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Is a Nitrogen Atom an Important Pharmacophoric Element in Sigma Ligand Binding?

Seth Y. Ablordeppey,[a,*] James B. Fischer[b] and Richard A. Glennon[c]

[a]*College of Pharmacy & Pharmaceutical Sciences, Florida A&M University, Tallahassee, FL 32307, USA*
[b]*Cambridge NeuroScience Inc., Cambridge, MA 02139, USA*
[c]*Dept. of Medicinal Chemistry, School of Pharmacy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298, USA*

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Abstract—A lingering question in σ receptor ligand development is whether a nitrogen atom serves as an important pharmacophoric element in binding affinity. To address this question, we have synthesized several phenylalkylpiperidines and phenylalkylpiperazines and demonstrated that removal of the N atom from a typical phenylalkylpiperidine led to little or no binding to σ receptors. In addition, where two N atoms occur in a compound, such as with phenylalkylpiperazines, the N atom on the longer alkyl chain appears to be more important. Thus, based on this study, the N atom is an important pharmacophoric element in the binding of phenylalkylpiperidines and phenylalkylpiperazines to σ receptors.

Although the initial excitement about sigma ligands has subsided somewhat, they continue to attract attention because of the involvement of sigma receptors in several physiological functions.[1–8] For example, several recent publications support the hypothesis that sigma ligands may serve as atypical antipsychotic agents with minimal extrapyramidal action.[9] In addition, sigma receptors appear to modulate K^+ channels,[10] and may serve as targets for drug design against certain tumor cells.[11] Unlike most neurotransmitter receptors, however, sigma receptors seemingly accommodate a very wide variety of structural classes.[1,2] This situation has made it difficult to obtain a unifying common pharmacophore model for each of the known receptor subtypes. Although several sigma receptor subtypes might remain to be identified, the presence of a nitrogen atom appears to be a common pharmacophoric element for most sigma ligands. Unfortunately, the role of the nitrogen atom as a required pharmacophoric element is clouded by the finding that steroid molecules lacking a basic nitrogen atom bind to sigma receptors with moderate affinity, and may effect several physiological actions through sigma receptors.[12–17]

In our work,[18–25] on developing structure–affinity relationship models for sigma receptor ligands, we have used the N atom as a primary pharmacophore element for alignment purposes.[18,19] On a number of such occasions, however, we were faced with the decision of selecting one nitrogen atom, among several, as the primary pharmacophoric element. We have previously shown that modifications around the nitrogen atom in phenylalkylamines and phenylpiperidines often led to changes in ligand binding affinity.[20,24,25] Therefore, we have sought a better understanding of the role and contributions of the nitrogen atom in sigma ligand binding because it might lead to the design and synthesis of new and more selective agents for sigma receptors. Thus, the primary purpose of this study was to evaluate the contributions of the N atom to sigma receptor binding using phenylpiperazines, phenylpiperidines and phenylalkylamines as the scaffold. It was also of interest to determine if the resulting modifications lead to more potent and selective σ ligands; consequently, both σ-1 and σ-2 receptor binding data were obtained on most of the compounds.

Chemistry

Standard synthetic methods were used to obtain most of the compounds examined in this investigation. Compound **1** was available commercially from Aldrich Chemicals. Both **3** and **4** were obtained by direct *N*-alkylation of **1** and **2**, respectively. Using the appropriate phenylalkyl

*Corresponding author. Tel.: +1-850-599-3834; fax: 1-850-599-3934; e-mail: sablordeppey@famu.edu

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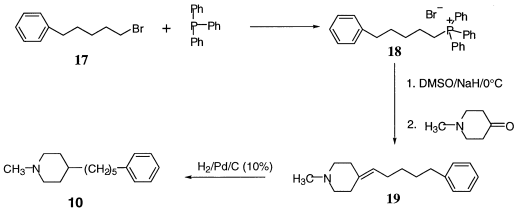
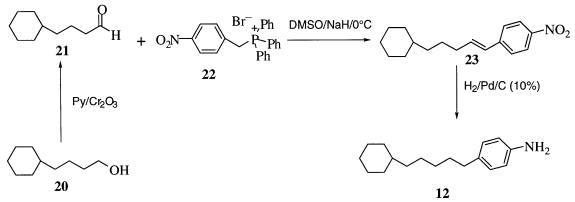
bromides generated from commercially available phenylalkanols, compounds **5**–**9** were similarly synthesized. The preparation of compound **10** (Scheme 1) required the use of the Wittig reaction. Thus, triphenylphosphine was alkylated with 5-phenylpentyl bromide[24] to obtain the phosphonium bromide **18**, which was subsequently reacted with sulfinyl carbanion generated from DMSO and sodium hydride. The resulting carbanion was reacted with *N*-methyl-4-piperidinone to form the corresponding olefin, **19**, which was catalytically reduced using palladium/carbon to yield the desired compound. Compound **11** was resynthesized using a published procedure.[24] To synthesize compound **12** (Scheme 2), the key intermediate, 4-cyclohexylbutanal (**21**), was obtained by oxidation of 4-cyclohexylbutanol (**20**) under Collins' conditions. The aldehyde was added to a mixture of 4-nitrobenzyltriphenylphosphonium bromide (**22**) and methyl sulfinyl carbanion, generated from dimethylsulfoxide and sodium hydride, to yield the required nitrophenyl olefin **23**. Hydrogenation under Parr conditions and in the presence of palladium and carbon yielded the desired compound, **12**.

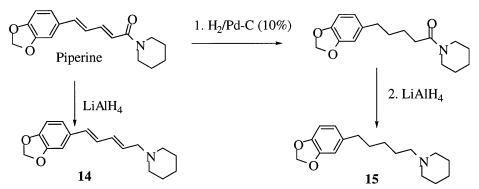
Compound **13** was obtained by nitration of **11** to form N-[5-(4-nitrophenyl)pentyl]piperidine and by the subsequent hydrogenation using 10% palladium on carbon. Compound **14** was obtained by the direct reduction of the commercially available natural product, piperine, while the synthesis of **15** (Scheme 3) was accomplished by hydrogenation of piperine, followed by lithium aluminum hydride reduction of the resulting amide. Finally, compound **16** was obtained by direct methylation of **7**.



Scheme 1. Synthesis of compound **10**.



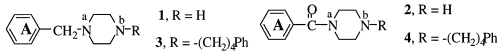
Scheme 2. Synthesis of compound **12**.



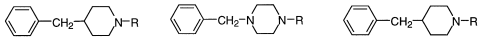
Scheme 3. Synthesis of compound **15**.

Results and Discussion

Radioligand binding data are shown in Table 1. 1-Benzylpiperazine (**1**) and 1-benzoylpiperazine (**2**) do not bind to σ receptors (i.e., $K_i > 10{,}000$ nM).[22] Alkylation of the terminal N atom with a Ph-$(CH_2)_4$-X (where X = halide) in each of these compounds, results in a dramatic increase in the σ-1 binding affinity of the resulting products. However, while there is at least a 50,000-fold increase from **1** to **3** ($K_i = 0.2$ nM), there is a much smaller increase from **2** to **4** ($K_i = 125$ nM).[22]



At least two possible hypotheses can be expounded for the significant difference in the binding affinities. A basic nitrogen atom is required at N(a) and the introduction of an amide N at that position therefore results in a lack of binding affinity. By implication, N(a) must be very important for binding to σ-1 receptors. A second hypothesis for the difference in binding affinity can be attributed to the change in the position of the phenyl-A ring brought about by the carbonyl group or by intolerance of the carbonyl oxygen itself. To further explore these issues, we synthesized compound **5** in which N(a) is replaced by CH in order to evaluate the importance of N(a) to binding. Compound **5** binds with a K_i of 0.8 nM; only a 4-fold difference with **3**. Inserting an additional methylene group in phenylalkyl derivatives **3** and **5** yielded similar results (i.e., **6** $K_i = 0.4$ nM, and **7** $K_i = 0.6$ nM, respectively). These results suggest that N(a) is not essential for high affinity binding to σ-1 receptors.



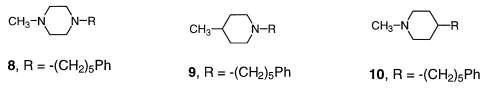
Interestingly, previous work in our laboratories has demonstrated that the phenyl-A ring plays essentially no role in the binding of phenylalkylamines or phenylpiperidines at sigma receptors. Thus, removal of the

Table 1. Sigma receptor ligand binding data

Compound	Binding constants, K_i (nM)		
	σ-1	σ-2	Ratio of σ-2/σ-1
1[a]	> 10,000	NT[d]	
2[a]	> 10,000	NT	
3	0.2 (0.1)[c]	NT	
4[a]	125	NT	
5	0.8 (0.2)	3.1 (0.6)	4
6	0.4 (0.2)	NT	
7	0.6 (0.2)	2.8 (0.8)	5
8	1.4 (0.4)	79 (13)	64
9	0.07 (0.02)	7 (2)	100
10	1.3 (0.3)	97 (18)	75
11[b]	0.48	50 (12)	125
12	> 36,000	54,500	
13	38 (8)	830 (132)	22
14	0.86 (0.24)	554 (180)	644
15	0.32 (0.10)	63 (12)	197
16[b]	2.7 (0.9)	NT	

[a]Previously reported as non-selective sigma receptor ligand; see ref 22.
[b]Previously reported in ref 20.
[c]SEM in parenthesis.
[d]NT = was not determined.

phenyl A ring from **6** and **7** led to compounds **8** (K_i = 1.4 nM) and **9** (K_i = 0.07 nM) with affinities consistent with our previous findings.



So far, the replacement of N(a) with CH in the piperazines series appears to have no serious bearing on the binding of these compounds to σ-1 receptors. The question then arises as to what role N(b) plays in the binding of the piperazines. We reasoned that if N(a) does not contribute significantly to binding at σ-1 receptors, and if a nitrogen atom is required, N(b) must be the essential N atom required for binding affinity. We therefore synthesized compound **10** in an attempt to answer this question. Compound **10** (K_i = 1.3 nM) binds as well as **8** although its binding affinity is 20-fold lower than that of **9**. We can deduce from these results that compounds in which N(a) is present bind to σ-1 receptors in essentially the same way notwithstanding the presence of other nitrogen atoms such as N(b). Furthermore, although N(b) provides a more effective binding to sigma receptors, the presence of other N atoms leads to additional interactions which somehow detracts from maximum binding. This observation is consistent with our previous suggestion that phenylalkylamines, phenylalkylpiperidines and phenylalkylpiperazines may utilize reverse modes of binding to σ-1 receptors[26] (see Fig. 1 below).

Compound **9** displays the highest affinity of the compounds examined. It is important, then, to examine the role the N atom plays in the binding of such compounds. We have previously reported that N-ω-phenylpentylpiperidine (**11**), a demethylated analogue of **9,** binds with high affinity at σ-1 receptors (K_i = 0.48 nM). In order to evaluate the necessity of the nitrogen atom for sigma binding, we wished to replace it with a methine group. However, this would likely result in a water-insoluble compound. To overcome this problem, we needed to attach a water-solubilizing group elsewhere in the molecule. We selected

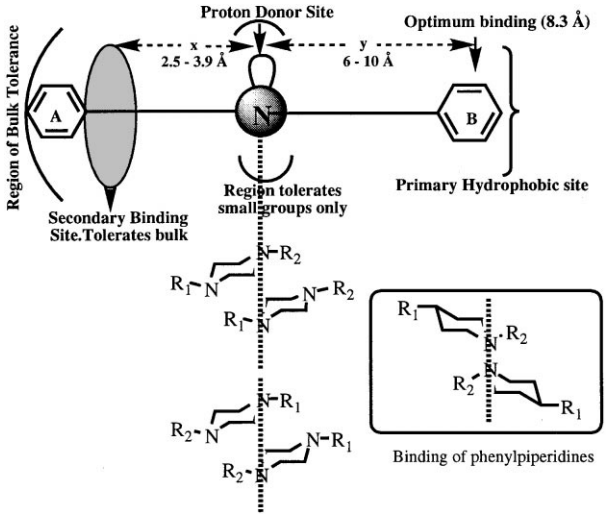
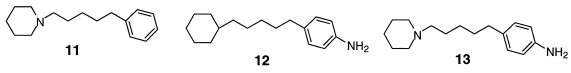
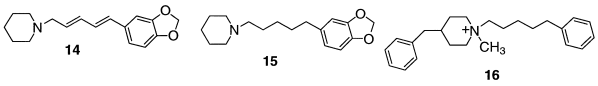


Figure 1. Proposed receptor features important for σ-1 binding[18,20] and the modes of binding of phenylpiperazines (4 modes) and phenylpiperidines (2 modes).

the *para* position of the phenyl ring based on previous results indicating that substitution at this position might be tolerated and affinity may not be significantly affected at σ-1 receptors.[25] We explored aromatic substitution to further evaluate this concept and found that aromatic substitution is tolerated and can, depending upon the substituents incorporated (e.g. **14** and **15**), actually enhance affinity and/or selectivity; this will be further discussed below. One of the compounds prepared and examined was the amino-substituted derivative **12** (K_i > 36,000 nM); compound **13** (K_i = 38 nM) was used as the positive control. Since compound **13** binds with moderate affinity, it can be concluded that replacement of the piperidine nitrogen atom with CH reduces affinity by at least a 1000-fold and, hence, a nitrogen atom is an essential pharmacophoric element in the binding of these and related ligands to σ-1 receptors. An alternative, but less satisfying, explanation is that compound **12** binds in an entirely different manner to result in low affinity.



In the course of exploring aromatic substitution, as described above, we prepared several aryl substituted compounds including **14** and**15**. The purpose of these studies was to determine the influence of aryl substituents on σ-1 binding and subsequently to determine if such substitutions detract from binding. Compounds **14** (K_i = 0.86 nM) and **15** (K_i = 0.32 nM) were both found to bind with high affinity. Comparing compounds **11** (K_i = 0.48 nM) and **15** (K_i = 0.32 nM) also supports the suggestion that aromatic substitution has little influence on σ-1 binding. Interestingly, compound **14** displayed the highest σ-2/σ-1 selectivity among the ligands evaluated in this study (**14:** σ-1 K_i = 0.86 nM; σ-2 K_i = 554 nM, for a ratio of 664).



Having established the importance of the N(b) atom for maximum binding, it was also of interest to examine the nature of the nitrogen atom as it binds the sigma receptors. In this regard, we wanted to know if a lone pair of electrons on the nitrogen was needed for binding or if protonation of the nitrogen occurs on binding. A very simple way to do this is to introduce a permanent positive charge on the nitrogen such as occurs in quaternization. We therefore prepared and examined the *N*-methylated analogue of compound **7** (i.e. compound **16**). Compound **16** binds with a K_i of 2.7 nM; implying that the lone pair of electrons on these compounds probably becomes protonated at σ-1 receptors and that a protonated N atom may be required for σ-1 receptor binding.

The binding affinities of several of the present compounds were also examined at σ-2 receptors (Table 1). All of the compounds investigated displayed (4- to > 600-fold) lower affinity at σ-2 than at σ-1 receptors. Comparing compounds **12** and **13**, it would appear that N(b) might also be an important feature for σ-2 binding. It also seems that

aromatic substitution (e.g., compare **11** with **13**–**15**) is not as well tolerated by σ-2 receptors as it is by σ-1 receptors.

Conclusion

In this study, it has been demonstrated that at least one N atom in phenylpiperidines and phenylpiperazines is important for binding at sigma receptors. In the case of the piperazines, the N atom attached to the longer chain, N(b), is more important than N(a). Furthermore, several modes of binding are available to each molecule at the binding site and therefore reversed modes of binding are possible as well. Thus, we have now extended our previously reported sigma ligand model to include the various modes of binding for phenylpiperidines and phenylpiperazines at the σ-1 receptors (Fig. 1). If the N atom is important in σ binding, how can we explain the binding of non-basic steroids to sigma receptors? At this point, it can only be speculated that steroids probably bind in a different manner at the σ receptors from the phenylalkylamines, such as the ones discussed in this manuscript.

Experimental

Syntheses

Proton and carbon magnetic resonance spectra were obtained on QE 300 (300 MHz/75 MHz) spectrometers with tetramethylsilane as internal standard. The unit of all *J* values shown in the experimentals is Hz, and all spectra are consistent with the assigned structures. Melting points were determined on a Thomas Hoover apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab and determined values are within 0.4% of calculated values. No attempt was made to optimize yields in any of the synthesis presented.

1-Benzyl-4-(5-phenylpentyl)piperazine (6) hydrochloride (method A). A stirred mixture of 1-benzylpiperazine (0.4 g, 2.3 mmol), 5-phenylpentyl bromide (0.5 g, 2.2 mmol), K_2CO_3 (0.6 g, 4.3 mmol), and KI (50 mg) in 1,2-dimethoxyethane (DME) (8 mL) was heated under reflux overnight (20 h) and allowed to cool to room temperature. The mixture was partitioned between Et_2O (30 mL) and aqueous ammonia solution (15 mL). The aqueous portion was reextracted with Et_2O (30 mL) and the combined organic portions were pooled, washed with H_2O (20 mL) and dried ($MgSO_4$). Solvent was removed under reduced pressure and the residue chromatographed over silica gel using 50% EtOAc/hexane to yield a yellowish oil, which was converted to the hydrochloride salt using ethereal HCl (156 mg, 18%): mp 253 °C (Dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.66 (2H, dd, *J* = 4, 3), 7.47 (3H, m), 7.27 (2H, d, *J* = 8), 7.18 (2H, dd, *J* = 8, 6), 7.14 (1H, d, *J* = 6 Hz), 4.34 (2H, s), 3.92 (4H, d, *J* = 9 Hz), 3.57 (2H, d, *J* = 9 Hz), 3.48 (2H, d, *J* = 9 Hz), 3.10 (2H, t, *J* = 8 Hz), 2.62 (2H, t, *J* = 6 Hz), 1.85 (2H, m), 1.66 (2H, m), 1.48 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.48, 26.00, 30.54, 35.42, 47.84, 57.03, 60.64, 125.96, 128.38, 129.60, 130.65, 131.30, 141.61. Anal. $C_{22}H_{32}N_2Cl_2 \cdot 0.2H_2O$, C, H, N.

4-Benzyl-1-(5-phenylpentyl)piperidine hydrochloride (7). A stirred mixture of 4-benzylpiperidine (1.0 g, 5.7 mmol), 5-phenylpentyl bromide (1.95 g, 8.6 mmol), K_2CO_3 (1.57 g, 11.4 mmol), and KI (100 mg) in 1,2-dimethoxyethane (DME) (10 mL) was heated under reflux over the weekend (60 h) and allowed to cool to room temperature. The mixture was partitioned between Et_2O (30 mL) and aqueous ammonia solution (15 mL). The aqueous portion was re-extracted with Et_2O (30 mL) and the combined organic portions were pooled, washed with H_2O (20 mL) and dried ($MgSO_4$). Solvent was removed under reduced pressure and the residue (335 mg, 19%) was converted to the hydrochloride salt using ethereal HCl: mp 163–165 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.42–7.10 (10 H, m), 3.76 (2H, t), 3.50 (2H, d, br), 3.28, (2H, m), 2.62 (4H, m), 2.20, (1H, br), 1.88–1.32, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.43, 26.36, 28.84 (2C), 30.65, 35.54, 36.62, 41.95, 52.92 (2C), 57.52, 125.85, 126.36, 128.36 (4C), 128.49, (2C), 128.98 (2C), 139.12, 141.80. Anal. $C_{23}H_{32}NCl$, C, H, N.

1-Methyl-4-(5-phenylpentyl)piperazine dihydrobromide (8). A stirred mixture of 1-methylpiperazine (0.3 g, 3.0 mmol), 5-phenylpentyl bromide (0.7 g, 3.1 mmol), K_2CO_3 (0.9 g, 6.2 mmol) and KI (100 mg) in 1,2-dimethoxyethane (DME) (8 mL) was heated under reflux for 5 h and allowed to cool to room temperature. The mixture was partitioned between Et_2O (30 mL) and 10% NaOH solution (15 mL). The aqueous portion was reextracted with Et_2O (30 mL) and the combined organic portions were pooled, washed with H_2O (20 mL) and dried ($MgSO_4$). A saturated hydrogen bromide solution in anhydrous Et_2O was added to obtain a salt, which was recrystallized from MeOH (0.51 g, 43%): mp 239 °C (Dec.). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.22 (2H, d, *J* = 9 Hz), 7.17 (1H, d, *J* = 7 Hz), 7.15 (2H, dd, *J* = 9, 7 Hz), 3.73 (4H, s, br), 3.39 (4H, s, br), 3.18 (2H, s, br), 2.88 (3H, s), 2.54 (2H, t, *J* = 7 Hz), 1.68 (2H, m), 1.56 (2H, m), 1.27 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.67, 30.58, 35.04, 38.96, 84.11, 125.94, 128.51, 142.17. Anal. $C_{16}H_{28}N_2Br_2$, C, H, N.

4-Methyl-1-(5-phenylpentyl)piperidine hydrogen oxalate (9). Method A was used. Product (1.2 g, 75%), mp 138–139 °C. Recrystallization solvent: MeOH/Et_2O. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.23 (2H, d, *J* = 9 Hz), 7.15 (2H, dd, *J* = 9, 7 Hz), 7.12 (1H, d, *J* = 7 Hz), 3.62 (2H, d, *J* = 15 Hz), 2.92 (2H, dd, *J* = 15, 9 Hz), 2.55 (2H, t, *J* = 7 Hz), 1.65 (11H, m), 1.29 (3H, d, *J* = 6 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.82, 23.38, 26.06, 29.14, 30.48, 30.84, 35.31, 53.09, 57.29, 125.64, 128.16, 141.63, 163.08. Anal. $C_{19}H_{29}NO_4$, C, H, N.

***N*-Methyl-4-(phenylpentyl)piperidine hydrogen oxalate (10).** A mixture of triphenylphosphine (3.8 g, 14.5 mmol) and 5-phenylpentyl bromide (3.1 g, 13.6 mmol) in dry benzene (20 mL) was allowed to reflux overnight (~24 h). After cooling to room temperature, anhydrous Et_2O was added, the solid was filtered and washed with several portions of anhydrous Et_2O and dried under reduced pressure at 50 °C overnight (6.1 g, 91%); mp 143–145 °C. A solution of methyl sulfinyl carbanion was obtained by stirring a mixture of dried DMSO (10 mL) and powdered

sodium hydride (0.2 g) under N_2 and heating at ~50 °C for 15 min. The resulting solution was cooled to 0 °C and a solution of 5-phenylpentyltriphenylphosphonium bromide (3 g, 6.1 mmol) in dry DMSO (10 mL) was added in a dropwise manner over 5 min. After 15 min of stirring at room temperature, a solution of 1-methyl-4-piperidone (0.6 g, 5.3 mmol) in dry DMSO (10 mL) was added in a dropwise manner over ~5 min. The resulting reddish yellow solution was allowed to stir at room temperature for ~20 h and then poured into H_2O (80 mL) and extracted with Et_2O (3×50 mL). The pooled Et_2O solution was washed with H_2O (2×50 mL) and dried ($MgSO_4$). Solvent was removed under reduced pressure and the residue was converted to the oxalate salt, which was recrystallized from abs EtOH (0.31 g, 17%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.19 (5H, m), 5.25 (1H, t, J = 9 Hz), 3.01 (4H, s, br), 2.65 (3H, s), 2.51(2H, t, J = 6 Hz), 2.32 (4H, m), 1.95 (2H, dt, J = 9, 9 Hz), 1.51 (2H, m), 1.27 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 25.01, 26.85, 29.21, 30.87, 32.23, 35.38, 42.28, 54.16, 54.82, 125.73, 126.03, 128.63, 130.54, 142.56, 165.20.

A solution of this product (0.25 g, 0.74 mmol) in abs EtOH (40 mL) was hydrogenated under Parr conditions (psi 50) for 3 h in the presence of Pd/C (10%) (0.1 g) catalyst. The mixture was filtered, the solvent was removed under reduced pressure and the residue was recrystallized from MeOH/anhydrous Et_2O/EtOAc (0.19 g, 77%); mp 142–144 °C. ^{1}HNMR (300 MHz, DMSO-d_6) δ 7.18 (5H, m), 3.25 (2H, d, J = 12 Hz), 2.78 (2H, t, J = 12 Hz), 2.62 (3H, s), 2.50 (2H, t, J = 9 Hz), 1.72 (2H, d, J = 15 Hz), 1.50 (2H, m), 1.23 (9H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.19, 29.12, 31.33, 32.67, 35.33, 35.52, 42.87, 53.49, 125.99, 128.63, 142.65, 165.27. Anal. $C_{19}H_{29}NO_4$, C, H, N.

1-(5-Phenylpentyl)piperidine (11). A stirred mixture of piperidine (1.1 g, 13 mmol), 5-phenylpentyl bromide[24] (3.1 g, 14 mmol) KI (20 mg) and K_2CO_3 (2 g, 15 mmol) in 1,2-dimethoxyethane (DME) (10 mL) was heated under reflux for 12 h and allowed to cool to room temperature. The mixture was partitioned between Et_2O (30 mL) and 10% NaOH solution (15 mL). The aqueous portion was reextracted with Et_2O (30 mL) and the combined organic portions were pooled, washed with H_2O (20 mL) and dried ($MgSO_4$). The oxalate salt was prepared and recrystallized from MeOH (3.1 g, 74%), mp 149–151 °C (lit.[24] 149 °C.)

5-(4-Aminophenyl)pentylcyclohexane hydrogen oxalate (12). A solution of pyridine chlorochromate (16.9 g) in CH_2Cl_2 (200 mL) was added in a portionwise manner to a stirred, ice-cold solution of 4-cyclohexyl-1-butanol (3.0 g, 19.2 mmol) in CH_2Cl_2 (50 mL). After addition was complete, the mixture was allowed to stir at room temperature for ~15 min. The mixture was washed with H_2O (2×50 mL), dried ($MgSO_4$) and solvent was removed under reduced pressure. Distillation (using Kulgerohl), under reduced pressure, afforded a colorless oil (1.1 g, 38%).

A solution of methyl sulfinyl carbanion, obtained by stirring a mixture of dried DMSO (10 mL) and powdered sodium hydride (0.25 g) under N_2 at ~50 °C for 15 min, was cooled to 0 °C and a solution of 4-nitrobenzyltriphenylphosphonium bromide (5 g, 10 mmol) in dry DMSO (10 mL) was added in a dropwise manner over 5 min. After 15 min of stirring at room temperature, a solution of 4-cyclohexyl-1-butanal (0.9 g, 6 mmol) in dry DMSO (5 mL) was added in a dropwise manner over ~5 min. The resulting deep red solution was allowed to stir at room temperature for ~6 h and then poured into H_2O (50 mL) and extracted with Et_2O (3×50 mL). The pooled Et_2O solution was washed with H_2O (30 mL) and dried ($MgSO_4$). Solvent was removed under reduced pressure and the residue was Kugelrohr distilled (1 mm, 240 °C) to afford the desired compound as a yellowish oil (1 g, 63%). A solution of this product (0.9 g) in MeOH (40 mL) was hydrogenated under Parr conditions (psi 55) for 7 h in the presence of Pd-C (10%) (0.2 g). The mixture was filtered and after the solvent was removed under reduced pressure, the residue was redissolved in Et_2O, dried ($MgSO_4$) and a saturated oxalic acid solution was added to form the salt. Recrystallization was achieved from MeOH/anhydrous Et_2O (0.6 g, 53%); mp 127–132 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40 (3H, s, br), 6.87 (2H, d, J = 6 Hz), 6.61 (2H, d, J = 6 Hz), 2.37 (2H, t, J = 6 Hz), 1.61 (6H, m), 1.43 (2H, m), 1.13 (9H, m , 0.87 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.12, 26.37, 26.48, 29.18, 31.62, 33.16, 34.66, 37.24, 116.17, 129.02, 133.15, 144.16, 162.07. Anal. $C_{19}H_{29}NO_4 \cdot 0.5H_2O$, C, H, N.

***N*-[5-(4-aminophenyl)pentyl]piperidine dihydrogen oxalate (13).** An ice-cold solution of compound **11** (0.5 g, 1.6 mmol), concd H_2SO_4 (2 mL) and glacial AcOH (3 mL) were mixed, and a mixture of concd H_2SO_4 (1 mL) and fuming HNO_3 (1 mL) was added in a dropwise manner to maintain the temperature at ~0 °C. Stirring was continued at room temperature for 0.5 h. Thereafter, the mixture was poured onto ice (50 g), basified by the addition of solid NaOH and extracted with Et_2O (2×50 mL). The pooled organic portion was dried ($MgSO_4$) and the oxalate salt was prepared and recrystallized from MeOH (205 mg, 37%), mp 159–161 °C. The resulting compound (0.38 g) in MeOH (50 mL) was hydrogenated at a pressure of 50 psi in a Parr bottle containing 10% Pd/C (0.2 g) until sufficient H_2 was taken up (24 h). The catalyst was removed by filtration, the solvent was removed under reduced pressure and the oxalate salt was prepared. Recrystallization was achieved from Abs EtOH/EtOAc (0.24 g, 51%), mp 99–103 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.87 (2H, d, J = 12 Hz), 6.58 (2H, d, J = 12 Hz), 3.31 (2H, s, br), 2.89 (2H, s, br), 2.72 (2H, s, br), 2.36 (2H, t, J = 9 Hz), 1.50 (10H, m), 1.19 (2H, s, br); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.23, 23.27, 23.85, 26.51, 31.63, 34.99, 52.82, 56.69, 116.55, 129.70, 164.03. Anal. $C_{16}H_{26}N_2 \cdot 2.1C_2H_2O_4 \cdot 1.0H_2O$, C, H, N.

1-[1-(3,4-Methylenedioxyphenyl)-2,4-pentadienyl]piperidine hydrochloride (14). A solution of piperine (1.0 g, 3.5 mmol) in Et_2O (50 mL) was added to a stirred suspension of $LiAlH_4$ (0.93 g, 7 equiv) in Et_2O (100 mL) and the mixture allowed to reflux under an atmosphere of N_2 for 24 h. The reaction was quenched by the addition of drops of H_2O (20 mL) and stirring was continued for an additional 0.5 h. The ethereal portion was decanted and 10% NaOH (60 mL) was added to the residue. The

resulting mixture was then filtered and the filtrate extracted with Et_2O (2×50 mL). The total combined organic phases was washed with H_2O (20 mL), dried ($MgSO_4$) and ethereal HCl was added to obtain a precipitate of the salt. The precipitate was recrystallized from EtOAc/MeOH to give yellow crystals, mp 174–176 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.10 (1H, s, br), 6.92 (1H, d, J=1.5 Hz), 6.82 (1H, d, J=1.5 Hz), 6.78 (1H, s), 6.65 (1H, dd, J=11, 16 Hz), 6.54 (1H, d, J=16 Hz), 6.48 (1H, dd, J=16, 12 Hz), 6.03 (1H, dt, J=16, 8 Hz), 5.98 (2H, s), 3.65 (2H, dd, J=4, 12 Hz), 3.51 (2H, d, J=12 Hz), 2.63 (2H, dt, J=12, 4 Hz), 2.25 (2H, m), 1.89 (2H, m), 1.42 (2H, m); ^{13}C NMR (300 MHz, $CDCl_3$) δ 21.91, 22.41, 52.26, 59.13, 101.05, 105.42, 108.26, 118.80, 121.76, 124.65, 130.54, 135.46, 139.99, 147.79, 148.00. Anal. $C_{17}H_{22}NO_2Cl{\cdot}0.15H_2O$, C, H, N.

1-[5-(3,4-Methylenedioxyphenyl)-pentyl]piperidine hydrogen oxalate (15). A mixture of piperine (1.0 g, 3.5 mmol), 10% Pd/C (0.24 g) and MeOH (50 mL) was hydrogenated at 50 psi for 2 h. The mixture was filtered, and solvent was removed in vacuo to afford an amide; IR $\sqrt{}$=1651 cm^{-1}. Without further purification, the amide was dissolved in Et_2O (50 mL) and added dropwise onto a suspension of $LiAlH_4$ (0.6 g, 0.01 mol) in Et_2O (50 mL). The mixture was allowed to reflux for 4 h after which it was cooled in ice and quenched by the addition of H_2O (3 mL), 10% NaOH (3 mL) and H_2O (10 mL). The resulting mixture was allowed to stir for additional 0.5 h, the organic phase was decanted, and the aqueous phase extracted with Et_2O (2×50 mL). The organic phases were pooled, dried ($MgSO_4$) and a saturated solution of oxalic acid was added to precipitate the salt. The salt was recrystallized from EtOAc/MeOH to give white crystals (0.90 g, 70%), mp 122–123 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.72 (1H, d), 6.63 (1H, s), 6.58 (1H, d), 5.91 (2H, s), 3.62 (2H, d), 2.95 (2H, m), 2.60 (2H, m), 2.50 (2H, t), 2.08–1.66 (7H, m), 1.58 (2H, m), 1.45–1.22 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.88, 22.51 (2C), 23.29, 25.90, 30.72, 35.01, 53.16 (2C), 57.17, 100.53, 107.91, 108.55, 120.88, 135.46, 163.08. Anal. $C_{19}H_{27}NO_6$, C, H, N.

4-Benzyl-1-(5-phenylpentyl)-1-methylpiperidinium chloride (16). A mixture of compound **7** (270 mg, 0.84 mmol) methyl iodide (1.5 mL) and acetonitrile (1 mL) was heated for 22 h, charged with additional methyl iodide (1.5 mL) and heated for 5 more hours. The solvent was removed, the residue was reconstituted in EtOAc/MeOH and allowed to crystallize in the refrigerator (261 mg, 65%), mp 144–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.25 (5H, m), 7.16 (5H, m), 3.62 (6H, m), 3.18 (3H, s), 2.67 (2H, d, J=8 Hz), 2.50 (2H, t, J=7 Hz), 2.09 (2H, m), 1.73 (7H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.11, 25.88, 26.07, 30.87, 34.59, 35.58, 41.50, 45.23, 60.83, 67.70, 125.83, 126.50, 128.38, 128.47, 128.56, 129.12, 138.43, 141.92. Anal. $C_{24}H_{34}NI{\cdot}0.75H_2O$, C, H, N.

Binding studies

The σ_1 radioligand-binding assay was carried out as previously reported[27] using (+)-[^{3}H]pentazocine as the radioligand. Approximately 100 μg of guinea pig brain membranes and (+)-[^{3}H]pentazocine (3–4 nM final concentration) in a final volume of 500 μL of 50 mM Tris–HCl buffer (pH 8.0). For the standard equilibrium essay, 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 spectrometer at an efficiency of about 50%. Nonspecific binding was determined in the presence of 10 μM haloperidol. IC_{50} values were determined from competitive curves using nonlinear least-squares regression analysis and converted to K_i values with the Cheng–Prusoff transformation. Each K_i value was determined from three to five separate determinations.

The σ-2 selective binding assay[27] was performed using about 2 nM [^{3}H]DTG as the radioligand in the presence of 200 nM (+)pentazocine to block the σ-1 sites, with 400 μg of guinea pig brain membranes in a total volume of 500 μL of 50 mM TRIS-HCl, 7.4. Nonspecific binding was determined in the presence 10 μM haloperidol. For the standard equilibrium assay the mixtures were incubated for 30 min at room temperature, filtered and the radioactivity determined as described for σ-1.

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