drastically when one of the phenyl group was replaced by its bioisoster, thiophene (**15e**). It was noteworthy that all compounds were selective $S1P_1$ agonists against $S1P_3$.

With the left & right hand molecule optimized, we then focused on optimizing the core. The aza-benzoxazole core proved to be labile in basic conditions (cleaved oxazole byproduct was observed in the ester hydrolysis). In order to improve its stability, we then tried to replace the core with other bicyclic heteroaromatic rings, while maintaining $S1P_1$ potency. As shown in Table 3, all the bicyclic cores (**16a–e**) resulted in 10- to 1000-fold loss of potency except the benzoxazole derivative **15f** (pEC_{50} 9.4). Compound **16f** demonstrated high $S1P_1$ potency comparable to that of the aza-benzoxazole lead compound (**15d**) and good selectivity over $S1P_3$. In addition, the benzoxazole core was observed to be more stable than the aza-benzoxazole structure under basic condition. With the successful optimization of left hand side (LHS) and core,

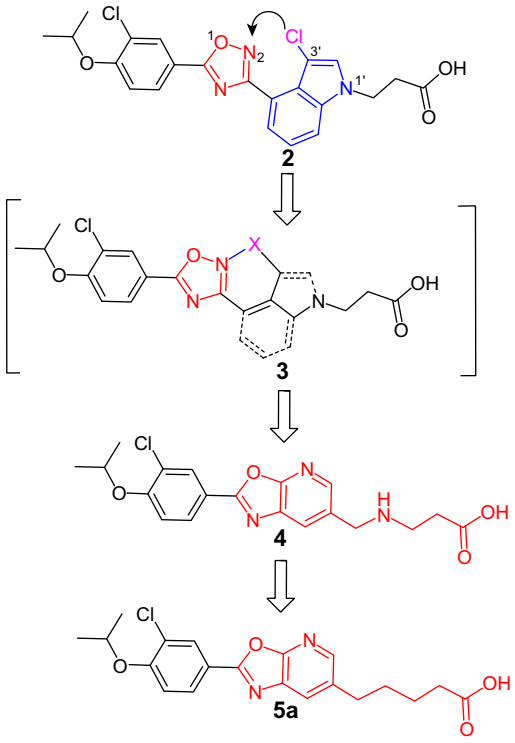
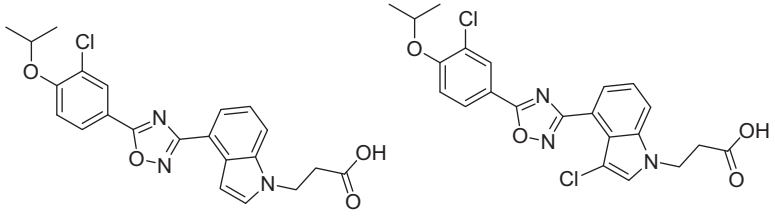
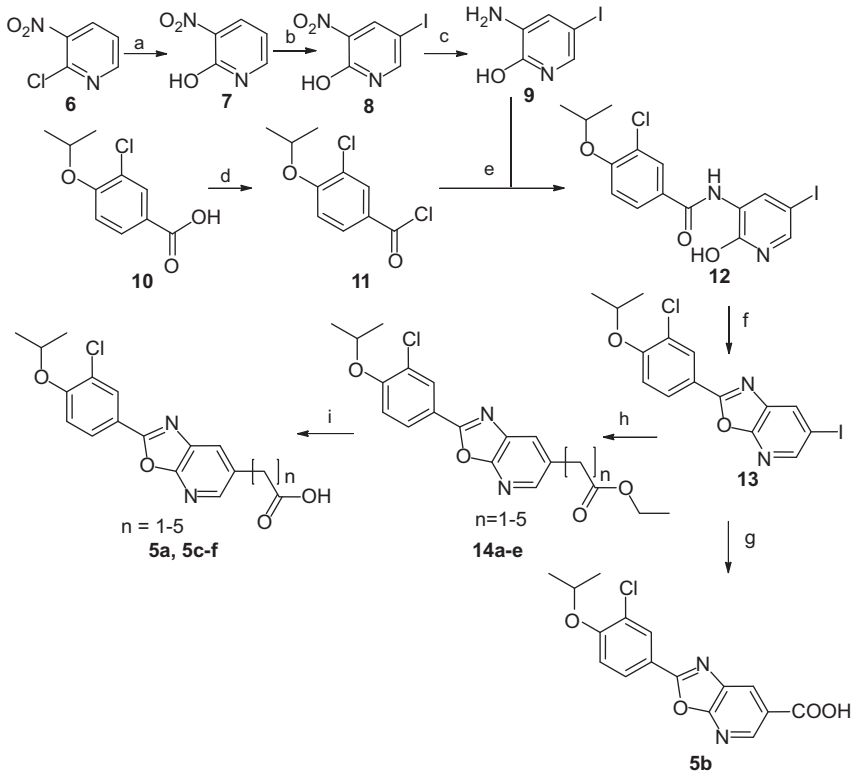


Figure 3. Design of aza-benzoxazole compound **5a**.



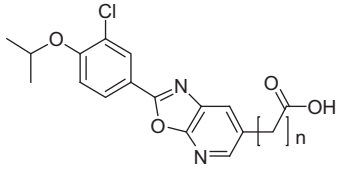
1 $S1P_1$ pEC_{50} = 11 **2** $S1P_1$ pEC_{50} = 10.6

Figure 2. The $S1P_1$ agonizing activity of compounds **1** and **2**.



Scheme 1. Synthesis of compound **5a** and its analogues. Reagents and conditions (a) concd HCl, 112 °C, 20 h, 100%; (b) I_2, H_5IO_6, AcOH, H_2SO_4, H_2O, 90 °C , 20 h, 95%; (c) Fe, HCl, MeOH, reflux, 1 h, 85%; (d) $(COCl)_2$, DMF, DCM, rt, 2 h; (e)TEA, DCM, rt, overnight; (f) Ph_3P, C_2Cl_6, TEA, DCM, rt, 50% of d, e and f; (g) nBuLi, dry ice, −78 °C, 26.5%; (h) $Pd_2(dba)_3$, Cs_2CO_3, (t-Bu)$_3$P-HBF$_4$, zinc reagent, THF, 50 °C; (i) NaOH, iPrOH, H_2O, rt, 4–35% of h and i.

Table 1
$S1P_1$ and $S1P_3$ agonizing activity of compounds **5a–f**



No.	n	$S1P_1$ pEC_{50}[a]	$S1P_3$ pEC_{50} [b]
5a	4	6.8	<5
5b	0	<5.5	<5
5c	1	<5.5	<5
5d	2	<5.5	<5
5e	3	6.0	<5
5f	5	6.8	<5

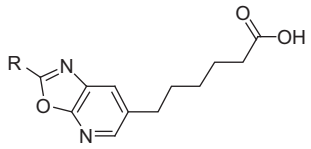
[a] The Tango assay[13] for $S1P_1$.
[b] $S1P_3$ Ca^{2+} mobilization in h$S1P_3$ assay (in 96-well microplates).[14]

the subsequent SAR study was thus focused back at the right hand side (RHS) of **16f** while fixing the LHS and the benzoxazole core.

To optimize RHS of **16f**, structure-based design was utilized. Without $S1P_1$ crystal structure at the time of the research, homology modeling was utilized.[15] The proposed binding mode of **16f** is shown in Figure 4.

The binding mode suggests that the carboxylic acid group of **16f** occupy the region of the lipid membrane close to the extracellular loops and form ionic interaction with Arg^{120}/Arg^{292} while the remaining ligand structure extend toward the center of the membrane. This binding mode allows the charged carboxylic acid moiety located in the shallow and less hydrophobic region of the pocket while the more hydrophobic aromatic ring system

Table 2
$S1P_1$ and $S1P_3$ agonizing activity of compounds **15a–e**



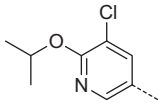
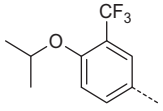
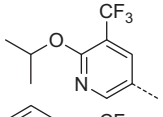
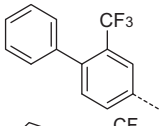
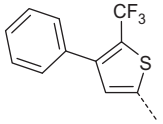
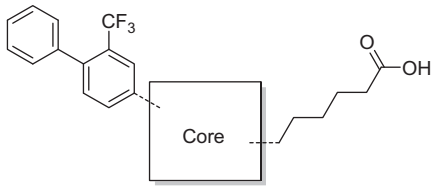
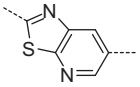
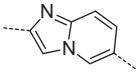
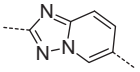
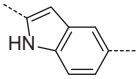
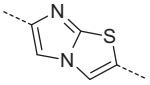
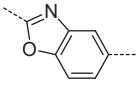
No.	R	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}
15a		6.0	<5
15b		7.7	<5
15c		8.2	<5
15d		9.5	<5
15e		6.4	<5

Table 3
$S1P_1$ and $S1P_3$ agonizing activity of core replacement compounds **16a–f**



No.	Core	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}
16a		8.0	<5.0
16b		6.4	<5.0
16c		6.9	<5.0
16d		6.9	<5.0
16e		7.2	<5.0
16f		9.4	<5.0

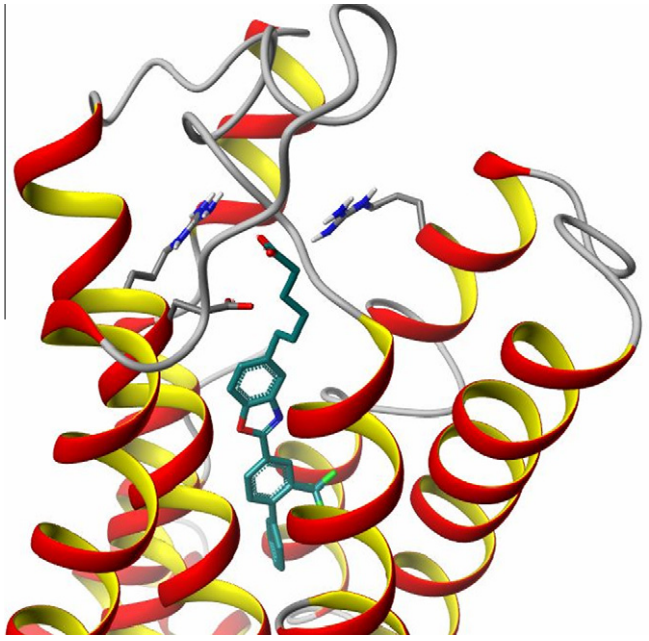
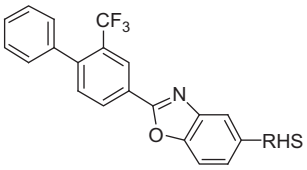
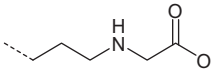
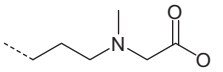
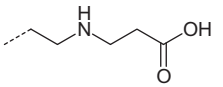
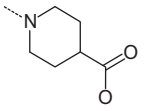
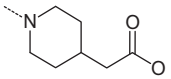
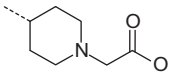
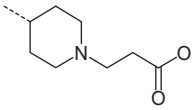
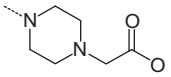
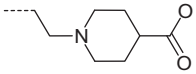
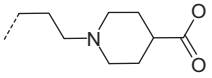
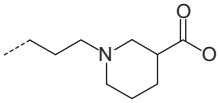
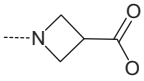
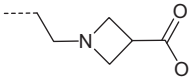
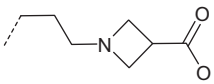


Figure 4. Predicted binding mode for **16f** in $S1P_1$ homology model.

Table 4
$S1P_1$ and $S1P_3$ activity of compounds **17a–n**



No.	RHS	$S1P_1$ pEC_{50}	$S1P_3$ pEC_{50}[a]
17a		10.5	6.6
17b		9.5	6.0
17c		10.5	6.7
17d		9.2	<5.5
17e		8.9	5.9
17f		9.0	5.7
17g		10.1	6.0
17h		9.4	5.7
17i		9.8	6.3
17j		9.1	6.3
17k		9.3	5.7
17l		8.0	5.7
17m		9.4	6.1
17n		10	6.9

[a] $S1P_3$ Ca^{2+} mobilization in $hS1P_3$ assay (in 384-well microplates).

penetrates into the more lipophilic region of the membrane. The binding mode is also consistent with those reported up to date.[16,17] Glu^{121}, a residue with negatively charged side chain, is located closely to Arg^{120}, and might interacts with Arg^{120} when Arg^{120} is free of contact. This observation suggests that a positively charged moiety, for example basic amine, in the RHS may even enhance ligand binding affinity in $S1P_1$ if accessible to Glu^{121}. Based on this finding, we tried to introduce a nitrogen atom into RHS chain to validate this hypothesis.

As shown in Table 4, insertion of N into the side chain provided analogue **17a** with a 10-fold increase in $S1P_1$ potency, while at the same time the undesirable $S1P_3$ activity was also increased by at least 10-fold. The $S1P_1$ selectivity over $S1P_3$ still met our criterion of over 1000-fold. The tertiary amine analogue (**17b**) showed similar $S1P_1$ potency to**16f**. Moving N from right to left side provided analogue **17c** showing similar profiles with its parent compound **17a**. The piperidine acid derivative (**17d**) proved to be potent

Table 5
The mouse PK, brain–blood ratio and lymphopenia

No.	Mouse PK (2 mg/kg)	Br/Br ratio	Lymphopenia
17d	$T_{1/2}$: 5.40 h; *F*: >100% C_{max} 1.07 μg/mL $AUC_{0-24\,h}$: 10.3 μg h/mL	1.45 (1 h), 3.73 (4 h)	37% (4 h), >100% (24 h)

$S1P_1$ agonist without $S1P_3$ agonistic activity. Extension of the acid side chain by one carbon (**17e**) resulted in lower $S1P_1$ potency. A similar $S1P_1$ potency was observed when the piperidine N was moved inside ring (**17f**). The potency was much improved by further extension of the side chain by one carbon (**17g**) but the compound was also suffered from undesirable $S1P_3$ activity. The piperazine analogue (**17h**) demonstrated comparable $S1P_1$ potency however still with $S1P_3$ activity. Similarly, the $S1P_3$ activity could not be mitigated by changing the side chain length (**17i** and **17j**) or moving the carboxylic acid around the piperidine ring (**17k**). The azetidine analogue (**17l**) was found to be much less potent compared with the piperidine derivative (**17d**), probably due to a shorter side chain. When the side chain length was increased by one or two carbon, the $S1P_1$ potency was improved drastically (**17m** and **17n**). Both compounds still showed significant $S1P_3$ agonistic activity. The amino acid compounds in Table 4 suggested that nitrogen atom in the proper position would facilitate the binding affinity and improve potency, which confirmed the modeling findings.

Compound **17d** was selected for further progress in in vivo studies due to its good $S1P_1$ potency (pEC_{50} = 9.2), minimal undesirable $S1P_3$ activity (pEC_{50} <5.5), and good overall physiochemical properties. In mouse CNS penetration study (iv dosing), compound **17d** showed good brain to blood ratio (BBR at 1 h and 4 h post dosing are 1.45 and 3.73, respectively; Table 5). The mouse PK study (p.o. dosing, 2 mg/kg) revealed that this compound had very good oral bioavailability (~100%) and a moderate half-life (5.40 h, p.o.) in correspondence with its low clearance (3.68 mL/min/kg, iv) and moderate volume of distribution (1.48 L/kg, iv). The compound was evaluated in the in vivo efficacy study. In the acute mouse lymphopenia model, **17d** turned out to be efficacious at 1 mg/kg (37% lymphocyte reduction at 4 h). And the lymphopenia effect was transient. The lymphocytes count measured at 24 h was back to normal (100% of baseline). Indeed, this transient lymphopenia profile was desired for MS treatment, and the sustained lymphopenia observed from FTY720 (blood lymphocytes were reduced to 20% of baseline and after drug cessation lymphocyte counts only return to baseline values after 4–8 weeks) was believed to be responsible for its serious infection side effects in clinical trial.[18,19]

In summary, we have discovered a novel benzoxazole series of selective $S1P_1$ agonist based on knowledge-based scaffold hopping combined with computational approaches. Among these analogues, compound **17d** was identified to have good $S1P_1$ agonistic potency with $S1P_3$ selectivity. Compound **17d** demonstrated high brain penetration and favorable pharmacokinetic properties in mice. Oral dosing of compound **17d** in mice resulted in a statistically significant reduction in circulating lymphocytes at 4 h post-dosing and back to normal at 24 h.

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