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Structure–activity relationship studies of sphingosine-1-phosphate receptor agonists with *N*-cinnamyl-β-alanine moiety

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ABSTRACT

Structure–activity relationship of sphingosine-1-phosphate receptor agonist was examined. In terms of reducing the flexibility of molecule, hit compound **1** was modified to improve $S1P_1$ agonistic activity as well as selectivity over $S1P_3$ agonistic activity. Novel S1P agonists with cinnamyl scaffold or 1,2,5,6-tetrahydropyridine scaffold were identified.

Sphingosine-1-phosphate (S1P) exerts a variety of biological activities, including vascular maturation and cell survival.[1] Five S1P receptor subtypes have been known as $S1P_{1-5}$, respectively. One significant achievement in S1P research field was published in 2002. Lynch and co-workers reported that FTY720 (Fingolimod, Fig. 1), which was developed as a novel immunomodulator[2] and was recently approved in Russia and USA 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 secondary lymph tissue compartment.[3] It is also reported that $S1P_1$ is essential for lymphocyte recirculation since $S1P_1$ modulates egress from thymus and peripheral lymphoid organs.[4]

It was reported that $S1P_3$ agonism does not relate to lymphocyte recirculation but links to bradycardia in rodents.[5] Asymptomatic bradycardia was also reported in clinical studies of FTY720.[6] Herein, we report our result to identify orally available $S1P_1$ agonists without $S1P_3$ agonism.

High Throughput Screening (HTS) campaign of our historical compound library yielded a hit compound **1**, which showed a submicromolar human $S1P_1$ agonistic activity with human $S1P_3$ and human $S1P_5$ agonistic activities. Additionally, oral administration of **1** in mice could induce peripheral lymphocyte lowering (PLL) with 50% effective dose (ED_{50}) of 26 mg/kg at 4 h after oral dosing (Fig. 2). Therefore, we decided to investigate the structure–activity relationship around **1** to improve $S1P_1$ agonistic activity and selectivity against $S1P_3$.

Compounds in this report were synthesized in Schemes 1–3 as shown below. Compound **1** was prepared in 21% yield and compounds **9a–9c** were synthesized by using a solid-phase method[7] from 4-(3-aminopropyl)phenol **2**. Starting from compound **2**, *N*-Boc-β-alanine derivative **5** was prepared in 56% yield. 2-Chlorotrityl resin was reacted with **5** and treated with the corresponding alcohol in the presence of tri-*n*-butylphosphine and *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD). The treatment with the acetic acid and 2,2,2-trifluoroethanol yielded Boc-protected β-alanine **8a–8c**, which was treated with HCl/EtOAc to give **9a**–**9c** (Scheme 1).

Cinnamyl derivatives **12a–12d** were synthesized by reductive alkylation of β-alanine with aldehydes[8] **11a–11d** in acceptable yields. Cinnamyl aldehyde **11a** was derived by reduction of the corresponding ester by $LiAlH_4$ in presence of Et_2NH[9] in 17% yield. (4-Hydroxy)-acetophenone **13c** was treated with diethyl (cyanomethyl)-phosphonate and the resulting α,β-unsaturated nitrile was converted to the corresponding aldehyde, which was treated with alkyl bromide and each regioiosomer generated under the reaction condition could be separated by column chromatography. The

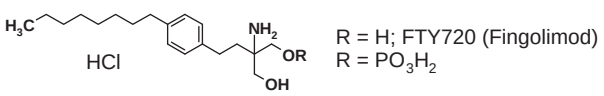

Figure 1. Structure of FTY720 (Fingolimod).

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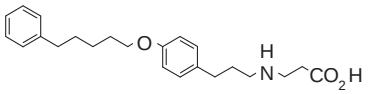
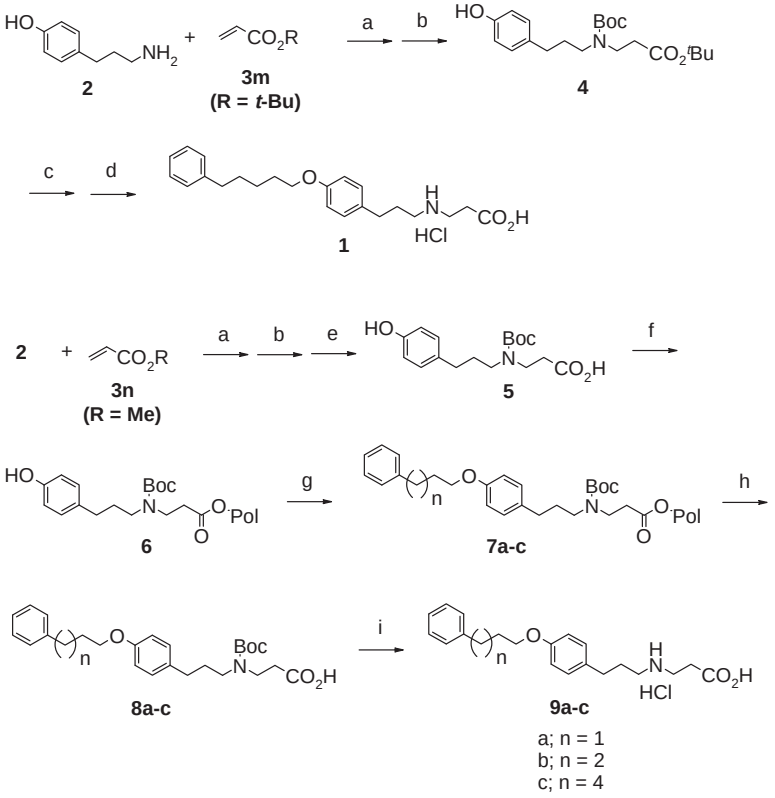


Hit compound 1

Ca Assay EC_{50} (uM)				Mouse PLL ED_{50} (mg/kg)
$hS1P_1$	$hS1P_2$	$hS1P_3$	$hS1P_5$	*p.o.* 4 hr
0.16	>10.0	4.2	0.17	26

Figure 2. Structure and pharmacological profiles of hit compound **1**.



Scheme 1. Reagent: (a) MeOH; (b) Boc_2O, THF; (c) $Ph(CH_2)_5Br$, K_2CO_3, DMF; (d) HCl/1,4-dioxane; (e) NaOH-aq, MeOH, THF; (f) Pol-Cl (Pol = 2-chlorotrityl resin), *i*-Pr_2NEt, CH_2Cl_2; (g) $Ph–(CH_2)_n–CH_2CH_2OH$, *n*-Bu_3P, TMAD, CH_2Cl_2, THF; (h) CF_3CH_2OH, AcOH, CH_2Cl_2; (i) HCl/EtOAc.

separated *trans*- and *cis*-α,β-unsaturated aldehydes **11c** and **11d** were reacted with β-alanine to yield **12c** and **12d**, respectively. (Scheme 2).

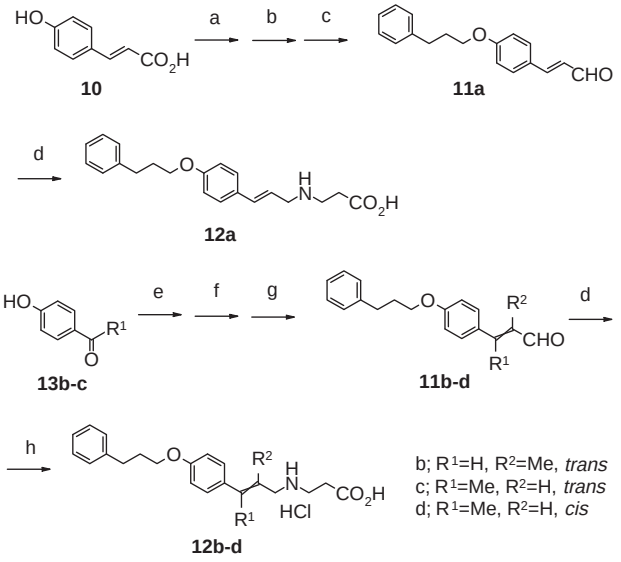
Tetrahydropyridine derivative **18** and piperidine derivative **19** were prepared via tetrahydro pyridine derivative **17**, which was obtained from the corresponding piperidone **15**. Piperidone **15** was reacted with lithiated phenylbromide **14** and then converted to tetrahydro pyridine **17** by HCl deprotection of Boc group followed by dehydration and Michael addition to *tert*-butyl acrylate in 52% yield. Tetrahydropyridine derivative **17** was converted to **18** by the treatment with trifluoroacetic acid (TFA) in 82% yield, and **19** by hydrogenation and subsequent treatment with TFA in 52% yield (Scheme 3).

Piperidine derivative **24** was prepared from 3-hydroxypiperidine **20**. Piperidine derivative **20** was alkylated and oxidised to give **21** in 77% yield. On the other hand, phosphonium salt **23** was obtained from benzyl alcohol **22** in 94% yield. Phosphonium salt **23** was treated with dimsyl anion to afford a phosphonium ylide and reacted with ketone **21** to yield the condensed product in regioisomeric mixture, which was deprotected by HCl and then separated by column chromatography to give the *trans* isomer **24** (Scheme 3).

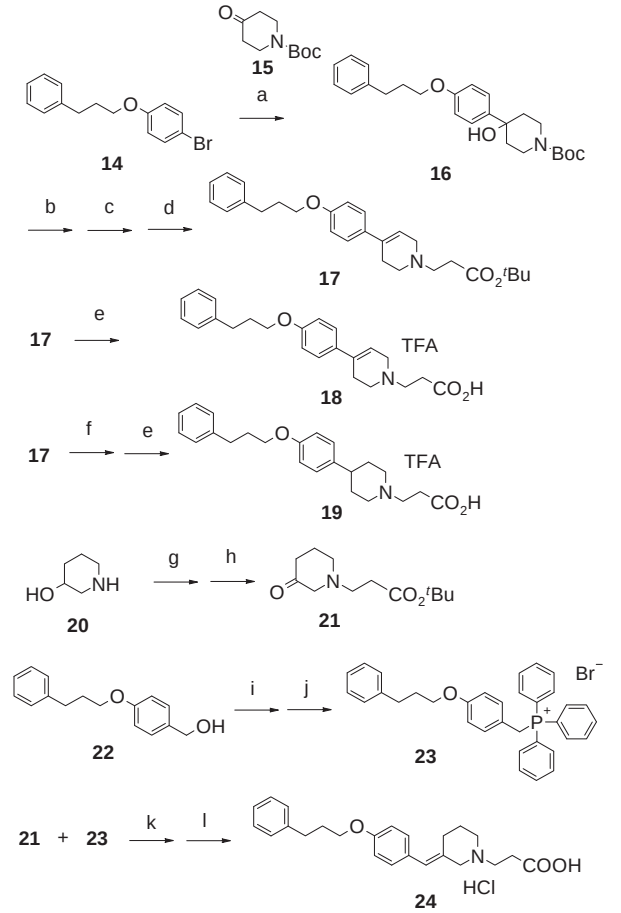
The effect of the length of alkylene linker between terminal phenyl and internal phenyl ring of hit compound **1** on its in vitro activity and oral exposure level was investigated. Although compound **1** showed the highest activity against $S1P_1$, shorter analogues **9a** and **9b** as well as longer analogue **9c** retained the $S1P_1$ agonistic activity. On the other hand, compound **9a** showed higher oral exposure level in rat than compound **1** (Table 1), which supports that the restriction of conformational flexibility improves the oral absorption.[10] We decided to use propylene linker for the further SAR studies.

Next we modified the propylene linker between the internal phenyl ring and nitrogen (NH) in compound **9a** by introducing a double bond. Cinnamylamino compound **12a** significantly improved not only $S1P_1$ agonist activity but also selectivity over $S1P_3$. Hale et al. reported the $S1P_1$ selective agonists with benzyl amine moiety at the γ-position of carboxylic acid.[11] By combining this information, it is supposed that the π-electron density at this position might be important for $S1P_1$ agonist activity (Table 2).

Introducing a methyl group on the double bond of the allyl group of **12a** was examined. Whereas compound **12b** with methyl group at the β-position of amine showed comparable $S1P_1$ agonistic activity and selectivity over $S1P_3$, compound **12c** with methyl group at the γ-position improved both $S1P_1$ activity and selectivity. Additionally, the activity to reduce the peripheral blood lymphocyte count was enhanced significantly. Compound **12d** with *cis*-double bond showed less $S1P_1$ agonistic activity than

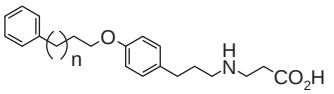


Scheme 2. Reagent: (a) $SOCl_2$, MeOH; (b) $Ph(CH_2)_3OH$, Ph_3P, DEAD, THF; (c) $LiAlH_4$, Et_2NH, *n*-hexane; (d) (i) $H_2NCH_2CH_2CO_2H$, NaOH, $HC(OMe)_3$, MeOH, THF; (ii) $NaBH_4$; (e) $(EtO)_2P(O)CHR^2CN$ (b: R^2 = Me, c: R^2 = H), NaH, THF; (f) DIBAH, THF; (g) (i) $Ph(CH_2)_3Br$, K_2CO_3, DMF; (ii) separation of regioisomer **11c** and **11d**; (h) HCl-aq/THF.



Scheme 3. Reagent: (a) *n*-BuLi, THF; (b) HCl/1,4-dioxane, CH_2Cl_2; (c) Et_3SiH, TFA, CH_2Cl_2; (d) $CH_2{=}CHCO_2Bu^t$, iPr_2NEt, DMF; (e) TFA, CH_2Cl_2; (f) H_2, 10% Pd-C, EtOAc; (g) $CH_2{=}CHCO_2{}^tBu$, MeOH; (h) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (i) CBr_4, Ph_3P, CH_2Cl_2; (j) Ph_3P, PhMe; (k) NaH, DMSO; (l) (i) HCl/EtOAc, (ii) separation of regioisomers.

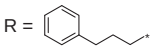
Table 1
The influence of length of linker towards $S1P_1$ and $S1P_3$ agonistic activities and oral exposure levels in rat

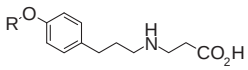
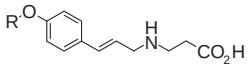


Compound	*n*	Ca assay[a] EC_{50} (μM)		Pharmacokinetic properties[b] in rat (30 mg/kg p.o.)		
		$hS1P_1$	$hS1P_3$	AUC_{inf} μg h/mL	C_{max} μg/mL	$T_{1/2}$ h
9a	1	2.6	9.0	174 (±22)	28 (±6.4)	3.8 (±0.4)
9b	2	1.5	>10	N.T.	N.T.	N.T.
1	3	0.16	4.2	11 (±1.1)	2.1 (±0.23)	3.8 (±0.1)
9c	4	2.7	>10	N.T.	N.T.	N.T.

[a] Agonistic activity was evaluated by the measurement of intracellular calcium concentration stimulated by the addition of each compound for human $S1P_1$ or human $S1P_3$ receptors stably expressed in Chinese Hamster Ovary (CHO) cell, respectively.
[b] Values are means of three experiments, standard deviation is given in parentheses (N.T. = not tested).

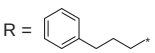
Table 2
Replacement of propylene linker to allyl linker

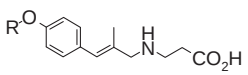
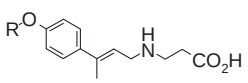
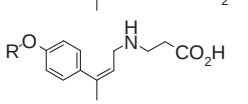


Compound	Structure	Ca assay[a] EC_{50} (μM)		Mouse PLL[b] ED_{50} (mg/kg)
		$hS1P_1$	$hS1P_3$	p.o. 4 h
9a		2.6	9.0	N.T.
12a		0.070	3.3	4.6

[a] See Table 1.
[b] Individual data points for dose–titrations were the average percentage decrease of peripheral blood lymphopocyte counts in $n = 5$ animals versus control ($n = 5$) 4 h after oral administration of the test compound (N.T. = not tested).

Table 3
Addition of a methyl group on double bond



Compound	Structure	Ca assay[a] EC_{50} (μM)		Mouse PLL[b] ED_{50} (mg/kg)
		$hS1P_1$	$hS1P_3$	p.o. 4 h
12b		0.033	1.9	N.T.
12c		0.021	>10.0	0.69
12d		0.93	>10.0	N.T.

[a,b] See Tables 1 and 2.

trans-congener **12c** (Table 3), which indicates the *trans* configuration might be beneficial to assist the basic nitrogen for interacting with $S1P_1$ receptor. This unexpected result prompted us to investigate further active conformations of compound **12a**.

Compound **12a** should have two distinct conformations around the allyl amine linker: extended conformer A and folded conformer

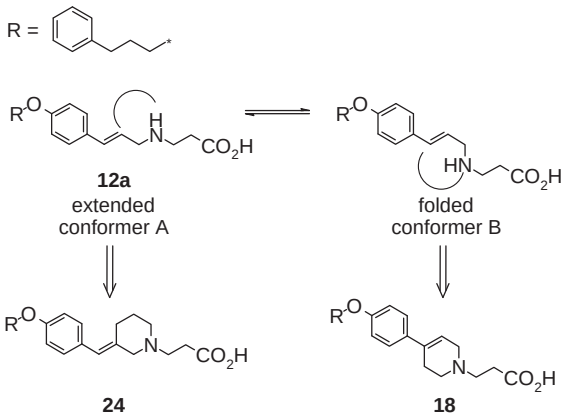
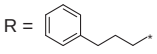
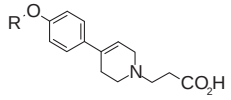
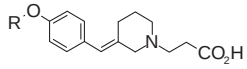
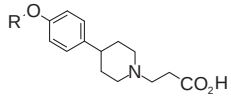


Figure 3. Conformational relationship between compounds **18** and **24**.

Table 4
Cyclic derivatives of compound **12a**



Compound	Structure	Ca assay[a] EC_{50} (μM)		Mouse PLL[b] ED_{50} (mg/kg)
		$hS1P_1$	$hS1P_3$	p.o. 4 h
18		0.045	1.2	1.4
24		1.5	>10.0	N.T.
19		1.6	>10.0	N.T.

[a,b] See Tables 1 and 2.

B (Fig. 3). Compounds **24** and **18** were designed to fix the extended conformation A and folded conformer B, respectively.

As a result, compound **18** showed much stronger $S1P_1$ activity than compound **24** (Table 4). This data strongly suggests that the folded conformer B would be an active conformation of compound **12a**. Additionally, the activity of compound **19**, having a single bond instead of double bond in the piperidine ring compared to compound **18**, was weaker than compound **18**, which indicates again that the π-electron density at this position might be important to activate $S1P_1$ receptor.

In summary, we explored SAR of hit compound **1** to identify a cinnamyl derivative **12a** with improved in vitro $S1P_1$ agonist activity as well as selectivity over $S1P_3$. Further modification of compound **12a** led to compound **12c** with strong $S1P_1$ agonism and selectivity over $S1P_3$. Compound **12c** also showed superior pharmacokinetic property with long half-time ($T_{1/2}$ = 7.4 h) in rat (data not shown). During the SAR study, the importance of π-electron density between the central benzene ring and nitrogen atom was also determined. Furthermore, we conclude that the active conformation of compound **12a** would be the folded conformer **18**. Further optimisation of compounds **12c** and **18** will be reported in due course.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.029. These data include MOL files and InChiKeys of the most important compounds described in this article.

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