Synthesis of 3-Carbamoylecgonine Methyl Ester Analogues as Inhibitors of Cocaine Binding and Dopamine Uptake

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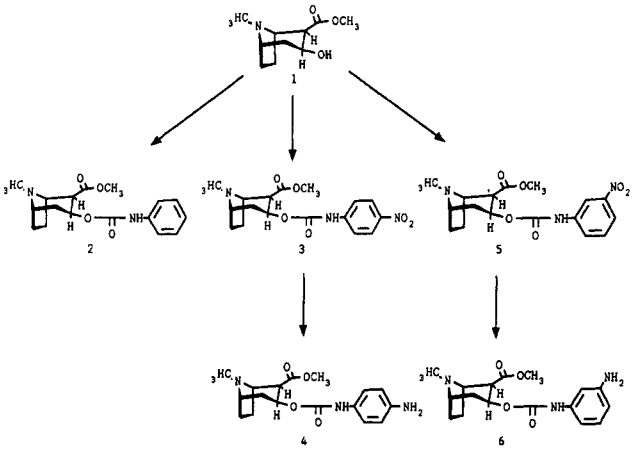
Five (1*R*-2-*exo*-3-*exo*)-3-(*N*-phenylcarbamoyl)ecgonine methyl ester analogues were synthesized and characterized by ^1H and ^{13}C NMR, IR, and thermospray MS. The compounds were synthesized in two or three steps as (-)-stereoisomers from (-)-ecgonine in good yield (56% overall). These cocaine derivatives were assessed for their ability to inhibit [^3H]cocaine binding to rat striatal tissue and to inhibit [^3H]dopamine uptake into synaptosomes prepared from the same tissue. The most potent of the analogues was (1*R*-2-*exo*-3-*exo*)-2-(carbomethoxy)-8-methyl-8-azabicyclo[3.2.1]octyl 3-*N*-(3'-nitrophenyl)carbamate. IC_{50} values for inhibition of cocaine binding and dopamine uptake were 37 and 178 nM, respectively. Amino derivatives were less active than the nitro and (1*R*-2-*exo*-3-*exo*)-2-(carbomethoxy)-8-methyl-8-azabicyclo[3.2.1]octyl 3-*N*-(4'-aminophenyl)carbamate had the lowest affinity for the receptor with IC_{50} values of 63 and >100 μM in the aforementioned assays, respectively.

Introduction

Cocaine is a potent central stimulant[1] which is abused by an alarming number of people in the U.S.[2] and has thus become a serious social problem.[3] The abuse of this extremely addictive drug[4] is generally believed to be related to its powerful positive reinforcing properties.[1] It is generally accepted that these properties arise as a result of the effect of cocaine, as well as other addictive drugs, on the central mesocorticolimbic dopamine (DA) system.[5-7] Specifically, cocaine inhibits the reuptake of DA into mesocorticolimbic dopaminergic nerve terminals and thus leads to exaggerated DA-like effects on the postsynaptic neurones carrying dopamine receptors.[8,9] Several groups have reported [^3H]cocaine binding associated with this system.[10-12] Our group utilizes assays for both inhibition of cocaine binding and dopamine uptake for characterization of the cocaine receptor.[13] Recently, it has become obvious that a greater number of cocaine analogues will be necessary for a more complete characterization and purification of the cocaine receptor.

One possible approach toward receptor purification is through affinity chromatography. Previously, Soffer and Schneider have reported the synthesis of the *p*-nitro- and *p*-aminobenzoylecgonine analogues of cocaine.[14] Conversion of the p-amino analogue to an isothiocyanate derivative by these authors gave a compound which bound readily to proteins. An advantage seen with these analogues is that they retain a close structural resemblance to cocaine; thus they would be expected to be better probes to use for labeling the cocaine receptor protein than non-cocaine ligands. A potential disadvantage is that the carboxylic ester moiety in the 3-position of the tropane ring would be susceptible to hydrolysis both in vitro and in vivo.

Scheme I



In a recent publication[15] we described the synthesis and pharmacological testing of a series of 3-arylecgonine methyl ester analogues. These compounds proved to be very promising as potential affinity ligands. First, at least one of them has already been shown to be a very potent central nervous system stimulant, as is cocaine.[16] Second, a phenyl moiety directly attached to the tropane ring chemically stabilizes the 3-position toward hydrolysis. Third, the compounds were shown to be potent inhibitors of [^3H]cocaine binding and [^3H]dopamine uptake.[15]

Although the β-phenyl isomers were the most pharmacologically active, as measured by [^3H]cocaine binding and [^3H]DA uptake assays,[15] there were disadvantages associated with the use of these analogues. The first disadvantage was that the synthetic route used produced them in the lowest yields (3:1 ratio, α:β; 2% overall yield from (-)-ecgonine). The second was that separation of the reaction products was tedious and time consuming. In an attempt to overcome these disadvantages, we synthesized the 3-carbamoylecgonine methyl ester analogues which are the subject of this report. These compounds are potent inhibitors of [^3H]cocaine binding to rat brain striatal tissue homogenate and [^3H]dopamine uptake into striatal synaptosomes. It was possible to obtain these compounds easily in two or three steps from (-)-ecgonine in good yield (56% overall yield from (-)-ecgonine). This synthesis provides compounds which more closely resemble the cocaine molecule than do the 3-aryl analogues. The benzoyl

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Table I. IC_{50} Data for Competitive Inhibition of [^{3}H]Cocaine Binding and [^{3}H]Dopamine Uptake in Rat Striatal Tissue

corresponding analogue	IC_{50},[a] μM	
	cocaine binding	dopamine uptake
cocaine	0.065 ± 0.010	0.213 ± 0.067
2	5.600 ± 0.7	52.600 ± 3
3	1.090 ± 0.25	5.700 ± 1.2
4	63.300 ± 12.2	>100
5	0.037 ± 0.010	0.178 ± 0.023
6	2.070 ± 0.34	23.100 ± 0.9

[a] Each value represents the mean ± SEM (n = 3 or 4).

ester side chain is isosterically replaced by carbamate, a group which is more resistant to hydrolysis than is the benzoyl substituent. Also, as with the other series of compounds mentioned, the amino derivatives produced can be investigated as potential affinity ligands.

Results and Discussion

Chemistry. (−)-Ecgonine methyl ester (**1**) was obtained from (−)-cocaine hydrochloride by using known procedures.[17,18] Thus, (−)-cocaine hydrochloride was first hydrolyzed in dilute HCl and then esterification of the resultant amino acid was performed in CH_3OH/H_2SO_4. Overall yield from (−)-cocaine hydrochloride to (−)-ecgonine methyl ester was 85%.

3-Phenylcarbamate analogue **2** as well as the two (nitrophenyl)carbamate derivatives **3** and **5** (Scheme I) were synthesized from **1** and the appropriately substituted isocyanates by using the general procedures of Nilsson et al. without modification.[19] Catalytic reduction (PtO_2/H_2) of **3** and **5** led to the desired amino compounds **4** and **6** (Scheme I).

Pharmacology. The synthesized compounds were tested for their ability to inhibit both [^{3}H]cocaine binding and [^{3}H]dopamine uptake. The reason for this is that the cocaine receptor, which mediates the reinforcing properties of the drug, is generally believed to be identical with or very closely associated with the dopamine transporter.[10-12] Evidence for this was presented in a previous publication.[15]

The more potent of the analogues was the m-NO_2 derivative **5** (Table I). This compound was found to be twice as potent in inhibiting [^{3}H]cocaine binding than (−)-cocaine itself. The p-NO_2 compound **3** was 30-fold less potent than **5**. The reason for this difference is unclear. It could be speculated that because meta substitution would more greatly restrain the conformational mobility of the carbamate side chain due to its proximity to the methyl ester in the 2-position of the tropane ring, the conformation of the entire molecule more closely fits the active site of the receptor.

The different analogues had a similar rank order of potency in inhibiting both [^{3}H]cocaine binding and [^{3}H]dopamine uptake (Table I). The correlation coefficient of inhibition in both assays by the different compounds was very high (Figure 1).

Another interesting observation is that the reduced amino derivatives showed a marked decrease in affinity for the receptor as compared to their nitro precursors. m-NH_2 analogue **6** was approximately 60 times less potent than **5**, and a corresponding decrease was noted with para isomers **3** and **4** (Table I). Despite this, the amino analogues may still be excellent affinity ligands. It may be that the topography of the receptor is sterically favorable

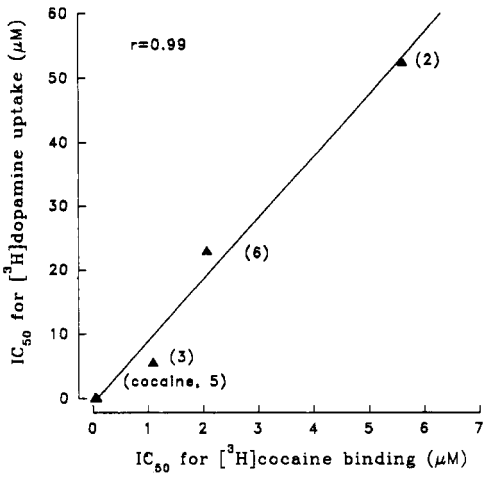

Figure 1. Correlation between IC_{50} values for inhibition of [^{3}H]dopamine uptake and [^{3}H]cocaine binding.

to the nitro derivatives. A more probable explanation for this phenomenon could be that the electron density of the phenyl ring greatly influences the drug–receptor interaction, the lower electron density ring being favored. However, since there are only two compounds in each series (meta and para), Hammett and/or Hansch correlations would not be meaningful. Recent papers by Ritz et al.[20] and by Madras et al.[21] have discussed structure–activity relationships of some cocaine analogues, but these were not mathematically correlated quantitative structure–activity studies.

Nitro compounds have been shown to bind to receptors by photo activation.[22] Compounds **3** and **5** may form photoaffinity ligands in a similar manner. It is more likely, however, that the amino compounds **4** and **6** will be useful as affinity ligands after binding to an Affi-gel support through a covalent amide linkage. Though amines **4** and **6** are considerably less potent than their nitro analogues **3** and **5**, the formation of an amide bond may negate the strong electron-donating effect of the basic amines and thus potentially enhance the affinity of the ligand for the desired transporter protein.

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were obtained with a General Electric QE-300 spectrometer. Spectra were obtained with $CDCl_3$ with TMS as an internal standard. Infrared spectra were recorded on an Analect FX-6160 FT-IR spectrophotometer with KBr disks. Mass spectra were performed with a quadrupole mass spectrometer. Compounds, dissolved in MeOH, were introduced via direct injection into a Vestec 201 thermospray interface using 0.1 M aqueous ammonium acetate at a flow rate of 1 mL/min. A discharge electrode was utilized. Elemental analyses performed (on samples dried to constant weight) by Micro Analysis Inc. agreed to within 0.4% of the calculated values.

General Method for the Synthesis of Carbamates 2, 3, and 5. The above compounds were made by the method of Nilsson et al.[19] Briefly, a mixture of ecgonine methyl ester[17,18] (1.0 mM) and the appropriate phenyl isocyanate (1.0 mM) in dry toluene was heated under reflux for 2 h. After cooling, the organic solution was extracted with 10% aqueous HCl (3 × 20 mL). The combined aqueous extract was made alkaline with ice-cold 10% NaOH

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solution and then extracted with $CHCl_3$ (3 × 20 mL). After drying ($MgSO_4$), the solvent was removed under vacuum and the residue crystallized from pentane/EtAc.

Methyl (1*R*-2-*exo*-3-*exo*)-8-Methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (1). Compound 1 was synthesized from (-)-cocaine hydrochloride using the procedures of Bell and Archer,[17] and Grundmann and Ottmann[18] in an overall yield of 85%.

(1*R*-2-*exo*-3-*exo*)-2-Carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octyl 3-*N*-Phenylcarbamate (2). This compound was synthesized according to the procedure of Nilsson et al.[19] (yield 58.3%). The product obtained was recrystallized from EtAc/pentane: mp = 164–165 °C (lit.[23] mp = 166–167 °C); $[\alpha]^{23}_D$ = -15.80; IR 3333, 3050, 2942, 1737, 1722, 1650, 1539, 1231 cm^{-1}; ^1H NMR δ 1.61–1.75 (m, 2 H, H_{6ax}, H_{7ax}), 1.78–1.85 (m, 1 H, H_{4eq}), 2.07–2.18 (m, 2 H, H_{6eq}, H_{7eq}), 2.21 (s, 3 H, NCH_3), 2.33–2.41 (dt, J = 3 Hz (vic), 12 Hz (gem), 1 H, H_{4ax}), 2.99–3.02 (m, 1 H, H_2), 3.22–3.28 (m, 1 H, H_5), 3.52–3.54 (m, 1 H, H_1), 3.71 (s, 3 H, OCH_3), 4.97–5.05 (m, 1 H, H_3), 6.85 (s (br), 1 H, NH), 7.01–7.67 (dd, J = 1.5 Hz (meta), 7 Hz (ortho), 1 H, $H_{4'}$), 7.26–7.38 (dt, J = 1.5 Hz (meta), 7 Hz (ortho), 4 H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$); ^{13}C NMR δ 24.70, 24.83 (C_6, C_7), 35.21 (C_4), 40.65 (NCH_3), 49.76 (C_2), 50.95 (OCH_3), 61.09 (C_5), 64.32 (C_1), 66.79 (C_3), 117.90 ($C_{4'}$), 122.79 ($C_{3'}$, $C_{5'}$), 128.49 ($C_{2'}$, $C_{6'}$), 137.35 ($C_{1'}$), 152.54 (C=O, carbamate), 170.57 (C=O, ester).

(1*R*-2-*exo*-3-*exo*)-2-Carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octyl 3-*N*-(4'-Nitrophenyl)carbamate (3). Compound 3 was synthesized by reacting 1 with 4-nitrophenyl isocyanate by using the procedure of Nilsson et al.[19] (yield 66%). Recrystallization from EtAc/pentane resulted in small pale yellow crystals: mp = 172–174 °C; $[\alpha]^{25}_D$ = -20.60° (c = 0.50, $CHCl_3$); IR 3338, 2952, 1740, 1731, 1554, 1515, 1332, 1225 cm^{-1}; ^1H NMR δ 1.67–1.71 (m, 2 H, H_{6ax}, H_{7ax}), 1.81–1.87 (m, 1 H, H_{4eq}), 2.06–2.19 (m, 2 H, H_{6eq}, H_{7eq}), 2.22 (s, 3 H, NCH_3), 2.32–2.41 (dt, J = 3 Hz (vic), 12 Hz (gem), 1 H, H_{4ax}), 3.00–3.03 (m, 1 H, H_2), 3.27–3.28 (m, 1 H, H_5), 3.54–3.56 (m, 1 H, H_1), 3.71 (s, 3 H, OCH_3), 5.00–5.08 (m, 1 H, H_3), 7.57–7.60 (d, J = 9 Hz, 2 H, $H_{2'}$, $H_{6'}$), 7.71 (s (br), 1 H, NH), 8.16–8.19 (d, J = 9 Hz, 2 H, $H_{3'}$, $H_{5'}$); ^{13}C NMR δ 24.73, 24.85 (C_6, C_7), 35.14 (C_4), 40.63 (NCH_3), 49.66 (C_2), 51.07 (OCH_3), 61.00 (C_5), 64.29 (C_1), 67.65 (C_3), 117.20 ($C_{2'}$, $C_{6'}$), 124.61 ($C_{3'}$, $C_{5'}$), 142.31 ($C_{1'}$), 143.68 ($C_{4'}$), 152.05 (C=O, carbamate), 170.48 (C=O, ester); MS m/z (relative intensity) 364 (MH^+, 4.5), 200 (100), 182 (40), 168 (4), 156 (21), 139 (6), 109 (10), 96 (5), 82 (22). Anal. ($C_{17}H_{21}N_3O_6$) C, H, N.

(1*R*-2-*exo*-3-*exo*)-2-Carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octyl 3-*N*-(4'-Aminophenyl)carbamate (4). This compound was obtained in near quantitative yield by reducing compound 3 in MeOH over PtO_2. Recrystallization of the crude product from EtAc/pentane resulted in light brown crystals: mp = 198–199.5 °C; $[\alpha]^{25}_D$ = -20.33° (c = 0.30, $CHCl_3$); IR 3405, 3341, 3058, 2955, 1734, 1708, 1553, 1517, 1231, 1072 cm^{-1}; ^1H NMR δ 1.62–1.73 (m, 2 H, H_{6ax}, H_{7ax}), 1.77–1.83 (m, 1 H, H_{4eq}), 2.05–2.14 (m, 2 H, H_{6eq}, H_{7eq}), 2.20 (s, 3 H, NCH_3), 2.30–2.39 (dt, J = 2.4 Hz (vic), 12 Hz (gem), 1 H, H_{4ax}), 2.97–3.00 (m, 1 H, H_2), 3.26 (s (m), 1 H, H_5), 3.51 (s (m), 1 H, H_1), 3.71 (s, 3 H, OCH_3), 4.95–5.03 (m, 1 H, H_3), 6.61–6.64 (d, J = 8.4 Hz, 2 H, $H_{3'}$, $H_{5'}$), 6.72 (s (br), 1 H, NH), 7.11–7.13 (d, J = 8.4 Hz, 2 H, $H_{2'}$, $H_{6'}$); ^{13}C NMR δ 24.69, 24.83 (C_6, C_7), 35.25 (C_4), 40.65 (NCH_3), 49.81 (C_2), 50.91 (OCH_3), 61.10 (C_5), 64.33 (C_1), 66.44 (C_3), 115.08 ($C_{3'}$, $C_{5'}$), 120.09 ($C_{2'}$, $C_{6'}$), 128.77 ($C_{4'}$), 142.04 ($C_{1'}$), 152.98 (C=O, carbamate), 170.61 (C=O, ester). The hydrochloride of compound 3 was prepared by using standard methods. Anal. ($C_{17}H_{23}N_3O_4 \cdot 2HCl \cdot H_2O$) C, H, N.

(1*R*-2-*exo*-3-*exo*)-2-Carbomethoxy-8-methyl-8-azabicyclo[3.2.1]octyl 3-*N*-(3'-Nitrophenyl)carbamate (5). Compound 5 was prepared by reacting 1 with 3-nitrophenyl isocyanate according to the method of Nilsson et al.[19] (yield 62%). The product was recrystallized from EtAc/pentane, resulting in pale yellow needles: mp = 195–197 °C; $[\alpha]^{25}_D$ = -14.40° (c = 0.50, $CHCl_3$); IR 3305, 3021, 2954, 1730, 1620, 1544, 1354 cm^{-1}; ^1H NMR δ 1.55–1.75 (m, 2 H, H_{6ax}, H_{7ax}), 1.80–1.87 (m, 1 H, H_{4eq}), 2.05–2.23 (m, 2 H, H_{6eq}, H_{7eq}), 2.21 (s, 3 H, NCH_3), 2.33–2.42 (m, J = 3 Hz (vic), 9 Hz (gem), 1 H, H_{4ax}), 3.01–3.04 (dd, J = 3.6 Hz, 1 H, H_2), 3.27–3.28 (m, 1 H, H_5), 3.55–3.57 (m, 1 H, H_1), 3.72 (s, 3 H, OCH_3), 5.00–5.08 (m, 1 H, H_3), 7.31 (s (br), 1 H, NH), 7.42–7.47 (dd, J = 8.4 Hz, 1 H, $H_{5'}$), 7.66–7.69 (dd, J = 1.5 Hz (meta), 8.4 Hz (ortho), 1 H, $H_{6'}$), 7.88–7.91 (dd, J = 1.5 Hz (meta), 8.4 Hz (ortho), 1 H, $H_{4'}$), 8.37 (s, 1 H, $H_{2'}$); ^{13}C NMR δ 24.75, 24.84 (C_6, C_7), 35.18 (C_4), 40.60 (NCH_3), 49.69 (C_2), 51.03 (OCH_3), 61.04 (C_5), 64.32 (C_1), 67.63 (C_3), 112.71 ($C_{5'}$), 117.39 ($C_{4'}$), 123.44 ($C_{6'}$), 129.19 ($C_{2'}$), 138.77 ($C_{1'}$), 148.24 ($C_{3'}$), 152.34 (C=O, carbamate), 170.45 (C=O, ester); MS m/z (relative intensity) 364 (MH^+, 6), 200 (100), 182 (40), 168 (3), 156 (25), 109 (19), 82 (6). Anal. ($C_{17}H_{21}N_3O_6$) C, H, N.

(1*R*-2-*exo*-3-*exo*)-2-Carbomethoxy-8-methyl-8-azabicylo-[3.2.1]octyl 3-*N*-(3'-Aminophenyl)carbamate (6). Compound 6 was synthesized in nearly quantitative yield by reducing compound 5 in MeOH over PtO_2. A pale brown powder was crystallized from EtAc/pentane: mp = 175–177 °C; $[\alpha]^{25}_D$ = -19.00° (c = 0.50, $CHCl_3$); ^1H NMR δ 1.65–1.74 (m, 2 H, H_{6ax}, H_{7ax}), 1.77–1.84 (m, 1 H, H_{4eq}), 2.00–2.17 (m, 2 H, H_{6eq}, H_{7eq}), 2.21 (s, 3 H, NCH_3), 2.31–2.40 (dt, J = 2.7 Hz (vic), 14.7 Hz (gem), 1 H, H_{4ax}), 2.97–3.00 (m, 1 H, H_2), 3.27 (s (m), 1 H, H_5), 3.52 (s (m), 1 H, H_1), 3.72 (s, 3 H, OCH_3), 4.96–5.04 (m, 1 H, H_3), 6.35–6.39 (m, J = 7.8 Hz, 1 H, $H_{4'}$), 6.57–6.60 (d (br), J = 7.8 Hz, 1 H, $H_{6'}$), 6.89 (s (br sh), 1 H, NH), 6.91 (s, 1 H, $H_{2'}$), 7.01–7.06 (dd, J = 7.8 Hz, 1 H, $H_{5'}$); ^{13}C NMR δ 24.70, 24.84 (C_6, C_7), 35.22 (C_4), 40.66 (NCH_3), 49.76 (C_2), 50.97 (OCH_3), 61.09 (C_5), 64.34 (C_1), 66.64 (C_3), 104.53 ($C_{5'}$), 108.06 ($C_{4'}$), 109.62 ($C_{6'}$), 129.23 ($C_{2'}$), 138.40 ($C_{3'}$), 146.73 ($C_{1'}$), 152.49 (C=O, carbamate), 170.60 (C=O, ester). Anal. ($C_{17}H_{23}N_3O_4$) C, H, N.

Biology. Membrane Preparation. All tissue samples were prepared with striatum dissected from rat brain. The dissected tissue included caudate, globus pallidus, putamen, as well as nucleus accumbens. All experiments were conducted with fresh tissue and all subsequent steps for membrane preparation were performed at 0–4 °C.

For [^{3}H]cocaine binding experiments, the striatal tissue was homogenized in ice-cold 10 mM Na_2HPO_4 buffer containing 0.25 M sucrose, pH = 7.4, using a glass Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 1000g for 10 min and the supernatant was centrifuged at 48000g for 20 min. The pellet was resuspended in the same buffer with a P-10 Kinematica Polytron homogenizer (setting 7) for 20 s. The homogenate was centrifuged at 48000g for 20 min. The resulting pellet was resuspended in 40 volumes (vol/wt) of the same phosphate–sucrose buffer to yield approximate protein concentrations of 1 mg/mL. The Lowry et al.[24] method was utilized to determine all final protein concentrations.

Tissue used in [^{3}H]dopamine uptake experiments was prepared according to the procedure of Richelson and Pfenning[25] with slight modifications. Homogenization of the striatal tissue was performed in ice-cold 0.25 M sucrose, 11 mM glucose (pH = 7.4) buffer using a glass Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 1000g in a Beckman centrifuge, and then the supernatant was decanted and centrifuged for 20 min at 4800g. The resultant P_2 pellet was washed by centrifugation in the same sucrose–glucose solution and the final pellet was resuspended in 20 volumes (vol/wt) of the same solution.

[^{3}H]Cocaine Binding. Binding of [^{3}H]cocaine (1-[benzoyl-3,4-^{3}H(N)], 29.7 Ci/mmol; New England Nuclear, Boston, MA) to rat striatal membranes was measured by filtration assay using a previously reported method.[13] At least eight concentrations of each ligand were tested, ranging from 10 nM to 10 μM. In most cases, these concentrations displaced specific [^{3}H]cocaine binding from 2 to 98%. The assay included 200 μL of membrane preparation (200 μg protein), 25 μL of [^{3}H]cocaine (6 nM), 2.5 μL of cocaine analogue in dimethyl sulfoxide (DMSO) or solvent alone, and buffer to reach a final volume of 250 μL. Nonspecific binding was defined as that measured in the presence of 100 μM unlabeled cocaine and was typically less than 15% of total binding (total dpm bound was 3900, dpm bound in the presence of 100 μM cocaine was 430). After a 20-min incubation, the reaction was

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terminated by the addition of 4 mL of ice-cold saline and filtration through Whatman GF/B filters which were presoaked in 0.05% polyethylenimine. Filters were washed once with 4 mL of ice-cold saline and the radioactivity remaining on the filters was counted.

[^{3}H]Dopamine Uptake. Uptake of [^{3}H]dopmine [(dihydroxyphenyl)ethylamine-3,4-t_2, 30 Ci/mmol; New England Nuclear, Boston MA] into rat striatal synaptosomes was measured at 37 °C by using a previously described method.[13] In brief, 50 μL of striatal synaptosomes (100 μg of protein) were preincubated with 5 μL of cocaine analogue in DMSO or solvent alone for 10 min in a final volume of 500 μL; uptake was initiated by the addition of 4 nM [^{3}H]dopamine and terminated after 5 min by the addition of 4 mL of ice-cold saline and filtration through Whatman GF/B filters. Nonspecific uptake was measured at 37 °C using 100 μM cocaine in buffer in which choline was substituted equimolar for sodium, which defined sodium-dependent, cocaine-sensitive [^{3}H]dopamine uptake. Nonspecific uptake was typically 3–5% of the total (total and nonspecific dpm were 38600 and 1360, respectively).

Data Analysis. All assays were performed in triplicate and the mean values of at least three separate experiments were used. The IC_{50} values for inhibition of [^{3}H]cocaine binding were determined with ligand EBDA, an iterative nonlinear curve-fitting routine for an IBM-PC.[26] The IC_{50} values for inhibition of [^{3}H]dopamine uptake were determined from the inhibition curves.

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Registry No. 1, 7143-09-1; 2, 29364-08-7; 3, 131013-13-3; 4, 131013-14-4; 4·2HCl, 131100-30-6; 5, 131013-15-5; 6, 131013-16-6; 4-nitrophenyl isocyanate, 100-28-7; 3-nitrophenyl isocyanate, 3320-87-4.

(26) Munson, P. J.; Rodbard, D. *Anal. Biochem.* **1980**, *107*, 220.

Structure–Affinity Relationships of 12-Sulfonyl Derivatives of 5,8,8a,9,10,11,12,12a,13,13a-Decahydro-6*H*-isoquino[2,1-*g*][1,6]naphthyridines at α-Adrenoceptors[1]

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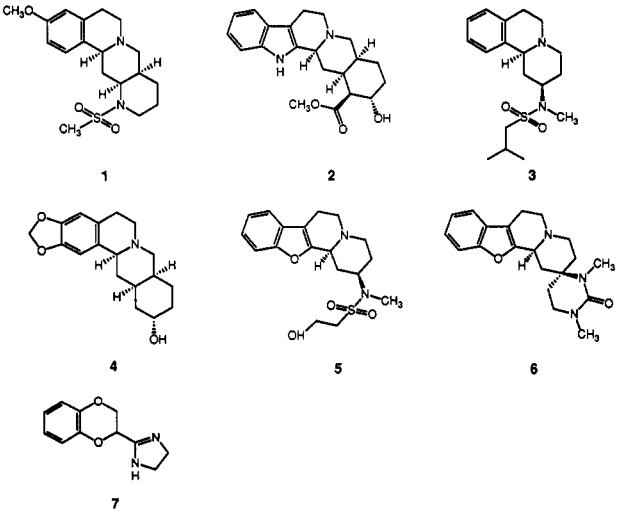
Analogues of the potent α_2-adrenoceptor antagonist (8a*R*,12a*S*,13a*S*)-5,8,8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(methylsulfonyl)-6*H*-isoquino[2,1-*g*][1,6]naphthyridine (**1b**) were prepared and evaluated for α_1- and α_2-adrenoceptor affinity. Affinity for α_2-adrenoceptors was assessed by displacement of [^{3}H]yohimbine from rat cerebral cortical membranes and although **1b** and close structural analogues demonstrated high affinity, none were selective for the α_{2A} or α_{2B} subtypes reputedly present in this tissue. All of the high affinity α_2-adrenoceptor ligands were, however, selective with respect to [^{3}H]prazosin (α_1) binding. Affinity for [^{3}H]yohimbine-labeled α_2-adrenoceptors was found to be highly dependent on the stereochemistry of the tetracyclic system. The 8aβ,12aα,13aα diastereomer of **1** (**56**) had moderate affinity for α_2-adrenoceptors while the 8aβ,12aβ,13aα diastereomer (**55**) had very low affinity. The affinity and selectivity of these agents for α_2-adrenoceptors was found to correspond to that observed for several isomeric yohimbine analogues which have similar relative and absolute stereochemistries. Deviation from the structure of **1** by opening the B ring, changing the position of the sulfonamide nitrogen, or changing the attachment of the D ring led to a dramatic decrease in α_2-adrenoceptor affinity. High binding affinity was found to correlate with functional antagonism in the guinea pig ileum. The reversal of clonidine-induced mydriasis in the rat was used to assess bioavailability and indicated that **1b** was a potent α_2-adrenoceptor antagonist in vivo.

We have previously described the synthesis and preliminary pharmacological profile of the potent and selective α_2-adrenoceptor antagonist **1**.[2] This compound is a representative of a class of α_2-adrenoceptor antagonists related to the early prototype rauwolscine (**2**).[3] Other members of this class include the hexahydrobenzo[*a*]quinolizine WY-26703 (**3**),[4] the berbane derivative CH-38083 (**4**),[5] and the hexahydro-2*H*-benzo[*b*]furo[2,3-*a*]quinolizines L-654,284 (**5**)[6] and L-657,743 (**6**).[7] These agents are structurally dissimilar to the imidazoline α_2-adrenoceptor antagonists typified by idazoxan (**7**).[8] The potential therapeutic utility of selective α_2-adrenoceptor antagonists, which may be principally in the treatment of depression, has been reviewed.[9]

We based the design of compound **1** on the structures of rauwolscine (**2**) and **3**. Of primary interest were the conformational constraints imposed by the D ring in **1** which would determine the orientation of the sulfonamido group. The sulfonamido group would therefore occupy the same region of space as the carbomethoxy group of rauwolscine. The critical importance of the spatial orientation of the carbomethoxy group in determining α_2-adrenoceptor



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