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2′-Substituted analogs of cocaine: synthesis and dopamine transporter binding potencies

A series of 2′-substituted cocaine analogs **(4–8)** was prepared and evaluated in an *in vitro* dopamine transporter (DAT) binding assay. Compounds **4–7** were prepared by esterifying the 3β-hydroxyl group of ecgonine methyl ester **(3)** using the appropriate acid chloride in the presence of Et_3N and benzene. Compound **3** was obtained from cocaine **(1)** by hydrolysis using 1N HCl to afford ecgonine·HCl which was subjected to acid catalyzed esterification using methanol saturated with HCl gas. Compound **8** was obtained by hydrogenation of **7** using H_2/Pd-C. The IC_{50} values were calculated from displacement experiment of the radioligand [^{3}H]WIN-35,428 **(2)**. 2′-Aminococaine **(8)** showed high binding affinity to the DAT (14- and 1.3-fold more active than cocaine and the radioligand **2**, respectively). These results, along with previous results, emphasize the importance of a hydrogen-bond donor group at the 2′-position of cocaine to enhance binding affinity to the DAT.

Key Words: Cocaine analogs; Substituted cocaines; Cocaine antagonists; Dopaminergic system

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Introduction

The natural component of coca leaves (*Erythroxylum coca*), known as (–)-cocaine, is a psychostimulant and a powerful reinforcer[1] known to bind to specific sites in mammalian brain [2–4]. Cocaine is a widely abused drug. Crack cocaine remains a serious problem in the United States. The estimated number of current crack users was about 604,000 in 1997, which does not reflect any significant change since 1988 [5]. A correlation of the potencies of cocaine and cocaine analogs in drug self-administration with their potencies to inhibit dopamine uptake and with their binding affinities has supported the existence of a cocaine receptor at the dopamine transporter [6]. While cocaine inhibits the neuronal uptake of dopamine (DA) [6], serotonin (5-HT) [7], and norepinephrine (NE) [8], the rewarding properties of cocaine require activation of the dopaminergic system and behaviors associated with cocaine addiction result, to a large extent, not from a direct message elicited by the binding of (–)-cocaine but rather from the accumulation of dopamine in the synapse and its action at one or more of the D_1–D_5 dopamine receptors [9].

In the past few years, substantial structural information has been obtained by structure-activity relationship (SAR) work done by many contributors such as Carroll and co-workers[10–15] and Kozikowski [16–18].

A few publications describe the effects of substitution at the 2′-position [19–22]. In contrast to most, recently we reported highly potent 2′-substituted cocaine analogs with binding potencies in the range of the 3-phenyltropanes WIN-35,428 **(2)** which is unusual for the benzoyl ester class of tropane [21–23]. For this reason, we began a limited characterization of the chemical nature of 2′-substituents on binding affinities to the DAT. In contrast to most substituted cocaines, certain substituents at 2′-position increase significantly the binding potency of cocaine for DAT [22].

In this manuscript we report the synthesis of certain 2′-substituted cocaine analogs as inhibitors of [^{3}H]WIN-35,428 uptake by the DAT.

The structures of cocaine, WIN-35,428 and the prepared compounds are shown in **Figure 1.**

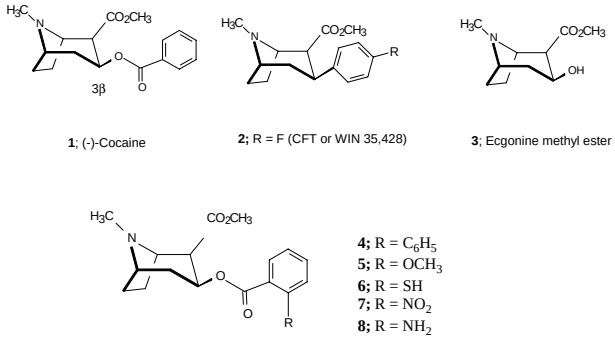

Figure 1

Results and discussion

Chemistry

The intermediate 2β-methyl ecgonine **(3)** was synthesized, following a reported procedure [21], via the hydrolysis of

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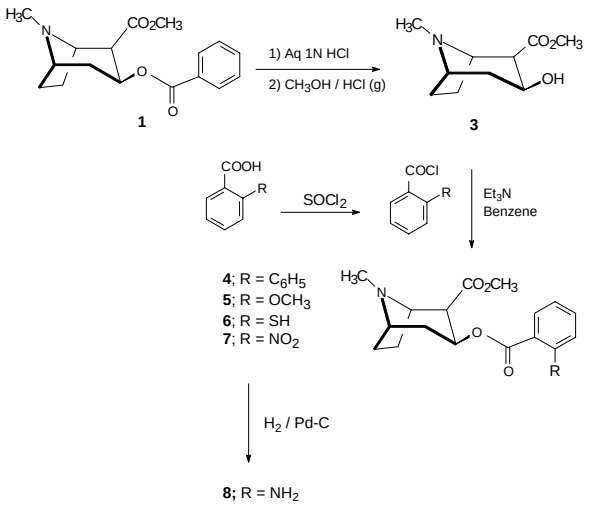

Scheme 1

cocaine·HCl **(1)** with aqueous 1N HCl, followed by acid catalyzed esterification of ecgonine·HCl using methanol and HCl gas. Compounds **4–7** were prepared, according to **Scheme 1**, by esterifying the 3β-hydroxyl group of **3** using the appropriate acid chloride in presence of triethylamine and benzene [21]. Compound **8** was obtained by hydrogenation of **7** using H_2/Pd-C.

Physical data are summarized in **Table 1**.

Table 1. Physical data of **4–8**.

Cpd	Mp °C	Column chromatography[a] (mobile phase)	Recryst Solvent	Yield %
4	103–104[b]	EtOAc : pet ether[c]	EtOH : Et_2O	64
5	88–90[d]	EtOAc : pet ether[c]	Isopropanol	71
6	178–180[b]	EtOAc : pet ether[c]	MeOH : Et_2O	65
7	95–97[d]	EtOAc	Isopropanol	87
8	80–82[d]	None	MeOH : Et_2O	97

[a]Silica gel, [c](1 : 1), [b]HCl salt and [d]tartrate salt.

Table 2. Dopamine transporter binding affinities of **4–8**.

Cpd	IC_{50} (nM)
Cocaine **(1)**	249 ± 37
[^{3}H]WIN-35,428 **(2)**	24 ± 4
4	8162 ± 152
5	46654 ± 840
6	3224 ± 172
7	8440 ± 986
8	18 ± 2

IC_{50} values are mean ± standard error of the mean (SEM) of two to three experiments performed in triplicate.

Dopamine transporter binding assays

Compounds **4–8** were tested for their abilities to displace [^{3}H]WIN-35,428 and the IC_{50} values for inhibiting 4 nM of the radioligand binding to DAT are listed in **Table 2.**

The prepared compounds had 2′-substituents of different chemical nature as follows: (a) a bulky group, **4**, (b) an electron-donating group, **5** and **6**, (c) an electron-withdrawing group, **7**, and (d) a hydrogen-bond donor, **8**.

Compounds **6** (2′-SH) and **8** (2′-NH_2) were prepared in view of the high binding potency shown by 2′-hydroxycocaine which has an IC_{50} (mean ± SEM) value of 25 ± 4 nM (about 10-fold more active than cocaine which has an IC_{50} value of 249 ± 37 nM) and is nearly of equal potency to the radioligand WIN-35,428 **(2)** [21,23]. As shown in **Table 2,** 2′-NH_2 cocaine had a binding potency with an IC_{50} value of 18 ± 2 nM, which is about 14- and 1.3-fold more active than cocaine and **2**, respectively. It was postulated that the hydroxyl group, and similarly the amino group, may engage in an intermolecular hydrogen-bonding with the serine residues at the acceptor site of dopamine transporter [21]. The high binding potency exhibited by 2′-OH and 2′-NH_2 (unusual for the benzoyl ester class of tropanes) emphasized the importance of a hydrogen-bond donor group at this position in enhancing binding to DAT. These results are in accord with previous work published by our group [23]. This work included a bulky group (2′-CH_3), an electron-donating group (2′-$OCOCH_3$), a hydrogen-bond acceptor group (2′-F) and a hydrogen-bond donor group (2′-OH), which had IC_{50} values (mean ± SEM) of 251 ± 96, 70 ± 1, 604 ± 67 and 25 ± 4 nM, respectively.

Acknowledgment

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Experimental

General

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Midwest Microlab LTD, Indianapolis, IN, USA. NMR spectra were recorded on a Varian XL-300 spectrometer. All organic reagents were obtained from Aldrich Co. and were used without further purification. Silica gel (200–400 mesh, 60 Å) used for column chromatography was obtained from Aldrich and silica gel chromatographic sheets with a fluorescent indicator used for thin layer chromatography (TLC) were obtained from Eastman Kodak Co., Rochester, NY. [^{3}H]WIN-35,428 was obtained from Dupont-New England Nuclear, Boston, MA. Homogenization of the striata was performed using a Polytron Homogenizer, Kinematic Kriens-Luzern, Switzerland. Centrifugation of the membrane homogenate was carried out using a Dupont Sorvel RC-5 superspeed refrigerated centrifuge (rotor SS-34). Filtration of the bound-membrane was carried out using a Brandel Cell Harvester. Counting of the activity of the receptor-bound ligand was performed using a Beckman LS 1701 liquid scintillation counter.

Chemistry

General procedures for the preparation of 2′-cocaine analogs [21]

*3β-[(2′-Substituted-benzoyl)oxy]-1R-(exo,exo)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester (**4–7**)*

Ecgonine methyl ester·HCl **(3)** was obtained from cocaine by hydrolysis with 1N HCl and the produced ecgonine·HCl was subjected to acid catalyzed esterification by stirring ecgonine·HCl overnight with MeOH saturated with HCl gas**.** To the free base of **3** (0.30 g, 1.50 mmol) dissolved in dry C_6H_6 (15 mL) was added Et_3N (1 mL, 10 mmol). To the stirred solution were added, under dry N_2, 2-phenyl-, methoxy-, mercapto-, or nitro benzoyl chloride (2.25 mmol). The reaction mixtures were stirred, under dry N_2, at 40 °C overnight. The reactions were stopped and the benzene layer was washed with H_2O (10 mL) and 5% aqueous Na_2CO_3 solution (3 × 5 mL) and dried over $MgSO_4$ (anhyd), and the solvent was removed under vacuum to give oils. The oils were purified on a silica gel column, cf. **Table 1**, to afford the pure oils. The oils were converted to the tartrate or HCl salts. Elemental analyses (C, H, and N) were within ± 0.4 % of calculated values.

^{1}*H-NMR (D$_2$O, δ)*

4: 7.66–7.63 (m, 1H, C(6′)H), 7.52–7.47 (m, 1H, C(4′)H), 7.35–7.30 (m, 1H, C(5′)H), 7.28 (s, 5H, C_6H_5), 7.15–7.12 (m, 1H, C(3′)H), 5.17–5.13 (m, 1H, C(3)H), 3.97–3.95 (m, 1H, C(1)H), 3.75 (m, 1H, C(5)H), 3.43 (s, 3H, $COOCH_3$), 3.26–3.24 (m, 1H, C(2)H), 2.64 (s, 3H, NCH_3), 2.27–2.16 (m, 4H, C(4,7)H), 1.96–1.89 (m, 2H, C(6)H).

5: 7.65–7.62 (dd, 1H, C(6′)H), 7.52–7.46 (m, 1H, C(4′)H), 7.35–7.30 (m, 1H, C(5′)H), 7.06–7.04 (d, 1H, C(3′))H), 6.95–6.90 (m, 1H, C(5′)H), 5.41–5.38 (m, 1H, C(3)H), 4.08–4.06 (m, 1H, C(1)H), 3.94–3.92 (m, 1H, C(5)H), 3.75 (s, 3H, $COOCH_3$), 3.50 (s, 3H, Ar-OCH_3), 3.26–3.24 (m, 1H, C(2)H), 2.72 (s, 3H, NCH_3), 2.33–2.22 (m, 4H, C(4,7)H), 1.93–1.89 (m, 2H, C(6)H).

6: 7.76 (m, 1H, C(6′)H), 7.50 (m, 2H, C(4′,5′)H), 7.34–7.31 (m, 1H, C(3′)H), 5.41–5.33 (m, 1H, C(3)H), 4.05–4.03 (m, 1H, C(1)H), 3.91–3.85 (m, 1H, C(5)H), 3.51 (s, 3H, $COOCH_3$), 3.47–3.45 (m, 1H, C(2)H), 2.66 (s, 3H, NCH_3), 2.29–2.05 (m, 4H, C(4,7)H), 2.04–1.98 (m, 2H, C(6)H).

7: 7.88–7.85 (m, 1H, (3′)H), 7.70–7.62 (m, 3H, (4′, 5′, 6′)H), 5.47–5.43 (m, 1H, C(3)H), 4.05–4.03 (m, 1H, C(1)H), 3.90 (m, 1H, C(5)H), 3.46 (s, 3H, COO**CH_3**), 3.33–3.00 (m, 1H, C(2)H), 2.67 (s, 3H, N**CH_3**), 2.30–2.28 (m, 4H, C(4, 7)H), 2.05–2.00 (m, 2H, C(6)H).

*3β-[(2′-Aminobenzoyl)oxy]-1R-(exo,exo)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester (**8**)*

To a 100 mL sealed round-bottomed flask under vacuum were added 0.3 g (0.86 mmol) of **7**, 30 mg of palladium on activated carbon and 40 mL cyclohexane. A hydrogen balloon was attached to the flask while it was under vacuum to allow hydrogen to be sucked into the flask. The mixture was stirred for 24 h. The crude product was filtered over celite and the solvent was evaporated to obtain (0.27 g, 97% yield) of a pure oil. The oil was converted to the tartrate salt and recrystallized from methanol/ether. Elemental analyses (C, H and N) were within ±0.4 % of calculated values.

^{1}*H-NMR (D$_2$O, δ)*

8: 7.57–7.54 (d, 1H, C(6′)H); 7.25–7.19 (t, 1H, C(4′)H); 6.72–6.69 (d, 1H, C(3′)H); 6.59–6.57 (t, 1H, C(5′)H); 5.42–5.34 (m, 1H, C(3)H); 4.04–4.02 (m, 1H, C(1)H); 3.88 (m, 1H, C(5)H); 3.74 (s, 3H, $COOCH_3$); 3.46–3.43 (m, 1H, C(2)H); 2.69 (s, 3H, NCH_3); 2.24–2.20 (m, 4H, C(4, 7)H; 2.01–2.00 (m, 2H, C(6)H).

Dopamine transporter binding assays

The binding was performed in rat striatal tissue using the method of Reith.[24]

Materials: [^{3}H]WIN-35,428 was obtained from Dupont-New England Nuclear, Boston, MA.

Preparation of test substances: stock solutions of the test substances were prepared freshly by dissolving them in the incubation buffer (phosphate buffer pH 7.4).

Phosphate buffer: obtained by mixing 35 mM NaH_2PO_4 and 17.5 mM Na_2HPO_4 to obtain pH 7.4.

Membrane preparation: Whole brains from male Sprague-Dawley rats weighing from 350–400 g (Sasco Inc, Wilmington, MA) were rapidly harvested after decapitation with a guillotine. The striata were isolated and homogenized using a Polytron Homogenizer (setting 6 for 15 s) in ice-cold 0.32 M sucrose solution (1.5 mL/100 mg of tissue). The homogenizer and blade were then rinsed with twice the volume of 0.32 M sucrose solution which was added to the homogenate. The combined mixture was centrifuged at 3,300 rpm for 10 min at 4 °C. The resulting supernatant was subsequently centrifuged at 13,800 rpm for 20 min at 4 °C to obtain a pellet (P_2), which was homogenized in ice-cold 35 mM phosphate buffer.

Each assay tube contained 130 μL buffer or buffer plus 10 ?mL of unlabeled test compound (1×10^{-10} to 100×10^{-6} M), [^{3}H]WIN-35,428 in the same buffer (20 μL, 4 nM) and 50 μL of membranes (4 mg/mL) to a total volume of 200 μL. (–)-Cocaine (100 μM) was used for non-specific binding. Assays performed in triplicate were incubated for 2 h in an ice bath and terminated by rapid filtration through Whatman GF/B glass fiber filters presoaked for 30 min in 0.05% polylysine solution. Membranes were rapidly washed three times with ice-cold buffer. Filters containing membrane-bound radioligand were added to vials containing 10 mL of scintillation fluid (Ecolume, Costa Mesa, CA), stored overnight, and counted for 5 min on a Beckman LS 1701 liquid scintillation counter.

Calculation of IC_{50}: IC_{50} values were determined from competition curves of twelve points using the curve-fitting program EBDA (Biosoft software, Ferguson, MO). Mean values and standard errors were calculated from two to three assays for each test compound.

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