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Electron-Donating Para-Methoxy Converts a Benzamide-Isoquinoline Derivative into a Highly Sigma-2 Receptor Selective Ligand

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Abstract

The sigma-2 (σ_2) receptor has been suggested to be a promising target for pharmacological interventions to curb tumor progression. Development of σ_2 -specific ligands, however, has been hindered by lack of understanding of molecular determinants that underlie selective ligand- σ_2 interactions. Here we have explored effects of electron donating and withdrawing groups on ligand selectivity for the σ_2 versus σ_1 receptor using new benzamide-isoquinoline derivatives. The electron-donating methoxy group increased but the electron-withdrawing nitro group decreased σ_2 affinity. In particular, an extra methoxy added to the para-position (**5e**) of the benzamide phenyl ring of **5f** dramatically improved (631 fold) the σ_2 selectivity relative to the σ_1 receptor. This para-position provided a sensitive site for effective manipulation of the sigma receptor subtype selectivity using either the methoxy or nitro substituent. Our study provides a useful guide for further improving the σ_2 -over- σ_1 selectivity of new ligands.

Keywords

Sigma-2 receptor; methoxy; electron-donating; para-position; selectivity; isoquinoline

1. INTRODUCTION

The σ_1 and σ_2 receptors are unique, non-opioid binding sites ubiquitously distributed in various tissues (see review ¹). These two subtypes are pharmacologically distinguishable ². With no homology to any known mammalian protein ³, the σ_1 receptor has been identified as a unique ligand-operated chaperone residing in the endoplasmic reticulum membrane ⁴. In contrast, however, little had been known about the molecular properties of the σ_2 receptor

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until most recently the progesterone receptor membrane component 1 (PGRMC1) protein complex was identified as a putative $\sigma 2$ receptor binding site⁵.

Sigma receptor ligands have been reported to have various therapeutic potentials. Numerous studies have proposed selective $\sigma 1$ agonists as therapeutic agents for anxiety, depression, psychosis, and learning and memory improvement, and recently, neuroprotective effects of $\sigma 1$ agonists have garnered increasing attention (see reviews⁶⁻⁹). Thus far, the most prominent function of the $\sigma 2$ receptor has been linked to tumor progression. It is known that the $\sigma 2$ receptor is highly expressed in various tumor cells¹⁰, and remarkably, proliferating tumor cells express nearly 10-fold higher amounts of the $\sigma 2$ receptor than quiescent tumor cells¹¹. Importantly, $\sigma 2$ receptor agonists have been found to induce apoptosis in different tumor cell lines¹²⁻¹⁶. These findings strongly support use of the $\sigma 2$ receptor as a biomarker for solid proliferating tumors and thereby selective $\sigma 2$ agonists as potential anti-cancer drugs. However, only a very few highly $\sigma 2$ selective drugs are now under development (see review¹⁷).

It is interesting to note that the putative $\sigma 2$ receptor (PGRMC1) has a molecular size (25kDa)⁵ distinct from the $\sigma 2$ binding sites previously observed through photoaffinity labeling (21.5 and/or 18 kDa)¹⁸⁻²¹. It remains to be clarified whether they are different splice variants. Therefore it is important to develop $\sigma 2$ selective ligands for understanding the enigmatic $\sigma 2$ receptor binding site(s). Ligand binding has been the main approach for detecting the $\sigma 2$ receptor. The commonly used ligands including DTG (1,3-di(2-tolyl)guanidine) and haloperidol, however, are not selective over the $\sigma 1$ receptor. Considerable effort has been paid to create $\sigma 2$ ligands with improved affinity and selectivity, some of which have been developed into imaging agents for proliferating tumor detection as well as for tissue and cellular distribution of this receptor (see review¹⁷). In spite of these advances, only a few compounds, such as CB-184²², PB28¹⁴, siramesine¹⁵, WC-26 and tetrahydroisoquinoline derivatives^{5, 23}, have shown excellent $\sigma 2$ over $\sigma 1$ selectivity.

A major barrier for discovery of $\sigma 2$ -selective ligands is the lack of understanding of the molecular determinants for ligand/ $\sigma 2$ interactions. Development of $\sigma 2$ -selective ligands has not been straightforward, and in some cases, identification of ligands with high affinity for the $\sigma 2$ receptor has been a serendipitous result of drug screening intended for other targets¹⁷. The purpose of the current study was to identify $\sigma 2$ -favoring molecular determinants in ligands. We have explored the contribution of electron-donating and electron-withdrawing groups to $\sigma 2$ receptor selectivity, through the synthesis and characterization of a series of new compounds featuring a benzamide moiety and an isoquinoline moiety linked by an alkyl chain. We found that adding an extra methoxy group (electron-donating) to the para-position (**5e**) of the benzamide phenyl ring of **5f** dramatically improved the $\sigma 2$ selectivity.

2. CHEMISTRY

A commercial *N*-(4-bromoalkyl) phthalimide (**1**) was treated with 1,2,3,4-tetrahydroisoquinoline (**2**) to yield an imide derivative (**3**). Hydrazinolysis of **3** afforded *N*-(3-aminoalkyl)-1,2,3,4-tetrahydroisoquinolines (**4a** and **4b**) which, by reaction with appropriate carboxylic acid in the presence of DCC in THF, were converted to (**5**) (Scheme 1).

3. RESULTS AND DISCUSSION

We have previously discovered that the electron-withdrawing nitro group greatly improved ligand affinity for the $\sigma 1$ receptor^{24, 25}. This prompted us to test the effects of electron-withdrawing, or conversely, electron-donating groups on ligand affinity for the $\sigma 2$ receptor. A class of isoquinoline derivatives, which have previously shown good $\sigma 2$ selectivity²³,

provided an ideal template for our experiments. The molecular structures of benzamide and isoquinoline groups linked by a butyl chain provided us the flexibility to modify the phenyl ring either on the isoquinoline side or on the benzamide side. We thus synthesized a series of new derivatives with electron withdrawing or donating substituents, and determined their affinities for the σ_2 receptor as well as the σ_1 receptor. The results are presented in Table 1.

The new finding from our data is that the electron-donating methoxy group favored σ_2 affinity, but conversely, the electron-withdrawing nitro group on the phenyl ring mitigated against ligand binding to the σ_2 receptor. The most interesting evidence was revealed by the comparison of **5e** to **5f**, which showed a dramatic difference of affinity for the σ_2 receptor. When an extra electron-donating methoxy was added to the para-position (**5e**), whereas σ_1 affinity was slightly reduced, σ_2 affinity was increased by 500 fold, and compound **5e** had thus acquired an ~400 fold selectivity for the σ_2 over the σ_1 receptor. Also supporting the σ_2 affinity-enhancing effect of electron-donating, iodine at the para-position (**5g**), which in this case is relatively electron-donating compared to the hydrogen (**5h**), increased σ_2 affinity by 4.5 fold in comparison to compound **5h**. On the contrary, however, the electron-withdrawing nitro group on the benzamide phenyl ring reduced σ_2 affinity. For example, a nitro group placed at the para-position (**5d**) or ortho-position (**5a**) led to a great decrease of affinity for the σ_2 receptor in comparison to compound **5c**. Interestingly, the nitro group on the isoquinoline side of compound **5h** also reduced σ_2 affinity as compared to **5i**. While the nitro group on the benzamide reduced σ_2 affinity, it significantly improved the σ_1 binding (see comparison of **5c** to **5d** and **5a**), consistent with our previous findings^{24, 25}. Moreover, the σ_1 -affinity enhancing effect was more profound when the nitro group was placed at the para-position (**5d**) than at the ortho-position (**5a**).

Thus, it appeared that the electron-donating methoxy group was favorable but the electron-withdrawing nitro group was unfavorable for σ_2 affinity, when placed either on the benzamide side or on the isoquinoline side. The para-position of the benzamide phenyl ring was a sensitive location for changing the σ_2/σ_1 selectivity by adding either an electron-donating group or an electron-withdrawing group.

It is also noteworthy that all the tested isoquinoline derivatives in Table 1 that contain an amide group generally showed impaired σ_1 affinity. This data is consistent with a previously reported observation that an amide group abrogates σ_1 binding²⁶. In our current study, including an amide group together with electron-donating groups likely facilitated higher σ_2 selectivity over σ_1 in some of the isoquinoline derivatives, such as compound **5e**. Additionally, compared to **5e**, replacing benzamide with phthalimide (**3b**) achieved a similar σ_2 affinity, suggesting that phthalimide may provide another good template for generating high affinity compounds for σ_2 .

To further confirm the σ_2 selectivity of **5e** shown by the binding assays (Table 1), this compound and two other new compounds (**3b** and **5f**) were used as competitors to block sigma receptor photoaffinity labeling by [¹²⁵I]-IAF (1-N-(2',6'-dimethyl-morpholino)-3-(4-azido-3-[¹²⁵I]iodo-phenyl propane) (Figure 1). As also shown in our previous studies^{20, 21}, [¹²⁵I]-IAF labeled both σ_1 and σ_2 in the absence of competitors (lane 1). As a control, haloperidol blocked photolabeling of both σ_1 and σ_2 receptors (lane 2). Consistent with the binding data (Table 1), compound **5e** eliminated σ_2 labeling but left σ_1 labeling essentially unaltered (lane 4). In contrast, compound **5f** which showed low affinities for both σ_1 and σ_2 , did not block the labeling of either receptor (lane 5). Compound **3b**, which was determined to have relative high affinities for both subtypes, however, diminished both bands (lane 3). Thus, the photolabeling data (Figure 1) agreed well with the competitive radioligand binding data (Table 1).

4. CONCLUSION

Our data indicate that in a class of new benzamide-isoquinoline derivatives, the electron-donating methoxy group favored σ_2 affinity but not σ_1 binding, thus improving σ_2 selectivity. Most remarkably, an extra methoxy group at the para-position of the benzamide phenyl ring (**5e**) dramatically increased σ_2 selectivity. This para-position appeared to be highly sensitive to molecular manipulations for the purpose of altering σ_2/σ_1 selectivity. The new information from our study offers a useful guide for designing σ_2 specific compounds.

5. EXPERIMENTAL SECTION

5.1. Chemistry

All yields refer to isolated products after purification. All products were characterized by their spectral (IR, $^1\text{H-NMR}$, CHN, TLC and GC) and physical data (melting and boiling points). The melting point for compound **2b** was measured on a Gallenkamp melting apparatus. The remainder compounds are oil. All the amine derivatives are free bases. $^1\text{H-NMR}$ spectra were recorded using 400 MHz in CDCl_3 solutions at room temperature (TMS was used as an internal standard) on a Bruker Avance 500MHz instrument (Rheinstetten, Germany) or Varian 400MHz NMR spectrometer. FT-IR spectra were recorded on a spectrophotometer (Jasco-680, Japan). Spectra of solids were carried out using KBr pellets. Vibrational transition frequencies are reported in the wave number (cm^{-1}). Furthermore, we used GC (BEIFIN 3420 Gas Chromatograph equipped a Varian CP SIL 5CB column- 30 m, 0.32 mm, 0.25 μm) for examination of reaction completion and yields. CHN analyses were measured by Isfahan University of Technology using a Vario EL III Element Analyzer (Germany). All of the starting materials were purchased from Merck or Sigma-Aldrich. TLC plates were from Merck.

5.1.1. Preparation of 7-nitro-1,2,3,4-tetrahydroisoquinoline (2b)—In a mortar 2 g of P_2O_5 /silica gel (65% w/w)¹ (10 mmol) and 1,2,3,4-tetrahydroisoquinoline (10 mmol, 1.33 g) (scheme 1) was triturated for 30 sec., and then 5 ml of HNO_3 65% was added drop-wise and the mixture was further triturated with a pestle at room temperature for 20 min until a deep-yellow color appeared, at which point TLC (n-hexane:EtOAc 70:30) showed complete disappearance of 1,2,3,4-tetrahydroisoquinoline (30 min). To the reaction mixture was added diethyl ether (50 ml) and the solid was separated through a short pad of silica gel and washed with diethyl ether (2×15 ml). The filtrate was washed with NaHCO_3 10% (20 ml) and dried (MgSO_4). The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (n-Hexane:EtOAc, 2:1), 7-nitro-1,2,3,4-tetrahydroisoquinoline (**2b**) was obtained (8 mmol, 1.4 g 80%) as a yellow solid, mp 121 $^\circ\text{C}$. $^1\text{H NMR}$, δ : 8.05 (m, 2 H), 7.60 (m, 1 H), 3.82 (s, 2 H), 3.38 (t, 2H, $J = 7.4$ Hz), 3.12 (t, 2H, $J = 7.4$ Hz), 2.83 (s, 1H). $^{13}\text{C NMR}$, δ : 150.6, 145.0, 140.3, 129.6, 122.6, 121.4, 46.9, 44.1, 28.1. EMS [M^+H^+] for $\text{C}_9\text{H}_{10}\text{N}_2\text{O}_2$, Calcd. 179.0740, Found, 179.1121.

5.1.2. Preparation of Compounds 3a and 3b—A mixture of *N*-(4-bromobutyl)-phthalimide (10 mmol) and isoquinoline derivative (10 mmol) and K_2CO_3 (20 mmol) in ethanol (100 ml) was refluxed for 12 h. The reaction mixture was then filtered off, and the solvent was evaporated to give a crude product, that was purified by column chromatography (silica gel, toluene:diethylamine, 20:1).

2-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)butyl)isoindoline-1,3-dione (3a): Oil, yield 75%; $^1\text{H NMR}$, δ : 7.8 (m, 6 H), 7.43 (d, 2 H, $J = 8.6$ Hz), 3.72 (s, 2 H), 3.61 (t, 2H, $J = 7.4$ Hz), 2.90 (t, 2H, $J = 7.4$ Hz), 2.58 (t, 2H, $J = 7.6$ Hz), 1.59 (m, 4H). $^{13}\text{C NMR}$, δ :

171.0, 149.8, 149.3, 132.2, 126.6, 123.7, 111.4, 108.3, 57.2, 56.1, 53.8, 37.2, 27.3, 25.6, 25.0. EMS $[M^+H^+]$ for $C_{23}H_{26}N_2O_4$, Calcd. 395.1863, Found, 395.1839.

2-(4-(7-Nitro-3,4-dihydroisoquinoline-2(1H)-yl)butyl)isoindoline-1,3-dione (3b): Oil, yield 72%; 1H NMR, δ : 8.3-7.8 (m, 3 H), 6.84 (s, 2 H), 3.83 (s, 6 H), 3.7 (s, 2 H), 3.61 (t, 2H, $J = 7.4$ Hz), 2.90 (t, 2H, $J = 7.4$ Hz), 2.58 (t, 2H, $J = 7.6$ Hz), 1.59 (m, 4H). ^{13}C NMR, δ : 171.0, 151.0, 144.6, 134.3, 129.4, 127.5, 122.4, 57.9, 56.8, 53.8, 37.2, 27.3, 25.6, 25.0. EMS $[M^+H^+]$ for $C_{21}H_{21}N_3O_4$, Calcd. 380.1503. Found, 380.1529.

5.1.3. Preparation of Compounds 4a and 4b—A solution of **3a** or **3b** (free bases, 1.2 mmol) and hydrazine monohydrate (0.05 ml, 15 mmol) in ethanol (95 %, 15 ml) was refluxed for 1 h. The reaction mixture was cooled and treated with an additional amount of ethanol (95 %, 15 ml) and concentrated HCl (1.3 ml). The reaction mixture was then refluxed for 4 h and left overnight in a refrigerator. The precipitate was filtered off, and the solvent was evaporated. The residue was treated with *n*-hexane (20 ml) and NH_3 (aqueous, 15 ml). The solution was extracted with $CHCl_3$ (3 $\times$ 15 ml), the organic layer was dried over anhydrous K_2CO_3 , and the solvents were evaporated to give **4a** or **4b** which were used without further purification.

4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)butan-1-amine (4a): Oil, yield 85%; 1H NMR, δ : 6.92 (s, 2 H, $J = 8.6$ Hz), 3.83 (s, 6 H), 3.7 (s, 2 H), 3.70 (t, 2H, $J = 7.4$ Hz), 2.95 (t, 2H, $J = 7.4$ Hz), 2.78 (t, 2H, $J = 7.6$ Hz), 2.63 (t, 2 H, $J = 7.4$ Hz), 2.46 (t, 2 H), 2.32 (s, 2 H), 1.72 (m, 2 H), 1.48 (m, 2H). ^{13}C NMR, δ : 148.2, 146.7, 126.9, 126.4, 111.4, 108.3, 57.2, 56.1, 53.7, 41.7, 27.3, 26.2, 25.4. EMS $[M^+H^+]$ for $C_{15}H_{24}N_2O_2$, Calcd. 265.1838, Found, 265.3521.

4-(7-Nitro-3,4-dihydroisoquinoline-2(1H)-yl)butan-1-amine (4b): Yellow oil, yield 79%; 1H NMR, δ : 8.07 (s, 1H), 7.02 (m, 1 H), 7.43 (m, 1 H), 3.75 (s, 2 H), 3.61 (t, 2H, $J = 7.4$ Hz), 2.95 (t, 2H, $J = 7.4$ Hz), 2.68 (t, 2H, $J = 7.6$ Hz), 2.65 (t, 2 H, $J = 7.6$ Hz), 2.54 (s, 2 H), 2.46 (t, 2 H, $J = 7.6$ Hz), 1.74 (m, 2 H), 1.54 (m, 2H). ^{13}C NMR, δ : 150.8, 142.3, 135.4, 129.7, 122.8, 122.3, 53.9, 53.5, 53.2, 41.7, 27.0, 26.4, 25.0. EMS $[M^+H^+]$ for $C_{13}H_{19}N_3O_2$, Calcd. 250.3089, Found, 250.1841.

5.1.4. Preparation of Compounds 5a-5i—To a solution of benzoic acid derivatives (1.1 mmol) in anhydrous THF (50 ml) was added dicyclohexylcarbodiimide (DCC, 1.1 mmol) in anhydrous THF (5 ml) and the reaction mixture was stirred at room temperature. After 5 min, a solution of the amine **4a** or **4b** in THF (5 ml) was added to this reaction mixture, and then was stirred at room temperature overnight. The precipitate was filtered off, and the solvent was evaporated. The organic layer was dried over anhydrous $MgSO_4$ and the solvents were evaporated to give **5a-5i**, which were purified using column chromatography (silica gel, $CHCl_3$: MeOH, 95:5).

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)butyl)-3-nitrobenzamide (5a): Yellow oil, yield 78%; 1H NMR, δ : 8.76 (s, 1 H), 8.48 (m, 3 H), 7.92 (m, 1 H), 6.92 (s, 2 H), 3.88 (s, 6 H), 3.68 (s, 2H, $J = 7.4$ Hz), 3.34 (t, 2H, $J = 7.4$ Hz), 2.98 (t, 2H, $J = 7.6$ Hz), 2.78 (t, 2H, $J = 7.6$ Hz, $J = 7.6$ Hz), 2.40 (t, 2H, $J = 7.6$ Hz), 1.70 (t, 2H, $J = 7.8$ Hz), 1.50 (t, 2H, $J = 7.8$ Hz). ^{13}C NMR, δ : 169.4, 148.2, 148.0, 135.4, 129.7, 126.9, 126.4, 111.3, 108.4, 57.2, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[M^+H^+]$ for $C_{22}H_{27}N_3O_5$, Calcd. 414.4669, Found, 414.2243.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)butyl)benzamide (5c): Oil, yield 75%; 1H NMR, δ : 8.40 (s, 1 H), 8.03 (m, 2 H), 7.75 (m, 3 H), 6.85 (m, 2 H), 3.80 (s, 6 H),

3.68 (s, 2H, J = 7.4 Hz), 3.34 (t, 2H, J = 7.4 Hz), 2.98 (t, 2H, J = 7.5 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.6 Hz), 1.70 (t, 2H, J = 7.8, J = 7.6 Hz), 1.50 (t, 2H). ^{13}C NMR, δ : 167.5, 148.2, 146.5, 134.4, 132.1, 128.9, 127.4, 111.3, 108.4, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_3$, Calcd. 369.4693. Found, 369.2639.

N-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)butyl)-4-nitrobenzamide (5d):

Oil, yield 69%; ^1H NMR, δ : 8.44 (s, 1 H), 8.40 (m, 3 H), 8.11 (d, 2 H, J = 8.6 Hz), 6.85 (m, 2 H), 3.80 (s, 6 H), 3.68 (s, 2H), 3.34 (t, 2H, J = 7.4 Hz), 2.98 (t, 2H, J = 7.4 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.6 Hz), 1.70 (t, 2H, J = 7.5 Hz), 1.50 (t, 2H, J = 7.6 Hz). ^{13}C NMR, δ : 167.5, 151.3, 148.2, 146.5, 133.2, 124.0, 128.9, 127.4, 111.3, 108.4, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{22}\text{H}_{27}\text{N}_3\text{O}_5$, Calcd. 414.4669. Found, 414.2436.

2,3,4-Trimethoxy-N-(4-(7-nitro-3,4-dihydroisoquinoline-2(1H)-yl)butyl)benzamide (5e):

Oil, yield 82%; ^1H NMR, δ : 8.44 (s, 1 H), 8.05 (m, 2 H), 7.37 (m, 1 H), 6.73 (m, 2 H), 3.88 (s, 3 H), 3.83 (s, 6 H), 3.76 (s, 2H), 3.34 (t, 2H, J = 7.4 Hz), 2.98 (t, 2H, J = 7.4 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.6 Hz), 1.70 (t, 2H, J = 7.5 Hz), 1.50 (t, 2H, J = 7.5 Hz). ^{13}C NMR, δ : 168.6, 160.2, 151.3, 143.2, 141.5, 134.2, 129.7, 122.9, 122.4, 120.3, 111.3, 104.4, 60.9, 60.8, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{23}\text{H}_{29}\text{N}_3\text{O}_6$, Calcd. 444.4929. Found, 444.2573.

2,3-Dimethoxy-N-(4-(7-nitro-3,4-dihydroisoquinoline-2(1H)-yl)butyl)benzamide (5f):

Oil, yield 82%; ^1H NMR, δ : 8.44 (s, 1 H), 8.05 (m, 2 H), 7.41 (m, 1 H), 7.13 (m, 1 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.76 (s, 2H), 3.34 (t, 2H, J = 7.4 Hz, J = 7.4 Hz), 2.98 (t, 2H, J = 7.6 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.4 Hz), 1.70 (t, 2H, J = 7.5 Hz), 1.50 (t, 2H, J = 7.5 Hz). ^{13}C NMR, δ : 168.6, 153.5, 150.3, 149.4, 141.5, 134.2, 129.7, 122.9, 122.4, 120.3, 111.3, 104.4, 60.9, 60.8, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{22}\text{H}_{27}\text{N}_3\text{O}_5$, Calcd. 414.4669. Found, 414.2247.

2-Hydroxy-4-iodo-3-methoxy-N-(4-(7-nitro-3,4-dihydroisoquinoline-2(1H)-yl)butyl)benzamide (5g):

Oil, Yield 75%; ^1H NMR, δ : 13.42 (s, 1 H), 8.28 (s, 1 H), 8.03 (m, 2 H), 7.43 (m, 1 H), 7.19 (m, 1 H), 3.84 (s, 3 H), 3.70 (s, 2H), 3.34 (t, 2H, J = 7.4 Hz), 2.98 (t, 2H, J = 7.4 Hz, J = 7.6 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.5 Hz), 1.70 (t, 2H, J = 7.5 Hz), 1.50 (t, 2H, J = 7.5 Hz). ^{13}C NMR, δ : 172.4, 155.2, 150.6, 141.1, 134.2, 129.7, 122.9, 122.4, 120.3, 111.3, 104.4, 91.03, 60.3, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{21}\text{H}_{24}\text{IN}_3\text{O}_5$, Calcd. 526.3368. Found, 526.3984.

2-Hydroxy-3-methoxy-N-(4-(7-nitro-3,4-dihydroisoquinoline-2(1H)-yl)butyl)benzamide (5h):

Light yellow oil, yield 79%; ^1H NMR, δ : 13.78 (s, 1 H), 8.50 (s, 1 H), 8.02 (m, 2 H), 7.42 (m, 3 H), 7.08 (m, 1 H), 3.86 (s, 3 H), 3.70 (s, 2H), 3.34 (t, 2H, J = 7.4 Hz), 2.98 (t, 2H, J = 7.4 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.6 Hz), 1.70 (t, 2H, J = 7.5 Hz), 1.50 (t, 2H, J = 7.4 Hz). ^{13}C NMR, δ : 171.4, 149.0, 148.4, 141.1, 134.2, 129.7, 122.9, 122.4, 120.3, 111.3, 104.4, 91.03, 60.3, 56.1, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{21}\text{H}_{25}\text{N}_3\text{O}_5$, Calcd. 400.4403. Found, 400.4913.

N-(4-(6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-yl)butyl)-2-hydroxy-3-methoxybenzamide (5I):

Oil, yield 83%; ^1H NMR, δ : 13.81 (s, 1 H), 8.44 (s, 1 H), 7.40 (m, 2 H), 7.04 (m, 2 H), 6.85 (m, 2 H), 3.88 (s, 6 H), 3.83 (m, 3 H), 3.70 (s, 2H), 3.34 (t, 2H, J = 7.4 Hz), 2.98 (t, 2H, J = 7.4 Hz), 2.78 (t, 2H, J = 7.6 Hz), 2.40 (t, 2H, J = 7.6 Hz), 1.70 (t, 2H, J = 7.5 Hz), 1.50 (t, 2H, J = 7.6 Hz). ^{13}C NMR, δ : 171.6, 149.0, 148.4, 146.7, 126.8, 126.5, 122.9, 122.4, 120.3, 111.3, 104.4, 91.03, 60.3, 56.6, 56.2, 53.9, 53.6, 39.4, 27.8, 27.4, 25.3. EMS $[\text{M}^+\text{H}^+]$ for $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_5$, Calcd. 415.44947. Found, 415.5231.

5.2. Preparation of rat liver membranes and RT4 cell membranes

Rat livers (65 g, Pel-Freez Biologicals) were minced in 100 ml homogenization buffer (20 mM Tris-HCl pH 8.0, 0.32M sucrose) that contains the Protease Inhibitor Cocktail (Sigma-Aldrich P8340-5ML, for use with mammalian cell and tissue extracts), and then homogenized on ice using a Brinkman Polytron Homogenizer (setting 6, 4 bursts of 10 sec each) followed by a glass homogenizer (Teflon pestle by 6 slow passes at 3000 rpm). The homogenized tissues were first centrifuged at 1,000 $\times g$ for 10 min, and the supernatant was then centrifuged at 100,000 $\times g$ for 1 h at 4°C. The membrane pellets were resuspended in the homogenization buffer, and used for competitive sigma receptor binding assays.

Human urinary bladder transitional papilloma RT4 cells (HTB-2, ATCC) were harvested from the cell culture, and lysed using a sonicator (Branson Sonifier, output 50%, duty cycle 50%, 6 $\times$ 10 sec) in the homogenization buffer. The cell homogenates were centrifuged at 1,000 $\times g$ for 10 min, and the supernatant was then centrifuged at 100,000 $\times g$ for 1 h at 4°C. The pelleted membranes were resuspended by brief sonication and used fresh for the photolabeling assays.

5.3. Sigma receptor radioligand binding assays

Competitive binding assays were performed to determine binding affinities of the new compounds for the σ_2 and σ_1 receptors as previously described^{24, 27}. Briefly, assays for σ_2 binding were performed using rat liver membranes (~50 μg of total proteins per reaction) and 10 nM [³H]-DTG (PerkinElmer, 58.1 Ci/mmol) in 50 mM Tris-HCl pH 8.0, and in the presence of 100 nM cold (+)-pentazocine to block σ_1 binding sites. σ_1 binding in rat liver membranes was assayed by using 10 nM [³H]-(+)-pentazocine (PerkinElmer, 34.8 Ci/mmol). Haloperidol (Sigma-Aldrich) of 10 μM was used to determine non-specific binding. Serial dilutions of the stocks (in DMSO) of compounds listed in Table 1 were added to the reactions and incubated for 1.5 h at 32°C. To assess the ability of the new compounds to displace radioligands from the sigma receptor subtypes, following the incubation, the samples were filtered through Brandel GF/B Fired Membranes (which were pretreated with 0.5% polyethyleneimine) using a Brandel Cell Harvester (M-48T). Radioactivity on the filters was counted using a Beckman Scintillation Counter (LS-6500) in an NEN formula 989 scintillation cocktail (Ultima Gold MV, PerkinElmer). Values were fit to a non-linear regression curve (one-site competition) using Graphpad Prism Version 4.0c. K_i was calculated using the Cheng-Prusoff equation²⁸. For the K_i calculation, a K_d of 50 nM was used for [³H]-DTG and a K_d of 25 nM was used for (+)-pentazocine in rat liver membranes.

5.4. Photoaffinity labeling of sigma receptors

Radiochemical synthesis of the sigma receptor photolabel [¹²⁵I]-IAF (1-N-(2',6'-dimethyl-morpholino)-3-(4-azido-3-[¹²⁵I]iodo-phenyl)propane) was performed as previously described²⁰. Fresh RT4 cell membranes were used for [¹²⁵I]-IAF photoaffinity labeling of sigma receptors since this cell line is highly enriched in the σ_2 receptor. To test the σ_2 or σ_1 binding specificity of the new compounds, compounds **3b**, **5e**, and **5f** were first preincubated with RT4 membranes (200 μg total proteins per reaction) in 50 mM Tris (pH 7.4) for 30 min at 32°C. [¹²⁵I]-IAF was then added to the membranes to a final concentration of 1 nM (final 1% ethyl acetate) and incubated for another 30 min at 32°C. Following the incubation, the [¹²⁵I]-IAF photoreactive label was activated by exposure for 6 sec to a high-pressure AH-6 mercury lamp (10 cm distance). The photolysis reactions were then quenched immediately with the sample buffer containing 200 mM β -mercaptoethanol and 1% SDS. Proteins were separated on a SDS-Tricine gel, and radiolabeled proteins were visualized using a PhosphorImager (Molecular Dynamics).

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ABBREVIATIONS

RT	room temperature
DCC	dicyclohexylcarbodiimide
THF	tetrahydrofuran
Ar	aromatic ring
OMe	methoxy
EtOH	ethanol
DTG	1,3-di(2-tolyl)guanidine
[¹²⁵I]-IAF	1-N-(2',6'-dimethyl-morpholino)-3-(4-azido-3-[¹²⁵ I]iodo-phenyl) propane

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Figure 1.

Sigma-2 receptor-specific protection of [¹²⁵I]-IAF photolabeling by compound **5e**
Photoaffinity labeling of RT4 cell membranes with 1 nM [¹²⁵I]-IAF was performed in the absence (lane 1) or presence of 10 μ M competing compounds: haloperidol (lane 2), **3b** (lane 3), **5e** (lane 4), or **5f** (lane 5).

Scheme 1.

Table 1

Characterization of new isoquinoline derivatives

Compounds	$\sigma 2$ K_i (nM)	$\sigma 1$ K_i (nM)	$\sigma 1/\sigma 2$
5e	26.78 (± 2.92)	10,320 (± 363)	385
5f	12,930 (± 55.84)	7,870 (264)	0.61
5g	866.70 (± 138.6)	74,680 (± 305)	86.17
5h	4,000 (± 177.9)	11,200 (± 469)	2.80
5i	1,400 (± 286)	67,800	48.40
5c	5,290 (± 408)	$>10^7$	
5d	152,000 ($\pm 4,106$)	14,690	0.096
5a	$>10^7$	1.21×10	
3b	21.26 (± 2.41)	87.5 (± 3.07)	4.12

 K_i is presented as a mean ($\pm$ SEM).