Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1115–1119
Bioorganic & Medicinal Chemistry Letters

Synthesis and biological properties of novel sphingosine derivatives

Teiichi Murakami,[a,*] Kiyotaka Furusawa,[a] Tadakazu Tamai,[b,*] Kazuyoshi Yoshikai[b] and Masazumi Nishikawa[b]

[a]*National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 5, Tsukuba, Ibaraki 305-8565, Japan*
[b]*Central Research Institute, Maruha Corporation, 16-2 Wadai, Tsukuba, Ibaraki 305-4295, Japan*

Received 25 October 2004; revised 2 December 2004; accepted 6 December 2004
Available online 28 December 2004

Abstract—Sphingosine-1-phosphate (S-1P) derivatives such as *threo*-(2*S*,3*S*)-analogues, which are C-3 stereoisomers of natural *erythro*-(2*S*,3*R*)-S-1P, have been synthesized starting from L-serine or (1*S*,2*S*)-2-amino-1-aryl-1,3-propanediols (**6**). *threo*-(1*S*,2*R*)-2-Amino-1-aryl-3-bromopropanols (HBr salt) have also been prepared from **6**. The *threo*-S-1Ps and the *threo*-amino-bromide derivatives have shown potent inhibitory activity against Ca^{2+} ion mobilization in HL60 cells induced by *erythro*-S-1P, suggesting that these compounds would compete with cell surface EDG/S1P receptors.

Sphingolipids, for example, sphingomyelin, cerebrosides, and gangliosides, are ubiquitous cell membrane components and are involved in many essential biological processes such as cell growth, cell differentiation, and adhesion.[1] Sphingolipid metabolites such as sphingosine and ceramide are emerging as a novel class of lipid second messengers.[2] Recently sphingosine-1-phosphate (S-1P), one of the metabolites, has attracted considerable attention[3] both as an intracellular second messenger[4] and as an intercellular mediator. It has been reported that S-1P binds to cell surface receptor EDG (endothelial differentiation gene)/S1P family (five subtypes: EDG-1/$S1P_1$, EDG-3/$S1P_3$, EDG-5/$S1P_2$, EDG-6/$S1P_4$, and EDG-8/$S1P_5$ have been identified[3]), which are coupled via plasma membrane G-protein to multiple effector systems.[5] Physiological significance of S-1P seems very important in the vascular system because blood platelets store S-1P abundantly and release this bioactive lipid extracellularly upon stimulation to bind surface receptors on vascular endothelial cells.[6] The receptors bound to S-1P would affect various biological responses, including mitogenesis, differentiation, proliferation, and apoptosis, and thus are supposed to be involved in a variety of pathological conditions such as angiogenesis, inflammation, and cardiovascular diseases.[7] S-1P is also suggested to be a central component of a complex network of cytokines and chemokines, which influence the responses of cells including immunosuppression.[8] Therefore, the search for agonists and antagonists toward EDG/S1P receptors would provide the basis for development of novel therapeutic agents for such diseases.[9]

Sphingoid bases are 2-amino-1,3-diols with a long-chain alkyl tail at C3. The alkyl tail may vary in chain length (from 16 to 24 carbon atoms), unsaturation (usually C4,5-*trans* olefin), and hydroxylation. The most common sphingoid base in mammalian tissues is D-*erythro*-C_{18}-sphingosine [(2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol]. Recently, Parrill and co-workers proposed that both the C1 phosphate group and C2 ammonium moiety of D-*erythro*-S-1P are critical for its specific binding to EDG-1/$S1P_1$ receptor based on their homology modeling and point mutation studies. However, the role of the C3 hydroxy group remains to be solved.[10] Chung and co-workers reported the synthesis of four stereoisomers of S-1P and the analogues, and their binding affinities to EDG-1, -3, and -5.[11a] Their results have suggested that (1) the D-*erythro* configuration of S-1P is important for a high affinity binding, (2) the phosphate group of S-1P is essential for ligand recognition by the receptors, (3) besides the C1 phosphate group and the C2 ammonium moiety, the presence and configuration of the C3 hydroxy group of S-1P appears to be very important for specific binding.

Encouraged by these studies, we planned to investigate agonist/antagosist activities of novel S-1P analogues

Keywords: Sphingosine-1-phosphate; Amino alcohols; EDG/S1P receptors; Antagonists.
* Corresponding authors. Tel.: +81 29 861 9394; fax: +81 29 861 4549; e-mail: t-murakami@aist.go.jp

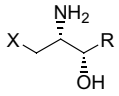
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.12.010

toward EDG/S1P receptors. Herein we report the synthesis of several analogues and their effects on Ca^{2+} ion mobilization in HL60 leukemia cells expressing these receptors.[12] In addition, their actions on the growth of vascular smooth muscle cells and on an inflammation model are reported.

We recently reported[13] a highly diastereoselective synthesis of both D-*erythro*- and L-*threo*-sphingosines from L-serinal derivative (Garner's aldehyde[14]) with 1-alkenyl nucleophiles prepared via hydrozirconation[15] of terminal alkynes. Thus, as shown in Scheme 1, *N*-Boc-sphingosine derivatives *erythro*-**3a**–**c** and *threo*-**3a**–**c** (**a**: natural length C18; **b**: shorter-chain homologue; **c**: styryl analogue) were prepared[13] from Garner's aldehyde (**1**) in a stereocontrolled manner via the *N*,*O*-isopropylidene acetal derivatives *erythro*-**2a**–**c** and *threo*-**2a**–**c**, respectively. Selective phosphorylation of C1 alcohol of **3** was achieved by a procedure of Bielawska and co-workers[16] with $(MeO)_3P$ and CBr_4 in pyridine.[9d,11] Treatment of the dimethyl phosphate **4** with trimethylsilyl bromide (TMSBr) followed by addition of water resulted in deprotection of both the Boc group and the phosphate dimethyl ester to afford S-1P derivative (*erythro*-**5a**–**c** and *threo*-**5a**–**c**). The spectral and physical data of these S-1P derivatives were identical with those reported[11a,16,17] and/or consistent with assigned structures.[18]

We then evaluated biological activities of the S-1P analogues by measuring Ca^{2+} ion mobilization in HL60 cells.[19] The bioassays have indicated that *erythro*-**5a**–**c** show the Ca^{2+} ion increasing activity comparable to natural S-1P, whereas *threo*-**5a**–**c** do not show that activity (data not shown). Interestingly, as shown in Table 1, *threo*-**5a**–**c** inhibit the natural S-1P induced-Ca^{2+} ion increase at rather low concentrations (IC_{50} = 0.015–0.037 μM).[20] Thus the *threo*-(2*S*,3*S*)-analogues might be recognized as a ligand by EDG/S1P receptors to show potent antagonist-like activities. Since subtype specific receptors were not used in this preliminary assay, these observations may reflect total affairs concerning with the receptors.

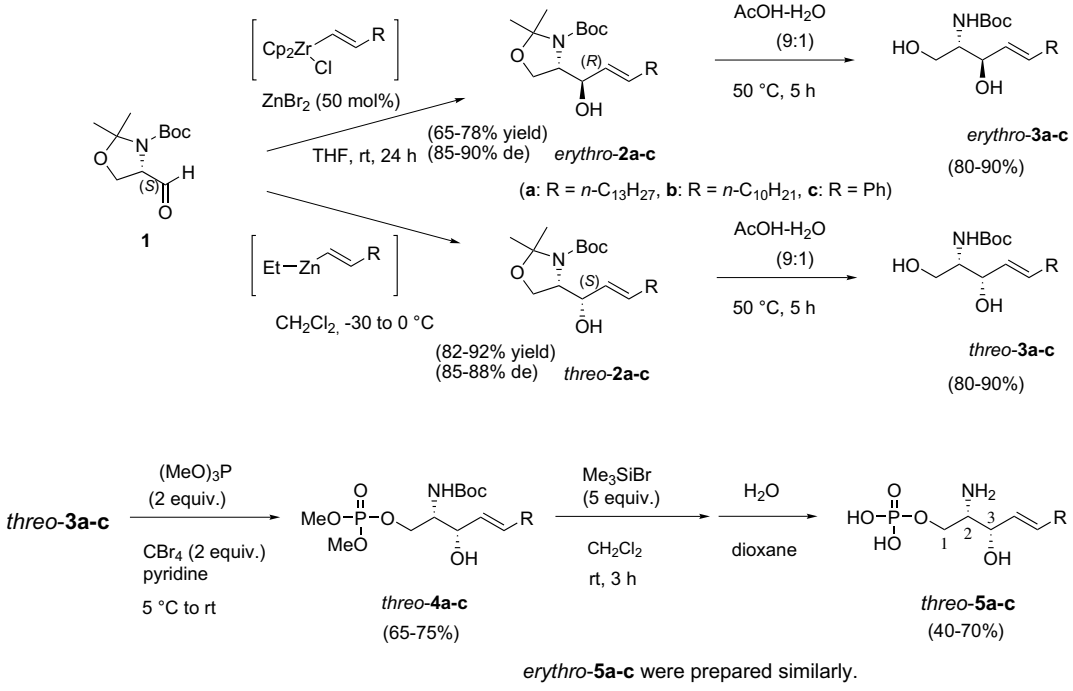
Table 1. Inhibition concentration (50%) of S-1P derivatives (*threo*-amino alcohols) against Ca^{2+} ion increase in HL60 induced by natural S-1P



Compound	R	X	IC_{50} (μM)[a]
threo-**5a**	(*E*)-*n*-Pentadec-1-enyl	OPO_3H_2	0.031
threo-**5b**	(*E*)-*n*-Dodec-1-enyl	OPO_3H_2	0.015
threo-**5c**	(*E*)-Styryl	OPO_3H_2	0.037
9d	Phenyl	OPO_3H_2	0.179
9e	*p*-Nitrophenyl	OPO_3H_2	0.056
9f	*p*-(Methylthio)phenyl	OPO_3H_2	0.015
11d[b]	Phenyl	Br	0.071
11f[b]	*p*-(Methylthio)phenyl	Br	0.018

[a] The values are the mean of duplicate experiments.
[b] HBr salt was used.

Since the *threo*-S-1Ps showed inhibitory effect, our attention was turned to readily available *threo*-amino alcohols. Commercially available *threo*-2-amino-1-aryl-1,3-propanediols (**6d**–**f**; **d**: phenyl; **e**: *p*-nitrophenyl; **f**: *p*-methylthiophenyl) are suitable for our purpose. Phosphorylation of the primary alcohol was carried out in a



Scheme 1. Synthesis of S-1P derivatives.