2-[(4-Phenylpiperazin-1-yl)methyl]imidazo(di)azines as Selective D_4-Ligands. Induction of Penile Erection by 2-[4-(2-Methoxyphenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (PIP3EA), a Potent and Selective D_4 Partial Agonist

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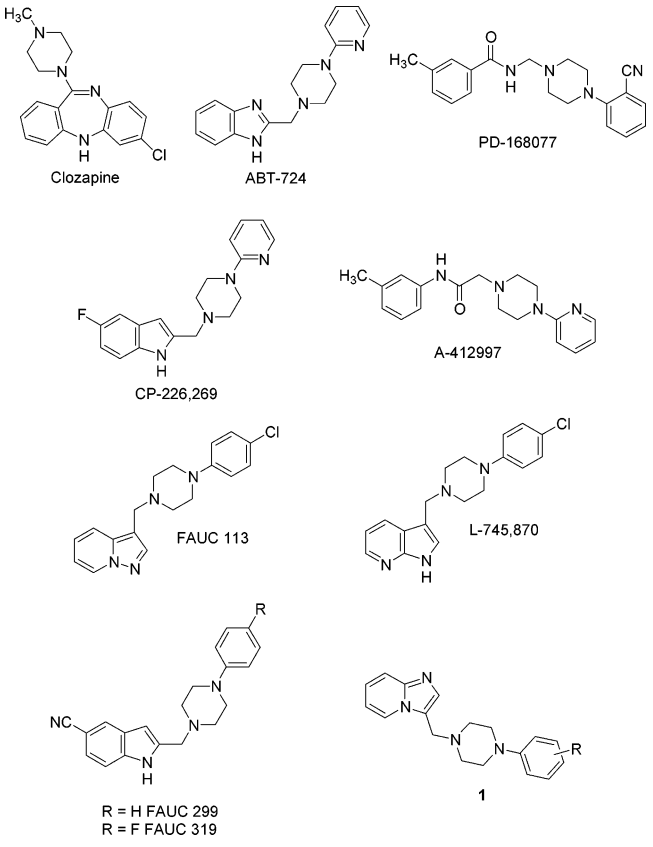
A series of novel 2-[(4-phenylpiperazin-1-yl)methyl]imidazoazines and aza-analogues were prepared and screened at selected dopamine, serotonin, and adrenergic receptor subtypes. 2-Substituted imidazopyridines and pyridazines presented high affinities and selectivities for D_4 dopamine receptors. Whereas functional experiments indicated neutral antagonists or weak partial agonist effects for most of the target compounds, the 2-methoxyphenyl substituted 2-piperazinylmethylimidazopyridine **3c** (PIP3EA) displayed substantial agonist efficacy in mitogenesis experiments and GTPγS binding tests, resulting in EC_{50} values of 3.0 (46%) and 4.5 nM (57%), respectively. Our D_4 agonist **3c** induced penile erection in vivo when administered to rats. This effect was inhibited by L-745,870 a D_4 selective antagonist, confirming the mechanistic pathway.

Introduction

Dopamine receptors are divided into D_1-like and D_2-like families.[1] From the recent progress in molecular cloning techniques, the existence of subtypes within these families was described. The D_1-like family comprises D_1 and D_5 subtypes, whereas the D_2-like family consists of D_2, D_3, and D_4 receptors.[2] In a recent review, Missale and coworkers reported the structure and function of these different subtypes in peripheral tissues as well as in the brain.[3] Nevertheless, the D_4 receptor remains a controversial target,[4] and the therapeutic potential of D_4 ligands in various psychiatric or neurological disorders, such as schizophrenia, ADHD (attention deficit hyperactivity disorders), mood disorders, and Parkinson's disease, is still under discussion.[5−7] Starting from the atypical antipsychotic clozapine, which presents a higher affinity for D_4 than for D_2 receptors,[8] several selective dopamine D_4 receptor ligands were synthesized.[9] SAR studies on D_4 antagonists[10] and D_4 agonists[11] have been recently reviewed indicating an *N*-arylpiperazinyl group as a common scaffold.

In recent years, it was demonstrated that D_4 agonists, such as ABT-724,[12] PD-168077,[13] CP-226,269,[14] and A-412997[15] (Chart 1), induce penile erection in rats. More recently, using PD-168077, the potential therapeutic use of D_4 agonists in preventing dermatoses, which are characterized by epidermal proliferation and epidermal barrier dysfunction, was demonstrated.[16] Unfortunately from these compounds, PD-168077 was demonstrated to be unstable in acidic solution, excluding oral administration.[17] Furthermore, Moreland and coworkers showed a poor selectivity of this compound toward the hα_1 receptor (K_ihα_1/K_ih$D_{4.4}$ = 8).[18]

Chart 1. Structure of Dopaminergic Agents Discussed in the Text



In our work on selective dopamine ligands, we reported that azaindoles bearing an arylpiperazinylmethyl moiety at the 3-position of the heterocyclic moiety (e.g., FAUC 113) show high affinity for dopamine D_4 receptors.[19] The comparison of the molecular electrostatic isopotential maps derived by ab initio molecular orbital calculations at the RFH (restricted Hartree−

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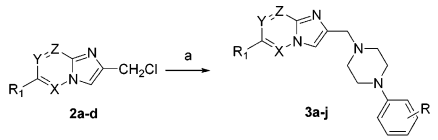
Scheme 1[a]



Starting Material	Compd	X	Y	Z	R_1	R	Yield (%)
2a	**3a**	CH	CH	CH	H	4-F	41
2a	**3b**	CH	CH	CH	H	3,4-diCl	83
2a	**3c**	CH	CH	CH	H	2-OCH_3	40
2a	**3d**	CH	CH	CH	H	4-Cl	55
2b	**3e**	N	CH	CH	Cl	4-F	91
2b	**3f**	N	CH	CH	Cl	3,4-diCl	92
2b	**3g**	N	CH	CH	Cl	2-OCH_3	96
2b	**3h**	N	CH	CH	Cl	4-Cl	55
2c	**3i**	CH	N	CH	H	4-F	83
2d	**3j**	CH	CH	N	H	4-F	72

[a] Reagents and conditions: (a) substituted phenylpiperazine (1 equiv), Na_2CO_3 (1 equiv), EtOH, reflux 3 h.

Fock) level of theory gave an explanation for the extraordinary D_4 selectivity of the aza-indoles L-745,870 and FAUC 113. Our very recent studies led us to the 2-substituted indoles FAUC 299 and FAUC 319 and 7a-aza-analogues displaying even higher affinities and subtype selectivities compared to those of the respective regioisomers.[20] The structural manipulations allowed us to perform a fine-tuned ligand efficacy.[21] In addition, further 3-substituted imidazo[1,2-*a*]pyridine analogues of type **1** have been investigated very recently.[22] Thus, to further increase D_4 ligand efficacy and selectivity toward the adrenergic subtype α_1, suitable structural modifications should be performed on the imidazoazine and diazine scaffolds. The evaluation of dopamine D_4 receptor affinities followed by binding studies, functional experiments, and in vivo investigations of the synthesized compounds should then lead to a final product of potential interest for the treatment of erectile dysfunction.

Results and Discussion

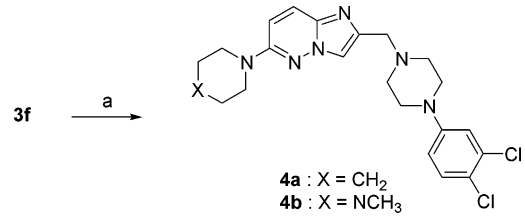
Chemistry. In the first part of this study, four suitably functionalized imidazoazine and diazines were obtained with various phenyl substituents introduced in the imidazo[1,2-*a*]-pyridine and [1,2-*b*]pyridazine series. Thus, the suitable 2-chloromethylimidazoazine and diazines derivatives **2a**−**d** were refluxed with *N*-(4-fluoro, 3,4-dichloro, 2-methoxy or 4-chlorophenyl)piperazine in ethanol in the presence of sodium carbonate to give **3a**−**j** in good yields (Scheme 1).

Then, the nature of the six-membered ring substitution was investigated. It is well established that chlorine in position 6 of the imidazo[1,2-*b*]pyridazine series easily gives nucleophilic substitutions.[23,24] In our case, the chlorine displacement was performed on **3f** using piperidine or 4-methylpiperazine, leading to the attempted compounds **4a**,**b** in good yields (Scheme 2).

In the same way, the six-membered ring substitution was investigated in the imidazo[1,2-*a*]pyridine series. To this end, the 6- or 8-halogenated starting materials **6a**−**d** were prepared by the condensation of 2-amino-3 or 5-halopyridines **5a**−**d** with 1,3-dichloroacetone. Then, the reaction with *N*-(2-methoxyphenyl)piperazine led to the attempted compounds **7a**−**d**. Nucleophilic substitution on **6a** using *N*-(3,4-dichlorophenyl)piperazine gave **7e** (Scheme 3).

Because nucleophilic substitution reactions are usually unsuccessful in positions 6 and 8 of the imidazo[1,2-*a*]pyridine series,

Scheme 2[a]



[a] Reagents and conditions: (a) piperidine or *N*-methylpiperazine, reflux 24 h.

we have recently decided to study the application of new metallo-catalyzed methodologies in this series.[25,26] These methods allow the introduction of various functionalities, and in regard to FAUC 299, we were first interested in the synthesis of cyano derivative **8a**. Indeed, to the best of our knowledge, the substitution using sodium or potassium cyanide on 6- or 8-halogenoimidazopyridine was inefficient. Thus, we focused our interest on a copper-based method described by Buchwald's group, starting from the bromo derivative through a one-step exchange with iodine and followed by cyanation using sodium cyanide.[27] Because iodinated starting material **7d** was available, the reaction was conducted using sodium cyanide with CuI as the catalyst and *N*,*N*′-dimethyl-1,2-diaminoethane as the ligand in toluene at 112 °C. In these conditions, nitrile **8a** was obtained in 70% yield (Scheme 4).

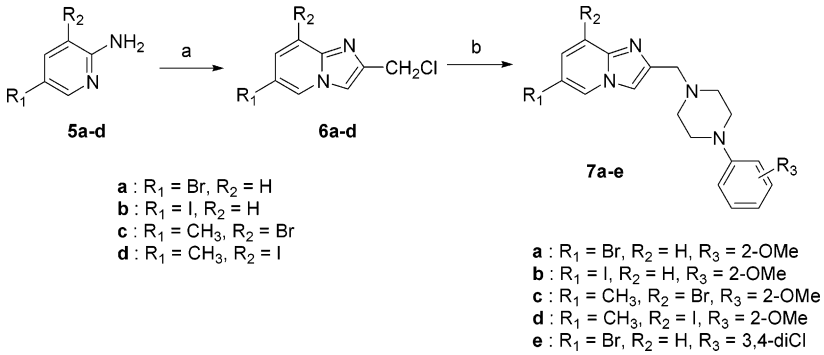
Lately, we reported that aminations using copper or palladium catalysts are efficient on 6- or 8-halogenoimidazo[1,2-*a*]-pyridine.[28,29] In these previously described conditions, **7d** was allowed to react with morpholine under copper catalysis using ethyleneglycol as the ligand in the presence of potassium phosphate in 2-propanol, leading to morpholino derivative **8b** in 72% yield.

We then focused on the introduction of an azole group. Buchwald and coworkers previously reported a methodology for the N-arylation of indole[30] and various nitrogen heterocycles,[31] which we extended to the functionalization of the 6-position of the imidazo[1,2-*a*]pyridine.[32] Thus, in the usual conditions (e.g., CuI, *N*,*N*′-dimethyl-1,2-diaminoethane, K_3PO_4 in toluene), pyrazolyl compound **8c** was obtained from **7d** in 42% yield (not optimized). Then, to evaluate the effect of the substitution pattern, the pyrazolyl group was introduced into position 5. Indeed, it was demonstrated in our laboratory that the reaction of the 6-halogenoimidazo[1,2-*a*]pyridine with an azole in DMF in the presence of cesium carbonate gives the *cine* substitution in position 5.[32] In these conditions, **7a** led to 5-pyrazolyl compound **8d** in only 27% yield, mixed with 50% of the remaining starting material.

Finally, we have recently reported that the Suzuki-type cross coupling reaction occurs on 8-halogenoimidazo[1,2-*a*]pyridine;[33] therefore, the reaction of **7c** with phenylboronic acid gave compound **8e** in 54% yield (not optimized) using tetrakis-(triphenylphosphine)palladium as the catalyst and sodium hydroxide as the base in DME/water. The crystal data for **3a** could be obtained.

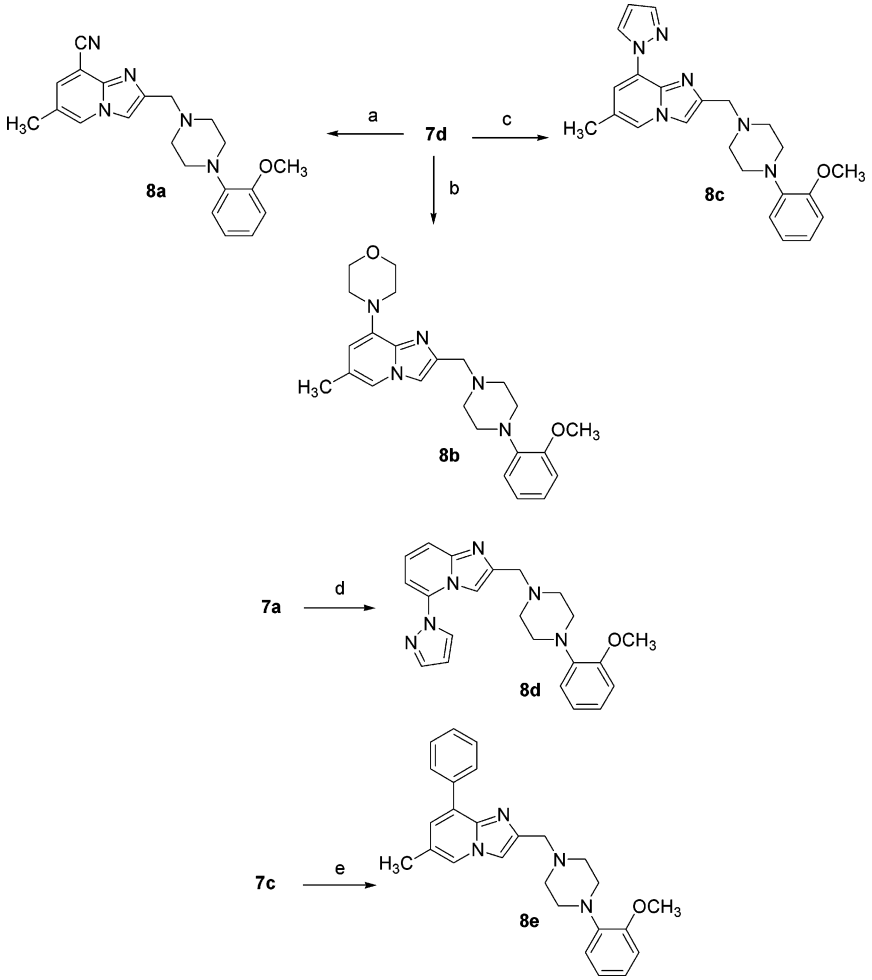
Biological Activity. Receptor binding profiles of the synthesized compounds were determined in vitro by measuring their ability to compete with [^{3}H]spiperone for the cloned human dopamine receptor subtypes D_{2long}, D_{2short}, D_3, and $D_{4.4}$ stably expressed in Chinese hamster ovary cells (CHO).[34−38] D_1 receptor affinity was measured by employing porcine striatal membranes and the D_1 selective radioligand [^{3}H]SCH 23390.[39] The investigation of serotonin 5-HT_{1A} and 5-HT_2 affinities was performed as described in the literature.[40]

Scheme 3[a]



[a] Reagents and conditions: (a) 1,3-dichloroacetone, EtOH, reflux; (b) substituted arylpiperazine (1 equiv), Na_2CO_3 (1 or 2 equiv for chlorhydrates), EtOH, reflux 3h.

Scheme 4[a]



[a] Reagents and conditions: (a) KCN (1.2 equiv), CuI (10 mol %), *N*,*N*′-dimethyl-1,2-diaminoethane (1 equiv), toluene, 112 °C; (b) morpholine (1.1 equiv), CuI (15 mol %), ethyleneglycol (2 equiv), K_3PO_4 (2 equiv), *i*PrOH, 85 °C; (c) pyrazole (1 equiv), CuI (5 mol %), *N*,*N*′-dimethyl-1,2-diaminoethane (15 mol %), K_3PO_4 (2.2 equiv), toluene, 112 °C; (d) pyrazole (1 equiv), Cs_2CO_3 (2.1 equiv), DMF, 112 °C; (e) phenylboronic acid (1 equiv), $Pd(PPh_3)_4$ (5 mol %), NaOH (2 equiv), DME/H_2O.

The K_i values of the test compounds are listed in Table 1 in comparison to those for FAUC 113, FAUC 299, and clozapine. The first general point that emerged from the data is a low D_1 affinity for all compounds investigated. A comparison of the 4-fluorophenyl derivatives **3a**, **3e**, **3i**, and **3j** shows that the number and position of nitrogen atoms within the heteroarene moiety strongly influences $D_{4.4}$ receptor recognition. Heteroatom exchange in positions 7 or 8 of the bicyclic heterocycle is detrimental for $D_{4.4}$ receptor affinity as previously observed for 3-substituted derivatives.[19] The highest affinities for the D_4 binding site were determined for the imidazo[1,2-*a*]pyridine and imidazo[1,2-*b*]pyridazine series with K_i values in the range of 1.2 to 7.0 nM for test compounds **3b**−**h**, although they present micromolar affinities at D_3. With respect to the phenyl substituent, a chlorine atom appeared to be superior to a fluorine atom in the para position as indicated by K_i values of 7 and 43 nM in the imidazo[1,2-*a*]pyridine series and 2 and 4 nM in the imidazo[1,2-*b*]pyridazine series. A second chlorine atom in the meta position even increased the $D_{4.4}$ affinity with K_i values of 1.2−1.3 nM in both series (compounds **3b** and **3f**). A 2-methoxy

Table 1. Receptor Binding Data of Different 2-[4-(Phenylpiperazin-1-yl)methyl]imidazoazines and Diazines Derivatives Employing Bovine D_1 and Human D_2, D_3, and $D_{4.4}$ Receptors[a]

	[^{3}H]SCH23390	[^{3}H]spiperone				[^{3}H]8-OH-DPAT	[^{3}H]ketanserin	[^{3}H]prazosin
compd	D_1	$D_{2\ Long}$	$D_{2\ short}$	D_3	$D_{4.4}$	5-HT_{1A}	5-HT_2	α1
3a	8400 ± 1100	21000 ± 4000	19000 ± 3500	3100 ± 750	44 ± 1.5	3600 ± 650	860 ± 25	260 ± 40
3b	2300 ± 50	3200 ± 730	2700 ± 650	1700 ± 170	1.4 ± 0.14	2300 ± 500	1200 ± 100	61 ± 7.3
3c	20000 ± 0	990 ± 120	930 ± 170	3900 ± 2300	2.8 ± 1.1	2200 ± 1000	5900 ± 1400	98 ± 4.5
3d	6700 ± 1100	15000 ± 2500	16000 ± 3500	8800 ± 2300	7.0 ± 1.3	1700 ± 730	1200 ± 100	60 ± 6.5
3e	7000 ± 0	9900 ± 1200	12000 ± 3400	5600 ±3400	4.0 ± 0.85	nd	nd[b]	nd
3f	1500 ± 250	1100 ± 100	670 ± 55	1500 ± 150	1.3 ± 0.51	360 ± 97	1200 ± 540	190 ± 23
3g	7700 ± 550	96 ± 4.5	79 ± 3.0	1400 ± 100	1.6 ± 0.49	24 ± 0.33	3400 ± 100	64 ± 3.6
3h	5500 ± 350	12000 ± 1000	8200 ± 150	7700 ± 950	2.0 ± 0.74	390 ± 90	910 ± 90	380 ± 55
3i	15000 ± 3000	64000 ± 3000	68000 ± 6000	42000 ± 3000	500 ± 75	nd	nd	nd
3j	47000 ± 22000	55000 ± 5500	49000 ± 5000	60000 ± 2300	2800 ±220	nd	nd	nd
4a	2200 ± 150	1100 ± 210	710 ± 65	850 ± 130	120 ± 20	nd	nd	nd
4b	13000 ± 11000	8000 ± 800	7800 ± 1100	880 ±120	1900 ± 640	nd	nd	nd
7a	7100 ± 100	130 ± 10	140 ± 15	800 ± 20	0.25 ± 0.040	nd	nd	nd
7b	6400 ± 800	73 ± 9.0	72 ± 6.0	600 ± 25	0.33 ± 0.040	nd	nd	nd
7c	5600 ± 1900	240 ± 25	150 ± 25	670 ± 110	0.99 ± 0.21	nd	nd	nd
7d	5900 ± 200	88 ± 12	97 ± 14	470 ± 55	0.53 ± 0.050	nd	nd	nd
7e	2500 ± 0.0	3500 ± 100	3700 ± 450	1000 ± 180	0.99 ± 0.12	nd	nd	nd
8a	40000 ± 10000	4200 ± 750	2700 ± 150	3500 ± 1200	1.3 ± 0.27	nd	nd	nd
8b	6400 ± 1200	210 ± 20	100 ± 17	2100 ± 500	2.3 ± 0.23	nd	nd	nd
8c	8700 ± 50	530 ± 10	280 ± 5.0	2400 ± 200	1.6 ± 0.32	nd	nd	nd
8d	27000 ± 0.0	590 ± 60	280 ± 5.0	1000 ± 200	0.94 ± 0.040	nd	nd	nd
8e	2900 ± 100	49 ± 10	28 ± 5.0	390 ± 110	0.84 ± 0.13	nd	nd	nd
clozapine	420 ± 50	41 ± 1.5	28 ± 0.50	960 ± 45	16 ± 0.50	nd	nd	nd
FAUC 113	12000 ± 500	3200 ± 400	4300 ± 650	5000 ± 650	3.6 ± 0.87	1400 ± 130	380 ± 140	240 ± 5.0
FAUC 299	13000 ± 500	3100 ± 550	290 ± 30	1700 ± 220	0.52 ± 0.050	nd	nd	nd
FAUC 319	8600 ± 2400	28000 ± 6500	19000 ± 4000	15000 ± 2000	1.0 ± 0.057	nd	nd	nd

[a] The K_i values (nM) are the means of 2−5 independent experiments each carried out in triplicate. [b] nd: values not determined.

group is also well tolerated with K_i values of 2.8 nM for **3c** and 1.6 nM for **3g**. In terms of selectivity, with the exception of **3e** ($K_ihD_3/K_ihD_{4.4}$ = 72), the 4-fluoro, 4-chloro, and 3,4-dichloro compounds presented high selectivities for D_4R toward D_1, D_{2L}, D_{2S}, and D_3 receptors ($K_iD/K_ihD_{4.4}$ >440). Changing to the 2-methoxy substitution gave a slightly decreased selectivity for imidazopyridine **3c** toward D_2R ($K_ihD_2/K_ihD_{4.4}$ = 332 for D_{2S} and 353 for D_{2L}) and an even more significant effect for **3g** ($K_ihD_2/K_ihD_{4.4}$ = 49 for D_{2S} and 60 for D_{2L}). Finally, a cyclic amine (piperidine **4a** and *N*-methylpiperazine **4b**) in the 6-position of the imidazo[1,2-*b*]pyridazine dramatically increased the K_i values toward $D_{4.4}$ receptors.

We evaluated the 5-HT_{1A}, 5-HT_2, and α_1 recognition for compounds **3a**−**d** and **3f**−**h** with the aid of [^{3}H]-8-OH-DPAT, [^{3}H]ketanserin, and [^{3}H]prazosin labeled porcine brain homogenate, respectively. With the exception of **3g**, which showed a good affinity for the 5-HT_{1A} receptor (K_i = 24 nM, $K_ip5\text{-}HT_{1A}/K_ihD_{4.4}$ = 15), a high selectivity was observed for all target compounds. Nevertheless, there is clear evidence for a loss of selectivity toward 5-HT_{1A} in the imidazo[1,2-*b*]pyridazine series. Interestingly, only moderate α_1 affinities (K_i = 60−260 nM) were observed. Thus, the very potent D_4 ligands turned out to also display also substantial selectivity over α_1.

From **7a**−**d**, iodine or bromine appeared to be well tolerated in positions 6 and 8 giving derivatives displaying the highest affinities (K_i < 1 nM). A comparison between **7a** and **7e**, confirmed a decrease of the selectivity toward D_2 receptors induced by the presence of 2-methoxy substitution. Introducing a cyano in position 8 gave compound **8a** with high affinity (K_i = 1.3 nM) and an interestingly recovered selectivity toward D_{2L} ($K_iD_{2L}/K_iD_{4.4}$ = 3230) and D_{2S} ($K_iD_{2S}/K_iD_{4.4}$ = 2076). Changing to a phenyl group enhanced the affinity when a loss of specificity toward D_2 was noted (K_iD_{2S}/K_iD_4 = 33). The same observation was made when introducing a morpholine or a pyrazol-1-yl group in position 8. When the pyrazolyl group was introduced in position 5, the affinity was conserved (K_i = 0.94 nM), and a good selectivity toward hD_{2S} was noted (K_ihD_{2S}/K_ihD_4 = 300).

Ligand efficacy of selected compounds was determined by mitogenesis assays, measuring the rate of [^{3}H]thymidine incorporation into growing CHO10001 cells stably expressing $D_{4.2}$.[20] Intrinsic activity was quantified by the determination of the effective concentration (EC_{50}) of a test compound and by comparing the maximal effect to that of a full agonist (Table 2).[39,40] The tested compounds (**3b**,**d**, **3f**−**h**, **7a**,**b**, and **7d**,**e**) were shown to be complete antagonists, whereas **3c** appeared to be a partial agonist with an EC_{50} value of 3.0 nM and an intrinsic effect of 46% compared to that of the nonselective full agonist quinpirole. To corroborate the functional activity of the test compounds using a second functional experiment, a [^{35}S]GTPγS binding assay was realized. Employing membrane preparations from human $D_{4.4}$ receptor expressing CHO cells, the dose dependent stimulation of [^{35}S]GTPγS binding was determined to distinguish between agonist and antagonist properties and to measure the potency and efficacy at the D_4 receptor.[41,42] Using this assay, agonist efficacy of target compound **3c** was confirmed revealing an EC_{50} value of 4.5 nM and an intrinsic activity of 57% when **3d** appeared to be a weak agonist (18%).

In addition, we investigated the affinity of the most interesting compound **3c** to the agonist binding site of the D_2 receptor. Using the agonist radioligand [^{3}H]-7-OH-DPAT for competition experiments with human D_{2long} and native porcine D_2 receptor-containing membranes, the K_i values for **3c** of 110 and 400 nM, respectively, were measured. Although these data may indicate the weak agonist effect of **3c** at D_2 receptors, a detailed analysis of the binding curves derived from competition experiments with the antagonist [^{3}H]spiperone did not give any evidence for a biphasic curve shape, which should be expected when measuring

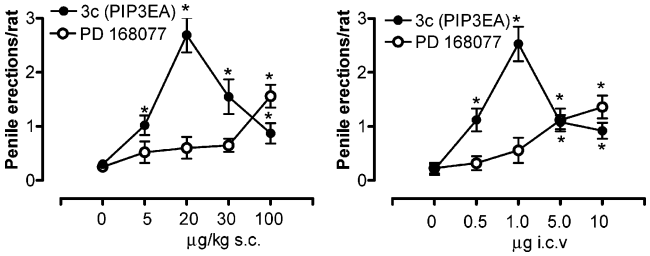


Figure 1. Effect of compound **3c**, administered by s.c. or icv injections, on penile erection in male rats: comparison with PD-186077. Compound **3c** and PD 168077 were dissolved in ethanol and diluted with distilled water to a final 0.3% ethanol concentration and injected s.cly in a volume of 0.2 mL/rat and icvly in a volume of 10 μL/rat. After treatment, the rats were individually placed in Plexiglas cages (30 × 30 × 30 cm^3) and observed for 60 min to count penile erection episodes. The values are means ± SEM of 6 rats per group. *P < 0.001 with respect to vehicle-treated rats (compound **3c** or PD 168077 = 0).

an agonist compound. To prove these findings, we performed a functional experiment by the determination of the agonist stimulated [^{35}S]GTPγS binding at the D_{2short} receptor; **3c** was not able to stimulate the receptor up to a concentration of 10 μM.

Because of the interest in recent studies on D_4 agonists in the treatment of erectile dysfunction,[12,13,15,43] compound **3c** was then tested in rats to study its ability to promote erection after systemic (subcutaneous, s.c.) or intracerebroventricular (icv) administration. When given subcutaneously (s.cly), **3c** induced penile erection with an inverted U dose−response curve (Figure 1). The minimal effective dose was 5 μg/kg of body weight, which induced penile erection in 60% of the treated rats and increased penile erection episodes from 0.3 ± 0.02 to 1.02 ± 0.05. The maximal effect was obtained with 20 μg/kg, which induced penile erection in all treated rats and increased penile erection episodes to 2.69 ± 0.43. A much lower response was seen with doses of 30 μg/kg or higher. Similar results were found with an icv injection of compound **3c**. The minimal effective dose was 0.5 μg/rat, which increased penile erection episodes from 0.14 ± 0.02 to 1.12 ± 0.32, whereas the maximal response (2.52 ± 0.61) was found with the dose of 1 μg/rat. A much lower response was found with the dose of 5 μg/rat or higher (Figure 1). A comparison of the dose−response curves of **3c** with those of PD-168077, another D_4 agonist that induces penile erection in rats,[13,15] given s.cly or intracerebroventricularly (icvly) revealed that **3c** was more effective than PD-168077 because compound **3c** induces a higher number of episodes of penile erections/rat, and higher doses of PD-168077 were necessary to induce penile erection when given either s.cly or icvly (Figure 1). Perhaps more important for this work, the pro-erectile effect of compound **3c** given s.cly (20 μg/kg) or icvly (1 μg/rat) was reduced by more than 80%, not only by clozapine and haloperidol, which block all dopamine receptor subtypes, but also by L-745,870, a selective D_4 antagonist,[10] all given intraperitoneally (i.p.) at the dose of 1 mg/kg, 15 min before compound **3c** (Table 3).

Conclusions

Herein we have presented a novel series of 2-(4-arylpiperazin-1-ylmethyl)imidazoazines and diazines. From the synthesized compounds, the imidazo[1,2-*a*]pyridine and imidazo[1,2-*b*]-pyridazine derivatives were found to present high affinity and selectivity for D_4R over D_1, D_{2L}, D_{2S}, and D_3 as well as 5-HT_2 and α_1 receptors. A good selectivity toward the 5-HT_{1A} receptors was also noticed with the imidazo[1,2-*a*]pyridine series, but it was decreased with the imidazo[1,2-*b*]pyridazine compounds,

Table 2. Intrinsic Activities of **3b**−**d**, **3f**−**h**, **7a**, **b**, **d**, and **e** as Well as Reference Compound Quinpirole Derived from the Stimulating Effect on the Mitogenesis of D_4 Receptor-Expressing Cells and the D_4 Receptor Mediated Binding of [^{35}S]GTPγS

	[^{3}H]thymidine uptake (mitogenesis)[a]		[^{35}S]GTP S binding[b]	
entry	EC_{50} (nM)[c]	intrinsic effect (% quinpirole)[d]	EC_{50} (nM)[e]	intrinsic effect (% quinpirole)[f]
3b		0	nd[g]	nd
3c	3.0	46	4.5	57
3d	1.8	6	2.6	18
3f		0	nd	nd
3g		0	nd	nd
3h		0	nd	nd
7a		0	nd	nd
7b	0.52	10	nd	nd
7d		0	nd	nd
7e		0	nd	nd
quinpirole	1.4	100	49	100

[a] Determined with CHO10001 cells stably expressing the human $D_{4.2}$ receptor. [b] Determined with CHO K1 cells stably expressing the human $D_{4.4}$ receptor. [c] The EC_{50} values were derived from a mean curve of 2−11 independent experiments. [d] The rate of incorporation of [^{3}H]thymidine as evidence for mitogenesis activity relative to the maximal effect of the full agonist quinpirole (=100%) used as a reference. [e] The EC_{50} values were derived from a mean curve of three or four independent experiments. [f] Maximum binding of [^{35}S]GTPγS induced by receptor activation at 1 μM relative to the effect of the reference agonist quinpirole (=100%) (at 10 μM) derived from six independent experiments. [g] nd: values not determined.

Table 3. Effect of L-745,870, Haloperidol, and Clozapine on Compound **3c**-induced Penile Erection[a]

treatment	penile erections/rat
i.p. saline (1 mL) + s.c. vehicle (0.2 mL)	0.31 ± 0.06
i.p. saline (1 mL)+ s.c. **3c** (20 μg/kg)	2.42 ± 0.51[b]
i.p. L-745,870 (1 mg/kg) + s.c. **3c** (20 μg/kg)	0.53 ± 0.12[c]
i.p. haloperidol (1 mg/kg) + s.c. **3c** (20 μg/kg)	0.49 ± 0.12[c]
i.p. clozapine (1 mg/kg) + s.c. **3c** (20 μg/kg)	0.50 ± 0.12[c]
i.p. saline (1 mL) + icv vehicle (10 μl)	0.26 ± 0.05
i.p. saline (1 mL) + icv **3c** (1 μg)	2.32 ± 0.32[b]
i.p. L-745,870 (1 mg/kg) + icv **3c** (1 μg))	0.67 ± 0.09[c]
i.p. haloperidol (1 mg/kg) + icv **3c** (1 μg))	0.59 ± 0.09[c]
i.p. clozapine (1 mg/kg) + icv **3c** (1 μg))	0.54 ± 0.09[c]

[a] Compound **3c** was dissolved in ethanol and then diluted with water to a final concentration of 0.3% of ethanol (vehicle). Haloperidol, clozapine or L-745,870 was dissolved in saline and administered by i.p. injection 15 min before compound **3c** was administered. After treatment, the rats were observed individually for 60 min to count penile erection episodes. The values are means ± SEM of 6 rats/group. [b] P < 0.001 with respect to saline + vehicle treated rats. [c] P < 0.001 with respect to compound **3c**-treated rats.

especially 2-methoxy derivative **3g**. With regard to phenyl substitution and its influence on D_4 affinity, all of the substituents tested (fluorine, chlorine, and methoxy) were well tolerated. Nevertheless, the introduction of a 2-methoxy group led to a decrease in selectivity for D_4 receptors toward D_2 subtypes.

According to mitogenesis and GTPγS assays, the 2-substituted imidazo[1,2-*a*]pyridine **3c** (PIP3EA) showed substantial agonist efficacy. In line with the above in vitro results, compound **3c** was shown to be capable of inducing penile erection in rats after s.c. and icv injections with an efficacy higher than that of PD-168077, a D_4 agonist previously shown to be capable of inducing penile erection in rats.[13,15] The reason PIP3EA, which presents an intrinsic activity of only 50% compared to that of full agonists in the above tests, shows such a good efficacy in the rat model of penile erection is unknown at present. However, similar low intrinsic activity was also found with other partial D_4 agonists that induce penile erection (e.g., ABT 724, intrinsic activity 60%). [13,15] Because the pro-erectile

effect of compound **3c** is reduced not only by haloperidol and clozapine, which block all dopamine receptor subtypes, but also by the selective D_4 antagonist L-745,870, these findings provide further support for the role of central D_4 receptors in the control of penile erection and the possible therapeutic use of D_4 agonists in the treatment of erectile dysfunctions.

Experimental Section

Chemistry Methods. All solvents were anhydrous reagents from commercial sources. Unless otherwise noted, all chemicals and reagents were obtained commercially and used without purification. NMR spectra were recorded at 200 MHz (^{1}H) or 50 MHz (^{13}C) on a Bruker DPX instrument or at 360 MHz (^{1}H) or 90 MHz (^{13}C) on a Bruker AM 360. The chemical shifts are reported in parts per million (ppm, δ) relative to residual deuterated solvent peaks. The possible inversion of two values in the NMR spectra is expressed by an asterisk. Known compounds were prepared according to a literature procedure: 2-chloromethylimidazo[1,2-*a*]pyridine (**2a**), 6-chloro-2-chloromethylimidazo[1,2-*b*]pyridazine (**2b**), 2-chloromethylimidazo[1,2-*a*]pyrazine (**2c**), and 2-chloromethylimidazo[1,2-*a*]pyrimidine (**2d**).[44−47]

2-[4-(4-Fluorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (3a). Typical procedure: a mixture of 2-chloromethylimidazoazine or diazine (5 mmol), 1-(substituted phenyl)piperazine (5 mmol), and sodium carbonate (5 mmol) in dry ethanol (20 mL) was refluxed for 3 h. After cooling, ethanol was removed in vacuo, and the residue was dissolved in water and extracted with dichloromethane. The organic layer was dried ($CaCl_2$), evaporated, and the residue purified by chromatography (neutral alumina−dichloromethane). Mp 124−126 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.73 (m, 4H, pip), 3.13 (m, 4H, pip), 3.76 (s, 2H, CH_2), 6.72 (td, 1H, J = 6.8−1.1 Hz, H-6), 6.80−6.97 (m, 4H, F−Ph), 7.11 (ddd, 1H, J = 8.9−6.8−1.1 Hz, H-7), 7.52 (s, 1H, H-3), 7.54 (bd, 1H, J = 8.9 Hz, H-8), 8.04 (dt, 1H, J = 6.8−1.1 Hz, H-5).

6-Chloro-2-[4-(4-fluorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*b*]pyridazine (3e). Mp 111−113 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.75 (m, 4H, pip), 3.16 (m, 4H, pip), 3.82 (s, 2H, CH_2), 6.84−7.01 (m, 4H, F−Ph), 7.05 (d, J = 9.4 Hz, H-7), 7.87 (d, J = 9.4 Hz, H-8), 7.92 (s, 1H, H-3).

2-[4-(4-Fluorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyrazine (3i). Mp 127−129 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.72 (m, 4H, pip), 3.12 (m, 4H, pip), 3.81 (s, 2H, CH_2), 6.82−6.97 (m, 4H, F−Ph), 7.66 (s, 1H, H-3), 7.83 (d, 1H, J = 4.5 Hz, H-5), 8.02 (dd, 1H, J = 4.5−1.5 Hz, H-6), 9.03 (m, 1H, H-8).

2-[4-(4-Fluorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyrimidine (3j). Mp 170−172 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.77 (m, 4H, pip), 3.13 (m, 4H, pip), 3.83 (s, 2H, CH_2), 6.82−6.98 (m, 5H, F−Ph, H-6), 7.55 (s, 1H, H-3), 8.44 (dd, 1H, J = 6.7−2.1 Hz, H-5), 8.49 (dd, 1H, J = 4.1−2.1 Hz, H-7).

2-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (3b). Mp 103−105 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.74 (m, 4H, pip), 3.21 (m, 4H, pip), 3.78 (s, 2H, CH_2), 6.73 (dd, 1H, J = 8.9−2.9 Hz, diCl-Ph-6), 6.79 (td, 1H, J = 6.8−1.2 Hz, H-6), 6.95 (d, 1H, J = 2.9 Hz, diCl-Ph-2), 7.16 (ddd, 1H, J = 9−6.8−1.2 Hz, H-7), 7.26 (d, 1H, J = 8.9 Hz, diCl-Ph-5), 7.56 (s, 1H, H-3), 7.58 (m, 1H, H-8), 8.09 (dt, 1H, J = 6.8−1.2 Hz, H-5). Anal. Calcd for $C_{18}H_{18}Cl_2N_4$: C, 59.84; H, 5.02; N, 15.51. Found: C, 59.81; H, 5.13; N, 15.43.

2-[4-(2-Methoxyphenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (3c). Mp 142−144 °C.^{1}H NMR (200 MHz, $CDCl_3$) δ 2.81 (m, 4H, pip), 3.13 (m, 4H, pip), 3.83 (s, 3H, CH_3O), 3.86 (s, 2H, CH_2), 6.76 (td, 1H, J = 6.6−1.2 Hz, H-6), 6.86 (m, 1H, CH_3O-Ph-3), 6.92−7.02 (m, 3H, CH_3O-Ph-4,5,6), 7.15 (ddd, 1H, J = 9−6.6−1.2 Hz, H-7), 7.56 (s, 1H, H-3), 7.59 (m, 1H, H-8), 8.08 (dt, 1H, J = 6.6−1.2 Hz, H-5).

2-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (3d). Mp 141−143 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.75 (m, 4H, pip), 3.20 (m, 4H, pip), 3.79 (s, 2H, CH_2), 6.77 (t, 1H, J = 6.8 Hz, H-6), 6.84 (d, 2H, J = 8.8 Hz, Cl-Ph-2,6), 7.17 (dd, 1H, J = 9−6.8 Hz, H-7), 7.20 (d, 2H, J = 8.8 Hz, Cl-Ph-3,5), 7.55 (s, 1H, H-3), 7.58 (d, 1H, J = 9 Hz, H-8), 8.08 (d, 1H, J = 6.8 Hz, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.46 (pip), 53.53 (pip), 56.97 (CH_2), 111.43 (C-3), 112.59 (C-6), 117.56 (Cl-Ph-2,6), 117.87 (C-8), 124.69 (Cl-Ph-4), 124.73 (C-7), 125.91 (C-5), 129.30 (Cl-Ph-3,5), 144.00 (C-8a*), 145.54 (C-2*), 150.40 (Cl-Ph-1).

6-Chloro-2-[4-(3,4-dichlorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*b*]pyridazine (3f). Mp 118−120 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.71 (m, 4H, pip), 3.21 (m, 4H, pip), 3.80 (s, 2H, CH_2), 6.72 (dd, 1H, J = 8.9−2.8 Hz, diCl-Ph-6), 6.94 (d, 1H, J = 2.8 Hz, diCl-Ph-2), 7.05 (d, 1H, J = 9.4 Hz, H-7), 7.25 (d, 1H, J = 8.9 Hz, diCl-Ph-5), 7.87 (d, 1H, J = 9.4 Hz, H-8), 7.90 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.91 (pip), 53.28 (pip), 56.92 (CH_2), 115.66 (diCl-Ph-6), 116.38 (C-3), 117.50 (diCl-Ph-2), 119.22 (C-7), 122.39 (diCl-Ph-4), 126.90 (C-8), 130.75 (diCl-Ph-5), 133.10 (diCl-Ph-3), 137.78 (diCl-Ph-1), 145.19 (C-8a*), 147.03 (C-2*), 151.01 (C-6).

6-Chloro-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]imidazo-[1,2-*b*]pyridazine (3g). The title compound was obtained as an oil.^{1}H NMR (200 MHz, $CDCl_3$) δ 2.82 (m, 4H, pip), 3.17 (m, 4H, pip), 3.86 (s, 2H, CH_2), 3.89 (s, 3H, CH_3), 6.87−7.06 (m, 4H, CH_3O-Ph), 7.07 (d, 1H, J = 9.4 Hz, H-7), 7.89 (d, 1H, J = 9.4 Hz, H-8), 7.95 (s, 1H, H-3).

6-Chloro-2-[4-(4-chlorophenyl)piperazin-1-ylmethyl]imidazo-[1,2-*b*]pyridazine (3h). Mp 149−151 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.75 (m, 4H, pip), 3.23 (m, 4H, pip), 3.83 (s, 2H, CH_2), 6.86 (d, 2H, J = 8.7 Hz, Cl-Ph-2,6), 7.07 (d, 1H, J = 9.4 Hz, H-7), 7.22 (d, 2H, J = 8.7 Hz, Cl-Ph-3,5), 7.89 (d, 1H, J = 9.4 Hz, H-8), 7.93 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 49.49 (pip), 53.54 (pip), 57.06 (CH_2), 116.40 (C-3), 117.66 (Cl-Ph-2,6), 119.17 (C-7), 124.90 (Cl-Ph-4), 126.93 (C-8), 129.34 (Cl-Ph-3,5), 137.82 (Cl-Ph-1), 145.43 (C-8a), 147.02 (C-2), 150.32 (C-6).

2-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]-6-(piperidin-1-yl)imidazo[1,2-*b*]pyridazine (4a). Typical procedure: a solution of **3f** (0.5 g, 1.26 mmol) in the suitable amine (5 mL) was refluxed for 24 h. After cooling, the mixture was diluted with water and extracted with dichloromethane. The organic layer was dried ($CaCl_2$), evaporated, and the residue purified by chromatography (neutral alumina−dichloromethane). Mp 135−137 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.71 (m, 6H, piperidine), 2.73 (m, 4H, pip), 3.22 (m, 4H, pip), 3.50 (m, 4H, piperidine), 3.75 (s, 2H, CH_2), 6.75 (dd, 1H, J = 8.9−2.9 Hz, diCl-Ph-6), 6.83 (d, 1H, J = 9.9 Hz, H-7), 6.96 (d, 1H, J = 2.9 Hz, diCl-Ph-2), 7.28 (d, 1H, J = 8.9 Hz, diCl-Ph-5), 7.64 (d, 1H, J = 9.9 Hz, H-8), 7.65 (s, 1H, H-3).^{13}C NMR (50 MHz, $CDCl_3$) δ 24.84 (piperidine), 25.79 (piperidine), 47.89 (piperidine), 48.99 (pip), 53.17 (pip), 57.18 (CH_2), 110.83 (C-7), 115.64 (diCl-Ph-6), 115.83 (C-3), 117.50 (diCl-Ph-2), 122.26 (diCl-Ph-4), 125.71 (C-8), 130.77 (diCl-Ph-5), 133.11 (diCl-Ph-3), 136.44 (diCl-Ph-1), 141.58 (C-8a*), 151.17 (C-2*), 155.58 (C-6).

2-[4-(3,4-Dichlorophenyl)piperazin-1-ylmethyl]-6-(4-methylpiperazin-1-yl)imidazo[1,2-*b*]pyridazine (4b). Mp 178−180 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3), 2.58 (m, 4H, 6-pip), 2.72 (m, 4H, 2-pip), 3.22 (m, 4H, 2-pip), 3.53 (m, 4H, 6-pip), 3.76 (s, 2H, CH_2), 6.75 (dd, 1H, J = 8.9−2.9 Hz, diCl-Ph-6), 6.83 (d, 1H, J = 9.9 Hz, H-7), 6.97 (d, 1H, J = 2.9 Hz, diCl-Ph-2), 7.28 (d, 1H, J = 8.9 Hz, diCl-Ph-5), 7.67 (s, 1H, H-3), 7.69 (d, 1H, J = 9.9 Hz, H-8). ^{13}C NMR (50 MHz,$CDCl_3$) δ 46.59 (CH_3), 46.67 (6-pip), 49.00 (2-pip), 53.19 (2-pip), 55.01 (6-pip), 57.15 (CH_2), 110.29 (C-7), 115.65 (diCl-Ph-6), 115.88 (C-3), 117.51 (diCl-Ph-2), 122.29 (diCl-Ph-4), 125.94 (C-8), 130.78 (diCl-Ph-5), 133.12 (diCl-Ph-3), 136.55 (diCl-Ph-1), 141.95 (C-8a*), 151.16 (C-2*), 155.31 (C-6).

6-Bromo-2-(chloromethyl)imidazo[1,2-*a*]pyridine (6a). Typical procedure: a mixture of the conveniently substituted 2-aminopyridine (50 mmol), 1,3-dichloroacetone (50 mmol), and dimethoxyethane (30 mL) was stirred overnight at room-temperature. After which time, the resulting solid was collected and washed with several milliliters of dimethoxyethane. The solid was then dissolved in 150 mL of ethanol and refluxed for 2 h. The solvent was then removed under reduced pressure, and the residue was suspended in H_2O, made alkaline with Na_2CO_3, and extracted with CH_2Cl_2.

After drying ($CaCl_2$), the organic layers were evaporated to dryness. The residue was chromatographed on neutral alumina eluting with CH_2Cl_2; 53% yield (not optimized). Mp 125 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.77 (s, 2H, CH_2), 7.28 (dd, 1H, J = 9.6−1.8 Hz, H-7), 7.50 (d, 1H, J = 9.6 Hz, H-8), 7.61 (s, 1H, H-3), 8.26 (m, 1H, H-5).

2-(Chloromethyl)-6-iodoimidazo[1,2-*a*]pyridine (6b). 48% yield (not optimized). Mp 161 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.76 (s, 2H, CH_2), 7.37 (bs, 2H, H-8, H-7), 7.58 (s, 1H, H-3), 8.36 (m, 1H, H-5).

8-Bromo-2-(chloromethyl)-6-methylimidazo[1,2-*a*]pyridine (6c). The compound was obtained in 36% yield (not optimized). Mp >250 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.34 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 7.36 (d, 1H, J = 1.2 Hz, H-7), 7.66 (s, 1H, H-3), 7.87 (m, 1H, H-5).

2-(Chloromethyl)-8-iodo-6-methylimidazo[1,2-*a*]pyridine (6d). The compound was obtained in 43% yield (not optimized). Mp 146 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.31 (s, 3H, CH_3), 4.82 (s, 2H, CH_2), 7.58 (d, 1H, J = 1.2 Hz, H-7), 7.70 (s, 1H, H-3), 7.87 (m, 1H, H-5).

6-Bromo-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]imidazo-[1,2-*a*]pyridine (7a). To a solution of 1.23 g (5 mmol) of 6-bromo-2-chloromethylimidazo[1,2-*a*]pyridine (**6a**) and 0.97 g (5 mmol) of 1-(2-methoxyphenyl)piperazine in anhydrous ethanol (25 mL) was added 0.55 g (5 mmol) of sodium carbonate. The suspension was refluxed for 3 h. After cooling, the ethanol was removed in vacuo, the residue dissolved in water, and then extracted with dichloromethane. The organic layer was dried over calcium chloride, filtered, and evaporated to dryness. The residue was chromatographed on silica gel, eluting with dichloromethane to give 1.62 g (81%) of **7a**. Mp 108−109 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.85 (m, 4H, pip), 3.15 (m, 4H, pip), 3.84 (s, 2H, CH_2), 3.88 (s, 3H, CH_3O), 6.86−7.04 (m, 4H, CH_3O-Ph), 7.23 (dd, 1H, J = 9.5−1.8 Hz, H-7), 7.50 (d, 1H, J = 9.5 Hz, H-8), 7.58 (s, 1H, H-3), 8.26 (m, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 50.85 (pip), 53.84 (pip), 55.74 (CH_2), 56.86 (CH_3O), 107.19 (C-6), 111.52 (CH_3O-Ph-6), 111.90 (C-3), 118.51 (C-8), 118.63 (CH_3O-Ph-3), 121.38 (CH_3O-Ph-4), 123.33 (CH_3O-Ph-5), 125.93 (C-5), 128.12 (C-7), 141.67 (CH_3O-Ph-1), 143.94 (C-8a*), 144.86 (C-2*), 152.64 (CH_3O-Ph-2).

6-Iodo-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]imidazo-[1,2-*a*]pyridine (7b). To a solution of 2 g (6.83 mmol) of 6-iodo-2-chloromethylimidazo[1,2-*a*]pyridine (**6b**) and 1.56 g (6.83 mmol) of 1-(2-methoxyphenyl)piperazine hydrochloride in anhydrous ethanol (25 mL) was added 1.45 g (13.66 mmol) of sodium carbonate. The suspension was refluxed for 3 h. After cooling, the ethanol was removed in vacuo and the residue dissolved in water, and then extracted with dichloromethane. The organic layer was dried over calcium chloride, filtered, and evaporated to dryness. The residue was chromatographed on silica gel, eluting with dichloromethane to give 2.23 g (73%) of **7b**. Mp 78−80 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.79 (m, 4H, pip), 3.14 (m, 4H, pip), 3.80 (s, 2H, CH_2), 3.86 (s, 3H, CH_3O), 6.86 (dd, 1H, J = 8.2−1.4 Hz, CH_3O-Ph-6), 6.90−7.02 (m, 3H, CH_3O-Ph-3,4,5), 7.31 (dd, 1H, J = 9.6−1.8 Hz, H-7), 7.38 (d, 1H, J = 9.6 Hz, H-8), 7.51 (s, 1H, H-3), 8.34 (m, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 50.48 (pip), 53.41 (pip), 55.24 (CH_2), 56.42 (CH_3O), 74.71 (C-6), 110.68 (C-3), 111.11 (CH_3O-Ph-6), 118.10 (C-8), 118.40 (CH_3O-Ph-3), 120.89 (CH_3O-Ph-4), 122.71 (CH_3O-Ph-5), 130.28 (C-5), 131.96 (C-7), 141.34 (CH_3O-Ph-1), 143.52 (C-8a*), 144.49 (C-2*), 152.18 (CH_3O-Ph-2).

8-Bromo-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]-6-methylimidazo[1,2-*a*]pyridine (7c). The title compound was prepared, following the procedure used for **7b**, in 83% yield. Mp 149−150 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.32 (s, 3H, CH_3), 2.82 (m, 4H, pip), 3.15 (m, 4H, pip), 3.88 (s, 3H, CH_3O), 3.89 (s, 2H, CH_2), 6.86−7.05 (m, 4H, CH_3O-Ph), 7.30 (s, 1H, H-7), 7.60 (s, 1H, H-3), 7.86 (m, 1H, H-5).^{13}C NMR (50 MHz, $CDCl_3$) δ 18.25 (CH_3), 51.04 (pip), 53.89 (pip), 55.75 (CH_2), 57.15 (CH_3O), 111.00 (C-8), 111.55 (CH_3O-Ph-6), 112.79 (C-3), 118.58 (CH_3O-Ph-3), 121.35 (CH_3O-Ph-4), 122.43 (C-6), 123.08 (C-5), 123.25 (CH_3O-Ph-5), 130.00 (C-7), 141.78 (CH_3O-Ph-1), 142.39 (C-8a*), 144.93 (C-2*), 152.67 (CH_3O-Ph-2).

8-Iodo-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]-6-methylimidazo[1,2-*a*]pyridine (7d). The title compound was prepared, following the procedure used for **7b**, in 81% yield. Mp 143 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.28 (s, 3H, CH_3), 2.81 (m, 4H, pip), 3.14 (m, 4H, pip), 3.86 (s, 5H, CH_2, CH_3O), 6.86 (dd, 1H, J = 7.8−1 Hz, CH_3O-Ph-6), 6.89−7.02 (m, 3H, CH_3O-Ph-3,4,5), 7.51 (m, 1H, H-7), 7.62 (s, 1H, H-3), 7.84 (m, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.43 (CH_3), 50.53 (pip), 53.37 (pip), 55.22 (CH_2), 56.63 (CH_3O), 83.00 (C-8), 111.07 (CH_3O-Ph-6), 112.39 (C-3), 118.05 (CH_3O-Ph-3), 120.83 (CH_3O-Ph-4), 122.44 (C-6), 122.67 (C-5), 123.39 (CH_3O-Ph-5), 136.39 (C-7), 141.29 (CH_3O-Ph-1), 143.34 (C-8a*), 144.35 (C-2*), 152.15 (CH_3O-Ph-2).

6-Bromo-2-[4-(3,4-dichlorophenyl)piperazin-1-ylmethyl]imidazo[1,2-*a*]pyridine (7e). The title compound was prepared following the procedure used for **7a** using 1-(3,4-dichlorophenyl)-piperazine in 87% yield. Mp 58−59 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.70 (m, 4H, pip), 3.19 (m, 4H, pip), 3.75 (s, 2H, CH_2), 6.71 (dd, 1H, J = 8.9−2.8 Hz, diCl-Ph-6), 6.93 (d, 1H, J = 2.8 Hz, diCl-Ph-2), 7.20 (dd, 1H, J = 9.6−1.8 Hz, H-7), 7.24 (dd, 1H, J = 8.9 Hz, diCl-Ph-5), 7.46 (d, 1H, J = 9.6 Hz, H-8), 7.51 (s, 1H, H-3), 8.22 (m, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 48.83 (pip), 53.24 (pip), 56.66 (CH_2), 107.33 (C-6), 111.91 (C-3), 115.70 (diCl-Ph-6), 117.56 (diCl-Ph-2), 118.44 (C-8), 122.43 (diCl-Ph-4), 125.99 (C-5), 128.35 (C-7), 130.80 (diCl-Ph-5), 133.10 (diCl-Ph-3), 143.97 (C-8a*), 144.44 (C-2*), 150.98 (diCl-Ph-1).

8-Cyano-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]-6-methylimidazo[1,2-*a*]pyridine (8a). A screw-cap test tube was charged with 0.23 g (0.5 mmol) of **7d**, 29.4 mg (0.6 mmol) of sodium cyanide, and 9.52 mg (10 mol %) of copper(I)iodide. A Teflon septum was attached, and the tube was evacuated and backfilled with argon. The evacuated/backfield sequence was repeated an additional time. Then, 44 mg (0.5 mmol, 53.2μL) of *N*,*N*′-dimethyl-1,2-diaminoethane and toluene (1 mL) were added by syringe under argon. The screw-cap test tube was sealed with a cap, and the reaction mixture was stirred magnetically at 112 °C for 24 h. After cooling, the suspension was diluted with dichloromethane and then washed with sodium carbonate solution followed by water. The organic layer was dried over calcium chloride and evaporated to dryness. The residue was chromatographed on neutral alumina eluted with ethyl acetate−hexane (50: 50 v/v) to give 126 mg (70%) of **8a**. Mp 149−150 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.36 (s, 3H, CH_3), 2.80 (m, 4H, pip), 3.12 (m, 4H, pip), 3.85 (s, 3H, CH_3O), 3.86 (s, 2H, CH_2), 6.84−7.01 (m, 4H, CH_3O-Ph), 7.44 (d, 1H, J = 1.4 Hz, H-7), 7.60 (s, 1H, H-3), 8.07 (m, 1H, H-5). ^{13}C NMR (50 MHz, $CDCl_3$) δ 17.72 (CH_3), 50.58 (pip), 53.47 (pip), 55.32 (CH_2), 56.44 (CH_3O), 101.69 (C-8), 111.19 (CH_3O-Ph-6), 112.05 (C-3), 115.11 (CN*), 118.16 (CH_3O-Ph-3), 120.94 (CH_3O-Ph-4), 120.99 (C-6*), 122.85 (CH_3O-Ph-5), 127.35 (C-5), 133.79 (C-7), 141.34 (CH_3O-Ph-1*), 141.47 (C-8a*), 145.92 (C-2*), 152.27 (CH_3O-Ph-2).

6-Methyl-2-[4-(2-methoxyphenyl)piperazin-1-ylmethyl]-8-*N*-morpholinoimidazo[1,2-*a*]pyridine (8b). A screw-cap test tube was charged with 231 mg (0.5 mmol) of **7d**, 14.3 mg (15 mol %) of copper(I)iodide, and 212.3 mg (1 mmol) potassium phosphate. A Teflon septum was attached, and the tube was evacuated and back-filled with argon. The evacuated/backfield sequence was repeated two additional times. Then 47.9 mg (0.55 mmol, 48 μL) of morpholine and 62 mg (1 mmol, 55 μL) of ethyleneglycol and 2-propanol (1 mL) were added by syringe under argon. The screw-cap test tube was sealed with a cap, and the reaction mixture was stirred magnetically at 85 °C for 48 h. After cooling, the suspension diluted with dichloromethane and washed with sodium carbonate solution followed by water. The organic layer dried over calcium chloride and evaporated to dryness. The residue was chromatographed on neutral alumina eluted with ethyl acetate−hexane (50: 50 v/v) to give 151 mg (72%) of **8b**. oil. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.58 (s, 3H, CH_3), 2.81 (m, 4H, pip), 3.13 (m, 4H, pip), 3.51 (m, 4H, mor), 3.81 (s, 2H, CH_2), 3.85 (s, 3H, CH_3O), 3.97 (m, 4H, mor), 6.21 (s, 1H, H-7), 6.84−7.01 (m, 4H, CH_3O-Ph),

7.41 (s, 1H, H-5), 7.53 (m, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.44 (CH_3), 49.88 (mor), 50.60 (pip), 53.38 (pip), 55.35 (CH_2), 56.71 (CH_3O), 66.86 (mor), 109.23 (C-3), 111.20 (CH_3O-Ph-6, C-7), 116.79 (C-5), 118.25 (CH_3O-Ph-3), 120.98 (CH_3O-Ph-4), 121.70 (C-6), 122.83 (CH_3O-Ph-5), 139.48 (C-8a*), 139.98 (C-2*), 141.39 (CH_3O-Ph-1*), 152.30 (CH_3O-Ph-2), C-8 not found.

2-[4-(2-Methoxyphenyl)piperazin-1-ylmethyl]-6-methyl-8-(pyrazol-1-yl)imidazo[1,2-*a*]pyridine (8c). A screw-cap test tube was charged with 231 mg (0.5 mmol) of **7d**, 34 mg (0.5 mmol) of pyrazole, 5 mg (5 mol %) of copper(I)iodide, and 223 mg (1.1 mmol) potassium phosphate. A Teflon septum was attached, and the tube was evacuated and back-filled with argon, and the evacuated/backfield sequence was repeated two additional times. Then, 6.61 mg (15 mol %, 8 μL) of *N*,*N*′-dimethyl-1,2-diaminoethane and toluene (1 mL) were added by syringe under argon. The screw-cap test tube was sealed with a cap, and the reaction mixture was stirred magnetically at 112 °C for 24 h. After cooling, the suspension was diluted with dichloromethane and washed with sodium carbonate solution followed by water. The organic layer was dried over calcium chloride and evaporated to dryness. The residue was chromatographed on neutral alumina, eluting with ethyl acetate−hexane (70:30 v/v) to give 84 mg (42%) of **8c**. Mp 152 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 2.84 (m, 4H, pip), 3.14 (m, 4H, pip), 3.85 (s, 5H, CH_2, CH_3O), 6.50 (dd, 1H, J = 2.5−1.8 Hz, Pyr-4), 6.84−7.02 (m, 4H, CH_3O-Ph), 7.57 (s, 1H, H-3), 7.76 (d, 1H, J = 1.8 Hz, Pyr-3*), 7.76 (s, 1H, H-7), 7.80 (m, 1H, H-5), 9.51 (d, 1H, J = 2.5 Hz, Pyr-5*). ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.66 (CH_3), 51.01 (pip), 53.84 (pip), 55.77 (CH_2), 56.99 (CH_3O), 107.57 (Pyr-4), 111.52 (CH_3O-Ph-6), 112.40 (C-3), 116.13 (C-7), 118.64 (CH_3O-Ph-3), 121.07 (C-5), 121.38 (CH_3O-Ph-4), 122.24 (C-6), 123.35 (CH_3O-Ph-5), 128.38 (C-8), 132.76 (Pyr-5*), 137.57 (C-8a), 141.54 (CH_3O-Ph-1*), 141.71 (Pyr-3*), 143.58 (C-2), 152.67 (CH_3O-Ph-2).

2-[4-(2-Methoxyphenyl)piperazin-1-ylmethyl]-5-(pyrazol-1-yl)imidazo[1,2-*a*]pyridine (8d). A screw-cap test tube was charged with 0.2 g (0.5 mmol) of **7a**, 34 mg (0.5 mmol) of pyrazole, and 342 mg (1.05 mmol) of cesium carbonate. A Teflon septum was attached, and the tube was evacuated and back-filled with argon, and the evacuated/backfield sequence was repeated an additional time. *N*,*N*-Dimethylformamide (1 mL) was added by syringe under argon. The screw-cap test tube was sealed with a cap, and the reaction mixture was stirred magnetically at 112 °C for 24 h. After cooling, the suspension was taken up in water, extracted with dichloromethane. The organic layer was washed three times with water, dried over calcium chloride, and evaporated to dryness. The residue was chromatographed on neutral alumina eluted with ethyl acetate−hexane (60:40 v/v) and then ethyl acetate to give the remaining 100 mg (50%) of starting material **7a** and 52 mg (27%) of **8d**. Mp 160−161 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.86 (m, 4H, pip), 3.17 (m, 4H, pip), 3.87 (s, 5H, CH_3O, CH_2), 6.62 (dd, 1H, J = 2.4−2.1 Hz, Pyr-4), 6.87 (d, 1H, J = 7.3 Hz, H-6), 6.85−7.05 (m, 4H, CH_3O-Ph-3,4,5,6), 7.29 (dd, 1H, J = 9−7.3 Hz, H-7), 7.69 (d, 1H, J = 9 Hz, H-8), 7.90−7.95 (m, 3H, Pyr-3,5, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 50.85 (pip), 53.78 (pip), 55.70 (CH_2), 56.87 (CH_3O), 106.81 (C-6), 108.34 (Pyr-4), 110.31 (C-3), 111.45 (CH_3O-Ph-6), 117.31 (C-8), 118.60 (CH_3O-Ph-3), 121.34 (CH_3O-Ph-4), 123.24 (CH_3O-Ph-5), 124.22 (C-7), 131.45 (Pyr-5*), 133.41 (C-5), 141.75 (CH_3O-Ph-1), 143.01 (Pyr-3*), 144.38 (C-2*), 146.65 (C-8a*), 152.61 (CH_3O-Ph-2).

2-[4-(2-Methoxyphenyl)piperazin-1-ylmethyl]-6-methyl-8-phenylimidazo[1,2-*a*]pyridine (8e). A mixture of 207.5 mg (0.5 mmol) of **7c**, 67 mg (0.5 mmol) of phenylboronic acid, 29 mg (5 mol %) of tetrakis(triphenylphosphine)palladium, and 40 mg (1 mmol) of sodium hydroxide was suspended in 1,2-dimethoxyethane (4 mL) and water (2 mL) and heated at 85 °C for 3 h. After cooling and stirring at room temperature for 17 h, dichloromethane was added, and the resulting mixture was washed with water. The organic layer was dried over calcium chloride and evaporated to dryness. The residue was chromatographed on neutral alumina eluted with ethyl acetate−hexane (70:30 v/v) to give 112 mg (54% not optimized) of **8e**. Mp 59−60 °C. ^{1}H NMR (360 MHz, $CDCl_3$) δ 2.38 (s, 3H, CH_3), 2.88 (m, 4H, pip), 3.18 (m, 4H, pip), 3.89 (s, 5H, CH_3, CH_2), 6.89 (d, 1H, J = 7.7 Hz, CH_3O-Ph-6), 6.94−7.08 (m, 3H, CH_3O-Ph-3,4,5), 7.14 (d, 1H, J = 1.4 Hz, H-7), 7.38−7.56 (m, 3H, Ph-3,4,5), 7.58 (s, 1H, H-3), 7.88 (H-5), 8.04 (m, 2H, Ph-2,6). ^{13}C NMR (50 MHz, $CDCl_3$) δ 18.61 (CH_3), 51.02 (pip), 53.81 (pip), 55.77 (CH_2), 57.15 (CH_3O), 111.56 (CH_3O-Ph-6), 111.67 (C-3), 118.66 (CH_3O-Ph-3), 121.39 (CH_3O-Ph-4), 122.06 (C-6), 122.78 (C-5), 123.29 (CH_3O-Ph-5), 126.35 (C-7), 128.59 (C-8), 128.92 (Ph-3,5), 129.30 (Ph-2,6), 129.73 (Ph-4), 137.03 (Ph-1), 141.78 (CH_3O-Ph-1), 143.24 (C-8a*), 143.73 (C-2*), 152.69 (CH_3O-Ph-2).

Receptor Binding Experiments. Receptor binding experiments were carried out as described in the literature.[37] In brief, the dopamine D_1 receptor assay was done with porcine striatal membranes at a final protein concentration of 40 μg/assay tube and the radioligand [^{3}H]SCH 23390 at 0.3 nM (K_d = 0.62−1.1 nM). Competition experiments with human D_{2long}, D_{2short}, D_3, and $D_{4.4}$ receptors were run with preparations of membranes from CHO cells expressing the corresponding receptor and [^{3}H]spiperone at a final concentration of 0.1 nM. The assays were carried out with a protein concentration of 3−20 μg/assay and K_d values of 0.06−0.21 nM for D_{2long}, 0.07−0.15 nM for D_{2short}, 0.10−0.35 nM for D_3, and 0.10−0.50 nM for $D_{4.4}$. The determination of the binding affinity to the agonist binding site of the D_2 receptor was done with the radioligand [^{3}H]7-OH-DPAT at 0.5 nM (K_d = 2.5 nM for D_{2long}; K_d = 1.6 nM for porcine D_2) and a protein concentration of 10 μg/assay and 100 μg/assay for D_{2long} and porcine D_2, respectively.

The investigation of 5-HT_{1A} and 5-HT_2 binding was performed as described in the literature.[40] In brief, porcine cortical membranes were subjected to the binding assay at a concentration of 80−110 μg/assay tube for the determination of 5-HT_{1A} and 5-HT_2 binding using [^{3}H]8-OH-DPAT and [^{3}H]ketanserin, each at a final concentration of 0.5 nM with K_d values of 1.1−4.1 nM (for 5-HT_{1A}) and 1.9−4.8 nM (for 5-HT_2). The protein concentration was established by the Lowry method using bovine serum albumin as the standard.[48]

Data analysis of the resulting competition curves was accomplished by nonlinear regression analysis using the algorithms in PRISM (GraphPad Software, San Diego, CA). The K_i values were derived from the corresponding EC_{50} data using the equation of Cheng and Prusoff.[49]

Mitogenesis Experiments. The determination of the stimulating effects of the test compounds on mitogenesis as a functional assay was done with the CHO 10001 cell line stably expressing the human dopamine $D_{4.2}$ receptor as referred to in the literature.[38,40] In detail, 5000 cells/well were grown for 72 h in a 96 well plate in the appropriate medium supplemented with serum. After washing and adding a serum free medium, the cells were incubated for 20 h with the test compound at 8 different concentrations in the range of 0.01−10 000 nM as hexaduplicates. After the addition of 0.02 μCi/well of [^{3}H]thymidine (specific activity 25 Ci/mmol, Amersham Biosciences), the incubation of the D_4 expressing cells was continued for 2 h. Finally, the cells were harvested, and the incorporated radioactivity was measured with a microplate scintillation counter. Experimental data resulting from the mitogenesis assay were each normalized and then combined to get a mean curve. A nonlinear regression analysis of this curve provided the EC_{50} values as a measure of potency. The top value of the curve represented the maximal intrinsic activity, which was correlated to the effect of the full agonist quinpirole (100%).

Determination of [^{35}S]GTPγS Binding. The [^{35}S]GTPγS binding assay was established on the basis of literature.[41] The preparation of membranes from CHO cells stably expressing the human $D_{4.4}$ and the human D_{2short} receptors, with a receptor density of B_{max} = 2.25 and 6.23 pmol/mg protein, were diluted in HEPES buffer (20 nM HEPES, 10 nM $MgCl_2$, 100 nM NaCl; pH 7.4) and incubated at 37 °C with 1 μM GDP in HEPES buffer and the test compounds (in HEPES buffer supplemented with 0.1 nM dithiothreitol) in eight different concentrations (0.01−10 000 nM) as hexaduplicates at a final volume of 200 μL in 96-well microplates. After 30 min, 0.1 nM [^{35}S]GTPγS (specific activity 1250 Ci/mmol, Perkin-Elmer)

was added, and incubation continued for a further 30 min. The experiment was terminated by rapid filtration through GF/B filters using an automated cell harvester (Brandel), and the filters were washed five times with ice-cold washing buffer (140 mM NaCl, 10 mM KCl, 1.5 mM KH_2PO_4, 8 mM Na_2HPO_4; pH 7.4) and dried at 60 °C for 3 h, and the trapped radioactivity was counted in a microplate scintillation counter (MicroBeta Trilux, Perkin-Elmer). To confirm the efficacy derived from the dose−response curves, the experiments were repeated with representative concentrations of the test compounds (0.01 and 1000 nM) in comparison with the effect of 10 000 nM quinpirole. For all experiments, the maximum stimulation induced with 10 000 nM quinpirole was about 35% and 150% over basal activity for the D_4 and D_{2short} receptors, respectively. Data analysis was done in the same manner described for the mitogenesis experiments (see above) comparing the derived EC_{50} values and efficacy to the effect of the reference compound quinpirole.

Penile Erection Experiments. The effect of **3c** on penile erection was studied in male rats as described in the literature.[50,51] Sprague−Dowley male rats (225−250 g, Harlan Nossan, Italy) were housed at 24 °C and 60% humidity, with water and standard laboratory food ad libitum. Compound **3c** and PD-168077 were dissolved in ethanol and diluted with distilled water (0.3% ethanol concentration) and injected s.cly in a volume of 0.2 mL/rat or icvly in a volume of 10 μL/rat. For icv injections, a chronic stainless guide cannula (28 gauge) was implanted stereotaxically in the skull of the animals (coordinates A 1, L 1, and V 5 [52]) under chloral hydrate anaesthesia three days before the experiments. The day of the experiments, the male rats were individually placed in Plexiglas cages ($30 \times 30 \times 30$ cm^3) for 30 min and injected s.cly or icvly and observed for 60 min to count penile erection episodes. When haloperidol, clozapine, or L-745,870 was used, the compound was dissolved in saline and administered by i.p. injections 15 min before the D4 agonists were administered. Penile erection was scored when the penis emerged from the penile sheath, which was usually accompanied by penile grooming and hip flexion. At the end of the experiments, the rats were sacrificed by decapitation, and the brains were removed and stored in 2% aqueous formaldehyde for 12−15 days. To localize the position of the tip of the injection cannula, 50 μm transverse brain sections were prepared by means of a freezing microtome stained with Neutral Red and inspected on a phase contrast microscope. The site of the probe tip was localized by following the probe tract through a series of brain sections. Only those animals found to have the probe tip correctly positioned icvly were considered for the statistical evaluation of the results (Student's *t* test).

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Supporting Information Available: X-ray crystallographic data for compound **3a** and purity data of compounds **3a**−**i**, **4a**−**b**, **7a**−**e**, and **8a**−**e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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