Novel Disease-Modifying Antirheumatic Drugs. I. Synthesis and Antiarthritic Activity of 2-(4-Methylphenyl)benzothiazoles

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A series of 2-(4-methylphenyl)benzothiazoles was synthesized and evaluated using an adjuvant-induced arthritic rat model. This class of desired compounds affecting the immune response was found using hemagglutination assay. 4-Acetoxy-2-(4-methylphenyl)benzothiazole (7m), KB-2683, was most potent in the adjuvant-induced arthritic rat model and selected for further evaluation. In contrast to nonsteroidal antiinflammatory drugs, compound 7m showed no antiinflammatory or analgesic activities. It did, however, show an immunomodulatory activity in enhanced delayed type hypersensitivity.

Keywords 2-(4-methylphenyl)benzothiazole; adjuvant arthritis; hemagglutination assay; immunomodulating activity

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic articular inflammation and destruction. Nonsteroidal antiinflammatory drugs (NSAIDs), the primary line of treatment, provide symptomatic relief for acute inflammation.¹⁾ Disease-modifying antirheumatic drugs (DMARDs) that affect the immune response, such as gold salts,²⁾ D-penicillamine,³⁾ and lobenzarit,⁴⁾ retard the underlying progression of the disease.¹⁾ However, there are few drugs that exhibit a truly effective therapy for RA. Accordingly, considerable effort has gone into research seeking a new type of DMARD

with greater efficacy.

We focused on the azole skeleton of Wy-18, 251 (1),⁵⁾ SK & F86002 (2),⁶⁾ and YM-11124 (3),⁷⁾ all of which possess antiarthritic activity, and synthesized several 2-(4-methylphenyl)benzothiazoles. A hemagglutination assay was selected as the first screening to find immunologically active compounds. The compounds that affected antibody formation in this screening system were examined for their ability to inhibit the development of rat adjuvant arthritis (AA). We found that 4-acetoxy-2-(4-methylphenyl)benzothiazole (7m), KB-2683, possessed antiarthritic activity

Chart 1

in the AA model.

In this article, we describe the synthesis, the suppressive activity on antibody formation, and the antiarthritic activity of 2-(4-methylphenyl)benzothiazoles.

Chemistry Two synthetic routes as shown in Chart 1 were used for the construction of the benzothiazole nucleus: (i) oxidative cyclization of a benzothioanilide compound, and (ii) condensation of an *o*-aminothiophenol compound with *p*-toluic acid. The required benzothioanilides (**6**) were synthesized by condensation of anilines (**4**) with *p*-toluoyl chloride, followed by sulfurization⁸⁾ with Lawesson's reagent in toluene. The benzothiazoles (**7b**, **7d**, and **7f**) were prepared by oxidative cyclization of the thioamides (**6**) with $K_3Fe(CN)_6$ in aqueous KOH solution. The hydroxy analogue (**7i**) was prepared from the methyl ether (**7f**) by demethylation⁹⁾ with AlI_3 in the presence of tetra-*n*-butylammonium iodide, and **7i** was then acetylated by refluxing in Ac_2O to give the ester analogue (**7m**). The chloro analogue (**7e**) was prepared by condensation of 4-chloro-2-aminothiophenol hydrochloride with *p*-toluic acid in polyphosphoric acid (PPA).

Synthesis of the other analogues (**7a**, **7c**, **7g**—**h**, **7j**—**l**, and **7n**—**q**) has been described previously.¹⁰⁾

Results and Discussion

The test compounds were administered intraperitoneally and then were evaluated for their effects on antibody formation in mice using the hemagglutination assay.¹¹⁾ The results are shown in Table I. In this screening system, 4-hydroxy-2-(4-methylphenyl)benzothiazole (**7f**) was found to suppress antibody formation; thus its substituent effects on the benzothiazole ring in 2-(4-methylphenyl)benzothiazole were tested. 4-Substituted compounds (**7b**, **7i**, and **7m**) also showed suppressive activity. Although the 6- and 7-methoxy compounds (**7g** and **7h**) also showed some suppressive activity, these were less active than 4-substituted compounds. The 5- and 6-fluoro (**7c** and **7d**), 6-hydroxy (**7k**), and 6-acetoxy (**7o**) compounds were inactive. These results taken together suggest that substitution at the 4-position in the benzothiazole ring is important for enhancement of the suppressive activity on antibody formation in the hemagglutination assay.

The thioamides (**6b** and **6f**), which have the open benzothiazole ring, were also tested. Compounds **6b** and **6f** were less active than **7b** and **7f**, respectively. These results suggest that the benzothiazole ring system is essential for the expression of the desired activity.

Compounds (**7f**, **7i**, and **7m**) which showed the suppressive activity in the hemagglutination assay were evaluated for their inhibitory activity in the rat adjuvant arthritis model.¹²⁾ Edema of both paws was measured on day 21 after the adjuvant injection (Table II). 4-Acetoxy compound (**7m**) administered orally (100 mg/kg) showed a significant inhibitory activity against edema in both the treated and untreated paws. 4-Hydroxy compound (**7i**) inhibited edema in the untreated paw, and 4-methoxy compound (**7f**) inhibited that in the treated paw, although these results were not statistically significant. The acetoxy (**7n**—**p**) and hydroxy (**7j**—**l**) compounds were tested to evaluate the inhibitory effects against the adjuvant-induced arthritic rat model of the position of the substituents on the

TABLE I. Suppressive Effect of 2-(4-Methylphenyl)benzothiazoles on Antibody Formation in Mice

Compd.	R	Dose (mg/kg, i.p.)	Hemagglutinin titer (log 2) (mean $\pm$ S.E.)	% inhibition ^{a)}
7a	H	Control	5.20 $\pm$ 0.20	
		100	4.80 $\pm$ 0.20	7.7
7b	4-F	Control	5.20 $\pm$ 0.20	
		100	4.20 $\pm$ 0.20	19.2 ^{b)}
7c	5-F	Control	6.50 $\pm$ 0.29	
		100	6.25 $\pm$ 0.48	3.8
7d	6-F	Control	7.00 $\pm$ 0.41	
		100	6.25 $\pm$ 0.25	10.7
7e	5-Cl	Control	6.60 $\pm$ 0.24	
		100	6.20 $\pm$ 0.20	6.1
7f	4-OMe	Control	7.25 $\pm$ 0.29	
		100	4.50 $\pm$ 0.29	37.9 ^{c)}
7g	6-OMe	Control	5.20 $\pm$ 0.20	
		100	4.20 $\pm$ 0.20	19.2 ^{b)}
7h	7-OMe	Control	5.20 $\pm$ 0.20	
		100	4.20 $\pm$ 0.20	19.2 ^{b)}
7i	4-OH	Control	5.25 $\pm$ 0.48	
		100	2.50 $\pm$ 0.29	52.4 ^{b)}
7k	6-OH	Control	6.50 $\pm$ 0.29	
		100	5.75 $\pm$ 0.48	11.5
7m	4-OAc	Control	7.00 $\pm$ 0.41	
		100	3.75 $\pm$ 0.25	46.4 ^{c)}
7o	6-OAc	Control	6.50 $\pm$ 0.29	
		100	6.25 $\pm$ 0.25	3.8
7q	6-NO ₂	Control	6.50 $\pm$ 0.29	
		100	5.25 $\pm$ 0.48	19.2
6b		Control	7.00 $\pm$ 0.41	
		100	6.25 $\pm$ 0.25	10.7
6f		Control	7.00 $\pm$ 0.41	
		100	5.25 $\pm$ 1.25	25.0

a) Significantly different from control by Student's *t*-test. b) $p < 0.01$; c) $p < 0.001$.

TABLE II. Antiarthritic Activity of 2-(4-Methylphenyl)benzothiazoles

Compound	R	Dose (mg/kg, <i>p.o.</i>)	<i>n</i>	Paw edema inhibition (%) ^{a)}	
				Treated paw	Untreated paw
7f	4-OMe	100	8	26.9	3.4
7i	4-OH	100	16	8.5	25.6
7j	5-OH	100	8	−7.1	−9.4
7k	6-OH	100	8	−2.2	−7.8
7l	7-OH	100	8	9.3	10.1
7m	4-OAc	100	24	31.8 ^{c)}	32.0 ^{b)}
7n	5-OAc	100	16	12.9	4.5
7o	6-OAc	100	8	−7.6	4.4
7p	7-OAc	100	24	16.6	8.9

a) Significantly different from control by Student's *t*-test. b) $p < 0.01$; c) $p < 0.001$.

benzothiazole ring. Substitution at the 5-, 6-, or 7-position resulted in a loss of the activity. These results suggest that the substituent at the 4-position played an important role

TABLE III. Effect of Compound **7m** on the Enhanced DTH by Cyclophosphamide

Dose (mg/kg)		<i>n</i>	Footpad swelling ($\times 10^{-2}$ mm, mean $\pm$ S.E.)	% inhibition ^{b)}
7m	CY ^{a)}			
1	75	7	37 $\pm$ 5	57.1 ^{c)}
3	75	7	35 $\pm$ 1	66.7 ^{d)}
10	75	7	31 $\pm$ 3	85.7 ^{d)}
30	75	7	33 $\pm$ 2	76.2 ^{d)}
100	75	7	37 $\pm$ 4	57.1 ^{c)}
—	—	7	28 $\pm$ 2 ^{e)}	—
—	75	7	49 $\pm$ 3	—

a) Cyclophosphamide. b) Significantly different from control by Student's *t*-test. c) $p < 0.05$; d) $p < 0.01$; e) $p < 0.001$.

TABLE IV. Effect of Compound **7m** on DTH

Dose (mg/kg)	<i>n</i>	Footpad swelling ($\times 10^{-2}$ mm, mean $\pm$ S.E.)	% inhibition
1	7	59 $\pm$ 5	0.0
3	7	63 $\pm$ 6	−6.8
10	7	57 $\pm$ 6	3.4
30	7	64 $\pm$ 6	−8.5
100	7	53 $\pm$ 4	10.2
—	7	59 $\pm$ 6	—

in the expression of antiarthritic activity.

On the other hand, it was found that compound **7m** had been rapidly metabolized to the deacetylated compound **7i** after oral administration of **7m** to rats.¹³⁾ From this data, we assumed that the lower potency of **7i** than **7m** in the AA model is partly due to the lower absorption of **7i** than of **7m**.

In the series of 2-(4-methylphenyl)benzothiazoles, compound **7m** showed the most potent antiarthritic activity in the AA model, and thus was selected for further biological evaluation.

The antiinflammatory activity of compound **7m** was examined using acute antiinflammatory and analgesic models. Compound **7m**, orally administered at 100 or 300 mg/kg, had no effect in the carrageenan-induced paw edema assay.¹⁴⁾ Also, **7m** at the same administrations showed no analgesic activity in the mouse acetic acid writhing assay.¹⁵⁾ We also examined the effect of **7m** on the delayed type hypersensitivity (DTH) in a normal immune reaction and in an enhanced immune reaction in mice. At oral doses of 1–100 mg/kg, compound **7m** inhibited DTH in an enhanced immune reaction with cyclophosphamide¹⁶⁾ (Table III). The inhibition pattern of **7m** on the enhanced DTH exhibited a bell shape, and was most active at 10 mg/kg. In contrast, at similar dosage levels it had no effect on DTH in a normal immune reaction (Table IV). Compound **7m** restored the immune response in the enhanced DTH to the normal level. These results suggest that **7m**, distinct from NSAIDs, possesses an immunomodulating activity and inhibits the development of arthritis through this immunomodulatory mechanism. Studies on its more detailed immunopharmacological properties are in progress. Coded KB-2683, compound **7m**, is under preclinical development in our laboratory.

Experimental

Melting points were determined on a Yamato MP-21 melting point apparatus and were uncorrected. ¹H-Nuclear magnetic resonance (¹H-NMR) spectra were determined on Hitachi R-24B, Nihon Denshi PS-100, and Bruker AM300 spectrometers, with tetramethylsilane as an internal standard. All compounds were analyzed for C, H, and N. The results were within $\pm 0.4\%$ of the theoretical values.

General Procedure for Benzanilides. 2'-Methoxy-4-methylbenzanilide (5f) A catalytic amount of *N,N*-dimethylformamide (DMF) (approximately 0.5 ml) was added to a mixture of *p*-toluic acid (33.0 g, 0.242 mol) and thionyl chloride (80 ml). The mixture was refluxed for 3 h and evaporated *in vacuo*. The residue was dissolved in tetrahydrofuran (40 ml) and added dropwise to a solution of *o*-anisidine (29.8 g, 0.242 mol) in pyridine (190 ml) at 5 to 10°C. After being stirred at room temperature for 1 h, the reaction mixture was poured into water (2.5 l). The precipitate was collected by filtration, washed with water, and dried. Recrystallization from cyclohexane produced **5f** (50.5 g, 86%); mp 72.5–74.5°C. NMR (300 MHz, CDCl₃) δ : 2.41 (3H, s), 3.90 (3H, s), 6.90 (1H, dd), 6.95–7.10 (2H, m), 7.27 (2H, d), 7.78 (2H, d), 8.40–8.60 (2H, m). *Anal.* Calcd for C₁₅H₁₅NO₂: C, 74.67; H, 6.27; N, 5.80. Found: C, 74.67; H, 6.21; N, 5.80.

2'-Fluoro-4-methylbenzanilide (5b) Amide **5b** was prepared from *o*-fluoroaniline with *p*-toluic acid: yield, 97%; mp 120.0–122.0°C (benzene). NMR (60 MHz, CDCl₃) δ : 2.40 (3H, s), 6.8–7.4 (5H, m), 7.72 (2H, d), 7.8–8.1 (1H, br), 8.2–8.6 (1H, m). *Anal.* Calcd for C₁₄H₁₂FNO: C, 73.35; H, 5.28; N, 6.11. Found: C, 73.46; H, 5.16; N, 6.08.

4'-Fluoro-4-methylbenzanilide (5d) Amide **5d** was prepared from *p*-fluoroaniline: yield, 81%; mp 182.0–184.0°C (benzene). NMR (60 MHz, CDCl₃) δ : 2.45 (3H, s), 6.9–8.0 (9H, m). *Anal.* Calcd for C₁₄H₁₂FNO: C, 73.35; H, 5.28; N, 6.11. Found: C, 73.52; H, 5.33; N, 6.04.

General Procedure for Benzothioanilides. 2'-Methoxy-4-methylbenzothioanilide (6f) To a solution of **5f** (50.0 g, 0.207 mol) in toluene (200 ml) was added Lawesson's reagent (46.1 g, 0.114 mol). The mixture was refluxed for 1 h. The reaction mixture was cooled to about 50°C, and water (200 ml) was added. The mixture was further refluxed for 2 h. The organic layer was separated, washed with water, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure. The residue was recrystallized from methanol and then cyclohexane to give **6f** (31.9 g, 60%); mp 91.0–93.0°C. NMR (300 MHz, CDCl₃) δ : 2.39 (3H, s), 3.91 (3H, s), 6.96 (1H, d), 7.03 (1H, dd), 7.10–7.30 (3H, m), 7.77 (2H, d), 9.14 (1H, d), 9.61 (1H, brs). *Anal.* Calcd for C₁₅H₁₅NOS: C, 70.01; H, 5.87; N, 5.44. Found: C, 69.98; H, 5.92; N, 5.42.

2'-Fluoro-4-methylbenzothioanilide (6b) Thioamide **6b** was prepared from **5b**: yield, 53%; mp 124.0–126.0°C (cyclohexane). NMR (60 MHz, CDCl₃) δ : 2.35 (3H, s), 7.0–7.4 (5H, m), 7.73 (2H, d), 8.3–8.8 (1H, br), 8.8–9.3 (1H, br). *Anal.* Calcd for C₁₄H₁₂FNS: C, 68.54; H, 4.93; N, 5.71. Found: C, 68.78; H, 4.83; N, 5.71.

4'-Fluoro-4-methylbenzothioanilide (6d) Thioamide **6d** was prepared from **5d**: yield, 78%; mp 167.0–168.0°C (benzene). NMR (100 MHz, CDCl₃) δ : 2.42 (3H, s), 6.9–7.3 (4H, m), 7.4–7.9 (4H, m), 8.7–9.2 (1H, br). *Anal.* Calcd for C₁₄H₁₂FNS: C, 68.54; H, 4.93; N, 5.71. Found: C, 68.64; H, 4.96; N, 5.62.

General Procedure for Benzothiazoles. 4-Methoxy-2-(4-methylphenyl)-benzothiazole (7f) To a solution of potassium hydroxide (21.8 g, 0.389 mol) and potassium ferricyanide (64.0 g, 0.194 mol) in water (2.5 l) was added **6f** (25.0 g, 97.1 mmol) with stirring. The mixture was stirred at room temperature for 6 h. The crystals were collected by filtration, washed with water, and dried. Recrystallization from cyclohexane produced **7f** (13.9 g, 56%); mp 98.0–100.0°C. NMR (300 MHz, CDCl₃) δ : 2.41 (3H, s), 4.08 (3H, s), 6.92 (1H, d), 7.27 (2H, d), 7.31 (1H, dd), 7.48 (1H, d), 8.02 (2H, d). *Anal.* Calcd for C₁₅H₁₃NOS: C, 70.56; H, 5.13; N, 5.49. Found: C, 70.67; H, 4.94; N, 5.61.

4-Fluoro-2-(4-methylphenyl)benzothiazole (7b) Compound **7b** was prepared from **6b**: yield, 41%; mp 119.5–121.0°C (*n*-hexane). NMR (100 MHz, CDCl₃) δ : 2.42 (3H, s), 7.0–7.4 (4H, m), 7.60 (1H, dd), 7.96 (2H, d). *Anal.* Calcd for C₁₄H₁₀FNS: C, 69.11; H, 4.14; N, 5.76. Found: C, 69.01; H, 3.97; N, 5.75.

6-Fluoro-2-(4-methylphenyl)benzothiazole (7d) Compound **7d** was prepared from **6d**: yield, 20%; mp 127.0–128.5°C (*n*-hexane). NMR (300 MHz, CDCl₃) δ : 2.42 (3H, s), 7.21 (1H, ddd), 7.29 (2H, d), 7.56 (1H, dd), 7.93 (2H, d), 7.99 (1H, dd). *Anal.* Calcd for C₁₄H₁₀FNS: C, 69.11; H, 4.14; N, 5.76. Found: C, 69.30; H, 4.31; N, 5.66.

4-Hydroxy-2-(4-methylphenyl)benzothiazole (7i) Aluminum powder (1.4 g, 51.9 mmol atom weight) and iodine (10.9 g, 42.9 mmol) were added to benzene (50 ml).⁹⁾ The mixture was refluxed in a stream of nitrogen

until the iodine color disappeared. Then a solution of **7f** (11.4 g, 44.6 mmol) and tetra-*n*-butylammonium iodide (31 mg, 0.084 mmol) in benzene (100 ml) was added dropwise. The mixture was refluxed for 7 h, poured into water (200 ml), and extracted with ethyl acetate three times. The extract was dried over anhydrous magnesium sulfate, and the solvent was evaporated *in vacuo*. The residue was washed with a small amount of chloroform and recrystallized from acetonitrile to give **7i** (7.8 g, 72%): mp 163.5–165.5 °C. NMR (300 MHz, CDCl₃) δ : 2.42 (3H, s), 6.80 (1H, s), 6.97 (1H, dd), 7.20–7.30 (3H, m), 7.39 (1H, dd), 7.94 (2H, d). *Anal.* Calcd for C₁₄H₁₁NOS: C, 69.68; H, 4.60; N, 5.80. Found: C, 69.72; H, 4.64; N, 5.71.

4-Acetoxy-2-(4-methylphenyl)benzothiazole (7m) To acetic anhydride (58 ml) was added **7i** (5.8 g, 24.0 mmol). The mixture was refluxed for 3 h and evaporated *in vacuo*. The residue was washed with a small amount of cyclohexane and recrystallized from cyclohexane to produce **7m** (4.8 g, 71%): mp 110.0–113.0 °C. NMR (300 MHz, CDCl₃) δ : 2.42 (3H, s), 2.48 (3H, s), 7.20 (1H, dd), 7.28 (2H, d), 7.36 (1H, dd), 7.76 (1H, dd), 7.96 (2H, d). *Anal.* Calcd for C₁₆H₁₃NO₂S: C, 67.82; H, 4.62; N, 4.94. Found: C, 68.11; H, 4.58; N, 5.07.

5-Chloro-2-(4-methylphenyl)benzothiazole (7e) *p*-Toluic acid (9.9 g, 72.7 mmol) was added to a mixture of 4-chloro-2-aminothiophenol hydrochloride (11.0 g, 56.0 mmol) and PPA (57 g). The mixture was stirred at 180 °C for 2 h. After cooling to 100 °C, the reaction mixture was poured into water (500 ml). The precipitate was collected by filtration, washed with water, and dried. Recrystallization from cyclohexane produced **7e** (7.0 g, 48%): mp 138.0–140.0 °C. NMR (300 MHz, CDCl₃) δ : 2.42 (3H, s), 7.29 (2H, d), 7.33 (1H, dd), 7.78 (1H, d), 7.95 (2H, d), 8.02 (1H, d). *Anal.* Calcd for C₁₄H₁₀ClNS: C, 64.73; H, 3.88; N, 5.39. Found: C, 64.80; H, 3.98; N, 5.27.

Hemagglutination Assay¹¹⁾ Male mice (5 weeks old) of BALB/c Crslc strains were immunized intravenously by a single cell suspension, which contained 0.2 ml of 2×10^8 sheep red blood cells (SRBC) in phosphate-buffered saline (PBS(-), pH 7.2) solution, on day 0. For 3 consecutive days after the SRBC injection, the animals were given intraperitoneally a suspension of the test compound in 1% gum arabic solution. The sera collected on day 4 were heat-inactivated at 56 °C for 30 min. The micro-HA assay was carried out using the U-bottom microplate. Five-hundredths ml of SRBC suspension (containing 2×10^8 in PBS(-)) was mixed with an equal volume of two-fold dilutions of serum in each well of the microplate, and incubated at 37 °C for 60 min (diluent and SRBC alone served as the control in all tests). Antibody titer was expressed as a reciprocal of the highest dilution giving a definite positive agglutination.

Adjuvant-Induced Arthritis in Rats¹²⁾ Arthritis was induced in male Fischer 344 rats at 8 weeks of age by injecting a heat-killed *Mycobacterium butyricum*. Each animal was given 0.1 ml of a 6 mg/ml adjuvant suspension in paraffin oil by subcutaneous injection into the footpad of the right hind paw. Animals were orally given a suspension of the test compound in 1% gum arabic solution 6 days a week for 3 weeks starting immediately after the adjuvant injection. The volumes of both hind paws were measured plethysmographically by displacement of water on day 21 after the adjuvant injection. The results were expressed as percent of inhibition of paw swelling in the drug-treated group compared to the vehicle-treated arthritic control.

Effect on Delayed-Type Hypersensitivity a) Effect on a normal immune reaction. BALB/c male mice (8 weeks old) were sensitized by injection into the tail vein with 0.2 ml of 5×10^6 /ml SRBC. Three days after the sensitization, 0.05 ml of 8×10^9 /ml SRBC was injected into the footpad of the right hind paw for elicitation of DTH. Footpad thickness was measured immediately before and 24 h after the elicitation with a dial thickness gauge. The level of DTH was expressed as footpad swelling (mm) at 24 h. The inhibition (%) of footpad swelling was calculated from the following equation.

The test compound was dissolved or suspended in a 1% gum arabic solution, and administered orally once a day over a period of 7 d starting from four days before the sensitization. A 1% gum arabic solution was administered to a control group instead of the test compound.

$$\text{footpad swelling inhibition (\%)} = \left(1 - \frac{T_{\text{exp}}}{T_{\text{cont}}}\right) \times 100$$

T_{exp} : mean of the footpad swelling in mice treated with the test compound.

T_{cont} : mean of the footpad swelling in mice of the control group.

b) Effect on enhanced immune reaction. Cyclophosphamide (75 mg/kg) was administered intraperitoneally to BALB/c male mice (8 weeks old), and four days later the mice were sensitized by injection into the tail vein with 0.2 ml of 5×10^7 /ml SRBC. Three days after the sensitization, 0.05 ml of 8×10^9 /ml SRBC was injected into the footpad of the right hind paw for elicitation of DTH. Twenty four hours later, footpad swelling (mm) was measured in the same way as in a) above, and the inhibition (%) of footpad swelling was calculated by the equation below.

The test compound was dissolved or suspended in a 1% gum arabic solution, and administered orally once a day over a period of 7 d starting from 4 d before the sensitization (immediately after administration of cyclophosphamide). A 1% gum arabic solution was administered to a control group instead of the test compound. A group administered physiological saline instead of cyclophosphamide and 1% gum arabic solution instead of test compound, respectively, served as a normal group.

$$\text{footpad swelling inhibition (\%)} = \left(\frac{T_{\text{cont}} - T_{\text{exp}}}{T_{\text{cont}} - T_{\text{nor}}}\right) \times 100$$

T_{cont} : mean of the footpad swelling in mice of the control group.

T_{exp} : mean of the footpad swelling in mice treated with the test compound.

T_{nor} : mean of the footpad swelling in mice of the normal group.

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