Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 954–958
Bioorganic & Medicinal Chemistry Letters

Hybrid angiogenesis inhibitors: Synthesis and biological evaluation of bifunctional compounds based on 1-deoxynojirimycin and aryl-1,2,3-triazoles

Ying Zhou,[b] Yunxue Zhao,[a] Kathy M. O' Boyle[a] and Paul V. Murphy[b,*]

[a]*UCD School of Biomolecular and Biomedical Science and the UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland*

[b]*UCD School of Chemistry and Chemical Biology and Centre for Synthesis and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland*

Received 22 November 2007; revised 13 December 2007; accepted 16 December 2007
Available online 23 December 2007

Abstract—Synthesis of hybrids of 1-deoxynojirimycin (DNJ) and 5-aryl-1,2,3-triazole as potential bifunctional inhibitors of angiogenesis is described. The DNJ component inhibits the biosynthesis of cell surface oligosaccharides necessary for angiogenesis, whereas the aryl-1,2,3-triazole inhibits methionine aminopeptidase II, a target in angiogenesis therapy. One bifunctional compound was a more potent inhibitor of angiogenesis in vitro than DNJ alone or the 5-aryl-1,2,3-triazole alone.

Angiogenesis[1] provides new blood vessels to growing and developing tissue including tumours. Pathological angiogenesis occurs in tumour formation, and also in a range of other 'angiogenesis-dependent diseases' that include rheumatoid arthritis, diabetic retinopathy, atherosclerosis, Crohn's disease and diseases of the nervous system.[2] Angiogenesis is a major factor affecting the metastatic spread of malignant tumour cells and so the development of angiogenesis inhibitors is of interest in cancer therapy. Drug discovery for angiogenesis dependent disease has been recently reviewed by Folkman.[3] Angiogenesis inhibitors that target a range of angiogenic proteins and/or pathways will be less susceptible to drug resistance than those agents that target a single protein or pathway. Therefore, it will be important for synthetic chemists to produce novel multifunctional pharmaceutical compounds that have potential to inhibit two or more biological targets relevant to angiogenesis.

Methionine aminopeptidase II (MetAP2) has been indentified[4] as the target for the angiostatic agents fumagillin and ovalicin and an inhibitor PPI-2458 is currently in clinical trials.[5] Recently aryl-1,2,3-triazoles **1** have been identified as inhibitors of both MetAP2 and angiogenesis.[6] Glycosidases are another relevant target; inhibitors of α-glucosidases such as *N*-methyl-1-deoxynojirimycin **2b**, castanospermine, or 1-deoxymannojirimycin alter the biosynthesis of glycans on endothelial cell surfaces that are required for angiogenesis.[7] Herein we describe the synthesis and evaluation of the hybrid angiogenesis inhibitors **3**–**5** (Chart 1) which have been designed to inhibit both α-glucosidase and MetAP2.

The design of **3**–**5** was based on combining the properties of glycosidase inhibition and MetAP2 inhibition into a single molecule to generate a hybrid angiogenesis

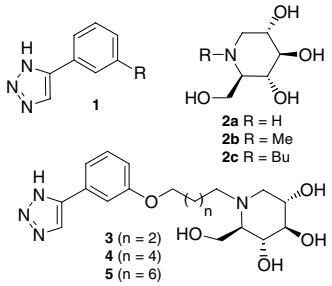


Chart 1. Structures of **1**–**5**.

Keywords: Angiogenesis; Inhibitor; Alpha-glucosidase; Methionine aminopeptidase; Aryl-1,2,3-triazole; 1-Deoxynojirimycin, Bifunctional compound; Hybrid.
* Corresponding author. E-mail: paul.v.murphy@ucd.ie

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.12.034

inhibitor. The ability of *N*-butyl-DNJ **2c** to inhibit α-glucosidases[8] indicated that an alkyl chain linker between the DNJ nitrogen atom and the aromatic residue would not have a deleterious effect on glycosidase activity. Secondly, the docking of a low energy conformational isomer of **5** to the active site of MetAP2, based on the crystal structure of the complex between an aryl-1,2,3-triazole derivative and MetAP2, indicated that **5** would adopt a conformation that would preserve the MetAP2 interactions and additionally bind the DNJ moiety. The DNJ moiety can form VDW interactions with the residues on the surface of the enzyme (Fig. 1).

The synthesis of **3**–**5** (Scheme 1) began with cleavage of the aromatic methoxy group of 3-methoxybenzaldehyde **6**[9] to give **7**. This was best achieved using thiophenol, potassium carbonate and *N*-methyl-pyrrolidinone.[10] These conditions were superior to the use of boron trichloride or to the use of boron trichloride–tetrabutylammonium iodide which have been applied previously for the demethylation of aromatic ethers. Next the selective alkylation of **7** using dialkyl bromides in acetone using potassium carbonate gave the aldehydes **8a**–**c**. The aldehyde groups of **8a**–**c** were then converted to the acetylenes **10a**–**c**, respectively, using the Ohira-Bestmann reagent **9**[11] under basic conditions.[12]

The alkylation of **2a**[13] or per-*O*-acetylated **2a** with the bromides **10a**–**c** under a variety of conditions (using the bases K_2CO_3, KOH, DIPEA, NaH, K_2CO_3–CuI, Et_3N in solvents MeCN, DMF or MeOH) gave either low yields or none of the desired products. To circumvent this problem the bromides **10a**–**c** were converted to the more reactive iodides **11a**–**c** by reaction with sodium iodide in acetone. These iodides were then reacted with DNJ **12** in DMF in the presence of K_2CO_3 to give **13a**–**c** in moderate to good yield. Attempts to carry out Huisgen 1,3-dipolar cycloaddition reactions of **12a**–**c** with benzyl azide or azidotrimethylsilane were unsuccessful. Conversely, the reaction of benzyl azide and acetylenes **13a**–**c**, obtained by per-*O*-acetylation of the DNJ hydroxyl groups of **12a**–**c**, gave the triazoles **14a**–**c** as mixtures of regioisomers.[14] Deacetylation of **14a**–**c** using sodium methoxide in methanol followed by catalytic hydrogenolysis gave the hybrids **3**–**5**.[15] In addition the aryl-1,2,3-triazole **15** was also prepared from the relevant aromatic aldehyde by first converting the aldehyde to the acetylene and the subsequent Huisgen reaction with azidotrimethylsilane.

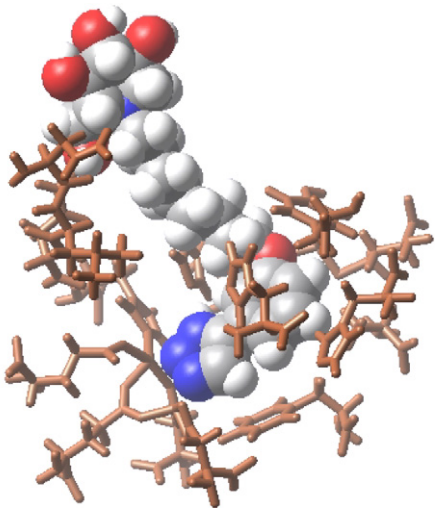
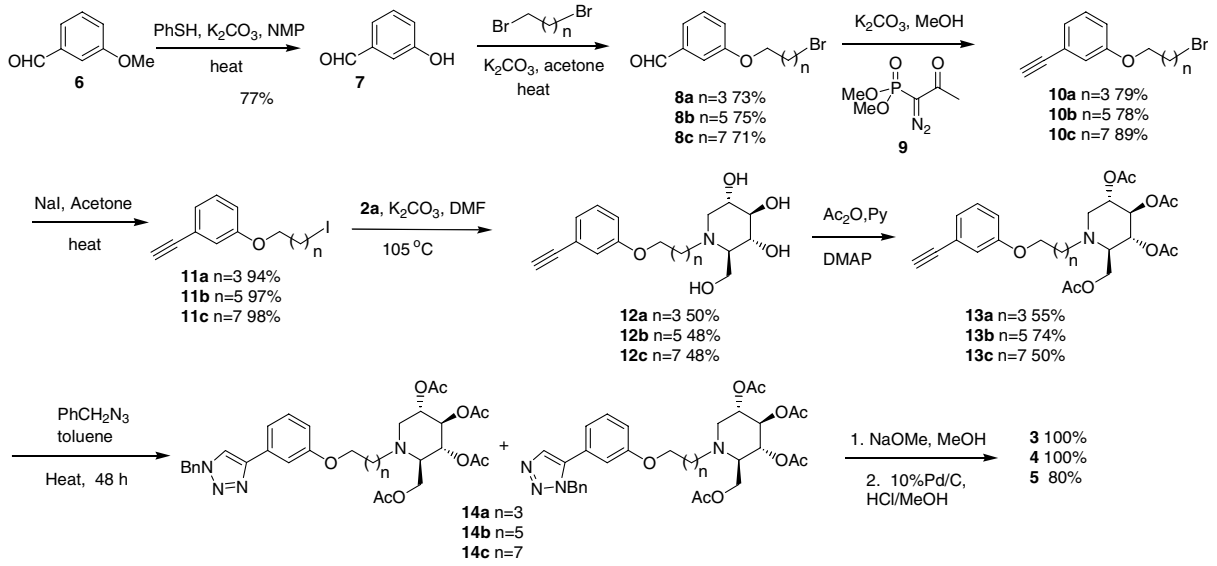


Figure 1. Hybrid **5** (CPK model) docked to the aryl-1,2,3-triazole binding site (brown tubes) of MetAP2. The MetAP2 amino acid residues shown are those within 4 Å of **5**.

Compounds were evaluated for their ability to inhibit α-glucosidase assay from bacillus stearothermophilus as described by Hakamata et al.[16] in order to test their enzyme inhibitory activity, and the results are provided in Table 1. The IC_{50} for **2a** was 1.67 μM. The hybrid compound **5** (IC_{50} = 1.15 μM) was more potent than **2a**, whereas compounds **3** and **4** were less potent (IC_{50} = 6.07 μM and 2.41 μM, respectively) than **2a**. Thus, the structural modification of incorporating the aryl triazole via an alkyl linker to **2a** did not lead to a significant reduction of the inhibition of α-glucosidase. In the case of **5**, the structural modification led to a modest enhancement of inhibitory properties, indicating that the N-substituent may have favourable interactions with the glucosidase. The aryl-1,2,3-triazole **15** (Chart 2) did not inhibit α-glucosidase.

The inhibition of BAEC growth by compounds was next determined using the MTT assay[17] and the results are also summarized in Table 1.[18] The iminosugar **2a** was inactive with no significant inhibition of proliferation being observed at concentrations up to 1 mM. However, the aromatic triazole **15** (IC_{50} = 0.347 mM) was able to inhibit growth of BAECs. The inhibition of human (HUVEC) and mouse (MS-1) endothelial cell proliferation by aryl-1,2,3-triazoles is consistent with the ability to inhibit MetAP2 and it can be inferred that inhibition of MetAP2 in BAECs would account for the activity observed for **15**. It is worth noting that aryl-1,2,3-triazole derivatives have IC_{50} values of 2–30 μM as inhibitors of HUVEC and MS-1 growth.[5] The hybrids **3**–**5** all inhibited BAEC proliferation with **5** being the most potent inhibitor (IC_{50} = 0.105 mM). Thus, introduction of the alkylated DNJ substituent to the 3-position of the benzene residue did not lead to a loss in ability of the aryl-1,2,3-triazole to inhibit endothelial cell growth; in the case of **5**, the structural modification led to an increase in potency.

As compounds **3**–**5** can inhibit α-glucosidase at the enzyme level, the effect on the cell surface oligosaccharide structure was investigated. Accordingly, BAECs were treated with compounds (50 μM) for 24 h and then the binding of specific fluorescein (FITC)-labelled lectins to the cells was monitored by fluorescence activated cell sorting (FACS) analysis in a flow cytometer.[19] In these studies the two lectins, concanavalin A (ConA) and phytohemagglutinin-L (L-PHA), were investigated. Con A recognises mannose and glucose residues, whereas L-PHA recognises complex branched chain oligosaccharides containing tri- and tetra-antennary β-linked disac-



Scheme 1.

Table 1. Inhibition of α-glucosidase and BAEC growth by compounds

Compound	α-Glucosidase inhibition IC_{50} (μM)	BAEC growth inhibition IC_{50} (mM)
2a	1.67	Not active
3	6.07	0.797
4	2.41	0.610
5	1.15	0.105
15	Not active	0.347

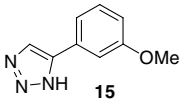


Chart 2. Structure of **15**.

charides of *N*-acetyl-lactosamine on mannose residues of asparagine linked oligosaccharides.[7a]

Following 24-h treatment with compounds, cells were labelled with ConA-FITC or L-PHA-FITC and then cells were sorted according to the intensity of the fluorescence they emitted. Results of the analyses are shown in Figure 2. As expected, BAECs that had been treated with **2a** for 24 h showed greater binding of ConA than untreated control cells, indicating increased levels of high mannose structures at the cell surface. The treatment of BAECs with **3**–**5** also led to increased binding of ConA to cell surfaces when compared to the control and **5** and **4** increased ConA binding more than **2a**, providing evidence that the hybrid compounds inhibit α-glucosidase at the cellular level. Further evidence to support this conclusion was obtained when the binding of L-PHA to BAECs was examined. It was found that, as expected, treatments that increased ConA binding decreased L-PHA binding. Thus, L-PHA binding to BAECs was reduced in cells that had been treated with **2a** compared to control, untreated cells. Compounds **3**–**5** lowered levels of L-PHA binding to BAECs and all three did this to a greater extent than **2a**. The hybrid **5**, the most potent α-glucosidase inhibitor, was the most potent inhibitor of glycoprocessing as indicated by the high level of ConA and low level of L-PHA binding to BAECs following treatment. Although compounds **3** and **4** are less potent α-glucosidase inhibitors than **2a**, they alter surface oligosaccharide expression to a greater extent than **2a**. This apparent anomaly may be explained by the possibility that they have increased cellular permeability compared to **2a**, thereby facilitating diffusion to the endoplasmic reticulum(ER) and enhancing access to the glycoprocessing enzymes therein. Satisfactory cellular permeability and its potency as an inhibitor of glucosidase activity would account for the activity displayed by **5**.

Compounds **3**–**5** were next evaluated for their ability to inhibit angiogenesis in vitro using the endothelial cell tube formation assay.[20] Endothelial cells which are induced to undergo tube formation change their architecture and form cell–cell contacts that lead to branched networks that are similar to capillary-like blood vessels. When BAECs are cultured on polymerized matrigel they organise into such tube-like structures (Fig. 3(a)). However, when BAECs were cultured on polymerized matrigel in the presence of **5** (0.2 mM, Fig. 3(b)) the cells failed to organise into these capillary-like structures. At this concentration none of the other compounds inhibited tube formation.

In summary, the synthesis of novel hybrids of **2a** and 5-aryl-1,2,3-triazoles has been achieved by a sequence that includes N-alkylation of **2a** and subsequent Huisgen 1, 3-dipolar cycloadditions of azides and alkynes. These novel hybrid compounds showed interesting biological properties when compared with **2a** and **15**. One hybrid showed increased glycosidase inhibitory activity and increased ability to alter the biosynthesis of oligosaccharides on endothelial cell surfaces when compared with **2a**. The same compound showed increased ability to in-

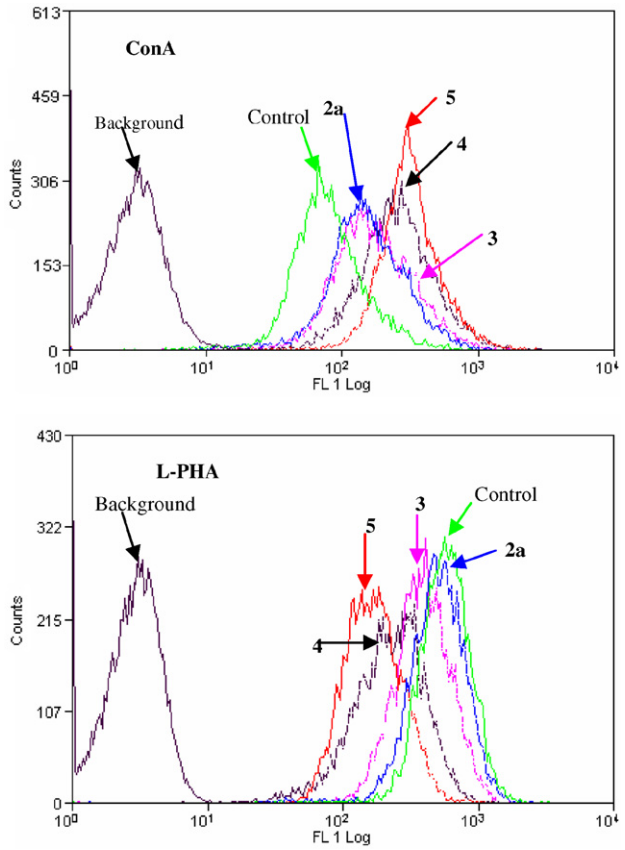


Figure 2. Effect of glycosidase inhibitors on cell surface BAEC oligosaccharide expression. BAECS were cultured for 24 h in the absence (control) or presence of 5×10^{-5} M DNJ and novel compounds. Cells were harvested with trypsin/EDTA, treated with Con A-FITC (top) or L-PHA-FITC (bottom) and analyzed by flow cytometry. Plots show the relationship between fluorescence intensity (FL1Log, *x*-axis) and cell number (counts, *y*-axis) for populations of cells treated with different compounds.

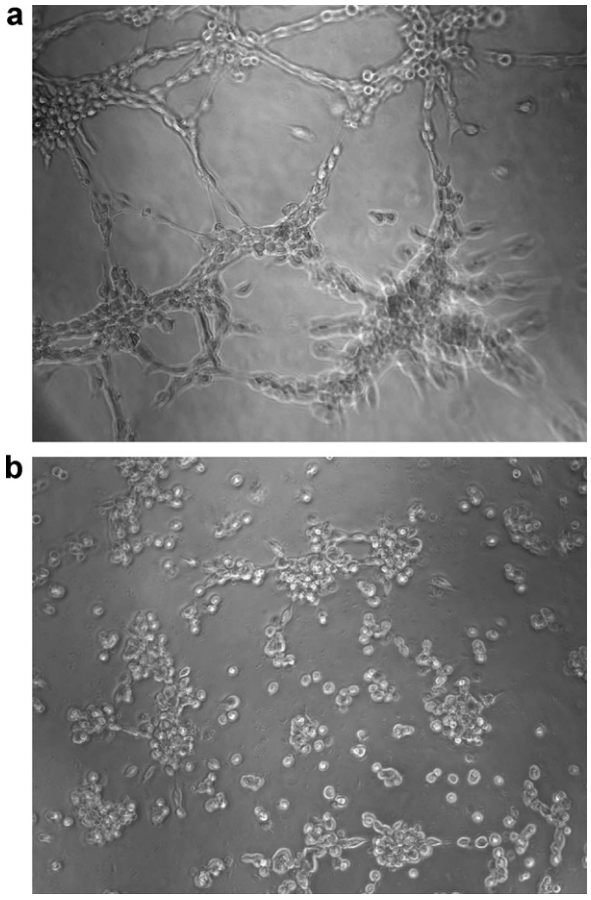


Figure 3. Tube formation. (a) BAECs cultured on polymerized matrigel organise into tube-like structures. (b) Hybrid **5** inhibits tube formation (2×10^{-4} M).

hibit the proliferation of BAECs when compared to **15**. Endothelial cell proliferation was not inhibited by **2a** at high concentrations and aryl-1,2,3-triazoles did not inhibit either α-glucosidase at the enzyme level or glycoprotein processing at the cellular level. Hybrid **5** completely inhibited capillary tube formation similar to capillary-like blood vessels that are formed in vivo during angiogenesis. Hybrid **5** displays properties desirable in the development of bifunctional inhibitors of angiogenesis.

Acknowledgments

The authors thank staff at the UCD NMR Centre and Dr. Dilip Rai. The research was funded by Science Foundation Ireland (03/IBN/B352).

Supplementary data

NMR spectra of **3**–**5**, and additional figures. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.034.

References and notes

1. (a) Liekens, S.; de Clercq, E.; Neyts, J. *Biochem. Pharm.* **2001**, *61*, 253; (b) Carmeliet, P.; Jain, R. K. *Nature* **2000**, *407*, 249.
2. Hanahan, D. *Nature Med.* **1998**, *4*, 13.
3. Folkman, J. *Nature Rev. Drug Discov.* **2007**, *6*, 273.
4. Griffith, E. C.; Su, Z.; Niwayama, S.; Ramsay, C. A.; Chang, Y.-H.; Liu, J. O. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15183.
5. Cooper, A. C.; Karp, R. M.; Clark, E. J.; Taghizadeh, N. R.; Hoyt, J. G.; Labenski, M. T.; Murray, M. J.; Hannig, G.; Westlin, W. F.; Thompson, C. D. *Clin. Cancer Res.* **2006**, *12*, 2583.
6. Kallander, L. S.; Lu, Q.; Chen, W.; Tomaszek, T.; Yang, G.; Tew, D.; Meek, T. D.; Hofmann, G. A.; Schulz-Pritchard, C. K.; Smith, W. W.; Janson, C. A.; Ryan, M. D.; Zhang, G.-F.; Johanson, K. O.; Kirkpatrick, R. B.; Ho, T. F.; Fisher, P. W.; Mattern, M. R.; Johnson, R. K.; Hansbury, M. J.; Winkler, J. D.; Ward, K. W.; Veber, D. F.; Thompson, S. K. *J. Med. Chem.* **2005**, *48*, 5644.
7. (a) Pili, R.; Chang, J.; Partis, R. A.; Mueller, R. A.; Chrest, F. J.; Passaniti, A. *Cancer Res.* **1995**, *55*, 2920; (b) Nguyen, M.; Folkman, J.; Bischoff, J. *J. Biol. Chem.* **1992**, *267*, 26157.
8. For reviews on glycosidase inhibition, see (a) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199; (b) Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340; (c) Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 750; (d)

Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515; (e) Greimel, P.; Spreitz, J.; Stutz, A. E.; Wrodnigg, T. M. *Curr. Topics. Med. Chem.* **2003**, *3*, 513; (f) Asano, N.; Nash, R. J.; Molyneaux, R. J.; Fleet, G. W. J. *Tetrahedron Asymmetry* **2000**, *11*, 1645.
9. Woodward, R. B. *Org. Synth.* **1945**, *25*, 55.
10. Chrakaborti, A. K.; Sharma, L.; Nayak, M. K. *J. Org. Chem.* **2002**, *67*, 6406.
11. Xu, Z. S.; Peng, Y. G.; Ye, T. *Org. Lett.* **2003**, *5*, 2821.
12. (a) Müller, S.; Liepold, B.; Roth, G. J.; Bestmann, H. J. *SynLett* **1996**, *6*, 521; (b) Roth, G. J.; Liepold, B.; Müller, S.; Bestmann, H. J. *Synthesis* **2004**, *1*, 59.
13. (a) Danieli, E.; Lalot, J.; Murphy, P. V. *Tetrahedron* **2007**, *63*, 6827; (b) McDonnell, C.; Cronin, L.; O' Brien, J. L.; Murphy, P. V. *J. Org. Chem.* **2004**, *69*, 3565.
14. A regioselective reaction was not required as the benzyl group was later removed.
15. Selected analytical data for **5**: ^{1}H NMR (CD_3OD, 500 MHz) δ 8.14 (s, 1H), 7.37 (m, 2H), 7.31 (t, 1H, *J* 7.8 Hz), 6.92 (d, 1H, *J* 7.7 Hz), 4.04 (t, 2H, *J* 6.40 Hz), 3.87 (d, 2H, *J* 2.6 Hz), 3.50 (dt, 1H, *J* 4.7 Hz, *J* 9.9 Hz), 3.38 (t, 1H, *J* 9.3 Hz), 3.16 (t, 1H, *J* 9.1 Hz), 3.04 (dd, 1H, *J* 4.9 Hz, *J* 11.3 Hz), 2.84 (m, 1H), 2.62 (m, 1H), 2.25 (t, 1H, *J* 11.00 Hz), 2.21 (d, 1H, *J* 9.5 Hz), 1.82 (dt, 2H, *J* 6.5 Hz, *J* 13.5 Hz), 1.55–1.29 (m, 10H); ^{13}C NMR (CD_3OD, 125 MHz) δ 159.9 (C), 131.3 (C), 129.9 (CH), 118.1 (CH), 114.7 (CH), 111.7 (CH), 79.2 (CH), 70.7 (CH), 69.3 (CH), 67.9 (CH_2), 66.2 (CH), 58.0 (CH_2), 56.3 (CH_2), 52.6 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 27.3 (CH_2), 25.9 (CH_2), 24.1 (CH_2); IR (cm^{-1}) ESI-HRMS: calcd for $C_{22}H_{35}N4O_5$ 435.2607, found *m/z* 435.2602 $[M + H]^+$.
16. Hakamata, W.; Nakanishi, I.; Masuda, Y.; Shimizu, T.; Higuchi, H.; Nakamura, Y.; Saito, S.; Urano, S.; Oku, T.; Ozawa, T.; Ikota, N.; Miyata, N.; Okuda, H.; Fukuhara, K. *J. Am. Chem. Soc.* **2006**, *128*, 6524, 4-Nitrophenyl α-D-glucopyranoside (PNP-G, 40 μL of 3 mM) in DPBS (pH 7.0), selected compound (5 μL) in DPBS and α-glucosidase (5 μL, Sigma, G-3651) were mixed. After incubation of this mixture for 20 min at 37 °C, the reaction mixture was added to 200 μL of 0.5 M Na_2CO_3 to stop the reaction, and the absorbance of 4-nitrophenol released from PNP-G at 405 nm was then measured. Each compound was tested in triplicate and the experiment was repeated at least twice. The concentrations of compound required to inhibit the α-glucosidase activity by 50% (IC_{50}) are shown in Table 1.
17. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55, BAECs were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 25 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin and maintained at 37 °C in a humidified atmosphere of 5% CO_2. Subcultures were created by passaging using a trypsin/EDTA mixture in phosphate-buffered saline. BAECs (3×10^3 cells) were seeded on 96-well microtiter plates in 1640 medium with 10% FBS and incubated overnight. The compounds (10^{-6}–10^{-3} M) were then added to the cells and cultured for another 48 h. MTT was added directly to the cell supernatant so as to give a final concentration of 0.5 mg/mL of MTT. After 3 h at 37 °C the cell culture medium was removed. Formazan crystals in adherent cells were dissolved in 200 μL DMSO and the absorbance of the formazan solution was measured at 570 nm. Each compound was tested in quadruplicate and the experiments were repeated at least twice. The IC_{50}s are shown in Table 1.
18. For other selected carbohydrate derived compounds that inhibit BAEC growth, see (a) Rawe, S. L.; Zaric, V.; O' Boyle, K. M.; Murphy, P. V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1316; (b) Murphy, P. V.; Pitt, N.; O' Brien, A.; Enright, P. M.; Dunne, A.; Wilson, S. J.; Duane, R. M.; O' Boyle, K. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3287.
19. Cultured BAECs (Ref. 17) were treated with compounds (50 μM) for 24 h in 6 well plate. Cells were then harvested and washed with DPBS. Approximately 5×10^5 cells were resuspended in test tubes and incubated in DPBS/1% BSA with 200 μL of 2 μg/ml Con A-FITC or L-PHA-FITC for 1 h at 4 °C. Cells were washed twice with DPBS, and fluorescence histogram profiles were determined using flow cytometry. Each experiment was carried out at least twice with each compound.
20. Tube-structure formation on Matrigel was conducted and modified as described previously. See Akalu, A.; Roth, J. M.; Caunt, M.; Policarpio, D.; Liebes, L.; Brooks, P. C. *Cancer Res.* **2007**, *67*, 4353, Briefly, 70 μL growth factor–reduced Matrigel was added to 96-well plates at 4 °C and then allowed to polymerize at 37 °C for 1 h. Cultured cells (Ref. 17) were treated with compounds (0.2 mM) for 24 h in 6-well plate. BAE cells were then harvested and suspended at a concentration of 3×10^4 cells/0.1 ml in RPMI 1640 containing 10 ng/mL bFGF and 0.2 mM compounds. Control cells were resuspended with 10 ng/ml bFGF alone. Cells were carefully layered on top of the polymerized gel and incubated for 8 h at 37 °C in 5% CO_2. Tube formation was observed and photographed under a microscope. At least five visual fields were counted and the average number of tubes per field was calculated using light microscope. The experiments were repeated at least twice for each compound.