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Discovery of Quinazolines as a Novel Structural Class of Potent Inhibitors of NF-κB Activation

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Abstract—We disclose here a new structural class of low-molecular-weight inhibitors of NF-κB activation that were designed and synthesized by starting from quinazoline derivative **6a**. Structure–activity relationship (SAR) studies based on **6a** elucidated the structural requirements essential for the inhibitory activity toward NF-κB transcriptional activation, and led to the identification of the 6-amino-4-phenethylaminoquinazoline skeleton as the basic framework. In this series of compounds, **11q**, containing the 4-phenoxyphenethyl moiety at the C(4)-position, showed strong inhibitory effects on both NF-κB transcriptional activation and TNF-α production. Furthermore, **11q** exhibited an anti-inflammatory effect on carrageenin-induced paw edema in rats.

Introduction

Autoimmune diseases and allergies, such as rheumatoid arthritis, septic shock, transplant rejection, asthma, and psoriasis, are considered to be caused by abnormalities of T cell immune responses.[1] In particular, the activation of T cells initiates a network of events that results in the overproduction of certain transcription factors and proinflammatory cytokines.[2] Transcription factors are DNA-binding proteins that regulate the production of proinflammatory cytokines as well as that of a variety of other cellular regulators.

Nuclear factor-κB (NF-κB) is a pivotal transcription factor that functions to enhance the transcription of proinflammatory cytokines including TNF-α, IL-1β, IL-6, and IL-8, which are thought to be important in the generation of acute inflammatory responses.[3] Activation and regulation of NF-κB are tightly controlled by members of a family of inhibitory proteins referred to as IκB. Several IκB proteins have been identified, including IκB-α, IκB-β, IκB-γ, IκB-ε, and Bcl-3.[4]

In resting cells, NF-κB is sequestered in the cytoplasm through its interaction with its inhibitor, IκB-α, IκB-β or IκB-ε. However, a large variety of inflammatory conditions, such as inflammatory cytokines, bacterial and viral infections, and certain chemical agents, induce NF-κB activity.[5] NF-κB activation involves signaled phosphorylation, ubiquitination, and proteolysis of IκB-α.[4] Liberated NF-κB migrates to the nucleus, where it stimulates the transcription of its target gene. The activation of NF-κB initiates both extracellular and intracellular regulatory events that result in autoregulation of the inflammatory cascade through modulation of NF-κB activation. The inhibition of NF-κB activation would lead to suppression of proinflammatory cytokines levels and be beneficial for the treatment of the above-mentioned diseases.[6]

Several disclosures have reported some compounds with the inhibitory activities toward NF-κB-mediated transcriptional activation. Low-molecular-weight compounds, such as MG-132 (**1**),[7] BAY 11-7085 (**2**),[8] and an indan derivative (**3**),[9] as well as natural products, such as caffeic acid phenylethyl ester (**4**)[10] and the sesquiterpene lactone helenalin (**5**),[11] have been shown to inhibit NF-κB activation (Fig. 1).

In order to find a new structural class of NF-κB activation inhibitors, we conducted a reporter gene-based screening featuring phorbol 12-myristate-13-acetate (PMA) plus phytohemagglutin (PHA) stimulation of human Jurkat T cells transfected with the reporter

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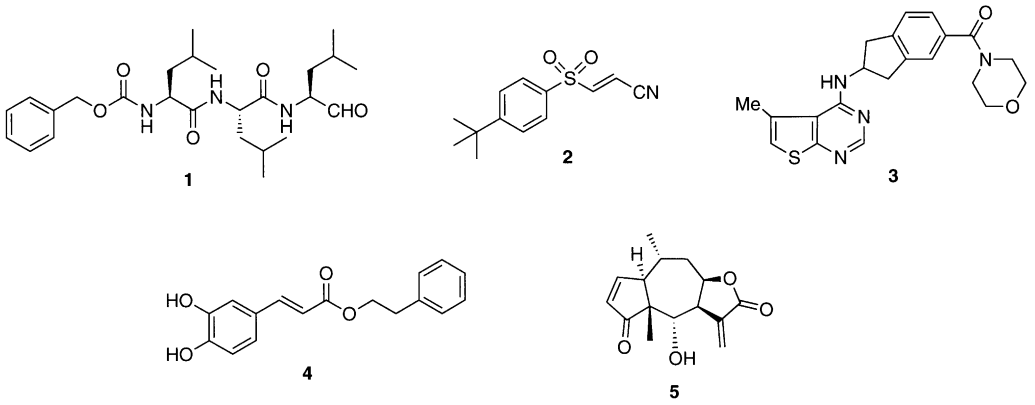


Figure 1. Several inhibitors of NF-κB-mediated transcriptional activation. (**1**) MG-132; (**2**) BAY 11–7085; (**3**) indan derivative; (**4**) caffeic acid phenylethyl ester; (**5**) helenalin.

DNA having the binding sequence for NF-κB and the luciferase gene.[12] This led to the identification of the quinazoline **6a** as a lead compound ($IC_{50} = 2381$ nM). To date, no one has reported quinazoline derivatives as NF-κB activation inhibitors. This situation prompted us to investigate structure–activity relationship (SAR) of quinazoline derivatives as inhibitors of NF-κB activation. In this paper, we describe the results of our preliminary SAR studies on the quinazolines, conducted on the basis of the lead compound, **6a**.

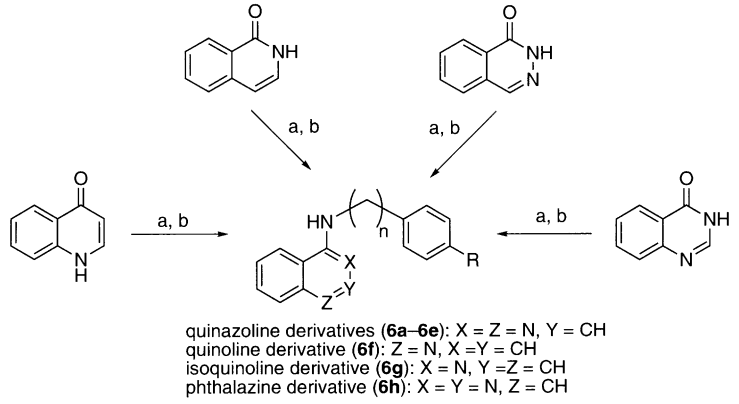
Chemistry

Procedures for the preparation of **6a**–**6h** having the 6–6 condensed-ring systems are shown in Scheme 1. Chlorination of 4-quinazolone with phosphorus oxychloride followed by the reaction of the corresponding amines provided the quinazolines **6a**–**6e**. The quinoline (**6f**), the isoquinoline (**6g**), and the phthalazine (**6h**) were also prepared by the same procedures used for the quinazolines.

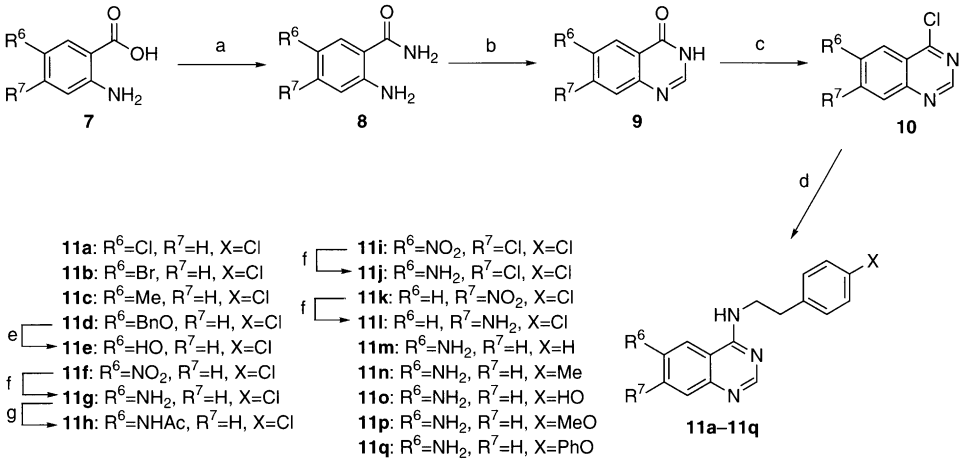
The general synthetic pathway to the phenethylaminoquinazolines is shown in Scheme 2. Treatment of the appropriate anthranilic acids (**7**) with EDC (*N*-ethyl-*N*′-[3-(dimethylamino)propyl] carbodiimide) and HOBt (1-hydroxybenzotriazole), followed by the addition of 28% ammonia, gave the corresponding 2-aminobenzamides (**8**). The benzamide (**8**) was cyclized to the 4-quinazolone (**9**) by using trimethyl orthoformate. Subsequently, chlorination of the 4-quinazolones (**9**) with thionyl chloride gave the corresponding 4-chloroquinazolines (**10**). Reaction of these compounds with phenethylamines in the presence of triethylamine provided compounds **11a**–**11d**, **f**, **i**, and **k**. Catalytic hydrogenation of **11d** gave the 6-hydroxyquinazoline, **11e**. Compound **11f** underwent iron dust reduction to provide the 6-aminoquinazoline, **11g**. Acetylation of **11g** with acetic anhydride in pyridine gave **11h**. Compounds **11j**, **l**, **m**–**q** were also prepared by the same procedure used for **11g**.

Results and Discussion

To screen compounds for their inhibitory potency toward NF-κB transcriptional activation, we set up a luciferase reporter gene assay using human Jurkat cells. Compounds were also evaluated for their inhibitory activities toward TNF-α production by using murine splenocytes stimulated with LPS. Cytotoxicity toward both human Jurkat cells and murine splenocytes was measured by using the MTS assay. The results obtained by using these tools to drive the SAR efforts are shown in Tables 1 and 2. Among NF-κB activation inhibitors,

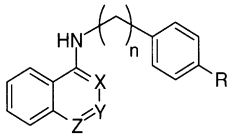


Scheme 1. Synthesis of compounds **6a**–**6h**. Reagents and conditions: (a) phosphorus oxychloride, 100 °C, 2–3 h.; (b) RNH_2, triethylamine, *i*-PrOH, reflux, 5–10 h.



Scheme 2. Synthesis of the 4-phenethylaminoquinazolines, **11a–11q**. Reagents and conditions: (a) 28% aqueous ammonia solution, EDC·HCl, HOBt, DMF, 3–5 h; (b) $HC(OMe)_3$, 12 N HCl, 1–3 h; (c) $SOCl_2$, DMF, reflux, 2–3 h; (d) phenethylamines, triethylamine, *i*-PrOH, 1–2 h; (e) H_2, Pd–C, MeOH, 5 h; (f) Fe, glacial acetic acid, EtOH–H_2O, reflux, 30 min; (g) Ac_2O, pyridine, DMF, 50 °C, 2 h.

Table 1. Inhibition of NF-κB activation and TNF-α production by compounds **6a–6h**



						NF-κB[a]	Toxicity[b]	TNF-α[c]	Toxicity[d]
Compd	X	Y	Z	n	R	IC_{50} (nM)	TC_{50} (nM)	IC_{50} (nM)	TC_{50} (nM)
6a	N	CH	N	3	H	2381	>10,000	7273	>10,000
6b	N	CH	N	3	Cl	1401	>10,000	3220	>10,000
6c	N	CH	N	2	Cl	630	>10,000	398	>10,000
6d	N	CH	N	1	Cl	1832	>10,000	>10,000	>10,000
6e	N	CH	N	0	Cl	>10,000	>10,000	>10,000	>10,000
6f	CH	CH	N	2	Cl	1392	>10,000	1643	5103
6g	N	CH	CH	2	Cl	>10,000	>10,000	>10,000	>10,000
6h	N	N	CH	2	Cl	>10,000	>10,000	>10,000	>10,000
MG-132						715	1141	809	1893

[a]IC_{50} for the inhibition of NFκB activation in human Jurkat cells transfected with pNFκB-Luc.
[b]TC_{50} for the growth inhibition of human Jurkat cells.
[c]IC_{50} for the inhibition of TNF-α production from murine splenocytes stimulated with LPS.
[d]TC_{50} for the growth inhibition of mouse splenocytes stimulated with LPS.

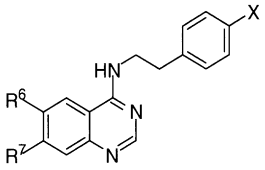
MG-132 (**1**) has been shown to be a proteasome inhibitor blocking NF-κB transcriptional activation in Hela cells stimulated by TNF-α ($IC_{50}=3$ μM).[7] The indan derivative (**3**) was also reported to inhibit NF-κB transcriptional activation, in A549 cells stimulated with TNF-α or IL-1β (TNF-α: $IC_{50}=89$ nM; IL-1β: $IC_{50}=51$ nM).[9] We found both compounds to block the activation of NF-κB transcriptional factor in our assay system (MG-132: $IC_{50}=715$ nM; **3**: $IC_{50}=38$ nM), and so we selected MG-132 and **3** as the positive controls in our system.

Our initial efforts focused on investigating the effects of varying the methylene chain length between the quinazoline and the 4-chlorophenyl group at the C(4)-position of the quinazoline (**6b–6e**) (Table 1). The maximum suppressing effect on NF-κB transcriptional activation was obtained when the spacer was the ethylene chain (**6c**). The optimal length was also confirmed to be 2 methylene units (**6c**) toward TNF-α inhibitory activity.

To establish the role of the quinazoline ring, we studied a set of the quinazoline and related heteroaromatic derivatives. The quinoline, **6f**, was 2-fold less active than the quinazoline **6c**; and the isoquinoline, **6g**, or the phthalazine, **6h**, showed severe loss of their inhibitory activities toward both NF-κB activation and TNF-α production. These results indicate that, according to the numbering of the quinazoline ring, the 1-nitrogen atom is essential to exhibit both inhibitory activities and the combination of 1- and 3-nitrogen atoms is more effective than that of 1- and 2-nitrogen atom. Therefore, we considered the quinazoline core to be the most favorable in this SAR study.

Next, we investigated the effect of C(6)- and C(7)-substituents on the quinazoline ring (Table 2). Among the C(6)-substituents, compounds **11a–c** and **e** were equipotent or 2-fold more potent than **6c** in their inhibitory activity toward NF-κB activation; whereas the benzyloxy (**11d**) and the nitro (**11f**) showed great loss of this inhibitory activity. Furthermore, as shown by **11g**, introduction of the amino group at the C(6)-position afforded a 3–4 fold increase in the inhibitory activities toward both NF-κB activation and TNF-α production over **6c**, whereas the 7-amino analogue (**11l**) displayed a loss of both inhibitory activities. Acetamidation (**11h**) resulted in a 26-fold loss in NF-κB inhibitory activity compared with **11g**. Additional introduction of a chloro group at the 7-position of **11g** also gave a less potent compound (**11j**). This trend was also observed in the case of **11i**. These results suggest that the presence of a basic nitrogen as well as a smaller substituent at the C(6)-position of the quinazoline ring was necessary to inhibit both NF-κB transcriptional activation and TNF-α production.

Table 2. Inhibition of NF-κB activation and TNF-α production by 4-phenethylaminoquinazolines **11a**–**11q**



				NF-κB[a]	Toxicity[b]	TNF-α[c]	Toxicity[d]
Compd	R^6	R^7	X	IC_{50} (nM)	TC_{50} (nM)	IC_{50} (nM)	TC_{50} (nM)
6c	H	H	Cl	630	>10,000	398	>10,000
11a	Cl	H	Cl	344	>10,000	5478	>10,000
11b	Br	H	Cl	851	>10,000	1763	>10,000
11c	Me	H	Cl	730	>10,000	358	>10,000
11d	BnO	H	Cl	7340	>10,000	5103	>10,000
11e	HO	H	Cl	313	>10,000	1376	>10,000
11f	NO_2	H	Cl	1515	>10,000	2031	>10,000
11g	NH_2	H	Cl	176	>10,000	142	>10,000
11h	NHAc	H	Cl	4562	>10,000	6540	>10,000
11i	NO_2	Cl	Cl	9060	>10,000	>10,000	>10,000
11j	NH_2	Cl	Cl	429	>10,000	781	>10,000
11k	H	NO_2	Cl	1765	>10,000	1763	6775
11l	H	NH_2	Cl	2202	>10,000	1376	6775
11m	NH_2	H	H	311	>10,000	1426	>10,000
11n	NH_2	H	Me	210	>10,000	138	>10,000
11o	NH_2	H	HO	3645	>10,000	1702	>10,000
11p	NH_2	H	MeO	90	>10,000	279	>10,000
11q	NH_2	H	PhO	11	>10,000	7	>10,000
3				38	>10,000	84	>1000
MG-132				715	1141	809	1893

[a–d]See corresponding footnotes in Table 1.

To improve the inhibitory activity toward NF-κB transcriptional activation, we next performed substitution of the chloro group on the terminal phenyl ring with several other substituents, keeping the 6-amino group constant. Removal of the chloro group from **11g** gave **11m**, which caused a slight loss of the inhibitory activity. Replacing the chloro group with the methyl group (**11n**) resulted in an IC_{50} value comparable to that of **11g** for the inhibitory activity. Substantially decreased potency was observed when the methyl group in **11n** was replaced with a hydroxyl group (**11o**). However, the methoxy (**11p**) showed a 2-fold higher inhibitory activity toward NF-κB transcriptional activation over **11g**. Replacement of the methoxy group in **11p** with the phenoxy group gave **11q** which displayed a remarkable improvement in the inhibition of both NF-κB transcriptional activation and TNF-α production compared with the inhibitory activities of **11g**. Moreover, **11q** showed 4- and 12-fold improvement of the inhibitory activities toward NF-κB activation and TNF-α production, respectively, compared with **3**. Finally, on the basis of the above-mentioned in vitro study, we selected compound **11q** and evaluated its anti-inflammatory effect in a rat carrageenin-induced paw edema model.[13] d paw swelling was induced by the injection of carrageenin into the right hind paw of rats. The resulting edema was quantified 2 h later by measuring the increase in the volume of the inflamed paw. As seen in Figure 2, compound **11q** inhibited edema formation dose-dependently and showed a more potent suppressing effect than **3** when both were tested at a dose of 1 mg/kg. In this model, the activation of NF-κB has been reported to play a key role in the development of inflammatory response.[14] Therefore, we consider that compound **11q** had a suppressing effect of the NF-κB mediated-inflammatory response. We are continuing further optimization study based on **11q**, especially focusing on the substitution at the terminal phenyl ring at the C(4)-position of the quinazoline ring. The results of this detailed SAR study will be reported in the near future.

Conclusions

Based on the lead compound **6a** obtained from random screening, we identified and synthesized a novel structural class of NF-κB activation inhibitors. Our SAR investigation allowed us to identify the 6-amino-4-phenethylaminoquinazoline skeleton as the basic framework and led to the discovery of **11q**, whose inhibitory activity toward NF-κB transcriptional activation represented a 2 order of magnitude increase relative to that of **6a**. Compound **11q** was also highly potent in the inhibition of TNF-α production ($IC_{50} = 7$ nM). In addition, **11q** demonstrated in vivo efficacy by reducing the edema formation seen in carrageenin-induced inflammation of the rat hind paw. Further SAR studies on 6-aminoquinazolines made on the basis of **11q** are ongoing and will be reported in due course.

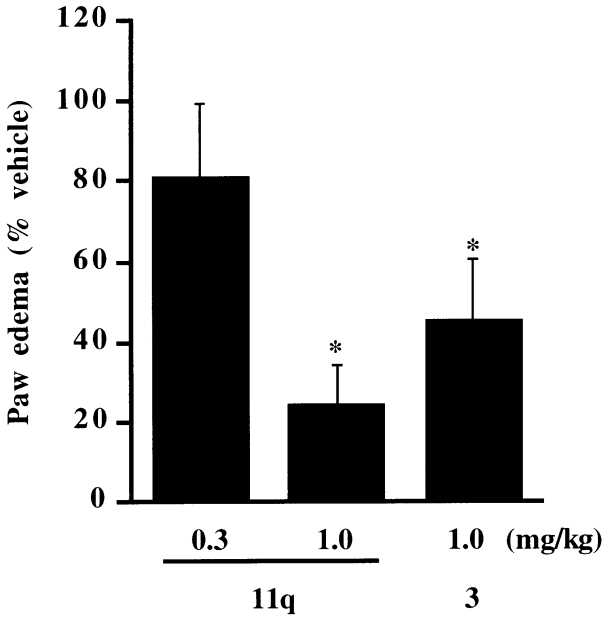


Figure 2. Effects of **11q** and **3** on carrageenin-induced paw edema in rats. Compound **11q** and **3** were intraperitoneally administered 15 min prior to the injection of carrageenin. The resulting edema was quantified 2 h later by measuring the increase in the volume of the inflamed paw. The results are expressed as the mean ± SEM of 5 rats per group. * $p < 0.05$ versus vehicle control (Dunnett's test).

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. ^{1}H NMR was recorded on a Jeol GSX270 FT NMR spectrometer. Chemical shifts were given in parts per million (ppm) using tetramethylsilane as the internal standard for spectra obtained in DMSO-d_6 and $CDCl_3$. TOFMS (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). Following abbreviations are used for solvents: AcOH (acetic acid), DMF (*N,N*-dimethylformamide), AcOEt (ethyl acetate), *i*-PrOH (2-propanol).

4-[3-(Phenyl)propylamino]quinazoline (6a). A suspension of 4-quinazolinol (300 mg, 2.05 mmol) in phosphorus oxychloride (6.0 mL) was heated at 100 °C for 2 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 5% aqueous $NaHCO_3$ solution. The organic layer was washed with water and brine, dried over Na_2SO_4. The solution was concentrated under reduced pressure to provide crude 4-chloroquinazoline, which was used directly. To a mixture of the crude chloro compound and triethylamine (571 μL, 4.10 mmol) in EtOH (15 mL) was added 3-(phenyl)propylamine (692 mg, 5.12 mmol). The resulting mixture was stirred at reflux for 5 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 evaporated in vacuo, and the residue was triturated with diethyl ether. The white solid was filtered and dried at 40 °C under high vacuum to give **6a** (309 mg, 57% yield for 2 steps from 4-quinazolinol): mp 116–117 °C; ^{1}H NMR (DMSO-d_6) d 8.45 (s, 1H), 8.29–8.23 (m, 2H), 7.79–7.65 (m, 2H), 7.53–7.47 (m, 1H), 7.32–7.15 (m, 5H), 3.59–3.52 (m, 2H), 2.69 (t, $J = 7.6$ Hz, 2H), 2.02–1.91 (m, 2H); MS (TOF) m/z 264 $(M + H)^+$; anal. calcd for $C_{17}H_{17}N_3$: C, 77.54; H, 6.51; N, 15.96. Found: C, 77.26; H, 6.46; N, 15.71.

Compounds **6b**–**6h** were also prepared by using the same procedure as for **6a**.

4-[3-(4-Chlorophenyl)propylamino]quinazoline (6b). White solid (49% yield for 2 steps from 4-quinazolinol): mp 151–153 °C; ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1H), 8.29–8.22 (m, 2H), 7.79–7.65 (m, 2H), 7.53–7.47 (m, 1H), 7.35–7.26 (m, 4H), 3.58–3.51 (m, 2H), 2.68 (t, $J = 7.6$ Hz, 2H), 2.00–1.89 (m, 2H); MS (TOF) m/z 298 $(M + H)^+$; anal. calcd for $C_{17}H_{16}ClN_3$: C, 68.57; H, 5.42; N, 14.11. Found: C, 68.53; H, 5.42; N, 14.03.

4-(4-Chlorophenethylamino)quinazoline (6c). White solid (39% yield for 2 steps from 4-quinazolinol): mp 192–194 °C; ^{1}H NMR (DMSO-d_6) δ 8.48 (s, 1H), 8.36 (t, $J = 5.4$ Hz, 1H), 8.19 (d, $J = 7.8$ Hz, 1H), 7.79–7.66 (m, 2H), 7.53–7.47 (m, 1H), 7.37–7.27 (m, 4H), 3.75 (dt, $J = 5.4$, 7.3 Hz, 2H), 2.97 (t, $J = 7.3$ Hz, 2H); MS (TOF) m/z 284 $(M + H)^+$; anal. calcd for $C_{16}H_{14}ClN_3$: C, 67.72; H, 4.97; N, 14.81. Found: C, 67.54; H, 4.98; N, 15.20.

4-(4-Chlorobenzylamino)quinazoline (6d). White solid (52% yield for 2 steps from 4-quinazolinol): mp 206–208 °C; ^{1}H NMR (DMSO-d_6) δ 8.88 (t, $J = 5.9$ Hz, 1H), 8.45 (s, 1H), 8.29 (d, $J = 7.8$ Hz, 1H), 7.82–7.69 (m, 2H), 7.57–7.51 (m, 1H), 7.37 (s, 4H), 4.77 (d, $J = 5.9$ Hz, 2H); MS (TOF) m/z 270 $(M + H)^+$; anal. calcd for $C_{15}H_{12}ClN_3 \cdot 0.1H_2O$: C, 66.35; H, 4.49; N, 15.48. Found: C, 66.01; H, 4.54; N, 15.78.

4-(4-Chlorophenylamino)quinazoline (6e). White solid (45% yield for 2 steps from 4-quinazolinol): mp 163–164 °C; ^{1}H NMR (DMSO-d_6) δ 9.89 (br s, 1H), 8.63 (s, 1H), 8.56 (d, $J = 8.1$ Hz, 1H), 7.97–7.92 (m, 2H), 7.89–7.80 (m, 2H), 7.69–7.63 (m, 1H), 7.49–7.43 (m, 2H); MS (TOF) m/z 256 $(M + H)^+$; anal. calcd for $C_{14}H_{10}ClN_3 \cdot 0.1H_2O$: C, 65.30; H, 3.95; N, 16.32. Found: C, 65.16; H, 4.05; N, 16.30.

4-(4-Chlorophenethylamino)quinoline (6f). White solid (58% yield for 2 steps from 4-quinolinol): mp 158–160 °C; ^{1}H NMR (DMSO-d_6) δ 8.39 (d, $J = 5.4$ Hz, 1H), 8.17 (d, $J = 7.8$ Hz, 1H), 7.77 (d, $J = 7.8$ Hz, 1H), 7.63–7.57 (m, 1H), 7.44–7.38 (m, 1H), 7.35 (s, 4H), 7.22 (t,

$J = 5.4$ Hz, 1H), 6.53 (t, $J = 5.4$ Hz, 1H), 3.50 (dt, $J = 5.4$, 7.3 Hz, 2H), 2.98 (t, $J = 7.3$ Hz, 2H); MS (TOF) m/z 283 $(M + H)^+$; anal. calcd for $C_{17}H_{15}ClN_2$: C, 72.21; H, 5.35; N, 9.91. Found: C, 72.00; H, 5.34; N, 9.78.

1-(4-Chlorophenethylamino)isoquinoline (6g). White solid (18% yield for 2 steps from isocarbostyril): mp 127–128 °C; ^{1}H NMR ($CDCl_3$) δ 9.21 (br s, 1H), 8.73 (d, $J = 7.8$ Hz, 1H), 7.79 (d, $J = 6.8$ Hz, 1H), 7.70–7.66 (m, 2H), 7.62 (d, $J = 6.8$ Hz, 1H), 7.19 (d, $J = 8.1$ Hz, 2H), 6.96 (d, $J = 8.1$ Hz, 2H), 6.91 (d, $J = 6.8$ Hz, 1H), 4.15–4.07 (m, 2H), 3.09 (t, $J = 7.3$ Hz, 2H); MS (TOF) m/z 283 $(M + H)^+$; anal. calcd for $C_{17}H_{15}ClN_2 \cdot 0.6H_2O$: C, 69.55; H, 5.36; N, 9.54. Found: C, 69.62; H, 5.28; N, 9.45.

1-(4-Chlorophenethylamino)phthalazine (6h). White solid (42% yield for 2 steps from 1(2*H*)-phthalazinone): mp 154–156 °C; ^{1}H NMR (DMSO-d_6) δ 8.91 (s, 1H), 8.25–8.22 (m, 1H), 7.94–7.83 (m, 3H), 7.55 (t, $J = 5.4$ Hz, 1H), 7.37–7.29 (m, 4H), 3.77 (dt, $J = 5.4$, 7.4 Hz, 2H), 3.02 (t, $J = 7.4$ Hz, 2H); MS (TOF) m/z 284 $(M + H)^+$; anal. calcd for $C_{16}H_{14}ClN_3 \cdot 0.2H_2O$: C, 66.88; H, 4.98; N, 14.62. Found: C, 66.80; H, 4.98; N, 14.58.

4-(4-Chlorophenethylamino)-6-nitroquinazoline (11f). To a solution of 2-amino-5-nitrobenzoic acid (10.0 g, 54.9 mmol) and HOBt (8.16 g, 60.4 mmol) in DMF (275 mL) was added EDC·HCl (11.6 g, 60.4 mmol). The mixture was stirred at ambient temperature for 2 h. The resulting solution was cooled to 0 °C, then 28% ammonia solution (5.5 mL) was added and temperature was allowed to rise to ambient temperature. After 1 h, the reaction mixture was concentrated under reduced pressure, and the residue was partitioned AcOEt 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 triturated with AcOEt/hexane (1:1). The pale yellow solid was filtered and dried at 40 °C under high vacuum to give 2-amino-5-nitrobenzamide (8.78 g, 88% yield).

Next, to a mixture of 2-amino-5-nitrobenzamide (8.78 g, 48.5 mmol) in trimethyl orthoformate (70 mL) was added 12 N HCl (6.0 mL) dropwise at 0 °C. The reaction mixture was stirred at ambient temperature for 1.5 h, and then was concentrated under reduced pressure. The residue was triturated with water, and was adjusted to pH 6 with 5 N NaOH. The precipitate was collected, washed with MeOH and water, and dried in air. The light yellow solid was filtered and dried at 40 °C under high vacuum to give 6-nitro-4-quinazolone (8.21 g, 89% yield).

Subsequently, a suspension of 6-nitro-4-quinazolone (1.00 g, 5.23 mmol) in thionyl chloride (30 mL) containing 1 drop of DMF was heated at reflux for 3 h to give a clear solution. Excess thionyl chloride was removed under reduced pressure to provide crude 4-chloro-6-nitroquinazoline, which was used directly. To a mixture of the crude chloro compound and triethylamine (875 μL, 6.28 mmol) in *i*-PrOH (50 mL) was added 4-chlorophenethylamine (878 μL, 6.28 mmol). The resulting mixture was stirred at ambient temperature 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 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 (1:4). The light yellow solid was filtered and dried at 40 °C under high vacuum to give **11f** (1.43 g, 83% yield for 2 steps from 6-nitro-4-quinazolone): mp 232–234 °C; ^{1}H NMR (DMSO-d_6) δ 9.33 (d, $J = 2.4$ Hz, 1H), 9.08 (br s, 1H), 8.61 (s, 1H), 8.49–8.45 (m, 1H), 7.85–7.81 (m, 1H), 7.37–7.28 (m, 4H), 3.82–3.77 (m, 2H), 2.99 (t, $J = 7.3$ Hz, 2H); MS (TOF) m/z 329 $(M + H)^+$; anal. calcd for $C_{16}H_{13}ClN_4O_2$: C, 58.45; H, 3.99; N, 17.04. Found: C, 58.29; H, 3.99; N, 16.95.

6-Chloro-4-(4-chlorophenethylamino)quinazoline (11a). Similarly to the procedure described for **11f**, the title compound was prepared starting from 2-amino-5-chlorobenzoic acid. After purification, **11a** was obtained as a white solid (20% yield for 4 steps): mp 192–194 °C; ^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.45 (t, $J = 5.3$ Hz, 1H), 8.37 (d, $J = 2.2$ Hz, 1H), 7.80–7.68 (m, 2H), 7.37–7.27 (m, 4H), 3.75 (dt, $J = 5.3$, 7.3 Hz, 2H), 2.96 (t, $J = 7.3$ Hz, 2H); MS (TOF) m/z 318 $(M + H)^+$; anal. calcd for $C_{16}H_{13}Cl_2N_3 \cdot 0.2H_2O$: C, 59.72; H, 4.13; N, 13.06. Found: C, 59.71; H, 4.08; N, 13.04.

6-Bromo-4-(4-chlorophenethylamino)quinazoline (11b). Similarly to the procedure described for **11f**, the title compound was prepared starting from 2-amino-5-bromobenzoic acid. After purification, **11b** was obtained as a white solid (37% yield for 4 steps): mp 209–211 °C; ^{1}H NMR (DMSO-d_6) δ 8.52–8.44 (m, 3H), 7.89 (dd, $J = 8.8$, 2.0 Hz, 1H), 7.63 (d, $J = 8.8$ Hz, 1H), 7.37–7.27 (m, 4H), 3.78–3.71 (m, 2H), 2.96 (t, $J = 7.4$ Hz, 2H); MS (TOF) m/z 362 $(M + H)^+$; anal. calcd for $C_{16}H_{13}BrClN_3 \cdot 0.1H_2O$: C, 52.73; H, 3.62; N, 11.53. Found: C, 52.34; H, 3.71; N, 11.87.

4-(4-Chlorophenethylamino)-6-methylquinazoline (11c). Similarly to the procedure described for **11f**, the title compound was prepared starting from 2-amino-5-methylbenzoic acid. After purification, **11c** was obtained as a white solid (35% yield for 4 steps): mp 153–154 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (s, 1H), 8.23 (t, $J = 5.3$ Hz, 1H), 8.00 (s, 1H), 7.62–7.56 (m, 2H), 7.37–7.27 (m, 4H), 3.74 (dt, $J = 5.3$, 7.3 Hz, 2H), 2.96 (t, $J = 7.3$ Hz, 2H), 2.46 (s, 3H); MS (TOF) m/z 298 $(M + H)^+$; anal. calcd for $C_{17}H_{16}ClN_3 \cdot 0.1H_2O$: C, 68.16; H, 5.42; N, 14.03. Found: C, 68.03; H, 5.37; N, 13.75.

6-Benzyloxy-4-(4-chlorophenethylamino)quinazoline (11d). Similarly to the procedure described for **11f**, the title compound was prepared starting from 2-amino-5-benzyloxybenzoic acid. After purification, **11d** was obtained as a white solid (28% yield for 4 steps): mp 182–184 °C; ^{1}H NMR (DMSO-d_6) δ 8.40 (s, 1H), 8.20 (t, $J = 5.4$ Hz, 1H), 7.79 (d, $J = 2.4$ Hz, 1H), 7.64 (d, $J = 9.2$ Hz, 1H), 7.54–7.28 (m, 10H), 5.19 (s, 2H), 3.76 (dt, $J = 5.4$, 7.4 Hz, 2H), 2.98 (t, $J = 7.4$ Hz, 2H); HR-FABMS m/z $(M + H)^+$; calcd for $C_{23}H_{20}ClN_3O$: 390.1373. Found: 390.1354.

4-(4-Chlorophenethylamino)-7-nitroquinazoline (11k). Similarly to the procedure described for **11f**, the title compound was prepared starting from 2-amino-4-nitrobenzoic acid. After purification, **11k** was obtained as a white solid (43% yield for 4 steps): mp 196–198 °C; ^{1}H NMR (DMSO-d_6) δ 8.81 (t, J = 4.9 Hz, 1H), 8.62 (s, 1H), 8.47 (d, J = 9.2 Hz, 1H), 8.41 (d, J = 2.2 Hz, 1H), 8.26 (dd, J = 2.2, 9.2 Hz, 1H), 7.36–7.27 (m, 4H), 3.78 (dt, J = 4.9, 7.2 Hz, 2H), 2.98 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 329 (M + H)$^+$; anal. calcd for $C_{16}H_{13}ClN_4O_2$: C, 58.45; H, 3.99; N, 17.04. Found: C, 58.09; H, 4.04; N, 16.95.

7-Chloro-4-(4-chlorophenethylamino)-6-nitroquinazoline (11i). A suspension of 7-chloro-6-nitro-4-quinazolone[15] (1.00 g, 4.43 mmol) in thionyl chloride (18 mL) containing 1 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 4,7-dichloro-6-nitroquinazoline, which was used directly. To a mixture of the crude chloro compound and triethylamine (742 μL, 5.32 mmol) in *i*-PrOH (22 mL) was added 4-chlorophenethylamine (745 μL, 5.32 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). The light yellow solid was filtered to give **11i** (1.34 g, 83% yield): mp 248–250 °C; ^{1}H NMR ($CDCl_3$) δ 8.75 (s, 1H), 8.27 (s, 1H), 7.99 (s, 1H), 7.32 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.1 Hz, 2H), 5.91 (t, J = 5.5 Hz, 1H), 3.95 (dt, J = 5.5, 7.0 Hz, 2H), 3.03 (t, J = 7.0 Hz, 2H); MS (TOF) m/z 364 (M + H)$^+$; anal. calcd for $C_{16}H_{12}Cl_2N_4O_2$: C, 52.91; H, 3.33; N, 15.43. Found: C, 53.23; H, 3.48; N, 15.50.

6-Amino-7-chloro-4-(4-chlorophenethylamino)quinazoline (11j). Iron powder (92 mg; freshly washed with 1 N HCl followed by distilled water) was added in portions to a refluxing solution of **11i** (150 mg, 0.41 mmol) in EtOH/H_2O (2:1, 18 mL) containing glacial AcOH (268 μL). The resulting suspension was heated at reflux with vigorous stirring for 30 min, then cooled, basified with concentrated NH_4OH and EtOH, and combined filtrate was concentrated under reduced pressure, diluted with water and extracted with AcOEt. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with AcOEt and the light yellow solid was filtered to give **11j** (28 mg, 20% yield): mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 8.31 (s, 1H), 8.26 (t, J = 5.3 Hz, 1H), 7.63 (s, 1H), 7.36–7.26 (m, 5H), 5.72 (br s, 2H), 3.72 (dt, J = 5.3, 7.3 Hz, 2H), 2.95 (t, J = 7.3 Hz, 2H); MS (TOF) m/z 333 (M + H)$^+$; anal. calcd for $C_{16}H_{14}Cl_2N_4 \cdot 0.9H_2O$: C, 55.00; H, 4.30; N, 16.03. Found: C, 55.08; H, 4.27; N, 15.82.

6-Amino-4-(4-chlorophenethylamino)quinazoline (11g). Similarly to the procedure described for **11j**, the title compound was prepared starting from **11f**. After purification, **11g** was obtained as a white solid (92% yield): mp 190 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H), 7.86 (t, J = 5.4 Hz, 1H), 7.44–7.26 (m, 5H), 7.13 (dd, J = 2.1, 8.9 Hz, 1H), 7.01 (d, J = 2.1 Hz, 1H), 5.45 (br s, 2H), 3.70 (dt, J = 5.4, 7.0 Hz, 2H), 2.95 (t, J = 7.0 Hz, 2H); MS (TOF) m/z 299 (M + H)$^+$; anal. calcd for $C_{16}H_{15}ClN_4 \cdot 0.8H_2O$: C, 61.36; H, 5.09; N, 17.89. Found: C, 61.67; H, 5.18; N, 17.89.

7-Amino-4-(4-chlorophenethylamino)quinazoline (11l). Similarly to the procedure described for **11j**, the title compound was prepared starting from **11k**. After purification, **11l** was obtained as a white solid (85% yield): mp 228–230 °C; ^{1}H NMR (DMSO-d_6) δ 9.59 (t, J = 5.4 Hz, 1H), 8.61 (s, 1H), 8.10 (d, J = 9.2 Hz, 1H), 7.37–7.27 (m, 4H), 6.95–6.88 (m, 3H), 6.66 (d, J = 1.9 Hz, 1H), 3.84 (dt, J = 5.4, 7.2 Hz, 2H), 2.97 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 299 (M + H)$^+$; anal. calcd for $C_{16}H_{15}ClN_4 \cdot 3H_2O$: C, 54.47; H, 5.14; N, 15.88. Found: C, 54.39; H, 5.12; N, 15.61.

4-(4-Chlorophenethylamino)-6-hydroxyquinazoline (11e). A mixture of **11d** (148 mg, 0.38 mmol) and 5% palladium on carbon (20 mg) in MeOH (5.0 mL) was stirred under an atmosphere of H_2 gas using a balloon reservoir. The reaction mixture was stirred at ambient temperature for 5 h. The catalyst was filtered off over a pad of Celite, and the pad was washed with MeOH. The combined filtrate was concentrated under reduced pressure. The residue was triturated with water, and the precipitate was collected, washed with water, and dried in air. The solid was filtered and dried at 40 °C under high vacuum to give **11e** (85 mg, 75% yield): mp 188–190 °C; ^{1}H NMR (DMSO-d_6) δ 9.95 (s, 1H), 8.37 (s, 1H), 8.17 (br s, 1H), 7.55 (d, J = 9.5 Hz, 1H), 7.42 (d, J = 2.4 Hz, 1H), 7.36–7.26 (m, 5H), 3.74–3.69 (m, 2H), 2.96 (t, J = 7.3 Hz, 2H); HR-FABMS m/z (M + H)$^+$; calcd for $C_{16}H_{14}ClN_3O$: 300.0903. Found: 300.0912.

6-Acetamido-4-(4-chlorophenethylamino)quinazoline (11h). To a solution of **11g** (570 mg, 1.91 mmol) in DMF (19 mL) were added acetic anhydride (270 μL, 2.86 mmol) and pyridine (231 μL, 2.86 mmol). The mixture was stirred at 50 °C for 2 h. The reaction mixture was concentrated under reduced pressure, and the residue was partitioned AcOEt and water. The organic layer was washed with brine, and then dried over Na_2SO_4. The solution was evaporated in vacuo, and the residue was triturated with hexane. The white solid was filtered and dried at 40 °C under high vacuum to give **11h** (470 mg, 72% yield): mp 253–255 °C; ^{1}H NMR (DMSO-d_6) δ 10.19 (s, 1H), 8.42–8.39 (m, 2H), 8.28 (t, J = 5.3 Hz, 1H) 7.72–7.62 (m, 2H), 7.36–7.27 (m, 4H), 3.73 (dt, J = 5.3, 7.3 Hz, 2H), 2.96 (t, J = 7.3 Hz, 2H), 2.10 (s, 3H); MS (TOF) m/z 341 (M + H)$^+$; anal. calcd for $C_{18}H_{17}ClN_4O \cdot 0.1H_2O$: C, 63.10; H, 5.03; N, 16.35. Found: C, 62.89; H, 5.01; N, 16.22.

6-Amino-4-(4-phenoxyphenethylamino)quinazoline (11q). A suspension of 6-nitro-4-quinazolone (300 mg, 1.57 mmol) in thiony chloride (10 mL) containing 1 drop of DMF was refluxed for 3 h until it became clear. The excess thiony chloride was removed under reduced pressure. To a mixture of the resulting crude 4-chloro-6-

nitroquinazoline and triethylamine (262 μL, 1.88 mmol) in *i*-PrOH (15 mL) was added 4-phenoxyphenethylamine (368 μL, 1.88 mmol). The resulting mixture was stirred at ambient temperature for 1 h and concentrated in vacuo. The residue was 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). The light yellow solid was filtered to give 6-nitro-4-(4-phenoxyphenethylamino)quinazoline (403 mg, 66% yield for 2 steps from 6-nitro-4-quinazolone).

Next, iron powder (58 mg; freshly washed with 1 N HCl followed by distilled water) was added in portions to a refluxing solution of the above 6-nitroquinazoline (100 mg, 0.26 mmol) in EtOH/H_2O (2:1, 12 mL) containing glacial AcOH (168 μL). The resulting suspension was heated at reflux with vigorous stirring for 30 min, then cooled, basified with concentrated NH_4OH and EtOH, and combined filtrate was concentrated under reduced pressure, diluted with water and extracted with AcOEt. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with AcOEt/hexane (1:1). The light yellow solid was filtered to give **11q** (72 mg, 77% yield): mp 168–170 °C; ^{1}H NMR (DMSO-d_6) δ 8.33 (br s, 2H), 7.45 (d, J = 8.9 Hz, 1H), 7.40–7.34 (m, 2H), 7.28 (d, J = 8.6 Hz, 2H), 7.20–7.07 (m, 3H), 6.98–6.92 (m, 4H), 5.59 (br s, 2H), 3.79–3.72 (m, 2H), 2.95 (t, J = 7.3 Hz, 2H); MS (TOF) m/z 357 (M + H)$^+$; anal. calcd for $C_{22}H_{20}N_4O \cdot 1.0H_2O$: C, 70.57; H, 5.65; N, 14.96. Found: C, 70.48; H, 5.60; N, 14.87.

6-Amino-4-(phenethylamino)quinazoline hydrochloride (11m). Similarly to the procedure described for **11q**, the crude compound was prepared starting from 6-nitro-4-quinazolone. To a mixture of the above compound in EtOH (14 mL) was added 12 N HCl (66 μL). The mixture was stirred at ambient temperature for 10 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 (63% yield for 4 steps from 6-nitro-4-quinazolone): mp 223–225 °C; ^{1}H NMR (DMSO-d_6) δ 9.90 (br s, 1H), 8.67 (s, 1H), 7.67 (d, J = 8.6 Hz, 1H), 7.42–7.21 (m, 7H), 3.93–3.86 (m, 2H), 3.00 (t, J = 7.3 Hz, 2H); MS (TOF) m/z 265 (M + H)$^+$; anal. calcd for $C_{16}H_{17}ClN_4 \cdot 2.0H_2O$: C, 57.06; H, 5.69; N, 16.63. Found: C, 56.97; H, 5.41; N, 16.56.

6-Amino-4-(4-methylphenethylamino)quinazoline hydrochloride (11n). Similarly to the procedure described for **11m**, the title compound was prepared starting from 6-nitro-4-quinazolone. After purification, **11n** was obtained as a white solid (54% yield for 4 steps from 6-nitro-4-quinazolone): mp 240 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 9.88 (t, J = 5.1 Hz, 1H), 8.67 (s, 1H), 7.67 (d, J = 8.9 Hz, 1H), 7.42–7.35 (m, 2H), 7.17–7.09 (m, 4H), 3.90–3.83 (m, 2H), 2.95 (t, J = 7.3 Hz, 2H), 2.26 (s, 3H); MS (TOF) m/z 279 (M + H)$^+$; anal. calcd for $C_{17}H_{19}ClN_4 \cdot 2.0H_2O$: C, 58.20; H, 6.03; N, 15.97. Found: C, 58.23; H, 5.80; N, 15.90.

6-Amino-4-(4-hydroxyphenethylamino)quinazoline (11o). Similarly to the procedure described for **11q**, the title compound was prepared starting from 6-nitro-4-quinazolone. After purification, **11o** was obtained as a white solid (21% yield for 3 steps from 6-nitro-4-quinazolone): mp 115–117 °C; ^{1}H NMR (DMSO-d_6) δ 9.17 (br s, 1H), 8.20 (s, 1H), 7.73 (t, J = 5.5 Hz, 1H), 7.40 (d, J = 8.9 Hz, 1H), 7.14–7.10 (m, 1H), 7.06–7.01 (m, 3H), 6.68 (d, J = 8.4 Hz, 2H), 5.40 (br s, 2H), 3.63 (dt, J = 5.5, 7.4 Hz, 2H), 2.81 (t, J = 7.4 Hz, 2H); HR-FABMS m/z (M + H)$^+$; calcd for $C_{16}H_{16}N_4O$: 281.1402. Found: 281.1410.

6-Amino-4-(4-methoxyphenethylamino)quinazoline (11p). Similarly to the procedure described for **11q**, the title compound was prepared starting from 6-nitro-4-quinazolone. After purification, **11p** was obtained as a white solid (17% yield for 3 steps from 6-nitro-4-quinazolone): mp 230 °C (Dec.); ^{1}H NMR (DMSO-d_6) δ 8.23 (s, 1H), 7.85 (t, J = 5.1 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.19–7.11 (m, 3H), 7.03 (d, J = 2.2 Hz, 1H), 6.87–6.84 (m, 2H), 5.44 (br s, 2H), 3.71 (s, 3H), 3.68–3.63 (m, 2H), 2.88 (t, J = 7.6 Hz, 2H); MS (TOF) m/z 295 (M + H)$^+$. Anal. calcd for $C_{17}H_{18}N_4O \cdot 0.7H_2O$: C, 66.52; H, 6.14; N, 18.25. Found: C, 66.74; H, 6.27; N, 18.04.

Biology

NF-κB assay.[12] Human Jurkat T cells (Riken, Japan) were cultured at 37 °C in a 5% CO_2 atmosphere in RPMI1640 containing 10% FCS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. The cells were plated in 6-well plates (2×10^6/well) and transiently transfected using the SuperFect Transfection Reagent (QIAGEN) with 1 μg of pNFκB-Luc (PathDetect Cis-Reporter Plasmid, STRATAGENE). After transfection, the cells were cultured at 37 °C overnight. They were then collected, resuspended in fresh medium, and plated in 96-well plates (2×10^5/well). Test compounds were dissolved in DMSO and added at the appropriate concentrations to the 96-well plates containing the cells, and the plates were then incubated at 37 °C for 1 h. For induction of transcription, 10 ng/mL of PMA and 100 μg/mL of PHA were added to each well, and the cells were incubated for an additional 6 h at 37 °C. The culture media were removed, and cell lysis buffer containing luciferase substrate (Bright-Glo Luciferase Assay System, Promega) was added to each well. The each portion was transferred to a black 96-well plate, and then luminescence was immediately measured with a Packard Topcount (Packard Instruments). The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method. To measure the cytotoxicity of test compounds toward Jurkat cells, we added the compounds to 96-well plates containing nontransfected cells (2×10^5/well), and incubated the plates at 37 °C for 24 h. Cell viability was measured by using the MTS assay (Promega).

Inhibition of LPS-induced TNF-α production by murine splenocytes. Splenocytes were prepared by mechanical disruption of the spleen from BALB/c mice with a metal

sieve, followed by hypoosmotic lysis of red blood cells, filtration through nylon gauze, and extensive washing with PBS. The cells were resuspended in RPMI1640 (10% FCS, 100 U/mL of penicillin, and 100 μg/mL of streptomycin) and plated in 96-well plates (1×10^6/well). Then, cells were cultured in the presence of 3 μg/mL LPS (*Escherichia coli*, 0111:B4, DIFCO) and test compounds for 18 h at 37 °C in a 5% CO_2 atmosphere. The culture media were stored at −20 °C until used for determination of TNF-α production. The levels of TNF-α in the culture media were determined by ELISA (Genzyme TECNE). The 50% inhibitory concentration (IC_{50}) values were calculated by a nonlinear regression method. Similarly to the method described for Jurkat cells, the cell viability for murine splenocytes was measured by using the MTS assay.

Anti-inflammatory effect on carrageenin-induced paw edema.[13] Paw edema was induced in male SD rats (7 weeks old, Charles River Japan Inc.). This was done by subcutaneously injecting a 1% suspension of carrageenin (type 1, Sigma) in saline (0.1 mL) into the plantar surface of the right hind paw. Compounds **3** and **11q** were intraperitoneally administered 15 min prior to the injection of the carrageenin. These compounds were administered as suspensions in 0.5% hydroxypropyl cellulose. The resulting edema was quantified 2 h later by measuring the increase in the volume of the inflamed paw. The ensuing paw swelling was measured by using a water-displacement plethysmograph (Ugo Basile).

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