126.4 (C-5), 129.3 (C-3), 130.7 (C-1), 134.7 (C-8), 147.3, 147.8 (C-5a, 10a), 152.7 (C-9), 166.4 (NHCO); mass spectrum, m/z 349 (M), 306 (M − $CH_2CH_2CH_3$) 277, 250 (100). Anal. ($C_{22}H_{27}N_3O$) C, H, N.

Preparation and Alkylation of Labeled DNA Fragment. The plasmid pBR322 DNA was obtained from Boehringer–Mannheim. The restriction enzymes, EcoRI and Bam HI, and the Klenow fragment of DNA polymerase I were all purchased from Pharmacia. EcoRI digested pBR322 was 3′-end labeled by using Klenow fragment in the presence of ^{32}P-dATP and cold mixture of dNTPs. The fragment was then further digested with BamHI and the 375 base-pair labeled fragment was isolated on a 4% nondenaturing polyacrylamide gel. The partial sequence is given in ref 7.

Appropriate conditions for DNA alkylation were established as previously described.[7] Labeled DNA (ca. 30 000 counts) was incubated with the compounds **5**, **7**, or **8** in the presence of 1 μg of sonicated calf thymus DNA for 30 minutes at 37 °C. The drug to base-pair ratio was adjusted against the calf thymus DNA. For Mg^{2+} experiments, the reactions were carried out in the presence of either 30 mM NaCl or 10 mM $MgCl_2$ (final ionic strength = 0.04).

The modified DNA was then precipitated with EtOH, dissolved in 100 μL of 0.01 SHE buffer (pH = 7.3), and heated at 90 °C for 10 min. This leads to depurination at alkylated purine sites.[7] The DNA was then again precipitated with EtOH, dissolved in 100 μL of freshly prepared 1 M piperidine and heated at 90 °C for 10 min which leads to strand breaks at all depurinated sites.[7] The sample was then lyophilized overnight, washed twice with 10 μL of distilled water, and lyophilized briefly. The pellet was dissolved in a sequencing dye (80% deionized formamide, 1% xylene cyanol, and 1% bromophenol blue) and denatured at 90 °C for 2 min before loading on a 0.4 mm thick, 8% polyacrylamide sequencing gel containing 8 M urea. The gel was run on a Sequi-Gen kit from Biorad for 2 h in the TBE buffer at a constant 2500 V. It was then transferred to 3-mm Whatman filter paper, covered with plastic wrap and dried in a Biorad gel drier model 1125B for 30 min at 80 °C. The gel was then placed in intimate contact with X-AR5 Kodak film and exposed for 10–15 h before developing. The densitometer scans were performed on a Zeineh soft laser scanning densitometer.

Helix Unwinding Assay. The covalently closed circular pBR322 plasmid (1 μg) was incubated with various concentrations of drug in 10 μl of 0.01 SHE for 30 min at 37 °C. The drug to base-pair ratios used are 0.0, 0.1, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.40. At the end of the incubation 2 μL of 1% bromophenol blue dye was added, and the solutions were directly loaded on to a nondenaturing 1-cm thick 1% agarose minigel (noncovalently bound drug molecules will quickly run out of the gel and hence they will not play a part in the unwinding of the superhelix). The gel was run for 90 min at 80 V. It was then stained with ethidium bromide and the pattern observed under a UV light was photographed.

Determination of in Vitro Cytotoxicity and in Vivo Antileukemic Activity. Cell lines were maintained in exponential growth phase by subculturing in RPMI 1640 (P388) or Alpha MEM (AA8, UV4) containing 10% fetal calf serum as previously described.[11,33] IC_{50} values were determined by using log phase cultures in 96-well microculture plates and are calculated as the nominal drug concentration required to reduce the cell density to 50% of control values, with eight control cultures used on each microplate. For P388 cultures, drug was present throughout the growth period (72 h) and final cell densities were determined with use of a minor modification of the MTT method of Mossman.[34] For AA8 and UV4 cultures, drug exposure was terminated after 18 h by washing three times with fresh medium. Cultures were grown for a further 72 h before determining cell density by staining with methylene blue.[35]

The in vivo antileukemic activities of the compounds were determined in mice bearing the P388 lymphocytic leukemia cell line,[36] by using a single-dose protocol (see Experimental Section).

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Dopamine Autoreceptor Agonists as Potential Antipsychotics. 2. (Aminoalkoxy)-4*H*-1-benzopyran-4-ones

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The synthesis and pharmacological properties of a novel type of [(arylpiperazinyl)alkoxy]-4*H*-1-benzopyran-4-ones with dopaminergic activity are described. The nature of the arylpiperazine (AP) moiety determines the dopamine (DA) agonist/antagonist character of this series of compounds; when the aryl portion of the AP is unsubstituted the compounds appear to be DA autoreceptor agonists while substituted aryl groups seem to impart DA antagonist activity. A heterocyclic piperazine, 7-[3-[4-(2-pyridinyl)-1-piperazinyl]propoxy]-4*H*-1-benzopyran-4-one (**31**, PD 119819) has been identified as an extremely selective DA autoreceptor agonist in tests that include [^{3}H]haloperiodol binding, inhibition of spontaneous locomotor activity, inhibition of brain DA synthesis, inhibition of brain DA neuronal firing, stereotypy assessment, and reversal of 6-hydroxydopamine (6-OHDA) induced akinesia in rats. In addition, **31** possesses good oral activity in the Sidman avoidance test in squirrel monkeys, a predictor of clinical antipsychotic efficacy. In another primate model, **31** has been found to lack the liability for extrapyramidal side effects observed with currently available antipsychotic drugs.

During recent years several research groups, including our own, have focused on the development of dopamine (DA) autoreceptor agonists, compounds with selectivity for central nervous system (CNS) presynaptic DA receptors, as potential agents for the treatment of schizophrenia.[1] This approach is based on evidence that DA auto-

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Scheme I[a]

5: X = H, Y = OH
6: X = OH, Y = H

7: X = H, Y = OH
8: X = OH, Y = H

9: X' = H, Y' = $O(CH_2)_nCl$
10: X' = $(OCH_2)_nCl$, Y' = H

[a] (a) $HC(OEt)_3$, 70% $HClO_4$; (b) H_2O, 100 °C; (c) $Cl(CH_2)_nBr$, K_2CO_3, acetone.

Scheme II[a]

[a] (a) $Cl(CH_2)_nBr$, K_2CO_3, acetone; (b) Na, HCO_2Et; (c) HCl, EtOH.

receptors serve an inhibitory feed-back function on brain DA neurotransmission. Activation of DA autoreceptors results in inhibition of DA neuronal firing as well as inhibition of brain DA synthesis and release.[2] On this basis, it has been proposed that functional modulation of brain DA systems by DA autoreceptor activation might provide a viable treatment for psychotic symptoms,[3] without the excessive inhibition of DA neuronal activity that has been implicated in the adverse side effects of classical DA antagonist antipsychotics.[4]

The DA autoreceptor agonists described to date can be divided into two general categories according to their structures. The first group of compounds bears an unmistakable resemblance to DA. These compounds, such as 3-PPP[5] or HW-365,[6] contain a phenethylamine moiety

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Scheme III

9, 10, or 13 → (HNR_1R_2, $NaHCO_3$, NaI, DMF, 80 °C) $R_1R_2N-(CH_2)_nO-$ 4

embedded into a more rigid ring system. The selectivity of these compounds for the DA autoreceptor has been attributed to their increased rigidity, which probably introduces steric bulk in volumes of the postsynaptic DA receptor where it is not allowed.[7]

The second group of compounds includes EMD 23448 (1),[8] OPC-4392 (2),[9] and 3, a compound recently described

EMD 23,448 (1)

OPC-4392 (2)

3

by us as an extremely selective DA autoreceptor agonist with efficacy in rodent and primate models of antipsychotic activity.[1] These compounds all possess an aromatic ring separated from a 4-phenyl-1,2,3,6-tetrahydropyridine or 4-arylpiperazine (AP) moiety by a flexible lipophilic spacer. In addition to the aforementioned DA agonists, many substituted and unsubstituted 4-arylpiperazines have been described as CNS-active agents. The main pharmacological actions of this broad family of compounds include DA antagonism,[10] adrenergic[11] and noradrenergic[12] antagonism, and serotonergic activity (both agonist and antagonist).[13] In order to explain its interaction with the

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monoamine neurotransmitter receptors, the AP moiety has been viewed as a bioisosteric replacement for the arylethylamine portion of those neurotransmitters.[14] Alternatively, early in our work we postulated that there might exist an AP binding site within the neurotransmitter receptors, perhaps allosteric to the binding site for the neurotransmitters themselves. If so, a cursory look at the published data on substituted arylpiperazines suggests that the nature of the aryl portion of the AP moiety is a main factor in determining its specificity for a given receptor.

We therefore set out to determine how the AP portion of compounds related to **1–3** influences their specificity for the DA autoreceptor and their DA agonist/antagonist properties. We decided to abandon the aniline moiety of **3** when it was determined that high doses of this compound produced convulsions in animals. In search for an alternative aromatic ring, we discovered that the aniline could be replaced by a number of other aromatic rings without loss of CNS activity.[15] One such acceptable replacement is the 4*H*-1-benzopyran-4-one (chromone) ring.[16] This paper describes the synthesis of a group of compounds of general formula **4** and their evaluation as dopaminergic agents and potential antipsychotic drugs.

Chemistry

The compounds prepared are described in Table I. The target 5-, 6-, and 7-substituted chromones were prepared according to Schemes I–III. The parent 6- and 7-hydroxychromones (**7** and **8**, respectively) were prepared in 60–75% yield from the corresponding dihydroxyacetophenones (**5** and **6**, respectively) by treatment with triethyl orthoformate and 70% perchloric acid followed by aqueous

Scheme IV[a]

[a] (a) $ICH_2CH_2CH_2Cl$, CH_3CN; (b) $NaBH_4$, H_2O, MeOH; (c) **7**, NaH, DMF.

hydrolysis of the intermediate perchlorate salts.[22] Alkylation of **7** and **8** with the appropriate 1-bromo-ω-chloroalkanes gave chromones **9** and **10** in 70–95% yield. Reaction of **9** and **10** with secondary amines was typically carried out in DMF in the presence of sodium bicarbonate and a catalytic amount of sodium iodide. The 5-substituted chromones were best prepared according to Scheme II. Thus, 2,6-dihydroxyacetophenone was easily monoalkylated with 1-bromo-3-chloropropane to give **11**. The pyranone ring was built by reaction of **11** with sodium metal in ethyl formate, followed by treatment of crude **12** with hydrochloric acid in refluxing ethanol, to give **13** in about 80% yield. Alkylation of **13** with secondary amines was carried out as described above for **9** and **10** (Scheme III). Our choice of amine moieties included variously substituted phenylpiperazines, heteroaromatic piperazines, and phenyltetrahydropyridines, as well as a small group of miscellaneous moieties (see entries **29**, **30**, and **39–45** in Table I) with structural similarity to the phenylpiperazine/phenyltetrahydropyridine ring systems. The synthesis of **42** is outlined in Scheme IV. Tetrahydropyridine **48** was prepared by alkylation of 3-phenylpyridine with 3-chloro-1-iodopropane, followed by reduction of the quaternary salt with sodium borohydride. Alkylation of **48** with the sodium salt of **7** in DMF produced **42** in 69% yield.

Results and Discussion

Our screening strategy for DA autoreceptor agonists was based on the early identification of compounds with affinity for DA receptors and CNS behavioral effects, followed by functional tests aimed at establishing DA agonist or antagonist mechanism of action. DA agonists were then evaluated for DA autoreceptor selectivity as well as predicted antipsychotic efficacy in behavioral tests. Table I shows the in vitro binding affinity of target compounds for rat striatal DA receptors using [^{3}H]haloperidol as the ligand[23] and their inhibition of exploratory locomotor activity in mice and rats. Locomotor inhibition and lack of stimulation were used as a preliminary behavioral indicator of DA autoreceptor agonist or postsynaptic DA antagonist activity.[24] As part of this test, compounds were also tested for impairment of motor coordination since it has been shown that DA autoreceptor agonists as well as antipsychotic agents inhibit locomotor activity without producing ataxia.[1,25] The data presented in Table I show that the

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majority of the compounds containing the phenylpiperazine moiety, both substituted and unsubstituted on the phenyl ring, inhibit dark-stimulated exploratory activity in rodents. Also active are some heteroaromatic piperazine compounds, such as the 4-(2-pyridyl)piperazine **31** and the 4-(2-pyrimidyl)piperazine **36**. Phenyltetrahydropyridine **14** was behaviorally equipotent with phenylpiperazines **15**, **34**, and **35**. Interestingly, replacement of the phenyltetrahydropyridine ring in **14** with a 4-phenylpiperidine produces a compound (**29**) with greatly reduced activity. This loss of activity is probably due to the more coplanar arrangement of the phenyl/tetrahydropyridine rings than the phenyl/piperidine rings. Since it has been suggested that the bioactive conformation of arylpiperazines is fully coplanar,[26] it seems reasonable to view the phenyltetrahydropyridine moiety as a more viable replacement for the AP system than the less coplanar phenylpiperidine. With the exception of the γ-carboline ring system (**44**) all other attempted replacements for the arylpiperazine group (**29–30**, **39–43**, **45**) failed to provide compounds with potent behavioral activity. The majority of the target compounds shown in Table I were found to inhibit [^{3}H]haloperidol binding at the screening concentration of 10^{-7} M; however, with few exceptions, most compounds were only moderately active in displacing haloperidol from brain DA receptors.

The DA autoreceptor agonist mechanism of action of behaviorally active compounds was determined by their ability to reverse the γ-butyrolactone (GBL) induced increase in DA synthesis in the corpus striatum (a major brain DA projection area) of conscious rats, as measured by the rate of DOPA formation following decarboxylase inhibition[27] (Table II). These results were corroborated by measuring the ability of selected compounds to inhibit the spontaneous firing of DA neurons in the substantia nigra (a major brain DA cell body region) of anesthetized rats[28] (Table II). Phenyltetrahydropyridine **14** as well as **15**, **34**, and **35**, which contain the phenylpiperazine moiety, 4-(2-pyridyl)piperazine **31**, and 4-(2-pyrimidyl)piperazine **36** were all found to be DA autoreceptor agonists; the last two compounds produced complete reversal of the GBL-induced increase in DA synthesis and complete inhibition of DA neuronal firing. Compound **38**, an analogue of **31**, was also active, suggesting that branching on the central connecting chain is not particularly detrimental to DA agonist activity. However, the remaining compounds, all possessing substituted phenylpiperazines, were found to be extremely weak at inhibiting brain DA synthesis (e.g., **18**, **24–26**, **37**) and some actually stimulated brain DA synthesis over the already elevated rate induced by the GBL pretreatment (e.g., **17**, **19**, **28**, **44**), an effect suggestive of DA receptor antagonism. Particularly remarkable in this respect were the large effects observed with two compounds, (4-fluorophenyl)piperazine **19** and 6-fluoro-γ-carboline **44**. In order to rule out the possibility that this effect might be an artifact produced by the GBL treatment, we studied the effects of **19** on DOPA accumulation in rats treated only with NSD 1015 (a DOPA decarboxylase inhibitor), a test that reveals DA antagonist-induced stimulation of DA synthesis (Table III). Like haloperidol, **19** produced a significant increase in the rate of DOPA accumulation. Although the magnitude of this effect was much smaller than that with haloperidol, these results indicate that **19** produces DA antagonistlike effects. In this model **31** inhibits DA synthesis, as would be expected of a DA autoreceptor agonist. The magnitude of the inhibition in this test is usually smaller than in the GBL test, where the baseline rate of DOPA accumulation is greatly enhanced.

It is remarkable that **15**, the desfluoro analogue of **19**, appears to be a DA agonist and that a simple substitution of hydrogen by fluorine can change a DA agonist into a DA antagonist. In fact, the results shown in Table II suggest that substitution on the phenyl ring of the phenylpiperazines is likely to reduce DA agonist activity and may even impart DA antagonist activity (e.g. **17**, **19**, **28**, and **44**). However, the group of substituents explored in this study is relatively limited and other substituents might not maintain this trend. Most likely, this is the result of a strict steric requirement at the aryl portion of the AP binding site that prevents (substituted-aryl)piperazines from exerting DA agonist actions. It can be seen in Tables II and III that, of all the AP groups tested, the 4-(2-pyridyl)piperazine and 4-(2-pyrimidyl)piperazine are optimal for DA autoreceptor agonist activity. A similar correlation has been made between the effect of aryl substitution and heteroaryl replacement on the agonist/antagonist actions of a group of arylpiperazines at human platelet 5-HT_2 receptors.[29] In terms of other structural features, side-chain attachment at the 6- and 7-positions of the chromone ring was found to be optimal, and both tri- and tetramethylene connecting chains were acceptable.

Compounds **14**, **31**, and **35** were selected as the most interesting DA autoreceptor agonists from this series. The postsynaptic activity of these compounds was evaluated by assessing their ability to induce stereotyped behavior in rats[30] with and without coadministration of the selective D_1 agonist SKF 38393 (Table IV). Since it has been shown that concurrent activation of postsynaptic D_1 and D_2 receptors is required in order to produce a full stereotypy profile,[31] the test with SKF 38393 should unmask any postsynaptic activity of selective D_2 agonists. As seen in Table IV, **14**, **31**, and **35** failed to produce any stereotypy in either of these tests, which indicates a lack of functional postsynaptic DA receptor activity. Thus, these compounds appear to be selective DA autoreceptor agonists. The DA autoreceptor agonist **1** was also inactive in this test. In contrast, the nonselective DA agonist apomorphine produced stereotypy in all the animals at 10 times its presynaptically active dose (0.03 mg/kg sc). The partial agonist (+)-3-PPP was weakly active by itself but it produced a full stereotypy profile when coadministered with SKF 38393.

Compound **31** (PD 119819) was selected from this group as the most likely compound for development. Further proof of its lack of postsynaptic DA agonist activity was obtained from locomotor activity experiments in 6-hydroxydopamine (6-OHDA) lesioned rats.[32] In this

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Table I. Physical Properties and Pharmacological Evaluation of Target Compounds

$R_1R_2N(CH_2)_nO$–

no.	X[a]	n	R_1R_2N	% yield	formula	mp, °C	[^{3}H]HAL[b] binding: % inhibn at 10^{-7} M[c] (IC_{50}, nM)	inhibn of mouse locomotor activity:[d,e] ED_{50} mg/kg ip	inhibn of rat locomotor activity:[d,e] ED_{50} mg/kg po
14	7	3	N	40	$C_{23}H_{23}NO_3$	134–135.5	(69)	0.7	5.5
15	7	3	N N	69	$C_{22}H_{24}N_2O_3 \cdot H_2O$	149–152	20	2.4	4.3
16	7	3	Cl N N	68	$C_{22}H_{23}ClN_2O_3 \cdot 1.5HCl \cdot H_2O$	201–203	59	>30	NT[f]
17	7	3	Cl N N	50	$C_{22}H_{23}ClN_2O_3 \cdot HCl \cdot 0.25H_2O$	>275	35	7.7	8.5
18	7	3	Cl– N N	35	$C_{22}H_{23}ClN_2O_3 \cdot 0.5H_2O$	160–163	10	11.1	18.6
19	7	3	F– N N	41	$C_{22}H_{23}FN_2O_3 \cdot 2HCl$	229–230	24	7.0	7.1
20	7	3	Cl Cl– N N	32	$C_{22}H_{22}Cl_2N_2O_3 \cdot HCl$	258–259	23	14.0	17.8
21	7	3	OMe N N	66	$C_{23}H_{26}N_2O_4 \cdot HCl \cdot 0.5H_2O$	193–195	48	7.7	>30
22	7	3	MeO– N N	58	$C_{23}H_{26}N_2O_4 \cdot 1.1HCl$	230–233 dec	25	30	>30
23	7	3	HO N N	63	$C_{22}H_{24}N_2O_4 \cdot HCl \cdot 0.4H_2O$	240–243 dec	29	>30	NT
24	7	3	Me N N	41	$C_{23}H_{26}N_2O_3 \cdot 2HBr$	243–244.5	29	2.0	>30
25	7	3	Me N N	45	$C_{23}H_{26}N_2O_3 \cdot 1.5HCl \cdot 0.5H_2O$	210–212	38	5.4	4.8
26	7	3	Me– N N	20	$C_{23}H_{26}N_2O_3 \cdot 2HCl \cdot 0.3H_2O$	211–213	36	6.1	22.3
27	7	3	Me Me N N	35	$C_{24}H_{28}N_2O_3 \cdot HBr \cdot 0.5H_2O$	255–257	0	11.2	13.2
28	7	3	Me Me– N N	65	$C_{24}H_{28}N_2O_3 \cdot 1.7HCl \cdot 0.3H_2O$	238–238.5	41	3.0	7.3
29	7	3	N	62	$C_{23}H_{25}NO_3 \cdot HCl \cdot H_2O$	182–184	40	14.8	>30
30	7	3	–CH$_2$–N N	28	$C_{23}H_{26}N_2O_3 \cdot 2HCl \cdot 0.5H_2O$	248–250	31	>30	NT
31	7	3	N –N N	97	$C_{21}H_{23}N_3O_3 \cdot 2HCl$	272–275	(1000)	1.3	1.7
32	7	3	N N –N N	40	$C_{20}H_{22}N_4O_3 \cdot 0.65C_2H_2O_4 \cdot 1.5H_2O$	178–182	0	12.4	15.6
33	7	3	N N –N N	66	$C_{20}H_{22}N_4O_3 \cdot 2.5HCl$	235–240	17	26.3	NT
34	7	4	–N N	55	$C_{23}H_{26}N_2O_3 \cdot HCl \cdot 0.25H_2O$	204–206	74	0.35	1.9
35	6	4	–N N	50	$C_{23}H_{26}N_2O_3 \cdot 1.8HCl \cdot H_2O$	193–195 dec	73	3.0	1.5

Table I (Continued)

no.	X[a]	n	R_1R_2N	% yield	formula	mp, °C	[^{3}H]HAL[b] binding: % inhibn at 10^{-7} M[c] (IC_{50}, nM)	inhibn of mouse locomotor activity:[d,e] ED_{50} mg/kg ip	inhibn of rat locomotor activity:[d,e] ED_{50} mg/kg po
36	6	4		20	$C_{21}H_{24}N_4O_3$·1.8HCl	189–191	(349)	3.6	8.2
37	5	3		25	$C_{22}H_{24}N_2O_3$	141–143	9	13.3	>30
38	7	g		20	$C_{22}H_{25}N_3O_3$·2.5HCl·2.5H_2O	–[h]	(437)	5.9	NT
39	7	3		53	$C_{21}H_{21}NO_3$·HCl·H_2O[i]	220–225	50	30	N.T.
40	7	3	(j)	33	$C_{22}H_{20}N_2O_5$·1.25HCl·1.75H_2O	218–220	0	>30	N.T.
41	7	3	(k)	33	$C_{23}H_{21}NO_4$·0.25H_2O	134–137	8	13.5	>30
42	7	3		69[l]	$C_{23}H_{23}NO_3$·HCl	201–203 dec	(243)	30.3	NT
43	7	3	(m)	50	$C_{23}H_{26}N_2O_3$·1.75HCl·1.5H_2O	190–200 dec	(884)	>30	NT
44	7	3	(n)	37	$C_{23}H_{21}FN_2O_3$·HCl·0.25H_2O	218–219	NT	7.5	5.5
45	7	3	(o)	40	$C_{23}H_{27}NO_3$	oil	26	>30	NT
apomorphine							(27)	6.4	>30
1							(16)	0.1	4.8
3							(138)	1.3	10.3

[a] Indicates the point of attachment of the side chain at the chromone ring. [b] [^{3}H]Haloperidol. [c] Assays were performed in duplicate. IC_{50} values were determined from four to five concentrations by a nonlinear-regression analysis. [d] ED_{50} values were generated from four to five doses; 5–12 animals were used per dose. [e] No ataxia was observed up to doses of 30 mg/kg. [f] NT = not tested. [g] The central chain is $CH_2CH(CH_3)CH_2$. See the Experimental Section for details. [h] Hygroscopic salt. [i] Cl: calcd, 9.09; found, 9.77. [j] For the synthesis of the amine see ref 17. [k] For the synthesis of the amine see ref 18. [l] See the Experimental Section for the synthesis of this compound. [m] For the synthesis of the amine see ref 19. [n] For the synthesis of the amine see ref 20. [o] For the synthesis of the amine see ref 21.

model, postsynaptic DA receptors have been rendered supersensitive by destruction of most afferent DA neurons. Under these conditions, the DA agonist activity of a compound is augmented and it manifests itself in the form of locomotor stimulation. As shown in Table V, **31** produced no stimulation when tested up to a dose of 4.5 mg/kg sc, which is 8 times greater than its ED_{50} for locomotor inhibition in naive rats by this route of administration (0.6 mg/kg sc). By contrast, **1**, apomorphine, and (+)-3-PPP all had ED_{200} values for locomotor stimulation that were far lower than their respective ED_{50} values for locomotor inhibition in naive animals. These results confirm that **31** is a selective DA autoreceptor agonist with no detectable postsynaptic activity even in very sensitive tests.

Table VI shows the affinity of **31** for a series of receptors in rat brain. In terms of DA receptors, **31** is selective for the D_2 type, to which the DA autoreceptor belongs. In agreement with the expected profile for an agonist, **31** displays greater potency against the agonist ligand *N*-propylnorapomorphine (NPA) than against the antagonist haloperidol. The compound also displays moderate affinity for the α_1 receptor (IC_{50} = 169 nM) and the 5-HT_2 receptor (IC_{50} = 1000 nM).

We have previously shown that selective DA autoreceptor agonists such as **3** are efficacious in the Sidman avoidance test in squirrel monkeys,[33] a model that is predictive of antipsychotic activity in man.[1] The effects of **31** in this test are shown in Table VII; **31** had an ED_{50} of 6.0 mg/kg po, comparable to that of the DA antagonist thioridazine (3.9 mg/kg po). Haloperidol-sensitized squirrel monkeys were used to determine the liability of **31** for extrapyramidal side effects (EPS).[34] Thioridazine induced clear dystonias in sensitized monkeys at doses lower than its behaviorally active dose. On the other hand, **31** did not produce any signs of EPS even at high multiples of its behaviorally active dose (80 mg/kg po).

In conclusion, we have identified a novel group of selective DA autoreceptor agonists. These compounds are 1-substituted-4-arylpiperazines and 1-substituted-4-phenyl-1,2,3,6-tetrahydropyridines. The DA agonist

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Table II. Effects of Selected Compounds on Rat Striatal DA Synthesis and DA Neuronal Firing

compd	% reversal of DOPA accumulation[a,b] ± SEM	% inhibn of DA neuronal firing[c] ± SEM (no. of neurons)
14	54 ± 4	90 ± 10 (2)
15	56 ± 2	68 ± 11 (2)
17	(−26)[d]	
18	IA[e]	
19	(−42)[d]	
24	IA[e]	
25	IA[e]	
26	IA[e]	
28	(−29)[d]	
31	100 ± 6	93 ± 7 (4)
34	63 ± 1	
35	84 ± 7	94 ± 4 (2)
36	100 ± 4	100 ± 0 (2)
37	IA[e]	
38	69 ± 4	53 ± 18 (3)
41	29 ± 10	
44	(−44)[d]	
apomorphine	104 ± 0.9[f]	100 ± 0 (5)[g]
haloperidol	IA[e]	IA[h]

[a] All compounds were administered at 30 mg/kg ip, except where noted otherwise. [b] Shown are the percent reversal of the increase in rat striatum DOPA accumulation produced by pretreatment with GBL (750 mg/kg). Average DOPA content for NSD 1015 (control) and NSD 1015/GBL treated animals was 1.07 ± 0.04 and 3.01 ± 0.11 μg/g, respectively. [c] All compounds were administered at 2.5 mg/kg ip unless noted otherwise. [d] The negative values in parentheses represent further percent increases in DOPA levels over the GBL-induced increase. [e] IA = inactive. DOPA levels in drug-treated animals were not significantly different from levels in the NSD/GBL group. [f] At 2.0 mg/kg ip. [g] At 0.25 mg/kg ip. [h] Average cell firing rate was 143% (±28) of control following administration of haloperidol (0.5 mg/kg ip).

Table III. Effects of Various Agents on Rat Striatal DA Synthesis (DOPA Accumulation after NSD 1015 Treatment)[a]

Expt	treatment	dose, mg/kg ip	DOPA level,[b] μg/g ± SEM
1	control		1.10 ± 0.04
	19	30	1.36 ± 0.03* (24% increase)
2	control		1.00 ± 0.06
	31	30	0.28 ± 0.03* (62% decrease)
3	control		1.11 ± 0.05
	haloperidol	0.5	2.72 ± 0.25* (145% increase)

[a] Animals were administered the test agents 30 min prior to the injection of NSD 1015 (100 mg/kg ip) and they were sacrificed 30 min after the latter injection, with the exception of haloperidol, which was administered 90 min before NSD 1015. Each value is the mean of four animals. [b] An asterisk indicates that $p < 0.05$ versus control.

character of these compounds is highly dependent on the absence of substituents on the aryl ring. DA autoreceptor agonist activity and selectivity are optimal in **31**, a compound that contains the (2-pyridyl)piperazine moiety as its pharmacophore. This compound is devoid of any detectable postsynaptic DA activity. In addition, **31** possesses good oral activity in a primate model of antipsychotic activity and it does not have the EPS liability of most available antipsychotic drugs.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were recorded on an IBM WP100SY NMR spectrometer (100 MHz) or a Varian XL200 NMR spectrometer (200 MHz) and were consistent with the proposed structures. The peaks are described in ppm downfield from TMS (internal standard). The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG Analytical 7070E/HF mass spectrometer. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values. TLC was carried out with 0.25 mm silica gel F254 (E. Merck) glass plates. Medium-pressure liquid chromatography (MPLC) was performed on silica gel (E. Merck, grade 60, 230–400 mesh, 60 Å) with a RB-SY pump (FMI).

Table IV. Comparative Effects of Several Agents on Behavioral Effects Mediated by Pre- and Postsynaptic Brain DA Receptors

no.	inhibn of rat locomotor activity:[a] ED_{50}, mg/kg po	stereotyped behavior in rats:[b] % animals (dose, mg/kg po) A[c]	B[d]
14	5.5	0 (110)	0 (55)
31	1.7	0 (30)	0 (17)
35	1.5	0 (30)	0 (15)
1	4.8	0 (30)	0 (30)
apomorphine	0.03[e]	100 (0.3)[e]	NT[f]
(+)-3-PPP	[g]	16 (30)[e]	100 (30)[e]

[a] ED_{50} values were taken from Table I. [b] Groups of six animals were tested at each dose level. The table shows the percentage of animals showing signs of stereotypy at the dose tested. [c] The test compound was administered to naive rats and the animals were observed for 60 min postdose for the presence of stereotyped behavior. [d] The test compound was administered to naive rats followed 1 h later by 10 mg/kg sc of SKF 38393 and the animals were observed for 60 min postdose for the presence of stereotyped behavior. [e] These drugs were administered sc. [f] NT = not tested. [g] The compound was not very active in this test. Maximal effects (40% inhibition of locomotor activity) were obtained at 3 mg/kg sc.

Table V. Additional Evaluation of **31** as a Selective DA Autoreceptor Agonist

no.	inhibn of rat locomotor activity: ED_{50},[a,b] mg/kg sc	reversal of 6-OHDA induced depression:[a] ED_{200},[c] mg/kg sc
31	0.58 ± 0.13	>5.0[d]
1	4.69 ± 1.10	0.29 ± 0.05
apomorphine	0.03 ± 0.005	0.005 ± 0.001
(+)-3-PPP	3.0 ± 0.58[e]	0.48 ± 0.09

[a] ±95% confidence limits. [b] ED_{50} values were generated from four to five doses; 5–12 animals were used per dose. Locomotor activity was measured immediately after dosing for a period of 30 min. [c] ED_{200} values were estimated from three or four doses: six animals were used per dose. Locomotor activity was measured immediately after dosing for a period of 30 min. [d] No stimulation was observed up to 5.0 mg/kg sc. [e] See footnote g in Table IV.

Table VI. Binding Profile of **31** in Rat Brain

receptor	ligand	IC_{50}[a] ± SEM, nM
D_1	[^{3}H]flupenthixol	21748 ± 2446
D_2	[^{3}H]haloperidol	1080 ± 103
D_1/D_2	[^{3}H]-*N*-propylnorapomorphine	59 ± 5
5-HT_2	[^{3}H]spiroperidol	1000
α_1	[^{3}H]prazocin	170
α_2	[^{3}H]-*p*-aminoclonidine	2600

[a] IC_{50} values were determined from four to five concentrations by a nonlinear-regression analysis. The [^{3}H]flupenthixol and [^{3}H]-*N*-propylnorapomorphine values are the result of four independent determinations each. The [^{3}H]haloperidol value is the result of two experiments. The remaining values were determined only once.

Table VII. Effects of **31** in the Sidman Avoidance and EPS Tests in Squirrel Monkeys

no.	inhibn of Sidman avoidance:[a] ED_{50}, mg/kg po ± SEM	EPS[b] (dose, mg/kg po)
31	6.0 ± 0.5	0/3 (80)
thioridazine	3.9 ± 0.4	3/3 (2.5)

[a] ED_{50} values were generated from three doses. Four animals were tested per dose. [b] Number of animals showing signs of EPS/number of animals tested.

The arylpiperazines and other amines used to prepare the compounds described in Table I were obtained from Aldrich Chemical Co. or prepared according to published procedures (see Table I). Appropriate literature references to starting amines are indicated in Table I as footnotes. The following compounds have been described in the literature, but our synthesis deviated from the published procedures or the original paper did not provide adequate experimental detail.

1-Phenyl-2,6-piperazinedione (46)[17a] (Starting Material for 40). A solution of 4-benzyl-1-phenyl-2,6-piperazinedione[17b] (32.44 g, 115 mmol) in methanol (400 mL) was treated with 20% Pd/C (1 g) and hydrogenated in a Parr shaker at room temperature until the theoretical amount of hydrogen was consumed. The catalyst was filtered and the methanol was evaporated. The yellowish solid residue was taken up into boiling methanol (400 mL). The solution was treated with activated charcoal and filtered. The volume was reduced to 300 mL on a steam bath, and the solution was allowed to cool. The product crystallized as thin needles: 13.1 g (60%); mp 200–202 °C; ^{1}H NMR ($CDCl_3$) δ 3.65 (4 H, s), 7.05–7.20 (2 H, m), 7.30–7.60 (3 H, m). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

***N*-Ethyl-3-benzenepropanamine (47).[21] (Starting Material for 45).** Benzenepropanamine (40.56 g, 300 mmol; Fluka) and triethylamine (35.30 g, 350 mmol) were dissolved in chloroform (500 mL) and cooled in an ice bath. Acetyl chloride (27.47 g, 350 mmol) was added dropwise. The mixture was stirred at room temperature overnight, followed by addition of 5% HCl (200 mL). The organic layer was evaporated in vacuo, and the residue was partitioned between water (200 mL) and ether (2 × 200 mL). The organic extracts were dried over $MgSO_4$ and evaporated in vacuo to leave 50 g of a thick oil; this crude amide was dissolved in anhydrous THF (400 mL) and was added dropwise to an ice-cold suspension of aluminum hydride [prepared by addition of a solution of aluminum chloride (20.0 g, 150 mmol) in ether (500 mL) to a suspension of lithium aluminum hydride (18.0 g, 500 mmol) in THF (400 mL) and ether (400 mL), followed by stirring at room temperature for 20 min]. The reaction mixture was refluxed under nitrogen for 1 h. It was then cooled in an ice bath and carefully quenched by sequential addition of water (100 mL), 30% NaOH (100 mL), and water again (100 mL). The precipitated salts were filtered through Celite, and the salts were thoroughly rinsed with fresh THF. The colorless solution was evaporated in vacuo. The oil obtained was added dropwise to 600 mL of a saturated solution of hydrogen chloride in ether. The hydrochloride salt of 47 crystallized as a colorless solid: mp 120–124 °C; yield 48.8 g (81.4% for two steps); ^{1}H NMR ($CDCl_3$) δ 1.20–1.40 (3 H, t, *J* = 7 Hz), 2.05–2.25 (2 H, m), 2.55–2.75 (2 H, m), 2.75–3.00 (4 H, m), 7.05–7.25 (5 H, m), 9.50 (2 H, br s, NH_2). Anal. ($C_{11}H_{17}N\cdot HCl$) C, H, N.

7-Hydroxy-4*H*-1-benzopyran-4-one (7).[22] A suspension of 2,4-dihydroxyacetophenone (100.0 g, 657 mmol) in triethyl orthoformate (580 mL) was placed in an open 4000-mL beaker. While the suspension was stirred with a magnetic stirrer, 70% perchloric acid (66 mL) was added from an addition funnel over a 3-min period. The thick dark solution that resulted was slightly warm to the touch, and it was stirred until it cooled to room temperature (about 40 min). Anhydrous ethyl ether (1750 mL) was added and a brown precipitate formed. This solid was filtered, dissolved in hot water (1000 mL), and heated at reflux for 5 min. The mixture was then allowed to cool to room temperature overnight. A dark solid was filtered and dissolved in boiling ethanol (500 mL). About 100 mL of hot water was added, and the solution was concentrated to a volume of 500 mL on a steam bath. The product (40.25 g) crystallized at room temperature overnight, mp 214–216 °C (lit.[22b] mp 215–216 °C). A second crop was obtained from the mother liquors (22.0 g): mp 213–216 °C; combined yield 62.25 g (59%); ^{1}H NMR (DMSO-d_6) δ 6.25 (1 H, d, *J* = 6.5 Hz), 6.80–7.00 (2 H, m), 7.90 (1 H, d, *J* = 7.5 Hz), 8.20 (1 H, d, *J* = 6.5 Hz); MS *m/z* 163 (M + 1, 100).

6-Hydroxy-4*H*-1-benzopyran-4-one (8).[22a] 2,5-Dihydroxyacetophenone (152.15 g, 1.0 mol) was treated with 70% perchloric acid (100 mL) and triethyl orthoformate (875 mL) in a manner similar to the synthesis of 7. In this case, however, a proportionally larger amount of ether (6000 mL) was required to precipitate the intermediate oxonium perchlorate salt. The hydrolysis of this salt was carried out in 1500 mL of water. The product was obtained as a dark brown solid (crude yield 123.0 g, 76%), mp 187–193 °C, that could be used in the next step without further purification. If desired, recrystallization could be done from 2-propanol/ethyl acetate to give a purple solid with mp 219–221 °C (lit.[22a] mp 244 °C) which was not analytically pure: ^{1}H NMR (DMSO-d_6) δ 6.20 (1 H, d, *J* = 6.5 Hz), 6.80–7.00 (2 H, m); 7.85 (1 H, d, *J* = 7.5 Hz), 8.15 (1 H, d, *J* = 6.5 Hz); MS *m/z* 163 (M + 1, 100).

7-(3-Chloropropoxy)-4*H*-1-benzopyran-4-one (9a). To a solution of 7 (250.0 g, 1.543 mol) in 5000 mL of acetone was added anhydrous potassium carbonate (302.0 g, 3.086 mol) and 1-bromo-3-chloropropane (486.0 g, 3.086 mol). The mixture was stirred mechanically and refluxed for 20 h. After cooling to room temperature, the mixture was filtered through a pad of Celite. The solvent was evaporated in vacuo and a semisolid, reddish residue was obtained. This crude product was triturated with 1000 mL of ether, filtered, and air-dried to yield 310.7 g (84.4%) of **9a**: mp 70–74 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (2 H, quintet, *J* = 7.5 Hz), 3.80 (2 H, t, *J* = 7.5 Hz), 4.23 (2 H, t, *J* = 7.5 Hz), 6.30 (1 H, d, *J* = 6.5 Hz), 6.85 (1 H, d, *J* = 2 Hz), 6.95 (1 H, dd, *J* = 7 Hz, 2 Hz), 7.80 (1 H, d, *J* = 7 Hz), 8.15 (1 H, d, *J* = 6.5 Hz); MS *m/z* 238 (M, 60), 162 (100). Anal. ($C_{12}H_{11}ClO_3$) C, H, Cl.

7-(4-Chlorobutoxy)-4*H*-1-benzopyran-4-one (9b). This compound was prepared in a manner analogous to that of **9a**. Starting with 7 (10.0 g, 61.7 mmol), there was obtained 14.2 g (91%) of **9b**: mp 75–78 °C. ^{1}H NMR ($CDCl_3$) δ 1.90–2.10 (4 H, m), 3.50–3.75 (2 H, m), 4.00–4.20 (2 H, m), 6.30 (1 H, d, *J* = 6.5 Hz), 6.82 (1 H, d, *J* = 2 Hz), 6.95 (1 H, dd, *J* = 6.5 and 2 Hz), 7.80 (1 H, d, *J* = 6.5 Hz), 8.13 (1 H, d, *J* = 6.5 Hz); MS *m/z* 252 (M, 15), 91 (100). Anal. ($C_{13}H_{13}ClO_3\cdot 0.15H_2O$) C, H, Cl.

6-(4-Chlorobutoxy)-4*H*-1-benzopyran-4-one (10a). This compound was prepared in a manner analogous to the synthesis of **9a**. Starting with 8 (32.4 g, 200 mmol), there was obtained 33.0 g (65%) of **10a**: mp 75–77 °C; ^{1}H NMR ($CDCl_3$) δ 1.85–2.10 (4 H, m), 3.50–3.75 (2 H, m), 4.00–4.20 (2 H, m), 6.25 (1 H, d, *J* = 6 Hz), 6.82 (1 H, d, *J* = 2 Hz), 6.95 (1 H, dd, *J* = 6 and 2 Hz), 7.76 (1 H, d, *J* = 6 Hz), 8.12 (1 H, d, *J* = 6 Hz); MS *m/z* 252 (M, 35), 91 (100). Anal. ($C_{13}H_{13}ClO_3$) C, H, Cl.

2-(3-Chloropropoxy)-6-hydroxyacetophenone (11a). A solution of 2,6-dihydroxyacetophenone (10.0 g, 65.5 mmol) in acetone (250 mL) was treated with 1-bromo-3-chloropropane (15.74 g, 100 mmol) and potassium carbonate (7.35 g, 150 mmol) and refluxed for 24 h. The mixture was filtered through Celite and the solvent was evaporated in vacuo. The solid residue was recrystallized from ethyl acetate/hexane to give **11a** as a colorless solid: mp 60.5–62.5 °C (15.0 g, 100%); ^{1}H NMR ($CDCl_3$) δ 2.31 (2 H, quintet, *J* = 6.3 Hz), 2.64 (3 H, s), 3.73 (2 H, t, *J* = 6.3 Hz), 4.20 (2 H, t, *J* = 6.3 Hz), 6.38 (1 H, d, *J* = 8.5 Hz), 6.55 (1 H, dd, *J* = 7.5 and 0.9 Hz), 7.31 (1 H, t, *J* = 8.4 Hz); MS *m/z* 229 (M, 100). Anal. ($C_{11}H_{13}ClO_3$) C, H, Cl.

5-(3-Chloropropoxy)-4*H*-1-benzopyran-4-one (13a). To a solution of **11a** (28.0 g, 131 mmol) in ethyl formate (100 mL) was added sodium (10.9 g, 474 mmol) in small pieces. The resulting mixture was refluxed under nitrogen for 1 h. After cooling to room temperature, the mixture was poured over crushed ice (200 g), and it was extracted with dichloromethane. The organic extract was dried over $MgSO_4$ and evaporated in vacuo. The residue was taken up into absolute ethanol (400 mL), treated with 2 mL of concentrated HCl, and refluxed for 1 h. Excess anhydrous K_2CO_3 was added to neutralize the acid and the mixture was filtered. The filtrate was evaporated and purified by MPLC (ethyl acetate/heptane 1:1) to yield **13a** as a reddish oil (26.0 g, 80%) that solidified in a freezer: mp 50–56 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (2 H, quintet, *J* = 6.1 Hz) 3.75 (2 H, t, *J* = 6.1 Hz), 4.23 (2 H, t, *J* = 6.1 Hz), 6.40 (1 H, d, *J* = 7.7 Hz), 6.50–6.60 (2 H, m), 7.22–7.37 (2 H, m). This material could not be obtained analytically pure but was suitable for the synthesis of **37** according to the general method described below.

General Method for the Synthesis of 4. Synthesis of 7-[3-[4-(2-Pyridinyl)-1-piperazinyl]propoxy]-4*H*-1-benzopyran-4-one (31). To a solution of **9a** (20.0 g, 84 mmol) and 1-(2-pyridinyl)piperazine (15.0 g, 92 mmol) in DMF (350 mL) was added sodium bicarbonate (20.0 g, 238 mmol) and sodium iodide (1.0 g, 6.6 mmol). The resulting mixture was heated at 85 °C under nitrogen for 10 h. The solvent was evaporated in vacuo, and the residue was partitioned between water and dichloromethane. The organic phase was dried over $MgSO_4$ and evaporated. The residue

was dissolved in hot ethanol (400 mL) and treated with an excess of hydrogen chloride in 2-propanol. The solution was refrigerated overnight, and the salt formed was filtered and washed with ethyl acetate. This salt was very hygroscopic initially; however, after drying in a vacuum oven at 50 °C for 24 h, a stable beige solid was obtained, mp 272–275 °C, which was characterized as the dihydrochloride salt of **31** (35.7 g, 97 %): ^{1}H NMR (DMSO-d_6) δ 2.23–2.37 (2 H, m), 3.00–3.50 (4 H, m), 3.50–3.80 (4 H, m), 4.26 (2 H, t, J = 5.8 Hz), 4.50 (2 H, m), 6.29 (1 H, d, J = 6 Hz), 6.96 (1 H, t, J = 6.2 Hz), 7.08 (1 H, dd, J = 9 and 2.3 Hz), 7.18 (1 H, d, J = 2.3 Hz), 7.29 (1 H, d, J = 9 Hz), 7.88–8.00 (2 H, m), 8.15 (1 H, dd, J = 5.6 and 1.4 Hz), 8.26 (1 H, d, J = 6 Hz); MS m/z 365 (M, 14), 107 (100). Anal. ($C_{21}H_{23}N_3O_3{\cdot}2HCl$) C, H, N, Cl.

7-[2-Methyl-3-[4-(2-pyridinyl)-1-piperazinyl]propoxy]-4*H*-1-benzopyran-4-one (38). A solution of **7** (6.0 g, 37 mmol) in acetone (150 mL) was treated with 1,3-dibromo-2-methylpropane[35] (15.0 g, 69.7 mmol) and anhydrous potassium carbonate (12.0 g, 87 mmol). The mixture was refluxed for 16 h and filtered through Celite while it was still hot. The Celite was rinsed with fresh acetone, and the combined filtrates were evaporated in vacuo. The residue was purified by MPLC (ethyl acetate/hexane 1:1), and 13.0 g of an oil was obtained. Even though TLC showed one single component, NMR analysis of this oil indicated a 2:1 mixture of 7-(2-methylprop-2-enoxy)-4*H*-1-benzopyran-4-one and 7-(3-bromo-2-methylpropoxy)-4*H*-1-benzopyran-4-one. This mixture of compounds was dissolved in DMF (75 mL), treated with 1-(2-pyridinyl)piperazine (4.08 g, 25 mmol) and sodium bicarbonate (2.5 g, 29.5 mmol), and heated at 80 °C for 6 h. The solvent was evaporated in vacuo, and the residue was partitioned between ethyl acetate and water. The organic layer was extracted with 10% HCl (2 × 100 mL), and the aqueous layer was then made basic with ammonium hydroxide and extracted with ethyl acetate. The organic extract was dried over $MgSO_4$ and evaporated. The residue was purified by MPLC (ethyl acetate/hexane/NH_4OH 75:24:1). The product was dissolved in ethyl acetate and treated with excess ethereal hydrogen chloride to obtain 0.9 g of a hygroscopic amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.19 (3 H, d, J = 6.8 Hz), 2.50–2.65 (1 H, m), 3.05–3.30 (4 H, m), 3.60–3.95 (4 H, m), 4.05–4.30 (2 H, m), 4.40–4.60 (2 H, m), 6.28 (1 H, d, J = 6.1 Hz), 7.01 (1 H, t, J = 6.5 Hz), 7.10 (1 H, dd, J = 8.8 and 2.3 Hz), 7.18 (1 H, d, J = 2.3 Hz), 7.39 (1 H, d, J = 9.3 Hz), 7.96 (1 H, d, J = 8.9 Hz), 8.02 (1 H, m), 8.13 (1 H, dd, J = 5.9 and 1.4 Hz), 8.25 (1 H, d, J = 6.0 Hz); MS m/z 379 (M, 6), 107 (100). Anal. ($C_{22}H_{25}N_3O_3{\cdot}2.5HCl{\cdot}2.5H_2O$) C, H, N, Cl.

7-[3-(3,6-Dihydro-5-phenyl-1(2*H*)-pyridinyl)propoxy]-4*H*-1-benzopyran-4-one (42). A solution of 3-phenylpyridine (7.76 g, 50 mmol) and 3-chloro-1-iodopropane (11.24 g, 55 mmol) in acetonitrile (100 mL) was refluxed for 5 h. The mixture was then cooled in an ice bath, and the yellow solid that formed was filtered and washed with ether (15.0 g, 83%, mp 213–225 °C). A solution of this salt in water (100 mL) and methanol (100 mL) was cooled in an ice bath and treated with sodium borohydride (8.0 g, 200 mmol) in small portions. After 30 min, the mixture was adjusted to pH 7 with 10% HCl, evaporated in vacuo, and partitioned between ethyl acetate and dilute ammonium hydroxide. The organic extract was dried over $MgSO_4$, evaporated in vacuo, and purified by MPLC (ethyl acetate/hexane 1:1) to give 2.7 g of **48** as a reddish oil: ^{1}H NMR ($CDCl_3$) δ 1.55–1.90 (2 H, m), 1.90–2.15 (2 H, m), 2.25–2.45 (4 H, m), 2.95–3.10 (2 H, m), 3.30 (2 H, t, J = 6.5 Hz), 5.80 (1 H, m), 6.90–7.30 (5 H, m).

A solution of **7** (1.94 g, 12 mmol) in DMF (5 mL) was added to a suspension of sodium hydride (0.32 g, 14 mmol) in DMF (5 mL) and was stirred at room temperature for 15 min. A solution of **48** (2.5 g, 10.6 mmol) in DMF (2 mL) was added to the reaction mixture, followed by sodium iodide (1.0 g, 6.6 mmol). The mixture was heated at 80 °C for 4 h. The solvent was evaporated in vacuo and the residue was partitioned between water and dichloromethane. The organic extract was dried over $MgSO_4$ and evaporated in vacuo. The residue was taken up into ethyl acetate (100 mL) and treated with 1 equiv of an ethereal hydrogen chloride solution. The solid formed was filtered and dried to give 2.9 g (69%) of the hydrochloride salt of **42**: mp 201–203 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.37–2.49 (2 H, m), 2.72–2.85 (1 H, m), 3.09–3.25 (1 H, m), 3.32–3.62 (4 H, m), 3.97–4.10 (1 H, m), 4.23–4.30 (3 H, m), 6.29 (1 H, d, J = 6 Hz), 6.39 (1 H, br s), 7.07 (1 H, dd, J = 8.8 Hz, 2.3 Hz), 7.16 (1 H, d, J = 2.3 Hz), 7.29–7.51 (5 H, m), 7.96 (1 H, d, J = 8.8 Hz), 8.25 (1 H, d, J = 6 Hz), 11.47 (1 H, br s, exchangeable with D_2O); MS m/z 361 (M, 56), 172 (100). Anal. ($C_{23}H_{23}NO_3{\cdot}HCl$) C, H, N, Cl.

Pharmacological Methods. Receptor Binding Assays. The affinities of compounds for several CNS receptors were determined by standard receptor binding assays[23] according to methods described previously.[36]

Inhibition of spontaneous locomotor activity and motor coordination[24] was carried out according to methods described previously.[24,36]

Inhibition of GBL-Stimulated DA Synthesis.[27] Compounds were administered to rats 1 h before sacrifice and γ-butyrolactone (GBL, 750 mg/kg ip) and NSD 1015 (100 mg/kg ip) were administered 30 and 25 min, respectively, before sacrifice. Brain levels of dihydroxyphenylalanine (DOPA) were analyzed by HPLC with electrochemical detection.[37]

Effects on the Firing Rate of Substantia Nigra DA Neurons.[28] The action potential of zona compacta DA cells was recorded in chloral-anesthetized rats by using standard extracellular recording techniques. DA cells were identified by waveform and firing pattern and recording sites were verified histologically. Drugs were administered intraperitoneally via an indwelling catheter. Baseline firing rate was calculated by averaging the rate over the 2 min prior to drug injection. Drug effects were determined by averaging the response during the 1-min period of maximal inhibition. Drug-induced inhibition of firing was reversed with the DA antagonist haloperidol to confirm a DA agonist mechanism.

Stereotypy Assessment in Rats. Compounds were tested in a two-part procedure. In part 1, compounds were administered po to individually housed naive rats (N = 6) at doses equivalent to 20 times the ED_{50} for inhibition of locomotion and the animals were observed for the presence of repetitive rearing, head-swaying, sniffing, licking, and gnawing of at least 5-s duration, at 60, 80, 100, and 120 min postdose. Data were expressed as percentage of rats showing signs of stereotypy. In part 2, compounds were administered po to naive rats at doses equivalent to 10 times the ED_{50} for inhibition of locomotion, followed 1 h later by the D_1 DA agonist SKF 38393 (10 mg/kg sc), and the animals were rated for stereotyped behavior as described above.

Effects on Spontaneous Locomotion in 6-OHDA-Lesioned Rats.[32] Drugs were administered subcutaneously to rats treated at least 1 month previously with central injections of 6-hydroxydopamine (6-OHDA, 20 μg icv) and systemic injections of pargyline (50 mg/kg ip) and desipramine (25 mg/kg ip) as described previously.[38] This treatment produced large selective depletion of brain DA (approximately 90%) as described previously[38] and as determined by brain DA determinations in representative animals. Locomotor activity was measured for 30 min beginning immediately after drug administration as described previously.[25,36]

Sidman avoidance procedure[33] **and extrapyramidal side effect test**[34] in mature squirrel monkeys were performed as described previously.[36]

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