VOL. 55 NO. 9, SEPT. 2002 THE JOURNAL OF ANTIBIOTICS pp. 829 — 831

COMMUNICATIONS TO THE EDITOR

Epoxyquinol B, a Fungal Metabolite with a Potent Antiangiogenic Activity

Sir:

Angiogenesis, the formation of new capillaries from preexisting vessels, is a prerequisite for many physiological processes, including embryonic development, wound healing, and female reproductive functions. Moreover, a number of pathological conditions such as cancer, rheumatoid arthritis, and other chronic inflammatory diseases are characterized by extensive angiogenesis.[1~5] Recent studies have revealed that vascular endothelial growth factor (VEGF) plays a pivotal role in pathological situations that involve neovascularization as well as enhanced vascular permeability. Additionally, VEGF has been shown to strongly induce cell migration, proliferation, and tube formation with a unique specificity for endothelial cells.[6,7] Consequently the concept that progression of various diseases may be halted by inhibiting endothelial cell functions has raised considerable interest.[8]

In a previous paper, we have already reported a novel fungal metabolite, epoxyquinol A (**1**), as an angiogenesis inhibitor.[9] The continuous bioassy-guided purification led to a more potent angiogenesis inhibitor, epoxyquinol B (**2**), a unique diastereomer of **1** in respect to the structure, biosynthesis, and biological properties. In this communication, the isolation, structure, and biological activities of epoxyquinol B (**2**) are reported.

The producing fungal strain was cultured in a 30-liter jar fermenter containing 18 liters of fermentation medium (glucose 1%, soluble starch 2%, soybean meal 1.5%, malt extract 0.5%, vegetable extract 10%, potato dextrose 2.5%, KH_2PO_4 0.05%, and $MgSO_4 \cdot 7H_2O$ 0.05%). The fermentation was carried out at 28°C, with a stirring speed of 350 rpm and an aeration rate of 10 liters/minute for 96 hours. The active principle was extracted with ethyl acetate from culture filtrate adjusted to pH 7.0. The solvent layer was dried over Na_2SO_4 and concentrated *in vacuo* to give an oily residue. This material was subjected to silica gel column chromatography with 0~50% methanol in chloroform stepwise. Epoxyquinol B (**2**) was eluted with 10% methanol in chloroform, and was purified on preparative HPLC using a reverse phase column (PEGASIL ODS, 20 i.d.×250 mm, Senshu Scientific Co. Ltd., Tokyo) with a gradient solvent system of CH_3CN-water (from 20 : 80 to 50 : 50). Final purification by preparative silica gel thin-layer chromatography ($CHCl_3$–MeOH=10 : 1) gave 50 mg of epoxyquinol B (**2**).

Epoxyquinol B (**2**) as a colorless oil was optically active, $[\alpha]_D^{21}$ +153.0° (*c* 0.315, MeOH). Epoxyquinol B (**2**) has the same molecular formula $C_{20}H_{20}O_8$ as that of epoxyquinol A (**1**), determined by HR-EIMS (Found: *m/z* 388.1135. Calcd: *m/z* 388.1158) and NMR spectra. UV spectrum showed absorption maxima (ε) at 205 (13140), 240 (5970), 320 (430) nm in MeOH. The IR spectrum (neat) showed characteristic absorption bands at 3425, 1685, 1675, 1340, 1190, and 1000 cm^{-1}, indicating the presence of hydroxyl

Table 1. ^{13}C and ^{1}H NMR data for epoxyquinol B (**2**).

No.	^{13}C (mult.) [a,b]	^{1}H (mult.) [a]	*J* (Hz)
1	73.14 (d)	5.06 (s)	
2	150.03 (s)		
3	63.75 (d)	4.83 (br d)	4.6
4	56.16 (d)	3.82 (dd)	3.4, 1.5
5	52.53 (d)	3.53 (dd)	3.4, 1.0
6	190.81 (s)		
7	132.86 (s)		
8	36.75 (d)	3.11 (dd)	2.7, 1.2
9	70.47 (d)	4.16 (dq)	1.2, 6.4
10	20.01 (q)	0.80 (d)	6.4
11	149.86 (d)	6.50 (s)	
12	105.73 (s)		
13	68.34 (d)	4.64 (ddd)	2.2, 2.0, 0.7
14	54.58 (d)	3.63 (dd)	3.2, 2.0
15	52.48 (d)	3.51 (dd)	3.2, 0.7
16	198.86 (s)		
17	51.58 (s)		
18	41.82 (d)	2.79 (dd)	6.6, 2.7
19	74.42 (d)	3.53 (dq)	6.6, 6.4
20	19.36 (q)	1.28 (d)	6.4
3-OH		4.37 (d)	4.6
13-OH		3.97 (d)	2.2

[a] Data collected at 600 MHz (^{1}H) and 150 MHz (^{13}C) in $CDCl_3$. Chemical shifts are in ppm relative to TMS (0 ppm) for ^{1}H or to the solvent $CDCl_3$ (77.0 ppm) for ^{13}C as internal references. [b] ^{13}C multiplicities were assigned from DEPT and PFG-HMQC spectra.

groups and both a ketone and unsaturated carbonyls. The ^{13}C and ^{1}H NMR data for epoxyquinol B (**2**) are summarized in Table 1.

The ^{1}H and ^{13}C NMR spectral analysis including 2D NMR techniques (PFG-DQFCOSY, PFG-HMQC, and PFG-HMBC) revealed that the plain structure of **2** was the same as that of **1**. Relative stereochemistry between hydroxy and epoxy groups of both epoxyquinol moieties of **2** was determined to be *trans* configuration from the small coupling constant values of $^{3}J_{H\text{-}3,H\text{-}4}$ and $^{3}J_{H\text{-}13,H\text{-}14}$. Significant NOEs observed between H-8 and H-19 as well as H-9 and H-18 confirmed the sequential stereochemistry at C-9, C-8, C-18, and C-19. Moreover, the isolated methine proton H-1 displayed significant NOEs with H-14, H-15, and 3-OH, thereby establishing the stereochemistry at C-1, C-17, C-13, and C-3. Thus, the relative stereochemistry of **2** was unambiguously determined as shown in Figure 1. As the asymmetric total synthesis of **1** and **2** has been completed,[10] this stereochemistry including their absolute stereochemistry is confirmed.

Epoxyquinol B (**2**) has the opposite configurations at C-17, C-18, and C-19 in comparison with those of **1**. Although several biosynthetic pathways for the production of **2** could be considered, one possible path is shown in Figure 2. Epoxyquinol A (**1**) would be generated by an *exo* intermolecular Diels-Alder reaction between **4a** and **4b** resulted from a key precursor **3** *via* an oxidative 6π-electrocyclization.[9] On the other hands, a Diels-Alder *endo* homodimerization of 2*H*-pyran monomer **4a** could generate **2** with the corresponding configuration, in which case both methyl groups could approach one another with the low steric hindrance.

We tested the effect of epoxyquinol B (**2**) on human umbilical vein endothelial cells (HUVECs) migration induced by VEGF. VEGF significantly stimulated the cell migration in the migration assay by using a CHEMOTAXICELL chamber.[9,11] Ten μM SU5614, a well-known inhibitor of VEGF receptor tyrosine kinase (VEGF-R2/KDR/Flk-1)[12,13] inhibited VEGF-induced endothelial migration. As shown in Figure 3, epoxyquinol B (**2**) inhibited the cell migration induced by VEGF in a dose-

Fig. 1. Structures of epoxyquinol A (**1**) and B (**2**).

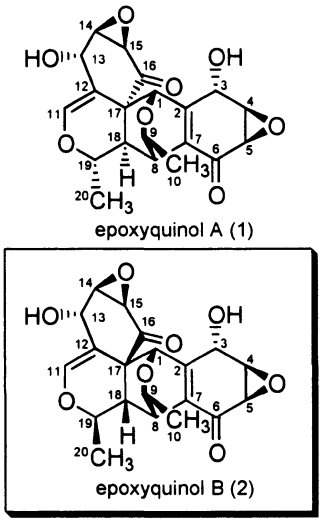


Fig. 2. Possible biosynthetic scheme for epoxyquinol B (**2**).

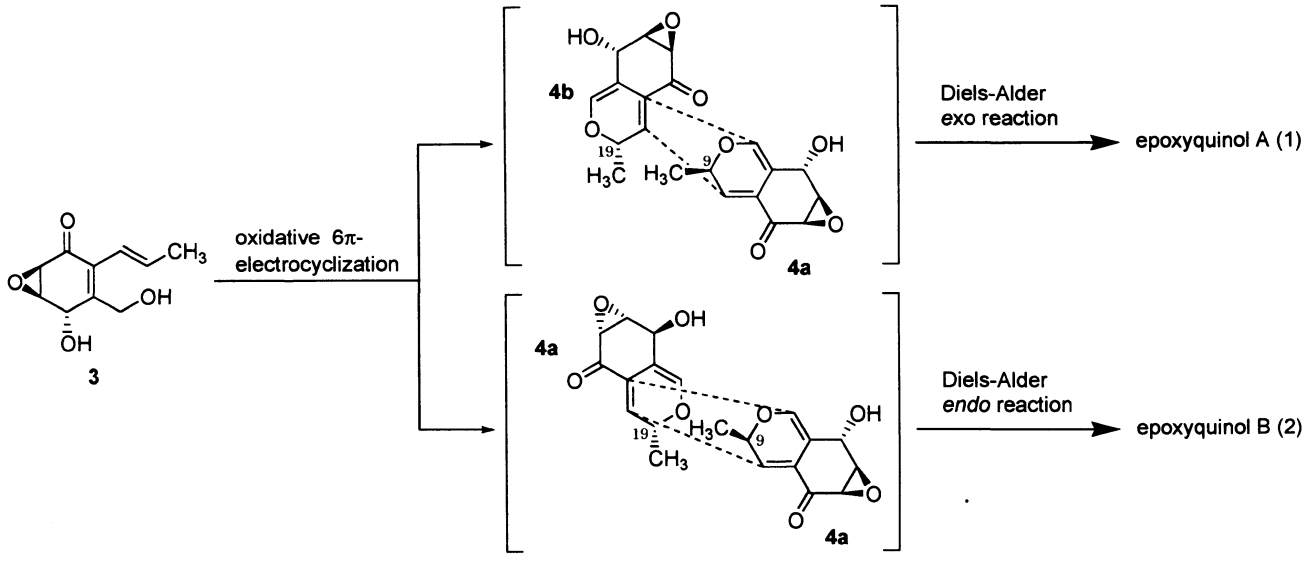
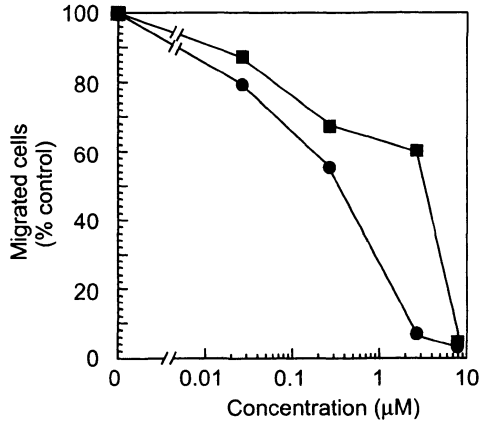


Fig. 3. Inhibition of VEGF-induced cell migration by epoxyquinol B (**2**) in HUVECs.



HUVECs (1×10^5) suspended in HuMedia-EG2 medium (KURABO, Osaka) with various concentrations of epoxyquinols A (■) or B (●) was added to upper compartment of a CHEMOTAXICELL chamber (KURABO, Osaka) and incubated with HuMedia-EG2 medium containing 12.5 ng/ml of VEGF in the lower compartment for 18 hours at 37°C in a 5% CO_2 atmosphere. The filter was fixed with MeOH and stained with hematoxylin. The cells on the upper surface of the filter were removed by wiping with cotton swabs. The cells that migrated through the filter to the various areas of the lower surface were counted manually under microscope at a magnification of ×100. Values are means for triplicate samples.

dependent manner. The treatment of epoxyquinol B (**2**) with 2.6 μM completely inhibited cell migration without showing significant cell toxicity, as estimated by a trypan blue dye exclusion assay. The IC_{50} value of **2** with 0.4 μM shows *ca.* ten times more potent than that of **1**. These results suggest that the differences of stereochemistry at C-17, C-18, and C-19 between **1** and **2** play an important role for showing the biological activity. Further studies on the structure-activity relationships and the biological activities of epoxyquinols A (**1**) and B (**2**) are in progress.

Acknowledgements

This work was supported in part by a Special Grant for Promotion of Research from RIKEN, and a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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(Received May 16, 2002)

References

1) FOLKMAN, J.: Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat. Med. 1: 27~31, 1995
2) FAN, T. P.; R. JAGGAR & R. BICKNELL: Controlling the vasculature: angiogenesis, anti-angiogenesis and vascular targeting of gene therapy. Trends Pharmacol. Sci. 16: 57~66, 1995
3) RISAU, W.: Mechanism of angiogenesis. Nature 386: 671~674, 1997
4) ISNER, J. M. & T. ASAHARA: Angiogenesis and vasculogenesis as therapeutic strategies for postnatal neovascularization. J. Clin. Invest. 103: 1231~1236, 1999
5) KUWANO, M.; J. FUKUSHI, M. OKAMOTO, A. NISHIE, H. GOTO, T. ISHIBASHI & M. ONO: Angiogenesis factors. Intern. Med. 40: 565~572, 2001
6) SHIBUYA, M.: Structure and dual function of vascular endothelial growth factor receptor-1 (Flt-1). Int. J. Biochem. Cell Biol. 33: 409~420, 2001
7) FERRARA, N. & T. DAVIS-SMYTH: The biology of vascular endothelial growth factor. Endocr. Rev. 18: 4~25, 1997
8) GASPARINI, G.: The rationale and future potential of angiogenesis inhibitors in neoplasia. Drugs 58: 17~38, 1998
9) KAKEYA, H.; R. ONOSE, H. KOSHINO, A. YOSHIDA, K. KOBAYASHI, S.-I. KAGEYAMA & H. OSADA: Epoxyquinol A, a highly functionalized pentaketide dimer with antiangiogenic activity isolated from fungal metabolites. J. Am. Chem. Soc. 124: 3496~3497, 2002
10) SHOJI, M.; J. YAMAGUCHI, H. KAKEYA, H. OSADA & Y. HAYASHI: Total synthesis of (+)-epoxyquinols A and B. Angew. Chem. Int. Ed. 41: 3192~3194, 2002
11) DVORAK, H. F.; L. F. BROWN, M. DETMAR & A. M. DVORAK: Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am. J. Path. 146: 1029~1039, 1995
12) SUN, L.; N. TRAN, F. TANG, H. APP, P. HIRTH, G. MCMAHON & C. TANG: Synthesis and biological evaluations of 3-substituted indolin-2-ones: a novel class of tyrosine kinase inhibitors that exhibit selectivity toward particular receptor tyrosine kinases. J. Med. Chem. 41: 2588~2603, 1998
13) SHAHEEN, R. M.; D. W. DAVIS, W. LIU, B. K. ZEBROWSKI, M. R. WILSON, C. D. BUCANA, D. J. MCCONKEY, G. MCMAHON & L. M. ELLIS: Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. Cancer Res. 59: 5412~5416, 1999

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