Synthesis and Receptor Binding of N-Substituted Tropane Derivatives. High-Affinity Ligands for the Cocaine Receptor§

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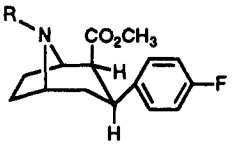
The synthesis and pharmacological characterization of a series of N-substituted 3-(4-fluorophenyl)tropane derivatives is reported. The compounds displayed binding characteristics that paralleled those of cocaine, and several had substantially higher affinity at cocaine recognition sites. Conjugate addition of 4-fluorophenyl magnesium bromide to anhydroecgonine methyl ester gave 2β-(carbomethoxy)-3β-(4-fluorophenyl)tropane (**4a**, designated CFT, also known as WIN 35,428) after flash chromatography. N demethylation of **4a** was effected by Zn/HOAc reduction of the corresponding 2,2,2-trichloroethyl carbamate to give 2β-carbomethoxy-3β-(4-fluorophenyl)nortropane (**5**), which was alkylated with allyl bromide to afford the *N*-allyl analogue, **6**. The *N*-propyl analogue, **7**, was prepared by catalytic reduction (Pd/C) of **6**. The most potent analogue, **4a**, was tritiated at a specific activity of 81.3 Ci/mmol. [^{3}H]**4a** bound rapidly and reversibly to caudate putamen membranes; the two-component binding curve typical of cocaine analogues was observed. Equilibrium was achieved within 2 h and was stable for at least 4 h. High- and low-affinity K_d values observed for [^{3}H]**4a** (4.7 and 60 nM, respectively) were more than 4 times lower than those for [^{3}H]cocaine, and the density of binding sites (B_{max} = 50 pmol/g, high, and 290 pmol/g, low) for the two drugs were comparable. Nonspecific binding of [^{3}H]**4a** was 5–10% of total binding.

The abuse of cocaine is a public health problem of great national concern. Despite the intensive efforts that have been made in this area, there remains a lack of adequate information about the neurochemical mechanisms that mediate cocaine's behavioral effects and abuse liability.[1] Selective, pharmacologically relevant binding sites for [^{3}H]cocaine have been identified in striatal tissue of rodents,[2-5] nonhuman primates,[6] and humans.[7] These sites are associated with the monoamine uptake system and display properties characteristic of a pharmacological receptor. Specifically, the sites bind [^{3}H]cocaine saturably and reversibly,[8,9] they display stereoselectivity for (-)-cocaine versus its enantiomer (+)-cocaine or its C-2 epimer, pseudococaine,[6] and they show a high degree of correspondence between the binding affinity of various cocaine analogues and their relative potencies for inhibiting dopamine uptake[6] and for producing cocaine-like behavioral effects such as behavioral stimulation,[10] intravenous self-administration,[11] and stereotypy.[2]

More detailed characterization and mapping of cocaine binding sites and investigation of their relationship to the dopamine reuptake system has been restricted by the limitations of [^{3}H]cocaine as a radioligand probe of the receptor complex. [^{3}H]Cocaine binds to the receptor with only modest affinity in all brain regions studied: $K_{0.50}$ = 280 nM in striatal tissue. Dissociation from the drug–receptor complex is rapid. The specific activity of commercially available [^{3}H]cocaine is also relatively low (25–30 Ci/mmol). These factors often result in unacceptable variability in replicate experiments and severely limit attempts to identify the receptor in brain regions with low receptor density or in solubilized tissue preparations.

The non-tropane dopamine uptake inhibitors mazindol (**9**) and GBR 12935 (**10**) label elements of the dopamine transport system with high affinity.[12] The population of striatal sites labeled by the agents is similar, but not identical, to those labeled by [^{3}H]cocaine. However, multiple binding components are observed for [^{3}H]cocaine in striatum, while mazindol and GBR 12935 appear to label a single site, or multiple sites with similar affinities. Furthermore, the dopamine uptake blockers do not fully displace [^{3}H]cocaine.[6]

Table I. N-Substituted-2-carbomethoxy-3β-(4-fluorophenyl)tropanes



no.	R	mp, °C	formula	anal.[a]
4a	CH_3	92–94	$C_{16}H_{20}FNO_2$	C,H,N
4a[b]	CH_3	202–204	$C_{26}H_{28}FNO_8S_2 \cdot H_2O$	C,H,S
4b[b]	CH_3	188–190	$C_{26}H_{28}FNO_8S_2 \cdot H_2O$	C,H,S
5	H	114–115	$C_{15}H_{18}FNO_2$	C,H,N
6	$CH_2CH{=}CH_2$	58–59	$C_{18}H_{22}FNO_2$	C,H,N
7[b]	C_3H_7	143–145	$C_{26}H_{32}FNO_8S_2 \cdot H_2O$	C,H,S

[a] Analytical results were within ±0.4% of theoretical values. [b] 1,5-Naphthalenedisulfonic acid salt.

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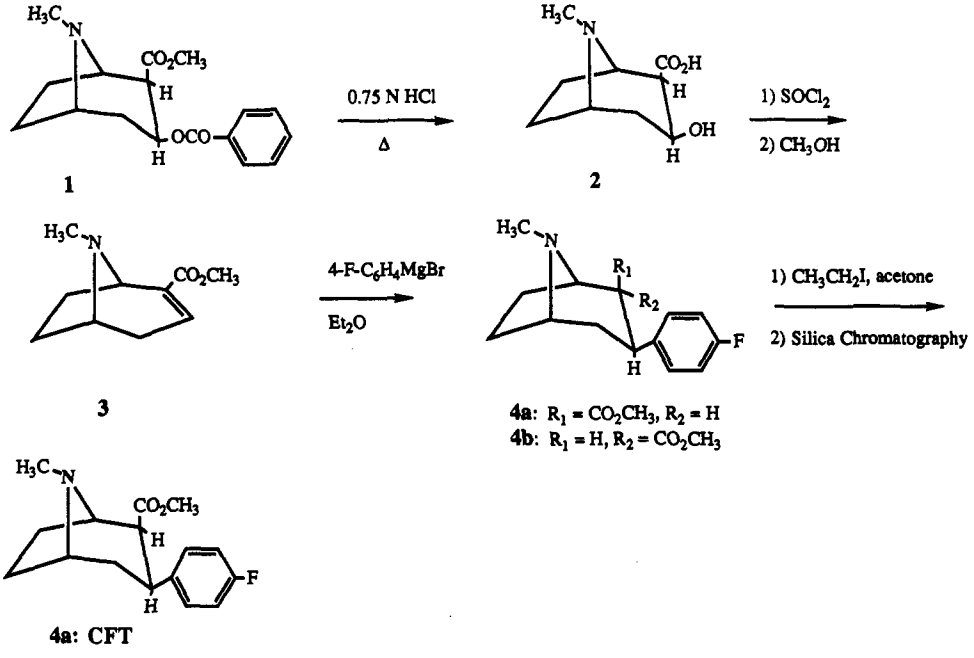
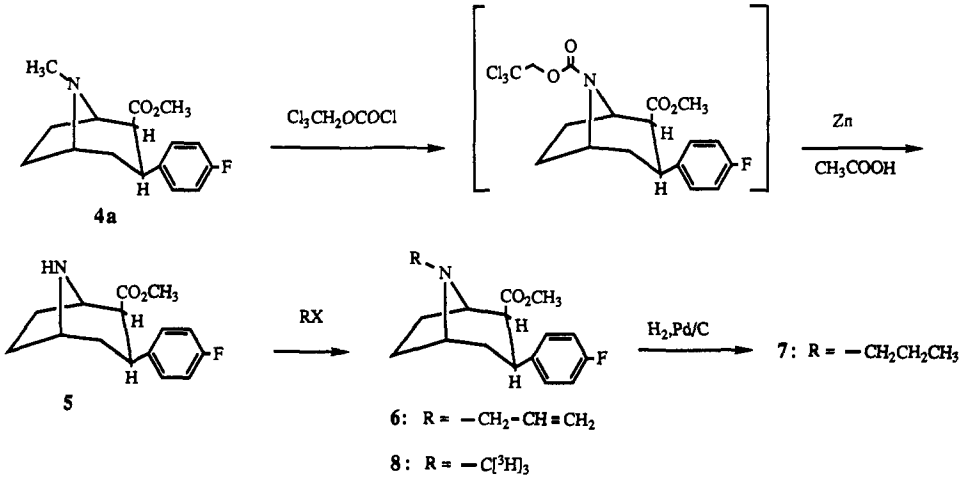
Scheme I. Synthesis of 2β-Carbomethoxy-3β-(4-fluorophenyl)tropane



Scheme II. Synthesis of N-Substituted-(4-fluorophenyl)tropane Derivatives



Cocaine congeners therefore appear to be the most appropriate starting point in the design of improved ligands for cocaine receptors. Clarke and co-workers[15] in 1973 synthesized a series of cocaine analogues lacking the ester function at C-3. Two of these, WIN 35,428 (**4a**, designated CFT) and WIN 35,065-2, were later found to possess potent cocaine-like activity in vivo.[17] We[18] and others[18] have prepared other compounds of this type in search of more potent and selective probes of cocaine receptor structure and function. In this communication, we report the synthesis and pharmacological characterization of a series of novel N-substituted 3-(4-fluorophenyl)tropane derivatives (Figure 1, **6–8**). These analogues have been evaluated at cocaine binding sites in vivo.[16] The compounds displayed binding characteristics that closely paralleled those of cocaine, and all had substantially higher affinity for the cocaine recognition site. The most potent analogue, 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (**4a**) was triti-

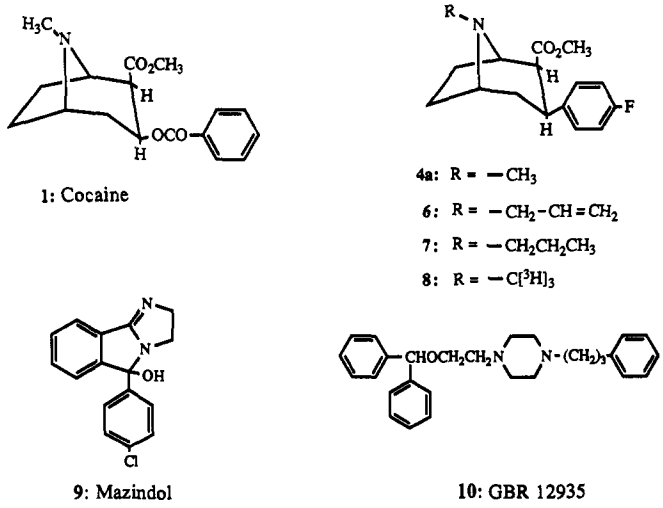


Figure 1.

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ated at a specific activity of 81.3 Ci/mmol.

We report that [^{3}H]**4a** is a superior probe for the cocaine receptor. This radioligand displayed an overall affinity 15 times higher than cocaine ($K_{0.50}$ = 18 vs 280 nM). Furthermore, the dissociation of [^{3}H]**4a** appears to be approximately 100 times slower than cocaine. Its higher affinity and specific activity represent a substantial improvement over [^{3}H]cocaine.

Chemistry

The N-substituted (fluorophenyl)tropane analogues **4–8** were synthesized as shown in Schemes I and II. Physical constants are given in Table I. Cocaine HCl (**1**) was hydrolyzed in refluxing 0.75 N HCl to produce ecgonine HCl (**2**) in 81% yield.[15] Treatment of **2** with $POCl_3$ gave anhydroecgonine acid chloride, which was not purified but treated with dry MeOH at 0 °C to give anhydroecgonine methyl ester[15] (**3**) in 60% yield after bulb-to-bulb distillation. Conjugate addition of 4-fluorophenyl magnesium bromide afforded a 4:1 (equatorial:axial) mixture of the C-2 epimers (**4a**, **4b**) of 2-carbomethoxy-3β-(4-fluorophenyl)tropane in 70% yield. The C-2 equatorial epimer can be selectively removed by conversion to its N-ethyl quaternary salt, thus permitting a greater than 4-fold enrichment of the desired C-2 axial epimer. Presumably, the axial carbomethoxy group in **4a** prevents access to the tropane nitrogen by the electrophile. Final purification was effected by flash chromatography and subsequent recrystallization to yield the crystalline 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (**4a**). The relative stereochemistry at C-2 and C-3 was confirmed by NMR spectroscopy (300 MHz). Clarke and co-workers[15] investigated a number of C-2-carbomethoxy-C-3-aryltropanes and found, in all cases, that the tropane *N*-methyl resonance for C-2β esters occurred 0.1–0.2 ppm upfield from the corresponding signal of the epimeric C-3α esters. In the present series, the *N*-methyl singlet occurred at 2.24 ppm for **4a** and at 2.43 ppm for **4b**.

N-Demethylation of **4a** was accomplished as outlined in Scheme II. Conversion of **4a** to its 2,2,2-trichloroethyl carbamate followed by reduction (Zn/acetic acid) afforded **5** in 63% yield after flash chromatography. The *N*-allyl analogue (**6**) was prepared by alkylation of **5** with allyl bromide in 47% yield after flash chromatography. Catalytic reduction of **6** over Pd/C afforded the *N*-propyl analogue (**7**) in 93% yield after flash chromatography. Alkylation of **5** with [^{3}H]CH_3I gave [^{3}H]**4a** (structure **8**, Figure 1), specific activity = 81.3 Ci/mmol, after purification by HPLC.

Pharmacology

The binding properties of the (fluorophenyl)tropane analogues **4a**, **5–7** were compared in competition experiments with [^{3}H]cocaine. A summary of these data appear in Table II. Detailed descriptions of these experiments have been described.[16] All four analogues displaced specifically bound [^{3}H]cocaine from caudate putamen membranes of cynomolgus monkeys with affinities exceeding that of cocaine. The two compounds with highest affinities, **4a** and **6** were about 3 times more potent than cocaine. The significantly higher activity of **6** compared with its saturated analogue, **7**, suggests that electronic as well as steric factors must be important in the interaction of tropane nitrogen substituents with the cocaine receptor.

The binding of [^{3}H]**4a** was investigated under conditions comparable to those used to characterize [^{3}H]cocaine binding. These kinetic studies and competition experiments have been described.[14] In summary, [^{3}H]**4a** bound rapidly and reversibly to caudate putamen membranes; equilibrium was achieved within 2 h and was stable for at least 4 h. Two binding components were observed in saturation experiments using increasing concentrations of [^{3}H]**4a**. High- and low-affinity K_d values observed for [^{3}H]**4a** (4.7 and 60 nM, respectively) were more than 4 times lower than those for cocaine, and the density of binding sites (B_{max} = 50 pmol/g, high, and 290 pmol/g, low) for the two drugs were comparable. Nonspecific binding (measured in the presence of 30 μM (−)-cocaine) of [^{3}H]**4a** was 5–10% of total binding.

Table II. Inhibition of Specifically Bound [^{3}H]Cocaine (2.7 nM) by (Fluorophenyl)tropane Analogues of Cocaine[6,16a]

drug	IC_{50}, nM ± SEM	nH ± SEM
cocaine (1)	67.8 ± 8.7	0.54 ± 0.10
CFT (**4a**)	17.5 ± 4.9	0.66 ± 0.10
norCFT (**5**)	36.4 ± 1.5	0.64 ± 0.07
N-allyl-norCFT (**6**)	22.6 ± 2.9	0.65 ± 0.08
N-propyl-norCFT (**7**)	43.0 ± 17.7	0.70 ± 0.09

[a] IC_{50} values and pseudo-Hill coefficients (nH) are means ±SEM from three or four independent experiments performed in triplicate.

Because of its high affinity, low dissociation, and high specific activity, [^{3}H]**4a** appears to be the radioligand of choice for the study of cocaine receptors. The IC_{50} of [^{3}H]**4a** is nearly 4 times lower that that of [^{3}H]cocaine, and the specific activity of the tritiated ligand is nearly 3 times higher than that attainable for [^{3}H]cocaine. The pharmacological specificity of **4a** binding sites closely parallels that of cocaine, and the rank order of potency of cocaine congeners and other dopamine uptake inhibitors at [^{3}H]**4a** binding sites is identical with their order of potency at [^{3}H]cocaine binding sites.[14] The pseudo-Hill coefficients are also consistent with multiple binding components. These data further confirm the close parallel between cocaine and **4a** binding. The availability of [^{3}H]**4a** should facilitate further studies on the molecular characterization of the cocaine receptor and ultimately provide greater insight for the broader issues of cocaine dependence and abuse.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on Varian XL-300 or T-60A spectrometers, with TMS an internal reference. Mass spectra were obtained on a Varian EM-360 spectrometer. Elemental analyses, performed by Atlantic Microlabs, Atlanta, GA, were within ±0.4% of theoretical values. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Cocaine HCl was obtained from Research Biochemicals Incorporated, Natick, MA. Ethyl iodide, *p*-fluoromagnesium bromide, 2,2,2-trichloroethyl chloroformate, allyl bromide, palladium on carbon, and naphthalene-1,5-disulfonic acid were obtained from Aldrich Chemical Company, Milwaukee, WI. [^{3}H]Cocaine was purchased from DuPont/NEN Research Products, Boston, MA. Thin-layer chromatograms employed Whatman PE SIL G/UV 250-μm plates; visualization was performed by illumination with UV light (254 nm), exposure to I_2 vapor, or spraying with Dragendorf reagent. **4a** and **5** were analyzed by HPLC by using isocratic elution with mixtures of acetonitrile and 0.05 M K_2HPO_4. HPLC apparatus consisted of a Rainin Rabbit-HP pump, Rheodyne injector, Waters μbondapak C-18 column, 3.9 × 300 nm, and LDC Spectromonitor II variable wavelength UV detector. Silica and organic solvents were obtained from VWR, Bedford, MA. Solvents were distilled and/or dried as required.

2β-Carbomethoxy-3β-(4-fluorophenyl)tropane (4a, CFT). The procedure of Clarke et al.[15] was followed with minor modifications. A 2 M ethereal solution of *p*-fluorophenylmagnesium bromide (83 mL, 166 mmol) in a 500-mL 3-neck round-bottom flask equipped with mechanical stirrer, addition funnel, and nitrogen inlet tube was diluted with 83 mL of anhydrous diethyl

ether and cooled to −20 °C under an atmosphere of dry nitrogen. A solution of anhydroecgonine methyl ester (**3**), prepared from cocaine (**1**) by the procedure previously described[15] (15 g, 82.8 mmol) in anhydrous ether (75 mL) was added dropwise. The heterogeneous mixture was stirred for 1 h at −20 °C, then poured into an equal volume of ice and water, and acidified by the dropwise addition of 2 M HCl. The aqueous layer was made basic by the addition of concentrated ammonium hydroxide, saturated with NaCl, and extracted with diethyl ether. The combined extracts were dried (Na_2SO_4) and concentrated in vacuo to give a brown viscous oil. Bulb-to-bulb distillation (70 °C, 0.9 Torr) of the crude product gave a pale yellow oil (16 g, 70%). TLC analysis of the oil (silica, pentane/diethyl ether/2-propylamine, 15:5:0.8) showed it to be a mixture of the C-2 epimers in a ratio of approximately 1:4 (axial/equatorial). The mixture could be substantially enriched in the desired C-2 axial epimer by selective quaternization of the equatorial epimer. Thus, stirring the product mixture with a full molar equivalent of ethyl iodide in dry acetone at 40 °C resulted in the precipitation of nearly pure *N*-ethyl 2α-carbomethoxy-3β-(4-fluorophenyl)troponium iodide, which was removed by filtration. The filtrate, which contained chiefly the 2β epimer, contaminated by less than 10% of the 2α epimer and two unidentified trace contaminants, was purified by flash chromatography (silica, pentane/diethyl ether/2-propylamine, 7:3:0.3). Crystallization from THF afforded 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (**4a**, 2.7 g, 11% from **3**): mp 92–94 °C (lit.[15] 94–96 °C); NMR (300 MHz, $CDCl_3$) 7.25 (2 H, m), 6.95 (2 H, m), 3.60 (1 H, m, C-3α), 3.51 (3 H, s, CO_2CH_3), 3.39 (1 H, br d, C-2α), 2.24 (3 H, s, NCH_3). Anal. C, H, N (Table I). The naphthalene-1,5-disulfonic acid salt (1:1) of **4a** was prepared and recrystallized from acetonitrile to give white needles, mp 202–204 °C. Anal. C, H, S (Table I).

2α-Carbomethoxy-3β-(4-fluorophenyl)tropane (4b). The C-2α epimer of **4a** (**4b**) was recovered from the Grignard reaction mixture by silica chromatography as described above. Crystallization from THF gave **4b**: NMR (300 MHz, $CDCl_3$) 7.25 (2 H, m), 7.00 (2 H, m), 3.55 (s, CO_2CH_3), 3.44 (1 H, br d, C-2β), 3.28 (1 H, m, C-3α), 2.43 (3 H, s, NCH_3). The naphthalene-1,5-disulfonic acid salt (1:1) of **4b** was prepared and crystallized from acetonitrile to give a white powder, mp 90–92 °C (lit.[15] 85–93 °C). Anal. C, H, S (Table I).

2β-Carbomethoxy-3β-(4-fluorophenyl)nortropane (5). A solution of **4a** (410 mg, 1.5 mmol) in 20 mL of toluene was treated with 1 mL of 2,2,2-trichloroethyl chloroformate (7.3 mmol). The mixture was heated at 120 °C for 1 h, cooled to room temperature, and evaporated to dryness in vacuo. The residue was partitioned between methylene chloride and water. The organic layer was separated, dried (Na_2SO_4), and concentrated in vacuo to give the trichloroethyl chloroformate as a dry foam. The crude carbamate was dissolved in 50% aqueous acetic acid, treated with 200 mg (0.0067 g-atom) of zinc dust, and stirred at room temperature for 16 h. The reaction mixture was filtered adjusted to pH 7 with concentrated ammonium hydroxide, saturated with NaCl, and extracted with diethyl ether. The extracts were combined, dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash chromatography (silica, pentane/diethyl ether/2-propylamine, 3:7:0.7) to afford 247 mg (63%) of **5** as a white solid: mp 114–115 °C: MS ($C_{15}H_{18}FNO_2$) *m/z* 263 (M^+); $[\alpha]_D$ −108.8° (*c* = 1, CH_2Cl_2); NMR (300 MHz, $CDCl_3$) 7.21 (2 H, m), 7.00 (2 H, m), 3.75 (2 H, m), 3.40 (3 H, s). Anal. C, H, N (Table I).

2β-Carbomethoxy-3β-(4-fluorophenyl)-8-allylnortropane (6). Allyl bromide (47 μL, 0.54 mmol) was added to a stirred solution of **5** (128 mg, 0.48 mmol) in ethanol (5 mL) in the presence of a catalytic amount of KI. The reaction mixture was stirred at room temperature for 16 h, then diluted with 10 volumes of water, and extracted with methylene chloride. The combined extracts were dried (Na_2SO_4) and concentrated in vacuo. The residual oil was purified by flash chromatography (silica, pentane/diethyl ether/2-propylamine, 7:3:0.3) to give 70 mg (47%) of **6** as a white solid: mp 58–59 °C; MS *m/z* 303 (M^+); $[\alpha]_D$ −3.0° (*c* = 1, CH_2Cl_2); NMR (60 mHz, $CDCl_3$) 6.5–7.0 (4 H, m), 4.7–5.0 (3 H, m), 3.6 (3 H, s). Anal. C, H, N (Table I).

2β-Carbomethoxy-3β-(4-fluorophenyl)-8-propylnortropane 1,5-Naphthalenedisulfonic Acid Salt (7). A mixture of **6** (32 mg, 0.10 mmol) and 5% Pd/C (20 mg) in ethanol (20 mL) was hydrogenated in a Parr shaker under 10 psi of hydrogen for 4 h. The reaction mixture was filtered through a Celite bed and concentrated in vacuo. The residue was purified by flash chromatography (silica, pentane/diethyl ether/2-propylamine, 7:3:0.3) to afford 30 mg (93%) of **7** as a colorless oil: MS *m/z* 305 (M^+); $[\alpha]_D$ −40° (*c* = 1, CH_2Cl_2); NMR (60 MHz, $CDCl_3$) 6.8–7.3 (4 H, m), 3.5 (3 H, s), 2.9 (2 H, t), 0.9 (3 H, t). The 1,5-naphthalene disulfonic acid salt (1:1) of **7** was prepared and recrystallized from acetonitrile to give a white crystalline solid, mp 143–145 °C. Anal. C, H, S (Table I).

[^{3}H]Cocaine Binding. Brain tissue of adult male and female cynomolgus monkeys (*Macaca facicularis*) was obtained from the brain bank of the New England Regional Primate Research Center. The caudate putamen was dissected from coronal slices and homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.4. The homogenate was centrifuged at 38700*g* for 20 min at 0–4 °C, and the pellet was resuspended in 40 volumes of buffer. This wash procedure was repeated twice. After washing, the membrane suspension (25 mg original wet weight of tissue/mL) was stored in small aliquots at −85 °C until use, generally within 2 weeks. Immediately before assay, the suspension was thawed, diluted to 12 mg/mL in buffer and dispersed with a polytron for 15 s.

The [^{3}H]cocaine binding assay used has been previously described.[6] [^{3}H]Cocaine (28.5 Ci/mmol; DuPont/NEN Research Products) was stored at −20 °C and diluted in buffer immediately prior to assay. Stock solutions of drugs were made in distilled water containing ethanol (up to 20%) and/or HCl (up to 0.01 M), if required. The stock solutions were diluted serially in Tris-HCl buffer to yield 10 or more concentrations, each of which was studied in triplicate in tissue from 3 or 4 individual brains.

Glass tubes (12 × 75 mm) received in the following order: 0.2 mL of 50 nM Tris-HCl buffer containing NaCl and test drug, 0.2 mL of 2.7 nM [^{3}H]cocaine, and 0.2 mL of tissue suspension (12 mg/mL original wet tissue weight). The final concentration of tissue was 4 mg/mL and the final concentration of NaCl was 100 mM in a total volume of 0.6 mL. Tubes were incubated for 60 min at 0–4 °C. Incubation was terminated by rapid filtration on glass fiber filters presoaked in 0.1% ice-cold bovine serum albumin. The filters were washed twice with 5 mL of Tris-HCl buffer at 4 °C under reduced pressure (Brandel Cell Harvester) and were stored overnight at room temperature in LSC vials containing 4 mL of fluor.

Radioactivity was monitored for 5 min by liquid scintillation spectrometry; cpm were converted to dpm following determination of the counting efficiency (45–50%) of each vial by external standardization. Nonspecific binding was defined as the dpm in the presence of an excess concentration (30 μM) of unlabeled (−)-cocaine. Specific binding was defined as the difference between total and nonspecific binding. In the present study, total binding ranged from 500–1000 dpm, and specific binding was approximately 85% of the total binding. Data were analyzed by the EBDA (Equilibrium Binding Data Analysis; Elsevier-Biosoft, U.K.) software program, which calculated IC_{50} values and pseudo-Hill coefficients (nH).

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Registry No. 1, 50-36-2; **3**, 50373-10-9; **4a**, 50370-59-7; **4a** 1,5-disulfonic acid salt, 50372-97-9; **4b**, 50370-57-5; **5**, 131488-15-8; **6**, 127648-29-7; **7**, 127627-50-3; (*p*-fluorophenyl)magnesium bromide, 352-13-6; *N*-ethyl 2α-carbomethoxy-3β-(4-fluorophenyl)troponium iodide, 131437-01-9.

Supplementary Material Available: Analytical data for compounds **4a**, **4b**, **5**, **6**, and **7** (1 page). Ordering information is given on any current masthead page.