using graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å) and an ω scan technique with a variable scan rate of 3.91–29.30°/min. Background counts were taken for half the scan time at each extreme of the scan range. All data (2630) having $h, k \geq 0$ with $3° \leq 2\theta \leq 114°$ were measured in this manner. Crystal decomposition was monitored throughout data collection by remeasuring two standard reflections after every 50 data measurements; no significant variations in intensity were observed. The intensities were reduced by applying Lorentz and polarization corrections. Empirical absorption corrections were applied on the basis of azimuthal scans of 15 reflections representing the range of 2θ values. The maximum and minimum transmission coefficients were 0.840 and 0.428, respectively. Systematically absent reflections were eliminated, and equivalent reflections were averaged to give 2364 unique data of which 2183 were considered to be observed [$F_o > 3\sigma(F_o)$].

The structure was solved by direct methods using the SHELXTL software. Following refinement of the carbon, nitrogen, and oxygen atoms with isotropic temperature factors, a different Fourier map displayed a number of large peaks near the inversion center. The residual peaks were assigned to a molecule of the solvent ethyl acetate which is disordered about the inversion center. In the final stages of refinement, the solvent atoms were refined with isotropic temperature factors and all other nonhydrogen atoms were refined with anisotropic temperature factors. The carboxylic acid proton was located on a difference map and fixed at the observed position. All other hydrogens were included at idealized positions (C–H 0.96 Å, C–CH 109° or 120°) and allowed to ride on the carbon to which they were attached. Solvent hydrogens were not included. Refinement converged (shift/error ≤ 0.05) at $R = 0.065$, where R is $\sum||F_o| - |F_c||/\sum|F_o|$, and $R_w = 0.084$, where R_w is $\sum[w(|F_o| - |F_c|)^2]^{1/2}/\sum(wF_o^2)^{1/2}$. The four largest peaks in a final difference map (e/Å^3 = 0.29–0.60) were in the vicinity of the disordered solvent molecule. The quantity minimized by the least-square program was $w(|F_o| - |F_c|)^2$ where w is the weight of a given observation ($w^{-1} = \sigma^2(|F_o|) + 0.0015|F_o|^2$). The analytical forms for the scattering factors of the neutral atoms were used.[17]

Acknowledgment. We express our appreciation to Dr. D. Cochran, Mr. A. Verwijs, and their associates for microanalytical and spectral data.

Supplementary Material Available: The atomic coordinates, anisotropic and isotropic thermal parameters, bond lengths, and bond angles for **25** (7 pages); calculated structure factors (13 pages). Ordering information is given on any current masthead page.

2-Phenyl-2-(1-hydroxycycloalkyl)ethylamine Derivatives: Synthesis and Antidepressant Activity

John P. Yardley,*,† G. E. Morris Husbands,† Gary Stack,† Jacqueline Butch,† James Bicksler,† John A. Moyer,† Eric A. Muth,† Terrance Andree,† Horace Fletcher, III,† Michael N. G. James,‡ and Anita R. Sielecki‡

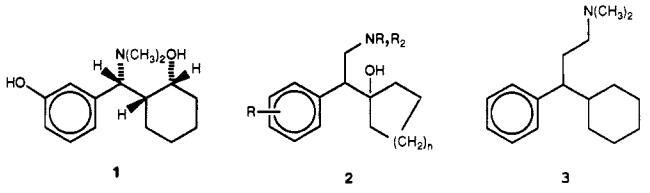
Wyeth-Ayerst Research, C.N. 8000, Princeton, New Jersey 08543-8000, and Department of Biochemistry, University of Alberta, Edmonton, Canada T6G2H7. *Received November 3, 1989*

A series of 2-phenyl-2-(1-hydroxycycloalkyl)ethylamine derivatives was examined for the ability to inhibit both rat brain imipramine receptor binding and the synaptosomal uptake of norepinephrine (NE) and serotonin (5-HT). Neurotransmitter uptake inhibition was highest for a subset of 2-phenyl-2-(1-hydroxycyclohexyl)dimethylethylamines in which the aryl ring has a halogen or methoxy substituent at the 3- and/or 4-positions. Potential antidepressant activity in this subset was assayed in three rodent models—the antagonism of reserpine-induced hypothermia, the antagonism of histamine-induced ACTH release, and the ability to reduce noradrenergic responsiveness in the rat pineal gland. An acute effect seen in the rat pineal gland with several analogues, including 1-[1-(3,4-dichlorophenyl)-2-(dimethylamino)ethyl]cyclohexanol (**23**) and 1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol (4), was taken as a possible correlate of a rapid onset of antidepressant activity. Compound 4 (venlafaxine) is presently undergoing clinical evaluation.

Introduction

Recent years have seen the development of a number of nontricyclic antidepressants with diminished cardiovascular and anticholinergic liability.[1] However, the goal of a rapid-onset antidepressant remains elusive. Indeed, the discrepancy in time course between the onset of clinical effectiveness and the pharmacologically observable increase in monoamine levels characteristic of most antidepressants has led to an extensive reappraisal of the simple "monoamine hypothesis" of depression. Current theories of antidepressant action focus on the gradual adaptational changes in neurotransmitter receptors, particularly the down-regulation of β-adrenoceptors coupled to adenylate cyclase, caused by chronic antidepressant therapy.[2]

The present study evolved from investigations on the mixed opiate agonist–antagonist ciramadol (**1**)[3] and was primarily aimed at structural simplification. Thus, the variants (**2**) identified in Table I contain a single stereogenic carbon, while a three-carbon interval between the amine and hydroxyl functions is maintained. The target structures proved inactive as analgesics; however, since



1 2 3

minor structural alterations can frequently induce profound changes in the profile of central nervous system drugs,[4] we conducted further studies. In particular, structural similarities to gamfexine (**3**)[5] suggested the evaluation of **2** for antidepressant activity. Demonstration

† Wyeth-Ayerst Research.
‡ University of Alberta.

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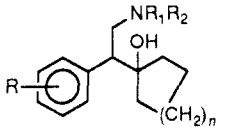
Table I. Phenylalkylamines



2

no.	R	NR_1R_2	n	mp, °C	formula[a]	method	% yield	imipramine binding; IC_{50}, nM (95% CI)
4	4-OCH_3	$N(CH_3)_2$	2	215–217	$C_{17}H_{27}NO_2$·HCl	A	24	90 (10–3000)
(+)-4[b]	4-OCH_3	$N(CH_3)_2$	2	240–242	$C_{17}H_{27}NO_2$·HCl	A[c]	33	109 (71–198)
				210–212	$C_{17}H_{27}NO_2$·HBr			
(−)-4[b]	4-OCH_3	$N(CH_3)_2$	2	240–242	$C_{17}H_{27}NO_2$·HCl	A[c]	31	140 (100–200)
5	4-OCH_3	NH_2	2	168–172	$C_{15}H_{23}NO_2$·HCl	A[c]	20	900
6	4-OCH_3	$NH(CH_3)$	2	164–166	$C_{16}H_{25}NO_2$·HCl	B	15	300
7	H	$N(CH_3)_2$	2	229–230	$C_{16}H_{25}NO$·HCl	B	15	300
8	4-OH	$N(CH_3)_2$	2	140–142	$C_{16}H_{25}NO_2$·$C_4H_4O_4$·H_2O[d]	B	25	60
9	4-Cl	$N(CH_3)_2$	2	228–230	$C_{16}H_{24}NOCl$·HCl	B	15	100
10	4-Br	$N(CH_3)_2$	2	239–241	$C_{16}H_{24}NOBr$·HCl	B	40	62 (35–100)
11	4-CH_3	$N(CH_3)_2$	2	238–239	$C_{17}H_{27}NO$·HCl	B	23	205 (46–540)
12	4-CF_3	$N(CH_3)_2$	2	238–240	$C_{17}H_{24}NOF_3$·HCl	B	27.5	88
13	4-Br	$N(CH_3)_2$	0	220–222	$C_{14}H_{20}NOBr$·HCl	B	18	435
14	4-OCH_3	$N(CH_3)_2$	1	193–195	$C_{16}H_{25}NO_2$·HCl	B	28	126
15	4-CF_3	$N(CH_3)_2$	1	208–210	$C_{16}H_{22}NOF_3$·HCl	B	50	NT[h]
16	4-OCH_3	$N(CH_3)_2$	3	175–177	$C_{18}H_{29}NO_2$·HCl·0.5H_2O	B	27	300
17	4-CF_3	$N(CH_3)_2$	3	223–225	$C_{18}H_{26}NOF_3$·HCl[e]	B	24	NT[h]
18	3-OCH_3	$N(CH_3)_2$	2	169–171	$C_{17}H_{27}NO_2$·HCl	B	21	150 (60–370)
19	3-Cl	$N(CH_3)_2$	2	214–216	$C_{16}H_{24}NOCl$·HCl	B	18	130 (95–175)
20	3-Br	$N(CH_3)_2$	2	198–201	$C_{16}H_{24}NOBr$·HCl	B	13	52 (42–69)
21	3-CF_3	$N(CH_3)_2$	2	194–196	$C_{17}H_{24}NOF_3$·HCl	B	43	360
22	3-OCH_3	$N(CH_3)_2$	1	166–168	$C_{16}H_{25}NO_2$·HCl	B	18	95 (44–175)
23	3-Cl, 4-Cl	$N(CH_3)_2$	2	241–244	$C_{16}H_{23}NOCl_2$·HCl	B	27	37 (28–48)
24	3-OCH_3, 4-OCH_3	$N(CH_3)_2$	2	210–215	$C_{18}H_{29}NO_3$·HCl	B	25	700 (415–2100)
25	2-Cl	$N(CH_3)_2$	2	205–206	$C_{16}H_{24}NOCl$·HCl	B	18	523
26	4-OCH_3	$N(CH_3)_2$	4	178–180	$C_{19}H_{31}NO_2$·HCl·0.25H_2O	B	50	>10 μM
27	2-Br	$N(CH_3)_2$	2	215–217	$C_{16}H_{24}NOBr$·HCl	B	18	260
28	2-$OCH_2C_6H_5$	$N(CH_3)_2$	2	150–151	$C_{23}H_{31}NO_2$·$C_2H_2O_4$[f,g]	B[c]	18	NT[h]
29	2-OH	$N(CH_3)_2$	2	188–189	$C_{16}H_{25}NO_2$·HCl	B	10	>10 μM
30	4-NH_2	$N(CH_3)_2$	2	105 dec	$C_{16}H_{26}N_2O$·2$C_2H_2O_4$[f]	B[c]	49	>10 μM
31	4-NO_2	$N(CH_3)_2$	2	211–212	$C_{16}H_{24}N_2O_3$·HCl	B[c]	44	250 (125–550)
32	4-OCH_3, 3-Br	$N(CH_3)_2$	2	218–220	$C_{17}H_{26}NO_2Br$·HCl	A	43	190
33	4-OCH_3		2	232–233	$C_{19}H_{29}NO_3$·HCl	B	10	>10 μM
34	4-OCH_3		2	205–206	$C_{19}H_{29}NO_2$·HCl	B	25	>10 μM
35	4-OCH_3		2	211–212	$C_{20}H_{31}NO_2$·HCl	B	16	>10 μM
imipramine								1.7 (1.6–1.9)
desipramine								130 (110–150)

[a] Analyses for C, H, N were within ±0.4 of the theoretical values. [b] (+) and (−) refers to the sign of rotation for the free base. [c] Modified synthesis described in the Experimental Section. [d] Fumarate salt. [e] C: calcd 59.01, found 59.61. [f] Oxalate salt. [g] C: calcd 67.68, found 67.18. [h] Not tested.

of a neurotransmitter uptake inhibitory profile combined with an ability to rapidly desensitize β-adrenoceptors led to further development of this series and the selection of venlafaxine (4) for clinical studies.

Chemistry

Variants of 2, evaluated as their water-soluble salt forms, are identified in Table I and were prepared by the straightforward methods depicted in Schemes I and II. Scheme I is the preferred route for the preparation of 4. The appropriately substituted phenylacetonitrile (I) was condensed with cyclohexanone following the procedure of Sauvetre et al.[6] Catalytic hydrogenation of the β-hydroxynitrile product (II) with rhodium on alumina catalyst[7] yielded primary amine III. N-methylation was accomplished by the Eschweiler–Clark procedure. The Tilford and Van Campen modification of this reaction[8] was necessary to convert the intermediate oxazine IV, isolable under normal Eschweiler–Clarke conditions, to product. Scheme I was unsuitable for derivatives containing ring-activating substituents at the meta position. In these cases, formation of tetrahydroisoquinolines by the reaction of the activated ring position with the electrophilic iminium intermediates of the Eschweiler-Clarke reaction occurred. Traces of oxazines were detected in these reactions also. The problem was circumvented in Scheme II. The starting material here was the appropriately substituted phenylacetic acid which was first converted to the required amide V, a procedure that also allowed variation of the amine portion of the structure. The amide was then condensed

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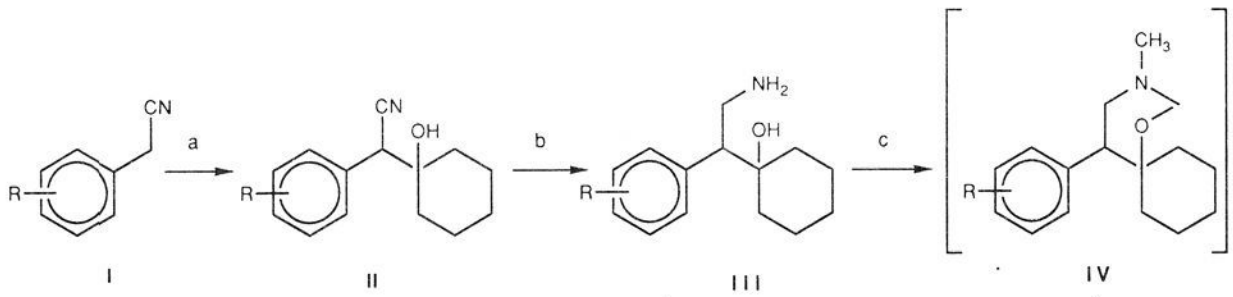
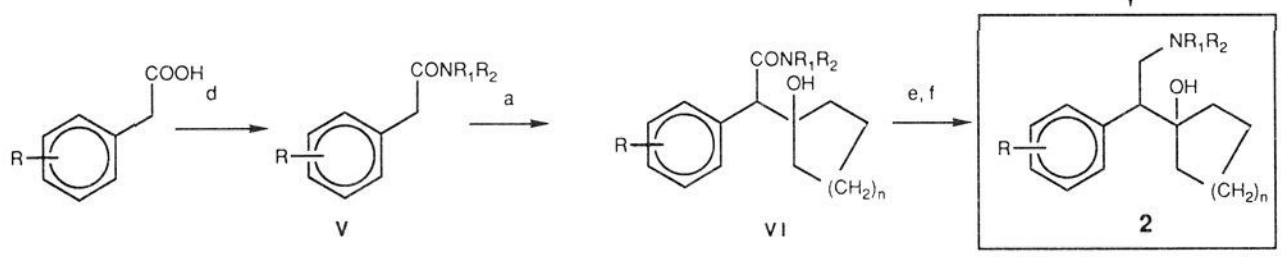
Scheme I[a]



Scheme II



[a] (a) LDA, cycloalkananone, −78 °C; (b) Rh/Al_2O_3, EtOH; (c) HCHO, HCOOH, H_2O, reflux; (d) $(COCl)_2$, R_1R_2NH, CH_2Cl_2; (e) borane/THF or AlH_3/THF, (f) H_2/Pd for R = OBz to R = OH conversion.

Figure 1. View of (*S*)-(+)-1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol hydrobromide [(+)-**4**·HBr].

with the cycloalkanone as shown, and the product VI was reduced to the tertiary amine using either aluminum hydride[9] or borane/THF complex.[10] The electrophilic reducing agents were employed to minimize the retrocondensation reaction which was significant in the case of phenylacetamides substituted with electron-withdrawing groups, e.g. VI, R = 3,4-dichloro. Synthesis of **30** utilized an in situ conversion of the 4-amino moiety to its tetramethyldisilethylene derivative[11] for temporary protection during the condensation step. 4-Nitro compound **31** was obtained by treatment of the diazonium salt of **30** with sodium nitrite in the presence of cuprous ion, since *N*,*N*-dimethyl-4-nitrobenzeneacetamide failed to condense with cyclohexanone.

The resolution of **4** was achieved with the chiral di-*p*-toluoyltartaric acids as resolving agents. The absolute configuration of (+)-**4** was established as *S* (see Figure 1) by a single-crystal X-ray analysis of the hydrobromide salt and the anomalous dispersion technique.

Results and Discussion

Inhibition of binding of [^{3}H]imipramine to rat brain tissue was used in the early stage of the program as a preliminary screen for antidepressant activity (Table I). While this property is not universal among the new generation of antidepressants, imipramine binding has been correlated with the ability of agents to inhibit monoamine uptake.[12] This screen led to the elimination of derivatives with amine functions other than those of the dimethylamine type. Imipramine receptor affinity was highest for the latter, with a six-membered cycloalkanol ring and an aryl moiety substituted with halogen or methoxy in the 3- and 4-positions, but not the 2-position. Three disubstituted derivatives were examined and the order of imipramine receptor affinity was as follows: 3,4-dichloro > 3-bromo-4-methoxy > 3,4-dimethoxy. However, while the imipramine assay was important in the early phases of this study, compound selection became increasingly dependent upon the ability to inhibit the uptake of tritiated norepinephrine (NE) and serotonin (5-HT) into rat brain synaptosomes. This was due, in part, to the nonlinear [^{3}H]imipramine binding observed. IC_{50} data with confidence intervals were frequently not obtainable and, in their absence, estimates were obtained by a graphical comparison of the displacement curves of test compounds with that of imipramine. Fluoxetine, paroxetine, norzimelidine, and citalopram, unlike classical tricyclic antidepressants, have also been reported to inhibit [^{3}H]imipramine binding in a complex manner.[13]

Neurotransmitter uptake inhibitory behavior is outlined in Table II. The majority of compounds showed only a modest neurotransmitter selectivity; however, (+)-**4** proved to be a relatively 5-HT-selective uptake inhibitor, whereas (−)-**4** matched the profile of the racemate. An interesting observation was that, unlike the case of many antidepressants, e.g., desipramine and fluoxetine, mono-*N*-methyl derivative **6** of this series showed minimal activity in both imipramine binding and synaptosomal uptake. The effect of increasing the cycloalkanol ring size on neurotransmitter uptake inhibition was examined in the series **14**, **4**, and **16**; NE uptake was particularly sensitive with maximal inhibitory effect seen with six-membered ring compound **4**. Some members of the series, e.g., **16** and **24**, with low affinity for the impramine receptor, showed strong inhibition of 5-HT uptake, a result that is contrary to the general belief that a relationship exists between the imipramine binding site and the 5-HT uptake mechanism.[13]

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Table II. Effects of Selected Compounds on NE and 5-HT Uptake Inhibition

no.	IC_{50}, μM (95% CI); synaptosomal uptake inhibition			
	NE		5-HT	
4	0.64	(0.50–0.84)	0.21	(0.15–0.28)
(+)-4[a]	3.14	(2.87–3.45)	0.10	(0.09–0.12)
(−)-4[a]	0.76	(0.61–0.99)	0.19	(0.16–0.23)
7	1.9	NA[b]	2.58	(0.71–4.81)
6	4.7	(3.5–7.0)	1.6	(1.41–1.91)
8	1.16	(1.05–1.28)	0.18	(0.13–0.23)
9	0.30	(0.24–0.37)	0.18	(0.11–0.27)
10	0.21	(0.18–0.26)	0.11	(0.09–0.13)
11	0.53	(0.47–0.61)	1.54	NA[b]
12	2.8	NA[b]	0.4	(0.31–0.54)
13	0.89	(0.61–1.65)	0.58	(0.39–1.05)
14	5.8	(3.1–14.3)	0.4	(0.28–0.53)
15	10.4	(9.1–12.1)	0.49	(0.37–0.68)
16	2.07	(1.22–4.66)	0.24	(0.22–0.27)
17	2.4	(2.0–3.0)	0.5	(0.47–0.68)
18	0.62	(0.46–0.95)	0.19	(0.16–0.22)
19	0.16	(0.14–0.18)	0.32	(0.30–0.35)
20	0.11	(0.09–0.12)	0.23	(0.20–0.26)
21	0.36	(0.26–0.56)	1.44	NA[b]
22	>10		0.68	(0.44–1.33)
23	0.07	(0.045–0.088)	0.08	(0.056–0.098)
24	1.38	(0.98–2.17)	0.13	(0.11–0.16)
32	0.14	(0.08–0.22)	0.2	(0.10–0.35)
desipramine	0.15	(0.07–0.38)	3.0	(1.1–1.3)
imipramine	0.26	(0.15–0.61)	0.12	(0.04–0.93)

[a] (+) and (−) refers to the sign of rotation for the free base. [b] Not available.

In parallel with the earlier imipramine binding studies, the subset of **2** comprised of 2-phenyl-2-(1-hydroxycyclohexyl)dimethylethylamines, substituted at the 3- and 4-positions with either chloro, bromo, or methoxy substituents, provided the most potent uptake inhibitors. Nine representatives of this subset, which included the enantiomers of **4** and disubstituted compounds **23** and **32**, were selected for further evaluation in in vivo models. Biological results for this series and for the standard desipramine are shown in Table III.

The primary in vivo model employed to assay potential antidepressant activity was the antagonism of reserpine-induced hypothermia.[14] All compounds showed significant activity but were less potent than desipramine. In addition, all compounds tested, with the exception of (+)-**4** and **19**, were active in antagonizing histamine-induced ACTH release; classical antidepressants are similarly active in this test.[15] All were finally examined for their ability to downregulate β-adrenergic responsiveness in the rat pineal gland. Compounds **4**, (−)-**4**, **18**, **23**, and **32** were found to significantly reduce responsiveness of the adenylate cyclase coupled β-adrenergic system on both single-dose treatment and on repeated administration. Many known antidepressants, including desipramine, which was used as a standard in this study, reduce noradrenergic responsiveness in rodents only after repeated administration.[16] While no obvious correlation of this property with neurotransmitter selectivity was apparent, we note that the three monosubstituted halo compounds were without a significant effect even after repeated administration.

Initial interest in the development of an antidepressant candidate centered on **23**, on the basis of its potent amine uptake inhibitor and in vivo profiles.[17] Additional in vitro testing suggested reduced cholinergic or antihistaminic side effects when compared with that of tricyclic antidepressants. In the muscarinic cholinergic ([^{3}H]quinuclidinyl benzilate) assay, **23** and **4** exhibited IC_{50} values greater than 10^{-5} M and thus were essentially inactive (imipramine IC_{50} = 37 nM, desipramine IC_{50} = 30 nM) and both failed to exhibit histamine-1 ([^{3}H]pyrilamine) binding (IC_{50} > 10^{-5} M).[17] However, a positive finding with **23** in the Ames test[18] using *Salmonella typhimurium* in tester strain TA 1537 at 1000 μg per plate without metabolic activation, refocused attention on the halogen-free compound **4**, which evinced no detectable activity in the Ames evaluation using tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 at doses up to 10 mg per plate in both direct plate and liver microsome assays. Pragmatic considerations such as ease of synthesis and lead time together with an indication of oral activity in the antagonism of reserpine-induced hypothermia model contributed to the selection of **4**. On further examination, **4** was found to have very weak affinities for α-adrenergic, serotonergic 5-HT_{1A} and 5-HT_2, dopamine-2, opiate, and benzodiazepine binding sites.[19] Many antidepressants have been shown to downregulate cortical β-adrenergic[20] and/or 5-HT_2[21] receptors following repeated administration to rats; however, **4** was without effect upon these densities following the 14-day protocol shown in Table IV.

Clinical studies are currently in progress with **4** and should determine what, if any, relationship exists between the acute reduction in noradrenergic responsiveness observed preclinically and the onset of antidepressant activity in humans.

Experimental Section

Chemistry. Melting points (uncorrected) were taken with a Thomas-Hoover capillary apparatus. Microanalyses agree with calculated values within ±0.4%, unless indicated otherwise. Infrared spectra were determined with a Perkin-Elmer Model 299 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian XL100 instrument and Mass Spectra were obtained with a Kratos MS 25 spectrometer. We are indebted to the Analytical Chemistry Department of Wyeth-Ayerst Research for the analytical services. TLC were run on precoated plates (Merck silica gel F_{254}).

Method A (General Procedure). 1-[Cyano(4-methoxyphenyl)methyl]cyclohexanol (II, R = 4-OCH_3). A solution of (*p*-methoxyphenyl)acetonitrile (100 g, 0.66 mol) in THF (250 mL) was cooled to −70 °C under nitrogen and a hexane solution of butyllithium (410 mL, 0.66 mol) was added. After the solution was stirred for 30 min, cyclohexanone (70 mL, 0.7 mol) was added. After stirring at −65 °C for 2 h, the mixture was poured into saturated NH_4Cl (200 mL) containing ice (300 g), water (500 mL) was added, and the precipitated product was filtered. The latter was washed with water and ether and was dried. A second crop was obtained from the THF layer: total yield 135 g (82.9%); mp 123–126 °C; ^{1}H NMR ($CDCl_3$) δ 7.32 and 6.95 (q, 4 H, Ar H), 3.8 (s, 3 H, OCH_3), 3.76 (s, 3 H, CHCN), 1.56 (m, 10 H, cyclohexyl H); MS *m/e* 246 (M^+ + 1; CIMS).

1-[2-Amino-1-(4-methoxyphenyl)ethyl]cyclohexanol (5). 1-[Cyano(4-methoxyphenyl)methyl]cyclohexanol (12 g, 0.05 mol) was dissolved in an ammonia/ethanol mixture (250 mL, 2:8 v/v) and hydrogenated over 5% rhodium on alumina (2.8 g). The catalyst was filtered and washed well with ethanol, and the

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Table III. In Vivo Studies of Selected Compounds

no.	antagonism of reserpine-induced hypothermia; MED, mg/kg ip	antagonism of HA/ACTH; % decrease[a] at 10 mg/kg ip	% change of noradrenergic responsiveness[a] at 10 mg/kg ip	
			single treatment	chronic treatment, 5 days bid
4	10[b]	26	-51[c]	-43[c]
(+)-4	30	+54	NSE[d]	NSE[d]
(-)-4	3	37	-53	-51
10	1	43	NSE[d]	NSE[d]
18	3	52	-79	-81
19	1	NSE[d]	NSE	NSE[d]
20	1	67	NSE	NSE[d]
23	3	55	-51	-74
32	1	34	-65	-82
desipramine	0.4	52	NSE[d]	-81

[a] Significantly different from saline-stimulated control at 10 mg/kg ip. [b] Also active orally at this dose. [c] Average of several experiments. [d] No significant effect.

Table IV. Effect of 2 Week Administration of 4 on β-Adrenergic (DHA) and 5-HT$_2$ (Spiperone) Receptor Binding

treatment: 10 mg/kg ip, 14 days bid	n	receptor binding (fmol/mg protein)			
		[^{3}H]DHA		[^{3}H]spiperone	
		1.25 nM	10 nM	0.4 nM	0.6 nM
saline	10	36.7 ± 2.1	61.4 ± 2.1	82.6 ± 1.5	235.6 ± 6.9
4	10	35.0 ± 0.9	61.8 ± 1.5	77.8 ± 1.1	250.6 ± 4.2
		(-4.6%)	(0.0%)	(-5.8%)	(+6.4%)

combined filtrates were evaporated to an oil (12 g). A solution of the oil in toluene (100 mL) was acidified to pH 2 (moist pH paper) with 2-propanolic HCl and diluted with ether (400 mL) to precipitate **5**·HCl as a crystalline solid (9 g, 57%): mp 168–172 °C; ^{1}H NMR (DMSO-d_6) δ 7.85 (s, 3 H, NH_3^+), 3.75 (s, 3 H, OCH_3), 3.20 (m, 3 H, $CHCH_2$), 1.35 (m, 10 H, cyclohexyl H); MS m/e 250 (M^+ + 1; CIMS). Anal. ($C_{15}H_{23}NO_2$·HCl) C, H, N.

1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol (4). A solution consisting of **5** (12 g), 33% aqueous formaldehyde (12.5 mL), 88% formic acid (16.5 mL), and water (115 mL) was refluxed overnight. The solution was evaporated to 30 mL, diluted to 200 mL with water, adjusted to pH 2 with concentrated HCl, and extracted with ethyl acetate to remove a pink impurity. The solution was basified with 50% NaOH and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), filtered, and evaporated to a residue. The residue as a solution in ethyl acetate (700 mL) was treated with 2-propanolic HCl to afford **4**·HCl (10.8 g, 80%): mp 207–208 °C; TLC R_f = 0.83 (cyclohexane/ethyl acetate/ethanol/concentrated NH_4OH 1:1:0.5:0.05 v/v). An analytical sample was recrystallized from methanol/ethyl acetate: mp 215–217 °C; ^{1}H NMR (DMSO-d_6) δ 7.32 and 6.98 (4 H, q, Ar H), 3.78 (s, 3 H, OCH_3), 3.64 (m, 2 H, $CH_2N(CH_3)_2$), 3.06 (m, 1 H, $CHCH_2N(CH_3)_2$), 2.74 (s, 6 H, $N(CH_3)_2$), 1.38 (m, 10 H, cyclohexyl H). Anal. ($C_{17}H_{27}NO_2$·HCl) C, H, N.

Method B. 4-Bromo-*N*,*N*-dimethylbenzeneacetamide (V, R = 4-Br, R_1 = R_2 = CH_3). A solution of *p*-bromophenylacetic acid (50 g, 0.233 mol) in methylene chloride (500 mL) was treated with oxalyl chloride (23.3 mL, 0.27 mol) and DMF (0.5 mL) and then stirred at room temperature during 4 h. The solvent was evaporated in vacuo to remove excess oxalyl chloride. The residue was dissolved in methylene chloride (300 mL) and treated with an excess of gaseous dimethylamine. The mixture was stirred overnight and the solvent was evaporated. The residue was dissolved in methylene chloride and the solution was washed with saturated $NaHCO_3$, 1 N HCl, and brine, dried ($MgSO_4$), and evaporated, yielding crystals (51.2 g, 97%): mp 73–76 °C; ^{1}H NMR ($CDCl_3$) δ 7.55 (4 H, q, Ar H), 3.65 (s, 2 H, $CH_2CON(CH_3)_2$), 2.95 (s, 6 H, $N(CH_3)_2$).

1-[(4-Bromophenyl)[(dimethylamino)carbonyl]methyl]cyclohexanol (VI, R = 4-Br, n = 2, R_1 = R_2 = CH_3). A solution of 4-bromo-*N*,*N*-dimethylbenzeneacetamide (15 g, 0.06 mol) in THF (250 mL) was treated with a hexane solution of butyllithium (43.3 mL, 0.06 mol) at −78 °C. The mixture was stirred for 20 min and cyclohexanone (7 mL, 0.07 mol) was added. This mixture was stirred for 50 min at −78 °C and poured into saturated NH_4Cl solution. The layers were separated, and the aqueous layer was extracted with ether. The combined organic solution was washed with brine, dried (K_2CO_3), and evaporated. The crystalline product was filtered, washed with 2-propanol, and dried to afford the product (9.8 g 44%): mp 140–144 °C; ^{1}H NMR ($CDCl_3$) δ 4.35 (m, 4 H, Ar H), 3.63 (s, 1 H, $CHCON(CH_3)_2$), 2.95 (s, 6 H, $N(CH_3)_2$), 1.45 (m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{22}NO_2Br$) N.

Reductions: (1) Aluminum Hydride Procedure. 1-[1-(4-Bromophenyl)-2-(dimethylamino)ethyl]cyclohexanol (10). Concentrated H_2SO_4 (0.5 mL) was cautiously added to an ice-cold suspension of lithium aluminum hydride (0.7 g) in THF (25 mL) and the mixture was stirred for 1 h. 1-[(4-Bromophenyl)[(dimethylamino)carbonyl]methyl]cyclohexanol (4 g, 0.012 mol) in THF (35 mL) was added dropwise and the reaction mixture was stirred at 0 °C for 1 h. A THF/water mixture (6 mL) was added cautiously followed by 10% NaOH solution (10 mL). The mixture was stirred for 15 min after which the white precipitate was removed by filtration. The filtrate was dried (K_2CO_3) and evaporated to an oil (3.5 g) which on treatment with 2-propanolic HCl gave **10**·HCl: mp 239–241 °C; ^{1}H NMR (DMSO-d_6) δ 7.4 (m, 4 H, Ar H), 3.55 (d, 2 H, $CHCH_2N(CH_3)_2$), 3.05 (t, 1 H, $CHCH_2N(CH_3)_2$), 2.63 (s, 6 H, $N(CH_3)_2$), 1.3 (m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{24}NOBr$·HCl) C, H, N.

Reductions: (2) Borane/THF Procedure. 1-[1-(3,4-Dichlorophenyl)-2-(dimethylamino)ethyl]cyclohexanol (23). To an ice-cold solution of borane/THF complex (152 mL, 152 mmol) was added a solution of 1-[(3,4-dichlorophenyl)[(dimethylamino)carbonyl]methyl]cyclohexanol (30 g, 90 mmol) in THF. The mixture was refluxed for 2 h and cooled in an ice bath, 2 N HCl (23 mL) was added, and the mixture was again refluxed for 1.5 h. The reaction mixture was allowed to cool overnight and basified to pH 14 with solid KOH, and the layers were separated. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated to a solid. This was filtered, washed with petroleum ether, and air-dried to yield 15.4 g; mp 128–130 °C. The solid was dissolved in ether and treated with 2-propanolic HCl to give **22**·HCl: mp 241–244 °C; ^{1}H NMR (DMSO-d_6) δ 7.5 (m, 4 H, Ar H), 3.45 (m, 2 H, $CHCH_2N(CH_3)_2$), 3.25 (t, 1 H, $CHCH_2N(CH_3)_2$), 2.65 (s, 6 H, $N(CH_3)_2$), 1.25 (m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{23}NO_2$·HCl) C, H, N.

1-[1-(4-Methoxyphenyl)-2-(methylamino)ethyl]cyclohexanol (6). To a solution of **5** (13 g, 0.052 mol) in methylene chloride (200 mL) was added $NaHCO_3$ (80 g), followed by ethyl chloroformate (10 mL, 0.1 mol). After stirring at room temperature during 20 h, the reaction mixture was washed with water, saturated $NaHCO_3$, water, 1 N HCl, brine, and dried (K_2CO_3). After filtration, the solution was concentrated in vacuo to a white solid (11.7 g). This intermediate was dissolved in THF (120 mL) and added under N_2 to an ice-cold suspension of lithium aluminum hydride (6 g) in THF (200 mL). After refluxing for 1.5 h the reaction mixture was cooled in an ice bath and a mixture of water and THF (200 mL, 1:1 v/v) was added followed by a 25% solution of NaOH (80 mL). The mixture was filtered and the filtrate was evaporated to an oil which on crystallization from 2-propanolic

HCl/ether yielded 6·HCl (8.2 g, 54%): mp 165–167 °C; ^{1}H NMR (DMSO-d_6) δ 7.3 and 6.96 (q, 4 H, Ar H), 3.34 (m, 2 H, $CHCH_2NHCH_3$), 3.08 (t, 1 H, $CHCH_2NHCH_3$), 2.46 (s, 3 H, $NHCH_3$)), 1.36 (m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{25}NO$·HCl) C, H, N.

1-[(4-Aminophenyl)[(dimethylamino)carbonyl]methyl]cyclohexanol (VI, R = 4-NH_2, n = 2, R_1 = R_2 = CH_3). 4-Amino-*N*,*N*-dimethylbenzeneacetamide (17 g, 95 mmol) in THF (500 mL) was cooled to −20 °C under N_2 and 1,1,4,4-tetramethyl-1,4-dichlorodisilethylene (23.6 g, 0.11 mmol) was added, followed by a solution of sodium bis(trimethylsilyl)amide (42 g, 0.23 mol) in THF (250 mL).[11] The mixture was allowed to warm to room temperature and was stirred for 18 h. It was then cooled to −78 °C and 1.6 N *n*-butyllithium (71.6 mL, 0.11 mol) in hexane was added. The reaction was stirred for 45 min at −78 °C and then cyclohexanone (20 mL, 0.19 mol) was added. After an additional 1 h of stirring at −78 °C, the reaction was poured into saturated aqueous ammonium chloride (100 mL). The organic phase was removed and the aqueous phase was extracted with diethyl ether. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to yield crude 1-[(4-aminophenyl)[(dimethylamino)carbonyl]methyl]cyclohexanol (20 g). Column chromatography on silica gel with 1% methanol in methylene chloride gave a white solid (16 g). The analytical sample was twice recrystallized from ethanol: mp 169–170 °C; ^{1}H NMR ($CDCl_3$) δ 7.2 and 6.65 (q, 4 H, Ar H), 4.15 (br s, 3 H), 3.55 (s, 1 H), 2.95 and 2.92 (s, 3 H each), 1.48 (br m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{24}N_2O_2$) C, H, N.

1-[1-(4-Aminophenyl)-2-(dimethylamino)ethyl]cyclohexanol (30). A solution of 1-[(4-aminophenyl)[(dimethylamino)carbonyl]methyl]cyclohexanol (5 g, 18 mmol) in THF (300 mL) was added dropwise to a mixture of lithium aluminum hydride (1.1 g, 29 mmol) and concentrated H_2SO_4 (8.0 mL) in THF (200 mL) at 0 °C. The mixture was stirred at 0 °C for 5 h and then the excess reagent was destroyed by the dropwise addition of THF/water (4 mL, 1:1 v/v), followed by 15% aqueous sodium hydroxide (4 mL), and finally water (4 mL). The mixture was filtered and the precipitate was washed several times with THF. The combined filtrates were evaporated, and the residue was recrystallized from 2-propanol to give **30** (3.8 g, 80%). Treatment of **30** with excess oxalic acid in ethyl acetate gave **30**·$2C_2H_2O_4$: mp 105 °C; ^{1}H NMR (DMSO-d_6) δ 7.0 and 6.56 (q, 4 H, Ar H), 3.54 (m, 2 H, $CH_2N(CH_3)_2$), 2.86 (m, 1 H, $CHCH_2N(CH_3)_2$), 2.65 (s, 6 H, $N(CH_3)_2$), 1.3 (br m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{26}N_2O$·$2C_2H_2O_4$) C, H, N.

1-[2-(Dimethylamino)-1-(4-nitrophenyl)ethyl]cyclohexanol (31). A solution of **30** (2.0 g, 7.6 mmol) in methylene chloride (50 mL) was added dropwise to a stirred solution of nitrosonium tetrafluoroborate (2.2 g, 19 mmol) in methylene chloride (25 mL) and the reaction was stirred at room temperature for 4 h. The methylene chloride was then evaporated and replaced with water (100 mL). This solution was added slowly to a slurry of copper powder (2 g) in 1 N aqueous sodium nitrite (200 mL) and the combination was stirred for 2 h at room temperature. Extraction with methylene chloride, drying ($MgSO_4$), and evaporation afforded **31** (2 g, 90%). Recrystallization from 2-propanol with addition of 4 N 2-propanolic HCl (5 mL) gave **31**·HCl: mp 211–212 °C; NMR (DMSO-d_6) δ 9.9 (br s, 1 H), 8.21 and 7.7 (q, 4 H, Ar H), 4.77 (br s, 1 H), 3.68 (m, 2 H, $CH_2N(CH_3)_2$), 3.29 (m, 1 H, $CHCH_2N(CH_3)_2$), 2.74 (s, 6 H, $N(CH_3)_2$), 1.4 (br m, 10 H, cyclohexyl H). Anal. ($C_{16}H_{24}N_2O_3$·HCl) C, H, N.

Resolution of 1-[2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol (4). A solution of **4** (61.8 g, 0.223 mol) in ethyl acetate (450 mL) was treated with a solution of di-*p*-toluoyl-L-tartaric acid monohydrate (43 g, 0.11 mol) in ethyl acetate (320 mL) and kept at room temperature overnight. The neutral di-*p*-toluoyl-L-tartrate salt was filtered, dried, and recrystallized three times from a mixture of ethyl acetate and methanol (6:1, v/v) to yield 44.3 g of white crystals: mp 126–128 °C; $[\alpha]^{25}_D$ −56.1° (*c* 0.99, EtOH).

The salt was added to 2 N NaOH and the free base was extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and evaporated to a crystalline residue (+)-4 (25.6 g): mp 102–104 °C; $[\alpha]^{25}_D$ +27.6° (*c* 1.07, 95% EtOH).

The free base (23.2 g) was dissolved in ether (500 mL) and treated with 4.5 N 2-propanolic HCl (25 mL). The precipitated solid was recrystallized from a mixture of methanol (100 mL) and ether (500 mL) to yield (+)-4·HCl (23.7 g, 68%): mp 240–240.5 °C; $[\alpha]^{25}_D$ −4.7° (*c* 0.945, EtOH). Anal. ($C_{17}H_{27}NO_2$·HCl) C, H, N.

A sample of the hydrobromide, for X-ray analysis, was obtained in a similar fashion with isopropanolic HBr and recrystallized from 2-propanol to give (+)-4·HBr: mp 210–212 °C; $[\alpha]^{25}_D$ −4.4° (*c* 0.64, EtOH). Anal. ($C_{17}H_{27}NO_2$·HBr) C, H, N, Br.

The combined mother liquors containing (−)-4 were treated with 1 N NaOH, washed with brine, dried ($MgSO_4$), and evaporated to a solid (30.4 g, 0.111 mol). The solid was dissolved in ethyl acetate (225 mL) and treated with di-*p*-toluoyl-D-tartaric acid monohydrate (22.24 g, 55 mmol) and kept at room temperature during 3 h. The crystalline precipitate was recrystallized twice from methanol/ethyl acetate to give (−)-4·di-*p*-toluoyl-D-tartrate (44.4 g): mp 124–126 °C; $[\alpha]^{25}_D$ +55.70° (*c* 0.97, EtOH).

The free base and hydrochloride salt (19.4 g, 55%) were obtained in the manner previously described.

(−)-4: mp 102–104 °C; $[\alpha]^{25}_D$ −27.1° (*c* 1.04, 95% EtOH).

(−)-4·HCl: mp 240–240.5 °C; $[\alpha]^{25}_D$ +4.6° (*c* 1.0, EtOH). Anal. ($C_{17}H_{27}NO_2$·HCl) C, H, N.

X-ray Crystallography of (+)-4·HBr. Crystals of the hydrobromide salt of (+)-1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol [(+)-4·HBr] were grown from ethanol by vapor diffusion of ether into the solution. The crystals were monoclinic, space group $P2_1$, with unit cell dimensions a = 5.905 (2) Å, b = 11.625 (3) Å, c = 13.552 (4) Å, and β = 97.68 (2)°. The measured crystal density, 1.30 g cm^{-3}, and unit cell volume indicate two formula units of $C_{17}H_{28}NO_2^+Br^-$ (MW 358.3) in the unit cell. The calculated density is 1.291 g cm^{-3}. The unit cell dimensions and intensity measurements were made on an Enraf-Nonius CAD4 diffractometer. The radiation used was Ni-filtered Cu Kα (λ = 1.5480 Å). A total of 1757 reflections were measured by the $\omega/2\theta$ scan mode in the range $4° \le 2\theta \le 120°$. Absorption[22] and radiation decay corrections were applied (max absorption factor 1.61). Following data reduction there were 1429 reflections with $I > \sigma(I)$ used for the structure solution and least-squares refinement.

The structure was solved by Patterson and heavy-atom Fourier methods. The parameters of the non-hydrogen atoms were refined by a full-matrix least-squares technique. The non-hydrogen atoms were assigned anisotropic thermal parameters. Toward the conclusion of the structure refinement, hydrogen atoms were included with the isotropic temperature factors of the bonded heavy atom but their parameters were not refined. The final agreement factor R ($=\sum||F_o| - |F_c||/\sum|F_o|$) for this model is 0.037. A final difference electron density map had peaks and troughs of ±1 eÅ^{-3} in the region of the Br$^-$. Computer programs used were from the XRAY70 system[23] with scattering factors for C, N, O, and Br atoms from Cromer[24] and for H atoms from Mason and Robertson.[25]

In order to determine the absolute configuration of the molecule, a set of 100 reflections to which Br′ made a positive contribution to the scattering intensity were remeasured along with their Freidel mates. For these data, the inclusion of the anomalous dispersion correction for Br′ resulted in R factors of 0.030 for S and of 0.050 for R enantiomorphs. The R factor ratio test (ratio = 1.163) shows that the hypothesis that the configuration at C1 is R can be rejected at better than the 0.005 significance level.[26] The complete set of data was used to test the same hypothesis (ratio of R factors 1.043) and confirms the previous conclusion obtained with the selected data set.

Tables of final atomic positional parameters, atomic thermal parameters, and bond distances and angles are submitted as supplementary material.

Pharmacology. Receptor Binding. In Vitro Affinities. Receptor affinities were assessed by methods described previously.[19] The displacement of the applicable tritium-labeled ligand present at the K_D concentration was measured in homogenates

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of rat brain tissue (0.2–0.3 mg protein/mL) in the presence of various concentrations of test compounds. Binding was measured by liquid-scintillation counting after standard vacuum filtration and separation of bound tissue from free radioactivity.[27]

Effects of Subacute Treatments on Receptor Binding. Two concentrations of [^{3}H]dihydroalprenolol (DHA) and [^{3}H]-spiperone were employed to estimate β-adrenergic and serotonergic (5-HT$_2$) receptor binding.

Three groups of 10 rats each were administered either 0.9% saline (1 mL/kg) or 4·HCl (10 mg/kg). All injections were given ip twice a day for 14 days; 24 h after the last injection, rats were decapitated and the frontal cortex (for 5-HT$_2$) and the remainder of the cortex (for β-adrenergic binding) were dissected out and frozen at −70 °C for subsequent assay in the appropriate in vitro receptor binding assay.

Synaptosomal Uptake. Rat brain cortical synaptosomes were prepared by modification of the sucrose density gradient/vertical rotor centrifugation method of Wood and Wyllie.[19,28] Uptake of [^{3}H]NE and [^{14}C]-5-HT was measured by incubating synaptosomes with both labeled substrates in the presence of varying concentrations of the test compound. After incubation, the mixtures were filtered through 0.45-μM cellulose acetate filters, and residual radioactivity was determined by liquid-scintillation counting. Uptake in the presence of test compound was expressed as a percent of basal uptake (without test compound) over 4 min at 37 °C. An IC$_{50}$ was calculated by determining the concentration of named test compound which resulted in 50% of basal uptake.

Antagonism of Reserpine-Induced Hypothermia. Test compounds, suspended or dissolved in 0.25% Tween 80 in water, were administered ip or po at several dose levels (1, 3, 10, 30, 100, mg/kg) to male mice which had been pretreated (18 h) with 5.0 mg/kg reserpine subcutaneously. Rectal temperatures were recorded at intervals of 1, 2, and 3 h after test-drug administration. Two-way analysis of variance with subsequent Dunnett's comparison to control tests were used to determine the minimally effective dose (MED) for antagonism of hypothermia.

Antagonism of Histamine-Induced ACTH Release. Male Sprague–Dawley rats (200–300 g, Charles River) were used for this assay, an adaptation of the procedure of Reilly and Sigg.[15] Test compounds (10 mg/kg) dissolved in saline, were administered ip 1 h before the injection of either saline or histamine (5 mg/kg, ip). Ten minutes following the second injection, the animals were decapitated and trunk blood samples were collected. Serum samples were prepared through two 2-min centrifugations at 4 °C, divided into 300 μM aliquots, and frozen at −60 °C until radioimmunoassay for ACTH concentrations. These were determined with a commercially available kit (Immuno Nuclear Corp., Stillwater, MN). Results were expressed as pg ACTH/mL serum and subjected to a two-way analysis of variance with Dunnett's comparison to control with Student–Neuman–Keuls multiple comparison tests.

Induction of Noradrenergic Subsensitivity in the Rat Pineal Gland. Male Sprague–Dawley rats were injected twice daily with either saline or a test compound (10 or 30 mg/kg, ip) for 5 days (total of nine injections) and maintained in continuous light throughout the experiment. Another group of rats received saline injections twice daily for 5 days, followed by a single injection of test compound (10 or 30 mg/kg, i.p.) on the 5th day. One hour following the final injection of the test compound or saline, animals were administered either 0.1% ascorbic acid (controls) or isoproterenol (2 μmol/kg in 0.1% ascorbic acid). Rats were decapitated 2.5 min later and the pineal glands were removed, homogenized in perchloric acid, and centrifuged. The cAMP content of the neutralized extract was measured by radioimmunoassay[29] with ^{125}I-labeled antigen and antiserum (New England Nuclear Corp., Boston, MA). Results were calculated as pmol cAMP/pineal and expressed as a percent change from the control isoproterenol-stimulated values. Analysis of variance with Student–Neuman–Keuls tests was used to determine significant differences from control values.

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Supplementary Material Available: Tables of final atomic positional parameters, atomic thermal parameters, and bond distances and angles for (+)-**4**·HBr (3 pages). Ordering information is given on any current masthead page.

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Synthesis and Activity of a Potent *N*-Methyl-D-aspartic Acid Agonist, *trans*-1-Aminocyclobutane-1,3-dicarboxylic Acid, and Related Phosphonic and Carboxylic Acids

Robin D. Allan,*,† Jane R. Hanrahan,† Trevor W. Hambley,‡ Graham A. R. Johnston,† Kenneth N. Mewett,† and Ann D. Mitrovic†

Department of Pharmacology and School of Chemistry, The University of Sydney, New South Wales 2006, Australia.
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We report the synthesis of a series of 3-carboxy-, 3-(carboxymethyl)-, 3-(ω-phosphonoalkenyl)-, and 3-(ω-phosphonoalkyl)-1-aminocyclobutane-1-carboxylic acids for evaluation as agonists or antagonists of neurotransmission at excitatory amino acid receptors, particularly *N*-methyl-D-aspartic acid (NMDA) receptors. The compounds were evaluated as agonists on their ability to depolarize the rat brain cortical wedge preparation or as antagonists of the actions of the selective agonists NMDA, quisqualic acid, and kainic acid. The chain-elongated glutamate derivatives with potential antagonist activity proved to be weak and frequently nonselective antagonists in this assay. The most noteworthy result was that trans isomer **7b** was a very potent agonist, approximately 20 times more active than NMDA at NMDA receptors, while the cis isomer was $^1/_3$ as potent as NMDA.

Of the ligands responsible for neurotransmission at excitatory amino acid receptors in the mammalian central nervous system (CNS), the excitatory amino acids L-glutamic acid and L-aspartic acid appear to be the most important.[1-3] They are, however, rather poor tools for the understanding of this phenomenon since they have other

† Department of Pharmacology.
‡ School of Chemistry.

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