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Structure–activity relationship studies of the lipophilic tail region of sphingosine kinase 2 inhibitors

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ABSTRACT

Sphingosine-1-phosphate (S1P) is a ubiquitous, endogenous small molecule that is synthesized by two isoforms of sphingosine kinase (SphK1 and 2). Intervention of the S1P signaling pathway has attracted significant attention because alteration of S1P levels is linked to several disease states including cancer, fibrosis, and sickle cell disease. While intense investigations have focused on developing SphK1 inhibitors, only a limited number of SphK2-selective agents have been reported. Herein, we report our investigations on the structure–activity relationship studies of the lipophilic tail region of **SLR080811**, a SphK2-selective inhibitor. Our studies demonstrate that the internal phenyl ring is a key structural feature that is essential in the **SLR080811** scaffold. Further, we show the dependence of SphK2 activity and selectivity on alkyl tail length, suggesting a larger lipid binding pocket in SphK2 compared to SphK1.

Sphingosine 1-phosphate (S1P) is both an intermediate in the catabolism of sphingolipids and an extracellular signaling molecule. The synthesis of S1P in vivo is controlled by two isoforms of sphingosine kinase (SphK1 and SphK2), which phosphorylate sphingosine (Sph) to S1P. S1P is involved in a variety of important intracellular and extracellular functions through a complex network of signaling pathways including G-protein coupled receptors S1P1–5. S1P signaling has been associated with a variety of diseases including cancer, fibrosis, multiple sclerosis, and sickle cell disease.[1–4] As a result of its key role in Sph and S1P metabolism, regulation of SphKs has attracted an increasing amount of attention as a therapeutic target. The ability to control SphK function would also aid in the understanding of their *in vivo* function as well as their effects in the sphingolipid signaling pathway.

Many differences exist between SphK1 and SphK2 including size, cellular localization, and intracellular roles.[5,6] While double knockout studies in mice suggests that SphKs are the sole source of S1P, some functional redundancy exists as SphK1 or SphK2 null mice are viable and fertile. Although inhibitor development towards SphK1 has been a focus of intense studies,[7] inhibitors of SphK2 are emerging (Fig. 1). For example, **ABC294640** (K_i = 10 μM) was the first inhibitor with SphK2 activity that has been deployed in a variety of disease models including lupus nephritis, diabetic nephropathy, Crohn's disease, ulcerative colitis, and osteoarthritis.[8,9] However, it was recently reported to inhibit estrogen receptors in breast cancer cells by acting as a partial agonist similar to tamoxifen.[10] Another inhibitor, thiazolidine-2,4-dione **K145** (K_i = 6.4 μM), which is an analog of sphingosine was recently reported as a selective SphK2 inhibitor.[11] **K145** was shown

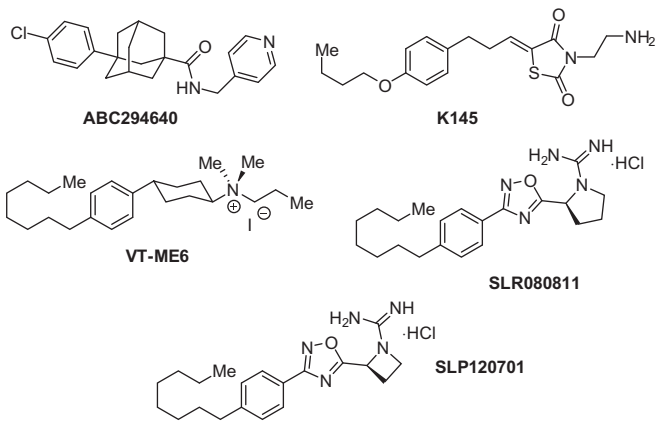

Figure 1. Structure of sphingosine kinase 2 inhibitors.

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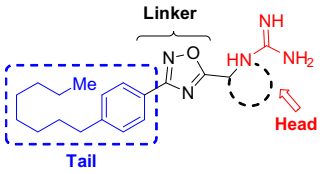
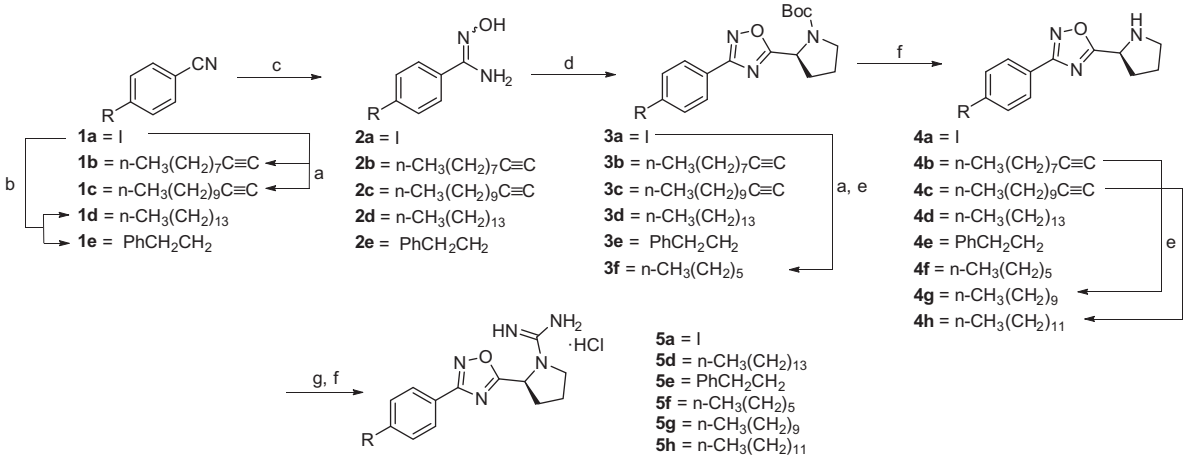


Figure 2. Pharmacophore of guanidine-based inhibitors.

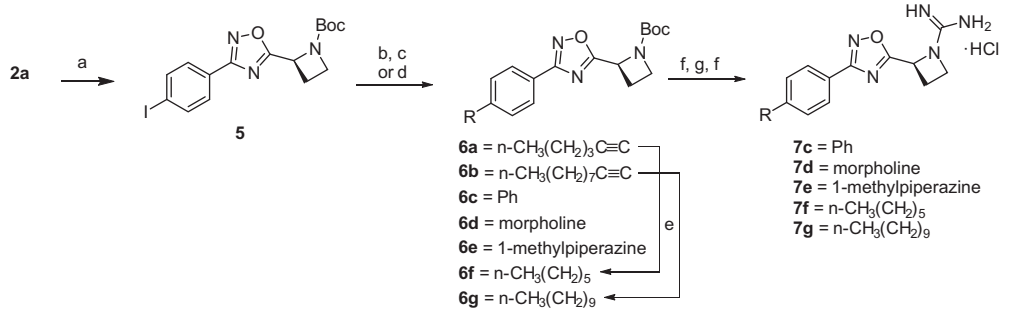
to inhibit leukemia cell growth in vitro as well as in a xenograft mouse model.

Due to our interest in understanding the in vivo function of SphK2 and the lack of highly potent and selective inhibitors,[12] we focused our studies in developing unique scaffolds to achieve our goals. Our first generation inhibitor, **VT-ME6**, contained a quaternary ammonium group as a warhead and established that a positively charged moiety is necessary for engaging key amino acid residues in the enzyme binding pocket.[13,14] This compound is moderately potent (K_i = 8 μM) and displays three-fold selectivity for SphK2 over SphK1. Subsequent improvement resulted in a scaffold that featured a 1,2,4-oxadiazole linker and guanidine as warhead: **SLR080811** possesses a K_i of 13.3 μM and 1.3 μM for SphK1 and SphK2, respectively.[15] A significant finding from these studies was that pharmacological inhibition of SphK2 resulted in elevated S1P levels in mice. Further structure–activity relationship studies on the guanidine core revealed that an azetidine-containing derivative **SLP1201701** improved the half-life to 8 h in mice.[16] In this report, we detail our investigations on the tail region of the scaffold (Fig. 2). Our studies demonstrate that the internal phenyl ring is essential to maintain inhibitory activity for SphK2 and that the alkyl tail length has a significant effect on the potency and selectivity towards SphK2.

The synthesis of **SLR080811** derivatives with varying alkyl length as well as heterocycles attached to the phenyl ring is shown in Schemes 1 and 2. In Scheme 1, 4-iodobenzonitrile was cross-coupled to a series of alkynes or hydroborated intermediates under standard Sonogashira or Suzuki–Miyaura conditions. Subsequent reaction with hydroxylamine afforded amidoximes **2a**–**e**, which were cyclized to 1,2,4-oxadiazoles **3a**–**f** in the presence of HCTU and Boc-L-Proline. Deprotection with HCl and reduction of alkynyl groups with tosylhydrazine at refluxing conditions yielded amines **4a**–**h**. To install the guanidine moiety, the amines were treated with DIEA and *N*,*N*′-Di-Boc-1*H*-pyrazole-1-carboxamidine for several days at room temperature and deprotected with HCl to produce the desired derivatives **5a**,**d**,**f**–**h**. A similar synthetic strategy was employed to access the remaining phenyl/alkyl derivatives (**7c** and **7f**–**g**); however, heterocycles **7d**–**e** were obtained via Buchwald–Hartwig coupling conditions as shown in Scheme 2. Similarly, Scheme 3 illustrates the synthesis of various



Scheme 1. (a) Alkyne (2 equiv), TEA (5 equiv), DMF, $PdCl_2(PPh_3)_2$ (0.05 equiv), CuI (0.03 equiv), 80 °C, 18 h, (72–93%); (b) (i) alkene, 0.5 M 9-BBN, in THF, rt, 12 h; (ii) $Pd(dppf)Cl_2$, Cs_2CO_3, DMF, 70 °C, 18 h, (75–93%); (c) $NH_2OH{\cdot}HCl$ (3 equiv), TEA (3 equiv), EtOH, 80 °C, 6 h, (43–95%); (d) Boc-L-Proline (1.4 equiv), DIEA (1.4 equiv), HCTU (1.8 equiv), DMF, 110 °C, 18 h, (25–65%); (e) DME (20 vol/wt), 4-toluenesulfonyl hydrazide (10 equiv), TEA (5 equiv), reflux, (67–71%); (f) HCl/MeOH, (35–100%); (g) DIEA (3 equiv), *N*,*N*′-Di-Boc-1*H*-pyrazole-1-carboxamidine (1.05 equiv), CH_3CN, rt, 3 days, (27–76%).



Scheme 2. (a) Boc-L-azetidine (1.4 equiv), DIEA (1.4 equiv), HCTU (1.8 equiv), DMF, 110 °C, 18 h, (63%); (b) alkyne (2 equiv), TEA (5 equiv), DMF, $PdCl_2(PPh_3)_2$ (0.05 equiv), CuI (0.03 equiv), 80 °C, 18 h, (33–57%); (c) phenylboronic acid (1.3 equiv), Cs_2CO_3 (equiv.), DMF, $PdCl_2(dppf)$ (0.04 equiv), 80 °C, 18 h, (91%); (d) amine, $Pd(dba)_3$, Cs_2CO_3, $PtBu_3$, toluene, 120 °C, 6 d, (81–83%); (e) DME (20 vol/wt), 4-toluenesulfonyl hydrazide (10 equiv), TEA (5 equiv), reflux, (60–71%); (f) HCl/MeOH, (78–96%); (g) DIEA (3 equiv), *N*,*N*′-Di-Boc-1*H*-pyrazole-1-carboxamidine (1.05 equiv), CH_3CN, rt, 3 days, (43–66%).