Discovery of 2-(4-Pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (ABT-724), a Dopaminergic Agent with a Novel Mode of Action for the Potential Treatment of Erectile Dysfunction

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A new class of agents with potential utility for the treatment of erectile dysfunction has been discovered, guided by the hypothesis that selective D_4 agonists are erectogenic but devoid of the side effects typically associated with dopaminergic agents. The lead agent 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (**1**, ABT-724) was discovered by optimization of a series of benzimidazole arylpiperazines. This highly selective D_4 agonist was found to be very potent and efficacious in vivo, eliciting penile erections in rats at a dose of 0.03 μmol/kg, with a positive response rate of 77% erectile incidence. Even at high doses, it was devoid of side effects in animal models of central nervous system behaviors, emesis, or nausea. The structure−activity relationship of the parent benzimidazole series leading to **1** is described, with the detailed in vitro and in vivo profiles described. Distinctive structural features were discovered that are associated with D_4 selective agonism in this series of analogues.

Introduction

Physicians and the public have recently acknowledged the need for safe and effective pharmacological treatments for erectile dysfunction (ED). This is a consequence of the increased recognition of the prevalence of ED in the population and the influence of the disorder on the lives of patients and their partners. We have discovered that selective dopaminergic D_4 agonists are highly efficacious in facilitating penile erections in animals and yet are free of the side effects of other known classes of dopaminergics. 2-(4-Pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (**1**, ABT-724) is a particularly potent and selective D_4 agonist in vitro and in vivo (Chart 1). The properties of this compound are described in detail, along with the SAR of the related series of benzimidazole analogues.

Dopamine (DA) agonists have been associated with the induction of erectile effects in animals[1] and in humans, but these agonists can also produce side effects that would compromise their clinical utility for treatment of this indication. For example, seven of eight dopamine agonists clinically in use or investigated for their beneficial effects on Parkinson's disease can induce nausea, among other side effects.[2] For example, L-DOPA, the anti-Parkinsonian dopamine precursor, has been reported to be associated with erectogenesis in humans.[1] Quinelorane (**2**, LY-163502) has been described as a D_2 agonist with erectogenic properties in primates, yet it failed to complete clinical trials for this indication because of side effects.[1,3] Apomorphine is the only dopaminergic agonist currently in clinical use for treating ED. This agent is a potent but nonselective dopamine agonist. It has long been recognized to induce erections in animals, yet in humans and animals there have been reports of the production of emetic effects.[4] The general opinion expressed in the literature[1,5] has been that the erectogenic effects of apomorphine and other dopaminergic agonists are mediated by D_2 receptors and that since stimulation of this receptor also induces nausea, dopaminergic agonists will by necessity be plagued by this side effect. Contrary to this, we have recently found that D_4 selective dopaminergic agonists are able to produce erectogenesis in animal models without nauseagenic or emetic side effects,[6] and in this report, we provide a specific demonstration with compound **1**.

Many classes of compounds have been described (Chart 1) with dopamine receptor modulating activity at one or more dopamine receptor subtypes (D_{1-5}). Examples include the selective D_2 agonist PNU-95666E (**3**), investigated as a treatment for Parkinson's disease,[7] spiperone (**4**), which is a D_2/D_4 antagonist, and clozapine (**5**), which is an antipsychotic agent with potent D_4 antagonist activity. In the past, most of the interest in D_4 ligands was motivated by the hypothesis that selective D_4 antagonists such as L-745870 (**6**) might treat symptoms of schizophrenia.[8] However, though many D_4 selective antagonists have been discovered, none of them have elicited the desired antipsychotic effects when tested in clinical trials, and indeed, the hypothesis has lately been questioned.

Our strategy for the design of potent and selective D_4 agonists was to examine the SAR of known classes of dopaminergic agents, to prepare and test analogues of these in in vitro functional assays, and to use the

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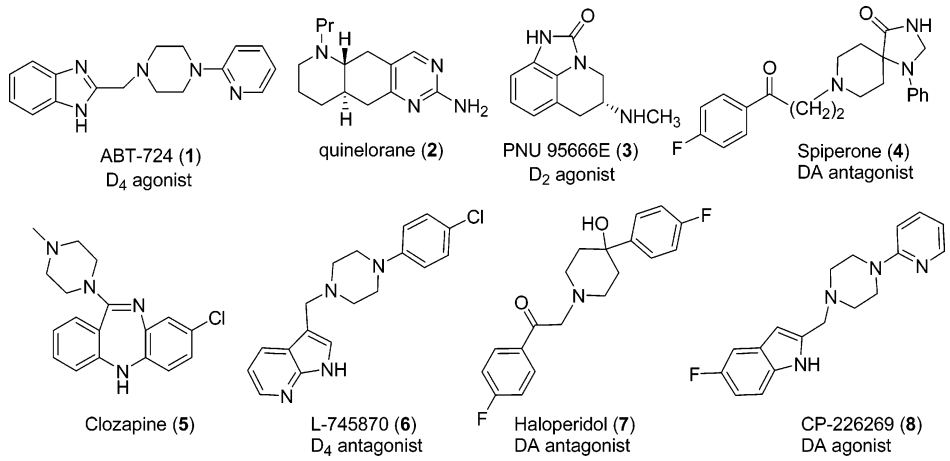
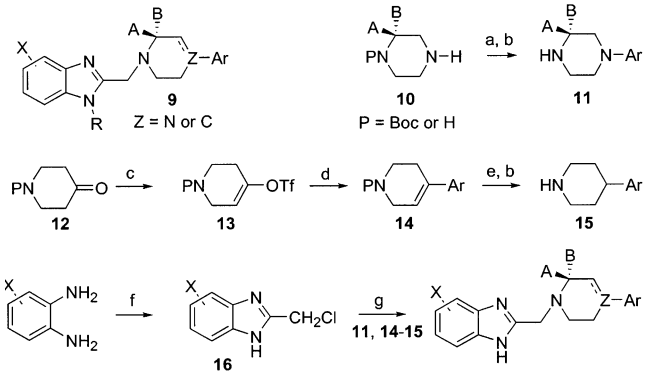
Chart 1. Structures of Dopaminergic Agents Discussed in the Text



Scheme 1[a]



[a] Reagents and conditions: (a) Pd_2dba_3, BINAP or P(*t*-Bu)$_3$, NaOtBu, Δ; (b) CF_3CO_2H; (c) LDA, −78 °C, $PhNTf_2$; (d) ArZnX, $Pd(PPh_3)_4$, Negishi cross-coupling conditions; (e) H_2, PtO_2; (f) CH_2ClCO_2H in aqueous HCl, Δ; (g) **11**, **14**, or **15**, DMF, Et_3N.

resulting data to elucidate (1) factors that differentiate agonists from antagonists and (2) factors that enhance D_4 receptor potency and selectivity.

Arylpiperazines and piperidines have a history as ligands of many types of G-protein-coupled receptors (GPCRs), for example, of serotonin, opiate, and dopamine receptors. The arylpiperazine moiety is found in the selective D_4 antagonist **6** and also in many other less selective dopamine antagonists such as haloperidol (**7**). CP-226269 (**8**) has been described in a patent application as a D_4 agonist,[9] though pharmacological data characterizing the agonism were not disclosed. We prepared a family of compounds (structure **9**, Scheme 1) in which an arylpiperazine/piperidine moiety was attached through a methylene chain to the 2-position of a benzimidazole[10] and found that compounds bearing substituents at the ortho position of the aryl moiety in **9** were potent D_4 agonists.

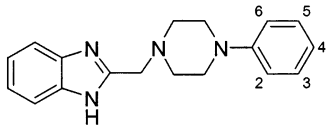
Results and Discussion

Chemistry. Compounds were synthesized by the methods depicted in Scheme 1. Many arylpiperazines (**11**) are commercially available; where they were not commercially available, they were easily prepared by heating either piperazine or BOC-protected piperazine with aryl halides or heteroaryl halides,[10] optionally in the presence of a palladium[11] source and base to give **11**. When BOC-piperazine was used as the starting material, treatment of the intermediate products with acid was required to liberate the arylpiperazines. The BOC-protected aryltetrahydropyridines were accessed by converting 4-oxopiperidine-1-carboxylic acid *tert*-butyl ester (**12**) to its *O*-triflyl enolate (**13**) and then subjecting the intermediates to a Pd-catalyzed Negishi cross-coupling to yield **14**. Hydrogenation of **14** gave the corresponding arylpiperidine, which, like **14**, was treated with acid to remove the BOC group prior to incorporation into the final product. The reaction of 2-chloromethylbenzimidazoles (**16**) with different arylpiperazines **11** gave analogues **17**−**45**, while reaction with **14** gave analogues **60** and **61** and reaction with **15** gave analogues **58** and **59**. Where substituted 2-chloromethylbenzimidazoles were required, but not commercially available, heating the appropriate 1,2-diaminobenzene in hydrochloric acid with chloroacetic acid gave the desired intermediates **16**, which were then treated with **11** to give analogues **46**−**48**. Alkylation of the benzimidazole nitrogen of compound **1** with base and alkyl halides gave **50**−**53**, while acylation of the benzimidazole nitrogen of **1** with alkyl chloroformates gave **54** and **55**.

Assessment of the in Vitro Activity of Compounds. The ability of compounds to act as D_4 agonists was tested in an in vitro Ca^{2+} flux assay based on FLIPR (fluorometric imaging plate reader) methodology. In this assay, D_4 agonists elicit a fluorescent response in cells cotransfected with human D_4 receptor[12] and a G protein ($G\alpha_{qo5}$) by inducing an intracellular Ca^{2+} accumulation and a subsequently measurable increase in the fluorescence of a Ca^{2+}-specific dye. In the analysis of the results of the assay seen in Table 1, it is noted that the interaction with an agonist ligand with a receptor has two very important but independently measurable features: (1) E_{max}, the agonist efficacy, and (2) EC_{50}, the agonist potency.[13,14] Table 1 shows the agonist efficacy as the maximal efficacy of analogues, with full efficacy (100%) standardized to 10 μM dopamine. Also seen is the compound potency expressed as the EC_{50}, the concentration of a compound giving half its maximal D_4 specific fluorescence.

SAR of Agonist Potency (Table 1). The agonist efficacy of a compound is a measure of its ability to activate the receptor in a particular assay system. Examination of the arylpiperazine moiety of the benzimidazoles **17**−**45** revealed a clear SAR for agonist

Table 1. In Vitro Activity in FLIPR Assays, Using HEK-293 Cells Cotransfected with Human $D_{4.4}$ Receptor and G-Protein $G\alpha_{qo5}$



	human D_4 FLIPR		aromatic ring substituent				
compd	$EC_{50} \pm$ SEM,[a] nM	% efficacy[b]	2	3	4	5	6
1	12.4 ± 1.0	61	aza				
17	30.6 ± 14.4	33					
18	14.6 ± 5.6	46	CN				
19	24 ± 7	27	aza				F
20	13.9 ± 4.3	50	aza				CN
21	7.2 ± 4.4	39	Cl				
22	3.9 ± 3.5	40	NO_2				
23	14.7 ± 7.3	42	F				
24	12.4 ± 4.5	48	OCH_3				
25	25[c]	31	OEt				
26	86 ± 40	42	SCH_3				
27	44.8 ± 12.7	49	aza				aza
28	136 ± 13	27	SO_2CH_3				aza
29	36.2 ± 9.9	36	aza	CH_3			
30		na	CH_2CH_3				
31		na	CH3				CH_3
32	antagonist, $K_i = 3.8$		CH_3			CH_3	
33	antagonist, $K_i = 1.5$			CH_3	CH_3		
34		na	OH				
35		na	aza	CF_3			aza
36		na	aza		Et		aza
37		na	aza	aza	Cl		
38		na	aza	aza			
39		na	aza				NO_2
40		na	aza				Cl
41		na	aza	aza	OH		
42		na	aza	SCH_3			
43		na	CH_2−2-pyridyl				
44		na	CH_2 CH_2−2-pyridyl				
45		na	cyclohexyl				

[a] EC_{50} (nM) for agonists (SEM, $n \geq 3$), K_i (nM) versus spiperone. [b] Efficacy relative to 10 μM dopamine (100%). na = not an agonist up to 10 μM. [c] Single determination; $n = 1$.

efficacy. The unsubstituted phenyl analogue **17** proved to be an agonist. Of the rest of the compounds, only the compounds bearing small ortho substituents or ortho ring nitrogens, or both, were found to be agonists. Compared to the unsubstituted phenyl ring analogue **17**, many of the ortho-substituted analogues had higher agonist efficacy. For example, while analogue **17** had an E_{max} of 33%, the 2-pyridyl analogue (**1**) was more than twice as effective ($E_{max} = 61\%$) in stimulating the receptor.

Although a small substituent or a nitrogen atom at the ortho position of the phenyl ring was necessary to induce agonist activity, that alone was insufficient for agonism. Benzimidazole analogues bearing a substituent or incorporating a nitrogen atom into the aromatic ring at the meta or the para position (**32**−**42**) were found to be nonagonists. Furthermore, the effect of such a substitution was dominant, overcoming even the most favorable ortho substituents. The only exception to this trend was seen in compound **29** ($E_{max} = 36\%$). Yet even here, the addition of a single methyl substituent reduced the agonist efficacy to only 36%, much lower than in compound **1** (61%).

SAR of Agonist Potency. The potency of the agonist interaction with the D_4 receptor is the second independently measurable parameter describing the interaction of the ligands with the receptor and is reported in the Table 1 as the EC_{50} value. Most of the ortho-substituted agonists had comparable potencies in the range 15−25 nM. The 2-Cl and 2-NO_2 substituted analogues, **21** and **22**, were somewhat more potent ($EC_{50} = 7.2$ and 3.9 nM, respectively), though they were not the most efficacious agonists in activating the receptor, having a maximal efficacy of 40%. The two least potent agonists, **26** (86 nM) and **28** (136 nM), were also the compounds with the largest ortho substituents on the phenylpiperazine moiety (SCH_3, SO_2CH_3), which may indicate that compounds with even larger substituents might be less potent agonists.

In the in vitro assay described here, none of the new compounds activated the receptor as effectively as the natural agonist dopamine; i.e., all agonist compounds were partial agonists. However, even though compounds that activated the receptor with efficacies of less than 25% were defined to be nonagonists, there is the possibility that some compounds could act as antagonists, potently binding to, but not activating, the receptor. When tested, some compounds were indeed able to antagonize receptor activation by dopamine (10 μM) in the FLIPR assay, with nanomolar IC_{50} values. For example, **32** ($IC_{50} = 1$ nM) and **33** ($IC_{50} = 6$ nM) were both able to antagonize receptor activation by dopamine. To better characterize the interaction of these two compounds with the $D_{4.4}$ receptor, the inhibition constants (K_i) of these were determined in a competitive binding assay against the known dopamine ligand [^{3}H]-

Chart 2. Benzimidazole Analogues of **1**

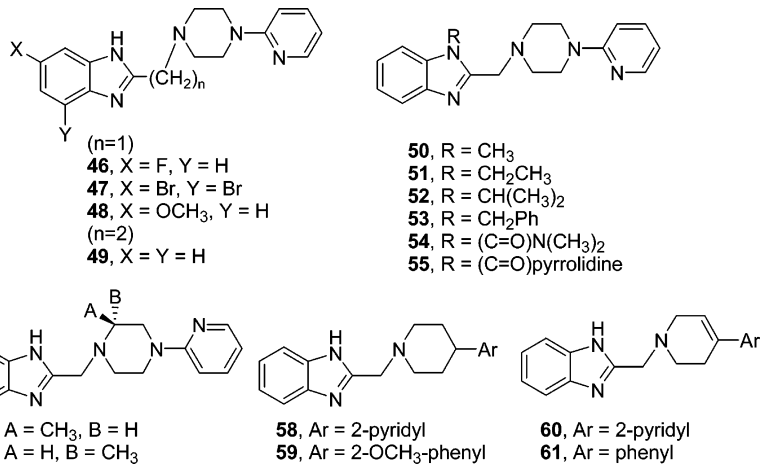


Table 2. In Vitro Activity in FLIPR Assays, Using HEK-293 Cells Cotransfected with Human $D_{4.4}$ Receptor and G-Protein $G\alpha_{qo5}$

	human D_4 FLIPR	
compd	EC_{50} ± SEM,[a] nM	% efficacy[b]
46	8 ± 4	57
47	416 ± 20	37
48		na
49	23 ± 13	62
50		na
51		na
52		na
53		na
54		na
55		na
56	52 ± 14	43
57	64 ± 21	34
58	14 ± 3	51
59	100 ± 64	34
60	11 ± 2	63
61	228 ± 80	48

[a] EC_{50} (nM) for agonists (SEM, $n \geq 3$). [b] Efficacy relative to 10 μM dopamine (100%). na = not an agonist up to 10 μM.

spiperone. Here, **32** showed a K_i of 3.8 nM, while **33** showed a K_i of 1.5 nM. Compounds **32** and **33** may be useful pharmacological tools for probing the physiological role of D_4 receptors, since they not only are potent and selective D_4 antagonists but have good oral bioavailability in rats as well (data not shown).

Benzimidazole analogues similar to those shown in Table 1 have also been reported by Pugsley et al.,[15] who determined the K_i of a series of six meta and para substituted benzimidazole analogues similar to those described. Interestingly, compound **17** was among the compounds tested, and it was found to competitively displace [^{3}H]-spiperone in a binding assay with a K_i of 33 nM at the $D_{4.2}$ receptor in expressed in a Chinese hamster ovary cell line.

SAR of Other Positions. To study the effect of substitution of the benzimidazole ring, several analogues were synthesized (**46**−**61**, Chart 2). The 5-fluorobenzimidazole analogue **46** (EC_{50} = 8 nM, 57% efficacy) had agonist activity comparable to analogue **1**, whereas the dibrominated benzimidazole **47** was both less potent and less efficacious (416 nM, 37%), as seen in Table 2. In contrast, the incorporation of the methoxy group in the benzimidazole ring of **48** eliminated agonist activity. Whether this effect was due to an electronic effect (fluorine is electron-withdrawing, while methoxy is electron-donating) or a steric effect remains to be investigated.

Insertion of one or two methylenes between the piperazine nitrogen and the pyridine ring of compound **1** also eliminated activity, as seen in compounds **43** and **44**. However, potent agonist activity was retained in compound **49** (23 nM, 62%), which has an extra methylene between the distal piperazine nitrogen and the benzimidazole ring. Compounds with alkyl groups (**50**−**52**) or with a benzyl group (**53**) on the benzimidazole nitrogen had no activity as agonists, and likewise, the benzimidazole N-substituted ureas **54** and **55** had no agonist activity. Appending a methyl group onto the piperazine ring allowed retention of receptor agonist activity, as seen in the enantiomers **56** (52 nM, 43%) and **57** (64 nM, 34%).

Typically, aminergic GPCR ligands require at least one basic amine, which is thought to interact with an aspartate in the receptor.[16,17] The weakly basic aniline nitrogen of the arylpiperazine ring was not found to be required, since the piperidine analogue **58** (Ar = 2-pyridyl, 14 nM, 51%) retained in vitro potency and efficacy comparable to those of **1** (12.4 nM, 61%). The 2-methoxyphenylpiperidine analogue **59** (100 nM, 34%) showed 8-fold lower potency compared to the 2-methoxyphenylpiperazine **24** (12.4 nM, 48%). The presence of the double bond in the tetrahydropiperidine ring was examined, where analogue **60** (11 nM, 63%) had in vitro potency and efficacy similar to those of **1**. However, the phenyltetrahydropyridine analogue **61** (228 nM, 48%) was about 7-fold less potent than the corresponding phenylpiperazine homologue **17**. These results support the hypothesis that only the distal, more basic nitrogen of the arylpiperazine moiety in compound **1** is required for activity.

In Vitro Properties at D_4 and at Other Receptors. Potent binding to the molecular target is a desirable feature of a drug candidate, as is high selectivity, to minimize the potential for off-target side effects. During the evaluation of reference compounds, two important in vivo findings provided the motivation to target agents with high selectivity for the D_4 receptor, compared to the D_2 receptor. First, nonselective dopaminergic agonists with both D_2 and D_4 agonistic activity were found to induce erections in rats, but they also induced emesis in a sensitive ferret model.[6,18] Second,

Table 3. In Vitro Agonist Activity in Cellular FLIPR Assays, Using HEK Cells Cotransfected with the Target Receptor and G Protein $G\alpha_{qo5}$

	$EC_{50} \pm SEM$,[a] nM					
agonist	human D_4	ferret D_4	rat D_4	human D_2	ferret D_2	rat D_2
1	12.4 ± 1.0 (61%)	23.2 ± 1.3 (64%)	14.3 ± 0.6 (70%)	na	na	n.a.
8	32 ± 7 (46%)	22 ± 3 (72%)	36 ± 3 (91%)	na	527 ± 88 (55%)	55 ± 5.3 (89%)
dopamine	2.2 ± 0.2 (100%)	2.7 ± 0.3 (100%)	2.4 ± 0.2 (100%)	18 ± 2 (100%)	8 ± 0.4 (100%)	1.1 ± 0.06 (100%)
apomorphine	4.3 ± 0.2 (84%)	1.5 ± 0.1 (84%)	5.5 ± 0.3 (87%)	5.8 ± 0.3 (86%)	1.8 ± 0.2 (91%)	0.4 ± 0.01 (103%)
3, PNU 95666E	n.a.	na	na	165 ± 11.5 (93%)	35 ± 4 (90%)	10.8 ± 1 (103%)

[a] % efficacy relative to 10 μM dopamine is indicated in parentheses. na = not an agonist up to 10 μM. Effects were not seen in untransfected cells.

it was found that D_2 selective agonists induced emesis in ferrets but did not induce erections in rats.[6] Taken together, the observations provided support for the hypothesis that a selective D_4 agonist would be erectogenic but free of emetic effects. It was found that all of the agonists in Table 1 selectively activated D_4 receptors, compared to D_2 receptors. Compound **1** was chosen for a more detailed examination of its selectivity as a D_4 agonist in FLIPR assays of human, rat, and ferret receptors (Table 3).

Many dopaminergic agonists described in the literature are active at the D_2 receptor, including apomorphine. On the basis of their emetic effects in ferrets, D_2 agonists were expected to produce nausea and other effects. However, antagonistic activity at D_2 receptors is also undesirable, since D_2 antagonists (e.g., typical antipsychotic agents) are associated with prolactin secretion and dyskinesias with chronic use in humans. The D_4 binding affinity of **1** was assessed by competitive displacement of the reference agent spiperone, where it was found that **1** displaced spiperone with a K_i of 160 ± 7 nM. In binding assays versus the radiolabeled D_2 antagonist 7-OH-PIPAT in the human long and short isoforms of the D_2 receptor, **1** showed negligible binding for the human D_{2s} and D_{2L} receptor isoforms (K_i = 51 000 nM and K_i = 15 000 nM, respectively). When screened for activity against more than 70 different receptors, ion channels, and neuronal receptors (CEREP), **1** was exceptionally selective in that it did not bind to other receptors (K_i > 10 μM) except for weak (K_i = 2780 ± 640 nM) binding to 5-HT_{1a} receptors. In contrast, other compounds showed less selectivity for D_4 receptors than for other receptors. For example, **8** (CP-226269) showed a K_i values of 120 nM for human D_2 receptors, 560 nM for D_3 receptors, 3.6 nM for $D_{4.4}$ receptors, 300 nM for κ opiate receptors, 200 nM for 5-HT_{1a} receptors, and 400 nM for α_2 receptors. In FLIPR assays (Table 3) compound **8** showed little selectivity for rat D_4 receptors (36 nM, 91%) over rat D_2 receptors (55 nM, 89%). The in vitro studies of D_4 versus D_2 selectivity in other agents also suggest an explanation for both the reported in vivo activity of quinelorane (**2**), an agent that entered trials as an anti-impotence agent, and its failure to advance. The potent human D_4 agonism of quinelorane (EC_{50} = 26.5 nM, 98% efficacy) would be consistent with the reported erectogenic ability.[3] However, in view of its even more potent human D_2 agonism (EC_{50} = 10.3 nM, 98% efficacy), it could also be predicted that unavoidable D_2-mediated side effects such as nausea and emesis would be present.

In Vivo Studies. Twelve of the benzimidazole analogues with potent and efficacious D_4 agonism in vitro were tested in vivo in a rat penile erection assay, where all increased the incidence of erections (Table 4). This assay[18] measures the ability of a test compound to increase the number of naturally occurring spontaneous erections in conscious, freely mobile rats. Animals (n = 8−30) are observed over 60 min, with or without drug (administered subcutaneously). The background incidence rate of rat penile erection observed in the absence of drug was 20%, and all of the D_4 agonist compounds significantly increased the rate of erectile incidence at maximally efficacious doses. The erectogenic activity of compounds was dose-dependent and reproducible. While different compounds did have different in vivo potencies, with the doses required to elicit the maximal increase in erectile incidence for different compounds of the series ranging widely, from 0.003 to 1 μmol/kg, all of the compounds tested reliably enhanced the erectile incidence to at least 60−80%. The highly selective D_4 agonist **1** induced a 77% incidence of erections in male rats at a maximally efficacious dose of 0.03 μmol/kg. In this model, the well-known nonselective dopamine agonist apomorphine also efficiently induced erections in rats at a high incidence rate (91%), with a maximally efficacious dose of 0.1 μmol/kg. Thus, **1** is effective in this model but 3-fold more potent than apomorphine. The erectogenic effects of compound **1** were determined to be due to activation of central[6] rather than peripheral D_4 receptors, since the erectile effects were completely prevented by coadministration of the central nervous system (CNS) permeating selective D_4 antagonist clozapine but not by the peripherally selective dopamine antagonist domperidone.[6a,c]

Table 4. In Vivo Proerectile Activity of Compounds in Rats after More Than 60 min Following Subcutaneous Injection

compd	maximally effective dose, μmol/kg, sc	maximal incidence of penile erections,[a] %
1	0.03	77***
apomorphine	0.1	91***
17	0.03	75**
18	0.003	65*
19	0.01	75*
23	0.01	70*
24	0.01	60*
27	0.1	85**
29	0.03	62
46	0.01	75*
47	0.03	60
60	0.03	75*
8	1	83***

[a] Level of statistical significance relative to control: (*) $p < 0.1$; (**) $p < 0.05$; (***) $p < 0.001$. The number of animals tested was 8−30.

While all of the benzimidazole agonists tested elicited erections when administered in vivo (55−85% erectile incidence), there was not an obvious correlation between the in vitro human D_4 FLIPR EC_{50} and the maximally

effective dose determined in vivo in rats. This is perhaps not surprising when three factors are considered. (1) The degree of SAR overlap between the human and rat D_4 receptors is unknown at present, so compounds could have different human and rat receptor D_4 potencies and efficacies. Furthermore, while in vivo efficacies were determined in rats, the in vitro FLIPR efficacies were determined in a cell line transfected with the human $D_{4.4}$ receptor because an assay to routinely measure agonist activity in the rat D_4 receptor was not readily available. (2) Differences in the pharmacokinetics and CNS penetration of compounds could lead to differences in brain tissue concentrations between drugs. (3) There are likely to be differences between the receptor/G-protein stoichiometry in the in vitro and the in vivo systems. The ability of the in vitro FLIPR-based assay to distinguish differences in agonist efficacy should depend on the ratio of transfected receptor to signaling G-protein. In vitro assay systems having excess G-protein should be the most sensitive in their ability to distinguish partial agonists. On the other hand, if D_4 receptors are present in excess over the signaling G-protein in vivo, then both full and "partial" agonists will be able to fully activate all of the available G-protein, thereby eliciting comparable maximal efficacies, though potencies should still differ.

In animal studies, the observed in vivo pharmacological effects of compound **1** were entirely confined to erectogenic activity, with no other observable side effects. Most significantly, no nausea or emesis was found in ferrets at any dose tested (0.03−3 μmol/kg, sc) so that there is a >100-fold window of selectivity for drug doses that induce erectogenesis, as opposed to emesis or nausea.[6c] Ferrets are especially sensitive to the emetic effects of drugs and are therefore used to gauge emetic liability.[4d] The unique profile of **1** can be contrasted to dopaminergic agents selective for other receptor subtypes or to those having agonist or antagonist activity at other subtypes. For example, the recently described D_2 selective agonist PNU-95666E[19] (**3**) was profiled in the FLIPR assay and found to be an extremely potent agonist at the D_2 receptor but without D_4 agonistic activity. This compound was not found to have erectogenic activity in the rat erectile incidence assay over the tested range of 0.1−3 μmol/kg sc, yet it induced nausea in all ferrets, an 80% incidence of emesis at 1 μmol/kg, and a 100% incidence of emesis at 3 μmol/kg.[6,18] Additional experiments with other selective dopaminergic agents further confirmed and extended these findings to other dopamine receptor subtypes.[6] For example, the mixed $D_2/D_3/D_4$ dopaminergic agonist apomorphine showed maximal rat erectogenic effects (91%) at 0.1 μmol/kg, but at the same dose (0.1−3 μmol/kg) it induced nauseagenic symptoms in ferrets, and at 0.3 μmol/kg it produced an 80% incidence of ferret emesis.

In a general screen of rat behavior (the Irwin assay), **1** induced no overt behavioral effects at subcutaneous doses ranging from 0.1 to 10 μmol/kg. Additional behavioral tests found no effects on spontaneous locomotor activity (0.003−1.0 μmol/kg) or EEG waves (3 μmol/kg). The selective induction of erectile activity over other behaviors may be a product of the high selectivity for **1** for D_4 receptors. Except for the observation that D_4 agonists facilitate erections, D_4 agonists do not appear to produce other obvious effects on rat behavior. Consistent with this, using two D_4 agonists, CP-226269[9] and PD-168077,[21] Clifford and Waddington[20] found no psychopharmacological pattern in rodents that could be attributed to D_4 agonism.

To rule out the possibility that **1** was inducing erections through inhibition of phosphodiesterases, **1** was screened against phosphodiesterases PDE-1, PDE-5, and PDE-6 at up to 10 μM; no enzyme inhibition was seen, precluding PDE inhibition as an explanation for the erectogenic properties of **1**. Because different classes of dopaminergic agents can induce both central and peripheral side effects such as pressor effects on the cardiovascular system, **1** was also profiled carefully in additional safety studies. When administered to rats at 3 μmol/kg iv, there was no change in heart rate, blood pressure, cardiac contractility (d*P*/d*T*), or systemic vascular resistance.[6c] Similarly, very high drug doses in canines produced no changes in cardiovascular parameters and no effect on the QT_c intervals (data not shown). Additionally, because nonselective dopaminergic agonists and dopaminergics possessing inherent D_4 agonism have been found to be safe after long-term clinical administration in Parkinson's disease patients,[22] chronic administration of D_4 selective agents may also be similarly benign.

Conclusion

A new class of dopaminergic agents has been discovered and optimized to produce highly selective D_4 agonists, exemplified by compound **1**. Among benzimidazole compounds of general structure **9**, the ortho-substituted aryl and heteroarylpiperazines showed pronounced efficacy as D_4 agonists. Substitution at the meta and para positions of the aryl ring of the arylpiperazine moiety negated the ability of compounds to act as D_4 agonists. All of the D_4 selective benzimidazole agonists that were tested in conscious male rats demonstrated erectogenic activity. Compound **1** potently induced a high incidence of erections in rats at a dose of 0.03 μmol/kg, yet in contrast to other less selective dopaminergic agents, it did not induce nausea or emesis in a sensitive animal model at any dose tested. Furthermore, this compound demonstrated no other overt behavioral effects, and no deleterious effects were noted in comprehensive safety assessments so that a high therapeutic index can be projected. The erectogenic activity of **1** was centrally mediated because the induction of erections could be prevented by coadministration with a centrally acting dopamine antagonist but not by a peripherally selective dopamine antagonist. Altogether, these observations suggest that not only does compound **1** robustly induce erectogenesis in rats without side effects but they also support the proposal that selective D_4 agonists may represent a novel approach to the safe and effective treatment of erectile dysfunction in humans.

Experimental Section

Chemistry Methods. All solvents were of anhydrous reagent grade from commercial sources. Unless otherwise noted, all chemicals and reagents were obtained commercially and used without purification. Unless otherwise noted, ^{1}H NMR spectra were obtained at 300 MHz on a Nicolet/GE QE300 spectrometer. Chemical shifts are reported in parts per

million (ppm, δ) relative to TMS or TSP as in internal standard. Mass spectra were obtained on a Kratos MS-50 instrument in DCI/NH_3 mode. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Flash chromatography was carried out using silica gel 60 (E. Merck, 230−400 mesh) or prepacked 40 mm silica gel columns from BioTage. Thin-layer chromatography was performed on 250 μM silica-coated glass plates from EM Science. Samples were analyzed by HPLC−MS ELSD on an Open Access Finnigan Navigator/Agilent 1100/Sedere Sedex 75 system using a Phenomenex Luna C_8 column (5 μm, 2.1 mm × 50 mm). The elution system used was a gradient of 10−100% over 4.5 min at 1.5 mL/min, and the solvent was either acetonitrile/0.1% aqueous TFA or acetonitrile/10 mM ammonium acetate. Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected.

2-(4-Pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (1). To a large round-bottom flask in a water bath containing a rapidly stirred solution of 5.9 g (36 mmol) of 2-pyridylpiperazine in 15 mL of DMF at 20 °C, was added 6 g (36 mmol) of solid 2-chloromethylbenzimidazole over 2 min. Et_3N (7.5 mL, 54 mmol) was added, and the reaction mixture was stirred for 16 h. The product was deposited as a solid in the DMF during the reaction. The reaction was worked up by treatment with 5 mL of Et_3N, followed by addition of 70 mL of H_2O. After 1 h, the solid product was collected by suction filtration, washed with 400 mL of H_2O, and dried to give 9 g of product. This was recrystallized twice from boiling *n*-BuOH to give 7.6 g (72%) of **1** as a buff powder: mp 220−221 °C; ^{1}H NMR (DMSO-d_6) δ 8.09 (dd, 1H, J = 4.5, 1.8 Hz), 7.41−7.58 (m, 3H), 7.14 (m, 2H), 6.81 (d, 1H, J = 8.7 Hz), 6.62 (dd, 1H, J = 6.6, 4.5 Hz), 3.77 (s, 2H), 3.52 (t, 4H, J = 4.5 Hz), 2.55 (t, 4H, J = 4.5 Hz); MS (DCI) m/z 294 $[MH]^+$. Anal. ($C_{17}H_{19}N_5$) C, H, N. The maleate salt was recrystallized from EtOH as a white powder, mp 189−190 °C. Anal. ($C_{17}H_{19}N_5 \cdot C_4H_4O_4$) C, H, N.

The following compounds were prepared after the manner of compound **1**.

2-[(4-Phenylpiperazin-1-yl)methyl]-1*H*-benzimidazole (17). Prepared in 79% yield; mp 258−260 °C; ^{1}H NMR (CD_3OD) δ 3.01 (m, 4H), 3.39 (m, 4H), 4.28 (s, 2H), 6.97 (m, 1H), 7.08 (m, 2H), 7.31 (m, 2H), 7.57 (m, 2H), 7.78 (m, 2H); MS (DCI) m/z 393 $(MH)^+$. Anal. ($C_{18}H_{20}N_4$) C, H, N.

2-[4-(1*H*-Benzimidazol-2-yl)methyl)piperazin-1-yl]benzonitrile (18). Prepared in 57% yield; mp 236−237 °C; ^{1}H NMR (CD_3OD) δ 2.77 (m, 4H), 3.27 (m, 4H), 3.89 (s, 2H), 7.07 (m, 1H), 7.15 (m, 1H), 7.23 (m, 2H), 7.56 (m, 4H); MS (DCI) m/z 318 $(MH)^+$. Anal. ($C_{19}H_{19}N_5$) C, H, N.

2-[4-(3-Fluoropyridin-2-yl)piperazin-1-ylmethyl]-1*H*-benzoimidazole (19). 2-Chloro-3-fluoropyridine. A −78 °C solution of DABCO (5.78 g, 51.5 mmol) and Et_2O (130 mL) was treated dropwise with *n*-BuLi (32.2 mL, 51.5 mmol, 1.6 M solution in hexane). The reaction mixture was warmed to −20 °C for 1 h and then cooled back to −78 °C before a solution of 3-fluoropyridine (5.0 g, 51.5 mmol) in Et_2O (5 mL) was added dropwise. The reaction mixture was stirred for 2 h at −78 °C, after which a solution of CCl_3CCl_3 (12.2 g, 51.5 mmol) in 24 mL of THF was added, and the mixture was stirred for 1 h at −78 °C before quenching with a solution of H_2O (15 mL) and THF (25 mL). The mixture was warmed to 0 °C for 30 min, and then additional H_2O and Et_2O were added. The aqueous phase was further extracted with Et_2O. The combined organic extract was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was chromatographed on flash silica gel (10% EtOAc/hexanes) to afford 3.5 g (52% yield) of 2-chloro-3-fluoropyridine. ^{1}H NMR (DMSO-d_6) δ 7.54 (m, 1H), 7.96 (m, 1H), 8.31 (m, 1H); MS (ESI) m/z 154 $(M + Na)^+$.

1-(3-Fluoropyridin-2-yl)piperazine. A 23 °C solution of 2-chloro-3-fluoropyridine (3.3 g, 0.025 mol) and *n*-BuOH (150 mL) was treated with piperazine (21.5 g, 0.25 mol), and the reaction mixture was heated at reflux for 3 days. The reaction mixture was cooled to 23 °C and concentrated in vacuo. The residue was slurried with H_2O and EtOAc. The EtOAc layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 3.3 g (73% yield) of 1-(3-fluoropyridin-2-yl)piperazine: ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.80 (m, 4H), 3.38 (m, 4H), 6.84 (m, 1H), 7.47 (m, 1H), 7.98 (m, 1H); MS (ESI) m/z 182 $(MH)^+$.

A mixture of 1-(3-fluoropyridin-2-yl)piperazine (0.50 g, 2.76 mmol), DMF (28 mL), 2-chloromethyl-1*H*-benzimidazole (0.48 g, 2.90 mmol), and Cs_2CO_3 (1.8 g, 5.52 mmol) was stirred for 1.25 h and then the DMF was removed in vacuo. The residue was rinsed with 10% CH_3OH/CH_2Cl_2, and the solid was removed by filtration. The filtrate was concentrated in vacuo, after which the residue was impregnated onto flash silica gel and chromatographed on flash silica gel (10% CH_3OH/CH_2Cl_2) to afford 311 mg (36% yield) of **19**: mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 2.62 (m, 4H), 3.43 (m, 4H), 3.78 (s, 2H), 6.87 (m, 1H), 7.14 (m, 2H), 7.50 (m, 3H), 8.00 (m, 1H); MS (APCI) m/z 312 $(MH)^+$. Anal. ($C_{17}H_{18}FN_5 \cdot 0.4H_2O$) C, H, N.

2-[4-(1*H*-Benzoimidazol-2-ylmethyl)piperazin-1-yl]nicotinonitrile (20). Prepared in 92% yield; mp 208−210 °C; ^{1}H NMR (CD_3OD) δ 2.72 (t, 4H, J = 6.0 Hz), 3.74 (t, 4H, J = 6.0 Hz), 3.87 (s, 2H), 6.87 (dd, 1H, J = 7.0, 6.0 Hz), 7.22 (m, 2H), 7.54 (m, 1H), 7.93 (dd, 1H, J = 7.0, 3.0 Hz), 8.35 (dd, 1H, J = 6.0, 3.0 Hz); MS (DCI) m/z 319 $(MH)^+$. Anal. ($C_{18}H_{18}N_6$) C, H, N.

2-[4-(2-Chlorophenyl)piperazin-1-yl]methyl-1*H*-benzimidazole (21). Prepared in 64% yield; mp 245−246 °C; ^{1}H NMR (CD_3OD) δ 3.02 (m, 4H), 3.20 (m, 4H), 4.29 (s, 2H), 7.04 (m, 1H), 7.17 (dd, 1H, J = 9.0, 2.0 Hz), 7.28 (m, 1H), 7.47 (dd, 1H, J = 9.0, 2.0 Hz), 7.55 (m, 2H), 7.75 (m, 2H); MS (DCI) m/z 327 $(MH)^+$. Anal. ($C_{18}H_{19}ClN_4$) C, H, N.

2-[4-(2-Nitrophenyl)piperazin-1-yl]methyl-1*H*-benzimidazole (22). Prepared in 55% yield; ^{1}H NMR (CD_3OD) δ 2.89 (m, 4 H), 3.20 (m, 4 H), 4.22 (s, 2 H), 7.17 (m, 1 H), 7.24 (m, 1H), 7.57 (m, 3H), 7.77 (m, 3H); MS (DCI) m/z 338 $(MH)^+$. Anal. ($C_{18}H_{19}N_5O_2$) C, H, N.

2-[4-(2-Fluorophenyl)piperazin-1-yl]methyl-1*H*-benzimidazole (23). Prepared in 73% yield; mp 245−246 °C; ^{1}H NMR (CD_3OD) δ 2.96 (m, 4H), 3.24 (m, 4H), 4.26 (s, 2H), 7.06 (m, 4H), 7.55 (m, 2H), 7.66 (m, 2H); MS (DCI) m/z 311 $(MH)^+$. Anal. ($C_{18}H_{19}FN_4$) C, H, N.

2-[4-(2-Methoxyphenyl)piperazin-1-yl]methyl-1*H*-benzimidazole (24). Prepared in 73% yield, purified by reversed-phase HPLC and isolated as the CF_3CO_2H salt; ^{1}H NMR (CD_3OD) δ 3.13 (m, 4H), 3.46 (m, 4H), 3.93 (s, 3H), 4.33 (s, 2H), 7.03 (m, 1H), 7.12 (m, 1H), 7.35 (m, 2H), 7.55 (m, 2H), 7.76 (m, 2H); MS (DCI) m/z 323 $(MH)^+$. Anal. ($C_{19}H_{22}N_4O \cdot CF_3CO_2H$) C, H, N.

2-[4-(2-Ethoxyphenyl)piperazin-1-yl]methyl-1*H*-benzimidazole (25). Prepared in 40% yield; mp 95−100 °C; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, J = 6.0 Hz), 2.39 (m, 4H), 3.33 (m, 4H), 4.03 (s, 2H), 4.07 (q, 2H, J = 6.0 Hz), 6.83−7.03 (m, 3H), 7.26 (m, 3H), 7.60 (m, 2H); MS (DCI) m/z 337 $(MH)^+$. Anal. ($C_{20}H_{24}N_4O \cdot 0.55H_2O$) C, H, N.

2-[4-(2-Methylthiophenyl)piperazin-1-ylmethyl]-1*H*-benzoimidazole (26). Prepared in 50% yield; mp 214−216 °C; ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3H), 2.77 (m, 4H), 3.07 (m, 4H), 3.94 (s, 2H), 7.06 (m, 1H), 7.12 (m, 3H), 7.25 (m, 2H), 7.59 (m, 2H); MS (DCI) m/z 339 $(MH)^+$. Anal. ($C_{19}H_{22}N_4S \cdot 0.25H_2O$) C, H, N.

2-[(4-Pyrimidin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole (27). Prepared in 15% yield; mp 198−200 °C; ^{1}H NMR (CD_3OD) δ 2.60 (t, 4H, J = 6.0 Hz), 3.86 (t, 4H, J = 6.0 Hz), 3.85 (s, 2H), 6.58 (t, 1H, J = 5.0 Hz), 7.23 (m, 2H), 7.52 (m, 2H), 8.30 (d, 2H, J = 5.0 Hz); MS (DCI) m/z 295 $(MH)^+$. Anal. ($C_{16}H_{18}N_6 \cdot 0.25$ hexane) C, H, N.

2-[4-(3-Methanesulfonylpyridin-2-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (28). A mixture of 0.7 g (3.7 mmol) of 2-chloro-3-methanesulfonylpyridine[23] and 0.67 g (7.5 mmol) of piperazine in 20 mL of *m*-xylene was heated at 120 °C for 12 h. The reaction mixture was then concentrated in vacuo, and the residue was passed through a silica gel column, eluting with 95:5 CH_2Cl_2/CH_3OH containing 0.5% NH_4OH. After the mixture was concentrated in vacuo, the product 1-(3-methanesulfonylpyridin-2-yl)piperazine was obtained and was used directly in the next step. Compound **28** was prepared in

32% yield by the method used to prepare **1**, substituting 1-(3-methanesulfonylpyridin-2-yl)piperazine as the amine: mp 133−136 °C; ^{1}H NMR (DMSO-d_6) δ 2.68 (t, 4H, J = 6.0 Hz), 3.26 (t, 4H, J = 6.0 Hz), 3.39 (s, 3H), 3.81 (s, 2H), 7.15 (m, 2H), 7.39 (dd, 1H, J = 7.0, 6.0 Hz), 7.45 (m, 2H), 7.54 (m, 1H), 8.28 (dd, 1H, J = 7.0, 3.0 Hz), 8.63 (dd, 1H, J = 6.0, 3.0 Hz); MS (DCI) m/z 372 $(MH)^+$. Anal. ($C_{18}H_{21}N_5O_2S\cdot 2H_2O$) C, H, N.

2-[4-(6-Methylpyridin-2-yl)piperazin-1-yl]methyl-1*H*-benzimidazole (29). Prepared in 86% yield, purified by reversed-phase HPLC, and isolated as the CF_3CO_2H salt; ^{1}H NMR (CD_3OD) δ 2.55 (s, 3H), 2.86 (t, 4H, J = 5.0 Hz), 3.83 (t, 4H, J = 5.0 Hz), 4.22 (s, 2H), 6.84 (d, 1H, J = 7.0 Hz), 7.17 (d, 1H, J = 9.0 Hz), 7.59 (m, 2H), 7.79 (m, 2 H), 7.92 (dd, 1H, J = 7.0, 9.0 Hz); MS (DCI) m/z 308 $(MH)^+$. Anal. ($C_{18}H_{21}N_5\cdot 2.3CF_3CO_2H$) C, H, N.

2-[4-(2-Ethylphenyl)piperazin-1-ylmethyl]-1*H*-benzimidazole (30). Prepared in 82% yield; mp 178−179 °C; ^{1}H NMR (CD_3OD) δ 1.22 (t, 3H, J = 7.5 Hz), 2.73 (q, 2H, J = 7.5 Hz), 3.08 (s, 6H), 4.33 (s, 2H), 7.05−7.25 (m, 3H), 7.52 (dd, 2H, J = 6.0, 3.0 Hz), 7.75 (dd, 2H, J = 6.0, 3.0 Hz); MS (DCI) m/z 321 $(MH)^+$. Anal. ($C_{20}H_{24}N_4$) C, H, N.

2-[4-(2,6-Dimethylphenyl)piperazin-1-ylmethyl]-1*H*-benzimidazole (31). Prepared in 57% yield; mp 203−205 °C; ^{1}H NMR (CD_3OD) δ 3.13 (t, 4H, J = 5.0 Hz), 3.32 (m, 4H), 4.4 (s, 2H), 6.95 (m, 3H), 7.49 (dd, 2H, J = 6.0, 3.0 Hz), 7.72 (dd, 2H, J = 6.0, 3.0 Hz); MS (DCI) m/z 321 $(MH)^+$. Anal. ($C_{20}H_{24}N_4$) C, H, N.

2-[4-(2,5-Dimethylphenyl)piperazin-1-ylmethyl]-1*H*-benzimidazole (32). Prepared in 66% yield, purified by reversed-phase HPLC and isolated as the CF_3CO_2H salt; ^{1}H NMR (CD_3OD) δ 2.25 (s, 3H), 2.29 (s, 3H), 3.10 (s, 8H), 4.35 (s, 2H), 6.82 (d, 1H, J = 7.5 Hz), 6.92 (s, 1H), 7.05 (d, 1H, J = 7.5 Hz), 7.51 (dd, 2H, J = 6.0, 3.0 Hz), 7.75 (dd, 2H, J = 6.0, 3.0 Hz); MS (DCI) m/z 321 $(MH)^+$. Anal. ($C_{20}H_{24}N_4\cdot 1.7CF_3CO_2H$) C, H, N.

2-[4-(3,4-Dimethylphenyl)piperazin-1-ylmethyl]-1*H*-benzimidazole (33). Prepared in 52% yield; mp 197−198 °C; ^{1}H NMR (CD_3OD) δ 2.25 (s, 3H), 2.29 (s, 3H), 3.08 (t, 4H, J = 5.0 Hz), 3.50 (m, 4H), 4.32 (s, 2H), 7.02 (dd, 1H, J = 9.0, 1.8 Hz), 7.10 (d, 1H, J = 1.8 Hz), 7.28 (d, 1H, J = 9.0 Hz), 7.55 (dd, 2H, J = 6, 3 Hz), 7.78 (dd, 2H, J = 6.0, 3.0 Hz); MS (DCI) m/z 321 $(MH)^+$. Anal. ($C_{20}H_{24}N_4$) C, H, N.

2-[4-(1*H*-Benzimidazol-2-ylmethyl)piperazin-1-yl]phenol (34). Prepared in 50% yield; mp 208−216 °C; ^{1}H NMR ($CDCl_3$) δ 2.78 (m, 4H), 2.97 (m, 4H), 3.93 (s, 2H), 6.83−6.95 (m, 2H), 7.05 (m, 1H), 7.14 (dd, 1H, J = 7.0, 2.0 Hz), 7.59 (m, 2H); MS (DCI) m/z 309 $(MH)^+$. Anal. ($C_{18}H_{20}N_4O\cdot 0.5H_2O$) C, H, N.

2-[4-(4-Trifluoromethylpyrimidin-2-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (35). Prepared in 29% yield; mp 217−218 °C; ^{1}H NMR (DMSO-d_6) δ 2.57 (t, 4H, J = 6.0 Hz), 3.81 (s, 2H), 3.85 (t, 4H, J = 6.0 Hz), 7.02 (d, 1H, J = 4.5 Hz), 7.15 (m, 2H), 7.45 (dd, 1H, J = 7.0, 6.0 Hz), 7.55 (m, 1H), 8.28 (d, 1H, J = 4.5 Hz), 12.43 (s, 1H); MS (DCI) m/z 363 $(MH)^+$. Anal. ($C_{17}H_{17}F_3N_6$) C, H, N.

2-[4-(5-Ethylpyrimidin-2-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (36). Prepared in 35% yield; mp 199−201 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (t, 3H, J = 7.5 Hz), 2.43 (q, 2H, J = 7.5 Hz), 2.65 (t, 4H, J = 6.0 Hz), 3.73 (t, 4H, J = 6.0 Hz), 3.81 (s, 2H), 7.15 (m, 2H), 7.43 (dd, 1H, J = 7.0, 6.0 Hz), 7.55 (m, 1H), 8.24 (s, 1H), 12.33 (s, 1H); MS (DCI) m/z 323 $(MH)^+$. Anal. ($C_{18}H_{22}N_6$) C, H, N.

2-[4-(6-Chloropyridazin-3-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (37). Prepared in 38% yield; mp 199−201 °C; ^{1}H NMR (DMSO-d_6) δ 2.58 (t, 4H, J = 6.0 Hz), 3.62 (t, 4H, J = 6.0 Hz), 3.79 (s, 2H), 7.13 (m, 2H), 7.36 (d, 1H, J = 9.0 Hz), 7.44 (dd, 1H, J = 7.0, 6.0 Hz), 7.53 (d, 1H, J = 9.0 Hz), 7.57 (m, 1H), 12.33 (s, 1 H); MS (DCI) m/z 329 $(M + H)^+$. Anal. ($C_{16}H_{17}ClN_6$) C, H, N.

2-(4-Pyridazin-3-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (38). Prepared in 56% yield; mp 90−94 °C; ^{1}H NMR (DMSO-d_6) δ 2.58 (t, 4H, J = 6.0 Hz), 3.64 (t, 4H, J = 6.0 Hz), 3.79 (s, 2H), 7.15 (m, 2H), 7.23 (dd, 1H, J = 7.0, 1.5 Hz), 7.38 (dd, 1H, J = 7, 6 Hz), 7.47 (dd, 1H, J = 7.0, 1.5 Hz), 7.57 (m, 1H), 12.34 (s, 1H); MS (DCI) m/z 295 $(MH)^+$. Anal. ($C_{16}H_{18}N_6\cdot H_2O$) C, H, N.

2-[4-(3-Nitropyridin-2-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (39). Prepared in 20% yield, purified by reversed-phase HPLC, and isolated as the CF_3CO_2H salt; ^{1}H NMR (DMSO-d_6) δ 3.02 (m, 4H), 3.55 (m, 4H), 4.35 (s, 2H), 7.00 (dd, 1H, J = 9, 6 Hz), 7.41 (dd, 2H, J = 6, 3 Hz), 7.72 (dd, 2H, J = 6, 3 Hz), 8.31 (dd, 1H, J = 9.0, 3.0 Hz), 8.46 (dd, 1H, J = 6.0, 3.0 Hz); MS (ESI) m/z 339 $(MH)^+$. Anal. ($C_{17}H_{18}N_6O_2\cdot 1.25CF_3CO_2H$) C, H, N.

2-[4-(3-Chloropyridin-2-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (40). Prepared in 18% yield, purified by reversed-phase HPLC, and isolated as the CF_3CO_2H salt; ^{1}H NMR (DMSO-d_6) δ 3.27 (m, 4H), 3.50 (m, 4H), 4.52 (s, 2H), 7.08 (dd, 1H, J = 9.0, 6.0 Hz), 7.36 (dd, 2H, J = 9.0, 6.0 Hz), 7.70 (dd, 2H, J = 6.0, 3.0 Hz), 7.85 (dd, 1H, J = 9.0, 3.0 Hz), 8.26 (dd, 1H, J = 6.0, 3.0 Hz); MS (ESI) m/z 328 $(MH)^+$. Anal. ($C_{17}H_{18}ClN_5\cdot 1.5CF_3CO_2H$) C, H, N.

6-[4-(1*H*-Benzoimidazol-2-ylmethyl)piperazin-1-yl]pyridazin-3-ol (41). Prepared in 42% yield; mp >220 °C; ^{1}H NMR (DMSO-d_6) δ 2.57 (t, 4H, J = 6.0 Hz), 3.23 (t, 4H, J = 6.0 Hz), 3.76 (s, 2H), 6.78 (d, 1H, J = 9.0 Hz), 7.15 (m, 2H), 7.45 (m, 1H), 7.49 (d, 1H, J = 9.0 Hz), 7.56 (m, 1H), 12.08 (s, 1H), 12.31 (s, 1H); MS (DCI) m/z 311 $(MH)^+$. Anal. ($C_{16}H_{18}N_6O\cdot H_2O$) C, H, N.

2-[4-(3-Methylsulfanylpyridin-2-yl)piperazin-1-ylmethyl]-1*H*-benzimidazole (42). Prepared in 31% yield; mp 191−192 °C; ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 2.63 (t, 4H, J = 6.0 Hz), 3.16 (m, 4H), 3.81 (s, 2H), 7.39 (dd, 1H, J = 7.0, 6.0 Hz), 7.15 (m, 2H), 7.45 (m, 1H), 7.54 (m, 2H), 8.05 (dd, 1H, J = 7.0, 3.0 Hz), 12.31 (s, 1H); MS (DCI) m/z 340 $(MH)^+$. Anal. ($C_{18}H_{21}N_5S$) C, H, N.

2-(4-Pyridin-2-ylmethylpiperazin-1-ylmethyl)-1*H*-benzimidazole (43). Prepared in 79% yield; ^{1}H NMR (CD_3OD) δ 2.50−2.65 (m, 8H), 3.65 (s, 2H), 3.82 (s, 2H), 7.18−7.24 (m, 2H), 7.30−7.35 (m, 1H), 7.60−7.65 (m, 3H), 7.75−7.85 (m, 1H), 8.42−8.44 (m, 1H); MS (DCI) m/z 308 $(MH)^+$; Anal. ($C_{18}H_{21}N_5$) C, H, N.

2-[4-(2-Pyridin-2-ylethyl)piperazin-1-ylmethyl]-1*H*-benzimidazole (44). Prepared in 21% yield, as the maleic acid salt; ^{1}H NMR (CD_3OD) δ 2.48−2.70 (m, 8H), 2.72−2.81 (m, 2H), 2.92−3.02 (m, 2H), 3.81 (s, 2H), 7.17−7.28 (m, 3H), 7.35 (d, 1H, J = 9.0 Hz), 7.51−7.76 (m, 2H), 7.70 (t, 1H, J = 9.0 Hz), 8.92 (d, 1H, J = 6.0 Hz); MS (DCI) m/z 322 $(MH)^+$. Anal. ($C_{19}H_{23}N_5\cdot C_4H_4O_4\cdot 0.50H_2O$) C, H, N.

2-(4-Cyclohexylpiperazin-1-ylmethyl)-1*H*-benzimidazole (45). Prepared in 60% yield; mp 176−177 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (m, 10 H), 1.54 (m, 1 H), 1.75 (m, 4 H), 3.04 (m, 4H), 3.71 (s, 2H), 7.14 (m, 2H), 7.45 (m, 2H), 12.28 (s, 1H); MS (DCI) m/z 299 $(MH)^+$. Anal. ($C_{18}H_{26}N_6\cdot 1.5H_2O$) C, H, N.

5-Fluoro-2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole (46). 2-Chloromethyl-5-fluoro-1*H*-benzimidazole. To a 250 mL round-bottom flask was added 4-fluoro-1,2-phenylenediamine (5.0 g, 39.7 mmol), chloroacetic acid 4.9 g (51.6 mmol), and 6 N HCl (25 mL). The mixture was heated at 95 °C for 12 h, then cooled to room temperature, and neutralized with 1 M aqueous K_2CO_3. After extraction with EtOAc (5 × 500 mL) and drying over $MgSO_4$, the combined organic layers were filtered and concentrated under reduced pressure. The product, 2-chloromethyl-5-fluoro-1*H*-benzimidazole, was purified by flash chromatography, eluting with 10% MeOH/CH_2Cl_2 to give 2.65 g of a brown foam (36%), which was immediately used in the next step: ^{1}H NMR (CD_3OD) δ 4.87 (s, 2H), 7.05 (td, 1H, J = 3.0, 9.0 Hz,), 7.27 (dd, 1H, J = 3.0, 9.0 Hz), 7.51−7.55 (m, 1H); MS m/z 185 $(MH)^+$.

Compound **46** was prepared in 24% yield by the method of compound **1**, substituting 5-fluoro-2-chloromethylbenzimidazole for 2-chloromethylbenzimidazole: ^{1}H NMR (CD_3OD) δ 2.62−2.69 (t, 4H, J = 5.8 Hz), 3.52−3.59 (t, 4H, J = 6.0 Hz), 3.84 (s, 2H), 6.77 (dd, 1H, J = 2.0, 6.0 Hz), 6.82 (d, 1H, J = 9.0 Hz), 7.02 (dt, 1H, J = 3.0, 9.0 Hz), 7.24 (dd, 1H, J = 2.0, 9.0 Hz), 7.48−7.59 (m, 2H), 8.05−8.10 (m, 1H); MS (DCI) m/z 312 $(MH)^+$. Anal. ($C_{17}H_{18}N_5F\cdot 0.2CH_3OH$) C, H, N.

5,7-Dibromo-2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole (47). 5,7-Dibromo-2-choromethylbenzimidazole. A suspension of 0.50 g (1.65 mmol) of 5,7-dibromo-1,2-phenylenediamine, chloroacetic acid (0.47 g, 3.3 mmol), and 5 N HCl (6 mL) was heated at reflux for 12 h. The mixture was cooled to 25 °C, and 10 mL of water was added. The resulting orange solid suspension was collected by suction filtration and dried under vacuum to give 0.55 g (92%) of the dihydrochloride as an orange powder, which was used immediately in the next step: ^{1}H NMR (CD_3OD/DMSO-d_6) δ 4.92 (s, 2H), 7.63 (d, 1H, J = 1.8 Hz), 7.79 (d, 1H, J = 1.8 Hz); MS (DCI) m/z 320, 321, 323, 326.7 $(MH)^+$.

Compound **47** was prepared in 80% yield from 5,7-dibromo-2-choromethylbenzimidazole by method described for compound **1**; ^{1}H NMR ($CDCl_3$) δ 2.70 (t, 4H, J = 6 Hz), 3.58 (t, 4H, J = 6 Hz), 3.90 (s, 2H), 6.67 (m, 2H), 7.53 (m, 2H), 7.65 (m, 1H), 8.18 (m, 1H); MS (DCI) m/z 450, 452, 454 $(MH)^+$. Anal. ($C_{17}H_{17}Br_2N_5$) C, H, N.

5-Methoxy-2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (48) was prepared after the manner of compound **46**, using 4-methoxy-1,2-phenylenediamine to give **48** in 28% yield: ^{1}H NMR (CD_3OD) δ 2.83−2.92 (m, 8H), 3.0 (s, 3H), 3.91 (s, 2H), 7.04 (t, 1H, J = 9.0 Hz), 7.18−7.28 (m, 2H), 7.45 (m, 1H), 7.68 (m, 1H), 7.93−8.01 (m, 1H), 8.03−8.12 (m, 1H); MS (DCI) m/z 323 $(MH)^+$. Anal. ($C_{18}H_{21}N_5O$·2HCl) C, H, N.

2-[2-(4-Pyridin-2-ylpiperazin-1-yl)ethyl]-1*H*-benzimidazole (49) was prepared after the manner of compound **46**, using 1,2-phenylenediamine and 3-chloropropionic acid, to give **49** in 28% yield as a foam: ^{1}H NMR (CD_3OD) δ 2.68 (t, 4H, J = 9.0 Hz), 2.93 (m, 2H), 3.15 (m, 2H), 3.53 (m, 4H), 6.62−6.71 (m, 1H), 6.81 (m, 1H), 7.13−7.24 (m, 2H), 7.43−7.60 (m, 3H), 8.03 (m, 1H); MS (DCI) m/z 323 $(MH)^+$. Anal. ($C_{18}H_{21}N_5$·2HCl) C, H, N.

1-Methyl-2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (50). To a solution of **1** (0.22 g, 0.80 mmol) in THF (8 mL) at 23 °C was added 0.8 mL (0.80 mmol) of a 1 M solution of NaHMDS in THF, and the reaction mixture was stirred for 10 min. To this mixture was added 0.8 g (0.80 mmol) of CH_3I, and the mixture was stirred for 12 h. The reaction mixture was concentrated in vacuo and purified on SiO_2, eluting with 10% CH_3OH/CH_2Cl_2 to give **50** as a white solid in 80% yield: ^{1}H NMR (CD_3OD) δ 2.67−2.75 (m, 4H), 3.54−3.63 (m, 4H), 3.90−3.95 (m, 5H), 6.60−6.68 (m, 2H), 7.27−7.42 (m, 3H), 7.50 (m, 1H), 7.78 (d, 1H, J = 9.0 Hz), 8.19 (d, 1H, J = 6.0 Hz); MS (DCI) m/z 308 $(MH)^+$. Anal. ($C_{18}H_{21}N_5$) C, H, N.

1-Ethyl-2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (51) was prepared in 66% yield after the manner of compound **50**, substituting CH_3CH_2I: ^{1}H NMR ($CDCl_3$) δ 1.48 (t, 3H, J = 9 Hz), 2.67 (t, 4H, J = 6 Hz), 3.52 (t, 4H, J = 6 Hz), 3.87 (s, 2H), 4.30−4.45 (m, 2H), 6.55−6.68 (m, 2H), 7.21−7.32 (m, 3H), 7.32−7.50 (m, 2H), 7.71−7.81 (m, 1H), 8.13−8.21 (m, 1H); MS (DCI) m/z 321 $(MH)^+$. Anal. ($C_{19}H_{23}N_5$) C, H, N.

1-Isopropyl-2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (52) was prepared in 37% yield after the manner of compound **50**, substituting $(CH_3)_2CHI$: ^{1}H NMR ($CDCl_3$) δ 2.65 (t, 4H, J = 9.0 Hz), 2.88 (s, 3H), 2.95 (s, 3H), 3.51 (t, 4H, J = 9.0 Hz), 3.87 (s, 2H), 5.03−5.18 (m, 1H), 6.58−6.69 (m, 2H), 7.19−7.29 (m, 2H), 7.43−7.51 (m, 1H), 7.52−7.61 (m, 1H), 7.73−7.80 (m, 1H), 8.15−8.21 (m, 1H); MS (DCI) m/z 335 $(MH)^+$. Anal. ($C_{20}H_{25}N_5$) C, H, N.

1-Benzyl-2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (53) was prepared in 68% yield after the manner of compound **50**, substituting $PhCH_2I$: ^{1}H NMR (CD_3OD) δ 2.57 (t, 4H, J = 9.0 Hz), 3.28−3.47 (m, 4H), 3.83 (s, 2H), 5.68 (s, 2H), 6.61−6.69 (m, 1H), 6.75 (d, 1H, J = 6.0 Hz), 7.13 (m, 2H), 7.22−7.25 (m, 5H), 7.23−7.45 (m, 1H), 7.47−7.56 (m, 1H), 7.62−7.70 (m, 1H), 8.01−8.03 (m, 1H); MS (DCI) m/z 383 $(MH)^+$. Anal. ($C_{24}H_{25}N_5$) C, H, N.

***N*,*N*-Dimethyl-2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole-1-carboxamide (54).** Similar to the method used to prepare **55**, compound **54** was prepared using *N*,*N*-dimethylaminocarbonyl chloride in 50% yield: mp 174−176 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (m, 4H), 2.93 (m, 3H), 3.21 (m, 3H), 3.48 (m, 4H), 3.71 (m, 1H), 4.25 (m, 1H), 6.64 (m, 2H), 7.29 (m, 3H), 7.48 (m, 1H), 7.76 (m, 1H), 8.18 (m, 1H); MS (DCI) m/z 365 $(MH)^+$. Anal. ($C_{20}H_{24}N_6O$·0.2H_2O) C, H, N.

2-[(4-Pyridin-2-ylpiperazin-1-yl)methyl]-1-(pyrrolidin-1-ylcarbonyl)-1*H*-benzimidazole (55). To a stirred solution of 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-benzimidazole (0.66 g, 2.2 mmol) in CH_2Cl_2 (7 mL) was added 1-pyrrolidinecarbonyl chloride (0.28 mL, 2.2 mmol) and Et_3N (0.63 mL, 4.5 mmol). The mixture was heated in a sealed vial for 17 h, allowed to cool to 23 °C, diluted with CH_2Cl_2, washed with 5% aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column on SiO_2, eluting with 20% hexane/EtOAC to give 0.4 g (40%) of **55**: mp 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 1.79−2.10 (m, 4H), 2.70 (m, 4H), 3.13 (m, 1H), 3.35−3.78 (m, 8H), 4.32 (m, 1H), 6.65 (m, 2 H), 7.30 (m, 3H), 7.49 (m, 1H), 7.76 (m, 1H), 8.28 (m, 1H); MS (DCI) m/z 391 $(M + H)^+$. Anal. ($C_{22}H_{26}N_6O$·0.5H_2O) C, H, N.

(3*S*)-3-Methyl-1-pyridin-2-ylpiperazine. A mixture of (*S*)-(+)-2-methylpiperazine (0.50 g, 5 mmol) and 5 mL (50 mmol) of 2-bromopyridine was stirred with heating at 120 °C for 14 h. After cooling to 23 °C, the reaction mixture was poured into H_2O and extracted with EtOAc. The aqueous layer was made basic to pH 11 by addition of Na_2CO_3 and saturated aqueous $NaHCO_3$ and was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2CO_3 and concentrated in vacuo to give the product, which was used directly in the next step. ^{1}H NMR (400 MHz) δ 1.02 (d, 3H, J = 6.0 Hz), 2.27 (dd, 1H, J = 10.0, 12.0 Hz), 2.67 (m, 3H), 2.92 (m, 1H), 4.07 (m, 2H), 6.58 (dd, 1H, J = 6.0, 8.0 Hz), 6.77 (d, 1H, J = 8.0 Hz), 7.49 (m, 1H), 8.08 (m, 1H); MS (ESI) m/z 178 $(MH)^+$.

2-[(2*S*)-2-Methyl-4-pyridin-2-ylpiperazin-1-yl]methyl-1*H*-benzimidazole (56). A mixture of 10 mL of DMF containing 0.24 g (1.33 mmol) of (3*S*)-3-methyl-1-pyridin-2-ylpiperazine, 0.21 g (1.27 mmol) of 2-chloromethyl-1*H*-benzimidazole, and 0.41 mmol (1.27 mmol) of Cs_2CO_3 was stirred at 23 °C for 3 h, then diluted with EtOAc. The reaction mixture was then washed with H_2O and saturated aqueous NaCl, dried over Na_2SO_4, and concentrated in vacuo. After purification by flash chromatography on silica gel, the product was obtained as a light-yellow solid in 46% yield: mp 149−151 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.18 (d, J = 6 Hz, 3H), 2.38 (m, 1H), 2.53 (m, 1H), 2.76 (dd, J = 8, 11.2 Hz, 1H), 2.83 (m, 1H), 3.03 (m, 1H), 3.69 (d, J = 14.0 Hz, 1H), 3.94 (m, 1H), 4.00 (m, 1H), 4.07 (d, J = 14.0 Hz, 1H), 6.60 (dd, J = 4.8, 6.4 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 7.13 (m, 2H), 7.60 (m, 3H), 8.08 (m, 1H), 12.22 (s, 1H); MS (ESI) m/z 308 $(MH)^+$. Anal. ($C_{18}H_{21}N_5$) C, H, N.

2-[(2*R*)-2-Methyl-4-pyridin-2-ylpiperazin-1-yl]methyl-1*H*-benzimidazole (57) was prepared in 89% yield in the same manner as **56**, from (*R*)-(−)-2-methylpiperazine, and had physical properties identical to those of **56**. Anal. ($C_{18}H_{21}N_5$) C, H, N.

(5-Fluoro-1*H*-indol-2-yl)-(4-pyridin-2-yl-piperazin-1-yl)methanone.[9] To a suspension of 4.00 g (22.0 mmol) of 5-fluoro-1*H*-indole-2-carboxylic acid and 4.29 g (22.0 mmol) of EDCI−HCl in 90 mL of dichloromethane was added 3.64 g (22.0 mmol) of 1-pyridin-2-ylpiperazine. After being stirred at 25 °C for 24 h, the reaction mixture was washed with 150 mL of H_2O and filtered to collect a white solid, which was then washed with 300 mL of water, then 250 mL of CH_2Cl_2 and 20 mL of EtOAc. After the sample was dried over Na_2SO_4, 4.61 g (64%) of a white solid was obtained, which was used directly in the next step: ^{1}H NMR (DMSO-d_6) δ 3.61 (m, 4H), 3.85 (m, 4H), 6.67 (dd, 1H, J = 7.5, 4.8 Hz), 6.85 (m, 2H), 7.06 (m, 1H), 7.40 (m, 2H), 7.65 (m, 1H), 8.04 (dd, 1H, J = 4.5, 1.8 Hz), 11.72 (s, 1 H); MS (DCI) m/z 325 $[M\ H]^+$.

5-Fluoro-2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1*H*-indole (8).[9] A well-stirred slurry of 3.50 g (10.8 mmol) of (5-fluoro-1*H*-indol-2-yl)-(4-pyridin-2-ylpiperazin-1-yl)methanone in 50 mL of THF in a 500 mL flask was immersed in a water bath 25 °C before addition of 30 mL of a 1.0 M solution of $LiAlH_4$. The reaction progress was monitored by TLC and,

on completion, was quenched by sequential addition of 1.1 mL of water, 1.1 mL of 15% aqueous NaOH, and then 3.4 mL of H_2O. The white solid deposited after stirring was removed by filtration. Ethanol was added to the filtrate, the filtrate was evaporated in vacuo, and then it was diluted with EtOAc and filtered through a plug of silica gel. The filtrate was purified by chromatography on silica gel, eluting with EtOAc containing 0.1% NH_4OH. The product was obtained as a solid (2.66 g, 80%): ^{1}H NMR (DMSO-d_6) δ 2.55 (m, 4H), 3.50 (t, 4H, J = 4.5 Hz), 3.65 (s, 2H), 6.30 (s, 1H), 6.63 (dd, 1H, J = 7.5, 4.8 Hz), 6.80 (d, 1H, J = 7.5 Hz), 6.85 (m, 1H), 7.20 (dd, 1H, J = 10.5, 2.4 Hz), 7.30 (dd, 1H, J = 9, 5.4 Hz), 7.50 (m, 1H), 8.09 (dd, 1H, J = 4.5, 1.8 Hz), 11.72 (s, 1H); MS (DCI) m/z 294 $[MH]^+$. The maleate was recrystallized from CH_3OH/toluene/Et_2O. Anal. ($C_{18}H_{19}N_4F\cdot C_4H_4O_4$) C,H, N.

1′,2′,3′,6′-Tetrahydro[2,4′]bipyridine.[10,24] To a solution of diisopropylamine (13.4 mL. 96 mmol) in THF (350 mL) at −78 °C was added 1.6 M *n*-BuLi in hexane (60 mL, 96 mmol). The reaction mixture was stirred for 5 min at −78 °C, then a solution of *N*-BOC-4-piperidone (16 g, 80 mmol) in THF (100 mL) was added, and the reaction mixture was stirred for 10 min. A solution of $PhN(Tf)_2$ (31.4 g, 88 mmol) in THF was added, and the reaction mixture was stirred at −78 °C for 30 min, after which the cooling bath was removed to warm to 23 °C. The reaction was quenched with saturated aqueous $NaHCO_3$, then the mixture extracted was with Et_2O. The organic layer was washed successively with 5% citric acid, 1 M NaOH (4 × 200 mL), water (2 × 200 mL), and brine (1 × 200 mL), dried over $MgSO_4$, and then concentrated in vacuo to give a yellowish oil. Purification by flash chromatography using hexane/EtOAc 8:2 as eluent gave 18 g (68%) of a colorless oil, 4-trifluoromethanesulfonyloxy-3,6-dihydro-2*H*-pyridine-1-carboxylic acid *tert*-butyl ester: ^{1}H NMR (DMSO-d_6) δ 1.41 (s, 9H), 2.41 (m, 2H), 3.54 (t, 2H, J = 5.7 Hz), 3.98 (dd, 2H, J = 6.0, 2.7 Hz), 6.02 (m, 1H). This material (18 g, 54 mmol) was dissolved in 200 mL of THF and treated with 124 mL (62.5 mmol) of 2-pyridylzinc bromide (0.5 M in THF), followed by 625 mg (0.54 mmol) of $Pd(PPh_3)_4$. The mixture was heated at 60 °C for 90 min. The reaction mixture was cooled and concentrated in vacuo, and then EtOAc (300 mL) and 1 M NaOH (200 mL) were added to the residue. The precipitated zinc salts were filtered, and the organic layer was separated and washed with brine (300 mL), then dried over $MgSO_4$, and concentrated on a rotary evaporator to give a brown oil. Purification by flash chromatography using hexane/EtOAc 6:4 gave 9.0 g (64%) of 3′,6′-dihydro-2′*H*-[2,4′]bipyridinyl-1′-carboxylic acid *tert*-butyl ester as a colorless oil: ^{1}H NMR (DMSO-d_6) δ 1.43 (s, 9H), 2.56 (m, 2H), 3.54 (t, 2H, J = 5.8 Hz), 4.04 (m, 2H), 6.08 (m, 1H), 7.25 (m, 1H), 7.56 (d, 1H, J = 9 Hz), 7.77 (m, 1H), 8.54 (m, 1H); MS (DCI) m/z 259 $(MH)^+$. A solution of dihydro-2′*H*-[2,4′]bipyridinyl-1′-carboxylic acid *tert*-butyl ester (1.0 g, 3.84 mmol) was treated with 10 mL of 4 M HCl in dioxane at 23 °C, stirred for 5 min, and then concentrated in vacuo from CH_3CN. The residue was triturated with Et_2O, and the solid product was collected by filtration to give 0.74 g of 1′,2′,3′,6′-tetrahydro[2,4′]bipyridinyl hydrochloride:[25] ^{1}H NMR (DMSO-d_6) δ 2.58 (m, 1H), 2.81 (m, 2H), 3.35 (m, 2H), 3.82 (m, 2H), 6.80 (m, 1H), 7.49 (dd, 1H, J = 6 Hz, 2.7 Hz), 7.79 (d, 1H, J = 9 Hz), 8.02 (m, 1H), 8.62 (m, 1 H), 9.45 (s, 1 H); MS m/z 161 (M H)$^+$. A solution of 322 mg (1.64 mmol) of 1′,2′,3′,6′-tetrahydro[2,4′]bipyridinyl hydrochloride in EtOH was hydrogenated using 250 mg of 10% Pd/C at 60 psi for 40 h at 50 °C to give 1′,2′,3′,4′,5′,6′-hexahydro[2,4′]bipyridine (150 mg, 88%): MS (DCI/NH_3) m/z (DCI) 163 $(MH)^+$. This was used without any further purification in the preparation of 2-[(4-pyridin-2-ylpiperidin-1-yl)methyl]-1*H*-benzimidazole (**58**).

2-[(4-Pyridin-2-ylpiperidin-1-yl)methyl]-1*H*-benzimidazole (58). 1′,2′,3′,4′,5′,6′-Hexahydro[2,4′]bipyridine (0.6 g, 0.36 mmol), 2-chloromethylbenzimidazole (0.62 g, 0.36 mmol), and Cs_2CO_3 (0.12 g, 0.36 mmol) in DMF (8 mL) were stirred at room temperature for 18 h. The reaction mixture was poured into water (30 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed with brine (2 × 30 mL), dried over $MgSO_4$, and filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography, eluting with 5% MeOH/CH_2Cl_2 to give the title compound (11.2 mg, 11%): ^{1}H NMR ($CDCl_3$) δ 2.0 (m, 5H), 2.51 (m, 2H), 2.79 (m, 1H), 3.14 (m, 2H), 4.01 (s, 2H), 7.09 (m, 3H), 7.29 (m, 1H), 7.55 (m, 3H), 8.49 (m, 1H); MS (DCI) m/z 293 $(MH)^+$. Anal. ($C_{18}H_{20}N_4\cdot 0.3H_2O$) C, H, N.

2-[4-(2-Methoxyphenyl)piperidin-1-yl-methyl]-1*H*-benzimidazole (59). A mixture of 4-(2-methoxyphenyl)piperidine (200 mg, 1.06 mmol), 2-chloromethylbenzimidazole (186 mg, 1.1 mmol), and Cs_2CO_3 (358 mg, 0.36 mmol) in DMF (8 mL) was stirred at °C for 18 h. The reaction mixture was poured into H_2O (30 mL) and extracted with EtOAc (20 mL). The organic layer was washed with brine (2 × 30 mL), dried over $MgSO_4$, filtered, concentrated in vacuo, and purified by flash chromatography (95:5 CH_2Cl_2/MeOH) to give 82 mg (25%) of product: ^{1}H NMR ($CDCl_3$)) δ 1.69 (m, 4H), 2.19 (m, 2H), 2.87(m, 1H), 2.96 (m, 2H), 3.75 (s, 2H), 3.77 (s, 3H), 6.92 (m, 2H), 7.15 (m, 4H), 7.45 (m, 1H), 7.55 (m, 1H), 12.26 (s, 1H); MS (DCI) m/z 322 $(MH)^+$; HRMS (FAB) m/z 322.1908 (322.1919 calcd for $C_{20}H_{23}N_3O$ $(MH)^+$). Anal. ($C_{20}H_{23}N_3O\cdot 0.3H_2O$) C, H, N.

1′-(1*H*-Benzoimidazol-2-ylmethyl)-1′,2′,3′,6′-tetrahydro-[2,4′]bipyridinyl (60). Prepared by the method described for compound **59**, using the 1′,2′,3′,6′-tetrahydro[2,4′]bipyridinyl hydrochloride[25] described above as the amine, to give the product in 38% yield as a foam: ^{1}H NMR (CD_3OD) δ 2.62 (m, 2H), 2.74 (t, 2H, J = 5.2 Hz), 3.24 (m, 2H), 3.84 (m, 2H), 6.69 (m, 1H), 7.13 (m, 2H), 7.23 (m, 2H), 7.44 (m, 1H), 7.55 (m, 1H), 7.75 (m, 1H), 8.52 (m, 1H); MS (DCI) m/z 290 $(M)^+$. Anal. ($C_{18}H_{18}N_4\cdot 0.2H_2O$) C, H, N.

2-[(4-Phenyl-3,6-dihydropyridin-1(2*H*)-yl)methyl]-1*H*-benzimidazole (61). A mixture of 4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (391 mg, 2.0 mmol), 2-chloromethylbenzimidazole (186, 2.0 mmol), and Cs_2CO_3 (651 mg, 2.0 mmol) in DMF (8 mL) was stirred at 23 °C for 18 h. The reaction mixture was poured into H_2O (50 mL) and EtOAc (50 mL). A white solid precipitated out of the biphasic mixture, which was collected by filtration and air-dried to give the pure desired product, 290 mg (50%): ^{1}H NMR (CD_3OD/5% TFA) δ 2.90 (m, 2H), 3.52 (t, 2H, J = 5.8 Hz), 3.92 (m, 2H), 4.7 (s, 2H), 6.14 (m, 1H), 7.34 (m, 3H), 7.46 (m, 2H), 7.52 (m, 2H), 7.78 (m, 2H); MS (DCI) m/z 290 $(MH)^+$. Anal. ($C_{19}H_{19}N_3\cdot 0.5H_2O$).

FLIPR Assay of Receptor Activation by Agonists. Human $D_{4.4}$ receptors were coexpressed with $G\alpha_{qo5}$ in HEK293 cells.[12,26] Cells were plated into 96-well, black-wall, clear-bottom microplates (Biocoat, Becton Dickinson, Boston, MA) at 20 000 cells per well. After 2 days, the culture medium was removed by aspiration and cells were incubated with 0.1 mL of DPBS (Dulbecco's phosphate buffered saline with D-glucose and sodium pyruvate) containing 0.04% Pluronic F-127 and 4 μM Fluo-4 fluorescent calcium indicator dye. After 1 h at 23 °C, the cells were washed four times with DPBS in a plate washer (Molecular Devices), and then 150 μL of DPBS was added to each well. Test compounds in 50 μL of buffer were added, and fluorescence readings were recorded over 3 min (every second for the first minute and every 5 s for the next 2 min) with a fluorometric imaging plate reader (FLIPR384, Molecular Devices). The fluorescent readings were normalized to give equivalent (0%) initial readings for all wells at time zero, and all the data were normalized (to 100%) with the response of 10 μM dopamine.

Radioligand Binding Assays. Cell membranes from human dopamine $D_{4.4}$ receptor transfected HEK-293 cells were isolated and stored at −80 °C until use.[27] Binding assays were initiated by addition of membrane to 0.1 nM [^{3}H]-spiperone, and the sample was incubated for 2 h in an incubation buffer of 50 mM Tris-HCl, pH 7.4, 5 mM KCl, 120 mM NaCl, 5 mM $MgCl_2$, and 1 mM EDTA. In competition binding studies, test compounds were prepared with 0.1% ascorbic acid in the buffer. Binding reactions were terminated by filtration through glass microfiber filters, washed three times with cold 50 mM Tris-HCl, pH 7.4, and the retained radioactivity was measured by scintillation counting.

Conscious Rat Penile Erection Model.[6,18] Male Wistar rats (Charles River, Portage, WI) were used as a primary animal model to study penile erection in vivo. All experiments were carried out between 9:00 a.m. and 3:00 p.m. in a diffusely illuminated testing room with a red light. Animals were weighed and allowed to adapt to the testing room for 60 min prior to the beginning of experiments. Rats were placed individually in a transparent cage (20 cm × 30 cm × 30 cm) after drug injection. The number of penile erections was recorded by direct observation for a period of 60 min after drug dosing, and the number of animals exhibiting 1 or more erections was expressed as the percent incidence.

Emesis Model in Ferrets.[6] Male Fitch ferrets (body weights of 1.0−1.5 kg, Marshall Farms) were fasted overnight before experimentation. Test compounds were administrated subcutaneously, and animals were carefully placed in individual observation cages and watched for any signs of drug-induced emesis and signs of nausea over 90 min. Nausea was characterized by behaviors such as licking, gagging, hacking, head burying, and intense abdominal grooming. When present, emesis was usually preceded by these behaviors and was characterized by rhythmic abdominal contractions that were associated with vomiting or retching movements.

Supporting Information Available: Elemental analysis data for the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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