Probes for the Cocaine Receptor. Potentially Irreversible Ligands for the Dopamine Transporter

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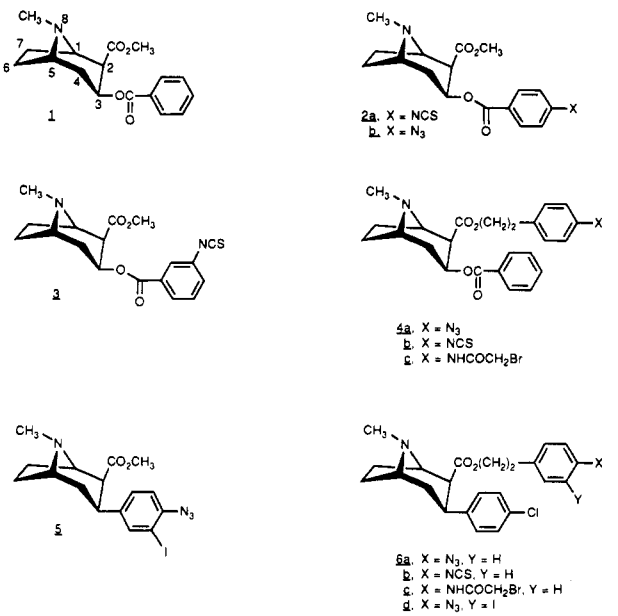
Several potentially irreversible ligands (i.e., wash-resistant binding inhibitors) for the cocaine receptor site on the dopamine transporter, derived from (−)-cocaine or 3β-phenyltropan-2β-carboxylic acid methyl ester (WIN 35,065-2), were prepared and shown to produce wash-resistant inhibition of [^{3}H]-3β-(*p*-fluorophenyl)tropan-2β-carboxylic acid methyl ester ([^{3}H]WIN 35,428) binding. All the compounds prepared had the same absolute configuration as cocaine; they include analogues possessing chemically reactive groups such as the isothiocyanato and bromoacetamido as well as photoactive azido groups. The potentially irreversible ligands, as well as all the intermediates prepared in this study, were evaluated for their ability to inhibit the binding of [^{3}H]WIN 35,428 in coincubation experiments. Of the potentially irreversible ligands, 3β-(*p*-chlorophenyl)tropan-2β-carboxylic acid 2-[*p*-(bromoacetamido)phenyl]ethyl ester (**6c**) had the highest apparent potency. The potentially irreversible ligands were also preincubated, and inhibition of [^{3}H]WIN 35,428 binding was determined both before and after washing the ligand-exposed tissues. The most effective ligands in this regard were 3β-(3-iodo-4-azidophenyl)tropan-2β-carboxylic acid methyl ester (**5**) and 3β-(*p*-chlorophenyl)tropan-2β-carboxylic acid 2-(3-iodo-4-azidophenyl)ethyl ester (**6d**). The structure–activity relationships of these data are discussed.

Progress in the isolation and characterization of the cocaine receptor protein(s) will be aided by the availability of suitable molecular probes. It has been shown by us[1] and others[2] that the dopamine transporter which has been referred to as a "cocaine receptor" is likely to be associated with the reinforcing properties of cocaine. Currently, numerous ligands of several structural types have been reported to bind at the dopamine uptake site, but it remains questionable whether their binding sites are identical to that of cocaine.[3] Therefore, molecular probes based on the molecular framework of cocaine are required to study the molecular sites associated with cocaine binding. Since irreversible ligands can bond covalently to or near the ligand recognition site of a receptor, these agents are particularly valuable as molecular probes. As part of our ongoing cocaine receptor studies, we have described the synthesis and use of *p*- and *m*-isothiocyanatococaines (*p*-isococ, **2a**, and *m*-isococ, **3**, respectively), and of the *p*-azidococaine analogue **2b** for potential covalent bonding to the cocaine receptor.[4,5] More recently, we reported the synthesis and in vitro binding properties of the azide **4a** and of the isothiocyanate and bromoacetamide analogues **4b** and **4c**.[6] In this study, we report the synthesis of the new, potentially irreversible ligands **5** and **6a–d** and describe the wash-resistant binding inhibition of **4a**, **4c**, **5**, and **6a–d**.



Results

Chemistry. All the compounds described in this study were prepared starting from natural cocaine. Thus, all are optically active and possess the same absolute configuration as natural cocaine. The synthesis of 3β-(3-iodo-4-azidophenyl)tropan-2β-carboxylic acid methyl ester (**5**) is shown in Chart I. Iodination of the 3β-(4-aminophenyl) compound **7**[7] with iodine chloride in acetic acid gave the 2-iodo-3-amino analogue **8**. Diazotization of **8** followed by treatment with sodium azide yielded **5**. Chart II outlines the synthesis of the analogues possessing the photolabile and electrophilic group in the 2β-position. Hydrolysis of **9**[7] in aqueous dioxane gave the carboxylic acid **10**. Treatment of **10** with thionyl chloride gave the acid chloride of **10**, which was converted without isolation into the *p*-nitrophenethyl ester **11**. Selective reduction of **11** using nickel boride in hydrochloric acid provided the amine **12**. Iodination of **12** with iodine chloride in acetic acid

† National Institute on Drug Abuse.

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Chart I

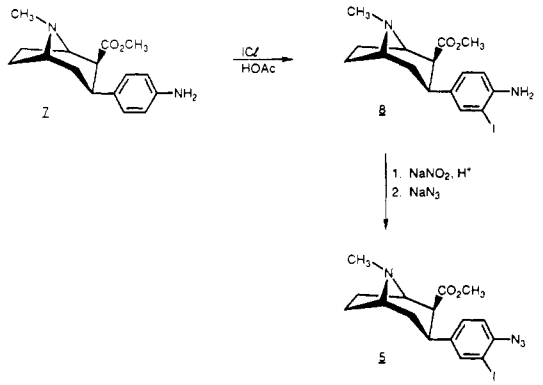
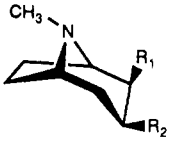


Table I. Potencies of Cocaine and Analogues in Inhibiting Binding of [^{3}H]-3β-(4-Fluorophenyl)tropan-2β-carboxylic Acid Methyl Ester (WIN 35,428)



compd	R_1	R_2	IC_{50} (nM)[a]
1	CO_2CH_3	$CO(O)C_6H_5$	102 ± 12[b]
4a	$CO_2(CH_2)_2C_6H_4$ 4-N_3	$CO(O)C_6H_5$	227 ± 19[b]
4b	$CO_2(CH_2)_2C_6H_4$ 4-NCS	$CO(O)C_6H_5$	196 ± 14[b]
4c	$CO_2(CH_2)_2C_6H_4$ 4-$NHCOCH_2Br$	$CO(O)C_6H_5$	61 ± 6[b]
5	CO_2CH_3	C_6H_3 3-I, 4-N_3	4.93 ± 0.32
6a	$CO_2(CH_2)_2C_6H_4$ 4-N_3	C_6H_4 4-Cl	6.17 ± 0.57
6b	$CO_2(CH_2)_2C_6H_4$ 4-NCS	C_6H_4 4-Cl	5.3 ± 0.6
6c	$CO_2(CH_2)_2C_6H_4$ 4-$NHCOCH_2Br$	C_6H_4 4-Cl	1.73 ± 0.06
6d	$CO_2(CH_2)_2C_6H_3$ 3-I, 4-N_3	C_6H_4 4-Cl	14.5 ± 0.94
7	CO_2CH_3	C_6H_4 4-NH_2	24.8 ± 1.3[c]
8	CO_2CH_3	C_6H_3 3-I, 4-NH_2	1.35 ± 0.11
9	CO_2CH_3	C_6H_4 4-Cl	1.17 ± 0.1[c]
11	$CO_2(CH_2)_2C_6H_4$ 4-NO_2	C_6H_4 4-Cl	2.71 ± 0.13
12	$CO_2(CH_2)_2C_6H_4$ 4-NH_2	C_6H_4 4-Cl	2.16 ± 0.25
13	$CO_2(CH_2)_2C_6H_3$ 3-I, 4-NH_2	C_6H_4 4-Cl	2.51 ± 0.25
14	$CO_2(CH_2)_2C_6H_4$ 4-NH_2	$CO(O)C_6H_5$	72 ± 0.007[b]
15	$CO_2(CH_2)_2C_6H_4$ 4-NO_2	$CO(O)C_6H_5$	601 ± 0.028[b]

[a] Mean ± standard error of four experiments performed in triplicate. [b] Taken from ref 6. [c] Taken from ref 7.

yielded the ortho iodo amino analogue **13**. Diazotization of **12** and **13** followed by treatment with sodium azide yielded **6a** and **6d**, respectively. Reaction of **12** with thiophosgene or bromoacetyl bromide afforded the *p*-isothiocyanate **6b** and the bromoacetyl amide **6c**, respectively.

Biological. Receptor Binding. Table I shows the inhibitory potencies of cocaine and several analogues to inhibit the binding of [^{3}H]-3β-(*p*-fluorophenyl)tropan-2β-carboxylic acid methyl ester (WIN 35,428) in rat striatal membranes. The apparent IC_{50} values for the new ligands **5** and **6a–d** ranged from 1.73 to 14.5 nM with the 4-bromoacetamido analogue **6b** and the 3-iodo-4-azido analogue **6d** being the most and least potent, respectively. Compounds **6a–d** have a β-(4-chlorophenyl) group at the 3-position of the tropane ring, whereas **4a–c** have a 3β-benzoyloxy moiety at this position. Compounds **6a–c** are approximately 36 times more potent than the corresponding analogues **4a–c**. Similarly, the p-amino intermediate **12** is 33 times more potent than the *p*-amino analogue **14**, which has a 3β-benzoyloxy group at position 3. These observations were expected since we had reported[8] that analogue **9** was 87 times more potent than cocaine.[7] It is somewhat surprising that the *p*-nitro analogue **11** is 220 times more potent than the corresponding compound **15**, which has a 3β-benzoyloxy substituent.

The addition of a 3-iodo group to **7** to give the 3β-(3-iodo-4-aminophenyl) analogue **8** resulted in an 18-fold increase in potency. In contrast, the addition of a 3-iodo group to **12** to give the analogue **13** resulted in a slight decrease in potency.

Table II. Wash-Resistant Binding of 0.5 nM [^{3}H]WIN 35,428 in Rat Striatal Membranes[a] Pretreated with Potentially Irreversible Ligands

	% of control[b]			
compd	no washes	one wash	two washes	three washes
cocaine	43 ± 9	85 ± 10	79 ± 1	97 ± 6
4a[c]	23 ± 5	30 ± 6	45 ± 13	49 ± 15
4c	22 ± 9	34 ± 12	33 ± 1	38 ± 18
5[c]	0 ± 5	2 ± 8	5 ± 6	5 ± 9
6a	21 ± 16	19 ± 7	24 ± 10	26 ± 12
6b	24 ± 9	29 ± 12	26 ± 9	30 ± 11
6c	14 ± 4	23 ± 17	26 ± 3	34 ± 8
6d[c]	14 ± 8	14 ± 5	22 ± 4	16 ± 6

[a] Membranes were prepared and radioligand binding was performed as described in the Experimental Section. Tissues were preincubated for 60 min with 10 times (cocaine, **4c**, **6b**, **6c**) or 500 times (**4a**, **5**, **6a**, **6d**; this was done due to the small amount of photoincorporation) the apparent IC_{50} concentration of the ligand or with buffer as control. [b] Mean ± standard error of three experiments performed in triplicate. [c] Irradiated with UV light (2800 Å) for 40 s prior to washing.

Irreversible Binding Studies. The ability of compounds **4b**, **4c**, **5**, and **6a–c** to function as apparent irreversible inhibitors of the cocaine binding site was evaluated in vitro by incubating rat striatal membranes with (10–500) × IC_{50} concentrations of each compound for 60 min at 0 °C. The azido compounds **4a**, **5**, and **6d** were exposed to UV light (2800 Å) for 40 s following the 60-min incubation period. Measurement of [^{3}H]WIN 35,428 binding demonstrated no further change after two washes (Table II). Compounds **4a** and **4c**, derived from (−)-cocaine, and compounds **5**, **6b**, and **6c**, derived from 3β-phenyltropan-2β-carboxylic acid methyl ester (WIN 35,065-2), could not be completely washed from the cocaine binding site labeled by [^{3}H]WIN 35,428, suggesting irreversible binding (Table II). (−)-Cocaine in the presence or absence of UV light failed to produce a significant irreversible effect on rat striatal [^{3}H]WIN 35,428 binding. Compounds **5** and **6d** when exposed to UV light reduced [^{3}H]WIN 35,428 binding to 5 and 16% of control, respectively.

Discussion

The concept of site-directed receptor probes of Baker[9] has been widely used in the studies of receptor systems.[10,11] The technique consists of selective covalent bond formation between a chemically reactive ligand and a group at, or close to, the active site of the receptor. The covalent bond can be established either by photoactivation of a

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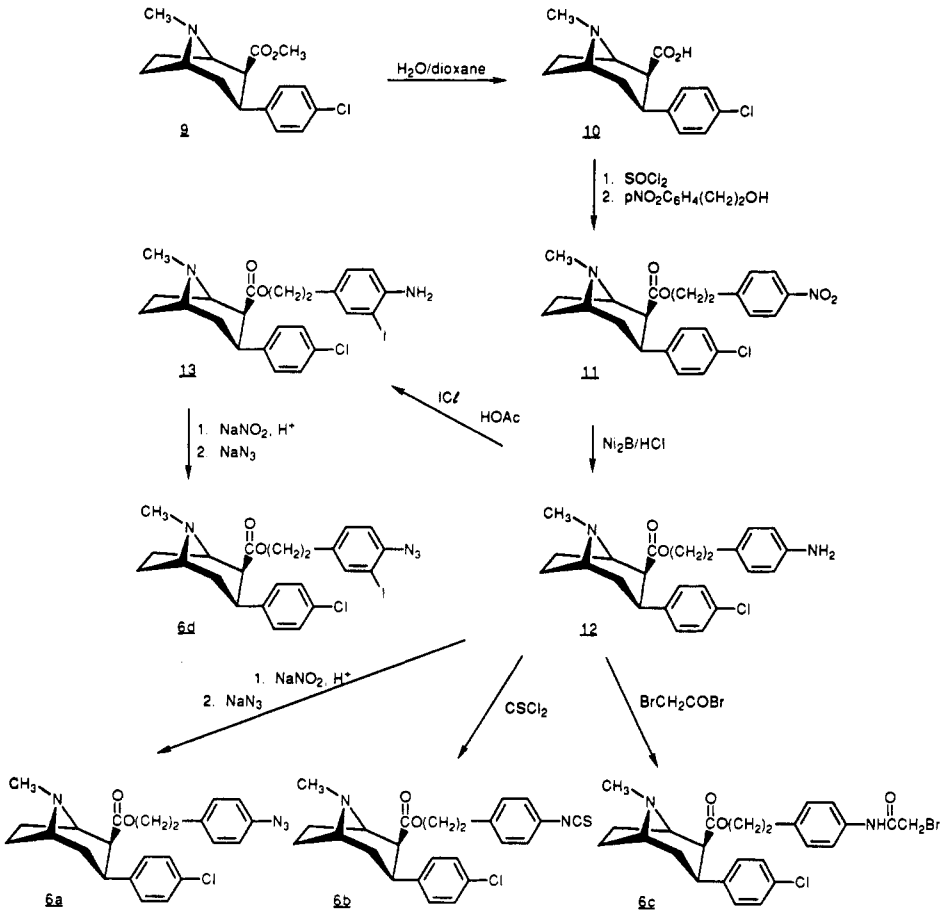
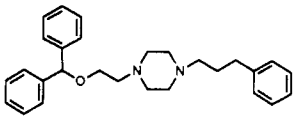
Chart II

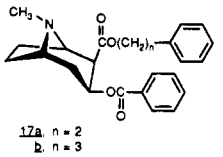


photoactive ligand at the receptor site or by chemical reaction between a protein bionucleophile in the vicinity of the binding site and a reactive substituent on the ligand. Typical photoactive moieties are azido groups, while isothiocyanato and bromoacetamido are some of the chemically reactive substrates which have been used. When radiolabeled, these probes can be used in attempts to purify and characterize the receptor protein(s).

This approach, utilizing two photoaffinity analogues of GBR12935 (**16**), has been used to obtain information about the dopamine transporter and has revealed some properties



16



17a, n = 2
b, n = 3

of a receptor protein associated with the GBR site.[12-16] However, since there is no a priori reason to expect these structurally dissimilar compounds to bind to the same site, irreversible probes with structures similar to cocaine may label other parts of the receptor protein. Recently, we reported that *p*-isococ (**2a**) inhibited binding at the cocaine receptor[4] in a wash-resistant fashion and blocked dopamine uptake by the transporter. Moreover, it blocked the high-affinity binding site in preference to the low-affinity site. This suggested that *p*-isococ may be a useful probe for studying the role of the high- versus the low-affinity site. However, the observation that *p*-isococ was 10 times less potent than cocaine prompted us to look for other ligands.[4] Our goal was to design selective, more potent, potentially irreversible ligands for the cocaine receptor. The observation that the methyl group of the 2-carbomethoxy moiety of cocaine could be replaced with larger groups without significant loss in binding affinity suggested this position for incorporation of electrophilic and photolabile functionalities.[6] In particular, the IC_{50}s of 248 and 139 nM for the phenethyl and phenpropyl analogues **17a** and **17b**, respectively, demonstrated that either of these analogues might be suitable for modification to irreversible ligands. Since the addition of an electrophilic or photoactive group to the phenyl ring of either **17a** or **17b** would increase the size of the group and thus might reduce binding potency, we decided to use the smaller phenethyl structure as the framework for the potentially irreversible ligands. Indeed, the *p*-azido, *p*-isothiocyanato, and *p*-bromoacetamido analogues **4a–c**, respectively, were found to have affinity for the receptor similar to that of cocaine

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(Table I).[6] Combining these results with our earlier report that 3β-(p-chlorophenyl)tropan-2β-carboxylic acid methyl ester (9) was 85 times more potent than cocaine[7] suggested that modification of 9 would lead to ligands of even greater potency. This expectation was realized with compounds 5 and 6a–d exhibiting apparent IC_{50} values between 1.73 and 14.5 nM.

Additional information concerning requirements for high binding affinity to the cocaine receptor at the dopamine transporter was also revealed in this study. Specifically, the observation that the addition of an m-iodo substituent to 3β-(4-aminophenyl)tropan-2β-carboxylic acid methyl ester (7)[7] to give 3β-(4-amino-3-iodophenyl) analogue 8 increased the binding potency 18-fold suggests that 3β-(3-substituted phenyl) and 3β-(3,4-disubstituted phenyl) 2β-carboxylic acid methyl esters should be investigated. Furthermore, a comparison of the IC_{50} value of 9 to those of the 2β-phenethyl analogues 6a–d and 11–13 shows that the tropane ring possesses high tolerance for steric variation in parallel to our observations for the analogous cocaine system.[6] However, the steric tolerance in the cocaine series appears to be greater than that in the 3β-(4-chlorophenyl)tropane series.

The wash-resistant inhibition of cocaine binding at the dopamine transporter produced by the cocaine analogues (Table II) demonstrates that some of these analogues may serve as useful molecular probes. The observation that analogues 5 and 6d, which are derivatives of 3β-phenyltropan-2β-carboxylic acid methyl ester (WIN 35,066-2), inhibited 90% (±5%) of [^{3}H]WIN 35,428 binding suggests their potential use, in radiolabeled form, for receptor protein characterization and possible isolation.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1-dm cell). NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. Thin-layer chromatography was carried out on Whatman silica gel 60 TLC plates using $CHCl_3$–MeOH–concentrated NH_4OH (40:9:1) unless otherwise noted. Visualization was accomplished under UV or in an iodine chamber. Since all of the compounds described were prepared starting from natural cocaine, they are all optically active and have the absolute configuration of natural cocaine. Microanalyses were carried out by Atlantic Microlab, Inc. [^{3}H]-3β-(p-Fluorophenyl)tropan-2β-carboxylic acid methyl ester with specific activity 83.1 Ci/mmol was purchased from Dupont-New England Nuclear (Boston, MA).

3β-(3-Iodo-4-aminophenyl)tropan-2β-carboxylic Acid Methyl Ester (8) Dihydrochloride. To a solution of 3β-(4-aminophenyl)tropan-2β-carboxylic acid methyl ester (7)[7] (300 mg, 1.09 mmol) in glacial AcOH (15 mL) was added dropwise ICl (195 mg, 1.2 mmol) at room temperature for 3 h under N_2. After removal of solvent, the residue was diluted with H_2O, and then basified with concentrated NH_4OH. The mixture was extracted with $CHCl_3$ which was washed with water and brine. After drying over $MgSO_4$, the solvent was evaporated to an oily product which was purified by flash chromatography (hexane–Et_2O, 4:1). The collected fraction was converted to HCl salt with HCl/Et_2O to yield 140 mg (29%) of 8·HCl: mp 170–173 °C; $[\alpha]^{25}_D$ –90.9° (c 0.055, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.65 (m, 3), 2.09 (m, 2), 2.2 (s, 3, NCH_3), 2.45 (m, 1), 2.75 (m, 1, H-2), 2.8 (m, 1, H-3), 3.3 (m, 1, H-5), 3.45 (m, 4, H-1, OCH_3), 3.95 (m, 2, NH_2), 6.65 (d, 1, J = 8.7 Hz, ArH), 7.05 (dd, 1, J = 8.7, J = 1.5 Hz, ArH), 7.42 (d, J = 1.5 Hz, 1, ArH). Anal. ($C_{16}H_{21}IN_2O_2$·2HCl·H_2O) C, H, N.

3β-(3-Iodo-4-azidophenyl)tropan-2β-carboxylic Acid Methyl Ester (5) Hydrochloride. To a solution of 3β-(3-iodo-4-aminophenyl)tropan-2β-carboxylic acid methyl ester dihydrochloride (8·2HCl) (90 mg, 0.19 mmol) in 1 mL of AcOH (3 M) was added an aqueous solution of $NaNO_2$ (17.3 mg, 0.266 mmol, in 0.5 mL of H_2O) at 0 °C. After 30 min at this temperature, NaN_3 (19 mg, 0.275 mmol) in 0.4 mL of H_2O was added dropwise to the reaction mixture and was stirred for 30 min at 0 °C and then 30 min at room temperature. After removal of all solvent by evaporation, the residue was dissolved in $CHCl_3$ and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated to give an oil which was converted to HCl salt to yield 64 mg (72.7%) of 5·HCl as a yellowish solid: mp 140–143 °C; $[\alpha]^{25}_D$ –97.4° (c 0.115, MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.51–1.73 (m, 3), 2.07–2.16 (m, 2), 2.19 (s, 3, NCH_3), 2.47 (m, 1), 2.80–2.93 (m, 2), 3.32 (m, 1, H-5), 3.51 (s, 3, OCH_3), 3.54 (m, 1, H-1), 7.01 (d, 1, J = 7.7 Hz, ArH), 7.28 (dd, 1, J = 7.7, J = 1 Hz, ArH), 7.60 (d, 1, J = 1 Hz, ArH). Anal. ($C_{16}H_{19}IN_4O_2$·HCl·H_2O) C, H, N.

3β-(p-Chlorophenyl)tropan-2-carboxylic Acid (10). A suspension of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid methyl ester (9) (5 g, 6.91 mmol) in a solution of 50 mL of dioxane–H_2O (1:1) was heated at 58–60 °C for 3 days. After removal of all solvent under vacuum, the residue was crystallized from H_2O to afford 3.5 g (74%) of 10 as an off-white solid: mp 300–301 °C; $[\alpha]^{25}_D$ –108.0° (c 0.10, MeOH); ^{1}H NMR of free base of 10 (250 MHz, $CDCl_3$) δ 1.57–1.9 (m, 4), 2.25 (m, 2), 2.45 (s, 3, NCH_3), 2.52 (m, 1), 3.12 (m, 1, H-2), 3.55 (m, 2, H-1, H-5), 7.19 (dd, 4, C_6H_6). Anal. ($C_{15}H_{18}ClNO_2$·0.25H_2O) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-(p-Nitrophenyl)ethyl Ester (11) Hydrochloride. 3β-(p-Chlorophenyl)tropan-2β-carboxylic acid (10) (2 g, 7.15 mmol) was stirred with $SOCl_2$ (25 mL) at 0 °C for 2 h. After dilution with toluene, the solution was evaporated to dryness. The residual oily acid chloride was dissolved in a solution of 0.5 mL of pyridine in 20 mL of dried $CHCl_3$. To this mixture was added dropwise 2-(p-nitrophenyl)ethyl alcohol (1.2 g, 7.18 mmol) in dried $CHCl_3$ (5 mL), and the solution was stirred at 0 °C for 2 h and at room temperature for 2 h. The reaction mixture was evaporated, and the residue was crystallized from EtOH to give 1.05 g (34%) of 11 as off-white crystals. The free base was dissolved in Et_2O, and HCl/Et_2O was added to yield 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-nitrophenyl)ethyl ester hydrochloride (11·HCl) as an off-white solid: mp 209–211 °C; $[\alpha]^{25}_D$ –69.5° (c 0.0475, MeOH); ^{1}H NMR (free base) (250 MHz, $CDCl_3$) δ 1.67 (m, 3), 2.13 (m, 5, NCH_3, H-4), 2.5 (m, 1), 2.89 (m, 4), 3.39 (m, 2, H-1, H-5), 4.17 (m, 1), 7.16 (dd, 4, J = 7.8 Hz, ArH), 7.25 (d, 2, J = 7.8 Hz, ArH), 8.1 (d, 1, J = 7.8 Hz, ArH). Anal. ($C_{23}H_{26}Cl_2N_2O_4$·0.75H_2O) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-(p-Aminophenyl)ethyl Ester (12) Hydrochloride. A mixture of Ni_2B (600 mg, 4.66 mmol) and 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-nitrophenyl)ethyl ester (11) (1 g, 2.33 mmol) in 20 mL of a solution of EtOH–H_2O–concentrated HCl (1:1:0.022) was stirred at 50 °C for 8 h, and was diluted with H_2O. After filtration (Celite) to remove unreacted catalyst, the solution was adjusted to basic pH with concentrated NH_4OH and extracted with Et_2O. The extract was evaporated to give a residue which was crystallized from EtOH–hexane to afford 710 mg (76%) of 12 as crystal. The free base was dissolved in Et_2O, and HCl/Et_2O was added to yield 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-aminophenyl)ethyl ester dihydrochloride (12·2HCl) as an off-white solid: mp 189–193 °C; $[\alpha]^{25}_D$ –57.5° (c 0.04, MeOH); ^{1}H NMR (free base) (250 MHz, $CDCl_3$) δ 1.67 (m, 4), 2.15 (m, 4, NCH_3, H-4), 2.5 (m, 1), 2.65 (t, 2), 2.82 (m, 1, H-2), 2.94 (m, 1, H-3), 3.85, 3.39 (m, 2, H-1, H-), 4.1 (m, 1), 6.6 (d, 2, J = 8 Hz, ArH), 6.9 (d, 1, J = 8 Hz, ArH), 7.16 (dd, 4, J = 8 Hz, ArH). Anal. ($C_{23}H_{27}ClN_2O_2$·2HCl·1.5H_2O) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-(p-Azidophenyl)ethyl Ester (6a) Hydrochloride. To a solution of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-aminophenyl)ethyl ester dihydrochloride (12) (50 mg, 0.106 mmol) in 0.5 mL of AcOH (3M) was added an aqueous solution of $NaNO_2$ (10.2 mg, 0.148 mmol, in 0.3 mL of H_2O) at 0 °C. After 30 min at this temperature, NaN_3 (10 mg, 0.154 mmol) in 0.3 mL of H_2O was added dropwise to the reaction mixture, and stirred for 30 min at 0 °C and then 30 min at room temperature. After removal of all solvent by evaporation, the residue was dissolved in $CHCl_3$ and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated to give an oil which was converted to HCl salt to yield 29 mg (59.3%) of 6a·HCl as a yellowish solid: mp 82–84 °C; $[\alpha]^{25}_D$ –66.2° (c 0.065, MeOH); ^{1}H NMR (250 MHz, CD_3OD)

δ 2.0–2.5 (m, 7), 2.7 (m, 1), 2.8 (s, 3, NCH_3), 3.1 (m, 1), 3.55 (m, 1), 3.85 (m, 1), 4.1 (m, 3), 6.9 (dd, 4, J = 7.7 Hz, ArH), 7.22 (dd, 4, J = 7.7 Hz, ArH). Anal. ($C_{23}H_{25}ClN_4O_2 \cdot HCl \cdot 1.5H_2O$) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-(3-Iodo-4-aminophenyl)ethyl Ester (13) Dihydrochloride. To a solution of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-aminophenyl)ethyl ester (12) (200 mg, 0.501 mmol) in glacial AcOH (10 mL) was added dropwise ICl (90 mg, 0.554 mmol) at room temperature over 3 h under N_2. After removal of solvent, the residue was diluted with H_2O and basified with concentrated NH_4OH. The mixture was extracted with $CHCl_3$ which was washed with water and brine. After drying over $MgSO_4$, the solvent was evaporated to an oily product which was purified by flash chromatography (hexane–Et_2O, 4:1). The collected fraction was converted to HCl salt with HCl–Et_2O to yield 205 mg (68%) of 13·2HCl: mp 158–160 °C; $[\alpha]^{25}_D$ –60.0° (c 0.055, MeOH); ^1H NMR (250 MHz, CD_3OD) δ 2.0–2.5 (m, 7), 2.7 (m, 1), 2.85 (s, 3, NCH_3), 3.15 (m, 1), 3.6 (m, 1), 3.9 (m, 1), 4.1 (m, 3), 7.05 (d, 1, ArH), 7.22 (m, 5, ArH), 7.6 (s, 2, ArH). Anal. ($C_{23}H_{28}Cl_3IN_2O_2$) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-(3-Iodo-4-azidophenyl)ethyl Ester (6d) Hydrochloride. To a solution of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(3-iodo-4-aminophenyl)ethyl ester dihydrochloride (13) (100 mg, 0.167 mmol) in 1 mL of AcOH (3 M) was added an aqueous solution of $NaNO_2$ (16.2 mg, 0.235 mmol) in 0.5 mL of H_2O at 0 °C. After 30 min at this temperature, NaN_3 (15.8 mg, 0.243 mmol) in 0.5 mL of H_2O was added dropwise to the reaction mixture and stirred for 30 min at 0 °C and then 30 min at room temperature. After removal of all solvent by evaporation, the residue was dissolved in $CHCl_3$ and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated to give an oil which was converted to HCl salt to yield 53 mg (54%) of **6d** as a yellowish solid: mp 136–138 °C; $[\alpha]^{25}_D$ –46.3° (c 0.095, MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 1.67 (m, 3), 2.15 (s, 5, NCH_3, H-4), 2.45 (m, 1), 2.7 (t, 2), 2.85 (m, 1, H-2), 2.9 (m, 1, H-3), 3.4 (m, 2, H-1, H-5), 4.1 (m, 2), 6.95 (d, 1, ArH), 7.2 (m, 5, ArH), 7.6 (s, 1, ArH). Anal. ($C_{23}H_{25}Cl_2IN_4O_2 \cdot H_2O$) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-(p-Isothiocyanatophenyl)ethyl Ester (6b) Hydrochloride. To a rapidly stirred solution of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-aminophenyl)ethyl ester hydrochloride (12) (25 mg, 0.053 mmol) and $NaHCO_3$ (28 mg, 0.331 mmol) in a mixture of H_2O–THF (1 mL, 2:1) was added a solution of thiophosgene (5.7 μL, 0.073 mmol) in THF (1 mL) at 0 °C. After 5 h at room temperature, the reaction appeared complete (TLC). The organic layer was separated, diluted to 20 mL with $CHCl_3$, and washed once with water. After drying over $MgSO_4$, the solvent was evaporated to afford 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-isothiocyanatophenyl)ethyl ester (**6b**) as a viscous oil which was converted to HCl salt to give 9 mg (36%) of product as a solid: mp 126–128 °C; $[\alpha]^{25}_D$ –61.8° (c 0.055, MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 2.20 (m, 5, NCH_3, H-4), 2.50 (m, 1), 2.77 (t, 2), 2.8 (m, 1, H-2), 2.95 (m, 1, H-3), 3.40 (m, 2, H-1, H-5), 4.13 (m, 2), 7.14 (m, 8, ArH). Anal. ($C_{24}H_{25}ClN_2O_2S \cdot HCl \cdot H_2O$) C, H, N.

3β-(p-Chlorophenyl)tropan-2β-carboxylic Acid 2-[p-(Bromoacetamido)phenyl]ethyl Ester (6c) Hydrochloride. To a solution of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-(p-aminophenyl)ethyl ester dihydrochloride (12) (50 mg, 0.125 mmol) in dry 1,2-dichloroethane was added dropwise 33 μL of bromoacetyl bromide (0.377 mmol) at 0 °C under N_2. Stirring was continued for 24 h, allowing the mixture to come to room temperature. After removal of solvent, the residue was diluted with H_2O and basified with concentrated NH_4OH. The mixture was extracted with Et_2O which was washed with H_2O. After drying over $MgSO_4$, the solvent was evaporated to afford 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-[p-(bromoacetamido)phenyl]ethyl ester (**6c**) as an oil which was subjected to purification by flash chromatography (silica gel, using hexane–Et_2O 4:1) to give 18 mg (26%) of 3β-(p-chlorophenyl)tropan-2β-carboxylic acid 2-[p-(bromoacetamido)phenyl]ethyl ester which was converted to the hydrochloride salt: mp 177–179 °C; $[\alpha]^{25}_D$ –64.4° (c 0.045, MeOH); ^1H NMR (250 MHz, $CDCl_3$) δ 1.25–1.68 (m, 3), 2.20 (m, 5, NCH_3, H-4), 2.52 (m, 1), 2.7 (t, 2), 2.8 (m, 1, H-2), 2.95 (m, 1, H-3), 3.45 (m, 2, H-1, H-5), 4.0 (s, 2, $BrCH_2$), 4.13 (m, 2), 7.14 (m, 6, ArH), 7.41 (d, 2, ArH). Anal. ($C_{25}H_{28}BrClN_2O_3 \cdot HCl \cdot 0.75H_2O$) C, H, N.

[^{3}H]WIN 35,428 Radioligand Binding. Rat striata from male Sprague–Dawley rats (250–350 g) were rapidly dissected, frozen, and stored at –70 °C until used. The frozen rat striata were homogenized in 20 volumes of 10 mM phosphate buffer (pH 7.4) containing 0.32 M sucrose using a polytron (setting 6) for 10 s. The homogenate was centrifuged for 10 min at 50000g, and the resulting pellet was washed in buffer, recentrifuged, and resuspended to a tissue concentration of 10.0 mg/mL. Binding assays were carried out in a total volume of 0.5 mL containing 0.5 nM [^{3}H]WIN 35,428 and 1.0 mg of tissue. The ligands were added as buffered solutions of the hydrochloride salts. The suspensions were incubated for 2 h on ice. Incubations were terminated by filtration with three 5-mL washes through Whatman GF/B filters previously soaked in 0.05% polyethylenimine using a Brandel M48R filtering manifold (Brandel Instruments, Gaithersburg, MD). Radioactivity was counted in 5 mL of scintillation cocktail in a Beckman LS 3801 liquid scintillation counter with an efficiency of approximately 50%. Nonspecific binding of [^{3}H]WIN 35,428 was defined by the presence of 30 μM (–)-cocaine. Under these conditions, nonspecific binding was approximately 5–8% of total binding. IC_{50} values were determined from competition curves of 10–12 points utilizing the curve fitting program EBDA.[17] Mean values and standard errors were calculated from three to four assays for each test drug.

Tissue Preincubation with Potentially Irreversible Agents. Tissue was prepared as described above, and the final homogenate was incubated for 60 min with either drug or vehicle as control for 60 min on ice in the above buffer. Following the 60-min incubation period, all homogenates involving a ligand with an azido group were then exposed to UV light (2800 Å) for 40 s. The incubation of all compounds was terminated by centrifugation at 50000g for 10 min. The resulting pellet was resuspended to a concentration of 10 mg/mL, and an aliquot was removed (zero washes). This procedure was repeated for a total of three washes. Residual [^{3}H]WIN 35,428 binding was detected as described above. Data are expressed as the percent of specific control binding.

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(17) Biosoft Software, Ferguson, MO.