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Angiogenesis Inhibitor Epoxyquinol A: Total Synthesis and Inhibition of Transcription Factor NF-κB

Chaomin Li,[†] Sujata Bardhan,[†] Emily A. Pace,[‡] Mei-Chih Liang,[‡] Thomas D. Gilmore,[‡] and John A. Porco, Jr.*[,†]

Department of Chemistry and Center for Streamlined Synthesis and Department of Biology, Boston University, Boston, Massachusetts 02215

porco@chem.bu.edu.

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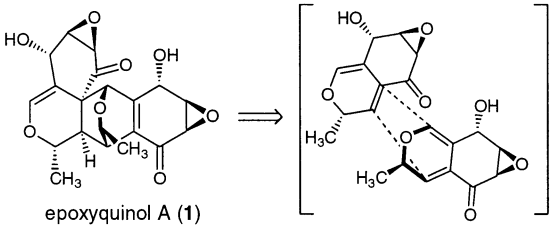
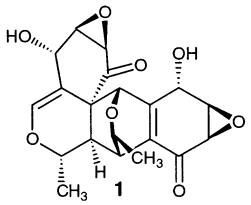
ABSTRACT



The asymmetric synthesis of the natural product (+)-epoxyquinol A (1) and related epoxyquinoid dimers, employing a cascade oxidation/electrocyclization/Diels–Alder dimerization sequence, is reported. In addition, we show that 1 and related molecules inhibit activation of the transcription factor NF-κB.

Angiogenesis, new blood vessel formation, involves a number of distinct processes, including endothelial cell migration, proliferation, and capillary tube formation, and is believed to be a key requirement for tumor growth and metastasis.[1] Angiogenesis inhibition thus represents an important approach to cancer chemotherapy, and several agents, including those derived from natural product leads, are now entering clinical development.[2] The pentaketide dimer (+)-epoxyquinol A (**1**) was recently isolated from an uncharacterized fungus by Osada and co-workers[3] and was shown to have potent antiangiogenic activity. The relative stereochemistry of **1** was determined by X-ray crystallography. We have previously reported syntheses of the dimeric epoxyquinone natural product torreyanic acid[4] and two monomeric epoxyquinol natural products (cycloepoxydon (inset, Figure 1)[5] and jesterone).[6] Thus far, chemical synthesis of an epoxyquinol dimer has not been reported. In this Letter, we report the synthesis and absolute stereochemical assignment of (+)-epoxyquinol A and four related epoxyquinoid dimers. In addition, we show that **1** and related molecules inhibit activation of transcription factor NF-κB.



A retrosynthetic analysis for (+)-epoxyquinol A is depicted in Figure 1. Compound **1** may be prepared by an

[†] Department of Chemistry and Center for Streamlined Synthesis.
[‡] Department of Biology.

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(2) For a review of natural products as angiogenesis inhibitors, see: Paper, D. H. *Planta Med.* **1998**, *64*, 686.

(3) Kakeya, H.; Onose, R.; Koshino, H.; Yoshida, A.; Kobayashi, K.; Kageyama, S.-I.; Osada, H. *J. Am. Chem. Soc.* **2002**, *124*, 3496.

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

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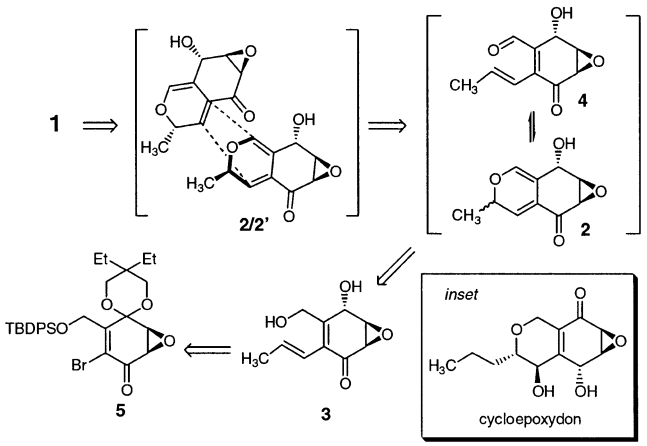


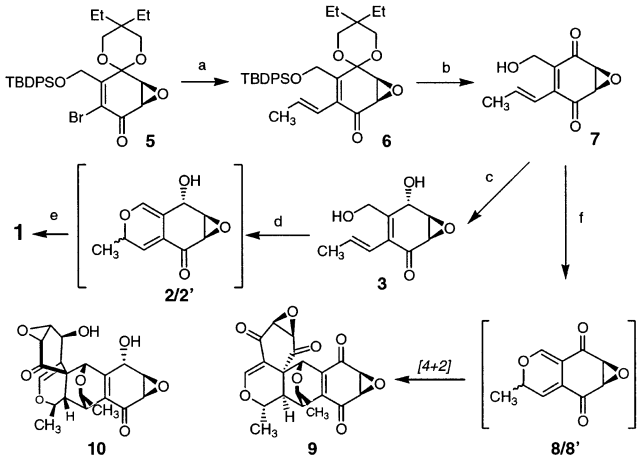
Figure 1. Retrosynthetic analysis for epoxyquinol A.

oxidation/electrocyclization/Diels−Alder dimerization of diastereomeric 2*H*-pyran monomers **2/2′**.[4,6] In this biomimetic, *endo*-selective[7] Diels−Alder heterodimerization, the methyl groups of the pyran are *anti* to one another and the dienophile approaches the diene *anti* to the epoxide moiety. Monomers **2/2′** may be derived from selective primary oxidation of epoxyquinol **3** (RK-302, isolated from the same fermentation broth as **1**[3]) followed by 6π-electrocyclic ring closure of the resulting dienal **4**. Compound **3** may be prepared from readily available chiral epoxide **5**, which is a known intermediate in our previous synthesis of cycloepoxydon.[5]

The synthesis was initiated by Stille coupling of **5** with (*E*)-tributyl-1-propenyl-stannane to afford α-propenyl enone **6** in near quantitative yield (Scheme 1).[8] Sequential hydrolysis of the cyclic acetal and silyl ether protecting groups (aqueous HF) afforded the quinone monoepoxide **7**. Regio- and stereoselective reduction of **7** using Kiyooka's conditions[9] (2 equiv of DIBAL-H, THF, −78 °C) afforded **3**[10] (72%) as the major diastereomer.

In line with our previous studies,[4,6] treatment of **3** with Dess−Martin periodinane afforded a crude product mixture, which was subjected to column chromatography to provide two major fractions. NMR analysis indicated that the major fraction contained 2*H*-pyrans **2/2′** and aldehyde **4** (cf. Figure 1), as well as the desired dimer **1**. The minor fraction comprised quinone epoxide **7**, the related quinone 2*H*-pyran monomers **8/8′**, and quinone dimer **9** (Scheme 1).[11] This result indicates that undesired oxidation of the secondary allylic alcohol was occurring under these conditions.[12] To

Scheme 1[a]



[a] Reagents and conditions: (a) (*E*)-tributyl-1-propenyl-stannane, Pd_2dba_3, $AsPh_3$, $PhCH_3$, 110 °C, 2 h (98%); (b) 48% HF, CH_3CN, rt, 1 h (84%); (c) DIBAL-H, THF, −78 °C, 10 min (72%); (d) O_2 (1 atm), TEMPO, CuCl, DMF, 3 h; (e) 40% MeOH−H_2O, 10 h, **1** (55%), **10** (14%); (f) Dess−Martin periodinane, CH_2Cl_2, 1.5 h; SiO_2, 2 h, 67%. DIBAL-H = diisobutylaluminum hydride, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy.

promote the desired electrocyclization/Diels−Alder dimerization, the major fraction was treated with silica gel in $CHCl_3$ for 20 h.[4] Epoxyquinol A was obtained in 26% yield after silica gel chromatography. Synthetic (+)-epoxyquinol A was confirmed to be identical to natural (+)-epoxyquinol A by ^1H and ^{13}C NMR, mass spectrum, $[\alpha]_D$ (+66° (*c* = 0.23, $CHCl_3$)), and TLC R_f values in three different solvent systems.

Alternative oxidation methods were next evaluated in order to obtain higher selectivity for oxidation of the primary vs secondary allylic alcohol of **3** (Scheme 1). We found that selective oxidation could be achieved using conditions reported by Semmelhack (CuCl (cat.), O_2, TEMPO, DMF).[13] Under these conditions, no oxidation of the secondary allylic alcohol or overoxidation to the carboxylic acid was detected. After oxidation, the crude product mixture[14] was dissolved in $CDCl_3$, and ^1H NMR analysis was used to monitor the disappearance of aldehyde and 2*H*-pyran. Use of this slightly acidic condition for dimerization afforded *endo* dimer **1** (36%) along with a related dimer **10** (30%) after column chromatography. In contrast, Diels−Alder dimerization in 40% aqueous MeOH (10 h) afforded epoxyquinol A and **10** in 55% and 14% yield, respectively.[15] The structure of **10** was determined to be an *exo*-dimer *of two identical* 2*H-pyran monomers*[16] by X-ray crystal structure analysis (Figure 2).

(7) In refs 3 and 4, epoxyquinol and epoxyquinone dimers were referred to as "*exo*." However, it is more appropriate to refer to the dimers as "*endo*" with respect to the carbonyl substituent on the 2*H*-pyran dienophile.

(8) Attempted Stille coupling using $Pd(PPh_3)_4$ as catalyst afforded the undesired 1,3-diketone as the main product; cf.: Suzuki, M.; Watanabe, A.; Noyori, R. *J. Am. Chem. Soc.* **1980**, *102*, 2095.

(9) Kiyooka, S.; Kuroda, H.; Shimasaki, Y. *Tetrahedron Lett.* **1986**, *27*, 3009.

(10) Synthetic **3** has the same HPLC retention time and the same R_f by TLC with the natural compound RK-302 (see Supporting Information).

(11) *Endo* quinone dimer **9** was also produced in 67% yield by Dess−Martin periodinane oxidation of **7**, followed by treatment with silica gel (Scheme 1). See Supporting Information for details.

(12) It is also conceivable that internal hydride transfer of dienal **4** to a quinone epoxide may occur. For a recent example of an internal hydride transfer, see: Szendi, Z.; Forgó, P.; Tasi, G.; Böcskel, Z.; Nyerges, L.; Sweet, F. *Steroids* **2002**, *67*, 31.

(13) Semmelhack, M. F.; Schmid, C. R.; Cortes, D. A.; Chou, C. S. *J. Am. Chem. Soc.* **1984**, *106*, 3374.

(14) ^1H NMR ($CDCl_3$) analysis of the crude reaction mixture indicates a ratio of dienal **4**:2*H*-pyran **2**:dimer **1**:dimer **10** of 1:4.9:7.9:7.7.

(15) For enhancement of *endo/exo* selectivity by water in Diels−Alder reactions, see: Otto, S.; Engberts, J. B. F. N. *Pure Appl. Chem.* **2000**, *72*, 1365.

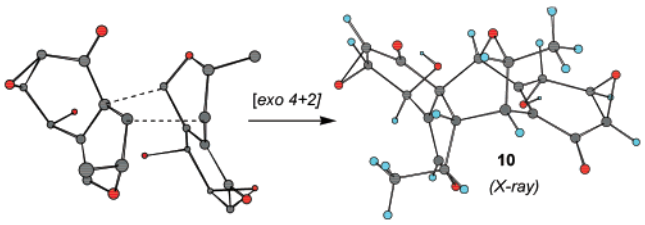


Figure 2. X-ray structure of dimer **10**.

With diastereomeric epoxyquinol dimers **1** and **10** in hand, we next investigated their reactivity to thermolysis. Surprisingly, both dimers were found to be stable at 80 °C in $CDCl_3$. Microwave irradiation was next used as a method to establish rapid thermodynamic equilibration of the compounds.[17] Treatment of **1** in the CEM Discover microwave system (chlorobenzene, 180 °C, 5 min) afforded *exo* dimer **10** (14%), dimer **11** (43%) (Figure 3), and **12** (10%). Extended

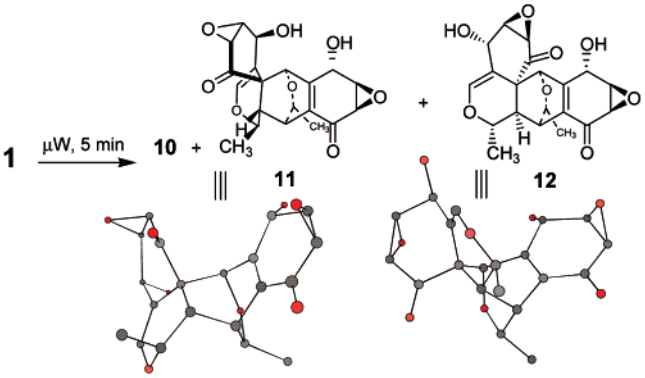


Figure 3. Microwave irradiation of epoxyquinol A and AM1 equilibrium geometries of **11** and **12** (PC Spartan Pro, v. 1.0.7).

microwave irradiation of **1** (180 °C, 2 × 5 min) led to the production of *exo* dimer **10** in higher yield (36%) along with **11** (25%) and **12** (10%). However, when dimer **10** was irradiated using the same conditions, starting material was recovered, indicating higher stability of **10**, possibly derived from intramolecular hydrogen bonding.[18] The structure of **11** was assigned by NOE difference NMR spectroscopy to be an *endo* dimer in which the dienophile *approaches the diene syn to the epoxide*. Dimer **12** was similarly determined to be an *exo* dimer in which the dienophile approaches the diene *syn* to the epoxide. Interestingly, dimer **11** is derived from Diels−Alder reaction of diastereomeric 2*H*-pyran monomers (cf. **1**), and **12** from identical 2*H*-pyran monomers (cf. **10**). Evidently, **11** and **12** are not kinetically favored products and may be obtained under microwave irradiation as a result of the higher activation energy provided under these conditions. On the basis of these results, three underlying rules for Diels−Alder dimerizations of 2*H*-pyran epoxyquinol monomers are thus apparent: (1) two identical or diastereomeric 2*H*-pyran monomers may undergo *endo*- or *exo*-Diels−Alder cycloaddition; (2) the methyl groups of the diene and dienophile tend to orient themselves away from one another, presumably to avoid steric interactions; and (3) kinetically, the dienophile approaches the diene *anti* to the epoxide moiety (cf. **1** and **10**),[19] whereas thermodynamically (by higher activation energy with microwave irradiation), the dienophile can approach the diene *syn* to the epoxide moiety.

Because other epoxyquinoids, including both natural[5,20] and synthetic[21] molecules, have been shown to inhibit activation of transcription factor NF-κB,[22] we determined whether epoxyquinol A and three synthetic derivatives also had this ability. As shown in Table 1 and Figure 4, dimers

Table 1. Inhibition of NF-κB DNA Binding by Epoxyquinol A (**1**) and Related Compounds

compound	IC_{50} (μM)
epoxyquinol A (**1**)	11[a]
quinone dimer (**9**)	10[a]
quinol monomer (**3**)	20[b]
quinone monomer (**7**)	2.3[b]

[a] Average of four experiments. [b] Average of two experiments.

1 and **9** and monomers **3** and **7** all inhibit tumor necrosis factor (TNF)-induced activation of NF-κB DNA binding in mouse 3T3 cells. In these assays, monomer **7** was approximately 5−10 times more effective than the others, with an IC_{50} of 2.3 μM. Of note, transcription of the gene encoding vascular endothelial growth factor (VEGF), one of the primary mediators of angiogenesis, is known to be controlled, at least in part, by NF-κB.[23] Indeed, the concentrations of **1**

(16) For *endo* Diels−Alder dimerization of two identical 2*H*-pyran monomers, see ref 4.

(17) For select examples of microwave irradiation of natural products, see: (a) Salmoria, G. V.; Dall'Oglio, E. L.; Zucco, C. *Synth. Commun.* **1997**, *29*, 4335. (b) Das, B.; Venkataiah, B. *Synth. Commun.* **1999**, *29*, 863. (c) Das, B.; Madhusudhan, P.; Venkataiah, B. *Synth. Commun.* **2000**, *30*, 4001. (d) Das, B.; Venkataiah, B.; Kashinatham, A. *Tetrahedron* **1999**, *55*, 6585.

(18) Dimer **10** shows a distance of 2.7−2.8 Å between the two hydroxyl oxygens (X-ray, two conformers), as well as an anomalous downfield shift for one hydroxyl proton (^{1}H NMR). For a recent example of intramolecular OH−OH hydrogen bonding, see: Vasquez, T. E., Jr.; Bergset, J. M.; Fierman, M. B.; Nelson, A.; Roth, J.; Khan, S. I.; O'Leary, D. J. *J. Am. Chem. Soc.* **2002**, *124*, 2931.

(19) For an example of Diels−Alder cycloaddition with π-facial selectivity *anti* to the diene epoxide moiety, see: Gillard, J. R.; Newlands, M. J.; Bridson, J. N.; Burnell, D. J. *Can. J. Chem.* **1991**, *69*, 1337.

(20) (a) Cycloepoxydon: Gehrt, A.; Erkel, G.; Anke, T.; Sterner, O. *J. Antibiot.* **1998**, *51*, 455. (b) Panepoxydon: Erkel, G.; Anke, T.; Sterner, O. *Biochem. Biophys. Res. Commun.* **1996**, *226*, 214.

(21) Umezawa, K.; Ariga, A.; Matsumoto, N. *Anticancer Drug Des.* **2000**, *15*, 239.

(22) For a review of natural products as modulators of the NF-κB signaling pathway, see: Bremner, P.; Heinrich, M. *J. Pharm. Pharmacol.* **2002**, *54*, 453.

(23) For recent reports on suppression of angiogenesis by inhibition of NF-κB in cancer cells, see: (a) Huang, S.; Robinson, J. B.; DeGuzman, A.; Bucana, C. D.; Fidler, I. J. *Cancer Res.* **2000**, *60*, 5334. (b) Huang, S.; Pettaway, C. A.; Uehara, H.; Bucana, C. D.; Fidler, I. J. *Oncogene* **2001**, *20*, 4188.

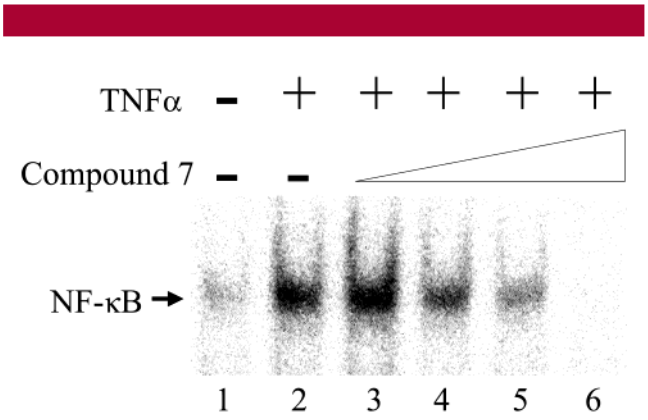


Figure 4. Compound **7** inhibits tumor necrosis factor-induced activation of NF-κB. Mouse 3T3 cells were untreated (lane 1) or were treated with TNF-α (lanes 2−6), and NF-κB DNA-binding activity was analyzed in an electrophoretic mobility shift assay. In lanes 3−6, cells were preincubated with 0.675, 1.25, 2.5, and 5 μM, respectively, **7** prior to treatment with TNF-α; in lanes 1 and 2, cells were preincubated with solvent (CH_3OH). The arrow indicates the induced NF-κB (p50-RelA) complex.

reported to inhibit cell migration induced by VEGF[3] (ED_{100} = 7 μM) and activation of NF-κB (Table 1) are similar. Thus, our demonstration that epoxyquinol A and related compounds inhibit induction of NF-κB may explain the molecular basis for inhibition of angiogenesis by epoxyquinol A. If so, one would predict that **7**, the most effective inhibitor of TNF-induced activation of NF-κB, would also be the most effective blocker of angiogenesis.

In summary, we have completed an asymmetric synthesis of epoxyquinol A employing a [4 + 2] dimerization of epoxyquinol 2*H*-pyran monomers. Additional epoxyquinone and epoxyquinol dimers have been produced as part of these studies, which further clarifies the underlying rules for Diels−Alder dimerizations of epoxide-containing 2*H*-pyran monomers. Notably, microwave irradiation of epoxyquinol A has been used to produce novel stereoisomers of **1** that are not accessible at ambient temperature. Future biological experiments will be aimed at identifying the molecular target(s) for epoxyquinol A mediated inhibition of NF-κB activation and angiogenesis. Similarly, it will be of interest to determine whether epoxyquinol A and related compounds also inhibit other biological processes, including inflammation and the growth of certain tumor cells, that are dependent on NF-κB.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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