Structure-Activity Relationships of Imidazo[4,5-f]quinoline Partial Structures and Analogs. Discovery of Pyrazolo[3,4-f]quinoline Derivatives as Potent Immunostimulants

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Structure-activity studies have been carried out on a series of imidazo[4,5-f]quinoline derivatives reported to have potent in vivo immunostimulatory activity. This activity has been confirmed, and subtle structure-activity relationships have been uncovered which resulted in the identification of novel analogs (pyrazolo[3,4-f]quinoline derivatives, **7a,b**) with potent in vivo effects in a mouse protection model. Regioisomeric pyrazolo[4,3-f]quinoline derivatives (**6a,b**) were shown to be inactive. Data are presented which support the notion that the in vivo activity is mediated by an immunostimulatory mechanism.

Immunocompromised individuals such as surgery patients, severe burn victims, AIDS patients, and cancer chemotherapy patients are susceptible to a range of opportunistic infections by bacteria, fungi, protozoa, and mycoplasma. Such infections represent a leading cause of morbidity and mortality among these patients and are difficult to treat with conventional antibiotic therapy or by immunization. Stimulation of the nonspecific immune response would be expected to significantly assist these patients in avoiding such infections (prophylactic treatment) as well as to control those already established (therapeutic treatment).

In addition to these important applications in human medicine, an immunostimulant could play an important role in livestock production. Periods of stress (e.g. shipping, weaning, handling, dietary changes, comingling) have been associated with increased incidences of bacterial disease in cattle and swine.[1] This is consistent with studies demonstrating stress to have a deleterious effect on the immune system. It is expected that an immunostimulant, alone or in conjuction with a conventional antibiotic, would provide an improved clinical outcome relative to antibiotic treatment alone, resulting in considerable economic benefit to the food producer.[1]

With these therapeutic applications as the driving force, research into the design, synthesis, and biological evaluation of novel immunostimulants continues to be an important area of medicinal chemistry and immunology research.[2] A recent report[3] of potent in vivo immunostimulatory activity of imidazo[4,5-f]quinoline derivatives lead to the evaluation of these compounds in our own in vivo model. Confirmation of these compounds as among the most potent low molecular weight, non-peptide immunostimulants we had evaluated provided the stimulus for the initiation of a synthetic program designed to explore the structural features necessary for activity and to probe possible mechanisms of the in vivo effects. The results of these studies are the subject of this report.

Results and Discussion

Strategy. Our strategy for exploration of the structural features necessary for biological activity consisted of targeting several partial structures of the imidazo[4,5-f]-quinoline nucleus as shown in Figure 1. The importance of the NH-linker from the imidazo[4,5-f]quinoline nucleus to the R_1 substituent was probed by preparing the corresponding sulfur and *N*-methyl linked compounds generically described by **3**. The R_1 and R_2 substituents in compounds **2-7** of Figure 1 were chosen from among the substituents giving potent activity in the parent system (**1**). The contribution of the pyridine portion of the imidazo[4,5-f]quinoline nucleus was tested by the 7-carbamoylbenzimidazole substructure **4**. The pyridine derivative **5** was targeted to assess the contribution of the intact tricycle and the nature of the hydrogen bond donor/acceptor characteristics of the X-H substituent. The 4-aminoquinoline derivative **2** was prepared to address the contribution of the imidazole portion of imidazo[4,5-f]quinoline nucleus. The regioisomeric pyrazoloquinoline derivatives **6** and **7** probe this structural feature in a more refined manner.

Chemistry. The synthesis of compounds **1a-f**, **6a,b**, and **7a,b** follows procedures described previously[3,4] and is illustrated for **7** (Scheme I). Condensation of 6-aminoindazole (**8**) with ethyl acetoacetate produces **9**, which cleanly undergoes thermal cyclization in boiling Dowtherm A to give **10**. Interestingly, none of the linearly fused tricycle is isolated.[5] Treatment with phosphorus oxychloride generates the key intermediate **11**, which is used in the preparation of **7a,b**. Reaction of the chloro compound with the appropriate aniline or amine derivative cleanly produces the targeted compounds, in this case, **7a,b**.

Compounds **2a-d** are conveniently prepared from commercially available 4-chloroquinaldine (**12**) by treatment with the appropriate aniline (Scheme II).

Compounds **3a,b** are prepared from 9-chloro-7-methyl-1*H*-imidazo[4,5-f]quinoline by treatment with the sodium

† Terre Haute, Indiana.

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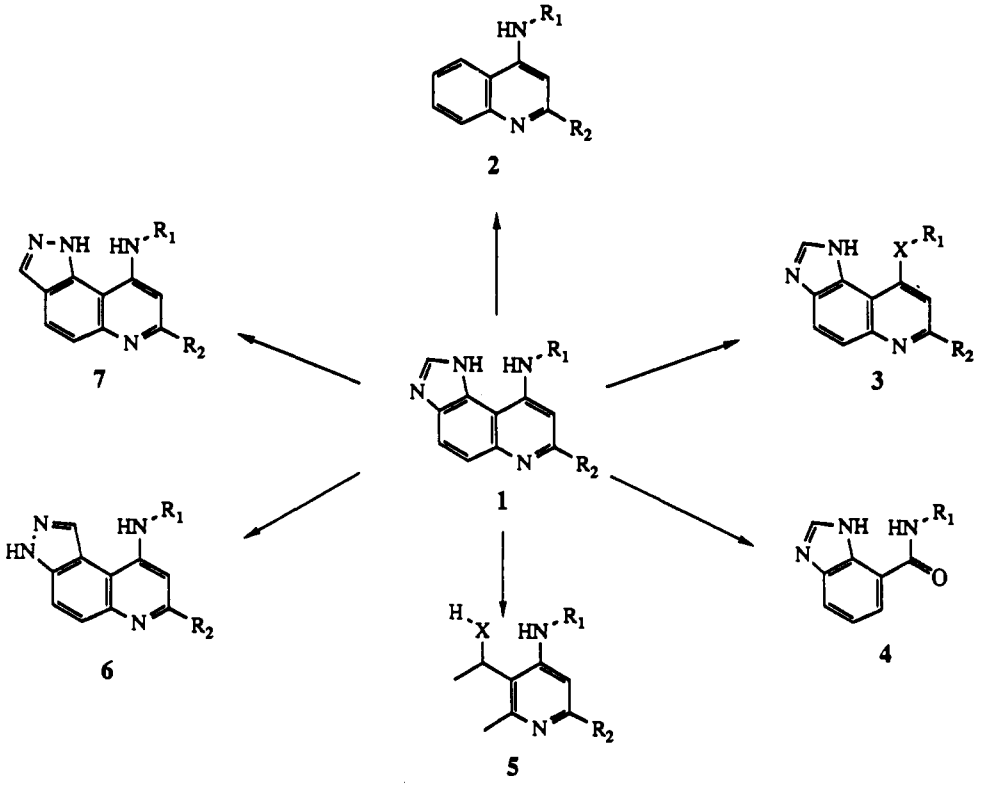
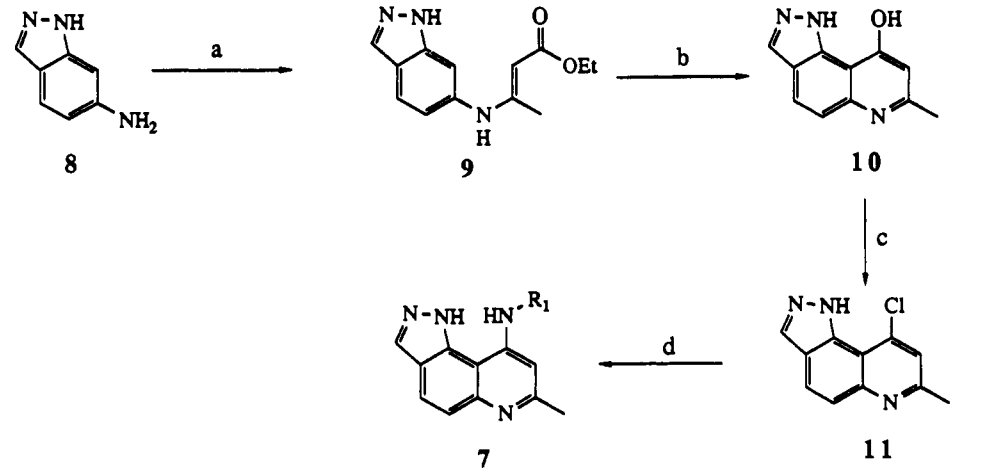


Figure 1.

Scheme I [a]



[a] (a) Ethyl acetoacetate, $CaSO_4$, HOAc, EtOH, reflux; (b) Dowtherm A, reflux; (c) $POCl_3$, DMF; (d) appropriate aniline derivative, EtOH, reflux.

salt of 3-methoxythiophenol or *N*-methyl-*m*-anisidine, respectively (Scheme III).

Synthesis of the benzimidazole derivatives **4a–d** begins with the conversion of 3-nitrophthalic anhydride to the ring-opened amide **14**[6] (Scheme IV). Treatment of **14** with sodium hypochlorite[6] produces **15**, which is converted to the diamine by reduction of the nitro group.[7] Formation of the benzimidazole nucleus is completed by treatment of **16** with formic acid.[8] The amides **4a–d** are prepared by activating the carboxyl group with isobutyl chloroformate followed by treatment with the appropriate aniline or amine derivative. The benzimidazole nucleus is N-acylated by the isobutyl chloroformate treatment; however, the acyl group is easily removed by alkaline hydrolysis to give the targeted compounds, **4a–d**.

Preparation of **5a–c** (Scheme V) proceeds from the known pyridone **18**[9] by treatment with phosphorus oxychloride to give the activated 4-chloropyridine derivative **19**. Treatment with *m*-anisidine produces **5a**, which serves

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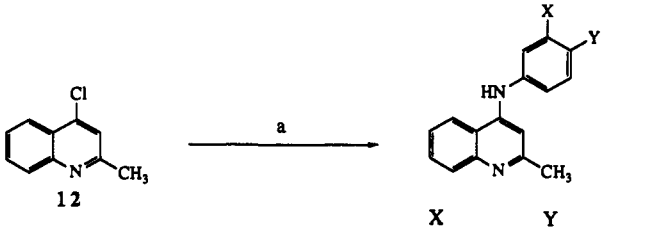
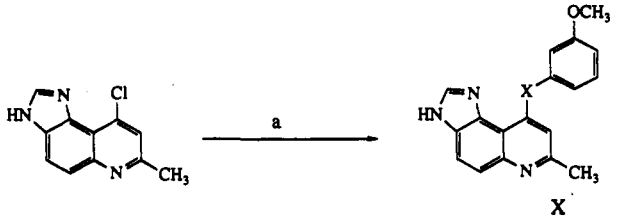
Scheme II [a]



	X	Y
2a	OCH_3	H
2b	H	$OCH_2C_6H_5$
2c	H	Bu
2d	H	OBu

[a] (a) Appropriate aniline derivative, EtOH, reflux.

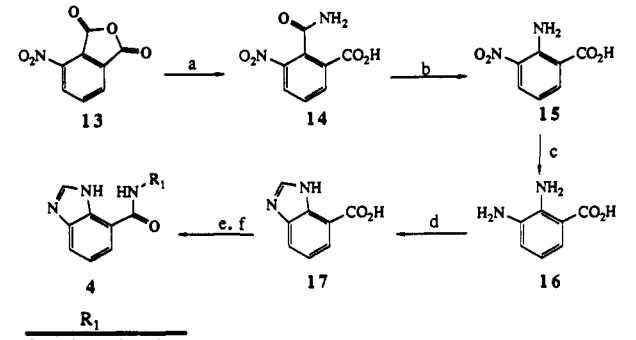
Scheme III [a]



	X
3a	S
3b	NCH_3

[a] (a) 3-Methoxythiophenol sodium salt, THF, or *N*-methyl-3-methoxyaniline, EtOH, reflux.

Scheme IV [a]



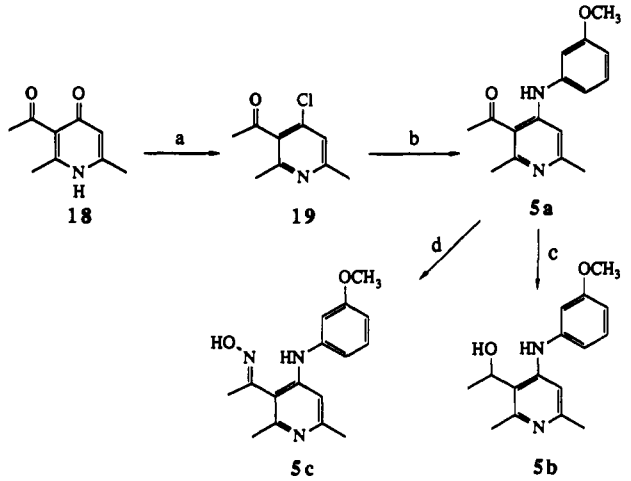
R_1	
a	3-methoxyphenyl
b	4-(cyclohexylmethoxy)phenyl
c	4-cyanophenyl
d	cyclohexyl

[a] (a) Concentrated ammonium hydroxide; (b) NaOCl, aqueous NaOH; (c) H_2, Pd/C, aqueous NaOH; (d) formic acid, reflux; (e) isobutyl chloroformate, Et_3N, THF followed by addition of the appropriate aniline or amine; (e) KOH, MeOH.

as the precursor to **5b,c**. These compounds are prepared by reduction of the ketone with sodium borohydride or by reaction with hydroxylamine to give the corresponding alcohol and oxime, respectively.

Biological Results. Immunostimulant activity was evaluated in a mouse protection assay in which increased survival of infected, drug-treated animals over infected, untreated controls was the endpoint. Briefly, mice were treated 24 h prior to a lethal inoculum of *Escherichia coli* which was followed by a subtherapeutic dose of antibiotic (gentamicin). The mice were monitored for 96 h and survivors in the drug-treated groups compared with survivors in the untreated control groups. The results of these evaluations are shown in Table I.

Scheme V [a]

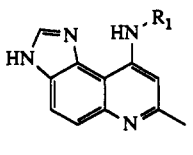


[a] (a) $POCl_3$, DMF; (b) 3-methoxyaniline, EtOH, reflux; (c) $NaBH_4$, EtOH; (d) hydroxylamine hydrochloride, aqueous NaOH, EtOH, reflux.

Table I. Protective Activity of Analogs in Systemic *E. coli* Mouse Protection Assay

compd	mp (°C)	MED[a] (mg/kg)	ED_{100}[b] (mg/kg)
1a	>300	6	50
1b	>300	1	5
1c	>300	5	15
1d	>300	5	15
1e	>300	2	2
1f	>300	6	6
2a	>250	>50	NA[c]
2b	>250	>50	NA
2c	>250	>50	NA
2d	>250	>50	NA
3a	>300	>50	NA
3b	>300	>50	NA
4a	187–188	>50	NA
4b	234–236	>50	NA
4c	269–272	>50	NA
4d	180–182	>50	NA
5a	*d*	>100	NA
5b	167–172	>100	NA
5c	220 dec	>100	NA
6a	>300	>50	NA
6b	>300	no dose response	NA
7a	>300	6	17
7b	>300	2	6

[a] Minimum effective dose is that dose (sc) which provides statistically significant ($p < 0.05$) protection as compared to control animals. [b] Dose at which 100% protection occurs. [c] Not applicable. [d] Extremely hygroscopic.



R_1	
a	3-methoxyphenyl
b	4-(cyclohexylmethoxy)phenyl
c	4-butoxyphenyl
d	4-butylphenyl
e	4-cyanophenyl
f	cyclohexyl

Figure 2.

The imidazo[4,5-*f*]quinoline derivatives **1a–f** (Figure 2) all provided potent protective effects in this model with minimum effective doses ranging from 1 mg/kg to 6 mg/kg. Importantly, these compounds were also able to provide complete protection at only slightly higher doses.

These results are comparable to the results reported previously[3] and justified the investigation of the structural features contributing to this potent immunostimulant activity.

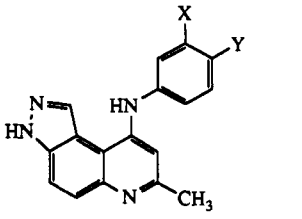
The nature of the linker between the imidazo[4,5-*f*]-quinoline nucleus and the C-4 substituent (quinoline numbering) is clearly important for activity as replacement of the N–H with either sulfur or *N*-methyl (**3a** and **3b**) significantly reduces activity. The lack of activity of **3b** was particularly surprising given the presumed similarity in steric requirements and in the electronic nature of **3b** and **1a**. All of the remaining analogs presented in this paper retain the N–H linker.

The benzimidazole derivatives **4a–d** represent an attempt to assess the contribution to activity of the pyridine portion of the imidazo[4,5-*f*]quinoline nucleus. These analogs are not ideal probes for this effect due to the likelihood that the preferred conformation of the amides places the nitrogen substituent in a different orientation relative to the remainder of the molecule than that of the parent compounds. With this caveat in mind, however, the lack of activity of **4a–d** implies that the pyridine portion also plays an important role in the activity of the imidazo-[4,5-*f*]quinoline derivatives.

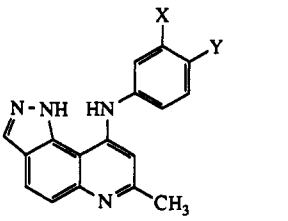
Pyridine analogs **5a–c** provided evidence for importance of the benzimidazole portion of the imidazo[4,5-*f*]quinoline nucleus. Clearly it is insufficient to have the 4-aminopyridine portion intact with hydrogen bond donor or acceptor functionality mimicking a portion of the imidazole ring of the parent compounds. Pinpointing the nature of the insufficiency was not possible with these analogs. One possibility is that the portion of the imidazole not mimicked by these analogs is important for activity. This possibility is addressed more directly with analogs discussed below. An alternative explanation is that the orientation of the substituents relative to the 4-amino group is nonoptimal. This possibility could be addressed by introducing constraints into the system which force the pyridine substituents to more closely approximate the orientation of the parent system.

Analogs **2a–d**, differing from the parent compounds only in their lack of the imidazole portion of the imidazo[4,5-*f*]quinoline nucleus, completely lacked immunostimulant activity. This clearly points to the importance of this region of the parent structure and prompted further investigation with analogs that more closely resemble the imidazo[4,5-*f*]quinoline compounds. The regioisomeric pyrazoloquinolines **6a,b** and **7a,b** (Figure 3) provide an interesting opportunity to study this region in a more refined manner. Surprisingly, the immunostimulant activity of the two regioisomeric pair of compounds was markedly different. Compounds **7a,b** are potent immunostimulants with activity comparable to the parent compounds, **1a,b**. Contrary to this is the lack of activity of **6a,b**, indicating an important functional role, and relatively tight SAR, for this region of the molecule. One postulate for the difference in activity would be the lack of a hydrogen bond donor substituent at the pyrazole position adjacent to the anilino substituent at C-9. However, in additional series of related immunostimulants, hydrogen-bonding potential at this position is not necessary for potent activity.[10]

Precise interpretation of the structure–activity relationships associated with these partial structures and



	X	Y
6a	OCH_3	H
6b	H	$OCH_2C_6H_5$



	X	Y
7a	OCH_3	H
7b	H	$OCH_2C_6H_5$

Figure 3.

Table II. Efficacy of Aminoquinolines in a Murine Systemic *E. coli* Infection with and without Subtherapeutic Gentamicin Treatment (0.5 mg/kg)[a]

compd	+ gentamicin	*N*	– gentamicin	*N*
1b	0.5	12	1.5	1
7a	4.2	10	9.8	3

[a] PD_{50} values (mg/kg) were calculated from pooled data by probit analysis (*N*: number of pooled experiments).

analogs is difficult without in vitro data as a correlate to the in vivo results presented. Obviously, the in vivo activity is a reflection not only of the inherent activity of the compounds, but pharmacokinetic/pharmacodynamic parameters as well. Thus, an inactive compound may lack inherent activity or lack the requisite pharmacokinetic characteristics. Identification of an in vitro immunostimulatory effect associated with these compounds will be required before this issue can be adequately addressed.

In addition to the uncertainty relative to an in vitro immunostimulatory mechanism, the in vivo protective activity described above could potentially have been mediated by a mechanism other than immunostimulation. This concern was heightened by the reported antibacterial[11] and anthelmintic[12] activity of members of this family of compounds. Experiments were carried out to establish whether the in vivo protective effects were, in fact, due to immunostimulation or an antibacterial effect.

As previously discussed, **1b** and **7a** were extremely effective at protecting mice from a systemic *E. coli* infection (Table II). Neither of the compounds demonstrated in vitro antibacterial activity, however (Table III). Addition of subtherapeutic gentamicin in vivo increased the potency

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Table III. In Vitro Antibacterial Activity of **1b** and **7a** and Their Effect on Sensitivity of *E. coli* N63 to Gentamicin

		MIC[a] of gentamicin in the presence of aminoquinolines; aminoquinoline concentration (μg/mL):							
compd	MIC[b]	100	50	25	12.5	6.25	3.13	1.56	0
1b	>100	0.063	0.063	0.125	0.125	0.25	0.25	0.25	0.50
7a	100		0.25	0.25	0.25	0.25	0.25	0.25	0.25

[a] MIC: minimum inhibitory concentration (μg/mL) against *E. coli* N63. [b] MIC of aminoquinoline alone against *E. coli* N63.

Table IV. Effect of Interval between Drug Treatment and Challenge on Activity of Aminoquinolines in the Mouse Protection Assay[a]

time of treatment (days prior to infection)	**7a** (25 mg/kg)	**1b** (1.7 mg/kg)
6	88	0
3	100	40
1	100	60
0	0	0

[a] Results are expressed as percent protection ([number dead in control − number dead in treated) + number dead in control] × 100).

of the aminoquinolines 2–3-fold, suggesting a potential for synergistic interaction between gentamicin and aminoquinolines. Compound **7a** did not affect the antibacterial activity of gentamicin, whereas high concentrations of **1b** (50–100 μg/mL) caused a moderate decrease in the MIC. Concentrations below 25 μg/mL caused only a 2–4-fold reduction in the MIC of gentamicin, a change which is within the experimental error for this assay. The high dose effect of **1b** is unlikely to have played a role in vivo as plasma and tissue levels never reach the high concentrations necessary to see an effect.[13] Thus, there is little in vitro evidence for an interaction between **1b** or **7a** and gentamicin at physiologically relevant concentrations. To test the possibility that a metabolite with antibacterial activity was being generated in vivo, serum was collected from mice 24 h after subcutaneous treatment with **1b** (15 mg/kg) and tested for antibacterial activity against *E. coli* N63 by means of a disc diffusion assay. Sterile paper discs containing 20 μL of serum were placed on BHI agar plates which had been inoculated with *E. coli* in such a manner as to form a confluent layer of bacteria on the plate. After overnight incubation at 37 °C, there were no zones of inhibition around any of the triplicate discs used, indicating that no antibacterial activity was present in the serum of mice 24 h after receiving **1b** (data not shown).

Treatment of mice at various times relative to infection provided further evidence of an immunostimulatory mechanism of action of aminoquinolines. An antibacterial mechanism of action would require the presence of the compound or its active metabolite at the time of challenge in the tissues or plasma at levels sufficient to kill or inhibit bacteria. Greater tissue levels of the compounds at challenge would be expected when the drugs are administered near the time of infection as compared to administration prior to challenge. Both compounds increased the number of surviving mice when administered 1 or 3 days prior to infection, but were inactive when administered 1 h before infection (Table IV). Thus, it seems likely that the aminoquinolines protected mice from infection by means of an immunomodulatory mechanism rather than an antibacterial effect.

Conclusions. The in vivo immunostimulatory activity of imidazo[4,5-*f*]quinoline derivatives has been shown to be quite sensitive to structural modifications as partial structures and analogs routinely showed significantly reduced activity. An important exception to this is the pyrazolo[3,4-*f*]quinoline subseries represented by **7a,b** which showed activity comparable to that of the parent series. Structure–activity studies have been carried out on this novel series of immunostimulants and will be presented in due course.

Several indirect lines of evidence were presented which suggest the in vivo protective effects are mediated by an immunostimulatory mechanism. Identification of an in vitro correlate to this activity would significantly solidify the mechanism of action as well as provide an important tool for increased understanding of in vivo structure–activity relationships.

Experimental Section

General Methods. Materials were obtained from commercial vendors and used without further purification. Thin-layer chromatography was used to monitor the progress of reactions and was performed with Analtech silica gel GF (250 μm) plates. Flash column chromatography was performed with J. T. Baker silica gel (40 μm). Melting points were determined on a Büchi 510 melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian XL-300 operating at 300 MHz and the data tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, coupling constants in hertz. Mass spectra were obtained with an A.E.I. MS-30 mass spectrometer or a Finnegan 4510 instrument. Elemental analyses were performed by the Microanalytical Laboratory operated by the Analytical Department, Pfizer Central Research and are within 0.4% of calculated values unless otherwise indicated. Those compounds for which high-resolution mass spectra were obtained were homogeneous by TLC analysis. Known compounds (**1a–f**) were characterized by ^{1}H NMR and mass spectral data which were fully in accord with the expected structures.

Compounds **1a–f**, **6a,b**, and **7a,b** were prepared by methods described in the literature[3,4] and which are illustrated by the preparation of **7a** below.

9-*m*-Anisidino-7-methyl-1*H*-pyrazolo[3,4-*f*]quinoline Hydrochloride (7a). To a suspension of 6-aminoindazole (40.0 g, 0.30 mol) in absolute ethanol (500 mL) were added ethyl acetoacetate (70.37 g, 0.54 mol), calcium sulfate (20 g), and acetic acid (2 mL). The reaction was heated to reflux, and after 24 h more $CaSO_4$ (10 g) and ethyl acetoacetate (19 mL) were added and the reflux continued for another 24-h period. It was necessary to add more $CaSO_4$ (5 g) and ethyl acetoacetate (10 mL) and to reflux the reaction an additional 24 h to complete the conversion of 6-aminoindazole to product. The solid was removed by filtration and the filtrate subjected to rotary evaporation. Ethanol was added and the slurry cooled in a refrigerator. The solid was collected by filtration, washed with hexanes, and dried in a vacuum oven to give the cyclization precursor **9** as a light brown solid (62.19 g, 84%).

A fraction of this material (30.00 g, 0.12 mol) was added to boiling Dowtherm A (600 mL) in portions. Upon completion of the addition, the reaction was continued for an additional 6 min. On cooling the reaction, a precipitate formed which was collected by filtration and washed thoroughly with hexanes to give 9-hydroxy-7-methyl-1*H*-pyrazolo[3,4-*f*]quinoline (**10**) as a tan solid (23 g, 94%): ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3 H), 6.10 (s, 1 H), 7.22 (d, 1 H, J = 8), 7.84 (d, 1 H, J = 8), 8.08 (s, 1 H).

To a suspension of **10** (8.00 g, 0.04 mol) in phosphorus oxychloride (61.6 g, 0.40 mol) was slowly added *N,N*-dimethylformamide (40 mL). After the addition was complete, the reaction

(13) Weber, F. H.; Rice, J. R.; Earley, D. L.; Moyer, M. P. Unpublished results.

was warmed to 80 °C for 1 h and then allowed to cool to room temperature. The reaction was poured onto ice, dissolved in water (2 L total volume), and neutralized to pH 7 with 20% aqueous NaOH. The precipitate which formed upon neutralization was collected by filtration, washed with water, and dried under vacuum to give 9-chloro-7-methyl-1*H*-pyrazolo[3,4-*f*]-quinoline (11) as a white solid (8.07 g, 92%): ^{1}H NMR (DMSO-d_6) δ 2.66 (s, 3 H), 7.61 (d, 1 H, *J* = 9), 7.72 (s, 1 H), 8.07 (d, 1 H, *J* = 9), 8.33 (s, 1 H).

To a solution of 11 (1.63 g, 7.5 mmol) in ethanol (60 mL) was added *m*-anisidine (1.11 g, 9.0 mmol) and the mixture heated at reflux overnight. The solvent was removed by rotary evaporation and the residue redissolved in methanol and treated with decolorizing carbon. After filtration through Celite, the filtrate was subjected to rotary evaporation and the resulting pale yellow solid recrystallized from methanol/ethyl ether to give 9-*m*-anisidino-7-methyl-1*H*-pyrazolo[3,4-*f*]quinoline hydrochloride (**7a**, 2.35 g, 92%) as pale yellow needles: ^{1}H NMR δ 2.72 (s, 3 H), 3.85 (s, 3 H), 7.05 (d, 1 H, *J* = 8), 7.12 (s, 1 H), 7.14–7.18 (m, 2 H), 7.50 (t, 1 H, *J* = 8), 7.72 (d, 1 H, *J* = 9), 8.34 (d, 1 H, *J* = 9), 8.84 (s, 1 H); mass spectrum *m/z* 304 (M$^+$). Anal. ($C_{18}H_{16}N_4O$·HCl·1.5H_2O) C, H, N.

1a: ^{1}H NMR (DMSO-d_6) δ 2.74 (s, 3 H), 3.86 (s, 3 H), 7.00 (d, 1 H, *J* = 8), 7.10 (s, 1 H), 7.16 (m, 2 H), 7.50 (t, 1 H, *J* = 8), 7.92 (br d, 1 H, *J* = 8), 8.26 (d, 1 H, *J* = 9), 8.76 (s, 1 H); mass spectrum *m/z* 304 (M$^+$).

1b: ^{1}H NMR (CD_3OD) δ 1.1–1.4 (m, 5 H), 1.7–1.95 (m, 6 H), 2.66 (s, 3 H), 3.86 (d, 2 H, *J* = 7), 6.80 (s, 1 H), 7.10 (d, 2 H, *J* = 9), 7.42 (d, 2 H, *J* = 9), 7.70 (d, 1 H, *J* = 7), 8.16 (d, 1 H, *J* = 7), 8.50 (s, 1 H); mass spectrum *m/z* 386 (M$^+$).

1c: ^{1}H NMR (CD_3OD) δ 1.03 (t, 3 H, *J* = 7), 1.57 (m, 2 H), 1.82 (m, 2 H), 2.65 (s, 3 H), 4.06 (t, 2 H, *J* = 7), 6.81 (s, 1 H), 7.11 (d, 2 H, *J* = 9), 7.43 (d, 2 H, *J* = 9), 7.70 (d, 1 H, *J* = 9), 8.17 (d, 1 H, *J* = 9), 8.52 (s, 1 H); mass spectrum *m/z* 346 (M$^+$).

1d: ^{1}H NMR (CD_3OD) δ 0.99 (t, 3 H, *J* = 8), 1.42 (m, 2 H), 1.68 (m, 2 H), 2.66 (s, 3 H), 2.71 (t, 2 H, *J* = 8), 6.92 (s, 1 H), 7.42 (overlapping doublets, 4 H), 7.71 (d, 1 H, *J* = 9), 8.17 (d, 1 H, *J* = 9), 8.52 (s, 1 H); mass spectrum *m/z* 330 (M$^+$).

1e: ^{1}H NMR (DMSO-d_6) δ 2.74 (s, 3 H), 7.33 (s, 1 H), 7.78 (d, 2 H, *J* = 8), 7.99 (overlapping doublets, 3 H), 8.23 (d, 1 H, *J* = 9), 8.50 (s, 1 H); mass spectrum *m/z* 299 (M$^+$).

1f: the cyclohexylamine addition is carried out in a sealed vessel at 150 °C; ^{1}H NMR (CD_3OD) δ 1.5–1.8 (m, 6 H), 1.95 (m, 2 H), 2.20 (m, 2 H), 3.99 (m, 1 H), 6.86 (s, 1 H), 7.67 (d, 1 H, *J* = 9), 8.15 (d, 1 H, *J* = 9), 8.50 (s, 1 H); mass spectrum *m/z* 280 (M$^+$).

6a: ^{1}H NMR (DMSO-d_6) δ 2.74 (s, 3 H), 3.88 (s, 3 H), 7.02 (m, 2 H), 7.16 (m, 2 H), 7.52 (t, 1 H, *J* = 8), 8.16 (br d, 1 H), 8.25 (d, 1 H, *J* = 8), 9.08 (br s, 1 H), 9.42 (br s, 1 H); mass spectrum *m/z* 304 (M$^+$). Anal. ($C_{18}H_{16}N_4O$·HCl·0.75H_2O) C, H, N.

6b: ^{1}H NMR (DMSO-d_6) δ 1.1–1.4 (m, 5 H), 1.7–1.95 (m, 6 H), 2.70 (s, 3 H), 3.92 (d, 2 H, *J* = 8), 6.76 (s, 1 H), 7.16 (d, 2 H, *J* = 9), 7.46 (d, 2 H, *J* = 9), 8.1 (br d, 1 H), 8.30 (d, 1 H, *J* = 8), 9.19 (br s, 1 H), 9.28 (br s, 1 H); mass spectrum *m/z* 386 (M$^+$). Anal. ($C_{24}H_{26}N_4O$·HCl) C, H, N.

7b: ^{1}H NMR (DMSO-d_6) δ 1.1–1.4 (m, 5 H), 1.7–2.0 (m, 6 H), 2.64 (s, 3 H), 3.83 (d, 2 H, *J* = 6), 6.86 (s, 1 H), 7.10 (d, 2 H, *J* = 9), 7.45 (d, 2 H, *J* = 9), 7.69 (d, 1 H, *J* = 9), 8.30 (d, 1 H, *J* = 9), 8.83 (s, 1 H); mass spectrum *m/e* 386 (M$^+$); high-resolution mass spectrum calcd for $C_{24}H_{26}N_4O$ 386.2107, found 386.2077.

4-*m*-Anisidino-2-methylquinoline Hydrochloride (2a). A mixture of 4-chloroquinaldine (0.20 g, 1.1 mmol), *m*-anisidine (0.14 g, 1.1 mmol), and ethanol (10 mL) was heated at reflux for 6 h at which time TLC analysis showed consumption of starting materials. The solvent was removed with a rotary evaporator and the residue recrystallized from ethanol/ether to give **2a** as a yellow crystalline solid (0.26 g, 78%): ^{1}H NMR (CD_3OD) δ 2.62 (s, 3 H), 3.86 (s, 3 H), 6.76 (s, 1 H), 7.02 (m, 3 H), 7.48 (t, 1 H, *J* = 7), 7.75 (t, 1 H, *J* = 7), 7.86 (d, 1 H, *J* = 9), 7.97 (t, 1 H, *J* = 7), 8.50 (d, 1 H, *J* = 9); mass spectrum *m/z* 264 (M$^+$). Anal. ($C_{17}H_{16}N_2O$·HCl) C, H, N, Cl.

Compounds **2b–d** were prepared in an analogous fashion from 4-chloroquinaldine and the appropriate aniline derivative.

2b: ^{1}H NMR (CD_3OD) δ 1.1–1.4 (m, 5 H), 1.7–1.95 (m, 6 H), 2.59 (s, 3 H), 3.85 (d, 2 H, *J* = 7), 6.58 (s, 1 H), 7.10 (d, 2 H, *J* = 9), 7.34 (d, 2 H, *J* = 9), 7.73 (t, 1 H, *J* = 7), 7.84 (d, 1 H, *J* = 8), 7.95 (t, 1 H, *J* = 7), 8.47 (d, 1 H, *J* = 8); mass spectrum *m/z* 346 (M$^+$). Anal. ($C_{23}H_{26}N_2O$·HCl) C, H, N.

2c: ^{1}H NMR (CD_3OD) δ 0.98 (t, 3 H, *J* = 8), 1.43 (m, 2 H), 1.67 (m, 2 H), 2.60 (s, 3 H), 2.71 (t, 2 H, *J* = 8), 6.68 (s, 1 H), 7.38 (overlapping doublets, 4 H), 7.74 (t, 1 H, *J* = 7), 7.86 (d, 1 H, *J* = 8), 7.97 (t, 1 H, *J* = 7), 8.50 (d, 1 H, *J* = 8); mass spectrum *m/z* 290 (M$^+$). Anal. ($C_{20}H_{22}N_2$·HCl) C, H, N.

2d: ^{1}H NMR (CD_3OD) δ 1.02 (t, 3 H, *J* = 7), 1.57 (m, 2 H), 1.82 (m, 2 H), 2.59 (s, 3 H), 4.06 (t, 2 H, *J* = 7), 6.59 (s, 1 H), 7.10 (d, 2 H, *J* = 9), 7.35 (d, 2 H, *J* = 9), 7.74 (t, 1 H, *J* = 7), 7.85 (d, 1 H, *J* = 8), 7.95 (t, 1 H, *J* = 7), 8.48 (d, 1 H, *J* = 8); mass spectrum *m/z* 306 (M$^+$). Anal. ($C_{20}H_{22}N_2O$·HCl·0.2H_2O) C, H, N.

9-(3-Methoxythiophenoxy)-7-methyl-1*H*-imidazo[4,5-*f*]-quinoline Hydrochloride (3a). To a suspension of a sodium hydride (32 mg, 1.3 mmol) in anhydrous DMF (2 mL) was added a solution of 3-methoxybenzenethiol (168 mg, 1.2 mmol) in anhydrous DMF (2 mL) and the mixture stirred at 0 °C until gas evolution ceases. A solution of 9-chloro-7-methyl-1*H*-imidazo-[4,5-*f*]quinoline (218 mg, 1.0 mmol) in DMF (5 mL) was added and the mixture stirred at 0 °C for 2 h and at room temperature overnight. The reaction was poured into water and extracted with ethyl acetate, and the combined organics were washed with water. After drying over sodium sulfate, the solvent was removed with a rotary evaporator and the residue purified by column chromatography (9:1 ethyl acetate/methanol) to give **3a** (151 mg, 47%). The hydrochloride salt was prepared by treatment of a methanolic solution of **3a** with HCl/ether: ^{1}H NMR (DMSO-d_6) δ 2.72 (s, 3 H), 3.83 (s, 3 H), 6.86 (s, 1 H), 7.25 (d, 1 H, *J* = 8), 7.33 (m, 2 H), 7.57 (t, 1 H, *J* = 8), 8.21 (d, 1 H, *J* = 9), 8.35 (d, 1 H, *J* = 9), 8.71 (s, 1 H); mass spectrum *m/z* 321 (M$^+$). Anal. ($C_{18}H_{15}N_3OS$·2HCl·0.8H_2O) C, H, N, S.

9-(*N*-Methyl-*m*-anisidino)-7-methyl-1*H*-imidazo[4,5-*f*]quinoline Hydrochloride (3b). To a solution of *N*-methyl-*m*-anisidine (0.33 g, 2.4 mmol) in dry ethanol (25 mL) was added 9-chloro-7-methyl-1*H*-imidazo[4,5-*f*]quinoline (0.44 g, 2.0 mmol) and the mixture heated at reflux overnight. The reaction was concentrated, and the residue was dissolved in methanol, treated with decolorizing carbon, and filtered through Celite. The filtrate was concentrated and the residue triturated with ethyl acetate to give **3b** (0.55 g, 78%) as a yellow solid. The product can be further purified by recrystallization from ethanol/ether. **3b**: ^{1}H NMR (DMSO-d_6) δ 2.79 (s, 3 H), 3.56 (s, 3 H), 3.70 (s, 3 H), 6.68 (m, 2 H), 6.77 (s, 1 H), 7.18 (t, 1 H, *J* = 8), 7.43 (s, 1 H), 8.08 (d, 1 H, *J* = 9), 8.28 (d, 1 H, *J* = 9), 8.44 (s, 1 H); mass spectrum *m/z* 318 (M$^+$). Anal. ($C_{19}H_{18}N_4O$·HCl·0.5H_2O) C, H, N.

4-[*N*-(3-Methoxyphenyl)carbamoyl]benzimidazole (4a). 3-Nitroanthranilic acid[6] (15, 4.00 g, 22 mmol) was dissolved in aqueous sodium hydroxide (0.97 g, 24 mmol) to which was added palladium/carbon (0.40 g) and the mixture hydrogenated on a Parr apparatus at atmospheric pressure. After 4 h the reaction was filtered and acidified with concentrated hydrochloric acid. Formic acid (96%, 2.5 mL) was added and the mixture refluxed for 2 h. The volume of solvent was reduced and the reaction cooled. The precipitate which formed was collected and air-dried to give **17** (2.54 g, 58%). Concentration and further acidification of the filtrate yielded another 0.98 g. The combined products were recrystallized from 0.5 M HCl to give pure **17** (2.62 g, 60%): ^{1}H NMR (DMSO-d_6) δ 7.66 (t, 1 H, *J* = 8), 8.10 (overlapping doublets, 2 H), 9.55 (s, 1 H); mass spectrum *m/z* 162 (M$^+$).

To a suspension of **17** (199 mg, 1.0 mmol) in anhydrous THF at 0 °C were added isobutyl chloroformate (300 mg, 2.2 mmol), triethylamine (405 mg, 4.0 mmol), and *m*-anisidine (271 mg, 2.2 mmol) as a solution in THF. The reaction was continued at 0 °C for 6 h at which time the reaction was poured into ethyl acetate (100 mL) and H_2O (20 mL). The layers were separated and the organic layer was washed with H_2O. After drying the organics over sodium sulfate, the solvent was removed with a rotary evaporator to give a dark yellow solid which was chromatographed (4:1 hexane/ethyl acetate) to give **4a** with an acylated (isobutoxycarbonyl)benzimidazole nucleus (229 mg, 62%). The acyl group was removed with methanolic KOH to give **4a** (84%): ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3 H), 6.74 (d, 1 H, *J* = 7), 7.30 (m, 2 H), 7.45 (t, 1 H, *J* = 9), 7.59 (s, 1 H), 7.86 (d, 1 H, *J* = 9), 8.01 (d, 1 H, *J* = 9), 8.63 (s, 1 H); mass spectrum *m/z* 267 (M$^+$). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

Compounds **4b–d** were prepared in an analogous fashion from **17** and the appropriate amine.

4b: ^{1}H NMR (DMSO-d_6) δ 1.0–1.4 (m, 5 H), 1.6–1.9 (m, 6 H), 3.82 (s, 3 H), 6.98 (d, 2 H, J = 9), 7.43 (t, 1 H, J = 8), 7.71 (d, 2 H, J = 9), 7.84 (d, 1 H, J = 8), 7.99 (d, 1 H, J = 8), 8.59 (s, 1 H); mass spectrum m/z 267 (M^+). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

4c: ^{1}H NMR (DMSO-d_6) δ 7.42 (t, 1 H, J = 8), 7.83 (m, 3 H), 7.96 (m, 3 H), 8.57 (s, 1 H); mass spectrum m/z 262 (M^+). Anal. ($C_{15}H_{10}N_4O\cdot0.3H_2O$) C, H, N.

4d: ^{1}H NMR (DMSO-d_6) δ 1.2–1.9 (cyclohexane envelope, 10 H), 4.92 (br m, 1 H), 7.33 (t, 1 H, J = 8), 7.70 (d, 1 H, J = 8), 7.85 (d, 1 H, J = 8), 8.46 (s, 1 H), 9.88 (br d, 1 H, J = 7); mass spectrum m/z 243 (M^+). Anal. ($C_{14}H_{17}N_3O$) C, H, N.

3-Acetyl-4-*m*-anisidino-2,6-dimethylpyridine (5a). To a solution of 18[9] (165 mg, 1.0 mmol) in phosphorus oxychloride was added DMF (1.5 mL) dropwise and the reaction stirred at room temperature for 1 h at which time **18** was consumed. The reaction was poured onto ice and the resulting solution carefully neutralized to pH 7 with 10 N sodium hydroxide. The aqueous mixture was extracted with ethyl acetate, the combined organics were dried over sodium sulfate, and the solvent was removed with a rotary evaporator to give **19** as a brown oil (39%) which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 3 H), 2.50 (s, 3 H), 2.53 (s, 3 H), 7.01 (s, 1 H); mass spectrum m/z 183 (M^+).

A solution of **19** (0.70 g, 3.8 mmol) and *m*-anisidine (0.56 g, 4.6 mmol) in ethanol (15 mL) was heated at reflux for 16 h at which time the solvent was removed by rotary evaporation and the residue purified by column chromatography (40% CH_2Cl_2/EtOAc then 5% CH_3OH/EtOAc) to give **5a** as a yellow solid (0.13 g, 13%): ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3 H), 2.63 (s, 3 H), 2.67 (s, 3 H), 3.82 (s, 3 H), 6.72 (m, 3 H), 7.26 (t, 1 H, J = 8), 8.00 (br s, 1 H); mass spectrum m/z 270 (M^+). Anal. ($C_{16}H_{18}N_2O_2\cdot0.25HCl\cdot H_2O$) C, H, N.

4-*m*-Anisidino-2,6-dimethyl-3-(2-hydroxyethyl)pyridine (5b). To a solution of **5a** (125 mg, 0.46 mmol) in ethanol (7 mL) was added sodium borohydride (32 mg, 0.83 mmol) and the reaction was stirred at room temperature for 1 h. The solvent was removed with a rotary evaporator and the residue partitioned between water and ethyl acetate. The aqueous layer was extracted with more ethyl acetate, the combined organics were dried (sodium sulfate), and the solvent was removed with a rotary evaporator to give **5b** as a yellow oil (80 mg, 63%) which solidified on standing: ^{1}H NMR ($CDCl_3$) δ 1.51 (d, 1 H, J = 7), 2.22 (s, 3 H), 2.30 (s, 3 H), 3.79 (s, 3 H), 5.21 (q, 1 H, J = 7), 6.58 (d, 1 H, J = 8), 6.66 (s, 1 H), 6.70 (d, 1 H, J = 8), 6.85 (s, 1 H), 7.20 (t, 1 H, J = 8), 8.45 (br s, 1 H); mass spectrum m/z 272 (M^+). Anal. $C_{16}H_{20}N_2O_2\cdot0.25H_2O$) C, H; N: calcd, 7.46; found, 7.00.

4-*m*-Anisidino-2,6-dimethyl-3-(2-oximinoethyl)pyridine (5c). To a solution of **5a** (0.15 g, 0.55 mmol) in 95% ethanol (8 mL) were added hydroxylamine hydrochloride (0.069 g, 1.0 mmol) and sodium hydroxide (0.05 g, 1.2 mmol) in water (2 mL), and the mixture was heated at reflux for 16 h. The reaction was concentrated on a rotary evaporator and the residue suspended in water, acidified with concentrated hydrochloric acid, and then neutralized with 10 N sodium hydroxide. The solid which precipitated was extracted into ethyl acetate, the combined organics were dried over sodium sulfate, and the solvent was removed with a rotary evaporator to give **5c** (80 mg, 51%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.01 (s, 3 H), 2.23 (overlapping s, 6 H), 3.70 (s, 3 H), 6.55 (d, 1 H, J = 8), 6.72 (m, 3 H), 7.16 (t, 1 H, J = 8), 7.58 (s, 1 H); high-resolution mass spectrum calcd for $C_{16}H_{19}N_3O_2$ 285.1477, found 285.1476.

Biological Methods. *Escherichia coli* N63 (serotype O18) was maintained as a frozen stock in bovine blood at −70 °C. On the day before challenge, stock culture was thawed and streaked for isolation on brain–heart infusion (BHI) agar. After overnight incubation, three 1-mL BBL Prompt (Becton-Dickinson Microbiology Systems, Cockeysville, MD) vials were prepared according to the manufacturers directions and used to inoculate 100 mL of L broth (Difco). The broth culture was incubated at 37 °C with shaking for approximately 3 h, at which time the optical density (600 nm) reached 0.3–0.4, corresponding to approximately 8×10^8 CFU/mL as determined by viable count. This culture was then diluted in cold L broth to achieve a concentration near 4×10^7 CFU/mL.

Female NSA (CF-1) mice weighing 11–16 g (Harlan Sprague Dawley, Indianapolis, IN) were infected by intraperitoneal (ip) injection of 0.5 mL of bacterial culture described above. Gentamicin was administered subcutaneously (sc) at 0.5, 4, and 24 h postinfection. Immunomodulators were prepared in pyrogen-free saline or water for injection (USP) and administered 24 h prior to infection unless otherwise noted. A hand-held glass or electric (Omni 1000) tissue homogenizer was used to prepare suspensions of drugs in saline. Ten mice were used per treatment group.

Inoculum for in vitro sensitivity studies was prepared by growing *E. coli* N63 overnight on BHI agar plates and then preparing a single 1-mL BBL Prompt. This cell suspension was used to inoculate Mueller-Hinton broth in microtiter trays containing serial 2-fold dilutions of test compounds and/or gentamicin. To test for interactions between aminoquinolines and gentamicin, a checkerboard dilution protocol was employed. The microtiter trays were incubated overnight at 37 °C. The turbidity in each well was then determined by measuring the absorbance at 600 nm. Uninoculated wells containing test compounds were used to correct for turbidity due to the presence of insoluble drugs.

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