SDP crystallographic package[32] with the physical constants tabulated therein.

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Supplementary Material Available: Full list of atomic coordinates, anisotropic thermal parameters, bond distances, bond angles, and an ORTEP drawing with complete labeling scheme (14 pages). Ordering information is given on any current masthead page.

(32) B. A. Frenz and Associates, SDP Plus Version 1.0, Enraf-Nonius, Delft, 1980.

Analogues of the 5-HT$_{1A}$ Serotonin Antagonist 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine with Reduced α_1-Adrenergic Affinity†

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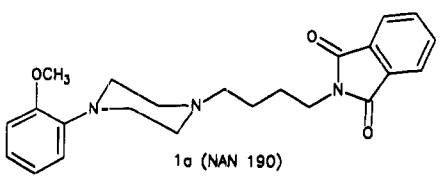
1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190; **1a**) is a putative postsynaptic 5-HT$_{1A}$ serotonin antagonist. This high affinity ligand (K_i = 0.6 nM), although selective for 5-HT$_{1A}$ versus other 5-HT receptors, binds with nearly equal affinity at α_1-adrenergic receptors (K_i = 0.8 nM). Structure–affinity relationship studies were conducted in order to achieve an improved selectivity. Replacement of the phthalimide moiety by substituted benzamides led to retention of 5-HT$_{1A}$ affinity but to no improvement in selectivity, whereas replacement by alkyl amides proved beneficial, leading to an improvement in affinity and selectivity. Branching α to the amide carbonyl group and increased bulkiness of the alkyl moiety further improved 5-HT$_{1A}$ affinity and selectivity. 4-[4-(1-Adamantanecarboxamido)butyl]-1-(2-methoxyphenyl)piperazine (**2j**) was found to bind at 5-HT$_{1A}$ sites with high affinity (K_i = 0.4 nM) and with a 160-fold selectivity over α_1-adrenergic sites. Preliminary studies show that this agent retains antagonist activity as determined in a 5-HT$_{1A}$-coupled adenylyl cyclase assay. Further functional studies are warranted to fully characterize this agent.

Introduction

Since the discovery of the heterogeneity of serotonin (5-hydroxytryptamine, 5-HT) receptors by Gaddum and Picarelli in 1957,[1] four major populations of serotonin receptors have been identified: 5-HT$_1$, 5-HT$_2$, 5-HT$_3$, and 5-HT$_4$ (for recent reviews, see refs 2 and 3). Of these, 5-HT$_1$ receptors are heterogeneous and are comprised of at least the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ subtypes.[2] The 5-HT$_{1A}$ subtype has been best characterized, owing largely to the availability of a selective and potent agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT).[4] However, research in the 5-HT$_{1A}$ area continues to be hampered by the lack of selective antagonists.

Investigations in our laboratory of the structure–affinity relationships (SAFIR) of arylpiperazines have led to the development of high-affinity ligand, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190; **1a**) that was reported to be the first antagonist at 5-HT$_{1A}$ sites.[5,6] Although, **1a** is selective for 5-HT$_{1A}$ sites over other serotonergic sites, it possesses an almost equal affinity for α_1-adrenergic receptors. The action of this ligand as an antagonist, or as a partial agonist, at 5-HT$_{1A}$ receptors is still under debate. Rydelek-Fitzgerald et al.[7] have proposed that **1a** is a partial agonist at postsynaptic sites with very low intrinsic activity, while Hjorth and Sharp[8] have suggested that **1a** is an antagonist at postsynaptic sites and a partial agonist at presynaptic sites. It has also



1a (NAN 190)

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‡ Medical College of Virginia, Virginia Commonwealth University.

§ Albany Medical College.

(1) Gaddum, J. H.; Picarelli, Z. P. Two Kinds of Tryptamine Receptor. *Br. J. Pharmacol.* **1957**, *12*, 323.
(2) Glennon, R. A.; Raghupathi, R. K. Serotonin Receptor Ligands. *Curr. CNS Pat.* **1990**, *1*, 323.
(3) Wikstrom, H.; Svensson, K. Advances in Central Serotonergics. *Ann. Rep. Med. Chem.* **1990**, *25*, 41.
(4) Middlemiss, D. N.; Fozard, J. R. 8-Hydroxy-2-(di-*n*-propylaminotetralin Discriminates between Subtypes of the 5-HT$_1$ Recognition Site. *Eur. J. Pharmacol.* **1983**, *90*, 151.
(5) Glennon, R. A.; Naiman, N. A.; Pierson, M. E.; Titeler, M.; Lyon, R. A.; Weisberg, E. NAN-190: An Arylpiperazine Analog that Antagonizes the Stimulus Effects of the 5-HT$_{1A}$ Agonist 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). *Eur. J. Pharmacol.* **1988**, *154*, 339.
(6) Glennon, R. A.; Naiman, N. A.; Pierson, M. E.; Titeler, M.; Lyon, R. A.; Herndon, J. L.; Misenheimer, B. Stimulus Properties of Arylpiperazines: NAN-190, a Potential 5-HT$_{1A}$ Serotonin Antagonist. *Drug Dev. Res.* **1989**, *16*, 335.
(7) Rydelek-Fitzgerald, L.; Teitler, M.; Fletcher, P. W.; Ismaiel, A. M.; Glennon, R. A. NAN-190: Agonist and Antagonist Interactions with Brain 5-HT$_{1A}$ Receptors. *Brain Res.* **1990**, *532*, 191.
(8) Hjorth, S.; Sharp, T. Mixed Agonist/Antagonist Properties of NAN-190 at 5-HT$_{1A}$ Receptors: Behavioral and In Vivo Brain Microdialysis Studies. *Life Sci.* **1990**, *46*, 955.

0022-2623/91/1834-2633$02.50/0

Scheme I

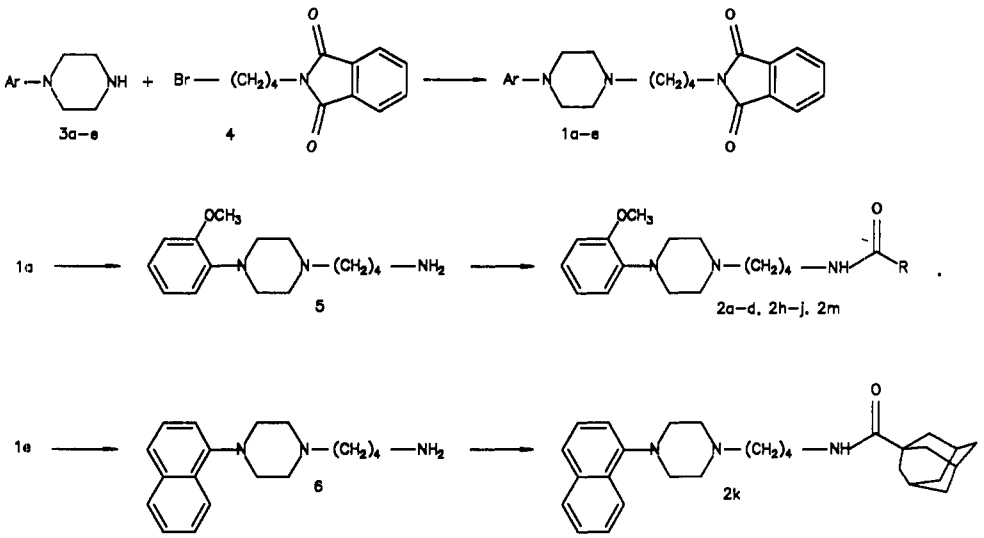
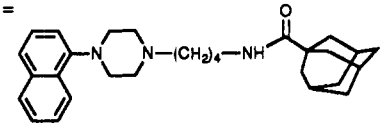


Table I. Properties of NAN-190 Analogues

	Ar/R	% yield	mp, °C	recrystn solvent	formula
1a	2-OCH_3-phenyl HBr		230–234[a]	abs EtOH	
1b	2,5-$(OCH_3)_2$-phenyl[b]	24	196–199	abs EtOH	$C_{24}H_{29}N_3O_4 \cdot 2HCl$[c]
1c	4-NO_2-phenyl	91[d]	206–207.5	MeCN	$C_{22}H_{24}N_4O_4 \cdot C_2H_2O_4$[e]
1d	2-NO_2-phenyl[f]	70	140–141.5	abs EtOH	$C_{22}H_{24}N_4O_4 \cdot C_4H_4O_4$[g]
1e	1-naphthyl[h]	67[d]	256–260	MeOH	$C_{26}H_{27}N_3O_2 \cdot HCl$
1f	4-(CH_3CONH)-phenyl	22	223–225	abs EtOH/Et_2O	$C_{48}H_{56}N_8O_6C_2H_2O_4$[i]
2a	4-Cl phenyl	31	202–204	abs EtOH	$C_{22}H_{28}ClN_3O_2 \cdot HCl$[j]
2b	3,4-$(Cl)_2$ phenyl	25	229–231	abs EtOH	$C_{22}H_{27}Cl_2N_3O_2 \cdot HCl$
2c	4-NO_2 phenyl	35	120–121	abs EtOH	$C_{22}H_{28}N_4O_4 \cdot HCl$[k]
2d	4-I-phenyl	46	201–202	abs EtOH	$C_{22}H_{28}IN_3O_2 \cdot HCl$[k]
2e	4-NH_2-phenyl	45	181–183	–[l]	$C_{44}H_{60}N_8O_4 \cdot C_4H_4O_4$[m]
2f	4-OH-phenyl	48	146–148 dec	abs EtOH	$C_{22}H_{29}N_3O_3 \cdot HCl$
2g	$(CH_2)_3$-phenyl	14	151	–[l]	$C_{25}H_{35}N_3O_2 \cdot 2HCl$[n]
2h	$(CH_2)_3CH_3$	14	109–110	Et_2O	$C_{20}H_{33}N_3O_2 \cdot C_4H_4O_4$[o]
2i	$C(CH_3)_3$	45	145	–[p]	$C_{20}H_{33}N_3O_2 \cdot C_4H_4O_4$[q]
2j	1-adamantyl	24	112–117 dec	THF	$C_{26}H_{39}N_3O_2 \cdot C_4H_4O_4$[r]
2k[t]	–	54[d]	185–186	abs EtOH/Et_2O	$C_{29}H_{39}N_3O \cdot C_4H_4O$[o]
2m	4-(BnO)-phenyl[s]	50	156–157.5	MeCN	–

[a] Literature[10] mp 239–242 °C. [b] Required the preparation of 1-(2,5-dimethoxyphenyl)piperazine.[11] [c] Crystallized with $1.0H_2O$. Anal. Calcd for $C_{24}H_{29}N_3O_4 \cdot 2HCl \cdot 1.0H_2O$: C, 56.01; H, 6.47; N, 8.17. Found: C, 55.93; H, 5.93; N, 8.06. [d] Yield of the free base. [e] Oxalate salt, crystallized with $0.1H_2O$. [f] Required the preparation of 1-(2-nitrophenyl)piperazine.[12] [g] Maleate salt. [h] Required the preparation of 1-(1-naphthyl)piperazine.[13] [i] Hemioxalate salt, crystallized from $1.25H_2O$. [j] Anal. Calcd for $C_{22}H_{28}ClN_3O_2 \cdot HCl \cdot 1.4H_2O$: C, 56.99; H, 6.91; N, 9.06. Found: C, 56.71; H, 6.48; N, 8.99. [k] Crystallized with $0.5H_2O$. [l] Free base was purified by column chromatography; salt was analytically pure without recrystallization (see the Experimental Section). [m] Hemifumarate salt, crystallized with $0.25H_2O$. [n] Crystallized with $0.75H_2O$. [o] Fumarate salt. [p] Free base was purified by acid–base extraction; salt was analytically pure without recrystallization (see the Experimental Section). [q] Fumarate salt, crystallized with $0.75H_2O$. [r] Fumarate salt, crystallized with 1.0THF. [s] Required the preparation of 4-(benzyloxy)benzoyl chloride.[23] [t] 2k =



been proposed that the presynaptic agonist activity of NAN-190 in some assays might be a consequence of its α_1-blocking properties since prazosin, an α_1-antagonist, reproduces some of the effects of NAN-190.[9] The development of a 5-HT_{1A} antagonist with reduced α_1-adrenergic affinity might help in clarifying some of these issues. Therefore, the specific goals of the present study were (a) to modify the structure of **1a** in order to decrease its α_1-adrenergic affinity, while maintaining its very high affinity for 5-HT_{1A} sites; (b) to determine how various structural features influence 5-HT_{1A}/α_1 selectivity; and (c) to obtain some preliminary functional data in order to determine if an analogue with greater selectivity retains 5-HT_{1A} antagonist activity in a functional assay.

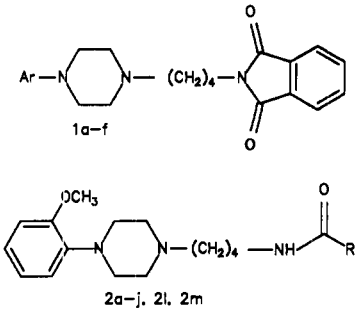
Chemistry

Appropriate arylpiperazines **3a–e** were alkylated with *N*-(4-bromobutyl)phthalimide (**4**) to yield products **1a–e** (Table I) as shown in Scheme I and as described in the Experimental Section for **1c**. Compound **1f** was prepared by catalytic reduction of **1c** in the presence of Ac_2O. Compound **5**, obtained by hydrazinolysis of **1a**,[10] was

(9) Claustre, Y.; Benavides, J.; Rouquier, L.; Serrano, A.; Scatton, B. Effects of the Putative 5-HT_{1a} Receptor Antagonist NAN-190 on Rat Brain Serotonergic Transmission. Abstracts of Papers, The Second IUPHAR Satellite Meeting on Serotonin, Basel, Switzerland, 1990, p 114.

(10) Glennon, R. A.; Naiman, N. A.; Lyon, R. A.; Titeler, M. Arylpiperazine Derivatives as High-Affinity 5-HT_{1A} Serotonin Ligands. *J. Med. Chem.* **1988**, *31*, 1968.

acylated with the appropriate aryl or alkyl acid chloride to yield products **2a–d**, **2h–j** and **2m** (Table I). Compound **2e** was prepared by catalytic hydrogenation of **2c**, and **2f** was prepared by catalytic hydrogenolysis of **2m**. Reaction of **5** with a mixture of phenylbutyric acid, ethyl chloroformate, and triethylamine afforded **2g**. Compound **6**, obtained by hydrazinolysis of **1e**, was acylated with 1-adamantanecarbonyl chloride to yield **2k** (Table I) as shown in Scheme I.



Results and Discussion

In order to improve the selectivity of **1a** for 5-HT_{1A} over α_1-adrenergic sites, structural alterations were required that would either increase affinity for 5-HT_{1A} sites or decrease affinity for α_1-adrenergic sites. Because of the already high affinity of **1a** for 5-HT_{1A} sites, the latter approach was selected. Structure–activity relationships (SARs) for adrenergic agents have been studied extensively (e.g. see refs 14–17). However, in many cases, extrapolation of these results to the present system proved to be difficult for several reasons, including nonavailability of complete pharmacological data; availability of binding data at α-adrenergic, but not α_1-adrenergic sites; and lack of binding data on closely related structures. Nevertheless, some indications were obtained as to what changes are detrimental for α_1-adrenergic affinity.

Arylpiperazine Portion (1b–f). A study by Mitani et al.[18] indicates that the following structural changes in systems analogous to arylpiperazines are detrimental for α-adrenergic activity: (a) dimethoxy substitution; (b) incorporation of electron-withdrawing substituents, such as a nitro group, at the 2-position; and (c) introduction of a nitro group at the 4-position. A study by Augstein et al.[19] further indicates that in systems analogous to arylpiperazines, a 2,5-dimethoxy substitution pattern, or replacement of a phenyl ring by a 1- or 2-naphthyl substituent, may be detrimental to α-adrenergic blocking activity. Accordingly, the 2,5-dimethoxy (**1b**), 2-nitro (**1d**), 4-nitro (**1c**), 4-acetamido (**1f**), and 1-naphthyl (**1e**) analogues were prepared.

Table II. Affinities of NAN-190 Analogues for 5-HT_{1A} and α_1 Sites

	Ar/R	K_i (nM) (±SEM) 5-HT_{1A}[a]	K_i (nM) (±SEM) α_1[b]	selectivity
1a	2-OCH_3-phenyl[d]	0.6	0.8	1
1b	2,5-$(OCH_3)_2$-phenyl	22 (±2)	15 (±2)	0.7
1c	4-NO_2-phenyl	1000 (±72)	378 (±66)	0.4
1d	2-NO_2-phenyl	4 (±1)	25 (±14)	6
1e	1-naphthyl	1.0 (±0.3)[d]	22 (±4)	22
1f	4-(CH_3CONH)-phenyl	213 (±30)	<10000	–
2a	4-Cl-phenyl	3.6 (±1.2)	23 (±3)	6
2b	3,4-Cl_2-phenyl	2.0 (±0.6)	21 (±3)	11
2c	4-NO_2-phenyl	4.4 (±0.3)	14 (±1)	3
2d	4-I-phenyl	4.0 (±0.3)	15 (±3)	4
2e	4-NH_2-phenyl	6.3 (±0.9)	23 (±0.5)	4
2f	4-OH-phenyl	5.4 (±1.2)	17 (±3)	3
2g	$(CH_2)_3$-phenyl	12 (±1)	84 (±3)	7
2h	$(CH_2)_3CH_3$	7.5 (±2.2)	84 (±9)	11
2i	$C(CH_3)_3$	1.0 (±0.2)	74 (±23)	74
2j	1-adamantyl	0.4 (±0.03)	64 (±6)	160
2k	1-adamantyl[e]	0.7 (±0.01)	109 (±20)	156
2l	phenyl		2[d]	

[a] Affinity at [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)-labeled 5-HT_{1A} sites. [b] Affinity at [^{3}H]WB-4101-labeled α_1-adrenergic sites. [c] Selectivity for 5-HT_{1A} over α_1-adrenergic sites: calculated as $K_i(\alpha_1)/K_i$(5-HT_{1A}). [d] Data from ref 10. [e] Note: the 2-methoxyphenyl portion of **2j** is replaced by a 1-naphthyl group.

2,5-Dimethoxy substitution (i.e., **1b**) of the arylpiperazine ring decreases both 5-HT_{1A} and α_1-adrenergic binding affinities leading to a nonselective compound (selectivity = 0.7, Table II) that has a lower affinity at 5-HT_{1A} sites (K_i = 22 nM) than NAN-190 (**1a**). The 4-nitrophenyl analogue **1c** also displays a decreased affinity both for 5-HT_{1A} and α_1-adrenergic sites (selectivity = 0.4) with the affinity at 5-HT_{1A} sites (K_i = 1000 nM) being much lower than that for **1a**. Although the 2-nitrophenyl analogue **1d** binds at 5-HT_{1A} sites with considerably greater affinity than **1c** (K_i = 4 nM), it displays little selectivity. The 4-acetamido analogue **1f** shows some selectivity for 5-HT_{1A} sites due to its low affinity at α_1-adrenergic sites; however, it binds at 5-HT_{1A} sites with 350 times lower affinity than **1a**. The 1-naphthyl analogue (i.e., **1e**) displays the best 5-HT_{1A} affinity/selectivity profile of the compounds in this series; it binds with high affinity (K_i = 1 nM) and a 22-fold selectivity at 5-HT_{1A} sites.

Amide Portion. (a) Benzamides. The simple benzamide analogue **2l** (K_i = 2 nM; Table II) binds at 5-HT_{1A} sites with an affinity comparable to that of **1a**. This simpler and more accessible system was therefore used as a starting point for molecular modification. From studies by Mitani et al.[18] and Campbell et al.,[20] generalizations may be made regarding modifications at the benzamido end of

(11) Brewster, K.; Coult, D. B.; Pinder, R. M. 1-Phenylpiperazines: Potential Antagonists of Lysergic Acid Diethylamide. *Chim. Ther.* **1972**, No. 2, 87.
(12) Schmutz, V. J.; Kunzle, F. Piperazino-1′,2′:1,2-Benzimidazole. *Helv. Chim. Acta* **1956**, *39*, 1144.
(13) Glennon, R. A.; Slusher, R. M.; Lyon, R. A.; Titeler, M.; McKenney, J. D. 5-HT_1 and 5-HT_2 Binding Characteristics of Some Quipazine Analogues. *J. Med. Chem.* **1986**, *29*, 2375.
(14) Werner, L. H.; Barrett, W. E. Adrenergic Blocking Agents. In *Antihypertensive Agents*; Schlittler, E., Ed.; Academic Press: New York, 1967; pp 331–392.
(15) Ghouri, M. S. K.; Haley, T. J. Structure–Activity Relationships in the Adrenergic-Blocking Agents. *J. Pharm. Sci* **1969**, *58*, 511.
(16) Timmermans, P. B. M. W. M. α-Adrenoreceptors. In *Receptor Pharmacology and Function*; Williams, M., Glennon, R. A., Timmermans, P. B. M. W. M., Eds.; Marcel Dekker: New York, 1989; pp 173–205.
(17) Ruffalo, R. R., Jr. Structure–Affinity Relationships of α-Adrenoceptor Agonists. In *Adrenoceptor and Catecholamine Action*; Kunos, G., Ed.; Wiley: New York, 1983; Vol. 1(B), pp 1–50.
(18) Mitani, K.; Sakurai, S.; Suzuki, T.; Morikawa, K.; Koshinata, E.; Kato, H.; Ho, Y.; Fujita, T. Novel Phenoxyalkylamine Derivatives. V.[1] Synthesis, α-Blocking Activity and Quantitative Structure-Activity Analysis of α-[(Phenoxyethylamino)-propyl]-α-phenylacetonitrile Derivatives. *Chem. Pharm. Bull.* **1988**, *36*, 4121.
(19) Augstein, J.; Austin, W. C.; Boscott, R. J.; Green, S. M.; Worthing, C. R. Some Cardiovascular Effects of a Series of (Aryloxy)alkylamines. 1. *J. Med. Chem.* **1965**, *8*, 356.
(20) Campbell, S. F.; Davey, M. J.; Hardstone, J. D.; Lewis, B. N.; Palmer, M. J. 2,4-Diamino-6,7-dimethoxyquinazolines. 1. 2-[4-(1,4-Benzodioxan-2-ylcarbonyl)piperazin-1-yl] Derivatives as α_1-Adrenoceptor Antagonists and Antihypertensive Agents. *J. Med. Chem.* **1987**, *30*, 49.

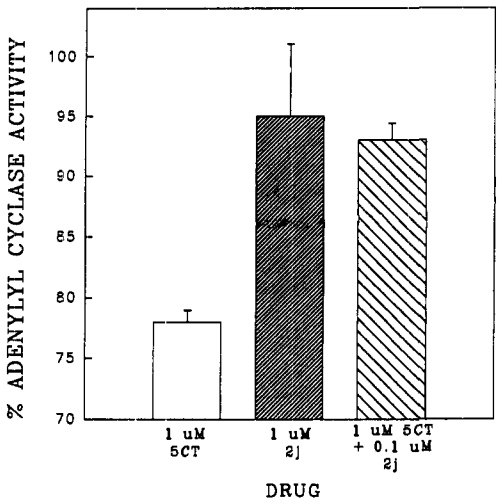


Figure 1. Effects of 5-carboxamidotryptamine (5-CT) and **2j** on forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes and the antagonism of 5-CT by **2j**. Results are expressed as percentage of adenylyl cyclase activity in the presence of 50 μM forskolin with the effect of 10 μM GTP as the base line. Values are means ±SEM obtained from three experiments.

the molecule. Electron-donating substituents are beneficial to α-adrenergic activity, whereas electron-withdrawing substituents give mixed results. Halogen substitution, for example, is detrimental to α-adrenergic activity, whereas introduction of a nitro group is not. For comparative purposes, and in order to generate SAFIR data at 5-HT_{1A} and α_1-adrenergic sites, the corresponding 4-chloro, 3,4-dichloro, 4-nitro, 4-iodo, 4-amino, and 4-hydroxy analogues **2a–f**, respectively, were prepared and evaluated.

Incorporation of substituents with wide-ranging electronic and lipophilic character into the benzamide portion of **2l** results in compounds (i.e., **2a–f**) with surprisingly little difference in affinity for 5-HT_{1A} sites (K_i = 2.0–6.3 nM) or α_1-adrenergic receptors (K_i = 14–23 nM). Consequently, these compounds display poor selectivities for 5-HT_{1A} over α_1-adrenergic receptors (ranging from 3 to 6, with the exception of the 3,4-dichloro analogue **2b**, which has a selectivity of 11). This suggests that simple, substituted benzamides are probably not the route to improved selectivity.

Amide Portion. (b) Alkyl Amides. A study by Alabaster et al.[21] suggests that alkyl amides may bind at α_1-adrenergic sites with lower affinity than benzamides. The 4-phenylbutyramide analogue **2g**, which incorporates a combination of an alkyl amide moiety and a lipophilic aromatic system, was synthesized. Although this compound binds at 5-HT_{1A} sites with lower affinity (K_i = 12 nM) than the benzamides, it is apparent that the alkyl amide reduces affinity for α_1-adrenergic receptors (selectivity = 7). Replacement of the phenyl group with a methyl (**2h**; K_i = 7.5 nM, selectivity = 11) slightly improves 5-HT_{1A} affinity and selectivity. Because there is evidence that bulk at this position may be tolerated by 5-HT_{1A} sites, we next prepared the α-branched alkyl amide **2i** (K_i = 1.0 nM, selectiity = 74). Since this substitution leads to improved 5-HT_{1A} affinity and selectivity, we examined the even bulkier, α-branched adamantyl amide **2j**; this compound binds with an affinity (K_i = 0.4 nM) slightly better than that of **1a**. Its affinity for α_1-adrenergic receptors is comparable to that of the other alkyl amides, resulting in a selectivity of 160. In a final step, we prepared an analogue incorporating the most successful modifications of the arylpiperazine portion (1-naphthyl, i.e., **1e**) and of the amide portion (1-adamantanecarboxamido, i.e., **2j**) with regard to 5-HT_{1A} affinity/selectivity. This analogue (**2k**) retains high affinity for 5-HT_{1A} receptors (0.7 nM). Although the selectivity (ratio = 156) is not as high as was expected from such a combination, it is comparable to **2j** (ratio = 160), and suggests that **1e**, **2j**, and **2k** may not bind in the same orientation at α_1-adrenergic receptors.

Table III. Binding Profile for **2j**

receptor	K_i (nM)[a]	receptor	K_i (nM)[a]
5-HT_{1A}	0.4 (±0.03)	5-HT_2	34 (±2)
α_1-adrenergic	65 (±6)	5-HT_3	>10000
5-HT_{1B}	890 (±160)	β-adrenergic	1020 (±130)
5-HT_{1C}	225 (±70)	D_1-dopaminergic	>1000
5-HT_{1D}	460 (±45)	D_2-dopaminergic	2.7 (±0.01)

[a] Values represent the means and the SEM of three individual experiments performed in triplicate.

Adenylyl Cyclase Assay. The adamantyl amide **2j** was examined for its ability to antagonize 5-HT_{1A}-coupled adenylyl cyclase (Figure 1). Forskolin-stimulated adenylyl cyclase assays were performed in rat hippocampal membranes to determine the activity of **2j** at postsynaptic 5-HT_{1A} receptors. As shown in Figure 1, 5-carboxamidotryptamine, a 5-HT_{1A} agonist, was potent at inhibiting forskolin-stimulated adenylyl cyclase activity. At a comparable concentration, compound **2j** showed no agonist activity, but, when examined in combination with 5-carboxamidotryptamine, was able to antagonize the agonist effect. These results suggest that **2j** retains antagonist properties at postsynaptic 5-HT_{1A} receptors and that additional functional studies are warranted.

Binding Profile of 2j. Affinities of compound **2j** (which displays a 160-fold selectivity for 5-HT_{1A} over α_1-adrenergic receptors) were determined at various serotonergic and other neurotransmitter receptors. A binding profile of **2j** (RK-153) is presented in Table III. By virtue of being one of the highest affinity 5-HT_{1A} ligands reported to date, it binds at 5-HT_{1A} receptors over other neurotransmitter receptors with considerable selectivity. Compound **2j** does, however, bind at 5-HT_2 receptors (K_i = 34 nM) with only about a 100-fold selectivity, and binds at D_2 dopamine receptors with even higher affinity (K_i = 2.7 nM). Nevertheless, **2j** should prove to be of value in those functional studies in which the α_1-adrenergic character of **1a** interferes with the assay.

In summary, we have demonstrated that (a) incorporation of various substituents into the arylpiperazine portion of **1a** leads to relatively little improvement in its selectivity for 5-HT_{1A} over α_1-adrenergic receptors; (b) replacement of the 2-methoxyphenyl portion of **1a** by a naphthyl group has no effect on 5-HT_{1A} affinity, but decreases α-adrenergic affinity by about 25-fold; however, in the analogue incorporating an adamantyl amide moiety, instead of a phthalimide group as in **1a**, replacement of the 2-methoxyphenyl portion by a naphthyl group (i.e., **2k**) has little effect on 5-HT_{1A} or α_1-adrenergic affinity; (c) replacement of the phthalimide portion of **1a** by a benzamide or substituted benzamides slightly reduces affinity for 5-HT_{1A} sites and does not result in a significant improvement in the 5-HT_{1A}/α_1-adrenergic selectivity; (d) replacement of the benzamides by alkyl amides tends to decrease α_1-adrenergic affinity but not 5-HT_{1A} affinity, thus leading to improved selectivity for 5-HT_{1A} receptors over α_1-adrenergic receptors; and (e) bulky amides branched on the carbon atom adjacent to the carbonyl group result in en-

(21) Alabaster, V. A.; Campbell, S. F.; Danilewicz, J. C.; Greengrass, C. W.; Plews, R. M. 2,4-Diamino-6,7-dimethoxyquinazolines. 2. 2-(4-Carbamoylpiperidino) Derivatives as α_1-Adrenoceptor Antagonists and Antihypertensive Agents. *J. Med. Chem.* 1987, *30*, 999.

hanced 5-HT_{1A} affinities and, consequently, better 5-HT_{1A}/α_1-adrenergic selectivity. The adamantyl derivative **2j** binds at 5-HT_{1A} sites with somewhat greater affinity and displays greater 5-HT_{1A}/α_1-adrenergic selectivity than **1a**. Its activity in the adenylyl cyclase assay suggests that this agent retains postsynaptic 5-HT_{1A} antagonist properties; additional studies are necessary to further characterize this agent.

Experimental Section

Synthesis. Proton magnetic resonance spectra were obtained with a JEOL FX90 spectrometer with tetramethylsilane as an internal standard; infrared spectra were recorded on a Nicolet 5ZDX FT-IR. Spectral data are consistent with assigned structures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Microanalysis was performed by Atlantic Microlab (Norcross, GA), and determined values are within 0.4% of the theory, except where noted. Column chromatography was performed with silica gel, particle size 150 Å, mesh = 60–200 (Davison Chemical Corp., Baltimore, MD). Flash column chromatography was performed with silica gel, particle size 60 Å, mesh = 230–400 (Aldrich Chemical Co.).

1-(4-Nitrophenyl)-4-[4-(2-phthalimido)butyl]piperazine Oxalate (1c). A solution of *N*-(4-bromobutyl)phthalimide (4) (2.04 g, 7.2 mmol) in MeCN (20 mL) was added to a refluxing mixture of 1-(4-nitrophenyl)piperazine (1.5 g, 7.2 mmol) and potassium carbonate (2 g, 14.5 mmol). The reaction mixture was heated at reflux for 8 h and filtered hot. The solvent was removed under reduced pressure to yield 2.7 g (91%) of the desired product as a yellow solid, mp 164–165 °C after recrystallization from absolute EtOH. The salt was prepared by the dropwise addition of a cold ethereal solution of the amine (0.42 g, 1 mmol) to a cold ethereal solution of oxalic acid (0.09 g, 1 mmol) to yield 0.1 g of **1c** as a yellow solid; recrystallization from a mixture of absolute EtOH and anhydrous ether afforded **1c** as yellow crystals, mp 206–207.5 °C (Table I).

Compounds **1a**, **1b**, **1d**, and **1e** (Table I) were prepared in a similar manner except that the reflux time for **1a** was 5 h and that for **1b** was 4.5 h.

1-(4-Acetamidophenyl)-4-[4-(2-phthalimido)butyl]-piperazine Hemioxalate (1f). Pd/C (10%, 0.07 g), moistened with a few drops of glacial HOAc, was added to a solution of acetic anhydride (0.1 g, 0.98 mmol) and 1-(4-nitrophenyl)-4-[4-(2-phthalimido)butyl]piperazine (**1c**) (0.15 g, 0.4 mmol) in glacial HOAc (60 mL), and hydrogenation (Parr hydrogenator; initial pressure ca. 35 psi) was carried out for 2 h. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (25 mL), and the $CHCl_3$ fraction was washed with water (25 mL) and dried ($MgSO_4$). The solvent was evaporated to dryness to yield 0.09 g of the desired product. The salt was prepared by the addition of an ethereal solution of the free base to a cold ethereal solution of oxalic acid; recrystallization from a mixture of absolute EtOH and anhydrous Et_2O afforded 0.04 g (22%) of **1f** as white crystals, mp 223–225 °C (Table I).

4-[4-(4-Nitrobenzamido)butyl]-1-(2-methoxyphenyl)-piperazine Hydrochloride (2c). A solution of 4-nitrobenzoyl chloride (0.49 g, 2.6 mmol) in dry THF (15 mL) was added dropwise to a solution of 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine[10] (**5**) (0.7 g, 2.7 mmol) and triethylamine (1 g, 10 mmol) in dry THF (20 mL) at 0 °C under a nitrogen atmosphere. The mixture was allowed to stir at 0 °C for 1 h and at room temperature for 45 min. The white precipitate formed was removed by filtration, and the filtrate was evaporated to dryness to yield a crude yellow solid. Recrystallization from MeCN afforded 0.7 g of yellow crystals. The hydrochloride salt was prepared by the dropwise addition of a cold saturated solution of dry HCl gas in anhydrous Et_2O to a cold ethanolic solution of the free base (0.15 g), and the crude salt was recrystallized from absolute EtOH to yield 0.09 g (35%) of **2c** as brown crystals, mp 120–121 °C (Table I).

Compound **2m** (Table I) was prepared in the same manner as **2c**. Compounds **2a**, **2b**, **2d**, **2h** (Table I) were prepared in a similar manner, the reaction mixture being allowed to stir at 0 °C for 30–60 min, and at room temperature for 30–90 min. In the case of **2i** and **2j** (Table I), the reaction mixture was allowed to stir at 0 °C for 30 min and at room temperature overnight. The free base of **2i** was purified by dissolving the crude oil obtained in $CHCl_3$ (50 mL) and extracting into 1 N HCl (3 × 50 mL). The aqueous fractions were pooled, the pH was increased to approximately 11, and the mixture was extracted with $CHCl_3$ (3 × 50 mL). The organic fractions were combined and dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield 1.18 g of the free base of **2i** as a yellow oil. The fumarate salt was prepared by the dropwise addition of a cold ethereal solution of the free base (0.24 g, 0.69 mmol) to a cold ethereal solution of fumaric acid (0.08 g, 0.7 mmol) to afford 0.22 g (45%) of the desired product **2i** as a white solid; **2i** was analytically pure without recrystallization.

4-[4-(4-Aminobenzamido)butyl]-1-(2-methoxyphenyl)-piperazine Hemifumarate (2e). Pd/C, (10%, 0.11 g) moistened with a few drops of EtOH was added to a solution of 4-[4-(4-nitrobenzamido)butyl]-1-(2-methoxyphenyl)piperazine (**2c**) (0.42 g, 1.0 mmol) dissolved in absolute EtOH (60 mL), and the mixture was subjected to hydrogenation (Parr hydrogenator; initial pressure ca. 35 psi) overnight. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure to yield a crude solid. The solid was purified by silica gel flash chromatography using a mixture of $CHCl_3$ and MeOH (4:1) as the eluent to yield 0.4 g of the free base of **2e**, mp 116–123 °C. The fumarate salt was prepared by the addition of an ethereal solution of fumaric acid (0.017 g, 0.15 mmol) to a cold, stirred solution of the amine (0.11 g, 0.28 mmol) in dry THF to afford 0.07 g (56%) of the salt as off-white crystals after washing with hot anhydrous Et_2O, mp 181–183 °C (Table I).

4-[4-(4-Hydroxybenzamido)butyl]-1-(2-Methoxyphenyl)-piperazine Hydrochloride (2f). Pd/C, (10%, 0.1 g) moistened with a few drops of MeOH was added to a solution of 4-[4-[4-(benzyloxy)benzamido]butyl]-1-(2-methoxyphenyl)piperazine (**2m**) (0.5 g, 1.1 mmol) dissolved in MeOH (100 mL), and hydrogenation (Parr hydrogenator; initial pressure ca. 35 psi) was carried out for 1.5 h. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure to yield 0.4 g of a yellow oil, which solidified on standing overnight. The salt was prepared by the dropwise addition of a cold saturated ethereal solution of HCl gas to an ethanolic solution of the amine. The solvents were removed under reduced pressure to yield 0.42 g of a yellowish-white solid; recrystallization from absolute EtOH afforded 0.21 g (48%) of the desired product **2f**, mp 146–148 °C dec (Table I).

1-(2-Methoxyphenyl)-4-[4-(4-phenylbutyramido)butyl]-piperazine Dihydrochloride (2g). Ethyl chloroformate (0.46 g, 4.2 mmol) was added dropwise to a stirred solution of 4-phenylbutyric acid (0.69 g, 4.2 mmol) and triethylamine (0.43 g, 4.25 mmol) in CH_2Cl_2 (30 mL). The resultant solution was allowed to stir for 30 min, and a solution of 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine[10] (**5**) (1.1 g, 4.2 mmol) in CH_2Cl_2 (10 mL) was added in a dropwise manner. After the addition was complete, the reaction mixture was allowed to stir at room temperature for 18 h. The reaction mixture was washed with water (3 × 50 mL), dilute HCl (0.5%, 2 × 50 mL), and again with water (50 mL). The CH_2Cl_2 layer was dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to yield 0.67 g of the free base as a pinkish-orange solid. The solid was purified by silica gel column chromatography using a mixture of $CHCl_3$ and MeOH (4:1) as the eluent. The salt was prepared by the addition of a cold saturated solution of dry HCl gas in anhydrous Et_2O to a solution of the amine (0.09 g, 0.2 mmol) in a mixture of absolute EtOH and anhydrous Et_2O to afford 0.04 g (14%) of the desired product, **2g**, after washing with hot anhydrous Et_2O, mp 151 °C (Table I).

4-[4-(1-Adamantanecarboxamido)butyl]-1-(1-naphthyl)-piperazine Fumarate (2k). A solution of hydrazine (0.1 g, 2 mmol) in absolute EtOH (5 mL) was added to a suspension of 1-(1-naphthyl)-4-[4-(2-phthalimido)butyl]piperazine[10] (**1e**) (0.27 g, 0.7 mmol) in absolute EtOH (10 mL), and the mixture was heated at reflux for 2 h. The volatiles were removed under reduced pressure, and the resultant solid was suspended in $CHCl_3$ (50 mL) and washed with water (2 × 25 mL). The $CHCl_3$ fraction was dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield 0.13 g (70%) of the desired intermediate 4-(4-

aminobutyl)-1-(1-naphthyl)piperazine (6) as a yellow oil, which was used in the next step without further purification.

A solution of 1-adamantanecarbonyl chloride (0.08 g, 0.4 mmol) in dry THF (5 mL) was added to a solution of 4-(4-aminobutyl)-1-(1-naphthyl)piperazine (6) (0.12 g, 0.4 mmol) and triethylamine (0.16 g, 1.6 mmol) in dry THF (5 mL) maintained at 0 °C under a nitrogen atmosphere. The reaction mixture was allowed to stir at 0 °C for 75 min and at room temperature for 2 h. The solids were removed by filtration, and the volatiles were removed under reduced pressure to yield 0.15 g of an oil, which was purified by column chromatography using a mixture of $CHCl_3$ and MeOH (9:1) as the eluent to yield 0.1 by (54%) of the desired product. The fumarate salt was prepared by the addition of an ethereal solution of the free base (0.1 g, 0.23 mmol) to an ethereal solution of fumaric acid (0.03 g, 0.23 mmol). Recrystallization from a mixture of absolute EtOH and anhydrous Et_2O afforded 0.03 g of the title compound as white crystals, mp 185-186 °C (Table I).

Forskolin-Stimulated Adenylyl Cyclase Assay. Forskolin-stimulated adenylyl cyclase assays were performed using Sprague Dawley rat hippocampi (Taconic Farms, Germantown, NY) as previously described.[7] Briefly, the assay incubation media contained 80 mM Tris-HCl (pH 7.5 at 22 °C), 100 mM NaCl, 2 mM $MgCl_2$, 5 mM creatine phosphate, 2 mM cAMP, 0.2 μg of myokinase, 10 μg of creatine phosphokinase, 3 units/mL of adenosine deaminase, 10 μM GTP, 50 μM forskolin, 60 mM sucrose, 0.2 mM EGTA, 1 mM Na_2EDTA, 0.2 mM dithiothreitol, 50-100 μg of membrane protein, and 0.1 mM ATP containing 1.5 μCi of [α-^{32}P]ATP. The conversion of [α-^{32}P]ATP to [^{32}P]cAMP was measured as described by Salomon.[22]

Radioligand Binding Assays. Brain homogenate preparation and radioligand binding assays were performed as previously described.[7] Briefly, 5-HT_{1A} receptor affinity was assayed using 0.1 nM [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), 6 mg of rat hippocampal homogenate, and 10 μM 8-OH-DPAT to define nonspecific binding. 5-HT_{1B} receptor binding was assayed using [^{3}H]-5-HT (2.0 nM), 5 mg of rat striatal homogenate, 100 nM 8-OH-DPAT, and 100 nM mesulergine. Nonspecific binding was defined using 10 μM 5-HT. 5-HT_{1C} affinity was assayed with 1.0 nM [^{3}H]mesulergine, 10 nM spiperone, 10 mg of homogenized rat frontal cortex, and 10 μM 5-HT to define nonspecific binding. 5-HT_{1D} receptor affinity was assayed with 2 nM [^{3}H]-5-HT, 10 μM pindolol, 100 nM mesulergine, 10 mg of homogenized calf caudate, and 10 μM 5-HT to define nonspecific binding. 5-HT_2 receptor affinity was assayed using [^{3}H]DOB (0.4 nM), 15 mg of homogenized rat frontal cortex, and 1 μM cinanserin to define nonspecific binding. 5-HT_3 receptor affinity was assayed with [^{3}H]GR-65630, 5 mg of homogenized calf area postrema, and 140 mM NaCl. ICS-205-930 (1 μM) was used to define nonspecific binding. α_1-Adrenergic receptor affinity was assayed using 1.0 nM [^{3}H]WB4101, 100 nM 8-OH-DPAT, 10 mg of rat frontal cortex, and 1 μM prazosin to define nonspecific binding. β-Adrenergic affinity was assayed using 1 nM [^{3}H]DHA and 5 mg of homogenized rat frontal cortex. Nonspecific binding was defined by 1 μM propranolol. D_1-Dopamine receptor affinity was assayed using 1 nM [^{3}H]SCH-23390, 20 nM ketanserin, 2 mg of homogenized rat striatum, and 1 μM SCH-23390 to define nonspecific binding. D_2-Dopamine receptor affinity was assayed using [^{3}H]NMSP (1 nM) and 2 mg of calf caudate in the presence of 140 mM NaCl. Sulpiride (10 μM) was used to define nonspecific binding.

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Registry No. **1a**, 102392-05-2; **1b**, 134390-52-6; **1b** free base, 134390-53-7; **1c**, 134390-55-9; **1c** free base, 134390-54-8; **1d**, 134390-57-1; **1d** free base, 134390-56-0; **1e**, 115338-33-5; **1e** free base, 115338-25-5; **1f**, 134390-59-3; **1f** free base, 134390-58-2; **2a**, 134390-60-6; **2b**, 134390-61-7; **2c**, 134390-62-8; **2d**, 134390-63-9; **2e**, 134390-65-1; **2e** free base, 134390-64-0; **2f**, 134390-66-2; **2g**, 134390-67-3; **2g** free base, 134390-68-4; **2h**, 134390-70-8; **2i**, 134390-71-9; **2i** free base, 133025-01-1; **2j**, 134390-73-1; **2k**, 134390-75-3; **2k** free base, 134390-74-2; **2l**, 115338-28-8; **2m**, 134390-76-4; **3a**, 35386-24-4; **3b**, 1019-06-3; **3c**, 6269-89-2; **3d**, 59084-06-9; **3e**, 57536-86-4; **4**, 5394-18-3; **5**, 21103-33-3; **6**, 134390-77-5; 4-nitrobenzoyl chloride, 122-04-3; 4-phenylbutyric acid, 1821-12-1; 1-adamantanecarbonyl chloride, 2094-72-6; 4-(benzyloxy)benzoyl chloride, 1486-50-6.

(22) Salomon, Y. Adenylate Cyclase Assay. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 35.
(23) Cavallito, C. J.; Buck, J. S. Synthesis of Phenolic Acid Esters. 1. Depsides. *J. Am. Chem. Soc.* **1943**, *65*, 2140.

Acyclic Analogues of 2-(4-Phenylpiperidino)cyclohexanol (Vesamicol): Conformationally Mobile Inhibitors of Vesicular Acetylcholine Transport

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Several 1,3-disubstituted propan-2-ols and one α,β-disubstituted ethanol (**11i**) were synthesized and evaluated as potential acyclic mimics of the vesicular acetylcholine transport inhibitor 2-(4-phenylpiperidinyl)cyclohexanol (**1**, vesamicol, AH5183). Analogues containing the 4-phenylpiperidyl fragment (**11a**, **11b**) were more potent than those containing the 4-phenylpiperazyl moiety (**11e**, **11f**). Substitution at the second terminal carbon of the propyl (or ethyl) fragment with simple lipophilic aryl substituents yielded potent inhibitors of vesicular acetylcholine storage, including (-)-**11a** and *d*-**11i**, which are equipotent with vesamicol. However, the activity of analogues containing bicyclic aryl groups was susceptible to aryl substitution patterns (**11g** vs **11h**), indicating a definite receptor site topography. In addition, the inhibitory activity of these acyclic analogues was enantioselective, exhibiting a preference, similar to the parent vesamicol, for the levorotatory isomer [(-)-**11a** vs (+)-**11a**]. Therefore, the simple lipophilic acyclic vicinal amino alcohols may successfully mimic the biological activity of vesamicol.

Introduction

The lipophilic amino alcohol *trans*-2-(4-phenylpiperidino)cyclohexanol (**1**, vesamicol, AH 5183) induces paralysis and death in rodents and other laboratory animals.[1,2] The biological activity of **1** is largely mediated

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(1) Brittain, R. T.; Levy, G. P.; Tyers, M. B. Observations on the neuromuscular blocking action of 2-(4-phenylpiperidino)-cyclohexanol (AH 5183). *Br. J. Pharmacol.* **1969**, *36*, 173-174.

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