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Synthesis and structure–activity relationships of 8-substituted-2-aryl-5-alkylaminoquinolines: Potent, orally active corticotropin-releasing factor-1 receptor antagonists

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ABSTRACT

We previously reported a series of 8-methyl-2-aryl-5-alkylaminoquinolines as a novel class of corticotropin-releasing factor-1 (CRF_1) receptor antagonists. A critical issue encountered for this series of compounds was low aqueous solubility at physiological pH (pH 7.4). To address this issue, derivatization at key sites (R^2, R^3, R^5, $R^{5'}$, and R^8) was performed and the relationships between structure and solubility were examined. As a result, it was revealed that introduction of a methoxy substituent at the C_8 position had a positive impact on the solubility of the derivatives. Consequently, through in vivo and in vitro biological studies, compound **21d** was identified as a potent, orally active CRF_1 receptor antagonist with improved physicochemical properties.

1. Introduction

Corticotropin releasing factor (CRF), isolated by Vale et al. in 1981 as a 41 amino acid peptide, is the primary regulator of hypothalamic-pituitary-adrenal (HPA) stress response.[1] It is known that CRF exerts its biological functions through binding to two GPCR subfamily receptors, the CRF_1 and CRF_2 receptors.[2] The CRF_1 receptor is abundantly found in the pituitary and is involved in the regulation of adrenocorticotropic hormone (ACTH), a key mediator of stress response.[3] There is much evidence that CRF and CRF_1 receptors are heavily involved in stress-related disorders such as depression and anxiety. In fact, it was shown that intracerebroventricular administration of CRF in rodents elicits behavioral and physiological effects identical to those caused by natural stressors.[4] Furthermore, elevated CRF concentration in CSF has been observed in patients with depression[5] and decreased expression of CRF receptor in the frontal cortex has been observed in suicide victims.[6] Therefore, several pharmaceutical research groups have focused on the discovery of CRF_1 receptor antagonists for the treatment of depression or other stress-related disorders. Meanwhile, the benefits of blocking the CRF_2 receptor remain uncertain. To date, several CRF_1 receptor antagonists have been reported and prototypical antagonists are illustrated in Figure 1. CRF_1 receptor antagonists **1** (R121919),[7] **2** (CP-154526),[8] **3** (DMP696),[9] **4** (NBI-27914),[10] and **5** (CP-316311)[11] exhibited high in vitro affinity to the receptor and significant activity in animal models. However, clinical studies remain ambiguous. Antagonist **1** demonstrated efficacy in treating depressed patients in an open-label phase 2 clinical trial,[12] but **5** was unsuccessful in a double-blind study for depression.[13] From these results, it is apparent that the discovery of structurally diverse CRF_1 receptor antagonists and the accumulation of clinical studies for clarifying the role of CRF in humans are essential.

Known CRF_1 receptor antagonists have common structural features as illustrated in Figure 2. Each has a top region occupied by an alkyl chain, a central ring system occupied by a mono- or biheteroaromatic core, a small pocket occupied by a methyl group, and a bottom region occupied by a substituted phenyl or heteroaromatic moiety. Accordingly, we hypothesized that these four structural features were essential for CRF_1 receptor antagonism. As a result of compound design based on this hypothesis, we previously

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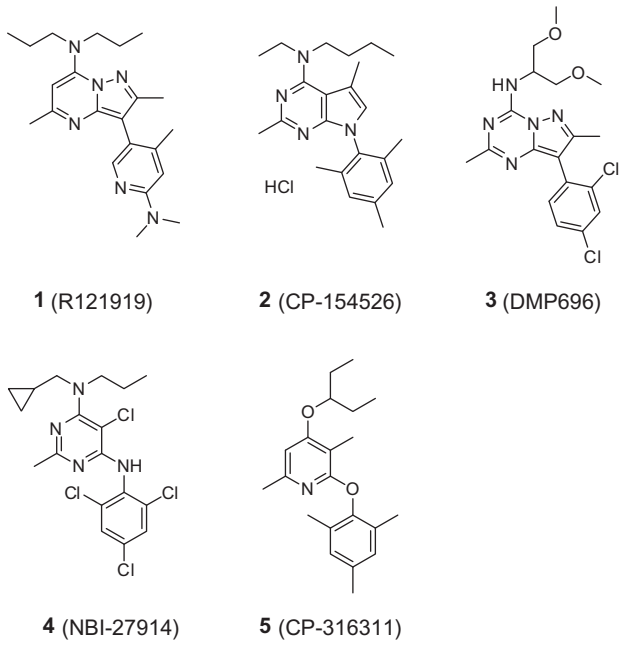


Figure 1. Known CRF_1 receptor antagonists.

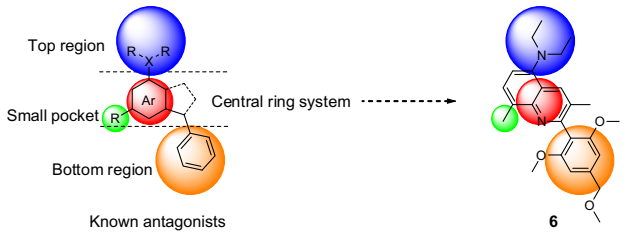


Figure 2. Design of a novel CRF_1 receptor antagonist.

reported compound **6** as a novel CRF_1 receptor antagonist (Fig. 2).[14] This compound exhibited potent in vitro activity and in vivo efficacy by oral administration. However, the unacceptable physicochemical profile (e.g., low aqueous solubility at pH 7.4) rendered this compound unsuitable for further development. Herein, we describe the results of our efforts to resolve this issue. Key sites in **6** were derivatized to generate a new series of compounds and the relationships between structure and solubility were examined. Studies of the structure–activity relationships, physicochemical properties, and electrostatic potentials of the derivatives are discussed. The biological activities of some of the new compounds were also examined, revealing a new analog with well-balanced solubility and in vitro efficacy.

2. Chemistry

Derivatives with a methyl substituent at the C_8 position were synthesized as shown in Scheme 1. Nitration of commercially available compound **7** afforded 5-nitroquinoline **8**. The position of the nitro group was determined by NMR analysis (Fig. 3). All of the proton and carbon signals of compound **8** were assigned by HMBC, COSY, and NOESY analysis. Next, the Suzuki–Miyaura cross-coupling reaction of **8** with [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid gave compound **9**. Reduction of the nitro group followed by reductive amination with aldehydes or substitution reaction with alkyl halide afforded derivatives **6** and **11a–c**.

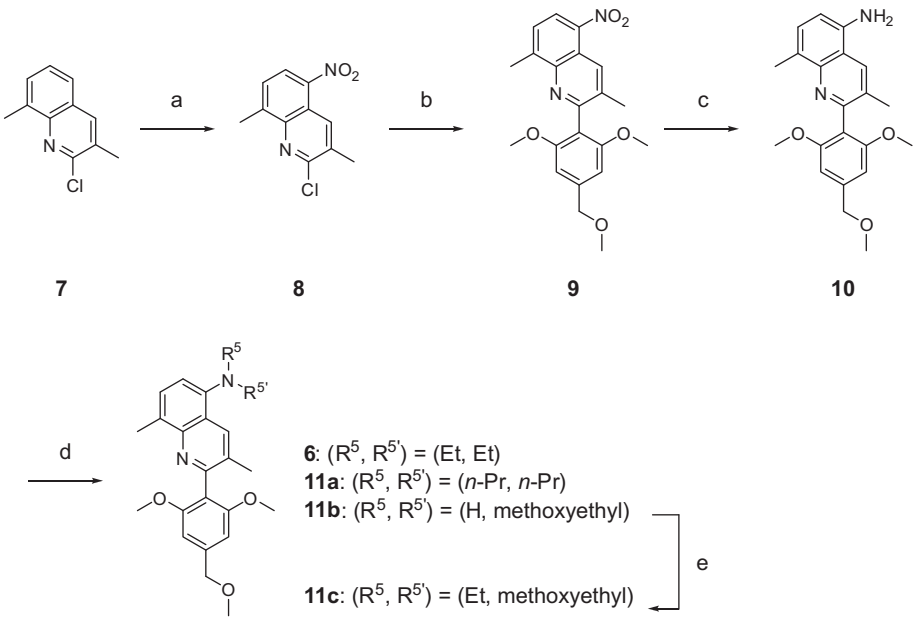
To easily prepare compounds derivatized at each site of the quinoline template, two different synthetic approaches were investigated. Derivatization at R^3 and R^8 was achieved as shown in Scheme 2. Compound **12d** was synthesized by the previously reported method using 3-chloroquinoline.[14] Nitration of 2-halo-3-substituted quinolines **12a–e** afforded corresponding 5-nitroquinolines **13a–e**, as confirmed by NMR analysis (Fig. 3). Subsequent Suzuki–Miyaura cross-coupling reaction with [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid afforded 2-aryl-quinolines **14a–e**. Reduction of the nitro group followed by reductive amination with acetaldehyde afforded intermediates **16a–e**. Halogenation of intermediate **16a** using N-chlorosuccinimide (NCS) and N-iodosuccinimide (NIS) afforded 8-haloquinolines **17** and **18**, respectively, and the positions of the halogen atoms were determined by NMR analysis (Fig. 3). The iodide of **18** was lithiated and transformed to aldehyde **19** and fluoride **21a**. Trifluoromethyl derivative **21b**, cyanide **21c**, and methoxide **21d** were also synthesized from iodide **18**. Aldehyde **19** was subsequently converted to difluoromethyl derivative **20a** in the presence of [bis(2-methoxyethyl) amino]sulfur trifluoride. Methoxymethyl derivative **20b** was also synthesized from aldehyde **19** using decaborane in methanol. Target compounds **23ba–ea** were synthesized from intermediates **16b–e** in a manner similar to that described for the synthesis of **21d**. The positions of the iodine atoms of **22b**, **22c**, **22d**, and **22e** were determined by NMR analysis (Fig. 3). Derivatives with methyl substituents at the C_8 position (**23bb–23db**) were synthesized from **22b–d** using $Me_2Zn/(tBu_3P)_2Pd$.

Compounds derivatized at R^2, R^5, and $R^{5'}$ were synthesized as shown in Scheme 3. First, 2-chloro-8-methoxy-3-methylquinoline **25** was synthesized by the method reported by Meth-Cohn et al.[15] starting with amide **24**. Nitration of **25** and the subsequent Suzuki–Miyaura cross-coupling reaction were conducted in a manner similar to that described for the synthesis of **14b** to afford 2-aryl-5-nitroquinline **27**. The position of the nitro group of **26** was determined by NMR analysis (Fig. 3). Reduction of the nitro group of **27** using Fe followed by reductive amination with aldehydes in the presence of reducing agent $NaBH(OAc)_3$ afforded target compound **29**. Compound **21d**, which was synthesized by the method described in Scheme 2, was also conveniently synthesized by this route. Boc protection of **28** followed by methoxyethylation afforded intermediate **31**. Deprotection of the Boc group followed by reductive amination with aldehydes afforded target compounds **33a–b**. Target compounds **36a–c** were synthesized from **26** in a manner similar to that described for the synthesis of **29**.

3. Results and discussions

The compounds in this study were first screened for their ability to inhibit [^{125}I] CRF binding to membranes of cells expressing the human CRF_1 receptor. Compounds with high binding affinity were then subjected to an in vitro functional assay to evaluate their antagonistic functions in the inhibition of CRF-induced cAMP (cyclic adenosine monophosphate) production in human CRF_1-receptor-expressed HEK293 cells.

The explorations initially performed were aimed at investigating the effects of substituents at the C_8 position on activity and aqueous solubility (pH 7.4). In order to clarify the effects of lipophilicity on aqueous solubility, C*log*P values were calculated (using Daylight software, version 4.94) and the results are shown in Table 1. Replacement of methyl with fluorine, difluoromethyl, trifluoromethyl, or methoxymethyl reduced binding affinity and antagonistic activity (**21a**, **20a**, **21b**, and **20b**, respectively). On the other hand, replacement with chlorine, cyano, or methoxy resulted in retained binding affinity (**17**, **21c**, and **21d**, respectively) compared with parent compound **6**. There seemed to be a strict



Scheme 1. Reagents and conditions: (a) HNO_3, H_2SO_4, −10 °C to RT; (b) [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid, $Pd(PPh_3)_4$, K_2CO_3, DME, H_2O, reflux; (c) Fe, saturated aqueous NH_4Cl, EtOH, reflux; (d) for **6**, acetaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT; for **11a**, propionaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT; for **11b**, 2-bromoethyl methyl ether, K_2CO_3, DMF, 100 °C; (e) acetaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT.

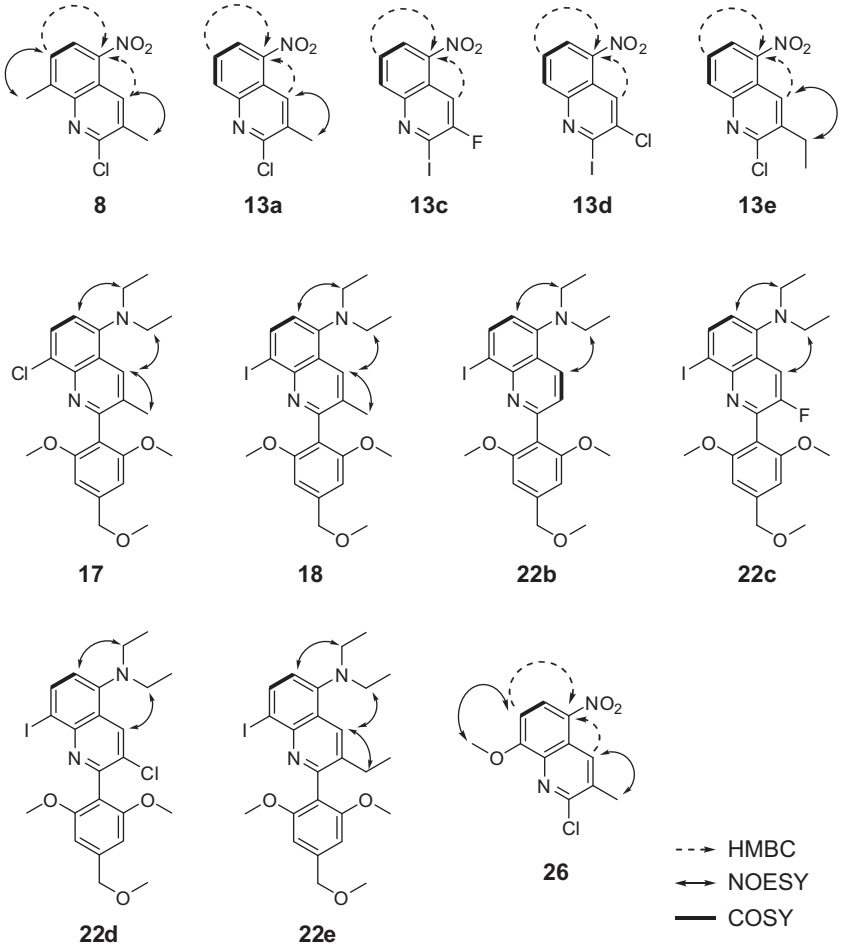
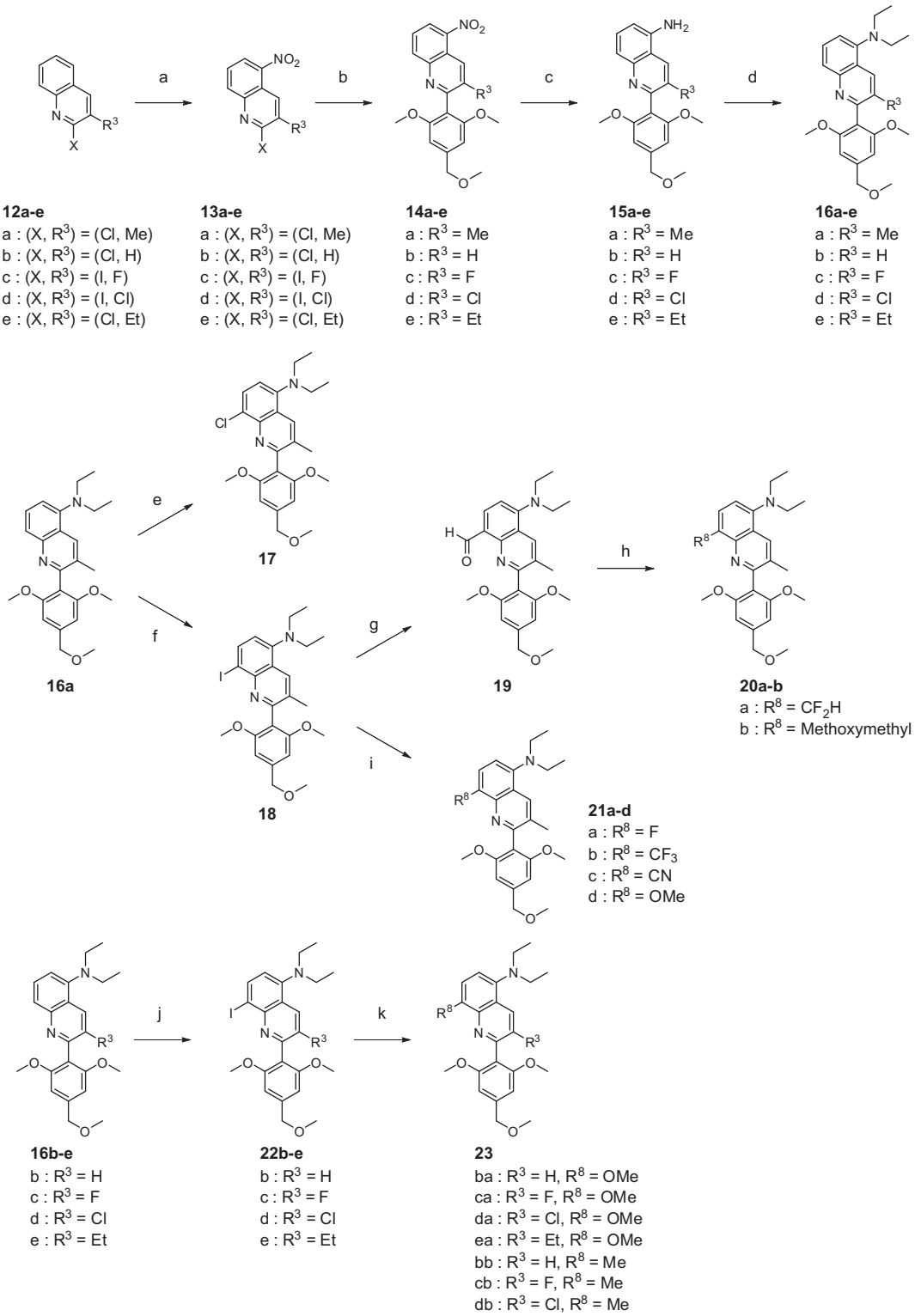
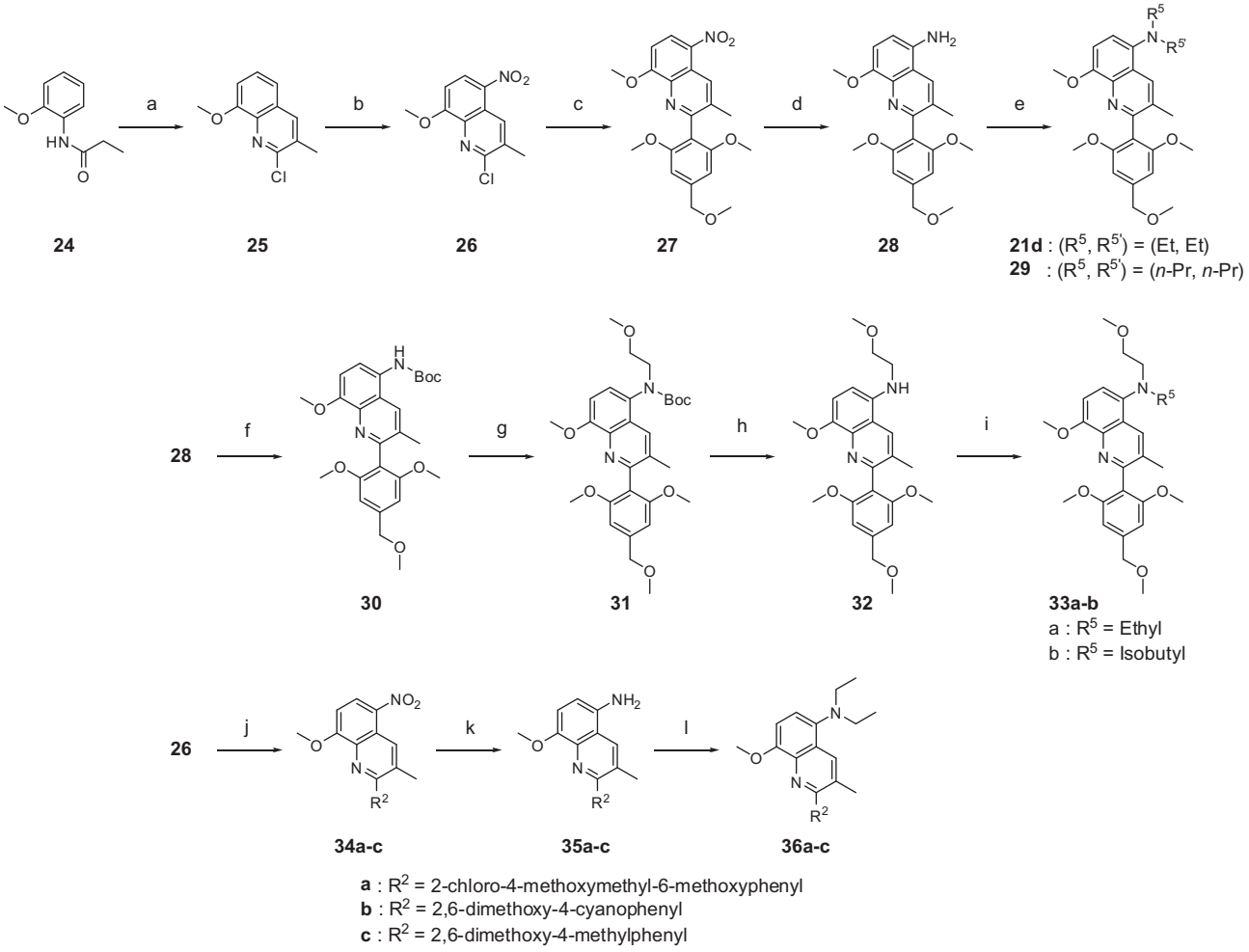


Figure 3. Results of the NMR analysis of derivatives of **6**.



Scheme 2. Reagents and conditions: (a) for **13a**, **13b**, and **13e**, HNO_3, H_2SO_4, −10 °C to RT; for **13c** and **13d**, HNO_3, fuming HNO_3, H_2SO_4, 0 °C; (b) for **14a**, [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid, $Pd(OAc)_2$, PPh_3, K_2CO_3, DME, H_2O, reflux; for **14b**, **14c**, and **14e**, [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, reflux; for **14d**, [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid, $Pd(PPh_3)_4$, 1 M aqueous Na_2CO_3, PhMe, EtOH, reflux; (c) for **15a**, H_2, Pd/C, ethyl acetate, RT; for **15b**, **15c**, **15d**, and **15e**, Fe, saturated aqueous NH_4Cl, EtOH, reflux; (d) acetaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT; (e) NCS, DMF, 60 °C; (f) NIS, DMF, RT; (g) DMF, *n*BuLi, THF, −78 °C; (h) for **20a**, [bis(2-methoxyethyl)amino]sulfur trifluoride, DCM, RT; for **20b**, decaborane, MeOH, RT; (i) for **21a**, *N*-fluorobenzenesulfonimide, *n*BuLi, THF, −78 °C; for **21b**, methyl fluorosulfonyldifluoroacetate, Cu, DMF, 90 °C; for **21c**, CuCN, DMF, 80 °C; for **21d**, CuBr, NaOMe/MeOH, ethyl acetate, reflux; (j) NIS, DMF, RT; (k) for **23ba**, **23ca**, **23da**, and **23ea**, CuBr, NaOMe/MeOH, ethyl acetate, reflux; for **23bb**, **23cb**, and **23db**, Me_2Zn, $(t\text{Bu}_3\text{P})_2\text{Pd}$, 1,4-dioxane, 80 °C.

requirement for the size of the substituent rather than its electronic effect at the C_8 position. No correlation between *C*log*P* and aqueous solubility was observed (Fig. 4). Compounds that had *C*log*P* values higher than **6** exhibited decreased solubility (**17** and **21b**). However, not all compounds that had *C*log*P* values lower than **6** exhibited improved solubility. For example,



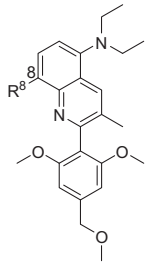
Scheme 3. Reagents and conditions: (a) $POCl_3$, DMF, 70 °C; (b) HNO_3, H_2SO_4, −10 °C to RT; (c) [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, reflux; (d) Fe, saturated aqueous NH_4Cl, EtOH, reflux; (e) for **21d**, acetaldehyde, $NaBH(OAc)_3$, AcOH, MeOH, THF, RT; for **29**, propionaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT; (f) Boc_2O, Et_3N, DCM, RT; (g) 2-bromoethyl methyl ether, NaH, DMF, 40 °C; (h) TFA, DCM, RT; (i) for **33a**, acetaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT; for **33b**, isobutyraldehyde, $NaBH(OAc)_3$, AcOH, THF, RT; (j) for **34a**, [2-chloro-6-methoxy-4-(methoxymethyl)phenyl]boronic acid, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, reflux; for **34b**, (4-cyano-2,6-dimethoxyphenyl)boronic acid, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, reflux; for **34c**, (2,6-dimethoxy-4-methylphenyl)boronic acid, $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, reflux; (k) Fe, saturated aqueous NH_4Cl, EtOH, reflux; (l) acetaldehyde, $NaBH(OAc)_3$, AcOH, THF, RT.

compound **21c**, which had the lowest *C*log*P* value of all the derivatives, did not exhibit much improved solubility compared with **6**. On the other hand, compound **21d**, which exhibited a slight decrease in *C*log*P* value compared to **6**, showed remarkably improved solubility.

To gain further insight into these results, pK_a values were determined for compounds **6**, **21c**, and **21d** (Table 2). Compound **21c** exhibited a remarkable decrease in pK_a relative to **6** (pK_a <3 for **21c**, pK_a = 5.86 for **6**), which might be caused by the introduction of the electron withdrawing cyano moiety. On the other hand, **21d** exhibited a slight increase in pK_a (pK_a = 6.01) relative to **6**. In order to confirm the effects of pK_a on aqueous solubility, the solubilities of **6**, **21c**, and **21d** were determined at various pH values (1.2, 5.0, and 9.0) and the results are shown in Table 2. The results were reasonable, indicating that the solubility of each compound increased below pH values approximately equal to their pK_a values. This indicates that for these three compounds, the effect of dissociation on solubility at pH 7.4 was negligible. In short, the difference in solubility observed at pH 7.4 in these compounds could not be explained by their lipophilicity (i.e., *C*log*P*) and their basicity (i.e., pK_a).

Next, to gain further insight into the relationships between structure and solubility, electrostatic potentials of **6**, **21c**, and **21d** were calculated because we speculated that the electronic state of the quinoline core could be influenced by substituents at the C_8 position. The calculations of electrostatic potential were performed using MOPAC (version 7.01, AM1 method) and the results are shown in Figure 5. Compared with **6**, compound **21d** possessed an area with higher electron density around the 8-methoxy moiety and the nitrogen atom of the quinoline core (Area A). The anionic area was thought to have a high affinity for water molecules in aqueous solutions, which may have led to the improved solubility observed for **21d**. On the other hand, the electrostatic potential of **21c** indicated that the electron density of the quinoline core was lower compared with **6** and **21d** owing to introduction of the cyano substituent. The electrostatic potential of **20b**, which has a methoxymethyl substituent at the C_8 position, was also calculated. An area with high electron density was not observed in this compound, unlike compound **21d**. Replacing methyl with methoxymethyl generally has a positive impact on aqueous solubility. However, **20b** did not exhibit improved solubility compared with **6**. This result indicates that simply introducing polar substituents at the C_8 position would not have a positive impact on aqueous solubility. These studies of electrostatic potential suggest that the good solubility of **21d** might result from the area with high electron density around the 8-methoxy moiety and the nitrogen atom

Table 1
Effects of substitution of R^8 on biological activity and physicochemical properties



No.	R^8	Binding IC_{50} (nM)	Functional IC_{50} (nM)	Solubility at pH 7.4[a] (μM)	*C*log*P*
6	Me	79	24	1.7	5.22
21a	F	226	67	10.5	4.94
17	Cl	62	28	<0.2	5.51
20a	CF_2H	414	NT	<0.2	4.92
21b	CF_3	288	259	<0.2	5.74
21c	CN	102	36	3.2	4.33
20b	Methoxymethyl	>1000	NT	2.9	4.52
21d	OMe	110	91	74.0	5.02

NT = not tested.
[a] pH 7.4: Dulbecco's phosphate-buffered saline (PBS).

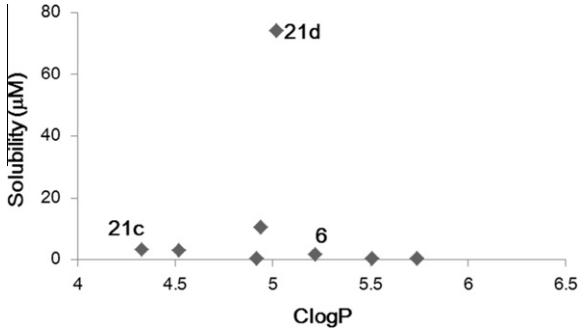


Figure 4. Correlation between *C*log*P* and solubility for compounds derivatized at C_8.

Table 2
Solubility and pK_a for **6**, **21c**, and **21d** at various pH values

No.	pK_a	pH 1.2 (μM)[a]	pH 5 (μM)[b]	pH 7.4 (μM)[c]	pH 9[b] (μM)
6	5.86	>100	22.0	1.7	2.0
21c	<3	>100	2.1	3.2	4.2
21d	6.01	>100	98.2	74.0	71.0

[a] pH 1.2: HCl/NaCl.
[b] pH 5 and pH 9: Britton–Robinson buffer, ionic strength 3 (acetate/phosphate/borate buffer containing KCl and adjusted with NaOH).
[c] pH 7.4: Dulbecco's phosphate-buffered saline (PBS).

of the quinoline core (Area A). As a result of derivatization at the C_8 position, it was determined that introduction of the methoxy substituent at C_8 resulted in well-balanced compatibility of activity and solubility. Thus, further derivatization was conducted with the methoxy substituent retained at the C_8 position in order to grasp the SAR and the relationship among structure, lipophilicity, and solubility.

The effect of R^3 was examined and the results are shown in Table 3. Derivatives with a methyl substituent at C_8 were also prepared to further clarify the effects of the methoxy substituent at C_8. Removing the methyl substituent resulted in lower binding affinity (**23ba**) and compounds with fluorine (**23ca**), chlorine (**23da**), and ethyl (**23ea**) at R^3 retained binding affinity. In functional assays, **23ca**, **23da**, and **23ea** exhibited a tendency for slightly improved antagonistic activity. This result indicated that introducing any substituent at the C_3 position effectively resulted in high binding affinity and potent antagonistic activity, although this position did not seem to be related to the pharmacophores shown in Figure 2. The dihedral angle between the quinoline and aromatic group in the bottom region might be important for activity because substituents at this position should have a significant impact on the angle. Furthermore, judging from the enhanced binding affinity and antagonistic activity observed for substituted derivatives (**21d**, **23ca**, **23da**, and **23ea**) relative to unsubstituted derivative **23ba,** a twisted conformation might be favored for the aryl moiety in the bottom region. The same tendency was observed for the 8-methylquinoline derivatives; substituted derivatives (**6**, **23cb**, and **23db**) displayed more potent activity than the unsubstituted derivative (**23bb**). The 8-methoxy derivatives (**21d**, **23ba**, **23ca**, and **23da**) exhibited comparable or slightly less activity than the 8-methyl derivatives (**6**, **23bb**, **23cb**, and **23db**). In terms of solubility, compound **23ba**, which exhibited a slightly lower *C*log*P* value than **21d**, had decreased solubility. We speculated that removing the methyl group from the C_3 position might increase the planarity of the molecule, which led to the decrease in solubility.[16] On the other hand, compounds **23ca** and **23da**, which had halogen atoms, exhibited remarkably lower solubility. In particular, the decreased solubility of compound **23ca**, which exhibited a *C*log*P* value comparable to that of **21d**, could not be explained by lipophilicity. Therefore, the electrostatic potentials of these two compounds were calculated (Fig. 6). Compared with **21d** (Fig. 5), electron density of Area A in **23ca** and **23da** was diffused by the electron-withdrawing effect of the halogen atoms at the C_3 position. We speculated that the lowered electron density weakened affinity toward water molecules, which led to decreased solubility. As for **23da**, both increased lipophilicity and electron density lowered by chloride atom might cause the decreased solubility. Compound **23ea** also exhibited lower solubility, which could be explained by its increased lipophilicity. On the other hand, solubility of 8-methylquinoline derivatives (**23bb**, **23cb**, and **23db**) did not seem to improve as a result of substitution at C_3. In summation, from comparison of the solubilities of 8-methoxy derivatives with those

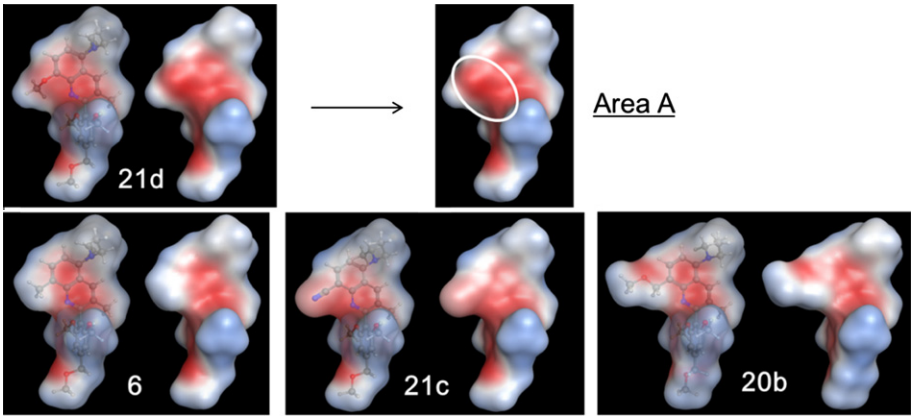
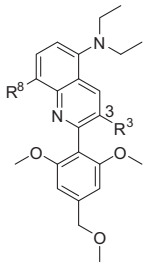


Figure 5. Electrostatic potential of compounds **6**, **20b**, **21c**, and **21d**.

Table 3
Effects of substitution of R^3 on biological activity and physicochemical properties



No.	R^8	R^3	Binding IC_{50} (nM)	Functional IC_{50} (nM)	Solubility at pH 7.4[a] (μM)	*C*log*P*
21d	OMe	Me	110	91	74	5.02
23ba	OMe	H	202	332	38.2	4.82
23ca	OMe	F	118	75	4.6	5.00
23da	OMe	Cl	144	57	1.9	5.32
23ea	OMe	Et	108	32	12.1	5.55
6	Me	Me	79	24	1.7	5.22
23bb	Me	H	184	381	<0.2	5.02
23cb	Me	F	144	131	<0.2	5.23
23db	Me	Cl	81	45	<0.2	5.55

[a] pH 7.4: Dulbecco's phosphate-buffered saline (PBS).

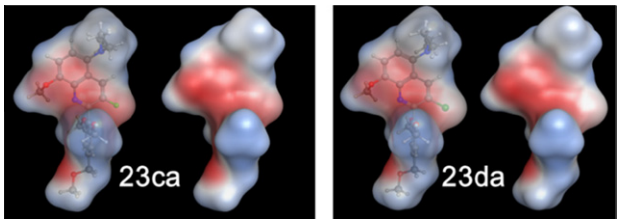


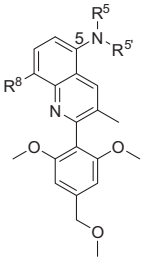
Figure 6. Electrostatic potential of compounds **23ca** and **23da**.

of 8-methyl derivatives, it was clear that introducing a methoxy substituent at the C_8 position had a significant impact on the solubility of the compounds.

The effects of R^5 and $R^{5'}$ were also examined, and the results are shown in Table 4. Compounds with a methyl substituent at the C_8 position were also prepared to clarify the effects of the methoxy substituent at C_8. The SAR study of our previously reported 8-methylquinoline series suggested that secondary amines and amines with saturated heterocycles such as tetrahydropyran were not tolerated, and that tertiary amines with chain-like alkyl or ether substituents were favored. Accordingly, a few derivatives with different R^5 and $R^{5'}$ substituents were prepared and SAR was briefly examined for the present 8-methoxyquinoline series. Di-*n*-propyl derivative **29** retained binding affinity and antagonistic activity compared with diethyl derivative **21d**. Replacement of ethyl with methoxyethyl resulted in fourfold lower binding affinity (**33a**), but the compound with isobutyl groups retained binding affinity and antagonistic activity (**33b**). In terms of solubility, a good correlation between solubility and lipophilicity was observed for these four derivatives as shown in Table 4. These results indicate that well-balanced compatibility of activity and solubility was achieved for compounds with a diethylamino substituent at the C_5 position. Comparing the solubilities of 8-methoxy derivatives with those of 8-methyl derivatives, it was again clear that introduction of the methoxy substituent at the C_8 position effectively enhanced the solubility of the compounds (**21d** vs **6** and **33a** vs **11c**). In terms of activity, derivatives with a methyl substituent at the C_8 position exhibited slightly more potent activity than those with a methoxy substituent, especially in the functional assay.

Table 5 summarizes the effects of various R^2 substituents. Replacement of methoxy at the *ortho*-position of the phenyl ring

Table 4
Effects of substitution of R^5 and $R^{5'}$ on biological activity and physicochemical properties



No.	R^8	R^5	$R^{5'}$	Binding IC_{50} (nM)	Functional IC_{50} (nM)	Solubility at pH 7.4[a] (μM)	*C*log*P*
21d	OMe	Et	Et	110	91	74	5.02
29	OMe	*n*Pr	*n*Pr	105	99	<0.2	6.08
33a	OMe	Et	Methoxyethyl	392	NT	89.4	4.52
33b	OMe	Isobutyl	Methoxyethyl	156	65	8.7	5.44
6	Me	Et	Et	79	24	1.7	5.22
11a	Me	*n*Pr	*n*Pr	88	70	2	6.23
11c	Me	Et	Methoxyethyl	109	23	7.2	4.71

NT = not tested.
[a] pH 7.4: Dulbecco's phosphate-buffered saline (PBS).

with chloride (**36a**) resulted in slightly higher binding affinity and fourfold more potent antagonistic activity than **21d**; however, its aqueous solubility was lower. The SAR of the *para*-position of the phenyl ring was also examined. Replacement of methoxymethyl with cyano or methyl resulted in retained binding affinity and slightly improved antagonistic activity (**36b** and **36c**, respectively), but the aqueous solubilities of these compounds were lower. To determine the cause of the lower solubility of **36b**, which exhibited a *C*log*P* value comparable to that of **21d**, the electrostatic potential of **36b** was calculated (Fig. 7). The results indicated that the electron density of the phenyl ring in the bottom region was remarkably lower and the electron density of Area A was also slightly influenced. As mentioned before, lower electron density in Area A might have a negative impact on the solubility of these compounds. Judging from the results of the electrostatic potential calculations for **36b**, the electronic state of the phenyl ring in the bottom region might also be important for better solubility.

To clarify the effects of lipophilicity on solubility in the 8-methoxyquinoline series, the correlation between *C*log*P* and solubility was analyzed (Fig. 8A). This figure shows the results for all of the 8-methoxyquinoline derivatives (eleven compounds). From the figure, it can be seen that coefficient determination was low (least-squares method, $R^2 = 0.44$) and no clear correlation was

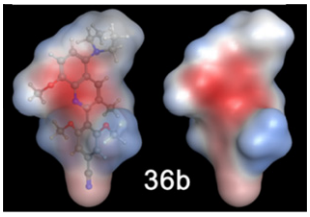
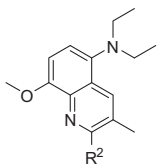


Figure 7. Electrostatic potential of compound **36b**.

found. As mentioned before, electronic density of aromatic groups might be a key factor for better solubility. Accordingly, compounds possessing electron-withdrawing groups (**23ca**, **23da**, **36a**, and **36b**) were excluded from the correlated calculation. Furthermore, compound **23ba** was also excluded because its lower solubility might be caused by molecular planarity. The result of the recalculation is shown in Figure 8B. This result indicates that there was a clear correlation between *C*log*P* and solubility in these six compounds (least-squares method, $R^2 = 0.86$). In short, these results confirm that the solubilities of the 8-methoxyquinoline derivatives were basically proportional to their *C*log*P* values and the lower solubility observed in the excluded compounds was caused by

Table 5
Effects of R^2 on biological activity and physicochemical properties



No.	R^2	Binding IC_{50} (nM)	Functional IC_{50} (nM)	Solubility at pH 7.4[a] (μM)	*C*log*P*
21d	2,6-Dimethoxy-4-methoxymethylphenyl	110	91	74.0	5.02
36a	2-Chloro-4-methoxymethyl-6-methoxyphenyl	67	22	3.7	6.06
36b	2,6-Dimethoxy-4-cyanophenyl	132	63	11	4.92
36c	2,6-Dimethoxy-4-methylphenyl	99	43	17.3	5.72

[a] pH 7.4: Dulbecco's phosphate-buffered saline (PBS).

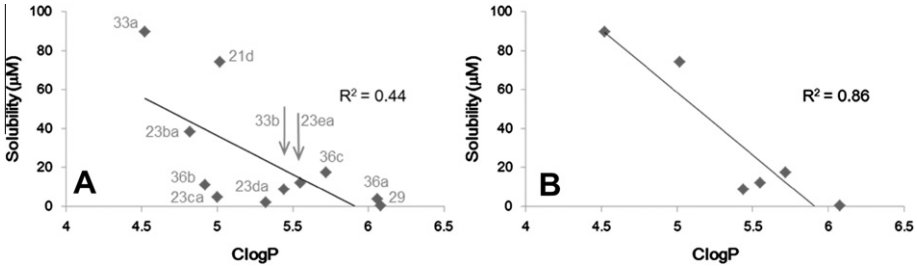


Figure 8. Correlation between *C*log*P* and solubility in 8-methoxyquinoline derivatives.

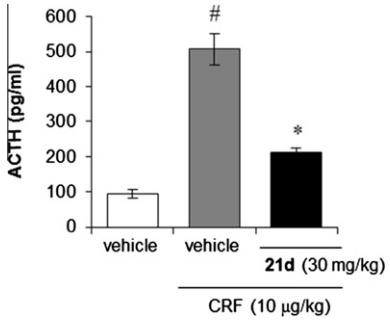


Figure 9. Effects of **21d** on the CRF-induced elevation of plasma ACTH concentration in F344 rats. The compound was administrated orally 60 min before subcutaneous injection of CRF. Plasma ACTH concentration was measured 30 min after CRF injection. Each value represents the mean ± S.E.M. of 5–6 rats. $^{\#}P$ <0.05 versus non-CRF control (Welch's *t*-test), $^{*}P$ <0.05 versus CRF control (unpaired *t*-test).

factors other than lipophilicity, such as electron density. Furthermore, it was revealed that lowering lipophilicity without lowering the electron density of the aromatic groups was crucial for obtaining highly soluble compounds in the 8-methoxyquinoline series.

Among the 8-methoxyquinoline derivatives, compound **21d** was tested in the CRF-induced ACTH release model. In this model, the effects of the compounds on CRF-induced ACTH release were evaluated. Figure 9 shows that subcutaneous CRF administration in rats (10 μg kg^{-1}) produced a robust increase in the plasma levels of ACTH. Orally administered, compound **21d** attenuated this effect at a 30 mg kg^{-1} dose and statistically significant reduction of the ACTH levels was observed ($^{*}P$ <0.05, unpaired *t*-test). These results indicate that in vivo functional activity of this compound confirmed that it is a CRF receptor antagonist.

Additionally, the anxiolytic efficacy of compound **21d** was evaluated in the light-dark test (Fig. 10). Compound **36a** was also assessed in this behavior model because this compound exhibited high binding affinity and potent antagonistic activity in the in vitro assays. In this model, time spent in the aversive parts of the apparatus (i.e., the illuminated box) and locomotor activity (i.e., the number of tunnel crossings) were evaluated in BALB/c mice. Compound **21d** (10 and 30 mg kg^{-1}) dose-dependently increased the time and the number of tunnel crossings compared with vehicle groups ($^{*}P$ <0.05, Dunnett multiple comparison test). Compound **36a** also exhibited dose-dependent increases of both the time and number of crossings, and statistically significant increase was observed at a 30 mg kg^{-1} dose ($^{*}P$ <0.05, Dunnett multiple comparison test). These results indicate that these compounds were effective at reducing anxiety-related responses in the light-dark test.

Table 6
Pharmacokinetic parameters for **21d** in rats

	iv (3 mg kg^{-1})[a]	p.o. (10 mg kg^{-1})[b]
$T_{1/2}$ (h)	1.73 ± 0.29	
C_{max} (μg mL^{-1})		0.305 ± 0.012
T_{max} (h)		0.500 ± 0.000
AUC inf. (μg mL^{-1} h^{-1})	1.542 ± 0.063	0.845 ± 0.036
VD_{ss} (mL kg^{-1})	2147.09 ± 449.80	
Cl tot. b (mL h^{-1} kg^{-1})	2637.40 ± 105.17	
BA (%)		16.4
B/P (1 h after p.o.)		0.61

Standard errors of the mean are reported (n = 3 rats).
[a] The vehicle for iv studies was 5% EtOH-0.02 mol/L HCl/5% glucose.
[b] The vehicle for p.o. studies was H_2O containing 5% DMSO, 5% Cremophor® EL and equivalent HCl.

The pharmacokinetic (PK) profile of compound **21d** was studied in rats (Table 6). The rats were dosed intravenously at 3 mg kg^{-1} and orally at 10 mg kg^{-1}. Total blood clearance (Cl tot. b) of this compound was high (2637.40 ± 105.17 mL h^{-1} kg^{-1}), which was approximately 80% of hepatic blood flow (55 mL min^{-1} kg^{-1}). Thus, the hepatic clearance was thought to be the main cause of the modest bioavailability (16.4%). Additionally, compound concentration in the brain was measured 1 h after p.o. administration. As a result, the brain-to-plasma (B/P) concentration ratio was 0.61. This PK study suggested that **21d** was a brain penetrant compound.

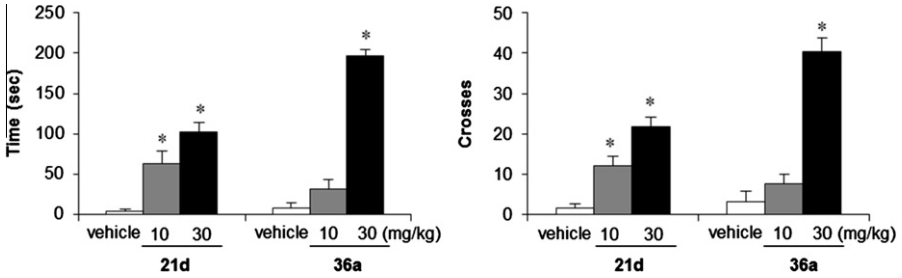


Figure 10. Effects of **21d** and **36a** on anxiety-related behavior in the light/dark test in BALB/c mice (n = 10). Compounds were administrated orally 60 min before the test and the behavior of the mice was observed for 5 min. Each value represents the mean ± S.E.M. $^{*}P$ <0.05 versus vehicle groups (Dunnett multiple comparison test).

4. Conclusions

Through the preparation and evaluation of compounds derivatized at each site (R^2, R^3, R^5, $R^{5'}$, and R^8), SAR and the relationships between structure and aqueous solubility were investigated. In the study of derivatization at the C_8 position, it was revealed that introduction of a methoxy substituent overcame the issue of low aqueous solubility observed in previously reported compound **6**, without loss of in vitro activity. In the 8-methoxyquinoline series, it was revealed that lowering lipophilicity without lowering the electron density of the aromatic groups was crucial for obtaining highly soluble compounds. Among the derivatives, compound **21d** exhibited activity in the CRF-induced ACTH release model with statistical significance by oral administration (30 mg kg^{-1}). Additionally, orally administered compounds **21d** and **36a** exhibited statistically significant anxiolytic effects in the light-dark test (10 mg kg^{-1} for **21d** and 30 mg kg^{-1} for **36a**). A PK study of **21d** was conducted and the results suggested that this compound was a brain penetrant. From these results, **21d** was identified as a promising new lead compound for clinical candidates for the treatment of stress-related disorders.

5. Experimental sections

5.1. Chemistry

5.1.1. General methods

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (operating at 600 MHz for ^1H and 151 MHz for ^{13}C). Chemical shifts were expressed in ppm (δ) from the residual $CHCl_3$ signal at δ_H 7.26 ppm and δ_C 77.0 ppm in $CDCl_3$ (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, and b = broad). Coupling constants (J) are given in Hertz (Hz). IR spectra were obtained using the KBr method and the attenuated total reflectance (ATR) method with an FT/IR-620 spectrometer (JASCO). Only the most significant absorption bands have been reported. High resolution mass spectra (HRMS) were recorded on a Thermo Fisher LTQ Orbitrap XL spectrometer using electrospray ionization (ESI) and a Waters GCT Premier using electron ionization (EI). LC–MS analyses were performed using a Shimadzu LCMS-2010 EV to determine the purities of the compounds tested. Column chromatography was carried out using a Hi-Flash™ column (40 μm, silica gel and NH-silica gel, Yamazen Corporation). Chemicals and solvents used in the study were commercially available.

5.1.2. 2-Chloro-3,8-dimethyl-5-nitroquinoline (8)

To a solution of **7** (7.00 g, 36.5 mmol) in H_2SO_4 (35 mL) was added HNO_3 at −10 °C and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was poured into crushed-ice, filtered, and washed with water to afford the title compound **8** as a light yellow solid (2.35 g, 9.93 mmol, 27.2%).

^1H NMR (600 MHz, $CDCl_3$): δ = 2.63 (s, 3H), 2.86 (s, 3H), 7.61 (d, J = 7.9 Hz, 1H), 8.30 (d, J = 7.9 Hz, 1H), 8.92 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.8, 20.6, 120.6, 124.8, 127.7, 133.7, 134.0, 143.1, 145.3, 145.4, 152.5; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{11}H_{10}ClN_2O_2^+$: 237.0425, found: 237.0427.

5.1.3. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3,8-dimethyl-5-nitroquinoline (9)

A mixture of **8** (1.75 g, 7.40 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (2.17 g, 9.61 mmol), $Pd(PPh_3)_4$ (427 mg, 370 μmol), and K_2CO_3 (3.07 g, 22.2 mmol) in a mixture of 1,2-dimethoxyethane (40 mL) and water (10 mL) was stirred at reflux temperature for 4 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9:1–5:5 n-heptane/ethyl acetate gradient) to afford the title compound **9** as a light yellow solid (2.41 g, 6.31 mmol, 85.0%).

^1H NMR (600 MHz, $CDCl_3$): δ = 2.30 (s, 3H), 2.86 (s, 3H), 3.51 (s, 3H), 3.74 (s, 6H), 4.55 (s, 2H), 6.72 (s, 2H), 7.54 (d, J = 7.9 Hz, 1H), 8.26 (d, J = 7.9 Hz, 1H), 8.87 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.3, 19.6, 56.1, 58.5, 75.0, 103.7, 117.5, 120.7, 124.1, 126.1, 130.9, 135.1, 140.7, 143.3, 145.6, 146.4, 156.6, 158.0; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{21}H_{23}N_2O_5^+$: 383.1601, found: 383.1600.

5.1.4. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3,8-dimethylquinolin-5-amine (10)

A mixture of **9** (2.36 g, 6.17 mmol) and Fe (1.64 g, 29.4 mmol) in a mixture of EtOH (26 mL) and saturated aqueous NH_4Cl (2.60 mL) was stirred at reflux temperature for 1 h. The reaction mixture was cooled to room temperature and filtered through a pad of NH-silica gel with ethyl acetate as the eluent to yield the crude title compound **10** as an orange solid (2.16 g, 6.13 mmol, 99.0%), which was used in the following reaction without further purification.

^1H NMR (600 MHz, $CDCl_3$): δ = 2.24 (s, 3H), 2.67 (s, 3H), 3.50 (s, 3H), 3.73 (s, 6H), 4.54 (s, 2H), 6.67–6.74 (m, 3H), 7.26 (d, J = 7.6 Hz, 1H), 8.01 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 18.0, 19.3, 56.2, 58.4, 75.1, 104.1, 109.8, 118.7, 127.7, 128.3, 129.1, 129.7, 139.6, 140.1, 146.3, 154.7, 158.2; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{21}H_{25}N_2O_3^+$: 353.1860, found: 353.1868.

5.1.5. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3,8-dimethylquinolin-5-amine (6)

A mixture of **10** (150 mg, 426 μmol), acetaldehyde (119 μL, 2.13 mmol), and $NaBH(OAc)_3$ (451 mg, 2.13 mmol) in a mixture of AcOH (0.2 mL) and THF (2 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (10:0–5:5 n-heptane/ethyl acetate gradient) to afford the title compound **6** as a colorless oil (60.8 mg, 149 μmol, 35.0%). Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 7.1 Hz, 6H), 2.23 (s, 3H), 2.72 (s, 3H), 3.19 (q, J = 7.1 Hz, 4H), 3.50 (s, 3H), 3.74 (s, 6H), 4.54 (s, 2H), 6.71 (s, 2H), 7.05 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 7.6 Hz, 1H), 8.40 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.3, 18.2, 19.4, 48.0, 56.2, 58.4, 75.1, 104.1, 117.7, 119.1, 125.8, 127.3, 130.1, 131.8, 132.3, 139.9, 145.1, 147.0, 154.5, 158.2; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{25}H_{33}N_2O_3^+$: 409.2486, found: 409.2487.

5.1.6. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3,8-dimethyl-*N*,*N*-dipropylquinolin-5-amine (11a)

A mixture of **10** (150 mg, 426 μmol), propionaldehyde (154 μL, 2.13 mmol), and $NaBH(OAc)_3$ (451 mg, 2.13 mmol) in a mixture of AcOH (0.2 mL) and THF (2 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (10:0–5:5 n-heptane/ethyl acetate gradient) to afford the title compound **11a** as a white solid (34.6 mg, 79.3 μmol, 18.6%). Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 0.89 (t, J = 7.0 Hz, 6H), 1.47–1.57 (m, 4H), 2.23 (s, 3H), 2.71 (s, 3H), 3.08 (t, J = 6.8 Hz, 4H), 3.50 (s, 3H), 3.74 (s, 6H), 4.54 (s, 2H), 6.71 (s, 2H), 7.08 (d, J = 7.2 Hz, 1H), 7.36 (d, J = 7.2 Hz, 1H), 8.42 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$):

δ = 11.8, 18.2, 19.4, 20.3, 56.3, 56.5, 58.4, 75.1, 104.2, 117.9, 119.1, 125.7, 127.3, 130.0, 131.8, 132.2, 139.9, 145.9, 147.0, 154.5, 158.2; HRMS (ESI+): *m*/*z* [*M*+H]$_+$ calcd for $C_{27}H_{37}N_2O_3^+$: 437.2799, found: 437.2798.

5.1.7. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*-(2-methoxyethyl)-3,8-dimethylquinolin-5-amine (11b)

A mixture of **10** (400 mg, 1.14 mmol), 2-bromoethyl methyl ether (533 μL, 5.68 mmol), and K_2CO_3 (1.57 g, 11.4 mmol) in DMF (8 mL) was stirred at 100 °C for 12 h. The reaction mixture was cooled to room temperature, quenched with water, and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (10:0–5:5 *n*-heptane/ethyl acetate gradient) to afford the title compound **11b** as a light yellow solid (302 mg, 736 μmol, 64.8%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.24 (s, 3H), 2.66 (s, 3H), 3.42–3.47 (m, 5H), 3.49 (s, 3H), 3.73 (s, 6H), 3.77 (t, *J* = 5.1 Hz, 2H), 4.53 (s, 2H), 6.56 (d, *J* = 7.8 Hz, 1H), 6.70 (s, 2H), 7.33 (d, *J* = 7.8 Hz, 1H), 8.09 (s, 1H); ^{13}C NMR (151 MHz, C_5D_5N): δ = 17.5, 18.5, 43.5, 55.3, 57.4, 57.8, 70.4, 74.2, 103.5, 103.6, 118.5, 118.5, 124.3, 128.7, 128.9, 129.2, 140.4, 142.3, 146.7, 154.7, 157.9; HRMS (ESI+): *m*/*z* [*M*+H]$_+$ calcd for $C_{24}H_{31}N_2O_4^+$: 411.2278, found: 411.2279.

5.1.8. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*-ethyl-*N*-(2-methoxyethyl)-3,8-dimethylquinolin-5-amine (11c)

A mixture of **11b** (120 mg, 292 μmol), acetaldehyde (33.0 μL, 585 μmol), and $NaBH(OAc)_3$ (124 mg, 585 μmol) in a mixture of AcOH (0.2 mL) and THF (2 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (10:0–7:3 *n*-heptane/ethyl acetate gradient) to afford the title compound **11c** as a colorless oil (95.7 mg, 218 μmol, 74.6%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, *J* = 7.0 Hz, 3H), 2.23 (s, 3H), 2.72 (s, 3H), 3.25 (q, *J* = 7.0 Hz, 2H), 3.31 (s, 3H), 3.35 (t, *J* = 6.0 Hz, 2H), 3.47 (t, *J* = 6.0 Hz, 2H), 3.50 (s, 3H), 3.74 (s, 6H), 4.54 (s, 2H), 6.71 (s, 2H), 7.13 (d, *J* = 7.4 Hz, 1H), 7.38 (d, *J* = 7.4 Hz, 1H), 8.45 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 18.3, 19.3, 49.6, 53.5, 56.2, 58.4, 58.8, 70.8, 75.1, 104.1, 118.1, 119.1, 125.8, 127.3, 130.3, 131.7, 132.8, 139.9, 144.9, 147.0, 154.7, 158.2; HRMS (ESI+): *m*/*z* [*M*+H]$_+$ calcd for $C_{26}H_{35}N_2O_4^+$: 439.2591, found: 439.2590.

5.1.9. 3-Chloro-4-iodoquinoline

A solution of diisopropylamine (720 μL, 5.14 mmol) in THF (6 mL) was cooled to −30 °C. To the solution was added *n*-BuLi (2.77 M in *n*-hexane, 1.46 mL, 4.04 mmol) and the mixture was stirred at −30 °C for 15 min. To the mixture was added a solution of 3-chloroquinoline (600 mg, 3.67 mmol) in THF (3 mL) at −78 °C and the mixture was stirred at −78 °C for 2 h. Then, a solution of I_2 (1.40 g, 5.51 mmol) in THF (3 mL) was added to the mixture at −78 °C and the mixture was stirred at −78 °C for 2.15 h. The reaction mixture was quenched with AcOH and concentrated under reduced pressure. The residue was extracted into ethyl acetate. The organic layer was purified by silica gel column chromatography (*n*-heptane) to afford the title compound as a white solid (515 mg, 1.78 mmol, 48.5%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.63–7.68 (m, 1H), 7.71–7.77 (m, 1H), 8.06 (d, *J* = 8.3 Hz, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 8.73 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 114.8, 129.2, 130.0, 130.0, 131.6, 132.5, 135.3, 145.6, 148.2; IR (KBr): $\tilde{\nu}$ = 1549, 1483, 1334, 1115, 866, 759 cm^{-1}, HRMS-ESI *m*/*z* [*M*+H]$^+$ calcd for $C_9H_6ClIN^+$: 289.9228, found: 289.9229.

5.1.10. 3-Chloro-2-iodoquinoline (12d)

A solution of diisopropylamine (322 μL, 2.30 mmol) in THF (10 mL) was cooled to −30 °C. To the solution was added *n*-BuLi (2.77 M in *n*-hexane, 651 μL, 1.80 mmol) and the mixture was stirred at −30 °C for 15 min. To the mixture was added a solution of 3-chloro-4-iodoquinoline (475 mg, 1.64 mmol) in THF (5 mL) at −78 °C and the resultant mixture was stirred at −78 °C for 2 h. Then, water (443 μL, 24.6 mmol) was added to the mixture at −78 °C and stirring was continued at −78 °C for 10 min. The reaction was quenched with AcOH and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (10:0–9:1 *n*-heptane/ethyl acetate gradient) to afford the title compound **12d** as a white solid (267 mg, 922 μmol, 56.2%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.54–7.66 (m, 1H), 7.67–7.79 (m, 2H), 8.06 (d, *J* = 7.9 Hz, 1H), 8.12 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 122.4, 126.8, 127.7, 128.1, 128.8, 130.4, 133.5, 134.1, 147.3; IR (KBr): $\tilde{\nu}$ = 1544, 1484, 1359, 1315, 1293, 1114, 961, 778, 756 cm^{-1}; HRMS-ESI *m*/*z* [*M*+H]$^+$ calcd for $C_9H_6ClIN^+$: 289.9228, found: 289.9228.

5.1.11. 2-Chloro-3-methyl-5-nitroquinoline (13a)

To a solution of **12a** (19.5 g, 110 mmol) in H_2SO_4 (100 mL) was added HNO_3 (14.3 mL, 226 mmol) at −10 °C and the reaction mixture was stirred at −10 °C for 20 min and at room temperature for 1 h. The mixture was poured into crushed-ice, filtered, and washed with water. The obtained crude product was dissolved in DCM and silica gel (40 g) was added to the solution. The solution was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (6:1–4:1 *n*-heptane/ethyl acetate gradient) to afford the title compound **13a** as a yellow solid (11.3 g, 50.8 mmol, 46.1%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.63 (s, 3H), 7.76 (dd, *J* = 8.3, 7.6 Hz, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.36 (d, *J* = 7.6 Hz, 1H), 8.87 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 20.7, 120.6, 124.9, 127.7, 133.7, 134.2, 135.2, 144.9, 146.6, 153.8; IR (KBr): $\tilde{\nu}$ = 3096, 3044, 1515, 1484, 1392, 1333, 1176, 1061, 937, 832, 740 cm^{-1}; HRMS-ESI *m*/*z* [*M*+H]$^+$ calcd for $C_{10}H_8ClN_2O_2^+$: 223.0269, found: 223.0264.

5.1.12. 2-Chloro-5-nitroquinoline (13b)

To a solution of **12b** (5.00 g, 30.6 mmol) in H_2SO_4 (15 mL) was added HNO_3 (2.04 mL, 45.9 mmol) at −10 °C and the reaction mixture was stirred at −10 °C for 20 min and at room temperature for 1 h. The mixture was poured into crushed-ice, filtered and, washed with water. The obtained crude title compound (5.64 g), which was used in the following reaction without further purification. (CAS Registry Number: 13067-94-2).

5.1.13. 3-Fluoro-2-iodo-5-nitroquinoline (13c)

To a solution of **12c** (5.00 g, 18.3 mmol) in H_2SO_4 (40 mL) was added HNO_3 (1.22 mL, 27.5 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 20 min. Fuming HNO_3 (1.14 mL, 27.5 mmol) was added and the mixture was stirred at the same temperature for 2 h. The mixture was poured into crushed ice and extracted into ethyl acetate. The organic layer was concentrated and the residue was purified by silica gel column chromatography (9:1–7:3 *n*-heptane/ethyl acetate gradient) to afford the title compound **13c** as a white solid (2.76 g, 8.68 mmol, 47.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.80 (dd, *J* = 8.3, 7.9 Hz, 1H), 8.41 (d, *J* = 8.3 Hz, 1H), 8.52 (d, *J* = 7.9 Hz, 1H), 8.68 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 113.1, 114.8, 121.7, 126.4, 127.5,

136.0, 144.6, 146.6, 156.8; IR (KBr): $\tilde{\nu}$ = 3092, 1516, 1396, 1335, 1212, 1054, 823 cm^{-1}; HRMS-EI: m/z $[M]_+$ calcd for $C_9H_4FIN_2O_2^+$: 317.9296, found: 317.9334.

5.1.14. 3-Chloro-2-iodo-5-nitroquinoline (13d)

The title compound was prepared from **12d** using a method analogous to that described for **13c** in 42.3% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.81 (dd, J = 8.3, 7.9 Hz, 1H), 8.37 (d, J = 8.3 Hz, 1H), 8.48 (d, J = 7.9 Hz, 1H), 9.06 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 120.8, 124.9, 126.1, 128.5, 130.3, 135.9, 137.8, 144.2, 146.9; IR (KBr): $\tilde{\nu}$ = 3077, 1522, 1351, 1327, 1282, 1128, 903, 822, 738 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_9H_5ClIN_2O_2^+$: 334.9079, found: 334.9087.

5.1.15. 2-Chloro-3-ethyl-5-nitroquinoline (13e)

The title compound was prepared from **12e** using a method analogous to that described for **13a** in 40.4% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.40 (t, J = 7.6 Hz, 3H), 2.99 (q, J = 7.6 Hz, 2H), 7.77 (dd, J = 8.3, 7.6 Hz, 1H), 8.31 (d, J = 8.3 Hz, 1H), 8.37 (d, J = 7.6 Hz, 1H), 8.86 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 13.4, 27.1, 120.7, 124.9, 127.7, 132.4, 135.2, 139.4, 145.0, 146.5, 153.5; IR (KBr): $\tilde{\nu}$ = 3089, 2966, 1518, 1395, 1335, 1172, 1064, 932, 914, 824, 737 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{11}H_{10}ClN_2O_2^+$: 237.0426, found: 237.0426.

5.1.16. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3-methyl-5-nitroquinoline (14a)

A mixture of **13a** (1.77 g, 7.95 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (2.70 g, 11.9 mmol), $Pd(OAc)_2$ (178 mg, 0.795 mmol), PPh_3 (1.04 g, 3.98 mmol), and K_2CO_3 (3.30 g, 23.9 mmol) in a mixture of 1,2-dimethoxyethane (60 mL) and H_2O (15 mL) was stirred at reflux temperature for 2 h. The mixture was cooled to room temperature, followed by addition of ethyl acetate. After thoroughly shaking the mixture, the organic layer was separated and the aqueous layer was extracted into ethyl acetate. The combined organic layers were concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (9:1–1:1 *n*-heptane/ethyl acetate gradient) to afford the title compound **14a** as a light yellow solid (2.56 g, 6.95 mmol, 87.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.29 (s, 3H), 3.46 (s, 3H), 3.72 (s, 6H), 4.53 (s, 2H), 6.68 (s, 2H), 7.69 (dd, J = 8.3, 7.6 Hz, 1H), 8.32 (d, J = 7.6 Hz, 1H), 8.45 (d, J = 8.3 Hz, 1H), 8.81 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.8, 55.9, 58.3, 74.9, 103.3, 116.3, 120.9, 124.1, 126.0, 130.8, 135.7, 136.5, 141.1, 145.1, 147.0, 157.8, 158.5; IR (KBr): $\tilde{\nu}$ = 2943, 2841, 1582, 1523, 1415, 1335, 1232, 1121, 822 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{20}H_{21}N_2O_5^+$: 369.1445, found: 369.1435.

5.1.17. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-5-nitroquinoline (14b)

A mixture of crude **13b** (5.64 g, 27 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (7.93 g, 35.1 mmol), $Pd(PPh_3)_4$ (1.56 g, 1.35 mmol), and Na_2CO_3 (7.15 g, 67.5 mmol) in a mixture of 1,2-dimethoxyethane (100 mL) and H_2O (20 mL) was stirred at reflux temperature for 4 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9:1–7:3 *n*-heptane/ethyl acetate gradient) to afford the title compound **14b** as a white solid (1.43 g, 4.04 mmol, 13.2% in two steps).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.45 (s, 3H), 3.74 (s, 6H), 4.52 (s, 2H), 6.68 (s, 2H), 7.64 (d, J = 9.1 Hz, 1H), 7.77 (dd, J = 8.3, 7.6 Hz, 1H), 8.35 (d, J = 7.6 Hz, 1H), 8.49 (d, J = 8.3 Hz, 1H), 8.98 (d, J = 9.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 56.0, 58.2, 74.8, 103.3, 117.3, 120.1, 124.1, 127.0, 127.6, 131.1, 136.7, 141.4, 145.6, 148.4, 157.1, 158.1; IR (KBr): $\tilde{\nu}$ = 2942, 2842, 1611, 1578, 1524, 1457, 1417, 1332, 1229, 1116, 1098, 825 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{19}H_{19}N_2O_5^+$: 355.1289, found: 355.1291.

5.1.18. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3-fluoro-5-nitroquinoline (14c)

The title compound was prepared from **13c** using a method analogous to that described for **14b** in 99.0% yield as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.45 (s, 3H), 3.75 (s, 6H), 4.53 (s, 2H), 6.69 (s, 2H), 7.75 (dd, J = 8.3, 7.9 Hz, 1H), 8.47 (d, J = 7.9 Hz, 1H), 8.51 (d, J = 8.3 Hz, 1H), 8.78 (d, J = 10.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 56.0, 58.3, 74.7, 103.1, 111.4, 115.4, 122.1, 125.5, 126.1, 136.9, 142.4, 144.8, 145.3, 149.1, 157.5, 158.5; IR (KBr): $\tilde{\nu}$ = 2935, 1582, 1527, 1461, 1413, 1340, 1231, 1129, 1105, 817 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{19}H_{18}FN_2O_5^+$: 373.1194, found: 373.1183.

5.1.19. 3-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-5-nitroquinoline (14d)

A mixture of **13d** (1.55 g, 4.63 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (1.57 g, 6.95 mmol), 1 M aqueous Na_2CO_3 (9.26 mL, 9.26 mmol), and $Pd(PPh_3)_4$ (535 mg, 0.463 mmol) in a mixture of toluene (20 mL) and ethanol (10 mL) was stirred at 100 °C for 2 h. The reaction mixture was cooled to room temperature and extracted into ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (3:1–1:2 *n*-heptane/ethyl acetate gradient) to afford the title compound **14d** as a light yellow solid (772 mg, 1.99 mmol, 42.9%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.46 (s, 3H), 3.74 (s, 6H), 4.54 (s, 2H), 6.68 (s, 2H), 7.77 (dd, J = 8.3, 7.6 Hz, 1H), 8.44 (d, J = 7.6 Hz, 1H), 8.48 (d, J = 8.3 Hz, 1H), 9.16 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 56.0, 58.3, 74.8, 103.2, 114.8, 121.1, 125.3, 127.2, 131.1, 134.3, 136.9, 141.9, 144.4, 146.5, 156.2, 158.1; IR (KBr): $\tilde{\nu}$ = 2926, 2850, 1583, 1528, 1417, 1348, 1232, 1123, 1094, 1079, 818, 736 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{19}H_{18}ClN_2O_5^+$: 389.0899, found: 389.0886.

5.1.20. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3-ethyl-5-nitroquinoline (14e)

The title compound was prepared from **13e** using a method analogous to that described for **14b** in 53.0% yield as a light brown oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.18 (t, J = 7.5 Hz, 3H), 2.60 (q, J = 7.5 Hz, 2H), 3.47 (s, 3H), 3.71 (s, 6H), 4.53 (s, 2H), 6.68 (s, 2H), 7.69 (dd, J = 8.3, 7.6 Hz, 1H), 8.33 (d, J = 7.6 Hz, 1H), 8.45 (d, J = 8.3 Hz, 1H), 8.83 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 13.8, 26.0, 55.8, 58.3, 74.9, 103.3, 116.3, 121.0, 124.1, 126.0, 129.3, 136.5, 141.0, 141.2, 145.2, 146.9, 157.9, 158.2; IR (KBr): $\tilde{\nu}$ = 2963, 2933, 2838, 1609, 1581, 1522, 1456, 1414, 1336, 1238, 1123, 1092, 824 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{21}H_{23}N_2O_5^+$: 383.1607, found: 383.1602.

5.1.21. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3-methylquinolin-5-amine (15a)

To a solution of **14a** (2.56 g, 6.95 mmol) in ethyl acetate (70 mL) was added 10% Pd-C (2.00 g) and the mixture was stirred under an atmosphere of hydrogen at room temperature for 2 h. The reaction mixture was filtered through Celite and the filtrate was concentrated under reduced pressure to afford the crude title compound

15a as a light yellow solid (2.33 g, 6.89 mmol, 99.1%), which was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.22 (s, 3H), 3.45 (s, 3H), 3.70 (s, 6H), 4.51 (s, 2H), 6.66 (s, 2H), 6.77 (d, J = 7.2 Hz, 1H), 7.41 (dd, J = 8.3, 7.2 Hz, 1H), 7.62 (d, J = 8.3 Hz, 1H), 7.97 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.4, 55.9, 58.2, 75.0, 103.5, 109.8, 117.6, 118.3, 120.4, 128.5, 128.8, 130.1, 140.3, 141.5, 147.6, 156.3, 157.9; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{20}H_{23}N_2O_3^+$: 339.1703, found: 339.1694.

5.1.22. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]quinolin-5-amine (15b)

A mixture of **14b** (1.30 g, 3.67 mmol) and Fe (1.64 g, 29.4 mmol) in a mixture of EtOH (26 mL) and saturated aqueous NH_4Cl (2.60 mL) was stirred at reflux temperature for 1 h. The reaction mixture was cooled to room temperature and filtered through a pad of NH-silica gel with ethyl acetate as the eluent to yield the crude title compound **15b** as a brown solid (1.18 g, 3.64 mmol, 99.1%), which was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.43 (s, 3H), 3.72 (s, 6H), 4.17 (br s, 2H), 4.51 (s, 2H), 6.65 (s, 2H), 6.80 (d, J = 7.2 Hz, 1H), 7.34 (d, J = 8.7 Hz, 1H), 7.48 (dd, J = 8.3, 7.2 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 8.17 (d, J = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 56.0, 58.1, 74.9, 103.5, 109.8, 117.4, 118.7, 120.6, 122.8, 128.9, 129.5, 140.5, 142.2, 149.1, 155.2, 158.3; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{19}H_{21}N_2O_3^+$: 325.1547, found: 325.1547.

5.1.23. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3-fluoroquinolin-5-amine (15c)

The crude title compound was prepared from **14c** using a method analogous to that described for **15b** in quantitative yield as a light yellow solid, which was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.43 (s, 3H), 3.74 (s, 6H), 4.52 (s, 2H), 6.66 (s, 2H), 6.83 (d, J = 7.6 Hz, 1H), 7.44 (dd, J = 8.3, 7.6 Hz, 1H), 7.66 (d, J = 8.3 Hz, 1H), 7.80 (d, J = 9.8 Hz, 1H); HRMS-ESI m/z [M+H]$^+$ calcd for $C_{19}H_{20}FN_2O_3^+$: 343.1453, found: 343.1445.

5.1.24. 3-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]quinolin-5-amine (15d)

The title compound was prepared from **14d** using a method analogous to that described for **15b** in 96.8% yield as a light yellow foam, which was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.44 (s, 3H), 3.73 (s, 6H), 4.13 (br s, 2H), 4.53 (s, 2H), 6.66 (s, 2H), 6.81 (d, J = 7.6 Hz, 1H), 7.47 (dd, J = 8.7, 7.6 Hz, 1H), 7.62 (d, J = 8.7 Hz, 1H), 8.22 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 56.0, 58.1, 74.9, 103.3, 110.6, 116.0, 118.7, 120.4, 128.6, 128.9, 129.7, 141.1, 141.4, 147.3, 153.7, 158.2; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{19}H_{20}ClN_2O_3^+$: 359.1157, found: 359.1146.

5.1.25. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-3-ethylquinolin-5-amine (15e)

The title compound was prepared from **14e** using a method analogous to that described for **15b** in 97.6% yield as a yellow solid, which was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.14 (t, J = 7.4 Hz, 3H), 2.54 (q, J = 7.4 Hz, 2H), 3.45 (s, 3H), 3.69 (s, 6H), 4.51 (s, 2H), 6.65 (s, 2H), 6.78 (d, J = 7.2 Hz, 1H), 7.41 (dd, J = 7.9, 7.2 Hz, 1H), 7.63 (d, J = 7.9 Hz, 1H), 7.99 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 14.1, 25.7, 55.8, 58.2, 75.0, 103.4, 109.8, 117.5, 118.4, 120.5, 127.2, 128.5, 135.8, 140.2, 141.6, 147.6, 156.0, 158.1; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{21}H_{25}N_2O_3^+$: 353.1860, found: 353.1866.

5.1.26. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-methylquinolin-5-amine (16a)

A mixture of **15a** (4.00 g, 11.8 mmol), acetaldehyde (1.99 mL, 35.4 mmol), and $NaBH(OAc)_3$ (7.50 g, 35.4 mmol) in a mixture of THF (100 mL) and AcOH (10 mL) was stirred at room temperature for 4.5 h. The mixture was concentrated under reduced pressure. The residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **16a** as a light yellow oil (4.08 g, 10.3 mmol, 87.6%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.08 (t, J = 6.9 Hz, 6H), 2.23 (s, 3H), 3.22 (q, J = 6.9 Hz, 4H), 3.45 (s, 3H), 3.71 (s, 6H), 4.52 (s, 2H), 6.66 (s, 2H), 7.12 (d, J = 7.2 Hz, 1H), 7.52 (dd, J = 8.3, 7.2 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 8.39 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 19.4, 47.8, 55.9, 58.2, 75.0, 103.5, 117.8, 124.7, 125.7, 127.4, 130.5, 131.9, 140.2, 147.2, 148.2, 156.0, 158.0; IR (KBr): $\tilde{\nu}$ = 2963, 2807, 1582, 1454, 1415, 1379, 1240, 1123, 1096, 823 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_3^+$: 395.2329, found: 395.2315.

5.1.27. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethylquinolin-5-amine (16b)

The title compound was prepared from **15b** using a method analogous to that described for **16a** in 71.7% yield as a light yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.08 (t, J = 7.2 Hz, 6H), 3.22 (q, J = 7.2 Hz, 4H), 3.44 (s, 3H), 3.73 (s, 6H), 4.51 (s, 2H), 6.66 (s, 2H), 7.16 (d, J = 7.6 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.59 (dd, J = 8.7, 7.6 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 8.60 (d, J = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.3, 47.8, 56.0, 58.1, 74.9, 103.5, 117.9, 118.8, 123.3, 124.8, 125.0, 128.5, 132.2, 140.4, 148.0, 149.6, 154.9, 158.3; IR (KBr): $\tilde{\nu}$ = 2970, 2933, 2838, 1607, 1582, 1456, 1415, 1230, 1124, 823 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{23}H_{29}N_2O_3^+$: 381.2173, found: 381.2174.

5.1.28. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-fluoroquinolin-5-amine (16c)

The title compound was prepared from **15c** using a method analogous to that described for **16a** in 71.5% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 6.9 Hz, 6H), 3.20 (q, J = 6.9 Hz, 4H), 3.44 (s, 3H), 3.75 (s, 6H), 4.53 (s, 2H), 6.67 (s, 2H), 7.21 (d, J = 7.2 Hz, 1H), 7.57 (dd, J = 8.7, 7.2 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H), 8.24 (d, J = 10.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.3, 47.9, 56.0, 58.1, 74.8, 103.3, 112.9, 115.5, 119.0, 124.9, 127.2, 127.5, 141.4, 146.2, 146.5, 147.6, 155.4, 158.7; IR (KBr): $\tilde{\nu}$ = 2964, 2934, 2822, 1583, 1459, 1409, 1359, 1230, 1128, 1101, 819 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{23}H_{28}FN_2O_3^+$: 399.2079, found: 399.2066.

5.1.29. 3-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethylquinolin-5-amine (16d)

The title compound was prepared from **15d** using a method analogous to that described for **16a** in 68.7% yield as a light yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.08 (t, J = 6.9 Hz, 6H), 3.21 (q, J = 6.9 Hz, 4H), 3.44 (s, 3H), 3.74 (s, 6H), 4.53 (s, 2H), 6.67 (s, 2H), 7.19 (d, J = 7.2 Hz, 1H), 7.59 (dd, J = 8.3, 7.2 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 8.65 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 47.9, 56.0, 58.1, 74.9, 103.3, 116.1, 119.0, 124.7, 126.3, 128.7, 129.4, 131.8, 141.0, 147.3, 147.8, 153.5, 158.2; IR (KBr): $\tilde{\nu}$ = 2928, 2837, 1582, 1461, 1415, 1361, 1228, 1126, 1103, 1069, 818 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{23}H_{28}ClN_2O_3^+$: 415.1783, found: 415.1766.

5.1.30. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*,3-triethylquinolin-5-amine (16e)

The title compound was prepared from **15e** using a method analogous to that described for **16a** in 69.6% yield as a light yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.10 (t, J = 7.1 Hz, 6H), 1.16 (t, J = 7.5 Hz, 3H), 2.54 (q, J = 7.5 Hz, 2H), 3.23 (q, J = 7.1 Hz, 4H), 3.45 (s, 3H), 3.70 (s, 6H), 4.52 (s, 2H), 6.65 (s, 2H), 7.12 (d, J = 7.6 Hz, 1H), 7.52 (dd, J = 8.3, 7.6 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 8.42 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.3, 14.1, 25.6, 47.8, 55.8, 58.2, 75.0, 103.5, 117.6, 117.7, 124.5, 125.7, 127.5, 130.2, 136.2, 140.1, 147.4, 148.1, 155.6, 158.1; IR (KBr): $\tilde{\nu}$ = 2967, 2933, 2871, 2838, 1610, 1582, 1456, 1415, 1230, 1123, 824 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{25}H_{33}N_2O_3^+$: 409.2486, found: 409.2487.

5.1.31. 8-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-methylquinolin-5-amine (17)

To a solution of **16a** (155 mg, 0.391 mmol) in DMF (3 mL) was added NCS (52.2 mg, 0.391 mmol) and the mixture was stirred at 60 °C for 2 h. The reaction mixture was quenched with H_2O and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–6:4 *n*-heptane/ethyl acetate gradient) to afford the title compound **17** as a colorless foam (4.1 mg, 9.56 μmol, 2.44%). Compound purity: 95%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 7.2 Hz, 6H), 2.23 (s, 3H), 3.20 (q, J = 7.2 Hz, 4H), 3.47 (s, 3H), 3.73 (s, 6H), 4.51 (s, 2H), 6.67 (s, 2H), 7.03 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 8.39 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 19.4, 47.8, 56.3, 58.3, 75.0, 104.1, 117.9, 118.2, 127.1, 127.4, 127.9, 131.6, 132.2, 140.3, 144.1, 146.4, 156.7, 158.2; IR (KBr): $\tilde{\nu}$ = 2970, 2840, 1583, 1444, 1416, 1233, 1121, 1062, 828 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{24}H_{30}ClN_2O_3^+$: 429.1940, found: 429.1926.

5.1.32. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-iodo-3-methylquinolin-5-amine (18)

To a solution of **16a** (4.00 g, 10.1 mmol) in DMF (80 mL) was added NIS (2.50 g, 11.1 mmol) at 0 °C and the mixture was stirred at room temperature for overnight. The reaction mixture was quenched with H_2O and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–6:4 *n*-heptane/ethyl acetate gradient) to afford the title compound **18** as a light red oil (3.44 g, 6.61 mmol, 65.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 7.0 Hz, 6H), 2.25 (s, 3H), 3.21 (q, J = 7.0 Hz, 4H), 3.49 (s, 3H), 3.76 (s, 6H), 4.52 (s, 2H), 6.70 (s, 2H), 6.86 (d, J = 7.9 Hz, 1H), 8.12 (d, J = 7.9 Hz, 1H), 8.31 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.1, 19.1, 47.7, 56.7, 58.4, 75.0, 96.9, 104.6, 118.7, 119.3, 126.3, 131.6, 132.2, 137.6, 140.2, 146.6, 148.3, 156.8, 158.3; IR (KBr): $\tilde{\nu}$ = 2968, 2813, 1581, 1433, 1415, 1234, 1120, 1091, 1061, 819 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{24}H_{30}IN_2O_3^+$: 521.1296, found: 521.1281.

5.1.33. 5-(Diethylamino)-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-3-methylquinoline-8-carbaldehyde (19)

To a solution of **18** (700 mg, 1.35 mmol) in THF was added *n*-BuLi (0.665 mL, 1.76 mmol, 2.64 M in *n*-hexane) at −78 °C and the mixture was stirred at the same temperature for 1 h. Next, DMF (0.178 mL, 2.29 mmol) was added at −78 °C and the mixture was stirred at the same temperature for 1 h. The reaction was quenched with AcOH and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9:1–5:5 *n*-heptane/ethyl acetate gradient) to afford the title compound **19** as a yellow oil (199 mg, 471 μmol, 34.9%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.15 (t, J = 6.9 Hz, 6H), 2.26 (s, 3H), 3.37 (q, J = 6.9 Hz, 4H), 3.49 (s, 3H), 3.74 (s, 6H), 4.54 (s, 2H), 6.71 (s, 2H), 7.11 (d, J = 7.9 Hz, 1H), 8.19 (d, J = 7.9 Hz, 1H), 8.29 (s, 1H), 11.25 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 19.5, 47.1, 56.2, 58.4, 75.0, 103.8, 115.9, 118.0, 123.7, 126.3, 128.2, 130.8, 132.2, 140.5, 148.1, 153.9, 156.8, 158.0, 193.1; IR (KBr): $\tilde{\nu}$ = 2958, 2932, 2837, 1667, 1563, 1453, 1414, 1225, 1121, 1097, 1068, 807 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{25}H_{31}N_2O_4^+$: 423.2279, found: 423.2263.

5.1.34. 8-(Difluoromethyl)-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*,-diethyl-3-methylquinolin-5-amine (20a)

A mixture of **19** (90 mg, 0.213 mmol) and [bis(2-methoxyethyl)amino]sulfur trifluoride (0.118 mL, 0.639 mmol) in DCM (3 mL) was stirred at room temperature for overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted into DCM. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (10:0–6:4 *n*-heptane/ethyl acetate gradient) to afford the title compound **20a** as a light yellow oil (33.1 mg, 74.5 μmol, 35.0%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.10 (t, J = 7.0 Hz, 6H), 2.24 (s, 3H), 3.27 (q, J = 7.0 Hz, 4H), 3.49 (s, 3H), 3.73 (s, 6H), 4.53 (s, 2H), 6.70 (s, 2H), 7.14 (d, J = 7.8 Hz, 1H), 7.80 (t, J = 36.0 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 8.34 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = δ = 12.1, 19.4, 47.4, 56.2, 58.4, 75.0, 104.0, 112.9, 116.7, 118.4, 124.7, 124.8, 126.2, 131.0, 131.9, 140.3, 145.4, 149.7, 156.0, 158.1; IR (KBr): $\tilde{\nu}$ = 2971, 2933, 2840, 1612, 1579, 1456, 1415, 1376, 1228, 1123, 1093, 1014, 823 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{25}H_{31}F_2N_2O_3^+$: 445.2297, found: 445.2282.

5.1.35. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-(methoxymethyl)-3-methylquinolin-5-amine (20b)

A mixture of **19** (90 mg, 0.213 mmol) and decaborane (23 mg, 0.213 mmol) in MeOH (3 mL) was stirred at room temperature for overnight. The reaction mixture was concentrated under reduced pressure and the residue was extracted into ethyl acetate. The organic layer was concentrated and the residue was purified by silica gel column chromatography (10:0–7:3 *n*-heptane/ethyl acetate gradient) to afford the title compound **20b** as a light yellow oil (33.1 mg, 75.5 μmol, 35.4%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 7.1 Hz, 6H), 2.22 (s, 3H), 3.20 (q, J = 7.1 Hz, 4H), 3.49 (s, 3H), 3.50 (s, 3H), 3.72 (s, 6H), 4.53 (s, 2H), 5.13 (s, 2H), 6.69 (s, 2H), 7.13 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 8.37 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 19.4, 47.8, 56.2, 58.4, 58.6, 70.7, 75.1, 104.0, 117.7, 118.9, 125.2, 125.3, 130.3, 131.7, 131.8, 140.0, 145.7, 146.3, 154.6, 158.2; IR (KBr): $\tilde{\nu}$ = 2969, 2930, 2818, 1580, 1455, 1415, 1375, 1228, 1122, 1066, 825 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{26}H_{35}N_2O_4^+$: 439.2592, found: 439.2579.

5.1.36. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-fluoro-3-methylquinolin-5-amine (21a)

To a solution of **18** (250 mg, 0.481 mmol) in THF was added *n*-BuLi (0.260 mL, 0.722 mmol, 2.77 M in *n*-hexane) at −78 °C and the mixture was stirred at the same temperature for 1 h. After a solution of *N*-fluorobenzenesulfonimide (303 mg, 0.962 mmol) in THF (5 mL) was added to the reaction mixture, the mixture was stirred at the same temperature for 2 h. The mixture was quenched with AcOH and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by NH-silica gel column chromatography (9:1–5:5 *n*-heptane/ethyl acetate

gradient) to afford the title compound **21a** as a colorless oil (3.2 mg, 7.76 μmol, 1.6%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.06 (t, J = 7.0 Hz, 6H), 2.24 (s, 3H), 3.28 (q, J = 7.0 Hz, 4H), 3.47 (s, 3H), 3.73 (s, 6H), 4.53 (s, 2H), 6.68 (s, 2H), 7.33 (dd, J = 11.5, 9.2 Hz, 1H), 7.94 (dd, J = 9.2, 4.5 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 13.5, 19.4, 48.1, 55.9, 58.2, 75.0, 103.5, 117.5, 118.4, 128.1, 129.2, 130.1, 131.7, 132.3, 140.3, 144.5, 155.4, 158.0, 158.4; IR (KBr): $\tilde{\nu}$ = 2975, 2925, 2850, 1609, 1580, 1463, 1415, 1382, 1225, 1123, 1097, 990, 823 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{30}FN_2O_3^+$: 413.2235, found: 413.2233.

5.1.37. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-methyl-8-(trifluoromethyl)quinolin-5-amine (21b)

A mixture of **18** (100 mg, 0.192 mmol), methyl fluorosulphonyldifluoroacetate (0.073 mL, 0.577 mmol), and copper (36.7 mg, 0.577 mmol) in DMF (2.14 mL) was stirred at 90 °C for 1.5 h. The reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9:1–7:3 *n*-heptane/ethyl acetate gradient) to afford the title compound **21b** as a light yellow foam (68.0 mg, 147 μmol, 76.6%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.11 (t, J = 6.9 Hz, 6H), 2.26 (s, 3H), 3.28 (q, J = 6.9 Hz, 4H), 3.49 (s, 3H), 3.73 (s, 6H), 4.52 (s, 2H), 6.70 (s, 2H), 7.03 (d, J = 7.9 Hz, 1H), 7.84 (d, J = 7.9 Hz, 1H), 8.33 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.0, 19.4, 47.3, 56.5, 58.4, 75.0, 104.6, 115.2, 118.9, 122.0, 124.6, 125.2, 126.2, 131.3, 131.8, 140.2, 144.8, 151.3, 156.3, 158.2; IR (KBr): $\tilde{\nu}$ = 2977, 2929, 2844, 1614, 1578, 1457, 1417, 1376, 1328, 1237, 1120, 1106, 822 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{25}H_{30}F_3N_2O_3^+$: 463.2203, found: 463.2189.

5.1.38. 5-(Diethylamino)-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-3-methylquinoline-8-carbonitrile (21c)

A mixture of **18** (100 mg, 0.192 mmol) and CuCN (22.4 mg, 0.25 mmol) in DMF (2 mL) was stirred at 80 °C for overnight. The reaction mixture was cooled to room temperature, quenched with H_2O, and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–7:3 *n*-heptane/ethyl acetate gradient) to afford the title compound **21c** as a light yellow foam (39.3 mg, 93.7 μmol, 48.8%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.13 (t, J = 7.1 Hz, 6H), 2.26 (s, 3H), 3.33 (q, J = 7.1 Hz, 4H), 3.48 (s, 3H), 3.75 (s, 6H), 4.52 (s, 2H), 6.69 (s, 2H), 7.01 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 7.9 Hz, 1H), 8.27 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.1, 19.5, 47.2, 56.5, 58.3, 74.9, 104.2, 106.1, 115.6, 118.0, 118.8, 124.3, 132.1, 132.1, 134.3, 140.5, 147.8, 152.4, 158.1, 158.1; IR (KBr): $\tilde{\nu}$ = 2970, 2871, 2220, 1569, 1463, 1416, 1378, 1244, 1223, 1120, 1101, 1066, 821 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{25}H_{30}N_3O_3^+$: 420.2282, found: 420.2267.

5.1.39. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methoxy-3-methylquinolin-5-amine (21d)

A mixture of **18** (120 mg, 0.230 mmol), CuBr (16.6 mg, 0.115 mmol), NaOMe (0.922 mL, 4.61 mmol, 28% methanol solution), and ethyl acetate (0.0227 mL, 0.23 mmol) was stirred at reflux temperature for 3 h. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted into ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by NH-silica gel column chromatography (9:1–4:6 *n*-heptane/ethyl acetate gradient) to afford the title compound **21d** as a light yellow solid (81.0 mg, 191 μmol, 83.0%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.03 (t, J = 7.0 Hz, 6H), 2.20 (s, 3H), 3.13 (q, J = 7.0 Hz, 4H), 3.44 (s, 3H), 3.69 (s, 6H), 3.99 (s, 3H), 4.50 (s, 2H), 6.62 (s, 2H), 6.89 (d, J = 8.1 Hz, 1H), 7.09 (d, J = 8.1 Hz, 1H), 8.42 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 19.5, 48.4, 55.9, 56.0, 58.1, 75.0, 103.6, 105.9, 118.3, 118.4, 127.4, 131.3, 131.8, 139.5, 139.6, 139.9, 152.3, 154.9, 158.3; IR (KBr): $\tilde{\nu}$ = 2962, 2835, 1582, 1462, 1415, 1240, 1124, 1090, 1065, 825 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{25}H_{33}N_2O_4^+$: 425.2435, found: 425.2415.

5.1.40. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-iodoquinolin-5-amine (22b)

The title compound was prepared from **16b** using a method analogous to that described for **18** in 79.2% yield as a light yellow oil.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 7.1 Hz, 6H), 3.21 (q, J = 7.1 Hz, 4H), 3.47 (s, 3H), 3.81 (s, 6H), 4.52 (s, 2H), 6.71 (s, 2H), 6.90 (d, J = 7.9 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 7.9 Hz, 1H), 8.50 (d, J = 8.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 47.8, 56.8, 58.3, 74.9, 97.1, 104.6, 119.3, 119.4, 124.4, 125.8, 132.3, 138.6, 140.6, 147.9, 149.0, 155.6, 158.6; IR (KBr): $\tilde{\nu}$ = 2975, 2932, 2824, 1579, 1455, 1415, 1401, 1355, 1232, 1127, 1099, 1065, 822 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{23}H_{28}IN_2O_3^+$: 507.1139, found: 507.1137.

5.1.41. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-fluoro-8-iodoquinolin-5-amine (22c)

The title compound was prepared from **16c** using a method analogous to that described for **18** in 75.3% yield as a light yellow foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.06 (t, J = 6.6 Hz, 6H), 3.18 (q, J = 6.6 Hz, 4H), 3.47 (s, 3H), 3.80 (s, 6H), 4.53 (s, 2H), 6.70 (s, 2H), 6.86–6.99 (m, 1H), 8.12–8.27 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 47.8, 56.6, 58.4, 74.9, 96.6, 104.2, 113.5, 115.7, 120.3, 127.5, 137.8, 141.5, 144.8, 146.9, 148.7, 155.9, 159.0; IR (KBr): $\tilde{\nu}$ = 2972, 2936, 2843, 1584, 1455, 1408, 1235, 1123, 1090, 908, 824 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{23}H_{27}FIN_2O_3^+$: 525.1045, found: 525.1056.

5.1.42. 3-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-iodoquinolin-5-amine (22d)

The title compound was prepared from **16d** using a method analogous to that described for **18** in 76.7% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.07 (t, J = 7.2 Hz, 6H), 3.20 (q, J = 7.2 Hz, 4H), 3.48 (s, 3H), 3.78 (s, 6H), 4.53 (s, 2H), 6.70 (s, 2H), 6.90 (d, J = 7.9 Hz, 1H), 8.19 (d, J = 7.9 Hz, 1H), 8.58 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.1, 47.8, 56.6, 58.4, 75.0, 96.4, 104.2, 116.7, 120.3, 126.7, 130.5, 132.0, 138.9, 141.0, 146.3, 148.3, 154.2, 158.5; IR (KBr): $\tilde{\nu}$ = 2970, 2932, 2838, 1579, 1453, 1415, 1381, 1235, 1125, 1091, 1070, 824 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{23}H_{27}ClIN_2O_3^+$: 541.0749, found: 541.0732.

5.1.43. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*,3-triethyl-8-iodoquinolin-5-amine (22e)

The title compound was prepared from **16e** using a method analogous to that described for **18** in 76.7% yield as a light yellow foam.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.10 (t, J = 6.8 Hz, 6H), 1.16 (t, J = 7.2 Hz, 3H), 2.58 (q, J = 7.2 Hz, 2H), 3.23 (q, J = 6.8 Hz, 4H), 3.51 (s, 3H), 3.76 (s, 6H), 4.54 (s, 2H), 6.71 (s, 2H), 6.87 (d, J = 7.6 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 8.35 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.2, 14.2, 25.3, 47.7, 56.6, 58.5, 75.1, 96.8, 104.6, 118.6, 119.0, 126.4, 130.5, 137.2, 137.6, 140.1, 146.5, 148.5, 156.4, 158.4; IR (KBr): $\tilde{\nu}$ = 2962, 2933, 2814, 1579, 1454,

1412, 1230, 1120, 817 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{25}H_{32}IN_2O_3^+$: 535.1452, found: 535.1445.

5.1.44. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methoxyquinolin-5-amine (23ba)

The title compound was prepared from **22b** using a method analogous to that described for **21d** in 95.2% yield as a light yellow solid. Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.03 (t, J = 6.7 Hz, 6H), 3.13 (q, J = 6.7 Hz, 4H), 3.43 (s, 3H), 3.72 (s, 6H), 4.02 (s, 3H), 4.50 (s, 2H), 6.63 (s, 2H), 6.97 (d, J = 8.1 Hz, 1H), 7.13 (d, J = 8.1 Hz, 1H), 7.38 (d, J = 8.7 Hz, 1H), 8.64 (d, J = 8.7 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.5, 48.5, 56.0, 56.1, 58.1, 74.9, 103.7, 107.1, 118.4, 119.6, 124.2, 126.7, 132.1, 140.1, 140.2, 141.0, 152.3, 153.8, 158.6; IR (KBr): $\tilde{\nu}$ = 2961, 2932, 2809, 1608, 1579, 1455, 1414, 1378, 1254, 1238, 1225, 1122, 1105, 984, 820 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{31}N_2O_4^+$: 411.2279, found: 411.2277.

5.1.45. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-fluoro-8-methoxyquinolin-5-amine (23ca)

The title compound was prepared from **22c** using a method analogous to that described for **21d** in 61.3% yield as a light yellow solid. Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.02 (t, J = 7.1 Hz, 6H), 3.10 (q, J = 7.1 Hz, 4H), 3.43 (s, 3H), 3.73 (s, 6H), 4.01 (s, 3H), 4.51 (s, 2H), 6.63 (s, 2H), 6.92 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 8.1 Hz, 1H), 8.28 (d, J = 10.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.5, 48.6, 56.0, 56.0, 58.1, 74.9, 103.4, 106.1, 113.3, 115.4, 119.9, 129.4, 137.8, 139.7, 141.1, 144.9, 152.5, 156.1, 159.0; IR (KBr): $\tilde{\nu}$ = 2971, 2819, 1586, 1454, 1407, 1321, 1127, 1097, 826 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{30}FN_2O_4^+$: 429.2184, found: 429.2168.

5.1.46. 3-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methoxyquinolin-5-amine (23da)

The title compound was prepared from **22d** using a method analogous to that described for **21d** in 76.2% yield as a light yellow solid. Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.02 (t, J = 7.1 Hz, 6H), 3.11 (q, J = 7.1 Hz, 4H), 3.43 (s, 3H), 3.72 (s, 6H), 4.00 (s, 3H), 4.51 (s, 2H), 6.63 (s, 2H), 6.96 (d, J = 8.1 Hz, 1H), 7.16 (d, J = 8.1 Hz, 1H), 8.69 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.5, 48.6, 56.0, 58.1, 74.9, 103.4, 107.2, 116.7, 119.8, 128.2, 130.7, 131.7, 139.1, 139.4, 140.7, 152.3, 152.3, 158.5; IR (KBr): $\tilde{\nu}$ = 2970, 2926, 2837, 1607, 1583, 1453, 1416, 1358, 1310, 1236, 1125, 1108, 1093, 907, 825 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{30}ClN_2O_4^+$: 445.1889, found: 445.1875.

5.1.47. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*,3-triethyl-8-methoxyquinolin-5-amine (23ea)

The title compound was prepared from **22e** using a method analogous to that described for **21d** in 65.4% yield as a white solid. Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.04 (t, J = 6.7 Hz, 6H), 1.14 (t, J = 7.4 Hz, 3H), 2.51 (q, J = 7.4 Hz, 2H), 3.14 (d, J = 6.7 Hz, 4H), 3.45 (s, 3H), 3.68 (s, 6H), 3.99 (s, 3H), 4.50 (s, 2H), 6.62 (s, 2H), 6.89 (d, J = 8.1 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.5, 14.1, 25.6, 48.4, 55.9, 55.9, 58.2, 75.1, 103.6, 106.0, 118.0, 118.3, 127.5, 130.2, 136.9, 139.5, 139.7, 139.8, 152.2, 154.5, 158.4; IR (KBr): $\tilde{\nu}$ = 2968, 2927, 2835, 1580, 1455, 1416, 1320, 1261, 1236, 1123, 1097, 826 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{26}H_{35}N_2O_4^+$: 439.2592, found: 439.2587.

5.1.48. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methylquinolin-5-amine (23bb)

A mixture of **22b** (100 mg, 197 μmol), Me_2Zn (394 μL, 394 μmol, 1.0 M in n-hexane), and $(t\text{-}Bu_3P)_2Pd$ (5.03 mg, 9.90 μmol) in 1,4-dioxane (2.5 mL) was stired at 80 °C for 1 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into DCM and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9:1–5:5 n-heptane/ethyl acetate gradient) to afford the title compound **23bb** as a yellow oil (73.7 mg, 187 μmol, 94.8%). Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.05 (t, J = 7.0 Hz, 6H), 2.74 (s, 3H), 3.17 (q, J = 7.0 Hz, 4H), 3.46 (s, 3H), 3.76 (s, 6H), 4.52 (s, 2H), 6.69 (s, 2H), 7.07 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.44 (d, J = 7.6 Hz, 1H), 8.60 (d, J = 8.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 18.2, 48.1, 56.4, 58.3, 74.9, 104.2, 117.8, 119.9, 123.2, 125.1, 128.3, 132.0, 132.4, 140.2, 145.9, 148.4, 153.4, 158.5; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{24}H_{31}N_2O_3^+$: 395.2329, found: 395.2329.

5.1.49. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-3-fluoro-8-methylquinolin-5-amine (23cb)

The title compound was prepared from **22c** using a method analogous to that described for **23bb** in 84.6% yield as a yellow oil. Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.05 (t, J = 7.1 Hz, 6H), 2.73 (s, 3H), 3.15 (q, J = 7.1 Hz, 4H), 3.47 (s, 3H), 3.77 (s, 6H), 4.53 (s, 2H), 6.70 (s, 2H), 7.12 (d, J = 7.6 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 8.25 (d, J = 10.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 18.2, 48.1, 56.3, 58.3, 74.9, 103.8, 114.0, 115.3, 119.2, 127.4, 127.5, 132.8, 141.1, 144.5, 145.3, 145.5, 155.4, 158.9; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{24}H_{30}FN_2O_3^+$: 413.2235, found: 413.2220.

5.1.50. 3-Chloro-2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methylquinolin-5-amine (23db)

The title compound was prepared from **22d** using a method analogous to that described for **23bb** in 98.3% yield as a yellow oil. Compound purity: >99%.

^1H NMR (600 MHz, $CDCl_3$): δ = 1.05 (t, J = 7.0 Hz, 6H), 2.70 (s, 3H), 3.16 (q, J = 7.0 Hz, 4H), 3.48 (s, 3H), 3.75 (s, 6H), 4.54 (s, 2H), 6.69 (s, 2H), 7.10 (d, J = 7.6 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 8.65 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.3, 18.1, 48.1, 56.3, 58.3, 75.0, 103.8, 117.3, 119.0, 126.6, 128.6, 129.3, 131.7, 132.6, 140.7, 145.2, 146.6, 151.9, 158.5; HRMS (ESI+): m/z $[M+H]_+$ calcd for $C_{24}H_{30}ClN_2O_3^+$: 429.1940, found: 429.1924.

5.1.51. 2-Chloro-8-methoxy-3-methylquinoline (25)

To $POCl_3$ (227 mL, 2.44 mol) was slowly added DMF (37.7 mL, 487 mmol) at 0 °C and the mixture was stirred at the same temperature for 15 min. To the mixture was added **24** (72.8 g, 0.406 mol) and the mixture was stirred at 70 °C for 2.5 h. The reaction mixture was cooled to room temperature, quenched with crushed-ice, neutralized with 5 M aqueous NaOH, and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (8:2–7:3 n-heptane/ethyl acetate gradient) to afford the title compound **25** as a white solid (54.8 g, 264 mmol, 65.0%).

^1H NMR (600 MHz, $CDCl_3$): δ = 2.53 (s, 3H), 4.05 (s, 3H), 7.01 (d, J = 7.6 Hz, 1H), 7.31 (d, J = 7.9 Hz, 1H), 7.43 (dd, J = 7.9, 7.6 Hz, 1H), 7.94 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 20.1, 56.0, 107.8, 118.5, 127.1, 128.8, 130.9, 137.8, 138.2, 151.1, 154.5; IR (KBr): $\tilde{\nu}$ = 3006, 2841, 1569, 1486, 1466, 1435, 1342, 1268, 1198, 1184, 1114, 1031, 761 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{11}H_{11}ClNO^+$: 208.0524, found: 208.0518.

5.1.52. 2-Chloro-8-methoxy-3-methyl-5-nitroquinoline (26)

To a solution of **25** (16.5 g, 79.5 mmol) in H_2SO_4 (80 mL) was added HNO_3 (5.29 mL, 119 mmol) at −10 °C and the mixture was stirred at room temperature for 3.5 h. The mixture was quenched with crushed-ice, filtered, and washed with water. The obtained

crude product was purified by NH-silica gel column chromatography (ethyl acetate) to afford the title compound **26** as a light yellow solid (6.90 g, 27.3 mmol, 34.4%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.63 (s, 3H), 4.17 (s, 3H), 7.04 (d, J = 8.7 Hz, 1H), 8.51 (d, J = 8.7 Hz, 1H), 9.08 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 20.7, 56.9, 105.6, 122.4, 127.8, 134.5, 134.9, 137.1, 137.6, 152.6, 160.0; IR (KBr): $\tilde{\nu}$ = 1557, 1487, 1311, 1266, 1108, 1060, 937, 822 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{11}H_{10}ClN_2O_3^+$: 253.0375, found: 253.0361.

5.1.53. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methyl-5-nitroquinoline (27)

A mixture of **26** (860 mg, 3.4 mmol), [2,6-Dimethoxy-4-(methoxymethyl)phenyl]boronic acid (1.15 g, 5.1 mmol), $Pd(PPh_3)_4$ (196 mg, 0.17 mmol), and K_2CO_3 (1.41 g, 10.2 mmol) in a mixture of 1,4-dioxane (20 mL) and H_2O (5 mL) was stirred at reflux temperature for 1.5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (8:2–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **27** as a light yellow solid (1.18 g, 2.96 mmol, 87.1%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.26 (s, 3H), 3.45 (s, 3H), 3.70 (s, 6H), 4.13 (s, 3H), 4.50 (s, 2H), 6.64 (s, 2H), 6.98 (d, J = 9.1 Hz, 1H), 8.47 (d, J = 9.1 Hz, 1H), 9.02 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.8, 55.9, 56.8, 58.3, 74.9, 103.4, 104.4, 116.8, 122.7, 127.0, 131.4, 136.2, 137.4, 138.0, 140.8, 156.8, 158.1, 161.2; IR (KBr): $\tilde{\nu}$ = 2926, 2837, 1583, 1550, 1504, 1454, 1308, 1238, 1192, 1126, 1094, 822 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{21}H_{23}N_2O_6^+$: 399.1551, found: 399.1559.

5.1.54. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methylquinolin-5-amine (28)

A mixture of **27** (1.10 g, 2.75 mmol) and Fe (1.23 g, 22.0 mmol) in a mixture of EtOH (20 mL) and saturated aqueous NH_4Cl (2 mL) was stirred at reflux temperature for 1 h. The reaction mixture was cooled to room temperature and filtered through a pad of NH-silica gel with ethyl acetate as the eluent to yield the crude title compound **28** as a light yellow solid (956 mg, 2.59 mmol, 94.4%), which was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.21 (s, 3H), 3.44 (s, 3H), 3.68 (s, 6H), 3.96 (s, 3H), 4.50 (s, 2H), 6.62 (s, 2H), 6.72 (d, J = 8.1 Hz, 1H), 6.81 (d, J = 8.1 Hz, 1H), 8.01 (s, 1H); HRMS-ESI m/z [M+H]$^+$ calcd for $C_{21}H_{25}N_2O_4^+$: 369.1809, found: 369.1798.

5.1.55. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methoxy-3-methylquinolin-5-amine (21d)

A mixture of **28** (100 mg, 0.271 mmol), acetaldehyde (0.0456 mL, 0.813 mmol), and $NaBH(OAc)_3$ (172 mg, 0.813 mmol) in a mixture of THF (2 mL), AcOH (0.2 mL), and MeOH (0.5 mL) was stirred at room temperature for 14.5 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **21d** as a light yellow solid (88 mg, 207 μmol, 76.5%).

5.1.56. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methyl-*N*,*N*-dipropylquinolin-5-amine (29)

A mixture of **28** (120 mg, 0.325 mmol), propionaldehyde (0.0938 mL, 1.3 mmol), and $NaBH(OAc)_3$ (276 mg, 1.3 mmol) in a mixture of THF (3 mL) and AcOH (0.3 mL) was stirred at room temperature for 14.5 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **29** as a white solid (18 mg, 39.8 μmol, 12.2%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.87 (t, J = 7.3 Hz, 6H), 1.43–1.54 (m, 4H), 2.20 (s, 3H), 3.02 (t, J = 7.3 Hz, 4H), 3.44 (s, 3H), 3.70 (s, 6H), 3.99 (s, 3H), 4.50 (s, 2H), 6.63 (s, 2H), 6.88 (d, J = 7.9 Hz, 1H), 7.11 (d, J = 7.9 Hz, 1H), 8.44 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 11.8, 19.6, 20.4, 55.9, 56.0, 56.9, 58.2, 75.0, 103.7, 106.0, 118.4, 127.3, 131.3, 131.8, 139.5, 139.9, 140.3, 152.2, 154.8, 158.3; IR (KBr): $\tilde{\nu}$ = 2956, 2927, 1583, 1454, 1417, 1319, 1237, 1123, 1097, 824 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{27}H_{37}N_2O_4^+$: 453.2748, found: 453.2743.

5.1.57. Tert-butyl *N*-{2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methylquinolin-5-yl}carbamate (30)

A mixture of **28** (500 mg, 1.36 mmol), Boc_2O (1.04 g, 4.76 mmol), and Et_3N (1.32 mL, 9.52 mmol) in DCM (10 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (9:1–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **30** as a white solid (390 mg, 832 μmol, 61.2%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.54 (br s, 9H), 2.20 (s, 3H), 3.44 (s, 3H), 3.67 (s, 6H), 4.01 (s, 3H), 4.49 (s, 2H), 6.40–6.56 (m, 1H), 6.61 (s, 2H), 6.92 (d, J = 8.7 Hz, 1H), 7.44–7.66 (m, 1H), 8.01 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.5, 28.4, 55.9, 56.1, 58.2, 75.0, 80.4, 103.5, 106.1, 117.8, 124.5, 129.4, 132.3, 138.8, 140.1, 153.8, 154.4, 155.3, 158.2; IR (KBr): $\tilde{\nu}$ = 3228, 2928, 2839, 1675, 1584, 1533, 1456, 1269, 1239, 1171, 1126, 1098, 1023, 827 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{26}H_{33}N_2O_6^+$: 469.2333, found: 469.2289.

5.1.58. Tert-butyl *N*-{2-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methylquinolin-5-yl}-*N*-(2-methoxyethyl)carbamate (31)

To a solution of **30** (340 mg, 0.726 mmol) in DMF (7 mL) was added NaH (60% oil dispersion, 43.6 mg, 1.09 mmol) at room temperature and the reaction mixture was stirred at the same temperature for 15 min. To the mixture was added 2-bromoethyl-methyl-ether (0.102 mL, 1.09 mmol) and the resultant mixture was stirred at 40 °C for 1 h. The reaction mixture was cooled to room temperature, quenched with H_2O, and extracted into diethyl-ether. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (9:1–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **31** as a colorless oil (374 mg, 710 μmol, 97.8%). The obtained product contained some impurities but was used in the following reaction without further purification.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.26 (br s, 9H), 2.18 (s, 3H), 3.30 (s, 3H), 3.44 (s, 3H), 3.49–3.56 (m, 2H), 3.67 (s, 3H), 3.69 (s, 3H), 3.73–3.84 (m, 1H), 3.95–4.08 (m, 4H), 4.50 (s, 2H), 6.62 (br s, 2H), 6.91 (br s, 1H), 7.21–7.38 (m, 1H), 7.89 (br s, 1H); HRMS-ESI m/z [M+H]$^+$ calcd for $C_{29}H_{39}N_2O_7^+$: 527.2752, found: 527.2739.

5.1.59. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-*N*-(2-methoxyethyl)-3-methylquinolin-5-amine (32)

A mixture of **31** (324 mg, 0.615 mmol) in a mixture of TFA (3 mL) and DCM (3 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by

silica gel column chromatography (7:3–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **32** as a yellow solid (192 mg, 450 μmol, 73.2%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.22 (s, 3H), 3.40 (t, J = 4.9 Hz, 2H), 3.44 (s, 6H), 3.68 (s, 6H), 3.75 (t, J = 4.9 Hz, 2H), 3.96 (s, 3H), 4.50 (s, 2H), 6.57 (d, J = 8.3 Hz, 1H), 6.62 (s, 2H), 6.89 (d, J = 8.3 Hz, 1H), 8.17 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.4, 44.5, 55.9, 56.3, 58.2, 58.8, 71.0, 75.0, 103.5, 105.3, 107.9, 117.6, 120.3, 129.6, 130.9, 136.9, 138.8, 140.3, 148.2, 154.6, 158.2; IR (KBr): $\tilde{\nu}$ = 3268, 2924, 2836, 1613, 1583, 1456, 1415, 1376, 1271, 1230, 1187, 1126, 1096, 790 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{24}H_{31}N_2O_5^+$: 427.2228, found: 427.2213.

5.1.60. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-*N*-ethyl-8-methoxy-*N*-(2-methoxyethyl)-3-methylquinolin-5-amine (33a)

A mixture of **32** (70 mg, 0.164 mmol), acetaldehyde (0.0184 mL, 0.328 mmol), and $NaBH(OAc)_3$ (104 mg, 0.492 mmol) in a mixture of THF (2 mL) and AcOH (0.2 mL) was stirred at room temperature for 15 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (9:1–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **33a** as a white solid (41.8 mg, 92.0 μmol, 56.1%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.03 (t, J = 6.8 Hz, 3H), 2.20 (s, 3H), 3.19 (q, J = 6.8 Hz, 2H), 3.28 (s, 3H), 3.29 (t, J = 5.9 Hz, 2H), 3.41 (t, J = 5.9 Hz, 2H), 3.44 (s, 3H), 3.69 (s, 6H), 4.00 (s, 3H), 4.50 (s, 2H), 6.63 (s, 2H), 6.89 (d, J = 8.3 Hz, 1H), 7.16 (d, J = 8.3 Hz, 1H), 8.47 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.5, 19.5, 50.0, 54.1, 55.9, 56.0, 58.2, 58.7, 70.8, 75.0, 103.6, 106.0, 118.3, 118.8, 127.5, 131.5, 131.8, 139.3, 139.4, 139.9, 152.6, 155.0, 158.3; IR (KBr): $\tilde{\nu}$ = 2925, 2839, 1582, 1455, 1418, 1374, 1319, 1237, 1121, 1094, 824 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{26}H_{35}N_2O_5^+$: 455.2541, found: 455.2527.

5.1.61. 2-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-8-methoxy-*N*-(2-methoxyethyl)-3-methyl-*N*-(2-methylpropyl)quinolin-5-amine (33b)

A mixture of **32** (50 mg, 0.117 mmol), isobutyraldehyde (12.7 mg, 0.176 mmol), and $NaBH(OAc)_3$ (37.2 mg, 0.176 mmol) in a mixture of THF (0.8 mL) and AcOH (0.08 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the residue was quenched with saturated aqueous $NaHCO_3$ and extracted into ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by NH-silica gel column chromatography (9:1–2:8 *n*-heptane/ethyl acetate gradient) to afford the title compound **33b** as a light yellow oil (29.6 mg, 61.3 μmol, 52.4%). Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.93 (d, J = 6.8 Hz, 6H), 1.72–1.81 (m, 1H), 2.20 (s, 3H), 2.95 (d, J = 6.8 Hz, 2H), 3.23 (t, J = 6.0 Hz, 2H), 3.28 (s, 3H), 3.43 (t, J = 6.0 Hz, 2H), 3.44 (s, 3H), 3.69 (s, 6H), 3.99 (s, 3H), 4.50 (s, 2H), 6.63 (s, 2H), 6.89 (d, J = 7.9 Hz, 1H), 7.18 (d, J = 7.9 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.5, 21.0, 26.7, 55.9, 55.9, 56.0, 58.2, 58.7, 62.8, 70.7, 75.0, 103.6, 106.1, 118.3, 119.0, 127.2, 131.4, 131.8, 139.4, 140.0, 140.1, 152.4, 155.0, 158.3; IR (KBr): $\tilde{\nu}$ = 2951, 2869, 2836, 1610, 1582, 1458, 1415, 1374, 1319, 1230, 1121, 1094, 823 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{28}H_{39}N_2O_5^+$: 483.2854, found: 483.2838.

5.1.62. 2-[2-Chloro-6-methoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methyl-5-nitroquinoline (34a)

A mixture of **26** (200 mg, 0.793 mmol), [2-chloro-6-methoxy-4-(methoxymethyl)phenyl]boronic acid (219 mg, 0.952 mmol), $Pd(PPh_3)_4$ (45.6 mg, 0.0395 mmol), and K_2CO_3 (328 mg, 2.37 mmol) in a mixture of 1,4-dioxane (6 mL) and H_2O (1.5 mL) was stirred at reflux temperature for 2.5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (8:2–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **34a** as a light yellow oil (280 mg, 695 μmol, 87.7%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.29 (s, 3H), 3.45 (s, 3H), 3.72 (s, 3H), 4.15 (s, 3H), 4.49 (s, 2H), 6.91 (s, 1H), 7.02 (d, J = 8.9 Hz, 1H), 7.08 (s, 1H), 8.51 (d, J = 8.9 Hz, 1H), 9.08 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.5, 56.0, 56.9, 58.4, 73.9, 104.8, 108.4, 120.8, 122.9, 127.0, 127.4, 132.1, 133.8, 135.4, 137.4, 137.9, 141.2, 156.6, 158.3, 161.2; IR (KBr): $\tilde{\nu}$ = 2840, 1608, 1555, 1507, 1462, 1403, 1312, 1261, 1194, 1092, 1053, 834, 821 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{20}H_{20}ClN_2O_5^+$: 403.1055, found: 403.1053.

5.1.63. 3,5-Dimethoxy-4-(8-methoxy-3-methyl-5-nitroquinolin-2-yl)benzonitrile (34b)

A mixture of **26** (300 mg, 1.19 mmol), (4-cyano-2,6-dimethoxyphenyl)boronic acid (296 mg, 1.43 mmol), $Pd(PPh_3)_4$ (68.4 mg, 0.0593 mmol), and K_2CO_3 (492 mg, 3.56 mmol) in a mixture of 1,4-dioxane (8 mL) and H_2O (2 mL) was stirred at reflux temperature for 1.5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (8:2–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **34b** as a light yellow solid (392 mg, 1.03 mmol, 86.8%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.25 (s, 3H), 3.73 (s, 6H), 4.14 (s, 3H), 6.94 (s, 2H), 7.02 (d, J = 8.7 Hz, 1H), 8.51 (d, J = 8.7 Hz, 1H), 9.07 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.5, 56.2, 56.9, 104.8, 108.2, 113.5, 118.8, 122.4, 122.9, 127.4, 132.0, 135.3, 137.4, 138.0, 154.7, 158.4, 161.1; IR (KBr): $\tilde{\nu}$ = 2227, 1574, 1555, 1506, 1468, 1413, 1308, 1241, 1194, 1123, 1093, 823 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{20}H_{18}N_3O_5^+$: 380.1241, found: 380.1245.

5.1.64. 2-(2,6-Dimethoxy-4-methylphenyl)-8-methoxy-3-methyl-5-nitroquinoline (34c)

A mixture of **26** (300 mg, 1.19 mmol), (2,6-dimethoxy-4-methylphenyl)boronic acid (280 mg, 1.43 mmol), $Pd(PPh_3)_4$ (68.4 mg, 0.0593 mmol), and K_2CO_3 (492 mg, 3.56 mmol) in a mixture of 1,4-dioxane (8 mL) and H_2O (2 mL) was stirred at reflux temperature for 2.5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was extracted into ethyl acetate and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (8:2–0:10 *n*-heptane/ethyl acetate gradient) to afford the title compound **34c** as a light yellow foam (380 mg, 1.03 mmol, 86.7%).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.28 (s, 3H), 2.41 (s, 3H), 3.67 (s, 6H), 4.12 (s, 3H), 6.47 (s, 2H), 6.97 (d, J = 9.1 Hz, 1H), 8.46 (d, J = 9.1 Hz, 1H), 9.01 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.9, 22.3, 55.8, 56.7, 104.4, 105.2, 115.0, 122.7, 126.9, 131.3, 136.4, 137.4, 138.1, 140.3, 157.1, 157.8, 161.2; IR (KBr): $\tilde{\nu}$ = 3004, 2838, 1612, 1554, 1504, 1466, 1403, 1306, 1265, 1243, 1194, 1123, 1093, 822, 810 cm^{-1}; HRMS-ESI m/z [M+H]$^+$ calcd for $C_{20}H_{21}N_2O_5^+$: 369.1445, found: 369.1445.

5.1.65. 2-[2-Chloro-6-methoxy-4-(methoxymethyl)phenyl]-8-methoxy-3-methylquinolin-5-amine (35a)

The title compound was prepared from **34a** using a method analogous to that described for **15b** in 83.4% yield as a yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.23 (s, 3H), 3.45 (s, 3H), 3.71 (s, 3H), 3.99 (s, 3H), 4.49 (s, 2H), 6.80 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 8.3 Hz, 1H), 6.89 (s, 1H), 7.06 (s, 1H), 8.08 (s, 1H); HRMS-ESI m/z $[M+H]^+$ calcd for $C_{20}H_{22}ClN_2O_3^+$: 373.1313, found: 373.1312.

5.1.66. 4-(5-Amino-8-methoxy-3-methylquinolin-2-yl)-3,5-dimethoxybenzonitrile (35b)

The title compound was prepared from **34b** using a method analogous to that described for **15b** in 61.3% yield as a light yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.18 (s, 3H), 3.70 (s, 6H), 3.96 (s, 3H), 6.76 (d, J = 8.1 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 6.91 (s, 2H), 8.02 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.2, 56.2, 56.2, 107.6, 108.2, 110.4, 112.9, 119.1, 120.4, 123.8, 129.7, 130.1, 134.3, 139.2, 149.5, 153.0, 158.5; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{20}H_{20}N_3O_3^+$: 350.1499, found: 350.1500.

5.1.67. 2-(2,6-Dimethoxy-4-methylphenyl)-8-methoxy-3-methylquinolin-5-amine (35c)

The title compound was prepared from **34c** using a method analogous to that described for **15b** in 94.6% yield as a yellow solid.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.22 (s, 3H), 2.40 (s, 3H), 3.66 (s, 6H), 3.96 (s, 3H), 6.45 (s, 2H), 6.71 (d, J = 7.8 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 8.06 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 19.5, 22.3, 55.9, 56.2, 105.3, 107.6, 109.9, 115.8, 120.2, 129.8, 131.1, 134.5, 138.9, 139.8, 149.2, 155.2, 158.0; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{20}H_{23}N_2O_3^+$: 339.1703, found: 339.1709.

5.1.68. 2-[2-Chloro-6-methoxy-4-(methoxymethyl)phenyl]-*N*,*N*-diethyl-8-methoxy-3-methylquinolin-5-amine (36a)

The title compound was prepared from **35a** using a method analogous to that described for **16a** in 79.6% yield as a light yellow foam. Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.04 (t, J = 6.9 Hz, 6H), 2.21 (s, 3H), 3.14 (q, J = 6.9 Hz, 4H), 3.44 (s, 3H), 3.71 (s, 3H), 4.01 (s, 3H), 4.44–4.52 (m, 2H), 6.88 (s, 1H), 6.93 (d, J = 8.3 Hz, 1H), 7.06 (s, 1H), 7.12 (d, J = 8.3 Hz, 1H), 8.47 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 19.2, 48.4, 56.0, 56.1, 58.3, 74.1, 106.3, 108.5, 118.7, 120.7, 127.6, 128.5, 130.6, 132.3, 134.2, 139.5, 139.6, 140.4, 152.3, 154.9, 158.5; IR (KBr): $\tilde{\nu}$ = 2965, 2923, 2817, 1604, 1567, 1466, 1402, 1368, 1258, 1187, 1101, 1045, 822 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{30}ClN_2O_3^+$: 429.1940, found: 429.1937.

5.1.69. 4-[5-(Diethylamino)-8-methoxy-3-methylquinolin-2-yl]-3,5-dimethoxybenzonitrile (36b)

The title compound was prepared from **35b** using a method analogous to that described for **16a** in 77.3% yield as a light yellow foam. Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.03 (t, J = 6.3 Hz, 6H), 2.18 (s, 3H), 3.13 (q, J = 6.3 Hz, 4H), 3.71 (s, 6H), 4.00 (s, 3H), 6.83–7.00 (m, 3H), 7.13 (d, J = 7.6 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 19.2, 48.4, 56.0, 56.2, 106.2, 108.3, 112.7, 118.8, 119.1, 124.1, 127.6, 130.4, 132.2, 139.5, 139.6, 152.2, 152.8, 158.6; IR (KBr): $\tilde{\nu}$ = 2970, 2833, 2225, 1601, 1572, 1465, 1412, 1243, 1122, 1096, 1064, 820 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{28}N_3O_3^+$: 406.2125, found: 406.2125.

5.1.70. 2-(2,6-Dimethoxy-4-methylphenyl)-*N*,*N*-diethyl-8-methoxy-3-methylquinolin-5-amine (36c)

The title compound was prepared from **35c** using a method analogous to that described for **16a** in 76.1% yield as a light yellow foam. Compound purity: >99%.

^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.03 (t, J = 6.7 Hz, 6H), 2.21 (s, 3H), 2.40 (s, 3H), 3.13 (q, J = 6.7 Hz, 4H), 3.67 (s, 6H), 3.99 (s, 3H), 6.46 (s, 2H), 6.88 (d, J = 8.1 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 8.41 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 12.4, 19.6, 22.3, 48.4, 55.9, 55.9, 105.3, 105.9, 116.4, 118.2, 127.4, 131.4, 131.7, 139.5, 139.5, 152.3, 155.1, 158.0; IR (KBr): $\tilde{\nu}$ = 2968, 2814, 1611, 1580, 1460, 1401, 1318, 1241, 1124, 1092, 813 cm^{-1}; HRMS-ESI m/z $[M+H]^+$ calcd for $C_{24}H_{31}N_2O_3^+$: 395.2329, found: 395.2331.

5.2. Biology

5.2.1. In vitro binding assay

For the binding assay and functional assay, HEK293 cells expressing the human CRF_1 receptor were cloned.[2a] Screening of CRF_1 receptor binding was performed using a scintillation proximity assay (SPA, Amersham Pharmacia, UK) using 96-well plates. Cell membrane (5 μg/well), wheat germ agglutinin coated SPA beads (1 mg/well), [^{125}I] human/rat CRF (0.1 nM), and a diluted test compound solution were suspended in 150 μL of assay buffer (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 10 mM $MgCl_2$, 2 mM EGTA, 1.5% bovine serum albumin (BSA), protease inhibitor cocktail (Roche Diagnostics GmbH), pH 7.0). Total binding and nonspecific binding were measured in the absence and presence of 0.4 μM unlabeled sauvagine, respectively. Plates were shaken gently and incubated for over 2 h at room temperature. The plates were centrifuged (260×*g*, 5 min, room temperature), and the radioactivity was detected using a TopCount scintillation counter (Perkin Elmer, MA, USA) with 1 min counting time per well. Each count was corrected by subtracting the non-specific binding and was represented as a percentage of total binding. The IC_{50} value of each compound was calculated using a concentration-response curve.

5.2.2. In vitro functional assay

To determine the activities of the antagonists, their effects on CRF-stimulated intracellular cyclic AMP (cAMP) accumulation were examined for HEK293 cells expressing the human CRF_1 receptor, as described previously.[2a] The accumulation of cAMP was measured using an enzyme immunoassay (EIA) kit (Amersham Pharmacia, UK). HEK293 cells expressing the human CRF_1 receptor were seeded in 96-well plates (5×10^4 cells/well) in Dulbecco's modified eagle medium (DMEM) containing 0.1% fetal bovine serum and 1 mM 3-isobutyl-1-methylxanthine, which is a phosphodiesterase inhibitor. After 30 min of preincubation, the diluted test compounds were added to the wells and incubated for another 30 min at 37 °C. The cells were then stimulated with 1 nM human/rat CRF for 30 min at 37 °C and collected by centrifugation (630×*g*, 5 min, 4 °C). After aspiration of the medium, the cells were lysed with the EIA kit lysis buffer. The amount of intracellular cAMP was measured according to the manufacturer's instructions. Basal levels of cAMP (i.e., in the absence of CRF) were subtracted from the measured values and these were then expressed as a percentage of total production. The IC_{50} value of each compound was calculated using a concentration-response curve.

5.2.3. CRF-induced ACTH release model

For CRF-induced ACTH elevation studies, male Fischer 344 rats weighing 152–177 g were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). Test compounds were orally administered to the rats 1 h before subcutaneous injection of CRF (10 μg kg^{-1}). Non-CRF control and CRF control groups received an equivalent volume of vehicle (5% DMSO/5% Cremophor®/90% saline). Thirty minutes after subcutaneous injection of CRF, blood samples of over 2 mL were obtained from the decapitated animals, stirred with 100 μL of EDTA 2Na (100 mg mL^{-1}), and kept on ice. The blood samples were centrifuged (1000×*g*, 4 C, 5 min), and 200 μL of plasma samples per animal were prepared in duplicate. Plasma ACTH concentrations were determined by using a radioim-

munoassay (RIA) kit (ACTH IRMA, Mitsubishi Chemical Corporation). The radioactivity of the bead was measured using a scintillation counter (ARC-1000M, Aloka Corp., Tokyo, Japan). ACTH concentration was calculated from a standard curve prepared using standard ACTH solutions.

5.2.4. Light–dark exploration tests

The apparatus consisted of a dark compartment (10 cm width (W), 15 cm depth (D), 20 cm height (H)) and a light compartment (20 cm W, 15 cm D, 20 cm H). The dark compartment had a lid on top and consisted of black Plexiglas® while the light compartment was open at the top and consisted of white Plexiglas®. A black Plexiglas® tunnel (7 cm W, 10 cm D, 4.5 cm H) separated the dark box from the light box. Light intensity in the experimental room was 150 lx. Mice were transferred and habituated to 10 lx light intensity at least 2 h before the test. Male BALB/c mice (n = 10/group) were p.o. administered 1 h before the test and kept in the 10-lx area until the experiment was started by placing the mouse in the dark compartment. Behavior was videotaped for a 5-min period, and the time spent in the light compartment and the number of tunnel crossings was measured after the experiments. A mouse with all four paws in the light compartment was considered to be in the light compartment fully. The vehicles of **21d** and **36a** were (0.5% MC containing HCl) and (5% DMSO, 5% Cremophor® EL, 90% saline, containing HCl), respectively.

5.3. Physicochemical properties

5.3.1. Aqueous solubility

The solubilities of the test compounds were determined using a high-throughput HPLC method (a few min/sample).[17] A 10 mM solution of test compound in DMSO was prepared and diluted to give a 1:100 test compound solution/DMSO in the microwell of the filter plate. After gyratory shaking of the filter plate for 15 min at room temperature, the solution was filtered. An aliquot of this filtered solution was injected into the HPLC system, which was equipped with an ODS column and UV detector. Mobile phase A (0.1% TFA, 1% MeCN in water) and mobile phase B (50:50 MeCN/EtOH, v/v) were pumped using a linear gradient program. The solubility of the compounds was determined by comparison to an external standard. The standard solution was prepared by diluting a 10 mM DMSO solution of test compound with DMSO to yield a 1:100 test compound solution/DMSO mixture in the well of the microwell plate.

5.3.2. Determination of pK_a

The pK_a values of the test compounds were determined using the same method reported by Ishihama et al.[18]

5.4. DMPK study

5.4.1. Rat pharmacokinetic studies

Fasted F344 rats (n = 3 at each dose, 189.4–205.9 g, obtained from Charles River Laboratories Japan, Inc.) were used for the study. The dosing solution containing the test compound (3 mg mL^{-1} for 3 mg kg^{-1} iv, 2 mg mL^{-1} for 10 mg kg^{-1} p.o.) was administered (dosing solution: 5% ethanol-0.02 mol L^{-1} HCl/5% glucose for iv; 5% DMSO/5% Cremophor® EL/equivalent HCl/H_2O for p.o.). Blood samples were collected at designated times: 0.083 (for iv), 0.25, 0.5, 1, 2, 4, 6, and 8 h (for iv and p.o.). Brain samples were collected 1 h after p.o. administration and homogenized in distilled water (1:4, v/v). A MeOH/MeCN (50:50, v/v, 0.25 mL) solution containing an internal standard (imipramine) was added to plasma or brain homogenate (0.05 mL), centrifuged (12,000 rpm, 5 min), and filtered. Then, the samples were examined using an LC–MS/MS methodology. Mobile phase A (distilled water containing 0.1% HCOOH) and mobile phase B (MeCN containing 0.1% HCOOH) were pumped at 0.5 mL min^{-1} using a linear gradient program [B%: 5% (0–1 min), 5–90% (1–4 min), 90% (4–6.5 min)]. Ionization was initiated using electrospray (positive mode) with monitoring of parent–daughter peaks of 425.25 > 381.2 for **21d** and 281.1 > 86.1 for the internal standard.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.028.

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