

**IMPROVED SYNTHESIS OF SUBSTITUTED
2-ARYL-N-[2-(DIMETHYLAMINO)ETHYL]QUINOLIN-4-AMINES
AND THEIR ACTIVITY AS ANTAGONISTS OF IMMUNOSTIMULATORY
CpG-OLIGODEOXYNUCLEOTIDES**

Ekaterina Paliakov,[#] Maged Henary,[#] Martial Say,[#] Lubomir Janda,[#] Lori Manzel,⁺ Donald E. Macfarlane,⁺
and Lucjan Streckowski,^{#,*}

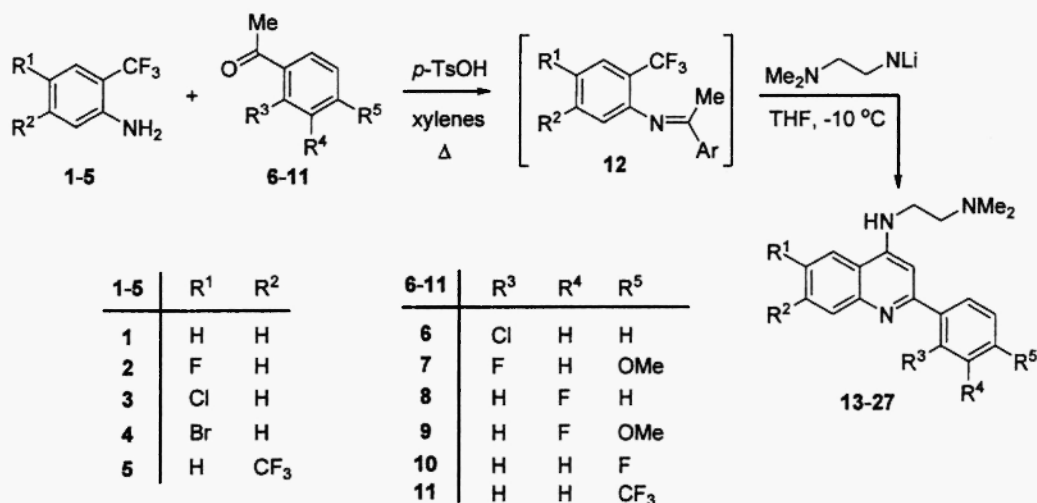
[#]Department of Chemistry, Georgia State University, Atlanta, Georgia 30302, USA
⁺Veterans Affairs Medical Center and University of Iowa, Iowa City, Iowa 52242, USA
lucjan@gsu.edu

Abstract : A general methodology for the synthesis of substituted quinolin-4-amines developed by us previously has been simplified. The synthesized compounds, depending on substituents, show activities in the range from 35 nM to 550 nM as antagonists of immunostimulatory oligodeoxynucleotides containing a CpG motif. A SAR analysis is presented.

CpG motifs in DNA of vertebrates contain mostly 5-methylcytosine.¹ By contrast, the CpG motifs with unmethylated cytosine are common in bacterial DNA, and stimulate immune responses in vertebrates.^{2,3} Single-stranded synthetic CpG-containing oligodeoxynucleotides and phosphorothioate oligodeoxynucleotides (CpG-ODN) are also immunostimulatory. This finding has led to the development of a simple assay *in vitro* for the search of compounds that inhibit the immunostimulatory effect.⁴ Several agents 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.^{5,6} In our previous work we synthesized and assayed many classes of compounds and showed that several substituted 2-arylquinolin-4-amines are the most potent antagonists of the immunostimulatory CpG-ODN found to date.⁷⁻¹⁰ The assayed quinolines contained diverse 2-aryl and 4-amino groups but had no substituents at other positions of the quinolines system. This report pertains to the synthesis and biological evaluation of a series of new 2-aryl-4-[[2-(dimethylamino)ethyl]amino]quinolines that contain an additional substituent at the 6 or 7 position (Scheme 1). The [2-(dimethylamino)ethyl]amino function was chosen for all quinolines because such derivatives, in general, show high activity. Pairs of quinolines without and with the 6- or 7-substituent were synthesized and assayed for a direct analysis of the effect of the substituent.

The desired compounds **13 – 27** (Scheme 1) were synthesized by condensation of 2-(trifluoromethyl)anilines **1-5** with aryl methyl ketones **6-11** followed by cyclization of the resultant ketimines **12** by treatment with a lithium derivative of 2-(dimethylamino)ethylamine. Previously,¹⁰⁻¹² this general methodology called for the use of analytically pure intermediate ketimines **12**. As part of this work it was found that prepurification by a simple bulb-to-bulb distillation, giving **12** of about 90% purity, was adequate. In addition, tetrahydrofuran was substituted for ether¹⁰⁻¹² as solvent in the subsequent cyclization reactions of **12**, which resulted in slightly increased yields of the quinoline products. Compounds **13 – 27** were obtained by using this modified methodology.

The effective concentrations (EC₅₀) for 50% inhibition of the CpG-ODN mediated effect vary from 35 nM for **14** to 550 nM for **24** and **25** (Scheme 1). Comparison of the EC₅₀ values for pairs of compounds composed of an identical 2-arylquinoline system, e.g. **14/18** and **15/20**, reveals that the presence of a fluorine atom at the position 6 of the quinolines decreases the activity. A similar effect is observed for 6-chloro substitution (compare **13/22** and **16/23**). However, the largest decrease in activity is seen for the 7-trifluoromethyl substituted quinolines (e.g. **14/24** and **15/26**). Interestingly, the 4-(trifluoromethyl)phenyl substituted quinolines **17** and **21** also exhibit relatively low activities. The highest EC₅₀ values were obtained for compounds **14 – 16** that are devoid of a trifluoromethyl group. These results have important ramifications for the design of new antagonists.



13-27	R ¹	R ²	R ³	R ⁴	R ⁵	EC ₅₀ , [nM]
13	H	H	Cl	H	H	160
14	H	H	F	H	OMe	35
15	H	H	H	F	OMe	40
16	H	H	H	H	F	50
17	H	H	H	H	CF ₃	155
18	F	H	F	H	OMe	140
19	F	H	H	F	H	280
20	F	H	H	F	OMe	80
21	F	H	H	H	CF ₃	410
22	Cl	H	Cl	H	H	470
23	Cl	H	H	H	F	55
24	H	CF ₃	F	H	OMe	550
25	H	CF ₃	H	F	H	550
26	H	CF ₃	H	F	OMe	410
27	H	CF ₃	H	H	F	490

Scheme-1 : Synthesis of quinolines 13 – 27 and their activities as Antagonists of Immunostimulatory CpG-Oligodeoxynucleotides^a

^a EC₅₀ is an effective concentration for 50% inhibition by quinoline antagonists of the immunostimulatory effect of CpG-ODN. The ability of test compounds to reverse the action of CpG-ODN on WEHI 231 murine lymphoma B-cells was assessed *in vitro* as described previously.^{4,10}

Experimental

General. Tetrahydrofuran was distilled from sodium benzophenone ketyl immediately before use. 2-(Dimethylamino)ethylamine was dried over solid KOH. Unless stated otherwise, ¹H NMR spectra (400 MHz) were obtained in DMSO-*d*₆ solutions. Electron-impact mass spectra were obtained at 70 eV.

Quinolines 13 – 27. A solution of a 2-(trifluoromethyl)aniline 1 – 5 (0.03 mmol), a ketone 6 – 11 (0.02 mmol), and a catalytic amount of p-toluenesulfonic acid (100 mg) in xylenes (75 mL) in a 250-mL flask equipped with a Dean-Stark water collector was heated under reflux for several hours until no ketone was detected by TLC analysis (silica gel; hexanes/ether, 9:1). After cooling, xylenes and the excess aniline were removed on a rotary evaporator, and the oily residue was distilled from the same flask by using bulb-to-bulb distillation (150 – 230 °C/0.5 mmHg) to give 12 of about 90% purity (GC analysis).

A solution of 2-(dimethylamino)ethylamine (0.66 mL, 6 mmol) in tetrahydrofuran (15 mL) was treated a solution of *n*-butyllithium in cyclohexane (2 M, 3 mL, 6 mmol) at -10 °C, and the resultant mixture was stirred at -10 °C for 20 min before treatment with a solution of ketimine 12 (1.5 mmol). The mixture was stirred at -10 °C for an additional 1 h and then quenched with water (0.5 mL). The organic layer was concentrated on a rotary evaporator, and the residue was purified by silica gel chromatography (hexanes/Et₃N/EtOH, 7:2:1). A simple removal of a colored polymeric material on a short silica gel column was sufficient for a crystalline product which was further purified by crystallization from hexanes or hexanes/AcOEt. The purification of an oily quinoline required the use of a conventional chromatographic procedure. The noncrystalline product was additionally purified by crystallization of its hydrobromide salt. Thus a solution of a quinoline in EtOH was treated with a solution of hydrobromic acid (3 molar equiv) in EtOH/H₂O (9:1), and the resultant mixture was concentrated to precipitate the hydrobromide salt. The salt was crystallized twice from EtOH or EtOH/hexanes. The composition was determined by elemental analysis.

2-(2-Chlorophenyl)-*N*-[2-(dimethylamino)ethyl]quinolin-4-amine (13). This compound has been characterized previously.¹²

***N*-[2-(Dimethylamino)ethyl]-2-(2-fluoro-4-methoxyphenyl)quinolin-4-amine (14).** Yield 80%; mp 96-98 °C; ¹H NMR (CDCl₃) δ 2.32 (s, 6H), 2.71 (t, J = 6 Hz, 2H), 3.36 (t, J = 6 Hz, 2H), 3.86 (s, 3H), 5.92 (br s, exchangeable with D₂O), 6.70 (m, 1H), 6.84 (m, 2H), 7.42 (m, 1H), 7.63 (m, 1H), 7.80 (d, J = 8 Hz, 1H), 8.04 (m, 2H); EI-MS m/z 58 (100), 339 (M⁺). Anal. calcd for C₂₀H₂₂FN₃O: C, 70.77; H, 6.53. Found: C, 70.63; H, 6.59.

***N*-[2-(Dimethylamino)ethyl]-2-(3-fluoro-4-methoxyphenyl)quinolin-4-amine (15).** Yield 70%; mp 130-131 °C; ¹H NMR (CDCl₃) δ 2.76 (s, 6H), 3.16 (t, J = 6 Hz, 2H), 3.81 (t, J = 6 Hz, 3H), 4.32 (s, 3H), 6.45 (br s, exchangeable with D₂O), 7.16 (s, 1H), 7.47 (t, J = 8 Hz, 1H), 7.83 (m, 1H), 8.05 (m, 1H), 8.21 (d, J = 8 Hz, 1H), 8.29 (m, 2H), 8.49 (m, 1H); EI-MS m/z 58 (100), 339 (M⁺). Anal. calcd for C₂₀H₂₂FN₃O: C, 70.77; H, 6.53. Found: C, 70.70; H, 6.54.

***N*-[2-(Dimethylamino)ethyl]-2-(4-fluorophenyl)quinolin-4-amine (16).** This compound has been characterized previously.¹¹

***N*-[2-(Dimethylamino)ethyl]-2-[4-(trifluoromethyl)phenyl]quinolin-4-amine dihydrobromide (17•2HBr•1.5H₂O).** Yield 88%; mp 198-200 °C; ¹H NMR δ 2.37 (s, 6H), 2.79 (t, J = 6 Hz, 2H), 3.44 (m, 2H), 6.13 (br s, exchangeable with D₂O), 6.82 (s, 1H), 7.47 (t, J = 8 Hz, 1H), 7.68 (t, J = 8 Hz, 1H), 7.74 (d, J = 8 Hz, 2H), 7.86 (d, J = 8 Hz, 1H), 8.09 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 2H). Anal. calcd for C₂₀H₂₀F₃N₃•2HBr•1.5H₂O: C, 43.81; H, 4.60; N, 7.66. Found: C, 43.94; H, 4.80; N, 7.61.

***N*-[2-(Dimethylamino)ethyl]-6-fluoro-2-(2-fluoro-4-methoxyphenyl)quinolin-4-amine dihydrobromide (18•2HBr).** Yield 76%; mp 268-270 °C; ¹H NMR (free base in CDCl₃) δ 2.33 (s, 6H), 2.71 (t, J = 6 Hz, 2H), 3.34 (t, J = 6 Hz, 2H), 3.86 (s, 3H), 5.75 (br s, exchangeable with D₂O), 6.70 (m, 1H), 6.85 (m, 2H), 7.40 (m, 2H), 8.01 (m, 2H); EI-MS (free base) m/z 58 (100), 357 (M⁺). Anal. calcd for C₂₀H₂₁F₂N₃O•2HBr: C, 46.26; H, 4.46; N, 8.09. Found: C, 46.00; H, 4.50; N, 8.15.

***N*-[2-(Dimethylamino)ethyl]-6-fluoro-2-(3-fluorophenyl)quinolin-4-amine (19).** Yield 77%; mp 135-136 °C; ¹H NMR (CDCl₃) δ 2.34 (s, 6H), 2.74 (t, J = 6 Hz, 2H), 3.38 (m, 2H), 6.82 (br s, exchangeable with D₂O), 6.80 (s, 1H), 7.12 (m, 1H), 7.43 (m, 3H), 7.82 (m, 2H), 8.05 (m, 1H); EI-MS m/z 58 (100), 99, 240, 269, 327 (M⁺). Anal. calcd for C₁₉H₁₉F₂N₃: C, 69.70; H, 5.85. Found: C, 69.49; H, 5.84.

***N*-[2-(Dimethylamino)ethyl]-6-fluoro-2-(3-fluoro-4-methoxyphenyl)quinolin-4-amine dihydrobromide (20•2HBr•1.5H₂O).** Yield 68%; mp 258-260 °C; ¹H NMR (free base in CDCl₃) δ 6.36 (s, 6H), 2.76 (t, J = 6 Hz, 2H), 3.39 (t, J = 6 Hz, 2H), 3.95 (s, 3H), 5.85 (br s, exchangeable with D₂O), 6.76 (s, 1H), 7.06 (t, J = 8 Hz, 1H), 7.41 (m, 2H), 7.84 (m, 2H), 8.04 (m, 1H); EI-MS (free base) m/z 58 (100), 357 (M⁺). Anal. calcd for C₂₀H₂₁F₂N₃O•2HBr•1.5H₂O: C, 43.97; H, 4.80; N, 7.69. Found: C, 43.90; H, 4.82; N, 7.64.

***N*-[2-(Dimethylamino)ethyl]-6-fluoro-2-[4-(trifluoromethyl)phenyl]quinolin-4-amine (21).** Yield 53%; mp 122-124 °C; ¹H NMR (CDCl₃) δ 2.35 (s, 6H), 2.76 (t, J = 6 Hz, 2H), 3.39 (t, J = 6 Hz, 2H), 5.85 (br s, exchangeable with D₂O), 6.84 (s, 1H), 7.44 (m, 3H), 7.74 (d, J = 8 Hz, 2H), 8.06 (dd, J₁ = 9 Hz, J₂ = 6 Hz, 1H), 8.18 (d, J = 8 Hz, 1H); EI-MS m/z 58 (100), 99 (25), 319 (40), 377 (M⁺). Anal. calcd for C₂₀H₁₉F₄N₃: C, 63.65; H, 5.07; N, 11.13. Found: C, 63.64; H, 5.03; N, 11.17.

***N*-[2-(Dimethylamino)ethyl]-6-chloro-2-(2-chlorophenyl)quinolin-4-amine dihydrobromide (22•2HBr•H₂O).** Yield 40%; mp 213-215 °C; ¹H NMR (CDCl₃) δ 2.32 (s, 6H), 2.69 (t, J = 6 Hz, 2H), 3.20 (t, J = 6 Hz, 2H), 5.83 (br s, exchangeable with D₂O), 6.07 (s, 1H), 7.36 (m, 2H), 7.48 (m, 1H), 4.60 (m, 2H), 7.79 (m, 1H), 7.98 (d, J = 9 Hz, 1H); EI-MS (free base) m/z 58 (100), 360 (M⁺). Anal. calcd for C₁₉H₁₉Cl₂N₃•2HBr•H₂O: C, 42.24; H, 4.29; N, 7.77. Found: C, 41.98; H, 4.32; N, 7.71.

***N*-[2-(Dimethylamino)ethyl]-6-chloro-2-(4-fluorophenyl)quinolin-4-amine (23).** Yield 65%; mp 179-180 °C; ¹H NMR (DMSO-*d*₆) δ 2.24 (s, 6H), 2.59 (t, J = 6 Hz, 2H), 3.52 (t, J = 6 Hz, 2H), 7.02 (s, 1H), 7.19 (br s, exchangeable with D₂O), 7.33 (m, 2H), 7.64 (d, J = 8 Hz, 1H), 7.85 (d, J = 9 Hz, 2H), 8.26 (d, J = 8 Hz, 1H), 8.38 (s, 1H). Anal. calcd for C₁₉H₁₈ClFN₃: C, 66.57; H, 5.29; N, 12.25. Found: C, 66.50; H, 5.58; N, 11.86.

***N*-[2-(Dimethylamino)ethyl]-7-trifluoromethyl-2-(2-fluoro-4-methoxyphenyl)quinolin-4-amine (24).** Yield 75%; mp 110-111 °C; ¹H NMR (CDCl₃) δ 2.56 (s, 6H), 2.75 (t, J = 6 Hz, 2H), 3.39 (t, J = 6 Hz, 2H), 3.87 (s, 3H), 6.05

(br s, exchangeable with D₂O), 6.72 (m, 1H), 6.86 (m, 1H), 6.95 (d, J = 2 Hz, 1H), 7.58 (m, 1H), 7.93 (d, J = 9 Hz, 1H), 8.05 (t, J = 9 Hz, 1H), 8.32 (s, 1H); EI-MS m/z 58 (100), 277 (55), 349 (85), 407 (M⁺). Anal. calcd for C₂₁H₂₁F₄N₃O: C, 61.90; H, 5.19; N, 10.31. Found: C, 61.83; H, 5.20; N, 10.31.

N-[2-(Dimethylamino)ethyl]-7-trifluoromethyl-2-(3-fluoro)phenylquinolin-4-amine (25). Yield 66%; mp 117-119 °C; ¹H NMR (CDCl₃) δ 2.37 (s, 6H), 2.78 (t, J = 6 Hz, 2H), 3.42 (t, J = 6 Hz, 2H), 6.15 (s, 1H), 6.87 (s, 1H), 7.14 (m, 1H), 7.47 (m, 1H), 7.60 (m, 1H), 7.89 (m, 3H), 8.35 (s, 1H); EI-MS m/z 58 (100), 377 (M⁺). Anal. calcd for C₂₀H₁₉F₄N₃: C, 63.65; H, 5.07. Found: C, 63.74; H, 5.07.

N-[2-(Dimethylamino)ethyl]-7-trifluoromethyl-2-[(3-fluoro-4-methoxy)phenyl]quinolin-4-amine dihydrobromide (26•2HBr•H₂O). Yield 40%; mp 245-247 °C; ¹H NMR (free base in CDCl₃) δ 2.37 (s, 6 H), 2.79 (t, J = 6 Hz, 2 H), 3.42 (t, J = 6 Hz, 2 H), 3.96 (s, 3 H), 6.25 (br s, exchangeable with D₂O), 6.83 (s, 1 H), 7.07 (t, J = 8 Hz, 1H), 7.57 (m, 1H), 7.89 (m, 3H), 8.13 (s, 1H); EI-MS (free base) m/z 58 (100), 277 (35), 349 (45), 407 (M⁺). Anal. calcd for C₂₁H₂₁F₄N₃O•2 HBr•H₂O: C, 42.94; H, 4.29. Found: C, 43.01; H, 4.35.

N-[2-(Dimethylamino)ethyl]-7-trifluoromethyl-2-[(4-fluoro)phenyl]quinolin-4-amine (27). Yield 74%; mp 123-125 °C; ¹H NMR (CDCl₃) δ 2.35 (s, 6 H), 2.76 (t, J = 6 Hz, 2H), 3.40 (t, J = 6 Hz, 2H), 6.10 (br s, exchangeable with D₂O), 6.85 (s, 1H), 7.19 (m, 2H), 7.58 (m, 1H), 7.92 (d, J = 8 Hz, 1H), 8.10 (m, 2H), 8.33 (s, 1H); EI-MS m/z 58 (100), 377 (M⁺). Anal. calcd for C₂₀H₁₉F₄N₃: C, 63.65; H, 5.07. Found: C, 63.63; H, 5.09.

Biological Evaluation

The ability of test compounds to reverse the action of CpG-ODN on WEHI 231 murine lymphoma B-cells was assessed as described previously.^{4,10}

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