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Structure–activity relationship studies of S1P agonists with a dihydronaphthalene scaffold

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ABSTRACT

Structure–activity relationship (SAR) of sphingosine-1-phosphate receptor agonists with a dihydronaphthalene scaffold was investigated. Compound **1** was modified to improve $S1P_1$ agonistic activity and in vivo peripheral lymphocyte lowering (PLL) activity without impairing selectivity over $S1P_3$ agonistic activity. A detailed SAR study of the terminal lipophilic part revealed that the introduction of substituents on the propylene linker and the terminal benzene ring influences in vitro and PLL activities. Compound **6n** bearing a (*S*)-methyl group at the 2-position on the propylene linker and chlorine at the *para*-position on the terminal benzene ring showed potent $hS1P_1$ agonistic activity with excellent selectivity over $hS1P_3$ and in vivo PLL activity in mice.

Sphingosine-1-phosphate (S1P) exerts a variety of biological activities including vascular maturation and cell survival.[1] S1P is the natural ligand of a specific family of G-protein coupled receptors known as $S1P_{1-5}$. A significant achievement in the S1P research field was published in 2002 by Lynch and co-workers. They reported that FTY720 (Fingolimod, Fig. 1), which was developed as a novel immunomodulator[2] and was recently approved in several countries for the treatment of multiple sclerosis, is metabolized across species to a monophosphate ester, which can activate four S1P receptors ($S1P_{1,3-5}$) to sequester lymphocytes from circulation to a secondary lymph tissue compartment.[3] It was also reported that $S1P_1$ is essential for lymphocyte recirculation since $S1P_1$ modulates egress from thymus and peripheral lymphoid organs.[4] On the other hand, in rodents, $S1P_3$ agonism is not related to lymphocyte recirculation but instead is linked to bradycardia.[5] Asymptomatic bradycardia was also observed in clinical studies with FTY720.[6] Hence agonists with selectivity for $S1P_1$ over $S1P_3$ are desired. Herein, we report our efforts to identify orally active $S1P_1$ agonists which are selective over $S1P_3$ with good oral bioavailability in rats.

In a previous report,[7] we described the identification of dihydronaphthalene derivative **1** as a potential scaffold with good $S1P_1$ agonist activity, selectivity over $S1P_3$ and in vivo efficacy. Oral administration of **1** in mice induced peripheral lymphocyte lowering (PLL) with a 50% effective dose (ED_{50}) of 0.16 mg/kg at 4 h and 1.9 mg/kg at 24 h after oral dosing (Fig. 2).[8] Therefore, we decided to investigate the structure–activity relationship around **1** to improve the $S1P_1$ agonistic activity and in vivo efficacy without impairing selectivity over $S1P_3$.

Compounds were synthesized as shown in Schemes 1–4. Compound **6a** was prepared by a linear synthetic procedure as described in a previous report.[7] 6-Hydroxytetralone **2** was treated with 3-phenylpropyl bromide in the presence of potassium carbonate in DMF to yield 6-(3-phenylpropyloxy)tetralone **3a** in 94% yield. The methyl group was incorporated using a methyl Grignard reagent to the ketone **3a** to yield tetrahydronaphthalenole **4**. Treat-

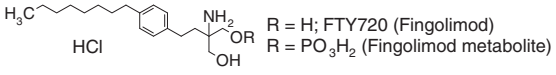

Figure 1. Structure of FTY720 (Fingolimod) and major metabolite.

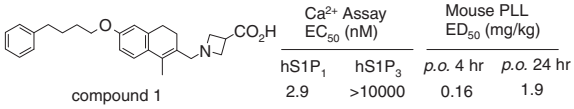

Figure 2. Structure and pharmacological profiles of dihydronaphthalene derivative **1**.

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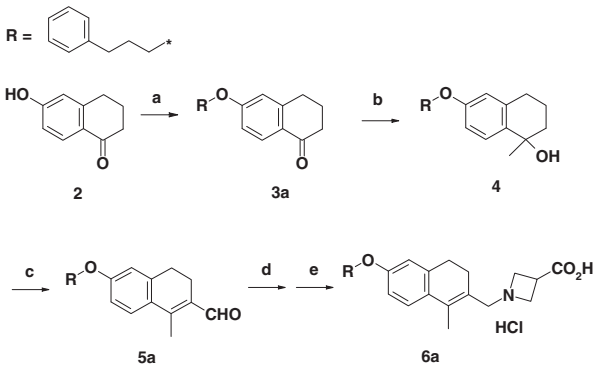
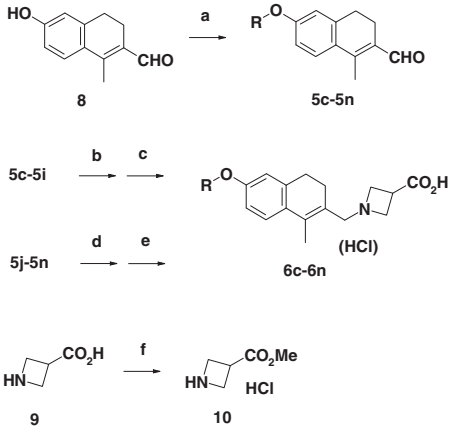


Scheme 1. Reagents: (a) $Ph(CH_2)_3Br$, K_2CO_3, DMF; (b) MeMgBr/Et_2O, THF; (c) $POCl_3$, DMF, CH_2Cl_2; (d) (i) 3-azetidine carboxylic acid, NaOH (powder), $HC(OMe)_3$, MeOH, THF; (ii) $NaBH_4$; (e) HCl-aq, THF.



Scheme 3. Synthesis of **6c**–**6n**. Reagents: (a) the corresponding alcohol **12c**–**12n**, DEAD (for **12c** and **12e**) or TMAD (for **12d** and **12f**–**12n**), PPh_3, THF; (b) 3-azetidine carboxylic acid **9**, NaOH (powder), MeOH, THF, $HC(OMe)_3$; (ii) $NaBH_4$; (c) HCl-aq, THF; (d) 3-azetidine carboxylic acid methyl ester **10**, $NaBH(OAc)_3$, Et_3N/THF (for **5j** and **5n**) or AcOH/DMF (for **5k**–**5m**); (e) NaOH-aq, MeOH; (f) $SOCl_2$, MeOH.

ment of alcohol **4** with Vilsmeier reagent generated by $POCl_3$ in DMF yielded dihydronaphthalene-2-carboaldehyde **5a** via dehydration in situ in 42% yield from **3a**.[9] Aldehyde **5a** was treated with 3-azetidine carboxylic acid **9** in the presence of NaOH powder and methyl orthoformate. The resulting iminium salt was reduced in situ by sodium borohydride to yield the amino acid,[10] which was subsequently treated with HCl-aq to be prepared as the HCl salt **6a** in 46% yield (Scheme 1).

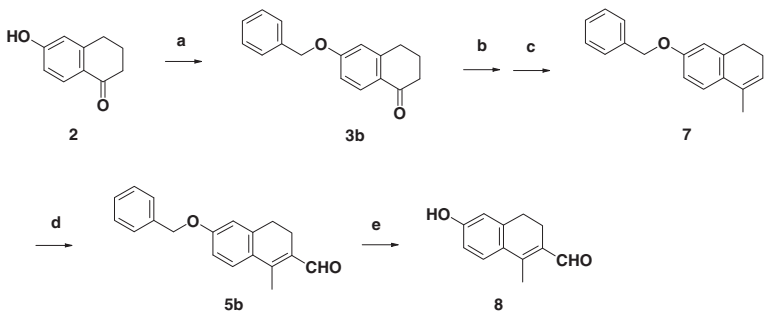
Compounds **6c–6n** were prepared by a convergent synthetic procedure using phenol-aldehyde **8** as a key intermediate. First, intermediate **8** was prepared by modifying the method of Scheme 1. 6-Benzyloxy tetralone **3b** was obtained by a similar procedure to **3a** in 87% yield. The methyl group at 1-position of dihydronaphthalene was incorporated by addition of a methyl Grignard reagent followed by dehydration with aqueous HCl to yield 6-benzyloxy dihydronaphthalene **7** in 38% yield. The benzyl group of compound **5b**, which was obtained in 62% yield from **7** by a similar procedure to aldehyde **5a**, was removed in TFA in the presence of thioanisole to yield the key intermediate **8** in 59% yield (Scheme 2).

The phenylpropyl moiety was incorporated into the phenol part of the key intermediate **8** by Mitsunobu reaction with the corresponding alcohol **12c–12n** to yield aldehydes **5c–5n** in 27% to quantitative yield. Finally, the amino acid moiety was incorporated by two procedures. Compounds **6c–6i** were obtained from **5c–5i** by a similar procedure to **6a** in 34–58% yield. Compounds **5j–5n** were treated with 3-azetidine carboxylic acid methyl ester hydrochloride **10** in the presence of sodium triacetoxyborohydride to yield the corresponding 3-azetidine carboxylates in 37–94% yield, which were converted by saponification to amino acids **6j–6n** in 42–89% yield (Scheme 3).

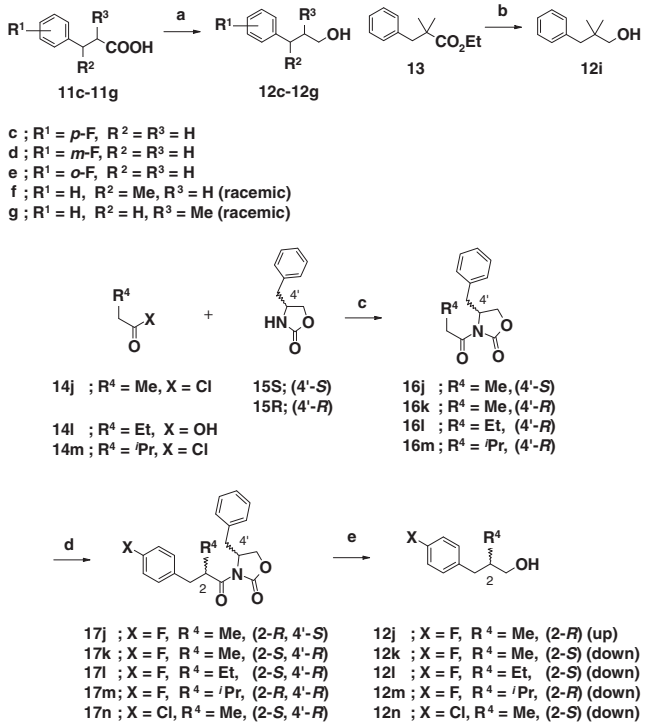
Since only 4-phenylbutan-2-ol **12h** was commercially available, the remaining corresponding alcohols **12c–12g** and **12i–12n** were prepared as shown in Scheme 4. 3-Phenylpropanoic acids **11c**–**11g** were treated with a BH_3 THF complex to yield alcohols **12c**–**12g** in 57% to quantitative yield. Ethyl ester **13** was treated with $LiAlH_4$ to yield alcohols **12i** in 94% yield. Evans' chiral oxazolidinone auxiliary[11] **15S** was treated with *n*-BuLi followed by acid chloride **14j** to yield 3′-acyloxazolidinone **16j** (4′-*S*) in quantitative yield, which was treated with sodium hexamethyldisilazane (NaHMDS) followed by *p*-fluorobenzyl bromide to yield 2-benzylated-3′-acyloxazolidinone **17j** (2-*R*, 4′-*S*) in 56% yield as a single diastereomer. Compound **17j** was treated with $LiBH_4$ to yield alcohol **12j** as an optically pure form in 86% yield. Other alcohols **12k–12n** were also prepared by a similar procedure to **12j** (Scheme 4).

First, the effect of introducing a fluorine substituent on the terminal phenyl ring of **6a** was investigated through the in vitro and in vivo activity. While introducing fluorine at the *meta* (**6d**) or *ortho* (**6e**) position on the terminal phenyl ring showed comparable in vitro activity, **6c** bearing a fluorine atom at the *para*-position showed an improved $S1P_1$ agonistic activity. Then, the in vivo PLL activity of **6c–6e** was examined. At 4 h after oral administration, compounds **6c–6e** showed improved activity by 3- to 7-fold compared to **6a** and at 24 h after oral administration they showed 3- to 8-fold better activity than **1** (Table 1).

Next, the effect of a methyl group on the alkylene linker between the aromatic rings of **6a** was investigated. Whereas **6h** bearing a methyl group at the 1-position of the alkylene linker showed modest $S1P_1$ activity, **6f** or **6g** bearing a methyl group at the 2- or 3-position improved $S1P_1$ agonistic activity by fivefold. Additionally, their selectivity over $S1P_3$ was improved by approximately



Scheme 2. Synthesis of the key intermediate **8**. Reagents: (a) benzyl bromide, potassium carbonate, acetone; (b) MeMgBr/Et_2O, THF; (c) EtOAc, HCl-aq; (d) $POCl_3$, DMF; (e) TFA, PhSMe.

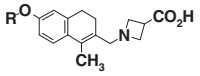


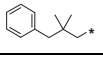
Scheme 4. Synthesis of **12c**–**12g** and **12i**–**12n**. Reagents: (a) BH_3·THF complex, THF; (b) $LiAlH_4$, THF; (c) *n*-BuLi, THF (for **14j** and **14m**) or DCC, DMAP, CH_2Cl_2 (for **14l**); (d) *p*-fluorobenzyl bromide (for **17j**–**17m**) or *p*-chlorobenzyl bromide (for **17n**), NaHMDS, THF; (e) $LiBH_4$, THF.

20-fold (**6a** vs **6f** or **6g**). In terms of the in vivo PLL activity, **6g** showed an improved in vivo PLL efficacy by twofold (**6a** vs **6g**) at 4 h after oral administration. **6i** with two methyl groups at the 2-position showed less $S1P_1$ agonistic activity than **6g** (Table 2).

Substitution of a fluorine atom at the *para*-position on the terminal phenyl ring was effective for improving in vitro $S1P_1$ agonist activity as well as in vivo efficacy. Introduction of a methyl group at the 2-position on the alkylene linker was effective for improving selectivity over $S1P_3$. Therefore, based on these data, introduction of both a fluorine atom at the *para*-position on the terminal phenyl ring and a methyl group at the 2-position on the alkylene linker was investigated. As expected, **6j** and **6k** showed better selectivity

Table 2
The influence of a methyl group(s) on the alkylene linker between the aromatic rings on $S1P_1$, $S1P_3$ agonistic activities and in vivo efficacy



Compound	R	Ca assay[a] EC_{50} (nM)		Mouse PLL[b] ED_{50} (mg/kg)
		$hS1P_1$	$hS1P_3$	po 4 h
6f		0.60	22,000	>0.3[c]
6g		0.66	20,800	0.19
6h		20	14,000	N.T.
6i		20	>30,000	N.T.

[a,b] See Table 1.
[c] Decreased by 20% at 0.3 mg/kg.

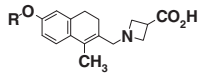
over $S1P_3$ activity than **6c**. Additionally, **6k** with the *S*-configuration showed twofold better $S1P_1$ activity, selectivity over $S1P_3$ and in vivo PLL activity than **6j** with the *R*-configuration.

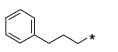
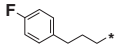
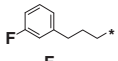
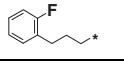
With an aim to further improve the activity and selectivity, we tested other substituents at the 2-position on the alkylene linker. Although the selectivity over $S1P_3$ was improved by introducing a more bulky substituent, the $S1P_1$ activity became weaker as the substituent became bulkier (**6k**, **6l** and **6m**).

Finally, another substituent at the *para*-position on the terminal phenyl ring was investigated. **6n** bearing a chlorine atom improved in vivo efficacy compared to **6k** by twofold without impairing selectivity over $S1P_3$ activity. **6n** showed efficacy at a dose of 0.095 mg/kg at 24 h after oral administration and 15,000-fold selectivity for $S1P_1$ over $S1P_3$ agonistic activity in the in vitro assay (Table 3).

We examined the pharmacokinetic profiles of representative compounds **6c**, **6k** and **6n** in rats. These three compounds showed good oral exposure and long half life. The results are summarized in Table 4 (the pharmacokinetic profile of compound **1** was also reported in a previous paper[7b]). Compared to compound **1**, **6c** and **6n** showed longer half-lives ($T_{1/2}$) and higher plasma concentrations at 24 h after oral dosing. This suggests that introducing a fluorine or chlorine atom on the terminal phenyl ring may be beneficial for the prevention of oxidative metabolism resulting in more potent in vivo PLL activity.

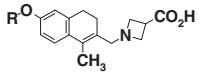
Table 1
The influence of the substitution of a fluorine atom on the terminal phenyl ring on $S1P_1$, $S1P_3$ agonistic activities and in vivo efficacy

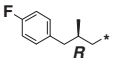
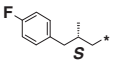
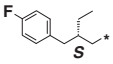
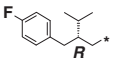
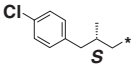


Compound	R	Ca assay[a] EC_{50} (nM)		Mouse PLL[b] ED_{50} (mg/kg)	
		$hS1P_1$	$hS1P_3$	po 4 h	po 24 h
6a		3.0	5000	0.40	N.T.
6c		0.84	3200	0.055	0.25
6d		4.0	3900	0.15	0.69
6e		2.7	2900	0.13	0.75

[a] Agonistic activity was evaluated by measuring intracellular Ca^{2+} concentration stimulation in Chinese Hamster Ovary (CHO) cells stably expressing human $S1P_1$ or $S1P_3$ receptors respectively.
[b] Peripheral Lymphocyte Lowering; Individual data points for dose-titrations were the average percentage decrease of peripheral blood lymphocyte counts in $n = 5$ animals versus control ($n = 5$) 4 h or 24 h after oral administration of the test compound (N.T. = not tested).

Table 3
The influence of the absolute configuration of a methyl group and steric hindrance at the 2-position on the alkylene linker and substituents at the *para*-position on the terminal phenyl ring on $S1P_1$, $S1P_3$ agonistic activities and in vivo efficacy



Compound	R	Ca assay[a] EC_{50} (nM)		Mouse PLL[b] ED_{50} (mg/kg)
		$hS1P_1$	$hS1P_3$	po 24 h
6j		0.85[d]	11,000[d]	0.55
6k		0.37[d]	9450[d]	0.22
6l		1.1	29,300	0.77
6m		4.5	>30,000	0.68
6n		0.55	8200	0.095

[a,b] See Table 1.
[d] Average of two assays.

Table 4
Pharmacokinetic profiles of **1**, **6c**, **6k** and **6n** in rat[a] (1 mg/kg)

Compound		AUC_{inf} (μg h/mL)	Plasma concentration (ng/mL)		CL_{tot} (mL/min/kg)	$T_{1/2}$ (h)	V_{ss} (L/kg)	BA (%)
			C_{max}[b]	24 h				
1	iv	4.4 (±1.2)	—	—	4.0 (±1.0)	11 (±4)	2.9 (±0.46)	85
	po	3.8 (±0.30)	181 (±33)	53 (±11)	—	17 (±9)	—	
6c	iv	5.4 (±0.81)	—	—	3.2 (±0.5)	21 (±2)	5.3 (±0.49)	106
	po	5.7 (±1.7)	159 (±79)	88 (±34)	—	24 (±9)	—	
6k	iv	2.6[c] (±0.33)	—	—	4.0 (±0.6)	19 (±2)	5.7 (±0.51)	75[c]
	po	2.0[c] (±0.22)	103 (±5)	69 (±19)	—	57 (±51)	—	
6n	iv	9.0 (±2.5)	—	—	2.0 (±0.6)	27 (±6)	3.9 (±0.48)	62
	po	5.5 (±1.1)	184 (±46)	87 (±33)	—	20 (±1)	—	

[a] Values are means of three experiments, standard deviation is given in parentheses.
[b] T_{max} for these four compounds are between 4.7 and 6.0 h.
[c] The data until 24 h was adopted.

In summary, we explored detailed structure–activity relationships (SAR) of substitutions on the alkylene linker and/or on the terminal phenyl ring on the activity of dihydronaphthalene derivatives with 3-phenylpropyloxy group at 6-position. These efforts identified the novel $S1P_1$ agonist **6n**, which showed sub-nanomolar $hS1P_1$ agonistic activity with excellent selectivity over $hS1P_3$ (15,000-fold), potent in vivo PLL activity in mice (ED_{50} = 0.095 mg/kg at po 24 h) and a good pharmacokinetic profile. This study also unveiled that the introduction of a substituent at the 2-position on the propylene linker is highly beneficial for the improvement of selectivity over $S1P_3$. This information should be useful for the consideration of the binding mode of these compounds with the $S1P_1$ or $S1P_3$ receptor.

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