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Discovery of novel sphingosine kinase 1 inhibitors

Yibin Xiang *, Gary Asmussen, Michael Booker, Bradford Hirth, John L. Kane Jr., Junkai Liao, Kevin D. Noson, Christopher Yee

Drug and Biomaterial R&D, Genzyme Corporation, 153 Second Avenue, Waltham, MA 02451, USA

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ABSTRACT

Sphingosine kinase 1 (SK1) is an important enzyme that regulates the balance between ceramide and sphingosine-1-phosphate (S1P). Potent and novel SK1 inhibitors (**6ag**, **9ab** and **12aa**) have been discovered through a series of modifications of sphingosine (**1**), the substrate of this enzyme.

A large body of evidence from various academic and industrial laboratories indicates that sphingolipid metabolites can play important roles in biological processes far beyond the confines of cellular membranes.[1] The balance between cellular levels of sphingolipids is important for regulating cell function. For instance, it has been reported that ceramide and sphingosine **(1)** induce apoptosis or growth arrest while sphingosine-1-phosphate (S1P, **2**) mediates proliferation and angiogenesis.[2] Sphingosine kinases (SKs), which mediate the conversion of **1** to **2** (Fig. 1), are important enzymes in the sphingolipid metabolic pathway as they sit in a crucial position to regulate the relative levels of S1P, sphingosine, and ceramide.[3]

To date, two mammalian sphingosine kinases (SK1 and SK2) have been identified. Although both enzymes are capable of phosphorylating sphingosine, there are reports indicating that they have different cellular functions.[4] SK1 promotes cell growth and proliferation,[5] whereas SK2 has the opposite effects.[6] Much of the research in this area has been directed towards understanding the mechanism of the cellular and physiological functions of these enzymes. Recently, SK1 has been implicated in several pathological states, including various immune-mediated diseases, inflammation and cancer.[7]

N,*N*-Dimethylsphingosine (DMS, **3**) has been widely used for modulating S1P biosynthesis, even though **3** is a relatively weak and non-selective SK inhibitor.[8] Identification of a more potent and selective SK1 inhibitor could provide a useful tool for studying the sphingolipid metabolism pathway, and also lead to new potential approaches to the treatment of cancer and/or immune-mediated diseases. Recently, Spiegel and co-workers revealed the structure of a SK1-selective inhibitor (SK1-I, **11**).[9] The authors report that this compound effectively reduced the growth and survival of human leukemia U937 cells in vitro. Furthermore, **11** has been reported to suppress U937 growth in a murine xenograft model.

In this Letter, we report our discovery of a novel class of SK1-selective inhibitors based on a modified sphingosine scaffold. Our original hypothesis for designing these inhibitors involved replacing the aminodiol headpiece of sphingosine with a serine amide. Given the complementary functionality, we believed that the

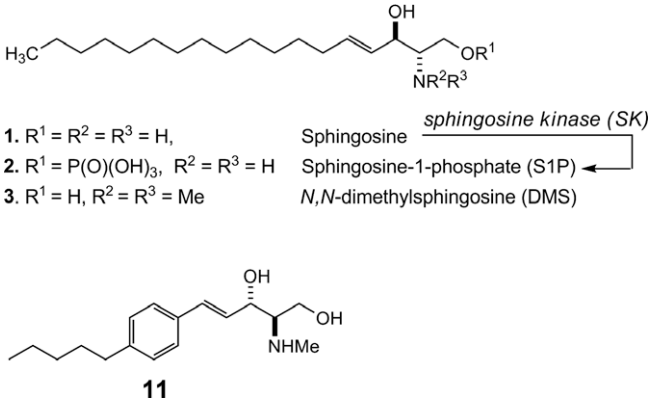

Figure 1.

* Corresponding author. Tel.: +1 781 434 3653; fax: +1 781 672 5823.
E-mail address: yibin.xiang@genzyme.com (Y. Xiang).

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new molecules generated by this modification would have affinity to the SK. Additionally, the carboxylic acid of serine provides a convenient synthetic handle that would allow for installing a variety of mimics for the lipophilic tail of sphingosine. A similar approach has been successfully employed by Macdonald and co-workers in their efforts to identify novel S1P receptor agonists.[10] Thus, a standard EDC-mediated amidation of *N*-*t*-Boc-L-serine with 4-octylaniline followed by removal of the carbamate protecting group with TFA afforded **6ab** (Scheme 1). This compound and its enantiomer, **6aa**, proved to be modest inhibitors of SK1, with potencies similar to DMS (Table 1).[11]

Derivatives **6ac–bl** were prepared with focusing on altering the serine headpiece. The assay results revealed that the substitution pattern had a significant impact on the potency of the compounds. Neither the *O*-methyl or α-methylated derivative (**6ad** or **6ac**) had activity against SK1, while the N-mono-methylated derivative **6af** demonstrated slightly improved potency relative to **6ab**. A substantial increase in activity was observed when L-threonine was incorporated as the polar headpiece. The resulting compound, **6ag**, was nearly 10-fold more potent than **6ab** (650 nM vs 4.3 μM). Similar levels of potency were also observed for the isomeric threonine analogs **6ah**–**6aj**, although in all cases, activity was reduced compared to **6ag**.

Compounds **9aa–9bc** were prepared to explore the impact of altering the distance between the terminal alcohol functionality and the amide group (Scheme 2). Interestingly, the homoserine derivative **9ac** was approximately 25 times more potent than the serine derivative **6ab** (180 nM vs 4.3 μM). In addition, the stereochemistry of the homoserine analogs had a significant impact on activity; the *S*-enantiomer **9ab** was about 40 times more potent than the *R*-enantiomer **9aa** (50 nM vs 2.2 μM). Further increases in the distance between the alcohol and amide groups, as demonstrated in compounds **9ad** and **9ae**, significantly decreased activity against SK1.

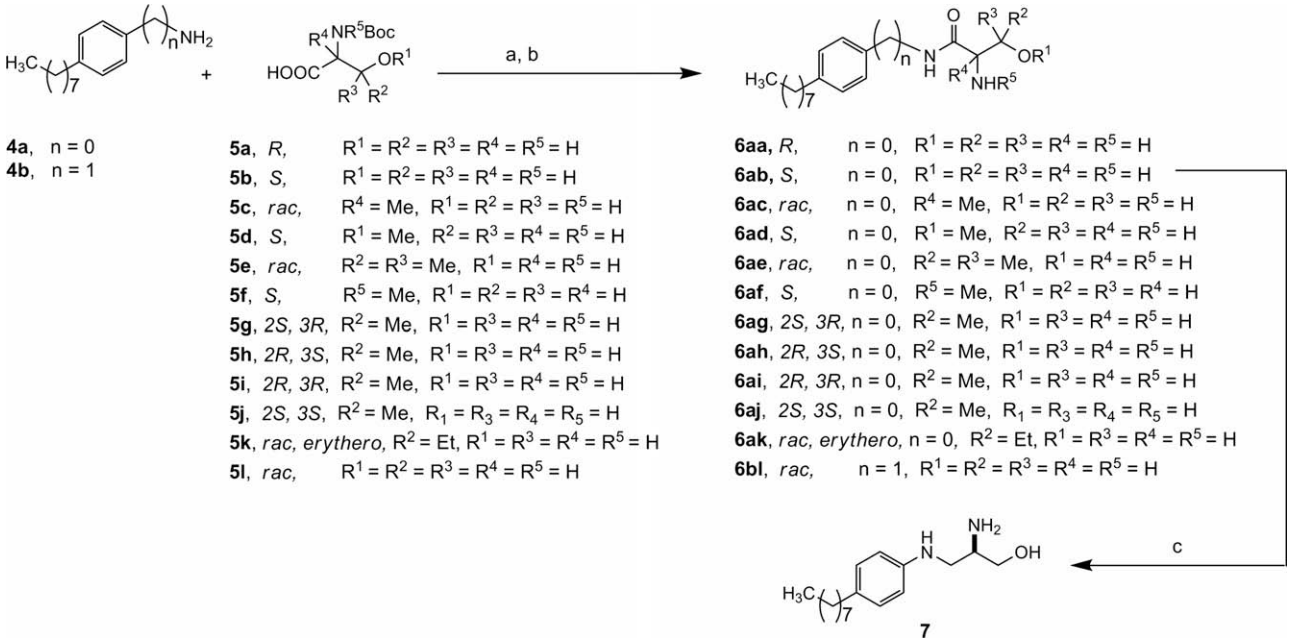
Encouraged by the results obtained with **6ag** and **9ab**, we made further modifications to the polar headpiece by preparing a series of 3-hydroxyproline analogs **12aa–ca** (Scheme 3). In this series, the most potent compound (**12aa**) was nearly 80-fold more active than **6ab** (62 nM vs 4.3 μM). Inversion of the stereochemistry at the 3-position (**12ab**) resulted in a marked decrease in potency as did the incorporation of an additional hydroxy substituent at the 4-position (**12ac**).

Table 1
IC_{50}s of sphingosine kinase 1 (SK1) inhibition[11]

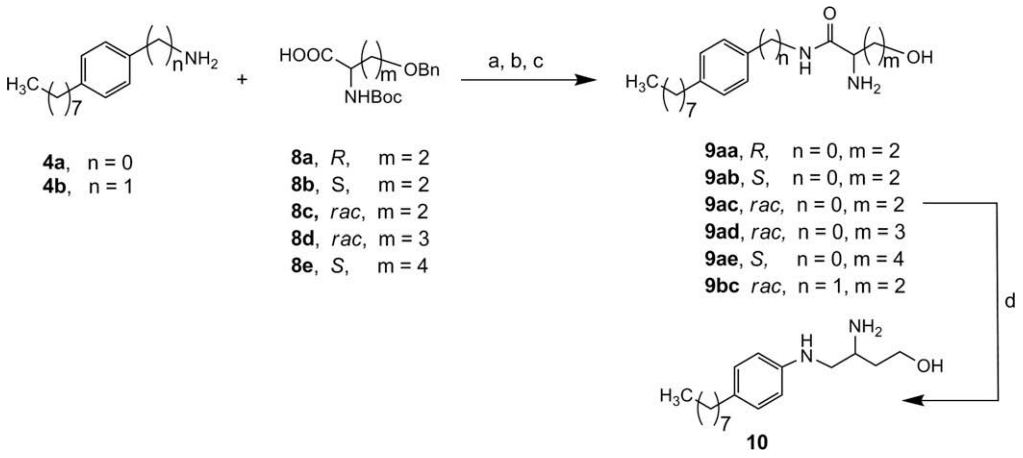
Compd#	IC_{50}* (μM)
3	5.7
6aa	5.0
6ab	4.3
6ac	>10
6ad	>10
6ae	2.1
6af	2.8
6ag	0.65
6ah	2.1
6ai	1.6
6aj	1.1
6ak	3.2
6bl	>10
7	5.0
9aa	2.2
9ab	0.05
9ac	0.18
9ad	>10
9ae	4.0
9bc	3.5
10	>10
12aa	0.062
12ab	3.4
12ac	8.3
12ba	0.43
12ca	6.2
13	0.74

* IC_{50}: Concentration of the testing compounds to inhibit 50% activity of the enzyme. The IC_{50}s were determination based on a method described in Ref. 11. All the values of IC_{50}s are the average of at least two times determinations.

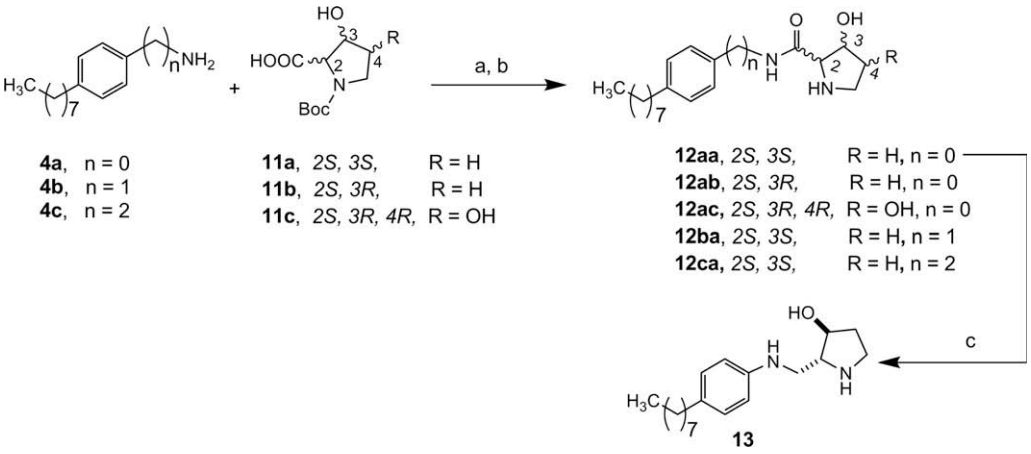
In all three series (**6**, **9**, **12**), the amide functionality was important for activity. Amine-containing analogs (prepared by borane reduction of the corresponding amides) had reduced activity against SK1 (cf. **6ab** vs **7**, **9ab** vs **10**, **12aa** vs **13**). Furthermore, the 4-octylanilide derivatives were more potent than the 4-octylbenzyl amide derivatives (cf. **6ab** vs **6bl**, **9ac** vs **9bc** and **12aa** vs **12ba**). None of the SK1 active compounds demonstrated any activity versus SK2 when screened at a concentration of 10 μM.



Scheme 1. Reagents: (a) EDC, DMAP, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) BH_3–SMe_2, THF.



Scheme 2. Reagents: (a) EDC, DMAP, CH_2Cl_2; (b) H_2, Pd/C, MeOH; (c) HCl, dioxane; (d) BH_3–SMe_2, THF.



Scheme 3. Reagents: (a) EDC, DMAP, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) BH_3–SMe_2, THF.

In conclusion, we have identified novel potent SK1 inhibitors through structural modification of sphingosine. Several of these compounds, including **9ab** and **12aa**, are significantly more potent than the previously reported SK1 inhibitor, *N*,*N*-dimethylsphingosine. Further optimization of the 3-hydroxyproline series with aims to improve in vitro activity and ADME properties will be reported in a separate publication.

References and notes

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11. The in vitro SK1 inhibition assays were conducted in the following manner: In a 384 well polystyrene plate, human sphingosine kinase 1 (300 ng/ml, from BPS Bioscience San Diego CA) was incubated in a 100 mM Hepes pH 7.5 buffer containing 2 μM fluorescein labeled sphingosine (Echelon Biosciences, Salt Lake City, UT), 25 μM ATP, 0.05% Triton X-100, 10% glycerol, 4 mM DTT, 20 mM $MgCl_2$ and compound with a final concentration of 4.35% DMSO for one hour at room temperature. The reaction was terminated by adding 40 mM EDTA in a 100 mM Hepes pH 7.5 buffer which contained 0.05% Triton X-100, 2.5 % glycerol, 0.3% CR-3 (Caliper Life Sciences, Hopkinton, MA) and 0.6% DMSO. The plate was run for one cycle on a LabChip 3000 (Caliper Life Sciences, Hopkinton, MA) in an off-chip mobility shift assay with an upstream voltage of −500 V, a downstream voltage of −2400 volts and a vacuum pressure of −2.1 psi. The sample sip time was 0.2 s. The LabChip separates and measures the amount of fluorescein labeled sphingosine and fluorescein labeled sphingosine-1-phosphate present in each well. Results are expressed as percent conversion of fluorescein labeled sphingosine by measuring peak height for both the substrate and product. On every plate 100% inhibition (substrate without enzyme) and 0% inhibition (substrate with enzyme and DMSO) controls were used to calculate percent inhibition of tested compounds.