(Benzoylphenyl)piperidines: A New Class of Immunomodulators

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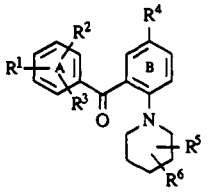
A series of (benzoylphenyl)piperidines has been synthesized and evaluated for activity as immunomodulators. Several of these compounds show good activity in primary screening on the basis of the lymphocytes mitogenic response to Con A, PHA, and PWM. A chloro group in position 4 of the benzoyl moiety as well as an amino group (or a carbamate derivative) para to the piperidine nucleus seems to be essential for activity. The depicted compounds may be considered as the first examples of a new series of immunomodulators.

The clinical need for therapeutic agents able to restore a normal immune response in immunocompromised patients (primary and acquired immunodeficiency, recurrent viral infections, autoimmune diseases, cancer) has led to the discovery of a number of substances collectively defined as immunomodulators. The word has been coined to emphasize the phase variation of most of these drugs, which may enhance or suppress the immune response according to their mode of adminstration.[1-3] Most of them are bacterial preparations or peptide hormones; only a few are synthetic, low molecular weight molecules.[4] As exemplified by the work performed with thymic hormones, one promising issue is to discover compounds acting on T lymphocytes either by enhancing T cell mediated activation or by favoring T cell differentiation.[5]

In this paper, we describe the synthesis and T cell immunomodulating properties of a series of (benzoylphenyl)piperidines (Table I) exemplified by structure I and structurally unrelated to other known immunomodulators.



R^1,R^2,R^3 = H,Cl,Br,F,OCH_3

R^4 = H,Cl,N,R^7,R^8

R^5,R^6 = H,CH_3,C_2H_5,nBu,tBu,Ph,CH_2Ph,OH

I

Chemistry

Aromatic Substitution of Ring A (Substituents R^1, R^2, R^3 Compounds 1–15). For all compounds, except the 2- and 3-monosubstituted examples, the benzophenone framework was constructed by Friedel–Crafts reaction of 6-chloro-3-nitrobenzoyl chloride (37) with a suitably substituted benzene 38 (Scheme I). The piperidine subunit 40 was then introduced by nucleophilic displacement of chlorine. Subsequent reduction of the nitro group of 41 with either Fe or $SnCl_2$ in acidic medium afforded the final compounds. However, because of the directional control of substitution in the Friedel–Crafts reaction, this route was not applicable for the 2- and the 3-monosubstituted compounds and alternative synthetic schemes had to be developed. According to a recent patent,[6] 2-halo derivatives of 2-amino-5-nitrobenzophenone may be obtained by Friedel–Crafts reaction of a 2-halobenzoyl chloride with 4-nitrobenzalinilides (Scheme II). In our hands, this reaction conducted between 42 and 43 was successful, but the adduct 44 was obtained in only moderate and nonreproducible yields. Replacement of the amino group by chlorine through diazotization with isoamyl nitrite,[7] followed by treatment with $CuCl_2$ led to 45. Selective displacement of the doubly activated chloro group and subsequent reduction to the nitro substituent then afforded the desired compound 12.

Scheme III describes the synthetic route used for preparation of the 3-monosubsubstituted derivatives. Again, with 6-chloro-3-nitrobenzoic acid as the starting material, the chlorine atom was first replaced by the piperidine unit, and the corresponding acid chloride 48 was then treated with the appropriate organocadmium reagent[8] 49. A final reduction (Fe/HCl) of the resulting nitrobenzophenones 50 gave the expected products (13, 14, and 15).

Aromatic Substitution of Ring B (Substituent R^4; Compounds 16–25). As a consequence of the synthetic approaches chosen, all compounds first synthesized bore an NH_2 group para to the piperidine ring. As it became desirable to investigate the influence of this R^4 substituent on the biological activity, modifications were performed on the NH_2 group as depicted in Scheme IV. These involved classical transformations which do not require further comment.

Compounds in which the NH_2 group had been replaced by hydrogen were obtained by a more straightforward route and with better yields by simply substituting the fluoro group of substituted 2-fluorobenzophenones (51) by the piperidine unit.

Modification of the Piperidine Ring (Substituents R^5, R^6; Compounds 26–35). A range of suitably substituted piperidines was also investigated. Most of the starting materials were commercial and only a few had to be prepared. Those piperidines bearing an ethyl or a *tert*-butyl group (52 and 53, respectively) at C-4 were obtained by catalytic hydrogenation of the corresponding pyridines by using slightly modified standard procedures[9] (see Experimental Section). Finally, the 4-butylpiperidine 55 was prepared in two steps from the commercially available *N*-benzyl-4-piperidone (54) according to Scheme V.[10]

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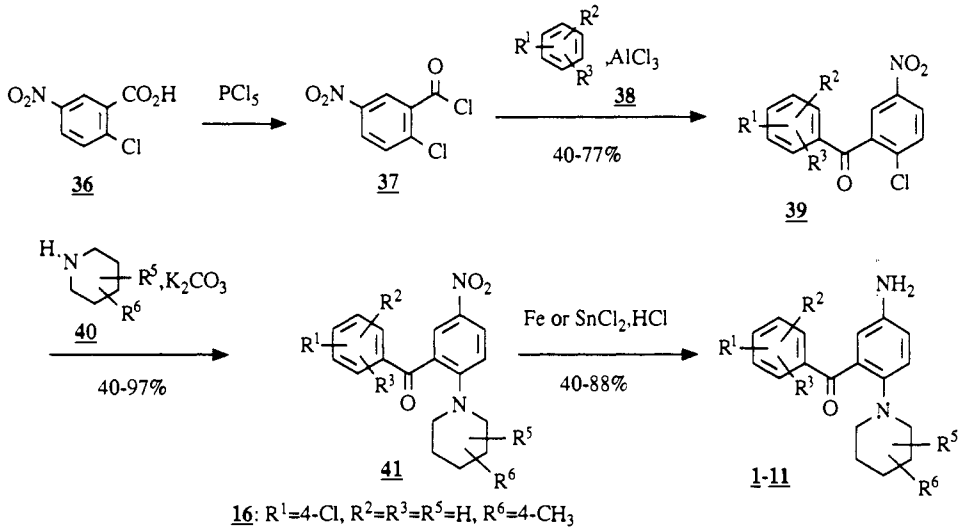
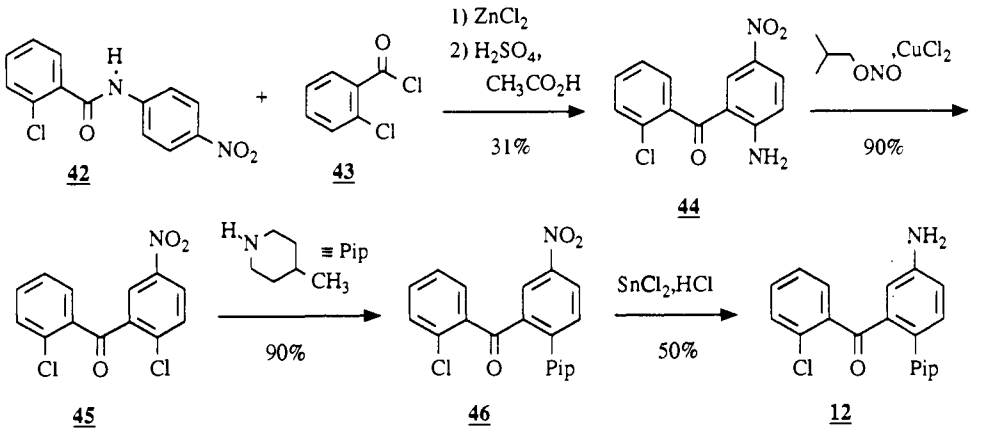
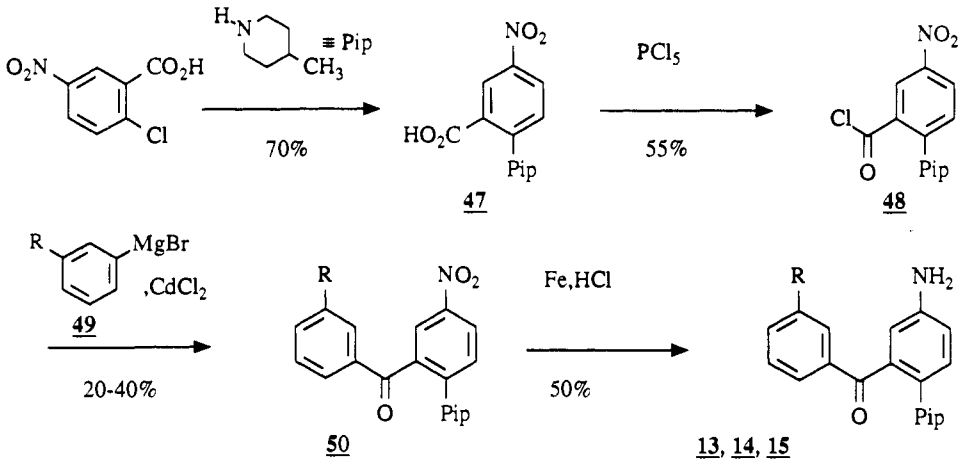
Scheme I



Scheme II



Scheme III



Results and Discussion

1. Proliferative Response to Mitogens. The biological activity of the (benzoylphenyl)piperidines described herein was first determined by the ability of these compounds to stimulate the proliferative response of spleen cells to mitogens, as this is the most commonly studied function of such cells. Augmentation of lymphoproliferative responses to mitogens has been consistently observed with such drugs as isoprinosine[13] and 2,2'-[ethylenebis(dithio)]bisethanol (ADA 202-718).[14] After having confirmed the lack of intrinsic mitogenic effect of our test compounds in the absence of lectin, the proliferative potential of each product was evaluated over the concentration range of 5 to 80 μg/mL. The results in Table II list the values obtained for each compound at their optimal stimulating concentration.

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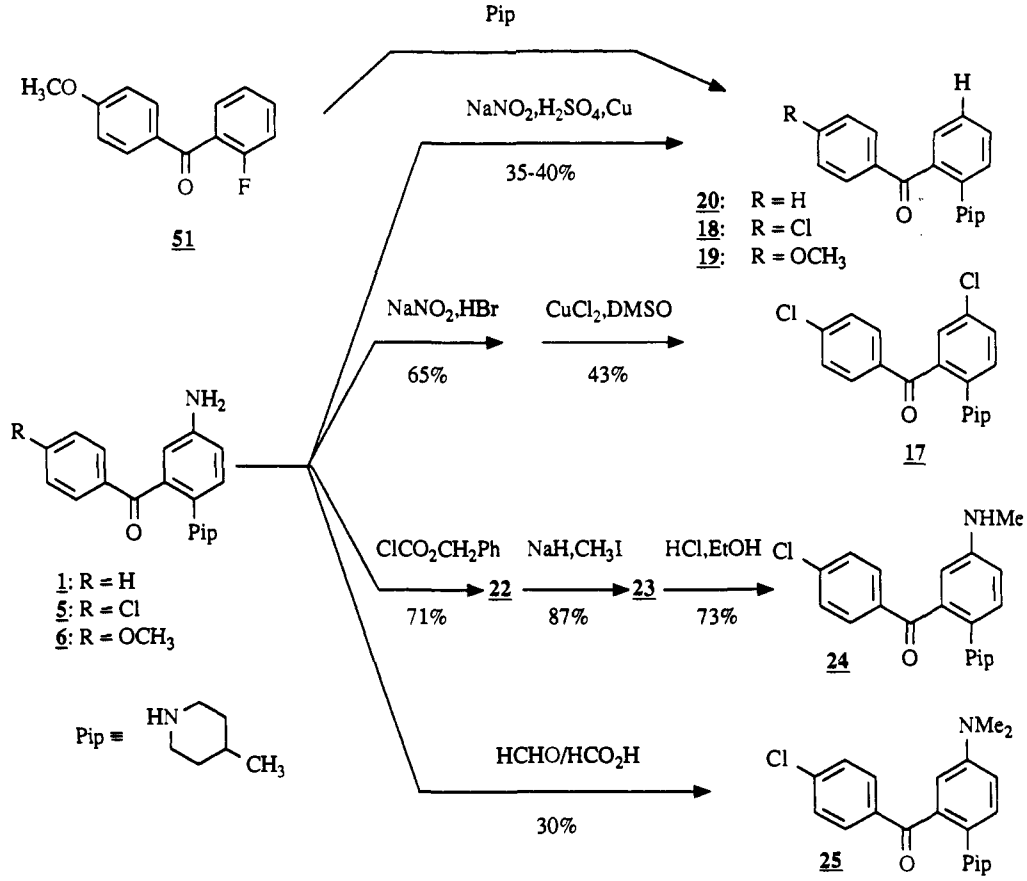
Table I. (Benzoylphenyl)piperidines Synthesized

no.	R^1	R^2	R^3	R^4	R^5	R^6	mp, °C	formula
1	H	H	H	NH_2	4-CH_3	H	108	$C_{19}H_{21}N_2O$
2	4-CH_3	H	H	NH_2	4-CH_3	H	78	$C_{20}H_{24}N_2O$
3	4-F	H	H	NH_2	4-CH_3	H	116	$C_{19}H_{21}N_2O$
4	4-Br	H	H	NH_2	4-CH_3	H	105	$C_{19}H_{21}BrN_2O$
5	4-Cl	H	H	NH_2	4-CH_3	H	89	$C_{19}H_{21}ClN_2O$
6	4-OCH_3	H	H	NH_2	4-CH_3	H	64	$C_{20}H_{24}N_2O_2$
7	2-CH_3	4-CH_3	H	NH_2	4-CH_3	H	90	$C_{21}H_{26}N_2O$
8	3-OCH_3	4-OCH_3	H	NH_2	4-CH_3	H	132	$C_{21}H_{26}N_2O_3$
9	3-Cl	4-Cl	H	NH_2	4-CH_3	H	94	$C_{19}H_{20}Cl_2N_2O$
10	2-Cl	4-Cl	H	NH_2	4-CH_3	H	117	$C_{19}H_{20}Cl_2N_2O$
11	3-CH_3	4-CH_3	5-CH_3	NH_2	4-CH_3	H	127	$C_{22}H_{28}N_2O$
12	2-Cl	H	H	NH_2	4-CH_3	H	94	$C_{19}H_{21}ClN_2O$
13	3-Cl	H	H	NH_2	4-CH_3	H	88	$C_{19}H_{21}ClN_2O$
14	3-CH_3	H	H	NH_2	4-CH_3	H	95	$C_{20}H_{24}N_2O$
15	3-CF_3	H	H	NH_2	4-CH_3	H	138	$C_{20}H_{21}F_3N_2O$
16	4-Cl	H	H	NO_2	4-CH_3	H	136	$C_{19}H_{19}ClN_2O_3$
17	4-Cl	H	H	Cl	4-CH_3	H	109	$C_{19}H_{19}ClN_2O_3$
18	4-Cl	H	H	H	4-CH_3	H	69	$C_{20}H_{20}ClNO$
19	4-OCH_3	H	H	H	4-CH_3	H	oil	$C_{20}H_{23}NO_2$
20	H	H	H	H	4-CH_3	H	82	$C_{19}H_{21}NO$
21	4-Cl	H	H	$NHCO_2Et$	4-CH_3	H	132	$C_{22}H_{25}ClN_2O_3$
22	4-Cl	H	H	$NHCO_2CH_2Ph$	4-CH_3	H	159	$C_{27}H_{27}ClN_2O_3$
23	4-Cl	H	H	$N(CH_3)CO_2CH_2Ph$	4-CH_3	H	117	$C_{28}H_{29}ClN_2O_3$
24	4-Cl	H	H	$NHCH_3$	4-CH_3	H	117	$C_{20}H_{23}ClN_2$
25	4-Cl	H	H	$N(CH_3)_2$	3-CH_3	H	99.5	$C_{21}H_{25}ClN_2O$
26	4-Cl	H	H	NH_2	H	H	96	$C_{18}H_{19}ClN_2O$
27	4-Cl	H	H	NH_2	3-CH_3	H	78	$C_{19}H_{21}ClN_2O$
28	4-Cl	H	H	NH_2	2-CH_3	H	92	$C_{19}H_{21}ClN_2O$
29	4-Cl	H	H	NH_2	4-CH_3	5-CH_3	103	$C_{20}H_{23}ClN_2O$
30	4-Cl	H	H	NH_2	4-*t*-Bu	H	109	$C_{22}H_{27}ClN_2O$
31	4-Cl	H	H	NH_2	4-C_2H_5	H	92	$C_{20}H_{23}ClN_2O$
32	4-Cl	H	H	NH_2	4-*n*-Bu	H	104	$C_{22}H_{27}ClN_2O$
33	4-Cl	H	H	NH_2	4-CH_2Ph	H	109	$C_{25}H_{25}ClN_2O$
34	4-Cl	H	H	NH_2	4-Ph	H	144	$C_{24}H_{23}ClN_2O$
35	4-Cl	H	H	NH_2	4-OH	H	71	$C_{18}H_{19}ClN_2O_2$

Scheme IV



Seven out of the 30 compounds evaluated in this test were chosen on the basis of their activity on at least one of the mitogen-induced responses: compounds **5**, **21**, **27**, **28**, **29**, **32**, and **34**. No distinction could be made, however,

Table II. Proliferative Response to Mitogens

	concanavaline A				PWM				PHA			
no.	dose: μg/mL (μM)	control blastogenesis[a]	stimulated blastogenesis[b]	stimulated index[c]	dose: μg/mL (μM)	control blastogenesis[a]	stimulated blatogenesis[b]	stimulated index[c]	dose: μg/mL (μM)	control blastogenesis[a]	stimulated blastogenesis[b]	stimulated index[c]
1	5 (17)	188.1 ± 1.6	167.0 ± 34.5	0.89	40 (136)	48.9 ± 5.2	55.7 ± 1.1	1.14				
2	5 (16)	179.7 ± 17.2	283.2 ± 19.4	1.58	40 (130)	53.8 ± 4.3	48.9 ± 1.4	0.91				
3	5 (17)	268.2 ± 49.9	165.6 ± 18.8	0.61	80 (273)	72.2 ± 7.8	69.9 ± 8.8	0.96				
4	5 (17)	118.7 ± 10.7	123.4 ± 16.6	1.04	40 (132)	40.5 ± 5.6	56.4 ± 13.2	1.39	5 (17)	7.0 ± 1.6	11.1 ± 0.6	1.59
5	5 (13)	194.5 ± 28.6	269.5 ± 45.7	1.39	40 (107)	36.5 ± 13.6	101.9 ± 3.3	2.81	10 (27)	12.8 ± 1.8	43.5 ± 9.2	3.4
6	5 (15)	322.1 ± 11.3	363.6 ± 11.3	1.13	10 (30)	103.3 ± 1.4	121.8 ± 2.4	1.18	5 (15)	36.3 ± 1.9	54.3 ± 3.7	1.5
7	5 (15)	279.6 ± 15.7	250.0 ± 19.6	0.89	80 (247)	68.8 ± 10.7	61.5 ± 5.9	0.89				
8	5 (14)	137.4 ± 23.0	158.4 ± 31.1	1.15	80 (226)	44.9 ± 3.5	48.8 ± 2.5	1.09				
9	5 (14)	136.6 ± 19.1	151.5 ± 19.0	1.11	80 (220)	48.4 ± 5.3	64.4 ± 8.7	1.33				
10	5 (14)	280.0 ± 20.3	246.2 ± 68.4	0.88	10 (27)	105.5 ± 2.2	115.9 ± 29.1	1.10	5 (14)	46.7 ± 8.2	46.4 ± 10.2	0.99
11	5 (15)	152.6 ± 10.6	206.0 ± 0.8	1.35	20 (60)	84.5 ± 4.1	77.1 ± 4.8	0.91				
12	5 (15)	150.0 ± 14.3	165.5 ± 8.7	1.1	20 (61)	80.0 ± 4.0	85.8 ± 17.8	1.07				
13	5 (15)	316.0 ± 56.2	343.8 ± 27.8	1.09	10 (30)	102.8 ± 7.2	105.0 ± 12.4	1.02	5 (15)	40.2 ± 3.9	40.9 ± 0.4	1.14
14	5 (16)	389.9 ± 35.9	414.1 ± 5.1	1.06	10 (32)	98.8 ± 3.7	135.9 ± 2.9	1.37	5 (16)	47.9 ± 0.7	62.4 ± 4.7	1.3
15	5 (14)	332.0 ± 25.6	297.7 ± 48.6	0.89	10 (28)	79.0 ± 7.3	79.9 ± 4.9	1.01	5 (14)	34.3 ± 4.6	36.0 ± 2.6	1.05
16	5 (14)	160.9 ± 8.8	93.7 ± 3.6	0.58	40 (112)	59.9 ± 10.6	42.7 ± 6.3	0.71				
17	5 (14)	55.6 ± 3.6	68.7 ± 4.4	1.23					7.5 (21)	14.6 ± 0.7	8.2 ± 1.0	0.56
18	5 (15)	92.7 ± 6.7	62.7 ± 5.8	0.68					7.5 (23)	17.4 ± 2.8	15.5 ± 1.0	0.89
19	5 (16)	127.5 ± 24.5	165.1 ± 25.7	1.29	10 (32)	20.8 ± 1.8	15.5 ± 2.4	0.74				
20	5 (18)	13.6 ± 1.3	9.0 ± 4.5	0.66	10 (36)	15.1 ± 0.9	24.7 ± 0.7	1.63				
21	5 (12)	8.0 ± 2.5	16.3 ± 1.9	2.03								
24	5 (15)	18.2 ± 0.6	21.3 ± 0.5	1.17	10 (31)	20.3 ± 0.6	19.5 ± 1.1	0.96				
25	5 (14)	17.6 ± 0.5	14.9 ± 1.5	0.85	10 (28)	21.4 ± 0.6	18.6 ± 1.2	0.87				
26	5 (16)	24.6 ± 3.1	21.3 ± 6.1	0.86	10 (32)	47.7 ± 4.4	44.8 ± 5.5	0.94				
27	5 (15)	124.4 ± 6.7	141.6 ± 20.1	1.14	80 (243)	41.3 ± 8.2	45.0 ± 10.5	1.07				
28	5 (16)	131.5 ± 13.3	294.8 ± 329	2.24	40 (130)	55.8 ± 2.4	58.1 ± 3.7	1.04				
29	5 (15)	48.6 ± 3.9	50.2 ± 3.9	1.03	40 (117)	67.3 ± 8.8	75.8 ± 2.6	1.13				
30	5 (13)	30.5 ± 11.6	31.9 ± 10.8	1.04	10 (30)	114.8 ± 11.7	137.5 ± 21.8	1.20	5 (13)	59.1 ± 8.8	55.0 ± 0.91	0.93
31	5 (15)	319.5 ± 22.0	348.0 ± 61.7	1.09	10 (29)	88.7 ± 27.7	114.6 ± 6.7	1.29	5 (15)	30.4 ± 2.6	37.4 ± 7.8	1.23
32	5 (14)	211.8 ± 40.1	289.2 ± 33.0	1.36	10 (27)	78.4 ± 13.9	81.7 ± 9.1	1.04	10 (27)	51.3 ± 10.0	59.1 ± 6.6	1.15
33	5 (12)	328.0 ± 41.0	308.4 ± 65.1	0.94	10 (25)	108.2 ± 12.8	79.5 ± 4.1	0.73	5 (12)	27.4 ± 6.5	35.1 ± 2.4	1.88
34	5 (13)	263.2 ± 23.4	294.1 ± 37.1	1.12	10 (26)	95.7 ± 4.8	115.3 ± 15.1	1.20	10 (26)	59.9 ± 5.3	54.4 ± 9.8	0.92
35	5 (15)	374.8 ± 53.5	281.1 ± 38.3	0.75	10 (30)	107.9 ± 7.1	88.7 ± 14.3	0.82	5 (15)	45.0 ± 3.3	52.6 ± 3.8	1.17

[a] Control blastogenesis: proliferation induced by the mitogen alone (Con A, PWM, PHA). [b] Stimulated blastogenesis: maximal proliferation induced by the test compound in the presence of the mitogen. [c] Results are expressed as the mean ± standard deviation after three different cultures.

Scheme V

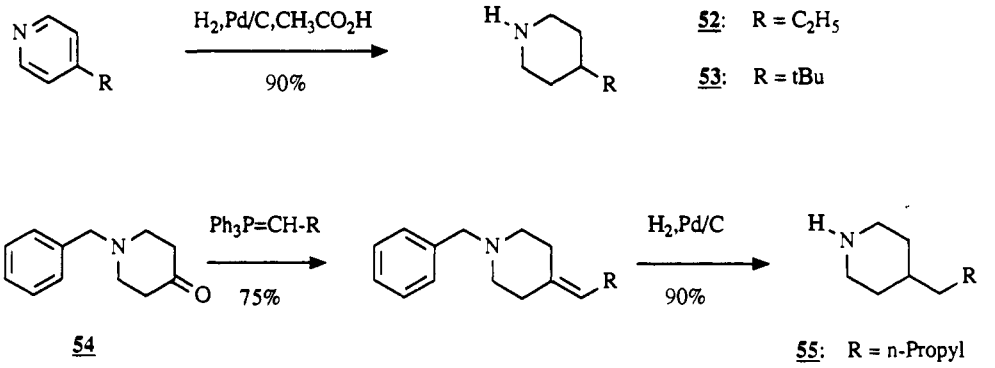


Table III. In Vitro Bone Marrow T Cell Precursors Differentiation

		% Thyl+ positive cells				stimulation index[a]	
		fraction A		fraction B			
no.	concn, μM	control (%)	treated	controls (%)	treated	fraction A	fraction B
5	7	5.6	11.5	5.0	11.4	2.05	2.28
21	2.5	6.1	11.6	6.1	5.5	1.90	0.90
34	3	3.8	7	5.2	6.4	1.84	1.23
32	3.2	3.8	4.2	5.2	6.4	1.10	1.23
29	2.9	6.1	8.8	6.1	3	1.44	0.49
28	2.7	5.6	6	5.0	4.4	1.07	0.88
27	2.6	5.0	6.7	3.8	5.0	1.34	1.43
thymosin		10.8	21.9	6.3	9.4	2.02	1.49

[a] Stimulation index = % Thyl+ treated cells/% Thyl+ control cells.

Table IV. Restoration of T Cell Markers in Nude Mice[a]

no.	% Thyl+ positive cells	% restoration	Thyl+ positive cells/spleen × 10^6	% restoration
control	6.0 ± 0.3		5.34	
5	6.9 ± 0.4	+14.3	6.65	+24.5
control	3.3 ± 0.2		2.42	
21	3.4 ± 0.2	+3.2	2.50	+3.3
control	4.4 ± 0.3		2.98	
34	5.5 ± 0.2	+12.5	3.20	+7.4
control	3.4 ± 0.4		2.04	
32	3.5 ± 0.2	+3.6	2.05	+0.6

[a] Results are expressed as mean value ± SD after individual measurement (5 animals per group).

on the basis of a preferential response to one type of lectin or another, although clearly, compound **5** is the most powerful agent acting on PWM and PHA proliferation and one of the best on Con A (respective optimal concentrations: 107, 27, and 13 μM).

2. In Vitro Differentiation of Bone-Marrow T Cell Precursors in Normal Mice. Thymic hormones[15,16] and other immunomodulators[13,17] are able to stimulate bone marrow stem cell to differentiate into mature T lymphocytes, a process usually induced by a functional thymus. Mature T lymphocytes bearing the Thyl+ antigen may be detected by immunofluorescence.

The previously selected compounds, **5**, **21**, **27**, **28**, **29**, **32**, and **34**, were incubated for 24 h with fractionated bone marrow from normal C57/B16 mice and the percentage of fluorescent Thyl+ cells was evaluated.[25] A stimulation index, defined as the ratio % of Thyl+ cells in treated marrow/% of Thyl+ cells in the control, was established, and this index for all of the compounds was compared to the score obtained with Thymosin Fraction V prepared according to the published procedure.[5] The results are presented in Table III, where A and B refer, respectively, to the fractions of lowest density, and second lowest density, from bone marrow separation in a gradient of bovine serum albumin.

Compound **5** is evidently the most powerful differentiator in this test and is very similar in this respect to Thymosin Fraction V.

3. Induction of T Cell Differentiation in the Athymic nu/nu Mouse (Nude). The congenitally athymic nu/nu mouse is considered to be a good model of primary immunodeficiency diseases involving such T cell defects as the di George Syndrome: the homozygous nu/nu mouse is almost entirely deprived of splenic mature T cells bearing the Thyl+ antigen, whereas the control heretozygous mouse exhibits a normal T cell population. It has been shown that in vivo treatment of nude mice with thymosin,[5,18] isoprinosine,[13] or levamisole[17] can restore a normal or subnormal Thyl+ expression.

Among the compounds selected, compound **5** and three analogues (**21**, **32**, and **34**) were tested in this model following oral adminstration.

As may be seen from Table IV, compound **5** displays very good activity with a score of nearly 25%, whereas **34** is less active, and **21** and **32** are totally inactive.

In conclusion, this promising profile of activities led to the selection of **5** (LF 1695) as a candidate for further pharmacological,[19,20] toxicological, and clinical studies.

Experimental Section

Chemistry. Melting points were determined with a Reichert apparatus and are uncorrected. Proton NMR spectra were recorded with a Bruker WP Model 80 spectrometer: chemical shifts (δ in ppm) quoted for multiplets are measured from the approximate center. IR spectra were obtained with a Perkin-Elmer Model 297 spectrophotometer. All spectral data for intermediates and products were consistent with the assigned structures. Microanalytical determinations were made by ATX SA Nanterre (France). Analyses indicated by symbols of the elements were within ±0.4% of the theoretical values.

All experiments were conducted under an inert atmosphere.

A. Construction of the Benzophenone Skeleton. 1. By Friedel–Crafts Acylation of Substituted Benzenes with 6-Chloro-3-nitrobenzoyl Chloride. The Friedel–Crafts acylations were performed by standard procedures. To avoid possible side reactions the 6-chloro-3-nitrobenzoyl chloride was always prepared by using the appropriate substituted benzene as solvent. This procedure is exemplified below.

2,4'-Dichloro-5-nitrobenzophenone (39), R^1 = Cl, R^2 = R^3 = H (Scheme I). A mixture of 60 g (0.298 mol) of 6-chloro-3-

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nitrobenzoic acid and 68.3 g (0.328 mol) of PCl_5 in 50 mL of chlorobenzene was stirred for 24 h at room temperature and then for 1 h at 50 °C to complete the reaction. Chlorobenzene was removed under diminished pressure, and the residue was triturated with hexane. The precipitate of the crude acid chloride was filtered off and washed with hexane to afford 55.5 g (85%) of a pale-yellow solid. To a solution of this crude acid chloride (3.12 g; 0.143 mol) in 50 mL of chlorobenzene was slowly added, under ice cooling, 22.9 g of $AlCl_3$. Upon completion of the addition, the mixture was allowed to reach room temperature, and stirring was continued for 24 h. The deep-red solution was then hydrolyzed with HCl and ice, and the resulting aqueous phase was extracted several times with ether. The combined organic extracts were back washed with 2% aqueous NaOH and then water. The organic phase was dried ($MgSO_4$) and the solvent removed in vacuo to give the crude benzophenone, which was recrystallized from 1:1 toluene–isopropyl ether to give 35 g (83%) of pure **39** (R^1 = Cl, R^2 = R^3 = H):[21] mp 95 °C; NMR ($CDCl_3$) δ 8.3 (dd, 1 H, J = 8 Hz, J = 2.6 Hz), 8.26 (br s, 1 H), 7.76 (d, 2 H, J = 8 Hz), 7.48 (d, 2 H, J = 8 Hz).

2,2′-Dichloro-5-nitrobenzophenone (45) (Scheme II). 2-Amino-5-nitro-2-chlorobenzophenone was prepared according to a patented procedure[6] which, in our hands, gave only low yields of the desired product (15–30% instead of the 60% yield claimed) and the final product had to be purified by column chromatography on silica gel (2:1 hexane–EtOAc).

To a mixture of 2.6 g (22 mmol) of isoamyl nitrite and 2.3 g (17 mmol) of $CuCl_2$ in 80 mL of acetonitrile at 65 °C was slowly added 4 g (14 mmol) of the foregoing aminobenzophenone and stirring was continued for 4 h at 65 °C and overnight at room temperature.[7] After the addition of water, the aqueous phase was extracted three times with ether, and the ether extracts were dried (Na_2SO_4) and evaporated in vacuo to leave 4.6 g of a crude yellow solid consisting mainly of the expected product **45** contaminated with a small amount of the deaminated 2-chloro-5-nitrobenzophenone. This compound was used in the next step without further purification.

2. By Reaction of an Organocadmium Derivative with an Acid Chloride (Scheme III). The details of the procedure used for the preparation of the *m*-chloro derivative **13** are described here. The same procedure was utilized for the *m*-methyl and *m*-trifluoromethyl derivatives **14** and **15**, respectively.

2-(4-Methyl-1-piperidinyl)-5-nitrobenzoyl Chloride (48). A mixture of 40.3 g (0.2 mol) of 2-chloro-5-nitrobenzoic acid and 39.6 g (0.22 mol) of 4-methylpiperidine was refluxed for 2 h and then hydrolyzed with dilute HCl. Extraction of the aqueous phase with CH_2Cl_2 followed by washing of the combined extracts with water, drying ($MgSO_4$), and evaporation of the solvent in vacuo afforded the crude acid **47** which was recrystallized from ethanol (38 g; 72%): mp 160 °C (lit.[22] mp 155 °C); NMR ($CDCl_3$) δ 9.1 (d, 1 H, J = 2.6 Hz), 8.4 (dd, 1 H, J = Hz), 7.6 (d, 1 H, J = 8 Hz), 3.2–2.9 (m, 4 H), 2–1.5 (m, 5 H), 1.08 (d, 3 H, J = 5.3 Hz). This acid (26.4 g, 0.1 mol) was heated with 21.9 g (0.109 mol) of PCl_5 in 70 mL of anhydrous benzene for 5 h at 60 °C. The solvent was then removed leaving a dark oil that crystallized upon treatment with hexane. Recrystallization from 1:1 benzene–hexane gave 15.4 g (55%) of **48** as light-brown crystals.

3-Chloro-5′-nitro-2′-(4-methyl-1-piperidinyl)benzophenone (50). Freshly cleaned magnesium turnings (0.96 g, 40 mmol) and a crystal of iodine were heated under reflux in 10 mL of anhydrous ether. A solution of 7.66 g (40 mmol) of 3-bromochlorobenzene in 30 mL of ether was then added dropwise and the mixture kept under reflux for 1.5 h. The mixture was cooled (ice bath) to 0 °C and 3 g (16 mmol) of $CdCl_2$ was added portionwise. After 2 h at room temperature, the ether was distilled off and replaced by 60 mL of anhydrous benzene. The acid chloride **48** (4 g, 14 mmol) in 50 mL of benzene was then added slowly at 0 °C. The mixture, which turned deep red, was stirred overnight and allowed to attain room temperature. Treatment with saturated aqueous NH_4Cl followed by several extractions with EtOAc, backwashing, drying of the organic phase, and removal of the solvent under vacuo gave 3.2 g (64%) of an orange-red oil which was purified by filtration through silica gel with toluene to afford the nitrobenzophenone **50** as orange crystals: mp 90 °C; NMR ($CDCl_3$) δ 8.27 (m, 2 H), 7.7–7.4 (m, 4 H), 7.0 (m, 1 H), 3.4 (m, 2 H), 2.8 (m, H), 1.5 (m, 3 H), 0.9 (m, 2 H), 0.82 (d, 3 H, J = 5 Hz). Anal. ($C_{19}H_{19}ClN_2O_3$) C, H, N, Cl.

B. Introduction of the Piperidine Ring. The most efficient procedure is detailed for the aromatic substitution of 2,4-dichloro-5-nitrobenzophenone **39** (R^1 = Cl) with 4-methylpiperidine, leading to compound **16** (Scheme I).

4-Chloro-5′-nitro-2′-(4-methyl-1-piperidinyl)benzophenone (16). 2,4-Dichloro-5-nitrobenzophenone (**39**) R^1 = Cl, R^2 = R^3 = H, 20.7 g, 70 mmol), 11.3 mL (100 mmol) of 4-methylpiperidine, and 9.6 g (70 mmol) of K_2CO_3 were heated under reflux in 150 mL of absolute EtOH for 2 h. The product crystallized on cooling, followed by refrigeration overnight at 0 °C. The orange-red crystals of **16** were collected by filtration (21.7 g, 86%): mp 137 °C; NMR ($CDCl_3$) δ 8.26 (m, 2 H, J = 9.3, J = 2.6 Hz), 7.75 (br d, 2 H, J = 8 Hz), 7.45 (br d, 2 H, J = 8 Hz), 7.0 (d, 1 H, J = 9.3 Hz), 3.4 (m, 2 H), 2.8 (m, 2 H), 1.45 (m, 3 H), 0.83 (m, 5 H, J = 5.3 Hz). Anal. ($C_{19}H_{19}ClN_2O_3$) C, H, N, Cl.

C. Preparation of the Piperidines Not Commercially Available (Scheme V). The 4-ethyl- and 4-*tert*-butylpiperidines (**52** and **53**, respectively) are known compounds and were obtained by catalytic hydrogenation of the corresponding pyridines in acetic acid by a slight modification of a procedure already described;[9] the pyridines were hydrogenated with 5% palladium on charcoal at 100 °C in acetic acid. Upon completion of the reaction, the catalyst was removed by filtration trough Celite, and the acetic acid was removed by distillation. The crude piperidine acetates were used without further purification in the aromatic substitution step.

The 4-butylpiperidine (**55**) was synthesized as follows: 1.8 g (76.4 mmol) of NaH was added to 250 mL of Me_2SO. After stirring for 20 min at room temperature, the solution was cooled to 10 °C and 30.5 g (76.4 mmol) of butyltriphenylphosphonium bromide added. Stirring was continued for 5 h while the mixture was allowed to warm to room temperature. The solution was again cooled at 10 °C before addition of 12 g (63.7 mmol) of *N*-benzyl-4-piperidinone (**54**). After stirring overnight at room temperature, the mixture was poured into ice and extracted with ether. The organic phase was washed with water and dried ($MgSO_4$) and the solvent evaporated under diminished pressure. Triphenylphosphine oxide was precipitated by the addition of cold pentane and removed by filtration. Finally the pure N-benzylated unsaturated piperidine was obtained (10.6 g; 73%) by distillation (bp 118–120 °C, 0.5 mmHg). This compound was then submitted to catalytic hydrogenation. In a 500-mL round-bottomed flask were introduced 10.6 g of the piperidine derivative, 2.2 g of 5% palladium on charcoal, and 100 mL of acetic acid. Hydrogen was then introduced and the mixture heated to 100 °C. After stirring for 12 h, the mixture was filtered through Celite and the solvent removed in vacuo. Distillation under slightly diminished pressure afforded 10.5 g of pure 4-butylpiperidine (**55**).

D. Reduction of the Nitro Group. All of the following reductions were performed either with Fe/HCl or with $SnCl_2$, HCl, although the former was usually preferred. As an example, the preparation of compound **5** (LF 1695) is described.

5′-Amino-4-chloro-2′-(4-methyl-1-piperidinyl)benzophenone (5). The nitro percursor **16** (7.2 g, 20 mmol), 11.2 g of iron powder, and 7 mL of 1 N HCl in 80 mL of absolute EtOH were boiled under reflux for 15 h. The mixture was then hydrolyzed with dilute aqueous $NaHCO_3$ and the aqueous phase filtered through Celite and extracted with EtOAc. The organic extracts were washed with water and dried ($NaSO_4$) and the solvent removed in vacuo, leaving an oil that crystallized upon standing. Recrystallization from hexane gave 4.3 g (65%) of **5** as yellow crystals: mp 89 °C; NMR ($CDCl_3$) δ 7.7 (br d, 2 H, J = 8 Hz), 7.35 (br d, 2 H, J = 8 Hz), 6.9 (m, 3 H, J = 8, J = 2.6 Hz), 3.58 (2 H, NH_2), 2.8 (m, 2 H), 2.5 (br t, 2 H), 1.37 (m, 2 H), 0.7 (m, 5 H, J = 5.3 Hz). Anal. ($C_{19}H_{21}ClN_2O$) C, H, N, Cl.

E. Transformation of the Amino Group. 1. Replacement by Hydrogen (Compounds 18, 19, and 20). The general procedure is described for the preparation of **18**.

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4-Chloro-2'-(4-methyl-1-piperidinyl)benzophenone (18). The aminobenzophenone **5** (6.9 g, 21 mmol) was dissolved in 80 mL of absolute EtOH. To the resulting solution were added, under ice cooling, first 25 mL of concentrated H_2SO_4 and then 2.8 g of sodium nitrite. The mixture was allowed to warm up to room temperature while under stirring for 1 h. Addition of activated copper then induced gas evolution. After heating at 60 °C for one additional hour to complete the reaction, the solution was neutralized with 4% NaOH and the aqueous phase extracted with EtOAc. Conventional processing gave 4.4 g (68%) of crude product which was chromatographed over silica gel to afford pure compound **18**: mp 69 °C; NMR ($CDCl_3$) δ 7.9 (m, 2 H), 7.7 (m, 2 H), 7.4–7.1 (m, 4 H), 3.19 (m, 2 H), 2.67 (m, 2 H), 1.5 (m, 3 H), 0.78 (m, 5 H). Anal. ($C_{19}H_{20}NOCl$) C, H, N.

4-Methoxy-2'-(4-methyl-1-piperidinyl)benzophenone (19). An alternative synthesis of **19** was performed as follows: 2-fluoro-4-methoxybenzopheonone[23] (**51**, 7 g, 30 mmol) was treated with 3.3 g (33 mmol) of 4-methylpiperidine and 4.1 g of K_2CO_3 in refluxing DMF for 30 h. Dilution with water, extraction with CH_2Cl_2, backwashing and drying of the organic phase, and evaporation of the solvent gave 9 g of crude product. Column chromatography on silica gel (hexane–EtOAc) afforded pure **19** as an oil: NMR ($CDCl_3$) δ 7.8 (br d, 2 H, J = 8 Hz), 7.35–6.83 (m, 6 H), 3.85 (s, 3 H), 3.15 (m, 2 H), 2.6 (broad t, 2 H), 1.34 (m, 3 H), 0.75 (m, 5 H, J = 5.3 Hz). Anal. ($C_{20}H_{23}NO_2$) C, H, N.

2. Replacement by Chlorine. 4,5'-Dichloro-2'-(4-methyl-1-piperidinyl)benzophenone (17). The aminobenzophenone **5** (9.9 g, 30 mmol) was dissolved in 50 mL of HBF_4.[24] To the resulting solution, cooled in an ice bath, was added dropwise a solution of 2.1 g (30 mmol) of $NaNO_2$ in 20 mL of water. After stirring for 0.5 h the precipitate was filtered off and washed successively with HBF_4, alcohol, and ether, affording 8.7 g (68%) of the crude salt. This product was taken up in Me_2SO and slowly added to a solution of 26.9 g (0.2 mL) of $CuCl_2$ in 100 mL of Me_2SO. The reaction was monitored by TLC and found to be complete after 0.5 h. The mixture was poured into water and the aqueous phase extracted with EtOAc. Conventional processing gave 3 g (43%) of the chloro compound **17** contaminated with a small amount of **18**. Column chromatography on silica gel with benzene as eluant was necessary for the purification of **17**: mp 109 °C; NMR ($CDCl_3$) δ 7.7 (br d, 2 H, J = 8 Hz), 7.36 (m, 4 H), 6.98 (br d, 1 H, J = 8 Hz), 3.0 (m, 2 H), 2.6 (br t, 2 H) 1.33 (m, 4 H), 0.76 (m, 4 H, J = 5.3 Hz). Anal. ($C_{19}H_{19}Cl_2NO$) C, H, N.

3. Monomethylation of the Amine. 4-Chloro-2'-(4-methyl-1-piperidinyl)-5'-[(benzyloxycarbonyl)amino]benzophenone (22). A mixture of 6.6 g (20 mmol) of **5**, 3 g of K_2CO_3, and 6.9 g (40 mmol) of benzyl chloroformate in 60 mL of toluene was stirred for 2 h at room temperature, where upon the solution was transferred to a separatory funnel and washed with water. Removal of the volatile material under diminished pressure gave 9.0 g of a product that crystallized on standing. The pure urethane **22** (6.6 g; 71%) was obtained by recrystallization from isopropyl alcohol: mp 159 °C; NMR ($CDCl_3$) δ 7.7 (br 2 H, J = 8 Hz), 7.37 (m, 8 H), 6.95 (m, 2 H + NH), 5.2 (s, 2 H), 2.94 (m, 2 H), 2.55 (br t, 2 H), 1.4 (m, 3 H), 0.73 (m, 5 H, J = 5.3 Hz). Anal. ($C_{27}H_{27}ClN_2O_3$) C, H, N.

4-Chloro-5'-(methylamino)-2'-(4-methyl-1-piperidinyl)-benzophenone (24). Compound **22** (5.5 g, 12 mmol) was added portionwise to a suspension of 0.3 g of NaH in 50 mL of dry THF at 0 °C. After stirring for 0.5 h a solution of 1 mL of CH_3I in 10 mL of THF was introduced dropwise, the temperature being kept at 0 °C during the addition. The ice bath was then removed and stirring continued overnight at room temperature. Standard workup gave 5 g (87%) of **23** which, after recrystallization had mp 117 °C; NMR ($CDCl_3$) δ 7.7 (br d, 2 H, J = 8 Hz), 7.3 (m, 9 H), 7.0 (br d, 1 H, J = 8 Hz), 5.15 (s, 2 H), 3.3 (s, 3 H), 3.1 (m, 2 H), 2.6 (br t, 2 H), 1.3 (m, 3 H), 0.7 (m, 5 H, J = 5.3 Hz). Anal. ($C_{28}H_{29}ClN_2O_3$) C, H, N.

The methylated urethane **23** (4.8 g, 10 mmol) was dissolved in a mixture of 25 mL of 5 N HCl in EtOH and the solution was boiled under reflux for 5 h. The alcohol was distilled off and the aqueous phase extracted with EtOAc brought to pH 9 with NaOH and extracted again with EtOAc. An oily residue (3 g) that crystallized on standing was obtained after drying of the organic phase and evaporation of the solvent in vacuo. Recrystallization from hexane gave 2.5 g (73%) of the *N*-methyl derivative **24**: mp 117 °C; NMR ($CDCl_3$) δ 7.7 (br d, 2 H, J = 8 Hz), 7.34 (br d, 2 H, J = 8 Hz), 7.04 (br d, 1 H, J = 8 Hz), 6.7 (m, 2 H), 3.5 (NH), 2.8 (s, 3 H + m, 2 H), 2.5 (br t, 2 H), 1.4 (m, 3 H), 0.75 (m, 5 H, J = 5.3 Hz). Anal. ($C_{20}H_{23}ClN_2OCl$) C, H, N.

4. Dimethylation of the Amine. 4-Chloro-5'-(dimethylamino)-2'-(4-methyl-1-piperidinyl)benzophenone (25). To a solution of 6.6 g (20 mmol) of the amine in 80 mL of DMF preheated at 80 °C was added dropwise a mixture of 9 mL of HCHO (30%) and 9 mL of HCO_2H (90%). After stirring for 2.5 h at 80 °C, the chilled reaction mixture was poured into aqueous $NaHCO_3$ and the aqueous phase was extracted with EtOAc. The organic extract was washed with water until the pH reached 7 and dried (Na_2SO_4) and the solvent removed under diminished pressure, leaving a black oily residue that was purified by column chromatography followed by recrystallization from hexane to give 2.1 g (30%) of **25**: mp 99.5 °C; NMR ($CDCl_3$) δ 7.7 (br d, 2 H, J = 8 Hz), 7.3 (br d, 2 H, J = 8 Hz), 6.9 (m, 3 H), 2.9 (s, 6 H + m, 2 H), 2.5 (br t, 2 H), 1.34 (m, 3 H), 0.7 (m, 5 H, J = 5.3 Hz). Anal. ($C_{21}H_{25}ClN_2O$) C, H, N.

Biology. Splenocytes from 6–9 weeks old femalemDBA/2 mice were obtained by gently teasing spleen tissue through a 30 gauge mesh, in hanks balanced salt solution (HBSS). Red blood cells present in the preparation were lysed by adding cold NH_4Cl (0.17 M) on a cell pellet and incubating the cells for 10 min at 4 °C. After two washings with HBSS, the cells were suspended in RPMI 1640 medium (GIBCO, UK) supplemented with 5% fetal calf serum at a concentration of 5×10^6 cells/mL. The spleen cell suspension (100 μL) was introduced into each well of microtiter plates. The test substances were first added in 25 μL of culture medium. In each experiment, the test compounds were evaluated for their proliferative activity over a concentration range of 5–80 μg/mL. Lectins were then added in the same volume [Concanavalin A (Con A) and Pokewed Mitogen (PWM) were purchased from I.B.F. (France) and phytohemagglutinin (PHA) from Sigma]. The concentration of lectins in the culture medium was adjusted for each batch in order to give an optimum proliferative response. Cells were cultured in an incubator for 72 h at 37 °C in a water-saturated, 5% CO_2 in air, atmosphere. At 24 h before cell harvesting 1 μCi of [6-^{3}H]thymidine (specific activity 5 Ci/mmol, Amersham, UK) was added to each well. Cells were harvested on glass-fiber paper by using a multiple-sample harvester, and the incorporated radioactivity was evaluated by scintillation counting by using the standard procedure.

Each measurement was made in triplicate. A nonstimulated culture (no lectins added) was run in parallel for each concentration of test substance. None of the compounds tested exhibited any significant intrinsic activity (results not shown). Stimulated indexes were calculated as the ratio of maximal radioactive incorporation in the presence of the test compound/corresponding control.

1. In Vivo Induction of Thy1$^+$ Antigen Expression on Murine Bone Marrow Cells. A modified Komuro–Boyse assay was used:[25] briefly, bone marrow cells from C57 BL/6 female mice, 5–8 weeks old were separated on a discontinuous bovine serum albumin (BSA) gradient.[26] Fractions A and B, corresponding, respectively, to the lowest and next lowest density fraction were collected and resuspended in HBSS supplemented with 5% BSA (Path-O-Cyte, Miles) at a concentration of 10^6 cells per mL. Culture was initiated in sterile plastic tubes containing 1 mL of cell suspension and one concentration of the tested compounds(1 Thy1$^+$ antigen expression was evaluated by a direct staining method with monoclonal anti Thy1$^+$ conjugated to FITC (Miles) and an epifluorescent microscope (Nikon Fluophot, Japan). The percentage of Thy1$^+$ positive cells was calculated for each concentration level and in a control culture.

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Thymosin fraction V was used as a positive control and was prepared according to Goldstein.[5]

2. In Vivo Induction of Thy1$^+$ Antigen Expression in nu/nu Mice Spleen Cells. nu/nu female mice, 6-9 weeks old, were treated by gastric tubing with the test compounds by using 0.2 mL of 2% (w/v) arabic gum containing 0.1% (v/v) Tween-80 as vehicle, and 24 h later the spleens were removed and spleen cells were prepared as already described. The percentage of Thy1$^+$ positive cells was evaluated as already described in treated and control (vehicle only) animals.

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Registry No. 1, 86188-04-7; 1 (nitro derivative), 113456-79-4; 2, 86187-96-4; 2 (nitro derivative), 113456-74-9; 3, 86187-98-6; 3 (nitro derivative), 132439-22-6; 4, 86188-07-0; 4 (nitro derivative), 113456-81-8; 5, 86187-86-2; 6, 86188-02-5; 6 (nitro derivative), 113456-78-3; 7, 86187-97-5; 7 (nitro derivative), 113456-75-0; 8, 86187-95-3; 8 (nitro derivative), 132439-23-7; 9, 86188-05-8; 9 (nitro derivative), 113456-80-7; 10, 86188-14-9; 10 (nitro derivative), 113456-84-1; 11, 86188-06-9; 11 (nitro derivative), 132439-24-8; 12, 86187-94-2; 13, 132439-15-7; 14, 132439-16-8; 15, 132439-17-9; 16, 86187-85-1; 17, 86187-90-8; 18, 86188-01-4; 19, 86188-03-6; 20, 86187-88-4; 21, 86187-87-3; 22, 86187-91-9; 23, 86187-92-0; 24, 86187-89-5; 25, 132439-18-0; 26, 86202-31-5; 27, 86250-95-5; 28, 86188-00-3; 29, 132439-19-1; 30, 86188-08-1; 31, 86188-09-2; 32, 86188-10-5; 33, 86188-11-6; 34, 86188-12-7; 35, 86188-13-8; 36, 2516-96-3; 37, 25784-91-2; 38 (R^1 = 4-Cl, R^2 = R^3 = H), 108-90-7; 38 (R^1 = R^2 = R^3 = H), 71-43-2; 38 (R^1 = 4-CH_3, R^2 = R^3 = H), 108-88-3; 38 (R^1 = 4-F, R^2 = R^3 = H), 462-06-6; 38 (R^1 = 4-Br, R^2 = R^3 = H), 108-86-1; 38 (R^1 = 4-OCH_3, R^2 = R^3 = H), 100-66-3; 38 (R^1 = 2-CH_3, R^2 = 4-CH_3, R^3 = H), 108-38-3; 38 (R^1 = 3-OCH_3, R^2 = 4-OCH_3, R^3 = H), 91-16-7; 38 (R^1 = 3-Cl, R^2 = 4-Cl, R^3 = H), 95-50-1; 38 (R^1 = 2-Cl, R^2 = 4-Cl, R^3 = H), 541-73-1; 38 (R^1 = 3-CH_3, R^2 = 4-CH_3, R^3 = H), 95-47-6; 39 (R^1 = 4-Cl, R^2 = R^3 = H), 70132-91-1; 40, 626-58-4; 42, 55501-45-6; 43, 609-65-4; 44, 2011-66-7; 45, 54534-72-4; 46, 113456-71-6; 47, 78243-27-3; 48, 132439-20-4; 50 (R = Cl), 113456-93-2; 51, 66938-29-2; 52 (R = C_2H_5), 3230-23-7; 53 (R = *t*-Bu), 1882-42-4; 54, 3612-20-2; 55 (R = *n*-propyl), 24152-39-4; ClC_6H_4-3-Br, 108-37-2; $CH_3C_6H_4$-3-Br, 591-17-3; $CF_3C_6H_4$-3-Br, 401-78-5; *N*-benzyl-4-butylidenepiperidine, 132439-21-5; benzyl chloroformate, 501-53-1; 4-*tert*-butylpyridine, 3978-81-2; butyltriphenylphosphonium bromide, 1779-51-7; 2-chloro-5-nitrobenzophenone, 34052-37-4; 4-ethylpyridine, 536-75-4; formaldehyde, 50-00-0.

DNA-Directed Alkylating Agents. 4. 4-Anilinoquinoline-Based Minor Groove Directed Aniline Mustards

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A series of 4-anilinoquinoline-linked aniline mustards of widely varying mustard reactivity were prepared and evaluated for their antitumor activity. The compounds were designed as minor groove binding agents, where the aniline mustard ring is itself part of the DNA-binding ligand. While there was a general trend for cytotoxicity to correlate with mustard reactivity, this was much less pronounced than with untargeted mustards. The compounds were much more cytotoxic than the parent diols, and were also at least 10-fold more cytotoxic than the corresponding aniline mustards themselves. Comparative cell line studies suggested that the mechanism of cytotoxicity varied with mustard reactivity. The most reactive mustards cross-linked DNA, while cell killing by the less reactive compounds appeared to be by the formation of bulky monoadducts. The compounds were active but not particularly dose-potent against P388 leukemia in vivo. The modest potency may be related to their poor aqueous solubility, since the more soluble methyl quaternary salts were equally active at much lower doses.

The majority of alkylating antitumor drugs, including the clinically used compounds chlorambucil, melphalan, and cyclophosphamide, alkylate DNA primarily at the N-7 position of guanine, in a reaction dominated by the molecular electrostatic potential of the DNA site.[1] Thus, reaction occurs on DNA preferentially in the middle of runs of guanines,[2] where this potential is at its most negative.[3] In terms of chemotherapy, there are major drawbacks with such a "bonding-dominated"[4] mechanism of alkylation. The sequence specificity of these compounds is limited, and the electrophilicity of the drug needs to be high, leading to rapid loss by reaction with other (non-DNA) cell nucleophiles[5] and thus low potency.

There has been recent interest in overcoming these deficiencies by the use of DNA-targeted alkylating agents,[6,7] using DNA-intercalating carriers to increase the "binding component" of the DNA interaction. We have recently shown[5] that a series of acridine-carried aniline mustards of carefully varied reactivity (1) are both more potent in vitro and more active in vivo than the untargeted mustards themselves. In the latter study there was also some evidence that DNA targeting permitted the use of less reactive alkylating moieties. However, the binding selectivity of DNA intercalators is low,[6] and a more promising approach to highly potent sequence-selective alkylators appears to be the use of minor groove binding structures as carriers. Compounds such as the tris(pyrrolecarboxamide) (2) show highly AT-specific reversible

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