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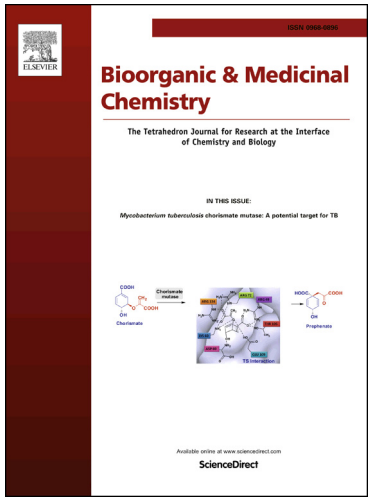

Identification of selective inhibitors of sphingosine kinases 1 and 2 through a structure–activity relationship study of 4-*epi*-jaspine B

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Identification of selective inhibitors of sphingosine kinases 1 and 2 through a structure–activity relationship study of 4-*epi*-jaspine B

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ABSTRACT

We recently reported that 4-*epi*-jaspine B exhibits potent inhibitory activity towards sphingosine kinases (SphKs). In this study, we investigated the effects of modifying the 2-alkyl group, as well as the functional groups on the THF ring of 4-*epi*-jaspine B using a diversity-oriented synthesis approach based on a late-stage cross metathesis reaction. The introduction of a *p*-phenylene tether to the alkyl group was favored in most cases, whereas the replacement of a carbon atom with an oxygen atom led to a decrease in the inhibitory activity. Furthermore, the introduction of a bulky alkyl group at the terminus led to a slight increase in the inhibitory activity of this series towards SphKs compared with 4-*epi*-jaspine B (the *Q* values of compound **13** for SphK1 and SphK2 were 0.2 and 0.4, respectively). Based on this study, we identified two isoform selective inhibitors, including the *m*-phenylene derivative **4** [IC_{50} (SphK1) = >30 μM; IC_{50} (SphK2) = 2.2 μM] and the methyl ether derivative **22** [IC_{50} (SphK1) = 4.0 μM; IC_{50} (SphK2) = >30 μM].

1. Introduction

Sphingosine kinases (SphKs) catalyze the conversion of sphingosine (Sph) to sphingosine 1-phosphate (S1P), which plays an important role in the regulation of numerous biological processes, including cell growth, proliferation, survival, cell death and migration.[1–3] SphKs exist in two isoforms in mammals, including SphK1 and SphK2. SphK1 is the smaller of these two proteins, and lacks the N-terminal nuclear localization sequence that is found in SphK2.[4] SphK1 is located primarily in the cytosol and overexpressed in many tumor tissues.[5-7] The S1P generated by SphK1 interacts with G-protein coupled sphingosine-1-phosphate receptors (S1P1–5) to elicit prosurvival and proliferative effects.[8] The knockdown of SphK1 leads to a reduction in the plasma levels of S1P, as well as a significant increase in cellular ceramides.[9] SphK2 in mainly located in the nucleus, but can also be found in several other organelles, including the endoplasmic reticulum and mitochondria. Notably, the expression of SphK2 has been linked to an increase in gene expression[10] and apoptosis.[11] Furthermore, it has been reported that the siRNA knockdown of SphK2 expression inhibited the proliferation of glioblastoma cells more effectively than the knockdown of SphK1.[12] S1P signaling is also involved in numerous diseases, such as cancer,[13,14] asthma,[15,16] atherosclerosis,[17] inflammation,[18] fibrosis[19] and sickle cell disease.[20,21] The development of effective SphK inhibitors, especially isoform-selective compounds, has therefore emerged as an important area of research in drug discovery.[2,22,23]

Several different types of SphK inhibitors have been developed during the last decade.[3] *N*,*N*-Dimethylsphingosine (DMS)[24] and SKI-II[25] inhibit both isoforms of the SphKs (Fig. 1).[26] PF-543,[27] oxadiazole **A**[28] and CB5468139[26] are potent SphK1 inhibitors that are more than 50-fold selective for this particular isoform over SphK2. In contrast, there have been very few reports to date pertaining to the development of SphK2-selective inhibitors, and the activities and/or selectivities of those inhibitors that have been reported are not high. For example, ABC294640,[29] which is currently in phase II clinical trials for the treatment of pancreatic cancer and solid tumors, exhibits moderate inhibitory activity and selectivity towards SphK2 [IC_{50} (SphK1) = >100 μM; IC_{50} (SphK2) = ~60 μM]. Several other SphK2 selective inhibitors have been reported, including K145,[30] (*R*)-FTY720-OMe[31] and SLP120701,[32] although these compounds typically only demonstrate 2- to 10-fold selectivity for SphK2 over SphK1. The difficulties associated with

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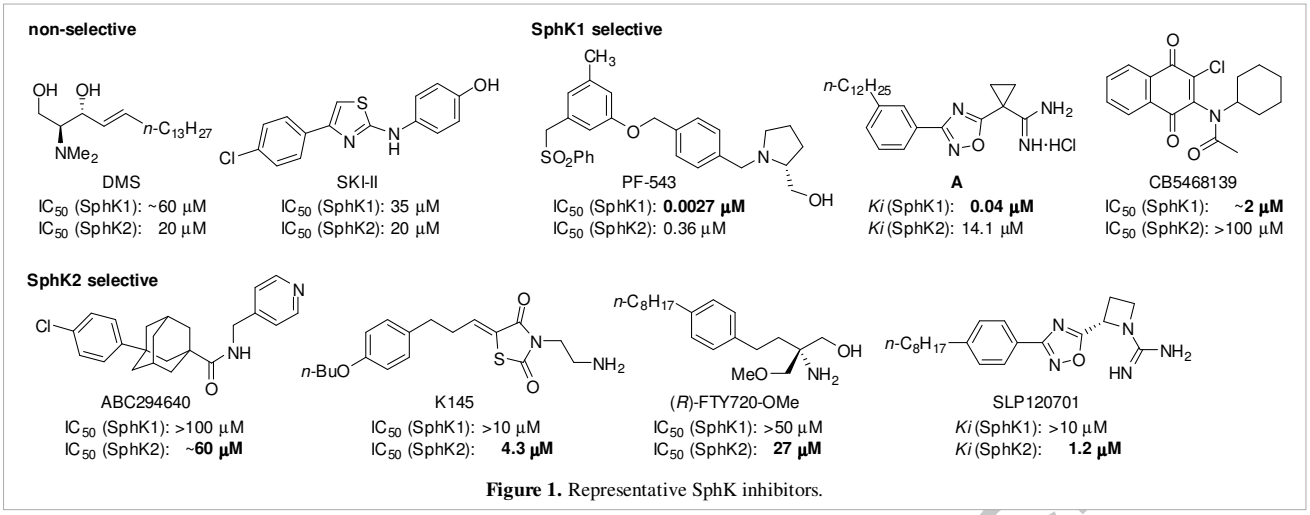


Figure 1. Representative SphK inhibitors.

development of highly potent SphK2-selective inhibitors can be attributed in part to the lack of structural information for SphK2, although homology models of this protein were reported in 2013[33] and 2014[34] based on the X-ray crystal structure of human SphK1.

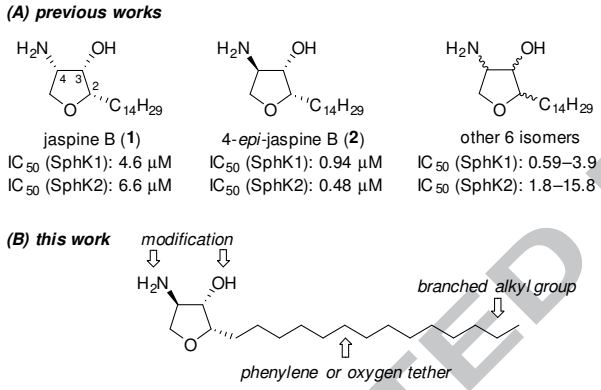


Figure 2. Our works on SphK inhibitory activities of jaspine B derivatives.

Our group has an ongoing interest in the development of SphK inhibitors based on the structure of the naturally occurring anhydrophytosphingosine derivative jaspine B (**1**) (Fig. 2A).[35-37] In terms of its main structural features, this compound consists of a functionalized THF moiety (polar head group) that is attached to an alkyl side chain (lipophilic tail).[38] Several synthetic[39-43] and structure–activity relationship studies[44-46] have been conducted around jaspine B and its derivatives. However, most of these studies have focused on the polar head group and very little is known about the impact of making changes to the lipophilic tail.[37,47,48] In our previous study, we synthesized all of the possible stereoisomers of jaspine B and evaluated their biological activities. The results revealed that all of the isomers exhibited inhibitory activities toward SphKs and that 4-*epi*-jaspine B (**2**) exhibited the most potent activities of all of the isomers tested towards SphKs 1 and 2.[36] The optimum length of the linear alkyl chain in epimer **2** for SphK inhibition was determined to be C14, which is the same length as the chain in natural jaspine B.[37] However, we did not investigate the impact of modifications of the alkyl side chain or the functional groups on the THF moiety. Herein, we report the results of our recent structure–activity relationship study on the alkyl side chain of jaspine B (*i.e.*, the introduction of a phenylene/heteroatom tether and bulky alkyl groups), as well as the effect of modifying the hydroxy and amino groups on the THF ring (Fig. 2B). Notably, this work led to the identification of several isoform selective inhibitors towards SphK1 and SphK2.

2. Results and discussion

2.1. Design and synthesis

We designed a new series of jaspine B derivatives **3**–**22** (Fig. 3) based on the SAR information obtained during our previous studies, as well as the structures of the known inhibitors shown in Fig. 1. Consideration of the structures of compound **A**, (*R*)-FTY720-OMe (and its parent compound FTY720) and SLP120701 suggested that the inclusion of a phenylene tether was tolerated, having no significant impact on the interaction between these inhibitors and the SphKs. These results also indicated that the positioning of the substituents on the phenyl ring could affect the isoform selectivity. With this in mind, we prepared a series of derivatives **3**–**11** containing a phenylene tether bearing an alkyl group (R) at the *o*-, *m*- or *p*-position. We reasoned that the alkyl chain length of the R group should be C8 or C9, given that the benzene ring would correspond to a C3 or C4 alkyl group. Bulky-alkyl derivatives **12**–**16** were designed to determine whether the introduction of a branched alkyl group would lead to an increase in the lipophilicity of the side chain without significantly affecting the chain length. The oxygen atom introduced in the alkyl chain of **12**–**16** was for ease of synthesis. The two ether derivatives **17** and **18** were also designed to assess the impact of incorporating an oxygen atom in the alkyl chain. Notably, the alkyl chain in these two analogs was the same length as that of jaspine B. The methoxy and guanidino groups in (*R*)-FTY720-OMe and SLP120701, respectively, prompted us to design derivatives **19**–**22** bearing guanidino, amidino, acetamido and methoxy moieties as the polar head groups, respectively.

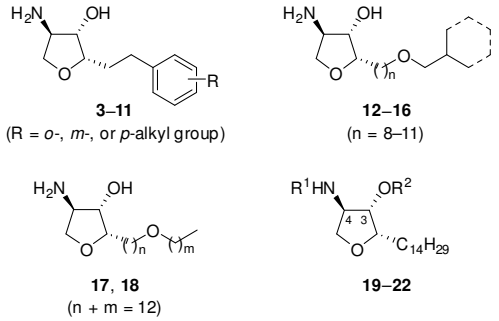
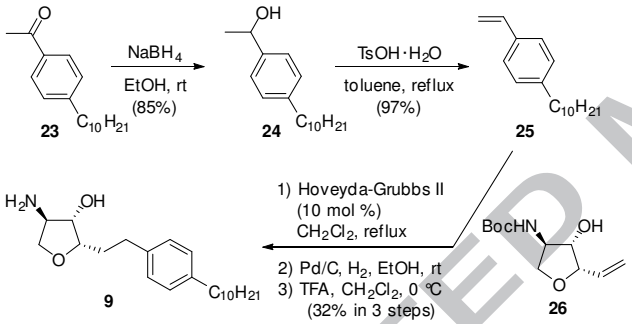


Figure 3. Design of SphK inhibitors **3**–**22**.

Compounds **3**–**22** were prepared according to our diversity-oriented synthesis of jaspine B derivatives, involving the use of a late-stage cross metathesis reaction.[37,49-51] A representative example of this process is shown in Scheme 1. The reduction of the known ketone **23** with $NaBH_4$, followed by the dehydration of the resulting alcohol with TsOH gave alkene **25**.[52] The subsequent cross metathesis of **25** with the known tetrahydrofuran derivative **26** using the Hoveyda–Grubbs second generation catalyst, followed by the hydrogenation of the resulting C=C double bond and the deprotection of the Boc group gave the desired phenylene derivative **9**. All of the other 4-*epi*-jaspine B derivatives in this series were readily prepared in a similar manner (see Supplementary material).

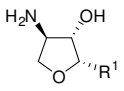


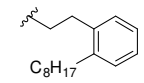
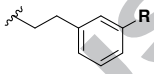
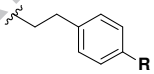
Scheme 1. Synthesis of 4-*epi*-jaspine B derivative **9**.

2.2. SphK inhibitory activity

The in vitro inhibitory activities of all of the 4-*epi*-jaspine B derivatives prepared in the current study were evaluated towards SphK1 and SphK2. The results for derivatives **3**–**11** bearing a phenylene tether are shown in Table 1. The *o*-substituted derivative **3** exhibited no inhibitory activity towards SphK1 or SphK2 at 30 μM. In contrast, the *m*-substituted derivatives **4**–**6** exhibited potent inhibitory activities toward SphK2 (IC_{50} = 1.7–2.2 μM), although their activities toward SphK1 were generally weak (>12 μM) and appeared to be dependent on the chain length of the R group. It is noteworthy that the heptyl derivative **4** (R = *n*-C_7H_{15}) exhibited 14-fold selectivity for SphK2 over SphK1. Of all the *p*-phenylene tether-containing derivatives prepared in the current study (**7**–**11**), compounds **8**–**10** bearing an alkyl group of the appropriate chain length exhibited potent inhibitory activities, especially towards SphK1 (0.42–1.4 μM). Furthermore, a slight improvement over the activity of 4-*epi*-jaspine B (**2**) was observed in some cases [*Q* (SphK1) for **9** = 0.38; *Q* (SphK2) for **8** = 0.76]. Interestingly, the inhibitory activities of **8**–**10** towards SphK2 (0.56–21 μM) were more sensitive to the alkyl chain length of the R group than their activities towards SphK1 (0.42–1.4 μM), which contrasted with the results observed for the *m*-phenylene derivatives. As anticipated, the use of too short or too long an alkyl chain, as exemplified by compounds **7** and **11**, respectively, resulted in a decrease in the inhibitory activity.

Table 1
SphK inhibitory activities of the phenylene tether derivatives **3–11**.

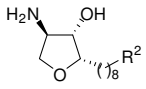


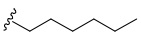
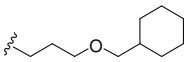
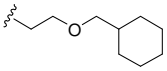
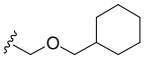
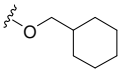
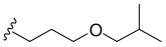
Compd	R^1	IC_{50} (μM)[a]		Q[b]	
		SphK1	SphK2	SphK1	SphK2
2	$C_{14}H_{29}$	0.82–1.1	0.60–0.74	1.0	1.0
3		>30	>30	–	–
4	 (**R** = *n*-C_7H_{15})	**>30**	**2.2**	**–**	**3.7**
5	(**R** = *n*-C_8H_{17})	16	2.1	20	2.8
6	(**R** = *n*-C_9H_{19})	12	1.7	11	2.8
7	 (**R** = *n*-C_6H_{13})	>30	8.3	–	14
8	(**R** = *n*-C_8H_{17})	1.4	0.56	1.7	0.76
9	(**R** = *n*-$C_{10}H_{21}$)	0.42	0.67	0.38	1.1
10	(**R** = *n*-$C_{12}H_{25}$)	0.88	21	0.80	35
11	(**R** = *n*-$C_{14}H_{29}$)	>30	>30	–	–

[a] IC_{50} values (i.e., the concentration required to inhibit the phosphorylation of sphingosine by SphK1 or SphK2 by 50%). [b] The *Q* values were calculated as follows: *Q* = IC_{50}(compound)/IC_{50}(4-*epi*-jaspine B).

The SphK inhibitory activities of the bulky alkyl derivatives **12**–**16** are shown in Table 2. Pleasingly, we found that the introduction of a cyclohexyl group bearing an oxygen atom-containing tether generally favored SphK inhibition. For example, we observed slight improvement in the inhibitory activities of compounds **13** [*Q* (SphK1) = 0.20; *Q* (SphK2) = 0.40] and **14** [*Q* (SphK1) = 0.61; *Q* (SphK2) = 0.76] compared with 4-*epi*-jaspine. However, the same tendency was not observed for compound **15** bearing a shorter alkyl chain (*Q* = >2.2 for both the isoforms). A comparison of the cyclohexyl analog **12** (IC_{50} = 0.38–0.78 μM) with the isopropyl analog **16** (IC_{50} = 0.71–0.84 μM) revealed that the inclusion of an isopropyl group as the terminal moiety was well tolerated.

Table 2
SphK inhibitory activities of the bulky-alkyl derivatives **12–16**

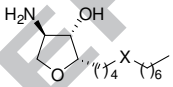


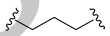
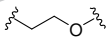
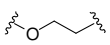
Compd	R^2	IC_{50} (μM)[a]		Q[b]	
		SphK1	SphK2	SphK1	SphK2
2		0.82–1.1	0.60–0.74	1.0	1.0
12		0.38	0.78	0.46	1.1
13		0.22	0.24	0.20	0.40
14		0.67	0.46	0.61	0.76
15		2.8	1.3	2.5	2.2
16		0.71	0.84	0.65	1.4

[a] IC_{50} values (i.e., the concentration required to inhibit the phosphorylation of sphingosine by SphK1 or SphK2 by 50%). [b] The Q values were calculated as $Q = IC_{50}$(compound)/IC_{50}(4-*epi*-jaspine B).

The introduction of an oxygen tether had an adverse effect on inhibitory activities of these compounds (Table 3). For example, the ether derivative **18** did not exhibit any inhibitory activity at 30 μM. In contrast, the introduction of an oxygen atom at a position further away from the polar head was better tolerated, although a considerable decrease was observed in the inhibitory activity (e.g., **17**, Q = 6.5–8.7).

Table 3
SphK inhibitory activities of heteroatom tether derivatives

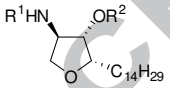


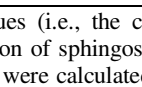
Compd	X	IC_{50} (μM)[a]		Q[b]	
		SphK1	SphK2	SphK1	SphK2
2		1.1–2.2	0.60–1.1	1.0	1.0
17		9.6	3.9	8.7	6.5
18		>30	>30	–	–

[a] IC_{50} values (i.e., the concentration required to inhibit the phosphorylation of sphingosine by SphK1 or SphK2 by 50%). [b] The Q values were calculated as $Q = IC_{50}$(compound)/IC_{50}(4-*epi*-jaspine B).

We also investigated the effect of modifying the amino and hydroxy groups on the THF ring (Table 4). Disappointingly, the introduction of a guanidino or amidino group (**19** and **20**, respectively); changes inspired by the substructures of SLP120701 and oxadiazole **A**, did not lead to any improvements in the inhibitory activity [Q (SphK1) = 1.0–2.8; Q (SphK2) = 3.2–16]. The acetylation of the amino group (**21**) resulted in a significant drop in the inhibitory activity towards both isoforms (IC_{50} = >30 μM). Given that the degree of steric hindrance observed in the modified amino group analogs **19**–**21** was almost identical, these results highlighted the importance of the cationic functional group in 4-*epi*-jaspine B (**2**). Pleasingly, methyl ether **22**, which was designed to mimics (*R*)-FTY720-OMe, inhibited the activity of SphK1 (IC_{50} = 4.0 μM) much more effectively than that of SphK2 (IC_{50} = >30 μM).

Table 4
SphK inhibitory activities of amino or hydroxy-modified derivatives



Compd	NHR^1	OR^2	IC_{50} (μM)[a]		Q[b]	
			SphK1	SphK2	SphK1	SphK2
2	NH_2	OH	1.1–2.8	0.32–0.60	1.0	1.0
19		OH	1.1	1.9	1.0	3.2
20		OH	3.1	9.4	2.8	16
21	NHAc	OH	>30	>30	–	–
22	NH_2	OMe	**4.0**	**>30**	3.6	–

[a] IC_{50} values (i.e., the concentration required to inhibit the phosphorylation of sphingosine by SphK1 or SphK2 by 50%). [b] The Q values were calculated as $Q = IC_{50}$(compound)/IC_{50}(4-*epi*-jaspine B).

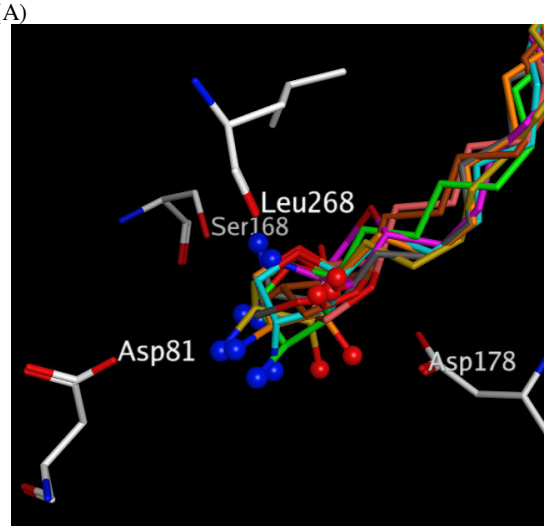
2.3. Docking mode analysis

To develop a deeper understanding of the SAR information and generate some new insights for further SAR studies, we investigated the binding modes of some jaspine B derivatives. Calculations were performed using MOE-Dock based on the crystal structures of SphK1 and the Sph complex (PDBID: 3VZB). It is noteworthy that we experienced some difficulty in quantifying these binding interactions because of the flexible nature of the alkyl side chain, which did not form any meaningful interactions. An RMSD value of 2.1 Å was obtained for the re-docking of Sph, highlighting the flexibility of these ligands.

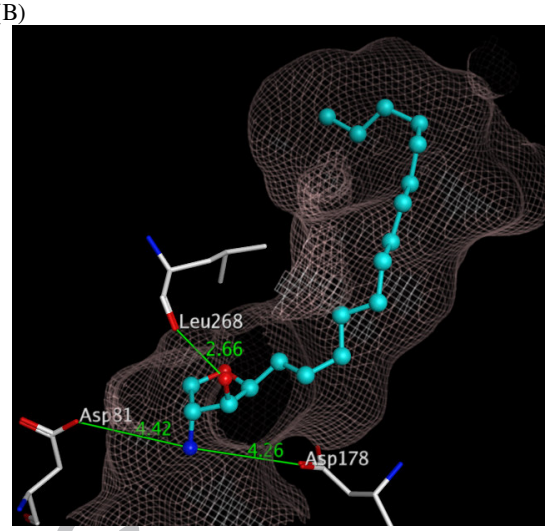
As shown in Fig. 4A, all the stereoisomers of jaspine B bound to the Sph binding site of SphK1 through several polar residues, including Asp81, Asp178, Leu268 and Ser168, regardless of the stereochemistries of the amino and hydroxy groups of the THF ring. The relatively high inhibitory activity of 4-*epi*-jaspine B (**2**) can be explained by the formation of favorable hydrophilic interactions without the need to undergo any conformational strain (i.e., the formation of a hydrogen bond from the hydroxy group to the carbonyl group of Leu268, and the formation of electrostatic interactions between the amino group and the carboxy groups of Asp178 and Asp81) (Fig. 4B). The corresponding interactions of jaspine B (**1**) are shown in Fig. 4C. The hydrophobic pocket of SphK1 contains additional space

around the terminal region of **2** (C8–C14). Notably, the docking simulation of the cyclohexyl analog **12** revealed the presence of a reasonable interaction, as shown in Fig. 4D. These calculations therefore provided a strong rationale for the slight improvements observed in the inhibitory activities of compounds bearing a branched alkyl group as their terminal moiety (Table 2). Taken together with their inhibitory activities, the results of this docking mode analysis suggest that further modifications could be made to the alkyl chain, as well as the functional groups on the THF to improve the isoform selectivity and inhibitory activity of these compounds.

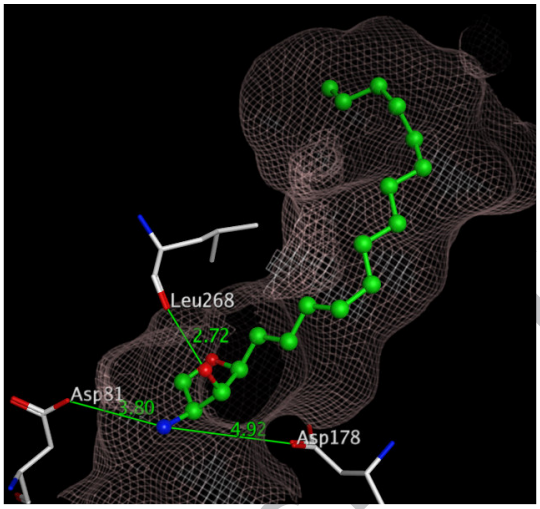
(A)



(B)



(C)



(D)

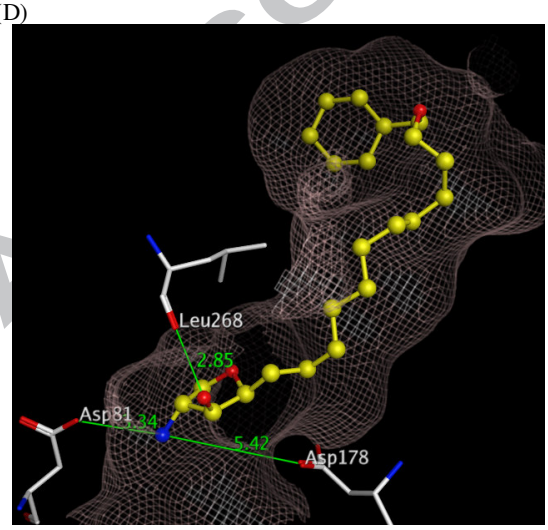


Figure 4. (A) Electrostatic interactions between 4-*epi*-jaspine B stereoisomers and SphK1. Each amino group and hydroxy group is shown in small sphere. (B) Predicted binding mode of 4-*epi*-jaspine B (**2**) (cyan). (C) Predicted binding mode of jaspine B (**1**) (green). (D) Predicted binding mode of **12** (yellow).

3. Conclusions

In this study, we investigated the effect of modifying the alkyl side chain, as well as the hydroxy and amino groups of 4-*epi*-jaspine B (**2**) on the inhibitory activity and isoform selectivity of this compound towards SphK1 and SphK2. The incorporation of a phenylene tether in the alkyl group resulted in the SphK2 selective inhibitor **4** with moderate activity and selectivity [IC_{50} (SphK1) = >30 μM; IC_{50} (SphK2) = 2.2 μM]. We also achieved a slight improvement in the inhibitory activity of this compound series following the introduction of a cyclohexyl group at the end of the alkyl chain, as exemplified by the *Q* values for **13**, which were 0.20 (SphK1) and 0.40 (SphK2). The methyl etherification of the hydroxy group in **2** led to the identification of **22**, which exhibited moderate selectivity [SphK1 (IC_{50} = 4.0 μM); SphK2 (IC_{50} = >30 μM)].

4. Experimental

General Methods. ^{1}H NMR spectra were recorded on a 500 MHz JEOL AL-500 spectrometer using $CDCl_3$ as a solvent. Chemical shifts (δ) were reported in ppm relative to Me_4Si, which was used as an internal reference standard. ^{13}C NMR spectra were recorded on a JEOL AL-500 spectrometer using $CDCl_3$ as a solvent. The signals were reported relative to the residual $CHCl_3$ signal. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. High resolution mass spectrometry (HRMS) analyses were conducted on a JMS-HX/HX 110A mass spectrometer or Shimadzu LC-ESI-IT-TOF-MS system. Purification by column chromatography was conducted using Wakogel C-300E (Wako), Chromatorex NH-DM1020 (Fuji Silysia) or Aluminum oxide 90 (Merck-Millipore). The purities of the compounds submitted for biological analysis were determined to be >95% by HPLC.

General procedure for the ketone reduction: synthesis of 1-(4-decylphenyl)ethan-1-ol (24). $NaBH_4$ (436 mg, 11.5 mmol) was added to a stirred solution of ketone **23** (2.50 g, 9.60 mmol) in EtOH (32 mL; 0.3 M) at 0 °C, and the resulting mixture was allowed to warm to room temperature with stirring for 2 h. The reaction was then cooled to 0 °C and quenched by the addition of a saturated aqueous solution of NH_4Cl. The solvent was removed under reduced pressure, and the resulting residue was dissolved in EtOAc. The resulting solution was washed sequentially with water and brine, before being dried over $MgSO_4$. The mixture was then evaporated to dryness under reduced pressure to give an oily residue, which was purified by flash column chromatography over silica gel eluting with *n*-hexane and EtOAc (10:1) to give **24** (2.14 g, 85% yield) as a colorless oil: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, *J* = 6.9 Hz, 3H), 1.22–1.34 (m, 14H), 1.49 (d, *J* = 6.3 Hz, 3H), 1.57–1.63 (m, 2H), 1.75 (d, *J* = 3.4 Hz, 1H), 2.59 (t, *J* = 7.7 Hz, 2H), 4.85–4.90 (m, 1H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.29 (d, *J* = 8.0 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 25.0, 29.3 (2C), 29.5, 29.58, 29.60, 31.5, 31.9, 35.6, 70.2, 125.3 (2C), 128.5 (2C), 142.2, 143.0; HRMS (FAB^+) calcd for $C_{18}H_{30}O$ [M^+], 262.2297; found, 262.2294.

General procedure for the acid-catalyzed dehydration of alcohols: synthesis of 1-decyl-4-vinylbenzene (25). $TsOH·H_2O$ (140 mg, 0.74 mmol) was added to a stirred solution of alcohol **24** (1.94 g, 7.39 mmol) in toluene (150 mL), and the resulting mixture was heated under reflux for 30 min. The mixture was then cooled to room temperature and basified by the addition of a saturated aqueous solution of $NaHCO_3$. The organic phase was separated and washed sequentially with water and brine, before being dried over $MgSO_4$. The solvent was removed under reduced pressure to give a residue, which was purified by flash column chromatography over silica gel eluting with *n*-hexane to give **25** (1.74 g, 97% yield) as a colorless oil. The ^{1}H and ^{13}C NMR spectra of **25** were consisted with those reported in the literature[52]: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, *J* = 6.9 Hz, 3H), 1.21–1.30 (m, 14H), 1.56–1.62 (m, 2H), 2.58 (t, *J* = 7.7 Hz, 2H), 5.18 (d, *J* = 10.9 Hz, 1H), 5.70 (d, *J* = 17.8 Hz, 1H), 6.69 (dd, *J* = 17.8, 10.9 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 14.1, 22.7, 29.30, 29.34, 29.5, 29.60, 29.62, 31.5, 31.9, 35.7, 112.8, 126.1 (2C), 128.6 (2C), 135.0, 136.7, 142.7.

General procedure for the synthesis of 4-*epi*-jaspine B derivatives through sequential cross-metathesis, hydrogenation and deprotection reactions: synthesis of (2*S*,3*S*,4*R*)-4-amino-2-(4-decylphenethyl)tetrahydrofuran-3-ol (9). To a stirred solution of **26** (100 mg, 0.436 mmol) in CH_2Cl_2 (9 mL) under argon at room temperature was added **25** (434 mg, 1.78 mmol), followed by the 2nd generation Hoveyda–Grubbs catalyst (27 mg, 0.044 mmol; 10 mol %), and the resulting mixture was heated under reflux for 1.5 h. The reaction was then cooled to room temperature and filtered through a short NH_2 silica gel column eluting with EtOAc. The solvent was removed under reduced pressure to give the crude metathesis product as a residue, which was dissolved in a 1:1 mixture of EtOH and THF (15 mL). The solution was treated with 10 wt% Pd/C (23 mg, 0.022 mmol; 5 mol%) at room temperature, and the resulting mixture was stirred at room temperature for 2 h under an atmosphere of H_2. The mixture was then filtrated through a short pad of Celite. The solvent was removed under vacuum to give the crude hydrogenation product, which was dissolved in CH_2Cl_2 (9 mL). This solution was cooled to 0 °C and treated with TFA (9 mL), and the resulting mixture was stirred at room temperature for 30 min. The reaction was then concentrated under reduced pressure to give an oily residue, which was purified by column chromatography over silica gel eluting with $CHCl_3$–MeOH–28% NH_4OH (94:5:1) to give **9** (48.4 mg, 32% yield in 3 steps) as a white solid: $[\alpha]^{29}_D$ –16.1 (*c* 1.08, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.88 (t, *J* = 6.9 Hz, 3H), 1.20–1.37 (m, 14H), 1.58 (tt, *J* = 7.4, 7.4 Hz, 2H), 1.65 (br s, 3H), 1.84–1.93 (m, 1H), 1.93-2.02 (m, 1H), 2.56 (t, *J* = 7.4 Hz, 2H), 2.61–2.69 (m, 1H), 2.79 (ddd, *J* = 14.3, 9.2, 5.2 Hz, 1H), 3.39 (dd, *J* = 9.2, 3.4 Hz, 1H), 3.42–3.47 (m, 1H), 3.77–3.80 (m, 1H), 3.89–3.94 (m, 1H), 4.20 (dd, *J* = 9.2, 5.7 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 2H), 7.13 (d, *J* = 8.0 Hz, 2H); ^{13}C-NMR ($CDCl_3$) δ: 14.1, 22.7, 29.3, 29.4, 29.5, 29.58, 29.59, 30.4, 31.6, 31.9, 32.1, 35.5, 60.0, 73.6, 79.6, 80.2, 128.2 (2C), 128.4 (2C), 138.9, 140.5; HRMS (FAB^+) calcd for $C_{22}H_{38}NO_2$ [$M + H]^+$, 348.2903; found, 348.2905.

Sphingosine kinase assay. SphK inhibitory activities were evaluated by the off-chip mobility shift assay by the QuickScout® service from Carna Bioscience (Kobe, Japan). SphK1(1-384) and SphK2(1-618) were expressed as N-terminal GST-fusion proteins using a baculovirus expression system. They were purified using glutathione sepharose chromatography. Each chemical in DMSO at different concentrations was diluted fourfold with reaction buffer [20 mM HEPES (pH 7.5), 0.01% Triton X-100, 2 mM DTT]. For SphK reactions, a combination of the compound, 1 μM Sph, 5 mM $MgCl_2$, ATP (25 μM for SphK1; 600 μM for SphK2) in reaction buffer (20 μL) were incubated with each SphK in 384-well plates at room temperature for 1 h (*n* = 2). The reaction was terminated by addition of 60 or 70 μL of termination buffer (Carna Biosciences). Substrate and product were separated by electrophoretic means using the LabChip system. The kinase reaction was evaluated by the product ratio, which was calculated from the peak heights of the substrate (S) and product (P): [P/(P+S)]. Inhibition data were calculated by comparing with no-enzyme controls for 100% inhibition and no-inhibitor reactions for 0% inhibition. IC_{50} values were calculated using GraphPad Prism 4 software (GraphPad Software, Incorporated, La Jolla, CA, USA).

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Supplementary data

Supplementary data associated with this article (provided by the authors including experimental procedures and

characterization data for all new compounds) can be found in the online version at http://dx.doi.org/10.1016/j.bmc

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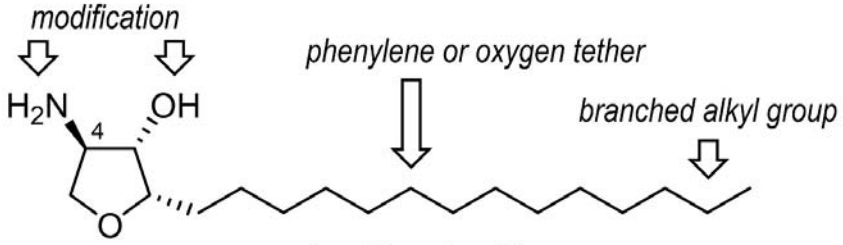
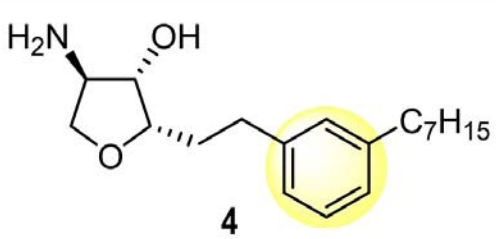


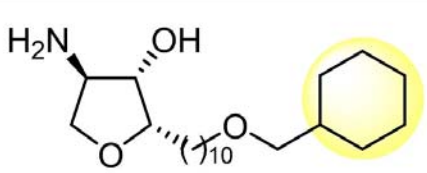
4-*epi*-jaspine B

IC_{50} (**SphK1**) = 0.82–2.8 μM; (**SphK2**) = 0.32–0.74 μM



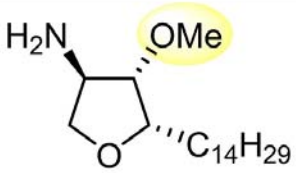
4

SphK1 >30 μM
SphK2 **2.2 μM** (Q = 3.7)



13

0.22 μM (Q = 0.20)
0.24 μM (Q = 0.24)



22

4.0 μM (Q = 3.6)
>30 μM