Redox Derivatives of Tranylcypromine: Syntheses, Properties, and Monoamine Oxidase Inhibitor Activity of Some Chemical Delivery Systems

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Abstract □ Several brain-targeting chemical delivery systems (CDS) based on a dihydropyridine ⟷ pyridinium salt type redox system were synthesized for the monoamine oxidase (MAO) inhibitor tranylcypromine (TCP). The dihydronicotinate moiety was chemically attached to the amino group of TCP by either an amide or substituted carbamate linkages. Physicochemical studies of the new derivatives, including chromatographic R_m determinations, were performed. Only the substituted carbamate-type derivatives manifested an increased lipophilicity relative to the parent compound. In vitro oxidation stability studies were also performed on selected derivatives using a ferricyanide-mediated method. Results of this assay showed that the dihydropyridine-type derivatives oxidized to the respective quaternary salt forms with stabilities which empirically correlated with other effective CDSs. Preliminary in vivo studies performed in rats indicated that some of the new derivatives exerted significant biological activity.

Monoamine oxidase (MAO) is a flavoprotein which is present in mitochondrial membranes and catalyzes the oxidative deamination of the primary amino group of neurotransmitters (noradrenaline, dopamine, serotonin) to the corresponding aldehydes. Other enzymes then convert the aldehyde to the corresponding alcohol or carboxylic acid. The MAO catabolizes excess neurotransmitters subsequent to induction of their action. Any interference with MAO activity prolongs the action of the transmitter, and such interferences have been found to be of therapeutic value in the treatment of depression.[1]

Monoamine oxidase inhibitors are a heterogenous group of drugs which block the oxidative deamination of naturally occurring monoamines.[2,3] These drugs, including hydrazines, hydrazides, amines, or acetylene derivatives, were the first clinically useful antidepressant drugs and as such have had an important impact on the development of modern biological psychiatry. However, their usefulness is tarnished because of their unpredictable efficacy since MAO inhibitors interact with many drugs and food-derived amines and therefore exert notorious side effects such as hepatic necrosis and orthostatic hypotension. As a result, they are generally used only when tricyclic antidepressants, which have a significantly greater therapeutic index, give unsatisfactory results and when electroconvulsive therapy is contraindicated or refused. However, the general opinion is that MAO inhibitors have been underrated and that novel, safer MAO inhibitors would lead to an increase in their use. A selective inhibitor of MAO, with potent central activity and minimal peripheral effects, would be a useful antidepressant.[4]

Some of the herein enumerated goals may be achieved by the application of the chemical delivery (CDS)[5-7] approach to certain MAO inhibitors. *trans*-(±)-2-Phenylcyclopropanamine (tranylcypromine, TCP; **1**)[3,8] is one of the few MAO inhibitor drugs still in use and was therefore considered for this study. Tranylcypromine is a mechanism-based (or suicide) inhibitor, meaning that the unreactive compound is converted by the enzyme into an active species which inhibits the enzyme via covalent interaction.[9,10] Unlike other MAO inhibitors, **1** produces an inhibition which is reversed more rapidly, since the bound drug can be hydrolyzed from the enzyme. In addition, **1** has a more rapid onset of action. Although withdrawn from the market in 1964 because of toxicity, **1** again became available for use under close medical supervision.[3] It is known that one of the main advantages of the dihydropyridine ⟷ pyridinium salt redox carrier system-based CDS approach (discussed in detail elsewhere)[5-7] is the ability to enhance the central nervous system (CNS) concentration of the drug via delivery of a biologically inactive derivative (the quaternary pyridinium salt-type metabolite of the CDS), which is simultaneously rapidly eliminated from the systemic circulation. This manipulation can lower the dosage of the drug and reduce peripheral toxic side effects, improving the therapeutic index of the drug.[11-14] The CDS approach was also deemed feasible since other drug latentation methods have already been applied to **1**, resulting in improved pharmacological action.[15,16] Interestingly, the tissue distribution of the drug is not preferential and neither an accumulation of the drug in brain nor the reduction of peripheral toxicity has been achieved using classical prodrug schemes.

The derivatization of **1** in order to generate the CDSs, a study of several physicochemical properties, as well as a preliminary study of the in vivo MAO inhibitory properties of these has been the goal of this work.

Results and Discussion

The various synthetic procedures which have been used in order to obtain the CDSs of **1** are summarized in Schemes I–V. The CDS **4**, the simple dihydrotrigonelline amide, was obtained by acylating **1** with nicotinoyl chloride, followed by the *N*-methylation of the resulting amide (**2**) with methyl iodide. Reduction of the resulting quaternary salt (**3**) with aqueous basic sodium dithionite produced **4** as shown in Scheme I. Another amide type of CDS (**8**), in which the pyridine moiety is attached to **1** as a substituted acetamide containing an ester linkage, was obtained by acylating **1** with bromoacetyl chloride, followed by reacting the resulting bromoacetamide **5** with a salt (triethylammonium) of nicotinic acid. The resulting nicotinate **6** was then *N*-methylated and the quaternary salt **7** was reduced with sodium dithionite (Scheme II). In **10a** and **10b**, the brain-targeting dihydronicotinate moiety is attached via the ring nitrogen as a substituted amide. Compound **10a** was obtained by reacting **5** with nicotinamide in a polar, aprotic solvent. Reduction of the resulting quaternary

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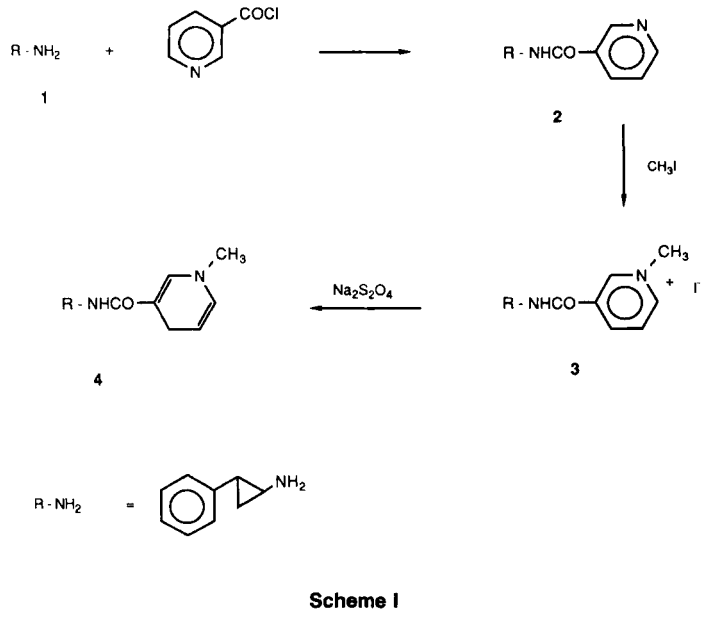
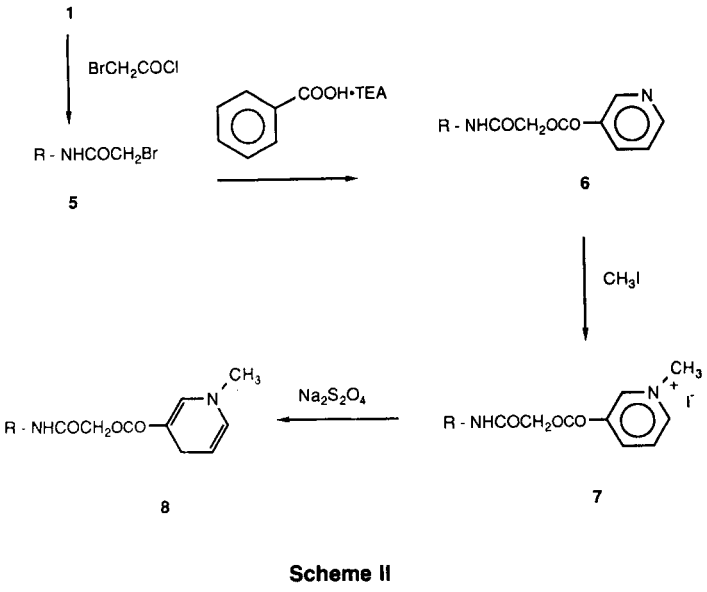
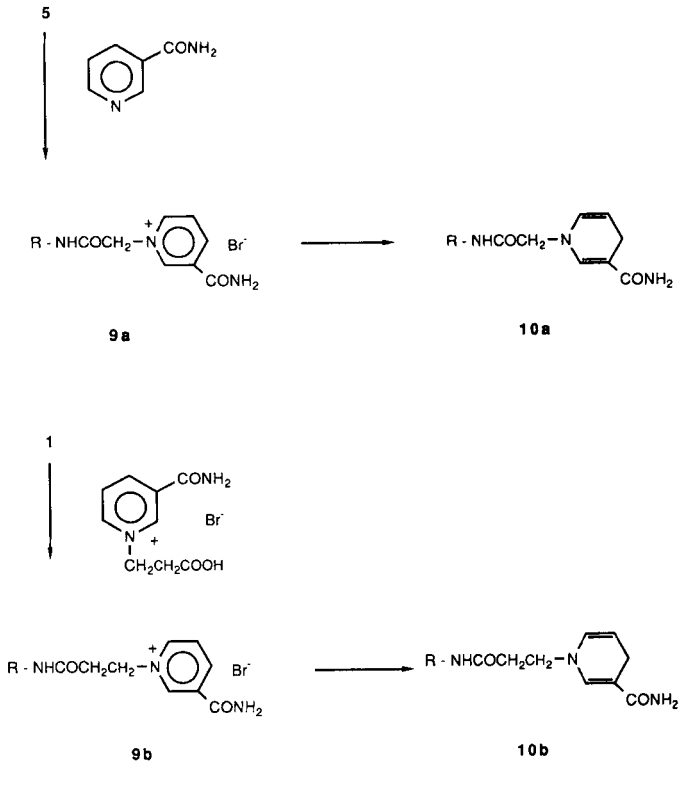


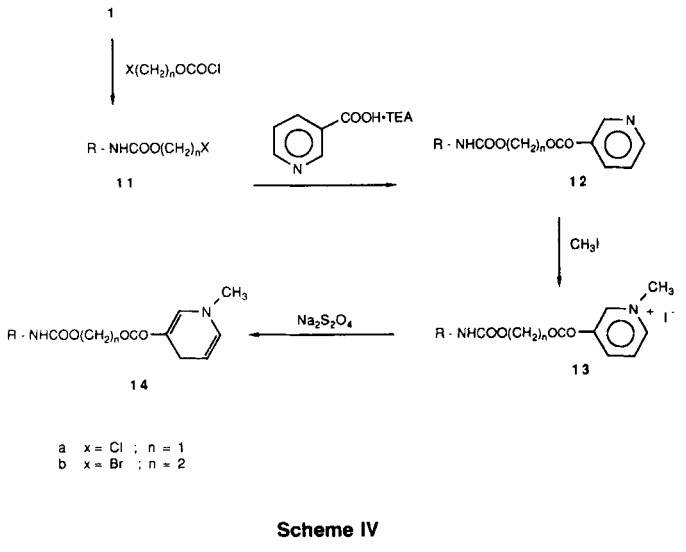
Scheme I



Scheme II



Scheme III



Scheme IV

salt **9a** with sodium dithionite resulted in the dihydropyridine. Compound **10b** was obtained by reacting **1** with 3-carbamoyl-1-carboxyethylpyridinium bromide in the presence of dicyclohexylcarbodiimide, which was used as dehydrating agent, and by reducing the resulting quaternary salt **9b** with sodium dithionite (Scheme III).

In Scheme IV, the synthesis of two CDSs in which the pyridine moiety is attached to the drug by a hydrolytically labile substituted carbamate linkage is described. The haloalkyl carbamate intermediates **11** were obtained by acylation of **1** with haloalkyl chloroformates. Chloromethyl chloroformate was synthesized by reacting formaldehyde with phosgene, while bromoethyl chloroformate was obtained from commercial sources. The haloalkylcarbamates were then reacted with a triethylammonium salt of nicotinic acid to give the carbonyloxyalkyl pyridine carboxylates of **1** (**12**). These were *N*-methylated and the resulting reduced quaternary salts (**13**) formed the CDSs **14**.

The general method used for reducing the pyridinium salts involved sodium dithionite reduction in a biphasic solvent system consisting of ethyl acetate and aqueous sodium bicarbonate. This approach is known to give regioselectively the 1,4-dihydropyridines isomers.[17]

The synthesized compounds were characterized by elemental analysis, MS, UV spectrophotometry, and ^{1}H NMR spectroscopy. Chromatographic techniques (TLC) indicated only one component in each case. The dihydropyridines were shown to be the 1,4-isomers by UV spectrophotometry and by ^{1}H NMR spectroscopy.

All dihydropyridines were shown to be converted to the corresponding quaternary salt via methanolic silver nitrate or H_2O_2 in the presence of catalytic cupric ions. A ferricyanide-mediated study was performed for various dihydronicotinates to compare their oxidative stability. The oxidations were monitored using a spectrophotometric method,[18] and the

data are summarized in Table I. The second-order oxidation rates were found to fall within the range 0.46–1.60 $s^{-1}M^{-1}$, indicating that the CDSs had adequate stability toward oxidation to be formulated and manipulated. The values correlated empirically with successful CDS examples.

Lipophilicity is an important factor controlling the interaction of drugs with biological systems. Lipophilic character is essential for a CDS to penetrate the blood–brain barrier (BBB).

Chromatographic R_m methods can be used as lipophilicity indices[19] since they are related to the partition coefficient between the mobile and stationary phase of a chromatographic system.[20–23] The R_m values were measured for a series of compounds by means of a reversed-phase TLC method in which varying concentrations of aqueous acetone were used as the mobile phase. The determined R_m values are shown in Table II. The lipophilic indices presented in Table III were calculated by extrapolation of the linear portion of R_m value curves to a 100% aqueous mobile phase. Tranylcypromine itself is a rather lipophilic molecule, which explains the good CNS uptake of the drug. It is not surprising then that most of the CDSs were less lipophilic relative to **1**. Only the two substituted carbamate-type CDSs (**14a** and **14b**) were more lipophilic, the substituted amide **8** was of about the same lipophilicity as the parent drug, while the simple nicotinamide (**4**) and the pyridine ring nitrogen attached carrier-type CDSs (**10a** and **10b**) had considerably lower lipophilicity. The relative lipophilicity increases in the following order: **4** < **10b** ≤ **10a** < **8a** ≤ **1** < **14b** < **14a**.

In the series of compounds produced, it is expected[24,25] that the amide type CDSs **4**, **8**, and **10a,b**, as well as their quaternary salt-type synthetic precursors and metabolic successors **3**, **7**, and **9a,b**, are stable toward both chemical and enzymatic hydrolysis and slowly release the parent drug. Compounds **8** and **7** should be hydrolyzed faster at the ester site, but the resulting hydroxyacetamide of **1** is probably stable toward further hydrolysis: **7** → $R\text{-}NHCOCH_2OH$ → $R\text{-}NH_2$. On the other hand, the substituted carbamate type CDSs **14a** and **14b** and the respective quaternary salts **13a** and **13b** should generate **1** more rapidly via both chemical and enzymatic hydrolysis as their labile sites are acyloxyalkyl ester linkages. The hydroxyalkoxycarbamates (hemiketals), which result as the first products of hydrolysis, are unstable in an aqueous environment and should decompose spontaneously to the parent compound through an intermediary carbamic acid. The side products of the reaction are *N*-methyl nicotinic acid, an aldehyde, and carbon dioxide: **13** → $R\text{-}NHCOO(CH_2)_nOH$ → $R\text{-}NHCOOH$ → $R\text{-}NH_2$.

The presented physicochemical study indicates that all CDSs possess the required properties to deliver the drug into the brain, across the BBB, and to generate by oxidation the quaternary salt type derivatives. However, the release of **1** from these polar species "locked-in" the CNS (but eliminated from periphery) by enzymatic hydrolysis is predicted to be faster in the case of **13a** and **13b**.

Preliminary Activity Study—A preliminary in vivo MAO inhibition study was performed using the synthesized derivatives of **1** in the rat. The design of the activity study was quantitation of the ability of **1** to inhibit the MAO capacity to oxidate endogenous dopamine (DA) to 3,4-dihydroxyphenylacetaldehyde, which in turn, generates 3,4-dihydroxyphenylacetic acid (DOPAC) under the catalytic influence of the NAD-linked aldehyde dehydrogenase. Rats were administered either **1** at a dose of 10 mg/kg or equimolar doses of **4**, **8**, **10**, or **14**, (dose range 19.15 to 25.9 mg/kg) via the tail vein. Drug was solubilized in dimethyl sulfoxide. Based on preliminary activity results which indicated that time of maximum MAO inhibition after administration of **1** was 30 min, rats were killed 30 min subsequent to dosing of **1** or various dihydropyridine derivatives, and concentrations of DA and DOPAC were determined in the paired striata (putamen and caudate nuclei) and cortex by HPLC with electrochemical detection. The DA and DOPAC levels, as well as the DA:DOPAC ratio, are indicated in Figures 1–3. A higher DA concentration value or DA:DOPAC ratio and a lower DOPAC concentration suggests a greater MAO inhibition effected by the administered compound. The DA:DOPAC ratio better illustrates the drug potency. Figures 1–3 indicate that the DA:DOPAC ratios of **14a** (substituted carbamate) and **8** are significantly ($p < 0.05$) higher than vehicle controls, indicating MAO inhibition activity at the 30-min timepoint. The activity of **14a** was expected since it was the most lipophilic of the CDSs and most likely to rapidly release **1**. The activity shown by **8** might suggest that the stability of the intermediate hydroxyacetamide of **1**, the probable metabolite of the hydrolysis catalyzed by esterases, is such that release of the parent derivative is rapid (compared with the case of desipramine[24]) and **1** was released.

No toxic side effects were registered with any of the dihydropyridine derivatives. However, a detailed toxicity study has not yet been done.

Table I—Second-Order Rate Constants (k) of Ferricyanide-Mediated Oxidation of Tranylcypromine Chemical Delivery Systems

Compound	k_o, $s^{-1}M^{-1}$	r[a]
8	1.562	0.999
14a	0.3510	1.000
14b	0.4587	0.980

[a] Correlation coefficient.

Table II—R_m Values for Trans-(±)-2-phenylcyclopropanamine Chemical Delivery Systems in Different Mobile Phases (Acetone:Water)

Compound	Acetone, %				
	30	50	60	70	80
4	0.6580	0.4280	0.0890	−0.2350	−0.5210
8a	1.3160	0.4878	0.1270	−0.2500	−0.5670
10a	0.6200	0	−0.3520	−0.6728	−0.9437
10b	0.6450	−0.0570	−0.3365	−0.6640	−0.9366
14a	0	0.8404	0.3420	−0.0768	−0.4180
14b	0	0.7830	0.2710	−0.1156	−0.4437
TCP (**1**)	1.4990	0.5840	0.3400	−0.0128	−0.2860

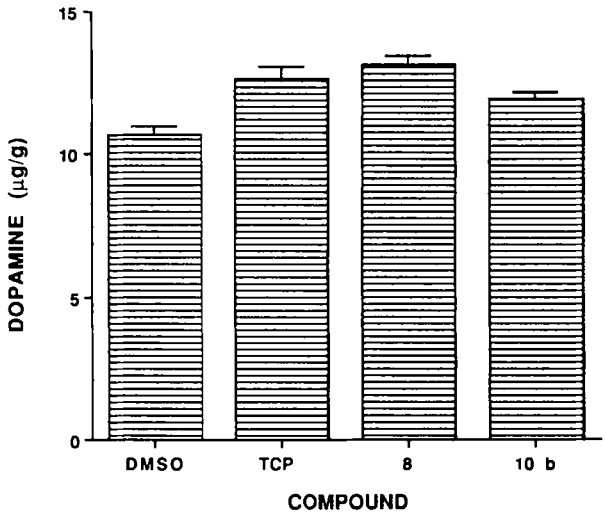
Table III—Lipophilic Indexes (Extrapolated R_m Values) for Tranylcypromine Chemical Delivery Systems

Compound	R_m[a]	r
4	1.49	0.9760
8a	2.41	0.9980
10a	1.57	0.9990
10b	1.56	0.9980
14a	2.89	0.9960
14b	2.76	0.9940
TCP (**1**)	2.47	0.9900

[a] $R_m = \log (1/R_f - 1)$.

Experimental Section

Uncorrected melting points were determined using an Electrothermal melting-point apparatus (Fisher Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA. Ultraviolet (UV) spectra were determined using a Hewlett-Packard 8451A diode array spectrophotometer. Proton nu-



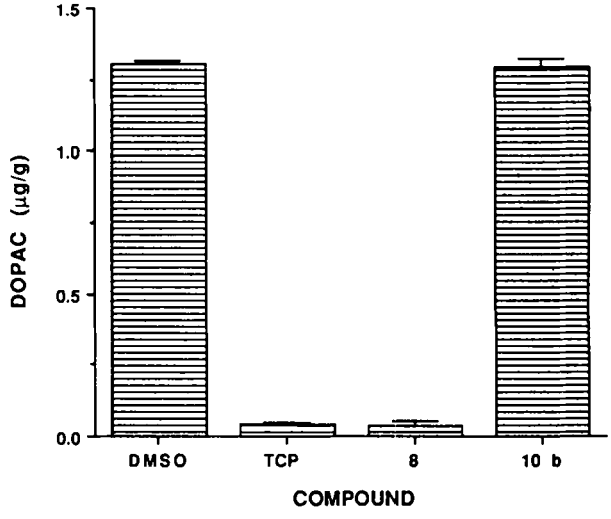
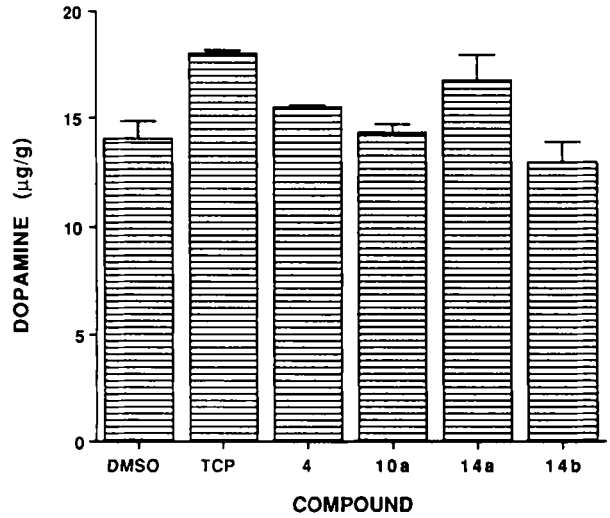


Figure 1—Dopamine (DA) levels in rat brain tissues 30 min after iv administration of 10 mg/kg of **1** or equimolar doses of CDSs.



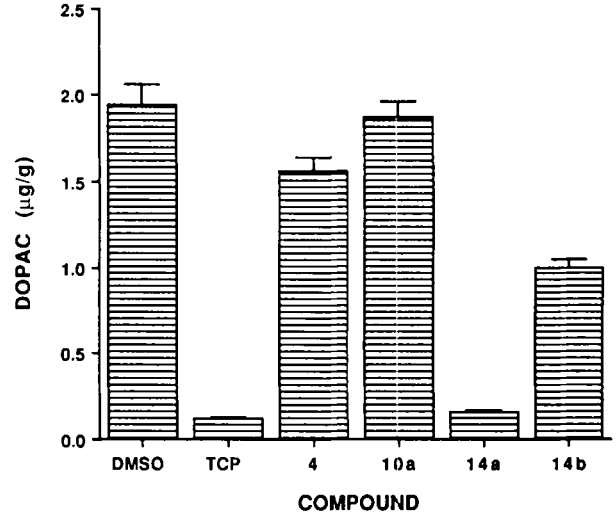


Figure 2—3,4-Dihydroxyphenylacetic acid (DOPAC) levels in rat brain tissue 30 min after iv administration of 10 mg of **1** or equimolar doses of various CDSs.

clear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian XL 200 (200-MHz; FT mode) spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported in parts per million (δ) relative to tetramethylsilane as an internal standard. Coupling constants (J) are reported in hertz. Mass spectra were recorded on a Kratos MS 80-RFA instrument. Fast atom bombardment (FAB) ionization was performed via xenon beam (6 KeV) impingement and the samples were dissolved in a glycerol matrix as described in the literature.[26] Thin-layer chromatography (TLC) was performed on EM Reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel 60 using methanol:chloroform (1:8) as the mobile phase. All chemicals were reagent grade. Tranylcypromine hydrochloride was obtained from Aldrich Chemical Company.

(±)-*N*-(*trans*-2-Phenylcyclopropyl)nicotinamide (2)—To a solution of 1.69 g (10 mmol) of the hydrochloride salt of **1** in 20 mL of dry pyridine, 1.95 g (11 mmol) of nicotinoyl chloride hydrochloride were added. The mixture was stirred at room temperature for 1 d, poured into 200 mL of water, and extracted twice with chloroform. The organic layer was collected, dried, and reduced in volume under reduced pressure. The residue (1.67 g, 70% yield) was an off-white precipitate, mp 145–147 °C; R_f = 0.33; ^{1}H NMR ($CDCl_3$): δ 1.26–1.33 (m, 2H), 2.16–2.18 (m, 1H); 3.06–3.07 (m, 1H); 7.11–7.38 (m, 6H); 8.15 (d, 1H, J = 7.8), 8.66 (d, 1H, J = 6.74); 9.03 (s, 1H).

Anal.—Calc. for $C_{15}H_{14}N_2O$: C, 75.64; H, 5.87; N, 11.74. Found: C, 75.54; H, 5.93; N, 11.73.

(±)-1-Methyl-3-[(*trans*-2-phenylcyclopropyl)carbamoyl]pyridinium Iodide (3)—To a solution of 1.00 g (4.2 mmol) of **2** in 15 mL of acetone and 3 mL of methanol were added 1.5 mL of methyl iodide after which the solution was stirred at room temperature for 1 d. After removing the solvent, the residue (yellow oil) was dissolved in methanol and ether was added to precipitate 1.44 g (90% yield) of an off-white material, mp 163–165 °C; UV_{max} (MeOH): 212, 268 nm; R_f = 0; MS (FAB) C^+ m/z: 253; ^{1}H NMR (DMSO-d_6): δ 1.33–1.39 (m, 2H), 2.17–2.18 (m, 1H), 3.08–3.10 (m, 1H), 4.42 (s, 3H), 7.21–7.47 (m, 5H), 8.27–8.73 (m, 1H), 8.89 (d, 1H, J = 6.12), 9.12 (d, 1H, J = 7.16), 9.31 (bs, 1H), 9.41 (s, 1H).

Anal.—Calc. for $C_{16}H_{17}IN_2O$: C, 50.86; H, 4.42; N, 7.38; I, 33.38. Found: C, 50.82; H, 4.52; N, 7.33; I, 33.31.

(±)-1,4-Dihydro-1-methyl-*N*-(*trans*-2-phenylcyclopropyl)nicotinamide (4)—To a solution of 0.40 g (1.05 mmol) of **3** in 100 mL of deaerated water and 100 mL of ethyl acetate was added 0.53 g (6.30 mmol) of sodium bicarbonate and 0.73 g (4.20 mmol) of sodium dithionite. The system was maintained under an argon stream and was stirred for 2 h. The organic layer was separated and the aqueous layer extracted with 2 × 50 mL of ethyl acetate. The combined organic layers were washed with 2 × 50 mL of cold deaerated water, dried, and evaporated under reduced pressure to give 0.19 g (70%) of a

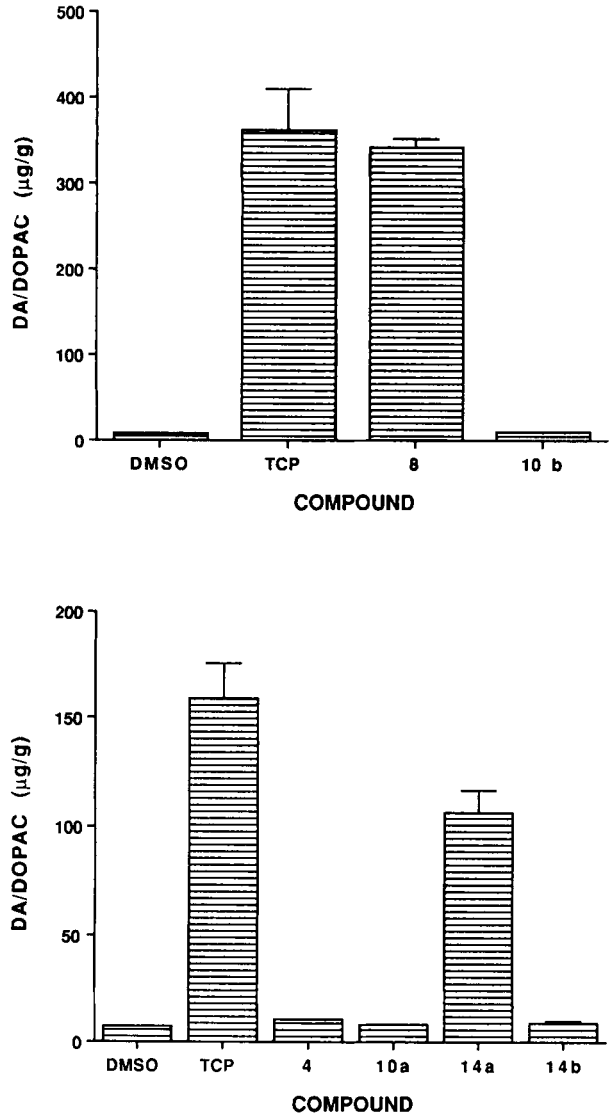


Figure 3—The DA:DOPAC ratio in rat brain tissues 30 min after iv administration of 10 mg/kg of **1** or various CDSs.

yellow oil, R_f = 0.61; UV_{max} (MeOH): 212, 358 nm; ^1H NMR (DMSO-d_6); δ 1.11–1.31 (m, 2H), 1.98–2.01 (m, 1H), 2.81–2.91 (m, 1H), 2.98 (s, 3H), 3.00–3.03 (m, 2H), 4.61–4.71 (m, 1H), 5.71 (d, 1H, J = 8.12), 7.02–7.78 (m, 6H).

Anal.—Calc. for $C_{16}H_{18}N_2O$: C, 67.14; H, 6.28; N, 9.79. Found: C, 67.41; H, 6.39; N, 9.61.

(±)-N-*trans*-(2-phenylcyclopropyl)bromoacetamide (5)—To a solution of 1.69 g (10 mmol) of **1** · HCl and 1.70 g (20 mmol) of sodium bicarbonate in 20 mL of chloroform were added 1.64 g (10.5 mmol) of bromoacetylchloride in 5 mL of chloroform at 0 °C. After 0.5 h, the ice bath was removed and the mixture was stirred for 2 d at room temperature and then poured into 50 mL of water; the organic phase was then washed, dried, and evaporated. The resulting brown oil was treated with acetone:hexane to result in the formation of 2.0 g (80%) of white solid, mp 63–65 °C; R_f = 0.65; ^1H NMR (DMSO-d_6): δ 1.12–1.16 (m, 2H), 1.90–1.92 (m, 1H), 2.90–2.92 (m, 1H), 3.85 (s, 2H), 7.11–7.31 (m, 5H).

Anal.—Calc. for $C_{11}H_{12}BrNO$: C, 51.98; H, 4.76; Br, 31.45; N, 5.55. Found: C, 51.95, H, 4.41; Br, 31.41; N, 5.54.

(±)-*trans*-2-Oxo-2-[(2-phenylcyclopropyl)amino]ethyl-3-pyridinecarboxylate (6)—To a solution of 2.53 g (10 mmol) of **5** in 15 mL of dimethylformamide were added 1.30 g (11 mmol) of nicotinic acid and 1.11 g (11 mmol) of triethylamine in 2 mL of dimethylformamide. The mixture was stirred at room temperature for 2 d and then poured into 10 g of ice and extracted twice with ethyl acetate. The organic phase was collected, dried, and evaporated resulting in 1.92 g (80%) of a yellow oil; R_f = 0.69; ^1H NMR (DMSO-d_6): δ 1.16–1.24 (m, 2H), 2.07–2.09 (m, 1H), 2.79–2.81 (m, 1H), 4.61 (s, 2H), 7.11–7.31 (m, 5H), 7.89–7.91 (m, 1H), 8.20–8.24 (m, 1H), 8.81 (d, 1H, J = 7.01), 9.12 (s, 1H).

(±)-*trans*-1-Methyl-3-[[2-oxo-2-[(2-phenylcyclopropyl)amino]ethoxy]carbonyl]pyridinium Iodide (7)—To a solution of 2.96 g (10 mmol) of **6** in 15 mL of acetone were added 2.84 g (20 mmol) of methyl iodide. The solution was stirred at room temperature for 1 d and was then treated with ether. The resulted yellow solid (3.94 g, 90%) was recrystallized from acetone:hexane, mp 175–178 °C; UV_{max} (MeOH): 226, 268 nm; R_f = 0; MS (FAB) C^+ *m/z*: 311; ^1H NMR (DMSO-d_6): δ 1.16–1.26 (m, 2H), 2.01–2.10 (m, 1H), 2.84–2.86 (m, 1H), 4.51 (s, 3H), 4.87 (s, 2H), 7.09–7.28 (m, 5H), 8.30–8.31 (m, 1H), 8.55 (d, 1H, J = 5.26), 9.05 (d, 1H, J = 6.85), 9.69 (s, 1H).

Anal.—Calc. for $C_{18}H_{19}IN_2O_3 \cdot H_2O$: C, 47.54; H, 4.52; I, 27.94; N, 6.16. Found: C, 47.56; H, 4.69; I, 28.00; N, 6.24.

(±)-*trans*-2-Oxo-2-[(2-phenylcyclopropyl)amino]ethyl 1,4-dihydro-1-methyl-3-pyridinecarboxylate (8)—First, 0.21 g (0.46 mmol) of **7** was dissolved in 100 mL of water containing 100 mL of ethyl acetate. Then, 0.32 g (1.84 mmol) of sodium dithionite and 0.23 g (2.76 mmol) sodium bicarbonate were added and the solution stirred for 2 h. A yellow solid (0.10 g, 70%) was obtained, mp 107–110 °C; R_f = 0.83; UV_{max} (MeOH): 213, 359 nm; ^1H NMR (DMSO-d_6): δ 1.10–1.23 (m, 2H), 1.98–2.01 (m, 1H), 2.89–2.92 (m, 1H), 3.01 (s, 3H), 3.07–3.11 (m, 2H), 4.51–4.67 (m, 3H), 5.84 (d, 1H, J = 7.11), 6.99–7.18 (m, 6H).

Anal.—Calc. for $C_{18}H_{20}N_2O_3$: C, 69.20; H, 6.45; N, 8.98. Found C, 68.87; H, 6.61; N, 8.71.

(±)-*trans*-3-(Aminocarbonyl)-1-[2-oxo-2-[(2-phenylcyclopropyl)amino]ethyl]pyridinium Bromide (9a)—A mixture of 2.53 g (10 mmol) of **5** and 1.22 g (10 mmol) of nicotinamide in 30 mL of acetonitrile was stirred at room temperature for 2 d. The solution was concentrated and treated with ether to generate 3.00 g (80%) of an off-white precipitate. The solid was recrystallized twice from methanol:hexane, mp 166–170 °C; UV_{max} (MeOH): 213, 268 nm; MS (FAB) C^+ (*m/z*): 296; ^1H NMR (DMSO-d_6): δ 1.19–1.34 (m, 2H), 2.08–2.12 (m, 1H), 2.90–2.92 (m, 1H), 5.63 (s, 2H), 7.07–7.18 (m, 5H), 8.00–8.17 (m, 1H), 8.20–8.24 (m, 1H), 8.67–8.69 (m, 1H), 9.19–9.25 (m, 3H), 9.65 (s, 1H).

Anal.—Calc. for $C_{17}H_{18}BrN_3O_2$: C, 54.28; H, 4.78; Br, 21.24; N, 11.17. Found: C, 54.26; H, 4.84; Br, 21.62; N, 11.14.

(±)-*trans*-3-(Aminocarbonyl)-1-[3-oxo-3-[(2-phenylcyclopropyl)amino]propyl]pyridinium Bromide (9b)—To 1.67 g (10 mmol) of **1** · HCl and 0.84 g (10 mmol) of sodium bicarbonate in 20 mL of dimethylformamide were added 2.75 g (10 mmol) of 3-carbamoyl-1-carboxyethyl pyridinium bromide and 2.06 g (10 mmol) of dicyclohexylcarbodiimide (DCC) at 0 °C. The mixture was allowed to warm to room temperature and was stirred for 2 d. The obtained precipitate was filtered off, the dimethylformamide solution was evaporated, and ether was added to the residue. The obtained hygroscopic precipitate (2.73 g; 70%) was recrystallized twice from methanol:ether, mp 170–171 °C; UV_{max} (MeOH): 210, 269 nm; MS (FAB) C^+ *m/z*: 310; ^1H NMR (DMSO-d_6): δ 1.01–1.11 (m, 2H), 1.82–1.84 (m, 1H), 2.95–2.98 (m, 1H), 3.02 (tr, 2H, J = 1.87), 4.91 (tr, 2H, J = 1.97), 6.96–7.13 (m, 5H), 7.91 (s, 1H), 8.08–8.17 (m, 1H), 8.49 (bs, 1H), 8.90 (d, 1H, J = 6.11), 9.78 (d, 1H, J = 5.97), 9.73 (s, 1H).

Anal.—Calc. for $C_{18}H_{20}BrN_3O_2$: C, 55.35; H, 5.16; Br, 70.41; N, 10.76. Found: C, 55.17, H, 5.75; Br, 70.27; N, 10.85.

(±)-*trans*-3-(Aminocarbonyl)-*N*-(2-phenylcyclopropyl)-1(4*H*)-pyridineacetamide (10a)—A 0.35-g sample (0.93 mmol) of **9a** was reduced with the mixture of 0.64 g (3.72 mmol) of sodium dithionite and 0.47 g (5.78 mmol) of sodium bicarbonate. Compound **10a** was obtained in a yield of 60% (0.17 g) as a yellow solid, mp 145–148 °C; UV_{max} (MeOH): 209, 348 nm; ^1H NMR (DMSO-d_6): δ 1.11–1.18 (m, 2H), 2.01–2.11 (m, 1H), 2.81–2.83 (m, 1H), 3.00 (bs, 2H), 3.23 (s, 2H), 4.61–4.62 (m, 1H), 5.73 (d, 1H, J = 7.01), 7.32–7.60 (m, 6H).

Anal.—Calc. for $C_{17}H_{19}N_3O_2 \cdot 0.5H_2O$: C, 66.64; H, 6.52; N, 13.17. Found: C, 66.29; H, 6.71; N, 12.95.

(±)-*trans*-3-(Aminocarbonyl)-*N*-(2-phenylcyclopropyl)-1(4*H*)-pyridinepropanamide (10b)—A 2.00-g sample (5.14 mmol) of **9b** was reduced in 100 mL of water and 100 mL of ethylacetate using 3.57 g (20.5 mmol) of sodium dithionite and 2.59 g of (30.8 mmol) sodium bicarbonate. Compound **10b** was obtained in a yield of 1.04 g (65%) as a yellow precipitate, mp 62–66 °C; UV_{max} (MeOH): 212, 354 nm; ^1H NMR (DMSO-d_6): δ 1.13–1.16 (m, 2H), 1.80–1.84 (m, 1H), 2.79–2.81 (m, 1H), 2.99–3.01 (m, 2H), 3.22–3.46 (m, 4H), 4.70–4.71 (m, 1H), 5.81

(d, 1H, J = 6.12), 7.09–7.14 (m, 6H).

Anal.—Calc. for $C_{18}H_{21}N_3O_2$: C, 69.42; H, 6.79; N, 13.49; Found: C, 69.65; H, 6.50; N, 13.81.

Chloromethyl (±)-*trans*-(2-phenylcyclopropyl)carbamate (11a)—To 1.67 g (10 mmol) of **1** · HCl and 2.10 g (20 mmol) of triethylamine in 40 mL of chloroform were added 1.54 g (12 mmol) of chloromethylchloroformate (obtained from phosgene and formaldehyde monomer as previously described[27]) in 5 mL of chloroform at 0 °C. The solution was stirred for 0.5 h at 0–5 °C and then for 1 d at room temperature. The solution was poured in 30 mL of water. The organic phase was separated, washed with water, dried, and evaporated to give an oil. The residue was crystallized from acetone:hexane, resulting in 1.57 g (60%) of **11a**, mp 76–78 °C; R_f = 0.90; ^{1}H NMR ($CDCl_3$): δ 1.19–1.27 (m, 2H), 2.02–2.12 (m, 1H), 2.78–2.80 (m, 1H), 5.30 (bs, 1H), 5.75 (s, 2H), 7.17–7.31 (m, 5H).

Anal.—Calc. for $C_{11}H_{12}ClNO_2$: C, 58.56; H, 5.32; Cl, 15.71; N, 6.10. Found: C, 58.58; H, 5.39; Cl, 15.77; N, 6.12.

2-Bromoethyl (±)-*trans*-(2-phenylcyclopropyl)carbamate (11b)—Compound **11b** was obtained in a yield of 65% by the same procedure as for **11a** (above), using bromoethylchloroformate, mp 54–56 °C; R_f = 0.90; ^{1}H NMR ($CDCl_3$): δ 1.17–1.18 (m, 2H), 1.99–2.01 (m, 1H), 2.74–2.81 (m, 1H), 3.71 (tr, 2H, J = 6.61), 4.21 (tr, 2H, J = 6.59), 5.81 (bs, 1H), 7.18–7.29 (m, 5H).

Anal.—Calc. for $C_{12}H_{14}BrNO_2$: C, 50.73; H, 4.92; Br, 78.12; N, 4.92. Found: C, 50.79; H, 4.96; Br, 78.06; N, 4.95.

(±)-*trans*-[[[(2-Phenylcyclopropyl)amino]carbonyl]oxy]methyl-3-pyridine Carboxylate (12a)—To 2.25 g (10 mmol) of **11a** in 15 mL of dimethylformamide were added 1.35 g (11 mmol) of nicotinic acid and 1.12 g (11 mmol) of triethylamine in 3 mL of dimethylformamide at 0 °C. The solution was stirred at room temperature until the reaction was completed as determined by TLC (~2 d). The system was subsequently concentrated and extracted with ethyl acetate and water. The organic layer was washed with water, dried, and evaporated to give 2.19 g (70%) of an oil, R_f = 0.69; MS [M + H]$^+$ m/z: 313.

(±)-*trans*-2-[[[(2-Phenylcyclopropyl)amino]carbonyl]oxy]ethyl-3-pyridine Carboxylate (12b)—Compound **12b** was obtained in a yield of 65% following the same procedure as described above for **12a**, R_f = 0.51; MS [M + H]$^+$ m/z: 327.

(±)-*trans*-1-Methyl-3-[[[[[(2-phenylcyclopropyl)amino]carbonyl]oxy]methoxy]carbonyl]pyridinium Iodide (13a)—To 0.31 g (1 mmol) of **12a** dissolved in 10 mL acetone were added 0.28 g (2 mmol) of methyl iodide. After stirring for 1 d at room temperature, the solution was treated with ether to give **13a** as a yellow substance in a yield of 3.63 g (80%) after two recrystallizations from acetone:hexane, mp 170–174 °C; UV_{max} (MeOH): 210, 266 nm; MS (FAB) C$^+$ m/z: 327; ^{1}H NMR (DMSO-d_6): δ 1.10–1.18 (m, 2H); 1.99–2.08 (m, 1H); 3.09–3.11 (m, 1H), 4.44 (s, 3H), 6.00 (s, 2H), 7.07–7.31 (m, 5H), 8.21–8.27 (m, 1H), 8.55 (d, 1H, J = 5.91), 8.98 (d, 1H, J = 6.03), 9.22 (bs, 1H), 9.56 (s, 1H).

Anal.—Calc. for $C_{18}H_{19}IN_2O_4 \cdot H_2O$: C, 45.47; H, 4.59; I, 26.87; N, 4.23. Found: C, 45.19; H, 4.71; I, 26.61; N, 4.14.

(±)-*trans*-1-Methyl-3-[[[[[(2-phenylcyclopropyl)amino]carbonyl]oxy]ethoxy]carbonyl]pyridinium Iodide (13b)—The synthesis of **13b** was similar to that of **13a**. The yield was 85%, mp 208–210 °C; UV_{max} (MeOH): 113, 265 nm; MS (FAB) C$^+$ m/z: 341; ^{1}H NMR (DMSO-d_6): δ 1.07–1.15 (m, 2H), 1.97–1.99 (m, 1H), 2.61–2.63 (m, 1H), 4.36 (tr, 2H, J = 6.11), 4.43 (s, 3H), 4.58 (tr, 2H, J = 6.09), 7.01–7.31 (m, 5H), 7.69 (bs, 1H), 8.23–8.31 (m, 1H), 8.94 (d, 1H, J = 7.51), 9.22 (d, 1H, J = 5.12), 9.55 (s, 1H).

Anal.—Calc. for $C_{19}H_{21}IN_2O_4 \cdot 0.5\ H_2O$: C, 47.71; H, 4.55; I, 26.68; N, 5.89. Found: C, 47.78; H, 4.61; I, 26.68; N, 5.87.

(±)-*trans*-[[[(2-Phenylcyclopropyl)amino]carbonyl]oxy]methyl-1,4-dihydro-1-methyl-3-pyridine Carboxylate (14a)—A 0.20-g sample (0.44 mmol) of **13a** was reduced using 0.22 g (1.26 mmol) of sodium dithionite and 0.22 g (2.64 mmol) of sodium bicarbonate in 100 mL of ethylacetate and 100 mL of water. Compound **14a** resulted as an oil (0.10 g, 70%), R_f = 0.88; UV_{max} (MeOH): 211, 362 nm; ^{1}H NMR (DMSO-d_6); δ 1.09–1.25 (m, 2H), 1.95–2.01 (m, 1H), 2.87–2.89 (m, 1H), 3.21 (s, 3H), 3.27–3.31 (m, 2H), 4.16–4.18 (m, 1H), 5.18 (d, 1H, J = 7.91), 5.80 (s, 2H), 7.01–7.26 (m, 6H).

Anal.—Calc. for $C_{18}H_{20}N_2O_4 \cdot 0.5H_2O$: C, 64.07; H, 6.27; N, 8.30. Found: C, 63.92; H, 6.31; N, 8.11.

(±)-*trans*-2-[[[(2-Phenylcyclopropyl)amino]carbonyl]oxy]ethyl-1,4-dihydro-1-methyl-3-pyridine Carboxylate (14b)—In the same way as described for **14a**, 0.61 g (1.28 mmol) of **13b** was treated with 0.89 g (5.12 mmol) of sodium dithionite and 0.65 g (7.68 mmol) of sodium bicarbonate giving **14b** as an oil (yield 70%), R_f = 0.64; UV_{max} (MeOH): 214, 360 nm; ^{1}H NMR (DMSO-d_6): δ 1.05–1.16 (m, 2H), 1.92–1.99 (m, 1H), 2.67–2.68 (m, 1H), 2.92 (s, 3H), 2.94–2.97 (m, 2H), 4.39 (tr, 2H, J = 6.09), 4.41–4.51 (m, 3H), 5.87 (d, 1H, J = 7.01), 7.06–7.23 (m, 6H).

Anal.—Calc. for $C_{19}H_{22}N_2O_4 \cdot H_2O$: C, 64.93; H, 6.59; N, 7.97. Found: C, 64.72; H, 6.71; N, 7.84.

Chemical Oxidation Studies—The rate of ferricyanide-mediated oxidation of dihydronicotinates was determined using a modification of published methods. In this procedure, the rate of decrease of the 358 nm absorbance band of CDSs was determined in buffered 20% aqueous acetonitrile solutions [0.1 mM $K_4Fe(CN)_6$, 60 mM KCl, and 1.0 mM K_2CO_3] containing various concentrations of $K_3Fe(CN)_6$ (1–50 mM). The dihydronicotinates (in acetonitrile) were added (via Hamilton syringe) to the test solutions which were maintained at 37 °C in a thermostated cell holder and contained in anaerobic screw-top cuvettes (Spectrocell, Inc.). For a given $Fe(CN)_6^{3-}$ concentration, the pseudo-first-order rate constant was determined and these values were plotted as a function of ferricyanide ion concentration. The second-order constant (k, $s^{-1}M^{-1}$) was obtained from the resulting slope.

Lipophilicity Measurements; Determination of R_m Values—Chromatography was performed on TLC plates [Baker, Si-C13F 19C, 20 × 20 glass plates coated with octadecylsilane (C_{18}) reversed-phase bonded to silica gel, ~20-μm particle size, 200-μm hard surface layer, with 254-nm fluorescent indicator and 19 channels, each of 3-mm width]. The compounds were dissolved in distilled water or acetone and 1 μL of a 3-mg/mL solution was applied to each channel along a line 2 cm above the bottom of the plate in random locations. A mobile phase of 200 mL of water or various concentrations of acetone in water was allowed to run 14 cm from the origin. The developed plates were dried and the compounds were detected by an UV illumination. The corresponding R_m values were calculated from the R_f values by means of the equation: $R_m = \log(1/R_f - 1)$. The theoretical values at 0% acetone in the mobile phase were calculated by the least-squares method from the R_m values within the linear range of the curves, which were plotted as R_m values versus acetone concentrations.

Activity Study—*Animals*—Male Sprague-Dawley rats weighing 175–200 g (Harlan) were maintained in a temperature- and humidity-controlled (22 °C and 50–70%) vivarium with a 14:10 h light:dark cycle. Adequate acclimation time was allowed prior to compound administration.

Drug Injection and Sample Collection—Groups of rats were administered a single iv (tail vein) injection of either **1** at a dose of 10.0 mg/kg or equimolar doses of CDSs **4**, **8**, **10a**, **10b**, **14a**, and **14b** (19.15 to 15.90 mg/kg). All drugs were solubilized in dimethyl sulfoxide (DMSO) and administered at a volume of 0.5 mL/kg. After sacrificing animals at the appropriate time (see below), paired striata (putamen and caudate nuclei) and cortex were rapidly removed and homogenized in 0.4 M perchloric acid solution containing 1.0 mM EDTA, 10.0 mM $NaHSO_3$, and the internal standard 3,4-dihydroxybenzylamine (200 ng/mL). The samples were then centrifuged and the supernatant analyzed by HPLC for DA and DOPAC.

Analytical Method—A method for reversed-phase (RP) HPLC with electrochemical (EC) detection (HPLC-EC) was developed for quantitative analysis. A modification of the procedure of Kissinger[28] was used. The system configuration included a PE series 400 pump, an electrochemical detector consisting of a BAS LC-4B amperometric controller and a LC-17 oxidative transducer with oxidative potential at +0.70 V, 200 nA/FS, and a PE LCI-100, 2V full scale, 0.5 cm/min integrator. Compounds were separated on a Spherisorb ODS-2, 4.6 mm ID × 25 cm, 5μ (S/N 082988 C-A) and S/N 070589 H-A) column with SSI inline filter and Alltech handpacked guard columns. The mobile phase consisted of a buffer containing 0.33 mM sodium octansulfonate, 0.1 M KH_2PO_4 (pH 3.13), and 150 mL of methanol to make 1 L of solution at room temperature. The flow rate was 1 mL/min and the injection volume was 20 μL.

The concentration of dopamine (DA) and 3,4-dihydroxyphenyl acetic acid (DOPAC) were determined. Each sampling was performed in duplicate. The relative standard deviation (% RSD) for the slopes of the calibration curves (run within 30 h) was <1.1% for the first group of determinations and 25% less for the second one (the two groups of experiments were run two months apart, using different electrochemical cells and columns). Correlation coefficients were >0.99. Standards of DA and DOPAC were prepared in the same perchlorate stock solution and used to calibrate each set of samples.

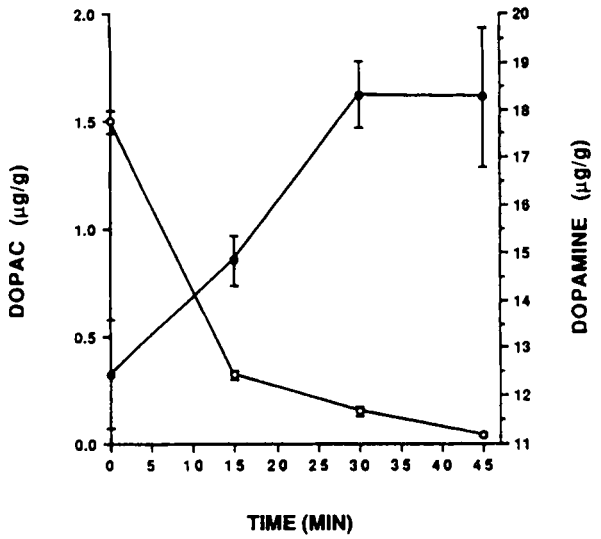


Figure 4—Dopamine (●) and DOPAC (○) concentrations in rat brain tissues after iv administration of a 10-mg/kg dose of **1** at various time points.

In the recovery from the matrices experiment, rat cortical tissue was used as the "blank" of the striatum. Recoveries of 89.5 and 86.8% for DA and DOPAC, respectively, were obtained.

Monoamine Oxidase Inhibition Activity—The time of maximum effect for TCP itself was determined by injecting **1** (10 mg/kg) and then sacrificing animals (3 per group) at 15, 30, 45 min post-drug administration. Brain tissues were analyzed by HPLC-EC. The concentration of DA and DOPAC were plotted as a function of time. The data indicated that at 30 min post-dosing, the maximum effect (maximum concentration of DA) of **1** was exerted (Figure 4). The activity of CDSs was then determined at 30 min after administration. The compounds were divided into two groups. Three animals were used for each compound and in each group there was a vehicle control and a positive **1** control. The average DA and DOPAC concentrations were obtained. In order to estimate the efficacy of CDSs compared with the parent compound, the DA and DOPAC concentrations were normalized to the concentration from the administration of **1**.

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