SUPPORTING INFORMATION

Structure Activity Relationships and Molecular Modeling of Sphingosine Kinase Inhibitors

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Sphingosine kinase activity assays.

For SK2 activity assays, sphingosine (Sph) was complexed with fatty acid free bovine serum albumin (final concentration, 0.2 mg/mL) in reaction buffer 1 containing 20 mM Tris (pH 7.4), 1 mM EDTA, 1 mM Na_3VO_4, 40 mM β–glycerophosphate, 1 mM NaF, 0.007% (v/v) β-mercaptoethanol, 20% (v/v) glycerol, 10 μg/mL aprotinin, 10 μg/mL soybean trypsin inhibitor, 1 mM PMSF, 0.5 mM 4-deoxypyridoxine, and 400 mM KCl. Inhibition of SK2 activity was determined by incubating 37 ng of purified SK2 for 30 min at 30 °C in the presence of 10 μM Sph, 250 μM of [γ-^{32}P]ATP (specific activity, 4.4×10^4 cpm/nmol) in 10 mM $MgCl_2$, and varying concentrations of the inhibitors dissolved in DMSO or control (5% v/v DMSO). For SK1 activity assays, Sph was solubilized in Triton X-100 (final concentration, 0.063% w/v) and combined with buffer 1 without KCl. SK1 activity was determined by incubating 30 μg of recombinant SK1 in lysates from HEK 293 cells for 30 min at 30 °C, in the presence of 3 μM Sph, 250 μM of [γ-^{32}P]ATP in 10 mM $MgCl_2$ with or without inhibitor dissolved in DMSO or control (5% v/v DMSO). SK1 and SK2 reactions were terminated by the addition of 500 μL of 1-butanol and were then mixed with 1 mL of 2 M KCl. The organic phase containing [^{32}P]-S1P was extracted by washing twice with 1 mL of 2 M KCl before quantification by Cerenkov counting.

Compounds bearing hydroxyl groups were assessed for their ability to act as substrates for SK1 and SK2. Of these compounds, only **RB-037**, **RB-041,** and **RB-043** were weak substrates for SK2 (Fig. S1).

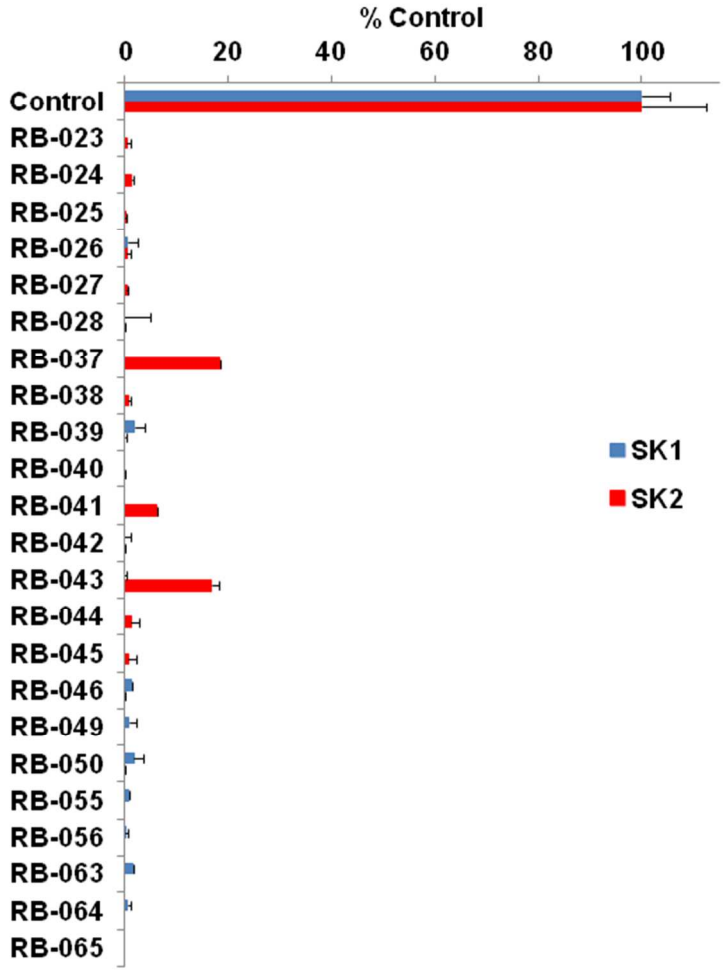

Fig. S1. Evaluation of compounds as putative substrates of SK1 and SK2. SK1 and SK2 activity was measured using 50 μM compound and 250 μM ATP in the absence of Sph (n = 3 for each compound). The results are expressed as % of control ± S.D. Control = activity using Sph alone (3 μM for SK1 and 10 μM for SK2) and is represented as 100%, against which each compound alone is compared.

As shown in Fig. S2, **RB-035** is predicted to utilize its carbonyl group to form hydrogen bonds with the hydroxyl group of S168 and water. Therefore, **RB-035** exhibits a different binding mode than **RB-005** to explain its inhibitory activity.

Fig. S2. Docking of **RB-035** into the active site of SK1.