Structure–Activity Relationships of 6-Nitroquinazolines: Dual-Acting Compounds with Inhibitory Activities toward both TNF-α Production and T Cell Proliferation

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We synthesized various 6-nitroquinazolines by modifying the structure of compound 1 and evaluated their inhibitory activities toward both TNF-α production and T cell proliferation responses. The presence of the unsubstituted piperazine ring at the C(7)-position was required for both inhibitory activities. In this series of compounds, 5d and 5f, containing the 4-fluorophenyl and 3,4-difluorophenyl moiety, respectively, at the C(4)-position, showed the suppressing effects toward both responses with low cell growth inhibition. Furthermore, the oral administration of these compounds mentioned above at doses of 30 and 100 mg/kg also resulted in significant inhibition of TNF-α production induced by LPS *in vivo*.

Key words TNF-α production; T cell proliferation; 6-nitroquinazoline

Tumor necrosis factor α (TNF-α), which is mainly produced by activated monocytes and macrophages,[1] plays an important role in the protective immune response of the host against bacterial and viral infections. For example, TNF-α is an essential substance for granuloma formation and the control of bacterial dissemination in experimental tuberculosis in mice.[2,3] Additionally, TNF-α added to infected cells inhibits the replication of both DNA and RNA viruses.[4,5]

On the other hand, TNF-α is a key cytokine in the inflammatory cascade[6]; and elevated TNF-α levels in serum are associated with autoimmune diseases such as rheumatoid arthritis (RA),[7,8] septic shock,[9] Crohn's disease,[10] and multiple sclerosis.[11—14] The best example for this evidence is the dramatic reduction in disease activity observed in RA[15,16] and Crohn's disease[17] after treatment of such patients with anti-TNF-α chimera antibody (Remicade™). Accordingly, anti-TNF-α therapy may be a hopeful treatment for these diseases. In addition, autoimmune diseases are considered to be caused by abnormalities of T cell immune responses. In particular, the activation of T cells (especially Th1) may be involved in a possible pathogenesis by which inflammation is accelerated in these diseases.[18—21] Therefore, the correction of the abnormal immune responses mediated by T cell is also regarded as a possible approach for treatment of these diseases.

In view of these reports, we concluded that ideal anti-autoimmune disease agents should possess inhibitory activities toward both TNF-α production and T cell proliferation. Although glucocorticoids show the inhibition toward both of these activities, the long-term use of them is well known to give rise to several side effects such as infection and osteoporosis. Therefore, there is a great need for new nonsteroidal compounds that have the same efficacy as steroids but without the side effects.

Recently, we reported that nonsteroidal compound **1** (Fig. 1) showed inhibitory activities toward both TNF-α production and T cell proliferation.[22] In this study, we examined further the structure–activity relationships of 6-nitroquinazolines by generating new drugs made on the basis of **1** by placing various substituents on the phenyl ring at the C(4)-position, replacing a piperazine at the C(7)-position with other heterocycles, such as a morpholine and piperidines, and varying the methylene chain length at the C(4)-position. In addition, we also evaluated the inhibitory effects of several compounds on TNF-α production *in vivo*.

Chemistry The general synthetic pathway to the 4,7-disubstituted-6-nitroquinazolines is shown in Chart 1. The key intermediate 4,7-dichloro-6-nitroquinazoline (**3**) was prepared by the chlorination of 7-chloro-6-nitro-4-quinazolone (**2**)[23] with thionyl chloride in good yield. Compound **3** was subsequently treated with the corresponding amines to give

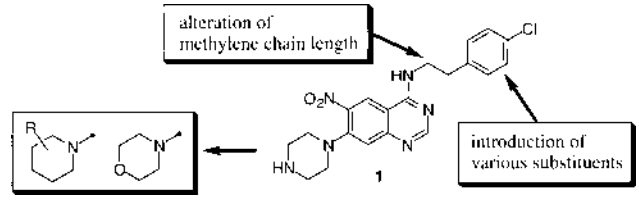
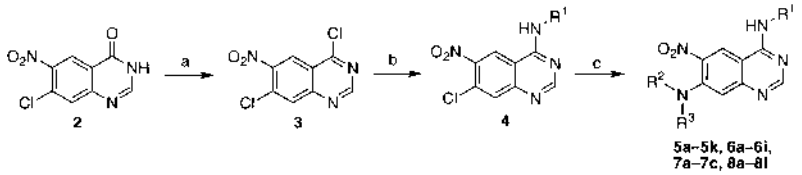


Fig. 1. Design of 6-Nitroquinazolines



Reagents and conditions: (a) $SOCl_2$, DMF, reflux; (b) R^1NH_2, triethylamine, *i*-PrOH; (c) R^2R^3NH, *N,N'*-diisopropylethylamine, *n*-BuOH, 110°C.

Chart 1. General Procedure for 4,7-Di-substituted-6-nitroquinazolines

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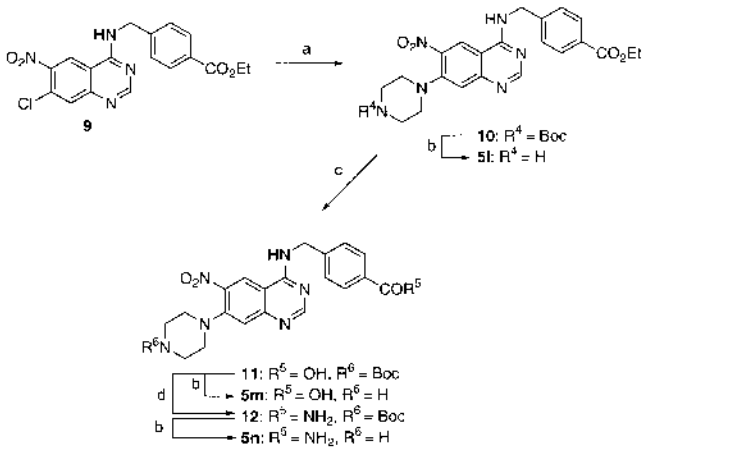
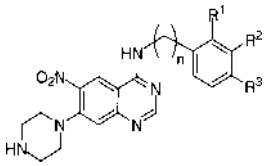


Reagents and conditions: (a) 1-(*tert* butoxycarbonyl)piperazine, *N,N'*-diisopropylethylamine, *n*-BuOH, 110°C; (b) 4 N HCl 1,4-dioxane; (c) 5 N NaOH, EtOH; (d) 28% aqueous ammonia solution, EDC•HCl, HOBt, DMF.

Chart 2. Synthesis of Compounds **5l**—**n**

Table 1. Inhibition of TNF-α Production and T Cell Proliferation by Compounds **5a**—**n**



Compound	R^1	R^2	R^3	*n*	IC_{50} (μM)		% Inhibition
					TNF-α[a]	Con A[b]	Cytotoxicity[c]
1	H	H	Cl	2	0.8	1.1	48.5
5a	H	H	Cl	1	0.7	2	46.3
5b	F	H	H	1	0.5	9.6	3.3
5c	H	F	H	1	0.4	4.5	3.6
5d	H	H	F	1	0.4	3	4.3
5e	F	H	F	1	0.5	4.5	3.8
5f	H	F	F	1	0.4	3.2	4.2
5g	H	H	H	1	0.9	6.3	2.5
5h	H	H	CF_3	1	2.4	3.7	17.7
5i	H	H	NO_2	1	2.4	7.8	20.1
5j	H	H	CN	1	0.8	>10	2.3
5k	H	H	NHAc	1	>10	>10	1.4
5l	H	H	CO_2Et	1	2.5	5.5	25.6
5m	H	H	CO_2H	1	>10	>10	1.5
5n	H	H	$CONH_2$	1	>10	>10	1.7
Dexamethasone					0.01	0.005	100

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*) Inhibition of the growth of human PBMCs stimulated by LPS at 10 μM test compound.

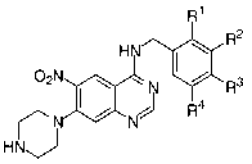
4-substituted-7-chloro-6-nitroquinazolines (**4**). Reaction of these compounds with amines, such as piperazines and piperidines, in the presence of Hunig's base provided compounds **5a**—**k**, **6a**—**i**, **7a**—**c**, and **8a**—**l**.

Compounds **5l**—**n** were prepared by following the reaction sequences described in Chart 2. Compound **9** was substituted with *N*-Boc-piperazine to give the key intermediate *N*-Boc-protected ester **10**. The *N*-Boc deprotection using 4 N HCl in dioxane afforded **5l** in 65% yield. Compound **10** was hydrolyzed to give **11**, which was then deprotected the Boc group to provide **5m**. Treatment of **11** with EDC (*N*-ethyl-*N'*-[3-(dimethylamino)propyl] carbodiimide), and HOBt (1-hydroxybenzotriazole) followed by the additon of 28% ammonia gave **12**, which was subsequently deprotected the Boc group to give the amide **5n**.

Results and Discussion

The compounds listed in Tables 1—4 were evaluated for their inhibitory activities toward both TNF-α production and T cell proliferation. The potency of the inhibition of TNF-α production was measured by ELISA using human peripheral blood mononuclear cells (PBMCs) stimulated by LPS, as previously reported.[24,25] The cell growth inhibition of human PBMCs was measured by the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] assay.[26] Suppression of concanavalin

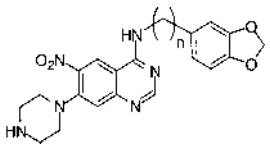
Table 2. Inhibition of TNF-α Production and T Cell Proliferation by Compounds **6a**—**i**



Compound	R^1	R^2	R^3	R^4	IC_{50} (μM)		% Inhibition
					TNF-α[a]	Con A[b]	Cytotoxicity[c]
6a	H	H	Me	H	1.1	1.9	50.4
6b	H	H	MeO	H	0.9	2.1	52.5
6c	H	MeO	H	H	1.7	5.6	16.1
6d	MeO	H	H	H	1.2	7.2	10.2
6e	H	H	MeO	MeO	5.2	7.6	9.2
6f	H	MeO	MeO	MeO	7.5	>10	11.8
6g	H	H	–OCH_2O–		0.08	2.7	51.1
6h	H	MeO	–OCH_2O–		1.5	6	8.1
6i	Cl	H	–OCH_2O–		0.7	3.8	10
Dexamethasone					0.01	0.005	100

a—*c*) See corresponding footnotes in Table 1.

Table 3. Inhibition of TNF-α Production and T Cell Proliferation by Compounds **7a**—**c**



Compound	*n*	IC_{50} (μM)		% Inhibition
		TNF-α[a]	Con A[b]	Cytotoxicity[c]
7a	0[d]	3.3	5.1	45.1
6g	1	0.08	2.7	51.1
7b	2	0.7	4.2	48.5
7c	3	1.1	6.4	10.2

a—*c*) See corresponding footnotes in Table 1. *d*) 0 means the anilino derivative.

A (Con A) induced proliferation of mouse spleen cells was determined by the MTS assay according to the previously described method.[27,28]

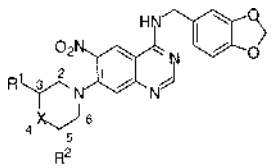
At first, comparing the results for **1** and **5a**, we found that these compounds had nearly equal inhibitory activity toward TNF-α production and T cell proliferation. On the basis of this data, we performed a substitution study on the phenyl group at the C(4)-position by using the benzyl type derivative. As shown in Table 1, the introduction of a fluorine atom on the phenyl group (**5b**—**f**) resulted in an approximately 2-fold greater potency against TNF-α inhibitory activity as compared with the potency of **1**. The change in the position of the fluorine atom from *para* (**5d**) to *ortho* (**5b**) or *meta* (**5c**) reduced the inhibitory activities toward T cell proliferation, whereas the substitution pattern (*ortho*, *meta*, *para*) had no affect on the TNF-α inhibitory activity. It should be noted that **5d** showed reduced cell growth inhibition; *i.e.*, the cell growth inhibition of compound **1** was 48.5% at 10 μM, whereas that of **5d** was only 4.3%. The activities of compounds having an electron-withdrawing moiety in the *para* position of the phenyl group (such as the trifluoromethyl (**5h**), the nitro (**5i**), the cyano (**5j**), and the ester (**5l**) analogues) were not improved toward either response. The introduction of polar, hydrogen bonding groups also diminished both inhibitory activities (**5k**, **m**, and **n**). These results suggest that the placement of a hydrophilic group on the phenyl group was not suitable to exert either inhibitory activity.

Secondly, we investigated the effect of an electron-donating moiety on the phenyl group (Table 2). Based on the fluorine substitution study, the same tendency was observed when the position of the methoxy group on the phenyl group was changed from *para* to *ortho* or *meta*. That is, the *para* substitution (**6b**) was suitable for maintaining both inhibitory activities. The 3,4-dimethoxy (**6e**) and the 3,4,5-trimethoxy (**6f**) analogues decreased both inhibitory activities. However, the replacement the 3,4-dimethoxy group with the 3,4-methylenedioxy one (**6g**) led to a 10-fold increase in the TNF-α inhibitory activity as compared with that of **1**. The comparison between **6f** and **6h** also showed that the substitution with the 3,4-methylenedioxy group was more preferable than that with the 3,4-dimethoxy group for the TNF-α inhibitory activity. These results indicate that the substituents on the phenyl group must be sterically compact to show a potent TNF-α inhibitory activity.

Thirdly, we explored the effects of varying the length of the linker between the quinazoline and the phenyl group at the C(4)-position of the quinazoline (Table 3). The maximum potency of TNF-α inhibitory activity was obtained when the spacer was the methylene chain (**6g**). The optimal length was also confirmed to be 1 methylene unit (**6g**) for suppressing T cell proliferation.

Finally, to further refine the framework at the C(7)-position, we investigated the effects of the *C*-methylpiperazines and the replacement of the piperazine ring with other heterocycles (Table 4). The 3-methylpiperazine analogues (**8a**, **b**) resulted in a 4—5-fold loss in TNF-α inhibitory activity as compared with the activity of **6g**. The 3,5-dimethypiperazine (**8c**) led to dramatically decreased inhibitory activities toward TNF-α production and T cell proliferation. These results suggest that the presence of the simple piperazine ring at the

Table 4. Inhibition of TNF-α Production and T Cell Proliferation by Compounds **8a**—**l**



Compound	X	R^1	R^2	IC_{40} (μM)		% Inhibition
				TNF-α[a]	Con A[b]	Cytotoxicity[c]
6g	NH	H	H	0.08	2.7	51.1
8a	NH	3(*S*)-Me	H	0.4	3.6	49.3
8b	NH	3(*R*)-Me	H	0.3	3.5	47.4
8c	NH	*cis*-3,5-dimethyl		>10	>10	5
8d	NCO_2Et	H	H	>10	>10	1.8
8e	O	H	H	6.9	>10	8.7
8f	CH_2	H	H	>10	>10	1.5
8g	CHOH	H	H	7.1	>10	6.8
8h	$CHCH_2OH$	H	H	>10	8.2	10
8i	$CHCO_2Et$	H	H	>10	>10	19.7
8j	$CHCO_2H$	H	H	>10	>10	12.3
8k	CH_2	OH	H	>10	>10	11.5
8l	CH_2	CH_2OH	H	>10	>10	5.9
Dexamethasone				0.01	0.005	100

a—*c*) See corresponding footnotes in Table 1.

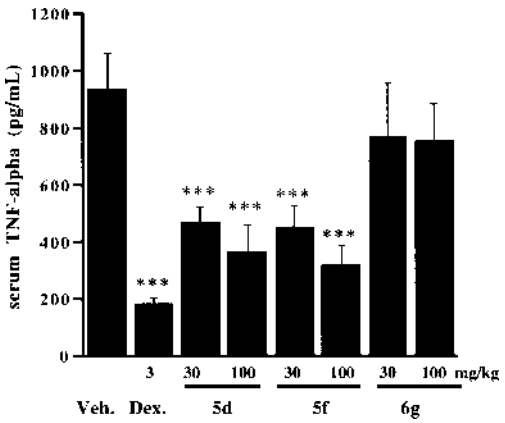


Fig. 2. Effects of **5d**, **5f**, and **6g** on LPS-Induced TNF-α Production in Mice

Compounds **5d**, **5f**, and **6g** were evaluated as their corresponding hydrochloride salts. The results are expressed as the mean±S.E.M. of 5 mice per group. ***$p<0.001$ *versus* vehicle control (Dunnett's test). Veh., vehicle control; Dex., dexamethasone.

C(7)-position was necessary to show both inhibitory activities. Compound **8d**, in which the NH group of the piperazine ring was blocked by the ethoxycarbonyl group, had notably decreased inhibitory activities toward both parameters. Moreover, other heterocycle analogues (**8e**—**l**) (morpholine and piperidine), which are lacking in a basic nitrogen, also showed loss of both inhibitory activities. The above-mentioned results support the possibility of the interaction between the distal piperazine nitrogen and some target molecules which we have not identified yet.

We selected 3 compounds (**5d**, **f**, **6g**) from the results of above *in vitro* study to further evaluate their activities toward TNF-α production *in vivo* (Fig. 2). By oral administration, both **5d** and **5f** showed almost the same inhibitory activity against LPS-induced TNF-α production, whereas **6g** was less active in spite of its highly inhibitory activity *in vitro*. These results suggest the poor oral bioavailability of **6g**. Additionally, **5d** and **6g** also showed potent inhibitory activities against B cell proliferation (**5d**, $IC_{50}=0.3\,\mu$M; **6g**, $IC_{50}=0.3\,\mu$M). This property suggests possible suppression of autoantibody production, which would make these compounds desirable drug candidates for RA. Therefore, to develop a novel compound exhibiting more potency and orally bioavailability than **6g**, we are planning further chemical modification of **6g**.

Conclusions

A number of 6-nitroquinazolines were synthesized and evaluated for their inhibitory activities toward both TNF-α production and T cell proliferation. We determined that the best framework at the C(7)-position was the simple piperazine ring. Furthermore, in this series of compounds, we found that the introduction of a fluorine atom on the phenyl group resulted in reduced cell toxicity as well as in improved inhibitory activities. Especially, **5d** and **5f** exhibited almost the same inhibitory activity toward both TNF-α production and T cell proliferation *in vitro*, and inhibited the LPS-induced TNF-α production when given by oral administration. Although **6g** exerted the most potent activity against TNF-α production ($IC_{50}=0.08\,\mu$M), it failed to inhibit this response by oral administration, probably due to its poor oral bioavailability. At present, we are continuing our study to obtain even more potent and orally active quinazoline derivatives.

Experimental

Chemistry 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. Proton NMR spectra were recorded on a JEOL GSX270 FT NMR spectrometer. Chemical shifts were given in δ values (ppm) using tetramethylsilane as an internal standard, and following abbreviations were used: s=singlet,

d=doublet, t=triplet, q=quartet, m=multiplet, bs=broad singlet, and dd=double doublet. Time-of-flight mass spectrometry (TOF-MS) was recorded on a KOMPACT MALDI III spectrometer. Optical rotations were determined by using a JASCO DIP-370 digital polarimeter. Elemental analyses were performed at the Toray Research Center. Wakogel C-200 (Wako; 70—150 μm) 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). Following abbreviations are used for solvents: DMF (*N*,*N*-dimethylformamide), AcOEt (ethyl acetate).

General Procedure for Preparation of 4,7-Di-substituted-6-nitroquinazolines. 4-Benzylamino-6-nitro-7-(1-piperazino)quinazoline (5g) A suspension of 7-chloro-6-nitro-4-quinazolone (**2**)[23] (250 mg, 1.11 mmol) in thionyl chloride (6.0 ml) containing 1 drop of *N*,*N*-dimethylformamide (DMF) was refluxed for 2 h to give a clear solution. Excess thionyl chloride was removed under reduced pressure to provide crude 4,7-dichloro-6-nitroquinazoline (**3**), which was used directly. To a mixture of **3** and triethylamine (185 μl, 1.33 mmol) in *i*-PrOH (12 ml) was added benzylamine (145 μl, 1.33 mmol). The resulting mixture was stirred at ambient temperature 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–hexane (1 : 1, v/v). The light yellow solid was filtered to give 4-benzylamino-7-chloro-6-nitroquinazoline (269 mg, 77% yield). Next, to a mixture of the above compound (150 mg, 0.48 mmol) and *N*,*N*′-diisopropylethylamine (502 μl, 2.88 mmol) in *n*-BuOH (9.5 ml) was added piperazine (248 mg, 2.88 mmol). The reaction was stirred at 110 °C for 10 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, dried over Na_2SO_4. The solution was evaporated *in vacuo*, and the yellow residue was suspended in CH_2Cl_2–hexane (1 : 1, v/v) until a solid formed. The light yellow solid was filtered to give **5g** (130 mg, 74% yield). mp 189—191 °C. ^{1}H-NMR ($CDCl_3$) δ: 8.66 (1H, s), 8.20 (1H, s), 7.41—7.36 (5H, m), 7.33 (1H, s), 5.92 (1H, t, J=4.9 Hz), 4.87—4.85 (2H, m), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m). TOF-MS *m*/*z*: 365 $(M+H)^+$. *Anal.* Calcd for $C_{19}H_{20}N_6O_2\cdot 0.1H_2O$: C, 62.31; H, 5.53; N, 22.95. Found: C, 62.17; H, 5.59; N, 22.84.

Similarly to the procedure described for **5g**, compounds **5a**—**f** and **5h**—**k** were prepared from **2**.

4-(4-Chlorobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5a) Yellow solid (30% yield for 3 steps from **2**); mp, 209—210 °C; ^{1}H-NMR (DMSO-d_6) δ: 9.08 (1H, t, J=5.9 Hz), 8.91 (1H, s), 8.44 (1H, s), 7.38 (4H, bs), 7.19 (1H, s), 4.74 (2H, d, J=5.9 Hz), 3.00—2.97 (4H, m), 2.83—2.79 (4H, m); TOF-MS *m*/*z*: 399 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{19}ClN_6O_2$: C, 57.22; H, 4.80; N, 21.07. Found: C, 57.03; H, 4.54; N, 21.24.

4-(2-Fluorobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5b) Yellow solid (18% yield for 3 steps from **2**); mp, 194—196 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.21 (1H, s), 7.48—7.31 (3H, m), 7.17—7.08 (2H, m), 5.99 (1H, t, J=3.5 Hz), 4.93 (2H, d, J=3.5 Hz), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 383 $(M+H)^+$+; *Anal.* Calcd for $C_{19}H_{19}FN_6O_2\cdot 0.1H_2O$: C, 59.40; H, 5.01; N, 21.87. Found: C, 59.21; H, 4.97; N, 21.92.

4-(3-Fluorobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5c) Yellow solid (11% yield for 3 steps from **2**); mp, 191—193 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.25 (1H, s), 7.39—7.30 (2H, m), 7.18—6.99 (3H, m), 6.05 (1H, t, J=5.4 Hz), 4.89—4.87 (2H, m), 3.14—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 383 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{19}FN_6O_2\cdot 0.3H_2O$: C, 58.85; H, 5.01; N, 21.67. Found: C, 58.71; H, 4.75; N, 21.42.

4-(4-Fluorobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5d) Yellow solid (50% yield for 3 steps from **2**); mp, 216—218 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.21 (1H, s), 7.40—7.33 (3H, m), 7.10—7.03 (2H, m), 5.92 (1H, t, J=3.8 Hz), 4.84 (2H, d, J=3.8 Hz), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 383 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{19}FN_6O_2$: C, 59.68; H, 5.01; N, 21.98. Found: C, 59.56; H, 5.02; N, 22.30.

Compound **5d** was converted to its hydrochloride salt according to the following procedure: To a suspension of **5d** (100 mg, 0.26 mmol) in EtOH (8 ml) was added 12 N HCl (65 μl). The mixture was stirred at ambient temperature for 4 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 pale yellow powder (102 mg, 94% yield). mp 251 °C (Dec.). ^{1}H-NMR (DMSO-d_6) δ: 10.80 (1H, bs), 9.39 (2H, bs), 9.29 (1H, s), 8.86 (1H, s), 7.49—7.44 (3H, m), 7.23—7.16 (2H, m), 4.90 (2H, d, J=5.4 Hz), 3.42—3.26 (8H, m); *Anal.* Calcd for $C_{19}H_{20}ClFN_6O_2\cdot 2.5H_2O$: C, 49.19; H, 4.89; N, 18.12. Found: C, 48.82; H, 4.78; N, 18.05.

4-(2,4-Difluorobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5e) Yellow solid (33% yield for 3 steps from **2**); mp, 182—184 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.21 (1H, s), 7.50—7.41 (1H, m), 7.33 (1H, s), 6.90—6.84 (2H, m), 5.97 (1H, t, J=4.1 Hz), 4.88 (2H, d, J=4.1 Hz), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 401 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{18}F_2N_6O_2$: C, 57.00; H, 4.53; N, 20.99. Found: C, 56.83; H, 4.83; N, 20.80.

4-(3,4-Difluorobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5f) Yellow solid (26% yield for 3 steps from **2**); mp, 203—205 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.24 (1H, s), 7.34 (1H, s), 7.24—7.12 (3H, m), 6.04 (1H, t, J=3.8 Hz), 4.84 (2H, d, J=3.8 Hz), 3.14—3.11 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 401 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{18}F_2N_6O_2$: C, 57.00; H, 4.53; N, 20.99. Found: C, 56.92; H, 4.56; N, 21.18.

Similarly to the procedure described for **5d**, compound **5f** was converted to its hydrochloride salt (92% yield); mp, 248 °C (Dec.); ^{1}H-NMR (DMSO-d_6) δ: 10.63 (1H, bs), 9.27—9.25 (3H, m), 8.82 (1H, s), 7.54—7.28 (4H, m), 4.88 (2H, d, J=5.1 Hz), 3.41—3.26 (8H, m); *Anal.* Calcd for $C_{19}H_{19}ClF_2N_6O_2\cdot 2.2H_2O$: C, 47.89; H, 4.48; N, 17.64. Found: C, 48.00; H, 4.27; N, 17.33.

6-Nitro-7-(1-piperazino)-4-(4-trifluoromethylbenzylamino)quinazoline (5h) Yellow solid (35% yield for 3 steps from **2**); mp, 215—217 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.25 (1H, s), 7.63 (2H, d, J=8.4 Hz), 7.51 (2H, d, J=8.4 Hz), 7.35 (1H, s), 6.06 (1H, t, J=4.6 Hz), 4.95 (2H, d, J=4.6 Hz), 3.14—3.11 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 433 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{19}F_3N_6O_2\cdot 0.2H_2O$: C, 55.09; H, 4.44; N, 19.28. Found: C, 54.90; H, 4.52; N, 19.43.

6-Nitro-4-(4-nitrobenzylamino)-7-(1-piperazino)quinazoline (5i) Yellow solid (18% yield for 3 steps from **2**); mp, 182—184 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.62 (1H, s), 8.35 (1H, s), 8.20 (2H, d, J=8.6 Hz), 7.55 (2H, d, J=8.6 Hz), 7.34 (1H, s), 6.49 (1H, t, J=5.9 Hz), 5.00 (2H, d, J=5.9 Hz), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 410 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{19}N_7O_4\cdot 0.9H_2O$: C, 53.62; H, 4.71; N, 23.04. Found: C, 53.76; H, 5.10; N, 22.98.

4-(4-Cyanobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5j) Yellow solid (23% yield for 3 steps from **2**); mp, 207—209 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.61 (1H, s), 8.34 (1H, s), 7.64 (2H, d, J=8.9 Hz), 7.50 (2H, d, J=8.9 Hz), 7.33 (1H, s), 6.45 (1H, t, J=5.7 Hz), 4.96 (2H, d, J=5.7 Hz), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS *m*/*z*: 390 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{19}N_7O_3\cdot 0.7H_2O$: C, 59.75; H, 4.94; N, 24.39. Found: C, 59.65; H, 5.08; N, 24.78.

4-(4-Acetamidobenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5k) Yellow solid (8% yield for 3 steps from **2**); mp, 253—255 °C; ^{1}H-NMR (DMSO-d_6) δ: 9.90 (1H, s), 9.02 (1H, t, J=5.4 Hz), 8.92 (1H, s), 8.45 (1H, s), 7.52 (2H, d, J=8.4 Hz), 7.28 (2H, d, J=8.4 Hz), 7.18 (1H, s), 4.70 (2H, t, J=5.4 Hz), 3.00—2.97 (4H, m), 2.83—2.80 (4H,m), 2.20 (3H, s); TOF-MS *m*/*z*: 422 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{23}N_7O_3\cdot 0.5H_2O$: C, 58.59; H, 5.50; N, 22.78. Found: C, 58.51; H, 5.56; N, 22.90.

7-[1-(4-*tert*-Butoxycarbonyl)piperazino]-4-(4-ethoxycarbonylbenzylamino)-6-nitroquinazoline (10) Similarly to the procedure described for **5g**, the title compound was prepared from 7-chloro-4-(4-ethoxycarbonylbenzylamino)-6-nitroquinazoline (**9**) (300 mg, 0.78 mmol) and 1-(*tert*-butoxycarbonyl)piperazine (433 mg, 2.33 mmol). After purification, **10** was obtained as a orange solid (270 mg, 65% yield); mp, 201—203 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.34 (1H, s), 7.97 (1H, s), 7.72 (2H, d, J=8.4 Hz), 7.12 (2H, d, J=8.4 Hz), 7.04 (1H, s), 5.76 (1H, t, J=3.2 Hz), 4.63 (2H, d, J=3.2 Hz), 4.06 (2H, q, J=7.0 Hz), 3.32—3.29 (4H, m), 2.81—2.77 (4H, m), 1.17 (9H, s), 1.07 (3H, t, J=7.0 Hz); TOF-MS *m*/*z*: 537 $(M+H)^+$; *Anal.* Calcd for $C_{27}H_{32}N_6O_6$: C, 60.44; H, 6.01 N, 15.66. Found: C, 60.10; H, 6.00; N, 15.37.

4-(4-Ethoxycarbonylbenzylamino)-6-nitro-7-(1-piperazino)quinazoline (5l) To a suspension of **10** (100 mg, 0.19 mmol) in 1,4-dioxane (3.0 ml) was added 4 N HCl–1,4-dioxane (1.3 ml) dropwisely at ambient temperature. The reaction mixture was stirred at the same temperature for 8 h, and was concentrated under reduced pressure. The 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, dried over Na_2SO_4. The solution was evaporated under reduced pressure, and the yellow residue was suspended in CH_2Cl_2–hexane (1 : 2, v/v). The light yellow solid was filtered to give **5l** (53 mg, 65% yield); mp, 179—181 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s),

8.25 (1H, s), 8.04 (2H, d, J=8.1 Hz), 7.45 (2H, d, J=8.1 Hz), 7.34 (1H, s), 6.06 (1H, t, J=3.8 Hz), 4.94 (2H, d, J=3.8 Hz), 4.38 (2H, q, J=7.2 Hz), 3.14—3.11 (4H, m), 3.06—3.03 (4H, m), 1.39 (3H, t, J=7.2 Hz); TOF-MS m/z: 437 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{24}N_6O_4$: C, 60.54; H, 5.54; N, 19.25. Found: C, 60.17; H, 5.53; N, 19.20.

4-[[7-[1-(4-*tert*-Butoxycarbonyl)piperazino]-6-nitro-4-quinazolinyl]-aminomethyl]benzoic Acid (11) A solution of **10** (270 mg, 0.50 mmol) in EtOH (20 ml) containing 5 N NaOH (500 μl) was stirred at ambient temperature for 10 h. The reaction mixture was neutralized with 6 N HCl and concentrated under reduced pressure. The residue was partitioned between AcOEt and 5% aqueous citric acid solution. The organic layer was washed with water and brine, dried over Na_2SO_4. The solution was evaporated *in vacuo*, and the yellow residue was triturated with AcOEt–hexane (5 : 1, v/v). The yellow solid was filtered to give **11** (212 mg, 83% yield); mp, 258—260 °C; ^{1}H-NMR (DMSO-d_6) δ: 12.87 (1H, bs), 9.21 (1H, t, J=5.8 Hz), 9.00 (1H, s), 8.46 (1H, s), 7.90 (2H, d, J=8.1 Hz), 7.46 (2H, d, J=8.1 Hz), 7.28 (1H, s), 4.83 (2H, d, J=5.8 Hz), 3.47—3.45 (4H, m), 3.09—3.07 (4H, m), 1.43 (9H, s); TOF-MS m/z: 509 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{28}N_6O_6$: C, 59.05; H, 5.55; N, 16.53. Found: C, 58.96; H, 5.56; N, 16.39.

4-[[6-Nitro-7-(1-piperazino)-4-quinazolinyl]aminomethyl]benzoic Acid (5m) Similarly to the procedure described for **5l**, the title compound was prepared from the *N*-Boc-protected acid **11** (70 mg, 0.14 mmol). After purification, **5m** was obtained as a yellow solid (50 mg, 89% yield); mp, 266 °C (Dec.); ^{1}H-NMR (DMSO-d_6) δ: 9.16 (1H, t, J=3.8 Hz), 8.94 (1H, s), 8.44 (1H, s), 7.89 (2H, d, J=8.1 Hz), 7.44 (2H, d, J=8.1 Hz), 7.21 (1H, s), 4.82 (2H, d, J=3.8 Hz), 3.02—3.00 (4H, m), 2.86—2.84 (4H, m); TOF-MS m/z: 409 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{20}N_6O_4\cdot 1.3H_2O$: C, 55.63; H, 4.97; N, 19.46. Found: C, 55.59; H, 5.18; N, 19.37.

4-[[7-[1-(4-*tert*-Butoxycarbonyl)piperazino]-6-nitro-4-quinazolinyl]-aminomethyl]benzamide (12) To a solution of **11** (200 mg, 0.39 mmol) and HOBt (64 mg, 0.47 mmol) in DMF (8 ml) was added EDC·HCl (90 mg, 0.47 mmol). The mixture was stirred at ambient temperature for 1 h. The resulting solution was cooled to 0 °C, then 28% ammonia solution was added and temperature was allowed to rise to ambient temperature. After 8 h, the reaction mixture was concentrated under reduced pressure, and the residue was partitioned CH_2Cl_2 and 5% aqueous $NaHCO_3$ solution. The organic layer was washed with water and brine, and then dried over Na_2SO_4. The solution was evaporated *in vacuo*, and the residue was purified by column chromatography on SiO_2 with CH_2Cl_2–MeOH (98 : 2, v/v) to give **12** as the pale yellow solid (157 mg, 78% yield); mp, 251—253 °C; ^{1}H-NMR (DMSO-d_6) δ: 9.18 (1H, t, J=5.9 Hz), 8.99 (1H, s), 8.46 (1H, s), 7.91 (1H, bs), 7.82 (2H, d, J=8.4 Hz), 7.41 (2H, d, J=8.4 Hz), 7.31 (1H, bs), 7.27 (1H, s), 4.81 (2H, d, J=5.9 Hz), 3.49—3.46 (4H, m), 3.09—3.06 (4H, m), 1.43 (9H, s); TOF-MS m/z: 508 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{29}N_7O_5$: C, 58.95; H, 5.76; N, 19.25. Found: C, 58.89; H, 5.87; N, 18.90.

4-[[6-Nitro-7-(1-piperazino)-4-quinazolinyl]aminomethyl]benzamide Hydrochloride (5n) To a suspension of **12** (100 mg, 0.20 mmol) in EtOH (2.0 ml) was added 4 N HCl in 1,4-dioxane (2.0 ml) dropwisely at ambient temperature for 10 h. The reaction mixture was concentrated under reduced pressure, and the residue was triturated with Et_2O. The pale yellow solid was filtered to give **5n** (76 mg, 87% yield); mp, 263 °C (Dec.); ^{1}H-NMR (DMSO-d_6) δ: 10.97 (1H, bs), 9.48 (2H, bs), 9.34 (1H, s), 8.86 (1H, s), 7.97 (1H, bs), 7.86 (2H, d, J=8.4 Hz), 7.52—7.46 (3H, m), 7.36 (1H, bs), 4.96 (2H, d, J=5.4 Hz), 3.43—3.26 (8H, m); *Anal.* Calcd for $C_{20}H_{22}ClN_7O_3\cdot 4.5H_2O$: C, 45.76; H, 5.09; N, 18.68. Found: C, 45.81; H, 5.31; N, 18.51.

Compounds **6a**—**i**, **7a**—**c**, **8a**—**i**, **8k**, and **8l** were also prepared by using the same procedure as for **5g**.

4-(4-Methylbenzylamino)-6-nitro-7-(1-piperazino)quinazoline (6a) Yellow solid (32% yield for 3 steps from **2**); mp, 227—229 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.18 (1H, s), 7.32 (1H, s), 7.29 (2H, d, J=8.4 Hz), 7.20 (2H, d, J=8.4 Hz), 5.87 (1H, t, J=3.5 Hz), 4.81 (2H, d, J=3.5 Hz), 3.13—3.09 (4H, m), 3.06—3.03 (4H, m), 2.37 (3H, s); TOF-MS m/z: 379 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{22}N_6O_2$: C, 63.48; H, 5.86; N, 22.21. Found: C, 63.14; H, 5.81; N, 22.18.

4-(4-Methoxybenzylamino)-6-nitro-7-(1-piperazino)quinazoline (6b) Yellow solid (11% yield for 3 steps from **2**); mp, 219—221 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.66 (1H, s), 8.18 (1H, s), 7.33 (2H, d, J=8.4 Hz), 7.32 (1H, s), 6.92 (2H, d, J=8.4 Hz), 5.84 (1H, t, J=4.9 Hz), 4.79—4.77 (2H, m), 3.82 (3H, s), 3.13—3.10 (4H,m), 3.06—3.03 (4H, m); TOF-MS m/z: 395 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{22}N_6O_3\cdot 0.5H_2O$: C, 59.54; H, 5.62; N, 20.83. Found: C, 59.67; H, 5.72; N, 20.80.

4-(3-Methoxybenzylamino)-6-nitro-7-(1-piperazino)quinazoline (6c) Yellow solid (39% yield for 3 steps from **2**); mp, 175—177 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.66 (1H, s), 8.20 (1H, s), 7.33 (1H, s), 7.31—7.28 (1H, m), 6.99—6.87 (3H, m), 5.91 (1H, t, J=3.5 Hz), 4.83 (2H, d, J=3.5 Hz), 3.82 (3H, s,), 3.14—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS m/z: 395 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{22}N_6O_3\cdot 0.5H_2O$: C, 59.54; H, 5.62; N, 20.83. Found: C, 59.70; H, 5.63; N, 20.50.

4-(2-Methoxybenzylamino)-6-nitro-7-(1-piperazino)quinazoline (6d) Yellow solid (26% yield for 3 steps from **2**); mp, 94—96 °C; ^{1}H-NMR (DMSO-d_6) δ: 8.97 (1H, s), 8.89 (1H, t, J=5.4 Hz), 8.42 (1H, s), 7.28—7.18 (3H, m), 7.01 (1H, d, J=8.4 Hz), 6.90—6.85 (1H, m), 4.70 (2H, d, J=5.4 Hz), 3.84 (3H, s), 3.00—2.97 (4H, m), 2.83—2.80 (4H, m); TOF-MS m/z: 395 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{22}N_6O_3\cdot 2.5H_2O$: C, 54.66; H, 5.62; N, 19.12. Found: C, 54.68; H, 5.66; N, 19.01.

4-(3,4-Dimethoxybenzylamino)-6-nitro-7-(1-piperazino)quinazoline (6e) Yellow solid (33% yield for 3 steps from **2**); mp, 229—231 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.66 (1H, s), 8.20 (1H, s), 7.33 (1H, s), 6.97—6.86 (3H, m), 5.90 (1H, t, J=5.4 Hz), 4.78 (2H, d, J=5.4 Hz), 3.89 (3H, s), 3.88 (3H, s), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS m/z: 425 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{24}N_6O_4\cdot 0.7H_2O$: C, 57.71; H, 5.70; N, 19.23. Found: C, 57.61; H, 5.88; N, 19.51.

6-Nitro-7-(1-piperazino)-4-(3,4,5-trimethoxybenzylamino)quinazoline (6f) Yellow solid (25% yield for 3 steps from **2**); mp, 204—206 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s), 8.25 (1H, s), 7.33 (1H, s), 6.61 (2H, s), 5.98 (1H, t, J=4.9 Hz), 4.77 (2H, d, J=4.9 Hz), 3.86 (6H, s), 3.85 (3H, s), 3.14—3.11 (4H, m), 3.07—3.04 (4H, m); TOF-MS m/z: 455 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{26}N_6O_5\cdot 0.2H_2O$: C, 57.68; H, 5.76; N, 18.35. Found: C, 57.87; H, 5.94; N, 18.11.

4-(3,4-Methylenedioxy)benzylamino-6-nitro-7-(1-piperazino)quinazoline (6g) Yellow solid (72% yield for 3 steps from **2**); mp, 217—219 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.19 (1H, s), 7.33 (1H, s), 6.88—6.79 (3H, m), 5.97 (2H, s), 5.87 (1H, t, J=3.2 Hz), 4.76 (2H, d, J=3.2 Hz), 3.12—3.10 (4H, m), 3.06—3.04 (4H, m); TOF-MS m/z: 409 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{20}N_6O_4\cdot 0.1H_2O$: C, 58.56; H, 4.94; N, 20.49. Found: C, 58.67; H, 4.92; N, 20.50.

Similarly to the procedure described for **5d**, compound **6g** was converted to its hydrochloride salt (89% yield); mp, 255 °C (Dec.); ^{1}H-NMR (DMSO-d_6) δ: 10.52 (1H, bs), 9.23 (3H, bs), 8.82 (1H, s), 7.41 (1H, s), 7.00 (1H, s), 6.89 (2H, s), 6.00 (2H, s), 4.80 (2H, d, J=5.4 Hz), 3.40—3.26 (8H, m); *Anal.* Calcd for $C_{20}H_{21}ClN_6O_4\cdot 2.5H_2O$: C, 49.03; H, 4.83; N, 17.15. Found: C, 49.04; H, 4.78; N, 17.01.

4-[(3-Methoxy-4,5-methylenedioxy)benzylamino]-6-nitro-7-(1-piperazino)quinazoline (6h) Yellow solid (49% yield for 3 steps from **2**); mp, 197—199 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.22 (1H, s), 7.33 (1H, s), 6.59—6.57 (2H, m), 5.98 (2H, s), 5.92 (1H, t, J=5.4 Hz), 4.74 (2H, d, J=5.4 Hz), 3.91 (3H, s), 3.14—3.10 (4H, m), 3.07—3.04 (4H, m); TOF-MS m/z: 439 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{22}N_6O_5\cdot 1.2H_2O$: C, 54.82; H, 5.08; N, 18.27. Found: C, 54.92; H, 5.29; N, 18.07.

4-[(2-Chloro-4,5-methylenedioxy)benzylamino]-6-nitro-7-(1-piperazino)quinazoline (6i) Yellow solid (43% yield for 3 steps from **2**); mp, 175—177 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.21 (1H, s), 7.32 (1H, s), 6.99 (1H, s), 6.88 (1H, s), 6.07 (1H, t, J=5.9 Hz), 5.98 (2H, s), 4.85 (2H, d, J=5.9 Hz), 3.13—3.10 (4H, m), 3.06—3.03 (4H, m); TOF-MS m/z: 443 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{19}ClN_6O_4\cdot 0.3H_2O$: C, 53.59; H, 4.34; N, 18.75. Found: C, 53.49; H, 4.36; N, 18.81.

4-(3,4-Methylenedioxyphenyl)amino-6-nitro-7-(1-piperazino)quinazoline (7a) Yellow solid (53% yield for 3 steps from **2**); mp, 222—223 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s), 8.39 (1H, s), 7.45 (1H, bs), 7.35 (1H, s), 7.31 (1H, d, J=2.4 Hz), 6.94 (1H, dd, J=8.4, 2.4 Hz), 6.84 (1H, d, J=8.4 Hz), 6.01 (2H, s), 3.16—3.12 (4H, m), 3.07—3.04 (4H, m); TOF-MS m/z: 395 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{18}N_6O_4\cdot 0.5H_2O$: C, 56.57; H, 4.62; N, 20.83. Found: C, 56.70; H, 4.77; N, 21.09.

4-[2-(3,4-Methylenedioxyphenyl)ethylamino]-6-nitro-7-(1-piperazino)quinazoline (7b) Yellow solid (30% yield for 3 steps from **2**); mp, 210—212 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.63 (1H, s), 8.10 (1H, s), 7.31 (1H, s), 6.79—6.67 (3H, m), 5.96 (2H, s), 5.79 (1H, t, J=5.7 Hz), 3.92—3.84 (2H, m), 3.11—3.09 (4H, m), 3.06—3.04 (4H, m), 2.95 (2H, t, J=6.8 Hz); TOF-MS m/z: 423 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{22}N_6O_4\cdot 0.5H_2O$: C, 58.64; H, 5.26; N, 19.48. Found: C, 58.65; H, 5.24; N, 19.37.

4-[3-(3,4-Methylenedioxyphenyl)propylamino]-6-nitro-7-(1-piperazino)quinazoline (7c) Yellow solid (38% yield for 3 steps from **2**); mp, 187—189 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.59 (1H, s), 7.95 (1H, s), 7.29 (1H, s), 6.75—6.66 (3H, m), 5.92 (2H, s), 5.58 (1H, t, J=5.1 Hz), 3.57—3.67 (2H, m), 3.11—3.09 (4H, m), 3.06—3.04 (4H, m), 2.72 (2H, t, J=7.2 Hz), 2.10—2.00 (2H, m); TOF-MS m/z: 437 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{24}N_6O_4\cdot 0.1H_2O$: C, 60.29; H, 5.54; N, 19.18. Found: C, 60.16; H, 5.61; N, 19.52.

(3*S*)-4-(3,4-Methylenedioxy)benzylamino-7-[1-(3-methyl)piperazino]-

6-nitroquinazoline (8a) Yellow solid (48% yield for 3 steps from **2**); mp, 214—216 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.20 (1H, s), 7.31 (1H, s), 6.88—6.79 (3H, m), 5.97 (2H, s), 5.91 (1H, bs), 4.75 (2H, d, J=5.4 Hz), 3.25—3.21 (2H, m), 3.11—3.04 (3H, m), 2.98—2.90 (1H, m), 2.62—2.54 (1H, m), 1.10 (3H, d, J=6.5 Hz); $[\alpha]_D^{20}$ −27.8° (c=2.0, MeOH); TOF-MS m/z: 423 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{22}N_6O_4\cdot 0.9H_2O$: C, 57.50; H, 5.26; N, 19.16. Found: C, 57.43; H, 5.35; N, 19.14.

(3*R*)-4-(3,4-Methylenedioxy)benzylamino-7-[1-(3-methyl)piperazino]-6-nitroquinazoline (8b) Yellow solid (37% yield for 3 steps from **2**); mp, 214—216 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.20 (1H, s), 7.32 (1H, s), 6.88—6.79 (3H, m), 5.97 (2H, s), 5.89 (1H, bs), 4.75 (2H, d, J=5.4 Hz), 3.25—3.21 (2H, m), 3.11—3.04 (3H, m), 2.98—2.92 (1H, m), 2.62—2.54 (1H, m), 1.10 (3H, d, J=6.5 Hz); $[\alpha]_D^{20}$ +27.8° (c=2.0, MeOH); TOF-MS m/z: 423 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{22}N_6O_4\cdot 1.2H_2O$: C, 56.80; H, 5.27; N, 18.93. Found: C, 56.89; H, 5.15; N, 18.84.

7-[1-(*cis*-3,5-Dimethyl)piperazino]-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8c) Yellow solid (44% yield for 3 steps from **2**); mp, 208—210 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.20 (1H, s), 7.31 (1H, s), 6.88—6.79 (3H, m), 5.97 (2H, s), 5.87 (1H, bs), 4.75 (2H, d, J=5.4 Hz), 3.23—3.09 (4H, m), 2.57—2.49 (2H, m), 1.10 (6H, d, J=6.5 Hz); TOF-MS m/z: 437 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{24}N_6O_4$: C, 60.54; H, 5.54; N, 19.25. Found: C, 60.42; H, 5.58; N, 19.14.

7-[1-(4-Ethoxycarbonyl)piperazino]-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8d) Yellow solid (36% yield for 3 steps from **2**); mp, 204—206 °C; ^{1}H-NMR (DMSO-d_6) δ: 9.05 (1H, t, J=5.1 Hz), 8.98 (1H, s), 8.48 (1H, s), 7.26 (1H, s), 6.95 (1H, s), 6.85 (2H, s), 5.97 (2H, s), 4.66 (2H, d, J=5.1 Hz), 4.07 (2H, q, J=7.2 Hz), 3.53—3.50 (4H, m), 3.10—3.07 (4H, m), 1.20 (3H, t, J=7.2 Hz); TOF-MS m/z: 481 $(M+H)^+$; *Anal.* Calcd for $C_{23}H_{24}N_6O_6\cdot 0.1H_2O$: C, 57.28; H, 5.04; N, 17.43. Found: C, 57.04; H, 4.99; N, 17.33.

4-(3,4-Methylenedioxy)benzylamino-7-morpholino-6-nitroquinazoline (8e) Yellow solid (66% yield for 3 steps from **2**); mp, 259—261 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.67 (1H, s), 8.22 (1H, s), 7.34 (1H, s), 6.89—6.80 (3H, m), 5.98 (2H, s), 5.86 (1H, t, J=5.4 Hz), 4.76 (2H, d, J=5.4 Hz), 3.90—3.86 (4H, m), 3.15—3.12 (4H, m); TOF-MS m/z: 410 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{19}N_5O_5$: C, 58.68; H, 4.68; N, 17.11. Found: C, 58.70; H, 4.82; N, 16.87.

4-(3,4-Methylenedioxy)benzylamino-6-nitro-7-piperidinoquinazoline (8f) Light yellow solid (52% yield for 3 steps from **2**); mp, 207—209 °C; ^{1}H-NMR (DMSO-d_6) δ: 9.76 (1H, t, J=4.9 Hz), 9.00 (1H, s), 8.63 (1H, s), 7.23 (1H, s), 6.97 (1H, s), 6.87 (2H, s), 5.98 (2H, s), 4.73 (2H, d, J=4.9 Hz), 3.10 (4H, bs), 1.61 (6H, bs); TOF-MS m/z: 408 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{21}N_5O_4\cdot 1.2H_2O$: C, 58.79; H, 5.22; N, 16.32. Found: C, 58.71; H, 5.02; N, 16.38.

7-(4-Hydroxypiperidino)-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8g) Yellow solid (52% yield for 3 steps from **2**); mp, 174—176 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.22 (1H, s), 7.33 (1H, s), 6.88—6.79 (3H, m), 5.97—5.93 (3H, m), 4.75 (2H, d, J=4.9 Hz), 3.98—3.92 (1H, m), 3.41—3.32 (2H, m), 3.04—2.95 (2H, m), 2.08—2.01 (2H, m), 1.83—1.71 (2H, m); TOF-MS m/z: 424 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{21}N_5O_5\cdot 1.2H_2O$: C, 56.67; H, 5.03; N, 15.74. Found: C, 56.71; H, 5.18; N, 15.77.

7-(4-Hydroxymethylpiperidino)-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8h) Yellow solid (36% yield for 3 steps from **2**); mp, 151—153 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.63 (1H, s), 8.21 (1H, s), 7.32 (1H, s), 6.88—6.78 (3H, m), 5.97—5.93 (3H, m), 4.75 (2H, d, J=5.4 Hz), 3.59—3.56 (2H, m), 3.43—3.39 (2H, m), 2.95—2.85 (2H, m), 1.87—1.66 (3H, m), 1.56—1.41 (3H, m); TOF-MS m/z: 438 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{23}N_5O_5\cdot 0.8H_2O$: C, 58.48; H, 5.31; N, 15.50. Found: C, 58.41; H, 5.44; N, 15.48.

7-(4-Ethoxycarbonylpiperidino)-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8i) Yellow solid (63% yield for 3 steps from **2**); mp, 176—178 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.23 (1H, s), 7.36 (1H, s), 6.88—6.79 (3H, m), 6.05—5.97 (3H, m), 4.76 (2H, d, J=4.1 Hz), 4.17 (2H, q, J=7.0 Hz), 3.42—3.35 (2H, m), 3.00—2.90 (2H, m), 2.56—2.45 (1H, m), 2.08—1.89 (4H, m), 1.28 (3H, t, J=7.0 Hz); TOF-MS m/z: 480 $(M+H)^+$; *Anal.* Calcd for $C_{24}H_{25}N_5O_6$: C, 60.12; H, 5.26; N, 14.61. Found: C, 60.06; H, 5.17; N, 14.43.

1-[4-(3,4-Methylenedioxy)benzylamino-6-nitro-7-quinazolinyl]-4-piperidinecarboxylic Acid (8j) A solution of **8i** (85 mg, 0.18 mmol) in EtOH (5 ml) containing 5 N NaOH (180 μl) was stirred at ambient temperature for 8 h. The reaction mixture was concentrated under reduced pressure. The residue was partitioned between AcOEt and 5% aqueous citric acid solution. The organic layer was washed with water and brine, dried over Na_2SO_4. The solution was evaporated under reduced pressure, and the yellow residue was suspended in AcOEt–hexane (10 : 7, v/v). The light yellow solid was filtered to give **8j** (68 mg, 85% yield); mp, 275 °C (Dec.); ^{1}H-NMR (DMSO-d_6) δ: 12.32 (1H, bs), 9.00 (1H, t, J=5.9 Hz), 8.93 (1H, s), 8.46 (1H, s), 7.21 (1H, s), 6.94 (1H, s), 6.88 (1H, s), 6.85 (1H, s), 5.97 (2H, s), 4.65 (2H, d, J=5.9 Hz), 3.25 (2H, bs), 2.95—2.87 (2H, m), 2.45—2.40 (1H, m), 1.99—1.90 (2H, m), 1.73—1.61 (2H, m); TOF-MS m/z: 452 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{21}N_5O_6$: C, 58.53; H, 4.69; N, 15.51. Found: C, 58.38; H, 4.73; N, 15.31.

7-(3-Hydroxypiperidino)-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8k) Yellow solid (44% yield for 3 steps from **2**); mp, 201—203 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.65 (1H, s), 8.23 (1H, s), 7.36 (1H, s), 6.88—6.79 (3H, m), 5.97 (3H, bs), 4.75 (2H, d, J=5.4 Hz), 3.99 (1H, bs), 3.28—3.22 (1H, m), 3.11—3.03 (3H, m), 2.06—1.95 (1H, m), 1.90—1.80 (1H, m), 1.76—1.67 (3H, m); TOF-MS m/z: 424 $(M+H)^+$; *Anal.* Calcd for $C_{21}H_{21}N_5O_5\cdot 0.4H_2O$: C, 58.57; H, 5.01; N, 16.26. Found: C, 58.69; H, 5.01; N, 16.11.

7-(3-Hydroxymethylpiperidino)-4-(3,4-methylenedioxy)benzylamino-6-nitroquinazoline (8l) Yellow solid (52% yield for 3 steps from **2**); mp, 199—201 °C; ^{1}H-NMR ($CDCl_3$) δ: 8.64 (1H, s), 8.18 (1H, s), 7.35 (1H, s), 6.88—6.79 (3H, m), 5.97 (2H, s), 5.85 (1H, bs), 4.75 (2H, d, J=5.4 Hz), 3.63—3.58 (2H, m), 3.44—3.40 (1H, m), 3.29—3.24 (1H, m), 2.97—2.76 (2H, m), 2.02—1.73 (4H, m), 1.30—1.27 (2H, m); TOF-MS m/z: 438 $(M+H)^+$; *Anal.* Calcd for $C_{22}H_{23}N_5O_5$: C, 60.40; H, 5.30; N, 16.01. Found: C, 60.35; H, 5.32; N, 15.92.

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.[24,25] 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.[27,28] Briefly, male BALB/c mice, 10 weeks of age, were sacrificed 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 d. The MTS assay was performed by using a commercial kit (Promega), and formazan dye products were measured by absorbance at 490 nm by 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.[29] Briefly, male BALB/c mice, 10 weeks of age, were sacrificed 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 d. 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 the test compounds against TNF-α production in LPS treated mice were evaluated according to a previously reported method.[30] Briefly, BALB/c mice (Charles River Japan Inc.) were used at 10 weeks of age. LPS from *Escherichia coli* (serotype 026:B6) was purchased from Difco Laboratories (Detroit, U.S.A.). A solution of a test compound in water was orally administered to mice 0.5 h prior to i.v. 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, U.S.A.).

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