has to be incubated for exactly the same time. The sequence of pipetting has to be the same for this and all the following steps.) The chromogenic substrate was dissolved in prewarmed substrate buffer (37 °C) and kept at 37 °C. A 100-μL portion of this solution was added to the wells, the solutions were carefully mixed, and the microtiterplate was incubated for 3 min at 37 °C. The reaction was stopped by addition of 100 μL of 25% aqueous CH_3COOH. The optical density at 405 nm was measured using an ELISA reader. Results represent the mean of three values.

ODC Assay. ODC activity was measured by following a published procedure,[23] with some modifications. RAW-264 cells (ATCC, Rockville, MD) were suspended in DMEM-H21 (with 15 mM HEPES and 1 mM sodium pyruvate, no serum) to a final cell number of 5.5×10^5 cells/mL. For each sample, 10 mL of the cell suspension was transferred to 50 mL of cell culture flasks and incubated overnight at 37 °C/5% CO_2. Then 2000 units/mL murine γ-IFN (Sigma) and the test substances, diluted with pyrogen-free water to the desired concentration, were added. The flasks were incubated for 4 h at 37 °C with 5% CO_2. The supernatants were discarded. The cells attached to the surface of the flasks were washed off and suspended in 1 mL of the following buffer: 50 mM Tris·HCl, 0.01 mM EDTA, 2 mM DTE, 5 mM NaF, 0.1% Brij 35, 1 mM phenylmethanesulfonyl fluoride, and 60 μM pyridoxal phosphate. The cell suspensions were sonicated for 15 s each and centrifuged for 15 min in an Eppendorf centrifuge. The supernatants, containing the ODC, were frozen in liquid N_2. The frozen samples were thawed, and 190 μL of each sample was mixed with 5 μL of aqueous 0.2 mCi/mL [^{3}H]-L-ornithine hydrochloride (NEN, Boston, MA) and 7 μL of 1 mM L-ornithine hydrochloride. The solution was incubated at 37 °C for 60 min. A 150-μL portion of each sample was pipetted onto a 2.5 × 2.5 cm Whatman P-81 phosphocellulose filter paper. The filters were washed three times in 500 mL of 0.1 N NH_3 to remove unreacted [^{3}H]L-ornithine and dried under an IR lamp. The [^{3}H]putrescine content was determined by the addition of 10 mL of scintillation fluid (Lumagel, Baker) and subsequent reading in a β-counter. The stimulation values are expressed as percentage of ODC stimulation obtained with 5 ng/mL LPS. At this concentration of LPS maximal stimulation of ODC is observed.

Pyrogenicity Test in Rabbits. This test was carried out as described in the European Pharmacopeia II-1971.

Acknowledgment. Pyrogenicity in rabbits was performed at the Versuchstierzucht und -haltung, University of Vienna. We thank Dr. Gerhard Schulz for recording and interpretation of NMR spectra.

Synthesis and Antiallergic Activity of 11-(Aminoalkylidene)-6,11-dihydrodibenz[*b*,*e*]oxepin Derivatives

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A new series of 11-substituted 6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic acid derivatives was synthesized and demonstrated to be orally active antiallergic agents. These compounds are structurally related to 1 (KW-4994), which we had reported previously to be a new antiallergic agent. Most compounds synthesized exhibited potent inhibitory effects on 48-h homologous passive cutaneous anaphylaxis (PCA) in rats and on IgG_1-mediated bronchoconstriction in guinea pigs. Additionally, compounds possessing a terminal carboxyl group at the 2-position of the dibenz[*b*,*e*]oxepin ring system exhibited inhibitory effects on specific [^{3}H]pyrilamine binding to guinea pig cerebellum histamine H_1 receptors, whereas these demonstrated negligible effects on specific [^{3}H]QNB binding to rat striatum muscarinic acetylcholine M_1 receptors. Structure–activity relationship studies revealed that the following key elements were required for enhanced antiallergic activities: (1) a 3-(dimethylamino)propylidene group as the side chain at the 11-position, (2) a terminal carboxyl moiety at the 2-position, and (3) a dibenzoxepin ring system. Among the compounds synthesized, (*Z*)-11-[3-(dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic acid hydrochloride (**16**) was selected for further evaluation. It had an ED_{50} value of 0.049 mg/kg po in the PCA test in rats and an ID_{50} value of 0.030 mg/kg po in inhibiting anaphylactic bronchoconstriction in guinea pigs. Furthermore, it had a K_i value of 16 ± 0.35 nM for the histamine H_1 receptor, while it exhibited negligible CNS side effects up to a dose of 600 mg/kg po. Compound **16** is now under clinical evaluation as KW-4679.

Introduction

Effective and orally active antiallergic agents with fewer side effects have been an attractive target for drug research in recent years.[2] Our research has been focused on the synthesis of a new series of benzoxepin derivatives and evaluation of their pharmacological properties.[3] In the course of our studies, we found that compound **1** (KW-4994, see Chart I) showed highly potent antiallergic activity with negligible central nervous system (CNS) side effects.[4]

Known antiallergic agents might be classified into two groups according to their chemical structures.[5] The basic agents such as **2** (ketotifen) elicit their antiallergic effects by mainly antagonizing histamine H_1 receptors. Acidic agents such as disodium cromoglycate (DSCG) predominantly work by inhibiting release of chemical mediators.

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(2) (a) Brandon, M. L. Newer Non-Sedating Antihistamines. Will They Replace Older Agents? *Drugs* **1985**, *30*, 377–381. (b) Adamus, W. S.; Oldigs-Kerber, J.; Lohmann, H. Pharmacodynamics of the New H_1-Antagonist 3-Amino-9,13b-dihydro-1*H*-dibenz[*c*,*f*]imidazo[1,5-*a*]azepine Hydrochloride in Volunteers. *Arzneim. Forsch.* **1987**, *37*, 569–572.
(3) Kumazawa, T.; Harakawa, H.; Obase, H.; Oiji, Y.; Tanaka, H.; Shuto, K.; Ishii, A.; Oka, T.; Nakamizo, N. Synthesis and Antiulcer Activity of 5,11-Dihydro[1]benzoxepino[3,4-*b*]pyridines. *J. Med. Chem.* **1988**, *31*, 779–785.

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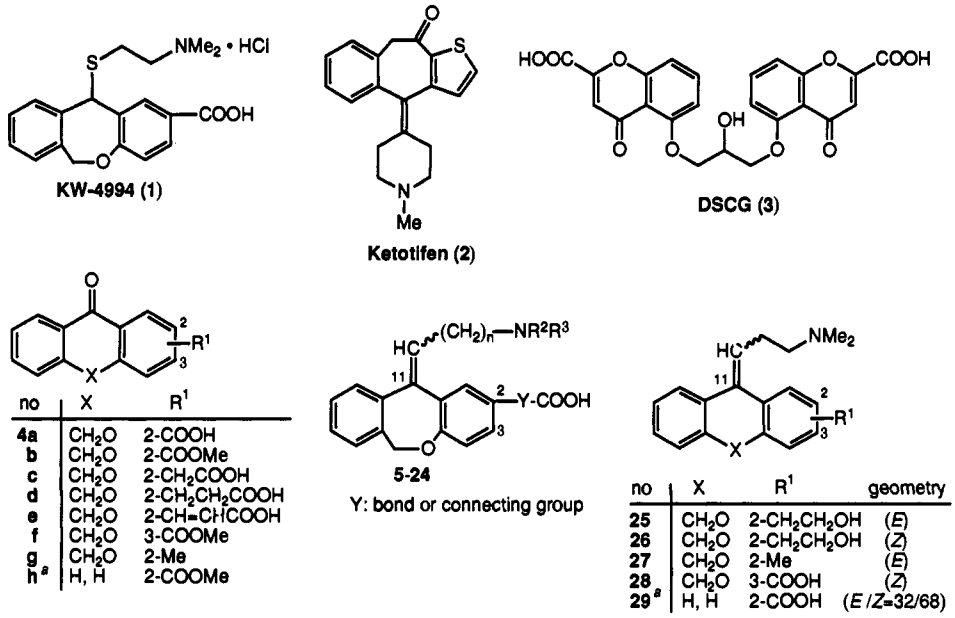
Chart I[a]

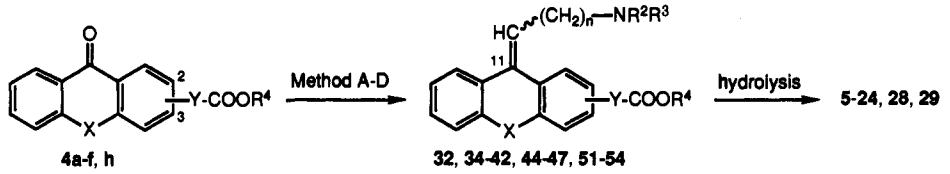


no	X	R^1
4a	CH_2O	2-COOH
b	CH_2O	2-COOMe
c	CH_2O	2-CH_2COOH
d	CH_2O	2-CH_2CH_2COOH
e	CH_2O	2-CH=CHCOOH
f	CH_2O	3-COOMe
g	CH_2O	2-Me
h[a]	H, H	2-COOMe

no	X	R^1	geometry
25	CH_2O	2-CH_2CH_2OH	(E)
26	CH_2O	2-CH_2CH_2OH	(Z)
27	CH_2O	2-Me	(E)
28	CH_2O	3-COOH	(Z)
29[a]	H, H	2-COOH	(E/Z=32/68)

[a] An open-chain analogue. Numbering of substitution positions is conveniently designated according to the general structure illustrated.

Chart II

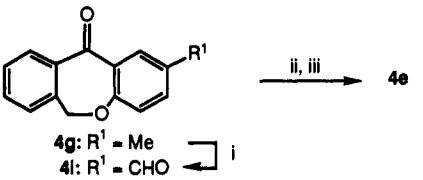


From this view point, compound 1 represents a new class of antiallergic agents possessing both acidic and basic moieties in one molecule. To our knowledge, relatively a few agents have been reported with these type of functional groups (e.g., acrivastine,[6] levocabastine,[7] and amoxanox[8]).

The present work has been carried out to define the structural requirements for antiallergic activity of this new series of dibenz[b,e]oxepin derivatives. In our previous paper,[4] we described the structure–activity relationships of 1. Two key elements were required both for enhanced antiallergic activity and for diminished CNS side effects: (1) a terminal dimethylamino group on the side chain, and (2) a carboxyl group connected to the 2-position of the oxepin ring by a bond or alkylene spacer. On the basis of these findings, we carried out further structural modification of 1 by replacing the sulfide linkage (–S–) with a carbon–carbon double bond. The optimum length (n) of the (dialkylamino)alkylidene side chain and significance of the dibenz[b,e]oxepin nucleus were also examined (Chart I).

In this paper, we describe the synthesis and structure–activity relationships of these new 11-substituted 6,11-dihydrodibenz[b,e]oxepin derivatives (**5–24**) and related compounds (**25–29**, see Chart I). Ketotifen (**2**), an antiallergic agent possessing a similar tricyclic skeleton, was used as a reference compound during our series of experiments.

Scheme I[a]



[a] Reagents: (i) $K_2S_2O_8$, $CuSO_4$, CH_3CN; (ii) $(EtO)_2P(O)CH_2COOEt$, NaH, THF; (iii) NaOH, H_2O, MeOH.

Chemistry

The 11-substituted 6,11-dihydrodibenz[b,e]oxepin-carboxylic acid derivatives (**5–24, 28, 29**) listed in Table I were prepared by saponification of the corresponding esters (also shown in Table I), which in turn were obtained from the 11-ketones **4** as demonstrated in Chart II.

(4) (a) Ohshima, E.; Kumazawa, T.; Takizawa, H.; Harakawa, H.; Sato, H.; Obase, H.; Oiji, Y.; Ishii, A.; Ishii, H.; Ohmori, K. A New Series of Antiallergic Agents. I. Synthesis and Activity of 11-(2-Aminoethyl)thio-6,11-dihydrodibenz[b,e]oxepin Derivatives. *Chem. Pharm. Bull.* **1991**, *39*, 2724–2728. (b) Kumazawa, T.; Ohshima, E.; Harakawa, H.; Sato, H.; Obase, H.; Oiji, Y.; Ishii, A.; Ishii, H.; Ohmori, K. A New Series of Antiallergic Agents. II. Synthesis and Activity of New 6,11-dihydrodibenz[b,e]oxepincarboxylic Acid Derivatives. *Chem. Pharm. Bull.* **1991**, *39*, 2729–2733.

(5) Tasaka, K. Anti-allergic Drugs. *Drugs Today* **1986**, *22*, 101–133.

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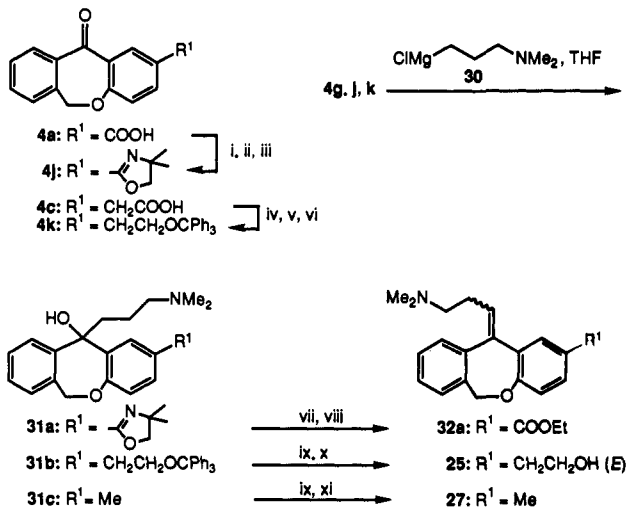
(8) Nohara, A.; Ishiguro, T.; Ukawa, K.; Sugihara, H.; Maki, Y.; Sanno, Y. Studies on Antianaphylactic Agents. 7. Synthesis of Antiallergic 5-oxo-5*H*-[1]benzopyrano[2,3-*b*]pyridines. *J. Med. Chem.* **1985**, *28*, 559–568.

Scheme II[a]

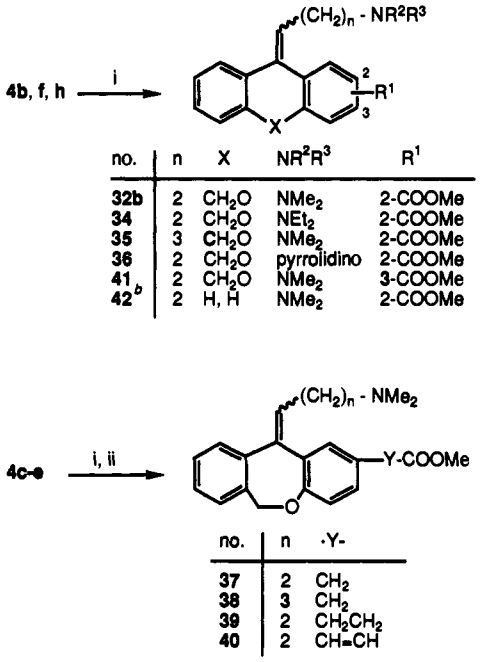


[a] Reagents: (i) $SOCl_2$, Py, CH_2Cl_2; (ii) $H_2NC(Me)_2CH_2OH$, toluene; (iii) $SOCl_2$, CH_2Cl_2; (iv) $LiAlH_4$, THF; (v) Ph_3CCl, Py; (vi) $KMnO_4$, $MgSO_4$, Na_2HPO_3, acetone, H_2O; (vii) *p*-TsOH, EtOH, H_2O; (viii) H_2SO_4, EtOH; (ix) *p*-TsOH, dioxane, H_2O; (x) fract crystn; (xi) fumaric acid, acetone.

The starting ketones (see Chart I), except **4e**, were synthesized according to the methods previously reported.[9,10] Ketone **4e** was prepared from the aldehyde **4i** (Scheme I). The four-step synthesis of **4i** starting from **4g** was known,[9e] but the total yield was low. Thus, an alternative method, in which the methyl group of **4g** is oxidized to furnish **4i** directly, was developed (65% yield).[11,12] Horner–Emmons reaction of **4i** and subsequent saponification afforded **4e** in high yield.

Double bond formation in the 11-position of the oxepin ring was accomplished by means of Grignard reaction (method A), Wittig reaction (method B), or an aminomethylation reaction of olefins (method C). Method A was depicted in Scheme II. The carboxyl group of **4a** was protected to provide **4j**, which was allowed to react with Grignard reagent **30**[13] to provide the alcohol **31a**. Simultaneous dehydration and cleavage of the oxazoline ring of **31a** and subsequent ester exchange were accomplished under acidic conditions to furnish **32a** (E/Z = 9/1). The esters (Chart II) were also prepared by Wittig reaction (method B) as depicted in Scheme III. Ylides generated by the treatment of Wittig reagents **33**[14] with *n*-BuLi were

Scheme III[a]

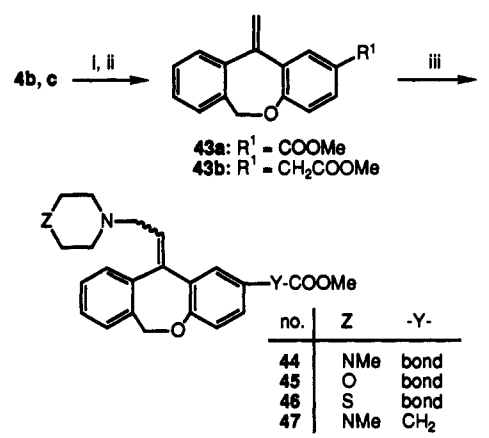


no.	n	X	NR^2R^3	R^1
32b	2	CH_2O	NMe_2	2-COOMe
34	2	CH_2O	NEt_2	2-COOMe
35	3	CH_2O	NMe_2	2-COOMe
36	2	CH_2O	pyrrolidino	2-COOMe
41	2	CH_2O	NMe_2	3-COOMe
42[b]	2	H, H	NMe_2	2-COOMe

no.	n	-Y-
37	2	CH_2
38	3	CH_2
39	2	CH_2CH_2
40	2	CH=CH

[a] Reagents: (i) Ph_3P^+–$CH_2(CH_2)_n$–$NR^2R^3Br^-$ (**33**), *n*-BuLi, THF; (ii) *p*-TsOH, MeOH. [b] An open-chain analogue. Numbering of substitution positions is conveniently designated according to the general structure illustrated.

Scheme IV[a]



no.	Z	-Y-
44	NMe	bond
45	O	bond
46	S	bond
47	NMe	CH_2

[a] Reagents: (i) Ph_3P^+–CH_3Br^-, NaH, THF; (ii, omitted in the case of **4b**) *p*-TsOH, MeOH; (iii) 1-methylpiperazine (**44**, **47**) or morpholine (**45**) or thiomorpholine (**46**), $(CH_2O)_n$, CF_3COOH, AcOH, dichloroethane.

(9) (a) Stach, K.; Spingler, H. New Heterocyclic Ring Systems. *Angew. Chem.* **1962**, *74*, 31–32. (b) Ueno, K.; Kubo, S.; Tagawa, H.; Yoshioka, T.; Tukada, W.; Tubokawa, M.; Kojima, H.; Kasahara, A. 6,11-Dihydrodibenz[*b*,*e*]oxepinacetic Acids with Potent Antiinflammatory Activity. *J. Med. Chem.* **1976**, *19*, 941–946. (c) Aultz, D. E.; Helsley, G. C.; Hoffman, D.; McFadden, A. R.; Lassman, H. B.; Wilker, J. C. Dibenz[*b*,*e*]-oxepinalkanoic Acids as Nonsteroidal Antiinflammatory Agents. 1. 6,11-Dihydro-11-oxodibenz[*b*,*e*]oxepin-2-acetic Acids. *J. Med. Chem.* **1977**, *20*, 66–70. (d) Aults, D. E.; McFadden, A. R.; Lassman, H. B. Dibenz[*b*,*e*]oxepinalkanoic Acids as Nonsteroidal Antiinflammatory Agents. 3. ω-(6,11-Dihydro-11-oxodibenz[*b*,*e*]oxepin-2-yl)alkanoic Acids. *J. Med. Chem.* **1977**, *20*, 1499–1501. (e) Yoshioka, T.; Kitagawa, M.; Oki, M.; Kubo, S.; Tagawa, H.; Ueno, K.; Tsukada, W.; Tubokawa, M.; Kasahara, A. Nonsteroidal Antiinflammatory Agents. 2. Derivatives/Analogues of Dibenz[*b*,*e*]oxepin-3-acetic Acid. *J. Med. Chem.* **1978**, *21*, 633–639. (f) Rokach, J.; Cragoe, E. J., Jr.; Rooney, C. S. Dibenz[*b*,*e*]oxepin Compounds. U.S. Patent 4,282,365; *Chem. Abstr.* **1982**, *96*, 35124c.

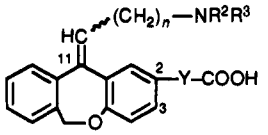
(10) Ketone **4f** was prepared by the acid catalyzed esterification of the corresponding carboxylic acid derivative.[9e] Similarly, ketone **4h** was prepared from 3-benzoylbenzoic acid (commercially available from Aldrich).

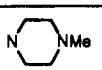
(11) Bhatt, M. V.; Perumal, P. T. Facile Conversion of Electron Rich Benzylic Hydrocarbons to Carbonyl Compounds by Peroxydisulphate and Copper Ions. *Tetrahedron Lett.* **1981**, *22*, 2605–2608.

(12) Stoichiometrical experiment reveaed that 2 equiv of $K_2S_2O_8$ provided **4i** in a 65% yield, while 5 equiv of the oxidant provided **4a**, a further oxidized product, in a 60%. *Caution: the reaction was exothermic and started at 60–70 °C. The internal temperature must be monitored carefully. Moreover, the oxidation of* **4i** *to* **4a** *may proceed violently unless controlled by a cautious addition of* $K_2S_2O_8$.

(13) Winthrop, S. O.; Davis, M. A.; Herr, F.; Stewart, J.; Gaudry, R. New Psychotropic Agents. III. Derivatives of 6,11-Dihydrodibenz[*b*,*e*]oxepine and 6,11-Dihydrodibenzo[*b*,*e*]thiepine. *J. Med. Pharm. Chem.* **1962**, *5*, 1207–1215.

Table I. Substituted 6,11-Dihydrodibenz[*b*,*e*]oxepin Derivatives



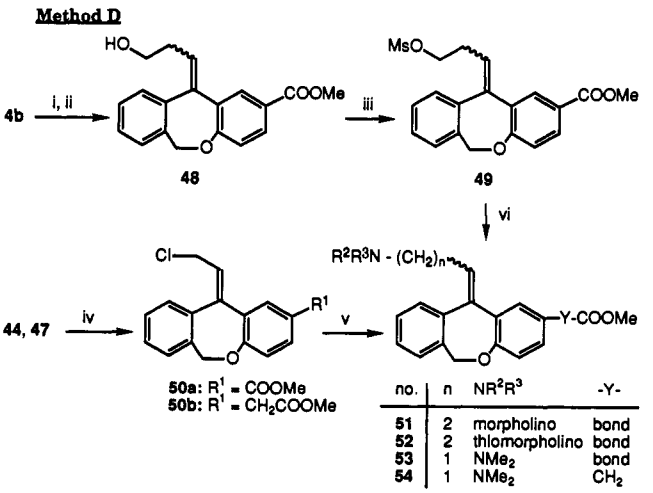
no.	*n*	NR^2R^3	Y-COOH	geometry[a]	precursor,[b] method[c]	mp, °C	recryst solvent[d]	formula[e]
5	1		COOH	*E*	44, C	266–268 dec	AC[f]	$C_{22}H_{24}N_2O_3 \cdot 2C_4H_4O_4^g \cdot 0.5H_2O$
6	1		CH_2COOH	*E*/*Z* = 88/12	47, C	108–110 dec	IP	$C_{23}H_{26}N_2O_3 \cdot C_4H_4O_4^g \cdot 0.6H_2O$
7	1		COOH	*E*/*Z* = 93/7	45, C	232–235 dec	IP	$C_{21}H_{21}NO_4 \cdot 0.5C_4H_4O_4^g \cdot 0.5H_2O$
8	2		COOH	*E*/*Z* = 13/87	51, D	130–131 dec	IP	$C_{22}H_{23}NO_4 \cdot C_3H_8O^h$
9	1		COOH	*E*	46, C	250–254 dec	IP	$C_{21}H_{21}NO_3S \cdot C_4H_4O_4^g$
10	2		COOH	*E*/*Z* = 10/90	52, D	201–205 dec	IP	$C_{22}H_{23}NO_3S \cdot 0.5H_2O$
11	1	NMe_2	COOH	*E*	53, D	216–217	IP	$C_{19}H_{19}NO_3 \cdot C_4H_4O_4^g$
12	1	NMe_2	CH_2COOH	*E*	54, D	208–209	IP	$C_{20}H_{21}NO_3 \cdot 0.75H_2O$
13	2	NMe_2	COOH	*E*	32, A,B	253–254	IP	$C_{20}H_{21}NO_3 \cdot 0.5C_4H_4O_4^g \cdot 0.2H_2O$
14	2	NMe_2	COOH	*Z*		162–164	IE[f]	$C_{20}H_{21}NO_3 \cdot 0.3H_2O$
15	2	NMe_2	CH_2COOH	*E*	37, B	158–160	AN	$C_{21}H_{23}NO_3 \cdot H_2O$
16	2	NMe_2	CH_2COOH	*Z*		248 dec	AC–W	$C_{21}H_{23}NO_3 \cdot HCl$
17	2	NMe_2	CH_2CH_2COOH	*E*	39, B	148–149	AN	$C_{22}H_{25}NO_3 \cdot 0.2H_2O$
18	2	NMe_2	CH_2CH_2COOH	*Z*		136–138	IE[f]	$C_{22}H_{25}NO_3$
19	2	NMe_2	CH=CHCOOH[i]	*Z*	40, B	176–178	IP[f]	$C_{22}H_{23}NO_3 \cdot C_4H_4O_4^g$
20	2		COOH	*E*/*Z* = 30/70	36, B	*j*	IE[f]	$C_{22}H_{23}NO_3 \cdot C_4H_4O_4^g \cdot xH_2O^j$
21	2	NEt_2	COOH	*Z*	34, B	100 dec	IE[f]	$C_{22}H_{25}NO_3 \cdot C_4H_4O_4^g \cdot 0.3H_2O$
22	3	NMe_2	COOH	*E*	35, B	222–223	IP	$C_{21}H_{23}NO_3$
23	3	NMe_2	COOH	*Z*		128–129	W	$C_{21}H_{23}NO_3 \cdot 1.8H_2O$
24	3	NMe_2	CH_2COOH	*E*/*Z* = 8/92	38, B	206–209	IP	$C_{22}H_{25}NO_3$
25[k]					–, B	96–97	EE	$C_{21}H_{25}NO_2$
26[k]					–, *l*	168–170	EE	$C_{21}H_{25}NO_2 \cdot H_2O$
27[k]					–, A	184–186	AC[f]	$C_{20}H_{23}NO \cdot 0.5C_4H_4O_4^g$
28[k]					41, B	251–252	IP	$C_{20}H_{21}NO_3$
29[k]					42, B	205–207	IP[f]	$C_{19}H_{21}NO_2$

[a] The ratios were obtained from HPLC analysis. [b] The corresponding ester. [c] The method for the introduction of the aminoalkylidene side chain in the 11-position. [d] EE, diethyl ether; IP, 2-propanol; AC, acetone; W, water; AN, acetonitrile; IE, diisopropyl ether. [e] All new compounds had C, H, N microanalyses within 0.4% of theoretical values. [f] Trituration solvent. [g] $C_4H_4O_4$, fumaric acid. [h] C_3H_8O, 2-propanol. [i] $E \geq 95\%$. [j] Extremely hygroscopic. [k] See Chart I. [l] See text.

allowed to react with **4b**, **4f**, and **4h** to furnish **32b**, **34–36**, **41**, and **42** in moderate yields. In a similar manner, the ketones possessing a carboxyl group connected by an alkyl or vinylene spacer (**4c–e**) were submitted to the Wittig reaction, and the crude products obtained were esterified to **37–40** for ease of purification. In this method, the *Z*-isomers were preferentially obtained (*E*/*Z* = 3/7). Attempts to prepare **37–40** directly under the same Wittig reaction conditions from the corresponding esters (e.g. R^1 = CH_2COOMe) resulted in the recovery of the starting materials.

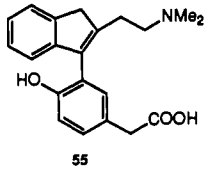
Compounds **44–47**, i.e., 11-(2-aminoethylidene)-6,11-dihydrodibenz[*b*,*e*]oxepin derivatives, were prepared by the method as depicted in Scheme IV (method C). The olefins **43**, which were obtained by Wittig olefination of the corresponding ketones, were treated with secondary amines under Mannich reaction conditions. Trifluoroacetic acid was best to promote this reaction, whereas the reaction was extremely sluggish or did not occur in the presence of acetic acid, trichloroacetic acid, or sulfuric acid. Moreover, the facility of the reaction critically depended upon the nature of the amine employed. Piperazine, morpholine, and thiomorpholine readily underwent reac-

Scheme V[a]



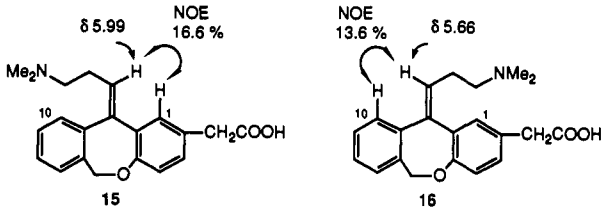
[a] Reagents: (i) Ph_3P^+–$CH_2CH_2CH_2OTHPBr^-$, *n*-BuLi, THF; (ii) *p*-TsOH, H_2O, dioxane; (iii) MsCl, Py; (iv) ClCOOEt, AcONa, dichloroethane; (v) HNR^2R^3, EtOH.

(14) Syntheses with aminoalkyl trisubstituted phosphonium salts: *Chem. Abstr.* **1965**, *63*, 16366a.



55

Figure 1.



15 16

Figure 2.

tion, whereas the starting olefin was recovered in the case of dimethylamine and pyrrolidine. In this method, the *E*-isomers were predominantly obtained (E/Z = 9/1).

Since the aminoalkylidene side chains of **51–54** could not be introduced by methods A–C, we developed method D (Scheme V) in which the terminal amino group was introduced in the final step. Preparations of intermediates **49** and **50** were key. Wittig olefination of the ketone **4b** in a similar procedure described in the method B provided **48**, which was mesylated to afford **49** (E/Z = 1/2). Alternatively, compounds **44** and **47** (E/Z = 9/1) were purified by fractional crystallization and the resulting *E*-isomers were treated with ethyl chloroformate to provide **50** without isomerization.[15] Compounds **49** and **50** were then treated with appropriate amines to furnish **51–54**.

The esters obtained by methods A–D described above were saponified without detectable isomerization to furnish the target carboxylic acids (Table I). Each geometrical isomer was isolated in pure form by chromatography and/or fractional crystallization. Chemical isomerization of **15** or **16** was effected in acetic acid in the presence of *p*-TsOH at 100 °C to furnish a 65:35 equilibrium mixture of **15** and **16**, irrespective of the starting geometry. On the other hand, treatment of **16** with 48% HBr at 100 °C for 2 h afforded an unstable rearrangement product **55** instead of **15** (Figure 1).[16] This type of ring transformation reaction has been reported previously.[17]

Compound **26** was prepared by the reduction of **16** with $LiAlH_4$, while compound **25** was preferentially synthesized by method A (Scheme I). The ketone **4c** was converted to **4k**, which was allowed to react with **30** to provide **31b**. The crude mixture (E/Z = 9/1) obtained by acid treatment of **31b** was purified to the single isomer by fractional crystallization. Similarly, compound **27** was prepared from **4g**.

The geometry about the olefin was determined by NOE experiments as shown in Figure 2, and the E/Z ratios were determined by HPLC.

(15) Tanabe, K.; Katagiri, T.; Tanaka, J. Highly Stereoselective Syntheses of Nerol and Geraniol. *Chem. Lett.* **1977**, 1025–1026.

(16) We could not purify **55** completely (purity 87%, HPLC calculation), because of its unstableness. However, the spectroscopic data of the crude **55** were compatible with its speculated structure: FABMS (glycerol + DMSO) m/z 338 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 2.11 (s, 6 H), 2.48–2.54 (m, 4 H), 3.42 (s, 2 H), 3.47 (s, 2 H), 6.88 (d, J = 8.1 Hz, 1 H), 6.99 (d, J = 2.2 Hz, 1 H), 6.9–7.2 (m, 4 H), 7.41 (d, J = 6.8 Hz, 1 H).

(17) Bickelhaupt, F.; Stach, H.; Thiel, M. Beiträge zur Entwicklung psychotroper Stoffe. 5. Mitt. Reactionen in der Dibenzo-[*b*,*e*]oxepin-Reihe. *Monatsh. Chem.* **1964**, *95*, 485–494.

Table II. Crystal Data and Data Collection Parameter for the Free Base of **16**

crystal data at 20 °C	
mol formula	$C_{21}H_{23}NO_3 \cdot 3H_2O$
a, Å	12.4158 (9)
b, Å	19.209 (2)
c, Å	8.7081 (7)
β, deg	92.924 (6)
Z	4
space group	$P2_1$
crystal size	0.4 × 0.3 × 0.3 mm
data measurement parameters	
radiation	graphite monochromated Cu Kα λ = 1.541 84 Å
diffractometer	Enraf-Nonius CAD-4
θ range, deg	2–75
unique reflections	4675
unique reflections with $I \geq 3.0\sigma(I)$	2537

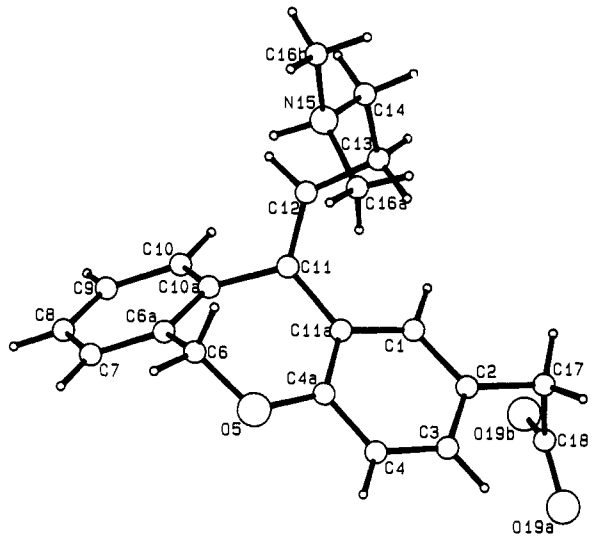


Figure 3. X-ray crystal structure of the free base of **16**.

Single-Crystal X-ray Analysis

The structure of **16** was confirmed unequivocally by X-ray crystallographic analysis. A summary of the crystal data and data collection parameters for the free base of **16** is listed in Table II. Colorless prismatic crystals were obtained by slow evaporation of an ethanol solution of the free base of **16**. Unit cell parameters were determined from angular settings of 25 carefully centered reflections. The intensities of three standard reflections were monitored periodically for stability control during data collection. Intensities were corrected for Lorentz and polarization and secondary extinction effects but not for absorption. A total of 2537 reflections with $I > 3\sigma(I)$ were used in the structure determination. The structure was solved by direct methods using MULTAN 11/82.[18] There are three water molecules in the crystal. The structure was refined by full-matrix least-squares methods. The final R and R_w values are 0.57 and 0.073, respectively. All crystallographic calculations were performed with CAD-4 SDP-PLUS.[19] PLUTO[20] drawing

(18) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J.-P.; Woofson, M. M. MULTAN 11/82. A system of computer programs for the automatic solution of crystal structures from X-ray diffraction data. Univs. of York, England, and Louvain, Belgium, 1982.

(19) Frenz, B. A. Enraf-Nonius structure determination package CAD4-SDP-PLUS V.1.0, 1982.

(20) Motherwell, S. Cambridge Crystallographic Files user manual (1976).

Table III. Biological Data for 5–29

	in vivo		in vitro	
no.	PCA: ED_{50},[a] mg/kg, po, or % inhibn[b]	bronchoconstriction: ID_{50},[c] mg/kg, po, or % inhibn[d] at 0.03 mg/kg	H_1 binding: K_i,[e] nM or % inhibn[f] at 0.1 μM	M_1 binding: % inhibn[g] at 10 μM
1 (KW-4994)	0.92 (0.41–2.6)	0.029 (10)	14 (1)	7 ± 0.5%
2 (ketotifen)	4.8 (2.2–9.3)	0.009 (10)	0.31 ± 0.018 (3)	55 ± 0.2%
5	10 ± 7.5% at 1	NT[h]	0%	2%[i]
6	44 ± 8.2% at 1	NT[h]	29%	3%[i]
7	12 ± 12% at 1	NT[h]	19%	2%[i]
8	74 ± 4.4% at 1	−1 ± 2.9%	24%	−2%[i]
9	24 ± 12.5% at 10	NT[h]	30%	3%[i]
10	60 ± 2.6% at 1	27 ± 23%	7.6 ± 0.43 (3)	27%[i]
11	19 ± 4.0% at 1	28 ± 11%	12 ± 1.7 (3)	−2%[i]
12	0.077 (0.03–0.13)	0.018 (3)	11 ± 0.9 (3)	1%[i]
13	0.74 (0.42–1.4)	0.021 (10)	4.2 ± 0.32 (3)	5 ± 0.5%
14	0.25 (0.004–16)	0.053 (10)	20 (1)	1 ± 0.3%
15	0.021 (0.015–0.030)	0.0041 (10)	5.2 ± 0.20 (3)	−1 ± 0.3%
16	0.049 (0.033–0.071)	0.030 (10)	16 ± 0.35 (3)	3 ± 0.4%
17	0.26 (0.16–0.48)	0.0064 (10)	4.2 ± 0.56 (3)	5 ± 0.4%
18	0.32 (0.12–0.88)	0.015 (10)	15 ± 1.9 (3)	2%[i]
19	1.0 (0.68–1.52)	1.1 ± 4.2%	29 ± 2.0 (3)	−1%[i]
20	53 ± 7.0% at 10	8.7 ± 13%	5.0 ± 0.27 (3)	3%[i]
21	0.12 (0.088–0.17)	19 ± 22%	10 (1)	3%[i]
22	1.2 (0.55–2.5)	1.4 ± 7.1%	6.3 ± 0.09 (3)	12%[i]
23	1.2 (0.73–1.9)	11 ± 27%	5.8 ± 0.09 (3)	2%[i]
24	55 ± 6.2% at 1	60 ± 12%	4.6 ± 0.15 (3)	2%[i]
25	0.14 (0.027–0.77)	0.0059 (10)	0.48 (1)	72 ± 0.1%
26	0.12 (0.33–0.41)	0.039 (10)	0.63 ± 0.19 (3)	80%[i]
27	20 ± 16% at 1	NT[h]	0.13 ± 0.010 (3)	80 ± 0.4%
28	19 ± 9.6% at 1	NT[h]	24 ± 1.5 (3)	−1%[i]
29	14 ± 9.1 at 100	NT[h]	10%	4%[i]

[a] ED_{50} and 95% confidence limits (in parentheses). [b] Mean ± SEM, $n = 3$. [c] ID_{50} and number of doses (in parentheses). Four to eight animals were used for each different dose. [d] Mean ± SEM, $n = 3$–10. [e] Mean ± SEM and number of determinations (in parentheses). [f] $n = 1$. [g] Mean ± SEM, $n = 3$–4. [h] Not tested. [i] $n = 1$–2.

of one of the two crystallographically independent molecules is shown in Figure 3.

Results and Discussion

The compounds synthesized were tested for their inhibitory effect on 48-h homologous passive cutaneous anaphylaxis (PCA) in rats. Compounds demonstrating sufficient antiallergic activity in the PCA test were evaluated for their ability to inhibit IgG_1-mediated bronchoconstriction in guinea pigs by the Konzett and Rössler method.[21] Each compound was administered orally 1 h before the antigen challenge and the result was represented by percent inhibition at a single dose (0.03 mg/kg po): ID_{50} values were determined for the more potent compounds. In order to elucidate the ability of the compounds to antagonize the action of histamine, one of the most prevalent chemical mediators in allergic reactions,[22] inhibitory effect on specific [^{3}H]pyrilamine binding to guinea pig cerebellum histamine H_1 receptors was tested.[23] The compound's inhibition of specific [^{3}H]QNB binding to rat striatum muscarinic acetylcholine M_1 receptors was also tested,[24] since the M_1 receptor antagonizing effect is considered to be one of the indices of side effects of the basic antiallergic agents (e.g., suppression of salivary secretion and mydriasis).[25] The biological results are summarized in Table III.

Most of the compounds synthesized exhibited potent antiallergic activities in the PCA test and experimental bronchoconstriction, and displayed significant H_1 receptor binding affinities. M_1 receptor binding affinities of the compounds possessing a carboxyl group at C-2 of the dibenzoxepin ring systems (i.e., **5–24**, **28**, and **29**) were negligible.

The effect of substitution around the amino group in the side chain was examined. From the data of compounds (**8**, **10**, **13**, **14**, **20**, and **21**) in which the numbers of the methylene groups were fixed (i.e., $n = 2$, Y = bond) the PCA inhibitory and H_1 receptor binding activities were irrespective of the substitution patterns. However, the nature of the terminal amino group was critical to the effect on bronchoconstriction. Compounds substituted with dimethylamino exhibited the highest activity: **13** and **14** showed 50 ± 14% ($n = 10$) and 40 ± 17% ($n = 14$) inhibitions, respectively, at 0.03 mg/kg po. Substitutions with diethylamine and thiomorpholine were tolerable, but compounds with morpholine and pyrrolidine were almost devoid of activity.

We optimized the length (n) of side chain in the 11-position. In a series of compounds possessing a dimethylamino group in the terminal of the side chain, the optimum side chain length (n) is two, irrespective of the geometry around the 11-position (**11** vs **13** vs **22**, **12** vs **15**, **14** vs **23**). Shortening and lengthening the side chain reduced the antiallergic activities. In the H_1 receptor binding assay, however, **23** was about 3-fold more potent than **14**.

(21) Konzett, H.; Rössler, R. Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmakol.* **1940**, *195*, 71–74.

(22) Gould, C. A. L.; Ollier, S.; Aurich, R.; Davies, R. J. A Study of the Clinical Efficacy of Azelastine in Patients with Extrinsic Asthma, and its Effect on Airway Responsiveness. *Br. J. Clin. Pharmacol.* **1988**, *26*, 515–525.

(23) Chang, R. S. L.; Tran, V. T.; Snyder, S. H. Characteristics of Histamine H_1-Receptors in Peripheral Tissues Labeled with [^{3}H]Mepyramine. *J. Pharmacol. Exp. Ther.* **1979**, *209*, 437–442.

(24) Landuron, P. M.; Verwimp, M.; Leysen, J. E. Stereospecific in vitro Binding of [^{3}H]Dexetimide to Brain Muscarinic Receptors. *J. Neurochem.* **1979**, *32*, 421–427.

(25) Kubo, N.; Shirakawa, O.; Kuno, T.; Tanaka, C. Antimuscarinic Effects of Antihistamines: Quantitative Evaluation by Receptor-Binding Assay. *Jpn. J. Pharmacol.* **1987**, *43*, 277–282.

Influence of the introduction of a connecting group (Y) between the carboxyl group and the dibenzoxepin skeleton was evaluated. Every compound possessing a 3-(dimethylamino)propylidene side chain (13–19), except 19, exhibited potent inhibitory effects on both PCA and bronchoconstriction. Among them, maximum antiallergic activities were observed when the connecting group (Y) was methylene, irrespective of the geometry of the side chain (15 vs 13 or 17, 16 vs 14 or 18 or 19). A similar result was observed for the ethylidenes in 11 and 12. Although compound 11 showed negligible antiallergic activity, one methylene elongation of the acidic moiety of 11 to provide 12 resulted in significant enhancement in antiallergic activity. However, no significant influence of the connecting group (Y) was observed on the H_1 receptor binding affinity.

The difference between each geometrical isomer was small with respect of the PCA inhibitory effect, although the activities of the *E*-isomer both in the bronchoconstriction and in the H_1 receptor binding were always higher than those of the *Z*-isomer (13 vs 14, 15 vs 16, 17 vs 18, 25 vs 26).

Compounds 25 and 26, which are hydroxyethyl derivatives of 15 and 16, recorded a greater than 10-fold H_1-binding affinity compared to the parents. Their antiallergic activities, however, were not significant in both models (bronchoconstriction and PCA). On the other hand, their M_1 receptor binding activities were enhanced and undesirable anticholinergic effects were observed in rats. Compound 28, possessing a carboxyl group at the 3-position, was compared with 14. Compound 28 maintained its H_1 receptor binding affinity similar to that of 14, whereas 28 displayed a marked decreased activity in the PCA test. It is interesting to note that 29, an open-chain analogue of the dibenzoxepins, was devoid of all activities.

From the results described above, the following three elements are critical for enhanced antiallergic activity: (1) a 3-(dimethylamino)propylidene group as the side chain in the 11-position, (2) a carboxyl moiety as the terminal of the substituent at the 2-position, and (3) the dibenzoxepin ring system. Although, H_1 receptor antagonizing activity was generally considered to be one of the mechanisms of the series of compounds, there was a lack of correlation between the H_1 receptor binding affinity and the antiallergic activity.

Among the compounds tested, 15 and 16 are the most promising. In the PCA test these compounds were approximately 100–200-fold more potent than 2 (ketotifen) and also approximately 15–40-fold more potent than 1. Additionally, these compounds exhibited potent inhibitory effects in IgG_1-mediated bronchoconstriction with ID_{50} values of 0.0041 and 0.030 mg/kg po, respectively. Compound 16 was selected for further pharmacological evaluation.

A beneficial duration of action is necessary for a compound to be useful for the clinical prophylaxis of allergic diseases. Comparative oral PCA duration studies for 16 and 2 (ketotifen) were performed in rats. The inhibitory effect of 16 was approximately 100-fold more potent than 2. Therefore, compound 16 and 2 were administered orally at 0.2 and 20 mg/kg, respectively. Each inhibition of the PCA response was measured at various times following drug administration. Compound 16 exhibited 74.9 ± 2.2%, 68.0 ± 3.0%, and 55.6 ± 5.5% inhibitors at 2, 9, and 16 h after dosing, respectively, whereas 2 exhibited 52.7 ± 8.6% and 39.4 ± 4.2% inhibitions at 2 and 9 h after dosing, respectively. Overall, the inhibitory effect of 16 was exerted rapidly and is superior to that of 2 (0.08–24 h after the administration of the compounds).

In conclusion, compound 16 exhibited strong preventive effects on experimental allergic models and had a long duration of action. Compound 16 (KW-4679) appears to be more promising as an antiallergic candidate than 1. It exhibited no sign of mutagenicity or teragenicity and is now under clinical evaluation. Its mechanism of action is now under extensive investigation in our research laboratories and a detailed pharmacological profile will be published.

Experimental Section

Melting points were determined with a Büchi-510 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Shimadzu IR-400 spectrometer. Proton nuclear magnetic resonance spectra (^1H NMR) were recorded on a JEOL PMX-60 (60 MHz), a Hitachi R-90H (90 MHz), or a JEOL GX-270 (270 MHz) spectrometer. All spectra were determined in $CDCl_3$ or DMSO-d_6. Chemical shifts are reported in δ units downfield from the internal standard tetramethylsilane (TMS). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; and dd, doublet of doublet. Mass spectra (MS) were recorded on a JEOL D300 mass spectrometer. Elemental analyses were performed by the analytical department of our laboratories. For column chromatography, silica gel [Kieselgel 60 (Merck, 70–230 or 230–400 mesh)] and highly porous synthetic resins [Diaion HP-10 and HP-40 (Mitsubishi Chem. Ind. Co., Ltd.)] were used. *E*/*Z* ratios were measured by HPLC [column, YMC A-312 (ODS, 6 mm × 150 mm); eluent, 0.01 M octanesulfonic acid in MeOH/H_2O (2/1)].

Ketones 4a-d,f-h. These compounds were synthesized according to the methods previously reported.[9,10]

11-Oxo-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carbaldehyde (4i). A mixture of 4g[9e] (80 g, 0.33 mol), $K_2S_2O_8$ (200 g, 0.74 mol), $CuSO_4$ (11.2 g, 0.07 mol), pyridine (80 mL), acetonitrile (2 L), and water (1.2 L) was stirred and *gradually warmed* to 65 °C (internal). When the color of the reaction mixture changed from blue to green, the heating bath was removed and gentle reflux was maintained for 2 h. *Caution: the reaction was exothermic and started at 60–70 °C. The internal temperature must be monitored carefully.* After being cooled, the reaction mixture was diluted with EtOAc. The organic phase was separated, washed successively with brine, 2 N HCl, and aqueous $NaHCO_3$, dried, and evaporated. The residue was chromatographed on silica gel with EtOAc as eluent to give 50.8 g (65%) of 4i: mp 171–172 °C (EtOAc); ^1H NMR ($CDCl_3$) δ 5.28 (s, 2 H), 6.98–8.11 (m, 6 H), 8.63 (d, J = 2.2 Hz, 1 H), 9.92 (s, 1 H); MS m/z 238 (M^+). Anal. ($C_{15}H_{10}O_3$) C, H.

(*E*)-11-Oxo-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acrylic Acid (4e). To a suspension of NaH (60% in oil, 1.4 g, 35 mmol) in THF (50 mL) was added triethyl phosphonoacetate (6.2 g, 27 mmol) at 0 °C, and the mixture was stirred at 0 °C for 30 min. A solution of 4i (5.5 g, 23 mmol) in THF (300 mL) was added. After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc. The solution was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel with hexane/EtOAc (1/1) as eluent to give 5.4 g (76%) of 56 (the ethyl ester of 4e): mp 115–116 °C (ether); ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7.1 Hz, 3 H), 4.24 (q, J = 7.1 Hz, 2 H), 5.14 (s, 2 H), 6.35 (d, J = 16.4 Hz, 1 H), 7.00 (d, J = 9.0 Hz, 1 H), 7.22–8.04 (m, 6 H), 8.31 (d, J = 2.0 Hz, 1 H); MS m/z 308 (M^+). Anal. ($C_{19}H_{16}O_4$) C, H. A mixture of the ester 56 (1.7 g, 5.5 mmol), NaOH (0.55 g, 13.8 mmol), MeOH (10 mL), and water (5 mL) was refluxed for 40 min. After being concentrated, the reaction mixture was diluted with water and then acidified to pH 2.0 with 4 N HCl. The resultant precipitate was collected and recrystallized from THF to give 1.3 g (87%) of 4e: mp 279–280 °C; ^1H NMR ($CDCl_3$) δ 5.20 (s, 2 H), 6.35 (d, J = 16.0 Hz, 1 H), 7.05 (d, J = 16.0 Hz, 1 H), 7.25–8.00 (m, 6 H), 8.30 (d, J = 3.0 Hz, 1 H); IR (KBr) 1710 cm^{-1}; MS m/z 280 (M^+). Anal. ($C_{17}H_{12}O_4$) C, H.

Method A. a. **2-(4,4-Dimethyl-2-oxazolin-2-yl)-11-oxo-6,11-dihydrodibenz[*b*,*e*]oxepin (4j).** To a solution of 4a (12.5 g, 49 mmol) in CH_2Cl_2 (300 mL) and pyridine (5 mL) was added $SOCl_2$ (8.9 g, 75 mmol) at 0 °C, and the mixture was stirred at room temperature for 2 h. After being concentrated, the residue

was treated with 2-amino-2-methylpropanol (32.4 g, 363 mmol) in toluene (100 mL) at 50 °C for 3 h. The reaction mixture was diluted with EtOAc, washed with brine, dried, and evaporated. The residue was recrystallized from toluene to give 8.3 g (52%) of *N*-(1,1-dimethyl-2-hydroxyethyl)-11-oxo-6,11-dihydrodibenz-[*b*,*e*]oxepin-2-carboxamide (**57**): mp 155–159 °C; ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 6 H), 3.53 (s, 2 H), 5.25 (s, 2 H), 6.91–8.68 (m, 7 H). Anal. ($C_{19}H_{19}NO_4$) C, H, N. The carboxamide **57** (8.0 g, 25 mmol) was suspended in CH_2Cl_2 (100 mL). To the suspension was added $SOCl_2$ (3.6 g, 30 mmol) at 0 °C, and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2, washed with brine, dried, and evaporated. The residue was chromatographed on silica gel with hexane/EtOAc (2/1) as eluent, and the crude product obtained was recrystallized from hexane to give 6.3 g (68%) of **4j**: mp 122 °C; ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 6 H), 4.06 (s, 2 H), 5.14 (s, 2 H), 6.84–8.89 (m, 7 H); MS *m*/*z* 307 (M^+). Anal. ($C_{19}H_{17}NO_3$) C, H, N.

b. 11-Oxo-2-[2-[(triphenylmethyl)oxy]ethyl]-6,11-dihydrodibenz[*b*,*e*]oxepin (4k). To a solution of **4c** (19 g, 71 mmol) in THF (500 mL) was added $LiAlH_4$ (6.0 g, 160 mmol) portionwise, and the mixture was stirred at room temperature for 1 h. A small amount of water was added to destroy any excess reagent, and the resultant insoluble inorganic salts were removed by filtration. The filtrate was concentrated to give 17.7 g (98%) of 11-hydroxy-2-(2-hydroxyethyl)-6,11-dihydrodibenz[*b*,*e*]oxepin (**58**): mp 132–136 °C; ^{1}H NMR (DMSO-d_6) δ 2.59 (t, *J* = 6.8 Hz, 2 H), 3.55 (t, *J* = 6.8 Hz, 2 H), 4.89 and 5.71 (AB, J_{AB} = 12.6 Hz, 2 H), 5.60 (s, 1 H), 6.46–7.49 (m, 7 H); MS *m*/*z* 256 (M^+). Anal. ($C_{16}H_{16}O_3$) C, H. To a solution of **58** (17.2 g, 67 mmol) in pyridine (50 mL) was added triphenylchloromethane (30 g, 108 mmol), and the mixture was stirred at 50 °C for 5 h. The reaction mixture was concentrated and then extracted with EtOAc. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel with hexane/EtOAc (3/1) as eluent to give 21.7 g (65%) of 11-hydroxy-2-[2-[(triphenylmethyl)-oxy]ethyl]-6,11-dihydrodibenz[*b*,*e*]oxepin (**59**) as an amorphous powder: ^{1}H NMR ($CDCl_3$ + D_2O) δ 2.47–2.95 (m, 2 H), 2.96–3.45 (m, 2 H), 4.87 and 5.71 (AB, J_{AB} = 13.2 Hz, 2 H), 5.43 (s, 1 H), 6.33–7.51 (m, 22 H). To a mixture of **59** (10 g, 20 mmol), saturated $MgSO_4$ solution (20 mL), Na_2HPO_4 (2 g, 14 mmol), acetone (800 mL), and water (1 L) was added dropwise a solution of $KMnO_4$ (2.6 g, 16.5 mmol) in water (300 mL), and the mixture was stirred at room temperature for 4.5 h. Methanol (100 mL) was added and the solution stirred under reflux for 3 h. Upon cooling, insoluble materials were filtered off, and the reaction mixture was diluted with EtOAc. The solution was washed with brine, dried, and evaporated. The residue was recrystallized from 2-propanol to give 8.0 g (81%) of **4k**: mp 132–134 °C; ^{1}H NMR ($CDCl_3$) δ 2.61–3.04 (m, 2 H), 3.05–3.46 (m, 2 H), 5.01 (s, 2 H), 6.63–8.07 (m, 22 H); MS *m*/*z* 496 (M^+). Anal. ($C_{35}H_{28}O_3$) C, H.

c. 11-[3-(Dimethylamino)propyl]-11-hydroxy-2-(4,4-dimethyl-2-oxazolin-2-yl)-6,11-dihydrodibenz[*b*,*e*]oxepin (31a). To a [3-(dimethylamino)propyl]magnesium chloride solution, which was prepared from 3-(dimethylamino)propyl chloride (6.0 g, 50 mmol) and magnesium (1.2 g, 50 mmol) in THF (8 mL) in the presence of a catalytic amount of dibromoethane,[13] was added a solution of **4j** (7.6 g, 25 mmol) in THF (80 mL), and the mixture was stirred at room temperature overnight. Aqueous NH_4Cl was added and the mixture was neutralized with 4 N HCl and concentrated. The residue was dissolved in water, acidified to pH 1 with 4 N HCl, and washed with ether. The aqueous layer was adjusted to pH 13 with 10 N NaOH and the crude product extracted with CH_2Cl_2. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel with hexane/EtOAc/triethylamine (10/10/1) as eluent and the product triturated with diisopropyl ether to give 6.1 g (65%) of **31a**: mp 166–167 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (br, 10 H), 2.18 (br, 8 H), 3.98 (s, 2 H), 4.97 and 5.46 (AB, J_{AB} = 15.0 Hz, 2 H), 6.65–8.49 (m, 7 H); MS *m*/*z* 394 (M^+). Anal. ($C_{24}H_{30}N_2O_3$) C, H, N.

Compounds **31b** and **31c** were prepared in a similar manner as described above from appropriate starting materials: **31b** (amorphous powder, 81%); ^{1}H NMR ($CDCl_3$) δ 0.85–1.83 (m, 4 H), 2.08 (s, 6 H), 2.67–3.44 (m, 6 H), 4.94 and 5.36 (AB, J_{AB} = 15.8 Hz, 2 H), 6.63–8.13 (m, 22 H); MS *m*/*z* 583 (M^+), **31c** (amorphous powder, 86%); MS *m*/*z* 311 (M^+).

d. (*E*,*Z*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Ethyl Ester (32a). A mixture of **31a** (6.1 g, 16 mmol), *p*-TsOH·H_2O (5.0 g, 26 mmol), EtOH (300 mL), and water (30 mL) was refluxed for 4 h. After being concentrated, the reaction mixture was dissolved in EtOH (300 mL) containing concentrated H_2SO_4 (20 mL), and the solution was refluxed for 15 h. Upon cooling, the reaction mixture was concentrated and then diluted with water. The solution was washed with ether and adjusted to pH 12 with 10 N NaOH. The crude product was extracted with CH_2Cl_2. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel with EtOAc/triethylamine (10/1) as eluent to give 1.4 g (25%) of **32a** as an oil: MS *m*/*z* 351 (M^+). The corresponding methyl ester (**32b**) was obtained as described in method B.a.

e. (*E*)-11-[3-(Dimethylamino)propylidene]-2-(2-hydroxyethyl)-6,11-dihydrodibenz[*b*,*e*]oxepin (25). A mixture of **31b** (0.92 g, 1.6 mmol), *p*-TsOH·H_2O (0.6 g, 3.2 mmol), dioxane (20 mL), and water (20 mL) was refluxed for 2 h. After being concentrated, the reaction mixture was diluted with EtOAc. The solution was washed with aqueous $NaHCO_3$, dried, and evaporated. The residue was chromatographed on silica gel with EtOAc/triethylamine (10/1) as eluent to give a mixture of geometrical isomers (*E*/*Z* = 9/1). The mixture was recrystallized from ether to give 0.4 g (68%) of **25** (*E* ≥ 99%): ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 6 H), 2.30–2.45 (m, 4 H), 2.77 (t, *J* = 6.5 Hz, 2 H), 3.81 (t, *J* = 6.5 Hz, 2 H), 4.50–5.70 (br, 2 H), 6.02 (t, *J* = 6.8 Hz, 1 H), 6.70 (d, *J* = 8.3 Hz, 1 H), 6.97 (dd, *J* = 2.2 and 8.3 Hz, 1 H), 7.12 (d, *J* = 2.2 Hz, 1 H), 7.20–7.40 (m, 4 H); MS *m*/*z* 323 (M^+). Anal. ($C_{21}H_{25}NO_2$) C, H, N.

Compound **27** was prepared by a similar acid catalyzed dehydration and subsequent treatment with fumaric acid in acetone (overall 41%).

Method B. a. (*E*,*Z*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (32b). To a suspension of [3-(dimethylamino)propyl]triphenylphosphonium bromide hydrobromide[14] (**33a**, 45 g, 88 mmol) in THF (200 mL) was added a 1.6 N solution of *n*-BuLi in hexane (82 mL, 130 mmol) under N_2 atmosphere at 0 °C, and the mixture was stirred under the same conditions for 1 h. A solution of **4b** (10 g, 37 mmol) in THF (200 mL) was added at 0 °C. The resultant mixture was stirred at room temperature for 2 h and then diluted with EtOAc and water. The organic phase was separated, washed with brine, dried, and evaporated. The residue was chromatographed on silica gel with EtOAc/triethylamine (10/1) as eluent to give 7.6 g (61%) of a mixture of geometrical isomers **32b** (*E*/*Z* = 1/2) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.19 (s, 6 H), 1.96–2.74 (m, 4 H), 3.80 (s, 3 H), 5.19 (br, 2 H), 5.70 (t, *J* = 6.6 Hz, 0.67 H, for *Z*-isomer), 6.06 (t, *J* = 6.6 Hz, 0.33 H, for *E*-isomer), 6.75–8.00 (m, 7 H); IR (neat) 1715, 1608, 1242, 1115, 1004 cm^{-1}; MS *m*/*z* 337 (M^+).

Compounds **34–36**, **41**, **42** were prepared in a similar manner as described above from appropriate starting materials: **34** (oil, 68%), **35** (oil, 89%), **36** (oil, 43%), **41** (oil, 16%), **42** (oil, 22%).

b. (*E*,*Z*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic Acid Methyl Ester (37). To a suspension of [3-(dimethylamino)propyl]triphenylphosphonium bromide hydrobromide (**33a**, 48 g, 93 mmol) in THF (200 mL) under N_2 atmosphere at 0 °C was added a 1.6 N solution of *n*-BuLi in hexane (90 mL, 144 mmol), and the solution was stirred under the same conditions for 1 h. A solution of **4c** (5.0 g, 19 mmol) in THF (120 mL) was added, and the resultant mixture was stirred at room temperature for 2 h. After being concentrated, the reaction mixture was diluted with water, washed with ether, and then neutralized. The solution was concentrated and the residue was dissolved in MeOH (400 mL) containing *p*-TsOH·H_2O (5.0 g, 26 mmol) and refluxed for 2 h. After being concentrated, the reaction mixture was diluted with EtOAc, washed with aqueous $NaHCO_3$, dried, and concentrated. The residue was chromatographed on silica gel with hexane/EtOAc/triethylamine (10/10/1) to give 4.0 g (61%) of a mixture of geometrical isomers **37** (*E*/*Z* = 1/2) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.06–2.67 (m, 4 H), 2.16 (s, 6 H), 3.46 (s, 2 H), 3.58 (s, 3 H), 5.08 (br, 2 H), 5.69 (t, *J* = 7.0 Hz, 0.67 H, for *Z*-isomer), 6.06 (t, *J* = 7.0 Hz, 0.33 H, for *E*-isomer), 6.53–7.30 (m, 7 H); IR (neat) 2950, 1740, 1495, 1230, 1020 cm^{-1}.

Compounds **38–40** were prepared in a manner similar to that described above from appropriate starting materials: **38** (oil, 67%), **39** (oil, 84%), **40** (oil, 69%).

Method C. a. 11-Methylene-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (43a). To a suspension of methyltriphenylphosphonium bromide (25 g, 70 mmol) in THF (100 mL) was added a 1.6 N solution of *n*-BuLi in hexane (40 mL, 64 mmol) under N_2 atmosphere at 0 °C, and the mixture was stirred under the same conditions for 0.5 h. A solution of **4b** (15 g, 56 mmol) in THF (250 mL) was added and the resultant mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc. The organic solution was washed with brine, dried, and concentrated. The residue was chromatographed on silica gel with hexane/EtOAc (3/1) to give 12.6 g (84%) of **43a**: mp 73–76 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3 H), 5.15 (s, 2 H), 5.29 (s, 1 H), 5.74 (s, 1 H), 6.69–8.22 (m, 7 H); MS *m*/*z* 266 (M^+). Anal. ($C_{17}H_{14}O_3$) C, H.

b. 11-Methylene-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic Acid Methyl Ester (43b). Crude 11-methylene-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic acid (**43c**) was prepared in a manner similar to that described above from **4c** (10 g, 37 mmol) and methyltriphenylphosphonium bromide (60 g, 168 mmol). It was esterified by the same method as described in the synthesis of **37** to give 7.1 g (68%) of **43b** as an oil: ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 2 H), 3.58 (s, 3 H), 5.02 (s, 2 H), 5.17 (s, 1 H), 5.59 (s, 1 H), 6.68 (d, *J* = 8.5 Hz, 1 H), 6.98 (dd, *J* = 2.2 and 8.5 Hz, 1 H), 6.99–7.42 (m, 5 H); MS *m*/*z* 280 (M^+).

c. (*E*)-11-[2-(4-Methylpiperazino)ethylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (44). A mixture of **43a** (80.3 g, 0.3 mol), 1-methylpiperazine (67 mL, 0.6 mol), paraformaldehyde (4.5 g), trifluoroacetic acid (230 mL), acetic acid (160 mL), and dichloroethane (1.6 L) was refluxed for 1 h. More paraformaldehyde (4.5 g) was added and the reflux was continued for 1 h. Paraformaldehyde (4.5 g) was added again and the reflux was maintained for another 1 h. After being concentrated, the reaction mixture was diluted with water and EtOAc. The organic phase was separated, washed with brine, dried, and concentrated. The residue (*E*/*Z* = 9/1) was purified by fractional crystallization with 2-propanol to give 110 g of trifluoroacetic acid salt of **44** (*E* > 99%). The salt was suspended in a mixture of EtOAc (1 L) and H_2O (1.2 L). The medium was cooled under 5 °C and adjusted to pH 10 with 2 N NaOH. The organic phase was separated, washed with brine, dried, and concentrated to give 79 g (71%) of **44** as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3 H), 2.21–2.71 (m, 8 H), 3.14 (d, *J* = 7.0 Hz, 2 H), 3.82 (s, 3 H), 4.7–5.4 (br, 2 H), 6.20 (t, *J* = 7.0 Hz, 1 H), 6.72 (d, *J* = 8.6 Hz, 1 H), 7.02–7.42 (m, 4 H), 7.72 (dd, *J* = 2.2 and 8.6 Hz, 1 H), 7.98 (d, *J* = 2.2 Hz, 1 H); IR ($CHCl_3$) 2945, 2810, 1710, 1250, 1111, 1010 cm^{-1}; MS *m*/*z* 378 (M^+).

Compounds **45–47** were prepared in a manner similar to that described above from appropriate starting materials: **45** (oil, 84%), **46** (oil, 66%), **47** (oil, 64%).

Method D. a. (*E*,*Z*)-11-(3-Hydroxypropylidene)-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (48). The corresponding tetrahydropyranyl ether of **48** was prepared from **4b** (15 g, 56 mmol) and [3-[(tetrahydro-2*H*-pyran-2-yl)oxy]propyl]triphenylphosphonium bromide[26] (40 g, 82 mmol) by the same method as described in the synthesis of **32b** (method B). The resultant ether was treated with a mixture of *p*-TsOH·H_2O (1 g, 0.5 mmol), dioxane (500 mL), and water (200 mL) and refluxed for 1 h. After being concentrated, the reaction mixture was diluted with EtOAc. The solution was washed successively with aqueous $NaHCO_3$ and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexane/EtOAc (1/1) to give 9.8 g (56%) of a mixture of geometrical isomers **48** (*E*/*Z* = 3/7) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.17–2.72 (m, 2 H), 3.37–3.76 (m, 2 H), 3.77 (s, 3 H), 4.68–5.43 (br, 2 H), 5.70 (t, *J* = 7.4 Hz, 0.7 H, for *Z*-isomer), 6.40 (t, *J* = 6.9 Hz, 0.3 H, for *E*-isomer), 6.52–8.12 (m, 7 H).

b. (*E*,*Z*)-11-[3-[(Methylsulfonyl)oxy]propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (49). To a solution of **48** (*E*/*Z* = 3/7, 2.0 g, 6.5 mmol) in pyridine (50 mL) was added methanesulfonyl chloride (1 mL, 13 mmol) at 0 °C, and the solution was stirred at room temperature for 30 min. A few pieces of crushed ice were added, and the mixture was stirred for 30 min. The reaction mixture was diluted with EtOAc. The organic solution was washed successively with 1 N HCl and brine, dried, and evaporated (<30 °C) to give crude **49** (*E*/*Z* = 3/7) as an oil: ^{1}H NMR ($CDCl_3$) δ 2.93 (s, 0.9 H, for *E*-isomer), 3.00 (s, 2.1 H, for *Z*-isomer). The unstable mesylate **49** was used without purification in the next reaction.

c. (*E*)-11-(2-Chloroethylidene)-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (50a). To a mixture of **44** (*E* > 99%, 31 g, 81 mmol), NaOAc (33 g, 406 mmol), and dichloromethane (460 mL) was added ClCOOEt (39 mL, 406 mmol) dropwise. After being stirred for 2 h at room temperature, the insoluble salts were filtered off. The filtrate was concentrated, diluted with CH_2Cl_2, washed with brine, dried, and concentrated. The residue was recrystallized from 2-propanol to give 9.2 g (58%) of **50a** as colorless needles: 134–135 °C; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3 H), 4.16 (d, *J* = 8.1 Hz, 2 H), 4.88 (br, 1 H), 5.57 (br, 1 H), 6.31 (t, *J* = 8.1 Hz, 1 H), 6.79–8.04 (m, 7 H); MS *m*/*z* 314 (M^+). Anal. ($C_{18}H_{15}ClO_3$) C, H.

Compound **50b** [mp 127–128 °C (2-propanol)] was prepared in the similar manner as described above from **47** (*E* > 99%), which was obtained by fractional crystallization of the fumaric acid salt of crude **47** (*E*/*Z* = 9/1) with 2-propanol.

d. (*E*)-11-[2-(Dimethylamino)ethylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-carboxylic Acid Methyl Ester (53). A mixture of **50a** (2.0 g, 6.4 mmol), 50% dimethylamine solution (2.9 mL, 32 mmol), and EtOH (100 mL) was refluxed for 2 h and then concentrated. The reaction mixture was diluted with EtOAc, washed with brine, dried, and concentrated. The residue was chromatographed on silica gel with hexane/EtOAc/triethylamine (10/10/1) as eluent to give 2.0 g (97%) of **53** as an oil: ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 6 H), 2.90–3.05 (m, 2 H), 3.87 (s, 3 H), 4.80–5.35 (br, 2 H), 6.24 (t, *J* = 6.8 Hz, 1 H), 6.77 (d, *J* = 8.6 Hz, 1 H), 7.00–7.35 (m, 4 H), 7.79 (dd, *J* = 2.2 and 8.6 Hz, 1 H), 8.03 (d, *J* = 2.2 Hz, 1 H); IR (neat) 2970, 1711, 1606, 1488, 1313, 1241, 1118, 1004 cm^{-1}; MS *m*/*z* 323 (M^+).

Compounds **51–52** and **54** were prepared in a manner similar to that described above from appropriate starting materials: **51** (oil, 66%), **52** (oil, 94%), **54** (oil, 80%).

Typical Procedure for Obtaining the Target Carboxylic Acids (5–24, 28, and 29) by Saponification: (*E*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic Acid (15). A mixture of **37** (1.7 g, 4.8 mmol, *E*/*Z* = 1/2), MeOH (50 mL), 10 N NaOH (1.5 mL, 15 mmol), and water (10 mL) was refluxed for 1 h and then concentrated. The residue (*E*/*Z* = 1/2) was diluted with H_2O and the solution was neutralized with 4 N HCl. The *E*-isomer (**15**) was separated by chromatography using HP-40 with MeOH/H_2O (1/1) as eluent to give 0.3 g (5.7%) of **15**: mp 158–160 °C (acetonitrile); ^{1}H NMR (DMSO-d_6) δ 2.17 (s, 6 H), 2.20–2.60 (m, 4 H), 3.48 (s, 2 H), 4.92 (br, 1 H), 5.43 (br, 1 H), 5.99 (t, *J* = 7.2 Hz, 1 H), 6.68 (d, *J* = 8.3 Hz, 1 H), 7.05 (dd, *J* = 2.2 and 8.3 Hz, 1 H), 7.20 (d, *J* = 2.2 Hz, 1 H), 7.28–7.55 (m, 4 H); MS *m*/*z* 337 (M^+). Anal. ($C_{21}H_{23}NO_3\cdot H_2O$) C, H, N.

(*Z*)-11-[3-(Dimethylamino)propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic Acid Hydrochloride (16). The crude mixture (26 g, 77 mmol, *E*/*Z* = 1/2) obtained from saponification of **37** described above and subsequent desalination with HP-10 (H_2O and then MeOH as eluent) was dissolved in 2-propanol (400 mL) containing *p*-TsOH·H_2O (14.7 g, 77 mmol). The solution was stirred at room temperature and the resultant precipitate was collected by filtration. The crude product was recrystallized from 2-propanol to give 24 g (62%) of (*Z*)-11-[3-(dimethylamino)-propylidene]-6,11-dihydrodibenz[*b*,*e*]oxepin-2-acetic acid *p*-toluenesulfonate: mp 185–187 °C. The salt was added portionwise to aqueous $NaHCO_3$ with ice cooling and the resultant solution was neutralized with 4 N HCl. The crude product was desalinated with HP-10 and recrystallized successively from 2-propanol and water to give 12.7 g (49%) of the free base of **16**: mp 188–189.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.15 (s, 6 H), 2.40–2.60 (m, 4 H), 3.45 (s, 2 H), 5.00–5.55 (br, 2 H), 5.66 (t, *J* = 6.7 Hz, 1 H), 6.75 (d, *J* = 8.1 Hz, 1 H), 7.0–7.1 (m, 2 H), 7.2–7.4 (m, 4 H); MS *m*/*z* 337 (M^+). Anal. ($C_{21}H_{23}NO_3\cdot 0.5H_2O$) C, H, N. To a solution of the

(26) Schow, S. R.; McMorris, T. C. Utility of the Wittig Reaction for the Construction of Side Chains of Steroids Starting from Pregnenolone. *J. Org. Chem.* **1979**, *44*, 3760–3765.

free base of **16** (12 g, 35 mmol) was added a 8 N solution of HCl in 2-propanol (8 mL, 64 mmol) and the mixture was stirred at room temperature. After being concentrated, the residue was recrystallized from acetone–water (2/1) to give 10.4 g (80%) of **16** as hydrochloride salt: mp 248 °C dec. Anal. ($C_{21}H_{23}ClNO_3$·HCl) C, H, N.

(*Z*)-11-[3-(Dimethylamino)propylidene]-2-(2-hydroxyethyl)-6,11-dihydrodibenz[*b*,*e*]oxepin (26). To a solution of the free base of **16** (0.9 g, 2.6 mmol) in THF (50 mL) was added $LiAlH_4$ (0.1 g, 2.6 mmol) at 0 °C, and the solution was stirred at room temperature for 4 h. Excess reagent was destroyed by the addition of water, and the resultant insoluble salts were filtered off. The filtrate was concentrated and submitted to chromatography on silica gel with $CHCl_3$/MeOH (10/1) as eluent. The crude product obtained was recrystallized from ether to give 0.1 g (11%) of **26**: mp 168–170 °C; ^1H NMR ($CDCl_3$) δ 2.39 (s, 6 H), 2.60–2.85 (m, 6 H), 3.84 (t, J = 6.1 Hz, 2 H), 5.17 (br, 2 H), 5.66 (t, J = 7.1 Hz, 1 H), 6.82 (d, J = 8.3 Hz, 1 H), 7.01 (dd, J = 2.2 and 8.3 Hz, 1 H), 7.08 (d, J = 2.2 Hz, 1 H), 7.2–7.4 (m, 4 H); MS m/z 323 (M^+). Anal. ($C_{21}H_{25}NO_2$·H_2O) C, H, N.

Acid-Catalyzed Isomerization of 15 and 16. A solution of 50 mg (0.14 mmol) of **15** (or **16**) in acetic acid (5 mL) containing 80 mg (0.14 mmol) of *p*-TsOH·H_2O was heated under Ar atmosphere at 100 °C for 21 h. The E/Z ratio was measured by analytical HPLC at 1-h intervals (0–5 h) and 4-h intervals (5–21 h). The ratio (E/Z = 65/35) did not change after 13 h in both of the separate experiments.

Biological Evaluation Procedures. Histamine-1 (H_1) Receptor Binding Assay. The H_1 binding assay was performed according to the previously reported method[23] with minor modification. The cerebellum of male Hartley guinea pigs was homogenized in 40 volumes (v/w) of ice-cold 50 mM sodium–potassium phosphate buffer, pH 7.5, by a polytron homogenizer (Kinematica). The homogenate was centrifuged at 35500*g* for 10 min at 4 °C and the precipitate was homogenized again in the same volumes of the buffer. The homogenate was centrifuged again at 35000*g* for 10 min. The resulting precipitate was resuspended in 100 volumes (v/w) of the buffer by a Teflon homogenizer. Tissue homogenates (10 mg wet weight), 3.8 nM of [^3H]pyrilamine, and 0.1 μM of the drug in the total volume of 1.1 mL of 50 mM sodium–potassium phosphate buffer, pH 7.5, were added to a polypropylene tube and incubated for 30 min at 25 °C. Nonspecific binding was determined in the presence of 1 μM astemizole. The reaction was terminated by the addition of ice-cold 50 mM sodium–potassium phosphate buffer, pH 7.5 (4 mL), and subsequent rapid filteration under reduced pressure over a Whatman GF/C glass fiber filter using a cell harvester (Brandel M-24-R). The filter was washed three times with 5 mL of the ice-cold buffer. The filter was transferred to a scintillation vial, to which 0.5 mL of methanol and 8 mL of scintisol EX-H (Wako Pure Chemicals) were added to determine radioactivity by a liquid scintillation counter (Packard 4530).

Muscarinic Acetylcholine (M_1) Receptor Binding Assay. The binding assay was carried out as in the previously described method[24] with minor modification. The striatum of rat was homogenized in 10 volumes (v/w) of distilled water with a Potter-El-vehjem homogenizer. This homogenate preparation was diluted to 200 volumes (v/w) with 50 mM sodium–potassium phosphate buffer, pH 7.4. The homogenate (5 mg wet weight), 1.26 nM of [^3H]quinuclidinyl benzilate, and 1 μM of the drug in the total volume of 1.1 mL of 50 mM sodium–potassium phosphate buffer, pH 7.4, were incubated at 37 °C for 60 min. Nonspecific binding was determined by the addition of 1 μM unlabeled dexetimide. The assay was terminated by rapid filtration under reduced pressure over a Whatman GF/B filter. The filters were washed three times with 5 mL of ice-cold 50 mM sodium–potassium phosphate buffer, pH 7.4, and the radioactivity was counted by liquid scintillation counter.

Effects on 48-h Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats. Rat reaginic antibody (IgE) raised to ovalbumin (OA) was prepared by the method reported previously.[27] Briefly, Wistar strain male rats were immunized by giving a subcutaneous injection of 1 mL of a suspension containing 1 mg of OA, 20 mg of aluminum hydroxide gel, and 10^{10} killed Bordetella pertussis organisms and then bled 14 days after this sensitization. The antiserum was separated and kept at −80 °C. Groups of three Wistar male rats were used and 0.05 mL of anti-OA rat serum, diluted 1:8 with 0.9% saline, was injected intradermally at two points on the dorsum. After 48 h, the PCA reaction was induced by intravenous administration of an aqueous solution containing 1 mg of OA and 5 mg of Evans blue. Test compounds were administered orally 1 h before injection of the antigen. After 30 min, the animals were anesthetized with ethyl ether, and the dorsal skin was removed to determine the extravasated dye at each reaction site. The amount of dye was extracted by the method of Katayama[28] and was quantified by spectrometry. The percent inhibition of the PCA reaction was then calculated. Animals having more than 50% inhibition on the amount of dye leakage compared to the control animals were judged to respond. The dose required for 50% inhibition (ED_{50} value) was calculated from the number of responded animals at each dose.

Effects on Anaphylactic Bronchoconstriction in Passively Sensitized Guinea Pigs. The experiment was performed according to the method of Konzett and Rössler.[21] Groups of 8–12 Hartley strain male guinea pigs were sensitized passively by giving intraperitoneally 1 mL of IgG-like guinea pig antiserum against OA. After 24 h, the animals were anesthetized with urethane (1.2 g/kg ip) and tracheotomized and ventilated by means of a respiratory pump (75 strokes/min, stroke volume 6 mL). After eliminating the spontaneous respiration by injection of gallamine triethiodide (10 mg/kg iv), initial airway resistance was kept constant at 10 cm H_2O pressure by means of a water valve. The airway was connected to a bronchospasm transducer (Type 7020, Ugo Basile, Milan, Italy). The animals were challenged with OA (5 mg/kg iv) and anaphylactic bronchoconstriction was recorded as the percent of maximal overflow obtained by clamping off the trachea. Test drugs were administered orally 1 h before the OA challenge. The preventive effect of the test drugs was expressed as percent inhibition as compared to the increase of overflow volume determined at 5 min later antigen challenge in control animals. The ID_{50} value, i.e., the dose required to 50% inhibition of the anaphylactic bronchoconstriction, was calculated from the relation between the logarithmic dose and the percent inhibition by the method of least squares.

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Registry No. **4a**, 66801-40-9; **4b**, 79669-87-7; **4c**, 55453-87-7; **4e**, 140439-54-9; **4g**, 23560-66-9; **4i**, 66801-46-5; **4j**, 113836-39-8; **4k**, 113836-41-2; **5**, 113805-88-2; **5**·2, 113836-17-2; (*E*)-**6**, 113835-71-5; (*E*)-**6**·fumarate, 113836-23-0; (*Z*)-**6**, 113835-70-4; (*Z*)-**6**·fumarate, 113836-24-1; (*E*)-**7**, 113805-92-8; (*E*)-**7**·$^1/_2$fumarate, 113836-18-3; (*Z*)-**7**, 113805-91-7; (*Z*)-**7**·$^1/_2$fumarate, 113836-19-4; (*E*)-**8**, 113835-84-0; (*E*)-**8**·*i*-PrOH, 140439-55-0; (*Z*)-**8**, 113835-83-9; (*Z*)-**8**·*i*-PrOH, 140439-56-1; **9**, 113805-96-2; **9**·fumarate, 113836-20-7; (*E*)-**10**, 113835-88-4; (*Z*)-**10**, 113835-87-3; **11**, 140439-57-2; **11**·fumarate, 140439-58-3; **12**, 140439-59-4; **13**, 109293-24-5; **13**·$^1/_2$fumarate, 140439-60-7; **14**, 109293-23-4; **15**, 113806-06-7; **16**, 113806-05-6; **16**·HCl, 140462-76-6; **17**, 113835-75-9; **18**, 113835-74-8; **19**, 113835-90-8; **19**·fumarate, 140439-61-8; (*E*)-**20**, 113805-81-5; (*E*)-**20**·fumarate, 113836-16-1; (*Z*)-**20**, 113805-80-4; (*Z*)-**20**·fumarate, 113836-15-0; **21**, 113805-77-9; **21**·fumarate, 113836-14-9; **22**, 113805-85-9; **23**, 113805-84-8; (*E*)-**24**, 113835-65-7; (*Z*)-**24**, 113806-09-0; **25**, 113835-77-1; **26**, 113835-76-0; **27**, 140439-62-9; **27**·$^1/_2$fumarate, 140439-63-0; **28**, 109293-26-7; (*E*)-**29**, 140439-64-1; (*Z*)-**29**, 140439-65-2; **31a**, 113877-82-0; **31b**, 140439-66-3; **31c**, 100212-92-8; (*E*)-**32a**, 113805-74-6; (*Z*)-**32a**, 113805-73-5; (*E*)-**32b**, 113805-72-4; (*Z*)-**32b**, 113805-71-3; **33a**, 27710-82-3; **34**, 113805-

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Supplementary Material Available: Listings of atomic parameters (2 pages); observed structure factors, calculated structure factors, and standard deviations (22 pages). Ordering information is given on any current masthead page.