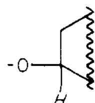
and evaporated in vacuo to a syrup (3.3 g), which was dissolved in 2-butanone (50 mL). Sodium iodide (1.0 g) was then added and the mixture was heated under reflux for 4 h. The solution was evaporated to a residue, which was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with 5% sodium thiosulfate and H_2O, dried, and evaporated to a crystalline mass. Recrystallization from *i*-PrOH gave **25** (2.6 g): mp 82-83 °C; NMR ($CDCl_3$) δ 5.37 (d, olefinic), 3.59 (t, J = 7.0 Hz, CCH_2O), 3.21 (m, iii), 3.23 (t, I CH_2C). Anal. ($C_{33}H_{53}IO$) C, H, I.



iii

6-(5-Cholesten-3β-yloxy)-3-hexynyl 1-Thio-β-L-fucopyranoside (7). A solution of 1 (0.5 g, 1.6 mmol) and **25** (0.94 g, 1.6 mmol) in CH_2Cl_2 (30 mL) containing Et_3N (0.17 g) was kept under N_2 for 2 days at room temperature. The solution was washed with dilute HCl, aqueous $NaHCO_3$, and H_2O. The solution was dried and evaporated to a residue, which was put on a column of silica gel and eluted with Et_2O-pet. Et_2O (30:70). The desired fractions were combined and evaporated to give 6-(5-cholesten-3β-yloxy)-3-hexynyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranoside (0.9 g, 37%): $[\alpha]_D^{27}$ +3.4° (*c* 1.5, $CHCl_3$). Deacetylation of this compound with NaOMe in MeOH afforded **7** in 65% yield: mp 137-138 °C (MeOH), $[\alpha]_D^{27}$ -6.5° (*c* 1.5, $CHCl_3$). Anal. ($C_{39}H_{64}SO_5 \cdot 0.5CH_3OH$) C, H, S.

Acknowledgment. The authors thank Dr. B. H. Arison and H. Flynn for recording 300-MHz spectra, J. Smith for mass-spectral measurements, J. P. Gilbert and his associates for microanalyses, and Dr. A. F. Woodhour for his advice on immunological studies. The authors also thank Dr. A. C. Herman for preparation of the influenza antigen and Dr. J. B. Meeker for statistical analysis. The technical assistance of J. J. Doyle, J. N. Armstrong, R. J. Cesarone, and Ms. K. J. Lalk is also acknowledged.

Substituted 6,7-Dihydroimidazo[1,2-*a*]purin-9(4*H*)-ones

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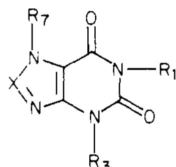
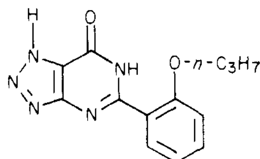
The synthesis of a series of substituted 6,7-dihydroimidazo[1,2-*a*]purin-9(4*H*)-ones is described. Several members of the series exhibit enhanced antiallergic and bronchodilator activity and reduced side effects as compared to theophylline. Structure-activity relationships and metabolic considerations are discussed for the series. Analogues substituted with a 4-(4-chlorobenzyl) moiety, such as **33** and **40**, show an optimal balance of antiallergic and bronchodilator activity and are of particular interest. Compound **33** is significantly more potent than theophylline against both metacholine- and antigen-induced bronchospasms, does not affect spontaneous motor activity, and shows minimal cardiovascular effects in the rat.

Theophylline (**1a**) is currently recognized as the drug



1a, $R_1 = R_3 = CH_3$; R_7 = H; X = CH
b, $R_1 = CH_3$; $R_3 = 4\text{-}NO_2C_6H_4CH_2$; R_7 = H; X = N
c, $R_1 = CH_3$; $R_3 = CH_3CH_2CH(CH_3)CH_2$; R_7 = H; X = CH_3C
d, $R_1 = n\text{-}C_3H_7$; $R_3 = CH_3$; $R_7 = CH_3CH(OH)(CH_2)_4$; X = CH



2

of choice for the maintenance therapy of asthma. The compound is an effective bronchodilator which has been shown to also inhibit the release of mediators of anaphylaxis from sensitized human lung and leukocytes.[1,2] Unfortunately, theophylline causes the limiting side effects of nausea, vomiting, and abdominal pains. Arrhythmias, CNS convulsions, and even death may occur if blood levels exceed the generally recommended 10-20 μg/mL.[3] Theophylline therapy is further complicated by patient to patient variability in rate of elimination and metabolism, which requires careful monitoring of serum theophylline levels in order to maximize benefit and minimize risk.[4,5]

In recent years considerable effort has been directed toward optimizing the bronchodilator and/or antiallergic activities inherent in the xanthine molecule while reducing undesirable side effects. For instance, 8-azatheophylline was shown to be more potent than theophylline in inhibiting the rat passive cutaneous anaphylaxis (PCA) reaction. This activity was maximized in the 3-(4-nitrobenzyl) derivative **1b**.[6] Further modification of the 8-azapurin-6-one nucleus led to the development of **2** (M&B 22948) as a potent antiallergic agent.[7-9] The xanthine derivatives 1c

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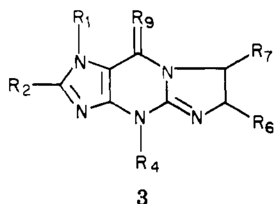
Table I. Intermediate 2,3-Dihydroimidazo[1,2-*a*]pyrimidin-5(8*H*)-ones

no.	X	R_4	R_6	R_7	% yield	mp, °C	formula[a]	recryst solvent	method
4	NO	4-$ClC_6H_4CH_2$	H	H	77	241 dec	$C_{13}H_{12}ClN_5O$	DMF-EtOH	A
5	NHCHO	4-$ClC_6H_4CH_2$	H	H	98	275 dec	$C_{14}H_{14}ClN_5O_2$	MeOH-*i*-PrOH	B
6	NO	4-$ClC_6H_4CH_2$	CH_3	H	26	200-221	$C_{14}H_{14}ClN_5O_2$	MeOH-*i*-PrOH	A
7	NHCHO	4-$ClC_6H_4CH_2$	CH_3	H	100	252-253 dec	$C_{15}H_{16}ClN_5O_2\cdot 0.5H_2O$[b]	MeOH-*i*-PrOH	C
8	NO	4-$ClC_6H_4CH_2$	$(CH_3)_2$	H	80	246-247 dec	$C_{15}H_{16}ClN_5O$		A
9	NHCHO	4-$ClC_6H_4CH_2$	$(CH_3)_2$	H	77	206-208 dec	$C_{16}H_{18}ClN_5O_2$[c]	MeCN	C
10	NO	4-$ClC_6H_4CH_2$	CH_3[d]	CH_3	36	237 dec	$C_{15}H_{16}ClN_5O_2$[e]	DMF	A
11	NO	3-$ClC_6H_4CH_2$	H	H	78	236 dec	$C_{13}H_{12}ClN_5O_2$	DMF-*i*-PrOH	A
12	NHCHO	3-$ClC_6H_4CH_2$	H	H	91	262 dec	$C_{14}H_{14}ClN_5O_2$	DMF-*i*-PrOH	B
13	NO	2-$ClC_6H_4CH_2$	H	H	96	235 dec	$C_{13}H_{12}ClN_5O_2$	DMF-*i*-PrOH	A
14	NHCHO	2-$ClC_6H_4CH_2$	H	H	87	247-249 dec	$C_{14}H_{14}ClN_5O_2$	EtOH-*i*-PrOH	B
15	NO	3,4-$Cl_2C_6H_3CH_2$	H	H	59	257 dec	$C_{13}H_{11}Cl_2N_5O_2$	DMF	A
16	NHCHO	3,4-$Cl_2C_6H_3CH_2$	H	H	64	275 dec	$C_{14}H_{13}Cl_2N_5O_2$	DMF	C
17	NO	4-$FC_6H_4CH_2$	H	H	100	223.5-225.5 dec	$C_{13}H_{12}FN_5O_2$	DMF-MeOH	A
18	NHCHO	4-$FC_6H_4CH_2$	H	H	90	262 dec	$C_{14}H_{14}FN_5O_2$	MeOH	B
19	NO	$C_6H_5CH_2$	H	H	68	242 dec	$C_{13}H_{13}N_5O_2$	DMF	A
20	NHCHO	$C_6H_5CH_2$	H	H	86	248-250 dec	$C_{14}H_{15}N_5O_2$	DMF-*i*-PrOH	C
21	NO	2-$C_5H_4NCH_2$	H	H	79	222 dec	$C_{12}H_{12}N_6O_2$	DMF	A
22	NHCHO	2-$C_5H_4NCH_2$	H	H	73	238 dec	$C_{13}H_{14}N_6O_2$	*i*-PrOH	C
23	NO	4-$ClC_6H_4(CH_2)_2$	H	H	32	251-253 dec	$C_{14}H_{14}ClN_5O_2$	DMF-MeCN	A
24	NHCHO	4-$ClC_6H_4(CH_2)_2$	H	H	49	229-239 dec	$C_{15}H_{16}ClN_5O_2$[f]	MeOH-MeCN	B
25	NO	$(CH_3)_2CHCH_2$	H	H	27	205-207	$C_{10}H_{16}N_5O_2\cdot H_2O$[b]	H_2O	A
26	NHCHO	$(CH_3)_2CHCH_2$	H	H	94	198 dec	$C_{11}H_{17}N_5O_2$[g]		B
27	NO	$(CH_3)_2CHCH_2$	CH_3	H	50	oil	$C_{11}H_{17}N_5O_2$[g]		A
28	NHCHO	$(CH_3)_2CHCH_2$	CH_3	H	20	221-222	$C_{12}H_{19}N_5O_2$	*i*-PrOH	C
29	NO	$C_6H_{11}CH_2$	H	H	55	228-229 dec	$C_{13}H_{19}N_5O_2\cdot 1.5H_2O$[b]	EtOH	A
30	NHCHO	$C_6H_{11}CH_2$	H	H	100	151-155 dec	$C_{14}H_{21}N_5O_2$[g]	$CHCl_3$	B
31	NO	*n*-C_8H_{17}	H	H	80	170	$C_{14}H_{23}N_5O_2$	*i*-PrOH	A
32	NHCHO	*n*-C_8H_{17}	H	H	53	218-219	$C_{15}H_{25}N_5O_2$[h]	DMF	B

[a] Unless otherwise indicated, all compounds had C, H, and N analyses within ±0.40% of theoretical values. [b] Level of hydration determined by Karl Fisher water analysis. [c] C: calcd, 55.25; found, 54.26. [d] This compound contains an isomer ratio of 35% cis, 65% trans. [e] C: calcd, 53.98; found, 53.44. N: calcd, 20.99; found, 20.39. [f] C: calcd, 53.98; found, 52.90. [g] Compound not analyzed. Crude product reacted in subsequent step. [h] C: calcd, 58.61; found, 57.07. H: calcd, 8.20; found, 7.62. N: calcd, 22.78; found, 21.98.

and **1d** have been recently reported to show an enhanced antiallergic/bronchodilator profile as compared to theophylline.[10,11]

In our search for compounds exhibiting potent bronchodilator and antiallergic activity with lessened side effects, we prepared a series of imidazo[1,2-*a*]purin-9-(4*H*)-one derivatives (**3**) in which the 2-carbonyl group of



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theophylline has been replaced with a 2-iminoalkylene moiety cyclized to the 1 position. It was intended that this polar imidazoline-fused system would impart increased metabolic stability and decreased CNS penetration to the molecule while maintaining the desired antiallergic and bronchodilator activities. Hardtmann has previously reported a series of 2,3-dihydroimidazo[2,1-*b*]quinazolin-5-(10*H*)-ones to possess bronchodilator activity without CNS or cardiovascular side effects.[12] In the present work, ai extensive investigation of the structural requirements fo selective antiallergic and bronchodilator activity in the titl series of compounds was accomplished. The possibilitie for biotransformation of the new heterofused system **3** wer also considered.

Theophylline is demethylated and oxidized in the : position to give 3-methylxanthine, 1,3-dimethyluric acid and 1-methyluric acid during its extensive biotransfor mation in man.[13] Clonidine, 2-[(2,6-dichlorophenyl)imi no)]-2-imidazoline, is metabolized through various path ways, including oxidation at the 4 and 5 positions of th imidazoline ring.[14,15] These observations suggested pos sible oxidative metabolism of **3** in the 2, 6, and 7 positions Therefore, several analogues of **3** with alkyl groups in thes three key positions were subsequently prepared.

Chemistry. The preparation of substituted 6,7-di hydroimidazo[1,2-*a*]purin-9(4*H*)-ones and intermediat substituted 2,3-dihydroimidazo[1,2-*a*]pyrimidin-5(8*H*)-one (Tables I and II) is outlined in Schemes I and II. Th

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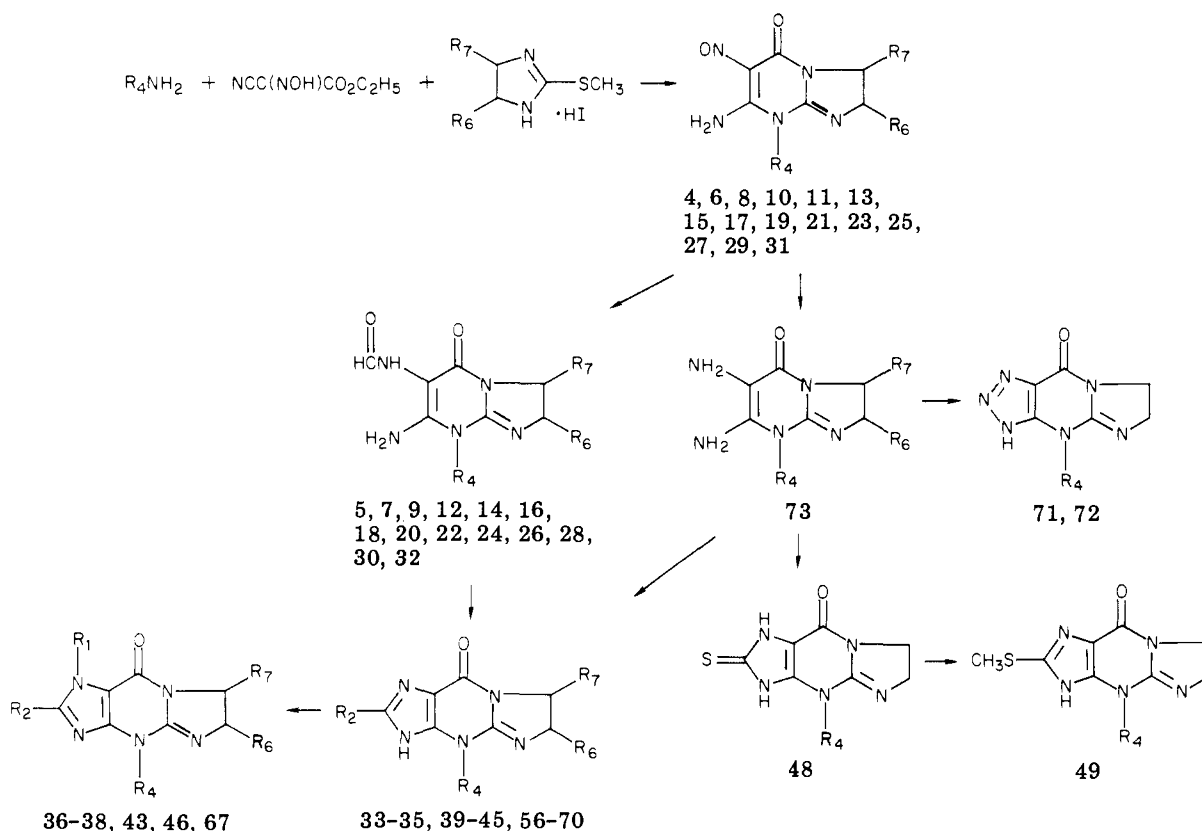
Scheme I



starting 2-(methylthio)-2-imidazolines were either commercially available or prepared by previously described methods.[12] Intermediate 8-substituted 7-amino-2,3-dihydro-6-nitrosoimidazo[1,2-*a*]pyrimidin-5(8*H*)-ones were prepared by an efficient "one pot" method (method A). Reaction of the appropriate amine (R_4-NH_2) with 2-(methylthio)-2-imidazoline hydriodide salt in boiling alcohol provided the corresponding substituted 2-aminoimidazoline, which was treated directly with ethyl oximinocyanoacetate and base to give the 7-amino-6-nitroso compounds in high yield. The alternate annelation product was not isolated from this reaction sequence. The formylamino derivatives listed in Table I were obtained directly by either catalytic (method B) or sodium dithionite (method C) reduction of the corresponding 6-nitroso derivatives in formic acid.

The final substituted 6,7-dihydroimidazo[1,2-*a*]purin-9(4*H*)-ones **33-70** were prepared by a variety of methods. Analogues substituted with a hydrogen at the 2 position were prepared by base-catalyzed cyclization (method D), triethyl orthoformate–acetic anhydride cyclization (method E), or pyrolysis (method F). Analogues substituted with alkyl groups in the 2 position were either prepared by a facile acyl transfer-cyclization reaction using the appropriate anhydride in pyridine (method G) or, alternately, by heating the crude diamine **73** with the desired anhydride (method H). In the case of the 6,7-dimethyl analogue, both the nitroso (**10**) and formylamino intermediates were carried through as 65% trans/35% cis mixtures. Cyclization (method D) gave a mixture of isomers, which was separated by chromatography to provide pure 6,7-*trans*- (**41**) and -*cis*-dimethyl (**42**) analogues (method Q). The imidazoline ring protons of **41** occurred as doublets at 3.55 and 3.97 ppm in the ^{1}H NMR (J = 4.7 Hz). The corresponding protons of **42** were observed as doublets at 4.13 and 4.51 ppm (J = 9.0 Hz). Reaction of **73** with carbon disulfide gave **48** (method K), which was methylated to give **49** (method L). Reaction of diamine **73** with nitrous acid provided the triazoles **71** and **72** (method J).

Scheme II

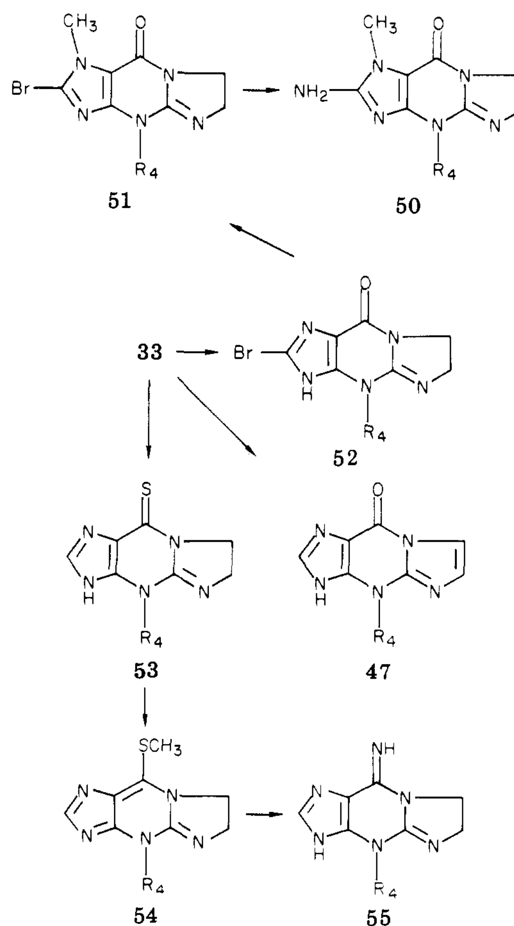
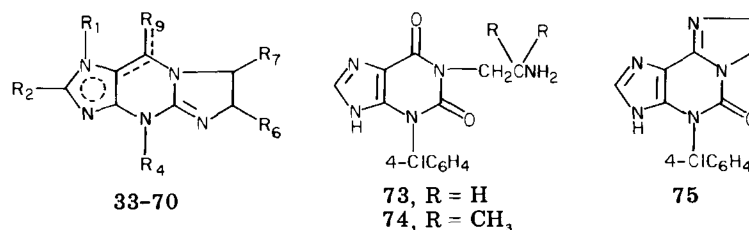


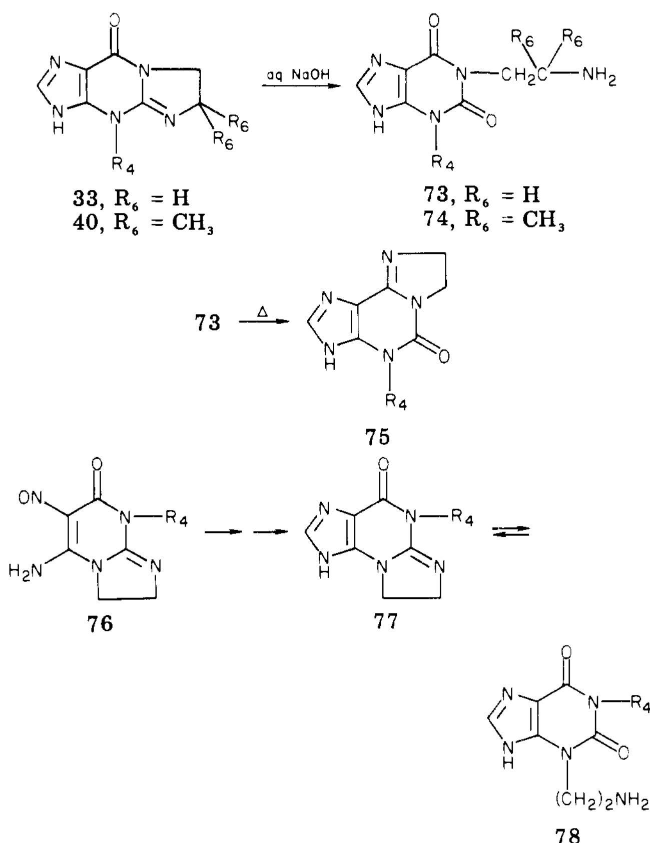
Table II. 6,7-Dihydroimidazo[1,2-*a*]purin-9(4*H*)-one Derivatives



no.	R_1	R_2	R_4	R_6	R_7	R_9	% yield	mp, °C	formula[a]	recryst solvent	method
33	H	H	4-$ClC_6H_4CH_2$	H	H	O	93	289–293	$C_{14}H_{12}ClN_5O$	DMF	E, F
34	H	CH_3	4-$ClC_6H_4CH_2$	H	H	O	55	312–316	$C_{15}H_{14}ClN_5O$	MeCN	G
35	H	C_2H_5	4-$ClC_6H_4CH_2$	H	H	O	64	278–279	$C_{16}H_{16}ClN_5O$	DMF–*i*-PrOH	G
36	CH_3	H	4-$ClC_6H_4CH_2$	H	H	O	100	182–184	$C_{15}H_{14}ClN_5O$	*i*-PrOH	I
37	*n*-C_4H_9	H	4-$ClC_6H_4CH_2$	H	H	O	28	205.5–206.5 dec	$C_{18}H_{20}ClN_5O \cdot HCl$	MeCN–EtOAc	I
38	CH_3	CH_3	4-$ClC_6H_4CH_2$	H	H	O	51	228.5–229.5	$C_{16}H_{16}ClN_5O$	EtOH	I
39	H	H	4-$ClC_6H_4CH_2$	CH_3	H	O	57	259–260.5	$C_{15}H_{14}ClN_5O$	MeOH–*i*-PrOH	D
40	H	H	4-$ClC_6H_4CH_2$	$(CH_3)_2$	H	O	30	222–223	$C_{16}H_{16}ClN_5O$	MeCN	E
41	H	H	4-$ClC_6H_4CH_2$	CH_3 [b]	CH_3 [b]	O	32	261–262	$C_{16}H_{16}ClN_5O$	EtOH	D, Q
42	H	H	4-$ClC_6H_4CH_2$	CH_3 [c]	CH_3 [c]	O	<10	244–246	$C_{16}H_{16}ClN_5O$	*i*-PrOH	D, Q
43	CH_3	H	4-$ClC_6H_4CH_2$	CH_3	H	O	55	151.5–152.5	$C_{16}H_{16}ClN_5O \cdot H_2O$[d]	*i*-PrOAc	I
44	H	CH_3	4-$ClC_6H_4CH_2$	CH_3	H	O	15	290–291	$C_{16}H_{16}ClN_5O$	MeOH–*i*-PrOH	G
45	H	CH_3	4-$ClC_6H_4CH_2$	$(CH_3)_2$	H	O	80	251–252	$C_{17}H_{18}ClN_5O$	MeOH	D
46	CH_3	CH_3	4-$ClC_6H_4CH_2$	CH_3	H	O	69	170–172	$C_{17}H_{18}ClN_5O$	*i*-PrOH	I
47	H	H	4-$ClC_6H_4CH_2$	dehydro		O	5	291–293 dec	$C_{14}H_{10}ClN_5O$	EtOH	P
48	H	SH	4-$ClC_6H_4CH_2$	H	H	O	67	232–235	$C_{14}H_{12}ClN_5OS \cdot H_2O$[d]	NaOH–HOAc	K
49	H	CH_3S	4-$ClC_6H_4CH_2$	H	H	O	55	296–297	$C_{15}H_{14}ClN_5OS$	$MeOCH_2CH_2OH$	L
50	CH_3	NH_2	4-$ClC_6H_4CH_2$	H	H	O	80	259–261 dec	$C_{15}H_{15}ClN_6O \cdot 0.25H_2O$[d]	MeOH	N
51	CH_3	Br	4-$ClC_6H_4CH_2$	H	H	O	71	206–207	$C_{15}H_{13}BrClN_5O$	$MeOCH_2CH_2OH$	I
52	H	Br	4-$ClC_6H_4CH_2$	H	H	O	100	247 dec	$C_{14}H_{11}BrClN_5O \cdot HBr$	DMF–MeCN	M
53	H	H	4-$ClC_6H_4CH_2$	H	H	S	52	257–260	$C_{14}H_{12}ClN_5S$	DMF–MeCN	O
54		H	4-$ClC_6H_4CH_2$	H	H	CH_3S	82	253–255 dec	$C_{15}H_{14}ClN_5S$	DMF	L
55	H	H	4-$ClC_6H_4CH_2$	H	H	NH	41	296–299 dec	$C_{14}H_{13}ClN_6 \cdot 0.25H_2O$[d]	DMF	N
56	H	H	3-$ClC_6H_4CH_2$	H	H	O	27	262–267	$C_{14}H_{12}ClN_5O$	DMF	E
57	H	H	2-$ClC_6H_4CH_2$	H	H	O	54	292–293	$C_{14}H_{12}ClN_5O$	DMF	E
58	H	H	3,4-$Cl_2C_6H_3CH_2$	H	H	O	40	278–280	$C_{14}H_{11}Cl_2N_5O$	MeOH	E
59	H	H	4-$FC_6H_4CH_2$	H	H	O	71	296–298	$C_{14}H_{12}FN_5O$	DMF	F
60	H	H	$C_6H_5CH_2$	H	H	O	40	262–264	$C_{14}H_{13}N_5O$	DMF–*i*-PrOH	E
61	H	H	2-$C_5H_4NCH_2$	H	H	O	50	258–260	$C_{13}H_{12}N_6O \cdot 0.5H_2O$[d]	DMF	F
62	H	H	4-$ClC_6H_4(CH_2)_2$	H	H	O	43	257–259	$C_{15}H_{14}ClN_5O$	DMF–MeCN	F
63	H	H	$(CH_3)_2CHCH_2$	H	H	O	34	222–223	$C_{11}H_{15}N_5O$	*i*-PrOH	F
64	H	CH_3	$(CH_3)_2CHCH_2$	H	H	O	17	234–236	$C_{12}H_{17}N_5O$	MeCN	H
65	H	C_2H_5	$(CH_3)_2CHCH_2$	H	H	O	25	242–243	$C_{13}H_{19}N_5O$	MeCN	H
66	H	$CH(CH_3)_2$	$(CH_3)_2CHCH_2$	H	H	O	4	244–248	$C_{14}H_{21}N_5O$	MeOH–MeCN	G
67	CH_3	CH_3	$(CH_3)_2CHCH_2$	H	H	O	56	133–135	$C_{13}H_{19}N_5O \cdot 0.25H_2O$[d]	*i*-Pr_2O	I
68	H	H	$(CH_3)_2CHCH_2$	CH_3	H	O	20	201–202	$C_{12}H_{17}N_5O$	MeCN	E
69	H	H	$C_6H_{11}CH_2$	H	H	O	20	245–249	$C_{14}H_{19}N_5O$	*i*-PrOH–MeCN	E
70	H	H	*n*-C_8H_{17}	H	H	O	87	149–150	$C_{15}H_{23}N_5O$	EtOAc	E
71	triazole		4-$FC_6H_4CH_2$	H	H	O	29	294–295 dec	$C_{13}H_{11}FN_6O$	1 N NaOH–HOAc	J
72	triazole		$(CH_3)_2CHCH_2$	H	H	O	93	288–290	$C_{10}H_{14}N_6O \cdot HCl$	*i*-PrOH	J
73							3.5	217–219	$C_{14}H_{14}ClN_5O_2$	*i*-PrOH–MeOH	R
74							4	295–300	$C_{16}H_{18}ClN_5O_2 \cdot HCl$	EtOH	R
75							35	309–312	$C_{14}H_{12}ClN_5O$	DMF–*i*-PrOH	S

[a] All compounds had C, H, and N analyses within ±0.40% of theoretical values. [b] Trans. [c] Cis. [d] Level of hydration determined by Karl Fisher analysis.

Scheme III[a]



[a] R_4 = 4-ClC$_4$H$_4$CH$_2$

Alkylation with the appropriate alkyl halide in aqueous base (method I) gave the 1-alkyl derivatives (e.g., **36**) as the sole products. The ^{13}C NMR chemical shifts of the methyl group of **36** and of the 7-methyl group of the model compound caffeine are 33.0 and 33.6 ppm, respectively, thus providing good evidence for **36** being the 1-methyl derivative.

The further synthetic elaboration of **33** is shown in Scheme II. Compound **33** is readily brominated with bromine in acetic acid to give **52** in high yield (method M). Unfortunately, **52** failed to react with amines under a variety of conditions, probably due to proton abstraction which inhibits attack by the nucleophile. Methylation of **52** (method I) gave the 2-bromo-1-methyl derivative **51**, which underwent reaction with ammonia to give **50** (method N). Conversion of **33** to the 9-thione **53** with phosphorus pentasulfide in pyridine (method O), methylation to **54** (method L), and reaction with ammonia provided the 9(4*H*)-imine **55** (method N). Manganese dioxide dehydrogenation of **33** gave **47** in low yield (method P).

There is literature precedent for the exclusive formation of imidazo[1,2-*a*]pyrimidin-5-ones from the reaction of 2-aminoimidazolines with difunctional reagents.[16] While we contend that the products we obtained from the reaction of amines, 2-(methylthio)imidazolines, and ethyl oximinocyanoacetate are also imidazo[1,2-*a*]pyrimidin-5-ones and not the isomeric imidazo[1,2-*a*]pyrimidin-7-ones, e.g., **73** (Scheme III), it is difficult to unequivocally verify the structures on the basis of their spectra. The possibility of isomer formation is increased when unsymmetrically substituted 2-(methylthio)imidazolines are employed as starting materials. Our isolation of single products (e.g., 8) in such cases can be rationalized as being due to steric control of the reaction which results in regioselective acylation of the imidazoline ring nitrogen remote from the alkyl substituents. Again, however, spectral assignment of structure is not unambiguous. Since the ring fusion and position of substituents of the ensuing imidazopurinones is determined by the structural features of their corresponding imidazopyrimidinone precursors, we sought to ellucidate the structural ambiguities via chemical means.

As shown in Scheme III, hydrolysis of **33** in aqueous base gave a low (3.5%) yield of the aminoethyl compound **73**. The latter was also isolated in low yield as a byproduct in a large-scale (2 kg) preparation of **33** from **5**. The methylene protons at side-chain carbons 1 and 2 of **73** were observed as triplets at 4.0 and 2.91 ppm, respectively (J = 6.5 Hz), in the ^{1}H NMR. The triplet at 2.91 ppm was broadened considerably in acidic solution due to interaction with the NH_3^+ protons. Heating **73** in 2-hydroxyethyl ether at 240 °C afforded **75**, an isomer of **33**. Compounds **33** and **75** are easily distinguished from each other by their ^{1}H NMR and IR spectra. In addition to aromatic protons, the ^{1}H NMR spectrum of **33** shows a one-proton singlet at 7.84 ppm (2-C*H*=N), a two-proton singlet at 5.04 ppm (benzyl CH_2), and a four-proton multiplet centered at 3.84 ppm (imidazoline ring protons). The corresponding protons of the isomer **75** appear at 7.64, 5.10, and 3.95 ppm, respectively. The infrared spectrum of **33** exhibits a carbonyl band at 1695 and C=N bands at 1635 and 1555 cm^{-1}; these bands appear at 1710, 1660, and 1550 cm^{-1} in the spectrum of **75**. The stepwise isomerization of **33** to **75** supports the imidazo[1,2-*a*]purinone structural assignment for **33** and related compounds; hydrolytic ring opening of the imidazo[2,1-*b*]purinone **77** would result in aminoethyl compound **78**, which could only revert to **77** upon recyclization.

Hydrolysis of the 6,6-dimethyl compound **40** gave a 4% yield of **74**. The side-chain methylene protons of **74** occurred as a singlet at 3.90 ppm and the signal was not effected by protonation. This shows that the *gem*-dimethyl substituents of **74** are upon the amino-substituted carbon and, hence, the latter must be derived from a 6-substituted imidazo[1,2-*a*]purinone.

While the chemical evidence cited above is not completely unequivocal, it is highly supportive of our structural assignments.

Structure–Activity Relationships. Table III lists biological data for the various 6,7-dihydroimidazo[1,2-*a*]-purin-9(4*H*)-one derivatives. In vitro bronchodilator activity was determined as that concentration of drug producing a 50% decrease in the spontaneous tonus of an isolated guinea pig tracheal spiral preparation. In vivo bronchodilator activity was assessed by the ability of a test drug administered intraduodenally (iduo) to antagonize methacholine (intravenous) induced bronchospasm in spontaneously respiring male Harlan–Sprague–Dawley rats. Passive cutaneous anaphylaxis (PCA) ED_{50} values were determined following oral administration to egg albumin sensitized, male, Harlan–Sprague–Dawley rats. Since PCA activity can only be construed as indicating cutaneous antiallergic activity, an additional test was used to determine "antiasthma" effects for selected analogues. Antiallergic/bronchodilator activity was determined as an intraduodenal ED_{50} dose inhibiting an allergen-induced (intravenous egg albumin) bronchospasm in male, Harlan–Wister rats.

For this discussion, the 4-chlorobenzyl analogue (**33**) will be used as a standard of comparison, since it showed a good level of activity in all four biological tests shown in Table

(16) H. Stähle, *Med. Chem., Proc. Int. Symp., Main Sect., 4th, 1974*, 1974, pp 95–99.

Table III. Antiallergic and Bronchodilator Activity of Substituted 6,7-Dihydroimidazo[1,2-*a*]purin-9(4*H*)-ones and Related Compounds

	bronchodilator act.		antiallergic act.	
compd	in vitro IC_{50}, μg/mL	in vivo (rat) ED_{50}: mean ± SE, mg/kg iduo	PCA[a,b] (rat) ED_{50}: mean ± SE, mg/kg po	allergic bronchospasm[a] (rat) ED_{50}: mean ± SE, mg/kg iduo
33	14	5.2 ± 1.3	33.7 ± 14.4	0.9 ± 0.5
34	5.3	1.30 ± 0.4		
35	13	>10[c]		
36	2.9	8.8 ± 1.1	I at 25[d]	
37	15.5	>10[c]		
38	8.4	5.7 ± 2.7		
39	3.7	2.1 ± 0.58	10.6 ± 4.4	1.5 ± 0.3
40	14.3	6.3 ± 2.6	35.3 ± 8.8	3.0 ± 1.4
41	1.1	5.0 ± 1.3	35.5 ± 13.8	2.8 ± 1.3
42	0.7	3.0 ± 0.8	28.5 ± 8.3	1.7 ± 0.5
43	2.3	1.1 ± 0.27	28.9 ± 11.2	3.4 ± 1.1
44	4.4	1.0 ± 0.34	30.2 ± 15.4	1.23 ± 0.34
45	1.7	7.3 ± 2.0	44.4 ± 11.4	1.08 ± 0.26
46	7.9	7.5 ± 2.0	38.7 ± 18.4	14.2 ± 3.7
47	4.1	8.1 ± 4.0		
49	13.7	>30[c]		
50	4.8	>30[c]		
51	3.9	I at 10		
52	54			
53	5.1	>30[c]		
54	8.1	>30[c]		
55	19.8	>30[c]		
56	12.5			
57	49			
58	12.5			
59	17.5			
60	22.5		I at 25	
61	128		>25[c]	
62	53.0		I at 25	
63	6.3		>25[c]	
64	2.8	3.3 ± 0.8	33.9 ± 9.8	5.4 ± 2.4
65	16.7			
66	11.5	9.5 ± 4.2	15.3 ± 7.3	
67	36.6			
68	14	4.6 ± 1.0	>30[c]	
69	10		I at 25	
70	24.5		I at 25	
71	>200[c]	>10[c]	17.7 ± 3.0	
72	~200[c]	>30[c]	>30[c]	
75		>50[c]		
theophylline	18.5	19.2 ± 0.64	41.5 ± 11.5	22.4 ± 5.9

[a] Drug administered 15 min prior to antigen challenge. [b] Under these test conditions, the intravenous ED_{50} for disodium cromoglycate was 0.6 (0.15–2.6) mg/kg. [c] Statistically significant inhibition of <50% at the stated dose. [d] Inactive at a dose of 25 mg/kg.

III. Compound **33** shows a similar level of in vitro bronchodilator activity and oral PCA activity to theophylline. However, compound **33** shows a 3.7-fold enhancement of intraduodenal bronchodilator activity against methacholine and a 24.9-fold enhancement of intraduodenal antiallergic/bronchodilator activity against antigen when compared to theophylline.

Because it was felt that alkyl substitution in the 2, 6, and 7 positions might markedly affect the biotransformation of these imidazo[1,2-*a*]purin-9(4*H*)-one derivatives, several such analogues were prepared, keeping the 4-chlorobenzyl group constant (**34**, **35**, **38–46**). A similar level of bronchodilator and, in most cases, PCA activity was retained by methyl substitution in the 1 (**36**), 2 (**34**), 6 (**39**), 6,6 (**40**), and 6,7 (**41**, **42**) positions. Combinations of methyl groups as seen in **38** and **43–46** gave compounds with similar potencies in several or all of the four test systems; however, alkyl groups larger than methyl in the 1 and 2 positions (1-*n*-butyl in **37** and 2-ethyl in **35**) significantly reduced intraduodenal bronchodilator activity. Thus, methyl substitution at potential sites of metabolism on **33** did not significantly affect the overall profle of activity. As a general rule, the 2-methyl analogues were more toxic (acute mouse toxicity) than the corresponding compounds not substituted in the 2 position. The 6,7-dehydro analogue **47** also retained potent bronchodilator activity.

Compounds **49–52** were prepared to determined the affects of substituents other than alkyl in the 2 position. The 2-(methylthio) analogue (**49**) retained in vitro bronchodilator activity but showed significantly reduced in vivo bronchodilator activity, as did the corresponding 2-amino-1-methyl and 2-bromo-1-methyl analogues (**50** and **51**, respectively). The 2-bromo-1-*H* analogue **52** showed considerably reduced activity in vitro.

Sulfur and nitrogen were evaluated as carbonyl oxygen replacements in the 9(4*H*)-imine (**55**) and 9(4*H*)-thione (**53**) derivatives. Both compounds showed significant in vitro but little in vivo bronchodilator activity. Similar results were obtained with the intermediate 9-(methylthio) derivative **54**.

Biological activity was strongly influenced by the nature of the 4-substituent. None of the other 4-benzyl analogues of **33** (**56–61**) showed any enhancement of in vitro bronchodilator activity. It is of interest to note that the unsubstituted benzyl analogue **60** was inactive in the PCA screen. Both the 4-(2-pyridylmethyl) analogue **61** and the

Table IV. Comparative Potencies of 33, Theophylline, and DSCG as Derived from Various Tests in Rats

test	drug	route	ED_{50}, mg/kg	rel potency
PCA reaction	33	po	33.7	1.4
	theophylline[a]		47.2	1.0
	33	iv	2.4	>3
	theophylline[a]		>7.9	1.0
	DSCG		0.29	>27
	33	iduo	8.4	1.4
	theophylline[a]		11.7	1.0
metacholine-induced bronchospasm	33	iduo	5.2	3.7
	theophylline[a]		19.2	1.0
antigen-induced bronchospasm	33	iduo	0.9	24.9
	theophylline[a]		22.4	1.0
antigen-induced histamine release from isolated mast cells	33	in vitro	490 μM[b]	5.1
	theophylline		2500 μM[b]	1.0
	DSCG		8 μM[b]	312.5

[a] Aminophylline, dose expressed as equivalent of theophylline free acid. [b] IC_{50} values.

Table V. Inhibition of Rat Bronchial Smooth-Muscle Phosphodiesterase by 33, Theophylline, and Papaverine

drug	K_i (cAMP), M	K_i (cGMP), M	K_i (cGMP)/K_i (cAMP), M
33	7.0×10^{-5}	2.60×10^{-4}	4.4
theophylline	5.23×10^{-4}	1.15×10^{-3}	2.6
papaverine	8.00×10^{-6}		

2-phenylethyl homologue **62** showed reduced in vitro bronchodilator and PCA activity.

Several of the 4-alkyl analogues (**63**–**70**) showed significant antiallergic and potent bronchodilator activities. For instance, the 2-methyl-4-isobutyl analogue **64** showed activity comparable to **33** in all four tests. Activity was significantly decreased in the 4-(*n*-octyl) derivative **70**.

The corresponding *v*-triazolo[4,5-*d*]pyrimidin-9(4*H*)-ones **71** and **72** showed significantly diminished in vitro bronchodilator activity; however, **71** retained potent oral PCA activity. Compound **75**, an isomer of **33**, showed considerably less bronchodilator activity than did the latter.

In summary, 6,7-dihydroimidazo[1,2-*a*]purin-9(4*H*)-one derivatives showed improved bronchodilator and antiallergic effects when compared to theophylline. The parent imidazopurine nucleus is tolerant to a variety of substitutions, with either alkyl or benzyl groups in the 4 position providing useful bronchodilator and antiallergic activity. Substitution of small alkyl groups at possible sites of metabolism (2, 6, and 7 positions) does not significantly diminish activity. Conversion of the imidazopurinone system to the more acidic triazolopyrimidinone system leads to diminished bronchodilator activity with retention of antiallergic activity.

4-[(4-Chlorophenyl)methyl]-6,7-dihydro-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (33). Compound **33** was selected for further biological evaluation and comparison with theophylline and disodium cromoglycate (DSCG). Figure 1 compares the dose–response curves for **33** for inhibition of allergen-induced bronchospasm, methacholine-induced bronchospasm, and the PCA reaction. The drug was administered by the intraduodenal route for all three studies. Compound **33** exhibited similar ED_{50} values for the inhibition of metacholine-induced bronchospasm (5.2 ± 1.3 mg/kg) and the PCA reaction (8.4 ± 1.1 mg/kg), while a lower ED_{50} value was obtained for inhibition of antigen-induced bronchospasm (0.9 ± 0.5 mg/kg). Inhibition of allergen-induced bronchospasm by **33** at lower doses than are required for the other two tests may relate to the dual mechanism of action of the compound. As may be seen from Table IV, theophylline (as aminophylline) showed a similar ED_{50} to **33** for inhibition of the PCA reaction but was significantly less active in inhibition of both methacholine and allergen-induced bronchospasms. Table IV compares the potencies of **33**, theophylline, and DSCG when administered by various routes in several rat tests. The intrinsic antiallergic activity of **33**, as evidenced by its intravenous potency in the rat PCA test (ED_{50} = 2.4 mg/kg), lies between that of DSCG (0.29 mg/kg) and theophylline (>7.9 mg/kg).

The relative potencies of **33**, theophylline, and DSCG as inhibitors of the antigen-induced release of histamine from passively sensitized rat peritoneal mast cells were determined. Although the rank-order potency in this test is similar to that observed for inhibition of the PCA reaction, DSCG was relatively much more potent than **33** and theophylline in vitro. All three compounds may exert their antiallergic activity through inhibition of mediator release from sensitized cells; the mechanism of mediator release inhibition may involve the inhibition of airway cAMP phosphodiesterase by these drugs. As shown in Table V, both **33** and theophylline inhibited cAMP and cGMP phosphodiesterases from rat whole lung homogenate noncompetitively as determined from the respective Dixon plots. DSCG has also been shown to be a potent

Table VI. Duration of Antiallergic Effects of 33 and Theophylline After Oral and Intraduodenal Administration to Anesthetized Rats

drug	dose, mg/kg (route)[a]	% inhibn of PCA reaction[b] 15 min	3 h	6 h	24 h
33	8 (iduo)	46.1 ± 5.5[d]	42.1 ± 6.6[d]		
	33 (po)	52.1 ± 4.7[d]	36.5 ± 4.3[d]	36.7 ± 5.2[d]	11.5 ± 9.6
theophylline	11.7 (iduo)[c]	42.4 ± 6.0[d]	25.1 ± 9.3		
	47.6 (po)[c]	51.9 ± 3.3[d]	52.5 ± 2.0[d]	13.0 ± 9.0	0 ± 8.3

[a] Approximate ED_{50} doses. [b] Mean ± SE for 10 animals per dose level. [c] Equivalent of free acid; animals were dosed with aminophylline. [d] Significantly different from zero at $p \leq 0.05$ by Dunnett's test.

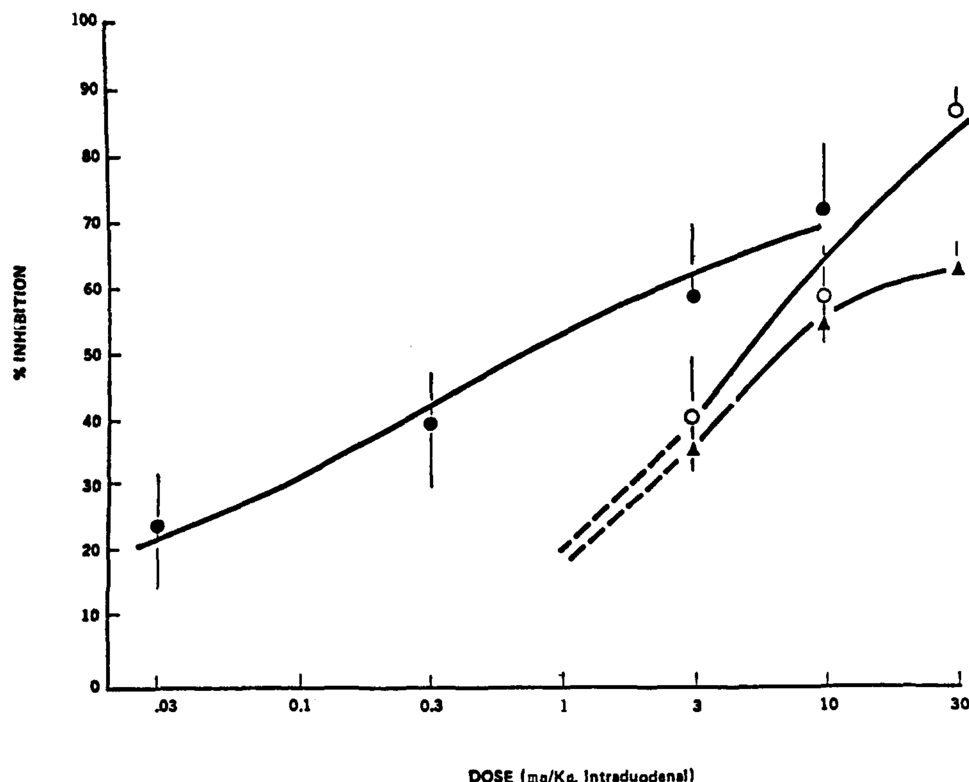


Figure 1. The effects of 33 on allergen-induced bronchospasm (●), methacholine-induced bronchospasm (○), and the PCA reaction (▲) in the rat. Drug effects are 15 min (or within 30 min) after intraduodenal administration to anesthetized rats. Vertical bars represent standard errors of the means.

inhibitor of cAMP phosphodiesterase.[17]

Table VI compares the duration of effects of ED_{50} doses of **33** and theophylline in the rat PCA test. Compound **33** evidenced a significantly longer duration of action by both oral and intraduodenal routes than did theophylline. This observation suggests that the imidazopurinone nucleus may be less susceptible to biotransformation than the corresponding xanthine nucleus or that **33** is less readily eliminated than theophylline. Since compound **33** cannot readily undergo the facile "1-dealkylation" shown by theophylline, this may play a major role in its increased duration of action. Compound **33** may also have a slower rate of oral absorption than that of theophylline.

Mean arterial blood pressure and heart rate were monitored during the bronchodilator experiments. Compound **33** caused no significant changes in mean arterial blood pressure, while theophylline caused a small to moderate decrease in the parameter. Neither drug caused appreciable increases in heart rate. The effects of **33** and theophylline (aminophylline) on spontaneous motor activity of the rat, as measured by the annular cage method, were compared. Doses of 10, 20, 40, 80, and 160 mg/kg orally of **33** did not increase or decrease spontaneous motor activity in the rat. However, aminophylline exhibited stimulant activity in that spontaneous motor activity was significantly increased at 28 mg/kg orally. Thus, **33** is devoid of CNS stimulant activity at levels well beyond effective therapeutic doses in rats.

Experimental Section

Chemistry. Melting points were taken in capillaries utilizing a Thomas-Hoover melting point apparatus and are uncorrected. The IR, NMR, and mass spectral data are consistent with the assigned structures. Analyses for C, H, and N were within ±0.4% of the calculated values unless otherwise noted in Tables I and II.

Method A. 7-Amino-2,3-dihydro-8-[(4-chlorophenyl)-methyl]-6-nitrosoimidazo[1,2-*a*]pyrimidin-5(8*H*)-one (4). To a solution of 4-chlorobenzylamine (62.30 g, 0.44 mol) in 500 mL of absolute EtOH was added 2-(methylthio)-2-imidazoline hydriodide (107.40 g, 0.44 mol). The mixture was heated at reflux for 2 h (**hood!**). The solution was added while still hot to 1.76 mol of NaOEt in 1650 mL of absolute EtOH. To the resulting stirred, basic solution of 2-[[(4-chlorophenyl)methyl]amino]-2-imidazoline was added ethyl oximinocyanoacetate[18] (61.85 g, 0.44 mol) in portions. The bright yellow solution was refluxed for 3 h and then cooled to room temperature. The yellow precipitate was collected, washed with *i*-PrOH, and partially air-dried. The damp sodium salt was dissolved in 2 L of H_2O and acidified with glacial HOAc. The bright pink precipitate was collected and oven-dried in vacuo at 100 °C to yield 103.05 g (77%) of pink powder, mp 239–241 °C dec. Recrystallization from DMF–EtOH gave red crystals, mp 241 °C.

Method B. 7-Amino-8-[(4-chlorophenyl)methyl]-6-(formylamino)-2,3-dihydroimidazo[1,2-*a*]pyrimidin-5(8*H*)-one (5). A sample of 4 (40.50 g, 0.133 mol) was dissolved in 950 mL of 97% HCOOH and 25.0 g of 5% Pd/C–50% H_2O was added under CO_2. The mixture was reduced on a Parr hydrogenation apparatus. The catalyst was filtered (Celite), and the resulting colorless solution concentrated in vacuo to a thick syrup. The syrup dissolved in 500 mL of H_2O and was neutralized with concentrated NH_4OH with cooling. The off-white solid was filtered and air-dried to yield 41.90 g (98%), mp 272–275 °C dec. Recrystallization from MeOH–*i*-PrOH gave white crystals, mp 275 °C dec.

Method C. 7-Amino-8-[(4-fluorophenyl)methyl]-6-(formylamino)-2,3-dihydroimidazo[1,2-*a*]pyrimidin-5(8*H*)-one (18). To a stirred solution of nitroso derivative 17 (9.79 g, 0.034 mol) in 100 mL of 97% HCOOH at room temperature was added 15.00 g (0.086 mol) of $Na_2S_2O_4$ in portions over about 5 min. The solution turns from dark purple to light yellow during the resulting exothermic reaction, and some yellow precipitate forms. The

(17) A. C. Roy and B. T. Warren, *Biochem. Pharmacol.*, 23, 917 (1974).

(18) C. O. Parker, *Tetrahedron*, 17, 109 (1962).

mixture was stirred for 10 min and then concentration in vacuo to about 25 mL. The residue was dissolved in 150 mL of H_2O, filtered (Celite), and neutralized with concentrated NH_4OH. The white precipitate was collected, slurried in hot MeOH, and filtered. Oven drying in vacuo yielded 9.25 g (90%) of white solid, mp 248–250 °C. Recrystallization from MeOH yielded white crystals, mp 262 °C dec.

Method D. 4-[(4-Chlorophenyl)methyl]-6,7-dihydro-6-methyl-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (39). To a stirred solution of NaOH (16 g, 0.40 mol) in 1.5 L of H_2O was added 7 (120 g, 0.36 mol). The mixture was heated until solution occurred. The solution was filtered (Celite), and the filtrate was cooled and neutralized with HOAc. Stirring the resultant gum with *i*-PrOH gave 65 g (57%) of solid. Recrystallization from methoxyethanol gave a beige solid, mp 259–260.5 °C dec.

Method E. 4-[(4-Chlorophenyl)methyl]-6,7-dihydro-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (33). A suspension of formylamino derivative 5 (45.88 g, 0.14 mol) in a mixture of 130 mL of Ac_2O and 65 mL of $(OEt)_3CH$ was refluxed for 5 h (a solution formed after 30 min). Concentration in vacuo to ~25% of the original volume produced an oil which dissolved in 300 mL of H_2O. The mixture was treated with Darco G-60 and filtered, and the clear filtrate was neutralized with concentrated NH_4OH. The white precipitate was filtered and oven dried in vacuo to yield 28.06 g (66%) of off-white solid, mp 285–290 °C. Recrystallization from DMF–*i*-PrOH gave off-white crystals, mp 289–293 °C.

Method F. Pyrolytic Preparation of 33. A slurry of 4 (7.20 g, 0.022 mol) in a small volume of DMF was inserted in an oil bath at 260 °C. The DMF evaporated rapidly, and the residual cake was heated for 12 min with constant agitation. The residual light-brown solid, mp 280–285 °C, weighed 6.36 g (93%). Recrystallization from DMF gave material identical with that obtained via method E.

Method G. 4-[(4-Chlorophenyl)methyl]-2-ethyl-6,7-dihydroimidazo[1,2-*a*]purin-9(4*H*)-one (35). A mixture of 4 (25.00 g, 0.078 mol) and 50 mL of dry pyridine in 50 mL of propionic anhydride was heated at reflux for 3 h. When the mixture cooled, a white solid precipitated, MeCN was added, and the white solid was filtered and air-dried to give 16.46 g (64%) of crystals, mp 278–279 °C.

Method H. 2-Ethyl-6,7-dihydro-4-(2-methylpropyl)-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (65). A mixture of 25 (9.5 g, 0.04 mol) in MeOH was reduced rapidly using a Parr apparatus on 5% palladium on carbon. The catalyst was removed and the solution concentrated in vacuo. The residual diamine was immediately treated with propionic anhydride (25.5 mL) at reflux for 1 h. The solvent was removed under reduced pressure and the residue was triturated with MeCN to give 3.67 g of off-white solid. Recrystallization from MeCN gave 2.5 g (25%) of white crystals, mp 242–243 °C.

Method I. 4-[(4-Chlorophenyl)methyl]-6,7-dihydro-1,6-dimethyl-1*H*-imidazo[1,2-*a*]purin-9(4*H*)-one Hydrate (43). To a solution of 39 (2.8 g, 0.0089 mol) and NaOH (0.8 g, 0.01 mol of 50% solution) in 30 mL of H_2O and 5 mL of EtOH was added iodomethane (3.2 g, 0.023 mol), and the mixture was stirred at room temperature overnight. The insolubles were collected, washed with H_2O, and dried to give 1.8 g of solid. The solid was recrystallized from *i*-PrOAc to give 1.5 g (55%) of product, mp 151.5–152.5 °C.

Method J. 4-[(4-Fluorophenyl)methyl]-6,7-dihydro-3*H*-imidazo[2′,1′:5,6]-*v*-triazolo[4,5-*d*]pyrimidin-9(4*H*)-one (71). A solution of 2.0 g (0.0069 mol) of 17 in 100 mL of 1 N HCl was hydrogenated with 2 g of 5% palladium on carbon. The catalyst was removed and the solution cooled to 0 °C. A solution of 0.48 g (0.0069 mol) of $NaNO_2$ in 20 mL of H_2O was added dropwise. The mixture was stirred for 1 h and concentrated in vacuo. The solid residue was dissolved in a minimum volume of H_2O and neutralized with NH_3 to give 1.5 g of solid. This material was dissolved in 1 N NaOH and precipitated with HOAc to give 0.56 g (29%) of yellow crystals, mp 294–295 °C dec.

Method K. 4-[(4-Chlorophenyl)methyl]-2,3,6,7-tetrahydro-2-thioxo-1*H*-imidazo[1,2-*a*]purin-9(4*H*)-one Monohydrate (48). A mixture of 5% palladium on carbon (1 g) and 4 (2.0 g, 0.0066 mol) in MeOH (50 mL) was hydrogenated until H_2 uptake ceased. The catalyst was removed and carbon disulfide (0.5 g, 0.0066 mol) was added. The solution was refluxed for 4 h and stirred at room temperature for 16 h. The mixture was concentrated in vacuo and the residue was suspended in H_2O (50 mL). The mixture was refluxed for 6 h and cooled to give 1.55 g of solid. The solid was dissolved in dilute base (Darco) and reprecipitated with HOAc to give 1.18 g (67%) of product, mp 232–235 °C.

Method L. 4-[(4-Chlorophenyl)methyl]-6,7-dihydro-2-(methylthio)-3*H*-imidazo[1,2-*a*]purin-9(5*H*)-one (49). To a solution of 48 (2.4 g, 0.0068 mol) in 0.1 N NaOH (85 mL) was added iodomethane (1.94 g, 0.0136 mol), and the mixture was stirred at room temperature for 16 h. The insolubles were collected and recrystallized from methoxyethanol to give 1.3 g (55%) of product, mp 296–297 °C.

Method M. 2-Bromo-4-[(4-chlorophenyl)methyl]-6,7-dihydro-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one Hydrobromide (52). Bromine (1.60 g, 0.010 mol) was added to a solution of 2.00 g (0.0066 mol) of 33 in 10 mL of HOAc, and the resulting solution was heated on a steam bath for 10 min. Yellow flakes precipitated and were filtered and air-dried to give 3.29 g (>100%, contains Br_2) of solid, mp 212 °C dec. Heating a suspension of the material in CH_3CN gave a white powder, mp 248 °C dec. Recrystallization from DMF–MeCN gave fine, white needles, mp 274 °C dec.

Method N. 2-Amino-4-[(4-chlorophenyl)methyl]-6,7-dihydro-1-methyl-1*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (50). A suspension of 0.50 g (0.0013 mol) of 51 in 50 mL of EtOH saturated with NH_3 was heated at 150 °C overnight in a bomb to give 0.5 g of solid, mp 205–209 °C dec. Recrystallization from MeOH gave 0.34 g (80%) of off-white solid, mp 259–261 °C dec.

Method O. 4-[(4-Chlorophenyl)methyl]-6,7-dihydro-3*H*-imidazo[1,2-*a*]purine-9(4*H*)-thione (53). A mixture of 6.02 g (0.02 mol) of 33 and 12.0 g (0.054 mol) of P_2S_5 in 125 mL of C_5H_5N was stirred at reflux for 5 h. The mixture was cooled and poured in H_2O (1 L) to give a brown solid. This material was dissolved in dilute NaOH (Darco) and reprecipitated with HOAc to give 3.27 g (52%) of yellow solid, mp 248–251 °C. Recrystallization from DMF–MeCN gave a buff solid, mp 257–260 °C.

Method P. 4-[(4-Chlorophenyl)methyl]-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (47). A suspension of 33 (5 g, 0.017 mol) and activated MnO_2 (13 g) in xylene (500 mL) was refluxed for 5 days. The mixture was concentrated in vacuo and the residue was extracted with acetone (700 mL). The acetone was concentrated in vacuo and the residue was triturated with *i*-PrOH to give 0.4 g of solid. This material was dissolved in dilute NaOH, reprecipitated with HOAc, and then recrystallized from EtOH to give 0.25 g (5%) of solid, mp 291–293 °C dec.

Method Q. *trans*- and *cis*-4-[(4-Chlorophenyl)methyl]-6,7-dihydro-6,7-dimethyl-3*H*-imidazo[1,2-*a*]purin-9(4*H*)-one (41 and 42). The nitroso derivative 10 (120.0 g, 0.36 mol) was converted using methods C and D to a mixture of trans and cis geometric isomers 41 and 42 [88.0 g (74%)]. Two recrystallizations from 95% EtOH gave 26.0 g of pure *trans*-41, mp 260–261 °C. The filtrates from the recrystallizations were combined and concentrated in vacuo. A portion of the resulting material was chromatographed on a silica gel column eluted with CH_2Cl_2–5% MeOH. Fractions shown to contain only 42 by TLC were combined, evaporated in vacuo, and recrystallized from *i*-PrOH to give 2.50 g of pure *cis*-42, mp 244–246 °C.

Method R. 1-(2-Aminoethyl)-3-[(4-chlorophenyl)methyl]-7(9*H*)-purine-2,6(1*H*,3*H*)-dione (73). A solution of 33·HCl (3.38 g, 0.01 mol) in 1 N NaOH (50 mL) was heated with stirring at 80 °C for 18 h. After cooling, 1 N HCl was added until pH 7, and the resulting precipitate was collected by filtration. A gummy solid, 0.22 g, came out of the filtrate after partial concentration and cooling. Recrystallization of the crude material from *i*-PrOH gave 0.11 g (3.5%) of white solid, mp 217–219 °C. Anal. ($C_{14}H_{14}ClN_5O_2$) C, H, N.

1-(2-Amino-2,2-dimethylethyl)-3-[(4-chlorophenyl)methyl]-7(9*H*)-purine-2,6(1*H*,3*H*)-dione (74). The preparation and workup for this compound is identical with that described for 73. From 40 (3.3 g, 0.01 mol) was obtained 0.2 g of crude 74 free base. Treatment with ethanolic HCl afforded 74·HCl (0.15 g, 4%), mp 295–300 °C. Anal. ($C_{16}H_{18}ClN_5O_2$·HCl) C, H, N.

Method S. 4-[(4-Chlorophenyl)methyl]-7,8-dihydro-3*H*-imidazo[2,1-*i*]purin-5(4*H*)-one (75). A solution of 73 (6.0 g, 0.019 mol) in 50 mL of 2-hydroxyethyl ether was heated at 240 °C for 5 min. The solution was cooled and diluted with H_2O. The

dark solid was collected and recrystallized from DMF–i-PrOH to give 2 g (35%) of tan solid, mp 309–312 °C. Anal. ($C_{14}H_{12}ClN_5O$) C, H, N.

Isolated Guinea Pig Tracheal Spiral Test. The method of Dungan and Lish[19] was used to determine that concentration of drug producing a 50% decrease in the spontaneous tonus of an isolated guinea pig tracheal spiral.

An adult English short-hair guinea pig (either sex, body weight 400–600 g) was killed by cervical dislocation, and the trachea was immediately exposed, excised, and divested of extraneous tissue. The tissue was cut spirally and suspended vertically in a tissue chamber containing continuously oxygenated, modified Tyrode's solution, maintained at 37.5 °C. One end of the spiral was attached in a fixed position to a glass rod in the bottom of the bath chamber while the upper, free end was threaded to a tension transducer connected to a polygraph, which afforded permanent tracings of the electrical analogues of relaxation or contraction of the tracheal smooth muscle. A sustained 1.0-g tension was exerted on the tissue throughout the test, and an equilibration period of 90–120 min was allowed for development of maximal spontaneous tonus prior to the addition of drug solution. Fifteen minutes after test drug addition, or a longer interval if necessary to observe maximal intrinsic test drug effect, *dl*-isoproterenol hydrochloride (0.01 μg/mL) was added to the tissue medium. In 12 control experiments, this concentration of isoproterenol produced 67% of maximal tracheal relaxation. Fifteen minutes after isoproterenol addition, the tissue was exposed to papaverine hydrochloride (10 μg/mL), and the experiment was terminated 20 min later to determine maximum tracheal relaxation. Each tracheal spiral was used for one test drug concentration only, and a minimum of three trials was obtained for each concentration.

Drug (test drug and/or isoproterenol) induced relaxation of a tracheal spiral was expressed as a percentage of that induced in the same spiral by the supramaximal papaverine concentration. The effect of prior β-adrenergic blockade on the tracheal relaxant action of a test drug was determined by exposure of the trachea (15-min interval) to a β-adrenergic blocking concentration of sotalol hydrochloride (10 μg/mL) before the addition of the test drug. Solutions of each test agent employed were added to the tissue medium at a constant volume dose of 0.02 mL/mL bath fluid; all drug solution concentrations are expressed as μg/mL bath fluid. Sufficient dose–response data were attained to permit statistical estimations (regression analyses) of test drug concentrations causing 50% of maximal tracheal relaxation.

Inhibition of Methacholine Bronchospasm in Rats. Male, Harlan–Sprague–Dawley strain rats, weighing 150–250 g each, were anesthetized with urethane (1.5 g/kg, ip) and prepared for physiologic recording. The trachea was cannulated with a 2-cm length of P.E. no. 200 tubing and connected to a glass t-tube of 1.8-mm i.d., which in turn was connected to a Statham P23-BB pressure transducer through a plastic vial-moisture trap containing $CaCl_2$ to obtain recordings of pulmonary ventilation pressure (PVP; inspiratory–expiratory pressure). A 3-cm segment of a 25-μL Corning sampling pipet inserted into the open side-arm of the t-tube served to partially restrict air flow into and out of the system during an animal's spontaneous breathing. The restriction provided a larger PVP excursion within the system to facilitate recording and placed a greater workload on the animal's breathing mechanics to better demonstrate changes in airway caliber.

A jugular vein was cannulated with P.E. no. 50 tubing to which was connected a 10-mL syringe locked in a Harvard no. 940 infusion pump. This system provided for induction of bronchospasm via iv methacholine infusion. A carotid artery was cannulated with P.E. no. 50 tubing and connected to a Statham P23Db pressure transducer for recording of blood pressure and pulse rate. The tubing and transducer were filled with heparinized saline.

The duodenum was exposed through a small abdominal incision and positioned in the abdominal cavity with a loose-braided cotton ligature in preparation for administration of drugs. Solutions of drugs were injected into the lumen of the duodenum in 2 mL/kg volumes using a syringe and 23-gauge needle.

A 150 μg/mL solution of methacholine was infused intravenously at a rate of 0.2 mL/min (30 μg/min) until the PVP was reduced by 50% and then the infusion was stopped. The final reduction of PVP was measured and the duration (in seconds) of the infusion was recorded. After recovery of the pulmonary and cardiovascular parameters to normal values, a second infusion of methacholine was made for the duration of the first infusion. The final PVP reduction was then measured. If the percent reduction in PVP resulting from the second methacholine infusion did not vary by more than 5% from that of the first infusion, the preparation was considered acceptable for drug testing and a control percent bronchoconstriction was established by averaging the PVP values for the first and second infusions.

When the pulmonary and cardiovascular systems had fully recovered from the second methacholine infusion, test drug was administered into the duodenum and then, at 5-, 15-, and 30-min intervals thereafter, methacholine was again infused. The percent bronchoconstriction resulting at each postdrug time interval was compared to the control (predrug) average value and the percent inhibition of methacholine-induced bronchospasm by drug was calculated. Changes in PVP, arterial blood pressure, and heart rate after test drug administration were examined to derive the drugs's intrinsic pharmacologic actions on these parameters. A minimum of three animals was employed at each test drug dose.

Inhibition of Allergic Bronchospasm in Sensitized Rats. The methods employed were similar to those previously described for this model.[20,21] Male Harlan–Wistar rats (225–275 g) were sensitized by treatment with egg albumin (EA) intramuscularly (1 mg/rat) and *B. pertussis* vaccine ip (2×10^{10} organisms/rat), thus generating IgE antibody.[22]

Thirteen to fifteen days after sensitization, the rats were anesthetized with urethane (1.5 g/kg, ip), and the duodenum was exposed through a small abdominal incision. For id administration, drugs in solution or suspension were injected directly into the lumen of the duodenun. A cannulated jugular vein was used for iv drug or EA administration. A cannulated carotid artery was connected to a Statham P23Db pressure transducer for blood-pressure measurements. The trachea was cannulated and connected to a Statham P23-BB pressure transducer to obtain measurements of pulmonary ventilation.

Rats were challenged with a 2 mg/rat dose of EA (iv), and the changes in pulmonary ventilation pressure and mean arterial blood pressure arising from the ensuing anaphylactic syndrome were measured. Drugs were administered (iv or id) at various time intervals prior to antigen challenge, employing a minimum of four animals per drug dose. Drug effects on the antigen-induced syndrome were derived by comparison of responses in individual drug-treated animals to a mean response obtained in a separate group of non-drug-treated (control) animals.

Rat Passive Cutaneous Anaphylaxis (PCA) Test. This test was performed by adaptations of literature procedures.[22,23] Adult male Harlan–Sprague–Dawley or Wistar rats (225–275 g) were injected with egg albumin (EA) im (1 mg/rat) and with killed *B. pertussis* vaccine (2×10^{10} organisms/rat) ip. The animals were bled 10 to 12 days later to afford antisera containing predominantly IgE antibody.

Male Harlan–Sprague–Dawley or Wistar rats (100–150 g) were passively sensitized with 0.1-mL intradermal injections of two dilutions (1:2.5 and 1:10) of the antiserum in saline. The lesser dilution should yield a 20–25-mm diameter spot and the greater one a 10–15-mm diameter spot.

After a 48-h latent period, the rats were treated with either test compound or vehicle and then challenged at an appropriate time; a minimum of five animals was used for each test drug dose. The PCA response was induced by iv injection of EA (25 mg/kg) and Evans blue dye (25 mg/kg) in saline. The PCA response was scored by measuring the spot diameter on the excised and reflected skin 20 to 30 min after challenge. Ten minutes prior to sacrifice, histamine was injected intradermally at a dose of 0.1 mg/0.1 mL

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saline in order to rule out antihistaminic activity of the test compound. Percent inhibition was determined from the difference between mean spot diameters in test drug-treated and control animals.

Inhibition of Anaphylactic Histamine Release from Rat Peritoneal Mast Cells. Male Harlan–Sprague–Dawley rats (200–300 g) were decapitated and injected ip with 10 mL of Hank's balanced salt solution (HBSS) containing 0.1% human albumin and 5 mM potassium phosphate. The peritoneum was massaged for 1 min and the lavage fluid aspirated and centrifuged at 350 g. The supernatant was discarded and the cell pellets were suspended in 2 mL of rat antisera (prepared as in the PCA test) containing 0.1 mg of heparin. The cells were centrifuged after sensitization by incubation at 37 °C for 2 h with shaking. The resulting cell pellets were resuspended and diluted to the final working volume in buffered HBSS. Aliquots (1.5 mL) of the sensitized cell suspension were challenged with 0.5 mL of EA (80 mg/mL). Drugs were tested at several different concentrations by adding them simultaneously with the antigen challenge, employing four trials at each drug concentration. After antigen and drug additions, the cells were incubated for 10 min and histamine was assayed fluorimetrically. Percent inhibition was determined by comparison with histamine release in the absence of drug.

Inhibition of Rat Lung Phosphodiesterases. Inhibition of rat whole lung cAMP and cGMP phosphodiesterases was determined on rat, whole lung homogenate using the method of Thompson and Appleman.[24] The phosphodiesterase assays were carried out using two substrate concentrations (2×10^{-6} and 1×10^{-6} M) of cAMP and cGMP and using four drug substrate concentrations bracketing the K_i values. The drug concentrations were chosen on the basis of a preliminary experiment. The drugs were allowed to incubate with the enzymes for 30 min at 30 °C prior to the addition of the substrate. The results were plotted according to the method of Dixon.[25]

Spontaneous Motor Activity in Rats. Male Charles River/Sprague–Dawley rats were dosed orally with test compounds and placed in annular activity cages as previously described.[26] One hour later their activity scores were compared with those of simultaneously tested control animals, employing groups of 20 rats per drug dose. A compound was considered a stimulant or depressant if the log motor activity scores were 0.3 above or below that of the control (AED ± 0.3).

Acknowledgment. The authors gratefully acknowledge the interest and assistance of Dr. Dan Lednicer in the completion of this effort. We also acknowledge Charles M. Combs who provided analytical data and spectral interpretation.

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Antimalarials. 12. Preparation of Carbon Isosteres of Selected 4-Pyridinemethanols as Suppressive Antimalarials

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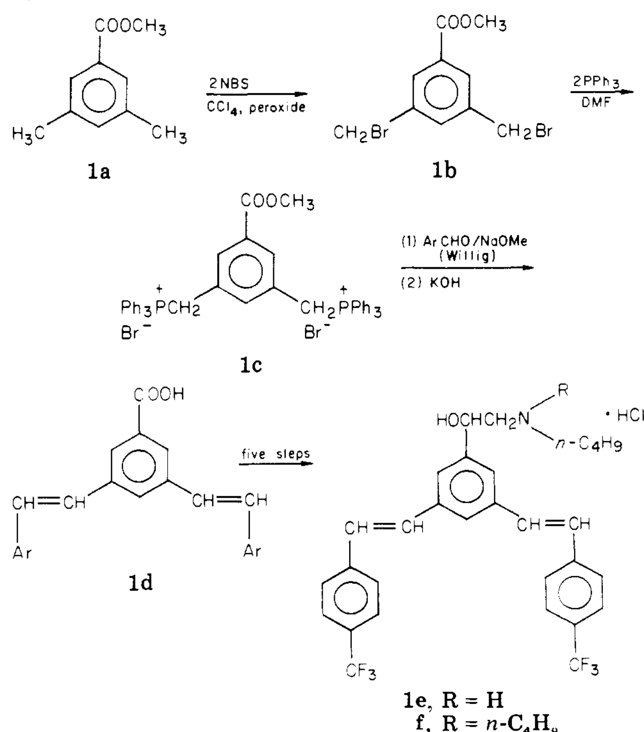
Four carbon isosteres related to the highly active 4-pyridylcarbinolamines were prepared and evaluated for suppressive antimalarial activity against *Plasmodium berghei* in mice. Three of the four examples possessed significant activity but were approximately one dose level less active than the corresponding pyridines.

We have previously reported the preparation of 2,6-bis(aryl)-4-pyridinemethanols[1] and 2,6-bis(styryl)-4-pyridinemethanols[2] which demonstrated a high level of suppressive antimalarial activity against *Plasmodium berghei* in mice. The four compounds reported herein were prepared in order to assess the effect of replacing the pyridine nitrogen atom with a carbon atom. Similar studies have previously been performed on the highly active 2-aryl-4-quinolinemethanols and corresponding carbon isosteres.[3]

Chemistry. Two examples were prepared in the 3,5-bis[4-(trifluoromethyl)styryl]phenylcarbinolamine series and the synthetic sequence is shown in Scheme I. Commericially available 3,5-dimethylbenzoic acid was converted to the methyl ester **1a**, which upon bromination with *N*-bromosuccinimide according to the procedure of Wenner[4] afforded methyl 3,5-bis(bromoethyl)benzoate (**1b**). Treatment of **1b** with triphenylphosphine[5a,b] afforded the bisphosphonium salt **1c**. Treatment of **1c** with 4-(trifluoromethyl)benzaldehyde under Wittig conditions followed by ester hydrolysis afforded 3,5-bis(styryl)benzoic acid (**1d**). Conversion of acid **1d** to the desired phenylcarbinolamines (**1e** and **1f**) involved the standard Lutz

Scheme I



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0022-2623/80/1823-1198$01.00/0 © 1980 American Chemical Society