was dissolved in ethyl acetate/hexanes (1:1, 2 mL). This solution was chromatographed via a circular silica gel plate (2 mm) spun by a Chromatotron. The product was eluted with hexanes/ethyl acetate [9:1 (130 mL), then 7:1 (100 mL)]. Fractions 3 and 4 were combined. After removal of the solvent, the resulting thick oil (0.27 g) solidified on standing. Recrystallization (hot 95% ethanol) gave, after washing (cold 95% ethanol) and drying under high vacuum [P_2O_5, room temperature (4 h) and 40 °C (1 h)], 143 mg (30.5%) of ester **3a** as colorless flakes: mp 100.3–100.8 °C; IR (KBr) 1717 cm^{-1} (C=O); ^1H NMR ($DCCl_3$) δ 1.38 [s, 6 H, $C(CH_3)_2$], 2.29 [d, J = 1.2 Hz, 3 H, $CH{=}C(CH_3)$], 3.93 (s, 3 H, OCH_3), 4.28 (s, 2 H, OCH_2), 6.77 [br s, 1 H, $CH{=}C(CH_3)$], 6.80 [d, J = 8.3 Hz, 1 H, H(7)], 7.28 [d, J = 2 Hz, 1 H, H(4)], 7.31 [dd, J = 8.3 Hz, J = 2 Hz, 1 H, H(6)], 7.41 (d, 2 H, Ar H), 8.03 (d, 2 H, Ar H); ^{13}C NMR ($DCCl_3$), majority of ^{13}C NMR signals are given Table I; nonprotonated and vinylic carbons (127.6, 136.4, 136.8, 139.6, 143.4). Anal. ($C_{21}H_{22}O_3$) C, H.

4-[2-(2,3-Dihydro-3,3-dimethyl-5-benzofuranyl)-1-propenyl]benzoic Acid (3b). Ester **3a** (85 mg, 0.26 mmol) was heated at reflux under N_2 for 5 h in a solution of NaOH (0.06 g, 1.5 mmol) in 95% C_2H_5OH (1.3 mL) and H_2O (2.8 mL). After cooling slowly to a few degrees above room temperature, the solution was acidified (litmus) with concentrated HCl. A white solid formed and was filtered, washed (H_2O, about 10 mL), and recrystallized (hot ethanol followed by a wash with dry hexane) to give after drying 41 mg (51%) of acid **3b** as white crystals: mp 197.0–198.6 °C; ^1H NMR ($DCCl_3$) δ 1.39 [s, 6 H, $(CH_3)_2C$], 2.30 [d, J = 1 Hz, 3 H, $CH{=}C(CH_3)$], 4.28 (s, 2 H, OCH_2), 6.78–6.83 [m, 2 H, $CH{=}C(CH_3)$ and H(7)], 7.29 [d, J = 2 Hz, 1 H, H(4)], 7.32 [dd, J = 8.2 Hz, J = 2 Hz, 1 H, H(6)], 7.46 (d, 2 H, Ar H], 8.12 (d, 2 H, Ar H); ^{13}C NMR ($DCCl_3$), the majority of ^{13}C NMR signals are given in Table I; nonprotonated and vinylic carbons (126.7, 136.4, 136.8, 140.0, 144.3). Anal. ($C_{20}H_{20}O_3$) C, H.

Biological Screening Procedures. The method for determining the effect of a retinoid upon the TPA-induced ODC activity in mouse epidermus has been fully described.[9,10] Briefly, the test sample in 0.2 mL of acetone is applied 1 h prior to application of 10 nmol of TPA to the mouse skin. The mice were sacrificed 5 h after treatment in preparation for the ODC assay. Epidermus was separated, homogenized, and centrifuged at 30000 g. Soluble epidermal ODC activity was assayed. Each value in Table II is the mean ± SE of determinations carried out on three groups of mice with three mice per group.

The experimental methodology for performing the assay with the HL-60 cell line have been detailed previously.[12,13] Briefly, HL-60 cells were grown in a serum-free defined medium consisting of RPMI 1640 supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 5 μg of insulin/mL, and 5 μg of transferrin/mL. The retinoids were dissolved in Me_2SO or ethanol and diluted into the culture medium so that the final concentration of the solvent did not exceed 0.1%, a concentration that has no effect on differentiation. After incubation for 4 days at 37 °C in a humidified atmosphere of 5% CO_2 in air, the capacity of the cells to reduce NBT was determined. Viable cells (10^6) were harvested by centrifugation and suspended in 0.5 mL of RPMI 1640 containing 20% fetal bovine serum. This cell suspension was mixed with an equal volume of phosphate buffered saline containing 1 mg of NBT/mL and 200 ng of TPA/mL. The reaction mixture was incubated at 37 °C for 25 min, and the reaction was terminated by cooling in an ice–water bath. Cytospin slides were prepared from a portion of this reaction mixture and stained with Wright-Giemsa. A minimum of 200 cells were counted under light microscopy to determine the percentage of cells with cell-associated formazan.

Acknowledgment. We gratefully acknowledge partial support by the College of Arts and Sciences in the form of salary (K.D.B.). We also gratefully acknowledged partial support of the National Science Foundation in the form of a Department grant for the purchase the XL-300 NMR spectrometer (CHE81-06157). We are pleased to acknowledge the aid of Dr. K. Loening, Director of Nomenclature at Chemical Abstracts, in deducing the correct names for the compounds cited herein. It is a pleasure to acknowledge the expert help of Beverly R. Keene at the National Institute of Health in performing the HL-60 assay.

Synthesis and Antidepressant Properties of Novel 2-Substituted 4,5-Dihydro-1H-imidazole Derivatives[1]

Mark P. Wentland,* Denis M. Bailey,* E. John Alexander, Michael J. Castaldi, Richard A. Ferrari, Dean R. Haubrich, Daniel A. Luttinger, and Mark H. Perrone

Departments of Medicinal Chemistry and Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.
Received November 25, 1986

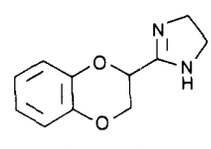
A unique combination of α_2-adrenoreceptor antagonist and serotonin-selective reuptake inhibitory activities has been identified in a series of 2-substituted 4,5-dihydro-1H-imidazole derivatives. This combination of blocking activities has provided one of these derivatives, napamezole hydrochloride (**2**), with potential as an antidepressant. A discussion of the syntheses of these compounds includes a convenient method for the conversion of nitriles to imidazolines with ethylenediamine and trimethylaluminum.

Inhibition of norepinephrine release in central nervous system adrenergic neurons via presynaptic stimulation of α_2-adrenoreceptors has been found to be blocked in vitro and in vivo by idazoxan,[2] mianserin,[3] yohimbine,[4] and other α_2-adrenoreceptor antagonists. The characterization and pharmacology of subtypes 1 and 2 of α-adrenoreceptors has been extensively reviewed.[5-8] The structure–activity relationships of α_2-adrenoreceptor antagonists has also been reviewed.[9]

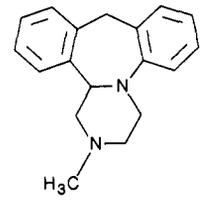
Since depletion of biogenic amines in the brain is thought to contribute to the symptomology of depression, α_2-adrenoreceptor antagonists have emerged as potential antidepressants having a novel mechanism.[3] These agents

(1) This work was presented in part at the 191st National Meeting of the American Chemical Society, April 13-18, 1986, New York, NY; MEDI 57.
(2) Chapleo, C. B.; Myers, P. L.; Butler, R. C. M.; Doxey, J. C.; Roach, A. G.; Smith, C. F. C. *J. Med. Chem.* **1983**, *26*, 823.
(3) Pinder, R. M. *Drugs Future* **1985**, *10*, 841.
(4) Goldberg, M. R.; Robertson, D. *Pharmacol. Rev.* **1983**, *35*, 143.
(5) Timmermans, P. B. M. W. M.; van Zwieten, P. A. *J. Med. Chem.* **1982**, *25*, 1389.
(6) Timmermans, P. B. M. W. M.; van Zwieten, P. A. *Drugs Future* **1984**, *9*, 41.
(7) Molinoff, P. B. *Drugs* **1984**, *28* (Suppl 2), 1.
(8) Gothert, M. *Arzneim.-Forsch./Drug Res.* **1985**, *35*, 1909.
(9) Clark, R. D.; Michel, A. D.; Whiting, R. L. *Prog. Med. Chem.* **1986**, *23*, 2.

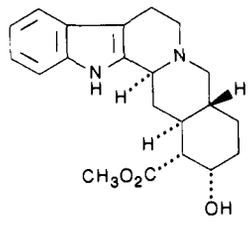
0022-2623/87/1830-1482$01.50/0



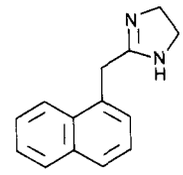
idazoxan



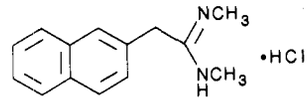
mianserin



yohimbine



naphazoline



napactadine

could very well compliment the existing clinical utility of inhibitors of monoamine reuptake (e.g., tricyclic antidepressants) and monoamine oxidase (e.g., pargyline).[10]

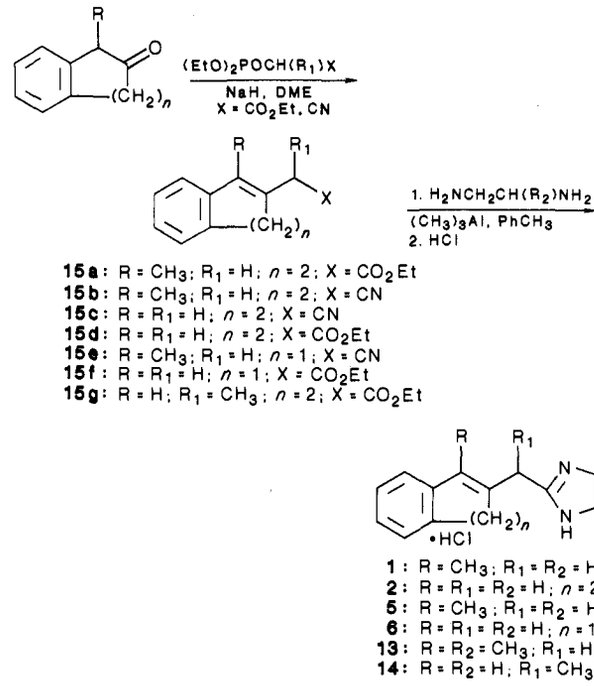
In this study we have prepared a series (**1**–**14**) of 2-substituted imidazolines as potential α_2-adrenoreceptor antagonists. These agents have structural features common to many α-adrenergic agents such as idazoxan and the α_1-adrenoreceptor agonist naphazoline. In addition to the selective α_2-adrenoreceptor antagonist properties identified in this series, several members also exhibited serotonin-selective reuptake inhibitory activity. This combination of blocking activities is, to our knowledge, unique and the first to be reported. A potential advantage of an agent having this dual mechanism might be a more rapid onset of the therapeutic effect. The delay in onset of the effects of tricyclic and monoamine oxidase inhibitor antidepressants is believed to be a consequence of a progressive decrease in the density of central β-adrenoreceptors.[11] Results from animal studies show that α_2-adrenoreceptor antagonists enhance the tricyclic antidepressant induced decrease in central β-adrenoreceptor density.[11-13] To our knowledge only one clinical study using such a combination (DMI and yohimbine) has been reported.[14] This combination was not efficacious in treating patients refractory to standard antidepressant therapy.

The norepinephrine reuptake inhibitory and antidepressant activities of a related series, which includes napactadine, was recently described.[15] No reference, however, was made to α-adrenergic testing of these compounds.

Chemistry

The structures of the derivatives prepared for biological testing are shown in Table I. Compounds **1**, **2**, **5**, **6**, **13**,

Scheme I



15a: R = CH_3; R_1 = H; n = 2; X = CO_2Et
15b: R = CH_3; R_1 = H; n = 2; X = CN
15c: R = R_1 = H; n = 2; X = CN
15d: R = R_1 = H; n = 2; X = CO_2Et
15e: R = CH_3; R_1 = H; n = 1; X = CN
15f: R = R_1 = H; n = 1; X = CO_2Et
15g: R = H; R_1 = CH_3; n = 2; X = CO_2Et

1: R = CH_3; R_1 = R_2 = H; n = 2
2: R = R_1 = R_2 = H; n = 2
5: R = CH_3; R_1 = R_2 = H; n = 1
6: R = R_1 = R_2 = H; n = 1
13: R = R_2 = CH_3; R_1 = H; n = 2
14: R = R_2 = H; R_1 = CH_3; n = 2

and **14** were prepared as shown in Scheme I[16] with either 2-tetralone, 1-methyl-2-tetralone, 2-indanone, or 1-methyl-2-indanone as starting material.[17] With the 1-methyl-2-tetralone example, its exposure to either triethyl phosphonoacetate or diethyl (cyanomethyl)phosphonate under standard Wadsworth–Emmons conditions gave the intermediates **15a** and **15b** in yields of 83% and 37%, respectively.

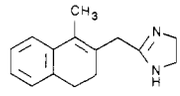
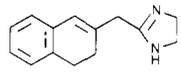
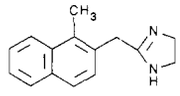
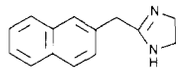
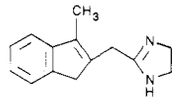
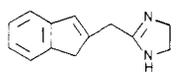
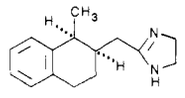
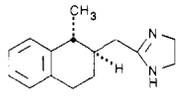
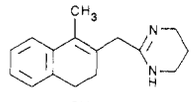
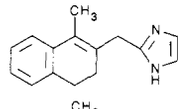
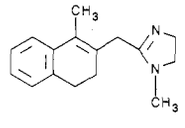
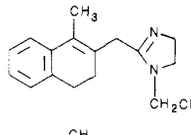
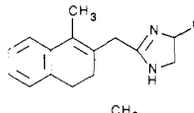
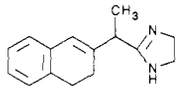
Of the many documented methods[18] for the synthesis of amidines and more specifically imidazolines, one recent report described the direct conversion of aliphatic and aromatic ethyl esters to imidazolines with ethylenediamine (EDA) and trimethylaluminum (TMA) in toluene.[19] As an example, ethyl phenylacetate was converted to 2-benzyl-4,5-dihydro-1*H*-imidazole in 89% yield. Application of this method to the conversion to **15a** to **1** proceeded smoothly in an unoptimized yield of 33% (includes conversion to a water-soluble HCl salt). Subsequent to this experiment we discovered that nitrile **15b** could also give **1** (41%) by using the TMA–EDA procedure. This method for the direct conversion of nitriles to imidazolines was extended to several additional examples.

Compound **2** was made from both nitrile **15c**[20] and ester **15d**[20] by using the TMA–EDA procedure. Crude nitrile **15e**, prepared from 1-methyl-2-indanone[17] and diethyl (cyanomethyl)phosphonate, was used to make **5**, 2-(1-methylnaphthyl)acetonitrile[21] was converted to **3**, and commercially available 2-naphthylacetonitrile provided the known compound **4**.[22]

Imidazolines **6**, **13**, and **14** were made from their corresponding ethyl esters **15f**,[23] **15a**, and **15g**[24] via the TMA–

(10) Enna, A. In *Antidepressants: Neurochemical, Behavioral, and Clinical Perspectives*; Enna, S. J., Malick, J. B., Richelson, E., Eds.; Raven; New York, 1981.
(11) Scott, J. A.; Crews, F. T. *J. Pharmacol. Exp. Ther.* **1983**, *224*, 640.
(12) Johnson, R. W.; Reisine, T.; Spotnitz, S.; Wiech, N.; Ursillo, R.; Yamamura, H. I. *Eur. J. Pharmacol.* **1980**, *67*, 123.
(13) Crews, F. T.; Paul, S. M.; Goodwin, F. K. *Nature (London)* **1981**, *290*, 787.
(14) Charney, D. S.; Price, L. H.; Heninger, G. H. *Arch. Gen. Psychiatry* **1986**, *43*, 1155.
(15) McCarthy, J. R.; Wright, D. L.; Schuster, A. J.; Abdallah, A. H.; Shea, P. J.; Eyster, R. *J. Med. Chem.* **1985**, *28*, 1721.
(16) See Experimental Section.
(17) Starting materials were commercially available except for 1-methyl-2-indanone: Edlund, U.; Bergson, G. *Acta Chem. Scand.* **1971**, *25*, 3625.
(18) Sandler, S. R.; Karo, W. *Organic Functional Group Preparations*; Academic: New York, 1972; Vol. III, p 205.
(19) Neef, G.; Eder, U.; Sauer, G. *J. Org. Chem.* **1981**, *46*, 2824.
(20) Smith, T. H.; Fijiwara, A. N.; Lee, W. W.; Wu, H. Y.; Henry, D. W. *J. Org. Chem.* **1977**, *42*, 3653.
(21) Nakazaki, M.; Isoe, S. *Bull. Soc. Chem. Jpn.* **1959**, *32*, 1202.
(22) Reynaud, P.; Dao, P. C.; Ismaili, A.; Trefouel, J. M. *C. R. Hebd. Seances Acad. Sci., Ser. C* **1969**, *268*, 432.

Table I. 2-[(2-Naphthalenyl)methyl]-4,5-dihydro-1*H*-imidazoles and Related Compounds

compd	structure	formula	mp, °C	in vitro α_2-adrenoreceptor activity[a]: [^{3}H]clonidine binding: K_i, nM	in vitro α_2-adrenoreceptor activity[a]: rat vas deferens pA_2 ± SE vs. clonidine
1		$C_{15}H_{18}N_2$·HCl	228–230	12	7.19 ± 0.16
2		$C_{14}H_{16}N_2$·HCl	235–237	23	7.76 ± 0.09
3		$C_{15}H_{16}N_2$·HCl	240–242	6.6	7.35 ± 0.13
4		$C_{14}H_{14}N_2$·HCl	262–264	48	7.67 ± 0.12
5		$C_{14}H_{16}N_2$·HCl	255 dec	6.9	7.71 ± 0.10
6		$C_{13}H_{14}N_2$·HCl	147 dec	76	7.19 ± 0.02
7		$C_{15}H_{20}N_2$·HCl	215–216	135	7.22 ± 0.07
8		$C_{15}H_{20}N_2$·HCl	151–152	401	NT[b]
9		$C_{16}H_{20}N_2$·HCl	201–203	334	IA[c]
10		$C_{15}H_{16}N_2$	209–211	88	6.92 ± 0.07
11		$C_{16}H_{20}N_2$·HCl·$^1/_3H_2O$	162–164	436	IA[c]
12		$C_{17}H_{22}N_2$·HCl	159–161	548	IA[c]
13		$C_{16}H_{20}N_2$·HCl	165–166	>800	IA[c]
14		$C_{15}H_{18}N_2$·HCl·$^1/_4H_2O$	182–185	32	7.43 ± 0.05
idazoxan				10	8.11 ± 0.07
yohimbine				78	7.91 ± 0.06
mianserin				76	6.80 ± 0.04

[a] See Experimental Section. [b] Not tested. [c] Inactive, no antagonism at 500 μM.

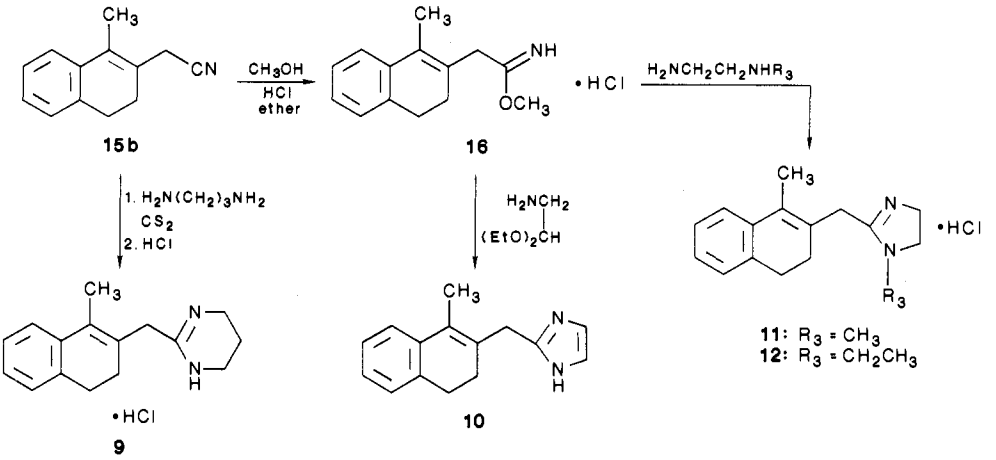
EDA route. Of particular interest is the synthesis of **13**, which utilizes a branched reagent, 1,2-diaminopropane.

The *cis*-tetrahydronaphthalene derivative **7** was made by the catalytic (H_2/Pd/C) reduction of **1**. Dissolving metal (Li/NH_3) reduction of **1** provided the trans analogue **8**.

The tetrahydropyrimidine homologue **9** was prepared by treating **15b** with 1,3-diaminopropane and catalytic CS_2 (Scheme II). Compound **15b** also served as starting material for derivatives **10–12**. Conversion of **15b** to its methyl imidate ester, **16**, was accomplished in ethereal HCl–methanol. The hydrolytically unstable **16** was sub-

(23) Ahmed, H.; Campbell, N. *J. Chem. Soc.* **1960**, 4115.
(24) Chatterjee, A.; Banerjee, D.; Mallik, R. *Tetrahedron* **1974**, *30*, 1073.

Scheme II



sequently treated with either aminoacetaldehyde diethyl acetal, *N*-methylethylenediamine, or *N*-ethylethylenediamine to give **10**, **11**, and **12**, respectively (Scheme II).

Results and Discussion

The in vitro α_2-adrenoreceptor potency of compounds **1–14** was characterized by using two assays and is summarized in Table I.[16] The affinity of the test compound for the α_2-receptor was measured in the tritiated clonidine binding assay. Compounds with low binding constants (K_i) efficiently displaced tritiated clonidine from rat brain homogenate with Hill coefficients not statistically different from unity. The antagonist properties of these compounds toward α_2-adrenoreceptors were measured in the isolated, electrically stimulated rat vas deferens and are expressed as pA_2 values.

The first six entries in Table I consist of three pairs of compounds; the difference within each pair being methyl vs. hydrogen substitution at position 1. The 1-methyl analogues **1**, **3**, and **5** have great affinities for the α_2-adrenoreceptor with K_i's near to that of idazoxan. Although compound **2** had about half the potency as its 1-methyl counterpart (**1**), the other 1-hydrogen analogues, **4** and **6**, had substantially weakened affinities relative to the methyl derivatives, **3** and **5**, respectively. Of the six analogues, the one with the weakest affinity for the α_2-adrenoreceptor, compound **6**, had a K_i very close to those of yohimbine and mianserin.

Upon comparing the antagonist potencies within each pair, an inverse correlation with the binding activity was evident in the first two pairs. Specifically, compounds **2** and **4** were significantly better antagonists than their 1-methyl counterparts, **1** and **3**, respectively. The reverse was true for the pair **5** and **6**. Within this group, the compounds (**2**, **4**, and **5**) having the best α_2-adrenoreceptor antagonist potencies (pA_2 = 7.67–7.76) were somewhat less potent than idazoxan (8.11) and yohimbine (7.91) and significantly more potent than mianserin (6.80).

From these data it was evident that, in most cases, the in vitro α_2-adrenoreceptor activity was not substantially changed by modifying one of the benzenoid rings. This trend was not sustained, however, upon testing **7** and **8**, two dihydro analogues of **1**. Compared to that of **1**, binding potency was decreased by 1 order of magnitude in the cis analogue **7**. The two did, however, have similar antagonist properties. The trans derivative **8**, had even poorer binding potency having a K_i value of 401.

The effect of modifying the imidazoline ring of **1** on activity was then studied. The tetrahydropyrimidine homologue **9** and the *N*-methyl- and *N*-ethylimidazolines **11** and **12** displayed very poor α_2-adrenoreceptor binding properties (K_i = 334–548) and were inactive as antagonists. Moderate activity in both the binding and antagonist assays were found for imidazole **10**.

Substitution of a methyl group at position 4 of the imidazoline of naphazoline was reported to impart antagonist activity to this α-adrenergic agonist.[25] When this same substitution was made with **1**, the resulting compound **13** was devoid of binding or antagonist activity. The decreased activity upon this type of substitution was similarly noted in a 3,4-dihydroxytolazoline study.[26] Another branched derivative **14** showed somewhat weaker binding and similar antagonist potency relative to its positional isomer, **1**.

Within this small series it is apparent that binding and antagonist activities remain relatively constant with changes in one of the benzenoid rings but are markedly affected by modifications of the imidazoline. Exceptions are the reduced derivatives **7** and **8** and imidazole **10**.

In the electrically and nonelectrically stimulated rat vas deferens, we never observed any evidence of agonist activity in the test compounds. In the absence of agonist activity, the correlations noted between binding and antagonist potencies might be a consequence of the different tissues (brain vs. vas deferens) used in the assays.

In order to determine if the α_2-adrenoreceptor antagonist properties identified in vitro could be translated to in vivo activity, the capacity of compounds **1–14** to antagonize clonidine-induced antinociception (ACA test) in mice was measured.[16] With the exception of derivative **2**, data from these tests provided little information and guidance due mainly to weak potency (ED_{50} > 100 mg/kg) upon oral and/or subcutaneous administration and to the lack of good dose–response relationships. Since several of the compounds were potent α_2-adrenoreceptor antagonists in vitro, their poor performance in the ACA test might be due to an undesirable pharmacokinetic profile (e.g., poor oral bioavailability, low plasma or brain levels, extensive metabolism). In light of these results, only the biological activities of **1**, **2**, and the appropriate reference compounds will be given in subsequent discussions. Compound **1** did show oral activity in other tests and thus is included in these comparisons even though it showed a poor dose response in the ACA test (maximal antagonism of 50% at 100 mg/kg po). The other oral ACA data summarized in Table II indicate that, for compound **2**, in vitro α_2-adre-

(25) Hsu, F.-L.; Hamada, A.; Booher, M. E.; Fuder, H.; Patil, P. N.; Miller, D. D. *J. Med. Chem.* **1980**, *23*, 1232.
(26) Miller, D. D.; Hamada, A.; Craig, E. C.; Christoph, G. G.; Gallucci, J. C.; Rice, P. J.; Banning, J. W.; Patil, P. N. *J. Med. Chem.* **1983**, *26*, 957.

Table II. Oral Activity in Rodents

compd	antagonism of clonidine-induced antinociception: mouse ED_{50}, mg/kg po[a,b]	inhibition of tetrabenazine-induced ptosis: mouse ED_{50}, mg/kg po[a,b]	inhibition of serotonin uptake, PCA method: rat MED, mg/kg po[a,d]
1	50% at 100[c]	18 (12–25)	30
2	36 (15–86)	35% at 30[c]	30
idazoxan	1.3 (0.5–3.8)	>30	>30
imipramine	NT[e]	4.1 (1.8–9.3)	30

[a] See Experimental Section. [b] (95% confidence limits). [c] Maximum response. [d] MED = minimum effective dose. [e] Not tested.

Table III. Receptor Selectivity of α-Adrenoreceptor Antagonists in vitro

compd	binding assay, K_i (nM) for displacement[a]		selectivity ratio α_1/α_2	antagonism of α-adrenoreceptors, rat vas deferens, $pA_2 \pm SE$[a]		selectivity ratio α_1/α_2[b]
	α_1([^{3}H]prazosin)	α_2([^{3}H]clonidine)		α_1(methoxamine)	α_2(clonidine)	
1	148	12	12.3	7.08 ± 0.38	7.19 ± 0.16	1.3
2	78	23	3.4	6.87 ± 0.12	7.76 ± 0.09	7.8
idazoxan	261	10	26.1	6.42 ± 0.07	8.11 ± 0.07	49

[a] See Experimental Section. [b] antilog pA_2.

Table IV. In Vitro Monoamine Uptake Inhibitory Activity

compd	inhibition of [^{3}H]catecholamine uptake into rat brain synaptosomes: IC_{50}, μM[a,b]		
	serotonin	norepinephrine	dopamine
1	0.4 (0.3–0.5)	60 (20–180)	26 (18–39)
2	0.4 (0.2–0.8)	2.9 (1.9–4.4)	3.3 (2.5–4.5)
imipramine	0.18 (0.14–0.22)	0.03 (0.01–0.07)	0.13 (0.09–0.18)
idazoxan	>30	13 (8.6–21)	>30

[a] See Experimental Section. [b] (95% confidence limits).

noreceptor antagonist activity was carried over to the in vivo system. The compound's potency, however, was substantially lower than that of idazoxan.

Compounds 1 and 2 were also examined for α_1-adrenoreceptor binding and antagonist properties.[16] These data as compared to idazoxan are summarized in Table III. The α_1-binding potency is expressed as a K_i value for displacement of tritiated prazocin from homogenized rat brain cortex. The α_1-antagonism is presented as a pA_2 value and measures the ability of the test compound to reverse methoxamine-induced contractions of unstimulated rat vas deferens. The selectivity ratio of the two binding assays, α_1 and α_2, indicate 1 to have good selectivity for the α_2-adrenoreceptor. It is approximately 4-fold more selective than 2 and half as selective as idazoxan. A reversal was noted upon comparison of the antagonist selectivity ratios of 1 and 2. In this case 2 is 6-fold more selective than 1 as an α_2-antagonist. Idazoxan exhibits a high degree of α_2-antagonist selectivity which is approximately 6-fold greater than that of 2.

Further in vitro pharmacological testing revealed the ability of 1 and 2 to block the neuronal uptake of the monoamine neurotransmitters norepinephrine, 5-hydroxytryptamine (5-HT, serotonin), and dopamine. These properties, common to tricyclic antidepressants (e.g., imipramine), were unexpected and in combination with their α_2-adrenoreceptor antagonist qualities provided a unique pharmacologic profile. Table IV summarizes these data for 1, 2, imipramine, and idazoxan.[16] Compounds 1 and 2 were potent inhibitors of tritiated serotonin uptake in rat brain synaptosomes and had IC_{50} values near that of imipramine. Against norepinephrine and dopamine the rank order of potency was imipramine ≫ 2 ≫ 1. Idazoxan, as anticipated, displayed poor activity in this assay. Compounds 1 and 2 were weaker inhibitors of the uptake of norepinephrine or dopamine compared to their ability to block the uptake of serotonin.

Two screens were used to measure in vivo inhibition of monoamine reuptake: the reversal of tetrabenazine-induced ptosis in mice and the blockade of *p*-chloroamphetamine (PCA) induced central depletion of serotonin in rats.[16] In the ptosis test, compound 1 had an oral ED_{50} approximately 4-fold larger than that of imipramine while compound 2 displayed a maximum response of 35% at 30 mg/kg po. The latter compound induced ptosis at higher doses, possibly due to its α_2-adrenoreceptor antagonist properties.[27] Idazoxan had no effect on mouse ptosis at 30 mg/kg.

In the PCA test, substances that block the in vitro uptake of serotonin (e.g., the tricyclic antidepressants) also block the in vivo central depletion of serotonin by PCA. Treatment of rats orally with 1, 2, and imipramine blocked the in vivo depletion of serotonin induced by PCA at the same minimum effective dose of 30 mg/kg. Idazoxan showed no activity at that dose in this test.

From this study, compound 2, napamezole hydrochloride, has emerged as a promising antidepressant and is currently undergoing phase 1 clinical evaluation. The combination of α_2-adrenoreceptor antagonist and serotonin-selective reuptake blocking activities identified in vitro and in vivo for napamezole hydrochloride is, to our knowledge, not a property of any other antidepressant reported.

Experimental Section

Elemental analyses, performed by Instranal Laboratories, Rensselaer, NY, and Galbraith Laboratories, Knoxville, TN, were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analyses were within ±0.4% of the theoretical values. Melting points were taken in capillary tubes and are not corrected. All compounds were routinely check by proton NMR (Varian HA-100 or IBM AM-200), IR (Perkin-Elmer 21), TLC (silica gel), and in most instances mass spectrometry (JEOLCO JMS-1-OCS).

Ethyl 3,4-Dihydro-1-methyl-2-naphthaleneacetate (15a). To a stirred 0 °C suspension of 33.6 g (0.70 mol) of hexane-rinsed NaH (50% oil dispersion) in 1 L of dimethoxyethane was added dropwise over 1.25 h a solution of 163.4 g (0.70 mol) of triethyl phosphonoacetate (96%) in 200 mL of DME. The mixture was

(27) Pettibone, D. J.; Pflueger, D. J.; Totaro, J. A. *Eur. J. Pharmacol.* **1984**, *102*, 431.

stirred 2 h at ambient temperature, cooled to 15 °C, and treated dropwise over 1 h with 96.0 g (0.54 mol) of 1-methyl-2-tetralone (90%) in 100 mL of DME. After being stirred overnight at 25 °C, the brown solution was cooled in ice and 31.5 mL of HOAc was added slowly. This was poured onto ice water, and the layers were separated. The aqueous layer was extracted three times with cyclohexane, and the combined organic layers were washed with water and brine and then dried ($MgSO_4$) and concentrated to give 128.5 g of crude material. Vacuum distillation of the resulting oil (bp 106–115 °C (0.05 mm)) gave 103 g (83%) of **15a** of sufficient purity to be used in the next step. Analytically pure material was obtained by silica gel chromatography of the distillate. Anal. ($C_{15}H_{18}O_2$) C, H.

Compounds **15d**,[20] **15f**,[23] and **15g**[24] were also made by using this procedure. Purification was achieved by silica chromatography and yields were 91%, 78%, and 48%, respectively.

3,4-Dihydro-1-methyl-2-naphthaleneacetonitrile (15b). To a stirred suspension of 7.2 g (0.15 mol) of NaH (50% oil dispersion) in 200 mL of DME at 25 °C was added dropwise over 10 min 28.1 g (0.15 mol) of diethyl (cyanomethyl)phosphonate. A mild exotherm was noted. The mixture was stirred 0.75 h with cooling to 25 °C. 1-Methyl-2-tetralene (90%, 25.0 g, 0.14 mol) was then added over 0.5 h and the resulting mixture was heated at 40 °C for 2 h, poured onto ice–water, and extracted three times with ether. The combined ether extracts were dried ($MgSO_4$) and concentrated to give a crude oil, which was dissolved in ether–hexane. The desired material, **15b**, crystallized and was collected and dried to give 9.6 g (37%), mp 48–49 °C. Anal. ($C_{13}H_{13}N$) C, H, N.

With use of this same procedure, nitriles **15c** (oil, 90%)[15] and **15e** were prepared. The latter was obtained in 37% yield after silica gel chromatography and was used in the next step without further purification.

2-[(3,4-Dihydro-1-methyl-2-naphthalenyl)methyl]-4,5-dihydro-1*H*-imidazole Monohydrochloride (1). Method A. Ester **15a** upon treatment with ethylenediamine and trimethylaluminum in toluene according to the literature procedure[19] provided a crude product. This was treated with a small excess of dilute HCl in *i*-PrOH to give a water soluble salt, **1** (33%), mp 228–230 °C. Anal. ($C_{15}H_{18}N_2$·HCl) C, H, N. Compounds **2** (29%), **6** (11%), and **14** (62%) were similarly prepared from esters **15d**,[20] **15f**,[23] and **15g**,[24] respectively, by using this TMA–EDA procedure. In several instances, no difference in yield was noted when the ratio of ester:TMA:EDA was raised from 1:1.6:1.6[19] to 1:2.1:2.1. 1,2-Diaminopropane and TMA were used in this procedure to convert **15a** to **13** (42%).

Method B. To a chilled (MeOH/ice) solution of 244 mL (0.488 mol) of 2 N trimethylaluminum (in toluene) and 490 mL of toluene under N_2 was added a solution of 29.3 g (0.488 mol) of ethylenediamine in 50 mL of toluene. The ice bath was removed and stirring was continued 1 h. Compound **15b** (42.0 g, 0.232 mol) in 200 mL of toluene was then added dropwise and the resulting mixture was refluxed for 18 h. After the mixture was cooled to 25 °C, 340 mL of MeOH, 35 mL of H_2O, and 340 mL of CH_2Cl_2 were added, and the slurry was refluxed for 30 min. The solids were filtered, and the filtrate was dried (Na_2SO_4) and concentrated. The resulting crude solid was taken up in EtOH and acidified with ethanolic HCl. A 24.9-g (41%) sample of **1** was obtained, identical with that prepared via method A.

In similar fashion, 2-(1-methylnaphthyl)acetonitrile,[21] 2-naphthylacetonitrile, and **15e** were converted to **3** (40%), **4** (33%), and **5** (25%), respectively.

***cis*-4,5-Dihydro-2-[(1,2,3,4-tetrahydro-1-methyl-2-naphthalenyl)methyl]-1*H*-imidazole Monohydrochloride (7).** A mixture of 2.0 g of **1**, 200 mg of 10% Pd on C, and 100 mL of H_2O was subjected to approximately 40 psi of H_2 in a Paar shaker for 18 h. The catalyst was filtered and the solution was concentrated. Recrystallization of the resulting solid from EtOH gave 1.7 g of **7** (84%), mp 215–216 °C. Anal. ($C_{15}H_{20}N_2$·HCl) C, H, N.

***trans*-4,5-Dihydro-2-[(1,2,3,4-tetrahydro-1-methyl-2-naphthalenyl)methyl]-1*H*-imidazole Monohydrochloride (8).** Liquid NH_3 (120 mL) was added to a cold (dry ice–acetone bath) suspension of 6.8 g (0.026 mol) of **1** in 23 mL of THF. To this was added 0.50 g (0.071 mol) of Li wire in 14 portions every 5 min. The blue mixture was stirred 30 min and 4.5 g of NH_4Cl was added. The ammonia was allowed to evaporate and the residue was partitioned between H_2O and ether. Three ether extracts were dried ($MgSO_4$) and concentrated to give 6.0 g of crude product. This was chromatographed on silica gel (5% isopropylamine–95% $CHCl_3$) to give 3.5 g of clean product, which was converted to 2.4 g (35%) of pure **8**, mp 151–152 °C. Anal. ($C_{15}H_{20}N_2$·HCl) C, H, N.

2-[(3,4-Dihydro-1-methyl-2-naphthalenyl)methyl]-1,4,5,6-tetrahydropyrimidine Monohydrochloride (9). To a mixture of 1.8 g of **15b** in 20 mL of 1,3-diaminopropane at 0 °C was added 2 drops of CS_2. The mixture was then heated at 150 °C for 2 h, 2 more drops of CS_2 were added, and reluxing was continued for 18 h. Upon removal of the solvent under reduced pressure a solid formed, which was collected and converted to HCl salt **9** (1.22 g, 44%), mp 201–203 °C. Anal. ($C_{16}H_{20}N_2$·HCl) C, H, N.

2-[(3,4-Dihydro-1-methyl-2-naphthalenyl)methyl]-1*H*-imidazole (10). A 0 °C solution of 10.0 g (0.054 mol) of **15b** in 10 mL of MeOH and 100 mL of ether was treated with a stream of HCl(g) for 10 min and was allowed to stand for 3 days at −10 °C. A white solid (**16**) was collected and briefly dried. The solid was then heated on a steam bath 4 h with 7.2 g (0.054 mol) of aminoacetaldehyde diethyl acetal and 5 mL of HOAc. To this mixture was added 25 mL of 6 N HCl and the mixture was heated for 2 h (steam bath) and then stirred 18 h at 25 °C. After concentration under reduced pressure, there remained an oil which was partitioned between CH_2Cl_2 and 2 N HCl. The aqueous layer was basified with saturated K_2CO_3 and the product was extracted with CH_2Cl_2. The CH_2Cl_2 extracts were dried ($MgSO_4$) and concentrated, giving a crude solid, which was chromatographed on silica gel (preparative TLC, 5% isopropylamine–95% $CHCl_3$) to give 0.61 g (5%) of **10**, mp 209–211 °C. Anal. ($C_{15}H_{16}N_2$) C, H, N.

2-[(3,4-Dihydro-1-methyl-2-naphthalenyl)methyl]-4,5-dihydro-1-methylimidazole Monohydrochloride Hydrate (3:1) (11). A solution of 4.5 g (0.018 mol) of **16** and 1.5 g (0.019 mol) of *N*-methylethylenediamine in 30 mL of MeOH was refluxed for 20 h and cooled. Ethanolic HCl was added, and the solvents were removed under reduced pressure. A solid obtained after tritiation with ether was recrystallized from CH_3CN–ether and then from CH_3CN–acetone to give 1.5 g (29%) of **11**, mp 162–164 °C. Anal. ($C_{16}H_{20}N_2$·HCl·$^1/_3H_2O$) C, H, N.

Compound **12** was prepared from **16** and *N*-ethylethylenediamine in a similar procedure (37%), mp 159–161 °C. Anal. ($C_{17}H_{22}N_2$·HCl) C, H, N.

α_2-Adrenergic Receptor Binding Affinity. Affinity for α_2-adrenergic receptors in brain was assessed by measuring the ability of test compounds to displace [^{3}H]clonidine from its receptors. In this assay, the cerebral cortex of rat brain was homogenized in 20 volumes of Tris buffer (50 mM, pH 7.7) with a 10-s burst from Brinkman PT-10 polytron homogenizer set at 6. The homogenate was centrifuged at 48000*g* for 10 min. The pellet was then resuspended in fresh buffer and centrifuged as before. The pellet was resuspended in 50 volumes of fresh buffer, and aliquots were incubated (2 mL final volume) with [^{3}H]clonidine (New England Nuclear Corp., 20–22 Ci/mmol) at a concentration of 0.4 nM in the presence of various concentrations of test compound. Unlabeled clonidine (1 μM) was added to correct for nonspecific binding in control tubes. The samples were incubated for 30 min at 25 °C and then the membranes were harvested by filtration of the samples through a Millipore sampling manifold. Radioactivity of the filters (GF/B) in the absence and presence of test compound was measured in 10 mL of Biofluor (NEN) by Packard 460 liquid scintillation spectrometry with 40% efficiency. All assays were performed in triplicate. Data from binding assays were plotted by the logit transformation. The IC_{50} values obtained were used to calculate apparent K_i values by the method of Cheng and Prusoff.[28]

α_2-Adrenergic Antagonist Activity. α_2-Adrenergic antagonist activity was assessed by measuring the ability of test compounds to block the clonidine-induced decrease in twitch height of the isolated, electrically stimulated rat vas deferens. Male albino rats (180–220 g) were killed by cervical dislocation. Both vasa de-

(28) Cheng, Y.-C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

ferentia were removed and dissected free of connective tissue. While immersed in Krebs solution, each tissue was stripped of associated blood vessels and the semen gently expressed from the lumen. Each tissue was then mounted separately under 1.0 g of tension in a 10-mL organ bath containing warmed (33–34 °C) oxygenated (95% O_2; 5% CO_2) Krebs solution having the following composition: NaCl (119 mM), KCl (4.7 mM), $CaCl_2$ (2.5 mM), KH_2PO_4 (1.2 mM), $MgSO_4$ (0.1 mM), $NaHCO_3$ (25 mM), dextrose (11.1 mM). Repetitive field stimulation was delivered by means of Grass stimulators (Model 8) connected to platinum wire ring electrodes positioned at the top and bottom of the bath. Pulses were delivered at 80 V for 1-ms duration at a frequency of 0.15 Hz. Regular contractions of the muscle were recorded with a Grass Model 8 polygraph via Grass FT .03 transducers. The tissue was allowed to equilibrate for 40–60 min prior to addition of drugs during which time the Krebs solution was changed every 10 min. Compounds were added in a volume of 2–30 μL. Cumulative concentration–response curves were generated for clonidine alone and in the presence of various concentrations of test compound. The magnitude of the shift in the clonidine dose–response curve is indicative of the affinity of the test compound for the α_2-receptor. The results are expressed as the pA_2, which is the negative logarithm (no units) of the molar concentration of test compound needed to shift the dose–response curve of the agonist by a factor of 2. The pA_2 values were calculated by the Schild method using a computer program.[29]

Antagonism of Clonidine-Induced Antinociception in the Mouse (ACA). The intraperitoneal administration of phenyl-*p*-quinone (PPQ) to mice elicits a nociceptive response that consists of abdominal writhing and extension of the hind limbs. This writhing response is prevented in mice treated with the α_2-adrenergic agonist clonidine. When an α_2-adrenergic antagonist is given prior to clonidine, the mice display the writhing response when PPQ is administered. For each experiment 10 mice/treatment (18–24 g; Swiss Webster from Taconic Farms) were evaluated in separate compartments of a Plexiglas box. Compounds dissolved in 0.9% NaCl were administered either subcutaneously (behind the neck) or orally (intubation). Clonidine (0.2 mg of base/kg) was administered orally when the test compound was given subcutaneously and subcutaneously when the test compound was administered orally. Clonidine was administered 10 min after administration of the test compound followed 20 min later by intraperitoneal administration of PPQ (3 mg/kg). Beginning 5 min after injection of PPQ, the mice were observed for writhing for a period of 5 min. The number of mice that writhed at least 2 min during the 5-min observation period was counted. In any single study, 90–100% (generally 100%) of the mice injected with vehicle plus PPQ writhed. In clonidine-treated animals, the writhing response to PPQ was never fully restored by administration of α_2-antagonists. Mice pretreated with antagonists always exhibit the abdominal constriction, but hind limb extension seldom occurred.

The number of mice that writhed was scored for each dose of the antagonist and the percentage reversal of clonidine-induced antinociception was calculated by dividing the number of animals writhing by the total number of tested animals and multiplying the quotient by 100. All compounds were administered in a volume of 10 mL/kg; all doses were administered in terms of the free base. ED_{50} values with 95% confidence limits were determined where possible with use of the computer program of Tallarida and Murray for the Litchfield–Wilcoxon test.[29]

α_1-Adrenergic Receptor Binding Affinity. Affinity for α_1-adrenergic receptors in brain was assessed by measuring the ability of test compounds to displace [^{3}H]prazosin from its receptors. In the assay, the cortex of rat brain was homogenized in 20 volumes of Tris buffer (50 mM, pH 7.7) by a 10-s burst of a Brinkman PT 10 Polytron set at 6. The homogenate was centrifuged at 48000*g* for 10 min. The pellet was resuspended in 10 volumes of fresh buffer and the procedure was repeated. The final resuspension was in 150 volumes of buffer. The homogenate was incubated (8 mL final volume) with [^{3}H]prazosin (Amersham, 18–20 Ci/mmol) at a concentration of 0.03 nM in the presence of various concentrations of test compound. Unlabeled phentolamine (10 μM) was added to correct for nonspecific binding in control tubes. The samples were incubated for 90 min at 25 °C and then the membranes were harvested by filtration of the samples through a Millipore sampling manifold. Radioactivity on the filter (GF/B) in the absence and presence of test compound was measured in 10 mL of Biofluor (NEN) by Packard 460 liquid scintillation spectrometry with 40% efficiency. All assays were performed in triplicate. Data from binding assays were plotted by logit transformation. The IC_{50} values obtained were used to calculate apparent K_i values by the method of Cheng and Prusoff.[28]

α_1-Adrenergic Antagonist Activity. α_1-Adrenergic antagonist activity was assessed by measuring the ability of test compounds to prevent contractions of the rat vas deferens induced by methoxamine, a selective α_1-adrenergic agonist. The experimental preparation was similar to that described above for measurement of α_2-receptors except that the vasa deferentia were not stimulated electrically. Instead, the tissues were first equilibrated for 30–40 min prior to addition of test compound, during which time the Krebs solution (maintained at 24 °C) was changed every 15 min. Methoxamine was then added (3–70 μL) to give final concentrations of 0.3, 1, 3, 10, 30, 100, 300, 1000, and 3000 μM, such that a cumulative concentration–response curve was generated for each tissue. When the maximal contraction was reached, i.e., when increasing the concentration of methoxamine caused no further contractions, the tissue was washed two to three times to remove the α_1-agonist. The test compound (10–100 μL) was then added, and after a 15-min equilibration period, another cumulative concentration–response curve for methoxamine was generated with the test compound present. This procedure was repeated until concentration–response curves for methoxamine were obtained with three different concentrations of the test compound. Contractions of the muscle were recorded with a Grass Model 8 polygraph via a Grass FT .03 transducer. Antagonist activity in the vas deferens was expressed as pA_2 values, which were calculated by the Schild method with a computer program.[29]

Synaptosomal Uptake of Monoamines. Male Sprague–Dawley rats (180–240 g) were decapitated, and the brain was rapidly removed. The cerebral cortex (for norepinephrine and 5-HT uptake) or the corpus striatum (for dopamine uptake) was dissected over ice and homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose with six up-and-down strokes of a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 1000*g*. The supernatant was decanted and recentrifuged at 48000*g* for 10 min. The supernatant from the second centrifugation was decanted and the resultant pellet (P2) was resuspended in 5–10 volumes of 0.32 M sucrose (w/v).

Uptake of biogenic amines was determined by modification of the method of Coyle and Snyder.[30] The following radioisotopes were obtained from New England Nuclear Corp. (Boston, MA): Hydroxytryptamine binoxalate, 5-[1,2-^{3}H(n)], 26.4 Ci/mmol ([^{3}H]-5-HT); norepinephrine, *levo*-5-[1,2-^{3}H(n)], 2.7 Ci/mmol ([^{3}H]NE); and dihydroxyphenylethylamine, 3,4-[8-^{3}H(n)], 10.1 Ci/mmol ([^{3}H]DA). Solutions of drugs were prepared in a modified Krebs–Ringer phosphate buffer containing ascorbic acid (11 mM), ethylenediamine tetraacetate (50 μM), and nialamide (12.5 μM). A 50-μL aliquot of the P2 fraction was incubated for 5 min at 25 °C in a shaking water bath. Uptake was initiated by addition of a 50-μL aliquot of the ^{3}H-labeled amine to yield a final concentration of 0.1 μM. After a further 5-min incubation, uptake was terminated by addition of 3 mL of ice-cold saline (0.9% NaCl) and vacuum filtration over GF/B filters with 2 × 3 mL washes of the filters with saline. Nonspecific uptake was determined in parallel incubations at 4 °C. The filters were emulsified in 10 mL of Biofluor, and the radioactivity in the samples was determined by liquid scintillation spectroscopy. IC_{50} values with 95% confidence limits were determined by computer analysis of quantal dose–response data as described by Tallarida and Murray.[29]

Prevention of Tetrabenazine-Induced Ptosis. A procedure essentially the same as one previously reported was used.[31] Male

(29) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacologic Calculations*; Springer-Verlag: New York, 1981.

(30) Coyle, J. T.; Snyder, S. H. *J. Pharmacol. Exp. Ther.* **1969**, *170*, 221.

Swiss Webster mice (Taconic Farms) were treated intraperitoneally with tetrabenazine methanesulfonate (50 mg/kg). The compound was solubilized in 0.9% NaCl with moderate heating. The extent of ptosis was evaluated subjectively and then scored on a rating scale of 0–4 with a score of 0 representing complete eye closure and a score of 4 representing no closure. Test compounds were dissolved in 0.9% NaCl (10 mL/kg) and administered po 30 min before tetrabenazine. Mice were evaluated for ptosis 30 min after treatment with tetrabenazine. ED_{50} values with 95% confidence limits were determined by computer analysis of quantal dose–response data as described by Tallarida and Murray.[29]

In Vivo Uptake of 5-HT in Rat Brain Using the *p*-Chloroamphetamine (PCA) Method. Modifications of published methods were used in this study to evaluate the inhibition of serotonin uptake in vivo.[32,33] Male Sprague–Dawley rats (160–200 g, Taconic Farms, Germantown, NY) were fasted overnight. They were orally medicated by intubation (10 mL/kg) with test compounds or vehicle (triple distilled water) 15 min prior to intraperitoneal injection with PCA (3 mg/kg; 1 mL/kg). This dose of PCA was chosen because it resulted in approximately 45% depletion of serotonin levels with no overt signs of toxicity. Three hours later animals were decapitated and the forebrains were quickly excised and homogenized in 10 volumes (w/v) of ice-cold acidified 1-butanol (0.85 mL of 12 N HCl/L of 1-butanol) with a 10-s burst of a Polytron homogenizer. The homogenates were centrifuged at 6000*g* for 10 min. A 4-mL aliquot of the supernatant was added to 4 mL of *n*-heptane and 1 mL of 0.1 N HCl and the mixture was transferred to glass-stoppered centrifuge tubes. Samples were shaken on a flat-bed shaker for 15 min and were then centrifuged for 5 min at 2000*g*. The organic phase (including the tissue disk at the organic/aqueous interface) was aspirated and discarded. Serotonin content in the aqueous phase was determined spectrofluorometrically after condensation with *o*-phthaladehyde (OPT). Briefly, a 0.2-mL aliquot of the aqueous phase was added with vortex mixing to 1.0 mL of a 10 mg % solution of OPT in 10 N HCl. The tubes were placed in boiling water for 10 min and were then cooled to room temperature in tap water. Fluorescence was measured on an Aminco-Bowman Spectrofluorometer (excitation, 360 nm; emission, 470 nm, uncorrected). Standard curves were generated from known amounts of 5-HT added to 4 mL of acidified 1-butanol just prior to the first shaking step. Percent recoveries were determined from 5-HT standards added to aqueous phase just prior to reaction with OPT. The percent blockade of the PCA depletion was calculated by the formula:

$$\%\ \text{block} = [(5\text{HT levels in presence of drug + PCA}) - (5\text{HT levels in presence of PCA alone})]/[(\text{control 5HT levels}) - (5\text{HT levels in presence of PCA})]$$

Data were analyzed by using a Duncan's multiple range *t* test using SAS software. A $p < 0.05$ was considered significant.

Acknowledgment. We thank the Analytical Chemistry and Chemical Development Departments of this Institute for spectral measurements and synthesis support.

(31) Barnett, A.; Taber, R. I.; Greenhouse, D. D. *Int. J. Neuropharmacol.* **1969**, *8*, 353.
(32) Miller, F. P.; Cox, R. H., Jr.; Snodgrass, W. F.; Maickle, R. P. *Biochem. Pharmacol.* **1970**, *19*, 435.
(33) Fuller, R. N.; Perry, K. W.; Baker, J. C.; Paeli, C. J.; Lee, N.; Day, W. A.; Molley, B. B. *Biochem. Pharmacol.* **1974**, *23*, 3267.

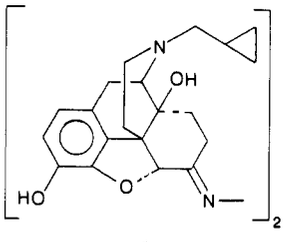
X-ray Crystal Structure of the Opioid Ligand Naltrexonazine

Z. Urbanczyk-Lipkowska,[1] A. W. Lipkowski,[†2] M. C. Etter,[‡] E. F. Hahn,[§] G. W. Pasternak,[∥] and P. S. Portoghese*[†]

Departments of Chemistry and Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, Laboratory of Biochemical Endocrinology, Rockefeller University, New York, New York 10021, and Department of Neurology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021. *Received August 8, 1986*

The anti–anti isomer of naltrexonazine (1) was synthesized, and its configuration was confirmed by X-ray crystallography. The syn–anti isomer is readily converted to 1 under acidic conditions. The apparent equal receptor binding of 1 and syn–anti isomer indicates that isomerization of the azine moiety may take place under the conditions of biological evaluation. Two possible explanations for wash-resistant binding of 1 to opioid receptors are presented. The first possibility involves a noncovalent interaction of the ligand with the opioid receptor, and the second considers covalent binding by a receptor-based sulfhydryl group.

Molecules that consist of two pharmacophores connected by a spacer chain have been termed bivalent ligands[3–9] and are of interest as probes for opioid receptors because of the possibility of "bridging" a subpopulation of vicinal receptors when the spacer is a specific length. The binding and biological activities of bivalent ligands derived from naloxone, naltrexone, and oxymorphone in which two alkaloid pharmacophores are connected by an azine moiety have been reported.[10–13] In this connection, the opioid receptor binding characteristics of naltrexonazine (1) and related azines to brain membranes have been investigated. On the basis of their reversible and wash-resistant binding components, Wolozin and Pasternak[14] divided μ receptors into two distinct μ subtypes. The naloxonazine-selective reversible and wash-resistant properties have been suggested as a means of distinguishing between receptors that mediate analgesia and those that produce respiratory depressant actions.[15,16]



1

Two questions concerning opioid azines are addressed in this paper; the first deals with the configuration and

[†]Department of Medicinal Chemistry, University of Minnesota.
[‡]Department of Chemistry, University of Minnesota.
[§]Rockefeller University.
[∥]Memorial Sloan-Kettering Cancer Center.

(1) Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland.
(2) Visiting professor from Department of Chemistry, Warsaw University, Warsaw, Poland.
(3) Erez, M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1982**, *25*, 847.
(4) Portoghese, P. S.; Ronsisvalle, G.; Larson, D. L.; Yim, C. B.; Sayre, L. M. *Life Sci.* **1982**, *31*, 1283.
(5) Costa, T.; Wuster, M.; Herz, A.; Shimohigashi, Y.; Chen, H. C.; Rodbard, D. *Biochem. Pharmacol.* **1985**, *34*, 25.
(6) Hazum, E.; Chang, K. J.; Leighton, H. J.; Lever, O. W.; Cuatrecasas, P. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 347.

0022-2623/87/1830-1489$01.50/0