Synthesis and Dopaminergic Activity of 3-Substituted 1-(Aminomethyl)-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyrans: Characterization of an Auxiliary Binding Region in the D1 Receptor

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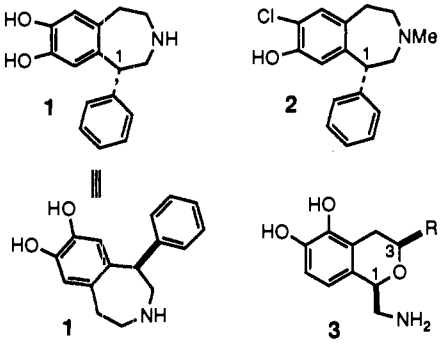
The synthesis and dopaminergic activity of a series of C3 and nitrogen-substituted 1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyrans (isochromans) is described. The synthesis of the compounds was stereospecific for the 1,3 cis isomer, and the enantioselective synthesis of both enantiomers of one of the analogoues (**20**) was achieved. It was determined that all of the dopaminergic activity resides in the [1*R*,3*S*] isomer. Generally, substitution at the C3 position provided compounds with very high potency (<10 nm EC_{50}) and selectivity for the D1 receptor, with a wide range of intrinsic activities (60–160%). Analogues containing C3 substituents including aryl, arylalkyl, and cyclic and acyclic alkyl groups showed a marked enhancement of dopaminergic activity compared to the unsubstituted compound. As a class, the drugs were orally active in the rat rotation model with a very long duration of action.

Introduction

The post-synaptic dopamine receptor is a member of a class of trans-membrane spanning, G-protein coupled receptors in the central nervous system. In 1979, a dual dopamine receptor theory was proposed by Kebabian and Calne.[1] The D1 and D2 receptors are distinguished on the basis of pharmacological differences, the most striking of these being their interaction with the enzyme adenylate cyclase.[3] A stimulatory response is generated upon activation of the D1 receptor which results in an increase in the production of cyclic adenosine monophosphate (cAMP). In contrast, certain biochemical models of the D2 receptor have shown that activation leads to an inhibition of adenylate cyclase. Recently, both receptors have been cloned and sequenced and distinct differences in their sequences can be seen.[4] The isolation and cloning of a putative D3 receptor[2] was reported although little is known about its pharmacology.

The availability of selective dopamine receptor ligands has allowed for investigations into the binding properties, as well as into functional roles of the individual receptors.[5] The benzazepines **1**[6a] (SKF38393) and **2**[6b] (SCH23390)



1 2 1 3

have been the most widely studied D1 selective agonist and antagonist, respectively. Several years ago, we began a program with the goal of developing a selective D1 agonist for the treatment of neurodegenerative disorders. We now wish to report on our work on the isochroman series of D1 agonists **3**. We, and others, have demonstrated that proper positioning of a phenyl substituent on a core dopaminergic compound can impart high D1 potency and selectivity. Because of this effect, the new binding region has been considered a phenyl-binding pocket.[5,8] However, in the isochroman series, a wide variety of substituents are tolerated, and thus in this publication the site will be referred to as the auxiliary binding region. When one overlaps the important heteroatoms in molecular models, substitution at the C1 position in the benzazepines places groups in similar regions in space to C3 substituents in the isochromans. The synthesis and dopaminergic activity of these C3 analogues as well as some amino-substituted derivatives will be presented here.

Chemistry

All of the compounds described herein were prepared by one of the methods shown in Scheme I. The most common literature method for the synthesis of isochromans is via cyclization of an intermediate phenethyl alcohol.[9]

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(6) (a) Weinstock, J.; Hieble, J. P.; Wilson, J. W., III *Drugs of the Future* **1985**, *10*, 649. (b) Iorio, L. C.; Houser, V.; Korduba, C. A.; Leitz, F. H.; Barnett, A. *Pharmacologist* **1981**, *23*, 136. (c) Iorio, L. C.; Barnett, A.; Leitz, F. H.; Houser, V. P.; Korduba, C. A. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 462.
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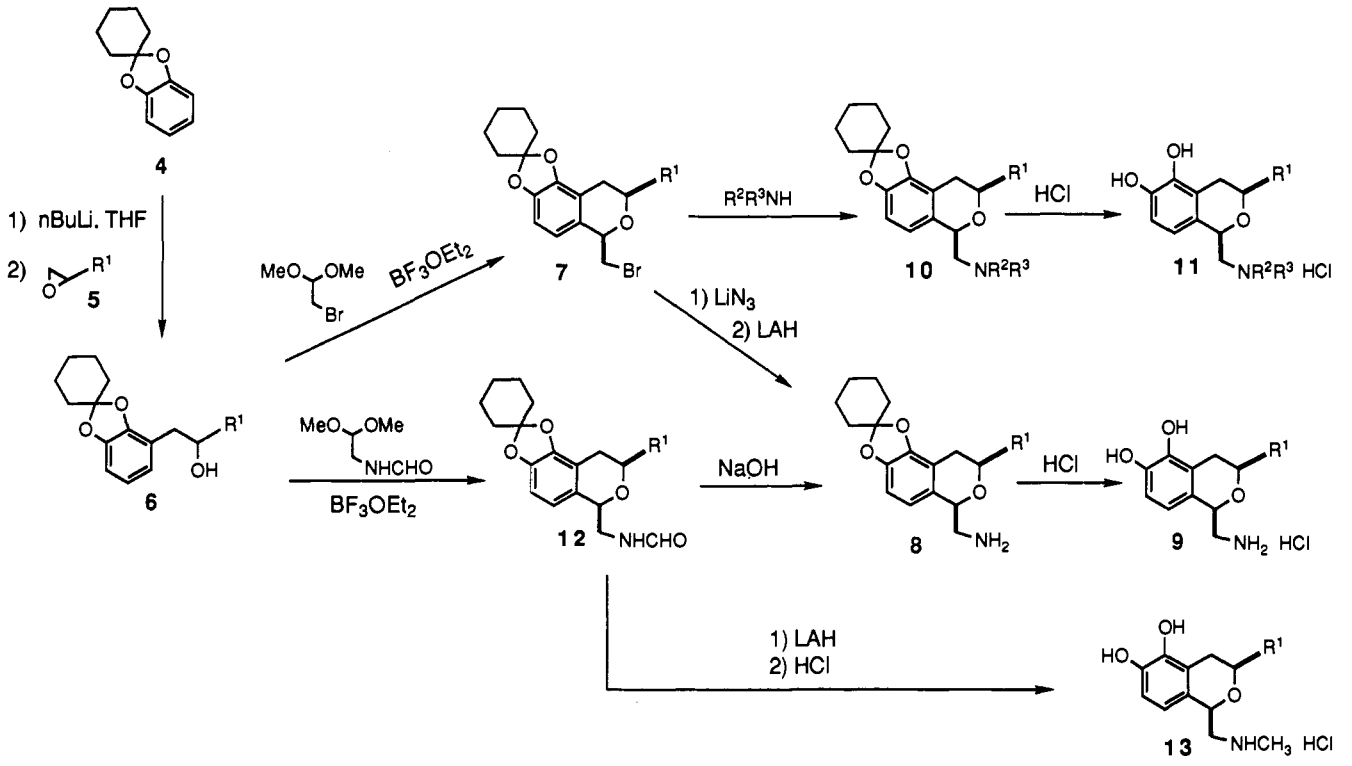
Scheme I



This route was attractive to us since a large number of phenethyl alcohols could be made by opening an epoxide with an aryl lithium derivative.[10] The first challenge faced in this scheme was to find a suitable catechol protecting group. A successful candidate would have to allow ortho lithiation of the aromatic ring, be stable to Lewis acids and base, and be readily cleaved in neutral or acidic medium. The cyclohexylidene protecting group[11] was chosen and proved to be ideal for this application. Treatment of **4** with *n*-butyllithium in tetrahydrofuran (THF) for 4 h afforded a homogeneous solution of the ortho-lithiated intermediate. Addition of an epoxide **5** (commercially available or prepared by standard methods from the aldehyde[12] or olefin[13]) produced the alcohol **6** in 40–80% yields. The key step in the synthesis was cyclization of the alcohol to the isochroman nucleus.[9] A second chiral center would be introduced in this process and the stereochemical control of such a reaction was uncertain. In the event, treatment of intermediate alcohol **6** with bromoacetaldehyde dimethyl acetal under boron trifluoride etherate (BF_3 OEt_2) catalysis afforded the cyclized product **7** in 50–80% yield. In every example but one (R^1 = ethyl) the 1,3 cis isomer was formed exclusively. The stereochemical assignment was based upon a strong NOE signal between the C1 and C3 diaxial protons. This result can be rationalized by a transition state such as **14** (Figure 1) where the C-3 substituent occupies a pseudoequatorial position in the *E* oxonium intermediate. The *trans*-isochroman would result from transition state **15** where the R^1 group is in a pseudoaxial position.[14] The importance of this stereochemical result

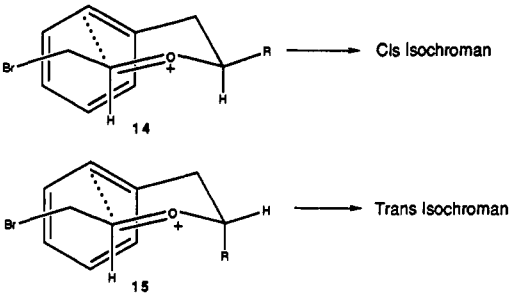


Figure 1.

was only later realized when it was determined that the cis isomer **34** was somewhat more potent than the trans isomer **35**. It was also discovered, quite surprisingly, that the trans isomer is the thermodynamically favored product.[15]

Bromide **7** was a versatile intermediate. Displacement of the bromide with lithium azide in dimethylformamide (DMF) at 70 °C afforded the azide which was cleanly reduced with lithium aluminum hydride (LAH) in ether to afford the amine **8** in good overall yield. Cleavage of the cyclohexylidene protecting group could be efficiently carried out by using anhydrous HCl in ethanol. In general, the catecholamine hydrochloride salt **9** could be isolated in analytically pure form directly from the reaction by a simple trituration with ether.

Alternatively, the bromide **7** could be displaced by a primary or secondary amine, giving rise to compound **10** in high yield. Deprotection as described above afforded

(10) See for example: (a) Lewis, B. A.; Raphael, R. A. *J. Chem. Soc.* **1962**, 4263. (b) Narasimhan, N. S.; Paradkar, M. V.; Alurkar, R. H. *Tetrahedron* **1971**, *27*, 1351. (c) Screttas, C. G. *J. Chem. Soc. Chem. Commun.* **1972**, 869. (d) Hay, J. V.; Harris, T. M. *Org. Synth.* **1973**, *53*, 56.

(11) Boeckmann, J.; Schill, G. *Chem. Ber.* **1977**, *110*, 703.

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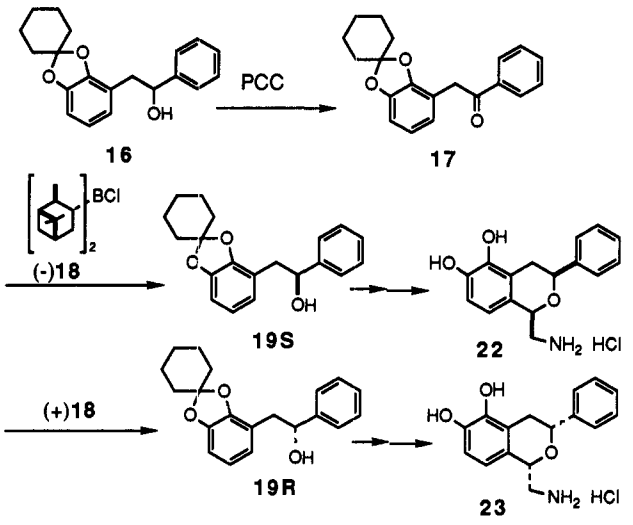
(13) Swern, D. *Organic Peroxides*; Vol. II, John Wiley and Sons: New York, 1971; Vol. II, p 355.

(14) The two alternate transition states from the Z-oxonium species are also disfavored due to 1,3-axial interactions. For a reference proposing a boat transition state for ring closure, see: Mohler, D. L.; Thompson, D. W. *Tetrahedron Lett.* **1987**, *28*, 2567.

(15) Deprotection of isochroman derivatives, such as **7**, (aqueous TFA, 6.5 h) results in an epimerization of the C1 center to afford mixtures (>7:1) favoring the trans isomer (unpublished results).

Scheme II



the N-substituted derivatives 11.

Another more efficient route to compound type 9 was developed. By utilizing (*N*-formylamino)acetaldehyde dimethyl acetal in the cyclization reaction, the formamide 12 could be isolated in high yield once again with complete control of stereochemistry. This reaction could be catalyzed by a number of Lewis acids including BF_3OEt_2 and trimethylsilyl triflate (TMSOTf). Both the amine and catechol protecting groups contained in 12 could be cleaved in one operation (HCl/ethanol); however, it was often desirable to proceed stepwise. Thus, cleavage of the formamide with sodium hydroxide gave the amine 8, which was followed by HCl deprotection as described previously to afford compound 9. Intermediate 12 was also useful for the preparation of *N*-methyl analogues 13 by a LAH–HCl sequence of reactions.

It is noted that in these reactions, the chiral center at C3 controls the center at C1, thereby simplifying an enantioselective synthesis of isochromans to the preparation of optically active versions of alcohol 6. Fortunately, there are several methods available for the asymmetric synthesis of alcohols.[16] One such method is described in the preparation of the enantiomeric pair 22 and 23 (Scheme II). Ketone 17 was produced from the racemic alcohol 16 by a pyridinium chlorochromate oxidation. Compound 16 could be reduced enantioselectively by using either the (-)- or (+)-B-chlorodiisopinocampheylborane reagent 18 developed by H. C. Brown[16h] to afford the **19S** and **19R** alcohol isomers, respectively.[17] The compounds were shown to be approximately 98% ee by analysis of their corresponding Mosher esters.[18] The synthesis was completed as described above and the final products, after recrystallization, were shown to be >98% ee by the Mosher amide method.[18]

In Vitro Pharmacology

The binding affinities of the synthetic analogues were determined for both the D1 and the D2 receptor. For the D1 receptor [^{125}I]SCH 23982[19] was used as the radiolabeled competitive binding ligand while [^{3}H]spiroperidol[20] was used in the D2 assay. The compounds were also screened in functional assays for the D1 and D2 receptor.[21] The D1 receptor assay measured the ability of the drug to stimulate cyclic AMP production. An EC_{50} value (concentration required for half maximal stimulation) and intrinsic activity (IA, the magnitude of the increase of cAMP production expressed as a percentage of the maximal response to dopamine) were determined. The tissue used in the assay was a cell-free homogenate of carp retina (D1C(R)) with selected additional tests using tissue from rat striatum (D1C(S)). Agonist activity at the D2 receptor was measured by the ability of a compound to inhibit a forskolin-stimulated increase in cyclic AMP production. Cells from the rat intermediate lobe[22] or MMQ cell line[23] were used in this assay. The results of these assays are shown in Table II. Certain compounds were also screened for their ability to bind to adrenergic and seretonergic receptors (Table III). Full details of the biochemical methods are given in the experimental section.

Behavioral Pharmacology

The screening protocol included an in vivo behavioral model which was used to test the most interesting analogues. A widely used model of dopaminergic activity, and the one we chose for our studies, is the rat rotation model.[24] It is well known that rats with unilateral 6-hydroxydopamine (6OHDA) lesions of the nigro-striatal bundle will rotate contralaterally to the lesion in response to administration of a direct D1 or D2 agonist and that this response can be blocked by an appropriate dopamine receptor antagonist. This assay was a valuable tool since rotational response following subcutaneous administration of an agonist gave some information about its ability to cross the blood–brain barrier. Comparisons of the subcutaneous and oral potency provided some insight into the oral absorption and metabolism of the drug. Some of the data for selected compounds are shown in Table III and are presented as the mean net number of contralateral rotations in 2 h following administration of the agonist by either the subcutaneous or oral route.

Discussion

The compounds presented here can be divided into four categories: (1) C-3 aryl, (2) C-3 arylalkyl, (3) C-3 alkyl, and (4) amino group substitutions. The in vitro pharmacology

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(17) The absolute configuration of **19S** was verified by its independent synthesis from the commercially available (*R*-(+)-styrene oxide.

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(21) (a) Watling, K. J.; Dowling, J. E. *J. Neurochem.* **1981**, *36*, 559. (b) Kebabian, J. W.; Petzold, G. L.; Greengard, P. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2145.

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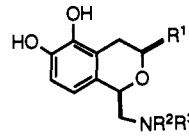
(24) (a) Ungerstedt, V.; Arbuthnott, G. W. *Brain Res.* **1970**, *24*, 485. (b) Arnt, J.; Hyttel, J. *Eur. J. Pharmacol.* **1984**, *102*, 349.

Table I. Physical and Analytical Data[a]



	R1	R2	R3	formula	mp, °C[b]	anal.[c]
20	H	H	H	$C_{10}H_{14}BrNO_3$	238	C,H,N
21	phenyl	H	H	$C_{16}H_{18}ClNO_3$	146	C,H,N
22	(1R,2S)-phenyl	H	H	$C_{16}H_{18}ClNO_3$	158	C,H,N
23	(1S,3R)-phenyl	H	H	$C_{16}H_{18}ClNO_3$	158	C,H,N
24	4-Br-phenyl	H	H	$C_{16}H_{17}BrClNO_3$	222 (d)	C,H,N
25	4-I-phenyl	H	H	$C_{16}H_{17}ClINO_3 \cdot 0.25H_2O$	257 (d)	C,H,N
26	3-OMe-phenyl	H	H	$C_{17}H_{20}ClNO_4$	250 (d)	C,H,N
27	3-OH-phenyl	H	H	$C_{16}H_{18}ClNO_4 \cdot 0.25H_2O$	245 (d)	C,H,N
28	benzyl	H	H	$C_{17}H_{20}ClNO_3$	242	C,H,N
29	4-OMe-benzyl	H	H	$C_{18}H_{22}ClNO_4$	257 (d)	C,H,N
30	phenethyl	H	H	$C_{18}H_{22}ClNO_3$	215 (d)	C,H,N
31	phenoxymethyl	H	H	$C_{17}H_{20}ClNO_4 \cdot 0.5H_2O$	230 (d)	C,H,N
32	(benzyloxy)methyl	H	H	$C_{16}H_{23}ClNO_4$	222 (d)	C,H,N
33	diphenylmethyl	H	H	$C_{23}H_{24}ClNO_3$	250 (d)	C,H,N
34	(cis)-ethyl	H	H	$C_{12}H_{18}ClNO_3$	204	C,H,N
35	(trans)-ethyl	H	H	$C_{12}H_{18}ClNO_3$	240	C,H,N
36	propargyl	H	H	$C_{13}H_{16}ClNO_3$	>250	C,H,N
37	*n*-butyl	H	H	$C_{14}H_{22}ClNO_3$	215 (d)	C,H,N
38	*n*-hexyl	H	H	$C_{16}H_{26}ClNO_3 \cdot 0.3H_2O$	205 (d)	C,H,N
39	cyclopentylmethyl	H	H	$C_{16}H_{24}ClNO_3$	265 (d)	C,H,N
40	spirocyclohexyl	H	H	$C_{15}H_{22}ClNO_3$	245 (d)	C,H,N
41	cyclohexyl	H	H	$C_{16}H_{24}ClNO_3$	225 (d)	C,H,N
42	cyclooctyl	H	H	$C_{18}H_{28}ClNO_3$	>250	C,H,N
43	*tert*-butyl	H	H	$C_{14}H_{22}ClNO_3$	258 (d)	C,H,N
44	1-adamantyl	H	H	$C_{20}H_{28}ClNO_3$	225 (d)	C,H,N
45	cyclohexyl	Me	H	$C_{17}H_{26}ClNO_3$	244 (d)	C,H,N
46	cyclohexyl	cyclopropyl	H	$C_{19}H_{28}ClNO_3$	200	C,H,N
47	cyclohexyl	benzyl	H	$C_{23}H_{30}ClNO_3$	242 (d)	C,H,N
48	cyclohexyl	allyl	H	$C_{19}H_{28}ClNO_3$	217	C,H,N
49	cyclohexyl	propargyl	H	$C_{19}H_{26}ClNO_3$	211	C,H,N
50	cyclohexyl	allyl	Me	$C_{20}H_{30}ClNO_3$	172	C,H,N
51	cyclohexyl	propargyl	Me	$C_{20}H_{28}ClNO_3$	216	C,H,N
52	cyclohexyl	3-propanol	H	$C_{19}H_{30}ClNO_4 \cdot 0.5H_2O$	115	C,H,N

[a] All compounds were characterized as their HCl salts except compound 20 (HBr). [b] A (d) following the melting point means the compound melts with decomposition. [c] Combustion analyses were within ±0.4% of the calculated values.

data for these derivatives is presented in Table II. To gauge the effect of the substituents, data are presented for the unsubstituted analogue **20**.[25] Our data for compound **1** are also given in the table.

As a class, the aryl-substituted derivatives were the most potent. The parent member of the series (phenyl), compound **21**,[7] had a D1 binding constant of 3.0 nM, an EC_{50} value of 2.1 nM and was a partial agonist with an intrinsic activity of 72%. The D1/D2 selectivity in the functional assays was greater than 1500:1. As anticipated, the enantiomers of compound **21** (**22** and **23**) displayed contrasting pharmacology. Compound **22** was very potent and selective for the D1 receptor while **23** was a very weak but full agonist at the same receptor. Nearly a 5000-fold difference in D1 potency was seen with these isomers. The substituted aryl derivatives **24–27** maintain very high potency and selectivity while demonstrating a marked increase in intrinsic activity over the phenyl compound **21**. The increase in D2 functional activity seen in the phenol **27** as compared with its methyl ether **26** suggests a possible hydrogen bonding interaction with the D2 receptor. Further evidence for this interaction has been seen with other derivatives which will be described in a future publication.

The second series of compounds was those where the aryl group was removed from the ring and a carbon or carbon–oxygen spacer was inserted. These compounds are shown as examples **28–33** in Table I. They show a decrease in potency as compared with the aryl-substituted derivatives, although they still show enhanced potency over the parent structure **20**. This class of C3 substituents further define the length (compound **32**) and breadth (compound **33**) of the auxiliary binding region.

A continuing trend that can be noted in these derivatives is a discrepancy between the binding and cyclase values. In general the compounds were 10- to 50-fold less potent in binding than in the functional assay. One hypothesis for the disagreement between the values was that different tissues from different species were used for the binding and cyclase assays. This theory did not prove true, however, since the functional activity for selected compounds was determined by using the same tissue as the binding assay (rat striatum). These results (column D1C(S) in Table II) compare well with those obtained from the fish retina. This disparity is not so unexpected since these assays are only simple biochemical models of the complex process of receptor-signal transduction. The reason for this discrepancy, and why some compounds (i.e. **21**) do not show it, is still not well understood. These questions may be answered when cell lines containing cloned receptors are available in which binding and cyclase activities can be determined in a homogeneous assay system.

The most remarkable series is the third class of derivatives which are the C-3 alkyl-substituted compounds. To our knowledge, there is no report in the literature where

(25) Kumar, A.; Khanna, J. M.; Jain, P. C.; Anand, N.; Srimal, R. C.; Kohli, J. D. *Ind. J. Chem.* **1987**, *26B*, 47.

the potency of a D1 agonist was greatly enhanced by the incorporation of an alkyl group in the auxiliary binding region. In fact, substitution of a cyclohexyl group for a phenyl in the benzazepine series decreased potency more than 100-fold.[5] However in the isochromans, this substitution was well tolerated. Indeed the C3 cyclohexyl-substituted derivative **41** is nearly as potent as the phenyl analogue **21**, with an EC_{50} value of 3.1 nM. As can be seen in Table II, a wide variety of cyclic and acyclic alkyl groups enhance the potency of the parent structure **20**. One interesting comparison is between the ethyl derivatives **34** and **35**. As described in the chemistry section, the preparation of compound **34** was not completely diastereoselective, thus allowing for the preparation and testing of the cis and trans isomers. The cis compound **34** is only 1.6-fold more potent than the trans isomer in the functional assay while displaying over a 20-fold greater binding affinity. These analogues may prove useful in studying the binding/cyclase discrepancy described earlier. Of all the compounds, the spirocyclohexyl derivative **40** has the most restricted conformation. The fact that it shows a large loss of potency (as compared to **41**) indicates that either the spiro ring is in the wrong orientation for a good interaction with the receptor or that the receptor cannot accommodate the increased steric bulk about the C3 carbon atom. Once again, the high potency of derivatives **42–44** attest to the size and lipophilic nature of the auxiliary binding region.

The last category of compounds studied is the amino-substituted analogues. Here, the C3 substituent was maintained as a cyclohexyl group and the substituents on amine were varied. In general, N-methylation (**45**) was the only substitution which did not drastically change the pharmacological profile of the compound. Compound **45** and the desmethyl analogue **41** have very similar dopaminergic activity, with the only significant difference being that **45** is a full agonist in the functional assay. The remaining compounds show a large decrease in D1 receptor binding affinity while maintaining fair to good potency in the cyclase assay.

To this point, the discussion of selectivity has been based upon the compound's relative affinity to the D1 and D2 receptors. However, dopaminergic compounds have been known to bind to other biogenic amine receptors. We have therefore included a broad-based screening protocol for the D1 agonist leads. The binding affinities of a representative series of compounds to six other receptors are given in Table III. Generally, the only other receptor which the isochromans showed affinity for was the $\alpha 2$; however, they bind with 25- to 60-fold more affinity to the D1 receptor.

As previously mentioned, selected compounds were screened in vivo by using the rat rotation model (Table IV). For general screening, the animals were dosed either orally or subcutaneously and were monitored for 2 h. For the most part, a compound's in vivo potency mirrored its in vitro activity. In one experiment, rats were pretreated with SCH23390, a selective D1 antagonist, prior to agonist dosing. As demonstrated for compound **21**, the pretreatment ameliorated the rotational response further verifying that the behavior was being mediated through D1 receptor activation.

The compounds were also shown to be orally active. The data for compounds **21** and **43** are given in the table. It is noted that the potency after oral dosing was substantially lower than the potency via subcutaneous route of administration, presumably due to low oral bioavailability. This theory has been supported by additional in vivo metabolism studies.[26] Despite this drawback, the agonists

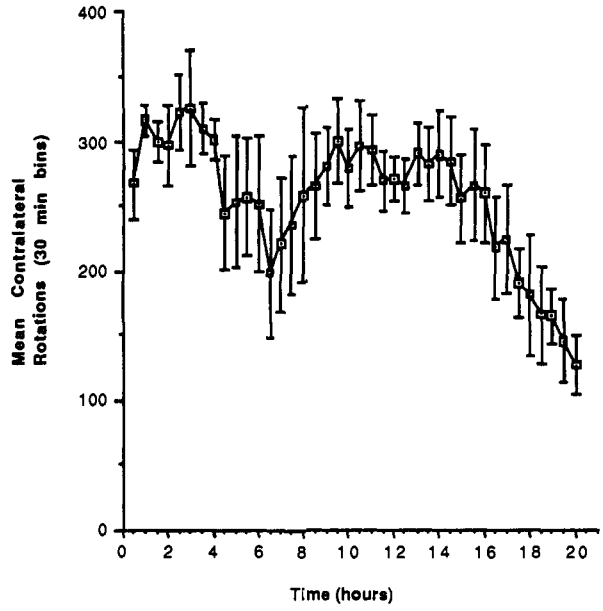


Figure 2. Duration of rotational response of compound **21** (3.2 μmol/kg, sc).

are still relatively potent and, more importantly, have a long duration of action. The duration of action of the drug was determined by monitoring the animals' rotational behavior for 20 h after dosing. A typical result is exemplified by compound **21** and is displayed in Figure 2. Remarkably, following a 3 μmol/kg sc dose, turning behavior was still observed at the 20-h time point. These data are in dramatic contrast to other selective D1 agonists, such as SKF38393, which are much shorter acting.[27] The reasons for the long duration of action are not well understood, particularly in light of the fact that catecholamines are notorious for being rapidly metabolized. One possible explanation is that the compound readily crosses the blood–brain barrier protecting it from peripheral metabolism. For this hypothesis to be true, the drug would have to be sequestered in the brain, and more importantly, not be a good substrate for metabolic enzymes in the CNS (i.e. COMT, MAOB, etc.).

In summary, we have demonstrated the 3-substituted isochroman agonists to be highly potent and selective for the D1 receptor subtype. The substituted aryl derivatives were full agonists while the remainder of the compounds displayed a wide range of intrinsic activities with no apparent correlation to their structure. The series was shown to have oral activity with a long duration of action. Because of the novel pharmacology of this class of D1 agonists as compared with previously studied drugs (i.e. compound **1**), further investigations are warranted.

Experimental Section

General. Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Flash chromatography was carried out with use of silica gel 60 (E. Merck 9285, 230–400 mesh). Anhydrous solvents were purchased from the Aldrich Chemical Co. and used without further drying. All reactions requiring anhydrous conditions were run in flame-dried glassware under nitrogen. All spectral and analytical data was obtained through the Abbott analytical department by using the following instruments: NMR, General Electric QE300; mass spectra, Finnigan MAT SSQ7; IR, Nicolet 5SXC FT-IR. Optical rotations were recorded on a Perkin-Elmer 241 spectrophotometer.

(26) Unpublished results.
(27) See for example: Morelli, M.; DiChiara, G. *Psychopharmacology* **1990**, *101*, 287.

Table II. In Vitro Pharmacology[a]

	D1B:[b] K_i, nM	D1C(R):[c] EC_{50}, nM	IA,[d] %	D1C(S):[e] EC_{50}, nM	IA, %	D2B:[b] K_i, nM	D2C: EC_{50}, nM	IA, %
1	64.1 (49–84)	386 (244–612)	61 ± 3			6870 (5350–8800)	>10000	–
20	1030 (681–1567)	490 (370–650)	71 ± 7.0			4610 (2290–9280)	2850 (2290–3550)	96 ± 2.5
21	3.0 (2.1–4.3)	2.1 (1.7–2.64)	72 ± 6.5	7.1 (5.3–9.5)	93 ± 8.0	776 (500–1200)	3910 (3110–4930)	101 ± 1.9
22	1.6 (1.1–2.4)	1.95 (1.5–2.5)	60 ± 3.4			807 (490–1340)	10200 (5920–17700)	98 ± 6.1
23	7200 (6580–7880)	8580 (7330–10030)	99 ± 8.6			4610 (2290–9280)	2850 (2290, 3550)	96 ± 2.5
24	52 (28.0–98.0)	1.02 (0.38–2.75)	106 ± 19			2090 (1160–3750)	358 (186–680)	94 ± 1.3
25	13.7 (11.0–17.3)	2.02 (0.79–5.01)	161 ± 29.3			1055 (736–1490)	>10000	–
26	310 (74–1290)	6.4 (4.6–8.7)	107 ± 4.3			764 (573–1000)	>10000	–
27	0.49 (0.39–0.62)	3.5 (2.6–4.6)	110 ± 9.0			590 (427–813)	192 (130–280)	111 ± 6.2
28	443 (290–575)	34.4 (26.0–46.2)	93 ± 5.3			1470 (820–3070)	>10000	–
29	2650 (1910–3310)	237 (162–340)	64 ± 12			2710 (1260–5000)	>10000	–
30	248 (200–305)	5.2 (3.5–7.8)	76 ± 6.5	18.9 (13.3–27.3)	78 ± 14	670 (280–1630)	>10000	–
31	374 (324–427)	24.0 (20.9–27.5)	51 ± 0.8	39.8	64	1280 (982–1690)	>10000	–
32	2290 (2160–2440)	89.0 (74–107)	107 ± 7.6			386 (232–653)	204 (170–232)	78 ± 19.8
33	530 (470–582)	100 (73–137)	66 ± 4.6			2610 (2000–3470)	>10000	–
34	167 (110–249)	142 (89–224)	89 ± 1.0			3530 (2820–4470)	10800 (5820–20700)	81 ± 4.3
35	1760 (1450–2180)	232 (225–244)	92 ± 6.7			>10000	5130 (2090–12600)	76 ± 8.9
36	407 (224–740)	67 (40–118)	135 ± 4.9			1070 (850–1350)	286 (182–460)	100 ± 5.7
37	870 (455–1670)	6.3 (4.4–9.0)	151 ± 47.0			825 (590–1180)	359 (248–485)	80 ± 13.2
38	704 (650–770)	13.6 (10.5–17.4)	90 ± 7.6	23.4	84	5540 (1870–16260)	782 (443–1430)	80 ± 7.3
39	30.4 (15.5–59)	6.7 (40–118)	124 ± 27			776 (630–960)	427 (320–570)	76 ± 12
40	479 (268–855)	68.7 (57–84)	65 ± 3.7			4680 (2710–8070)	6680 (2990–14600)	56 ± 2.2
41	5.4 (3.3–8.8)	3.1 (1.9–5.3)	71 ± 2.7	1.7 (1.2–2.5)	74 ± 9.2	1120 (890–1410)	7030 (4940–10400)	85 ± 1.2
42	88.7 (70.1–113)	5.8 (3.7–8.9)	122 ± 3.7			342 (198–580)	760 (565–1020)	70 ± 7.3
43	31.8 (22.0–45.5)	16.4 (10.3–25.5)	67 ± 6.1	52.3 (43.2–63.8)	140 ± 8.0	8190 (5840–11300)	>10000	–
44	31.7 (26.6–37.6)	5.1 (4.3–6.1)	92 ± 6.5	8.3 (4.7–14.8)	139 ± 9.7	1290 (1030–1610)	>10000	–
45	14.1 (11.9–16.8)	1.3 (1.1–1.5)	106 ± 2.5			724 (543–966)	>10000	–
46	329 (210–525)	124 (62.5–242)	71 ± 6.5			636 (330–1200)	>10000	–
47	>10000	2730 (2110–3600)	78 ± 4.9			2450 (2140–2820)	>10000	–
48	1540 (1290–1860)	46.5 (32.1–68.1)	60 ± 8.9	30.3 (13.3–68.7)	73 ± 8.0	650 (440–950)	>10000	–
49	4730 (3510–6240)	12.5 (1.87–84.7)	87 ± 10.8			11500 (10000–13200)	>10000	–
50	3190 (2300–4360)	650 (270–1550)	64 ± 17.6			2600 (1460–4520)	>10000	–
51	2510 (1590–3980)	37.6 (19.1–72.3)	66 ± 12.8			>10000	>10000	–
52	3140 (2700–3700)	1740 (1200–2520)	58 ± 6.3			12300 (9770–16200)	>10000	–

[a] Values are reported as the mean with the range of the SEM in parentheses. [b] D1B and D2B refer to competitive binding assays with [^{125}I]SCH23982 and [^{3}H]spiperone used as the radioactive ligands for the D1 and D2 receptor, respectively. [c] D1C(R) refers to the D1 receptor functional cyclase assay using a cell-free homogenate of carp retina. [d] IA = intrinsic activity (relative to dopamine). [e] D1C(S) refers to the D1 receptor functional cyclase assay using a cell-free homogenate of rat striatum tissue. See the biochemical methods section for complete details.

The experimental procedures for the chemistry described in the text for a typical compound (R^1 = cyclohexyl) are given below.

2-Cyclohexyl-1-spiro[1,3-benzodioxole-2,1′-cyclohexane]-2-ethanol (6) (R^1 = cyclohexyl). nBuLi (48.2 mL of

Table III. Binding Affinities of Selected Compounds to Other Receptors

compd	receptor binding: K_i, nM[a]					
	α1	α2	β	5HT1a	5HT1c	5HT2
21	10800 (10350–11100)	122 (68–224)	>10000	18100 (15700–21100)	9300 (8650–10100)	960 (450–2030)
22	>10000	40 (25–63)	>10000	2550 (1480–4470)	>10000	4940 (4330–5810)
23	6060 (5750–6310)	2500 (1480–4270)	>10000	>20000	1590 (1230–2040)	782 (664–910)
41	>10000	271 (257–355)	>10000	>20000	736 (690–800)	>20000
43	>10000	450 (350–575)	>10000	>20000	>10000	>20000
48	>10000	2200 (1860–2570)	>10000	>20000	1260 (1000–1590)	>20000

[a] K_i values are reported as the mean with the range of the SEM in parentheses. The radioactive ligands for the binding assays were as follows: α1, [^{3}H]prazosin; α2, [^{3}H]rauwolscine; β, [^{125}I]iodocyanopindolol; 5HT1a, [^{3}H]8-OH-DPAT; 5HT1c, [^{125}I]-SCH23982; 5HT2, [^{3}H]-ketanserin. See biochemical methods section for complete details.

Table IV. Rotational Behavior

compd	dose, μmol/kg	rte[a]	mean net contralateral[b] rotations per 2 h (±SEM)
20	65	sc	93 (±66)
	130	sc	286 (±119)
	260	sc	584 (±161)
21	0.41	sc	192 (±83)
	1.2	sc	895 (±185)
	4.1	sc	1554 (±194)
	20	po	326 (±117)
	40	po	1066 (±308)
	1.0[c]	sc	53 (±20)
22	0.2	sc	348 (±210)
	1.0	sc	1113 (±227)
	2.0	sc	1525 (±384)
23	2.0	sc	-22 (±13)
	20	sc	-24 (±10)
31	15.0	sc	467 (±160)
	30.0	sc	514 (±232)
43	0.18	sc	354 (±231)
	0.36	sc	671 (±163)
	1.1	sc	661 (±95)
	11	po	176 (±125)
	18.0	po	689 (±242)
	36.0	po	932 (±136)
48	1.3	sc	667 (±221)
	2.5	sc	1056 (±152)

[a] Rte route of administration: sc = subcutaneous; po = oral. [b] A negative value corresponds to ipsilateral rotation. [c] Predosed with SCH23390 (3.2 μmol/kg).

a 2.4 M solution in hexanes, 0.116 mol) was added to a solution of the protected catechol 4[11] (22 g, 0.116 mol) in THF (200 mL) at 0 °C, then warmed to room temperature. After 4 h at room temperature, the solution was cooled to 0 °C and cyclohexyl ethyleneoxide was added dropwise. The reaction mixture was warmed to room temperature for 2 h and then poured into NH_4Cl solution (200 mL). The mixture was extracted with ether (3 × 100 mL) and the combined ethereal layers were washed with brine. The solution was dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified through a short column of silica gel (2, then 10% EtOAc/Hex) to afford 17.5 g alcohol as a viscous oil (50%). DCI MS: 334 $(M + NH_4)^+$, 317 $(M + H)^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.68 (m, 5 H), 3.64 (m, 1 H), 2.82 (dd, 1 H, J = 15.0, 4.0 Hz), 2.62 (dd, 1 H, J = 15.0, 8.0 Hz), 2.0–1.0 (m, 21 H). Anal. ($C_{20}H_{28}O_3$) C, H.

***cis*-1-(Bromomethyl)-3-cyclohexyl-5,6-(cyclohexylidenedioxy)-3,4-dihydro-1*H*-2-benzopyran (7) (R^1 = cyclohexyl).** BF_3OEt_2 (18.45 mL, 0.15 mol) was added dropwise to a solution of alcohol 6 (16.1 g, 0.051 mol) and bromoacetaldehyde dimethyl acetal (7.1 mL, 0.06 mol) in dry ether (110 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 24 h, at which time the product had precipitated. The product was filtered and washed with cold ether to afford 16.5 g of bromide 7 as a colorless solid (77%), mp 141–143 °C. DCI MS: 438 $(M + NH_4)^+$, 421 $(M + H)^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.6 (d, 1 H, J = 8.0 Hz), 6.5 (d, 1 H, J = 8.0 Hz), 4.86 (m, 1 H), 3.88 (dd, 1 H, J = 11.0, 3.0 Hz), 3.52 (dd, 1 H, J = 11.0, 7.5 Hz), 3.34 (m, 1 H), 2.7 (dd, 1 H, J = 16.0, 3.0 Hz), 2.5 (dd, 1 H, J = 16.0, 11.0 Hz), 2.15 (m, 1 H), 2.0–1.0 (m, 20 H). Anal. ($C_{22}H_{29}BrO_3$) C, H.

***cis*-1-(Aminomethyl)-3-cyclohexyl-5,6-(cyclohexylidenedioxy)-3,4-dihydro-1*H*-2-benzopyran (8) (R^1 = cyclohexyl).** LiN_3 (1.05 g, 22 mmol) was added to a solution of bromide 7 (1.8 g, 4.28 mmol) in DMF (6 mL). The solution was heated to 70 °C for 3 h, then cooled to room temperature and poured into water (50 mL). The mixture was extracted with ether (3 × 50 mL) and the combined ethereal layers were washed with water (1 × 50 mL) and brine (1 × 50 mL). The solution was dried ($MgSO_4$), filtered, and concentrated in vacuo to give an oil. The product was purified by flash chromatography (3% EtOAc/Hex) to afford 1.23 g of azide as a colorless solid (75%), mp 88–89 °C. DCI MS: 401 $(M + NH_4)^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.6 (d, 1 H, J = 7.0 Hz), 6.42 (d, 1 H, J = 7.0 Hz), 4.88 (m, 1 H), 3.08 (dd, 1 H, J = 12.0, 3.0 Hz), 3.45 (dd, 1 H, J = 12.0, 7.5 Hz), 3.4 (m, 1 H), 2.67 (dd, 1 H, J = 16.0, 4.0 Hz), 2.57 (dd, 1 H, J = 16.0, 11.0 Hz), 2.06 (bd, 1 H, J = 12.0 Hz), 2.0–1.0 (m, 20 H). Anal. ($C_{22}H_{29}N_3O_3$) C, H, N.

LAH (1.25 mL of a 1 M solution in ether, 1.25 mmol) was added to a solution of the azide from the previous step (480 mg, 1.25 mmol) in dry ether (8 mL) at 0 °C. The reaction was quenched after 1 h by the sequential addition of H_2O (50 μL), 15% aqueous NaOH (50 μL) and H_2O (150 μL). The mixture was diluted with ether (20 mL) and CH_2Cl_2 (10 mL) and dried with $MgSO_4$. The suspension was filtered and washed with 1:1 ether/methylene chloride. The filtrate was concentrated in vacuo to afford 440 mg amine 8 as a colorless solid (98%), mp 162–163 °C. DCI MS: 358 $(M + H)^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.6 (d, 1 H, J = 8.0 Hz), 6.5 (d, 1 H, J = 8.0 Hz), 4.6 (m, 1 H), 3.35 (ddd, 1 H, J = 12.0, 7.0, 3.0 Hz), 3.19 (dd, 1 H, J = 13.0, 3.0 Hz), 2.92 (dd, 1 H, J = 13.0, 7.0 Hz) 2.69 (dd, 1 H, J = 18.0, 3.0 Hz), 2.5 (dd, 1 H, J = 18.0, 13.0 Hz), 2.18 (bd, 1 H, J = 13.0 Hz), 2.0–1.0 (m, 20 H). Anal. ($C_{22}H_{31}NO_3$) C, H, N.

***cis*-1-(Aminomethyl)-3-cyclohexyl-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyran Hydrochloride (41).** Amine 8 (500 mg, 1.4 mmol) was added to a 5 M solution of $HCl_{(g)}$ in anhydrous ethanol (10 mL). The mixture was heated to reflux temperature for 3 h, cooled and concentrated to ~1 mL. Ether was added (5 mL) and the resulting precipitate was filtered and washed with ether, and dried in a vacuum oven overnight (80 °C) to afford 380 mg product as a colorless solid (87%), mp 225 °C dec. DCI MS: 278 $(M + H)^+$. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.3 (bs, 1 H), 8.5 (bs, 1 H), 7.95 (bs, 2 H), 6.65 (d, 1 H, J = 7.0 Hz), 6.5 (d, 1 H, J = 7.0 Hz), 4.8 (m, 1 H), 3.45 (m, 1 H), 3.3 (m, 1 H), 2.83 (m, 1 H) 2.7 (dd, 1 H, J = 15.0, 3.0 Hz), 2.3 (dd, 1 H, J = 15.0, 12.0 Hz), 2.1 (bd, 1 H, J = 13.0 Hz), 1.8–1.0 (m, 10 H). Anal. ($C_{16}H_{24}ClNO_3$) C, H, N.

***cis*-3-Cyclohexyl-5,6-(cyclohexylidenedioxy)-3,4-dihydro-1-[(formylamino)methyl]-1*H*-2-benzopyran (12) (R^1 = cyclohexyl).** BF_3OEt_2 (2.26 mL, 18.4 mmol) was added to a solution of alcohol 6 (1.94 g, 6.13 mmol) and [(formylamino)methyl]-acetaldehyde dimethyl acetal (0.97 g, 7.3 mmol) in ether (35 mL) at 0 °C. The mixture was allowed to warm to room temperature

and stirred for 24 h. The precipitate was filtered and washed with ether to afford a quantitative yield of the title compound as a stable BF_3 adduct. The complex can be broken up by dissolving in methylene chloride and washing with $NaHCO_3$ solution to afford a colorless solid (2.1 g, 90%). The compound exists as a 4:1 mixture of rotomer isomers. Evidence for the presence of rotamers was obtained by a variable-temperature NMR study in which the peaks coalesced at ~130 °C, mp 195–196 °C. DCI MS: 386 $(M + H)^+$. ^1H NMR (300 MHz, $CDCl_3$) (major rotomer) δ 8.18 (d, 1 H, J = 2 Hz), 6.6 (m, 2 H), 5.95 (bs, 1 H), 4.72 (bd, 1 H, J = 7.0 Hz), 4.1 (m, 1 H), 3.4 (m, 2 H), 2.7 (dd, 1 H, J = 18.0, 4.0 Hz), 2.5 (dd, 1 H, J = 18.0, 14.0 Hz), 2.02 (bd, 1 H, J = 13.0 Hz), 1.9–1.0 (m, 20 H). Anal. ($C_{23}H_{31}NO_4$) C, H, N.

***cis*-1-(Aminomethyl)-3-cyclohexyl-5,6-(cyclohexylidenedioxy)-3,4-dihydro-1*H*-2-benzopyran (8) (R^1 = cyclohexyl).** The *N*-formyl derivative **12** from above (2.1 g, 5.5 mmol) was dissolved in THF (10 mL) and methanol (10 mL). A 15% aqueous solution of NaOH (5 mL) was added, and the solution was heated to a gentle reflux. After 4 h, the volatile solvents were removed in vacuo and the residue was diluted with water (50 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (1 × 50 mL) and dried (Na_2SO_4). The mixture was filtered and concentrated to afford 1.95 g as a colorless solid (100%) identical in all respects with compound **8** prepared above.

***cis*-3-Cyclohexyl-3,4-dihydro-5,6-dihydroxy-1-(methylamino)methyl-1*H*-2-benzopyran Hydrochloride (45).** LAH (175 mg, 4.6 mmol) was added to a solution of formamide **12** (885 mg, 2.3 mmol) in THF (10 mL) at room temperature. The solution was heated at reflux temperature for 3 h, cooled to 0 °C, and quenched by the sequential addition of H_2O (175 μL), 15% aqueous NaOH (175 μL), and H_2O (525 μL). The mixture was diluted with ether (20 mL) and dried with $MgSO_4$. The solids were filtered and washed with ether. The filtrate was concentrated in vacuo to give an oil which was used directly in the next step. The deprotection was carried out by using the same procedure used to prepare compound **41** to afford 600 mg of catechol amine **45** as a colorless solid (80% over two steps), mp 244 °C. DCI MS 292 $(M + H)^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 9.3 (bs, 1 H), 8.5 (bs, 1 H), 8.5 (bs, 1 H), 8.4 (bs, 1 H), 6.65 (d, 1 H, J = 7.0 Hz), 6.5 (d, 1 H, J = 7.0 Hz), 4.89 (d, 1 H, J = 7.0 Hz), 3.6 (bd, 1 H, J = 13.0 Hz), 3.3 (m, 1 H), 3.0 (dd, 1 H, J = 12.0, 10.0 Hz) 2.72 (dd, 1 H, J = 15.0, 2.0 Hz), 2.6 (bs, 3 H), 2.3 (dd, 1 H, J = 15.0, 12.0 Hz), 2.1 (bd, 1 H, J = 13.0 Hz), 1.8–1.0 (m, 10 H). Anal. ($C_{17}H_{26}ClNO_3$) C, H, N.

***cis*-1-[(Allylamino)methyl]-3-cyclohexyl-5,6-(cyclohexylidenedioxy)-3,4-dihydro-1*H*-2-benzopyran (10) (R^1 = cyclohexyl).** Bromide **7** (1.1 g, 2.6 mmol) was dissolved in 10 mL of allyl amine. The solution was heated to reflux temperature for 5 h and then cooled and concentrated in vacuo. The residue was dissolved in ethyl acetate (50 mL) and washed with $NaHCO_3$ solution (2 × 50 mL) and brine (1 × 25 mL). The organic solution was dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (30% EtOAc/Hex) to give 928 mg of compound **10** as a colorless solid (90%). DCI MS: 398 $(M + H)^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 6.58 (d, 1 H, J = 7.0 Hz), 6.5 (d, 1 H, J = 7.0 Hz), 5.95 (m, 1 H), 5.2 (m, 2 H), 4.7 (bd, 1 H, J = 7.5 Hz), 3.48 (m, 3 H), 3.18 (dd, 1 H, J = 10.0, 3.0 Hz), 2.82 (d, 1 H, J = 10.0, 7.5 Hz), 2.7 (dd, 1 H, J = 13.5, 2.8 Hz), 2.5 (dd, 1 H, J = 13.5, 9.0 Hz), 2.4 (bs, 1 H), 2.05 (bd, 1 H, J = 11.0 Hz), 2.0–1.0 (m, 20 H).

***cis*-1-[(Allylamino)methyl]-3-cyclohexyl-3,4-dihydro-5,6-dihydroxy-1*H*-2-benzopyran Hydrochloride (48).** Compound **10** was deprotected by the same procedure as described for compound **8**. Compound **48** was isolated in 72% yield as an off-white powder, mp 217–219 °C. DCI MS: 318 $(M + H)^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 9.32 (s, 1 H), 9.2 (bs, 1 H), 8.7 (bs, 1 H), 8.52 (s, 1 H), 6.68 (d, 1 H, J = 7.0 Hz), 6.48 (d, 1 H, J = 7.0 Hz), 5.95 (m, 1 H), 5.42 (m, 2 H), 4.92 (bd, 1 H, J = 7.5 Hz), 3.65 (bd, 2 H, J = 7.0 Hz), 3.55 (bd, 1 H, J = 13.0 Hz) 3.3 (m, 1 H), 2.9 (d, 1 H, J = 10.0, 8.5 Hz), 2.7 (dd, 1 H, J = 13.5, 2.8 Hz), 2.3 (dd, 1 H, J = 13.5, 8.5 Hz), 2.1 (bd, 1 H, J = 11.0 Hz), 1.8–1.0 (m, 10 H). Anal. ($C_{19}H_{28}ClNO_3$) C, H, N.

2-[2′,3′-(Cyclohexylidenedioxy)phenyl]-1-phenylethanone (17). A solution of alcohol **16** (15.5 g, 50 mmol) in methylene chloride (60 mL) was added to a mixture of PCC (60 g, 28 mmol) and 50 g of Celite in CH_2Cl_2 (300 mL) at room temperature. After 4 h, the reaction mixture was diluted with ether (200 mL), and the granular precipitate was removed by filtration through silica gel with copious ether washings. The filtrate was concentrated in vacuo to give 14 g (90% yield) of ketone **17** as a viscous syrup. DCI MS: 326 $(M + NH_4)^+$; 309 $(M + H)^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 8.05 (m, 2 H), 7.53 (m, 1 H), 7.42 (m, 2 H), 6.7 (m, 3 H), 4.2 (s, 2 H), 1.9–1.4 (m, 10 H).

(1*S*)-2-[2′,3′-(Cyclohexylidenedioxy)phenyl]-1-phenylethanol (19S). A solution of ketone **17** (754 mg, 2.45 mmol) in THF (1 mL) was added to a solution of (+)-B-chlorodiisopinocampheylborane (936 mg, 2.9 mmol commercially available from the Aldrich Chemical Co.) in THF (3 mL) at −20 °C. After the solution was stored at −15 °C for 12 h, the solvent was evaporated and the residue was dissolved in ether (15 mL), and 565 mg of diethanolamine was added. The mixture was stirred for 30 min and the resultant precipitate was filtered through Celite with ether washes. The filtrate was concentrated and the residue was purified by flash chromatography (100:20:1 CH_2Cl_2/hexane/ether) to afford 546 mg of chiral alcohol **19S** (72% yield) as a viscous syrup. $[\alpha]_D$ = −4.8° (*c* 2.1, $CHCl_3$). DCI MS: 328 $(M + NH_4)^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 7.3 (m, 5 H), 6.62 (m, 3 H), 4.98 (dd, 1 H, J = 7.5, 5.0 Hz), 3.0 (m, 2 H), 2.3 (bs, 1 H), 1.9–1.4 (m, 10 H).

The enantiomeric purities of compounds **19S** and **19R** were determined by quantitative conversion to the corresponding Mosher ester[18] derivatives ((*R*)-(+) Mosher acid, DCC, DMAP, CH_2Cl_2) and integration of the benzylic methine protons in the 500-MHz ^1H NMR spectra (δ 6.15 and 6.3, respectively).

(1*R*,3*S*)-1-(Aminomethyl)-3,4-dihydro-5,6-dihydroxy-3-phenyl-1*H*-2-benzopyran Hydrochloride (22). The chiral alcohol **19S** from above was carried on as described for compound **41**. The product **22** was recrystallized twice from ethanol/ether, mp 158 °C. $[\alpha]_D$ = −109° (*c* 0.35, 1 N HCl). DCI MS: 272 $(M + H)^+$. ^1H NMR (300 MHz, DMSO-d_6) δ 9.3 (bs, 1 H), 8.5 (bs, 1 H), 7.9 (bs, 2 H), 7.59 (m, 2 H), 7.4 (m, 3 H), 6.72 (d, 1 H, J = 8.0 Hz), 6.6 (d, 1 H, J = 8.0 Hz), 5.08 (bd, 1 H, J = 7.5 Hz), 4.72 (dd, 1 H, J = 11.0, 4.0 Hz), 3.5 (dd, 1 H, J = 11.0, 3.0 Hz), 3.02 (m, 2 H), 2.55 (dd, 1 H, J = 17.0, 12.0 Hz). Anal. ($C_{16}H_{18}ClNO_3$) C, H, N.

The enantiomeric purities of compounds **22** and **23** were determined by integration of the ^{19}F NMR signals (140 and 160 MHz, respectively) of their corresponding Mosher amides[18] (prepared by treatment of the catecholamines with Mosher's acid chloride and DMAP in DMF).

Biochemical Methods. General. Binding tissues were obtained from Zivic-Miller (Zelienople, PA) except where noted. The radioligands were obtained from New England Nuclear (Boston, MA) except [^{3}H]spiperone which was obtained from Amersham (Arlington Heights, IL). Miscellaneous ligands were obtained from Research Biochemicals, Natick, MA, (SCH23390, propranolol, and yohimbine), Sigma Chemical Co., St. Louis, MO, (phentolamine), or the Yamanouchi Pharmaceutical Company Ltd., Tokyo, Japan (YM09151-2). For the binding assays, the K_i's were determined from the IC_{50} values as described by Cheng and Prusoff.[28]

Radioligand Binding Assays. Binding to the D1 dopamine receptor was determined as follows: Frozen rat striata were thawed and homogenized, by using a Brinkmann Polytron, in 50 vol of assay buffer containing 50 mM Tris, pH 7.4, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 0.02% ascorbic acid and then spun at 20000*g* for 10 min. All centrifugations were performed at 4 °C. The pellet was resuspended in 50 vol of assay buffer and 50 μL was added to 50 μL of buffer or drug dissolved in buffer and 50 μL of [^{125}I]SCH-23982. Final concentration of label was 150 pM and nonspecific binding was defined with use of 1 μM SCH-23390. The mixture was incubated at 37 °C for 50 min and the reaction terminated by filtration with ice cold 0.9% NaCl used as the wash buffer.

For D2 dopamine binding, the striatal pellets were resuspended in 200 volumes of buffer and 500 μL was added to 50 μL of buffer or drug dissolved in buffer and 200 μL of [^{3}H]spiperone. Final concentration of label was 300 pM and nonspecific binding was defined by 10 μM YM-09151-2. The mixture was incubated at

(28) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.

37 °C for 20 min and terminated by filtration.

Binding to β-adrenergic receptors was assayed in striatal homogenates essentially according to Zahniser et al.[29] with some modifications. Briefly, membranes prepared as above were resuspended in 300 vol of assay buffer and 50 μL was added to 50 μL of buffer or drug dissolved in buffer and 50 μL of [^{125}I]iodocyanopindolol. Final concentration of label was 50 pM and nonspecific binding was defined with use of 10 μM propranolol. The mixture was incubated at 37 °C for 1 h and terminated by filtration.

Binding to α2-adrenergic receptors was performed according to Summers et al.[30] with some modifications. Briefly, frozen rat cortex was homogenized in 10 vol of 50 mM Tris-HCl, pH 7.4 and 5 mM EDTA and spun at 300*g* for 10 min. The supernatant was then spun at 48000*g* for 20 min and the pellet resuspended in 50 vol of assay buffer used in the above assays and 500 μL was added to 50 μL of buffer or drug dissolved in buffer and 200 μL of [^{3}H]rauwolscine. The mixture was incubated for 45 minutes at 37 °C and the reaction terminated by filtration. Final concentration of label was 325 pM and nonspecific binding was defined with use of 10 μM yohimbine.

Binding to α1-adrenergic receptors was performed as for α2-adrenergic binding except that the radioligand was [^{3}H]prazosin. Final concentration was 1.0 nM and nonspecific binding was defined with use of 10 μM phentolamine.

Binding to 5-HT$_{1A}$ and 5-HT$_2$ receptors was performed essentially as described by Hoyer et al.[31a] and Pazos et al.[31b] Briefly, the anterior third of rat cortex was homogenized in 0.32 M sucrose and spun at 900*g* for 10 min. The supernatant was then spun at 48000*g* for 20 min and the pellet resuspended in 20 vol of incubation buffer containing 50 mM Tris-HCl, pH 7.7, 4 mM $CaCl_2$, and 10 μM pargyline. The resuspension was then incubated at 37 °C for 30 min and then spun at 48000*g*. The final pellet was resuspended in 20 vol of incubation buffer and 500 mL added to 50 μL of buffer or drug dissolved in buffer and 200 μL of radioligand which was [^{3}H]8-OH-DPAT (final concentration = 800 pM) for 5-HT$_{1A}$ sites and [^{3}H]ketanserin (final concentration = 400 pM) for 5-HT$_2$ sites. Nonspecific binding was defined by 10 μM spiroxatrine for 5-HT$_{1A}$ receptors and 10 μM mianserin for 5-HT$_2$ receptors.

The 5-HT$_{1c}$ binding assay was performed by using procedures described by Hoyer and Karpf.[32] In brief, membranes of the porcine choroid plexus were prepared by homogenizing the tissue (obtained frozen from a slaughter house) in 10 vol (w/v) of cold 0.32 M sucrose and centrifuging for 10 min at 950*g*. The resulting supernatant fluid was centrifuged for 15 min at 65000*g*. The resulting pellet was resuspended in 10 vol of 50 mM Tris-HCl, pH 7.5, incubated for 15 min at 37 °C, and then centrifuged for 15 min at 65000*g*. The resulting pellet was resuspended in 10 vol of 50 mM Tris-HCl, containing 4 mM $CaCl_2$ and 0.1% ascrobic acid (Tris-calcium buffer). The binding assays were performed in Tris-calcium buffer to which was added 150 pM [^{125}I]SCH23982, particulate material from 10 mg of choroid plexus tissue, and experimental drugs (highest concentration tested 10 μM). The assay system was incubated for one hour at 37 °C; nonspecific binding was defined with 1 μM SCH23390. Bound and free radiolabel were separated by filtration with ice-cold Tris-calcium buffer used as the washing solution.

Adenylate Cyclase Assays. Agonist activity at the D1 dopamine receptor was modeled by determination of adenylate cyclase activity in cell-free homogenates of goldfish retina and rat striatum as described previously.[33] Agonist activity at the D2 dopamine receptor was modeled by determination of adenylate cyclase activity in cell-free homogenates of the intermediate lobe of the rat pituitary gland as described by Kerkman et al.[33] or using the MMQ cell line as described below.

MMQ cells are a clonal, nonadherent cell line derived from the 7135a rat anterior pituitary tumor and express functional D2 dopamine receptors.[23] MMQ cells (obtained from Dr. Robert MacLeod, University of Virginia) were maintained at 37 °C under 95% air/5% CO_2 in RPMI 1640 medium containing 2.5% fetal bovine serum, 7.5% horse serum, 2.0 mM L-glutamine, 1 × antibiotic–antimycotic solution, and 10 mg of gentamycin sulfate. Cells grown to a concentration of approximately 2×10^6/mL were pelleted and resuspended in a preincubation medium which consists of RPMI1640 media containing 0.5 mM IBMX plus 0.02% ascorbic acid and were preincubated for 5 min. Two hundred μL of the cell suspension was added to 12 × 75 mm tubes containing 100 μL of assay medium (which consists of the preincubation medium containing 300 nM forskolin) or drug dissolved in the assay medium and the mixture was incubated for 15 min. The reaction was terminated with 1 mL of 0.2 N HCl and the sample acetylated by addition of 50 μL of triethylamine/acetic anhydride (2.5:1). Acetylated samples were then spun and an aliquot of the supernatant was applied to the Gammaflow[34] automated radioimmunoassay system for the measurement of cAMP.

Behavioral Pharmacology. The rat rotation experiments were performed as previously described[33] with use of 6-hydroxy dopamine lesioned rats obtained from Zivic-Miller.

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