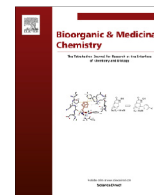
Bioorganic & Medicinal Chemistry 22 (2014) 5354–5367

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc



Design, synthesis and evaluation of 2-aminothiazole derivatives as sphingosine kinase inhibitors

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ARTICLE INFO

Article history:
Received 16 April 2014
Revised 23 July 2014
Accepted 28 July 2014
Available online 6 August 2014

Keywords:
Sphingosine-1-phosphate
Sphingolipids
Sphingosine
ST-1803
Enzyme inhibitor

ABSTRACT

Sphingosine kinases (SphK1, SphK2) are main regulators of sphingosine-1-phosphate (S1P), which is a pleiotropic lipid mediator involved in numerous physiological and pathophysiological functions. SphKs are targets for novel anti-cancer and anti-inflammatory agents that can promote cell apoptosis and modulate autoimmune diseases. Herein, we describe the design, synthesis and evaluation of an aminothiazole class of SphK inhibitors. Potent inhibitors have been discovered through a series of modifications using the known **SKI-II** scaffold to define structure–activity relationships. We identified *N*-(4-methylthiazol-2-yl)-(2,4′-bithiazol)-2′-amine (**24**, **ST-1803**; IC_{50} values: 7.3 μM (SphK1), 6.5 μM (SphK2)) as a promising candidate for further in vivo investigations and structural development.

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1. Introduction

Sphingolipids represent a major class of lipids that are ubiquitous localized and essential constituents of eukaryotic cells. Besides structural roles in membrane formation, they act as modulators in cell signaling processes. Sphingolipid metabolism displays a complex network of enzymatically controlled reactions with ceramide (Cer) in the center of biosynthesis and catabolism.[1] Most important sphingolipid mediators are sphingosine-1-phosphate (S1P), sphingosine (Sph) and Cer.[2] The balance between cellular concentrations of Cer and S1P (called 'sphingolipid rheostat') has been proposed to determine the physiological fate of cells. Cer and S1P elicit opposing cellular fates, for example, growth arrest and apoptosis versus proliferation and survival. Changes of intracellular S1P levels also affect the levels of Cer and Sph. The bioactive S1P is a potent mitogenic and migratory signaling lipid. It has been linked to the development and progression of numerous hyperproliferative and inflammatory diseases including cancer, asthma, atherosclerosis, sepsis, inflammatory bowel disease, rheumatic arthritis and multiple sclerosis.[3,4]

Therapeutic opportunities targeting the S1P/Cer rheostat are manifold. The major objective of drug discovery has focused on molecules that are capable of agonizing or antagonizing S1P receptors. S1P receptor modulator Fingolimod (FTY720), a Sph analogue, is the first oral therapeutic approved for the treatment of multiple sclerosis.[5] Another approach is to reduce the bioavailability of S1P at its receptors using S1P neutralizing antibodies. The anti-S1P monoclonal antibody Sonepcizumab (LT1009) is currently in clinical trials phase I for age-related macular degeneration (iSONEP™) and for advanced solid tumors (ASONEP™).[6] Sphingosine kinase (SphK) inhibitors are another therapeutic option.[7]

Sph is phosphorylated by SphK to form S1P. SphK exists in two isoforms, SphK1 and SphK2, which differ in their substrate preferences, subcellular localizations and tissue distributions, suggesting that they perform different physiological roles.[8] Studies using isoform-specific siRNA and knock-out mice have indicated that SphK1 and SphK2 have distinct and non-redundant functions involved in (patho)physiology. This promoted the search for isoform-specific inhibitors of SphK1 and SphK2.[9] The actions of SphK1 and S1P are complex and far away from being fully understood, especially with regard to the involvement in inflammation and cancer. Cancers of stomach, lung, brain, colon, kidney and breast as well as non-Hodgkin's lymphoma have increased SphK1 expression.[10] Up-regulation of SphK1 increases S1P production and correlates

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; Cer, ceramide; ERK-1/2, extra-cellular signal-regulated kinase 1/2; HDAC, histone deacetylase; LDH, lactate dehydrogenase; S1P, sphingosine-1-phosphate; SAR, structure–activity relationship; Sph, sphingosine; SphK1, sphingosine kinase 1; SphK2, sphingosine kinase 2; TRAF2, TNFα receptor associated factor 2; TNFα, tumor necrosis factor alpha; TPSA, topological polar surface area.

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http://dx.doi.org/10.1016/j.bmc.2014.07.044
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with poor cancer prognosis.[11] The activity and expression of SphK1 is increased in response to growth factors and pro-inflammatory cytokines.[8] TNFα-induced interaction of SphK1 with TNFα receptor associated factor 2 (TRAF2) is required for the extracellular signal-regulated kinase (ERK-1/2)-mediated phosphorylation of SphK1 and subsequent translocation of SphK1 to the plasma membrane, where it interacts with phosphatidylserine.[12] The physiological and pathophysiological relevance of this translocation remains to be determined. It might be required for the so-called 'inside-out' signaling of S1P.[13]

Less is known about the role and regulation of SphK2. Some studies have supported a role for SphK2 in survival and migration.[14] By contrast, other studies have demonstrated the suppression of cell cycle arrest and apoptosis.[15] The ambiguous nature might be determined by the subcellular localization of SphK2 and resulting intracellular S1P pools.[16] Recently, histone deacetylase (HDAC) has been claimed as an intracellular target for nuclear localized SphK2-derived S1P. S1P binds to and inhibits HDAC1 and HDAC2 within the repressor complexes that are enriched at the promoters of genes encoding the transcriptional regulator c-fos and cyclin-dependent kinase inhibitor p21. S1P promotes their expression by inhibiting HDACs and increasing histone acetylation (epigenetic S1P effect).[17]

There is a need for specific inhibitors of SphK1 and/or SphK2 to determine whether strategies targeting the isoenzymes alone or in combination offer the best therapeutic option for the diseases mentioned above. A major difficulty in the development of isoenzyme-selective inhibitors has been the lack of structural information of substrate recognition and catalysis. The recent elucidation of the X-ray crystal structure of SphK1 in 2013[18] will lead to a better understanding of the enzyme functionality. The crystal structure of SphK2 remains to be solved and would accelerate the development of selective inhibitors for therapeutic uses.

To date, most SphK inhibitors exhibit K_i or IC_{50} values in the micromolar range.[7] The roles of SphKs and S1P in different pathologies were first elucidated by the discovery of the non-selective SphK inhibitor *N*,*N*-dimethylsphingosine (DMS)[19] (Fig. 1). Due to its high structural similarity to the endogenous Sph, the therapeutic potential of this substrate analogue is limited. A milestone in the development of more 'drug like' molecules was the synthesis and evaluation of non-selective SphK inhibitor **SKI-II** (4-((4-(4-chlorophenyl)-2-thiazolyl)amino)phenol, compound **1**)[20] (Fig. 1). **SKI-II** has been used for in vitro and in vivo studies.[21,22] It reduces intracellular S1P, inhibits proliferation and induces apoptosis in various cancer cell lines.[23] **SKI-II** is orally bioavailable.[22] Its described potency may depend beside several non-Sph-related effects in part on its ability to induce the proteasomal[24] or lysosomal[25] degradation of SphK1. **PF-543** (**42**) is the first nanomolar SphK1-selective inhibitor that rapidly reduces S1P in cells (Fig. 1).[26] It appears to be a useful tool for inhibiting SphK1 in vitro, but its in vivo effects were not described so far.

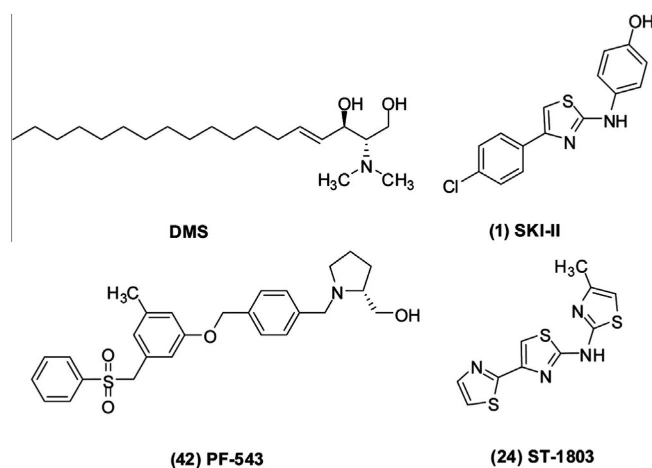

Figure 1. Representative SphK inhibitors and screening hit ST-1803.

We focused on **SKI-II** as promising lead compound for the development of a small library of 2-aminothiazole derivatives as SphK inhibitors with different selectivity ratios. 2-Aminothiazoles and derivatives provide a wide spectrum of biological activities[27] and are seen in many bioactive scaffolds as 'privileged structures'.[28,29]

2. Chemistry

Compounds **1**–**37** were prepared as shown in Scheme 1. We started with the bromination of the corresponding ketone derivatives (**A**) that gave α-bromoketones (**B**). The substituted thioureas (**E**) that were not commercially available were synthesized by condensation of the appropriate aniline or amine derivative (**C**) with benzoyl chloride in the presence of ammonium thiocyanate, followed by saponification of the resultant *N*-aryl-*N*′-benzoylthioureas (**D**) to remove the benzoyl group.[30,31] In the final step, microwave-assisted Hantzsch thiazole synthesis, the condensation of α-bromoketones (**B**) and N-substituted thioureas (**E**), provided the desired 2-aminothiazole derivatives (**1**–**37**).[32]

For the synthesis of compounds **38**–**41** we used different amide bond formation procedures to couple 2-aminothiazole to activated cinnamic acid derivatives.[33] Acyl chlorides (for **38**) or carboxylic acids after previous activation by DIC/HOBt (**39**) or EDC/HOBt (**40**, **41**) were coupled to the aromatic amine.[34] Reference compound **PF-543** (**42**) was synthesized as described by Schnute et al. (cf. Supplementary material).[26]

3. Results and discussion

3.1. Biological evaluation

Compounds were screened for SphK inhibitory activity at 10 μM (Table 1). An ADP-detecting fluorescence assay has been used as ADP and S1P are equimolar products of the enzymatic phosphorylation reaction. Inhibition of SphK1 or SphK2 was determined by incubating SphK1 or SphK2 in the presence of sphingosine, ATP and inhibitor or control (DMSO). The inhibitory potential of the lead structure **SKI-II** (**1**) has been shown to be only moderate under these assays conditions (15–25% inhibition), whereas **PF-543** (**42**) was able to inhibit SphK1 almost quantitatively at this concentration (94%).

The initial round of lead compound **SKI-II** (**1**) modifications aimed at improving its inhibitory potential at SphK1 and/or SphK2 via structural variations at R^3. Small functional groups were attached at different positions of the aromatic ring to affect the hydrogen-bond donor/acceptor ability of the moiety, thereby playing a role in the interactions with the target binding site.

The hydroxy group in 4-position of the phenyl ring seems to be crucial for inhibitory potential, as both its shift to the 2- and 3-position (**3**, **4**) and methylation (**6**) led to loss of inhibition. The thioether derivative **12** nevertheless was able to selectively inhibit SphK1. The insertion of a methylene spacer (**5**) between hydroxyl moiety and aromatic ring produced an inactive compound. Interestingly, the methoxy-group in 2-position of the phenyl ring (**7**) showed a slight tendency towards SphK1-selective inhibition. Analogues containing alkyloxy-substituents in 4- and 3-position as dimethoxy (**8**) as well as methylene or ethylene linked diethers (**10**, **11**) lost their ability to inhibit SphKs. By introducing a third methoxy group in 5-position (**9**, **ST-1780**), a substantial increase

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