Antipsychotic Properties of New *N*-(4-Substituted-1-Piperazinylethyl)- and *N*-(4-Substituted-1-Piperidinylethyl)-Phthalimides

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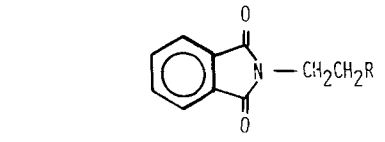
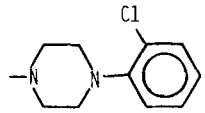
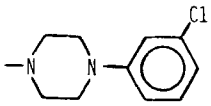
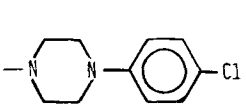
Abstract □ A series of *N*-(4-phenyl- and 4-pyridyl-1-piperazinylethyl)- and *N*-(4-phenyl-1-piperidinylethyl)-phthalimides were synthesized and tested for antipsychotic activity. All compounds suppressed the spontaneous motor activity and the apomorphine-induced climbing in mice and pergolide-induced locomotor activity in rats, demonstrating psychotropic properties equal to the corresponding properties of sulpiride. Although the compounds, like sulpiride, were less potent than haloperidol in blocking the locomotor activites, they caused no catalepsy, a major side effect following treatment with conventional antipsychotic agents. It is likely that the new compounds produce their neuroleptic activities through inhibition of limbic dopamine receptors.

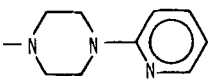
One of the major drawbacks following treatment with neuroleptic drugs is the occurrence of extrapyramidal side effects. In recent years studies have been directed towards the synthesis of drugs with greater clinical efficacy and/or reduced liability to produce extrapyramidal side effects. A number of agents, belonging to diverse chemical classes, have been reported to produce such atypical antipsychotic properties.[1-11] Sulpiride, a substituted benzamide, is one of the most studied agents,[8,9] and other benzamides were shown to possess similar properties.[10,11] Substituted aliphatic cyclic imides have also been reported to have atypical antipsychotic activity.[6,7] As a part of the effort to develop atypical antipsychotic agents, we have prepared a series of *N*-(4-phenyl- and 4-pyridyl-1-piperazinylethyl)- and *N*-(4-phenyl-1-piperidinylethyl)-substituted aromatic cyclic imides as potential neuroleptic agents.

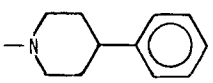


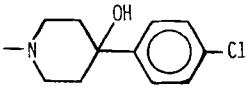
1 R = 

2 R = 

3 R = 

4 R = 

5 R = 

6 R = 

Experimental Section

Chemicals—Apomorphine hydrochloride (Sigma Chemical Company, St. Louis, MO), pergolide mesylate (Eli Lilly, Basingstoke, England), sulpiride (Delagrange, Paris, France), and haloperidol (Lusofarmaco, Italy) were used. All other chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI).

Apparatus—Melting points were determined using a Mettler FP5 melting point apparatus and are uncorrected. The IR spectra were run on a Perkin-Elmer 580 B infrared spectrometer as KBr discs. The ^{1}H NMR spectra were recorded on a Jeol FX100 (100 MHz) instrument, with tetramethylsilane as an internal standard. Elemental analyses were obtained using a Perkin-Elmer 240 B elemental analyzer.

Solutions—Apomorphine hydrochloride was dissolved in 0.1% sodium metabisulfite solution. Sulpiride was dissolved in 0.9% NaCl with the aid of a small amount of tartaric acid. All other drugs were dissolved in 0.9% NaCl solution in water.

Chemistry—The *N*-substituted imides were prepared from *N*-(2-bromoethyl)-phthalimide (10 mmol) and the appropriately substituted piperazine or piperidine (10 mmol) dissolved in freshly distilled *n*-butanol (10 mL). Anhydrous Na_2CO_3 (15 mmol) was added to the solution and the mixture was refluxed for 24 h. The reaction mixture was then filtered while hot to remove inorganic salts. After cooling, the crude product separated as a solid which was collected by filtration. The crude product was then recrystallized from ethanol, except for 2 which was recrystallized from benzene. The purity of each product was tested with TLC using silica gel and either chloroform or a chloroform:ethanol mixture as the solvent system. The hydrochloride salts of **1–3**, **5**, and **6** were prepared by passing ethereal hydrogen chloride into the ethanolic solutions of the bases. The same procedure was used for preparing the hydrochloride salt of **4** except that the base was dissolved in chloroform. The resulting hydrochloride salts were then recrystallized from ethanol.

Toxicity—Male Albino Wistar mice (25–30 g) were divided into six groups of 10 mice each. Increasing doses of test compounds were given ip at increments of 50 or 100 μmol/kg, and then death in each group was scored. The mean mortality was calculated and multiplied by the dose increments used. A special formula of Karber[12] was then used to calculate the LD_{50} of each compound.

Apomorphine-Induced Climbing Behavior—Female Albino Wistar mice weighing 30 ± 2.1 g were placed in individual climbing

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cages with 1-cm diameter wire mesh sides (12 cm long, 6 cm wide, 9 cm high). Following a short habituation period (10–15 min), each animal was removed and 0.5 mg/kg of apomorphine hydrochloride was administered sc in a volume of 0.5 mL per 100 g of body weight. The animals were then returned to the cages and the ability to climb was measured at 5-min intervals from 5 to 30 min following apomorphine administration.[13,14] For measuring climbing behavior, an Opto Varimex activity meter (Columbus Instruments) equipped with a sensor to detect vertical activity was used. The climbing cage was placed in front of the sensors every time climbing was measured. Test drugs were injected ip 30 min before apomorphine administration. The number of climbing activities was recorded automatically on a timer-controlled printer at 5-min intervals for a period of 30 min. Groups of six mice were used for each dose of test drug.

Spontaneous Locomotor Activity—Female Albino Wistar mice weighing 30 ± 3.2 g were injected ip with drug solutions and placed in an Opto Varimex activity meter (Columbus Instruments). Four groups of six mice each were used for each test and for saline-treated controls. Experiments were run at the same time of the day and at the same temperature, and noise and lighting were kept at the same level each day. Activity counts were recorded automatically on a timer-controlled printer at 10-min intervals for a period of 2 h. The effects of the test drugs on locomotor activity were compared with appropriate controls at each time interval and recorded.

Pergolide-Induced Locomotor Activity—Locomotor activity was assessed in perspex activity cages (38 cm long, 25 cm wide, 23 cm high), that were fitted with grid bars running parallel to each other. When rats traverse these bars they activate a circuit which transforms these movements into signals that can be registered as counts per a preset time. The instrument used was UGO Basile Biological Research Apparatus (Comerio, Italy). Monitoring commenced immediately following the introduction of animals into individual cages. The total number of counts during each 20-min period was recorded. This behavior was monitored for 2 h for male Albino Wistar rats (190–240 g) which had been injected ip with pergolide (2.55 μmol/kg) or vehicle. Antagonists were injected 30 min prior to pergolide administration and then activity was measured for 2 h. Then, the same rats were kept drug free for a week before they were pretreated ip with different doses of test drug (10, 20, and 30 μmol/kg). These steps were repeated on a fresh group of rats (n = 6) whenever a new compound was tested for its effect on locomotor activity.

Catalepsy—The ability of the test compounds to induce a cataleptic state in male Albino Wistar rats (220–250 g) was assessed by application of a scoring system which allows the demonstration of a dose dependency for the cataleptic action of haloperidol (0.38–5 μmol/kg ip gave scores ranging from 1 to 5). Catalepsy was measured by carefully placing an animal with its front limbs extended over a 10-cm high bar and noting the time the animal maintained the imposed position.[15] Animals were tested every 10–30 min after drug administration and the catalepsy was scored as follows: 0 = no catalepsy; 1 = 1.0–3.1 min; 2 = 3.2–5.0 min; 3 = 5.1–10.0 min; 4 = 10.01–25.0 min; 5 = >25 min.

Antimuscarinic Activity—Male Dunkin Hartley guinea pigs were killed, the abdomens were opened, and pieces of the ileum (2 cm long) were removed and suspended under a tension of 1.0 g in Tyrode's solution (pH 7.4, 37 °C).[16] The bathing fluid was oxygenated with a gas mixture of 5% CO_2 in O_2. Each tissue was allowed to equilibrate in the bathing fluid for 30 min with two changes of bathing fluid before addition of any drug. Isometric tension was recorded using a Washington MD2R recorder and a Washington type D1 transducer. Three minutes were allowed before any successive two additions of acetylcholine. The compounds, dissolved in saline, were added to the bathing fluid in a volume of 0.2 mL and left in contact with the tissue for 15 min before addition of acetylcholine.

Statistics—A *t* test was used for the statistical analyses when comparing the effects of these compounds with the standard drugs; where appropriate, analysis of variance was used.

Results

Chemistry—The title compounds were generally synthesized by direct reaction of *N*-(2-bromoethyl)-phthalimide with the appropriately 4-substituted piperazine or piperidine. Sodium carbonate was used to neutralize the HCl generated in the reaction. The compounds were purified by repeated recrystallization using ethanol or benzene as the solvent. The hydrochloride salts were prepared and purified as described in the *Experimental Section*. The purity of the compounds was tested using silica gel thin-layer chromatography. Characterization of the products was achieved through IR and NMR spectrophotometry. The IR and ^1H NMR spectra of all compounds were in full agreement with the proposed structures. Further identification was obtained using elemental analysis (Table I).

Effect on Apomorphine-Induced Climbing—All compounds caused inhibition of the apomorphine-induced climbing in mice. The percent inhibition was calculated for various doses of each compound, log dose–effect curves were constructed, and the ID_{50} was determined (Table II).

Effect on Spontaneous Locomotor Activity—All compounds caused considerable reduction in the spontaneous locomotor activity in mice in the first hour of observation as compared with the activity of control mice receiving saline only. The mean of the total activity counts during the first hour following drug administration was expressed as a percentage of the mean activity counts of the saline-treated group. The effect of each compound was expressed as a percentage of the mean activity counts of the saline-treated group. The ID_{50} value of each compound was calculated from the log concentration–effect curves (Table II).

Effect on Pergolide-Induced Locomotor Activity—The test compounds significantly inhibited the pergolide-induced locomotor activity (Table II). The magnitude of inhibition of the substituted aromatic cyclic imides was similar to that of sulpiride, but significantly less than that of haloperidol.

Catalepsy—The ip injection of the test compounds, in a dose range of 25 to 125 μmol/kg, and sulpiride (15–90 μmol/kg) into different groups of rats produced no cataleptic response. Haloperidol, at a dose of 5 μmol/kg, showed maximum catalepsy in rats (Table II).

Antimuscarinic Activity—Compounds 2–4 showed weak antimuscarinic activity by inhibiting the acetylcholine-induced contractions of the guinea pig ileum. A representative trace is shown in Figure 1.

Toxicity—The acute lethality of the compounds was determined in male Albino Wistar mice after ip dosing. The median lethal dose (LD_{50}) was shown to be lower in those compounds having a 4-substituted piperidinyl group as the basic part of the molecule (Table II).

Discussion

The compounds prepared by a nucleophilic substitution of the properly substituted piperazines and piperidines with the *N*-(2-bromoethyl)imide were screened for their anticipated antipsychotic activity as well as for possible toxicity. The pharmacological properties of the compounds were compared with those of haloperidol and sulpiride. Psychosis is believed to be ameliorated by agents that block dopamine receptors in limbic regions of the brain. Concomitant blockade of dopa-

Table I—Physical and Analytical Data

Compound	Yield, %[a]	mp, °C[a]	Formula[b]
1	64	112–114	$C_{20}H_{21}Cl_2N_3O_2$
2	38	164–165	$C_{20}H_{21}Cl_2N_3O_2$
3	61	165–166	$C_{20}H_{21}Cl_2N_3O_2$
4	55	144–145	$C_{19}H_{21}ClN_4O_2$
5	52	128–129	$C_{21}H_{22}ClN_2O_2$
6	26	162–164	$C_{21}H_{22}Cl_2N_2O_3$

[a] Yield and mp are those of the purified bases. [b] All compounds were analyzed as the hydrochloride salts for C, H, and N; the values were ±0.4% of the theoretical value.

Table II—Pharmacological and Toxicological Data for the New Compounds

Compound	LD_{50}, mmol/kg (Mean ± SE; n = 10)	Inhibition of Apomorphine-Induced Climbing, ID_{50}, μmol/kg (Mean ± SE; n = 6)	Inhibition of Spontaneous Locomotor Activity, ID_{50}, μmol/kg (Mean ± SE; n = 4)	Catalepsy Score (n = 6)[c]	Inhibition of Pergolide-Induced Locomotor Activity, Total Activity Counts in 2 h (Mean ± SE; n = 6)[f]
1	1.25[a]	42.4 ± 6.8	32.4 ± 4.1	0	754.1 ± 61.3[d]
2	1.25 ± 0.041	21.2 ± 1.6	64.0 ± 9.1	0	860.6 ± 72.1[d]
3	1.25[a]	39.6 ± 7.2	50.4 ± 6.1	0	990.6 ± 112.1[e]
4	1.05 ± 0.032	28.1 ± 3.4	135.1 ± 6.2	0	904.2 ± 161.3[e]
5	0.36 ± 0.011	33.4 ± 5.6	43.7 ± 5.6	0	544.6 ± 25.7[d]
6	0.53 ± 0.017	35.6 ± 4.9	110.0 ± 12.1	0	1845.3 ± 114.8[e]
Sulpiride	—	44.7 ± 4.6	17.6 ± 4.4	0	1034.0 ± 81.1
Haloperidol	—	0.11 ± 0.01[b]	2.1 ± 0.01[b]	5	61.9 ± 8.5[b]
Saline only	—	—	—	—	76.4 ± 8.3[b]
Pergolide only	—	—	—	—	3248.3 ± 212.9

[a] Insoluble at higher concentrations. [b] Significantly different ($p < 0.001$). [c] 0 = No catalepsy; 5 = maximum catalepsy. [d] Significantly different from values of pergolide ($p < 0.01$). [e] Significantly different from values of pergolide ($p < 0.05$). [f] Dosages: haloperidol, 0.5 μmol/kg; sulpiride or test compound, 30 μmol/kg.

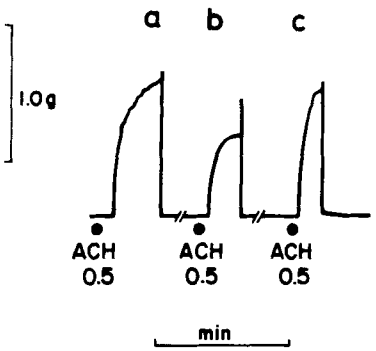

Figure 1—*The inhibitory effect of* **4** *(50 nM) on acetylcholine-induced contractions of the guinea pig ileum. Compound* **4** *was added between peaks* a *and* b. *The response to acetylcholine (0.5 nM) was attenuated by* **4** (b) *and returned to normal after washing* (c).

mine receptors in the striatum leads to extrapyramidal side effects.[17,18] Therefore, preferential blockade of limbic dopamine receptors might lead to a lower tendency to produce extrapyramidal side effects. Behavioral studies are generally conducted to identify dopamine antagonists selectively acting on the limbic system. Inhibition of dopamine agonist-induced locomotor activity is thought to result from limbic dopamine receptor blockade.[19–21] It follows, therefore, that blockade of limbic dopamine receptors, and presumably antipsychotic activity, would be indicated by inhibition of locomotor activity, while decreased striatal dopamine receptor blockade would be predicted by the absence of catalepsy.[22] To test their neuroleptic effects, the title compounds were subjected to three different behavioral experiments in mice and rats (i.e., spontaneous motor activity, apomorphine-induced climbing, and pergolide-induced locomotor activity). Pergolide (1 mg/kg, 2.55 μmol/kg) was reported to produce strong locomotor stimulation in rats which lasted for several hours.[23]

From an examination of the data in Table II, it is apparent that all compounds caused considerable inhibitory effects in the three behavioral test models. Although they exhibited potencies weaker than that of haloperidol, they demonstrated inhibitory properties equal to or better than those of sulpiride (Table II). Sulpiride, classified as an atypical neuroleptic drug, inhibited apomorphine-induced climbing without inducing catalepsy.[8] The ability or inability of the new compounds to induce catalepsy was also tested in rats, using haloperidol as a standard. No cataleptic response was observed for any of the test compounds at the dose levels used, while haloperidol, at much lower doses, produced a strong catalepsy (Table II).

Our results using behavioral studies suggest that the test compounds possess antipsychotic properties in mice and rats that are comparable to those of sulpiride, probably by selective blockade of limbic dopamine receptors. The fact that none of the compounds produced catalepsy in rats suggests little or no interaction with striatal dopamine receptors. It has been reported that the antimuscarinic potency of some antipsychotic agents is inversely correlated with their tendency to produce extrapyramidal side effects.[24,25] Thus, an intrinsic antimuscarinic property will attenuate the effects of dopamine antagonists in the striatum. The antimuscarinic activity associated with **2**–**4**, though weak compared with atropine (Figure 1), may contribute, in part, to their lack of cataleptic behavior. From the statistical data at hand, no structure–activity correlation could be made. It seems, however, that chlorosubstitution at the ortho, meta, or para positions of the phenyl moiety produces no significant difference in the neuroleptic potency or toxicity. Likewise, isosteric replacement of the phenyl group with a pyridyl moiety did not significantly alter the antipsychotic potency. Compounds **5** and **6**, having substituted piperidine, showed equal potencies to those containing substituted piperazine, but exhibited higher toxicity.

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