Novel 4-Anilinoquinazolines with C-7 Basic Side Chains: Design and Structure Activity Relationship of a Series of Potent, Orally Active, VEGF Receptor Tyrosine Kinase Inhibitors

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We have previously shown that 4-anilinoquinazolines can be potent inhibitors of vascular endothelial growth factor (VEGF) receptor (Flt-1 and KDR) tyrosine kinase activity. A novel subseries of 4-anilinoquinazolines that possess basic side chains at the C-7 position of the quinazoline nucleus have been synthesized. This subseries contains potent, nanomolar inhibitors of KDR (median IC_{50} 0.02 μM, range 0.001−0.04 μM), which are comparatively less potent vs Flt-1 tyrosine kinase (median IC_{50} 0.55 μM, range 0.02−1.6 μM). The compounds also retain some inhibitory activity against the tyrosine kinase associated to the endothelial growth factor receptor (EGFR) (median IC_{50} 0.2 μM, range 0.075−0.8 μM) but demonstrate selectivity vs that associated to the FGF receptor 1 (median IC_{50} 2.5 μM, range 0.9−19 μM). This selectivity profile is also evident in a growth factor-stimulated human endothelial cell (HUVEC) proliferation assay (i.e., inhibition of VEGF > EGF > FGF), with inhibition of VEGF-induced proliferation being achieved at nanomolar concentrations (median IC_{50} 0.06 μM). Further examination of compound **2** (ZD6474) in recombinant enzyme assays revealed excellent selectivity for the inhibition of KDR tyrosine kinase (IC_{50} 0.04 μM) vs the kinase activity of erbB2, MEK, CDK-2, Tie-2, IGFR-1R, PDK, PDGFRβ, and AKT (IC_{50} range: 1.1 to >100 μM). Anilinoquinazolines possessing basic C-7 side chains exhibited markedly improved aqueous solubility over previously described anilinoquinazolines possessing neutral C-7 side chains (up to 500-fold improvement at pH 7.4). In addition, aqueous solubility of the neutral fraction present at pH 7.4 of the basic subseries of anilinoquinazoline proved to be higher than that of the neutral analogue **1** (ZD4190). Oral administration of representative compounds to mice (50 mg/kg) produced plasma levels between 0.2 and 3 μM at 24 h after dosing. Our development candidate **2** demonstrated a very attractive in vitro profile combined with excellent solubility (330 μM at pH 7.4) and good oral bioavailability in rat and dog (>80 and >50%, respectively). This compound demonstrated highly significant, dose-dependent, antitumor activity in athymic mice. Once daily oral administration of 100 mg/kg of compound **2** for 21 days inhibited the growth of established Calu-6 lung carcinoma xenografts by 79% ($P < 0.001$, Mann Whitney rank sum test), and substantial inhibition (36%, $P < 0.02$) was evident with 12.5 mg/kg/day.

Introduction

Angiogenesis is an important component of certain normal physiological processes such as embryogenesis, wound healing, and the female reproductive cycle but also contributes to some pathological disorders and in particular to tumor growth.[1,2] VEGF-A (vascular endothelial growth factor A) has been identified as a key factor promoting neovascularization of many tumors.[3−7] VEGF activates endothelial cells by signaling through two high affinity receptors, the fms-like tyrosine kinase receptor, Flt-1, and the kinase insert domain-containing receptor, KDR.[8,9] These signaling responses are critically dependent upon receptor dimerization and activation of intrinsic receptor tyrosine kinase (RTK) activity.

Several approaches, currently being evaluated in the clinic, are directed at blocking either the interaction of VEGF with its receptors (VEGF-Ab,[10] VEGF-receptor-Ab)[11,12] or the intracellular signaling process that follows receptor activation (RTK inhibitors).

Three orally bioavailable small molecules that can interfere with VEGF RTK activity, the phthalazine CGP79787/PTK787 (Flt-1 ∼ KDR),[13,14] the indolinone SU6668[15,16] (predominantly a c-kit, PDGFRβ inhibitor; PDGFR ≫ KDR), and the anilinoquinazoline **2** (ZD6474),[17] are being examined in patients with several types of cancer (Figure 1). The discovery and properties of compound **2** are described in this paper.

We recently reported[18,19] that 4-anilinoquinazolines can be potent nanomolar inhibitors of the Flt-1 and KDR enzymes. We showed that both of these enzymes are quite tolerant with regard to the nature and size of the substituent present at the C-7 position of the quinazoline nucleus (Figure 2). In this paper, we focus on the

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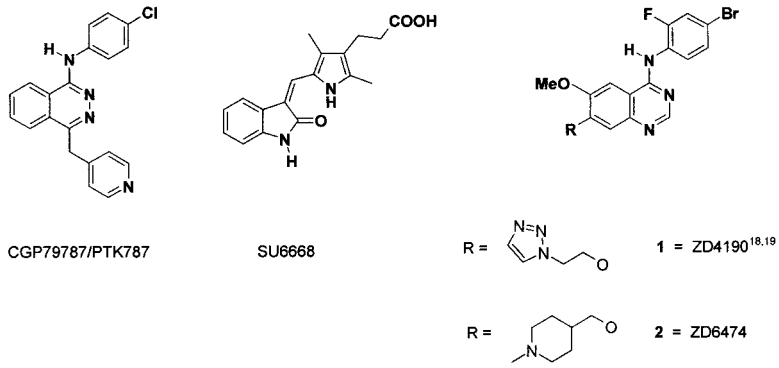


Figure 1.

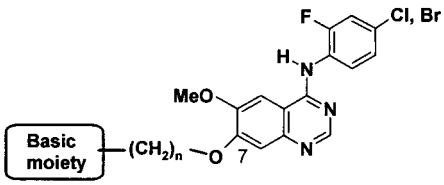


Figure 2.

physicochemical properties, in vitro profile, and in vivo activity of a series of anilinoquinazolines that possess basic side chains at the C-7 position of the quinazoline nucleus (Figure 2).

Chemistry

The compounds described in Table 1 were usually prepared by two main routes described in Schemes 1−3. The first route involves the coupling of 7-hydroxy anilinoquinazoline **39**,[18] **40**,[18] **44**,[18] and **41**−**43** with a range of alcohols R^2-H (see Table 1) under Mitsunobu conditions. The second route is based on displacing the chlorine atom of the C-4-activated quinazoline **64** and **65** (Scheme 3) with different anilines (see Table 1).

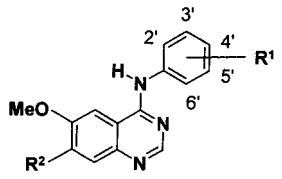
The 7-benzyloxy-4-chloro-6-methoxyquinazoline (**32**)[18] was reacted with anilines under acid catalysis in a protic solvent in the case of anilines with high or moderate nucleophilic character (2′-F, 4′-Cl; 2′-F, 4′-Br; 2′-F, 4′-Me). In the case of weakly nucleophilic anilines (2′,6′-F_2, 4′-Cl; 2′,6′-F_2, 4′-Br; 2′-F, 4′-CN), the sodium salts of the anilines were preformed in situ (NaH, dimethylformamide (DMF)) and then reacted with **32**[18] to give the corresponding C-7-benzyloxyanilinoquinazolines **33**,[18] **34**,[18] **38**,[18] and **35**−**37** (Scheme 1). Deprotection of the C-7-benzyl moiety was subsequently achieved using trifluoroacetyl (TFA) and led to the key intermediate 7-hydroxy-4-anilinoquinazolines **39**,[18] **40**,[18] **44**,[18] and **41**−**43** (Scheme 1). Reaction of **39**−**43** with a range of alcohols under Mitsunobu conditions using either diethyl or di-*tert*-butyl azodicarboxylate or the more hydrophilic 1,1′-(azodicarbonyl)dipiperidine led to **6**, **10**−**12**, and **29**−**31** (Scheme 2). Using similar reaction conditions, the N-protected piperidines **52**[20] and **53**[20] were coupled with **39**−**41**, **43**, and **44**[18] to give **21** and **46**−**49**. Compound **45** was obtained by coupling the 7-hydroxy-anilinoquinazoline **40**[18] with the tosylate piperidine **54**[21] under alkylation conditions (K_2CO_3, DMF). The *t*-BOC protecting group of **45**−**49** was subsequently removed by treatment with TFA to release the basic piperidine nitrogen (Scheme 2). Compounds **2**, **15**−**20**, and **26** were prepared as described in Scheme 3. The N-protected 4-piperidine methanol side chains **52** and **53**[20] or their tosylate-activated counterparts **54**[21] and **55**[22] were first coupled at the C-7 position of the N-3-POM-protected quinazolone **51**[18] to give **56** and **57**. Subsequent selective deprotection of the *t*-Boc group of **56** and **57** led to **58** and **59**. N-methylation of the piperidine nitrogen using formaldehyde under reducing conditions led, respectively, to **60** and **61**. The quinazolone moiety of **60** and **61** was then unmasked using ammonia in methanol to give **62** and **63**, and the C-4 position of these quinazolones was subsequently chlorinated using thionyl chloride and DMF to give **64** and **65** (Scheme 3). Reaction of **64** and **65** with a range of aniline under either acid-catalyzed or basic conditions as described previously led to **2**, **15**−**20**, and **26** (Scheme 3). Compound **27** was obtained directly from **28** by methylating the piperidine nitrogen under Eschweiler−Clarke conditions (HCOOH, HCHO, reflux; Scheme 4). The anilinoquinazolines **4** and **5** were synthesized from the pivotal intermediates **39**[18] and **40.**[18] Alkylation of the C-7 position of **39**[18] and **40**[18] with 1-bromo-3-chloropropane gave **66** and **67**, respectively. Subsequent nucleophilic displacement of the aliphatic chlorine of **66** and **67** by heating in an excess of *N*-methylpiperazine led to the expected anilinoquinazolines (Scheme 5).

Results and Discussion

For clarity of discussion, data on only a limited, representative set of compounds are presented in Table 1 and used to describe the structure−activity relationship (SAR). When some trends are exemplified by single pairs of compounds, it is to be understood that more examples[23−25] exist to support the SAR described below. Where cited, selectivity ratios refer to the quotients of the respective IC_{50} values for enzyme (or cell growth) inhibition.

i. Inhibition of Receptor Kinase Activity in Vitro. As previously reported,[18] one of the preferred patterns of substitution of the aniline ring to optimize inhibition of the target enzymes consists of a small lipophilic, electron-deficient substituent at the C-2′ position combined with a larger lipophilic, electron-withdrawing atom at the C-4′ position such as chlorine or bromine. The same patterns were apparent with basic C-7 side chains. As shown by comparison of the 4′-cyano and 4′-bromo derivatives **12** and **11** (Table 1), replacement of hydrophilic electron-withdrawing substituents that possess marked π-character, by more lipophilic substituents with moderate electron-withdrawing char-

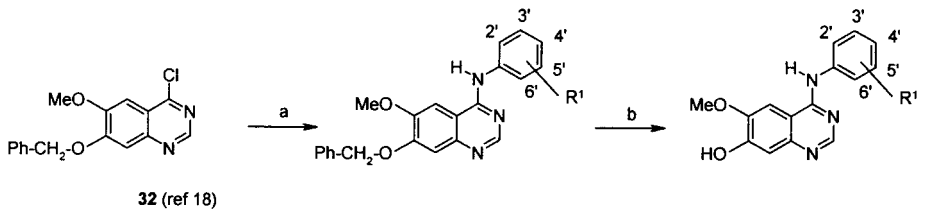
Table 1. Role of the C-7 Side Chain and Aniline Substitution on Enzyme Inhibition and HUVEC Activity



	R′							enzyme inhibition (2 μM ATP) IC_{50} μM [b]				inhibition of HUVEC growth IC_{50} μM [c]		
												stimulated by		unstimulated
no.	2′	4′	5′	6′	R^2	proced	formula [a]	Flt-1	KDR	FGFR	EGFR	VEGF	EGF	Basal
1	F	Br	H	H	1-(1,2,3-triazolyl)-$(CH_2)_2O$	ref 18	ref 18	0.7	0.03	>100	0.4	0.05	0.05	>10
2	F	Br	H	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	A	$C_{22}H_{24}N_4O_2BrF$, $0.5H_2O$, 1.8HCl	1.6	0.04	3.6	0.5	0.06	0.17	>3
3	F	Cl	H	H	$MeO(CH_2)_2O$	ref 18	ref 18	0.4	0.007	27	0.1	0.04	0.1	5
4	F	Cl	H	H	4-Me-piperazinyl-$(CH_2)_3O$	B	$C_{23}H_{27}N_5O_2ClF$, $0.7H_2O$, 2.75HCl	0.6	0.02	2.1	0.075	0.05	0.1	5
5	F	Br	H	H	4-Me-piperazinyl-$(CH_2)_3O$	B	$C_{23}H_{27}N_5O_2BrF$, $1H_2O$, 2.6HCl	0.55	0.009	2.0	0.1	0.03	0.09	>1
6	F	Cl	H	H	4-Me-piperazinyl-$(CH_2)_2$-O	C	$C_{22}H_{25}N_5O_2ClF$	0.3	0.03	8	0.2	0.1	0.2	10
7	F	Cl	H	H	4-morpholinyl-$(CH_2)_3O$	ref 18	ref 18	0.2	0.009	6.7	0.1	0.05	0.2	2
8	F	Cl	H	H	4-morpholinyl-$(CH_2)_2O$	ref 18	ref 18	1.1	0.04	19	0.4	0.3	0.3	5
9	F	Cl	H	H	1-pyrrolidinyl-$(CH_2)_3O$	D	$C_{22}H_{24}N_4O_2ClF$, $1.8H_2O$, 1.5HCl	0.2	0.03	4.2	0.1	0.008	0.1	5
10	F	Cl	H	H	$(CH_2)_4N-CH_2CH{=}CH-CH_2O$	E	$C_{23}H_{24}N_4O_2ClF$, $0.6H_2O$, 1.85HCl	0.75	0.04	1.9	0.09	0.04	0.08	1
11	F	Br	H	H	$(CH_2)_4N-CH_2CH{=}CH-CH_2O$	F	$C_{23}H_{24}N_4O_2BrF$, $0.5H_2O$, 2HCl	0.65	0.008	2.1	0.1	0.03	0.09	5
12	F	CN	H	H	$(CH_2)_4N-CH_2CH{=}CH-CH_2O$	E	ND[d]	3.9	0.23	19	0.8	0.5	1.4	10
13	F	Cl	H	H	4-pyridyl-N(Me)-$(CH_2)_2O$	ref 18	ref 18	0.1	0.006	1.6	0.3	0.2	0.2	5
14	F	Cl	H	H	$MeN(CH_2CH_2)_2CH\text{-}O$	ref 18	ref 18	1.5	1.5	50				
15	F	Cl	H	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	A	$C_{22}H_{24}N_4O_2ClF$, $0.4H_2O$, 2HCl	2	0.015	4.3	0.3	0.05	0.1	5
16	F	Me	H	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	G	$C_{23}H_{27}N_4O_2F$, $0.3H_2O$	0.5	0.025	13.5	1	0.065	0.4	>5
17	F	Cl	OH	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	A	$C_{22}H_{24}N_4O_3ClF$, 2HCl, $0.5C_3H_8O$, $0.8\ H_2O$	0.02	0.008	0.9	0.1	0.003	0.07	0.5
18	F	Me	OH	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	A	$C_{23}H_{27}N_4O_3F$, 1.95HCl, $0.65C_3H_8O$, $1H_2O$	0.025	0.007	1.5	0.3	0.003	0.2	0.8
19	F	Cl	H	F	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	H	$C_{22}H_{23}N_4O_2ClF_2$	0.3	0.025	1.7	0.45	0.06	0.3	>5
20	F	Br	H	F	$MeN(CH_2CH_2)_2CH\text{-}CH_2O$	C	$C_{22}H_{23}N_4O_2BrF_2$	0.5	0.01	1.5	0.3	0.1	0.4	>1
21	F	Cl	H	H	$HN(CH_2CH_2)_2CH\text{-}CH_2O$	I	$C_{21}H_{22}N_4O_2ClF$, 2.25HCl	1	0.05	5.0	0.3	0.06	0.1	5
22	F	Br	H	H	$HN(CH_2CH_2)_2CH\text{-}CH_2O$	J	$C_{21}H_{22}N_4O_2BrF$	0.9	0.07	6	0.2	0.025	0.1	5
23	F	Me	H	H	$HN(CH_2CH_2)_2CH\text{-}CH_2O$	J	ND[d]	0.7	0.03	12.3	0.5	0.25	0.45	>5
24	F	Cl	H	F	$HN(CH_2CH_2)_2CH\text{-}CH_2O$	J	ND[d]	0.45	0.04	2.5	0.5	0.1	0.6	>1
25	F	Br	H	F	$HN(CH_2CH_2)_2CH\text{-}CH_2O$	J	ND[d]	0.8	0.015	1.9	0.45	0.07	0.4	3
26	F	Cl	H	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2CH_2O$	A	ND[d]	0.5	0.055	4.1	0.3	0.07	0.2	3
27	F	Br	H	H	$MeN(CH_2CH_2)_2CH\text{-}CH_2CH_2O$	K	$C_{23}H_{26}N_4O_2BrF$, $0.1H_2O$, $0.15C_4H_{10}O$	0.15	0.006	2.1	0.2	0.06	0.15	3
28	F	Br	H	H	$HN(CH_2CH_2)_2CH\text{-}CH_2CH_2O$	L	$C_{22}H_{24}N_4O_2BrF$, $0.54H_2O$, $0.26CH_4O$	0.2	0.04	2.7	0.2	0.04	0.08	3
29	F	Cl	H	H	(R) $MeN(CH_2)(CH_2)_3CH\text{-}CH_2O$	E	$C_{22}H_{24}N_4O_2ClF$[e]	0.7	0.005	1.8	0.1	0.08	0.15	5
30	F	Br	H	H	(R) $MeN(CH_2)(CH_2)_3CH\text{-}CH_2O$	E	$C_{22}H_{24}N_4O_2BrF$	0.5	0.001	1.8	0.2	0.06	0.15	10
31	F	Br	H	H	(S) $MeN(CH_2)(CH_2)_3CH\text{-}CH_2O$	E	$C_{22}H_{24}N_4O_2BrF$	0.5	0.01	5.0	0.1	0.04	0.15	1

[a] C, H, and N analyses were obtained for every compound and were within ±0.4% of the theoretical value unless otherwise stated. [b] Values are averages from at least three independent dose−response curves; variation was generally ±10% for Flt-1 and ±20% for KDR, EGFR, and FGFR. [c] Values are averages from at least three independent dose−response curves; variation was generally ±15% for VEGF and EGF. [d] ND, not determined. Purity was assessed as >98 ± 3% by ^1H NMR at 400 MHz. [e] C: calcd, 61.3%; found, 60.7%.

Scheme 1[a,b]



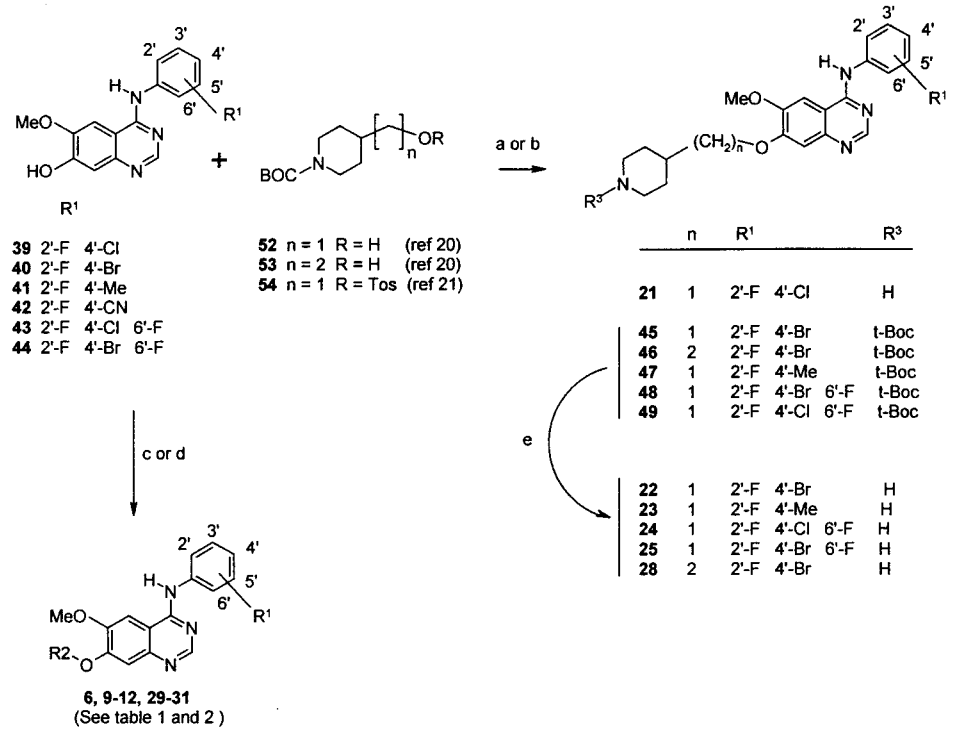
32 (ref 18)

	R1		
33	2'-F	4'-Cl	(ref 18)
34	2'-F	4'-Br	(ref 18)
35	2'-F	4'-Me	
36	2'-F	4'-CN	
37	2'-F	4'-Cl	6'-F
38	2'-F	4'-Br	6'-F (ref 18)

	R1		
39	2'-F	4'-Cl	(ref 18)
40	2'-F	4'-Br	(ref 18)
41	2'-F	4'-Me	
42	2'-F	4'-CN	
43	2'-F	4'-Cl	6'-F
44	2'-F	4'-Br	6'-F (ref 18)

[a] Reagents: (a) R^1-Ar-NH_2/*i*PrOH/HCl/reflux (procedure A or G) or R^1-Ar-NH_2/NaH/DMF (procedure H). [b] TFA (procedure L or J).

Scheme 2[a]



39 2'-F 4'-Cl
40 2'-F 4'-Br
41 2'-F 4'-Me
42 2'-F 4'-CN
43 2'-F 4'-Cl 6'-F
44 2'-F 4'-Br 6'-F

52 n = 1 R = H (ref 20)
53 n = 2 R = H (ref 20)
54 n = 1 R = Tos (ref 21)

	n	R1		R3
21	1	2'-F	4'-Cl	H
45	1	2'-F	4'-Br	t-Boc
46	2	2'-F	4'-Br	t-Boc
47	1	2'-F	4'-Me	t-Boc
48	1	2'-F	4'-Br 6'-F	t-Boc
49	1	2'-F	4'-Cl 6'-F	t-Boc
22	1	2'-F	4'-Br	H
23	1	2'-F	4'-Me	H
24	1	2'-F	4'-Cl 6'-F	H
25	1	2'-F	4'-Br 6'-F	H
28	2	2'-F	4'-Br	H

6, 9-12, 29-31
(See table 1 and 2)

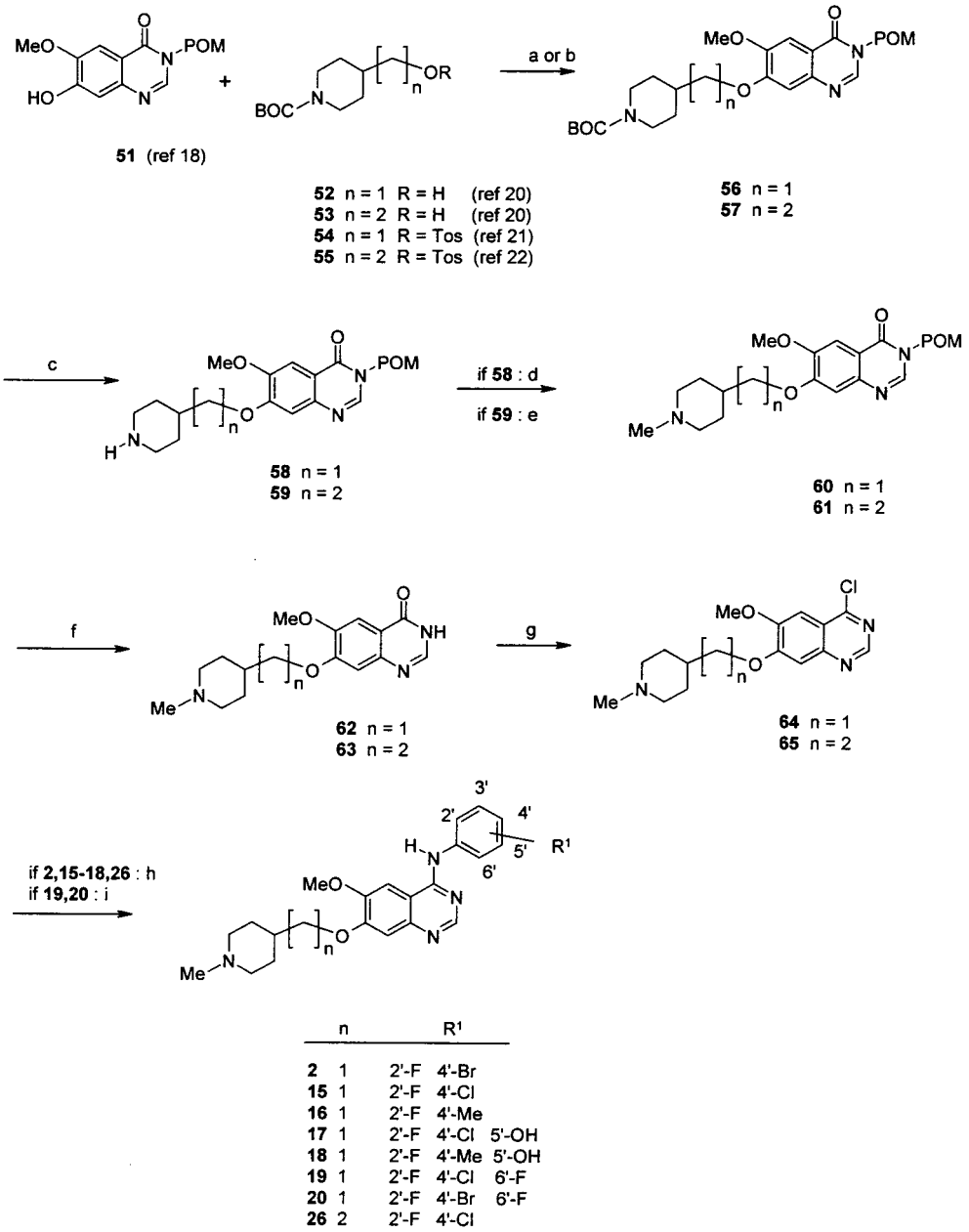
[a] Reagents: (a) DMF/K_2CO_3 (procedure M). (b) i. Ph_3P/DEAD; ii. TFA (procedure I). (c) R^2OH (see Table 1)/*n*-Bu_3P/ADDP (procedure C) or Ph_3P/DEAD (procedure E or F). (d) 1-(3-Chloropropyl)pyrrolidine/DMF/K_2CO_3 (procedure D). (e) TFA (procedure J or L).

acter, led to marked improvement in both Flt-1 and KDR inhibition (up to 350-fold). A previous development candidate (**1**, ZD4190;[18] Table 1) possessed one of the preferred substitution patterns (C-2′-F, C-4′-Br) and proved to be a very potent inhibitor of KDR (Table 1). It also inhibited, but to a lesser extent, the TK associated with the endothelial growth factor receptor (EGFR) (KDR:EGFR selectivity ratio ~ 13). This pattern of substitution also led in the C-7 neutral series to high selectivity for KDR against FGFR-1 (fibroblast growth factor receptor TK) (**1** and **3**: selectivity ratios > 1000; Table 1). Introduction of a hydroxyl substituent at the meta position of the aniline led to an improvement in Flt-1 inhibition by 20−100-fold (comparison of **15**−**18**) while improving KDR inhibition by only 2−4-fold. Introduction of an additional fluorine atom at the C-6′ position of the aniline in general increased the activity against Flt-1 over KDR (2−7-fold; comparison of **19**, **20**, and **24** and **15**, **2**, and **21**).

The 1,2,3-triazole-ethoxy side chain present at the C-7 position of **1** is a typical neutral side chain commonly used in medicinal chemistry. This side chain confers on **1** high and sustained plasma levels in mouse following oral administration (13 μM at 24 h following 100 mg/kg).[18,19] Such plasma levels combined with nanomolar inhibition of growth factor-stimulated endothelial cell proliferation led to profound inhibition of growth of human tumor xenografts in athymic mice with once daily chronic oral administration.[18,19] However, the neutral nature of the 1,2,3-triazole-ethoxy side chain conferred on **1** suboptimal physicochemical properties that we believe were principally responsible for variable pharmacokinetic properties across species (see the sections on physicochemical properties and PK properties, Tables 3−5). We therefore turned our attention to C-7 side chains that might provide VEGF RTK inhibitors with improved physical properties.

Introduction of nitrogen-containing, basic residues in

Scheme 3[a]

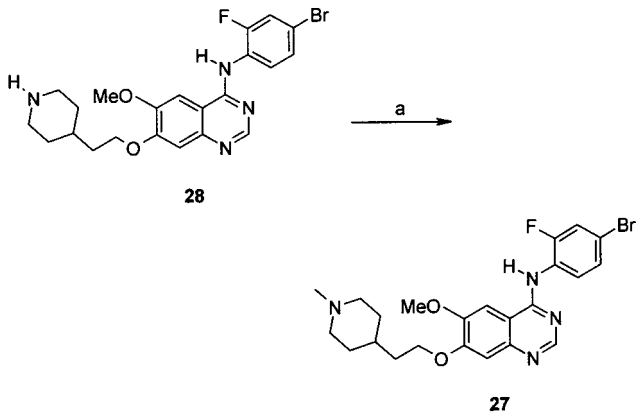


n		R^1
2	1	2'-F 4'-Br
15	1	2'-F 4'-Cl
16	1	2'-F 4'-Me
17	1	2'-F 4'-Cl 5'-OH
18	1	2'-F 4'-Me 5'-OH
19	1	2'-F 4'-Cl 6'-F
20	1	2'-F 4'-Br 6'-F
26	2	2'-F 4'-Cl

[a] Reagents: (a) **54**: Ph_3P/CH_2Cl_2/DEAD. (b) **55**: K_2CO_3/DMF. (c) TFA. (d) MeOH/THF/HCHO/$NaBH_3CN$. (e) HCHO/$NaB(OAc)_3$/AcOH. (f) MeOH/NH_3. (g) $SOCl_2$/DMF. (h) R^1-Ar-NH_2/*i*PrOH/HCl (procedure A or G). (i) NaH/DMF (procedure H).

the C-7 side chain covering a range of pK_a's from 7 to 9.5 (Table 3), as illustrated by the morpholines (**7** and **8**),[18] the pyrrolidine (**9**), and the piperidine (**30**, **2**), retained an excellent level of KDR inhibition (median IC_{50} 0.02 μM, range 0.001−0.03 μM; Table 1). The KDR enzyme proved to be consistently more sensitive than Flt-1 (median IC_{50} 0.55 μM, range 0.02−1.6 μM) with Flt-1:KDR selectivity ratios ranging from 5 to 500. The most marked separation was observed with the cyclic *N*-methylpiperidine, and in particular piperidine methoxy derivative **30**, due to a slightly weaker activity against Flt-1 (comparison of **15** and **2** with **9** and **24**). C-7 side chains containing nitrogens that can be protonated or hydrated tend to be slightly more potent against Flt-1 (comparison of **1** and **3** with **7**, **4**, **9**, and **24**). Basic heteroaromatic nuclei, as illustrated by the pyridine-containing derivative **13**,[18] were also potent inhibitors of KDR.

Scheme 4[a]



[a] HCOOH, HCHO, reflux (procedure K).

Table 2. Selectivity Profile in a Panel of Kinase Enzymes of Compounds **1** and **2**

	enzyme inhibition[a] (IC_{50} μM[b])											
no.	Flt-1	KDR	EGFR	Tie-2	FGFR-1	PDGFRβ	erbB2	MEK	CDK2	IGF-1R	AKT	PDK
1	0.7 ± 0.06	0.03 ± 0.004	0.4 ± 0.1	>100	>100	3.4 ± 0.3	>100	>10	>10	>20	>20	>20
2	1.6 ± 0.4	0.04 ± 0.01	0.5 ± 0.1	2.5 ± 1.2	3.6 ± 0.9	1.1 ± 0.3	>20	>20	>20	>20	>20	>20

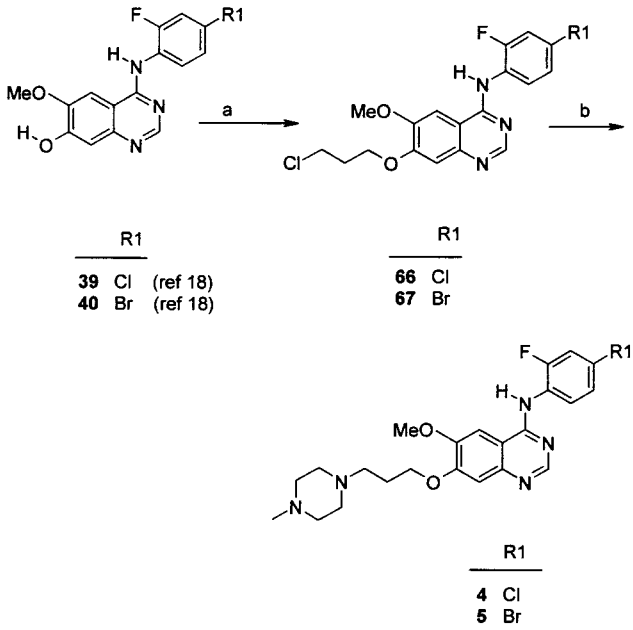
[a] ATP concentrations used are all <Km and ranged from 0.2 to 8 μM; for details for each enzyme, see Experimental Section. [b] Values are averages of at least three independent dose−response curves.

Table 3. Physicochemical Parameters of Representative Anilino-Quinazolines

no.	C7 side chain functionality	pK_a's	LogD[a]	LogP[b]	aqueous solubility at pH 7.4[c,d] (μM)	relative calculated solubility of the neutral form[e]
1	triazole	5.3	4.6	4.6	0.7	1
7	morpholine	7.2−5.2	3.4	3.6	20	
4	piperazine	7.9−5.4	2.4[f]	3.3	60[g]	
2	piperidine	9.4−5.3	2.6[f]	5.0	330	17[h]

[a] Measured at pH 7.4 unless otherwise stated. [b] Calculated from LogD and pK_a. [c] Average of at least three measurements using the free base. [d] Measured at 3 days unless otherwise stated. [e] Solubility of the neutral form normalized to the solubility of the neutral form of **1**. [f] Measured at pH 7. [g] Measured at 1 day. [h] Solubility of the neutral form of compound **2** was measured at pH 12.7.

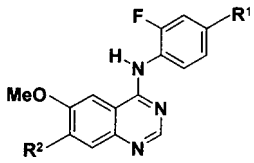
Scheme 5[a]



[a] Reagents: (a) Cl-$(CH_2)_3$-Br/K_2CO_3/DMF. (b) *N*-Methylpiperazine/100 °C (procedure B).

The *N*-methylpiperidine nucleus proved to be particularly interesting. When directly linked to the C-7 position of the quinazoline, it resulted in a weaker inhibitor of both Flt-1 and KDR (compound **14**:[18] IC_{50} 1.5 μM). An increase in flexibility of the side chain, resulting from the insertion of a single methylene between the piperidine ring and the C7−O atom, provided a 38−100-fold increase in potency against KDR with no enhancement of activity against Flt-1 (comparison of **14**[18] with **2** and **15**); insertion of two methylenes (**27**) increased potency against KDR 250-fold and Flt-1 by 10-fold. 3-Piperidinyl isomers were more potent than their 4-isomers (compare **2**, **30**, and **31**), and chirality of the 3-piperidine side chain also influenced potency, the (*R*)-enantiomer **30** being 10-fold more potent of an inhibitor than the (*S*)-enantiomer **31**. In the 2′-F, 4′-Cl, or 4′-Br series, alkylation of the C-7 side chain piperidine−nitrogen suggests an increase in selectivity of 3−7-fold for KDR over Flt-1 (Flt-1/KDR IC_{50} ratio's, respectively, 40- and 13-fold for **2** and **22**; 133- and 20-fold for **15** and **21**; and 25- and 5-fold for **27** and **28**). This effect is not apparent in the difluorinated series (2′-F, 4′-F, 4′-Cl, or 4′-Br series: comparison of **19** and **24** and **20** and **25**).

Table 4. Mouse Plasma Levels Following Administration of 50 or 100 mg/kg Po



			dose	plasma levels (μM)[a]	
no.	R^1	R^2	(mg/kg)	at 6 h	at 24 h
1	Br	1-(1,2,3-triazolyl)-$(CH_2)_2O$	50	14	0.2
2	Br	MeN$(CH_2CH_2)_2$CH-CH_2O	50	5.5	3
3	Cl	MeO-$(CH_2)_2$-O	100	12	<0.1
4	Cl	4-Me-piperazinyl-$(CH_2)_3O$	50	5	3
7	Cl	4-morpholinyl-$(CH_2)_3O$	100	4	0.8
8	Cl	4-morpholinyl-$(CH_2)_2O$	100	12	0.5
13	Cl	4-pyridyl-N(Me)-$(CH_2)_2O$	100	4.6	2
27	Br	MeN$(CH_2CH_2)_2$CH-CH_2CH_2O	50	3	2
30	Br	(*R*)-MeN$(CH_2)(CH_2)_3$CH-CH_2O	50	5.5	2.5

[a] Variation was generally ±15%.

Table 5. Pharmacokinetic Parameters in Rat and Dog of **1** and **2**

	rat [a]		dog[a]	
no.	IV $t_{1/2}$ (h)	F (%)	IV $t_{1/2}$ (h)	F (%)
1	~1.3 ± 0.2[b]	>80[b]	1.1 ± 0.2[c]	<5[c]
2	~15 ± 0.2[b]	>50[b]	8.3 ± 1.8[b]	>50[b]

[a] Measured on the free base. For dose and vehicles, see Experimental Section. [b] Average of three males. [c] Average of four males.

All of the compounds described in this paper are also inhibitors of the EGFR enzyme (median IC_{50} 0.2 μM, range 0.075−0.8 μM), but in all cases, potency against KDR was greater (KDR:EGFR selectivity ratios 3−200). Inhibition of the FGFR-1 enzyme was consistently weaker (median IC_{50} 2.5 μM, range 0.9−19 μM; KDR:FGFR selectivity ratios 33−1800; Table 1), but by comparison with the C-7 neutral side chain series (e.g., **1** and **3**), selectivity with respect to FGFR is slightly reduced in the basic side chain series.

Selectivity of representative compounds, and **2** in particular, against a broad panel of kinases, has also been investigated. As shown in Table 2, compounds such

as **2** and **1** possess good selectivity profiles and are devoid of significant activity against kinases such as MEK, AKT, and erbB2 or kinases involved in cell cycle regulation such as CDK-2. Similarly, no significant activity was detected against the kinase enzymes associated with the angiopoietin receptor (Tie-2) and the IGF-1 receptor.

ii. Inhibition of Endothelial Cell Proliferation. Selectivity for the VEGF, EGF, and FGF pathways in whole endothelial cells was investigated using growth factor-stimulated human endothelial cells (HUVECs). Inhibition of proliferation was again achieved with nanomolar concentrations (median IC_{50} vs VEGF 0.06 μM, range 0.003−0.5 μM), and the selectivity profile observed in the kinase assays was reasonably conserved in HUVECS (VEGF > EGF; Table 1), the median IC_{50} vs EGF being 0.15 μM (range 0.07−1.4 μM). The best selectivity ratios were observed with the *meta*-hydroxy derivatives **17** and **18** (VEGF:EGF ratios of 23 and 66, respectively). Selectivity for VEGF vs FGF inhibition was also confirmed in these cells. In the case of **1** and **2**, concentrations of 1.5 and 0.8 μM were necessary to reduce FGF-stimulated growth by 50% giving VEGF: FGF selectivity ratios of 30 and 13, respectively. The slight changes in selectivity occasionally observed between recombinant enzyme and whole cell data can be attributed to differences (e.g., ATP concentration) between the two assays.

The compounds presented in this paper do not significantly affect basal, unstimulated growth of HUVECs (IC_{50} range: 1 to >10 μM; Table 1), which clearly suggests that activity is specifically directed to the receptor-activated signaling pathways and that the compounds do not impart any direct cytostatic or cytotoxic effect.

iii. Physicochemical Properties. As shown in Table 3, the lipophilic character of **1** as well as the neutral nature of its C-7 side chain (1-(1,2,3-triazolyl)-ethoxy) confers low aqueous solubility on this compound (∼0.7 μM). Nevertheless, good plasma levels were seen in mouse following oral dosing.[18,19] Its bioavailability in rats was also good, but in higher species, it was found to be low and variable. The low aqueous solubility of **1** was thought to be the major factor contributing to this inconsistency (see the section on pharmacokinetics). C-7 basic side chains proved extremely effective for increasing both solubility and dissolution rate. As expected, aqueous solubility increased proportionally to the pK_a of the basic function present at C-7. Side chains such as the morpholinyl-propoxy and the *N*-methylpiperazinyl-propoxy (compounds **4** and **7**, Table 3) led to 30- and ∼80-fold, respectively, increases in solubility relative to **1**. This is attributed to partial protonation at pH 7.4 and a reduction in overall lipophilicity of these molecules as compared to **1**. Introducing more basic functions, such as *N*-methylpiperidine methoxy (compound **2**, Table 3), that are almost completely protonated at physiological pH increased aqueous solubility even more markedly. For compound **2**, solubility (>5 mM at pH 3.6 and ∼330 μM at pH 7.4) was ∼470-fold that of **1**. As compounds are absorbed through the gut wall predominantly in the nonprotonated form, it was also advantageous if the solubility of the neutral form present at the site of absorption was improved over compounds such as **1**. As shown in Table 3, the 4-methylpiperidine-methoxy side chain in **2** also provided a 17-fold improvement in aqueous solubility of the neutral form as compared to **1**. At intestinal pH (∼6.5), the solubility of **2** is ∼650 μM (310 μg/mL).

iv. Serum Protein Binding (SPB) in Different Species. Introduction of a basic function at the C-7 position of the anilinoquinazoline nucleus, such as the 4-methylpiperidine-methoxy (pK_a, 9.4) of **2**, led to good levels of free drug in rodent plasma, in part by virtue of the positive charge present on the C-7 side chain at physiological pH. Comparison of the neutral (**1**) and basic (**2**) anilinoquinazolines indicates a 2-fold higher level of unbound basic drug despite a slight (∼0.4 unit) increase in logP (Mouse SPB: **1**, 98 ± 0.5%; **2**, 96 ± 0.5%. Rat SPB: **1**, 95 ± 0.5%; **2**,[26] 90 ± 0.5%; mean ± SE, n = 5). Free plasma levels were consistently about 2-fold higher in rat than in mouse.

v. Blood Levels in Mice Following Oral and Intravenous Administration. We reported that anilinoquinazolines with neutral or weakly basic C-7 side chains and **1** in particular were orally bioavailable in mice.[18] Anilinoquinazolines possessing more basic C-7 moieties also yielded good plasma levels in mice following oral administration (Table 4), which declined more slowly than those with neutral or weakly basic side chains (compare 24 h levels of **1**, **3**, and **8** with those of **2**, **4**, **13**,[18] and **30**). As we previously reported,[19] prolonged exposure combined with good intrinsic potency was particularly important for good efficacy in the Calu-6 tumor xenograft model with once daily oral administration.

vi. Bioavailability and Half-Lives in Rat and Dog. The pharmacokinetic profile of **1** in the mouse following oral dosing at 50 mg/kg suggested rapid elimination in this species. This was confirmed in the rat where rapid clearance (CL ∼ 3.2 L/hr/kg), moderate plasma half-life ($t_{1/2}$ ∼ 1.3 h), and a low volume of distribution (∼1.2 L/kg) were observed. The half-life of **1** was also short in the dog (Table 5), and its bioavailability was low (<5%) and variable. Attempts to increase the aqueous solubility and bioavailability of **1** by making salts was successful in these respects but introduced stability and hygroscopicity issues that did not auger well for development.

In the case of the more soluble 4-anilinoquinazolines possessing C-7 basic side chains such as **2**, the improvement in physicochemical properties, in particular better aqueous solubility, was accompanied by good (>50%) and more consistent bioavailability in rat and dog (Table 5). Moreover, and probably as a consequence of protonation of the C-7 basic moiety, plasma half-lives in rat and dog proved to be longer than those observed in the neutral side chain series. For example, in rat and dog, **2** had half-lives 12- and 8-fold, respectively, higher than **1**.

vii. In Vivo Efficacy in Mouse. We have previously reported that **1** was effective at reducing the growth of a number of human tumor xenografts grown subcutaneously in athymic mice.[18,19] The antitumor activity of the anilinoquinazolines reported here was routinely examined using a Calu-6 lung tumor xenograft model. As shown in Table 6, once daily oral administration of **2** for 21 days inhibited the growth of established (∼0.2

Table 6. Growth Inhibition of Established Calu-6 Tumor Xenografts Implanted Subcutaneously in Athymic Mice, Measured after 21 Days of Dosing of **2**

	% inhibition of tumor growth @ mg/kg/day[a] po			
no.	100	50	25	12.5
2	79[b]	67[b]	51[b]	36[c]

[a] Percent inhibition represents the difference between the median of 10 control (vehicle-treated) and 9−10 compound-treated mice, following 21 days of treatment; measured from start of treatment. [b] $p < 0.001$. [c] $p < 0.02$ (Mann Whitney rank sum one-tailed test).

cm^3) Calu-6 tumors in a dose-dependent manner. Tumor growth was almost completely arrested at a dose of 100 mg/kg/day and more than halved at 25 mg/kg/day. Compound **2** has been evaluated against a range of human tumor xenografts including colon, lung, breast, prostate, and ovary tumors and has demonstrated highly significant activity in each model.

Compound **2** does not inhibit the growth of Calu-6 cells directly in vitro (IC_{50} 14 ± 0.4 μM; mean ± SE, $n = 3$) at concentrations of compound likely to be freely available in vivo. Hence, the tumor growth inhibition observed in vivo, even taking into account possible accumulation of the compound at the highest doses evaluated, cannot be attributed to a direct cytotoxic or cytostatic effect on the tumor cells. Furthermore, we have shown that selective inhibitors of VEGF receptor kinases, completely devoid of EGFR inhibition (IC_{50} > 10 μM), inhibit the growth of Calu-6 tumors when administered orally,[23,24,26] whereas, over a similar dose range, selective EGFR inhibitors devoid of VEGF receptor inhibition (IC_{50} > 10 μM) do not. The activity observed in the Calu-6 xenograft model with a compound possessing activity against VEGF and EGF tyrosine kinase such as **2** is thus most likely attributable to the inhibition of the VEGF signaling pathway.

Conclusions

Anilinoquinazolines possessing C-7 basic side chains are potent nanomolar inhibitors of KDR tyrosine kinase, and some are also potent inhibitors of Flt-1 tyrosine kinase. Their enzyme inhibition profiles translate very well into endothelial cells as demonstrated by nanomolar inhibition of VEGF-stimulated HUVEC proliferation. The presence of a basic side chain confers excellent physicochemical properties, in particular, good aqueous solubility, improved bioavailability in rodent and dog, and reduced protein binding. The plasma half-life of this subseries of anilinoquinazolines is generally longer than that of anilinoquinazolines with neutral side chains. In view of its excellent physicochemical properties, selectivity profile, pharmacokinetics, and antitumor activity in preclinical models, **2** was preferred over **1** and replaced it in development. Compound **2** is currently undergoing phase I clinical evaluation.[17]

Experimental Section

For general procedures, see the Supporting Information section.

***N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (2). Procedure A.** A mixture of 4-chloro-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazoline **(64**; 200 mg, 0.62 mmol) and 4-bromo-2-fluoroaniline (142 mg, 0.74 mmol) in 2-propanol (3 mL) containing 6 M hydrogen chloride in 2-propanol (110 μL, 0.68 mL) was heated at reflux for 1.5 h. The mixture was cooled, and the solid was filtered, washed with 2-propanol (2 mL) followed by diethyl ether (2 mL), and dried under vacuum overnight at 50 °C to give 304 mg of **2** as an hydrochloride salt (90%).

The nuclear magnetic resonance (NMR) spectrum of the protonated form of **2** hydrochloride shows the presence of two forms (A and B) in a ratio A:B of approximately 9:1 attributable to the protonation of the basic side chain. ^{1}H NMR: δ 1.60−1.78 (m, form A 2H), 1.81−1.93 (br s, form B 4H), 1.94−2.07 (d, form A 2H), 2.08−2.23 (br s, form A 1H), 2.29−2.37 (br s, form B 1H), 2.73 (d, form A 3H), 2.77 (d, form B 3H), 2.93−3.10 (q, form A 2H), 3.21 (br s, form B 2H), 3.27 (br s, form B 2H), 3.42−3.48 (d, form A 2H), 4.04 (s, 3H), 4.10 (d, form A 2H), 4.29 (d, form B 2H), 7.49 (s, 1H), 7.53−7.61 (m, 2H), 7.78 (d, 1H), 8.47 (s, 1H), 8.81 (s, 1H), 10.48 (br s, form A 1H), 10.79 (br s, form B 1H), 11.90 (br s, 1H). Anal. ($C_{22}H_{24}N_4O_2$-BrF, 0.5 H_2O, 1.8 HCl, 0.08 2-propanol) C, H, N.

For another NMR reading, some solid potassium carbonate was added into the dimethyl sulfoxide (DMSO) solution of the **2** hydrochloride described above, to release the free base in the NMR tube. The NMR spectrum was then recorded again and showed only one form as described below. ^{1}H NMR (DMSO-d_6; solid potassium carbonate): δ 1.30−1.45 (m, 2H), 1.75 (d, 2H), 1.70−1.90 (m, 1H), 1.89 (t, 2H), 2.18 (s, 3H), 2.80 (d, 2H), 3.98 (s, 3H), 4.0 (d, 2H), 7.20 (s, 1H), 7.48 (d, 1H), 7.55 (t, 1H), 7.68 (d, 1H), 7.80 (s, 1H), 8.35 (s, 1H), 9.75 (s, 1H).

A sample of **2** (free base) was generated from the **2** hydrochloride, prepared as described above, as follows: **2** hydrochloride (50 mg) was suspended in methylene chloride (2 mL) and was washed with saturated sodium hydrogen carbonate. The methylene chloride solution was dried ($MgSO_4$), and the volatiles were removed by evaporation to give **2** (free base). The NMR of **2** (free base) so generated shows only one form, the NMR spectrum of which is identical to that described above. ^{1}H NMR: δ 1.30−1.45 (m, 2H), 1.76 (d, 2H), 1.70−1.90 (m, 1H), 1.90 (t, 2H), 2.19 (s, 3H), 2.80 (d, 2H), 3.95 (s, 3H), 4.02 (d, 2H), 7.20 (s, 1H), 7.48 (d, 1H), 7.55 (t, 1H), 7.68 (dd, 1H), 7.80 (s, 1H), 8.38 (s, 1H), 9.55(br s, 1H).

For another NMR reading, some CF_3COOD was added into the NMR DMSO solution of **2** (free base) described above and the NMR spectrum was recorded again. The spectrum of the protonated form of the trifluoroacetate salt of **2** so obtained shows the presence of two forms (A and B) in a ratio A:B of approximately 9:1. ^{1}H NMR (DMSO-d_6; CF_3COOD): δ 1.50−1.70 (m, form A 2H), 1.93 (br s, form B 4H), 2.0−2.10 (d, form A 2H), 2.17 (br s, form A 1H), 2.35 (br s, form B 1H), 2.71 (s, form A 3H), 2.73 (s, form B 3H), 2.97−3.09 (t, form A 2H), 3.23 (br s, form B 2H), 3.34 (br s, form B 2H), 3.47−3.57 (d, form A 2H), 4.02 (s, 3H), 4.15 (d, form A 2H), 4.30 (d, form B 2H), 7.2 (s, 1H), 7.3−7.5 (m, 2H), 7.60 (d, 1H), 7.90 (s, 1H), 8.70 (s, 1H).

A similar procedure was used to prepare **15**, **17**, **18, 26**, and **35**.

***N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-[3-(4-methylpiperazin-1-yl)propoxy]quinazolin-4-amine (4). Procedure B.** A suspension of 4-(4-chloro-2-fluoroanilino)-7-(3-chloropropoxy)-6-methoxyquinazoline **(66**; 150 mg, 0.38 mmol) in 1-methylpiperazine (2 mL) was heated at 100 °C for 3 h. The mixture was cooled and was partitioned between an aqueous solution of sodium carbonate (pH 11.5) and ethyl acetate. The organic layer was separated, washed with brine, and dried ($MgSO_4$), and the volatiles were removed by evaporation. The residue was dissolved in methylene chloride, and diethyl ether was added. The precipitate was filtered, washed with diethyl ether (2 mL), and dried. The solid was dissolved in methylene chloride, and 2.2 M hydrogen chloride in diethyl ether (1 mL) was added. After the solution was concentrated to half of its initial volume, the precipitate was filtered, washed with diethyl ether (2 mL), and dried under vacuum to give 158 mg of **4** hydrochloride (75%). ^{1}HNMR (DMSO-d_6; CF_3-COOD; 60 °C): δ 2.35 (m, 2H), 2.95 (s, 3H), 3.43 (t, 2H), 3.52−3.70 (m, 8H), 4.03 (s, 3H), 4.34 (t, 2H), 7.41 (s, 1H), 7.45 (d,

1H), 7.60−7.70 (m, 2H), 8.11 (s, 1H), 8.80 (s, 1H). MS−EI *m/z*: 460 $[MH]^+$. Anal. ($C_{23}H_{27}N_5O_2ClF$, 0.7 H_2O, 2.75 HCl) C, H, N.

A similar procedure was used to prepare **5**.

***N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-[2-(4-methylpiperazin-1-yl)ethoxy]quinazolin-4-amine (6). Procedure C.** A solution of 2-(4-methylpiperazin-1-yl)ethanol (112 mg, 0.78 mmol) in methylene chloride (1 mL) was added to a stirred suspension of *N*-(4-chloro-2-fluorophenyl)-7-hydroxy-6-methoxy-4-quinazolinylamine **(39**;[18] 225 mg, 0.7 mmol) and tributylphosphine (420 mg, 2.1 mmol) in methylene chloride (10 mL). 1,1′-(Azodicarbonyl)dipiperidine (525 mg, 2.1 mmol) was then added in portions to the mixture. The resulting clear, pale yellow solution was stirred for 3.5 h and then allowed to stand overnight. The reaction mixture was quenched with diethyl ether (8 mL), and the precipitate was filtered. The solvent was removed from the filtrate by evaporation, the residue was dissolved in acetone (5 mL), and 1 M hydrogen chloride in diethyl ether was added until the hydrochloride salt precipitated. The precipitate was collected by filtration, dissolved in methanol (3 mL), and then basified with excess triethylamine. The volatiles were removed by evaporation, and the residue was purified by column chromatography eluting with methylene chloride/methanol/aqueous ammonia (100/8/1). The resulting oil was triturated with diethyl ether (1 mL), collected by filtration, and dried to give 79 mg of **6** (25%) as a white solid; mp 173−175 °C. ^{1}H NMR: δ 2.10 (s, 3H), 2.30 (m, 4H), 2.50 (m, 4H), 2.75 (t, 2H), 3.95 (s, 3H), 4.25 (t, 2H), 7.21 (s, 1H), 7.30 (dd, 1H), 7.50 (d, 1H), 7.55 (dd, 1H), 7.75 (s, 1H), 8.30 (s, 1H), 9.50 (s, 1H). MS−ESI *m/z*: 446 $[MH]^+$. Anal. ($C_{22}H_{25}N_5O_2ClF$).

***N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-(3-pyrrolidin-1-ylpropoxy)quinazolin-4-amine (9). Procedure D.** A solution of 1-(3-chloropropyl)pyrrolidine (230 mg, 0.96 mmol) was added to *N*-(4-chloro-2-fluorophenyl)-7-hydroxy-6-methoxy-4-quinazolinylamine **(39**;[18] 295 mg, 0.92 mmol) and potassium carbonate (130 mg, 0.94 mmol) in DMF (8 mL). The mixture was heated at 100 °C for 90 min and allowed to cool. The volatiles were removed by evaporation, and the residues were partitioned between water (10 mL) and methylene chloride (10 mL). The organic phase was separated and passed through phase-separating paper, and the solvent was removed under reduced pressure. The residue was dissolved in acetone (2 mL), and 1 M hydrogen chloride in diethyl ether (2 mL) was added. The mixture was stirred at ambient temperature for 30 min, and the resulting precipitate was collected by filtration and dried to give 320 mg of **9** as a hydrochloride (67%). ^{1}H NMR: δ 1.80−2.0 (m, 6H), 3.0−3.60 (m, 6H), 4.05 (s, 3H), 4.30 (t, 2H), 7.40 (m, 2H), 7.55 (d, 1H), 7.60 (m, 1H), 8.40 (s, 1H), 8.80 (s, 1H). MS−ESI *m/z*: 431 $[MH]^+$. Anal. ($C_{22}H_{24}N_4O_2ClF$, 1.8 H_2O, 1.5 HCl) C, H, N.

***N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-{[(2*E*)-4-pyrrolidin-1-ylbut-2-enyl]oxy}quinazolin-4-amine (10). Procedure E.** Diethyl azodicarboxylate (5.91 mL, 37 mmol) was added dropwise to a stirred mixture of (*E*)-4-(pyrrolidin-1-yl)-but-2-en-1-ol **(68**; 3.97 g, 28 mmol), **39**[18] (3.0 g, 9 mmol), and triphenylphosphine (9.84 g, 38 mmol) in methylene chloride (300 mL). The reaction mixture was stirred for 18 h at ambient temperature. The volatiles were removed by evaporation, and the residue was purified by column chromatography eluting with methylene chloride/methanol (a gradient from 80/20 to 70/30). The purified product was dissolved in methylene chloride/methanol (10 mL/10 mL), and 1 M hydrogen chloride in diethyl ether (25 mL) was added. The precipitated product was collected by filtration, washed with diethyl ether (2 mL), and dried to give 1.62 g of **10** hydrochloride (33%). ^{1}H NMR (DMSO-d_6; CF_3COOD): δ 1.85−1.95 (m, 2H), 2.0−2.15 (m, 2H), 3.0−3.10 (m, 2H), 3.50−3.60 (m, 2H), 3.95 (d, 2H), 4.10 (s, 3H), 4.95 (d, 2H), 6.10 (td, 1H), 6.35 (td, 1H), 7.40 (s, 1H), 7.45 (dd, 1H), 7.60−7.70 (m, 2H), 8.15 (s, 1H), 8.90 (s, 1H). MS−EI *m/z*: 443 $[MH]^+$. Anal. ($C_{23}H_{24}N_4O_2ClF$, 0.6 H_2O, 1.85 HCl) C, H, N.

A similar procedure was used to prepare **12**, **29**−**31**, **46**, **47**, and **49**.

***N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-{[(2*E*)-4-pyrrolidin-1-ylbut-2-enyl]oxy}quinazolin-4-amine (11). Procedure F.** Diethyl azodicarboxylate (1.55 mL, 9.89 mmol), *N*-(4-bromo-2-fluorophenyl)-7-hydroxy-6-methoxy-4-quinazolinylamine **(40**;[18] 1.2 g, 3.3 mmol), and a solution of **68** (697 mg, 4.9 mmol) in methylene chloride (5 mL) were added successively to a solution of triphenylphosphine (2.59 g, 9.89 mmol) in methylene chloride (150 mL) cooled at 5 °C. The mixture was stirred at ambient temperature for 10 min, and then, methylene chloride (100 mL) was added followed successively by triphenylphosphine (432 mg, 1.6 mmol), (*E*)-4-(pyrrolidin-1-yl)but-2-en-1-ol (232 mg, 1.6 mmol), and diethyl azodicarboxylate (246 μL, 1.6 mmol). The mixture was stirred at ambient temperature for 30 min, and then, the solvent was removed by evaporation. The residue was purified by column chromatography eluting with methylene chloride/methanol (80/20 followed by 70/30 and 60/40). The semipurified product was repurified by column chromatography eluting with methylene chloride/methanol (80/20 followed by 75/25). The purified product was dissolved in methylene chloride, 3.7 M hydrogen chloride in diethyl ether (3 mL) was added, and the volatiles were removed by evaporation. The residue was triturated with diethyl ether, collected by filtration, and dried under vacuum to give 600 mg of **11** hydrochloride (32%). ^{1}H NMR: (DMSO-d_6; CF_3COOD): δ 1.80−1.90 (m, 2H), 2.0−2.10 (m, 2H), 3.0−3.10 (m, 2H), 3.45−3.55 (m, 2H), 3.88 (d, 2H), 4.01 (s, 3H), 4.90 (d, 2H), 6.0 (td, 1H), 6.30 (td, 1H), 7.41 (s, 1H), 7.5−7.65 (m, 2H), 7.82 (d, 1H), 8.13 (s, 1H), 8.88 (s, 1H). MS−EI *m/z*: 487 $[M.]^+$. Anal. ($C_{23}H_{24}N_4O_2BrF$, 0.5 H_2O, 2 HCl) C, H, N.

A similar procedure was used to prepare **48**.

***N*-(4-Methyl-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (16). Procedure G.** A suspension of 4-chloro-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazoline **(64**; 200 mg, 0.622 mmol) and 2-fluoro-4-methylaniline (94 mg, 0.764 mmol) in 2-propanol (5 mL) containing 6.2 M hydrogen chloride in 2-propanol (110 μL) was stirred at 80 °C for 1.5 h. After the solution was cooled, the precipitate was collected by filtration, washed with 2-propanol (2 mL) followed by diethyl ether (2 mL), and dried under vacuum. The solid was dissolved in the minimum volume of methanol/methylene chloride/methanol (saturated with ammonia) and stirred for 5 min at ambient temperature. The volatiles were removed under vacuum. The solid was purified by column chromatography, eluting with methylene chloride/methanol (90/10) followed by methylene chloride/methanol containing 5% ammonia (90/10). Evaporation of the fractions containing the expected product gave 170 mg of **16** (61%). ^{1}H NMR: δ 1.30−1.45 (m, 2H), 1.80 (d, 2H), 1.70−1.90 (m, 1H), 1.90 (t, 2H), 2.20 (s, 3H), 2.35 (s, 3H), 2.80 (d, 2H), 3.95 (s, 3H), 4.01 (d, 2H), 7.10 (d, 1H), 7.13 (d, 1H), 7.16 (s, 1H), 7.40 (t, 1H), 7.81 (s, 1H), 8.32 (s, 1H), 9.40 (s, 1H). MS−ESI *m/z*: 411 $[MH]^+$. Anal. ($C_{23}H_{27}N_4O_2F$, 0.3 H_2O) C, H, N.

A similar procedure was used to prepare **36**.

***N*-(4-Chloro-2,6-difluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine (19). Procedure H.** Under argon, sodium hydride (60%, 76.5 mg, 1.9 mmol) was added to a solution of 4-chloro-2,6-difluoroaniline (250 mg, 1.53 mmol) in DMF (9 mL). After the solution was stirred for 30 min at ambient temperature, **64** (246 mg, 0.764 mmol) was added and stirring was continued for a further 20 h. The mixture was poured onto water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed with water and brine and dried ($MgSO_4$), and the volatiles were removed by evaporation. The residue was purified by column chromatography on silica, eluting with methylene chloride/methanol (95/5) followed by methylene chloride/methanol containing 1% ammonia (90/10). The fractions containing the expected product were combined and evaporated. The residue was triturated with diethyl ether (2 mL), filtered, washed with diethyl ether (2 mL), and dried under vacuum at 50 °C to give 210 mg of **19** (61%). ^{1}H NMR: δ 1.30−1.45 (m, 2H), 1.80 (d, 2H), 1.70−1.90 (m, 1H), 1.90 (t, 2H), 2.20 (s, 3H), 2.80 (d, 2H), 3.96 (s, 3H), 4.02 (d, 2H), 7.21 (s, 1H), 7.52 (s, 1H), 7.54 (s,

1H), 7.82 (s, 1H), 8.35 (s, 1H). MS−ESI *m/z*: 449−451 $[MH]^+$. Anal. ($C_{22}H_{23}N_4O_2ClF_2$) C, H, N.

A similar procedure was used to prepare **20** and **37**.

***N*-(4-Chloro-2-fluorophenyl)-6-methoxy-7-(piperidin-4-ylmethoxy)quinazolin-4-amine (21). Procedure I.** *tert*-Butyl 4-(hydroxymethyl)piperidine-1-carboxylate (**52**;[20] 590 mg, 2.75 mmol) followed by triphenylphosphine (1.2 g, 4.58 mmol) and diethyl azodicarboxylate (0.72 mL, 4.58 mmol) were added to a solution of **39**[18] (585 mg, 1.83 mmol) in methylene chloride (20 mL). After the solution was stirred for 1 h at ambient temperature, further triphenylphosphine (239 mg, 0.91 mmol) and diethyl azodicarboxylate (0.14 mL, 0.91 mmol) were added. After the solution was stirred for 1.5 h, the volatiles were removed under vacuum and the residue was purified by column chromatography eluting with ethyl acetate/methylene chloride (1/1). The crude product was used directly in the next step.

A solution of the crude product in methylene chloride (15 mL) containing TFA (4.5 mL) was stirred at ambient temperature for 1.5 h. The volatiles were removed under vacuum. The residue was partitioned between water and ethyl acetate. The aqueous layer was adjusted to pH 9.5 with 2 M sodium hydroxide. The organic layer was separated, washed with water followed by brine, dried ($MgSO_4$), and evaporated to give **21** free base. ^{1}H NMR: δ 1.10−1.30 (m, 2H), 1.75 (d, 2H), 1.85−2.0 (br s, 1H), 2.55 (d, 2H), 2.95 (d, 2H), 3.95 (s, 3H), 4.0 (d, 2H), 7.20 (s, 1H), 7.35 (dd, 1H), 7.55 (dd, 1H), 7.60 (t, 1H), 7.80 (s, 1H), 8.35 (s, 1H), 9.55 (s, 1H). MS−ESI *m/z*: 417−419 $[MH]^+$. The hydrochloride salt was made as follows: **21** free base was dissolved in a mixture of methanol and methylene chloride (5 mL/5 mL), and 6 M hydrogen chloride in diethyl ether was added. The volatiles were removed under vacuum, and the residue was triturated with diethyl ether (5 mL), collected by filtration, washed with diethyl ether (2 mL), and dried under vacuum to give 390 mg of **21** hydrochloride (47% over 2 steps). Anal. ($C_{21}H_{22}O_2N_4ClF$, 2.25 HCl)

***N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-(piperidin-4-ylmethoxy)quinazolin-4-amine (22). Procedure J.** TFA (3 mL) was added to a suspension of *tert*-butyl 4-[({4-[(4-bromo-2-fluorophenyl)amino]-6-methoxyquinazolin-7-yl}oxy)methyl]-piperidine-1-carboxylate **(45**; 673 mg, 1.2 mmol) in methylene chloride (10 mL). After the suspension was stirred for 1 h at ambient temperature, the volatiles were removed under vacuum. The residue was triturated with a mixture of water and diethyl ether. The organic layer was separated, and the aqueous layer was washed again with diethyl ether. The aqueous layer was adjusted to pH 10 with 2 M aqueous sodium hydroxide and then extracted with methylene chloride. The combined organic layers were dried ($MgSO_4$), and the solvent was removed under vacuum. The solid was triturated with a mixture of diethyl ether and petroleum ether (1/1), filtered, washed with diethyl ether (2 mL), and dried under vacuum to give 390 mg of **22** (70%). ^{1}H NMR: δ 1.13−1.30 (m, 2H), 1.75 (d, 2H), 1.87−2.0 (m, 1H), 2.50 (d, 2H), 3.0 (d, 2H), 3.96 (s, 3H), 3.98 (d, 2H), 7.20 (s, 1H), 7.50 (dd, 1H), 7.55 (t, 1H), 7.68 (dd, 1H), 7.80 (s, 1H), 8.36 (s, 1H), 9.55 (br s, 1H). MS−ESI *m/z*: 461−463 $[MH]^+$. Anal. ($C_{21}H_{22}N_4O_2BrF$) C, H, N.

A similar procedure was used to prepare **23**−**25**, **42**, and **43**.

***N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-[2-(1-methylpiperidin-4-yl)ethoxy]quinazolin-4-amine (27). Procedure K.** Thirty-seven percent aqueous formaldehyde (480 μL) was added to a solution of *N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-(piperidin-4-ylmethoxy)quinazolin-4-amine **(28**; 480 mg, 1.01 mmol) in formic acid (4.8 mL). The mixture was heated at 95 °C for 4 h. The volatiles were removed under vacuum, and the residue was partitioned between water and ethyl acetate. The pH of the aqueous layer was adjusted to 11 with an aqueous solution of 2 M NaOH. The organic layer was separated, washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography, eluting with methylene chloride/methanol containing 1%ammonia (90/10). The fractions containing the expected product were combined and evaporated to give 386 mg of **27** (78%). ^{1}H NMR: δ 1.15−1.30 (m, 2H), 1.40−1.50 (m, 1H), 1.65−1.80 (m, 4H), 1.85 (t, 2H), 2.15 (s, 3H), 2.75 (d, 2H), 3.95 (s, 3H), 4.20 (t, 2H), 7.20 (s, 1H), 7.50 (d, 1H), 7.55 (dd, 1H), 7.80 (s, 1H), 8.38 (s, 1H), 9.55 (s, 1H). MS−ESI *m/z*: 489 $[MH]^+$. Anal. ($C_{23}H_{26}N_4O_2BrF$, 0.1 H_2O, 0.15 Et_2O) C, H, N.

***N*-(4-Bromo-2-fluorophenyl)-6-methoxy-7-(piperidin-4-ylmethoxy)quinazolin-4-amine (28). Procedure L.** A solution of *tert*-butyl 4-[2-({4-[(4-bromo-2-fluorophenyl)amino]-6-methoxyquinazolin-7-yl}oxy)ethyl]piperidine-1-carboxylate (**46**; 2.5 g, 4.3 mmol; ∼80% of purity) in methylene chloride (50 mL) containing TFA (12 mL) was stirred at ambient temperature for 1.5 h. The volatiles were removed under vacuum, and the residue was partitioned between methylene chloride (10 mL) and water (10 mL). The aqueous layer was separated, and the pH was adjusted to 11 with 2 M aqueous sodium hydroxide and then extracted with methylene chloride. The combined organic layers were washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography eluting with methylene chloride/methanol (90/10) followed by methylene chloride/methanol/methanol containing ammonia (1%, 90/5/5) followed by 80/10/10. The fractions containing the expected product were combined and evaporated. The residue was triturated with diethyl ether (10 mL), filtered, washed with diethyl ether (5 mL), and dried under vacuum to give 840 mg of **28** (42%). ^{1}H NMR (DMSO-d_6, CD_3COOD): δ 1.30−1.50 (m, 2H), 1.80 (m, 3H), 1.90−2.0 (m, 2H), 2.90 (t, 2H), 3.30 (d, 2H), 3.95 (s, 3H), 4.20 (bs, 2H), 7.22 (s, 1H), 7.50 (d, 1H), 7.55 (dd, 1H), 7.65 (d, 1H), 7.80 (s, 1H), 8.38 (s, 1H). MS−ESI *m/z*: 475−477 $[MH]^+$. Anal. ($C_{22}H_{24}N_4O_2BrF$, 0.54 H_2O, 0.26 CH_4O) C, H, N.

A similar procedure was used to prepare **41**.

***tert*-Butyl 4-[({4-[(4-bromo-2-fluorophenyl)amino]-6-methoxyquinazolin-7-yl}oxy)methyl]piperidine-1-carboxylate (45). Procedure M.** Potassium carbonate (414 mg, 3 mmol) was added to a suspension of *N*-(4-bromo-2-fluorophenyl)-7-hydroxy-6-methoxy-4-quinazolinylamine **(40**;[18] 546 mg, 1.5 mmol) in DMF (5 mL). After the suspension was stirred for 10 min at ambient temperature, *tert*-butyl 4-({[(4-methylphenyl)sulfonyl]oxy}methyl)piperidine-1-carboxylate (**54**;[21] 636 mg, 1.72 mmol) was added and the mixture was heated at 95 °C for 2 h. After the mixture was cooled, the mixture was poured onto cooled water (20 mL). The precipitate was collected by filtration, washed with water, and dried under vacuum to give 665 mg of **45** (79%). ^{1}H NMR: δ 1.15−1.30 (m, 2H), 1.46 (s, 9H), 1.80 (d, 2H), 2.0−2.10 (m, 1H), 2.65−2.90 (m, 2H), 3.95 (s, 3H), 4.02 (br s, 2H), 4.05 (d, 2H), 7.20 (s, 1H), 7.48 (d, 1H), 7.55 (t, 1H), 7.65 (d, 1H), 7.80 (s, 1H), 8.35 (s, 1H), 9.55 (br s, 1H). MS−ESI *m/z*: 561−563 $[MH]^+$.

(*R*)-(1-Methylpiperidin-3-yl)methanol (50). (*R*)-Ethyl nipecotate (5.7 g, 365 mmol) was dissolved in aqueous formaldehyde (38%, 45 mL) and formic acid (90 mL), and the mixture was heated at reflux for 18 h. The mixture was allowed to cool and was added dropwise to a cooled saturated aqueous sodium hydrogen carbonate solution. The mixture was adjusted to pH 12 by the addition of saturated aqueous sodium hydroxide, and the mixture was extracted with methylene chloride (50 mL). The organic extract was washed with brine and dried ($MgSO_4$), and the solvent was removed by evaporation to give 4.51 g of (*R*)-ethyl 1-methylpiperidine-3-carboxylate (73%) as a colorless oil. MS−ESI *m/z*: 172 $[MH]^+$.

A solution of (*R*)-ethyl 1-methylpiperidine-3-carboxylate (5.69 g, 33 mmol) in diethyl ether (20 mL) was added dropwise to a stirred solution of 1 M lithium aluminum hydride in THF (36.6 mL, 36.6 mmol) in ether (85 mL) and cooled to maintain the reaction temperature at 20 °C. The mixture was stirred for 1.5 h at ambient temperature, and then, water (1.4 mL), aqueous sodium hydroxide (15%, 1.4 mL), and water (4.3 mL) were added. The precipitate was removed by filtration, and the volatiles were removed from the filtrate by evaporation to give 4.02 g of **50** (94%). ^{1}H NMR: δ 1.06 (q, 1H), 1.51−1.94 (m, 5H), 2.04 (s, 3H), 2.34 (br s, 1H), 2.62 (m, 1H), 2.78 (d, 1H), 3.49 (m, 1H), 3.59 (m, 1H). MS−ESI *m/z*: 130 $[MH]^+$.

7-(1-(*tert*-Butoxycarbonyl)piperidin-4-ylmethoxy)-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-

4-one (56). Triphenylphosphine (1.7 g, 6.5 mmol) was added under nitrogen to a suspension of 7-hydroxy-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one **(51**;[18] 1.53 g, 5 mmol) in methylene chloride (20 mL), followed by the addition of *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate **(52**;[20] 1.29 g, 6 mmol) and by a solution of diethyl azodicarboxylate (1.13 g, 6.5 mmol) in methylene chloride (5 mL). After the solution was stirred for 30 min at ambient temperature, the reaction mixture was poured onto a column of silica and was eluted with ethyl acetate/petroleum ether (1/1) followed by 6/5, 6/4, and 7/3. Evaporation of the fractions containing the expected product led to an oil that crystallized following trituration with pentane (5 mL). The solid was collected by filtration and dried under vacuum to give 2.32 g of **56** (92%). ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 9H), 1.20−1.35 (m, 2H), 1.43 (s, 9H), 1.87 (d, 2H), 2.05−2.20 (m, 1H), 2.75 (t, 2H), 3.96 (d, 2H), 3.97 (s, 3H), 4.10−4.25 (br s, 2H), 5.95 (s, 2H), 7.07 (s, 1H), 7.63 (s, 1H), 8.17 (s, 1H). MS−ESI *m*/*z*: 526 $[MNa]^+$. Anal. ($C_{26}H_{37}N_3O_7$) C, H, N.

7-(2-(1-*tert*-Butoxycarbonylpiperidin-4-yl)ethoxy)-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (57). A suspension of **51**[18] (7 g, 23 mmol) and *tert*-butyl 4-(2-{[(4-methylphenyl)sulfonyl]oxy}ethyl)piperidine-1-carboxylate **(55**;[22] 11.4 g, 30 mmol) in DMF (70 mL) containing potassium carbonate (6.32 g, 46 mmol) was stirred at 100 °C for 3 h. After the suspension was cooled, the volatiles were removed under vacuum and the residue was partitioned between diethyl ether and water. The organic layer was separated, washed with water and brine, dried ($MgSO_4$), and evaporated. The solid was triturated with pentane, filtered, and dried under vacuum to give 10.5 g of **57** (88%). ^{1}H NMR ($CDCl_3$): δ 1.20 (s, 9H), 1.15−1.25 (m, 2H), 1.48 (s, 9H), 1.65−1.75 (m, 1H), 1.70 (d, 2H), 1.90 (dd, 2H), 2.72 (t, 2H), 4.0 (s, 3H), 4.0−4.20 (m, 2H), 4.20 (t, 2H), 5.95 (s, 2H), 7.10 (s, 1H), 7.65 (s, 1H), 8.20 (s, 1H). MS−ESI *m*/*z*: 540 $[MNa]^+$.

6-Methoxy-7-(piperidin-4-ylmethoxy)-3-((pivaloyloxy)-methyl)-3,4-dihydroquinazolin-4-one (58). A solution of **56** (2.32 g, 4.6 mmol) in methylene chloride (23 mL) containing TFA (5 mL) was stirred at ambient temperature for 1 h. The volatiles were removed under vacuum. The residue was partitioned between ethyl acetate (20 mL) and sodium hydrogen carbonate. The organic solvent was removed under vacuum, and the residue was filtered. The precipitate was washed with water and dried under vacuum. The solid was azeotroped with toluene and dried under vacuum to give 1.7 g of **58** (92%). ^{1}H NMR (DMSO-d_6; CF_3COOD): δ 1.15 (s, 9H), 1.45−1.60 (m, 2H), 1.95 (d, 2H), 2.10−2.25 (m, 1H), 2.95 (t, 2H), 3.35 (d, 2H), 3.95 (s, 3H), 4.10 (d, 2H), 5.95 (s, 2H), 7.23 (s, 1H), 7.54 (s, 1H), 8.45 (s, 1H). MS−ESI *m*/*z*: 404 $[MH]^+$. Anal. ($C_{21}H_{29}N_3O_5$, 1 H_2O, 0.8 CF_3COOH) C, H, N.

A similar procedure was used to prepare **59**.

6-Methoxy-7-(1-methylpiperidin-4-ylmethoxy)-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (60). A 37% aqueous solution of formaldehyde (501 μL, 6 mmol) followed by sodium cyanoborohydride (228 mg, 3.6 mmol) was added in portions to a solution of **58** (1.21 g, 3 mmol) in a mixture of THF and methanol (10 mL/10 mL). After the solution was stirred for 30 min at ambient temperature, the organic solvents were removed under vacuum and the residue was partitioned between methylene chloride (20 mL) and water (20 mL). The organic layer was separated, washed with water and brine, and dried ($MgSO_4$), and the volatiles were removed by evaporation. The residue was triturated with diethyl ether (5 mL), and the resulting solid was collected by filtration, washed with diethyl ether (3 mL), and dried under vacuum to give 1.02 g of **60** (82%). ^{1}H NMR ($CDCl_3$): δ 1.19 (s, 9H), 1.40−1.55 (m, 2H), 1.90 (d, 2H), 2.0 (t, 2H), 1.85−2.10 (m, 1H), 2.30 (s, 3H), 2.92 (d, 2H), 3.96 (s, 3H), 3.99 (d, 2H), 5.94 (s, 2H), 7.08 (s, 1H), 7.63 (s, 1H), 8.17 (s, 1H). MS−ESI *m*/*z*: 418 $[MH]^+$. Anal. ($C_{22}H_{31}N_3O_5$, 0.5 H_2O) C, H, N.

7-(2-(1-Methylpiperidin-4-yl)ethoxy)-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one (61). To a solution of 7-(2-(piperidin-4-yl)ethoxy)-6-methoxy-3-((pivaloyloxy)methyl)-3,4-dihydroquinazolin-4-one **(59**; 6 g, 14.4 mmol) in methanol (30 mL) and methylene chloride (60 mL) was added aqueous formaldehyde (37%, 2.2 mL, 28.9 mmol) followed by acetic acid (990 μL, 17.3 mmol). Sodium borohydride triacetate (4.6 g, 21.6 mmol) was added in portions. After the solution was stirred for 1 h at ambient temperature, the volatiles were removed under vacuum and the residue was partitioned between water (50 mL) and methylene chloride (50 mL). The pH of the aqueous layer was adjusted to 7, washed with water and brine, dried ($MgSO_4$), and evaporated. The solid was triturated with diethyl ether (5 mL), filtered, washed with diethyl ether (5 mL), and dried under vacuum to give 4.2 g of **61** (68%). ^{1}H NMR ($CDCl_3$): δ 1.22 (s, 9H), 1.68 (br s, 3H), 1.90 (m, 4H), 2.32 (br s, 2H), 2.52 (s, 3H), 3.18 (d, 2H), 4.0 (s, 3H), 4.20 (t, 2H), 5.95 (s, 2H), 7.10 (s, 1H), 7.65 (s, 1H), 8.20 (s, 2H). MS−ESI *m*/*z*: 432 $[MH]^+$.

6-Methoxy-7-[(1-methylpiperidin-4-yl)methoxy]-3,4-dihydroquinazolin-4-one (62). A saturated solution of ammonia in methanol (14 mL) was added to a solution of **60** (1.38 g, 3.3 mmol) in methanol (5 mL). After the solution was stirred for 20 h at ambient temperature, the suspension was diluted with methylene chloride (10 mL) and filtered. The filtrate was evaporated under vacuum, and the residue was triturated with diethyl ether (3 mL), filtered, washed with diethyl ether (2 mL), and dried under vacuum to give 910 mg of **62** (83%). ^{1}H NMR: δ 1.30−1.45 (m, 2H), 1.75 (d, 2H), 1.70−1.85 (m, 1H), 1.90 (t, 2H), 2.20 (s, 3H), 2.80 (d, 2H), 3.90 (s, 3H), 4.0 (d, 2H), 7.13 (s, 1H), 7.45 (s, 1H), 7.99 (s, 1H). MS−ESI *m*/*z*: 304 $[MH]^+$.

A similar procedure was used to prepare **63**.

4-Chloro-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazoline (64). A solution of **62** (2.8 g, 9.24 mmol) in thionyl chloride (28 mL) containing DMF (280 μL) was refluxed at 85 °C for 1 h. After the solution was cooled, the volatiles were removed by evaporation. The precipitate was triturated with diethyl ether (5 mL), filtered, washed with diethyl ether (3 mL), and dried under vacuum. The solid was dissolved in methylene chloride (15 mL), and saturated aqueous sodium hydrogen carbonate was added. The organic layer was separated, washed with water and brine, dried ($MgSO_4$), and evaporated to give 2.9 g of **64** (98%). ^{1}H NMR: δ 1.30−1.50 (m, 2H), 1.75−1.90 (m, 3H), 2.0 (t, 1H), 2.25 (s, 3H), 2.85 (d, 2H), 4.02 (s, 3H), 4.12 (d, 2H), 7.41 (s, 1H), 7.46 (s, 1H), 8.90 (s, 1H). MS−ESI *m*/*z*: 322 $[MH]^+$. Anal. ($C_{16}H_{20}N_3O_2Cl$, 0.3 H_2O, 0.1 CH_2Cl_2) C, H, N.

A similar procedure was used to prepare **65**.

***N*-(4-Chloro-2-fluorophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine (66).** A mixture of **39**[18] (957 mg, 3 mmol), 1-bromo-3-chloropropane (2.36 g, 15 mmol), and potassium carbonate (2.1 g, 15 mmol) in DMF (20 mL) was heated at 40 °C for 1.5 h. The mixture was cooled, diluted with water, and extracted with ethyl acetate. The organic extracts were combined, washed with water and brine, and dried ($MgSO_4$), and the volatiles were removed by evaporation. The residue was triturated with hexane/ethyl acetate (5 mL/5 mL), filtered, and dried under vacuum to give 650 mg of **66** (55%). ^{1}H NMR: δ 2.26 (m, 2H), 3.82 (t, 2H), 3.95 (s, 3H), 4.26 (t, 2H), 7.20 (s, 1H), 7.32 (dd, 1H), 7.48−7.60 (m, 2H), 7.80 (s, 1H), 8.35 (s, 1H), 9.52 (s, 1H). MS−EI *m*/*z*: 396 $[MH]^+$.

***N*-(4-Bromo-2-fluorophenyl)-7-(3-chloropropoxy)-6-methoxyquinazolin-4-amine (67).** A solution of **40**[18] (0.5 g, 1.37 mmol) and 1-bromo-3-chloropropane (0.165 mL, 1.65 mmol) in DMF (7.5 mL) containing potassium carbonate (0.475 g, 3.43 mmol) was stirred at ambient temperature overnight. The mixture was poured onto water. The precipitate was filtered, washed with water followed by diethyl ether (3 mL), and dried under vacuum to give 425 mg of **67** (70%). ^{1}H NMR (DMSO-d_6; CF_3COOD): δ 2.30−2.40 (m, 2H), 3.85 (t, 2H), 4.02 (s, 3H), 4.35 (t, 2H), 7.37 (s, 1H), 7.52−7.64 (m, 2H), 7.82 (d, 1H), 8.09 (s, 1H), 8.88 (s, 1H). MS−ESI *m*/*z*: 441 $[M.]^+$.

(*E*)-4-(Pyrrolidin-1-yl)but-2-en-1-ol (68). A solution of 4-pyrrolidin-1-ylbut-2-yn-1-ol[27] (4.3 g, 31 mmol) in THF (20 mL) was added dropwise to a suspension of lithium aluminum hydride (2.35 g, 62 mmol) in anhydrous THF (8 mL), and the mixture was stirred and heated at 60 °C for 2 h. The mixture

was cooled to 5 °C, and 2 M aqueous sodium hydroxide solution (28 mL) was added dropwise. The resulting suspension was filtered, and the filtrate was evaporated under vacuum. The residue was dissolved in a mixture of methylene chloride/ethyl acetate (20 mL/20 mL) and dried ($MgSO_4$), and the solvent was removed by evaporation. The residue was purified by column chromatography on aluminum oxide eluting with methylene chloride/methanol (97/3) to give 3.09 g of **68** (70%). ^{1}H NMR ($CDCl_3$): δ 1.82 (m, 4H), 2.61 (m, 4H), 3.17 (m, 2H), 4.13 (s, 2H), 5.84 (m, 2H).

Biological Evaluation. IC_{50} values reported are the average of 3−5 independent measurements, depending on compound potency. Experimental details for the in vitro receptor TK inhibition assays, in vitro HUVEC proliferation assay, mouse plasma pharmacokinetic assay, and human tumor xenograft test have been previously reported.[18,19] For the in vitro HUVEC proliferation assay, EGF 0.3 ng/mL was used. Adenosine 5′-triphosphate (ATP) concentrations used in the enzyme assays were 2 μM for Flt-1, KDR, EGFR, PDGFRβ, FGFR1, and erbB2; 0.2 μM for CDK2; 0.8 μM for AKT; 2.5 μM for IGF-1R; 5 μM for Tie-2; 7.5 μM for PDK; and 8 μM for MEK. The concentration of ATP used was below the respective Km for ATP in all cases.

Alignment of protein sequences indicates a very high degree of homology between the kinase domain of the human KDR receptor and the murine equivalent (from *mus musculus*; similarity >96% and identity > 95%) as well as between the human Flt-1 receptor and the murine equivalent (from *mus musculus*; similarity > 94% and identity > 92%).

Rat and Dog Pharmacokinetics. Compounds were dosed as free bases. Rat: **1** and **2** were dosed iv and po to groups of 3 males. Compound **1** was dosed iv at 5 mg/kg using a 2 mg/mL solution in 25% HP β cyclodextrin and po at 50 mg/kg using a 2 mg/mL ball-milled suspension in poly(vinylpyrrolidone) (PVP)/sodium lauryl sulfate (SLS) solution. Compound **2** was dosed iv at 5 mg/kg using a solution in DMSO (10%), cremophor (10%), and physiological saline (80%) and po at 50 mg/kg of a suspension of 5 mg/mL in polysorbate 80 (1%). Dog: **1** was dosed iv and po to 4 males, and **2** was dosed iv and po to 3 males. Compound **1** was dosed iv at 5 mg/kg of a 3.5 mg/mL solution in 45% HP β-cyclodextrin and po at 30 mg/kg of a 10 mg/mL suspension in PVP (1 mg/mL)/SLS (0.2 mg/mL) solution. Compound **2** was dosed iv at 5 mg/kg of a 5 mg/mL solution in 45% (w/v) HP β-cyclodextrin in pH 7 Sorenson's phosphate buffer and po at 25 mg/kg of a 5 mg/mL milled suspension in HPMC/polysorbate 80.

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Supporting Information Available: A description of the general procedures and the complete experimental procedures for the synthesis of as well as the corresponding ^{1}H NMR spectra, MS-ESI, and elemental analyses (16 pages) for **5**, **12**, **15**, **17**, **18**, **20**, **23**−**26**, **29**−**31**, **35**−**37**, **41**−**43**, **46**−**49**, **59**, **63**, and **65**. This material is available free of charge via the Internet at http://pubs.acs.org.

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