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Article

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## Just Accepted





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# Structure Activity Relationships and Molecular Modeling of Sphingosine Kinase Inhibitors

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**Abstract**

The design, synthesis, and evaluation of the potency of new isoform-selective inhibitors of sphingosine kinase 1 and 2 (SK1 and SK2), the enzyme that catalyzes the phosphorylation of D-*erythro*-sphingosine to produce the key signaling lipid, sphingosine 1-phosphate, are described. Recently, we reported that 1-(4-octylphenethyl)piperdin-4-ol (**RB-005**) is a selective inhibitor of SK1. Here we report the synthesis of 43 new analogues of **RB-005**, in which the lipophilic tail, polar headgroup, and linker region were modified to extend the structure-activity relationship profile for this lead compound, which we explain using modeling studies with the recently published crystal structure of SK1. We provide a basis for the

key residues targeted by our profiled series, and provide further evidence for the ability to discriminate between the two isoforms using pharmacological intervention.

## Introduction

The lipid kinase, sphingosine kinase (SK), plays a myriad of roles in regulating cell survival, growth, and migration of mammalian cells through its product, sphingosine 1-phosphate (S1P). S1P is a ligand for five cell-surface G-protein coupled receptors and for several intracellular targets, such as histone deacetylase 1 and 2 (HDAC1/2, which regulate gene expression).[1] There are two isoforms of sphingosine kinase, SK1 and SK2, that are encoded by different genes and which exhibit distinct biochemical properties, substrate and inhibitor sensitivities, and sub-cellular distribution. SK1 and SK2 have redundant roles to some extent as knockout of both genes is embyronically lethal in mice, whereas animals survive when either gene is removed alone.[2] Moreover, there is evidence that both SK1 and SK2 play a similar role in certain cancers.[3,4] Surprisingly, SK1 and SK2 may have opposing roles in inflammation, being largely pro-inflammatory and anti-inflammatory, respectively. On the other hand, a pro-inflammatory role for SK2 in other cell types has also been reported.[3] The differing roles of SK1 and SK2 in inflammatory disease and supporting data from knockout mice make a compelling case for the development of isoform-selective inhibitors in order to elucidate the functions and roles of each isozyme and for designing drugs for therapeutic intervention in pathophysiological processes such as inflammatory diseases and cancer.

Various SK inhibitors have been identified (see Fig. 1 for examples). Enzyme kinetic studies show that many of these are competitive with sphingosine (Sph). The initial SK inhibitors were Sph analogues, such as D,L-*threo*-dihydrosphingosine which has a $K_i$ of ~5 μM.[3] *N*,*N*-Dimethylsphingosine also inhibits both SK isoforms. The first SK1-selective inhibitor to be reported was the water-soluble Sph analogue

**SK1-I** (also known as **BML-258**; $K_i$ ~10 μM).[5] The most potent nanomolar SK1-selective inhibitor is **PF-543**.[6] However, this compound is also a substrate for SK1, thereby compromising data interpretation. Analogues of the immunosuppressive agent FTY720 (Gilenya) have been prepared using a synthetic route starting with 4-octylphenethyl alcohol and were found to be SK1-selective inhibitors (e.g., **RB-005**).[7] FTY720 is an inhibitor of SK1,[8] and also inhibits other sphingolipid-metabolizing enzymes, such as ceramide synthase and S1P lyase.[9,10] We also recently showed that SK1 contains an allosteric site.[11] Replacement of the amino group in (*S*)-FTY720-vinylphosphonate with an azido group changes this compound from an allosteric inhibitor to an activator of SK1.[12] Therefore, allosteric inhibitors of SK1 are also an exciting option for future study. With regard to SK2-selective inhibitors, **ABC294640**,[13] (*R*)-FTY720 methyl ether (**ROMe**),[14] **K145** (3-(2-amino-ethyl)-5-[3-(4-butoxyl-phenyl)-propylidene]-thiazolidine-2,4-dione),[15] and **SLR080811** ((*S*)-2-[3-(4-octylphenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-carboximidamide)[16] have $K_i$ values in the range of ~1-10 μM. Taken together, it is clear that there is still a need to develop new SK1 and SK2 inhibitors, both to increase the number of selective tools that can be used to interrogate the biology of these enzymes and to increase the possibility of having useful new therapeutic agents to treat disease.

In a recent study, we showed that **RB-005** is a highly selective SK1 inhibitor.[7] Herein, we describe the synthesis of analogues of the known SK1 inhibitors **RB-005**, **FTY720**,[8] **SKi**,[17] compounds **36a**[18,19] and **82**,[20] **BML-258**,[5] **CB5468139**,[21] and **SLR080811**[16] These new analogues, which were designed to possess some degree of structural similarity to the known inhibitors, were evaluated through enzyme activity studies as inhibitors of SK1 and SK2. Structure-activity relationship (SAR) studies are discussed, which are supported by an analysis of molecular modeling poses of the inhibitors in the active site of human SK1.

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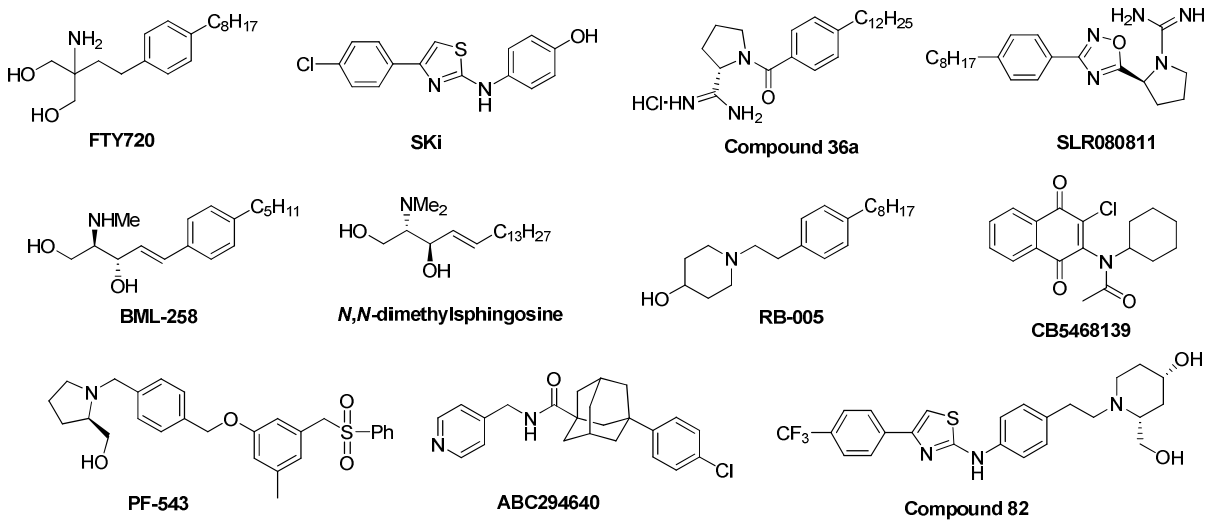



**Fig. 1.** Structures of selected SK inhibitors.

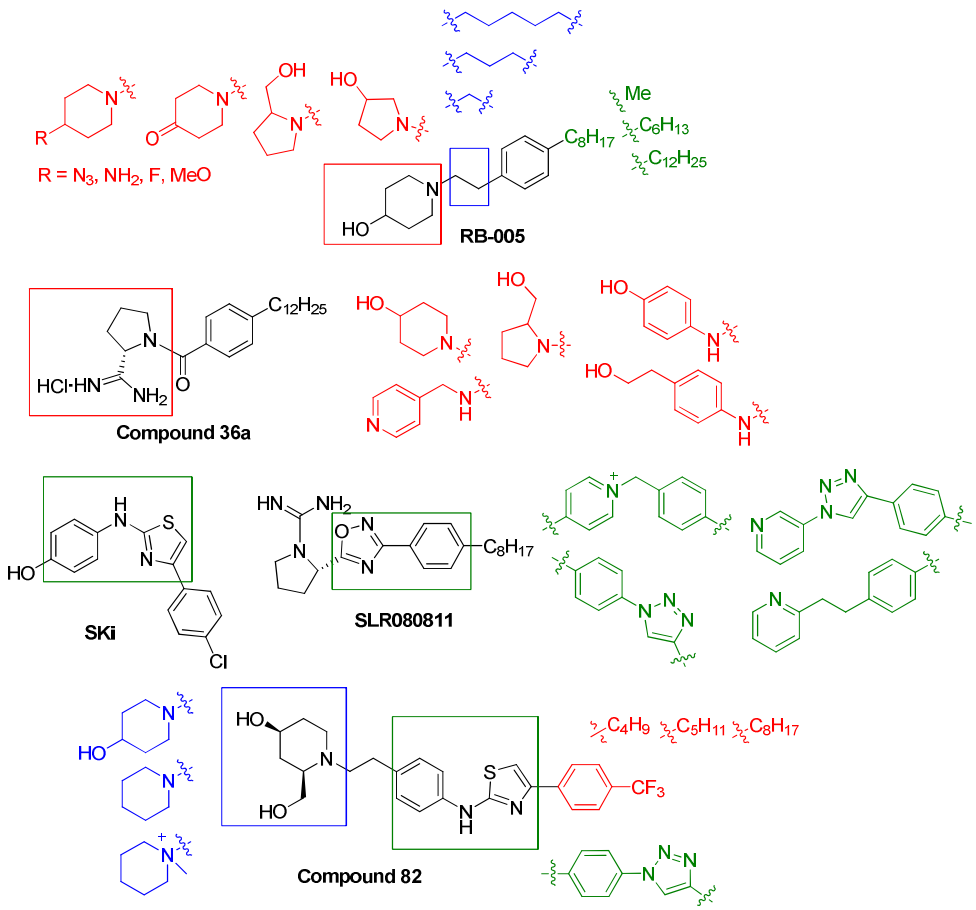
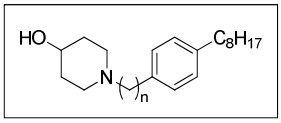


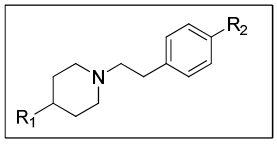

**Fig. 2.** Modifications introduced into the known SK inhibitors **RB-005**, **SKi**, **SLR080811**, **36a** (**VPC96091**), and **82**.

## Results and Discussion

The structures of the new compounds and their key structural modifications are shown in Tables 1-4.

**Table 1** Piperidyl analogues of **RB-005** synthesized for evaluation as SK inhibitors



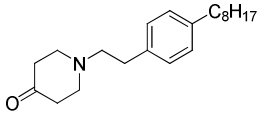

| Compound | Structure | Compound | Structure |
|---|---|---|---|
| **RB-023** | n = 1 | **RB-032** | $R_1 = NH_2$, $R_2 = C_8H_{17}$ |
| **RB-024** | n = 3 | **RB-033** | $R_1 = NH_2$, $R_2 = C_{12}H_{25}$ |
| **RB-025** | n = 4 | **RB-034** | $R_1 = F$, $R_2 = C_8H_{17}$ |
| **RB-026** | $R_1 = OH$, $R_2 = CH_3$ | **RB-035** |  |
| **RB-027** | $R_1 = OH$, $R_2 = C_6H_{13}$ | **RB-036** | $R_1 = OMe$, $R_2 = C_8H_{17}$ |
| **RB-028** | $R_1 = OH$, $R_2 = C_{12}H_{25}$ | | |
| **RB-029** | $R_1 = N_3$, $R_2 = CH_3$ | | |
| **RB-030** | $R_1 = N_3$, $R_2 = C_8H_{17}$ | | |
| **RB-031** | $R_1 = NH_2$, $R_2 = CH_3$ | | |



**Table 2** Pyrrolidine analogues of **RB-005** synthesized for evaluation as SK inhibitors

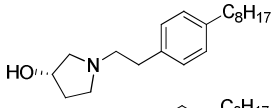
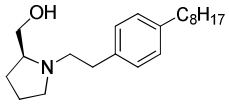
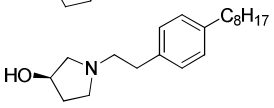
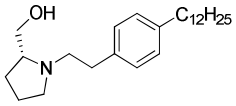
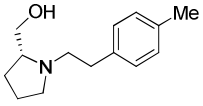
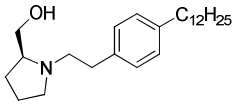
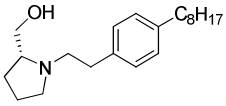
| Compound | Structure | Compound | Structure |
|---|---|---|---|
| **RB-037** |  | **RB-041** |  |
| **RB-038** |  | **RB-042** |  |
| **RB-039** |  | **RB-043** |  |
| **RB-040** |  | | |

**Table 3** Benzamide analogues of **RB-005** synthesized for evaluation as SK inhibitors

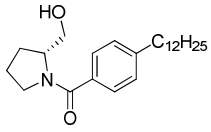
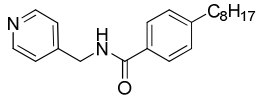
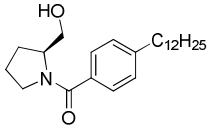
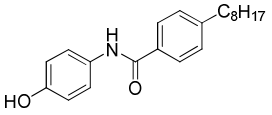
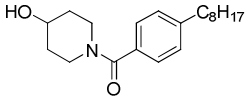
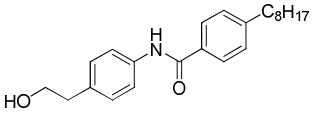
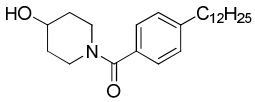
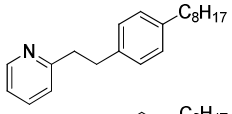
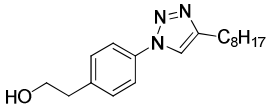
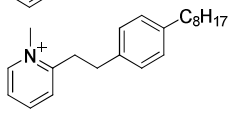
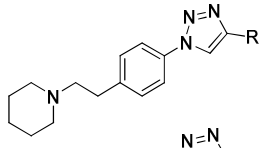
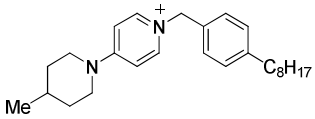
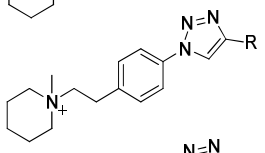
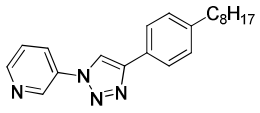
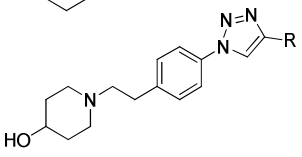
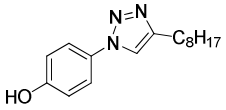
| Compound | Structure | Compound | Structure |
|---|---|---|---|
| **RB-044** |  | **RB-048** |  |
| **RB-045** |  | **RB-049** |  |
| **RB-046** |  | **RB-050** |  |
| **RB-047** |  | | |

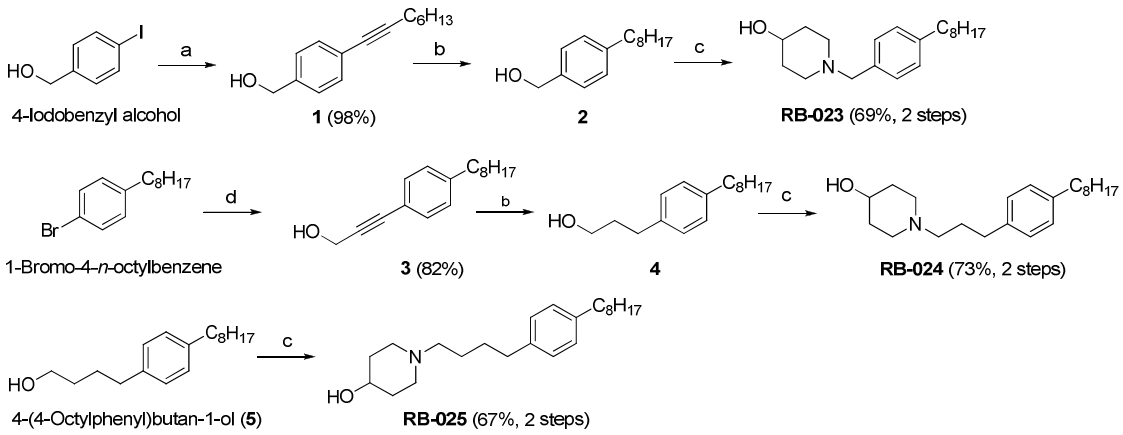
**Table 4** Pyridine, pyridinium, and triazole analogues of **RB-005** synthesized for evaluation as SK inhibitors

| Compound | Structure | Compound | Structure |
|---|---|---|---|
| **RB-051** |  | **RB-056** |  |
| **RB-052** |  | **RB-057** R = $C_4H_9$<br>**RB-058** R = $C_5H_{11}$<br>**RB-059** R = $C_8H_{17}$ |  |
| **RB-053** |  | **RB-060** R = $C_4H_9$<br>**RB-061** R = $C_5H_{11}$<br>**RB-062** R = $C_8H_{17}$ |  |
| **RB-054** |  | **RB-063** R = $C_4H_9$<br>**RB-064** R = $C_5H_{11}$<br>**RB-065** R = $C_8H_{17}$ |  |
| **RB-055** |  | | |

**Chemical Synthesis.**

*Linker Length.* The synthetic routes we employed to prepare compounds with one-, three-, and four-carbon tethers are displayed in Scheme 1. The sole carbon atom of the hydroxymethyl group of 4-iodobenzyl alcohol provided the tether in **RB-023**, whereas the three carbons of propargyl alcohol were the source of the tether in **RB-024**. In each of these compounds, a Sonogashira reaction followed by catalytic hydrogenation of the alkyne intermediate (**1** and **3**) and a $S_N2$ reaction of a mesylate intermediate derived from **2** and **4** with 4-hydroxypiperidine gave the desired compounds. We used 4-(4-octylphenyl)butan-1-ol (**5**)[22] as the starting material for the preparation of **RB-025**.

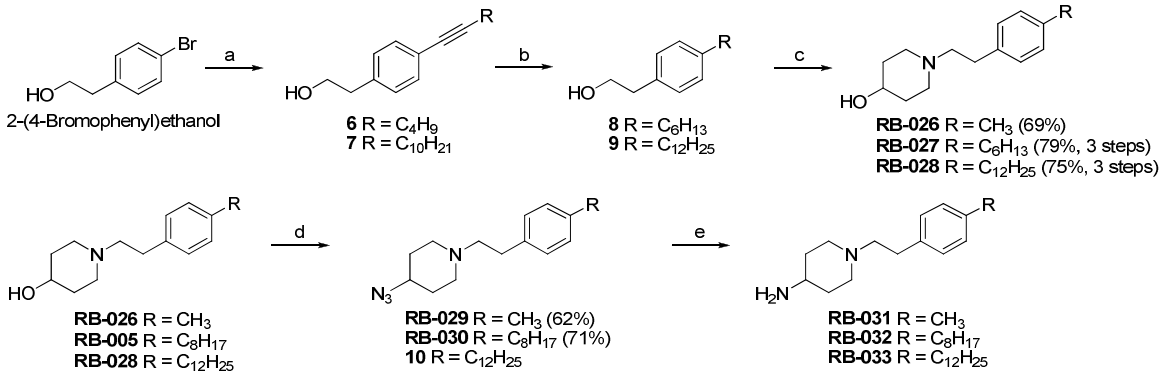
**Scheme 1**[a] Synthesis of **RB-023** – **RB-025**




[a]Reagents and conditions: (a) 1-octyne, $Pd(PPh_3)_4$, CuI, $Et_3N$, 50 °C, 12 h; (b) Pd/C, $H_2$, EtOAc, rt, 12 h; (c) i) MsCl, $Et_3N$, $CH_2Cl_2$, 3 h, rt; ii) 4-hydroxypiperidine, MeCN, 50 °C, 12 h; (d) propargyl alcohol, $Pd(PPh_3)_4$, CuI, $Et_3N$, 50 °C, 12 h.

*Modifications of the 4-Alkylphenyl and the Piperidyl Groups*. Scheme 2 shows the synthetic pathways employed to prepare **RB-026** – **RB-033**. After a Sonogashira reaction was used to install an alkynyl group with six to twelve carbons, catalytic hydrogenation of **6** and **7** afforded alkyl derivatives **8** and **9**, and $S_N2$ displacement as in Scheme 1 gave the desired compounds in good yield. To assess the role of the hydroxyl group in **RB-005**, **RB-026**, and **RB-028** in inhibition of SK, we replaced this group with an azido group via mesylation of the alcohol and reaction with sodium azide in DMF to obtain **10**, **RB-029**, and **RB-030**. Reduction of the azide afforded the amino derivatives, **RB-031**, **RB-032**, and **RB-033**.

**Scheme 2**[a] Synthesis of **RB-026** – **RB-033**

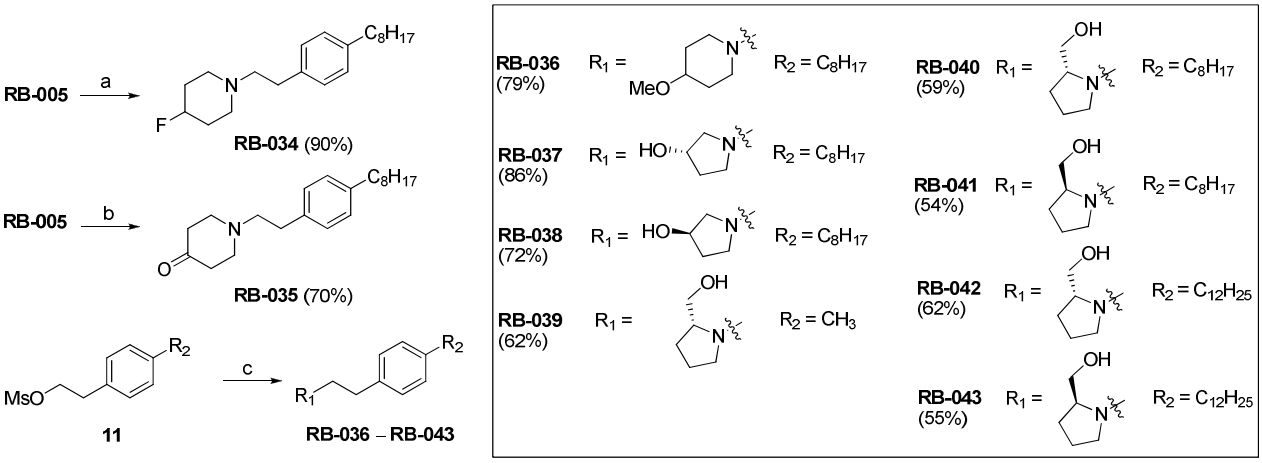



[a]Reagents and conditions: (a) 1-hexyne or 1-dodecyne, $Pd(PPh_3)_4$, CuI, $Et_3N$, 50 °C, 12 h; (b) Pd/C, $H_2$, EtOAc; (c) i) MsCl, $Et_3N$, $CH_2Cl_2$; ii) 4-hydroxypiperidine, MeCN, 50 °C, 12 h; (d) i) MsCl, $Et_3N$, $CH_2Cl_2$, 3 h, rt; ii) $NaN_3$, DMF, 80 -100 °C, 12 h; (e) Pd/C, $H_2$, $CH_2Cl_2$/MeOH (1:3), rt, 12 h.

Fluorination of **RB-005** with diethylaminosulfur trifluoride (DAST) gave **RB-034** in 90% yield (Scheme 3). The 4-keto derivative **RB-035** was synthesized by oxidation of the 4-hydroxyl group of **RB-005** with pyridinium chlorochromate. Reaction of mesylate **11**[7] with 4-methoxypiperidine provided **RB-036**. Similarly, reaction of commercially available chiral pyrrolidines containing a 3-hydroxyl or a

2-hydroxymethyl substituent gave **RB-037** – **RB-043**.

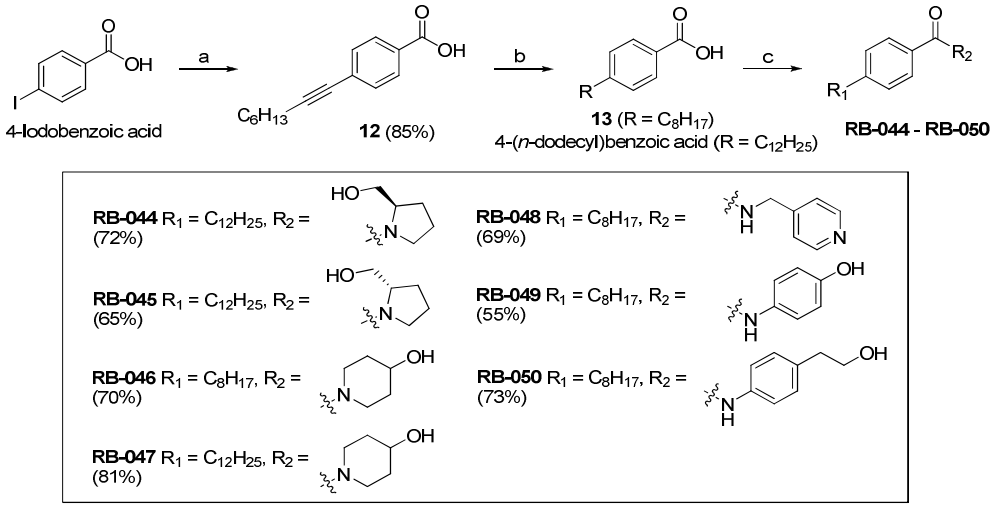
**Scheme 3**[a] Synthesis of **RB-034** – **RB-043**




[a]Reagents and conditions: (a) DAST, $CH_2Cl_2$, 0 °C - rt, 5 h, rt; (b) PCC, $CH_2Cl_2$, 4 h, rt; (c) cyclic amine, MeCN, 50 °C, 12 h.

*Benzamide derivatives.* Benzamide-containing analogues **RB-044** – **RB-050** were prepared from 4-iodobenzoic acid as outlined in Scheme 4. Alkyne intermediate **12** was reduced to carboxylate **13**, which was converted to the acyl chloride with thionyl chloride and then treated with the desired cyclic amine in the presence of potassium carbonate.

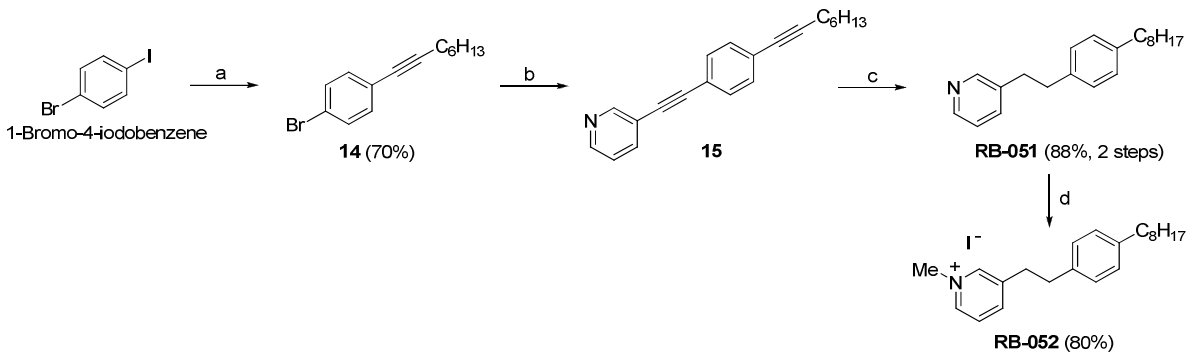
**Scheme 4**[a] Synthesis of **RB-044** – **RB-050** from 4-iodobenzoic acid




[a]Reagents and conditions: (a) 1-octyne, $Pd(PPh_3)_4$, CuI, $Et_3N$, 60 °C, 12 h; (b) Pd/C, $H_2$, EtOAc, 12 h, rt; (c) i) $SOCl_2$, $CH_2Cl_2$, reflux, 12 h; ii) cyclic amine, $K_2CO_3$, MeCN, 50 °C, 12 h.

*Quaternary Ammonium Derivatives.* To synthesize **RB-052**, which contains a pyridinium ion in the polar headgroup, we used the halogens in 1-bromo-4-iodobenzene to carry out two separate Sonogashira reactions, as shown in Scheme 5. First, we used 1-octyne to prepare alkyne **14**, which reacted with 3-ethynylpyridine to give dialkyne **15**. Reduction of **15** yielded **RB-051**, and *N*-alkylation with methyl iodide (5 equiv) in acetonitrile afforded the *N*-methylpyridinium salt **RB-052**.

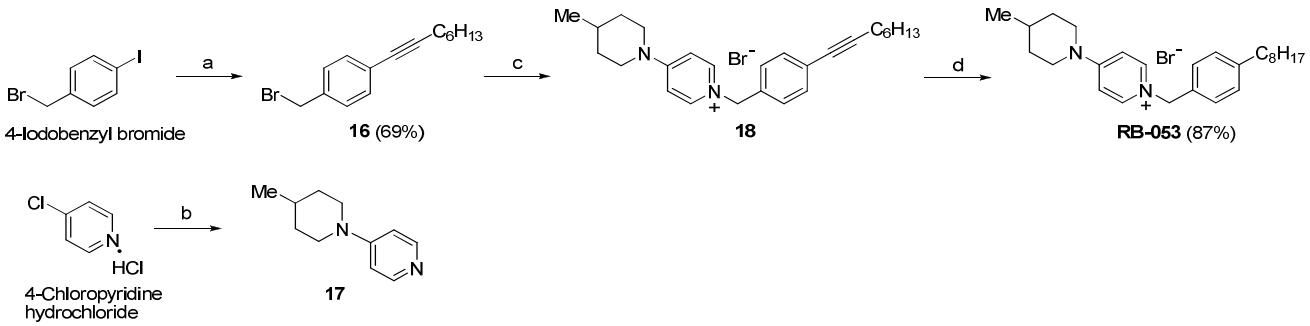
**Scheme 5**[a] Synthesis of **RB-051** and **RB-052** from 1-bromo-4-iodobenzene




[a]Reagents and conditions: (a) 1-octyne, $Pd(PP_3)_4$, CuI, $Et_3N$, 50°C, 12 h; (b) 3-ethynylpyridine, $Pd(PPh_3)_4$, CuI, $Et_3N$, 80 °C, 3 d; (c) Pd/C, $H_2$, EtOAc, 12 h, rt; (d) $K_2CO_3$, MeI, MeCN, overnight, rt.

Pyridinium salt **RB-053** was prepared by a similar route, as shown in Scheme 6. A Sonogashira reaction of 4-iodobenzyl bromide with 1-octyne provided alkyne **16**; displacement of bromide ion with 4-(4-methylpiperidin-1-yl)pyridine (**17**) afforded alkyne **18**, which provided **RB-053** on catalytic hydrogenation in $MeOH/CH_2Cl_2$ (1:3).

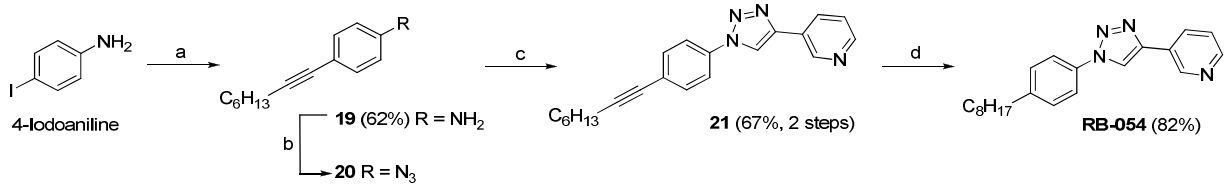
**Scheme 6**[a] Synthesis of pyridinium salt **RB-053** from 4-iodobenzyl bromide and of 4-(4-methylpiperidin-1-yl)pyridine (**17**) from 4-chloropyridine hydrochloride




[a]Reagents and conditions: (a) 1-octyne, $Pd(PPh_3)_4$, CuI, $Et_3N$; (b) 4-methylpiperidine, DIPEA, MeCN, microwave heating, 160 °C, 1 h; (c) **17**, 2-butanone, 100 °C, 3 d; (d) Pd/C, $H_2$, $MeOH/CH_2Cl_2$ (1:3).

*Triazole Derivatives*. A Pd/Cu-catalyzed Sonogashira reaction followed by a Cu(I)-catalyzed azide-alkyne 1,3-dipolar addition (click reaction) were employed to prepare **RB-054**, in which a triazole ring bearing a pyridine substituent is the headgroup (Scheme 7). Thus, Sonogashira coupling of 1-octyne with 4-iodoaniline afforded alkynyl-aniline derivative **19**. After conversion of amine **19** to aryl azide **20**, the triazole ring was installed in a click reaction with 3-ethynylpyridine; finally, catalytic hydrogenation of alkyne **21** gave **RB-054**.

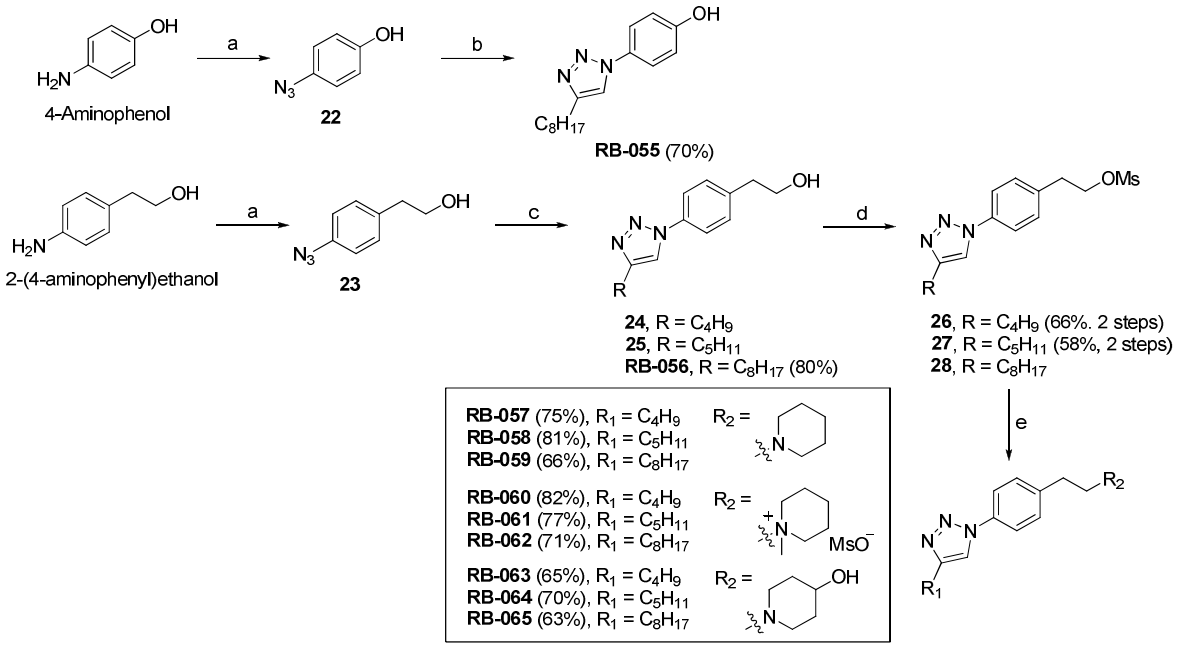
**Scheme 7** Synthesis of **RB-054** from 4-iodoaniline




[a]Reagents and conditions: (a) 1-octyne, $Pd(PPh_3)_4$, CuI, $Et_3N$, 60 °C, 12 h; (b) i) 10% aq. HCl, $NaNO_2$; ii) $NaN_3$; (c) 3-ethynylpyridine, sodium ascorbate, $CuSO_4$, *t*-BuOH/$H_2O$ (1:1); (d) Pd/C, $H_2$, EtOAc, rt, 2 d.

We reversed the location of the triazole in analogues **RB057** – **RB-065**. As shown in Scheme 8, the headgroup in these analogues is a piperidyl derivative and the lipophilic tail contains an alkyl-substituted triazole. Azides **22** and **23** were prepared from commercially available 4-aminophenol and 2-(4-aminophenyl)ethanol, respectively. The click reaction with terminal alkynes afforded triazoles **RB-055**, **24**, **25**, and **RB-056**. To prepare **RB-059** – **RB-065**, alcohols **24**, **25**, and **RB-056** were converted to their corresponding mesylates **26, 27**, and **28**, which were treated with piperidine, 1-methylpiperidine, or 4-hydroxypiperidine.

**Scheme 8**[a] Synthesis of **RB-055** – **RB-065**




[a]Reagents and conditions: (a) i) 10% aq. HCl, $NaNO_2$; ii) $NaN_3$; (b) 1-decyne, sodium ascorbate, $CuSO_4$, *t*-BuOH/$H_2O$ (1:1), 12 h, rt; (c) acetylenic substrate (**24**: 1-hexyne; **25**: 1-heptyne, **RB-056**: 1-decyne), sodium ascorbate, $CuSO_4$, *t*-BuOH, $H_2O$; (d) MsCl, $Et_3N$, $CH_2Cl_2$, 0 °C - rt, 5 h; (e) piperidine, MeCN (**RB-059** – **RB-061**), 1-methylpiperidine, MeCN (**RB-060** – **RB-062**) or 4-hydroxypiperidine, MeCN, 50 °C, 12 h (**RB-063** – **RB-065**).

**Effects on SK1 and SK2 Activity and SAR.**

We previously demonstrated the importance of the 4-hydroxypiperidinyl group in the selective inhibition of SK1,[7] which was subsequently confirmed by Gustin et al., who generated chiral piperidyl analogues bearing hydroxyl and hydroxymethyl groups.[20] To examine SAR among a panel of related compounds, we prepared a series of analogues bearing a 4-hydroxypiperidinyl group but varied the linker length between the aryl group and the piperidine. We also assessed the role of the alkyl substituent in the aryl group. To evaluate compound selectivity against SK1 or SK2, the assays were performed (see

Supporting Information) using Sph at concentrations of 3 and 10 μM (the $K_m$ values of SK1 and SK2, respectively), which corresponds to 50% substrate saturation and enables a qualitative estimation of selectivity by comparing the % inhibition of each kinase using a fixed concentration of inhibitor. We consider this approach to be an appropriate comparison of selectivity since both enzymes exhibit 50% occupancy with the substrate. Compounds that were found to be effective inhibitors were then analyzed in more detail by performing dose-response curves.

We have previously shown that **RB-005** (the 'parent compound') is a selective inhibitor of SK1 and exhibits an $IC_{50} = 3.6 \pm 0.38$ μM at 3 μM Sph (which corresponds to the $K_m$ of SK1) and reduces SK1 activity by ~90% at 50 μM **RB-005**.[7] The effect of linker length on potency was assessed by comparing the % inhibition of SK1 and SK2 obtained with **RB-023** (which has a one-carbon tether), **RB-024** (three-carbon tether), and **RB-025** (four-carbon tether). The linker length did not significantly alter the ability of **RB-023**, **RB-024**, and **RB-025** to inhibit SK1 activity (Fig. 3). **RB-023** – **RB-025** also retained selectivity for SK1 over SK2.

The aliphatic chain at the *para* position of the benzene ring of FTY720 is $C_8H_{17}$, which is known to be optimal for the action of FTY720 on its targets such as S1P receptors.[23] Knott et al.[24] reported that the ability of quaternary ammonium salts with a phenyl-substituted cyclohexylamine scaffold to inhibit SK2 was affected by the alkyl chain length. To examine the role of the alkyl substituent on the benzene ring of RB-005, and thus the lipophilicity of the molecule, we compared the inhibitory activity of **RB-026** (which has a methyl group as the alkyl substituent), **RB-027** (which has a *n*-hexyl group), **RB-005** (which has a *n*-octyl group), and **RB-028** (which has a *n*-dodecyl group). SK1 inhibition was decreased by more than 6-fold in **RB-026** compared with **RB-023**. The almost complete lack of inhibition displayed by **RB-026** against SK1 indicates that a larger alkyl group than a methyl group is required for inhibitory activity. We also evaluated the effect of alkyl chain length in the lipophilic tail of compounds

in which the 4-hydroxypiperidinyl group was replaced by a 4-aminopiperidinyl group (see below). Changing the *n*-octyl group of **RB-032** to a methyl or *n*-dodecyl group gave **RB-031** and **RB-033**, respectively, and eliminated the inhibitory activity toward SK1. These results confirm the critical requirement for the *n*-octyl group.

Next, we probed the role of the 4-hydroxyl group of **RB-005** by replacing it with an azido, amino, fluoro, keto, or methoxy group (**RB-029** – **RB-036**). Azido replacement (**RB-029**, **RB-030**) reduced SK1 inhibition markedly, while replacement of the 4-hydroxyl group with an amino group (**RB-032**) diminished the potency of SK1 inhibition (Fig. 4). The isoform selectivity of SK1 over SK2 was retained for **RB-032**, suggesting that the amino group replacement maintains efficient binding to SK1. Replacement of the 4-hydroxyl group of **RB-005** with a fluoro (**RB-034**) or methoxy group (**RB-036**) eliminated inhibitory activity against SK1, while replacement with a keto group to produce **RB-035** increased inhibition of SK2 and maintained inhibition of SK1 but eliminated the isoform selectivity.

To examine the role of the piperidyl group in inhibition of SK, we replaced it with a pyrrolidine ring; the hydroxyl-containing substituent was retained (as either a chiral hydroxyl or a chiral hydroxymethyl group) but its orientation was varied, as shown in compounds **RB-037** – **RB-043**. **RB-037** and **RB-038** retained inhibitory activity against SK1 despite having opposite configurations at C-3 of the pyrrolidin-3-ol group. Stereoisomers **RB-040** and **RB-042**, which differ in the length of the aliphatic chain ($C_8H_{17}$ vs. $C_{12}H_5$) but possess the *R* configuration at C-2 of the 2-hydroxymethyl pyrrolidinyl group, were equipotent inhibitors of SK1 and SK2 (Fig. 3 and Fig. 5A,B). The corresponding *S* enantiomers **RB-041** and **RB-043** were much less active (Fig. 3). To establish whether **RB-041** and **RB-043** were capable of inhibiting SK1 and SK2 activity in a concentration-dependent manner, we used a higher concentration of each (100 μM, compared to the 50 μM concentration data shown in Fig. 3) and found that the inhibition of SK1 and SK2 with **RB-041** was 72.2 ± 5.9% and 45.7 ± 2.6%, respectively, whereas with **RB-043** the

inhibition of SK1 and SK2 was 49.9 ± 6.2% and 49.7 ± 7%, respectively. These findings indicate that **RB-041** and **RB-043** can inhibit SK1 and SK2, but that the sensitivity of inhibition compared with **RB-040** and **RB-042** is considerably reduced. Interestingly, the *S* enantiomers **RB-041** and **RB-043** are substrates for SK2 (see Supporting Information, Fig. S1).

To further examine the influence of the length of the alkyl substituent on the benzene ring on SK activity, we assessed the extent of SK inhibition afforded by pyrrolidine derivatives **RB-039**, **RB-042**, and **RB-043**. The ability of the compound to inhibit SK1 is abolished in **RB-039** and **RB-043**, which have a methyl and a *n*-dodecyl group in the lipophilic tail, respectively.

An amidine or proline headgroup incorporated into a benzamide-containing scaffold was shown to provide potent SK1 inhibitors,[18,19] as in compound **36a** (Fig. 1). When we replaced the methylene linker between the aryl group and the heterocycle with a keto group to produce the benzamide analogues **RB-044** – **RB-050**, inhibition of SK1 was effectively abolished (Fig. 3), as were the pyridine derivatives (**RB-048** and **RB-051**). The report that quaternary ammonium salts are selective SK2 inhibitors[25] prompted us to prepare equivalent derivatives **RB-052**, **RB-053**, **RB-060**, **RB-061**, and **RB-062**, which were ineffective as SK inhibitors, although **RB-053** demonstrated a moderate selectivity for SK2 (Fig. 3). We also prepared aliphatic quaternary ammonium salts (not shown) that were inactive.

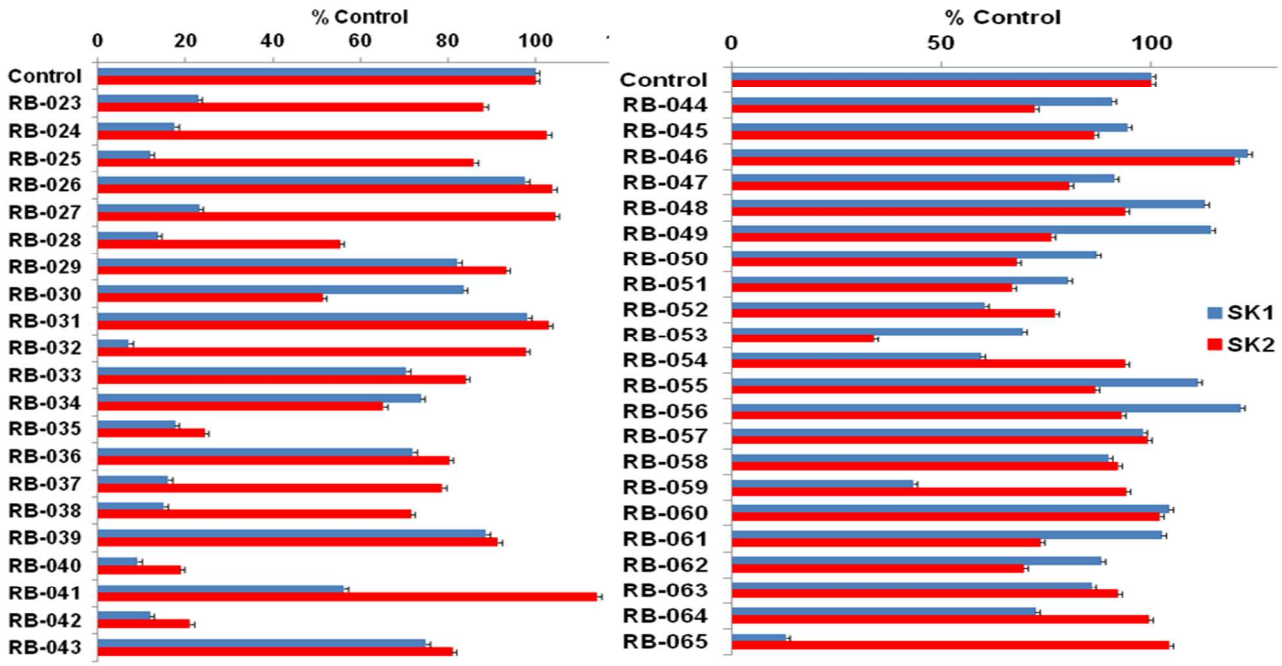



**Fig. 3.** Effect of inhibitors on SK1 or SK2 activity. SK1 activity was measured using 3 μM Sph and 250 μM ATP. SK2 activity was assayed using 10 μM Sph and 250 μM ATP (n = 3 for each compound, results are expressed as % of control ± S.D.). RB series compounds were used at 50 μM. BML-258 (50 μM) inhibited SK1 activity by 74.5 ± 3.3 % (n = 3). The control is 100% and equals activity against Sph alone.

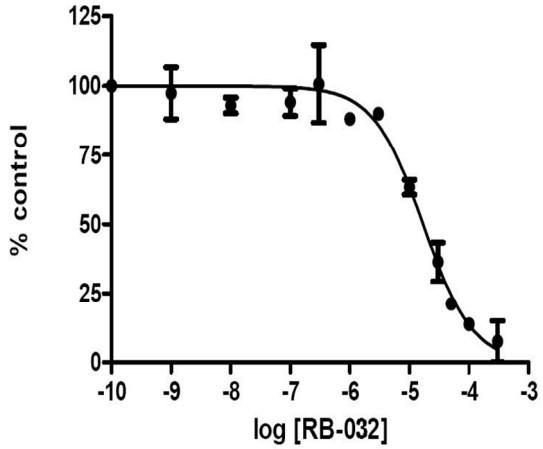



**Fig. 4.** Effect of **RB-032** on SK1 activity. Concentration-dependent inhibition of SK1 activity by **RB-032** using 3 μM Sph and 250 μM ATP. The results are expressed as % of control ± S.D.; n = 3; the control is 100% and equals activity against Sph alone. **RB-032** inhibits SK1 activity with an $IC_{50}$ = 16.9 ± 1.6 μM; **RB-005** inhibits SK1 activity with an $IC_{50}$ = 3.6 ± 0.4 μM.[7]

SK inhibitors containing a central thiazole group have been reported (e.g., **SKi** and compound **82**, Fig. 1). 1,2,3-Triazoles are mimics of thiazoles, and are easily prepared by Cu(I)-catalyzed azide/alkyne click chemistry. In our series of triazole analogues of **RB-005** (**RB-054** – **RB-065**), we found that **RB-065** was a highly selective SK1 inhibitor, whereas the other ten triazole analogues, all of which lack the 4-hydroxypiperidinyl group, were inactive (Fig. 3).

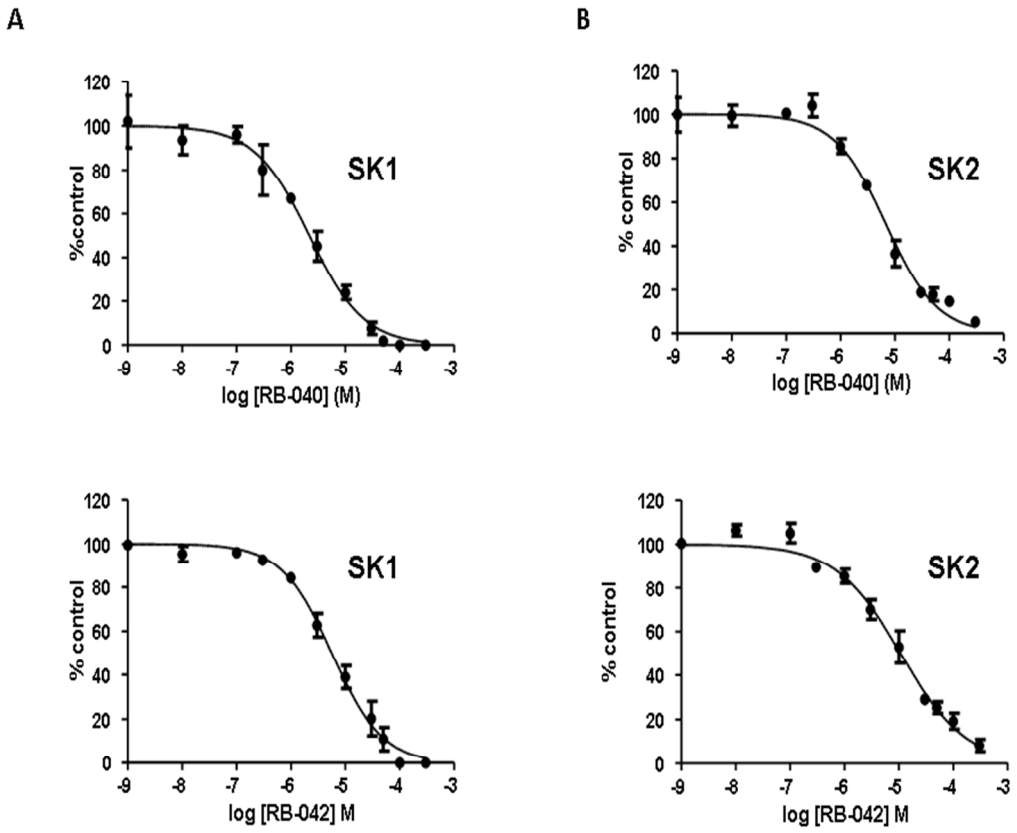



**Fig. 5.** Effect of **RB-040** and **RB-042** on (A) SK1 activity and (B) SK2 activity. Concentration-dependent inhibition of SK activity by **RB-040** and **RB-042** using 3 μM Sph (SK1) or 10 μM Sph (SK2) and 250 μM ATP. The results are expressed as % of control ± S.D. (n = 3). The control is 100% and equals activity against Sph alone. **RB-040** inhibits SK1 activity with an $IC_{50}$ = 2.2 ± 0.22 μM, and SK2 with an $IC_{50}$ = 5.2 ± 0.82 μM (Fig. 5). **RB-042** inhibits SK1 activity with an $IC_{50}$ = 5.3 ± 0.5 μM and SK2 with an $IC_{50}$ = 5.0 ± 1.3 μM.[7]

**Modeling the Inhibitors in the Atomic Structure of SK1.**

The crystal structures of human SK1 in a complex with ADP and SKi were determined recently.[26] We demonstrate here that the chemical modifications of the highly selective SK1 inhibitor **RB-005** produced SAR that can be explained using this crystal structure. Fig. 6A displays the result of a

modeling analysis of **RB-005** in the active site of human SK1; the piperidyl hydroxyl group is hydrogen bonded to D81 and the protonated amine of the head group forms a salt bridge with the carboxylate of D178. Fluoro- (**RB-034**) or methoxy-(**RB-036**) containing compounds do not exhibit inhibitory activity against SK1 (Fig. 3), as these groups no longer have hydrogen bond donating capacity, suggesting that the interaction of the 4-hydroxypiperidinyl group of **RB-005** is with a hydrogen bond acceptor in the protein. The lack of SK1 inhibitor activity of the azide-containing compounds (**RB-029**, **RB-030**) supports this possibility. One of the two oxygens of the carboxylate ion of D81 is hydrogen bonded to the backbone NH of L116, and the other is hydrogen bonded to the backbone NH of A115; these interactions prevent the carboxylate of D81 from being catalytic and shift the catalytic role to D178 (Fig. 7). The latter carboxylate oxygen also forms a hydrogen bond to the hydroxyl group of the inhibitor. If these compounds formed hydrogen bonds with the side chain of S168, then **RB-029**, **RB-034**, and **RB-036** could also bind to the donor/acceptor hydroxyl group of S168 or water and therefore act as inhibitors. Since **RB-029**, **RB-034**, and **RB-036** cannot form hydrogen bonds with D81 and are not inhibitors, we propose that the key interaction of the hydroxyl group of **RB-005** (Fig. 6A), **RB-025** (Fig. 6B), and **RB-028** (Fig. 6C) is with D81 and not with S168. In contrast, modeling of RB-035 (which contains a 4-keto group instead of a 4-hydroxyl group, yet maintains inhibition of SK1) suggests that the carbonyl group can form a hydrogen bond with the hydroxyl group of S168 and water (see Supporting Information, Fig. S2).

The inhibitory effect of **RB-032** (Fig. 6D) and its absence for **RB-033** (Fig. 6E) can be explained by protonation of the primary amine. When the protonated primary amine rather than the piperidyl group forms a salt bridge with D178, the inhibitors are pushed deeper into the J channel, which was identified by Wang et al.[26] as the region that accommodates the alkyl chain of Sph. Since **RB-032** has a shorter alkyl chain than **RB-033**, it can be accommodated in the substrate pocket, whereas the dodecyl group of

**RB-033** cannot fit into the channel formed when the protonated primary amine forms a salt bridge with D178. Therefore, **RB-033**, which is inactive, does not bind to D81, D178, S168 or L268, indicating that these are key amino-acid residues required for binding SK inhibitors.

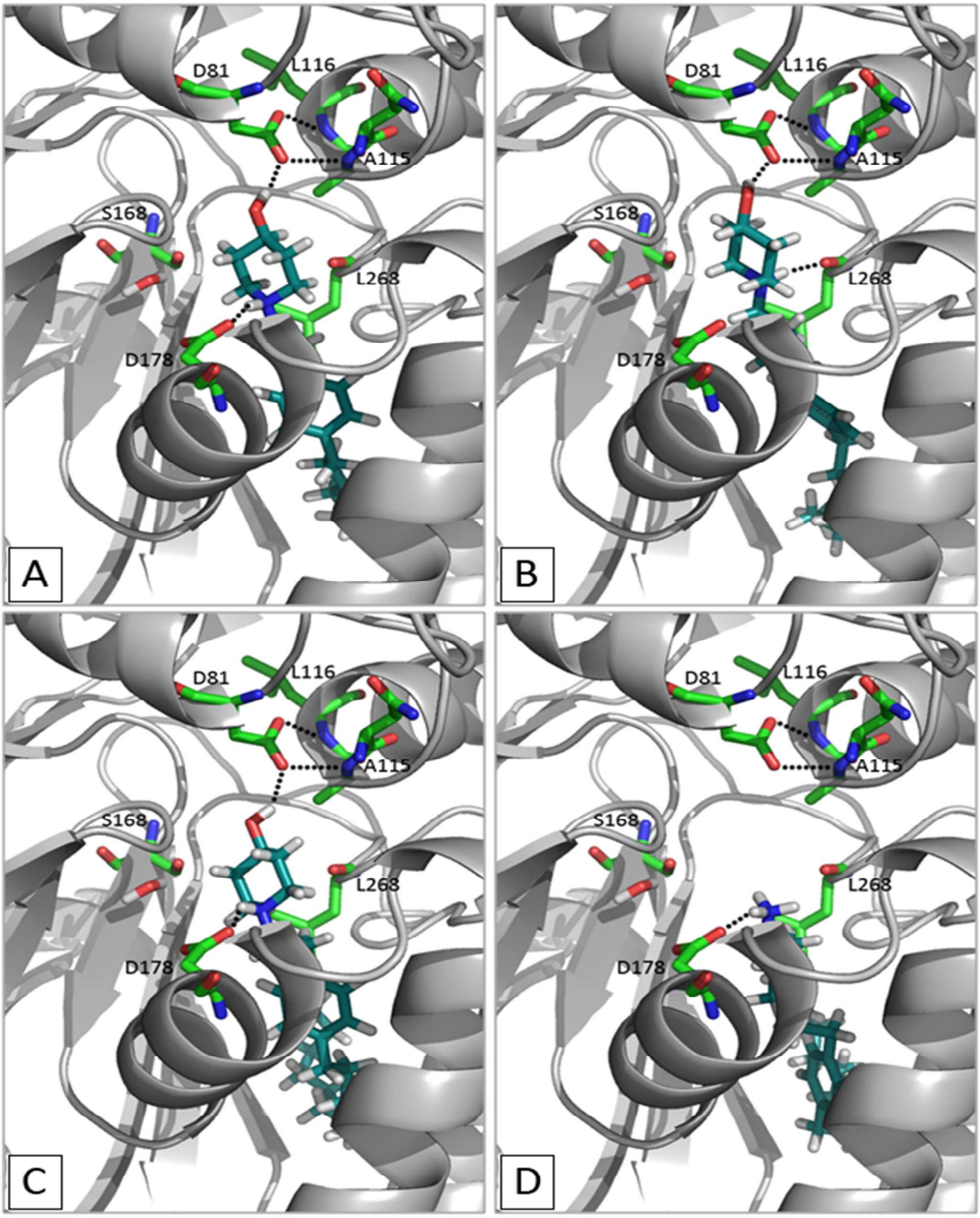
The salt bridge between the $NH_3^+$ group of **RB-032** and D178 (Fig. 6D) is the only polar interaction with the protein, which might explain the lower SK1 inhibitor activity when compared with **RB-005**; the latter forms a salt bridge *and* hydrogen bonds with D81. Interestingly, the *R*-enantiomers **RB-040** and **RB-042** are equipotent inhibitors of SK1 and SK2, while the *S*-enantiomers **RB-041** and **RB-043** are weak substrates for SK2, implying that the spatial orientation of the hydroxyl group in **RB-041** and **RB-043** required for catalysis is different in SK2 compared with SK1. The protonated amine in **RB-040** (Fig. 6F) and **RB-042** (Fig. 6G) can form a salt bridge with D178 and can also form a hydrogen bond with the carbonyl oxygen of L268. Both inhibitors can orientate the hydroxymethyl group of the pyrrolidine (*R* enantiomer) to also form a hydrogen bond with the side chain of D81. The protonated amino group of **RB-041** and **RB-043** can form a salt bridge with D178 but, because of the orientation of the hydroxymethyl group of the pyrrolidine (*S* enantiomer), cannot form a hydrogen bond between their hydroxyl group and D81, as found in our modeling study. Instead, the hydroxymethyl group could form a hydrogen bond to D178. As the experimental evidence shows that **RB-041** and **RB-043** do not inhibit SK1, this suggests that dynamic factors (accessing the binding site), which are not taken into account by docking studies, prevent the binding of these compounds.

**RB-044**-**RB-050** are ineffective inhibitors of SK1. There are three possible explanations: first, the nitrogen in an amide cannot be protonated, thus preventing salt bridge formation. Second, the link between nitrogen and phenyl is constrained and planar compared with a methylene group, which prevents optimization of the hydrogen bonding network with the hydroxyl group. Third, the carbonyl group of the amide would be proximal to the side chain of D178, which would result in electrostatic

repulsion.

The pyridinium salts **RB-052** and **RB-053** and the quaternary ammonium salts **RB-060**, **RB-061**, and **RB-062** were also ineffective SK1 inhibitors. The absence of a hydroxyl group in these compounds rules out hydrogen bonding with D81 or D178. The triazole moiety in **RB-065** forms a hydrogen bond with T196 (Fig. 6H) and, furthermore, adds a kink in the chain that helps orientate the alkyl group into the J channel, which may account for its SK1 inhibitory activity.

Our modeling studies suggest that **RB-005** (Fig. 6A), **RB-025** (Fig. 6B), and **RB-028** (Fig. 6C) interact with D81 and not with S168. These findings are consistent with D81 not acting as a base, because **RB-005**, **RB-025**, or **RB-028** are not substrates for SK1. As depicted in Fig. 7, modeling of Sph into the catalytic site of SK1 suggests that S168 can form hydrogen bonds with the $NH_3^+$ group of Sph and, via a water molecule, with the secondary hydroxyl group of Sph. The water molecule also hydrogen bonds with A339, thereby linking this amino acid residue to the secondary hydroxyl group of Sph (Fig. 7). Thus D178 functions as the deprotonating base in this model to enable nucleophilic attack by Sph on the γ-phosphate group of ATP, with subsequent transfer of this phosphate to Sph.


D81
L116
A115
S168
L268
D178
A
B
C
D

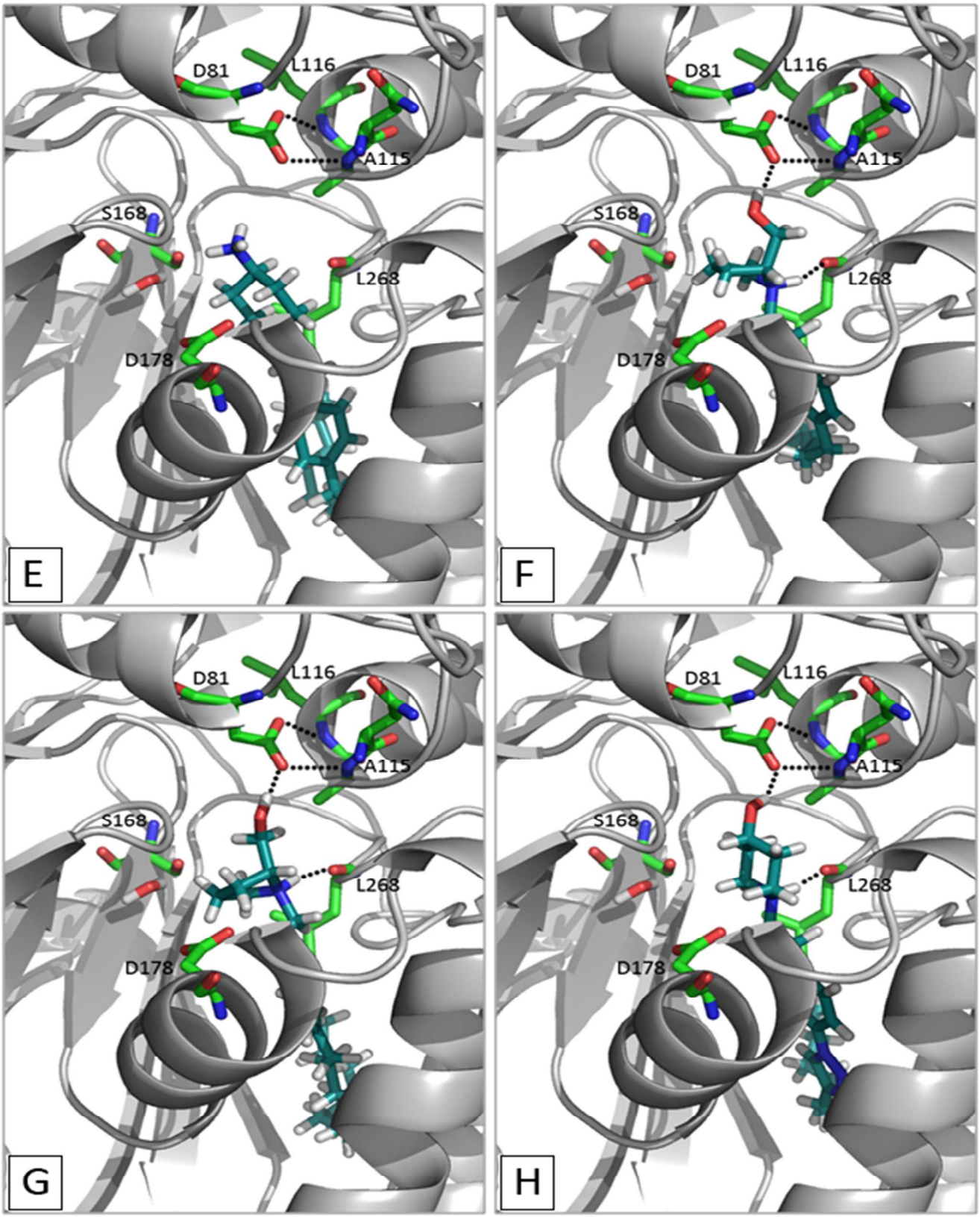

D81
L116
A115
S168
L268
D178
E
D81
L116
A115
S168
L268
D178
F
D81
L116
A115
S168
L268
D178
G
D81
L116
A115
S168
L268
D178
H

**Fig. 6.** Various modeled poses of SK1 inhibitors in the catalytic site of SK1. (A) **RB-005**, (B) **RB-025**, (C) **RB-028**, (D) **RB-032**, (E) **RB-033**, (F) **RB-040**, (G) **RB-042**, and (H) **RB-065**.

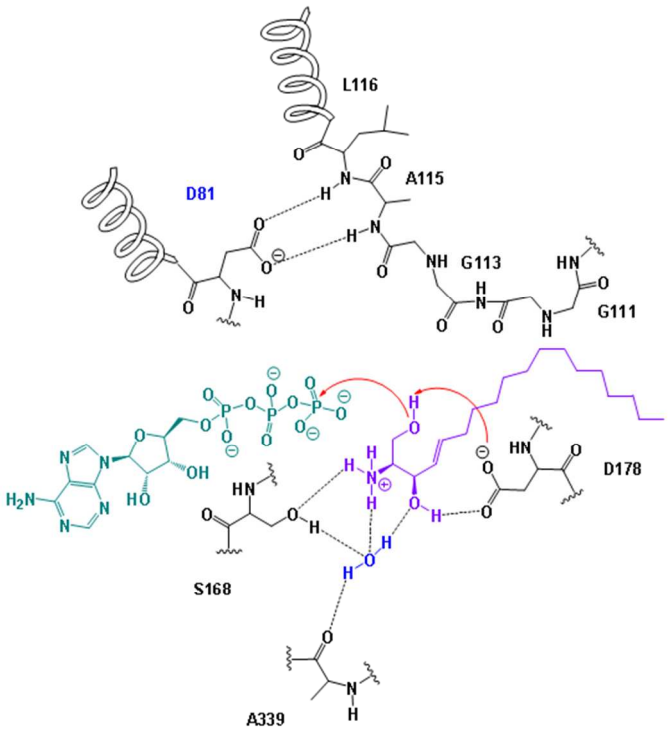



**Fig. 7.** Schematic model of the proposed mechanism of the phosphorylation of Sph catalyzed by SK1.

## Conclusion

In this study we have identified a series of SK1-selective inhibitors and have used molecular modeling to define their interactions with the catalytic site of the enzyme. These studies reveal a substantial flexibility in the catalytic site in terms of binding SK1 inhibitors. For instance, **RB-005** is proposed to interact with D81 and D178, while **RB-025** appears to interact with D81 and L268. The findings obtained from the modeling study fully account for the SAR of the inhibitors and explain why some of these compounds are inactive. These findings also reveal the architecture of the SK1 catalytic site and suggest a major role for D178 as the deprotonating base that facilitates phosphorylation of Sph

by ATP. In summary, the novel information presented here should enable development of new SK1 inhibitors with improved potency and selectivity. Similarly, resolution of the atomic structure of SK2 (yet to be achieved) along with information provided herein will enable better insights into the molecular basis of the selectivity of these inhibitors for SK1 over SK2.

## Experimental Section

**Docking studies**. The crystal structure of SK1 in complex with Sph (PDB entry 3VZB) was used for docking studies. Chain A of the complex was kept along with a single water molecule found to be tightly bound to the complex which hydrogen bonds to the side chain hydroxyl group of S168, the backbone –NH of G342, and the secondary hydroxyl group of Sph (water number 680). Hydrogen atoms were added to the protein and water using Accelrys Discovery studio 3.1 (Accelrys Software, San Diego, CA), and all of the inhibitors presented in this study were docked using GOLD 5.1 for Windows (Cambridge Crystallographic Data Centre, Cambridge, UK). Default software settings were used, keeping ChemPLP as a scoring function after redocking Sph in place in the 3VZB crystal structure as well as the SKi inhibitor in the 3VZD pdb entry (RMSD of 1.8 and 0.2 Å, respectively) as validation.

**Synthesis. General Methods.** All chemicals were reagent grade and used as purchased. Reactions were run under nitrogen and were monitored by TLC using silica gel 60 $F_{254}$ aluminum-backed plates. Flash column chromatography was performed on silica gel grade 60 (230–400 mesh). THF was distilled over sodium/benzophenone immediately prior to use; dichloromethane was distilled over $CaH_2$; $Et_3N$ was distilled over KOH pellets. All other solvents were of anhydrous quality and were used as received. $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker Avance I spectrometer, and chemical shifts

are reported in δ units relative to deuterated solvents, which served as internal references, at 400 and 100 MHz, respectively. High-resolution mass spectra (HRMS) were recorded at the CUNY Mass Spectrometry Facility on an Agilent Technologies G6520A Q-TOF mass spectrometer using electrospray ionization (ESI). Microwave reactions were performed in a Biotage Emrys Creator Synthesizer. HPLC was carried out using a reverse-phase column with a gradient of acetonitrile/water from 50/50 to 90/10, with detection at 214 and 254 nm. Elemental analysis was performed at Columbia Analytical Services, Tucson, AZ. All compounds were ≥95% pure as determined by examining their HRMS and $^1$H NMR spectra.

**(4-(Oct-1-ynyl)phenyl)methanol (1)**

To a solution of 4-iodobenzyl alcohol (200 mg, 0.85 mmol), bis(triphenylphosphine)palladium dichloride (49 mg, 0.040 mmol), and copper(I) iodide (7.6 mg, 0.04 mmol) in anhydrous triethylamine (10 mL) was added 1-octyne (283 mg, 2.56 mmol) at rt. After the reaction mixture was heated at 50 °C for 12 h, saturated aqueous ammonium chloride solution was added, and the mixture was extracted with EtOAc. The combined solution was washed with water, brine, and dried. Flash column chromatography with hexanes/EtOAc (5:1) as the eluent gave alkyne **1** (180 mg, 98%) as a yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, $J$ = 6.9 Hz, 3H), 1.28–1.36 (m, 4H), 1.41–1.49 (m, 2H), 1.60 (quin, $J$ = 7.3 Hz, 2H), 1.89 (br s, 1H), 2.40 (t, $J$ = 7.1 Hz, 2H), 4.65 (s, 2H), 7.25 (d, $J$ = 8.1 Hz, 2H), 7.37 (d, $J$ = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.4, 22.6, 28.6, 28.7, 31.4, 65.0, 80.3, 90.6, 123.4, 126.8, 131.7, 140.1; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{15}H_{21}O$ 217.1592, found 217.1588.

**(4-Octylphenyl)methanol (2)**

Compound **1** (180 mg, 0.83 mmol) was dissolved in EtOAc (10 mL), and 10% Pd/C (90 mg, 50 wt %) was added. The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration

through a pad of Celite, which was rinsed with EtOAc. Product **2** was obtained, without purification, as a colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.6 Hz, 3H), 1.26–1.30 (m, 10H), 1.56–1.63 (m, 2H), 1.78 (br s, 1H), 2.59 (t, *J* = 7.7 Hz, 2H), 4.64 (s, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 35.7, 65.3, 127.1, 128.6, 138.1, 142.6; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{15}H_{25}O$ 221.1905, found 221.1887.

**1-(4-Octylbenzyl)piperidin-4-ol (RB-023)**

To a solution of **2** (37 mg, 0.17 mmol) and triethylamine (0.23 mL, 1.7 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added methanesulfonyl chloride (40 μL, 0.50 mmol). After being stirred at rt for 3 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. To a solution of the reaction mixture (0.17 mmol) in MeCN (3 mL) was added 4-hydroxypiperidine (86 mg, 0.85 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (5:1), gave 35 mg (69%, 2 steps) of **RB-023** as a colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.23–1.30 (m, 10H), 1.56–1.73 (m, 4H), 1.97–2.06 (m, 2H), 2.35–2.47 (m, 2H), 2.59 (t, *J* = 7.7 Hz, 2H), 2.85–2.90 (m, 2H), 3.64 (s, 2H), 3.78–3.81 (m, 1H), 7.15 (d, *J* = 7.9 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.5, 31.9, 33.2, 35.7, 50.1, 62.2, 128.5, 129.7, 137.2, 142.8; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{20}H_{34}NO$ 304.2640, found 304.2637.

**3-(4-Octylphenyl)prop-2-yn-1-ol (3)**

Compound **3** was prepared from 1-bromo-4-*n*-octylbenzene and propargyl alcohol according to a Sonogashira reaction procedure similar to that described for **1**; yield = 82%; $^1$H NMR (400 MHz, $CDCl_3$)

δ 0.86 (t, *J* = 6.8 Hz, 3H), 1.22–1.30 (m, 10H), 1.57–1.60 (m, 2H), 2.59 (t, *J* = 7.7 Hz, 2H), 4.49 (s, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 7.7 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.2, 29.4, 31.2, 31.9, 35.9, 51.7, 85.9, 86.5, 119.6, 128.4, 131.6, 143.7; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{17}H_{25}O$ 245.1905, found 245.1903.

**3-(4-Octylphenyl)propan-1-ol (4)**

Compound **4** was prepared from **3** by a catalytic hydrogenation procedure similar to that described for **2**; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.24–1.30 (m, 10H), 1.59 (quin, *J* = 7.4 Hz, 2H), 1.88 (quin, *J* = 6.5 Hz, 2H), 2.56 (t, *J* = 7.7 Hz, 2H), 2.67 (t, *J* = 7.7 Hz, 2H), 3.66 (t, *J* = 6.4 Hz, 2H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.7, 31.9, 34.3, 35.6, 62.4, 128.3, 128.4, 138.9, 140.5; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{17}H_{29}O$ 249.2218, found 249.2210.

**1-(3-(4-Octylphenyl)propyl)piperidin-4-ol (RB-024)**

Compound **RB-024** was prepared from **4** according to a procedure similar to that described for **RB-023**; yield = 73%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.25–1.30 (m, 10H), 1.55–1.65 (m, 4H), 1.79–1.92 (m, 4H), 2.16–2.20 (m, 2H), 2.40–2.43 (m, 2H), 2.53–2.63 (m, 4H), 2.80–2.83 (m, 2H), 3.67–3.69 (m, 1H), 7.08 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 28.5, 29.3, 29.4, 29.5, 31.6, 31.9, 33.3, 34.0, 35.6, 50.9, 57.9, 128.2, 128.4, 138.9, 140.4; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{22}H_{38}NO$ 332.2953, found 332.2951.

**1-(4-(4-Octylphenyl)butyl)piperidin-4-ol (RB-025)**

Compound **RB-025** was prepared from 4-(4-octylphenyl)butan-1-ol (**5**)[22] according to a procedure

similar to that described for compound **RB-023**; yield = 67% (2 steps); $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.24–1.33 (m, 10H), 1.55–1.68 (m, 4H), 1.80–1.88 (m, 4H), 2.21–2.26 (m, 2H), 2.56 (t, *J* = 7.8 Hz, 2H), 2.61 (t, *J* = 7.5 Hz, 2H), 2.71–2.89 (m, 4H), 3.11 (t, *J* = 9.0 Hz, 2H), 3.97–4.01 (m, 1H), 7.05 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 28.9, 29.3, 29.4, 29.5, 31.6, 31.9, 34.9, 35.6, 57.5, 128.2, 128.5, 138.6, 140.7; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{23}H_{40}NO$ 346.3110, found 346.3107.

**2-(4-(Hex-1-ynyl)phenyl)ethanol (6)**

Compound **6** was prepared from 2-(4-bromophenyl)ethanol and 1-hexyne according to a Sonogashira procedure similar to that described for **1**; yield = 60%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.43–1.52 (m, 2H), 1.55–1.62 (m, 2H), 2.40 (t, *J* = 7.0 Hz, 2H), 2.85 (t, *J* = 6.5 Hz, 2H), 3.84 (t, *J* = 6.5 Hz, 2H), 7.14 (d, *J* = 8.1 Hz, 2H), 7.34 (t, *J* = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.7, 19.1, 22.0, 29.7, 30.9, 39.0, 63.5, 80.3, 90.2, 122.3, 128.9, 131.7, 137.9; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{14}H_{19}O$ 203.1436, found 203.14333.

**2-(4-(Dodec-1-ynyl)phenyl)ethanol (7)**

Compound **7** was prepared from 2-(4-bromophenyl)ethanol and 1-decyne according to a procedure similar to that described for **1**; yield = 62%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.23–1.30 (m, 12H), 1.40–1.45 (m, 2H), 1.59 (quin, *J* = 7.3 Hz, 2H), 2.38 (t, *J* = 7.1 Hz, 2H), 2.81 (t, *J* = 6.6 Hz, 2H), 3.79 (t, *J* = 6.5 Hz, 2H), 7.11 (d, *J* = 8.2 Hz, 2H), 7.32 (d, *J* = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.4, 22.7, 24.9, 28.8, 29.0, 29.2, 29.4, 29.6, 31.9, 39.0, 63.4, 80.4, 90.2, 122.3, 128.6, 131.4, 138.0; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{20}H_{31}O$ 287.2375, found 287.2371.

**2-(4-Hexylphenyl)ethanol (8)**

Compound **8** was prepared from **6** according to a procedure similar to that described for **2**; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.6 Hz, 3H), 1.22–1.37 (m, 6H), 1.55–1.63 (m, 2H), 2.57 (t, $J$ = 7.8 Hz, 2H), 2.84 (t, $J$ = 6.6 Hz, 2H), 3.84 (t, $J$ = 6.5 Hz, 2H), 7.13 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 29.0, 31.5, 31.7, 35.6, 38.8, 63.8, 128.6, 128.9, 135.5, 141.2; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{14}H_{23}O$ 207.1749, found 207.1725.

**2-(4-Dodecylphenyl)ethanol (9)**

Compound **9** was prepared from **7** according to a procedure similar to that described for **2**; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.23–1.31 (m, 18H), 1.57–1.60 (m, 2H), 2.56 (t, $J$ = 7.8 Hz, 2H), 2.81 (t, $J$ = 6.6 Hz, 2H), 3.81 (t, $J$ = 6.6 Hz, 2H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.4, 29.6, 29.7, 31.6, 31.9, 35.6, 38.8, 63.7, 128.6, 128.9, 135.5, 141.2; $^{13}$C NMR (100 MHz, $CDCl_3$) δ 21.0, 32.3, 33.1, 39.5, 50.5, 59.9, 66.0, 128.6, 129.3, 135.9, 136.0; ESI-HRMS $(M+Na)^+$ $m/z$ calcd for $C_{20}H_{34}ONa$ 313.2507, found 313.2502.

**1-(4-Methylphenethyl)piperidin-4-ol (RB-026)**

Compound **RB-026** was prepared from 2-(4-methylphenyl)ethanol according to a procedure similar to that described for **RB-023**; yield = 69%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 1.69–1.77 (m, 2H), 1.99–2.04 (m, 2H), 2.31 (s, 3H), 2.48–2.52 (2H), 2.72–2.76 (m, 2H), 2.79 (s, 1H), 2.84–2.88 (m, 2H), 2.98–3.03 (m, 2H), 3.78–3.84 (m, 1H), 7.10 (s, 4H); ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{14}H_{22}NO$ 220.1701, found 220.1699.

**1-(4-Hexylphenethyl)piperidin-4-ol (RB-027)**

Compound **RB-027** was prepared from **8** according to a procedure similar to that described for **RB-023**; yield = 79%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.5 Hz, 3H), 1.26–1.36 (m, 6H), 1.58 (quin, $J$ = 7.4 Hz, 2H), 1.75–1.80 (m, 2H), 2.08–2.13 (m, 2H), 2.56 (t, $J$ = 7.7 Hz, 2H), 2.74–2.77 (m, 2H), 2.90–2.94 (m, 2H), 3.00–3.04 (m, 2H), 3.86–3.90 (m, 1H), 7.11 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 29.0, 31.5, 31.7, 35.6, 50.1, 59.9, 128.5, 128.6, 141.2; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{19}H_{32}NO$ 290.2484, found 290.2478.

**1-(4-Dodecylphenethyl)piperidin-4-ol (RB-028)**

Compound **RB-028** was prepared from **9** according to a procedure similar to that described for **RB-023**; yield = 75%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.6 Hz, 3H), 1.23–1.33 (m, 18H), 1.56–1.60 (m, 2H), 1.67–1.72 (m, 2H), 1.98–2.01 (m, 2H), 2.34–2.39 (m, 2H), 2.56 (t, $J$ = 7.4 Hz, 2H), 2.64–2.68 (m, 2H), 2.81–2.84 (m, 2H), 2.91–2.95 (m, 2H), 3.75–3.79 (m, 1H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.4, 29.5, 29.6, 29.7, 31.6, 31.9, 32.8, 35.6, 50.6, 60.3, 128.5, 136.7, 140.9; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{25}H_{44}NO$ 374.3423, found 374.3414.

**4-Azido-1-(4-methylphenethyl)piperidine (RB-029)**

To a solution of **RB-026** (115 mg, 0.52 mmol) and triethylamine (0.73 mL, 5.24 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added methanesulfonyl chloride (0.12 mL, 1.57 mmol). After being stirred at rt for 4 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. To a solution of reaction mixture in 5 mL of DMF was added sodium azide (170 mg, 2.62 mmol). The reaction mixture was stirred at 80 °C for 12 h and then concentrated. The residue was dissolved in EtOAc and the organic phase was evaporated and dried.

Purification by silica gel chromatography, eluting with hexane/EtOAc (1/1), gave 79 mg (62%) of **RB-029** as a colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 1.69–1.78 (m, 2H), 1.96–1.99 (m, 2H), 2.30–2.35 (m, 2H), 2.31 (s, 3H), 2.60–2.65 (m, 2H), 2.76–2.80 (m, 2H), 2.86–2.89 (m, 2H), 3.44–3.48 (m, 1H), 7.09 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 21.0, 29.4, 30.5, 33.0, 50.9, 57.3, 60.4, 128.6, 129.2, 132.4, 135.7, 136.7; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{14}H_{21}N_4$ 245.1766, found 245.1763.

**4-Azido-1-(4-octylphenethyl)piperidine (RB-030)**

Compound **RB-030** was prepared from **RB-005**[7] according to a procedure similar to that described for **RB-029**; yield = 71%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.24–1.31 (m, 10H), 1.58 (quin, *J* = 7.2 Hz, 2H), 1.67–1.76 (m, 2H), 1.93–1.97 (m, 2H), 2.24–2.29 (m, 2H), 2.54–2.61 (m, 4H), 2.75–2.79 (m, 2H), 2.84–2.90 (m, 2H), 3.40–3.46 (m, 1H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.7, 30.3, 30.7, 31.6, 31.9, 33.2, 35.6, 51.1, 57.6, 60.5, 128.5, 137.2, 140.8; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{21}H_{35}N_4$ 343.2862, found 343.2857.

**1-(4-Methylphenethyl)piperidin-4-amine (RB-031)**

To a solution of **RB-029** (30 mg, 0.12 mmol) in MeOH/$CH_2Cl_2$ (1/3, 3 mL) was added 10% Pd/C (50 wt %). The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with MeOH/$CH_2Cl_2$ (3/1). The residue was washed with EtOAc/hexane (1/1), evaporated, and dried. **RB-031** was obtained as a white solid; $^1$H NMR (400 MHz, $CD_3OD$) δ 1.82–1.90 (m, 2H), 2.17 (d, *J* = 10.2 Hz, 2H), 2.28 (s, 3H), 2.34 (t, *J* = 10.6 Hz, 2H), 2.68–2.71 (m, 2H), 2.79–2.83 (m, 2H), 3.13 (d, *J* = 11.2 Hz, 2H), 3.13–3.20 (m, 1H), 7.07 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 21.0, 30.7, 32.4, 48.2, 51.4, 59.8, 128.6, 129.2, 135.8, 136.0; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{14}H_{23}N_2$ 219.1861, found 219.1858.

**1-(4-Octylphenethyl)piperidin-4-amine (RB-032)**

Compound **RB-032** was prepared from **RB-030** according to a procedure similar to that described for **RB-031**; $^{1}$H NMR (400 MHz, $CD_3OD$) δ 0.91 (t, *J* = 6.8 Hz, 3H), 1.28–1.34 (m, 10H), 1.61 (quin, *J* = 6.5 Hz, 2H), 2.06–2.15 (m, 2H), 2.32 (d, *J* = 13.2 Hz, 2H), 2.60 (t, *J* = 7.52 Hz, 2H), 3.08–3.14 (m, 2H), 3.24 (t, *J* = 11.5 Hz, 2H), 3.35–3.38 (m, 2H), 3.51–3.58 (m, 1H), 3.76 (d, *J* = 11.8 Hz, 2H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 14.4, 23.7, 30.3, 30.4, 30.6, 31.1, 32.7, 33.0, 36.5, 36.9, 129.8, 129.9, 134.7, 143.2; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{21}H_{37}N_2$ 317.2957, found 317.2951.

**1-(4-Dodecylphenethyl)piperidin-4-amine (RB-033)**

To a solution of **RB-028** (20 mg, 0.050 mmol) and triethylamine (70 μL, 0.54 mmol) in $CH_2Cl_2$ (3 mL) at 0 °C was added methanesulfonyl chloride (10 μL, 0.15 mmol). After being stirred at rt for 4 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. To a solution of the reaction mixture in 3 mL of DMF was added sodium azide (10 mg, 0.16 mmol). The reaction mixture was stirred at 100 °C for 12 h, and then concentrated. The residue was dissolved in EtOAc and the organic phase was evaporated and dried. To a solution of residue in MeOH/$CH_2Cl_2$ (3/1, 3 mL) was added 10% Pd/C (50 wt %). The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with MeOH/$CH_2Cl_2$ (3/1). The residue was washed with EtOAc/hexane (1/1), evaporated, and dried, affording **RB-033** as a white solid; $^{1}$H NMR (400 MHz, $CD_3OD$) δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.27–1.32 (m, 18H), 1.58–1.62 (m, 2H), 2.00–2.10 (m, 2H), 2.29 (d, *J* = 13.2, 2H), 2.58–2.62 (m, 2H), 3.05–3.09 (m, 2H), 3.15 (d, *J* = 13.6 Hz, 2H), 3.28–3.33 (m, 2H), 3.46–3.54 (m, 1H),

3.71 (d, $J$ = 10.9 Hz, 2H), 7.17 (d, $J$ = 7.9 Hz, 2H), 7.22 (d, $J$ = 7.9 Hz, 2H); $^{13}$C NMR (100 MHz, $CD_3OD$) δ 14.5, 23.8, 29.9, 30.3, 30.5, 30.6, 30.7, 30.8, 30.9, 31.3, 32.8, 33.1, 36.5, 36.9, 129.9, 130.1, 133.1, 143.1; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{25}H_{45}N_2$ 373.3583, found 373.3576.

**4-Fluoro-1-(4-octylphenethyl)piperidine (RB-034)**

To a solution of **RB-005** (12 mg, 0.040 mmol) in $CH_2Cl_2$ (3 mL) at 0 °C was added diethylaminosulfur trifluoride (DAST, 15 μL, 0.12 mmol). After being stirred at rt for 5 h, the reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (10:1), gave 11 mg (90%) of **RB-034** as a colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, $J$ = 6.8 Hz, 3H), 1.25–1.30 (m, 10H), 1.54–1.62 (m, 2H), 1.90–2.00 (m, 4H), 2.48–2.63 (m, 6H), 2.66–2.71 (m, 2H), 2.76–2.80 (m, 2H), 4.61–4.66 (m, 0.5H), 4.74–4.78 (m, 0.5H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.7, 33.2, 35.6, 49.4, 60.5, 128.5, 137.2, 140.8; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{21}H_{35}FN$ 320.2754, found 320.2751.

**1-(4-Octylphenethyl)piperidin-4-one (RB-035)**

To a solution of **RB-005** (25 mg, 0.080 mmol) in $CH_2Cl_2$ (3 mL) at 0 °C was added pyridinium chlorochromate (PCC, 25 mg, 0.12 mmol). After being stirred at rt for 4 h, the reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, evaporated, and dried. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (3:1), gave 17 mg (70%) of **RB-035** as a colorless oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, $J$ = 6.6 Hz, 3H), 1.22–1.30 (m, 10H), 1.58–1.60 (m, 2H), 2.47–2.52 (m, 4H), 2.57 (t, $J$ = 7.7 Hz, 2H), 2.72–2.74 (m, 2H), 2.81–2.86 (m, 6H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 29.7, 31.6,

31.9, 33.7, 35.6, 36.0, 41.2, 53.1, 59.4, 128.6, 137.0, 141.0, 178.0; ESI-HRMS $(M+H)^+$ *m/z* calcd for $C_{21}H_{34}$ NO 316.2640, found 316.2635.

**4-Methoxy-1-(4-octylphenethyl)piperidine (RB-036)**

To a solution of **11**[7] (17 mg, 50 μmol) in MeCN (3 mL) was added at rt. After the suspension was stirred for 10 min, 4-methoxypiperidine (19 mg, 0.16 mmol) was added. The reaction mixture was stirred at 50 °C for 12 h. The solvent was evaporated and the residue was purified by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (5:1), to give 14 mg (79%) of **RB-036** as a yellow liquid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.5 Hz, 3H), 1.23–1.31 (m, 10H), 1.56–1.61 (m, 2H), 1.67–1.72 (m, 2H), 1.95–2.00 (m, 2H), 2.30–2.37 (m, 2H), 2.56 (t, *J* = 7.7 Hz, 2H), 2.62–2.64 (m, 2H), 2.79–2.85 (m, 4H), 3.25–3.30 (m, 1H), 3.34 (s, 3H), 7.10 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 35.6, 50.8, 55.6, 60.5, 128.5, 128.6, 140.8; ESI-HRMS $(M+H)^+$ *m/z* calcd for $C_{22}H_{38}$NO 332.2953, found 332.2948.

**(*S*)-1-(4-Octylphenethyl)pyrrolidin-3-ol (RB-037)**

To a solution of **11** (20 mg, 0.064 mmol) in MeCN (4 mL), $K_2CO_3$ (44 mg, 0.32 mmol) was added at rt. After the suspension was stirred for 10 min, (*S*)-pyrrolidine-3-ol hydrochloride (79 mg, 0.64 mmol) was added. The reaction mixture was stirred at 50 °C for 12 h. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (3:1), gave 17 mg (86%) of **RB-037** as a yellow liquid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.22–1.32 (m, 10H), 1.58 (quin, *J* = 7.3 Hz, 2H), 1.85 (quin, *J* = 6.7 Hz, 1H), 2.19–2.28 (m, 1H), 2.46–2.54 (m, 2H), 2.56 (t, *J* = 7.8 Hz, 2H), 2.68 (dd, *J* = 5.1, 10.4 Hz, 1H), 2.78–2.88 (m, 4H), 2.91 (d, *J* =10.4 Hz,

1H), 3.07–3.13 (m, 1H), 4.38–4.41 (m, 1H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 34.3, 34.7, 35.6, 52.6, 57.8, 62.9, 71.1, 128.5, 128.6, 136.5, 141.0; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{20}H_{34}NO$ 304.2640, found 304.2639.

**(*R*)-1-(4-Octylphenethyl)pyrrolidin-3-ol (RB-038)**

Compound **RB-038** was prepared from **11** according to a coupling procedure similar to that described for **RB-036**, using (*R*)-pyrrolidine; yield = 72%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.26–1.29 (m, 10H), 1.56–1.59 (m, 2H), 1.94–2.01 (m, 1H), 2.22–2.31 (m, 1H), 2.56 (t, *J* = 7.9 Hz, 2H), 2.74–2.76 (m, 1H), 2.91–2.99 (m, 4H), 3.14–3.24 (m, 2H), 3.30–3.35 (m, 1H), 4.47–4.50 (m, 1H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 29.3, 29.4, 29.5, 31.5, 31.9, 33.4, 34.2, 35.6, 52.9, 57.9, 62.7, 70.4, 128.5, 128.7, 135.2, 141.5; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{20}H_{34}NO$ 304.2640, found 304.2637.

**(*R*)-(1-(4-Methylphenethyl)pyrrolidin-2-yl)methanol (RB-039)**

Compound **RB-039** was prepared from 2-(4-methylphenyl)ethanol according to a coupling procedure similar to that described for **RB-037**, using D-prolinol; yield = 62%; $^1$H NMR (400 MHz, $CDCl_3$) δ 1.84–1.91 (m, 1H), 1.97–1.21 (m, 1H), 2.03–2.13 (m, 2H), 2.31 (s, 3H), 2.83–2.89 (m, 1H), 2.96–3.08 (m, 2H), 3.13–3.21 (m, 1H), 3.34–3.41 (m, 1H), 3.49–3.56 (m, 1H), 3.72–3.77 (m, 1H), 3.85 (d, *J* = 5.3 Hz, 2H), 7.12 (dd, *J* = 1.9, 8.4 Hz, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 21.0, 23.6, 26.5, 32.0, 39.4, 50.7, 55.0, 57.9, 61.1, 69.5, 128.6, 129.5, 133.7, 136.7; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{14}H_{22}NO$ 220.1701, found 220.1698.

**(*R*)-(1-(4-Octylphenethyl)pyrrolidin-2-yl)methanol (RB-040)**

Compound **RB-040** was prepared from **11** according to a coupling procedure similar to that described for **RB-037**, using D-prolinol; yield = 59%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.22–1.31 (m, 10H), 1.54–1.62 (m, 2H), 1.84–2.05 (m, 4H), 2.56 (t, *J* = 7.8 Hz, 2H), 2.64–2.70 (m, 1H), 2.85–3.00 (m, 3H), 3.08–3.13 (m, 1H), 3.32–3.36 (m, 1H), 3.57–3.62 (m, 1H), 3.67 (dd, *J* = 5.5, 13.7, Hz, 1H), 3.78 (dd, *J* = 3.2, 12.2 Hz, 1H), 7.11 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.8, 26.9, 29.3, 29.5, 29.7, 31.0, 31.5, 31.9, 35.6, 54.5, 56.9, 61.4, 66.3, 128.5, 128.7, 135.5, 141.5; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{21}H_{36}NO$ 318.2797, found 318.2792.

**(*S*)-(1-(4-Octylphenethyl)pyrrolidin-2-yl)methanol (RB-041)**

Compound **RB-041** was prepared from **11** according to a coupling procedure similar to that described for **RB-037**, using L-prolinol; yield = 54%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.22–1.31 (m, 10H), 1.58 (quin, *J* = 7.3 Hz, 2H), 1.80–1.96 (m, 4H), 2.52–2.55 (m, 1H), 2.56 (t, *J* = 7.8 Hz, 2H), 2.73 (td, *J* = 11.6, 2.7 Hz, 1H), 2.86–2.96 (m, 3H), 3.18–3.25 (m, 1H), 3.47 (quin, *J* = 4.7 Hz, 1H), 3.56 (dd, *J* = 4.4, 11.7 Hz, 1H), 3.70 (dd, *J* = 3.2, 11.8 Hz, 1H), 7.11 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.8, 27.1, 29.3, 29.4, 29.5, 29.7, 31.5, 31.9, 33.7, 35.6, 54.3, 57.0, 61.5, 66.9, 128.5, 128.8, 135.8, 141.2; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{21}H_{36}NO$ 318.2797, found 318.2791.

**(*R*)-(1-(4-Dodecylphenethyl)pyrrolidin-2-yl)methanol (RB-042)**

Compound **RB-042** was prepared from **9** according to a coupling procedure similar to that described for **RB-037**, using D-prolinol; yield = 62%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.6 Hz, 3H), 1.22–1.31 (m, 18H), 1.56–1.60 (m, 2H), 2.00–2.16 (m, 4H), 2.56 (t, *J* = 7.7 Hz, 2H), 2.82–2.88 (m, 1H), 3.03–3.09 (m, 2H), 3.22–3.30 (m, 1H), 3.35–3.39 (m, 1H), 3.46–3.53 (m, 1H), 3.75–3.80 (m, 1H), 3.86

(dd, $J$ = 6.5, 12.7 Hz, 1H), 3.95 (dd, $J$ = 2.1, 12.0 Hz, 1H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 24.0, 26.5, 29.3, 29.4, 29.5, 29.6, 29.7, 31.5, 31.8, 31.9, 35.5, 54.7, 58.0, 60.9, 70.2, 128.5, 128.9, 133.6, 142.0; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{25}H_{44}NO$ 374.3423, found 374.3418.

**(*S*)-(1-(4-Dodecylphenethyl)pyrrolidin-2-yl)methanol (RB-043)**

Compound **RB-043** was prepared from **9** according to a coupling procedure similar to that described for **RB-037**, using L-prolinol; yield = 55%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.6 Hz, 3H), 1.23–1.30 (m, 18H), 1.56–1.62 (m, 2H), 1.93–2.11 (m, 4H), 2.56 (t, $J$ = 7.8 Hz, 2H), 2.98–3.06 (m, 2H), 3.14–3.22 (m, 1H), 3.28–3.32 (m, 1H), 3.41–3.49 (m, 1H), 3.65–3.68 (m, 1H), 3.71–3.73 (m, 1H), 3.79–3.83 (m, 1H), 3.89 (dd, $J$ = 2.5, 12.0 Hz, 1H), 7.12 (s, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 23.9, 26.8, 29.4, 29.5, 29.6, 29.7, 31.5, 31.9, 35.6, 54.5, 57.5, 61.1, 70.1, 128.5, 128.8, 134.1, 141.7; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{25}H_{44}NO$ 374.3423, found 374.3415.

**4-(Oct-1-ynyl)benzoic acid (12)**

To a deaerated solution of 4-iodobenzoic acid (500 mg, 2.02 mmol), bis(triphenylphosphine)palladium dichloride (116 mg, 0.10 mmol), and copper(I) iodide (19 mg, 0.10 mmol) in anhydrous triethylamine (15 mL) was added 1-octyne (0.89 mL, 6.05 mmol) at rt. The reaction mixture was heated at 60 °C for 12 h. After saturated aqueous ammonium chloride solution was added, the product was extracted with EtOAc. The combined solution was washed with water, brine, and dried. Purification by silica gel chromatography, eluting with hexane/EtOAc (3:1), gave 395 mg (85%) of alkyne **12** as a yellow liquid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, $J$ = 6.7 Hz, 3H), 1.31–1.35 (m, 4H), 1.43–1.50 (m, 2H), 1.58–1.66 (m, 2H), 2.43 (t, $J$ = 7.1 Hz, 2H), 7.47 (d, $J$ = 8.4 Hz, 2H), 8.02 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.8, 19.3, 22.3, 28.3, 31.1, 79.8, 94.4, 127.6, 129.6, 129.7,

131.3, 171.8; ESI-HRMS $(M-H)^-$ *m*/*z* calcd for $C_{15}H_{17}O_2^-$ 229.1234, found 229.1237.

**4-Octylbenzoic acid (13)**

Compound **12** (300 mg, 1.30 mmol) was dissolved in EtOAc (10 mL), and 10% Pd/C (150 mg, 50 wt %) was added. The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with EtOAc, affording **13** as a yellow solid without purification; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.22–1.32 (m, 10H), 1.61–1.64 (m, 2H), 2.64 (t, *J* = 7.7 Hz, 2H), 7.25 (d, *J* = 8.1 Hz, 2H), 8.02 (d, *J* = 8.2 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 31.1, 31.9, 36.1, 126.9, 128.6, 130.3, 149.6, 172.6; ESI-HRMS $(M-H)^-$ *m*/*z* calcd for $C_{15}H_{21}O_2^-$ 233.1547, found 233.1548.

**(*R*)-(4-Dodecylphenyl)-(2-(hydroxymethyl)pyrrolidin-1-yl)methanone (RB-044)**

To a solution of 4-(*n*-dodecyl)benzoic acid (10 mg, 0.034 mmol) in $CH_2Cl_2$ (3 mL), thionyl chloride (0.25 mL, 0.34 mmol) was added at rt. The reaction mixture was heated at reflux for 12 h. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. To a solution of residue in MeCN (3 mL) was added $K_2CO_3$ (24 mg, 0.17 mmol) at rt. After the suspension was stirred for 10 min, D-prolinol (10 mg, 0.10 mmol) was added. The reaction mixture was stirred at 50 °C for 12 h. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (10:1), gave 9 mg (72%) of **RB-044** as a yellow liquid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 3H), 1.23–1.34 (m, 18H), 1.59–1.64 (m, 4H), 1.70–1.77 (m, 1H), 1.84–1.89 (m, 1H), 2.14–2.21 (m, 1H), 2.62 (t, *J* = 7.6 Hz, 2H), 3.46–3.59 (m, 2H), 3.71–3.82 (m, 2H), 4.39–4.44 (m, 1H), 7.20 (d, *J* = 7.9 Hz, 2H), 7.43 (d, *J* = 7.9 Hz,

2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.1, 28.6, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.3, 31.9, 35.8, 51.3, 61.6, 67.6, 127.2, 128.3, 133.8, 145.5, 172.5; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{24}H_{40}NO_2$ 374.3059, found 374.3055.

**(*S*)-(4-Dodecylphenyl)(2-(hydroxymethyl)pyrrolidin-1-yl)methanone (RB-045)**

Compound **RB-045** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using L-prolinol instead of D-prolinol; yield = 65%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.23–1.30 (m, 18H), 1.58–1.64 (m, 4H), 1.70–1.77 (m, 1H), 1.84–1.89 (m, 1H), 2.14–2.21 (m, 1H), 2.62 (t, *J* = 7.7 Hz, 2H), 3.47–3.59 (m, 2H), 3.71–3.82 (m, 2H), 4.38–4.44 (m, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.43 (d, *J* = 7.9 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.1, 28.6, 29.2, 29.4, 29.5, 29.6, 29.7, 31.2, 31.9, 35.8, 51.3, 61.6, 67.5, 127.2, 128.3, 133.8, 145.5, 172.5; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{24}H_{40}NO_2$ 374.3059, found 374.3058.

**(4-Hydroxypiperidin-1-yl)(4-octylphenyl)methanone (RB-046)**

Compound **RB-046** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-hydroxypiperidine; yield = 70%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.26–1.30 (m, 10H), 1.51–1.62 (m, 4H), 1.80–1.97 (m, 2H), 2.63 (t, *J* = 7.7 Hz, 2H), 3.21-3.36 (m, 2H), 3.67–3.76 (m, 1H), 3.93–4.00 (m, 1H), 4.18–4.26 (m, 1H), 7.19 (d, *J* = 8.2 Hz, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.2, 29.3, 29.4, 31.3, 35.8, 67.4, 126.9, 128.5, 130.2, 133.2, 144.8, 170.7; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{20}H_{32}NO_2$ 318.2428, found 318.2432.

**(4-Dodecylphenyl)(4-hydroxypiperidin-1-yl)methanone (RB-047)**

Compound **RB-047** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-hydroxypiperidine; yield = 81%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.22–1.37 (m, 18H), 1.58–1.63 (m, 4H), 1.84–1.95 (m, 2H), 2.61 (t, *J* = 7.7 Hz, 2H), 3.21–3.34 (m, 2H), 3.67–3.75 (m, 1H), 3.95 (sep, *J* = 3.9 Hz, 1H), 4.18–4.23 (m, 1H), 7.19 (d, *J* = 7.9 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 29.3, 29.4, 29.5, 29.6, 29.7, 31.3, 31.9, 35.8, 67.2, 126.9, 128.5, 133.1, 144.9, 170.8; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{24}H_{40}NO_2$ 374.3059, found 374.3055.

**4-Octyl-*N*-(pyridin-4-ylmethyl)benzamide (RB-048)**

Compound **RB-048** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-(aminomethyl)pyridine; yield = 69%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.07–1.30 (m, 10H), 1.59–1.63 (m, 2H), 2.65 (t, *J* = 7.7 Hz, 2H), 4.63 (d, *J* = 6.0 Hz, 2H), 6.97 (t, *J* = 5.6 Hz, NH), 7.24 (d, *J* = 8.0 Hz, 4H), 7.74 (d, *J* = 8.2 Hz, 2H), 8.51–8.56 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 21.1, 22.7, 29.2, 29.4, 29.7, 31.2, 31.8, 35.8, 42.7, 60.4, 127.1, 128.5, 128.7, 131.2, 147.4, 147.8, 149.9, 167.7, 171.2; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{21}H_{29}N_2O$ 325.2274, found 325.2277.

***N*-(4-Hydroxyphenyl)-4-octylbenzamide (RB-049)**

Compound **RB-049** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 4-aminophenol; yield = 55%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.11–1.29 (m, 10H), 1.54–1.60 (m, 2H), 2.67 (t, *J* = 7.1 Hz, 2H), 6.83 (d, *J* = 7.8 Hz, 2H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.43 (d, *J* = 6.8 Hz, 2H), 7.80 (d, *J* = 6.8 Hz, 2H); $^{13}$C NMR (100

MHz, $CDCl_3$) δ 14.3, 22.9, 27.6, 29.6, 29.7, 30.0, 30.4, 31.6, 32.2, 36.2, 123.2, 127.5, 129.0, 132.5, 147.6, 167.8; ESI-HRMS $(M+H)^+$ *m/z* calcd for $C_{21}H_{28}NO_2$ 326.2115, found 326.2118.

**_N_-(4-(2-Hydroxyethyl)phenyl)-4-octylbenzamide (RB-050)**

Compound **RB-050** was prepared from 4-(*n*-dodecyl)benzoic acid according to a coupling procedure similar to that described for **RB-044**, using 2-(4-aminophenyl)ethanol; yield = 73%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.24–1.32 (m, 10H), 1.51–1.60 (m, 2H), 2.67 (t, *J* = 7.7 Hz, 2H), 2.87 (t, *J* = 6.5 Hz, 2H), 3.86 (t, *J* = 6.5 Hz, 2H), 7.23 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.58 (d, *J* = 8.4 Hz, 2H), 7.76 (br s, NH), 7.78 (d, *J* = 8.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 27.3, 29.2, 29.4, 29.7, 31.2, 31.9, 38.6, 63.7, 120.5, 127.0, 128.9, 129.7, 132.3, 134.6, 136.5, 147.4, 165.7; ESI-HRMS $(M+Na)^+$ *m/z* calcd for $C_{23}H_{31}NO_2Na$ 376.2247, found 376.2251.

**1-Bromo-4-(oct-1-ynyl)benzene (14)**

Compound **14** was prepared from 1-bromo-4-iodobenzene according to a procedure similar to that described for **1**; yield = 70%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.1 Hz, 3H), 1.29–1.34 (m, 4H), 1.40–1.47 (m, 2H), 1.59 (quin, *J* = 7.3 Hz, 2H), 2.37 (t, *J* = 7.1 Hz, 2H), 7.24 (dt, *J* = 8.3, 2.0 Hz, 2H), 7.39 (dt, *J* = 8.6, 2.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.5, 22.6, 28.8, 31.4, 79.6, 91.8, 121.5, 123.1, 131.4, 133.0; ESI-HRMS $(M)^+$ *m/z* calcd for $C_{14}H_{17}Br$ 264.0514, found 264.0508.

**4-(4-Octylphenethyl)pyridine (RB-051)**

To a deaerated solution of **14** (100 mg, 0.38 mmol), bis(triphenylphosphine)palladium dichloride (22 mg, 0.010 mmol), and copper(I) iodide (4 mg, 10 μmol) in anhydrous triethylamine (8 mL) was added 3-ethynylpyridine (78 mg, 0.75 mmol) at rt. The reaction mixture was heated at 80 °C for 3 d. After

saturated ammonium chloride solution was added, the product was extracted with EtOAc. The combined solution was washed with water, brine, and dried. The catalyst was removed by filtration through a pad of Celite, which was rinsed with hexanes/EtOAc (3:1). 3-((4-(Oct-1-ynyl)phenyl)ethynyl)pyridine (**15**) (53 mg, 0.18 mmol) was dissolved in EtOAc (8 mL), and 10% Pd/C (53 mg, 100 wt %) was added. The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with EtOAc. Flash column chromatography with hexanes/EtOAc (1:1) as eluent gave **RB-051** (48 mg, 88%) as a yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.27–1.34 (m, 10H), 1.59 (quin, $J$ = 7.3 Hz, 2H), 2.56 (t, $J$ = 7.8 Hz, 2H), 2.86–2.92 (m, 4H), 7.05 (d, $J$ = 8.1 Hz, 2H), 7.09 (d, $J$ = 8.1 Hz, 2H), 7.17 (dd, $J$ = 4.8, 7.7 Hz, 1H), 7.43 (dt, $J$ = 7.8, 1.8 Hz, 1H), 8.42–8.44 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.2, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 35.0, 35.6, 37.1, 123.2, 128.3, 128.5, 135.9, 137.0, 140.8, 147.5, 150.0; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{21}H_{30}N$ 296.2378, found 296.2374.

**1-Methyl-4-(4-octylphenethyl)pyridinium iodide (RB-052)**

To a solution of **RB-051** (32 mg, 0.11 mmol) in MeCN (5 mL) was added $K_2CO_3$ (75 mg, 0.54 mmol). After the suspension was stirred at rt for 10 min, MeI (30 μL, 0.54 mmol) was added. The reaction mixture was stirred overnight at rt. The reaction mixture was diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. The residue was washed with hexane to give 38 mg (80%) of **RB-052** as a yellow solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.9 Hz, 3H), 1.26–1.30 (m, 10H), 1.56 (quin, $J$ = 7.3 Hz, 2H), 2.54 (t, $J$ = 7.8 Hz, 2H), 3.02 (t, $J$ = 7.6 Hz, 2H), 3.17 (t, $J$ = 7.6 Hz, 2H), 4.60 (s, 3H), 7.07 (s, 4H), 7.92 (dd, $J$ = 6.2, 7.7 Hz, 1H), 8.06 (d, $J$ = 8.0 Hz, 1H), 9.12 (d, $J$ = 6.5 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.3, 29.4, 29.5, 31.6, 31.9, 34.4, 35.5, 35.7, 49.2, 127.6, 128.6, 128.7, 136.1, 141.4, 142.8, 143.0, 145.1, 145.2;

ESI-HRMS $(M)^+$ *m*/*z* calcd for $C_{22}H_{32}N^+$ 310.2535, found 310.2533.

**1-(Bromomethyl)-4-(oct-1-ynyl)benzene (16)**

Compound **16** was prepared from 4-iodobenzyl bromide according to a procedure similar to that described for **1**; yield = 69%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.9 Hz, 3H), 1.27–1.30 (m, 8H), 2.38 (t, *J* = 6.8 Hz, 2H), 4.44 (s, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.3, 22.4, 28.4, 28.7, 31.2, 33.3, 80.0, 91.2, 124.3, 128.9, 131.8, 136.9; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{15}H_{20}Br$ 279.0748, found 279.0730.

**4-(4-Methylpiperidin-1-yl)-1-(4-(oct-1-ynyl)benzyl)pyridinium bromide (18)**[27]

To a Pyrex vessel charged with a magnetic stirring bar was added a suspension of 4-chloropyridine hydrochloride (0.20 g, 1.3 mmol) in dry acetonitrile (4 mL). *N*,*N*-Diisopropylethylamine (DIPEA, 0.7 mL, 4.0 mmol) was added, followed by 4-methylpiperidine (0.16 mL, 1.3 mmol). The reactor was placed in a microwave apparatus and irradiated at 160 ºC for 1 h. After the reaction mixture was cooled rt, EtOAc was added, and the solution was washed with water and brine, dried ($Na_2SO_4$), and concentrated in vacuo. Ether (3 mL) was added to the resulting crude oil, and the inorganic precipitate was removed by filtration. Evaporation of the solvent gave 4-(4-methylpiperidin-1-yl)pyridine (**17**). To a solution of **16** (100 mg, 0.35 mmol) in 5 mL of 2-butanone was added **17** (124 mg, 0.71 mmol) in a sealed tube. The reaction mixture was stirred at 100 °C for 3 d and concentrated. The residue was washed with EtOAc to give 125 mg (78%) of **18** as a slightly yellow solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.4 Hz, 3H), 1.15–1.32 (m, 6H), 1.40–1.47 (m, 2H), 1.59 (quin, *J* = 7.28 Hz, 2H), 1.74–1.79 (m, 1H), 1.84 (d, *J* = 13.2 Hz, 2H), 2.39 (t, *J* = 7.1 Hz, 2H), 3.12 (td, *J* = 12.9, 2.1 Hz, 2H), 4.04 (d, *J* = 13.4 Hz, 2H), 5.60 (s, 2H), 6.95 (d, *J* = 7.1 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H),

7.43 (d, $J$ = 8.1 Hz, 2H), 8.56 (d, $J$ = 7.1 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.2, 19.4, 21.3, 22.6, 28.6, 29.7, 30.5, 31.3, 33.5, 47.4, 60.3, 79.8, 92.2, 108.4, 125.3, 128.9, 132.4, 133.1, 143.0, 155.2; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{26}H_{36}N_2^+$ 376.2873, found 376.2871.

**4-(4-Methylpiperidin-1-yl)-1-(4-octylbenzyl)pyridinium bromide (RB-053)**

Compound **18** (10 mg, 0.02 mmol) was dissolved in MeOH/$CH_2Cl_2$ (1/3, 3 mL), and 10% Pd/C (10 mg, 100 wt %) was added. The reaction mixture was hydrogenated at rt for 12 h. The catalyst was removed by filtration through a pad of Celite, which was rinsed with $CH_2Cl_2$ and concentrated. The residue was washed with hexane to give 9 mg (87%) of **RB-053** as a white solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, $J$ = 7.0 Hz, 3H), 0.98 (d, $J$ = 6.4 Hz, 3H), 1.16–1.33 (m, 10H), 1.55–1.60 (m, 4H), 1.72–1.78 (m, 1H), 1.84 (d, $J$ = 13.8 Hz, 2H), 2.58 (d, $J$ = 7.6 Hz, 2H), 3.13 (td, $J$ = 12.5, 2.4 Hz, 2H), 4.08 (d, $J$ = 13.5 Hz, 2H), 5.47 (s, 2H), 7.02 (d, $J$ = 7.6 Hz, 2H), 7.18 (d, $J$ = 8.0 Hz, 2H), 7.34 (d, $J$ = 8.0 Hz, 2H), 8.46 (d, $J$ = 7.6 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 21.3, 22.7, 29.2, 29.3, 29.4, 29.7, 30.3, 30.5, 31.4, 31.9, 33.5, 35.7, 47.5, 60.8, 108.5, 128.8, 129.5, 130.9, 142.8, 144.5, 155.2; ESI-HRMS $(M)^+$ $m/z$ calcd for $C_{26}H_{39}N_2^+$ 379.3113, found 379.3108.

**4-(Oct-1-ynyl)aniline (19)**

Compound **19** was prepared from 4-iodoaniline according to a procedure similar to that described for **1**; yield = 62%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, $J$ = 7.0 Hz, 3H), 1.26–1.35 (m, 4H), 1.40–1.47 (m, 2H), 1.58 (quin, $J$ = 7.4 Hz, 2H), 2.37 (t, $J$ = 7.1 Hz, 2H), 6.57 (d, $J$ = 8.6 Hz, 2H), 7.19 (d, $J$ = 8.6 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.5, 22.6, 28.6, 29.0, 31.4, 80.7, 87.9, 113.7, 114.8, 132.7, 145.9; ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{14}H_{20}N$ 202.1596, found 202.1589.

**3-(1-(4-(Oct-1-ynyl)phenyl)-1H-1,2,3-triazol-4-yl)pyridine (21)**[28]

To a solution of **19** (157 mg, 0.78 mmol) in 2 mL of 10% aqueous HCl was added $NaNO_2$ (65 mg, 0.94 mmol) in 1 mL of water at 0 ºC. After the solution was stirred for 30 min, $NaN_3$ (61 mg, 0.94 mmol) in 1 mL of water was added at 0 ºC, with stirring for another hour. The reaction mixture was warmed to 25 ºC, diluted with EtOAc, washed with water and brine, dried ($Na_2SO_4$), and concentrated in vacuo, affording **20**. Without purification, **20** (43 mg, 0.19 mmol) and 3-ethynylpyridine (39 mg, 0.38 mmol) were dissolved in *t*-BuOH/$H_2O$ (3 mL, 1:1), and $CuSO_4$ (30 mg, 0.19 mmol) and sodium ascorbate (37 mg, 0.19 mmol) were added at rt. The reaction mixture was stirred for 2 d and then was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel chromatography, eluting with hexanes/EtOAc (1:1), gave 50 mg (67%, 2 steps) of **21** as a white solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, $J$ = 6.9 Hz, 3H), 1.29–1.38 (m, 4H), 1.47 (quin, $J$ = 7.3 Hz, 2H), 1.63 (quin, $J$ = 7.3 Hz, 2H), 2.44 (t, $J$ = 7.1 Hz, 2H), 7.41 (dd, $J$ = 4.8, 7.9 Hz, 1H), 7.57 (d, $J$ = 8.6 Hz, 2H), 7.74 (d, $J$ = 8.6 Hz, 2H), 8.27–8.30 (m, 2H), 8.61–8.63 (m, 1H), 9.08 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.5, 22.6, 28.5, 28.6, 31.4, 79.3, 93.0, 117.8, 120.2, 123.1, 123.9, 125.2, 126.4, 133.0, 133.3, 135.6, 139.5, 145.4, 147.1, 149.6, 153.2, ESI-HRMS $(M+H)^+$ $m/z$ calcd for $C_{21}H_{23}N_4$ 331.1923, found 331.1919.

**3-(1-(4-Octylphenyl)-1H-1,2,3-triazol-4-yl)pyridine (RB-054)**

Compound **RB-054** was prepared from **21** according to a procedure similar to that described for **2**; yield = 82%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, $J$ = 6.8 Hz, 3H), 1.25–1.35 (m, 10H), 1.62–1.68 (m, 2H), 2.69 (t, $J$ = 7.7 Hz, 2H), 7.36 (d, $J$ = 8.3 Hz, 2H), 7.42 (dd, $J$ = 4.80, 7.82 Hz, 1H), 7.69 (d, $J$ = 8.3 Hz, 2H), 8.25 (s, 1H), 8.30 (d, $J$ = 7.86 Hz, 1H), 8.61–8.63 (m, 1H), 9.08 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 29.2, 29.3, 29.4, 29.7, 31.4, 31.9, 35.5, 118.1, 120.6, 123.9, 126.6, 129.8, 133.2,

134.7, 144.4, 145.2, 147.1, 149.4; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{21}H_{27}N_4$ 335.2236, found 335.2232.

**4-(4-Octyl-1*H*-1,2,3-triazol-1-yl)phenol (RB-055)**

To a solution of 4-aminophenol (100 mg, 0.92 mmol) in 2 mL of 10% aqueous HCl was added $NaNO_2$ (76 mg, 1.10 mmol) in 1 mL of water at 0 ºC. After the solution was stirred for 30 min, $NaN_3$ (72 mg, 1.10 mmol) in 1 mL of water was added at 0 ºC, with stirring for another hour. The reaction mixture was warmed to 25 ºC, diluted with EtOAc, washed with water and brine, dried ($Na_2SO_4$), and concentrated in vacuo, affording 4-azidophenol (**22**). Without purification, **22** (20 mg, 0.15 mmol) and 1-decyne (61 mg, 0.44 mmol) were dissolved in *t*-BuOH/$H_2O$ (5 mL, 1:1), and $CuSO_4$ (35 mg, 0.22 mmol) and sodium ascorbate (44 mg, 0.22 mmol) were added at rt. The reaction mixture was stirred for 2 d and then was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (10:1), gave 26 mg (70%, 2 steps) of **RB-055** as a white solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.7 Hz, 3H), 1.23–1.38 (m, 10H), 1.72 (t, *J* = 8.6 Hz, 2H), 2.79 (t, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 7.6 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.68 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.5, 29.2, 29.3, 29.4, 31.8, 116.7, 119.6, 122.3, 129.7, 148.8, 157.7; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{16}H_{24}N_3O$ 274.1914, found 274.1918.

**2-(4-(4-Octyl-1*H*-1,2,3-triazol-1-yl)phenyl)ethanol (RB-056)**

To a solution of **23**[26] (200 mg, 1.23 mmol) and 1-decyne (508 mg, 3.68 mmol) in *t*-BuOH/$H_2O$ (6 mL, 1:1) were added $CuSO_4$ (196 mg, 1.23 mmol) and sodium ascorbate (243 mg, 1.23 mmol). The reaction mixture was stirred at rt for 12 h and then was diluted with EtOAc and washed with brine. The aqueous

layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (10:1), gave 296 mg (80%) of **RB-056** as a white solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.27–1.42 (m, 10H), 1.71 (quin, *J* = 7.5 Hz, 2H), 2.76 (t, *J* = 7.7 Hz, 2H), 2.92 (t, *J* = 6.6 Hz, 2H), 3.89 (t, *J* = 6.6 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 7.61 (d, *J* = 8.5 Hz, 2H), 7.70 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.6, 29.1, 29.2, 29.3, 29.4, 31.8, 38.7, 63.1, 118.9, 120.4, 130.2, 135.6, 139.7, 149.1; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{18}H_{28}N_3O$ 302.2227, found 302.2230.

**4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)phenethyl methanesulfonate (26)**

To a solution of **23**[26] (200 mg, 1.23 mmol) and 1-hexyne (0.42 mL, 3.68 mmol) in *tert*-BuOH/$H_2O$ (6 mL, 1:1) were added $CuSO_4$ (196 mg, 1.23 mmol) and sodium ascorbate (243 mg, 1.23 mmol). The reaction mixture was stirred at rt for 12 h and then was diluted with EtOAc and washed with brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. Compound **24** was obtained, without purification, as a yellow liquid. To a solution of **24** (1.23 mmol) and triethylamine (0.86 mL, 6.15 mmol) in $CH_2Cl_2$ (10 mL) at 0 °C was added methanesulfonyl chloride (0.29 mL, 3.69 mmol). After being stirred at rt for 5 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated. Purification by silica gel chromatography, eluting with hexanes/EtOAc (1:2), gave 253 mg (66%, 2 steps) of **26** as a white solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, *J* = 7.3 Hz, 3H), 1.43 (sex, *J* = 7.5 Hz, 2H), 1.72 (quin, *J* = 7.6 Hz, 2H), 2.80 (t, *J* = 7.7 Hz, 2H), 2.92 (s, 3H), 3.12 (t, *J* = 6.7 Hz, 2H), 4.45 (t, *J* = 6.7 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.73 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.3, 25.3, 31.5, 31.6, 35.1, 37.4, 69.7, 118.8, 120.6, 130.3, 136.3, 136.9, 149.2; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{15}H_{22}N_3O_3S$ 324.1382, found

324.1382.

**4-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)phenethyl methanesulfonate (27)**

Compound **27** was prepared from **23**, via **25**, according to a coupling procedure similar to that described for **26**, using 1-heptyne; yield = 58% (2 steps); $^1$H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.1 Hz, 3H), 1.36–1.40 (m, 4H), 1.74 (quin, *J* = 7.5 Hz, 2H), 2.79 (t, *J* = 7.7 Hz, 2H), 2.93 (s, 3H), 3.12 (t, *J* = 6.7 Hz, 2H), 4.46 (t, *J* = 6.7 Hz, 2H), 7.38 (d, *J* = 8.5 Hz, 2H), 7.69 (d, *J* = 8.5 Hz, 2H), 7.74 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.4, 25.6, 29.1, 31.4, 31.6, 35.1, 37.4, 60.4, 69.7, 118.8, 120.6, 130.3, 136.2, 136.9, 149.3; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{16}H_{24}N_3O_3S$ 338.1538, found 338.1536.

**1-(4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidine (RB-057)**

To a solution of **26** (50 mg, 0.15 mmol) in 3 mL of acetonitrile was added piperidine (150 μL, 1.54 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (5:1), gave 11 mg (75%) of **RB-057** as a slightly yellow waxy solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, *J* = 7.4 Hz, 3H), 1.42 (sex, *J* = 7.4 Hz, 2H), 1.51–1.55 (m, 2H), 1.72 (quin, *J* = 7.6 Hz, 2H), 1.80 (quin, *J* = 5.4 Hz, 4H), 2.74–2.83 (m, 8H), 3.04–3.08 (m, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.71 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.3, 23.5, 24.7, 25.3, 29.7, 31.5, 31.9, 54.1, 60.0, 118.8, 120.5, 130.0, 135.7, 139.6, 149.1; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{19}H_{29}N_4$ 313.2392, found 313.2385.

**1-(4-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidine (RB-058)**

Compound **RB-058** was prepared from **27** according to a coupling procedure similar to that described for **RB-057**; yield = 81%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, *J* = 6.9 Hz, 3H), 1.36–1.43 (m, 4H),

1.47–1.52 (m, 2H), 1.67–1.77 (m, 6H), 2.53–2.61 (m, 4H), 2.65–2.69 (m, 2H), 2.78 (d, $J$ = 7.7 Hz, 2H), 2.92–2.96 (m, 2H), 7.34 (d, $J$ = 8.4 Hz, 2H), 7.62 (d, $J$ = 8.4 Hz, 2H), 7.69 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.4, 24.0, 25.5, 25.7, 29.1, 31.5, 32.7, 54.4, 60.7, 118.8, 120.5, 129.9, 135.6, 140.6, 149.1; ESI-HRMS $(M+H)^+$ *m/z* calcd for $C_{20}H_{31}N_4$ 327.2549, found 327.2543.

**1-(4-(4-Octyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidine (RB-059)**

To a solution of **RB-056** (50 mg, 0.17 mmol) and triethylamine (116 μL, 0.83 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added methanesulfonyl chloride (39 μL, 0.51 mmol). After being stirred at rt for 5 h, the reaction mixture was evaporated, diluted with water, and the product was extracted with EtOAc. The extract was washed with brine, dried, and evaporated to afford **28** as a yellow liquid. To a solution of 65 mg (0.17 mmol) of **28** (without purification) in 3 mL of acetonitrile was added piperidine (168 μL, 1.70 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. Purification by silica gel chromatography, eluting with $CH_2Cl_2$/MeOH (5:1), gave 11 mg (66%) of **RB-059** as a slightly yellow waxy solid; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, $J$ = 6.8 Hz, 3H), 1.25–1.39 (m, 10H), 1.58–1.62 (m, 2H), 1.72 (quin, $J$ = 7.3 Hz, 2H), 1.89 (quin, $J$ = 5.1 Hz, 4H), 2.78 (t, $J$ = 7.7 Hz, 2H), 2.96–3.04 (m, 6H), 3.14–3.18 (m, 2H), 7.40 (d, $J$ = 8.1 Hz, 2H), 7.64 (d, $J$ = 8.1 Hz, 2H), 7.67 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.6, 22.8, 23.9, 25.6, 29.1, 29.2, 29.3, 30.9, 31.8, 53.8, 59.1, 118.9, 120.6, 130.0, 135.8, 138.5, 149.1, 173.3; ESI-HRMS $(M+H)^+$ m/z calcd for $C_{23}H_{37}N_4$ 369.3013, found 369.3015; $(M+H-N2)^+$ m/z calcd for $C_{23}H_{35}N_2$ 341.2951, found 341.2954.

**1-(4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)phenethyl)-1-methylpiperidinium methanesulfonate (RB-060)**

To a solution of **26** (15 mg, 46 μmol) in 3 mL of acetonitrile was added 1-methylpiperidine (23 mg, 0.23 mmol). The reaction mixture was stirred at 50 °C for 12 h and concentrated. The residue was

washed with hexane to give 16 mg (82%) of **RB-060** as a yellow oil; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.41 (sex, *J* = 7.5 Hz, 2H), 1.66–1.74 (m, 4H), 1.80–1.87 (m, 4H), 2.77 (s, 3H), 2.74–2.84 (m, 2H), 3.13–3.17 (m, 2H), 3.26 (s, 3H), 3.57 (t, *J* = 5.5 Hz, 4H), 3.71–3.76 (m, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.5 Hz, 2H), 7.76 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.9, 20.2, 20.8, 21.5, 22.3, 22.8, 25.3, 27.8, 31.5, 39.6, 44.2, 55.4, 61.0, 119.1, 120.6, 120.7, 136.1, 136.2, 149.2; ESI-HRMS $(M)^+$ *m*/*z* calcd for $C_{20}H_{31}N_4^+$ 327.2549, found 327.2546.

**1-Methyl-1-(4-(4-pentyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidinium methanesulfonate (RB-061)**

Compound **RB-061** was prepared from **27** according to a coupling procedure similar to that described for **RB-060**; yield = 77%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.2 Hz, 3H), 1.34–1.39 (m, 4H), 1.68–1.75 (m, 4H), 1.81–1.86 (m, 4H), 2.76 (s, 3H), 2.74–2.83 (m, 2H), 3.13–3.18 (m, 2H), 3.28 (s, 3H), 3.59 (t, *J* = 5.5 Hz, 4H), 3.75–3.79 (m, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 2H), 7.76 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.0, 20.2, 20.8, 21.5, 22.4, 22.8, 25.6, 27.9, 29.1, 31.5, 39.7, 44.2, 55.3, 61.0, 119.0, 120.6, 120.8, 130.3, 130.8, 136.0, 136.2, 149.3; ESI-HRMS $(M)^+$ *m*/*z* calcd for $C_{21}H_{33}N_4^+$ 341.2705, found 341.2704.

**1-Methyl-1-(4-(4-octyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidinium methanesulfonate (RB-062)**

Compound **RB-062** was prepared from **28** according to a coupling procedure similar to that described for **RB-060**; yield = 71%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.1 Hz, 3H), 1.27–1.49 (m, 10H), 1.71–1.78 (m, 4H), 1.85–1.89 (m, 4H), 2.76 (s, 3H), 2.80–2.90 (m, 2H), 3.14–3.18 (m, 2H), 3.35 (s, 3H), 3.48 (t, *J* = 5.5 Hz, 4H), 3.67–3.85 (m, 2H), 7.60 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 8.5 Hz, 2H), 7.83 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.2, 20.5, 20.9, 21.4, 22.7, 23.0, 25.7, 27.9, 28.9, 29.1, 29.3,

29.4, 29.5, 31.9, 39.6, 44.0, 55.0, 61.1, 119.1, 120.4, 120.8, 130.2, 130.8, 136.1, 136.3, 149.3; ESI-HRMS $(M)^+$ m/z calcd for $C_{24}H_{39}N_4^+$ 383.3169, found 383.3174.

**1-(4-(4-Butyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidin-4-ol (RB-063)**

Compound **RB-063** was prepared from **26** according to a coupling procedure similar to that described for **RB-057**, using 4-hydroxypiperidine; yield = 65%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, *J* = 7.4 Hz, 3H), 1.42 (sex, *J* = 7.4 Hz, 2H), 1.60–1.75 (m, 4H), 1.93–1.97 (m, 2H), 2.26 (t, *J* = 9.4 Hz, 2H), 2.60–2.65 (m, 2H), 2.79 (t, *J* = 7.7 Hz, 2H), 2.85–2.89 (m, 4H), 3.75 (sep, *J* = 4.4 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.69 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 13.9, 22.3, 25.3, 29.7, 31.5, 33.3, 34.4, 51.1, 60.0, 67.6, 118.8, 120.4, 129.9, 135.5, 141.0, 149.1; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{19}H_{29}N_4O$ 329.2341, found 329.2336.

**1-(4-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidin-4-ol (RB-064)**

Compound **RB-064** was prepared from **27** according to a coupling procedure similar to that described for **RB-057**, using 4-hydroxypiperidine; yield = 70%; $^1$H NMR (400 MHz, $CDCl_3$) δ 0.90 (t, *J* = 7.1 Hz, 3H), 1.34–1.42 (m, 4H), 1.61–1.77 (m, 4H), 1.93–1.97 (m, 2H), 2.26 (t, *J* = 9.5 Hz, 2H), 2.61–2.65 (m, 2H), 2.78 (t, *J* = 7.7 Hz, 2H), 2.85–2.89 (m, 4H), 3.75 (sep, *J* = 4.2 Hz, 1H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.69 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.0, 22.4, 25.6, 29.1, 31.4, 33.3, 34.4, 51.0, 60.0, 67.6, 118.8, 120.4, 129.9, 135.5, 141.0, 149.1; ESI-HRMS $(M+H)^+$ *m*/*z* calcd for $C_{20}H_{31}N_4O$ 343.2498, found 343.2495.

**1-(4-(4-Octyl-1*H*-1,2,3-triazol-1-yl)phenethyl)piperidin-4-amine (RB-065)**

Compound **RB-065** was prepared from **28** according to a coupling procedure similar to that described

for **RB-057**, using 4-hydroxypiperidine; yield = 63%; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, $J$ = 6.7 Hz, 3H), 1.27–1.39 (m, 12H), 1.71 (quin, $J$ = 7.5 Hz, 2H), 1.77–1.84 (m, 2H), 2.10–2.15 (m, 2H), 2.77 (t, $J$ = 7.7 Hz, 4H), 2.91–2.95 (m, 2H), 3.05–3.07 (m, 2H), 3.09–3.14 (m, 2H), 3.89–3.93 (m, 1H), 7.37 (d, $J$ = 8.4 Hz, 2H), 7.63 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.6, 25.6, 29.1, 29.2, 29.3, 29.4, 31.7, 50.0, 58.9, 118.9, 120.6, 130.0, 135.8, 138.9, 149.2; ESI-HRMS $(M+H)^+$ m/z calcd for $C_{23}H_{37}N_4O$ 385.2962, found 385.2965.

## ASSOCIATED CONTENT

### Supporting Information

Evaluation of compounds as putative substrates of SK1 and SK2 and a molecular docking pose of **RB-035** in the SK1 binding pocket. This information is available free of charge via the Internet at http://pubs.acs. org.

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### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS USED

SK1, sphingosine kinase 1; SK2, sphingosine kinase 2; Sph, sphingosine

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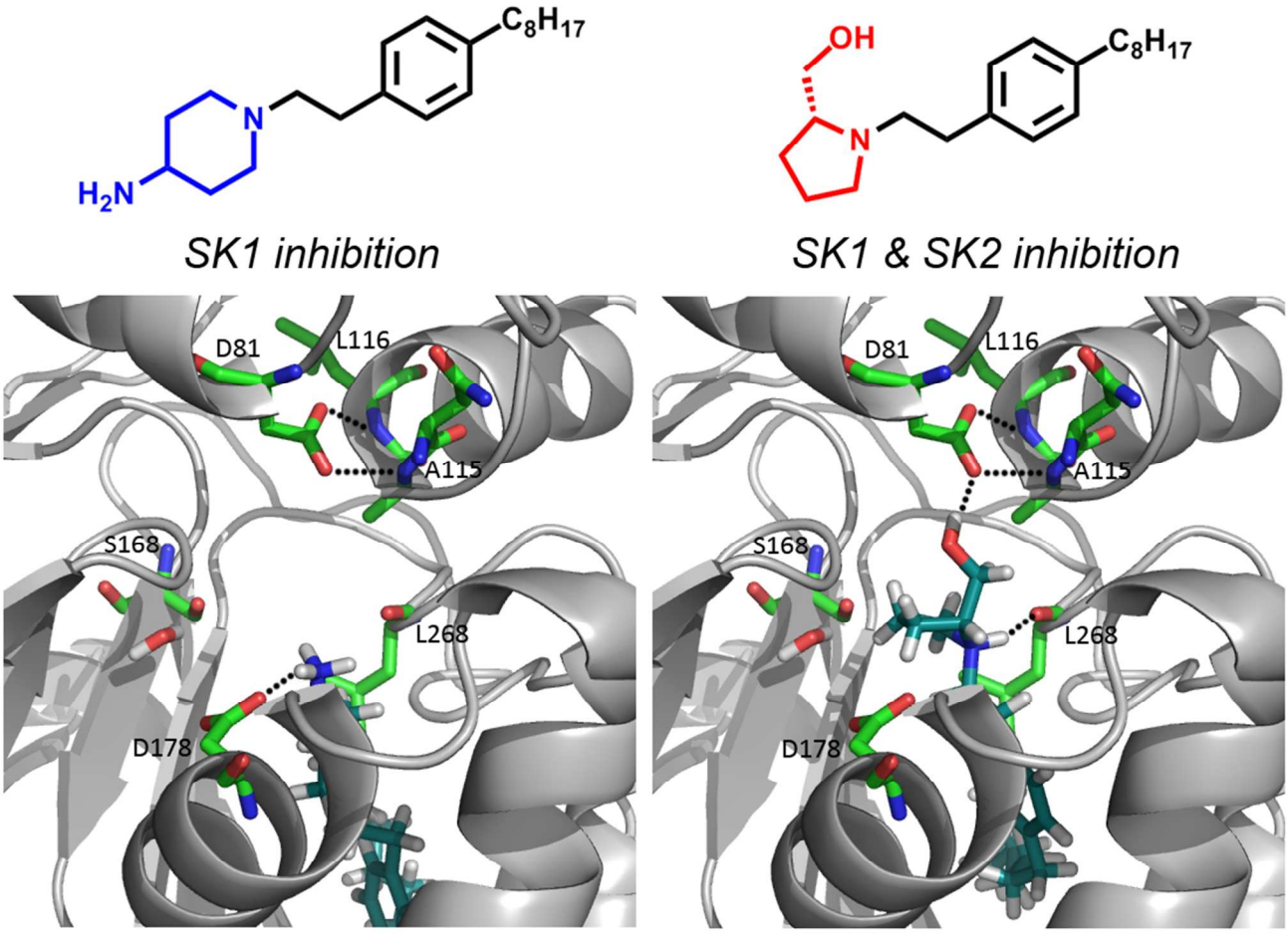
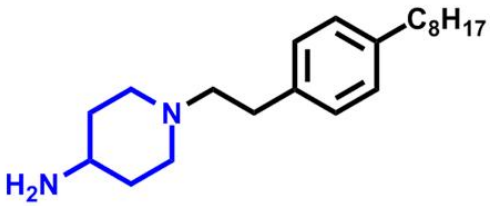
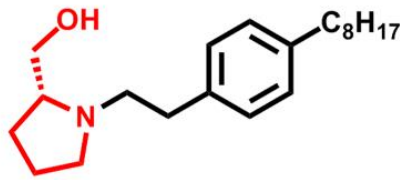
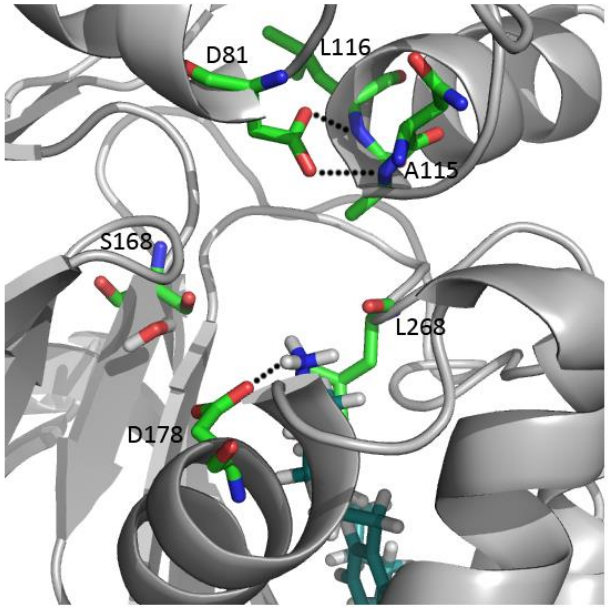
**Table of Contents graphic**






*SK1 inhibition*




*SK1 & SK2 inhibition*