Preparation of radioactively labeled dehydroxymethylepoxyquinomicin, an NF-κB function inhibitor

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Abstract: Dehydroxymethylepoxyquinomicin (DHMEQ), a synthetic derivative of epoxyquinomicin C, is a potent and specific inhibitor of NF-κB in cultured cells. Tritium-labeled DHMEQ was synthesized by reduction reaction between epoxyquinone 5 and sodium borotritium. Specific radioactivity of the synthesized tritium-labeled DHMEQ was 15.45 mCi/mmol. It should be useful to study the mechanism of action and the stability of DHMEQ in cultured cells.

Introduction: Nuclear factor-κB (NF-κB) is a transcription factor typically consisting of p65 and p50. It induces inflammatory cytokines and anti-apoptotic proteins and plays a key role in immune reactions, inflammatory responses, and progression of neoplastic cells (1-4). NF-κB is often constitutively activated in human carcinomas (5). Therefore, inhibitors of NF-κB functions should be useful as anti-inflammatory and anticancer agents. Previously, we designed and synthesized an NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ) (6). It inhibited NF-κB activity in human T-cell leukemia Jurkat cells by inhibiting the nuclear translocation of p65 (7). It showed in vivo anti-inflammatory activity and no prominent toxicity in mice (6). Recently, it was found to suppress the growth of human prostate carcinoma in mice (8).
We also studied the structure-activity relationship of DHMEQ analogues as inhibitors of NF-κB functions and found that 2-hydroxyl group of the benzamide ring of DHMEQ was shown to be essential for the expression of NF-κB inhibitory activity (9). Therefore, DHMEQ may be useful as anti-inflammatory and anticancer agents.

The radioactively labeled DHMEQ could be developed as an anticancer or antiinflammatory agent. In the present study, tritium-labeled DHMEQ was synthesized to study the accumulation, stability and the mechanism of action.

![Fig. 1 Structure of dehydroxymethylepoxyquinomicin](image)

**Methodology:** Preparation of 2-hydroxy-N-(2-hydroxy-2-tritium-5-oxo-7-oxa-bicyclo[4.1.0]hept-3-en-3-yl)-benzamide (6). N-(2,5-Dioxo-7-oxa-bicyclo[4.1.0]hept-3-en-3-yl)-2-hydroxy-benzamide (5) was synthesized as described before (6). To a stirred solution of compound 5 (32.7 mg, 0.13 mmol) in methanol was added a mixture of sodium borotritium (370 mCi/mmol, 0.5 mg, 1.35 x 10^{-2} mmol) and sodium borohydride (0.5 mg, 1.35 x 10^{-2} mmol) at 0 °C. The reaction was allowed to warm to room temperature over 30 min. The reaction was quenched by concentration under reduced pressure. The residue was purified by prep-TLC with CHCl₃:MeOH (10:1) as eluent, afforded tritium-labeled DHMEQ (6) of 19.0 mg (54%). Tritium-labeled DHMEQ (6) was confirmed by its Rₘ value. It showed the Rₘ value of 0.45 (CHCl₃:MeOH = 10:1) which is identical to the value of authentic DHMEQ.

**Results, Discussion and Conclusion:** The synthetic procedures of tritium-labeled DHMEQ are shown in Figure 2. 2,5-Dimethoxyaniline (1) was coupled with acetylsalicyloyl chloride to afford compound 2. Oxidation of compound 2 with hypervalent iodine in methanol gave the quinine monoketal, compound 3. Epoxidation of compound 3 with alkaline hydrogen peroxide gave compound 4 in 80% yield. Deprotection of the acetal group of compound 4 with boron trifluoride etherate in dichloromethane produced compound 5 in 70% yield. In the final step, compound 5 was reduced by a mixture of sodium borotritium (370 mCi/mmol) and sodium borohydride to afford tritium-labeled DHMEQ (6) in 54% yield. Specific radioactivity of the synthesized tritium-labeled DHMEQ (6) was 15.45 mCi/mmol or 1.32 x 10⁸ dpm/mg.
Preparation of radioactive DHMEQ enabled us to study the accumulation and stability of DHMEQ in cultured cells.

References
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Keywords: dehydroxymethylepoxyquinomicin, DHMEQ, NF-κB