Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3175–3180

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STRUCTURE-ACTIVITY RELATIONSHIP OF SHORT-CHAIN SPHINGOID BASES AS INHIBITORS OF SPHINGOSINE KINASE

Steven De Jonghe[#], Ilse Van Overmeire[#], Samantha Poulton[§], Chris Hendrix[¶], Roger Busson[¶], Serge Van Calenbergh[#], Denis De Keukeleire[#], Sarah Spiegel[§] and Piet Herdewijn[#,¶,*]

[#] *University of Gent, Faculty of Pharmaceutical Sciences, Laboratory for Medicinal Chemistry, Harelbekestraat 72, B-9000 Gent, Belgium;* [§] *Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, D.C. 20007, USA;* [¶] *Catholic University of Leuven, Rega Institute, Laboratory for Medicinal Chemistry, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*

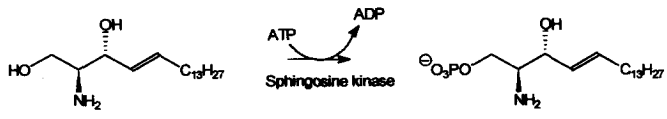
Received 31 August 1999; accepted 4 October 1999

Abstract: Short-chain sphinganine analogues **8**, **9**, **18**, and **19**, as well as 3-fluoro-sphingosine analogues **25** and **26** were synthesized. Their potential as sphingosine kinase inhibitors was investigated, in combination with previously synthesized sphingosine and fluorinated sphinganine analogues.

Introduction

Sphingosine-1-phosphate, formed by phosphorylation of sphingosine involving sphingosine kinase as catalyst (Figure 1), was originally described as an intermediate in the catabolism of sphingolipids. However, in recent years, it has become clear that sphingosine-1-phosphate exhibits several important biological functions. It has been implicated as a second messenger in cellular proliferation and survival induced by platelet-derived growth factor, fetal calf serum[1] and nerve growth factor.[2] In addition, sphingosine-1-phosphate protects cells from ceramide-induced apoptosis[3] and intervenes as an intercellular signaling molecule (first messenger) by binding to the specific G-protein coupled receptors EDG-1, EDG-3, and EDG-5.[4] Examples of such processes include inhibition of chemotactic cell motility and invasiveness of various tumor cells[5], induction of platelet activation[6], and retraction of neurites in neuronal cells.[7]

Figure 1



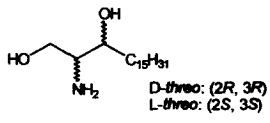
Sphingosine Sphingosine-1-phosphate

* Fax. +32 (0)16/33.73.87; e-mail: Piet.Herdewijn@rega.kuleuven.ac.be

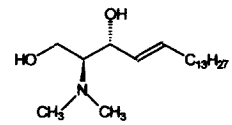
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00554-5

D,L-*threo*-dihydrosphingosine (or D,L-*threo*-sphinganine)[8] and *N,N*-dimethylsphingosine[9] are known competitive inhibitors of sphingosine kinase (Figure 2). To further explore the structural requirements, necessary for inhibition of sphingosine kinase, we previously synthesized sphingosine analogues[9] and fluorinated sphinganine analogues.[10] We have now prepared a new series of sphinganine derivatives and 3-fluoro-sphingosine analogues in order to comparatively assess these compounds as potential inhibitors of sphingosine kinase. Such inhibitors could be considered as potential biochemical tools to further elucidate the biological significance of sphingosine-1-phosphate and to characterize features related to substrate specificity of the kinase.

Figure 2



D,L-*threo*-dihydrosphingosine
(= D,L-*threo*-sphinganine)

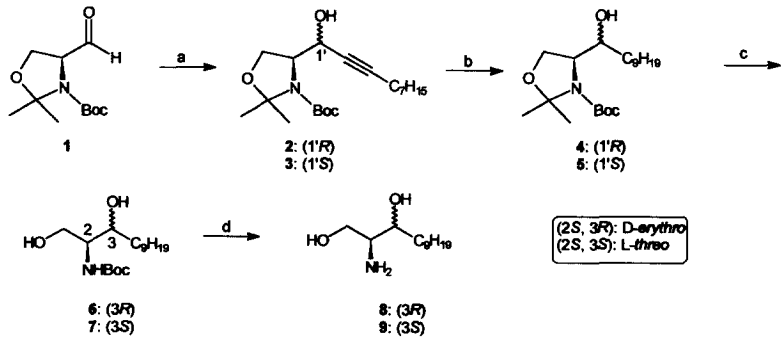


N,N-dimethylsphingosine

Synthesis

The synthesis of the sphinganine analogues was accomplished using a modified procedure originally described by Herold (Scheme 1).[12] Diastereoselective addition reactions of alkynyl lithium to the well-known Garner aldehyde **1** (synthesized from L-serine, according to an established protocol[13]) may furnish either *erythro*-alkynol **2** or *threo*-alkynol **3**, depending on the reaction conditions. While the use of hexamethylphosphoramide (HMPA) as a cosolvent accounts for high *erythro*-selectivity, addition of anhydrous zinc dibromide in diethyl ether mainly led to *threo*-alkynol **3**.

Scheme 1



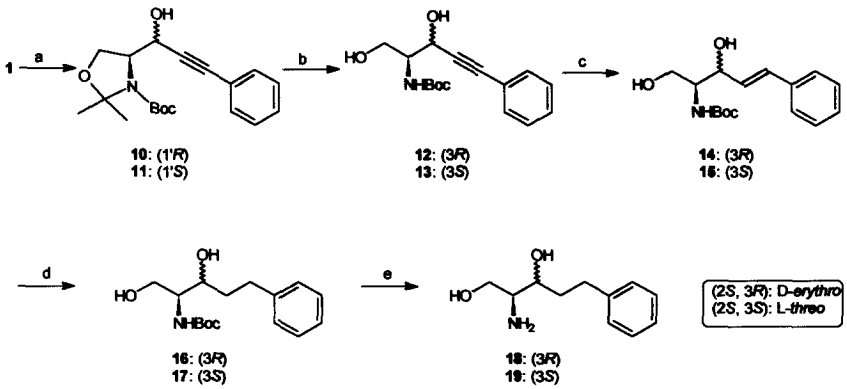
a: *n*-BuLi, $CH_3(CH_2)_6C{\equiv}CH$, HMPA, THF, -78 °C (for **2**: 84%) or *n*-BuLi, $CH_3(CH_2)_6C{\equiv}CH$, $ZnBr_2$, Et_2O, -78 °C to rt (for **3**: 64%);
b: H_2, Pt/C, MeOH, rt (**4**: 98%, **5**: 92%); c: TosOH, MeOH, rt (**6**: 76%, **7**: 68%); d: HCl (37%), EtOAc, rt (**8**: 74%, **9**: 80%).

The diastereoselectivity is convincingly accounted for by considering the preferred transition state in each reaction. According to the well-known Felkin-Anh model, the nucleophile attacks preferentially at the *re*-face

of aldehyde **1** thereby leading to the *erythro*-configuration. The diastereoselectivity is reversed in the presence of a Lewis acid (such as zinc dibromide), since chelation control in the transition state results in nucleophilic reaction from the *si*-face to give the *threo*-epimer. Catalytic hydrogenation of propargyl alcohols **2** and **3** yielded alcohols **4** and **5**, respectively. Cleavage of the oxazolidine by treatment with TosOH resulted in formation of the *N*-protected sphinganine analogues **6** and **7**. Deprotection of the *tert*-Boc group with concentrated hydrogen chloride gave access to the C_{12}-sphinganine analogues **8** and **9**.[14]

The synthesis of sphinganine analogues possessing an aromatic residue is outlined in Scheme 2. We applied a sequence, which was slightly adapted from that described above. Diastereoselective addition of the lithium salt of phenylacetylene to the Garner aldehyde **1** yielded alkynols **10** and **11**, respectively. As the catalytic hydrogenation of the propargyl alcohols **10** and **11** did not proceed as expected, we followed a two-step reduction route. First, the alkynyl group was reduced to the *trans*-alkenes **14** and **15** using Red-Al, subsequent to cleavage of the oxazolidine. Next, catalytic hydrogenation yielded the *N*-protected sphinganine analogues **16** and **17**. Acid-assisted removal of the *tert*-Boc group afforded sphinganine analogues **18** and **19**.[15]

Scheme 2

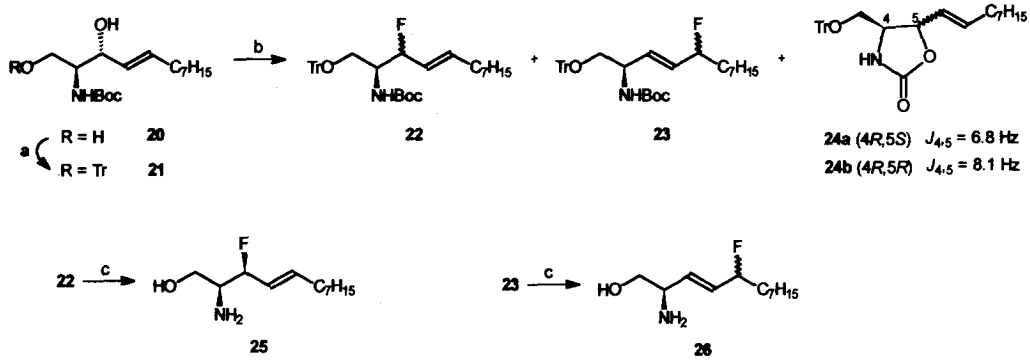


a: *n*-BuLi, $C_6H_5C{\equiv}CH$, HMPA, THF, -78 °C (for **10**: 81%) or *n*-BuLi, $C_6H_5C{\equiv}CH$, $ZnBr_2$, Et_2O, -78 °C to rt (for **11**: 69%); b: TosOH, MeOH, rt (**12**: 67% **13**: 60%); c: Red-Al, Et_2O, 0 °C to rt (**14**: 68%, **15**: 73%); d: H_2, Pt/C, MeOH (**16**: 97%, **17**: 92%); e: HCl (1 N), dioxane, 100 °C (**18**: 78%, **19**: 71%).

The fluorinated sphingosine analogues **25** and **26** were synthesized from the *N-tert*-Boc-protected sphingoid **20**.[9] After protection of the primary hydroxyl group of **20** as a trityl ether, reaction of **21** with diethylaminosulfur trifluoride (DAST) gave the fluorinated compounds **22** and **23** together with oxazolidinones **24a** and **24b** as side products (Scheme 3). Compounds **22** and **23** occurred as epimeric mixtures, which could not be separated at this stage. The epimeric ratios, as determined by NMR, were 7:3 (*threo*/*erythro*) for **22** and 1:1 for **23**. The stereochemistry of **24a** and **24b** was assigned based upon literature data for $J_{4,5}$. The isomer exhibiting the largest $J_{4,5}$ coupling constant is the *erythro* (4*R*,5*R*) compound.[16]

Deprotection of the trityl and *N-tert*-Boc protecting groups of **22** and **23** by treatment with 1 N hydrogen chloride and chromatographic purification gave the L-*threo* fluorinated sphingoid **25** and the rearranged fluoride **26** (epimeric mixture), respectively.[17]

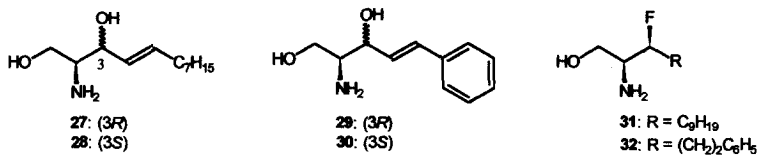
Scheme 3



a: TrCl, pyridine, 100 °C (93%); b: DAST, CH_2Cl_2, -78 °C (22: 26%, 23: 43%, 24a: 18%, 24b: 13%); c: 1 N HCl, dioxane, 100 °C (25: 34%, 26: 59%).

The synthesis of sphingosine analogues **27-30**[10] and of the fluorinated sphinganine analogues **31-32**[11] has been described previously (Figure 3).

Figure 3

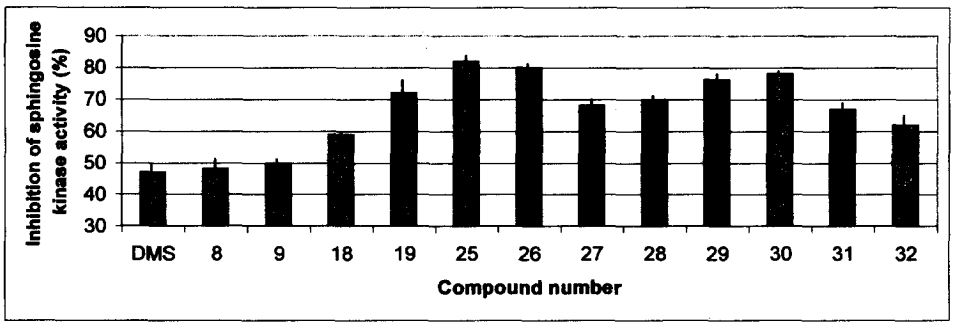


Biological evaluation and discussion

Compounds **8**, **9**, **18**, **19**, and **25-32** were evaluated as potential inhibitors of sphingosine kinase. The data, shown in Figure 4, include *N,N*-dimethylsphingosine (DMS, the most potent sphingosine kinase inhibitor known hithereto[18]) as a reference. HEK 293 cells were transfected with a sphingosine kinase expression vector. The sphingosine kinase activity was measured in the presence of each of the analogues at a concentration of 10 μM, using 10 μM D-*erythro*-sphingosine as substrate.[17] Racemic D,L-*threo*-dihydrosphingosine is known to competitively inhibit recombinant sphingosine kinase activity.[17] To establish firm structure-activity relationships of sphinganine analogues as sphingosine kinase inhibitors, the use of enantiopure sphinganines is required. Compounds **8** and **9**, having a shortened sphingoid base backbone (C_{12}), were as potent as DMS in inhibiting sphingosine kinase. As there was almost no difference in activity between the *erythro*- and the *threo*-epimers, the stereochemistry at position 3 has only a marginal effect on the potency of sphinganine derivatives to inhibit sphingosine kinase. Compounds **27** and **28** were examined to determine the effect of the 4,5-*trans* double bond on sphingosine kinase inhibition. These compounds are much more potent sphingosine kinase inhibitors than their saturated counterparts **8** and **9**, respectively, indicating the significance of the double bond in the inhibition. Again, the stereochemistry at position 3 has only a small effect on the activity.

Substitution of the alkyl sphingoid base backbone for an aromatic residue (compounds **18**, **19**, **29**, and **30**) resulted in an increased inhibitory capacity when compared to the corresponding C_{12}-analogues. Regarding the sphingosine analogues with an aromatic side chain, the difference in inhibitory capacity between the D-*erythro* (compound **29**) and the L-*threo* epimer (compound **30**) is only marginal, while for the aromatic sphinganine analogues, the *threo*-epimer **19** is the more potent inhibitor, when compared to its *erythro*-epimer **18**. This may be related to the unnatural configuration of the *threo*-epimer **19**. The aromatic sphingosine analogues **29** and **30** are stronger inhibitors than the sphinganine analogues **18** and **19**, although this effect is less pronounced in comparison to the C_{12}-analogues. In addition, we evaluated the fluorine-containing isosteres **25**, **26**, **31**, and **32** as sphingosine kinase inhibitors. The introduction of a fluorine atom for a hydroxyl group resulted in increased inhibitory activity for the sphingosine and C_{12}-sphinganine analogues. In contrast, for the sphinganine analogue **32** bearing an aromatic side chain, the isosteric substitution led to a decrease of the inhibitory capacity.

Figure 4: **Inhibition of recombinant sphingosine kinase activity by various sphingosine and sphinganine analogues.** Sphingosine-1-phosphate formation was determined with D-*erythro*-sphingosine as substrate (10 μM), dissolved in 5% Triton X-100 (final concentration 0.25%), and [^{32}P]ATP (10 μCi, 1 mM) containing $MgCl_2$ (10 mM) after 10 min incubations at 37 °C as previously described.[17] Cytosolic fractions from HEK 293 cells overexpressing sphingosine kinase were used as a source of the enzyme (0.125 μg). Results are expressed as percentage of the activity determined in the presence of vehicle only. Data are means ± S.D.



Conclusion

The potential of various sphingosine and sphinganine analogues to inhibit sphingosine kinase was investigated. All the new compounds are stronger sphingosine kinase inhibitors than DMS. Replacement of the alkyl chain by an aromatic residue or introduction of a fluorine atom for the 3-hydroxyl group leads, in general, to strong sphingosine kinase inhibitors. The difference in activity between the *erythro*- and the *threo*-epimers is negligible, except for compounds **18** and **19**, where the *threo*-epimer **19** is the stronger inhibitor. The presence of the 4,5-*trans* double bond enhanced the capacity to inhibit sphingosine kinase activity.

Acknowledgments

This work was supported by doctoral fellowships offered by the Special Research Fund of the University of Gent (BOZF 01101497, to S.D.J) and the Flemish Institute for the Promotion of Scientific-Technological

Research in Industry (IWT, Brussels, Belgium, to I.V.O.) and by Research Grants from the National Institutes of Health (GM43880 and CA61774 to S.S). We thank Prof. P. Van Veldhoven for helpful discussions.

References and Notes

(1) Olivera, A. & Spiegel, S. *Nature* **1993**, *365*, 557-559.
(2) Edsall, L.C.; Pirianov, G.G. & Spiegel, S. *J. Neurosci.* **1997**, *17*, 6952-6960.
(3) Cuvillier, O.; Pirianov, G.; Kleuser, B.; Vanek, P.G.; Coso, O.A.; Gutkind, J.S. & Spiegel, S. *Nature* **1996**, *381*, 800-803.
(4) Van Brocklyn, J.R.; Lee, M.J.; Menzeleev, R.; Olivera, A.; Edsall, L.; Cuvillier, O.; Thomas, D.M.; Coopman, P.J.P.; Thangada, S.; Hla, T. & Spiegel, S. *J. Cell. Biol.* **1998**, *142*, 229-240.
(5) Sadahira, Y.; Ruan, F.; Hakomori, S. & Igarashi, Y. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9686-9690.
(6) Yatomi, Y.; Ruan, F.; Hakomori, S. & Igarashi, Y. *Blood* **1995**, *86*, 193-202.
(7) Van Brocklyn, J.R.; Tu, Z.; Edsall, L.C.; Schmidt, R.R. & Spiegel, S. *J. Biol. Chem.* **1999**, *274*, 4626-4632.
(8) Buehrer, B.M. & Bell, R.M. *J. Biol. Chem.* **1992**, *267*, 3154-3159.
(9) Edsall, L.C.; Van Brocklyn, J.R.; Cuvillier, O.; Kleuser, B. & Spiegel, S. *Biochemistry* **1998**, *37*, 12892-12898.
(10) Van Overmeire, I.; Boldin, S.A.; Dumont, F.; Van Calenbergh, S.; Slegers, G.; De Keukeleire, D.; Futerman, A.H. & Herdewijn, P. *J. Med. Chem.* **1999**, *42*, 2697-2705.
(11) De Jonghe, S.; Van Overmeire, I.; Gunst, J.; De Bruyn, A.; Hendrix, C.; Van Calenbergh, S.; Busson, R.; De Keukeleire, D.; Philippé, J. & Herdewijn, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3159-3164.
(12) Herold, P. *Helv. Chim. Acta* **1988**, *71*, 354-362.
(13) Garner, P. & Park, J.M. *J. Org. Chem.* **1987**, *52*, 2361-2364.
(14) ^{1}H-NMR data of **8** and **9** (500 MHz, CD_3OD): (2*S*,3*R*)-2-Aminododecane-1,3-diol (**8**): δ 0.90 (3 H, t, J = 6.7 Hz, CH_3), 1.25-1.40 (14 H, m, 7 CH_2), 1.45-1.55 (2 H, m, CH_2), 3.15 (1 H, m, H-C(2)), 3.68 (1 H, dd, J = 8.7 Hz and 11.5 Hz, H_a-C(1)), 3.75 (1 H, m, H-C(3)), 3.82 (1 H, dd, J = 4 Hz and 11.5 Hz, H_b-C(1)). (2*S*, 3*S*)-2-Aminododecane-1,3-diol (**9**): δ 0.90 (3 H, t, J = 6.9 Hz, CH_3), 1.25-1.40 (14 H, m, 7 CH_2), 1.45-1.55 (2 H, m, CH_2), 3.0 (1 H, m, H-C(2)), 3.65 (2 H, dd, J = 6.9 Hz and 11.7 Hz, 2 H-(C(1)), 3.75 (1 H, dd, J = 4.1 Hz and 11.7 Hz, H-C(3)).
(15) ^{1}H-NMR data of **18** and **19** (500 MHz, CD_3OD): (2*S*,3*R*)-2-Amino-5-phenylpentane-1,3-diol (**18**): δ 1.68-1.75 (1 H, m, H_a-C(4)), 1.80-1.90 (1 H, m, H_b-C(4)), 2.60-2.68 (1 H, m, H_a-C(5)), 2.80 (1 H, m, H-C(2)), 2.85 (1 H, m, H_b-C(5)), 3.48 (1 H, dd, J = 7.6 Hz and 11.0 Hz, H_a-C(1)), 3.53 (1 H, m, H-C(3)), 3.75 (1 H, dd, J = 4.2 Hz and 11.0 Hz, H_b-C(1)), 7.15 (1 H, m, arom H), 7.20-7.25 (4 H, m, arom H). (2*S*,3*S*)-2-Amino-5-phenylpentane-1,3-diol (**19**): δ 1.75-1.85 (2 H, m, 2 H-C(4)), 2.61-2.68 (1 H, m, H_a-C(5)), 2.73 (1 H, m, H-C(2)), 2.78-2.85 (1 H, m, H_b-C(5)), 3.49 (1 H, dd, J = 11.0 Hz and 6.7 Hz, H_a-C(1)), 3.50-3.63 (2 H, m, H_b-C(1) and H-C(3)), 7.13 (1 H, m, arom H), 7.20-7.25 (4 H, m, arom H).
(16) Futagawa, S.T.I. & Shiba, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3308-3310; Rich, D.H. & Sun, E.T.O. *J. Med. Chem.* **1980**, *23*, 27-33.
(17) The synthesis of L-*threo*-3-fluoro-sphingosine and its allylic rearranged isomer has been described using 2-chloro-1,1,2-trifluorotriethylamine as fluorinating agent (Kozikowski, A.P. & Wu, J.P. *Tetrahedron Lett.* **1990**, *31*, 4309-4312). However, on using DAST, a higher yield of the desired 3-fluorinated compound was obtained (26% vs. 13%).
(18) Kohama, T.; Olivera, A.; Edsall, L.; Nagiec, M.M.; Dickson, R. & Spiegel, S. *J. Biol. Chem.* **1998**, *273*, 23722-23728.