Synthesis and Receptor Binding Properties of Fluoro- and Iodo-Substituted High Affinity σ Receptor Ligands: Identification of Potential PET and SPECT σ Receptor Imaging Agents

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Unlabeled fluoro- and iodo-substituted ligands exhibiting very high affinity and selectivity for σ receptors were synthesized based on three different structural classes of σ receptor ligands. These compounds were evaluated for σ receptor affinity and specificity in order to assess their potential as PET/SPECT imaging agents. Thus, (+)- and (−)-*N*-(5-fluoro-1-pentyl)normetazocines [(+)- and (−)-**4**] based on the (+)-benzomorphan class of σ ligands were synthesized via N-alkylation of optically pure (+)- and (−)-normetazocine with 5-[(methylsulfonyl)oxy]-1-pentyl fluoride (**11**). (+)- and (−)-**4** displaced [^{3}H](+)-3-PPP with K_i values of 0.29 and 73.6 nM and [^{3}H](+)-pentazocine with K_i values of 10.5 and 38.9 nM, respectively. The second class of PET/SPECT ligands was based upon the *N*-(arylethyl)-*N*-alkyl-2-(1-pyrrolidinyl)ethylamine class of σ ligands; *N*-[2-(3,4-dichlorophenyl)-1-ethyl]-*N*-(3-fluoro-1-propyl)-2-(1-pyrrolidinyl)ethylamine (**5**) was obtained via N-alkylation of *N*-[2-(3,4-dichlorophenyl)-1-ethyl]-2-(1-pyrrolidinyl)ethylamine (**14**) with 3-fluoropropyl *p*-toluenesulfonate. **5** exhibited K_i values of 4.22 and 5.07 nM for displacement of [^{3}H](+)-3-PPP and [^{3}H](+)-pentazocine, respectively, comparable with the parent *N*-propyl compound. Attempts to synthesize *N*-[2-(3,4-dichlorophenyl)-1-ethyl]-*N*-[3-[(methylsulfonyl)oxy]-1-propyl]-2-(1-pyrrolidinyl)ethylamine (**26**), a precursor to **5** that could conceivably be converted to [^{18}F]-**5** by treatment with $^{18}F^-$, proved unsuccessful. The sequence of regioselective nitration, catalytic hydrogenation, and diazotization followed by NaI quench of *N*-[2-(3,4-dichlorophenyl)-1-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**2**) afforded the iodinated ethylenediamine *N*-[2-(2-iodo-4,5-dichlorophenyl)-1-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**8**), a potential SPECT ligand for σ receptors. This compound showed an affinity of 0.54 nM ([^{3}H](+)-3-PPP) comparable with the parent compound **2** (K_i = 0.34 nM, [^{3}H](+)-3-PPP). Ligand **8** exhibited a similar potency against [^{3}H](+)-pentazocine. The third class of high-affinity σ receptor ligands was rationalized based on rearrangement of the bonds in ethylenediamine **2** to give 1-[2-(3,4-dichlorophenyl)-1-ethyl]-4-(1-propyl)piperazine (**3**). This compound exhibited very high affinity (K_i = 0.31 nM, [^{3}H](+)-3-PPP) and selectivity for σ receptors. Compound **3** was synthesized in three steps from commercially available 1-propylpiperazine and served as a template for the synthesis of PET and SPECT compounds 1-[3-fluoro-(1-propyl)]-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (**6**) (K_i = 4.24 nM, [^{3}H](+)-3-PPP), 1-(5-fluoro-1-pentyl)-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (**7**) (K_i = 0.86 nM, [^{3}H](+)-3-PPP), and 1-(3-iodo-1-propyl)-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (**9**) (K_i = 1.32 nM, [^{3}H](+)-3-PPP). Compound **7** was synthesized via N-alkylation of 1-(3,4-dichlorophenylacetyl)piperazine (**19**) with **11**, followed by AlH_3 reduction. Ligands **6** and **9** were obtained via reaction between 1-[3-[(methylsulfonyl)oxy]-1-propyl]-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (**22**) and the corresponding anions F^- (in acetonitrile) and I^- (in acetone). The ease of generation of **6** and **9** from methanesulfonate ester **22** and of **8** from the corresponding aniline **29** combined with their very high affinity identifies them as potentially useful PET/SPECT ligands. All compounds **4**–**9** displayed very high σ receptor affinity and showed low to negligible affinity for several receptor systems which commonly cross-react with σ ligands (κ, phencyclidine, D_2-dopamine, and muscarinic/cholinergic). In general, it appeared that the K_i values for displacement of [^{3}H](+)-3-PPP correlated with those obtained for displacement of [^{3}H](+)-pentazocine, suggesting that these compounds interact with the same population of σ receptors. However, exceptions observed with (+)-**4** and **6** suggest a more complex interaction with σ sites.

Introduction

σ receptors are non-opioid, non-dopaminergic binding sites for haloperidol and other neuroleptics, and have been the focus of intense study because of their potential to offer new insights into the mechanisms of psychoses, movement disorders, and neurodegeneration. These sites have been implicated in genetic movement disorders such as dystonia, as well as motor side effects of antipsychotic drugs (ref 1 for a review). A possible role in psychoses[3,4] is suggested by the high affinity of σ sites for several typical neuroleptics and antidepressants, as well as affinity for ligands related to cocaine.[2] Very recently, neuroprotective effects have been identified among structurally diverse classes of high affinity σ receptor ligands,[5] and there is evidence that the σ receptor may be associated with an NMDA-like calcium channel.[6] σ receptors have also been shown to be involved in numerous biochemical and pharmacological effects which include negative modulation of cholinergic agonist-stimulated phosphoinositide turnover,[7] effect on serotonin and electrically induced smooth muscle contraction,[8] and release of neurotransmitter from smooth muscle preparations.[9]

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Despite the recent focus of attention, the functional role of the σ receptor remains unclear, in part due to the relative shortage of ligands exhibiting both high affinity and selectivity[10] and difficulties with making clear assignments of compounds as agonists or antagonists.[1] However, this problem is beginning to be addressed with the development of superpotent and specific ligands for this binding site.[1] The association of elevated σ receptor binding in certain tumors[11] and the presence of σ receptors in certain peripheral tissues such as the liver and gonads[12] offers a novel use of σ receptor ligands as agents for imaging tumors and other structures. Positron emission computerized tomography (PET) and single photon emission computed tomography (SPECT) are two techniques that were originally developed for the evaluation of cerebral bloodflow and metabolism,[13] but more recently have been adapted to the study of receptor localization. Thus, PET and SPECT ligands specific for σ receptors would be of great value as diagnostic tools for movement disorders and certain psychotic illnesses, for understanding the mechanisms of action of atypical antipsychotic drugs, and for shedding light on the role of this receptor. PET and SPECT imaging agents for σ receptors would allow binding and localization studies of these receptors in their native or unaltered states since tissue homogenization techniques often alter the architectural configuration and membrane properties of receptors.

PET involves labeling of a drug or ligand with a positron-emitting atom such as ^{11}C or ^{18}F and detection of the isotope by coincidence detection. In contrast, SPECT utilizes the single monoenergetic photons emitted from the decay of ^{123}I. PET and SPECT are complimentary techniques since the longer half-life (13.2 h) of ^{123}I allows more time for radiosynthesis and observation of receptor localization whereas the shorter half-life of positron-emitting isotopes such as ^{18}F (110 min) allows multiple studies to be performed in the same subject. PET ligands have been developed for numerous receptor systems that include phencyclidine,[14] benzodiazepine,[15] and dopamine.[16] In our laboratory, the first successful images of opiate receptors in primates were obtained using the PET ligand, [^{18}F]-acetylcyclofoxy.[17] SPECT has been successfully employed in receptor localization studies and offers the advantage over PET that it is readily adaptable to the small hospital setting. SPECT has been successfully employed in the imaging of several receptor systems which include monoamine uptake,[18] muscarinic,[19] and benzodiazepine.[20]

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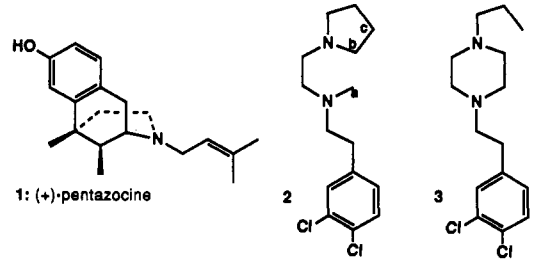
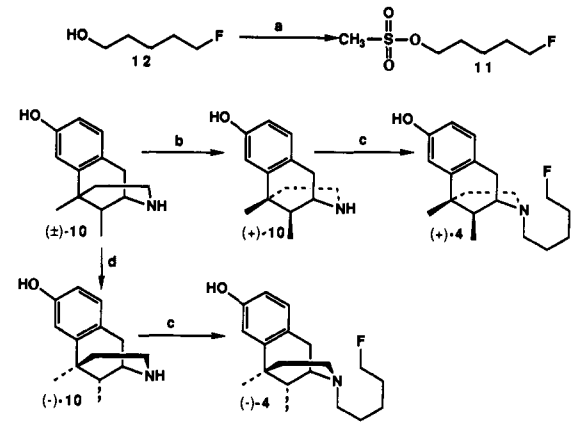
Until very recently,[21] no ligands were available for PET and SPECT imaging of σ receptors. The synthesis of potent and specific σ receptor ligands from diverse structural classes that are readily amenable to labeling with ^{123}I or positron-emitting isotopes is important in identifying ligands suitable for receptor imaging. In the present study, we wished to identify potential PET/SPECT ligands from three different classes of super-high-affinity σ receptor ligands. These include the (+)-benzomorphans,[22,23] *N*-(arylethyl)-*N*-alkyl-2-(1-pyrrolidinyl)ethylamines[24] and the *N*-alkyl-*N'*-(2-arylethyl)piperazines. Pentazocine (1)[23] is representative of the benzomorphans and has been shown to have very high affinity and selectivity for the σ receptor (K_i = 1.2 nM; [^{3}H](+)-3-PPP).[25,26] We have proposed the existence of multiple σ receptor subtypes.[1,26] (+)-Pentazocine is selective for the σ-1 receptor subtype.[26] Our



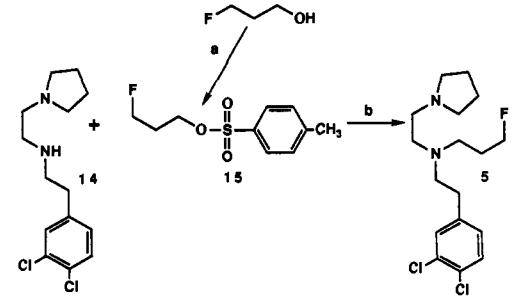
recently identified *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (2) (K_i = 0.34 nM; [^{3}H](+)-3-PPP)[24] is representative of the second class of σ receptor ligands, and preliminary data suggest that this compound is also specific for the σ-1 receptor subtype;[27] the neuroprotective properties of 2 and related compounds is noteworthy[5] since a PET or SPECT ligand derived from these compounds may offer insights into the involvement of σ receptors in neuroprotection. Compound 3 is representative of the latter class. The rationale for this compound was joining atoms a and b in 2 and breaking the bond between b and c. Compound 3, which we report here for the first time, was shown to have similar affinity (K_i = 0.31 nM; [^{3}H](+)-3-PPP) as the parent compound 2. We report here the synthesis, σ receptor binding, and receptor selectivity of potential PET ligands (+)-4, (−)-4, 5–7 and potential SPECT ligands 8–9 from three different classes of compounds. Compounds (+)-4 and (−)-4 are pentazocine- or benzomorphan-based ligands. Compound (−)-4 was synthesized in order to test the σ receptor enantiospecificity of (+)-4 since benzomorphan-based σ ligands have been shown to have a relatively high degree of enantiospecificity.[28] Demonstration of enantiospecificity is

Scheme I[a]

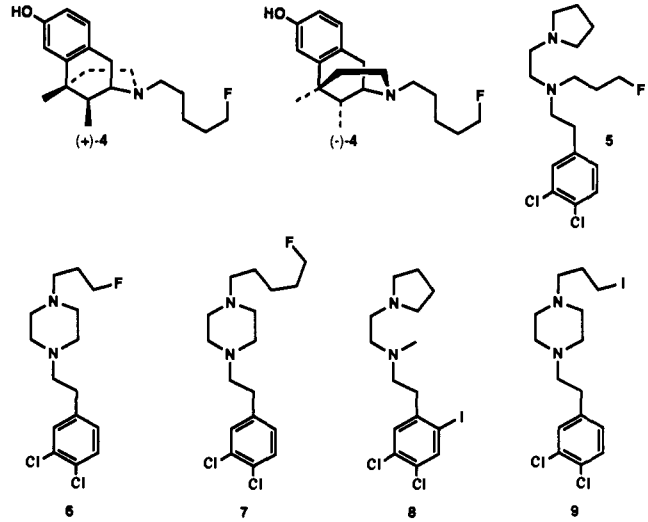


[a] (a) $MeSO_2Cl$, Et_3N, THF, room temperature; (b) (−)-tartaric acid, H_2O; (c) 5-[(methanesulfonyl)oxy]-1-pentyl fluoride, $NaHCO_3$, DMF, 40 °C; (d) (+)-tartaric acid, H_2O.

Scheme II[a]



[a] (a) TsCl, Et_3N, THF, room temperature; (b) 3-[(*p*-toluenesulfonyl)oxy]-1-propyl fluoride, $NaHCO_3$, DMF, 55 °C.



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often a useful indicator of specific binding in PET and SPECT studies. Compound 5 is based upon the *N*-propyl analogue of 2. We have previously shown that replacement of the *N*-methyl group of 2 (K_i = 0.34 nM) with an *N*-(*n*-propyl) group results in only a slight reduction in affinity (K_i for *N*-propyl homologue = 1.35 nM).[24] Compound 6 is based upon piperazine 3. Compound 7 (Scheme III) served to investigate the effect of further extension of the fluoroalkyl sidechain of 6 on receptor binding affinity and selectivity. The potential SPECT ligands 8 and 9 are based upon 2 and 3, respectively. In compounds 4–9, the position of the halogen was selected such that no new chiral centers were introduced.

Chemistry

Racemic *N*-normetazocine [(±)-10] (Scheme I) was resolved to optically purity using the procedure of Tuller et al.[23] (+)-4 was obtained in 33% purified yield by treatment of (+)-10 with 5-[(methylsulfonyl)oxy]-1-pentyl fluoride (11) (generated by treatment of 5-fluoropentanol (12) with methanesulfonyl chloride in THF/Et_3N (see Scheme I) for 28 h at 40 °C. Compound (−)-4 was similarly obtained in starting with (−)-10. The low yield in the formation of (+)- and (−)-4 can be accounted for by the formation of the corresponding *N*-methyl derivatives (+)- and (−)-13 (metazocines) as side products due to the presence of a MeOH impurity in the commercially available 5-fluoro-1-pentanol.

Synthesis of potential PET ligand 5 was achieved starting with the previously reported 14.[24] Treatment of 14 with 3-(tosyloxy)-1-propyl fluoride (15) (Scheme II) in dry DMF afforded 5 in 91% yield (based on the amount of recovered starting material). The reaction was found to be incomplete after 19 h at 61 °C. However, no attempt was made to force the reaction to completion because of competing quaternization of the N-atoms of 5 and 14. Piperazine 3 was obtained by the sequence of DCC coupling (quantitative) of commercially available *N*-(*n*-propyl)piperazine (16) with 3,4-dichlorophenylacetic acid followed by AlH_3 reduction[24,29] of the amide linkage in THF (76%) (Scheme III). Compound 6 was obtained starting from piperazine 18 (Scheme III). Treatment of an excess of piperazine (5 equiv) with the complex generated by treatment of 3,4-dichlorophenylacetic acid with DCC afforded monoamide 19 in 50% yield. Treatment of 19 with excess ethyl acrylate in boiling toluene resulted in alkylated intermediate 20. AlH_3 reduction of 20 in THF afforded 21 in 75% yield. Reaction of 21 with methanesulfonyl chloride in $CHCl_3$ in the presence of Et_3N gave methanesulfonate ester 22 together with approximately 20% yield of the corresponding chloro compound 23 as a side product. However, a new set of reaction conditions using methanesulfonic anhydride instead of methanesulfonyl chloride gave 22 as the only product in 85% yield. Compound 22 was found to be unstable on prolonged storage as the free base. It was, however, found to be indefinitely stable as the crystalline bismethanesulfonate salt. Treatment of either the free base or methanesulfonate salt of 22 with excess tetrabutylammonium fluoride in CH_3CN afforded an 80% yield of the desired 6. Preliminary studies to introduce ^{18}F into 22 by nucleophilic displacement with $^{18}F^-$ have routinely given [^{18}F]-6 with radiochemical yields of approximately 50%.[30] Introduction of ^{18}F into drug molecules via generation and use of $^{18}F^-$ in nucleophilic displacement reactions on suitable precursors is advantageous over synthesizing for example [^{18}F]-3-(tosyloxy)-1-propyl fluoride and alkylating for example a secondary amine such as 19 with this reagent. Thus, we also hoped to synthesize a methanesulfonate ester precursor to 5 that could conceivably be transformed to the fluoro analogue as for 6. Treatment of *N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)ethylamine (14)[24] with excess ethyl acrylate in refluxing toluene during 72 h afforded ester 24 in quantitative yield (Scheme IV). Reduction of the ester group of 24 with AlH_3 in THF afforded alcohol 25 in 75% yield. Unfortunately, unlike alcohol 21 (Scheme III), treatment of 25 with methanesulfonic anhydride in the presence of Et_3N resulted only in aqueous soluble products; no trace of methanesulfonate ester 26 could be detected. The greater flexibility of 26 compared with 22 may help promote its self-quaternization. The relatively more constrained 22 is less amenable to self-quaternization. Fluoropentyl derivative 7 was synthesized (Scheme III) by the sequence of alkylation of 19 with methanesulfonate 11 to give 27 (57%) followed by AlH_3 reduction (85%). The low yield formation of intermediate 27 is due to competing N-methylation as we have observed previously for (+)- and (−)-4 (Scheme I). Iodide 9 was obtained (Scheme III) by treatment of 22 with sodium iodide in acetone at 50 °C and was isolated as its bishydroiodide salt. Attempts to generate 9 by treatment of alcohol 21 with 57% aqueous HI at 70 °C resulted only in unchanged starting material. Iodide 8 was synthesized (Scheme V) starting from 2. Treatment of 2 with HNO_3/H_2SO_4/AcOH mixture at 4–5 °C resulted in mononitro derivative 28 as the only product. Catalytic reduction of 28 by treatment with hydrogen gas in the presence of Adams catalyst gave a 62% yield of aniline 29. The diazonium cation 30 generated by reaction of 29 with nitrous acid was quenched into excess aqueous NaI to give 8 in 20% yield.

Results and Discussion

By synthesizing and evaluating the binding affinity of unlabeled fluoro and iodo derivatives derived from three different classes of high affinity σ receptor ligands, we have identified several potentially useful PET and SPECT imaging agents for this receptor. Thus, the *N*-(3-fluoropropyl) analogue of our previously reported *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (2) (K_i = 0.34 nM, [^{3}H](+)-3-PPP) exhibited a σ receptor affinity of 4.22 nM ([^{3}H](+)-3-PPP) as well as high selectivity for the σ receptor. Selectivity ranged from 740-fold for σ over D_2-dopamine receptors to 2500 for κ receptors to 7600 for muscarinic cholinergic receptors and negligible affinity for PCP receptors (Table I). D_2-dopamine, κ opioid, PCP, and muscarinic cholinergic receptors were investigated since several prototypic σ ligands have been shown to cross-react to some extent with one or more of these receptors.[1,5] The ortho-iodo derivative of 2, a

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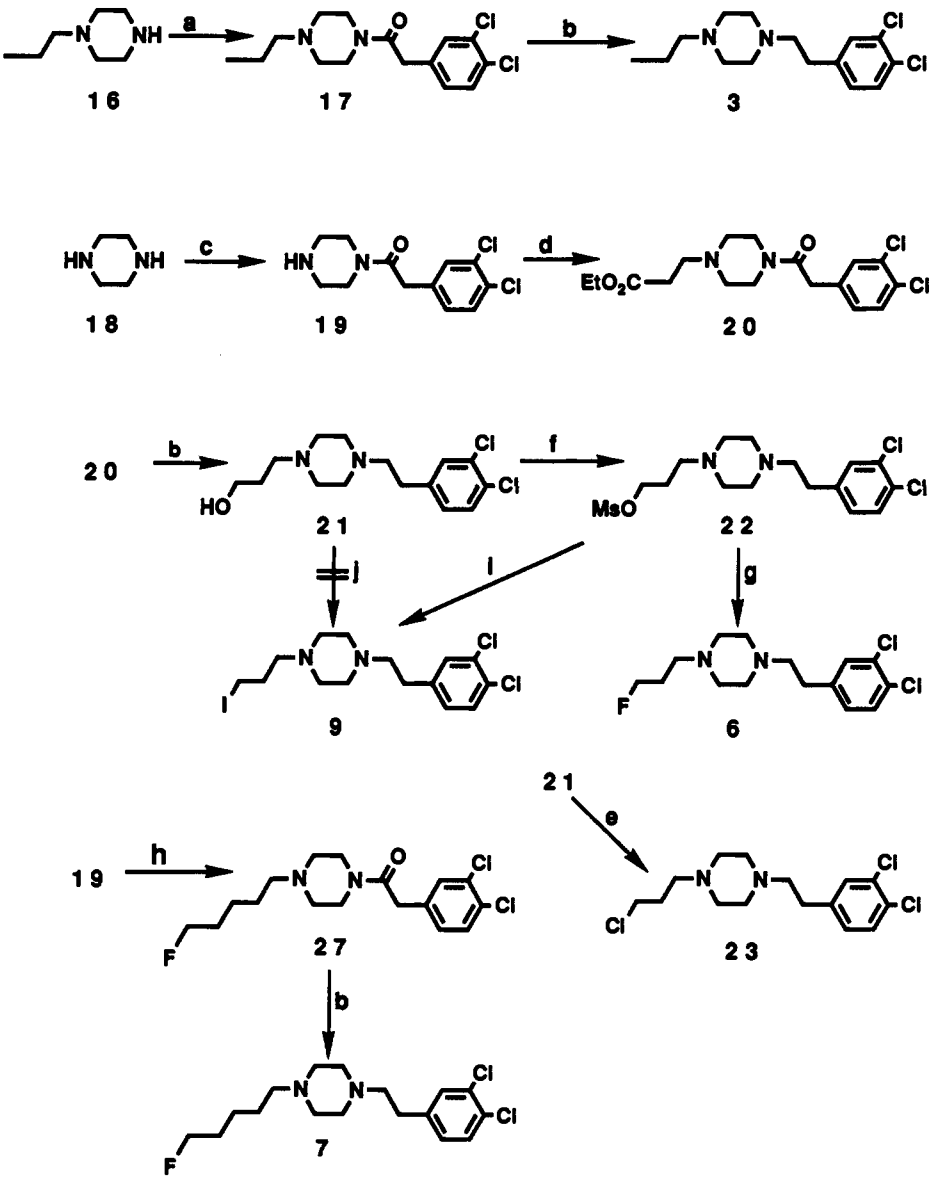
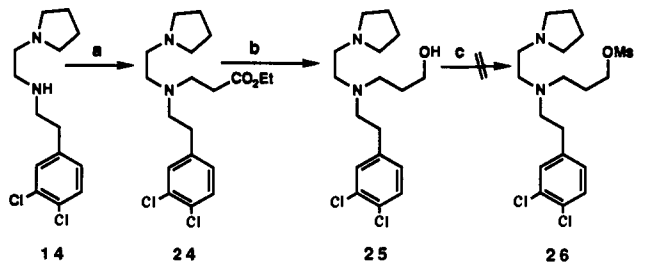
Scheme III[a]



[a] (a) 3,4-Dichlorophenylacetic acid, DCC, CH_2Cl_2; (b) AlH_3, THF, room temperature; (c) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2, 5 equiv piperazine; (d) ethyl acrylate, toluene, reflux; (e) CH_3SO_2Cl, Et_3N, $CHCl_3$; (f) $(CH_3SO_2)_2O$, Et_3N, $CHCl_3$; (g) nBu$_4$NF, CH_3CN, room temperature, 1 h; (h) 5-[(methanesulfonyl)oxy]-1-pentyl fluoride, $NaHCO_3$, DMF, 40 °C; (i) NaI, acetone, 50 °C, 30 min; (j) 57% aqueous HI, 70 °C, 24 h.

Scheme IV[a]



[a] (a) Ethyl acrylate, toluene, reflux, 72 h; (b) AlH_3, THF, room temperature; (c) $(CH_3SO_2)_2O$, Et_3N, $CHCl_3$.

potential SPECT imaging agent, exhibited a σ receptor affinity of 0.54 nM ([^{3}H](+)-3-PPP) which is comparable to the parent compound. The selectivity ratio for this compound ranged from 2300 for σ vs D_2-dopamine to 43 000 for PCP receptors and 13 000 for muscarinic cholinergic receptors.

Among the (+)-benzomorphans, replacement of the prenyl group of (+)-pentazocine (1) with a 1-(5-fluoropentyl) group gave (+)-4. This compound (K_i = 0.29 nM; [^{3}H](+)-3-PPP) exhibited a 4-fold increase in affinity compared with (+)-pentazocine and is sufficiently potent for consideration as a (+)-benzomorphan-based PET imaging agent. The selectivity of (+)-4 for the other receptors ranged from 6800 for σ vs κ receptors to 196 000-fold for cholinergic receptors versus σ receptors. Compound (+)-4, the only chiral member of the series exhibited an enantioselectivity ratio of 253-fold in favor of the (+)-enantiomer for σ receptors. This enantioselectivity ratio is significantly higher than the 73-fold ratio observed with the enantiomers of pentazocine.[26]

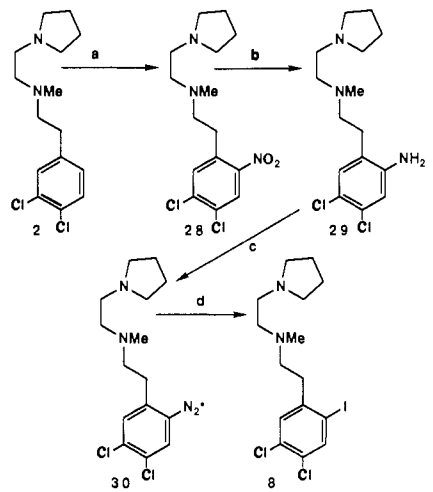
The third (piperazine) class of compounds was identified in the present study. Thus, modification of **2** by breakage of the bond between the C2 and C3 carbon atoms of the pyrrolidine ring and formation of a new bond between the *N*-methyl group and pyrrolidine C2 atom gave rise to 1-(1-propyl)-4-[2-(3,4-dichlorophenyl)-1-ethyl]piperazine (**3**).

Table I. Receptor Binding Properties of Potential σ Receptor Imaging Agents[a]

	K_i (nM)					
	σ		κ:	PCP:	D_2-dopamine:	muscarinic:
compd	[^{3}H](+)-3-PPP	[^{3}H]pent	[^{3}H]brem	[^{3}H]TCP	[^{3}H]sulp	[^{3}H]QNB
2	0.34 ± 0.07	ND	no inhibn[b]	no inhibn[b]	1112 ± 74	ND
3	0.31 ± 0.03	ND	no inhibn[b]	no inhibn[b]	2902 ± 157	ND
(+)-4	0.29 ± 0.10	10.5 ± 0.57	1988 ± 233	9036 ± 80	2533 ± 503	56 850 ± 2150
(−)-4	73.6 ± 7.1	38.9 ± 9.4	ND	ND	ND	ND
5	4.22 ± 0.84	5.07 ± 0.84	10 478 ± 39	no inhibn[b]	3111 ± 543	32 030 ± 840
6	4.24 ± 0.26	0.39 ± 0.002	no inhibn[b]	no inhibn[b]	11 993 ± 1115	682 500 ± 13 600
7	0.86 ± 0.18	0.52 ± 0.02	ND	ND	ND	ND
8	0.54 ± 0.03	1.23 ± 0.02	18 537 ± 481	24 900 ± 1664	1311 ± 195	7000
9	1.32 ± 0.42	1.19 ± 0.06	ND	ND	ND	ND

[a] Sigma binding affinities were determined by incubating the appropriate σ receptor probe in the presence of 12 concentrations of test ligand in one of three concentration ranges: 0.0005–100 nM, 0.005–1000 nM, or 0.05–10 000 nM. In order to obtain an initial estimate of binding affinity at other receptors, three concentrations of each compound (100, 1000, and 10 000 nM) were incubated with the indicated radioligand for dopamine-D_2, κ opiate, PCP, and cholinergic receptors. Compounds eliciting >30% inhibition were investigated further using 12 concentrations of unlabeled ligand ranging from 0.0005–100 μM or 0.05–5000 μM. All assay conditions were as described in Experimental Section. Values are the average of two to three experiments ± SEM, each carried out in duplicate. The CDATA iterative curve fitting program (EMF Software, Inc., Baltimore, MD) was used to determine IC_{50} values. The Cheng–Prusoff equation[33] was then used to convert IC_{50} values to apparent K_i values. The following K_d values (as determined in independent experiments) were employed to calculate K_i: [^{3}H](+)-3-PPP (guinea pig brain), K_d = 27.4 nM; [^{3}H](+)-pentazocine (guinea pig brain), K_d = 4.8 nM; [^{3}H](−)-sulpiride (rat brain), K_d = 10.3 nM; [^{3}H](−)-bremazocine (guinea pig brain), K_d = 0.64 nM; [^{3}H]TCP (guinea pig brain), K_d = 25 nM; [^{3}H]QNB (rat brain), K_d = 0.3 nM. [b] No IC_{50} or K_i value was determined since the compound produced less than 30% inhibition of control binding at a concentration of 10 000 nM. ND: value not determined in this case.

Scheme V[a]



[a] (a) HNO_3, H_2SO_4, AcOH; (b) H_2, PtO_2; (c) HONO; (d) aqueous NaI.

This compound proved to be equipotent (K_i = 0.31 nM; [^{3}H](+)-3-PPP) to the parent compound **2** and exhibited high selectivity for σ versus other receptor types. Compound **2** served as a template for the development of **6**, **7**, and **9**. The 1-[3-fluoro-(1-propyl)]piperazine derivative **6** of **3** exhibited an affinity of 4.24 nM for displacement of [^{3}H](+)-3-PPP. The selectivity ratio of this compound ranged from 2800 for σ versus D_2-dopamine to negligible affinity at κ opioid, PCP, and muscarinic receptors. The corresponding 1-(5-fluoropentyl) derivative **7** showed a σ affinity (K_i = 0.86 nM, [^{3}H](+)-3-PPP) somewhat more potent than the parent compound **3** and 5-fold improved over the 1-(3-fluoropropyl) derivative **6**. The 1-[3-iodo-(1-propyl)]piperazine derivative **9**, a potential SPECT imaging agent exhibited a σ receptor affinity of 1.32 nM ([^{3}H](+)-3-PPP).

Under the conditions of the σ receptor assay described here (i.e. guinea pig brain), [^{3}H](+)-3-PPP and [^{3}H](+)-pentazocine label predominantly the putative σ-1 subtype of the σ receptor.[1,26] In general the K_i values from displacement of [^{3}H](+)-3-PPP correlated closely with those obtained from displacement of [^{3}H](+)-pentazocine, the latter being a much more selective probe for the σ-1 site.[25,26,31] However, there are two exceptions: Compound (+)-**4** exhibited 36 times higher affinity at sites labeled by [^{3}H](+)-3-PPP compared to [^{3}H](+)-pentazocine, while compound **6** exhibited 11-fold higher affinity at sites labeled by [^{3}H](+)-pentazocine compared to [^{3}H](+)-3-PPP. The reason for these discrepancies is not clear, but might suggest either differential interaction with σ-1 and σ-2 subtypes or complex interactions of these particular compounds with σ sites. The σ-2 binding properties of these compounds is currently under investigation.

The exceptional potency and selectivity of compounds **5–9** identifies them as potential agents for PET and SPECT imaging of σ receptors. The relatively high polarity of these compounds suggests that nonspecific labeling as a result of association of these compounds with lipid may be less of a problem. Potential SPECT ligands **8** and **9** both exhibited very high σ receptor affinity. However, because **9** is a primary alkyl iodide, it is likely that it may exhibit lower in vivo stability than aryl iodide **8**. Further information concerning the suitability of **4–9** as PET and SPECT ligands for imaging of σ receptors in living subjects as well as their in vivo stability awaits their radiolabeling to high specific activity.

High densities of σ binding sites have been found in the liver and gonads of the rat.[12] The densities in these tissues are in fact 10 to 20 times higher than in rat brain.[27,34] This

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may limit the utility of radiolabeled σ ligands for imaging in the human due to possible delivery of high doses of radioactivity to these sensitive tissues. However, the development of σ subtype-specific imaging agents might reduce exposure due to the presence of σ subtypes in these tissues. For example, we have observed that σ binding in rat liver is comprised of 25% σ-1 sites and 75% σ-2 sites.[34] Therefore, ligands selective for σ-1 sites would expose the liver to much lower doses of radioactivity than a non-subtype-selective σ ligand. Another issue is that of metabolism of the radioligand in vivo by the liver. This is particularly germane since it has been proposed that at least one of the σ binding sites may be related to a type of drug-metabolizing enzyme.[35] This aspect will be the topic of future studies using radiolabeled ligands. Despite these potential problems for σ imaging in humans, these ligands should prove quite useful in animal studies and will aid in elucidating functions of σ sites.

Experimental Section

Materials and Methods. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Specific rotation determinations at the sodium-D line were obtained in a 1-dm cell using a Perkin-Elmer 241-MC polarimeter. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Molecular formula followed by the symbols of C, H, N indicates that elemental analyses were found to be within ±0.4% of the theoretical values for C, H, and N. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high resolution mass measurements (HRMS) were obtained using a VG-Micro Mass 7070F mass spectrometer. ^{1}H-NMR spectra were obtained from $CDCl_3$ solutions using a Varian XL-300 spectrometer. Thin-layer chromatography (TLC) was performed on 250-μm Analtech GHLF silica gel plates. No attempt was made to optimize the yields. Solvent system A corresponds to concentrated aqueous NH_3–MeOH–$CHCl_3$ (1:9:90). Solvent system B corresponds to concentrated aqueous NH_3–MeOH–$CHCl_3$ (2:18:80). Solvent system C corresponds to concentrated aqueous NH_3–MeOH–$CHCl_3$ (0.5:4.5:95).

5-[(Methylsulfonyl)oxy]-1-pentyl Fluoride (11). Methanesulfonyl chloride (6.48 g, 56.6 mmol, 1.2 equiv) in dry THF (20 mL) was added dropwise at 0 °C to a stirred mixture of 5-fluoropentanol (5.00 g, 47.2 mmol) and triethylamine (19.7 mL, 141 mmol, 3 equiv) in dry THF (100 mL). After the addition was complete (10 min), the reaction mixture was allowed to warm to room temperature and stirred for a further 10 min. The copious white precipitate of Et_3N·HCl was filtered, and the filtercake was washed with a small amount THF. The combined filtrate and washings were evaporated in vacuo at not more than 40 °C, and the oily residue was dissolved in $CHCl_3$ (50 mL) and stirred in the presence of saturated aqueous $NaHCO_3$ (50 mL) for 1 h at room temperature. The $CHCl_3$ layer was separated and diluted to 100 mL with $CHCl_3$, and the solution was backwashed with water (50 mL). The $CHCl_3$ layer was evaporated in vacuo (<40 °C) and dried by azeotropic distillation with hydrocarbon-stabilized $CHCl_3$ (3 × 10 mL). Evaporation of the solvent afforded 11 as a colorless oil: 5.7 g (66%); ^{1}H-NMR ($CDCl_3$) δ 4.46 (dt, J = 47, 5.8 Hz, 2 H), 4.25 (t, J = 6.4 Hz, 2 H), 3.01 (s, 3 H), 1.50–1.89 (complex m, 6 H). No attempt was made to further purify this compound because of its lability. The low yield is due to the presence of MeOH as an impurity in the starting material.

(+)-*N*-(5-Fluoro-1-pentyl)normetazocine [(+)-4]. To a stirred solution of optically pure (+)-normetazocine base [(+)-10] (0.50 g, 2.30 mmol)[31] and $NaHCO_3$ (1.00 g, 11.9 mmol, 5 equiv) in dry DMF (10 mL) at 40 °C was added 11 (0.51 g, 2.72 mmol, 1.2 equiv), and the solution was stirred for 28 h at 40 °C when TLC (solvent system A) indicated that the reaction was complete. The reaction mixture was poured into 10% aqueous citric acid solution (100 mL), the solution was extracted with ether (3 × 50 mL), and the combined ether extract was discarded. The aqueous layer was basified to pH = 9 by addition of excess concentrated aqueous NH_3 solution. The basified solution was extracted with ether (3 × 50 mL), the combined ether layer was backwashed with water (50 mL), and then the solvent was evaporated in vacuo to give the product as an oily residue which was dried by azeotropic distillation with ethanol (2 × 50 mL) to give 0.44 g of crude product. This was separated by chromatography on silica gel eluting with solvent system C. Evaporation of the earlier fractions afforded (+)-4 (0.23 g, 33%) while the later fractions afforded (+)-metazocine (formed as a result of the presence of an $MeSO_2Me$ impurity in the 11). Crystalline (+)-4 was obtained by dissolving the oily material in ethyl acetate (0.8 mL) and diluting with hexanes (4.0 mL): mp 135.5–137 °C; ^{1}H-NMR ($CDCl_3$) δ 6.93 (d, J = 8.2 Hz, 1 H), 6.71 (d, J = 2.5 Hz, 1 H), 6.60 (dd, J = 8.2, 2.5 Hz, 1 H), 4.44 (dt, J = 47, 6.1 Hz, 2 H), 2.90 (m, 2 H), 2.42–2.69 (complex m, 4 H), 2.06 (m, 1 H), 1.37–1.95 (complex m, 10 H), 1.33 (s, 3 H), 0.84 (d, J = 7.0 Hz, 3 H); $[\alpha]_D$ = +95° (c = 1.01, $CHCl_3$); CIMS (MH^+ calcd for $C_{19}H_{28}FNO$) 306; found, 306. Anal. ($C_{19}H_{28}FNO$) C, H, N.

(−)-*N*-(5-Fluoro-1-pentyl)normetazocine [(−)-4]. Compound (−)-4 was similarly obtained starting with 0.82 g (3.78 mmol) of (−)-10,[31] 0.49 g (2.66 mmol, 0.70 equiv) of 11, and 1.58 g (18.9 mmol) of $NaHCO_3$: yield, 0.32 g (28%); 135.5–137 °C; $[\alpha]_D$ = −96° (c = 0.98, $CHCl_3$); CIMS (MH^+ calcd for $C_{19}H_{28}FNO$) 306; found, 306. Anal. ($C_{19}H_{28}FNO$) C, H, N. The ^{1}H-NMR spectrum was identical to that of (+)-4 above.

3-(Tosyloxy)-1-propyl Fluoride (15). To a stirred solution of 3-fluoropropanol (14.69 g, 188 mmol) and triethylamine (52.5 mL, 377 mmol, 2.0 equiv) in dry THF (200 mL) at room temperature was added toluenesulfonyl chloride (39.49 g, 207 mmol, 1.1 equiv) dissolved in THF (100 mL). The reaction mixture was stirred overnight at room temperature, the copious precipitate of Et_3N·HCl was filtered, and the filtercake was washed with a small amount of THF. The solvent was evaporated in vacuo, and the residue was taken up in CH_2Cl_2 (200 mL) and washed with saturated $NaHCO_3$ (100 mL) and water (100 mL). Evaporation of the solvent afforded 15 (43.7 g, quantitative) as a reddish oil: ^{1}H-NMR ($CDCl_3$) δ 7.80, 7.36 (ABq, J = 7.9 Hz, 4 H), 4.48 (dt, J = 47, 5.6 Hz, 2 H), 4.16 (t, J = 6.1 Hz, 2 H), 2.45 (s, 3 H), 2.04 (doublet of quintets, J = 26, 5.5 Hz, 2 H).

***N*-[2-(3,4-Dichlorophenyl)-1-ethyl]-*N*-(3-fluoro-1-propyl)-2-(1-pyrrolidinyl)ethylamine (5).** A stirred mixture of 14 (base) (1.92 g, 6.68 mmol),[24] DMF (60 mL), and 15 (1.86 g, 8.02 mmol, 1.2 equiv) was stirred and heated at 60 °C for 19 h when TLC (solvent system A) indicated that the reaction was 50% complete. No attempt was made to leave the reaction longer because of competing quaternization of the tertiary nitrogen atoms. The reaction mixture was cooled, poured into cold water (200 mL) and extracted with ether (200 mL). The ethereal layer was separated and backwashed with water (50 mL), and the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel eluting with solvent system C to give 5 (0.50 g, 91% based on amount of recovered 14) as a colorless oil. Treatment of 5 in EtOH with 48% aqueous HBr afforded 5·HBr. Crystallization was facilitated by cooling of the solution. 5·HBr: mp 199–201 °C; ^{1}H-NMR ($CDCl_3$) δ 7.33 (d, J = 8.2 Hz, 1 H), 7.29 (d, J = 2.0 Hz, 1 H), 7.02 (dd, J = 8.2, 2.0 Hz, 1 H), 4.45 (dt, J = 47, 5.8 Hz, 2 H), 2.59–2.72 (complex m, 10 H), 2.49–2.59 (m, 4 H), 1.70–1.88 (m, 6 H); CIMS (MH^+ calcd for $C_{17}H_{25}Cl_2FN_2$) 347; found, 347. Anal. ($C_{17}H_{27}Br_2Cl_2FN_2$) C, H, N.

1-[(3,4-Dichlorophenyl)acetyl]-4-(1-propyl)piperazine (17). To a stirred solution of the complex formed by addition of DCC (11.8 g, 57.2 mmol, 2.0 equiv) to a solution of 3,4-dichlorophenylacetic acid (8.8 g, 43.1 mmol, 1.5 equiv) in CH_2Cl_2 (200 mL) was added 1-propylpiperazine dihydrobromide (8.3 g, 28.6 mmol). To the reaction mixture was finally added triethylamine (16 mL, 115 mmol, 4 equiv), and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was filtered to remove the precipitated *N*,*N*′-dicyclohexylurea (DCU) and the filtercake was washed with a little ether. The combined filtrate and washings were evaporated in vacuo, and the residue was partitioned between 10% citric acid (200 mL) and ether (200 mL). The ethereal layer was separated, and the aqueous layer was washed with a further 2 × 200 mL of ether. The combined ether

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washings were discarded, and the aqueous layer was basified by the addition of excess concentrated aqueous ammonia solution and then extracted with CH_2Cl_2 (2 × 200 mL). The CH_2Cl_2 extract was backwashed with water (50 mL), and the solvent was evaporated in vacuo to give **17** (9.0 g, quantitative) as a crystalline solid. A portion of the material was recrystallized from isooctane: mp 54–55 °C; ^{1}H-NMR ($CDCl_3$) δ 7.39 (d, J = 8.1 Hz, 1 H), 7.34 (d, J = 2.0 Hz, 1 H), 7.08 (dd, J = 8.1, 2.0 Hz, 1 H), 3.66 (s, 2 H), 3.65 (m, 2 H), 3.46 (m, 2 H), 2.40 (m, 2 H), 2.33 (m, 2 H), 2.29 (t, J = 7.8 Hz, 2 H), 1.49 (sextet, J_{app} = 7.5 Hz, 2 H), 0.90 (t, J = 7.4 Hz, 3 H); CIMS (MH^+ calcd for $C_{15}H_{20}Cl_2N_2O$) 315; found, 315. Anal. ($C_{15}H_{20}Cl_2N_2O$) C, H, N.

1-[2-(3,4-Dichlorophenyl)-1-ethyl]-4-(1-propyl)piperazine (3). To a stirred solution of freshly prepared AlH_3 (101 mL of a 1.0 M solution in THF, 5 equiv)[24,29] was added dropwise at room temperature a solution of **17** (6.4 g, 20.3 mmol) in THF (20 mL). The reaction was allowed to stir for 20 min at room temperature and then quenched into 15% NaOH (200 mL). The aqueous mixture was extracted with $CHCl_3$ (3 × 200 mL), and the combined extract was allowed to dry over anhydrous K_2CO_3. Evaporation of the solvent in vacuo gave the product in quantitative yield as a colorless oil. A solution of the oil in MeOH (100 mL) was warmed to boiling point and then treated (to pH = 3) with 48% aqueous HBr. Crystallization occurred on cooling to room temperature. The first crop afforded 6.34 g (67%) of **3**·HBr. Recovery of the second and third crystal crops increased the yield to 76%. **3**·HBr: mp 290–292 °C dec; ^{1}H-NMR ($CDCl_3$) δ 7.33 (d, J = 8.1 Hz, 1 H), 7.30 (d, J = 1.9 Hz, 1 H), 7.04 (dd, J = 8.1, 1.9 Hz, 1 H), 2.76 (m, 2 H), 2.39–2.63 (complex m, 10 H), 2.31 (m, 2 H), 1.52 (sextet, J_{app} = 7.6 Hz, 2 H), 0.90 (t, J = 3.6 Hz, 3 H); CIMS (MH^+ calcd for $C_{15}H_{22}Cl_2N_2$) 301; found, 301. Anal. ($C_{15}H_{24}Br_2Cl_2N_2$) C, H, N.

1-[(3,4-Dichlorophenyl)acetyl]piperazine (19). To a stirred solution of anhydrous piperazine (105.1 g, 1.22 mol, 5.0 equiv) in CH_2Cl_2 (600 mL) was added the complex generated from mixing a solution of 3,4-dichlorophenylacetic acid (50 g, 244 mmol, 1.0 equiv) in CH_2Cl_2 (300 mL) with DCC (67.1 g, 325 mmol, 1.33 equiv) in CH_2Cl_2 (300 mL). The reaction mixture was allowed to stir overnight at room temperature when TLC (solvent system A) indicated the reaction to be complete. The solvent was evaporated in vacuo and the residue was partitioned between ether (800 mL) and water (500 mL). The organic layer was washed with a further 500 mL of water, and the combined aqueous washings were discarded. The ethereal layer was washed well with 10% aqueous citric acid (500 mL). The aqueous acidic extract was washed with ether (2 × 500 mL), and the combined ether washings were discarded. The acidic extract was basified by addition of excess concentrated aqueous ammonia solution and extracted with CH_2Cl_2 (2 × 500 mL). The CH_2Cl_2 layer was dried (Na_2SO_4) and evaporated to afford 33.5 g (50%) of crude **19** as a colorless oil. **19**·fumarate crystallized from MeOH to give analytically pure material: mp 189–190 °C; ^{1}H-NMR ($CDCl_3$) δ 7.39 (d, J = 8.3 Hz, 1 H), 7.35 (d, J = 2.0 Hz, 1 H), 7.09 (dd, J = 8.3, 2.0 Hz, 1 H), 3.66 (s, 2 H), 3.61 (m, 2 H), 3.42 (m, 2 H), 2.83 (m, 2 H), 2.75 (m, 2 H), 1.25 (br s, 1 H); CIMS (MH^+ calcd for $C_{12}H_{14}Cl_2N_2O$) 273; found, 273. Anal. ($C_{16}H_{18}Cl_2N_2O_5$) C, H, N.

1-[3-(Ethoxypropionyl)-4-[(3,4-dichlorophenyl)acetyl]piperazine (20). To a solution of **19** base (3.6 g, 49.3 mmol) in toluene (40 mL) was added ethyl acrylate (7.1 mL, 5.0 equiv), and the solution was boiled under reflux for 48 h when TLC (solvent system A) indicated complete reaction. The reaction mixture was cooled, diluted with ether (100 mL), and extracted with 10% aqueous citric acid (200 mL). The ethereal layer was discarded, and the aqueous layer was washed with a further 2 × 200 mL of ether. The aqueous layer was basified with excess concentrated aqueous ammonia solution and extracted with CH_2Cl_2 (2 × 200 mL). The combined CH_2Cl_2 extract was backwashed with water (100 mL) and evaporated to give **20** as a colorless crystalline solid (4.3 g, 87%). Recrystallization from hot isooctane afforded an analytically pure sample of **20**: mp 81–82 °C; ^{1}H-NMR ($CDCl_3$) δ 7.39 (d, J = 8.1 Hz, 1 H), 7.34 (d, J = 2.0 Hz, 1 H), 7.08 (dd, J = 8.1, 2.0 Hz, 1 H), 4.14 (q, J = 7.1 Hz, 2 H), 3.66 (s, 2 H), 3.63 (m, 2 H), 3.44 (m, 2 H), 2.69 (t, J = 7.1 Hz, 2 H), 2.47 (t, J = 7.1 Hz, 2 H), 2.43 (m, 2 H), 2.36 (m, 2 H), 1.26 (t, J = 7.1 Hz, 3 H); CIMS (MH^+ calcd for $C_{17}H_{22}Cl_2N_2O_3$) 373; found, 373. Anal. ($C_{17}H_{22}Cl_2N_2O_3$) C, H, N.

1-(3-Hydroxy-1-propyl)-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (21). To a stirred solution of AlH_3 in THF[24,29] (40 mL of a freshly prepared 1.0 M solution, 40 mmol, 5.0 equiv) was added dropwise at room temperature a solution of **20** (3.00 g, 8.04 mmol). TLC (solvent system B) analysis of the reaction mixture after 10 min at room temperature indicated that the reaction was complete. The reaction was quenched (care!) into 200 mL of 15% aqueous NaOH. The aqueous mixture was extracted with $CHCl_3$ (200 mL), and the extract was dried (Na_2SO_4) and evaporated to give the crude product as a colorless oil. The oil was dissolved in EtOH (50 mL), and the solution was heated to boiling point. To this solution was added 48% aqueous HBr (to pH = 3). Crystallization occurred spontaneously as soon as all of the HBr solution had been added. The crystallization mixture was set aside to cool to room temperature and then allowed to cool to 4 °C. The first crop afforded **21**·HBr (2.50 g, 65%). The yield could be raised to 75% by collecting the second and third crystal crops: mp 265–266 °C dec; ^{1}H-NMR ($CDCl_3$) δ 7.34 (d, J = 8.3 Hz, 1 H), 7.29 (d, J = 2.0 Hz, 1 H), 7.03 (dd, J = 8.3, 2.0 Hz, 1 H), 3.81 (t, J = 5.1 Hz, 2 H), 2.74 (m, 2 H), 2.40–2.69 (complex m, 10 H), 2.63 (t, J = 5.7 Hz, 2 H), 1.73 (quintet, J_{app} = 5.5 Hz, 2 H); CIMS (MH^+ calcd for $C_{15}H_{22}Cl_2N_2O$) 317; found, 317. Anal. ($C_{15}H_{24}Br_2Cl_2N_2O$) C, H, N.

1-[3-[(Methylsulfonyl)oxy]-1-propyl]-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (22). Methanesulfonic anhydride was added in one portion to a stirred solution of **21** (base) (0.53 g, 1.67 mmol) and Et_3N (1.0 mL, 7.19 mmol, 4.3 equiv) in hydrocarbon-stabilized $CHCl_3$ (20 mL). TLC (solvent system A) indicated the reaction to be complete after 10 min at room temperature. The reaction mixture was diluted to 50 mL with $CHCl_3$ and poured into saturated $NaHCO_3$ (50 mL). The aqueous layer was discarded, the organic layer was washed with a further 50 mL of water and dried (Na_2SO_4), and the solvent was evaporated to afford the crude product as a yellow oil. The oil was dissolved in 2-propanol (10 mL) and treated with methanesulfonic acid to pH = 3. Crystallization was induced by scratching with a glass rod. The crystallization mixture was set aside at 4 °C, filtered, washed twice with cold 2-propanol followed by ether, and oven dried at room temperature overnight. Yield = 0.83 g (85%). **22**·CH_3SO_3H: mp 162–163 °C; ^{1}H-NMR ($CDCl_3$) δ 7.34 (d, J = 8.2 Hz, 1 H), 7.29 (d, J = 1.9 Hz, 1 H), 7.03 (dd, J = 8.2, 1.9 Hz, 1 H), 4.30 (t, J = 6.3 Hz, 2 H), 3.01 (s, 3 H), 2.75 (m, 2 H), 2.41–2.62 (complex m, 12 H), 1.93 (quintet, J_{app} = 6.7 Hz, 2 H); CIMS (MH^+ calcd for $C_{16}H_{24}Cl_2N_2O_3S$) 395; found, 395. Anal. ($C_{18}H_{32}Cl_2N_2O_9S_3$) C, H, N. Use of methanesulfonyl chloride instead of methanesulfonic anhydride afforded a 4:1 (^{1}H-NMR) mixture of the desired **22** together with the corresponding chloro compound **23**: ^{1}H-NMR ($CDCl_3$) δ 3.62 (t, J = 6.0 Hz, 2 H, CH_2Cl); CIMS (MH^+ calcd for $C_{15}H_{21}{}^{35}Cl_3N_2$) 335; found, 335.

1-(3-Fluoro-1-propyl)-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (6). Tetrabutylammonium fluoride trihydrate (4.39 g, 14 mmol, 5.0 equiv) was azeotropically dried by distilling the toluene (4 × 20 mL). To the residue was added dry acetonitrile (20 mL) followed by **22** (base) (1.1 g, 2.78 mmol). After 1 h at room temperature, TLC (solvent system A) indicted disappearance of the starting material (**22**) and formation of a new, less polar spot. The solvent was evaporated, and the oil residue was taken up in water (100 mL) and rendered basic by addition of excess concentrated aqueous ammonia. The solution was extracted with ether (6 × 100 mL), the combined ether layer was dried over Na_2SO_4, and the solvent was evaporated to give the product as an oil (0.70 g, 80%). The HBr salt crystallized from hot MeOH/EtOH (1:1). **6**·HBr: mp 252–254 °C then resolidifies and remelts with decomposition at 281–282 °C; ^{1}H-NMR ($CDCl_3$) δ 7.37 (d, J = 8.1 Hz, 1 H), 7.33 (br s, 1 H), 7.07 (d, J = 8.1 Hz, 1 H), 4.54 (dt, J = 5.9, 47 Hz, 2 H), 2.79 (m, 2 H), 2.48–2.66 (complex m, 12 H), 1.93 (doublet of quintets, J = 25, 6.7 Hz, 2 H); CIMS (MH^+ calcd for $C_{15}H_{21}Cl_2FN_2$) 319; found, 319. Anal. ($C_{15}H_{23}Br_2Cl_2FN_2$) C, H, N.

1-(3-Iodo-1-propyl)-4-[2-(3,4-dichlorophenyl)ethyl]piperazine (9). To a stirred solution of **22** (base) (135 mg, 0.34 mmol) in dry acetone (4 mL) was added NaI (200 mg). The reaction mixture was heated to 50 °C for 30 min when TLC (solvent system C) indicated formation of a less polar spot together with quaternized side products at the baseline. The reaction mixture was poured into water (50 mL) and extracted with ether

(50 mL). The ether layer was backwashed with water (50 mL) and then diluted to 100 mL by addition of MeOH. The solution was then treated with 70% aq HI to pH = 3, and the MeOH/ether was replaced with EtOH by distillation and addition of EtOH. Spontaneous crystallization occurred when the volume of the EtOH had reached 20 mL. The solution was set aside to cool slowly to room temperature, and the crystals were filtered, washed with a little cold EtOH, and oven-dried overnight in vacuo, yield = 54 mg (23%). **9**·HI: mp 244–245 °C dec; ^{1}H-NMR ($CDCl_3$) δ 7.34 (d, J = 8.0 Hz, 1 H), 7.30 (d, J = 2.2 Hz, 1 H), 7.04 (dd, J = 8.0, 2.2 Hz, 1 H), 3.23 (t, J = 6.9 Hz, 2 H), 2.76 (m, 2 H), 2.40–2.66 (complex m, 12 H), 2.00 (m, 2 H); CIMS (MH^+ calcd for $C_{15}H_{21}Cl_2IN_2$) 427; found, 427. Anal. ($C_{15}H_{23}Cl_2I_3N_2$) C, H, N.

1-(5-Fluoro-1-pentyl)-4-[(3,4-dichlorophenyl)acetyl]-piperazine (27). A mixture of **19** base (1.0 g, 3.66 mmol), **11** (0.78 g, 4.24 mmol, 1.2 equiv), and $NaHCO_3$ (2.3 g, 27.4 mmol, 7.5 equiv) in dry DMF (15 mL) was heated and stirred overnight at 50 °C as described previously for (+)-**4** and purified by column chromatography on silica gel eluting with solvent system A to give **27** (0.76 g, 57%) as a colorless oil: ^{1}H-NMR ($CDCl_3$) δ 7.38 (d, J = 8.2 Hz, 1 H), 7.34 (d, J = 2.0 Hz, 1 H), 7.1 (dd, J = 8.2, 2.0 Hz, 1 H), 4.43 (dt, J = 47, 6.0 Hz, 2 H), 3.66 (s, 2 H), 3.64 (m, 2 H), 3.46 (m, 2 H), 2.39 (m, 2 H), 2.33 (m, 4 H), 1.35–1.85 (complex m, 6 H); CIMS (MH^+ calcd for $C_{17}H_{23}Cl_2FN_2O$) 361; found, 361.

1-(5-Fluoro-1-pentyl)-4-[2-(3,4-dichlorophenyl)ethyl]-piperazine (7). Amide **27** (160 mg, 0.44 mmol) was reduced to the corresponding amine **7** using a 1.0 M solution of AlH_3 in THF (1.32 mL, 1.32 mmol, 3.0 equiv)[24,29] as described above for **3** to give **7** in quantitative yield as a colorless oil. This was further purified by crystallization of the HBr salt from EtOH to give **7**·HBr (130 mg, 85%): mp 283 °C dec; ^{1}H-NMR ($CDCl_3$) δ 7.34 (d, J = 8.2 Hz, 1 H), 7.30 (d, J = 1.9 Hz, 1 H), 7.04 (dd, J = 8.2, 1.9 Hz, 1 H), 4.45 (dt, J = 47, 6.1 Hz, 2 H), 2.76 (m, 2 H), 2.40–2.66 (complex m, 8 H), 2.36 (m, 2 H), 1.35–1.82 (complex m, 8 H); CIMS (MH^+ calcd for $C_{17}H_{25}Cl_2FN_2$) 347; found, 347. Anal. ($C_{17}H_{27}Br_2Cl_2FN_2$) C, H, N.

***N*-[2-(3,4-Dichlorophenyl)-1-ethyl]-*N*-[1-(ethoxypropionyl)]-2-(1-pyrrolidinyl)ethylamine (24).** A solution of **14** (0.96 g, 3.34 mmol)[24] in a mixture of toluene (20 mL) and ethyl acrylate (14 mL) was boiled under reflux for 72 h when TLC (solvent system A) indicated the reaction to be complete. The product was isolated as for **20** to give **24** (1.3 g, quantitative) as a pale yellow oil. For the purposes of characterization, the fumarate salt was crystallized from EtOAc. **24**·fumarate: mp 130–131 °C; ^{1}H-NMR ($CDCl_3$) δ 7.33 (d, J = 8.1 Hz, 1 H), 7.28 (d, J = 2.1 Hz, 1 H), 7.02 (dd, J = 8.1, 2.1 Hz, 1 H), 4.13 (q, J = 7.1 Hz, 2 H), 2.85 (t, J = 7.1 Hz, 2 H), 2.49–2.81 (complex m, 12 H), 2.42 (t, J = 7.1 Hz, 2 H), 1.78 (m, 4 H), 1.25 (t, J = 7.1 Hz, 3 H); CIMS (MH^+ calcd for $C_{19}H_{28}Cl_2N_2O_2$) 387; found, 387. Anal. ($C_{27}H_{36}Cl_2N_2O_{10}$) C, H, N.

***N*-[2-(3,4-Dichlorophenyl)-1-ethyl]-*N*-[1-(3-hydroxypropyl)]-2-(1-pyrrolidinyl)ethylamine (25).** Ester **24** (1.00 g, 2.58 mmol) was reduced with 10 mL (4 equiv) of a 1.0 M solution of AlH_3 in THF[24,29] as described for **21** to give **25** as a colorless oil in quantitative yield. Crystallization of the HBr salt from hot 2-propanol afforded **25**·HBr (0.98 g, 75%): mp 196–197 °C; ^{1}H-NMR 7.34 (d, J = 8.2 Hz, 1 H), 7.28 (d, J = 2.0 Hz, 1 H), 7.02 (dd, J = 8.2, 2.0 Hz, 1 H), 3.70 (t, J = 5.2 Hz, 2 H), 2.60–2.78 (complex m, 10 H), 2.53 (m, 4 H), 1.79 (m, 4 H), 1.64 (quintet, J_{app} = 5.4 Hz, 2 H); CIMS (MH^+ calcd for $C_{17}H_{26}Cl_2N_2O$) 345; found, 345. Anal. ($C_{17}H_{28}Br_2Cl_2N_2O$) C, H, N.

***N*-[2-(2-Nitro-4,5-dichlorophenyl)-1-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (28).** Compound **2** (base) (9.92 g, 33.0 mmol) was dissolved in AcOH (30 mL) and cooled down to 4–5 °C, and H_2SO_4 (50 mL) was added slowly. To this solution was added dropwise 90% HNO_3 (3 mL, 66 mmol). The reaction was found to be complete at the end of the addition. The mixture was poured over 500 mL of crushed ice, and NaOH pellets (100 g) were added slowly. The basified solution was extracted with $CHCl_3$ (3 × 150 mL), the combined organic extract was dried (Na_2SO_4), and the solvent was evaporated in vacuo. The crude nitro compound was purified by crystallization of the HCl salt from EtOH–H_2O (1:1) to give **28**·HCl (8.88 g, 64%): mp 214–215 °C; ^{1}H-NMR δ 8.03 (s, 1 H), 7.50 (s, 1 H), 3.02 (t, J = 7 Hz, 2 H), 2.65 (t, J = 7 Hz, 2 H), 2.45–2.60 (m, 8 H), 2.30 (s, 3 H), 1.78 (m, 4 H); CIMS (MH^+ calcd for $C_{15}H_{21}Cl_2N_3O_2$) 346; found, 346. Anal. ($C_{15}H_{23}Cl_4N_3O_2$) C, H, N.

***N*-[2-(2-Amino-4,5-dichlorophenyl)-1-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (29).** Compound **28**·HCl (8.1 g, 19.3 mmol) was dissolved in a 1:1 mixture of EtOH–H_2O (160 mL), PtO_2 (1 g) was added, and the mixture was stirred under an atmosphere of H_2 (1 atm) for 24 h at room temperature when TLC (solvent system A) indicated the reaction to be complete. After filtration through Celite, the solvent was partially evaporated in vacuo, and the resulting aqueous solution was basified with NaOH and extracted with $CHCl_3$ (3 × 80 mL). The combined organic extract was dried (Na_2SO_4), and the solvent was evaporated in vacuo. The amino compound was crystallized as the oxalate salt from EtOH to give 5.96 g (62%): mp 174–177 °C dec; ^{1}H-NMR δ 7.02 (s, 1 H), 6.72 (s, 1 H), 2.33–2.72 (m, 12 H), 2.30 (s, 3 H), 1.75 (m, 4 H). This compound failed to afford a satisfactory elemental analysis due to extensive solvation. HRMS (M^+ calcd for $C_{15}H_{23}Cl_2N_3$) 315.1269; found, 315.1265.

***N*-[2-(2-Iodo-4,5-dichlorophenyl)-1-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (8).** Intermediate **29** base (0.47 g, 1.5 mmol) was dissolved in a mixture of concentrated HCl (0.5 mL) and deionized H_2O (4.0 mL) and cooled to 0 °C. A solution of $NaNO_2$ (0.115 g, 1.7 mmol) in 2 mL of H_2O was added dropwise, and the mixture was warmed up to 5 °C and stirred for 2 h. The mixture was filtered and cooled down to 0 °C, and the diazonium salt was quenched with a solution of NaI (0.23 g, 1.5 mmol) in H_2O (1.5 mL). Addition of the aqueous NaI resulted in a precipitate. The mixture was basified with aqueous NaOH and extracted with $CHCl_3$ (3 × 7 mL). After drying with Na_2SO_4, the solvent was evaporated in vacuo. The crude iodo compound was purified by column chromatography eluting with solvent system A to yield **8** (120 mg, 20%). The oily product was further purified by crystallization of the oxalate salt from MeOH: mp 217–218 °C dec; ^{1}H-NMR δ 7.83 (s, 1 H), 7.30 (s, 1 H), 2.82 (m, 2 H), 2.45–2.65 (m, 10 H), 2.32 (s, 3 H), 1.75 (m, 4 H); CIMS (MH^+ calcd for $C_{15}H_{21}Cl_2IN_2$) 427; found, 427. Anal. ($C_{17}H_{23}Cl_2IN_2O_4$) C, H, N.

Biological Materials and Methods. Membrane Preparation. Receptor binding assays were performed using the crude synaptosomal (P_2) membrane fraction of guinea pig brain (σ, κ, and PCP receptors) or rat brain (dopamine-D_2 and muscarinic cholinergic receptors).

Crude P_2 membrane fractions were prepared from frozen (−80 °C) guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebella. After removal of cerebella, brains were allowed to thaw slowly on ice and placed in ice-cold 10 mM Tris-HCl, pH 7.4 containing 320 mM sucrose (Tris-sucrose buffer). Brains were then homogenized in a Potter-Elvehjem homogenizer by 10 strokes of a motor driven Teflon pestle in a volume of 10 mL/gm tissue wet weight. The homogenate was centrifuged at 1000g for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in 2 mL/g ice cold Tris-sucrose and centrifuged again at 1000g for 10 min. The combined 1000g supernatant was centrifuged at 31 000g for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/gm of 10 mM Tris-HCl, pH 7.4, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31 000g for 15 min, the pellets were resuspended by gentle Potter-Elvehjem homogenization to a final volume of 1.53 mL/gm in 10 mM Tris-HCl, pH 7.4. Aliquots were stored at −80 °C until use. Protein concentration was determined by the method of Lowry et al.[32] using bovine serum albumin (BSA) as standard.

To prepare rat brain crude P_2 membranes, male Sprague-Dawley rats (150–200 g, Charles River, Boston, MA) were killed by decapitation. Brains (minus cerebella) were then treated as described above.

Receptor Binding Assays. σ Receptors. σ receptors were labeled with [^{3}H](+)-3-PPP [1-(1-propyl)-3-(3-hydroxyphenyl)-piperidine] (109 Ci/mmol) using membranes from guinea pig brain. Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 500 μg of membrane protein and 3 nM [^{3}H](+)-3-PPP. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass fiber filters (Schleicher and Schuell, Keene, NH). Filters were then washed twice with 5 mL

of ice-cold Tris-HCl buffer. Filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25 °C prior to use.

σ receptors were also labeled using [^{3}H](+)-pentazocine (specific activity = 52 Ci/mmol) prepared as previously described.[31] Briefly, guinea pig brain membranes were incubated with 3 nM [^{3}H](+)-pentazocine using 500 μg of membrane protein in a volume 500 μL of 50 mM Tris-HCl, pH 8.0. Incubations were carried out for 120 min at 25 °C. Nonspecific binding was carried out in the presence of 10 μM unlabeled (+)-pentazocine. Assays were terminated by dilution with 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and vacuum filtration through glass fiber filters (Scleicher and Schuell, Keene, NH). Filters were then washed twice with 5 mL of ice-cold Tris-HCl buffer. The filters were pretreated with polyethyleneimine as described above.

κ Opiate Receptors. κ receptors were labeled with [^{3}H](-)-bremazocine (17.3 Ci/mmol) in the presence of [D-Ala^2,-*N*-methyl-Phe^4,Gly-ol^5]enkephalin (DAMGO) and [D-Ser^2,Leu^5,Thr^6]enkephalin (DSTLE) as μ and δ opiate receptor blockers, respectively. Incubations were carried out in 0.5 mL of 10 mM Tris-HCl, pH 7.4, for 90 min at 25 °C with 500 μg of guinea pig brain membrane protein, 100 nM (DAMGO), 100 nM DSTLE, and 2 nM [^{3}H](-)-bremazocine. Assays were terminated by the addition of 5 mL of ice-cold buffer and filtration through glass fiber filters (Schleicher and Schuell) under reduced pressure. Filters were then washed twice with 5 mL ice-cold buffer. Nonspecific binding was determined in the presence of 10 μM levallorphan.

Phencyclidine (PCP) Receptors. PCP [1-(1-phenylcyclohexyl)piperidine] receptors were labeled using [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) (48.9 Ci/mmol) and membranes from guinea pig brain. Incubations were carried out in 5 mM Tris-HCl, pH 7.4, for 60 min at 4 °C in a volume of 0.5 mL with 500 μg of membrane protein and 5 nM [^{3}H]TCP. Assays were terminated by addition of 5 mL of ice-cold buffer and filtration through glass fiber filters under reduced pressure. Filters were then washed twice with 5 mL ice-cold buffer. Filters were soaked in 0.3% polyethyleneimine for at least 30 min at 25 °C prior to use. Nonspecific binding was determined in the presence of 10 μM (±)-cyclazocine.

Dopamine-D_2 Receptors. Dopamine-D_2 receptors were labeled with 5 nM [^{3}H](-)-sulpiride (73.1 Ci/mmol) using rat brain membranes. Incubations were carried out for 60 min at 25 °C in 0.5 mL of 50 mM Tris-HCl, pH 7.7, containing 120 mM NaCl and 500 μg of membrane protein. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by the addition of 5 mL of ice-cold incubation buffer and vacuum filtration through glass fiber filters (Schleicher and Schuell). Filters were then washed twice with ice-cold incubation buffer.

Muscarinic Cholinergic Receptors. Muscarinic Cholinergic receptor binding was performed using rat brain membranes. [^{3}H]QNB (32.9 Ci/mmol) (0.3 nM) was incubated with about 500 μg membrane protein in 0.5 mL of Krebs-Henseleit/HEPES, pH 7.4, for 60–90 min at 37 °C. Nonspecific binding was determined in the presence of 10 μM QNB. Assays were terminated by dilution with 5 mL ice-cold phosphate-buffered saline (PBS), pH 7.4, and vacuum filtration through glass fiber filters (Schleicher and Schuell). Filters were then washed twice with ice cold buffer.

Chemicals. All scintillation counting was performed with a Packard Model 4450 scintillation spectrometer using Ecoscint cocktail (National Diagnostics, Manville, NJ) after overnight extraction of the counts from the filters. All filtration was carried out using a Brandel cell harvester (Gaithersburg, MD). Haloperidol, polyethyleneimine, and Tris were purchased from Sigma Chemicals (St. Louis, MO). Cyclazocine and levallorphan were obtained from the National Institute on Drug Abuse (Rockville, MD). [^{3}H](+)-3-PPP, [^{3}H](-)-bremazocine, [^{3}H]TCP, [^{3}H](-)-sulpiride and [^{3}H]QNB were purchased from Dupont–New England Nuclear, Boston, MA. [^{3}H](+)-Pentazocine (52 Ci/mmol) was synthesized as described previously by us.[31]

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