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THE SYNTHESIS AND IDENTIFICATION OF 4,6-DIAMINOQUINOLINE DERIVATIVES AS POTENT IMMUNOSTIMULANTS

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Abstract: The synthesis of a number of 4,6-diaminoquinoline derivatives is described as well as their evaluation in a mouse protection model designed to identify immunostimulant activity. These compounds represent a novel series of potent immunostimulants.

Immunocompromised individuals are subject to a range of opportunistic infections which represent a leading cause of morbidity and mortality among these patients. Stimulation of the non-specific immune response would be expected to significantly assist these patients in avoiding such infections as well as to control those already established.[1-3] In addition to these important applications in human medicine, an immunostimulant could play an important role in livestock production where bacterial infections associated with immunosuppression result in considerable economic loss to the food producer.[4]

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.[5,6] Recent reports of the potent immunostimulant activity of aminoquinoline derivatives[7,8] led to the evaluation of 4,6-diaminoquinoline derivatives in our in vivo model of immunostimulant activity. The synthesis and evaluation of this novel class of immunostimulants is the subject of this report.

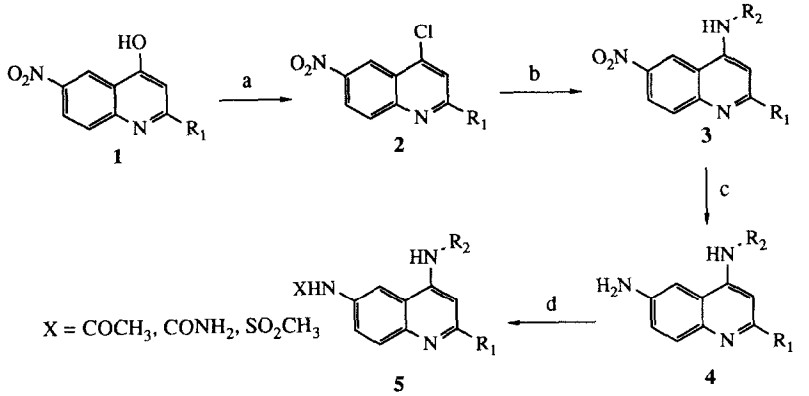
Chemistry:

Those compounds for which R_1 = phenyl were conveniently synthesized from commercially available 4-hydroxy-6-nitro-2-phenylquinoline (Scheme 1). The 4-position was activated by conversion to the chloro-derivative which smoothly underwent displacement reactions with various amines. Catalytic reduction of the nitro group cleanly produced **4** which could be derivatized by standard conditions to give **5**.

The preparation of those compounds for which R_1 = methyl or hydrogen required the synthesis of the appropriate hydroxyquinoline derivative as shown in Scheme 2. Treatment of 4-nitroaniline with ethyl acetoacetate or diethyl ethoxymethylenemalonate gave condensation products **7** and **9**, respectively, which were cyclized in Dowtherm A to give **8** and **10**.[9,10] Removal of the ethyl ester of **10** was accomplished by alkaline hydrolysis to the acid

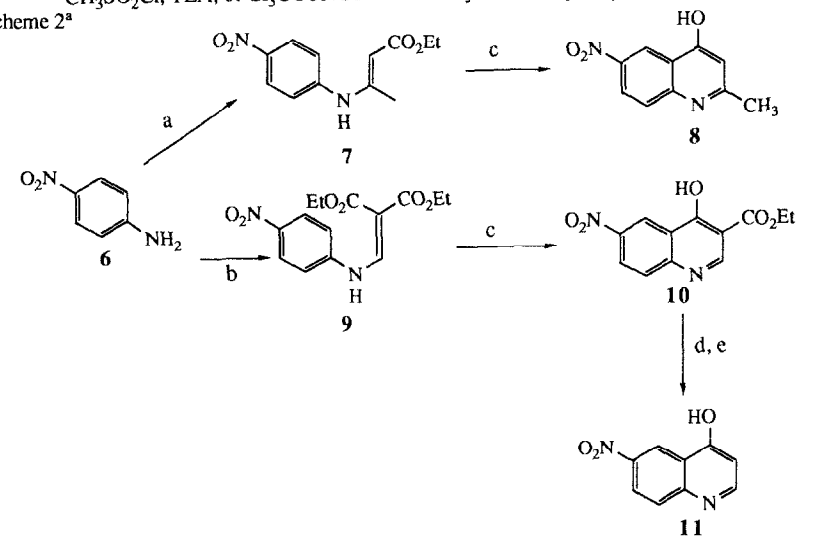
followed by decarboxylation in boiling Dowtherm A. Completion of analog synthesis from **8** and **11** proceeded exactly as described in Scheme 1.

Scheme 1[a]



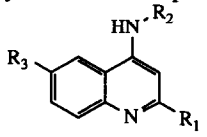
[a](a) $POCl_3$, DMF (b) R_2NH_2, EtOH (c) H_2, Pd(C) (d) Ac_2O, TEA, DMAP, CH_2Cl_2; CH_3SO_2Cl, TEA; or $Cl_3CCONCO$ followed by alkaline hydrolysis

Scheme 2[a]



[a](a) ethyl acetoacetate, CaSO4, HOAc, EtOH (b) diethyl ethoxymethylenemalonate, toluene (c) Dowtherm A (d) KOH/methanol (e) Dowtherm A

Table 1 Immunostimulant Activity of 4,6-Diaminoquinoline Derivatives



Compound	R_1	R_2	R_3	MED* (mg/kg)
12	Ph	4-($C_6H_{11}CH_2O$)-C_6H_4	NO_2	>50
13	Ph	4-($C_6H_{11}CH_2O$)-C_6H_4	NH_2	5.6
14	Ph	4-Cl-C_6H_4	NO_2	>50
15	Ph	4-Cl-C_6H_4	NH_2	5.6
16	Ph	3,4-di F-C_6H_3	NH_2	6.25
17	Ph	C_6H_{11}	NH_2	>100
18	Ph	4-($C_6H_{11}CH_2O$)-C_6H_4	$NHSO_2CH_3$	12.5
19	Ph	4-($C_6H_{11}CH_2O$)-C_6H_4	$NHCONH_2$	6.25
20	CH3	4-($C_6H_{11}CH_2O$)-C_6H_4	NH_2	6.25
21	CH3	3,4-di F-C_6H_3	NH_2	12.5
22	CH3	4-($C_6H_{11}CH_2O$)-C_6H_4	$NHCOCH_3$	12.5
23	CH3	4-($C_6H_{11}CH_2O$)-C_6H_4	$NHCONH_2$	50
24	H	4-BuO-C_6H_4	NH_2	25
25	H	4-EtO-C_6H_4	NH_2	6.25
26	H	4-($C_6H_{11}CH_2O$)-C_6H_4	NH_2	12.5
27	H	4-($C_6H_{11}CH_2O$)-C_6H_4	$NHCOCH_3$	6.25

*Minimum effective dose (MED) is the dose (given s.c.) which provides statistically significant ($p < 0.05$) protection as compared to control animals.

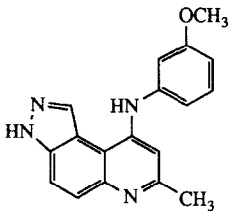
Biology:

Immunostimulant activity was evaluated in a mouse protection assay in which increased survival over non-drug treated controls was the endpoint. Briefly, mice were dosed at -24 h with drug and at time 0 h with a lethal inoculum of *E. coli* and a sub-therapeutic dose of antibiotic (gentamicin). The mice were monitored for 96 h and survivors in the drug treated groups compared with survivors in the control groups (no drug treatment).[11] The results of these evaluations are shown in Table 1.

Results and Discussion:

Several trends become apparent upon examination of Table 1.[12] The functional group at C-6 of the quinoline nucleus (R3 of Table 1) clearly plays an important role in the immunostimulant activity. Nitro compounds **12** and **14** are devoid of activity whereas amines, sulfonamides, ureas and amides all lead to active compounds. A hypothesis derived from these data is that a hydrogen-bond donating group is required at C-6 for the compounds to exhibit immunostimulant activity. This theory is contradicted by our work in the pyrazoloquinoline area where it was discovered that the regioisomeric pyrazoloquinolines **28** and **29** showed marked differences in activity. The inactive regioisomer, **28**, contains a hydrogen-bond donating group at what would be C-6 of the quinoline nucleus. The active regioisomer, **29**, has no hydrogen-bond donating potential at C-6 of the quinoline nucleus.[8] Further work is necessary to understand this divergent SAR.

Figure 1



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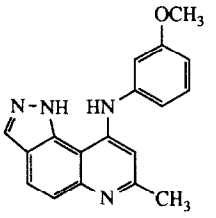

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The R1 position tolerates a hydrogen, methyl or phenyl equally well, suggesting this position is not important for interacting with the molecular target of these compounds. Consequently, this position may be suitable for use in modulating the physicochemical properties of these compounds.

Aromatic groups containing both electron-donating and electron-withdrawing substituents produce potent immunostimulant activity when incorporated at R_2. Contrary to this is the lack of activity of the cyclohexyl substituent, a group which produces good activity in related series' of compounds.

Precise interpretation of the structure-activity relationships associated with these 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.

A novel series of 4,6-diaminoquinoline derivatives has been shown to possess potent in vivo immunostimulatory activity which significantly protects mice from a lethal bacterial challenge. Compounds with this type of activity could play an important role in the treatment of infectious diseases in humans and food-producing animals.

Acknowledgment

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10. Dowtherm A is a eutectic mixture of diphenyl ether and biphenyl and boils at approximately 250 ^{0}C.

11. *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 agar (BHI). After overnight incubation, three 1-ml Prompt-innoc® (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 hours, at which time the optical density (600 nm) reached 0.3-0.4, corresponding to approximately 8 x 10^8 CFU/ml as determined by viable count. This culture was then diluted in cold L broth to achieve a concentration near 4 x 10^7 CFU/ml.

    Female NSA (CF-1) mice weighing 11-16 grams (Harlan Sprague Dawley, Indianapolis, IN) were infected by intraperitoneal (i.p.) injection of 0.5 ml of bacterial culture as described above. Gentamicin was administered subcutaneously (s.c.) at 0.5, 4, and 24 hours post-infection. Immunomodulators were prepared in pyrogen-free saline or water for injection (USP) and administered 24 hours 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.

12. All final compounds were characterized by ^{1}H NMR, combustion analysis and/or mass spectral data which were fully in accord with expected structures. Data for Compound 16 (dihydrochloride salt) is included as a representative example. ^{1}H NMR (DMSO-d_6) ∂ 6.97 (s, 1H), 7.36-7.74 (m, 8H), 7.92 (m, 2H), 8.20 (d, 1H, J = 9 Hz); mass spectrum, *m/z* 347 (M^+); Anal. calcd for $C_{21}H_{15}N_3F_2$•2HCl: C, 60.01; H, 4.08; N, 10.00. Found: C, 60.07; H, 4.21; N, 9.79.