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Probing the Proposed Phenyl-A Region of the Sigma-1 Receptor

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Abstract—The proposed phenyl-A region of sigma (σ) receptors accommodates several structural features. In this study we explored the possibility that appropriate structural features located at the phenyl-A region of σ receptor sites could lead to more potent and selective agents for the sigma receptor subtypes. By keeping the phenyl-B substituent as the optimum ω-phenylpentyl moiety, and varying substituents in the phenyl-A region, we have observed changes in binding potency and selectivity at the σ receptor subtypes. SAR for the binding of these compounds at σ-2 sites was also examined.

Sigma (σ) receptors were of interest about a decade ago; but, with growing uncertainty regarding the functional significance and therapeutic utility of σ receptor ligands, enthusiasm began to wane. However, interest in σ ligands appears to be on the rebound. This may be related to the identification of multiple σ binding sites (e.g., σ-1 and σ-2) and of newly identified physiological roles for σ receptors (for a recent review, see Bowen[1]). For example, several recent publications support the hypothesis that σ-2 ligands may have potential utility in the therapy of certain carcinomas[2] (through induction of apoptosis) and antipsychotic-induced motor side effects.[1] The σ-1 receptor has been shown, among others, to modulate the synthesis and release of dopamine[3] and acetylcholine,[4] negatively modulate agonist-stimulated phosphoinositide turnover,[5] modulate NMDA-type glutamate receptor electrophysiology,[6] modulate opioid analgesia,[7] and possess neuroprotective and anti-amnesic activity.[8] Sigma-1 receptors also produce alterations in cocaine-induced locomotor activity and toxicity,[9] modulate NMDA-induced activation of pyramidal neurons in the hippocampus of the rat[10] and based on this property, several compounds are now classified as either putative receptor agonists or antagonists.[11] A primary impediment to a more detailed understanding of the role of σ receptors in mammalian physiology remains the lack of high-affinity and selective agents for σ-1 and σ-2 receptors.

Amine-substituted 5-(phenyl)pentylamines represent high-affinity σ receptor ligands.[12] We have previously proposed a model to account for the binding of ligands at σ-1 receptors[13–16] (Fig. 1). In this model, shortening of a phenyl-B to amine alkyl chain reduces affinity; the pentyl chain appears to be an optimal spacer between the primary N atom and the phenyl-B ring. Much less is known regarding the phenyl-A region as this area appears to tolerate various structural features and chain lengths. We have proposed that there exists a secondary hydrophobic binding region within about 4 Å from the amine site and that a region of bulk tolerance extends beyond this. In this study we explore, using phenylalkylamines as probes, the possibility that this region of the

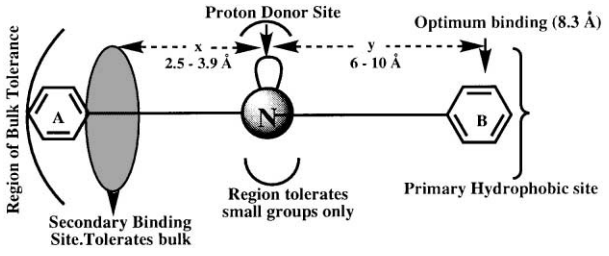

Figure 1. Pictorial representation of proposed features for σ-1 binding.[13–15]

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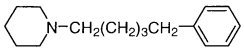
σ receptors may afford opportunities for developing more potent and selective ligands for the σ receptor subtypes. Athough σ-binding data have been already reported for certain of the compounds described herein, this study brings together the results in a more systematic and comprehensive manner, and addresses issues of σ-1 versus σ-2 selectivity.

Chemistry

All of the compounds in this study were obtained by standard synthetic techniques and several were previously reported. Compound **2** was obtained by direct alkylation of the commercially available 3-azabicyclo[3.2.2]nonane with the previously reported 5-phenylpentyl bromide.[17] The syntheses of compounds **1**, **5**, **6a**, **6b**, **8a**, **8b**, **10a**, **10b**, **11**, and **20** were previously reported (Chart 1),[14,18] and compound **7** was re-synthesized.[14] To obtain the phenyl or benzyl piperidines, the corresponding phenyl or benzylpyridines were hydrogenated on Pd–C in gl. AcOH and the resulting piperidines were either directly alkylated with 5-phenylpentyl bromide or converted to the amide using ethyl chloroformate, Et_3N and 5-phenylpentanoic acid.



1 σ-1,Ki = 0.48 nM
σ-2,Ki = 50 nM

Once obtained, the amides were reduced using lithium aluminum hydride to produce the desired ω-phenylpentyl piperidines, **12**, **13**, **15**, and **16** (Scheme 1). Compounds **14** and **17** (Chart 2) were also previously synthesized and their binding affinities were reported.[15,16] Compound **18** was synthesized from tetralone by reductive amination with methyl amine to produce the secondary amine which was subsequently alkylated with 5-phenylpentyl bromide to produce the desired amino tetraline, **18** (Scheme 2).

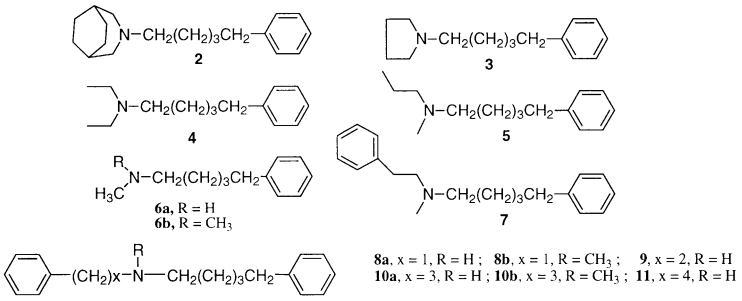


Chart 1. Compounds **2**–**11** discussed in this article.

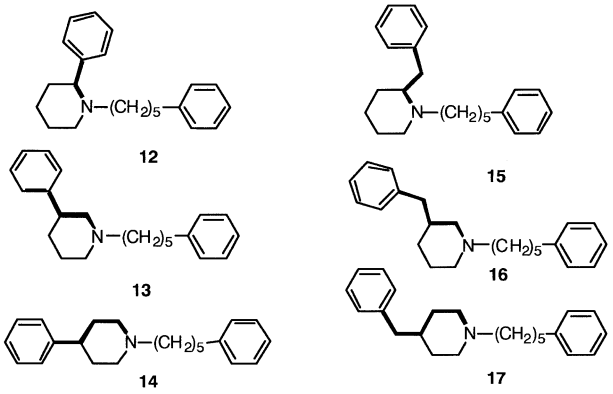
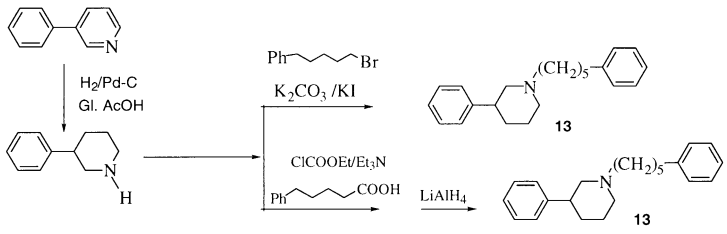
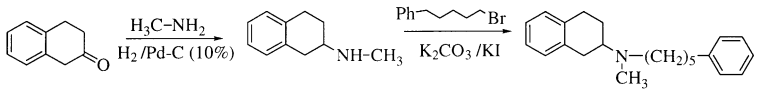


Chart 2. Conformationally constrained analogues discussed in this article.



Scheme 1. Synthesis of ω-phenylpentyl piperidines, **12**–**16**.



Scheme 2. Synthesis of aminotetralin **18**.

Results and Discussion

σ-1 Structure–activity relationships

1-(5-Phenylpentyl)piperidine (**1**) binds at σ-1 receptors with high affinity (σ-1 $K_i = 0.48$ nM), but only with moderate affinity at σ-2 receptors (σ-2 $K_i = 50$ nM).[16] The high σ-1 affinity of **1** indicates that a phenyl-A ring is not required for high affinity binding. To understand the role of the piperidine ring of **1** in the binding to sigma receptors, and to further probe the phenyl-A region, we synthesized compounds **2**–**5** in which the σ-phenylpentyl moiety was held constant while substitution on the N-atom in the direction of the phenyl-A region was varied (see Table 1 for binding data).

Replacement of the piperidine ring with a 3-azabicyclo[3.2.2]nonane ring results in compound **2** (σ-1 $K_i = 1.0$ nM) while reduction of the ring size of **1** to a five-membered pyrrolidine ring yields compound **3** (σ-1 $K_i = 0.76$ nM). Both changes resulted only in a minimal decrease in binding affinity.

These results are consistent with the proposed model. The N-substituents of **1**–**3** appear to optimally utilize a limited hydrophobic region situated between the amine and the region of bulk tolerance. Increasing the lipophilic character of **1** to **2** had little effect on σ-1 affinity.

Table 1. Radioligand binding data

	K_i (nM)[a]		σ-1 Selectivity (σ-2 K_i/σ-1 K_i)
	σ-1 Sites	σ-2 Sites	
1	0.48[d]	50[d]	104
2	1.0	—[b]	—
3	0.76[c]	70	92
4	6.0	89	13
5	0.25[c]	—[b]	—
6a	418[c]	7920	19
6b	14.0[c]	965	69
7	0.25[c]	5.0	20
8a	0.17[c]	34	200
8b	0.19[c]	13	68
9	0.17[c]	15	88
10a	0.28[c]	9.8	35
10b	0.36[c]	6.3	18
11	0.48[c]	68	142
12	0.17	4.7	28
13	0.14	5.3	38
14	0.16[d]	—[b]	
15	0.40	10	25
16	0.076	4.2	55
17	0.58[d]	2.8	5
18	0.60	170	283

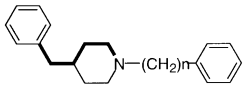
[a]Values were determined from the averages of at least three separate experiments, and SEM was typically $< \pm 20\%$.
[b]K_i value not determined.
[c]K_i value for σ-1 subtype was previously reported.[14]
[d]K_i value was previously reported.[16]

The next issue to be addressed was: what little is required of amine substituents to retain high affinity? Opening the pyrrolidine ring symmetrically (**4**, σ-1 $K_i = 6.0$ nM) or asymmetrically (**5**, σ-1 $K_i = 0.25$ nM) supports prior claims that a 3-carbon amine-substituent in the direction of the phenyl-A region is an optimum requirement for binding at σ-1 receptors.[14] Further truncation of chain length, comparing **5** with **6b** (σ-1 $K_i = 14$ nM; σ-2 $K_i = 965$ nM), results in a significant reduction in binding affinity at both receptor subtypes. Thus, overall, these results suggest that binding affinity is modulated by varying substituents in the phenyl-A region of sigma receptors. In addition, while the piperidine ring of **1** is not essential for high affinity, the number of carbon atoms on the N atom appears to influence binding affinity.

Replacing the terminal methyl group of compound **5** with a phenyl ring (**7**, σ-1 $K_i = 0.25$ nM) should not significantly enhance σ-1 affinity if the phenyl ring extends into the region of bulk tolerance. This was found to be the case; this structural alteration did not result in further increase in binding affinity. Removal of one of the methyl groups from compound **6b** to form the monomethyl analogue (**6a**, σ-1 $K_i = 418$ nM; σ-2 $K_i = 7920$ nM) suggests a minimum number of carbons are required in the direction of the phenyl-A region to bind to sigma receptors. To eliminate the possibility that this reduction in affinity might be associated with the simple transformation of a tertiary amine to a secondary amine, we examined the secondary amine counterpart of **7**, that is, compound **9** (σ-1 $K_i = 0.17$ nM; σ-2 $K_i = 15$ nM). The high affinity binding of **9** suggests that a tertiary amine is not an absolute requirement for σ-1 or σ-2 binding. This supports previous claims to this effect.[14] [See other examples of secondary (**a**) and tertiary amine (**b**) comparisons below; compounds **8a** and **8b**, and **10a** and **10b**.] Decreasing the spacer length between the N atom and the phenyl-A ring (**8a**, σ-1 $K_i = 0.32$ nM: **8b**, σ-1 $K_i = 0.19$ nM), or increasing the spacer length to three carbon atoms, (**10a**, σ-1 $K_i = 0.28$ nM: **10b**, σ-1 $K_i = 0.36$ nM) and to 4 carbon atoms, **11** (σ-1 $K_i = 0.48$ nM), did not significantly affect binding to σ-1 receptors. This, too, is consistent with the proposed region of bulk tolerance shown in Figure 1.

It was of interest to further probe the space around the piperidine ring in the phenyl-A direction to determine if there are preferred areas for sigma binding. Compounds **12**–**17** might be viewed as analogues of **7**, **8**, **10**, and **11** where some conformational restraint has been imposed. Compounds **13** (σ-1 $K_i = 0.14$ nM; σ-2 $K_i = 5.3$ nM) and **15** (σ-1 $K_i = 0.4$ nM; σ-2 $K_i = 10$ nM) are conformationally restricted analogues of **7** with the phenyl rings fixed in different locations around the piperidine ring. Only minimal changes occurred in binding affinity at both σ-1 and σ-2 receptors. A similar observation

Table 2. Binding affinity of compounds **17**–**20**



Compd	*n*	K_i (nM)[a]		σ-1 Selectivity
		σ-1 Sites	σ-2 Sites	σ-2 K_i/σ-1 K_i)
17	5	0.6	2.8	4.7
20	4	0.8	3.1	3.7
21	3	0.4	3.3	8.3

[a]Values were determined from the averages of at least three separate experiments, and SEM was typically $< \pm 20\%$.

was made for the restricted analogues of **10**, that is, compounds **14** (σ-1 $K_i = 0.16$ nM; σ-2 K_i = NA) and **16** (σ-1 $K_i = 0.076$ nM; σ-2 $K_i = 4.2$ nM). Interestingly, compound **16** binds with a 6-fold higher affinity than **1** and is the highest affinity σ-1 analogue in this series; the added substituent might be availing itself of some new binding feature. This will require further investigation.

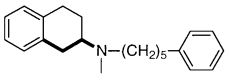
The conformationally restricted analogues of compounds **8** and **11**, that is, **12** (σ-1 $K_i = 0.17$ nM; σ-2 $K_i = 4.7$ nM) and **17** (σ-1 $K_i = 0.58$ nM; σ-2 $K_i = 2.8$ nM), were also synthesized and evaluated for their binding to the sigma receptors. The results show that while σ-1 binding is only minimally affected by restriction of the phenyl ring, σ-2 binding is increased substantially by restricting the phenyl ring when it is close to the N atom as in **8** and **12**. Comparing **11** and **17** similarly shows that σ-1 binding is not affected by restriction of the phenyl ring while σ-2 binding is significantly increased by restriction. In fact, compound **17** has the highest affinity for the σ-2 receptor in this series.

Finally, the racemic aminotetralin **18** can be considered as a restricted analogue of **7** (**18**, σ-1 $K_i = 0.6$ nM; σ-2 $K_i = 170$ nM). Compound **18**, while differing little from **7** in terms of σ-1 affinity, displays >280-fold selectivity for σ-1 versus σ-2 sites. It is interesting to note that **18** is a close structural relative of *N*-allylnormetazocine (**19**)—one of the first σ ligands reported.[19]

σ-2 Structure–activity relationships

While there have been several σ-1 structure–activity studies, there is a paucity of σ-2 SAR. Hence, we evaluated whether there are structural differences associated with σ-2 receptor binding in the phenyl-A region of the receptor. As with σ-1 binding, contraction of the piperidine ring of **1** to a pyrrolidine ring has little effect on σ-2 affinity (**1**, $K_i = 50$ nM; **3**, $K_i = 70$ nM) (Table 1). Symmetrical opening of the pyrrolidine ring of **3** to **4** ($K_i = 89$ nM) also had no effect on affinity, whereas this same structural modification reduced σ-1 affinity by one order of magnitude. The *N*-methyl-*N*-(2-phenylethyl) derivative **7** ($K_i = 5.0$ nM) binds with high affinity at σ-2 sites. Shortening the carbon chain of **7** by a single methylene unit (i.e., **8b**, $K_i = 13$ nM) halves affinity, whereas chain lengthening by the same amount (**10b**, $K_i = 6.3$ nM) has little effect. A similar trend is observed with the corresponding secondary (i.e., des-methyl) amines (**9**, $K_i = 15$ nM; **8a**, $K_i = 34$ nM; **10a**, $K_i = 9.8$ nM), but the secondary amines bind with about half the affinity of their *N*-methyl tertiary amine derivatives **7**, **8b**, and **10b**, respectively. Further extension of the methylene chain, as with **11** ($K_i = 68$ nM) reduces σ-2 affinity.

A phenyl or benzyl group was attached to the piperidine moiety of **1** (**12**, **13**, and **15**–**17**) (Table 1); each of the compounds displayed enhanced affinity ($K_i \leq 10$ nM), with compound **17** ($K_i = 2.8$ nM) being optimal. Because we have previously shown that an *n*-pentyl chain in the direction of the 'phenyl-B' site is optimal for σ-1 binding, the 4-benzylpiperidine moiety of **17** was held constant and chain length in the direction of the phenyl-B site was shortened with the expectation that if σ-2 affinity was retained, selectivity would be enhanced. Table 2 shows that shortening of this chain from *n*-pentyl to *n*-butyl or *n*-propyl (**17**–**20** or **21**, $K_i = 3.1$ and 3.3 nM, respectively) did indeed result in retention of σ-2 affinity. However, because σ-1 affinity was also retained, selectivity was not achieved. In retrospect, such a result might have been expected. That is, compounds **20** and/or **21** might be binding in a flipped mode where the so-called 'phenyl-A' phenyl ring is binding at the phenyl-B site and vice versa. We have previously encountered this problem due to the nearly symmetrical nature of many of the σ ligands.[14,16] Future studies will require evaluation of less symmetrical aryl-substituted derivatives to address this situation.



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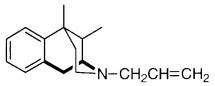

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Conclusions

By holding the optimum ω-phenylpentyl moiety constant, it has been possible to explore the phenyl-A region of the sigma receptors in an incremental manner. For the most part, the compounds investigated in this study are consistent with the proposed σ-1 model. The changes introduced beyond three carbon atoms in the phenyl-A region of the σ-1 receptor generally have little effect on the binding affinity of the ligands; these substituents likely spill over to a region of bulk tolerance. In this manner, compound **16** was identified as a very high-affinity ($K_i = 0.076$ nM) σ-1 ligand with modest (55-fold) selectivity over σ-2 sites. However, truncation of the number of carbons below three leads to a decrease in binding affinity at both receptor subtypes; hence, there seems to be a minimal chain-length requirement for binding. The seemingly large region of bulk tolerance in the phenyl-A region of the sigma receptors allows for further elaboration of amine substituents. However, σ-2 receptors appear to be more sensitive (in binding affinity) to changes made in the phenyl-A region. Using this approach, compound **18** was developed as a high-affinity σ-1 agent with nearly 290-fold selectivity over σ-2 sites. In addition, it appears that phenyl piperidines bind with a higher affinity at σ-2

receptors compared to the corresponding phenylalkylamines. It might be possible in the future to exploit some of these differences to develop agents with enhanced selectivity for σ-1 versus σ-2 binding.

Experimental

Syntheses

Proton and carbon magnetic resonance spectra were obtained on QE 300 (300 MHz) spectrometer with tetramethylsilane as internal standard. 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 below.

Method A

1-(5-Phenylpentyl)-3-azabicyclo[3.2.2]nonane hydrochloride (2). A stirred mixture of 3-azabicyclo[3.2.2]nonane (0.5 g, 4.0 mmol), 5-phenylpentyl bromide (1.1 g, 4.9 mmol) KI (20 mg) and K_2CO_3 (1.3 g, 6.2 mmol) 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 extracted with Et_2O (30 mL) and the combined organic portions were pooled, washed with H_2O (20 mL) and dried ($MgSO_4$). A saturated ethereal solution of HCl was added to obtain a salt, which was recrystallized from MeOH/Et_2O (1.2 g, 97%): mp 239 °C (Dec.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.11 (m, 5H), 3.72 (m, 2H), 2.98 (m, 2H), 2.78 (t, 2H), 2.62 (t, 2H), 2.50 (d, 2H), 2.16 (s, br, 2H), 1.95 (m, 2H), 1.78–1.12 (m, 8H), 1.36 (q, 2H). Anal. $C_{19}H_{30}NCl$. C, H, N.

1-(5-Phenylpentyl) pyrrolidine hydrogen oxalate (3). A mixture of 5-phenylpentanol tosylate (96 mg, 0.3 mmol), pyrrolidine (1 mL), and TEA (0.3 mmol), were combined in dioxane (15 mL) and heated to reflux for 2 h. The reaction mixture was partitioned between Et_2O (15 mL) and water (10 mL). The organic fraction was separated, washed with water (10 mL) and dried ($MgSO_4$). The oxalate salt was made and recrystallized from EtOH to afford the desired compound (60 mg, 50%); mp 143–145 °C. Anal. $C_{17}H_{25}NO_4$ C, H, N.

***N*,*N*-Diethyl-5-phenylpentylamine hydrogen oxalate (4).** A solution of $ClCO_2Et$ (1.0 g, 9.2 mmol) in CH_2Cl_2 (10 mL) was added in a dropwise manner to a stirred ice-cooled solution of 5-phenylpentanoic acid (1.6 g, 9 mmol) and Et_3N (2 g) in dry CH_2Cl_2 (90 mL). The addition was done under N_2, over 10 min. Stirring was continued for 30 min before Et_2NH HCl (1.0 g, 9.1 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 5 min. Stirring was continued overnight (~12 h) before the mixture was heated to reflux for 3 h. After cooling to room temperature, the reaction mixture was washed successively with H_2O (60 mL), 3 N HCl (60 mL), H_2O (60 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure to afford an oil (2.1 g, 100%). Without further purification, a solution of the amide (2.6 g) in THF (20 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (1.0 g) in THF (60 mL). The reaction mixture was heated under reflux, in a stream of N_2, for 3 h and excess $LiAlH_4$ was decomposed by the gentle addition of H_2O (2 mL) and 10% NaOH (2 mL) solution. Stirring was continued for 30 min, solid matter was removed by filtration, and the solvent was removed in vacuo to obtain an oily residue. The residue was dissolved in Et_2O (20 mL) and a saturated solution of oxalic acid was added to yield a salt (1.54 g, 55%). Recrystallization from EtOH/EtOAc afforded the purified product; mp 81–83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (s, b, N^+H), 7.32–7.12 (m, 5H), 3.22–3.10 (q, 4H), 3.00 (t, 2H), 2.62 (t, 2H), 1.68 (m, 4H), 1.38 (q, 2H), 1.30 (t, 6H). Anal. $C_{17}H_{27}NO_4$ C, H, N.

1-Methyl-1-(2-phenethyl)-5-phenylpentylamine hydrogen oxalate (7). A solution of ethyl chloroformate (1.0 g, 9.2 mmol) in methylene chloride (10 mL) was added in a dropwise manner to a stirred ice-cooled solution of 5-phenylvaleric acid (1.3 g, 7.4 mmol) and Et_3N (1.0 g, 9.2 mmol) in dry methylene chloride (80 mL) under N_2, over 10 min. Stirring was continued for 30 min and *N*-methylphenylethylamine (1.0 g, 7.4 mmol) in methylene chloride (10 mL) was added dropwise over 5 min. Stirring was allowed to continue overnight (~12 h) and heated to reflux for 3 h. After cooling to room temperature, the reaction mixture was washed successively with H_2O (20 mL), 5% NaOH (10 mL), 5% AcOH (10 mL), H_2O (15 mL) and dried ($MgSO_4$). Solvent was removed under reduced pressure to afford an oil. Without further purification, a solution of the amide (2.8 g) in THF (20 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (1.4 g) in THF (60 mL). The reaction mixture was heated under reflux in a stream of N_2 for 12 h. Excess $LiAlH_4$ was decomposed by the gentle addition of H_2O (2 mL) and 10% NaOH (2 mL) solution and stirring was continued for 30 min. Solid matter was removed by filtration and solvent was removed in vacuo to obtain an oily residue. The residue was taken up in Et_2O (30 mL), dried ($MgSO_4$) and a saturated solution of oxalic acid was added. The resulting salt was recrystallized from MeOH (1.3 g, 87%); mp 141–142 °C (lit[14] 140–142 °C).

Method B

1-(5-Phenylpentyl)-2-phenylpiperidine (12). A mixture of 2-phenylpyridine (2.0 g, 16 mmol), glacial acetic acid (25 mL) and 10% Pd/C (0.4 g) was hydrogenated at psi 50 in a Parr bottle until sufficient H_2 was taken up (24 h). The catalyst was removed by filtration, the solvent was removed under reduced pressure and the residue was chromatographed over silica gel to yield an oil. The oil was used without further purification or characterization. A solution of ethyl chloroformate (0.8 g) in CH_2Cl_2 (10 mL) was added in a dropwise manner to a stirred ice-cooled solution of 5-phenylpentanoic acid (1.1 g, 6.2 mmol) and Et_3N (0.7 g) in dry CH_2Cl_2

(50 mL). The addition was done under N_2, over 10 min. Stirring was continued for 30 min before 2-phenypiperidine obtained above (1.0 g, 6.2 mmol) in CH_2Cl_2 (10 mL) was added dropwise over 5 min. Stirring was continued overnight (~12 h) before the mixture was heated to reflux for 3 h. After cooling to room temperature, the reaction mixture was washed successively with H_2O (20 mL), 5% NaOH (10 mL), 5% AcOH (10 mL), H_2O (15 mL), and dried ($MgSO_4$). The solvent was removed under reduced pressure to afford an oil (2 g, 100%). Without further purification, a solution of the amide (1 g, 6.2 mmol) in THF (20 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (0.8 g) in THF (60 mL). The reaction mixture was heated under reflux, in a stream of N_2, for 12 h and excess $LiAlH_4$ was decomposed by the gentle addition of H_2O (2 mL) and 10% NaOH (2 mL) solution. Stirring was continued for 30 min before solid matter was removed by filtration and solvent removed in vacuo to obtain an oily residue. The oily residue was purified by distillation (0.05 mm of Hg, at 190 °C) to yield a colorless oil. All attempts to form the hydrochloride or oxalate salts failed. Anal. $C_{22}H_{29}N$, C, H, N.

Method C

1-(5-Phenylpentyl)-3-phenylpiperidine (13). A mixture of 3-phenylpyridine (2.5 g), glacial acetic acid (25 mL) and 10% Pd/C (0.4 g) was hydrogenated at psi 50 in a Parr bottle for 40 h. The catalyst was removed by filtration and the filtrate was added onto ice (150 g). The resulting solution was basified with solid NaOH and extracted with Et_2O (3×40 mL). The pooled organic component was washed with H_2O (40 mL), dried ($MgSO_4$) and a saturated solution of oxalic acid was added to precipitate the salt. The salt was recrystallized from MeOH/Et_2O to give the desired intermediate (3.4 g, 85%); mp 111–113 °C.

A stirred mixture of 3-phenylpiperidine (0.7 g, 4.4 mmol), 5-phenylpentyl bromide (1.3 g, 1.3 equiv), KI (20 mg) and K_2CO_3 (1.2 g) in 1,2-dimethoxyethane (DME) (5 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 extracted 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/Et_2O to yield the desired product (0.8 g, 47%); mp 143–145 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40–7.04 (m, 10H), 3.36 (t, 2H), 3.10–2.68 (m, 6H), 2.50 (t, 2H), 1.82 (d, 2H), 1.70 (m, 2H), 1.50 (quin, 2H), 1.20 (quin, 2H). Anal. $C_{24}H_{31}NO_4$ C, H, N.

1-(5-Phenylpentyl)-2-benzylpiperidine hydrobromide (15). Method B was used. 2-Benzylpiperidine (2.0 g) was used to obtain a final product of the HBr salt (440 mg,% yield): mp 94–96 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.12 (m, 10H), 3.62 (m, 1H), 3.54–3.35 (m, 1H), 3.32–3.12 (m 2H), 3.40 (q, 2H), 2.06 (m, 2H), 1.85 (m, 2H), 1.60–1.30 (m, 2H). Anal. $C_{23}H_{32}NBr$ C, H, N.

1-(5-Phenylpentyl)-3-benzylpiperidine (16). Method C was used. 3-Benzylpiperidine was obtained in 92% yield from 3-benzylpyridine. The oxalate salt of the final product was obtained: mp 171–172 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.08 (m, 10H), 3.28 (m, 2H), 2.88 (t, 2H), 2.65 (t, 1H), 2.5 (m, 5H), 2.02 (s, br, 1H), 1.78–1.44 (m, 7H), 1.20 (m, 2H), 1.05 (q, 1H). Anal. $C_{25}H_{33}NO_4$ C, H, N.

1-(5-Phenylpentyl)-1-methyl-2-aminotetraline dihydrogen oxalate (18). A stirred mixture of 2-tetralone (1 g, 6.8 mmol) methylamine hydrochloride (0.5 g, mmol) 10% Pd/C (0.4 g) and absolute EtOH (30 mL) was hydrogenated at 55 psi over the week-end (48 h). The catalyst was removed by filtration, the solvent removed under reduced pressure and the residue was chromatographed over silica gel to yield an oil. Repeated crystallization from MeOH/Et_2O yielded the hydrochloride salt (0.2 g, 12%).

A stirred mixture of *N*-methyl-2-aminotetraline (0.25 g, 1.6 mmol), 5-phenylpentyl bromide (0.4 g, 1.6 mmol) and K_2CO_3 (0.5 g) in 1,2-dimethoxyethane (DME) (3 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 extracted 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 recrystalized from MeOH (3.1 g, 74%), mp 125–126 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.0–7.15 (m, 9H), 3.28 (t, 1H), 3.02–2.78 (m, 7H), 2.70 (s, 3H), 2.54 (t, 2H), 2.18 (d, 1H), 1.50–1.80 (m, 5H), 1.24 (q, 2H). Anal. $C_{24}H_{31}NO_4$ C, H, N.

4-Benzyl-1-[3-(*N*-phenyl)propyl]piperidine hydrochloride (21). Method A was used except for the use of acetonitrile as solvent instead of DME. Using 4-benzylpiperidine and 1-bromo-3-phenylpropane as reactants, the product was obtained in 90% yield; mp 187–189 °C. Anal. $C_{21}H_{27}N{\cdot}HCl$ C, H, N.

Binding studies

The σ_1 radioligand-binding assay was carried out as previously reported[16] 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 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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