Structure−Activity Relationships of Novel 2-Substituted Quinazoline Antibacterial Agents

Pei-Pei Kung,* Martin D. Casper, Kimberley L. Cook, Laura Wilson-Lingardo, Lisa M. Risen, Timothy A. Vickers, Ray Ranken, Lawrence B. Blyn, Jacqueline R. Wyatt, P. Dan Cook, and David J. Ecker

Ibis Therapeutics, a Division of Isis Pharmaceuticals, and Medicinal Chemistry, Isis Pharmaceuticals, 2292 Faraday Avenue, Carlsbad, California 92008

Received July 8, 1999

High-throughput screening of in-house compound libraries led to the discovery of a novel antibacterial agent, compound **1** (MIC: 12−25 μM against *S. pyogenes*). In an effort to improve the activity of this active compound, a series of 2-substituted quinazolines was synthesized and evaluated in several antibacterial assays. One such compound (**22**) displayed improved broad-spectrum antibacterial activity against a variety of bacterial strains. This molecule also inhibited transcription/translation of bacterial RNA, suggesting a mechanism for its antibiotic effects. Structure−activity relationship studies of **22** led to the synthesis of another 24 compounds. Although some of these molecules were found to be active in bacterial growth assays, none were as potent as **22**. Compound **22** was tested for its ability to cure a systemic *K. pneumonia* infection in the mouse and displayed moderate effects compared with a control antibiotic, gentamycin.

Introduction

The spread of antibiotic resistance among pathogenic bacteria has become a serious problem for the clinical management of infectious diseases and has resulted in a clear need for novel antibacterial agents other than analogues of existing antibiotics.[1] In an effort to identify such novel agents, our antibacterial program routinely screens large in-house libraries of small synthetic compounds for active leads. Compound **1** (Figure 1, Table 1) was indicated from these screening efforts to have moderate antibacterial activity against the Gram-positive bacterium, *Streptococcus pyogenes*. Intrigued by the novel structure of **1**, we subsequently conducted structure−activity relationship (SAR) studies to (1) define the functionalities responsible for the observed antibacterial activity and (2) further improve the antibiotic potency of the molecule. In this work, we report on the initial SAR studies of compound **1** which led to improved broad-spectrum antibacterial activity.

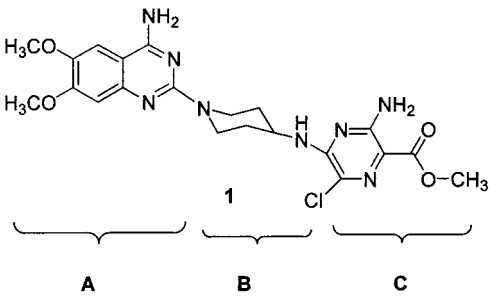
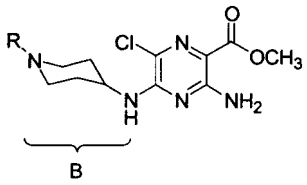
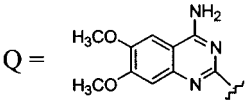


Figure 1. Structure of antibacterial lead from high-throughput screening.

Table 1. SAR of Compound **1** with Fixed "C" Portion



compd	R	B	*S. pyogenes* (#14289) (μM)	*E. coli* imp− (μM)
1	Q	Pip	12−25	>25
7	Q	HN−NH	>50	>50
35	Boc	Pip	>25	>25
36	H	Pip	>25	>25
37	$PhCH_2$	Pip	>50	>50



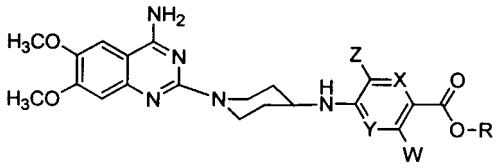
Pip = 4-aminopiperidine

Results and Discussion

Inspection of the chemical structure of **1** suggested that the compound could be divided into three subunits: the quinazoline fragment (A), the 4-aminopiperidine linker (B), and the pyrazine moiety (C) (Figure 1). Initial SAR studies were performed by modification of the parent compound to determine if any of the subunits which comprise **1** displayed antibacterial activity. As seen in Table 1, replacement of the 4-aminopiperidine linker B with a simple hydrazine moiety resulted in complete loss of antibacterial activity (compound **7**). Replacement of the quinazoline fragment (A) with either Boc, hydrogen, or benzyl functionalities (compounds **35**−**37**, respectively) resulted in similar loss of activity. Therefore, we concluded that both A and B fragments were required for antibacterial activity, and subsequent SAR studies were conducted with molecules containing such moieties.

10.1021/jm9903500 CCC: $18.00
Published on Web 10/14/1999

Table 2. SAR of Compound **1** with Fixed "A" and "B" Portions



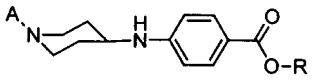
compd	X	Y	Z	W	R	*S. pyogenes* (#14289) (μM)	*E. coli* imp− (μM)
1	N	N	Cl	NH_2	CH_3	12−25	>25
2	N	N	H	NH_2	CH_3	>50	>50
3	CH	N	H	H	CH_3	25−50	>50
4	CH	CH	H	H	CH_3	5−25	5−25
5	CH	CH	H	H	$C(CH_3)_3$	3−6	6−12
6	CH	CH	NO_2	H	$C(CH_3)_3$	50−100	25−50
18	N	N	Cl	NH_2	H	>100	>100
19	N	N	H	NH_2	H	>25	>25
20	CH	N	H	H	H	>50	>50
21	CH	CH	NO_2	H	H	>20	>20
22	CH	CH	H	H	H	>25	0.4−2

Modifications to the pyrazine moiety present in **1** (the C fragment) were then undertaken (Table 2). Removal of the 6-chloro substituent from the pyrazine ring resulted in diminished antibacterial activity relative to **1** (compound **2**). However, removal of the 3-amino group and substitution of a CH moiety for the nitrogen at the 1 position of the dehalogenated pyrazine returned antibacterial activity to nearly that displayed by compound **1** (compare pyridine **3** with **1**). Additional CH for N substitution further improved antibacterial activity relative to **1** when the resulting compound (**4**) was tested against both *S. pyogenes* and *Escherichia coli*, imp− bacterial strains. This dual-strain antibiotic effect could be further improved by incorporation of a *tert*-butyl ester moiety into the compound structure (**5**) but was impaired when a nitro group was introduced on the benzene C ring (compound **6**).

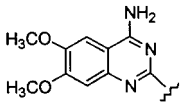
In addition to the molecules described above bearing C rings with ester moieties, we also examined the corresponding carboxylic acid-containing compounds (compounds **18**−**22**, Table 2). In most cases, replacement of the ester functional group with the free acid resulted in loss of or no improvement in antibacterial activity (compare **18** with **1**, **19** with **2**, and **20** with **3**). However, in the example of compound **5**, such acid-for-ester substitution resulted in reduced activity against *S. pyogenes* but substantially *improved* activity against *E. coli*, imp− (compound **22**). Therefore, compounds containing C rings comprised of substituted benzoic acids and/or esters were examined in greater detail with an additional SAR study.

Several truncated molecules related to **4**, **5**, and **22** were examined to confirm that the antibacterial activity exhibited by these compounds was related to all three subunits (A, B, and C, Figure 1) of the molecules (Table 3). Replacement of the quinazoline moiety (A fragment) of **4**, **5**, and **22** with a benzyl group resulted in loss of antibacterial activity (compounds **46**, **48**, and **50**, respectively). A similar loss in activity was noted for a molecule related to **5** which contained no A fragment (compound **49**). Thus, as was observed for the pyrazine-containing molecules above, the quinazoline fragment could not be removed from compounds incorporating benzoic acids/esters without negatively affecting their antibacterial activity.

Table 3. SAR with Fixed "B" Portion



compd	A	R	*S. pyogenes* (#14289) (μM)	*E. coli* imp− (μM)
4	Q	CH_3	5−25	5−25
5	Q	$C(CH_3)_3$	3−6	6−12
22	Q	H	>25	0.4−2
46	$PhCH_2$	CH_3	>25	>25
48	$PhCH_2$	$C(CH_3)_3$	>25	>25
49	H	$C(CH_3)_3$	>25	>25
50	$PhCH_2$	H	>25	>25

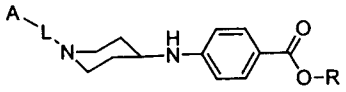
Q = 

We next attempted modification of the quinazoline A fragment contained in the most active compounds **5** and **22** (Table 4). Replacement of the quinazoline moiety of either **5** or **22** with simple (less functionalized) aromatic groups resulted in loss of antibacterial activity (compare compounds **8**, **11**, and **12** with **5** and compare **23**, **26**, and **27** with **22**). Variation of the linking functionality between one such simple aromatic group (naphthyl) and the 4-aminopiperidine moiety did not restore the lost antibacterial activity (compounds **9**, **10**, **24**, and **25**). Modification of the 4-amino substituent on the quinazoline ring predominately afforded less active antibacterial agents (compare **13**−**15** with **5** and compare **28**−**31** with **22**) although amine substitution was tolerated in one case (compare **16** to **5**). Similar reduction in antibacterial potency was observed when the 6- and 7-methoxy substituents were removed from the quinazoline ring (compounds **17** and **32**).

Having completed the above SAR studies, three of the most potent compounds (**4**, **5**, and **22**) were selected to be screened against additional bacterial strains as shown in Table 5. In these assays, **22** displayed moderate activity against the pathogenic bacteria, *Klebsiella pneumoniae* (6−12 μM) and *Staphylococcus aureus* (6−12 μM), but was inactive against several other strains. Compounds **4** and **5** exhibited somewhat less potent broad-spectrum activity than **22**, and the latter molecule was therefore selected for in vivo testing. In the event, compound **22** displayed moderate antibacterial activity in mice infected with *K. pneumoniae* and afforded a 40% survivor rate when administrated at 100 mg/kg. This result was compared with that provided by the known antibiotic, gentamycin, which afforded a 70% survivor rate when dosed at 3 mg/kg.

In addition, compound **22** inhibited a luciferase-based bacterial transcription/translation assay with an IC_{50} = 12−12.5 μM. Further testing of compound **22** in the transcription/translation assay showed that the response was dose-dependent (Figure 2a) and that the compound did not directly inhibit luciferase (data not shown). This inhibition was specific to bacterial transcription/translation as shown by the inability of the compound to effectively inhibit a rabbit reticulocyte

Table 4. SAR of Compounds **5** and **22** with Different "A" Portion



compd	A	L	R	*S. pyogenes* (#14289) (μM)	*E. coli* imp− (μM)
5	Q		$C(CH_3)_3$	3−6	6−12
8	Naph		$C(CH_3)_3$	>25	>25
9	Naph	SO_2	$C(CH_3)_3$	>25	>25
10	Naph	CO	$C(CH_3)_3$	>25	>25
11	Pyr		$C(CH_3)_3$	>25	>25
12	Bnim		$C(CH_3)_3$	>100	>100
13	QH		$C(CH_3)_3$	>20	>20
14	QME		$C(CH_3)_3$	<100[a]	<100[a]
15	QOH		$C(CH_3)_3$	>100	>100
16	QPH		$C(CH_3)_3$	2.5−5	10−20
17	QDME		$C(CH_3)_3$	<100[a]	<100[a]
22	Q		H	>25	0.4−2
23	Naph		H	>25	>25
24	Naph	SO_2	H	>25	>25
25	Naph	CO	H	>25	>25
26	Pyr		H	>25	>25
27	Bnim		H	>25	>25
28	QH		H	>20	>20
29	QME		H	>20	>20
30	QOH		H	>20	>20
31	QPH		H	>20	>20
32	QDME		H	>100	>100

[a] About 90% inhibition at 100 μM.

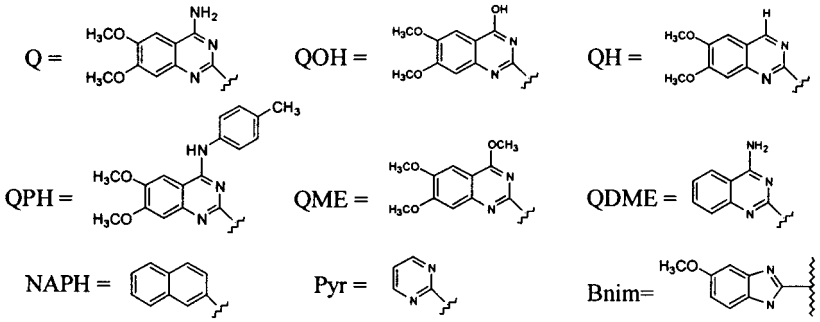
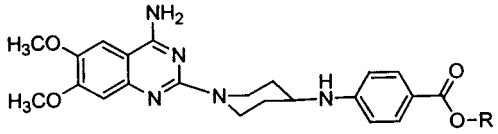


Table 5. Comparison of Lead Compounds in Broad-Spectrum Antibacterial Screening



compd	R	*K. pneumoniae* (#13883) (μM)	*E. coli* (#25922) (μM)	*S. pyogenes* (#49399) (μM)	*S. aureus* (#13709) (μM)	*E. faecalis* (#29212) (μM)
4	CH_3	>100	>100	5−25	>100	20−40
5	$C(CH_3)_3$	>100	>100	6−12	>100	6−12
22	H	6−12	60−80	>25	6−12	>100

transcription/translation system (Figure 2b, $IC_{50} = 190$ μM). To provide information about the specific mechanism of action of compound **22**, we looked at its ability to inhibit incorporation of [^{3}H]UTP into RNA (Figure 3). Even at the highest doses, there was no effect on RNA synthesis, indicating that the compound worked at the translational level. Interestingly, incorporation of tritiated amino acids into luciferase was not significantly inhibited by compound **22**. This result suggested that compound **22** might function by allowing misincorporation of amino acids into the luciferase protein. Indeed, compound **22** provided a 50% signal increase in an amino acid misincorporation[1d,2] experiment when tested at 500 μM. The difference in compound concentration required to see results in the two assays may be due, in part, to the high sensitivity of luciferase to small changes in protein sequence and the relatively high tolerance to amino acid substitution by the type of misincorporation assay used in this work. Together, these results suggest that the antibacterial effects exhibited by **22** may be due to a mechanism in which the compound causes misincorporation during translation.

To conclude, this work describes the initial SAR study of quinazoline-containing antibacterial agents which resulted in the identification of several moderately potent broad-spectrum antibacterial compounds of novel structure. One such compound (**22**) was active in an in

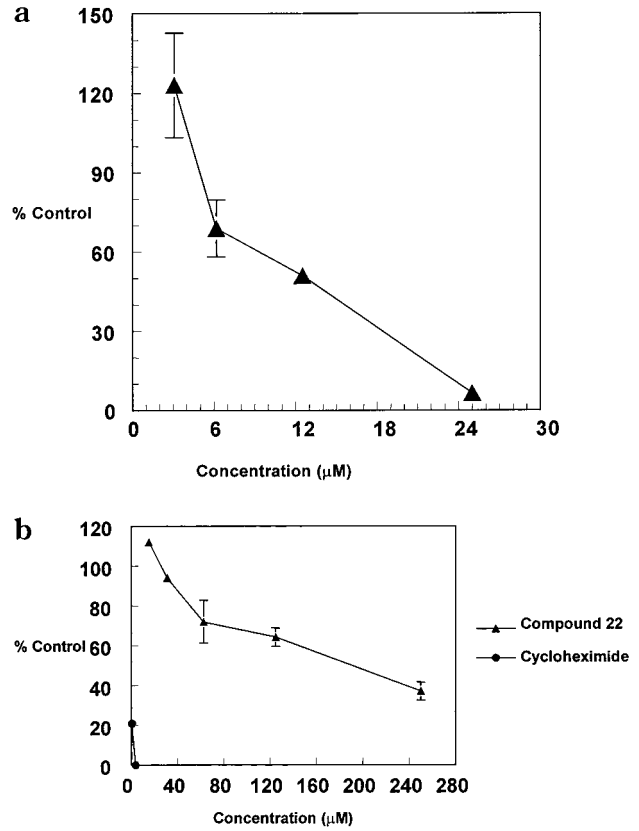


Figure 2. Specificity of compound **22** for inhibition of bacterial transcription/translation. (a) Compound **22** was tested at increasing doses for its ability to inhibit a bacterial cell-free coupled transcription/translation system. The IC_{50} of compound **22** in this system was ~12−12.5 μM. (b) Compound **22** was tested at increasing doses for its ability to inhibit a rabbit reticulocyte cell-free coupled transcription/translation system. The IC_{50} of compound **22** in this system was ~190 μM.

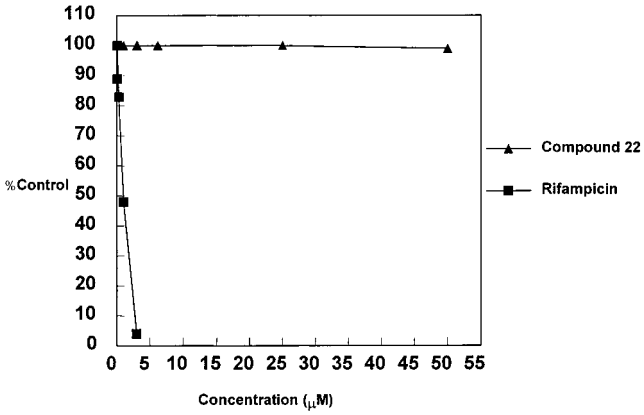


Figure 3. Effect of compound **22** on incorporation of [^{3}H]UTP into cellular RNA. The effect of compound **22** on bacterial transcription was measured by the incorporation of [^{3}H]UTP into cellular RNA. Increasing concentrations of compound **22** were compared to a rifampicin control. Rifampicin had an IC_{50} of 1 μM, while compound **22** had no detectable IC_{50} in this assay.

vivo model of bacterial infection and was suggested to exert its antibacterial effects by altering RNA translation. Additional studies of these novel antibacterial agents will be reported in due course.[3]

Chemistry

The synthetic routes used to prepare the compounds described in this work are depicted in Schemes 1−4. In general, the B−C fragments of these molecules were assembled using either aromatic nucleophilic substitution chemistries (SNAr)[4] or palladium-catalyzed cross-coupling methods.[5] An example of the former methodology is given by the synthesis of compounds **1**−**3** and **18**−**20** (Scheme 1). Thus, SNAr coupling of commercially available pyrazine **34** with the known[6] Boc-protected 4-aminopiperidine (**41**) afforded the B−C product **35** in moderate yield. Removal of the Boc moiety and subsequent coupling of the resulting amine hydrochloride salt (**36**) with 4-amino-2-chloro-6,7-dimethoxyquinazoline then provided the antibacterial agent **1**.[7] The carboxylic acid (**18**) corresponding to **1** was prepared by basic hydrolysis of the latter.

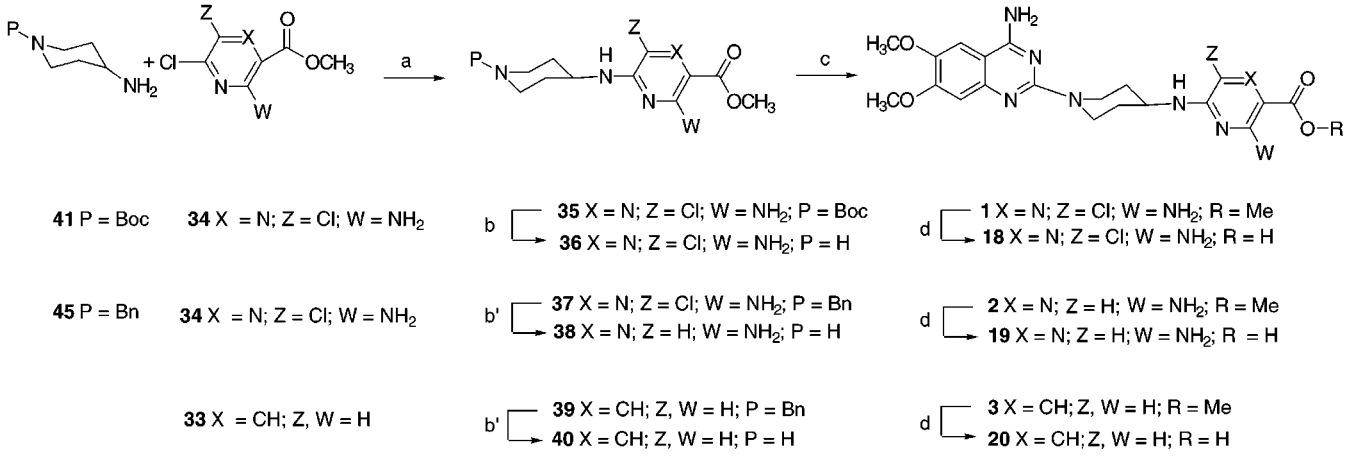
A second example of SNAr synthetic methodology is provided by the preparation of compounds **2**, **3**, **19**, and **20** (Scheme 1). In this example, commercially available 4-amino-1-benzylpiperidine (**45**) was derivatized via SNAr chemistries with either pyrazine **34** or pyridine **33** to provide the corresponding coupling products (**37** and **39**, respectively). These intermediates were debenzylated, and the resulting free amines (**38** and **40**) were condensed with 4-amino-2-chloro-6,7-dimethoxyquinazoline to provide antibacterial agents **2** and **3** in moderate yields. As described for the preparation of compound **18** above, basic hydrolysis of the ester moiety present in **2** and **3** afforded the corresponding carboxylic acids **19** and **20**, respectively.

A final example of the SNAr synthetic approach is given by the preparation of compounds **6** and **21** (Scheme 2). Thus, SNAr coupling of protected piperidine **41** with the chlorinated benzoic acid ester **42** prepared by reacting the corresponding acid chloride with *tert*-butyl alcohol provided intermediate **43** in moderate yield. Boc deprotection was then effected in the presence of the *tert*-butyl ester moiety,[8] and the resulting amine hydrochloride salt was condensed with 4-amino-2-chloro-6,7-dimethoxyquinazoline to afford compound **6**. The carboxylic acid corresponding to **6** (**21**) was subsequently prepared by acidic deprotection of the *tert*-butyl ester moiety.

An alternate method of assembling the B−C fragments of the compounds described in this work involved use of palladium-catalyzed cross-coupling methodology.[5] An example of such an alternative synthetic method is given in Scheme 3 by the preparation of compounds **4**, **5**, and **22**. Thus, Pd-mediated coupling of 4-bromobenzoic acid methyl ester with 4-amino-1-benzylpiperidine (**45**) provided either methyl ester **46** or *tert*-butyl ester **48** depending on the reaction conditions.[10] The benzyl protecting group in both products was subsequently removed, and the resulting amines (**47** and **49**) were condensed with 4-amino-2-chloro-6,7-dimethoxyquinazoline to afford compounds **4** and **5** in moderate yields. The carboxylic acid **22** was prepared by acidic deprotection of compound **5**.

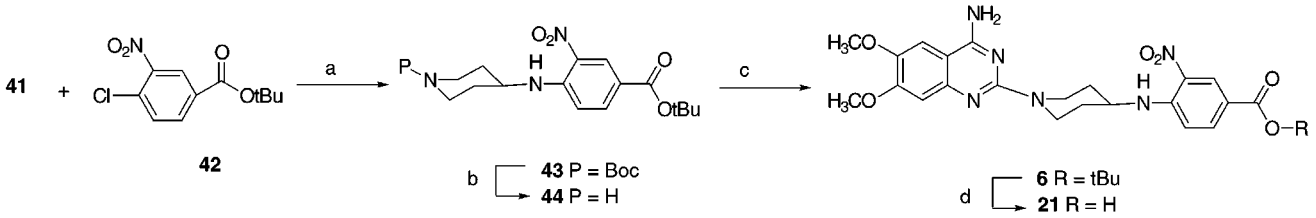
Scheme 4 illustrates the preparation of several compounds described in this work which were synthesized by modifications of the methods depicted above. Thus, the key intermediate **49** (described in Scheme 3 above) was independently derivatized with 2-naphthalenesulfonyl chloride or 2-naphthoyl chloride to afford coupling products **9** and **10**, respectively. The *tert*-butyl esters present in these compounds were subsequently

Scheme 1[a]



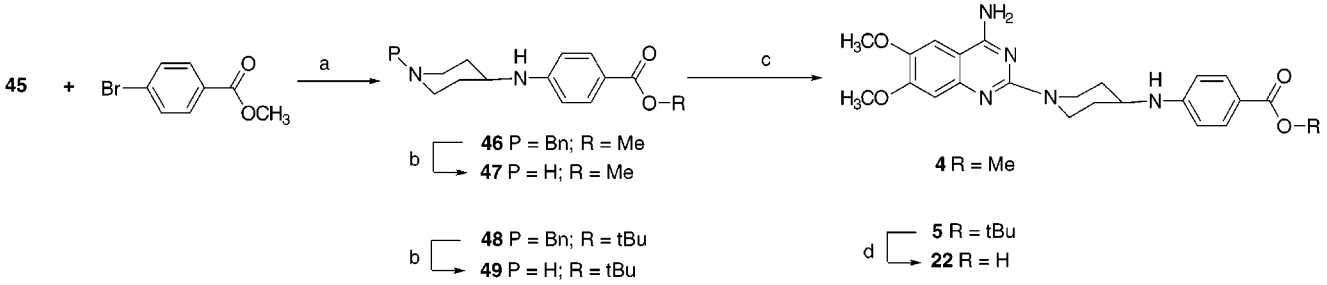
[a] Reagents and conditions: (a) 5.5 equiv *N*,*N*′-diisopropylethylamine, DMF, 25 °C, 12 h, 78−99%; (b) HCl in 1,4-dioxane, 25 °C, 12 h; (b′) 10 equiv ammonium formate, ethanol, 80 °C, 12 h, quantitative yield 86%; (c) 1.0 equiv 4-amino-2-chloro-6,7-dimethoxyquinazoline, isoamyl alcohol, 120 °C, 12 h, 49−68%; (d) potassium hydroxide, 1:9 MeOH:H_2O, 85 °C, 12 h, HCl, 93−99%.

Scheme 2[a]



[a] Reagents and conditions: (a) 2.7 equiv *N*,*N*′-diisopropylethylamine, DMF, 110 °C, 24 h, 55%; (b) HCl in 1,4-dioxane, 25 °C, 12 h, 59%; (c) 1.0 equiv 4-amino-2-chloro-6,7-dimethoxyquinazoline, *n*-pentanol, 120 °C, 12 h, 64%; (d) TFA, 25 °C, 12 h, 78%.

Scheme 3[a]



[a] Reagents and conditions: (a) 0.003 equiv $Pd_2(dba)_3$, 0.01 equiv (±)-BINAP, 1.3 equiv cesium carbonate, toluene, 80 °C, 12 h, 35%; 0.003 equiv $Pd_2(dba)_3$, 0.01 equiv (±)-BINAP, 2.6 equiv sodium *tert*-butoxide, toluene, 80 °C, 12 h, 49%; (b) 10 equiv ammonium formate, ethanol, 80 °C, 12 h, 92−94%; (c) 1.0 equiv 4-amino-2-chloro-6,7-dimethoxyquinazoline, *tert*-butyl alcohol, 120 °C, 12 h, 59−83%; (d) HCl in 1,4-dioxane, 25 °C, 12 h, 91%.

removed to provide carboxylic acids **24** and **25**. Alternatively, **49** could be coupled with a variety of heterocycles (2-bromonaphthalene, 2-bromopyrimidine, and 2-chloro-5-methoxybenzimidazole) to give products **8**, **11**, and **12** in moderate yields. As described above, the *tert*-butyl esters present in these molecules were subsequently removed to afford carboxylic acids **23**, **26**, and **27**, respectively.

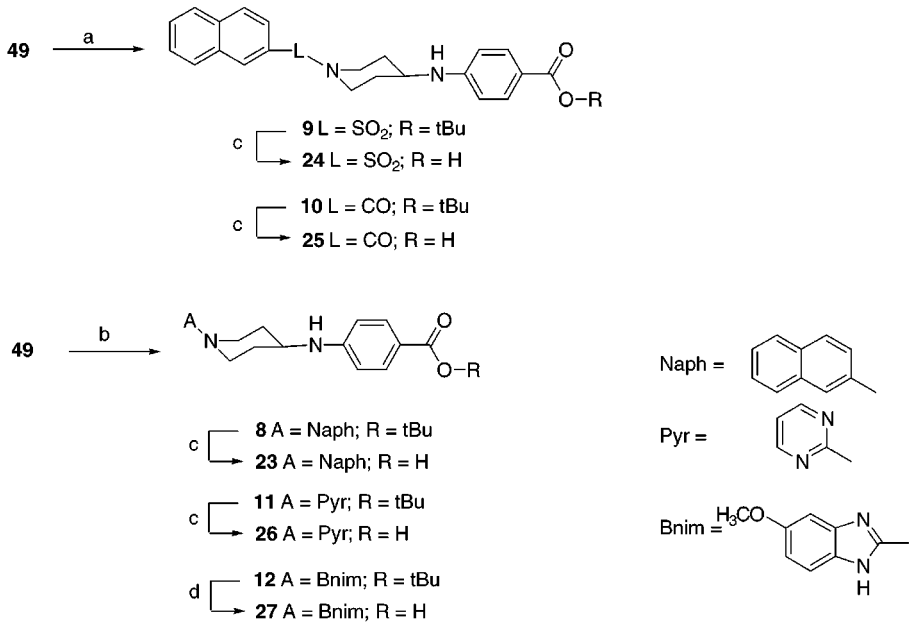
Experimental Section

General. Chemicals, reagents, and solvents were purchased and used as received unless otherwise noted. Flash column chromatography[16] was performed using silica gel 60 (Merck Art 9385). Proton and carbon NMR spectra were recorded at 200 MHz utilizing a Varian Gemini spectrometer. Chemical shifts are reported in ppm (δ) downfield relative to internal tetramethylsilane, and coupling constants are given in Hertz.

2-Chloro-4-substituted-quinazolines (QH, QME) were prepared from 2,4-dichloro-6,7-dimethoxyquinazoline as described in the literature.[11,12] Sodium borohydride reduction of 2,4-dichloro-6,7-dimethoxyquinazoline gave QH in about 37% yield,[11] and compound **52** (2-chloro-4-hydroxyl-6,7-dimethoxyquinazoline) was obtained as a major side product due to sodium hydroxide contamination in the commercially available sodium borohydride. 2,4-Dichloro-6,7-dimethoxyquinazoline was treated with sodium methoxide to give QME in quantitative yield.[12] Compounds QPH and QDME were gifts from Dr. Karl Hargrave of Boehringer Ingelheim, and their preparation procedures are reported in the literature.[13,14] SNAr chemistry similar to that depicted in Scheme 3 was used to prepare compounds **13**, **14**, **16**, and **17** which correspond to compounds QH, QME, QPH, and QDME, respectively**.** Compound **15** was obtained in the course of synthesizing compound **14** through in situ hydrolysis of the quinazoline 4-methoxy group.[15] Compounds **13**−**17** were converted to the corresponding carboxylic acid derivatives **28**−**32** by treatment with trifluoroacetic acid.

General Procedure for Coupling with 4-Amino-2-chloro-6,7-dimethoxyquinazoline and Derivatives. Com-

Scheme 4[a]



[a] Reagents and conditions: (a) 3 equiv 2-naphthalenesulfonyl chloride, 5 equiv triethylamine, CH_2Cl_2, 25 °C, 12 h, 93%; 3 equiv 2-naphthoyl chloride, 5 equiv triethylamine, CH_2Cl_2, 25 °C, 12 h, 50%; (b) 1 equiv 2-bromonaphthalene, 0.005 equiv $Pd_2(dba)_3$, 0.014 equiv (±)-BINAP, 2.6 equiv sodium *tert*-butoxide, toluene, 80 °C, 12 h, 59%; 1 equiv 2-bromopyrimidine, *tert*-butyl alcohol, 25 °C, 12 h, 89%; 1 equiv 2-chloro-5-methoxybenzimidazole, *n*-pentanol, 25 °C, 12 h, 83%; (c) HCl in 1,4-dioxane, 25 °C, 12 h; (d) TFA, 25 °C, 12 h.

pound **49** (**36**, **38**, **40**, **44**, **47**) was added to a solution of 4-amino-2-chloro-6,7-dimethoxyquinazoline or derivatives (0.9 equiv) in isoamyl alcohol (3-methyl-1-butanol) or *n*-pentanol (10 mL/mmol) at 25 °C. The mixture was then stirred at 120 °C for 12 h, cooled to room temperature, and filtered and rinsed with acetone to give the desired compounds (**1**−**6**, **11**−**14**, **16**, **17**).

3-Amino-5-[1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-piperidin-4-ylamino]-6-chloropyrazine-2-carboxylic acid methyl ester (1): ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 1H), 6.82 (s, 1H), 5.44 (d, 1H, J = 7.8), 5.21 (br s, 2H), 4.77 (d, 2H, J = 13.7), 4.19 (m, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.12 (t, 2H, J = 11.3), 2.12 (d, 2H, J = 12.6), 1.56 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.6, 160.7, 158.4, 155.6, 155.4, 150.8, 149.6, 146.1, 121.5, 110.6, 105.8, 102.8, 101.7, 56.3, 56.1, 51.9, 48.9, 43.0, 31.8; HRMS (FAB) m/z 621.0766 $(M + Cs)^+$ ($C_{21}H_{25}N_8O_4Cl$ requires 621.0742). Anal. ($C_{21}H_{25}N_8O_4Cl \cdot 1/2HCl \cdot 1/4CH_3OH$) C, H, N.

3-Amino-5-[1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-piperidin-4-ylamino]pyrazine-2-carboxylic acid methyl ester (2): ^{1}H NMR (DMSO) δ 7.59 (d, 1H, J = 7.7), 7.41 (s, 1H), 7.22 (s, 1H), 7.15 (br s, 4H), 6.73 (s, 1H), 4.63 (d, 2H, J = 13.2), 4.10 (m, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 3.69 (s, 3H), 2.99 (t, 2H, J = 9.8), 1.90 (d, 2H, J = 10.0), 1.39 (m, 2H); ^{13}C NMR (DMSO) δ 167.0, 161.1, 158.0, 156.6, 154.7, 154.2, 144.8, 123.0, 109.5, 104.9, 103.7, 102.6, 98.4, 55.8, 55.4, 50.8, 47.1, 42.4, 31.2; HRMS (FAB) m/z 455.2143 $(M + H)^+$ ($C_{21}H_{26}N_8O_4$ requires 455.2155). Anal. ($C_{21}H_{26}N_8O_4 \cdot 1/2H_2O \cdot 1/2CH_3OH$) C, H, N.

6-[1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperidin-4-ylamino]nicotinic acid methyl ester (3): ^{1}H NMR (DMSO) δ 12.37 (s, 1H), 8.74 (d, 2H, J = 6.3), 8.57 (d, 1H, J = 2.2), 7.80 (m, 1H), 7.76 (s, 1H), 7.62 (s, 1H), 7.60 (d, 1H, J = 8.8), 6.56 (d, 1H, J = 8.9), 4.57 (m, 2H), 4.20 (m, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.76 (s, 3H), 3.42 (m, 2H), 2.03 (m, 2H), 1.51 (m, 2H); ^{13}C NMR (DMSO) δ 165.6, 161.3, 160.2, 155.1, 151.0, 150.6, 146.6, 136.8, 136.4, 113.1, 107.9, 105.2, 101.5, 99.3, 56.2, 55.9, 51.2, 46.6, 43.4, 30.9; HRMS (FAB) m/z 439.2083 $(M + H)^+$ ($C_{22}H_{26}N_6O_4$ requires 439.2094). Anal. ($C_{22}H_{26}N_6O_4 \cdot 1HCl$) C, H, N.

4-[1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperidin-4-ylamino]benzoic acid methyl ester (4): ^{1}H NMR (DMSO) δ 12.15 (s, 1H), 8.75 (d, 2H), 7.73 (s, 1H), 7.69 (d, 2H, J = 8.7), 7.51 (s, 1H), 6.66 (d, 2H, J = 8.4), 6.57 (d, 1H, J = 7.3), 4.52 (d, 2H, J = 13.2), 3.87 (s, 3H), 3.83 (s, 3H), 3.82 (m, 1H), 3.73 (s, 3H), 3.43 (m, 2H), 2.05 (m, 2H), 1.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.3, 161.4, 155.2, 151.7, 151.0, 146.7, 136.0, 131.0, 115.8, 111.3, 104.9, 101.6, 99.2, 56.2, 56.0, 51.2, 47.9, 43.4, 31.0. Anal. ($C_{23}H_{27}N_5O_4 \cdot 1HCl$) C, H, N.

4-[1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperidin-4-ylamino]benzoic acid *tert*-butyl ester (5): ^{1}H NMR (DMSO) δ 12.20 (br s, 1H), 8.66 (br, 2H), 7.76 (s, 1H), 7.66 (d, 2H, J = 8.7), 7.53 (s, 1H), 6.67 (d, 2H, J = 8.7), 6.51 (d, 1H, J = 7.4), 4.56 (d, 2H, J = 13.6), 3.90 (s, 3H), 3.86 (s, 3H), 3.76 (m, 1H), 3.46 (m, 2H), 2.07 (d, 2H, J = 11.1), 1.52 (s, 9H), 1.44 (m, 2H); ^{13}C NMR (DMSO) δ 165.3, 161.4, 155.3, 151.4, 151.1, 146.8, 136.4, 131.0, 117.8, 111.3, 105.0, 101.6, 99.2, 79.1, 56.3, 56.1, 48.0, 43.4, 31.0, 28.1; HRMS (FAB) m/z 480.2616 $(M + H)^+$ ($C_{26}H_{33}N_5O_4$ requires 480.2611). Anal. ($C_{26}H_{33}N_5O_4 \cdot 1HCl$) C, H, N.

4-[1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperidin-4-ylamino]-3-nitrobenzoic acid *tert*-butyl ester (6): ^{1}H NMR (DMSO) δ 12.23 (s, 1H), 8.89 (s, 1H), 8.65 (s, 1H), 8.56 (s, 1H, J = 2.0), 8.23 (d, 1H, J = 7.8), 7.94 (d, 1H, J = 9.2), 7.74 (s, 1H), 7.55 (s, 1H), 7.34 (d, 1H, J = 9.3), 4.62 (d, 2H, J = 13.3), 4.15 (m, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.35 (t, 2H, J = 11.6), 2.10 (d, 2H, J = 11.3), 1.74 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (DMSO) δ 163.5, 161.4, 155.2, 151.2, 146.8, 146.2, 136.3, 135.8, 130.6, 128.2, 117.9, 115.1, 104.9, 101.6, 99.1, 80.8, 56.2, 56.0, 48.8, 43.5, 30.5, 27.8; HRMS (FAB) m/z 525.2456 $(M + H)^+$ ($C_{26}H_{32}N_6O_6$ requires 525.2454). Anal. ($C_{26}H_{32}N_6O_6 \cdot 1HCl$) C, H, N.

4-[1-(5-Methoxy-1*H*-benzimidazol-2-yl)piperidin-4-ylamino]benzoic acid *tert*-butyl ester (12): ^{1}H NMR (DMSO) δ 13.40 (br s, 1H), 7.63 (d, 2H, J = 8.3), 7.29 (d, 1H, J = 8.7), 6.91 (s, 1H), 6.83 (d, 1H, J = 8.4), 6.65 (d, 2H, J = 8.3), 6.50 (s, 1H), 4.13 (d, 2H, J = 12.7), 3.77 (s, 3H), 3.73 (m, 1H), 3.47 (m, 4H), 2.06 (d, 2H, J = 11.0), 1.48 (s, 9H); ^{13}C NMR (DMSO) δ 165.2, 156.1, 151.3, 149.8, 131.0, 130.9, 124.0, 117.8, 111.8, 111.3, 110.2, 96.4, 79.0, 55.7, 47.3, 45.6, 30.2, 30.0; HRMS (FAB) m/z 423.2388 $(M + H)^+$ ($C_{24}H_{30}N_4O_3$ requires 423.2389). Anal. ($C_{24}H_{30}N_4O_3 \cdot 1HCl \cdot 1/2H_2O$) C, H, N.

4-[1-(6,7-Dimethoxy-4-*p*-tolylaminoquinazolin-2-yl)piperidin-4-ylamino]benzoic Acid *tert*-Butyl Ester (16). Compound QPH (BI2758-159C) (0.099 g, 0.3 mmol) was added to a solution of **49** (0.10 g, 0.37 mmol) in *n*-pentanol (5 mL) at 25 °C. The mixture was heated to 120 °C and maintained at

that temperature for 12 h. The reaction mixture was then cooled to room temperature and the desired product was filtered and rinsed with acetone to give 0.146 g (85.3%) of the title compound: TLC (R_f = 0.40; 5% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO) δ 12.41 (br s, 1H), 10.71 (br s, 1H), 8.08 (s, 1H), 7.63 (d, 2H, J = 8.7), 7.57 (s, 1H), 7.55 (d, 2H, J = 8.3), 7.24 (d, 2H, J = 8.3), 6.63 (d, 2H, J = 8.8), 6.44 (d, 1H, J = 6.9), 4.41 (d, 2H, J = 12.9), 3.91 (s, 3H), 3.89 (s, 3H), 3.73 (m, 1H), 3.42 (m, 2H), 2.31 (s, 3H), 2.03 (d, 2H, J = 10.8), 1.49 (s, 9H), 1.41 (m, 2H); ^{13}C NMR (DMSO) δ 165.2, 157.2, 155.2, 151.3, 150.7, 146.9, 136.6, 134.7, 130.9, 129.0, 124.0, 117.7, 111.2, 104.6, 102.3, 99.3, 79.0, 56.5, 56.1, 47.9, 43.7, 30.8, 28.0, 20.6; HRMS (FAB) m/z 570.3070 (M + H)$^+$ ($C_{33}H_{39}N_5O_4$ requires 570.3080). Anal. ($C_{33}H_{39}N_5O_4$·1HCl) C, H, N.

4-[1-(4-Aminoquinazolin-2-yl)piperidin-4-ylamino]benzoic Acid *tert*-Butyl Ester (17). Compound QDME (BI3163-162A) (0.070 g, 0.39 mmol) was added to a solution of **49** (0.13 g, 0.47 mmol) in *n*-pentanol (5 mL) at 25 °C. The mixture was heated to 120 °C and was maintained at that temperature for 12 h. The reaction mixture was then cooled to room temperature and concentrated to a brown yellow oily residue. Purification of the residue by flash column chromatography (gradient elution 0→5% MeOH in CH_2Cl_2) provided the title compound (0.12 g, 73%): TLC (R_f = 0.33; 5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 2H, J = 8.7), 7.51 (m, 3H), 7.10 (t, 1H, J = 6.6), 6.57 (d, 2H, J = 8.8), 5.35 (br s, 2H), 4.81 (d, 2H, J = 13.6), 4.00 (br s, 1H), 3.60 (m, 1H), 3.14 (t, 2H, J = 14.0), 2.13 (d, 2H, J = 13.2), 1.57 (s, 9H), 1.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.1, 161.6, 158.8, 152.0, 150.4, 133.2, 131.5, 125.9, 121.8, 121.3, 120.3, 111.8, 109.7, 79.9, 50.3, 42.8, 32.3, 28.3. Anal. ($C_{24}H_{29}N_5O_2$·1/4HCl) C, H, N.

General Procedure for *tert*-Butyl Ester Cleavage. Hydrogen chloride (4 M in 1,4-dioxane) or trifluoroacetic acid in excess amount was added to the substrate and the reaction mixture was capped with a drying tube and stirred at 25 °C for 12 h. The reaction mixture was concentrated to give the product as either HCl salt or TFA salt.

General Procedure for Methyl Ester Hydrolysis. An excess of potassium hydroxide was added to a solution of the substrate in MeOH/H_2O (10%; v/v) at 25 °C. The reaction mixture was heated to 85 °C for 12 h then cooled to room temperature. The mixture was concentrated and acidified with HCl (aq) and the desired product was filtered and rinsed with cold H_2O.

3-Amino-5-[1-(4-amino-6,7-dimethoxyquinazolin-2-yl)-piperidin-4-ylamino]-6-chloropyrazine-2-carboxylic acid (18): ^{1}H NMR (DMSO) δ 12.20 (br s, 1H), 8.50 (br s, 1H), 7.69 (s, 1H), 7.40 & 7.31 (br s, 2H),7.16 (d, 1H, J = 7.2), 4.70 (d, 2H, J = 11.5), 4.24 (m, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.10 (m, 2H), 1.92 (m, 2H), 1.66 (m, 2H); ^{13}C NMR (DMSO) δ 167.4, 161.3, 155.7, 155.1, 150.6, 146.5, 119.0, 109.2, 104.8, 101.7, 56.2, 55.9, 47.7, 43.9, 30.4; HRMS (FAB) m/z 475.1589 (M + H)$^+$ ($C_{20}H_{23}N_8O_4Cl$ requires 475.1604). Anal. ($C_{20}H_{23}N_8O_4Cl$·2HCl·1/2H_2O) C, H, N.

6-[1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperidin-4-ylamino]nicotinic acid (20): ^{1}H NMR (DMSO) δ 12.36 (br s, 1H), 9.56 (br s, 1H), 8.78 (br s, 2H), 8.36 (s, 1H), 8.07 (d, 1H, J = 9.2), 7.75 (s, 1H), 7.61 (s, 1H), 7.11 (d, 1H, J = 9.3), 4.64 (m, 2H), 4.29 (m, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.37 (m, 2H), 2.12 (m, 2H), 1.60 (m, 2H); ^{13}C NMR (DMSO) δ 164.7, 161.3, 155.2, 154.4, 151.0, 146.7, 141.8, 140.1, 136.3, 115.2, 112.4, 105.2, 101.6, 99.3, 56.3, 56.0, 48.4, 43.3, 30.4; HRMS (FAB) m/z 425.1948 (M + H)$^+$ ($C_{21}H_{24}N_6O_4$ requires 425.1937). Anal. ($C_{21}H_{24}N_6O_4$·2HCl·2H_2O) C, H, N.

4-[1-(4-Amino-6,7-dimethoxyquinazolin-2-yl)piperidin-4-ylamino]benzoic acid (22): ^{1}H NMR (DMSO) δ 12.08 (br s, 2H), 8.85 (br s, 1H), 8.64 (br s, 1H), 7.73 (s, 1H), 7.67 (d, 2H, J = 8.7), 7.48 (s, 1H), 6.65 (d, 2H, J = 8.8), 6.47 (br s, 1H), 4.51 (d, 2H, J = 13.2), 3.88 (s, 3H), 3.84 (s, 3H), 3.71 (m, 1H), 3.35 (t, 2H, J = 12.0), 2.06 (d, 2H, J = 10.4), 1.47 (m, 2H); ^{13}C NMR (DMSO) δ 167.8, 161.6, 155.7, 151.7, 151.1, 147.1, 136.2, 131.6, 117.1, 111.6, 105.0, 101.7, 99.1, 56.5, 56.4, 48.1, 43.6, 31.2; HRMS (FAB) m/z 424.2003 (M + H)$^+$ ($C_{22}H_{25}N_5O_4$ requires 424.1985). Anal. ($C_{22}H_{25}N_5O_4$·2HCl) C, H, N.

4-(1-Naphthalen-2-ylpiperidin-4-ylamino)benzoic acid (23): ^{1}H NMR (DMSO) δ 8.31 (s, 1H), 8.06 (m, 5H), 7.70 (d, 2H, J = 8.7), 7.60 (m, 2H), 6.70 (d, 2H, J = 8.8), 3.76 (m, 5H), 2.17 (m, 4H); ^{13}C NMR (DMSO) δ 167.4, 151.2, 132.7, 131.2, 129.9, 128.0, 127.8, 127.4, 119.3, 117.4, 111.4, 66.3, 29.0; HRMS (FAB) m/z 347.1770 (M+H)$^+$ ($C_{22}H_{22}N_2O_2$ requires 347.1760). Anal. ($C_{22}H_{22}N_2O_2$·2HCl) C, H, N.

4-[1-(Naphthalene-2-sulfonyl)piperidin-4-ylamino]benzoic acid (24): ^{1}H NMR (DMSO) δ 8.44 (s, 1H), 8.20 (m,1H), 8.09 (m, 1H), 7.72 (m, 4H), 7.57 (d, 2H, J = 5.7), 6.50 (d, 2H, J = 6.8), 3.60 (m, 2H), 3.25 (m, 1H), 2.55 (m, 2H), 1.93 (d, 2H, J = 11.9), 1.45 (m, 2H); ^{13}C NMR (DMSO) δ 167.4, 151.3, 134.4, 132.7, 131.9, 131.1, 129.3, 129.0, 128.6, 127.9, 122.9, 111.2, 47.2, 45.0, 30.6; HRMS (FAB) m/z 411.1366 (M + H)$^+$ ($C_{22}H_{22}N_2O_4S$ requires 411.1379). Anal. ($C_{22}H_{22}N_2O_4S$·1/2HCl·1/2H_2O) C, H, N.

4-[1-(Naphthalene-2-carbonyl)piperidin-4-ylamino]benzoic acid (25): ^{1}H NMR (DMSO) δ 7.98 (m, 4H), 7.66 (d, 2H, J = 8.7), 7.55 (m, 3H), 6.65 (d, 2H, J = 8.8), 4.41 (m, 2H), 3.67 (m, 1H), 3.15 (m, 2H), 1.95 (m, 2H), 1.40 (m, 2H); ^{13}C NMR (DMSO) δ 169.0, 167.4, 151.1, 133.6, 133.1, 132.3, 131.1, 128.3, 128.1, 127.7, 127.1, 126.8, 126.1, 124.3, 117.3, 111.6, 48.7, 45.9, 31.2; HRMS (FAB) m/z 375.1693 (M + H)$^+$ ($C_{23}H_{22}N_2O_3$ requires 375.1709). Anal. ($C_{23}H_{22}N_2O_3$·11/2HCl·1/4H_2O) C, H, N.

4-[1-(5-Methoxy-1*H*-benzoimidazol-2-yl)piperidin-4-ylamino]benzoic acid (27): ^{1}H NMR (DMSO) δ 13.00 (br s, 1H), 7.68 (d, 2H, J = 8.6), 7.30 (d, 1H, J = 8.7), 6.92 (d, 1H, J = 2.2), 6.85 (dd, 1H, J = 8.0, 2.3), 3.99 (d, 2H, J = 13.3), 3.78 (s, 3H), 3.70 (m, 1H), 3.46 (t, 2H, J = 11.2), 2.09 (d, 2H, J = 10.7), 1.56 (m, 2H); ^{13}C NMR (DMSO) δ 167.4, 156.2, 151.3, 150.0, 131.2, 131.0, 124.0, 117.1, 111.9, 111.3, 110.3, 96.6, 55.7, 47.3, 45.4, 30.2; HRMS (FAB) m/z 367.1777 (M + H)$^+$ ($C_{20}H_{22}N_4O_3$ requires 367.1765). Anal. ($C_{20}H_{22}N_4O_3$·1CF_3COOH·3/4H_2O) C, H, N.

4-[1-(6,7-Dimethoxy-4-*p*-tolylaminoquinazolin-2-yl)piperidin-4-ylamino]benzoic acid (31): ^{1}H NMR (DMSO) δ 12.10 (br s, 1H), 10.56 (br s, 1H), 7.92 (s, 1H), 7.67 (d, 2H, J = 8.5), 7.51 (d, 2H, J = 8.3), 7.27 (s, 1H), 7.26 (d, 2H, J = 7.9), 6.64 (d, 2H, J = 8.4), 4.32 (d, 2H, J = 12.6), 3.91 (s, 3H), 3.89 (s, 3H), 3.68 (m, 1H), 3.36 (t, 2H, J = 10.7), 2.32 (s, 3H), 2.05 (d, 2H, J = 8.0), 1.47 (m, 2H); ^{13}C NMR (DMSO) δ 167.4, 157.2, 155.4, 151.4, 150.7, 147.0, 136.4, 135.0, 134.6, 131.2, 129.1, 124.0, 117.0, 111.2, 104.3, 102.2, 99.1, 56.3, 56.1, 47.9, 43.5, 30.8, 20.6; HRMS (FAB) m/z 514.2459 (M + H)$^+$ ($C_{29}H_{31}N_5O_4$ requires 514.2454). Anal. ($C_{29}H_{31}N_5O_4$·1CF_3COOH·11/4H_2O) C, H, N.

4-[1-(4-Aminoquinazolin-2-yl)piperidin-4-ylamino]benzoic acid (32): ^{1}H NMR (DMSO) δ 11.8 (br s, 1H), 9.07 & 8.95 (br s, 2H), 8.20 (d, 1H, J = 7.8), 7.82 (t, 1H, J = 7.8), 7.68 (d, 3H, J = 8.5), 7.42 (t, 1H, J = 7.4), 6.65 (d, 2H, J = 8.8), 4.47 (d, 2H, J = 13.2), 3.73 (m, 1H), 3.42 (t, 2H, J = 11.3), 2.07 (d, 2H, J = 10.7), 1.50 (m, 2H); ^{13}C NMR (DMSO) δ 167.4, 162.1, 151.4, 139.8, 135.4, 131.2, 124.6, 120.0, 117.3, 117.0, 111.2, 109.1, 47.8, 43.4, 30.9. Anal. ($C_{20}H_{21}N_5O_2$·11/2CF_3-COOH) C, H, N.

General Procedure for Aromatic Nucleophilic Substitution Reaction. Compound **41** or **45** and *N*,*N*′-diisopropylethylamine (5.5 equiv) were added sequentially to a solution of compound **33**, **34**, or **42** (0.9 equiv) in DMF (3 mL/mmol) at 25 °C. The mixture was stirred at 25 °C for 12 h then was concentrated and the residue was diluted with a mixture of H_2O/EtOAc (v/v, 50:50). The aqueous layer was extracted with more EtOAc. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a dark brown solid residue. Purification of the residue was effected by flash column chromatography.

3-Amino-5-(1-*tert*-butoxycarbonylpiperidin-4-ylamino)-6-chloropyrazine-2-carboxylic acid methyl ester (35): ^{1}H NMR ($CDCl_3$) δ 6.50 (br, 2H), 5.46 (d, 1H, J = 7.4), 4.13 (m, 1H), 4.08 (m, 2H), 3.89 (s, 3H), 2.93 (t, 2H, J = 11.7), 2.03 (d,

2H, J = 12.0), 1.47 (s, 9H), 1.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.4, 155.4, 154.5, 150.6, 121.2, 110.5, 79.6, 51.8, 48.3, 42.5, 31.6, 28.3; HRMS (FAB) m/z 408.1429 $(M + Na)^+$ ($C_{16}H_{24}N_5O_4Cl$ requires 408.1415).

3-Amino-5-(1-benzylpiperidin-4-ylamino)-6-chloropyrazine-2-carboxylic acid methyl ester (37): ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 5H), 5.42 (d, 1H, J = 7.4), 3.90 (m, 1H), 3.88 (s, 3H), 3.53 (s, 2H), 2.84 (d, 2H, J = 11.8), 2.17 (t, 2H, J = 11.2), 1.99 (d, 2H, J = 9.3), 1.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.5, 155.6, 150.9, 138.4, 128.9, 128.2, 127.0, 121.4, 110.0, 63.0, 52.0, 51.7, 48.2, 31.9; HRMS (FAB) m/z 376.1549 $(M)^{+\cdot}$ ($C_{18}H_{22}N_5O_2Cl$ requires 376.1540). Anal. ($C_{18}H_{22}N_5O_2Cl$) C, H, N.

6-(1-Benzylpiperidin-4-ylamino)nicotinic acid methyl ester (39): ^{1}H NMR ($CDCl_3$) δ 8.73 (d, 1H, J = 2.1), 7.95 (m, 1H), 7.30 (m, 5H), 6.32 (d, 1H, J = 8.8), 5.01 (d, 1H, J = 7.8), 3.85 (s, 3H), 3.71 (m, 1H), 3.52 (s, 2H), 2.85 (m, 2H), 2.18 (m, 2H), 1.20 (m, 2H), 1.57 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.4, 160.1, 151.6, 138.3, 129.0, 128.1, 127.6, 127.0, 115.0, 105.8, 63.0, 52.0, 51.4, 48.4, 32.3; HRMS (FAB) m/z 326.1879 $(M + H)^+$ ($C_{19}H_{23}N_3O_2$ requires 326.1869).

4-(4-*tert*-Butoxycarbonyl-2-nitrophenylamino)piperidine-1-carboxylic acid *tert*-butyl ester (43): ^{1}H NMR ($CDCl_3$) δ 8.81 (s, 1H), 8.36 (d, 1H, J = 7.3), 8.02 (d, 1H, J = 8.3), 6.88 (d, 1H, J = 9.3), 4.05 (d, 2H, J = 13.3), 3.73 (m, 1H), 3.06 (t, 2H, J = 11.2), 2.07 (d, 2H, J = 11.1), 1.59 (s, 9H), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 164.2, 154.6, 146.3, 136.4, 131.3, 129.5, 119.3, 113.4, 81.4, 80.0, 49.6, 42.0, 31.6, 28.4, 28.2; HRMS (FAB) m/z 422.2304 $(M + H)^+$ ($C_{21}H_{31}N_3O_6$ requires 422.2291).

4-(1-Benzylpiperidin-4-ylamino)benzoic Acid Methyl Ester (46). 4-Bromomethylbenzoate (4.3 g, 20 mmol) was dissolved in 40 mL of anhydrous toluene. 4-Amino-1-benzyl piperidine (4.8 mL, 22 mmol), tris(dibenzylideneacetone)-dipalladium(0) (60 mg, 0.07 mmol), (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl [(±)-BINAP] (124 mg, 0.2 mmol), and cesium carbonate (9.2 g, 28 mmol) were added sequentially at 25 °C. The resulting mixture was stirred and heated to 80 °C, maintained at that temperature for 12 h, and then cooled to 25 °C. The reaction mixture was diluted with a mixture of MeOH/CH_2Cl_2 (150 mL, v/v, 50:50). The diluted mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The resulting residue was partitioned between EtOAc (200 mL) and H_2O (200 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a deep yellow solid residue. Purification of the residue by flash column chromatography (gradient elution 30→40% EtOAc in hexanes) provided the title compound as a pale yellow solid residue (2.26 g, 35%): TLC (R_f = 0.40; 60% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.84 (d, 2H, J = 8.8), 7.31 (s, 5H), 6.52 (d, 2H, J = 8.9), 4.04 (d, 1H, J = 7.8), 3.84 (s, 3H), 3.53 (s, 2H), 3.40 (m, 1H), 2.85 (d, 2H, J = 11.9), 2.16 (t, 2H, J = 12.7), 1.99 (m, 2H), 1.52 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 167.3, 150.9, 138.2, 131.6, 129.1, 128.2, 127.1, 118.0, 111.7, 63.1, 52.1, 51.5, 49.5, 32.3. Anal. ($C_{20}H_{24}N_2O_2$) C, H, N.

4-(1-Benzylpiperidin-4-ylamino)benzoic Acid *tert*-Butyl Ester (48). Sodium *tert*-butoxide (5.6 g, 58 mmol) was added to a solution of 4-bromomethylbenzoate (4.3 g, 20 mmol) in 40 mL of anhydrous toluene at 25 °C. The resulting mixture was heated to 80 °C and stirred for 15 min. 4-Amino-1-benzylpiperidine (4.8 mL, 22 mmol), tris(dibenzylideneacetone)dipalladium(0) (60 mg, 0.07 mmol), and (±)-BINAP (124 mg, 0.2 mmol) were added sequentially. The reaction mixture was maintained at 80 °C for 12 h, then cooled to 25 °C, and diluted with a mixture of MeOH/CH_2Cl_2 (150 mL, v/v, 50:50). The diluted mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The resulting residue was partitioned between CH_2Cl_2 (150 mL) and H_2O (150 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a deep yellow solid residue. This residue was stirred for 30 min with a mixture of EtOAc/hexanes (100 mL, 30/70, v/v) and the desired product was filtered as a yellowish powder (3.57 g, 49%): TLC (R_f = 0.51; 50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.79 (d, 2H, J = 8.7), 7.31 (s, 5H), 6.51 (d, 2H, J = 8.8), 3.90 (m, 1H), 3.52 (s, 2H), 2.85 (d, 2H, J = 12.1), 2.15 (t, 2H, J = 11.5), 1.99 (m, 2H), 1.56 (s, 9H), 1.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.1, 150.5, 138.3, 131.4, 129.1, 128.2, 127.1, 120.1, 111.6, 79.8, 63.1, 52.2, 49.6, 32.3, 28.3. Anal. ($C_{23}H_{30}N_2O_2\cdot 1/2\ H_2O$) C, H, N.

4-(Piperidin-4-ylamino)benzoic Acid *tert*-Butyl Ester (49). Ammonium formate (4.2 g, 66 mmol) was added to a solution of **48** (2.7 g, 7.3 mmol) in 120 mL of absolute ethanol. The mixture was purged with argon; then palladium (1 g, 5% on activated carbon) was added. The resulting mixture was heated to 80 °C and maintained at that temperature for 12 h. After cooling to 25 °C, the mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was cooled to 0 °C, saturated Na_2CO_3 (100 mL) was added, and the resulting mixture was stirred for 30 min. The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated in vacuo to give 1.85 g (92%) of the title compound: TLC (R_f = 0.20; 10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 2H, J = 8.7), 6.53 (d, 2H, J = 8.8), 4.10 (m, 1H), 3.40 (m, 1H), 3.11 (d, 2H, J = 12.7), 2.70 (t, 2H, J = 11.9), 2.04 (m, 2H), 1.79 (s, 1H), 1.56 (s, 9H), 1.35 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.0, 150.3, 131.2, 119.9, 111.5, 79.6, 49.9, 45.3, 33.5, 28.2. Anal. ($C_{16}H_{24}N_2O_2\cdot 1/4H_2O$) C, H, N.

4-(1-Naphthalen-2-ylpiperidin-4-ylamino)benzoic Acid *tert*-Butyl Ester (8). Sodium *tert*-butoxide (0.4 g, 4 mmol) was added to a solution of 2-bromonaphthalene (0.3 g, 1.48 mmol) and **49** (0.4 g, 1.48 mmol) in 5 mL of anhydrous toluene at 25 °C. Tris(dibenzylideneacetone)dipalladium(0) (6 mg, 0.007 mmol) and (±)-BINAP (12.4 mg, 0.02 mmol) were added sequentially. The reaction mixture was heated to 80 °C, maintained at that temperature for 12 h, then was cooled to 25 °C, and diluted with a mixture of CH_3OH/CH_2Cl_2 (150 mL, v/v, 50:50). The diluted mixture was filtered through Celite and the filtrate was concentrated in vacuo. The resulting residue was partitioned between CH_2Cl_2 (150 mL) and H_2O (150 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated in vacuo to give a deep yellow solid residue. Purification of the residue by flash column chromatography (gradient elution 0→25% EtOAc in hexanes) provided the title compound (0.35 g, 59%): TLC (R_f = 0.80; 40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 2H, J = 8.4), 7.70 (m, 3H), 7.32 (m, 3H), 7.15 (d, 1H, J = 2.1), 6.56 (d, 2H, J = 8.7), 4.02 (d, 1H, J = 7.8), 3.76 (d, 2H, J = 12.7), 3.54 (m, 1H), 2.98 (t, 2H, J = 12.0), 2.20 (d, 2H, J = 10.7), 1.67 (m, 2H), 1.57 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 166.0, 150.4, 149.1, 134.7, 131.5, 128.7, 128.5, 127.4, 126.7, 126.3, 123.4, 120.6, 119.8, 111.9, 110.7, 79.8, 49.6, 48.8, 32.1, 28.4; HRMS (FAB) m/z 402.2321 $(M)^{+\cdot}$ ($C_{26}H_{30}N_2O_2$ requires 402.2307).

In Vitro Antibacterial Activity. 1. Bacterial Strains. *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus faecalis* used in the antibacterial activity studies (ATCC 14289, 49399, 13883, 25922, 13709, and 29212, respectively) were obtained from the American Type Culture Collection, Rockville, MD. *E. coli* imp− is a mutant strain of wild-type *E. coli* with increased outer membrane permeability and was a gift of Spenser Benson (University of Maryland at College Park).[17] In initial screens, *E. coli* imp− and *S. pyrogenes* (ATCC 14289) were used. The *E. coli* imp− strain was grown in LB broth, and the *S. pyogenes* strain was grown in Todd−Hewitt broth. Interesting compounds were further evaluated in screens against other bacterial strains. *S. aureus* was grown in trypticase soy broth, *E. faecalis* in Todd−Hewitt broth, and wild-type *E. coli* and *K. pneumoniae* in nutrient broth. All bacteria were grown at 37 °C.

2. Determination of Minimum Inhibitory Concentrations (MICs). Assays were carried out in 150 μL volume in duplicate in 96-well clear flat-bottom plates. The bacterial suspension from an overnight culture growth in appropriate medium was added to a solution of test compound in 4% DMSO in water. Final bacterial inoculum was approximately 10^5−10^6 CFU (colony forming units)/well. The percent growth of the bacteria in test wells relative to that observed for a well

containing no compound was determined by measuring absorbance at 595 nm (A_{595}) after 24 h. The MIC was determined as a range of single compound concentration where the complete inhibition of growth was observed at the higher concentration and cells were viable at the lower concentration. Both ampicillin and tetracycline were used as antibiotic-positive controls in each screening assay for *S. pyogenes*, *E. coli* imp−, *E. coli*, *S. aureus*, *E. faecalis*, *K. pneumoniae*.

3. Animals and in Vivo Studies. Male ICR mice were fed with autoclaved commercial food pellets and sterile water ad libitum. Mean weight at arrival was 20 g. Animals were inoculated intraperitoneally with 8.0×10^6 CFU/0.5 mL/mouse of *K. pneumoniae* (ATCC 10031) in BHI containing 5% mucin. Ten animals each were randomly assigned to either control or treatment groups. Compound **22** (DMSO solution, 100, 33.3, and 3.3 mg/kg) and gentamycin (3 mg/kg, included as a positive control) were both administered subcutaneously 1 h after infection. Compound **22** was administered as a solution in DMSO (100%) and 50 μL/mouse. Gentamycin was administered as an aqueous buffer solution (phosphate-buffered saline (PBS), pH = 7.4).

4. Coupled Bacterial Transcription/Translation Assay. The DNA template, pBestLuc (Promega), was a plasmid containing a reporter gene for firefly luciferase fused to a strong *tac* promoter and ribosome binding site. One microgram of pBestLuc was transcribed and translated in *E. coli* S30 bacterial extract in the presence or absence of test compound.[18] Compounds were tested in a black 96-well microtiter plate with an assay volume of 35 μL. Each test well contained 5 μL of test compound, 13 μL of S30 premix (Promega), 4 μL of 10X complete amino acid mix (1 mM each), 5 μL of *E. coli* S30 extract, and 8 μL of 0.125 μg/μL pBestLuc. The transcription/translation reaction was incubated for 35 min at 37 °C followed by detection of functional luciferase with the addition of 30 μL of LucLite (Packard). Light output was quantitated on a Packard TopCount.

5. Amino Acid Misincorporation Assay.[19] A mutant form of ubiquitin devoid of the amino acid tyrosine was produced in vitro in *E. coli* S30 extracts in the presence of a tritiated tyrosine.[20] Since ubiquitin has no tyrosine in the sequence, if tyrosine was used as the labeled amino acid, any incorporated counts above background were assumed to be due to the misincorporation of the tritiated amino acid. The labeled protein was captured via a ubiquitin antibody which was associated with anti-rabbit SPA beads. Altered ubiquitin molecules are not efficiently captured by the antibody. Compounds were tested in 96-well microtiter plate in an assay volume of 10 μL. Control experiments using the antibiotics kanamycin, novabiocin, monensin, gentamicin, neomycin, and tetracycline were run at 5 μM of each antibiotic. Compounds **4** and **22** were both tested at 5, 50, and 500 μM.

Acknowledgment. We are grateful for many helpful discussions throughout the course of this work with Prof. Michael E. Jung. We also thank Dr. Karl D. Hargrave of Boehringer Ingelheim for providing compounds QPH and QDME. This work was supported by a grant from the Defense Advanced Research Projects Agency.

References

(1) (a) Gold, H. S.; Moellering, R. C., Jr. Antimicrobial-Drug Resistance. *N. Engl. J. Med.* **1996**, *335*, 1445−1453. (b) Davies, J. Inactivation of Antibiotics and the Dissemination of Resistance Genes. *Science* **1994**, *264*, 375−382. (c) Spratt, B. G. Resistance to Antibiotics Mediated by Target Alterations. *Science* **1994**, *264*, 388−393. (d) Pratt, W. B.; Fekety, R. *The Antimicrobial Drugs*; Oxford University Press: New York, 1986; Chapter 7, pp 153−183.

(2) Tai, P.-C.; Wallace, B. J.; Davis, B. D. Streptomycin Causes Misreading of Natural Messenger by Interacting with Ribosomes after Initiation. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 275−279.

(3) Blyn, L.; Risen, L.; et al. Unpublished results.

(4) (a) Koeppe, H.; Speck, G.; Stockhaus, K. New Pyrazine Derivatives. Their Preparation and their Use as Ingredients in Drugs. PCT Int. Appl., WO 9304048. (b) Miyamoto, T.; Egawa, H.; Matsumoto, J.-I. Pyridonecarboxylic Acids as Antibacterial Agents. VIII. An Alternative Synthesis of Enoxacin via Fluoronicotinic Acid Derivatives. *Chem. Pharm. Bull.* **1987**, *35*, 2280−2285. (c) Kukla, M. J.; Breslin, H. J.; Pauwels, R.; Fedde, C. L.; Miranda, M.; Scott, M. K.; Sherrill, R. G.; Raeymaekers, A.; Van Gelder, J.; Andries, K.; Janssen, M. A. C.; De Clerq, E.; Janssen, P. A. J. Synthesis and Anti-HIV-1 Activity of 4,5,6,7-Tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepin-2(1*H*)-one (TIBO) Derivatives. *J. Med. Chem.* **1991**, *34*, 746−751.

(5) (a) Wagaw, S.; Buchwald, S. L. The Synthesis of Aminopyridines: A Method Employing Palladium-Catalyzed Carbon−Nitrogen Bond Formation. *J. Org. Chem.* **1996**, *61*, 7240−7241. (b) Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. An Improved Catalyst System for Aromatic Carbon−Nitrogen Bond Formation: The Possible Involvement of Bis(Phosphine) Palladium Complexes as Key Intermediates. *J. Am. Chem. Soc.* **1996**, *118*, 7215−7216.

(6) Mach, R. H.; Luedtke, R. R.; Unsworth, C. D.; Boundy, V. A.; Nowak, P. A.; Scripko, J. G.; Elder, S. D.; Jackson, J. R.; Hoffman, P. L.; Evora, P. H.; Rao, A. V.; Molinoff, P. B.; Childers, S. R.; Ehrenkaufer, R. L. ^{18}F-Labeled Benzamides for Studying the Dopamine D_2 Receptor with Positron Emission Tomography. *J. Med. Chem.* **1993**, *36*, 3707−3720.

(7) (a) Giardina, D.; Gulini, U.; Massi, M.; Piloni, M. G.; Pompei, P.; Rafaiani, G.; Melchiorre, C. Structure−Activity Relationships in Prazosin-Related Compounds. 2. Role of the Piperazine Ring on α-Blocking Activity. *J. Med. Chem.* **1993**, *36*, 690−698. (b) Althuis, T. H.; Hess, H.-J. Synthesis and Identification of the Major Metabolites if Prazosin Formed in Dog and Rat. Synthesis and Identification of the Major Metabolites if Prazosin Formed in Dog and Rat. *J. Med. Chem.* **1977**, *20*, 146−149.

(8) Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. Selective Removal of an *N*-BOC Protecting Group in the Presence of a *tert*-Butyl Ester and Other Acid-Sensitive Groups. *J. Org. Chem.* **1994**, *59*, 3216−3218.

(9) Taylor, E. C.; Wong, G. S. K. Convergent and Efficient Palladium-Effected Synthesis of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF). *J. Org. Chem.* **1989**, *54*, 3618−3624.

(10) Wolfe, J. P.; Buchwald, S. L. Improved Functional Group Compatibility in the Palladium-Catalyzed Amination of Aryl Bromides. *Tetrahedron Lett.* **1997**, *38*, 6359−6362.

(11) (a) Awaya, A.; Nakano, T.; Kobayashi, H.; Tan, K.; Horikomi, K.; Sasaki, T.; Yokoyama, K.; Ohno, H.; Kato, K. Preparation of 2-(1-piperazinyl)pyrimidines as Agents for Treating Neuropathy. PCT Int. Appl., WO 8704928. (b) Yokoyama, K.; Kato, K.; Kitahara, T.; Ohno, H.; Nishina, T.; Awaya, A.; Nakano, T.; Watanabe, K.; Saruta, S.; Kumakura, M. Quinazoline Derivatives and Antihypertensive Preparations Containing Them. Eur. Pat. Appl., EP 188094.

(12) Hess, H. J.; Cronin, T. H.; Scriabine, A. Antihypertensive 2-amino-4(3H)-quinazolines. *J. Med. Chem.* **1968**, *11*, 130−136.

(13) Gazit, A.; Chen, J.; App, H.; McMahon, G.; Hirth, P.; Chen, I.; Levitzki, A. Tyrphostins IV−Highly Potent Inhibitors of EGF Receptor Kinase. Structure−Activity Relationship Study of 4-Anilidoquinazolines. *Bioorg. Med. Chem.* **1996**, *4*, 1203−1207.

(14) Lacefield, W. B. 2,4-Diaminoquinazolines as Antithrombotic Agents. U.S. 3956495.

(15) Kuzmich, S.; Marky, L. A.; Jones, R. A. Specifically Alkylated DNA Fragments. Synthesis and Physical Characterization of d[CGC(O^{6}Me)GCG] and d[CGT(O^{6}Me)GCG]. *Nucleic Acids Res.* **1983**, *11*, 3393−3404.

(16) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(17) Sampson, B. A.; Misra, R.; Benson, S. A. Identification and Characterization of a New Gene of *Escherichia coli* K-12 Involved in Outer Membrane Permeability. *Genetics* **1989**, *122*, 491−501.

(18) Lesley, S. A.; Brow, M. A. D.; Burgess, R. R. Use of *inVitro* Protein Synthesis from Polymerase Chain Reaction-generated Templates to Study Interaction of *Escherichia coli* Transcription Factors with Core RNA Polymerase and for Epitope Mapping of Monoclonal Antibodies. *J. Biol. Chem.* **1991**, *266*, 2632−2638.

(19) (a) Grise-Miron, L.; Noreau, J.; Melancon, P.; Brakier-Gingras, L. Comparison of the Misreading Induced by Streptomycin and Neomycin. *Biochim. Biophys. Acta* **1981**, *656*, 103−110. (b) Davies, J.; Davis, B. D. Misreading of Ribonucleic Acid Code Words Induced by Aminoglycoside Antibiotics (The Effect of Drug Concentration). *J. Biol. Chem.* **1968**, *243*, 3312−3316.

(20) Ecker, D. J.; Butt, T. R.; Marsh, J.; Sternberg, E. J.; Margolis, N.; Monia, B. P.; Jonnalagadda, S.; Khan, M. I.; Weber, P. L.; Mueller, L.; Crooke, S. T. Gene Synthesis, Expression, Structures, and Functional Activities of Site-specific Mutants of Ubiquitin. *J. Biol. Chem.* **1987**, *262*, 14213−14221.

JM9903500