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Synthesis and structure–activity relationships of *N*-(3-phenylpropyl)-*N*′-benzylpiperazines: Potent ligands for σ_1 and σ_2 receptors

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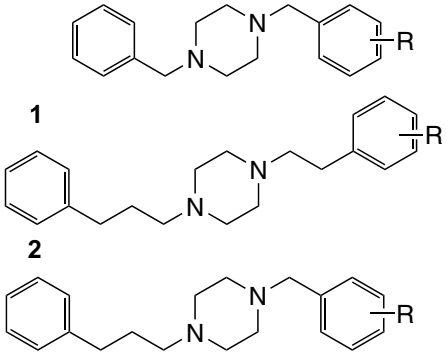
Abstract—Ten *N*-(3-phenylpropyl)-*N*′-benzylpiperazines having different substituents on the benzyl moiety were synthesized and evaluated for σ_1 and σ_2 receptor binding. The σ_1 affinities were 0.37–2.80 nM, σ_2 affinities were 1.03–34.3 nM, and selectivities, as σ_2/σ_1 affinity ratios, ranged from 1.4 to 52. Three compounds tested in a phenytoin shift binding assay profiled as probable σ_1 antagonists. Quantitative structure–activity relationships depended on π_x, MR or E_s and Hammett σ values. The hydrophobicity term is negative for σ_1 binding but positive for σ_2 binding, indicating a major difference between the pharmacophores.

1. Introduction

Recent reports indicate that sigma (σ) receptor antagonists attenuate the effects of (−)-cocaine in animal models.[1] Anti-cocaine activity correlates with affinities for both σ_1 and σ_2 receptors, but the σ_1 relationships appear stronger and (−)-cocaine itself displays 10-fold higher affinity for σ_1 over σ_2 receptors.[1–3] Notable active series (Fig. 1) include *N*-benzyl-*N*′-benzylpiperazines[2] (**1**) and *N*-phenylpropyl-*N*′-phenethylpiperazines[3] (**2**) although the structural features that confer antagonist activity are not well defined. In the present study, we investigated quantitative structure–activity relationships (QSARs) for the σ_1 and σ_2 receptor binding of *N*-phenylpropyl-*N*′-benzylpiperazines (**3**) that represent hybrids of **1** and **2**, and might also be potent σ receptor antagonists.



3 **a**, R = H; **b**, 2-Br; **c**, 2-NO_2; **d**, 3-I; **e**, 3-F; **f**, 3-OCH_3; **g**, 3-NO_2; **h**, 4-OCH_3; **i**, 4-NO_2; **j**, 4-CH_3

Figure 1. Prototype sigma ligands **1** and **2**; ligands (**3**) synthesized in this study.

The parent compound, **3a**, was shown by Younes and colleagues[4] to exhibit an IC_{50} of 20 nM against the binding sites labeled by [^{3}H](+)PPP, but the σ_1/σ_2 receptor selectivity and functional activity were not defined. Substituted benzyl derivatives of this lead compound

Abbreviations: QSAR, Quantitative structure–activity relationship; (+)PPP, (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; SA4503, 1-(2-(3,4-dimethoxy-phenyl)ethyl)-4-(3-phenylpropyl)piperazine; DAMGO, [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin; U69,593, (+)-(5α,7α,8β)-*N*-methyl-*N*-[7-(pyrrolidin-1-yl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide.

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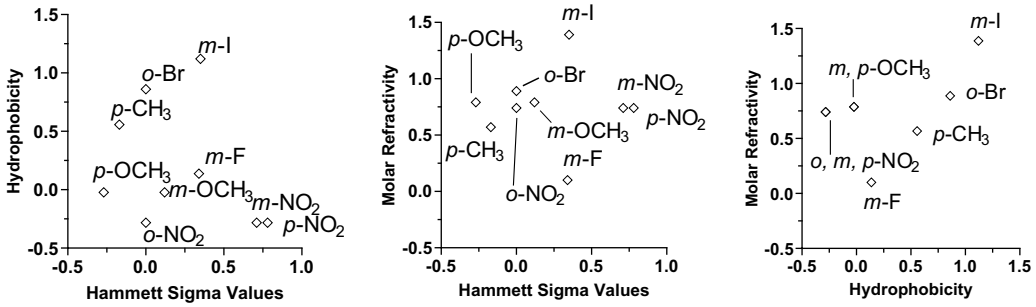

Scheme 1. Craig plots for σ, π, and MR values for substituents in calibration sets.

have not been reported, but might provide sharp correlations in QSAR studies.[5] Thus, we selected a set of congeners (**3a**–**3j**, Fig. 1) with substituents chosen according to the factorial design method[6] for optimal coverage of descriptor space for physicochemical parameters representing hydrophobic contributions (π_x), electronic characteristics (σ, Hammett constant), and Molar Refractivity (MR). We tested the data according to classical statistics' notions (average, variance, standard deviation, skewness, kurtosis, linear pair correlation coefficient) in order to ensure that the values are well dispersed, follow a normal distribution, and that the chosen descriptors series are totally independent of each other so that consequently the final results will be statistically sound, and fairly representative. The results of the tests (data not shown) confirmed that the compounds selected to build the correlation equation are statistically sound, and therefore, proceeding in this study is feasible. In Scheme 1, Craig plots illustrate the dispersion in the dual parameter space of hydrophobicity and Hammett sigma values, Molar Refractivity and Hammett sigma values, and Molar Refractivity and hydrophobicity, respectively. Determination of the σ receptor affinities for the series allowed fitting of robust models for σ_1 and σ_2 binding, and complemented previous qualitative studies of related compounds[7a,b] and quantitative[8a,b] studies of the σ_1 receptor binding of several different structural classes. We also evaluated three representative compounds (**3a**, **d**, **h**) for binding against [^{3}H](+)-pentazocine in the presence of the positive allosteric modulator phenytoin to determine if the series was likely to exhibit σ_1 receptor antagonism.[9a,b]

2. Results and discussion

2.1. Receptor binding and qualitative SAR

All of the *N*-phenylpropyl-*N*′-benzylpiperazines displayed high affinity for σ_1 sites (Table 1). Seven of the 10 compounds exhibited subnanomolar apparent affinities (K_i values), with 3-iodo (**3d**) and 4-nitro (**3i**) substitution exerting particularly strong effects. A wider range of affinities was noted for σ_2 receptor binding, with the most potent compound **3d** (K_i 1.0 nM) having a 3-iodo substituent, and the least potent compounds being the parent **3a** and the 4-methoxy analog **3h** (K_i 33–34 nM). Thus, σ_1/σ_2 selectivity is dominated by the relative σ_2 affinity, with 40- to 50-fold σ_1 selectivities noted for **3a** and **3h**. Haloperidol and SA4503 (*N*-phenylpropyl-*N*′-3,4-dimethoxyphenethyl piperazine) were included for reference in these assays, and gave K_i values near those reported.[10] Previous studies[4] showed excellent binding selectivity for compound **3a** to σ receptors (20 nM) over serotonin 5-HT$_{1A}$ and dopamine D$_2$ receptors (IC$_{50}$ values >100 μM). Affinities for additional recognition sites (e.g., monoamine transporters) do not appear to have been studied. However, some structurally related compounds, such as SA4503,[11] do exhibit high and preferential binding to σ receptors over a number of other receptors and ion channels. In the present work, congeners **3a**–**3j** were tested in binding assays for the classical opioid receptors (μ, δ, and κ) and uniformly exhibited poor affinities (<5% displacement of radioligand binding at 1–2 μM, data not shown).

Table 1. In vitro affinity and selectivity of compounds **3a**–**3j** for σ_1 and σ_2 receptors[a]

	K_i (nM) σ_1	K_i (nM) σ_2	Selectivity $K_i\sigma_2/K_i\sigma_1$
3a (lead, R = H)	0.66 ± 0.06	34.33 ± 2.69	52.0
3b (R = 2-Br)	0.60 ± 0.01	4.12 ± 0.19	6.9
3c (R = 2-NO$_2$)	2.80 ± 0.04	3.79 ± 0.23	1.4
3d (R = 3-I)	0.39 ± 0.05	1.03 ± 0.08	2.6
3e (R = 3-F)	1.36 ± 0.08	13.85 ± 0.98	10.2
3f (R = 3-OCH$_3$)	0.87 ± 0.01	14.19 ± 0.26	16.3
3g (R = 3-NO$_2$)	0.93 ± 0.02	1.59 ± 0.08	1.7
3h (R = 4-OCH$_3$)	0.76 ± 0.07	32.81 ± 2.93	43.2
3i (R = 4-NO$_2$)	0.37 ± 0.01	3.29 ± 0.34	8.9
3j (R = 4-CH$_3$)	1.17 ± 0.01	17.51 ± 2.51	15.0
Haloperidol	0.83 ± 0.03	9.58 ± 0.98	11.5
SA4503	4.34 ± 0.31	89.51 ± 7.97	20.6

[a] Mean ± SEM; n = 3–6.

The σ_1 receptor binding proved sensitive to the position of nitro substitution, with an 8-fold increase in affinity observed over the ortho-, meta-, and para-isomers (**3i** > **3g** > **3c**). By contrast, notable changes in σ_2 affinity were not observed. Having electron-donating methoxy and methyl groups in the *para*-position decreased both σ_1 and σ_2 receptor affinity with respect to the electron-withdrawing *para*-nitro substituent, with a greater detrimental effect on σ_2 binding than σ_1 binding. The halogen series showed a qualitative relationship, regardless of position of substitution, for higher σ_1 and σ_2 receptor affinity with increasing size, hydrophobicity, and polarizability (**3d** > **3b** > **3e**).

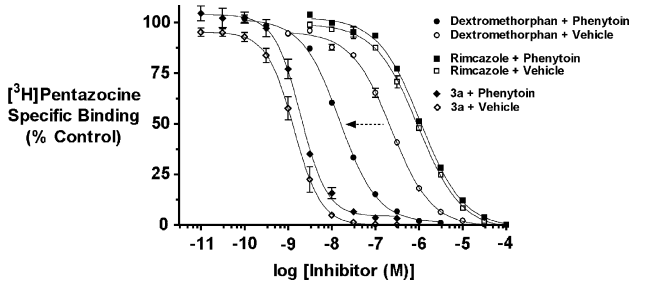

Figure 2. Phenytoin causes a 15-fold shift (arrow) to higher affinity in GPB membranes for the σ_1 agonist dextromethorphan, but only a small shift, to lower affinity, for the σ_1 antagonist rimcazole. *N*-Phenylpropyl-*N*′-benzylpiperazine (**3a**) behaves like an antagonist in this assay. Points are means ± SEM for 3–5 trials, each performed in duplicate.

2.2. Agonist/antagonist profiling

Cobos et al.[9a,b] reported competition binding assays in guinea pig brain (GPB) homogenates that use [^{3}H](+)-pentazocine in the presence of the allosteric modulator phenytoin to differentiate σ_1 receptor agonists from antagonists. In brief, known agonists exhibit major shifts to higher affinity, while known antagonists show either little change or modest shifts to lower affinity. As shown in Figure 2, binding parameter changes for the lead compound (**3a**, R = H) under these conditions were consistent with designation as an antagonist. The IC_{50}s were not significantly different ($p > 0.05$) in the presence and absence of phenytoin, but the ratio (1.47 ± 0.29 nM/1.89 ± 0.08 nM; vehicle/phenytoin) was less than unity (0.8) as previously found for a series of σ_1 receptor antagonists including haloperidol, NE100, BD1063, BD1047, and progesterone.[9a,b] Similar results were obtained for the 3-iodo (**3d**) and 4-methoxy (**3h**) analogs (data not shown). In validation studies (Fig. 2), we find the previously reported[9a,b] 15-fold shift to higher affinity for the σ_1 agonist dextromethorphan; viz., IC_{50} 232 ± 8.8 nM (Hill slope 0.95 ± 0.01) in the absence of phenytoin and 15.2 ± 0.5 nM (Hill slope 1.00 ± 0.04) in the presence of phenytoin. We also tested rimcazole, a low affinity σ_1 receptor antagonist and dopamine transporter inhibitor that can block (−)-cocaine effects in vivo.[12] Binding parameter changes in the presence (IC_{50} 1087 ± 241 nM; Hill slope 0.89 ± 0.05) or absence (IC_{50} 960 ± 77 nM; Hill slope 0.90 ± 0.05) of phenytoin were not significant ($p > 0.05$), and the IC_{50} ratio was less than unity (0.9) as expected. Although agonist and antagonist properties are best determined in functional assays, this series of *N*-phenylpropyl-*N*′-benzylpiperazines profiles as a set of probable σ_1 receptor antagonists that might have the functional ability to block some actions of (−)-cocaine in vivo.

2.3. Quantitative SAR

We employed the Multiple Linear Regression (MLR) approach, using the ordinary least squares method, that is especially useful for analyzing data when the number of physicochemical descriptors is smaller than the number of compounds.[13] While a multitude of regression equations were judged significant, the equations shown below represent the highest correlation coefficients (r^2) and *F* statistics. The number of compounds (*n*) used to establish the equation is listed, and values in parentheses are the 95% confidence interval of the regression coefficients. The *t* parameter gave decisive information regarding the importance of a single independent variable in a model involving all other independent variables (Table 2). The standard error of the multiple linear regression, *s*, was also taken in consideration. The symbol *q* denotes the predictive effectiveness of the model and is the square root of the cross-validated coefficient of determination, q^2.

Eq. 1 shows that σ_1 binding affinity is dependent on the hydrophobicity (π_x), Molar Refractivity (MR), and the sigma Hammett constant values for the corresponding *meta* and *para*-substituted compounds ($\sigma_{m,p}$).

$$\begin{aligned} \mathrm{Log}(1/K_i) = &-0.63(\pm 0.47) - 1.54(\pm 1.45)(\pi_x)^2 \\ &+ 1.26(\pm 1.01)(\pi_x) \\ &+ 0.96(\pm 0.79)\mathrm{MR} \\ &+ 0.62(\pm 0.37)\sigma_{m,p}, \end{aligned} \quad (1)$$

$$n = 9;\ r^2 = 0.89;\ F_{4,8} = 7.93;\ s = 0.13;$$

$$q^2 = 0.43;\ 0.01 < P < 0.05$$

The inclusion of compound **3a** (R = H) gave an equation having less statistical significance. Interestingly, the negative dependence of π and binding affinity is similar to the relationship described by Mascarella and co-workers for the σ_1 binding of a series of normetazocines.[8a] Utilizing the Molar Refractivity term gave a better correlation than the Taft Steric Effect (E_s) (data not shown). This implies that increasing the substituent size enhances the binding potency, and that the polarizability component of Molar Refractivity also plays a role in binding. The dependence on sigma Hammett values ($\sigma_{m,p}$) suggests that electron withdrawing groups in the *meta*- and *para*-positions improve σ_1 receptor affinity. The use of σ_p instead of σ^- for compound **3i**

Table 2. Values for *t* parameters

Variable	Eq. 1		Eq. 2		Eq. 3	
	Number (significance)	*t*	Number (significance)	*t*	Number (significance)	*t*
Constant	−0.630 (0.020)	−3.728	−1.495 (0.000)	−11.227	−1.495 (0.114)	−13.071
π	1.255 (0.026)	3.453	−0.968 (0.037)	−2.830	−1.001 (0.279)	−3.586
π^2	−1.535 (0.042)	−2.937	1.502 (0.019)	3.391	1.493 (0.374)	3.990
MR/E_s	0.965 (0.027)	3.399	−0.405 (0.084)	−2.155	−0.370 (0.162)	−2.281
σ	0.617 (0.011)	4.486	0.505 (056)	2.477	0.548 (0.173)	3.174

(R = 4-NO_2) decreases the correlation coefficient and the statistical significance of the model. This suggests that a nitro group in the *para*-position increases the binding affinity dramatically due to geometric requirements, or favorable charge interactions with an unknown binding region of the protein. These results agree with those from Ruoho's group on the σ_1 receptor binding of (−)-cocaine derivatives, where reduced π electron density caused by a *para*-nitro substituent on the phenyl ring promotes high-affinity binding through favorable interactions with electron-rich residues of the steroid binding domain-like regions.[14] Further, differential binding data across the present nitro series indicate that the precise orientation of the dipole moment is an important contributor to the σ_1 receptor–ligand binding interaction. In general, the statistical parameters of Eq. 1 may have been more robust had the K_i values been dispersed over a broader range.

Observed K_i values for all ten compounds, ranging from 1.0 to 34.3 nM, yielded a quality correlation equation for σ_2 binding affinity where substituent hydrophobicity proved a major contributor (Eq. 2).

$$\begin{aligned}\mathrm{Log}(1/K_i) = &-1.50(\pm0.34) + 1.50(\pm1.14)(\pi_x)^2 \\ &- 0.96(\pm0.88)(\pi_x) \\ &- 0.41(\pm0.48)E_s \\ &+ 0.50(\pm0.53)\sigma_{m,p}, \\ n = 10;\ r^2 = 0.95;\ F_{4,9} = 22.22;\ s = 0.17;& \\ q^2 = 0.79;\ P < 0.01&\end{aligned} \quad (2)$$

Introducing Hammett sigma values for the two *ortho*-substituted compounds gave a poorer performance in the equation derived for σ_1 binding, but a better performance for σ_2 binding as shown below Eq. 3.

$$\begin{aligned}\mathrm{Log}(1/K_i) = &-1.45(\pm0.30) + 1.48(\pm0.97)(\pi_x)^2 \\ &- 0.99(\pm0.72)(\pi_x) \\ &- 0.38(\pm0.42)E_s \\ &+ 0.55(\pm0.45)\sigma_{o,m,p}, \\ n = 10;\ r^2 = 0.96;\ F_{4,9} = 29.95;& \\ s = 0.15;\ q^2 = 0.86;\ P < 0.001&\end{aligned} \quad (3)$$

The positive parabolic form of the hydrophobicity term of Eqs. 2 and 3 as opposed to a negative sign in the case of Eq. 1 constitutes a major binding pharmacophore difference between the two σ receptors. The second apparent difference stems from utilizing E_s in Eqs. 2 and 3 instead of MR, suggesting the polarizability of the substituent has a pronounced effect on σ_2, but not σ_1, receptor binding. Further, both equations describe a binding affinity increase for electron-withdrawing groups in the *meta*- and *para*-positions. However, including σ_o values for the *ortho* substituents improves the quality of the correlation equation for σ_2 binding. This third differentiation indicates that *ortho* substituents interact favorably with the σ_2 receptor, leading to moderately improved ligand binding. A good correlation is observed between the actual pK_i values obtained and the values derived from Eqs. 1 and 3 for the σ_1 and σ_2 receptor subtypes, respectively (Fig. 3).

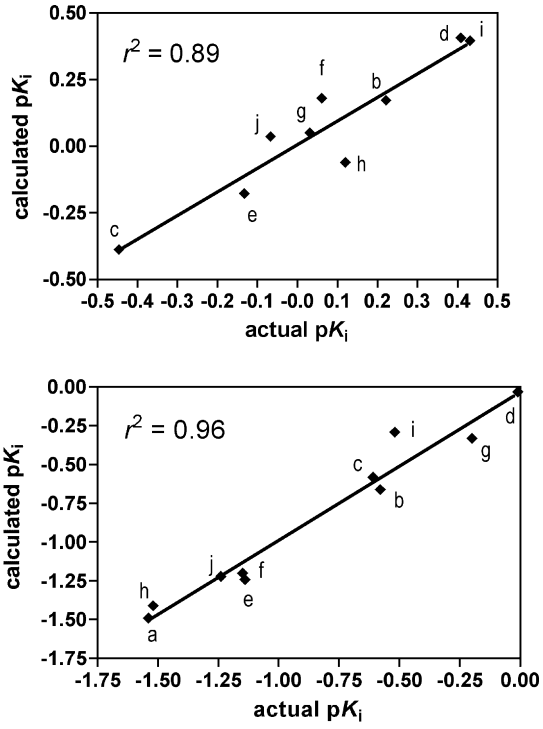

Figure 3. Actual pK_i versus calculated pK_i for σ_1 receptor binding affinity using Eq. 1 (upper panel). Actual pK_i versus calculated pK_i for σ_2 receptor binding affinity using Eq. 3 (lower panel).

3. Conclusion

A foundation has been established to correlate structure with σ_1 and σ_2 receptor binding affinity for a series of *N*-(3-phenylpropyl)-*N*′-benzylpiperazines. The results revealed a number of potent ligands, in particular for the σ_1 subtype. The predictive ability of these equations is of good statistical quality and showed interesting results regarding the quantified dependence of the binding affinity upon the studied physicochemical parameters. The hydrophobicity terms in the equations exhibited opposite signs for σ_1 and σ_2, which can be relied upon to design selective ligands, and a better understanding of the binding pharmacophore difference between the two subtypes.

4. Experimental

4.1. General procedures

^{1}H NMR spectra were determined on Bruker 250 or 300 MHz spectrometers. Chemical shifts are reported as parts per million (δ) relative to internal Me_4Si in $CDCl_3$, with coupling constants (J) given in hertz (Hz). Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA), and were in agreement with calculated values (C, H, N: ±0.4%). Short-path silica gel (Merck 7729, <230 mesh) chromatography was

conducted under N_2 pressure. Analytical TLC was performed with Macherey-Nagel silica gel 60 UV-254 plates (250 μm). *N*-Phenylpropylpiperazine was prepared as previously described.[15] OptiPhase® HiSafe 2 scintillation cocktail, [^{3}H](+)-pentazocine ([^{3}H](+)-PTZ, 34 Ci/mmol), [^{3}H]1,3-di(2-tolyl)guanidine ([^{3}H]DTG, 58 Ci/mmol), and [^{3}H]naltrindole ([^{3}H]NTI, 20 Ci/mmol) were from Perkin-Elmer Life Sciences, Inc. (Boston, MA), while [^{3}H]DAMGO (50 Ci/mmol) and [^{3}H]U69,593 (50 Ci/mmol) were from GE Healthcare Bio-Sciences Corp. (Piscataway, NJ). Other chemicals and solvents were of the best grades available, and were used as received from commercial sources.

4.2. General method for the preparation of compounds 3a–3j

The appropriate benzyl bromide (1.0 mmol), *N*-phenylpropylpiperazine (1.0 mmol), NaI (150 mg, 1.0 mmol), and K_2CO_3 (0.41 g, 3.0 mmol) were heated in DMF (10 mL) for 2 h at 60 °C. The mixture was filtered, and then concentrated under reduced pressure at 60 °C. The residue was partitioned between water and EtOAc, and the organic layer washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Column chromatography using a gradient of *n*-hexane/EtOAc (5:1–1:4) gave the target compounds in 70–88% yields as colorless to pale yellow oils that were stored in free base form. Analytical TLC R_f values ranged from 0.2 to 0.4 (1:1, *n*-hexane/EtOAc). ^{1}H NMR and elemental analysis data agreed with the assigned structures. Analytical reversed-phase HPLC, using a Symmetry C18 column (4.6 × 150 mm, 5 μm; Waters Corp., Milford, MA) and a ternary mobile phase of MeOH (25%), CH_3CN (25%), and water (50%) containing Et_3N (1.5%) and HOAc (2%) at a flow rate of 1 mL/min with detection at 254 nm, showed ⩾98% purity for each compound and provided retention times (t_R) and capacity factors (k').

5. Chemistry

A common precursor, *N*-phenylpropylpiperazine, was prepared according to the literature procedure.[15] Alkylation by the corresponding benzyl bromide, in the presence of potassium carbonate and KI, provided **3a**–**3j** as oils in 70–88% yields.

5.1. 1-Benzyl-4-(3-phenylpropyl)piperazine (3a)

^{1}H NMR δ 1.85 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.42 (2H, t, J = 7.5 Hz, –CH_2–CH_2–Ph), 2.53 (8H, m, Pip), 2.67 (2H, t, J = 7.5 Hz, –N–CH_2–CH_2–), 3.56 (2H, s, Ph–CH_2–N), 7.19–7.38 (10H, m, Ph). Yield: 78%. Anal ($C_{20}H_{26}N_2$). Theory: C, 81.59; H, 8.90; N, 9.51. Found: C, 81.22; H, 8.95; N, 9.59. HPLC: t_R = 5.1, k' = 2.0.

5.2. 1-(2-Bromobenzyl)-4-(3-phenylpropyl)piperazine (3b)

^{1}H NMR δ 1.85 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.4 (2H, t, J = 7.5 Hz, –CH_2–CH_2–Ph), 2.52 (4H, m, Pip), 2.6 (4H, m, Pip), 2.67 (2H, t, J = 7.5 Hz, –N–CH_2–CH_2–), 3.64 (2H, s, Ph–CH_2–N–), 7.09–7.55 (8H, m, Ph), 7.57 (1H, d, *ortho* to Br, J = 9 Hz). Yield: 70%. Anal ($C_{20}H_{25}N_2Br$). Theory: C, 64.34; H, 6.75; N, 7.50. Found: C, 64.05; H, 6.77; N, 7.49. HPLC: t_R = 9.3, k' = 4.8.

5.3. 1-(2-Nitrobenzyl)-4-(3-phenylpropyl)piperazine (3c)

^{1}H NMR δ 1.75 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.35 (2H, t, J = 7.5 Hz, –CH_2–CH_2–Ph), 2.47 (8H, m, Pip), 2.65 (2H, t, J = 7.5 Hz, –N–CH_2–CH_2–), 3.8 (2H, s, Ph–CH_2–N–), 7.15–7.79 (8H, m, Ph), 7.82 (1H, d, *ortho* to NO_2, J = 12.5 Hz). Yield: 83%. Anal ($C_{20}H_{25}N_3O_2$). Theory: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.50; H, 7.48; N, 12.26. HPLC: t_R = 6.1, k' = 2.6.

5.4. 1-(3-Iodobenzyl)-4-(3-phenylpropyl)piperazine (3d)

^{1}H NMR δ 1.85 (2H, apparent pentet, J = 7.6 Hz, –CH_2–CH_2–CH_2–), 2.4 (2H, t, J = 7.6 Hz, –CH_2–CH_2–Ph), 2.49 (8H, m, Pip), 2.65 (2H, t, J = 7.6 Hz, –N–CH_2–CH_2–), 3.46 (2H, s, Ph–CH_2–N–), 7.02–7.31 (7H, m, Ph), 7.60 (1H, d, *meta* to I, J = 7.8 Hz), 7.71 (1H, s, *ortho* to I and CH_2–). Yield: 76%. Anal ($C_{20}H_{25}N_2I$). Theory: C, 57.15; H, 5.99; N, 6.66. Found: C, 57.03; H, 5.95; N, 6.61. HPLC: t_R = 13.1, k' = 6.7.

5.5. 1-(3-Fluorobenzyl)-4-(3-phenylpropyl)piperazine (3e)

^{1}H NMR $CDCl_3$ δ 1.86 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.42 (2H, t, J = 7.5 Hz, –CH_2–CH_2–Ph), 2.52 (8H, m, Pip), 2.69 (2H, t, J = 7.5 Hz, –N–CH_2–CH_2–), 3.59 (2H, s, Ph–CH_2–N–), 6.95–7.36 (9H, m, Ph). Yield: 88%. Anal ($C_{20}H_{25}N_2F$). Theory: C, 76.84; H, 8.07; N, 8.97. Found: C, 76.91; H, 8.08; N, 9.10. HPLC: t_R = 5.9, k' = 2.5.

5.6. 1-(3-Methoxybenzyl)-4-(3-phenylpropyl)piperazine (3f)

^{1}H NMR δ 1.85 (2H, apparent pentet, J = 7.6 Hz, –CH_2–CH_2–CH_2–), 2.4 (2H, t, J = 7.6 Hz, –CH_2–CH_2–Ph), 2.51 (8H, m, Pip), 2.63 (2H, t, J = 7.6 Hz, –N–CH_2–CH_2–), 3.51 (2H, s, Ph–CH_2–N–), 3.83 (3H, s, Ph–OCH_3), 6.78–7.34 (9H, m, Ph). Yield: 84%. Anal ($C_{21}H_{28}N_2O$). Theory: C, 76.67; H, 8.73; N, 8.52. Found: C, 76.82; H, 8.73; N, 8.53. HPLC: t_R = 5.2, k' = 2.1.

5.7. 1-(3-Nitrobenzyl)-4-(3-phenylpropyl)piperazine (3g)

^{1}H NMR δ 1.81 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.39 (2H, t, J = 7.5 Hz, –CH_2–CH_2–Ph), 2.50 (8H, m, Pip), 2.64 (2H, t, J = 7.5 Hz, –N–CH_2–CH_2–), 3.6 (2H, s, Ph–CH_2–N–), 7.16–7.68 (7H, m, Ph), 8.12 (1H, d, *meta* to NO_2, J = 9.6 Hz), 8.21 (1H, s, *ortho* to NO_2 and CH_2–). Yield: 70%. Anal ($C_{20}H_{25}N_3O_2$). Theory: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.31; H, 7.50; N, 12.12. HPLC: t_R = 5.6, k' = 2.3.

5.8. 1-(4-Methoxybenzyl)-4-(3-phenylpropyl)piperazine (3h)

^{1}H NMR δ 1.85 (2H, apparent pentet, J = 7.6 Hz, –CH_2–CH_2–CH_2–), 2.39 (2H, t, J = 7.6 Hz, –CH_2 –CH_2–Ph), 2.48 (8H, m, Pip), 2.64 (2H, t, J = 7.6 Hz, –N–CH_2–CH_2–), 3.46 (2H, s, Ph–CH_2–N–), 3.81 (3H, s, Ph–OCH_3), 6.78–7.31 (9H, m, Ph). Yield: 84%. Anal ($C_{21}H_{28}N_2O$·0.5H_2O). Theory: C, 75.68; H, 8.55; N, 8.40. Found: C, 76.06; H, 8.60; N, 8.25. HPLC: t_R = 5.0, k' = 1.9.

5.9. 1-(4-Nitrobenzyl)-4-(3-phenylpropyl)piperazine (3i)

^{1}H NMR δ 1.84 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.37 (2H, t, J = 7.5 Hz –CH_2 –CH_2–Ph), 2.5 (8H, m, Pip), 2.67 (2H, t, J = 7.5 Hz, –N–CH_2–CH_2–), 3.6 (2H, s, Ph–CH_2–N–), 7.2–7.31 (5H, m, Ph), 7.52 (2H, d, *ortho* to –CH_2, J = 8.7 Hz), 8.18 (2H, d, *ortho* to NO_2, J = 8.7 Hz). Yield: 70%. Anal ($C_{20}H_{25}N_3O_2$). Theory: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.65; H, 7.51; N, 12.20. HPLC: t_R = 6.5, k' = 2.8.

5.10. 1-(4-Methylbenzyl)-4-(3-phenylpropyl)piperazine (3j)

^{1}H NMR δ 1.85 (2H, apparent pentet, J = 7.5 Hz, –CH_2–CH_2–CH_2–), 2.35 (2H, s, Ph–CH_3), 2.39 (2H, t, J = 7.5 Hz, –CH_2–CH_2–Ph), 2.65 (2H, t, J = 7.5 Hz, N–CH_2–CH_2–), 3.49 (2H, s, Ph–CH_2–N), 7.12–7.29 (9H, m, Ph). Yield: 82%. Anal ($C_{21}H_{28}N_2$). Theory: C, 81.77; H, 9.15; N, 9.08. Found: C, 81.91; H, 9.30; N, 9.19. HPLC: t_R = 5.6, k' = 2.3.

6. Receptor binding assays

Competition binding assays for ligands at σ_1 and σ_2 receptors were performed using [^{3}H](+)-PTZ (σ_1), [^{3}H]DTG/ 200 nM (+)-PTZ (σ_2), and membranes from fresh-frozen, male English Hartley guinea pig brains (Rockland Immunochemicals, Inc.; Gilbertsville, PA) as previously described.[10] Experiments were performed in duplicate and repeated 3–6 times. K_i values were calculated from the inhibition data using the Cheng-Prusoff equation,[16] and a σ_1 K_d of 2.3 nM for [^{3}H](+)-PTZ and a σ_2 K_d of 23.9 nM for [^{3}H]DTG.[10] Phenytoin modulation of ligand binding to σ_1 receptors was investigated using minor modifications of reported procedures.[9] Assays were conducted as described above for [^{3}H](+)-PTZ, except DPH (50 μL, 20 mm) in NaOH vehicle (0.15 M) was added to every tube. Control experiments, where only NaOH (50 μL, 0.15 M) was added, also were conducted. The incubation medium for these assays had pH 7.44 at 37 °C, while the medium had pH 7.06 at 37 °C for [^{3}H](+)-PTZ assays done in the absence of NaOH or DPH/NaOH. Opioid receptor binding assays were conducted in membranes from guinea pig (μ,κ) or mouse (δ) brains using [^{3}H]NTI (δ), [^{3}H]DAMGO (μ), and [^{3}H]U69,593 (κ) as previously reported.[17]

7. QSAR analysis

The QSAR of compounds **3a**–**3j** were analyzed by the Hansch–Fujita method,[18] using physicochemical descriptors that represent hydrophobic, electronic, and steric effects. Physicochemical parameters were taken from Hansch et al.[19] and we employed published σ values[20a,b] for 2-Br and 2-NO_2 in Eq. 3. We utilized Multiple Linear Regression analysis with SAS statistical software (v. 9.0) to generate regression equations that were evaluated by their correlation coefficients (r), F statistics, t parameters, and standard errors, s.

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References and notes

1. Matsumoto, R. R.; Liu, Y.; Lerner, M.; Howard, E. W.; Brackett, D. J. *Eur. J. Pharmacol.* **2003**, *469*, 1–12.
2. Foster, A.; Wu, H.; Chen, W.; Williams, W.; Bowen, W. D.; Matsumoto, R. R. *Coop, A* **2003**, *13*, 749–751.
3. Matsumoto, R. R.; Hourcade-Potelleret, F.; Mack, A.; Pouw, B.; Zhang, Y.; Bowen, W. D. *Pharmacol. Biochem. Behav.* **2004**, *77*, 775–781.
4. Younes, S.; Labssita, Y.; Baziard-Mouysset, G.; Payard, M.; Rettori, M.; Renard, P.; Pfeiffer, B.; Caignard, D. *Eur. J. Med. Chem.* **2000**, *35*, 107–121.
5. Leo, A. L.; Hansch, C. *Perpect. Drug Discov. Design* **1999**, *17*, 1–25.
6. *Methods and Principles in Medicinal Chemistry 2: Chemometric Methods in Molecular Design*; Mannhold, R., Krogsgaard-Larsen, P., Timmerman, H., Van de Waterbeemd, H., Eds.; Springer: Germany, 1995 (*2*).
7. (a) Glennon, R. A. *Mini-Rev. Med. Chem.* **2005**, *5*, 927–940; (b) Glennon, R. A. *Brazil J. Pharm. Sci.* **2005**, *41*, 1–12.
8. (a) Mascarella, S. W.; Bai, X.; Sine, B.; Bowen, W. D.; Carroll, F. I. *J. Med. Chem.* **1995**, *38*, 565–569; (b) Gund, T. M.; Floyd, J.; Jung, D. *J. Mol. Graph. Model.* **2004**, *22*, 221–230.
9. (a) Cobos, E. J.; Baeyens, J. M.; Del Pozo, E. *Synapse* **2005**, *55*, 192–195; (b) Cobos, E. J.; Lucena, G.; Baeyens, J. M.; Del Pozo, E. *Synapse* **2006**, *59*, 152–161.
10. Lever, J. R.; Gustafson, J. L.; Xu, R.; Allmon, R. L.; Lever, S. Z. *Synapse* **2006**, *59*, 350–358.
11. Matsuno, K.; Nakazawa, M.; Okamoto, K.; Kawashima, Y.; Mita, S. *Eur. J. Pharmacol.* **1996**, *306*, 271–279.
12. Matsumoto, R. R.; Hewett, K. L.; Pouw, B.; Bowen, W. D.; Husbands, S. M.; Cao, J. J.; Hauck Newman, A. *Neuropharmacology* **2001**, *41*, 878–886.
13. Hadjipavlou-Litina, D.; Garg, R.; Hansch, C. *Chem. Rev.* **2004**, *104*, 3751–3793.
14. Chen, Y.; Hajipour, A. R.; Sievert, M. K.; Arbabian, M.; Ruoho, A. E. *Biochemistry* **2007**, *46*, 3532–3542.
15. Kawamura, K.; Elsinga, P. H.; Kobayashi, T.; Ishii, S.-I.; Wang, W.-F.; Matsuno, K.; Vaalburg, W.; Ishiwata, K. *Nucl. Med. Biol.* **2003**, *30*, 273–284.
16. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
17. Duval, R. A.; Allmon, R. L.; Lever, J. R. *J. Med. Chem.* **2007**, *50*, 2144–2156.

18. Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616–1626.
19. Hansch, C.; Leo, A.; Hoekman, D. *Hydrophobic, Electronic, and Steric Constants in Exploring QSAR. Fundamentals and Applications in Chemistry and Biology*; American Chemical Society: Washington, DC, 1995.
20. (a) Smith, G. G.; Lum, K. K. *Chem. Commun. (London)* **1968**, *20*, 1208; (b) Pressman, D.; Brown, D. H. *J. Am. Chem. Soc.* **1943**, *65*, 540–543.