Study on Zwitter-Ionization of Drugs. II.[1] Synthesis and Pharmacological Activity of Some *N*-[3-(5*H*-Dibenzo[*a*, *d*]cyclohepten-5-ylidene)propyl]-*N*-methylamino- and *N*-[3-(6*H*-Dibenz[*b*, *e*]oxepin-11-ylidene)propyl]-*N*-methylamino-alkanoic Acid Derivatives and Related Compounds

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A series of *N*-[3-(5*H*-dibenzo[*a*, *d*]cyclohepten-5-ylidene)propyl]-*N*-methylamino- (6a) and *N*-[3-(6*H*-dibenz-[*b*, *e*]oxepin-11-ylidene)propyl]-*N*-methylamino-alkanoic acid derivatives (6b) and related compounds (6c—f) were synthesized and examined for pharmacological activities *in vitro*, *i.e.*, inhibitory effect on monoamine [noradrenaline (NA) and 5-hydroxytryptamine (5-HT)] uptake, inhibitory effect on 5-HT-, histamine-, acetylcholine- and NA-induced contraction, and binding affinity for α_2-adrenoceptor and dopamine D_2-receptor. *In vitro* tests indicated that zwitter-ionization was capable of maintaining H_1-antihistaminic activity while greatly reducing other pharmacological activities. Further, 6a—f showed much stronger inhibitory effects on compound 48/80-induced lethality in rats than did the corresponding *N*,*N*-dimethylamines (2a—f). 3-[*N*-[3-(6*H*-Dibenz[*b*,*e*]oxepin-11-ylidene)propyl]-*N*-methylamino]-propionic acid (6b-2), selected as a candidate antiallergic agent of a new type, equally potent in rats and guinea-pigs, exhibited strong inhibitory effects on 48 h homologous passive cutaneous anaphylaxis (PCA) in rats (ED_{50} = 0.019 mg/kg, *p.o.*) and on histamine-induced bronchoconstriction in anesthetized guinea-pigs (ED_{50} = 0.0067 mg/kg, *p.o.*).

Keywords zwitter-ionization; dibenzo[*a*, *d*]cycloheptene; dibenz[*b*, *e*]oxepin; *N*-alkylcarboxy group; pharmacological activity; antiallergic agent

Zwitter-ionization of drugs by introducing *N*-alkylcarboxy groups instead of *N*-alkyl (especially *N*-methyl) groups and its influence on pharmacological activity have been studied. In our previous paper,[1] zwitter-ionized derivatives of tricyclic antipsychotic agents (**1a**—**c**) were synthesized and examined for modification of the pharmacological activities, such as inhibitory effect on monoamine uptake, binding affinity for monoamine receptor and antiallergic activity. It was found that this zwitter-ionization resulted in retention of H_1-antihistaminic activity while greatly reducing other pharmacological activities, and the derivatives showed enhanced inhibitory effects on 48 h homologous passive cutaneous anaphylaxis (PCA) in rats, compared with the corresponding *N*-methylamines (**1a**—**c**). The zwitter-ionization would thus appear to be an effective approach to obtain antiallergic agents of a novel type.

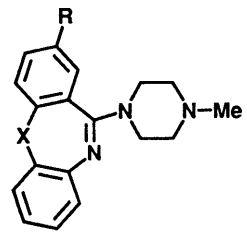
To examine the generality of the zwitter-ionization effect, we synthesized various zwitter-ionized derivatives of a tricyclic muscle relaxant, cyclobenzaprine (**2a**),[2] and a tricyclic antidepressant, doxepin (**2b**),[3] and examined the alterations of their pharmacological activities, such as inhibitory effect on monoamine uptake, binding affinity for monoamine receptor and H_1-antihistaminic activity (Table IV).

Synthesis

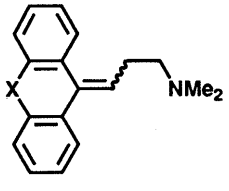
The compounds tested were synthesized by the methods shown in Chart 2. *N*,*N*-Dimethylamines (**2a**—**b**) were treated with ethyl chloroformate[4] in toluene or 1,2-dichloroethane to give the corresponding ethyl carbamates (**3a**—**b**), which were subsequently hydrolyzed with alkali, yielding *N*-methylamines (**4a**—**b**). Compounds **4a**—**b** were alkylated with ethyl bromoacetate, ethyl acrylate, ethyl 4-bromobutyrate, ethyl 5-bromovalerate and ethyl 6-bromohexanoate to give the corresponding ethyl *N*-alkylcarboxylates (**5a**—**b**); subsequent hydrolysis with 2 N NaOH afforded the corresponding *N*-alkylcarboxylic acids (**6a**—**b**), whose methylene chain varied in length. Compounds **3**—**6b** were mixtures of geometrical isomers (*E* and *Z*). Physicochemical data are given in Tables I, II and III.

Results and Discussion

We initially examined the changes of pharmacological profile caused by zwitter-ionization, by comparing **6a**—**b** with the corresponding *N*,*N*-dimethylamines (**2a**—**b**). Compounds **6a**—**b** were tested for the following pharmacological activities *in vitro*: (1) inhibitory effect on monoamine [noradrenaline (NA) and 5-hydroxytryptamine



1a: X = O, R = Cl
1b: X = S, R = Cl
(clothiapine)
1c: X = CH_2, R = H
(perlapine)



2a: X = CH=CH
(cyclobenzaprine)
2b: X = CH_2O
(doxepin, *E*,*Z*-mixture)

Chart 1

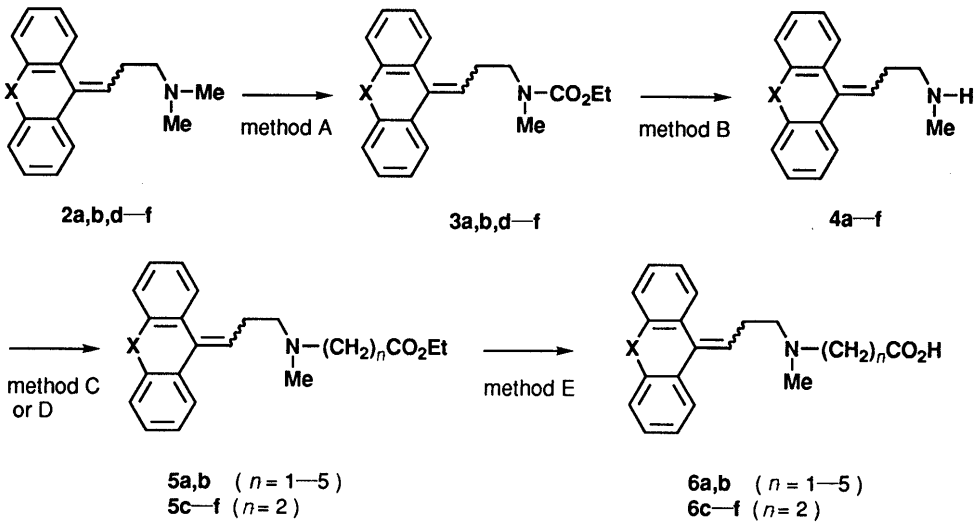
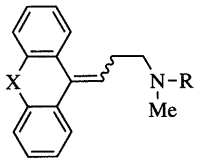


Chart 2

TABLE I. Physicochemical Data for Ethyl Carbamates (**3**) and *N*-Methylamines (**4**)

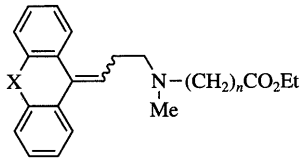


Compd. No.	X	R	Geometry[a] *E*/*Z*	mp, °C (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
3a	CH=CH	CO_2Et		Oil	89	$C_{22}H_{23}NO_2$	333.1729[c] (333.1720)		
3b	CH_2O	CO_2Et	85/15[b]	Oil	80	$C_{21}H_{23}NO_3$	337.1678[c] (337.1643)		
3d	CH_2S	CO_2Et	94/ 6[b]	Oil	92	$C_{21}H_{23}NO_2S$	353.1449[c] (353.1445)		
3e	O	CO_2Et		Oil	80	$C_{20}H_{21}NO_3$	323.1521[c] (323.1524)		
3f	S	CO_2Et		Oil	98	$C_{20}H_{21}NO_2S$	339.1293[c] (339.1296)		
4a	CH=CH	H		182.5—183.5 (CH_2Cl_2–C_6H_6)	80	$C_{19}H_{19}N \cdot HCl$	76.62 (76.48	6.77 6.82	4.70 4.68)
4b	CH_2O	H	70/30	224—227 (CH_2Cl_2–C_6H_6)	50	$C_{18}H_{19}NO \cdot HCl$	71.63 (71.74	6.68 6.64	4.64 4.56)
4d	CH_2S	H	86/14	220—223 (EtOH–Et_2O)	77	$C_{18}H_{19}NS \cdot HCl$	68.01 (67.98	6.34 6.24	4.41 4.49)
4e	O	H		183—185.5 (iso-PrOH)	48	$C_{17}H_{17}NO \cdot HCl$	70.95 (70.76	6.30 6.30	4.87 4.74)
4f	S	H		223—226 (iso-PrOH)	71	$C_{17}H_{17}NS \cdot HCl$	67.20 (67.20	5.97 5.89	4.61 4.55)

a) *E*/*Z* ratios were determined by HPLC. *b*) *E*/*Z* ratios were determined by ^{1}H-NMR. *c*) High-resolution MS data.

(5-HT)] uptake, a characteristic property of tricyclic antidepressants, (2) inhibitory effects on 5-HT- and histamine-induced contraction as an index of antiallergic activity, (3) inhibitory effects on acetylcholine- and NA-induced contraction, and binding affinity for α_2-adrenoceptor and dopamine D_2-receptor as indices of effects on the central nervous system (CNS). The results are shown in Table IV.

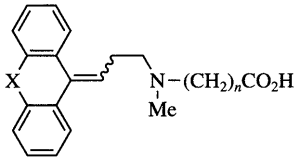
TABLE II. Physicochemical Data for Ethyl *N*-Alkylcarboxylates (**5**)



Compd. No.	X	*n*	Geometry[a] *E*/*Z*	Appearance	Yield (%)	Formula	HR-MS[b] Calcd (Found)
5a-1	CH=CH	1		Oil	54	$C_{23}H_{25}NO_2$	345.1729 (345.1777)
5a-2	CH=CH	2		Oil	89	$C_{24}H_{27}NO_2$	361.2042 (361.1970)
5a-3	CH=CH	3		Oil	67	$C_{25}H_{29}NO_2$	375.2198 (375.2175)
5a-4	CH=CH	4		Oil	77	$C_{26}H_{31}NO_2$	389.2355 (389.2323)
5a-5	CH=CH	5		Oil	68	$C_{27}H_{33}NO_2$	403.2551 (403.2530)
5b-1	CH_2O	1	70/30	Oil	64	$C_{22}H_{25}NO_3$	351.1835 (351.1803)
5b-2	CH_2O	2	70/30	Oil	89	$C_{23}H_{27}NO_3$	365.1991 (365.1977)
5b-3	CH_2O	3	70/30	Oil	66	$C_{24}H_{29}NO_3$	377.1991 (377.1989)
5b-4	CH_2O	4	70/30	Oil	64	$C_{25}H_{31}NO_3$	391.2148 (391.2142)
5b-5	CH_2O	5	70/30	Oil	60	$C_{26}H_{33}NO_3$	407.2461 (407.2482)
5c-2	CH_2CH_2	2		Oil	95	$C_{24}H_{29}NO_2$	363.2198 (363.2210)
5d-2	CH_2S	2	86/14	Oil	98	$C_{23}H_{25}NO_2S$	381.1763 (381.1763)
5e-2	O	2		Oil	93	$C_{22}H_{25}NO_3$	351.1834 (351.1812)
5f-2	S	2		Oil	72	$C_{22}H_{25}NO_2S$	367.1606 (367.1610)

a) *E*/*Z* ratios were determined by ^{1}H-NMR. *b*) High-resolution MS data.

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



Compd. No.	X	*n*	Geometry[a] *E*/*Z*	mp, °C (Recryst. solvent)	Yield (%)	Formula	Analysis Calcd (Found)		
							C	H	N
6a-1	CH=CH	1		97—98 (EtOH)	59	$C_{21}H_{21}NO_2$	78.97 (78.82)	6.63 6.88	4.39 4.44)
6a-2	CH=CH	2		119—120 (aq. EtOH)	74	$C_{22}H_{23}NO_2$	79.25 (79.20)	6.95 7.01	4.20 3.98)
6a-3	CH=CH	3		Oil	88	$C_{23}H_{25}NO_2$	347.1885[b] (347.1862)		
6a-4	CH=CH	4		133—134 (aq. EtOH)	74	$C_{24}H_{27}NO_2$	79.74 (79.55	7.53 7.80	3.87 3.65)
6a-5	CH=CH	5		129.5—130.5 (aq. EtOH)	52	$C_{25}H_{29}NO_2$	79.96 (80.11	7.78 7.99	3.73 3.96)
6b-1	CH_2O	1	70/30	Oil	78	$C_{20}H_{21}NO_3$	323.1552[b] (323.1527)		
6b-2	CH_2O	2	70/30	Oil	90	$C_{21}H_{23}NO_3$	337.1678[b] (337.1740)		
6b-3	CH_2O	3	70/30	Oil	88	$C_{22}H_{25}NO_3$	351.1834[b] (351.1818)		
6b-4	CH_2O	4	70/30	150—151 ($MeOH–Et_2O$)	45	$C_{23}H_{27}NO_3$	75.59 (75.60	7.45 7.43	3.83 4.02)
6b-5	CH_2O	5	70/30	Oil	77	$C_{24}H_{29}NO_3$	379.2148[b] (379.2146)		
6c-2[c]	CH_2CH_2	2		Oil	Quant.	$C_{22}H_{25}NO_2$	335.1885[b] (363.1885)		
6d-2	CH_2S	2	86/14	Oil	Quant.	$C_{23}H_{25}NO_2S$	353.1450[b] (353.1454)		
6e-2	O	2		Oil	99	$C_{20}H_{21}NO_3$	323.1521[b] (323.1515)		
6f-2	S	2		Oil	87	$C_{20}H_{21}NO_2S$	339.1293[b] (339.1286)		

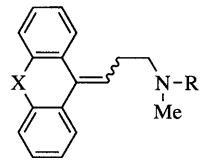
a) *E*/*Z* ratios were determined by HPLC. *b*) High-resolution MS data. *c*) Reference 7.

As regards inhibitory effect on monoamine uptake, anti-5-HT activity, anti-α_1 activity, and D_2-receptor binding affinity, **6a—b** showed much weaker activities than **2a—b**. These activities were not affected by alkylene chain length (*n*). Compounds **6a—b** also exhibited markedly decreased antimuscarinic (M) activity and α_2-binding affinity, which increased with elongation of the alkylene chain. On the contrary, the anti-H_1 activity was unaffected by zwitter-ionization, except in the case of acetic acid derivatives ($n=1$). Zwitter-ionized compounds (**6a—b**) thus appear capable of separating anti-H_1 activity from other pharmacological activities such as CNS effects. Similar results

TABLE IV. *In Vitro* Pharmacological Data for Dibenzo[*a*,*d*]cycloheptene (**2a**, **6a**) and Dibenz[*b*,*e*]oxepin Derivatives (**2b**, **6b**)

Compd. No.	Inhibition of NA uptake (pIC_{50})	Inhibition of 5-HT uptake (pIC_{50})	Anti-5-HT activity (pK_B)	Anti-H_1 activity (pK_B)	Anti-M activity (pK_B)	Anti-α_1 activity (pK_B)	α_2 binding affinity (pIC_{50})	D_2 binding affinity (pIC_{50})
2a	6.76	5.81	7.79	9.32	8.12	6.85	6.61	5.91
6a-1	5.27	<5	5.60	7.50	<5	<5	<5	<5
6a-2	<5	<5	5.92	8.16	<5	<5	<5	<5
6a-3	5.47	<5	6.06	8.37	5.59	<5	5.14	<5
6a-4	5.67	<5	5.90	8.21	6.04	5.35	5.33	<5
6a-5	<5	<5	6.03	7.92	6.26	5.60	5.59	<5
2b	6.74	5.29	7.51	10.11	7.23	7.02	7.23	5.65
6b-1	5.10	<5	5.25	7.81	<5	<5	<5	<5
6b-2	5.11	<5	5.65	8.51	5.15	<5	<5	<5
6b-3	5.27	<5	5.62	8.46	5.14	<5	5.30	<5
6b-4	<5	<5	5.60	8.36	5.00	5.63	5.55	<5
6b-5	<5	<5	5.49	8.11	5.50	5.76	6.04	<5

TABLE V. Inhibitory Effect of *N*,*N*-Dimethylamines (**2**) and *N*-Propionic Acids (**6**) on Compound 48/80-Induced Lethality



Compd. No.	X	R	Geometry[a] *E*/*Z*	Compound 48/80-induced lethality in rats (mg/kg, *p.o.*) Inhibition, % ($n=5$)				
				0.001	0.01	0.1	1	10
2a	CH=CH	Me					0	100
6a-2	CH=CH	$(CH_2)_2CO_2H$		0	20	100	100	
2b	CH_2O	Me	85/15[c]				0	100
6b-2[b]	CH_2O	$(CH_2)_2CO_2H$	70/30	20	60	100	100	
2c	CH_2CH_2	Me				0	40	40
6c-2	CH_2CH_2	$(CH_2)_2CO_2H$		0	20	80	100	
2d	CH_2S	Me	95/ 5[c]				0	40
6d-2	CH_2S	$(CH_2)_2CO_2H$	86/14		0	80	100	
2e	O	Me				0	20	40
6e-2	O	$(CH_2)_2CO_2H$					0	100
2f	S	Me				0	40	100
6f-2	S	$(CH_2)_2CO_2H$		0	20	100	100	

a) *E*/*Z* ratios were determined by HPLC. *b*) Although geometrical isomers of **6b-2** were similarly prepared from the geometrical isomers of **4b** according to the literature,[8] no significant difference between *E*- and *Z*-isomers was observed. *c*) *E*/*Z* ratios were determined by ^{1}H-NMR.

were observed in our previous study.[1]

From the viewpoint of the degree of separation between anti-H_1 activity and other pharmacological activities, propionic acid derivatives ($n=2$) were chosen for further evaluation of antiallergic activity. To optimize the tricyclic systems, the influence of various linkages (X) was examined. By the same methods as used for **6a**—**b**, the propionic acid derivatives of **6d** (X=CH_2S), **6e** (X=O), and **6f** (X=S) were synthesized from the corresponding *N*,*N*-dimethylamines (**2d**—**f**).[5,6] The propionic acid derivative of **6c** (X=CH_2CH_2) was prepared from 3-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)-*N*-methylpropylamine (nortryptyline, **4c**) obtained commercially (Tables I, II, and III). Antiallergic activity was initially assessed in terms of the inhibitory effect on compound 48/80-induced lethality in rats (Table V).

The propionic acid derivatives of **6a**—**d** and **6f**, but not that of **6e**, showed much stronger (100—1000 times greater) inhibotory effects than the corresponding *N*,*N*-dimethylamines (**2a**—**d**, **2f**). Saturation of the double bond at the 10,11-position of **6a-2** to give **6c-2** and substitution of an oxygen atom for a sulfur atom (**6b-2**→**6d-2**) led to slight loss of activity. Contraction of the seven-membered ring to the six-membered analogue (**6b-2**→**6e-2**) resulted in loss of activity, although **2e** exhibited stronger activity than **2b** in the *N*,*N*-dimethylamine series. Ring contraction increased the activity in the case of the thioether derivative (**6d-2**→**6f-2**).

Compound **6b-2**, exhibiting the strongest inhibitory effect on compound 48/80-induced lethality in rats, was selected for further evaluation, for comparison with ketotifen and terfenadine as reference compounds (Table VI). Antiallergic activity was assessed in terms of inhibitory effects on PCA in rats and on histamine-induced bronchoconstriction in anesthetized guinea-pigs. CNS side-effects were assessed in terms of effect on hexobarbital-induced anesthesia in mice, locomotor activity in mice, and general behavior in mice and rats. Compound **6b-2** showed greatly increased anti-PCA activity compared to the *N*,*N*-dimethylamine (**2b**) and was as potent as ketotifen in terms of its inhibito-

TABLE VI. Pharmacological Data for Compound **6b-2**

Compound No.	PCA in rats ED_{50} (mg/kg, *p.o.*)	Histamine-induced bronchoconstriction in G.P. ED_{50} (mg/kg, *p.o.*)	Hexobarbital-induced anesthesia in mice ID_{50} (mg/kg, *p.o.*)	Animex in mice MNED (mg/kg, *p.o.*)	General symptoms in mice MNED (mg/kg, *p.o.*)	General symptoms in rats MNED (mg/kg, *p.o.*)
2b	5.1	NT	30	10	10	30
6b-2	0.019	0.0067	17	30	10	>100
Ketotifen	0.43	0.0050	13	30	30	30
Terfenadine	9.0	0.33	76	>100	100	>100

G.P.: guinea-pigs. MNED: maximum no-effect dose. NT: not tested.

ry effect on histamine-induced bronchoconstriction. Thus, **6b-2** is promising candidate as an antiallergic agent of a new type, with equal potency in rats and guinea-pigs.

In conclusion, our results indicate that zwitter-ionization is an effective approach to the design of antiallergic agents, because of the separation of antihistaminic activity from other pharmacological activities such as CNS effects, as well as enhancement of antiallergic activity in both rats and guinea-pigs. Further studies are under way.

Experimental

All melting points were measured on a micro Yanagimoto melting point apparatus, without correction. Spectral data were obtained as follows: ^{1}H-NMR spectra with JEOL JNM-PMX 60 (60 MHz), JEOL FX-90Q (90 MHz) and JEOL A-500 (500 MHz) spectrometers, with 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 Perkin-Elmer 230 C and Yanagimoto MT-3 or MT-5 elemental analysis apparatus. HPLC was performed with a JASCO 880 pumping system and 870 ultraviolet detector. Column chromatography was carried out with silica gel [Kieselgel 60 (Merck)] or aluminum oxide [Al_2O_3 90 (Merck)]. TLC was conducted on a 0.25 mm pre-coated silica gel plate ($60F_{254}$, Merck) or a 0.20 mm pre-coated aluminum oxide plate ($60F_{254}$, Merck).

The following known intermediates were prepared essentially according to the literature: 3-(6*H*-dibenzo[*b,e*]thiepin-11-ylidene)-*N,N*-dimethylpropylamine (dothiepin, **2d**),[5] *N,N*-dimethyl-3-(9*H*-xanthen-9-ylidene)-propylamine (**2e**),[6] *N,N*-dimethyl-3-(9*H*-thioxanthen-9-ylidene)propylamine (**2f**).[6] 3-(5*H*-Dibenzo[*a,d*]cycloheptен-5-ylidene)-*N,N*-dimethylpropylamine (**2a**), 3-(6*H*-dibenz[*b,e*]oxepin-11-ylidene)-*N,N*-dimethylpropylamine (**2b**), and **4c** were commercial products, used as supplied.

Method A. Ethyl *N*-[3-(5*H*-Dibenzo[*a,d*]cycloheptен-5-ylidene)propyl]-*N*-methylcarbamate (3a) Saturated $NaHCO_3$ was added to a solution of **2a** hydrochloride (10 g, 32 mmol) in $CHCl_3$ (100 ml), and the mixture was stirred at room temperature for a few minutes. The $CHCl_3$ layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was diluted with toluene (50 ml). To this solution, ethyl chloroformate (17 g, 157 mmol) was added dropwise at 80 °C. The reaction mixture was refluxed for 6 h, washed with water, dried over Na_2SO_4, and evaporated to leave a yellow oil. The residue was purified by column chromatography [SiO_2, *n*-hexane–AcOEt (3 : 1)] to afford **3a** as a pale yellow oil (9.5 g, 89%). IR (film): 1702 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (3H, t, J=7.5 Hz, $CO_2CH_2C\underline{H}_3$), 2.29 (2H, q, J=7 Hz, CH_2) 2.72 (3H, s, CH_3), 3.27 (2H, t, J=7 Hz, CH_2), 4.05 (2H, q, J=7.5 Hz, $CO_2C\underline{H}_2CH_3$), 5.50 (1H, t, J=7 Hz, CH), 6.83 (2H, s, CH=CH), 7.00—7.60 (8H, m, Ar-H). MS *m/z*: 333 (M^+).

Compounds **3b**, **3d**, **3e** and **3f** were prepared by a similar method to that described above from the corresponding *N,N*-dimethylamines (**2**).

Method B. 3-(5*H*-Dibenzo[*a,d*]cycloheptен-5-ylidene)-*N*-methylpropylamine (4a) A mixture of **3a** (10 g, 30 mmol) and KOH (8.4 g, 150 mmol) in *n*-BuOH (50 ml) was refluxed for 4 h. The reaction mixture was poured into water, and extracted with $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography [SiO_2, $CHCl_3$–MeOH (10 : 1)] to afford **4a** as pale yellow crystals (6.3 g, 80%). ^{1}H-NMR ($CDCl_3$) δ: 1.56 (1H, s, NH, disappeared on adding D_2O), 2.00—2.92 (4H, m, CH_2CH_2), 2.29 (3H, s, N-CH_3), 5.52 (1H, t, J=7 Hz, CH), 6.85 (2H, s, CH=CH), 7.08—7.58 (8H, m, Ar-H). MS *m/z*: 261 (M^+). The free base was converted to the hydrochloride by the usual method.

Hydrochloride: Colorless crystals, mp 182.5—183.5 °C (CH_2Cl_2–benzene) [lit.[9] 183—184 °C (EtOH–Et_2O)]. IR (KBr): 2960 cm^{-1} (N–H).

3-(6*H*-Dibenz[*b,e*]oxepin-11-ylidene)-*N*-methylpropylamine (**4b**), 3-(6*H*-dibenzo[*b,e*]thiepin-11-ylidene)-*N*-methylpropylamine (**4d**), *N*-methyl-3-(9*H*-xanthen-9-ylidene)propylamine (**4e**) and *N*-methyl-3-(9*H*-thioxanthen-9-ylidene)propylamine (**4f**) were prepared in a manner similar to that described for **4a**.

Method C. Ethyl [*N*-[3-(5*H*-Dibenzo[*a,d*]cycloheptен-5-ylidene)propyl]-*N*-methylamino]acetate (5a-1) A mixture of **4a** (2.6 g, 10 mmol), ethyl bromoacetate (2.0 g, 12 mmol) and K_2CO_3 (1.4 g, 10 mmol) in dimethylformamide (DMF) (13 ml) was heated at 70 °C for 4 h with stirring. The reaction mixture was diluted with water and extracted with Et_2O. The organic layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was separated by column chromatography [Al_2O_3, *n*-hexane–Et_2O (1 : 1)] to afford **5a-1** as a pale yellow oil (1.9 g, 54%). IR (film): 1734 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$), 1.98—2.73 (4H, m, CH_2CH_2N), 2.28 (3H, s, CH_3), 3.16 (2H, s, NCH_2), 4.13 (2H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$), 5.56 (1H, t, J=7 Hz, CH), 6.85 (2H, s, CH=CH), 7.21—7.41 (8H, m, Ar-H). High resolution MS *m/z*: Calcd for $C_{23}H_{25}NO_2$ 345.1729. Found: 345.1777 (M^+).

Compounds **5a-3**, **5a-4**, **5a-5**, **5b-1**, **5b-3**, **5b-4** and **5b-5** were prepared by a similar method to that described above from the corresponding *N*-methylamines (**4**).

Method D. Ethyl 3-[*N*-[3-(5*H*-Dibenzo[*a,d*]cycloheptен-5-ylidene)propyl]-*N*-methylamino]propionate (5a-2) A solution of **4a** (2.6 g, 10 mmol) and ethyl acrylate (1.2 g, 12 mmol) in EtOH (13 ml) was refluxed for 2 h. The reaction mixture was evaporated and the residue was separated by column chromatography [Al_2O_3, *n*-hexane–Et_2O (1 : 1)] to afford **5a-2** as a pale yellow oil (3.2 g, 89%). IR (film): 1736 cm^{-1} (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$), 1.96—2.81 (8H, m, $CH_2NCH_2CH_2$), 2.15 (3H, s, CH_3), 4.11 (2H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$), 5.53 (1H, t, J=7 Hz, CH), 6.84 (2H, s, CH=CH), 7.23—7.42 (8H, m, Ar-H). High resolution MS *m/z*: Calcd for $C_{24}H_{27}NO_2$ 361.2042. Found: 361.1970 (M^+).

Compounds **5b-2**, **5c-2**, **5d-2**, **5e-2** and **5f-2** were prepared by a similar method to that described above from the corresponding *N*-methylamines (**4**).

Ethyl 3-[*N*-[3-(6*H*-Dibenz[*b,e*]oxepin-11-ylidene)propyl]-*N*-methylamino]propionate (5b-2) ^{1}H-NMR ($CDCl_3$) δ: 1.20 (0.9H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$ for *Z*-isomer), 1.21 (2.1H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$ for *E*-isomer), 2.03—2.78 (11H, m, $CH_2CH_2NCH_2CH_2$, CH_3), 4.07 (0.6H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$ for *Z*-isomer), 4.09 (1.4H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$ for *E*-isomer), 4.60—5.80 (2H, m, OCH_2), 5.68 (0.3H, t, J=7.5 Hz, CH for *Z*-isomer), 6.00 (0.7H, t, J=7.5 Hz, CH for *E*-isomer), 6.70—7.40 (8H, m, Ar-H).

Ethyl 3-[*N*-[3-(6*H*-Dibenzo[*b,e*]thiepin-11-ylidene)propyl]-*N*-methylamino]propionate (5d-2) ^{1}H-NMR ($CDCl_3$) δ: 1.20 (0.42H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$ for *Z*-isomer), 1.21 (2.58H, t, J=7 Hz, $CO_2CH_2C\underline{H}_3$ for *E*-isomer), 2.05—2.18 (2H, m, $=CHCH_2$), 2.12 (3H, s, CH_3), 2.35—2.75 (6H, m, $=CHCH_2C\underline{H}_2NC\underline{H}_2C\underline{H}_2$), 3.35 (1H, d, J=13.5 Hz, SC$\underline{H}$H), 4.07 (0.28H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$ for *Z*-isomer), 4.09 (1.72H, q, J=7 Hz, $CO_2C\underline{H}_2CH_3$ for *E*-isomer), 4.95 (1H, d, J=13.5 Hz, SCH$\underline{H}$), 5.64 (0.14H, t, J=7.5 Hz, CH for *Z*-isomer), 5.92 (0.86H, t, J=7.5 Hz, CH for *E*-isomer), 6.94—7.30 (8H, m, Ar-H).

Method E. [*N*-[3-(5*H*-Dibenzo[*a,d*]cycloheptен-5-ylidene)propyl]-*N*-methylamino]acetic Acid (6a-1) A solution of **5a-1** (1.7 g, 5 mmol) and 2 N NaOH (5 ml, 10 mmol) in MeOH (17 ml) was refluxed for 30 min and then evaporated. The residue was dissolved with water and neutralized with 2 N HCl (5 ml, 10 mmol). Deposited crystals were collected by filtration and recrystallized from aqueous EtOH to afford **6a-1** as colorless

crystals (0.94 g, 59%), mp 97—98 °C. IR (KBr): 1624 cm^{-1} (C=O). ^{1}H-NMR (CD_3OD) δ: 2.24—2.59 (2H, m, $=CHCH_2CH_2$), 2.66 (3H, s, CH_3), 2.99—3.23 (2H, s, $=CHCH_2CH_2$), 3.44 (2H, s, NCH_2), 5.50 (1H, t, J=7 Hz, CH), 6.91 (2H, s, CH=CH), 7.26—7.50 (8H, m, Ar-H). MS m/z: 319 (M^+). *Anal.* Calcd for $C_{21}H_{21}NO_2$: C, 78.97; H, 6.63; N, 4.39. Found: C, 78.82; H, 6.88; N, 4.40.

Compounds **6a-2**, **6a-3**, **6a-4**, **6a-5**, **6b-1**, **6b-2**, **6b-3**, **6b-4**, **6b-5**, **6c-2**, **6d-2**, **6e-2** and **6f-2** were prepared by a similar method to that described above from the corresponding ethyl *N*-alkylcarboxylate (**5**).

3-[*N*-[3-(6*H*-Dibenz[*b*,*e*]oxepin-11-ylidene)propyl]-*N*-methylamino]-propionic Acid (6b-2) ^{1}H-NMR ($CDCl_3$) δ: 2.36 (2.1H, s, CH_3 for *E*-isomer), 2.42—2.95 (8H, m, $CH_2CH_2NCH_2CH_2$), 2.51 (0.9H, s, CH_3 for *Z*-isomer), 4.60—5.70 (2H, m, OCH_2), 5.64 (0.3H, t, J=7.5 Hz, CH for *Z*-isomer), 5.95 (0.7H, t, J=7.5 Hz, CH for *E*-isomer), 6.70—7.43 (8H, m, Ar-H).

3-[*N*-[3-(6*H*-Dibenzo[*b*,*e*]thiepin-11-ylidene)propyl]-*N*-methylamino]-propionic Acid (6d-2) ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.12 (2H, m, $=CHCH_2$), 2.10 (3H, s, CH_3), 2.31 (1.72H, t, J=7 Hz, CH_2CO_2H for *E*-isomer), 2.35 (0.28H, t, J=7 Hz, CH_2CO_2H for *Z*-isomer), 2.42—2.53 (2H, m, $=CHCH_2CH_2N$), 2.58 (1.72H, J=7 Hz, $CH_2CH_2CO_2H$ for *E*-isomer), 2.64 (0.28H, t, J=7 Hz, $CH_2CH_2CO_2H$ for *Z*-isomer), 3.55—3.69 (1H, m, SCHH), 4.72—4.85 (1H, m, SCHH), 5.60 (0.14H, t, J=7.5 Hz, CH for *Z*-isomer), 5.92 (0.86H, t, J=7.5 Hz, CH for *E*-isomer), 6.92—7.42 (8H, m, Ar-H).

HPLC Analysis Chromatographic conditions were as follows. Condition A: column, TSK gel ODS-80TM (4.6 mm i.d. × 150 mm); column temperature, 30 °C; mobile phase, 0.01 M $KH_2PO_4 \cdot Na_2HPO_4$ buffer (pH 6.0)–MeOH (3:2); flow rate, 1.0 ml/min; detection, UV at 225 nm, t_R: *E*-**6b-2**, 28.1 min, *Z*-**6b-2**, 31.7 min. Condition B: column, TSK gel ODS-80TM (4.6 mm i.d. × 150 mm); column temperature, 30 °C; mobile phase, 0.01 M $KH_2PO_4 \cdot Na_2HPO_4$ buffer (pH 6.0)–MeOH (3:2); flow rate, 1.0 ml/min; detection, UV at 302 nm, t_R: *E*-**6d-2**, 44.8 min, *Z*-**6d-2**, 48.2 min.

Pharmacological Evaluation Procedures. Effects on NA and 5-HT Uptake Male Wistar rats (7—9 weeks of age) were decapitated and the hypothalamus was dissected out according to the method of Gowinski and Iversen.[10] The hypothalamus was weighed and homogenized with 10 (v/w) volumes of ice-cold 0.32 M sucrose in a glass Potter homogenizer with a Teflon pestle. The homogenates were centrifuged at 3000 × *g* for 10 min at 4 °C. The supernatant was decanted, and to it was added 20 (v/w) volumes of modified Krebs–Henseleit solution (containing NaCl 118 mM, KCl 4.70 mM, $CaCl_2 \cdot 2H_2O$ 1.25 mM, $MgSO_4 \cdot 7H_2O$ 1.20 mM, KH_2PO_4 1.20 mM, $NaHCO_3$ 25.0 mM, glucose 1.00 g/l, ascorbic acid 0.20 g/l, EDTA·2Na 0.05 g/l, and pargyline hydrochloride 60 μM). The solution was bubbled with 95% O_2 and 5% CO_2 gas mixture. The suspension was gently stirred to make it uniform, and distributed into test tubes in aliquots of 0.80 ml. Then 0.10 ml of various concentrations of test compound was added to each tube and the whole was allowed to equilibrate for 5 min at 37 °C. The reaction was started by adding 0.10 ml of radiolabelled amine such as *l*-[7,8-^{3}H]noradrenaline (Amersham) or 5-[1,2-^{3}H(N)]hydroxytryptamine to give a final concentration of 50 nM at 37 °C. After 5 min, the suspension was spplied to glass filters using a cell harvester and the filters were washed with 4 ml × 3 times of Krebs–Henseleit solution. Radioactivity on the filters was determined by liquid scintillation counting. Diffusion blanks were kept at 37 °C for 5 min, and their radioactivity was subtracted from all experimental samples as non-specific uptake. IC_{50} value was determined graphically by log–logit plot.

Effect of Contractile Responses Induced by NA and 5-HT in Isolated Rabbit Aorta (Anti-α_1- and Anti-5-HT Activity) Male Japanese White rabbits (2—4 months of age) were killed and the thoracic aorta was excised. Helical strips of the thoracic aorta were mounted vertically in an organ bath containing 10 ml of Krebs–Henseleit solution (NaCl 118 mM, KCl 4.70 mM, $CaCl_2 \cdot 2H_2O$ 2.55 mM, $MgSO_4 \cdot 7H_2O$ 1.18 mM, KH_2PO_4 1.18 mM, $NaHCO_3$ 24.9 mM, glucose 11.1 mM) continuously bubbled with 95% O_2 and 5% CO_2 gas mixture at 37 °C. Each strip was secured to the bottom of the organ bath and the other end was attached to a force-displacement transducer. Isometric tension was recorded on a recticorder. The length of strips was adjusted several times until a stable tension of 2 g was attained. The concentration–contractile response curves to NA and 5-HT were constructed before and after 30 min treatment with a test compound. In the case of noradrenaline, Krebs–Henseleit solution contained 10^{-6} M propranolol to block β-adrenoceptors. The dissociation constant (K_B value) of each test compound was calculated according to the method of Furchgott.[11]

Effect on Contractile Responses Induced by Histamine and Acetylcholine in Isolated Guinea-Pig Ileum (Anti-H_1 and Anti-M Activity) Male Hartley guinea-pigs (5—8 weeks of age) were killed and the ileum was excised. An approximately 20 mm strip of isolated ileum was mounted vertically under a 0.5 g load in an organ bath containing 10 ml of Locke–Ringer solution (NaCl 154 mM, KCl 5.60 mM, $CaCl_2 \cdot 2H_2O$ 2.20 mM, $MgCl_2 \cdot 6H_2O$ 2.10 mM, $NaHCO_3$ 5.90 mM, glucose 2.80 mM) maintained at 28 °C and bubbled with 95% O_2 and 5% CO_2 gas mixture. The contractile responses were recorded on a recticorder *via* an isotonic transducer. The concentration-contractile response curves to histamine and acetylcholine were constructed before and after 30 min contact with test compounds. The dissociation constant (K_B value) of each test compound was calculated according to the method of Furchgott.[11]

Adrenaline α_2 Receptor Binding Assay All adrenaline α_2 receptor binding assays were done in duplicate, using rat cortex homogenates and ^{3}H-rauwolscine, according to published methodology.[12] Non-specific binding was defined in the presence of 10 μM phentolamine. IC_{50} value (the concentration of the test compound that caused 50% inhibition of specific ^{3}H-rauwolscine binding) was calculated by nonlinear curve fitting techniques.

Dopamine D_2 Receptor Binding Assay All dopamine D_2 receptor binding assays were done in duplicate, using rat striatal homogenates and ^{3}H-spiperone, according to published methodology.[13] Non-specific binding was defined in the presence of 10 μM sulpiride. IC_{50} value (the concentration of the test compound that caused 50% inhibition of specific ^{3}H-spiperone binding) was calculated by nonlinear curve fitting techniques.

Effect on Compound 48/80-Induced Lethality in Rats[14] 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.*[15] 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-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. ED_{50} value (dose which produced 50% inhibition of the PCA) was calculated according to the probit method.

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.*[16] 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. ED_{50} value (dose which produced 50% inhibition of histamine-induced bronchoconstriction to that of control) was determined in each case.

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:

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

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 was determined.

Effect on General Behavior in Mice and Rats Male ddY mice (weighing 20 to 28 g) and male Wistar rats (weighing 190 to 230 g) were used. The general behavior of animals treated orally with test compounds was observed using a modification of the method of Irwin.[17] The maximum no-effect dose was determined.

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