Bioorganic & Medicinal Chemistry 19 (2011) 1852–1859

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Effect of structural modification in the amine portion of substituted aminobutyl-benzamides as ligands for binding σ_1 and σ_2 receptors

Kuo-Hsien Fan [a], John R. Lever [b,c,d], Susan Z. Lever [a,e,*]

[a] Department of Chemistry, University of Missouri-Columbia, Columbia, MO, USA
[b] Department of Radiology, University of Missouri-Columbia, Columbia, MO, USA
[c] Department of Medical Pharmacology and Physiology, University of Missouri-Columbia, Columbia, MO, USA
[d] Harry S. Truman Veterans Administration Medical Center, Columbia, MO, USA
[e] Research Reactor Center, University of Missouri-Columbia, Columbia, MO, USA

ARTICLE INFO

Article history:
Received 22 December 2010
Revised 29 January 2011
Accepted 3 February 2011
Available online 12 February 2011

Keywords:
Sigma receptors
Structure–activity relationships
Aminobutyl-benzamides

ABSTRACT

5-Bromo-*N*-[4-(6,7-dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-butyl)]-2,3-dimethoxy-benzamide (**1**) is one of the most potent and selective σ_2 receptor ligands reported to date. A series of new analogs, where the amine ring fused to the aromatic ring was varied in size (**5**–**7**) and the location of the nitrogen in this ring was modified, has been synthesized and assessed for their σ_1/σ_2 binding affinity and selectivity. The binding affinity of an open-chained variant of **1** was also evaluated. Only the five-membered ring congener of **1** displayed a higher σ_1/σ_2 selectivity, derived from a higher σ_2 affinity and a lower σ_1 affinity. Positioning the nitrogen adjacent to the aromatic ring in the five-membered and six-membered ring congeners dramatically decreased affinity for both subtypes. Thus, location of the nitrogen within a constrained ring is confirmed to be key to the exceptional σ_2 receptor binding affinity and selectivity for this active series.

1. Introduction

It has been approximately 20 years since the identification of two subtypes to the sigma receptor.[1] Advances have been made with regard to the σ_1 subtype, including cloning,[2–4] characterization of the binding site,[5,6] the search for putative endogenous ligands,[7] and the development of a knockout mouse.[8] Such progress has not yet been made with the σ_2 receptor. Both subtypes, however, are implicated in a variety of health related functions, including addiction and cancer.[9,10] Thus, this is a research area of much current interest.

Glennon has reviewed structure–affinity relationships (SAR) for σ_1 and σ_2 sites.[11,12] Important features of the Glennon pharmacophore for both σ_1 and σ_2 sites are an amine binding site flanked, on each side, by hydrophobic binding pockets that display bulk tolerance. More recently, a model has been proposed based on 19 benzo[*d*]oxazol-2(3*H*)-one derivatives for the σ_2 binding site.[13] In this model, fundamental attributes include an atom capable of accepting a positive charge, and three hydrophobic areas, one of which is aromatic and one which is aliphatic in nature. Although a number of studies have investigated the effects of structure on relative σ_1/σ_2 receptor binding affinity and selectivity, few truly σ_2 selective compounds are known. Mach et al.[14] have identified a class of tetrahydroisoquinolinyl benzamide ligands that rank among the most selective for the σ_2 receptor subtype. Benzamide **1** (Fig. 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. Analogs of **1** reported by this research group have focused on substitutions of the benzamide portion of the molecule, including **1a** which exhibits a higher selectivity of 8190.[15–17] By contrast, we have explored variations in the tetrahydroisoquinoline ring.[18] In the first study, the two methoxy groups were replaced with methylene-, ethylene- and propylenedioxy rings. 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 poorer σ_1 affinities were then noted with increasing ring size. Of more import, when the tetrahydroisoquinoline ring of **1** was opened to study the effects of conformational flexibility on σ receptor binding (Fig. 1, compound **2**), there was no change in σ_1 affinity, but a dramatic decrease (1700-fold) in the σ_2 affinity was observed. Thus, the constrained ring system appears to be a crucial component to the exceptional σ_2 receptor binding affinity and selectivity observed for this active series.

In order to gain a better understanding of the contribution of the tetrahydroisoquinoline moiety to σ_2 affinity and selectivity, we have extended our study to focus on structural modifications at the amine portion of **1**. The first goal was to modulate the conformational freedom of the amine-containing ring fused to the aromatic ring. We thus included the five-membered and

* Corresponding author. Tel.: +1 573 882 8395; fax: +1 573 882 2754.
E-mail address: levers@missouri.edu (S.Z. Lever).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.02.006

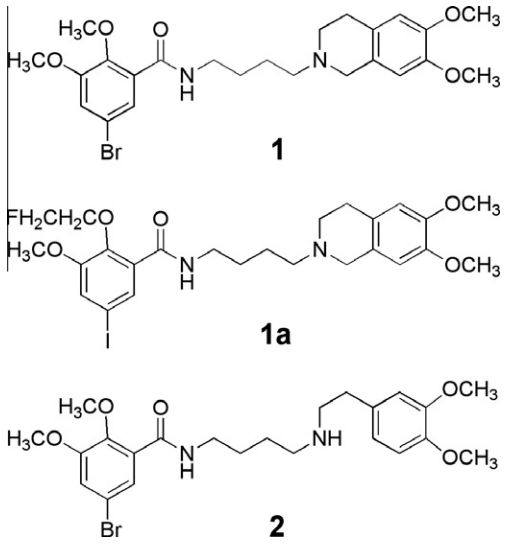


Figure 1. Lead compounds as subject of this study.

seven-membered variants of **1** (Fig. 2, compounds **3** and **4**). Nitrogen can occupy more than one isomeric position in the five-, and seven-membered rings, as well as in the six-membered ring of **1**, so we prepared these analogs as well (Fig. 2, compounds **5**–**8**). Compounds **3**–**8** represent all the unique possibilities for one nitrogen atom in the fused ring, in addition to **1**. From these compounds, effects on σ_1/σ_2 affinity and selectivity from either the orientation of the nitrogen in each respective ring or the inherent basicity of the nitrogen, can be determined. The second goal of the study was to prepare and evaluate **9**, the *N*-methyl analog of **2**. The rationale for this structure is that it maintains the conformational fluidity of **2**, while it re-introduces the tertiary nitrogen that might be required for optimum binding to the receptor.

2. Chemistry

In all cases, the ultimate step for the synthesis of compounds **3**–**9** is coupling 5-bromo-2,3-dimethoxybenzoyl chloride (**32**) to the appropriate primary butylamine derivative. The requisite butylamine precursors (Scheme 1) were prepared through three different two-step routes. The first route paralleled that reported for compound **1**; that is, alkylation with 4-bromobutryonitrile followed by lithium aluminum hydride reduction. This route proved successful in the synthesis of **12** in 68% yield. However, in the preparation of other amines, this route was plagued by formation of by-products that complicated purification (data not shown). Therefore, an alternate route, alkylation of **14**, **17**, **23** (accomplished through L-proline assisted cuprous iodide coupling[19]), or **27** by *N*-(4-bromobutyl) phthalimide, followed by treatment with hydrazine was utilized. Yields in this two-step route ranged from 29% for **28** to 63% for **24**. The last route, specifically used for the preparation of amine **21**, concomitantly formed the indoline ring and installed the 4-azidobutyl moiety with reaction of 4-azidobutylamine and 1,2-bis(bromomethyl)-4,5-dimethoxybenzene. The azide was then reduced to form amine **21** in 71% yield for the two-step sequence. For the synthesis of **31**, (Scheme 2) alkylation of 2-(3,4-dimethoxyphenyl)ethanamine with 4-bromobutryonitrile was followed by formylation. Lithium aluminum hydride achieved reduction of the *N*-formyl group to the *N*-methyl group as well as the nitrile moiety to the primary amine in 47% yield for the three-step sequence.

3. Stability of compounds in assay solutions

All compounds for in vitro binding assays were first formulated at a millimolar concentration in water containing 1% ethanol with a small percent of acetic acid (0.1% for **4** and **9**; 2.5% for **3**, **5**, **6**, **7**, and **8**). Purity was assessed by HPLC and compounds **3**, **4**, **6**, **7** and **9** exceeded 95% after 25 days. However, it was found that compound **5** was not stable during the monitoring period (86% remaining after 16 h and totally decomposed after 25 days). In addition, compound **8** was found to be unstable during the assay period (90% remaining after 16 h, 38% left after 44 h and totally decomposed after 25 days). For both of these compounds, binding assays used freshly prepared serial dilutions to insure accuracy of the results obtained from the binding studies. Solutions of these compounds in deuterated solvents followed by analysis by ^{1}H NMR and LC/MS provided analytical support for demethylation of the methoxy group *ortho* to the benzamide moiety in compounds **5**

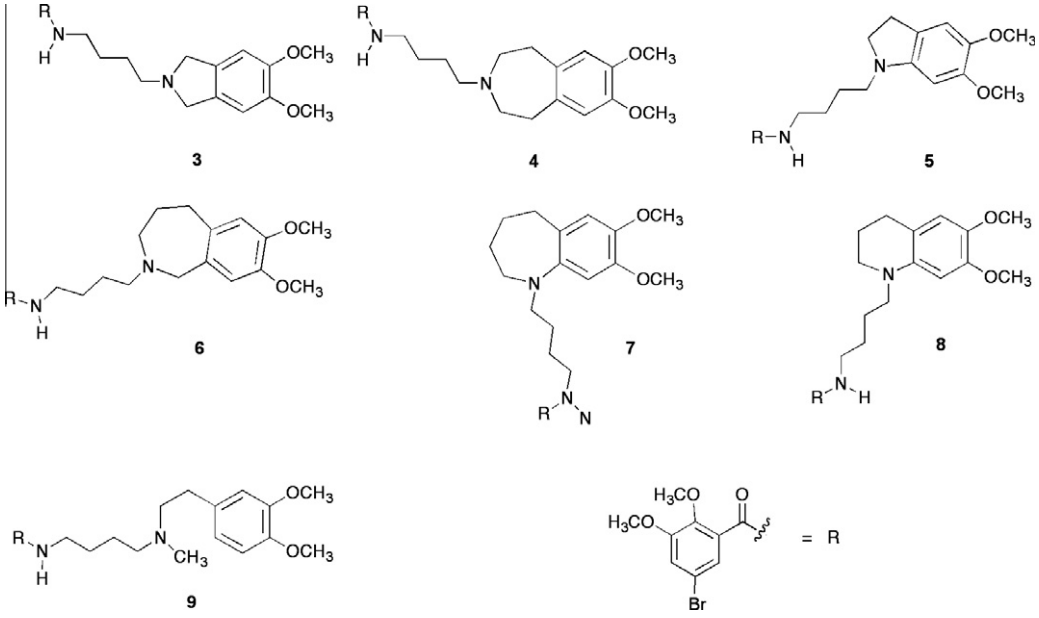
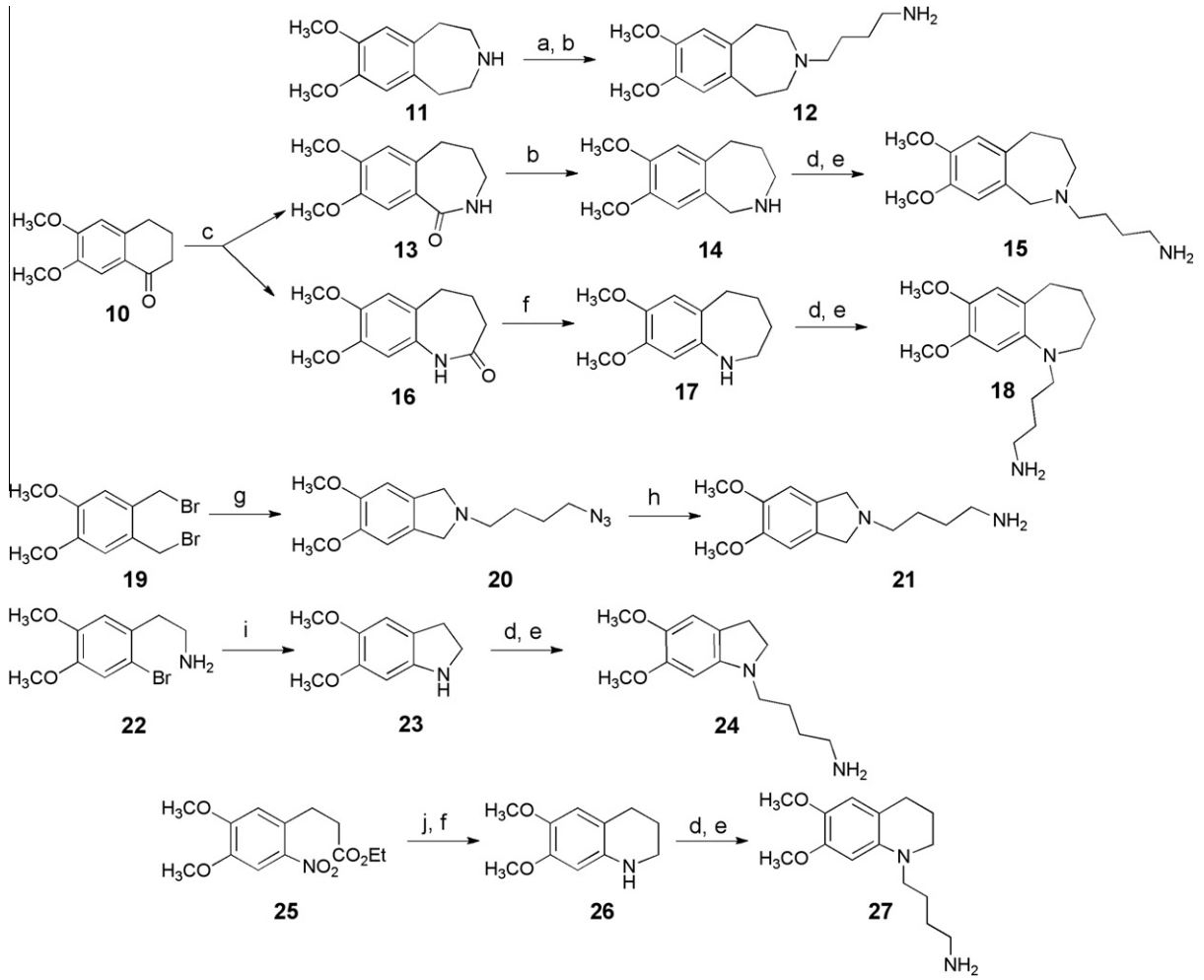
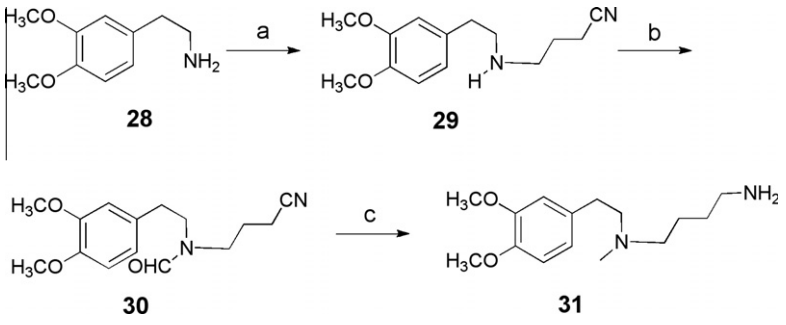


Figure 2. Compounds synthesized and evaluated in this study.



Scheme 1. Reagents: (a) $Br(CH_2)_3CN$; (b) $LiAlH_4$/THF; (c) NaN_3/HCl; (d) *N*-(4-bromobutyl)phthalimide; (e) $NH_2NH_2{\cdot}xH_2O$; (f) $BH_3{\cdot}$THF; (g) 4-azidobutylamine; (h) PPH_3/THF/H_2O; (i) 10 mol % CuI, 20 mol % L-proline, DMSO; (j) $Fe_{(s)}$/AcOH.



Scheme 2. Reagents: (a) $Br(CH_2)_3CN$; (b) HCO_2H, Ac_2O; (c) $LiAlH_4$/THF.

and **8** (spectra and LC/MS results are found in the Supplementary data). It is not obvious why only these two compounds would decompose; however, these results underscore the need to monitor stability of all compounds prior to binding assays.

4. Receptor binding assays

In vitro binding affinities to the σ_1 receptor for the series of compounds varied over an order of magnitude, from 583 ± 28 nM to 5073 ± 82 nM (Table 1). The range of affinities observed for the σ_2 receptor was much larger, on the order of 11,000. The overall selectivity for the σ_2 receptor based on the results for each analog was quite modest overall (0.27–8.5), with the exception for compound **3**, which exhibited a selectivity of 1758.

5. Discussion

Based upon previous work,[18] it is known that opening the ring of the tetrahydroisoquinolinyl ring ablates σ_2 affinity. In this study, modulating the conformational freedom and seeing its effect on binding affinity, especially the σ_2 affinity, was one of the major goals. The effect of ring size of the amine-containing component

Table 1
In vitro affinity and selectivity of compounds **3**–**9** for σ_1 and σ_2 receptors[a]

	K_i (nM) σ_1	K_i (nM) σ_2	Selectivity $K_i\sigma_1/K_i\sigma_2$
1 Mach lead	12,900	8.2	1573[b]
	881 ± 15	2.7 ± 0.1	326[c]
2 (Open-ring)	880 ± 60	4616 ± 247	0.2[c]
3 (5R-2)	1442 ± 88	0.82 ± 0.06	1758
4 (7R-3)	5073 ± 82	734 ± 50	8.5
5 (5R-1)	4521 ± 45	9681 ± 522	0.47
6 (7R-2)	2068 ± 60	315 ± 15	6.58
7 (7R-1)	2564 ± 175	8957 ± 335	0.29
8 (6R-1)	4499 ± 182	5823 ± 224	0.77
9 (Open-ring-Me)	583 ± 28	2126 ± 240	0.27
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; $n = 4$.
[b] From Ref. 14.
[c] From Ref. 18.

fused to the aromatic ring on the binding affinity was investigated by preparing a seven-membered variant and a five-membered variant. In the case of the seven-membered ring congener, **6** (7R-2), results supported the hypothesis that increasing conformational freedom in this portion of the molecule had a negative effect on binding to the σ_2 subtype. A negative effect on the σ_1 subtype was seen as well; and overall, only a modest selectivity for the σ_2 subtype was observed. Also, in support of the hypothesis that conformational constraint is helpful in binding to the σ_2 subtype, decreasing the ring size from 6 to 5 in **3** (5R-2) improved σ_2 affinity over that of **1** by 70%. The decrease in σ_1 affinity was not that dramatic, approximately 50%, with the overall effect of improving the selectivity over that of the lead compound.

As mentioned previously, isomeric compounds could be synthesized where the position of nitrogen in the fused ring varied. For the five- and six-membered rings, two isomers are possible and for the seven-membered ring, three isomers are possible. Binding results suggest the position of the nitrogen in the smaller ring structures is critical to binding to both subtypes, but much more sensitive for affinity to the σ_2 subtype. For example, in **8** (6R-1), the isomer of the Mach Lead (**1**), σ_1 affinity was decreased by a factor of 5 and σ_2 affinity was totally ablated; **7** (7R-1) versus **6** (7R-2) 20% poorer for σ_1 and almost 30 times poorer for σ_2; **3** (5R-2) versus **5** (5R-1) three times poorer for σ_1 and over 11,000 times poorer for σ_2.

In the seven-membered ring isomeric cases, there is no trend related to the position of the nitrogen. In σ_1 affinities, **4** (7R-3) had poorest affinity by a factor of 2 compared to **6** (7R-2) and **7** (7R-1); whereas, for σ_2, **4** (7R-3) had a poorer affinity that **6** (7R-2), but more than ten times higher than that of **7** (7R-1). Of these three isomers, **6** (7R-2), where the position of the nitrogen to the aromatic ring is similar to that of the Mach lead **1** and **3** (5R-2), has the best affinity for the σ_2 subtype. These results suggest that the structural position of the amine relative to the aromatic ring is important for optimum binding for the σ_2 subtype.

Introduction of a methyl group on the open-ring compound (**9** over **2**) improved affinity for both σ_1 and σ_2 subtypes. These results support the need for a tertiary nitrogen for binding to the receptor; however, in this series of compounds, this modification does not counteract the structural flexibility provided by the open chain structure, as affinity was still quite low and selectivity was poor.

Qualitatively, three-dimensional models can provide a preliminary rationale to explain the differences observed in the in vitro results in the pairs of the compounds (**1** (Mach lead) and **8** (6R-1) (Fig. 3a), **3** (5R-2) and **5** (5R-1) (Fig. 3b) and **1** and **9** (Open-ring-Me) (Fig. 3c). Each compound was first subjected to energy minimization by the MMFF94 force field built in ChemBio 3D (Cambridge Soft) and then each pair was overlaid. Clearly, compound **1** can extend itself linearly; whereas, the placement of the nitrogen next to the aromatic ring in **8** puts a definite twist in the molecule. This effect is also seen when comparing compounds **3** and **5**. Compound **3**, the compound with the highest σ_2 affinity and selectivity is extended like compound **1**. Compound **5**, similarly as seen for **8**, the placement of the nitrogen puts a twist into the heterocyclic portion of the molecule, and the observed affinities are much worse. These twists in the molecule may help explain the exceedingly poor affinity of **8** and **5** to the σ_2 receptor. In the comparison of **1** with **9**, the open-ringed analog with the tertiary amine, the conformational freedom inherent in the latter compound permits some rotation of the dimethoxy-substituted aryl ring out of plane and may impact the binding affinity. Taken together, the relative binding properties noted indicate the importance of these structural features to binding to the σ_1 and σ_2 receptor subtypes.

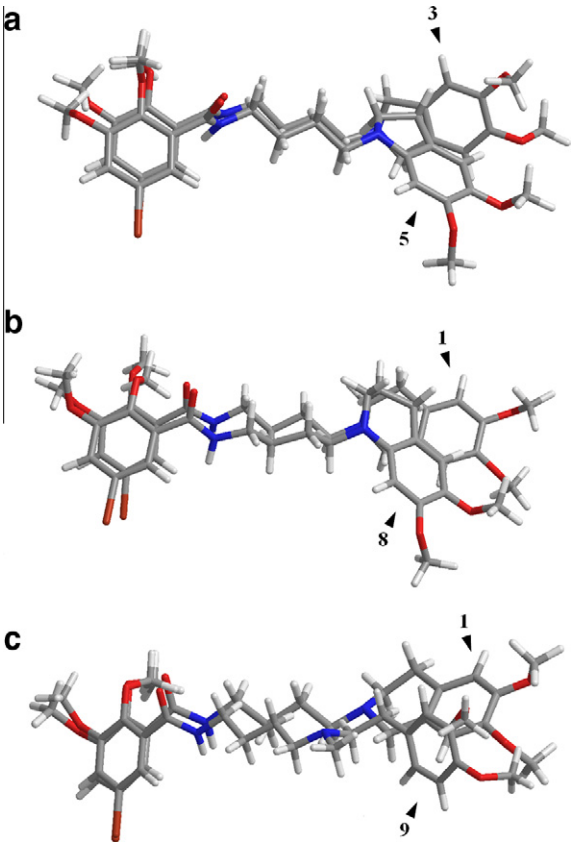


Figure 3. (a) Overlap of minimized structures **1** and **8**; (b) overlap of minimized structures **3** and **5**; (c) overlap of minimized structures **1** and **9**.

6. Conclusions

Binding affinities of a novel set of ligands are affected dramatically by the structural modification in the amine portion of the parent molecule. The excellent binding affinity of compound **3** only to σ_2 receptor subtypes provides a highly selective σ_2 receptor subtype ligand ($K_i\sigma_1/K_i\sigma_2 = 1758$), and these results corroborate the need of rigidity in this portion of this class of ligands. In addition, structural features were identified that result in extremely poor affinity and selectivity for both the σ_1 and σ_2 receptor subtypes.

7. Experimental

7.1. General procedures

^{1}H NMR spectra were determined on Bruker 250, 300, or 500 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 determined by Atlantic Microlab, Inc. (Norcross, GA), and were in agreement with calculated values (C, H, N: ±0.4%) with the exception of compounds **6** and **9** (H ±0.46%). 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 mm). OptiPhase® HiSafe 2 scintillation cocktail, [^{3}H](+)-pentazocine ([^{3}H](+)-PTZ, 29 Ci/mmol) and [^{3}H]1,3-di-(2-tolyl)guanidine ([^{3}H]DTG, 48.7 Ci/mmol) were from Perkin–Elmer Life Sciences, Inc. (Boston, MA). Other chemicals and solvents were the best grades available, and were used as received from commercial sources.

7.1.1. 4-(7,8-Dimethoxy-4,5-dihydro-1*H*-benzo[*d*]azepin-3(2*H*)-yl)butan-1-amine (12)

To a stirred solution of 7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine[20] (**11**) (1.36 g, 6.6 mmol) and 4-bromobutyronitrile (0.98 g, 6.6 mmol) in DMF (40 mL), NaI (1.00 g, 6.6 mmol) and K_2CO_3 (2.75 g, 19.9 mmol) were added and the mixture was stirred at 60 °C overnight. The solvent was removed under reduced pressure and the residue was diluted with H_2O (150 mL) and extracted with ethyl acetate (3 × 100 mL) to give 4-(7,8-dimethoxy-4,5-dihydro-1*H*-benzo[*d*]azepin-3(2*H*)-yl)butanenitrile (1.66 g, 92%) as a yellow oil. ^{1}H NMR ($CDCl_3$) d: 6.64 (s, 2H), 3.85 (s, 6H), 2.86–2.82 (m, 4H), 2.63–2.59 (m, 4H), 2.58 (t, J = 6.8 Hz, 2H), 2.47 (t, J = 6.8 Hz, 2H), 1.84 (tt, J = 6.9 Hz, 6.9 Hz, 2H). This material (1.58 g, 5.7 mmol) was used without further purification and dissolved in dry THF (20 mL) for the following reaction. A solution of $LiAlH_4$ (0.65 g, 17.1 mmol) in dry THF (20 mL) was added dropwise at 0 °C. The mixture was stirred at rt overnight under N_2. The mixture was cooled to 0 °C and quenched by adding H_2O (1 mL), 10% aqueous NaOH (2 mL), and H_2O (2.5 mL) successively. The inorganic salts were washed with EtOAc and filtered. The filtrate was evaporated under reduced pressure to give **12** (1.18 g, 75%) as yellow oil. ^{1}H NMR ($CDCl_3$) d: 6.64 (s, 2H), 3.85 (s, 6H), 2.87–2.83 (m, 4H), 2.72 (t, J = 6.9 Hz, 2H), 2.64–2.60 (m, 4H), 2.48 (t, J = 6.9 Hz, 2H), 1.57–1.45 (m, 4H).

7.1.2. 7,8-Dimethoxy-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-1-one (13) and 7,8-dimethoxy-4,5-dihydro-1*H*-benzo[*b*]azepin-2(3*H*)-one (16)

The isomeric amides **13** and **16** were prepared as a mixture by a literature procedure[21] and purified by short path chromatography EtOAc/$CHCl_3$ (9:1) to give **13** (2.77 g, 52%) and **16** (1.22 g, 23%) as white solids. **13**: ^{1}H NMR ($CDCl_3$) d: 7.27 (s, 1H), 6.68 (s, 1H), 6.48 (br t, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 3.15 (dt, J = 6.3, 6.6 Hz, 2H), 2.82 (t, J = 6.9 Hz, 2H), 2.02 (tt, J = 6.9, 6.9 Hz, 2H). Compound **16**: ^{1}H NMR ($CDCl_3$) d: 7.4 (br s, 1H), 6.72 (s, 1H), 6.53 (s, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 2.74 (t, J = 6.9 Hz, 2H), 2.36–2.32 (m, 2H), 2.26–2.20 (m, 2H). The amide **13** was reduced according to the literature procedure[21] to provide **14.**

7.1.3. 4-(7,8-Dimethoxy-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)butan-1-amine (15)

To a flask, *N*-(4-bromobutyl)-phthalimide (1.29 g, 4.58 mmol), **14** (0.95 g, 4.58 mmol), NaI (0.67 g, 4.58 mmol) and K_2CO_3 (0.93 g, 13.4 mmol) were added. The resulting mixture was stirred at 60 °C for 2 h and then the solvent was removed under reduced pressure. Water (100 mL) was added to the residue and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated to give 2-(4-(7,8-dimethoxy-4,5-dihydro-1*H*-benzo[*c*]azepin-2(3*H*)-yl)butyl)isoindoline-1,3-dione (1.55 g, 83%) as a yellow gum. ^{1}H NMR ($CDCl_3$) d: 7.84–7.79 (m, 2H), 7.73–7.68 (m, 2H), 6.66 (s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.80 (s, 2H), 3.69 (t, J = 7.0 Hz, 2H), 3.09–3.04 (m, 2H), 2.83–2.79 (m, 2H), 2.40 (t, J = 7.2, 2H), 1.74–1.62 (m, 4H), 1.55–1.50 (m, 2H). This material was used without further purification or characterization and was treated with hydrazine hydrate (0.33 g, 6.60 mmol) in dry MeOH (10 mL) and the mixture was refluxed for 3 h. The mixture was cooled and the MeOH and hydrazine were removed under reduced pressure. Diethyl ether (50 mL) was added to the residue, filtered and the filtrate was evaporated to give **15** (0.46 g, 44%) as a colorless oil. ^{1}H NMR ($CDCl_3$) d: 6.67 (s, 1H), 6.66 (s, 1H), 4.90 (br s, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.83 (s, 2H), 3.09–3.07 (m, 2H), 2.83–2.80 (m, 2H), 2.74 (t, J = 6.5 Hz, 2H), 2.34 (t, J = 6.5 Hz, 2H), 1.72–1.69 (m, 2H), 1.59–1.55 (m, 4H).

7.1.4. 7,8-Dimethoxy-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepine (17)

To a stirred solution of **16**[21] (0.82 g, 3.7 mmol) in dry THF (10 mL), BH_3·THF solution (20 mL, 1 M) was added dropwise at 0 °C and stirred at rt for 1.5 h. The reaction mixture was cooled to 0 °C and then quenched with HCl (4 N) until no H_2 gas further evolved. The solution was stirred at rt for 0.5 h and then the pH of the solution was adjusted with 10% aqueous NaOH to pH >14. The resulting solution was extracted with $CHCl_3$ (3 × 50 mL) to give **17** (0.66 g, 87%) as a white solid. ^{1}H NMR ($CDCl_3$) d: 6.65 (s, 1H), 6.34 (s, 1H), 3.82 (s, 6H), 3.02–2.99 (m, 2H), 2.72–2.68 (m, 2H), 1.80–1.76 (m, 2H), 1.62–1.59 (m, 2H).

7.1.5. 4-(7,8-Dimethoxy-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-1-yl)butan-1-amine (18)

To a stirred solution of **17** (155 mg, 0.75 mmol) in DMF (10 mL), *N*-(4-bromobutyl)-phthalimide (211 mg, 0.75 mmol), NaI (0.11 g, 0.75 mmol) and K_2CO_3 (0.31 g, 2.2 mmol) were mixed according to the procedure described in the preparation of **15**. After a short path chromatography column (EtOAc/CH_2Cl_2 1:1), 2-(4-(7,8-dimethoxy-2,3,4,5-tetrahydrobenzo[*b*]azepin-1-yl)butyl)isoindoline-1,3-dione (166 mg, 55%) was isolated as a yellow gum. ^{1}H NMR ($CDCl_3$) d: 7.87–7.79 (m, 2H), 7.73–7.67 (m, 2H), 6.59 (s, 1H), 6.50 (s, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.70 (t, J = 7.0, 2H), 3.11 (t, J = 6.6 Hz, 2H), 2.85–2.81 (m, 2H), 2.71–2.66 (m, 2H), 1.85–1.77 (m, 4H), 1.67–1.56 (m, 4H). This material (160 mg, 0.39 mmol) was added to dry MeOH (10 mL), and hydrazine hydrate (34 mg, 0.69 mmol) was added. The mixture was refluxed for 3 h, after which the MeOH and hydrazine were removed under reduced pressure and diethyl ether (50 mL) was added to the residue. The resulting mixture was filtered and the filtrate was evaporated to give **18** (61 mg, 56%) as a light yellow oil. ^{1}H NMR ($CDCl_3$) d: 6.66 (s, 1H), 6.55 (s, 1H), 3.85 (s, 3H), 3.83 (s, 3H), 3.09 (t, J = 6.6 Hz, 2H), 2.87–2.84 (m, 2H), 2.73–2.69 (m, 4H), 1.71–1.66 (m, 2H), 1.61–1.54 (m, 8H).

7.1.6. 2-(4-Azidobutyl)-5,6-dimethoxyisoindoline (20)

To a stirred solution of **19**[22] (0.48 g, 1.5 mmol) and 4-azidobutylamine[23] (0.17 g, 1.5 mmol) in DMF (15 mL), NaI (0.45 g, 3 mmol) and K_2CO_3 (3.7 g, 9 mmol) were added. The resulting mixture was stirred at 60 °C for 3 h and then the solvent was removed under reduced pressure. Water (50 mL) was added to the residue and then extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated to give **20** (0.38 g, 93%) as a dark red oil. ^{1}H NMR ($CDCl_3$) d: 6.75 (s, 2H), 3.93 (s, 4H), 3.86 (s, 6H), 3.34 (t, J = 6.3 Hz, 2H), 2.77 (t, J = 6.9 Hz, 2H), 1.73–1.66 (m, 4H).

7.1.7. 4-(5,6-Dimethoxyisoindolin-2-yl)butan-1-amine (21)

To a stirred solution of **20** (0.53 g, 1.9 mmol) in THF (10 mL) and H_2O (0.2 mL) was added PPh_3 (0.61 g, 2.3 mmol) slowly and the resulting mixture was stirred at rt for 24 h. The mixture was extracted with 1 M aqueous HCl (3 × 15 mL) and the organic phase was discarded. The combined aqueous phases were washed with CH_2Cl_2 (2 × 50 mL). The washed aqueous layer was basified with 2.5 M aqueous NaOH to pH >10 and then extracted with CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated to give **21** (0.36 g, 76%) as a brown oil. ^{1}H NMR ($CDCl_3$) d: 6.74 (s, 2H), 3.88 (s, 4H), 3.86 (s, 6H), 2.75 (t, *J* = 6.9 Hz, 2H), 2.72 (t, *J* = 8.4 Hz, 2H), 1.62–1.51 (m, 6H).

7.1.8. 5,6-Dimethoxyindoline (23)

A sealed round bottomed flask was charged with CuI (0.11 g, 0.55 mmol), L-proline (0.13 g, 1.1 mmol) and K_2CO_3 (1.62 g, 11.1 mmol). The flask was evacuated and then backfilled with N_2 (two cycles). A solution of 2-(2-bromo-4,5-dimethoxyphenyl)-ethanamine (**22**)[24] (1.45 g, 5.5 mmol) in DMSO (5.5 mL) was added to the flask and the mixture was stirred at 70 °C under N_2 for 45 h. Water (100 mL) was added to the mixture and extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, concentrated and applied to a short path chromatography column $CHCl_3$/MeOH (60:1) to give **23** (0.36 g, 36%) as a pale brown solid. ^{1}H NMR ($CDCl_3$) d: 6.76 (s, 1H), 6.35 (s, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.54 (t, *J* = 8.4 Hz, 2H), 2.98 (t, *J* = 8.4 Hz, 2H). This material corresponded to the spectral data reported for the compound prepared by a different method.[25]

7.1.9. 4-(5,6-Dimethoxyindolin-1-yl)butan-1-amine (24)

The indoline **23** (152 mg, 0.85 mmol) and *N*-(4-bromobutyl)-phthalimide (239 mg, 0.85 mmol) were mixed according to the procedure described in the preparation of **15**. After a short path column with $CHCl_3$/MeOH (40:1), **24** (240 mg, 74%) was obtained as a dark brown oil. ^{1}H NMR ($CDCl_3$) d: 7.86–7.81 (m, 2H), 7.74–7.70 (m, 2H), 6.72 (s, 1H), 6.19 (s, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.74 (t, *J* = 6.3 Hz, 2H), 3.26 (t, *J* = 8.1 Hz, 2H), 3.03 (t, *J* = 7.2 Hz, 2H), 2.87 (t, *J* = 8.1 Hz, 2H), 1.86–1.78 (m, 2H), 1.70–1.62 (m, 2H). This material was used without further purification and mixed with hydrazine hydrate (54.6 mg, 1.10 mmol) in the procedure used for the preparation of **15**. After filtration, **24** (133 mg, 85%) was obtained as a light brown oil. ^{1}H NMR ($CDCl_3$) d: 6.75 (s, 1H), 6.18 (s, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 3.27 (t, *J* = 8.1 Hz, 2H), 3.00 (t, *J* = 7.2 Hz, 2H), 2.88 (t, *J* = 8.1 Hz, 2H), 2.77 (t, *J* = 6.9, 2H), 1.68–1.53 (m, 4H), 1.26 (br s, 2H).

7.1.10. 6,7-Dimethoxy-1,2,3,4-tetrahydroquinoline (26)

6,7-Dimethoxy-3,4-dihydroquinolin-2(1*H*)-one, was first prepared by a literature procedure for a similar compound.[26] To a solution of **25**[27] (1.98 g, 7.0 mmol) in glacial acetic acid (40 mL) at 80 °C was added iron powder (7.0 g, 125.3 mmol) and the resulting mixture was stirred manually at 80 °C for 30 min. After stirring, the mixture was filtered through Celite and the filtrate was diluted with water (150 mL). The diluted filtrate was extracted with $CHCl_3$ (3 × 150 mL) and evaporated to give 6,7-dimethoxy-3,4-dihydroquinolin-2(1*H*)-one as a pale yellow solid (1.11 g, 77%). ^{1}H NMR ($CDCl_3$) d: 8.90 (s, 1H), 6.69 (s, 1H), 6.40 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 2.90 (t, *J* = 7.5 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H). A sample of this material (0.58 g, 2.8 mmol) was treated with BH_3·THF (20 mL, 1 M) following the procedure described for the preparation of **17**. After extraction, **26** (0.37 g, 68%) was obtained as a brown oil. ^{1}H NMR ($CDCl_3$) d: 6.51 (s, 1H), 6.09 (s, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.26–3.22 (m, 2H), 2.68 (t, *J* = 6.5 Hz, 2H), 1.95–1.86 (m, 2H). These spectral data are consistent with that reported by Toda et al.[28] for this compound prepared in a different manner.

7.1.11. 4-(6,7-Dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)butan-1-amine (27)

The two-step procedure to introduce the *N*-butylamino moiety onto **18** was followed. From **26** (175 mg, 0.91 mmol) and *N*-(4-bromobutyl)-phthalimide (257 mg, 0.91 mmol), after a short path column with ethyl acetate/hexane (2:1), 2-(4-(6,7-dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)butyl)isoindoline-1,3-dione (158 mg, 44%) was obtained as an orange oil. ^{1}H NMR ($CDCl_3$) d: 7.86–7.82 (m, 2H), 7.73–7.70 (m, 2H), 6.52 (s, 1H), 6.22 (s, 1H), 3.86 (s, 3H), 3.80 (s, 3H), 3.73 (t, *J* = 7 Hz, 2H), 3.25–322 (m, 2H), 3.19–3.17 (m, 2H), 2.67 (t, *J* = 6.5 Hz, 2H), 1.94–1.86 (m, 2H), 1.80–1.73 (m, 2H), 1.67–1.55 (m, 2H). This material was used without further characterization in the reaction with hydrazine hydrate (34.7 mg, 0.70 mmol). After filtration, **27** (70 mg, 67%) was obtained as a pale yellow oil. ^{1}H NMR ($CDCl_3$) d: 6.54 (s, 1H), 6.23 (s, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.23–3.18 (m, 4H), 2.74 (t, *J* = 7 Hz, 2H), 2.68 (t, *J* = 6.5 Hz, 2H), 1.95–1.90 (m, 2H), 1.65–1.59 (m, 2H), 1.53–1.47 (m, 2H).

7.1.12. 4-(3,4-Dimethoxyphenethylamino)butanenitrile (29)

The alkylation of 2-(3,4-dimethoxyphenyl)ethanamine (1.81 g, 10 mmol) (**28**) with 4-bromobutanenitrile (1.48 g, 10 mmol) followed the procedure described in the preparation of **12** and yielded **29** (2.31 g, 93%) as a yellow oil. ^{1}H NMR ($CDCl_3$) d: 6.83–6.71 (m, 3H), 3.88 (s, 3H), 3.86 (s, 3H), 2.81–2.83 (m, 2H), 2.77–2.72 (m, 4H), 2.42 (t, *J* = 7.2 Hz, 2H), 1.83–1.73 (m, 2H).

7.1.13. *N*-(3,4-Dimethoxyphenethyl)-*N*-(3-cyanopropyl)-formamide (30)

Formic acid (12.56 g, 285.5 mmol) and acetic anhydride (9.35 g, 8.57 mmol) were added to **29** (2.31 g, 9.29 mmol) and the mixture was stirred at 70 °C for 1 h. After the acid was evaporated under reduced pressure, the residue was diluted with H_2O (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). After a short path column with ethyl acetate, **30** (1.75 g, 68%) was obtained as a light yellow oil. ^{1}H NMR ($CDCl_3$) d: 8.11, 7.87 (s, total 1H), 6.83–6.34 (m, 3H), 3.88, 3.87, 3.86 (s, total 6H), 3.55–3.41 (m, 3H), 2.86–2.77 (m, 2H), 2.38 (t, *J* = 7.2 Hz, 1H), 2.33–2.29 (m, 1H), 1.96–1.92 (m, 1H), 1.86–1.73 (m, 1H).

7.1.14. N^1-(3,4-Dimethoxyphenethyl)-N^1-methylbutane-1,4-diamine (31)

With the procedure described for the preparation of **12, 30** (1.46 g, 5.3 mmol) was reduced with 1 M $LiAlH_4$ solution in THF (15 mL, 15 mmol). After filtration, **31** (1.1 g, 75%) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) d: 6.81–6.73 (m, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 2.78–2.64 (m, 4H), 2.62–2.54 (m, 2H), 2.44–2.38 (m, 2H), 2.30 (s, 3H), 1.55–1.37 (m, 6H).

7.2. General procedure for preparation of amides 3–9

To a stirred solution of amine (**12, 15, 18, 21, 24, 27, 31**) and equimolar amount of Et_3N in dry CH_2Cl_2, an equimolar amount of acid chloride **32**[28,29] in dry CH_2Cl_2 was added dropwise. The mixture was stirred at rt for overnight and then the solvent was removed under reduced pressure.

7.2.1. 5-Bromo-*N*-(4-(5,6-dimethoxyisoindolin-2-yl)butyl)-2,3-dimethoxybenzamide (3)

Compound **3** was prepared according to the general procedure with **21** (136 mg, 0.55 mmol). After a short path column with $CHCl_3$/MeOH (10:1), **3** (147 mg, 59.8%) was obtained as a light yellow gum. ^{1}H NMR ($CDCl_3$) d: 8.09 (br t, 1H), 7.77 (d, *J* = 2.4 Hz, 1H), 7.09 (d, *J* = 2.4 Hz, 1H), 6.73 (s, 2H), 3.88 (s, 4H), 3.87 (s, 6H), 3.86 (s, 3H), 3.85 (s, 3H), 3.50 (dt, *J* = 6.3, 6.3 Hz, 2H), 2.76 (t, *J* = 6.6 Hz, 2H), 1.74–1.67 (m, 4H). ^{13}C NMR ($CDCl_3$) d: 163.84, 153.16, 148.24,

146.38, 131.46, 128.42, 125.15, 118.06, 116.96, 105.71, 61.29, 59.11, 56.20, 56.00, 55.66, 39.69, 27.36, 26.40. Anal. Calcd for $C_{23}H_{29}BrN_2O_5$: C, 55.59; H, 5.92; N, 5.68. Anal. Calcd for $C_{23}H_{29}BrN_2O_5 \cdot H_2O$ (corrected): C, 54.02; H, 6.11; N, 5.48. Found: C, 54.32; H, 5.79; N, 5.48.

7.2.2. 5-Bromo-*N*-(4-(7,8-dimethoxy-4,5-dihydro-1*H*-benzo[*d*]-azepin-3(2*H*)-yl)butyl)-2,3-dimethoxybenzamide (4)

Compound **4** was prepared according to the general procedure with **12** (0.36 g, 1.3 mmol). The residue was applied to a short path chromatography column and eluted with acetone to give **4** (0.30 g, 45%) as a pale yellow gum. The free base was converted into the HCl salt and recrystallized from a mixture of ethanol and diethyl ether. Mp 135–137 °C. ^{1}H NMR of the free base ($CDCl_3$) d: 7.93 (br t, 1H), 7.81 (d, J = 2.4 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 6.63 (s, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.85 (s, 6H), 3.48 (dt, J = 6, 6 Hz, 2H), 2.85–2.81 (m, 4H), 2.63–2.60 (m, 4H), 2.51 (t, J = 6.6 Hz, 2H), 1.64–1.60 (m, 4H). ^{13}C NMR of the free base ($CDCl_3$) d: 163.71, 153.18, 146.60, 134.13, 128.23, 125.31, 118.19, 117.08, 112.81, 61.28, 58.51, 56.24, 55.91, 55.49, 39.62, 36.04, 27.55, 24.34. Anal. Calcd for $C_{25}H_{33}BrN_2O_5 \cdot HCl$: C, 53.82; H, 6.14; N, 5.02. Anal. Calcd for $C_{25}H_{33}BrN_2O_5 \cdot HCl \cdot 1.5\ H_2O$ (corrected): C, 51.33; H, 6.38; N, 4.79. Found: C, 51.40; H, 6.29; N, 4.83.

7.2.3. 5-Bromo-*N*-(4-(5,6-dimethoxyindolin-1-yl)butyl)-2,3-dimethoxybenzamide (5)

Compound **5** was prepared according to the general procedure with **24** (133 mg, 0.53 mmol). After a short path column with $CHCl_3$/MeOH (55:1), **5** (128 mg, 49%) was obtained as a light brown gum. ^{1}H NMR ($CDCl_3$) d: 7.93 (br t, 1H), 7.82 (d, J = 2.4 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 6.74 (s, 1H), 6.18 (s, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.52 (dt, J = 6.3, 6.3 Hz, 2H), 3.27 (t, J = 8.1 Hz, 2H), 3.03 (t, J = 6.6 Hz, 2H), 2.88 (t, J = 8.1 Hz, 2H), 1.77–1.69 (m, 4H). ^{13}C NMR ($CDCl_3$) d: 163.73, 153.18, 148.94, 147.13, 146.44, 141.59, 127.98, 125.32, 120.54, 118.26, 117.09, 110.54, 94.08, 61.26, 57.12, 56.23, 56.16, 53.99, 50.18, 39.50, 28.40, 27.22, 25.11. Anal. Calcd for $C_{23}H_{29}BrN_2O_5$: C, 55.99; H, 5.92; N, 5.68. Anal. Calcd for $C_{23}H_{29}BrN_2O_5 \cdot 0.25H_2O$ (corrected): C, 55.48; H, 5.97; N, 5.63. Found: C, 55.42; H, 5.64; N, 5.58.

7.2.4. 5-Bromo-*N*-(4-(7,8-dimethoxy-4,5-dihydro-1*H*-benzo[*c*]-azepin-2(3*H*)-yl)butyl)-2,3-dimethoxybenzamide (6)

Compound **6** was prepared according to the general procedure with **15** (306 mg, 1.1 mmol). After a short path column with $CHCl_3$/MeOH (10:1), **6** (480 mg, 92%) was obtained as a light yellow gum. ^{1}H NMR ($CDCl_3$) d: 7.96 (br t, 1H), 7.78 (d, J = 2.4 Hz, 1H), 7.12 (d, J = 2.4 Hz, 1H), 6.67 (s, 1H), 6.66 (s, 1H), 3.88 (s, 6H), 3.86 (s, 6H), 3.84 (s, 2H), 3.45 (dt, J = 5.7, 5.7 Hz, 2H), 3.15–3.11 (m, 2H), 2.85–2.81 (m, 2H), 2.46 (t, J = 6.6 Hz, 2H), 1.75–1.72 (m, 2H), 1.63–1.61 (m, 4H). ^{13}C NMR ($CDCl_3$) d: 163.73, 153.19, 149.59, 146.53, 146.45, 134.99, 128.16, 125.25, 118.19, 117.01, 113.80, 112.80, 61.41, 58.97, 58.25, 56.35, 56.13, 55.99, 52.22, 39.65, 35.52, 27.43, 24.67, 24.53. Anal. Calcd for $C_{25}H_{33}BrN_2O_5$: C, 57.58; H, 6.38; N, 5.37. Anal. Calcd for $C_{25}H_{33}BrN_2O_5 \cdot 1.5H_2O$ (corrected): C, 54.75; H, 6.62; N, 5.11. Found: C, 54.84; H, 6.16; N, 5.10.

7.2.5. 5-Bromo-*N*-(4-(7,8-dimethoxy-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-1-yl)butyl)-2,3-dimethoxybenzamide (7)

Compound **7** was prepared according to the general procedure with **18** (61 mg, 0.22 mmol). After a short path column with CH_2Cl_2/ethyl acetate (2:1), **7** (81 mg, 81%) was obtained as a light yellow gum. ^{1}H NMR ($CDCl_3$) d: 7.86 (br t, 1H), 7.81 (d, J = 2 Hz, 1H), 7.12 (d, J = 2.5 Hz, 1H), 6.65 (s, 1H), 6.54 (s, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.47 (dt, J = 6.5, 6.5 Hz, 2H), 3.12 (t, J = 6.5 Hz, 2H), 2.86–2.84 (m, 2H), 2.71–2.69 (m, 2H), 1.73–1.61 (m, 6H), 1.57–1.55 (m, 2H). ^{13}C NMR ($CDCl_3$) d: 163.63, 153.18, 146.95, 146.41, 145.34, 143.34, 129.12, 128.06, 125.34, 118.20, 117.07, 113.72, 103.73, 61.21, 56.23, 56.14, 56.07, 54.61, 53.48, 39.59, 34.27, 30.44, 27.08, 26.28, 26.03. Anal. Calcd for $C_{25}H_{33}BrN_2O_5$: C, 57.58; H, 6.38; N, 5.37. Found: C, 57.85; H, 6.52; N, 5.36.

7.2.6. 5-Bromo-*N*-(4-(6,7-dimethoxy-3,4-dihydroquinolin-1(2*H*)-yl)butyl)-2,3-dimethoxybenzamide (8)

Compound **8** was prepared according to the general procedure with **27** (70 mg, 0.26 mmol). After a short path column with CH_2Cl_2/ethyl acetate (1:1), **8** (66 mg, 62%) was obtained as a light yellow gum. ^{1}H NMR ($CDCl_3$) d: 7.91 (br t, 1H), 7.82 (d, J = 2.5 Hz, 1H), 7.13 (d, J = 2.5 Hz, 1H), 6.54 (s, 1H), 6.22 (s, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.51 (dt, J = 6.5, 6.5 Hz, 2H), 3.27–3.24 (m, 2H), 3.20–3.18 (m, 2H), 2.68 (t, J = 6.5 Hz, 2H), 1.95–1.90 (m, 2H), 1.71–1.64 (m, 4H). ^{13}C NMR ($CDCl_3$) d: 163.69, 153.18, 148.15, 146.44, 140.07, 139.85, 127.90, 125.34, 118.29, 117.10, 114.36, 114.16, 97.57, 61.23, 56.76, 56.24, 56.15, 51.84, 49.31, 39.54, 27.45, 27.38, 24.02, 22.39. Anal. Calcd for $C_{24}H_{31}BrN_2O_5$: C, 56.81; H, 6.16; N, 5.52. Found: C, 56.88; H, 6.15; N, 5.53.

7.2.7. 5-Bromo-*N*-(4-((3,4-dimethoxyphenethyl)(methyl)-amino)butyl)-2,3-dimethoxybenzamide (9)

Compound **9** was prepared according to the general procedure with **31** (0.32 g, 1.2 mmol). After a short path column with CH_2Cl_2/ethyl acetate (1:1), **9** (112 mg, 11%) was obtained as a yellow oil. The free base was converted into the oxalate salt and recrystallized from a mixture of ethanol, ethyl acetate and diethyl ether. Mp 115–117 °C. ^{1}H NMR of the oxalate salt ($CDCl_3$) d: 8.05 (t, J = 6 Hz, 1H), 7.77 (d, J = 2.5 Hz, 1H), 7.14 (d, J = 2.5 Hz, 1H), 6.81–6.71 (m, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H), 3.49 (dt, J = 6.5, 6.5 Hz, 2H), 3.24 (br s, 2H), 3.19 (br s, 2H), 3.00 (t, J = 8.5 Hz, 2H), 2.84 (s, 3H), 1.89–1.82 (m, 2H), 1.69 (tt, J = 6.5, 6.5 Hz, 2H). ^{13}C NMR of the oxalate salt ($CDCl_3$) d: 164.25, 163.09, 153.26, 149.22, 148.19, 146.58, 128.10, 127.40, 125.13, 120.55, 118.55, 117.06, 111.71, 111.44, 61.33, 57.65, 56.27, 55.87, 55.84, 55.81, 40.09, 38.21, 30.02, 27.00, 20.93. Anal. Calcd for $C_{24}H_{33}BrN_2O_5 \cdot COOH$: C, 54.16; H, 6.18; N, 5.05. Anal. Calcd for $C_{24}H_{33}BrN_2O_5 \cdot COOH \cdot 1.5H_2O$ (corrected): C, 51.64; H, 6.41; N, 4.82. Found: C, 51.72; H, 5.95; N, 4.55.

7.3. 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/500 nM (+)-PTZ (σ_2) and membranes from fresh-frozen, male English Hartley guinea pig brains (Rockland Immunochemicals, Inc., Gilbertsville, PA) as previously described.[30] Experiments were performed in duplicate, and repeated four times. K_i values were calculated from the inhibition data using the Cheng–Prusoff equation,[31] 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.[30]

7.4. Compound stability studies for in vitro binding assays

All analogs except **4** and **9** were dissolved in stock buffer (2.5% AcOH, 1% EtOH in water) at the concentration of 10^{-3} M. The 2.5% AcOH was used to increase solubility of the compounds in aqueous solution. Because **4** and **9** were prepared as the HCl and oxalate salt, respectively, they were dissolved in less concentrated acidic stock buffer (0.1% AcOH, 1% EtOH in water) at the concentration of 10^{-3} M. After 25 days in the stock buffer, each compound's purity was analyzed by analytical HPLC.

Acknowledgements

We acknowledge a grant award from the National Institute on Drug Abuse (DA028477, J.R.L., PI) for partial support of this research. We also acknowledge grant awards from the National Science Foundation (US) and the National Institutes of Health (US) (NSF CHE-89-08304, CHE-95-31247, CHE-92-2183526 and NIH 1S10RR11962-01) which supported the purchase of the NMR spectrometers.

Supplementary data

Supplementary data (^{1}H NMR spectra of intermediates, as well as ^{1}H NMR and ^{13}C NMR spectra of final compounds, are included in the supplementary data. In addition, HPLC chromatograms of compounds **3, 4, 6, 7** and **9** in stock buffer after 25 days can be found. HPLC chromatograms and ^{1}H NMR spectra that monitor the decomposition of compounds **5** and **8** as a function of time are included, along with LC/MS analyses of the decomposition products) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.006.

References and notes

1. Hellewell, S. B.; Bruce, A.; Feinstein, G.; Orringer, J.; Williams, W.; Bowen, W. D. *Eur. J. Pharmacol.* **1994**, *268*, 9.
2. Hanner, M.; Moebius, F. F.; Flandorfer, A.; Knaus, H. G.; Striessnig, J.; Kempner, E.; Glossmann, H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 8072.
3. Seth, P.; Leibach, F.; Ganapathy, V. *Biochem. Biophys. Res. Commun.* **1997**, *241*, 535.
4. Mei, J.; Pasternak, G. W. *Biochem. Pharmacol.* **2001**, *62*, 349.
5. Chen, Y.; Jahipour, A. R.; Sievert, M. K.; Arbabian, M.; Ruoho, A. E. *Biochemistry* **2007**, *46*, 3532.
6. Fontanilla, D.; Hajipour, A. R.; Pal, A.; Chu, U. B.; Arbabian, M.; Ruoho, A. E. *Biochemistry* **2008**, *47*, 7205.
7. Fontanilla, D.; Johannessen, M.; Hajipour, A. R.; Cozzi, N. V.; Jackson, M. B.; Ruoho, A. E. *Science* **2009**, *323*, 934.
8. Langa, F.; Codony, X.; Tovar, V.; Lavado, A.; Giménez, E.; Cozar, P.; Cantero, M.; Dordal, A.; Hernández, E.; Pérez, R.; Monroy, X.; Zamanillo, D.; Guitart, X.; Montoliu, L. *Eur. J. Neurosci.* **2003**, *18*, 2188.
9. Maurice, T.; Su, T.-P. *Pharmacol. Ther.* **2009**, *124*, 195.
10. Cobos, E. J.; Entrena, J. M.; Nieto, F. R.; Cendán, C. M.; Del Pozo, E. *Curr. Neuropharmacol.* **2008**, *6*, 344.
11. Glennon, R. A. *Mini-Rev. Med. Chem.* **2005**, *5*, 927.
12. Glennon, R. A. *Braz. J. Pharm. Sci.* **2005**, *41*, 1.
13. Laurini, E.; Zampieri, D.; Mamolo, M. G.; Vio, L.; Zanette, C.; Florio, C.; Posocco, P.; Fermeglia, M.; Pricl, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2954.
14. Mach, R. H.; Huang, Y.; Freeman, R. A.; Wu, L.; Vangveravong, S.; Luedtke, R. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 195.
15. Hou, C.; Tu, Z.; Mach, R.; Kung, H. F.; Kung, M.-P. *Nucl. Med. Biol.* **2006**, *33*, 203.
16. Tu, Z.; Xu, J.; Jones, L. A.; Li, S.; Dumstorff, C.; Vangveravong, S.; Chen, D. L.; Wheeler, K. T.; Welch, M. J.; Mach, R. H. *J. Med. Chem.* **2007**, *50*, 3194.
17. Tu, Z.; Xu, J.; Jones, L. A.; Li, S.; Zeng, D.; Kung, M.-P.; Kung, H. F.; Mach, R. H. *Appl. Radiat. Isot.* **2010**, *68*, 2268.
18. Xu, R.; Lever, J. R.; Lever, S. Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2594.
19. Zhang, H.; Cai, Q.; Ma, D. *J. Org. Chem.* **2005**, *70*, 5164.
20. Toda, J.; Ichikawa, T.; Saitoh, T.; Horiguchi, Y.; Sano, T. *Heterocycles* **2000**, *53*, 2009.
21. Tafesse, L.; Kyle, D. J. *Comb. Chem. High Throughput Screening* **2004**, *7*, 151.
22. Dalence-Guzmán, M. F.; Berglund, M.; Skogvall, S.; Sterner, O. *Bioorg. Med. Chem.* **2008**, *16*, 2499.
23. Lee, J. W.; Jun, S. I.; Kim, K. *Tetrahedron Lett.* **2001**, *42*, 2709.
24. Appukkuttan, P.; Orts, A. B.; Chandran, R. P.; Goeman, J. L.; Van der Eycken, J.; Dehaen, W.; Van der Eycken, E. *Eur. J. Org. Chem.* **2004**, 3277.
25. Chavdarian, C. G.; Karashima, D.; Castagnoli, N., Jr.; Hundley, H. K. *J. Med. Chem.* **1978**, *21*, 548.
26. Bethuel, Y.; Gademann, K. *J. Org. Chem.* **2005**, *70*, 6258.
27. Walker, G. N. *J. Am. Chem. Soc.* **1956**, *78*, 3698.
28. Toda, J.; Sakagami, M.; Sano, T. *Chem. Pharm. Bull.* **1999**, *47*, 1269.
29. Tiwari, S. B.; Kohli, D. V. *Med. Chem. Res.* **2008**, *17*, 386.
30. Lever, J. R.; Gustafson, J. L.; Xu, R.; Allmon, R. L.; Lever, S. Z. *Synapse* **2006**, *59*, 350.
31. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.