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Synthesis and in vitro evaluation of tetrahydroisoquinolinyl benzamides as ligands for σ receptors

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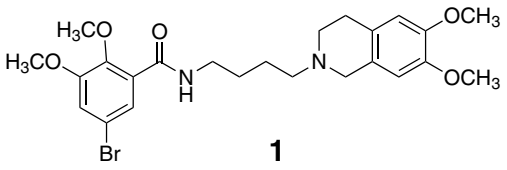
Abstract—5-Bromo-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)-butyl)]-2,3-dimethoxy-benzamide (**1**) is one of the most potent and selective σ_2 receptor ligands reported to date. Previous structure-activity relationship studies of such tetrahydroisoquinolinyl benzamides have focused on the linker that connects the ring systems and the effects of benzamide ring substituents. The present study explores the effects of fusing methylene-, ethylene-, and propylenedioxy rings onto the tetrahydroisoquinoline in place of the two methoxy groups. These modifications decreased σ_2 affinity by 8- to 12-fold, with no major differences noted with ring size. By contrast, the methylenedioxy analog showed a 10-fold greater σ_1 affinity than **1**, and progressively lower σ_1 affinities were then noted with increasing ring size. We also opened the tetrahydroisoquinoline ring of **1** to study the effects of greater conformational fluidity on σ receptor binding. The σ_2 affinity of the open-ring compound decreased by 1700-fold, while σ_1 affinity was not changed. Thus, a constrained tetrahydroisoquinoline ring system is key to the exceptional σ_2 receptor binding affinity and selectivity of this active series.

Functional sigma (σ) receptors are located throughout the brain and periphery, and can be differentiated into σ_1 and σ_2 subtypes.[1–4] These subtypes play distinct functional roles and have different pharmacological characteristics. Both σ_1 and σ_2 subtypes are involved in central nervous system disorders such as schizophrenia, depression, and dementia.[1,2] Certain σ receptor antagonists can ameliorate the effects of cocaine and other psychostimulant drugs of abuse, and have potential as medications.[1–3] Moreover, σ receptors are over-expressed by many cancers.[4] Some σ receptor ligands induce apoptosis in cancer cells,[5–7] and one is in a clinical trial for prostate cancer treatment.[8] Thus, there is much interest in subtype selective σ receptor ligands as molecular probes and as therapeutic agents.

A variety of structural classes are avid binders to both σ receptor subtypes which has hampered the development of selective binding models.[9,10] Although a number of studies have investigated the effects of structure on relative σ_1/σ_2 receptor binding affinity and selectivity, few truly selective compounds are known. Recently, Mach and colleagues[11] identified a series of tetrahydroisoquinolinyl benzamides that rank among the most selective σ_2 receptor ligands known to date. For example, **1** displays high apparent affinity, $K_i = 8.2$ nM, for σ_2 sites in vitro accompanied by 1573-fold selectivity over σ_1 sites.



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Published structural modifications have concentrated on the length of the alkyl spacer connecting the two different ring systems, and the effects of various benzamide substituents.[11–13] To extend the structure-activity relationships for this active series, we report on the effects

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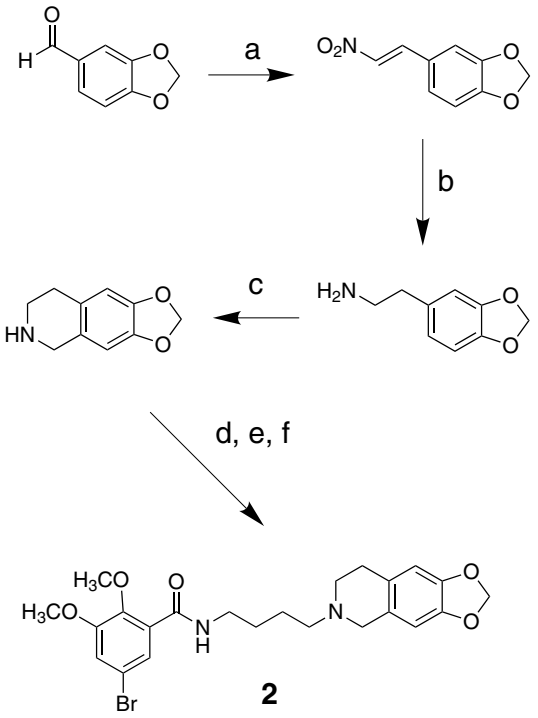
of fusing methylene-, ethylene-, and propylenedioxy rings onto the tetrahydroisoquinoline. This stems from work on 1,4-disubstituted piperazines, where we found that σ receptor subtype affinity and selectivity can be modulated by similar manipulations of dimethoxybenzene moieties.[14] In addition, we noticed that C–N bond rotation in **1** is limited by the tetrahydroisoquinoline ring. Thus, we wished to open this ring to gain insight into the contributions of conformational fluidity to σ receptor binding.

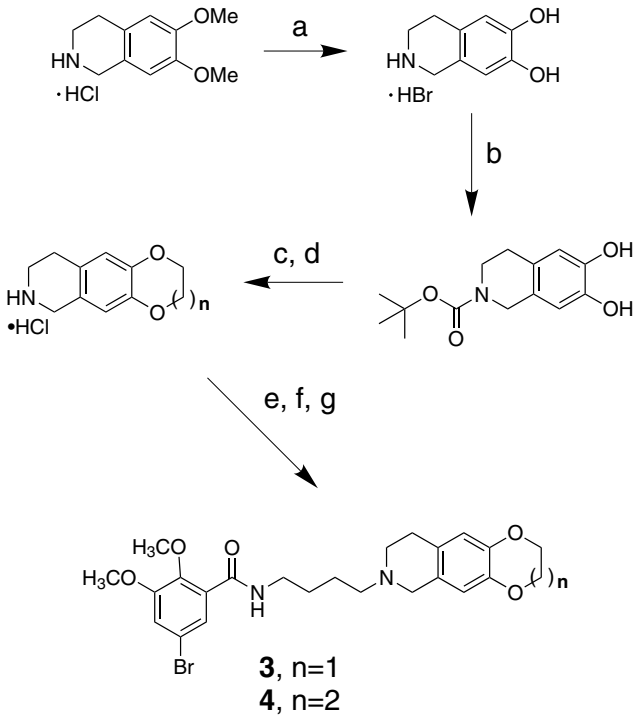
Compound **1** was obtained for reference using the reported methods.[11] The novel congeners were prepared as shown in Schemes 1–3. For methylenedioxy analog **2**, the corresponding tetrahydroisoquinoline was synthesized from piperonal using an established route that culminates with the Pictet–Spengler reaction.[15–17] Alkylation with 4-bromobutanenitrile, followed by reduction and amidation with 5-bromo-2,3-dimethoxybenzoyl chloride, afforded **2** which was characterized as the oxalate salt (Scheme 1).[18]

Ethylenedioxy (**3**) and propylenedioxy (**4**) analogs were synthesized in parallel fashion from their corresponding tetrahydroisoquinolines (Scheme 2). In turn, these three-ring heterocycles were obtained from *N*-Boc protected tetrahydroisoquinoline diol by base-promoted cycloalkylation with the appropriate dibromoalkane catalyzed by tetrabutylammonium bromide (Scheme 2).

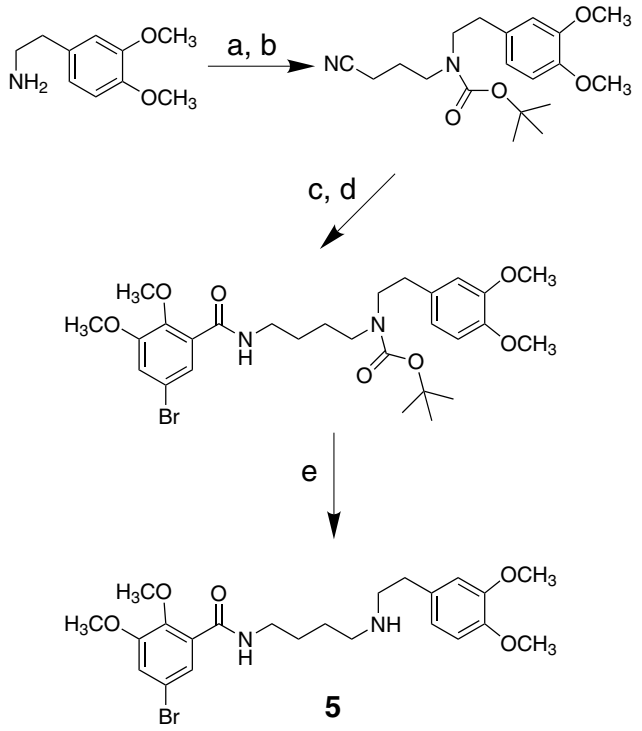
As shown in Scheme 3, open-ring compound **5** was prepared by alkylation of the commercially available 2-(3,4-dimethoxyphenyl)ethanamine, followed by *N*-Boc protection, reduction, amidation, and deprotection.



Scheme 1. Reagents: (a) CH_3NO_2, MeOH, NaOH; (b) $LiAlH_4$; (c) paraformaldehyde; (d) 4-bromobutanenitrile, K_2CO_3, NaI, DMF; (e) $LiAlH_4$; (f) 5-bromo-2,3-dimethoxybenzoyl chloride.



Scheme 2. Reagents and conditions: (a) 48% HBr, 120 °C, 2 h; (b) $(Boc)_2O$, MeOH, Et_3N; (c) Br-$(CH_2)_n$-Br: n = 2, 3, TBAB; (d) 4 N HCl; (e) 4-bromobutanenitrile, K_2CO_3, NaI, DMF; (f) $LiAlH_4$; (g) 5-bromo-2,3-dimethoxybenzoyl chloride.



Scheme 3. Reagents: (a) 4-bromobutanenitrile; (b) $(Boc)_2O$, MeOH, Et_3N; (c) $LiAlH_4$; (d) 5-bromo-2,3-dimethoxybenzoyl chloride; (e) 10% TFA, CH_2Cl_2.

Table 1. Binding properties of compounds **1**–**5** at σ_1 and σ_2 receptors[19]

Compound	K_i (nM)		Ratio σ_1/σ_2
	σ_1	σ_2	
1	881 ± 15	2.7 ± 0.1	326
2	82.2 ± 5.6	20.7 ± 2.0	4
3	338 ± 8.4	21.7 ± 1.2	16
4	1430 ± 36	32.6 ± 1.5	44
5	880 ± 60	4616 ± 247	0.2

Values are means ± SEM (n = 3–5) from competition assays against [^{3}H](+)-pentazocine (σ_1) and [^{3}H]DTG/(+)-pentazocine (σ_2) in membranes from male guinea pig brains.

As expected, compound **1** displayed very high affinity and selectivity for σ_2 sites in vitro (Table 1). The degree of σ_2 selectivity, based upon K_i ratios, was somewhat less than previously found[11] as a consequence of a higher apparent affinity for σ_1 sites. The σ_1 receptor assay in guinea pig brain membranes is susceptible to slight changes in conditions. So, we also tested **1** using the previously reported regimen (pH 8.0 vs pH 7.4 buffer, 3.0 nM vs 1.0 nM [^{3}H](+)-pentazocine, 25 °C vs 37 °C, 120 min vs 150 min, and 10 μM (+)-pentazocine vs 1.0 μM haloperidol to define nonspecific binding). The σ_1 receptor IC_{50} value of 1273 ± 22 nM found for **1** under the present conditions increased substantially, about 50%, to 1895 ± 110 nM. Comparing this lower affinity σ_1 receptor IC_{50} with the σ_2 receptor IC_{50} of 3.0 ± 0.11 for **1** under the present conditions would double the selectivity assigned. Also, the σ_2 receptor binding was assessed using rat liver membranes in the previous work, while guinea pig brain membranes were employed in the present study. In such ways, experimental factors can impact the σ_1/σ_2 subtype selectivity determinations from various laboratories.

Replacement of the two methoxy groups by a methylene-, ethylene- or propylenedioxy ring decreased σ_2 affinity by 8- to 12-fold, with no major effects attributable to the specific sizes of the rings (Table 1). By contrast, methylenedioxy analog **2** showed a 10-fold greater σ_1 affinity than the parent scaffold **1**. Further effects of ring size were well defined, with progressively 4-fold lower σ_1 affinities noted for the ethylenedioxy (**2**) and propylenedioxy (**3**) analogs. Thus, σ_1 binding exhibits the most sensitivity to these perturbations. Together, the data indicate that σ_1/σ_2 receptor binding affinity and selectivity can be modulated by subtle changes in molecular volumes, ring conformations, and the precise orientations of the oxygen atoms in this region.

Remarkably, the σ_2 affinity of open-ring compound **5** decreased by 1700-fold, while the σ_1 affinity was not changed (Table 1). It is difficult to provide a molecular explanation for such an interesting result. Nevertheless, this observation may aid in developing σ receptor binding models for tetrahydroisoquinolinyl benzamides. Clearly, the greater conformational freedom of **5** with respect to **1** is detrimental to σ_2 receptor binding but has no influence on binding interactions with σ_1 receptors. The effect is pronounced and leads to a low affinity compound having 5-fold selectivity for binding to σ_1 receptors. Thus, the constrained tetrahydroisoquinoline ring is critically important to high σ_2 receptor binding affinity and selectivity.

In conclusion, we determined that modifications of the two methoxy groups of the tetrahydroisoquinolinyl benzamides can be used to modulate the relative affinities and selectivities of ligand binding to σ_1 and σ_2 receptor subtypes. We also demonstrated that a constrained tetrahydroisoquinoline ring system is key to the exceptional σ_2 receptor binding affinity and selectivity observed for this active series.

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18. Data for **2**. ^{1}H NMR (free base, $CDCl_3$, δ) 1.67 (m, 4H, CH_2); 2.51 (t, 2H, CH_2); 2.65 (t, 2H, CH_2); 2.75 (t, 2H, CH_2); 3.44–3.48 (m, 4H, CH_2); 3.84–3.86 (2s, 6H, OCH_3); 5.86 (s, 2H, OCH_2O); 6.44 (s, 1H, aromatic CH); 6.52 (s, 1H, aromatic CH); 7.08 (d, 1H, CH); 7.74 (d, 1H, aromatic CH); 8.01 (t, 1H, amide NH). Mp. 174–175 °C (mono-oxalate salt); Anal. Calcd for $C_{23}H_{27}BrN_2O_5 \cdot C_2H_2O_4$: C, 51.64; H, 5.03; N, 4.82. Found: C, 51.80; H, 5.15; N, 4.80.
Data for **3**. ^{1}H NMR (free base, $CDCl_3$, δ) 1.68 (p, 4H, CH_2); 2.52 (t, 2H, CH_2); 2.66 (t, 2H, CH_2); 2.75 (t, 2H, CH_2); 3.49 (m, 4H, CH_2). 3.86 (2s, 6H, OCH_3); 4.20 (s, 4H, OCH_2CH_2O); 6.49 (s, 1H, CH); 6.57 (s, 1H, CH); 7.10 (d, 1H, CH); 7.75 (d, 1H, CH); 7.99 (t, 1H, amide NH). Mp. 146–147 °C (mono-oxalate salt); Anal. Calcd for $C_{24}H_{29}BrN_2O_5 \cdot C_2H_2O_4$: C, 52.45; H, 5.25; N, 4.70. Found: C, 52.66; H, 5.30; N, 4.61.
Data for **4**. ^{1}H NMR (free base, $CDCl_3$, δ) 1.66 (m, 4H, CH_2); 2.13 (m, 2H, CH_2); 2.50 (t, 2H, CH_2); 2.65 (t, 2H, CH_2); 2.77 (t, 2H, CH_2); 3.40–3.48 (m, 4H, CH_2). 3.84–3.86 (2s, 6H, OCH_3); 4.12 (s, 4H, CH_2O); 6.61 (s, 1H, CH); 6.69 (s, 1H, CH); 7.10 (d, 1H, CH); 7.75 (d, 1H, CH); 7.98 (t, 1H, amide NH). Mp. 163–164 °C (mono-oxalate salt); Anal. Calcd for $C_{25}H_{31}BrN_2O_5 \cdot C_2H_2O_4$: C, 53.21; H, 5.46; N, 4.60. Found: C, 53.26; H, 5.48; N, 4.60.
Data for **5**. ^{1}H NMR: (free base, $CDCl_3$, δ) 1.66 (br m, 4H, CH_2); 2.85 (t, 2H, CH_2); 2.96 (t, 2H, CH_2); 3.43 (t, 2H, CH_2); 3.83–3.86 (4s, 12H, OCH_3); 5.75 (br s, 1H, NH); 6.70–6.80 (m, 3H, aromatic CH); 7.11 (d, 1H, aromatic CH); 7.74 (d, 1H, aromatic CH); 7.99 (t, 1H, amide NH). Mp. 176–177 °C (mono-oxalate salt); Anal. Calcd for $C_{23}H_{31}BrN_2O_5 \cdot C_2H_2O_4$: C, 51.29; H, 5.68; N, 4.79. Found: C, 51.07; H, 5.72; N, 4.75.
19. The σ receptor binding assays were performed as previously described in detail,[20] except membranes were prepared exclusively from male guinea pig brains. In brief, σ_1 assays used [^{3}H](+)-pentazocine (1.0 nM) in 50 mM Tris–HCl buffer (pH 7.4, 25 °C) with nonspecific binding defined by haloperidol (1.0 μM). Assay tubes were incubated for 150 min at 37 °C using 0.25 mg protein in a final volume of 1.0 mL. The σ_2 assays used [^{3}H]ditolylguanidine ([^{3}H]DTG, 3.0 nM) with 200 nM (+)-pentazocine added as a σ_1 receptor mask. Incubations were performed using 50 mM Tris–HCl buffer (pH 8.0, 25 °C) with nonspecific binding defined by DTG (100 μM). Assay tubes were incubated for 120 min at 25 °C using 0.25 mg protein in a final volume of 0.5 mL. Test compounds were dissolved in water containing 0.1% HOAc and 1.0% EtOH, and comprised 10% of the final assay volumes. Ten concentrations were used that were centered on the IC_{50} and spaced equally on log scale. Assays were terminated by addition of ice-cold incubation buffer followed by rapid filtration through Whatman GF/B glass fiber filters presoaked in 0.5% polyethylenimine using a Brandel cell harvester. Filters were washed three times with 3–4 mL of ice-cold buffer, dried, and extracted with Hi-Safe 2 scintillation cocktail. Radioactivity was measured using a Wallac 1409 liquid scintillation counter at a tritium efficiency of 44%. Binding data were analyzed with curve-fitting programs Prism 4.0b and Radlig 6.0. K_i values were computed from IC_{50}'s using the Cheng-Prusoff relationship, with K_d input values of 2.3 nM for [^{3}H](+)-pentazocine and 23.9 nM for [^{3}H]DTG.[20]
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