# Inhibitory effects of sulfobacin B on DNA polymerase and inflammation

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Received June 14, 2010; Accepted August 12, 2010

DOI: 10.3892/ijmm_00000522

**Abstract.** The sulfonolipid, sulfobacin B, is isolated from *Chryseobacterium* sp. and functions both as a von Willebrand factor receptor antagonist and a DNA polymerase (pol) α inhibitor. Previously, we chemically synthesized sulfobacin B by starting from L-cysteine. In this study, we investigated the inhibitory effects of chemically synthesized sulfobacin B on the activity of pols and other DNA metabolic enzymes. Sulfobacin B selectively inhibited the activity of all animal pol species: Among the pols tested, the inhibitory effect of the compound on pol λ activity was the strongest with $IC_{50}$ values of 1.6 $\mu$M. However, sulfobacin B did not influence the activity of plant or prokaryotic pols, or that of the other DNA metabolic enzymes such as primase of pol α, RNA polymerase, polynucleotide kinase or deoxyribonuclease I. As we previously found a positive relationship between pol λ inhibition and anti-inflammation, we examined whether sulfobacin B could inhibit inflammatory responses. The compound caused a marked reduction in 12-*O*-tetradecanoylphorbol-13-acetate-induced acute inflammation in the mouse ear. In a cell culture system using mouse macrophages, sulfobacin B strongly inhibited the production of tumor necrosis factor (TNF)-α and the action of nuclear factor-κB induced by lipopolysaccharide (LPS). In an *in vivo* mouse model of LPS-induced acute inflammation, the intraperitoneal injection of sulfobacin B to mice led to the suppression of serum TNF-α production. These results indicate that sulfobacin B is a potential chemotherapeutic agent for inflammation.

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*Abbreviations:* pol, DNA polymerase (E.C. 2.7.7.7); TPA, 12-*O*-tetradecanoylphorbol-13-acetate; TNF, tumor necrosis factor; NF, nuclear factor; LPS, lipopolysaccharide; TdT, terminal deoxynucleotidyl transferase; dTTP, 2'-deoxythymidine 5'-triphosphate; DMSO, dimethyl sulfoxide; IE, inhibitory effect; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; BW, body weight; BER, base excision repair; dRP, 5'-deoxyribose-5-phosphate; AP, apurinic/apyrimidinic

*Key words:* sulfobacin B, DNA polymerases, enzyme inhibitor, 12-*O*-tetradecanoylphorbol-13-acetate, lipopolysaccharide, anti-inflammation

## Introduction

Sulfobacin B was first isolated from the culture broth of *Chryseobacterium* sp. in 1995 (1), and has been shown to have bioactivity both as a von Willebrand factor receptor antagonist (1) and a DNA polymerase (pol) α inhibitor (2). Sulfobacin B is a sulfonic acid belonging to a class of unusual sphingosine relatives, and we have chemically synthesized it successfully, as previously described (3,4).

The human genome encodes at least 15 pols that conduct cellular DNA synthesis (5,6). Eukaryotic cells contain 3 replicative pols (α, δ and ε), mitochondrial pol γ, and at least 11 non-replicative pols [ß, ζ, η, θ, ι, κ, λ, $\mu$, ν, terminal deoxynucleotidyl transferase (TdT) and REV1] (5-7). Pols have a highly conserved structure, which means that on the whole, their overall catalytic subunits vary very little from species to species. A conserved structure usually indicates that the protein has an important, irreplaceable function in the cell, the maintenance of which provides evolutionary advantages. On the basis of sequence homology, eukaryotic pols can be divided into 4 main different families, termed A, B, X and Y (8). Family A includes mitochondrial pol γ, as well as pols θ and ν. Family B includes 3 replicative pols (α, δ and ε) and pol ζ. Family X comprises of pols ß, λ and $\mu$, as well as TdT,

and lastly, family Y includes pols η, ι and κ, in addition to REV1. We have been studying the selective inhibitors of each pol from natural materials for >15 years (9,10), and we have found that pol λ selective inhibitors, such as curcumin derivatives (11-13), have 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced anti-inflammatory activity (14-16).

Although tumor promoters are classified as compounds that promote tumor formation (17), they also cause inflammation and are commonly used as artificial inducers of inflammation in order to screen for anti-inflammatory agents (18). Tumor promoter-induced inflammation can be distinguished from acute inflammation, which is exudative and is accompanied by fibroblast proliferation and granulation. The tumor promoter, TPA, is frequently used to search for new types of anti-inflammatory compound. TPA not only causes inflammation, but also influences mammalian cell growth (19), suggesting that the molecular basis of the inflammation stems from the pol reaction related to cell proliferation. This relationship, however, needs to be investigated more closely.

In many inflammatory responses, the activation of nuclear factor (NF)-κB is the rate-limiting step of the inflammatory mechanism (20). The 5 members of the mammalian NF-κB family, namely p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2), exist in unstimulated cells as homodimers or heterodimers bound to the proteins of the IκB family (21). The binding of NF-κB to IκB prevents NF-κB from translocating to the nucleus, thereby maintaining NF-κB in an inactive state. NF-κB proteins are characterized by the presence of a conserved 300-amino acid Rel homology domain located in the N-terminus of the protein, and this domain is responsible for dimerization with NF-κB, interaction with IκB and binding to DNA (21). The translocated NF-κB proteins function as transcription factors and regulate the expression of various genes that encode pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-12, which have been shown to play important roles in sustaining inflammatory responses (22-24).

In this study, we investigated the inhibitory effects (IEs) of sulfobacin B, a selective pol inhibitor, on inflammatory responses *in vitro* and *in vivo*. We found that this compound suppresses NF-κB activation induced by lipopolysaccharide (LPS) in mouse macrophage cells. Moreover, we also demonstrate that sulfobacin B exerts IEs against TNF-α production in an animal model of LPS-induced acute inflammation.

## Materials and methods

*Materials*. Sulfobacin B was chemically synthesized as previously described (3,4), and its structure is shown in Fig. 1. The purification grade of synthesized sulfobacin B was >98% pure (data not shown). Nucleotides, such as [$^3$H]-deoxythymidine 5'-triphosphate (dTTP) (43 Ci/mmol), and chemically synthesized DNA template, such as poly(dA), were purchased from GE Healthcare Bio-Sciences (Little Chalfont, UK). DNA primers, such as oligo(dT)$_{18}$, were customized by Sigma (Hokkaido, Japan). LPS was purchased from Sigma (St. Louis, MO, USA). For Western blot analysis, anti-NF-κB p65 antibody and horseradish peroxidase-conjugated anti-rabbit IgG antibody (i.e. secondary antibody) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Thermo Scientific (Kanagawa, Japan), respectively. All other reagents were of analytical grade and were purchased from Nacalai Tesque Inc. (Kyoto, Japan).

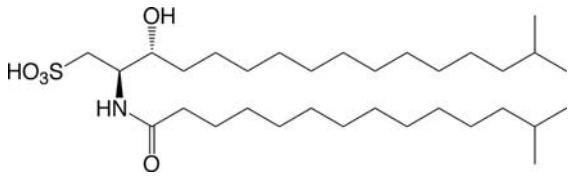


Figure 1. Structure of sulfobacin B.

*Pols and other DNA metabolic enzyme assays*. Pols from mammals, a fish (cherry salmon), an insect (fruit fly) and a plant (cauliflower) were purified, and prokaryotic pols and other DNA metabolic enzymes were purchased as described in our previous study (25). The activities of all the pols and other DNA metabolic enzymes were measured as described in our previous studies (25-27).

The components of the pol assay were poly(dA)/oligo(dT)$_{18}$ and dTTP as the DNA template-primer and as 2'-deoxyribonucleoside 5'-triphosphate (dNTP) substrate, respectively. Sulfobacin B was dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 sec. The sonicated samples (4 μl) were mixed with 16 μl of each pol enzyme (final amount, 0.05 units) in 50 mM Tris-HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM EDTA, and kept at 0˚C for 10 min. These inhibitor-enzyme mixtures (8 μl) were added to 16 μl of each standard enzyme reaction mixture [final concentration of 50 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1 mM $MgCl_2$, 15% glycerol, 10 μM poly(dA)/oligo(dT)$_{18}$ and 10 μM [$^3$H]-dTTP], and incubation was carried out at 37˚C for 60 min, except for *Taq* pol, which was incubated at 74˚C for 60 min. Activity without the inhibitor was considered to be 100%, and the activity remaining at each concentration of inhibitor was determined relative to this value. One unit of pol activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol dNTP (dTTP) into the synthetic DNA template-primer [poly(dA)/oligo(dT)$_{18}$, A/T=2/1] in 60 min at 37˚C under normal reaction conditions for each enzyme (scintillation counts: Approximately 1 pmol of incorporated radioactive nucleotides = 100 cpm) (26,27).

*Animal experiments*. All animal studies were performed according to the guidelines outlined in the 'Care and Use of Laboratory Animals' of Kobe-Gakuin University. The animals were anesthetized with pentobarbital before undergoing cervical dislocation. Male 8-week-old C57BL/6 mice that had been bred in-house with free access to food and water were used for all the experiments. All of the mice were maintained under a 12-h light/dark cycle and housed at a room temperature of 25˚C.

*Mouse TPA-induced anti-inflammatory assay*. The mouse inflammatory test was performed according to the method described by Gschwendt *et al* (28). In brief, a methanol solution of the test compound (250 or 500 μg/20 μl) was

applied to the inner part of the mouse ear. Thirty minutes after the test compound was applied, a TPA solution (0.5 μg/20 μl of acetone) was applied to the same part of the ear. Methanol, followed by TPA solution, was applied as the control to the other ear of the same mouse. After 7 h, a disk (6 mm in diameter) was obtained from the ear and weighed. The IE is presented as the ratio of the increase in weight of the ear disks: IE: {[(TPA only)-(tested compound plus TPA)] / [(TPA only)-(vehicle)] x 100}.

*Cell culture of mouse macrophages*. A mouse macrophage cell line, RAW264.7, was obtained from the American Type Culture Collection (VA, USA). The cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 4.5 g of glucose per liter plus 10% fetal calf serum, 5 mM L-glutamine, 50 units/ml penicillin and 50 units/ml streptomycin. The cells were cultured at 37˚C in standard medium in a humidified atmosphere of 5% $CO_2$/95% air.

*Investigation of cultured RAW264.7 cell growth*. A high concentration (10 mM) of sulfobacin B was dissolved in DMSO and stored. RAW264.7 cells, at ~5x$10^3$ cells per well were placed in 96-well micro-plates, and then the compound stock solution was diluted to various concentrations and applied to each well. After incubation for 24 h, the survival rate was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (29).

*Preparation of peritoneal macrophages*. The mice were injected intraperitoneally with phosphate buffered saline (PBS), and the peritoneal cavity of the mice was washed with PBS. PBS was collected and the peritoneal macrophages were separated from the PBS by centrifugation at 300 x g for 5 min.

*Measurement of TNF-α secreted from peritoneal macrophages*. The peritoneal macrophages were placed in a 12-well plate at 1x$10^5$ cells/well and incubated for 24 h. The cells were pre-treated with 0-10 μM sulfobacin B for 30 min and then stimulated with 100 ng/ml LPS. After 24 h, the cell culture medium was collected in order to measure the amount of TNF-α secreted. The concentration of TNF-α in the culture medium was quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) development system (Bay Bioscience Co., Ltd., Kobe, Japan) following the manufacturer's instructions.

*Cell treatment and preparation of nuclear and whole cellular proteins*. The RAW264.7 cells were pre-treated with 10 μM sulfobacin B for 30 min and then treated with 100 ng/ml LPS. After 30 min, the nuclear proteins and whole cell lysates were isolated from the RAW264.7 cells as described in a previous study (30). The protein concentration of the nuclear function and whole cell lysates were measured using a BCA™ Protein Assay Kit (Pierce, Rockford, IL) following the manufacturer's instructions. In brief, 25 μl of each sample or 2 mg/ml BSA solution as a standard, were added to the wells in a 96-well microplate, and then 200 μl of BCA working reagent was added to each well. The microplate was incubated at 37˚C for 30 min, and the protein concentration was determined after the measurement of the absorbance at 575 nm. The nuclear proteins and whole cell lysates were subjected to Western blotting in order to evaluate the nuclear translocation of NF-κB.

*Western blotting*. The nuclear proteins (30-50 μg protein) and whole cell lysates (50 μg proteins) were boiled in a quarter volume of sample buffer (1 M Tris-HCl, pH 7.5, 640 mM 2-mercaptoethanol, 0.2% bromophenol blue, 4% SDS and 20% glycerol) and then separated on 10% SDS polyacrylamide gels. The proteins in the gels were transferred to a PVDF membrane. The membrane was blocked with 1% skimmed milk in TBS-T (10 mM Tris-HCl, 100 mM NaCl and 0.5% Tween-20) and probed with anti-NF-κB p65 antibody (1:1000) before the reaction with the horseradish peroxidase-conjugated secondary antibody. The protein-antibody complex was detected using ChemiLumiONE (Nacalai Tesque) and an Image Reader (LAS-3000 imaging system, Fuji Photo Film, Tokyo, Japan). The intensity of each band was analyzed using ImageJ, which was developed at the National Institutes of Health.

*In vivo LPS-induced inflammatory experiment*. The mice were intraperitoneally injected with 5 mg/kg body weight (BW) of sulfobacin B dissolved in corn oil, or 200 μl of corn oil as the vehicle control. After 30 min, the mice were intraperitoneally injected with 250 μg/kg BW LPS dissolved in PBS or 200 μl of PBS as the vehicle control. After 1 h, the mice were sacrificed, and blood samples were collected. The blood serum was separated by centrifugation at 15,000 x g for 10 min at 4˚C. The TNF-α level in the serum was measured using ELISA.

*Statistical analysis*. All data are expressed as the means ± SE of at least 3 independent determinations for each experiment. Statistical significance was analyzed using the Student's t-test, and a level of probability of 0.05 was used as the criterion of significance.

## Results

*Effects of sulfobacin B on pols and other DNA metabolic enzymes*. As described previously (2), sulfobacin B acts as a pol α inhibitor, and therefore we first investigated the biochemical actions of chemically synthesized sulfobacin B *in vitro*. As described briefly in the Introduction, we succeeded in obtaining 10 eukaryotic pol species including pols α, ß, γ, δ, ε, ι, η, κ and λ, as well as TdT. However, pols ζ, θ, μ and ν, and REV1 are not yet available (Table I). Eukaryotes express at least 15 species of pols (5,6), and we are still in an era when most pols are very difficult to obtain in their purified form in a laboratory. Table I shows the IE ($IC_{50}$ value) of sulfobacin B against these various pol species. This compound inhibited the activity of all of the pols from mammals, fish and insects, and 50% inhibition was observed at a dose of 1.6-5.7 μM. The dose-response inhibition curves of sulfobacin B against human pol γ, calf pol α, and human pols λ and κ, which are representative of the A, B, X and Y families of pols, respectively, are shown in Fig. 2. Sulfobacin B showed the strongest inhibition of pol λ among the pols

Table I. $IC_{50}$ values of sulfobacin B against the activity of various DNA polymerases and other DNA metabolic enzymes.

| Enzyme | $IC_{50}$ value of sulfobacin B ($\mu$M) |
|---|---|
| Mammalian pols | |
| A family of pols | |
| Human pol γ | 4.4±0.22 |
| B family of pols | |
| Calf pol α | 2.5±0.12 |
| Human pol δ | 3.0±0.16 |
| Human pol ε | 3.1±0.17 |
| X family of pols | |
| Rat pol ß | 1.9±0.11 |
| Human pol λ | 1.6±0.09 |
| Calf TdT | 2.1±0.12 |
| Y family of pols | |
| Human pol η | 4.6±0.24 |
| Mouse pol ι | 4.5±0.22 |
| Human pol κ | 4.1±0.21 |
| Fish pols | |
| Cherry salmon pol δ | 3.5±0.19 |
| Insect pols | |
| Fruit fly pol α | 4.3±0.22 |
| Fruit fly pol δ | 5.7±0.30 |
| Fruit fly pol ε | 4.8±0.25 |
| Plant pols | |
| Cauliflower pol α | >200 |
| Prokaryotic pols | |
| *E. coli* pol I | >200 |
| *Taq* pol | >200 |
| T4 pol | >200 |
| Other DNA metabolic enzymes | |
| Calf primase of pol α | >200 |
| T7 RNA polymerase | >200 |
| T4 polynucleotide kinase | >200 |
| Bovine deoxyribonuclease I | >200 |

Sulfobacin B was incubated with each enzyme. Enzymatic activity was measured as described in Materials and methods. Enzyme activity in the absence of the compounds was taken as 100%. Data are expressed as the means ± SEM of 3 independent experiments.

investigated, with an $IC_{50}$ value of 1.6 $\mu$M. The IE of sulfobacin B on the X family of pols, such as pols ß and λ and TdT, was stronger than that on the A, B and Y families of pols (Table I).

On the contrary, sulfobacin B had no effect on plant (cauliflower) pol α or prokaryotic pols, such as *E. coli* pol I, *Taq* pol or T4 pol (Table I). The 3-dimensional structures of eukaryotic pols are likely to differ greatly from those of prokaryotic pols. Sulfobacin B did not inhibit the activity of other DNA metabolic enzymes, such as calf primase pol α, T7 RNA polymerase, T4 polynucleotide kinase, or bovine deoxyribonuclease I. These results suggest that sulfobacin B could be a selective inhibitor of animal pols, especially the X family of pols containing pol λ.

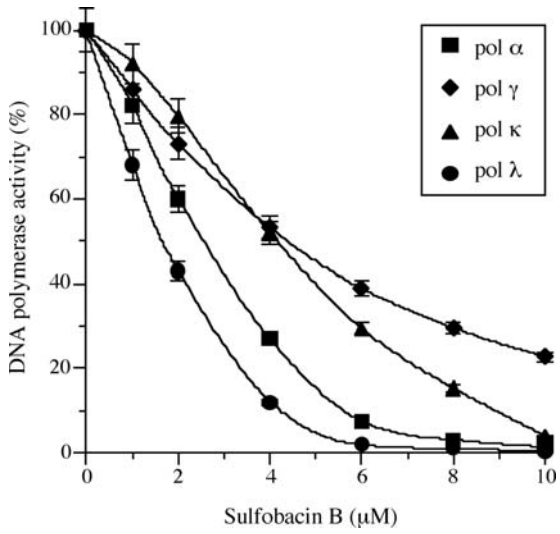


Figure 2. Mammalian pol inhibition dose-response curves of sulfobacin B. Sulfobacin B (0-10 $\mu$M) was incubated with calf pol α (B family of pols, square), human pol γ (A family of pols, diamond), human pol κ (Y family of pols, triangle) or human pol λ (X family of pols, circle) (each at 0.05 units). Pol activity was measured as described in Materials and methods. Pol activity in the absence of the compounds was taken as 100%. Data are expressed as the means ± SE of 3 independent experiments.

In order to test whether sulfobacin B is an intercalating agent that distorts DNA and subsequently inhibits enzyme activity, we measured the thermal transition of DNA in the presence or absence of this compound. The thermal transition profile of DNA was the same with or without sulfobacin B (data not shown). Therefore, the inhibition of pols by sulfobacin B is not due to DNA distortion, but due to the direct effect of this compound on the enzymes themselves.

*Effect of sulfobacin B on TPA-induced anti-inflammatory activity*. In a pol inhibitor study, we previously found that a relationship exists between pol λ inhibitors and TPA-induced acute anti-inflammatory activity (10,14,15). Therefore, using the mouse ear inflammatory test, we examined the anti-inflammatory activity of sulfobacin B. The application of TPA (0.5 $\mu$g) to the mouse ear induced edema, and the increase in weight of the ear disk 7 h after application was 241%. As shown in Table II, the pre-treatment with 250 and 500 $\mu$g of sulfobacin B suppressed the inflammation, with an IE of 48 and 81.5%, respectively. The application of sulfobacin B resulted in stronger reduction of TPA-induced inflammation than the one observed with curcumin, a major anti-inflammatory compound (31). We previously reported that curcumin has specific inhibitory activity against the mammalian pol λ, and 50% inhibition was observed at a concentration of 7 $\mu$M (10,14,15). The inhibition of pol λ was more profound by sulfobacin B than by curcumin. Thus,

Table II. Anti-inflammatory activity of sulfobacin B and curcumin in the mouse ear inflammation test.

| Compound | IE (%) | |
|---|---|---|
| | 250 $\mu$g/ear | 500 $\mu$g/ear |
| Sulfobacin B | 48.0±6.3 | 81.5±11.6 |
| Curcumin | 36.2±5.4 | 67.8±9.5 |

A sample of the test compound (250 or 500 $\mu$g) was applied to 1 mouse ear and, after 30 min, TPA (0.5 $\mu$g) was applied to both ears of the mouse. The edema was evaluated after 7 h, the IE being expressed as the percentage ratio of the edema. Data are expressed as the means ± SE of 6 mice used for each experiment.

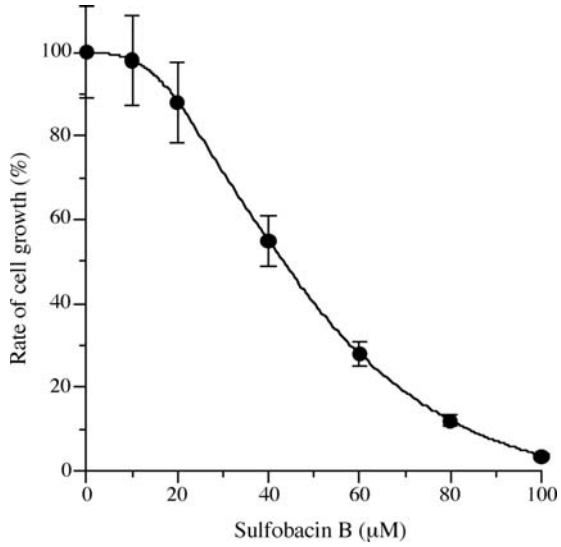


Figure 3. Macrophage cell growth inhibition dose-response curves of sulfobacin B. Various concentrations (0-100 $\mu$M) of sulfobacin B were incubated with mouse RAW264.7 macrophage cells for 24 h. The rate of cell growth was determined by MTT assay (29). Data are expressed as the means ± SE of 5 independent experiments.

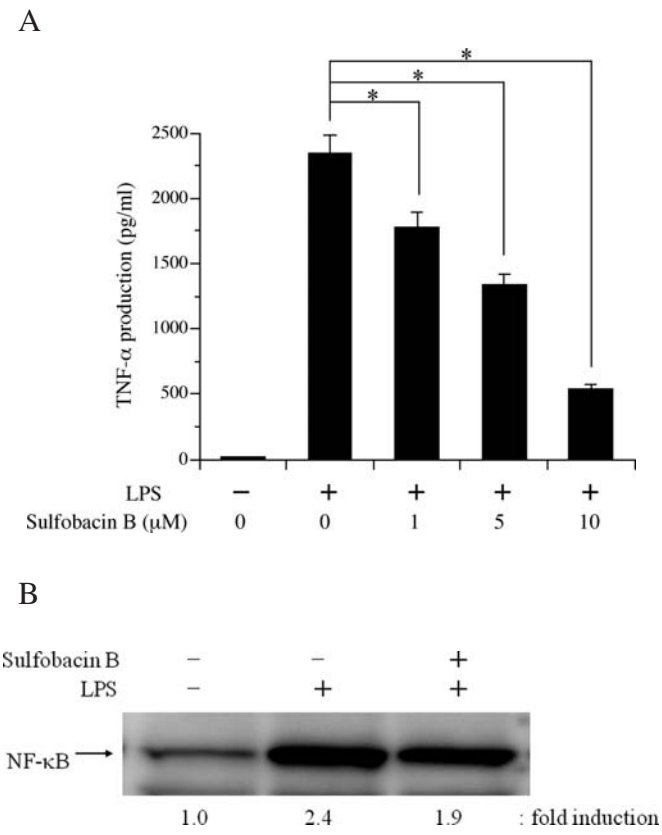


Figure 4. IEs of sulfobacin B on LPS-induced production of TNF-α in peritoneal macrophages and nuclear translocation of NF-κB in RAW264.7 cells. (A) The peritoneal macrophages were pre-treated with 0-10 $\mu$M sulfobacin B as the vehicle control for 30 min and then treated with 100 ng/ml LPS for 24 h, and the TNF-α concentration in the cell medium was measured by ELISA. Data are expressed as the means ± SE (n=4). *Significant difference according to the Student's t-test ($p<0.05$). (B) RAW264.7 cells were incubated with 10 $\mu$M sulfobacin B or DMSO (none), as the vehicle control for 30 min, and then treated with 100 ng/ml LPS for 30 min. The nuclear proteins were prepared from the cells and were subjected to Western blot analysis for the evaluation of the nuclear translocation of NF-κB p65. The intensity of each band was analyzed, and the values relative to treatment without LPS (negative control) are represented at the lower edge of the image.

these results suggest that the inhibition of the activity of pol λ has a positive correlation with anti-inflammatory activity.

*Effects of sulfobacin B on mouse macrophage cell growth*. In order to clarify the cytological effects of sulfobacin B, the influence of this compound on mouse macrophage RAW264.7 cultured cell growth was investigated, whereby the cells were incubated with sulfobacin B for 24 h. As shown in Fig. 3, sulfobacin B had no effect on the proliferation of this cell line at low concentrations up to 10 $\mu$M. However, concentrations >10 $\mu$M were found to dose-dependently suppress the growth of RAW264.7 cells, with an $LD_{50}$ value of 43.2 $\mu$M. This value is ~10-fold the $IC_{50}$ value of sulfobacin B against the activity of mammalian pols including pol λ, which is a repair and/or recombination pol (32,33), and this inhibition is mostly mediated through the function of these pols. Curcumin, which is a pol-λ-specific inhibitor, also suppressed RAW264.7 cell growth, and 50% inhibition was observed at a concentration of 62.5 $\mu$M. These results suggest that sulfobacin B could penetrate these macrophage cells and reach the nucleus, thereby inhibiting the activities of mammalian pols including pol λ.

*Inhibitory effects of sulfobacin B on LPS-induced inflammatory responses in cultured macrophage cells and peritoneal macrophages*. Next, we investigated whether sulfobacin B can inhibit both the reduction of TNF-α production in peritoneal macrophages and the nuclear translocation of NF-κB p65 induced by LPS stimulation in RAW264.7 cells. The inflammatory cytokine, TNF-α, activates the NF-κB signaling pathway by binding to the TNF-α receptor, and thereby initiating an inflammatory response, resulting in various inflammatory diseases (34). In RAW264.7 cells, the cytotoxicity of sulfobacin B at 10 $\mu$M was not observed (Fig. 3). As shown in Fig. 4A, the cells produced 2300 pg/ml of TNF-α after LSP treatment, and sulfobacin B (0-10 $\mu$M) dose-dependently suppressed this LPS-stimulated production

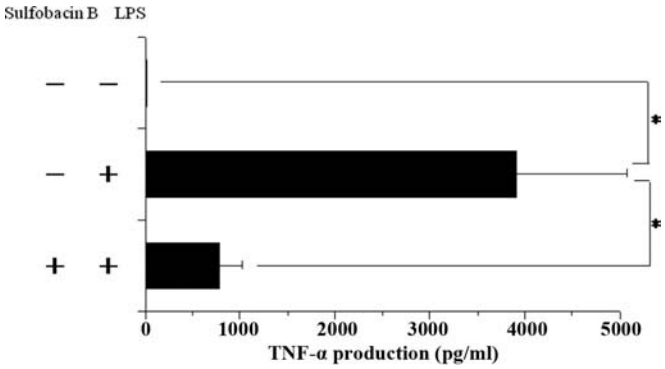


Figure 5. The inhibitory activity of sulfobacin B against LPS-induced inflammation *in vivo*. Male C57BL/6 mice were intraperitoneally injected sulfobacin B at 5 mg/kg BW or corn oil as a vehicle control. After 30 min, the mice were intraperitoneally injected with LPS at 250 $\mu$g/kg BW or saline as a vehicle control. One hour after the LPS injection, the mice were sacrificed, and the TNF-α level in serum was measured using ELISA. Data value are expressed as the mean ± SE of duplicate measurements of 3 experiments. Asterisks indicate a significant difference ($p<0.05$). Treatment with corn oil and LPS was a positive control (TNF-α level, 3,899 pg/ml), and that with corn oil and saline was a negative control (TNF-α level, 17 pg/ml).

of TNF-α. At 10 $\mu$M, the significant suppressive effect of the compound on TNF-α production was confirmed, with a reduction of 77%.

NF-κB is known to be the rate-controlling factor for inflammatory responses. Therefore, we examined the IEs of sulfobacin B on the LPS-induced nuclear translocation of NF-κB in RAW264.7 cells (Fig. 4B). By Western blot analysis, it was revealed that the amount of NF-κB nuclear translocation in RAW264.7 cells was 2.4-fold higher after LPS treatment, and that 10 $\mu$M sulfobacin B inhibited this LPS-stimulated nuclear translocation of NF-κB. These results demonstrate that sulfobacin B can suppress the nuclear translocation of NF-κB by inhibiting the production of TNF-α.

*Inhibitory effect of sulfobacin B on LPS-induced inflammation in vivo*. In order to assess the anti-inflammatory effects of sulfobacin B *in vivo*, we investigated the inhibitory activity of this compound against LPS-induced acute inflammation (Fig. 5). Treatment with 250 $\mu$g/kg BW of LPS significantly increased the serum TNF-α level, and the intraperitoneal injection of 5 mg/kg BW of sulfobacin B decreased this LPS-induced TNF-α production to 79.9%. Thus, the *in vivo* data obtained from the mouse show the same tendency as the data obtained from cultured mouse macrophage cells (Fig. 4).

## Discussion

We have shown here that synthetic sulfobacin B, a sulfonolipid (Fig. 1) selectively inhibits the activity of animal pols, especially the X family of pols such as pol λ, among the DNA metabolic enzymes tested (Table I and Fig. 2), and that this compound could be a novel therapeutic agent for chronic inflammation (Table II, Figs. 4 and 5). As reported previously, a phenolic compound, curcumin, which is a known anti-inflammatory agent, is a pol λ-specific inhibitor (10,14,15). The fact that the major molecular target of sulfobacin B is pol λ, is of great interest.

Eukaryotic cells reportedly contain 15 pol species consisting of 4 families: Namely, family A (pols γ, θ and ν), family B (pols α, δ, ε and ζ), family X (pols ß, λ, $\mu$ and TdT) and family Y (pols η, ι, κ and REV1) (5-8). Among the X family of pols, the biochemical function of pol λ is not yet clear. However, pol λ functions in a manner similar to pol ß (33). Pol ß not only is involved in the short-patch base excision repair (BER) pathway (35-38), but also plays an essential role in neural development (39). Pol λ has been found to contain 5'-deoxyribose-5-phosphate (dRP), but no apurinic/apyrimidinic (AP) lyase activity (40), and to be able to substitute for pol ß during *in vitro* BER, suggesting that pol λ also participates in BER. Northern blot analysis has indicated that transcripts of pol ß are abundantly expressed in the testis, thymus and brain in rats (41), whereas pol λ is efficiently transcribed mostly in the testis (32). Bertocci *et al* have reported that mice in which pol λ is knocked down, are not only viable and fertile, but also display a normal hypermutation pattern (42).

TPA not only causes inflammation, but also influences cell proliferation and has physiological effects on cells as it is a tumor promoter (19). Therefore, anti-inflammatory agents are expected to suppress both mammalian cell proliferation and DNA replication/repair in the nuclei in relation to the action of TPA. As pol λ is a repair-related pol (33), our finding that the molecular target of sulfobacin B is pol λ is in agreement with this expected mechanism of anti-inflammatory agents. As a result, a pol λ inhibitor could also be an inhibitor of chronic inflammation.

In this study, our results also revealed that sulfobacin B suppressed LPS-evoked inflammatory responses *in vitro* and *in vivo*. Our study is the first to demonstrate that sulfobacin B reduces NF-κB activation and TNF-α production. The molecular mechanism linking the LPS-induced inflammatory response and anti-inflammatory activity in the model of TPA-induced ear edema is not yet known. As the activated NF-κB has been observed in a model of TPA-induced ear edema (43), the anti-inflammatory effects of sulfobacin B could at least in part be dependent on the inhibition of NF-κB activation. Our study indicates that sulfobacin B is useful as an NF-κB inhibitor and could be a potent chemopreventive agent against inflammation.

In conclusion, we investigated the mode of action of the sulfobacin B inhibition of pols, which are responsible for DNA replication leading to cell proliferation and DNA repair/recombination, as well as for the relationship between the degree of the cell growth IE and anti-inflammatory activity. As a result, we found a positive relationship between the pol inhibitory and anti-inflammatory activities. The correlation between these activities could be useful as a new and convenient *in vitro* assay to screen for novel anti-inflammatory compounds.

## Acknowledgements

We are grateful for the donations of calf pol α by Dr M. Takemura of the Tokyo University of Science (Tokyo, Japan), rat pol ß, and human pols δ and ε by Dr K. Sakaguchi of the Tokyo University of Science (Chiba, Japan), human pol γ by Dr M. Suzuki of the Nagoya University School of Medicine

(Nagoya, Japan), human pols η and ι by Drs F. Hanaoka and C. Masutani of Osaka University (Osaka, Japan), human pol κ by Dr H. Ohmori of Kyoto University (Kyoto, Japan), and human pol λ by Dr O. Koiwai of the Tokyo University of Science (Chiba, Japan). This study was supported in part by the Global COE Program 'Global Center of Excellence for Education and Research on Signal Transduction Medicine in the Coming Generation' from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT) (T.A. and M.Y.), and a Grant-in-Aid for 'Academic Frontier' Project for Private Universities: Matching fund subsidy from MEXT, 2006-2010 (H.Y. and Y.M.). Y.M. acknowledges a Grant-in-Aid for Young Scientists (A) (no. 19680031) from MEXT.

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