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3-Amino-chromanes and Tetrahydroquinolines as Selective 5-HT$_{2B}$, 5-HT$_7$, or σ_1 Receptor Ligands

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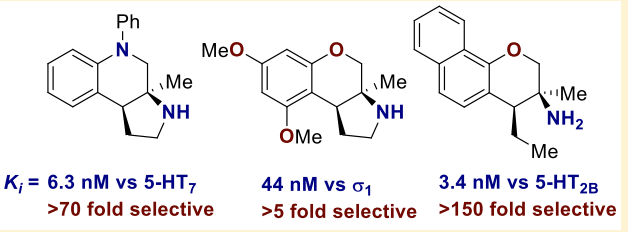
Supporting Information

ABSTRACT: The phenethylamine backbone is a privileged substructure found in a wide variety of G protein-coupled receptor (GPCR) ligands. This includes both endogenous neurotransmitters and active pharmaceutical agents. More than 20 structurally unique heterocyclic phenethylamine derivatives were broadly evaluated for GPCR affinity. Selective ligands for the 5-HT$_{2B}$, 5-HT$_7$, and σ_1 receptors were identified, each with low nanomolar binding affinities. The σ_1 receptor affinity was supported in a cellular assay that provided evidence for increased cell survival under oxidative stress.



KEYWORDS: *5-HT$_7$, serotonin receptors, σ receptor, σ$_1$ receptor, G protein-coupled receptors, neuroprotection*

G protein-coupled receptors (GPCRs) are a prominent pharmacological target. More than 30% of FDA approved drugs target at least one GPCR.[1,2] Worldwide, more than 25% of drug sales come from GPCR modulating compounds.[3] GPCR modulators are used to treat a wide variety of diseases and disorders including allergies,[4] schizophrenia,[5] depression,[6] pain management,[7] and asthma.[8] Common GPCR targeting pharmaceuticals[9] include the antihistamine loratadine,[4] antidepressants fluoxetine and sertraline,[6] antipsychotics aripiprazole, clozapine, and haloperidol,[5] the opioids morphine, hydrocodone, and fentanyl,[7] and bronchodilators salbutamol and tiotropium bromide.[10] The phenethylamine core is a privileged substructure in GPCR ligands (Figure 1). This is partially because several endogenous neurotransmitters or neuromodulators are phenethylamines including dopamine[11] and epinephrine[12] (Figure 1a). The phenethylamine backbone is also found in a wide variety of GPCR targeting active pharmaceutical ingredients including morphine, salbutamol, and pseudoephedrine (Figure 1b).

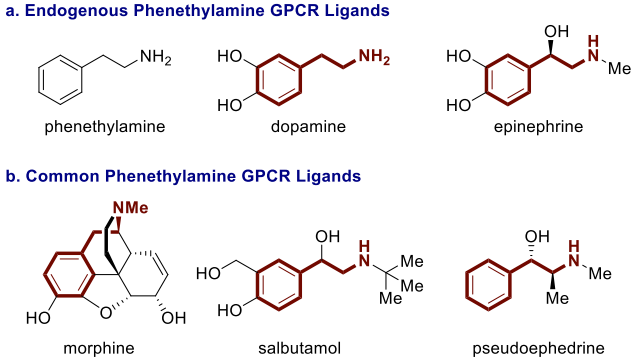
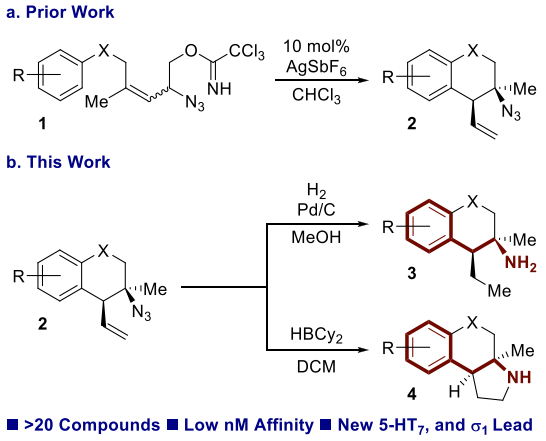


Figure 1. Phenethylamine containing GPCR ligands.

Scheme 1. Source of Novel Phenethylamines



Our recently developed a tandem Winstein rearrangement Friedel−Crafts alkylation that enabled the synthesis of differentially functionalized heterocyclic tertiary azides from

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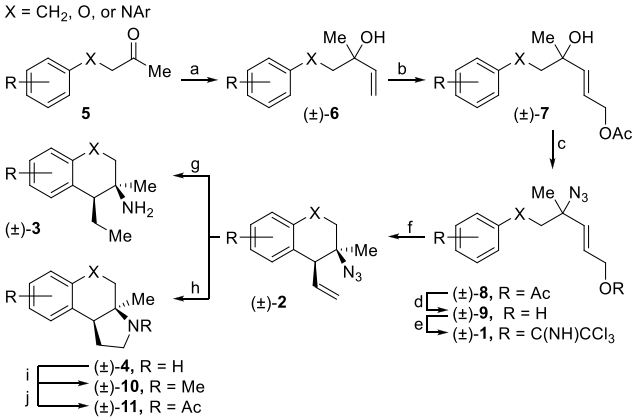
Scheme 2. Synthesis of (±)-GPCR Ligands[a]



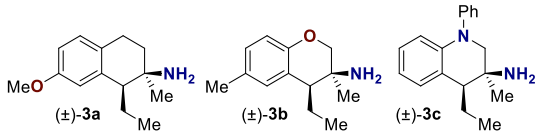
[a]Reagents and conditions: (a) vinyl MgCl, THF, 0 °C, 30 min, 83−91%; (b) *cis*-1,4-diacetoxy-2-butene, 1−2 mol % Hoveyda−Grubbs second generation catalyst, 40 °C, 18 h, 68−86%; (c) $TMSN_3$, 10 mol % $Zn(OTf)_2$, rt, 90 min, 29−87%; (d) K_2CO_3, MeOH, rt, 30 min, 97%−quant.; (e) $NCCCl_3$, 20 mol % DBU, rt, 90 min, 73−97%; (f) 10 mol % $AgSbF_6$, $CHCl_3$, 40−60 °C, 24 h, 39−94%; (g) H_2, 10% w/w Pd/C, MeOH, 18 h, 52%-quant; (h) $HBCy_2$, DCM, 0 °C to rt, 18 h, 35−84%; (i) aq. CH_2O, $NaBH_3CN$, HOAc, $NCCH_3$, 0 °C to rt, 30 min, 70−89%; (j) Ac_2O, TEA, DMAP, 0 °C to rt, 18 h 73−96%.

an equilibrating mixture of allylic azides (Scheme 1a).[13] This cascade was synthetically attractive because it constructed tetralins, chromanes, and tetrahydroquinolines featuring a tetra-substituted stereocenter while maintaining a diversifiable vinyl group. The heterocyclic products could be readily converted into substituted phenethylamine derivatives containing either a primary amine or pyrrolidine motif (Scheme 1b). It seemed prudent to investigate the potential GPCR affinity of these molecules because of their rigid structure, small molecular weight, and phenethylamine backbone. This report describes the results of an initial screen, which identified several individual molecules each with low nM binding affinity for 5-HT_{2B}, 5-HT_7, or σ_1 receptor. Furthermore, the small family of compounds screened demonstrates divergent selectivity across the GPCR family. A new lead compound was identified for the 5-HT_7 receptor, and a second lead compound was identified for the σ_1 receptor. The σ_1 receptor affinity was further supported by cellular assays.

The synthesis of these compounds began with the addition of vinyl Grignard to ketone **5**, forming tertiary allylic alcohol **6** (Scheme 2). Subsequent cross metathesis with *cis*-1,4-but-2-enediol diacetate and Hoveyda−Grubbs second generation catalyst afforded allylic acetate **7**. Azidation occurred upon exposure to $TMSN_3$ and catalytic $Zn(OTf)_2$ to generate allylic azide **8** as a mixture of equilibrating isomers. Methanolysis cleaved the acetate, and resulting alcohol **9** was activated as the trichloroacetimidate **1**. The dynamic cyclization was performed with catalytic $AgSbF_6$, yielding tertiary azide **2**, typically in >25:1 dr. Global reduction with H_2 and palladium on carbon afforded primary amine **3**. Alternatively, exposure to $HBCy_2$ resulted in pyrrolidine **4**. The pyrrolidine could be subjected to *N*-methylation (**10**) or acetylation (**11**).

The GPCR binding affinity of these compounds was assessed through the Psychoactive Drug Screening Program.[14] Initially, three primary amines were screened (Table 1, see the Supporting Information for selected binding curves). Compounds **3a**−**3c** represent one example each of the three

Table 1. Initial GPCR Screen[a]



	pK_i[a]		
receptor	compound **3a**	compound **3b**	compound **3c**
5-HT_{1A}	6.2 ± 0.1[b]	5.7 ± 0.1[b]	*7.13 ± 0.06*[b]
5-HT_{1B}	<5	<5	<5
5-HT_{1D}	<5	<5	<5
5-HT_{1E}	<5	<5	<5
5-HT_{2A}	<5	<5	<5
5-HT_{2B}	*6.84 ± 0.06*[b]	*6.75 ± 0.06*[b]	6.1 ± 0.1[b]
5-HT_{2C}	5.6 ± 1.0[b]	5.6 ± 3.5[b]	5.4 ± 1.3[b]
5-HT_3	<5	<5	<5
5-HT_{5A}	5.7 ± 1.5[b]	<5	<5
5-HT_6	<5	<5	<5
5-HT_7	<5	<5	6.0 ± 0.1[b]
$Alpha_{1A}$	<5	<5	5.8 ± 0.1[b]
$Alpha_{1B}$	<5	<5	<5
$Alpha_{1D}$	<5	<5	<5
$Alpha_{2A}$	5.2 ± 0.2[b]	<5	6.4 ± 0.1[b]
$Alpha_{2B}$	5.3 ± 0.1[b]	<5	6.4 ± 0.1[b]
$Alpha_{2C}$	<5	<5	<5
$Beta_1$	<5	<5	<5
$Beta_2$	5.3 ± 0.1[b]	<5	<5
$Beta_3$	<5	<5	6.0 ± 0.1[b]
BZP	5.8 ± 0.1[b]	<5	5.1 ± 0.1[b]
D_1	<5	<5	<5
D_2	<5	<5	<5
D_3	<5	<5	<5
D_4	<5	<5	5.7 ± 0.1[b]
D_5	<5	<5	<5
DAT	5.3 ± 0.2[b]	<5	5.5 ± 0.2[b]
$GABA_A$	<5	<5	<5
H_1	5.5 ± 0.5[b]	<5	<5
H_2	<5	<5	<5
H_3	<5	<5	5.4 ± 0.2[b]
H_4	<5	<5	<5
M_1	<5	<5	<5
M_2	<5	<5	<5
M_3	<5	<5	<5
M_4	<5	<5	<5
M_5	<5	<5	<5
NET	5.6 ± 0.1[b]	6.5 ± 0.1[b]	6.4 ± 0.1[c]
δ-OR	<5	<5	<5
κ-OR	*6.5 ± 0.1*[b]	<5	<5
μ-OR	5.7 ± 0.1[b]	<5	<5
PBR	<5	<5	<5
σ_1	*6.9 ± 0.1*[b]	6.0 ± 0.1[b]	6.5 ± 0.1[b]
σ_2	<5	<5	6.2 ± 0.1[b]

[a]For pK_i values reported as <5, the compound did not demonstrate ≥50% binding in an initial assay at 10 μM concentration. [b]These data reflect triplicate measurements of a single experiment. The error reflects the error in the sigmoidal curve fit. [c]These data reflect the average of triplicate experiments run in triplicate. The error reflects the standard deviation of the three calculated pK_i values. Values in italic type are noted for the reader's convenience.

synthetically accessible cores: tetralin (**3a**), chromane (**3b**), and tetrahydroquinoline (**3c**). These molecules were screened across a wide range of human GPCR including serotonin (5-

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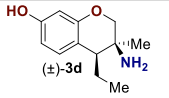
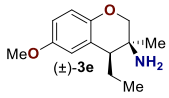
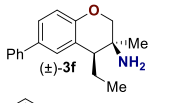
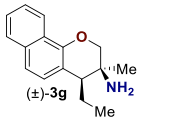
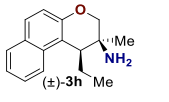
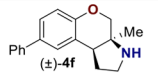
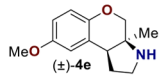
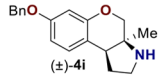
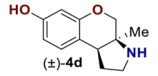
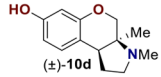
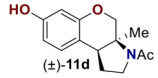
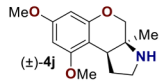
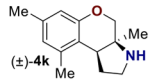
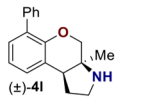
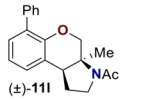
Table 2. K_i Data for Primary Amino-chromanes[a]

Compound	pK_i[a] 5-HT$_{1A}$	5-HT$_{2B}$	5-HT$_7$	σ_1	σ_2
 (±)-3d	n.d.	< 5	n.d.	< 5	< 5
 (±)-3e	5.6 ±0.1[b]	6.6 ±0.1[c]	n.d.	6.4 ±0.1[c]	< 5
 (±)-3f	5.7 ±1.9[b]	6.9 ±0.1[c]	n.d.	7.9 ±0.2[c]	6.5 ±0.2[b]
 (±)-3g	5.6 ±2.1[b]	8.6 ±0.2[b]	6.00 ±0.02[c]	5.5 ±0.4[b]	6.5 ±0.6[b]
 (±)-3h	6.5 ±0.1[b]	7.7 ±0.2[c]	6.16 ±0.03[c]	6.0 ±0.3[b]	5.7 ±0.2[b]

[a]For pK_i values reported as <5, the compound did not demonstrate ≥50% binding in an initial assay at 10 μM concentration. [b]These data reflect triplicate measurements of a single experiment. The error reflects the error in the sigmoidal curve fit. [c]These data reflect the average of triplicate experiments run in triplicate. The error reflects the standard deviation of the three calculated pK_i values. Values highlighted in red are noted for the reader's convenience. Values not determined are noted as n.d.

Table 3. K_i Data for Pyrrolidine Containing Chromanes[a]

Compound	pK_i[a] 5-HT$_{1A}$	5-HT$_{2B}$	5-HT$_7$	σ_1	σ_2
 (±)-4f	n.d.	6.6 ±0.1[d]	6.0 ±0.1[b]	7.9±0.1[d]	7.1 ±0.2[b]
 (±)-4e	n.d.	6.2 ±0.1[d]	6.7 ±0.1[b]	6.9 ±0.1[d]	5.5 ±0.2[b]
 (±)-4i	n.d.	6.9 ±0.1[d]	n.d.	8.1 ±0.1[d]	7.0 ±0.3[b]
 (±)-4d	n.d.	< 5	n.d.	< 5	6.0 ±0.4[b]
 (±)-10d	n.d.	6.0 ±0.1[d]	n.d.	7.3 ±0.1[d]	5.4 ±0.2[b]
 (±)-11d	n.d.	< 5	n.d.	< 5	< 5
 (±)-4j	6.0 ±0.1[b]	6.6 ±0.1[d]	5.9 ±0.1[b]	7.4 ±0.1[b]	< 5
 (±)-4k	n.d.	7.1 ±0.1[c]	5.9 ±0.1[d]	7.2±0.1[d]	6.5 ±0.2[b]
 (±)-4l	6.7 ±0.1[b]	6.8 ±0.1[d]	7.63 ±0.04[b]	7.4 ±0.1[b]	6.9 ±0.1[b]
 (±)-10l	6.7 ±0.2[b]	6.6 ±0.1[d]	7.7±0.1[d]	7.3±0.1[d]	6.5 ±0.2[b]
 (±)-11l	n.d.	6.3 ±0.1[d]	n.d.	< 5	5.9 ±0.2[b]
 (±)-4m	6.0 ±0.1[b]	7.7±0.1[d]	6.4 ±0.2[b]	6.3 ±0.1[d]	6.2 ±0.2[b]

[a]For pK_i values reported as <5, the compound did not demonstrate ≥50% binding in an initial assay at 10 μM concentration. [b]These data reflect triplicate measurements of a single experiment. The error reflects the error in the sigmoidal curve fit. [c]These data reflect the average duplicate experiments run in triplicate. The error reflects one-half the difference between the two calculated pK_i values. [d]These data reflect the average of triplicate experiments run in triplicate. The error reflects the standard deviation of the three calculated pK_i values. Values highlighted in red are noted for the readers convenience. Values not determined are noted as n.d.

HT), α- and β-adrenergic (Alpha and Beta), dopamine (D), histamine (H), muscarinic (M), opioid (OR), sigma (σ), and others (Table 1). Of the three compounds assayed, all exhibited at least moderate binding ($K_i \leq 200$ nM) against one or more receptors. Tetralin **3a** was the only compound to exhibit any affinity against the δ-, μ-, or κ-opioid receptor. This is consistent with the structures of other phenethyl opioid ligands that contain a cyclohexyl backbone (e.g., morphine, Figure 1b).[7,15] Compound **3a** exhibited moderate affinity toward the 5-HT$_{2B}$ and σ_1 receptors. Chromane **3b** exhibited moderate affinity against the 5-HT$_{2B}$ receptor but lacked any κ-opioid binding. Tetrahydroquinoline **3c** exhibited the strongest initial hit, with a K_i of 74 nM against the 5-HT$_{1A}$ receptor. Based on these initial results and lack of opioid affinity, a subsequent structure activity relationship (SAR) study was conducted on the chromane and tetrahydroquinoline scaffolds, screening against the 5-HT$_{1A}$, 5-HT$_{2B}$, 5-HT$_7$, σ_1, and σ_2 receptors.

Assay results for five additional primary amine analogues are displayed (Table 2). Amines **3d** and **3e** did not demonstrate a K_i below 100 nM for any of the GPCRs assayed. Amine **3f** bound the σ_1 receptor with a K_i of 16 nM and demonstrated reasonable selectivity relative to the 5-HT$_{2B}$ receptor (10-fold), which was the second most sensitive receptor assayed. Amines **3g** and **3h** have higher affinities toward the 5-HT$_{2B}$ receptor (K_i = 3.5 and 20 nM, respectively). Compound **3g** demonstrated ~150-fold selectivity relative to the σ_2 receptor, which was the second most sensitive GPCR assayed. The 5-HT$_{2B}$ receptor is a member of the 5-HT family of receptors that is known to be an essential receptor during development.[16] Long-term consumption of 5-HT$_{2B}$ agonists can induce potentially fatal myofibroblast proliferation and valvular heart disease.[17,18] Thus, the 5-HT$_{2B}$ receptor is considered an antitarget. Therefore, further optimization of this amine class was not pursued, opting instead to evaluate the σ_1 affinity demonstrated by amine **3f** on more rigid pyrrolidine analogues.

The σ receptors were initially thought to be members of the opioid receptor family.[19] Since, it has been shown that σ_1 acts as a chaperone protein localized in the endoplasmic reticulum,[20] and it affects a wide variety of cellular functions including regulation of opioid receptors,[20] kinases,[21] TRPV1,[22] dopamine receptors,[23] apoptosis,[24] as well as cellular calcium and potassium levels.[25] Modulating intracellular calcium levels implicated σ_1 as a target for treating colon and breast cancer.[26,27] Modulating the σ_1 receptor also effects alcohol abuse,[28] pain management,[29] opioid analgesia,[30,31] and neuroprotection in models of retinal neural degradation.[32,33]

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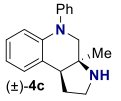
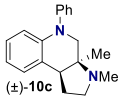
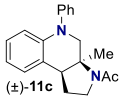
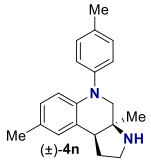
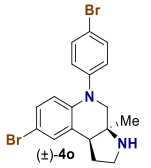
Table 4. K_i Data for Tetrahydroquinolines[a]

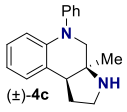
Compound	pK_i[a] 5-HT$_{1A}$	5-HT$_{2B}$	5-HT$_7$	σ_1	σ_2
 (±)-**4c**	< 5	6.3 ±0.1[b]	**8.2 ±0.1**[b]	6.3 ±0.1[b]	6.2 ±0.1[b]
 (±)-**10c**	6.7 ±0.1[b]	6.7 ±0.1[c]	**8.57 ±0.04**[b]	6.79 ±0.02[c]	6.8 ±0.2[b]
 (±)-**11c**	n.d.	< 5	n.d.	< 5	5.9 ±0.3[b]
 (±)-**4n**	n.d.	6.5 ±0.1[c]	6.7 ±0.1[b]	6.6 ±0.1[c]	6.5 ±0.2[b]
 (±)-**4o**	n.d.	6.5 ±0.1[c]	6.7 ±0.1[b]	6.8 ±0.1[c]	**7.1 ±0.2**[b]

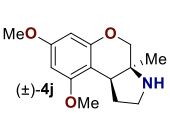
[a]For pK_i values reported as <5, the compound did not demonstrate ≥50% binding in an initial assay at 10 μM concentration. [b]These data reflect triplicate measurements of a single experiment. The error reflects the error in the sigmoidal curve fit. [c]These data reflect the average of triplicate experiments run in triplicate. The error reflects the standard deviation of the three calculated pK_i values. Values highlighted in red are noted for the reader's convenience. Values not determined are noted as n.d.

Pyrrolidine containing chromanes were assessed for σ_1 binding (Table 3). A direct analogue of amine **3f** was essentially equipotent against σ_1 (**4f**), while replacing the phenyl group with a methoxy group was disadvantageous (**4e**). Exploring substitution around the arene provided mixed results. Benzyloxy compound **4i** was a high-affinity σ_1 ligand. Removing the benzyl group was detrimental to affinity (**4d**). Some affinity could be restored through *N*-methylation (**10d**). Unsurprisingly, masking the basic amine as an amide removed affinity in both cases examined (**11d** and **11l**). Compound **4j** could be a lead compound. While compound **4j** demonstrated reduced affinity relative to amine **3f** as a σ_1 ligand, it shows >200-fold selectivity versus the σ_2 receptor. Changing the methoxy groups for methyl groups enhanced 5-HT$_{2B}$ binding (**4k**), and the other compounds assayed provided both reduced σ_1 affinity and selectivity (**4l** and **4m**).

With an attempt to evaluate the 5-HT$_{1A}$ affinity demonstrated by amine **3c** (Table 1), other tetrahydroquinolines were investigated (Table 4). As a direct comparison to amine **3c** (Table 1), pyrrolidine **4c** (Table 4) was assayed. While primary amine **3c** was ∼15-fold selective for 5-HT$_{1A}$ over 5-HT$_7$, pyrrolidine **4c** was not a suitable ligand for 5-HT$_{1A}$ (K_i > 10 000 nM) and was instead a high-affinity 5-HT$_7$ ligand (K_i = 6.3 nM). Therefore, the relatively small structural change resulted in more than a 20 000-fold relative difference in the 5-HT$_{1A}$ versus 5-HT$_7$ selectivity. The 5-HT$_7$ receptor has been implicated in the regulation of multiple biological functions including sleep, circadian rhythm, and mood.[16] Various 5-HT$_7$ antagonists have been investigated for depression treatment along with other disorders.[34] The 5-HT$_7$ affinity could be further enhanced though *N*-methylation (**10c**), while acylation removed affinity (**11c**). Other substituted arenes displayed reduced 5-HT$_7$ affinity (**4n** and **4o**). Even though compound **4c** has a weaker affinity than compound **10c**, compound **4c** was selected for further assay because it exhibited enhanced selectivity, having a ∼75-fold lower affinity for the next most sensitive receptor, σ_1.

Table 5. GPCR Screen for Selectivity[a]

 (±)-**4c**

 (±)-**4j**

receptor	pK_i[a] compound **4c**	compound **4j**
5-HT$_{1A}$	<5	6.0 ± 0.1
5-HT$_{1B}$	<5	<5
5-HT$_{1D}$	<5	<5
5-HT$_{1E}$	<5	<5
5-HT$_{2B}$	6.3 ± 0.1	6.6 ± 0.1
5-HT$_{2C}$	<5	5.8 ± 0.1
5-HT$_3$	<5	<5
5-HT$_6$	<5	<5
5-HT$_7$	*8.2 ± 0.1*	5.9 ± 0.1
Alpha$_{1A}$	<5	<5
Alpha$_{1B}$	<5	<5
Alpha$_{1D}$	6.0 ± 0.1	<5
Alpha$_{2B}$	<5	<5
Alpha$_{2C}$	6.0 ± 0.1	<5
Beta$_1$	5.5 ± 0.1	6.0 ± 0.1
Beta$_2$	<5	6.24 ± 0.03
Beta$_3$	<5	<5
BZP	<5	5.2 ± 0.1
D$_1$	<5	<5
D$_2$	<5	<5
D$_3$	<5	<5
D$_4$	<5	<5
D$_5$	<5	<5
DAT	<5	<5
GABA$_A$	<5	<5
H$_1$	<5	<5
H$_2$	5.7 ± 0.1	<5
H$_4$	<5	<5
M$_1$	<5	<5
M$_2$	<5	<5
M$_3$	<5	<5
M$_4$	<5	<5
M$_5$	<5	<5
NET	5.8 ± 0.1	<5
δ-OR	<5	<5
κ-OR	<5	<5
μ-OR	<5	<5
PBR	<5	<5
σ_1	6.3 ± 0.1	*7.4 ± 0.1*
σ_2	6.0 ± 0.1	<5

[a]For pK_i values reported as <5, the compound did not demonstrate ≥50% binding in an initial assay at 10 μM concentration. These data reflect triplicate measurements of a single experiment. The error reflects the error in the sigmoidal curve fit. Values in italic type are noted for the reader's convenience.

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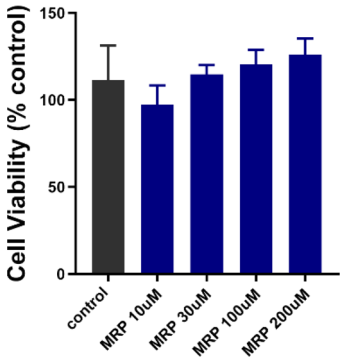

Figure 2. Cytotoxicity assay of compound **4j** in 661W cells. 661W cells were treated with compound **4j** (10, 30, 100, and 200 μM) for 24 h. Cell viability was assessed using the MTT assay. No significant difference in viability was observed at the **4j** concentrations tested compared with nontreated cells. MRP = compound **4j**.

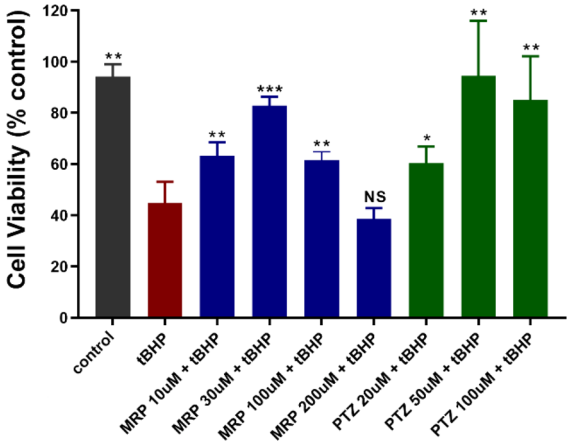

Figure 3. Enhanced 661W cell viability with compound **4j** upon *t*BHP exposure. 661W cells were treated with *t*BHP in the presence/absence of increasing concentrations of compound **4j** (10–200 μM) or the σ_1 receptor ligand PTZ (20–100 μM) for 24 h before cell viability assessment. Cell viability was assessed using the MTT assay. Data are presented as mean ± standard deviation (SD) of triplicate measurements; *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant as compared to the *t*BHP treatment. (*t*BHP = *tert*-butyl hydroperoxide; MRP = compound **4j**; PTZ = (+)-pentazocine.)

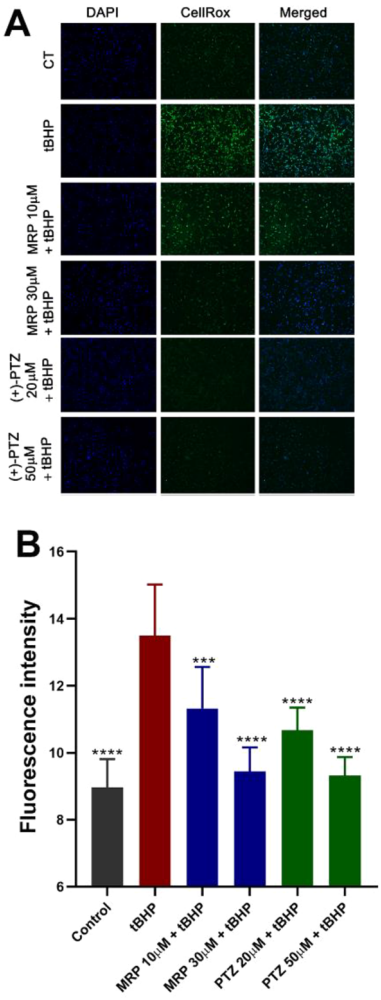

Figure 4. Attenuated oxidative stress induced by *t*BHP in 661W cells upon treatment with compound **4j** or PTZ. 661W cells were seeded on coverslips for 18 h. Cells either were or were not (control) exposed for 2 h to *t*BHP (55 μM) in the presence/absence of compound **4j** or PTZ. (A) Representative immunofluorescent images of cells incubated with CellROX green reagent to detect ROS; green fluorescent signals indicated ROS as visualized by epifluorescence. DAPI was used to label nuclei (blue). (B) Quantification of fluorescent intensity reflecting ROS levels of data shown in panel A. Data are presented as mean ± SD. Data represent three independent experiments performed in duplicate. Significant differences are indicated: *** $p < 0.001$; ****$p < 0.0001$ as compared to the *t*BHP treatment. (CT = control, *t*BHP = *tert*-butyl hydroperoxide; MRP = compound **4j**; PTZ = (+)-pentazocine.)

Based on the results outlined in Tables 1–4, compounds **4c** and **4j** were screened more broadly against a wider array of GPCRs (Table 5). Gratifyingly, only minimal affinity was observed across the additional GPCRs that were investigated. This indicates that compound **4c** could be considered a new lead targeting 5-HT$_7$ due to the low nM binding affinity (K_i = 6.3 nM) and selectivity (~75-fold versus next most sensitive receptor). Compound **4j** could be considered a new lead targeting σ_1 due to its respectable affinity (K_i = 44 nM), good σ_1 versus σ_2 selectivity (>200-fold), and good selectivity versus other GPCRs (>5-fold versus 5-HT$_{2B}$, and >20-fold versus 38 other GPCRs).

The σ_1 receptor is a target for protecting retinal cells from neural degradation.[32,33,35] To date, the most studied agent is (+)-pentazocine (PTZ), which has shown promise in mice.[36,37] Unfortunately, PTZ is a potent opioid and is probably unsuitable for clinical use as a treatment for retinopathy.[7] The protective effects of compound **4j** were demonstrated in the well-characterized cone photoreceptor cell line 661W.[38] Treatment of 661W cells with up to 200 μM of compound **4j** for 24 h did not alter cell viability as measured using the MTT assay (Figure 2). This indicated that compound **4j** was not cytotoxic to 661W cells at the concentrations assayed.

Exposing 661W cells to *tert*-butyl hydroperoxide (*t*BHP, 55 μM) for 24 h induced oxidative stress, which decreased cell viability by more than 50% (Figure 3). Cotreatment with compound **4j** resulted in a dose-dependent improvement in cell viability (see the Supporting Information for additional concentrations). At a **4j** concentration of 30 μM, cell viability was significantly greater than *t*BHP-exposed cells and was >80% of nontreated cells. The beneficial effects of compound **4j** (at 30 μM) were similar to those observed in cells treated

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with PTZ (50−100 μM, Figure 3). At the highest concentration tested (200 μM), the beneficial effects of compound **4j** were no longer observed. The beneficial effects of compound **4j** (30 μM) could be counteracted by the addition of BD1063 (10 μM), which is a known σ_1 receptor antagonist (see the Supporting Information).

Earlier studies demonstrated the beneficial effects of σ_1 receptor modulation by PTZ in attenuating oxidative stress in 661W cells.[39] Compound **4j** was evaluated for similar effects. Reactive oxygen species (ROS) were detected using the CellROX green reagent in an immunocytochemical assay upon exposure of 661W cells to *t*BHP (55 μM). CellROX is a cell-permeant dye that is weakly fluorescent in a reduced state but exhibits bright green photostable fluorescence upon oxidation by ROS. Cells that received no *t*BHP showed minimal fluorescence, while cells exposed to *t*BHP showed a marked increase in green fluorescence (Figure 4A). Treatment with compound **4j** resulted in decreased green fluorescence, especially at the 30 μM concentration. These data were similar to the fluorescence suppression observed with PTZ treatment (Figure 4A). Quantification of fluorescence intensity indicated a marked increase in ROS in the *t*BHP-exposed cells, whereas the level of ROS was significantly reduced in *t*BHP-exposed cells treated with compound **4j** or PTZ (Figure 4B).

In conclusion, the GPCR affinity was investigated for a variety of new phenethylamine-based primary amines and pyrrolidines. Several compounds demonstrated significant and selective binding against the 5-HT_{2B}, 5-HT_7, or σ_1 receptor. Compound **4j** protected against oxidative stress when assayed in 661W cells. The potency of compound **4j** was comparable to PTZ in these assays.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.9b00225.

Experimental procedures, characterization data, and additional information on assays (PDF)

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Author Contributions

MP performed the synthesis of new compounds. HX and JW performed assays with 661W cells. The project was directed by JT and SS. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare the following competing financial interest(s): A provisional patent has been filed by the University of Minnesota on the substructure disclosed in this report; M.R.P. and J.J.T. filed US patent No. 16/428,343, submitted 5/31/2019, on the compounds in this article.

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