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Synthesis of NF-κB Activation Inhibitors Derived from Epoxyquinomicin C

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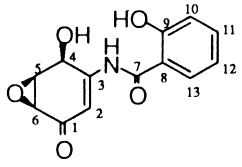
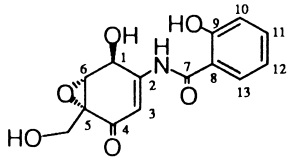
Abstract—In order to develop new inhibitors of NF-κB activation, we designed and synthesized dehydroxymethyl derivatives of epoxyquinomicin C, namely, DHM2EQ and its regioisomer DHM3EQ. These derivatives were synthesized from 2,5-dimethoxyaniline in 5 steps. Since DHM2EQ was more active and less toxic than DHM3EQ, its stereochemical configuration was determined by X-ray crystallographic analysis. Each enantiomer of the protected DHM2EQ was separated by a chiral column and deprotected. DHM2EQ inhibited TNF-α-induced activation of NF-κB in human T cell leukemia cells, and also inhibited collagen-induced arthritis in a rheumatoid model in mice.

Introduction

NF-κB is a transcription factor that mediates the expression of a variety of cellular genes regulating the inflammatory response. For example, the expression of various cytokines such as IL-1, IL-2, IL-8 and TNF-α is regulated by NF-κB. NF-κB is located in the cytoplasm along with its endogenous inhibitor, I-κB. Stimulatory signals such as TNF-α and phorbol esters induce degradation of I-κB, and NF-κB consequently activated enters the nucleus to bind to the κB site of DNA. Inhibitors of this process of NF-κB activation are likely to become new anti-inflammatory and anti-rheumatoid agents.[1] Recently, panepoxydone[2] and cycloepoxydon[3] were reported to inhibit NF-κB activation, both of which have the 4-hydroxy-5,6-epoxycyclohexenone structure. We previously isolated four novel 5,6-epoxycyclohexenone compounds named epoxyquinomicins from *Amycolatopsis* sp. MK299-95F4 as antibiotics and anti-inflammatory agents.[4,5] Epoxyquinomicin C was the simplest among them, having a 4-hydroxy-5,6-epoxycyclohexenone structure, however, it did not inhibit activation of NF-κB. Epoxyquinomicin C has an additional hydroxymethyl group compared with panepoxydone that inhibits NF-κB. Therefore, we designed and synthesized 5-dehydroxymethyl derivatives of epoxyquinomicin C.



(-)-DHM2EQ (**1**)



epoxyquinomicin C

Synthesis

The synthetic route for DHM2EQ and DHM3EQ is outlined in Scheme 1. We employed the Wipf method[6] with modifications for the preparation of the quinone monoketal structure. Commercially available 2,5-dimethoxyaniline, **3**, and acetylsalicyloyl chloride were coupled in pyridine to give salicylamide **4**, which was subsequently oxidized by iodobenzenediacetate in methanol to yield quinone monoketal **5**, in 50% yield. Epoxidation of **5** with alkaline hydrogen peroxide in aqueous THF gave epoxide **6** in 53% yield concomitant with deprotection of the phenolic acetyl group. Although the condition was strongly basic, no

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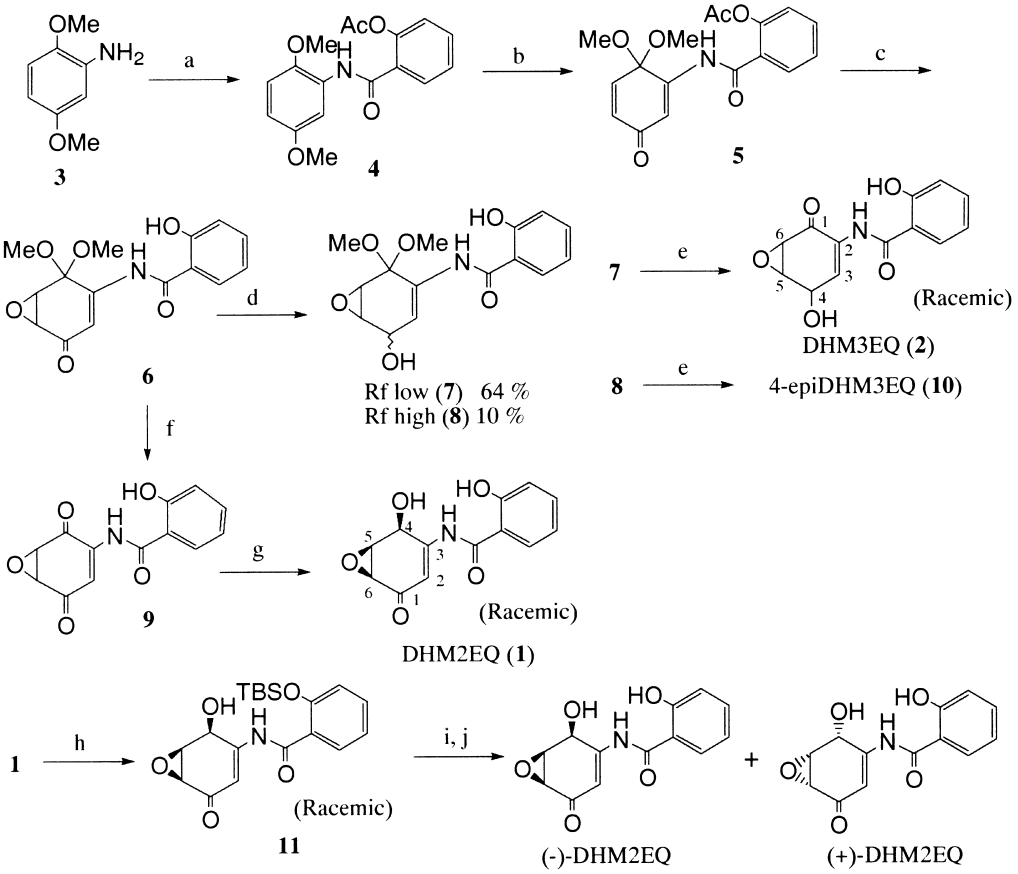

Scheme 1. Reagents and conditions: (a) acetylsalicyloyl chloride, pyridine, 0 °C, 30 min, quant.; (b) $PhI(OAc)_2$, MeOH, 0 °C, 1 h, 50%; (c) 30% H_2O_2, 1N NaOH, 0 °C, 2 h, 53%; (d) $NaBH_4$, 0 °C, 10 min; (e) *p*-TsOH, RT, 1 h; (f) *p*-TsOH, 60 °C, 1.5 h, 81%; (g) $NaBH(OAc)_3$, RT, 86%, 10 min; (h) TBDMS-Cl, *N,N*-diisopropylethylamine, 62%; (i) optical resolution; (j) HF, RT, 2 hr, 75% for (−)-**1**, 79% for (+)-**1**.

significant *N*-desalicylation was observed in **5**, unlike in the case for the acetamide reported by Wipf et al.[6] Reduction of epoxide **6** with $NaBH_4$ gave **7** in 64% yield and its epimer **8** in 10% yield after isolation. Deprotection of the major isomer **7** with *p*-TsOH at 0 °C smoothly proceeded and afforded DHM3EQ (**2**) in 74% yield. Similarly, minor product **8** was deprotected to 4-epiDHM3EQ (**12**). Dimethylketal **6** was treated with *p*-toluenesulfonic acid at 70 °C to give epoxyquinone **9** in 81% yield. Reduction of **9** with $NaBH(OAc)_3$ regioselectively occurred, and DHM2EQ (**1**) was obtained in 86% yield. The relative stereochemistry of DHM2EQ was determined by X-ray crystallographic analysis. Although the crystals of **1** were too fine, after monosilylation of **1**, satisfactory crystals of **11** for X-ray analysis were grown from the ethanol solution.

A colorless prism crystal of **11** having approximate dimensions of 0.06×0.10×0.15 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-K_α radiation. The following crystal data were obtained: empirical formula, $C_{19}H_{25}NO_5Si$; formula weight, 375.50; crystal system, triclinic; space group, $P\bar{1}$(#2); lattice parameters, $a = 9.9408(6)$Å, $b = 13.604(1)$Å, $c = 7.6298(6)$Å, $\alpha = 98.075(7)°$, $\beta = 90.118(6)°$, $\gamma = 69.577(5)°$, $V = 956.2(1)$Å^3; Z value, 2; D_{calc}, 1.304 g/cm^3; $\mu(Cu K_\alpha)$, 13.38 cm^{-1}. The reflection data were collected at a temperature of 20 ± 1 °C using the ω-2θ scan technique to a maximum 2θ value of 130.1°. Of the 3527 reflections collected, 3256 were unique. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods (SIR92)[7] and expanded using Fourier techniques.[8] The non-hydrogen atoms were refined anisotropically, whereas hydrogen atoms were not. The final cycle of full-matrix least-squares refinement was based on 2386 observed reflections ($I > 2.00\sigma(I)$) and 235 variable parameters and converged with unweighted and weighted agreement factors of $R = 0.040$ and $R_w = 0.056$. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.21 and $-0.18e^-/$Å^3, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation. From these results, orientation between the 4-hydroxyl and the epoxide in **11** was *cis* as displayed in Figure 1.

The NMR spectroscopic studies failed to determine the relative stereochemistry of **11** or that of **1** because of the lack of definitive NOE between H-4 and H-6. We did not determine the stereochemistry of **2** because we found a cytotoxicity as described bellow. Next we focused on enantiomeric separation of **1**. The optical resolution of **11** was successful by passage of **11** through a chiral stationary phase column, Daicel Chiralpak AS,

with methanol as an eluent. Figure 2 shows the HPLC profile of **11**. Twenty mg of racemic **11** was subjected to the chiral column in 20 portions to give 9.5 and 9.7 mg of the corresponding (+)- and (−)-enantiomers, respectively. The former showed optical rotation of $[\alpha]_D^{23}$ +198°(*c* 0.8 methanol); and the latter, optical rotation of $[\alpha]_D^{24}$ −201°(*c* 0.8 methanol). Enantiomeric purities of both separated enantiomers were >98% based on HPLC analyses. After desilylation of both enantiomers with HF in AcCN, (+)-DHM2EQ and (−)-DHM2EQ were obtained with optical rotation of $[\alpha]_D^{24}$ +224°(*c* 0.1 methanol) and $[\alpha]_D^{24}$ −242°(*c* 0.1 methanol), respectively.

Absolute stereochemistry was determined by comparing the circular dichroism of (+)-DHM2EQ with that of 1-epiepoxyquinomicin C, which was obtained by reduction of natural epoxyquinomicin B with $NaBH(OAc)_3$. (+)-DHM2EQ possessed one peak at 340 nm ($\Delta\epsilon = +9.54$) with a positive Cotton effect and one peak at 295 nm ($\Delta\epsilon = -6.76$) with a negative one (*c* 0.01 dioxane). While 1-epiepoxyquinomicin C showed one peak at 347 nm ($\Delta\epsilon = +12.8$) with a positive Cotton effect and one peak at 296 nm ($\Delta\epsilon = -10.3$) with a negative one (*c* 0.005 dioxane). The CD spectrum pattern of (+)-DHM2EQ was also close to that of epoxydon reported previously.[9,10] These indicate that the CD spectrum pattern of (+)-DHM2EQ is closely related to that of 1-epiepoxyquinomicin C. Therefore, the stereochemistry of C-4, C-5 and C-6 in (+)-DHM2EQ was assigned as *R*, *R*, and *R*, respectively.

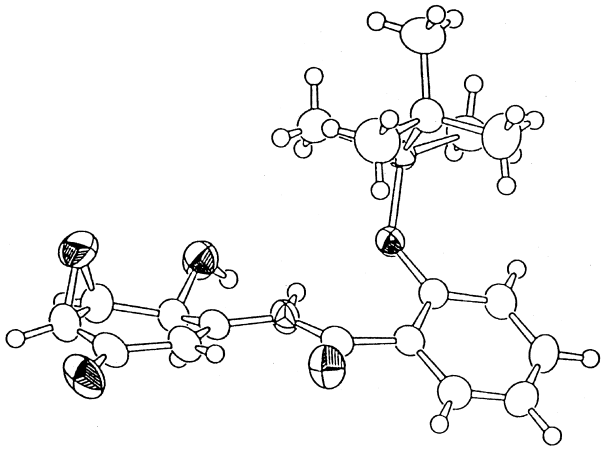

Figure 1. ORTEP drawing of **11**.

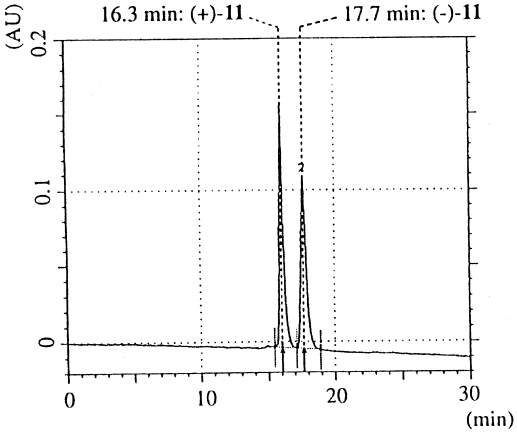

Figure 2. HPLC profile of **11**. Column: Daicel Chiralpak AS, column size: 10 mm I.D.×250 mm; eluent: methanol; flow rate: 1.0 mL/min; detection: 260 μm,.

Biological activity

Human T cell leukemia Jurkat cells were used for the assay of NF-κB activity. The cells were transfected with 2μg of DNA by the DEAE-Dextran method. The transfected cells were seeded into 12-well plates at 1×10^6/well. Chemicals dissolved in DMSO and TNF-α were added at 14 and 16 h, respectively. Six hours after this TNF-α addition, the cells were harvested and lysed; and the lysate was used for the luciferase assay with luciferin and ATP (Promega Luciferase Assay Systems). Luminescence was measured with a Lumat 9501 (Berthold). Each value was corrected by the transfection efficiency obtained from the β-galactosidase assay.

TNF-α induces degradation of I-κB and activation of NF-κB in human T cell leukemia Jurkat cells. The activity of NF-κB can be detected by transient transfection of the reporter DNA having the binding sequence for NF-κB and the luciferase gene. Figure 3 shows inhibition of TNF-α-induced NF-κB activation by the racemic DHM2EQ, DHM3EQ and the DHM3EQ epimer. DHM2EQ was more potent and less toxic than DHM3EQ. 4-epiDHM3EQ showed inhibition of NF-κB activation, but it was even more toxic than DHM3EQ. After enantiomeric separations, (−)-DHM2EQ was slightly more effective than (+)-DHM2EQ, as shown in Figure 4. Thus, DHM2EQ's were found to be unique inhibitors of NF-κB activation.

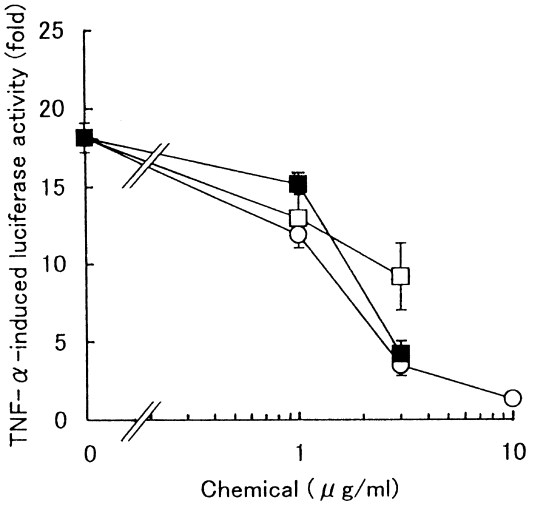

Figure 3. Effect of racemic DHM2EQ and its isomers on TNF-α-induced activation of NF-κB in Jurkat cells. The cells were treated with DHM2EQ **1** (○), DHM3EQ **2** (□) or 4-epiDHM3EQ **10** (■) with or without 20 ng/mL TNF-α. DHM3EQ and 4-epiDHM3EQ were toxic at 10 μg/ml. The values indicate the fold of luciferase activity induced by TNF-α, and are means ±SD of triplicate determinations.

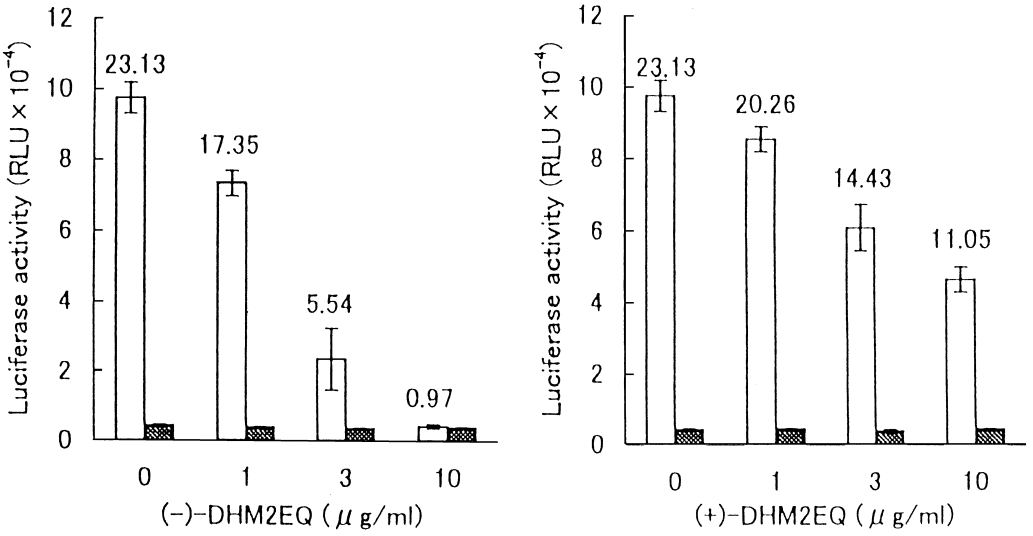

Figure 4. Inhibition of TNF-α-induced activation of NF-κB by (−)- and (+)-DHM2EQ in Jurkat cells. The cells were treated with the chemical with (white column) or without (dark column) 20 ng/ml TNF-α. The values are means ±SD of triplicate determinations. The figures indicate the fold increase in luciferase activity.

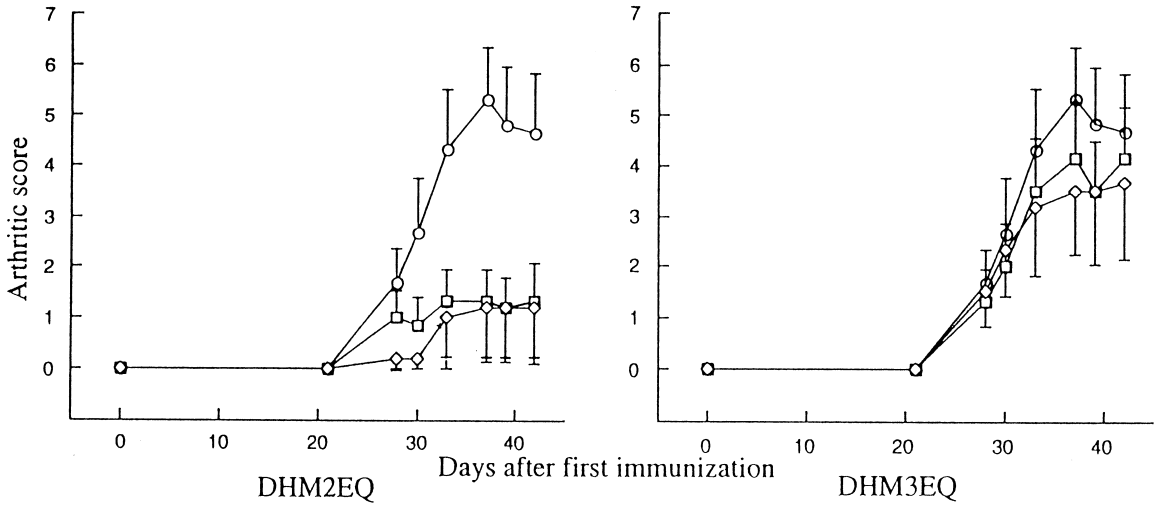

Figure 5. Inhibition of type-II collagen-induced rheumatoid arthritis in mice. Mice were immunized by intravenous administration of type-II collagen on Day 0. The collagen was intraperitoneally injected on Day 21. DHM2EQ or DHM3EQ was administered intraperitoneally at 0(○), 2(□), or 4(◇) mg/kg chemicals three times a week. The grade of rheumatoid arthritis was scored as described in ref 8.

We further examined the antiarthritic effects of DHM2EQ and DHM3EQ on type-II collagen-induced arthritis in DBA1/J mice. This animal model is widely used for evaluation of antirheumatic drugs because of its pathological similarities to human rheumatoid arthritis. The arthritis was elicited and scored as described previously.[5] As shown in Figure 5, DHM2EQ markedly inhibited type-II-collagen-induced arthritis in mice, whereas DHM3EQ tended to only slightly inhibit it. The order for the potency of the antiarthritic effect was parallel to that for the inhibitory effect on NF-κB activation, suggesting that the observed antiarthritic action by DHM2EQ may be, at least in part, due to the inhibition of NF-κB activation.

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