Combined Analgesic/Neuroleptic Activity in *N*-Butyrophenone Prodine-like Compounds

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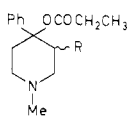
Some 4-phenyl-4-piperidinols, corresponding esters, and related compounds with a *p*-fluorobutyrophenone chain on nitrogen were synthesized and evaluated in in vitro and in vivo tests in order to examine their ability to interact contemporaneously with opioid and dopamine receptors. The propionyloxy derivatives showed a good combination of analgesic and neuroleptic activity. With a 3-methyl substituent on the piperidine ring, the β-configuration was the more active form not only for analgesic activity, as expected from previous results on prodines, but also for neuroleptic activity. Haloperidol and its propionate were also tested as reference compounds.

In the last few years the search for a drug that combines analgesic with neuroleptic or antidepressant properties has greatly increased,[1-4] due mainly to several reports emphasizing the need for such a drug in the clinic.[5-8]

Since it is well known that prodines (**1**) are potent analgesics[9,10] and piperidinylbutyrophenones (**2**) are potent neuroleptic agents,[11] both possessing a common 4-phenyl-4-piperidinol nucleus, we have thought to combine in one molecule (**6–8**) the essential structural features of **1** and **2** and to investigate the effects of this combination on the analgesic and neuroleptic properties of the hybrid molecule.



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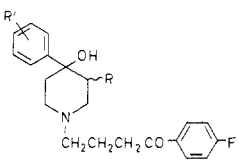
α-prodine, R = Me, *trans* Ph : Me
β-prodine, R = Me, *cis* Ph : Me



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Haloperidol, R' = *p*-Cl, R = H

Furthermore, to see the effects of other substitutions, we prepared compounds lacking alternatively the 4-phenyl or the 4-hydroxy substituent on the piperidine ring.

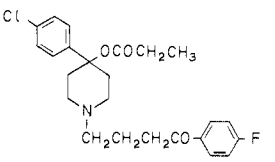
Comprehensive reviews on haloperidol and related butyrophenones[11] and on prodines[12] are available in the literature. *N*-Butyrophenone-substituted norpethidines[13] or norketobemidones[14] were also studied with the intent to correlate structural variations (particularly in the N-side chain) with analgesic potency.

This paper describes the synthesis and the pharmacological profiles of the piperidinylbutyrophenone derivatives shown in Scheme I.

Chemistry

The synthesis of the compounds, outlined in Scheme I, was accomplished by standard procedures. The (α)- and (β)-3-methyl-4-phenyl-4-piperidinols and the 3-desmethyl analogue were obtained by catalytic debenzylation of the corresponding *N*-benzyl derivatives of known stereochemistry.[15] After N-alkylation with γ-chloro-*p*-fluorobutyrophenone, they were isolated as piperidinols (**3–5**) or converted to esters with propionic anhydride (**6–8**).

1-[3-(*p*-Fluorobenzoyl)propyl]-4-phenylpiperidine (**9**) was obtained by N-alkylation of 4-phenylpiperidine, obtained, in turn, by dehydration of 1-benzyl-4-phenyl-4-piperidinol followed by catalytic hydrogenation of the Δ^3 double bond with contemporaneous N-debenzylation. The piperidinol **10** was obtained by N-alkylation of 4-hydroxypiperidine. Esterification of **10** with propionic anhydride gave **11**, and in a similar manner haloperidol propionate (**12**) was obtained from haloperidol.



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Results and Discussion

The affinities of the tested compounds for opioid receptors compared to their analgesic activities in vivo are summarized in Table I. The esters **6–8** displayed good affinity for the receptors and haloperidol propionate (**12**) was about 10 times less potent. The piperidinols **4** and **5** did not bind; **3** and haloperidol only bound weakly.

Interaction of neuroleptic drugs with opioid receptors (50% inhibition of specific binding of [^{3}H]naloxone) was investigated by Creese et al.[16] For haloperidol they reported an IC_{50} of 2650 nM, in absence of sodium chloride, a value that approximates those found for **3** and haloperidol in our experiments with [^{3}H]dihydromorphine.

Compound **9** was inactive and **10** showed very low affinity, whereas **11**, which lacked the 4-phenyl ring but retained the 4-propionyloxy substituent, showed a weak but consistent affinity.

In vivo (hot plate), analgesic activity in mice was shown by the esters **6–8**. They showed fast onset of effect (5 min),

†Recipient of a UNIDO fellowship (1984).

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Scheme I

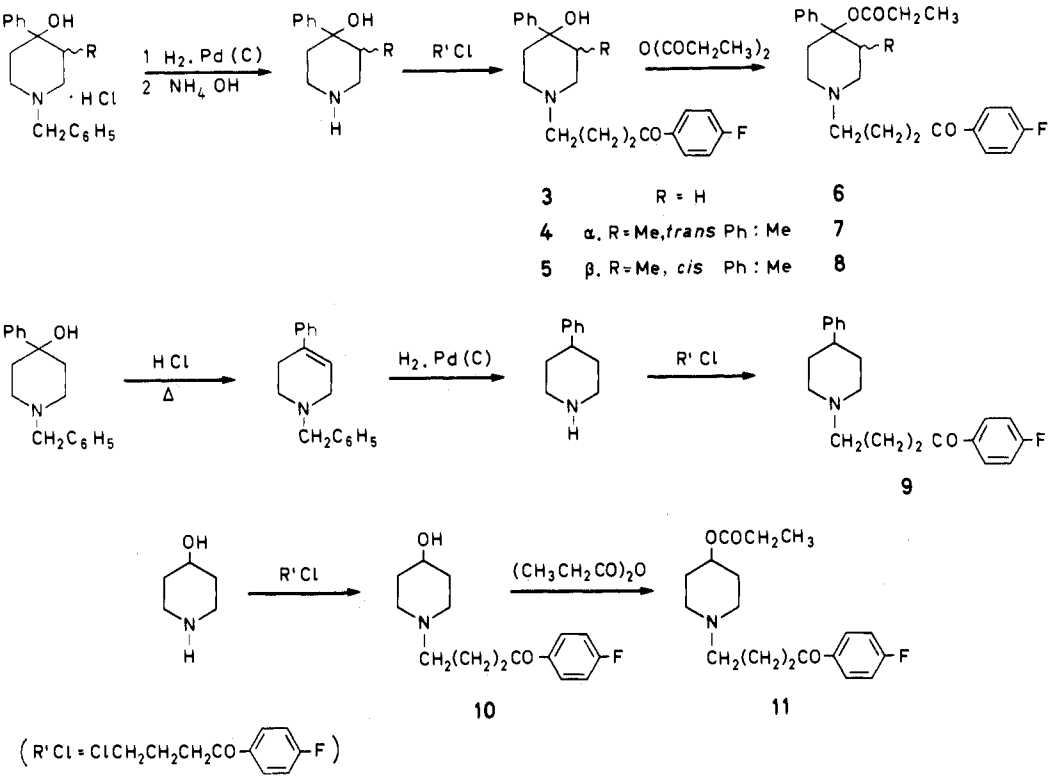

Table I.

compounds[a]	inhibn of [^{3}H]dihydromorphine binding:[b] IC_{50}, nM	analgesic activity (hot plate) in mice: ED_{50}, mg/kg sc	inhibn of [^{3}H]spiroperidol binding:[c] IC_{50}, nM	inhibn of adenylate cyclase:[d] IC_{50} μM	antagonism to apomorphine-induced circling behavior:[e] ED_{50}, mg/kg sc
3	3215 (2410–4544)	>5[f]	47.09 (33.30–58.10)	2.80 (2.6–3.0)	0.35 (0.30–0.39)
4	>10000	>5[f]	80.18 (55.54–124.62)	2.65 (1.7–3.6)	0.93 (0.87–0.99)
5	>10000	>5[f]	54.71 (48.23–62.61)	1.65 (1.5–1.8)	1.54 (1.39–1.68)
6	2.96 (2.71–3.28)	0.32 (0.21–0.48)[g]	28.80 (21.17–41.98)	2.40 (2.3–2.5)	0.20 (0.16–0.24)
7	8.67 (7.77–9.80)	0.65 (0.36–1.15)	46.94 (35.82–64.28)	3.20 (2.6–3.8)	1.59 (1.40–1.81)
8	3.33 (2.78–4.29)	0.23 (0.17–0.32)	33.53 (17.91–88.89)	2.60 (2.4–2.8)	0.22 (0.19–0.25)
9	>10000	*h*	69.91 (53.06–95.96)	7.50 (5.0–10.0)	1.31 (1.11–1.55)
10	5500 (2500–8000)	*i*	>5000		5.84 (5.13–6.64)
11	598 (320–829)	*h*	>50000		4.82 (4.10–5.65)
morphine	1.60 (1.10–2.10)	1.20 (0.90–1.30)			
haloperidol	5687 (5316–5838)	>3[f]	4.24 (3.85–4.72)	0.55 (0.40–0.70)	0.056 (0.050–0.062)
12[j]	23.5 (17.8–28.9)	>5[f]	41 (16–87)	1.5 (1.4–1.8)	0.12 (0.08–0.18)
dopamine			3045 (2474–3856)		
apomorphine			302 (239–384)		

[a] Administered in aqueous solution as hydrochloride salts. [b] IC_{50} is the concentration required to inhibit the specific binding of [^{3}H]dihydromorphine in rat brain membrane preparation by 50%. Values in parentheses in this and the other columns represent 95% confidence limits. [c] IC_{50} represents the concentration that reduces by 50% the rat striatal dopamine-stimulated adenylate cyclase activity. [e] ED_{50} is the dose required to reduce the number of apomorphine-induced rotations by 50% in rats with monolateral lesion of nigrostriatal tracts. [f] Apparent analgesic activity not affected by naloxone. After signs of excitement sedation occurred accompanied by ptosis, ataxia, and incoordination. [g] ED_{50} values were 0.92 (0.73–1.17) and 0.18 (0.13–0.23) for α- and β-prodine (1), respectively, and 0.85 (0.64–1.11) for the 3-desmethyl analogue. Data taken from ref 10. [h] At dose levels of 5–20 mg/kg (compound 9) and 5–40 mg/kg (compound 11), the mice were first excited and then depressed with decrease of spontaneous motor activity, ataxia, and ptosis. [i] Inactive at 25 mg. [j] Haloperidol propionate.

peak effect and duration being about 20 and 100 min, respectively, in the ED_{50} range. There was also a close correlation between in vivo and in vitro tests.

The β-isomer **8**, was about 3 times as active as the α-isomer **7**, confirming previous findings about the superiority of the β over the α geometry for analgesic activity in the 3-methyl-substituted prodines.[9,10,12]

The piperidinols **3–5** were also studied as eventual central analgesics with four dose levels 2.5, 5.0, 7.5, and 10 mg/kg sc. At 7.5 mg/kg all three compounds caused evident signs of toxicity in mice: in the arc of the first 15 min, they were excited and tried to jump out of the restrained glass cylinder when dropped on the hot plate; successively a sedation phase occurred, accompanied by ptosis, ataxia, and incoordination. At 5 mg/kg, where these signs were less evident, these compounds showed a modest analgesic activity that was shown to be not affected by naloxone, administred at the dose of 1 mg/kg ip, 15 min before the tested compounds. The treatment with naloxone did not modify even the behavioral profile of the drugs. At no dose level were the Straub phenomena seen. In contrast, naloxone administered as above completely abolished the analgesic activity of **6** injected at 0.25–0.5 mg/kg sc. Compounds **9** and **11** tested at dose levels of 5–20 and 5–40 mg/kg sc in mice also alternate a depressant to an exciting phase, as evidenced by ataxia and a decrease

in spontaneous motor activity after 45 min of treatment. Haloperidol and its propionate **12** showed a behavioral profile comparable with that of piperidinols **3–5**.

The antipsychotic potential of the new compounds was determined by measuring their potency in displacing [^{3}H]spiroperidol binding from rat striatal membranes (Table I). This binding is believed to involve mainly the dopamine D_2 postsynaptic receptors, which mediate many behavioral effects of neuroleptic drugs.[17,18] In this test both piperidinols and esters showed about the same activity, the esters being only a little more active than the corresponding piperidinols. It is significative that the order of activity: 3-desmethyl > β-isomer > α-isomer, found for the piperidinols (**3–5**) and the esters (**6–8**), was the same order of affinity for the opioid receptors found in the ester series. Considering also the order of potency in the hot-plate test, one can assume that an equatorially placed 3-methyl substituent (α-isomer, eq-4-Ph/eq-3-Me, preferred conformation) leads to a significant reduction of activity, while potency is retained or even increased by an axial 3-methyl substituent (β-isomer, eq-4-Ph/ax-3-Me, preferred conformation).

Compound **9** (OH replaced by H) retained activity in the DA receptor binding, while **10** and **11**, both lacking the 4-phenyl ring, were inactive, thus supporting previous findings about the importance of an aromatic ring for neuroleptic activity in this series of compounds.[11]

Compounds exhibiting good affinity for the D_2 receptors were also examined as inhibitors of the DA-mediated adenylate cyclase activity in homogenates of striatal brain tissue (Table I).[19,20]

Multiple receptor sites for dopamine (DA) in the central nervous system have been proposed:[21] the D_1 site is linked to the adenylate cyclase whose activation enhances the formation of cyclic AMP. This system is also sensitive to inhibition by antipsychotic drugs in a dose-dependent manner, and constitutes an efficient test for in vitro evaluation of these drugs.

In the latter test, however, there is less variation in activity, piperidinols and esters having potencies in the same range.

All the compounds were also evaluated for potential antipsychotic activity in rats with unilateral lesions of the nigrostriatal tract, a behavioural model developed by Ungerstedt.[22] In these rats, haloperidol and generally neuroleptic drugs antagonized, in a dose-dependent manner, the turning behavior induced by apomorphine. The pharmacological results are summarized in Table I. The activity of the piperidinol **3** and the two esters **6** and **8**, to block apomorphine-induced rotation, was in the same dose range, and about 4–6 times lower than that of haloperidol, which, in turn, was about twice more active than the corresponding propionate **12**. All the other listed compounds have reduced activity.

Here again the order of activity in the ester series **6** > **8** > **7** correlates positively with the inhibition of [^{3}H]-spiroperidol (D_2 receptors) and with in vitro and in vivo analgesic tests (Table I).

Conclusion

The pharmacological results obtained in this series of compounds suggested that a combination of neuroleptic and analgesic activity can be achieved by simply esterifying the 4-hydroxy function of piperidinols (**3–5**) with propionic anhydride to give the propionate **6–8**. Haloperidol propionate (**12**) also showed a good affinity to opioid receptors, but it was about 10 times weaker than **6** and **8**. This finding could be interpreted as a consequence of steric effects of the chlorine atom hindering a close fit of the phenyl ring with the complementary site of the receptor. In the in vivo hot-plate test, its analgesic activity is masked by the prevailing neuroleptic activity; in fact its behavioral profile resembles more those of haloperidol and piperidinols **3–5**.

The combined pharmalogical profile of the esters **6** and **8** is worth further consideration. Evaluation of the abuse potential of these two esters is under investigation.

Experimental Section

Melting points (uncorrected) were taken in a Büchi–Tottoli capillary melting 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. Silica gel (Stratocrom SIF_{254}, C. Erba), 5 × 10 cm, was used for TLC. Spots were visualized under UV light (short wavelength) or by spraying with Dragendorff's reagent. The α- and β-diastereoisomers were all evaluated as racemates. Haloperidol, from Sigma Chemical Co., was used for the preparation of **12**. Serenase (1 mg mL^{-1} of haloperidol hydrochloride), from Lusofarmaco, was used in pharmacological tests.

(±)-(α)- and -(β)-1-benzyl-3-methyl-4-phenyl-4-piperidinols were prepared as described previously.[15] The two isomers were obtained in pure form by fractional crystallization of the hydrochloride with ethanol; α-HCl, mp 186–187 °C (lit.[23] mp 188–189 °C); β-HCl, mp 228–229 °C. Mixed fractions were chromatographed on a column of silica gel (0.04–0.06 mm) with chloroform–acetone (2:1) as eluent: α, Rf 0.37; β, R_f 0.53.

Catalytic Hydrogenation of *N*-Benzylpiperidinols. (±)-(α)-3-Methyl-4-phenyl-4-piperidinol, (±)-β-isomer, and the 3-desmethyl analogue were obtained by catalytic hydrogenation at 55–60 °C (2 atm) over 10% Pd(C) of the corresponding *N*-benzyl derivatives, as hydrochloride salts, in 80% aqueous ethanol (for 5 g of the starting piperidinol in 100 mL of solvent, 1 g of catalyst was used). After 24 h of hydrogenation in a Parr hydrogenator, in the above conditions, TLC (SiO_2, chloroform–acetone, 2:1) revealed the absence of the starting material. The resulting secondary amines did not move from the point of application. The product was filtered and the filtrate evaporated to give a solid residue, which was converted to the free base with aqueous ammonia and extracted with chloroform. The analytical samples were crystallized from methanol–ether. (±)-(α)-3-Methyl-4-phenyl-4-piperdinol, mp 131–133 °C. Anal. ($C_{12}H_{17}NO$) C, H, N. (±)-(β)-3-Methyl-4-phenyl-4-piperidinol, mp 149–150 °C. Anal. ($C_{12}H_{17}NO$) C, H, N. 4-Phenyl-4-piperidinol, mp 158–160 °C (lit.[24,25] mp 159–160 °C).

Isomeric (±)-(α)- and -(β)-1-[3-(*p*-Fluorobenzoyl)-propyl]-3-methyl-4-phenyl-4-piperidinols (4, 5). Compounds **4** and **5** were obtained with some modifications of the method adopted by Janssen et al.[25] for the preparation of **3**.

To (±)-(α)-3-methyl-4-phenyl-4-piperidinol (3 g, 15.7 mmol) in 150 mL of toluene were added γ-chloro-*p*-fluorobutyrophenone (4.7 g, 24 mmol), potassium iodide (60 mg), and anhydrous sodium

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carbonate (5 g, 50 mmol), and the mixture was heated at reflux temperature on an oil bath for 48 h, with vigorous magnetic stirring (TLC monitoring, SiO_2 F_{254}, $CHCl_3$–MeOH–NH_4OH, 95:5:0.5; starting material, R_f 0.00; **4**, R_f (0.30). After cooling, the mixture was filtered from the inorganic salts, and the solid was washed with chloroform. The filtrate and washings were evaporated to give a residue, which was converted to the hydrochloride with ethereal HCl and crystallized from ethanol (yield 4.2 g, 69%), mp 169–171 °C. Anal. ($C_{22}H_{26}FNO_2$·HCl) C, H, N.

The β-isomer **5** was prepared similarly; yield 68%, hydrochloride, mp 253–255 °C dec from methanol. Anal. ($C_{22}H_{26}FNO_2$·HCl) C, H, N.

Isomeric (±)-(α)- and -(β)-1-[3-(*p*-Fluorobenzoyl)propyl]-3-methyl-4-phenyl-4-(propionyloxy)piperidines (7, 8). The α-piperidinol 4 as free base (3.5 g), propionic anhydride (10 mL), and pyridine (10 mL) were refluxed until TLC (SiO_2, $CHCl_3$–MeOH–NH_4OH, 95:5:0.5) indicated that all the starting piperidinol had been converted (ca. 2 h). The dark reaction mixture was then cooled, diluted with aqueous ammonia, and extracted with chloroform. The chloroform extract was washed three times with small portions of water, dried, and thoroughly evaporated on a rotavapor. Crystallization of the residue from ethyl acetate–ether gave **7**, 2.8 g, 68%, mp 85–86 °C. Anal. ($C_{25}H_{30}FNO_3$) C, H, N.

Other esters were prepared in the same way and characterized as follows. The β-ester **8** was prepared from **5**; yield 49%, mp 98–99 °C, from ethyl acetate–ether. Anal. ($C_{25}H_{30}FNO_3$) C, H, N. Hydrochloride, mp 141–143 °C, from ethanol–ether. Anal. ($C_{25}H_{30}FNO_3$·HCl·H_2O) C, H, N. TLC (SiO_2, $CHCl_3$–MeOH–NH_4OH, 95:5:0.5): **7**, R_f 0.51; **8**, R_f 0.79.

The 3-desmethyl ester **6** prepared from **3** was isolated as the hydrochloride, mp 176–178 °C from ethanol–ether. Anal. ($C_{24}H_{28}FNO_3$·HCl) C, H, N. The base melted at 81–82 °C from ethanol–heptane. Anal. ($C_{24}H_{28}FNO_3$) C, H, N.

1-[3-(*p*-Fluorobenzoyl)propyl]-4-(*p*-chlorophenyl)-4-(propionyloxy)piperidine (**12**) (haloperidol propionate), obtained from haloperidol, was isolated as the hydrochloride; mp 212–213 °C from ethanol; yield 79%. Anal. ($C_{24}H_{27}ClFNO_3$·HCl) C, H, N.

1-Benzyl-4-phenyl-1,2,5,6-tetrahydropyridine. A mixture of 1-benzyl-4-phenyl-4-piperdinol (4 g, 15 mmol), concentrated HCl (15 mL), and glacial acetic acid (54 g) was heated under reflux temperature for 10 h. Most of the solvent was removed under vacuum, and the concentrated solution was cooled, made alkaline with aqueous ammonia, and extracted with ether. After evaporation of the dried (Na_2SO_4) ether solution, the residue was converted to its hydrochloride salt with ethereal HCl and crystallized from ethanol–ether, mp 204 °C (yield amost theoretical)(lit.[26] mp 203 °C). Anal. ($C_{18}H_{19}N$·HCl) C, H, N.

4-Phenylpiperidine. 1-Benzyl-4-phenyl-1,2,5,6-tetrahydropyridine hydrochloride (4 g) was dissolved in 50 mL of 80% aqueous ethanol and hydrogenated in a Parr apparatus at 50–60 °C (1.5 atm) for ca. 5 h in the presence of 5% Pd(C) (1 g). The mixture was filtered through a pad of Celite and evaporated to dryness to give a solid, which was crystallized from ethanol–ether; yield 2.2 g, 79%; mp 164–165 °C. Anal. ($C_{11}H_{15}N$·HCl·0.5H_2O). The base melted at 79–81 °C from heptane (lit.[24] mp 77–78 °C).

1-[3-(*p*-Fluorobenzoyl)propyl]-4-phenylpiperidine (9). A mixture of 4-phenylpiperidine (3 g, 18.6 mmol), γ-chloro-*p*-fluorobutyrophenone (6 g, 30 mmol), potassium iodide (40 mg), and anhydrous sodium carbonate (6.4 g, 60 mmol) in 100 mL of toluene was vigorously stirred at reflux temperature on an oil bath for 48 h. The inorganic salt was filtered and washed with ether. The filtrate and washings were evaporated to give a residue that was converted to its hydrochloride salt with ethereal HCl and crystallized from ethanol; yield 2.9 g, 43%, mp 236–237 °C. Anal. ($C_{21}H_{24}FNO$·HCl) C, H, N.

1-[3-(*p*-Fluorobenzoyl)propyl]-4-hydroxypiperidine (10). Treatment of 4-hydroxypiperidine as described for **4** gave **10** in 50% yield after crystallization from ethanol–heptane, mp 84–85 °C. Anal. ($C_{15}H_{20}FNO_2$) C, H, N.

1-[3-(*p*-Fluorobenzoyl)propyl]-4-(propionyloxy)piperidine (11). Treatment of **10**, as described for **7**, gave **11**, which was isolated as the hydrochloride salt, mp 171–172 °C from ethanol–ether. Anal. ($C_{18}H_{24}FNO_3$·HCl) C, H, N. The base melted at 42–42.5 °C from ethanol–heptane. Anal. ($C_{18}H_{24}FNO_3$) C, H, N.

Pharmacology Methods. [^{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.[27] 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 concentration 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 coktail (Packard) with an efficiency of about 60%.

Hot-Plate Test. The antinociceptive effect was evaluated according to the hot plate assay procedure for the opioids as described by Atwell and Jacobson.[28] The plate temperature was set at 55 °C, and ED_{50} values and its 95% confidence limits were calculated by proibit analysis.

[^{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.[17] 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 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 Watman GF/B glass fiber 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 coktail (Packard) with an efficiency of 60%.

Adenylate Cyclase Assay. Rat striatal dopamine-sensitive adenylate cyclase was measured as described by Salomon et al.[19] with some modifications. Striata from male Sprague–Dawley rats were homogenized in Tris–maleate (80 mM), pH 7.4 (3 mg tissue/mL), containing $MgSO_4$ (5 mM), EGTA (1 mM), and dithiothreitol (1 mM). Aliquots of this homogenate (0.05 mL) were incubated at 30 °C for 3 min in 0.1 mL of Tris–maleate buffer in the presence of 20 mM creatine phosphate, 100 units/mL creatine kinase, 10 mM theophilline, 0.5 mM ATP, and about 500 000 cpm of [^{14}C]ATP (Amersham, specific activity 55 mCi/mmol).

The inhibitor effect of the drugs was estimated in the presence of 5×10^{-5} M dopamine, the concentration able to induce a 60–70% stimulation over the basal enzymatic activity. Reaction was stopped by boiling the samples for 5 min, and [^{14}C]cyclic AMP that formed was separated by the two-step chromatographic method (Alumina/Dowex AG 50 × 8 columns) as described in the original method.[19] The radioactivity in the eluate (4 mL) was measured by liquid scintillation coktail (LUMAGEL) with an efficiency of about 60%.

Antagonism of Apomorphine-Induced Circling Behavior. Potential neuroleptic activity in vivo of the synthesized compounds was investigated by studying their antagonism on apo-

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morphine-induced circling behavior in rats with monolateral lesion of nigrostriatal tract as described by Ungerstedt.[22] Male Sprague–Dawley rats weighing 140–180 g at the beginning of the experiments were anesthetized with sodium pentobarbital (40 mg/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/5 min) directly into substantia nigra. Coordinates were taken from ref 29. Ten days after surgery all the animals were checked for their sensitivity to dopaminergic stimulation by measuring the number of revolution/60 min occurring after the subcutaneous injection of 2 mg/kg apomorphine. For further circling studies, only those responding with, at least, 200 turns/60 min after apomorphine injection were used. Approximately 40% of the operated animals failed at 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 produces 50% of inhibition of rotational behavior induced by apomorphine for 60 min.

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Registry No. **2**, 52-86-8; **3**, 3109-12-4; **3**·HCl, 4021-57-2; (±)-**4**, 109765-70-0; (±)-**4**·HCl, 109765-72-2; (±)-**5**, 109765-71-1; (±)-**5**·HCl, 109765-79-9; **6**, 109765-76-6; **6**·HCl, 109765-77-7; (±)-**7**, 109765-73-3; (±)-**7**·HCl, 109765-83-5; (±)-**8**, 109765-74-4; (±)-**8**·HCl, 109765-75-5; **9**, 73962-26-2; **9**·HCl, 109765-82-4; **10**, 19695-21-7; **10**·HCl, 19668-15-6; **11**, 109765-80-2; **11**·HCl, 19668-16-7; **12**, 109765-78-8; **12**·HCl, 109765-81-3; (±)-(α)-1-benzyl-3-methyl-4-phenyl-4-piperidinol hydrochloride, 109765-66-4; (±)-(β)-1-benzyl-3-methyl-4-phenyl-4-piperidinol hydrochloride, 109765-67-5; 1-benzyl-4-phenyl-4-piperdinol hydrochloride, 88783-32-8; (±)-(α)-3-methyl-4-phenyl-4-piperidinol, 109765-68-6; (±)-(β)-3-methyl-4-phenyl-4-piperidinol, 109765-69-7; 4-phenyl-4-piperidinol, 40807-61-2; γ-chloro-*p*-fluoro-butyro-phenone, 3874-54-2; 1-benzyl-4-phenyl-1,2,5,6-tetrahydropyridine, 94163-98-1; 4-phenylpiperidine, 771-99-3; 4-phenylpiperidine hydrochloride, 10272-49-8; 4-hydroxypiperidine, 5382-16-1.

Effect of Reductive Alkylation of Lysine in Positions 6 and/or 8 on the Histamine-Releasing Activity of Luteinizing Hormone-Releasing Hormone Antagonists[1]

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The solid-phase reductive alkylation of the Lys side chain in positions 6 (D) and 8 (L) and position 8 alone of the LH-RH antagonist [*N*-Ac-D-Nal[1],D-Ph[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH was investigated in an attempt to reduce the histamine-releasing activity inherent to most potent antagonists while retaining high antiovulatory activity. The protected parent analogues were prepared by conventional solid-phase peptide synthesis. After selective removal of the Lys Fmoc side chain protection, the resin-bound peptides were readily and conveniently alkylated at the ε amino groups with various aldehydes and ketones in the presence of $NaBH_3CN$. The analogues were then cleaved from the resin with simultaneous deprotection by anhydrous hydrogen fluoride and purified to homogeneity in two stages: gel permeation followed by preparative reversed-phase liquid chromatography. The analogues were assayed in standard rat antiovulatory and in vitro histamine-release assays.

Two aims for the synthesis of antagonists of luteinizing hormone-releasing hormone (LH-RH), a decapeptide with the sequence Glp-His-Tyr-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2,[2] are the control of fertility by the blockade of ovulation and the control of hormone-dependent tumors. Toward these goals, over 1000 analogues have been synthesized, internationally, in the last several years in the search for ever more potent antagonists. Currently, the most active analogues are characterized by distinct hydrophobic and hydrophilic regions and are typified by the antagonist [*N*-Ac-D-Nal[1],D-Phe[2,3],D-Arg[6],Phe[7],D-Ala[10]]LH-RH.[3] Recently, however, the antagonists, when injected subcutaneously into rats at 50–100 times the effective antiovulatory dose, have been shown to cause transient edema of the face and extremities.[4] Additionally, many compounds are mast cell secretagogues, release histamine, and are able to induce a cutaneous anaphylactoid-like response in rats, causing a dose-related wheal reaction.[5] Other peptides, most notable those containing several closely spaced basic residues, are also known to cause the release of histamine including substance P, somatostatin, and neurotensin, and the phenomenon is clearly linked to the presence of highly basic Lys and Arg residues.[6–8] In this study of the structure–activity relationship of the histamine-releasing activity of the LH-RH antagonists, we decided to investigate the effects of changing the hydrophobicity and basicity of the basic side chains at positions 8 and 6, and position 8 alone.

Results and Discussion

Chemistry. Since only a limited number of naturally occurring basic amino acids are available commercially, we

(1) Abbreviations used in this paper for amino acids, protecting groups, and peptides follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature and Symbols as described in the following: *Eur. J. Biochem.* **1972**, *27*, 201. *J. Biol. Chem.* **1975**, *250*, 3215. Glp, pyroglutamic acid; D-Nal, 3-(2-naphthyl)-D-alanine.

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