



Efficient synthesis of (±)-parasitenone, a novel inhibitor of NF-κB

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ABSTRACT

Dehydroxymethylepoxyquinomicin (DHMEQ, **1**) is a novel nuclear factor-κB (NF-κB) inhibitor that inhibits DNA binding of NF-κB components including p65. To inspect its biological activity of **1**, we synthesized parasitenone (**3**), possessing the common epoxy cyclohexenone moiety of **1**. Assessment of the inhibitory activity against NF-κB indicated that the epoxy cyclohexenone moiety is the most essential element for the NF-κB inhibitory activity and the salicylic acid moiety may contribute the binding efficiency and specificity.

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Binding of nuclear factor-κB (NF-κB) to the κB sequence on target genes induces the transcription of several inflammatory cytokines, such as interleukins, TNF-α, and antiapoptotic proteins. Therefore, NF-κB inhibitors were expected to be novel candidates as anti-inflammatory and anticancer agents.

DHMEQ (**1**), designed based on the structure of epoxyquinomicin C (**2**) and synthesized by Umezawa and co-workers,¹ exhibited a remarkable inhibitory effect (Fig. 1). The known NF-κB inhibitors, such as panepoxydon (**4**) and cycloepoxydon (**5**), inhibit the activation of NF-κB by interference with the degradation process of IκB-α and activation of IKK kinase.^{2,3} Despite the structural similarity to **4** and **5**, **1** showed a completely different mode of action: **1** specifically deactivates NF-κB by inhibiting the DNA binding of NF-κB components.⁴

In the previous study, it was observed that the epoxy cyclohexenone moiety of **1** covalently binds the 38th cysteine residue of p65 to appear the inhibitory activity against NF-κB.⁴ To understand the detailed mode of action, and develop more effective inhibitor, biological activities of the epoxy cyclohexenone were investigated. The appropriate substrate carrying the same *cis* relationship of the corresponding OH and epoxide groups as that of **1** was parasitenone (**3**), isolated from the marine algicolic fungus *Aspergillus parasiticus*.⁵ It showed mild scavenging activity of free radicals, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH, IC₅₀ = 57.0 μM) and peroxynitrite (ONOO⁻) (IC₅₀ = 52.6 μM). The

same epoxy cyclohexenone structure as **1** prompted us to initiate its synthesis and assess the NF-κB inhibitory activity.

Selective reduction of phillostin (**6**),⁶ assisted with the neighboring primary hydroxyl group, was expected to be a good approach to the target molecule **3** (Scheme 1). The asymmetric structure of **6** was accessible by selective epoxidation to the sterically less hindered site of **7** or **8**, which was readily produced by anodic oxidation of **9**, followed by selective hydrolysis. According to the synthetic plan mentioned above, the synthesis was commenced by NaBH₄ reduction of commercially available 2,5-dimethoxybenzaldehyde (**10**), followed by protection of the benzyl alcohol to afford the siloxy ether **9** (98% in two steps).

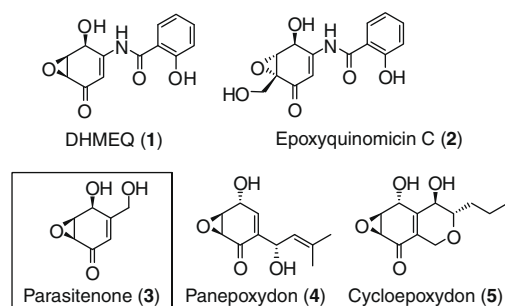
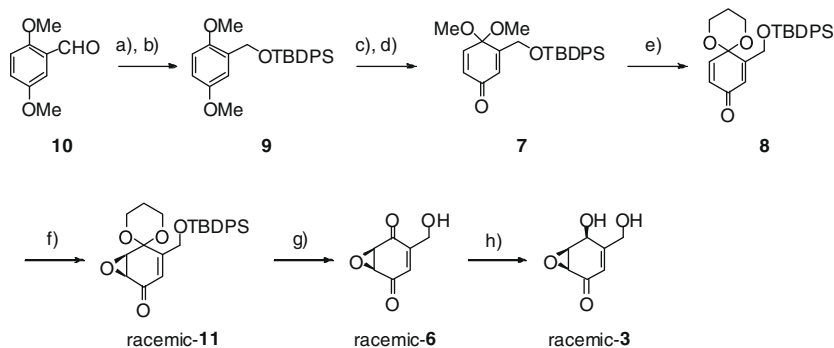


Figure 1. Bioactive compounds with the epoxy cyclohexenone moiety.

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Scheme 1. Reagents and conditions: (a) NaBH₄, MeOH, 0 °C; (b) TBDPSCl, Imid., DMF, rt, 98% in two steps; (c) C.P.E. (1.15 V vs SCE.), 1% KOH/ MeOH, platinum wire (cathode)—platinum net (anode), 0 °C; (d) 5% AcOH, acetone, quant. in two steps; (e) propane-1,3-diol, PPTS, benzene, reflux, 90%; (f) TBHP, *t*BuOK, THF, −78 to −35 °C, 57%, (**8**, 39%), (g) HF-pyr., CH₃CN; (h) NaBH(OAc)₃, MeOH, 0 °C, 43% in two steps.

Anodic oxidation of **9** in 1% KOH–MeOH⁷ provided the corresponding bisdimethylacetal as the two-electron oxidation product, which on regioselective hydrolysis⁸ yielded the vinylogous dimethylacetal **7** (100% in two steps). A number of efforts for direct epoxidation were unsuccessful. Thus, the substrate of the epoxidation reaction was changed to **8** (90%) by the Porco proto-

col.⁹ Epoxidation of **8** with *t*BuOOH and *t*BuOK provided the desired epoxide **11** (57%, conversion yield: 98%), which on treatment with HF-pyr. provided phillostin (**6**). Finally, NaB–H(OAc)₃ reduction of **6** effected the hydride-attack only from the desired side to produce (±)-parasitenone (**3**) as a single diastereomer (43% in two steps). The spectral data of synthetic **3** was identical to the reported data.¹⁰

DHMEQ (**1**) suppressed the LPS (lipopolysaccharide)-induced secretion of inflammatory mediators and cytokines, such as iNOS, COX-2, IL-6, and TNF-α, in the mouse macrophage cell line RAW264.7.¹¹ We evaluated the inhibitory activities of parasitenone (**3**) against secretion of inflammatory mediators and cytokines. Thus, **3** was subjected to the assay of NO production, which is an inflammatory mediator in LPS-stimulated RAW264.7 cells.

As shown in Figure 2, **3** lowered cell viability at 30 μg/mL, while at 10 μg/mL it only inhibited cell growth. Compound **3** clearly suppressed NO production in a dose-dependent manner (Fig. 3A). In contrast to salicylic acid, at 10 μg/mL, **3** decreased NO production to the control level. NO is produced by inducible NO synthase (iNOS), the expression of which is mediated by NF-κB. Compound **3** also diminished iNOS protein in a dose-dependent manner (Fig. 3B). Accordingly, we assessed the effects of racemic **1** and **3** on the transcriptional activity of NF-κB in RAW264.7 cell line. In Figure 4, compound **3** inhibited LPS-induced transcription of NF-κB, although it did so more weakly than **1**.

Parasitenone (**3**) was shown to covalently bind to the NF-κB component p65 in MALDI-TOF MS analysis (Fig. 5), although the binding was weak and non-specific compared with DHMEQ (**1**).⁴

In conclusion, effective synthesis of racemic parasitenone (**3**) was accomplished. Assessment of **3** by NO production, iNOS induction, and NF-κB activation, indicated that the epoxycyclohexenone core is the crucial factor for the inhibitory activity against NF-κB, while salicylic acid showed no inhibition of NF-κB (Fig. 3A). Despite lower activities than those of **1**, the dose-dependent inhibition of **3** was consistent with our previous observation that the epoxycyclohexenone core may react with the 38th cysteine residue.⁴ However, the biological effect and the p65 binding ability were lower than DHMEQ. Therefore, the salicylic acid moiety is likely to be required for more efficient and specific covalent-binding to the cysteine residue.

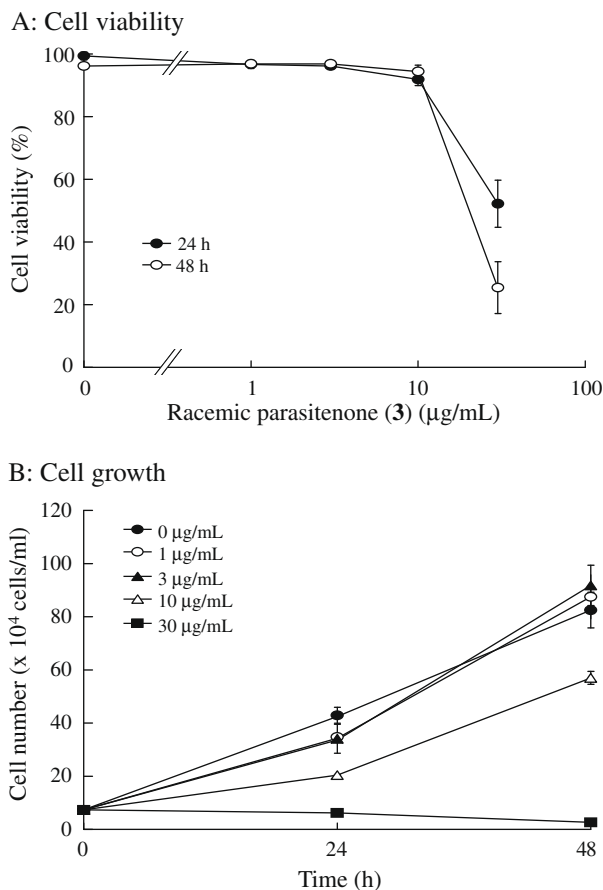


Figure 2. Effect of parasitenone (**3**) on cell viability (A) and cell growth (B) in RAW264.7 cells. The cell viability was assessed by trypan blue dye exclusion.

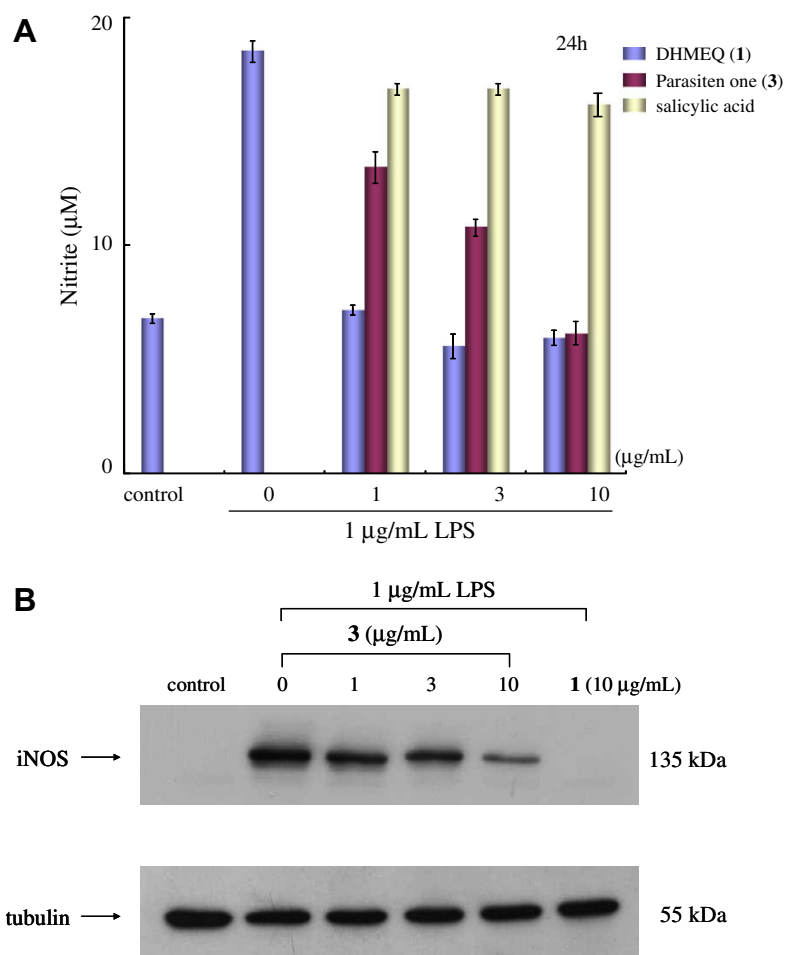


Figure 3. Inhibition of NO production and iNOS expression by parasitenone (**3**). (A) Effect on NO secretion in RAW264.7 cells. Cells were incubated without or with chemicals at various concentrations for 1 h, stimulated or not 1 μg/mL LPS for 24 h. NO secretion was assessed by Griess reaction. (B) Effect on iNOS expression. Cells were incubated with or without parasitenone (**3**) at various concentration for 1 h, then stimulated with 1 μg/mL LPS for 24 h. Total cell lysates were subjected to SDS-PAGE and immunoblotted with anti-iNOS anti-body. (Tubulin was used as a control.)

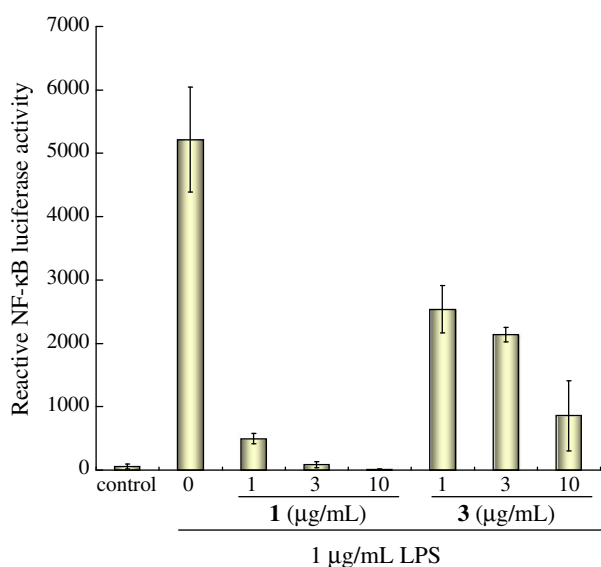


Figure 4. Effect of parasitenone (**3**) on transcription activity of NF-κB in RAW264.7 cells. Cells were transfected by diethylaminoethyl–dextran method and incubated for 24 h. Transfected cells were treated with the indicated concentrations of racemic **1** and **3** for 1 h, then stimulated with 1 μg/mL LPS for 6 h. Cells lysates were prepared and assayed for luciferase activity. Each value is the mean ± SD of triplicate determinations.

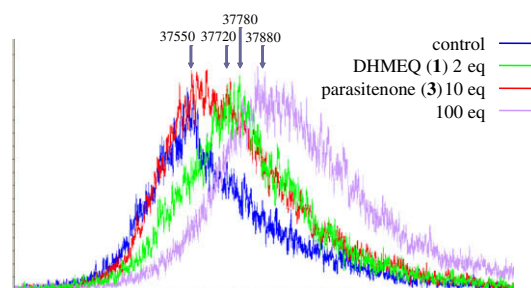


Figure 5. MALDI-TOF MS analysis of p65(1–325) with parasitenone (**3**) and DHMEQ (**1**). The p65(1–325) protein (20 μM) was treated with several equiv of racemic parasitenone (**3**) and racemic DHMEQ (**1**) for 1 h. After incubation, the proteins were used for the MALDI-TOF MS analysis.

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