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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 (σ2) receptor has been suggested to be a promising target for pharmacological interventions to curb tumor progression. Development of σ2-specific ligands, however, has been hindered by lack of understanding of molecular determinants that underlie selective ligand-σ2 interactions. Here we have explored effects of electron donating and withdrawing groups on ligand selectivity for the σ2 versus σ1 receptor using new benzamide-isoquinoline derivatives. The electron-donating methoxy group increased but the electron-withdrawing nitro group decreased σ2 affinity. In particular, an extra methoxy added to the *para*-position (**5e**) of the benzamide phenyl ring of **5f** dramatically improved (631 fold) the σ2 selectivity relative to the σ1 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 σ2-over-σ1 selectivity of new ligands.

1. Introduction

The σ1 and σ2 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 σ1 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 σ2 receptor until most recently the progesterone receptor membrane component 1 (PGRMC1) protein complex was identified as a putative σ2 receptor binding site.[5]

Sigma receptor ligands have been reported to have various therapeutic potentials. Numerous studies have proposed selective σ1 agonists as therapeutic agents for anxiety, depression, psychosis, and learning and memory improvement, and recently, neuroprotective effects of σ1 agonists have garnered increasing attention (see reviews[6–9]). Thus far, the most prominent function of the σ2 receptor has been linked to tumor progression. It is known that the σ2 receptor is highly expressed in various tumor cells,[10] and remarkably, proliferating tumor cells express nearly 10-fold higher amounts of the σ2 receptor than quiescent tumor cells.[11] Importantly, σ2 receptor agonists have been found to induce apoptosis in different tumor cell lines.[12–16] These findings strongly support use of the σ2 receptor as a biomarker for solid proliferating tumors and thereby selective σ2 agonists as potential anti-cancer drugs. However, only a very few highly σ2 selective drugs are now under development (see review[17]).

It is interesting to note that the putative σ2 receptor (PGRMC1) has a molecular size (25 kDa)[5] distinct from the σ2 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 σ2 selective ligands for understanding the enigmatic σ2 receptor binding site(s). Ligand binding has been the main approach for detecting the σ2 receptor. The commonly used ligands including DTG (1,3-di(2-tolyl)guanidine) and haloperidol, however, are not selective over the σ1 receptor. Considerable effort has been paid to create σ2 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 review[17]). In spite of these advances, only a few compounds, such as CB-184,[22] PB28[14], siramesine,[15] WC-26 and tetrahydroisoquinoline derivatives,[5,23] have shown excellent σ2 over σ1 selectivity.

A major barrier for discovery of σ2-selective ligands is the lack of understanding of the molecular determinants for ligand/σ2

Abbreviations: RT, room temperature; DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran; Ar, aromatic ring; OMe, methoxy; EtOH, ethanol; DTG, 1,3-di(2-tolyl)guanidine; [^{125}I]-IAF, 1-*N*-(2′,6′-dimethyl-morpholino)-3-(4-azido-3-[125I]iodo-phenyl propane.

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interactions. Development of σ2-selective ligands has not been straightforward, and in some cases, identification of ligands with high affinity for the σ2 receptor has been a serendipitous result of drug screening intended for other targets.[17] The purpose of the current study was to identify σ2-favoring molecular determinants in ligands. We have explored the contribution of electron-donating and electron-withdrawing groups to σ2 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 σ2 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

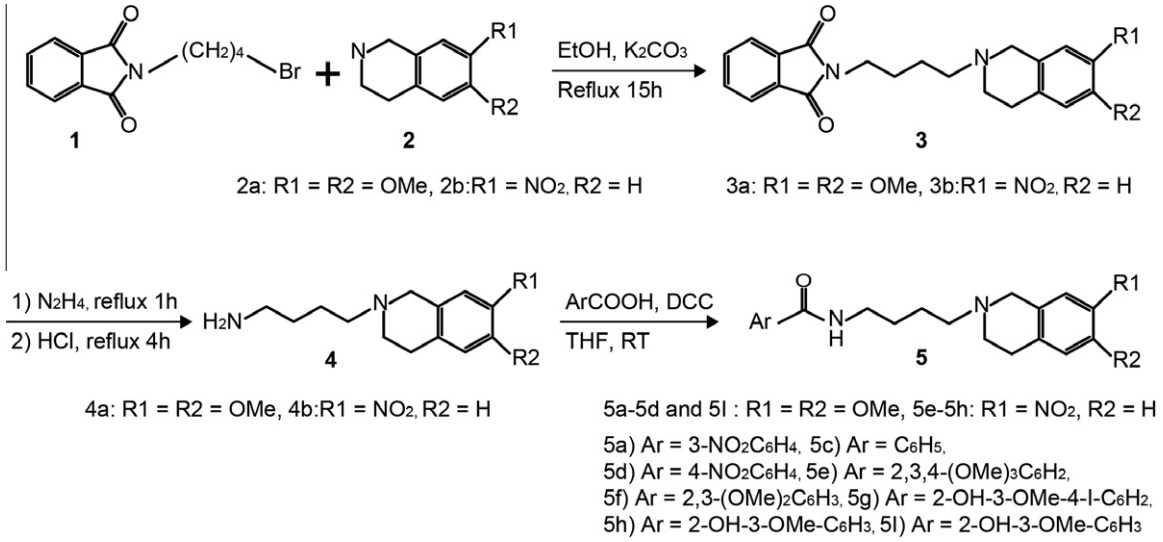
We have previously discovered that the electron-withdrawing nitro group greatly improved ligand affinity for the σ1 receptor.[24,25] This prompted us to test the effects of electron-withdrawing, or conversely, electron-donating groups on ligand affinity for the σ2 receptor. A class of isoquinoline derivatives, which have previously shown good σ2 selectivity,[23] 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 σ2 receptor as well as the σ1 receptor. The results are presented in Table 1.

The new finding from our data is that the electron-donating methoxy group favored σ2 affinity, but conversely, the electron-withdrawing nitro group on the phenyl ring mitigated against ligand binding to the σ2 receptor. The most interesting evidence was revealed by the comparison of **5e** to **5f**, which showed a dramatic difference of affinity for the σ2 receptor. When an extra electron-donating methoxy was added to the *para*-position (**5e**), whereas σ1 affinity was slightly reduced, σ2 affinity was increased by 500 fold, and compound **5e** had thus acquired an ~400 fold selectivity for the σ2 over the σ1 receptor. Also supporting the σ2 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 σ2 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 σ2 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 σ2 receptor in comparison to compound **5c**. Interestingly, the nitro group on the isoquinoline side of compound **5h** also reduced σ2 affinity as compared to **5l**. While the nitro group on the benzamide reduced σ2 affinity, it significantly improved the σ1 binding (see comparison of **5c** to **5d** and **5a**), consistent with our previous findings.[24,25] Moreover, the σ1-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 σ2 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 σ2/σ1 selectivity by adding either an electron-donating group or an electron-withdrawing group.

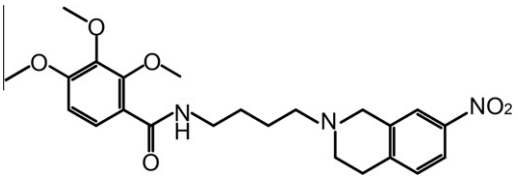
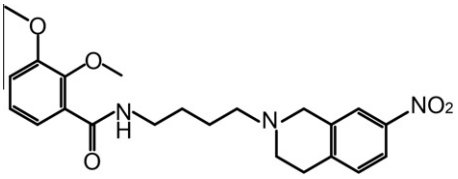
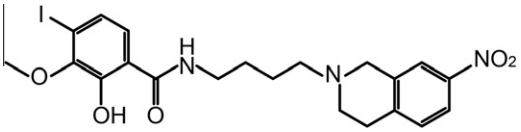
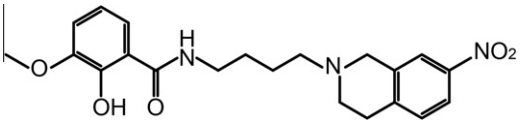
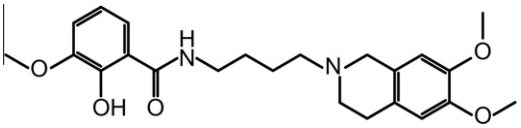
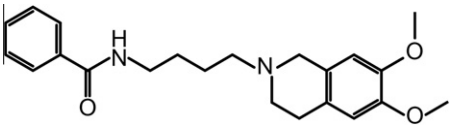
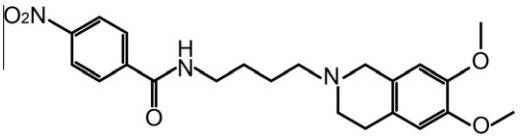
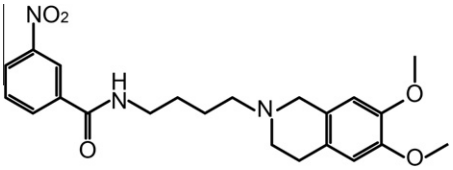
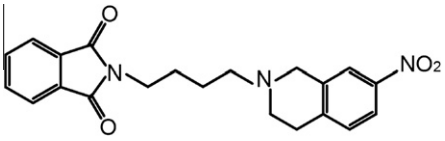
It is also noteworthy that all the tested isoquinoline derivatives in Table 1 that contain an amide group generally showed impaired σ1 affinity. This data is consistent with a previously reported observation that an amide group abrogates σ1 binding.[26] In our current study, including an amide group together with electron-donating groups likely facilitated higher σ2 selectivity over σ1 in some of the isoquinoline derivatives, such as compound **5e**. Additionally, compared to **5e**, replacing benzamide with phthalimide (**3b**) achieved a similar σ2 affinity, suggesting that phthalimide may provide another good template for generating high affinity compounds for σ2.

To further confirm the σ2 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 [^{125}I]-IAF (1-*N*-(2′,6′-dimethylmorpholino)-3-(4-azido-3-[125I]iodo-phenyl propane) (Fig. 1). As



Scheme 1.

Table 1
Characterization of new isoquinoline derivatives

	Compounds	σ2 K_i (nM)	σ1 K_i (nM)	σ1/σ2
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 (±4,155)	48.40
5c		5,290 (±408)	$>10^7$	
5d		152,000 (±4,106)	14,690 (±1,121)	0.096
5a		$>10^7$	1.21×10^6	
3b		21.26 (±2.41)	87.5 (±3.07)	4.12

K_i is presented as a mean (±SEM).

also shown in our previous studies[20,21], [^{125}I]-IAF labeled both σ1 and σ2 in the absence of competitors (lane 1). As a control, haloperidol blocked photolabeling of both σ1 and σ2 receptors (lane 2). Consistent with the binding data (Table 1), compound **5e** eliminated σ2 labeling but left σ1 labeling essentially unaltered (lane 4). In contrast, compound **5f** which showed low affinities for both σ1 and σ2, 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 (Fig. 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 σ2 affinity but not σ1 binding, thus improving σ2 selectivity. Most remarkably, an extra methoxy group at the *para*-position of the benzamide phenyl ring (**5e**) dramatically increased σ2 selectivity. This *para*-position appeared to be highly sensitive to molecular manipulations for the purpose of altering σ2/σ1 selectivity. The new information from our study offers a useful guide for designing σ2 specific compounds.

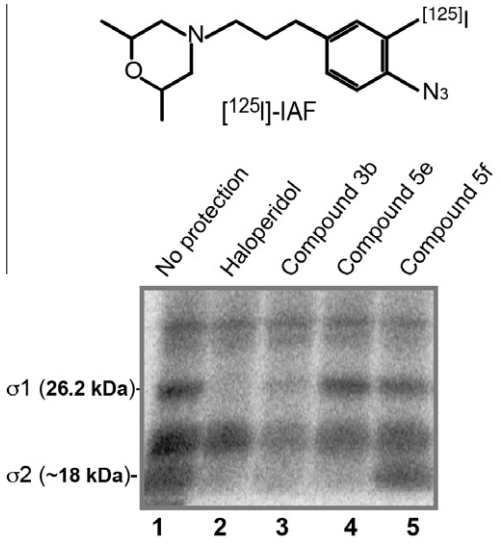


Figure 1. Sigma-2 receptor-specific protection of [^{125}I]-IAF photolabeling by compound **5e** photoaffinity labeling of RT4 cell membranes with 1 nM [^{125}I]-IAF was performed in the absence (lane 1) or presence of 10 μM competing compounds: haloperidol (lane 2), **3b** (lane 3), **5e** (lane 4),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

5. Experimental section

5.1. Chemistry

All yields refer to isolated products after purification. All products were characterized by their spectral (IR, ^{1}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}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 500 MHz instrument (Rheinstetten, Germany) or Varian 400 MHz 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 s, 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 °C. ^{1}H NMR, δ: 8.05 (m, 2H), 7.60 (m, 1H,), 3.82 (s, 2H), 3.38 (t, 2H, J = 7.4 Hz), 3.12 (t, 2H, J = 7.4 Hz), 2.83 (s, 1H). ^{13}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 $C_9H_{10}N_2O_2$, 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).

5.1.3. 2-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-yl)-butyl)isoindoline-1,3-dione (3a)

Oil, yield 75%; ^{1}H NMR, δ: 7.8 (m, 6 H), 7.43 (d, 2H, J = 8.6 Hz), 3.72 (s, 2H), 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, 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.

5.1.4. 2-(4-(7-Nitro-3,4-dihydroisoquinoline-2(1*H*)-yl)butyl)-isoindoline-1,3-dione (3b)

Oil, yield 72%; ^{1}H NMR, δ: 8.3–7.8 (m, 3H), 6.84 (s, 2H,), 3.83 (s, 6 H), 3.7 (s, 2H), 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.

5.1.5. 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 m) 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 × 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.

5.1.6. 4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-yl)-butan-1-amine (4a)

Oil, yield 85%; ^{1}H NMR, δ: 6.92 (s, 2H, J = 8.6 Hz), 3.83 (s, 6H), 3.7 (s, 2H), 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, 2H, J = 7.4 Hz), 2.46 (t, 2H), 2.32 (s, 2H), 1.72 (m, 2H), 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.

5.1.7. 4-(7-Nitro-3,4-dihydroisoquinoline-2(1*H*)-yl)butan-1-amine (4b)

Yellow oil, yield 79%; ^{1}H NMR, δ: 8.07 (s, 1H), 7.02 (m, 1H), 7.43 (m, 1H,), 3.75 (s, 2H), 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, 2H, J = 7.6 Hz), 2.54 (s, 2H), 2.46 (t, 2H, J = 7.6 Hz), 1.74 (m, 2H),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.

5.1.8. 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).

5.1.9. *N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-yl)-butyl)-3-nitrobenzamide (5a)

Yellow oil, yield 78%; ^{1}H NMR, δ: 8.76 (s, 1H), 8.48 (m, 3H), 7.92 (m, 1H), 6.92 (s, 2H,), 3.88 (s, 6 H), 3.68 (s, 2H, J = 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.

5.1.10. *N*-(4-(6,7-Dimethoxy-3,4-dihydroisoquinoline-2(1*H*)-yl)-butyl)benzamide (5c)

Oil, yield 75%; ^{1}H NMR, δ: 8.40 (s, 1H), 8.03 (m, 2H), 7.75 (m, 3H), 6.85 (m, 2H,), 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 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 [M^+H^+] for $C_{22}H_{28}N_2O_3$, Calcd 369.4693. Found, 369.2639.

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

Oil, yield 69%; ^{1}H NMR, δ: 8.44 (s, 1H), 8.40 (m, 3H), 8.11 (d, 2H, J = 8.6 Hz), 6.85 (m, 2H), 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 [M^+H^+] for $C_{22}H_{27}N_3O_5$, Calcd 414.4669. Found, 414.2436.

5.1.12. 2,3,4-Trimethoxy-*N*-(4-(7-nitro-3,4-dihydroisoquinoline-2(1*H*)-yl)butyl)benzamide (5e)

Oil, yield 82%; ^{1}H NMR, δ: 8.44 (s, 1H), 8.05 (m, 2H), 7.37 (m, 1H), 6.73 (m, 2H,), 3.88 (s, 3H), 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 [M^+H^+] for $C_{23}H_{29}N_3O_6$, Calcd 444.4929. Found, 444.2573.

5.1.13. 2,3-Dimethoxy-*N*-(4-(7-nitro-3,4-dihydroisoquinoline-2(1*H*)-yl)butyl)benzamide (5f)

Oil, yield 82%; ^{1}H NMR, δ: 8.44 (s, 1H), 8.05 (m, 2H), 7.41 (m, 1H), 7.13 (m, 1H,), 3.88 (s, 3H), 3.83 (s, 3H), 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 [M^+H^+] for $C_{22}H_{27}N_3O_5$, Calcd 414.4669. Found, 414.2247.

5.1.14. 2-Hydroxy-4-iodo-3-methoxy-*N*-(4-(7-nitro-3,4-dihydroisoquinoline-2(1*H*)-yl)butyl)benzamide (5g)

Oil, Yield 75%; ^{1}H NMR, δ: 13.42 (s, 1H), 8.28 (s, 1H), 8.03 (m, 2H), 7.43 (m, 1H), 7.19 (m, 1H,), 3.84 (s, 3H), 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 [M^+H^+] for $C_{21}H_{24}IN_3O_5$, Calcd 526.3368. Found, 526.3984.

5.1.15. 2-Hydroxy-3-methoxy-*N*-(4-(7-nitro-3,4-dihydroisoquinoline-2(1*H*)-yl)butyl)benzamide (5h)

Light yellow oil, yield 79%; ^{1}H NMR, δ: 13.78 (s, 1H), 8.50 (s, 1H), 8.02 (m, 2H), 7.42 (m, 3H), 7.08 (m, 1H,), 3.86 (s, 3H), 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 [M^+H^+] for $C_{21}H_{25}N_3O_5$, Calcd 400.4403. Found, 400.4913.

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

Oil, yield 83%; ^{1}H NMR, δ: 13.81 (s, 1H), 8.44 (s, 1H), 7.40 (m, 2H), 7.04 (m, 2H), 6.85 (m, 2H,), 3.88 (s, 6 H), 3.83 (m, 3H), 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, $\delta\delta$: 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 [M^+H^+] for $C_{23}H_{30}N_2O_5$, 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, 0.32 M 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 s each) followed by a glass homogenizer (Teflon pestle by 6 slow passes at 3000 rpm). The homogenized tissues were first centrifuged at 1,000 *g* for 10 min, and the supernatant was then centrifuged at 100,000×*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 × 10 s) in the homogenization buffer. The cell homogenates were centrifuged at 1,000×*g* for 10 min, and the supernatant was then centrifuged at 100,000×*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 σ2 and σ1 receptors as previously described.[24,27] Briefly, assays for σ2 binding were performed using rat liver membranes (~50 μg of total proteins per reaction) and 10 nM [^{3}H]-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 σ1 binding sites. σ1 binding in rat liver membranes was assayed by using 10 nM [^{3}H]-(+)-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. *Ki* was calculated using the Cheng-Prusoff equation.[28] For the K_i calculation, a K_d of 50 nM was used for [^{3}H]-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 [^{125}I]-IAF (1-*N*-(2′,6′-dimethyl-morpholino)-3-(4-azido-3-[125I]iodophenyl propane) was performed as previously described.[20] Fresh RT4 cell membranes were used for [^{125}I]-IAF photoaffinity labeling of sigma receptors since this cell line is highly enriched in the σ2 receptor. To test the σ2 or σ1 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. [^{125}I]-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 [^{125}I]-IAF photoreactive label was activated by exposure for 6 s 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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References and notes

1. Su, T. P.; Hayashi, T.; Maurice, T.; Buch, S.; Ruoho, A. E. *Trends Pharmacol. Sci.* **2010**, *31*, 557.
2. Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. *Trends Pharmacol. Sci.* **1992**, *13*, 85.
3. 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.
4. Hayashi, T.; Su, T. P. *Cell* **2007**, *131*, 596.
5. Xu, J.; Zeng, C.; Chu, W.; Pan, F.; Rothfuss, J. M.; Zhang, F.; Tu, Z.; Zhou, D.; Zeng, D.; Vangveravong, S.; Johnston, F.; Spitzer, D.; Chang, K. C.; Hotchkiss, R. S.; Hawkins, W. G.; Wheeler, K. T.; Mach, R. H. *Nat. Commun.* **2011**, *2*, 380.
6. Cobos, E. J.; Entrena, J. M.; Nieto, F. R.; Cendan, C. M.; Del Pozo, E. *Curr. Neuropharmacol.* **2008**, *6*, 344.
7. Maurice, T.; Su, T. P. *Pharmacol. Ther.* **2009**, *124*, 195.
8. Narayanan, S.; Mesangeau, C.; Poupaert, J. H.; McCurdy, C. R. *Curr. Top. Med. Chem.* **2011**, *11*, 1128.
9. Fishback, J. A.; Robson, M. J.; Xu, Y. T.; Matsumoto, R. R. *Pharmacol. Ther.* **2010**, *127*, 271.
10. Vilner, B. J.; John, C. S.; Bowen, W. D. *Cancer Res.* **1995**, *55*, 408.
11. Mach, R. H.; Smith, C. R.; al-Nabulsi, I.; Whirrett, B. R.; Childers, S. R.; Wheeler, K. T. *Cancer Res.* **1997**, *57*, 156.
12. Crawford, K. W.; Bowen, W. D. *Cancer Res.* **2002**, *62*, 313.
13. Crawford, K. W.; Coop, A.; Bowen, W. D. *Eur. J. Pharmacol.* **2002**, *443*, 207.
14. Azzariti, A.; Colabufo, N. A.; Berardi, F.; Porcelli, L.; Niso, M.; Simone, G. M.; Perrone, R.; Paradiso, A. *Mol. Cancer Ther.* **2006**, *5*, 1807.
15. Ostenfeld, M. S.; Fehrenbacher, N.; Hoyer-Hansen, M.; Thomsen, C.; Farkas, T.; Jaattela, M. *Cancer Res.* **2005**, *65*, 8975.
16. Zeng, C.; Vangveravong, S.; Xu, J.; Chang, K. C.; Hotchkiss, R. S.; Wheeler, K. T.; Shen, D.; Zhuang, Z. P.; Kung, H. F.; Mach, R. H. *Cancer Res.* **2007**, *67*, 6708.
17. Mach, R. H.; Wheeler, K. T. *Cent. Nerv. Syst. Agents Med. Chem.* **2009**, *9*, 230.
18. Hellewell, S. B.; Bowen, W. D. *Brain Res.* **1990**, *527*, 244.
19. Hellewell, S. B.; Bruce, A.; Feinstein, G.; Orringer, J.; Williams, W.; Bowen, W. D. *Eur. J. Pharmacol.* **1994**, *268*, 9.
20. Pal, A.; Hajipour, A. R.; Fontanilla, D.; Ramachandran, S.; Chu, U. B.; Mavlyutov, T.; Ruoho, A. E. *Mol. Pharmacol.* **2007**, *72*, 921.
21. Fontanilla, D.; Johannessen, M.; Hajipour, A. R.; Cozzi, N. V.; Jackson, M. B.; Ruoho, A. E. *Science* **2009**, *323*, 934.
22. Bowen, W. D.; Bertha, C. M.; Vilner, B. J.; Rice, K. C. *Eur. J. Pharmacol.* **1995**, *278*, 257.
23. 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. *J. Med. Chem.* **2007**, *50*, 3194.
24. Hajipour, A. R.; Fontanilla, D.; Chu, U. B.; Arbabian, M.; Ruoho, A. E. *Bioorg. Med. Chem.* **2010**, *18*, 4397.
25. Chen, Y.; Hajipour, A. R.; Sievert, M. K.; Arbabian, M.; Ruoho, A. E. *Biochemistry* **2007**, *46*, 3532.
26. Abate, C.; Mosier, P. D.; Berardi, F.; Glennon, R. A. *Cent. Nerv. Syst. Agents Med. Chem.* **2009**, *9*, 246.
27. Matsumoto, R. R.; Bowen, W. D.; Tom, M. A.; Vo, V. N.; Truong, D. D.; De Costa, B. R. *Eur. J. Pharmacol.* **1995**, *280*, 301.
28. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.