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Sphingosine kinase inhibitors: a review of patent literature (2006–2015)

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Abstract

Introduction—Sphingosine kinase (SphK1 & SphK2) is the sole source of the pleiotropic lipid mediator, sphingosine-1-phosphate (S1P). S1P has been implicated in a variety of diseases such as cancer, Alzheimer’s disease, sickle cell disease and fibrosis and thus the biosynthetic route to S1P is a logical target for drug discovery.

Areas covered—In this review, the authors consider the SphK inhibitor patent literature from 2006–2016 Q1 with the emphasis on composition of matter utility patents. The Espacenet database was queried with the search term “sphingosine AND kinase” to identify relevant literature.

Expert opinion—Early inhibitor discovery focused on SphK1 with a bias towards oncology indications. Structurally, the reported inhibitors occupy the sphingosine ‘J-shaped’ binding pocket. The lack of cytotoxicity with improved SphK1 inhibitors raises doubt about the enzyme as an oncology target. SphK2 inhibitors are featured in more recent patent applications. Interestingly, both SphK1 and SphK2 inhibition and gene ‘knockout’ share opposing effects on circulating S1P levels: SphK1 inhibition/gene ablation decreases, while SphK2 inhibition/gene ablation increases, blood S1P. As understanding of S1P’s physiological roles increases and more drug-like SphK inhibitors emerge, inhibiting one or both SphK isotypes could provide unique strategies for treating disease.

Keywords

Sphingosine kinase; sphingosine-1-phosphate; kinase inhibitor; sphingosine kinase 1; sphingosine kinase 2; cancer therapeutics

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Declaration of Interests

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

1. Introduction

Sphingosine kinases (SphKs) catalyze the transfer of a phosphate group from ATP to sphingosine to form ADP and the pleiotropic lipid mediator, sphingosine 1-phosphate (S1P). In mammals, two unlinked genes encode the SphK isotypes (SphK1, SphK2), which are the sole sources of S1P [1]. S1P levels are high in circulation relative to tissue, and this differential, which has been termed the S1P vascular gradient [2], helps both to maintain endothelial barrier integrity and to promote T-lymphocyte egress from secondary lymphoid tissues [3]. S1P1 receptor agonist drugs, e.g. fingolimod (Gilenya™), render lymphocytes insensitive to the lymph / lymph node S1P differential and thereby modulate immune function [4]. There have been many suggestions in the literature that S1P synthesis could likewise be a drug target, with a particular emphasis regarding SphK1 inhibition for oncology indications [5].

Two pharmaceutical companies, Amgen and Pfizer, have described SphK inhibitors in the scientific literature but no patent applications claiming these chemical entities have been published as of this writing (March 2016). The Amgen contributions have been particularly significant; they include a high resolution structure of human SphK1 with the native sphingosine substrate bound as well as an early generation SphK inhibitor, **SKI-II** [6,7], bound and a series of inhibitors [8,9], most of which were selective for SphK1. Pfizer published a study with a single compound, **PF-543** (see Figure 1), which is the most potent human SphK1 inhibitor described to date [10]. Subsequently, an academic group has published a high resolution human SphK1 crystal structure with **PF-543** bound [11]. Although **PF-543** has not appeared in the patent literature, it provided a lead for a set SphK1 inhibitors claimed by Ajinomoto (see below). Because the Pfizer and Amgen papers were published more than 18 months ago, prior art considerations presumably exclude patenting the published chemical matter. Both the Pfizer and Amgen papers document that their respective SphK1 inhibitors are not cytotoxic for a variety of cancer cells lines. The inability to recapitulate the cytotoxicity observed with early generation SphK1 inhibitors presumably influenced the decisions not to pursue patent coverage.

A search of the Espacenet database in March 2016 using the query “sphingosine AND kinase” (title and/or abstract) yields almost 250 returns. However, less than 10% of these are patents or patent applications claiming composition of matter, and only those, with several exceptions, that have priority dates from 2006 through the first quarter of 2016 are the focus of our review. The eleven issued composition of matter patents claiming SphK inhibitors are listed in Table 1.

2. Patent Evaluation

2.1 Apogee Biotechnology

The first patents issued wherein SphK inhibitors are claimed are assigned to Apogee Biotechnology. The first patent lists 12 chemical species that are described as SphK inhibitors without mention of isotype selectivity [12]. Although no claim is made as to a use of these SphK inhibitors, the abstract mentions the use of such inhibitors for hyperproliferative diseases. This patent is notable because it claims a widely used,

commercially available SphK inhibitor named **SKI-II** [6]. Three subsequent patents assigned to Apogee [13–15] make claims to several related aryladamantyl series (Figure 2). Notable among the species claimed is **ABC294640** that has appeared numerous times in the scientific literature, where it is reported to have K_I of 10 μM at human SphK2 [16]. The oft-repeated contention that **ABC294640** is SphK2 selective (vs. SphK1) appears to be based on an assay where the IC_{50} value at hSphK2 was reported as 60 μM while there was no observed inhibition in the assay at hSphK1 at concentrations up to 100 μM [16]. This compound was recently documented to affect estrogen receptor signaling in breast cancer cells [17]. **ABC294640** has been documented to be efficacious in numerous disease models including liver ischemia reperfusion injury [18], Crohn's disease [19], osteoarthritis [20] and others. **ABC294640** has the distinction among SphK inhibitors of having been administered to humans. Two phase I clinical trials have been reported (clinicaltrials.gov #NCT01488513, #NCT02229981). One was a dose escalation study that enrolled patients with solid tumors and was completed in July 2015. A second trial seeks to enroll patients with refractory diffuse large B cell lymphoma. A fifth issued US patent claims pro-drugs of aryladamantyl compounds including the active drug, **ABC294735** [21]. A patent application that has been abandoned makes method of use claims regarding the use of SphK inhibitors, including Apogee's aryladamantyl compounds, for treating ischemia reperfusion injury to heart, brain, kidney, lung and liver as a result of trauma, surgery, etc. [22] (Table 3).

2.2 Merck Serono

A second set of three issued US patents claiming a structurally related set of human SphK1 inhibitors are assigned to Merck Patent GmbH (Merck Serono) (Figure 3). The earliest of these claims a set of SphK1 inhibitors that are based on a 2-piperidine thiazole scaffold **1** [23]. Nearly all of the 107 chemical species listed feature a 5,5,8,8-tetramethyl tetralin linked at the 4 position of the thiazole ring. The species exemplified are classed by IC_{50} values (in μM : 0.01–1, 1–10, >10) at SphK1. As is often the case in the patent and scientific literature on SphK inhibitors, there is no IC_{50} value with a known SphK inhibitor and no K_I values are presented; thus, it is difficult to know how the potencies of the featured compounds compare to an external standard. The four claims make no mention of a use of these SphK1 inhibitors, but the background of the invention mentions that SphK1 inhibitors are particularly “suitable for the therapy of cancer diseases”. The second patent in the Merck series [24] makes composition of matter claims for SphK1 inhibitors that are an expansion of the 2-piperidine thiazole scaffold. Specifically, although the 2,4-disubstituted thiazole is featured in many of the species exemplified and the four position is often occupied by 5,5,8,8-tetramethyl tetralin, the thiazole is replaced by a variety of 5-membered heteroaryls including oxazole, oxadiazole and thiadiazole. Additional diversity is realized by linking the piperidine through the 1-position, replacing the piperidine with a piperazine and a variety of alkyl groups (**2**). As a group, these species are more potent at human SphK1 than those in the earlier species, being segregated into three potency groups (IC_{50} in nM: 1–100, 100–1000, > 1000). The eight claims are directed to 102 species and general aspects of their formulation. The third patent in the series [25] features 162 human SphK1 inhibitors wherein the pedant 5,5,8,8-tetramethyl tetralin group is nearly universal but a six membered heteroaryl, usually a 2,6-disubstituted pyridine (**3**), replaces the 5-membered heteroaryl group in the previous patents (Figure 3). This final patent is notable also for including a

Markush group in the claim set and 10 (of 22) claims are directed for the use of these SphK1 inhibitors in treatment of rheumatoid arthritis. None of the three Merck patents state the selectivity of the claimed chemical matter for SphK1 vs. SphK2, and it is not made clear whether this class of SphK1 inhibitors is competitive with ATP or sphingosine, or non-competitive.

2.3 Enzo Therapeutics

Two closely related patents, which arose from a common provisional application (61/048,638; 2008-04-29), issued to Enzo Therapeutics claims a small set of SphK1 inhibitors. Both applications focus on a single chemical entity that is an analog of *D-erythro*-sphingosine (Figure 4). This compound, which has appeared in the academic literature as **SK1-I** (BML-258), is a SphK1 selective inhibitor with a K_I value of 10 μ M [26]. The first Enzo patent claims **SK1-I** and a small set of related compounds [27]. The six claims include two that indicate the use of these SphK1-selective inhibitors for treatment of high grade astrocytomas. The second Enzo patent features only SKI-1 and its five claims do not address any uses [28].

2.4 University of Virginia

The final issued US patent making composition of matter claims to SphK inhibitors is assigned to the University of Virginia [29], which is the first of a series of three related filings. The patent features a set of 17 species with a common scaffold, *i.e.* a terminal, alpha-substituted amidino group linked to a 4-alkyl phenyl (**VPC96091**) or straight chain alkyl (**4**) group via an amide linkage (Figure 5). Many of these species are dual SphK1/2 inhibitors while others are selective for SphK1. The inventors on this, and the related patent applications, have published a number of papers on SphK inhibitors in the scientific literature, wherein they reveal that the most potent of these compounds have K_I values of 200 and 500 nM at recombinant human SphK1 and SphK2, respectively [30]. The amidino SphK inhibitor series is expanded in a patent application ([31] and Table 2), which includes additional species as well as the amidoxime (**5**) analogs, which is a known maneuver to make orally available prodrugs of compounds with carboximidamide groups [32]. One species, **VPC96091**, is featured in a publication where it is reported to have a K_I of 100 nM at SphK1 and 1,500 nM at SphK2 and is shown to decrease circulating S1P levels upon injection in mice [33]. Other species in that patent application are presented in a second paper, which includes the docking of a generic scaffold to a homology model of SphK1 [34]. This modeling predicts that the carboximidamide group chelates the gamma phosphate of the ATP to effect inhibition. A third patent application in this series features an expansive generic (Markush) structure [35]. The 84 species exemplified all have the pendant carboximidamide in common with the previous applications, but include numerous guanidine as well as amidine species. Two species in this application feature prominently in scientific publications. Species 50 (**SLR080801**) is a 10-fold selective SphK2 inhibitor that raises circulating S1P on administration to mice [36]. Species 64 (**SLP7111228**), which is a selective, potent (K_I 50 nM) SphK1 inhibitor was reported to lower circulating S1P to 20 % of normal after a single dose in rats [37]. These inhibitors demonstrate that engagement of the target is indexed by blood S1P levels.

The increase in blood S1P accumulation in response to inhibition, or gene deletion, of one of its synthetic enzymes (i.e. SphK2) is curious and the underlying mechanism is unclear. The increase could be due to increased activity of SphK1, which is responsible for blood S1P synthesis, and / or SphK2 somehow functioning in removal of S1P from blood. The latter is suggested by both the slowed clearance of mass labeled S1P injected intravenously (i.v.) in Sphk2 null vs. wt mice [38,39] and slowed clearance of mass labeled S1P injected i.v. into SphK2 inhibitor-treated vs. vehicle-treated mice [39]. A role for increased SphK1 activity is suggested by the observation that subjecting either humans or mice to hypoxic (10% oxygen) environments results in a pronounced rise in erythrocyte S1P that correlates with increased SphK1 activity in these cells [40].

2.5 Additional patent applications

Almac Discovery, the City University of New York together with the University of Strathclyde (UK), Ajinomoto and the University of South Australia have applied for patents claiming SphK inhibitors (Table 2). The Almac application [41] claims a series of SphK1 inhibitors, many of which are remarkably similar, but not overlapping, with those claimed in the most recent Merck Serono application (Figures 3 and 6). To our knowledge, neither the Merck Serono nor the Almac Discovery compounds have appeared in scientific publications. The City University of New York and the University of Strathclyde have applied for a patent claiming a disparate set of compounds [42]. Several broad Markush structures are drawn; one prominent group is a terminal vinyl phosphonate (**8**, Figure 7) and several compounds contain thiourea linkages. Interestingly, claims are made to both SphK inhibitors and SphK1 activators. The inventors have published a report [43] classifying their inhibitors as sphinganine-like, phytosphinganine-like and pachastrissamine-like (pachastrissamine is a cyclic phytosphingosine analog isolated from a marine sponge [44]). An intriguing aspect of the reported SphK1 inhibitors is that these evoke a degradation of SphK1, which is a property shared by SKI-II [45]. The other claims include substituted piperidine (**9**) and pyrrolidine head groups (**10**). The inventors reported in a publication that **9** is a selective inhibitor of SphK1 with an IC_{50} 3.6 μ M [46]. Additional medicinal chemistry work resulted in **10**, which converted the SphK1 selective inhibitor to a dual inhibitor (IC_{50} for both isotypes is 2 μ M) [46].

Ajinomoto has applied for a patent [47] claiming a series of SphK1 inhibitors that appear to have used the aforementioned SphK1 inhibitor, **PF-543** (Figure 1) [10], as the lead structure (Figure 8). All the compounds claimed and **PF-543** have a 1-substituted-2-methanol pyrrolidine head group. A structural feature of the Ajinomoto patent is the exchange of the phenyl ring linker in **PF-543** with an indole ring with 2,5-substituents attached (**11–14**). The presence of a terminal primary hydroxyl group as found in **PF-543**, Ajinomoto, Merck-Serono, and Almac compounds raises the formal possibility of the compounds being a SphK substrates and the phosphorylated product interacting with S1P receptors. Substrate activity was tested in the case of **PF-543** and, although the compound was phosphorylated by SphK1, the activity was minimal [10]. The most recent (as of March 2016) patent application published is from the University of South Australia [48]. The 13 species exemplified share a common N-(2-aminophenyl)-4-methylbenzenesulfonamide core (Figure 9). An associated publication reveals that one species, **15**, is both a dual SphK inhibitor and

is competitive with ATP with single digit micromolar potency [49]. This application is the first series of inhibitors reported to presumably dock in the ATP binding site.

2.6 Abandoned Applications

There are several composition of matter applications describing SphK inhibitors that have been abandoned by various applicants (Table 3). The most complete of these was filed by the Genzyme Corp [50]; there are two associated papers that present a characterization of these compounds [51,52]. Genzyme's papers and their patent application present a series of SphK1 inhibitors including several compounds with significant metabolic stability. Similar to **PF-543**, the Ajinomoto series and Amgen compounds, the Genzyme structures **16–17** (Figure 10) have a pendant pyrrolidine group, but it is substituted with hydroxyl rather than methanol. The modestly potent SphK2 inhibitor, **K145** (Figure 11), was featured in a Virginia Commonwealth University patent application that was not pursued.

3. Expert Opinion

Several patterns emerge in studying the SphK patent literature and the associated scientific publications. Structurally, two themes are the length and shape of the inhibitors – most are linear molecules with a backbone of about 18 atoms, to which the natural substrate, sphingosine, conforms. The scaffolds, which were arrived at independently, can be justified with the recent SphK1 crystal structure (and the homology models of SphK2), which indicate that sphingosine (and inhibitors such as **PF-543**) occupies a “J-shaped” tunnel. A second common feature is the presence of a terminal 1- or 2- substituted pyrrolidine group. The crystal structures of **PF-543** and Amgen **82** (Figure 11) [7], bound to SphK1 reveal that the aminoalcohol portion is directed towards the ATP binding pocket and interacts via hydrogen bond with two key aspartate residues that are critical for enzymatic activity. Not surprisingly, the endogenous ligand, sphingosine, is predicted to contact the same amino acid residues in a similar manner. Interestingly, the imidamide inhibitors from the University of Virginia, which lack the hydroxyl group, are situated near the ATP binding pocket and likely form a network of hydrogen bonds with the gamma phosphate of ATP as predicted by molecular modeling studies.

In the older patent literature, there was a focus on the use of SphK1 inhibitors for oncology. The earliest sphingosine kinase inhibitors, which have double digit micromolar potency and were not drug-like, were insufficiently characterized and often cytotoxic. The latter property, coupled with observations that elevated SphK1 expression correlates with poorer survival in some human cancers and certain biologic activities of S1P, e.g. cell migration, encouraged the thinking that SphK1 could be an oncology target. Later generation SphK1 inhibitors, including the aforementioned **PF-543**, the Amgen inhibitors and **VPC96091**, which are far more potent and more drug-like, are not notably cytotoxic. The lack of patent applications claiming SphK inhibitors by Pfizer and Amgen and the abandonment of the Genzyme application can be interpreted as decreased interest by Pharma in SphK1 as an oncology target.

In contrast with SphK1 inhibitors, SphK2 inhibitors along with the corresponding patent applications have emerged more recently. **ABC294640** was the first reported SphK2 inhibitor.

However, it is only moderately potent (10 μM K_i) and selective. While it has been deployed in a variety of animal model of diseases, the results cannot be confidently attributed to inhibition of SphK2 as off-target effects have recently been detected (for example, the estrogen receptor). A group at Virginia Commonwealth University published thiazolidine-2,4-dione analog, **K145**, that is a SphK2 inhibitor (6.5 μM K_i) and has not been documented to affect S1P levels *in vivo*. The latest generation of SphK2 inhibitors, which contain a 2-substituted guanidyl pyrrolidine head group, are more potent and selective [39]. In contrast with **K145** and **ABC294640**, these inhibitors are not cytotoxic in cultured cells at concentrations that inhibit the target. An interesting property of modulating SphK2 with selective inhibitors is their ability to *increase* the circulating S1P levels *in vivo*—a marked contrast with inhibiting SphK1, which results in decreased S1P levels [36,37]. Thus it appears that SphK1 and SphK2 exert a ‘yin-yang’ effect regarding S1P tone in blood. It is possible that as the physiological functions of SphK2 come into light, novel disease targets will arise. However, the therapeutic benefit of inhibiting either or both isotypes remains to be determined.

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WL Santos and KR Lynch are among the co-founders of SphynKx Therapeutics LLC, which was created to commercialize S1P-related discoveries, including SphK inhibitors, discovered and characterized in their laboratories. SB Thorpe is an employee at SphynKx Therapeutics. The three University of Virginia Patent Foundation patents mentioned are currently licensed to SphynKx.

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Reference annotations

* Of interest

** Of considerable interest

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Article highlights

- Sphingosine kinase 1 was initially targeted for oncology indications; however, recent studies with improved SphK1 inhibitors have not reproduced the cytotoxicity for cancer cells reported with early generation SphK1 inhibitors.
- Crystal structures of human SphK1 bound to sphingosine kinase inhibitors (SKI-II, PF-543) reveal a 'J-shaped' binding pocket that is predicted to bind sphingosine also. The tested inhibitors occupy this pocket with a positively charged nitrogen pointing toward the ATP-binding region.
- Blood S1P levels, which change rapidly, provide a convenient pharmacodynamic index of SphK engagement *in vivo*. Inhibition of SphK 1 lowers blood S1P level while SphK2 inhibition increases it. The mechanism of the latter phenomenon is unknown at present.
- A therapeutic niche for selective or dual SphK inhibitors remains to be determined.

Figure 1.
Structure of Pfizer's SphK1 selective inhibitor and lead compound SKI-II.

Figure 2.
SphK2 selective inhibitors patented by Apogee Biotechnology

Figure 3.
SphK1 selective inhibitors patented by Merck Serono

Figure 4.
A selective SphK1 inhibitor from Enzo Therapeutics

Figure 5.
Dual and selective SphK inhibitors from the University of Virginia

Figure 6.
Selective SphK1 inhibitors from Almac Discovery

Figure 7.
SphK inhibitors from the City University of New York and University of Strathclyde (UK).

Figure 8.
SphK1 inhibitors from Ajinomoto

15 (MP-A08)

Figure 9.

ATP competitive SphK1 inhibitor from University of South Australia and Central Adelaide Local Health Network, Inc.

Figure 10.
SphK1 inhibitors from Genzyme Corporation

Figure 11.
SphK inhibitors from Amgen and Virginia Commonwealth University

Table 1Issued patents claiming SphK^{*} inhibitors

Title	US patent	Assignee
SphK inhibitors	7,220,764	Apogee Biotechnology
SphK inhibitors and methods of their use	7,338,961	Apogee Biotechnology
SphK inhibitors and methods of their use	8,063,248	Apogee Biotechnology
SphK inhibitors and methods of their use	8,577,800	Apogee Biotechnology
SphK inhibitor prodrugs	8,685,936	Apogee Biotechnology
Thiazolyl piperidine derivatives	8,436,186	Merck Patent GmbH
Inhibitors of SphK	8,907,098	Merck Patent GmbH
Inhibitors of SphK	9,062,015	Merck Patent GmbH
SphK1 inhibitors and processes using the same	8,314,151	Enzo Therapeutics
Novel SphK1 inhibitors, compositions and processes	8,372,888	Enzo Therapeutics
Compositions and methods for inhibiting SphK	8,686,066	University of Virginia

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SphK = sphingosine kinase

Table 2

Pending Applications Claiming SphK inhibitors

Title	application #	applicant
Pharmaceutical compounds as inhibitors of SphK	WO 2012/069852	Almac Discovery Ltd.
Imidamide SphK inhibitors	WO 2011/020116	University of Virginia
Long chain base SphK inhibitors	WO 2013/119946	University of Virginia
Selective inhibitors and allosteric activators of SphK	WO 2014/118556	City Univ New York & Univ Strathelyde (UK)
SphK inhibitor	WO 2014/157382	Ajinomoto
Benzene sulfonamide-based inhibitors of SphK	WO 2016/007993	Univ South Australia

Table 3

Abandoned Applications Claiming SphK inhibitors

Title	application #	applicant
Inhibitors of SphK1	WO 2010/033701	Genzyme Corp
Sphingo-guanidines and their use as inhibitors of SphK	WO 2010/078247	Med Univ S Carolina
3-(2-amino-ethyl-alkylidene)-thiazolidine-2,4-dione and 1-(2-amino-ethyl)-alkylidene-1,3-dihydro-indol-2-one derivatives as selective SphK2 inhibitors	WO 2013/119774	VA Commonwealth U
Treatment of ischemia reperfusion injury	WO 2010/129954	Apogee Biotechnology