Bioorganic & Medicinal Chemistry 24 (2016) 3218–3230

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Development of hydroxy-based sphingosine kinase inhibitors and anti-inflammation in dextran sodium sulfate induced colitis in mice

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ARTICLE INFO

Article history:
Received 11 April 2016
Revised 20 May 2016
Accepted 21 May 2016
Available online 24 May 2016

Keywords:
SphK
DSS
Inflammation
Colitis

ABSTRACT

Sphingosine kinase (SphK)-catalyzed production of sphingosine-1-phosphate (S1P) regulates cell growth, survival and proliferation as well as inflammatory status in animals. In recent study we reported the *N*′-(3-(benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide scaffold as a potent SphK inhibitor. As a continuation of these efforts, 51 derivatives were synthesized and evaluated by SphK1/2 inhibitory activities for structure–activity relationship (SAR) study. Among them, **33** was identified as the most potent SphK inhibitor. Potency of **33** was also observed to efficiently decrease SphK1/2 expression in human colorectal cancer cells (HCT116) and significantly inhibit dextran sodium sulfate (DSS)-induced colitis as well as the decreased expression of interleukin (IL)-6 and cyclooxygenase-2 (COX-2) in mouse models. Collectively, **33** was validated as an effective SphK inhibitor, which can be served as anti-inflammatory agent to probably treat inflammatory bowel diseases in human.

1. Introduction

The conserved lipid kinase, sphingosine kinase (SphK), catalyzes the transfer of phosphate from ATP to the 1-OH in sphingosine (Sph) to form sphingosine 1-phosphate (S1P), which plays an important role in regulating cell growth, survival, proliferation, neovascularization, and migration.[1–3] There are two known isoforms of SphK exist in mammals, SphK1 and SphK2. The amino acid sequence of these two kinases is 80 percent similar and 45 percent overall identical.[4] SphK has been involved with a diverse range of disease including sickle cell disease,[5,6] cancer,[7,8] atherosclerosis,[9,10] asthma,[11,12] and inflammation,[13,14] among others.

SphK1's role in colitis is widely studied, where correlation between elevated expression and severity of disease has been reported. Deletion of SphK1 reduces colitis severity.[15] However, SphK2 knockout induces increased severe colitis. It is thought that the two enzymes have some compensatory mechanism. SphK2 knockout up-regulating SphK1 expression is one main reason.[16] Studies in mice indicate a redundant role of SphK1 and SphK2 because the individual knockout remains viable, but double knockouts are embryonically lethal.[4,17] In addition, there are also differences in their subcellular localization and structure elucidation: SphK1 is mainly localized in cytosol while SphK2 is mainly in nuclei;[18] a high resolution X-ray crystal structure of human SphK1 was announced but the structure of SphK2 was not resolved.[19,20]

At present, there are several classes of SphK inhibitors reported: (1) SphK-nonselective inhibitors, such as *N*,*N*-dimethylsphingosine (DMS),[21] SKI-II[22] and ABC294735;[21] (2) SphK1-selective inhibitors, such as PF-543,[19] Compound **56** (derived from VPC94075),[18,23] RB005[24,25] and SLP7111228;[17] (3) SphK2-selective inhibitors, such as ABC294640,[26] SG-12[27] and SLP120701[17] (Fig. 1). Some inhibitors are reported to exert anti-inflammatory activities in animal models.[28–30] For example, ABC747080, a derivative of known SKI-II, was reported to have influence on the expression of S1P levels and on inflammation progression including colonic levels of tumor necrosis factor (TNF) α, interleukin (IL)-1β, IL-6 in dextran sodium sulfate (DSS)-induced colitis in mice.[4,31] In this model, decreased severity of DSS-induced colitis was observed when SphK was inhibited. Actually, this model is often used to evaluate potency against colitis and elucidate mechanisms involved in colitis, being considered as one of the most widely used models for understand-

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http://dx.doi.org/10.1016/j.bmc.2016.05.047
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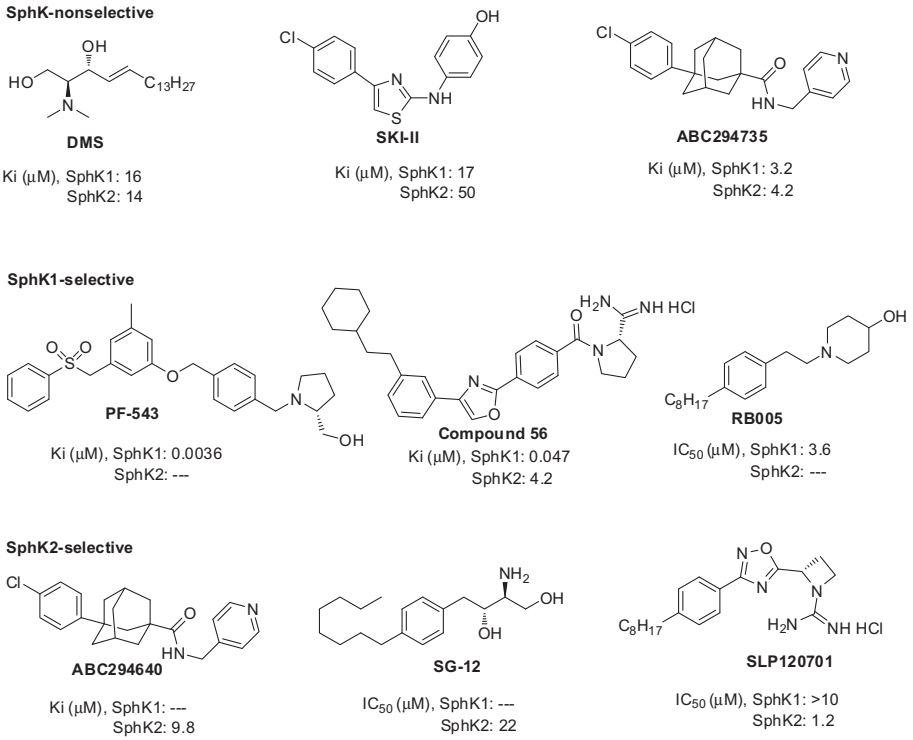

Figure 1. Representative structures and potencies of SphK inhibitors.

ing inflammatory bowel disease in human.[32,33] Herein, targeting the SphK pathway can treat colitis progression with concomitant decrease in inflammatory cytokines. Considering the importance of SphK inhibition in the therapy of colitis, novel SphK inhibitors are urgently needed to develop and attract the attention of medicinal chemists throughout the world.

In this study, we report a series of 51 compounds derived from *N*′-(3-(benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide scaffold. Among these compounds, **1** was first identified via structure-based hierarchical screening in recent study.[34] It showed potent activity against SphK in vitro. Thus **1** was selected as a hit for further optimization. Other 50 derivatives were designed, synthesized, and evaluated their ability to decrease SphK1 and SphK2 levels. Structure–activity relationship (SAR) studies indicated a more promising compound **33** for further analysis. It showed potent activity against SphK in cellular level and suppressed colitis in DSS mouse models. As far as we know, these compounds are first confirmed as SphK inhibitors by us, exhibiting favorable suppressive activity and serving as an ideal lead compound for further development of anti-inflammatory agents.

2. Results and discussion

2.1. Chemical synthesis

The synthetic route we employed to synthesize a class of aromatic compounds **1**–**51** was shown in Scheme 1. Accordingly, 3- or 4-(substituted benzyloxy)benzaldehyde (**III**) was synthesized via substitution reaction of 3- or 4-hydroxybenzaldehyde (**II**) with substituted benzyl bromide (**I**).[35,36] Treatment of substituted carboxylate (**IV**) with hydrazine monohydrate (**V**) in refluxing ethanol afforded the common intermediate or substituted carbohydrazide (**VI**).[37] Subsequent condensation was accomplished with **III**, which produced the corresponding desired target compounds **1**–**51** at reflux in excellent yield.[38,39]

2.2. Inhibitory activity on SphK1 and SphK2 and preliminary SAR

In an effort to discover new SphK inhibitors, we performed an enzyme-based screening for each compound to evaluate their ability to decrease SphK1 and SphK2 activity in 384-well plates.[40–42] Compound **33** showed the best inhibitory effect on SphK1 and SphK2 activity followed by **37** and **20**. In comparison, the positive control DMS displayed modest activity (Table 1).

To determine the effect of different alkyl or halogen substituent on ring A toward SphK1 activity, a set of compounds (**1**–**21**) around the phenyl ring were afforded to assess the extent of SphK1 inhibition. First, several examples incorporating either –F or –Cl atom were prepared. The great lack of inhibitory activity displayed by –F or –Cl substitution against SphK1 indicated that the halogen group was not tolerated except 2-Cl substituent (**11**). We also assessed the role of electron-drawing groups in the lipophilic tail of compounds. Changing the $-CF_3$ to –CN substitution at *ortho*- or *para*-position, the inhibitory activity against SphK1 was generally eliminated. The result confirmed that $-CF_3$ substituent played a more important role in increasing activity. However, it is worth mentioning that the $-CF_3$ substituents (**6**) at *meta*-position was inactive and –CN substituents (**16**) showed little inhibition. At this position, the introduction of electron-drawing groups exerted a negative influence on inhibition.

To further evaluate the effect of the electron-donating group on the ring A against SphK1 activity, we designed and synthesized some derivatives with $-CH_3$, $-C(CH_3)_3$ or $-OCH_3$ substitution. The compound with 3-OCH_3 substitution (**20**) increased SphK1 inhibition remarkably, suggesting that the 3-OCH_3 substituent maintained an efficient binding with SphK1 and had a positive effect on activity. When 4-OCH_3 group (**21**) was substituted, the activity was abolished completely, indicating that the substitution position was responsible for mediating the SphK1 activity. At *para*-position, when a $-C(CH_3)_3$ substituent (**14**) was further shortened to a $-CH_3$