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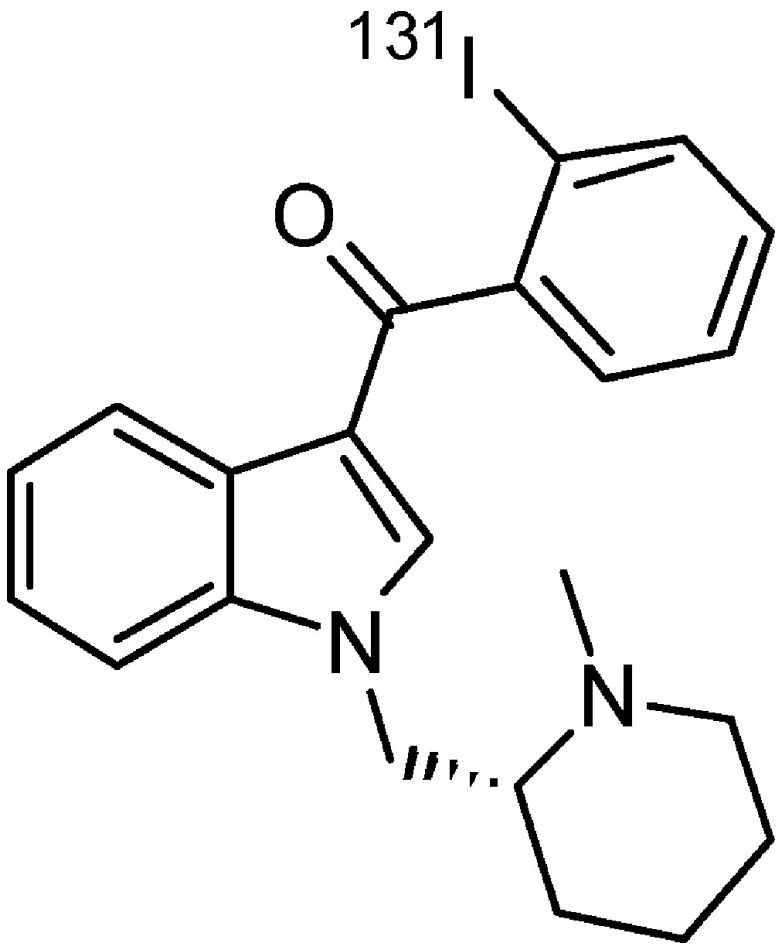

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Potent Cannabinergic Indole Analogues as Radioiodinatable Brain Imaging Agents for the CB1 Cannabinoid Receptor

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A series of novel aminoalkylindoles was synthesized in an effort to develop compounds that are potent agonists at the CB1 cannabinoid receptor and that are also easily labeled with radioisotopes of iodine for biochemical and imaging studies. 2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (**8**, AM2233) had a very high affinity for the rat CB1 receptor, with most of the affinity residing with the (*R*)-enantiomer. Radioiodinated **8**, (*R*)-**8**, and (*S*)-**8** were prepared by radioiododestannylation of the tributyltin analogues in high yields, radiochemical purities, and specific radioactivities. In a mouse hippocampal membrane preparation with [^{131}I](*R*)-**8** as radioligand, racemic **8** exhibited a K_i value of 0.2 nM compared with 1.6 nM for WIN55212-2. In autoradiographic experiments with mouse brain sections, the distribution of radioiodinated **8** was consistent with that of brain CB1 receptors. Again, very little specific binding was seen with the (*S*)-enantiomer [^{131}I](*S*)-**8** and none occurred with the (*R*)-enantiomer [^{131}I](*R*)-**8** in sections from CB1 receptor knockout mice. Radioiodinated **8** thus appears to be a suitable radioligand for studies of CB1 cannabinoid receptors.

Introduction

The CB1 cannabinoid receptor is abundant in the brain and is the receptor responsible for the psychotropic and central nervous system (CNS) effects of marijuana.[1] Radioligand binding studies of these G-protein coupled receptors (GPCRs) have been of great value in addressing a number of research questions. These include investigations of the degree of occupancy of cannabinoid receptors required to elicit biological responses, determining whether new therapeutic agents possess significant binding to cannabinoid receptors in vivo, determining if cannabinoid receptors are up- or down-regulated as a result of chronic drug use or psychiatric conditions, and monitoring the loss of neuronal cell types possessing cannabinoid receptors. The principal classes of ligands that have been found to interact with CB1 receptors are the classical and nonclassical cannabinoids, the endocannabinoids, the aminoalkylindoles, and the diarylpyrazoles.[2] The original characterization of cannabinoid receptors was accomplished by use of the tritium-labeled nonclassical cannabinoid [^{3}H]CP-55,940.[3] However, labeling with iodine radioisotopes including iodine-123, -124, -125 and -131 can be preferable to tritium labeling for certain purposes.[4] All these isotopes offer much higher specific radioactivity than tritium, as well as avoidance of liquid scintillation counting, and each has particular applications, for example, iodine-123 for single photon emission computed tomography (SPECT).[5] In the present study we employed iodine-131 because of its regulatory convenience for institutional radiation control.

Our previous studies of radioiodinated cannabinoid receptor ligands have involved pyrazole compounds that are antagonists and/or inverse agonists at the CB1 receptor and have included AM281 and AM251.[6] Pyrazoles are much less lipophilic than classical or nonclassical cannabinoids (both of which generally have log *P* values above 6[7]) and offer advantages in formulation, especially for animal studies. Aminoalkylindoles also have a relatively low lipophilicity and generally act as agonists at CB1 receptors.[8] For example, the full agonist aminoalkylindole WIN55212-2 (**1**) is the most commonly used cannabinergic ligand for laboratory studies.[9] The information obtained from pyrazole and aminoalkylindole radiotracers may therefore be different, since agonists mostly bind to GPCRs in the high affinity state whereas antagonists bind to both high and low affinity states.[10] In the present study, we report the design and synthesis of a group of indoles substituted at the 1-position with a *N*-methylpiperidin-2-ylmethyl group and bearing iodobenzoyl or iodonaphthoyl groups at the 3-position. Enantiomers of 2-iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (**8**, AM2233), the most potent of these compounds, were prepared in radioiodinated form for preliminary mouse hippocampal membrane homogenate binding and mouse brain autoradiographic studies.

Chemistry

Compounds in this study were generally synthesized by modifications of published procedures, as outlined in Schemes 1−4. 6-Nitroindole was treated with methylmagnesium bromide, zinc chloride, and then reacted with 1-naphthoyl chloride to give 3-naphthoylindole (**9**)

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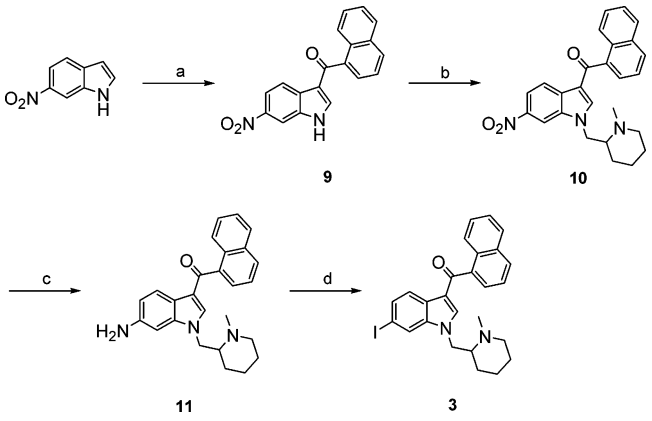
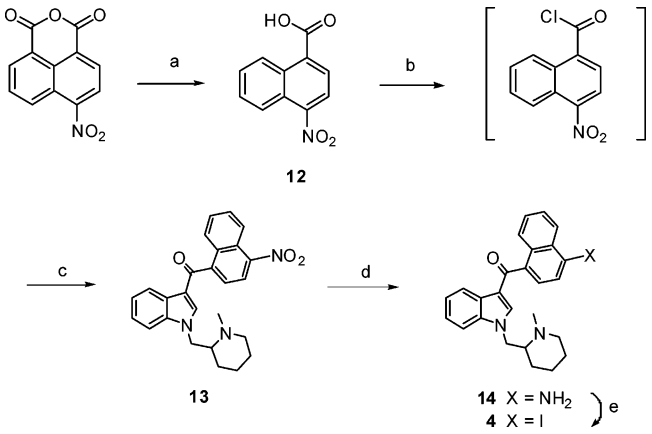
Scheme 1[a]



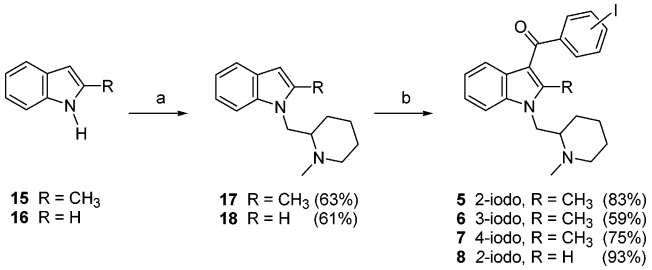
[a] Reagents and conditions: (a) methylmagnesium bromide, ethyl ether, 0 °C, 15 min; zinc chloride, 30 min; 1-naphthoyl chloride, 2 h, 40%; (b) NaH, DMF, 2-chloromethyl-1-methylpiperidine, 60−70 °C, overnight, 44%; (c) hydrazine, ethanol, Raney Ni, 2 h, 83%; (d) HCl, 1 h; $NaNO_2$, 0 °C, 1 h; KI, 84%.

Scheme 2[a]



[a] Reagents and conditions: (a) 50% aqueous NaOH; AcOH; $Hg(OAc)_2$, reflux, 4 days; 6 M HCl, 1 h; (b) $SOCl_2$, toluene, reflux, 6 h; (c) $AlCl_3$, CH_2Cl_2, 1-(1-methylpiperidin-2-ylmethyl)-1*H*-indole (**18**), 55 °C, 6 h, 35% from **12**; (d) hydrazine, ethanol, Raney Ni, 3 h, 91%; (e) HCl, 1 h; $NaNO_2$, 0 °C, 1 h; KI, 56%.

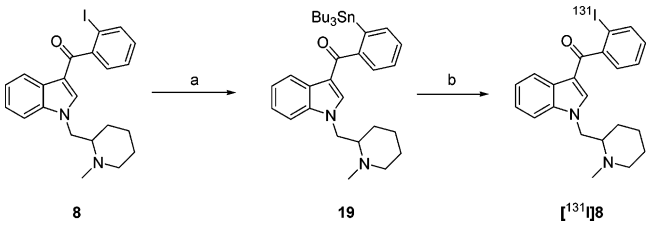
Scheme 3[a]



[a] Reagents and conditions: (a) KOH or NaH, 2-chloromethyl-1-methylpiperidine, DMF, 65−85 °C; (b) $AlCl_3$, CH_2Cl_2, iodobenzoyl chloride, 40 °C.

(Scheme 1),[11] which was then alkylated at the indole 1-position to afford **10**. Reduction of the 6-nitro group and subsequent conversion of the aryl amino analogue **11** via treatment of its corresponding aryldiazonium salt[12] with potassium iodide afforded the target compound **3**. 4-Nitro-1-naphthoic acid (**12**), prepared from 4-nitro-1,8-naphthalic acid anhydride,[13] was converted to its acid chloride and used to acylate 1-(1-methylpiperidin-2-ylmethyl)-1*H*-indole (**18**) at the 3-position to give **13** (Scheme 2). The 4-nitro group was converted to

Scheme 4[a]



[a] Reagents and conditions: (a) hexabutylditin, $Pd(PPh_3)_4$, toluene, reflux, 18 h, 93%; (b) chloramine-T, $Na^{131}I$, aqueous HCl, EtOH, 5 min, 40−60%.

an iodo group through the amine intermediate **14** by diazotization and reaction with potassium iodide to give **4** (AM1297). An X-ray crystal structure confirmed that the iodo substitution was at the 4-position on the naphthalene ring (see Supporting Information). By use of the corresponding iodo-substituted benzoyl chlorides as acylating reagents and either 1-(1-methylpiperidin-2-ylmethyl)-1*H*-indole **18** or the 2-methyl derivative **17**, four 3-iodobenzoyl analogues (**5**, **6**, **7**, and **8**) were synthesized in the same manner as **4** (Scheme 3). The chirally pure enantiomers (*R*)-**8** and (*S*)-**8** were first obtained through resolution of the racemic mixture by a chiral high-performance liquid chromatography (HPLC) method[14] and subsequently by synthesis from optically pure pipecolinic acids[15] (see Supporting Information) as starting materials. Racemic compound **8** and its two pure enantiomers were radiolabeled by successive stannylation[16] and modified radioiododestannylation[17] reactions as outlined in Scheme 4.

Results of the single-crystal X-ray study of **8** are shown in Figure 1. The solid-state structure outlines some important conformational features of the molecule. First, the *N*-methylpiperidinyl group exists in a chair conformation, with the *N*-methyl group being equatorial

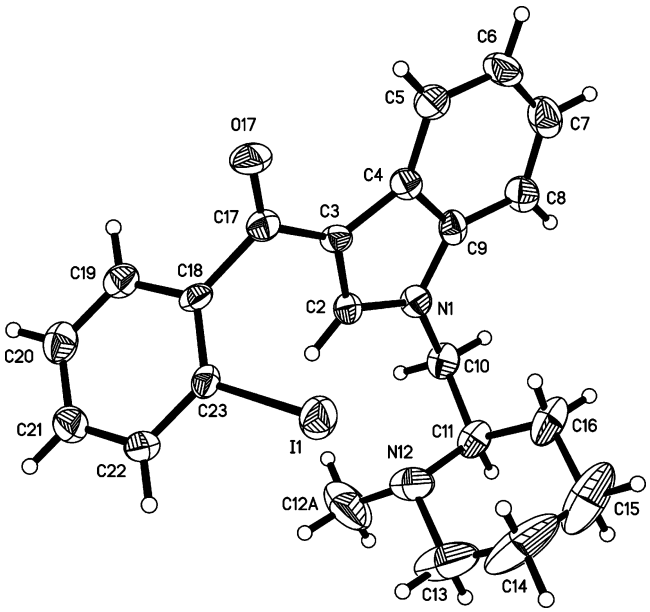
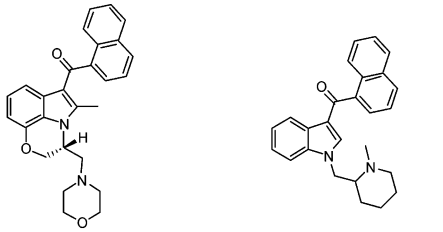


Figure 1. The results of the X-ray structural study of compound **8**, which crystallized in a centrosymmetric space group with both enantiomers present in the unit cell and related by crystallographic symmetry. Depicted is the 3-dimensional representation of the (*R*)-enantiomer. Thermal ellipsoids are at the 30% level. The highest peaks in the final difference map were located close to the iodine atom.

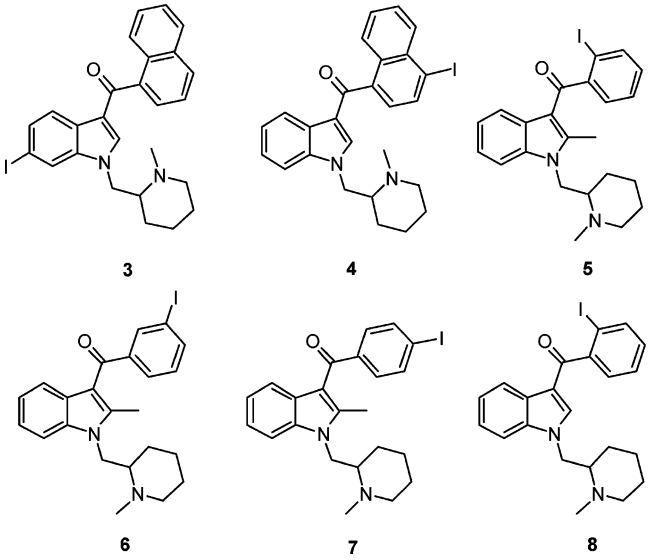
Chart 1. Binding Affinities (K_i) for Rat Forebrain (CB1)



1
K_i (CB1) = 1.89 nM
WIN55212-2

2
K_i (CB1) = 0.75 nM

Chart 2



3 4 5 6 7 8

and in close proximity to the iodo substituent of the benzoyl ring. The carbonyl group lies in the plane with indole in an anti conformation with respect to the indole C2−C3 bond. Conversely, the 2-iodophenyl ring is gauche with its plane forming a 73° angle with the plane of the indole.

Biological Results and Discussion

In Vitro Binding Studies. WIN55212-2 (**1**) and [1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]naphthalen-1-ylmethanone (**2**) are among the most potent previously described indole cannabimimetics (Chart 1).[18,19] With **2** as a lead compound, two analogues **3** and **4** were prepared bearing iodo groups on the indole and naphthalene rings, respectively (Chart 2). As can be seen from the data in Table 1, compound **3**, with an iodo substituent at the indole 6-position, has an affinity of 18 nM for CB1 and 9.8 nM for CB2, whereas compound **4**, iodinated at the 4-position of the 1-naphthoyl group, has affinities of 10 nM and 5.3 nM for the CB1 and CB2 receptors, respectively. In both cases, the affinities for the CB1 and CB2 receptors were reduced. More serious reductions in bindings were observed with CB1.

The most striking SAR data were observed with the iodobenzoyl analogues. Introduction of iodo substituents at the meta and para positions (compounds **6** and **7**, respectively) led to analogues with very low affinities for both CB1 and CB2. Conversely, ortho substitution provided a ligand with the highest affinities for both CB1 and CB2. This high affinity of the 2-iodobenzoyl ligand **5** was accentuated in the corresponding analogue **8**, which lacks the 2-methyl substituent on the indole. Compound **8** exhibited high affinities toward both cannabinoid receptors [K_i (CB1) = 2.8 nM in rat forebrain and K_i (CB2) = 2.9 nM in mouse spleen] that were comparable to those of its isosteric 1-naphthoyl analogue **2**. It can be argued that this reflects the presence of a hydrophobic binding pocket within both the CB1 and CB2 binding sites capable of accommodating bulky substituents at the 2-position of the benzoyl group. On the other hand, improvements in affinities for both CB1 and CB2 compared to its 2-methyl analogue can be associated with the higher conformational freedom of the iodobenzoyl group of **8**. This would enhance its ability to assume its pharmacophoric conformation at the active sites of CB1 and CB2.

Table 1. CB1 and CB2 Affinities of Aminoalkylindoles

compd	K_i[a] (nM) CB1	CB2
2	0.75 (0.63, 0.90)	1.91 (1.63, 2.23)
3	18 (15, 22)	9.8 (8.2, 12)
4	10 (8.9, 12)	5.3 (4.4, 6.3)
5	34 (29, 39)	34 (30, 39)
(+)-**5**	6.7 (5.7, 7.9)	10 (8.4, 12)
(−)-**5**	1200 (1000, 1500)	83 (69, 100)
6	2900 (2400, 3500)	9600 (8300, 11000)
7	1800 (1400, 2300)	790 (630, 1000)
8	2.8 (2.3, 4.0)	2.9 (2.3, 3.5)
(*R*)-(+)-**8**	1.80 (1.6, 2.4)	2.2 (1.8, 2.6)
(*S*)-(−)-**8**	560 (470, 670)	580 (480, 710)

[a] Binding affinities (K_i) of compounds for CB1 (rat forebrain) and CB2 (mouse spleen) cannabinoid receptors. The 95% confidence limits are given in parentheses.

Chiral resolution of **5** showed that most of the affinity resided with the (+)-enantiomer. Resolution of **8** also showed that the (*R*)-(+)-enantiomer has a very high affinity for the CB1 receptor, 300-fold greater than the (*S*)-(−)-enantiomer. The absolute configurations of the enantiomers of **8** were confirmed by chirospecific syntheses from the respective pipecolinic acids.

Additional binding assays in mouse hippocampal membranes showed that racemic **8** was approximately 8-fold more potent than the CB1 agonist WIN55212-2 **(1)** in displacing [^{131}I](*R*)-**8** binding [K_i (**8**) = 0.2 nM and K_i (**1**) = 1.6 nM; Figure 2A]. Racemic **8** was also approximately 15-fold more effective than WIN55212-2 (**1**) in displacing the antagonist [^{3}H]SR141716A binding [K_i (**8**) = 1.3 nM and K_i (**1**) = 20 nM, Figure 2B]. Of note in these studies is that both **8** and WIN55212-2 **(1)** showed a significantly greater potency at displacing agonist [^{131}I](*R*)-**8** binding than at displacing antagonist [^{3}H]SR141716A binding. This is consistent with previous studies where WIN55212-2 **(1)** and other cannabinoid agonists were more potent at displacing the binding of a radiolabeled agonist than at displacing binding of the antagonist [^{3}H]SR14176A.[20] These data were confirmed by use of the superfused rat hippocampal synaptosomal model previously described.[21] Compound **8** (racemic) produced the same maximal inhibition of calcium-stimulated acetylcholine release as WIN55212-2 **(1)** with slightly greater potency.[22] Furthermore, when injected intravenously in mice, compound **8** induced a marked catalepsy and inhibition of

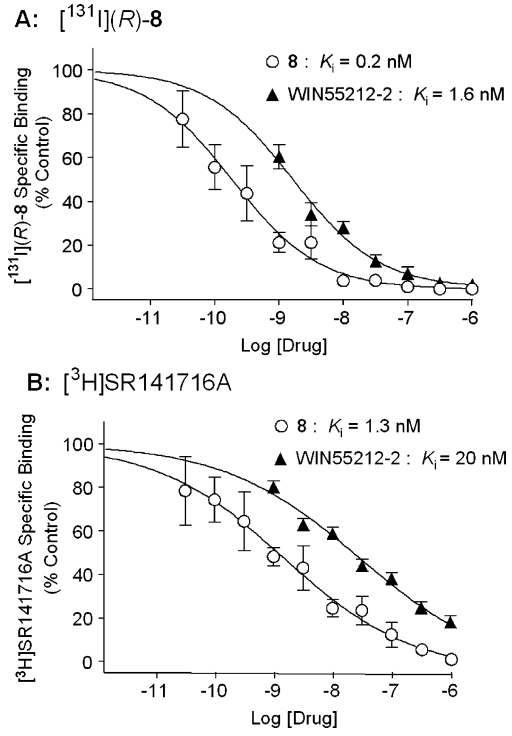


Figure 2. Displacement of (A) [^{131}I](*R*)-8 and (B) [^{3}H]-SR141716A in mouse brain homogenates by racemic compound **8** and WIN55212-2 (**1**). Data points are means ± SEM of four separate experiments.

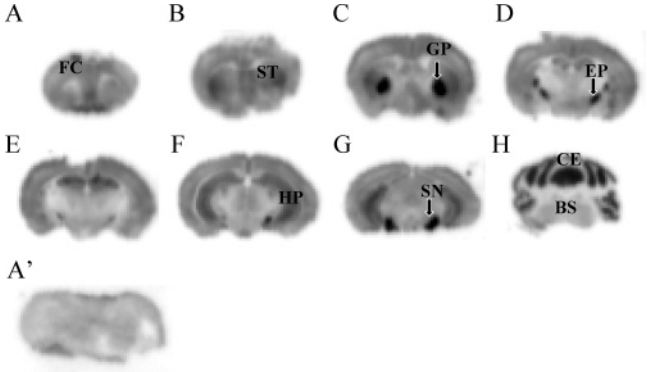


Figure 3. In vitro autoradiography with [^{131}I](*R*)-8 in mouse brain sections. Sections were approximately at the following levels relative to the interaural line (mm): (A) 5.5, (B) 5.2, (C) 3.5, (D) 2.1, (E) 1.8, (F) 1.1, (G) 0.7, (H) −2.0. Section A′ was cut at the level of the hippocampus from a CB1 receptor knockout mouse and incubated with [^{131}I](*R*)-8. Abbreviations: BS, brain stem; CE, cerebellum; GP, globus pallidus; EP, entopeduncular nucleus; FC, frontal cortex; HP, hippocampus; SN, substantia nigra; ST, striatum.

activity interspersed with brief bouts of rapid uncoordinated locomotion, as expected for a CB1 receptor agonist.[22]

In Vitro Autoradiography. Radioiodinated **8** was evaluated by in vitro autoradiography on slide-mounted mouse brain sections. [^{131}I](*R*)-**8** gave images consistent with binding to brain CB1 receptors (Figure 3). Binding of [^{131}I](*S*)-**8** in sections was much reduced compared with that of the labeled racemate. Selective binding of [^{131}I](*R*)-**8** was absent in sections prepared from a CB1 receptor knockout mouse, confirming that binding in vitro was due to CB1 receptors.

Conclusions

The present study describes the synthesis of a potent iodinated aminoalkylindole cannabinoid receptor ligand **8** and presents binding in rat forebrain (CB1) and mouse hippocampal (CB1) homogenates as well as mouse brain autoradiographic data documenting the suitability of this radioiodinated compound for biological studies. The lower K_i value (0.2 nM) measured for **8** binding against [^{131}I](*R*)-**8** versus that (1.3 nM) measured for **8** binding against antagonist [^{3}H]SR141716A, and the similarity in the binding behavior of **8** to that of WIN55212-2 **(1)**, suggest that **8** is a CB1 receptor agonist. The more active (*R*)-enantiomer of **8** possesses a much higher affinity for the CB1 receptor in mouse than the commonly used tritiated radioligand WIN55212-2 **(1)**. The availability of the (*S*)-enantiomer of **8** may in some circumstances provide a convenient way of evaluating the nonspecific binding of (*R*)-**8**, since it has a 300-fold lower affinity for the CB1 receptor (Table 1). The lack of selectivity between CB1 and CB2 receptors would not limit the usefulness of radioiodinated (*R*)-**8** in tissues containing both receptors, provided that highly selective nonradioactive receptor blocking drugs (e.g., SR141716A and SR144528 for CB1 and CB2, respectively) are added to incubation media. For studies of CB1 receptors in the central nervous system, the high affinity toward CB2 receptors should not be problematical since the latter are absent in the brain.[23]

While our study was in progress, Tamagnan et al.[24] reported the synthesis of an analogue of WIN55212-2 **(1)** with an iodine atom at the 4′ position. The affinities of this compound for CB1 and CB2 receptors were much lower (190 nM and 11 nM, respectively) than those of the parent compound WIN55212-2 (**1**).

Experimental Section

General Methods. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on either a Bruker WP-211SY 200 MHz or a Bruker DMX-500 500 MHz spectrometer. Chemical shifts are reported in ppm (parts per million) relative to tetramethylsilane as the internal standard, and signals are quoted as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), t (triplet), q (quartet), or m (multiplet). High-resolution mass spectra (HRMS) were recorded on a Kratos MS-902 instrument at 70 eV. Elemental analyses were performed by Baron Consulting Company (Milford, CT). WIN55212-2 (**1**) was purchased from Tocris Cookson, Inc. (Ballwin, MO). [^{3}H]SR141716A (46 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Swiss Webster mice were obtained from Taconic Farms (Germantown, NY). CB1 knockout mice were bred from a strain developed by Drs. C. Ledent and M. Parmentier (Universite Libre de Bruxelles, Belgium).

Naphthalen-1-yl-(6-nitro-1*H*-indol-3-yl)methanone (9). 6-Nitroindole (1.62 g, 10 mmol) suspended in ethyl ether (15 mL, freshly distilled from sodium) was added with stirring to a solution of methylmagnesium bromide (3.5 mL, 3 M solution in ethyl ether, 10.5 mmol) at 0 °C. After 15 min of stirring, $ZnCl_2$ (10 mL, 1 M solution in ethyl ether) was added and the mixture was continuously stirred for 30 min. Then to this reaction mixture was added, dropwise, 1-naphthoyl chloride (2.0 g, 10.5 mmol) in ethyl ether (2 mL) followed by stirring for another 2 h. Workup was with aqueous ammonium chloride and the resulting mixture was extracted with dichloromethane. The organic layer was dried over anhydrous Na_2SO_4, and solvent was removed by rotary evaporation under vacuum. The residue was chromatographed twice on silica gel columns. The

first column was eluted with petroleum ether/ethyl acetate (5:1∼2:1 v/v) and the second column was eluted with petroleum ether/acetone (3:1 v/v) to provide **9** as a yellow solid (871 mg, 28%). Recrystallization from ethyl acetate afforded **9** as yellow crystals, mp 2−3 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51−7.60 (m, 2H), 7.66 (d, J = 7.5 Hz, 1H), 7.76 (d, J = 6.8 Hz, 1H), 8.04 (t, J = 6.1 Hz, 2H), 8.10 (s, 1H), 8.14 (d, J = 8.7 Hz, 1H), 8.18 (d, J = 8.7 Hz, 1H), 8.44 (s, 1H), 8.46 (d, J = 8.8 Hz, 1H), 12.68 (br s, 1H). Anal. ($C_{19}H_{12}N_2O_3$) C, H, N.

[1-(1-Methylpiperidin-2-ylmethyl)-6-nitro-1*H*-indol-3-yl]naphthalen-1-ylmethanone (10). To a suspension of **9** (1.48 g, 4.68 mmol) and NaH (281 mg, 60% in mineral oil, 7.02 mmol) in *N*,*N*-dimethylformamide (DMF; 5 mL) was added dropwise a solution of 2-chloromethyl-1-methylpiperidine (691 mg, 4.68 mmol; see Supporting Information) in DMF (5 mL). The reaction was heated at 60∼70 °C overnight and then diluted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4, and the ethyl acetate was removed by rotary evaporation under vacuum. The residue was chromatographed twice on silica gel flash columns; the first column was eluted with toluene/triethylamine (90:10 v/v) and the second column was eluted with petroleum ether/ethyl acetate/triethylamine (3.5:1:0.3 v/v/v) to afford **10** as a yellow solid (863 mg, 44%), mp 194−195 °C (recrystallization from ethanol/hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12 (m, 2H), 1.25 (m, 1H), 1.46 (m, 1H), 1.61 (m, 2H), 2.14 (t, J = 10.5 Hz, 1H), 2.40 (s, 4H), 2.86 (m,1H), 4.02 (dd, J = 8.2 and 14.3 Hz, 1H), 4.53 (dd, J = 4.0 and 14.2 Hz, 1H), 7.52 (m, 3H), 7.67 (d, J = 6.0 Hz, 1H), 7.69 (s, 1H), 7.93 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 8.5 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 8.36 (s, 1H), 8.54 (d, J = 8.7 Hz, 1H). Anal. ($C_{26}H_{25}N_3O_3$) C, H, N.

[6-Amino-1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]naphthalen-1-ylmethanone (11). To a solution of **10** (863 mg, 2.02 mmol) and hydrazine (0.41 mL) in ethanol (80 mL) was added a suspension of Raney Ni (wet, ∼90 mg). The resulting suspension was stirred vigorously at room temperature for 1 h and was then carefully filtered. The solvent was removed by rotary evaporation under vacuum and the residue was recrystallized from ethyl acetate/hexane to afford **11** as a pale solid (665 mg, 83%), mp 198−199 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.06 (m, 3H), 1.26 (br s, 1H), 1.58 (m, 2H), 2.11 (m, 1H), 2.33 (br s, 1H), 2.38 (s, 3H), 2.83 (m, 1H), 3.70 (dd, J = 9.3 and 14.0 Hz, 1H), 4.43 (dd, J = 4.1 and 14.0 Hz, 1H), 6.40 (d, J = 1.7 Hz, 1H), 6.78 (dd, J = 1.7 and 8.4 Hz, 1H), 7.17 (s, 1H), 7.45−7.55 (m, 3H), 7.66 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 7.3 Hz, 1H), 7.96 (dd, J = 8.0 Hz, 1H), 8.19 (br s, 1H), 8.25 (d, J = 8.2 Hz, 1H). Anal. ($C_{26}H_{27}N_3O\cdot1.25H_2O$) C, H, N.

[6-Iodo-1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]naphthalen-1-ylmethanone (3). A solution of **11** (449 mg, 1.13 mmol) in 3 N HCl (2.4 mL) was stirred at room temperature for 1 h and then cooled to 0 °C, and 1 M $NaNO_2$ (1.4 mL) was added slowly. The reaction mixture was continuously stirred for another 1 h followed by the addition of sulfamic acid until starch-KI test paper did not change to blue. KI (750 mg, 4.52 mmol) was added with stirring at 0 °C for 30 min and then at room temperature for 1 h. The reaction was quenched by the addition of saturated aqueous Na_2CO_3. After extraction with dichloromethane, the organic layer was dried over Na_2SO_4 and the solvent was removed by rotary evaporation under vacuum. The residue was purified by chromatography on a silica gel column eluting with toluene/diisopropylamine (10:1 v/v) and afforded **3** as a pale solid (350 mg, 61%), mp 204−205 °C (recrystallization from ethyl acetate/hexane). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (br s, 2H), 1.23 (br s, 1H), 1.47 (m, 1H), 1.60 (m, 2H), 2.13 (m, 1H), 2.33 (br s, 1H), 2.38 (s, 3H), 2.83 (m, 1H), 3.82 (dd, J = 8.9 and 13.9 Hz, 1H), 4.43 (dd, J = 3.6 and 13.9 Hz, 1H), 7.32 (s, 1H), 7.52 (m, 3H), 7.63 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 6.9 Hz, 1H), 7.75 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.20 (d, J = 8.7 Hz, 1H). Anal. ($C_{26}H_{25}IN_2O$) C, H, N: calcd, C 61.43, H 4.96, N 5.51; found, C 61.90, H 5.44, N 5.54.

[1-(1-Methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]-4-nitronaphthalen-1-ylmethanone (13). The crude 4-nitro-1-naphthalic acid (**12**) (0.52 g, 2.4 mmol) prepared from 4-nitro-1,8-naphthalic anhydride[13] was treated with thionyl chloride (5 mL) and heated at reflux for 3 h. After removal of the excess amount of $SOCl_2$, the residue was transferred to a suspension of aluminum chloride in dichloromethane, which had been stirred at room temperature for 15 min. The suspension was stirred for another 30 min and then was added to a solution of racemic 1-(1-methylpiperidin-2-ylmethyl)-1*H*-indole (**18**) (0.54 g, 2.4 mmol; see Supporting Information) in dichloromethane. The reaction mixture was stirred at 55 °C for 6 h followed by the addition of 2 N NaOH until no further precipitate formed. The mixture was extracted with dichloromethane and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by flash column chromatography (silica gel, toluene/diisopropylamine (10:1 v/v) and recrystallization from ethanol to afford **13** (360 mg, 35%), mp 174−175 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04−1.26 (m, 3H), 1.49−1.60 (m, 3H), 2.11 (m, 1H), 2.37 (br s, 4H), 2.84 (m, 1H), 3.85 (dd, J = 9.0 and 14.1 Hz, 1H), 4.50 (dd, J = 4.1 and 14.1 Hz, 1H), 7.32 (s, 1H), 7.40 (m, 3H), 7.59 (t, J = 7.4 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.74 (t, J = 7.8 Hz, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 7.5 Hz, 1H), 8.46 (br s, 1H), 8.55 (d, J = 8.7 Hz, 1H). Anal. ($C_{26}H_{25}N_3O_3$) C, H, N.

4-Aminonaphthalen-1-yl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (14). A mixture of **13** (320 mg, 0.468 mmol), hydrazine (63.3 μM, 2.02 mmol), Raney nickel (wet, 12 mg), and ethanol (10 mL) was stirred at room temperature for 3 h. The insoluble material was removed by filtration and the filtrate was concentrated by rotary evaporation under vacuum to afford the crude product (170 mg, 91%), which was used for the next reaction without further purification. The analytical sample was obtained by flash column chromatography (silica gel; CH_2Cl_2/MeOH, 10:1 v/v) followed by recrystallization from ethyl acetate/hexane, mp 75−76 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.04−1.35 (m, 3H), 1.39−1.62 (m, 3H), 2.04−2.40 (m, 1H), 2.43 (br s, 4H), 2.83 (m, 1H), 3.85 (dd, J = 9.0 and 14.1 Hz, 1H), 4.57 (dd, J = 4.1 and 14.1 Hz, 1H), 5.45 (br s, 2H), 6.77 (d, J = 7.8 Hz, 1H), 7.31−7.41 (m, 3H), 7.45 (s, 1H), 7.49 (d, J = 3.1 Hz, 1H), 7.52 (d, J = 3.2 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.87 (dd, J = 3.0 and 6.5 Hz, 1H), 8.43 (m, 2H). Anal. ($C_{26}H_{27}N_3O\cdot0.56C_4H_8O_2$) C, H, N: calcd, C 75.91, H 7.10, N 9.40; found, C 75.94, H 7.57, N 9.23.

4-Iodonaphthalen-1-yl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (4). With **14** (170 mg, 0.43 mmol) as starting material, **4** was prepared analogously to the synthesis of **3**. The crude sample was purified by flash chromatography (silica gel; toluene/triethylamine, 100:4 v/v) to afford **4** as a white solid (121 mg, 56%), mp 161.5−162.0 °C (recrystallization from ethanol). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.04−1.35 (m, 3H), 1.39−1.62 (m, 3H), 2.04−2.40 (m, 1H), 2.40−2.42 (s, 4H), 2.84 (m, 1H), 3.78−3.89 (m, 1H), 4.47−4.56 (m, 1H), 7.31−7.64 (m, 8H), 8.17−8.21 (m, 2H), 8.45−8.48 (m, 1H). Anal. ($C_{26}H_{25}IN_2O$) C, H, N.

2-Iodophenyl-[2-methyl-1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (5). To anhydrous dichloromethane (10 mL) was added aluminum chloride (2.0 g, 15 mmol), and the mixture was stirred for 15 min. To this suspension was slowly added a solution of 2-iodobenzoyl chloride (1.8 g, 6.75 mmol) in dichloromethane (5 mL), and stirring was continued for another 30 min. Compound **17** (1.48 g, 6.0 mmol; see Supporting Information) in dichloromethane (10 mL) was then added to the above mixture carefully in order to maintain the reaction temperature below 40 °C. The reaction was stirred overnight before being quenched with 2 N sodium hydroxide. The organic layer was separated, washed with brine, dried over sodium sulfate, and the solvent was removed by rotary evaporation under vacuum to give the crude product, which was further purified by silica gel chromatography (toluene/triethylamine, 10:1 v/v) to afford **5** as a white solid (2.0 g, 83%), mp 153−154 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.12−1.29 (m, 3H), 1.59−1.72 (m, 3H), 2.19 (m, 1H), 2.47 (m, 1H), 2.55 (s, 3H), 2.60 (s, 3H), 2.95 (m, 1H), 4.07 (dd, J = 14.2 and 10.5 Hz, 1H), 4.59 (dd, J = 13.7 and 4.3 Hz, 1H), 7.10 (t,

J = 7.3 Hz, 1H), 7.15−7.24 (m, 3H), 7.35−7.40 (m, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.97 (d, J = 7.9 Hz, 1H). HRMS ($C_{23}H_{25}IN_2O$): calcd 472.1012, found 472.1010. Anal. ($C_{23}H_{25}IN_2O$) C, H, N.

The resolution of the enantiomers of **5** was performed on a Beckman HPLC system with a Chiralcel OD column (10 mm × 250 mm) as the stationary phase and 12% ethanol in hexane as the mobile phase (flow rate = 2.0 mL/min). Compound **5** (50 mg) was dissolved in ethanol (1.0 mL), and 0.1 mL was used per injection. The compounds were detected by UV absorption at 254 nm. The (+)-enantiomer with a retention time of 46 min showed $[\alpha]_D^{26.5}$ = +43.89° (c = 0.157 in EtOH), and the (−)-enantiomer with a retention time of 51 min showed $[\alpha]_D^{26.5}$ = − 43.14° (c = 0.105 in EtOH).

3-Iodophenyl-[2-methyl-1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (6). Compound **6** was prepared from **17** and 3-iodobenzoyl chloride analogously to the synthesis of **5** and was a white solid (1.67 g, 59%) after recrystallization from ethyl acetate, mp 168.5−169 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.12−1.29 (m, 3H), 1.59−1.72 (m, 3H), 2.22 (m, 1H), 2.43 (m, 1H), 2.50 (s, 3H), 2.67 (s, 3H), 2.92 (m, 1H), 4.10 (dd, J = 10.8 and 10.8 Hz, 1H), 4.55 (dd, J = 14.4 and 4.1 Hz, 1H), 7.23 (t, J = 7.7 Hz, 1H), 7.29 (t, J = 8.5 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.76 (d, J = 7.5 Hz, 1H), 7.87 (s, 1H), 7.90 (m, 1H), 8.09 (d, J = 8.3 Hz, 1H). HRMS ($C_{23}H_{25}IN_2O$): calcd 472.1012, found 472.1009. Anal. ($C_{23}H_{25}IN_2O$) C, H, N.

4-Iodophenyl-[2-methyl-1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (7). Compound **7** was prepared from **17** and 4-iodobenzoyl chloride analogously to **5** and was a white solid (63 mg, 75%) after recrystallization from ethyl acetate, mp 160−161 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90−1.40 (m, 7H), 2.05 (m, 1H), 2.50 (s, 3H), 2.55 (s, 3H), 2.90 (m, 1H), 3.95 (dd, J = 14.0 and 9.0 Hz, 1H), 4.55 (dd, J = 14.0 and 4.0 Hz, 1H), 7.05 (t, J = 8.0 Hz, 1H), 7.25 (t, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 8.0 Hz, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H). HRMS ($C_{23}H_{25}N_2OI$): calcd 472.1012, found 472.1005.

2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone (8). Compound **8** was prepared from **18** and 2-iodobenzoyl chloride analogously to the synthesis of **5** in 93% yield and was a white solid. Recrystallization was performed from ethanol to provide cubic crystals, mp 146−147 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15−1.30 (m, 3H), 1.60−1.69 (m, 3H), 2.15 (td, J = 12.1 and 3.2 Hz, 1H), 2.42 (m, 1H), 2.44 (s, 3H), 2.83 (m, 1H), 3.87 (dd, J = 9.3 and 14.2 Hz, 1H), 4.56 (dd, J = 14.0 and 4.2 Hz, 1H), 7.14−7.21 (m, 1H), 7.31−7.49 (m, 6H), 7.94 (d, J = 8.0 Hz, 1H), 8.30 (br s, 1H). Anal. ($C_{22}H_{23}IN_2O$) C, H, N.

***(R)*-(+)-2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone [(*R*)-(+)-8]**. With (*R*)-(+)-1-(1-methylpiperidin-2-ylmethyl)-1*H*-indole [(*R*)-(+)-**18**; see Supporting Information] and 2-iodobenzoyl chloride as starting materials, (*R*)-(+)-**8** was prepared analogously to the synthesis of racemic **8** as a gel-like material (81.5 mg, 95%), $[\alpha]_D^{25}$ = +43.48° (c 0.133, EtOH).

***(S)*-(−)-2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone [(*S*)-(−)-8].** Compound (*S*)-(−)-8 was obtained by chiral HPLC resolution of racemic compound 8.[14]

[1-(1-Methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]-2-(tributylbutylstannylphenyl)methanone (19). Dried nitrogen gas was bubbled through a solution of **8** (9.2 mg, 0.02 mmol) in anhydrous toluene (2.5 mL) for 15 min. Hexabutylditin (23.7 μL, 0.047 mmol) was added through a syringe, followed by the addition of tetrakistriphenylphosphine palladium [$Pd(PPh_3)_4$, 0.70 mg, 0.00061 mmol]. The reaction mixture was heated at 120 °C for 18 h and then purified by chromatography (silica gel; toluene/triethylamine, 15:1 v/v) to afford **19** as a semisolid (11.6 mg, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.77 (t, J = 7.4 Hz, 9H), 1.02 (m, 6H), 1.19−1.30 (m, 8H), 1.31 (br s, 1H), 1.43−1.49 (m, 7H), 1.50−1.69 (m, 2H), 2.16 (dt, J = 11.5 and 2.7 Hz, 1H), 2.42 (br s, 1H), 2.47 (s, 3H), 2.89 (m, 1H), 3.92 (dd, J = 9.1 and 14.1 Hz, 1H), 4.60 (dd, J = 14.1 and 4.1 Hz, 1H), 7.25−7.41 (m, 4 H), 7.49 (t, J = 7.3 Hz, 1H), 7.59 (s, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.74 (d, J = 7.2 Hz, 1H), 8.34 (d, J = 7.1 Hz, 1H). HRMS ($C_{34}H_{50}N_2OSn$): calcd 623.3023 (MH^+), found 623.3023.

***(R)*-(+)-[1-(1-Methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]-2-(tributylstannylphenyl)methanone [(*R*)-(+)-19].** Compound (*R*)-(+)-**19** was prepared from (*R*)-(+)-**8** analogously to the synthesis of racemic **19** in 60% yield. Additional purification was performed on a HPLC system: Phenomenex normal-phase semipreparative column (10 mm × 250 mm) with ethanol/heptane (15:85) as the mobile phase (flow rate = 4 mL/min) and UV detection at 254 nm. HRMS ($C_{34}H_{50}N_2OSn$): calcd 623.3023 (MH^+), found 623.3023.

***(S)*-(−)-[1-(1-Methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]-2-(tributylstannylphenyl)methanone [(*S*)-(−)-19].** Compound (*S*)-(−)-**19** was prepared from (*S*)-(−)-**8** analogously to the synthesis of racemic **19** in 64% yield. Additional purification was performed by HPLC: Phenomenex normal-phase semipreparative column (10 mm × 250 mm), ethanol/heptane (15:85), UV detection at 254 nm. HRMS ($C_{34}H_{50}N_2OSn$): calcd 623.3023 (MH^+), found 623.3028.

[^{131}I]2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone ([^{131}I]8). Fifty microliters of the tributyltin precursor (**19**, 1 mg/mL in ethanol), 10 μL of chloramine-T (0.2 mg/mL), and 10 μL of 0.5 M HCl were added to 2 mCi of sodium [^{131}I]iodide (Dupont NEN) in a sealed reaction vial. After 5 min the reaction mixture was injected directly into a HPLC equipped with an Econosil C-18 column, and the reaction products were separated by a mobile phase consisting of 60% acetonitrile and 40% aqueous ammonium formate (2 g/L). Fractions containing the radiolabeled [^{131}I]**8** were pooled and the radioactivity was extracted into ether. The ether was then evaporated to dryness and the product was redissolved in a small quantity of ethanol to give [^{131}I]**8** in 44% radiochemical yield and >97% radiochemical purity.

[^{131}I](*R*)-2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]methanone ([^{131}I](*R*)-8). [^{131}I](*R*)-**8** was prepared from [^{131}I](*R*)-**19** analogously to the method for [^{131}I]**8** in 62% yield and >97% radiochemical purity.

[^{131}I](*S*)-2-Iodophenyl-[1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl]-m ethanone ([^{131}I](*S*)-8). [^{131}I](*S*)-**8** was prepared from [^{131}I](*S*)-**19** analogously to the method for [^{131}I]**8** in 59% yield and >97% radiochemical purity.

Radioligand Binding Assays. Radioligand binding assays were conducted with rat forebrain (CB1), mouse spleen (CB2), and mouse hippocampal (CB1) homogenate assay systems.

[^{3}H]CP-55,940 in Rat Forebrain and Mouse Spleen Membrane Homogenates. A reported procedure was followed to obtain the K_i values on CB1 and CB2 receptors for the novel iodinated compounds in this study.[25]

[^{3}H]SR141716A and [^{131}I](*R*)-8 in Mouse Hippocampal Membrane Homogenates. Mouse hippocampuses were homogenized in 5 mL of ice-cold buffer containing 50 mM Tris, 5 mM $MgCl_2$, 0.1% ethylenediaminetetraacetic acid (EDTA), and 1% bovine serum albumin, pH 7.4. The homogenate was then centrifuged and the membrane pellet was resuspended in fresh buffer and centrifuged a second time before being resuspended again in ice-cold buffer. Binding assays were conducted in a total incubation volume of 2 mL per tube with approximately 1 mg (original wet weight) of membrane homogenate per tube. Radioligands for the binding assays were [^{131}I](*R*)-**8** (approximately 0.2 μCi/assay tube) and [^{3}H]-SR141716A (0.08 μCi/assay tube). Following incubation at 30 °C for 90 min, the tubes were filtered through GF/B and washed three times with 5 mL of ice-cold buffer containing 0.25% bovine serum albumin. Filters were counted for ^{131}I by scintillation counting. After the ^{131}I was allowed to decay through 4−5 half-lives, the samples were then counted again for ^{3}H. Nonspecific binding was determined by use of 3 μM AM281 and was 2.5−3% of the total binding with [^{131}I]**8** as the radioligand and 16−22% with [^{3}H]SR141716A as the radioligand.

In Vitro Autoradiography. Cryostat cut sections of mouse brain were incubated for 1 h in [^{131}I](*R*)-**8** (1 μCi/mL in 50

mM Tris, 5 mM $MgCl_2$, 0.1% EDTA, and 1% bovine serum albumin, pH 7.4) followed by washing in ice-cold buffer for 1 h. The sections were then dried and apposed to a phosphor imaging plate.

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Supporting Information Available: X-ray crystal structure data for **4** and **8**, the details for the synthesis of **17** and **18**, and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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