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1*H*-Imidazo[4,5-*c*]quinoline Derivatives as Novel Potent TNF-α Suppressors: Synthesis and Structure–Activity Relationship of 1-, 2-and 4-Substituted 1*H*-imidazo[4,5-*c*]quinolines or 1*H*-imidazo[4,5-*c*]pyridines

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Abstract—Structural modification of imiquimod (**1**), which is known as an interferon-α (IFN-α) inducer, for the aim of finding a novel and small-molecule tumor necrosis factor-α (TNF-α) suppressor and structure–activity relationship (SAR) are described. Structural modification of a imiquimod analogue, 4-amino-1-[2-(1-benzyl-4-piperidyl)ethyl-1*H*-imidazo[4,5-*c*]quinoline (**2**), which had moderate TNF-α suppressing activity without IFN-α inducing activity, led to a finding of 4-chloro-2-phenyl-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (**10**) with potent TNF-α suppressing activity. The relation between conformational direction of 2-(4-piperidyl)ethyl group at position 1 and TNF-α suppressing activity is also demonstrated by NMR.

Introduction

Immunostimulants have a rationale for use in primary or acquired immunodeficiency. In particular, interferon (IFN) inducers are examples of this approach. Imiquimod (**1**) has been known as a low molecular weight IFN-α inducer, and was approved by the US Food and Drug Administration (FDA) in 1997 for the topical treatment of external genital warts (Fig. 1).[1] The drug **1** is recognized by antigen presenting cells including monocytes, macrophages, B-cells and dendritic cells and induces these cells to produce cytokines including IFN-α and tumor necrosis factor-α (TNF-α) and others.[1] For the cytokine inducer like imiquimod, however, the selectivity against a kind of proinflammatory cytokine such as TNF-α is sometimes critical issue. On the other hand, TNF-α was defined as an endotoxin-induced serum factor, which produced necrosis of tumors both in vitro and in vivo, and identified as a proinflammatory cytokine produced in response to infection and other cellular stresses.[2] Overproduction of TNF-α can lead to the aggravation of the immune diseases such as rheumatoid arthritis (RA), Crohn's disease (CD) and endotoxic shock.[2] At present, infliximab (Remicade™), a chimeric monoclonal anti-TNF-α antibody, and etanercept (Enbrel™), a recombinant human soluble TNF-α p75 receptor fusion protein, have been used for treatment of RA and it has been proved that anti-TNF-α therapy was effective against RA.[3] Therefore, low molecular weight TNF-α suppressors seemed to be promising drugs for treatment of RA. By the way, the structural resemblance between agonist and antagonist was well known, for instance, the theory of Ariens,[4] antimetabolites,[5] conversion of muscarinic or γ-aminobutylic acid (GABA) receptor agonists into antagonists.[6] From this point of view, we counterscreened analogues without IFN-α inducing activity of **1** for lipopolysaccharide (LPS)-stimulated TNF-α production in human peripheral blood mononuclear cells (PBMCs) and consequently found that 4-amino-1-[2-(1-benzyl-4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (**2**) possessed weak suppressing effect on the TNF-α production ($IC_{50}=4810$ nM). This paper describes further structural modification of **2** for lead-finding of a novel and low molecular weight TNF-α suppressor and their structure–activity relationship (SAR) on TNF-α suppressing activity.

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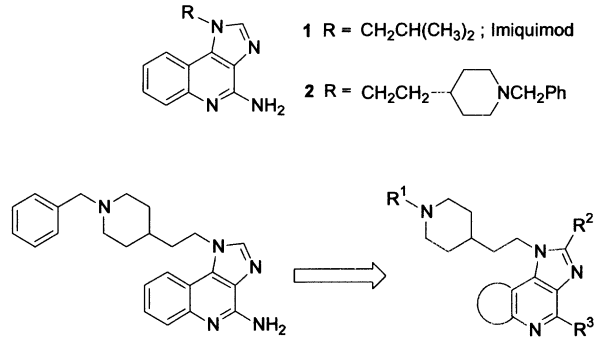
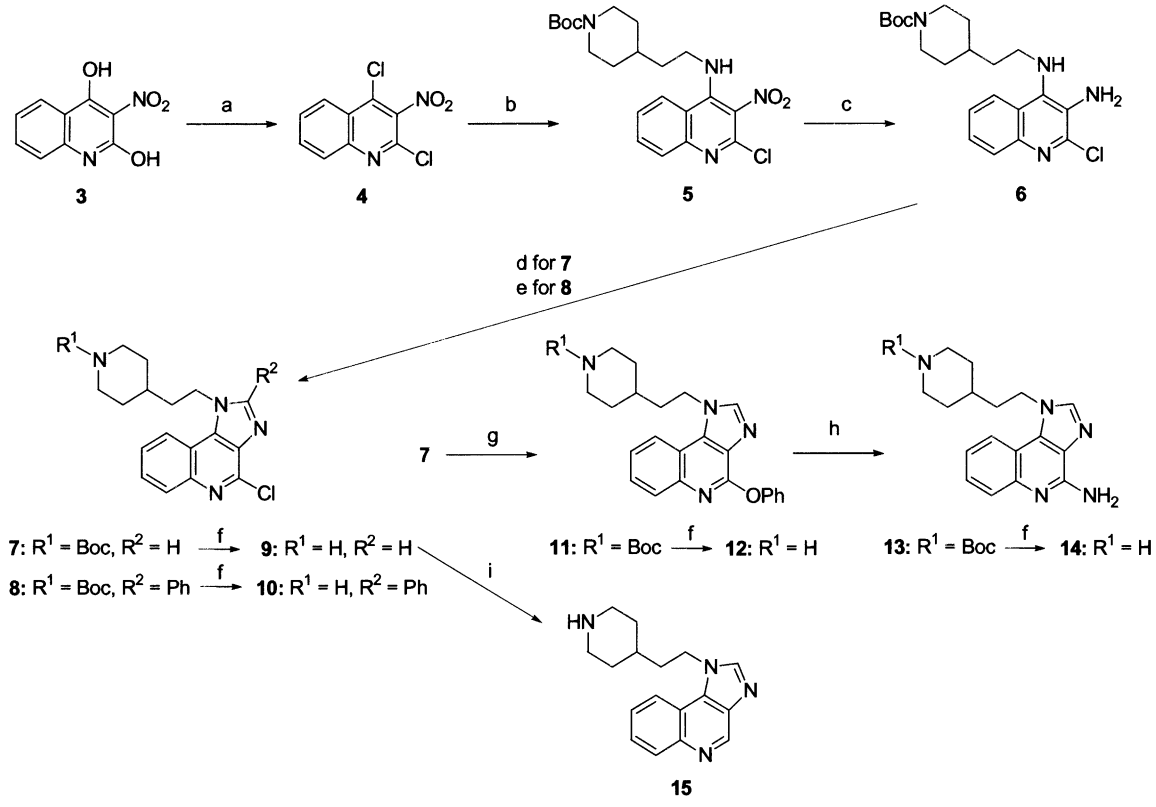


Figure 1.

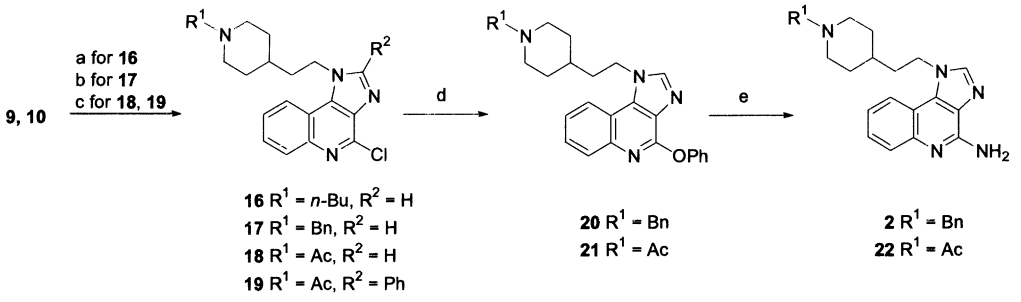
Chemistry

The synthetic methods of 1*H*-imidazo[4,5-*c*]quinolines and 1*H*-imidazo[4,5-*c*]pyridines are illustrated in Schemes 1–3.

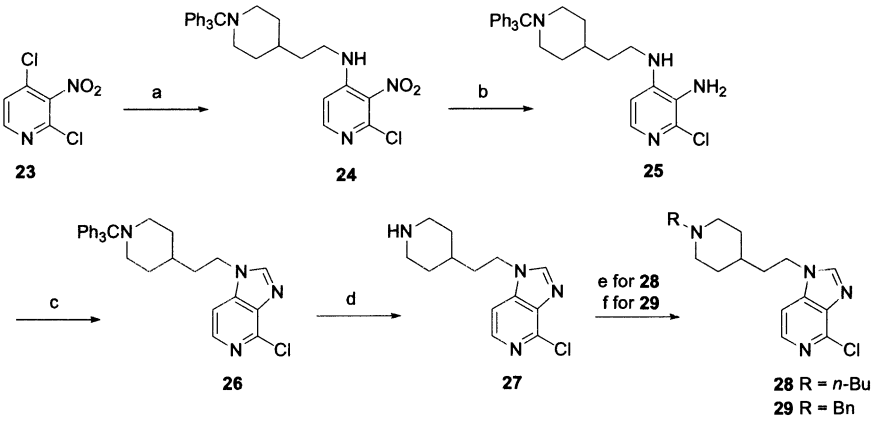
Compounds bearing an unsubstituted piperidine ring (**9**, **10**, **12**, **14** and **15**) were prepared as shown in Scheme 1. Dichlorination of 2,4-dihydroxy-3-nitroquinoline (**3**)[7] with phosphorus oxychloride gave 2,4-dichloro-3-nitroquinoline (**4**)[8] with a large amount of overchlorinated byproduct, 2,3,4-trichloroquinoline. In contrast, the reaction of **3** with phenylphosphonic dichloride ($PhPOCl_2$) gave **4** in 90% yield without any significant byproducts. The dichloride **4** was condensed with *tert*-butyl 4-(2-aminoethyl)-1-piperidinecarboxylate[9] to afford **5**, followed by the reduction with nickel(II) chloride hexahydrate ($NiCl_2{\cdot}6H_2O$) and sodium borohydride ($NaBH_4$) to give **6**. Cyclization of **6** with triethyl orthoformate [$HC(OEt)_3$] or triethyl orthobenzoate [$PhC(OEt)_3$] in the presence of catalytic amount of *p*-toluenesulfonic acid monohydrate (*p*-$TsOH{\cdot}H_2O$) gave compounds **7** or **8**, respectively. The reaction of **7** with phenol (PhOH) and potassium hydroxide (KOH) yielded 4-phenoxylated compound (**11**). Conversion of



Scheme 1. Reagents and conditions: (a) $PhPOCl_2$; (b) *tert*-butyl 4-(2-aminoethyl)-1-piperidinecarboxylate, Et_3N, DMF; (c) $Ni(II)Cl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, THF; (d) $HC(OEt)_3$, *p*-$TsOH{\cdot}H_2O$; (e) $PhC(OEt)_3$, *p*-$TsOH{\cdot}H_2O$; (f) TFA, 1,2-dichloroethane; (g) PhOH, KOH; (h) NH_4OAc; (i) H_2, Pd/C, MeOH.



Scheme 2. Reagents and conditions: (a) *n*-BuBr, K_2CO_3, DMF; (b) BnBr, K_2CO_3, DMF; (c) Ac_2O, pyridine; (d) KOH, PhOH; (e) NH_4OAc.



Scheme 3. Reagents and conditions: (a) 1-triphenylmethyl-2-(4-piperidyl)ethylamine, Et_3N, DMF; (b) $Ni(II)Cl_2{\cdot}6H_2O$, $NaBH_4$, MeOH, THF; (c) $HC(OEt)_3$, *p*-$TsOH{\cdot}H_2O$; (d) TFA, 1,2-dichloroethane; (e) *n*-BuBr, K_2CO_3, DMF; (f) BnBr, K_2CO_3, DMF.

11 into 4-amino compound (**13**) was achieved with ammonium acetate (NH_4OAc). Final deprotection of *tert*-butoxycarbonyl (Boc) group of the compounds (**7**, **8**, **11** or **13**) with trifluoroacetic acid (TFA) afforded the desired compounds (**9**, **10**, **12** or **14**). On the other hand, 4-dechlorinated compound (**15**) was prepared from **9** by catalytic hydrogenolysis.

Compounds with substituted piperidine rings (**2** and **16**–**22**) were prepared as shown in Scheme 2. Alkylation of secondary amines (**9** or **10**) with *n*-butyl bromide or benzyl bromide, and acetylation with acetic anhydride and pyridine afforded compounds **16**–**19**. The reaction of **17** or **18** with PhOH and KOH yielded 4-phenoxylated compounds (**20** or **21**), which were easily converted into 4-amino compounds (**2** or **22**) as described above.

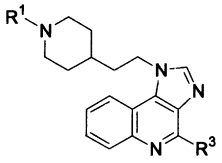
Imidazo[4,5-*c*]pyridine derivatives were prepared as shown in Scheme 3. The condensation of 2,4-dichloro-3-nitropyridine (**23**)[10] with 1-triphenylmethyl-2-(4-piperidyl)ethylamine gave the nitro compound (**24**). The reduction with $NiCl_2{\cdot}6H_2O$ and $NaBH_4$, the cyclization with triethyl orthoformate and the following acidic deprotection afforded the secondary amine (**27**), which was treated with *n*-butyl or benzyl bromide to give **28** or **29**, respectively.

Results and Discussion

A series of 1*H*-imidazo[4,5-*c*]quinolines or 1*H*-imidazo[4,5-*c*]pyridines were evaluated for the inhibitory activity of LPS-stimulated TNF-α production in human PBMCs and their potencies were expressed by IC_{50} (inhibitory concentration 50%) values.

First of all, we assessed some *C*-2 unsubstituted compounds (**9**, **12**, **14**–**18** and **22**) for SARs regarding the substituents at piperidyl nitrogen atom and *C*-4 position of **2** (Table 1). Debenzylation at piperidyl nitrogen atom (**14**) provided 3.5 times improved TNF-α suppressing activity. Although substitution of amino group for phenoxy group (**12**) or hydrogen atom (**15**) at position 4

Table 1. In vitro inhibitory activity of TNF-α production for 1*H*-imidazoquinolines



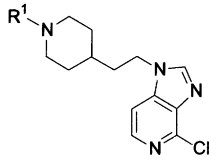
Compd	R^1	R^3	Inhibition of TNF-α production IC_{50} (nM)
2	Benzyl	NH_2	4810
9	H	Cl	205
12	H	OPh	3660
14	H	NH_2	1380
15	H	H	3680
16	*n*-Butyl	Cl	8020
17	Benzyl	Cl	2670
18	Acetyl	Cl	>10,000
22	Acetyl	NH_2	>10,000
1			>10,000

showed similar activities, that for chlorine atom (**9**) enhanced approximately 23-fold TNF-α suppressing activity as compared to **2**. As for the substitution at the piperidyl nitrogen atom, *n*-butyl (**16**), benzyl (**17**) or acetyl compound (**18**) showed 13 times or more decreased activity as compared to **9**. These results suggested that the combination of the unsubstituted piperidine and *C*-4 chlorine atom would be optimal for TNF-α suppressing activity.

Next, we compared the 1*H*-imidazo[4,5-*c*]quinoline compound with the corresponding 1*H*-imidazo[4,5-*c*]pyridine one (Table 2). However, none of 1*H*-imidazo[4,5-*c*]pyridine compounds (**27**–**29**) showed significant TNF-α suppressing activity. The fused benzene ring with imidazo[4,5-*c*]pyridine nucleus would be essential for TNF-α suppressing activity.

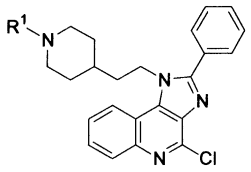
As concerned with 1*H*-imidazo[4,5-*c*]pyridine compounds without TNF-α suppressing activity, we inferred that the fused benzene ring contributed to stabilizing the

Table 2. In vitro inhibitory activity of TNF-α production for 1*H*-imidazopyridines



Compd	R^1	Inhibition of TNF-α production IC_{50} (nM)
27	H	> 10,000
28	*n*-Butyl	> 10,000
29	Benzyl	> 10,000

Table 3. In vitro inhibitory activity of TNF-α production for 2-phenyl-1*H*-imidazoquinolines



Compd	R^1	Inhibition of TNF-α production IC_{50} (nM)
10	H	52
19	Acetyl	611

spatial and conformational direction of 2-(4-piperidyl)ethyl group at position 1. In order to elucidate the relation between the conformational direction of *N*-1 substituent and TNF-α suppressing activity, we moved on to the introduction of phenyl ring at *C*-2 position, which made 2-(4-piperidyl)ethyl group at position 1 to be conformational restricted (Fig. 2 and Table 3). As we had expected, 2-phenyl-1*H*-imidazoquinolines (**10** and **19**) showed improved TNF-α suppressing activity, compared to *C*-2 unsubstituted compounds (**9** and **18**). In particular, **10** showed the highest TNF-α suppressing activity ($IC_{50} = 52$ nM) with no significant IFN-α inducing activity at the concentration of 10^{-6} M in human PBMCs (data not shown). Compound **10** also showed a moderate interleukin-1β (IL-1β) inhibitory activity (data not shown). These results indicated that a kind of conformational restriction of 2-(4-piperidyl)ethyl group at position 1 would be considerably related with TNF-α suppressing activity. We would suggest that both *C*-2 phenyl ring and *C*-9 proton on the fused benzene ring can stabilize the conformation of the 2-(4-piperidyl)ethyl group, and prevent bending to the unfavorable direction of that.

In order to demonstrate the conformational restriction of 2-(4-piperidyl)ethyl group at position 1, we measured the nuclear Overhauser effect (NOE) of compounds **9**, **10** and **27** in the DMSO solution with the 2 dimensional NMR (NOESY) experiments (Fig. 3). Judging from the line width or link number of the NOESY spectra for the *C*-2′ methylene proton resonance (δ 1.89) of **9**, the NOE with *C*-9 proton signal (δ 8.35) was observed more strongly than that of *C*-2 proton (δ 8.52). Furthermore, the long-range NOEs of **9** indicated that four equatorial protons (δ 1.92 and δ 3.28) of the piperidine ring would not be spatially located near *C*-9 proton but *C*-2 proton. The 2-(4-piperidyl)ethyl group of **9** was considered to fold to the imidazole ring side as shown in Figure 3. On the other hand, apparent conformational differences of *N*-1 aminoalkyl moiety between **10** and **27** were observed. In contrast with compound **9**, compound **27** showed a long-range NOE between *C*-2′ methylene proton resonance (δ 1.80) and *C*-7 proton signal (δ 7.74), but no long-range NOE between the *C*-2′ protons and *C*-2 proton (δ 8.49). Besides, the medium-range NOEs between *C*-6 proton signal (δ 8.16) and piperidine NH and two equatorial protons adjacent to nitrogen atom (δ 8.45 and δ 3.15–3.30, respectively) were also observed. From the above results, it was suggested that the conformation of *N*-1 side chain of **27** would be near to the left side of pyridine ring. Regarding **10**, there were three long-range NOEs. One NOE is observed between *C*-2′ methylene protons (δ 1.75) and *C*-9 proton signal (δ 8.36–8.42). The second one is observed between ortho and meta *C*-2 phenyl proton signals (δ 7.60–7.68 and δ 7.75–7.85) and two equatorial proton signals of the piperidine ring adjacent to nitrogen atom (δ 3.03). The other is between ortho *C*-2 phenyl proton signal (δ 7.75–7.85) and two axial proton signals of the piperidine ring adjacent to nitrogen atom (δ 2.58). In summary, the piperidine ring of **10** was in the relatively near to the *C*-2 phenyl ring, similarly to compound **9**. The TNF-α suppressing activity of **9**, **10** and **27** seemed to be significantly affected by the spatial position of piperidyl nitrogen atom. In other word, we proved our hypothesis that the fused benzene ring of **9** and **10** would hinder the 2-(4-piperidyl)ethyl group at position 1 from approaching to the tricyclic core.

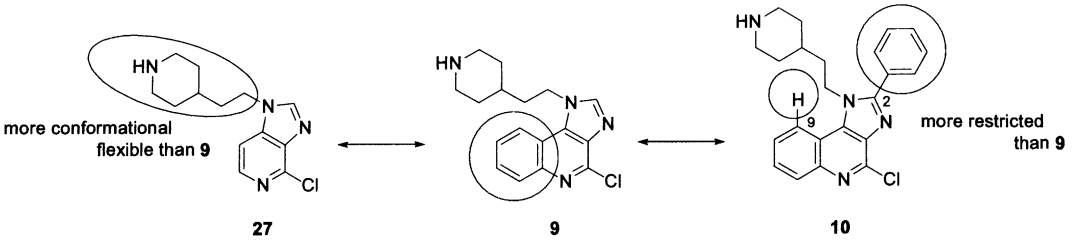


Figure 2.

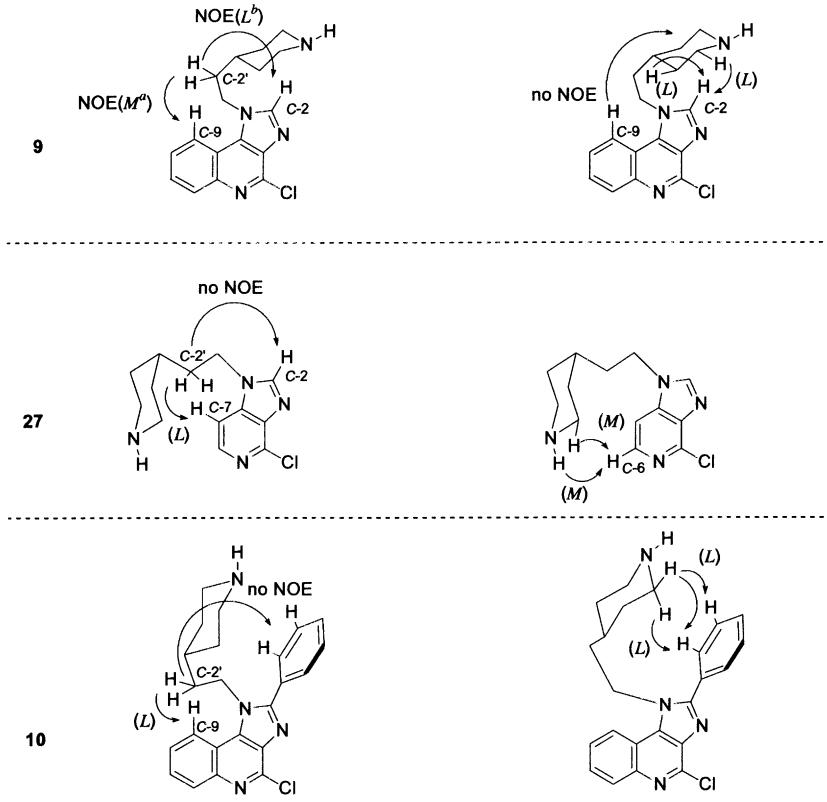


Figure 3. NOESY Experiments for compounds **9**, **10** and **27**. [a]*M* represents the medium-range NOE judged from links whose sequence separation is three or four. [b]*L* represents the long-range NOE judged from links whose sequence separation is one or two.

Conclusion

We developed modification of substituents at piperidyl nitrogen atom, position 2 and 4 of compound **2**, which was a imiquimod analogue without IFN-α inducing activity, and found a promising lead compound of TNF-α suppressor, 4-chloro-2-phenyl-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (**10**), which had about 100-fold TNF-α suppressing activity in comparison with **2**. In addition, compound **10** showed a moderate IL-1β suppressing activity and no significant IFN-α inducing activity at the concentration of 10^{-6} M in human PBMCs. On the other hand, NMR experiments indicated that *C*-2 phenyl ring and *C*-9 proton on the fused benzene ring could contribute the conformational restriction of 2-(4-piperidyl)ethyl group at position 1 related with TNF-α suppressing activity. Further detailed optimization with **10** is under investigation.

Experimental

Chemistry

All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured with JEOL Lambda-300 (300 MHz) or JEOL Alpha-500 (500 MHz) spectrometer; chemical shifts are expressed as δ (ppm) values with tetramethylsilane (TMS) as an internal standard (in NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). IR spectra were measured with Hitachi 270-30 spectrometer. Mass spectra (MS) were recorded with JEOL JMS-DX 300 or Waters 2690 mass spectrometer. Elemental analyses were performed using Yanagimoto MT-5 or MT-6 elemental analysis apparatus, and analytical results were within ±0.4% of theoretical values. Column chromatography was carried out with Kieselgel 60 (Merck) or Aluminum oxide 90 (Merck). TLC was conducted on a 0.25 mm pre-coated silica gel plate ($60F_{254}$, Merck), and spots were detected by inspection under short (254 nm) wavelength UV light, or by the colors developed with iodine. Organic extracts were dried over anhydrous Na_2SO_4. The solvent was concentrated under reduced pressure.

The following known compounds were prepared essentially according to the literature: 2,4-dihydroxy-3-nitroquinoline (**3**),[7] *tert*-butyl 4-(2-aminoethyl)-1-piperidinecarboxylate,[9] 2,4-dichloro-3-nitropyridine (**24**).[10]

2,4-Dichloro-3-nitroquinoline (4).[8] A mixture of **3** (0.50 g, 2.43 mmol) and $PhPOCl_2$ (1.40 mL, 10.1 mmol) was stirred at 140 °C for 3 h. The reaction mixture was poured into water. The resulting precipitate was collected by filtration and washed with water to give 0.53 g (90%) as a brown crystal, which was used for the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, t, *J* = 8 Hz), 7.95 (1H, t, *J* = 8 Hz), 8.12 (1H, d, *J* = 8 Hz), 8.28 (1H, d, *J* = 8 Hz); IR ν (KBr) cm^{-1}: 1546, 1296.

***tert*-Butyl 4-[2-[(2-chloro-3-nitroquinoline-4-yl)amino]ethyl]-1-piperidinecarboxylate (5).** To a mixture of **4**

(4.52 g, 18.6 mmol) and triethylamine (Et_3N) (2.80 mL, 20.1 mmol) in *N,N*-dimethylformamide (DMF) (11 mL), a solution of *tert*-butyl 4-(2-aminoethyl)-1-piperidinecarboxylate (4.30 g, 18.8 mmol) in DMF (3 mL) was added under ice cooling, followed by stirring at room temperature for 1 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate (EtOAc). The extract was washed with water, dried and concentrated. The residue was washed with a mixture of EtOAc and diisopropyl ether (*iso*-Pr_2O) to give 6.85 g (85%) of **5** as a yellow crystal, which was recrystallized from a mixture of EtOAc and *iso*-Pr_2O to afford yellow crystals: mp 133–134 °C; ^{1}H NMR ($CDCl_3$) δ 1.10–1.20 (2H, m), 1.46 (9H, s), 1.50–1.60 (1H, m), 1.60–1.75 (4H, m), 2.60–2.80 (2H, m), 3.45–3.55 (2H, m), 4.00–4.20 (2H, m), 5.80–5.90 (1H, m), 7.50–7.60 (1H, m), 7.70–7.80 (1H, m), 7.85–7.95 (2H, m); IR ν (KBr) cm^{-1}: 1660. Anal. calcd for $C_{21}H_{27}ClN_4O_4$: C, 57.99; H, 6.26; N, 12.88. Found: C, 57.99; H, 6.34; N, 12.85.

2-Chloro-3-nitro-4-[2-(1-triphenylmethyl-4-piperidyl)ethyl]amino]pyridine (24). By using the similar procedure as for **5**, compound **24** was prepared in 97% yield from **23**, yellow crystals (*iso*-Pr_2O): mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.15–1.45 (3H, m), 1.45–1.75 (6H, m), 3.00–3.20 (2H, m), 3.25 (2H, q, $J=7$ Hz), 6.50–6.60 (1H, m), 6.59 (1H, d, $J=6$ Hz), 7.10–7.20 (3H, m), 7.20–7.30 (6H, m), 7.35–7.60 (6H, m), 8.01 (1H, d, $J=6$ Hz); IR ν (KBr) cm^{-1}: 1602, 1518. Anal. calcd for $C_{31}H_{31}ClN_4O_2$: C, 70.64; H, 5.93; N, 10.63. Found: C, 70.70; H, 6.03; N, 10.52.

***tert*-Butyl 4-[2-[(3-amino-2-chloroquinolin-4-yl)amino]-ethyl]-1-piperidinecarboxylate (6).** To a mixture of $NiCl_2{\cdot}6H_2O$ (1.85 g, 7.78 mmol) and $NaBH_4$ (0.30 g, 7.93 mmol) in methanol (MeOH) (140 mL), a solution of **5** (6.75 g, 15.5 mmol) in tetrahydrofuran (THF) (20 mL) was added under ice-cooling. To the mixture, $NaBH_4$ (1.76 g, 46.5 mmol) was added portionwise, and then the reaction mixture was stirred at room temperature for 0.5 h. The precipitate was filtered off, and the filtrate was concentrated. To the resulting residue, EtOAc and 10% aqueous ammonium chloride (NH_4Cl) was added and passed through a pad of Celite. The organic layer of the filtrate was separated, washed successively with 10% aqueous NH_4Cl and saturated aqueous sodium chloride (NaCl), dried and concentrated. The resulting residue was washed with diethyl ether (Et_2O) to give 5.04 g (80%) of **6** as a pale brown crystal, which was recrystallized from a mixture of EtOAc and *iso*-Pr_2O to afford colorless crystals: mp 115.5–116 °C; ^{1}H NMR ($CDCl_3$) δ 1.10–1.20 (2H, m), 1.45 (9H, s), 1.50–1.70 (5H, m), 2.60–2.75 (2H, m), 3.25–3.35 (2H, m), 3.75–3.80 (1H, m), 4.00–4.20 (4H, m), 7.46 (1H, td, $J=8.5$, 2 Hz), 7.50 (1H, td, $J=8.5$, 2 Hz), 7.75 (1H, dd, $J=8.5$, 2 Hz), 7.90 (1H, dd, $J=8.5$, 2 Hz); IR *ν* (KBr) cm^{-1}: 3436, 3376, 3312, 1682. MS *m/z* (3:1, M)$^+$ 404, 406. Anal. calcd for $C_{21}H_{29}ClN_4O_2$: C, 62.29; H, 7.22; N, 13.84. Found: C, 61.99; H, 7.28; N, 13.73.

3-Amino-2-chloro-4-[2-(1-triphenylmethyl-4-piperidyl)-ethyl]amino]pyridine (25). By using the similar procedure as for **7**, compound **25** was prepared in 72% yield from **24**, colorless crystals [ethanol (EtOH)]: mp 206.5–208.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.15–1.40 (3H, m), 1.49 (2H, q, $J=11$ Hz), 1.54 (2H, q, $J=7$ Hz), 1.68 (2H, d, $J=11$ Hz), 2.85–3.00 (2H, m), 3.10 (2H, q, $J=7$ Hz), 4.62 (2H, s), 5.51 (1H, t, $J=7$ Hz), 7.15 (3H, t, $J=7.5$ Hz), 7.28 (6H, t, $J=7.5$ Hz), 7.25–7.35 (1H, m), 7.35–7.45 (6H, m), 7.38 (1H, d, $J=5.5$ Hz); IR ν (KBr) cm^{-1}: 3460, 3360. Anal. calcd for $C_{31}H_{33}ClN_4O_2{\cdot}1/2H_2O$: C, 73.57; H, 6.77; N, 11.07. Found: C, 73.61; H, 6.55; N, 10.92.

***tert*-Butyl 4-[2-(4-chloro-2-phenyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidinecarboxylate (8).** A mixture of **6** (21.1 g, 52.1 mmol), $PhC(OEt)_3$ (23.6 mL, 104 mmol) and *p*-$TsOH{\cdot}H_2O$ (0.99 g, 5.20 mol) in toluene (100 mL) was refluxed for 5 h. The reaction mixture was concentrated and the residue was washed with Et_2O to afford 21.1 g (83%) of **8** as a colorless crystal, which was recrystallized from a mixture of EtOAc and *n*-heptane to afford colorless crystals: mp 159–161 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (2H, qd, $J=12.5$, 3 Hz), 1.23–1.34 (3H, m), 1.36 (9H, s), 1.74 (2H, q, $J=7.5$ Hz), 2.48–2.57 (2H, m), 3.76 (2H, d, $J=12.5$ Hz), 4.71 (2H, t, $J=7.5$ Hz), 7.62–7.67 (3H, m), 7.75–7.83 (4H, m), 8.10–8.15 (1H, m), 8.37–8.43 (1H, m); IR ν (KBr) cm^{-1}: 1696; MS *m/z* (3:1, M)$^+$ 490, 492. Anal. calcd for $C_{28}H_{31}ClN_4O_2$: C, 68.49; H, 6.36; N, 11.41. Found: C, 68.36; H, 6.27; N, 11.37.

***tert*-Butyl 4-[2-(4-chloro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidinecarboxylate (7).** By using the similar procedure as for **8**, compound **7** was prepared in 96% yield from **6**, colorless crystals (EtOAc): mp 188–189 °C; ^{1}H NMR ($CDCl_3$) δ 1.20–1.35 (2H, m), 1.46 (9H, s), 1.50–1.60 (1H, m), 1.70–1.80 (2H, m), 1.98 (2H, q, $J=7.5$ Hz), 2.65–2.80 (2H, m), 4.00–4.25 (2H, m), 4.63 (2H, t, $J=7.5$ Hz), 7.67 (1H, td, $J=8.5$, 1.5 Hz), 7.72 (1H, td, $J=8.5$, 1.5 Hz), 7.97 (1H, s), 8.11 (1H, dd, $J=8.5$, 1.5 Hz), 8.21 (1H, dd, $J=8.5$, 1.5 Hz); IR ν (KBr) cm^{-1}: 1682; MS *m/z* (3:1, M)$^+$ 414, 416. Anal. calcd for $C_{22}H_{27}ClN_4O_2$: C, 63.68; H, 6.56; N, 13.50. Found: C, 63.45; H, 6.60; N, 13.40.

4-Chloro-1-[2-(1-triphenylmethyl-4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]pyridine (26). By using the similar procedure as for **8**, compound **26** was prepared in 80% yield from **25**, pale brown crystals [*iso*-propyl alcohol (*iso*-PrOH)]: mp 202–203 °C; ^{1}H NMR (DMSO-d_6) δ 1.00–1.10 (1H, m), 1.20–1.30 (2H, m), 1.51 (2H, q, $J=11$ Hz), 1.67 (2H, d, $J=11$ Hz), 1.78 (2H, q, $J=7$ Hz), 2.85–3.00 (2H, m), 4.29 (2H, t, $J=7$ Hz), 7.15 (3H, t, $J=7.5$ Hz), 7.29 (6H, t, $J=7.5$ Hz), 7.30–7.45 (6H, m), 7.69 (1H, d, $J=5.5$ Hz), 8.12 (1H, d, $J=7.5$ Hz), 8.44 (1H, s). Anal. calcd for $C_{32}H_{31}ClN_4$: C, 75.80; H, 6.16; N, 11.05. Found: C, 75.66; H, 6.23; N, 10.99.

4-Chloro-2-phenyl-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (10). A mixture of **8** (15.4 g, 31.4 mmol), TFA (46 mL) and 1,2-dichloroethane (77 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated and the residue was washed with a mixture of *iso*-PrOH and EtOAc to give the trifluoroacetate as a colorless crystal, which was

added to a mixture of MeOH/1,2-dichloroethane (1:10) and water, and adjusted to pH 10 with 5% aqueous sodium hydroxide (NaOH). The organic layer was separated, washed with saturated aqueous NaCl, dried and concentrated to afford pale brown oil. The residue was converted to the fumarate in the usual manner to afford 13.9 g (87%) of **10** as a colorless crystal, which was recrystallized from MeOH to afford colorless crystals: mp 185.5–186.5 °C (dec.); ^{1}H NMR (DMSO-d_6) δ 1.15 (2H, q, J = 12.5 Hz), 1.32–1.46 (3H, m), 1.75 (2H, q, J = 7.5 Hz), 2.58 (2H, t, J = 12.5 Hz), 3.03 (2H, d, J = 12.5 Hz), 4.71 (2H, t, J = 7.5 Hz), 6.44 (2H, s), 7.60–7.68 (3H, m), 7.75–7.85 (4H, m), 8.10–8.16 (1H, m), 8.36–8.42 (1H, m); IR ν (KBr) cm^{-1}: 3464, 1712; MS m/z (3:1, M + 1)$^+$ 391, 393. Anal. calcd for $C_{23}H_{23}ClN_4 \cdot C_4H_4O_4 \cdot H_2O$: C, 61.77; H, 5.57; N, 10.67. Found: C, 62.04; H, 5.40; N, 10.70.

4-Chloro-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (9). By using the similar procedure as for **10**, compound **9** was prepared in 98% yield as the trifluoroacetate from **7**, colorless crystals (EtOH/*iso*-Pr_2O): mp 182.5–185.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.40 (2H, qd, J = 13.0, 3.5 Hz), 1.61–1.72 (1H, m), 1.89 (2H, q, J = 7.5 Hz), 1.92 (2H, d, J = 13 Hz), 2.87 (2H, t, J = 13 Hz), 3.28 (2H, d, J = 13 Hz), 4.76 (2H, t, J = 7.5 Hz), 7.76 (2H, td, J = 7, 1.5 Hz), 8.10 (1H, dd, J = 7, 1.5 Hz), 8.35 (1H, dd, J = 7.0, 1.5 Hz), 8.52 (1H, s); IR ν (KBr) cm^{-1}: 3448, 1682. Anal. calcd for $C_{17}H_{19}ClN_4 \cdot C_2HF_3O \cdot 1/4H_2O$: C, 52.66; H, 4.77; N, 12.93. Found: C, 52.43; H, 4.77; N, 12.76.

4-Phenoxy-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (12). By using the similar procedure as for **10**, compound **12** was prepared in 100% yield as the trifluoroacetate from **11**, colorless crystals (EtOH/CH_2Cl_2): mp 211–216 °C (*dec.*); ^{1}H NMR (DMSO-d_6) δ 1.62–1.73 (1H, m), 1.39 (2H, qd, J = 12, 4 Hz), 1.93 (2H, q, J = 7 Hz), 2.88 (2H, t, J = 12 Hz), 3.28 (2H, d, J = 12 Hz), 4.75 (2H, t, J = 7 Hz), 7.30 (1H, d, J = 7.5 Hz), 7.47 (2H, t, J = 7.5 Hz), 7.54–7.62 (2H, m), 7.74 (1H, d, J = 7.5 Hz), 8.26 (1H, d, J = 7.5 Hz), 8.42 (1H, s); IR ν (KBr) cm^{-1}: 1676; MS m/z (M)$^+$ 372. Anal. calcd for $C_{23}H_{24}ClN_4O \cdot C_2HF_3O \cdot 1/4H_2O$: C, 61.16; H, 5.23; N, 11.41. Found: C, 61.26; H, 5.05; N, 11.47.

4-Amino-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (14). By using the similar procedure as for **10**, compound **14** was prepared in 86% yield as the hydrochloride from **13**, colorless crystals (EtOH): mp 243–244 °C (*dec.*); ^{1}H NMR (DMSO-d_6) δ 1.40–1.50 (2H, m), 1.60–1.70 (1H, m), 1.85 (2H, q, J = 7.5 Hz), 1.89 (2H, d, J = 13 Hz), 2.83 (2H, td, J = 13, 2.5 Hz), 3.23 (2H, d, J = 13 Hz), 4.65 (2H, t, J = 7.5 Hz), 6.99 (2H, br-s), 7.34 (1H, t, J = 7 Hz), 7.51 (1H, t, J = 7 Hz), 7.69 (1H, d, J = 7 Hz), 8.06 (1H, d, J = 7 Hz), 8.30 (1H, s), 8.87 (2H, br-s); IR ν (KBr) cm^{-1}: 3376, 3320, 3204; MS m/z (M)$^+$ 295. Anal. calcd for $C_{17}H_{21}N_5 \cdot HCl \cdot 3/4H_2O$: C, 59.12; H, 6.86; N, 20.28. Found: C, 59.10; H, 6.83; N, 20.30.

4-Chloro-1-[2-(4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]pyridine (27). By using the similar procedure as for **10**, compound **27** was prepared quantitatively as the trifluoroacetate from **26**, pale brown crystals (*iso*-PrOH): mp 149–150 °C; ^{1}H NMR (DMSO-d_6) δ 1.33 (2H, q, J = 12 Hz), 1.43–1.55 (1H, m), 1.80 (2H, q, J = 7.5 Hz), 1.86 (2H, d, J = 12 Hz), 2.83 (2H, t, J = 12 Hz), 3.15–3.30 (2H, m), 4.34 (2H, t, J = 7.5 Hz), 7.74 (1H, d, J = 5.5 Hz), 8.16 (1H, d, J = 5.5 Hz), 8.19 (1H, br-s), 8.45 (1H, br-s), 8.49 (1H, s); IR ν (KBr) cm^{-1}: 3460, 1682; MS m/z (3:1, M + 1)$^+$ 265, 267. Anal. calcd for $C_{13}H_{17}ClN_4 \cdot 3/2C_2HF_3O$: C, 44.10; H, 4.28; N, 12.86. Found: C, 44.15; H, 4.43; N, 12.88.

***tert*-Butyl 4-[2-(4-phenoxy-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidinecarboxylate (11).** A mixture of **7** (4.46 g, 10.7 mmol), PhOH (10.1 g, 107 mmol) and KOH (1.80 g, 32.1 mmol) was heated at 120 °C for 7 h. The reaction mixture was basified with 10% aqueous NaOH, and extracted with EtOAc. The extract was washed successively with 10% aqueous NaOH and saturated aqueous NaCl, dried and concentrated to give a brown oil. The residue was purified by column chromatography (SiO_2, EtOAc) to afford 3.59 g (71%) of **11** as a colorless solid, which was recrystallized from a mixture of EtOAc and *n*-hexane to afford colorless crystals: mp 130.5–132.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.22–1.33 (2H, m), 1.47 (9H, s), 1.52–1.62 (2H, m), 1.68–1.82 (1H, m), 2.00 (2H, q, J = 7.5 Hz), 2.66–2.78 (2H, m), 4.13 (2H, br-s), 4.64 (2H, t, J = 7.5 Hz), 7.26 (1H, t, J = 7.5 Hz), 7.40 (2H, d, J = 7.5 Hz), 7.45 (2H, t, J = 7.5 Hz), 7.51 (1H, td, J = 8, 1 Hz), 7.57 (1H, td, J = 8, 1 Hz), 7.91 (1H, dd, J = 8, 1 Hz), 7.95 (1H, s), 8.06 (1H, dd, J = 8, 1 Hz); IR ν (KBr) cm^{-1}: 1694; MS m/z (M)$^+$ 472. Anal. calcd for $C_{28}H_{32}N_4O_3$: C, 71.16; H, 6.83; N, 11.86. Found: C, 71.10; H, 7.10; N, 11.69.

1-[2-(1-Benzyl-4-piperidyl)ethyl]-4-phenoxy-1*H*-imidazo[4,5-*c*]quinoline (20). By using the similar procedure as for **11**, compound **20** was prepared in 87% yield from **17**, colorless crystals (MeOH): mp 152.5–153.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.40–1.50 (3H, m), 1.70–1.80 (2H, m), 1.95–2.05 (4H, m), 2.92 (2H, d, J = 11 Hz), 3.51 (2H, s), 4.61 (2H, t, J = 7.5 Hz), 7.20–7.35 (6H, m), 7.40 (2H, d, J = 7.5 Hz), 7.44 (2H, t, J = 7.5 Hz), 7.49 (1H, td, J = 8.5, 1 Hz), 7.56 (1H, td, J = 8.5, 1 Hz), 7.90 (1H, dd, J = 8.5, 1 Hz), 7.93 (1H, s), 8.06 (1H, dd, J = 8.5, 1 Hz); MS m/z (M)$^+$ 462. Anal. calcd for $C_{30}H_{30}N_4O$: C, 77.89; H, 6.54; N, 12.11. Found: C, 78.00; H, 6.29; N, 12.05.

4-[2-(4-Phenoxy-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidineacetamide (21). By using the similar procedure as for **11**, compound **21** was prepared in 82% yield from **18**, colorless crystals (EtOAc/*iso*-Pr_2O): mp 187–189.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (2H, qd, J = 13, 4.5 Hz), 1.60–1.72 (1H, m), 1.79 (1H, td, J = 13 Hz), 1.85 (1H, d, J = 13 Hz), 2.01 (2H, q, J = 7.5 Hz), 2.10 (3H, s), 2.56 (1H, td, J = 13, 2.5 Hz), 3.05 (1H, td, J = 13, 2.5 Hz), 3.84 (1H, d, J = 13 Hz), 4.58–4.72 (3H, m), 7.26 (1H, t, J = 7 Hz), 7.40 (2H, d, J = 7 Hz), 7.45 (2H, t, J = 7 Hz), 7.50 (1H, td, J = 8, 1 Hz), 7.57 (1H, td, J = 8, 1 Hz), 7.91 (1H, dd, J = 8, 1 Hz), 7.94 (1H, s), 8.04 (1H, dd, J = 8, 1 Hz); IR ν (KBr) cm^{-1}: 1640; MS m/z (M)$^+$ 414. Anal. calcd for $C_{25}H_{26}N_4O_2$: C, 72.44; H, 6.32; N, 13.52. Found: C, 72.35; H, 6.26; N, 13.42.

***tert*-Butyl 4-[2-(4-amino-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidinecarboxylate (13).** A mixture of **11** (4.40 g, 9.31 mmol) and NH_4OAc (34.5 g, 448 mmol) was heated at 140 °C for 3 h. The reaction mixture was adjusted to pH 9 with 10% aqueous NaOH and extracted with CH_2Cl_2. The extract was washed with saturated aqueous NaCl, dried and concentrated. The residue was purified by column chromatography [Al_2O_3, CH_2Cl_2/MeOH (100:1–20:1)] and washed with *iso*-Pr_2O to afford 1.88 g (51%) of **13** as a colorless crystal, which was recrystallized from EtOAc to afford colorless crystals: mp 193–193.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.20–1.30 (2H, m), 1.46 (9H, s), 1.50–1.60 (1H, m), 1.60–1.80 (2H, m), 1.96 (2H, q, J = 7.5 Hz), 2.60–2.80 (2H, m), 4.00–4.25 (2H, m), 4.56 (2H, t, J = 7.5 Hz), 5.42 (2H, br-s), 7.35 (1H, t, J = 8 Hz), 7.55 (1H, t, J = 8 Hz), 7.79 (2H, s), 7.84 (1H, d, J = 8 Hz), 7.93 (1H, d, J = 8 Hz); IR ν (KBr) cm^{-1}: 3476, 1694; MS m/z $(M)^+$ 395. Anal. calcd for $C_{22}H_{29}N_5O_2$: C, 66.81; H, 7.39; N, 17.71. Found: C, 66.93; H, 7.48; N, 17.66.

1-[2-(1-Benzyl-4-piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline-4-amine (2). By using the similar procedure as for **13**, compound **2** was prepared in 89% yield from **20**, colorless crystals (EtOH): mp 191.5–192 °C; ^{1}H NMR (DMSO-d_6) δ 1.20–1.40 (3H, m), 1.70 (2H, d, J = 11.5 Hz), 1.81 (2H, q, J = 7 Hz), 1.92 (2H, t, J = 11.5 Hz), 2.78 (2H, d, J = 11.5 Hz), 3.20 (2H, t, J = 11.5 Hz), 3.44 (2H, s), 4.61 (2H, t, J = 7 Hz), 6.41 (2H, s), 7.20–7.35 (6H, m), 7.43 (1H, td, J = 8, 1 Hz), 7.62 (1H, dd, J = 8, 1 Hz), 8.01 (1H, dd, J = 8, 1 Hz), 8.18 (1H, s); IR ν (KBr) cm^{-1}: 3360, 3304; MS m/z $(M)^+$ 385. Anal. calcd for $C_{24}H_{27}N_5$: C, 74.77; H, 7.06; N, 18.17. Found: C, 74.87; H, 7.18; N, 18.06.

4-[2-(4-Amino-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidineacetamide (22). By using the similar procedure as for **13**, compound **22** was prepared in 48% yield from **21**, colorless crystals (MeOH): mp 231.5–232.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.00–1.25 (2H, m), 1.55–1.65 (1H, m), 1.70–1.80 (2H, m), 1.82 (2H, q, J = 7.5 Hz), 1.97 (3H, s), 2.52 (1H, d, J = 12.5 Hz), 2.99 (1H, t, J = 12.5 Hz), 3.77 (1H, d, J = 12.5 Hz), 4.34 (1H, d, J = 12.5 Hz), 4.63 (2H, t, J = 7.5 Hz), 6.42 (2H, br-s), 7.27 (1H, td, J = 8, 1 Hz), 7.44 (1H, td, J = 8, 1 Hz), 7.63 (1H, dd, J = 8, 1 Hz), 8.02 (1H, dd, J = 8, 1 Hz), 8.20 (1H, s); IR ν (KBr) cm^{-1}: 3480, 3284, 1638; MS m/z $(M)^+$ 337. Anal. calcd for $C_{19}H_{23}N_5O$: C, 67.63; H, 6.87; N, 20.76. Found: C, 67.46; H, 6.79; N, 20.63.

1-[2-(4-Piperidyl)ethyl]-1*H*-imidazo[4,5-*c*]quinoline (15). A mixture of trifluoroacetate **9** (1.10 g, 2.57 mmol) and 5% palladium on carbon (0.55 g) in MeOH (100 mL) was stirred at room temperature and an atmospheric pressure of H_2 for 4 h. The catalyst was removed by filtration and the filtrate was concentrated. The resulting residue was dissolved in a small amount of water and washed with EtOAc. Then the aqueous layer was adjusted to pH 10 with 10% aqueous NaOH and extracted with a mixture of MeOH and 1,2-dichloroethane (1:10). The extract was washed with saturated aqueous NaCl, dried and concentrated to afford pale brown oil. The residue was converted to the hydrochloride in the usual manner to afford 0.47 g (52%) of **15** as a colorless crystal, which was recrystallized from a mixture of MeOH and 1,2-dichloroethane (1:10) to afford colorless crystals: mp 230–233 °C (*dec.*); ^{1}H NMR (DMSO-d_6) δ 1.48 (2H, q, J = 12 Hz), 1.65–1.77 (1H, m), 1.88–1.97 (4H, m), 2.84 (2H, q, J = 12 Hz), 3.25 (2H, d, J = 12 Hz), 4.86 (2H, t, J = 7.5 Hz), 7.97 (1H, t, J = 8 Hz), 8.02 (1H, t, J = 8 Hz), 8.49 (1H, d, J = 8 Hz), 8.57 (1H, d, J = 8 Hz), 8.83 (2H, br-s), 8.99 (1H, br-s), 9.70 (1H, s); IR ν (KBr) cm^{-1}: 3416; MS m/z $(M)^+$ 280. Anal. calcd for $C_{17}H_{20}N_4 \cdot 2HCl \cdot 1/2H_2O$: C, 56.36; H, 6.40; N, 15.46. Found: C, 56.36; H, 6.18; N, 15.35.

1-[2-(1-*n*-Butyl-4-piperidyl)ethyl]-4-chloro-1*H*-imidazo[4,5-*c*]quinoline (16). To a suspension of trifluoroacetate of **9** (1.20 g, 2.80 mmol) and potassium carbonate (K_2CO_3) (0.77 g, 5.57 mmol) in DMF (6 mL), *n*-butyl bromide (0.30 mL, 2.80 mmol) was added and stirred at room temperature for 5 h. The reaction mixture was adjusted to pH 10 with 10% aqueous NaOH and extracted with EtOAc. The extract was washed successively with water and saturated aqueous NaCl, dried and concentrated to give pale brown oil. The residue was dissolved in THF and passed through a pad of SiO_2. The filtrate was concentrated to give 0.87 g (84%) of **16** as a colorless solid, which was converted to the hydrochloride in the usual manner and recrystallized from a mixture of MeOH and EtOAc to afford colorless crystals: mp 144–146 °C; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, t, J = 7.5 Hz), 1.32 (2H, sextet, J = 7.5 Hz), 1.55–1.72 (5H, m), 1.88 (2H, q, J = 7.5 Hz), 1.94 (2H, d, J = 11 Hz), 2.83 (2H, q, J = 11 Hz), 2.91–2.98 (2H, m), 3.43 (2H, d, J = 11 Hz), 4.77 (2H, t, J = 7.5 Hz), 7.75 (1H, td, J = 7.5, 2 Hz), 7.78 (1H, td, J = 7.5, 2 Hz), 8.10 (1H, dd, J = 7.5, 2 Hz), 8.36 (1H, dd, J = 7.5, 2 Hz), 8.54 (1H, s), 10.19 (1H, br-s); MS m/z $(M)^+$ 370, 372. Anal. calcd for $C_{21}H_{27}ClN_4 \cdot 2HCl \cdot 1/2H_2O$: C, 55.70; H, 6.68; N, 12.37. Found: C, 55.80; H, 6.65; N, 12.44.

1-[2-(1-*n*-Butyl-4-piperidyl)ethyl]-4-chloro-1*H*-imidazo[4,5-*c*]pyridine (28). By using the similar procedure as for **16**, compound **28** was prepared in 69% yield from **27**, pale brown prisms (*iso*-Pr_2O): mp 107.5–109 °C; ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.5 Hz), 1.10–1.25 (3H, m), 1.26 (2H, sextet, J = 7.5 Hz), 1.37 (2H, quintet, J = 7.5 Hz), 1.60–1.70 (2H, m), 1.70–1.80 (4H, m), 2.20 (2H, t, J = 7.5 Hz), 2.78 (2H, d, J = 11 Hz), 4.32 (2H, t, J = 7.5 Hz), 7.71 (1H, d, J = 5.5 Hz), 8.14 (1H, d, J = 5.5 Hz), 8.47 (1H, s); MS m/z $(M)^+$ 320, 322. Anal. calcd for $C_{17}H_{25}ClN_4$: C, 63.64; H, 7.85; N, 17.46. Found: C, 63.56; H, 7.71; N, 17.46.

1-[2-(1-Benzyl-4-piperidyl)ethyl]-4-chloro-1*H*-imidazo[4,5-*c*]quinoline (17). To a suspension of trifluoroacetate of **9** (0.50 g, 1.17 mmol) and K_2CO_3 (0.32 g, 2.32 mmol) in DMF (3 mL), benzyl bromide (0.14 mL, 1.18 mmol) was added and stirred at room temperature for 3 h. The reaction mixture was basified with 10% aqueous NaOH and extracted with EtOAc. The extract was washed successively with water and saturated aqueous NaCl, dried and concentrated to give a pale brown oil. The residue was purified by column chromatography [SiO_2, *n*-heptane/THF (4:1)] to afford

0.41 g (87%) of **17** as a colorless crystal, which was recrystallized from a mixture of EtOAc and *iso*-Pr_2O to afford colorless crystals: mp 116.5–118 °C; ^{1}H NMR ($CDCl_3$) δ 1.38–1.48 (3H, m), 1.74 (2H, d, J = 11 Hz), 1.92–2.03 (4H, m), 2.93 (2H, d, J = 11 Hz), 3.51 (2H, s), 4.60 (2H, t, J = 8 Hz), 7.23–7.28 (1H, m), 7.30–7.35 (4H, m), 7.64 (1H, td, J = 7, 1.5 Hz), 7.71 (1H, td, J = 7, 1.5 Hz), 7.96 (1H, s), 8.11 (1H, dd, J = 7, 1.5 Hz), 8.20 (1H, dd, J = 7, 1.5 Hz); MS m/z (3:1, M)$^+$ 404, 406. Anal. calcd for $C_{24}H_{25}ClN_4$: C, 71.19; H, 6.22; N, 13.84. Found: C, 70.97; H, 6.44; N, 13.69.

1-[2-(1-Benzyl-4-piperidyl)ethyl]-4-chloro-1*H*-imidazo[4,5-*c*]pyridine (29). By using the similar procedure as for **17**, compound **29** was prepared in 48% yield from **27**, colorless prisms (toluene): mp 150–151 °C; ^{1}H NMR (DMSO-d_6) δ 1.15–1.30 (3H, m), 1.60–1.70 (2H, m), 1.76 (2H, q, J = 7 Hz), 1.87 (2H, t, J = 11 Hz), 2.76 (2H, d, J = 11 Hz), 3.42 (2H, s), 4.31 (2H, t, J = 7 Hz), 7.15–7.35 (5H, m), 7.71 (1H, d, J = 6 Hz), 8.14 (1H, d, J = 6 Hz), 8.47 (1H, s); MS m/z (3:1, M)$^+$ 354, 356. Anal. calcd for $C_{20}H_{23}ClN_4$: C, 67.69; H, 6.53; N, 15.79. Found: C, 67.65; H, 6.50; N, 15.73.

4-[2-(4-Chloro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)ethyl]-1-piperidineacetamide (18). A mixture of trifluoroacetate of **9** (0.60 g, 1.40 mmol), acetic anhydride (2.00 mL, 21.2 mmol) and pyridine (4.00 mL, 49.5 mmol) was stirred at room temperature for 1 h. The reaction mixture was concentrated, and the resulting residue was washed with a mixture of *iso*-PrOH and *iso*-Pr_2O to give 0.45 g (90%) of **18** as a colorless crystal, which was recrystallized from a mixture of CH_2Cl_2 and *iso*-Pr_2O to afford colorless crystals: mp 183–186.5 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (2H, qd, J = 12.5, 4 Hz), 1.61–1.71 (1H, m), 1.79 (1H, d, J = 12.5 Hz), 1.85 (1H, d, J = 12.5 Hz), 1.99 (2H, q, J = 7 Hz), 2.10 (3H, s), 2.55 (1H, td, J = 12.5, 2.5 Hz), 3.06 (1H, td, J = 12.5, 2.5 Hz), 3.84 (1H, d, J = 12.5 Hz), 4.58–4.72 (3H, m), 7.67 (1H, td, J = 8, 1 Hz), 7.73 (1H, td, J = 8, 1 Hz), 7.98 (1H, s), 8.09 (1H, dd, J = 8, 1 Hz), 8.21 (1H, dd, J = 8, 1 Hz); IR ν (KBr) cm^{-1}: 1642; MS m/z (3:1, M)$^+$ 356, 358. Anal. calcd for $C_{19}H_{21}ClN_4O$: C, 63.95; H, 5.93; N, 15.70. Found: C, 63.81; H, 5.87; N, 15.61.

4-[2-(4-Chloro-2-phenyl-1*H*-imidazo[4,5-*c*]quinolin-1-yl)-ethyl]-1-piperidineacetamide (19). By using the similar procedure as for **18**, compound **19** was prepared in 48% yield from **10**, colorless crystals (CH_2Cl_2/*iso*-Pr_2O): mp 154.5–156 °C; ^{1}H NMR (DMSO-d_6) δ 0.76–0.88 (1H, m), 0.90–1.02 (1H, m), 1.25–1.43 (3H, m), 1.71–1.82 (2H, m), 1.91 (3H, s), 2.33 (1H, t, J = 12 Hz), 2.83 (1H, t, J = 12 Hz), 3.63 (1H, d, J = 12 Hz), 4.18 (1H, d, J = 12 Hz), 4.72 (2H, t, J = 7.5 Hz), 7.63–7.67 (3H, m), 7.77–7.82 (4H, m), 8.11–8.15 (1H, m), 8.37–8.42 (1H, m); IR ν (KBr) cm^{-1}: 1642; MS m/z (3:1, M)$^+$ 432, 434. Anal. calcd for $C_{25}H_{25}ClN_4O \cdot 1/4H_2O$: C, 68.64; H, 5.88; N, 12.81. Found: C, 68.78; H, 5.78; N, 12.71.

Biology

Preparation of PBMCs for culture. About 50 mL of whole blood was collected from adult healthy volunteers by venipuncture, and poured into plastic tubes which contains 170 μL of Novo-heparin 1000 (Novo-Nordisk A/S). Then, PBMCs were separated using Lymphoprep™ (NYCOMED PHARMAAS) lymphocyte separation tube, and cultured with RPMI-1640 medium (Nissui Pharmaceutical Co.) containing 2 mM L-glutamine (Life Technologies), 2.5 U/mL penicillin-2.5 μg/mL streptomycin solution (Life Technologies) supplemented with 10% fetal calf serum (Intergen Company) at 1.11×10^6 cells/mL (final 1×10^6 ceus/mL).

Preparation of test compounds. Test compounds were dissolved in distilled ultra-pure water, DMSO or 0.1 N hydrochloric acid at 20 μM and were diluted with saline sequentially. Test compounds were evaluated at concentrations from 10^{-10} M to 10^{-5} M.

Treatment of cells with test compounds. 180 μL of prepared human PBMCs was added to each well of 96-well plate (MicroTest III™ tissue culture plate; FALCON) containing 10 μL of adequate concentrations solution of test compounds or solvent. Then after 30 min, 10 μL of 20 μg/mL LPS (final 1 μg/mL) was added to each well, and cultured for 16 h at 37 °C in humidified atmosphere of 5% CO_2 and 95% air.

Measurement of human TNF-α. The level of human TNF-α in culture supernatant was measured by constructed sandwich enzyme-linked immunosorbent assay method. 96-well microtiter plates were coated with anti-cytokine antibody (first-antibody) by introducing adequately diluted capture antibody. After washing the wells, the culture supernatant was diluted appropriately, added to each well and incubated. Then second-antibody against cytokine and third-antibody against second-antibody were introduced to wells sequentially, with interposed washing periods. After the final washing period, reaction of colorimetric quantification was started by addition of tetramethylbenzidine solution (DAKO) to each well. Optical absorbance at 450 nm of each well was measured by M-Vmax™ kinetic microplate reader (Molecular Devices) after the quenching by addition of 1 N sulfuric acid. Concentrations of cytokines were determined by Softmax™ (Molecular Devices) quantification software in comparison with calibration curve derived from corresponding recombinant cytokines as standard. For measurement of human TNF-α, monoclonal anti-human TNF-α (ENDOGEN), polyclonal rabbit anti-human TNF-α (Pharma Biotechnologie Hannover), peroxidase conjugated donkey anti-rabbit IgG (Jackson ImmunoRes. Labs.), and recombinant human TNF-α (INTERGEN Company) were used for first-, second-, third-antibody and standard for calibration curve, respectively. In measurement of effect on TNF-α, the activities of test compounds are shown as percentages of cytokine production with being treated by LPS and test compounds against by LPS only.

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