Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 609–616

BIOORGANIC & MEDICINAL CHEMISTRY

Structure–Activity Relationships of 6-Fluoroquinazolines: Dual-Acting Compounds with Inhibitory Activities Toward Both TNF-α Production and T Cell Proliferation

Masanori Tobe, Yoshiaki Isobe, Hideyuki Tomizawa, Takahiro Nagasaki, Fumihiro Obara and Hideya Hayashi*

Pharmaceuticals and Biotechnology Laboratory, Japan Energy Corporation, Toda-shi, Saitama 335-8502, Japan

Received 18 July 2002; accepted 22 July 2002

Abstract—We synthesized various 6-fluoro-7-(1-piperazino)quinazolines based on the structure of **1** and evaluated their inhibitory activities toward both TNF-α production and T cell proliferation responses. Among these compounds, **7a**, having the 3,4-(methylenedioxy)phenyl moiety at the C(4)-position of the quinazoline ring, showed both inhibitory activities. Furthermore, the oral treatment with **7a** exhibited an anti-inflammatory effect in rats with adjuvant arthritis as well as an inhibitory activity toward LPS-induced TNF-α production.

Introduction

The demonstration of dichotomy between Th1 and Th2 cells represents one of the most important advances in immunology. T cells within the rheumatoid arthritis (RA) synovium show mainly a Th1 pattern of cytokine production.[1] Therefore, Th1 cells play an important role in the pathogenesis of RA, and their activation has been shown to be a possible mechanism by which inflammation is enhanced in RA.[2]

Among the proinflammatory cytokines, tumor necrosis factor-α (TNF-α) has been shown to play an essential role in the disease process of RA.[3] The blockade of TNF-α by anti-TNF-α antibody (Remicade™) and soluble TNF-α receptor (Enbrel™) has been proved to be dramatically effective in improving both subjective and objective findings in RA patients.[4]

Based on the above information, we speculated that it is necessary for anti-rheumatic agents to possess the ability to block abnormal T cell-mediated immune responses as well as to inhibit TNF-α activity. Glucocorticoids show inhibitory activities toward both TNF-α production and T cell proliferation. However, the long-term use of steroids is well known to give rise to several side effects including infection and osteoporosis.

In a previous paper, we demonstrated that 6-nitroquinazoline derivative **1** exhibited inhibitory activities toward both TNF-α production and T cell proliferation; however, compound **1** did not show an oral activity on TNF-α production, presumably due to its poor bioavailability.[5] In order to find novel quinazoline derivatives exhibiting oral activities, we performed further modifications starting from **1** by focusing on the replacement of the nitro group with the fluoro group, which is an electron-withdrawing group similar to the nitro one, at the C(6)-position of the quinazoline ring.

This paper describes the results of our study on the structure–activity relationship of 6-fluoroquinazolines, which was conducted on the basis of the replacement of the phenyl group at the C(4)-position of **1** with other heteroaryl groups and alteration of the methylene chain length (Fig. 1). In addition, we report the anti-inflammatory effect of a selected compound on a rat model of adjuvant arthritis.

*Corresponding author. Tel.: +81-6-6466-5189; fax: +81-6-6466-5483; e-mail: hayashih@sumitomopharm.co.jp

0968-0896/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00338-3

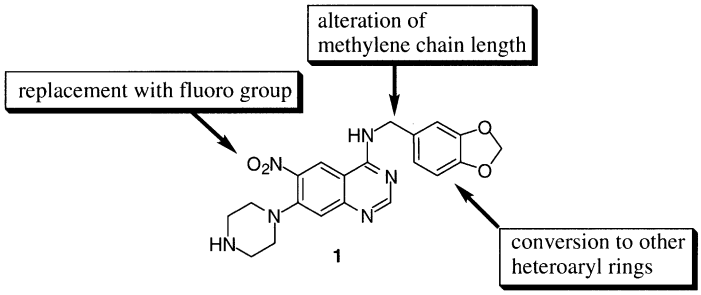
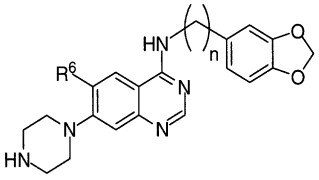


Figure 1. Design of 6-fluoro-7-(1-piperazino)quinazolines.

Table 1. Inhibition of TNF-α production and T cell proliferation by compounds **7a–7d**



Compd	R^6	*n*	IC_{50} (μM)		
			TNF-α[a]	Con A[b]	MTS[c]
1	NO_2	1	0.08	2.1	7.2
7a	F	1	0.5	5.1	>30
7b	F	0[d]	4.7	7.8	>30
7c	F	2	2.1	6.2	>30
7d	F	3	3.2	8.4	>30
Rolipram			0.5	>10	>30
Dexamethasone			0.01	0.005	0.04

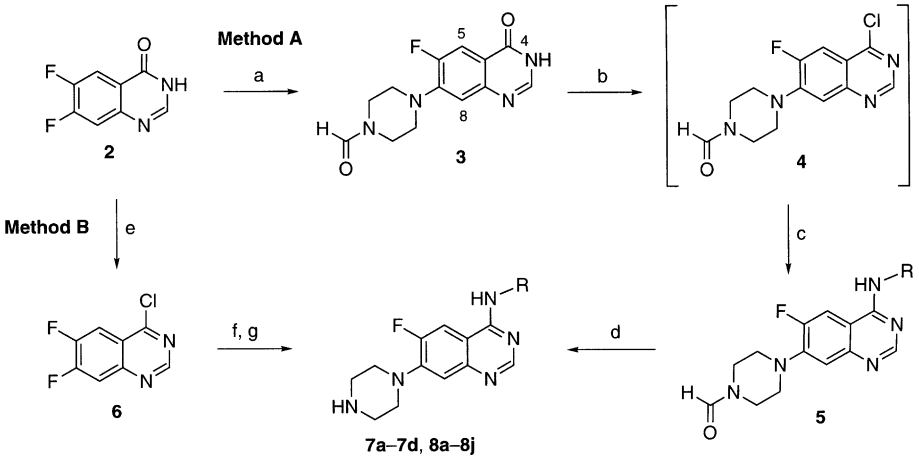
[a]IC_{50} for inhibition of TNF-α production from human PBMCs stimulated by LPS.
[b]IC_{50} for inhibition of Con A-induced proliferation of mouse spleen cells.
[c]IC_{50} for the growth inhibition of human PBMCs stimulated by LPS.
[d]0 means the anilino derivative.

Chemistry

The 6-fluoro-7-(1-piperazino)quinazolines described in this study are listed in Tables 1 and 2, and the general synthesis of the compounds is shown in Scheme 1. Regioselective displacement of the 7-fluorine atom in **2** with 1-formylpiperazine gave compound **3**. The structure of **3** was established on the basis of the long-range correlations observed in the COLOC spectrum of **3**: H-5 (δ 8.14, d, $J_{H-F} = 12.2$ Hz) correlated with C-4 (δ 160.8, s), whereas H-8 (δ 7.45, d, $J_{H-F} = 6.2$ Hz) did not.

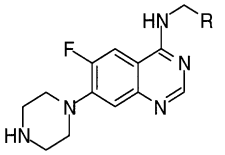
Chlorination of **3** with phosphorus oxychloride gave **4**, which was treated with the corresponding amines to provide **5** in low yields (3–8%) through two steps. The reason for this poor yield was due to the production of many by-products. Deprotection of the *N*-formyl group of **5** using 5 N NaOH–EtOH led to the 7-(1-piperazino)quinazolines (Method A).

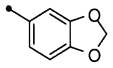
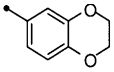
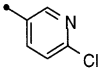
From the above-mentioned result, we considered that Method A was a rather troublesome procedure. Therefore, we selected alternatively a more convenient and facile procedure for the synthesis of the 7-(1-piperazino)quinazolines (Method B). The key intermediate **6** was prepared by the chlorination of **2** with thionyl chloride. Compound **6** was subsequently treated with the corresponding amines to give 4-substituted-6,7-difluoroquinazolines. Reaction of these compounds with piperazine in the presence of Hunig's base provided the objective compounds (**7a–7d** and **8a–8j**) in good yield. Regioselectivity in Method B was confirmed by another route of synthesis, Method A. The same compounds were afforded by Methods A and B in several cases.



Scheme 1. Synthesis of compounds **7a–7d** and **8a–8j**. Reagents and conditions: (a) 1-formylpiperazine, *N*,*N*-diisopropylethylamine, *i*-PrOH, 70 °C, 6 h; (b) phosphorus oxychloride, 100 °C, 30 min; (c) RNH_2, triethylamine, EtOH, reflux, 4–8 h; (d) 5 N NaOH, EtOH, reflux, 3–4 h; (e) $SOCl_2$, DMF, reflux, 2 h; (f) RNH_2, triethylamine, *i*-PrOH, 6–10 h; (g) piperazine, *N*,*N*-diisopropylethylamine, *n*-BuOH, 110 °C, 30–40 h.

Table 2. Inhibition of TNF-α production and T cell proliferation by compounds **8a–8j**



Compound	R	IC_{50} (μM)		
		TNF-α[a]	Con A[b]	MTS[c]
7a		0.5	5.1	>30
8a		1.2	8.2	>30
8b		2.2	8.1	>30
8c		1.3	6.2	27.2
8d		6.5	>10	>30
8e		2.6	>10	>30
8f		11.2	>10	>30
8g		4.4	>10	>30
8h		3.7	>10	>30
8i		2.5	8	>30
8j		0.8	4.8	6.4
Rolipram		0.5	>10	>30
Dexamethasone		0.01	0.005	0.04

[a]IC_{50} for inhibition of TNF-α production from human PBMCs stimulated by LPS.
[b]IC_{50} for inhibition of Con A-induced proliferation of mouse spleen cells.
[c]IC_{50} for the growth inhibition of human PBMCs stimulated by LPS.

Results and Discussion

The compounds listed in Tables 1 and 2 were evaluated for their abilities to inhibit both TNF-α production and T cell proliferation, as previously reported.[5] Also included in Tables 1 and 2 are the results of cytotoxicity experiments using the MTS assay in human PBMCs.[6]

In general, some compounds having the nitro group are not preferable for a drug candidate because of the possibility that the nitro group might change into the mutagenetic nitroso one in vivo. Thus, our initial efforts focused on investigating the effects of the replacement of the nitro group with the fluorine atom at the C(6)-position in **1** (Table 1). The inhibitory activities of the 6-fluoro derivative (**7a**) were several fold weaker than those of the 6-nitro one (**1**); however, the oral bioavailability (46%) of **7a** was much greater than that of **1** (1%). Moreover, **7a** showed reduced cell growth inhibition in human PBMCs stimulated by LPS. On the basis of these data, we performed the structure–activity relationship study by using the 6-fluoroquinazolines.

To explore the effects of varying the length of the linker between the quinazoline and the phenyl group at the C(4)-position, we prepared compounds **7b–7d** (Table 1). The maximum inhibitory activities toward TNF-α production and T cell proliferation were obtained when the spacer was the methylene chain (**7a**).

Next, we investigated the structure–activity relationship at the C(4)-position by replacing the 3,4-methylenedioxyphenyl group with several heteroaryl substituents (Table 2). The benzyl analogues (**8a–8c**) resulted in a 2–4-fold loss in TNF-α inhibitory activity as compared with the activity of **7a**. The pyridyl (**8d–8g**), the furyl (**8h**), and the thienyl (**8i**) analogues also exhibited decreased or diminished activities. The naphthyl analogue (**8j**) potently inhibited TNF-α production with an IC_{50} value of 0.8 μM, while this analogue showed cell growth inhibition (IC_{50} = 6.4 μM).

We selected compound **7a** from the results of the abovementioned in vitro study and evaluated its activity on TNF-α production in vivo (Table 3).[5] Also included in Table 3 are the results of the pharmacokinetic study on compounds **1** and **7a** conducted on male rats. Orally administered compound **7a** showed inhibitory activity toward LPS-induced TNF-α production, with an ED_{50} of 28 mg/kg, whereas compound **1** exhibited low oral activity in this model. Compound **7a** showed a plasma concentration of over 1.0 μM, and had 46% bioavail-

Table 3. Effects of **1** and **7a** on LPS-induced TNF-α production in mice, and pharmacokinetic profile in rats

Compd	ED_{50} or % of TNF-α inhibition (mg/kg/po)[a]	Pharmacokinetic profile[b]			
		C_{max} (μM)	T_{max} (h)	*AUC* (μM min)	*F* (%)
1	18% @ 30	0.04	2	40	1
7a	28	1.4	3	324	46

[a]ED_{50} value was determined from dose–response curve of TNF-α inhibition. Compounds were evaluated as their corresponding hydrochroride salts, and administered orally to mice 0.5 h prior to the LPS challenge.
[b]Compounds were examined for pharmacokinetics when orally administered to rats in water (30 mg/kg).

ability after oral administration in rats. On the other hand, the plasma level of **1** was very low ($C_{max} = 0.04\,\mu M$), and its oral bioavailability was poor (1%). Therefore, we considered that the improved oral bioavailability of **7a** compared with that of **1** reflects its greater inhibitory activity on TNF-α production in vivo.

To further confirm the anti-inflammatory effect of **7a**, we evaluated it using a rat model of adjuvant-induced arthritis (Fig. 2).[7] Compound **7a** (30 mg/kg, po) significantly reduced the increase in hind paw volume on day 15. In this model, elevated TNF-α levels and T cell activation have been observed in serum and joint tissue.[8] Moreover, **7a** also showed an inhibitory activity on B cell proliferation ($IC_{50} = 0.57\,\mu M$).[9] This pharmacological profile suggests that **7a** would be desirable for the therapy of RA.

As the mechanistic target for **7a** is unknown, a further evaluation of its pharmacological characteristics was undertaken. Nuclear factor-κB (NF-κB) is essential for the transcriptional regulation of the proinflammatory cytokines such as TNF-α, IL-1, and IL-6.[10] However, **7a** did not influence NF-κB gene expression when tested at 10 μM in the luciferase assay system.[11] Inhibition of phosphodiesterase 4 (PDE 4) is also one of the mechanisms by which TNF-α production may be reduced.[12] Indeed, **7a** showed a moderate inhibitory activity toward PDE 4 isolated from U937 cells ($IC_{50} = 1.6\,\mu M$). However, the IC_{50} of **7a** for PDE 4 inhibition was higher than that for TNF-α inhibition in vitro (cell culture). Although rolipram, which is well known as a specific PDE 4 inhibitor, inhibited TNF-α production in our assay systems, it did not suppress T cell proliferation. These results suggest that the biological properties of **7a** are different from those of PDE 4 inhibitors. In addition, **7a** did not show any inhibitory activity toward cyclooxygenase-1 (−5%), cyclooxygenase-2 (2%), constitutive NO synthase (−7%), or inducible NO synthase (−7%); the binding affinity for the adenosine A_{2A} receptor was zero at 10 μM in vitro.[13] Therefore, we are currently investigating some mechanistic targets of **7a** other than the above-mentioned molecular targets in more detail.

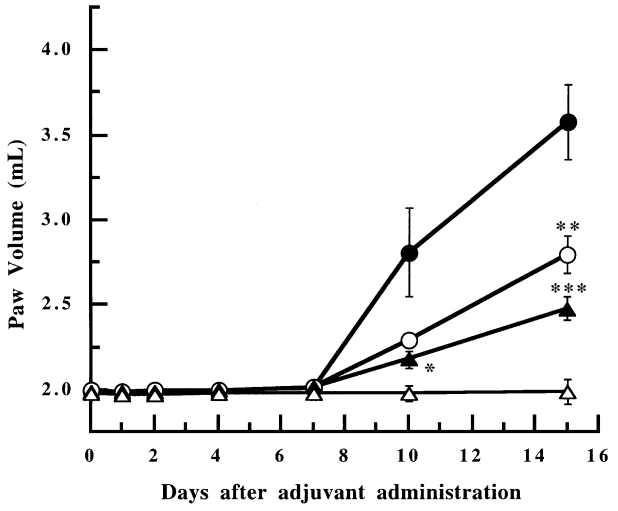


Figure 2. Effect of **7a** on hind paw swelling in rats with adjuvant-induced arthritis. Compound **7a** (HCl salt, 30 mg/kg) or indomethacin (1 mg/kg) was orally administered for 15 days after adjuvant injection into the rat's right hind paw. Each volume was obtained by comparison between the left paw volume in the arthritic group and that in the control group. The results are expressed as the mean ± SEM of eight rats per group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus arthritis control (Dunnett's test). ●, Arthritis control; ○, **7a**; ▲, indomethacin; △, normal control.

Conclusions

A series of 6-fluoro-7-(1-piperazino)quinazolines were synthesized and evaluated for their inhibitory activities toward both TNF-α production and T cell proliferation. We found that introduction of a fluorine atom in place of the nitro group at the C(6)-position resulted in slightly weaker activity in vitro, but exhibited a good oral bioavailability. Among them, **7a**, having both inhibitory activities in vitro, showed inhibition of TNF-α production in vivo. Furthermore, **7a** (30 mg/kg, po) exhibited an anti-inflammatory effect in a rat model of adjuvant arthritis and also had an inhibitory effect on B cell proliferation. This pharmacological profile of **7a** suggests that this compound would be desirable for use in the therapy of RA. We are continuing to search for more potent analogues and to elucidate their mechanisms of action.

Experimental

Chemistry

General. All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were measured with a Büchi 535 melting point apparatus and were uncorrected. ^1H NMR and ^{13}C NMR were recorded on a JEOL GSX270 FT NMR spectrometer. Chemical shifts are given in parts per million (ppm) using tetramethylsilane as the internal standard for spectra obtained in DMSO-d_6 and CF_3CO_2D. TOF MS (time-of-flight mass spectrometry) was recorded on a Kompact MALDI 3 V 4.0.0 spectrometer. High-resolution mass spectra were obtained on a JEOL JMS-700 mass spectrometer. Elemental analyses were performed at the Toray Research Center. Wakogel C-200 (Wako; 70–150 mm) was used for column chromatography. Monitoring of reactions was carried out using Merck 60 F_{254} silica gel, glass-supported TLC plates, and visualization with UV light (254 and 365 nm).

6,7-Difluoro-4-quinazolone (2). To a mixture of 2-amino-4,5-difluorobenzamide (13.0 g, 75.5 mmol) in trimethyl orthoformate (490 mL) was added 12 N HCl (25.2 mL, 302 mmol) dropwise at 0 °C. The reaction was stirred at ambient temperature for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was poured into 5% aqueous $NaHCO_3$ solution. The precipitate was collected, washed with MeOH and water, and dried in air. The white solid was filtered and dried at 40 °C under high vacuum to give **2** (12.2 g, 89%

yield): ^{1}H NMR (DMSO-d_6) δ 12.48 (br s, 1H, CONH), 8.15 (s, 1H, H-2), 8.03 (dd, J_{H-F} = 10.5, 9.2 Hz, 1H, H-5), 7.76 (dd, J_{H-F} = 11.3, 7.3 Hz, 1H, H-8); MS (TOF) m/z 183 $(M+H)^+$. Anal. calcd for $C_8H_4F_2N_2O$: C, 52.76; H, 2.21; N, 15.38. Found: C, 52.80; H, 2.25; N, 15.56.

6-Fluoro-4-(3,4-methylenedioxybenzylamino)-7-(1-piperazino)quinazoline (7a). Example of general method A. To a mixture of **2** (1.47 g, 8.07 mmol) and *N*,*N*-diisopropylethylamine (7.04 mL, 40.4 mmol) in *i*-PrOH (20 mL) was added 1-formylpiperazine (4.61 g, 40.4 mmol). The reaction was stirred at 70 °C for 6 h under a nitrogen atmosphere. The reaction mixture was cooled to ambient temperature, and then concentrated under reduced pressure. The residue was poured into water, and then the precipitate was collected, washed with MeOH and water. The white solid was filtered to give 6-fluoro-7-[1-(4-formyl)piperazino]-4-quinazolone (**3**) (1.48 g, 66% yield): ^{1}H NMR (CF_3CO_2D) δ 12.31 (s, 1H, CONH), 9.31 (s, 1H, H-2), 8.47 (s, 1H, CHO), 8.14 (d, J_{H-F} = 12.2 Hz, 1H, H-5), 7.45 (d, J_{H-F} = 6.2 Hz, 1H, H-8), 4.06–3.97 (m, 4H, piperazinyl methylene), 3.76–3.72 (m, 4H, piperazinyl methylene); ^{13}C NMR (CF_3CO_2D) δ 169.6 (s, CHO), 160.8 (s, C4), 157.5 (d, J_{C-F} = 256.7 Hz, C6), 150.3 (s, C2), 150.2 (d, J_{C-F} = 9.8 Hz, C7), 137.1 (s, C9), 116.8 (d, J_{C-F} = 26.5 Hz, C5), 115.2 (d, J_{C-F} = 9.5 Hz, C10), 109.5 (d, J_{C-F} = 3.7 Hz, C8), 51.4 (d, J_{C-F} = 5.0 Hz, piperazine), 50.3 (d, J_{C-F} = 4.2 Hz, piperazine), 49.4 (s, piperazine), 43.7 (s, piperazine); MS (TOF) m/z 277 $(M+H)^+$.

A suspension of **3** (500 mg, 1.81 mmol) in phosphorus oxychloride (15 mL) was heated at 100 °C for 30 min, when a clear solution was obtained. The reaction mixture was cooled to ambient temperature, and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and 5% aqueous $NaHCO_3$ solution. The organic layer was washed with water and brine, then dried over Na_2SO_4. The solution was concentrated under reduced pressure to provide crude 4-chloro-6-fluoro-7-[1-(4-formyl)piperazino]quinazoline (**4**), which was used directly. To a mixture of the crude chloro compound and triethylamine (379 μL, 2.72 mmol) in EtOH (18 mL) was added piperonylamine (339 μL, 2.72 mmol). The resulting mixture was stirred at reflux for 2 h and concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and 5% aqueous citric acid solution. The organic layer was washed with water and brine, and then dried over Na_2SO_4. The solution was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel eluting with 1:25 MeOH/CH_2Cl_2 to provide 37 mg (5%) of 6-fluoro-7-[1-(4-formyl)piperazino]-4-[(3,4-methylenedioxy)benzylamino]quinazoline [**5**: R = (3,4-methylenedioxy)benzyl]: mp 190–192 °C; ^{1}H NMR (DMSO-d_6) δ 8.47 (t, J = 5.4 Hz, 1H, N*H*CH_2), 8.39 (s, 1H, H-2), 8.13–8.07 (m, 2H, H-5 and CHO), 7.16 (d, J_{H-F} = 8.9 Hz, 1H, H-8), 6.92 (s, 1H, ArH), 6.86–6.80 (m, 2H, ArH), 5.97 (s, 2H, OCH_2O), 4.65 (d, J = 5.4 Hz, 2H, NHCH_2), 3.60–3.55 (m, 4H, piperazinyl methylene), 3.21–3.11 (m, 4H, piperazinyl methylene); MS (TOF) m/z 410 $(M+H)^+$. Anal. calcd for $C_{21}H_{20}N_5FO_3{\cdot}0.8H_2O$: C, 59.51; H, 4.95; N, 16.52. Found: C, 59.42; H, 5.11; N, 16.40.

A suspension of the above compound (30 mg, 0.07 mmol) in EtOH (3 mL) containing 5 N NaOH (3 mL) was heated at reflux for 3 h, when a clear solution was obtained. The reaction mixture was cooled to ambient temperature, and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and water. The organic layer was washed with water and brine, then dried over Na_2SO_4. The solution was evaporated in vacuo, and the residue was suspended in CH_2Cl_2/hexanes (1:1) until a solid formed. The white solid was filtered to give **7a** (23 mg, 85% yield): mp 192–193 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (t, J = 5.4 Hz, 1H, N*H*CH_2), 8.37 (s, 1H, H-2), 8.05 (d, J_{H-F} = 14.0 Hz, 1H, H-5), 7.08 (d, J_{H-F} = 8.4 Hz, 1H, H-8), 6.92 (s, 1H, ArH), 6.86–6.80 (m, 2H, ArH), 5.97 (s, 2H, OCH_2O), 4.64 (d, J = 5.4 Hz, 2H, NHCH_2), 3.09–3.05 (m, 4H, piperazinyl methylene), 2.88–2.85 (m, 4H, piperazinyl methylene); MS (TOF) m/z 382 $(M+H)^+$. Anal. calcd for $C_{20}H_{20}N_5FO_2{\cdot}0.2H_2O$: C, 62.39; H, 5.29; N, 18.19. Found: C, 62.33; H, 5.35; N, 18.11.

4-Benzylamino-6-fluoro-7-(1-piperazino)quinazoline (8b). Example of general method B. A suspension of **2** (250 mg, 1.37 mmol) in thionyl chloride (6 mL) containing one drop of DMF was heated at reflux for 2 h to give a clear solution. Excess thionyl chloride was removed under reduced pressure to provide crude 4-chloro-6,7-difluoroquinazoline (**6**), which was used directly. To a mixture of the crude chloro compound and triethylamine (229 μL, 1.64 mmol) in *i*-PrOH (12 mL) was added benzylamine (179 μL, 1.64 mmol). The resulting mixture was stirred at ambient temperature for 8 h and concentrated in vacuo, and partitioned between CH_2Cl_2 and 5% aqueous citric acid solution. The organic layer was washed successively with 1 N NaOH, water, and brine, and then dried over Na_2SO_4. The solution was concentrated under reduced pressure and the residue was triturated with CH_2Cl_2/hexanes (1:1). The light yellow solid was filtered to give 4-benzylamino-6,7-difluoroquinazoline (188 mg, 51% yield). To a mixture of the above compound (150 mg, 0.55 mmol) and *N*,*N*-diisopropylethylamine (1.15 mL, 6.60 mmol) in *n*-BuOH (6 mL) was added piperazine (569 mg, 6.60 mmol). The reaction was stirred at 110 °C for 30 h under a nitrogen atmosphere. The reaction mixture was cooled to ambient temperature, and then concentrated under reduced pressure. The brown residue was partitioned between CH_2Cl_2 and 5% aqueous citric acid solution. The aqueous layer was adjusted to pH 9 with 5 N NaOH, and extracted with CH_2Cl_2. The organic layer was washed with water and brine, then dried over Na_2SO_4. The solution was evaporated in vacuo, and the residue was suspended in CH_2Cl_2/hexanes (1:1) until a solid formed. The white solid was filtered to give **8b** (56 mg, 30% yield): mp 213–215 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (t, J = 5.9 Hz, 1H, N*H*CH_2), 8.36 (s, 1H, H-2), 8.07 (d, J_{H-F} = 14.9 Hz, 1H, H-5), 7.36–7.20 (m, 5H, ArH), 7.09 (d, J_{H-F} = 8.9 Hz, 1H, H-8), 4.75 (d, J = 5.9 Hz, 2H, NHCH_2), 3.09–3.06 (m, 4H,

piperazinyl methylene), 2.89–2.85 (m, 4H, piperazinyl methylene); MS (TOF) m/z 338 $(M+H)^+$. Anal. calcd for $C_{19}H_{20}N_5F{\cdot}0.4H_2O$: C, 66.22; H, 5.97; N, 20.32. Found: C, 66.10; H, 6.02; N, 20.10.

Similarly to the procedure described for **8b**, compounds **7a–7d**, **8a**, and **8c–8j** were prepared from **2**.

6-Fluoro-4-(3,4-methylenedioxybenzylamino)-7-(1-piperazino)quinazoline hydrochloride (HCl salt of 7a). Compound **7a** was obtained as a white solid (48% yield for three steps from **2**), and then was converted to its hydrochloride salt according to the following procedure: To a suspension of **7a** (30 mg, 0.08 mmol) in EtOH (3 mL) was added 12 N HCl (20 μL). The mixture was stirred at ambient temperature for 3 h, and then concentrated under reduced pressure. The residue was triturated with diethyl ether, and the precipitated solid was collected by filtration. The obtained solid was dried in vacuo to give the hydrochloride salt as a white powder (25 mg, 76% yield): mp 260 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 10.38 (br s, 1H, N*H*CH_2), 9.27 (br s, 2H, NH·HCl), 8.83 (s, 1H, H-2), 8.49 (d, $J_{H-F}=14.0$ Hz, 1H, H-5), 7.33 (d, $J_{H-F}=7.8$ Hz, 1H, H-8), 7.00 (s, 1H, ArH), 6.88 (s, 2H, ArH), 5.99 (s, 2H, OCH_2O), 4.80 (d, $J=5.4$ Hz, 2H, NHCH_2), 3.51–3.36 (m, 8H, piperazinyl methylene). Anal. calcd for $C_{20}H_{21}N_5ClFO_2{\cdot}2.5H_2O$: C, 51.89; H, 5.12; N, 15.13. Found: C, 51.95; H, 5.02; N, 15.09.

6-Fluoro-4-(3,4-methylenedioxyphenyl)amino-7-(1-piperazino)quinazoline (7b). White solid (55% yield for three steps from **2**): mp 259–261 °C; ^{1}H NMR (DMSO-d_6) δ 9.41 (s, 1H, ArNH), 8.46 (s, 1H, H-2), 8.28 (d, $J_{H-F}=14.6$ Hz, 1H, H-5), 7.50 (d, $J=2.2$ Hz, 1H, ArH), 7.18 (dd, $J=8.4$, 2.2 Hz, 1H, ArH), 7.14 (d, $J_{H-F}=9.7$ Hz, 1H, H-8), 6.92 (d, $J=8.4$ Hz, 1H, ArH), 6.03 (s, 2H, OCH_2O), 3.13–3.09 (m, 4H, piperazinyl methylene), 2.90–2.87 (m, 4H, piperazinyl methylene); MS (TOF) m/z 368 $(M+H)^+$. Anal. calcd for $C_{19}H_{18}N_5FO_2{\cdot}0.1H_2O$: C, 61.81; H, 4.94; N, 18.97. Found: C, 61.85; H, 4.97; N, 18.76.

6-Fluoro-4-[2-(3,4-methylenedioxyphenyl)ethylamino]-7-(1-piperazino)quinazoline (7c). White solid (15% yield for three steps from **2**): mp 189–191 °C; ^{1}H NMR (DMSO-d_6) δ 8.39 (s, 1H, H-2), 8.03 (t, $J=5.9$ Hz, 1H, N*H*CH_2), 8.00 (d, $J_{H-F}=14.6$ Hz, 1H, H-5), 7.08 (d, $J_{H-F}=8.4$ Hz, 1H, H-8), 6.85–6.80 (m, 2H, ArH), 6.71–6.67 (m, 1H, ArH), 5.96 (s, 2H, OCH_2O), 3.67 (dt, $J=7.0$, 5.9 Hz, 2H, NHC$H_2$$CH_2$), 3.13–3.09 (m, 4H, piperazinyl methylene), 2.94–2.91 (m, 4H, piperazinyl methylene), 2.85 (t, $J=7.3$ Hz, 2H, NHCH_2CH_2); MS (TOF) m/z 396 $(M+H)^+$. Anal. calcd for $C_{21}H_{22}N_5FO_2{\cdot}0.7H_2O$: C, 61.81; H, 5.61; N, 17.16. Found: C, 62.02; H, 5.63; N, 16.80.

6-Fluoro-4-[3-(3,4-methylenedioxyphenyl)propylamino]-7-(1-piperazino)quinazoline (7d). White solid (20% yield for three steps from **2**): mp 144–146 °C; ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H, H-2), 8.01 (d, $J_{H-F}=14.6$, Hz, 1H, H-5), 7.90 (t, $J=5.4$ Hz, 1H, N*H*CH_2), 7.06 (d, $J_{H-F}=8.4$ Hz, 1H, H-8), 6.83–6.79 (m, 2H, ArH), 6.69–6.66 (m, 1H, ArH), 5.95 (s, 2H, OCH_2O), 3.48 (dt, $J=7.2$, 5.4 Hz, 2H, NHCH_2), 3.08–3.05 (m, 4H, piperazinyl methylene), 2.89–2.86 (m, 4H, piperazinyl methylene), 2.59 (t, $J=7.6$ Hz, 2H, ArCH_2), 1.89 (tt, $J=7.6$, 7.2 Hz, 2H, ArCH_2CH_2); MS (TOF) m/z 410 $(M+H)^+$. Anal. calcd for $C_{22}H_{24}N_5FO_2{\cdot}0.4H_2O$: C, 63.42; H, 5.90; N, 16.81. Found: C, 63.29; H, 6.01; N, 16.61.

4-(3,4-Ethylenedioxybenzylamino)-6-fluoro-7-(1-piperazino)quinazoline (8a). White solid (12% yield for three steps from **2**): mp 188–190 °C; ^{1}H NMR (DMSO-d_6) δ 8.41 (t, $J=5.9$ Hz, 1H, N*H*CH_2), 8.36 (s, 1H, H-2), 8.05 (d, $J_{H-F}=14.0$ Hz, 1H, H-5), 7.07 (d, $J_{H-F}=8.4$ Hz, 1H, H-8), 6.83–6.79 (m, 3H, ArH), 4.61 (d, $J=5.9$ Hz, 2H, NHCH_2), 4.19 (s, 4H, OCH_2CH_2O), 3.08–3.05 (m, 4H, piperazinyl methylene), 2.88–2.85 (m, 4H, piperazinyl methylene; MS (TOF) m/z 396 $(M+H)^+$. Anal. calcd for $C_{21}H_{22}N_5FO_2{\cdot}1.5H_2O$: C, 59.70; H, 5.61; N, 16.58. Found: C, 59.89; H, 5.97; N, 16.44.

6-Fluoro-4-(4-fluorobenzylamino)-7-(1-piperazino)quinazoline (8c). White solid (20% yield for three steps from **2**): mp 222–224 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (t, $J=5.4$ Hz, 1H, N*H*CH_2), 8.36 (s, 1H, H-2), 8.06 (d, $J_{H-F}=14.6$ Hz, 1H, H-5), 7.41–7.36 (m, 2H, ArH), 7.18–7.08 (m, 3H, ArH and H-8), 4.72 (d, $J=5.4$ Hz, 2H, NHCH_2), 3.11–3.07 (m, 4H, piperazinyl methylene), 2.91–2.88 (m, 4H, piperazinyl methylene); MS (TOF) m/z 356 $(M+H)^+$. Anal. calcd for $C_{19}H_{19}N_5F_2{\cdot}0.3H_2O$: C, 63.25; H, 5.39; N, 19.41. Found: C, 63.25; H, 5.31; N, 19.18.

6-Fluoro-7-(1-piperazino)-4-(2-pyridylmethylamino)quinazoline (8d). White solid (36% yield for three steps from **2**): mp 154–156 °C; ^{1}H NMR (DMSO-d_6) δ 8.62 (t, $J=5.9$ Hz, 1H, N*H*CH_2), 8.52–8.50 (m, 1H, pyridine), 8.33 (s, 1H, H-2), 8.10 (d, $J_{H-F}=14.0$ Hz, 1H, H-5), 7.74–7.68 (m, 1H, pyridine), 7.32–7.23 (m, 2H, pyridine), 7.10 (d, $J_{H-F}=8.6$ Hz, 1H, H-8), 4.82 (d, $J=5.9$ Hz, 2H, NHCH_2), 3.10–3.07 (m, 4H, piperazinyl methylene), 2.89–2.86 (m, 4H, piperazinyl methylene); HR-FABMS m/z $(M+H)^+$: calcd for $C_{18}H_{19}N_6F$: 339.1733. Found: 339.1723.

6-Fluoro-7-(1-piperazino)-4-(3-pyridylmethylamino)quinazoline (8e). White solid (15% yield for three steps from **2**): mp 188–190 °C; ^{1}H NMR (DMSO-d_6) δ 8.60–8.53 (m, 2H, pyridine and N*H*CH_2), 8.46–8.44 (m, 1H, pyridine), 8.38 (s, 1H, H-2), 8.05 (d, $J_{H-F}=14.6$ Hz, 1H, H-5), 7.76–7.73 (m, 1H, pyridine), 7.36–7.32 (m, 1H, pyridine), 7.10 (d, $J_{H-F}=8.1$ Hz, 1H, H-8), 4.76 (d, $J=5.9$ Hz, 2H, NHCH_2), 3.11–3.07 (m, 4H, piperazinyl methylene), 2.91–2.87 (m, 4H, piperazinyl methylene); HR-FABMS m/z $(M+H)^+$: calcd for $C_{18}H_{19}N_6F$: 339.1733. Found: 339.1743.

6-Fluoro-7-(1-piperazino)-4-(4-pyridylmethylamino)quinazoline (8f). White solid (55% yield for three steps from **2**): mp 226–228 °C; ^{1}H NMR (DMSO-d_6) δ 8.60 (t, $J=5.9$ Hz, 1H, N*H*CH_2), 8.49–8.47 (m, 2H, pyridine), 8.34 (s, 1H, H-2), 8.07 (d, $J_{H-F}=14.3$ Hz, 1H, H-5), 7.31 (d, $J=5.9$ Hz, 2H, pyridine), 7.11 (d, $J_{H-F}=8.9$ Hz, 1H, H-8), 4.76 (d, $J=5.9$ Hz, 2H, NHCH_2), 3.10–3.07 (m,

4H, piperazinyl methylene), 2.89–2.86 (m, 4H, piperazinyl methylene); HR-FABMS m/z $(M+H)^+$: calcd for $C_{18}H_{19}N_6F$: 339.1733. Found: 339.1732.

4-(6-Chloro-pyridin-3-yl)methylamino-6-fluoro-7-(1-piperazino)quinazoline (8g). White solid (36% yield for three steps from **2**): mp 164–166 °C; ^{1}H NMR (DMSO-d_6) δ 8.55 (t, $J=5.9$ Hz, 1H, NHCH$_2$), 8.42 (d, $J=1.9$ Hz, 1H, pyridine), 8.37 (s, 1H, H-2), 8.02 (d, $J_{H-F}=14.9$ Hz, 1H, H-5), 7.84–7.80 (m, 1H, pyridine), 7.46 (d, $J=8.4$ Hz, 1H, pyridine), 7.10 (d, $J_{H-F}=8.9$ Hz, 1H, H-8), 4.74 (d, $J=5.9$ Hz, 2H, NHCH_2), 3.09–3.06 (m, 4H, piperazinyl methylene), 2.88–2.85 (m, 4H, piperazinyl methylene); HR-FABMS m/z $(M+H)^+$: calcd for $C_{18}H_{18}N_6ClF$: 373.1343. Found: 373.1333.

6-Fluoro-4-furfurylamino-7-(1-piperazino)quinazoline (8h). White solid (21% yield for three steps from **2**): mp 122–124 °C; ^{1}H NMR (DMSO-d_6) δ 8.43–8.40 (m, 2H, H-2 and NHCH$_2$), 8.06 (d, $J_{H-F}=14.6$ Hz, 1H, H-5), 7.58–7.57 (m, 1H, furan), 7.09 (d, $J_{H-F}=8.9$ Hz, 1H, H-8), 6.40–6.38 (m, 1H, furan), 6.31–6.30 (m, 1H, furan), 4.73 (d, $J=5.4$ Hz, 2H, NHCH_2), 3.09–3.06 (m, 4H, piperazinyl methylene), 2.89–2.85 (m, 4H, piperazinyl methylene); MS (TOF) m/z 327 $(M+H)^+$. Anal. calcd for $C_{17}H_{18}FN_5O\cdot1.2H_2O$: C, 58.51; H, 5.55; N, 20.07. Found: C, 58.61; H, 5.72; N, 20.09.

6-Fluoro-7-(1-piperazino)-4-[(thiophen-2-yl)methylamino]-quinazoline (8i). White solid (21% yield for three steps from **2**): mp 202–204 °C; ^{1}H NMR (DMSO-d_6) δ 8.58 (t, $J=5.9$ Hz, 1H, NHCH$_2$), 8.42 (s, 1H, H-2), 8.01 (d, $J_{H-F}=14.0$ Hz, 1H, H-5), 7.37–7.35 (m, 1H, thiophene), 7.11–7.06 (m, 2H, H-8 and thiophene), 6.97–6.94 (m, 1H, thiophene), 4.90 (d, $J=5.9$ Hz, 2H, NHCH_2), 3.09–3.05 (m, 4H, piperazinyl methylene), 2.88–2.85 (m, 4H, piperazinyl methylene); MS (TOF) m/z 344 $(M+H)^+$. Anal. calcd for $C_{17}H_{18}N_5FS\cdot0.3H_2O$: C, 58.53; H, 5.29; N, 20.08. Found: C, 58.69; H, 5.28; N, 19.96.

6-Fluoro-4-(1-naphthylmethylamino)-7-(1-piperazino)quinazoline (8j). White solid (61% yield for three steps from **2**): mp 222–224 °C; ^{1}H NMR (DMSO-d_6) δ 8.51 (t, $J=5.1$ Hz, 1H, NHCH$_2$), 8.39 (s, 1H, H-2), 8.19–8.16 (m, 1H, ArH), 8.13 (d, $J_{H-F}=14.0$ Hz, 1H, H-5), 7.98–7.95 (m, 1H, ArH), 7.87–7.82 (m, 1H, ArH), 7.59–7.52 (m, 2H, ArH), 7.50–7.42 (m, 2H, ArH), 7.11 (d, $J_{H-F}=8.6$ Hz, 1H, H-8), 5.21 (d, $J=5.1$ Hz, 2H, NHCH_2), 3.10–3.07 (m, 4H, piperazinyl methylene), 2.90–2.87 (m, 4H, piperazinyl methylene); MS (TOF) m/z 388 $(M+H)^+$. Anal. calcd for $C_{23}H_{22}N_5F\cdot0.7H_2O$: C, 69.05; H, 5.72; N, 17.51. Found: C, 69.06; H, 5.58; N, 17.32.

Biology

LPS-induced TNF-α production in human PBMCs. Inhibition of TNF-α production was measured by ELISA using human PBMCs stimulated by LPS, as previously reported.[14] Briefly, human PBMCs from healthy volunteers were seeded (3×10^5 cells/mL RPMI 1640, 10% FCS/well, 100 U/mL of penicillin, and 100 μg/mL of streptomycin) into 96-well culture plates, and 100 μL of medium containing 20 ng/mL LPS (*Escherichia coli* 0111:B4, Difco) and test compounds were then added. Cultures were incubated at 37 °C for 16 h, and thereafter the supernatants were collected for the determination of the TNF-α level by ELISA. The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method.

T cell proliferation assay. T cell proliferation was determined by the MTS assay using Con A-stimulated mouse spleen cells, as previously described.[15] Briefly, male BALB/c mice, 10 weeks of age, were killed by cervical dislocation, and their spleens were removed, mashed in PBS, and filtered through a nylon mesh. The cell suspension was washed twice with RPMI 1640 medium and resuspended to 8×10^6 cells/mL in RPMI 1640 containing 10% FCS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Splenocytes (8×10^6 cells/mL) were cultured in 96-well plates in the presence of Con A (5 μg/mL) with test compounds at 37 °C under a 5% CO_2 atmosphere for 3 days. The MTS assay was performed by using a commercial kit (Promega), and formazan dye products were measured by absorbance at 490 nm using a microplate reader (Model 450, Bio-Rad). The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method.

B cell proliferation assay. Mouse spleen cell proliferation assay, with proliferation induced by LPS, a well-known inducer of mouse B cell proliferation, was performed according to the method previously described with minor modification.[9] Briefly, male BALB/c mice, 10 weeks of age, were killed by cervical dislocation, and their spleens were removed, mashed in PBS, and filtered through a nylon mesh. The cell suspension was washed twice with RPMI 1640 medium and resuspended to 8×10^5 cells/mL in RPMI 1640 containing 10% FCS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Splenocytes (8×10^5 cells/mL) were cultured in 96-well plates in the presence of LPS (3 μg/mL, serotype 0111:B4, DIFCO) with test compounds at 37 °C under a 5% CO_2 atmosphere for 3 days. Next, [^{3}H]thymidine at 50 μCi/mL was added into the medium, and the cells were incubated for 4 h at 37 °C under a 5% CO_2 atmosphere. Then the cells were harvested by trypsinization, and radioactive thymidine incorporation into DNA was determined by scintillation counting. The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method.

LPS-induced TNF-α production in mice. Inhibitory effects of **1** and **7a** against TNF-α production in LPS-treated mice were evaluated according to a previously reported method.[16] Briefly, BALB/c mice (Charles River Japan Inc.) were used at 10 weeks of age. LPS from *E. coli* (serotype 026:B6) was purchased from Difco Laboratories (Detroit, USA). A solution of a test compound in water was orally administered to mice 0.5 h prior to iv injection of the LPS (25 μg/mouse). Blood samples were obtained 2 h after the LPS injection. Amounts of TNF-α in the blood were determined by a specific ELISA kit (Genzyme Techne, USA).

Pharmacokinetic analysis. The pharmacokinetic parameters of **1** and **7a** were studied in rats. Briefly, in the rat iv or po administration studies, three male SD rats received either a 10 mg/kg intravenous dose or a 30 mg/kg oral dose. Blood samples were obtained after dosing at appropriate times and analyzed by reverse-phase HPLC. The pharmacokinetic parameters for the compounds were estimated by a non-compartmental method.

Anti-inflammatory effect on adjuvant arthritis.[7] Adjuvant arthritis was induced in male SD rats (7 weeks old; Charles River Japan Inc.). This was done by injecting Freund's complete adjuvant [a 0.6% suspension of *Mycobacterium butyricum* (Difco Labs) in liquid paraffin] (0.1 mL) into the right hind paw. A solution of **7a** in water (30 mg/kg) was orally administered once a day for 14 days. The administration was started just before sensitization (day 0). The severity of the induced adjuvant disease was followed by measurement of the volume of the non-injected left hind paw by using aqueous plethysmography.

Acknowledgements

We are grateful for all of the help provided by Dr. Tsutomu Mimoto and Dr. Satoru Misawa. In addition, we would like to express our thanks to Mr. Mitsuhiro Matsumoto, Mr. Toshiyuki Sato, Mr. Toru Negishi, and Mr. Tominaga Fukazawa for their technical assistance in obtaining the biological data.

References and Notes

1. (a) Dolhain, R. J. E. M.; Van Der Heiden, A. N.; Ter Haar, N. T.; Breedveld, F. C.; Miltenburg, A. M. M. *Arthritis Rheum.* **1996**, *39*, 1961. (b) Kusaba, M.; Honda, J.; Fukuda, T.; Oizumi, K. *J. Rheumatol.* **1998**, *25*, 1466.
2. (a) Miltenburg, A. M. M.; Van Laar, J. M.; De Kuiper, R.; Daha, M. R.; Breedveld, F. C. *Scand. J. Immunol.* **1992**, *35*, 603. (b) Quayle, A. J.; Chomarat, P.; Miossec, P.; Kjeldsen-Kragh, J.; Førre, Ø.; Natvig, J. B. *Scand. J. Immunol.* **1993**, *38*, 75. (c) Simon, A. K.; Seipelt, E.; Sieper, J. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8562. (d) Schulze-Koops, H.; Lipsky, P. E.; Kavanaugh, A. F.; Davis, L. S. *J. Immunol.* **1995**, *155*, 5029. (e) Van Roon, J. A. G.; Van Roy, J. L. A. M.; Duits, A.; Lafeber, F. P. J. G.; Bijlsma, J. W. J. *Ann. Rheum. Dis.* **1995**, *54*, 836.
3. Bemelmans, M. H. A.; Van Tits, L. J. H.; Buurman, W. A. *Crit. Rev. Immunol.* **1996**, *16*, 1.
4. (a) Maini, R. N.; St. Clair, E. W.; Breedveld, F. C.; Furst, D. E.; Kalden, J. R.; Weisman, M.; Smolen, J. S.; Emery, P.; Harriman, G. R.; Feldmann, M.; Lipsky, P. E. *Lancet* **1999**, *354*, 1932. (b) Lipsky, P. E.; Van Der Heijde, D. M. F. M.; St. Clair, E. W.; Furst, D. E.; Breedveld, F. C.; Kalden, J. R.; Smolen, J. S.; Weisman, M.; Emery, P.; Feldmann, M.; Harriman, G. R.; Maini, R. N. *N. Engl. J. Med.* **2000**, *343*, 1594. (c) Markham, A.; Lamb, H. M. *Drugs* **2000**, *59*, 1341. (d) Weinblatt, M. E.; Kremer, J. M.; Bankhurst, A. D.; Bulpitt, K. J.; Fleischmann, R. M.; Fox, R. I.; Jackson, C. G.; Lange, M. L. M.; Burge, D. J. *N. Engl. J. Med.* **1999**, *340*, 253. (e) Moreland, L. W.; Schiff, M. H.; Baumgartner, S. W.; Tindall, E. A.; Fleischmann, R. M.; Bulpitt, K. J.; Weaver, A. L.; Keystone, E. C.; Furst, D. E.; Mease, P. J.; Ruderman, E. M.; Horwitz, D. A.; Arkfeld, D. G.; Garrison, L.; Burge, D. J.; Blosch, C. M.; Lange, M. L. M.; McDonnell, N. D.; Weinblatt, M. E. *Ann. Intern. Med.* **1999**, *130*, 478. (f) Bathon, J. M.; Martin, R. W.; Fleischmann, R. M.; Tesser, J. R.; Schiff, M. H.; Keystone, E. C.; Genovese, M. C.; Wasko, M. C.; Moreland, L. W.; Weaver, A. L.; Markenson, J.; Finck, B. K. *N. Engl. J. Med.* **2000**, *343*, 1586.
5. Tobe, M.; Isobe, Y.; Tomizawa, H.; Nagasaki, T.; Obara, F.; Matsumoto, M.; Hayashi, H. *Chem. Pharm. Bull.* **2002**, *50*, 1073.
6. For the purpose of evaluating the cytotoxicity of the compounds, we assessed cell viability by the MTS assay when we assayed TNF-α production.
7. Perper, R. J.; Alvarez, B.; Colombo, C.; Schroder, H. *Proc. Soc. Exp. Biol. Med.* **1971**, *137*, 506.
8. (a) Holoshitz, J.; Matitiau, A.; Cohen, I. R. *J. Clin. Invest.* **1984**, *73*, 211. (b) Van Eden, W.; Holoshitz, J.; Nevo, Z.; Frenkel, A.; Klajman, A.; Cohen, I. R. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5117. (c) Lider, O.; Karin, N.; Shinitzky, M.; Cohen, I. R. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4577. (d) Smith-Oliver, T.; Noel, L. S.; Simpson, S. S.; Yarnall, D. P.; Connolly, K. M. *Cytokine* **1993**, *5*, 298. (e) DiMartino, M.; Wolff, C.; High, W.; Stroup, G.; Hoffman, S.; Laydon, J.; Lee, J. C.; Bertolini, D.; Galloway, W. A.; Crimmin, M. J.; Davis, M.; Davies, S. *Inflamm. Res.* **1997**, *46*, 211.
9. Mouse spleen cell proliferation assay, with proliferation induced by LPS, a well-known inducer of mouse B cell proliferation, was performed according to the method previously described with minor modification; see: Apfel, C.; Bauer, F.; Crettaz, M.; Forni, L.; Kamber, M.; Kaufmann, F.; LeMotte, P.; Pirson, W.; Klaus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *4*, 7129.
10. Vossen, A. C. T. M.; Savelkoul, H. F. J. *Mediat. Inflamm.* **1994**, *3*, 403.
11. Gerlag, D. M.; Ransone, L.; Tak, P. P.; Han, Z.; Palanki, M.; Barbosa, M. S.; Boyle, D.; Manning, A. M.; Firestein, G. S. *J. Immunol.* **2000**, *165*, 1652.
12. Souness, J. E.; Griffin, M.; Maslen, C.; Ebsworth, K.; Scott, L. C.; Pollock, K.; Palfreyman, M. N.; Karlsson, J.-A. *Br. J. Pharmacol.* **1996**, *118*, 649.
13. These biological assays were performed by the MDS Pharma Services.
14. (a) Henry, J. R.; Rupert, K. C.; Dodd, J. H.; Turchi, I. J.; Wadsworth, S. A.; Cavender, D. E.; Fahmy, B.; Olini, G. C.; Davis, J. E.; Pellegrino-Gensey, J. L.; Schafer, P. H.; Siekierka, J. J. *J. Med. Chem.* **1998**, *41*, 4196. (b) Henry, J. R.; Rupert, K. C.; Dodd, J. H.; Turchi, I. J.; Wadsworth, S. A.; Cavender, D. E.; Schafer, P. H.; Siekierka, J. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3335.
15. (a) Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207. (b) Yamahara, J.; Shimoda, H.; Matsuda, H.; Yoshikawa, M. *Biol. Pharm. Bull.* **1996**, *19*, 1241.
16. Estrada, A.; Van Kessel, A.; Yun, C. H.; Li, B. *Immunopharmacol. Immunotoxicol.* **1998**, *20*, 217.