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A Novel Structural Class of Potent Inhibitors of NF-κB Activation: Structure–Activity Relationships and Biological Effects of 6-Aminoquinazoline Derivatives

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Abstract—In this study, we have investigated the roles of substituents on the terminal phenyl ring at the C(4)-position of the quinazoline core to complete the structure–activity relationships (SARs) of our NF-κB activation inhibitors. Among them, compound **12j** afforded highly potent inhibitory activity toward NF-κB transcriptional activation with IC_{50} value of 2 nM, along with an excellent in vivo efficacy by reducing the edema formation seen in carrageenin-induced inflammation of the rat hind paw.

Introduction

Nuclear factor kappa B (NF-κB) is composed of two subunits and is normally sequestered in the cytoplasm through being associated with an IκB protein. When the cell is exposed to activation signals, such as endotoxin or TNF-α binding to cell surface receptors, the IκB protein is phosphorylated, then ubiquinated and broken down in proteosomes. After being freed from association with IκB, the NF-κB complex moves to the nucleus where it binds to specific sequences in the promoter/enhancer regions of genes.[1]

Under baseline conditions, in the healthy human, NF-κB functions in regulating the expression of genes involved in normal immunologic responses, such as generation of antibody light chains and other immunoregulatory molecules. Rapid mobilization of an acute inflammatory response is necessary for defense against external biologic assaults to the organism, such as bacterial infection. However, excessive activation of NF-κB results in enhanced expression of pro-inflammatory cytokines, such as TNF-α, and IL-1β, and immunoregulatory mediators that then leads to inflammatory diseases, such as septic shock, psoriasis, asthma, and rheumatoid arthritis.[2]

The key role that NF-κB plays in controlling the expression of multiple inflammatory and immune genes involved in above mentioned diseases makes this factor a central and favorable target for therapeutic intervention of diseases. From this view point, an inhibitor of NF-κB activation would be expected to possess a therapeutic potential in the treatment of inflammatory diseases.[3]

To our knowledge, several inhibitors of NF-κB transcriptional activation have been previously reported, MG-132 (**1**),[4] an indan derivative (**2**),[5] or BAY 11-7085 (**3**),[6] which are illustrated in Figure 1. Recently, we reported the identification of a series of 6-aminoquinazoline derivatives represented by compounds **4a**–**4e** as the novel structural class of NF-κB activation inhibitors (Fig. 2).[7] Our initial structure–activity relationship (SAR) studies revealed that the following key elements were required for enhanced inhibitory activity toward NF-κB activation: (1) a quinazoline ring system, (2) a basic nitrogen as well as a smaller substituents at the C(6)-position, and (3) an ethylene chain as a spacer between the quinazoline ring and the phenyl one at the C(4)-position.

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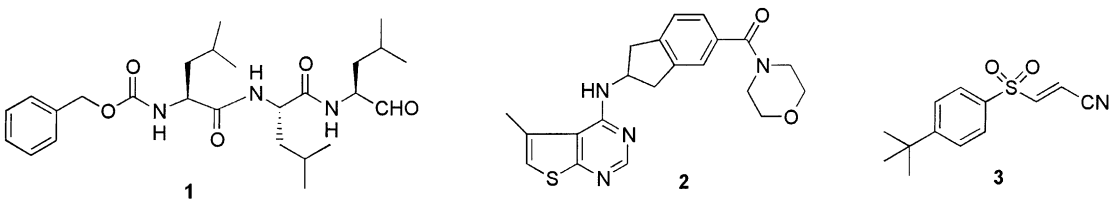


Figure 1. Several inhibitors of NF-κB-mediated transcriptional activation: **1**, MG-132; **2**, indan derivative; **3**, BAY 11-7085.

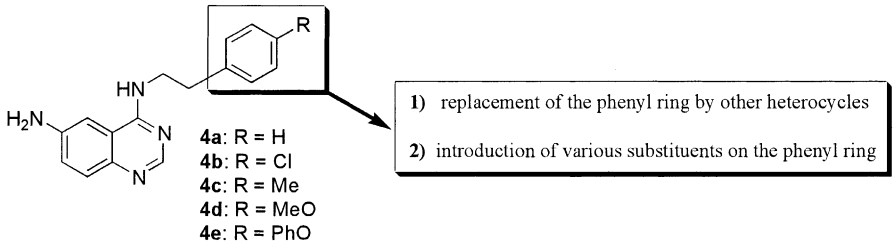


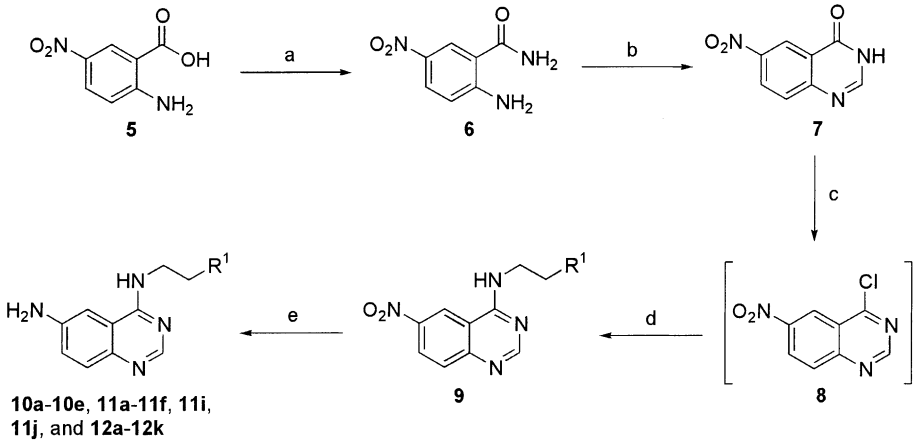
Figure 2. Structures of 6-amino-4-phenethylaminoquinazolines.

In the present study, we investigated further attempts to refine the basic framework by replacing the phenyl ring, which was connected with an ethylene chain at the C(4)-position, with the cyclohexyl ring and several heterocyclic rings, and by placing various substituents on the phenyl ring (Fig. 2). Extensive optimization of these new quinazoline derivatives led us to identify potent NF-κB activation inhibitors, with nanomolar IC_{50}'s toward NF-κB transcriptional activation in human Jurkat cells. Furthermore, selected analogues, such as **12d** and **12j**, demonstrated excellent in vivo efficacy by reducing the edema formation seen in carrageenin-induced inflammation of the rat hind paw. Herein, we wish to describe the SAR revealed on the way to the discovery of these highly potent NF-κB activation inhibitors.

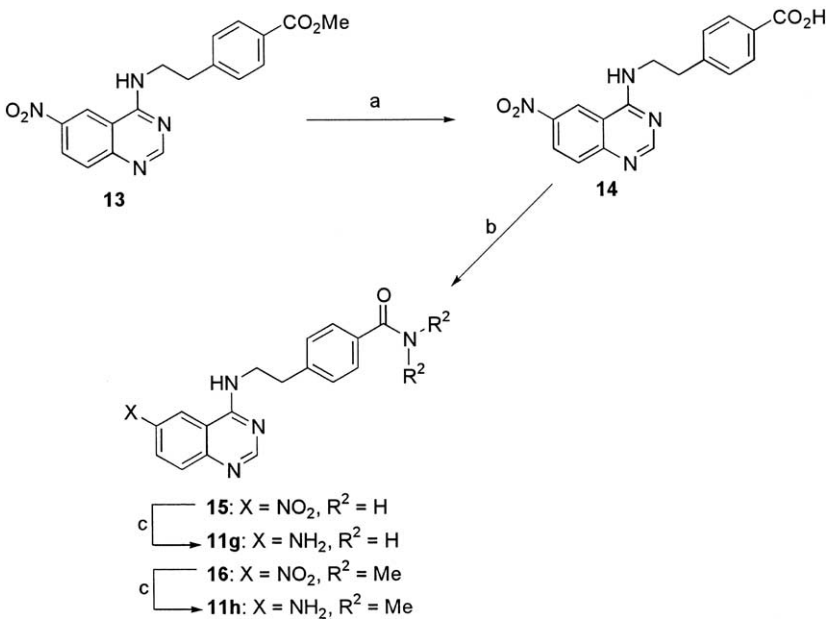
Chemistry

The general synthetic pathway to the 6-aminoquinazolines is shown in Scheme 1. Condensation of 5-nitroanthranilic acid (**5**) with 28% ammonia in the presence of 1-ethyl-3-[(dimethylamino)propyl]carbodiimide (WSCD) and 1-hydroxybenzotriazole (HOBt) followed by cyclization with trimethyl orthoformate gave the 6-nitro-4-quinazolone (**7**). Subsequently, chlorination of the 4-quinazolone (**7**) with thionyl chloride gave the corresponding 4-chloroquinazoline (**8**). Reaction of **8** with appropriate phenethylamines in the presence of triethylamine provided the corresponding 6-nitro-4-phenethylaminoquinazolines (**9**). Reduction of **9** with iron dust gave the target compounds **10a–10e**, **11a–11f**, **i**, **j**, and **12a–12k**.

Procedures for the preparation of **11g** and **11h** are shown in Scheme 2. The acid (**14**) was obtained by saponification of the methyl ester (**13**). Treatment of **14** with WSCD and HOBt followed by the addition of the corresponding amines gave the amides **15** and **16**. Compounds **15** and **16** underwent iron reduction to give the target 6-aminoquinazolines **11g** and **11h**, respectively.



Scheme 1. General procedure for 4-substituted-6-aminoquinazolines. Reagents and conditions: (a) 28% aqueous ammonia solution, WSCD, HOBt, DMF, 4 h; (b) $HC(OMe)_3$, 12 N HCl, 1 h; (c) $SOCl_2$, DMF, reflux, 3 h; (d) phenethylamines, triethylamine, *i*-PrOH, 2–3 h; (e) Fe, glacial acetic acid, EtOH–H_2O, reflux, 30 min.



Scheme 2. Synthesis of the 6-aminoquinazolines, **11g** and **11h**. Reagents and conditions: (a) 5 N NaOH, EtOH, 2 h; (b) 28% aqueous ammonia solution or 40% Me_2NH aqueous solution, WSCD, HOBt, DMF, 3–5 h; (c) Fe, glacial acetic acid, EtOH–H_2O, reflux, 30 min.

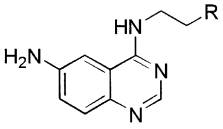
Results and Discussion

The above quinazoline derivatives were synthesized and evaluated for their inhibitory activities toward NF-κB transcriptional activation in the luciferase reporter gene assays featuring phorbol 12-myristate-13-acetate (PMA) plus phytohemagglutin (PHA) stimulation of human Jurkat T cells. Reporter gene assays were performed as described in the Experimental. Inhibition of LPS-induced TNF-α production was conducted in a manner similar to that described previously using mouse splenocytes.[7] Cytotoxicity toward both human Jurkat cells and murine splenocytes was measured by using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt] assay. The results are summarized in Tables 1–3.

With the aim to confirm the importance of the phenyl ring at the C(4)-position in **4a**, we prepared compounds **10a**–**10e** by replacing the phenyl ring with the cyclohexyl ring and several heterocycles, respectively. As shown in Table 1, the cyclohexyl (**10a**) showed a 2-fold loss in the inhibitory activities toward both NF-κB activation and TNF-α production as compared with the phenyl ring (**4a**). Moreover, the pyridyl (**10b**) showed a 27-fold loss in the inhibition of NF-κB activation as well as led to decreased inhibitory activity toward TNF-α production. The morpholino (**10c**), the piperidino (**10d**), and pyrrolidino (**10e**) also exhibited decreased or diminished the activities. These results suggest that the phenyl ring would be preferable for the inhibitory activities toward both NF-κB activation and TNF-α production.

We next focused on the substitution studies on the phenyl ring at the C(4)-position in more detail. As shown in Table 2, the role of the *para*-chlorophenethylamine substitution pattern at the 4-position

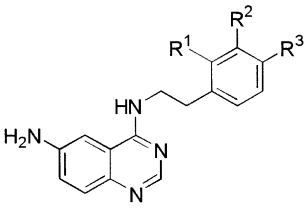
Table 1. Inhibition of NF-κB activation and TNF-α production by compounds **10a**–**10e**



Compd	R	NF-κB[a]	Toxicity[b]	TNF-α[c]	Toxicity[d]
		IC_{50} (nM)	TC_{50} (nM)	IC_{50} (nM)	TC_{50} (nM)
4a		311	> 10,000	1426	> 10,000
10a		685	> 10,000	3240	> 10,000
10b		8271	> 10,000	> 10,000	> 10,000
10c		> 10,000	> 10,000	nt	nt
10d		> 10,000	> 10,000	nt	nt
10e		3694	> 10,000	> 10,000	> 10,000
MG-132 (**1**)		715	1141	809	1893

nt, not tested.
[a]IC_{50} for the inhibition of NF-κB 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.

Table 2. Inhibition of NF-κB activation and TNF-α production by compounds **11a–11j**



Compd	R^1	R^2	R^3	NF-κB[a] IC_{50} (nM)	Toxicity[b] TC_{50} (nM)	TNF-α[c] IC_{50} (nM)	Toxicity[d] TC_{50} (nM)
11a	Cl	H	H	254	>10,000	275	>10,000
11b	H	Cl	H	389	>10,000	243	>10,000
4b	H	H	Cl	176	>10,000	142	>10,000
11c	MeO	H	H	155	>10,000	312	>10,000
11d	H	MeO	H	210	>10,000	366	>10,000
4d	H	H	MeO	90	>10,000	279	>10,000
11e	H	H	Br	207	>10,000	128	>10,000
11f	H	H	F	574	>10,000	510	>10,000
11g	H	H	$CONH_2$	7333	>10,000	nt	>10,000
11h	H	H	$CONMe_2$	2594	>10,000	nt	>10,000
11i	H	H	NHAc	5979	>10,000	nt	>10,000
11j	H	H	SO_2NH_2	>10,000	>10,000	nt	>10,000
MG-132 (**1**)				715	1141	809	1893

nt, not tested.
[a–d]See corresponding footnotes in Table 1.

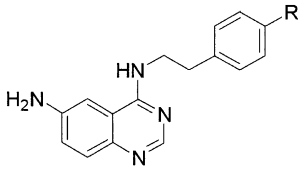
was studied by comparison with the corresponding *meta*- and *ortho*-analogues (**4b** vs **11a** and **11b**). Moving the chloro atom from a *para*- to a *ortho*- or a *meta*-position decreased the inhibitory activities toward both NF-κB activation and TNF-α production. Moreover, the same tendency was observed when the position of the methoxy group on the phenyl ring was changed from a *para*- to an *ortho*- or a *meta*-position (**4d** vs **11c** and **11d**).

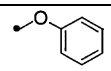
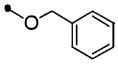
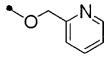
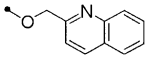
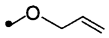
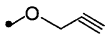
On the basis of these data, we performed a *para*-substitution study on the phenyl ring at the C(4)-position of the quinazoline ring. The bromo atom (**11e**) was comparable to the chloro atom (**4b**) in their inhibitory activities toward NF-κB activation, whereas the fluorine atom (**11f**) resulted in a 3-fold loss in activity compared with **4b**. The same trend was observed when these compounds were evaluated for TNF-α inhibitory activities. The introduction of polar, hydrogen bonding groups (**11g–11j**) had less potent inhibitory activity on NF-κB activation. In particular, the sulfonamide (**11j**), which is an electron-withdrawing substituent similar to the fluorine atom (**11f**), led to a substantial decrease in activity. From these results, it was found that placement of a hydrophilic group as well as an electron-withdrawing one on the phenyl ring was unfavorable for increasing activity.

Next, we investigated the effect of an electron-donating group on the phenyl ring. In our previous paper, we reported that the electron-donating groups, such as the methyl (**4c**), the methoxy (**4d**), and the phenoxy (**4e**) analogues, were found to be potent inhibitors for NF-κB activation.[7] Among these compounds, **4e** showed the most potent inhibitory activity toward both NF-κB activation and TNF-α production. With these data in mind, we focused on the bulky phenoxy analogue and synthesized the related aryloxy or heteroaryloxy compounds (**12a–12c**) listed in Table 3. The benzyloxy (**12a**), which was added to 1 methylene unit in **4e**, resulted in an IC_{50} value comparable to that of **4e** for the inhibitory activity. As shown by the pyridylmethoxy (**12b**), introduction of a nitrogen atom within the phenyl ring in **12a**, displayed similar inhibitory activity toward NF-κB activation over **12a**, whereas this compound exhibited a 4-fold loss in TNF-α inhibitory activity. Replacement of the monocyclic rings with the condensed ring provided the quinolinylmethoxy (**12c**) which led to decreased both inhibitory activities. Furthermore, substitution of the phenoxy group in **4e** with simplified substituents, keeping an electron rich feature constant, afforded the allyloxy (**12d**) which retained good inhibitory activity. Although the propargyloxy (**12e**) somewhat reduced NF-κB inhibitory activity, the high potency shown by the allyloxy group prompted us to further investigate such simplified alkoxy analogues in more detail (Table 3).

The ethoxy (**12f**) exhibited a 4.5- and 16-fold improvement of the inhibitory activities toward NF-κB activation and TNF-α production, respectively, compared with the methoxy (**4d**). The *n*-propoxy (**12g**) showed a potent inhibitory activity toward NF-κB activation similar to **12f**, whereas the iso-propyl (**12h**) displayed a loss of its inhibitory activity. From these results, we considered the hypothesis that the enhancement of lipophilicity would lead to an active compound.

Table 3. Inhibition of NF-κB activation and TNF-α production by compounds **12a**–**12k**



Compd	R	NF-κB[a]	Toxicity[b]	TNF-α[c]	Toxicity[d]
		IC_{50} (nM)	TC_{50} (nM)	IC_{50} (nM)	TC_{50} (nM)
4e		11	> 10,000	7	> 1000
12a		14	> 10,000	9	> 1000
12b		12	> 10,000	41	> 1000
12c		93	> 10,000	100	> 1000
12d		12	> 10,000	18	> 1000
12e		40	> 10,000	59	> 1000
4c	Me	210	> 10,000	138	> 10,000
4d	MeO	90	> 10,000	279	> 10,000
12f	EtO	21	> 10,000	17	> 1000
12g	*n*-PrO	20	> 10,000	15	> 1000
12h	*i*-PrO	40	> 10,000	23	> 1000
12i	*n*-BuO	11	> 10,000	4	> 1000
12j	*n*-PenO	2	> 10,000	3	> 1000
12k	*n*-HexO	4	> 10,000	8	> 1000
MG-132 (**1**)		715	1141	809	1893
2		38	> 10,000	84	> 1000

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

Therefore, in order to increase the lipophilicity, we prepared larger alkoxy analogues, such as *n*-butoxy (**12i**), *n*-pentyloxy (**12j**), and *n*-hexyloxy (**12k**) analogues. The *n*-butoxy (**12i**) led to a 2-fold increase in the NF-κB inhibitory activity as compared with that of **12g**. Furthermore, compounds **12j** and **12k** exhibited highly potent inhibitory activities toward both NF-κB activation and TNF-α production, respectively. As we expected, the enhancement of lipophilicity of compounds **12i**–**12k** compared with that of **4d** reflects these greater inhibitory activities.

Finally, we selected three compounds (**12a**, **12d**, and **12j**) from the results of the above-mentioned in vitro study to further evaluate their anti-inflammatory effects. In our previous study, compound **4e** showed a more potent suppressing effect on edema formation than the indan derivative (**2**) in a rat carrageenin-induced paw edema model.[7] On the basis of these data, we performed the comparative test of **4e** and the selected compounds using the same model when these compounds were tested at a ip dose of 1 mg/kg. The results of the reducing effects on edema formation for these compounds were summarized in Figure 3. Compounds **12a**, **12d**, and **12j** inhibited edema formation and showed more potent suppressing effects than **2**. Suppressing effect of the benzyloxy analogue (**12a**) on edema formation was comparable to that of **4e**. The allyloxy analogue (**12d**) displayed a more potent suppressing effect than **12a**. Moreover, the *n*-pentyloxy (**12j**) afforded low nanomolar IC_{50}'s toward NF-κB activation and TNF-α production along with further enhancement of in vivo activity. It has been reported that the activation of NF-κB may be involved in a pathogenesis by which inflammation is accelerated in the carrageenin-induced paw edema model.[8] Therefore, we consider that the above-mentioned compounds have a reducing effect of NF-κB mediated-inflammatory response.

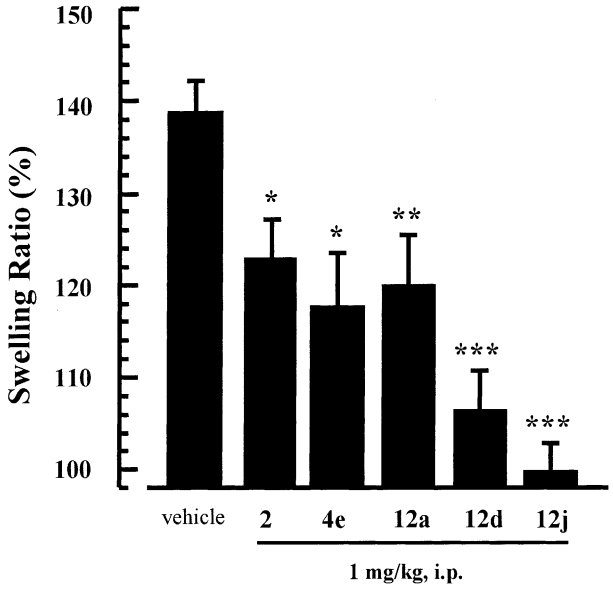


Figure 3. Effects of **12a**, **12d**, and **12j** on carrageenin-induced paw edema in rats. Test compounds were intraperitoneally administered 15 min prior to the injection of carrageenin. The resulting edema was quantified 2 h later by measuring the increase in the volume of the inflamed paw. The results are expressed as the mean ± SEM of five rats per group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus vehicle control (Dunnett's test).

Conclusions

In this study we have investigated the roles of substituents on the terminal phenyl ring at the C(4)-position of the quinazoline ring to complete the SAR of our NF-κB activation inhibitors. Since replacement of the phenyl ring with the cyclohexyl ring and heterocycles (**10a–10e**) reduced activity, we established that the best basic framework was the 6-amino-4-phenethylaminoquinazoline skeleton. The para substitution pattern on the phenyl ring at the C(4)-position was suitable for potent inhibitory activities toward both NF-κB activation and TNF-α production. Introduction of the hydrophilic groups (**11g–11j**) on the phenyl ring reduced the activity, whereas that of the larger alkoxy groups (**12i–12k**) was found to be optimal for potent activity. In particular, the *n*-pentyloxy (**12j**) afforded highly potent inhibitory activities toward both NF-κB activation and TNF-α production, with IC_{50} values of 2 and 3 nM, respectively, along with an excellent in vivo efficacy by reducing the edema formation seen in carrageenin-induced inflammation of the rat hind paw. Therefore, the representative compound **12j** is expected to be a candidate drug for use in the therapy of various inflammatory diseases. The details of pharmacological studies on compound **12j** and related compounds are currently underway.

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 $CDCl_3$. 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. 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).

6-Amino-4-(4-bromo)phenethylaminoquinazoline (11e). A suspension of 6-nitro-4-quinazolone (**7**)[7] (300 mg, 1.57 mmol) in thionyl chloride (10 mL) containing 1 drop of DMF was heated at reflux for 3 h. After cooling to ambient temperature, excess thionyl chloride was removed under reduced pressure to give crude 4-chloro-6-nitroquinazoline (**8**), which was used directly. To a mixture of **8** and triethylamine (262 μL, 1.88 mmol) in *i*-PrOH (15 mL) was added (4-bromo)phenethylamine (292 μL, 1.88 mmol). The resulting mixture was stirred at ambient temperature for 2 h and then was concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and 5% aqueous citric acid solution. The organic layer was washed with 1 N NaOH and brine, and then dried over Na_2SO_4. The solution was concentrated under reduced pressure, and then the residue was triturated with CH_2Cl_2/hexane (1:1). The light yellow solid was filtered to give 4-(4-bromo)phenethylamino-6-nitroquinazoline (**9**: R^1 = 4-bromophenyl) (373 mg, 64% yield for two steps from **7**). Next, iron powder (60 mg) was added to a refluxing solution of the above 6-nitroquinazoline (100 mg, 0.27 mmol) in EtOH/H_2O (2:1, 12 mL) containing glacial AcOH (185 μL). The resulting suspension was heated at reflux with vigorous stirring for 30 min, then cooled, basified with concentrated NH_4OH solution and EtOH, and combined filtrate was evaporated under reduced pressure, diluted with water, and extracted with AcOEt. The organic layer was 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 (**11e**) (30 mg, 32% yield): mp 112–114 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 7.76 (t, J = 5.3 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.6 Hz, 1H), 7.22 (d, J = 8.4 Hz, 2H), 7.12 (dd, J = 2.4, 8.6 Hz, 1H), 6.99 (d, J = 2.4 Hz, 1H), 5.41 (br s, 2H), 3.68 (dt, J = 5.3, 7.2 Hz, 2H), 2.92 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 343 $(M+H)^+$. Anal. calcd for $C_{16}H_{15}BrN_4 \cdot 0.5H_2O$: C, 54.56; H, 4.58; N, 15.91. Found: C, 54.66; H, 4.59; N, 15.67.

6-Amino-4-[2-(cyclohexyl)ethylamino]quinazoline (10a). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **10a** was obtained as a light yellow solid (39% yield for three steps from **7**): mp 130–132 °C; ^{1}H NMR

(DMSO-d_6) δ 9.12 (t, J = 5.0 Hz, 1H), 8.51 (s, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.27 (dd, J = 2.2, 8.6 Hz, 1H), 7.21 (d, J = 2.2 Hz, 1H), 5.89 (br s, 2H), 3.67–3.60 (m, 2H), 1.77–1.51 (m, 7H), 1.37–1.10 (m, 4H), 0.99–0.86 (m, 2H); HR-FABMS m/z $(M+H)^+$; calcd for $C_{16}H_{22}N_4$: 271.1923. Found: 271.1919.

6-Amino-4-[2-(3-pyridyl)ethylamino]quinazoline hydrochloride (10b). Similarly to the procedure described for **11e**, 4-[2-(3-pyridyl)ethylamino]-6-nitroquinazoline (**9**: R^1 = 3-pyridyl) was prepared starting from **7**. A mixture of the above 6-nitroquinazoline (150 mg, 0.51 mmol) and 5% palladium on carbon (15 mg) in MeOH (20 mL) containing 12 N HCl (55 μL) was stirred under H_2 atmosphere. After 5 h at ambient temperature, the catalyst was filtered off over a pad of Celite, and the pad was washed with MeOH. The combined filtrates were concentrated under reduced pressure to give crude compound, which was used directly. To a mixture of the crude compound in EtOH (5 mL) was added 12 N HCl (64 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 pale yellow powder (153 mg, 38% yield for 4 steps from **7**): mp 277–278 °C; ^{1}H NMR (DMSO-d_6) δ 9.94 (t, J = 5.4 Hz, 1H), 8.93 (s, 1H), 8.81 (d, J = 5.4 Hz, 1H), 8.65 (s, 1H), 8.52 (d, J = 8.1 Hz, 1H), 8.03–8.00 (m, 1H), 7.71 (d, J = 8.6 Hz, 1H), 7.42–7.32 (m, 2H), 4.05–3.98 (m, 2H), 3.25 (t, J = 6.3 Hz, 2H); MS (TOF) m/z 266 $(M+H)^+$. Anal. calcd for $C_{15}H_{16}ClN_5 \cdot 4.2H_2O$: C, 47.73; H, 5.02; N, 18.34. Found: C, 47.73; H, 5.39; N, 18.56.

6-Amino-4-[2-(1-morpholinyl)ethylamino]quinazoline hydrochloride (10c). Similarly to the procedure described for **10b**, the title compound was prepared starting from **7**. After purification, **10c** was obtained as a white solid (61% yield for four steps from **7**): mp 221–223 °C; ^{1}H NMR (DMSO-d_6) δ 10.06 (t, J = 5.3 Hz, 1H), 8.70 (s, 1H), 7.74 (d, J = 8.9 Hz, 1H), 7.40–7.36 (m, 2H), 4.11–3.92 (m, 6H), 3.53–3.37 (m, 6H); MS (TOF) m/z 274 $(M+H)^+$. Anal. calcd for $C_{14}H_{20}ClN_5O \cdot 3.5H_2O$: C, 45.10; H, 6.35; N, 18.78. Found: C, 45.49; H, 6.56; N, 18.45.

6-Amino-4-[2-(1-piperidinyl)ethylamino]quinazoline hydrochloride (10d). Similarly to the procedure described for **10b**, the title compound was prepared starting from **7**. After purification, **10d** was obtained as a white solid (54% yield for four steps from **7**): mp 183–185 °C; ^{1}H NMR (DMSO-d_6) δ 10.18 (t, J = 5.3 Hz, 1H), 8.72 (s, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.44 (dd, J = 8.9, 2.2 Hz, 1H), 4.10–4.08 (m, 2H), 3.59–3.55 (m, 2H), 3.41–3.35 (m, 2H), 3.02–2.88 (m, 2H), 1.86–1.68 (m, 5H), 1.47–1.32 (m, 1H); MS (TOF) m/z 272 $(M+H)^+$. Anal. calcd for $C_{15}H_{22}ClN_5 \cdot 5.0H_2O$: C, 45.28; H, 6.84; N, 17.60. Found: C, 45.22; H, 6.81; N, 17.56.

6-Amino-4-[2-(1-pyrrolidinyl)ethylamino]quinazoline hydrochloride (10e). Similarly to the procedure described for **10b**, the title compound was prepared starting from **7**. After purification, **10e** was obtained as a white solid (53% yield for four steps from **7**): mp 162–164 °C; ^{1}H NMR (DMSO-d_6) δ 10.21 (t, J = 5.3 Hz, 1H), 8.74 (s, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.59 (d, J = 1.9 Hz, 1H), 7.49 (dd, J = 8.9, 1.9 Hz, 1H), 4.06–4.04 (m, 2H), 3.72–3.65 (m, 2H), 3.56–3.40 (m, 2H), 3.11–3.00 (m, 2H), 2.03–1.87 (m, 4H); MS (TOF) m/z 258 $(M+H)^+$. Anal. calcd for $C_{14}H_{20}ClN_5 \cdot 5.2H_2O$: C, 43.40; H, 6.56; N, 18.07. Found: C, 43.34; H, 6.35; N, 17.79.

6-Amino-4-(2-chloro)phenethylaminoquinazoline (11a). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **11a** was obtained as a light yellow solid (28% yield for three steps from **7**): mp 178–180 °C; ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H), 8.20 (t, J = 4.9 Hz, 1H), 7.46–7.40 (m, 2H), 7.36–7.33 (m, 1H), 7.30–7.24 (m, 2H), 7.17 (dd, J = 2.3, 9.0 Hz, 1H), 7.05 (d, J = 2.3 Hz, 1H), 5.55 (br s, 2H), 3.76 (dt, J = 4.9, 7.2 Hz, 2H), 3.09 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 299 $(M+H)^+$. Anal. calcd for $C_{16}H_{15}ClN_4 \cdot 0.7H_2O$: C, 61.72; H, 5.08; N, 17.79. Found: C, 62.02; H, 5.09; N, 17.72.

6-Amino-4-(3-chloro)phenethylaminoquinazoline (11b). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **11b** was obtained as a light yellow solid (53% yield for three steps from **7**): mp 101–103 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 7.78 (t, J = 5.3 Hz, 1H), 7.41 (d, J = 8.9 Hz, 1H), 7.33–7.20 (m, 4H), 7.12 (dd, J = 2.4, 8.9 Hz, 1H), 7.00 (d, J = 2.4 Hz, 1H), 5.42 (br s, 2H), 3.71 (dt, J = 5.3, 7.2 Hz, 2H), 2.96 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 299 $(M+H)^+$. Anal. calcd for $C_{16}H_{15}ClN_4 \cdot 1.0H_2O$: C, 60.66; H, 5.41; N, 17.69. Found: C, 60.34; H, 5.39; N, 17.72.

6-Amino-4-(2-methoxy)phenethylaminoquinazoline (11c). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **11c** was obtained as a light yellow solid (46% yield for three steps from **7**): mp 167–169 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (s, 1H), 7.94 (t, J = 5.3 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 7.33–7.12 (m, 3H), 7.05 (d, J = 2.4 Hz, 1H), 6.98–6.95 (m, 1H), 6.89–6.83 (m, 1H), 5.47 (br s, 2H), 3.79 (s, 3H), 3.72–3.64 (m, 2H), 2.94 (t, J = 7.3 Hz, 2H); HR-FABMS m/z $(M+H)^+$; calcd for $C_{17}H_{18}N_4O$: 295.1559. Found: 295.1555.

6-Amino-4-(3-methoxy)phenethylaminoquinazoline (11d). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **11d** was obtained as a light yellow solid (42% yield for three steps from **7**): mp 166–168 °C; ^{1}H NMR (DMSO-d_6) δ 8.40 (t, J = 5.7 Hz, 1H), 8.36 (s, 1H), 7.46 (d, J = 8.9 Hz, 1H), 7.24–7.17 (m, 2H), 7.08 (d, J = 2.4 Hz, 1H), 6.84–6.75 (m, 3H), 5.63 (br s, 2H), 3.80–3.71 (m, 2H), 3.67 (s, 3H), 2.94 (t, J = 7.3 Hz, 2H); HR-FABMS m/z $(M+H)^+$; calcd for $C_{17}H_{18}N_4O$: 295.1559. Found: 295.1564.

6-Amino-4-(4-fluorophenethylamino)quinazoline hydrochloride (11f). Similarly to the procedure described for **10b**, the title compound was prepared starting from **7**.

After purification, **11f** was obtained as a white solid (47% yield for four steps from **7**): mp 231–233 °C; ^{1}H NMR (DMSO-d_6) δ 9.87 (t, J = 5.3 Hz, 1H), 8.66 (s, 1H), 7.67 (d, J = 8.9 Hz, 1H), 7.41–7.27 (m, 4H), 7.16–7.08 (m, 2H), 3.92–3.85 (m, 2H), 3.00 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 283 $(M+H)^+$. Anal. calcd for $C_{16}H_{16}ClFN_4 \cdot 2.0H_2O$: C, 54.16; H, 5.11; N, 15.79. Found: C, 54.12; H, 5.11; N, 15.79.

4-(4-Acetamido)phenethylamino - 6 - aminoquinazoline (11i). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **11i** was obtained as a white solid (41% yield for three steps from **7**): mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 9.86 (s, 1H), 8.21 (s, 1H), 7.75 (t, J = 5.3 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.9 Hz, 1H), 7.17 (d, J = 8.4 Hz, 2H), 7.14–7.10 (m, 1H), 7.01 (d, J = 2.2 Hz, 1H), 5.41 (br s, 2H), 3.66 (dt, J = 5.3, 7.3 Hz, 2H), 2.88 (t, J = 7.3 Hz, 2H), 2.02 (s, 3H); MS (TOF) m/z 322 $(M+H)^+$. Anal. calcd for $C_{18}H_{19}N_5O \cdot 1.7H_2O$: C, 61.42; H, 5.93; N, 19.90. Found: C, 61.57; H, 6.15; N, 19.80.

6-Amino - 4 - (4 - sulfonamido)phenethylaminoquinazoline (11j). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **11j** was obtained as a light yellow solid (15% yield for three steps from **7**): mp 233–235 °C; ^{1}H NMR (DMSO-d_6) δ 8.22 (s, 1H), 7.81 (t, J = 5.1 Hz, 1H), 7.74 (d, J = 8.4 Hz, 2H), 7.46–7.40 (m, 3H), 7.29 (br s, 2H), 7.15–7.11 (m, 1H), 7.00 (d, J = 2.2 Hz, 1H), 5.43 (br s, 2H), 3.72 (dt, J = 5.1, 7.0 Hz, 2H), 3.03 (t, J = 7.0 Hz, 2H); MS (TOF) m/z 344 $(M+H)^+$. Anal. calcd for $C_{16}H_{17}N_5O_2S \cdot 0.8H_2O$: C, 53.71; H, 5.01; N, 19.57. Found: C, 53.57; H, 5.17; N, 19.30.

6-Amino - 4 - (4 - benzyloxy)phenethylaminoquinazoline (12a). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12a** was obtained as a light yellow solid (17% yield for three steps from **7**): mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ 9.66 (t, J = 5.0 Hz, 1H), 8.63 (s, 1H), 7.59 (d, J = 8.9 Hz, 1H), 7.44–7.31 (m, 6H), 7.23–7.16 (m, 3H), 6.94 (d, J = 8.6 Hz, 2H), 6.06 (br s, 2H), 5.06 (s, 2H), 3.87–3.80 (m, 2H), 2.92 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 371 $(M+H)^+$. Anal. calcd for $C_{23}H_{22}N_4O \cdot 3.6H_2O$: C, 63.46; H, 5.93; N, 12.87. Found: C, 63.71; H, 5.94; N, 12.52.

6-Amino-4-[4-(2-pyridylmethyloxy)]phenethylamino]quinazoline (12b). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12b** was obtained as a light yellow solid (16% yield for three steps from **7**): mp 173–174 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (t, J = 5.0 Hz, 1H), 8.63 (s, 1H), 8.58–8.56 (m, 1H), 7.86–7.80 (m, 1H), 7.58 (d, J = 8.9 Hz, 1H), 7.49 (d, J = 8.9 Hz, 1H), 7.36–7.30 (m, 2H), 7.22–7.17 (m, 3H), 6.95 (d, J = 8.6 Hz, 2H), 6.06 (br s, 2H), 5.14 (s, 2H), 3.85–3.83 (m, 2H), 2.92 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 372 $(M+H)^+$. Anal. calcd for $C_{22}H_{21}N_5O \cdot 3.2H_2O$: C, 61.58; H, 5.68; N, 16.32. Found: C, 61.72; H, 5.59; N, 16.03.

6-Amino-4-[4-(2 - quinolylmethyloxy)]phenethylamino] quinazoline (12c). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12c** was obtained as a light yellow solid (44% yield for three steps from **7**): mp 211–213 °C; ^{1}H NMR (DMSO-d_6) δ 9.31 (t, J = 4.6 Hz, 1H), 8.55 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.03–7.98 (m, 2H), 7.82–7.76 (m, 1H), 7.67–7.53 (m, 3H), 7.31–7.27 (m, 1H), 7.21–7.17 (m, 3H), 7.00 (d, J = 8.4 Hz, 2H), 5.94 (br s, 2H), 5.34 (s, 2H), 3.84–3.77 (m, 2H), 2.91 (t, J = 7.3 Hz, 2H); MS (TOF) m/z 422 $(M+H)^+$. Anal. calcd for $C_{26}H_{23}N_5O \cdot 3.0H_2O$: C, 65.67; H, 5.51; N, 14.72. Found: C, 65.48; H, 5.65; N, 14.37.

4-(4-Allyloxy)phenethylamino-6-aminoquinazoline (12d). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12d** was obtained as a light yellow solid (17% yield for three steps from **7**): mp 207–209 °C; ^{1}H NMR (DMSO-d_6) δ 9.68 (t, J = 5.1 Hz, 1H), 8.63 (s, 1H), 7.59 (d, J = 8.9 Hz, 1H), 7.33 (dd, J = 2.2, 8.9 Hz, 1H), 7.23 (d, J = 2.2 Hz, 1H), 7.17 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.09–5.95 (m, 3H), 5.41–5.21 (m, 2H), 4.54–4.51 (m, 2H), 3.88–3.80 (m, 2H), 2.92 (t, J = 7.4 Hz, 2H); MS (TOF) m/z 321 $(M+H)^+$. Anal. calcd for $C_{19}H_{20}N_4O \cdot 3.5H_2O$: C, 59.51; H, 6.18; N, 14.61. Found: C, 59.26; H, 6.31; N, 14.71.

6-Amino-4-(4-propargyloxy)phenethylaminoquinazoline (12e). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12e** was obtained as a light yellow solid (25% yield for three steps from **7**): mp 201–203 °C; ^{1}H NMR (DMSO-d_6) δ 9.69 (t, J = 5.1 Hz, 1H), 8.63 (s, 1H), 7.61 (d, J = 8.9 Hz, 1H), 7.33 (dd, J = 2.2, 8.9 Hz, 1H), 7.24–7.18 (m, 3H), 6.91 (d, J = 8.4 Hz, 2H), 6.06 (br s, 2H), 4.75 (d, J = 2.2 Hz, 2H), 3.88–3.81 (m, 2H), 3.56 (t, J = 2.2 Hz, 1H), 2.93 (t, J = 7.2 Hz, 2H); MS (TOF) m/z 319 $(M+H)^+$. Anal. calcd for $C_{19}H_{18}N_4O \cdot 3.2H_2O$: C, 60.69; H, 5.68; N, 14.90. Found: C, 60.50; H, 5.71; N, 14.97.

6-Amino-4-(4-ethoxy)phenethylaminoquinazoline (12f). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12f** was obtained as a light yellow solid (8% yield for three steps from **7**): mp 243–245 °C; ^{1}H NMR (DMSO-d_6) δ 9.69 (t, J = 5.0 Hz, 1H), 8.64 (s, 1H), 7.58 (d, J = 9.2 Hz, 1H), 7.33 (dd, J = 1.9, 9.2 Hz, 1H), 7.22 (d, J = 1.9 Hz, 1H), 7.16 (d, J = 8.6 Hz, 2H), 6.84 (d, J = 8.6 Hz, 2H), 6.07 (br s, 2H), 3.97 (q, J = 7.0 Hz, 2H), 3.88–3.80 (m, 2H), 2.92 (t, J = 7.3 Hz, 2H), 1.30 (t, J = 7.0 Hz, 3H); MS (TOF) m/z 309 $(M+H)^+$. Anal. calcd for $C_{18}H_{20}N_4O \cdot 2.1H_2O$: C, 62.45; H, 6.43; N, 16.18. Found: C, 62.36; H, 6.13; N, 16.30.

6-Amino - 4 - (4 - *n* - propoxy)phenethylaminoquinazoline (12g). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12g** was obtained as a light yellow solid (20% yield for three steps from **7**): mp 127–129 °C; ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1H), 7.88 (t, J = 5.1 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H), 7.17–7.12 (m, 3H), 7.03 (d,

$J=2.2$ Hz, 1H), 6.84 (d, $J=8.6$ Hz, 2H), 5.45 (br s, 2H), 3.88 (t, $J=6.6$ Hz, 2H), 3.70–3.63 (m, 2H), 2.87 (t, $J=7.4$ Hz, 2H), 1.74–1.64 (m, 2H), 0.96 (t, $J=7.6$ Hz, 3H); MS (TOF) m/z 323 $(M+H)^+$. Anal. calcd for $C_{19}H_{22}N_4O\cdot0.8H_2O$: C, 67.75; H, 6.82; N, 16.63. Found: C, 67.63; H, 6.84; N, 16.39.

6-Amino - 4 - (4 - *iso* - propoxy)phenethylaminoquinazoline (12h). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12h** was obtained as a light yellow solid (13% yield for three steps from **7**): mp 213–215 °C; ^{1}H NMR (DMSO-d_6) δ 9.46 (t, $J=5.0$ Hz, 1H), 8.58 (s, 1H), 7.57 (d, $J=8.9$ Hz, 1H), 7.30 (dd, $J=2.2$, 8.9 Hz, 1H), 7.20 (d, $J=2.2$ Hz, 1H), 7.15 (d, $J=8.6$ Hz, 2H), 6.83 (d, $J=8.6$ Hz, 2H), 5.99 (br s, 2H), 4.59–4.50 (m, 1H), 3.85–3.78 (m, 2H), 2.90 (t, $J=7.4$ Hz, 2H), 1.23 (d, $J=5.9$ Hz, 6H); MS (TOF) m/z 323 $(M+H)^+$. Anal. calcd for $C_{19}H_{22}N_4O\cdot3.0H_2O$: C, 60.62; H, 6.69; N, 14.88. Found: C, 60.81; H, 6.62; N, 14.97.

6-Amino-4-(4-*n*-butoxy)phenethylaminoquinazoline (12i). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12i** was obtained as a light yellow solid (24% yield for three steps from **7**): mp 177–179 °C; ^{1}H NMR (DMSO-d_6) δ 8.46 (br s, 1H), 8.37 (s, 1H), 7.47 (d, $J=8.9$ Hz, 1H), 7.22–7.09 (m, 4H), 6.84 (d, $J=8.6$ Hz, 2H), 5.65 (br s, 2H), 3.91 (t, $J=6.5$ Hz, 2H), 3.75–3.68 (m, 2H), 2.88 (t, $J=7.3$ Hz, 2H), 1.72–1.62 (m, 2H), 1.49–1.35 (m, 2H), 0.92 (t, $J=7.3$ Hz, 3H); MS (TOF) m/z 337 $(M+H)^+$. Anal. calcd for $C_{20}H_{24}N_4O\cdot3.0H_2O$: C, 61.52; H, 7.74; N, 14.35. Found: C, 61.71; H, 7.70; N, 14.62.

6-Amino - 4 - (4 - *n* - pentyloxy)phenethylaminoquinazoline (12j). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12j** was obtained as a light yellow solid (32% yield for three steps from **7**): mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ 9.71 (t, $J=5.1$ Hz, 1H), 8.64 (s, 1H), 7.60 (d, $J=8.9$ Hz, 1H), 7.33 (dd, $J=2.2$, 8.9 Hz, 1H), 7.23 (s, 1H), 7.16 (d, $J=8.6$ Hz, 2H), 6.84 (d, $J=8.6$ Hz, 2H), 6.07 (br s, 2H), 3.93–3.83 (m, 4H), 2.92 (t, $J=7.2$ Hz, 2H), 1.71–1.66 (m, 2H), 1.36–1.32 (m, 4H), 0.89 (t, $J=7.2$ Hz, 3H); MS (TOF) m/z 351 $(M+H)^+$. Anal. calcd for $C_{21}H_{26}N_4O\cdot3.0H_2O$: C, 62.35; H, 7.23; N, 13.85. Found: C, 62.23; H, 7.26; N, 13.83.

6-Amino - 4 - (4 - *n* - hexyloxy)phenethylaminoquinazoline (12k). Similarly to the procedure described for **11e**, the title compound was prepared starting from **7**. After purification, **12k** was obtained as a light yellow solid (11% yield for three steps from **7**): mp 145–147 °C; ^{1}H NMR (DMSO-d_6) δ 8.27 (s, 1H), 8.03 (t, $J=4.9$ Hz, 1H), 7.43 (d, $J=8.6$ Hz, 1H), 7.17–7.13 (m, 3H), 7.05–7.04 (m, 1H), 6.84 (d, $J=8.4$ Hz, 2H), 5.50 (br s, 2H), 3.91 (t, $J=6.5$ Hz, 2H), 3.72–3.64 (m, 2H), 2.87 (t, $J=7.3$ Hz, 2H), 1.71–1.63 (m, 2H), 1.42–1.26 (m, 6H), 0.87 (t, $J=6.8$ Hz, 3H); MS (TOF) m/z 365 $(M+H)^+$. Anal. calcd for $C_{22}H_{28}N_4O\cdot0.8H_2O$: C, 69.74; H, 7.66; N, 14.79. Found: C, 69.87; H, 7.68; N, 14.75.

4-(4-Carboxy)phenethylamino - 6 - nitroquinazoline (14). Similarly to the procedure described for **11e**, 4-(4-methoxycarbonyl)phenethylamino - 6 - nitroquinazoline (**13**) was prepared starting from **7**. A solution of **13** (1.30 g, 3.69 mmol) in EtOH (34 mL) containing 5 N NaOH (3.4 mL) was stirred at ambient temperature for 2 h. The reaction mixture was neutralized with 6 N HCl and concentrated under reduced pressure. The residue was triturated with MeOH–H_2O (1:1, v/v), and then the yellow solid was filtered to give **14** (1.05 g, 84% yield); mp, 290–292 °C; ^{1}H NMR (DMSO-d_6) δ 12.82 (br s, 1H), 9.33 (d, $J=2.4$ Hz, 1H), 9.12 (t, $J=5.4$ Hz, 1H), 8.63 (s, 1H), 8.48 (dd, $J=2.4$, 9.2 Hz, 1H), 7.89–7.82 (m, 3H), 7.40 (d, $J=8.4$ Hz, 2H), 3.84 (dt, $J=5.4$, 7.3 Hz, 2H), 3.07 (t, $J=7.3$ Hz, 2H); HR-FABMS m/z $(M+H)^+$; calcd for $C_{17}H_{14}N_4O_4$: 339.1093. Found: 339.1096.

6-Amino - 4 - (4 - carboxamido)phenethylaminoquinazoline (11g). To a solution of **14** (150 mg, 0.44 mmol) and HOBt (65 mg, 0.48 mmol) in DMF (9 mL) was added WSCD (92 mg, 0.48 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 1 h, the reaction mixture was poured into CH_2Cl_2/5% aqueous $NaHCO_3$ solution (1:1), and then the precipitated solid was collected by filtration. The obtained solid was washed with water, and then dried in vacuo to give **15** as a pale yellow solid (134 mg, 91% yield): ^{1}H NMR (DMSO-d_6) δ 9.34 (d, $J=2.4$ Hz, 1H), 9.12 (t, $J=5.3$ Hz, 1H), 8.63 (s, 1H), 8.48 (dd, $J=2.4$, 9.2 Hz, 1H), 7.91 (br s, 1H), 7.85–7.80 (m, 3H), 7.35 (d, $J=8.1$ Hz, 2H), 7.30 (br s, 1H), 3.83 (dt, $J=5.3$, 7.2 Hz, 2H), 3.05 (t, $J=7.3$ Hz, 2H); MS (TOF) m/z 338 $(M+H)^+$.

Similarly to the procedure described for **11e**, the title compound was prepared starting from **15**. After purification, **11g** was obtained as a light yellow solid (34% yield): mp 116–118 °C; ^{1}H NMR (DMSO-d_6) δ 8.22 (s, 1H), 7.90 (br s, 1H), 7.82–7.76 (m, 3H), 7.41 (d, $J=8.6$ Hz, 1H), 7.32 (d, $J=8.4$ Hz, 2H), 7.28 (br s, 1H), 7.12 (dd, $J=2.2$, 8.6 Hz, 1H), 7.01 (d, $J=2.2$ Hz, 1H), 5.41 (br s, 2H), 3.76–3.69 (m, 2H), 3.00 (t, $J=7.2$ Hz, 2H); MS (TOF) m/z 308 $(M+H)^+$. Anal. calcd for $C_{17}H_{17}N_5O\cdot0.5H_2O$: C, 64.54; H, 5.73; N, 22.14. Found: C, 64.60; H, 5.69; N, 22.01.

6-Amino - 4 - (4 - *N,N* - dimethylcarboxamido)phenethylaminoquinazoline (11h). Similarly to the procedure described for **11g**, the title compound was prepared starting from **14**. After purification, **11h** was obtained as a light yellow solid (38% yield for two steps from **14**): mp 294–296 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (s, 1H), 7.80 (t, $J=5.4$ Hz, 1H), 7.41 (d, $J=8.6$ Hz, 1H), 7.32 (s, 4H), 7.13 (dd, $J=2.2$, 8.6 Hz, 1H), 7.01 (d, $J=2.2$ Hz, 1H), 5.42 (br s, 2H), 3.76–3.69 (m, 2H), 3.01–2.91 (m, 8H); MS (TOF) m/z 337 $(M+H)^+$. Anal. calcd for $C_{19}H_{21}N_5O\cdot0.5H_2O$: C, 66.26; H, 6.29; N, 20.33. Found: C, 66.45; H, 6.51; N, 19.97.

Biology

NF-κB assay.[9] 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 six-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 non-transfected 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.[10] 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 l, Sigma) in saline (0.1 mL) into the plantar surface of the right hind paw. Compounds **2**, **4e**, **12a**, **12d**, and **12j** 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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