Synthesis and Structure−Activity Relationship in a Class of Indolebutylpiperazines as Dual 5-HT$_{1A}$ Receptor Agonists and Serotonin Reuptake Inhibitors

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Received February 23, 2004

Systematic structural modifications of indolealkylphenylpiperazines led to improved selectivity and affinity within this class of 5-HT$_{1A}$ receptor agonists. Introduction of electron-withdrawing groups in position 5 on the indole raises serotonin transporter affinity, and the cyano group proved to be the best substituent here. 5-Fluoro and 5-cyano substituted indoles show comparable results in in vitro and in vivo tests, and bioisosterism between these substituents was supported by calculation of the molecular electrostatic potentials and dipole moments. Compounds showing promising in vitro data were further examined in ex vivo (*p*-chloroamphetamine assay) and in vivo (ultrasonic vocalization) tests. Optimization of the arylpiperazine moiety indicated that the 5-benzofuranyl-2-carboxamide was best suited to increase 5-HT transporter and 5-HT$_{1A}$ receptor affinity and to suppress D$_2$ receptor binding. 5-{4-[4-(5-Cyano-3-indolyl)butyl]-1-piperazinyl}benzofuran-2-carboxamide **29** (vilazodone, EMD 68843) was identified as a highly selective 5-HT$_{1A}$ receptor agonist [GTPγS, ED$_{50}$ = 1.1 nM] with subnanomolar 5-HT$_{1A}$ affinity [IC$_{50}$ = 0.2 nM] and as a subnanomolar 5-HT reuptake inhibitor [RUI = 0.5 nM] showing a great selectivity to other GPCRs (e.g., D$_2$, IC$_{50}$ = 666 nM).

Introduction

Selective 5-HT$_{1A}$ receptor full agonists did not prove to be effective as antidepressants in the clinic so far, although results from some animal models predictive for antidepressive activity in man indicate that selective presynaptic 5-HT$_{1A}$ agonists act as anxiolytics and that postsynaptic 5-HT$_{1A}$ agonists show antidepressive effects.[1] Clinical trials with quite selective 5-HT$_{1A}$ agonists such as ipsapirone[2] or eptapirone[3] (Chart 1) could not support this hypothesis. As a consequence, compounds possessing simultaneous activity at serotonergic and/or noradrenergic receptors and/or transporters are under consideration.[4] In particular, the combination of simultaneous 5-HT$_{1A}$ receptor affinity and selective serotonin reuptake inhibition is the focus of research because addition of pindolol (5-HT$_{1A}$ autoreceptor antagonist, Chart 1) to the current therapy with SSRIs showed promising results in the clinic.[5]

From our previous work with the optimized 5-HT$_{1A}$ receptor agonist class of indolebutylpiperazines, we knew that such agonist with simultaneous inhibition of serotonin reuptake (RUI) is able to increase the serotonin level in important brain areas (e.g., frontal cortex, ventral hypocampus). We have already reported the optimization within the structural class of roxindole toward selectivity of 5-HT$_{1A}$ binding, and Chart 2 summarizes the observed structure-related results:[6−8]

(1) In particular, hydroxy, methoxy, or carboxamide in position 5 of the indole moiety yields high-affinity 5-HT$_{1A}$ ligands, tolerating a diversity of substituents on the piperazine.[6]

Chart 1. Selection of 5-HT$_{1A}$ Receptor Ligands

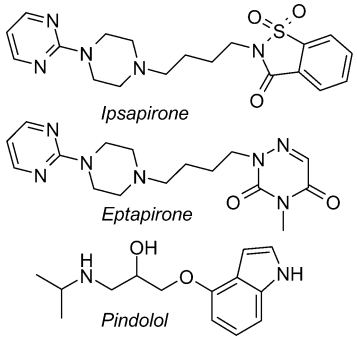
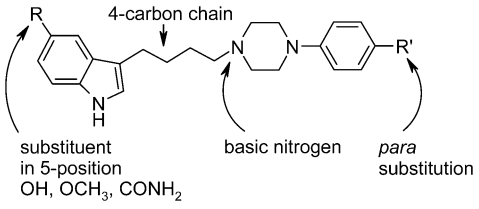


Chart 2. High Potential 5-HT$_{1A}$ Receptor Binding Pharmacophore



(2) A chain of four saturated carbon centers between the indole and the basic nitrogen is an optimum for 5-HT$_{1A}$ binding.[8]

(3) The introduction of a residue in the para position of the aromatic system can reduce dopaminergic activity.[6]

Apparently the proper selection of substituents on the two aromatic systems can be used to fine-tune receptor affinity and selectivity.[9] On this basis, further optimization was performed to find a dual selective 5-HT$_{1A}$ agonist and 5-HT reuptake inhibitor having both activi-

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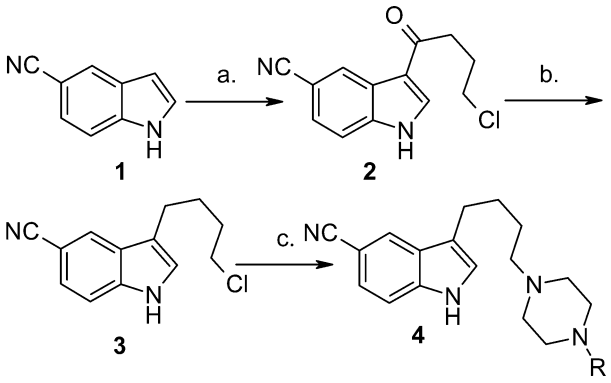
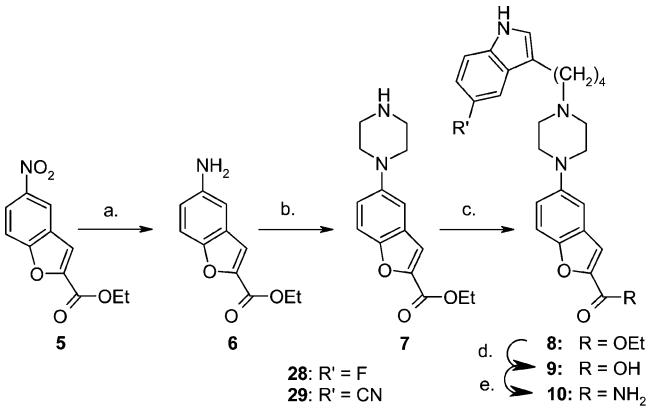
10.1021/jm040793q CCC: $27.50
Published on Web 08/10/2004

Scheme 1. Synthesis of 3-(Piperazin-1-yl-butyl)-5-cyanoindoles[a]



[a] (a) 4-Chlorobutyryl chloride, isobutyl-$AlCl_2$; (b) 2-(methoxyethoxy)aluminum hydride; (c) R-piperazine, DMF, K_2CO_3; R = 2,3-dihydrobenzo[1,4]dioxin-6-yl for Table 1 and see Tables 2, 5, and 6.

Scheme 2. Syntheses of Benzofuran-2-carboxamides **28** and **29**[a]



[a] (a) Pd/C, H_2; (b) bis(2-chloroethyl)ammonium chloride; (c) 5-CN- or 5-F-4-chlorobutylindole, K_2CO_3, NMP; (d) KOH, methanol, 80 °C; (e) 1-methyl-2-chloropyridinium iodide, NH_3 gas.

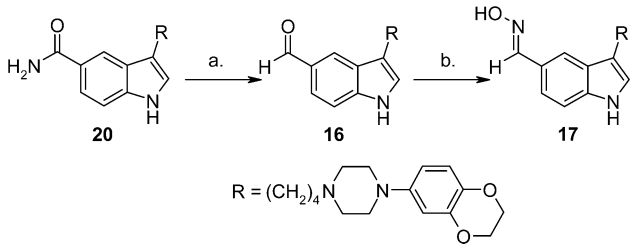
ties in the same order of magnitude within a nanomolar range. We found that both the methyl ether (inductive donor) and the carboxamide (mesomeric acceptor) in position 5 of the indole resulted in reliable 5-HT_{1A} binding.[6] Because we expected better metabolic stability from an electron-deficient indole, we took electron-withdrawing groups (EWG) under closer consideration.

Chemistry

The 5-cyanoindole derivatives were prepared as indicated in Scheme 1. Acylation of commercially available 5-cyanoindole **1**[10] in the presence of isobutyl-$AlCl_2$ with 4-chlorobutyryl chloride led to the chlorobutanoylindole **2** (73%). After selective desoxygenation of the keto function with sodium bis(2-methoxyethoxy)aluminum hydride (Vitride/Red-Al) to building block **3** in moderate yield, the chlorine was substituted with the appropriate piperazines either in NMP or in DMF in the presence of inorganic carbonates to yield scaffold **4** (55−60%). The phenylpiperazines used are commercially available or the synthesis is known from the literature.[8,11,12] The 5-(piperazin-1-yl)benzofuran-2-carboxamides **28** and **29** were synthesized according to Scheme 2.

Commercially available ethyl 5-nitrobenzofuran-2-carboxylate **5** was hydrogenated at a Raney nickel

Scheme 3. Reduction of Carboxamide **20** to Aldehyde **16** and Oxime **17**[a]



[a] (a) toluene, Vitride, room temp → 40 °C; (b) $H_2NOH{\cdot}HCl$, methanol, reflux.

contact in methanol to the corresponding amine **6**. The piperazine **7** was formed by reaction of **6** with bis(2-chloroethyl)ammonium chloride, and subsequently **7** was alkylated by the indole derivative **3** to form ethyl ester **8**. The ester **8** was saponified with potassium carbonate in methanol. The resulting acid **9** was activated with the Mukaiyama reagent[13] and transferred into the corresponding amide **10** with gaseous ammonia. The synthesis of the 5-fluoro- **14** and the 5-chloroindole **15** has been published previously.[6,7,14] The 5-carbaldehyde **16** was available by reduction of the corresponding carboxamide with Vitride in 30% yield. The aldehyde **16** was transferred into the aldoxime **17** by reaction with hydroxylammonium chloride in 80% yield (Scheme 3). The chemical methodology for the syntheses of the acid **18**, the ester **19**, and the carboxamide **20** has already been described by us.[6]

Biology

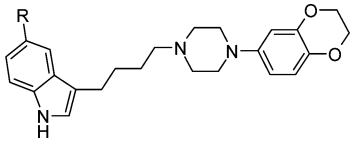
The affinity of the compounds toward the dopamine D_2 receptor subtype was evaluated by their ability to displace [^{3}H]spiperone (dopamine antagonist)[15,16] in rat striatal membranes. Alternatively, cloned human receptors were applied with spiperone as ligand. In all cases there was no significant difference for one substance in the described assays. Serotonergic activity of the compounds was measured in rat striatal membranes with [^{3}H]-8-OH-DPAT as ligand.[14] In vitro reuptake inhibition of [^{3}H]-5-HT was evaluated in rat synaptosomes,[17] and ex vivo after *p*-chloroamphetamine induced 5-HT depletion (PCA assay) in rats.[18] The intrinsic activity of the compounds as 5-HT_{1A} agonists or antagonists was determined in functional GTPγS assays with recombinant human 5-HT_{1A} receptors stably expressed in membranes of Chinese hamster ovary (CHO) cells.[19] In vivo the 5-HT_{1A} agonistic activity was determined in the rat ultrasonic vocalization test[20,21] and the D_2 antagonistic activity in the mouse climbing test.[22,23]

Results and Discussion

As a starting point for our investigation, the benzo[1,4]dioxane derivatives **11**−**21** with different indole 5-residues were prepared to evaluate the influence of these substituents on 5-HT reuptake inhibition in relation to 5-HT_{1A} and D_2 receptor affinity (Table 1).

Compounds **12**, **14**, **16**, **17**, and **21** show nanomolar or even subnanomolar binding to the 5-HT_{1A} receptor and equally potent 5-HT reuptake inhibition. The 5-HT reuptake inhibition property does not seem to affect the in vivo activity at 5-HT_{1A} receptors in this series; e.g., **13** and **20** had similar potency to inhibit ultrasonic

Table 1. 5-HT_{1A} and D_2 Receptor Binding and 5-HT Reuptake Inhibition of 3-{4-[4-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)-piperazin-1-yl]butyl}-1*H*-indol-5-yls with Different Residues on Position 5 (IC_{50} ± SEM (nM))[a]



compd	R	5-HT_{1A}	RUI	D_2
11	H	8 ± 4	10 ± 11	>100
12	OH	0.2 ± 0.15	0.5 ± 0.3	77 ± 36
13	OCH_3	0.5 ± 0.2	100 ± 29	120 ± 35
14	F	5 ± 2.3	1[b] ± 0.3	130 ± 61
15	Cl	40 ± 17	20 ± 21	>100
16	CHO	0.7 ± 0.4	1 ± 0.6	<100
17	C=N−OH	0.4 ± 0.2	5 ± 2	9.4 ± 4.9
18	CO_2H	4 ± 3	n.d.	>100
19	CO_2CH_3	0.6 ± 0.3	50 ± 23	<100
20	$CONH_2$	0.2 ± 0.2	60 ± 22	40 ± 17
21	CN	1 ± 0.7	0.4[c] ± 0.3	5.6 ± 0.6

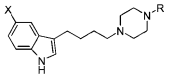
[a] IC_{50} values were obtained from 5−10 concentrations of the compound, each in triplicate, and are defined as the concentration (nM) resulting in 50% inhibition of binding. [b] PCA: 0.3 mg/kg po; 10% antagonist, 210 min. ED_{50} = 0.44 mg/kg, 210 min, po. [c] PCA: 0.3 mg/kg po; 13% antagonist, 210 min.

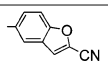
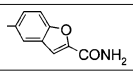
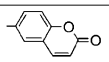
vocalization (USV, ED_{50} = 0.2 mg/kg sc for both). Because of its potential for phase II metabolism, the 5-hydroxy derivative **12** was not examined further.[6,8] The pharmaceutically unacceptable aldehyde **16** and the strong dopamine D_2 binding aldoxime **17** confirm that EWGs contribute to improve 5-HT reuptake inhibition. So consequently the fluorine (**14**) and the cyano (**21**) scaffolds were subsequently evaluated in more detail. Both derivatives show pronounced D_2 receptor affinity; the fluorinated compound **14** is a factor of 20 less potent. In the *p*-chloroamphetamine assay (PCA)[18] the results suggest a very similar ex vivo profile for **14** and **21** (10% or 13% antagonism, respectively, at 0.3 mg/kg po after 210 min). To further verify the assumption of bioisosterism of F and CN as substituents at the indole in position 5 with the 5-HT receptor subtype 1A and the 5-HT transporter as target, the following series was prepared and tested (Table 2).

Though there is one exception (5-HT_{1A} affinity of **22** is by a factor of 40 lower than that of **23**), it is obvious that F- and CN-indole derivatives behave much the same way. The comparison of **24**/**25**, **26**/**27**, **28**/**29**, and **30**/**31** revealed analogous properties in vitro. For benzofuran derivatives **28** and **29**, the inspection is also extended to ex vivo experiments and both compounds show the same result in the PCA (ED_{50} = 3.8 mg/kg, po). The analogy was also reflected in vivo in the ultrasonic vocalization test (Table 3).

The benzodioxols **24** and **25** show comparable single-digit nanomolar results independent from the application, and the chromenones **30** and **31** give similar inhibitory doses after po application. These results substantiate the suggested bioisosterism between F and CN substituents, and we were interested to see if theoretical considerations might support this hypothesis. Hardness has been discussed in the literature to be an acceptable descriptor of bioisosteric activity or even predictivity,[24] but for quantification, calculation of the energies of the highest occupied and lowest unoccupied molecular orbital is problematic for larger

Table 2. Comparison of 5-F and 5-CN Indolyl Derivatives (IC_{50} ± SEM (nM))[a]



cmpd	R	X	5-HT_{1A}	RUI	D_2
22		F	40 ± 15	2.0 ± 0.6	> 100
23		CN	1.1 ± 0.2	1.3 ± 0.9	255 ± 45
24		F	4 ± 2	1 ± 0.5	< 100
25		CN	0.7 ± 0.4	0.6 ± 0.06	4.9 ± 1.2
26		F	5 ± 2	3 ± 1	> 100
27		CN	0.4 ± 0.3	1 ± 0.7	> 630
28		F	3.0 ± 0.9	0.3[b] ± 0.2	> 100
29		CN	0.3 ± 0.06	0.5 ± 0.4	666 ± 75
30		F	0.7 ± 0.2	0.9 ± 0.3	> 100
31		CN	0.1 ± 0.06	0.7 ± 0.5	740 ± 232

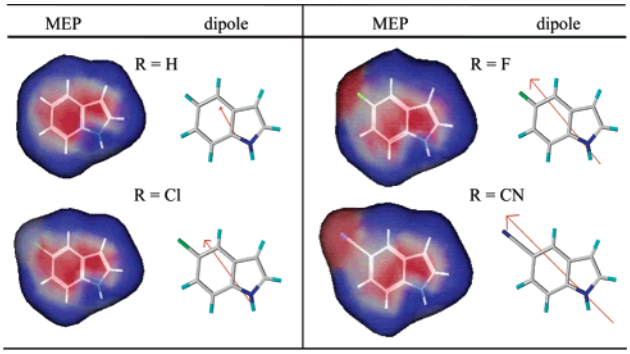
[a] IC_{50} values were obtained from 5−10 concentrations of the compound, each in triplicate, and are defined as the concentration (nM) resulting in 50% inhibition of binding. [b] PCA: ED_{50} = 3.8 mg/kg, po, 210 min.

Table 3. In Vivo 5-HT_{1A} Agonistic Activity in the Ultrasonic Vocalization Test of Selected 5-F and 5-CN Indolyl Derivatives (ID_{50} (mg/kg))

	24	**25**	**30**	**31**
po	7	4	18	4
sc	3	1	n.d.[a]	n.d.[a]

[a] n.d. = not determined.

Table 4. Visualization of MEP and Dipole Moment for Selected 5-Substituted Indoles



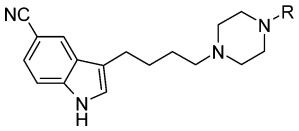
molecules. Therefore, we used the molecular electrostatic potentials (MEP) and dipole moments of indole fragments not to quantify but rather to visualize possible comparability. The results are shown in Table 4.[25]

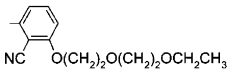
As can be seen from the illustrations in Table 4, the MEP (two negative areas, red) and the dipole vector (length and direction) are better comparable for CN and F than for Cl and F as substituents. The chlorine derivative shows properties similar to the unsubstituted indole, which is in accord with the results for **11** and **15** from Table 1.

For further investigation, the 5-cyanoindole moiety was chosen for closer examination with a series of differently substituted arylpiperazines (Tables 5 and 6). First, a series of monocyclic substituted piperazines was examined for the 5-HT_{1A} receptor binding and 5-HT reuptake inhibition (Table 5).

Besides the unsubstituted phenyl compound **32**, phenyl derivatives with electron-donating residues (**33**−**35**, **48**) as well as with electron-withdrawing groups (**36**−**38**, **45**−**47**) were prepared. In addition to these, we

Table 5. 5-CN Indole Derivatives with Different Arylpiperazine Residues (IC_{50} ± SEM (nM))[a]



compd	R	5-HT_{1A}		RUI		D_2	
32	C_6H_5	400	± 250	1	± 0.7	3.8	± 2.1
33	4-OH C_6H_4	6	± 2	0.5	± 0.2	35	± 3
34	4-OCH_3 C_6H_4	1	± 1	0.5	± 0.4	46	± 9
35	2-OCH_3 C_6H_4	1	± 0.2	3.0	± 0.1	0.7	± 0.5
36	4-F C_6H_4	2	± 0.6	1.0	± 0.5	4.2	± 1.9
37	3,4-$(CN)_2$ C_6H_3	12	± 7	2.0	± 0.6	n.d.	
38	2,6-$(CN)_2$ C_6H_3	40	± 18	2	± 0.9	< 100	
39	4-CN-3-OCH_3 C_6H_3	14	± 14	3.4	± 1.4	n.d.	
40	4-CN-2-OCH_3 C_6H_3	8	± 0.6	4.1	± 1.7	6.0	± 2.9
41	2-CN-3-OCH_3 C_6H_3	5.6	± 1.7	3.9	± 1.1	1.4	± 0.3
42		2	± 0.6	3.2	± 1.3	28	± 6
43	3-CN-4-OH C_6H_3	400	± 200	0.8	± 0.1	43	± 21
44	5-Cl-2-OCH_3 C_6H_3	30	± 12	6.8	± 3.4	n.d.	
45	4-CN-3,5-F_2 C_6H_2	36	± 18	5.4	± 1.8	n.d.	
46	4-$CONH_2$ C_6H_4	118	± 75	0.6	± 0.3	> 270	
47	2-$CONH_2$ C_6H_4	6.4	± 0.7	16	± 6	47	± 20
48	3,4-$(OCH_3)_2$ C_6H_3	60	± 32	0.3	± 0.1	40	± 18

[a] IC_{50} values were obtained from 5−10 concentrations of the compound, each in triplicate, and are defined as the concentration (nM) resulting in 50% inhibition of binding. n.d. = not determined.

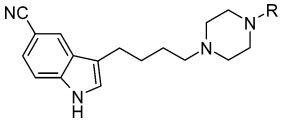
synthesized further derivatives with a more sophisticated substitution pattern (**39**−**44**). Results are in the range of what we strive for (dual nanomolar activity); however, the D_2 binding affinity is too pronounced for nearly all members of Table 5. Only the carboxamide **46** shows weak D_2 data as desired, but for this compound the 5-HT_{1A} affinity is not strong enough (vide infra). To optimize 5-HT_{1A} binding and the selectivity to the D_2 receptor, bicyclic aryl substituents on the piperazine were prepared and tested (Table 6).

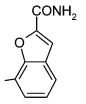
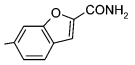
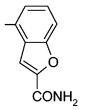
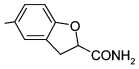
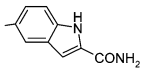
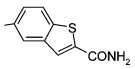
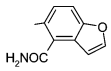
Dihydrobenzopyran **49**, dihydrobenzofuran **50** and its 2-carboxamide **58**, 2,1,3-benzothiadiazoles **51** and **52**, indole **53** and its 2-carboxamide **59**, benzothiophen-2-carboxamide **60**, and benzofuran **54** with a series of corresponding carboxamides with a close relationship to the benchmark **29** (**55**−**57**, **61**) were synthesized and analyzed for their receptor profiles.

Summarizing the data in Tables 2, 5, and 6, compounds containing the 5-cyanoindole-3-butylamine with a basic nitrogen almost guarantee effective serotonin reuptake inhibition. Comparable data were found for the monocyclic derivatives (**32**−**48**) and the bicyclic arylpiperazines (**49**−**61**). However, in most cases D_2 receptor affinity is too strong. The exception is the dihydrobenzofuran-2-carboxamide **58** showing low nanomolar in vitro data with a perfect selectivity to D_2 dopamine receptor binding affinity (>1 μmol/L). Nevertheless, compound **58** is still weaker than **29** on both serotonin targets by a factor of 10. All other derivatives and isomers do not differ significantly in their in vitro profile and thus do not show advantages compared to compound **29**, and none of them reached the potent activity of vilazodone **29**.

The hypothesis that para-substituted arylpiperazines in indolyl-5-carboxamides have reduced D_2 affinity[6] is not valid for the 5-cyanoindoles. With regard to the nanomolar dopaminergic receptor affinities found for the set of compounds **21**, **33**, **34**, **36**, **40**, **51**, **53**, **54**, and **56** in comparison to the unsubstituted core structure **32**, it is obvious that for the 5-cyanoindole derivatives para

Table 6. 5-CN Indole Derivatives with Heterobicyclic Arylpiperazine Substitution (IC_{50} ± SEM (nM))[a]



cmpd	R	5-HT_{1A}	RUI	D_2
49		5.0 ± 2.3	0.9 ± 0.6	< 42
50		4.0 ± 2.0	1.0 ± 0.6	13 ± 3
51		2.0 ± 1.5	3.1 ± 1.1	31 ± 12
52		1.0 ± 0.4	1.5 ± 0.9	1.4 ± 1.0
53		1.6 ± 0.3	2.4 ± 0.7	5.7 ± 2.7
54		8.7 ± 0.9	3.9 ± 0.8	8 ± 4
55		1.9 ± 0.7	2.6 ± 0.9	39 ± 25
56		4.4 ± 2.3	1.1 ± 0.5	95 ± 27
57		1.4 ± 0.2	8.5 ± 6	6.6 ± 2.5
58		3.2 ± 0.4	3.1 ± 1.2	1400
59		14 ± 8	2 ± 1.4	140 ± 47
60		3.1 ± 2.8	0.3 ± 0.1	n.d.[b]
61		3.3 ± 1.3	5.5 ± 2.6	220 ± 99

[a] IC_{50} values were obtained from 5−10 concentrations of the compound, each in triplicate, and are defined as the concentration (nM) resulting in 50% inhibition of binding.

substitution does not inherently reduce D_2 binding. In this context, the high dopaminergic activity of benzodioxane **21** is surprising. Within this group of benzodioxanepiperazines, the cyano group (**21**) and the corresponding oxime **17** show the strongest dopaminergic D_2 binding. The corresponding benzodioxole **25** shows equally high potency to this receptor, and as for **17** or **21**, this is astonishing because other comparable compounds in Table 1 lack this D_2 receptor affinity. It can be speculated that these activities might be linked to the different three-dimensional structures of the ligands and their different orientations at the D_2 receptor site[26] because these compounds can form a multitude of different conformations due to the flexible four-carbon chain.

For a couple of compounds the intrinsic activity on the 5-HT_{1A} receptor was determined in vitro by determining [^{35}S]GTPγS binding (Table 7). The potential of methyl ethers (**35** and **40**) to antagonize 5-HT induced [^{35}S]GTPγS binding (antagonism assay) was found to be very low, indicating that the compounds act as agonists. The 2,6-bis-cyan derivative **38** binds with an IC_{50} of 40 nM at the 5-HT_{1A} receptor and does not show full agonism at the receptor up to 250 nM. The compounds **23**, **29**, **30**, **42**, **51**, **54**−**56**, **58**, and **60** show full agonism at the 5-HT_{1A} receptor with nanomolar or even subnanomolar EC_{50} values. The 4-benzofuranyl derivative **57**, a regioisomer of **29** (5-benzofuranyl), is the only exception showing merely partial agonism in this functional assay for 5-HT_{1A} receptor activity.

Table 7. Intrinsic 5-HT_{1A} Receptor Activity of Some 5-Cyanoindole Derivatives in GTPγS Binding (EC_{50} (nM))

23	**29**	**30**	**35**[a]	**38**	**40**[a]	**42**	**51**	**52**	**54**	**55**	**56**	**57**[b]	**58**	**60**
15.2	1.1	9.3	51	250	85	11	8	6	6.2	0.4	3.8	54	3.8	1.1

[a] % control at 0.1 μM compound concentration. [b] % control at 1 μM compound concentration.

Table 8. In Vivo Activity of Selected 5-CN Indole Derivatives (ID_{50} (mg/kg))

	administration route	**23**	**25**	**29**	**31**	**36**	**51**
inhibition of ultrasonic vocalization	po	14	4	13	4	7	n.d.
	sc	n.d.	1	4	0.5	3	3
inhibition of climbing	po	n.d.	7	>30	n.d.	6	n.d.

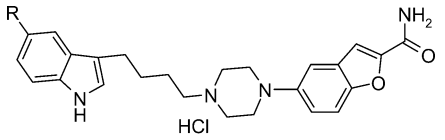
In vivo 5-HT_{1A} agonism was measured via inhibition of ultrasonic vocalization (USV) in rats and D_2 antagonism by inhibition of apomorphine-induced climbing in mice. The results are shown in Table 8. Compatible with the in vitro binding profile and the 5-HT_{1A} agonistic properties determined in the GTPγS assay, compounds **23**, **25**, **29**, **31**, **36**, and **51** are active in vivo. The electrically induced ultrasonic vocalization is inhibited by these derivatives, in good correlation with the in vitro results. A good bioavailability can be predicted from the ratio of the po versus sc ID_{50} values. In line with the determined affinity to D_2 receptors, compounds **25** and **36** also inhibited apomorphine-induced climbing behavior, indicating dopamine antagonistic properties, whereas benzofurancarboxamide **29**, without affinity for the dopamine D_2 receptor, was inactive.

In this report a series of indolealkylamines was tested with emphasis on three targets (5-HT_{1A}, D_2, 5-HT reuptake transporter). Other relevant G-protein-coupled receptors (GPCRs) have been tested for those compounds with promising profiles. For example, histaminergic activity has to be precluded to avoid any sedative side effects. In this context, the attractive bis-cyano derivative **23** has been evaluated (5-HT_{1A}, IC_{50} = 1 nM; USV, ID_{50} = 14 mg/kg po; RUI, IC_{50} = 1.3 nM; D_2, IC_{50} > 250 nM) and was found to bind with two-digit nanomolar affinity to the histaminergic receptor 1 (H_1, IC_{50} = 55 nM).

Because of its outstanding affinity and selectivity profile, compound **29** (vilazodone; 5-HT_{1A}, IC_{50} = 0.2 nM; RUI, IC_{50} = 0.5 nM; D_2, IC_{50} = 666 nM) was selected for further development (Chart 3).

In Table 9 a more comprehensive binding data collection is given for vilazodone **29**, and as a resumption and validation of the bioisosterism discussion above, the data for the fluorine analogue **28** are given in parentheses if available.

Chart 3



R = F : **28**
R = CN: **29** *Vilazodone*

Table 9. Receptor Binding Profile of Vilazodone **29** and Its Fluorine Analogue **28**[a]

target	IC_{50} (nM)	target	IC_{50} (nM)
5-HT_{1A}	0.2 (3.0)	RUI	0.5 (0.3)
GTPγS	1.1	PCA	3.8[b] (3.8[b])
5-HT_{1B}	5000	D_2	666 (>100)
5-HT_{1D}	4000 (>1000)	D_3	71
5-HT_{2A}	1510	D_4	3400
5-HT_{2C}	2000 (>1000)	α_1	1980 (3000)
5-HT_3	4300	α_2	6000 (9000)
5-HT_4	252	H_1	317
5-HT_6	3000	H_2	>1000
5-HT_7	3900	κ	>1000

[a] Data for **28** in parentheses. [b] ED_{50} (mg/kg).

The pharmacological profile of **29** as dual subnanomolar 5-HT reuptake inhibitor with a selective presynaptic 5-HT_{1A} receptor agonistic property was previously described.[20,27] Vilazodone was found to increase 5-HT levels in rat brain frontal cortex to an extent not reached with selective serotonin reuptake inhibitors[28] and therefore is an interesting compound to further examine the impact of increased serotonin levels in important brain areas on mood and mood disorders.

Experimental Section

Melting points were determined on a Büchi 535 melting point apparatus and are uncorrected. IR, ^{1}H NMR, and mass spectra are in agreement with the structures and were recorded on a Bruker IFS 48 IR spectrophotometer, a Bruker AMX 300 MHz or DRX 500 MHz NMR spectrometer (TMS as an internal standard), and Vaccum Generators VG 70-70 or 70-250 at 70 eV. Elemental analyses (obtained with a Perkin-Elmer 240 BCHN analyzer) for the final products were within 0.4% of theoretical values if not otherwise stated. All reactions were followed by TLC carried out on Merck KGaA F254 silica gel plates. Solutions were dried over Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure. Phenylpiperazines were available from EMKA.

General Methods. 3-{4-[4-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl]butyl}-1*H*-indole-5-carbaldehyde (16). A solution of sodium bis(2-methoxyethoxy)aluminum hydride (Vitride, 23.6 mL/70% in toluene, 120 mmol) in 25 mL of toluene was added dropwise to a suspension of 19.7 g (45 mmol) of 3-{4-[4-(2,3-dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl]butyl}indol-5-carboxamide in 400 mL of THF at room temperature. After 12 h a second portion of 24 mL of Vitride was added and stirring continued at 40 °C until the carboxamide had reacted completely (TLC). For workup, the batch was cooled with ice and an amount of 30 mL of water was added slowly. After filtration of the precipitate the solution was concentrated in vacuo and purified by chromatography on silica gel. The product fractions were collected and after evaporation the product was crystallized from acetonitrile giving 5.8 g (30%) of white crystals of **16**: mp 131−135 °C; ^{1}H NMR (DMSO-d_6) δ 9.97 (s, 1H), 8.18 (s, 1H), 7.63 (dd, 1H, J = 1.5 Hz and J = 8.5 Hz), 7.49 (d, 1H, J = 8.5 Hz), 7.31−7.16 (m, 2H), 6.71 (dd, 1H, J = 1.0 Hz and J = 8.0 Hz), 6.42 (m, 2H), 4.18 (m, 4H), 2.97 (m, 4H), 2.79 (t, 2H, J = 7.3 Hz), 2.58−2.32 (m, 6H), 1.79−1.66 (m, 2H), 1.62−1.52 (m, 2H). Anal. ($C_{25}H_{29}N_3O_3$) H. C: calcd, 71.6; found, 71.1. N: calcd, 10.0; found, 10.5.

3-{4-[4-(2,3-Dihydrobenzo[1,4]dioxin-6-yl)piperazin-1-yl]butyl}-1*H*-indole-5-carbaldehyde Oxime (17). A suspension of 1 g (2.4 mmol) of **16** in 20 mL of methanol was treated with 170 mg (2.4 mmol) of hydroxylammonium chloride and heated to reflux for 2 h. After the solution was cooled to room temperature, some yellowish precipitate was formed. The compound was filtered and washed with acetone, yielding 800 mg (80%) of **17** hydrochloride: mp 237−238 °C; ^{1}H NMR (DMSO-d_6) δ 10.98 (s, 1H), 10.73 (s, 1H), 10.45 (br s, 1H), 8.15 (s, 1H), 7.67 (s, 1H), 7.48−7.30 (m, 2H), 7.18 (d, 1H, J = 2.5 Hz), 6.73 (dd, 1H, J = 2.2 and J = 9.3 Hz), 6.46 (m, 2H), 4.17 (m, 4H), 3.50 (m, 4H), 3.03 (m, 6H), 2.74 (t, 2H, J = 8.4 Hz), 1.72 (m, 4H). Anal. ($C_{25}H_{30}N_4$·HCl) C, H, N. Cl: calcd, 7.5; found, 6.9.

3-[4-(4-Benzo[1,3]dioxol-5-ylpiperazin-1-yl)butyl]-1*H*-indole-5-carbonitrile (25). A solution of 14.2 g (0.1 mol) of 5-cyanoindole **1**[10] and 23.1 mL (0.2 mol) of 4-chlorobutyric acid chloride in 250 mL of dichloromethane was cooled to 15 °C, and 31.9 g (0.2 mol) of isobutylaluminum chloride were added drop by drop so that the temperature did not exceed 30 °C. The precipitate was collected by filtration and stirred in 500 mL of water. After exhaustive extraction with ethyl acetate, the combined organic phases were dried and cautiously concentrated. The precipitate **2** was filtered, washed with methyl *tert*-butyl ether, and dried: yield 17.9 g; 73%; mp 169−170 °C; ^{1}H NMR (DMSO-d_6) δ 12.47 (br s, 1H), 8.55 (s, 2H), 7.69−7.57 (m, 2H), 3.74 (t, 2H, J = 6.9 Hz), 3.07 (t, 2H, J = 6.9 Hz), 2.12 (quint, 2H, J = 6.9 Hz). An amount of 95 g (0.4 mol) of the previously prepared 3-(4-chlorobutyryl)-1*H*-indole-5-carbonitrile **2** was suspended in 900 mL of THF and cooled to 0 °C. A solution of 171 g of sodium bis(2-methoxyethoxy)-aluminum hydride (245 mL/70% in toluene) in 250 mL of toluene was added dropwise, and stirring was continued for an additional 2 h. The reaction was terminated by the addition of 200 mL of water. After phase separation and solvent evaporation, the residue was filtered over silica gel and the resulting material was washed with 2-propanol. The resulting crystals **3** were purified by chromatography over silica gel: yield 23.3 g; 26%; mp 99−99.5 °C; ^{1}H NMR (DMSO-d_6) δ 11.33 (br s, 1H), 8.06 (s, 1H), 7.50 (d, 1H, J = 8.4 Hz), 7.38 (dd, 1H, J = 1.5 Hz and J = 8.4 Hz), 7.32 (d, 1H, J = 2.1 Hz), 3.67 (m, 2H), 2.75 (m, 2H), 1.78 (m, 4H). A suspension of 114 g (0.5 mol) of 3-(4-chlorobutyl)-1*H*-indole-5-carbonitrile **3**, 119 g (0.5 mol) of 1-benzo[1,3]dioxol-5-yl-piperazine,[29] 135 g (1 mol) of K_2CO_3, and 81 g (0.5 mol) of KI in 900 mL of DMF was refluxed for 12 h. Subsequently the suspension was stirred with charcoal at room temperature and filtered over silica gel. After removal of the solvent, the residue was dissolved in acetone and purified via chromatography. The resulting crude product was recrystallized from methanol, yielding 115 g (60%) of **25**: mp 154−156 °C. A solution of 34 g (0.29 mol) of succinic acid and 116 g (0.29 mol) of the previously prepared base **25** was stirred at 35 °C until crystallization began. Crystallization was continued at 10 °C and the crystals were filtered and washed with ethyl ether and acetone, yielding 125 g (84%) of **25** as a succinate: mp 227−228 °C; ^{1}H NMR (DMSO-d_6) δ 11.32 (br s, 1H), 8.05 (s, 1H), 7.51−7.31 (m, 3H), 6.73 (d, 1H, J = 10.5 Hz), 6.63 (d, 1H, J = 2.9 Hz), 6.30 (dd, 1H, J = 2.9 Hz and J = 10.5 Hz), 5.89 (s, 2H), 2.99 (m, 4H), 2.73 (t, 2H, J = 8.9 Hz), 2.37−2.40 (m, 6H), 1.63 (m, 4H). Anal. ($C_{24}H_{26}N_4O_2$·$C_4H_6O_4$) C, H, N.

5-{4-[4-(5-Cyano-1*H*-indol-3-yl)butyl]piperazin-1-yl}-benzofuran-2-carboxamide (29). A mixture of 812 g (3.45 mol) of ethyl 5-nitrobenzofuran-2-carboxylate **5** and 300 g of wet Raney Nickel in 8 L of methanol were reacted with 232.1 L of H_2 at 28 °C for 27 h. Filtration, evaporation, and recrystallization from methanol yielded 664.6 g (93%) of the amine **6**: mp 59−60 °C; ^{1}H NMR (DMSO-d_6) δ 11.80 (br s, 2H), 7.87 (m, 3H), 7.55 (dd, 1H, J = 2.1 Hz and J = 8.9 Hz), 4.40 (q, 2H, J = 7.1 Hz), 1.37 (t, 3H, J = 7.1 Hz). A suspension of 48.3 g (0.2 mol) of ethyl 5-aminobenzofuran-2-carboxylate **6**, 37.7 g (0.2 mol) of bis(2-chloroethyl)ammonium chloride, and 15.2 g (0.1 mol) of potassium carbonate were heated to reflux in 250 mL of 1-butanol for 48 h. The hot suspension was

decanted and filtered. After evaporation the crude product was recrystallized from methanol, yielding 9.86 g (27%) of **7**·hydrochloride: mp 242−244 °C; ^{1}H NMR (DMSO-d_6) δ 9.30 (br s, 2H), 7.63 (m, 2H), 7.28 (m, 2H), 4.36 (q, 2H, J = 7.1 Hz), 3.37 (m, 4H), 3.24 (m, 1H), 1.34 (t, 3H, J = 7.1 Hz). Anal. ($C_{15}H_{18}N_2O_3$·HCl) C, H, N. Cl: calcd, 11.4; found, 11.0. A suspension of 4.9 g (16 mmol) of the previously prepared ethyl 5-(piperazin-1-yl)benzofuran-2-carboxylate hydrochloride **7**·HCl, 3.7 g (16 mmol) of 3-(4-chlorobutyl)-1*H*-indole-5-carbonitrile **3**, 2.2 g (16 mmol) of potassium carbonate, and 2.8 mL (16 mmol) of triethylamine in 120 mL of acetonitrile was heated to reflux for 12 h. After the mixture was cooled and evaporated, the residue was stirred in ethyl acetate and water. After the organic phase was dried and evaporated, an amount of 8 g of reddish oil was left. After chromatography an amount of 2.6 g of a yellow oil was obtained, which was transformed into the corresponding hydrochloride by dissolving the oil in 120 mL of acetone and adding 2 mL of HCl-saturated ethanol. The resulting crystals were washed with acetone and ether and dried, yielding 2.5 g (32%) of **8**·dihydrochloride as colorless crystals: mp 221−223 °C; ^{1}H NMR (DMSO-d_6) δ 11.49 (s, 1H), 10.91 (br s, 1H), 8.10 (s, 1H), 7.64 (m, 2H), 7.51 (d, 1H, J = 8.4 Hz), 7.40 (m, 2H), 7.31 (m, 2H),4.35 (q, 2H, J = 7.1 Hz), 3.74 (m, 2H), 3.59 (m, 2H), 3.18 (br s, 6H), 2.78 (t, 2H, J = 7.2 Hz), 1.76 (m, 4H), 1.34 (t, 3H, J = 7.1 Hz). Anal. ($C_{28}H_{30}N_4O_3$·2HCl) C, H, N, Cl. A suspension of 1.5 g (2.8 mmol) of ethyl 5-{4-[4-(5-cyano-3-indolyl)butyl]-1-piperazinyl}benzofuran-2-carboxylate **8** and 0.63 g (11.2 mmol) of KOH in 100 mL of methanol was heated to reflux for 3 h. The solvent was evaporated, and the crude residue was dissolved in water. By additio of 1 N HCl, the pH was adjusted to 7 and the precipitate was filtered and dried, yielding 1 g (80%) of 5-{4-[4-(5-cyano-1*H*-indol-3-yl)butyl]piperazin-1-yl}benzofuran-2-carboxylic acid **9** as tetrahydrate: mp 189−192 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.37 (br s, 2H), 8.07 (s, 1H), 7.52 (m, 2H), 7.37 (m, 3H), 7.17 (m, 2H), 3.13 (br s, 8H), 2.75 (t, 2H, J = 7.1 Hz), 2.61 (m, 8H), 3.24 (m, 4H), 1.62 (m, 4H). Anal. ($C_{26}H_{26}N_4O_3$·4H_2O) H, N. C: calcd, 60.7; found, 59.7. After exhaustive drying, 1 g (2.2 mmol) of the previously prepared acid **9** and 1.4 g (5.5 mmol) of 1-methyl-2-chloropyridinium iodide were suspended in 20 mL of NMP. While introducing NH_3 gas via a cannula, 2.6 mL of ethyldiisopropylamine were added drop by drop. The temperature rose to 41 °C and dropped to room temperature again after 15 min. The reaction mixture was poured onto water and extracted exhaustively with ethyl acetate, yielding 0.7 g (72%) of the free base **29**. The compound was dissolved in 30 mL of hot 2-propanol. At room temperature, HCl-saturated 2-propanol was added slowly until complete precipitation occurred, yielding 0.6 g (79%) of **29** as the hydrochloride: mp 277−279 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (d, 1H, J = 1.9 Hz), 10.80 (br s, 1H), 8.07 (d, 1H, J = 1.5 Hz), 7.57 (br s, 1H), 7.50 (d, 1H, J = 8.4 Hz), 7.49 (d, 1H, J = 8.4 Hz), 7.42 (d, 1H, J = 1 Hz), 7.37 (dd, 2H, J = 1.5 Hz and J = 8.4 Hz), 7.24 (d, 1H, J = 2.5 Hz), 7.18 (dd, 1H, J = 2.5 Hz and J = 9.1 Hz), 3.71 (m, 2H), 3.53 (m, 4H), 3.12 (m, 4H), 2.75 (t, 2H, J = 7.2 Hz), 1.79 (m, 2H), 1.67 (m, 2H). Anal. ($C_{26}H_{27}N_5O_2$·HCl) C, H, N, Cl.

Pharmacological Methods. Radioligand Binding Assay at Rat Hippocampus 5-HT$_{1A}$ Serotonergic Receptors. The method was adapted from Cossery et al.[30] Rat hippocampus membranes (0.2 mg of protein/tube) were incubated with 0.5 nM [^{3}H]-8-OH-DPAT in a total volume of 0.5 mL at 25 °C for 30 min. Nonspecific binding was determined in the presence of 1 μM serotonin.

Stimulation of [^{35}S]GTPγS binding at Cloned 5-HT$_{1A}$ Receptors. The effects of different compounds tested on [^{35}S]-GTPγS binding were evaluated according to the method of Newman-Tancredi et al.[19] with modifications. Membranes of Chinese hamster ovary (CHO) cells stably expressing the recombinant human 5-HT$_{1A}$ receptor were obtained from NEN (catalog no. CRM035, GenBank no. X13556). The membranes were stored at −70 °C. Prior to use, membranes were thawed and rehomogenized in assay buffer ($MgCl_2$, NaCl, and EDTA in Tris-HCl, pH 7.4). Membranes (~10 μg of protein) were incubated at 37 °C for 30 min (shaking water bath) in duplicate in a total volume of 800 μL of buffer containing $MgCl_2$ (3 mM), NaCl (120 mM), EDTA (0.2 mM), GDP (10 μM), [^{35}S]GTPγS (0.1 nM), Tris (50 mM), and test compounds. Prior to addition to the incubation mixture, the test compounds were dissolved in twice distilled water. DMSO was used to aid in solubilizing certain compounds. Nonspecific binding was defined with 0.1 μM GTPγS. 5-HT was tested as standard in each experiment at concentrations of 100, 30, and 10 nM. Experiments were terminated by rapid filtration through Whatman GF/B filters using a Brandel cell harvester. Subsequently, the filters were rinsed twice with 5 mL of ice-cold Tris-HCl and placed in scintillation vials. Radioactivity was extracted in 4 mL of scintillation fluid (Ultima Gold, Packard Instruments, Frankfurt, Germany) and determined by liquid scintillation counting. Binding isotherms were analyzed by nonlinear regression. Agonist efficacy (=E_{max}) is expressed relative to that of 5-HT (=100%), which was tested at a maximally active concentration (0.1 mM) in each experiment. EC_{50} values were defined as the concentration of the compound at which 50% of its own maximal stimulation was obtained.

[^{3}H]-5-HT Reuptake Inhibition Assay. Crude synaptosomal fraction (P_2 fraction) of rat cerebral tissue was prepared according to Whittaker et al.,[31] giving a suspension enriched in synaptosomes of 3 mg of protein/mL. The uptake was determined in a total volume of 570 μL, which contained 12 nM [^{3}H]-5-HT. Incubation was performed at 37 °C for 4 min in Krebs−Ringer buffer (126 mM NaCl, 1.4 mM $MgCl_2$, 4.8 mM KCl, 15.8 mM Na_2HPO_4, 11 mM glucose, 0.9 mM $CaCl_2$, pH 7.4).

Effects of Drugs on PCA (*p*-Chloroamphetamine) Induced 5-HT Depletion in Rat Hypothalamus. The experiments were essentially carried out as previously described.[32] Male rats (135−160 g body weight) were used. Brain regions were dissected out on ice[33] and immediately processed for HPLC analysis. PCA (5 mg/kg ip in saline) was given 3 h and drugs 3.5 or 5 h prior to decapitation. Brain tissue 5-HT was determined by an automated reversed phase/ion pair, direct injection HPLC method[32] within a 25 min run. *N*-Methyldopamine or *N*-ω-methylserotonin was used as an internal standard and the recoveries were >95%.

Ultrasonic Vocalization Test. Male Sprague Dawley rats (180−280 g) from Charles River (Sulzfeld, Germany) were used. Ultrasonic vocalization was measured in a sound-attenuated test chamber (width of 24 cm, length of 22 cm, height of 22 cm) with a grid floor for delivery of foot shock (scrambled shock of 0.2 mA for 0.5 s, shocker Getra BN 2002). Ultrasonic vocalization was recorded (microphone 4004, Bruel and Kjær) and processed by an interface (developed at Merck, Darmstadt) to select 22 ± 4 kHz signals and to digitize the resulting signals for automatic processing in a personal computer. In a priming phase, each rat was placed in the test chamber. After a 2 min time period, a series of at most 10 shocks (trials), 1.8 mA for 0.3 s separated by 20 s shock-free intervals, was delivered via the grid floor of the test chamber. In the shock-free intervals, the occurrence of ultrasonic vocalization was automatically recorded and the duration of ultrasonic vocalization was calculated immediately. The priming session was terminated either when the rat constantly vocalized at least for 10 s on three consecutive trials or after the tenth trial. Rats that did not respond with ultrasonic vocalization on three consecutive trials were excluded from further testing. In the actual test performed on the next day, each rat received five initial shocks (1.8 mA for 0.3 s, separated by 20 s shock-free intervals) in the test chamber, and the duration of ultrasonic vocalization was recorded during the following 3 min period. Animals were tested 2 h after administration of compounds. ID_{50} values (=calculated dose for half-maximal inhibition of ultrasonic vocalization) were determined from the dose−response curves.

Climbing Test. Male NMRI mice (24−34 g) from Charles River (Sulzfeld, Germany) were used. Climbing was induced by injection of 1.25 mg/kg apomorphine sc and 2 min later assessed in cylindrical wire mesh cages (11.5 cm diameter, 18

cm height) by scoring every 2 min for a period of 20 min as follows: (0) no climbing behavior, (1) at least two forelimbs on the wire mesh, (2) all four forelimbs on the wire mesh and climbing for at least 30 s (maximal score was 20). Drugs were administered 1 h prior to apomorphine.

Acknowledgment. We thank Horst Hochstätter, Marion Sturm, Claudia Daum, Bernd Arzt, Beate Opelt, Andreas Jonke, Uwe Eckert, and Kurt Schuster for the preparation of the compounds, and we thank Christl Roos and Herbert Ziegler for performing pharmacological tests.

Supporting Information Available: Spectral data, elemental analysis results, and melting points for compounds **11**−**61**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Schreiber, R.; De Vry, J. 5-HT_{1A} Receptor Ligands in Animal Models of Anxiety, Impulsivity and Depression: Multiple Mechanisms of Action? *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1993**, *17*, 87−104. (b) Murasaki, M.; Miura, S. The Future of 5-HT Receptor Agonists (Aryl-Piperazine Derivatives). *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1992**, *16*, 833−845.

(2) (a) Lapierre, Y. D.; Silverstone, P.; Reesal, R. T.; Saxena, B.; Turner, P.; Bakish, D.; Plamondon, J.; Vincent, P. M.; Remick, R. A.; Kroft, C.; Payer, R.; Rosales, D.; Lam, R.; Bologa, M. A Canadian Mulitcenter Study of Three Fixed Doses of Controlled-Release Ipsapirone in Outpatiants with Moderate to Severe Major Depression. *J. Clin. Psychopharmacol.* **1998**, *18*, 268−273. (b) Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Tortorella, V.; Fornaretto, M. G.; Caccia, C.: McArthur, R. A. Structure−Activity Relationship Studies on the 5-HT_{1A} Receptor Affinity of 1-Phenyl-4-[ω-(α- or β-tetralinyl)alkyl]piperazines. *J. Med. Chem.* **1996**, *39*, 4928−4934.

(3) Koek, W.; Patoiseau, J.-F.; Assie, M.-B.; Cosi, C.; Kleven, M. S. F 11440, a Potent, Selective, High Efficacy 5-HT_{1A} Receptor Agonist with Marked Anxiolytic and Antidepressant Potential. *J. Pharmacol. Exp. Ther.* **1998**, *287*, 266−283.

(4) (a) Spinks, D.; Spinks, G. Serotonin Re-Uptake Inhibition: An Update on Current Research Strategies. *Curr. Med. Chem.* **2002**, *9*, 799−810. (b) Pacher, P.; Kohegyi, E.; Kecskemeti, V.; Furst, S. Current Trends in the Development of New Antidepressants. *Curr. Med. Chem.* **2001**, *8*, 89−100. (c) Schechter, L. E.; McGonigle, P.; Barrett, J. E. Serotonergic Antidepressants: Current and Future Perspectives. *Curr. Opin. Cent. Peripher. Nerv. Syst. Invest. Drugs* **1999**, *1*, 432−447. (d) Evrard, D. A.; Harrison, B. L. Recent Approaches to Noval Antidepressant Therapy. *Annu. Rep. Med. Chem.* **1999**, *34*, 1−10. (e) Arborelius, L. 5-HT_{1A} Receptor Antagonists as Putative Adjuvants to Antidepressants: Clinical and Preclinical Evidence. *IDrugs* **1999**, *2*, 121−128.

(5) (a) Takeuchi, K.; Kohn, T. J.; Honigschmidt, N. A.; Rocco, V. P.; Spinazze, P. G.; Koch, D. J.; Nelson, D. L.; Wainbscott, D. B.; Ahmad, L. J.; Shaw, J.; Threlkeld, P. G.; Wong, D. T. Advances toward New Antidepressants beyond SSRIs: 1-Aryloxy-3-piperidinylpropan-2-ols with Dual 5-HT_{1A} Receptor Antagonism/SSRI Activities. Part 1. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1903−1905. (b) Martinez, J.; Perez, S.; Oficialdegui, A. M.; Heras, B.; Orus, L.; Villanueva, H.; Palop, J. A.; Roca, J.; Mourelle, M.; Bosch, A.; DelCastillo, J. C.; Lasheras, B.; Tordera, R.; Del Rio, J.; Monge, A. New 3-[4-(Aryl)piperazin-1-yl]-1-(benzo[*b*]thiophen-3-yl)propane Derivatives with Dual Action at 5-HT_{1A} Serotonin Receptors and Serotonin Transporter as a New Class of Antidepressants. *Eur. J. Med. Chem.* **2001**, *36*, 55−61. (c) Martinez-Esparza, J.; Oficialdegui, A.-M.; Perez-Silanes, S.; Heras, B.; Orus, L.; Palop, J.-A.; Lasheras, B.; Roca, J.; Mourelle, M.; Bosch, A.; Del Castillo, J.-C.; Tordera, R.; Del Rio, J.; Monge, A. New 1-Aryl-3-(4-arylpiperazin-1-yl)propane Derivatives, with Dual Action at 5-HT_{1A} Serotonin Receptors and Serotonin Transporter, as a New Class of Antidepressants. *J. Med. Chem.* **2001**, *44*, 418−428. (d) Meagher, K. L.; Mewshaw, R. E.; Evrard, D. A.; Zhou, P.; Smith, D. L.; Scerni, R.; Spangler, T.; Abulhawa, S.; Shi, X.; Schechter, L. E.; Andree, T. H. Studies towards the Next Generation of Antidepressants. Part 1: Indolylcyclohexylamines as Potent Serotonin Reuptake Inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1885−1888. (e) Orús, L.; Perez-Silanes, S.; Oficialdegui, A.-M.; Martinez-Esparza, J.; Del Castillo, J.-C.; Mourelle, M.; Langer, T.; Guccione, S.; Donzella, G.; Krovat, E. M.; Poptodorov, K.; Lasheras, B.; Ballaz, S.; Hervias, I.; Tordera, R.; Del Rio, J.; Monge, A. Synthesis and Molecular Modeling of New 1-Aryl-3-[4-arylpiperazin-1-yl]-1-propane Derivatives with High Affinity at the Serotonin Transporter and at 5-HT_{1A} Receptors. *J. Med. Chem.* **2002**, *45*, 4128−4139. (f) Tordera, R. M.; Monge, A.; Del Río, J.; Lasheras, B. Antidepressant-like activity of VN2222, a serotonin reuptake inhibitor with high affinity at 5-HT_{1A} receptors. *Eur. J. Pharmacol.* **2002**, *442*, 63−71.

(6) Heinrich, T.; Böttcher, H.; Bartoszyk, G. D.; Greiner, H. E.; Seyfried, C. A.; van Amsterdam, C. Indolebutylamines as Selective 5-HT_{1A} Agonists. *J. Med. Chem.* **2004**, *47*, 4677−4683.

(7) (a) Böttcher, H.; Barnickel, G.; Hausberg, H.-H.; Haase, A. F.; Seyfried, C. A.; Eiermann, V. Synthesis and Dopaminergic Activity of Some 3-(1,2,3,6-Tetrahydro-1-pyridylalkyl)indoles. A Novel Conformational Model To Explain Structure−Activity Relationships. *J. Med. Chem.* **1992**, *35*, 4020−4026. (b) Seyfried, C. A.; Fuxe, K.; Wolf, H.-P.; Agnati, L. F. Demonstration of a New Type of Dopamine Receptor Agonist: An Indole-3-butylamine. Action at Intact Versus Supersensitive Dopamine Receptors in the Rat Forebrain. *Acta Physiol. Scand.* **1982**, *116*, 465−468. (c) Hausberg, H.-H.; Böttcher, H.; Fuchs, A.; Gottschlich, R.; Koppe, V.; Minck, K.-O.; Pötsch, E.; Saiko, O.; Seyfried, C. A. Indole-alkyl-piperidines, a New Class of Dopamine Agonists. *Acta Pharm. Suec. (Suppl.)* **1983**, *2*, 213-217.

(8) Seyfried, C. A.; Böttcher, H. Central D_2-Autoreceptor Agonists, with Special Reference to Indol-butyl-amines. *Drugs Future* **1990**, *15*, 819−832.

(9) Oh, S. J.; Ha, H.-J.; Chi, D. Y.; Lee, H. K. Serotonin Receptor and Transporter Ligands−Current Status. *Curr. Med. Chem.* **2001**, *8*, 999−1034.

(10) Clark, R. D.; Repke, D. B. Some Observations on the Formation of Hydroxy-Indoles in the Leimgruber−Batcho Indole Synthesis. *J. Heterocycl. Chem.* **1985**, *22*, 121−125.

(11) Egawa, H.; Kataoka, M.; Shibamori, K.-i.; Miyamoto, T.; Nakano, J.; Matsumoto, J.-i. Pyridonecarboxylic Acid Antibacterial Agents. Part 7. A New Synthetic Route to 7-Halo-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-caroxylic Acid, an Intermediate for the Synthesis of Quinolone Antibacterial Agents. *J. Heterocycl. Chem.* **1987**, *24*, 181−185.

(12) Prelog, V.; Driza, G. J. Bis(β-haloethyl)amines III. *N*-Phenyl-Piperazines. *Collect. Czech. Chem. Commun.* **1933**, *5*, 497−502.

(13) Mukaiyama, T. New Synthetic Methods. 29. New Syntheses with Onium Salts of Azaarenes. *Angew. Chem.* **1979**, *91*, 798−812 [*IE* **1979**, *18*, 707−721].

(14) Matzen, L.; van Amsterdam, C.; Rautenberg, W.; Greiner, H. E.; Harting, J.; Seyfried, C. A.; Böttcher, H. 5-HT Reuptake Inhibitors with 5-$HT_{1B/1D}$ Antagonistic Activity: A New Approach toward Efficient Antidepressants. *J. Med. Chem.* **2000**, *43*, 1149−1157.

(15) Urwyler, S.; Markstein, R. Identification of Dopamine D_3 and D_4 Binding Sites Labeled with [^{3}H] 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene as High Agonist Affinity States of the D_1 and D_2 Dopamine Receptors, Respectively. *J. Neurochem.* **1986**, *46*, 1058−1067.

(16) Crees, I.; Schneider, R.; Snyder, S. H. ^{3}H-Spiroperidol Labels Dopamine Receptors in Pituitary and Brain. *Eur. J. Pharmacol.* **1977**, *46*, 377−381.

(17) Wong, D. T.; Bymaster, F. P.; Mayle, D. A.; Reid, L. R.; Krushinski, J. H. Robertson, D. W. LY248686, a New Inhibitor of Serotonin and Norephedrine Uptake. *Neuropsychopharmacology* **1993**, *8*, 23−33.

(18) Fuller, R. W.; Snoddy, H. D.; Snoddy, A. M.; Hemrick, S. K.; Wong, D. T.; Molloy, B. B. *p*-Iodoamphetamine as a Serotonin Depletor in Rats. *J. Pharmacol. Exp. Ther.* **1980**, *212*, 115−119.

(19) Newman-Tancredi, A.; Chaput, C.; Verrièle, L.; Millan, M. J. S15535 and WAY100,635 Antagonize 5-HT-Stimulated [^{35}S] GTPγS Binding at Cloned Human 5-HT_{1A} Receptors. *Eur. J. Pharmacol.* **1996**, *307*, 107−111.

(20) Bartoszyk, G. D.; Hegenbart, R.; Ziegler, H. EMD 68843, a Serotonin Reuptake Inhibitor with Selective Presynaptic 5-HT_{1A} Receptor Agonistic Properties. *Eur. J. Pharmacol.* **1997**, *322*, 147−153.

(21) De Vry, J.; Benz, U.; Schreiber, R.; Traber, J. Shock-Induced Ultrasonic Vocalization in Young Adult Rats: A Model for Testing Putative Anti-Anxiety Drugs. *Eur. J. Pharmacol.* **1993**, *249*, 331−339.

(22) Protais, P.; Costentin. J.; Schwartz, J. C. Climbing Behaviour Induced by Apomorphine in Mice, a Simple Test for the Study of Dopamine Receptors in Striatum. *Psychopharmacology* **1976**, *50*, 1−6.

(23) Bartoszyk, G. D.; Harting, J.; Minck, K.-O. Roxindole: Psychopharmacological Profile of a Dopamine D_2 Autoreceptor Agonist. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 41−48.

(24) (a) Graham, J. E.; Ripley, D. C.; Smith, J. T.; Vedene, H., Jr.; Weaver, D. F. Theoretical Studies Applied to Drug Design. Ab Initio Electronic Distributions in Bioisosters. *THEOCHEM* **1995**, *343*, 105−109. (b) Parr, R. G.; Pearson, R. G. Absolute Hardness: Companion Parameter to Absolute Electronegativity. *J. Am. Chem. Soc.* **1983**, *105*, 7512−7516. (c) Thornber, C. W. Isosterism and Molecular Modification in Drug Design. *Chem Soc. Rev.* **1979**, *8*, 563−580.

(25) Stewart, J. PM3 calculation. *Mopac*, version 6.00 (*QCPE* no. 455) distributed with *Sybyl*, version 6.8 (Tripos Associates).
(26) (a) Chidester, C. G.; Lin, C.-H.; Lahti, R. A.; Haadsma-Svensson, S. R.; Smith, M. W. Comparison of 5-HT_{1A} and Dopamine D_2 Pharmacophores. X-Ray Structures and Affinities of Conformationally Constrained Ligands. *J. Med. Chem.* **1993**, *36*, 1301−1315. (b) Homan, E. J.; Wikström, H. V.; Grol, C. J. Molecular Modeling of the Dopamine D_2 and Serotonin 5-HT_{1A} Receptor Binding Modes of the Enantiomers of 5-OMe-DPAT. *Bioorg. Med. Chem. Lett.* **1999**, *7*, 1805−1820.
(27) Bartoszyk, G. D.; Barber, A.; Böttcher, H.; Greiner, H. E.; Leibrock, J.; Martinez, J. M.; Seyfried, C. A. *Soc. Neurosci. Abstr.* **1996**, *22*, 613.
(28) Page, M. E.; Cryan, J. F.; Sullivan, A.; Dalvi, A.; Saucy, B.; Manning, D. R.; Lucki, I. Behavioral and Neurochemical Effects of 5-{4-[4-(5-Cyano-3-indolyl)-butyl)-butyl]-1-piperazinyl}-benzofuran-2-carboxamide (EMD 68843): A Combined Selective Inhibitor of Serotonin Reuptake and 5-Hydroxytryptamine 1A Receptor Partial Agonist. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 1220−1228.
(29) Nishiyama, M.; Yamamoto, T.; Koie, Y. Synthesis of *N*-Arylpiperazines from Aryl Halides and Piperazine under a Palladium Tri-*tert*-butylphosphine Catalyst. *Tetrahedron Lett.* **1998**, *39*, 617−620.
(30) Cossery, J. M.; Gozlan, H.; Spampinato, U.; Perdicakis, C.; Guillaumet, G.; Pichat, L.; Hamon, M. The Selective Labeling of Central 5-HT_{1A} Receptor Binding Sites by [^{3}H]5-Methoxy-3-(di-*n*-propylamino)chroman. *Eur. J. Pharmacol.* **1987**, *140*, 143−155.
(31) Whittaker, V. P. The synaptosome. In *Handbook of Neurochemistry*; Laiths, A., Ed.; Plenum Press: London and New York, 1969; pp 327−364.
(32) Seyfried, C. A.; Greiner, H. E.; Haase, A. F. Biochemical and Functional Studies on EMD 49980: A Potent, Selectively Presynaptic D-2 Dopamine Agonist with Actions on Serotonin Systems. *Eur. J. Pharmacol.* **1989**, *160*, 31−41.
(33) Glowinski, J.; Iversen, L. L. Catechol Amines in Rat Brain. I. Disposition of Norepinephrine-[^{3}H], Dopamine-[^{3}H] and Dopa-[^{3}H] in Various Regions of the Brain. *J. Neurochem.* **1966**, *13*, 655−669.

JM040793Q