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Syntheses of sphingosine-1-phosphate analogues and their interaction with EDG/S1P receptors

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Dedicated to Prof. Yong Hae Kim on the occasion of his 65th birthday

Abstract—Sphingosine-1-phosphate (S1P) is an important regulator of a wide variety of biological processes acting as an endogenous ligand to EDG/S1P receptors. In an effort to establish structure–activity relationship between EDG/S1P and ligands, we report herein homology modeling study of EDG-1/$S1P_1$, syntheses of S1P analogues, and cell based binding affinity study for EDG/S1P receptors.

Sphingosine-1-phosphate (S1P), one of the sphingolipid metabolites, is known to act as both an extracellular mediator and an intracellular second messenger. S1P-activated extracellular effects are mediated via plasma membrane G protein-coupled receptor EDG/S1P family, which include EDG-1/$S1P_1$, EDG-3/$S1P_3$, EDG-5/$S1P_2$, EDG-6/$S1P_4$, and EDG-8/$S1P_5$ subtypes, whereas specific intracellular targets remain to be identified.[1] S1P-activated EDG/S1P receptors are linked to diverse biological responses, such as mitogenesis, differentiation, migration, and apoptosis, and thus are believed to be involved in a variety of pathological conditions including angiogenesis, inflammation, and cardiovascular diseases, etc.[2] Therefore, S1P analogues with different specificities and affinities for the different EDG/S1P receptors would be extremely valuable in studying which receptor subtypes mediate which biological responses to S1P. More specifically, discovery of S1P agonists or antagonists might also provide the basis for development of novel therapeutic agents. However, the medicinal chemistry of S1P is not yet well developed, and there are no selective agonists or antagonists reported to date. Thus, it is deemed highly desirable to obtain some key structure–activity relationship (SAR) for S1P with respect to each individual EDG/S1P receptor.

Recently, we reported syntheses of S1P stereoisomers and derivatives, and their interaction with EDG/S1P receptors.[3] It was shown that D-*erythro* forms of S1P and dihydro-S1P have higher affinities than the other stereoisomers, indicating that 3D spatial orientations of key functionalities, that is, the C2-amino and C3-hydroxyl groups of S1P, are very important for the specific binding to EDG/S1P receptors. However, the possible interaction mode between the C3-hydroxyl group of S1P and EDG/S1P receptors remains to be defined. In order to shed a further light to these issues, and to prepare a basis for rational design of potential agonists and antagonists for EDG/S1P receptors, we have carried out a study on the computer modeling of EDG-1/$S1P_1$ docked with S1P, syntheses of a number of S1P analogues, and their interaction with EDG/S1P receptors.

Thus, we have built a homology model of EDG-1/$S1P_1$ receptor, and used it in docking studies with S1P to understand their specific interactions, specifically the role of the C3-hydroxyl group of S1P.[4] The computer model has shown three different ion-pairing interactions

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between EDG-1/S1P$_1$ receptor and the S1P head group; two ion pairs between Arg^{120} and Arg^{292} and the anionic phosphate of S1P; a single ion pair between Glu^{121} and the C2-amino group of S1P, observations that are in accord with the previous computational modeling study by Parrill et al.[5] In addition to these proposed interactions, the fourth interaction has also been noted: the interactions between the C3-hydroxyl group of S1P and the backbone carbonyl group of Phe^{296} and the hydroxyl group of Tyr^{98}, both within 3.2 Å distance (Fig. 1).

Since our previous studies[3] and the homology modeling clearly suggest that the C3-hydroxyl group should play an important role for the specific binding, we have prepared some C3-deoxy-S1P analogues. Garner aldehyde **1a**[6] was elaborated via Wittig condensation, hydrogenation, and phosphorylation to provide compounds **8a** and **9a**. By employing the same procedures on the enantiomeric aldehyde **1b**, the corresponding enantiomer **8b** was also obtained (Scheme 1).

The theoretical 3-D modeling of EDG-1/S1P$_1$ docked with S1P also suggests that the positively charged ammonium group of S1P is essential for the specific binding. Therefore, it was envisioned that N-alkylation of S1P might possibly differentiate its binding affinity and specificity to EDG/S1P subtypes. However, N,N-disubstitution is clearly not desirable because one hydrogen on the C2-amino group appears necessary to interact with the carboxylate of Glu^{121}. Thus, the two hydroxyl groups of *N*-Boc-sphingosine **10**, an intermediate in the S1P synthesis,[3] were protected with TBS and the product was alkylated with alkyl iodide in the presence of NaH/DMF to give **11a–c**. Selective deprotection of C1-*O*-silyl group was achieved by treatment of **11a–c** with HF/pyridine solution at 0 °C, under which the C3-*O*-silyl group remained intact.[7] Phosphorylation of compounds **12a–c**, followed by deprotection yielded the desired *N*-alkyl-S1P **14a–c** (Scheme 2).

The observation that 4,5-dihydro-S1P is less potent than S1P itself in its binding affinity, suggests that the double bond somehow plays some important role in the binding.[3] Thus, we substituted phenyl moieties for the C4–5 double bond of S1P. Synthesis of the phenyl-incorporated S1P analogues was accomplished via compounds **17a–d** following the literature method described by Chun et al.[8] Compounds **18a–d** were prepared using the procedure employed for the preparation of **8a** from **2a** (Scheme 3).[3,9]

The computational model also suggests that the aliphatic tail part of S1P should positively interact with the hydrophobic area formed by the seven transmembrane spanning helices in the EDG-1/S1P$_1$. In order to examine the hydrophobic interaction involving the tail part, we synthesized a series of S1P derivatives in which the *n*-$C_{13}H_{27}$ aliphatic long chain was replaced by the shorter chains such as *n*-$C_{11}H_{23}$, *n*-C_7H_{15}, cyclohexyl, and phenyl-containing side chains including 3-decyloxyphenyl, 4-buthoxyphenyl, and 4-benzyloxyphenyl. The synthesis started with the β-keto-phosphonate **19**,

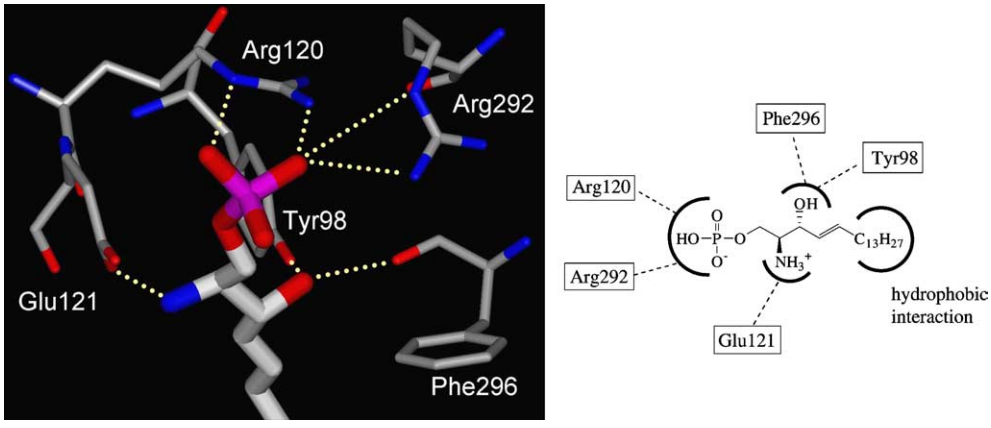
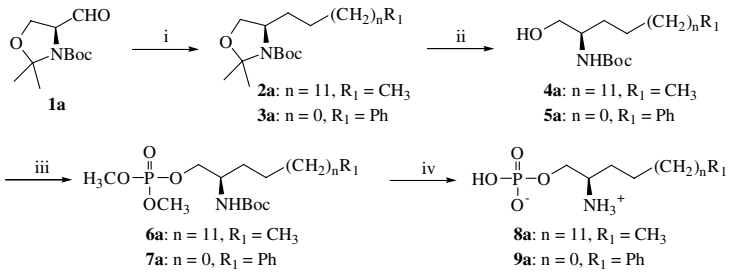
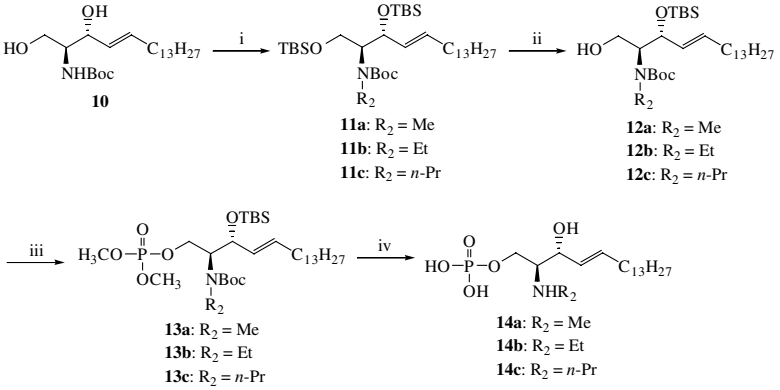


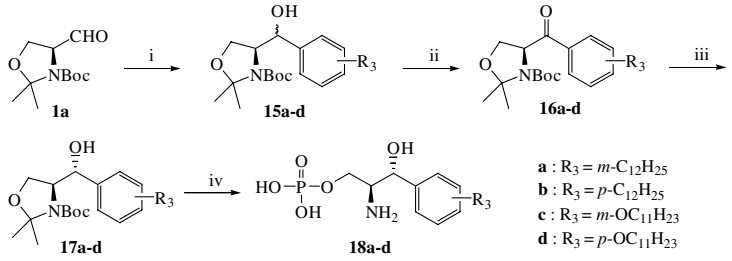
Figure 1. Theoretical binding conformation of D-*erythro* S1P in the EDG-1/S1P$_1$ active site. Hydrogen bonds are indicated as dashed lines.



Scheme 1. Reagents and conditions: (i) (a) $Ph_3P^+C_{15}H_{31}Br^-$/LHMDS for **2a** and **2b**, $Ph_3P^+BnBr^-$/LHMDS for **3a**, THF, −78 °C–rt, 91%, (b) H_2, 10% Pd/C, EtOAc, rt, 96%; (ii) LiCl, 90% aq AcOH, rt, 87%; (iii) $P(OCH_3)_3$, CBr_4, pyridine, 0 °C, 84%; (iv) TMSBr, CH_2Cl_2, 0 °C, 58%.



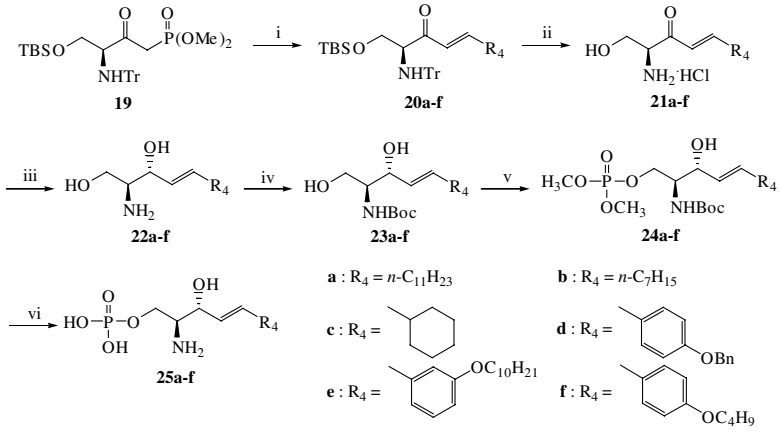
Scheme 2. Reagents and conditions: (i) (a) TBSCl, imidazole, DMF, rt, quant, (b) alkyl iodide, NaH, DMF, rt, 89–91%; (ii) HF, pyridine, THF, 0 °C, 63–88%; (iii) $P(OCH_3)_3$, CBr_4, pyridine, 0 °C, quant; (iv) (a) TBAF, THF, rt, 60–65%, (b) TMSBr, CH_2Cl_2, rt, 43–54%.



Scheme 3. Reagents and conditions: (i) *n*-BuLi, aryl bromide, THF, −42 °C, 60–80%; (ii) PCC, CH_2Cl_2, rt, 80–87%; (iii) DIBAL-H, THF, 0 °C, 93–98%; (iv) (a) LiCl, 90% aq AcOH, rt, 87–92%, (b) $P(OCH_3)_3$, CBr_4, pyridine, 0 °C, 79–90%, (c) TMSBr, CH_2Cl_2, 0 °C, 40–58%.

which was an intermediate in the earlier sphingosine synthesis in our laboratory.[10] The Horner–Wadsworth–Emmons (HWE) condensation of the phosphonate **19** with various aldehydes provided the corresponding enones **20a**–**f** in good yields. Removal of the two protecting groups (*N*-trityl and *O*-TBS) of **20a**–**f** with 2 N HCl in MeOH–THF under reflux gave 3-ketosphingosine derivatives **21a**–**f**. Enones **21a**–**f** were selectively reduced with $Zn(BH_4)_2$ to afford the *anti* form of sphingosine analogues **22a**–**f**.[10] *N*-Boc protection of **22a**–**f**, followed by phosphorylation and deprotections provided the desired tail-modified S1P analogues **25a**–**f** (Scheme 4).

The binding affinities of the synthetic S1P analogues were evaluated in vitro for the EDG/S1P receptors by measuring their ability to displace radioligand, [^{3}H]-S1P from EDG-1/$S1P_1$, EDG-3/$S1P_3$, and EDG-5/$S1P_2$, which were expressed in CHO cells.[11,12] The results are shown in Figure 2. Among the C3-deoxy-S1P analogues having an aliphatic or aromatic side chain, compound **8a** having (2*R*)-configuration and 16 carbon chain length displayed selective binding affinities for EDG-3/$S1P_3$ (82%) and EDG-5/$S1P_2$ (84%), ca. three times higher affinity than the 18 carbon analogue.[3] The enantiomeric compound **8b** did not show much of the



Scheme 4. Reagents and conditions: (i) For the reaction with aliphatic aldehydes, R_3CHO, DBU, LiCl, THF, rt, 90–95%, for the reaction with aromatic aldehydes, R_3CHO, NaH, THF, rt, quant; (ii) 2 N HCl, THF–MeOH, 40 °C, 80–85%; (iii) $Zn(BH_4)_2$, THF, −78 °C, 75%; (iv) Boc_2O, 1 N NaOH, dioxane–H_2O, rt, 85–93%; (v) $P(OCH_3)_3$, CBr_4, pyridine, 0 °C, 73–85%; (vi) TMSBr, CH_2Cl_2, rt, 43–54%.

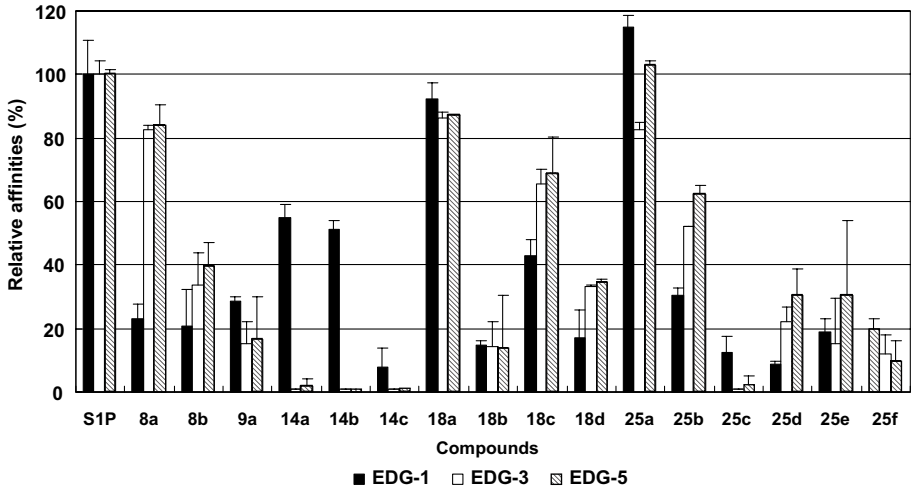


Figure 2. Relative binding affinity of S1P derivatives on specific binding of D-*erythro* S1P to EDG-1, -3, and -5 receptors. CHO cells transfected with Edg-1, -3, and -5, respectively, were incubated in the presence of 2 nM [^{3}H]-S1P with or without 1 μM of the indicated compounds. Results are means ± standard deviation of duplicate determinations.

binding affinity, indicating that 3D configuration of C2-amino group is important even in the C3-deoxy-S1P analogues.

Very interestingly, *N*-Me–S1P **14a** and *N*-Et–S1P **14b** showed highly selective affinity for EDG-1/S1P$_1$ (55.1% and 51.4%, respectively), and they were completely nonbinding to EDG-3/S1P$_3$ and EDG-5/S1P$_2$, whereas *N*-Pr–S1P **14c** showed negligible affinity to all subtypes. On the basis of the 3-D modeling of EDG-1/S1P$_1$ docked with S1P, it was initially thought that there was not enough room for the *N*-alkyl group to occupy. However, the flexible movement of the active site may allow the *N*-methyl or *N*-ethyl group, although the space surrounding the C2-amino group of S1P appears relatively tight; *N*-propyl–S1P may be just too bulky to be accommodated. None of the *N*-alkyl substituted S1P analogues was found to bind to EDG-3/S1P$_3$ or EDG-5/S1P$_2$. The high selectivity of *N*-Me–S1P **14a** and *N*-Et–S1P **14b** might be significant in the sense that it can provide a direction to find selective agonists or antagonists to EDG-1/S1P$_1$ subtype for the first time.

In the case of substituted phenyl-incorporated compounds, compound **18a** containing *m*-$C_{12}H_{25}$ substituted phenyl moiety showed almost the same affinities to S1P, albeit nonselectively for three EDG/S1P subtypes, whereas *p*-substituted one **18b** had much weaker affinities. This observation demonstrates that C4–5 double bond could be replaced with a phenyl group substituted with *m*-alkyl group without decrease of affinities for EDG/S1Ps.[13] Compounds **18c–d** with a substituted alkoxy group showed relatively weak affinities.

We have also tested the tail-modified S1P analogues. First, the aliphatic long chain of S1P, *n*-$C_{13}H_{27}$ was replaced with a shorter chain such as *n*-$C_{11}H_{23}$ or *n*-C_7H_{15}. Compound **25a** having *n*-$C_{11}H_{23}$ was found to have equipotent affinities for S1P (115% for EDG-1/S1P$_1$, 83% for EDG-3/S1P$_3$, and 103% for EDG-5/S1P$_2$, respectively). However, introducing a shorter alkyl group, *n*-C_7H_{15} (**25b**) substantially reduced binding affinities, especially for EDG-1/S1P$_1$ subtype (31% for EDG-1/S1P$_1$, 52% for EDG-3/S1P$_3$, and 63% for EDG-5/S1P$_2$, respectively). The replacement of the *n*-$C_{13}H_{27}$ in S1P with cyclohexyl (**25c**), 4-benzyloxyphenyl (**25d**), 3-decyloxyphenyl (**25e**), or 4-buthoxyphenyl (**25f**) resulted in marked decreases of the binding affinity. These results suggest that the hydrophobic pocket located in the transmembrane helices region of EDG/S1P receptors is somewhat narrow and long, and the hydrophobic interaction plays an important role for efficient binding. Thus, seemingly minor structural changes in the tail part of S1P can have substantial consequences in the interaction with the hydrophobic pocket of the receptors.

In summary, we have carried out a homology modeling study of EDG-1/S1P$_1$, prepared a series of S1P analogues, and evaluated their binding affinity for EDG/S1P receptors. Based on these results, it might be concluded that (1) the C3-hydroxyl group plays important role in binding to EDG/S1Ps, specifically to EDG-1/S1P$_1$, (2) the tail portion (olefin and *n*-$C_{13}H_{27}$) of S1P can be replaced by *m*-alkylphenyl group without much impact on the binding affinity, (3) *N*-Me–S1P and *N*-Et–S1P show selective binding affinity for EDG-1/S1P$_1$, thus providing a possibility to find selective agonists or antagonists to EDG-1/S1P$_1$ for the first time, and (4) a fine-tuning of the long alkyl chain may be possible in terms of the selectivity and the absolute affinity. These findings are expected to substantially contribute to the development of potent and selective agonists and antagonists for EDG/S1P receptors. Further works along these lines are currently in progress.

Acknowledgements

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12. Binding assay protocol: [^{3}H]-S1P was purchased from American Radiolabeled Chemicals, Inc. CHO cells (CHO/Edg-1, CHO/Edg-3, CHO/Edg-5, and CHO/Mock) in confluent six multiplates were washed twice with the ice-cold binding buffer consisting of 20 mM Tris–HCl (pH 7.5), 100 mM NaCl, 15 mM NaF, and 0.4% (w/v) BSA, and then incubated with the same buffer containing 2 nM [^{3}H]-S1P (about 40,000 dpm per well) and 1 μM of S1P or S1P derivatives in a final volume of 1.0 mL. The plates were kept in ice for 90 min, and the cells were washed twice with the same ice-cold binding buffer to remove unbounded ligand. The cells were solubilized with the solubilizing solution composed of 0.1% SDS, 0.4% NaOH, and 2% Na_2CO_3, and the radioactivity was measured by a liquid scintillation counter after the addition of scintillation cocktail solution. The relative binding affinities of each compound to each EDG/S1P receptor were presented as percentage to S1P.
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14. The rhodopsin model (PDB code 1boj) used as the template in this study is not based on a crystal structure but rather a theoretical model. Because of the potential problems associated with this discrepancy, a homology modeling study based on a rhodopsin crystal structure is in progress.