Amphoteric Drugs. II.[1] Synthesis and Antiallergic Activity of [4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidino]alkanoic Acid Derivatives and Related Compounds

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A simple method of transforming classical tricyclic antihistaminics into nonsedative antiallergic agents with equal potency in rats and guinea-pigs is described. A series of [4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-piperidino]alkanoic acid derivatives (6a) and related compounds (6b—f) were synthesized and examined for anti-allergic and antihistaminic activities and effects on the central nervous system (CNS) in comparison with the corresponding *N*-methyl derivatives (2a—f). *N*-Alkylcarboxylic acids (6a—f) showed stronger inhibitory effects on 48 h homologous passive cutaneous anaphylaxis (PCA) in rats than 2a—f, and also were less effective in prolongation of the sleeping time on hexobarbital-induced anesthesia in mice in comparison with 2a—f. As a result of further modification, it was found that introduction of an oxygen atom into the central ring of the tricyclic system in amphoteric compounds enhanced their antiallergic and antihistaminic activities. 3-[4-(6*H*-Dibenz[*b*,*e*]oxepin-11-ylidene)piperidino]propionic acid (6c) exhibited strong inhibitory effects on 48 h homologous PCA in rats (ED_{50} = 0.067 mg/kg, *p.o.*) and on histamine-induced bronchoconstriction in anesthetized guinea-pigs (ED_{50} = 0.0085 mg/kg, *p.o.*), and thus is a promising candidate as an antiallergic agent.

Keywords amphoteric drug; zwitter-ionization; antiallergic agent; classical tricyclic antihistaminic; dibenzo[*a*,*d*]cycloheptene; dibenz[*b*,*e*]oxepin

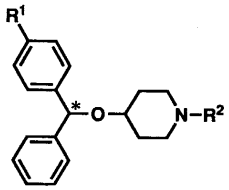
Much effort has been directed to the development of nonsedative antihistaminics and antiallergic agents, and several compounds have been shown to be effective clinically in the treatment of allergic disorders.[2] In the preceding paper,[1] we synthesized zwitter-ionized derivatives of representative antihistaminics bearing a diphenyl system, such as diphenylpyraline (**1a**), and examined them for modification of both the antiallergic activity and the effect on the central nervous system (CNS). This zwitter-ionization resulted in great enhancement of the inhibitory effect on 48 h homologous passive cutaneous anaphylaxis (PCA) in rats and a marked reduction of CNS side-effects, exemplified by prolongation of sleeping time on hexobarbital-induced anesthesia in mice. The propionic acid derivative (**1b**) was found to be a potent nonsedative antiallergic agent.

To extend the scope of this approach to the design of antiallergic agents, we synthesized a series of amphoteric compounds derived from classical tricyclic antihistaminics (cyproheptadine, **2a**),[3] and examined them for antiallergic activities in comparison with the corresponding *N*-methyl derivatives.

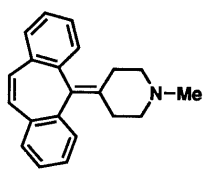
Synthesis

The compounds tested were prepared by the methods shown in Chart 2. *N*-Methylamines (**2**) obtained by the usual methods[3] were treated with ethyl chloroformate in toluene to give the corresponding ethyl carbamates (**3**), which were hydrolyzed to afford secondary amines (**4**). Compounds **4** were alkylated with ethyl bromoacetate, ethyl 3-bromopropionate (or ethyl acrylate), ethyl 4-bromobutyrate, ethyl 5-bromovalerate or methyl 6-bromohexanoate to give the corresponding *N*-alkylcarboxylates (**5**), hydrolysis of which with 2 N NaOH afforded the corresponding *N*-alkylcarboxylic acids (**6**) with various methylene chain lengths.

In the case of the xanthene derivative (X = O), hydrolysis of the ethyl carbamate (**3e**) under a strongly alkaline condition gave an approximately 1 : 1 mixture of **4e** and **4e′** (migration of double bond). These secondary amines



1a: R^1 = H, R^2 = Me (diphenylpyraline)
1b: R^1 = Me, R^2 = $(CH_2)_2CO_2H$ ((+)-isomer)



2a (cyproheptadine)

Chart 1

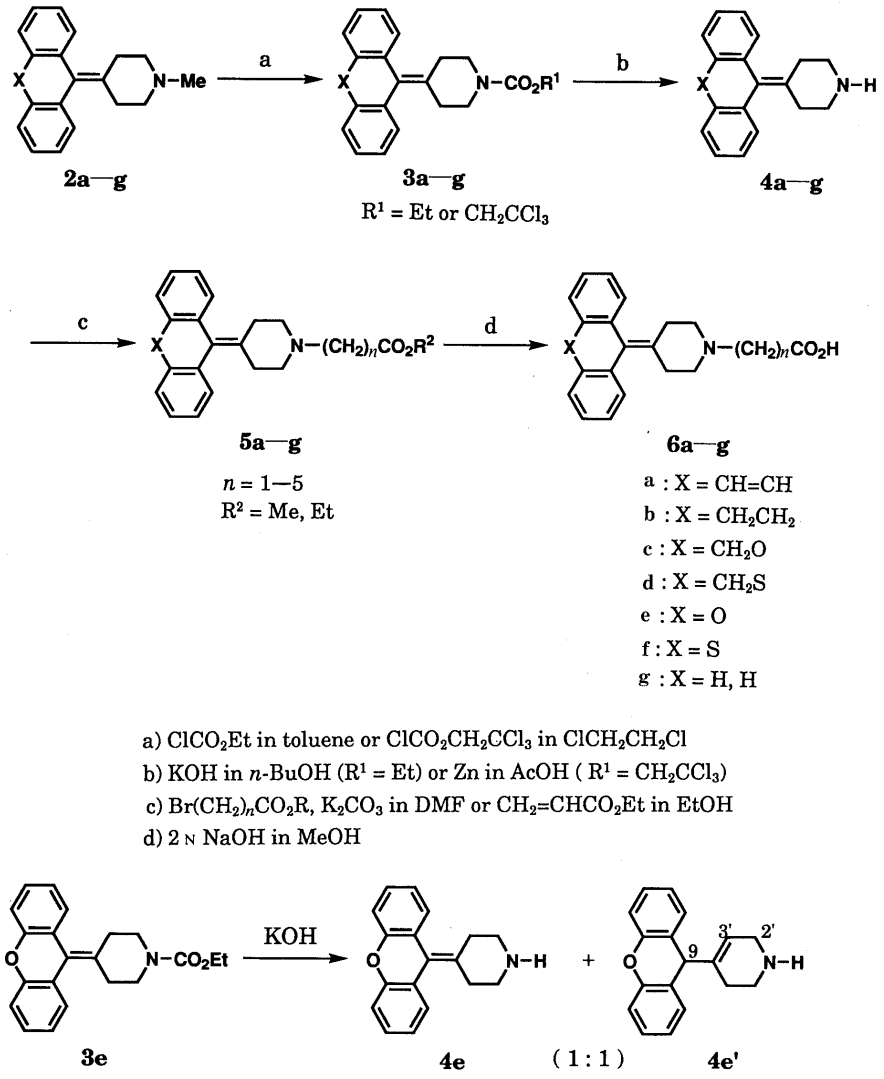


Chart 2

(**4e** and **4e'**) were separated by fractional recrystallization of the fumarate. The ^{1}H-NMR spectrum [dimethyl sulfoxide-d_6 (DMSO-d_6)] of **4e'** (fumarate) exhibited a singlet at 5.75 ppm due to the C-3' vinylic proton, a singlet at 4.84 ppm due to the C-9 bridgehead methine proton and a broad singlet at 3.49 ppm due to the C-2' allylic proton. The assignments were confirmed by the 2D correlation spectroscopy (H–H COSY) spectrum. Alternatively, treatment of 2,2,2-trichloroethyl carbamate, formed from **2e**, with Zn powder in aqueous AcOH provided the desired **4e** in 62% yield from **2e** as a single product. Physicochemical data are given in Table I.

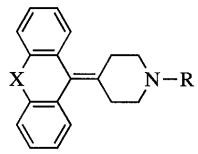
Results and Discussion

We initially examined the changes of antiallergic activity and undesirable CNS side-effects caused by zwitterionization, by comparing **6a** with cyproheptadine (**2a**). Histamine (H_1) antagonistic action was measured in terms of inhibitory effect on specific [^{3}H]mepyramine binding to guinea-pig cortex histamine receptors. Antiallergic activity was evaluated in terms of inhibitory effect on compound 48/80-induced lethality in rats. The prolongation of sleeping time on hexobarbital-induced anesthesia in mice was taken as an index of CNS side-effects. The results are summarized in Table II.

Except for the case of acetic acid derivative (**6a**-1), *N*-alkylcarboxylic acids (**6a**) showed stronger inhibitory effects on compound 48/80-induced lethality *in vivo* than cyproheptadine (**2a**), in spite of a reduction in H_1 binding affinity *in vitro*. Although the influence of difference in species should be naturally considered, zwitter-ionization may change the mechanism of action. As for the prolongation of sleeping time on hexobarbital-induced anesthesia, all *N*-alkylcarboxylic acids (**6a**) were much weaker than **2a**.

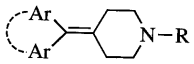
The influence of the methylene chain length (n) in **6a** was examined by comparison with open-chain analogues of 4-(diphenylmethylene)piperidinoalkanoic acids (**6g**), prepared previously.[1] In terms of effect on hexobarbital-induced anesthesia, **6a**-2 ($n=2$) was the weakest among all *N*-alkylcarboxylic acids (**6a**), although no obvious difference was found in antiallergic activity. For maximum separation between antiallergic activity and CNS side-effects, the optimum length (n) in the tricyclic system was found to be two (propionic acid derivative), while no

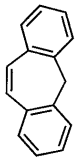
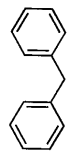
TABLE I. Physicochemical Data for *N*-Alkylcarboxylic Acids (**6**)



No.	X	R	mp °C (Recryst. solvent)	Yield (%)	Formula	Analysis Calcd (Found)		
						C	H	N
6a-1	CH=CH	CH_2CO_2H	162—165 (EtOH–Et_2O)	61	$C_{22}H_{21}NO_2 \cdot HCl \cdot 1/2H_2O$	70.11 (70.29	6.15 6.23	3.72 3.51)
6a-2	CH=CH	$(CH_2)_2CO_2H$	199—201 (aq. DMF)	88	$C_{23}H_{23}NO_2 \cdot 2H_2O$	72.42 (72.23	7.13 6.94	3.67 3.67)
6a-3	CH=CH	$(CH_2)_3CO_2H$	237—239 (H_2O)	79	$C_{24}H_{25}NO_2 \cdot HCl \cdot 1/2H_2O$	71.19 (71.46	6.72 6.82	3.46 3.37)
6a-4	CH=CH	$(CH_2)_4CO_2H$	229—230 (EtOH–Et_2O)	75	$C_{25}H_{27}NO_2 \cdot HCl$	73.25 (73.25	6.88 6.84	3.42 3.30)
6a-5	CH=CH	$(CH_2)_5CO_2H$	213—214 (EtOH–Et_2O)	52	$C_{26}H_{29}NO_2 \cdot HCl$	73.66 (73.37	7.13 7.25	3.30 3.26)
6b	CH_2CH_2	$(CH_2)_2CO_2H$	189—191 (aq. DMF)	87	$C_{23}H_{25}NO_2 \cdot 5/2H_2O$	70.38 (70.70	7.70 7.52	3.57 3.63)
6c	CH_2O	$(CH_2)_2CO_2H$	142—143 (aq. DMF)	73	$C_{22}H_{23}NO_3 \cdot 2H_2O$	68.55 (68.82	7.06 6.97	3.63 3.62)
6d	CH_2S	$(CH_2)_2CO_2H$	207—209 (EtOH–Et_2O)	55	$C_{22}H_{23}NO_2S \cdot HCl$	65.74 (65.54	6.02 6.06	3.48 3.60)
6e	O	$(CH_2)_2CO_2H$	197—199 (CH_2Cl_2–MeOH)	87	$C_{21}H_{21}NO_3 \cdot 3/2H_2O$	69.60 (69.32	6.67 6.66	3.86 3.90)
6f	S	$(CH_2)_2CO_2H$	233—236 (aq. DMF)	88	$C_{21}H_{21}NO_2S \cdot 9/4H_2O$	64.34 (64.56	6.56 6.29	3.57 3.38)

TABLE II. Pharmacological Data for Dibenzo[*a*,*d*]cycloheptene Derivatives (**2a**, **6a**) and Open-Chain Analogues (**2g**, **6g**)



No.		H_1-binding pIC_{50}	Compound 48/80-induced lethality in rats (mg/kg, *p.o.*) inhibition, % ($n=5$)				Hexobarbital-induced anesthesia in mice[a] (*p.o.*)
			0.01	0.1	1	10	
2a		8.9		0	60	100	++++
6a-1		6.6	0	20	60	100	+
6a-2		7.0	20	40	100		−
6a-3		6.9	20	40	80	100	++
6a-4		7.1		0	100		+
6a-5		6.7		0	100		++
2g		8.5				0	++++
6g-1		<5				0	++
6g-2		6.8		0	60	100	++
6g-3		6.4	0	20	40	60	+++
6g-4		6.8		0	40	100	+++
6g-5		6.6			0	60	++

a) The symbols have the following meanings: ++++, percent increase of sleeping time at 3 mg/kg of test compound is 50% or above; +++, percent increase of sleeping time at 10 mg/kg of test compound is 50% or above; ++, percent increase of sleeping time at 30 mg/kg of test compound is 50% or above; +, percent increase of sleeping time at 100 mg/kg of test compounds is 50% or above; −, percent increase of sleeping time at 100 mg/kg of test compound is less than 50%.

significant correlation was observed in open-chain analogues. Thus, zwitter-ionization was more effective in the tricyclic system.

We next focused our attention on the influence of various linkages (X) in the tricyclic system. A systematic study on antihistaminic and antiserotonin activities of a series of compounds related to cyproheptadine (**2a**) has been reported by Engelhardt *et al.*[3] They described the effects of structural variations including saturation of the 10,11 double bond of **2a** and replacement of the dibenzo[*a*,*d*]cycloheptene nucleus by xanthene, thioxanthene or fluorene, and found that the antihistaminic and antiserotonin activity of the thioxanthene congener was closely similar to that of cyproheptadine. Taking their

TABLE III. Pharmacological Data for *N*-Methylamines (**2**) and *N*-Propionic Acids (**6**)

No.	H_1-binding pIC_{50}	PCA in rats ED_{50} (mg/kg *p.o.*)	Histamine-induced bronchoconstriction in guinea-pigs ED_{50} (mg/kg, *p.o.*)	Hexobarbital-induced anesthesia in mice ID_{50} (mg/kg, *p.o.*)	Locomotor activity in mice MNED[a] (mg/kg, *p.o.*)
2a	8.9	0.56	NT[b]	2	0.3
6a-2	7.0	0.081	0.047	105	>100
2b	8.4	3.3	NT[b]	2	0.1
6b	6.5	0.12	0.096	210	>100
2c	9.5	1.1	NT[b]	1	3
6c	6.5	0.067	0.0085	16	>100
2d	8.3	2.3	NT[b]	8	0.3
6d	6.8	0.52	NT[b]	115	>100
2e	8.8	0.52	NT[b]	3	10
6e	7.4	0.018	NT[b]	11	10
2f	8.4	0.057	NT[b]	3	0.3
6f	6.8	0.054	0.097	80	>100
Ketotifen	9.1	0.43	0.0050	13	30
Terfenadine	6.8	9.0	0.33	76	>100

a) Maximum no-effect dose. *b*) Not tested.

results into consideration, we undertook structural conversion of the central seven-membered ring of **6a**-2. Antiallergic activity was evaluated in terms of inhibitory effect on 48 h homologous PCA in rats and undesirable CNS side-effects were assessed in terms of the effect on hexobarbital-induced anesthesia and locomotor activity in mice. Ketotifen and terfenadine were used as reference compounds. The results are summarized in Table III.

As expected, the *N*-propionic acids (**6b**—**f**) showed stronger inhibitory effects on PCA and weaker CNS side-effects than the corresponding *N*-methylamines (**2b**—**f**). As regards anti-PCA activity, saturation of the double bond at the 10,11-position (**6a**-2→**6b**) resulted in slight loss of activity. Introduction of an oxygen atom into the central seven-membered ring (**6b**→**6c**) enhanced anti-PCA activity. In contrast, introduction of a sulfur atom (**6b**→**6d**) reduced the activity. Contraction of the seven-membered ring to a six-membered analogue (**6c**→**6e**, **6d**→**6f**) led to great enhancement of the potency. Engelhardt *et al.*[3] reported that the replacement of the dibenzo[*a*,*d*]cycloheptene nucleus of **2a** by the xanthene system greatly decreased antihistaminic activity, but this replacement in our system (**6a**-2→**6e**), on the contrary, resulted in enhancement of anti-PCA activity and H_1 binding affinity. This indicates that structure–activity relationships of amphoteric derivatives (**6a**—**f**) are not necessarily consistent with those of the *N*-methyl compounds (**2a**—**f**).

As for the effect on hexobarbital-induced anesthesia, compound **6b** showed the weakest effect as regards prolongation of sleeping time among all the *N*-propionic acids (**6a**—**f**). Conversion of the ethylene bridge into a double bond (**6b**→**6a**-2) and introduction of an oxygen (**6b**→**6c**) or a sulfur atom (**6b**→**6d**) failed to reduce effectively the undesirable CNS side-effect. Thus, it appeared that a shorter linkage (X) and introduction of an oxygen atom in the amphoteric compounds increased the sleeping time in hexobarbital-induced anesthesia as well as the anti-PCA activity.

Among the *N*-propionic acids (**6a**—**f**), compound **6e** showed the strongest anti-PCA activity, but still possessed a strong sedative action (decrease in locomotor activity). We therefore selected **6c** and **6f** as candidate agents, because of the superior separation of antiallergic activity from the undesirable CNS side-effects, and we examined their inhibitory effect on histamine-induced bronchoconstriction in guinea-pigs. Compounds **6c** and **6f** were effective with ED_{50} values of 0.0085 and 0.097 (mg/kg, *p.o.*), respectively. In particular, **6c** was approximately 10-fold more potent than ketotifen in the PCA test and as potent as ketotifen (ED_{50} = 0.0050 mg/kg) in the effect on histamine-induced bronchoconstriction. Compound **6c** exhibited strong antihistaminic and antiallergic effects on rat and guinea-pig models at the same oral dose. It had little sedative action (decrease in locomotor activity).

In conclusion, it was demonstrated that a simple zwitter-ionization was capable of transforming classical tricyclic antihistaminics into nonsedative antiallergic agents with equal potency in rats and guinea-pigs. More detailed evaluation of compound **6c** is in progress.

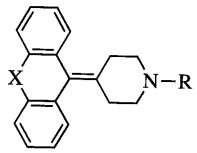
Experimental

All melting points were measured on a Yanagimoto melting point apparatus without correction. Spectral data were obtained as follows: ^{1}H-NMR spectra with JEOL FX-90Q (90 MHz) and JEOL A-500 (500 MHz) spectrometers, using tetramethylsilane (TMS) as an internal standard; mass spectra (MS) with a JEOL JMS-DX 300 mass spectrometer; IR spectra with a Hitachi 270-30 spectrometer. Elemental analyses were performed with a Yanagimoto MT-3 or MT-5 elemental analysis apparatus. Column chromatography was carried out with silica gel [Kieselgel 60 (Merck)]. TLC was conducted on 0.25 mm pre-coated silica gel plates ($60F_{254}$, Merck).

The following known intermediates were prepared essentially according to the literature: 4-(5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine (cyproheptadine, **2a**),[3] 4-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-methylpiperidine (**2b**),[3] 4-(6*H*-dibenz[*b*,*e*]oxepin-11-ylidene)-1-methylpiperidine (**2c**),[4] 4-(6*H*-dibenzo[*b*,*e*]-thiepin-11-ylidene)-1-methylpiperidine (**2d**),[5] 4-(9*H*-xanthen-9-ylidene)-1-methylpiperidine (**2e**)[3] and 4-(9*H*-thioxanthen-9-ylidene)-1-methylpiperidine (**2f**).[3]

Ethyl 4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)-1-piperidinecarboxylate (3a) Ethyl chloroformate (74.9 g, 0.69 mol) was added dropwise to a solution of **2a** (33.0 g, 0.11 mol) in toluene (170 ml) at room temper-

TABLE IV. Physicochemical Data for *N*-Alkylcarboxylates (**5**)



No.	X	R	mp °C (Recryst. solvent)	Yield (%)	Formula	Analysis Calcd (Found)		
						C	H	N
5a-1	CH=CH	CH_2CO_2Et	Oil	65	$C_{24}H_{25}NO_2$		359.1885[a] (359.1883)	
5a-2	CH=CH	$(CH_2)_2CO_2Et$	66—67 (*n*-Hexane)	97	$C_{25}H_{27}NO_2$	80.40 (80.35)	7.29 7.39	3.75 3.77)
5a-3	CH=CH	$(CH_2)_3CO_2Et$	Oil	96	$C_{26}H_{29}NO_2$		387.2198[a] (387.2201)	
5a-4	CH=CH	$(CH_2)_4CO_2Et$	185.5—186.5 (EtOH–Et_2O)	92	$C_{27}H_{31}NO_2 \cdot HCl$	74.04 (74.04	7.36 7.22	3.20 3.14)
5a-5	CH=CH	$(CH_2)_5CO_2Me$	200—201 (EtOH)	94	$C_{27}H_{31}NO_2 \cdot HCl$	74.04 (73.82	7.36 7.42	3.20 3.10)
5b	CH_2CH_2	$(CH_2)_2CO_2Et$	198—202 (EtOH–Et_2O)	70	$C_{25}H_{29}NO_2 \cdot HCl \cdot 1/4H_2O$	72.10 (72.04	7.38 7.41	3.36 3.33)
5c	CH_2O	$(CH_2)_2CO_2Et$	Oil	80	$C_{24}H_{27}NO_3$		377.1990[a] (377.1988)	
5d	CH_2S	$(CH_2)_2CO_2Et$	Oil	94	$C_{24}H_{27}NO_2S$		393.1763[a] (393.1764)	
5e	O	$(CH_2)_2CO_2Et$	Oil	90	$C_{23}H_{25}NO_3$		363.1834[a] (363.1826)	
5f	S	$(CH_2)_2CO_2Et$	185—188 (Acetone–Et_2O)	75	$C_{23}H_{25}NO_2S \cdot HCl \cdot 1/4H_2O$	65.70 (65.90	6.35 6.39	3.33 3.13)

a) High-resolution MS data. The upper values are calculated and the lower ones are those found.

ature, and the mixture was refluxed for 5 h. It was then washed with diluted hydrochloric acid and water, dried over Na_2SO_4 and evaporated. The oily residue was triturated in *n*-hexane to give **3a** (37.4 g, 94%). Recrystallization from EtOH afforded pale yellow needles, mp 123—124 °C [lit.[6] 116 °C (MeOH)]. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$), 1.92—2.52 (4H, m, $CH_2 \times 2$), 2.88—3.30 (2H, m, CH_2), 3.40—3.87 (2H, m, CH_2), 4.11 (2H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$), 6.91 (2H, s, CH=CH), 7.08—7.52 (8H, m, Ar-H). MS *m/z*: 345 (M^+). *Anal.* Calcd for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05. Found: C, 80.24; H, 6.73; N, 3.95.

4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine (4a) A mixture of **3a** (65.6 g, 0.19 mol) and KOH (32.0 g, 0.57 mol) in *n*-BuOH (250 ml) was refluxed for 2 h. After removal of the solvent under reduced pressure, the residue was diluted with water and extracted with toluene. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to afford **4a** as pale yellow crystals (47.9 g, 92%). The free base was converted to the hydrochloride by the usual method.

Hydrochloride: Colorless needles, mp >300 °C (MeOH) [lit.[3] 290—292 °C (dec.)]. ^{1}H-NMR (DMSO-d_6) δ: 1.95—3.20 (8H, m, $CH_2 \times 4$), 6.98 (2H, s, CH=CH), 7.13—7.58 (8H, m, Ar-H), 9.36 (2H, br s, N^+H_2). MS *m/z*: 273 (M^+ (free)). *Anal.* Calcd for $C_{20}H_{19}N \cdot HCl$: C, 77.53; H, 6.51; N, 4.52. Found: C, 77.79; H, 6.49; N, 4.48.

4-(10,11-Dihydro-5*H*-dibenzo[*a*,*d*]cycloheptene-5-ylidene)piperidine (**4b**), 4-(6*H*-dibenz[*b*,*e*]oxepin-11-ylidene)piperidine (**4c**), 4-(6*H*-dibenzo-[*b*,*e*]thiepin-11-ylidene)piperidine (**4d**) and 4-(9*H*-thioxanthen-9-ylidene)-piperidine (**4f**) were prepared similarly.

4-(9*H*-Xanthen-9-ylidene)piperidine (4e) A solution of **2e** (4.00 g, 14 mmol), 2,2,2-trichloroethyl chloroformate (5.96 ml, 43 mmol) and triethylamine (2.61 ml, 19 mmol) in 1,2-dichloroethane (60 ml) was stirred at room temperature overnight. The reaction mixture was washed with water, dried over Na_2SO_4 and evaporated to give crude 2,2,2-trichloroethyl carbamate as a colorless oil. Zinc powder (6.84 g, 104 mmol) was added to a solution of the crude 2,2,2-trichloroethyl carbamate in 90% AcOH (140 ml) at room temperature, and the mixture was stirred at room temperature for 30 min. The insoluble materials were filtered off. The filtrate was made alkaline with aqueous K_2CO_3 solution and then extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to afford **4e** as pale brown crystals (2.30 g, 62%). The free base was converted to the hydrochloride by the usual method.

Hydrochloride: Pale brown crystals, mp 290—293 °C (EtOH) [lit.[7] (free base) 147—149 °C]. ^{1}H-NMR (DMSO-d_6) δ: 2.85—3.00 (4H, m, $CH_2 \times 2$), 3.04—3.19 (4H, m, $CH_2 \times 2$), 7.11—7.55 (8H, m, Ar-H). MS *m/z*: 263 (M^+ (free)). *Anal.* Calcd for $C_{18}H_{17}NO \cdot HCl$: C, 72.11; H, 6.05; N, 4.67. Found: C, 72.07; H, 6.13; N, 4.59.

Ethyl [4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidino]acetate (5a-1) A mixture of **4a** (3.01 g, 11 mmol), ethyl bromoacetate (2.21 g, 13 mmol) and K_2CO_3 (1.52 g, 11 mmol) in *N*,*N*-dimethylformamide (DMF) (20 ml) was heated at 70 °C for 6 h. The reaction mixture was diluted with water (60 ml) and extracted with Et_2O. The organic layer was washed with water, dried over Na_2SO_4, and evaporated. The oily residue was purified by column chromatography [SiO_2, *n*-hexane–AcOEt (3:1)] to afford **5a**-1 as a pale brown oil (2.56 g, 65%). IR (liq.): 1746 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J= 7.5 Hz, $CO_2CH_2C\underline{H}_3$), 1.98—2.92 (8H, m, $CH_2 \times 4$), 3.17 (2H, s, CH_2), 4.16 (2H, q, J=7.5 Hz, $CO_2C\underline{H}_2CH_3$), 6.91 (2H, s, CH=CH), 7.08—7.46 (8H, m, Ar-H). High-resolution MS *m/z*: Calcd for $C_{24}H_{25}NO_2$: 359.1885. Found: 359.1883.

Ethyl 3-[4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidino]propionate (5a-2) A mixture of **4a** (4.00 g, 15 mmol) and ethyl acrylate (2.06 ml, 19 mmol) in EtOH (20 ml) was refluxed for 1 h. After removal of the solvent under reduced pressure, the oily residue was purified by column chromatography [SiO_2, $CHCl_3$] to afford **5a**-2 as colorless crystals (5.30 g, 97%). Recrystallization from *n*-hexane afforded colorless crystals, mp 66—67 °C. IR (KBr): 1738 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$), 1.95—2.80 (12H, m, $CH_2 \times 6$), 4.11 (2H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$), 6.90 (2H, s, CH=CH), 7.07—7.48 (8H, m, Ar-H). MS *m/z*: 373 (M^+). *Anal.* Calcd for $C_{25}H_{27}NO_2$: C, 80.40; H, 7.29; N, 3.75. Found: C, 80.35; H, 7.39; N, 3.77.

The free base was converted to the hydrochloride by the usual method.

Hydrochloride: Colorless needles, mp 151—152 °C (acetone–Et_2O). *Anal.* Calcd for $C_{25}H_{27}NO_2 \cdot HCl \cdot H_2O$: C, 70.16; H, 7.07; N, 3.27. Found: C, 70.26; H, 6.99; N, 3.01.

Other *N*-alkylcarboxylates (**5**) were prepared in a manner similar to

that described for **5a**-1 or **5a**-2 from corresponding secondary amines (**4**). Physicochemical data for *N*-alkylcarboxylates (**5**) are summarized in Table IV.

3-[4-(5*H*-Dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidino]propionic Acid (6a-2) A mixture of **5a**-2 hydrochloride (3.70 g, 9 mmol) and 2 N NaOH (13.6 ml, 27 mmol) in MeOH (35 ml) was refluxed for 1.5 h. After removal of the solvent under reduced pressure, the residue was diluted with water and adjusted to pH 4 with diluted hydrochloric acid. The resulting crystals were collected by filtration. Recrystallization from aqueous DMF afforded **6a**-2 as pale yellow needles (3.01 g, 88%), mp 199—201 °C. IR (KBr): 1606 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.80 (12H, m, $CH_2 \times 6$), 6.95 (2H, s, CH=CH), 7.08—7.52 (8H, m, Ar-H). MS *m/z*: 345 (M^+). *Anal.* Calcd for $C_{23}H_{23}NO_2 \cdot 2H_2O$: C, 72.42; H, 7.13; N, 3.67. Found: C, 72.23; H, 6.94; N, 3.67.

Hydrolysis of Ethyl 4-(9*H*-Xanthen-9-ylidene)-1-piperidinecarboxylate (3e) with Alkali A mixture of **3e** (41.6 g, 0.12 mol) and KOH (27.8 g, 0.50 mol) in *n*-BuOH (160 ml) was refluxed for 1 h. After removal of the solvent under reduced pressure, the residue was diluted with water and extracted with toluene. The organic layer was washed with water, dried over Na_2SO_4 and evaporated to leave a yellow oil, which was triturated in *n*-hexane to give a pale yellow solid (29.4 g) as an approximately 1 : 1 mixture of 4-(9*H*-xanthen-9-ylidene)piperidine (**4e**) and 1,2,3,6-tetrahydro-4-(9*H*-xanthen-9-yl)pyridine (**4e'**). Fumaric acid (0.44 g) was added to a solution of the above mixture (2.0 g) in EtOH (15 ml) at room temperature. Deposited crystals were collected by filtration and recrystallized from MeOH, yielding pure **4e** fumarate (0.66 g). The filtrate was evaporated to dryness. The residue was recrystallized from EtOH to afford pure **4e'** fumarate (0.12 g).

4e (1/2 Fumarate): Colorless needles, mp 234—235 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.61—2.78 (4H, m, $CH_2 \times 2$), 2.78—2.94 (4H, m, $CH_2 \times 2$), 6.45 (1H, s, fumarate), 7.10—7.48 (8H, m, Ar-H). MS *m/z*: 263 (M^+ (free)).

4e' (3/2 Fumarate): Colorless needles, mp 194.5—196 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.78 (2H, br s, CH_2), 2.90 (2H, t, J=6 Hz, CH_2), 3.49 (2H, br s, CH_2), 4.84 (1H, s, CH), 5.75 (1H, s, CH), 6.47 (1.5H, s, fumarate), 7.00—7.34 (8H, m, Ar-H). MS *m/z*: 263 (M^+ (free)).

Pharmacological Evaluation Procedures. Histamine-1 (H_1) Receptor Binding Assay Male Hartley guinea-pigs (weighing 420 to 560 g) were decapitated and the brain cortex was isolated. The cortex was homogenized in 20 volumes of 50 mM Na/K phosphate buffer (pH 7.4) using a Polytron at setting 7 with two 10-s bursts separated by a 30-s pause. The homogenates were centrifuged at 50000 × *g* for 15 min at 4 °C. The pellets were washed twice and the final pellets resuspended in cold 50 mM Na/K phosphate buffer. For the [^{3}H]mepyramine binding assay, each assay tube received 50 μl of radioligand ([^{3}H]mepyramine, 917.6 GBq/mmol, NEN), 50 μl of the test compound or buffer and 0.3 ml of the membrane suspension. The binding assay was initiated by the addition of the membrane suspension and assay tubes were kept at room temperature for 1 h. The reaction was terminated by rapid vacuum filtration over GF/B glass fiber filters (Whatman) using a cell harvester (M-24R, Brandel). The filters were transferred to vials containing 7 ml of Aquasol-2 and the radioactivity was counted (Model 3385, Packard). The specific binding of [^{3}H]mepyramine was estimated as the difference between radioactivity bound in the absence and in the presence of 1 μM promethazine. The IC_{50} values (concentration which produced 50% inhibition of the specific binding of [^{3}H]mepyramine) were determined.

Effect on Compound 48/80-Induced Lethality in Rats[8] Male Wistar rats (starved for 24 h, 6 weeks of age) were used. Compound 48/80 (formaldehyde condensation product of *p*-methoxy-*N*-methylphenethylamine) was administered intravenously at a lethal dose of 1 mg/kg. Survival for more than 2 h was selected as an all-or-none criterion. Test compounds were given orally 1 h before compound 48/80 administration.

Effect on 48 h Homologous PCA in Rats The induction and evaluation of allergic reaction were done according to the method of Makino *et al.*[9] Male Wistar rats (starved for 20 h, 6 weeks of age) were passively sensitized by intracutaneous injection on the back at a volume of 0.1 ml of 20- or 40-fold-diluted anti-2,4-dinitrophenylated ascaris extract (DNP-As) rat serum. After 48 h, the animals were challenged by an intravenous injection of 0.5 ml of saline solution containing 1 mg of DNP-As and 5 mg of Evans blue. The animals were killed 30 min after the challenge and the extravasated dye was extracted with 1 N KOH and acetone, neutralized with 1 N H_3PO_4 and determined from the absorbance at 620 nm (U-2000, Hitachi). Test compounds were administered orally 1 h before antigen challenge. The inhibitory activity of the test compound was expressed as percent inhibition of PCA as compared with the control group. The ED_{50} value (dose which produced 50% inhibition of the PCA) was calculated according to the probit method.

Effect on Hexobarbital-Induced Anesthesia in Mice Male ddY mice (starved for 20 to 24 h, weighing 19 to 27 g) were treated orally with test compounds or vehicle. Thirty minutes later, hexobarbital sodium (80 mg/kg, i.p.) was injected into the animals and the duration of loss of righting reflex was observed and taken as the sleeping time. The percent increase of sleeping time was calculated by using the following formula:

$$\text{percent increase} = \frac{\text{sleeping time of drug-treated} - \text{sleeping time of vehicle-treated}}{\text{sleeping time of vehicle-treated}} \times 100$$

The ID_{50} (mg/kg) value (dose which produced 50% increase of sleeping time relative to that of the vehicle-treated group) was determined for each compound.

Effect on Locomotor Activity in Mice Male ddY mice (starved for 24 h, weighing 20 to 30 g) were used. Locomotor activity was recorded with an Animex activity meter (MK-110, Muromachi Kikai) for 4 h after oral administration of each test compound. The maximum no-effect dose (MNED) was determined.

Effect on Histamine-Induced Bronchoconstriction in Anesthetized Guinea-Pigs The induction and evaluation of activity were done according to the method of Makino *et al.*[10] Male Hartley guinea-pigs (starved for 24 h, weighing 350 to 450 g) were anesthetized with urethane (1.5 g/kg, i.p.). The carotid artery and jugular vein were cannulated for measurement of arterial blood pressure and for intravenous histamine administration. The trachea was cannulated and the animals were ventilated using a respiratory pump (60 strokes/min; 4 ml/stroke). Changes in insufflation pressure at a constant airflow induced by the administration of histamine (20 μg/kg, i.v.) were expressed as a percentage of the maximum pressure (100%) (Bronchospasm transducer 7020, Ugo Basile). Test compounds were given orally 2 h before the administration of histamine. The ED_{50} value (dose which produced 50% inhibition of histamine-induced bronchoconstriction to that of control) was determined in each case.

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