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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 epoxycyclohexenone moiety of **1**. Assessment of the inhibitory activity against NF-κB indicated that the epoxycyclohexenone moiety is the most essential element for the NF-κB inhibitory activity and the salicylic acid moiety may contribute the binding efficiency and specificity.

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 epoxycyclohexenone 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 epoxycyclohexanone 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 algicolous fungus *Aspergillus parasiticus*.[5] It showed mild scavenging activity of free radicals, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH, IC_{50} = 57.0 μM) and peroxynitrite ($ONOO^-$) (IC_{50} = 52.6 μM). The same epoxycyclohexenone 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 $NaBH_4$ 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).

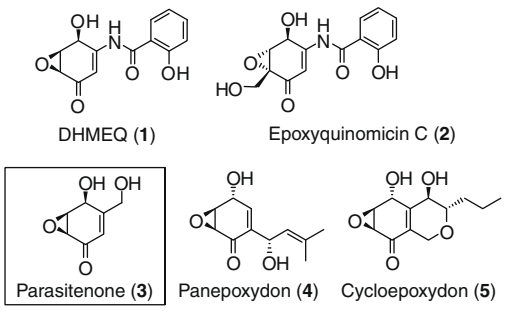

Figure 1. Bioactive compounds with the epoxycyclohexenone moiety.

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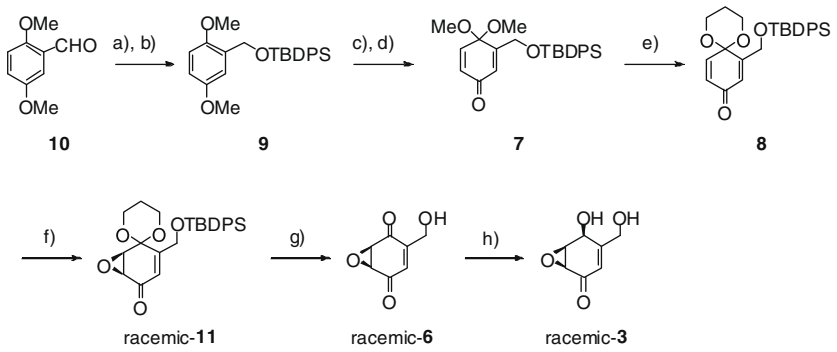

Scheme 1. Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C; (b) TBDPSCl, Imid., DMF, rt, 98% in two steps; (c) C.P.E. (1.15 V vs SCE.), 1% KOH/ MeOH, platinum wire (cathode)—platinum net (anode), 0 °C; (d) 5% AcOH, acetone, quant. in two steps; (e) propane-1,3-diol, PPTS, benzene, reflux, 90%; (f) TBHP, *t*BuOK, THF, −78 to −35 °C, 57%, (**8**, 39%), (g) HF-pyr., CH_3CN; (h) $NaBH(OAc)_3$, MeOH, 0 °C, 43% in two steps.

Anodic oxidation of **9** in 1% KOH–MeOH[7] provided the corresponding bisdimethylacetal as the two-electron oxidation product, which on regioselective hydrolysis[8] 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 protocol.[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, $NaBH(OAc)_3$ 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]

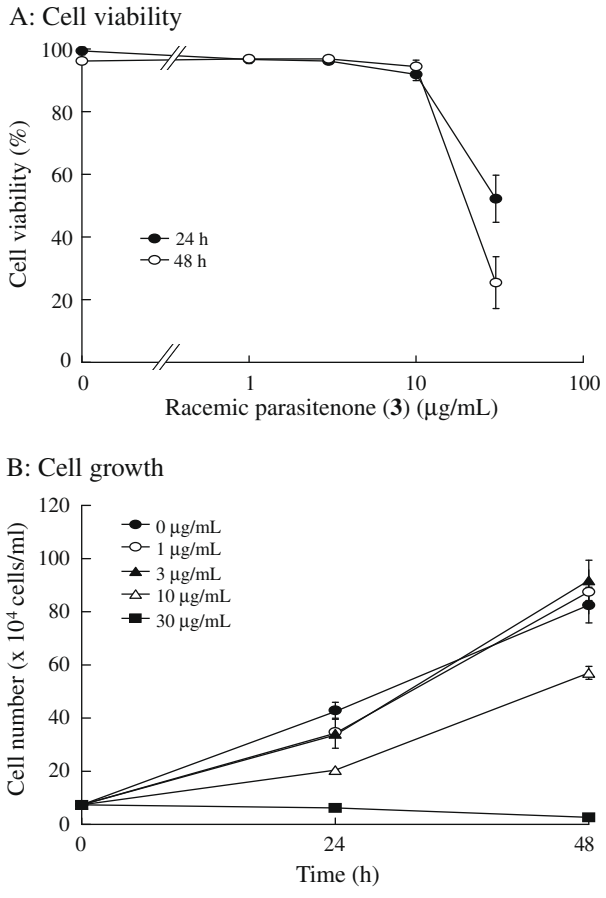

Figure 2. Effect of parasitenone (**3**) on cell viability (A) and cell growth (B) in RAW264.7 cells. The cell viability was assessed by trypan blue dye exclusion.

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.

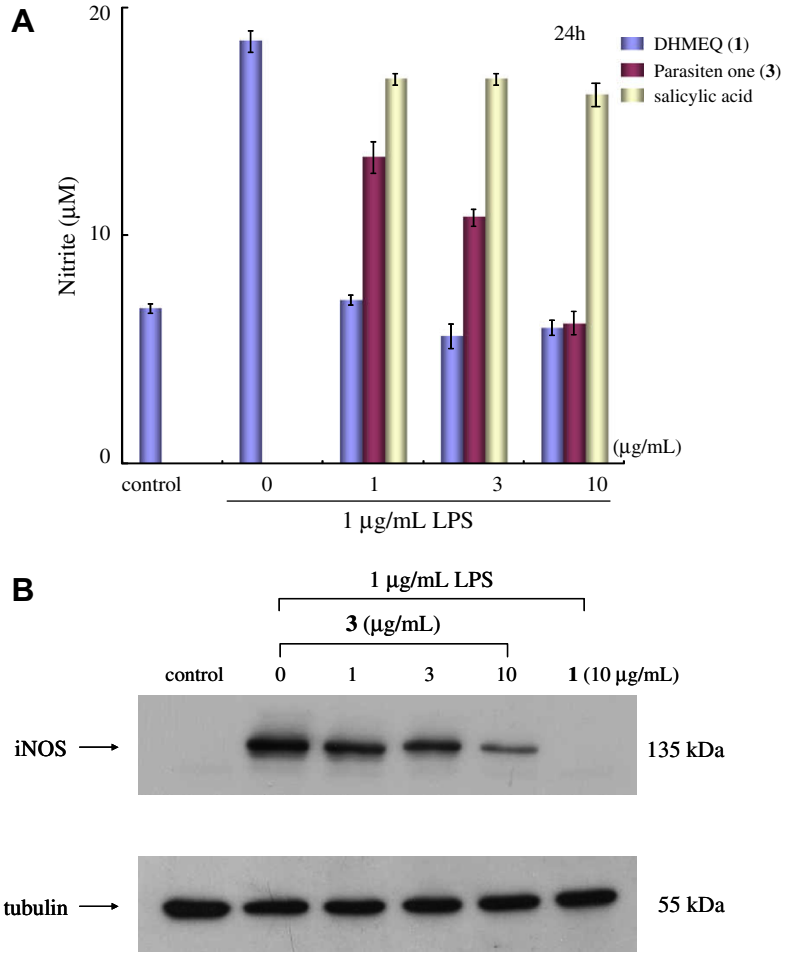

Figure 3. Inhibition of NO production and iNOS expression by parasitenone (**3**). (A) Effect on NO secretion in RAW264.7 cells. Cells were incubated without or with chemicals at various concentrations for 1 h, stimulated or not 1 μg/mL LPS for 24 h. NO secretion was assessed by Griess reaction. (B) 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. (Tublin was used as a control.)

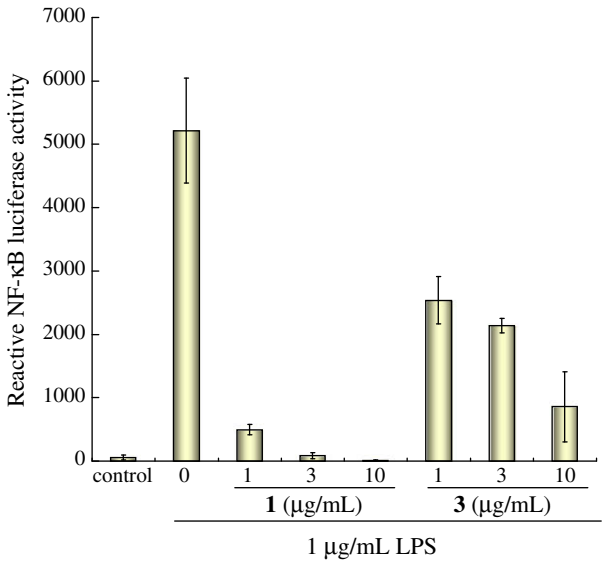

Figure 4. Effect of parasitenone (**3**) on transcription activity of NF-κB in RAW264.7 cells. Cells were transfected by diethylaminoethyl–dextran method and incubated for 24 h. Transfected cells were treated with the indicated concentrations of racemic **1** and **3** for 1 h, then stimulated with 1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

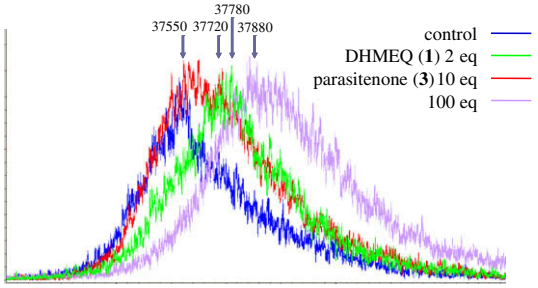

Figure 5. MALDI-TOF MS analysis of p65(1-325) with parasitenone (**3**) and DHMEQ (**1**). The p65(1-325) protein (20 μM) was treated with several equiv of racemic parasitenone (**3**) and racemic DHMEQ (**1**) for 1 h. After incubation, the proteins were used for the MALDI-TOF MS analysis.

Acknowledgments

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