N-(1-Arylpropionyl)-4-aryltetrahydropyridines, a New Class of High-Affinity Selective σ Receptor Ligands

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A series of *N*-(1-arylpropionyl)-4-aryl-1,2,3,6-tetrahydropyridines, prepared by simple Mannich condensations, have been found by radioligand binding assays to have moderate to high affinity (IC_{50} 0.5–500 nM) for bovine cerebellar σ receptor/binding sites and no measurable affinity (IC_{50} > 5000 nM) for bovine striatal D_2 receptors. The most active of these compounds rival in potency the most active σ ligands previously reported. Three of these σ-active compounds were screened for pharmacological activity under the NIMH-NovaScreen program and showed moderate affinity only for D_2 and 5-HT_2 receptors among the 40 sites assayed. Since these *N*-(1-arylpropionyl)-4-aryltetrahydropyridines are structurally related to other potent σ receptor ligands, in particular haloperidol and 4-phenylpiperidines, these data provide insights into the nature of the essential pharmacophore of the σ receptor. The selective affinity of these materials for σ receptors indicates they have potential as prototypes of novel psychotherapeutic medicinal agents, particularly as antipsychotic drugs which would be devoid of debilitating side effects associated with blockade of D_2 receptors.

Introduction

The σ receptor/binding site has been the subject of considerable attention in recent years. The σ receptor was first postulated by Martin et al.[1] to account for the psychotomimetic effects of *N*-allylnormetazocine (NANM, also known as SKF-10,047, 1) and other racemic benzomorphans in dogs. Although it was originally believed that the σ receptor was an opiate receptor subtype with particular affinity for phencyclidine (PCP),[2] selective radioligand displacement studies[3] and determination of σ receptor distribution by autoradiography[4] conclusively demonstrated that the σ receptor was an entity distinct from the PCP binding site. The σ receptor is now recognized as the high-affinity binding site of [^{3}H]NANM and shows preferential affinity for the (+) vs the (−) isomer of NANM and other benzomorphans. Since the classical neuroleptic agent haloperidol also shows high affinity for this particular site,[5] the site is also known as the σ-haloperidol receptor/binding site. The low-affinity NANM site was subsequently identified as the PCP binding site.[3]

Although their functional role is still unclear, σ receptors have been concluded to be biologically significant.[6,7] A large variety of physiological effects which are thought to be mediated by σ receptors were recently reviewed by Su,[8] who summarized the evidence that σ receptors are found in endocrine and immune tissues as well as in the central nervous system and the peripheral system.[9] With respect to several σ-ligand-induced responses, including psychotomimetic effects,[8] proconvulsant behavior in rats[10] and potentiation of neuronal firing induced by *N*-methyl-D-aspartate,[11] some σ ligands (e.g., (+)-NANM, dextromethorphan, cocaine, (+)-pentazocine, and di-*o*-tolylguanidine (DTG)) act as agonists and others (haloperidol, remoxipride, BMY-14802) as antagonists. However, all σ-ligands appear to act as agonists with respect to a set of other responses, which include blockade of tonic potassium channels in NCB-20 cells,[12] induction of dystonia,[13] and potentiation of twitches in guinea pig vas deferens.[14] Since steroids interact with σ receptors in the brain,[15] Su has suggested that σ receptors may represent links between nervous, endocrine, and immune systems.[8]

σ receptors have also been implicated as a potential site of action for neuroleptics.[7] In addition to typical neuroleptic agents such as haloperidol and perphenazine, a number of atypical neuroleptics, such as rimcazole, remoxipride, and BMY 14802, show high affinity for the σ receptor binding site. The latter group of pharmacological agents show neuroleptic activity in animal models and bind with much higher affinity to σ than to dopamine D_2 and PCP sites. New compounds with similar pharmacological profiles have been recently reported by several groups.[16–18] Gilligan *et al.*[16] have discussed the *in vitro* and *in vivo* evidence that selective σ receptor ligands may represent a new class of potential antipsychotic drugs which would be free of the disturbing extrapyramidal side effects and tardive dyskinesia associated with classical antipsychotic drugs, which act pharmacologically principally as D_2 receptor antagonists.[17]

It has also been suggested that the σ receptor site may play a role in regulation of motor behavior and in mediation of the side effects associated with the clinical use of antipsychotic drugs.[13] σ receptors modulate the actions of excitatory amino acids such as NMDA in hippocampal slice preparations, which has led to the speculation that σ-ligands may possess neuroprotective properties.[6,19] Wolfe has presented evidence that the ability of PCP to suppress a variety of immune functions *in vivo* may be mediated by the actions of PCP on σ receptors rather than on [^{3}H]TCP-labeled PCP receptors, at least in human peripheral leukocytes.[9a]

An unusually large variety of compounds have been shown to have affinity for σ receptors.[6,20] These include butyrophenones,[7,8] tricyclic analogues of 3-PPP,[6,20,21] arylpiperidines and arylpiperazines,[16,22] PCP analogues,[18] *N*,*N*-di-*o*-tolylguanidines,[23] and a series of benzeneacetamides.[24] Glennon has demonstrated that the 1-phenyl-

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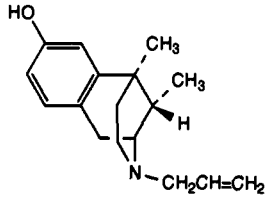
2-aminopropane portion (structure 2) of the benzomorphan (NANM) structure is sufficient for effective binding at σ sites, provided that the terminal nitrogen is substituted, and has argued that the primary pharmacophore of σ-opiates is most likely the phenethylamine moiety.[25] Some of the most potent compounds of this type, such as 3, have no measurable affinity for PCP or D_2 receptor sites.[25] Glennon has discovered some new 1-phenylpiperazines and 4-phenylpiperidines, illustrated by 4 and 5, which also show selective affinity for σ receptor sites and essentially no affinity for PCP, D_1, or D_2 sites.[22] Some of the phenylpiperidines made in Glennon's study[22] are structurally related to haloperidol (6), but their receptor pharmacology turns out to be quite different. We have recently prepared some tetracyclic analogues of 3-PPP which also show selective affinity for σ receptors.[26]



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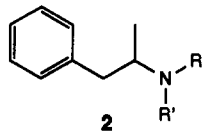

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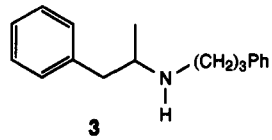

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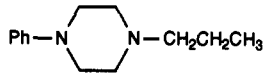

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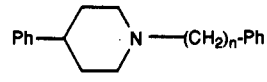

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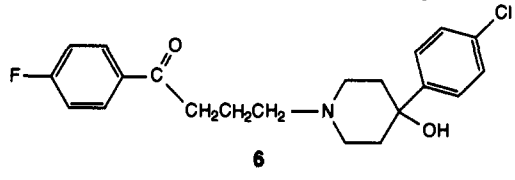

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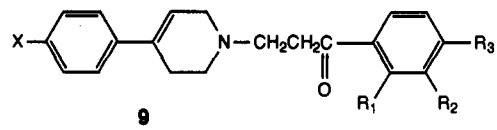
Finally, Gilligan *et al.* prepared a large number of compounds of general structure 7 which are also being promoted as potential antipsychotic drugs because of their pharmacological profiles.[16] The most selective of these compounds, **8a,b**, have K_i values of 10 nM for interaction at σ receptors and low affinity (K_i > 1000 nM) for D_2 receptors. Their behavioral profile classifies them as σ-1 antagonists, since they block rotation in rats induced by (+)-NANM, show strong activity in mouse antimescaline and antiaggression tests, and block the effects of the σ-ligand 3-PPP on dopamine neuronal firing rates. However, they show only weak activity in tests of D_2 activity, such as apomorphine-induced climbing and conditioned avoidance response in rats.

The structural diversity of σ-ligands has complicated attempts to define the essential pharmacophore(s) of these compounds.[16,20,22,25,27] In the course of preparing some materials which might serve as ligands for affinity purification and photoaffinity labeling of σ receptors, we have discovered some simple compounds which have unusually high affinity and selectivity for these receptors. Given the attention currently being given to new σ-ligands as potential medicinal agents, we are prompted to report the details of this study.

Table I. Physical Data for New 4-Aryltetrahydropyridines



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compd	X	R_1	R_2	R_3	mp, °C	analysis of HCl salt C, H, N
9a	H	H	H	H	175–178	$C_{20}H_{22}ClNO·0.3H_2O$
9b	F	H	H	H	210–212	$C_{20}H_{21}ClFNO$
9c	Cl	H	H	H	184–186	$C_{20}H_{21}Cl_2NO$
9d	Cl	H	H	F	206–208	$C_{20}H_{20}Cl_2FNO$
9e	Cl	H	H	Br	207–209	$C_{20}H_{20}BrCl_2NO$
9f	Cl	H	H	Cl	208–210	$C_{20}H_{20}Cl_3NO$
9g	Cl	H	OCH_3	OCH_3	193–195	$C_{22}H_{25}Cl_2NO_3$
9h	Cl	H	F	F	204–206	$C_{20}H_{19}Cl_2F_2NO$
9i	Cl	F	H	H	194–196	$C_{20}H_{20}Cl_2FNO$
9j	Cl	H	$-OCH_2O-$		198–200	$C_{21}H_{21}Cl_2NO_3$
9k	Cl	H	Cl	Cl	199–202	$C_{20}H_{19}Cl_4NO$
9l	Cl	Cl	H	H	148–151	$C_{20}H_{20}Cl_3NO$
9m	F	H	H	F	199–201	$C_{20}H_{20}ClF_2NO·0.2H_2O$
9n	F	H	H	Cl	201–204	$C_{20}H_{20}Cl_2FNO·1.0H_2O$
9o	F	H	H	Br	196–198	$C_{20}H_{20}BrClFNO·0.5H_2O$
11	H	H	H	H	155–157	$C_{20}H_{24}ClNO·1.0H_2O$

Results

Synthesis. The *N*-(1-arylpropionyl)-4-aryl-1,2,3,6-tetrahydropyridines **9a–o** shown in Table I were all prepared in a single step from corresponding commercially available 4-aryltetrahydropyridines **10** by Mannich condensation with acetophenone or substituted acetophenones and paraformaldehyde. In many cases, the products could be purified simply by recrystallization from ethanol, but occasionally column chromatography was required to obtain analytically pure samples. The identity of the products was established by their ^{1}H-NMR spectra, supported in some instances by ^{13}C-NMR and mass spectra. Satisfactory analytical data were obtained for all new compounds.

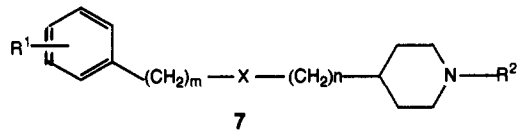
Radioligand Binding Assays. The affinity of the new compounds for bovine cerebellar σ receptors was assayed by competitive displacement of 2.0 or 4.0 nM [^{3}H]-haloperidol in the presence of 25 nM unlabeled spiperone to block association of the radioligand with dopamine D_2 sites. Nonspecific binding was defined using 1 μM unlabeled haloperidol. The protocol is based on that described by Tam and Cook[5] and Largent *et al.*,[28] except that the buffer was modified to include salts to more closely simulate physiological conditions. The precise assay conditions are described in detail in the Experimental Section and have been demonstrated to be reliable using appropriate control procedures and test ligands. Binding data are shown in Table II for compounds **9a–o**, the starting 4-aryltetrahydropyridines **10a–c**, the 4-arylpiperidine **11**, and several simple Mannich bases **12** lacking the tetrahydropyridine moiety. The IC_{50} values reported represent the mean of at least three separate assays for each compound, each of which involved triplicate determinations at a minimum. The dopamine D_2 binding data in Table II were obtained on bovine striatal homogenates using [^{3}H]spiperone (200 pM) as the radioligand and 250 nM unlabeled ketanserin to block association of the radioligand with 5-HT_2 receptors.[29]

The affinity of a group of the aryltetrahydropyridines (**9b,c,f**) for a large variety of other receptor sites was determined. In these assays, obtained through the NIMH-NovaScreen program, the initial percent inhibition of specific binding by 10 μM of the test compound is measured

Table II. Inhibition of Binding to σ and Dopamine D_2 Receptors by *N*-(1-Arylpropionyl)-4-aryltetrahydropyridines and Related Compounds[a]

	IC_{50}, nM[b]			IC_{50}, nM[b]	
compd	σ	D_2	compd	σ	D_2
9a	8.0	>5000	**9l**	105	
9b	4.0	>5000	**9m**	23	>10000
9c	0.5	>5000	**9n**	25	>15000
9d	500	>5000	**9o**	66	>13000
9e	100	>5000	**10a**	1000	>5000
9f	158	>5000	**10c**	50	>5000
9g	495	>5000	**11**	7.0	>5000
9h	235	>5000	**12a**	>5000	>5000
9i	400	>5000	**12b**	1250	>5000
9j	281	>5000	**12c**	1250	>5000
9k	158	>5000			

[a] Assays for binding of ligands to the σ receptor were performed using 2.0 nM [^{3}H]haloperidol (18.8 Ci/mmol) in the presence of 25 nM unlabeled spiperone. Dopamine D_2 binding assays were carried out using [^{3}H]spiperone (21.2 Ci/mmol) at a concentration of 200 pM. See the Experimental Section for details. [b] All IC_{50} values were based upon triplicate replications and were defined with the following approximate precision (SEM): >5000 nM, ±1000 nM; >1000 nM, ±100 nM; 100–1000 nM, ±40 nM; 10–100 nM, ± 4 nM; below 10 nM, ± 0.4 nM.



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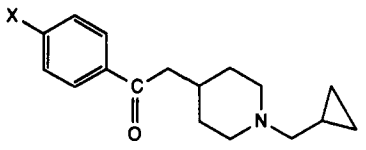
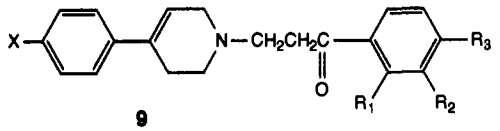


8 a) X = F b) X = CN



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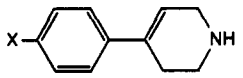
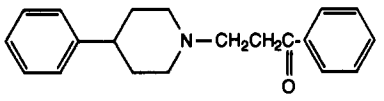


10 a) X = H b) X = F c) X = Cl



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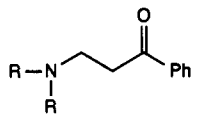

12 a) R = Me b) R = Et c) R = n-Pr

at 40 different receptor sites. These include receptors for 10 neurotransmitters (adenosine, dopamine 1 and 2, GABA A and B, serotonin 1 and 2, NMDA, kainate, and quisqualate), 5 regulatory sites (benzodiazepine, glycine [2 different assays], PCP, and MK-801), 11 brain/gut peptides (angiotensin, arg-vasopressin V1, bombesin, CCK central and peripheral, substances P and K, NMY, neurotensin, somatostatin, and VIP), growth factors and peptides (ABF1, EGF, NGF), ion channels (calcium, chloride and potassium), and second messengers (forskolin, phorbol ester, and inositol triphosphate). Only values for percent inhibition of specific binding greater than 50% under these assay conditions are considered to represent activity. The data for inhibition of radioligand binding to D_2, 5-HT_1, 5-HT_2, D_1 and PCP receptors are presented in Table III. The test ligands were inactive at all of the other binding sites in these assays.

Table III. Results of Receptor Binding Assays Performed by NovaScreen on *N*-(1-Arylpropionyl)-4-aryltetrahydropyridines

	% inhibition of binding[a]				
compd	D_2[b]	5-HT_1[c]	5-HT_2[d]	D_1[e]	PCP[f]
9b	86.7 ± 6.7	64.5 ± 1.3	94.8 ± 6.4	23.9	0
9c	92.7 ± 3.1	72.4 ± 1.0	96.9 ± 2.5	19.0	0
9f	71.8 ± 9.0	49.5	84.0 ± 11.2	31.6	5.8

[a] Percent inhibition of binding of radioligand by 10^{-5} M of the test compound. [b] 0.5 mM [^{3}H]sulpiride to rat striatal membranes, nonspecific binding defined using 10 μM (±)-sulpiride. [c] 3.0 nM [^{3}H]-5-hydroxytryptamine binoxalate ([^{3}H]-5-HT) to rat caudate membranes, nonspecific binding defined using 10 μM unlabeled 5-HT. [d] 1.0 nM [^{3}H]ketanserin to rat cortical membranes, nonspecific binding defined using 10 μM methysergide. [e] 500 pM [^{3}H]SCH 23390 to rat striatal membranes, nonspecific binding defined with 100 nM unlabeled SCH 23390. [f] 2 nM [^{3}H]TCP, binding defined with 1 μM PCP.

Discussion

The *N*-(1-arylpropionyl)-4-aryltetrahydropyridines prepared in this study had IC_{50} values ranging from 0.5 to 500 nM at σ receptors in the bovine cerebellum. The most active compounds (**9a–c**) were those lacking substituents on the phenyl ring attached to the carbonyl group on the side chain. Substitution by chlorine or fluorine at C-4 on the phenyl ring attached to the heterocyclic ring had only a small effect on σ activity. The next most active members of this class of compounds (**9m,n**) have a fluorine group on the phenyl ring at C-4 and either fluorine or chlorine at the para position of the benzoyl phenyl group. The presence of the double bond in the heterocyclic ring does not seem essential for σ activity, based on the activity observed for **11**. The simple Mannich bases **12a–c** with an acyclic instead of an aryltetrahydropyridine side chain were much less active pharmacologically than any of the compounds of structure **9**, as well as the simple aryltetrahydropyridine analogs **10**. All of these compounds, without exception, were devoid of activity at dopaminergic D_2 receptors in our assays. Some very modest D_2 activity for compounds **9b,c,f** was noted in the NovaScreen assay where the ligand concentration was 10 μM, and comparable activity was observed at 5-HT_2 sites. In both instances, this probably corresponds to IC_{50} values >5000 nM. Otherwise, these very active σ receptor ligands were devoid of significant activity at D_1, 5-HT_1, PCP, cholinergic, neuropeptide, and other neurotransmitter and neuroregulatory receptor binding sites (see Table III). Since the NovaScreen program does not include assays of activity at α- and β-adrenergic receptors, the activity of compounds **9–12** at such receptors remains to be determined.

It is not possible to state on the basis of these binding data whether these compounds are acting as agonists or antagonists at σ receptor sites. This has traditionally been a troublesome aspect of σ receptor pharmacology, as discussed earlier.[6,8,16,20] Because of the structural analogy with haloperidol, it is expected that compounds **9** would be more likely to act as antagonists rather than as agonists with respect to σ-induced behavioral effects, such as (+)-NANM-induced rotation, antimescaline, and antiaggression tests in rats,[16] and psychotomimetic effects in general.[8] In these behavioral tests, haloperidol and atypical antipsychotic drugs (e.g., remoxipride, BMY-14802) act as

antagonists, while (+)-NANM, dextromethorphan, cocaine, (+)-pentazocine, and steroids act as agonists.[8]

It is particularly instructive to compare the σ receptor affinities of the compounds in the present study and the 4-phenylpiperidines of structure **5** reported by Glennon *et al.*[22] For compounds **5** with n = 3, 4, and 5, K_i values of *ca.* 1 nM were determined in σ receptor assays in guinea pig brain membranes using [^{3}H]DTG as the radioligand. Although direct comparison of these data with data for compounds **9** is complicated by the fact that the radioligands and tissue source of σ receptors in the two studies are different, it appears that the σ potency of **9a–c** and of compounds **5** with n = 3–5 are similar. Thus, the presence of the carbonyl group in the side chain and of halogens on the phenyl group attached to the piperidine ring at C-4 does not have a significantly deleterious effect on σ receptor affinity. However, their affinity for D_2 receptors is strikingly different: IC_{50} values for compounds **5** vary from 165 to 335 nM while the values for **9a–c** are all >5000 nM. While both sets of compounds are essentially inactive at D_1 and PCP receptors, compounds **9b** and **9c** were determined to be inactive at a whole host of other receptors. Thus, the *selectivity* of compounds **9b** and **9c** for σ receptors is much superior to that of compounds of structure **5**[22] and is comparable to or better than that of most σ-ligands reported thus far in the literature.[6,16–18,20–25]

Some comments are in order concerning the structural and pharmacological relationship of the compounds **9** and **11** with haloperidol (**6**). Glennon has previously pointed out that certain of the substituents in haloperidol actually detract from its potency as a σ but not as a D_2 receptor ligand,[22] which is supported by the results of the present study. Thus, the number of carbons separating the piperidine nitrogen from the distal aromatic ring, which is 4 in haloperidol and 3 in the case of **9** and **11**, does not appear to be critical and can be allowed to vary over a narrow range, as shown by Glennon[22] in the case of **5**. The hydroxyl group in haloperidol is clearly not essential for σ activity and can be replaced by a carbon–carbon double bond and even (as in **5** and **11**) by a saturated piperidine ring. Neither of the halogens in haloperidol is critical for σ activity, but based on our findings the fluorine in the benzoyl group may be particularly detrimental in this regard. It would be most interesting to test the σ and D_2 potency of an analogue of haloperidol with hydrogen in place of fluorine.

These structural considerations will be incorporated into a new approach to computer modeling of the essential pharmacophore of σ receptor ligands that has been initiated in our laboratory and will be reported in due course.[30]

In conclusion, the pharmacological profiles of **9b** and **9c** suggest that these and probably other members of this easily synthesized series of compounds have excellent potential as antipsychotic drugs devoid of complications associated with blockade of D_2 receptors, such as extrapyramidal side effects and tardive dyskinesia.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Mr. R. Buzolich at Rockefeller University. ^{1}H and ^{13}C NMR spectra were obtained on a GE-Nicolet QE-300 spectrometer. All NMR spectra were obtained in CD_3OD, and chemical shifts are given in ppm relative to tetramethylsilane. GC/mass spectra were obtained using a Hewlett-Packard 5992 GC/MS system. Starting materials and solvents obtained from Aldrich were used without further purification.

General Procedures for Synthesis of *N*-(1-Arylpropionyl)-4-aryl-1,2,3,6-tetrahydropyridines. These compounds were prepared from commercially available (Lancaster) 4-aryl-1,2,3,6-tetrahydropyridines (**10**, X = H, Cl, F) by Mannich condensation with the appropriately substituted acetophenones and formaldehyde. Two typical procedures are described in detail. The first is for the preparation of *N*-(1-phenylpropionyl)-4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine (**9c**). A mixture of acetophenone (252 mg, 2.1 mmol), 4-(*p*-fluorophenyl)-1,2,3,6-tetrahydropyridine hydrochloride **10b** (426 mg, 2 mmol), and paraformaldehyde (90 mg, 3 mmol) in 6 mL of absolute ethanol containing 3 drops of concentrated HCl was heated under reflux for 2 h. An additional 60 mg (2 mmol) of paraformaldehyde was added before heating was continued for another 2 h. The mixture was allowed to cool, and 12 mL of purified ethyl ether was added, leading to formation of a precipitate (0.61 mg) of the desired product as the hydrochloride salt. This material was taken up in 10 mL of absolute ethanol, and the mixture was heated at reflux for 20 min and was then filtered while hot to remove any undissolved material. The crystals which formed when the solution was allowed to stand overnight were collected and washed with a little cold ethanol. The recovery of purified product was about 50% of the crude material.

More severe conditions were required to prepare the parent compound **9a**. A mixture of acetophenone (126 mg, 1.05 mmol), 4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride **10a** (195 mg, 1 mmol), and paraformaldehyde (45 mg, 1.5 mmol) in 4 mL of absolute ethanol containing 2 drops of concentrated HCl was heated at reflux for 4 h. An additional 30 mg (1 mmol) of paraformaldehyde was added, and the mixture was heated for an additional 16 h. The solution was kept in a refrigerator overnight, affording 50 mg of crystals which were purified as described above.

The purity of the new materials was assessed by TLC in at least two different solvent systems. The identity of these compounds was confirmed by ^{1}H-NMR spectra (300 MHz), supported in some cases by ^{13}C-NMR and mass spectral data. The purity of the final crystalline hydrochloride salts was demonstrated by their elemental analysis (Rockefeller University Microanalysis Facility), which was within acceptable limits in every case. Melting points of the salts are given in Table I, and spectral data for all new compounds are summarized below.

***N*-(1-Phenylpropionyl)-4-phenyl-1,2,3,6-tetrahydropyridine (9a).** ^{1}H NMR (CD_3OD): 7.99 (d, J = 8.0, 2H; $H_{17,21}$), 7.58 (t, J = 7.5, 1H; H_{19}), 7.46 (dd, J = 7.8, 7.5, 2H; $H_{18,20}$), 7.40 (d, J = 7.2, 1H; H_3), 7.23–7.31 (m, 4H; $H_{1,2,4,5}$), 6.06 (br d, 1H; H_8), 3.96 (br d, 2H; H_9), 3.50 (br d, 4H; $H_{11,13}$), 3.21 (br d, 2H; H_{12}), 2.85 (br d, 2H; H_{14}).

***N*-(1-Phenylpropionyl)-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine (9b).** ^{1}H NMR (CD_3OD): 8.05 (d, 2H, J = 7.2), 7.63 (t, 1H, J = 7.2), 7.52 (t, 2H, J = 7.2), 7.10 (t, 2H, J = 8.7), 6.09 (br m, 1H), 4.01 (br m, 2H), 3.67 (br s, 4H), 3.27 (m, 2H), 2.88 (br, 2H). MS: m/e 310 (parent pyridinium ion), 178 (loss of side chain), 133 (phenylpropionyl side chain).

***N*-(1-Phenylpropionyl)-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9c).** ^{1}H NMR (CD_3OD): 8.05 (d, 2H, J = 7.5), 7.63 (t, 1H, J = 7.5), 7.52 (t, 2H, J = 7.5), 7.46 (d, 2H, J = 8.7), 7.35 (d, 2H, J = 8.7), 6.15 (m, 1H), 4.01 (br, 2H), 3.67 (m, 4H), 3.27 (m, 1H), 2.88 (br, 2H). MS: m/e 326, 328 (parent pyridinium ion), 194 (loss of side chain), 133 (phenylpropionyl side chain).

***N*-[1-(4-Fluorophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9d).** ^{1}H NMR (CD_3OD): 8.14 (dd, 2H, J = 8.7, 5.4), 7.48 (d, 2H, J = 8.4), 7.37 (d, 2H, J = 8.4), 7.27 (t, 2H, J = 8.7), 6.18 (m, 1H), 4.02 (m, 2H), 3.7 (m, 4H), 3.28 (m, 2H), 2.9 (m, 2H). MS: m/e 344, 346 (pyridinium ion).

***N*-[1-(4-Bromophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9e).** ^{1}H NMR (CD_3OD): 7.97 (d, 2H, J = 8.4), 7.72 (d, 2H, J = 8.4), 7.48 (d, 2H, J = 8.7), 7.37 (d, 2H, J = 8.7), 6.14 (m, 1H), 4.04 (m, 2H), 3.68 (m, 4H), 3.29 (m, 2H), 2.91 (m, 2H).

***N*-[1-(4-Chlorophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9f).** ^{1}H NMR (CD_3OD): 8.06 (d, 2H, J = 8.4), 7.56 (d, 2H, J = 8.4), 7.48 (d, 2H, J = 8.7), 7.37

(d, 2H, J = 8.7), 6.18 (m, 1H), 4.03 (m, 1H), 3.68 (m, 4H), 3.29 (m, 2H), 2.90 (m, 2H).

***N*-[1-(3,4-Dimethoxyphenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9g).** ^{1}H NMR (CD_3OD): 7.75 (dd, 1H, J = 8.4, 1.8), 7.59 (d, 1H, J = 1.8), 7.48 (d, 2H, J = 8.4), 7.38 (d, 2H, J = 8.4), 7.07 (d, 1H, J = 8.4), 6.18 (m, 1H), 4.01 (m, 2H), 3.91 (s, 3H), 3.88 (s, 3H), 3.65 (m, 4H), 3.29 (m, 2H), 2.90 (m, 2H).

***N*-[1-(3,4-Difluorophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9h).** ^{1}H NMR (CD_3OD): 7.9 (m, 2H), 7.46 (d, 2H, J = 8.7), 7.35 (d, 2H, J = 8.7) superimposed on 7.4 (m, 1H), 6.15 (m, 1H), 4.01 (m, 2H), 3.64 (m, 4H), 3.27 (m, 2H), 2.88 (m, 2H).

***N*-[1-(2-Fluorophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9i).** ^{1}H NMR (CD_3OD): 8.00 (m, 1H), 7.68 (m, 1H), 7.50 (d, 2H, J = 8.4), 7.39 (d, 2H, J = 8.4) superimposed on 7.5 (m, 1H), 6.19 (m, 1H), 4.05 (m, 2H), 3.69 (m, 4H), 3.32 (m, 2H), 2.92 (m, 2H).

***N*-[1-[3,4-(Methylenedioxy)phenyl]propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9j).** ^{1}H NMR (CD_3OD): 7.71 (dd, 1H, J = 8.1, 1.2), 7.48 (d, 2H, J = 8.4), 7.45 (d, 1H, J = 1.2), 7.38 (d, 2H, J = 8.4), 6.96 (d, 1H, J = 8.1), 6.17 (m, 1H), 6.07 (s, 2H), 4.01 (m, 2H), 3.60 (m, 4H), 3.29 (m, 2H), 2.88 (m, 2H).

***N*-[1-(3,4-Dichlorophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9k).** ^{1}H NMR (CD_3OD): 8.20 (d, 1H, J = 1.8), 7.98 (dd, 1H, J = 1.8, 8.4), 7.38, 7.494 (AB quartet, 4H, J = 8.7), 6.18 (m, 1H), 4.03 (m, 2H), 3.68 (m, 4H), 3.29 (m, 2H), 2.90 (m, 2H).

***N*-[1-(2-Chlorophenyl)propionyl]-4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine (9l).** ^{1}H NMR (CD_3OD): 7.73 (d, 1H, J = 7.5), 7.38–7.53 (m, 3H), 7.46 (d, 2H, J = 8.4), 7.35 (d, 2H, J = 8.4), 6.15 (m, 1H), 4.07 (m, 2H), 3.65 (m, 4H), 3.27 (m, 2H), 2.88 (m, 2H).

***N*-[1-(4-Fluorophenyl)propionyl]-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine (9m).** ^{1}H NMR (CD_3OD): 8.29 (dd, J = 5.2, 8.7, 2H), 7.66 (dd, J = 5.2, 8.7, 2H), 7.43 (t, J = 8.7, 2H), 7.26 (J = 8.7, 2H), 6.27 (br d, 1H), 4.18 (m, 2H), 3.83 (m, 4H), 3.45 (m, 2H), 3.06 (m, 2H).

***N*-[1-(4-Chlorophenyl)propionyl]-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine (9n).** ^{1}H NMR (CD_3OD): 8.12 (d, J = 7.2, 2H), 7.55–7.65 (m, 4H), 7.15 (t, J = 7.5), 6.04 (br, 1H), 4.10 (br, 2H), 3.70 (br, 4H), 3.50 (br, 2H), 2.90 (br, 2H).

***N*-[1-(4-Bromophenyl)propionyl]-4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine (9o).** ^{1}H NMR (CD_3OD): 8.05 (d, J = 7.2, 2H), 7.80 (d, J = 7.2, 2H), 7.58 (dd, J = 8.7, 5.0, 2H), 7.15 (t, J = 7.5, 2H), 6.20 (br, 1H), 4.10 (br, 2H), 3.85 (br, 4H), 3.30 (br, 2H), 2.90 (br, 2H).

***N*-(1-Phenylpropionyl)-4-phenylpiperidine (11).** This compound was prepared by hydrogenation of **12a** in methanol over palladium on charcoal. ^{1}H NMR (CD_3OD): 7.99 (d, J = 7.5, 2H), 7.57 (t, J = 7.5, 1H), 7.46 (t, J = 7.5, 2H), 7.11–7.26 (m, 5H), 3.50–3.70 (m, 6H), 3.14 (t, J = 11, 2H), 2.84 (m, 2H).

Radioligand Binding Assays. Ten-point assays for binding of ligands to the σ receptor were performed using [^{3}H]haloperidol (specific activity 18.8 Ci/mmol, New England Nuclear) at a concentration of 2.0 nM (or 4.0 nM in some later assays, to increase the number of counts) in the presence of 25 nM unlabeled spiperone (Janssen) to inhibit association of the radioligand with the dopamine D_2 site. The concentration of the displacing ligand generally ranged from 0.2 nM to 16 μM. The tissue used in this study was the P3 synaptosomal fraction of bovine cerebellar homogenate. Incubations were carried out in 50 mM Tris-HCl containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM EDTA, pH 8.0. Nonspecific binding was defined in the presence of 1 μM unlabeled haloperidol. Each assay tube contained about 150 μg of protein in a total volume of 2.00 mL. Tubes were incubated for a 1-h period with continuous shaking at 25 °C. The incubation was terminated by rapid filtration through Schleicher and Schuell glass fiber filters (no. 32) which had been previously soaked for a 2-h period in a 0.1% (w/v) polyethyleneamine (Sigma) solution. The filters were washed three times with 2.0-mL aliquots of ice-cold wash buffer (10 mM Tris-HCl, pH 7.7) to remove loosely bound membranes. Samples were analyzed by liquid scintillation counting. Binding data were analyzed by classical graphing techniques as well as by the iterative computer programs LIGAND and EBDA (MacPherson); all methods yielded essentially equivalent results. Each assay was done in triplicate at a minimum. The data reported in Table II represent the mean of at least three separate experiments.

Dopamine D_2 binding assays were carried out in a similar manner on bovine striatal homogenates, using [^{3}H]spiperone (New England Nuclear, 21.2 Ci/mmol) at a concentration of 200 pM, and an incubation buffer (pH 7.40) containing 250 nM unlabeled ketanserin (Janssen) to inhibit association of the ligand with 5-HT_2 receptors. Nonspecific binding was defined using 10 μM (+)-sulpiride.

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