Structural Features Important for σ_1 Receptor Binding

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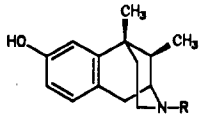
Received November 29, 1993®

Two problems that have hampered σ receptor research are (i) a lack of high-affinity agents and (ii) the recent identification of multiple populations of σ receptors (i.e., σ_1 and σ_2 sites). Recently, several high-affinity σ ligands have been identified, and the term *superpotent σ ligands* has been coined to describe agents with K_i values of <1 nM. We have previously shown that appropriately N-substituted phenylalkylamines bind at σ receptors with high affinity. In the present investigation, we examine the structure–affinity relationships of these phenylalkylamine derivatives for σ_1 binding and describe some of the first superpotent σ_1 ligands. A binding model was developed to account for the structural features of the phenylalkylamines that appear to be important for the interaction of these agents with σ_1 sites.

Current interest in σ receptors is related to their possible involvement in psychiatric disorders and regulation of motor behavior and because σ ligands may have potential application as neuroprotective agents (reviewed[1]). Relatively few agents bind at σ receptors with high affinity and selectivity. de Costa and co-workers[2] have recently defined the term *superpotent σ ligands* as compounds that bind at σ sites with subnanomolar affinity. Perhaps one reason for the necessity of such a term is because the prototypic σ ligand (+)-*N*-allylnormetazocine (NANM, SKF 10,047; 1) and some other older σ ligands bind with affinities of hundreds of nanomolar and, in some cases, in the micromolar range.[1] Over the last several years, we have identified a number of novel high-affinity ligands that bind at σ sites labeled by tritiated ditolylguanidine ([^{3}H]DTG) and have explored their structure–affinity relationship.[3-7] Recently, Hellewell and Bowen[8] described two separate populations of σ receptors: σ_1 and σ_2 receptors. The primary pharmacological distinction between these two sites is the affinities of certain (+)-benzomorphan derivatives; for example, (+)-pentazocine (2) binds with >100-fold selectivity for σ_1 versus σ_2 sites.



1 R = $-CH_2CH{=}CH_2$

2 R = $-CH_2CH{=}C(CH_3)_2$

We have found that DTG binds nearly equally well at both populations of sites (DTG: σ_1 K_i = 41 nM; σ_2 K_i = 49 nM), whereas (+)-pentazocine binds with considerable difference (σ_1 K_i = 1.7 nM; σ_2 K_i = 860 nM).[9] Consequently, much of our earlier work using [^{3}H]DTG as radioligand probably reflects a composite of binding at both populations of sites. The clinical relevance of σ_1 and σ_2 receptors is unknown at this time. This is due, in part, to a lack of high-affinity selective ligands. As a prelude to the design of such agents, more needs to be known about the structural requirements of each of the σ subpopulations. We report now on the structure–affinity relationships of a series of phenylalkylamine σ ligands with respect to their binding at σ_1 sites. The synthesis (and "overall σ-binding data") of many of the compounds used in the present investigation has been already reported; however, several new compounds were synthesized to further examine structure–affinity relationships at σ_1 sites.

Table 1. Physicochemical Properties of Novel Sigma Ligands

compd	method	yield (%)	RS[a]	mp/bp (°C) (torr)	mol formula
(-)5	A	80	E	185–187	$C_{18}H_{23}N{\cdot}C_2H_2O_4$
(+)6	B	58	A/T	87–88	$C_{19}H_{25}N{\cdot}C_4H_4O_4$
(+)7	C	49		82–85	$C_{25}H_{29}N$
(-)8	D	34	M/A	154–156	$C_{20}H_{28}NI$
15	D	23	M/A	161–163	$C_{22}H_{32}NI$
(-)16	E	60	E/W	155–157	$C_{19}H_{25}N{\cdot}C_2H_2O_4$
17	E	23	P/T	81–82[b]	$C_{21}H_{29}N{\cdot}HCl$
(-)18	E	65	E/W	141–143	$C_{20}H_{27}N{\cdot}C_2H_2O_4$
31	F	67	E/T	140–142	$C_{20}H_{27}N{\cdot}C_2H_2O_4$
37	A	47	M/A	280–282[c] (dec)	
40	F	74	M	167–169[d]	$C_{12}H_{19}N{\cdot}C_2H_2O_4$
41	F	50	E	143–145	$C_{15}H_{23}N{\cdot}C_2H_2O_4$
45	F	24	E	225–227	$C_{14}H_{22}N_2{\cdot}2C_2H_2O_4$

[a] Recrystallization solvents: E = absolute EtOH, A = EtOAc, T = anhydrous Et_2O, M = MeOH, W = H_2O, and P = 2-propanol. [b] Crystallized with 0.5 mol of H_2O. [c] Lit.[15] mp 269–272 °C. [d] Previously reported as free base[16] but no melting point provided.

Chemistry

Standard synthetic procedures were used to obtain the new compounds prepared for this study. (*R*)-(-)-Amphetamine and phenylacetaldehyde were condensed under reductive alkylation conditions, and subsequent N-alkylation of the resulting secondary amine under Eschweiler–Clarke conditions afforded (-)5 (method A, Table 1). Compound 37 was prepared in a similar manner. To obtain compounds (+)6, (-)16, 17, and (-)18, acylation of the appropriate amine followed by reduction of the intermediate amide using $LiAlH_4$ afforded the desired compounds (methods B and E). The first step of method C involved reductive alkylation using (*S*)-(+)-amphetamine and hydrocinnamaldehyde; the second step was a direct alkylation of the resulting secondary amine with benzyl bromide to obtain (+)-7. The quaternary compounds (-)8 and 15 were prepared by direct alkylation of the requisite amine with methyl iodide (method D). Compounds 31, 40, 41, and 45

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® Abstract published in *Advance ACS Abstracts*, March 15, 1994.

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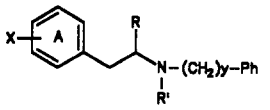
Table 2. Radioligand-Binding Data for Derivatives of (−)-3 at σ_1 Sites



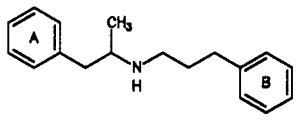
compd	X	R	R′	y	K_i, nM (SEM)	ref[a]
(−)4	H	Me (−)	H	2	43.7 (2.1)	3
(+)4	H	Me (+)	H	2	13.6 (1.8)	3
(−)5	H	Me (−)	Me	2	16.4 (1.8)	
(+)5	H	Me (+)	Me	2	5.8 (0.7)	3
(−)3	H	Me (−)	H	3	10.8 (2.1)	3
(+)3	H	Me (+)	H	3	39.0 (9.9)	3
(+)6	H	Me (+)	Me	3	1.3 (0.2)	
(+)7	H	Me (+)	Bz	3	130 (25)	
(−)8	H	Me (−)	Me_2^+	3	135 (6)	
9	3-Br	Me (±)	H	3	9.7 (2.4)	5
10	4-Br	Me (±)	H	3	12.0 (2.9)	5
(−)11	4-I	Me (−)	H	3	17.7 (3.6)	5
12	3-CF_3	Me (±)	H	3	8.8 (4.0)	5
13	4-OH	Me (±)	H	3	25.7 (6.3)	5
14	4-OEt	Me (±)	H	3	3.4 (0.3)	5
15	H	Pr (±)	Me_2^+	3	335 (27)	
(−)16	H	Me (−)	H	4	7.4 (1.6)	
(+)16	H	Me (+)	H	4	19.4 (5.0)	3
17	H	Pr (±)	H	4	2.7 (0.6)	
(−)18	H	Me (−)	H	5	0.5 (0.2)	
(+)18	H	Me (+)	H	5	0.9 (0.2)	3

[a] Literature reference for synthesis and overall σ-binding data.

were prepared by alkylation of the corresponding secondary amines using the appropriate alcohol tosylates (method F). Reference is made in the other tables to the appropriate literature for previously synthesized compounds.

Results and Discussion

Binding data for N-substituted phenylaminopropane derivatives are shown in Table 2. The 3-phenylpropyl derivative (*R*)(−)PPAP [(−)3] was the first compound reported in this series;[10] as such, it has served as an arbitrary standard to which other derivatives have been compared. The present investigation began with an examination of derivatives of (−)3.



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Stereochemistry. Comparing (−)3 (K_i = 10.8 nM) and its enantiomer (+)3 (K_i = 39.0 nM), it appears that stereochemistry plays only a small role (*S*/*R*-enantiomeric potency ratio = 3.6) (Table 2). However, with the 2-carbon alkyl-chain pair, the *S*-(+)-isomer (+)4 (K_i = 13.6 nM) binds with slightly more than 3 times the affinity of its *R*-(−)-enantiomer (−)4 (K_i = 43.7 nM), and similar results were obtained comparing (+)5 with (−)5. With the 4-carbon and 5-carbon alkyl-chain pairs [i.e., (−)16 versus (+)16 and (−)18 versus (+)18], there is little difference in the affinity of the *R*- and *S*-isomers (Table 2), but, similar to what was seen with 3, the (−)-isomers bind with about twice the affinity of the (+)-isomers. Although the enantioselective reversal seen for the binding of 3, 16, and 18 relative to 4 and 5 may be real and although we have previously raised the possibility of reversed modes of binding for certain phenylalkylamines at σ receptors,[4] for the isomeric pairs examined, the role of stereochemistry appears minimal when the α-substituent is a methyl group.

Table 3. Binding Data for Several Fused Derivatives of (−)3

X-$(CH_2)_x$-CH(R)-N(R′)-$(CH_2)_y$-Y

compd	X	x	R	R′	y	Y	K_i, nM (SEM)	ref[a]
19	1-NP[b]	1	Me (±)	H	3	phenyl	9.3 (0.8)	5
20	2-NP	1	Me (±)	H	3	phenyl	31.0 (13.0)	5
21	2-NP	2	H	Me	3	2-NP	30.0 (11.1)	4
(−)22	phenyl	1	Me (−)	H	3	1-NP	8.6 (1.4)	13
(−)23	phenyl	1	Me (−)	H	3	2-NP	5.7 (1.7)	13

[a] Literature reference for synthesis and overall σ-binding data. [b] NP = naphthyl.

Nevertheless, because 4 and 5 possess equivalent-length alkyl chains on both sides of the amine group, the reversed enantioselectivity may reflect somewhat greater tolerance by the receptor of an α-methyl group on one side of the amine versus the other. In contrast, when the substituents α to the amine are larger than methyl, such as with (+)-NANM (1; K_i = 150 nM)[9] and its isomer (−)NANM (K_i = 3600 nM)[9] or with (+)-pentazocine (K_i = 1.7 nM)[9] and its isomer (−)-pentazocine (K_i = 110 nM),[9] stereochemistry plays a greater role. A similar trend was noted with the overall σ binding of these agents, and it has been suggested that the rigid framework of the benzomorphans may impart directionality to the lone-pair nitrogen electrons or that the added steric bulk of the tricyclic ring system contributes to enantioselectivity.[11]

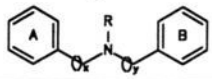
N-Methylation. N-Methylation of (+)3 (K_i = 39 nM) to afford (+)6 (K_i = 1.3 nM) results in a 30-fold increase in affinity (Table 2), whereas N-methylation of (−)4 [i.e., (−)5] and (+)4 [i.e., (+)5] only doubles affinity. The effect of N-monomethylation in the 5-phenylpentylamine series is also variable (see below discussion). N,N-Dimethylation of (−)3 (K_i = 10.8 nM) to afford the quaternary amine derivative (−)8 (K_i = 135 nM) decreases affinity by more than an order of magnitude. The quaternary amine analogue 15 also binds with low affinity. Evidently, tertiary and secondary amines are well tolerated, but quaternary amines are not readily accommodated by σ_1 receptors. For the one case examined, the *N*-benzyl analogue of (+)3 [i.e., (+)7; K_i = 130 nM] binds with only one-fourth the affinity of its parent and about one-tenth the affinity of (−)3.

Alkyl Chain "*y*". There is little effect (less than 3-fold) in increasing the length of the alkyl *y*-chain from 2 [(+)4; K_i = 13.6 nM] to 3 [(+)3; K_i = 39 nM] to 4 [(+)16; K_i = 19.4 nM] methylene units (Table 2). A parallel trend is seen with the (−)-isomers, but (−)16 binds with nearly 6 times the affinity of (−)4. However, the 5-membered chain derivatives (+)18 and (−)18 (K_i = 0.9 and 0.5 nM, respectively) bind with significantly higher affinity (Table 2). This same trend is also seen with the α-desmethyl derivatives (described below).

Aromatic Substituents. Comparing 9–14 (Table 2), it appears that aromatic substituents have relatively little effect on affinity with K_i values (3.4–25.7 nM) spanning about a 10-fold range. All of the substituted derivatives bind within several-fold of the affinity of the parent unsubstituted derivative (−)3 (K_i = 10.8 nM).

Where the *y*-chain is held constant at 3 methylene units, replacement of the phenyl-A ring (19–21), the phenyl-B ring [(−)22, (−)23], or both (i.e., 21) with a 1-naphthyl or 2-naphthyl group has little effect on affinity (K_i values range from 5.7 to 31 nM) (Table 3). Thus, although bulk appears to be tolerated, no affinity-enhancing effect is evident.

Table 4. Binding Data for Alkyl-Chain Variants



compd	x	R	y	K_i, nM (SEM)	ref[a]
24	2	H	3	11.3 (1.0)	4
25	3	H	3	11.4 (1.8)	4
26	1	H	4	9.6 (2.9)	4
27	2	H	4	2.6 (0.3)	4
28	1	H	5	0.32 (0.10)	4
29	1	Me	5	0.19 (0.04)	14
30	2	H	5	0.17 (0.0)	4
31	2	Me	5	0.25 (0.06)	
32	3	H	5	0.28 (0.03)	4
33	3	Me	5	0.38 (0.14)	14
34	4	H	5	0.48 (0.05)	4
35	1	H	7	2.3 (1.0)	4
36	2	H	7	1.5 (0.6)	4

[a] Literature reference for synthesis and overall σ-binding data.

Table 5. Binding Data for 5-Phenylpentylamine-Related Derivatives

X-CH_2-CH_2-Z-CH_2-CH_2-Y

compd	X	Z	Y	K_i, nM (SEM)	ref[a]
37	phenyl-CH_2CH_2-NH-	CH_2	H	48.2 (3.4)	
33	phenyl-$CH_2CH_2CH_2$-N(CH_3)-	CH_2	phenyl	0.38 (0.14)	14
38	CH_3-CH_2-CH_2-N(CH_3)-	CH_2	phenyl	0.29 (0.05)	14
39	CH_3-N(CH_3)-	CH_2	phenyl	11.7 (3.5)	14
40	CH_3-NH-	CH_2	phenyl	418 (17)	
41	1-pyrrolidinyl	CH_2	phenyl	0.76 (0.16)	14
42	1-piperidinyl	CH_2	phenyl	0.48 (0.01)	7
45	1-pyrrolidinyl	-NH-	phenyl	83 (15)	
46	phenyl-CH_2-NH-	CH_2	cyclohexyl	0.81 (0.17)	14
47	CH_3-N(CH_3)-	CH_2	cyclohexyl	0.26 (0.03)	14
48	CH_3-NH-	CH_2	cyclohexyl	6.8 (0.9)	14
49	H_2N-	CH_2	cyclohexyl	190 (17)	14

[a] Literature reference for synthesis and overall σ-binding data.

α-Desmethyl Derivatives. The presence of the α-methyl group makes only a negligible contribution to affinity; for example, the α-desmethyl counterpart of (−)**3** (K_i = 10.8 nM) is **24** (K_i = 11.3 nM). For this reason, and to eliminate the need to examine pairs of optical isomers, a series of α-desmethyl derivatives was examined (see Table 4).

With the *x*-chain held constant at 2 (i.e., an ethyl group), increasing the length of the *y*-chain from 3 (**24**; K_i = 11.3 nM) to 4 (**27**; K_i = 2.6 nM) to 5 (**30**; K_i = 0.17 nM) methylene units results in a progressive increase in affinity. The trend seems to terminate when the chain is longer than a pentyl group; for example, when the *y*-chain is 7 methylene units (**36**; K_i = 1.5 nM), affinity is decreased relative to that of **30**. Nevertheless, compound **36** still binds at σ_1 sites with high affinity. Shortening the *x*-chain to 1 (i.e., a methylene group) and lengthening the *y*-chain from 4 (**26**; K_i = 9.6 nM) to 5 (**28**; K_i = 0.32 nM) to 7 (**35**; K_i = 2.3 nM) methylene units provides the same trend. Via lengthening the *x*-chain to 3, again the 5-membered *y*-chain derivative (**32**; K_i = 0.28 nM) binds with higher affinity than its 3-membered chain counterpart (**25**; K_i = 11.4 nM). With an *x*-chain of 4, **34** binds with half the affinity of **32**.

Unlike what was observed above with (+)**3** (Table 2), N-monomethylation of **28**, **30**, and **32** (i.e., **29**, **31**, **33**, respectively) has no substantive effect on affinity. In contrast, N-monomethylation of pentylamine **40** (K_i = 418 nM; Table 5) to **39** (K_i = 11.7 nM) enhances affinity by about 30-fold. At this time, we can not satisfactorily explain the variable effect of N-methylation on σ_1 binding.

Desphenyl Derivatives. Evidently, and consistent with what we have previously reported,[4] there is something unique about the 5-membered *y*-chain derivatives. Viewing these results from another perspective where the *y*-chain is held constant at 5 methylene units and the *x*-chain is varied from 1 (**28**; K_i = 0.32 nM) to 2 (**30**; K_i = 0.17 nM) to 3 (**32**; K_i = 0.28 nM) to 4 (**34**; K_i = 0.48 nM) methylene units, it is evident that the length of the *x*-chain does not have much of an effect on binding. Because the *x*-chain serves as a spacer between the amine and the phenyl-A ring, the distance between these two features does not seem important and raises the question of whether the phenyl-A ring is necessary for binding. A comparison of **33** (K_i = 0.38 nM) with its desphenyl-A (i.e., *N*-*n*-propyl) counterpart **38** (K_i = 0.29 nM) reveals that the phenyl-A ring is not required for binding (see Table 5). However,

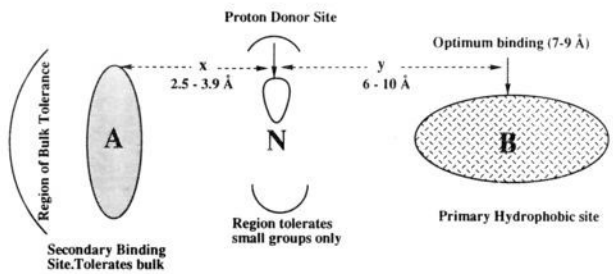


Figure 1. Receptor features presumed to be important for the binding of ligands at σ_1 receptors.

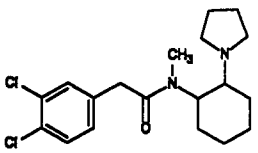
shortening the residual *n*-propyl substituent to methyl (**39**; K_i = 11.7 nM) reduces affinity by 40-fold. It would seem that the *x*-chain functions not as a simple spacer but that it interacts in a productive manner with a binding feature of the receptor (see Figure 1). This (presumably hydrophobic) interaction is also evident with the shortest possible *x*-chain derivative ..... the *N*-benzyl derivative **28** (K_i = 0.32 nM), where the phenyl-A group seemingly interacts with the same binding feature utilized by a portion of the *n*-propyl chain of **38** (see site A, Figure 1). An adjacent region of bulk tolerance allows for extension of the *x*-chain beyond the length of an *n*-propyl group, as with **34** (K_i = 0.48), with little to no effect on affinity. The presence of a region of bulk tolerance is also consistent with the finding that benz-fusion of the phenyl-A ring to naphthyl (e.g., **19**, **20**) has little impact on binding. In contrast, the desphenyl-B analogue **37** (K_i = 48.2 nM) binds with 280-fold lower affinity than its parent **30** (K_i = 0.17 nM), indicating the importance of the phenyl-B ring to binding. Thus, the alkyl chain length and/or the presence of a phenyl group (or reduced phenyl group; see below) may be determinants for specific modes of receptor interaction. Figure 1 is a schematic representation of some features that appear to be important for σ_1 binding. Site A must be located at a distance from the amine site such that it optimally accommodates an *n*-propyl group (3.9 Å). Comparing the progressively increasing affinities of **24**, **27**, **30**, and **36** (all of which possess a common 2-phenylethyl substituent and bind with K_i values of <12 nM), optimal affinity is associated with a 5-phenylpentyl group (calculated aromatic centroid to amine distance = 8.3 Å). Other features of this binding site require further investigation.

Cyclization of the terminal amine substituents of **38** (K_i = 0.29 nM) to a pyrrolidine (**41**; K_i = 0.76 nM) or piperidine

ring (**42**; K_i = 0.48 nM) has relatively little effect on binding. The distance between the pyrrolidine nitrogen atom and the furthest ring carbon in **41** (2.4 Å) probably sets the lower limit for the site A to amine distance shown in Figure 1.

It might be noted that as soon as the binding model in Figure 1 had been developed, Gilligan et al.[12] proposed a rather similar model to account for the binding of a series of N,C4-disubstituted piperidine derivatives at [^{3}H](+)-NANM-labeled σ (i.e., σ_1) sites. Their idealized σ ligand possesses two hydrophobic groups: one (the "proximal" group) lies about 3 ± 1 Å away from a basic amine, and a second (the "distal" group) is located 6 ± 2 Å away. These binding features correspond rather nicely with sites A and B, respectively, of the model shown in Figure 1, even though different chemical classes of compounds were used in the two investigations. Their ligand model differs from our binding model in that it proposes a hydrogen-bonding group 3 Å from the amine in the direction of the distal hydrophobic group. Because the ligand model reflects structural components that "contribute to optimal binding affinity *and* oral activity" in two in vivo functional assays, the necessity for this hydrogen-bonding moiety specifically for receptor binding is unknown. Our model differs from the ligand model in that it proposes a region of bulk tolerance. Our model also suffers from the same limitations as the ligand model in that both investigations utilized compounds with substantial conformational flexibility; thus, the three-dimensional relationship of the various binding sites can not be identified with any certainty.

The pyrrolidine derivative **41** is structurally similar to a fairly new class of σ ligands recently described by de Costa et al.[2] This latter class of agents was developed by the systematic structural modification of the κ-opiate agonist U50,488 (**43**) and includes compounds **44a** (R = H) and **44b** (R = 3,4-diCl) (K_i = 7.4 and 0.34 nM, respectively, at [^{3}H](+)3-PPP-labeled σ sites).[2] Interest-



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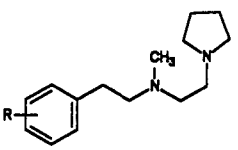

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ingly, these agents possess a 5-atom chain separating a phenyl group from a terminal amine, the one major difference between the present compounds and the U50,488 derivatives being that the latter possess a nitrogen atom in place of one of the alkyl-chain methylene groups. In order to determine the influence of a chain nitrogen atom in our series, compound **41** was compared with **45** (K_i = 83 nM) where the central methylene of **41** was replaced by an amino nitrogen. Although compound **45** does not possess those structural features optimal for σ binding in the U50,488 class of compounds in that N-demethylation of **44** reportedly reduces affinity by 3-fold,[2] it does allow for direct comparison between the two series. As shown in Table 5, introduction of this nitrogen atom into the chain decreases σ_1 affinity by 100-fold. If site B (Figure 1) represents a hydrophobic region, N-methylation and the presence of the dichloro substituents could account for the higher affinity of **44b** relative to **45**. Thus, it is likely that the U50,488-type compounds represent structural variants of the phenylpentylamine pharmacophore.

Cyclohexyl Derivatives. *N*-Benzyl-5-phenylpentylamine (**28**; K_i = 0.32 nM) binds with high affinity. Reduction of the phenyl-B ring to a cyclohexyl group (**46**; K_i = 0.81 nM) has little effect on binding affinity (Table 5). However, whereas **28** binds with more than 40 times the affinity of the corresponding *N,N*-dimethyl derivative **39**, the *N*-benzyl cyclohexyl analogue **46** binds with about half the affinity of its *N,N*-dimethyl derivative **47** (K_i = 0.26 nM). It should be noted, however, that in both cases (i.e., **39** → **40** and **47** → **48**), N-monodemethylation reduces affinity by 30-fold. Furthermore, although **46** and **28** bind with similar affinity, the cyclohexyl derivatives **47** and **48** bind with 50–60 times the affinity of their aromatic counterparts (i.e., **39** and **40**, respectively). This lack of parallelism of substituent effects raises questions about the mode of binding of the aromatic versus cyclohexyl derivatives.

Structure–Affinity Summary. There is a curious similarity between structure–affinity relationships for σ_1 binding (this investigation) and overall σ binding using [^{3}H]DTG as radioligand.[3-7] The results, although not necessarily quantitatively identical, are, nevertheless, qualitatively comparable. Specifically: (a) phenylethylamine derivatives typically bind with high affinity when the terminal amine bears an ω-phenylalkyl substituent of 2–5 methylene groups; (b) stereochemistry about the carbon atom adjacent to the amine appears to play a minimal role when the phenethylamines possess an α-methyl group, and larger substituents have a greater effect on enantioselectivity; (c) removal of this α-methyl group has little to no effect on binding; (d) aromatic substitution in the phenyl-A ring does not have a significant effect on binding; (e) with the phenyl-A alkyl chain (i.e., *x*-chain) held constant, optimal affinity is associated with a *y*-chain (separating the amine from phenyl-B) of 5 carbons in length; (f) N-monomethylation has a variable effect on affinity; and (g) with 5-phenylpentylamines, the length of the phenyl-A to amine-alkyl spacer does not seem to be all that important, but simple *N*-methyl and *N,N*-dimethyl amine derivatives do not bind with high affinity. Additional findings from the present investigation are that quaternary amines bind with low affinity and that cyclic amine derivatives such as the pyrrolidinyl and piperidinyl derivatives of the 5-phenylpentylamines bind with subnanomolar affinity. Cyclohexyl derivatives also bind with high affinity, but additional compounds will need to be examined in order to formulate more complete structure–affinity relationships. A binding model (Figure 1) was proposed to account for many of these findings.

While these binding studies were in progress, de Costa et al.[2] described some of their work on derivatives of the κ-opiate agonist U50,488. With appropriate structural modification, novel derivatives were identified that lack affinity for κ-opiate sites but possess high affinity for σ sites. Their binding studies employed (+)[^{3}H]3-PPP (i.e., 3-phenyl-*N*-*n*-propylpiperidine) as radioligand. Like [^{3}H]-DTG, (+)[^{3}H]3-PPP binds both at σ_1 and σ_2 sites. Under our assay conditions, the two compounds [DTG and (+)3-PPP] bind at σ_1 sites with comparable affinity; however, (+)3-PPP displays a 10-fold selectivity for σ_1 versus σ_2 sites [(+)3-PPP: σ_1 K_i = 48 nM; σ_2 K_i = 470 nM].[9] Thus, it is likely that the data reported by de Costa et al.[2] reflect primarily σ_1 properties. In fact, their structure–affinity

results are not vastly different from our findings, and it would appear that U50,488 derivatives and phenylalkylamines belong to the same general class of σ ligands.

We have previously reported that N-substituted phenylalkylamines constitute the primary pharmacophore of benzomorphans and certain other agents that bind at σ receptors.[3,5] It seems likely that these phenylalkylamines also constitute pharmacophores for σ_1 binding. Structural features important for σ_1 binding have been identified and are summarized in Figure 1. Many of the present compounds can be termed superpotent σ_1 ligands in that they bind with K_i values of <1 nM; indeed, **29–31**, for example, bind at σ_1 sites with affinities of nearly 1000 times that of (+)SKF 10,047 (K_i = 150 nM)[9] and comparable to that of (+)-pentazocine (K_i = 1.7 nM),[9] and they represent the highest affinity σ_1 ligands reported to date.

Experimental Section

Synthesis. Proton magnetic resonance spectra were obtained with a JEOL FX90Q spectrometer with TMS as an internal standard. Spectral data are consistent with the assigned structures. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab and are within 0.4% of theory. Each of the experiments below illustrates one of the methods mentioned in Table 1 (see Table 1 for details).

***(R)*-(−)-*N*-Methyl-*N*-(2-phenylethyl)-1-phenyl-2-aminopropane Hydrogen Oxalate [(−)5] (Method A).** A mixture of (*R*)-(−)-amphetamine (394 mg, 2.9 mmol), phenylacetaldehyde (350 mg, 2.9 mmol), and 10% Pd/C (160 mg) in 95% EtOH (25 mL) was hydrogenated on a Paar hydrogenator at ambient temperature for 2 h. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was redissolved in anhydrous Et_2O (30 mL), and a saturated solution of oxalic acid (200 mg) in Et_2O (30 mL) was added to obtain the salt.[3] The free base of the product (239 mg, 0.1 mmol) was treated with 37% HCHO (2 mL) and 98% HCOOH (3 mL) and heated at reflux for 18 h. After addition of concentrated HCl (1 mL), the volatile substances were removed under reduced pressure and H_2O (50 mL) was added to the residue. The solution was made alkaline by the addition of 30% NaOH, and the mixture was extracted with Et_2O (3 × 20 mL). The ethereal portions were pooled, washed with H_2O (10 mL), and dried ($MgSO_4$). The hydrogen oxalate salt was prepared and recrystallized from absolute EtOH (274 mg, 80%); mp 185–187 °C; $[\alpha]^{23}$ (2%, MeOH) −16°.

***(S)*-(+)-*N*-Methyl-*N*-(1-methyl-2-phenylethyl)-3-phenyl-1-aminopropane Hydrogen Maleate [(+)6] (Method B).** A solution of hydrocinnamoyl chloride (0.85 g, 5 mmol) in CH_2Cl_2 (10 mL) was added in a dropwise manner to a stirred ice-cold mixture of (+)-methamphetamine hydrochloride (0.95 g, 5 mmol) and triethylamine (1.0 g, 10 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was allowed to stir overnight at room temperature and then washed with H_2O (20 mL). The organic portion was dried ($MgSO_4$), and the solvent was removed under reduced pressure to afford the amide as an oil (1.2 g, 85%). Without further purification, a solution of the amide (0.7 g, 2.5 mmol) in anhydrous Et_2O (10 mL) was added in a dropwise manner to a suspension of $LiAlH_4$ (0.4 g, 10.5 mmol) in anhydrous Et_2O (40 mL) under a stream of N_2. The reaction mixture was allowed to stir under reflux for 3 h and cooled to 0 °C, and excess $LiAlH_4$ was destroyed by the addition of H_2O (2 mL) and 10% NaOH (2 mL). Solids were removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in Et_2O and dried ($MgSO_4$), and an ethereal solution of maleic acid was added. The salt was recrystallized from EtOAc/anhydrous Et_2O to give the desired product (0.55 g, 58%); mp 87–88 °C; $[\alpha]^{23}$ (2%, MeOH) +13.5°.

***(S)*-(+)-*N*-Benzyl-*N*-(1-methyl-2-phenylethyl)-3-phenyl-1-aminopropane [(+)7] (Method C).** A solution of (*S*)-(+)-amphetamine (1.35 g, 10 mmol) and hydrocinnamaldehyde (1.4 g, 10.5 mmol) in absolute EtOH (50 mL) was hydrogenated in a Paar bottle containing 10% Pd/C (0.65 g) until sufficient H_2 was consumed. The catalyst was removed by filtration; the filtrate was concentrated to about 10 mL under reduced pressure, and HCl was added to pH 1–2. The mixture was evaporated to dryness under reduced pressure, and the solid residue was recrystallized from 2-PrOH/Et_2O (1.5 g, 52%); mp 217 °C. A stirred mixture of the free base (0.75 g, 3.0 mmol), benzyl bromide (0.5 g, 2.9 mmol), and anhydrous K_2CO_3 (0.4 g, 2.9 mmol) in MeCN (30 mL) was heated under reflux for 5 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was partitioned between 10% HCl (15 mL) and Et_2O (20 mL), and the free base was generated by addition of 10% NaOH solution. The mixture was extracted with EtOAc (2 × 20 mL). The organic portion was washed with H_2O (10 mL) and dried ($MgSO_4$), and the solvent was removed under reduced pressure to obtain an oil that distilled at 82–85 °C (0.05 mmHg) to give the desired product (0.5 g, 49%); $[\alpha]^{23}$ (2%, MeOH) +28.8°.

***(R)*-(−)-*N*-Methyl-*N*-(1-methyl-2-phenylethyl)-3-phenyl-1-aminopropane Methiodide [(−)8] (Method D).** Methyl iodide (0.2 mL) was added to (*R*)-(−)-*N*-(1-methyl-2-phenylethyl)-3-phenyl-1-aminopropane[3] (0.2 g, 0.80 mmol) in a small reaction vial, and the mixture was allowed to stir at room temperature for 20 h. The reaction mixture was treated with chloroform (4 mL), H_2O (2 mL), and 12% NaOH solution (8 drops). The chloroform layer was separated, and the aqueous portion was extracted with additional chloroform (3 × 4 mL). The combined chloroform extracts were dried ($MgSO_4$), and the solvent was removed to give a semisolid product that solidified upon stirring with anhydrous Et_2O (4 mL). Recrystallization from MeOH/EtOAc gave 0.11 g (34%) of the desired product as colorless crystals; mp 154–156 °C.

***(R)*-(−)-*N*-(1-Methyl-2-phenylethyl)-5-phenyl-1-aminopentane Hydrogen Oxalate [(−)18] (Method E).** A solution of ethyl chloroformate (0.34 g, 3.15 mmol) in CH_2Cl_2 was added in a dropwise manner to a stirred ice-cooled solution of 5-phenylvaleric acid (0.51 g, 2.87 mmol) and Et_3N (0.29 g, 2.87 mmol) in dry CH_2Cl_2 (10 mL) over a 10-min period. Stirring was continued for 30 min, and (*R*)-(−)-amphetamine (0.39 g, 2.87 mmol) in CH_2Cl_2 (10 mL) was added in a dropwise manner. The reaction mixture was allowed to stir for an additional 3 h, after which the solution was washed with water (30 mL) and dried ($MgSO_4$). Solvent was removed under reduced pressure to obtain 0.77 g of a solid product; mp 48–50 °C (95% EtOH). A solution of the amide (0.31 g, 1.05 mmol) in anhydrous THF (10 mL) was added in a dropwise manner to a suspension of $LiAlH_4$ (0.42 g, 10.6 mmol) in anhydrous THF (20 mL). The reaction mixture was heated under reflux in a stream of N_2 for 7 h and allowed to cool, and the flask was then immersed in an ice bath. Excess $LiAlH_4$ was decomposed by the gentle addition of H_2O (2 mL) and 10% NaOH (2 mL). The solid material was removed by filtration, and the solvent was evaporated under reduced pressure to obtain an oil. The oxalate salt was prepared and recrystallized from EtOH/H_2O to give white crystals (0.25 g, 65%); mp 141–143 °C; $[\alpha]^{23}$ (1%, EtOH) −7.2°.

***N*-Methyl-*N*-(2-phenylethyl)-5-phenyl-1-aminopentane Hydrogen Oxalate (31) (Method F).** A mixture of 5-phenylpentanol tosylate (159 mg, 0.5 mmol), *N*-methyl-2-phenylethylamine (67 mg, 0.5 mmol), K_2CO_3 (69 mg), and dioxane (10 mL) was heated at reflux for 3 h. The solvent was removed under reduced pressure, and the residue was partitioned between Et_2O and 10% NaOH solution. The organic portion was washed with H_2O and dried ($MgSO_4$). The oxalic acid salt was prepared and recrystallized from absolute EtOH/anhydrous Et_2O to afford the target compound (125 mg, 67%); mp 140–142 °C.

Binding. The σ_1-radioligand-binding assay was conducted as previously reported.[9] In brief, the σ_1-selective binding assay was performed using (+)-[^{3}H]pentazocine as the radioligand (3–4 nM final concentration) and approximately 100 μg of guinea pig brain membranes (prepared as previously described[3]) in a final volume of 0.5 mL of 50 mM Tris-HCl buffer (pH 8.0). For the standard equilibrium assay, the mixtures were incubated for 4 h at 37 °C, the reactions quenched with 4 mL of ice-cold incubation buffer, and the mixtures rapidly filtered over Whatman GF/B or Schleicher & Scheull no. 32 glass fiber filters followed by three 4-mL rinses with additional ice-cold buffer. The radioactivity

on the filters was determined by scintillation spectrometry at an efficiency of about 50%. Nonspecific binding was determined in the presence of 10 μM haloperidol. IC_{50} values were determined from competition curves using nonlinear least-squares regression analysis and converted to K_i values with the Cheng–Prusoff equation. Each K_i value was determined from three to five separate determinations.

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