by silica gel chromatography using dichloromethane–methanol (25:1) as eluant to give isomer 1 (206 mg, 0.398 mmol, 52%) and isomer 2 (106 mg, 0.205 mmol, 27%).

Isomer 1 (0.42 g, 0.81 mmol) and *p*-toluenesulfonic acid monohydrate (0.155 g, 0.816 mmol) were dissolved in propan-2-ol (10 mL). The solvent was removed in vacuo and the residue was recrystallized from ether–propan-2-ol (7:1) to give **15** as white needles (0.51 g, 0.74 mmol, 91%): IR (film) 3468 cm^{-1}; NMR δ ($CDCl_3$, 300 MHz) 10.30 (1 H, br s, NH), 7.74 (2 H, apparent (app) d, J = 8, 2 C-H, tosylate), 7.54 (1 H, d, J = 8, C_7-H), 7.28 (1 H, t, J = 8, C_6-H), 7.16 (1 H, d, J = 8, C_5-H), 7.11 (2 H, app d, J = 8, 2 C-H tosylate), 4.65 (1 H, m, CHN), 4.39 (1 H, t, J = 5, CHCON), 4.11 (1 H, m, CHN), 3.85 (2 H, m, CH_2O), 3.65 (1 H, m, CHN), 3.45 (1 H, m, CHN), 3.29 (3 H, s, NCH_3), 3.05 (1 H, m, CHN), 2.93 (1 H, m, CHN), 2.71 (2 H, m, CH_2Ar), 2.33 (3 H, s, Ar-CH_3 tosylate), 1.5–2.2 (16 H, m); MS m/e (EI^+) 519 (91), 517 (90), 448 (74), 446 (74), 325 (100); $[\alpha]^{20}_D$ = −60° (c = 0.78, CH_2Cl_2).

Isomer 2 (0.27 g, 0.52 mmol) and *p*-toluenesulfonic acid monohydrate (99 mg, 0.52 mmol) were dissolved in propan-2-ol (10 mL). The solvent was removed in vacuo and the residue was recrystallized from ether–propan-2-ol (7:1) to give **16** as a white powder (0.32 g, 0.46 mmol, 88%): IR (film) 3467, 1641 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 10.30 (1 H, br s, NH), 7.75 (2 H, app′ d, J = 8, 2 C-H tosylate), 7.54 (1 H, d, J = 8, C_7-H), 7.18 (2 H, app′ d, J = 8, 2 C-H tosylate), 7.16 (1 H, t, J = 8, C_6-H), 6.80 (1 H, d, J = 8, C_5-H), 4.92 (1 H, m, CHN), 4.25 (1 H, m, CHCON), 4.18 (1 H, m, CHN), 3.85 (2 H, m, CH_2O), 3.65 (2 H, m, 2 CHN), 3.29 (3 H, s, NCH_3), 3.15–3.03 (2 H, m, 2 CHN), 2.80 (1 H, m, CHAr), 2.37 (3 H, s, Ar-CH_3 tosylate), 2.5–1.6 (17 H, m); MS m/e (EI^+) 519 (100), 517 (93), 448 (79), 446 (79), 325 (56); $[\alpha]^{20}_D$ = +39° (c = 0.63, CH_2Cl_2).

4,5-Dihydro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-3*H*-naphtho[1,8-*bc*]thiophene-5-carboxamide *p*-Toluenesulfonate (Isomer 1) (17). The bromo amide **15** (335 mg, 0.486 mmol) was dissolved in tetrahydrofuran (50 mL), cooled to −78 °C, and treated with *n*-butyllithium (1.6 M in hexane, 0.85 mL, 1.4 mmol). After 2 h at −78 °C the mixture was rapidly poured into 1% aqueous sodium carbonate (500 mL) at 25 °C with stirring. The mixture was extracted with dichloromethane (2 × 150 mL), and the organic fractions were concentrated in vacuo and purified by silica gel chromatography using dichloromethane–methanol (25:1) as eluant to give an oil (123 mg, 0.280 mmol). This oil (123 mg, 0.280 mmol) and *p*-toluenesulfonic acid monohydrate (55 mg, 0.29 mmol) were dissolved in propan-2-ol (1 mL) and recrystallized from ether–propan-2-ol (5:1) to give **17** (145 mg, 0.237 mmol, 49% from the bromo amide **15**) as colorless needles: IR (neat) 3401, 1637 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 10.20 (1 H, br s, NH), 7.74 (2 H, app′ d, J = 8, 2 C-H tosylate), 7.68 (1 H, d, J = 8, C_7-H), 7.24 (1 H, d, J = 8, C_5-H), 7.18 (1 H, t, J = 8, C_6-H), 7.10 (2 H, app′ d, J = 8, 2 C-H tosylate), 6.95 (1 H, s, C_2-H), 4.62 (1 H, m, CHN), 4.40 (1 H, m, CHCON), 4.15 (1 H, m, CHN), 3.85 (2 H, m, CH_2O), 3.75 (1 H, m, CHN), 3.51 (1 H, m, CHN), 3.29 (3 H, s, NCH_3), 3.10 (1 H, m, CHN), 3.0–2.85 (3 H, m, CHN and CH_2Ar), 2.32 (3 H, s, Ar-CH_3 tosylate), 2.25–1.6 (16 H, m); MS m/e (FAB) 439 (100), 368 (34), 325 (8), 217 (90); $[\alpha]^{20}_D$ = −43° (c = 0.68, CH_2Cl_2).

4,5-Dihydro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-3*H*-naphtho[1,8-*bc*]thiophene-5-carboxamide *p*-Toluenesulfonate (Isomer 2) (18). The method is the same as described for compound **17** above. The bromo amide **16** (335 mg, 0.486 mmol) was converted into **18** (75 mg, 0.12 mmol, 25% from **16**) as colorless diamonds: IR (neat) 3468, 1642 cm^{-1}; NMR δ ($CDCl_3$, 300 MHz) 10.3 (1 H, br s, NH), 7.75 (2 H, app′ d, J = 8, 2 C-H tosylate), 7.68 (1 H, d, J = 8, C_7-H), 7.18 (1 H, t, J = 8, C_6-H), 7.16 (2 H, app′ d, J = 8, 2 C-H tosylate), 6.96 (1 H, s, C_2-H), 6.79 (1 H, d, J = 8, C_5-H), 4.95 (1 H, m, CHN), 4.35 (1 H, m, CHCON), 4.20 (1 H, m, CHN), 3.88 (2 H, m, CH_2O), 3.78 (1 H, m, CHN), 3.56 (1 H, m, CHN), 3.29 (3 H, s, NCH_3), 3.10 (2 H, m, 2 CHN), 2.95 (1 H, m, CHAr), 2.68 (1 H, m, CHAr), 2.35 (3 H, s, Ar-CH_3 tosylate), 2.4–1.6 (16 H, m); MS m/e (FAB) 439 (10), 368 (3), 325 (9), 217 (100); $[\alpha]^{20}_D$ = +42° (c = 0.37, CH_2Cl_2).

Biological Assay. μ and κ opioid receptor binding assays and analgesia assay were performed as previously described.[3] δ opioid receptor binding assays were performed by the method of Paterson.[15]

Acknowledgment. We thank Dr. I. Pattison for expert technical assistance.

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Common Stereospecificity of Opioid and Dopamine Systems for *N*-Butyrophenone Prodine-like Compounds

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The two optical isomers of 1-[3-(*p*-fluorobenzoyl)propyl]-3-methyl-4-phenyl-4-propionoxypiperidine (FPP) were obtained by resolution of (±)-*r*-3-methyl-4-phenyl-*c*-4-piperidinol followed by N-alkylation and O-propionylation. These, as well as the racemate, were evaluated for their antinociceptive, opioid, and neuroleptic properties using in vivo and in vitro test systems. The results are remarkable in two respects, namely, the dextrorotatory isomer is consistently the most potent on all tests, and it acts on both opioid (μ) and neuroleptic (D_2) receptors.

In a recent communication, we reported the synthesis of certain 4-phenyl-4-piperidinols, corresponding esters, and related compounds with an *N*-fluorobutyrophenone chain on the nitrogen atom.[1] The synthesis was prompted by the increased interest in compounds with combined analgesic and neuroleptic properties.[2,3] Results of in vivo and in vitro testing revealed that the racemates of two propionoxy derivatives with 3-methyl-4-phenyl relationships like α- and β-prodine, respectively, had potent (μ opioid) analgesic and neuroleptic (D_2) activities.[1] Because the results of biological studies in which racemates are used may be misleading,[4] the optical isomers were prepared for study. Our objective was to ascertain whether the opioid

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Scheme I

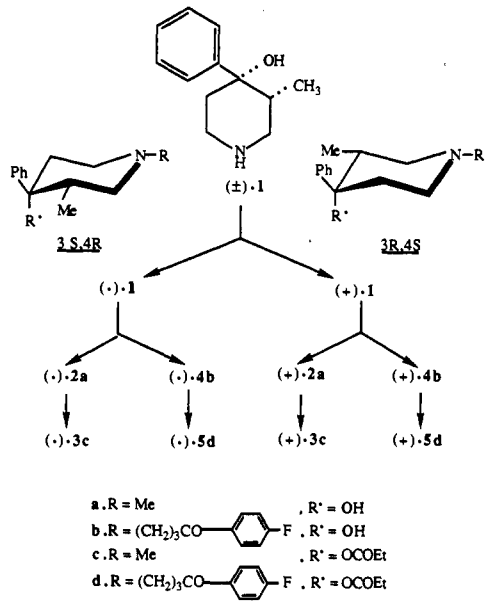

Table I. Comparison of the Agonist/Antagonist Antinociceptive Profile of Activity of the Racemate and Optical Isomers of 1-[3-(*p*-Fluorobenzoyl)propyl]-3-methyl-4-phenyl-4-(propionyloxy)-piperidine and Morphine

		ED_{50} (95% confidence limits), μmol/kg sc		
compd[a]	tailflick[b]	antagonist action in tail-flick antagonism vs morphine[b]	PPQ[b]	hot plate[c]
(±)-**5d**	1.7 (0.8–3.7)	>60.8	0.2 (0.1–0.9)	1.6 (0.9–2.8)
(+)-**5d**	0.8 (0.5–2.4)	>60.8	0.2 (0.05–0.5)	0.9 (0.8–1.1)
(–)-**5d**	>60.8	NA	2.9 (1.1–7.3)	>72.9
morphine	10.6 (10.3–10.8)	NA	0.4 (0.4–0.5)	2.9 (2.2–3.2)

[a] All doses expressed as free base. [b] Vehicle: 10% dimethyl sulfoxide and water. Vehicle inactive in all assays. [c] Vehicle: water.

and dopaminergic effects were associated with the same enantiomer. In this report, the results of the optical isomers with the α-prodine relationship are described.

Chemistry

All compounds in their racemic forms were reported previously.[1] Resolution of the target compound (+)- and (–)-**5d** was achieved by a classical recrystallization procedure of diastereoisomeric tartrates of the precursor secondary amine (±)-1, followed by N-alkylation and O-propionylation, and is summarized in Scheme I. By a different N-alkylation, (–)-α-prodine [(–)-**3c**] and (+)-α-prodine [(+)-**3c**] were prepared for determination of the absolute configuration of the *N*-butyrophenone analogues. In fact, compounds **3c** and **5d** differ only by the *N*-alkyl substituent, a difference which does not affect the integrity of the chiral centers.[5] Furthermore, (+)- and (–)-**5d** were converted to the fumarate salts, to avoid hydrolysis of the ester group under stronger acid conditions.

The two optical isomers (+)-**5d** (with the 3*R*,4*S* configuration) and (–)-**5d** (3*S*,4*R*) and the racemate were evaluated for antinociceptive, opioid, and neuroleptic properties by using in vivo and in vitro test systems. The tail-flick, tail-flick vs morphine, paraphenylquinone (PPQ), and hot-plate assays were used to determine the agonist/antagonist and antinociceptive profile of activity. Opioid activity was assessed as follows: (1) [^{3}H]dihydromorphine binding assay, (2) naloxone prevention of antinociception in the tail-flick test, and (3) substitution for morphine in withdrawn morphine-dependent rhesus monkeys. Neuroleptic activity was measured by using the [^{3}H]spiroperidol receptor binding assay and antagonism of apomorphine-induced circling behavior.

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Table II. Summary of Opioid and Neuroleptic Activity of the Racemate and Optical Isomers of 1-[3-(*p*-Fluorobenzoyl)propyl]-3-methyl-4-phenyl-4-(propionyoxy)piperidine and Reference Compounds

		neuroleptic activity	
compd[a]	opioid activity: inhibn of [^{3}H]dihydro-morphine binding;[b] IC_{50}, nM	inhibn of [^{3}H]-spiroperidol binding;[b] IC_{50}, nM	apomorphine-induced circling behavior;[b] ED_{50}, μmol/kg sc
(±)-**5d**	9.5 (7.3–11.0)	53.8 (42.3–65.0)	3.9 (3.4–4.4)
(+)-**5d**	6.1 (4.3–7.9)	43.3 (35.8–50.8)	1.6 (1.2–1.8)
(–)-**5d**	1789 (1320–2081)	146.7 (131.7–166.4)	>12.0
morphine	1.60 (1.10–2.10)		
haloperidol		4.24 (3.86–4.72)	0.1 (0.1–0.2)
dopamine		3045 (2474–3856)	
apomorphine		302 (239–384)	

[a] Compounds (+)-**5d** and (–)-**5** were administered in aqueous solution as acid fumarate salts and doses are expressed as free base. [b] Values in parentheses represent 95% confidence limits.

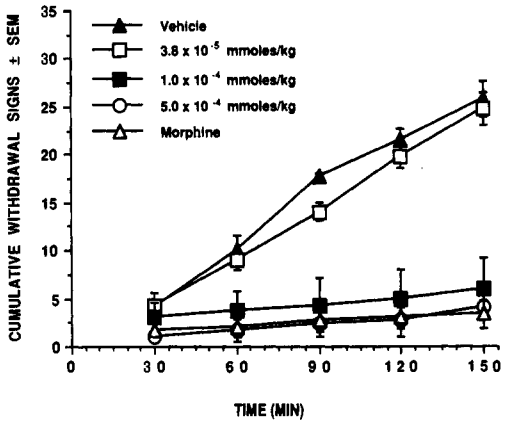

Figure 1. The effect of (+)-FPP on the suppression of withdrawal signs in withdrawn, morphine-dependent monkeys. Doses of morphine and vehicle were 3.0 mg/kg and 1.0 mL/kg, respectively.

As shown in Table I, (+)-**5d** was more potent than either (±)-**5d** or morphine on all antinociceptive tests. Except for some activity in the PPQ test, (–)-**5d** was essentially inactive. All the compounds lacked opioid antagonist properties. The enatiomeric discrimination of the opioid receptors for (+)- and (–)-**5d** was not unexpected since (+)-α-prodine [(+)-**3c**] with the same absolute configuration (3*R*,4*S*) possessed significantly more antinociceptive activity than the corresponding 3*S*,4*R* enantiomer in the hot-plate test in mice.[5] Further, in the hot-plate test, (–)-**5d** up to 72.9 μmol/kg was not only inactive but it also did not display any of the toxic signs which were evident with (±)-**5d** at 36.5–48.6 μmol/kg (ataxia and catalepsy).

Regarding opioid activity, morphine was the most potent, followed by (+)-**5d** in the [^{3}H]dihydromorphine binding assay. The levorotatory isomer was only weakly active when compared with the dextrorotatory isomer (Table II). In the single-dose-suppression test in withdrawn, morphine-dependent monkeys, (+)-**5d** substituted completely for morphine at 0.6 and 0.1 μmol/kg (Figure 1). In one monkey receiving 2.4 μmol/kg, body and jaw sag, staring, ataxia, eyelid ptosis and slowing were noted (data not shown). A challenge dose of 0.1 μmol/kg of naloxone eliminated these signs, implying opioid activity. Regarding the racemate, complete suppression of morphine

abstinence was observed at 0.6 μmol/kg but not at 0.1 μmol/kg.

The results of neuroleptic activity as measured by the dopamine D_2 receptor binding assay (inhibition of [^{3}H]-spiroperidol binding) and the Ungerstedt test (antagonism of apomorphine circling behavior) are summarized in Table II. Again, the dextrorotatory isomer was more active than either the levorotatory isomer or racemate.

In conclusion, opioid (μ type) receptors and dopamine D_2 receptors share the same enantospecificity in this class of compounds.

Experimental Section

Melting points were determined with a Büchi-Tottoli apparatus; Microanalyses were performed by the Microanalytical Section of our institute under the direction of Dr. A. Mazzeo-Farina. All compounds were analyzed for C, H, N; analytical results were within ±0.3% of the theoretical values. Optical rotations were obtained (at 20–22 °C) with 1% solutions in MeOH using a Perkin-Elmer 141 polarimeter and 1-dm microcell.

Resolution of (±)-*r*-3-Methyl-4-phenyl-*c*-4-piperidinol [(±)-1]. Racemic α-norprodinol[1] (5.7 g, 30 mmol) in 10 mL of hot MeOH was added to L-(+)-tartaric acid (4.5 g, 1 equiv) in 10 mL of hot MeOH. After 1 week at room temperature, the salt was isolated and recrystallized to constant rotation from MeOH: 2.5 g; mp 210–212 °C; $[\alpha]_D$ = +13.4°. The base was regenerated from an aqueous solution of the salt with excess NH_3 and extracted with $CHCl_3$. The solvent was evaporated, giving 1.4 g of crystalline (−)-1: mp 111–113 °C; $[\alpha]_D$ = −5.4°.

The combined mother liquors of the above tartrate were evaporated to dryness under reduced pressure. The residue in aqueous NH_3 was extracted with $CHCl_3$ and the extract was evaporated to dryness. The remaining solid residue (3 g) was dissolved in 10 mL of MeOH and 5 mL of water and treated with 2.4 g (1 equiv) of D-(−)-tartaric acid dissolved in 10 mL of MeOH. After being heated at reflux for 10 min, the homogeneous solution was allowed to stand for 2 days; the crystals were collected and recrystallized to optical purity from MeOH, giving 2.6 g of D-(−)-tartrate: mp 214–216 °C; $[\alpha]_D$ = −13.2°. The pure acid tartrate was treated with aqueous NH_3 and the liberated base was extracted with $CHCl_3$. The solvent was evaporated, giving 1.45 g of crystalline (+)-1: mp 111–112 °C; $[\alpha]_D$ = +5.2°. Anal. ($C_{12}H_{17}NO$) C, H, N. The racemate melted at 131–133 °C.[1]

(−)-1,*r*-3-Dimethyl-4-phenyl-*c*-4-piperidinol [(−)-2a]. A mixture of (−)-1 (0.28 g, 1.5 mmol), 35% CH_2O (0.56 g), and 90% HCOOH (0.66 g) was heated on a water bath for 6 h. The solution was adjusted to pH 9 with aqueous NaOH and extracted with ether. Evaporation of the solvent gave (−)-2a in theoretical yield; mp 90–91 °C; $[\alpha]_D$ −7.24° [lit.[5] mp 90–91 °C; lit. $[\alpha]_D$ = −12.0° (*c* 1, acetone)].

(+)-1,*r*-3-Dimethyl-4-phenyl-*c*-4-piperidinol [(+)-2a] was obtained from (+)-1 in the same manner as (−)-2a: mp 90–91 °C; $[\alpha]_D$ = +7.29 °C [lit.[4] mp 89–90 °C; lit.[4] $[\alpha]_D$ = +11.8° (*c* 1, acetone)].

(3*S*,4*R*)-(−)-α-Prodine [(−)-3c] was prepared by heating under reflux for 2 h a mixture of (−)-2a (0.2 g), propionic anhydride (1 mL), and pyridine (0.5 mL). The mixture was partitioned between dilute aqueous NH_3 and ether. After evaporation of the ether, the residue was converted to the hydrochloride with ethereal HCl and crystallized from EtOH–ether: mp 189–190 °C; $[\alpha]_D$ −31.6° [lit.[5] mp 196–197 °C; lit.[5] $[\alpha]_D$ = −27.9° (c 1, EtOH)].

(3*R*,4*S*)-(+)-Prodine hydrochloride [(+)-3c] was obtained similarly from (+)-2a: mp 190–191 °C; $[\alpha]_D$ +31.7° [lit.[5] mp 196–197 °C; lit.[5] $[\alpha]_D$ = −26.5° (c 1, EtOH)].

(−)-1-[3-(*p*-Fluorobenzoyl)propyl]-*r*-3-methyl-4-phenyl-*c*-4-piperidinol [(−)-4b] was obtained as its racemate[1] starting from (−)-1 and was characterized as the hydrochloride: mp 172–173 °C; $[\alpha]_D$ = −6.80°; (+)-4b was obtained similarly starting from (+)-1·HCl: mp 172–173 °C; $[\alpha]_D$ = +6.77°. Anal. ($C_{22}H_{27}ClFNO_2$) C, H, N.

(3*S*,4*R*)-(−)-1-[3-(*p*-Fluorobenzoyl)propyl]-*r*-3-methyl-4-phenyl-*c*-4-(propionyloxy)piperidine [(−)-5d] was obtained by esterification of (−)-4b with propionic anhydride in pyridine as described for the racemate.[1] The final product was passed through a silica gel column with $CHCl_3$ containing 1% MeOH and 0.1% NH_3 as eluant, and then converted to the fumarate by dissolving equimolar amounts of (−)-5d and fumaric acid in hot acetonitrile. After cooling, a crystalline acid fumarate precipitated: mp 144–145 °C; $[\alpha]_D$ = −29.2°. Anal. ($C_{29}H_{34}FNO_7$) C, H, N.

(3*R*,4*S*)-(+)-1-[3-(*p*-Fluorobenzoyl)propyl]-*r*-3-methyl-4-phenyl-*c*-4-(propionyloxy)piperidine [(+)-5d] was obtained from (+)-4b in the same manner as (−)-5d and converted to the acid fumarate: mp 144–145 °C; $[\alpha]_D$ = +30.67°. Anal. ($C_{29}H_{34}FNO_7$) C, H, N. The acid fumarate of (+)-5d melted at the same temperature. Both (+)-5d and (−)-5d as free bases did not crystallize and also remained as viscous residues on standing for a prolonged period of time; the free base as the racemate melted at 85–86 °C.[1]

Pharmacological Methods. A.1. In Vitro Assays. [^{3}H]-Dihydromorphine Binding Assay. The binding of [^{3}H]dihydromorphine to opiate receptors was performed on rat brain membranes as described by Mack et al.[6] Brains without cerebella from male Sprague–Dawley rats were homogenized in approximately 10 volumes of 50 mM Tris·HCl buffer, pH 7.4, and centrifuged at 50000*g* for 30 min. The pellet was resuspended in the same buffer and recentrifuged as described above. After preincubation of the membranes in 50 mM Tris·HCl, pH 7.4 (1 mg of protein/mL), at 25 °C for 60 min, [^{3}H]dihydromorphine (New England Nuclear, specific activity 85.1 Ci/mmol) was added to give a final concentration of 1 μM, in the absence and presence of different concentrations of drugs. Nonspecific binding was estimated in the presence of 10^{-5} M naloxone. After incubation at 25 °C for 60 min, samples were filtered through Whatman GF/B glass-fiber filters and rinsed twice with 5 mL of Tris buffer, and the radioactivity was measured by liquid scintillation spectrometry in 20 mL of Filtercount scintillation cocktail (Packard) with an efficiency of about 60%.

A.2. [^{3}H]Spiroperidol Receptor-Binding Assay. The binding of [^{3}H]spiroperidol to the D_2 dopamine receptors was performed in rat striatal membranes as described by Creese et al.[7] Striata from male Sprague–Dawley rats were homogenized in approximately 50 volumes of cold 50 mM Tris·HCl buffer, pH 7.7. The homogenate was centrifuged twice at 50000*g* for 10 min with rehomogenization of the intermediate pellet in fresh buffer. The final pellet was homogenized in 50 mM Tris·HCl buffer (1.4 mg of protein/mL) containing 0.1% ascorbic acid, 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.1. Aliquots of this homogenate (0.5 mL) were incubated in a total volume of 1 mL with 0.3 μM [^{3}H]spiroperidol (Amersham, specific activity 24.5 Ci/mmol) for 20 min, at 37 °C in the absence and presence of different concentrations of test drugs. Specific binding was defined as the difference between total binding and nonspecific binding estimated in the presence of 10^{-6} M haloperidol. After incubation, samples were filtered through Whatman GF/B glass filters and washed three times with 5 mL of Tris buffer, and radioactivity was measured by liquid scintillation spectrometry in 20 mL of Filtercount scintillation cocktail (Packard) with an efficiency of 60%.

B. In Vivo Assays. B.1. Antagonism of Apomorphine-Induced Circling Behavior. Potential neuroleptic activity in vivo of the synthesized compounds was investigated by studying their antagonism on apomorphine-induced circling behavior rats with a monolateral lesion of the nigrostriatal tract as described by Ungerstedt.[8] Male Sprague–Dawley rats weighing 140–180 g at the beginning of the experiments were anesthetized with sodium pentobarbital (0.16 mmol/kg ip) and placed in a David Kopf stereotaxic frame. Chemical denervation was induced by slow infusion of 6-OH-DOPA (8 μM/5 μL per 5 min) directly into substantia nigra. Coordinates were taken from Konig and Klippel.[9] Ten days after surgery all the animals were checked for their sensitivity to dopaminergic stimulation by measuring the number of revolutions/60 min occurring after the subcutaneous injection of 7.4×10^{-3} mmol/kg apomorphine. For further

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circling studies, only those responding with, at least 200 turns/60 min after apomorphine injection were used. Approximately 40% of the lesioned animals failed this criterion and were discarded. Rotational behavior was evaluated by a rotamer apparatus. The circling behavior was measured as the number of turns performed by the animal every 5 min after apomorphine injection and the extent of circling was recorded for 60 min. Each compound was administered subcutaneously 10 min before apomorphine injection. The results are expressed as the inhibitory dose that produced 50% of inhibition of rotational behavior induced by apomorphine at 60 min.

B.2. Mouse Antinociception Tests. Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or 10% DMSO, a few drops of H_3PO_4, and water. At least three doses were tested, and 6-10 animals per dose were used. ED_{50}'s were calculated by using computerized probit analysis.

B.2.1. Tail-Flick Assays. The procedure and modifications were described (D'Amour and Smith[10] and Dewey et al.[11]) in the literature. Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 s under control conditions. Mice were injected sc with drug or vehicle and tested 20 min later. In the assay for antagonism of the analgesic effect, the potential antagonists were administered sc 10 min before the agonist, and evaluation occurred 20 min later.

B.2.2. Phenylquinone Abdominal-Stretching Assay. The procedure was reported previously (Pearl and Harris).[12] The mice were injected sc with drugs and 10 min later received 10.9 μmol/kg ip of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. At 10 min after the PPQ injection, the total number of stretches per group were counted over a 1-min period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

B.2.3. Hot-Plate Assay. The method was also reported previously (Eddy and Leimbach[13] and Atwell and Jacobson[14]). The hot plate was held at a constant 55 °C. Mice were placed on the hot plate and activity was scored if the animal jumped or licked its paws after a delay of 5 s or more, but no more than 30 s beyond the control time.

B.2.4. Substitution for Morphine (SDS) in Morphine-Dependent Monkeys. Male and female rhesus monkeys (*Macaca mulatta*) in the weight range of 2.5-7.5 kg were used, and they received 3.9 μmol/kg sc of morphine sulfate every 6 h for at least 90 days. All the animals had received morphine for at least 3 months. Each animal was tested with a new compound with a minimum of 2 weeks recuperation period between tests. At least three monkeys per dose were used. The assay (Aceto et al.)[15,16] was initiated by the subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 h and showed definite signs of withdrawal. Each animal was randomly allocated to receive one of the following treatments: (a) a dose of the compound under investigation; (b) morphine control, 4.4×10^{-3} mmol/kg; and (c) vehicle control, 1 mL/kg. The animals were scored for suppression of withdrawal signs during a 2.5-h observation period. The observer was "blind" regarding the allocation of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative withdrawal signs ± SEM were calculated and the data illustrated in figure form.

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Aldophosphamide Acetal Diacetate and Structural Analogues: Synthesis and Cytotoxicity Studies[1]

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The synthesis of aldophosphamide acetal diacetate and a number of structural analogues is described. These compounds are designed to undergo biotransformation to the corresponding aldehydes in the presence of carboxylate esterases, enzymes that are ubiquitous in mammalian tissue. Several of these aldehydes can theoretically exist in pseudoequilibrium with the 4-hydroxyoxazaphosphorine tautomers; others lack this capability. The half-lives of the acetals in 0.05 M phosphate buffer, pH 7.4, at 37 °C ranged from 1 to 2 days. In the presence of 2 unit equiv of porcine liver carboxylate esterase, all of the compounds were hydrolyzed with half-lives of less than 1 min. Although closely structurally related, the compounds exhibited a wide range of cytotoxicities to L1210 murine leukemia cells in vitro.

Cyclophosphamide is one of the most extensively used drugs in medical oncology.[2-7] It has a better therapeutic index than most other nitrogen mustards and a much broader spectrum of autitumor activity. Although the principal biotransformation pathways of cyclophosphamide are well-characterized, its mechanism of tumoricidal selectivity remains controversial. Two major viewpoints have emerged on this question. One is that phosphoramide

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