Brief Articles

Cyanoindole Derivatives as Highly Selective Dopamine D_4 Receptor Partial Agonists: Solid-Phase Synthesis, Binding Assays, and Functional Experiments

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Traceless linking of diethoxymethyl (DEM)-protected 5- and 6-cyanoindoles and subsequent incorporation of phenylpiperazine derivatives led to the 2- and 3-piperazinylmethyl-substituted cyanoindoles **3a**−**m**. Dopamine receptor binding studies on the final products **3a**−**m** clearly indicated strong and selective recognition of the D_4 subtype which is known as a promising target for the treatment of neuropsychiatric disorders. The most interesting binding properties were observed for the 2-aminomethyl-5-cyanoindoles FAUC 299 (**3f**) and FAUC 316 (**3j**) (K_i = 0.52 and 1.0 nM, respectively) when the fluoro derivative **3j** proved extraordinary selectivity over D_1, D_{2long}, D_{2short}, and D_3 (>8600). To determine ligand efficacy, mitogenesis experiments were performed indicating partial agonist effects for the test compounds **3f**,**j** (35% and 30%, when compared to the full agonist quinpirole).

Introduction

Recent advances in molecular cloning techniques have led to the characterization of a number of dopamine receptor subtypes which can be divided into the D_1-like and D_2-like families.[1] Whereas the D_1-like family comprises D_1 and D_5 subtypes, the D_2-like family consists of D_2, D_3, and D_4 receptors.[2] Due to the preferred expression of messenger RNA for dopamine D_4 receptors in the frontal cortical and mesolimbic areas and the finding that the neuroleptic drug clozapine binds preferentially to D_4 receptors,[3] considerable interest has been focused on selective D_4 antagonists.[4] Furthermore, associations are emerging between D_4 receptors and specific personality traits such as novelty-seeking.[5] According to very recent neuropathological and genetic studies,[6] selective dopamine D_4 receptor agonists, partial agonists, or antagonists might be of interest for the treatment of neuropsychiatric disorders including attention-deficit hyperactivity, mood disorders, and Parkinson's disease.

Thus, SAR studies with respect to D_4 affinity, selectivity, and ligand efficacy are of special current interest. On the basis of our previous comparisons of molecular electrostatic potential maps, we recognized that the D_4 preference of test compounds of type **1** strongly depends on a large negative region which is obviously not tolerated by D_1, D_2, and D_3 binding sites.[7] Thus, the substantially higher selectivity of the 7-azaindole L-745,-870[8] and the 3a- and 7a-isomers compared to indole analogues can be explained. Employing a 2,2-dicyanovinyl moiety as a nonaromatic bioisostere for the six-membered aromatic subunit of the lead structure **1**, we have recently developed strong D_4 receptor ligands of type **2** (Chart 1).[9] The high affinity of the test compounds (K_i values: 3.9−11 nM) preferentially adopting *s-trans* geometry can be explained by the structural similarity of the *cis*-cyanovinyl subunit to positions 4−7 of the lead structure **1**. On the other hand, we expected the negative region induced by a *trans*-positioned cyano group to be responsible for the high subtype selectivity. As a consequence, cyanoindole derivatives of type **3** were selected as promising candidates being capable of strong and selective dopamine D_4 receptor recognition.

In this paper, we report the synthesis of the target compounds **3** involving an application of our solid-phase methodology for the traceless linking of indoles.[10] The results of dopamine receptor binding studies considering the subtypes D_1, D_{2long}, D_{2short}, D_3, and D_4 as well as functional experiments to determine ligand efficacy are presented.

Results and Discussion

The solid-phase synthesis of the target compounds **3a**−**e** was started from polymer-bound indole **5**, which was prepared according to our recently described methodology for traceless linking of indoles.[10] Conversion of the immobilized indole **5** into the 3-substituted aminomethyl derivatives **6a**−**e** was accomplished under Mannich conditions (aqueous formaldehyde, CH_2Cl_2, and HOAc) employing a series of five representative 1-phenylpiperazines (Scheme 1). Subsequent hydrolysis of the acetal linkage and release of the piperazinylmethylindoles **3a**−**e** was accomplished in the presence of 1,4-dioxane and 2 N HCl, followed by treatment with 2 N NaOH. Applying this reaction sequence, the final products **3b**−**e** were obtained in good yields, and the purity of the cleaved products, verified by ^{1}H NMR, was found to be in the range of 81−98%. On the other hand, yield and purity of the phenylpiperazine **3a** were only

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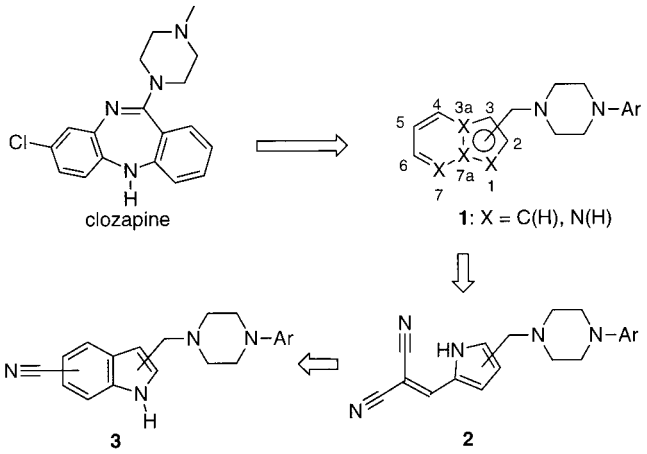
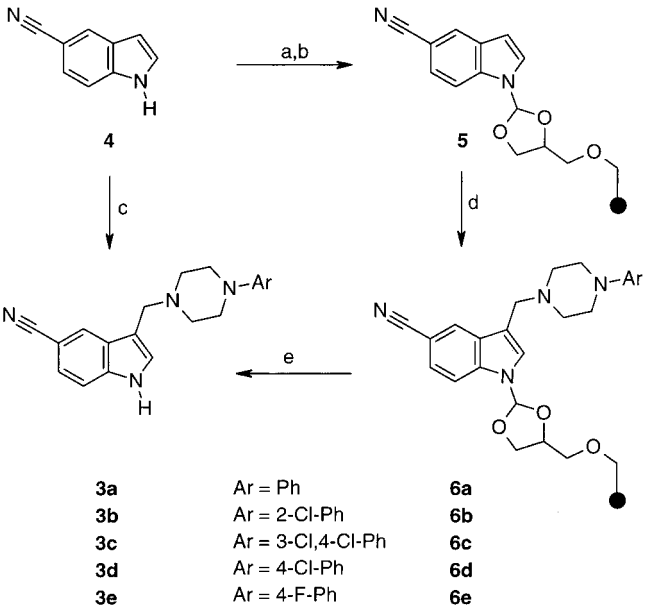
Chart 1

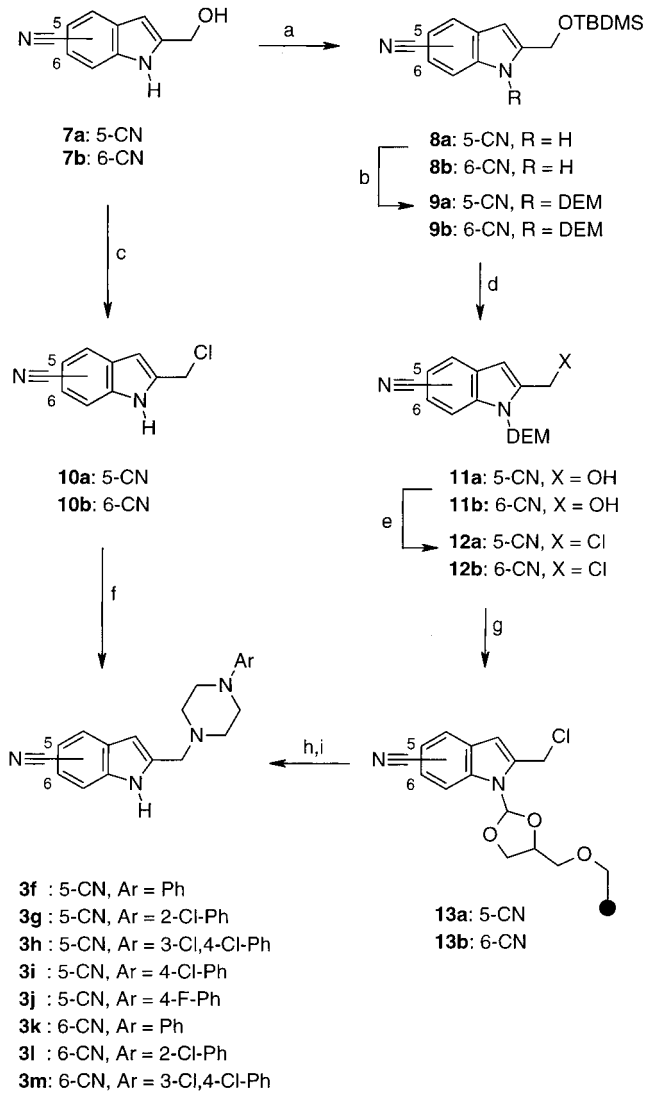


Scheme 1[a]



[a] Reagents and conditions: (a) $HC(OEt)_3$, 160 °C, 24 h;[10] (b) polymer-bound 3-benzyloxypropane-1,2-diol,[12] 1,4-dioxane, *p*-TosOH, rt, 3 h; (c) HNR_2, aq CH_2O, CH_2Cl_2/HOAc (4:1), rt, 0.5−6 h; (d) HNR_2, aq CH_2O, CH_2Cl_2/HOAc (1:4), 40 °C, 64 h; (e) 1. 1,4-dioxane/2 N HCl (1:1), 40 °C, 3 h, 2. 2 N NaOH, rt, 0.5 h.

Scheme 2[a]



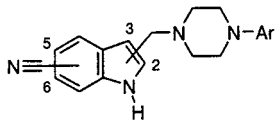
[a] Reagents and conditions: (a) TBDMSCl, imidazole, DMF, 0 °C, 0.5 h; (b) $HC(OEt)_3$, 160 °C, 16−24 h; (c) CCl_4, PPh_3, DMF, rt, 16 h; (d) Bu_4NF, THF, 0 °C, 0.5 h; (e) CCl_4, PPh_3, DMF, rt, 0.5 h; (f) HNR_2, DMF, 60 °C, 2 h; (g) polymer-bound 3-benzyloxypropane-1,2-diol,[12] 1,4-dioxane, *p*-TosOH, rt, 3 h; (h) HNR_2, DMF, 40 °C, 48 h; (i) 1. 1,4-dioxane/2 N HCl (1:1), 40 °C, 3 h, 2. 2 N NaOH, rt, 0.5 h.

modest. Besides the solid-phase synthesis of aminomethylindoles of type **3**, allowing the generation of indole-based combinatorial libraries, we also performed a solution-phase synthesis when aminomethylation of **4** afforded the target compounds **3a**−**e**.

Having successfully used our traceless linking methodology for the synthesis of the 3-substituted compounds **3a**−**e**, we worked out a solid-phase synthetic route for the preparation of the 5- and 6-cyanoindole derivatives **3f**−**m**, bearing the respective phenylpiperazinylmethyl moiety in the indole 2-position (Scheme 2). Starting from the alcohols **7a,b**,[11] reaction with TBDMSCl and imidazole afforded the *O*-protected products **8a,b** in 92% yield which were subsequently heated in neat triethyl orthoformate to give the *N*-diethoxymethyl-substituted indoles **9a,b**. Fluoride-induced cleavage of the silyl ethers **9a,b** resulted in formation of the 2-hydroxymethylindoles **11a,b** that could be transformed into the chlorides **12a,b** upon treatment with CCl_4 and PPh_3. An alternative approach to the intermediates **12a,b** by diethoxymethylation of the indoles **10a,b**, which were readily available from the primary alcohols **7a,b**,[11] failed. The DEM (diethoxymethyl)-protected indoles **12a,b** were loaded onto the 1,2-diol functionalized Merrifield resin[12] to give the polymer-bound electrophiles **13a,b**. Treatment of **13a,b** with different 1-phenylpiperazines, followed by resin cleavage, afforded the target compounds **3f**−**m** in excellent yields (88−100%) and high purity (86−98%). A solution-phase synthesis of the final products **3f**−**m** by nucleophilic displacement reactions was performed based on the chlorides **10a,b**.

Receptor binding properties of the test compounds of type **3** were determined in vitro by measuring their ability to compete with [^{3}H]spiperone for the cloned human dopamine receptor subtypes D_{2long}, D_{2short},[13] D_3,[14] and $D_{4.4}$[15] stably expressed in Chinese hamster ovary cells (CHO).[16] D_1 affinities were assessed via competition experiments using bovine striatal membrane preparations and the D_1 selective radioligand [^{3}H]SCH 23390.

Table 1. Binding Data of the Cyanoindoles **3a**−**m** and Clozapine to Human and Bovine Dopamine Receptors[a]



	positions			K_i (nM) ± SEM				
				[^{3}H]SCH 23390	[^{3}H]spiperone			
compd	CN	$CH_2N<$	Ar	bovine D_1	human D_{2long}	human D_{2short}	human D_3	human $D_{4.4}$[b]
3a	5	3	Ph	3000 ± 750	34000 ± 6000	3500 ± 950	4400 ± 1200	50 ± 3.5 (n = −0.9)
3b	5	3	2-Cl-Ph	510 ± 50	1400 ± 570	540 ± 230	550 ± 47	17 ± 1.0 (n = −1.0)
3c	5	3	3,4-diCl-Ph	450 ± 85	790 ± 70	480 ± 45	870 ± 140	22 ± 3.5 (n = −1.1)
3d	5	3	4-Cl-Ph	550 ± 15	4300 ± 650	2600 ± 1100	6400 ± 250	29 ± 7.5 (n = −0.9)
3e	5	3	4-F-Ph	490 ± 35	2400 ± 150	1800 ± 50	5200 ± 250	110 ± 21 (n = −0.9)
3f	5	2	Ph	13000 ± 500	3100 ± 550	290 ± 30	1700 ± 220	0.52 ± 0.050 (n = −1.0)
3g	5	2	2-Cl-Ph	7500 ± 750	2300 ± 50	950 ± 30	1700 ± 600	1.4 ± 0.33 (n = −0.9)
3h	5	2	3,4-diCl-Ph	11000 ± 2700	43000 ± 21000	36000 ± 12000	20000 ± 5000	7.5 ± 2.0 (n = −1.3)
3i	5	2	4-Cl-Ph	5300 ± 100	7900 ± 2100	7600 ± 1800	13000 ± 1500	2.1 ± 0.29 (n = −1.0)
3j	5	2	4-F-Ph	8600 ± 2400	28000 ± 6500	19000 ± 4000	15000 ± 2000	1.0 ± 0.057 (n = −1.0)
3k	6	2	Ph	5300 ± 1700	5600 ± 2600	2500 ± 1200	3100 ± 650	3.6 ± 0.43 (n = −0.9)
3l	6	2	2-Cl-Ph	1900 ± 0.0	500 ± 10	220 ± 15	920 ± 45	3.4 ± 0.87 (n = −1.0)
3m	6	2	3,4-diCl-Ph	880 ± 65	2000 ± 100	1400 ± 400	33000 ± 8500	9.0 ± 1.2 (n = −1.2)
clozapine				420 ± 50	41 ± 1.5	28 ± 0.50	960 ± 45	16 ± 0.50 (n = −0.9)

[a] K_i values are the means of 2−4 independent experiments ± SEM using 8 different concentrations each in triplicate. [b] Steepness of the competition curves and the derived Hill slopes gave a one-site binding model. Unless mitogenesis experiments showed partial agonist effects for **3f**,**j** indicating that the K_i values actually represent $K_{0.5}$ values, high- and low-affinity binding sites could not be resolved using the methodology described.

The K_i values of the test compounds were compared to those of the atypical antipsychotic drug clozapine which is known for its D_4 preference. The characterization of the test compounds was initiated by investigating the 3-aminomethyl-substituted derivatives **3a**−**e** when the 4-chlorophenylpiperazine **3d** showed the highest degree of similarity to the most potent derivatives of the lead compounds of type **1**. The binding data depicted in Table 1 indicate substantial (K_i = 29 nM) and selective D_4 recognition for **3d**. Exchange of the 4-chlorophenyl group by phenyl, 2-chlorophenyl, 3,4-dichlorophenyl, and 4-fluorophenyl resulting in analogues **3a**−**c**,**e** led only to a slight improvement of the binding affinities. Interestingly, the test compounds **3b**,**c** showed binding data for the D_1, D_3, and D_4 subtypes that are comparable to that for clozapine; however, higher selectivity over the D_2 isoforms was determined. To improve the D_4 potency, we modified the relative topicity of the cyano and aminomethyl substituents. In contrast to recent SAR studies with azaindoles, describing loss of affinity after modification of the attachment position,[17] strong enhancement of D_4 binding was observed. Thus, the (di)-chlorophenyl derivatives **3g**−**i** showed 3−14-fold higher potency when compared with the respective regioisomers **3b**−**d**. Interestingly, the improvement was even greater for the phenylpiperazine **3f** and the 4-fluoro derivative **3j** (96- and 110-fold based on **3a**,**e**, respectively). The K_i values determined for **3f** clearly demonstrate extraordinarily high D_4 affinity (K_i = 0.52 nM) and a 25000-, 6000-, 550-, and 3300-fold selectivity. It is worthy to note that the phenylpiperazines **3a**,**f** gave an approximately 10-fold difference between the affinities toward the isoforms D_{2long} and D_{2short} that we had not noticed before in the literature and within the data of our test compound library. Recent SAR studies with 3-[(*N*-phenylpiperazinyl)methyl]azaindoles indicated that 4-chloro or 4-iodo substituent exerts an enhancement of D_4 potency and selectivity. In this case, only the selectivity (especially over D_{2short} and D_3) is positively influenced by the electronegative chloro function. The reduced affinity of the chloro derivatives might be explained by steric interactions preventing a perfect fit of the 2-aminomethyl derivatives. As a consequence, a fluoro group combining highly electronegative properties and minimal steric demand was expected to be the optimal substituent. In practice, the 4-fluorophenylpiperazine **3j** turned out to represent both high D_4 receptor affinity (1.0 nM) and selectivity (>8600). Finally, the 6-cyanoindoles **3k**−**m** were evaluated revealing K_i values of 3.6, 3.4, and 9.0 nM at the D_4 subtype and moderate to strong selectivity over D_1−D_3.

Agonist activation of dopamine receptors is known to increase mitogenesis in heterologously transfected cell lines.[18] This stimulating effect can be determined by measuring the rate of [^{3}H]thymidine incorporation into growing cells. The functional experiment can be quantified by determination of the effective test compound concentration (EC_{50}) and by comparing the stimulating effect to the [^{3}H]thymidine incorporation that is caused by a full agonist.

To investigate the intrinsic effects of **3f** (FAUC 299) and **3j** (FAUC 316), which emphasized the most promising D_4 ligands in the series of tested cyanoindoles, CHO10001 cells stably expressing the human $D_{4.2}$ receptor were established for a mitogenesis assay.[19] Figure 1 shows the dose−response curves of **3f**,**j** indicating a partial agonist effect for both cyanoindoles with an efficacy of about one-third (35% for **3f** and 30% for **3j**) of the effect of quinpirole, which is known as a full agonist. Very recently, partial agonist effects were also reported for the D_4 ligands L-745,870 (3-[4-(4-chlorophenyl)piperazin-1-ylmethyl]-1*H*-pyrrolo[2,3-*b*]pyridine), U-101958 ((1-benzylpiperidin-4-yl)(3-isopropoxypyridin-2-yl)methylamine), and NGD 94-1 (2-[4-(2-phenyl-3-imidazol-4-ylmethyl)piperazin-1-yl]pyrimidine).[20−22] The EC_{50} values for **3f**,**j** (Table 2), determined to be 1.5 and 9.4 nM, respectively, demonstrated a good correlation to the corresponding K_i values derived from receptor binding experiments (see Table 1). It is interesting to note that the steepness of the competition curves and

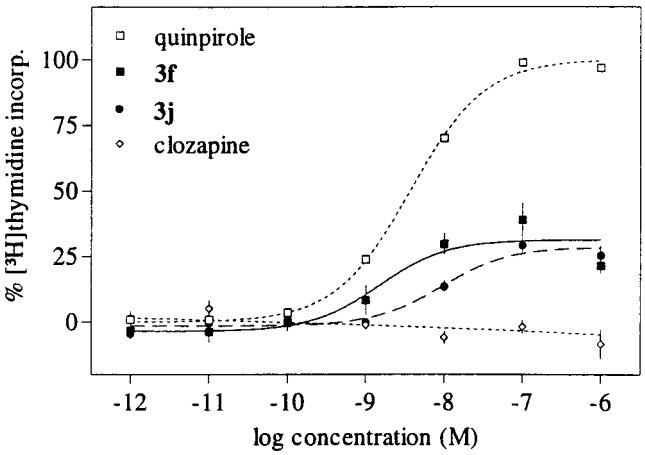


Figure 1. Stimulation of mitogenesis as a functional assay to assess the agonist effects of **3f**,**j** at the human dopamine $D_{4.2}$ receptor in relation to the full agonist quinpirole and the antagonist clozapine.

Table 2. Agonist Effects of the Cyanoindoles **3f**,**j**, Quinpirole, and Clozapine at the Dopamine $D_{4.2}$ Receptor Investigated by Measuring the Stimulation of Mitogenesis

	test compounds			
	3f	**3j**	quinpirole	clozapine
agonist effect (%)[a]	35	30	100	0
EC_{50} (nM)[b]	1.5	9.4	3.7	nd

[a] Rate of incorporation of [^{3}H]thymidine (in %) as evidence for mitogenetic activity relative to the maximal effect of the full agonist quinpirole (100%); the results are the means of quadruplicates from 6 experiments. [b] EC_{50} values derived from the mean curves of 6 experiments; nd, not determined.

the derived Hill slopes within the receptor binding studies gave a one-site binding model. Unless mitogenesis experiments showed partial agonist effects for **3f**,**j** indicating that the K_i values actually represent $K_{0.5}$ values, high- and low-affinity binding sites could not be identified using the methodology described.

In conclusion, it could be demonstrated that the traceless linking methodology of indoles, which was recently established in our laboratory, can be successfully applied for SAR studies and the synthesis of potential drug candidates. Biological investigation of a representative library of 13 indole derivatives showed highly potent and selective dopamine D_4 receptor binding profiles, when positions 2 and 5 proved highly suitable attachment positions for the aminomethyl and cyano groups, respectively. Ligand efficacy of the test compounds FAUC 299 (**3f**) and FAUC 316 (**3j**) was discovered by mitogenesis experiments. Selective D_4 partial agonists could serve as an interesting tool for the treatment of neuropsychiatric disorders such as attention-deficit hyperactivity.

Experimental Section

Solvents were purified and dried by standard procedures. If not otherwise stated reactions were performed under dry N_2. Melting points: Büchi melting point apparatus, uncorrected. IR: Jasco FT/IR 410. MS and HRMS were run on Finnigan MAT TSQ 70 and 8200 spectrometers, respectively, by EI (70 eV) with solid inlet. ^{1}H NMR spectra were obtained on a Bruker AM 360 (360 MHz) spectrometer, if not otherwise stated in $CDCl_3$ relative to TMS. Purity of the compounds prepared on solid phase was determined by ^{1}H NMR analysis of the crude product. Yields of solid-phase products **3a**−**m** are based on a loading of 0.72 mmol/g for resin **5**[10] and 0.31 mmol/g for resins **13a,b**. Chromatographic purification was performed using silica gel 60 (Merck).

3-(4-Phenylpiperazin-1-ylmethyl)indole-5-carbonitrile (3a). Method A: Resin **5** (188 mg, 0.72 mmol/g) was suspended in HOAc (4 mL) and CH_2Cl_2 (1 mL), treated with formaldehyde (0.6 mL, 37% in H_2O) and 1-phenylpiperazine (224 mg, 1.4 mmol) and stirred for 64 h at 40 °C. Then, the resin was filtered off, washed with EtOH−2 N NaOH, EtOH, CH_2Cl_2 and Et_2O and dried in vacuo to give 174 mg of **6a**. For hydrolysis, resin **6a** (172 mg) was suspended in a mixture of 1,4-dioxane (5 mL) and 2 N HCl (5 mL) and stirred at 40 °C for 3 h, followed by addition of 2 N NaOH (10 mL) and stirring at room temperature for 0.5 h. The resin was filtered off and washed with EtOAc. The organic layer was dried (Na_2SO_4) and evaporated to give crude **3a** (19 mg, 44%; purity: 45%).

Method B: To a solution of **4** (149 mg, 1 mmol) in CH_2Cl_2 (4 mL) and HOAc (1 mL) were added 1-phenylpiperazine (162 mg, 1 mmol) and formaldehyde (0.1 mL, 37% in H_2O). After being stirred at room temperature for 6 h the solution was basified with 2 N NaOH and extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (CH_2Cl_2−MeOH 95:5) to give **3a** (166 mg, 50%) as a colorless solid: mp 212 °C; IR 3320, 2815, 2220, 1600, 800, 760 cm^{-1}; ^{1}H NMR δ 2.61−2.70 (m, 4H), 3.18−3.26 (m, 4H), 3.76 (s, 2H), 6.85 (t, 1H, J = 7.2 Hz), 6.89−6.96 (m, 2H), 7.21−7.30 (m, 3H), 7.38−7.46 (m, 2H), 8.18 (s, 1H), 8.39 (br s, 1H); EIMS 316 (M^+). Anal. ($C_{20}H_{20}N_4$) C,H,N.

3-[4-(2-Chlorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3b). 5 (210 mg, 0.72 mmol/g), formaldehyde (0.6 mL, 37% in H_2O) and 1-(2-chlorophenyl)piperazine (297 mg, 1.5 mmol) were reacted and worked up as described for **6a** (method A) to give **6b** (190 mg). Hydrolysis of **6b** (160 mg) was performed as described for **3a** to give crude **3b** (20 mg, 45%; purity: 94%). In analogy to **3a**, a solution-phase synthesis was also performed (see method B) to give **3b** (51%) as a colorless solid: mp 193 °C; IR 2925, 2850, 1460, 750, 700 cm^{-1}; ^{1}H NMR δ 2.61−2.79 (m, 4H), 3.05−3.19 (m, 4H), 3.76 (s, 2H), 6.96 (td, 1H, J = 7.8, 1.5), 7.06 (dd, 1H, J = 7.8, 1.5), 7.22 (td, 1H, J = 7.8, 1.5), 7.25−7.28 (m, 1H), 7.35 (dd, 1H, J = 7.8, 1.5), 7.39−7.47 (m, 2H), 8.20 (s, 1H), 8.40 (br s, 1H); EIMS 352 (M^+), 350 (M^+). Anal. ($C_{20}H_{19}N_4Cl$) C,H,N.

3-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3c). 5 (220 mg, 0.72 mmol/g), formaldehyde (0.6 mL, 37% in H_2O) and 1-(3,4-dichlorophenyl)piperazine (405 mg, 1.7 mmol) were reacted and worked up as described for **6a** (method A) to give **6c** (205 mg). Hydrolysis of **6c** (205 mg) was performed as described for **3a** to give crude **3c** (40 mg, 66%; purity: 98%). In analogy to **3a**, a solution-phase synthesis was also performed (see method B) to give **3c** (76%) as a colorless solid: mp 174 °C; IR 3320, 2825, 2220, 1480 cm^{-1}; ^{1}H NMR δ 2.58−2.64 (m, 4H), 3.14−3.20 (m, 4H), 3.75 (s, 2H), 6.72 (dd, 1H, J = 8.9, 2.7 Hz), 6.95 (d, 1H, J = 2.7 Hz), 7.23−7.28 (m, 2H), 7.42−7.46 (m, 2H), 8.18 (s, 1H), 8.38 (br s, 1H); EIMS 388 (M^+), 386 (M^+), 384 (M^+). Anal. ($C_{20}H_{18}N_4Cl_2$) C,H,N.

3-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3d). 5 (185 mg, 0.72 mmol/g), formaldehyde (0.6 mL, 37% in H_2O) and 1-(4-chlorophenyl)piperazine (297 mg, 1.5 mmol) were reacted and worked up as described for **6a** (method A) to give **6d** (170 mg). Hydrolysis of **6d** (170 mg) was performed as described for **3a** to give crude **3d** (27 mg, 55%; purity: 93%). In analogy to **3a**, a solution-phase synthesis was also performed (see method B) to give **3d** (52%) as a colorless solid: mp 189 °C; IR 3320, 2820, 2220, 1495, 1240 cm^{-1}; ^{1}H NMR δ 2.60−2.67 (m, 4H), 3.13−3.20 (m, 4H), 3.75 (s, 2H), 6.80−6.86 (m, 2H), 7.16−7.22 (m, 2H), 7.27 (s, 1H), 7.39−7.46 (m, 2H), 8.17−8.19 (m, 1H), 8.42 (br s, 1H); EIMS 352 (M^+), 350 (M^+). Anal. ($C_{20}H_{19}N_4Cl$) C,H,N.

3-[4-(4-Fluorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3e). 5 (180 mg, 0.72 mmol/g), formaldehyde (0.6 mL, 37% in H_2O) and 1-(4-fluorophenyl)piperazine (270 mg, 1.4 mmol) were reacted and worked up as described for **6a** (method A) to give **6e** (167 mg). Hydrolysis of **6e** (167 mg) was

performed as described for **3a** to give crude **3e** (26 mg, 60%; purity: 81%). In analogy to **3a**, a solution-phase synthesis was also performed (see method B) to give **3e** (48%) as a colorless solid: mp 179 °C; IR 3320, 2820, 2220, 1510, 1235 cm^{-1}; ^1H NMR δ 2.61−2.69 (m, 4H), 3.09−3.16 (m, 4H), 3.76 (s, 2H), 6.84−6.90 (m, 2H), 6.91−6.98 (m, 2H), 7.27 (s, 1H), 7.38−7.46 (m, 2H), 8.19 (s, 1H), 8.39 (br s, 1H); EIMS 334 (M^+). Anal. ($C_{20}H_{19}N_4F$) C,H,N.

2-(4-Phenylpiperazin-1-ylmethyl)indole-5-carbonitrile (3f). Method A: Resin **13a** (70 mg, 0.31 mmol/g) was suspended in DMF (2 mL), treated with 1-phenylpiperazine (113 mg, 0.7 mmol) and stirred for 48 h at 40 °C. Then, the resin was filtered off and washed with EtOH−2 N NaOH, EtOH, CH_2Cl_2 and Et_2O. The resulting resin was suspended in a mixture of 1,4-dioxane (5 mL) and 2 N HCl (5 mL) and stirred at 40 °C for 3 h, followed by addition of 2 N NaOH (10 mL) and stirring at room temperature for 0.5 h. The resin was filtered off and washed with EtOAc. The organic layer was dried (Na_2SO_4) and evaporated to give crude **3f** (6 mg, 88%; purity: 96%).

Method B: To a solution of **10a** (48 mg, 0.25 mmol) in DMF (1 mL) was added 1-phenylpiperazine (81 mg, 0.50 mmol). After being stirred at 60 °C for 2 h, 2 N NaOH, water and EtOAc were added. The organic layer was washed with water, dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (EtOAc) to give **3f** (72 mg, 90%) as a colorless solid: mp 208 °C; IR 3345, 2830, 2220, 1600,1320, 1230 cm^{-1}; ^1H NMR δ 2.63−2.69 (m, 4H), 3.19−3.25 (m, 4H), 3.74 (s, 2H), 6.46 (d, 1H, J = 1.7 Hz), 6.84−6.96 (m, 3H), 7.23−7.31 (m, 2H), 7.38−7.41 (m, 2H), 7.89−7.91 (m, 1H), 8.91 (br s, 1H); EIMS 316 (M^+). Anal. ($C_{20}H_{20}N_4$) C,H,N.

2-[4-(2-Chlorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3g). 13a (70 mg, 0.31 mmol/g) and 1-(2-chlorophenyl)piperazine (139 mg, 0.7 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3g** (7 mg, 92%; purity: 96%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3g** (89%) as a colorless solid: mp 179 °C; IR 3420, 2820, 2220, 1480, 750 cm^{-1}; ^1H NMR δ 2.63−2.74 (m, 4H), 3.09−3.15 (m, 4H), 3.74 (s, 2H), 6.44−6.47 (m, 1H), 6.98 (td, 1H, J = 7.9, 1.4 Hz), 7.04 (dd, 1H, J = 7.9, 1.4), 7.22 (td, 1H, J = 7.9, 1.4), 7.36 (dd, 1H, J = 7.9, 1.4), 7.38−7.41 (m, 2H), 7.90 (s, 1H), 8.93 (br s, 1H); EIMS 352 (M^+), 350 (M^+). Anal. ($C_{20}H_{19}N_4Cl$) C,H,N.

2-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3h). 13a (160 mg, 0.31 mmol/g) and 1-(3,4-dichlorophenyl)piperazine (340 mg, 1.4 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3h** (19 mg, 99%; purity: 98%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3h** (93%) as a colorless solid: mp 178 °C; IR 3330, 2825, 2220, 1480 cm^{-1}; ^1H NMR δ 2.61−2.66 (m, 4H), 3.16−3.21 (m, 4H), 3.74 (s, 2H), 6.46 (d, 1H, J = 1.7 Hz), 6.73 (dd, 1H, J = 8.7, 2.7), 6.95 (d, 1H, J = 2.7), 7.27 (d, 1H, J = 8.7), 7.38−7.41 (m, 2H), 7.89−7.91 (m, 1H), 8.81 (br s, 1H); EIMS 388 (M^+), 386 (M^+), 384 (M^+). Anal. ($C_{20}H_{18}N_4Cl_2$) C,H,N.

2-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3i). 13a (59 mg, 0.31 mmol/g) and 1-(4-chlorophenyl)piperazine (119 mg, 0.6 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3i** (6 mg, 93%; purity: 86%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3i** (81%) as a colorless solid: mp 174 °C; IR 3360, 2820, 2220, 1600, 1500 cm^{-1}; ^1H NMR δ 2.61−2.67 (m, 4H), 3.14−3.21 (m, 4H), 3.74 (s, 2H), 6.55 (d, 1H, J = 1.7), 6.80−6.86 (m, 2H),7.17−7.22 (m, 2H), 7.38−7.40 (m, 2H), 7.88−7.90 (m, 1H), 8.92 (br s, 1H); EIMS 352 (M^+), 350 (M^+). Anal. ($C_{20}H_{19}N_4Cl$) C,H,N.

2-[4-(4-Fluorophenyl)piperazin-1-ylmethyl]indole-5-carbonitrile (3j). 13a (60 mg, 0.31 mmol/g) and 1-(4-fluorophenyl)piperazine (108 mg, 0.6 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3j** (6 mg, 96%; purity: 86%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3j** (88%) as a colorless solid: mp 232 °C; IR 3320, 2820, 2220, 1510 cm^{-1}; ^1H NMR δ 2.63−2.69 (m, 4H), 3.10−3.16 (m, 4H), 3.74 (s, 2H), 6.44−6.47 (m, 1H), 6.83−6.90 (m, 2H),6.93−7.01 (m, 2H), 7.38−7.41 (m, 2H), 7.89−7.91 (m, 1H), 8.87 (br s, 1H); EIMS 334 (M^+). Anal. ($C_{20}H_{19}N_4F$) C,H,N.

2-(4-Phenylpiperazin-1-ylmethyl)indole-6-carbonitrile (3k). 13b (63 mg, 0.31 mmol/g) and 1-phenylpiperazine (113 mg, 0.7 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3k** (6 mg, 97%; purity: 96%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3k** (90%) as a colorless solid: mp 59 °C; IR 3390, 2820, 2220, 1600, 1495 cm^{-1}; ^1H NMR δ 2.63−2.70 (m, 4H), 3.20−3.26 (m, 4H), 3.76 (s, 2H), 6.44−6.47 (m, 1H), 6.88 (t, 1H, J = 7.2), 6.90−6.95 (m, 2H), 7.24−7.28 (m, 2H), 7.32 (dd, 1H, J = 8.2, 1.4), 7.60 (d, 1H, J = 8.2), 7.66−7.68 (m, 1H), 8.90 (br s, 1H); EIMS 316 (M^+). Anal. ($C_{20}H_{20}N_4$) C,H,N.

2-[4-(2-Chlorophenyl)piperazin-1-ylmethyl]indole-6-carbonitrile (3l). 13b (73 mg, 0.31 mmol/g) and 1-(2-chlorophenyl)piperazine (140 mg, 0.7 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3l** (8 mg, 100%; purity: 98%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3l** (82%) as a colorless solid: mp 58−62 °C; IR 3420, 2820, 2220, 1475, 1455 cm^{-1}; ^1H NMR δ 2.65−2.74 (m, 4H), 3.06−3.15 (m, 4H), 3.78 (s, 2H), 6.46 (d, 1H, J = 1.0), 6.98 (td, 1H, J = 7.8, 1.4), 7.05 (dd, 1H, J = 7.8, 1.4), 7.19−7.25 (m, 1H), 7.32 (dd, 1H, J = 8.2, 1.4), 7.36 (dd, 1H, J = 7.8, 1.4), 7.60 (d, 1H, J = 8.2), 7.68 (br s, 1H), 8.88 (br s, 1H); EIMS 352 (M^+), 350 (M^+). Anal. Calcd for $C_{20}H_{19}N_4Cl$: C, 68.47; H, 5.46; N, 15.97. Found: C, 67.88; H, 5.99; N, 15.72.

2-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]indole-6-carbonitrile (3m). 13b (155 mg, 0.31 mmol/g) and 1-(3,4-dichlorophenyl)piperazine (340 mg, 1.4 mmol) were reacted and worked up as described for **3f** (method A) to give crude **3m** (19 mg, 100%; purity: 98%). In analogy to **3f**, a solution-phase synthesis was also performed (see method B) to give **3m** (91%) as a colorless solid: mp 132−134 °C; IR 3335, 2825, 2220, 1480 cm^{-1}; ^1H NMR δ 2.61−2.68 (m, 4H), 3.16−3.23 (m, 4H), 3.76 (s, 2H), 6.44−6.47 (m, 1H), 6.72 (dd, 1H, J = 8.9, 2.8), 6.95 (d, 1H, J = 2.8), 7.27 (d, 1H, J = 8.9), 7.33 (dd, 1H, J = 8.4, 1.2), 7.60 (d, 1H, J = 8.4), 7.67 (br s, 1H), 8.81 (br s, 1H); EIMS 384 (M^+), 386 (M^+), 388 (M^+). Anal. ($C_{20}H_{18}N_4Cl_2$) C,H,N.

2-(*tert*-Butyldimethylsilanyloxymethyl)indole-5-carbonitrile (8a). To a solution of **7a** (1.90 g, 11 mmol)[11] in DMF (40 mL) were added imidazole (1.49 g, 22 mmol) and TBDMSCl (1.83 g, 12 mmol) at 0 °C. After being stirred at 0 °C for 0.5 h aqueous saturated NH_4Cl, water and EtOAc were added. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc 7:3) to give **8a** (2.91 g, 92%) as a colorless solid: mp 81 °C; IR 3300, 2950, 2930, 2850, 2220, 840 cm^{-1}; ^1H NMR δ 0.17 (s, 6H), 0.98 (s, 9H), 4.93 (s, 2H), 6.39−6.42 (m, 1H), 7.41 (dd, 1H, J = 8.6, 1.4), 7.46 (d, 1H, J = 8.6), 7.94 (s, 1H), 8.64 (br s, 1H); EIMS 286 (M^+); HREIMS (M^+) 286.1506 (286.1502 calcd for $C_{16}H_{22}N_2OSi$).

2-(*tert*-Butyldimethylsilanyloxymethyl)indole-6-carbonitrile (8b). 7b (2.93 g, 17 mmol),[11] imidazole (2.31 g, 34 mmol) and TBDMSCl (2.83 g, 19 mmol) were reacted and worked up as described for **8a** to give **8b** (4.47 g, 92%) as a colorless solid: mp 102−104 °C; IR 3340, 2950, 2930, 2850, 2220, 840 cm^{-1}; ^1H NMR δ 0.17 (s, 6H), 0.98 (s, 9H), 4.91 (s, 2H), 6.37−6.39 (m, 1H), 7.32 (dd, 1H, J = 8.2, 1.4), 7.60 (d, 1H, J = 8.2), 7.69−7.71 (m, 1H), 8.62 (br s, 1H); EIMS 286 (M^+); HREIMS (M^+) 286.1506 (286.1502 calcd for $C_{16}H_{22}N_2$-OSi).

2-(*tert*-Butyldimethylsilanyloxymethyl)-1-diethoxymethylindole-5-carbonitrile (9a). A solution of **8a** (645 mg, 2.3 mmol) in triethyl orthoformate (5 mL, 30 mmol) was stirred at 160 °C for 16 h. The mixture was concentrated and the residue was purified by flash chromatography (petroleum ether−EtOAc 4:1) to give **9a** (646 mg, 74%) as a colorless solid: mp 58 °C; IR 2955, 2935, 2220, 1110, 1070 cm^{-1}; ^1H NMR δ 0.12 (s, 6H), 0.92 (s, 9H), 1.21 (t, 6H, J = 7.1), 3.45 (dq, 2H, J = 9.3, 7.1), 3.70 (dq, 2H, J = 9.3, 7.1), 4.88 (s, 2H),

6.41 (s, 1H), 6.47 (s, 1H), 7.41 (dd, 1H, J = 8.6, 1.7), 7.85 (d, 1H, J = 8.6 Hz), 7.87−7.90 (m, 1H); EIMS 388 (M^+); HREIMS (M^+) 388.2181 (388.2182 calcd for $C_{21}H_{32}N_2O_3Si$).

2-(*tert*-Butyldimethylsilanyloxymethyl)-1-diethoxymethylindole-6-carbonitrile (9b). A solution of **8b** (1.71 g, 6 mmol) and triethyl orthoformate (10 mL, 60 mmol) was stirred at 160 °C for 24 h. The mixture was worked up as described for **9a** to give **9b** (2.07 g, 90%) as a colorless solid: mp 87 °C; IR 2930, 2860, 2220, 1100, 1070 cm^{-1}; ^1H NMR δ 0.15 (s, 6H), 0.92 (s, 9H), 1.22 (t, 6H, J = 7.1 Hz), 3.45 (dq, 2H, J = 9.3, 7.1 Hz), 3.70 (dq, 2H, J = 9.3, 7.1), 4.88 (s, 2H), 6.40 (s, 1H), 6.47 (s, 1H), 7.32 (dd, 1H, J = 8.2, 1.4), 7.57 (d, 1H, J = 8.2), 8.14−8.16 (m, 1H); EIMS 388 (M^+); HREIMS (M^+) 388.2185 (388.2182 calcd for $C_{21}H_{32}N_2O_3Si$).

2-Chloromethylindole-5-carbonitrile (10a). To a solution of **7a** (600 mg, 3.8 mmol)[11] in DMF (5 mL) and CCl_4 (2 mL) was added PPh_3 (1.186 g, 4.5 mmol). After stirring for 16 h at room temperature water and EtOAc were added. The organic layer was washed with water, dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc 6:4) to give **10a** (294 mg, 40%) as a colorless solid: mp 110 °C; IR 3290, 2220, 1320, 1260, 715 cm^{-1}; ^1H NMR δ 4.80 (s, 2H), 6.57−6.59 (m, 1H), 7.39−7.47 (m, 2H), 7.92−7.94 (m, 1H), 8.61 (br s, 1H); EIMS 192 (M^+), 190 (M^+); HREIMS (M^+) 190.0299 (190.0297 calcd for $C_{10}H_7N_2Cl$).

2-Chloromethylindole-6-carbonitrile (10b). 7b (1.050 g, 6.7 mmol),[11] CCl_4 (2 mL) and PPh_3 (1.860 g, 7.1 mmol) were reacted and worked up as described for **10a** to give **10b** (540 mg, 42%) as a colorless solid: mp 112 °C; IR 3300, 2220, 1310, 1260 cm^{-1}; ^1H NMR δ 4.79 (s, 2H), 6.57−6.60 (m, 1H), 7.36 (dd, 1H, J = 8.2, 1.4), 7.62 (d, 1H, J = 8.2), 7.70−7.72 (m, 1H), 8.69 (br s, 1H); EIMS 192 (M^+), 190 (M^+); HREIMS (M^+) 190.0310 (190.0297 calcd for $C_{10}H_7N_2Cl$).

1-Diethoxymethyl-2-hydroxymethylindole-5-carbonitrile (11a). To a solution of **9a** (769 mg, 2 mmol) in THF (15 mL) was added Bu_4NF (2.2 mL, 1 M in THF) at 0 °C. After being stirred at 0 °C for 0.5 h aqueous saturated $NaHCO_3$ and EtOAc were added. The organic layer was dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc 1:1) to give **11a** (516 mg, 95%) as a colorless solid: mp 74−76 °C; IR 3455, 2980, 2220, 1320, 1100, 1065 cm^{-1}; ^1H NMR δ 1.25 (t, 6H, J = 7.1), 3.49 (dq, 2H, J = 9.3, 7.1), 3.74 (dq, 2H, J = 9.3, 7.1), 4.86 (s, 2H), 6.44 (s, 1H), 6.56 (s, 1H), 7.43 (dd, 1H, J = 8.6, 1.4), 7.68 (d, 1H, J = 8.6), 7.89 (d, 1H, J = 1.4); EIMS 274 (M^+); HREIMS (M^+) 274.1316 (274.1317 calcd for $C_{15}H_{18}N_2O_3$).

1-Diethoxymethyl-2-hydroxymethylindole-6-carbonitrile (11b). 9b (2.81 g, 7.3 mmol) and Bu_4NF (8.0 mL, 1 M in THF) were reacted and worked up as described for **11a** to give **11b** (1.95 g, 97%) as a colorless solid: mp 55 °C; IR 3450, 2980, 2220, 1100, 1065 cm^{-1}; ^1H NMR δ 1.25 (t, 6H, J = 7.1), 3.53 (dq, 2H, J = 9.2, 7.1), 3.75 (dq, 2H, J = 9.2, 7.1), 4.85 (s, 2H), 6.42 (s, 1H), 6.56 (s, 1H), 7.35 (dd, 1H, J = 8.2, 0.9), 7.61 (d, 1H, J = 8.2), 7.99 (d, 1H, J = 0.9); EIMS 274 (M^+); HREIMS (M^+) 274.1313 (274.1317 calcd for $C_{15}H_{18}N_2O_3$).

2-Chloromethyl-1-diethoxymethylindole-5-carbonitrile (12a). To a solution of **11a** (516 mg, 1.9 mmol) in DMF (4 mL) and CCl_4 (1 mL) was added PPh_3 (560 mg, 2.1 mmol). After stirring for 0.5 h at room temperature water and EtOAc were added. The organic layer was washed with water, dried (Na_2SO_4) and evaporated and the residue was purified by flash chromatography (petroleum ether−EtOAc 4:1) to give **12a** (250 mg, 45%) as a colorless solid: mp 76 °C; IR 2980, 2890, 2220, 1325, 1100, 1070 cm^{-1}; ^1H NMR δ 1.25 (t, 6H, J = 7.1 Hz), 3.51 (dq, 2H, J = 9.3, 7.1), 3.75 (dq, 2H, J = 9.3, 7.1), 4.82 (s, 2H), 6.38 (s, 1H), 6.66 (s, 1H), 7.45 (dd, 1H, J = 8.6, 1.4), 7.85 (d, 1H, J = 8.6), 7.90 (d, 1H, J = 1.4); EIMS 294 (M^+), 292 (M^+); HREIMS (M^+) 292.0989 (292.0979 calcd for $C_{15}H_{17}N_2ClO_2$).

2-Chloromethyl-1-diethoxymethylindole-6-carbonitrile (12b). 11b (548 mg, 2.0 mmol) CCl_4 (1 mL) and PPh_3 (560 mg, 2.1 mmol) were reacted and worked up as described for **12a** to give **12b** (354 mg, 60%) as a colorless solid: mp 57 °C; IR 2980, 2220, 1320, 1100, 1070 cm^{-1}; ^1H NMR δ 1.25 (t, 6H, J = 7.1), 3.54 (dq, 2H, J = 9.3, 7.1), 3.75 (dq, 2H, J = 9.3, 7.1), 4.85 (s, 2H), 6.38 (s, 1H), 6.65 (s, 1H), 7.35 (dd, 1H, J = 8.0, 1.4), 7.60 (d, 1H, J = 8.0 Hz), 8.14−8.17 (m, 1H); EIMS 294 (M^+), 292 (M^+); HREIMS (M^+) 292.0984 (292.0979 calcd for $C_{15}H_{17}N_2ClO_2$).

Polymer-Bound 2-Chloromethylindole-5-carbonitrile (13a). A mixture of **12a** (700 mg, 2.5 mmol), polymer-bound 3-benzyloxypropane-1,2-diol[12] (480 mg) and *p*-TosOH (100 mg) in 1,4-dioxane (5 mL) was stirred at room temperature for 3 h, filtered, subsequently washed with 1,4-dioxane, EtOH−2 N NaOH, EtOH and Et_2O, and dried in vacuo to give **13a** (480 mg).

Polymer-Bound 2-Chloromethylindole-6-carbonitrile (13b). 12b (350 mg, 1.2 mmol), polymer-bound 3-benzyloxypropane-1,2-diol[12] (240 mg) and *p*-TosOH (50 mg) were reacted and worked up as described for **13a** to give **13b** (257 mg).

Receptor Binding Studies. Receptor binding assays at the dopamine D_1 receptor were carried out using bovine striatal membranes with a final protein concentration of 25 μg/assay tube and a K_d value of 0.27−0.32 nM considering the radioligand [^{3}H]SCH 23390 as previously described.[16] Preparations of membranes from CHO cells expressing human dopamine D_{2long}, D_{2short}, D_3 and $D_{4.4}$ receptors were employed for competition binding analysis displacing the radioligand [^{3}H]-spiperone according to literature.[16] The assays were run with a protein concentration of 5−25 μg/assay tube, with K_d values being 0.10−0.20, 0.10, 0.20 and 0.15−0.30 nM for the D_{2long}, D_{2short}, D_3 and $D_{4.4}$ receptors, respectively. Protein concentration was established by the method of Lowry using bovine serum albumin as standard.[23]

Mitogenesis Assay. A CHO10001A cell line stably transfected with the human dopamine $D_{4.2}$ receptor was provided from Dr. R. Huff (Pharmacia & Upjohn, Inc., Kalamazoo, MI).[19] Cells were grown in MEM α-medium (without nucleosides) supplemented with 10% fetal calf serum, 2 mM L-glutamine and 500 U/mL hygromycin B (Chalbiochem-Novabiochem, Bad Soden, Germany) at 37 °C under a humidified atmosphere of 5% CO_2−95% air in the presence of 100 U/mL penicillin G and 100 μg/mL streptomycin.

CHO cells were seeded in a 96-well plate with a density of 10 000 cells/well and were grown in 200 μL of MEM α-medium, containing 10% fetal calf serum, at 37 °C for 75 h. Before incubation with the test compounds the growth medium was removed and the cells were rinsed twice with serum free medium. Incubation was started by adding seven different concentrations of the test compounds (with a final concentration of 0.001−1000 nM) diluted in 10 μL of sterile water to each well containing 90 μL of serum-free medium. Eight wells of every plate received 100 μL of serum-free medium or medium supplemented with 10% fetal calf serum, respectively, to control stimulation of growth. After the plate was cultured for 16−18 h, 0.25 μCi of [^{3}H]thymidine (specific activity 2.0 Ci/mmol; Amersham Pharmacia Biotech, Freiburg, Germany) in 10 μL of serum-free medium was added to each well for 2 h at 37 °C. Finally, cells were trypsinized and harvested onto GF/C filters using an automated cell harvester (Inotech, Dottikon, CH). Filters were washed four times with ice-cold PBS buffer and counted in a MicroBeta Trilux (Wallac ADL, Freiburg, Germany).

Data Analysis. The resulting competition curves of the receptor binding experiments were analyzed by nonlinear regression using the algorithms in PRISM (GraphPad Software, San Diego, CA). The data were fit in accordance to a sigmoid model to provide an IC_{50} value, representing the concentration corresponding to 50% of maximal inhibition, and then transformed to K_i values applying the equation of Cheng and Prusoff.[24] Data resulting from experiments investigating the stimulation of mitogenesis were each normalized and then combined to get a mean curve. Analyzing this curve as described above yielded an EC_{50} value expressing the concentration which caused half of the maximal rate of incorporation of [^{3}H]thymidine. The top value of the curve expressed the

maximal rate of uptake of the radioactive marker to derive the rate of agonistic effect for each test compound in correlation to the reference agonist quinpirole.

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