evaporated to give a crude solid. This was chromatographed on silica gel to give 12 g (78%) of 17 and 18.

NMR indicated two major isomers, (2*Z*,4*E*)-17 and (2*E*,4*E*)-18, in a 4:1 ratio, respectively: ^{1}H NMR ($CDCl_3$) for 17 δ 6.95 (d, J = 15 Hz, 1 H, C-5 H), 6.72 (dd, J_{H-F} = 1 Hz, J_{H-H} = 15 Hz, 1 H, C-4 H), 6.58 (s, 1 H, aromatic H), 4.29 (q, 2 H, $COOCH_2CH_3$), 3.78 (s, 3 H, OCH_3), 2.35 (d, J_{H-F} = 2.8 Hz, 3 H, C-3 CH_3), 2.28, 2.23, and 2.13 (singlets, 9 H, 3 CH_3), 1.35 (t, 3 H, $COOCH_2CH_3$); ^{19}F NMR −128.1; ^{1}H NMR ($CDCl_3$) for 18 δ 7.46 (dd, J_{H-F} = 1.5 Hz, J_{H-H} = 18 Hz, 1 H, C-4 H), 6.92 (d, J = 18 Hz, 1 H, C-5 H), 6.55 (s, 1 H, aromatic), 4.29 (q, 2 H, $COOCH_2CH_3$), 2.28, 2.23, and 2.13 (singlets, 9 H, 3 CH_3), 2.13 (3 H, C-3 CH_3); ^{19}F NMR −123.6 ppm. Anal. ($C_{18}H_{23}FO_3$) C, H, F.

(2*Z*,4*E*)- and (2*E*,4*E*)-2-Fluoro-3-methyl-5-(4-methoxy-2,3,6-trimethylphenyl)-2,4-pentadien-1-ol (19 and 20). The ester mixture 17 and 18 (8 g, 26 mmol) was dissolved in 150 mL of ether and reduced with *i*-Bu_2AlH (see procedure above for **9**). The resulting mixture of isomers was separated by silica gel chromatography. The major component proved to be (2*Z*,4*E*)-**19**: mp 65–68 °C; ^{1}H NMR ($CDCl_3$) δ 6.71 (dd, J_{H-F} = 1 Hz, J_{H-H} = 16 Hz, 1 H, C-4 H), 6.54 (d, J = 16 Hz, 1 H, C-5 H), 4.36 (d, J_{H-F} = 22 Hz, 2 H, CH_2OH), 3.78 (s, 3 H, CH_3O), 2.27, 2.21, and 2.13 (singlets, 9 H, 3 CH_3), 1.90 (d, J_{H-F} = 2.9 Hz, 3 H, C-3 CH_3); ^{19}F NMR −117.9 ppm (t, J_{H-F} = 22 Hz). Anal. ($C_{16}H_{21}FO_2$) C, H, F.

The minor compound, (2*E*,4*E*)-**20**: ^{1}H NMR ($CDCl_3$) δ 6.63 (d, J = 16 Hz, C-5 H), 6.56 (s, 1 H, aromatic), 6.25 (dd, J_{H-H} = 16 Hz, J_{H-F} = 2 Hz, 1 H, C-4 H), 4.33 (d, J_{H-F} = 23 Hz, 2 H, CH_2OH), 3.76 (s, 3 H, OCH_3), 2.24, 2.18, and 2.11 (singlets, 9 H, 3 CH_3), 1.94 (d, J_{H-F} = 4 Hz, 3 H, C-3 CH_3). This is in agreement with that previously reported for **20**.[6]

(2*Z*,4*E*)-2-Fluoro-3-methyl-5-(4-methoxy-2,3,6-trimethylphenyl)-2,4-pentadien-1-al (21). A solution of 5 g (16 mmol) of **19** in 50 mL of ether was added to a suspension of 30 g of manganese dioxide in 400 mL of hexane. After 14 h, the mixture was filtered and the filtrate was evaporated to give a yellow solid. Recrystallization (ether) gave 4.1 g (82%) of **21**: NMR ($CDCl_3$) δ 9.83 (d, J_{H-F} = 16.5 Hz, 1 H, CHO), 7.13 (d, J = 16 Hz, 1 H, C-5 H), 6.79 (dd, J_{H-F} = 1.5 Hz, J_{H-H} = 16 Hz, 1 H, C-4 H), 6.58 (s, 1 H, aromatic), 3.78 (s, 3 H, CH_3O), 2.31 (d, 3 H, C-3 CH_3), 2.30, 2.23, and 2.13 (singlets, 9 H, 3 CH_3); ^{19}F NMR −133.5 ppm (d, J_{H-F} = 16.5 Hz). Anal. ($C_{16}H_{19}FO_2$) C, H, F.

Methyl (2*E*,4*E*,6*Z*,8*E*)-3,7-Dimethyl-6-fluoro-9-(2,3,6-trimethyl-4-methoxyphenyl)-2,4,6,8-nonatetraenoate (15f). A solution of 3 g (11 mmol) of **21** in 10 mL of dry DMF was added to the anion prepared from 3.5 g (13 mmol) of **11**[15] and 500 mg (12 mmol) of NaH in 15 mL of DMF at 0 °C. Workup as described[6] and silica gel chromatography provided 2 g (50%) of **15f**, which was identical in all respects with that previously reported.[6]

Acknowledgment. We thank Drs. P. W. Trown, L. J. Machlin, and their associates for carrying out the antipapilloma and hypervitaminosis A tests, respectively. We also thank the Physical Chemistry Department, Hoffmann-La Roche Inc., Nutley, NJ, for the spectral data (in particular, the NMR measurements) and microanalytical determinations reported in this work.

1-(Alkylamino)isochromans: Hypotensives with Peripheral and Central Activities

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A series of 1-[1-(3,4-dihydro-6,7-dimethoxy-1*H*-2-benzopyran-1-yl)alkyl]-4-arylpiperazines that shows hypotensive activity in the conscious rat has been investigated. Structure–activity relationships are described. A typical example that was investigated in greater detail is 1-[2-(3,4-dihydro-6,7-dimethoxy-1*H*-2-benzopyran-1-yl)ethyl]-4-(4-fluorophenyl)piperazine. This compound decreases sympathetic nerve activity recorded from the external carotid and splanchnic nerves of baroreceptor-denervated cats and, therefore, has a central component to its mechanism of action. It also blocks pressor effects of norepinephrine and phenylephrine and is thus an α-adrenergic antagonist. Binding data characterize this as α_1-adrenergic receptor blockade.

Humber has reported the synthesis of 1-(haloalkyl)-6,7-dimethoxyisochromans and the reaction of these halides with simple alphatic amines.[1] Recently, a patent issued to Takeda named as hypotensives the adducts of various arylpiperazines and 1-(1-bromomethyl)- and 1-(2-chloroethyl)-6,7-dimethoxyisochromans.[2] This prompted us to report our own work in this area. We now report as hypotensives a variety of 6,7-dimethoxyisochromans which are substituted at C-1 via a one- to three-carbon chain with arylpiperazines (compounds **12–18**, Scheme I). In addition, C-1 has been optionally substituted by *p*-fluorophenyl or methyl, and C-3 and C-4 are optionally substituted by methyls. Most of these piperazines are novel, although a few which are referenced are described in the Takeda patent of ref 2. These isochromans are hypotensives which lower blood pressure presumably by both peripheral and central α-adrenoreceptor blockade. Structure–activity relationships (SAR) have been developed. The mechanism of action of these isochromans has been investigated by both in vitro receptor binding analysis and whole animal pharmacology. In addition, receptor binding information is reported for 1-[2-(1,3,4,5-tetrahydro-7,8-dimethoxy-2-benzoxepin-1-yl)ethyl]-4-(4-fluorophenyl)piperazine, a compound which we have already described.[3]

Chemistry. Our synthetic strategy is outlined in Scheme I. The isochroman ring was formed by the method of Humber.[1,4] Compounds **5** and **6** are literature compounds,[1] while the analogues **7–11** are novel although well precedented. During the synthesis of **7** and **8**, methyl substitution at isochroman position C-3 and C-4 was established in the 2-(3,4-dimethoxyphenyl)ethanol intermediates **4** and **3b**. These methyl-substituted phenylethanols were then cyclized to the corresponding 3,3- and 4,4-dimethylisochromans **8** and **7**. Methyl and *p*-fluorophenyl substitutions at isochroman C-1 were made by reacting alcohol **3a** with 4-chloro-*p*-fluorobutyrophenone, 5-chloro-2-pentanone, or ethyl acetoacetate.[1,4] 4-Arylpiperazines reacted readily with the 1-(haloalkyl)-6,7-dimethoxyisochromans **5–11** to yield compounds of generic structures **12–18**. These adducts were evaluated for hy-

(1) Humber, L. G. *J. Heterocycl. Chem.* **1975**, *12*, 591.
(2) Takeda Chemical Industries U.S. Patent 4 066 648, Jan 3, 1978.
(3) TenBrink, R. E.; McCall, J. M.; Pals, D. T.; McCall, R. B.; Orley, J.; Humphrey, S. J.; Wendling, M. G. *J. Med. Chem.* **1981**, *24*, 64.
(4) Humber, L. G. *J. Heterocycl. Chem.* **1975**, *12*, 937.

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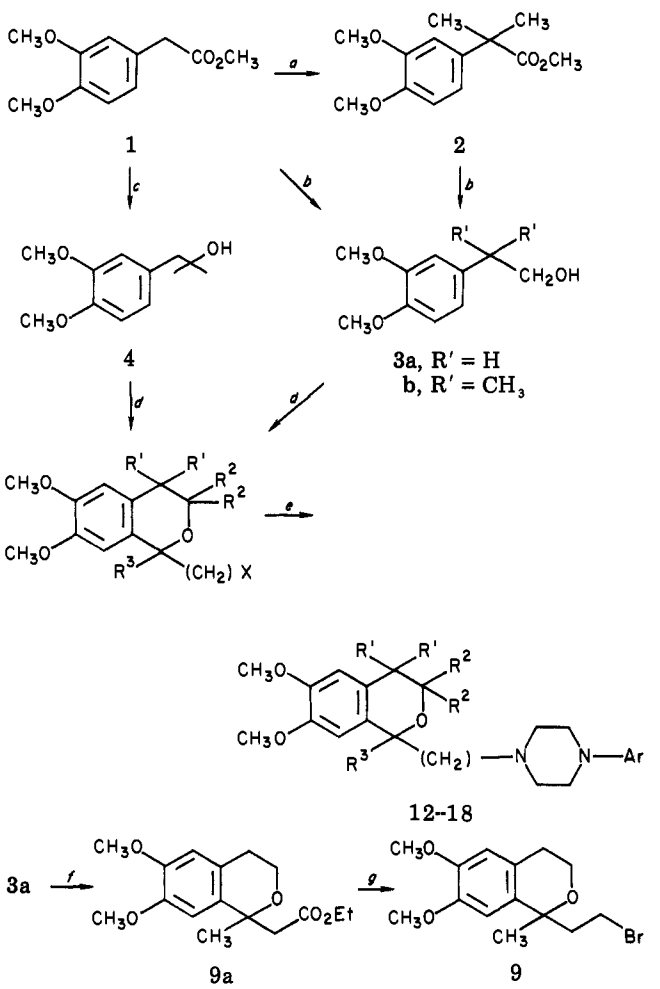
Scheme I. Synthesis of 1-(Haloalkyl)-6,7-dimethoxyisochromans



haloalkyls	alkylamines	n	R′	R^2	R^3
5 (X = Br)	12	1	H	H	H
6 (X = Cl)	13	2	H	H	H
7 (X = Cl)	14	2	Me	H	H
8 (X = Cl)	15	2	H	Me	H
9 (X = Br)	16	2	H	H	Me
10 (X = Cl)	17	3	H	H	Me
11 (X = Cl)	18	3	H	H	*p*-FPh

[a] LDA, MeI, LDA, MeI. [b] $BH_3 \cdot Me_2S$. [c] CH_3MgBr. [d] $X(CH_2)_nCH(OEt)_2$ or $X(CH_2)_nC(=O)R^3$, $BF_3 \cdot Et_2O$, CH_3NO_2. [e] HNR_2, $HOCH_2CH_2OH$. [f] $CH_3C(=O)CH_2CO_2Et$, $BF_3 \cdot Et_2O$. [g] $LiAlH_4$, Ph_3P, Br_2, DMF.

potensive activity. The majority of the isochroman arylpiperazines belong to structural groups 12–14. A few examples of groups 15–18 were prepared in order to establish structure–activity relationships (SAR).

Pharmacology. The oral hypotensive activity of compounds of type 12–18 was evaluated at 4 and 24 h in restrained, normotensive conscious rats.[5] This model is described under Experimental Section, and the results are reported in Table I. Certain rough generalizations can be made about the structure–activity relationships of these isochromans. Hypotensive responses at 4 h rather than 24 h after oral dosing are chosen for comparisons because they are less likely to reflect metabolic complication. Conclusions about SAR have been drawn only for isochromans which are identical except for a single structural

(5) Weeks, J. R.; Jones, J. A. *Proc. Soc. Exp. Biol. Med.* **1960**, *104*, 646.

Table I. Hypotensive Activity for 1-(Alkylamino)isochromans 12–18

Ar[a]	series[b]	ΔBP, mmHg (dose, mg/kg)[c,e] 12	13	14	15	16	17	18
phenyl	a	−20/−5 (50)[d]	−13/−10 (50)[d]	−16/−14 (50)		−17/−9 (50)	−4/−2 (50)	−19/−19 (50)
								−16/−10 (15)
2-chlorophenyl	b		+3/+2 (50)			−3/−1 (50)		
4-chlorophenyl	c	−13/−19 (50)[d]	−19/−21 (50)[d]	−13/−2 (50)		−8/−10 (50)	−8/−1 (50)	
3-chlorophenyl	d		+1/0 (50)	−4/−10 (50)				
4-fluorophenyl	e	0/−14 (50)	−20/−18 (15)		−16/−26 (50)	−16/−26 (50)	−10/−3 (50)	−22/−25 (50)
			−9/−3 (5)					−16/+3 (15)
2-methoxyphenyl	f	−15/−3 (50)			−17/−16 (50)	−9/−10 (50)		
					−15/+3 (15)			
3-(trifluoromethyl)phenyl	g	+2/+2 (50)	−2/+2 (50)	0/−1 (50)				
2-tolyl	h	−24/−5 (50)	−19/−9 (50)	−18/−28 (50)	−17/−20 (50)		−26/−21 (50)	
				−8/−10 (15)			−24/−5 (15)	
2-pyridinyl	i		−12/−5 (50)[d]				−11/−16 (50)	−19/−21 (50)
			−13/−14 (15)					

[a] The aryl substituent of arylpiperazines 12–18. [b] Each series letter designates all isochromans with a particular amine substituent. [c] ΔBP (dose) refers to the average change in mean arterial blood pressure observed 4 and 24 h after oral administration. The average changes observed at 4 h are entered prior to the 24-h changes and separated by a slash line. [d] Reported in ref 2. [e] Prazosin has been evaluated in this model and shows the following blood pressure effects: −26/+2 (1.5), −24/+3 (0.5), −14/+1 (0.05), −1/+2 (0.015).

Table II. Melting Points, Elemental Analyses, and Yields

no.	formula	anal.[a]	recrystn solvent	mp, °C	yield,[b] %
4	$C_{12}H_{18}O_3$	C, H	Et_2O/hexane	64-65	
9	$C_{14}H_{19}BrO_3$	C, H		oil	
7	$C_{15}H_{21}ClO_3$			50-52	
10	$C_{15}H_{21}ClO_3$			77-79	
11	$C_{20}H_{22}ClO_3F$		EtOAc/Skelly B	97-99	
12a[c]	$C_{22}H_{28}N_2O_2 \cdot 2HCl$	C, H, N	EtOH	259-273	60
13a[c]	$C_{23}H_{30}N_2O_3$	C, H, N	EtOAc/Skelly B	117-118	42
14a	$C_{25}H_{34}N_2O_3 \cdot 2H_2O$	C, H, N	MeOH/EtOAc	188-190	27
16a	$C_{24}H_{32}N_2O_3$	C, H, N	CH_2Cl_2/Skelly B	107.5-108.5	96
17a	$C_{25}H_{34}N_2O_3 \cdot HCl$	C, H, N, Cl	MeOH/EtOAc	209-211	27
18a	$C_{30}H_{35}FN_2O_3 \cdot HCl \cdot H_2O$	C, H, N, Cl	MeOH	153-155	25
13b	$C_{23}H_{29}N_2ClO_3 \cdot HCl \cdot MeOH$	C, H, N	MeOH/Et_2O	134.5-136	25
14b	$C_{25}H_{33}N_2ClO_3 \cdot 2HCl$		EtOAc/Et_2O	148-149.5	
16b	$C_{24}H_{31}ClN_2O_3 \cdot HCl$	C, H, N, Cl	EtOH	265-266	96
12c	$C_{22}H_{27}N_2O_3Cl$	C, H, N	Et_2O/Skelly B	104-105	23
13c[c]	$C_{23}H_{28}N_2ClO_3$	C, H, N	EtOAc/Skelly B	114-115	45
14c	$C_{25}H_{33}N_2O_3Cl \cdot HCl \cdot 0.5H_2O$	C, H, N	MeOH/Et_2O	198-200	33
16c	$C_{24}H_{31}ClN_2O_3$	C, H, N	CH_2Cl_2/Skelly B	127-127.5	71
17c	$C_{25}H_{33}ClN_2O_3 \cdot HCl$	H, N; C[d]	MeOH/EtOAc	196-198	60
13d[c]	$C_{23}H_{29}N_2ClO_3 \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	MeOH/Et_2O	156-158	44
12e	$C_{22}H_{27}N_2O_3F \cdot 2HCl$	H, N; C[e]	EtOAc/*i*-PrOH	267 dec	58
13e	$C_{23}H_{29}N_2O_3F \cdot 2HCl$	C, H, N, Cl	MeOH/EtOAc	198-200	33
15e	$C_{25}H_{33}FN_2O_3 \cdot HCl \cdot 0.5H_2O$	C, H, N	EtOH		60
16e	$C_{24}H_{31}FN_2O_3$	C, H, N	CH_2Cl_2/Skelly B	153-155	77
17e	$C_{25}H_{33}FN_2O_3 \cdot 2HCl \cdot 0.5H_2O$	C, N; H[f]	MeOH/Et_2O	240-242	30
18e	$C_{30}H_{34}F_2N_2O_3 \cdot HCl \cdot H_2O$	C, H, N, Cl	MeOH/Et_2O	140-142	36
12f	$C_{23}H_{30}N_2O_4 \cdot HCl$	C, H, N, Cl	EtOH	201-202.5	50
15f	$C_{26}H_{36}N_2O_4 \cdot 2HCl$	C, H, N	Et_2O	187-189	60
16f	$C_{25}H_{34}N_2O_4 \cdot 2HCl$	C, H, N	EtOH	240-242 dec	98
12g	$C_{23}H_{27}N_2F_3O_3 \cdot HCl$	C, H, N, Cl	Et_2O	258-260 dec	48
13g	$C_{24}H_{29}N_2F_3O_3 \cdot 2HCl$	C, H, N, Cl	MeOH/Et_2O	175-177	27
14g	$C_{26}H_{33}N_2F_3O_3 \cdot HCl$	C, H, N	EtOH/Et_2O	162-163.5	28
12h	$C_{23}H_{30}N_2O_3$	C, H, N	Et_2O/Skelly B	93-94.5	39
13h	$C_{24}H_{32}N_2O_3 \cdot HCl \cdot 0.5H_2O$	C, N, Cl; N[g]		143-146	36
14h	$C_{26}H_{36}N_2O_3 \cdot HCl$	H, N, Cl; C[h]	MeOH/Et_2O	243-245 dec	35
15h	$C_{26}H_{36}NO_3 \cdot HCl$	C, H, N	EtOH,EtOAc	222-223	42
17h	$C_{26}H_{36}N_2O_3 \cdot HCl$	C, H, N, Cl	MeOH/EtOAc	206-208	35
13i[c]	$C_{22}H_{29}N_3O_3$	C, H, N	Et_2O	87-89	37
17i	$C_{24}H_{33}N_3O_3 \cdot HCl \cdot MeOH$	C, N, Cl; H[i]	MeOH/EtOAc	230-231	31
18i	$C_{26}H_{33}NO_3 \cdot HCl$	C, H, N, Cl	MeOH/EtOAc	197-199	41
13j	$C_{18}H_{28}N_2O_3 \cdot 2HCl \cdot H_2O$	C, H, N, Cl	MeOH/Et_2O	247-249 dec	65
17j	$C_{20}H_{32}N_2O_3 \cdot HCl$	C, H, N, Cl	MeOH/Et_2O	186-188	36

[a] Elemental analyses are within ±0.4% of the theoretical values unless otherwise indicated. [b] Yield for reaction *f* in Scheme I. [c] Reference 3. [d] C: calcd, 62.36; found, 61.62. [e] C: calcd, 57.50; found, 56.49. [f] H: calcd, 7.25; found, 6.78. [g] N: calcd, 6.34; found, 6.88. [h] C: calcd, 67.73; found, 67.29. [i] H: calcd, 7.61; found, 7.10.

variation. However, the conclusions are necessarily qualitative because we are comparing hypotensive responses in the rat at fixed doses rather than at ED_{50} values.

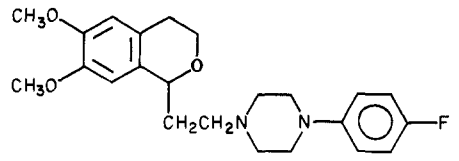
In these isochromans, hypotensive activity is influenced by (a) length of the alkyl chain at isochroman C-1, (b) aromatic ring substitution in the 4-arylpiperazines, and (c) methylation at C-1, C-3, or C-4. These structural features are discussed below.

(a) Chain Length. For a given amine, generic structure **12** is identical with structure **13**, except for the length of the alkyl chain at C-1 (n = 1 vs. n = 2, respectively). Likewise, structure **16** is identical with **17**, except for chain length (n = 2 vs. n = 3) (see Scheme I). The influence of the alkyl chain length (n) can be assessed by comparing **12** to **13** and **16** to **17** for particular amines. No clear trend emerges for n = 1 vs. n = 2. However, n = 2 is generally more potent than n = 3. In general, although structural variables are no longer tightly controlled, isochromans with methyl and ethyl chains (n = 1 and 2, compounds **12–16**) are more hypotensive than the propyl-substituted (n = 3) isochromans (**17** and **18**).

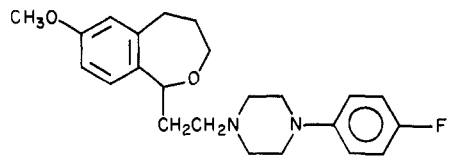
(b) Arylpiperazines. Each unique isochroman skeleton is substituted with various amines. For a given skeleton (e.g., **12**), these various amine derivatives show different hypotensive activities. From the hypotensive data of Table I, some general conclusions can be made about the effect of the aryl group of these arylpiperazines on activity in this series. In general, a *m*-(trifluoromethyl)phenyl and an *o*- or *m*-chlorophenyl reduce hypotensive activity. *p*-Methoxyphenyl has a modest activity. Interestingly, among the halophenyls, the *p*-fluoro- and *p*-chlorophenylpiperazine-substituted isochromans are quite active. Good hypotensive activity was also present for phenyl-, *o*-methoxyphenyl-, and 2-pyridinylpiperazines. In general, *o*-tolylpiperazine was the best amine for hypotensive activity.

(c) Methyl Substitution. For particular amines, isochroman skeletons **14**, **15**, and **16**, which bear methyls at C-1, C-3, and C-4, can be compared to isochroman **13**, which bears no methyls. Each of these isochromans has an ethyl chain at C-1 and identical skeleton, except for the methyl substitution of the isochroman ring. If a trend in the influence of alkyl substitution on hypotensive activity is meaningful and general, it should be valid for most amines. The relatively prolonged hypotensive activity of **14h** and **15h** relative to **13h** suggests that for *o*-tolylpiperazine, ring methylation may delay metabolic deactivation. The *p*-fluorophenylpiperazines cannot be compared because of dosage differences.

Compound **13e** was selected for further study because it is a potent hypotensive and because we have already studied and reported a direct analogue, 1-[2-(1,3,4,5-



13e



19

tetrahydro-7,8-dimethoxy-2-benzoxepin-1-yl)ethyl]-4-(4-fluorophenyl)piperazine (19).[3] Briefly, from our work in the rat, the dog, and the cat we found that 19 was an α blocker which resembled prazosin[3,6,7] in mechanism of action. Both central and peripheral sympatholytic actions on blood pressure were identified. We have compared 13e to 19 and have extended our work on compound 19.

A receptor-binding technique was used to determine the interaction between compounds 13e and 19 and α-adrenergic receptors. [^{3}H]Prazosin was used as the ligand for α_1-adrenergic receptors, while [^{3}H]clonidine was used to identify α_2-adrenoreceptors. The results of these studies are illustrated in Table III. Compounds 13e and 19 bind selectively to the α_1-adrenoreceptor as indicated by the low K_i for [^{3}H]prazosin binding. However, compound 13e has a 5 times greater affinity for the α_2-adrenergic receptor than compound 19. Prazosin and clonidine have selective affinities for the α_1- and α_2-adrenergic receptors, respectively, while phentolamine binds to both α_1- and α_2-adrenoreceptors (Table III).

Although the binding data indicate that compound 13e interacts with α-adrenergic receptors, the studies do not indicate that this compound acts as an α-adrenergic agonist or antagonist. Therefore, the effect of compound 13e on mean arterial pressure (MAP) and heart rate (HR), and the pressor effects of intravenous norepinephrine (NE), phenylephrine (PE), and angiotensin I were studied in the anesthetized rat. Intravenous administration of compound 13e (1 mg/kg iv) resulted in an immediate and significant decrease in MAP (−19 mmHg) which was maintained throughout a 1-h observation period (Table IV). The hypotension produced by compound 13e was not accompanied by a change of HR. In contrast to the action of compound 13e, administration of the citric acid vehicle failed to alter either MAP or HR in control rats. Compound 13e also produced a long-lasting α-receptor blockade in these animals. This is indicated by the fact that the pressor effects of intravenous NE and PE were significantly reduced following administration of compound 13e (Table IV). In contrast, the pressor effect of angiotensin I (0.1 μg/kg iv) was not altered by compound 13e. Previous studies indicate that compound 19 also possesses α-adrenergic receptor blocking activity.

TenBrink et al. have previously shown that compound 19 also acts in the central nervous system to reduce sympathetic nervous discharge (SND).[3] Therefore, the role of the central nervous system in the hypotensive action of compound 13e was assessed by recording peripheral sympathetic nerve activity in the external carotid nerve of six anesthetized, baroreceptor-denervated cats and three

Table III. Inhibition of Specific [^{3}H]Clonidine or [^{3}H]Prazosin Binding to Crude Rat Brain Membrane Preparation by Adrenergic Compounds

drug	K_i,[a] nM	
	[^{3}H]clonidine	[^{3}H]prazosin
clonidine	1.7	572.6
phentolamine	9.3	9.8
prazosin	1800	0.16
13e	62	4
19	307	4

[a] K_i values were determined on the basis of three experiments for each compound.

Table IV. Effect of Compound 13e on Mean Arterial Pressure (MAP), Heart Rate (HR), and Agonist Pressor Responses in Eight Anesthetized Rats[a]

time, min	MAP, mm/Hg	HR, beats/min	agonist pressor response, mmHg	
			norepinephrine (0.1 μg/kg)	phenylephrine (2.5 μg/kg)
0	105 ± 5	417 ± 18	+19 ± 3	+48 ± 2
60	87 ± 3	426 ± 17	+7 ± 1	+16 ± 1

[a] All values represent mean ± SEM ($p < 0.05$).

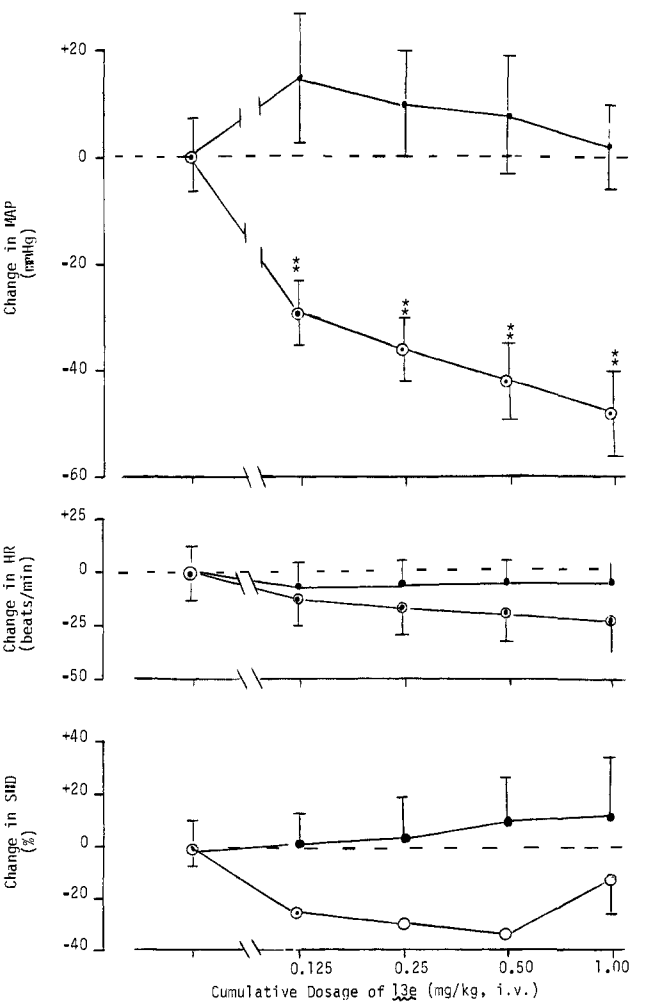


Figure 1. Effects of cumulative doses of vehicle (●) and 13e (○) on mean arterial pressure (MAP), heart rate (HR), and sympathetic nervous discharge (SND). 13e reduced MAP, HR, and SND.

baroreceptor-intact cats. In this regard, Gebber and Barman[8] have found that the activity of central sympa-

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thetic neurons cross-correlate with activity recorded from peripheral sympathetic nerves. Thus, activity recorded from peripheral nerves is an accurate reflection of activity in central sympathetic pathways. Figure 1 summarizes the effects of compound **13e** in comparison to the 0.1 M citric acid vehicle on MAP, HR, and SND in this preparation. In these experiments, compound **13e** was given sequentially at 30-min intervals until a cumulative dose of 1 mg/kg was attained. Intravenous administration of compound **13e** decreased MAP in a dose-related manner. MAP decreased from a pretreatment value of 136 ± 6 mmHg to 88 ± 8 mmHg following the 1 mg/kg dose of compound **13e**. Compound **13e** also produced a slight, statistically insignificant, bradycardia (−18 beats/min) in these animals. In contrast, the vehicle failed to significantly alter either MAP or HR. At low doses (0.125–0.50 mg/kg) compound **13e** significantly reduced SND. SND was maximally reduced by 35% at the 0.50 mg/kg dose. However, at a cumulative dose of 1.0 mg/kg iv, SND returned to pretreatment levels. This result may reflect a biphasic effect of compound **13e** on SND or, alternatively, the increase in SND may be secondary to a deterioration of the preparation over time. Therefore, the effect of a single 1 mg/kg dose of compound **13e** on SND was studied in six additional animals. Although compound **13e** reduced MAP in these animals (−46 mmHg), SND was not significantly altered. These data suggest that low doses of compound **13e** act in the central nervous system to reduce SND, while high doses have little effect on sympathetic nerve activity.

The mechanism of the hypotensive action of compound **13e** appears to be due to α-adrenergic receptor blockade. α-Adrenergic receptors have been divided into two categories: α_1-Receptors mediate the postsynaptic effects of norepinephrine, while α_2-receptors regulate the release of norepinephrine from nerve terminals. The binding data indicate that compound **13e** interacts with α-adrenergic receptors and that this compound has a 15 times higher potency in displacing [^{3}H]prazosin than [^{3}H]clonidine. The fact that compound **13e** blocks the pressor effects of intravenous norepinephrine and phenylephrine indicates that this compound acts as an α-adrenergic antagonist. Unlike many α-receptor antagonists,[7] the hypotension produced by compound **13e** in baroreceptor-intact cats was not accompanied by a tachycardia or an expected reflex increase in SND. In this regard, compound **13e** appears to be similar to prazosin and compound **19**.[3,7] Interestingly, it has been shown that these three compounds act in the central nervous system to reduce SND.[3,7] This suggests that the lack of tachycardia seen with these three α-adrenergic blockers might result from a central sympatholytic action. Graham and Pettinger have suggested that antagonists which are selective for α_1-receptors (e.g., prazosin, **13e**, and **19**) inhibit the release of additional norepinephrine from peripheral sympathetic neurons because of a lack of α_2-adrenergic antagonist activity.[9] However, this explanation does not take into account the expected baroreceptor-mediated increase in SND that should occur during the hypotension produced by prazosin. In this respect, direct-acting vasodilators, such as minoxidil and hydralazine, markedly increase HR. In any case, compound **13e** appears to resemble compound **19** and prazosin in that these agents decrease MAP without producing a tachycardia. Table III shows that compounds **13e**, **19**, and prazosin are selective α_1-antagonists. However, the α_1/α_2 ratio is much greater for compound **19** and prazosin than it is for compound **13e**. This indicates that compound **13e** has substantially greater α_2-blocking activity. In this regard it has been demonstrated that mixed α_1/α_2- or selective α_2-adrenergic receptor blocking agents fail to reduce SND via a central mechanism.[7] This may explain the fact that low doses of compound **13e** reduced SND, while high doses had no effect on sympathetic nerve activity. Further experiments are required to determine the relative importance of the central sympatholytic effect of low doses of compound **13e** in the hypotensive action of this drug. Recently, P. Mouille et al. have reported a new α_1-receptor antagonist, 2-[2-[4-(*o*-methoxyphenyl)piperazine-1-yl]-ethyl]-4,4-dimethyl-1,3(2*H*,4*H*)isoquinolinedione, that lowers blood pressure by both peripheral and central mechanisms.[10] Thus, this compound is pharmacologically similar to our **13e** and **19**.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Elemental analyses and melting points are recorded in Table II. NMR spectra of $CDCl_3$ solutions were recorded on a Varian HFT-80 and are consistent. Medium-pressure liquid chromatographies were run on EM silica gel 60 and EM RP2 silica gel. 1-(Bromomethyl)-6,7-dimethoxyisochroman (**5**) and 1-(2-chloroethyl)-6,7-dimethoxyisochroman (**6**) have been described by Humber.[1]

1-Methyl-1-(2-bromomethyl)-6,7-dimethoxyisochroman (9). To 20.0 g (0.107 mol) of 1-(3,4-dimethoxyphenyl)ethanol, 14.0 mL (0.107 mol) of ethyl acetoacetate, and 300 mL of nitromethane was added 13.1 mL (0.092 mol) of boron trifluoride etherate. The mixture was stirred at room temperature for 1 h and then partitioned between methylene chloride and aqueous sodium bicarbonate. The organic layer was concentrated. The residue was chromatographed on silica gel (20% ethyl acetate/Skelly Solve B) to yield 29.4 g (93%) of ethyl 3,4-dihydro-6,7-dimethoxy-1-methyl-1*H*-2-benzopyran-1-acetate (**9a**) as a clear liquid. A solution of 29.4 g (0.100 mol) of isochroman **9a** in 250 mL of ether was treated with 3.8 g of lithium aluminum hydride. After 40 min, the reaction was treated sequentially with 3.8 mL of water, 3.8 mL of 15% aqueous sodium hydroxide, and 11.4 mL of water. The mixture was filtered through Celite. The filtrate was washed with brine, concentrated, and chromatographed on silica gel (2% methanol in methylene chloride) to yield 20.75 g (82%) of 1-methyl-1-(2-hydroxyethyl)-6,7-dimethoxyisochroman. A 19.8 g (0.078 mol) sample of this alcohol and 22.64 g (0.086 mol) of triphenylphosphine in 500 mL of DMF was treated at 0 °C with 13.8 mL of bromine in 20 mL of DMF. After 1 h the reaction was heated at 55 °C for 16.5 h. The mixture was partitioned between ether and brine. The organic layer was concentrated. The residue was chromatographed on silica gel (15% ethyl acetate in Skelly Solve B) to yield 22.67 g (92%) of bromide **9** as an oil.

1-(2-Chloroethyl)-4,4-dimethyl-6,7-dimethoxyisochroman (7). An 89.6 g (0.476 mol) sample of 21.3% potassium hydride in mineral oil was washed with hexane. To this was added dropwise with ice cooling a solution of 200 mL of THF, 50.0 g (0.238 mol) of methyl 2-(3,4-dimethoxyphenyl)acetate, and 67.25 g (0.476 mol) of methyl iodide. The reaction was stirred at 22 °C for 17 h and then partitioned between aqueous sodium bicarbonate and methylene chloride. The organic phase was dried over sodium sulfate and concentrated. The residue was dissolved in 400 mL of ethyl ether and treated with 40 mL (0.4 mol) of 10 M borane methyl sulfide. After 60 h, the mixture was stirred with 2-propanol for 1 h and then partitioned between aqueous sodium bicarbonate and methylene chloride. The residue was crystallized from ether and hexane to yield 34 g (68% for the alkylation and reduction) of **4**. A solution of 27.3 g (0.131 mol) of **4**, 26.1 g (0.157 mol) of 3-chloropropionaldehyde diethyl acetal, and 3.5 mL of boron trifluoride etherate in 300 mL of nitromethane was stirred for 3 h at 22 °C. The mixture was partitioned between methylene chloride and aqueous sodium bicarbonate. The organic phase was

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concentrated and the residue was chromatographed on silica gel (5% ethyl acetate/Skelly Solve B) to yield 35.18 g (84%) of a yellow oil 7, which crystallized on standing.

1-(2-Chloroethyl)-3,3-dimethyl-6,7-dimethoxyisochroman (8). An ice-cooled solution of 40.5 g (0.193 mol) of methyl 2-(3,4-dimethoxyphenyl)acetate (1) in 1000 mL of THF was treated dropwise with 140 mL (0.405 mol) of 2.9 M methylmagnesium bromide. After 20 h, the mixture was poured onto ice and extracted with ether. The organics were then extracted with brine. The organic layer was concentrated to yield 36.6 g (90%) of alcohol 4. A solution of 2.10 g (0.01 mol) of alcohol 4, 2.00 mL (0.012 mol) of β-chloropropionaldehyde diethyl acetal, and 0.1 mL of boron trifluoride etherate in 125 mL of nitromethane was stirred for 1.3 h. The mixture was then partitioned between methylene chloride and aqueous sodium bicarbonate. The organic layer was concentrated and chromatographed on silica gel (15% ethyl acetate/Skelly Solve B) to yield 2.40 g (84%) of isochroman 8.

1-(3-Chloropropyl)-1-methyl-6,7-dimethoxyisochroman (10). A solution of 1.72 g (0.01 mol) of 3,4-dimethoxyphenethyl alcohol (3a), 1.64 g (0.01 mol) of 5-chloro-2-pentanone ethylene ketal, and 0.5 mL of boron trifluoride etherate was stirred at 22 °C for 4 h. The reaction was partitioned between methylene chloride and aqueous sodium bicarbonate. The organic phase was concentrated and the residue was chromatographed on silica gel (10% ethyl acetate/Skelly Solve B) to yield 1.2 g (43%) of the crystalline 10, mp 77–79 °C.

1-(4-Fluorophenyl)-1-(3-chlorophenyl)-6,7-dimethoxyisochroman (11). A mixture of 1.72 g (0.01 mol) of 3,4-dimethoxyphenethyl alcohol and 2.86 g (0.01 mol) of 4-chloro-*p*-fluorobutyrophenone dimethyl propylene ketal was converted to 2.26 g (60%) of 11 via the method for the preparation of 10, mp 97–99 °C.

General Procedure. Compounds 12–18. A mixture of the 1-(haloalkyl)-6,7-dimethoxyisochroman, 1–2 equiv of an arylpiperazine, and sometimes 1–4 equiv of triethylamine was stirred in ethylene glycol at 50–70 °C for 20 h. The reaction mixture was partitioned between methylene chloride and aqueous potassium carbonate. The organic phase was concentrated and the residue was chromatographed to yield the arylpiperazine product, which was either crystallized or converted to its hydrochloride salt by treating an ethyl acetate solution of the free base with 3.3 N hydrogen chloride in ethyl ether. Several examples of this preparation are given below. Physical data and reaction yields are cited in Table II.

1-[(3,4-Dihydro-6,7-dimethoxy-1*H*-2-benzopyran-1-yl)-methyl]-4-(2-methoxyphenyl)piperazine Dihydrochloride (12f). A solution of 1.48 g (0.0052 mol) of 6,7-dimethoxy-1-(bromomethyl)isochroman (5), 1.19 g (0.0062 mol) of (2-methoxyphenyl)piperazine, and 0.7 g (0.0069 mol) of triethylamine in 5 mL of ethylene glycol was heated at 50 °C for 20 h. The mixture was partitioned between methylene chloride and 10% aqueous potassium carbonate. The organic phase was dried over sodium sulfate and concentrated. The residue was chromatographed on silica gel (5% methanol, 0.5% ammonium hydroxide in methylene chloride) to yield 1.53 g (50%) of product. This was converted to the dihydrochloride salt, which was crystallized from ethyl acetate and ethanol, mp 201–202.5 °C. Compound 13e is cited as an example.

1-[2-(3,4-Dihydro-6,7-dimethoxy-1*H*-2-benzopyran-1-yl)-ethyl]-4-(4-fluorophenyl)piperazine Dihydrochloride (13e). A solution of 1.3 g (0.005 mol) of 1-(2-chloroethyl)-6,7-dimethoxyisochroman (6), 1.8 g (0.01 mol) of *p*-fluorophenylpiperazine, and 1.01 g (0.01 mol) of triethylamine in 20 mL of ethylene glycol was heated at 50 °C for 20 h. The reaction was worked up in the usual way. Compound 13e was isolated in 33% yield as the dihydrochloride salt. Compound 16e is cited as an example.

1-[2-(3,4-Dihydro-6,7-dimethoxy-3,3-dimethyl-1*H*-2-benzopyran-1-yl)ethyl]-4-(4-fluorophenyl)piperazine Dihydrochloride (16e). A solution of 1.00 g (0.0035 mol) of 3,3-dimethyl-1-(2-chloroethyl)-6,7-dimethoxyisochroman (8) and 1.40 g (0.0070 mol) of *p*-fluorophenylpiperazine in 5 mL of ethylene glycol was stirred at 70 °C for 20 h. The mixture was partitioned with methylene chloride and aqueous sodium carbonate. The organic phase was concentrated to yield 0.91 g (60%) of 16e. This was converted to the dihydrochloride salt, which was crystallized from ethanol to yield 0.38 g of 16e.

Methods for Receptor Binding. A receptor-binding technique was used to determine the interaction between compounds 13e and 19 and adrenergic receptors. [^{3}H]Clonidine and [^{3}H]-prazosin (New England Nuclear) were used as the ligands for the α_2- and α_1-adrenergic receptors, respectively. Briefly, the receptor-binding assays were carried out as follows: Male Sprague–Dawley rats were sacrificed by decapitation, and the brains were quickly removed from the skull and chilled on ice. Whole brain minus cerebellum was homogenized in 25 mL of ice-cold 50 mM Tris-HCl buffer, pH 7.4 at 25 °C, using a Brinkman polytron, PCU-2-110 homogenizer for 25 s at setting no. 6. The homogenate was centrifuged at 48000*g* for 10 min, and the pellet was washed once by resuspension and recentrifugation as described above. The final pellet was suspended in 50 volumes of the same buffer. The following solutions were added to a 13 × 100 mm disposable tube: 100 μL of either [^{3}H]clonidine or [^{3}H]prazosin (final concentration about 2–3 and 2.05 nM, respectively), 100 μL of water (for total binding) of drug solution (for inhibition curve or nonspecific binding), 0.8 mL of buffer, and 1.0 mL of cold membrane preparation to give a final volume of 2.0 mL. The samples were incubated for 30 min at 25 °C. The incubation was terminated by filtering the samples, under vacuum, through a Whatman GF/B glass–fiber filter disk 3.7 cm in diameter. Each tube was rinsed with 5.0 mL of cold buffer, which was also filtered. The filter disk was then washed 3 times using 5.0 mL of buffer for each wash. The filter paper was placed in a scintillation vial containing 15.0 mL of ACS scintillation cocktail. The radioactivity was counted by liquid scintillation spectrometry. Specific binding was defined as the total binding minus binding in the presence of either 10 μM clonidine or prazosin.

IC_{50}'s were obtained from a logit–log plot of the data. Inhibition constants were calculated from the following equation:

$$K_i = IC_{50}/1 + (c/K_d)$$

where c is the concentration of ligand, and K_d is the dissociation constant of the ligand.

Hypotensive Activity in Conscious Rat. The blood pressure of restrained female Sprague–Dawley rats was measured directly from chronic indwelling aortic cannulas exteriorized from the nape of the neck. In order to obtain a high level of sympathetic tone, rats were restrained in a towel during the period of blood pressure measurement via a Statham transducer (P23G) and a Grass Model 5 polygraph.[5] Measurements were made before, as well as 4 and 24 h after, the oral administration of each compound suspended in a carboxymethylcellulose vehicle at 10 mL/kg. Blood pressure values of two animals were averaged at each of the three measurement times. An average change of at least 5 mmHg was required posttreatment for statistical significance ($p < 0.05$) to be attained.

Methods for Anesthetized Rats. The initial cardiovascular evaluation of compound 13e was carried out in female Sprague–Dawley rats (200–300 g) that were anesthetized with an intravenous injection of α-chloralose (40 mg/kg), urethane (40 mg/kg), and sodium pentobarbital (15 mg/kg). Polyethylene catheters were inserted into the right common carotid artery and left external jugular vein to monitor arterial blood pressure and to administer drugs, respectively. Blood pressure and heart rate were measured via a Statham transducer and a Grass Model 7 polygraph. The pressor effects of intravenous norepinephrine (0.1 μg/kg), phenylephrine (2.5 μg/kg), and angiotensin I (0.1 μg/kg) were determined prior to and 15, 30, 45, and 60 min after the administration of compound 13e (1.0 mg/kg iv). Compound 13e was dissolved in 0.1 M citric acid at a concentration of 1 mg/mL.

Methods for Baroreceptor-Denervated Cats. Cats of either sex were anesthetized with a mixture of sodium diallylbarbituric acid (60 mg/kg), ethylurea (240 mg/kg), and urethane (240 mg/kg) administered intraperitoneally. Each animal was positioned in a Kopf stereotaxic apparatus, and a trachea tube was surgically inserted. A femoral artery and vein were cannulated to monitor arterial blood pressure via a Statham transducer (P23Gc) and a Grass polygraph, while heart rate was recorded continuously with a Grass tachograph triggered by the electrocardiogram. Carotid sinus, aortic depressor, and vagus nerves were exposed and sectioned after reflection of a portion of the trachea and esophagus. The sympathetic postganglionic external carotid nerve was isolated

distal to its junction with the superior cervical ganglion. Nerve potentials were recorded monophasically under oil with a bipolar platinum electrode after capacity-coupled preamplification (low and high half-amplitude responses at 1 and 500 Hz). Nerve activity was displayed on the polygraph and quantitated using a Grass 7P10B cumulative integrator. Compound 13e was dissolved in 0.1 M citric acid at a concentration of 1 mg/mL.

Statistical Analysis. Statistical analysis for most experiments was performed using the Student's t test for unpaired comparisons. The values obtained at each time period in the drug-treated groups were compared to the corresponding values in the vehicle group. The 0.05 level of probability was used to indicate statistical significance.

Acknowledgment. The authors are indebted to Michael G. Wendling and John E. Rogers who collected the hypotensive data in the conscious rat on the phenylpiperazine isochromans, to Lubomir Baczynskyj and James R. Boal who ran and interpreted mass spectra, and to Upjohn's Physical and Analytical Chemistry Department for elemental analyses and IR spectroscopy.

Notes

Syntheses and Activities of Antioxidant Derivatives of Retinoic Acid

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The syntheses of six antioxidant derivatives (butylated hydroquinone, ethoxyquin, and d-α-tocopherol) of retinoic acid are reported. These derivatives were examined for activity in terms of "chemoprevention" of cancer by measuring the reverse keratinization of epithelial cells in hamster trachael organ cultures. Ester **2A** was observed to be active in 100% of the cultures examined at 10^{-9} M, relative to 88.4% activity for (*all-E*)-retinoic acid at 10^{-9} M.

Vitamin A (retinol) is an essential nutritional substance which is supplied in the diet basically from natural and/or synthetic retinyl esters and/or β-carotene. The active form of vitamin A appears to differ depending on target tissues.[1] Retinol, which is required for healthy reproductive functions,[2] is reversibly oxidized to retinal, which is utilized in visual proteins as photoreceptor molecules.[3] Retinal is then further oxidized, irreversibly, to retinoic acid which exhibits hormonal-like properties in controlling the normal growth, development, and differentiation of epithelial tissues.[4-6] These epithelia make up the membranes that cover, enclose, and protect the major organs of the body. Well over half of cancer begins in the epithelial tissues of the bladder, breast, colon, lung, prostate, skin, stomach, and uterus.[7] Natural as well as synthetic retinoid analogues have been shown to prevent or delay the onset of certain forms of epithelial cancer, such as bladder, breast, lung, and skin, in animals which previously were given doses of chemical carcinogens, radiation, or viral transforming factors.[8-11] Natural retinoids have limited usefulness for "chemoprevention"[12] of cancer, because of excessive toxicity and inadequate tissue distribution. Therefore, it would be advantageous to explore the possibility of utilizing new synthetic retinoid derivatives with proper therapeutic indexes and pharmacokinetics in order to prevent or delay the onset of epithelial malignancies.

Antioxidants such as butylated hydroxyanisole (BHA) and ethoxyquin (Santoquin)[13] have been observed to inhibit the formation of neoplasia in animals treated with several chemical carcinogens.[14] Antioxidants such as BHA, ethoxyquin, and d-α-tocopherol (vitamin E)[15,16] are known to function as efficient inhibitors of lipid peroxidation, and as such they may serve to protect cellular membranes from the effects of various carcinogenic sub-

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0022-2623/82/1825-0081$01.25/0