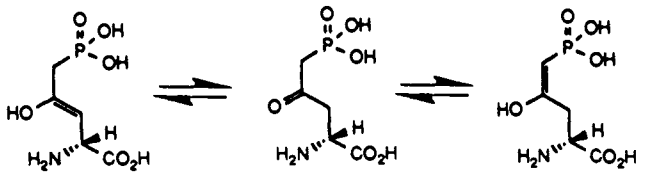

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In conclusion, this β-ketophosphonate represents an important new example of an NMDA receptor glutamate antagonist, combining good binding affinities with effective in vivo activity.

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Articles

Antiarthritic and Suppressor Cell Inducing Activity of Azaspiranes: Structure–Function Relationships of a Novel Class of Immunomodulatory Agents

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Spirogermanium (1; 8,8-diethyl-*N*,*N*-dimethyl-2-aza-8-germaspiro[4.5]decane-2-propanamine dihydrochloride) is a potent cytotoxic agent in vitro which has demonstrated limited activity in experimental animal tumor models. Subsequently, it has been reported that spirogermanium has antiarthritic and suppressor cell-inducing activity. We have synthesized a series of substituted 8-hetero-2-azaspiro[4.5]decane and 9-hetero-3-azaspiro[5.5]undecane analogues of spirogermanium to identify the heteroatom requirements for in vivo antiarthritic and suppressor cell-inducing activity. This structure–activity relationship study has identified that appropriately substituted silicon and carbon analogues of spirogermanium retain both antiarthritic and immunosuppressive activity, with the 8,8-dipropyl (carbon) analogue being among the most active. Following the identification of *N*,*N*-dimethyl-8,8-dipropyl-2-azaspiro[4.5]decane-2-propanamine dihydrochloride (**9**) as a more active analogue than spirogermanium, a series of 8,8-dipropyl analogues with various amine substituents were synthesized. A number of these analogues had activity similar to that of **9**. A correlation between activity in the adjuvant arthritic rat and the ability to induce suppressor cells ($r = 0.894$, $p < 0.001$) suggests an association between the two pharmacologic effects. While the precise biochemical mechanism(s) for the pharmacological activity is unclear, these data suggest that compounds within this series, e.g., *N*,*N*-dimethyl-8,8-dipropyl-2-azaspiro[4.5]decane-2-propanamine dihydrochloride, may provide effective therapy in diseases of autoimmune origin and/or the prevention of rejection in tissue transplantation.

Introduction

The demonstration of disorders of suppressor cells in animal models of autoimmune disease[1-4] and in patients with autoimmunity[5-9] suggests that their regulation may be a rational approach for immunotherapy. If the loss of suppressor cells in patients with autoimmunity is an important link in the causation of the disease(s), therapy directed toward augmenting suppressor mechanisms may be effective in treating the manifestations of autoimmune diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus, autoimmune diabetes) and rejection following tissue/organ transplantation. Suppressor cells are introduced by most, if not all, forms of immunogenic challenge and can be antigen-specific T cells, as first described by Gershon and Kondo,[10] or they can be nonspecific in nature and of various phenotypes.[11-14]

Suppressor cells can also be induced by a wide variety of compounds and treatments, one of the most notable of which is cyclosporin A (for review see[15]), a fungal metab-

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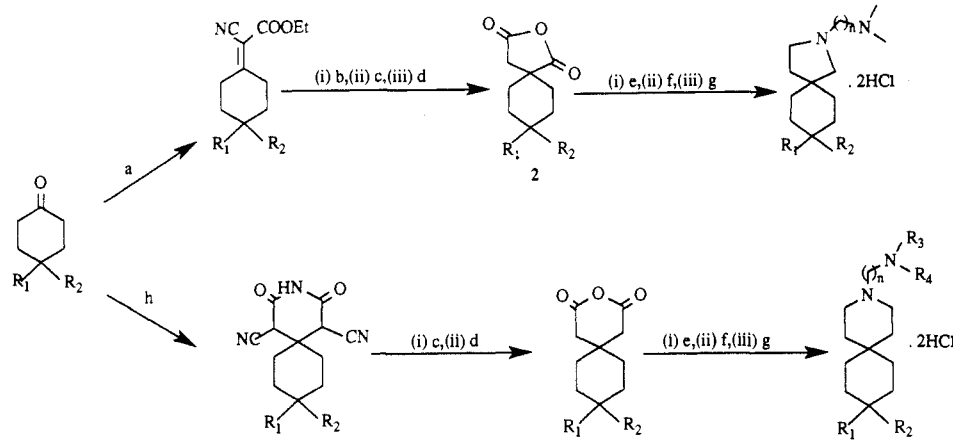
Scheme I[a,b]

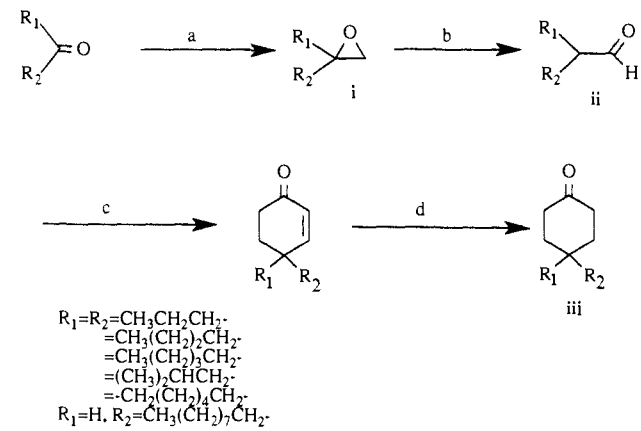


[a] Reagents: (a) EtO_2CCH_2CN, HOAc, NH_4OAc, toluene, (b) KCN, EtOH, H_2O; (c) HCl, HOAc, H_2O; (d) Ac_2O; (e) $H_2N(CH_2)_nNR_3R_4$,[c] toluene, $-H_2O$; (f) $LiAlH_4$, Et_2O; (g) HCl, EtOH; (h) EtO_2CCH_2CN, NH_3, EtOH. [b] R_1, R_2, R_3, R_4 substituents and n are as referred to in Table I. [c] For the synthesis of analogue **34** $H_2NCH_2CH_2CN$ was used.

olite which has found widespread use as an immunosuppressive agent in human organ transplantation[16] and is currently being evaluated as a treatment for autoimmune disease.[17] The immunosuppressive effects of cyclosporin A are thought to result, in part, from the generation of antigen specific suppressor cells via an inhibition of a regulatory subset of T lymphocytes.[18-20] However, the use of cyclosporin A, both in organ transplantation and autoimmune disease, is limited by the presence of significant nephrotoxicity.[21] Another example of an immunosuppressive therapeutic regimen that results in the induction of suppressor cells is total lymphoid irradiation (TLI), originally used for the treatment of Hodgkin's disease.[22] The precise cellular/molecular mechanism(s) responsible for the induction of suppressor cells by TLI has not been defined. The cells have been classified as "natural" suppressor cells as they do not have the characteristics of mature T cells, B cells, or macrophages and resemble the suppressor cells which have been described as occurring in neonatal spleen.[14] TLI has been evaluated in patients with refractory rheumatoid arthritis and lupus nephritis[23-26] and recent reports suggest that a significant number of these patients have demonstrated considerable improvement in their joint disease.

We have previously demonstrated that spirogermanium (1; 8,8-diethyl-*N*,*N*-dimethyl-2-aza-8-germaspiro[4.5]de-

Scheme II[a]



[a] Reagents: (a) $((CH_3)_3SO)^+I^-$, NaH, DMSO; (b) BF_3OEt_2, benzene; (c) methyl vinyl ketone, H_2SO_4 (cat.), benzene; (d) H_2, 10% Pd/C, EtOAc.

cane-2-propanamine dihydrochloride) induces a population(s) of suppressor cells characteristic of the "natural" suppressor cell induced by TLI and inhibits the hind paw inflammatory lesions of adjuvant arthritic rats.[27-30] It has also been demonstrated that this compound suppresses experimental autoimmune encephalomyelitis in the Lewis rat.[30,31] Spirogermanium (1) is a member of a class of azaspirane compounds first synthesized and characterized by Rice et al.[32] and which have been reported to possess a wide range of other pharmacological activities (including cardiovascular, ganglionic blockage, cytotoxic, antineoplastic, and antimalarial).[33-37] The structure-activity

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relationship (SAR) pertaining to their immunosuppressive and antiarthritic properties is not known. Toward this end we have synthesized a variety of azaspirane congeners and evaluated them for immunomodulatory activity in rat models of adjuvant-induced arthritis and suppressor cell induction. The chemistry and immunomodulatory activities of 43 azaspirane analogues are described.

Chemistry

The synthesis of substituted 2-azaspiro[4.5]decanes and 3-azaspiro[5.5]undecanes from appropriately substituted cyclohexanones has been described by Rice et al.[38] and is outlined in Scheme I. A number of procedural modifications were developed to improve the yields of each step and are described in the Experimental Section. All analogues synthesized are listed in Table I.

Access to noncommercially available 4,4-dialkylcyclohexanone intermediates required for the synthesis of both 8,8-dialkyl-2-azaspiro[4.5]decanes and 9,9-dialkyl-3-azaspiro[5.5]undecanes was less straightforward. Scheme II outlines the synthetic pathway used to synthesize these intermediates. α-Alkylaldehydes (ii) were required for condensation with methyl vinyl ketone to give 4,4-disubstituted cyclohexenones. These α-alkylaldehydes were synthesized by a two-step procedure (Scheme II). Initial Corey epoxidation[39] of commercially available ketones gave the desired epoxides (i). Boron trifluoride catalyzed rearrangement of these epoxides yielded the desired α-alkylaldehydes (ii). By the method of Flaugh et al.[40] for the acid-catalyzed annelation of α-alkyl aldehydes and α,β-unsaturated ketones, 4,4-disubstituted cyclohexanones were obtained in good yields following hydrogenation (65–75% overall yield).

The 4,4-dimethyl- and 4,4-diethyl-4-silacylcohexanones, intermediates in the syntheses of silaazaspiranes **4** and **5**, respectively, were synthesized by the method of Soderquist et al.[41] 8-Alkyl-2-azaspiro[4.5]decane analogues **13**, **14**, and **16** were isolated and tested as a mixture of isomers.[42] Analogues **13** and **16** were prepared from commercially available 4-*tert*-butylcyclohexanone and 4-phenylcyclohexanone, respectively. Analogue **14** was synthesized from 4-decylcyclohexanone which was prepared as in Scheme II from dodecanal.

Reduction of tertiary amine imide intermediates with $LiAlH_4$ proceeded smoothly to give the desired diamines in 95% yield. However $LiAlH_4$ reduction of the nitrile imide and the secondary amine imide to the desired diamines, required for the syntheses of analogues **34** and **35**, respectively, were contaminated with cyclic amine byproducts. These impurities were probably produced by intramolecular cyclization of an amine on an imine intermediate. The desired diamines were purified by chromatography.

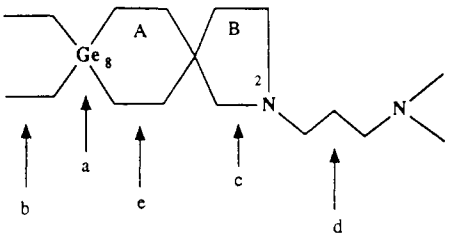

Figure 1. Structural modifications of the spirogermanium nucleus.

All azaspirane derivatives were amorphous stable solids. Cold-temperature high-field proton NMR of **9** indicates that the carbon derivatives, like spirogermanium itself, are conformationally mobile (unpublished results).

Pharmacological Testing

The relative activities of azaspirane analogues following oral administration were determined with respect to spirogermanium (**1**) in both the adjuvant arthritic rat and suppressor cell induction assays. In the adjuvant arthritic (AA) rat model, analogues were initially dosed at 30 mg/kg from day 0 (adjuvant administration) to day 10. At this dose spirogermanium has optimal activity against immune-mediated inflammation of the right hind paw in Lewis rats. If compounds were toxic at this dose they were reevaluated at 15 and 7.5 mg/kg; compounds inactive at 30 mg/kg were reevaluated at 60 and 90 mg/kg when adequate supplies were available. Suppressor cell induction was determined in normal rats by a splenic cell coculture assay following administration of the azaspirane analogue for 11 days at the optimal dose found in the adjuvant arthritic rat assay (see the Experimental Section for details). The results are presented in Table I.

Structure–Activity Relationship

Five areas on the spirogermanium nucleus were selected to develop a structure–activity relationship (Figure 1): (a) heteratom variation at position 8, (b) alkyl substitution at position 8, (c) B-ring size, (d) (*N*,*N*-dialkylamino)alkyl variation, and (e) A-ring modification.

It was initially found that sulfur analogue **6** was inactive but that the 8,8-diethylcarbon analogue **3** and the 8,8-diethylsilicon analogue **4** respectively had 57% and 92% of the activity of spirogermanium at an equivalent dose (30 mg/kg) in the adjuvant arthritic rat model. As a result of the activity of **3** and the synthetic facility of carbon analogues, all subsequent structural modifications were done with carbon at position 8.

The nature of the alkyl substitution at C-8 of azaspiro[4.5]decane-2-propanamine analogues (e.g. **3** and **7–16**) had a dramatic effect on activity and potency in the adjuvant arthritic rat model. A minimum of four carbons substituted at C-8 was required for activity. The 8,8-dipropyl analogue **9** was more active than spirogermanium, demonstrating optimal adjuvant arthritic rat activity of the analogues in this series. Increasing the chain length on 8,8-disubstituted analogues greater than propyl led to increased toxicity with decreased efficacy at the maximally tolerated doses (e.g. **10** and **11**). Analogues **9–12** also demonstrated suppressor cell induction greater than spi-

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(42) The isomeric mixture arose during the addition of cyanide to the cyano ester as outlined below:

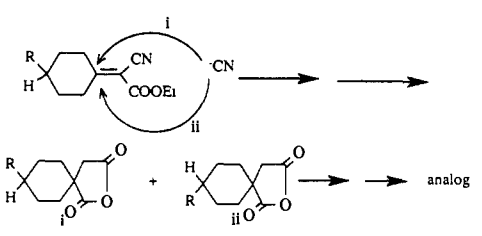
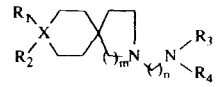
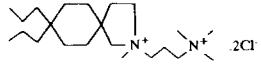
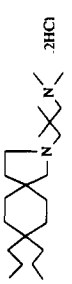
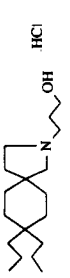
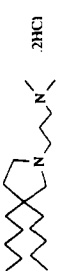
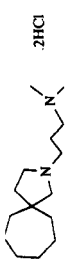
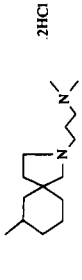
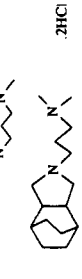
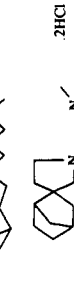


Table I. Physical and Biological Data of Azaspirane Analogues[a]



no.	X	m	n	R_1	R_2	R_3	R_4	mp, °C	formula	analysis[b]	dose,[c] mg/kg	surv[d]	AA[e,l]	SC[f,l] AUC
1	Ge	1	3	CH_3CH_2	CH_3CH_2	CH_3	CH_3				60	0/7	–	
											30	7/7	active*	100*
											15	7/7	inactive	ND[g]
3	C	1	3	CH_3CH_2	CH_3CH_2	CH_3	CH_3	298–299	$C_{18}H_{36}N_2 \cdot 2HCl$	C, H, N, Cl	90	0/7	–	
											30	7/7	0.57*	88*
											10	7/7	0.10	ND
4	Si	1	3	CH_3CH_2	CH_3CH_2	CH_3	CH_3	300–302	$C_{17}H_{36}N_2Si \cdot 2HCl$	C, H, N, Cl	30	8/8	0.92*	ND
											15	8/8	0.27	63*
5	Si	1	3	CH_3	CH_3	CH_3	CH_3	310–311	$C_{15}H_{32}N_2Si \cdot 2HCl$	C, H, N, Cl[h]	30	8/8	0	0
6	S	1	3			CH_3	CH_3	276–277	$C_{13}H_{26}N_2S \cdot 2HCl$	C, H, N, Cl	25	7/7	0	ND
7	C	1	3	H	H	CH_3	CH_3	276–278	$C_{14}H_{28}N_2 \cdot 2HCl$	C, H, N, Cl[i]	30	8/8	0.27	22 NS[g]
8	C	1	3	CH_3	CH_3	CH_3	CH_3	316–317	$C_{16}H_{32}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.12	0
9	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	299–301	$C_{20}H_{40}N_2 \cdot 2HCl$	C, H, N, Cl	60	0/6	–	
											30	8/8	1.35*	172*
											15	8/8	0.18	ND
10	C	1	3	$CH_3(CH_2)_3$	$CH_3(CH_2)_3$	CH_3	CH_3	299–301	$C_{22}H_{44}N_2 \cdot 2HCl$	C, H, N	30	0/8	–	
											15	2/8	–	
											7.5	8/8	0.41*	118*
12	C	1	3	$(CH_3)_2CHCH_2$	$(CH_3)_2CHCH_2$	CH_3	CH_3	297–298	$C_{22}H_{44}N_2 \cdot 2HCl$	C, H, N, Cl	30	7/8	0.97*	162*
13	C	1	3	H	$(CH_3)_3C$	CH_3	CH_3	325–326	$C_{18}H_{36}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.31*	21 NS
14	C	1	3	H	$CH_3(CH_2)_9$	CH_3	CH_3	297–298	$C_{24}H_{48}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.78*	96*
15	C	1	3	$-CH_2(CH_2)_4CH_2-$		CH_3	CH_3	305–307	$C_{20}H_{38}N_2 \cdot 2HCl$	C, H, N, Cl	60	5/8	1.70*	ND
											30	8/8	0.22	1 NS
16	C	1	3	H	C_6H_5	CH_3	CH_3	287–289	$C_{20}H_{32}N_2 \cdot 2HCl$	C, H, N	30	8/8	0.25	0
17	C	2	3	H	H	CH_3	CH_3	307–309	$C_{15}H_{30}N_2 \cdot 2HCl$	C, H, N, Cl[j]	30	8/8	0.20	ND
18	C	2	3	H	CH_3	CH_3	CH_3	323–235	$C_{16}H_{32}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.25	0
19	C	2	3	CH_3CH_2	CH_3CH_2	CH_3	CH_3	306–307	$C_{19}H_{38}N_2 \cdot 2HCl$	C, H, N, Cl	90	0/7	–	
											30	7/7	0.30	20 NS
											10	7/7	0.03	ND
20	C	2	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	295–296	$C_{21}H_{42}N_2 \cdot 2HCl$	C, H, N, Cl	30	3/8	1.02*	ND
21	C	2	3	$CH_3(CH_2)_4$	$CH_3(CH_2)_4$	CH_3	CH_3	308–310	$C_{25}H_{50}N_2 \cdot 2HCl$	C, H, N, Cl	30	0/8	–	
											15	8/8	0.11	ND
											7.5	8/8	0.18	ND
22	C	2	3	$(CH_3)_3C$	H	CH_3	CH_3	329–330	$C_{19}H_{38}N_2 \cdot 2HCl$	C, H, N, Cl	90	0/7	–	
											30	7/7	0.33	22 NS
											10	7/7	0.04	ND
23	C	1	2	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	270–272	$C_{19}H_{38}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0	62*
24	C	1	4	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	266–268	$C_{21}H_{42}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.46*	177*
25	C	1	5	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	274–276	$C_{22}H_{44}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.82*	177*
26	C	1	6	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	259–261	$C_{23}H_{46}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	1.20*	141*
27	C	1	7	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3	CH_3	245–246	$C_{24}H_{48}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	1.57*	217*
28	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	CH_3CH_2	CH_3CH_2	251–253	$C_{22}H_{44}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	1.42*	276*
29	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	172–174	$C_{24}H_{48}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.43*	89*
30	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	$CH_3(CH_2)_3$	$CH_3(CH_2)_3$	146–149	$C_{26}H_{52}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	0.07	28 NS
31	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	$-CH_2(CH_2)_3CH_2-$		285–286	$C_{23}H_{44}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	1.70*	241*
32	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	$-CH_2(CH_2)_2CH_2-$		284–286	$C_{22}H_{42}N_2 \cdot 2HCl$	C, H, N, Cl	30	8/8	1.03*	139*
33	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	$-CH_2CH_2OCH_2CH_2-$		165–167	$C_{22}H_{42}N_2 \cdot 2HCl$	C, H, N, Cl	30	0/7	0	ND
											15	1/8	0	
34	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	H	H	246–249	$C_{18}H_{36}N_2 \cdot 2HCl$	C, H, N, Cl	30	5/6	0.91*	158*
35	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	H	CH_3	259–261	$C_{19}H_{38}N_2 \cdot 2HCl$	C, H, N, Cl	30	6/6	1.55*	217*
36	C	1	3	$CH_3(CH_2)_2$	$CH_3(CH_2)_2$	H	$COCH_3$	163–165	$C_{20}H_{38}N_2O \cdot HCl$	C, H, N, Cl	30	6/6	0.24	101*
37								286–287	$C_{22}H_{46}N_2 \cdot 2Cl$	C, H, N, Cl	30	8/8	0.05	ND

38		197–199	$C_{22}H_{44}N_2{\cdot}2HCl$	C, H, N, Cl	30	8/8	0.32	31 NS
39		128–129	$C_{18}H_{34}NO{\cdot}HCl$	C, H, N, Cl	60	8/8	0.31	ND
					30	8/8	0.07	22 NS
40		274–276	$C_{19}H_{40}N_2{\cdot}2HCl$	C, H, N, Cl	30	8/8	0.19	48*
41		298–299	$C_{15}H_{30}N_2{\cdot}2HCl$	C, H, N	30	8/8	0.20	22 NS
42		267–269	$C_{15}H_{30}N_2{\cdot}2HCl$	C, H, N	30	8/8	0	0
43		289–291	$C_{15}H_{28}N_2{\cdot}2HCl$	C, H, N*	30	8/8	0.35	48 NS
44		293–296	$C_{15}H_{28}N_2{\cdot}2HCl$	C, H, N, Cl	60	8/8	0.10	ND
					30	8/8	0	

[a] X in the generic structure indicates position 8 in the 2-azaspiro[4.5]decane nucleus ($m = 1$) and position 9 in the 3-azaspiro[5.5]undecane nucleus ($m = 2$). [b] Analogues gave elemental analyses within 0.4% of calculated values unless otherwise indicated. [c] Oral administration, given daily on days 0–10. [d] Surviving animals on day 16/total starting number of animals. [e] Adjuvant arthritic rat (AA) activity normalized to spirogermanium (1). [f] Suppressor cell activity (SC); AUC = area under curve; see the Experimental Section for details. [g] ND, not done; NS, not significant. [h] H: calc, 10.04; found 9.59. [i] C: calc, 56.56; found, 55.94. [j] C: calc, 57.87; found, 57.14. [k] C: calc, 58.25; found, 57.52. [l] An asterisk denotes statistically significant activity; $p < 0.01$; *Biologically* significant activity is considered to be >0.30 for AA rat activity and AUC > 70 for suppressor cell activity.

rogermanium at an equivalent (**9** and **12**) or lower dose (**10** and **11**).

8-Monoalkyl-substituted 2-azaspiro[4.5]decane derivatives, e.g. **13** and **14**, were not as active as **9** in either the adjuvant arthritis or the suppressor cell models. 8-Phenyl analogue **16** was inactive in both screens.

In a series of 3-azaspiro[5.5]undecane-3-propanamine derivatives (e.g. **17–22**) dialkyl substitution at C-9 resulted in analogues that had a similar profile of activity in both the adjuvant arthritic rat and suppressor cell induction assays as the 8,8-dialkyl-2-azaspiro[4.5]decane analogues. Direct comparison between similarly substituted 2-azaspiro[4.5]decane and 3-azaspiro[5.5]undecane analogues (e.g. **3** vs **19**, **9** vs **20**, **11** vs **21**) demonstrated that the former series were more active in each case. As demonstrated with dialkyl substitution at C-8 of the 2-azaspiro[4.5]decane-2-propanamine series, optimal activity in the 9,9-dialkyl-3-azaspiro[5.5]undecane-3-propanamine series was obtained with C-9 dipropyl substitution. However it should be noted that **20** was toxic at 30 mg/kg and the compound was not reevaluated at lower doses.

Following the identification of **9** as more active than spirogermanium (**1**), the effects of varying the chain length from two to seven methylenes between the cyclic and terminal nitrogens of 8,8-dipropyl-2-azaspiro[4.5]decanes was examined. Separation of the cyclic and terminal nitrogens by two methylenes resulted in **23**, which was inactive in the adjuvant rat model and had weak suppressor cell induction activity. The other derivatives, e.g. **24–27**, all showed significant activity in both assays.

Derivatives incorporating straight chain and cyclic alkyl substitutents on the terminal nitrogen of the 8,8-dipropyl-2-azaspiro[4.5]decane-2-propanamine nucleus were also synthesized and tested. It was observed that **9** and the *N,N*-diethyl analogue **28** possessed equal activity in the adjuvant arthritic rat assay but that **28** had superior suppressor cell inducing activity. The *N,N*-dipropyl substitution (**29**) gave minimal activity in both assays and the *N,N*-dibutyl analogue **30** was inactive. Substitution of a pyrrolidinyl or piperidinyl ring on the terminal nitrogen gave compounds **31** and **32**, respectively, that were more active than **9** in both assays.

Putative metabolites of **9**, primary amine **34** and *N*-methyl **35**, showed significant activity in both models and acetamide analogue **36** was inactive in the adjuvant arthritic rat model but showed suppressor cell activity equal to that of spirogermanium.

Bisquaternary amine analogue **37** was inactive in the adjuvant rat model. This compound resembles the bisquaternary amines which have ganglionic blocking activity by virtue of their interactions with nicotinic receptors.[43] As many bisquaternary amines are poorly absorbed by the oral route, the compound was also evaluated when administered intraperitoneally. The compound produced lethality at 10 mg/kg and was inactive at doses below this. At 30 and 15 mg/kg spirogermanium (**1**) was active following intraperitoneal administration and **9** was active as low as 5 mg/kg when administered by this route (data not shown).

A compound with *gem*-dimethyl substitution at C-2 of the propyl chain between the cyclic and terminal nitrogens, analogue **38**, was inactive in both screens, indicating that substitution on the chain is not tolerated. Replacement of the terminal dimethylamino moiety with a hydroxyl function (**39**) also led to loss of activity. A number of

(43) Gyermek, L. *Drugs Affecting the Peripheral Nervous System*; Burger, A., Ed.; Marcel Dekker Inc.: New York, 1967; Vol. 1, Chapter 4, pp 149–326.

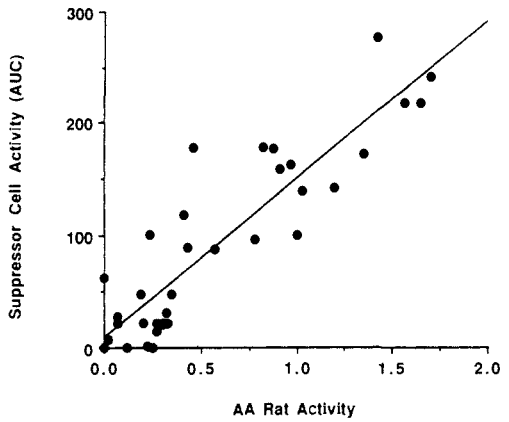


Figure 2. Correlation between the level of activity in the AA rat and ability to induce suppressor cells in normal rats by azaspirane analogues. Suppressor cell activity is units of suppression (AUC) and AA activity is relative to the activity of spirogermanium. The correlation is calculated from the linear regression data, $r = 0.894$, $p < 0.001$.

azaspirane analogues in which the A-ring was varied were synthesized to examine the degree to which this ring could be modified and activity retained. None of the compounds synthesized within this series (e.g. **40–44**) demonstrated any significant activity at a dose of 30 mg/kg.

In summary, 43 compounds were evaluated in the adjuvant rat model and 35 were also examined for their ability to induce suppressor cells in the spleens of normal rats. As shown in Figure 2 a significant correlation between the level of activity in the adjuvant arthritic rat and the units of suppressor cell activity was observed ($r = 0.894$, $p < 0.001$).

Discussion

The hypothesis that disorders in suppressor cell function/numbers may play a role in autoimmune disease has been the subject of considerable investigation. The clinical relevance of this hypothesis is that augmentation of suppressor cells could then be effective therapy in autoimmunity and/or tissue/organ transplantation and provides a rational approach for immunotherapy of these conditions. Our previously reported findings that spirogermanium (**1**) caused the generation of suppressor cells[27-30] in addition to the therapeutic effects of the compound in animal models of autoimmunity, support a link between suppressor cell generation and therapeutic activity. Following the identification of the immunomodulatory properties of spirogermanium, we synthesized a large number of azaspirane analogues based on the 2-azaspiro[4.5]decane nucleus and developed a structure–activity relationship. It is evident from the SAR that the immunomodulatory activity seen with spirogermanium does not require the presence of germanium within the azaspirane nucleus and that the mechanism(s) of biological activity of this class of compounds is distinct from other organogermanium complexes.[44] We have demonstrated, in fact, that replacement of the 8,8-diethylgermanium moiety with an 8,8-dipropylcarbon group leads to increased activity in both the adjuvant arthritic rat and suppressor cell induction models.

In our evaluation of the in vivo pharmacology of the azaspiranes there was no evidence of central nervous system (CNS) toxicity with the carbon analogues (data not shown) whereas spirogermanium was reported to produce CNS effects in animals when administered at and below lethal doses. The dose-limiting toxicity of the drug in humans during clinical trials for antitumor activity was somnolence and other indications of CNS depression.[34,45] Recent studies in our laboratory have demonstrated that **1** and other azaspiranes bind to specific sites on cellular membranes.[46] While the precise nature of this binding site is not defined, binding is saturable, is unaffected by nicotinic or muscarinic agonists/antagonists, and is displaced by active but not inactive azaspirane analogues (manuscript in preparation). In addition **1** and other azaspiranes can cause a decrease in the rise in intracellular Ca^{2+} levels in a number of cell types in response to a variety of stimuli.[47] The relationships of these physical and pharmacological properties, determined in vitro, to the immunomodulatory effects of the compounds in vivo is unknown. The elucidation of these relationships and further definition of the therapeutic utility of this class of compounds (in particular analogue **9**[48]) should be greatly enhanced through further SAR studies with these and other azaspirane analogues.

Experimental Section

Chemistry. ^1H NMR were recorded in $CDCl_3$ (unless otherwise indicated) on an 80 MHz IBM AF-80 spectrometer. IR spectra were recorded on a Perkin-Elmer 598 infrared spectrometer. NMR and IR spectra were obtained on all intermediates and final azaspirane analogues and were consistent with assigned structure. All final products were analyzed for C, H, N and in most cases Cl and the analytical results were within +0.4% of the theoretical values (see Table I).

The synthesis of 3-azaspiro[5.5]undecane analogues (Table I; $m = 2$) from appropriately substituted cyclohexanones was accomplished according to the methods of Rice et al.[38] The preparation of **9**, as a representative example of the synthesis of 2-azaspiro[4.5]decane analogues, is described in detail below. The overall yield of azaspirane analogues from the requisite cyclohexanone (seven steps) was 25–50% in all cases.

2-Propyl-1,2-epoxypentane. Dimethyl sulfoxide (320 mL) was slowly added to a mechanically stirred mixture of sodium hydride (10.1 g, 420 mmol) and trimethylsulfoxonium iodide (86.7 g, 394 mmol) under an inert atmosphere. Vigorous hydrogen evolution ensued and on completion of gas evolution heptan-4-one (30.0 g, 263 mmol) was added and the reaction mixture was heated at 70 °C for 5 h. The reaction mixture was poured into water (3–5 volumes) and extracted with ethyl acetate. The organic extract was washed with 1 volume of water, 1 volume of brine, dried with magnesium sulfate, filtered, and concentrated to give the desired epoxide: 28.5 g; 85% yield; ^1H NMR δ 0.75–1.05 (m, 6 H), 1.08–1.78 (m, 8 H), 2.56 (s, 2 H). The product was used without further purification.

2-Propylpentanal. To a vigorously stirred solution of 2-propyl-1,2-epoxypentane (28.2 g; 220 mmol) in benzene (400 mL) cooled to 5 °C with a water bath was added a solution of boron trifluoride etherate (15.6 g, 110 mmol) in benzene (30 mL). The reaction mixture was stirred for 30–60 s and was quenched by the addition of water. Following separation of the phases, the organic phase was washed with saturated aqueous sodium bicarbonate and water, dried with magnesium sulfate, filtered, and concentrated to give a colorless oil. The oil was distilled to give the desired product as a colorless oil: 22.8 g; 81% yield; bp 66–78 °C (28 mm); IR (neat) 1724 (s) cm^{-1}; ^1H NMR δ 0.7–1.1 (m, 6 H),

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(45) Mattson, W. A. *Proc. Am. Assoc. Cancer Res. ASCO* **1980**, *21*, 194.
(46) Sung, C. M.; Drake, F. H.; Sarau, H. M.; Brophy, L. P.; Badger, A. M.; Olivera, D.; Heyer, R.; Mirabelli, C. K. *FASEB. J.* **1989**, *3* (3), A278.
(47) Mirabelli, C. K.; Foley, J. J.; Sung, C. M.; Aiyer, N.; Sarau, H. M. *FASEB. J.* **1989**, *3* (4), 1292.
(48) Badger, A. M.; DiMartino, M. J.; Talmadge, J. E.; Picker, D. H.; Schwartz, D. A.; Dorman, J. W.; Mirabelli, C. K.; Hanna N. *Int. J. Immunopharm.* **1989**, *11*, 839.

1.15–1.90 (m, 8 H), 2.25 (m, 1 H), 9.56 (d, 1 H J = 3.2 Hz).

4,4-Dipropylcyclohex-2-en-1-one. To a solution of 2-propylpentanal (22.4 g, 175 mmol) and methyl vinyl ketone (12.2 g, 175 mmol) in benzene (120 mL) was added concentrated sulfuric acid (0.75 mL). The reaction mixture was heated under reflux with a Dean-Stark water trap. Reflux was continued for 3–5 h and a second equivalent of methyl vinyl ketone was added and reflux was continued for a further 3–5 h until no further separation of water was observed. The black reaction mixture was cooled to room temperature and washed with saturated sodium bicarbonate. The organic phase was dried with magnesium sulfate, filtered, and concentrated to give a dark brown oil. The oil was Kugelrohr distilled to give the desired product as a colorless oil: 21.5 g; 68% yield; bp 75–85 °C (0.2 mm); IR (neat) 1680 cm^{-1}; ^1H NMR δ 0.75–1.1 (m, 6 H), 1.15–1.7 (m, 8 H), 1.85 (t, 2 H, J = 7.7 Hz), 2.44 (t, 2 H, J = 7.7 Hz). 5.84 (d, 1 H, J = 5.2 Hz), 6.68 (d, 1 H, J = 5.2 Hz).

4,4-Dipropylcyclohexanone. To a suspension of 10% palladium-on-carbon (0.5 g) in ethyl acetate (220 mL) was added 4,4-dipropylcyclohex-2-enone (21.0 g, 117 mmol). The reaction mixture was hydrogenated at 50 psi hydrogen in a Parr hydrogenation apparatus at room temperature until hydrogen uptake had ceased (5 h). The catalyst was removed by filtration through Celite and the filtrate was concentrated to give the desired product as a colorless oil: 20.8 g; yield 98%; IR (neat) 1715(s) cm^{-1}; ^1H NMR δ 0.8–1.1 (m, 6 H), 1.1–1.5 (m, 12 H), 1.63 (t, 2 H, J = 7.2 Hz), 2.30 (t, 2 H, J = 7.2 Hz). The product was used without further purification.

Ethyl α-Cyano-α-(4,4-dipropylcyclohexylidene)acetate. To a solution of 4,4-dipropylcyclohexanone (20.6 g, 113 mmol) in toluene (350 mL) was added ethyl cyanoacetate (13.0 g, 115 mmol), acetic acid (1.4 mL, 22.6 mmol), and ammonium acetate (0.9 g, 11.3 mmol). The mixture was heated at reflux by employing a Dean–Stark apparatus to collect the water azeotropically removed from the reaction. Following collection of 1 equiv of water, the reaction mixture was cooled and washed with water and saturated aqueous sodium bicarbonate solution. The organic phase was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by distillation using a Kugelrohr apparatus to give the desired product as a colorless oil: 29.0 g; 89% yield; bp 95–101 °C (0.12 mm); IR (neat) 2220 (s), 1728 (s) cm^{-1}; ^1H NMR δ 0.76–1.05 (m, 6 H), 1.06–1.7 (m, 15 H), 2.65 (t, 2 H, J = 7.0 Hz), 2.98 (t, 2 H, J = 7.0 Hz), 4.27 (q, 2 H, J = 7.0 Hz).

4,4-Dipropyl-1-carboxycyclohexane-1-acetic Acid. To a solution of ethyl α-cyano-α-(4,4-dipropylcyclohexylidene)acetate (28.5 g, 99.2 mmol) in ethanol (350 mL) was added a solution of potassium cyanide (7.1 g, 109.1 mmol) in water (15 mL). The reaction mixture was heated at reflux for 5 h and then concentrated to dryness. The residue was cautiously treated with hydrochloric acid and extracted with ethyl acetate. The organic extract was dried over magnesium sulfate, filtered, and concentrated. The residue was dissolved in a mixture of acetic acid/hydrochloric acid/water (450 mL/200 mL/20 mL) and heated at reflux for 3.5 days. The volatiles were removed under reduced pressure, and the solid residue was partitioned between water and ethyl acetate. The organic extract was dried with magnesium sulfate, filtered, and concentrated to give the desired diacid as a white solid. Recrystallization from ethyl acetate gave a white, crystalline solid: 21.0 g; 78% yield; mp 130—131 °C; IR (KBr) 1700 (s) cm^{-1}; ^1H NMR δ 0.65–1.05 (m, 6 H), 1.06–2.2 (m, 16 H), 2.68 (s, 2 H).

4,4-Dipropyl-1-carboxycyclohexane-1-acetic Acid Anhydride (2; R = $CH_3CH_2CH_2$). 4,4-Dipropylcyclohexane-1-carboxy-1-acetic acid (20.8 g, 76.9 mmol) was dissolved in acetic anhydride (100 mL) and refluxed for 4 h. The excess acetic anhydride was removed by distillation under reduced pressure and the residue was recrystallized from hexanes: mp 94.5–95 °C; 17.3 g; 89% yield; IR (KBr) 1834 (m), 1860 (m), 1766 (s) cm^{-1}; ^1H NMR δ 0.8–1.05 (m, 6 H), 1.06–2.30 (m, 16 H), 2.79 (s, 2 H).

2-[3-(Dimethylamino)propyl]-8,8-dipropyl-2-azaspiro[4.5]decane-1,3-dione. To a solution of 4,4-dipropylcyclohexane-1-carboxy-1-acetic acid anhydride (17.0 g, 67.4 mmol) in toluene (350 mL) was added 3-(dimethylamino)propylamine (7.0 g, 68.7 mmol) and the reaction mixture was heated at reflux with a Dean–Stark trap. Following collection of water (1 equiv) in the trap, the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residue (22.7 g, quantitative yield) was used directly without further purification; IR (neat) 1681 (s), 1768 (m) cm^{-1}; ^1H NMR δ 0.75–1.08 (m, 6 H), 1.09–2.08 (m, 18 H), 2.18 (s, 6 H), 2.25 (t, 2 H, J = 7.0 Hz), 2.48 (s, 2 H), 3.55 (t, 2 H, J = 7.0 Hz).

2-[3-(Dimethylamino)propyl]-8,8-dipropyl-2-azaspiro[4.5]decane Dihydrochloride (9). To a mixture of lithium aluminum hydride (6.8 g, 181 mmol) in diethyl ether (500 mL) was added dropwise a solution of 2-[3-(dimethylamino)propyl]-8,8-dipropyl-2-azaspiro[4.5]decane-1,3-dione (22.5 g, 66.9 mmol) in diethyl ether. The reaction mixture was stirred for 3 h following completion of addition. The excess hydride was quenched with sodium sulfate decahydrate and the resulting mixture was filtered. The filtrate was concentrated to give the desired amine as a colorless oil: 19.5 g; 95% yield.[1] The oil was dissolved in a minimum of anhydrous ethanol and a cooled solution of hydrogen chloride in ethanol was added. On addition of a large volume of ether, a white precipitate formed which was isolated by filtration. The white solid was recrystallized from ethanol: 21.9 g; 91% yield; mp 299–300.5 °C dec; H NMR δ (D_2O) 0.75–1.1 (m, 6 H), 1.15–1.95 (m, 20 H), 2.21 (s, 6 H), 2.2–2.65 (m, 8 H).

2-(2-Cyanoethyl)-8,8-dipropyl-2-azaspiro[4.5]decane-1,3-dione. To a solution of **2** (R = $CH_3CH_2CH_2$; 11.5 g, 45.6 mmol) in toluene (250 mL) was added 3-aminopropionitrile (3.51 g, 45.6 mmol). The reaction mixture was heated at reflux with a Dean–Stark trap until 1 equiv of water was collected in the trap. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (hexane/ethyl acetate (2/1); R_f 0.58) to give the desired imide as a white solid: 13.2 g; yield 90%; mp 117–117.5 °C; ^1H NMR δ 0.7–1.05 (m, 6 H), 1.–5–2.28 (m, 16 H), 2.57 (s, 2 H), 2.72 (t, 2 H, J = 7.0 Hz), 3.79 (t, 2 H, J = 7.0 Hz).

2-(3-Aminopropyl)-8,8-dipropyl-8-azaspiro[4.5]decane Dihydrochloride (34). To a mixture of $LiAlH_4$ (4.54 g, 0.112 mol) in ether (300 mL) was added the above imide (6.75 g, 22.2 mmol) portionwise. The reaction mixture was stirred for 4 h following completion of addition. The excess hydride was quenched with sodium sulfate decahydrate and the resulting mixture was filtered and the filtrate was concentrated to give 6.1 g of a colorless oil. The resulting liquid was purified by preparative liquid chromatography (silica gel; ammonium hydroxide/methanol (2.5/97.5); R_f 0.31) to give the desired diamine as a colorless oil: 4.53 g; 73% yield; ^1H NMR δ 0.88 (br t, 6 H), 1.02–1.84 (m, 22 H), 2.33 (s, 2 H), 2.36–2.88 (m, 6 H). The oil (4.0 g) was dissolved in anhydrous anhydrous ethanol and a solution of hydrogen chloride in ethanol was added. The solution was concentrated to give 4.8 g of white solid: yield 95%; mp 245–249 °C dec; ^1H NMR δ (D_2O) 0.66–1.02 (m, 6 H), 1.02–1.74 (m, 4 H), 2.78–3.84 (m, 8 H).

2-[3-(Methylamino)propyl]-8,8-dipropyl-2-azaspiro[4.5]decane-1,3-dione. To a solution of anhydride **2** (R = $CH_3CH_2CH_2$; 3.02 g, 12.0 mmol) in toluene (100 mL) was added 3-(methylamino)propylamine (1.32 mL, 12.0 mmol). The reaction mixture was heated at reflux with a Dean–Stark trap until 1 equiv of water was collected in the trap. The reaction mixture was cooled to room temperature and concentrated under reduced pressure to give the desired imide as a colorless oil: 3.97 g; yield 99%; ^1H NMR 0.72 (m, 6 H), 1.05–2.26 (m, 18 H), 2.39 (s, 3 H), 2.50 (s, 2 H), 3.56 (t, 2 H, J = 7.0 Hz). The product was used without further purification.

2-[3-(Methylamino)propyl]-8,8-dipropyl-2-azaspiro[4.5]decane Dihydrochloride (35). To a mixture of $LiAlH_4$ (2.72 g, 71.8 mmol) in ether (200 mL) was added dropwise a solution of the above imide (6.44 g, 20.0 mmol) in ether (40 mL). The reaction mixture was stirred at room temperature for 4 h and worked up as described above. The crude product (3.50 g) was purified by flash chromatography [silica gel; ammonium hydroxide/methanol (0.5/99.5] to give the desired diamine as a colorless oil: 2.42 g; 67% yield; ^1H NMR δ 0.87 (br t, 6 H), 1.02–1.86 (m, 21 H), 2.22–2.94 (m, 11 H). The oil was dissolved in anhydrous ethanol and a solution of hydrogen chloride in ethanol was added. A white precipitate formed upon cooling which was isolated by filtration: mp 258–260 °C dec; yield 84%.

Biological Evaluation. Adjuvant-Induced Arthritis. AA was induced by a single injection of 0.75 mg of *Mycobacterium*

butyricum suspended in paraffin oil into the left hindpaw footpad of male Lewis rats (160–180 g). Hindpaw volumes were measured by a mercury displacement method.[49] The inflammation induced in the injected paw on day 3 is designated as the primary lesion. The inflammation of the noninjected paw on day 16 is designated as the secondary lesion. Unless otherwise indicated, test compounds were homogenized in aqueous 0.5% gum tragacanth and administered po in a volume of 10 mL/kg of body weight, on days 0–10. For the purpose of these studies, antiarthritic activity was measured on the secondary lesion (day 16) in the treated animals relative to the controls (non drug treated). In this model spirogermanium's activity ranged from 50 to 100% activity (mean of 15 experiments—68%) and in the results section is designated as 1.00 (at 30 mg/kg). Statistically significant activity in the biological assays was determined using the Student's *t* test.

Suppressor Cell Coculture Assay. Spleen cells from azaspirane analogue treated or control animals were established in RPMI with 10% fetal bovine serum (RPMI-10) at 5×10^6/mL. Coculture experiments were carried out by first adding varying numbers of the putative suppressor cells (0.15 to 5×10^5) to wells of 96-well round-bottomed microtiter plates (Linbro, Flow Laboratories) in 100 μL of RPMI-10. These were then irradiated (2000 rad) in a γ cell 40 with a Cesium-137 source. To these cultures were added 5×10^5 normal cells and an optimal concentration of Con A (5 μg/mL), and the final volume was adjusted to 200 μL. Cell cultures were incubated for 72 h at 37 °C in a 5% CO_2 atmosphere and pulsed with 0.5 μCi [^{3}H]thymidine (specific activity 1.9 μCi/mmol; Schwarz/Mann, Orangeburg, NY) for the last 18 h of culture. The cells were harvested on an automated multiple sample harvester and cell-associated radioactivity was counted in a Beckman liquid-scintillation counter. Suppressor cell activity is determined by calculating the percent inhibition of proliferation compared to control cultures. For comparison of azaspirane analogues, a program was developed that could be used to compare suppressor cells generated in different experiments and the activities of different compounds. This was calculated in the following manner. A plot of percent suppression at different cell concentrations (dependent variable) vs the logarithm (base *e*) of the number of suppressor cells (independent variable) was generated and the area under the curve (AUC) represented by the data points of this plot was determined by the trapezoidal rule. The trapezoidal rule provides AUC by means of the summation of the areas of the trapezoids whose vertices are located at adjacent values of the independent variable and the corresponding values of the dependent variable. Spirogermanium (1) at 30 mg/kg for 11 days, administered po, assayed on day 16 gives an average value of 100 units by this method (mean value derived from three experiments). All unit values for analogues were standardized to the activity of spirogermanium. On the basis of our experience with these compounds we do not consider an AUC of less than 70 for suppressor cells to have meaningful biological activity, whereas values above this correspond to activity in the AA rat (and other autoimmune disease models). In addition, we consider that it requires a change of approximately 70 units in suppressor cell activity (AUC) to result in a meaningful change in the immune profile of treated animals.

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Supplementary Material Available: A table of the bp/mp of novel cyclohexenone and anhydride derivatives is included (2 pages). Ordering information is given on any current masthead page.

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Synthesis, Biological Evaluation, and Quantitative Structure–Activity Relationship Analysis of [β-(Aroylamino)ethyl]piperazines and -piperidines and [2-[(Arylamino)carbonyl]ethyl]piperazines, -piperidines, -pyrazinopyridoindoles, and -pyrazinoisoquinolines. A New Class of Potent H_1 Antagonists[1]

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Some [β-(Aroylamino)ethyl]piperazines and -piperidines and [2-[(Arylamino)carbonyl]ethyl]piperazines, -piperidines, -pyrazinopyridoindoles, and -pyrazinoisoquinolines have been synthesized and their H1-antagonistic activity studied in isolated guinea pig ileum. Quantitative structure–activity relationship analysis indicates that the hydrophobicity of the side chain of these compounds plays a major role in their activity while steric and electronic factors are of secondary importance. All these compounds act on a common receptor and appear to interact similarly with the receptor.

Earlier work in this laboratory on 2-substituted 1,2,3,4,6,7,12,12a-octahydropyrazino[2′,1′:6,1]pyrido[3,4-*b*]indoles (1)[2,3] had shown that the presence of a β-(aroylamino)ethyl side chain at the 2-position results in potent H_1-antagonistic activity. The contributions of the substructure $(CH_2)_2$ NHCOAr and of hydrophobic interactions to the activity were quantified. It was suggested that a hydrophobic substituent at the ortho (π_o) or para (π_p) positions of 1 contributes more to the activity than a substituent at the meta (π_m) position and that the activity is enhanced by a bulky substituent at the ortho position of the side-chain phenyl ring. This effect was similar to the effect of a bulky substituent in one of the phenyl rings of diphenhydramine (2, R = H) observed by Kutter and Hansch.[4] Since 1 and 2 produce a similar biological re-

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