Novel Potent Orally Active Selective VEGFR-2 Tyrosine Kinase Inhibitors: Synthesis, Structure−Activity Relationships, and Antitumor Activities of *N*-Phenyl-*N*′-{4-(4-quinolyloxy)phenyl}ureas

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N-Phenyl-*N*′-{4-(4-quinolyloxy)phenyl}ureas were found to be a novel class of potent inhibitors for the vascular endothelial growth factor receptor 2 (VEGFR-2) tyrosine kinase through synthetic modifications of a lead compound and structure−activity relationship studies. A representative compound **6ab**, termed Ki8751, inhibited VEGFR-2 phosphorylation at an IC_{50} value of 0.90 nM, and also inhibited the PDGFR family members such as PDGFRα and c-Kit at 67 nM and 40 nM, respectively. However, **6ab** did not have any inhibitory activity against other kinases such as EGFR, HGFR, InsulinR and others even at 10000 nM. **6ab** suppressed the growth of the VEGF-stimulated human umbilical vein endothelial cell (HUVEC) on a nanomolar level. **6ab** showed significant antitumor activity against five human tumor xenografts such as GL07 (glioma), St-4 (stomach carcinoma), LC6 (lung carcinoma), DLD-1 (colon carcinoma) and A375 (melanoma) in nude mice and also showed complete tumor growth inhibition with the LC-6 xenograft in nude rats following oral administration once a day for 14 days at 5 mg/kg without any body weight loss.

Introduction

Angiogenesis play an important role in the growth of most solid tumors and the progression of metastasis.[1,2] Recently, it has been reported that the specific inhibition of tumor-induced angiogenesis suppresses the growth of many types of solid tumors. For this reason, it is considered that the inhibition of angiogenesis is a novel therapeutic approach against such tumors.[3−5]

The vascular endothelial growth factor (VEGF) is a key angiogenic factor and is secreted by malignant tumors; it induces the proliferation and the migration of vascular endothelial cells.[6−10] A number of compounds inhibiting the biological activity of VEGF has been produced, e.g., antibodies to VEGF[11] or its receptor,[12] small molecule receptor tyrosine kinase inhibitors,[13,14] such as the 3-substituted indolinones, i.e., SU5416[15,16] and SU6668,[17,18] the 4-anilinoquinazolines, such as ZD4190[19,20] and ZD6474,[21,22] and the anilinophthalazines, such as CGP79787/PTK787.[23,24] These agents have been evaluated in a clinical trial. Recently, an anti-VEGF antibody has shown significant effects in patients with colon and renal cancers.[25,26]

We also tried to find a novel tyrosine kinase inhibitor for VEGFR-2. The lead compound were found in a screening with in-house compounds which were synthesized as PDGFR tyrosine kinase inhibitors.[27−30] In this paper, we report the synthesis, structure−activity relationships (SAR) and biological activities for *N*-phenyl-*N*′-{4-(4-quinolyloxy)phenyl}urea derivatives.

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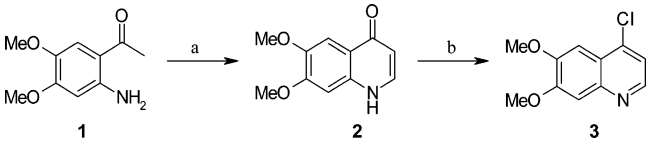
Scheme 1. Synthesis of 6,7-Dimethoxy-4-chloroquinoline[a]



[a] Reagents and conditions: (a) ethyl formate, NaOMe, dimethoxyethane, rt; (b) $POCl_3$, reflux.

Chemistry

4-Quinolone (**2**) was synthesized using the conventional Price method,[31] but a long time was needed to obtain **2** and its intermediates were difficult to handle. Accordingly, we developed a simple synthetic method as shown in Scheme 1. 2-Amino-4,5-dimethoxyacetophenone (**1**) was treated with 3 equivalents of NaOMe followed by 5 equivalents of ethyl formate at room temperature to give **2** in >95% yield. 4-Chloroquinoline (**3**) was prepared by the chlorination of **2** with phosphorus oxychloride. Most of the compounds reported in this paper were prepared by the general route outlined in Scheme 2. The 4-(4-nitrophenoxy)quinolines (**4**) were coupled with 4-nitrophenols at 150−190 °C or prepared by 4-nitrophenols with 2 equivalents of DMAP in xylene under reflux. They were reduced with Pd/C under a hydrogen atmosphere to obtain the anilino compound (**5**). In the case where the substituents on the nitrophenols were fluorine (**4b** and **4c**), **5b** and **5c** were prepared by the reduction with zinc powder in MeOH under reflux. **5** could be also prepared by direct coupling with the aminophenols and **3**. The ureas (**6**) were formed from **5** and the desired anilines with triphosgene and

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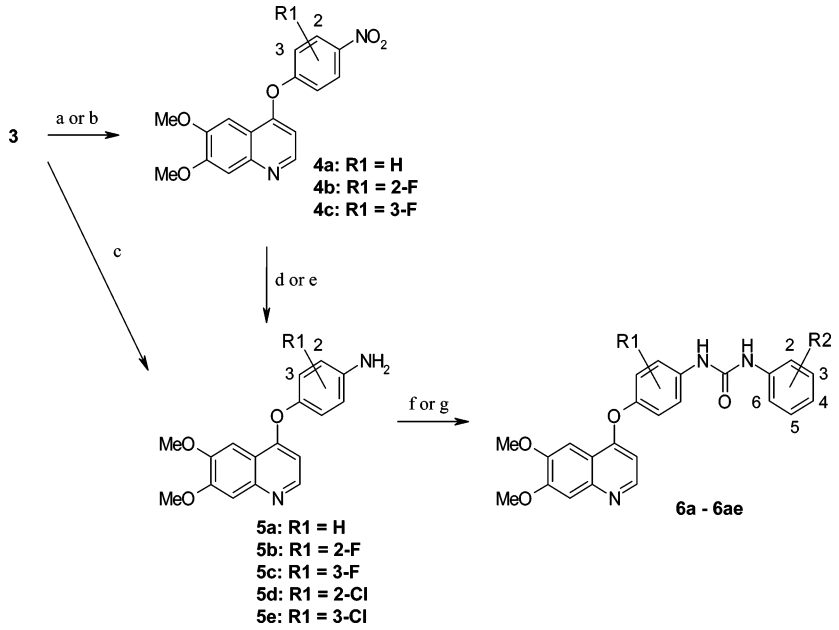
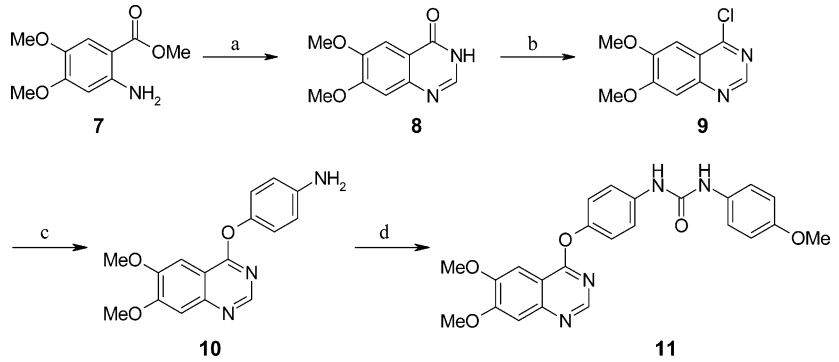
Scheme 2. Synthesis of Quinoline Derivatives[a]



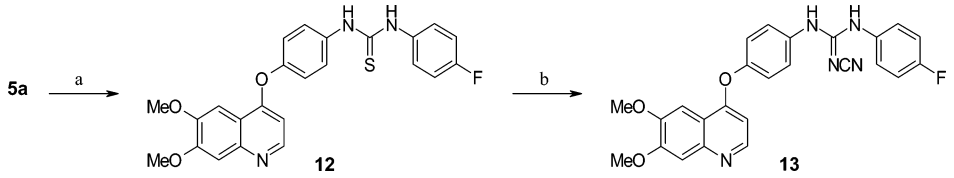
[a] Reagents and conditions: (a) 4-nitrophenols, 150−190 °C, di(ethylene glycol) dimethyl ether; (b) 4-nitrophenols, DMAP, xylene, reflux; (c) 4-aminophenols, NaH, 110 °C, DMSO; (d) Zn, NH_4Cl, MeOH, reflux; (e) palladium hydroxide on carbon, H_2, Et_3N, DMF, rt; (f) phenyl isocyanates, toluene, reflux; (g) anilines, triphosgene, Et_3N, $CHCl_3$.

Scheme 3. Synthesis of a Quinazoline Derivative[a]



[a] Reagents and conditions: (a) formamide, 160 °C; (b) $POCl_3$, toluene reflux; (c) 4-aminophenol, NaH, DMSO, 100 °C; (d) 4-methoxyphenyl isocyanate, toluene, reflux.

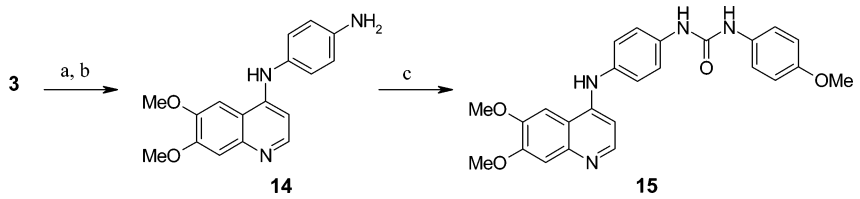
Scheme 4. Synthesis of Quinoline Derivatives[a]



[a] Reagents and conditions: (a) 4-fluorophenyl isothiocyanate, toluene, reflux; (b) DCC, cat. diisopropylethylamine, CH_2Cl_2, rt, then cyanamide, THF, rt.

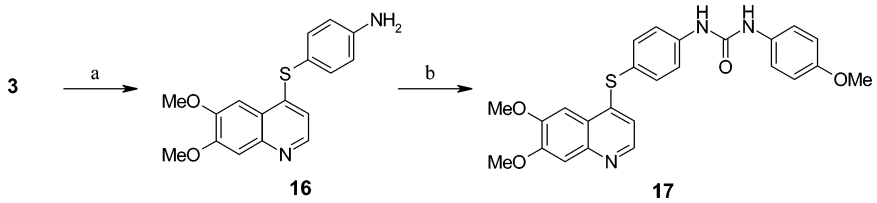
Et_3N in dry $CHCl_3$ or reacted with phenyl isocyanates in toluene under reflux. The quinazoline derivative (**11**) was prepared by the general method[32] in Scheme 3 as follows. Quinazolone (**8**) was prepared by cyclization of methyl 2-amino-4,5-dimethoxybenzoate with formamide at 150 °C. Next, we prepared 4-chloroquinazoline (**9**) using the same method as for **3**. Compound **9** was then coupled with 4-aminophenol treated with NaH at 100 °C in DMSO to obtain the aniline (**10**). Urea (**11**) was prepared with **10** in the same way as shown in Scheme 2. In addition to the urea moieties, we also synthesized the thiourea and cyanoguanidine derivatives for comparison purposes, as shown in Scheme 4. Thiourea (**12**) was prepared by the coupling with **5a** and isothiocyanate, followed by condensation with cyanamide using DCC to produce cyanoguanidine (**13**). We also modified the linker oxygen between the quinoline and the middle phenyl group with nitrogen and sulfur for comparison purpose as shown in Schemes 5 and 6. Compound **3** was coupled with 4-aminoactanilide at 160 °C followed by treating with concentrated HCl to give the phenylenediamine derivative (**14**) in good yield. Urea (**15**) was prepared by the reaction of **14** and 4-methoxy isocyanate. For the introduction of sulfur, **3** was coupled with

Scheme 5. Synthesis of a Quinoline Derivative[a]



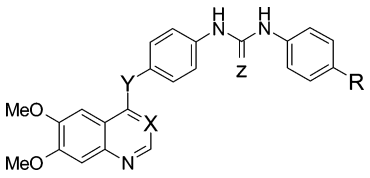
[a] Reagents and conditions: (a) 4-aminoacetanilide, 160 °C; (b) concentrated HCl aq, EtOH, reflux, (c) 4-methoxyphenyl isocyanate, $CHCl_3$, Et_3N, rt.

Scheme 6. Synthesis of a Quinoline Derivative[a]



[a] Reagents and conditions: (a) 4-aminothiophenol, DMF, rt; (b) 4-methoxyphenyl isocyanate, toluene, reflux.

Table 1. Inhibitory Activities of *N*-Phenyl-*N*′-{4-(4-quinolyloxy)phenyl}ureas and Related Compounds for VEGFR-2 and PDGFRα



entry	no.	X	Y	Z	R	IC_{50}[a] (nM) VEGFR-2	PDGFRα
1	**6a**	CH	O	O	OMe	1.1	2.0
2	**11**	N	O	O	OMe	2.2	3.6
3	**17**	CH	S	O	OMe	5.9	14
4	**15**	CH	NH	O	OMe	>100	21
5	**6b**	CH	O	O	F	0.4	5.8
6	**12**	CH	O	S	F	>100	52
7	**13**	CH	O	NCN	F	55	490

[a] IC_{50} values were averaged values determined by at least two independent experiments. ND: not detected.

aminothiophenol in DMF at room temperature to give the sulfur-containing compound (**16**) in good yield. The addition of 4-methoxyphenylisocyanate to **16** then gave the corresponding urea (**17**).

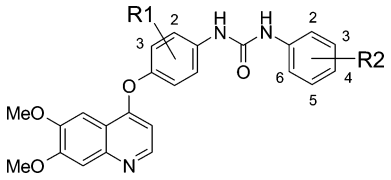
Results and Discussion

The structures of the *N*-phenyl-*N*′-{4-(4-quinolyloxy)-phenyl}ureas and related compounds prepared for this study are shown in Tables 1 and 2. The IC_{50} values for VEGFR-2 and the PDGFRα phosphorylations were measured by cell-based assay.

At first, partial modification of the lead compound (**1**) was performed as shown in Table 1. The introduction of a quinazoline ring instead of the quinoline ring, compound **11**, had almost the same inhibitory activities for VEGFR-2 and PDGFRα.

Next, the effect of the linker atom between the quinoline ring and the phenyl group was evaluated. Compound **17**, which had been substituted with a sulfur atom, was shown to have a potent inhibitory activity but which was slightly decreased when compared to that of compound **6a**. Compound **15** substituted with nitrogen showed no inhibition of VEGFR-2 even at 100 nM.

Table 2. Inhibitory Activities for VEGFR-2 and PDGFRα of Six Analogues



entry	no.	R1	R2	IC_{50}[a] (nM) VEGFR-2	PDGFRα
1	**6c**	H	H	0.2	3.6
2	**6d**	H	2-OMe	0.2	5.8
3	**6e**	H	3-OMe	<1.0	2.0
4	**6a**	H	4-OMe	1.1	4.4
5	**6f**	H	2-Me	2.3	3.6
6	**6g**	H	3-Me	0.2	3.3
7	**6h**	H	4-Me	0.5	36
8	**6i**	H	2-NO_2	19	6.8
9	**6j**	H	3-NO_2	1.1	18
10	**6k**	H	4-NO_2	5.6	17
11	**6l**	H	2-F	0.5	3.9
12	**6m**	H	3-F	1.2	6.4
13	**6b**	H	4-F	0.4	5.8
14	**6n**	H	3-Cl	1.6	24
15	**6o**	H	4-Cl	0.2	10
16	**6p**	H	2,3-F_2	0.7	1.6
17	**6q**	H	2,4-F_2	0.7	3.4
18	**6r**	H	2,5-F_2	0.4	4.7
19	**6s**	H	2,6-F_2	1.8	3.5
20	**6t**	H	3,4-F_2	1.1	6.8
21	**6u**	H	3,5-F_2	1.8	10
22	**6v**	H	2,3-Cl_2	27	24
23	**6w**	H	2,4-Cl_2	5.6	37
24	**6x**	H	2,5-Cl_2	6.0	43
25	**6y**	H	2,6-Cl_2	37	<10
26	**6z**	H	3,4-Cl_2	3.7	55
27	**6aa**	H	3,5-Cl_2	8.9	75
28	**6ab**	2-F	2,4-F_2	0.9	67
29	**6ac**	2-Cl	2,4-F_2	0.4	<3.0
30	**6ad**	3-F	2,4-F_2	<1.0	17
31	**6ae**	3-Cl	2,4-F_2	2.6	13

[a] IC_{50} values were averaged values determined by at least two independent experiments.

In recent reports about 4-anilinoquinazoline related compounds, the nitrogen atom formed bonds with the neighboring carbons as sp^2-like orbitals.[33] It could be considered that one of the reasons for the loss of activity

Table 3. Inhibitory Activities of **6ab** against Various Kinases by Intact-Cell Assays

kinase	IC_{50}[a] (nM)
VEGFR-2	0.9
PDGFRα	67
c-Kit	40
FGFR-2	170
EGFR	>10000
HGFR	>10000
InsulinR	>10000

[a] IC_{50} values were averaged values determined by at least two independent experiments.

for VEGFR-2 of **15** is caused by the conformational restriction for this bond type of nitrogen.

The thiourea and cyanoguanidine derivatives, which were considered as the bioisosters of urea, were also evaluated. The introduction of the thiourea group, compound **12**, resulted in a significant decrease in the inhibitory activities for VEGFR-2, but a good inhibitory activity for PDGFRα remained. The cyanoguanidine derivatives, compound **13**, showed a loss of activity for PDGFRα, but a good inhibitory activity for VEGFR-2 could be observed. When comparing the three compounds, **6b**, **12** and **13**, it suggests that the oxygen of the urea strongly interacts with the binding site of the receptors and the NH moiety of urea also has significant interactions based on previous investigation.[30] We think that the introduction of the urea group is the key modification to increase the activity for VEGFR-2 and PDGFRα.

These results indicate that the compounds containing the 4-quinolyloxy group and 1,3-diphenylurea group such as compounds **6a** and **6b** would have one of the most favorable structures to provide a potent inhibitory activity for VEGFR-2.

The effects of substituents on the terminal phenyl group were investigated as shown in Table 2. The compounds substituted with MeO, Me and a halogen at the 2-, 3- and 4-positions on the phenyl group showed potent inhibitory activities for VEGFR-2 and PDGFRα. In this study, it seemed that the substituents on the terminal phenyl group had hardly any influence on the inhibitory activities for VEGFR-2. In the case of the steric hindering substituents such as NO_2 and Cl positioned at the 2-position on the terminal phenyl group, such as **6k**, **6v**, **6w**, **6x** and **6y,** had decreased inhibitory activity for VEGFR-2.

We also investigated the effects of the substituents of fluorine or chlorine on the middle phenyl group, such as **6ab**, **6ac**, **6ad** and **6ae**, because halogenated compounds showed good in vivo antitumor activity (data not shown). All of these compounds showed potent inhibitory activity for VEGFR-2.

We selected **6ab** as one of a candidate and performed more detailed tests. Incidentally, a well-known compound as VEGFR-2 inhibitor, SU5416, inhibited at the IC_{50} of 55 nM in the assay.

The inhibitory activities of **6ab** toward the other receptor tyrosine kinases in cell-based assays, c-Kit, c-Fms, EGFR, HGFR and InsulinR, were investigated as shown in Table 3. **6ab** showed a potent inhibitory activity for the class III kinases such as c-Kit and PDGFRα and also good inhibition for FGFR-2. No inhibitory activities were observed against EGFR, HGFR and InsulinR even at 10000 nM.

Table 4. Inhibitory Activities of **6ab** against Various Kinases by Cell-Free Assays

kinase	IC_{50}[a](nM)
VEGFR-2	4.0
EGFR	>10000
c-Src	>10000
PKA	>10000
PKCα	>10000
MEK1	>10000
MAPK	>10000

[a] IC_{50} values were averaged values determined by at least two independent experiments.

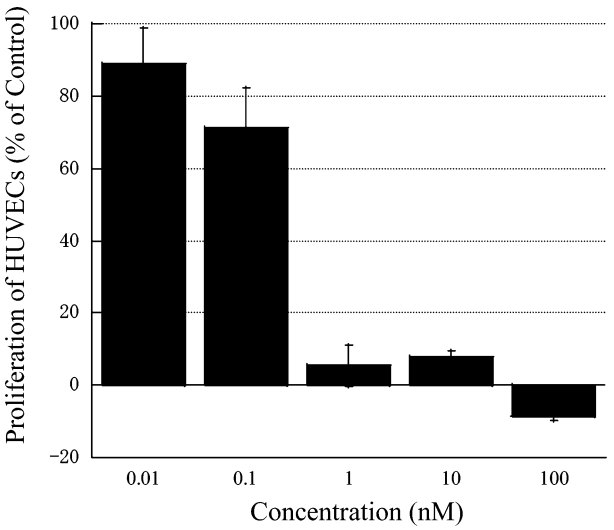


Figure 1. Effect of **6ab** on VEGF-stimulated HUVEC proliferation.

The inhibitory activities for VEGFR-2, EGFR, c-Src, PKA, PKCα, MEK1 and MAPK in cell-free assays were also investigated as shown in Table 4. A potent inhibitory activity was shown against VEGFR-2 at the IC_{50} of 4.0 nM, but no inhibitory activities were observed against the other kinases at 10000 nM. It was revealed that compound **6ab** possessed an excellent selectivity.

We evaluated **6ab** for its ability to inhibit the VEGF-stimulated proliferation of human umbilical vein endothelial cells (HUVEC), which naturally expressed the VEGFR-2 receptor. In this assay, growth-arrested HUVEC were treated with vehicle (control) or **6ab** (at concentrations of 0.01, 0.1, 1, 10, 100 nM), and then stimulated by VEGF to induce their growth in serum. The results shown in Figure 1 revealed that **6ab** inhibits HUVEC growth in a dose-dependent manner and completely suppresses it at 1 nM. In the treatment at 100 nM, a tendency to decrease the number of HUVEC was observed, considering that the compound might have a direct cytotoxic effect at this concentration.

The antitumor activities of **6ab** were measured against various tumor cell lines, i.e., GL07 human glioma, St-4 human stomach carcinoma, LC-6 human lung carcinoma, DLD-1 human colon carcinoma and A375 human melanoma, using xenografts in nude mice. The compounds were orally administered to mice in experimental groups of four animals once a day for 9 consecutive days at 20 mg/kg, and the vehicle was administered to the control animals as shown in Table 5. We showed the TGI % for the most potent antitumor activity in the course. Significant antitumor activities were observed

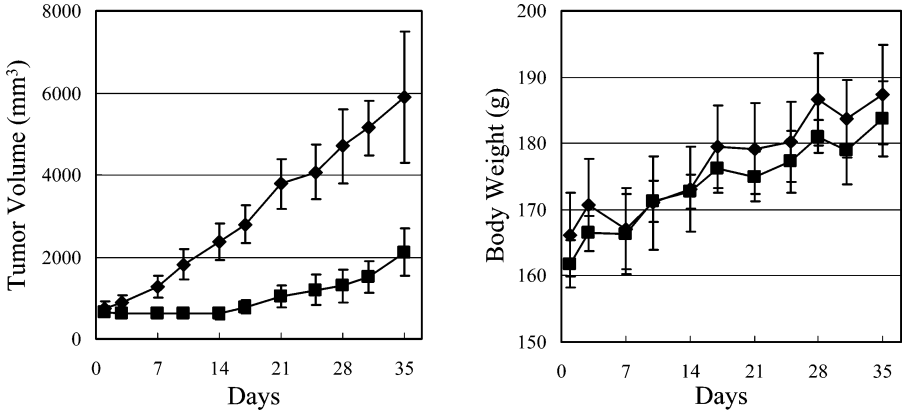


Figure 2. Antitumor activity and body weight change in treatment with **6ab** (■, 5 mg/kg/day, po) or vehicle (◆) in human lung carcinoma (LC-6) xenografts in nude rats. Values shown are the mean ±SE obtained in three experiments.

Table 5. Antitumor Activities of **6ab** against Various Tumors

tumor	tissue type	TGI[a] (%) 20 mg/kg/day, po
GL07	glioma	83
St-4	stomach	68
LC-6	lung	82
DLD-1	colon	58
A375	melanoma	43

[a] Compounds were administered orally to mice once a day for 9 consecutive days at 20 mg/kg. TGI means tumor growth inhibition ratio. See Experimental Section.

in all the tumor cell lines. Tumor regrowth was observed after the finish of the **6ab** administration in all cases.

To rule out the possibility that **6ab** had a direct inhibitory effect on the proliferation of tumor cells, its in vitro growth inhibition of selected tumor cell lines, A375 and DLD-1 that used in the in vivo studies was tested. The growth inhibition of the tumors without VEGF-stimulation was not observed at 3 μM (data not shown). Because the inhibitory activity for HUVEC growth was 1000-fold more potent than that for the tumor's unstimulated growth described above, we concluded that the inhibitory effect of **6ab** would be generated by an effect on the angiogenic process associated with tumor growth.

Compound **6ab** was also evaluated for its tumor inhibitory activity in vivo using LC-6 human tumor xenografts in nude rats following oral administration once a day for 14 days at 5 mg/kg, and the models were observed up to the 35th day as shown in Figure 2. Complete inhibition of tumor growth was seen in the treatment with **6ab**, but after the treatment with **6ab**, tumor regrowth was observed. During the course of the test schedule, no significant change in body weight was observed in comparison with the control.

Conclusions

We discovered that the *N*-phenyl-*N*′-{4-(4-quinolyloxy)-phenyl}ureas are some of the most potent and high selective VEGFR-2 inhibitors. The representative compound **6ab**, termed Ki8751, showed a potent inhibitory activity for VEGFR-2 at the IC_{50} of 0.9 nM and a high selectivity for many other kinases over 1000-fold. It is noted that **6ab** showed an excellent antitumor activity against some human tumor xenografts in nude mice and rats following oral administration without significant toxicity. Accordingly, we consider that a new anti-angiogenic agent for in vitro and in vivo studies could provide. We have been continuous studying the *N*-phenyl-*N*′-{4-(4-quinolyloxy)phenyl}urea structure in detail to find a clinical candidate.

Experimental Section

General Considerations. Melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA400 (400 MHz) or a JEOL JNM-A500 (500 MHz). Chemical Shifts (δ) are given in ppm downfield from tetramethylsilane as the internal standard. MS spectra were collected with a MICROMASS PLATFORM-LC or a JEOL JMS SX/SX-102. Column chromatography was carried out on silica gel 60 (70−230 mesh, KANTO Chemical) or preparative thin-layer chromatography (PLC plates; MERCK). Elemental analysis was performed by TORAY Research Center, Inc.

6,7-Dimethoxy-4-quinolone (2). To a solution of 2-amino-4,5-dimethoxyacetophenone (**1**) (10.0 g, 51 mmol) in dimethoxyethane (250 mL) was added NaOMe (8.3 g, 154 mmol) and the mixture stirred at room temperature for 70 min. Ethyl formate (21 mL, 261 mmol) was then added and stirred at room temperature for 2 h. To the resulting solution water (10 mL) was added and stirred for 5 min, and the solution was neutralized with 10% aqueous HCl solution. The resulting solid was collected by filtration and washed with water (50 mL ×2), dioxane (50 mL) and AcOEt (50 mL). The solid was dried under vacuum to obtain 10.0 g (95%) of **2**. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.82 (s, 3H), 3.86 (s, 3H), 5.95 (d, J = 4.6 Hz, 1H), 6.69 (s, 1H), 7.43 (s, 1H), 7.78 (d, J = 4.6 Hz, 1H), 8.13 (s, 1H); MS (ESI, *m/z*): 206 ($M^+ + 1$).

***N*-{4-[(6,7-Dimethoxy-4-quinazolinyl)oxy]phenyl}-*N*′-(4-methoxyphenyl)urea (11).** A suspension of **10** (81 mg, 0.27 mmol) in toluene (5 mL) was stirred under reflux and dissolved; 4-methoxyphenyl isocyanate (0.29 mL, 2.2 mmol) was then added and stirred under reflux for 40 min. The resulting solid was collected by filtration and washed with toluene to obtain 60 mg of **11** (49%). mp 240 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.79 (s, 3H), 4.06 (s, 3H), 4.07 (s, 3H), 6.48 (s, 1H), 6.64 (s, 1H), 6.91 (d, J = 9.2 Hz, 2H), 7.20 (d, J = 8.6 Hz, 2H), 7.27 (d, J = 8.6 Hz, 2H), 7.32 (s, 1H), 7.47 (d, J = 9.2 Hz, 2H), 7.55 (s, 1H), 8.60 (s, 1H); MS (ESI, *m/z*): 447 ($M^+ + 1$). Anal. ($C_{24}H_{22}N_4O_5 \cdot 0.5H_2O$) C, H, N.

***N*1-(6,7-Dimethoxy-4-quinolyl)-1,4-benzenediamine hydrochloride (14).** A mixture of **3** (10.0 g, 44.7 mmol) and 4-aminoacetanilide (6.7 g, 44.7 mmol) was heated at 160 °C for 1h. The reaction mixture was dissolved in EtOH (170 mL) and concentrated HCl (34 mL) was added. The solution was stirred under reflux for 4h and cooled to room temperature. The resulting solid was collected by filtration, washed with EtOH and dried under vacuum to obtain 13.2 g (80%) of **14**. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.99 (s, 3H), 4.00 (s, 3H), 6.69 (d, J = 7.1 Hz, 1H), 7.27 (d, J = 8.5 Hz, 2H), 7.40 (s, 1H), 7.43 (d, J = 8.5 Hz, 1H) 8.08 (s, 1H), 8.33 (d, J = 7.1 Hz, 1H), 10.57 (s, 1H); MS (ESI, *m/z*): 296 ($M^+ + 1$).

***N*-{4-[(6,7-Dimethoxy-4-quinolyl)amino]phenyl}-*N'*-(4-methoxyphenyl)urea (15).** To a solution of **14** (100 mg, 0.27 mmol) and Et_3N (0.3 mL, 2.2 mmol) in $CHCl_3$ was added 4-methoxyphenyl isocyanate (48 mg, 0.32 mmol) and the mixture stirred at room temperature for 5 h. The resulting solid was collected by filtration and washed with $CHCl_3$. The resulting solid was purified by column chromatography eluting with $CHCl_3$/MeOH (10/1) to obtain 54 mg g of **15** (31%). mp 255 °C (decomposition); ^{1}H NMR (CD_3OD, 400 MHz): δ 3.78 (s, 3H), 4.04 (s, 3H), 4.04 (s, 3H), 6.75 (d, J = 6.8 Hz, 1H), 6.89 (d, J = 9.0 Hz, 2H), 7.23 (s, 1H), 7.33 (d, J = 9.3 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 9.0 Hz, 2H), 7.81 (s, 1H), 8.16 (d, J = 6.8 Hz, 1H); MS (ESI, *m/z*): 445 ($M^+ + 1$); Anal. ($C_{25}H_{24}N_4O_4 \cdot 2.0H_2O$) C, N; H: calcd, 5.87, found, 5.39.

4-[(6,7-Dimethoxy-4-quinolyl)sulfanyl]aniline (16). A solution of **3** (1.00 g, 4.5 mmol) and 4-aminothiophenol (565 mg, 4.5 mmol) in DMF was stirred at room temperature for 2 h. The reaction mixture was separated between saturated aqueous $NaHCO_3$ and $CHCl_3$. The organic layer was washed with brine and dried over Na_2SO_4. The resulting solution was concentrated and purified by column chromatography on silica gel eluting with $CHCl_3$/acetone (4/1) to obtain 1.3 g of **16** (95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.77 (brs, 2H), 4.05 (s, 3H), 4.07 (s, 3H), 6.59 (d, J = 8.5 Hz, 2H), 7.25 (d, J = 7.3 Hz, 2H), 7.36 (d, J = 4.9 Hz, 1H) 7.42 (s, 1H), 7.43 (s, 1H), 8.53 (d, J = 4.9 Hz, 1H); MS (ESI, *m/z*): 313 ($M^+ + 1$).

***N*-{4-[(6,7-Dimethoxy-4-quinolyl)sulfanyl]phenyl}-*N'*-(4-methoxyphenyl)urea (17).** To a solution of **16** (57 mg, 0.18 mmol) in toluene (5 mL) was added 4-methoxyphenyl isocyanate (0.2 mL, 1.54 mmol) and the mixture stirred under reflux for 15 min. The resulting solid was collected by filtration and washed with toluene. The solid was dried under vacuum to obtain 69 mg of **17** (yield: 83%). mp 255 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.73 (s, 3H), 3.94 (s, 3H), 3.94 (s, 3H), 6.64 (d, J = 4.9 Hz, 1H),6.89 (d, J = 9.0 Hz, 2H), 7.33 (s, 1H), 7.37 (d, J = 8.3 Hz, 2H) 7.38 (s, 1H), 7.53 (d, J = 8.3 Hz, 2H), 7.64 (d, J = 8.8 Hz, 2H), 8.41 (d, J = 5.1 Hz, 1H), 8.57 (s, 1H), 8.92 (s, 1H); MS (ESI, *m/z*): 462 ($M^+ + 1$). Anal. ($C_{25}H_{23}N_3O_4S$) C, H, N.

4-(3-Fluoro-4-nitrophenoxy)-6,7-dimethoxyquinoline (4b). A suspension of **3** (10.2 g, 45.7 mmol) and 3-fluoro-4-nitrophenol (14.4 g, 91.5 mmol) in chlorobenzene (100 mL) was heated under reflux for 10 h. The resulting solid was collected by filtration, washed with toluene and dried under vacuum. The solid was mixed with 10% NaOH aq, and the suspension was stirred at room temperature for 1 h. The resulting solid was collected by filtration, washed with dioxane and AcOEt, and dried under vacuum to obtain 14.19 g of **4b** (90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.05 (s, 3H), 4.13 (s, 3H), 6.82 (d, J = 5.9 Hz, 1H), 7.11−7.18 (m, 2H), 7.42 (s, 1H), 7.87 (s, 1H), 8.27 (t, J = 8.5 Hz, 1H), 8.65 (d, J = 5.9 Hz, 1H).

4-[(6,7-Dimethoxy-4-quinolyl)oxy]-2-fluoroaniline (5b). A mixture of **4b** (10.00 g, 2.9 mmol), Zn powder (1.90 g, 29 mmol) and NH_4Cl (620 mg, 11.6 mmol) in MeOH (150 mL) was stirred under reflux for 10 h. The reaction mixture was filtered with Celite and washed with MeOH and $CHCl_3$. The resulting solution was washed with 10% NaOH aq (100 mL) and brine. The solution was dried over Na_2SO_4 and concentrated. The resulting solid was collected by filtration, washed with AcOEt and dried under vacuum to obtain 831 mg of **5b** (91%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.06 (s, 3H), 4.07 (s, 3H), 6.50 (d, J = 5.6 Hz, 1H), 6.80−6.96 (m, 3H), 7.53 (s, 1H), 7.55 (s, 1H), 8.48 (d, J = 5.4 Hz, 1H); MS (ESI, *m/z*): 315 ($M^+ + 1$).

***N*-(2,4-Difluorophenyl)-*N'*-{4-[(6,7-dimethoxy-4-quinolyl)-oxy]-2-fluorophenyl}urea (6ab).** Preparation from **5b** and 2,4-difluorophenyl isocyanate as described for **6c** yielded 2.52 g (yield: 84%) of **6ab**. mp 239 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.55 (d, J = 5.1 Hz, 1H), 7.04−7.12 (m, 2H), 7.28−7.35 (m, 2H), 7.40 (s, 1H), 7.49 (s, 1H), 8.11 (dt, J = 6.1, 9.3 Hz, 1H), 8.24 (t, J = 9.3 Hz, 1H), 8.50 (d, J = 5.4 Hz, 1H), 8.98 (s, 1H), 9.04 (s, 1H); MS (FD, *m/z*): 469 (M^{2+}). Anal. ($C_{24}H_{18}N_3O_4F_3 \cdot 0.5H_2O$) C, H, N.

***N*-(4-Fluorophenyl)-*N'*-(4-[(6,7-dimethoxy-4-quinolyl)-oxy]phenyl)thiourea (12).** A solution of **5a** (100 mg, 0.34 mmol) and 4-fluorophenyl isothiocyanate (52 mg, 0.34 mmol) in toluene (10 mL) was stirred under reflux for 24 h. The solution was evaporated and purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 32 mg of **12** (yield: 21%). mp 153 °C; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.95 (s, 3H), 6.50 (d, J = 4.9 Hz, 1H), 7.15−7.25 (m, 4H), 7.40 (s, 1H), 7.47−7.50 (m, 3H), 7.59 (d, J = 8.6 Hz, 2H), 8.50 (d, J = 5.5 Hz, 1H), 9.80 (brs, 1H), 9.84 (brs, 1H); MS (ESI, *m/z*) 450 ($M^+ + 1$). Anal. ($C_{24}H_{20}N_3O_3SF \cdot 1.7H_2O$) C, N; H: calcd., 4.90, found, 4.46.

1-(4-Fluorophenyl)-2-cyano-3-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}guanidine (13). A slurry of **12** (33 mg, 0.07 mmol) and a catalytic amount of diisopropylethylamine in CH_2Cl_2 (10 mL) was stirred at room temperature. To this solution, a solution of cyanamide (16 mg) in THF (1 mL) was added, and the reaction mixture was stirred at room-temperature overnight. The solution was evaporated, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 34 mg of **13** (yield: 99%). mp 163 °C;.^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.03 (s, 3H), 4.05 (s, 3H), 6.50 (d, J = 5.1 Hz, 1H), 7.15 (t, J = 8.5 Hz, 2H), 7.22 (d, J = 8.8 Hz, 2H), 7.34 (dd, J = 4.9, 8.8 Hz, 2H), 7.42 (d, J = 9.5 Hz, 2H), 7.43 (s, 1H), 7.49 (s, 1H), 8.52 (d, J = 5.4 Hz, 1H); MS (ESI, *m/z*) 458 ($M^+ + 1$). Anal. ($C_{25}H_{20}N_5O_3F \cdot 2.3H_2O$) C, H; N: calcd., 14.02, found, 13.54.

SU5416 was prepared using previous report.[15]

Cellular Kinase Assays. NIH3T3 cells[34] prepared by transfection of human KDR were cultured in a DMEM medium containing 10% FCS within a 5% CO_2 incubator. The cells were cultured in a collagen type I coated 96-well plate in an amount of 1.5×10^4 per well. The medium was then replaced by a DMEM medium containing 0.1% FCS. A solution of the test compound in DMSO was added to each well and cultured. rhVEGF was added to a final concentration of 100 ng/mL, and the stimulation of cells was carried out at 37 °C. The cells were washed with phosphate buffer saline (pH 7.4), 50 mL of a solubilization buffer (20 mM HEPES (pH 7.4), 150 mM NaCl, 0.2% Triton X-100, 10% glycerol, 5 mM Na_3VO_4, 5 mM disodium ethylenediamine tetraacetate, and 2 mM $Na_4P_2O_7$) was then added and a cell extract was prepared. Separately, phosphate buffered saline (50 μL, pH 7.4) containing 5 μg/mL of antiphosphotyrosine antibody (PY20) was added to a microplate for ELISA. After washing of the plate, 300 μL of a blocking solution was added. The cell extract was transferred to the plate. An anti-VEGFR-2 antibody and a peroxidase-laveled antirabbit Ig antibody were added. Next, a chromophoric substrate for peroxidase was added, and the absorbance at 450 nm was measured with microplate reader. The VEGFR-2 phosphorylation activity for each well was determined by presuming the absorbance with the addition of VEGF and without the addition of the test sample to be 100% VEGFR-2 phosphorylation activity and VEGF to be 0% VEGFR-2 phosphorylation activity. The concentration of the inhibition (%) of VEGFR-2 Phosphorylation was determined for each case, and IC_{50} value was calculated. Other assays were performed in a similar manner using indicated cell lines as previously reported.[28] The assays were performed for c-Kit and PDGFRα, using HMC-1 and G292 cells, respectively, for FGFR-2 and insulinR using normal human dermal fibroblast cells and HepG2 cells, respectively, and for HGFR and EGFR using A431 cells.

Cell-Free Kinase Assays. VEGFR-2 kinase assay is described below; GST-fusion proteins of KDR (Flk-1: cytoplasmic domain) were produced in the baculo-virus expression system. GST-KDR was premixed with a serial dilution of compound **6ab** in the kinase buffer consisting of 100 mM HEPES, 50 mM NaCl, 40 μM Na_3VO_4, and 0.02%(w/v) BSA. The kinase reaction was initiated by the addition of 2 μM ATP in a solution of $MnCl_2$. After 20 min incubation at room temperature, the reaction was stopped with an addition of EDTA. Phosphorylation levels of GST-KDR were detected by immunoblotting with anti-phosphotyrosine monoclonal antibodies (PY20) and detected by ECL fluorography as previously reported.[28] Similar assays were performed against other kinases as previously

reported.[30] All assays were carried out in quadruplicate with 1−10 μM ATP.

Inhibition of VEGF-Stimulated HUVEC Proliferation. HUVECs were placed at a density of 4000 cells/200 μL/well on collagene type I precoated 96-well plates in M199 medium with 5% fetal bovine serum (FBS). After 24 h, the cells were incubated for 1 h in the presence or absence of **6ab**; then the cells were stimulated by rhVEGF (20 ng/mL). The cultures were incubated at 37 °C for 72 h, then pulsed with 1 μCi/well [^{3}H]thymidine and reincubated for 14 h. Cells were assayed for the incorporation of tritium using a beta counter.

Antitumor Assays Using Human Tumor Xenografts in Nude Mice. Human glioma (GLO7) was transplanted into nude mice. When the tumor grew to a volume of about 100 mm^3, the nude mice were divided into several groups of four animals so as to equalize the average tumor volumes of each group. The compounds were administered orally to mice in the experimental groups once a day for 9 consecutive days at 20 mg/kg, and the vehicle was administered to control animals. Tumor volumes were monitored twice weekly by measuring the perpendicular diameters (taking the length to be the longest diameter (L), the width to be the shortest diameter (W) and the height (H) to be the longest perpendicular diameter) and calculated using the formula ($L \times W \times H$)/2. The tumor growth inhibition (TGI) was calculated as follows: TGI(%) = 1 − (RTV of the treated group at the day of measurement)/(RTV of control-group at the day of measurement) × 100. RTV = (Tumor volume at the day of measurement)/(Tumor volume at the initial day)

To determine the effects of **6ab** on tumor growth, its effects were tested against various tumors, human stomach carcinoma (St-4), human lung carcinoma (LC-6), human colon carcinoma (DLD-1) and human melanoma (A375), using human tumor xenografts in nude mice.

Antitumor Assay Using Human Xenograft in Nude Rats. Human lung carcinoma (LC-6) was transplanted into nude rats. When the tumor grew to a volume of about 700 mm^3, the nude rats were divided into several groups of three animals so as to equalize the average tumor volumes of each group. Body weights were also measured twice weekly at the same time of measurement of the tumor volume.

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Supporting Information Available: Analysis data not included in the Experimental Section and a table of elemental analyses data. This material is available free of charge via the Internet at http//pubs.acs.org.

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