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Epoxyquinol A, a Highly Functionalized Pentaketide Dimer with Antiangiogenic Activity Isolated from Fungal Metabolites

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Angiogenesis is a complex process involving several distinct and sequential steps such as membrane degradation, migration (chemotaxis), proliferation, and formation of capillary tubes in endothelial cells. Abnormal angiogenesis often occurs in pathological conditions such as cancer, rheumatoid arthritis, diabetic retinopathy, and other chronic inflammatory diseases. An important step in the development of pathological angiogenesis is thought to involve the production of vascular endothelial growth factor (VEGF) by normal and tumor cells, and the subsequent hyperactivation of downstream signaling pathways. Inhibition of angiogenesis is emerging as a promising strategy for the treatment of angiogenesis-related diseases including cancer.[1] Indeed, several natural product and synthetic angiogenesis inhibitors (small molecules and peptides) are currently undergoing clinical trials.[2] Nevertheless, the identification of novel angiogenesis inhibitors with different chemical structures and biological properties would provide a range of therapeutic tools for pathologic angiogenesis. Such inhibitors could also be used as powerful bioprobes to dissect the multiple stages leading to angiogenesis. In our screening program aimed at identifying angiogenesis inhibitors of microbial origin, we found a novel pentaketide dimer designated as epoxyquinol A (1, Figure 1) produced by an uncharacterized fungus isolated from a soil sample. In this communication, the structural determination and biological activities of **1** are reported.

The molecular formula of **1**, $[\alpha]^{21}_{D}$ +61.0° (*c* 0.146) and mp 186 °C dec, was determined to be $C_{20}H_{20}O_8$ by high-resolution EIMS (Found: *m*/*z* 388.1148, Calcd: *m*/*z* 388.1158). In the IR spectrum, characteristic absorption bands at 3350, 1705, and 1680 cm^{-1} indicated the presence of hydroxyl and saturated and unsaturated ketone carbonyl groups. The ^{13}C NMR spectrum confirmed the presence of 20 carbons including two ketones, three sp^2 quaternary carbons, one sp^2 methine carbon, eleven sp^3 methines, one sp^3 quaternary carbon, and two methyl carbons. The PFG-HMQC spectrum established all one-bond $^1H-^{13}C$ connectivities (Table 1). PFG-DQFCOSY spectral data suggested the presence of partial structures consisting of four sequential methine groups with two terminal methyl groups, and two similar parts with two epoxy methines and adjacent hydroxyl methine carbons. Later partial structures were confirmed by large $^1J_{CH}$ values of 184.5, 184.5, 183.0, and 186.0 Hz for epoxide methines C-4, C-5, C-14, and C-15, respectively. Two methine groups are isolated, and one of them, H-11 (δ 6.73), was assigned to an enol ether group at C-11 (δ 142.43, $^1J_{CH}$ = 187.5 Hz). Connectivities of those partial structures and quaternary carbons were determined by analyses of

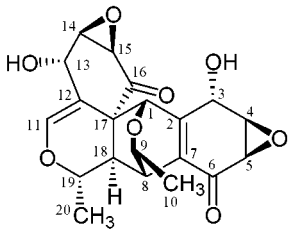

Figure 1. Structure of epoxyquinol A (**1**).

Table 1. ^{13}C (150 MHz) and ^1H (600 MHz) NMR Data for Epoxyquinol A (**1**) in Acetone-d_6

pos.	^{13}C(mult.)[a]	^1H(mult.)[a]	*J*(Hz)	pos.	^{13}C(mult.)[a]	^1H(mult.)[a]	*J*(Hz)
1	72.40 (d)	5.23 (s)		12	115.06 (s)		
2	153.52 (s)			13	64.15 (d)	4.94 (br s)	
3	63.92 (d)	4.69 (br d)	8.8	14	66.65 (d)	3.75 (d)	3.4
4	58.76 (d)	3.74 (dd)	3.4, 0.7	15	56.34 (d)	3.39 (d)	3.4
5	53.61 (d)	3.44 (dd)	3.4, 0.7	16	200.73 (s)		
6	190.19 (s)			17	50.66 (s)		
7	134.38 (s)			18	38.02 (d)	2.44 (dd)	1.5, 1.0
8	39.29 (d)	3.10 (dd)	1.5, 1.2	19	74.79 (d)	4.43 (dq)	1.0, 6.6
9	66.99 (d)	4.30 (dq)	1.2, 6.4	20	20.26 (q)	1.00 (d)	6.6
10	20.92 (q)	0.71 (d)	6.4	3-OH		4.67 (br d)	8.8
11	142.43 (d)	6.73 (d)	1.0	13-OH		4.94 (br s)	

[a] Chemical shifts are in ppm downfield of internal TMS in acetone- d_6.

H−C long-range correlations of PFG-HMBC spectral data. Important long-range correlations to be determined the right part of **1** are as follows: (i) from H-1, H-5, and H-8 to C-6 and C-7, (ii) from H-1, H-3, H-4, and H-8 to C-2, and (iii) from H-1, H-8 to C-17. The significant correlations from H-8, H-11, H-13, H-15, H-18, and H-19 to C-17, from H-15 to C-16, and from H-1, H-11, H-13, H-14, and H-18 to C-12 established the left part of **1**. The presence of two ether bridges between C-1 and C-9 and also between C-11 and C-19 for the bicyclic ring was confirmed by PFG-HMBC data. Thus, the planar structure of **1** was elucidated as illustrated in Figure 2a.

Relative stereochemistry between hydroxyl and epoxy groups of both epoxyquinol parts of **1** was determined to be *trans* configuration from the small coupling constant values of $^3J_{H-3,H-4}$ = 0.7 Hz and $^3J_{H-13,H-14}$ = ~0 Hz.[3] Additionally NOE differential spectra of **1** afforded several significant data on the relative stereochemistry as shown in Figure 2b. In particular, NOEs observed from H-19 to H-8 and H-9 as well as from H-18 to H-8 and H-20 established the sequential stereochemistry at C-9, C-8, C-18, and C-20. The proton on C-1 displayed a significant NOE with H-13, thereby allowing the stereochemistry at C-1, C-17, and C-13 to be determined. Finally the relative configuration of **1** was unambiguously determined by the application of X-ray crystallographic analysis.[4] The relative stereochemistry of two epoxyquinol

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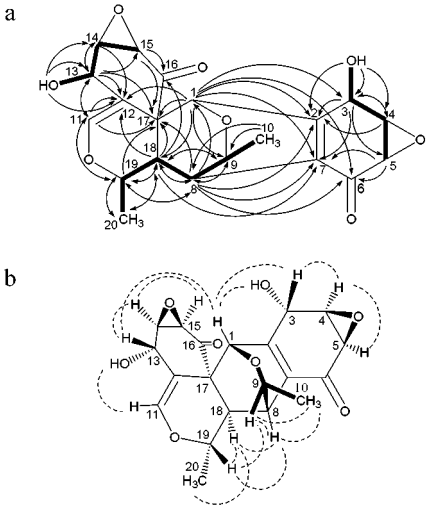
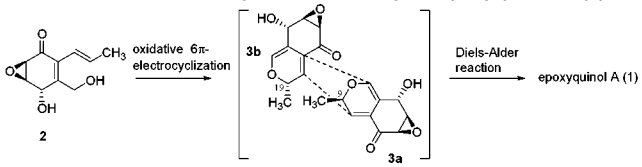


Figure 2. (a) Significant correlation in PFG-DQFCOSY and PFG-HMBC spectra in epoxyquinol A (**1**). (Bold lines show proton spin networks, and arrows show $^{1}H-^{13}C$ long-range correlations.) (b) NOE data summary for epoxyquinol A (**1**). (Dotted lines indicate significant NOEs).

Scheme 1. Possible Biosynthetic Pathway of Epoxyquinol A (**1**)



parts is the same, but that of methyl groups at C-9 and C-19 is the opposite configuration.

Epoxyquinol A (**1**) possesses a unique structure consisting of a highly functionalized cyclohexenone moiety. A possible biosynthetic scheme for the formation of **1** is shown in Scheme 1.[5] Epoxyquinol A is composed fundamentally of two related 2*H*-pyran monomers, **3a** and **3b** via the oxidative 6π-electrocyclic ring-closure of a related monomeric intermediate (**2**). The 2*H*-pyran monomers, **3a** and **3b**, of the opposite relative configurations for C-9 and C-19 might be fused via an *exo* intermolecular Diels−Alder reaction through sterically favored *anti* stereochemistry at both methyl positions.[6,7] In fact, **2** was isolated from the same fermentation broth as **1**, which supports the proposed biosynthesis of **1** via the oxidative dimerization of a monomeric intermediate. To date there have been several reports of quinone dimers cyclized via an intramolecular or intermolecular Diels−Alder reaction. These include longithorone A from the Tunicate *Aplydium longithorax*[8] and torreyanic acid from the fungus *Pestalotiopsis microspora*.[9] However, the formation of an epoxyquinol dimer product via an intermolecular [4 + 2] oxidative dimerization as for **1** has not been described previously.

We performed a chemotactic cell migration assay in a CHEMOTAXICELL chamber to examine the effect of **1** on human umbilical vein endothelial cells (HUVECs) migration induced by VEGF. In this assay, HUVECs in the top chamber migrated and penetrated the filters to enter the lower chamber supplemented with 12.5 ng/mL of VEGF-containing medium. VEGF significantly stimulated the cell migration (95.7±29.6 migrated cells/field), whereas 10 μM of SU5614, a well-known inhibitor of VEGF receptor tyrosine kinase (VEGF-R2/KDR/Flk-1),[10] inhibited the VEGF-induced cell migration (3.0 ± 3.0 migrated cells/field). Epoxyquinol A also inhibited the cell migration induced by VEGF in a dose-dependent manner. Three μg/mL (ED_{100}) of **1** completely inhibited cell migration (5.3 ± 1.8 migrated cells/field) without showing significant cell toxicity, as estimated by a trypan blue dye exclusion assay.

Although several angiogenesis inhibitors from natural products and chemical synthesis, such as endostatin,[11] TNP-470,[12] and thalidomide,[13] have been developed, **1** differs from these molecules in both structure and biological activity. Mechanistic studies to determine cellular target(s) of **1**, as well as the effect on endothelial cells would provide new insights into both the molecular mechanism of angiogenesis and potential for drug development related to angiogenesis therapy. Further studies on the in vitro and in vivo biological activities are in progress.

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Supporting Information Available: Fermentation, purification procedure, crystallographic data, and the ORTEP view of epoxyquinol A (**1**), and VEGF-induced migration assay protocol (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Folkman, J. *J. Natl Cancer Inst.* **1990**, *82*, 4−6. (b) Klagsbrun, M.; Moses, M. A. *Chem. Biol.* **1999**, *6*, R217−R224. (c) Carmeliet, P. *Nat. Med.* **2000**, *6*, 389−395. (d) Shibuya, M. *Cell Struct. Funct.* **2001**, *26*, 25−35. (e) Fan, T. P.; Jaggar, R.; Bicknell, R. *Trends Pharmacol. Sci.* **1995**, *16*, 57−66.

(2) Gasparini, G. *Drugs* **1998**, *58*, 17−38.

(3) Edwards, R. L.; Maitland, D. J.; Scowen, I. J.; De Sousa, A. J. T.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. 1* **2001**, 537−542.

(4) (a) Two hydroxyl groups in **1** participate in the hydrogen bonds. The epoxyquinol A (1) and EtOAc molecules were connected by an intermolecular hydrogen bond between 13-O*H* of **1** and a carbonyl group of EtOAc. Additionally, another intermolecular hydrogen bond between 13-O*H* and 3-O*H* in the neighboring epoxyquinol A molecule formed the layer arrays of the molecules along the *b*-axis. (b) X-ray crystal analysis was performed with a colorless needle crystal (0.65 × 0.03 × 0.02 mm) obtained from a solvent of EtOAc−CH_2Cl_2-MeOH. X-ray diffractometer with graphite monochromated Cu−Kα radiation. The structure was solved by direct methods and all non-H atoms were refined anisotropically by full-matrix least-squares techniques. All calculations were performed using the teXan crystallographic software package of Molecular Structure Corporation. The crystal data were $C_{20}H_{20}O_8 \cdot C_4H_8O_2$, monoclinic, $P2_1$, a=10.577(2) Å, b=8.278(2) Å, c=14.010(1) Å, β=109.38(1)°, Z=2, R=0.055, Rw=0.079 for 1256 independent reflections. See the Supporting Information for the ORTEP view for **1**.

(5) Reduction of the corresponding quinone dimer for **1** could be also envisioned to produce **1**.

(6) Li, C.; Lobkovsky, E.; Porco, J. A. Jr. *J. Am. Chem. Soc.* **2000**, *122*, 10484−10485.

(7) Hu, Y.; Li, C.; Kulkarni, B. A.; Strobel, G.; Lobkovsky, E.; Torczynski, R. M.; Porco, J. A. Jr. *Org. Lett.* **2001**, *3*, 1649−1652.

(8) Fu, X.; Hossain, M. B.; von der Helm, D.; Schmitz, F. J. *J. Am. Chem. Soc.* **1995**, *116*, 12125−12126.

(9) Lee, J. C.; Strobel, G. A.; Lobkovsky, E., Clardy, J. *J. Org. Chem.* **1996**, *61*, 3232−3233.

(10) Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; Mcmahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2588−2603.

(11) O′Reilly, M. S.; Boehm, T.; Shing, Y.; Fukai, N.; Vasios, G.; Lane, W. S.; Flynn, E.; Birkhead, J. R.; Olsen, B. R.; Folkman, J. *Cell* **1997**, *88*, 277−285.

(12) (a) Zhang, Y.; Griffith, E. C.; Sage, J.; Jacks, T.; Liu, J. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6427−6432. (b) Seki, M.; Toi, M.; Kobayashi, K.; Shitara, K.; Umezawa, K.; Seon, B. K.; Kan, M.; Rhim, J. S. *Int. J. Oncol.* **2000**, *3*, 525−533.

(13) D'Amato, R. J.; Loughnan, M. S.; Flynn, E.; Folkman, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4082−4085.

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