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STRUCTURE–ACTIVITY RELATIONSHIP ANALYSIS OF SUBSTITUTED 4-QUINOLINAMINES, ANTAGONISTS OF IMMUNOSTIMULATORY CpG-OLIGODEOXYNUCLEOTIDES

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Abstract: On the basis of a systematic SAR analysis of substituted quinolines, a derivative **32** was synthesized that shows half-maximal inhibition of the immunostimulatory effect of CpG-oligodeoxynucleotides in vitro at the concentration of 0.24 nM.

Bacterial DNA, oligodeoxynucleotides, and phosphorothioate oligodeoxynucleotides with CpG-motifs (CpG-ODN) are immunostimulatory by an unknown mechanism.[1] Chloroquine (structure in Table 1) and its structural analogs including quinacrine inhibit the immunostimulatory effect at nanomolar concentrations in a test system that quantifies the ability of the compounds to block an effect of CpG-ODN on WEHI 321 murine B-cells (inhibition of growth arrest induced by engagement of surface immunoglobulin).[1] Evidence has been accumulating that this in vitro assay may provide the basis for a search for drug candidates for treating rheumatoid arthritis and systemic lupus erythematosus. For example, a number of quinoline derivatives that are active in this in vitro assay have also been shown to induce remission of rheumatoid arthritis and lupus erythematosus.[2,3]

In this report we present for the first time a detailed SAR analysis of quinoline antagonists of immunostimulatory CpG-ODN's. The structures of compounds **1–32** are shown in Tables 1–5. The synthesis work together with SAR analysis of the synthesized quinolines culminated in the finding of an extremely active agent **32**. We also comment on a possible mechanism.

Substituted quinolines **1**,[4] **3**,[4] **4**,[5] **6**[6] (Table 1), **7**,[4] **9**,[4] **10**[4] (Table 2), and **25**[7] (Table 4) were available from other studies. Compounds **2** and **5** were obtained by using a general procedure.[8] Compounds **11** and **14** (Table 3) were obtained by treatment of 4-chloroquinoline with the corresponding amine.[7] An efficient synthesis of 2-aryl-4-chloroquinolines has recently been reported.[7] These compounds served as substrates for **8**, **12**, **13**, **15**, and **16**, **20–24**, and **26–32** (Table 5). This chemistry is illustrated in Scheme 1 for the preparation of **28** by the reaction of 4-chloro-2-naphthylquinoline with 4-hydroxyaniline. A subsequent Mannich reaction of **28** with formaldehyde and N-methylpiperazine furnished **32**. Compounds **29–31** were prepared in a similar manner. Finally, the quaternary compounds **17–19** (Table 3) were synthesized by the reaction of the corresponding aminoquinolines with MeI in DMF. The initially formed iodide of **17** [**15** (0.12 mmol), MeI (0.10 mmol), Na_2CO_3 (0.24 mmol), DMF (2 mL), stirring at 23 °C for 12 h] resisted all attempts of crystallization. A bromide/

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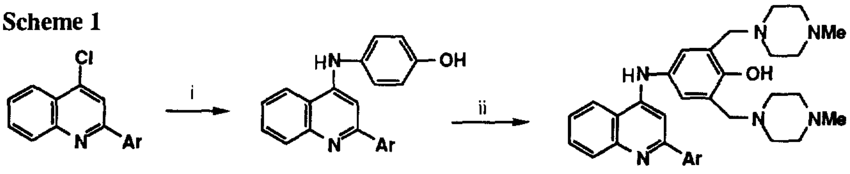
Scheme 1



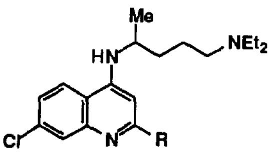
Ar = 2-naphthyl 28 32

(i): p-$H_2NC_6H_4OH$ (3 equiv), 130 °C, 4 h, then silica gel chromatography (Et_2O); (ii): **28** (1 mmol), *N*-methylpiperazine (5 mmol), aq CH_2O (13 M, 5 mmol), EtOH (20 mL), reflux, 24 h; then silica gel chromatography (AcOEt/Et_3N, 3/2).

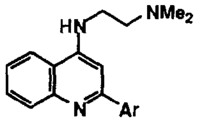
hydrobromide derivative of **17** crystallized following treatment of a solution of the iodide in MeOH with HBr (48%, 3 mmol). Dibromides **18** and **19** were prepared in a similar way by the reaction of the respective quinolines **15** and **14** (0.12 mmol) with MeI (2 mmol) followed by treatment with HBr. Solid quinolines were purified by crystallization, and oily derivatives were transformed into solid salts by using a general procedure[5] and the salts were crystallized. All compounds gave satisfactory results of elemental analysis and their structures were fully consistent with ^1H and ^{13}C NMR data.[9]

The SAR studies were initiated following our finding that a 2-(2-naphthyl)quinoline **1** is more active than chloroquine and 2-phenylchloroquine (Table 1). It was of interest, therefore, to analyze analogs of **1** containing different groups at position 2 of the quinoline. In comparison to **1**, the activity remains unchanged for **2**, which contains a larger 3-phenanthryl group and is slightly decreased for the 1-naphthyl derivative **3** with a severe steric hindrance around the inter-ring bond. Interesting results are the high efficacy of quinoline **4** that is substituted with a relatively small *para*-tolyl group and an even greater activity for compound **5**, which contains a styryl moiety. Comparison of compounds **4** and **6** reveals that the electron-withdrawing trifluoromethyl group in **6** exerts a strongly negative effect on the activity.

Table 1. Activities of Chloroquine,[a] 2-Phenylchloroquine,[a] and 2-Substituted 4-[2-(Dimethylamino)ethyl]quinolines **1–6**



Chloroquine: R=H (EC_{50} = 110 nM)[a]
2-Ph-chloroquine: R = Ph (EC_{50} = 51.3 nM)[a]



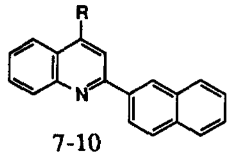
1-6

No	Ar	EC_{50} (nM)
1	2-naphthyl	9.1
2	3-phenanthryl	11.0
3	1-naphthyl	39.8
4	4-MePh	12.3
5	*trans*-CH=CHPh	6.5
6	4-CF_3Ph	155

[a]Taken from ref 1. Compounds 1–6 were assayed under the same conditions. The EC_{50} is the concentration required for half-maximal inhibition of CpG-ODN effect on thymidine uptake by WEHI 231 B-cells in the presence of α-sIgM. The estimated experimental error is ±15%.

The effect of basicity of the ring nitrogen atom in selected quinolines **1** and **7–10** was investigated (Table 2). As discussed previously,[4] the given pK_a values are functions of electronic effects of the 4-substituent at the quinoline including inhibition of the conjugation effect in **7** due to steric hindrance. As can be seen from Table 2, the pK_a values of **1**, **7–10** parallel nicely the respective EC_{50} values.

Table 2. The Experimental pK_a Values of Quinolines **1**, **7–10**[a] and Their Activity



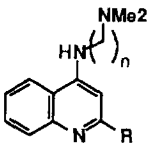
7-10

No.	R	pKa	EC_{50} (nM)
1	$NH(CH_2)_2NMe_2$	7.1	9.1
7	$N(Me)(CH_2)_2NMe_2$	6.2	416
8	$O(CH_2)_2NMe_2$	6.1	478
9	$S(CH_2)_2NMe_2$	4.4	2400
10	$C(O)NH(CH_2)_2NMe_2$	2.9	>10,000

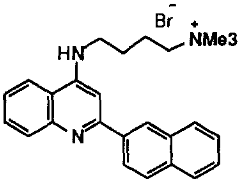
[a]The values for **1**, **7**, **9**, and **10** are taken from ref. 4.

Further analogs of **1** are analyzed in Table 3. These are quinolines with and without aryl substitution, with an increasing length of the (dimethylamino)polymethylene chain, and several quaternized derivatives in which a positive charge is permanently fixed. There is a dramatic increase in activity, due to 2-aryl substitution, as can be seen from comparison of **11** to **12** and **13** and comparison of **14** to **15**. On the other hand, the activities of compounds **4** and **1** containing the same (dimethylamino)dimethylene side chain are similar to those of the respective (dimethylamino)trimethylene analogs **12** and **13**. As the chain length increases in the series of compounds **13**, **15**, and **16**, which contain the same 2-(2-naphthyl)quinolin-4-amine core, the activity reaches a maximum for a tetramethylene derivative **15** and then is slightly decreased for compound **16** which has a hexamethyl-

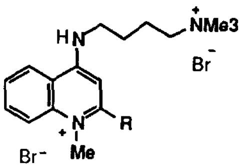
Table 3. Activities of Quinolin-4-amines **11–18** and Quaternary Derivatives **17–19**



11-16



17



18, 19

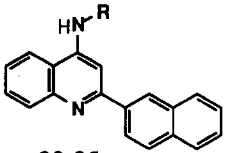
No.	R	n	EC_{50} (nM)
11	H	3	4400
12	*p*-tolyl	3	11.5
13	2-naphthyl	3	11.0
14	H	4	316
15	2-naphthyl	4	4.0

No.	R	n	EC_{50} (nM)
16	2-naphthyl	6	5.9
17			>10,000
18	2-naphthyl		7600
19	H		>10,000

ene linker. To our surprise, methylation of the terminal dimethylamino group of **15** rendered the resultant trimethylammonium derivative **17** completely inactive. An additional methylation of **17** at the ring nitrogen atom restored some activity in the resultant dication **18**. However, a dication **19** devoid of the naphthyl group was completely inactive again. Our additional studies (not shown) consistently indicated that compound **1** and its numerous analogs with a severely sterically hindered amino function at the terminus of the side chain showed comparable activities. Accordingly, the lack of activity of **17** and **19** cannot be explained in terms of an increased bulkiness of the trimethylammonium substituent in comparison to that of the dimethylamino group.

Analogs of **1** containing an alkyl group at N4 of the quinoline were inactive as well (not shown; see, however, **26** in Table 5). On the other hand, quinolines that contain groups capable of a hydrogen bonding interaction, such as a urethane in **20** or a hydroxy group in **21–24** (Table 4) show some activity. The activity of **25**, the side chain of which contains additional amino functions, is greater.

Table 4. Activities of *N*-Substituted Quinolin-4-amines

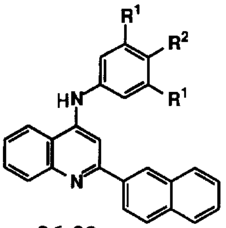


20-25

No.	R	EC_{50} (nM)
20	$(CH_2)_2NHCO_2Bu$-*t*	570
21	$CH(Me)(CH_2)_3C(Me_2)OH$	1150
22	CH(Me)CH(Ph)OH (erythro)	1000
23	$(CH_2)_2O(CH_2)_2OH$	330
24	$(CH_2)_6OH$	170
25	$(CH_2)_3N(CH_2CH_2)_2N(CH_2)_3NHC(O)(CH_2)_3OH$	25.1

This activity pattern is retained in a series of substituted 4-anilinoquinolines **26–32** (Table 5). Compound **32** has a relatively basic quinoline (pK_a = 6.9, a calculated value), contains hydroxy and amino functions, and is the most potent antagonist of immunostimulatory CpG-ODN's found to date.

Table 5. Activities of 4-(Substituted Anilino)quinolines



26-32

No.	R^1	R^2	EC_{50} (nM)
26	H	*n*-C_4H_9	>10,000
27	H	CH_2CH_2OH	510
28	H	OH	320
29	morpholinomethyl	OH	10.0
30	piperidinomethyl	OH	1.5
31	pyrrolidinomethyl	OH	1.2
32	*N*-methylpiperazinomethyl	OH	0.24

In order to better understand the requirements for activity, eleven 2-(2-naphthyl)quinolin-4-amines of Tables 4 and 5, all containing a hydroxy group at the side chain and occasionally substituted with additional amino groups, were subjected to correlation analysis by using calculated connectivity indices. These parameters, depending on their order, encode the length and the size of a substituent and also the type and position of branching. The lipophilicity, length, and branching of hydrocarbon substituents are well characterized by the set of ${}^3X_p{}^v$ indices.[10]

A statistically significant QSAR correlation for the eleven substituents, all containing free (non-protonated) amino groups at the side chain, was obtained (eq 1).

$$\log (1/EC_{50}) = 0.534 (\pm 0.08)\ {}^3X_p{}^v + 5.600 (\pm 0.27) \quad (1)$$

n = 11 (C4 groups of **21-25** and **27-32**), r = 0.934, s = 0.49, $F_{0.01}$ = 61.09

A slightly inferior correlation was obtained for the same set of substituents with protonated amino groups excepting the N4 atom at the quinoline which is non-basic due to the strong conjugation effect (not shown).

However, surprising as it may seem, these results and the experimental results obtained with quaternary compounds **17–19** suggest that the basic amino group (pK_a > 8) at the side chain of an active agent is not protonated in the compound-bioreceptor complex. On the other hand, the observed pK_a correlation (Table 2) and the activity of the N1-quaternized quinoline **18**[11] are consistent with protonation of the relatively less basic ring nitrogen (pK_a < 8). It can be suggested that the free amino group is located in a lipophilic microenvironment of the bioactive complex and is involved in hydrogen-bonding interaction with a hydrogen donor of the receptor. This hypothesis is consistent with our finding that hydroxy-substituted derivatives also show some activity. Proteins or phospholipids are likely candidates as the bioreceptor.

Since nucleic acids are involved and several compounds of this study have been shown previously to strongly stabilize a triple-helix DNA structure, it was hypothesized initially that stabilization of the triple helix complex is part of the mechanism. However, while the isomeric compounds **1** and **3** are antagonists of the CpG-induced effect and compound **1** is one of the best triplex DNA stabilizing agents known to date, the sterically hindered isomer **3** does not promote triplex DNA formation and does not bind to the triple DNA structure.[4] While compound **1** binds weakly to duplex DNA, compound **3** does not bind at all. The two compounds do not stabilize a quadruplex DNA as well. In addition, compound **1** binds weakly to RNA.[12] Thus, it can be suggested that the mechanism of quinoline antagonists does not involve binding to CpG-ODN.

4-Quinolinamines which inhibit CpG-ODN-induced effects are weak bases. Weak bases tend to partition into acidified vesicles within cells.[13] Chloroquine has been well studied in this regard. It concentrates in lysosomes and collapses the pH gradient,[14] leading to the hypothesis that organelle acidification is required for intracellular recognition of CpG-ODN.[15] However, this hypothesis is not consistent with the activity of a diquaternary derivative **18** which is not a base. In addition, a number of independent experiments consistently indicated that the active compounds do not inhibit the action of CpG-ODN by interfering with the acidification of vesicles, nor with the uptake or subcellular distribution of CpG-ODN (D.E. Macfarlane, manuscript in preparation). In summary, it seems most likely that these agents inhibit the detector system for CpG-ODN by a specific mechanism, not by a bulk effect.

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References and Notes

1. Macfarlane, D. E.; Manzel, L. *J. Immunol.* **1998**, *160*, 1122; and references cited therein.
2. Fox, R. *Semin. Arthritis Rheum.* **1993**, *23*, 82.
3. Wallace, D. J. *Rheum. Dis. Clin. North Amer.* **1994**, 20, 243.
4. Strekowski, L.; Gulevich, Y.; Baranowski, T. C.; Parker, A. N.; Kiselyov, A. S.; Lin, S.-Y.; Tanious, F. A.; Wilson, W. D. *J. Med. Chem.* **1996**, *39*, 3980.
5. Strekowski, L.; Mokrosz, J. L.; Honkan, V. A.; Czarny, A.; Cegla, M. T.; Wydra, R. L.; Patterson, S. E.; Schinazi, R. F. *J. Med. Chem.* **1991**, *34*, 1739.
6. Strekowski, L.; Janda, L.; Patterson, S. E.; Nguyen, J. *Tetrahedron* **1996**, *52*, 3273.
7. Strekowski, L.; Zegrocka, O.; Windham, C.; Czarny, A. *Org. Process Res. Dev.* **1997**, *1*, 384.
8. Strekowski, L.; Patterson, S. E.; Janda, L.; Wydra, R. L.; Harden, D. B.; Lipowska, M.; Cegla, M. T. *J. Org. Chem.* **1992**, *57*, 196.
9. Compound, mp (crystallization solvent): **2**•2HBr•$2H_2O$, 264-267 °C (95% EtOH); **5**•2HBr, 231-233 °C (95% EtOH); **8**, 52-54 °C (CH_2Cl_2/Et_2O); **11**•$2H_3PO_4$•H_2O, 222-225 °C (95% MeOH); **12**•2HBr•H_2O, 243-245 °C (95% MeOH); **13**•2HBr•H_2O, 249-250 °C (95% MeOH); **14**•2HCl•H_2O, 144-147 °C (95% EtOH); **15**•2HBr, 205-207 °C (95% MeOH); **17**, 214-216 °C (30% MeOH); **18**, 209-212 (acetone); **19**•H_2O, 239-241 °C (95% EtOH); **20**, 87-89 °C (AcOEt); **21**, 65-67 °C (AcOEt); **22**, 189-190 °C (Et_2O); **23**•HBr, 220-222 °C (95% MeOH); **24**•HCl•$2H_2O$, 122-129 °C (95% MeOH); **26**•H_2O, 173-175 °C (95% MeOH); **27**•HCl, 267-272 °C (95% MeOH); **28**•HCl•$0.5H_2O$, >300 °C (95% EtOH); **29**•3HCl•$2.5H_2O$, 224-226 °C (95% EtOH); **30**•$4H_2O$, 161-168 °C (95% MeOH); **31**•3HCl•$2H_2O$, 213-216 °C (95% EtOH/*t*-BuOMe); **32**•5HCl•$5H_2O$, 218-222 °C (95% EtOH). ^1H NMR for **32**•5HCl•$5H_2O$ (DMSO-d_6, 400 MHz): δ 2.74 (s, 6H), 3.35–3.75 (m, 16H), 4.45 (s, 4H), 7.17 (s, 1H), 7.67 (m, 2H), 7.81 (t, J = 8 Hz, 1H), 7.87 (s, 2H), 8.02–8.23 (m, 5H), 8.46 (d, J = 8 Hz, 1H), 8.82 (s, 1H), 8.95 (d, J = 8 Hz, 1H), 11.21 (s, exchangeable with D_2O, 1H), 12.05 (br s, exchangeable with D_2O, 1H). ^{13}C NMR for **32**•5HCl•$5H_2O$ (DMSO-d_6, 75 MHz): δ 41.8, 47.5, 49.9, 54.0, 99.4, 116.3, 119.7, 120.6, 123.5, 125.1, 127.2, 127.4, 127.8, 128.5, 128.8, 129.0, 129.2, 129.4, 129.5, 131.2, 132.4, 134.2, 134.3, 138.9, 152.9, 154.7, 155.4. Spectral data for other compounds will be published in due course.
10. Mokrosz, J. L.; Duszynska, B.; Strekowski, L. *Pharmazie* **1992**, *47*, 538; and references cited therein.
11. The inactive compounds **17**, **19** and others (EC_{50} > 10,000 nM) were assayed at the highest concentration of 10,000 nM and showed no effect on the cell culture. The EC_{50} value for **18** is 7600 ± 1200 nM.
12. Wilson, W. D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. *Biochemistry* **1993**, *32*, 4098.
13. de Duve, C.; de Barsy, T.; Poole, B.; Trouet, A.; Tulkens, P.; van Hoof, F. *Biochem. Pharm.* **1974**, *23*.
14. Ohkuma, S.; Poole, B. *J. Cell Biol.* **1981**, *90*, 656.
15. Yi, A. K.; Tuetken, R.; Redford, T.; Waldschmidt, M.; Kirsch, J.; Krieg, A. M. *J. Immunol.* **1998**, *160*, 4755.