Synthesis and Activity of Substituted 2-Phenylquinolin-4-amines, Antagonists of Immunostimulatory CpG-Oligodeoxynucleotides†

Lucjan Strekowski,*,# Martial Say,# Maged Henary,# Patricia Ruiz,# Lori Manzel,‡ Donald E. Macfarlane,‡ and Andrzej J. Bojarski§

Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, Department of Medicine, Veterans Affairs Medical Center and University of Iowa, Iowa City, Iowa 52242, and Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, Krakow, Poland

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Fifty-seven 2-phenylquinolines substituted at the phenyl group and C4 of the quinoline were synthesized and analyzed for inhibition of the immunostimulatory effect of oligodeoxynucleotides with a CpG-motif. The Fujita-Ban variant of the classical Free-Wilson analysis gave a highly significant correlation for a series of 48 relatively small molecules demonstrating that (i) the partial contributions of substituents to biological activity (EC_{50}) are additive and (ii) assuming similar bioavailability for all quinolines studied, the larger molecules cannot be accommodated within a still unknown biological receptor. The results suggest interaction of a basic antagonist molecule with weakly acidic groups in the antagonist−receptor complex. *N*-[2-(Dimethylamino)-ethyl]-2-[4-(4-methylpiperazino)phenyl]quinolin-4-amine (**50**) is the most effective antagonist found in this study (EC_{50} = 0.76 nM).

Introduction

The CpG motifs with unmethylated cytosine are more common in bacterial DNA than in vertebrate DNA, and stimulate a wide range of immune responses in vertebrates.[1−6] Many single-stranded CpG-containing oligodeoxynucleotides and phosphorothioate oligodeoxynucleotides (CpG-ODN) are also immunostimulatory.[7] This finding has led to the development of a simple, highly reliable assay in vitro for the search of compounds that inhibit the immunostimulatory effect.[7−10] Basic quinolin-4-amines are the most active agents of the many classes of compounds that have been assayed.[9−11] More important, a number of quinoline derivatives that are active in this in vitro assay induce remissions of rheumatoid arthritis and systemic lupus erythematosus, suggesting the involvement of CpG-DNA in these autoimmune diseases.[12,13] It has also been suggested that such compounds may be useful in the prevention of graft-versus-host disease in bone marrow transplant patients.[14]

The mechanism by which quinolin-4-amines inhibit the immune stimulation by CpG-ODN is not known. No correlation has been found between published antimalarial activity and the ability to block CpG-ODN-induced effects.[9] Limited mechanistic studies conducted to date have revealed that CpG-ODN are accumulated in acidified perinuclear vesicles of the cells and suggested that this uptake is necessary for immunostimulatory activity.[15] The basic quinolin-4-amines are concentrated in acidified peripheral organelles and the cellular uptake of CpG-ODN is not inhibited by the quinolines. In addition, the quinoline uptake does not correlate with inhibition of CpG-ODN-induced effects and does not affect the pH (about 6.2, as measured by us and others) of the intracellular environment of CpG-ODN at the concentration required for inhibition.[10] Additional studies have shown that the quinoline agents do not bind with CpG-ODN,[10] and their affinities toward duplex-DNA, triplex-DNA, and RNA of diverse sequences do not correlate with their activity as antagonists of the immunostimulatory effect.[11] On the other hand, it remains to be seen whether the quite specific binding of a quinoline antagonist of immunostimulatory CpG-ODN to phosphatidylcholine vesicles to form a complex of a well-defined stereochemical structure contributes to the inhibitory activity.[10]

In the search for highly active quinoline antagonists of immunostimulatory CpG-ODN, we have undertaken fundamental synthetic and SAR work.[9−11] Our studies have revealed that basicity of the quinoline N1 atom parallels the activity. The biological response is strongly increased for quinolin-4-amines that contain an additional basic aminoalkyl function linked to the N^4 atom of the quinoline. The best agents found previously are *N*-substituted derivatives of 2-(2-naphthyl)quinolin-4-amine.[11] Since naphthalenes are not biostable and are potentially toxic, the 2-(2-naphthyl)quinolin-4-amines, although highly active in vitro, are unlikely to be developed into practical immunosuppressive agents. Accordingly, in this paper we report synthesis and SAR analysis of potentially more biostable and less toxic, functionalized 2-phenylquinolines.

Results and Discussion

Design of Functionalized 2-Phenylquinolines. The design work was guided by our first SAR analysis of selected quinolines as antagonists of immunostimulatory CpG-oligodeoxynucleotides[9] and the subsequent synthesis and biological evaluation of a limited number of 2-(2-naphthyl)quinolines.[10,11] Fifty-seven 2-phenylquinolines were synthesized as part of this work. The

† Dedicated to the memory of Dr. Maria J. Mokrosz and Professor Stanislaw Misztal, Institute of Pharmacology, Polish Academy of Sciences.

* To whom correspondence should be addressed. Tel: 404-651-0999. Fax: 404-651-1416. E-mail: Lucjan@gsu.edu.
# Georgia State University.
‡ University of Iowa.
§ Institute of Pharmacology.

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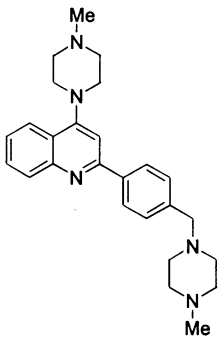
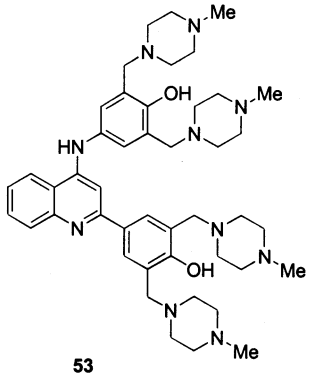
substituents R_{Ph} at the phenyl group, the substituents R_Q at position 4 of the quinoline, and a complete set of the functionalized compounds **1**−**51** designed for a Free-Wilson analysis are given in Table 1. The additional derivatives **52**−**57** for SAR analysis to better understand the structure of the unknown pharmacophore are shown as a block of structures.

As can be seen from Table 1, the phenyl group contains a methyl ($R_{Ph}4$, $R_{Ph}5$) or fluoro ($R_{Ph}2$) substituent, and in $R_{Ph}1$ and $R_{Ph}6$ the methyl is additionally functionalized with a piperazine for an increased total basicity of the molecule. The 4-methylpiperazino substituent ($R_{Ph}7$) also contains an additional basic center (MeN), and the piperazine nitrogen atom linked directly to the phenyl increases basicity of the quinoline N1 atom by an electronic conjugation effect. This effect is also operative for a piperidino group $R_{Ph}3$. Nevertheless, the most dramatic increase in basicity of the quinoline ring nitrogen is observed for 2-arylquinolines that are substituted at position 4 of the quinoline with a primary amino group.[11] Accordingly, the vast majority of quinolines designed for this work contain a primary alkylamino or arylamino group (R_Q) at position 4. Compounds with somewhat less conjugated alkoxy (R_Q2) and dialkylamino (compound **52**) groups are included for SAR analysis. The R_Q substituents are additionally functionalized with a hydroxy group (R_Q1, R_Q10, R_Q17), and most of the substituents contain an additional amino group or several amino groups. We have shown previously that these structural features often increase activity, although the relationship has not been clear. The most basic quinoline **53** is pentaprotonated at pH 7 and is expected to be even more cationic under lower pH conditions. The molecule **53** is also substantially larger than other quinolines discussed above.



52
$\log(1/EC_{50}) = 7.23$

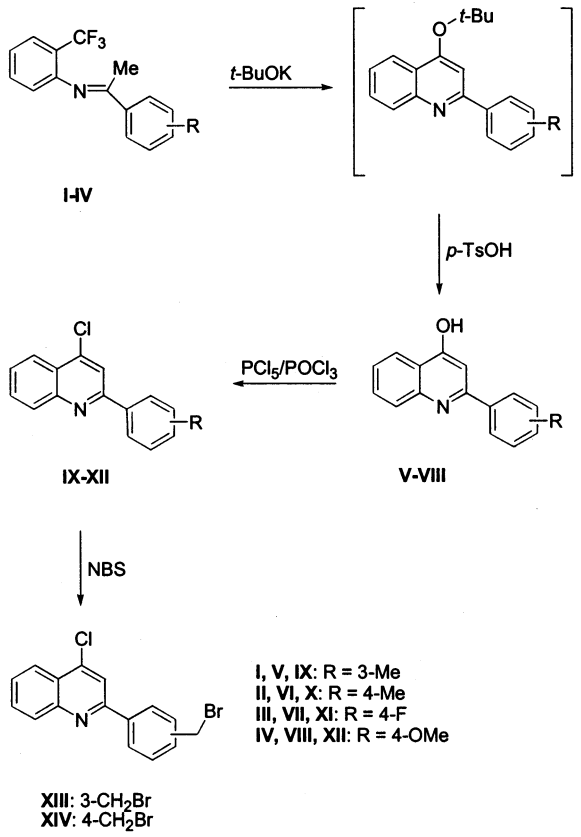


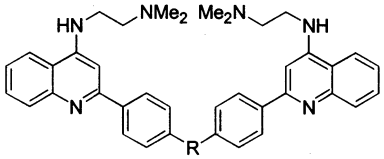
53
$\log(1/EC_{50}) = 6.67$

It should be noted that compounds with an unsubstituted phenol moiety are easily oxidized to a toxic quinoid-type derivative, and the mechanism involves an irreversible Michael-type addition of a thiol group of protein to the oxidized structure. A solution to this problem is to block the two ortho positions of the phenol, which sterically inhibits the nucleophilic addition.[16] Accordingly, the phenolic groups in **53** and the phenolic group R_Q17 in other quinolines are 2,6-disubstituted to decrease the potential toxicity. Quinolines with a potentially more toxic R_Q10 substituent are included for SAR purposes.

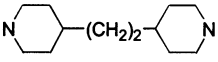
In the design of bis-quinolines **54**−**56** it was reasoned that these compounds might show increased binding to a receptor, due to a concentration factor. More specifically, the reversible monoquinoline−receptor interaction of the bis-quinoline composed of two quinoline subunits might be favored in comparison to the interaction of a corresponding monomeric quinoline molecule. However, this assumption proved to be incorrect (vide infra).

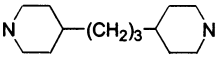
Scheme 1





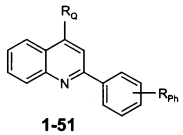
54: R =  $\log(1/EC_{50}) = 7.68$

55: R =  $\log(1/EC_{50}) = 6.88$

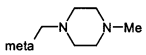
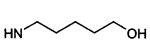
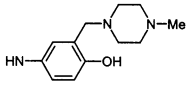
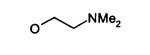
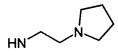
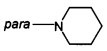
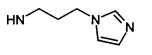
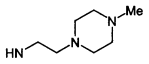
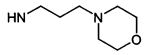
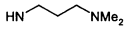
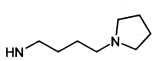
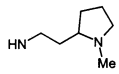
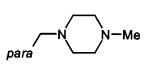
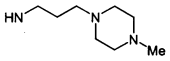
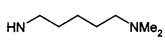
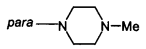
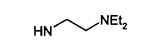
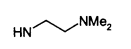
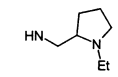
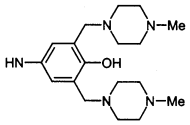
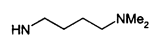
56: R =  $\log(1/EC_{50}) = 7.68$

Chemistry. Note: For the sake of clarity of the presentation, all intermediate compounds are labeled with Roman numbers, as opposed to Arabic numbers assigned to quinoline products used for SAR analyses. The construction of a quinoline ring system was conducted by using chemistry[17,18] developed by us previously (Scheme 1). Briefly, *t*-BuOK mediated cyclization of ketimines **I**−**IV** derived from 2-(trifluoromethyl)-aniline and an aryl methyl ketone followed by one-pot hydrolysis of the resultant 4-*tert*-butoxyquinolines furnished the corresponding 4-hydroxyquinolines **V**−**VIII**. These compounds, in turn, were efficiently transformed into 4-chloroquinolines **IX**−**XII**. As an extension of this synthetic methodology it was found that 4-chloro-2-

Table 1. Structure of Quinolines **1**−**51** and Activity Contribution α of Substituents R_{Ph} and R_Q According to Equation 3



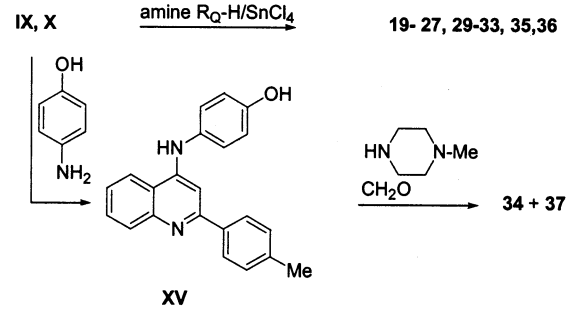
1-51

Substituent R_{Ph}		Contribution $\alpha_{R_{Ph}}$	Substituent R_Q		Contribution α_{R_Q}	Substituent R_Q		Contribution α_{R_Q}
$R_{Ph}1$		-0.170(0.039)	R_Q1		-2.478(0.039)	R_Q10		-0.164(0.039)
$R_{Ph}2$	*para*-F	-0.038(0.235)	R_Q2		-2.269(0.039)	R_Q11		-0.142(0.059)
$R_{Ph}3$		-0.032(0.059)	R_Q3		-1.151(0.059)	R_Q12		-0.116(0.039)
$R_{Ph}4$	*meta*-Me	0.000	R_Q4		-0.508(0.078)	R_Q13		-0.074(0.118)
$R_{Ph}5$	*para*-Me	0.033(0.412)	R_Q5		-0.419(0.039)	R_Q14		-0.069(0.059)
$R_{Ph}6$		0.415(0.078)	R_Q6		-0.335(0.059)	R_Q15		-0.066(0.039)
$R_{Ph}7$		0.939(0.196)	R_Q7		-0.284(0.039)	R_Q16		0.000
			R_Q8		-0.244(0.059)	R_Q17		0.902(0.059)
			R_Q9		-0.226(0.059)			

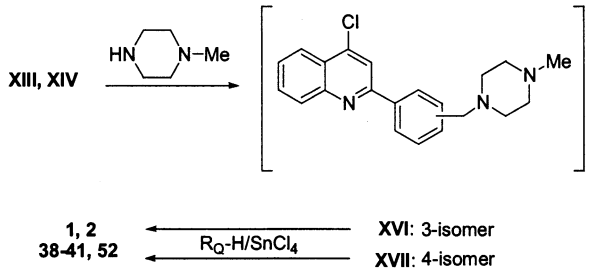
No	R_{Ph}	R_Q	No	R_{Ph}	R_Q	No	R_{Ph}	R_Q
1	R_{Ph} 1	R_Q 3	**18**	R_{Ph}4	R_Q 2	**35**	R_{Ph} 5	R_Q 13
2	R_{Ph} 1	R_Q 14	**19**	R_{Ph}4	R_Q 3	**36**	R_{Ph} 5	R_Q 16
3	R_{Ph} 2	R_Q 1	**20**	R_{Ph}4	R_Q 4	**37**	R_{Ph} 5	R_Q 17
4	R_{Ph} 2	R_Q 4	**21**	R_{Ph}4	R_Q 6	**38**	R_{Ph} 6	R_Q 6
5	R_{Ph} 2	R_Q 5	**22**	R_{Ph}4	R_Q 7	**39**	R_{Ph} 6	R_Q 11
6	R_{Ph} 2	R_Q 8	**23**	R_{Ph}4	R_Q 8	**40**	R_{Ph} 6	R_Q 13
7	R_{Ph} 2	R_Q 9	**24**	R_{Ph}4	R_Q 13	**41**	R_{Ph} 6	R_Q 16
8	R_{Ph} 2	R_Q 10	**25**	R_{Ph}4	R_Q 14	**42**	R_{Ph} 7	R_Q 4
9	R_{Ph} 2	R_Q 11	**26**	R_{Ph}4	R_Q 16	**43**	R_{Ph} 7	R_Q 5
10	R_{Ph} 2	R_Q 12	**27**	R_{Ph} 5	R_Q 1	**44**	R_{Ph} 7	R_Q 9
11	R_{Ph} 2	R_Q 13	**28**	R_{Ph} 5	R_Q 2	**45**	R_{Ph} 7	R_Q 11
12	R_{Ph} 2	R_Q 14	**29**	R_{Ph} 5	R_Q 3	**46**	R_{Ph} 7	R_Q 12
13	R_{Ph} 2	R_Q 15	**30**	R_{Ph} 5	R_Q 4	**47**	R_{Ph} 7	R_Q 13
14	R_{Ph} 2	R_Q17	**31**	R_{Ph} 5	R_Q 6	**48**	R_{Ph} 7	R_Q 14
15	R_{Ph}3	R_Q 9	**32**	R_{Ph} 5	R_Q 7	**49**	R_{Ph} 7	R_Q 15
16	R_{Ph}3	R_Q 13	**33**	R_{Ph} 5	R_Q 8	**50**	R_{Ph} 7	R_Q 16
17	R_{Ph}3	R_Q 16	**34**	R_{Ph} 5	R_Q 10	**51**	R_{Ph} 7	R_Q 17

(tolyl)quinolines **IX**, **X** are easily brominated at a methyl group by treatment with NBS to give bromomethyl derivatives **XIII**, **XIV**. The desired quinolines **1**−**53** were obtained by functionalization of the intermediate

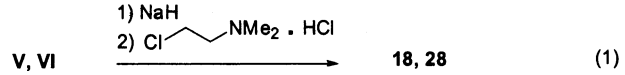
Scheme 2



Scheme 3



compounds **V**−**XIV**. Thus, alkylation of 4-hydroxyquinolines **V** and **VI** with 2-(dimethylamino)ethyl chloride under basic conditions furnished the respective 4-alkoxy derivatives **18** and **28** (eq 1). A one-step synthesis of compounds **19**−**27**, **29**−**33**, **35**, and **36** from the corresponding 4-chloro-2-(tolyl)quinolines **IX**, **X** is depicted in Scheme 2. In a similar way, treatment of **X** with 4-hydroxyaniline gave an intermediate anilino derivative **XV** which was subjected to Mannich reaction with *N*-methylpiperazine and formaldehyde to give the desired compounds **34** and **37**. A nucleophilic displacement of benzylic bromide in 2-[(bromomethyl)phenyl]-4-chloroquinolines **XIII**, **XIV** is selective as shown in Scheme 3 by the preparation of difunctionalized quinolines **1**, **2**, and **38**−**41**, **52** through the intermediary of **XVI** and **XVII**, respectively.



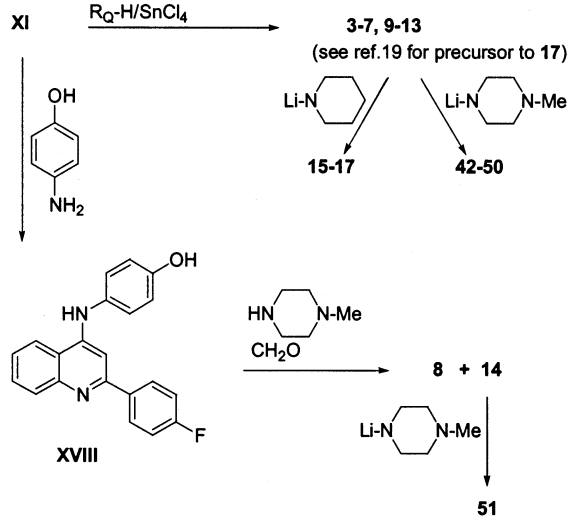
(1)

A selective displacement of chloride from 4-chloro-2-(4-fluorophenyl)quinoline (**XI**) furnished **3**−**7**, **9**, **10**, and **11**−**13** (Scheme 4). Treatment of these products and an additional 4-fluorophenyl derivative reported previously[19] with lithium reagents derived from piperidine and *N*-methylpiperazine resulted in *ipso*-substitution of fluoride[19] to give **15**−**17** and **42**−**50**. The preparation of Mannich bases **8** and **14** through the intermediary of **XVIII** utilized a synthetic pathway already mentioned in Scheme 2. Again, compound **51** was obtained in an *ipso*-substitution reaction of **14** with lithium 4-methylpiperizide.

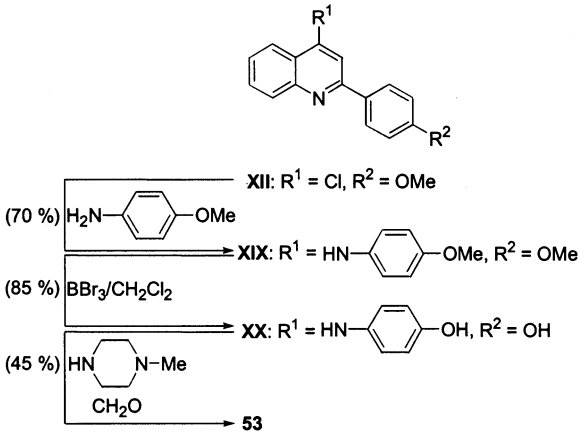
A synthetic pathway to the tetrapiperazino derivative **53** starting with 4-chloro-2-(4-methoxyphenyl)quinoline (**XII**) and through the intermediary of **XIX** and **XX** is given in Scheme 5. In addition to the transformations discussed above, this preparation involves efficient demethylation of **XIX** by the reaction with boron tribromide.

In an attempted synthesis of bis-quinolines **54**−**56** a bromophenyl derivative **XXI** or its fluorophenyl ana-

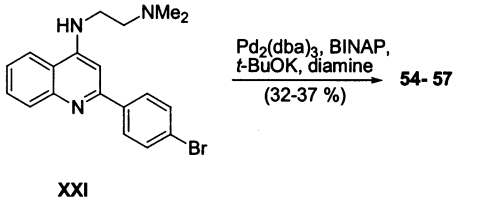
Scheme 4



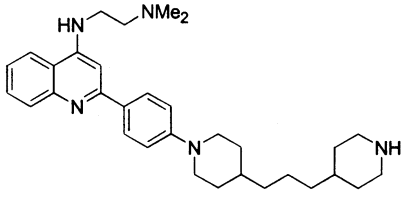
Scheme 5



logue (not shown) was treated with a dilithio reagent derived from the corresponding diamine. Despite the successful similar chemistry presented in Scheme 4 the desired bis-quinolines could not be obtained by this approach. Compounds **54**−**56** were obtained by palladium(0) catalyzed amination of **XXI** with a diamine in the presence of 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) and potassium *tert*-butoxide[20] (eq 2). The quinoline **57** was a byproduct from the synthesis of bis-quinoline **56**.



(2)



57

log(1/EC_{50}) = 7.84

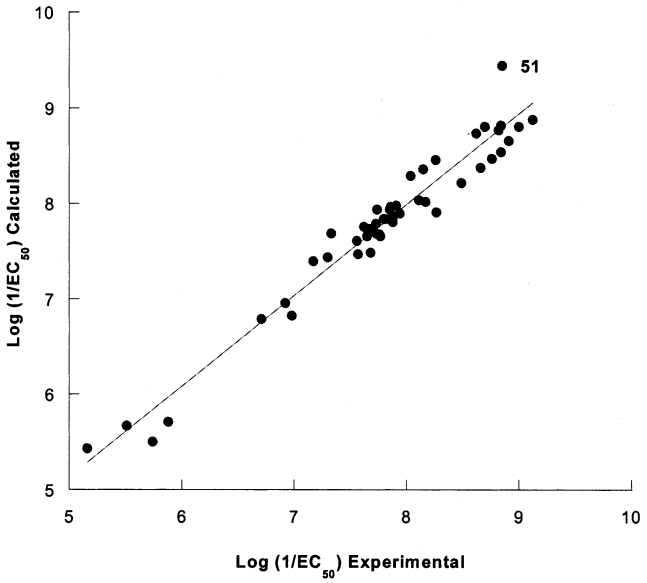


Figure 1. Plot of calculated vs observed activity for compounds **1**−**51** according to eq 3.

QSAR. The Fujita-Ban variant[21,22] of the classical Free-Wilson analysis[22,23] (eq 3 and Figure 1) was conducted with a series of compounds **1**−**51** (Table 1) for which the 2-phenylquinoline system is substituted with groups R_{Ph} and R_Q, each present in at least two molecules. For α_{RPh} and α_{RQ}, see Table 1. $n = 51$

$$\log(1/EC_{50}) = 7.94 + \alpha_{RPh} + \alpha_{RQ} \qquad (3)$$

(compounds **1**−**51**), $r = 0.976$, $s = 0.20$, $F = 149$.

In this nonparameter analysis, for every compound of the series the values EC_{50} used in the logarithmic scale are expressed as the sum of the activity contributions α of the substituents R_{Ph} and R_Q, referring to theoretical biological activity of the arbitrarily chosen reference compound **26** [$\log(1/EC_{50}) = 7.74$]. Accordingly, the substituent contributions of **26**, $\alpha_{RPh}4$ and $\alpha_{RQ}16$, are taken as zero (Table 1). Although 2-phenylquinoline, the unsubstituted analogue, would normally be a choice of reference for this analysis, its low activity could not be measured accurately.

This function (eq 3) shows that the partial contributions of substituents R_{Ph} and R_Q to the overall biological response of the quinolines are additive and can be quantified. Despite the excellent correlation obtained, the relatively high residual value of −0.60 for quinoline **51** substituted with a bulky group R_Q17 can be noted. Analogue **51** is the largest molecule of the series. As expected, elimination of the R_Q17-substituted derivatives from the analysis improved the quantitative relationship (eq 3, $n = 48$, $r = 0.980$, $s = 0.17$, $F = 360$). It was hypothesized that the receptor binding site for the quinolines is so relatively small in size that it can barely accommodate the large molecule **51**. Indeed, the larger analogue **53** shows the lowest activity of all quinolin-4-amines substituted with cationic side group(s). Additional suggestion for a limited size of the receptor site comes from comparison of a 2-(4-piperidinophenyl)quinoline **17** with its sterically bulkier analogues **54**−**57**, all these quinolines being substituted with the same small group R_Q16. As can be seen, the activity of **54**−**57** is substantially diminished relative to that of **17**. The relatively low activity of bis-quinolines **54**−**56** is consistent with a concept of the single-quinoline receptor.

The quinoline antagonists of immunostimulatory CpG-ODN are all basic molecules, and the most active quinolines contain a strongly basic ring nitrogen atom. The N1 basicity is a function of conjugation of a group at position 4 of the quinoline with the quinoline ring system.[24] Accordingly, compounds with a primary alkylamino group of low steric requirements around the C4 atom of the quinoline are strongly conjugated and, as a result, are strongly basic. By contrast, secondary dialkylamino analogues, for steric reasons, show a reduced conjugation effect and are less basic and less active. This is illustrated by comparison of a primary alkylamino derivative **41** [estimated[24,25] $pK_a(N1) \sim 7$, $\log(1/EC_{50}) = 8.15$] and its closely related piperazino analogue **52** [estimated [24] $pK_a(N1) < 6$, $\log(1/EC_{50}) = 7.23$]. In comparison to 4-aminoquinolines, the conjugation effect is less significant for 4-alkoxyquinolines. Thus, R_Q2-substituted compound **18** [estimated [24] $pK_a(N1) \sim 5$] shows the lowest activity of all quinolines **18**−**26** that contain the same group $R_{Ph}4$, and a similar phenomenon is observed for R_Q2- and $R_{Ph}5$-containing derivative **28** in the series **28**−**37**. Interestingly, quinoline **50** has a highly basic N1 atom [experimental [25] pK_a (N1) = 7.8] and is also the most active agent of all compounds studied. The biological activity data for all quinolines **1**−**51** is listed in Table S1 of Supporting Information.

There is clearly no correlation between the total number of strongly basic sites in the molecules and their activities. Thus, the most active compound **50** [$\log(1/EC_{50}) = 9.12$] is a trication at pH 7, and so is its closely related analogue **45** with $\log(1/EC_{50})$ of 8.62. Excluding possible protonation of the quinoline N1 atom in **53**, this compound is tetracationic and is a much worse antagonist than many quinolines with a smaller number of cationic groups. In a similar way, a tricationic quinoline **57** is less active than its dicationic analogue **17**. Many additional examples of the lack of correlation can be found within a series of compounds (Table 1) with the expected identical charge under physiological conditions (+2 or +3). Since the antagonists of immunostimulatory CpG-ODN must contain at least two basic sites including the N1 quinoline atom, it is concluded again that strong biological response is induced only for basic quinolines of a relatively small molecular size. Both steric and electronic factors appear to play a role in the antagonist−receptor interaction.

The conjugated acids of the R_Q substituent and the quinoline N1 atom in most of the analyzed compounds exhibit pK_a values above 7 and, as such, the compounds are expected to be protonated under physiological pH conditions. In addition, as already mentioned, the quinoline antagonists are concentrated in acidified organelles of cells. It can be concluded that the quinoline antagonists of CpG-ODN do not exert their activity in the acidified microenvironment of the cell. If that were the case, the low pH value that renders extensive protonation of the basic groups would result in a similar biological response to all compounds of similar molecular size and with the same number of basic sites in the molecule. It is most likely that in the active complex the basic R_Q group interacts with a weakly acidic group

of the receptor binding site, such as a carboxylic acid or a phenol. This may involve proton transfer for electrostatic interaction or formation of a strong hydrogen bond. This suggestion is fully consistent with our previous finding that methylation of the basic nitrogen atom in R_Q-substituted quinolines renders the resultant cationic tetraalkylammonium derivatives completely inactive.[11]

Conclusions

Although the mechanism of the inhibition of the immunostimulatory effect of CpG-ODN by 4-aminoquinolines is still not known, progress has been made toward understanding of the structure of the biological receptor. The QSAR analysis results clearly provide guidelines for the design of new 2-phenylquinolin-4-amine antagonists of the CpG-ODN mediated effect. The activity in vitro of the best 2-phenylquinolin-4-amine agent **50** ($EC_{50} = 0.76$ nM) compares favorably with that of a potentially toxic 2-(2-naphthyl)quinoline derivative ($EC_{50} = 0.24$ nM) prepared by us previously.[11] Preliminary data show that **50** has excellent bioavailability, and is well tolerated in acute studies in mice.

Experimental Section

General. Preparations of intermediate products **III**,[23] **VII**,[18] **XXI**,[23] and quinolines **36**,[23] **50**,[19] have been reported previously. All new compounds, for the sake of brevity of presentation, are characterized below by mp's only (uncorrected, Pyrex capillary). The ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and elemental analysis (C, H, N) or HRMS data are listed in the Supporting Information.

Ketimines I, II, and IV. Condensations of 2-(trifluoromethyl)aniline with aryl methyl ketones were conducted by using a general procedure.[19,23] The oily products **I** and **II** were purified by Kugelrohr distillation (100−150 °C/0.1−0.5 mmHg): **I**, yield 76%; **II**, yield 80%; **IV**, yield 84%, mp 70−71 °C (from hexanes).

2-Aryl-4-hydroxyquinolines V, VI, and VIII. These compounds were obtained by using a general procedure[17] and crystallized from MeOH. Compound, yield, mp: **V**, 38%, 179−181 °C; **VI**, 40%, 288−290 °C; **VIII**, 40%, 298−300 °C.

2-Aryl-4-chloroquinolines IX-XII. The reactions of **V**−**VII** with $PCl_5/POCl_3$ were conducted by using a general procedure.[17] The products were crystallized from hexanes. Compound, yield, mp: **IX**, 85%, 74−76 °C; **X**, 88%, 68−70 °C; **XI**, 78%, 90−92 °C; **XII**, 84%, 84−85 °C.

2-[(Bromomethyl)phenyl]-4-chloroquinolines XIII (meta) and XIV (para). A mixture of **IX** or **X** (1.2 g, 4.7 mmol), NBS (0.8 g, 4.7 mmol), and benzoyl peroxide (0.15 g) in CCl_4 (50 mL) was heated under reflux under a nitrogen atmosphere for 6 h and then cooled. The resultant precipitate of succinimide was filtered off and the solution was concentrated. The solid residue was crystallized from hexanes. Compound, yield, mp: **XIII**, 58%, 89−91 °C; **XIV**, 63%, 116−117 °C.

4-Chloro-2-[[(4-methylpiperazino)methyl]phenyl]-quinolines XVI (meta) and XVII (para). A mixture of **XIII** or **XIV** (0.2 g, 0.6 mmol) and *N*-methylpiperazine (2 mL) was stirred at 23 °C for 6 h under a nitrogen atmosphere and then treated with a saturated solution of $NaHCO_3$ (20 mL). The mixture was extracted with AcOEt (3 × 20 mL), and the extract was dried ($MgSO_4$) and concentrated. The oily residue of **XVI** and **XVII** was used for further transformations without purification.

2-Aryl-4-(4-hydroxyanilino)quinolines XV and XVIII. A mixture of **X** or **XI** (1 mmol) and 4-aminophenol (0.33 g, 3 mmol) was heated to 130 °C under a nitrogen atmosphere for 5 h and then cooled and treated with a solution of $NaHCO_3$ (10%, 10 mL). Extraction with AcOEt (4 × 10 mL) followed by washing of the extract with water (2 × 10 mL), drying ($MgSO_4$), and concentration under a reduced pressure gave a solid residue. The crude product was crystallized twice from AcOEt. Compound, yield, mp: **XV**, 83%, 252−254 °C; **XVIII**, 63%, 100−102 °C.

***N*,2-Bis(4-methoxyphenyl)quinolin-4-amine (XIX).** A mixture of **XII** (0.43 g, 1.6 mmol), 4-methoxyaniline (1.0 g, 8 mmol), and anhydrous $SnCl_4$ (0.2 mL) was stirred and heated to 130 °C under a nitrogen atmosphere for 4 h. After cooling, water (4 mL) was added, and the resultant solution was extracted with AcOEt (3 × 15 mL). The extract was dried ($MgSO_4$), and concentrated. The crude bis-methoxyphenyl derivative **XIX** was purified by silica gel chromatography eluting with AcOEt/MeOH (10:1) followed by crystallization from AcOEt/hexanes; yield 70%, mp 158−160 °C.

***N*,2-Bis(4-hydroxyphenyl)quinolin-4-amine (XX).** A mixture of **XIX** (0.2 g, 0.56 mmol), dichloromethane (25 mL), and a solution of BBr_3 in dichloromethane (1 M, 5.6 mL, 5.6 mmol) was stirred at 23 °C for 40 h. The addition of a solution of $NaHCO_3$ (10%, 25 mL) gave a precipitate of **XX** which was filtered, washed with water and EtOH, and dried; yield 85%, mp 356−360 °C.

4-[2-(Dimethylamino)ethoxy]-2-(tolyl)quinolines 18 (meta) and **28** (para). A solution of sodium 2-(dimethylamino)-ethoxide generated from 2-(dimethylamino)ethanol (0.90 g, 10 mmol) and NaH (0.24 g, 10 mmol) in DMF (10 mL) was treated with **V** or **VI** (0.24 g, 1 mmol), and the mixture was heated to 100 °C for 5 h under a nitrogen atmosphere. After cooling, the precipitate of NaCl was filtered off and the solution was concentrated under a reduced pressure. Crude product **18** or **28** was purified by silica gel chromotography eluting with dichloromethane/ether to give an oil. A hydrobromide salt was obtained by using a general procedure[23] and crystallized from AcOEt. Compound, yield, mp: **18**·2HBr·$2H_2O$, 60%, 199−201 °C; **28**·2HBr·$2H_2O$, 75%, 223−224 °C.

Quinolin-4-amines 1−7, 9−13, 19−27, 29−33, 35, 36, 38−41, and 52. A mixture of a 4-chloroquinoline **IX**−**XI**, **XVI**, or **XVII** (0.2 g), an amine R_Q-H (1 mL) and anhydrous $SnCl_4$ (2 drops) was stirred under a nitrogen atmosphere and heated to 130 °C for 4 h. The resultant brown oil was cooled and treated with water (20 mL). The product was isolated by extraction with AcOEt (4 × 10 mL), drying ($MgSO_4$), and concentration of the extract under a reduced pressure followed by silica gel chromotography of the residue eluting with AcOEt/pentane (4:1). Solid products were crystallized from MeOH or AcOEt. Oily compounds were transformed into hydrobromide, hydrochloride, or phosphate salts by using a general procedure,[23] and the salts were crystallized from EtOH or EtOH/AcOEt. Compound, yield, mp: **1**·$4H_3PO_4$·$3H_2O$, 35%, >180 °C (decomp); **2**·3HBr·$1.5H_2O$, 40%, 180−182 °C; **3**, 63%, 141−142 °C; **4**, 59%, 167−169 °C; **5**·2HBr·$0.5H_2O$, 75%, 218−220 °C; **6**·2HBr·H_2O, 61%, 278−280 °C; **7**·2HBr·$2H_2O$, 53%, 239−240 °C; **9**·2HBr, 72%, 279−281 °C; **10**·3HBr·$5H_2O$, 45%, 260−263 °C; **11**·2HBr·$2.5H_2O$, 31%, 218−220 °C; **12**·2HBr·$0.5H_2O$, 65%, 266−267 °C; **13**·2HBr·$2H_2O$, 60%, 259−260 °C; **19**·$0.25H_2O$, 65%, 184−186 °C; **20**, 52%, 136−138 °C; **21**·3HBr·$5H_2O$, 75%, 222−224 °C; **22**·2HBr·$2.25H_2O$, 70%, 296−298 °C; **23**·2HBr·$0.25H_2O$, 40%, 304−306 °C; **24**·2HBr·$1.5H_2O$, 57%, 122−124 °C; **25**·2HBr·$0.75H_2O$, 50%, 251−253 °C; **26**·2HBr·$2H_2O$, 55%, 218−220 °C; **27**, 70%, 126−128 °C; **29**, 72%, 169−171 °C; **30**, 75%, 146−148 °C; **31**·$0.25H_2O$, 85%, 140−141 °C; **32**·2HBr·$0.5H_2O$, 75%, 308−309 °C; **33**·2HBr·$0.5H_2O$, 45%, 325−327 °C; **35**·2HBr·H_2O, 68%, 246−248 °C; **36**·2HBr·$2H_2O$, 74%, 305−307 °C; **38**, 39%, 153−155 °C; **39**·4HBr·$1.5H_2O$, 46%, 156−159 °C; **40**·3HBr·$3.5H_2O$, 48%, 257−259 °C; **41**·3HBr·$2.5H_2O$, 44%, 248−251 °C; **52**·4HBr·H_2O, 50%, 266−268 °C.

Mannich Bases 8, 14, 34, 37, and 53. A solution of **XV** or **XVIII** (1 mmol), *N*-methylpiperazine (0.8 mL, 7 mmol), and aqueous formaldehyde (13 M, 0.5 mL, 7 mmol) in EtOH (10 mL) was heated under reflux for 30 h. A similar reaction of **XX** (0.23 g, 0.75 mmol) with *N*-methylpiperazine (1.3 mL, 12 mmol), and aqueous formaldehyde (13 M, 1 mL, 12 mmol) in EtOH (10 mL) was conducted for 40 h. Concentration under reduced pressure followed by silica gel chromatography gave

8 or **34** (AcOEt/MeOH, 4:1) and **14** or **37** (AcOEt/MeOH/Et_3N, 3:1:1). Compound **53** (from **XX**) was eluted with AcOEt/EtOH/Et_3N (15:3:1). Salts were obtained by using a general procedure[23] and crystallized from EtOH/AcOEt. Compound, yield, mp: **8**·3HCl·2.5H_2O, 10%, 278−281 °C; **14**·5HCl·3.5H_2O, 50%, 242−245 °C; **34**·3HBr·2H_2O, 40%, 225−228 °C; **37**·3HBr·5H_2O, 38%, 233−236 °C; **53**·9HCl·3H_2O, 45%, 256−259 °C.

2-(4-Piperidinophenyl)quinolin-4-amines 15−17 and 2-[4-(4-Methylpiperazino)phenyl]quinolin-4-amines 42−51. A solution of a lithium amide reagent prepared by stirring piperidine (0.50 mL, 5 mmol) or *N*-methylpiperazine (0.54 mL, 5 mmol) and *n*-BuLi (2 M in cyclohexane, 2.5 mL, 5 mmol) in anhydrous THF (10 mL) under a nitrogen atmosphere at −10 °C for 30 min was treated with a solution of a 2-(4-fluorophenyl)quinolin-4-amine (0.5 mmol) in anhydrous THF (2 mL). After the sample was stirred at 23 °C for 20 h, the mixture was quenched with water (0.2 mL), and the organic layer was concentrated under a reduced pressure. Compounds were purified by silica gel chromatography eluting with AcOEt/Et_3N (25:1) followed by crystallization of solid samples from AcOEt/hexanes. Oily products were transformed into salts,[23] and the salts were crystallized from 95% EtOH. Compound, yield, mp: **15**·3H_2O, 72%, 112−114 °C; **16**, 85%, 134−135 °C; **17**·2HBr·2H_2O, 40%, 175−176 °C; **42**·2H_2O, 75%, 144−145 °C; **43**·3HBr·H_2O, 65%, 269−272 °C; **44**·3HBr·2.5H_2O, 88%, 239−241 °C; **45**, 40%, 172−173 °C; **46**·4HBr·4.5H_2O, 50%, 280−283 °C; **47**·3HBr·2H_2O, 61%, 313−315 °C; **48**·3HBr·1.5H_2O, 68%, 260−261 °C; **49**·3HBr·3H_2O, 81%, 210−212 °C; **50**,[19] 76%, 132−133 °C; **51**·7HCl·2H_2O, 40%, 250−252 °C.

Bis-quinolines 54−56 and Quinoline 57. A solution of tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$, 4.5 mg, 0.005 mmol] and 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP, 12.2 mg, 0.02 mmol) in dioxane (20 mL) under a nitrogen atmosphere was stirred at 23 °C for 5 min before the addition of a 2-(4-bromophenyl)quinolin-4-amine **XXI** (0.35 g, 0.94 mmol) and piperazine, 4,4′-ethylenedipiperidine or 4,4′-trimethylenedipiperidine (0.4 mmol). After the sample was stirred for 5 min, the resultant yellow solution was treated with *t*-BuOK (0.27 g, 2.8 mmol). The deep-red mixture was stirred and heated to 80 °C for 40 h, then cooled, and treated with AcOEt (50 mL) and filtered. The solution was washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by silica gel chromatography eluting with AcOEt/Et_3N/EtOH (30:3:1) and then crystallized from EtOH/hexanes. The hydrobromide of **57** was crystallized from 95% EtOH. Compound, yield, mp: **54**, 37%, 257−259 °C; **55**, 32%, 240−243 °C; **56**, 34%, 198−200 °C; **57**·3HBr·4H_2O, 10%, 217−220 °C.

Biological Evaluation. The ability of test compounds to reverse the action of CpG-ODN on WEHI 231 murine B-cell lymphoma cells was assessed in vitro as described previously.[8−11] Briefly, these cells are killed by anti-surface IgM, an effect that is reversed by CpG-ODN. Test compounds were added in triplicate at 3-fold dilutions together with 6 μg/mL CpG-ODN 1760 with or without 10 μg/mL anti-surface-IgM to WEHI 231 cells at 2×10^5 per mL. The cells were incubated 16 h at 37 °C before the addition of 0.5 μCi ^{3}H thymidine for an additional 4 h. The concentration test compound that reduces the effect of CpG-ODN by half was estimated graphically from log dose−response plots. Each assay included quinacrine as an internal control, which established that the assay was both highly reproducible and did not change with time.

QSAR. A model was constructed using 24 variables (17 and 7 different substituents at position 4 and the phenyl group of 2-phenylquinoline, respectively) and the experimental activities [log(1/EC_{50}), molar concentrations] of 51 compounds. To determine how the substituents contribute to the biological activity, the partial least-squares analysis was carried out as implemented in the QSAR/CoMFA on SYBYL program. The model was cross-validated.

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Supporting Information Available: Characterization of all new compounds (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and elemental analysis) and experimental and calculated log(1/EC_{50}) values of quinolines **1**−**51** are available free of charge via the Internet at http://pubs.acs.org.

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