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BRIEF ARTICLE

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Novel Potent and Selective σ Ligands: Evaluation of Their Agonist and Antagonist Properties

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Supporting Information

ABSTRACT: Novel enantiomers and diastereoisomers structurally related to σ ligand (+)-MR200 were synthesized to improve σ_1/σ_2 subtype selectivity. The selective σ_1 ligand (−)-**8** showed an antagonist profile determined by phenytoin differential modulation of binding affinity in vitro, confirmed in vivo by an increase of κ opioid analgesia. The σ_2 ligand (−)-**9** displayed agonist properties in an in vitro isolated organ bath assay and antiproliferative effects on LNCaP and PC3 prostate cancer cell lines.

INTRODUCTION

The classification of σ receptors into two distinct subtypes, σ_1 and σ_2,[1] based on molecular weight (25 and 18−21.5 kDa, respectively), tissue distribution, and subcellular localization has prompted interest in investigating the functional roles of these two receptor sites.

The σ_1 receptor is an intracellular chaperone protein associated with endoplasmic reticulum and mitochondrial membranes.[2] Ligands of σ_1 receptors have been shown to prevent neuronal death related to glutamate toxicity,[3] play a role in memory processes,[4] and be involved in the development of cocaine-induced rewarding properties.[5] Moreover, several studies have shown a relationship between σ_1 and opioid receptors in pain modulation, which suggests that a tonically active antiopioid σ_1 system markedly influences the sensitivity toward opioid analgesia, especially κ-opioid receptor mediated.[6,7] Recently, studies have reported that a nonpolymorphic mutation (c.672*51G) in the 3′-untranslated region of the σ_1 receptor gene is involved in frontotemporal lobar degeneration−motor neuron disease, which is the most common cause of early onset dementia.[8]

Although the σ_2 receptor subtype has not been cloned, σ_2 agonists have been shown to cause phosphatidylserine translocation, DNA fragmentation, and chromatin condensation, which indicates that σ_2 receptors induce apoptosis in diverse tumor cell types.[9] The potential role of the σ_2 receptor in regulating cellular proliferation has led to an increased interest in investigating the biological function of this receptor. Furthermore, σ_2 selective ligands could be used as imaging agents for measuring the proliferative status of breast tumors by positron emission tomography analysis.[3,10]

Because the specific pharmacological roles of the two receptor subtypes are still being defined, the design of selective σ subtype receptor ligands is the subject of pharmaceutical studies.

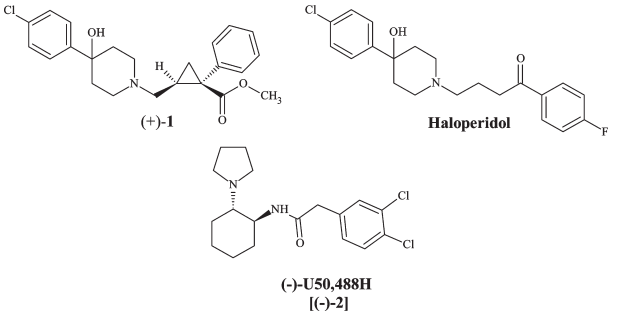

Figure 1. Structures of (+)-**1**, haloperidol, and (−)-**2**.

Recently, we reported the synthesis and pharmacological evaluation of (+)-methyl (1*R*,2*S*)-2-{[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]methyl}-1-phenylcyclopropanecarboxylate [(+)-MR200, (+)-**1**]. This new σ ligand, which is structurally related to haloperidol (Figure 1), has enhanced selectivity to different transporter and receptor systems.[11]

In vivo evaluation of the ability of (+)-**1** to increase μ-, δ-, and κ-opioid receptor analgesia provided evidence of an antagonistic σ_1 profile, but as previously reported, the σ_1/σ_2 selectivity of (+)-**1** was not very high.[7] To obtain compounds with improved selectivity, we synthesized new enantiomers and diastereoisomers with some modifications of the amino moiety of (+)-**1**. We eliminated the 4-chloro substituent from (+)-**1**; moreover, we synthesized tropane analogues to increase the structural rigidity and steric hindrance around the basic piperidine nitrogen atom. This last modification has already been reported for 3-(ω-aminoalkyl)-1*H*-indole σ ligands,

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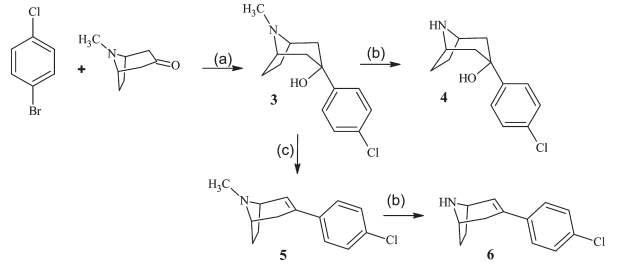
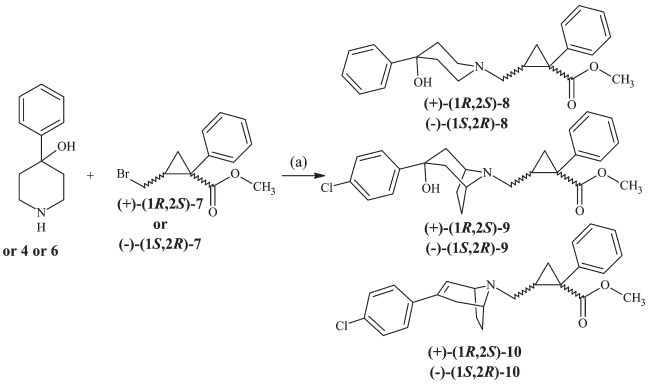
Scheme 1[a]



[a] Reagents and conditions: (a) *n*-BuLi, THF, −70 °C; (b) 1,2-dichloroethane, 1-chloroethyl chloroformate, reflux, 24 h; (c) CH_3COOH, HCl (37%), reflux, 25 min.

Scheme 2[a]



[a] Reagents and conditions: (a) $NaHCO_3$, DMF, 60 °C.

which provided a compound with high affinity and selectivity for σ_2 subtype receptors.[12] All compounds were tested to determine σ_1 and σ_2 binding affinity and selectivity, and ligands with desirable values were also evaluated in other transporter and receptor systems. The pharmacological activity of the more selective σ_1 ligand was assessed by evaluating the phenytoin differential modulation of binding affinity in guinea pig brain.[13] Moreover, we analyzed the σ_1 analgesic modulation of the κ agonist (−)-U50,488H [*trans*-(1*S*,2*S*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide, (−)-**2**] in rats.[7,14] The activity of the compound with the best σ_2 affinity and selectivity was evaluated by the inhibition of electrically evoked contractions in guinea pig ileum with desensitized σ_1 receptor.[15] In addition, we evaluated the antiproliferative effects on hormone sensitive LNCaP and hormone refractory PC3 prostate tumor cell lines, where the presence of σ_2 receptors has been already demonstrated.[16,17]

CHEMISTRY

The synthesis of the amines (3-*exo*)-3-(4-chlorophenyl)-8-azabicyclo[3.2.1]octan-3-ol (**4**) and (±)-3-(4-chlorophenyl)-8-azabicyclo[3.2.1]oct-2-ene (**6**) originated from the treatment of commercially available tropine-3-one with 1-bromochlorobenzene and *n*-butyllithium at −70 °C (Scheme 1). Acidic dehydration of alcohol **3** with acetic acid and HCl (37%) generated alkene **5**. Demethylation of **3** and **5** with 1-chloroethyl chloroformate provided the intermediate amines (for details, see Supporting Information). The reaction between commercially available amine 4-phenylpiperidin-4-ol, (3-*exo*)-3-(4-chlorophenyl)-8-azabicyclo[3.2.1]octan-3-ol (**4**), or (±)-3-(4-chlorophenyl)-8-azabicyclo[3.2.1]oct-2-ene (**6**) with the enantiomers (+)-(1*R*,2*S*) and (−)-(1*S*,2*R*)-methyl 2-(bromomethyl)-1-phenylcyclopropanecarboxylate[18−20] (**7**) provided **8**, **9**, or **10**, respectively (Scheme 2).

Table 1. σ_1 and σ_2 Binding Affinities of Synthesized Compounds, Haloperidol, Haloperidol Metabolite II, and 1,3-Di-(2-tolyl)guanidine (DTG)

	$K_i \pm$ SD (nM)[a]		
compd	σ_1	σ_2	σ_1/σ_2 [b]
(+)-**1**	3.95 ± 0.56	21.9 ± 2.55	5.5
(−)-**1**	5.61 ± 1.33	23.4 ± 2.77	4.2
(+)-**8**	5.02 ± 1.26	28.2 ± 2.55	5.6
(−)-**8**	7.01 ± 0.37	571 ± 4.77	81.4
(+)-**9**	44.0 ± 4.23	58.3 ± 4.95	1.3
(−)-**9**	520 ± 8.87	25.2 ± 3.22	0.05
(+)-**10**	397 ± 5.56	943 ± 9.67	2.4
(−)-**10**	198 ± 4.66	565 ± 7.56	2.8
haloperidol	2.20 ± 1.22	16.0 ± 2.68	7.3
haloperidol metabolite II	5.4 ± 0.67	0.98 ± 0.23	0.18
DTG	69.4 ± 0.56	23.0 ± 0.56	0.33

[a] Each value is the mean ± SD of three determinations. [b] This value is derived from the K_i for σ_2 binding affinity divided by the K_i for σ_1 binding affinity. Values of >1 indicate selectivity for σ_1 over σ_2. Values of <1 indicate selectivity for σ_2 over σ_1.

RESULTS AND DISCUSSION

Binding affinities for σ_1 and σ_2 receptors of the newly synthesized **8**−**10** and the two enantiomers (+)-**1** and (−)-**1** are reported in Table 1. Compounds (+)-**8** and (−)-**8** showed σ_1 receptor affinities (K_i of 5.02 and 7.01 nM, respectively) similar to those of their respective enantiomers of **1** [i.e., (+)-**4**-chloro substituted (+)-**1** (K_i = 3.95 nM) and (−)-**1** (K_i = 5.61 nM)]. Although the (+)-**8** enantiomer displayed a comparable σ_2 receptor affinity with respect to (+)-**1**, the (−)-**8** enantiomer possessed a lower σ_2 binding affinity (K_i = 571 nM), which suggested that the (−)-**8** enantiomer had increased σ_1 subtype selectivity compared with (+)-**1** and (−)-**1**. Compounds (+)-**9** and (−)-**9**, which had increased structural rigidity and steric hindrance on the amino moiety, showed reduced σ_1 binding affinity (K_i of 44.0 and 520 nM, respectively). Conversely, for σ_2 receptor subtypes, we only observed a slight reduction for the (+)-**9** isomer (K_i = 58.3 nM). Interestingly, the affinity of the (−)-**9** enantiomer (K_i = 25.2 nM) did not differ from the affinity of (−)-**1**. The elimination of the hydroxyl group on (+)-**10** and (−)-**10** significantly decreased the affinity to both σ receptors, which was probably related to an additional structural rigidity that was not suitable for interactions with σ receptors. The present binding results revealed that (−)-**8** was selective for the σ_1 receptor and (−)-**9** was moderately selective for the σ_2 receptor. Therefore, we also investigated the affinity of these compounds for other neurotransmitter systems (see Supporting Information Table 2). Similar to the parent compound (+)-**1**,[11] (−)-**8** and (−)-**9** showed very low affinity for opioid, dopamine (D_1, D_2, D_3, $D_{4.2}$), $5HT_{2A}$, and α_1 receptors compared with haloperidol. In addition, they did not show any significant affinities for other receptors or transporters tested.

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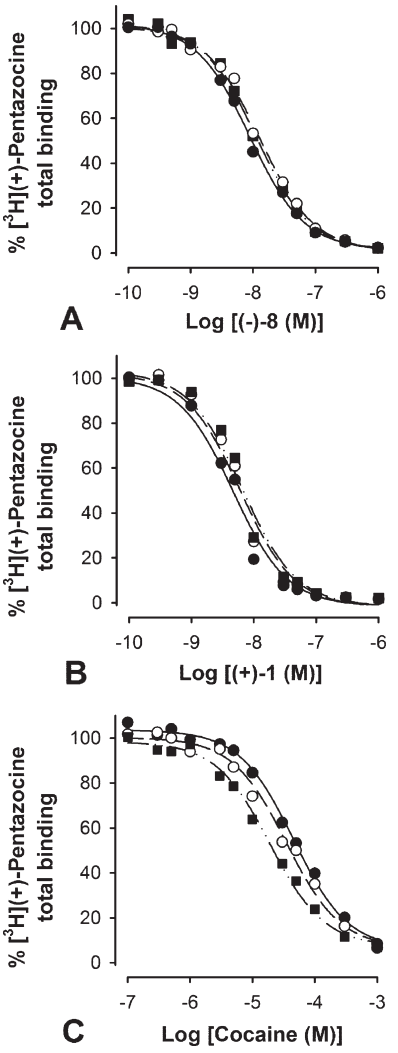

Figure 2. Inhibition of [^{3}H](+)-pentazocine binding by (−)-**8** (A), (+)-**1** (B), and cocaine (C) in the absence (●) or presence of 250 μM (○) or 1 mM (■) phenytoin.

In our studies, phenytoin (250 μM and 1 mM) did not significantly modify the inhibition curves of (−)-**8** (Figure 2A). The ratios of the control K_i with respect to those obtained in the presence of phenytoin were slightly lower than unity (see Supporting Information Table 3), which has previously been reported for several known σ_1 antagonists, including the structurally related compound haloperidol.[13,21] Similar results were obtained with the σ_1 antagonist parent (+)-**1**[7] (Figure 2B). To further validate this assay, we also tested the putative σ_1 agonist cocaine,[3,22] and incubation with phenytoin markedly shifted the inhibition curves of cocaine to the left in a concentration-dependent manner (Figure 2C).[13,21] Hill analysis of all competition curves yielded straight lines (r^2 = 0.97−0.99) with slopes or pseudo-Hill coefficients (n'_H) close to unity for all drugs tested (in the presence or absence of phenytoin; see Supporting Information Table 3). This confirms the existence of a single population of binding sites with an n'_H that does not change in the presence of phenytoin.

In vivo results showed that (−)-**8** (1 mg/kg sc), which was the dose used in previous experiments with (+)-**1**),[7] did not affect basal tail flick latency (TFL), expressed as the mean area under the curve (MAUC), during the entire time of observation (60 min).

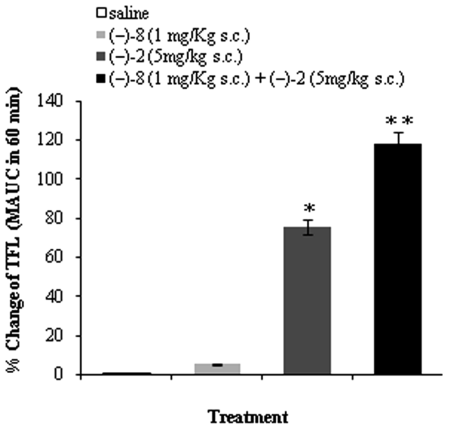

Figure 3. Effect of (−)-**8** (1 mg/kg sc) on (−)-**2** (5 mg/kg sc) analgesia. Results are expressed as MAUC (after the last injection) over the 60 min testing period. Columns represent the mean ± SD: ∗, p < 0.05 vs saline-treated rats (n = 8−10); ∗∗, p < 0.05 vs (−)-**2**-treated rats (n = 8−10).

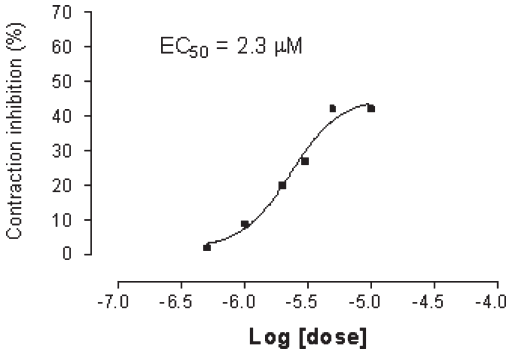

Figure 4. Representative dose-response curve of (−)-**9** in guinea pig ileum.

Injection of the κ agonist (−)-**2** (5 mg/kg sc) significantly increased the nociceptive latency following thermal stimulation, which clearly demonstrated an analgesic effect. Indeed, the percent change from basal level of TFL, compared with the group of saline-treated rats, was increased from 1.07% to 75.6%. Pretreatment with (−)-**8** (1 mg/kg sc) 45 min prior to (−)-**2** (5 mg/kg sc) increased the antinociceptive effect of the opioid agonist (Figure 3). In particular, the calculated value of MAUC (118.2%) was significantly higher than the corresponding value obtained with (−)-**2** (75.6%). Similar to haloperidol and (+)-**1**,[6,7] (−)-**8** demonstrated σ_1 antagonist actions, which confirmed the in vitro results obtained with the phenytoin binding assays.

In an ex vivo isolated organ bath experiment, (−)-**9** displayed σ_2 partial agonist activity (α = 0.4) in inhibiting twitch contraction with an EC_{50} of 2.3 μM[23] (Figure 4).

In LNCaP and PC3 cells, incubation with compound (−)-**9** (100 μM) for 48 h caused an antiproliferative effect that was more pronounced in LNCaP cells (Figure 5). A similar pattern was observed for the reference compounds haloperidol and haloperidol metabolite II. Although LNCaP and PC3 cells have similar σ_1/σ_2 receptor density, (−)-**9** did not exert the same activity in the two cell lines, which could be ascribed to the presence of some efflux pumps, such as P-glycoprotein (P-gp), in the hormone refractory PC3 cell line.[24]

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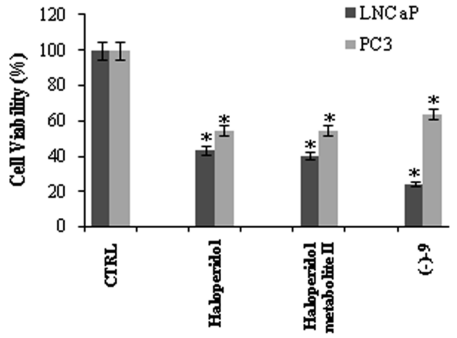

Figure 5. Effects of haloperidol, haloperidol metabolite II, and (−)-**9** (100 μM) on LNCaP and PC3 cells after 48 h of exposure: *, $p < 0.05$ vs CTRL.

In summary, we reported the synthesis of new σ ligands that are structurally related to the σ_1 antagonist (+)-**1**. Compounds (−)-**8** and (−)-**9** showed the best affinity and selectivity for σ_1 and σ_2 receptors, respectively, and did not show any significant affinity for other receptor or transporter systems. In vitro and in vivo pharmacological evaluations provided evidence of antagonist profile for (−)-**8** and agonist activity for (−)-**9**. In light of the emerging implications of σ subtype receptors in drug abuse,[25] neuropathic pain,[26] frontotemporal lobar degeneration−motor neuron disease,[8] and anticancer activity,[16] the two selective compounds (−)-**8** and (−)-**9** could be considered as new pharmacological speculative tools.

EXPERIMENTAL SECTION

For details, see the Supporting Information.

General Details. Reagents used for synthesis were purchased from Sigma-Aldrich (Milan, Italy) unless otherwise specified. The course of the reaction was monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 aluminum sheets (Merck, Darmstadt, Germany). Visualization was performed under ultraviolet (UV) light or in an iodine chamber. Merck silica gel 60, 230−400 mesh, was used for flash column chromatography. Melting points were obtained in open capillary tubes with a Büchi 530 apparatus (Büchi Italia, Assago, Italy) and are uncorrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a Varian Inova 200 MHz spectrometer (Varian, Leini, Italy). Chemical shifts are reported in δ values (ppm) relative to an internal standard of tetramethylsilane. Optical rotations were determined in MeOH ($c = 1$) with a Perkin-Elmer 241 polarimeter. Elemental analyses (C, H, N) were determined on an elemental analyzer, Carlo Erba model 1106 (Carlo Erba, Milan, Italy), and the results were within 0.4% of the theoretical values (purities of tested compound were ≥99%).

(+)-Methyl (1*R*,2*S*)-2-[(4-Hydroxy-4-phenylpiperidin-1-yl)-methyl]-1-phenylcyclopropanecarboxylate (8). A mixture of 4-phenyl-4-hydroxypiperidine (300 mg, 1.69 mmol), methyl (+)-(1*R*,2*S*)-2-(bromomethyl)-1-phenylcyclopropanecarboxylate (**7**) (455 mg, 1.69 mmol), and $NaHCO_3$ (213 mg, 2.53 mmol) in dry dimethylformamide (DMF) (15 mL) was heated at 60 °C for 6 h. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ and washed with a solution of 4% $NaHCO_3$. The organic layers were dried over anhydrous Na_2SO_4, and after evaporation of the solvent, the crude product was purified by flash column chromatography using cyclohexane/ethyl acetate (1:1) as eluant. The resulting colorless oil (355 mg) was dissolved in diethyl ether and treated with a solution of oxalic acid in diethyl ether to give the oxalate salts as a white solid. The analytically pure sample was obtained by crystallization from methanol/diethyl ether: yield 57.0%; mp 200−202 °C; $[\alpha]_{20}^{D} = +30$ (c 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 1.50 (dd, 1H, $J = 4.8, 9.0$ Hz), 1.66 (dd, 1H, $J = 4.8, 7.0$ Hz), 1.70−1.92 (m, 2H), 1.92−2.12 (m, 1H), 2.18−2.42 (m, 2H), 3.12−3.50 (m, 6H), 3.58 (s, 3H), 6.368 (s, br, 5H), 7.20−7.55 (m, 10H); ^{13}C NMR (DMSO-d_6) (oxalate salts) δ 20.26, 22.65, 33.74, 35.08, 47.95, 48.17, 52.56, 54.21, 68.07, 124.64, 126.85, 127.36, 128.14, 128.28, 129.90, 139.32, 147.97, 164.63, 171.82; Anal. $C_{23}H_{27}NO_3 \cdot 0.8\ C_2H_2O_4 \cdot 1.2\ H_2O$.

Compounds (−)-(1*S*,2*R*)-**8**, (+)-(1*R*,2*S*)-**9**, (−)-(1*S*,2*R*)-**9**, (+)-(1*R*,2*S*)-**10** and (−)-(1*S*,2*R*)-**10** were synthesized analogously.

(−)-Methyl (1*S*,2*R*)-2-[(4-Hydroxy-4-phenylpiperidin-1-yl)-methyl]-1-phenylcyclopropanecarboxylate (8). Yield 55.0%; mp 199−201 °C; $[\alpha]_D^{20}$ −30 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 1.50 (dd, 1H, $J = 4.8, 9.0$ Hz), 1.66 (dd, 1H, $J = 4.8, 7.0$ Hz), 1.70−1.92 (m, 2H), 1.92−2.12 (m, 1H), 2.18−2.42 (m, 2H), 3.12−3.50 (m, 6H), 3.58 (s, 3H), 6.368 (s, br, 5H), 7.20−7.55 (m, 10H); ^{13}C NMR (DMSO-d_6) (oxalate salts) δ 20.26, 22.65, 33.74, 35.08, 47.95, 48.17, 52.56, 54.21, 68.07, 124.64, 126.85, 127.36, 128.14, 128.28, 129.90, 139.32, 147.97, 164.63, 171.82. Anal. $C_{23}H_{27}NO_3 \cdot 0.8C_2H_2O_4 \cdot 1.2H_2O$.

(+)-Methyl (1*R*,2*S*)-2-{[(3-*endo*)-3-(4-Chlorophenyl)-3-hydroxy-8-azabicyclo[3.2.1]oct-8-yl]methyl}-1-phenylcyclopropanecarboxylate (9). Yield 52.0%; mp 130−132 °C; $[\alpha]_D^{20}$ +34 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 1.33 (dd, 1H, $J = 5.0, 9.2$ Hz), 1.62−2.45 (m, 10H), 2.64 (dd, 1H, $J = 7.6, 13.0$ Hz), 2.79 (dd, 1H, $J = 5.6, 13.0$ Hz), 3.23−3.57 (m, 3H), 3.60 (s, 3H), 7.10−7.50 (m, 9H); ^{13}C NMR ($CDCl_3$) (free base) δ 20.18, 25.85, 26.12, 29.25, 33.71, 46.02, 46.16, 50.23, 52.28, 58.57, 59.38, 73.12, 126.06, 127.05, 128.15, 130.24, 132.26, 140.80, 148.98, 173.06. Anal. $C_{25}H_{28}ClNO_3 \cdot C_2H_2O_4 \cdot 0.5H_2O$.

(−)-Methyl (1*S*,2*R*)-2-{[(3-*endo*)-3-(4-Chlorophenyl)-3-hydroxy-8-azabicyclo[3.2.1]oct-8-yl]methyl}-1-phenylcyclopropanecarboxylate (9). Yield 53.7%; mp 130−132 °C; $[\alpha]_D^{20}$ −35 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 1.33 (dd, 1H, $J = 5.0, 9.2$ Hz), 1.62−2.45 (m, 10H), 2.64 (dd, 1H, $J = 7.6, 13.0$ Hz), 2.79 (dd, 1H, $J = 5.6, 13.0$ Hz), 3.23−3.57 (m, 3H), 3.60 (s, 3H), 7.10−7.50 (m, 9H); ^{13}C NMR ($CDCl_3$) (free base) δ 20.18, 25.85, 26.12, 29.25, 33.71, 46.02, 46.16, 50.23, 52.28, 58.57, 59.38, 73.12, 126.06, 127.05, 128.15, 130.24, 132.26, 140.80, 148.98, 173.06. Anal. $C_{25}H_{28}ClNO_3 \cdot C_2H_2O_4 \cdot 0.4H_2O$.

(+)-Methyl (1*R*,2*S*)-2-{[(1*R*,5*S*/1*S*,5*R*)-3-(4-Chlorophenyl)-8-azabicyclo[3.2.1]oct-2-en-8-yl]methyl}-1-phenylcyclopropanecarboxylate (10). Diastereoisomeric mixture was obtained from (±)-3-(4-chlorophenyl)-8-azabicyclo[3.2.1]octan-2-ene (**6**) and (+)-methyl (1*R*,2*S*)-2-(bromomethyl)-1-phenylcyclopropanecarboxylate (**7**): yield 67.0%; mp 126−128 °C; $[\alpha]_D^{20}$ +40 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 1.00−2.30 (m, 8H), 2.50−2.95 (m, 3H), 3.48 (s, 3H), 3.55−3.65 (m, 2H), 6.20 (d, 1H, $J = 5.4$), 7.00−7.50 (m, 9H); ^{13}C NMR ($CDCl_3$) (free base) δ 19.74, 20.65, 28.58, 28.88, 29.50, 29.57, 33.42, 33.61, 33.97, 47.19, 47.34, 52.21, 52.26, 53.40, 56.06, 56.51, 56.98, 57.65, 125.91, 127.02, 127.06, 127.89, 128.13, 128.15, 128.31, 130.13, 130.17, 131.53, 131.80, 132.66, 138.55, 140.69, 172.81, 172.91. Anal. $C_{25}H_{26}ClNO_2 \cdot C_2H_2O_4 \cdot 0.7H_2O$.

(−)-Methyl (1*S*,2*R*)-2-{[(1*R*,5*S*/1*S*,5*R*)-3-(4-Chlorophenyl)-8-azabicyclo[3.2.1]oct-2-en-8-yl]methyl}-1-phenylcyclopropanecarboxylate (10). Diastereoisomeric mixture was obtained from (±)-3-(4-chlorophenyl)-8-azabicyclo[3.2.1]octan-2-ene (**6**) and (−)-methyl (1*S*,2*R*)-2-(bromomethyl)-1-phenylcyclopropanecarboxylate (**7**): yield 65.0%; mp 127−129 °C; $[\alpha]_D^{20}$ −39 (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) (free base) δ 1.00−2.30 (m, 8H), 2.50−2.95 (m, 3H), 3.48 (s, 3H), 3.55−3.65 (m, 2H), 6.20 (d, 1H, $J = 5.4$), 7.00−7.50 (m, 9H); ^{13}C NMR ($CDCl_3$) (free base) δ 19.74, 20.65, 28.58, 28.88, 29.50, 29.57, 33.42, 33.61, 33.97, 47.19, 47.34, 52.21, 52.26, 53.40, 56.06, 56.51, 56.98, 57.65, 125.91, 127.02, 127.06, 127.89, 128.13, 128.15, 128.31, 130.13, 130.17, 131.53, 131.80, 132.66, 138.55, 140.69, 172.81, 172.91. Anal. $C_{25}H_{26}ClNO_2 \cdot C_2H_2O_4 \cdot 0.7H_2O$.

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■ ASSOCIATED CONTENT

Supporting Information. Synthesis of **3**−**6**, elemental analysis results, binding affinity data, binding parameters, and biological experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

HPLC, high performance liquid chromatography; MAUC, mean area under the curve; NMR, nuclear magnetic resonance; PET, positron emission tomography; sc, subcutaneous; SD, standard deviation; TFL, tail flick latency; TLC, thin-layer chromatography; UV, ultraviolet

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