Novel Cerebroprotective Agents with Central Nervous System Stimulating Activity. 2. Synthesis and Pharmacology of the 1-(Acylamino)-7-hydroxyindan Derivatives

Yasuo Oshiro,* Youji Sakurai, Tatsuyoshi Tanaka, Tetsuro Kikuchi, Tsuyoshi Hirose, and Katsura Tottori

Otsuka Pharmaceutical Company Ltd. Third Institute of New Drug Research. 364-10 Kagasuno. Kawauchi-cho. Tokushima. Japan 771-01. *Received August 6, 1990*

In a continuous search for a novel cerebroprotective drug with a central nervous system (CNS) stimulating activity, a series of 1-(acylamino)-7-hydroxyindan derivatives has been synthesized and tested for its dual activities. The cerebroprotective activities of the compounds in this series were evaluated in terms of their effect on the survival of mice in hypoxic conditions (210 mmHg), and their CNS stimulating activities were examined by evaluating their promotional effects on the recovery from coma induced by cerebral concussion in mice. Several compounds prolonged the survival of mice in the hypoxic conditions at a dose of 30 mg/kg po. Four compounds in this series showed the CNS-stimulating effect at the same dose. Among them, 7-hydroxy-1-[[4-(3-methoxyphenyl)-1-piperazinyl]-acetyl]amino]-2,2,4,6-tetramethylindan (18, OPC-14117), which was active in the two tests with no side effect up to 500 mg/kg po daily for 2 weeks of administration to rats, was selected for preclinical investigations.

The generation of oxygen radicals and the process of lipid peroxidation have become a focus of attention for investigators in the fields of central nervous system (CNS) trauma and stroke.[1] Under such ischemic–anoxic conditions, the generation of oxygen radicals and the subsequent formation of lipid peroxide are likely to be involved in tissue damage in the brain.[2-7] An antioxidant, vitamin E (α-tocopherol), has been reported to protect against brain damage due to hypoxia in the rat by preventing the formation of the lipid peroxide.[8] Steroids methyl prednisolone[9] and hydrocortisone[10] have been known to show an improving effect on the neurological outcome after CNS injury. The effects were suggested to be due to their weak inhibition of lipid peroxidation.[1,9] Recently, a potent inhibitor of iron-dependent lipid peroxidation, U-74006F, one of the series of 21-amino steroids, has been reported to be effective in models of head and spinal cord injury (Figures 1 and 2).[11]

We have been interested in developing a novel cerebroprotective drug with a CNS-stimulating effect. The ideal drug would prevent tissue damage in the brain during the hypoxic conditions by its cerebroprotective effect derived from its inhibitory effect on the formation of the oxygen radicals and the lipid peroxides and would accelerate recovery from coma by its CNS-stimulating effect. To find our target compounds, two pharmacological tests, the test for the antihypoxic effect and for a promoting effect on recovery from coma, were used as the primary screening tests. The test for antihypoxic effect has been known as one of the experimental models for cerebral anoxia, and pentobarbital sodium, which is used as the cerebroprotective drug, has been reported to be active in the test.[12] The test for promoting effect on recovery from coma has been proposed as an experimental model for a cerebral trauma,[13] and the CNS activator, thirotropine release hormone (TRH), which is used in clinical treatment of symptoms at the chronic stage of stroke and traumatic insults has been reported to show the effects.[14] The known radical scavengers such as an artifical antioxidant, 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT), and an endogenous antioxidant, vitamin E, have a phenolic hydroxyl group in their chemical structure. A series of the compounds with a phenolic hydroxyl group was synthesized and examined for the dual activities in the two tests. As described in the previous paper,[15] the 1-amino-7-hydroxyindan derivatives (**1a–j**) showed potent activities in the two tests. However, the derivatives possessed undesirable side effects, tremor and hypothermia. In our continuing search for the compound with the dual activities and less undesirable side effects, 7-hydroxy-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-2,2,4,6-tetramethylindan (**18**) has been found. The compound showed cerebroprotection against hypoxia under low-pressure oxygen (210 mmHg) loading and it accelerated recovery from coma induced by cerebral concussion in mice. The compound did not show any undesirable side effect up to 500 mg/kg in repeated oral administration to rats for 2 weeks.

In this paper, we will describe the synthesis of a series of 1-(acylamino)-7-hydroxyindan derivatives by chemical modifications of several 1-amino-7-hydroxyindan derivatives (**1a–j**) and their pharmacological activity.

Chemistry

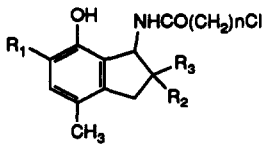
The 1-[[(4-phenyl-1-piperazinyl)acetyl]amino]-7-hydroxyindans listed in Table II and the 1-[[(cyclic amino)acetyl]amino]-7-hydroxyindans listed in Table III were prepared as shown in Scheme I. Chemical modifications of these derivatives are outlined in Scheme II. Preparations of the intermediates, 1-amino-7-hydroxyindan derivatives, have been reported in the preceding paper.[15]

Scheme I shows the preparations of the compounds listed in Tables I and II. Chloroacetylation of the 1-amino-7-hydroxyindans (**1a–j**) in the presence of tri-

(1) Braughler, J. M.; Hall, E. D. *Free Radical Med.* **1989**, *6*, 289.
(2) Goldstein, A., Jr.; Well, B. A.; Keats, A. S. *Arch. Int. Pharmacodyn.* **1966**, *161*, 128.
(3) Yatsu, F. M.; Diamond, I.; Graziano. G.; Lindquist, P. *Stroke* **1972**, *3*, 726.
(4) Smith, A. L.; Hoff, J. T.; Nielsen, S. L.; Larson, C. P. *Stroke* **1974**, *5*, 1.
(5) Yasuda. H.; Shimada, O.: Nakajima, A.; Asano. T. *J. Neurochem.* **1978**, *37*, 934.
(6) Johnson, G.; Marcoux, F. W. *Annu. Rep. Med. Chem.* **1986**, *21*, 109.
(7) Safar, P.: Bleyaert, A.; Nemoto, E. M.; et al. *Brain and Heart Infarct II*; Springer Verlag: New York, 1977; p 251.
(8) Yamada, M.; Shima, T.; Uozumi, T.; Sogabe. T.; et al. *Stroke* **1983**, *14*, 977.
(9) Giannota, S. L.; Weiss, M. H.; Apuzzo, M. L. J.; et al. *Neurosurgury* **1984**, *15*, 497.
(10) Hashi, K.: Takakura, K.; Sano, K.; et al. *No To Shinkei* **1988**, *40*, 373.
(11) Ayer, E. D.; VanDoornik, F. J.; Palmer, J. R.; et al. *J. Med. Chem.* **1990**, *33*, 1145.
(12) (a) Nakanishi, M.; Yasuda, H.; Tsumagari, T. *Life Sci.* **1973**, *13*, 467. (b) Yasuda, H.; Shuto, S.: Tsumagari, T.; Nakajima. A. *Arch. Int. Pharmacodyn.* **1978**, *233*, 136.
(13) Manaka, S.; Sano, K. *Igaku No Ayumi* **1977**, *102*, 867.
(14) Miyamoto, M.; Fukuda, N.; Narumi, S.; et al. *Life Sci.* **1981**, *28*, 861.
(15) Oshiro, Y.; Sakurai, Y.; et al. *J. Med. Chem.*, preceding paper in this issue.

0022-2623/91/1834-2014$02.50/0

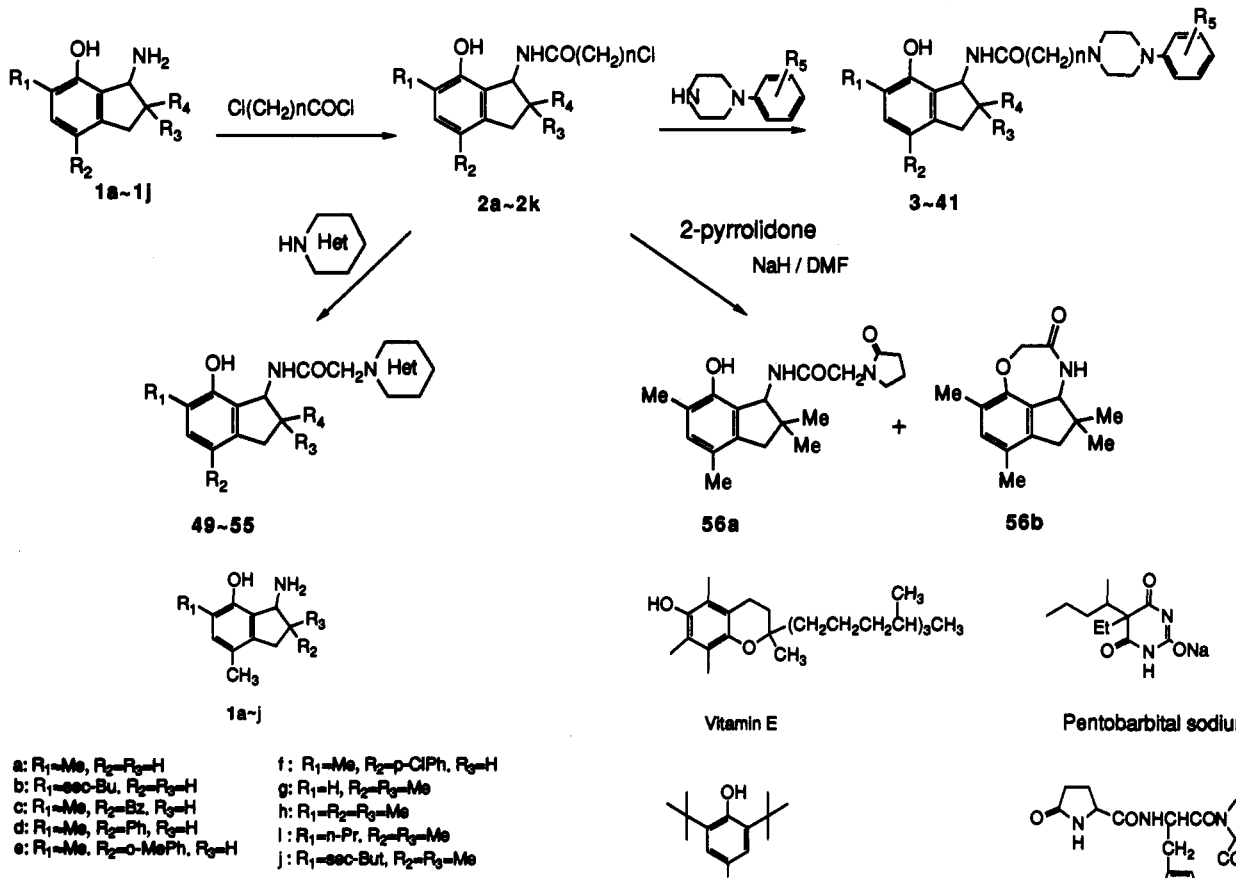
Table I. 1-[(Chloroacyl)amino]-4-methylindan Derivatives[a]



no.	R_1	R_2	R_3	n	% yield[b]	mp, °C	formula	elemental analyses[c]
2a	Me	H	H	1	81	131–132	$C_{13}H_{16}NO_2Cl$	C, H, N
2b	*s*-Bu	H	H	1	61	112–114	$C_{16}H_{22}NO_2Cl$	C, H, N
2c	Me	CH_2Ph	H	1	76	154–155	$C_{20}H_{22}NO_2Cl$	C, H, N
2d	Me	Ph	H	1	64	123–124	$C_{19}H_{20}NO_2Cl$	C, H, N
2e	Me	*o*-MeC_6H_4	H	1	93	oil	$C_{20}H_{22}NO_2Cl$	*d*
2f	Me	*p*-ClC_6H_4	H	1	68	186–187	$C_{19}H_{19}NO_2Cl_2$	C, H, N
2g	H	Me	Me	1	63	oil	$C_{14}H_{16}NO_2Cl$	*d*
2h	Me	Me	Me	1	85	200–201	$C_{15}H_{20}NO_2Cl$	C, H, N
2i	*n*-Pr	Me	Me	1	99	oil	$C_{17}H_{24}NO_2Cl$	*d*
2j	*s*-Bu	Me	Me	1	61	158–160	$C_{16}H_{26}NO_2Cl$	C, H, N
2k	Me	Me	Me	2	78	193–194	$C_{16}H_{22}NO_2Cl$	C, H, N

[a] All compounds were recrystallized from EtOH. [b] Yields were not optimized in most cases. [c] C, H, N analyses were within ±0.4% of the calculated value. [d] This compound was isolated, but not purified or analyzed, before use in the next step; see the Experimental Section.

Scheme I



a: R_1=Me, R_2=R_3=H
b: R_1=sec-Bu, R_2=R_3=H
c: R_1=Me, R_2=Bz, R_3=H
d: R_1=Me, R_2=Ph, R_3=H
e: R_1=Me, R_2=o-MePh, R_3=H
f: R_1=Me, R_2=p-ClPh, R_3=H
g: R_1=H, R_2=R_3=Me
h: R_1=R_2=R_3=Me
i: R_1=n-Pr, R_2=R_3=Me
j: R_1=sec-But, R_2=R_3=Me

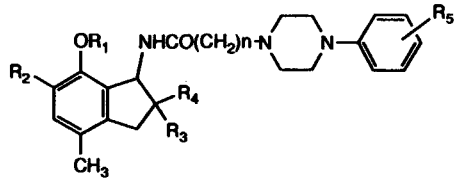
Figure 1. 1-Amino-7-hydroxyindan derivatives.

Figure 2. Structures of antioxidants and a CNS depressant and activator.

ethylamine (TEA) in chloroform gave the 1-[(chloroacetyl)amino]-7-hydroxyindans (**2a–j**) with a good yield. Reaction of compounds **2a–j** with various 1-phenylpiperazine derivatives in the presence of a base in acetonitrile afforded the 1-[[(4-phenyl-1-piperazinyl)acetyl]amino]-7-hydroxyindans (**3–39**) listed in Table II. Analogously, acylation of **1h** with 3-chloropropionyl chloride and subsequently amination with the 1-phenylpiperazines gave the 1-[[3-(4-phenyl-1-piperazinyl)propionyl]amino]-7-hydroxyindan derivatives (**40** and **41**). The 1-[[(cyclic amino)acetyl]amino]-7-hydroxyindans (**49–56**) listed in Table III were prepared by reacting **2h** with various cyclic amines by the same procedure as described for **3–39**. Reaction of **2h** with 2-pyrrolidone in the presence of sodium hydride (NaH) in dimethylformamide (DMF) gave a mixture of **56a** and intramolecular condensation product (**56b**) that was easily separated by silica gel column chromatography. Simple 1-(acylamino)-7-hydroxyindan derivatives were prepared by Shotten–Baumann reaction of **1a** with decanoyl chloride or *p*-toluoyl chloride in aqueous NaOH solution (**43** and **44**).

Scheme II shows the preparation of the 7-methoxy derivatives of compound **18** and chemical modifications of

Table II. 1-(Acylamino)indan Derivatives and Their Biological Activities[a]

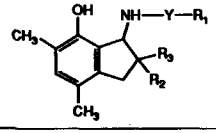


no.	R_1	R_2	R_3	R_4	R_5	n	% yield	recryst[b] solvents	mp, °C	formula[c]	protective effect: SVT[d] (n = 10)[e] ip[i]	protective effect: SVT[d] (n = 10)[e] po[j]	promotive effect RRT[f] po[j]	promotive effect SMT[g] po[j]	n[h]
3	H	Me	H	H	3-Cl	1	53	A	174–176	$C_{23}H_{28}N_3O_2Cl$·HCl	168.9 ± 25.5*,k	99.2 ± 9.9	72.1 ± 14.8	77.4 ± 15.3	10
4	H	Me	H	H	3-OMe	1	36	A	152–153	$C_{24}H_{31}N_3O_3$	nt	77.2 ± 3.6	64.2 ± 12.3*	54.7 ± 14.0	8
5	H	Me	H	H	2,3-Me_2	1	30	A	196–198	$C_{25}H_{32}N_3O_2$·HCl	134.3 ± 19.0	nt	nt	nt	
6	H	*s*-Bu	H	H	H	1	15	B	129–131	$C_{26}H_{35}N_3O_2$[l]	89.3 ± 9.2	85.9 ± 7.2	80.3 ± 8.2	78.2 ± 8.7	9
7	H	*s*-Bu	H	H	3-Me	1	22	C	106–107	$C_{27}H_{37}N_3O_2$·HCl	172.2 ± 72.7	110.5 ± 17.0	91.9 ± 10.6	125.1 ± 17.0	9
8	H	*s*-Bu	H	H	3-OMe	1	70	B	186–188	$C_{27}H_{35}N_3O_3$·2HCl	218.5 ± 57.4*	162.3 ± 21.8*	99.1 ± 17.6	103.5 ± 10.5	9
9	H	*s*-Bu	H	H	3-Cl	1	38	B	184.5–186	$C_{26}H_{34}N_3O_2Cl$·HCl	224.8 ± 86.8**	144.5 ± 11.9*	90.6 ± 14.1	81.9 ± 10.2	10
10	H	*s*-Bu	H	H	2,3-Cl_2	1	64	A	149–150	$C_{26}H_{33}N_3O_2Cl_2$	122.4 ± 11.8	119.2 ± 12.5	98.0 ± 20.0	117.4 ± 13.8	7
11	H	*s*-Bu	H	H	3,5-Cl_2	1	24	A	154–156	$C_{26}H_{33}N_3O_2Cl_2$	139.4 ± 11.7*	113.2 ± 26.3	114.0 ± 12.5	100.6 ± 9.8	9
12	H	Me	CH_2Ph	H	3-OMe	1	69	A	201–203	$C_{31}H_{37}N_3O_3$·HCl	nt	123.1 ± 19.4	nt	nt	
13	H	Me	Ph	H	3-OMe	1	84	A	143–144	$C_{31}H_{37}N_3O_3$	nt	78.3 ± 3.8	82.0 ± 15.3	80.6 ± 10.6	9
14	H	Me	2-MeC_6H_4	H	3-Cl	1	60	E	187–189	$C_{30}H_{37}N_3O_2Cl$	nt	123.7 ± 14.2	75.1 ± 16.4	66.0 ± 15.4	10
15	H	Me	4-ClC_6H_4	H	3-OMe	1	84	A	178.5–179	$C_{31}H_{37}N_3O_3Cl$[m]	nt	91.7 ± 9.2	80.1 ± 12.0	94.3 ± 13.5	9
16	H	Me	4-ClC_6H_4	H	3-Cl	1	86	A	204–205	$C_{26}H_{30}N_3O_2Cl_2$	nt	81.3 ± 9.2	98.0 ± 11.7	99.4 ± 18.6	10
17	H	Me	Me	Me	2-OMe	1	73	A	168–170	$C_{26}H_{35}N_3O_3$	nt	94.4 ± 8.9	97.2 ± 11.7	117.3 ± 16.0	10
18	H	Me	Me	Me	3-OMe	1	69	A	146–147	$C_{26}H_{35}N_3O_3$	169.1 ± 23.6*	135.4 ± 11.6*	48.3 ± 9.1**	46.0 ± 6.2**	9
19	H	Me	Me	Me	4-OMe	1	50	A	104–108	$C_{26}H_{35}N_3O_3$	nt	100.6 ± 9.1	82.3 ± 11.1	63.1 ± 6.6	8
20	H	Me	Me	Me	3,4-$(OMe)_2$	1	75	A	205–207	$C_{27}H_{37}N_3O_4$	nt	85.7 ± 10.1	59.5 ± 12.6**	81.0 ± 23.4	10
21	H	Me	Me	Me	2-OEt	1	83	A	172–174	$C_{27}H_{37}N_3O_3$	nt	130.4 ± 17.0	90.3 ± 9.0	71.8 ± 6.5	10
22	H	Me	Me	Me	2-Cl	1	76	A	176–177	$C_{25}H_{32}N_3O_2Cl$	nt	117.3 ± 6.4	86.5 ± 17.7	77.2 ± 11.0	8
23	H	Me	Me	Me	3-Cl	1	78	C	125–126	$C_{25}H_{32}N_3O_2Cl$	456.6 ± 114.0**	147.9 ± 19.1*	88.4 ± 13.5	128.1 ± 26.0	8
24	H	Me	Me	Me	2-F	1	79	A	165–166	$C_{25}H_{32}N_3O_2F$	nt	99.9 ± 9.3	89.0 ± 17.2	123.8 ± 29.2	9
25	H	Me	Me	Me	3-F	1	78	A	155–156	$C_{25}H_{32}N_3O_2F$	nt	122.7 ± 11.1	82.8 ± 15.6	72.3 ± 12.0	10
26	H	Me	Me	Me	4-F	1	39	A	166–167	$C_{25}H_{32}N_3O_2F$	nt	96.2 ± 8.3	110.5 ± 22.3	121.4 ± 17.8	10
27	H	Me	Me	Me	3-Br	1	69	A	104–108	$C_{25}H_{32}N_3O_2Br$[n]	nt	83.2 ± 3.4	81.8 ± 4.7	91.0 ± 9.9	10
28	H	Me	Me	Me	3-CF_3	1	51	D	81–84	$C_{26}H_{32}N_3O_2F_3$	nt	107.8 ± 10.0	89.5 ± 10.5	83.7 ± 11.0	10
29	H	Me	Me	Me	4-NO_2	1	75	A	201–202	$C_{25}H_{32}N_4O_4$	nt	107.9 ± 18.5	99.5 ± 12.3	90.6 ± 10.6	9
30	H	Me	Me	Me	2,3-Cl_2	1	86	A	178–179	$C_{25}H_{31}N_3O_2Cl_2$	nt	94.6 ± 10.6	99.2 ± 9.6	126.6 ± 28.3	7
31	H	Me	Me	Me	2,5-Cl_2	1	71	A	197–198.5	$C_{25}H_{31}N_3O_2Cl_2$	nt	100.0 ± 15.2	73.6 ± 16.9	69.4 ± 14.5	10
32	H	Me	Me	Me	3,5-Cl_2	1	72	A	125–126	$C_{25}H_{31}N_3O_2Cl_2$	nt	106.4 ± 9.2	76.1 ± 7.8	71.1 ± 14.0	9
33	H	Me	Me	Me	3,4-Cl_2	1	72	A	177–178	$C_{25}H_{31}N_3O_2Cl_2$	nt	100.1 ± 9.8	93.2 ± 8.6	108.2 ± 13.4	9
34	H	Me	Me	Me	3-Cl,4-Me	1	64	A	160–161.5	$C_{26}H_{34}N_3O_2Cl$	nt	90.5 ± 9.6	103.9 ± 20.8	117.7 ± 25.0	10
35	H	Me	Me	Me	2-Me,5-Cl	1	69	A	187–188	$C_{26}H_{34}N_3O_2Cl$	nt	103.2 ± 10.2	91.7 ± 15.2	74.0 ± 11.0	9
36	H	Me	Me	Me	2,4,6-Me_3	1	86	A	210–211	$C_{28}H_{39}N_3O_2$	nt	100.3 ± 39.6	87.6 ± 17.2	84.0 ± 13.9	7
37	H	H	Me	Me	3-OMe	1	78	A	167–168	$C_{25}H_{33}N_3O_3$	nt	97.2 ± 7.1	62.9 ± 7.6*	64.7 ± 7.5*	9
38	H	*n*-Pr	Me	Me	3-OMe	1	30	A	135–137	$C_{28}H_{29}N_3O_3$	nt	95.0 ± 12.5	114.6 ± 9.0	107.2 ± 13.8	10

39	H	*s*-Bu	Me	Me	3-Cl	1	88	A	130–131	$C_{28}H_{39}N_3O_2Cl$	147.7 ± 10.0*	nt	99.4 ± 12.1	80.3 ± 8.2	10
40	H	Me	Me	Me	3-Cl	2	86	A	157–158	$C_{26}H_{34}N_3O_2Cl$	79.5 ± 6.4	nt	95.4 ± 12.1	74.0 ± 9.7	9
41	H	Me	Me	Me	3-OMe	2	80	A	152–153	$C_{27}H_{37}N_3O_3$	nt	91.8 ± 7.7	98.0 ± 19.4	92.4 ± 25.0	10
42	Me	Me	Me	Me	3-OMe	1	72	A	142–143	$C_{27}H_{37}N_3O_3$	nt	93.4 ± 9.4	72.6 ± 9.4	61.8 ± 8.1	10
1b											276.5 ± 88.1**[o]	166.5 ± 12.3**	44.2 ± 2.9**	48.9 ± 15.3**	10
1d											185.7 ± 26.5*	114.1 ± 7.6	34.3 ± 8.9**	28.9 ± 6.4**	9
1h											298.2 ± 90.8**[o]	142.1 ± 9.8**	37.2 ± 12.3**	42.0 ± 10.0**	9
2a											116.4 ± 12.4	nt	nt	nt	
vitamin E											96.7 ± 12.7[k]	178.1 ± 34.6[k]	92.2 ± 8.9[k]	83.5 ± 9.2[k]	10
BHT											358.8 ± 37.1**[k]	87.6 ± 14.6[k]	69.6 ± 15.2[k]	80.9 ± 15.0[k]	10
pentobarbital sodium											306.7 ± 46.0**[p]	nt	494.0 ± 0.0**[p]	283.0 ± 0.0**[p]	5
TRH											43.4 ± 2.0**[s]	nt	40.8 ± 7.2**[r]	40.9 ± 9.3**[r]	10

[a] All values are represented as percent of control ± standard error (SEM); significance determined by Wilcoxon sum test, $*p < 0.05$, $**p < 0.01$, nt = not tested. [b] A = EtOH, B = acetone, C = hexane, D = EtOH/ether, E = hexane/AcOEt. [c] C, H, N analyses were within ±0.4% of the calculated value. [d] Survival time. [e] Numbers of animals used in the test. [f] Time for recovery of righting reflex (in seconds). [g] Time for recovery of spontaneous movement (in seconds). [h] Numbers of mice used. [i] 30 mg/kg ip unless otherwise indicated. [j] 30 mg/kg po unless otherwise indicated. [k] 100 mg/kg. [l] H: calcd, 8.37; found, 8.78. [m] C: calcd, 69.28; found, 69.77. [n] C: calcd, 61.73; found, 62.47. [o] 30 mg/kg sc. [p] 40 mg/kg ip. [q] 3 mg/kg ip. [r] 2.5 mg/kg iv.

Table III. 1-Amino-4,6-dimethylindan Derivatives and Their Biological Activities[a]



no.	Y	R	R_2	R_3	% yield	recryst[b] solvents	mp, °C	formula[c]	protective effect: SVT[d] (n = 10)[e] ip[i]	protective effect: SVT[d] (n = 10)[e] po[j]	promotive effect RRT[f] po[j]	promotive effect SMT[g] po[j]	n[h]
43	$-COCH_2-$	$(CH_2)_8CH_3$	H	H	48	B	103–104	$C_{21}H_{33}NO_2$	94.5 ± 8.9	nt	nt	nt	
44	$-CO-$	*p*-MeC_6H_4	H	H	48	C	230–232	$C_{19}H_{21}NO_2$	68.8 ± 8.5	nt	nt	nt	
45	$-CH_2CH_2-$	$N(CH_2CH_2)_2N$·(3-$OMeC_6H_4$)	Me	Me	20	A	122–125	$C_{28}H_{37}N_3O_2$·C_4H_4O·$0.5H_2O$[k]	120.8 ± 15.0	nt	110.0 ± 17.3	112.1 ± 19.6	9
46	$-CH_2CH_2-$	$N(CH_2CH_2)_2N$·(3-ClC_6H_4)	Me	Me	38	A	144–146	$C_{26}H_{34}N_3OCl$·$0.5C_4H_4O_4$·$0.75H_2O$	141.3 ± 17.0	nt	64.8 ± 19.5	59.9 ± 12.9	6
47	$-CH_2CO-$	$N(CH_2CH_2)_2N$·(3-ClC_6H_4)	Me	Me	21	A	144–145.5	$C_{26}H_{32}N_3O_2Cl$	146.8 ± 21.6	nt	116.3 ± 7.2	95.2 ± 7.0	7
48	$-CH_2CO-$	$N(CH_2CH_2)_2N$·(3-$OMeC_6H_4$)	Me	Me	23	A	164.5–166	$C_{26}H_{35}N_3O_3$	97.7 ± 11.3	nt	84.0 ± 7.8	111.0 ± 17.0	10
49	$-COCH_2-$	$N(CH_2CH_2)_2NCH_3$	Me	Me	54	A	189–190	$C_{20}H_{31}N_3O_2$	145.6 ± 15.6*	86.4 ± 9.0	54.9 ± 5.0**	43.1 ± 6.3**	9
50	$-COCH_2-$	$N(CH_2CH_2)_2O$	Me	Me	85	A	168–169	$C_{19}H_{28}N_3O_3$	104.3 ± 11.5	nt	106.9 ± 32.7	92.2 ± 19.1	7
51	$-COCH_2-$	$N(CH_2CH_2)_2S$	Me	Me	80	A	198–199	$C_{19}H_{28}N_3O_2S$	100.8 ± 10.0	nt	110.8 ± 11.0	119.9 ± 14.9	10
52	$-COCH_2-$	$N(CH_2CH_2)_2CH_2$	Me	Me	68	A	147.5–148	$C_{20}H_{28}N_3O_2$	112.7 ± 21.5	nt	100.7 ± 13.0	84.2 ± 8.0	9
53	$-COCH_2-$	$N(CH_2CH_2)_2$	Me	Me	81	A	127–128	$C_{19}H_{28}N_3O_2$	134.2 ± 17.1*	nt	85.8 ± 12.0	66.4 ± 9.0	10
54	$-COCH_2-$	$NCH(COOH)CH_2CH_2CH_2$	Me	Me	17	A	>211 (dec)	$C_{20}H_{28}N_2O_4$	nt	92.3 ± 40.1	103.3 ± 13.0	150.3 ± 26.2	9
55	$-COCH_2-$	$C_3H_3N_2$[i]	Me	Me	67	A	242 (dec)	$C_{19}H_{28}N_3O_2$·$0.1H_2O$	nt	71.8 ± 12.6	76.8 ± 11.1	66.4 ± 7.2	9
56a	$-COCH_2-$	$NCOCH_2CH_2CH_2$	Me	Me	49	A	165–168	$C_{19}H_{28}N_2O_2$	135.3 ± 16.3*	nt	89.2 ± 16.0	88.8 ± 16.4	5

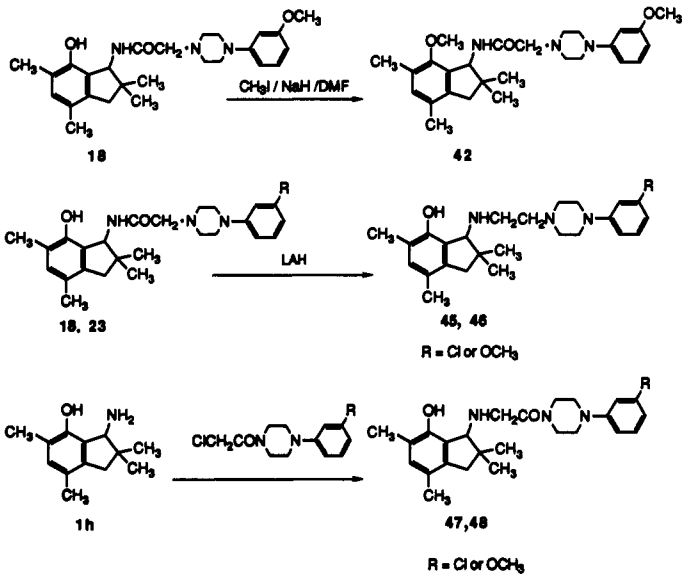
[a–j] See footnotes in Table II. [b] A = EtOH, B = hexane, C = ether/hexane. [k] $C_4H_4O_4$ represents fumaric acid. [i] $C_3H_3N_2$ represents imidazolyl group.

Table IV. Pharmacological Activities of Selected Compounds and Reference Drugs[a]

compd	effect: SVT dose, mg/kg ip	SVT (n = 10)	effect: RRT and SMT dose, mg/kg		RRT	SMT (n)[b]	effect: anesthetic dose, mg/kg		sleeping time
1h	3	156.2 ± 24.6*	po	1	62.8 ± 16.3	92.3 ± 27.5 (8)	sc	3	112.0 ± 12.0
	10	217.0 ± 69.0		3	66.2 ± 16.7	105.8 ± 34.0 (10)		10	82.1 ± 9.6
	30	298.1 ± 90.5*		10	54.8 ± 12.3*	62.5 ± 12.0* (9)		30	76.1 ± 3.4*
18	3	153.5 ± 34.5	po	0.3	72.7 ± 8.4	66.5 ± 7.5* (10)	po	1	92.7 ± 3.6
	10	176.4 ± 24.0*		1	58.0 ± 9.5*	56.2 ± 9.8* (8)		3	84.8 ± 5.0
	30	206.7 ± 33.1**		3	43.6 ± 8.5**	45.3 ± 8.7** (10)		10	71.4 ± 6.5*
49	3	93.0 ± 6.3	po	10	61.2 ± 10.1	60.9 ± 7.5 (10)	ip	3	92.7 ± 8.9
	10	92.5 ± 5.0		30	45.7 ± 10.3*	49.8 ± 6.2* (9)		10	78.8 ± 6.9
	30	145.2 ± 8.1*		100	25.8 ± 5.4**	36.2 ± 2.8* (7)		30	84.3 ± 9.3
barbital Na[d]	10	134.9 ± 25.0	ip	10	175.5 ± 39.8	176.3 ± 29.1* (5)	ip	5	133*[c]
	20	218.5 ± 21.8**		30	494 ± 0**	283 ± 0** (5)			
	40	443.6 ± 76.2**		100	494 ± 0**	283 ± 0** (5)			
TRH	0.3	83.8 ± 12.8	iv	0.625	100.1 ± 24.7	118.2 ± 21.0 (10)	ip	0.3	83.1 ± 8.8
	1	47.1 ± 5.0*		1.25	34.1 ± 7.3**	48.2 ± 8.3* (10)		1	70.7 ± 1.8**
	3	43.4 ± 2.0**		2.5	40.8 ± 7.2**	40.9 ± 7.3** (10)		3	73.2 ± 2.2**
BHT	10	140.3 ± 27.6	ip	10	96.4 ± 12.4	110.4 ± 19.2 (10)			
	30	109.4 ± 13.9		30	74.5 ± 8.4	114.7 ± 26.1 (10)		nt	
	100	583.9 ± 99.1*		100	98.7 ± 15.8	115.7 ± 23.4 (10)			

[a] See footnotes in Table II. [b] Numbers of mice used in this test. [c] From ref 13. [d] Pentobarbital sodium.

Scheme II



the bridge portions in compounds 18 and 23. Methylation of 18 with methyl iodide in the presence of NaH in DMF gave 7-methoxy derivative 42. In order to examine the effect of the bridge portion in compound 18, compounds 45–48 listed in Table II were prepared. Reduction of the 1-[[(4-phenyl-1-piperazinyl)acetyl]amino]-7-hydroxyindan derivatives with lithium aluminum hydride gave 1-[[2-(4-phenyl-1-piperazinyl)ethyl]amino]-7-hydroxyindans (45 and 46). Reaction of 1h with the 1-(chloroacetyl)-4-phenylpiperazines in the presence of TEA in acetonitrile gave the 1-[[(7-hydroxy-1-indanyl)amino]acetyl]-4-phenylpiperazine derivatives in low yield (47 and 48). The typical procedures for the preparation of these compounds are described in the Experimental Section.

Pharmacology

The test for antihypoxic effect and the test for promotional effect on recovery from coma were used as the primary screens. In the test for antihypoxic effect, the cerebroprotective effect of a series of 1-(acylamino)indan derivatives prepared was examined for its effect on the survival time of mice with cerebral hypoxia under low-pressure oxygen (210 mmHg) loading. The CNS-stimulating effect of the compound was evaluated in terms of its promoting effect on the recovery of mice from coma induced by cerebral concussion. The reference drugs pentobarbital sodium, TRH, vitamin E, and BHT were also examined. The results are summarized in Tables II and III.

The CNS-stimulating activity of the selected compound was confirmed by evaluating its effects on the sleeping time of mice anesthetized with halothane.[16] The dose dependence of the activities of the selected compounds in those tests was also examined and the results are summarized in Table IV.

Furthermore, the selected compound and two antioxidants were tested for their effects on the formation of lipid peroxide in rat brain homogenates.[17]

Results and Structure–Activity Relationships

Acyl derivatives of 1-amino-4,6-dimethyl-7-hydroxyindans 2a, 3, 5, 43, and 44 were initially examined for their cerebroprotective activities in the test for antihypoxic effect and for CNS-stimulating activities in the test for promotional effect on recovery from coma. Among them, only compound 3 with [(4-phenyl-1-piperazinyl)acetyl]amino moiety at the 1-position prolonged survival of the mice, indicating its cerebroprotective effect against hypoxia when administered by ip injection. The compound did not show the activity in the test for promoting effect on recovery from coma. In order to find our target compound, various modifications in the substituents were carried out on 3.

Replacement of the chlorine substituent on the phenylpiperazinyl moiety in 3 with a methoxy group revealed the activity in the test for promotional effect (4). However, 4 was inactive in the test for antihypoxic effect. Replacement of the methyl substituent on the indan nucleus in 3 with a more bulky *sec*-butyl group enhanced the potency in the cerebroprotection (9).

Several substituent effects in the phenylpiperazinyl moiety in the 6-*sec*-butylindan series on the protective effect were examined. Introduction of another chlorine substituent to the 2- or 5-position in the 4-(3-chlorophenyl)-1-piperazinyl moiety in compound 9 reduced the potency (10 and 11) and replacement of the chlorine

(16) Turnbull, M. J.; Watkins, J. W. *Br. J. Pharmacol.* **1976**, *58*, 27.
(17) Stockes, J.; Gutterdge, J. M. C.; Dormandy, T. L. *Clin. Sci. Mol. Med.* **1974**, *47*, 215.

substituent at the 3-position on the phenylpiperazinyl moiety in **9** with a more electron-donating methoxy group did not change the potency (**8**). The compound with no substituted phenylpiperazinyl moiety did not show any activity in the test for antihypoxic effect (**6**).

In the test for promoting effect on recovery from coma, none of the compounds were found to be active in this 6-*sec*-butylindan series.

The effects of substitutions in the 2-position of compounds **3** and **4** were examined. Introduction of a single benzyl and phenyl substituent to the 2-position in **3** and **4**, respectively, did not render any orally active compounds (**12–16**). Introduction of two methyl substituent to the 2-position in **4** resulted in our target compound showing the activity in both tests (**18**). Further structure–activity relationships were carried out on the 2,2-dimethyl-substituted indan series with the data shown in Tables II and III.

Test for Antihypoxic Effect

First, the positional effect of the methoxy group in the phenylpiperazinyl moiety in compound **18** was examined, and it was found that change in the position of the methoxy group on the phenylpiperazinyl moiety in **18** from the 3-position to the 2- and 4-positions gave inactive compounds (**17** and **19**). Incorporation of another methoxy substituent at the 4-position on the phenylpiperazinyl moiety in **18** also gave an inactive compound (**20**).

The effects of replacement of the methoxy group with various functional substituents on the phenylpiperazinyl moiety in **18** were then examined. Replacement of the methoxy group with a chlorine substituent at the 3-position on the phenylpiperazinyl moiety in the compound was found to greatly increase the potency (**23**), and compound **23** showed more potent cerebroprotection that sodium pentobarbital when administered by ip injection. The compound was also active by oral administration. Change in the position of the chlorine substituent on the phenylpiperazinyl moiety in compound **23** from position 3 to 2 (**22**) and replacement of the chlorine substituent with a more electron-negative group, such as a fluorine (**24–26**) or trifluoromethyl group (**28**), and with a more bulky bromine substituent (**27**) gave inactive compounds. Introduction of an additional chlorine or methyl group to the 2-, 4-, and 5-position on the 4-(3-chlorophenyl)-1-piperazinyl moiety in **23** also gave inactive compounds (**30–33**, or **34** and **35**).

The effects of alkyl substitution of the indan nucleus in compounds **18** and **23** were then examined. The 6-unsubstituted compound was inactive (**37**). Replacement of the methyl group at the 6-position in **18** and **23** with a more bulky propyl and *sec*-butyl substituent respectively diminished the potency (**38** and **39**).

The effect of the hydroxyl group at the 7-position in **18** was next examined, and it was found that replacement of the hydroxyl group with a methoxy moiety gave an inactive compound (**42**).

The effects of modifications of the bridge portion between the phenylpiperazinyl moiety and indan nucleus in **18** and **23** were then examined, and it was found that replacement of the acetyl with a propionyl moiety in the bridge portion gave inactive compounds (**40** and **41**). The compounds with ethyl (**45** or **46**) or carbonyl methyl moiety (**47** or **48**) in the bridge portion were also inactive in this test.

Finally, the effect of replacement of the phenylpiperazinyl moiety in **18** with various cyclic amino and cyclic amide moieties was examined. In this series, a few compounds with a 4-methyl-1-piperazinyl (**49**), pyrrolidinyl (**53**), and 2-pyrrolidon-1-yl moiety (**56a**) showed activity with low potency only when administered by ip injection, whereas the compounds with other cyclic amino and cyclic amide moiety were inactive in the test. No compound showing higher potency than that of compound **18** was found in this series. Findings for the structure–activity relationships in the test for antihypoxic effect were (1) several compounds having a phenylpiperazinyl moiety with a chlorine or methoxy substituent showed higher potency than that of the compounds with cyclic amino or amide moiety, and the chlorine or methoxy substituent should be located at the 3-position on the phenylpiperazinyl moiety, (2) the hydroxyl group at the 7-position in the indan nucleus was necessary, (3) acetyl moiety in the bridge portion was required to possess the activity.

Activity in the Test for Promotional Effect on Recovery from Coma

Compound **18** showed promotional effect equipotent to that of its parent compound **1h**. The substituent effect on the phenylpiperazinyl moiety and on the indan nucleus in compound **18** was examined, and then the effect of the bridge portion between phenylpiperazinyl moiety and the indan nucleus in compounds **18** and **23** was examined. Lastly, the effect of the hydroxyl group at the 7-position in compound **18** was examined. We found that minor changes in the structure in this series greatly diminished the potency or gave inactive compounds, and we found strict structure requirements for possession of the activity. Findings for the structure–activity relationships on the promotional effect in the series of compounds were (1) the methoxy substituent on the phenylpiperazinyl moiety was required for possession of the activity, and it should be located at the 3-position on the phenylpiperazinyl moiety; (2) with respect to the substituent located at the 6-position, the compound with methyl substituent showed the highest potency and the compound with no substitution was less potent; the compound with *n*-propyl substituent was inactive in this test; (3) dimethyl substitution at the 2-position in the indan nucleus was preferable to no substitution and to benzyl or phenyl substitution; (4) only the compound with acetyl moiety in the bridge portion showed activity, whereas other compounds with a propionyl, ethyl, and carbonylmethyl moiety were inactive; and (5) a hydroxyl group at the 7-position in compound **18** was necessary.

After the structure–activity studies, compounds **18** and **49**, which possessed the dual activities in the two tests, were selected for further investigations.

The dose-dependent effect of the two compounds on the CNS-stimulating activity and cerebroprotection was examined in comparison with that of the parent compound **1h**, pentobarbital sodium, TRH, and BHT, as shown in Table IV. Table IV also shows the effect of the compounds on the sleeping time of mice anesthetized with halothane. It was reported that the sleeping time was directly related to the sensitivity of the CNS to the anesthetic. The typical CNS stimulator amphetamine was reported to shorten the sleeping time, whereas the CNS depressant pentobarbital sodium prolonged the sleeping time.[16] As shown in Table IV, the CNS activator TRH and compound **18** were found to shorten sleeping time induced by halothane in a dose-dependent manner, whereas compound **49** did not change the sleeping time at doses up to 30 mg/kg. The CNS-stimulating activity of **18** was thus confirmed by this experiment.

The compounds were also investigated in toxicological studies. Each compound was administered orally to rats for 2 weeks (10, 30, 150, and 500 mg/kg daily), and the

general symptoms and mortality were examined. All rats examined were alive after 2 weeks of administration for 18. However, one of six rats was dead with 49 at a dose of 500 mg/kg day after 2 weeks. No general symptoms were observed for 18 even at maximal dosage for two weeks, whereas administration of 49 caused salivation and emaciation at a dose of 500 mg/kg day. Compound 18 was found to have less side effects than compound 49.

Compound 18 was examined for its effect on lipid peroxidation in rat brain homogenates. Lipid peroxide (LPO) produced in the brain homogenates was determined according to the method reported.[17-19] When rat brain homogenates were incubated at 37 °C, the level of malondialdehyde (MDA), a degradation product of LPO, increased time dependently. The compound inhibited the production of LPO in a concentration-dependent manner at 1–100 μM. Complete inhibition was observed at a concentration of 100 μM. The concentration giving 50% inhibition (IC_{50}) was estimated to be 10.7 μM. Vitamin E and BHT also inhibited the formation of LPO in this assay, and the IC_{50} was estimated to be 200 and 2.5 μM, respectively. The results in this experiment were fairly compatible with those in the test for antihypoxic effect (Table II) and suggested that the mechanism of the cerebroprotection and the brain function activating effects of the compound might be attributable to its potent inhibition of the formation of lipid peroxidation and to its radical-scavenging activity.

The radical scavenging activity of 18 was also confirmed by an electron spin resonance (ESR) study using potassium super anion radical in the presence of 18-crown-6 in dimethyl sulfoxide.[20]

Conclusion

In a search for a novel cerebroprotective drug with CNS-stimulating activity, a series of 1-(acylamino)-7-hydroxyindan derivatives was synthesized and tested for its dual activities in the two tests described in this paper. After examination of the structure–activity relationships, only compound 18 in this series showed the dual activities. The compound also showed an antilipid peroxidative activity with a higher potency than that of vitamin E. Compound 18 (OPC-14117) was selected for further preclinical investigations because of its higher potency in the three pharmacological examinations in this paper and its higher safety margin.

In the preclinical studies, the compound was examined to evaluate the potential clinical effects on impaired brain function resulting from stroke and cerebral trauma, and its brain function stimulating activity and cerebroprotection were confirmed. The compound showed brain function stimulating activity in the effect of accelerating awakening of mice from coma induced by cerebral concussion (0.3–100 mg/kg po), by loading of 100% nitrogen gas (3 mg/kg po or higher dosage), or by loading of potassium cyanide (3 and 10 mg/kg) (unpublished result). The compound (10 mg/kg or higher dosage) shortened the halothane-induced sleeping time in mice and rats.

The compound (10 or 30 mg/kg po) in repeated administration improved neurological signs, abnormal electroencephalograph (EEG) patterns, and learning and memory impairment in rats with cerebral infarction induced by microsphere injection. The compound (60 mg/kg po) in repeated administration improved neurological signs, learning, and memory impairments in rats with occluded left middle cerebral artery and improved cerebral metabolic disturbance induced by bilateral ligation of the common carotid arteries in stroke-prone spontaneous hypertensive rats (unpublished result).

The mechanism of action of compound 18 in these cerebroprotection and brain function activating effects has not been clarified, but we believe one mechanism of action might be attributable to its potent antilipid peroxidative activity and radical scavenging activity.

The pharmacological results and the mechanism of action will be published separately.

Experimental Section

Melting points were determined by a Yanagimoto Micro Melting Point Apparatus and were uncorrected. ^{1}H-NMR spectra were recorded on a Varian EM 390 NMR Spectrometer using teteramethylsilane (TMS) or 3-(trimethylsilyl)propionic acid-d_5 (TSP) as an internal standard. Elemental analyses for carbon, hydrogen and nitrogen were carried out on a Yanaco MT-5 CHN Corder. Where analyses are indicated only a symbols of elements, the obtained analytical results are within ±0.4% of the theoretical value. All compounds were routinely checked by TLC with Merck Silica Gel 60 F254 Precoated Plates.

Preparation of the 1-[(Chloroalkanoyl)amino]-7-hydroxyindans Listed in Table I. 1-[(Chloroacetyl)amino]-4,6-dimethyl-7-hydroxyindan (2a). To a solution of **1a** (3.54 g, 20 mmol) and triethylamine (TEA) (3.5 mL, 25 mmol) in chloroform (100 mL) was added chloroacetyl chloride (2.82 g, 25 mmol) in drops at ice-cooled temperature. After 2 h of stirring, the mixture was washed (diluted HCl and water), dried (anhydrous $MgSO_4$), and evaporated to dryness. Recrystallization from EtOH gave **2a** (4.1 g, 81%) as colorless needles. Mp: 131–132 °C. ^{1}H NMR ($CDCl_3$): δ 2.15 (3 H, s, CH_3), 2.20 (3 H, s, CH_3), 2.00–3.20 (4 H, m, 2,3-H), 4.10 (2 H, s, CH_2), 5.15–5.40 (1 H, m, 1-H), 6.85 (1 H, s, 5-H), 6.80–7.30 (1 H, br, NH), 8.40 (1 H, s, OH). Anal. ($C_{13}H_{16}NO_2Cl$): C, H, N.

Analogously, compounds **2b–d,f,h–j** were prepared starting from **1b–d,f,h–j**, respectively.

***cis*-1-[(Chloroacetyl)amino]-4,6-dimethyl-7-hydroxy-2-(2-methylphenyl)indan (2e).** This compound was prepared in a manner similar to that of **2a** by reacting **cis**-1-amino-4,6-dimethyl-7-hydroxy-(2-methylphenyl)indan (**1e**) (0.53 g, 2 mmol) with chloroacetyl chloride (0.71 g, 6.3 mmol) in the presence of TEA (1 mL, 7 mmol) in chloroform (10 mL). The compound was obtained as an oil, and it was used directly in the next step without further purifications. Yield: 5.7 g (93%). ^{1}H NMR ($CDCl_3$): δ 2.20 (6 H, s, CH_3), 2.33 (3 H, s, CH_3), 3.00 (1 H, dd, J = 15 Hz, 7.5 Hz, 2-H), 3.23–4.40 (4 H, m, CH_2 and 3-H), 5.20 (1 H, t, J = 7.5 Hz, 1-H), 6.70–7.55 (6 H, m, aromatic H, and NH), 8.83 (1 H, br, OH).

1-[(Chloroacetyl)amino]-7-hydroxy-2,2,4-trimethylindan (2g). This compound was obtained as an oily product by a manner similar to that of **2a** by reacting 1-amino-7-hydroxy-2,2,4-trimethylindan (6.3 g, 33 mmol) with chloroacetyl chloride (4.96 g, 44 mmol) in the presence of TEA (12.5 mL), and it was used in the next step without further purifications. Yield: 5.7 g (63%). ^{1}H NMR ($CDCl_3$): δ 1.11 (3 H, s, CH_3), 1.30 (3 H, s, CH_3), 2.15 (3 H, s, CH_3), 2.60 (1 H, d, J = 15 Hz, 3-H), 2.85 (1 H, d, J = 15 Hz, 3-H), 4.10 (2 H, s, CH_2), 4.75 (1 H, d. J = 7.5 Hz, 1-H), 6.60 (1 H, d, J = 9 Hz. 6-H), 6.95 (1 H, d, J = 9 Hz, 5-H), 7.20 (1 H, br d, J = 7.5 Hz, NH), 8.20 (1 H, br, OH).

1-[(3-Chloropropionyl)amino]-7-hydroxy-2,2,4,6-tetramethylindan (2k). To a solution of **1h** (2.08 g, 10 mmol) and TEA (2.86 mL, 20 mmol) in chloroform (100 mL) 3-chloropropionyl chloride (1.30 g, 10 mmol) was added in drops at an ice-cooled temperature. After 2 h of stirring, the mixture was washed, dried, and evaporated to dryness. Recrystallization from EtOH gave **2k** (2.30 g, 78%) as colorless needles. Mp: 193–194 °C. ^{1}H NMR ($CDCl_3$): δ 1.14 (3 H, s, CH_3). 1.27 (3 H, s, CH_3), 2.11 (3 H, s, CH_3), 2.19 (3 H, s, CH_3), 2.60 (1 H, d, J = 15 Hz, 3-H), 2.75 (1 H, d, J = 15 Hz, 3-H), 2.60–2.73 (2 H, m, CH_2), 3.70–3.90 (2 H, m, CH_2), 4.80 (1 H, d, J = 6 Hz, 1-H), 6.20–6.40 (1 H, br, NH), 6.83 (1 H, s, 5-H), 8.30 (1 H, s, OH). Anal. ($C_{16}H_{22}NO_2Cl$): C, H, N.

(18) Suno, S.; Nagaoka, A. *Jpn. J. Pharmacol.* **1984**, *35*, 196.
(19) Ohkawa, H.; Ohishi, N.; Yagi, K. *Anal. Biochem.* **1979**, *95*, 351.
(20) Jinno, J.; Mori, H.; Sakurai, H. 12th Annual Meeting of Magnetic Resonance in Medicine; May 1990, Tokyo, Japan.

Preparation of the 1-[[(4-Phenyl-1-piperazinyl)acetyl]amino]-7-hydroxyindans Listed in Table II. 1-[[[4-(3-Chlorophenyl)-1-piperazinyl]acetyl]amino]-4,6-dimethyl-7-hydroxyindan Hydrochloride (3). A solution of **2a** (1.52 g, 6 mmol) and 1-(3-chlorophenyl)piperazine hydrochloride (1.97 g, 10 mmol) and TEA (2.8 mL, 20 mmol) in acetonitrile (50 mL) was refluxed for 2 h and then evaporated to dryness. The residue was dissolved in chloroform (200 mL), and insoluble materials were filtered off. The filtrate was concentrated under reduced pressure and passed through a silica gel column (eluent: chloroform/MeOH 10:1). The second eluates were evaporated and the residue was dissolved in EtOH. The EtOH solution was acidified by addition of a HCl gas saturated EtOH solution and then evaporated. Recrystallization from EtOH gave **3** (2.38 g, 53%) as a colorless powder. Mp: 174–176 °C. ^{1}H NMR ($CDCl_3$): δ 2.10 (3 H, s, CH_3), 2.15 (3 H, s, CH_3), 2.00–3.30 (4 H, m, 2,3-H), 3.30–3.80 (8 H, m), 3.90–4.15 (2 H, m, CH_2), 5.00–5.30 (1 H, m, 1-H), 6.60–7.20 (5 H, m, aromatic H), 8.30–8.60 (1 H, br, NH), 9.60–9.90 (1 H, br, OH). Anal. ($C_{23}H_{28}N_3O_2Cl$·HCl): C, H, N.

Analogously, compounds 4–11 were prepared by reacting **2a** or **2b** with the corresponding phenylpiperazine derivatives.

***cis*-2-Benzyl-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-4,6-dimethyl-7-hydroxyindan Hydrochloride (12).** This compound was prepared in a manner similar to that of **3**, starting from *cis*-1-[(chloroacetyl)amino]-2-benzyl-4,6-dimethyl-7-hydroxyindan (**2c**) (1 g, 2.9 mmol), 1-(3-methoxyphenyl)piperazine (0.67 g, 3.48 mmol), and TEA (0.58 mL, 4 mmol). Recrystallization from EtOH gave a colorless powder. Yield: 1.07 g (69%). Mp: 201–203 °C. ^{1}H NMR (DMSO-d_6): δ 2.06 (3 H, s, CH_3), 2.12 (3 H, s, CH_3), 2.40 (1 H, d, J = 17.5 Hz, 3-H), 2.56–3.01 (4 H, m), 3.10–3.70 (12 H, m), 3.74 (3 H, s, OCH_3), 3.75–3.92 (2 H, br, CH_2), 3.95–4.20 (2 H, br, CH_2), 4.98–5.02 (1 H, m, 1-H), 6.44–6.60 (3 H, m, aromatic H), 6.83 (1 H, s, 5-H), 7.14–7.37 (6 H, m, aromatic H), 8.47 (1 H, br, OH), 9.53 (1 H, br d, 7.5 Hz, NH), 10.50 (1 H, br, NH^+). Anal. ($C_{31}H_{37}N_3O_3$·HCl·0.5H_2O): C, H, N.

1-[[[4-(3-Chlorophenyl)-1-piperazinyl]acetyl]amino]-4,6-dimethyl-7-hydroxy-2-(2-methylphenyl)indan (14). A mixture of crude **2e** (0.56 g, 1.6 mmol), 1-(3-chlorophenyl)piperazine (1 g, 5 mmol), and TEA (1 mL, 7 mmol) in acetonitrile (10 mL) was refluxed for 2 h and then cooled to room temperature. The mixture was filtered and evaporated under reduced pressure to give crude **14**, which was purified by passing through a silica gel column (eluent: *n*-hexane/AcOEt 10:1). Recrystallization from AcOEt/*n*-hexane gave **14** (0.49 g, 60%) as colorless needles. Mp: 187–189 °C. ^{1}H NMR ($CDCl_3$): δ 1.95–2.50 (4 H, m, CH_2), 2.21 (6 H, s, CH_3), 2.50–3.20 (6 H, m, CH_2), 3.50 (1 H, dd, J = 15 Hz, 7.5 Hz, 3-H), 3.90–4.27 (2 H, m, 2 and 3-H), 5.45 (1 H, t, J = 7.5 Hz, 1-H), 6.55–7.60 (10 H, m, aromatic H, and NH), 9.30 (1 H, s, OH). Anal. ($C_{30}H_{37}N_3O_2Cl$): C, H, N.

Analogously, compounds **13**, **15**, and **16** were prepared by reacting **2d** or **2f** with the corresponding 1-phenylpiperazine derivative.

7-Hydroxy-1-[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]-2,2,4,6-tetramethylindan (18). A solution of **2h** (4 g, 14 mmol) and 1-(3-methoxyphenyl)piperazine (5.46 g, 0.028 mol) in acetonitrile (60 mL) was refluxed for 3 h and then worked up by using a procedure similar to that for **3**. Recrystallization from EtOH gave **18** (4.26 g, 69%) as colorless granulars. Mp: 143–147 °C. ^{1}H NMR ($CDCl_3$): δ 1.12 (3 H, s, CH_3), 1.23 (3 H, s, CH_3), 2.03 (3 H, s, CH_3), 2.10 (3 H, s, CH_3), 2.18 (3 H, s, CH_3), 2.45–2.93 (6 H, m), 3.16 (6 H, t, J = 4.5 Hz), 3.77 (3 H, s, OCH_3), 4.69 (1 H, d, J = 7.5 Hz, 1-H), 6.32–6.57 (3 H, m), 6.80 (1 H, s, 5-H), 7.05–7.23 (1 H, m), 7.90–8.07 (1 H, m), 8.04 (1 H, br, OH). Anal. ($C_{28}H_{35}N_3O_3$): C, H, N.

Analogously, compounds **17** and **19–36** were prepared by reacting **2h** with the corresponding phenylpiperazine derivatives.

7-Hydroxy-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-2,2,4-trimethylindan Hemifumarate (37). This compound was prepared in a manner similar to that of **18**, starting from crude **2g** (2.0 g, 7.5 mmol) and 1-(3-methoxyphenyl)-piperazine (2.9 g, 15 mmol) in acetonitrile (50 mL) and was converted its fumarate by addition of a ethanol solution of fumaric acid. Recrystallization from EtOH gave **37** (2.47 g, 78%) as a colorless powder. Mp: 167–168 °C. ^{1}H NMR ($CDCl_3$): δ 1.10 (6 H, s, CH_3), 2.10 (3 H, s, CH_3), 2.50–2.90 (8 H, m, CH_2, and 3-H), 3.00–3.35 (4 H, m, CH_2), 3.75 (3 H, s, OCH_3), 4.85 (1 H, d, J = 7.5 Hz, 1-H), 6.30–6.60 (4 H, m, aromatic H, and CH=CH), 6.85 (1 H, d, J = 9 Hz, 5-H), 7.10 (1 H, t, J = 9 Hz, aromatic H), 7.75 (1 H, d, J = 9 Hz, aromatic H), 9.20–11.00 (3 H, br, NH_2^+ and OH). Anal. ($C_{25}H_{33}N_3O_3$·0.5$C_4H_4O_4$): C, H, N.

Analogously, compounds **38** and **39** were prepared by reacting **2i** or **2j** with the corresponding 1-phenylpiperazine derivatives.

Preparation of 7-Hydroxy-1-[[3-[4-(3-chlorophenyl)-1-piperazinyl]propionyl]amino]-2,2,4,6-tetramethylindan (40). A solution of **2k** (2.5 g, 9 mmol) and 1-(3-chlorophenyl)piperazine hydrochloride (3.55 g, 18 mmol) and TEA (8.65 mL, 60 mmol) in acetonitrile (50 mL) was refluxed for 2 h and then evaporated to dryness. The residue was dissolved in chloroform (200 mL), and insoluble materials were filtered off. The chloroform solution was concentrated under reduced pressure and then passed through a silica gel column (eluent: chloroform/MeOH 10:1). The second eluate was evaporated to dryness, and then recrystallized from EtOH to give **40** (3.52 g, 86%) as a colorless powder. Mp: 157–158 °C. ^{1}H NMR ($CDCl_3$): δ 1.07 (3 H, s, CH_3), 1.25 (3 H, s, CH_3), 2.06 (3 H, s, CH_3), 2.18 (3 H, s, CH_3), 2.36–2.86 (10 H, m), 2.97–3.22 (4 H, m), 4.70 (1 H, d, J = 7.5 Hz, 1-H), 6.63–6.90 (4 H, m), 7.04–7.13 (1 H, m), 8.88 (1 H, s, OH), 8.98 (1 H, d, J = 7.5 Hz, NH). Anal. ($C_{26}H_{34}N_3O_2Cl$): C, H, N.

Analogously, compound **41** was prepared starting from **2k** and 1-(3-methoxyphenyl)piperazine.

Preparation of 7-Methoxy-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-2,2,4,6-tetramethylindan (42). To a solution of **18** (4.35 g, 10 mmol) in DMF (100 mL) was added 60% sodium hydride (0.5 g, 12.5 mmol) in a small portions at room temperature and the mixture was stirred for 0.5 h. Methyl iodide (1.10 g, 11 mmol) was added to this mixture. After 4 h of stirring at 60 °C, the reaction mixture was poured into water and then extracted with AcOEt (200 mL). The extract was washed, dried, and evaporated to dryness. The residue was purified by passing through a silica gel column (eluent: AcOEt/hexane 1:10). Recrystallization from EtOH gave **42** (2.7 g, 53%) as colorless flakes. Mp: 142–143 °C. ^{1}H NMR ($CDCl_3$): δ 1.04 (3 H, s, CH_3), 1.26 (3 H, s, CH_3), 2.16 (3 H, s, CH_3), 2.21 (3 H, s, CH_3), 2.59 (1 H, d, J = 15 Hz, 3-H), 2.68 (1 H, d, J = 15 Hz, 3-H), 2.60–2.85 (4 H, m, CH_2), 3.00–3.30 (6 H, m, CH_2), 3.68 (3 H, s, OCH_3), 3.79 (3 H, s, OCH_3), 5.38 (1 H, d, J = 7.5 Hz, 1-H), 6.40–6.60 (3 H, m, aromatic H), 6.89 (1 H, s, 5-H), 7.17 (1 H, t, J = 9 Hz, aromatic H), 7.26 (1 H, m, NH). Anal. ($C_{27}H_{37}N_3O_3$): C, H, N.

Preparation of the 1-(Acylamino)-7-hydroxy-2,2,4,6-tetramethylindans Listed in Table III. 1-(Decanoylamino)-4,6-dimethyl-7-hydroxyindan (43). To a mixture of **1a** (1.77 g, 10 mmol) and NaOH (0.8 g, 20 mmol) in water (50 mL) was added decanoyl chloride (2.86 g, 15 mmol) in drops at 5–10 °C and the mixture was stirred for 1 h at room temperature, acidified by addition of concentrated HCl, and then extracted with chloroform (150 mL). The extract was washed, dried, and evaporated to dryness. Recrystallization from hexane gave **43** (1.58 g, 48%) as colorless needles. Mp: 103–104 °C. ^{1}H NMR ($CDCl_3$): δ 0.75–1.10 (3 H, m, CH_3), 1.20–1.80 (14 H, m, CH_2), 1.80–3.20 (6 H, m, 2,3-H and CH_2), 2.10 (3 H, s, CH_3), 2.18 (3 H, s, CH_3), 5.10–5.35 (1 H, m, 1-H), 5.90–6.20 (1 H, br, NH), 6.80 (1 H, s, 5-H), 8.95 (1 H, s, OH). Anal. ($C_{21}H_{33}NO_2$): C, H, N.

Analogously, compound **44** was prepared starting from **1a** and *p*-toluoyl chloride.

Preparation of 1-[[2-[4-(3-Chlorophenyl)-1-piperazinyl]-ethyl]amino]-7-hydroxy-2,2,4,6-tetramethylindan Fumarate (45). Lithium aluminum hydride (4.3 g, 11 mmol) was added in small portions to a solution of **23** (5.0 g, 11 mmol) in diglime (100 mL) and the mixture was refluxed for 3 h. After addition of ice, the mixture was filtered and the filtrate was extracted with CH_2Cl_2 (200 mL). The extract was washed, dried, and evaporated under reduced pressure to give the crude product, which was converted to its fumarate by addition of an ethanolic solution of fumaric acid and then recrystallized from EtOH to give **45** (2.3 g, 38%) as a colorless powder. Mp: 144–146 °C. ^{1}H NMR (DMSO-d_6): δ 1.03 (3 H, s, CH_3), 1.24 (3 H, s, CH_3), 2.02 (3 H, s, CH_3), 2.04 (3 H, s, CH_3), 2.40–3.33 (15 H, m), 4.13 (1 H, s, 1-H), 6.58 (2 H, s, CH=CH), 6.63–7.33 (5 H, m). Anal. ($C_{25}H_{30}N_3OCl$·$C_4H_4O_4$): C, H, N.

Analogously, compound **46** was prepared by starting from compound **18** (1.5 g, 3.4 mmol).

Preparation of 1-[[(7-Hydroxy-2,2,4,6-tetramethyl-1-indanyl)amino]acetyl]-4-(3-chlorophenyl)piperazine Fumarate (47). To a mixture of 1-(3-chlorophenyl)piperazine hydrochloride (1.97 g, 10 mmol) and TEA (2.84 mL, 20 mmol) in chloroform (20 mL) chloroacetyl chloride (1.21 g, 11 mmol) was added in drops at an ice-cooled temperature. After 2 h of stirring, the mixture was extracted with chloroform (200 mL). The extract was washed, dried, and concentrated under reduced pressure to give 1-(chloroacetyl)-4-(3-chlorophenyl)piperazine (2.64 g, 97%) as a pale yellow oil, which was used directly in the next step as the crude product without further purification.

A mixture of **2h** (2.45 g, 10 mmol), TEA (4.24 mL, 30 mmol), and the crude 1-(chloroacetyl)-4-(3-chlorophenyl)piperazine (2.64 g, 9.7 mmol) in acetonitrile (30 mL) was refluxed for 6 h, evaporated under reduced pressure, and extracted with CH_2Cl_2. The extracts were washed, dried, and evaporated under reduced pressure to give crude **47**, which was purified by passing it through a silica gel column (eluent: AcOEt/hexane 1:2). Recrystallization from EtOH gave **47** (0.96 g, 21%) as colorless needles. Mp: 144–145 °C. ^{1}H NMR ($CDCl_3$): δ 1.11 (3 H, s, CH_3), 1.24 (3 H, s, CH_3), 2.08 (3 H, s, CH_3), 2.19 (3 H, s, CH_3), 2.57 (1 H, d, J = 15 Hz, 3-H), 3.19 (5 H, m, 3-H and CH_2), 3.41–3.92 (6 H, m, CH_2), 3.97 (1 H, s, 1-H), 6.67–6.95 (4 H, m, aromatic H), 7.09 (1 H, s, 5-H), 7.18–7.30 (1 H, m, NH). Anal. ($C_{25}H_{32}N_3O_2Cl$): C, H, N.

Analogously, compound **48** was prepared starting from 1-(3-methoxyphenyl)piperazine (2.0 g, 10 mmol), chloroacetyl chloride (1.3 g, 12 mmol), and **2h** (2.50 g, 10 mmol).

7-Hydroxy-1-[[(4-methyl-1-piperazinyl)acetyl]amino]-2,2,4,6-tetramethylindan Dihydrochloride (49). A mixture of 1-methylpiperazine (1.07 g, 10 mmol) and **2h** (1.50 g, 5.3 mmol) in acetonitrile (24 mL) was refluxed for 2 h. The mixture was then evaporated under reduced pressure to dryness and extracted with chloroform. The extracts were washed, dried, and evaporated to dryness. Recrystallization from EtOH gave **49** (1.20 g, 65%) as colorless needles. Mp: 189–190 °C. ^{1}H NMR ($CDCl_3$): δ 1.13 (3 H, s, CH_3), 1.25 (3 H, s, CH_3), 2.11 (3 H, s, CH_3), 2.18 (3 H, s, CH_3), 2.27 (1 H, d, J = 15 Hz, 3-H), 2.30–2.83 (10 H, m), 3.03 (2 H, d, J = 1.5 Hz), 4.67 (1 H, d, J = 7.5 Hz, 1-H), 6.80 (1 H, s, 5-H), 7.85–8.13 (1 H, br, NH), 8.82 (1 H, s, OH). Anal. ($C_{20}H_{31}N_3O_2$): C, H, N.

Analogously, compounds **50–55** were prepared by starting from **2h** and the corresponding cyclic amines.

Preparation of 7-Hydroxy-1-[[(2-pyrrolidon-1-yl)acetyl]-amino]-2,2,4,6-tetramethylindan (56a). Sodium hydride (60%, 1.71 g, 43 mmol) was added in small portions to a solution of 2-pyrrolidone (3.63 g, 43 mmol) in DMF (40 mL) at room temperature and the mixture was stirred for 0.5 h. To this mixture was added **2h** (4.00 g, 14 mmol), and the mixture was stirred for another 0.5 h. The reaction mixture was poured into ice/water (100 mL) and extracted with CH_2Cl_2 (200 mL). The extract was washed, dried, and evaporated to dryness. The residue was subjected to silica gel column chromatography (eluent: AcOEt/*n*-hexane 1:2 and AcOEt). The AcOEt eluate was evaporated, and then recrystallized from EtOH to give **56a** (2.27 g, 48%) as colorless needles. Mp: 165–168 °C. ^{1}H NMR ($CDCl_3$): δ 1.08 (3 H, s, CH_3), 1.19 (3 H, s, CH_3), 1.83–2.33 (10 H, m), 2.47 (1 H, d, J = 16.5 Hz, 3-H), 2.75 (1 H, d, J = 16.5 Hz, 3-H), 3.44 (2 H, t, J = 6 Hz, CH_2), 3.72 (1 H, d, J = 15 Hz), 4.05 (1 H, d, J = 15 Hz), 4.66 (1 H, d, J = 6.5 Hz, 1-H), 6.78 (1 H, s, 5-H), 7.33 (1 H, br-d, J = 6.5 Hz, NH), 8.27 (1 H, s, OH). Anal. ($C_{19}H_{28}N_2O_3$): C, H, N.

Intramolecular condensation products were obtained from the prior run with AcOEt/hexane (1:2) eluent. Recrystallization from EtOH gave **56b** (0.88 g, 25%) as colorless needles. Mp: 207–208 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (3 H, s, CH_3), 1.33 (3 H, s, CH_3), 2.12 (3 H, s, CH_3), 2.14 (3 H, s, CH_3), 2.63 (2 H, s), 4.41 (1 H, dd, J = 1.5 Hz, 13.5 Hz), 4.85 (1 H, d, J = 13.5 Hz), 4.86 (1 H, s, 1-H), 6.28–6.43 (1 H, m, NH), 6.77 (1 H, s, 5-H). Anal. ($C_{15}H_{19}NO_2$): C, H, N.

Pharmacology. Test for Antihypoxic Effect. The test was conducted by a procedure similar to that reported.[12] ICR strain male mice (20–30 g) were used as test animals. The test compounds administered to a group of 10 mice 0.25 h (iv, sc, or ip) or 1 h (po) before the test, as indicated in Table III. The groups of mice were placed in a glass desiccator. Inside pressure of the desiccator was reduced to 210 mmHg by sucking out the inside air with a vacuum pump, then the stop bulb was closed.

The survival time of the test mice was determined as the length of time between the beginning of the vacuum pump operator and the cessation of respiration of the mice. Under the above hypoxic condition, the survival times of control animals were between 130 and 180 s. For convenience, the survival time of mice which lived longer than 900 s was taken as 900 s. Activity of the test compound was defined as the ratio (%) of the survival time of the test group to that of the control group. The results are shown in Table I as SVT. Statistical analyses were carried out by the Wilcoxon sum test (number of animals, n = 10).

Test for Promoting Effects on Recovery from Coma. The procedure used was similar to that reported as an experimental head injury model.[13,14] ICR strain male mice (20–30 g) were used as test animals. The head of the mouse was fixed on a pillow made of foamed polystyrene resin by holding the cervical skin of the mouse. A plastic tube, 22 mm i.d., was placed vertically over the head, and the centriciput of the mouse was shocked by the dropping of an acrylate cylinder rod (20 g) through the tube from a height of 40 cm to strike the vertex. Clonic convulsion occurred for 1–10 s, followed by a loss of consciousness (righting, reflex, RR) and then the mouse remained motionless in a crouching or prone position for some period. The length of time for reappearance of the righting reflex (RRT) after giving the shock and the length of time for reappearance of spontaneous movement (SMT) after recovery of righting reflex were used as indications of the effect on recovery from coma.

Each of the test compounds was administered orally or by ip injection 1 h before giving the shock. To the test mice of the control group, the same amount of physiological saline was administered. After the test, the brains of all the tested mice were subjected to postmortem examination, and those mice showing contused wounds in the brain were excluded from the determination. The numbers of animals used in the determination are shown in Tables II–IV. Activity of the test compound in this test was represented as the ratio (%) to the control group. Statistical analyses were carried out by the Wilcoxon sum test.

Effects on Sleeping Time of Mice Anesthetized by Halothane. This test was conducted by a procedure similar to that reported.[16] Male ICR strain mice (20–29 g, 4–5 weeks of age) were used. The gas flow from an anesthetic apparatus was passed through an acrylate resin box (13 × 14 × 25 cm). Oxygen containing 4% v/v halothane was passed through the box at a flow rate of 2 L/min for 3 min. During this period, the loss of righting reflex of the mice was observed. The mice were then removed from the box and placed on cotton wool. The mice were stimulated by picking them up and replacing them on the cotton wool at ca. 20-s intervals and the time at which the righting reflex of mice was recovered was noted with a stop watch for each animal. Sleeping time was defined as the length of time from the loss of righting reflex to the time of recovery of righting reflex. The effect on sleeping time was represented as the ratio (%) to the control group as shown in Table IV. Statistical analyses were carried out by the Wilcoxon sum test (number of animals, n = 10).

Test for Effects on Lipid Peroxidation of Rat Brain Homogenate. Lipid peroxide (LPO) produced in the brain homogenate was determined according to the method reported.[17,18] Brain tissue from male Wister rats (about 10 weeks of age) was obtained after decapitation and homogenated in an ice-cooled phosphate buffer (50 mmol, pH 7.4). The homogenate (1 mL) was incubated at 37 °C for 30 min with or without the testing compound dissolved in 10 μL of dimethyl sulfoxide. The reaction was stopped by adding 200 μL of 35% $HClO_4$, and the mixture was centrifuged at 1300 rps for 10 min. The LPO of the supernatant was measured by the thiobarbituric acid (TBA) method[19] and expressed as malondialdehyde (MDA)/mg of protein.

Acknowledgment. We express our appreciation to Mr. M. Ohsaki and Mr. J. Kunisaki, for their assistance of preparation of compounds, and Mrs. K. Ito for elemental analyses.

Registry No. **1a**, 93747-21-8; **1b**, 93747-31-0; **1c**, 133794-87-3; **1d**, 103246-79-3; **1e**, 133794-88-4; **1f**, 103246-81-7; **1g**, 133794-89-5; **1h**, 93747-39-8; **1i**, 133794-90-8; **1j**, 133794-91-9; **2a**, 93747-97-8; **2b**, 103234-04-4; **2c**, 133794-92-0; **2d**, 103247-04-7; **2e**, 133794-93-1; **2f**, 103247-05-8; **2g**, 133794-94-2; **2h**, 103234-03-3; **2i**, 133794-95-3;

2j, 103234-05-5; 2k, 133794-96-4; 3, 103233-91-6; 3·HCl, 103247-16-1; 4, 103247-26-3; 5, 103233-92-7; 5·HCl, 103247-17-2; 6, 103247-07-0; 7, 103247-08-1; 7·HCl, 133794-97-5; 8, 103233-89-2; 8·2HCl, 103247-09-2; 9, 103233-90-5; 9·HCl, 103247-11-6; 10, 103247-12-7; 11, 103247-10-5; 12, 133794-98-6; 12·HCl, 133794-99-7; 13, 103247-37-6; 14, 133795-00-3; 15, 103247-28-5; 16, 103247-25-2; 17, 103247-48-9; 18, 103233-65-4; 19, 103247-33-2; 20, 103247-47-8; 21, 103247-57-0; 22, 103247-50-3; 23, 103233-66-5; 24, 103247-53-6; 25, 103247-41-2; 26, 103247-54-7; 27, 103247-43-4; 28, 103247-29-6; 29, 103247-15-0; 30, 103247-55-8; 31, 103247-56-9; 32, 103247-44-5; 33, 103247-49-0; 34, 103247-45-6; 35, 103247-46-7; 36, 103247-14-9; 37, 103247-38-7; 38, 133795-01-4; 39, 103247-13-8; 40, 103247-06-9; 41, 133795-02-5; 42, 103247-22-9; 43, 133795-03-6; 44, 93747-96-7; 45, 103233-69-8; 45·$C_4H_4O_4$, 103233-70-1; 46, 103233-67-6; 46·$^1/_2C_4H_4O_4$, 133795-04-7; 47, 133795-05-8; 48, 133795-06-9; 49, 103233-56-3; 50, 103233-57-4; 51, 103233-58-5; 52, 103233-60-9; 53, 103233-61-0; (L)-54, 103233-62-1; 55, 103233-63-2; 56a, 103233-55-2; 56b, 133795-07-0; chloroacetyl chloride, 79-04-9; 3-chloropropionyl chloride, 625-36-5; 1-(3-chlorophenyl)piperazine, 6640-24-0; 1-(3-methoxyphenyl)piperazine, 16015-71-7; 1-(2,3-dimethylphenyl)piperazine, 1013-22-5; 1-phenylpiperazine, 92-54-6; 1-(3-methylphenyl)piperazine, 41186-03-2; 1-(2,3-dichlorophenyl)piperazine, 41202-77-1; 1-(3,5-dichlorophenyl)piperazine, 55827-50-4; 1-(2-methoxyphenyl)piperazine, 35386-24-4; 1-(4-methoxyphenyl)piperazine, 38212-30-5; 1-(3,4-dimethoxyphenyl)piperazine, 16015-73-9; 1-(2-ethoxyphenyl)piperazine, 13339-01-0; 1-(2-chlorophenyl)piperazine, 39512-50-0; 1-(2-fluorophenyl)piperazine, 1011-15-0; 1-(3-fluorophenyl)piperazine, 3801-89-6; 1-(4-fluorophenyl)piperazine, 2252-63-3; 1-(3-bromophenyl)piperazine, 31197-30-5; 1-(3-trifluoromethylphenyl)-piperazine, 15532-75-9; 1-(4-nitrophenyl)piperazine, 6269-89-2; 1-(2,5-dichlorophenyl)piperazine, 1013-27-0; 1-(3,4-dichlorophenyl)piperazine, 57260-67-0; 1-[(3-chloro-4-methyl)phenyl]-piperazine, 3606-03-9; 1-[(5-chloro-2-methyl)phenyl]piperazine, 76835-20-6; 1-(2,4,6-trimethylphenyl)piperazine, 91904-13-1; decanoyl chloride, 112-13-0; p-toluoyl chloride, 874-60-2; 1-(chloroacetyl)-4-(3-chlorophenyl)piperazine, 70395-06-1; 1-methylpiperazine, 109-01-3; 2-pyrrolidone, 616-45-5; morpholine, 110-91-8; thiomorpholine, 123-90-0; piperidine, 110-89-4; pyrrolidine, 123-75-1; (L)-proline, 147-85-3; imidazole, 288-32-4.

Octoclothepin Enantiomers. A Reinvestigation of Their Biochemical and Pharmacological Activity in Relation to a New Receptor–Interaction Model for Dopamine D-2 Receptor Antagonists

Klaus P. Bøgesø,*,† Tommy Liljefors,‡ Jørn Arnt,† John Hyttel,† and Henrik Pedersen†

Research Laboratories, H. Lundbeck A/S, Ottiliavej 7-9, DK-2500 Copenhagen-Valby, Denmark and Department of Organic Chemistry 3, Chemical Center, University of Lund, S-221 00 Lund, Sweden. *Received November 8, 1990*

Octoclothepin (1) was resolved into its *R* and *S* enantiomers via the diastereomeric tartaric acid salts. The enantiomers were shown to be of high optical purity by ^1H NMR with use of the chiral shift reagent (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol. Pharmacological and biochemical testing confirmed that (*S*)-1 is the more potent dopamine (DA) D-2 antagonist both in vitro and in vivo, although the *R* enantiomer still has significant D-2 antagonistic activity. In contrast, both enantiomers were equally active in test models detecting activity at D·1 receptors, serotonin·2 ($5\text{-}HT_2$) receptors and α_1 adrenoceptors. Contrary to a previous prediction, it was found that norepinephrine (NE) uptake inhibition was confined solely to the *S* enantiomer. Overall, (*S*)-1 has a "classical" neuroleptic profile, while the *R* enantiomer has a more "atypical" profile. These pharmacological profiles seem to be in agreement with the reported clinical profiles of the two enantiomers. A previous conformational study was revised in light of the biochemical test results with enantiomers of known optical purity. Their relative D-2 receptor affinity corresponded well with the calculated conformational energy difference between their "active conformations" deduced from a previously reported new D-2 receptor model. Also the high enantioselectivity of (*S*)-1 at the NE uptake site could be explained after a detailed conformational analysis showing strict requirements for the orientation of the piperazine lone-pair direction at the NE uptake site.

We have previously reported extensive conformational analysis and least-squares molecular superimposition studies of the enantiomers of the two neuroleptic compounds octoclothepin (1) and tefludazine (2) (Figure 1).[1] In contrast to previous assumptions we concluded, that the conformation of (*S*)-1 which is responsible for the dopamine D-2 (DA D-2) receptor antagonism is significantly different from the one observed in the crystal.[2] The superimposition of the suggested active conformations of (*S*)-1 and (1*R*,3*S*)-2 (Figure 2) defines the spatial relationships of the pharmacophore elements (the phenyl rings, the nitrogen atom and the nitrogen atom–nitrogen lone pair (or in the protonated case the N–H) vector). These spatial relationships represent in our opinion a contemporary DA D-2 receptor interaction model. D-2 antagonists from various chemical classes (thioxanthenes, phenothiazines, butyrophenones, and benzamides) can be accomodated into the model in low-energy conformations.[3a] Furthermore, Froimowitz has successfully employed this model in a study of dibenzocycloheptene and cyproheptadine derivatives.[3b]

The affinity of (*R*)-1 for the D-2 receptor has been reported by Seeman et al.[4] to be only nine times lower than that of (*S*)-1. Valchář have reported a stereoselectivity ratio of 38 in favor of the *S* enantiomer.[5] We explained this relatively low stereoselectivity by pointing out that also (*R*)-1 could be accommodated in the D-2 model in a conformation with a steric energy that was 2.2 kcal/mol higher than that of the suggested active conformation of (*S*)-1.

In contrast to the rather low stereoselectivity observed in vitro for the enantiomers of 1, they have been reported to show a *high* stereoselectivity in certain in vivo models for neuroleptic activity. These include catelepsy, antagonism of apomorphine-induced gnawing, chewing or agi-

† H. Lundbeck A/S.
‡ University of Lund.

(1) Liljefors, T.; Bøgesø, K. P. *J. Med. Chem.* **1988**, *31*, 306.
(2) Jaunin, A.; Petcher, T. J.; Weber, H. P. *J. Chem. Soc., Perkin Trans. 2* **1977**, 186.
(3) (a) Liljefors, T.; Petterson, I.; Palm, J. University of Lund, unpublished results. (b) Froimowitz, M. *BioTechniques* **1990**, *8*, 640.
(4) Seeman, P.; Westman, K.; Protiva, M.; Jílek, J.; Jain, P. C.; Saxena, A. K.; Anand, N.; Humber, L.; Phillip, A. *Eur. J. Pharmacol.* **1979**, *56*, 247.
(5) Valchář, M. *Activ. Nerv. Sup. (Praha)* **1984**, *26*, 28.

0022-2623/91/1834-2023$02.50/0