Synthesis and Antiarrhythmic Activity of 5,11-Dihydro[1]benzoxepino[3,4-*b*]pyridines

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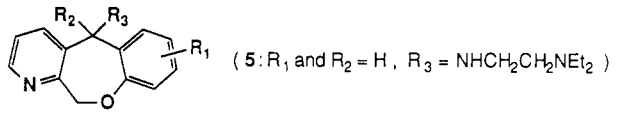
During further modification of the new antiulcer agent **5** (KW-5805), a 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine derivative, we found that some new derivatives had antiarrhythmic activity. So we continued synthesis and evaluation of a series of 5-substituted 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridines for antiarrhythmic activity in chloroform-induced ventricular arrhythmias in mice and in ouabain-induced ventricular arrhythmias in dogs. In chloroform-induced ventricular arrhythmias, the 7-methoxy group played an important role in activity and the type of terminal side chain at position 5 had not obvious effect on potency. On the other hand, in ouabain-induced ventricular arrhythmias, the structure–activity relationship was highly specific and only four compounds, **9**, **30**, **34**, and **35**, were effective. Compound **9**, 5-[[2-(diethylamino)ethyl]amino]-7-methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine 1.5-fumarate, which exhibited low affinity for muscarinic acetylcholine receptors and a high ED_{100}(mydriasis)/ED_{50}(antiarrhythmic activity) ratio, was selected for further development and clinical evaluation as KW-3407. The synthesis and antiarrhythmic activity of optically active **9** is described. The order of potency of antiarrhythmic activity in ouabain-induced ventricular arrhythmias in dogs was (−)-**9**, (±)-**9**, and (+)-**9**.

The risk of sudden death following a myocardial infraction has not been reduced in the past decade in patients with complex ventricular arrhythmias. Because current antiarrhythmic therapy is often ineffective and associated with adverse effects, the development of safe and effective agents for the treatment of arrhythmias, particularly against complex ventricular arrhythmias, remains an important pharmacological and therapeutic goal.[1]

We recently reported the synthesis and biological properties of a series of 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridines, from which **5** (KW-5805) was identified as a

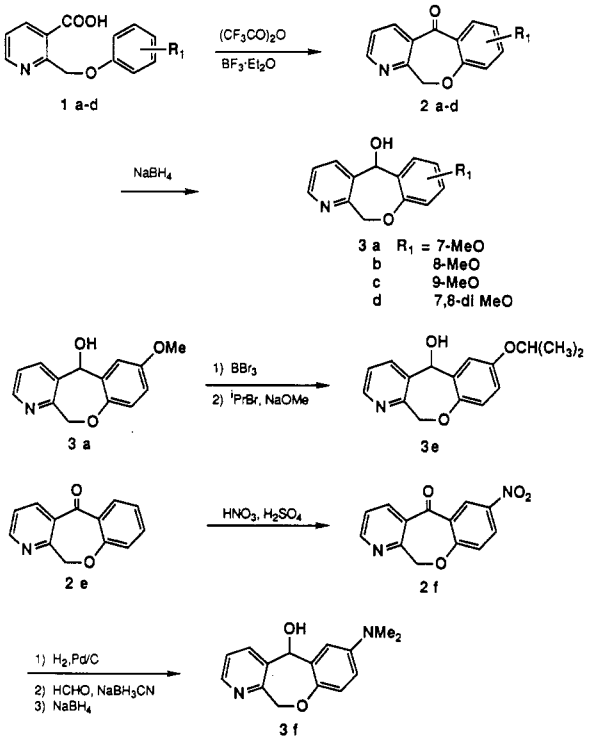


I

highly promising potent antiulcer agent.[2] During further modification of the series, we found that some new derivatives had antiarrhythmic activity. So, we continued synthesis and evaluation of a series of 5-substituted 5,11-dihydro[1]benzoxepino[3,4-*b*]pyridines (I) for antiarrhythmic activity. In addition, we also tested them for antimuscarinic effects, a frequently observed adverse effect of antiarrhythmic agents. As a result, 5-[[2-(diethylamino)ethyl]amino]-7-methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine 1.5-fumarate (**9**) was significantly effective in our arrhythmic models and selected for further development and clinical evaluation.

Chemistry. The 5,11-dihydro[1]benzoxepino[3,4-*b*]-pyridine derivatives (I) were synthesized by several routes starting from the 5,11-dihydro[1]benzoxepino[3,4-*b*]-pyridin-5-ones **2** and 5-ols **3**. Since the intermediate ketone **2a** couldn't be obtained in the manner described previously, a new method was developed (Scheme I). Thus nicotinic acid **1** was cyclized with trifluoroacetic anhydride and boron trifluoride etherate to afford ketone **2**, which was reduced with sodium borohydride to afford alcohol **3**. Alcohol **3a** was treated with boron tribromide to afford the phenol, which was alkylated with isopropyl bromide to alcohol **3e**. Nitration of ketone **2e**, followed by hydrogenation with Pd/C and reductive alkylation with formaldehyde in the presence of sodium cyanoborohydride, afforded dimethylamine **3f**.

Scheme I



The general synthetic method for compounds I listed in Table I is shown in Scheme II. Alcohol **3** was converted to the chloride with thionyl chloride, which was treated with amine or thiol to afford amines **9–14**, **16–22**, and **26–31** (method A). Amines **23–25** were obtained by alkylation of amine **4**, which was prepared by amination of the chloride obtained in method A with NH_3 (method B). Amine **4** was acylated with α-chloroacetyl chloride and then treated with amine to afford amide **33**. Condensation of ketone **2a** with amine in the presence of titanium tetrachloride, followed by reduction with sodium cyanoborohydride, afforded amine **32**. Amine **9** was demeth-

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(1) (a) Steinberg, M. I.; Lacefield, W. B.; Robertson, D. W. *Ann. Rep. Med. Chem.* **1986**, *21*, 95. (b) Douglas, P. Z. *Circulation* **1985**, *72*, 949.

(2) Kumazawa, T.; Harakawa, H.; Obase, H.; Oiji, Y.; Tanaka, H.; Shuto, K.; Ishii, A.; Oka, T.; Nakamizo, N. *J. Med. Chem.* **1988**, *31*, 779.

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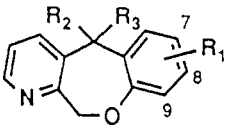
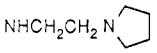
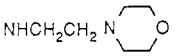
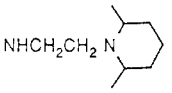
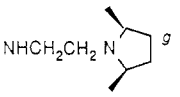
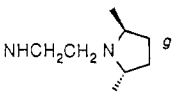
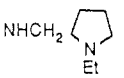
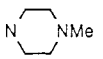
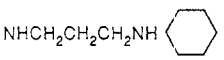
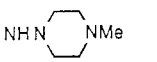
Table I. Substituted 5,11-Dihydro[1]benzoxepino[3,4-*b*]pyridine Derivatives



5: R^1 = H, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
6: R^1 = 7-Me, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
7: R^1 = 7-F, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
8: R^1 = 7-Cl, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$

compd	R^1	R^2	R^3	% yield,[a]	mp, °C	formula[b]	recrystn solvent[c]
9	7-OMe	H	$NHCH_2CH_2NEt_2$	86	128–129	$C_{20}H_{27}N_3O_2 \cdot 1.5C_4H_4O_4$[f]	IPA
10	8-OMe	H	$NHCH_2CH_2NEt_2$	86	132.5–134	$C_{20}H_{27}N_3O_2 \cdot C_4H_4O_4$[f]	IPA–IPE
11	9-OMe	H	$NHCH_2CH_2NEt_2$	43	161–165 dec	$C_{20}H_{27}N_3O_2 \cdot 3HCl \cdot 0.5C_3H_8O$[e]$\cdot H_2O$	IPA
12	7,8-$(OMe)_2$	H	$NHCH_2CH_2NEt_2$	78	166–169 dec	$C_{21}H_{29}N_3O_3 \cdot C_4H_4O_4$[f]	IPA–IPE
13	7-O-*i*-Pr	H	$NHCH_2CH_2NEt_2$	88	183–184 dec	$C_{22}H_{31}N_3O_2 \cdot 3HCl \cdot H_2O$	IPA
14	$7\text{-}NMe_2$	H	$NHCH_2CH_2NEt_2$	53	179–181 dec	$C_{21}H_{30}N_4O \cdot 3HCl \cdot 3H_2O$	IPA[d]
15	7-OH	H	$NHCH_2CH_2NEt_2$	80	191–193 dec	$C_{19}H_{25}N_3O_2 \cdot 3HCl$	IPA
16	7-OMe	H	$NHCH_2CH_2NMe_2$	53	190–191.5 dec	$C_{18}H_{23}N_3O_2 \cdot 3HCl$	IPA
17	7-OMe	H	$NHCH_2CH_2CH_2NMe_2$	52	193–195 dec	$C_{19}H_{25}N_3O_2 \cdot 3HCl \cdot 2H_2O$	IPA–IPE
18	7-OMe	H	$NHCH_2CH_2CH_2NEt_2$	78	115–120 dec	$C_{21}H_{29}N_3O_2 \cdot 3HCl \cdot H_2O$	IPA[d]
19	7-OMe	H	$NHCH_2CH_2N\text{-}i\text{-}Pr_2$	80	166–169 dec	$C_{22}H_{31}N_3O_2 \cdot 3HCl \cdot C_3H_8O$[e]$\cdot 0.5H_2O$	IPA–acetone
20	7-OMe	H	$SCH_2CH_2NEt_2$	93	193–196 dec	$C_{20}H_{26}N_2O_2S \cdot 2HCl$	IPA–IPE
21	7-OMe	H		64	172–175 dec	$C_{20}H_{25}N_3O_2 \cdot 3HCl \cdot 0.5C_3H_8O$[e]$\cdot H_2O$	IPA
22	7-OMe	H		75	159–162 dec	$C_{20}H_{25}N_3O_3 \cdot 2HCl \cdot H_2O$	IPA
23	7-OMe	H		67	204–205 dec	$C_{23}H_{31}N_3O_2 \cdot 3HCl \cdot 0.5H_2O$	IPA[d]
24	7-OMe	H	 [g]	38	205–207 dec	$C_{22}H_{29}N_3O_2 \cdot 3HCl$	IPA[d]
25	7-OMe	H	 [g]	21	173–175 dec	$C_{22}H_{29}N_3O_2 \cdot 3HCl \cdot H_2O$	CH_3CN
26 (α)	7-OMe	H	 α[h]	37	185–187 dec	$C_{21}H_{27}N_3O_2 \cdot 3HCl \cdot H_2O$	IPA
27 (β)	7-OMe	H	β[h]	33	160–162 dec	$C_{21}H_{27}N_3O_2 \cdot 3HCl \cdot H_2O$	acetone–IPA
28	7-OMe	H		56	101–102	$C_{19}H_{25}N_3O_2$	hexane
29	7-OMe	H		90	236–237 dec	$C_{23}H_{31}N_3O_2 \cdot 3HCl$	IPA[d]
30	7-OMe	H		38	125–128 dec	$C_{19}H_{24}N_4O_2 \cdot C_4H_4O_4$[f]	acetone
31	7-OMe	H	$NHCH_2CH_2NH_2$	67	170–173 dec	$C_{16}H_{19}N_3O_2 \cdot 3HCl \cdot H_2O$	CH_3CN[d]
32	7-OMe	H	$NHCH_2CH_2NHEt$	68	201–203 dec	$C_{18}H_{23}N_3O_2 \cdot 3HCl \cdot H_2O$	IPA[d]
33	7-OMe	H	$NHCOCH_2NEt_2$	67	204–205 dec	$C_{23}H_{31}N_3O_2 \cdot 3HCl \cdot 0.5H_2O$	IPA
34	H		$CHCH_2CH_2NEt_2$ (*E*)	34	198–202 dec	$C_{20}H_{24}N_2O \cdot 2HCl$	IPA[d]
35	H		$CHCH_2CH_2NEt_2$ (*Z*)[i]	30	186–193 dec	$C_{20}H_{24}N_2O \cdot 2HCl \cdot 0.67H_2O$	IPA–acetone
36	7-OMe		$CHCH_2CH_2NEt_2$ (*E*)	34	180–181 dec	$C_{21}H_{26}N_2O_2 \cdot 2HCl \cdot 0.33H_2O$	IPA–IPE
37	7-OMe		$CHCH_2CH_2NEt_2$ (*Z*)	37	203–204.5 dec	$C_{21}H_{26}N_2O_2 \cdot 2HCl \cdot 0.33H_2O$	IPA

[a] As free base. [b] All new compounds had C, H, and N microanalyses within 0.4% of theoretical values. [c] IPA, isopropyl alcohol; IPE, isopropyl ether. [d] Trituration solvent. [e] C_3H_8O, isopropyl alcohol. [f] $C_4H_4O_4$, fumaric acid. [g] Starting material was a mixture of cis and trans isomers. [h] Obtained as a mixture of diastereomeric isomers. [i] Contained 13% **34**.

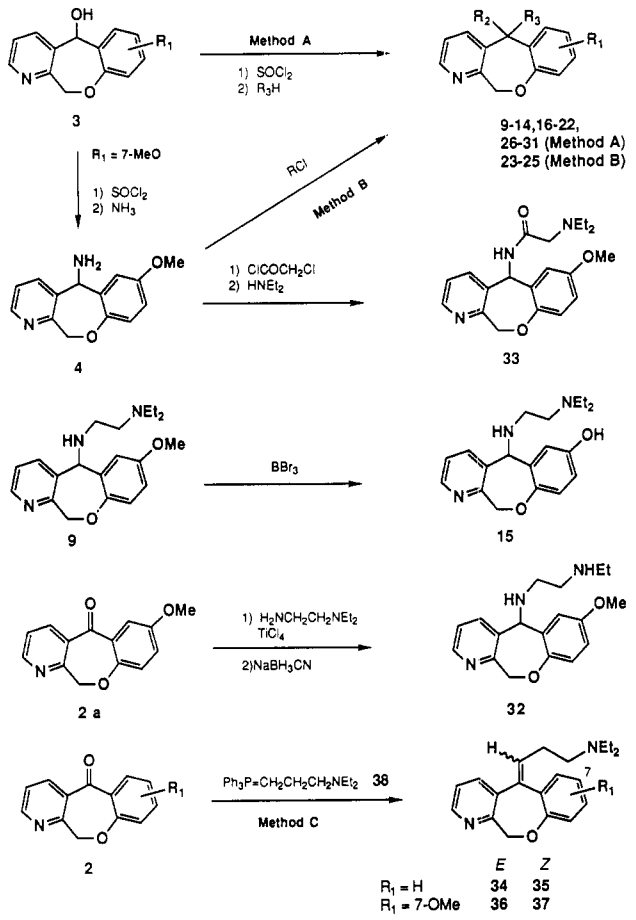
ylated with boron tribromide to afford phenol **15**. Condensation of ketone **2** (**a** and **e**) with Wittig reagent **38** afforded mixtures of *E* and *Z* stereoisomers, which were separated by silica gel chromatography to afford **34**–**37**, respectively (method C).

Since **9** possessed the most favorable combination of desired activities and low side effects, optically active **9** was prepared to examine the activities of the resolved stereoisomers. Optically active **9** was obtained by the optical resolution of **9** with L- and D-tartaric acid. The precipitated salt obtained by treatment of the free base **9** with L-tartaric acid was recrystallized to afford the L-tartarate of (+)-**9**, which was converted to its fumarate (+)-**9**. The mother liquor was basified and treated with D-tartaric acid to afford the D-tartarate of (−)-**9**. The recrystallized salt was converted to (−)-**9** in a manner similar to that used for (+)-**9**.[3]

Biological Tests. The compounds were screened for antiarrhythmic activity in chloroform-induced ventricular

(3) The enantiomeric excess was determined by HPLC (column: Shimadzu CLC-ODS): (+)-**9**, 98.4%; (−)-**9**, 97.1%.

Scheme II



arrhythmias in mice and ouabain-induced ventricular arrhythmias in dogs.[4,5] In the former test, ED_{50} values were calculated when the compounds tested were effective at a dose less than 100 mg/kg (po). In the latter test, when the compounds tested were effective in more than half of the animals, they were judged to be effective. The compounds were also tested for muscarinic acetylcholine receptor affinities, since this could lead to adverse effects. The binding assay was carried out in a previously described method, using rat homogenated striatum.[6]

Results and Discussion

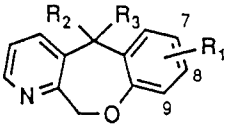
The results of antiarrhythmic testing and muscarinic acetylcholine receptor binding assays are shown in Tables II and III.

Several compounds tested showed antiarrhythmic activity in chloroform-induced ventricular arrhythmias in mice. The nature of the substituent on the benzene ring influenced the activity, and 7-alkoxylated **9** and **13** were effective. The type of the terminal side chain amine at position 5 had no obvious effect on potency.

On the other hand, in ouabain-induced ventricular arrhythmias in dogs, only **9**, **29**, **34**, and **35** showed significant activities. Thus, in this test, the SAR of this series of compounds was highly specific. Of the four compounds, **29**, **34**, and **35** showed undesirable side effects (e.g., ataxia). Compound **9** showed antiarrhythmic activities in both models and was selected for further evaluation.

(4) Block, A. J. *Life Sci.* **1981**, *28*, 2623.
(5) Lucchesi, B. R.; Hardman, H. F. *J. Pharmacol. Exp. Ther.* **1961**, *132*, 372.
(6) Laduron, P. M.; Verwimp, M.; Leysen, J. E. J. *Neurochem.* **1979**, *32*, 421.

Table II. Antiarrhythmic Activities and Affinities to the Muscarinic Receptor of Substituted 5,11-Dihydro[1]benzoxepino[3,4-*b*]pyridine Derivatives



5: R^1 = H, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
6: R^1 = 7-Me, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
7: R^1 = 7-F, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
8: R^1 = 7-Cl, R^2 = H, R^3 = $NHCH_2CH_2NEt_2$
9–37: R groups as in Table I

	antiarrhythmic activity		% inhibn of muscarinic receptor binding[d]	
compd	chloroform-induced ventricular arrythmia,[a] ED_{50} (mg/kg)	ouabain-induced ventricular arrythmia,[b] no. positive cases/ no. cases tested	10^{-5} M	10^{-6} M
5	93 (57–152)	0/3[c]	26	4
6	>100	NT	38	8
7	>100	0/2	37	4
8	>100	NT	46	10
9	62 (43–91)	3/6	8	−3
10	>100	0/2	7	14
11	>100	0/2	37	12
12	>100	0/2	2	7
13	53 (21–133)	0/2	12	3
14	>100	0/2[c]	19	4
15	>100	1/7	−1	0
16	>100	0/2	15	3
17	>100	0/2	5	1
18	50 (15–157)	1/3	24	4
19	>100	0/2	17	4
20	31 (16–59)	0/3	20	1
21	>100	0/3	12	3
22	>100	0/2	0	0
23	>100	1/3	11	2
24	>100	1/3	15	3
25	100 (44–225)	0/1	6	−2
26	61 (41–91)	0/2	23	2
27	58 (38–86)	1/3	25	3
28	81 (45–142)	0/2	11	0
29	>100	3/3	−1	−1
30	>100	0/2	7	2
31	>100	0/2	5	−1
32	>100	0/3	1	2
33	>100	0/2	1	−3
34	17 (5–59)	2/2	80	28
35	59 (33–104)	2/3	45	6
36	14 (8–21)	1/4	26	5
37	>100	0/4	27	3
disopyramide	35 (27–45)	6/6	7	−1
mexiletine	71 (48–105)	5/8	−3	−1

[a] N = 10; mice, po; 95% confidence limits of the mean ED_{50} are shown in parentheses. [b] Dogs, 5 mg/kg iv. [c] Dogs, 10 mg/kg iv. [d] This percent inhibition was measured in a single experiment. Atropine sulfate showed 104% inhibition at 10^{-6} M.

Table III. Antiarrhythmic Activities of Optically Active **9**

compd	% inhibn of ouabain-induced ventricular arrhythmia in dogs:[a] 10 mg/kg
(+)-**9**	51 ± 18
(−)-**9**	94 ± 6
(±)-**9**	76 ± 17

[a] Mean ± SE value from six determinations.

The antiarrhythmic activities of optical isomers of **9** were examined in ouabain-induced ventricular arrhythmias in dogs (Table III). The order of activity was (−)-**9**, (±)-**9**, and (+)-**9**.

Significant undesirable side effects of many antiarrhythmic agents are often caused by their anticholinergic activity. For example, suppression of salivary secretion and mydriasis are the typical ones. Most of the tested compounds exhibited low affinity for muscarinic acetyl-

Table IV. Comparison of Potencies of Compound **9**, Disopyramide, and Mexiletine

compd	chloroform-induced ventricular arrhythmia;[a] ED_{50}, mg/kg (A)	mydriasis[b] mice; ED_{100}, mg/kg po (B)	ratio B/A
9	62 (43–91)[c]	194 (175–211)[c]	4.0
disopyramide	35 (27–45)	20 (18–22)	0.6
mexiletine	71 (48–105)	138 (124–157)	1.7

[a] See Table II. [b] ED_{100} was defined as a dose needed to double the pupil size. [c] 95% confidence limits are shown in parentheses.

choline receptors. The selected compound **9** showed a high ED_{100}(mydriasis)/ED_{50}(antiarrhythmic activity) ratio compared to disopyramide and mexiletine (Table IV).

Although the structure of **9** is similar to that of antiulcer agent **5** which we have previously reported,[2] the pharmacological properties of these compounds are different. Compound **5** did not show antiarrhythmic activity in oubain-induced ventricular arrhythmias in dogs. The antiulcer activity of **9** in water immersion/restrained stress ulcer assay in rats was weak compared to that of **5** (percent inhibition at 30 mg/kg, po: **5** 85%; **9**, 48%).

In conclusion, we have described a new class of antiarrhythmic agents, the 5,11-dihydro[1]benzoxepino[3,4-*b*]-pyridine derivatives. Among the compounds tested, **9** has potent antiarrhythmic activities, exhibiting low anticholinergic side effects in experimental animal models (suppression of salivary secretion and mydriasis in mice) and low toxicity, and was selected for further development and clinical evaluation as KW-3407. Compound **9** displayed a class IA electrophysiological profile and the detailed pharmacology and mechanism of action will be published elsewhere.

Experimental Section

Melting points were determined with a Büchi-510 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a JASCO IR-810 spectrometer. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL PMX-60 or a JEOL JNM GX-270 spectrometer with Me_4Si as internal standard. Elemental analyses were performed by the analytical department of our laboratories.

Chemistry. 7-Methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridin-5-ol (3a). To a suspension of 100.0 g (0.39 mmol) of **1a** in 1.5 L of 1,1,2,2-tetrachloroethane was added 136 mL (0.96 mol) of trifluoroacetic anhydride and the mixture was stirred at room temperature for 40 min. Then, 38 mL (0.31 mol) of boron trifluoride etherate was added and the mixture was heated at 100–110 °C for 4 h. Upon cooling, the mixture was poured into ice–water and basified with 4 N NaOH. After filtration, the organic layer was dried and concentrated in vacuo. The residue was chromatographed on silica gel with hexane–AcOEt (2:1) to give 67.0 g (72%) of **2a** as crystals: mp 79–80.5 °C (*i*-Pr_2O); IR (KBr) 1643, 1610, 1489, 1330 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3 H), 5.24 (s, 2 H), 6.93–7.70 (m, 4 H), 8.25 (dd, 1 H, J = 2 and 8 Hz), 8.63 (dd, 1 H, J = 2 and 5 Hz). To a solution of 10.0 g (41 mmol) of **2a** in 300 mL of EtOH–THF (2:1) was added 1.0 g (25 mmol) of $NaBH_4$ at 0 °C and the mixture was stirred at room temperature for 3 h. After concentration in vacuo, 50 mL of water was added, and the mixture was extracted with AcOEt, dried, and concentrated in vacuo to give crude **3a**. Recrystallization from toluene gave 7.1 g (71%) of **3a**: mp 128–130.5 °C; IR (KBr) 3150, 1590, 1490, 1435, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.69 (s, 3 H), 5.07 and 5.30 (q, 2 H, AB type, J = 16 Hz), 5.92 (d, 1 H, J = 6 Hz), 6.18 (d, 1 H, J = 6 Hz), 6.48–7.28 (m, 4 H), 7.94 (dd, 1 H, J = 2 and 8 Hz), 8.22 (dd, 1 H, J = 2 and 5 Hz).

7-(Isopropyloxy)-5,11-dihydro[1]benzoxepino[3,4-*b*]-pyridin-5-ol (3e). To a solution of 5.2 mL (55 mmol) of boron tribromide in 50 mL of CH_2Cl_2 was added a solution of 6.7 g (27.5 mmol) of **3a** at −60 °C and the mixture was gradually warmed to room temperature with stirring. After being stirred at room temperature overnight, 50 mL of water was added and the mixture was adjusted to pH 5 with 10 N NaOH. The resultant crystalline product was collected by filtration and dried to give 5.17 g (82%) of the phenol: mp >250 °C; IR (KBr) 3450, 1580, 1460, 1200, 1040 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.01 and 5.30 (q, 2 H, AB type, J = 16 Hz), 6.12 (bs, 2 H), 6.42–7.33 (m, 4 H), 7.95 (dd, 1 H, J = 2 and 8 Hz), 8.26 (dd, 1 H, J = 2 and 5 Hz), 8.95 (s, 1 H). To a solution of 4.06 g (17.7 mmol) of the obtained phenol in 120 mL of MeOH was added 3.4 g (17.7 mmol) of 28% NaOMe–MeOH and the mixture was concentrated in vacuo. The residue was dissolved in 200 mL of EtOH and 6.5 g (53 mmol) of isopropyl bromide was added. The mixture was refluxed for 7 h and then concentrated in vacuo. The residue was dissolved in AcOEt and washed with water, dried, and concentrated in vacuo. The residue was chromatographed on silica gel with hexane–AcOEt (2:1) to give 3.0 g (62.5%) of **3e** as crystals: mp 108.5–109.5 °C (*i*-Pr_2O); IR (KBr) 3150, 2975, 1580, 1490, 1200, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 6 H, J = 6 Hz), 4.11–4.63 (m, 1 H), 5.08 (bs, 1 H), 5.18 (s, 2 H), 5.96 (s, 1 H), 6.15–7.27 (m, 4 H), 7.87 (dd, 1 H, J = 1.5 and 8 Hz), 8.20 (dd, 1 H, J = 1.5 and 5 Hz).

7-(Dimethylamino)-5,11-dihydro[1]benzoxepino[3,4-*b*]-pyridin-5-ol (3f). To a solution of 5.0 g (23.7 mmol) of **2e** in 36 mL of concentrated H_2SO_4 was added dropwise a mixture of 2.4 g of concentrated HNO_3 and 1.2 mL of concentrated H_2SO_4 at −30 °C. The mixture was gradually warmed to room temperature over 4 h with stirring and then poured into ice–water. After basification with 10 N NaOH, the resultant crystalline product was collected by filtration, washed with water and ether, and dried to give 4.69 g (77%) of **2f**: mp 205–207 °C; IR (KBr) 1660, 1610, 1580, 1518, 1340, 1080 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.58 (s, 2 H), 7.42 (d, 1 H, J = 9 Hz), 7.67 (dd, 1 H, J = 5 and 8 Hz), 8.25 (dd, 1 H, J = 1.5 and 8 Hz), 8.43 (dd, 1 H, J = 3 and 9 Hz), 8.84 (dd, 1 H, J = 1.5 and 8-Hz), 8.90 (d, 1 H, J = 3 Hz). To a solution of 2.70 g (10.5 mmol) of **2f** in EtOH–H_2O (4:1) was added 3.2 mL of concentrated HCl and 0.27 g of 10% Pd–C. The mixture was stirred under an H_2 atmosphere for 1 h. After filtration, the filtrate was basified with 1 N NaOH. The resultant crystalline product was collected by filtration and dried to give 1.86 g (78%) of the aniline: mp 149–151.5 °C (CH_3CN); IR (KBr) 3400, 3330, 1650, 1618, 1490, 1320 cm^{-1}; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) δ 5.19 (s, 2 H), 6.84 (d, 2 H, J = 2 Hz), 7.23–7.59 (m, 2 H), 8.22 (dd, 1 H, J = 2 and 8 Hz), 8.62 (dd, 1 H, J = 2 and 5 Hz). To a solution of 2.0 g (8.8 mmol) of the obtained aniline, 2.75 g (44 mmol) of $NaBH_3CN$, and a small amount of bromocresol green in 70 mL of MeOH was added HCl-*i*-PrOH until the solution became yellow. Then, 3.76 g (44 mmol) of 35% HCHO was added dropwise at room temperature and the mixture was stirred for 2 h. The mixture was concentrated in vacuo and water was added. After basification with 10 N NaOH, the mixture was extracted with AcOEt, dried, and concentrated in vacuo to give crude **3f**. Recrystallization from *i*-PrOH gave 1.72 g (77%) of **3f**: mp 154–157 °C; IR (KBr) 3150, 1615, 1580, 1510, 1220, 1050 cm^{-1}; ^{1}H NMR (DMSO-d_6 + $CDCl_3$) δ 2.80 (s, 6 H), 5.03 and 5.26 (q, 2 H, AB type, J = 15 Hz), 5.89 (bs, 1 H), 6.14 (s, 1 H), 6.33–7.26 (m, 4 H), 7.94 (dd, 1 H, J = 2 and 8 Hz), 8.22 (dd, 1 H, J = 2 and 5 Hz).

Method A. 5-[[2-(Diethylamino)ethyl]amino]-7-methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine 1.5-Fumarate (9). To a solution of 6.0 g (24.7 mmol) of **3a** in 100 mL of CH_2Cl_2 was added 2.7 mL (37.1 mmol) of $SOCl_2$ at 0 °C. After being stirred at 0 °C for 30 min and then at room temperature for 1 h, the mixture was concentrated in vacuo. The residue was dissolved in 30 mL of CH_2Cl_2 and added to a solution of 14.50 g (125 mmol) of *N,N*-diethylethylenediamine in 120 mL of CH_2Cl_2 at 0 °C. The mixture was stirred at 0 °C for 30 min and then at room temperature for 1 h. After addition of 100 mL of CH_2Cl_2, the mixture was washed with water, dried, and concentrated in vacuo. The residue was chromatographed on silica gel with AcOEt–Et_3N (20:1) to give 7.21 g (85%) of **9** as crystals: mp 85–86.5 °C; IR (KBr) 3300, 2968, 1575, 1495, 1450, 1208, 1043 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 6 H, J = 7 Hz), 2.07–2.74 (m, 9 H), 3.71 (s, 3 H), 4.45 (s, 1 H), 5.00 and 5.88 (q, 2 H, AB type, J = 14 Hz), 6.38–7.27 (m, 4 H), 7.55 (dd, 1 H, J = 2 and 8 Hz), 8.36 (dd, 1 H, J = 2 and 5 Hz). To a solution of 1.0 g (2.9 mmol) of **9** in 20 mL of *i*-PrOH was added 0.51 g (4.4 mmol) of fumaric acid. The mixture was stirred at room temperature overnight. The resulstant crystalline was collected by filtration and dried to give crude crystals. Recrystallization from *i*-PrOH gave 1.07

g (71%) of **9** 1.5-fumarate (Table I).

Method B. 5-[[2-(2,6-Dimethylpiperidino)ethyl]amino]-7-methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine (23). To a solution of 5.0 g (20.6 mmol) of **3a** in 120 mL of CH_2Cl_2 was added a solution of 1.6 mL (22.7 mmol) of $SOCl_2$ in 20 mL of CH_2Cl_2 at 0 °C. After being stirred at 0 °C for 30 min and then at room temperature for 1 h, the mixture was concentrated in vacuo. The residue was dissolved in 100 mL of CH_2Cl_2 and added to 200 mL of saturated NH_3 in CH_2Cl_2 at 0 °C. After being stirred at 0 °C for 1 h and then at room temperature for 1 h, the mixture was washed with water, dried, and concentrated in vacuo to afford 4.87 g (crude 98%) of crude **4** as a viscous oil, which was used for next reaction without further purification: IR (liquid film) 3370, 2950, 1580, 1500, 1205, 1042 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.21 (s, 2 H), 3.71 (s, 3 H), 4.86 (s, 1 H), 5.07 and 5.37 (q, 2 H, AB type, J = 17.5 Hz), 6.55–7.24 (m, 4 H), 7.66 (dd, 1 H, J = 2 and 7.5 Hz), 8.35 (dd, 1 H, J = 2 and 5 Hz). A mixture of 2.0 g (8.3 mmol) of **4**, 2.60 g (12.4 mmol) of 2-(2,6-dimethylpiperidino)ethyl chloride hydrochloride, 3.5 mL (24.9 mmol) of NEt_3, and catalyc amount of NaI in 60 mL of toluene was refluxed for 10 h. Upon cooling, 60 mL of toluene was added, and the mixture was washed with water, dried, and concentrated in vacuo. The residue was chromatographed on silica gel with AcOEt–Et_3N (10:1) to give 2.11 g (67%) of **23** as an oil; IR (liquid film) 3350, 2930, 1580, 1500, 1210, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.81–2.81 (m, 19 H), 3.73 (s, 3 H), 4.42 (s, 1 H), 4.99 and 5.48 (q, 2 H, AB type, J = 16 Hz), 6.60–7.23 (m, 4 H), 7.61 (dd, 1 H, J = 2 and 7 Hz), 8.36 (dd, 1 H, J = 2 and 5 Hz). This oil was converted to hydrochloride salt in a usual manner (Table I).

5-[[(*N*,*N*-Diethylamino)acetyl]amino]-7-methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine Dihydrochloride (33). To a solution of 2.0 g (8.3 mmol) of **4** and 1.2 mL (8.3 mmol) of NEt_3 in 50 mL of Et_2O was added dropwise a solution of 0.93 g (8.3 mmol) of α-chloroacetyl chloride in 5 mL of Et_2O at 0 °C, and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with AcOEt and washed with aqueous $NaHCO_3$, water, and brine. The organic layer was dried and concentrated in vacuo. A mixture of the residue and 3.0 g (41.5 mmol) of $NHEt_2$ in 70 mL of toluene was refluxed for 4 h. Upon cooling, the mixture was diluted with AcOEt and washed with water, dried, and concentrated in vacuo. The residue was chromatographed on silica gel with AcOEt–hexane–NEt_3 (10:5:1) to give 2.35 g (80%) of **33** as an oil: IR (liquid film) 3350, 2970, 1670, 1500, 1210, 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, 6 H, J = 7 Hz), 2.42 (q, 4 H, J = 7 Hz), 2.96 (s, 2 H), 3.73 (s, 3 H), 5.0 and 5.44 (q, 2 H, AB type, J = 16.5 Hz), 5.94 (d, 1 H, J = 10 Hz), 6.61–7.34 (m, 4 H), 7.81 (dd, 1 H, J = 2 and 8 Hz), 8.39 (dd, 1 H, J = 2 and 5 Hz), 8.63 (d, 1 H, J = 9 Hz). This oil was converted to hydrochloride salt in a usual manner.

5-[[2-(Ethylamino)ethyl]amino]-7-methoxy-5,11-dihydro-[1]benzoxepino[3,4-*b*]pyridine Trihydrochloride (32). To a solution of 6.03 g (25 mmol) of **2a** and 20.5 mL (195 mmol) of *N*-ethylethylenediamine in 30 mL of CH_2Cl_2 was added dropwise a solution of 4.3 mL (39 mmol) of $TiCl_4$ in 20 mL of CH_2Cl_2 at 0 °C, and the mixture was stirred at room temperature overnight. The reaction mixture was poured into cooled 1 N NaOH and then filtered. The filtrate was separated, and the organic layer was dried and concentrated in vacuo to afford 7.79 g of the crude amine as an oil. To a solution of this crude oil and a small amount of bromocresol green was added HCl–*i*-PrOH until the solution became yellow. Then, 1.65 g (25 mmol) of $NaBH_3CN$ was added, and the mixture was stirred at room temperature for 8 h. After addition of 200 mL of water, the mixture was basified with 10 N NaOH and extracted with AcOEt. The organic layer was washed with water, dried, and concentrated in vacuo. The residue was chromagraphed on silica gel with AcOEt–MeOH–NEt_3 (20:2:1) to afford 5.34 g (68%) of **32** as an oil; IR (liquid film) 3300, 2900, 1495, 1450, 1260, 1205 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7 Hz), 2.57 (q, 2 H, J = 7), 2.65 (s, 4 H), 3.71 (s, 3 H), 4.45 (s, 1 H), 5.02 and 5.51 (q, 2 H, AB type, J = 16.5 Hz), 6.62–7.19 (m, 4 H), 7.62 (dd, 1 H, J = 2 and 7.5 Hz), 8.39 (dd, 1 H, J = 2 and 4.5 Hz). This oil was converted to its hydrochloride salt in a usual manner.

Method C. 5-[3-(Diethylamino)propylidene]-7-methoxy-5,11-dihydro[1]benzoxepino[3,4-*b*]pyridine (36 and 37). To a suspension of 45.0 g (83 mmol) of [3-(diethylamino)propyl]-triphenylphosphonium bromide hydrogen bromide in 300 mL of THF was added 116 mL (174 mmol) of 1.5 M BuLi–hexane solution dropwise at 3 °C. After being stirred at room temperature for 2 h, a solution of 10.0 g (41.5 mmol) of **2a** in 160 mL of THF was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt, washed with water and saturated brine, dried, and concentrated in vacuo. The residue was chromatographed on silica gel with hexane–AcOEt–Et_3N (10:5:1) to afford 4.73 g (34%) of **36** (*E*) and 5.21 g (37%) of **37** (*Z*) as an oil, respectively. **36**: IR (liquid film) 2960, 1570, 1490, 1200, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (t, 3 H, J = 7 Hz), 2.06–2.86 (m, 8 H), 3.71 (s, 3 H), 5.20 (s, 2 H), 6.02 (t, 1 H, J = 7 Hz), 6.60–7.27 (m, 4 H), 7.61 (dd, 1 H, J = 2 and 8 Hz), 8.32 (dd, 1 H, J = 2 and 5 Hz). **37**: IR (liquid film) 2960, 1570, 1490, 1200, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, 6 H, J = 7 Hz), 2.05–2.73 (m, 8 H), 3.70 (s, 3 H), 5.21 (s, 2 H), 6.02 (t, 1 H, J = 7 Hz), 6.56–7.50 (m, 5 H), 8.37 (dd, 1 H, J = 2 and 4 Hz). These oils were converted to hydrochloride salt in a usual manner (Table I).

Optical Resolution of 9. To a solution of 341.6 g (1 mol) of the free base of **9** in 12.8 L of MeOH was added 300.2 g (2 mol) of L-tartaric acid, and the mixture was stirred at 65 °C to dissolve. Upon cooling, the resultant crystals were collected by filtration and dried. Recrystallization from MeOH three times afforded 116.5 g (18%) of (+)-**9** di-L-tartrate, $[\alpha]^{23}_D$ +100.6° (*c* 1, H_2O). The di-L-tartarate of (+)-**9** (116.5 g, 0.18 mol) was neutralized with aqueous NaOH and extracted with CH_2Cl_2. The extracts were washed with water, dried, and concentrated in vacuo. The residue was dissolved in 700 mL of THF, and 31.6 g (0.27 mol) of fumaric acid was added. The mixture was heated to dissolve and then stirred at 5 °C overnight. The resulting crystals were collected by filtration, washed with THF, and dried to afford 83.5 g (89%) of (+)-**9** 1.5-fumarate, $[\alpha]^{23}_D$ +112.5° (*c* 1, H_2O). The filtrate of the di-L-tartarate was neutralized and extracted with CH_2Cl_2 to afford 203.7 g of (−)-rich free base of **9**. This was dissolved in 6 L of MeOH, and 179.1 g (1.2 mol) of D-tartaric acid was added. The mixture was heated to reflux. Upon cooling, the resulting crystals were collected by filtration and dried. Recrystallization from MeOH twice afforded 161.0 g (62%) of di-D-tartarate, $[\alpha]^{23}_D$ −101.7° (*c* 1, H_2O). This salt was converted to 101.3 g (78%) of (−)-**9** 1.5-fumarate in a similar manner as that for (+)-**9**: $[\alpha]^{23}_D$ −114.7° (*c* 1, H_2O).

Biology. Chloroform-Induced Ventricular Arrhythmias in the Mouse. Male mice (ddY, 18–23 g) were used. According to the reported method,[4] ED_{50} values were calculated for protection from ventricular tachycardia induced by exposure to chloroform vapors. The compounds tested were orally administrated and 1 h later mice were exposed to chloroform.

Ouabain-Induced Ventricular Arrhythmias in the Dog. According to the reported method,[5] ventricular arrhythmias were evoked by intravenous administration of ouabain (80 mg/kg) in mongrel dogs of either sex (8–15 kg) anesthetized with penatobarbital sodium (35 mg/kg iv). Ten minutes after persistent appearance of ventricular arrhythmias, the compounds tested were injected intravenously within 10 s. The severity of arrhythmia was expressed by arrhythmic ratio: the number of ventricular ectopic beats (total beats minus sinus beats) divided by the total beats. The ventricular ectopic beats were judged by the different shape of the ventricular complex from the normal QRS complex on lead II ECG. The positive case means complete restoration of the sinus rhythm.

Muscarinic Acetylcholine Receptor Binding Assay. The binding assay was carried out as in the previously described method with minor modification.[6] The striatum of the rat was homogenized in 10 volumes of distilled water with a Potter–Elvehjem homogenizer. This homogenate preparation was diluted to 200 volumes of the wet tissue weight with 50 mM sodium–potassium buffer solution (pH 7.4). And then 50 μM of [^{3}H]-quinuclidinyl benzilate solution (final concentration 1.26 nM) and the drug solution (10% ethanol, 50 μL) were added to 1 mL of the homogenate (corresponds to 5 mg of wet tissue) and incubated at 37 °C for 1 h. Nonspecific binding was determined by addition of unlabeled doxetimide (10% ethanol solution, final concentration 1 μM). The assay was terminated by rapid filtration under reduced pressure over Whatman GF/B filter. The filters were rinsed three times with 5 mL of ice-cold 50 mM sodium–potassium

phosphate buffer (pH 7.4), transferred to counting vials containing 7 mL of scintillator (Scintisole EX-H, Wako), and counted by liquid-scintillation spectrometry (Packard Tri-Carb 330).

Mydriasis. For each dose 10 male mice of ddY strain weighing 18–23 g were used. Pupil size was microscopically measured under 1000 lx lighting before and 30 min after oral administration of drugs. ED_{100} was a dose required to double the predrug pupil size.

Synthesis and Evaluation of N-Substituted *cis*-*N*-Methyl-2-(1-pyrrolidinyl)cyclohexylamines as High Affinity σ Receptor Ligands. Identification of a New Class of Highly Potent and Selective σ Receptor Probes

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Certain benzeneacetamides [(−)- and (+)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide] were recently reported to be potent σ receptor ligands. In order to determine whether efficacy for the σ receptor could be improved, a series of compounds related to the benzeneacetamides, *N*-substituted *cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamines, were synthesized and their structure–activity requirements were determined. The compounds were synthesized by starting with the previously reported (±)-, 1*S*,2*R*-(+)-, and 1*R*,2*S*-(−)-*cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamines. Analysis of σ ([^{3}H](+)-3-PPP), κ ([^{3}H]bremazocine and [^{3}H]U69,593), dopamine-d_2 ([^{3}H](−)-sulpiride), and phencyclidine (PCP) ([^{3}H]TCP) receptor binding in guinea pig brain revealed a number of highly potent and selective σ receptor ligands. Notably, 1*S*,2*R*-*cis*-(−)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-(2-naphthyl)acetamide [(−)-**29**] (K_i = 8.66 ± 0.35 nM), (±)-*cis*-2-amino-4,5-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(±)-**17**] (K_i = 11 ± 3 nM), 1*S*,2*R*-(−)-*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-**44**] (K_i = 1.3 ± 0.3 nM), and 1*R*,2*S*-(+)-*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine. [(+)-**44**] (K_i = 6 ± 3 nM) exhibited very high affinity at σ receptors, by displacement of [^{3}H]-(+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ([^{3}H]-(+)-3-PPP). These compounds showed insignificant affinity for κ, dopamine, or PCP receptors, making them valuable tools for the study of σ receptors. Furthermore, these compounds also exhibited enantioselectivity ranging from 5-fold for (+)- and (−)-**44** to 160-fold for (+)- and (−)-**29**. Several other compounds showed equivalent selectivity but displayed lower σ receptor affinity.

Introduction

The σ receptor was first proposed by Martin et al., in 1976,[1] as an opioid-receptor type on the basis of the ability of the 6,7-benzomorphan opioid (±)-*N*-allylnormetazocine [(±)-SKF10,047] to produce effects different from those of other opioids in the chronic spinal dog.[1,2] However, several later studies suggested that this classification had to be extensively modified.

The first modification came from studies on the mechanism of action of the psychotomimetic drug phencyclidine (PCP). It was found that PCP and (+)-SKF10,047 may share a common binding site in brain.[3-6] [^{3}H]PCP is displaced from the PCP binding site by the 6,7-benzomorphans (+)-SKF10,047 and (+)-cyclazocine. Furthermore, binding sites for [^{3}H]cyclazocine and [^{3}H]-PCP have a similar regional distribution in brain.[5] Later work with enantiomeric 6,7-benzomorphans revealed that at least some of the PCP-like effects of SKF10,047 were associated with the dextrorotatory enantiomer.[4,7] Other studies examining similar behavioral effects produced by PCP and 6,7-benzomorphans such as (+)-SKF10,047 suggested that these compounds may exert their effects through the same site and that the σ receptor and PCP receptor are identical.[8-11]

However, it is now clear that (+)-6,7-benzomorphans bind to at least two distinct receptor sites in brain.[12] [^{3}H](+)-SKF10,047 is potently displaced by neuroleptic

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