phosphate buffer (pH 7.4), transferred to counting vials containing 7 mL of scintillator (Scintisole EX-H, Wako), and counted by liquid-scintillation spectrometry (Packard Tri-Carb 330).

Mydriasis. For each dose 10 male mice of ddY strain weighing 18–23 g were used. Pupil size was microscopically measured under 1000 lx lighting before and 30 min after oral administration of drugs. ED_{100} was a dose required to double the predrug pupil size.

Synthesis and Evaluation of N-Substituted *cis*-*N*-Methyl-2-(1-pyrrolidinyl)cyclohexylamines as High Affinity σ Receptor Ligands. Identification of a New Class of Highly Potent and Selective σ Receptor Probes

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Certain benzeneacetamides [(−)- and (+)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide] were recently reported to be potent σ receptor ligands. In order to determine whether efficacy for the σ receptor could be improved, a series of compounds related to the benzeneacetamides, *N*-substituted *cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamines, were synthesized and their structure–activity requirements were determined. The compounds were synthesized by starting with the previously reported (±)-, 1*S*,2*R*-(+)-, and 1*R*,2*S*-(−)-*cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamines. Analysis of σ ([^{3}H](+)-3-PPP), κ ([^{3}H]bremazocine and [^{3}H]U69,593), dopamine-d_2 ([^{3}H](−)-sulpiride), and phencyclidine (PCP) ([^{3}H]TCP) receptor binding in guinea pig brain revealed a number of highly potent and selective σ receptor ligands. Notably, 1*S*,2*R*-*cis*-(−)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-(2-naphthyl)acetamide [(−)-**29**] (K_i = 8.66 ± 0.35 nM), (±)-*cis*-2-amino-4,5-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(±)-**17**] (K_i = 11 ± 3 nM), 1*S*,2*R*-(−)-*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-**44**] (K_i = 1.3 ± 0.3 nM), and 1*R*,2*S*-(+)-*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine. [(+)-**44**] (K_i = 6 ± 3 nM) exhibited very high affinity at σ receptors, by displacement of [^{3}H]-(+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ([^{3}H]-(+)-3-PPP). These compounds showed insignificant affinity for κ, dopamine, or PCP receptors, making them valuable tools for the study of σ receptors. Furthermore, these compounds also exhibited enantioselectivity ranging from 5-fold for (+)- and (−)-**44** to 160-fold for (+)- and (−)-**29**. Several other compounds showed equivalent selectivity but displayed lower σ receptor affinity.

Introduction

The σ receptor was first proposed by Martin et al., in 1976,[1] as an opioid-receptor type on the basis of the ability of the 6,7-benzomorphan opioid (±)-*N*-allylnormetazocine [(±)-SKF10,047] to produce effects different from those of other opioids in the chronic spinal dog.[1,2] However, several later studies suggested that this classification had to be extensively modified.

The first modification came from studies on the mechanism of action of the psychotomimetic drug phencyclidine (PCP). It was found that PCP and (+)-SKF10,047 may share a common binding site in brain.[3-6] [^{3}H]PCP is displaced from the PCP binding site by the 6,7-benzomorphans (+)-SKF10,047 and (+)-cyclazocine. Furthermore, binding sites for [^{3}H]cyclazocine and [^{3}H]-PCP have a similar regional distribution in brain.[5] Later work with enantiomeric 6,7-benzomorphans revealed that at least some of the PCP-like effects of SKF10,047 were associated with the dextrorotatory enantiomer.[4,7] Other studies examining similar behavioral effects produced by PCP and 6,7-benzomorphans such as (+)-SKF10,047 suggested that these compounds may exert their effects through the same site and that the σ receptor and PCP receptor are identical.[8-11]

However, it is now clear that (+)-6,7-benzomorphans bind to at least two distinct receptor sites in brain.[12] [^{3}H](+)-SKF10,047 is potently displaced by neuroleptic

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drugs such as haloperidol and perphenazine[13] while these compounds exhibit little or no ability to displace radiolabeled PCP-related compounds.[14] Furthermore, PCP-related compounds displace [^{3}H]TCP with greater potency than [^{3}H](+)-SKF10,047.[14] The high affinity [^{3}H](+)-SKF10,047 binding site, which also exhibits high affinity for neuroleptics, is now called σ.[12,15] σ sites commonly defined as non-dopaminergic and non-opioid, are distinct from PCP receptors. The binding site for which [^{3}H]-(+)-SKF10,047 has lower affinity is synonymous with the PCP receptor and is associated with the ion channel of the NMDA-type glutamate receptor.[12] It is likely that the "σ-opioid" site originally defined by Martin is actually the PCP receptor, though more work is needed to verify this.

The functional role of σ receptors is not fully understood at the present time. However, several studies have shown σ sites to be involved in a variety of pharmacological and physiological effects. σ receptors affect (1) electrically stimulated and serotonin induced contraction of smooth muscle;[16-18] (2) release of neurotransmitter from smooth muscle preparations;[17] and (3) stimulation of phosphoinositide turnover by cholinergic agonists.[19,20] They have also been implicated in the regulation of motor behavior[21,22] and may mediate the motor side effects of antipsychotic drugs. This receptor may also be involved in various dystonic disorders.[23]

Several compounds which bind to σ sites cross-react with either dopamine-d_2, PCP, or opioid receptors. Although the 6,7-benzomorphan (+)-pentazocine is a potent and selective σ ligand,[24] 6,7-benzomorphans as a class tend to exhibit cross-reactivity with PCP receptors. Neuroleptics such as haloperidol, though binding with high affinity to σ sites, bind with nearly equal affinity to dopamine-d_2 receptors. Two other compounds which have been shown to bind with lower affinity to σ sites than (+)-pentazocine are (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP],[25] originally developed as a dopamine autoreceptor agonist, and 1,3-di(*o*-tolyl)guanidine (DTG).[26]

The evaluation of the biochemical, physiological, and behavioral effects mediated by σ receptors has thus been greatly hindered by the lack of σ ligands which possess both very high affinity and selectivity.

We recently identified a new class of σ receptor ligands among the benzeneacetamides, and identified the cis diastereomers (+)-1 and (−)-1 of the κ selective ligand, U50,488 to be potent ligands at the σ receptor.[27] However, (+)-1 and (−)-1 also exhibited moderate affinity for κ receptors labeled by [^{3}H]U69,593 but showed no affinity for κ receptors labeled by [^{3}H]bremazocine ([^{3}H]brem). In order to further our knowledge of the structure–activity requirements for increasing both the affinity and the selectivity at the σ receptor, we synthesized a number of analogues of (+)-1 and (−)-1 by varying the arylacetyl portion and leaving the rest of the molecule unchanged. We hoped to find compounds with high affinity at the σ receptor and little or no cross-reactivity with the PCP and dopamine-d_2 sites, or the two κ sites delineated by [^{3}H]-bremazocine and [^{3}H]U69,593. Our approach in this study was to synthesize the series in racemic form and examine their binding to the σ receptor and to κ receptors labeled by both [^{3}H]bremazocine and [^{3}H]U69,593. The enantiomers of the most interesting and potent members of the series would be separated and tested for their affinities for all of the various noted binding sites.

We found that the minimum structural requirements of a molecule necessary to elicit σ receptor binding activity could be represented by the phenylacetamide (±)-**3**. The aromatic substitution pattern on the phenylacetamide (±)-**3** was then varied, generating *o*-, *m*-, *p*-monosubstituted analogues with electron-withdrawing, neutral, and electron-donating groups (Table II). We then examined the effect of isosteric replacement of the carbonyl group, and the introduction of an ethylene moiety to create a more rigid analogue (Table III). A series of substituted phenoxyacetamides and analogous compounds (Table IV) were synthesized. Finally, we inspected various miscellaneous compounds with a variety of linkages between the aromatic and *N*-methyl groups as well as different aromatic moieties (Table V). Selected compounds were examined for their affinity for the various receptors, their enantiomers were separated, and these were explored for enantioselectivity (Table VI). Compounds 1*S*,2*R*-(−)-**44** and (±)-**29** were tested for behavioral activity in vivo and the results compared with their σ receptor binding potency.

Chemistry

The starting material, (±)-*cis*-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(±)-**4**] and its enantiomers (+)-**4** and (−)-**4** were prepared in optically pure form as previously described.[27] The substituted benzeneacetamides (±)-**1**, (±)-**3**, (±)-**5**–(±)-**19** (Table II), and (+)- and (−)-**51** (Table VI) were prepared in 52–99% yield (Table I) by dicyclohexylcarbodiimide (DCC) coupling of (±)-**4** with the respective phenylacetic acids in CH_2Cl_2 at room temperature or by direct condensation of (±)-, (+)-, or (−)-**4** with the corresponding acid chlorides (1.0 mol equiv) in dry chloroform. Similarly, the phenoxyacetamides and related compounds (±)-**20**–(±)-**27** (Table IV) were synthesized in 64–98% yield (Table I). Arylacetamides and miscellaneous compounds (±)-**2**, (±)-**29**–(±)-**38**, (+)-**29**,

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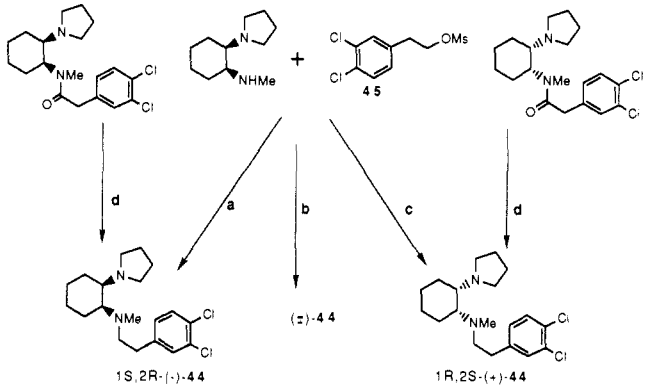
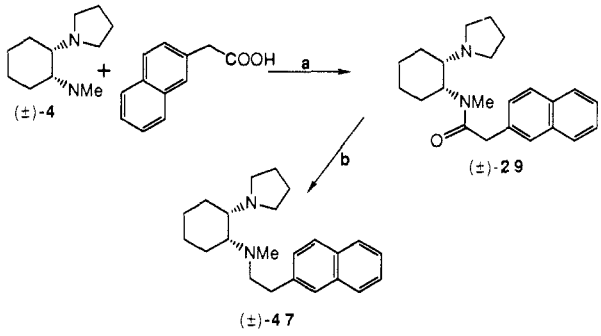
Scheme I[a]



[a] Reagents and conditions: a. 1*S*,2*R*-(+)-*cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamine, K_2CO_3, DMF; b. (±)-*cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamine, K_2CO_3, DMF; c. 1*R*,2*S*-(-)-*cis*-2-(1-pyrrolidinyl)-*N*-methylcyclohexylamine, K_2CO_3, DMF; d. AlH_3/THF/20 °C.

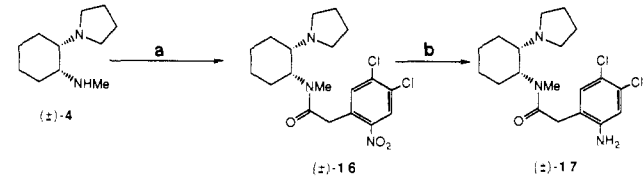
Scheme II[a]



[a] Reagents and conditions: a. DCC, pyridine, CH_2Cl_2, room temperature; b. LAH, THF, room temperature.

(-)-**29**, (+)-**51**, and (-)-**51** (Tables V and VI) were synthesized either by DCC coupling of (±)-, (+)-, or (-)-**4** with the respective carboxylic acids or with 1 mol equiv of the corresponding acid chloride in dry CH_2Cl_2. Carbamate (±)-**28** (Table IV) was obtained in 89% yield (Table I) by reaction of (±)-**4** with benzyl chloroformate in dry chloroform. Urea derivative (±)-**39** (Table III) was synthesized in 98% yield (Table I) by reaction of (±)-**4** with 3,4-dichlorophenyl isocyanate in dry chloroform. The thiourea derivative (±)-**40** (Table III) was synthesized similarly (Table I), starting with 3,4-dichlorophenyl isothiocyanate (**41**) (formed from treatment of 3,4-dichloroaniline hydrochloride with thiophosgene in refluxing toluene). Dichlorocinnamide [(±)-**42**] (Table III) was obtained in 79% yield (Table I) by DCC coupling of (±)-**4** with 3,4-dichlorocinnamic acid in CH_2Cl_2. The sulfonamide (±)-**43** (Table V) was prepared in 79% yield (Table I) by direct condensation of 1 mol equiv of phenylsulfonyl chloride with (±)-**4** in dry chloroform. The diamines (±)-**44**, 1*R*,2*S*-(+)-**44** and 1*S*,2*R*-(-)-**44** (Tables V and VI) were obtained either by N-alkylation of (±)-**4**, 1*R*,2*S*-(-)-**4**, and 1*S*,2*R*-(+)-**4** with 2-(3,4-dichlorophenyl)ethyl methanesulfonate (**45**) [formed in high yield by methanesulfonylation of 2-(3,4-dichlorophenyl)ethanol (**46**)] at 60 °C in dry DMF (Table I) or by alane (AlH_3) reduction of the corresponding acetamides (±)-**1**, (+)-**1** and (-)-**1** (Scheme I). Diamines (±)-**47** and (±)-**48** (Table V) and 1*R*,2*S*-(+)-**50** and 1*S*,2*R*-(-)-**50** (Table VI) were obtained by $LiAlH_4$ reduction (Scheme II) of the corresponding acetamides in THF (Table I). Aniline (±)-**17** (Tables II and VI) (Scheme III) was synthesized in 63% yield (Table I) by catalytic hydrogenation of the corresponding nitro

Scheme III[a]

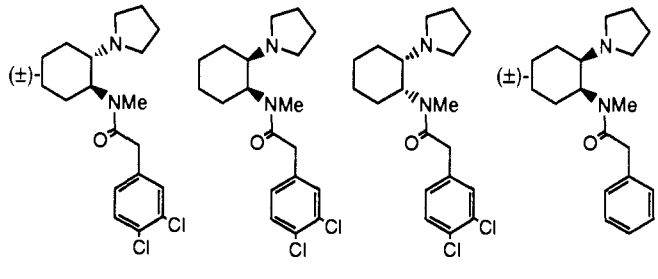


[a] Reagents and conditions: a. 2-nitro-4,5-dichlorophenylacetic acid, pyridine, DCC, CH_2Cl_2; b. H_2, PtO_2, MeOH.

compound (±)-**16** (Table II) over PtO_2 in MeOH solution. Use of 10% Pd-C instead of PtO_2 resulted in hydrogenolysis of the aromatic chlorine atoms. Acetamide (±)-**49** (Table V) was prepared by treatment of (±)-**4** with CH_3COBr in dry $CHCl_3$.

Results and Discussion

In order to determine the structure–activity requirements (SAR) of the benzeneacetamides for affinity and selectivity at the σ receptor, a number of structural alterations were made to the parent molecule (±)-**1** and the resulting analogues were then examined for σ receptor activity by in vitro competition studies against [^{3}H](+)-3-PPP (Tables II–VI). Furthermore, the analogues were also tested for in vitro κ receptor activity against κ receptors labeled by both [^{3}H]bremazocine and [^{3}H]U69,593 (Tables II–VI). The more potent members of the series were examined for σ receptor selectivity by examination of their in vitro binding characteristics across a number of receptor types; the binding sites examined (Table VI) were those labeled by [^{3}H](+)-3-PPP (σ), [^{3}H]sulpiride (dopamine-d_2), [^{3}H]bremazocine (κ), [^{3}H]U69,593 (κ), and [^{3}H]TCP (phencyclidine). The classes of compounds synthesized were the benzeneacetamides (Table II), urea and cinnamide (Table III), phenoxyacetamide and related compounds (Table IV), arylacetamide and miscellaneous derivatives (Table V) and, finally, miscellaneous racemic and enantiomeric compounds (Table VI).



Unsubstituted benzeneacetamide [(±)-**3**] (Table II) showed considerably reduced affinity (6-fold) for σ receptors compared with the 3,4-dichloro substituted parent compound [(±)-**1**]. This indication of the importance of hydrophobic functions at the 3- and 4-positions of the aromatic ring of (±)-**3** for increased affinity at the σ receptor was corroborated by examination of the 3- and 4-monochloro-substituted compounds (±)-**6** and (±)-**7**. These compounds exhibited a 4–5-fold increased affinity for σ receptors compared with (±)-**3** (Table II). The 2-chloro-substituted compound (±)-**5** (Table II) showed only marginally increased affinity for σ receptors compared to (±)-**3**, while the 2,6-dichloro-substituted analogue (±)-**15** (Table II) exhibited lower affinity, indicating the detrimental effect of hydrophobic groups at the ortho position on σ receptor binding. The dichloro derivative (±)-**1** (IC_{50} = 194 ± 87 nM) was only slightly more potent at the σ

Table I. Physical and Chemical Data of Compounds and Intermediates

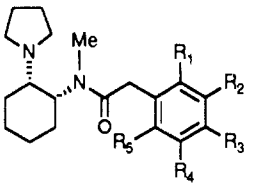
compd	method	molecular formula	recryst. solvent	% yield	mp, °C
(±)-**1**·HCl	B[a]				
(±)-**2**·HCl	A	$C_{18}H_{25}Cl_3N_2O$	EtOAc	97	229–230
(±)-**3**·HCl	A	$C_{19}H_{29}ClN_2O{\cdot}0.5H_2O$	EtOAc	95	160–161
(±)-**5**·HCl	B	$C_{19}H_{28}Cl_2N_2O{\cdot}0.5H_2O$	EtOAc	85	198–199
(±)-**6**·HCl	B	$C_{23}H_{31}ClN_2O_5$	*i*-PrOH	87	172–173
(±)-**7**·fumarate	B	$C_{23}H_{31}ClN_2O_5$	EtOAc	93	163–164
(±)-**8**·fumarate	B	$C_{23}H_{31}N_3O_7$	EtOAc	95	164–165
(±)-**9**·fumarate	B	$C_{23}H_{31}N_3O_7$	*i*-PrOH	96	161–163
(±)-**10**·oxalate	B	$C_{21}H_{29}N_3O_7{\cdot}2CH_3OH$	EtOH/MeOH	52	178–180 dec
(±)-**11**·HCl	B	$C_{20}H_{31}ClN_2O_2{\cdot}0.33H_2O$	EtOAc	85	186–188
(±)-**12**·fumarate	B	$C_{24}H_{34}N_2O_6$	EtOAc	74	155–156
(±)-**13**·HCl	B	$C_{20}H_{31}ClN_2O_2$	i-PrOH/EtOAc	77	187.5–188
(±)-**14**·fumarate	B	$C_{23}H_{30}Cl_2N_2O_5$	i-PrOH	97	199–200
(±)-**15**·HCl	B	$C_{19}H_{27}Cl_3N_2O$	EtOAc	85	233–235
(±)-**16**·HCl	B	$C_{19}H_{26}Cl_3N_3O_3$	*i*-PrOH	89	232–233 dec
(±)-**17**·HCl	F	$C_{19}H_{28}Cl_3N_3O$	i-PrOH	63	234–236
(±)-**18**·fumarate	B	$C_{26}H_{38}N_2O_8$	i-PrOH	99	157–158
(±)-**19**·fumarate	B	$C_{23}H_{31}BrN_2O_5$	i-PrOH	92	158–160
(±)-**20**·HCl	A	$C_{19}H_{29}ClN_2O_2{\cdot}0.5H_2O$	EtOAc	98	191–193
(±)-**21**·HCl	B	$C_{19}H_{29}ClN_2OS$	EtOAc	76	184.5–185
(±)-**22**·HCl	A	$C_{20}H_{31}ClN_2O$	EtOAc	87	185–187
(±)-**23**·fumarate	B	$C_{27}H_{40}N_2O_8{\cdot}H_2O$	EtOAc	77	95–97
(±)-**24**·HCl	B	$C_{19}H_{27}Cl_3N_2O_2{\cdot}0.5H_2O$	i-PrOH	74	210–211
(±)-**25**·fumarate	B	$C_{23}H_{30}Cl_2N_2O_6$	EtOAc	91	177–179
(±)-**26**·HCl	B	$C_{19}H_{27}Cl_3N_2O_2$	EtOAc	80	204–205
(±)-**27**·fumarate	B	$C_{23}H_{29}Cl_3N_2O_6$	EtOAc	64	178–179
(±)-**28**·HCl	C	$C_{19}H_{29}ClN_2O_2$	EtOAc	89	180–181
(±)-**29**·fumarate	B	$C_{27}H_{34}N_2O_5$	i-PrOH	85	181–182
1*S*,2*R*-(−)-**29**·fumarate	B[b]	$C_{27}H_{34}N_2O_5$	i-PrOH	94	199–200
1*R*,2*S*-(+)-**29**·fumarate	B[c]	$C_{27}H_{34}N_2O_5$	i-PrOH	92	199–200
(±)-**30**·fumarate	B	$C_{27}H_{34}N_2O_5{\cdot}0.5H_2O$	*i*-PrOH	75	166–167
(±)-**31**·HCl	B	$C_{22}H_{29}ClN_2O{\cdot}H_2O$	*i*-PrOH	86	250–251
(±)-**32**·HI	B	$C_{21}H_{31}N_2IO$	i-PrOH	72	230–231
(±)-**33**·HCl	B	$C_{21}H_{31}Cl_3N_2O_2{\cdot}0.5H_2O$	EtOAc	69	140–145
(±)-**34**·HCl	A	$C_{20}H_{29}ClN_2O$	EtOAc	77	225–226
(±)-**35**·HCl	B	$C_{17}H_{27}ClN_2OS{\cdot}0.5H_2O$	EtOAc	69	165–166
(±)-**36**·HCl	B	$C_{17}H_{27}ClN_2OS{\cdot}0.5H_2O$	EtOAc	74	155–156
(±)-**37**·fumarate	B	$C_{22}H_{31}N_3O_5$	i-PrOH	89	167–169
(±)-**38**·fumarate	B	$C_{21}H_{29}N_3O_5$	EtOAc	71	181–183
(±)-**39**·HCl	D	$C_{18}H_{26}Cl_3N_3O$	i-PrOH	98	207–208
(±)-**40**·HCl	D	$C_{18}H_{26}Cl_3N_3S$	i-PrOH	85	184.5–185
(±)-**42**·$MeSO_3H$	A	$C_{21}H_{30}Cl_2N_2O_4S$	EtOAc	79	185–187
(±)-**43**·HBr	E	$C_{17}H_{27}BrN_2O_2S$	*i*-PrOH	79	241–242
(±)-**44**·oxalate	H, I	$C_{21}H_{30}Cl_2N_2O_4$	i-PrOH	50	120–120.5
1*S*,2*R*-(−)-**44**·2HBr	H, I[d]	$C_{19}H_{30}Br_2Cl_2N_2$	*i*-PrOH	58	215–217
1*R*,2*S*-(+)-**44**·2HBr	H, I[e]	$C_{19}H_{30}Br_2Cl_2N_2$	*i*-PrOH	65	217–218
(±)-**47** (base)	G[f]	$C_{23}H_{32}N_2$	oil	67	–
(±)-**48** (base)	G[g]	$C_{19}H_{30}N_2$	oil	78	–
(±)-**49**·HBr	A	$C_{13}H_{25}BrN_2O$	EtOAc	71	182–182.5
1*S*,2*R*-(−)-**50**·2HBr	G[h]	$C_{20}H_{32}Br_2N_2O_2$	MeOH/*i*-PrOH	96	248–249
1*R*,2*S*-(+)-**50**·2HBr	G[i]	$C_{20}H_{32}Br_2N_2O_2$	MeOH/*i*-PrOH	96	248–249
1*R*,2*S*-(+)-**51**·fumarate	B[j]	$C_{24}H_{32}N_2O_7$	EtOAc	88	184–185
1*S*,2*R*-(−)-**51**·fumarate	B[k]	$C_{24}H_{32}N_2O_7$	EtOAc	86	184–185

[a] Synthesized as described previously.[27] [b] $[\alpha]^{23}_D$ = −35.8° (*c* 1.46, MeOH). [c] $[\alpha]^{23}_D$ = +32° (*c* 0.87, MeOH). [d] $[\alpha]^{23}_D$ = −8.6° (*c* 0.84, MeOH). [e] $[\alpha]^{23}_D$ = +8.0° (*c* 0.57, MeOH). [f] Compound was an oil and failed to yield a satisfactory elemental analysis; M^+ (calcd for $C_{23}H_{32}N_2$) = 336.2565, M^+ (found) = 336.2561. [g] Compound was an oil and failed to yield a satisfactory elemental analysis; M^+ (calcd for $C_{19}H_{30}N_2$) = 286.2409, M^+ (found) = 286.2420. [h] $[\alpha]^{23}_D$ = −13.0° (*c* 3.99, H_2O). [i] $[\alpha]^{23}_D$ = +15.2° (*c* 2.16, H_2O). [j] $[\alpha]^{23}_D$ = +43.5° (*c* 1.44, MeOH). [k] $[\alpha]^{23}_D$ = −41.5° (*c* 0.93, MeOH).

receptor than the monochloro compounds. (±)-**6** (IC_{50} = 311 ± 12 nM) and (±)-**7** (IC_{50} = 224 ± 18 nM)] (Table II). A change in the type of halide in the 4-position, from Br [(±)-**19**] to Cl [(±)-**7**] does not appear to produce a significant effect on receptor binding (Table II).

The effect of a nitro moiety as an electron-withdrawing group on the aromatic ring was also examined (Table II). A 3-nitro group [(±)-**9**] failed to produce a significant increase in σ receptor affinity compared with the unsubstituted compound (±)-**3**, while the 4-nitro group of (±)-**10** resulted in a 2-fold decrease in affinity for σ receptors. The most dramatic effect, however, was observed with the 2-nitro-substituted compound (±)-**8** which exhibited almost no affinity (IC_{50} > 10000 nM) for the σ receptor. The damaging effect of the nitro group was also seen in (±)-**16** where addition of the 2-nitro group to (±)-**1** reduced affinity for σ receptors 30-fold. Not surprisingly, then, the electron donating 2-amino function of (±)-**17** afforded an increase in affinity of 16-fold compared with the parent compound (±)-**1**, and a 500-fold increase in affinity compared with the corresponding nitro compound (±)-**16**; this dramatic difference between (±)-**16** and (±)-**17** reveals that aromatic electron-donating groups in the 2-position of (±)-**1** are capable of producing major increases in affinity at this receptor. The result is further exemplified by compound (±)-**11** containing an electron-donating methoxy group in the 2-position. This compound showed a 6-fold increase in affinity for the σ receptor when compared with

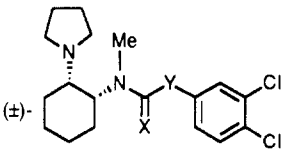
Table II. Binding Affinities of Benzeneacetamide Derivatives at the σ Receptor[a]



compd	R_1	R_2	R_3	R_4	R_5	IC_{50}, nM [^{3}H](+)-3-PPP	% control[c] [^{3}H]brem	% control[c] [^{3}H]U69,593
(±)-1	H	Cl	Cl	H	H	194 ± 87	96.24	9.60
(±)-3	H	H	H	H	H	1155 ± 353	108.88	69.55
(±)-5	Cl	H	H	H	H	870 ± 482	104.32	51.04
(±)-6	H	Cl	H	H	H	311 ± 12	106.71	42.70
(±)-7	H	H	Cl	H	H	224 ± 18	107.59	39.57
(±)-8	NO_2	H	H	H	H	no inhibn[b]	109.75	78.26
(±)-9	H	NO_2	H	H	H	1609 ± 258	101.75	28.01
(±)-10	H	H	NO_2	H	H	2374 ± 594	109.15	51.60
(±)-11	OMe	H	H	H	H	208 ± 16	110.34	71.22
(±)-12	H	OMe	H	H	H	211 ± 8	107.19	65.04
(±)-13	H	H	OMe	H	H	196 ± 25	108.01	90.83
(±)-14	Cl	H	Cl	H	H	278 ± 22	92.99	27.21
(±)-15	Cl	H	H	H	Cl	1385 ± 234	103.67	18.85
(±)-16	H	Cl	Cl	H	NO_2	5952 ± 237	92.20	3.78
(±)-17	H	Cl	Cl	H	NH_2	12 ± 3	95.20	8.18
(±)-18	H	OMe	OMe	OMe	H	302 ± 5	107.10	75.81
(±)-19	H	H	Br	H	H	228 ± 34	101.53	32.78

[a] The IC_{50} binding data for Tables II–V were generated as described in the methods section. In order to obtain an initial estimate of the binding affinity of the compounds, each compound was initially tested at 3 concentrations in the σ assay (100, 1000, 10000 nM). If a compound elicited >30% inhibition of binding of the test ligand ([^{3}H](+)-3-PPP) at 10000 nM, a 12-point curve using unlabeled test ligand concentrations ranging from 0.05–10000 nM (0.005–1000 nM for the most potent compounds) was conducted. The CDATA (EMF Software, Inc., Baltimore, MD) iterative curve-fitting program was used to analyze the results. The above results are the results of two, or in some cases three experiments (±SEM), each carried out in duplicate. [b] No IC_{50} values could be determined in these cases since the compounds produced less than 30% inhibition of control binding at a concentration of 10 μM. [c] For κ receptor binding, the percentage of specific [^{3}H]U69,593 and [^{3}H]bremazocine binding spared by 10 μM each compound was derived from a single experiment (as described in methods) performed in quadruplicate. Because a single experiment was performed, no error bars are indicated.

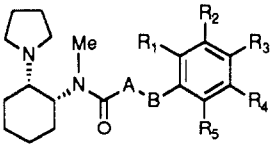
Table III. Binding Affinities of Benzeneacetamides at the σ Receptor[a]



compd	X	Y	IC_{50}, nM [^{3}H](+)-3-PPP	% control [^{3}H]brem	% control [^{3}H]U69,593
(±)-39	O	NH	5303 ± 730	102.95	43.94
(±)-40	S	NH	3267 ± 2464	100.31	68.67
(±)-42	O	*trans*-CH=CH	3650 ± 114	91.03	99.02

[a] See footnote of Table II.

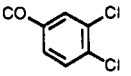
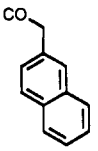
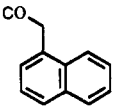
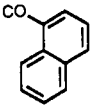
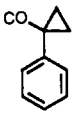
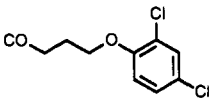
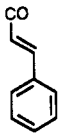
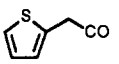
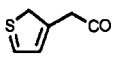
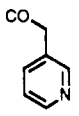
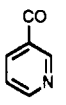
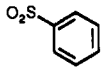
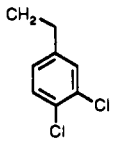
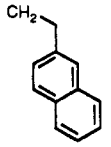
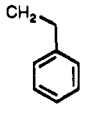
Table IV. Binding Affinities of Benzeneacetamide Derivatives at the σ Receptor[a]



compd	A	B	R_1	R_2	R_3	R_4	R_5	IC_{50}, nM [^{3}H](+)-3-PPP	% control [^{3}H]brem	% control [^{3}H]U69,593
(±)-20	CH_2	O	H	H	H	H	H	1247 ± 38	105.37	70.06
(±)-21	CH_2	S	H	H	H	H	H	200 ± 40	88.98	58.41
(±)-22	CH_2	CH_2	H	H	H	H	H	1333 ± 205	108.17	93.77
(±)-23	CH_2	CH_2	H	OMe	OMe	OMe	H	2670 ± 451	106.18	90.71
(±)-24	CH_2	O	H	H	Cl	Cl	H	110 ± 8	64.67	9.53
(±)-25	CH_2	O	Cl	Cl	H	H	H	42 ± 3	91.04	3.17
(±)-26	CH_2	O	Cl	H	Cl	H	H	76 ± 9	95.84	35.24
(±)-27	CH_2	O	Cl	H	Cl	Cl	H	128 ± 33	45.60	6.94
(±)-28	O	CH_2	H	H	H	H	H	928 ± 298	98.33	95.03

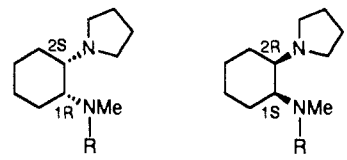
[a] See footnote on Table II.

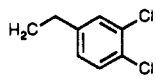
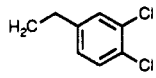
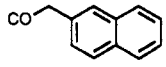
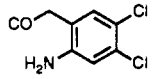
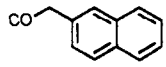
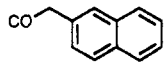
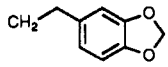
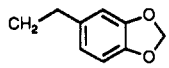
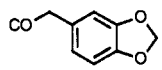
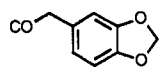
Table V. Binding Affinities of Miscellaneous Compounds at the σ Receptor[a]

compd	(±)- R	IC_{50}, nM [^{3}H](+)-3-PPP	% control [^{3}H]brem	% control [^{3}H]U69,593
(±)-2		7551 ± 496	100.97	44.40
(±)-29		13 ± 1	88.02	31.73
(±)-30		77 ± 0.4	96.14	16.06
(±)-31		2593 ± 290	102.78	78.66
(±)-32		6058 ± 321	106.81	86.35
(±)-33		418 ± 3	88.16	43.37
(±)-34		no inhibn[b]	108.48	99.36
(±)-35		1516 ± 435	105.99	84.57
(±)-36		2100 ± 652	104.85	73.88
(±)-37		no inhibn[b]	93.29	91.80
(±)-38		no inhibn[b]	105.15	105.07
(±)-43		966 ± 70	106.49	98.53
(±)-44		2 ± 0.4	99.20	78.98
(±)-47		37 ± 5	86.49	91.47
(±)-48		125 ± 9	ND	ND
(±)-49	$COCH_3$	no inhibn[b]	111.88	96.45

[a,b] See footnotes on Table II.

Table VI. Selectivity of σ Receptor Ligands across Receptor Types[a]



compd	R group	K_i, nM [^{3}H](+)-3-PPP σ	[^{3}H](−)-sulpdopamine-d_2	[^{3}H]brem κ	[^{3}H]U69,593 κ	[^{3}H]TCP phencyclidine
1*S*,2*R*-(−)-**44**		1.3 ± 0.3	no inhibn[b]	no inhibn[b]	no inhibn[b]	no inhibn[b]
1*R*,2*S*-(+)-**44**		6 ± 3	no inhibn[b]	no inhibn[b]	3793 ± 1046	no inhibn[b]
(±)-**29**		11 ± 1	no inhibn[b]	no inhibn[b]	1618 ± 219	no inhibn[b]
(±)-**17**		11 ± 3	1539 ± 258	no inhibn[b]	523 ± 5.75	no inhibn[b]
1*S*,2*R*-(−)-**29**		8.66 ± 0.35	no inhibn[b]	no inhibn[b]	1667 ± 80.5	no inhibn[b]
1*R*,2*S*-(+)-**29**		1372 ± 322	7352 ± 1238	1444 ± 614	2213 ± 80.5	no inhibn[b]
1*S*,2*R*-(−)-**50**		89.5 ± 20	no inhibn[b]	no inhibn[b]	no inhibn[b]	no inhibn[b]
1*R*,2*S*-(+)-**50**		30.5 ± 0.05	no inhibn[b]	no inhibn[b]	no inhibn[b]	no inhibn[b]
1*S*,2*R*-(−)-**51**		456 ± 72	no inhibn[b]	6986	3630 ± 407	no inhibn[b]
1*R*,2*S*-(+)-**51**		7212	no inhibn[b]	no inhibn[b]	no inhibn[b]	no inhibn[b]

[a] Competition data was generated as described in the methods section. Each compound was tested at three concentrations (100, 1000, 10000 nM) of unlabeled test ligand in the dopamine-d_2, κ, PCP, and σ assays. If a compound elicited >30% inhibition at 10000 nM, a 12-point curve using unlabeled test ligand concentrations ranging from 0.05–10000 nM (in σ assay 0.005–1000 nM for the most potent compounds) was conducted. The CDATA (EMF Software, Inc., Baltimore, MD) iterative curve-fitting program was used to analyze the results. The Cheng–Prusoff equation was used to convert IC_{50} values to apparent K_i values.[33] The above values are the result of two or three experiments, each carried out in duplicate. In order to calculate the K_i values, the following K_d values (as determined in independent experiments) were employed: [^{3}H]bremazocine (guinea pig) K_d = 0.64 nM; [^{3}H]TCP (guinea pig) K_d = 25 nM; [^{3}H](−)-sulpiride (rat) K_d = 10.3 nM, [^{3}H](+)-3-PPP (guinea pig) K_d = 27.4 nM, [^{3}H]U69,593 (guinea pig) K_d = 2.7 nM. For κ receptors labeled by [^{3}H]U69,593, the IC_{50} was determined for select compounds by fitting displacement curves to the two parameter logistic equation (Munson and Rodbart),[32] with use of a nonlinear-least-squares curve-fitting program for the best fit estimates of the IC_{50}. The IC_{50} values were converted to K_i values by use of the Cheng–Prusoff equation[33] with the experimentally determined K_d = 2.7 nM for [^{3}H]U69,593. These data points were the result of duplicate experiments, each performed in triplicate. [b] No IC_{50} or K_i values could be determined in these cases since the compounds produced less than 30% inhibition of control binding at a concentration of 10000 nM.

the unsubstituted compound (±)-**3**. Similar increases in affinity relative to that of (±)-**3** were observed with the 3- and 4-monomethoxy-substituted compounds [(±)-**12** and (±)-**13**].

None of the compounds in Table II exhibited significant affinity for κ receptors labeled by [^{3}H]bremazocine. However, the compounds did show some displacement of [^{3}H]U69,593 (Table II) at a 10 μM concentration. Compound (±)-**1** displaced 90.4% of [^{3}H]U69,593 binding at the 10 μM concentration, the increased potency perhaps following from the similarity of its structure to the κ selective ligand U50488.

The second group studied were urea and thiourea derivatives (±)-**39**, (±)-**40**, and (±)-**42** (Table III), which are all more rigid analogues of (±)-**1**. These compounds showed a 17–27-fold reduced affinity for σ receptors compared with (±)-**1**. Additionally, they also showed a significant decrease in affinity for the κ site labeled by [^{3}H]U69,593 at 10 μM compared with (±)-**1**. The lack of potency of these compounds may be attributable to the increased rigidity of the unit between the aromatic ring and cyclohexanediamine moiety which may place a part of the pharmacophore that is necessary for binding into an undesirable area within the receptor. The effect of an increase in length of this unit was investigated by evaluation of the phenoxyacetamides and related compounds shown in Table IV. In the unsubstituted analogue [(±)-**20**] (Table IV), there was no increase in σ receptor binding affinity compared with the unsubstituted benzeneacetamide [(±)-**3**] (Table II). However, simple isosteric replacement of oxygen with sulfur gave (±)-**21**, which exhibited a 6-fold increase in σ receptor binding compared with (±)-**20**. This result suggests that a more lipophilic sidechain may increase σ receptor potency. However, the

result may also be due to subtle changes in aromatic ring electron density of (±)-21. Of interest, also, is the phenylpropionamide (±)-22 which showed no advantage over (±)-20 at σ receptor binding, implying that factors more complex than a simple change in polarity or length of the sidechain may be involved in enhancement of σ receptor affinity. The most dramatic increases in σ receptor binding activity of the compounds in Table IV were produced by substitution of the aromatic ring of (±)-20 with two chlorine atoms as in (±)-24, (±)-25, and (±)-26. Compound (±)-25 showed a 30-fold increase in σ receptor activity compared with (±)-20. With these compounds a hydrophobic substituent in the 2-position on the aromatic ring enhanced activity. The 2,3-dichloro substitution pattern produced a greater increase in affinity at the σ receptor than the 3,4-dichloro substitution pattern. In contradistinction to the benzeneacetamides in Table II, 2,4-dichloro substitution resulted in a greater increase in σ receptor binding activity [e.g., in Table IV, (±)-26 exhibited a 16-fold increase in σ receptor binding compared with unsubstituted (±)-20, whereas, in Table II, (±)-14 showed a 4-fold increase in σ binding over (±)-3]. Furthermore, the electron-rich compound (±)-23 (Table IV) had a lower affinity for the σ receptor than the parent compound (±)-22 [compare (±)-18 to (±)-13 in Table II]. Carbamate (±)-28 exhibited only marginally improved affinity for σ receptors when compared with its amide isomer [(±)-20].

Only two chlorinated phenoxyacetamides (±)-24 and (±)-27 were able to displace [^{3}H]bremazocine from the κ receptor at concentrations of 10 μM (Table IV). These compounds were the only members of the entire series (Table I) that exhibited the capacity to displace [^{3}H]bremazocine. They share the common feature among the compounds in Table IV of having chlorine atoms in the 3- and 4-positions of the aromatic ring, a feature also shared by the κ receptor selective agonist U50,488. It is possible that the 3,4-chlorine atoms of (±)-24 and (±)-27 may share a similar spatial orientation to those of U50,488 at the κ receptor labeled by [^{3}H]bremazocine. In contrast, the highest potency for κ receptors labeled by [^{3}H]U69,593 of the compounds in Table IV was exhibited by the 2,3-dichloro compound [(±)-25]. Compounds (±)-24 and (±)-27 were also able to displace [^{3}H]U69,593 from κ receptors.

Of the miscellaneous compounds shown in Table V, the most potent compound in the entire series (Table I) at displacing [^{3}H](+)-3-PPP was diamine (±)-44. The compound (±)-44 (Table V) was 100 times more potent than the parent compound [(±)-1] (Table II). This enormous increase in binding to the σ receptor can be rationalized by the presence of an additional ionic binding site created by changing monoamine (±)-1 to diamine (±)-44. A 10 μM concentration of (±)-44 failed to displace [^{3}H]bremazocine and did not displace [^{3}H]U69,593, unlike the parent compound [(±)-1].

Other large increases in σ receptor binding activity were shown by the 2-(2-naphthyl)acetamide [(±)-29] (Table V) which exhibited a 17-fold increase in σ receptor binding compared with (±)-1 (Table II). This increase in σ receptor affinity was accompanied by a slight decrease in affinity for κ receptors labeled by [^{3}H]U69,593 (Table V). Anticipating a similar increase in σ receptor binding activity as was seen in the change from (±)-1 to (±)-44 (Table V), we reduced the amide portion of (±)-29 to give diamine (±)-47. However, a 3-fold decrease in σ affinity was observed, rather than the expected increase (Table V), indicating that more complex factors may be involved in the high affinity of diamines related to (±)-44 than simple addition of an extra ionic binding interaction. Diamine (±)-48 (Table V) showed a 9-fold increase in affinity relative to its precursor (±)-3 (Table II). This increase is consistent with the change from (±)-1 to (±)-44. All diamines [(±-44, (±)-47, and (±)-48] showed reduced affinities for κ receptors compared with their amide precursors. The relative increase or decrease in in vitro σ receptor binding following conversion of the amide to the diamine appears to be highly dependent on the nature of the aromatic group.

Miscellaneous compounds which showed decreased affinity for the σ receptor compared with (±)-1 include the 3-pyridyl-based amides (±)-37 and (±)-38 (Table V), thiophene acetamides (±)-35 and (±)-36 (Table V), sulfonamide (±)-43 (Table V), 1-naphthamide [(±)-31], and 3,4-dichlorobenzamide [(±)-2]. We also found that the anticonvulsant drug U54,494A [(±)-2],[28] which falls within this series of compounds, fails to show significant interaction with the σ binding site (Table V). The lack of activity of (±)-2 at the σ receptor suggests that the distance between the 3,4-dichlorophenyl moiety and the cyclohexanediamine portion may be important for σ receptor activity. In general, longer chain lengths do not seem to be as detrimental to σ receptor binding within this series as do shorter ones. The phenoxyacetamides (Table IV) and phenoxybutanamide [(±)-33, Table V] had appreciable activity compared with (±)-1. Compound (±)-49 (Table V) served as a control for the aromatic ring; its complete lack of activity quite clearly demonstrates the importance of the aromatic system.

Selected enantiomeric compounds, and a few racemic compounds, were evaluated for selectivity across receptor types (Table VI). The most potent and selective σ receptor ligands were found to be the (3,4-dichlorophenyl)ethyl diamines 1*R*,2*S*-(+)-44 and 1*S*,2*R*-(−)-44. The more potent (−)-44 displaced [^{3}H](+)-3-PPP with a K_i of 1.3 ± 0.3 nM, exhibiting a 5-fold increase in potency relative to its enantiomer (+)-44. Since (−)-44 exhibited no cross-reactivity with any of the other receptor systems tested, it represents a novel, highly potent, and selective σ receptor probe. Its less potent enantiomer, (+)-44, was almost equally selective, except for possessing affinity for κ receptors labeled by [^{3}H]U69,593 (K_i = 3793 ± 1046 nM). The only high affinity σ ligand to exhibit affinity for dopamine-d_2 receptors was (±)-17 which was 140-fold more selective for σ receptors than dopamine-d_2 receptors. This compound also exhibited weak affinity for κ sites labeled with [^{3}H]-U69,593. Interestingly, the degree of enantioselectivity at σ receptors was greater for the acetamides than for the diamines; for example, (+)-29 and (−)-29 exhibited a 160-fold enantioselectivity while (+)-44 and (−)-44 exhibited only a 5-fold enantioselectivity. Similarly, (+)-51 and (−)-51 exhibited a 16-fold enantioselectivity while the respective diamines (+)-50 and (−)-50 showed only a 3-fold enantioselectivity. Increase or decrease in receptor affinity in going from acetamides to their respective diamines appeared to follow no general rule. Subtle modifications of structure can dramatically alter binding affinity.

Examination of the in vivo potency of (±)-29 and (−)-44 indicated that the diamine (−)-44 (K_i = 1.3 ± 0.3 nM for sigma receptors) failed to produce behavioral effects in rats after intracerebroventricular (ICV) injection at concentrations up to 100 nM/rat. In contrast, the acetamide (±)-29 (IC_{50} = 13 ± 1 nM) produced stereotyped behavior (35 ± 5% at 125 nmol/rat and 67 ± 4% at 250 nmol/rat) and ataxia (50 ± 6% and 80 ± 0% at 125 and 250

(28) Von Voightlander, P. F.; Hall, E. D.; Ochoa, M. C.; Lewis, R. A.; Triezenberg, H. J. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 542–547.

nmol/rat, respectively) when administered ICV, but failed to have any effect by interperitoneal (ip) or subcutaneous (sc) administration. DTG (K_i = 49 ± 8.9 nM, [^{3}H](+)-3-PPP) potently produced stereotyped behavior [(ED_{50} = 55 (45–67) nmol/rat] and ataxia [(ED_{50} = 55 (45–67) nmol/rat]. The significance of these differences is not understood at the present time.

In summary, we have shown that some *cis*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]arylacetamides and their corresponding arylethylamines possess high affinity for σ receptors. Furthermore, we have obtained, to the best of our knowledge, the most potent and selective σ receptor ligand known, the arylethylamine 1*S*,2*R*-(−)-*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(−)-**44**] and consequently identified a new class of sigma ligands. These classes of compounds will provide a base for development of better tools with which to study σ receptor biochemistry and pharmacology.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Specific rotation determinations at the sodium D line were obtained on a 1-dM cell with use of a Perkin-Elmer 241-MC polarimeter. Gas chromatographic (GC) analyses were performed on a Hewlett-Packard 5880A instrument fitted with a 30 M SE-30 capillary column and a flame ionization detector. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Chemical-ionization mass spectra (CIMS) were obtained by using a Finnigan 1015 mass spectrometer. Electron-ionization mass spectra (EIMS) and high-resolution mass measurements (HRMS) were obtained by using a VG-Micro Mass 7070F mass spectrometer. ^{1}H NMR spectra were obtained from $CDCl_3$ solutions by using a Varian XL-300 spectrometer. Infrared (IR) spectra were determined with use of a Beckman 4230 IR spectrophotometer; spectra were taken from KBr pellets. Thin-layer chromatography (TLC) was performed on 250 μM Analtech GHLF silica gel plates. TLC system A corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (90:9:1) TLC system B corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (80:18:2).

(±)-*cis*-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(±)-3]. Procedure A. To a stirred solution of (±)-**4** (0.88 g, 4.83 mmol) in pentene-stabilized chloroform (50 mL) was added dropwise at room temperature phenylacetyl chloride (0.89 g, 5.76 mmol). The reaction was judged complete by TLC (system A) after 5 min at room temperature. The solvent was evaporated in vacuo and the residue was crystallized from 30 mL of ethyl acetate (Table I).

(±)-*cis*-2-Chloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(±)-5]. Procedure B. To a stirred solution of o-chlorophenylacetic acid (1.40 g, 8.23 mmol), dry pyridine (0.22 g, 2.74 mmol), and *N*,*N*′-dicyclohexylcarbodiimide (2.27 g, 10.9 mmol) in dry CH_2Cl_2 (50 mL) was added (±)-**4** (1.00 g, 5.48 mmol). TLC (System A) indicated that reaction was complete after stirring for 10 min at room temperature. The precipitated *N*,*N*′-dicyclohexylurea (DCU) was removed by filtration and the filter cake washed with ether (20 mL). The filtrate was diluted to 100 mL with ether and extracted with 10% citric acid (100 mL). The aqueous layer was washed with ether (2 × 50 mL) and the ether extract discarded. The aqueous layer was basified by addition of excess aqueous ammonia solution and extracted with CH_2Cl_2 (2 × 100 mL). The CH_2Cl_2 layer was washed with water (50 mL), dried over Na_2SO_4, and evaporated to afford (±)-**5** as an oil. The HCl salt of (±)-**5** was crystallized from ethyl acetate (Table I).

(±)-*cis*-*N*-Methyl-*N*-(benzyloxycarbonyl)-2-(1-pyrrolidinyl)cyclohexylamine [(±)-28]. Procedure C. Benzyl chloroformate [0.91 mL (6.03 mmol) of 95% (tech)] was added via syringe to a stirred solution of (±)-**4** (1.00 g, 5.48 mmol) in dry chloroform (5 mL). After 10 min at room temperature, TLC (System A) indicated that the reaction was complete. Evaporation of the solvent afforded (±)-**28**·HCl as an oil which crystallized from 20 mL of ethyl acetate (Table I).

(±)-*cis*-*N*-(3,4-Dichlorophenyl)-*N*′-[2-(1-pyrrolidinyl)cyclohexyl]urea [(±)-39]. Procedure D. To a stirred solution of (±)-**4** (1.00 g, 5.48 mmol) in dry chloroform (20 mL) was added 3,4-dichlorophenyl isocyanate (1.14 g, 6.06 mmol). After 10 min at room temperature, TLC (System A) indicated that the reaction was complete. Evaporation of the solvent afforded the crude product in quantitative yield. The HCl salt crystallized from ethyl acetate (Table I).

(±)-*cis*-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzenesulfonamide [(±)-43]. Procedure E. A stirred solution of (±)-**4** (1.00 g, 5.48 mmol) in 10 mL of dry chloroform was treated with benzenesulfonyl chloride (1.35 g, 7.64 mmol) and the solution stirred for 1 h at room temperature. Evaporation of the solvent afforded the HCl salt of the product which failed to crystallize. The HCl salt was dissolved in water (100 mL) and treated with excess concentrated aqueous ammonia to liberate free (±)-**43**. The aqueous mixture was extracted with CH_2Cl_2 (2 × 50 mL), and the organic extract was dried over Na_2SO_4 and evaporated to give the crude product as an oil. The HBr salt crystallized from 2-propanol (10 mL) (Table I).

(±)-*cis*-2-Amino-4,5-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(±)-17]. Procedure F. (±)-**16**·HCl (2.00 g, 4.45 mmol) was dissolved in a mixture of water (10 mL) and methanol (20 mL) and hydrogenated over Adams catalyst at atmospheric pressure. Reaction was complete after a 2-h reaction time. The reaction mixture was filtered through Celite and the filtrate evaporated to dryness. The product crystallized from hot 2-propanol (10 mL) (Table I).

(±)-*cis*-*N*-Methyl-*N*-(2-phenylethyl)-2-(1-pyrrolidinyl)cyclohexylamine [(±)-48]. Procedure G. (±)-**3** (1.00 g, 3.50 mmol) was added dropwise to a 1.00 M solution of LAH in THF (7.00 mL) at room temperature and the solution boiled under reflux until TLC (System B) indicated reaction to be complete. The solution was treated dropwise at 0 °C with water (0.27 mL) followed by the same volume of 15% NaOH (aqueous). The reaction mixture was finally treated with additional water (0.80 mL) and then filtered. Evaporation of the aqueous layer afforded the crude product as an oil. Numerous salts of this compound failed to crystallize (Table I).

(±)-*cis*-*N*-Methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(±)-44]. Procedure H. To a solution of (±)-**4** (3.00 g, 16.46 mmol) in dry DMF (70 mL) at 60 °C was added 2-(3,4-dichlorophenyl)ethyl methanesulfonate (14 g, 49.1 mmol) during 3 days. The reaction mixture was diluted to 500 mL with water and extracted with chloroform (3 × 100 mL). The organic layer was back extracted with water (50 mL) and then acidified (to pH 2) by addition of a solution of HBr gas in MeOH. Most of the solvent ($CHCl_3$) was evaporated in vacuo and the DMF-containing residue was dried by heating to 60 °C under high vacuum. Trituration of the residue with 2-propanol (100 mL) afforded a crystalline solid which was allowed to stand for 10 min and then filtered. The crystalline solid was washed once with cold 2-propanol and then recrystallized from 2-propanol (50 mL) (Table I). ^{1}H NMR ($CDCl_3$): δ 7.32 (d, J = 8.2 Hz, 1 H), 7.29 (d, J = 2.0 Hz, 1 H), 7.01 (dd, J = 2.0 Hz, 8.2 Hz, 1 H), 3.09 (m, 2 H), 2.67 (br s, 2 H), 2.5–2.67 (m, 4 H), 2.45 (m, 3 H), 2.35 (s, 3 H), 1.83 (m, 2 H), 1.65 (m, 2 H), 1.65–1.25 (m, 7 H). CIMS: M + H (m/z) (calcd for $C_{19}H_{29}Cl_2N_2$) = 355, found M + H = 355.

1*R*,2*S*-(+)-*cis*-*N*-Methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamine [(+)-44]. Procedure I. 1*R*,2*S*-(+)-1-(+)-tartrate·monohydrate[27] (5.14 g, 9.57 mmol) was converted to its free base by partitioning between ether (100 mL) and 10% aqueous NaOH (50 mL). The free base was azeotroped with toluene (3 × 40 mL) prior to use: A solution of this base in dry THF (50 mL) was added dropwise at 20 °C to 72 mL of a stirred solution of AlH_3 (0.66 M) in THF[34] (see below). After 20 min, the solution was poured into 15% aqueous NaOH (200 mL) and extracted with ether (2 × 200 mL). The combined ether extract was back-washed with water (50 mL) and evaporated, and the residue was dried under high vacuum. The oily residue was converted to its HBr salt in 50 mL of 100% ethanol. Crystallization was induced by scratching with a glass rod. The first crop afforded 3.01 g (61%) of analytically pure material (Table I).

2-(3,4-Dichlorophenyl)ethanol (46). Excess (2 mol equiv) of borane–THF complex (1.0 M) in THF was added dropwise during 20 min to a stirred and cooled (ice bath) solution of 3,4-dichlorophenylacetic acid (50 g, 243.8 mmol) in THF (200 mL). After the addition was complete, the reaction was quenched into 200 mL of water (care) and excess sodium hydroxide pellets were added, followed by ether (500 mL). The ether layer was retained and washed with water (100 mL) and evaporated to afford crude product. Distillation (110–119 °C at 0.1 mmHg) afforded **46** (45.2 g, 97% yield) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.37 (d, J = 8 Hz, 1 H), 7.32 (d, J = 2.1 Hz, 1 H), 7.06 (dd, J = 2.1 Hz, 8.2 Hz, 1 H), 3.82 (br s, 2 H), 2.81 (t, J = 6.4 Hz, 2 H), 1.67 (br s, 1 H). IR (film): 3600, 3350, 2950, 2880, 1595, 1560, 1470, 1395, 1130, 1030, 820 cm^{-1}. HRMS: M^+ (m/z) (calcd for $C_8H_8{}^{35}Cl_2O$) = 189.9952, found M^+ = 189.9959.

2-(3,4-Dichlorophenyl)ethyl Methanesulfonate (45). To a stirred solution of **46** (20.12 g, 105.3 mmol) and methanesulfonyl chloride (8.97 mL, 115.9 mmol) in THF (100 mL) was added, dropwise during 10 min, triethylamine (TEA) (27 mL, 193.7 mmol). During the addition of the TEA, the solution became warm and required cooling (ice bath). The TEA·HCl was filtered off and washed with THF (30 mL). Evaporation of the solvent in vacuo afforded a quantitative yield of **45** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 7.38 (d, J = 8.1 Hz, 1 H), 7.32 (d, J = 2.1 Hz, 1 H), 7.07 (dd, J = 2.1, 8.3 Hz, 1 H), 4.38 (t, J = 6.8 Hz, 2 H), 3.01 (t, J = 6.8 Hz, 2 H), 2.92 (s, 3 H). IR (film): 3020, 2940, 1595, 1560, 1470, 1350, 1170, 950, 905, 815 cm^{-1}. EIMS: M^+ (m/z) (calcd for $C_9H_{10}{}^{35}Cl_2O_3S$) = 268, found M^+ = 268.

3,4-Dichlorophenyl Isothiocyanate (41). To a vigorously stirred and refluxed suspension of 3,4-dichloroanilinium hydrochloride (5.39 g, 33.06 mmol) in dry toluene (30 mL), was added, dropwise during 20 min, a solution of freshly redistilled thiophosgene (3.03 mL, 1.2 equiv in toluene (7 mL). HCl gas was evolved during the addition. Stirring and refluxing was continued until all of the salt had dissolved. The toluene and excess thiophosgene was removed by distillation at atmospheric pressure through a short vigreaux column, and the oily residue remaining was distilled under high vacuum (120 °C at 2.0 mmHg) to give 5.28 g (78%) of pure **41** as a colorless oil. ^{1}H NMR: δ 7.41 (d, J = 8.6 Hz, 1 H), 7.31 (d, J = 2.4 Hz, 1 H), 7.06 (dd, J = 2.4 Hz, J = 8.6 Hz, 1 H). IR (film): 3080, 2060, 1580, 1550, 1460, 1120, 1030, 965, 865, 810, 780 cm^{-1}. EIMS: M^+ (m/z) (calcd for $C_7H_3{}^{35}Cl_2NS$) = 203, found M^+ = 203.

Preparation of 0.66 M Alane (AlH_3) in THF.[34] A 1 M solution of LAH in THF (200 mL) was diluted to 303 mL by addition of freshly dried and redistilled THF. To the stirred solution was added, dropwise, concentrated H_2SO_4 (5.35 mL), at such a rate that the temperature did not rise above 20 °C, and the solution was maintained at or below 20 °C by periodic immersion in an ice bath. Vigorous evolution of hydrogen occurred during the addition. The solution was stirred for 1 h at 20 °C and then allowed to stand for 4 h at 20 °C for the precipitated Li_2SO_4 to settle. The clear upper layer was decanted and stored at 0 °C prior to use.

Biological Materials and Methods. Membrane Preparation. Receptor binding assays were performed by using the crude synaptosomal (P_2) membrane fraction of guinea pig brain (σ, κ, and PCP receptors) or rat brain (dopamine-d_2 receptors).

Crude P_2 membrane fractions were prepared from frozen (−80 °C) guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebellum. 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/g tissue wet weight. The homogenate was centrifuged at 1000g for 10 min at 4 °C and the supernatants 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 supernatants were centrifuged at 31000g for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/g of 10 mM Tris-HCl, pH 7.4, and the suspension allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000g for 15 min, the pellets were resuspended by gentle Potter–Elvehjem homogenization to a final volume of 1.53 mL/g 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.[29] with bovine serum albumin 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 cerebellum) were then treated as described above.

σ Receptor Assay. σ receptors were labeled with [^{3}H](+)-3-PPP (98.9 Ci/mmol). 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), which were soaked in 0.5% poly(ethylenimine) for at least 30 min at 25 °C prior to use. Filters were then washed twice with 5 mL of ice cold Tris-HCl buffer.

Dopamine-d_2 Assay. Dopamine-d_2 receptors were labeled with 5 nM [^{3}H](−)-sulpiride (78.6 Ci/mmol). Incubations were carried out for 1 h 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 ice cold incubation buffer and vacuum filtration through glass fiber filters. Filters were then washed twice with ice cold incubation buffer.

κ Receptor Displacement ([^{3}H]U69,593 and [^{3}H]Bremazocine) Assays. κ binding assays were performed as previously described by Rothman et al.[30,31] κ sites were labeled with [^{3}H]-U69,593[30] (Tables II–VI) and [^{3}H]bremazocine[31] (Tables II–V) by using membranes pretreated with 1 μM 2-(4-ethoxybenzyl)-1-[(diethylamino)ethyl]-5-isothiocyanatobenzimidazole (BIT) and 1 μM N-phenyl-N-[1-[2-(4-isocyanatophenyl)-ethyl]-4-piperidinyl]propanamide (FIT) to deplete μ an δ receptors.[31] Membranes prepared from frozen guinea pig brains were incubated with a 10 μM concentration of test compound in 1-mL final volume containing approximately 1 mg protein. Incubations were terminated by rapid filtration through glass fiber filters followed by two washes with 5 mL of ice cold buffer. In Tables II–V, the percentage of specific [^{3}H]U69,593 and [^{3}H]bremazocine binding spared by a 10 μM concentration of each compound was derived from a single experiment performed in quadruplicate.

Also in Table VI, κ receptors were labeled with [^{3}H]bremazocine (17.3 Ci/mmol) in the presence of DAGO and DSTLE as μ and δ 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 membrane protein, 100 nM DSTLE, 100 nM DAGO, and 2 nM [^{3}H]bremazocine. Assays were terminated by the addition of 5 mL ice cold buffer and filtration through glass fiber filters under reduced pressure. Filters were then washed twice with 5 mL of ice cold buffer. Nonspecific binding was determined in the presence of 10 μM levallorphan.

Phencyclidine (PCP) Receptor Assay. PCP receptors were labeled by using [^{3}H]TCP (48.9 Ci/mmol). 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 of ice cold buffer. Filters were soaked in 0.3% poly(ethyleneimine) for at least 30 min at 25 °C prior to use. Nonspecific binding was determined in the presence of 10 μM (±)-cyclazocine.

All scintillation counting was performed with a Packard model 4450 scintillation spectrometer using Ecoscint cocktail (National Diagnostics, Manville, NJ) after an overnight extraction of the

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counts from the filters. Ligands were obtained from DuPont/New England Nuclear (Boston, MA). Haloperidol, poly(ethylenimine), and Tris were obtained from Sigma Chemicals (St. Louis, MO). Cyclazocine and levallorphan were obtained from the National Institute on Drug Abuse (Rockville, MD).

Behavioral Assays. Male Sprague-Dawley rats were placed individually into cages and allowed to acclimate to the new environment for at least 1 h. Drugs were dissolved in saline or sodium acetate buffer and administered such that 1 mL/kg was administered ip or sc, or 5 μL/rat was administered icv. The animals were rated by using a scale described by Sturgeon et al.,[35] and the ratings determined at the time of peak effect were used to generate dose-response curves. Briefly, the stereotyped behavioral rating is as follows: (0) inactive or in-place nonrepetitive activity, (1) sniffing, grooming, or rearing more frequently than the control; (2) nondirectional movements, occasional reciprocal forepaw treading, frequency of sniffing, rearing and grooming greater than (1); (3) turning or backpeddling; (4) rapid and continuous turning, backpeddling, assuming a praying posture and gagging; and (5) dyskinetic extension and flexion of limbs, head and neck, weaving greater than (4). The ataxial rating scale is as follows: (0) inactive or coordinated movements; (1) awkward or jerky movements or loss of balance while rearing; (2) moderate rate of falling; (3) frequent falling or partial impairment of antigravity reflexes; (4) cannot move beyond a small area, may support weight on stomach or haunches; and (5) unable to move except for twitching movements. A rating of 5 was considered a 100% response. Ratings for each animal were taken every 5 min after drug administration until ratings returned to control level. The ratings taken at the time of peak effect were used to evaluate the potency of each drug. ED_{50} values were determined by using a computerized Finney analysis.[36]

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Potent Anticonflict Activity and Lessening of Memory Impairment with a Series of Novel [1]Benzothieno[2,3-*c*]pyridines and 1,2,3,4-Tetrahydro[1]benzothieno[2,3-*c*]pyridines†

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[1]Benzothieno[2,3-*c*]pyridines (**10a-c**, **11**, **12a-t**, and **13a,b**) and 1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridines (**3a-c**, **7**, **8a-c**, and **9**) were synthesized. The compounds are bioisosteres of β-carbolines and 1,2,3,4-tetrahydro-β-carbolines where the indole nitrogen is replaced by sulfur. Their pharmacological activity was evaluated in a water lick conflict test in rats and a passive avoidance test in mice. In the 1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine series, the presence of ethyl ester (**3b**) or cyclohexyl carboxamide (**7**) groups at C-3 conferred good anticonflict activity and lessening of memory impairment, while N-acylation of **3b** abolished activity. In the [1]benzothieno[2,3-*c*]pyridine series, the aminoethyl carboxamide (**12a**) group at C-3 also conferred activity, but other amides studied were not active. The most potent compounds (**3b**, **7**, and **12a**) were also administered orally and had potent anticonflict and antiscopolamine amnesia-reversal activity. These compounds did not bind to the BZP receptor in spite of having structures similar to those of β-carbolines. Compound **7** bound strongly to 5-HT_{1A} receptors and would be expected to be a novel anxiolytic.

Introduction

Benzodiazepines have been widely used for the treatment of anxiety. They produce their pharmacological effects by interacting with central BZP receptors.[1] Whereas BZPs are safe and effective drugs, they produce sedation and muscle relaxation which may be undesirable in certain situations and potentiate the action of CNS depressants.[2] In addition, an amnesia[3,4] following their administration has been observed in men and experimental animals. Recently β-carbolines have been reported to be modulators[5,6] of anxiety states in the brain. β-Carboline methyl ester has been classified as an inverse agonist and reported to exert a proconflict effect.[7,8] In contrast, β-carbolines have been shown to produce a syndrome reminiscent of anxiety.[9,10]

In this paper, we report our successful efforts to design selective anxiolytics by structural modification of β-carboline to afford [1]benzothieno[2,3-*c*]pyridines. We describe the synthesis of novel [1]benzothieno[2,3-*c*]pyridines and 1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridines as well as their anticonflict activities, lessening of memory impairments, and structure-activity relationships. Interestingly the most potent compounds (**3b**, **7**, and **12a**) were different from BZP in that they did not bind to the

† Abbreviations: 5-HT, serotonin; BZP, benzodiazepine; CNS, central nervous system; DPPA, diphenyl phosphorazidate; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene.

‡ Synthetic Section.

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