Studies on Disease-Modifying Antirheumatic Drugs. II.[1] Synthesis and Activity of the Metabolites of Ethyl 4-(3,4-Dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (TAK-603)

Atsuo BABA,* Haruhiko MAKINO, Yoshikazu OHTA, and Takashi SOHDA

Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532-8686, Japan. Received February 10, 1998; accepted March 27, 1998

The metabolites of ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (1, TAK-603), which is under clinical evaluation as a new type of disease-modifying antirheumatic drug (DMARD), were prepared to confirm their structures and to study their pharmacological properties. Of the metabolites identified, the 4-(4-hydroxy-3-methoxyphenyl) derivative (2c, M-I) was found to have an anti-inflammatory effect in an adjuvant arthritic rat model, although its potency in this model was slightly lower than that of the parent compound.

Key words TAK-603; DMARD; metabolite; 4-phenylquinoline; anti-inflammatory effect

In a previous paper,[1] we reported a novel quinoline derivative, ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**1**, TAK-603, Fig. 1), and its potent anti-inflammatory effect. TAK-603 is under clinical evaluation as a new type of disease-modifying antirheumatic drug (DMARD)[2] characterized by an inhibitory effect on bone resorption and a protective effect against cartilage breakdown.[3] In preclinical and clinical studies, the metabolic fate[4] and mechanism of action[5] of TAK-603 have been studied in several animal species and in humans. Analytical evaluation using high performance liquid chromatography (HPLC)–mass spectrometry (LC-MS) suggested a monodemethylated structure for two of the three metabolites (M-I, M-III) of TAK-603 and that the other metabolite (M-II) is a carboxylic acid derivative.[6] Thus, four monodemethylated compounds **2a—d** and the carboxylic acid derivative **3** (Fig. 1) were prepared in order to confirm the structures of the metabolites and to study their pharmacological properties.

Chemistry The carboxylic acid derivative **3** was prepared by alkaline hydrolysis of **1** (see Experimental).

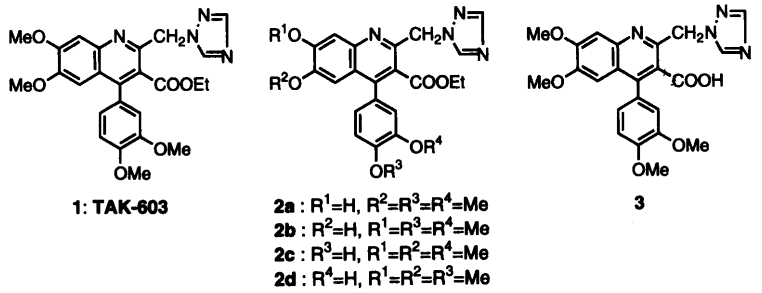

Fig. 1

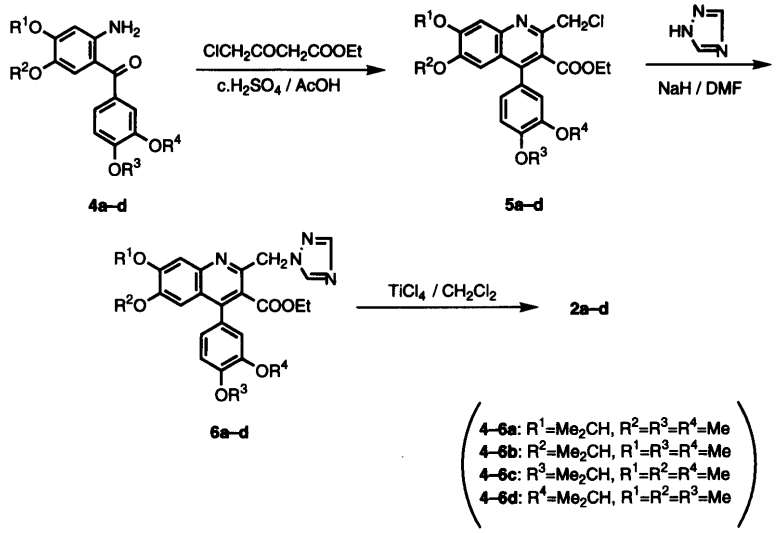

Chart 1

* To whom correspondence should be addressed.

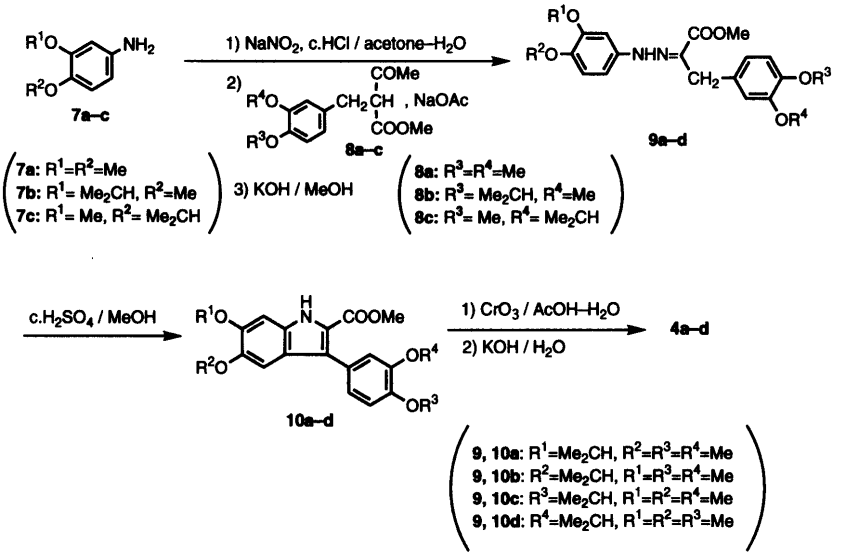

Chart 2

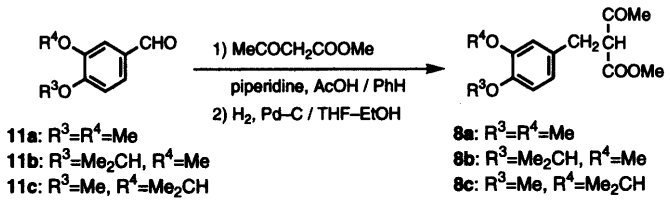

Chart 3

The mono-demethylated compounds **2** were synthesized starting from 2-aminobenzophenones **4** bearing an isopropoxy moiety (Chart 1). Treatment of **4** using the same procedure as for preparation of **1**[1] gave 2-(1,2,4-triazol-1-ylmethyl)quinolines **6**. Conversion of the isopropoxy moiety into the hydroxy moiety was effected by $TiCl_4$ to yield the corresponding phenolic compounds **2**.[7]

The requisite 2-aminobenzophenones **4** were obtained *via* indole intermediates as shown in Chart 2. Diazo coupling[8] of the diazonium salts obtained from the anilines **7** with the keto esters **8** followed by hydrolysis gave hydrazones **9**, which were converted to the indoles **10** by Fisher's method.[9] Oxidative cleavage of the C2–C3 position of the indole ring and successive hydrolysis yielded the 2-aminobenzophenones **4**.[10] The keto esters **8** were synthesized by condensation of aldehydes **11** with methyl acetoacetate followed by catalytic hydrogenation (Chart 3).

Biological Results The metabolites of TAK-603 were confirmed to be **2c** (M-I), **3** (M-II) and **2a** (M-III) by direct comparison with the authentic compounds using HPLC.[6]

The anti-inflammatory activities of the compounds prepared were evaluated using an adjuvant arthritic rat model (Table 1).[11] Although somewhat less potent than TAK-603, compound **2c** (M-I) was active, while the other mono-demethylated compound **2a** (M-III) and carboxylic acid **3** (M-II) exhibited reduced activities. *In vitro*, **2c** suppressed mitogen-induced mouse spleen cell proliferation in a dose-dependent manner, suggesting that it has a biological profile similar to that of the parent compound (Table 2). Since **2c** is abundant in rat and human plasma,[4] these findings suggest that the metabolite contributes, at

Table 1. Anti-inflammatory Effects of **2a**, **c** and **3** in Adjuvant Arthritic Rat Model (*p.o.* 14 d)

Compound	Dose (mg/kg)	Paw volume (% inhibition)
2a (M-III)	12.5	23
2c (M-I)	12.5	33**
3 (M-II)	25.0	<15
1 (TAK-603)	12.5	65**

Statistically significant at $^{**}p<0.01$ by Dunnet's test.

Table 2. Suppressive Activities of **1** and **2c** to LPS- and ConA-Induced Proliferation of Mouse Spleen Cells

	Conc. μM	LPS		Con A	
		dpm	% inh.	dpm	% inh.
Medium		30424±1490		52891±1745	
1 (TAK-603)[a]	0.3	23265±367**	24	46120±2427*	13
	1	18717±716**	38	38003±1740**	28
	3	14460±825**	52	25985±639**	51
	10	8359±210**	73	11351±628**	79
		IC_{50}: 2.2 μM		IC_{50}: 2.6 μM	
2c (M-I)	0.3	22215±414**	27	52709±2496	0
	1	16785±745**	45	38500±1865**	27
	3	11346±749**	63	28956±873**	45
	10	7122±454**	77	13267±758**	75
		IC_{50}: 1.4 μM		IC_{50}: 3.2 μM	

Data are the mean±S.E. values of triplicate experiments.
Statistically significant at $^{*}p<0.05$, $^{**}p<0.01$ *vs.* medium control by Dunnet's test.
a) Data concerning **1** is cited from Ref. 12.

least in part, to the pharmacological effects of **1**.

Experimental

Chemistry Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Elemental analyses (C, H, N) was carried out by the Analytical Department of Takeda Chemical Industries, Ltd. ^{1}H-NMR spectra of deuteriochloroform or dimethyl sulfoxide (DMSO)-d_6 solutions (internal standard tetramethylsilane (TMS), δ 0) were recorded on a Varian Gemini-200 spectrometer. Infrared (IR) spectra were recorded on a Hitachi IR-215 spectrometer. All compounds exhibited ^{1}H-NMR, IR, and analytical data consistent with the proposed structures. Column chromatography was done with E. Merck Silica gel 60 (0.063—0.200 mm).

General Procedure for Ethyl 2-Chloromethyl-4-phenylquinoline-3-carboxylate Derivatives 5. Ethyl 2-Chloromethyl-4-(3,4-dimethoxyphenyl)-7-isopropoxy-6-methoxyquinoline-3-carboxylate (5a) A mixture of **4a** (22.4 g, 64.8 mmol), ethyl 4-chloroacetoacetate (11.7 g, 71.3 mmol), concentrated H_2SO_4 (636 mg, 6.5 mmol) and acetic acid (200 ml) was stirred at 90 °C for 2.5 h and concentrated *in vacuo*. The residue was alkalinized with 2 N aqueous NaOH and extracted with $CHCl_3$. The extract was washed successively with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (350 g) with $CHCl_3$–Et_2O (10 : 1) to give crystals. Recrystallization from EtOH gave **5a** as colorless prisms (17.0 g, 55%), mp 150—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, t, J=7.2 Hz), 1.49 (6H, d, J=6.0 Hz), 3.78 (3H, s), 3.87 (3H, s), 3.97 (3H, s), 4.09 (2H, q, J=7.2 Hz), 4.82 (1H, septet, J=6.0 Hz), 4.92 (1H, d, J=11.4 Hz), 4.99 (1H, d, J=11.4 Hz), 6.90—7.02 (4H, m), 7.44 (1H, s). IR (KBr) ν: 1720 cm^{-1}. *Anal.* Calcd for $C_{25}H_{28}ClNO_6$: C, 63.36; H, 5.95; N, 2.96. Found: C, 63.32; H, 6.07; N, 2.78.

Ethyl 2-Chloromethyl-4-(3,4-dimethoxyphenyl)-6-isopropoxy-7-methoxyquinoline-3-carboxylate (**5b**): Colorless prisms (yield: 67%), mp 134—135 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, t, J=7.0 Hz), 1.34 (6H, d, J=6.2 Hz), 3.87 (3H, s), 3.98 (3H, s), 4.03 (3H, s), 4.09 (2H, q, J=7.0 Hz), 4.44 (1H, septet, J=6.2 Hz), 4.94 (1H, d, J=11.2 Hz), 5.00 (1H, d, J=11.2 Hz), 6.87—7.02 (4H, m), 7.45 (1H, s). IR (KBr) ν: 1719 cm^{-1}. *Anal.* Calcd for $C_{25}H_{28}ClNO_6$: C, 63.36; H, 5.95; N, 2.96. Found: C, 63.54; H, 5.77; N, 3.12.

Ethyl 2-Chloromethyl-4-(4-isopropoxy-3-methoxyphenyl)-6,7-dimethoxyquinoline-3-carboxylate (**5c**): Colorless prisms (yield: 48%), mp 149—150 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, t, J=7.2 Hz), 1.42 (3H, d, J=5.8 Hz), 1.45 (3H, d, J=5.8 Hz), 3.80 (3H, s), 3.85 (3H, s), 4.05 (3H, s), 4.08 (2H, q, J=7.2 Hz), 4.64 (1H, septet, J=5.8 Hz), 4.93 (1H, d, J=11.0 Hz), 5.00 (1H, d, J=11.0 Hz), 6.86—7.04 (4H, m), 7.46 (1H, s). IR (KBr) ν: 1710 cm^{-1}. *Anal.* Calcd for $C_{25}H_{28}ClNO_6$: C, 63.36; H, 5.95; N, 2.96. Found: C, 63.40; H, 5.72; N, 2.77.

Ethyl 2-Chloromethyl-4-(3-isopropoxy-4-methoxyphenyl)-6,7-dimethoxyquinoline-3-carboxylate (**5d**): Colorless prisms (yield: 50%), mp 126—127 °C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, J=7.2 Hz), 1.38 (6H, d, J=6.2 Hz), 3.79 (3H, s), 3.94 (3H, s), 4.05 (3H, s), 4.08 (2H, q, J=7.2 Hz), 4.52 (1H, septet, J=6.2 Hz), 4.92 (1H, d, J=11.2 Hz), 4.99 (1H, d, J=11.2 Hz), 6.83—7.01 (4H, m), 7.45 (1H, s). IR (KBr) ν: 1718 cm^{-1}. *Anal.* Calcd for $C_{25}H_{28}ClNO_6$: C, 63.36; H, 5.95; N, 2.96. Found: C, 63.18; H, 6.03; N, 2.68.

General Procedure for Ethyl 4-Phenyl-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate Derivatives 6. Ethyl 4-(3,4-Dimethoxyphenyl)-7-isopropoxy-6-methoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (6a) A solution of 1*H*-1,2,4-triazole (2.56 g, 37.1 mmol) in *N*,*N*-dimethylformamide (DMF) (150 ml) was treated with NaH (60% in oil, 1.62 g, 40.5 mmol) at room temperature for 15 min, and then **5a** (16.0 g, 33.7 mmol) was added. The mixture was stirred at 80 °C for 45 min, poured into H_2O, and extracted with AcOEt. The extract was washed successively with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (400 g) with $CHCl_3$–MeOH (30 : 1) to give crystals. Recrystallization from EtOH gave **5a** as colorless prisms (9.64 g, 56%), mp: 151—153 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.2 Hz), 1.49 (6H, d, J=6.0 Hz), 3.77 (3H, s), 3.86 (3H, s), 3.94 (2H, q, J=7.2 Hz), 3.96 (3H, s), 4.81 (1H, septet, J=6.0 Hz), 5.73 (2H, s), 6.84—7.01 (4H, m), 7.40 (1H, s), 7.93 (1H, s), 8.26 (1H, s). IR (KBr) ν: 1697 cm^{-1}. *Anal.* Calcd for $C_{27}H_{30}N_4O_6$: C, 64.02; H, 5.97; N, 11.06. Found: C, 63.89; H, 6.02; N, 10.82.

Ethyl 4-(3,4-Dimethoxyphenyl)-6-isopropoxy-7-methoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**6b**): Colorless prisms (78%), mp 99—100 °C (Et_2O–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.2 Hz), 1.33 (6H, d, J=6.0 Hz), 3.85 (3H, s), 3.93 (2H, q, J=7.2 Hz), 3.96 (3H, s), 4.02 (3H, s), 4.43 (1H, septet, J=6.0 Hz), 5.68 (1H, d, J=14.8 Hz), 5.77 (1H, d, J=14.8 Hz), 6.82—7.01 (4H, m), 7.41 (1H, s), 7.93 (1H, s), 8.27 (1H, s). IR (KBr) ν: 1716 cm^{-1}. *Anal.* Calcd for $C_{27}H_{30}N_4O_6$: C, 64.02; H, 5.97; N, 11.06. Found: C, 63.75; H, 5.91; N, 10.95.

Ethyl 4-(4-Isopropoxy-3-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**6c**): Colorless prisms (75%), mp 165—166 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t, J=7.4 Hz), 1.41 (3H, d, J=6.4 Hz), 1.44 (3H, d, J=6.4 Hz), 3.80 (3H, s), 3.84 (3H, s), 3.95 (2H, q, J=7.4 Hz), 4.05 (3H, s), 4.63 (1H, septet, J=6.4 Hz), 5.74 (2H, s), 6.83—6.90 (2H, m), 6.95 (1H, s), 7.00 (1H, d, J=8.0 Hz), 7.42 (1H, s), 7.94 (1H s), 8.28 (1H, s). IR (KBr) ν: 1700 cm^{-1}. *Anal.* Calcd for $C_{27}H_{30}N_4O_6$: C, 64.02; H, 5.97; N, 11.06. Found: C, 63.97; H, 5.90; N, 10.79.

Ethyl 4-(3-Isopropoxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**6d**): Colorless prisms (65%), mp 186—187 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t, J=7.2 Hz), 1.37 (6H, d, J=6.2 Hz), 3.79 (3H, s), 3.93 (3H, s), 3.95 (2H, q, J=7.2 Hz), 4.05 (3H s), 4.51 (1H, septet, J=6.2 Hz), 5.74 (2H, s), 6.88—7.01 (4H, m), 7.42 (1H, s), 7.94 (1H, s). 8.28 (1H, s). IR (KBr) ν: 1716 cm^{-1}. *Anal.* Calcd for $C_{27}H_{30}N_4O_6$: C, 64.02; H, 5.97; N, 11.06. Found: C, 63.80; H, 5.68; N, 10.96.

General Procedure for Phenolic Quinoline Derivaties 2. Ethyl 4-(3,4-Dimethoxyphenyl)-7-hydroxy-6-methoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (2a) A solution of $TiCl_4$ (20.2 g, 0.11 mol) in CH_2Cl_2 (15 ml) was added dropwise to a stirred and ice-cooled solution of **6a** (9.0 g, 0.018 mol) in CH_2Cl_2 (200 ml). The mixture was stirred at room temperature for 7 h, poured into H_2O, and extracted with $CHCl_3$. The aqueous layer was neutralized with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were washed successively with saturated aqueous $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was chromatographed on SiO_2 (150 g) with $CHCl_3$–AcOEt (5 : 1) to give crystals. Recrystallization from AcOEt–hexane gave **2a** as colorless prisms (4.5 g, 55%), mp 211—213 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.2 Hz), 3.84 (3H, s), 3.86 (3H, s), 3.95 (2H, q, J=7.2 Hz), 3.97 (3H, s), 5.73 (2H, s), 6.00 (1H, br s), 6.84—7.02 (4H, m), 7.52 (1H, s), 7.94 (1H, s), 8.30 (1H, s). IR (KBr) ν: 3300, 1705 cm^{-1}. *Anal.* Calcd for $C_{24}H_{24}N_4O_6$: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.87; H, 5.22; N, 12.01.

Ethyl 4-(3,4-Dimethoxyphenyl)-6-hydroxy-7-methoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**2b**): Colorless prisms (79%), mp 215—216 °C ($CHCl_3$–MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t, J=7.0 Hz), 3.85 (3H, s), 3.94 (2H, q, J=7.0 Hz), 3.95 (3H, s), 4.07 (3H, s), 5.73 (2H, s), 6.20 (1H, br s), 6.82—6.98 (3H, m), 7.08 (1H, s), 7.42 (1H, s), 7.93 (1H, s), 8.27 (1H, s). IR (KBr) ν: 3398, 1720 cm^{-1}. *Anal.* Calcd for $C_{24}H_{24}N_4O_6$: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.90; H, 5.06; N, 12.06.

Ethyl 4-(4-Hydroxy-3-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**2c**): Colorless needles (89%), mp 176—178 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.4 Hz), 3.80 (3H, s), 3.88 (3H, s), 3.91 (2H, q, J=7.4 Hz), 4.05 (3H, s), 5.73 (2H, s), 5.83 (1H, br s), 6.81—6.90 (2H, m), 6.93 (1H, s), 7.04 (1H, d, J=8.2 Hz), 7.41 (1H, s), 7.94 (1H, s), 8.28 (1H, s). IR (KBr) ν: 3400, 1730 cm^{-1}. *Anal.* Calcd for $C_{24}H_{24}N_4O_6$: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.77; H, 5.36; N, 11.93.

Ethyl 4-(3-Hydroxy-4-methoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate (**2d**): Colorless needles (62%), mp 230—231 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=7.0 Hz), 3.80 (3H, s), 3.97 (3H, s), 3.98 (2H, q, J=7.0 Hz), 4.04 (3H, s), 5.73 (2H, s), 5.88 (1H, br s), 6.80 (1H, dd, J=8.4, 2.0 Hz), 6.90—6.97 (3H, m), 7.40 (1H, s), 7.94 (1H, s), 8.28 (1H, s). IR (KBr) ν: 3400, 1718 cm^{-1}. *Anal.* Calcd for $C_{24}H_{24}N_4O_6$: C, 62.06; H, 5.21; N, 12.06. Found: C, 61.79; H, 5.26; N, 11.98.

General Procedure for 3-Phenyl-2-(phenylhydrazono)propionic Acid Methyl Ester Derivatives 9. Methyl 3-(3,4-Dimethoxyphenyl)-2-[(3-isopropoxy-4-methoxyphenyl)hydrazono]propionate (9a) 1) A solution of $NaNO_2$ (25.8 g, 0.37 mol) in H_2O (80 ml) was added to a stirred mixture of **7b** (67.8 g, 0.37 mol), concentrated HCl (93.5 ml, 1.12 mol), acetone (450 ml) and H_2O (70 ml) at 0—5 °C, and the mixture was stirred at the same temperature for 1 h. 2) **8a** (104.6 g, 0.39 mol) was added followed by NaOAc (98.2 g, 1.20 mol). After stirring at room temperature

for 2 h, the reaction mixture was poured into H_2O and extracted with $CHCl_3$. The extract was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. 3) To a stirred and ice-cooled solution of the residue obtained above in MeOH (350 ml) was added a solution of KOH (21.9 g, 0.39 mol) in MeOH (150 ml), and the mixture was stirred at the same temperature for 1 h. The precipitated crystals were collected by filtration. Recrystallization from MeOH gave **9a** as colorless prisms (98.4 g, 63%): mp 134—135 °C; ^{1}H-NMR ($CDCl_3$) δ: 1.36 (6H, d, J=6.2 Hz), 3.79 (3H, s), 3.84 (3H, s), 3.86 (3H, s), 3.90 (3H, s), 3.97 (2H, s), 4.55 (1H, septet, J=6.2 Hz), 6.46 (1H, dd, J=8.6, 2.6 Hz), 6.70—6.82 (5H, m), 7.92 (1H, br s). IR (KBr) ν: 1700 cm^{-1}. *Anal.* Calcd for $C_{22}H_{28}N_2O_6$: C, 63.45; H, 6.78; N, 6.73. Found: C, 63.72; H, 6.64; N, 6.75.

Methyl 3-(3,4-Dimethoxyphenyl)-2-[(4-isopropoxy-3-methoxyphenyl)hydrazono]propionate (**9b**): Colorless prisms (yield: 64%), mp 123—124 °C (MeOH). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (6H, d, J=5.8 Hz), 3.84 (3H, s), 3.86 (6H, s), 3.90 (3H, s), 3.98 (2H, s), 4.37 (1H, septet, J=5.8 Hz), 6.38 (1H, dd, J=8.8, 2.2 Hz), 6.70—6.85 (5H, m), 7.96 (1H, br s). IR (KBr) ν: 1718 cm^{-1}. *Anal.* Calcd for $C_{22}H_{28}N_2O_6$: C, 63.45; H, 6.77; N, 6.73. Found: C, 63.63; H, 6.80; N, 6.85.

Methyl 2-[(3,4-Dimethoxyphenyl)hydrazono]-3-(4-isopropoxy-3-methoxyphenyl)propionate (**9c**): Colorless prisms (yield: 51%), mp 93—94 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (6H, d, J=6.2 Hz), 3.81 (3H, s), 3.82 (3H, s), 3.88 (3H, s), 3.90 (3H, s), 3.98 (2H, s), 4.48 (1H, septet, J=6.2 Hz), 6.39 (1H, dd, J=8.6, 2.0 Hz), 6.68—6.87 (5H, m), 7.96 (1H, br s). IR (KBr) ν: 1718 cm^{-1}. *Anal.* Calcd for $C_{22}H_{28}N_2O_6$: C, 63.45; H, 6.77; N, 6.73. Found: C, 63.27; H, 6.80; N, 6.56.

Methyl 2-[(3,4-Dimethoxyphenyl)hydrazono]-3-(3-isopropoxy-4-methoxyphenyl)propionate (**9d**): Colorless prisms (yield: 50%), mp 110—111 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (6H, d, J=6.0 Hz), 3.83 (6H, s), 3.89 (3H, s), 3.90 (3H, s), 3.96 (2H, s), 4.49 (1H, septet, J=6.0 Hz), 6.40 (1H, dd, J=8.4, 2.4 Hz), 6.71—6.85 (5H, m), 7.93 (1H, br s). IR (KBr) ν: 1700 cm^{-1}. *Anal.* Calcd for $C_{22}H_{28}N_2O_6$: C, 63.45; H, 6.77; N, 6.73. Found: C, 63.18; H, 6.70; N, 6.73.

General Procedure for Methyl 3-Phenylindole-2-carboxylate Derivatives

10. Methyl 3-(3,4-Dimethoxyphenyl)-6-isopropoxy-5-methoxyindole-2-carboxylate (10a) A mixture of **9a** (160.9 g, 0.39 mol), concentrated H_2SO_4 (75 g, 0.77 mol) and MeOH (1300 ml) was stirred under reflux for 10 h, poured into H_2O, and extracted with $CHCl_3$. The organic extract was washed successively with saturated aqueous $NaHCO_3$, H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give crystals. Recrystallization from MeOH gave **10a** as colorless prisms (93.0 g, 61%), mp 149—151 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (6H, d, J=6.2 Hz), 3.81 (3H, s), 3.83 (3H, s), 3.91 (3H, s), 3.96 (3H, s), 4.59 (1H, septet, J=6.2 Hz), 6.88 (1H, s), 6.95—7.03 (2H, m), 7.07—7.17 (2H, m), 8.79 (1H, s). IR (KBr) ν: 3348, 1718 cm^{-1}. *Anal.* Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.24; H, 6.42; N, 3.27.

Methyl 3-(3,4-Dimethoxyphenyl)-5-isopropoxy-6-methoxyindole-2-carboxylate (**10b**): Colorless prisms (yield: 74%), mp 169—170 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (6H, d, J=6.2 Hz), 3.80 (3H, s), 3.90 (3H, s), 3.92 (3H, s), 3.96 (3H, s), 4.43 (1H, septet, J=6.2 Hz), 6.85 (1H, s), 6.98 (1H, d, J=8.8 Hz), 7.05—7.15 (3H, m), 8.79 (1H, s). IR (KBr) ν: 3360, 1728 cm^{-1}. *Anal.* Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.21; H, 6.42; N, 3.55

Methyl 3-(4-Isopropoxy-3-methoxyphenyl)-5,6-dimethoxyindole-2-carboxylate (**10c**): Colorless prisms (yield: 73%), mp 155—156 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.44 (6H, d, J=6.0 Hz), 3.81 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 3.96 (3H, s), 4.62 (1H, septet, J=6.0 Hz), 6.85 (1H, s), 6.99 (1H, d, J=7.4 Hz), 7.01 (1H, d, J=1.8 Hz), 7.09 (1H, dd, J=7.4, 1.8 Hz), 7.12 (1H, s), 8.82 (1H, s). IR (KBr) ν: 3300, 1720 cm^{-1}. *Anal.* Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 65.98; H, 6.11; N, 3.49.

Methyl 3-(3-Isopropoxy-4-methoxyphenyl)-5,6-dimethoxyindole-2-carboxylate (**10d**): Colorless prisms (yield: 54%), mp 120—121 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.41 (6H, d, J=6.2 Hz), 3.80 (3H, s), 3.86 (3H, s), 3.94 (3H, s), 3.96 (3H, s), 4.56 (1H, septet, J=6.2 Hz), 6.86 (1H, s), 6.97—7.01 (2H, m), 7.07—7.17 (2H, m), 8.82 (1H, s). IR (KBr) ν: 3340, 1710 cm^{-1}. *Anal.* Calcd for $C_{22}H_{25}NO_6$: C, 66.15; H, 6.31; N, 3.51. Found: C, 66.13; H, 6.35; N, 3.41.

General Procedure for 2-Aminobenzophenone Derivatives

4. 2-Amino-4-isopropoxy-3′,4′,5-trimethoxybenzophenone (4a) 1) A solution of CrO_3 (67.5 g, 0.68 mol) in H_2O (67 ml) was added dropwise to a stirred suspension of **12a** (90.0 g, 0.23 mol) in AcOH (450 ml) at 20—25 °C with ice-water cooling. After stirring at 50 °C for 4 h, the mixture was poured into ice-water. The precipitated crystals were collected by filtration, and washed sufficiently with H_2O–MeOH (10 : 1). 2) A mixture of the crystals obtained above (46.9 g, 0.108 mol), KOH (18.3 g, 0.326 mol) and H_2O (350 ml) was stirred under reflux for 7 h, and then extracted with CH_2Cl_2. The organic extract was washed successively with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give crystals. Recrystallization from AcOEt–hexane gave **4a** as yellow prisms (23.4 g, 30%), mp 89—90 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, d, J=6.2 Hz), 3.67 (3H, s), 3.92 (3H, s), 3.95 (3H, s), 4.62 (1H, septet, J=6.2 Hz), 5.96 (2H, br s), 6.21 (1H, s), 6.90 (1H, d, J=8.6 Hz), 7.03 (1H, s), 7.22—7.30 (2H, m). IR (KBr) ν: 3448, 3330, 1619 cm^{-1}. *Anal.* Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.06; H, 6.58; N, 4.02.

2-Amino-5-isopropoxy-3′,4,4′-trimethoxybenzophenone (**4b**): Yellow prisms (yield: 43%), mp 127—128 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (6H, d, J=6.2 Hz), 3.88 (3H, s), 3.92 (3H, s), 3.96 (3H, s), 4.19 (1H, septet, J=6.2 Hz), 6.00 (2H, br s), 6.21 (1H, s), 6,90 (1H, d, J=8.6 Hz), 7.07 (1H, s), 7.21—7.27 (2H, m). IR (KBr) ν: 3450, 3348, 1620 cm^{-1}. *Anal.* Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.09; H, 6.62; N, 4.12.

2-Amino-4′-isopropoxy-3′,4,5-trimethoxybenzophenone (**4c**): Yellow prisms (yield: 41%), mp 120—121 °C (AcOEt–iso-Pr_2O). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (6H, d, J=6.0 Hz), 3.70 (3H, s), 3.89 (3H, s), 3.91 (3H, s), 4.62 (1H, septet, J=6.0 Hz), 6.01 (2H, br s), 6.22 (1H, s), 6.92 (1H, d, J=8.0 Hz), 7.04 (1H, s), 7.21—7.30 (2H, m). IR (KBr) ν: 3470, 3353, 1622 cm^{-1}. *Anal.* Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.07; H, 6.72; N, 3.90.

2-Amino-3′-isopropoxy-4,4′,5-trimethoxybenzophenone (**4d**): Yellow prisms (yield: 50%), mp 93—94 °C (AcOEt–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (6H, d, J=6.0 Hz), 3.69 (3H, s), 3.90 (3H, s), 3.92 (3H, s), 4.58 (1H, septet, J=6.0 Hz), 6.00 (2H, br s), 6.21 (1H, s), 6.91 (1H, d, J=8.8 Hz), 7.01 (1H, s), 7.21—7.32 (2H, m). IR (KBr) ν: 3475, 3350, 1637 cm^{-1}. *Anal.* Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.00; H, 6.75; N, 4.00.

General Procedure for Methyl 2-Acetyl-3-phenylpropionate Derivatives

8. Methyl 2-Acetyl-3-(3,4-dimethoxyphenyl)propionate (8a) 1) A mixture of 3,4-dimethoxybenzaldehyde (200 g, 1.20 mol), methyl acetoacetate (153 g, 1.32 mol), piperidine (20.5 g, 0.24 mol), acetic acid (144 g, 2.40 mol) and benzene (1500 ml) was stirred under reflux equipped with Dean–Stark trap for 6 h. After cooling, the mixture was washed successively with H_2O, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo* to give an oil. 2) A mixture of the oil obtained above (133 g, 0.50 mol), 5% Pd–C (45 g), tetrahydrofuran (THF) (150 ml) and EtOH (1200 ml) was hydrogenated under atmospheric pressure at room temperature. The catalyst was filtered off, and the filtrate was concentrated *in vacuo*. The residue was chromatographed on SiO_2 (1000 g) with AcOEt–hexane (1 : 1) to give **8a** as a colorless oil (81.5 g, 61%). ^{1}H-NMR ($CDCl_3$) δ: 2.18 (3H, s), 3.11 (2H, d, J=7.6 Hz), 3.70 (3H, s), 3.77 (1H, t, J=7.6 Hz), 3.85 (3H, s), 3.86 (3H, s), 6.68—6.80 (3H, m). IR (neat) ν: 1748, 1719 cm^{-1}.

Methyl 2-Acetyl-3-(4-isopropoxy-3-methoxyphenyl)propionate (**8b**): Colorless oil (62%). ^{1}H-NMR ($CDCl_3$) δ: 1.34 (6H, d, J=6.0 Hz), 2.18 (3H, s), 3.10 (2H, d, J=7.6 Hz), 3.70 (3H, s), 3.77 (1H, t, J=7.6 Hz), 3.82 (3H, s), 4.47 (1H, septet, J=6.0 Hz), 6.63—6.73 (2H, m), 6.80 (1H, d, J=8.0 Hz). IR (neat) ν: 1740, 1715 cm^{-1}.

Methyl 2-Acetyl-3-(3-isopropoxy-4-methoxyphenyl)propionate (**8c**): Colorless oil (65%), ^{1}H-NMR ($CDCl_3$) δ: 1.35 (6H, d, J=6.2 Hz), 2.18 (3H, s), 3.09 (2H, d, J=7.6 Hz), 3.70 (3H, s), 3.76 (1H, t, J=7.6 Hz), 3.82 (3H, s), 4.51 (1H, septet, J=6.2 Hz), 6.67—6.82 (3H, m). IR (neat) ν: 1748, 1717 cm^{-1}.

4-(3,4-Dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)-quinoline-3-carboxylic Acid (3) A mixture of **1** (10.0 g, 20.9 mmol), 3 N aqueous NaOH (27.5 ml, 83.6 mmol) and EtOH (150 ml) was stirred under reflux for 6 h. After cooling, the reaction mixture was neutralized with 3 N aqueous HCl, and then concentrated *in vacuo*. The residue was chromatographed on SiO_2 (150 g) with $CHCl_3$–EtOH (4 : 1) to give crystals. Recrystallization from EtOH–Et_2O gave **3** as colorless prisms (4.2 g, 42%), mp 213—214 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 3.66 (3H, s), 3,74 (3H, s), 3.82 (3H, s), 3.89 (3H, s), 5.61 (2H, s), 6.90 (1H, s), 6.96—7.10 (3H, m), 7.16 (1H, s), 7.92 (1H, s), 8.63 (1H, s). IR (KBr) ν: 3680—3025, 1618 cm^{-1}. *Anal.* Calcd for $C_{23}H_{22}N_4O_6 \cdot 2.2H_2O$: C, 56.37; H, 5.43; N, 11.43. Found: C, 56.31; H, 5.12; N, 11.38.

Biological Procedure 1. Anti-inflammatry Effect in Adjuvant Arthritis: Male Lewis rats (7 weeks old; Charles River Japan Inc.) (n=6—7) were sensitized by injecting Freund's complete adjuvant (a 0.5%

suspension of killed *Mycobacterium tubeculosis* (H37 RA, Difco) in liquid paraffin) (0.05 ml) intradermally at a plantar site on the right hind leg. A suspension of a test compound in 0.5% methylcellulose was orally administered once a day for 14 d. The administration was started just before sensitization (day 0). The left hind paw volume was measured before sensitization (day 0) and on day 14, and the plantar edema inhibitory rate was determined by comparison with a nonsensitized group.

2. Suppressive Effect to the Lipopolysaccharide (LPS)- and Concanavalin A (ConA)-Induced Proliferation of Mouse Spleen Cells: Spleen cells were isolated from Balb/c mice (male, 8—12 wks), and 3×10^5 cells (per well of a 96-well microplate) were cultured in RPMI-1640 medium containing 5% fetal calf serum (FCA) with or without LPS (1.25 μg/ml, Difco) or ConA (0.31 μg/ml, Difco) for 72 h. During the last 6 h, [^{3}H]thymidine was added. The cells were harvested and the incorporated radioactivity was measured. Drug was added at the beginning of the culture period.

References and Notes

1) Part I : Baba A., Kawamura N., Makino H., Ohta Y., Taketomi S., Sohda T., *J. Med. Chem.*, **39**, 5176—5182 (1996).
2) *a*) McCulloch J., Lydyard P. M., Rook G. A. W., *Clin. Exp. Immunol.*, **92**, 1—6 (1993); *b*) Wick I., McColl G., Harrison L., *Immunol. Today*, **15**, 553—556 (1994).
3) *a*) Makino H., Ohta Y., Baba A., Sohda T., *Rheumatoid Arthritis ID Research Alert*, **1**, 573—582 (1997); *b*) Ohta Y., Yamane M., Sohda T., Makino H., *Immunology*, **92**, 75—83 (1997); *c*) Ohta Y., Fukuda S., Makino H., *Immunopharmacology*, **37**, 167—174 (1997).
4) Tagawa Y., Kiyota Y., Yoshimura Y., Motohashi M., Tanayama S., *Arzneim.-Forsch./Drug Res.*, in press.
5) Tagawa Y., Miwa K., Yamashita K., Tanayama S., Yoshimura Y., Tanigawa Y., 8th North American ISSX Meeting, International Society for the Study of Xenobiotics, Hilton Head, South Carolina, Oct. 26—30, 1997; Abstract pp 123.
6) Kiyota Y., coworkers, unpublished results.
7) *a*) Sala T., Sargent M. V., *J. Chem. Soc., Parkin Trans 1*, **1979**, 2593—2598; *b*) Bhatt M. V., Kulkarni S. U., *Synthesis*, **1983**, 249—282.
8) Phillips R. R., *Org. React.*, **10**, 143—178 (1959).
9) Robinson B., *Chem. Rev.*, **63**, 373—401 (1963).
10) *a*) Schofield K., Theobald R. S., *J. Chem. Soc.*, **1950**, 1505—1509; *b*) Yamamoto H., Saito C., Inaba S., Awata H., Yamamoto M., Sakai Y., Komatsu T., *Arzneim.-Forsh./Drug Res.*, **23**, 1266—1271 (1973).
11) Pearson C. M., *Proc. Soc. Exp. Biol. Med.*, **91**, 95—101 (1956).
12) Ohta Y., Fukuda S., Baba A., Nagai H., Tsukuda R., Sohda T., Makino H., *Immunopharmacology*, **34**, 17—26 (1996).