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Systematic in Vivo Screening of a Series of 1-Propyl-4-arylpiperidines against Dopaminergic and Serotonergic Properties in Rat Brain: A Scaffold-Jumping Approach

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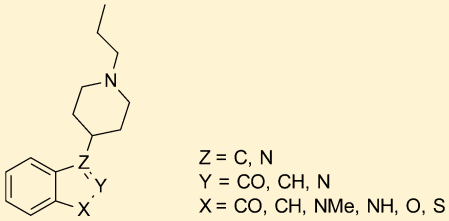
Supporting Information

ABSTRACT: A series of 1-propyl-4-arylpiperidines were synthesized and their effects on the dopaminergic and serotonergic systems tested in vivo and in vitro. Scaffold jumping among five- and six-membered bicyclic aryl rings attached to the piperidine ring had a marked impact on these effects. Potent and selective dopamine D_2 receptor antagonists were generated from 3-indoles, 3-benzoisoxazoles, 3-benzimidazol-2-one, and 3-benzothiophenes. In contrast, 3-benzofuran was a potent and selective inhibitor of monoamine oxidase (MAO) A. The effects of the synthesized compounds on 3,4-dihydroxyphenylacetic acid (DOPAC) levels correlated very well with their affinity for dopamine D_2 receptors and MAO A. In the 4-arylpiperidine series, the most promising compound for development was the 6-chloro-3-(1-propyl-4-piperidyl)-1*H*-benzimidazol-2-one (**19**), which displayed typical dopamine D_2 receptor antagonist properties in vivo but produced only a partial reduction on spontaneous locomotor activity. This indicates that the compound may have a lower propensity to induce parkinsonism in patients.



INTRODUCTION

Dopamine type 2 (D_2) receptors are located primarily in the basal ganglia of the mammalian brain but also occur in other structures of the brain, such as the cortex. The receptors, which are located at the neuronal membrane, belong to the monoamine subclass of the G-protein-coupled seven-transmembrane receptors (GPCRs).[1] In the brain, dopamine (**1**, Figure 1) is released at synaptic and extrasynaptic sites, affecting postsynaptic, presynaptic, and dendritic dopamine D_2 receptor populations. Synaptic dopamine release is followed by fast reabsorption or degradation, which terminates dopamine signaling. Drugs that interact with the agonist-binding site of dopamine D_2 receptors can be described as antagonists, partial agonists, or full agonists, and a number of these drugs have well-established applications in the treatment of various neurological and psychiatric disorders.[2] Recently, a new class of dopamine D_2 ligands known as dopidines has been discovered. These compounds act as dopaminergic stabilizers, and the most clinically advanced is pridopidine[3−5] (ACR16, **2**, Figure 1), currently in phase III development for the treatment of motor symptoms associated with Huntington's disease (HD). In patients with HD, **2** (45 mg, twice daily) displayed an adverse-effect profile similar to that of placebo.[6] Furthermore, this adverse-effect profile was relatively benign compared with that of classical dopamine D_2 receptor antagonists (such as haloperidol),[7] which are associated with severe adverse effects such as acute extrapyramidal symptoms (EPS).[8] The primary site of action of dopaminergic stabilizers is at dopamine D_2 receptors, where they display surmountable antagonism and fast-off kinetic properties.[3,5,9,10] From an in vivo perspective, these compounds stabilize dysregulated psychomotor functions (i.e., they reverse behavioral states originating from both hypo- and hyperdopaminergia while having only subtle effects on normal psychomotor activity).[4,11] The surmountable and fast-off receptor kinetics may account for the lack of reduction of spontaneous locomotor activity (LMA) across the full dose range (3.7−300 μmol/kg, in rats) by allowing dopamine receptors to regain responsiveness to dopamine rapidly.[3,5] Neurochemical analysis of post-mortem brain tissue from freely moving rats shows that dopaminergic stabilizers induce an increase in the synthesis and release of dopamine in the basal ganglia (e.g., the striatum),[4,11] a hallmark of dopamine D_2 receptor antagonism. This further supports the hypothesis that dopaminergic stabilizers lack intrinsic activity at dopamine D_2 receptors. This unique mechanism of action contrasts with that of classical dopamine D_2 receptor antagonists (i.e., haloperidol and olanzapine)[7,12] and partial dopamine D_2 agonists (i.e., aripiprazole and bifeprunox).[4,5,11,13] Historically, dopaminergic stabilizers (**2** and (−)-OSU6162 (**3**), Figure 1) were developed using agonist-like structural motifs that retained the hydrophilic nature of the agonist.[14] However, careful modification of the pharmacophore elements essential for intrinsic activity at dopamine D_2 receptors produced compounds displaying fast-off kinetics and surmountable antagonist properties.[15,3] From structure−activity relationship (SAR) investigations, it has been demonstrated that dopaminergic stabilizer properties are favored by a powerful electron-withdrawing substituent in meta position of the aryl (like the methylsulfone group; see compounds **2** and **3**) and a propyl substituent on the basic nitrogen.[3,14] It is interesting to note that the propyl substituent on the amine is also favored in several dopamine D_2 receptor agonists and has

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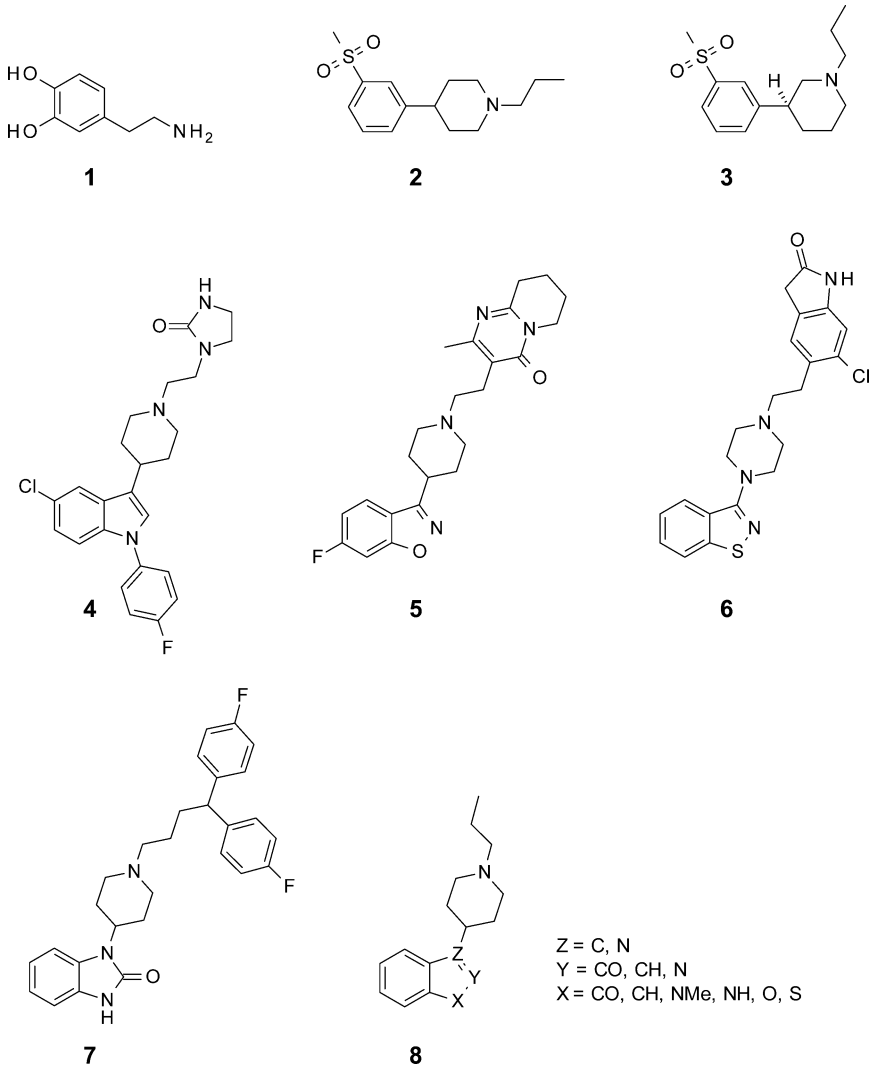


Figure 1. Dopamine D_2 receptor ligands: dopamine (**1**), the dopaminergic stabilizers pridopidine (**2**) and *S*-(−)-OSU6162 (**3**), atypical antipsychotics sertindole (**4**), risperidone (**5**), and ziprasidone (**6**), typical antipsychotic pimozide (**7**), generic structure of 1-propyl-4-arylpiperidines (**8**).

been referred to the propyl binding "pocket".[16,17] In the search for new chemical scaffolds to serve as starting points for development of dopaminergic stabilizers, we examined whether it would be possible to start from a dopamine D_2 receptor antagonist (rather than agonist) motif. We also investigated whether chemical manipulations, such as reducing size and lipophilicity, could produce new dopaminergic stabilizers. Furthermore we focused our investigations on bicyclic cores within typical/atypical antipsychotics (i.e., dopamine D_2 receptor antagonists), such as sertindole (**4**),[18] risperidone (**5**),[19,20] ziprasidone (**6**),[21] and pimozide (**7**, Figure 1).[22] These compounds are all high-affinity dopamine D_2 receptor ligands that have been optimized using the conventional methodology of increasing size and lipophilicity to improve affinity. These large lipophilic compounds are believed to interact with hydrophobic residues in the receptor cavity that are not involved in agonist interactions, thereby stabilizing the inactive state of dopamine D_2 receptors.[23,24]

However, we hypothesized that the hydrophilic nature of the five-membered heteroaryl ring with a piperidine ring attached could serve as a starting point for the development of new dopaminergic stabilizers (**4**−**7**, Figure 1). In addition, by removal of the cyclic "alkyl/aryl" ring(s) in the side chain attached to the basic amine, the propyl group known to be "optimal" for dopaminergic stabilizer properties would be retained (**8**, Figure 1).[3,14] In order to fully explore the SAR for 1-propyl-4-arylpiperidines, a wide spectrum of core building blocks were included in the data set (Figure 2). Many of these building blocks are often included in compounds with known effects on the dopaminergic and the serotonergic (5-hydroxytryptamine, 5-HT) systems in the brain.[25−33] However, they have been imbedded in larger compounds and it is therefore harder to judge the contribution that each core building block makes with regard to SAR on the dopaminergic system (i.e., dopamine D_2 receptors). A few examples with small alkyl groups on the nitrogen are published, and generally these compounds have been built on 3-substituted indoles.

Guillaume et al.[34] have reported the tetrahydropyridine RU 24969 (**25**, Figure 3) as a mixed dopamine and serotonin receptor agonist in vivo, although its affinity for dopamine receptors was very low (IC_{50} > 5000 nM) and later reported to lack effects on dopamine synthesis in striatal regions in the rat brain.[35] The reported agonist-like effects on the dopamine receptor may therefore be questioned, and other mechanisms may underlie these effects. A report from Hunt et al. within tetrahydropyridine/piperidine indoles (**25**, **26**) also supports

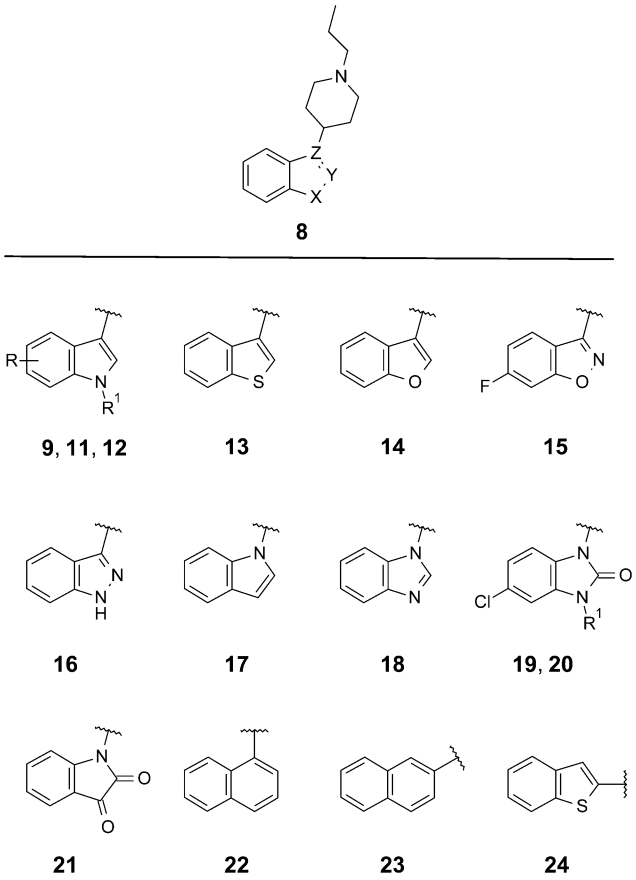


Figure 2. Overview of 1-propyl-4-arylpiperidines and their bicyclic aryl core building blocks: **9**, 6-F, R^1 = H; **11**, 6-F, R^1 = methyl; **12**, 5-F, R^1 = methyl; **19**, R^1 = H; **20** R^1 = methyl.

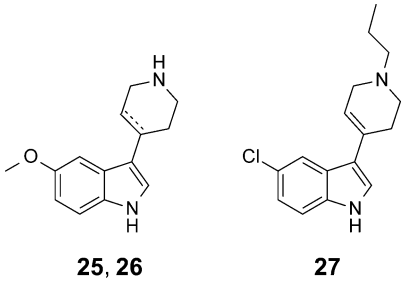


Figure 3. Serotonin 5-$HT_{1A/1B}$ receptor agonist RU 24969 (tetrahydropyridine, **25**), RU23686 (piperidine, **26**), and the dopamine D_2 receptor antagonist RU 27592 (**27**).

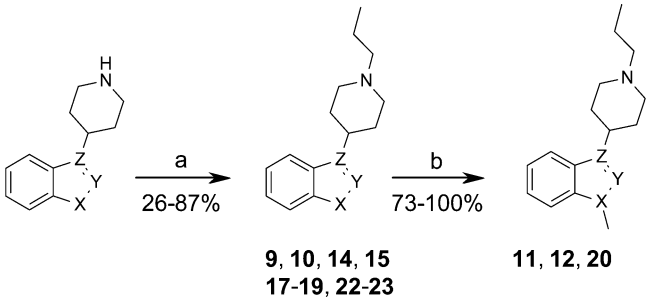
that these effects are not mediated via the dopamine system but are instead mediated via the 5-HT system.[36] Currently, **25** is classified as a serotonin 5-$HT_{1A/1B}$ agonist.[37] The corresponding propyl analogue RU 27592 (**27**, Figure 3) was reported by Guillaume et al. to be a dopamine antagonist with affinity for both dopamine and 5-HT receptors (80 and 260 nM, respectively).[34] Other 3-substituted indoles, 1*H*-indazoles and 1,2-benzisoxazoles with a piperidine or piperidene ring, and small alkyl groups on the nitrogen have been reported to display effects on serotonin transporter protein (SERT),[38,39] as well as on serotonin 5-HT_{1D},[40−42] 5-$HT_{1E/1F}$,[43] 5-HT_{1F},[27,44] 5-$HT_{1A/2A}$,[45−47] 5-$HT_{2A/2C}$,[48] 5-HT_{2A},[49] and 5-HT_6 receptors.[50−52]

On the basis of the generic structure **8** and the building blocks listed in Figure 2, we hereby report a systematic in vivo screening of 15 new compounds for their effects on dopamine and serotonin synthesis and turnover (i.e., effects on 3,4-dihydroxyphenylacetic acid [DOPAC] and 5-hydroxyindoleacetic acid [5-HIAA]) in rat brain and their effects on LMA. The new compounds were also tested for in vitro affinity for dopamine D_2, dopamine type 3 receptor (D_3), dopamine type 4 receptor (D_4), serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and adrenergic type 2A (α_{2A}) receptor, and dopamine transporter protein (DAT) and SERT. During the in vivo screening process we discovered that some compounds displayed a "dopamine agonist"-like effect on brain neurochemistry (i.e., a decrease in DOPAC levels), which was later revealed to be an effect of inhibition of flavin-containing monoamine oxidase A (MAO A), which metabolizes dopamine to DOPAC. Compounds' affinity for MAO A was therefore also included in the in vitro test panel. The synthesis and SAR of these new compounds will be discussed in this paper.

CHEMISTRY

The desired core piperidine building blocks have been used frequently before for synthesis and are described in the literature,[19,29,32,34,53−58] but many of them are also commercially available (Scheme 1): 6-fluoro-3-(4-piperidyl)-1*H*-indole, 5-fluoro-3-(4-piperidyl)-1*H*-indole, 4-(benzofuran-3-yl)-piperidine, 6-fluoro-3-(4-piperidyl)-1,2-benzoxazole, 1-(4-piperidyl)indole, 1-(4-piperidyl)benzimidazole, 6-chloro-3-(4-piperidyl)-1*H*-benzimidazol-2-one, 4-(2-naphthyl)piperidine, and 4-(1-naphthyl)piperidine. The piperidines were alkylated with iodopropane, affording the desired products **9**, **10** (5-fluoro-3-(1-propyl-4-piperidyl)-1*H*-indole), **14**, **15**, **17**−**19**, and **22**−**23** in moderate−good yields (26−87%, Scheme 1). The indoles **9**, **10** and benzimidazol-2-one **19** were further methylated and yielded products **11**, **12**, and **20** in good yields (73−100%, Scheme 1). Synthesis of compound **21** was performed according to Scheme 2 where the 1-(4-piperidyl)indolin-2-one was first alkylated (**28**) followed by oxidation to the corresponding isatin by treatment with hydrochloric acid (HCl)/ethanol (EtOH), yielding **21** in moderate yield (31%).[59]

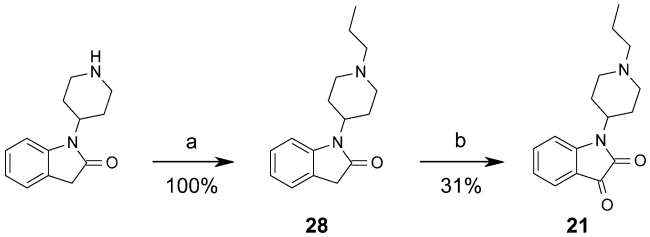
Scheme 1. General Synthesis of 1-Propyl-4-arylpiperidines Derivatives[a]



[a]Reagents and conditions: (a) 1-iodopropane (PrI), K_2CO_3, acetonitrile (ACN), Δ; (b) NaH, iodomethane, DMF.

Compound **16** (Scheme 3) was synthesized from 3-bromo-1*H*-indazole by lithiation with a mixture of *n*-butyllithium (*n*-BuLi)/*tert*-butyllithium (*t*-BuLi), generating the dianion of indazole at −78 °C, and quenching with 1-propylpiperidin-4-one yielded **29** in moderate yield (32%).[60] Subsequent treatments with trifluoroacetic acid (TFA) in a CH_2Cl_2 solution gave **30** in excellent yield (100%). The tetrahydropyridine **30** was

Scheme 2. Synthesis of 1-(1-Propyl-4-piperidyl)indoline-2,3-dione (21)[a]



[a]Reagents and conditions: (a) PrI, K_2CO_3, ACN, Δ; (b) EtOH, HCl.

reduced by catalytic hydrogenation (Pd/C), affording the piperidine derivative **16** in moderate yield (46%, Scheme 3).

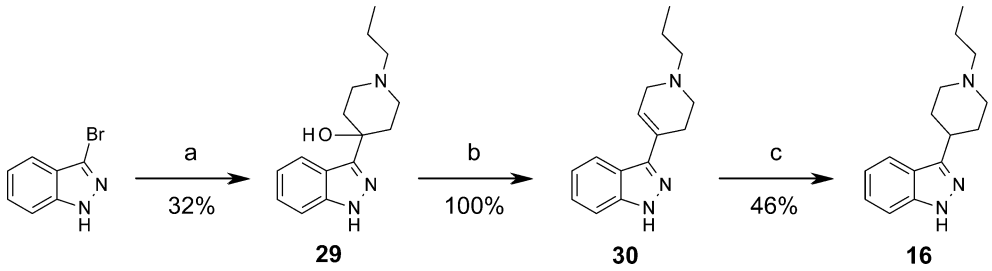
Compound **13** (Scheme 4) was synthesized from 3-bromobenzothiophene by lithiation with *n*-BuLi and quenching with 1-Boc-4-piperidone. Subsequent treatment with TFA in a CH_2Cl_2 solution (for Boc deprotection and elimination of hydroxy group) yielded 3-substituted benzothiophene **31** in moderate yield (35%).[29] The secondary amine **31** was treated with iodopropane and afforded **32** in excellent yield (100%) followed by reduction with catalytic hydrogenation (Pd/C), which afforded the piperidine derivative **13** in moderate yield (38%, Scheme 4). The 2-substituted benzothiophen derivate **24** (Scheme 4) was synthesized by the same sequence, by lithiation with *n*-BuLi regioselective at the 2-position, from benzothiophene (room temperature). Subsequent treatment with TFA yielded **33** in moderate yield (39%).[32] The secondary amine **33** was treated with iodopropane and afforded **34** in excellent yield (100%), followed by reduction with catalytic hydrogenation (Pd/C), affording the piperidine derivative **24** in moderate yield (22%, Scheme 4).

RESULTS

In Vitro. All target compounds (**9** and **11**−**24**) were evaluated in competition binding assays in human embryonic kidney (HEK) 293 cells transfected with human dopamine D_2 short form (D_{2S}) receptors using two different ligands: the antagonist [^{3}H]methylspiperone, which labels the low-affinity dopamine D_2 receptor state (D_2^{Low}), and the agonist [^{3}H]7-OH DPAT (7-hydroxy-2-*N*,*N*-dipropyl-2-aminotetralin), which labels the high-affinity dopamine D_2 receptor state (D_2^{High}).[61] In addition, the compounds were tested for affinity to the human SERT, using [^{3}H]imipramine as the ligand in Chinese hamster ovary (CHO) cells[62] and affinity for MAO A from rat cerebral cortex, using [^{3}H]Ro 41-1049 as the ligand (Table 1).[63] Some compounds were also further screened for affinity to serotonin 5-HT_{1A},[64] 5-HT_{2A},[65] 5-HT_6,[66] D_3,[67] D_4,[68] adrenergic α_{2A} receptors[69] and DAT[70] (Tables 3 and 4). The agonist affinity state of dopamine D_2 receptors (D_2^{High} or D_2^{Low}) is dependent on the degree of guanine nucleotide-binding protein (G-protein) coupling.[71−73] Antagonists are thought to bind approximately equally well to both receptor states.[74] A dopamine D_2 receptor that is uncoupled from a G-protein is considered to be in its low-affinity state, whereas coupling of the G-protein (a process promoted by agonists) gives a high-affinity state. By use of an agonist and antagonist as the [^{3}H]ligand, the affinity for D_2^{High} and D_2^{Low} can be determined, and the ratio between these two affinities (K_i^{Low}/K_i^{High}) correlates with the intrinsic activity of the compound (antagonists display ratios around 1 and agonists >50).[3,74] From the results listed in Table 1, it can be concluded that in general, the target compounds bind with slightly higher affinity to the dopamine D_2^{High} state than to the dopamine D_2^{Low} state. However, none of them displayed a high enough ratio to produce intrinsic activity that could be detected in vivo by measuring effects on DOPAC levels (Table 2). It can also be concluded that compounds **14**, **23**, and **24** bind with high affinity to MAO A while remaining compounds lack affinity (Table 1). In addition, most of the target compounds bind with fairly high affinity for SERT, except for **15**, **16**, and **19**−**21** (Table 1). From the results in Table 3, none of the tested compounds (**9**, **14**, **15**, **23**, **24**) bind with high affinity to 5-HT_{1A}, dopamine D_4 and DAT (less than 67% displacement at 1 μM), while nearly 100% displacement (at 1 μM) was obtained at the 5-HT_{2A} receptor (**9**, **23**, **24**). In addition, compound **9** was found to have affinity for α_2 receptors (91% displacement) and compound **15** for dopamine D_3 receptors (83%).

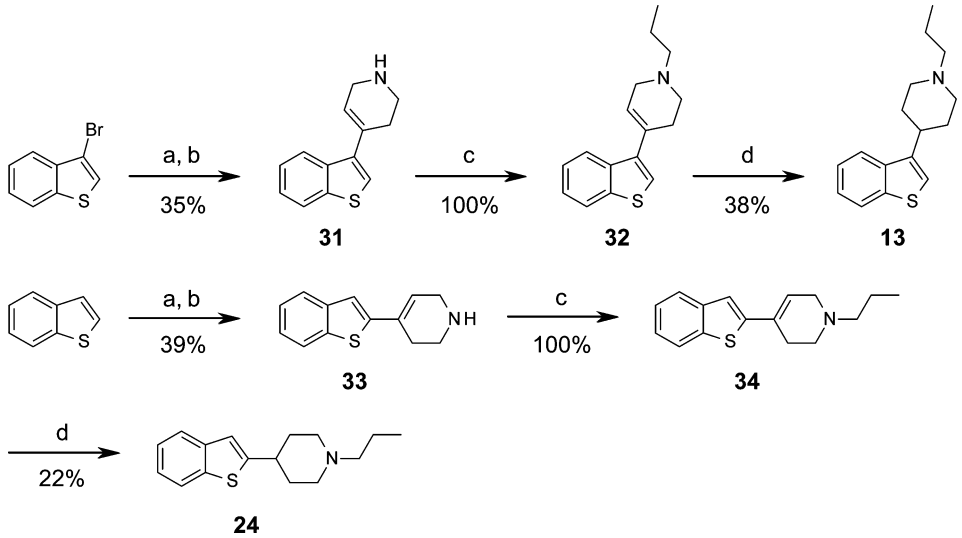
In Vivo. The typical in vivo effects of dopamine D_2 receptor antagonists are dose-dependent increases in the synthesis and release of dopamine in the striatum, measured as an increase in DOPAC levels (up to a maximum of 300−400% of control), plus a concomitant potent reduction in spontaneous LMA in partly habituated rats, which is a hallmark for a potential risk for EPS in patients (Figure 4 and Table 2, risperidone, pimozide, and ziprasidone). Generally, they also bind with high affinity to dopamine D_2 receptors (K_i < 12 nM, Table 1). All target compounds (**9** and **11**−**24**) were evaluated for dose−response effects on DOPAC and 5-HIAA levels and LMA (Figure 4 and Table 2; the effect on LMA is reported at the dose when the compound reaches its maximal effect on DOPAC). In addition, the reported effect on LMA is during the last 45 min of the behavioral session, which is regarded as the hypoactive state of the animal (and is the point during which dopaminergic stabilizers increase LMA compared with dopamine D_2 receptor antagonists, which decrease LMA; Table 2).

Scheme 3. Synthesis of 3-(1-Propyl-4-piperidyl)-1*H*-indazole (16)[a]



[a]Reagents and conditions: (a) *n*-BuLi (1 equiv), *t*-BuLi (2 equiv), 1-propylpiperidin-4-one, THF; (b) TFA, CH_2Cl_2, Δ; (d) Pd/C, H_2, EtOH.

Scheme 4. Synthesis of 4-(Benzothiophen-3-yl)-1-propylpiperidine (13) and 4-(Benzothiophen-2-yl)-1-propylpiperidine (24)[a]



[a]Reagents and conditions: (a) *n*-BuLi, 1-Boc-4-piperidone, diethyl ether, THF; (b) TFA, CH_2Cl_2, Δ; (c) PrI, K_2CO_3, ACN, Δ; (d) Pd/C, H_2, methanol, HAc, HCl.

DISCUSSION

Effects on Dopamine D_2 Receptors in Vitro and in Vivo. Changing the bicyclic ring structure of **8** was found to have a marked impact on the dopaminergic and serotonergic system (i.e., effects on DOPAC and 5-HIAA levels, binding to dopamine D_2 and SERT receptors) and affinity for MAO A (Figure 4, Tables 1 and 2). As can be seen in Figure 4 and Table 2, the indole structures (**9** and **12**) and 1,2-benzisoxazole (**15**) were potent and efficacious dopamine D_2 receptor antagonists, inducing an increase in DOPAC levels with an ED_{50} of 3−4 μmol/kg (Figure 4 and Table 2) and a strong reduction of LMA (<10% of control). These effects were similar to those of the classical dopamine D_2 receptor antagonists risperidone, pimozide, and ziprasidone, which have comparable potency on DOPAC levels and LMA (pimozide and ziprasidone have an ED_{50} of 1.6 and 1.2 μmol/kg; risperidone is slightly more potent at 0.5 μmol/kg). The binding affinities for dopamine D_2 receptors further supported the potency of these new compounds, with an affinity of 27, 32, and 194 nM for **9**, **12**, and **15**, respectively, for D_2^{Low} (Table 1). However, compound **15** had a binding affinity of 34 nM for D_2^{High}, which correlates better with its in vivo potency than the affinity for D_2^{Low}. It is noteworthy that the reported in vitro affinity of pimozide, ziprasidone, and risperidone for D_2 receptors is 11.7, 8.5, and 2.7 nM, respectively (D_2^{Low}, Table 1). This means that dopamine D_2 receptor affinity correlates very well with the different in vivo potencies observed, including also **9**, **12**, and **15** (R^2 = 0.92 between in vitro dopamine D_2^{High} receptor affinity and ED_{50} for DOPAC for these six compounds, Figure 1S). Risperidone is approximately 10-fold more potent in vitro than **9**, **12**, and **15**, and this is in agreement with a 6- to 8-fold greater potency in vivo.

However, there is one compound in this new series that does not show a correlation between in vitro and in vivo potency: the benzimidazole-2-one **19** (ED_{50} = 7 μmol/kg for DOPAC), which was found to be more or less equally potent and efficacious as, for example, **9** in vivo (ED_{50} = 4.1 μmol/kg for DOPAC). In contrast, its affinity in vitro for dopamine D_2 receptors was 10-fold lower (and 32- to 137-fold lower compared with the classical dopamine D_2 receptor antagonists). Compound **19** was one of the most hydrophilic compound in this series (clogP = 3.0), and it is possible that the more lipophilic compounds (**9**, **12**, and **15**) have a higher protein-binding degree, distributing to lipophilic compartments other than the brain, meaning that higher doses are needed to reach the target (i.e., dopamine D_2 receptors). As such, the "true" difference in potency may be masked. An alternative explanation could be differences in metabolic stability, but CYP450 turnover indicated that these compounds had the same stability (data not shown). Furthermore, given that all compounds were administered subcutaneously, the likelihood of problems with absorption or effects on first pass metabolism is minimized. One further interesting observation for **19** was that despite having similar potency and efficacy on DOPAC levels as **9**, **12**, and **15**, it displayed only a weak effect on spontaneous LMA. A reduction in spontaneous LMA can be caused by many substances that are not dopamine D_2 receptor antagonists, including histamine type 1 (H_1) and adrenergic α_1 receptor blockers and reserpine (by depleting dopamine levels).[77] By itself, this is therefore indicative only of general central nervous system depression. The dopamine D_3 receptor has been suggested to be involved in the control of LMA as a postsynaptic inhibitory receptor, and thereby dopamine D_3 agonists induce a decrease and antagonists an increase in LMA.[78−81] It is interesting to note that compound **19** displays some affinity for dopamine D_3 (6-fold lower than for dopamine D_2, Table 4), and this may partly explain the lower potency in decreasing LMA (dopamine D_3 counteracting the effects on dopamine D_2). But since compound **15** binds to dopamine D_3 as well (Table 3) and since risperidone, ziprasidone, and pimozide are known to bind with high affinity to dopamine D_3 receptors,[82,83] it seems to be an unlikely explanation that the dopamine D_3 receptor affinity will contribute to the different effects on LMA for **15**, **19**, risperidone, pimozide, and ziprasidone. However, we believe that the reduction in LMA seen with these compounds is mainly related to in vivo blocking of dopamine D_2 receptors, and therefore, a different explanation is needed for why **19** only partially reduced the LMA compared with pimozide, risperidone, ziprasidone, **9**, **12**, and **15**. We demonstrated recently that there is a correlation between affinity for dopamine D_2^{Low} and effects on spontaneous

Table 1. In Vitro Data for Compounds 9 and 11−24 and Reference Compounds[e]

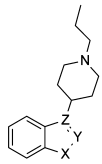
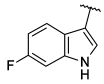
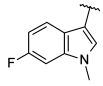
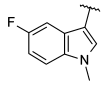
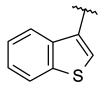
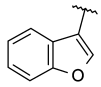
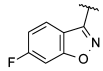
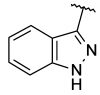
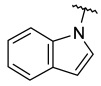
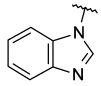
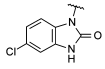
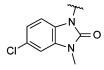
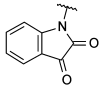
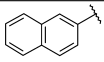
Compound		K_i D_{2S}^{High} (nM)[a]	K_i D_{2S}^{Low} (nM)[a]	D_{2S} K_i^{Low}/K_i^{High}	K_i MAO A (nM)[a]	K_i SERT (nM)[a]
9		43	27	0.6	15780	45
11		157	275	1.7	4053	45
12		19	32	1.7	6863	58
13		26	121	4.6	13990	6.5
14		803	2717	0.3	92	827
15		34	194	5.7	2232	29830
16		187	1066	5.7	7442	5638
17		109	1136	10	74900	54
18		10820	>580000[d]	n.c.[g]	>580000[d]	122
19		456	371	0.8	>580000[d]	3512
20		5182	2002	0.4	>580000[d]	2241
21		12830	12160	0.9	>580000[d]	23830
22		41	112	2.7	5417	52
23		1535	2870	1.9	63	127

Table 1. continued

Compound		K_i D_{2S}^{High} (nM)[a]	K_i D_{2S}^{Low} (nM)[a]	D_{2S} K_i^{Low}/K_i^{High}	K_i MAO A (nM)[a]	K_i SERT (nM)[a]
24		574	2325	4.0	18	130
2[f]		7521	17550	2.3	NT	NT
3[f]		755	3884	5.1	NT	NT
Risperidone (**5**)		NT	2.7[b]	n.c.[g]	NT	NT
Ziprasidone (**6**)		NT	8.50[b]	n.c.[g]	NT	NT
Pimozide (**7**)		NT	11.70[b]	n.c.[g]	NT	NT
Moclobemide		NT	NT	n.c.[g]	11500[c]	NT

[a]Binding affinities (apparent K_i) with [^{3}H]7-OH-DPAT as ligand for dopamine D_{2S} receptor (ag) (h), [^{3}H]methylspiperone as ligand for D_{2S} receptor (ant.) (h), [^{3}H]Ro 41-1049 as ligand for MAO A (rat), and [^{3}H]imipramine as ligand for SERT (h). [b]From Kongsamut et al., CHO cells, dopamine D_{2L} receptor (ant.) binding with [^{3}H]methylspiperone.[75] [c]From Di Santo et al.[76] [d]IC_{50} less than 50% displacement at the highest concentration tested (1.0×10^{-4} M). [e]Confidence intervals are reported in Supporting Information. [f]Data from Pettersson et al.[3] [g]Not calculated because of missing binding values. Abbreviations: [^{3}H]7-OH-DPAT, [^{3}H]7-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; Ro 41-1049, *N*-(2-aminoethyl)-5-(*m*-fluorophenyl)-4-thiazole carboxamide HCl; ag, agonist; ant., antagonist; CHO, Chinese hamster ovary; D_{2L}, dopamine type 2 long receptor; D_{2S}^{Low} dopamine type 2 short receptor low-affinity state; D_{2S}^{High} dopamine type 2 short receptor high-affinity state; MAO A, monoamine oxidase A enzyme; h, human; NT, not tested; n.c., not calculated.

LMA.[3] A compound such as the dopaminergic stabilizer **2** has a very low affinity for dopamine D_2^{Low} (K_i = 175 50 nM)[3] but induces an increase in DOPAC to the same extent as the most potent and efficacious dopamine D_2 receptor antagonists. However, in sharp contrast to these compounds, **2** induces an increase in spontaneous LMA (Table 2). Its unique mechanism of action (surmountable, low affinity, and fast-off receptor kinetics) may account for the increase in spontaneous LMA, since it is believed to allow dopamine receptors to rapidly regain responsiveness to the released dopamine.[3,5,9] Tighter binding to dopamine D_2 receptors therefore means that responsiveness to dopamine is reduced, which consequently reduces spontaneous LMA. In agreement with this, compound **19** binds moderately to dopamine D_2^{Low} (K_i = 371 nM) and demonstrates only a partial reduction in LMA. We have not measured the receptor dissociation kinetics for **19**, but we predict fast-off receptor kinetics, based on the chemical properties for **19** and the overlap with properties reported by Tresadern et al., to be crucial.[15] However, despite the predicted fast-off kinetics, compound **19** does not share the unique effects seen for the dopaminergic stabilizer **2** (i.e., an increase in LMA when reaching maximal effects on DOPAC), and **19** is therefore not regarded as a new dopaminergic stabilizer. On the basis of this finding, we can conclude that in addition to the surmountable antagonism and fast-off kinetics, a low affinity for dopamine D_2 receptors is also needed for a compound to be classified as a dopaminergic stabilizer. Additional notable SAR for the new series of compounds was that methylation of the indole nitrogen slightly decreased the affinity for dopamine D_2 receptors, which was further supported by lesser effects on DOPAC levels (comparing **9** and **11**). However, moving the fluoro atom from position 6 to 5 recovered the affinity and in vivo potency/efficacy (**12**). The reversed indole (**17**) and 1-naphthyl (**22**) were found to have weak effects on DOPAC levels compared with the indoles (**9**, **11**, **12**) and 1,2-benzisoxazole (**15**), and for these compounds the highest dose (100 μmol/kg) may not have been sufficient to reach the possible maximal effect on DOPAC levels (300−400% increase). In addition, the effect on LMA was also weak, if any. It is interesting to note that 1-naphthyl **22** bound with high affinity to dopamine D_2^{High} (41 nM, Table 1), although this did not correlate well with in vivo potency. The corresponding benzimidazole (**18**) and isatin (**21**) were found to be completely devoid of effects on DOPAC levels, which correlates with their very low affinity for dopamine D_2 receptors.

In Vivo and in Vitro Effects on MAO A and DOPAC Levels. The most surprising effects among the target compounds were those observed in compounds that induced a dose-dependent decrease in DOPAC levels (**14**, **23**, and **24**, Figure 4 and Table 2). Dopamine D_2 receptor agonists are known to reduce DOPAC levels, but when we investigated further the effects in vivo (e.g., effects on 3-methoxytyramine, 3-MT), the profiles of these three compounds were shown to be very similar to that of moclobemide (Table 2), a known selective and reversible inhibitor of MAO A.[84,85] This was further supported by subsequent affinity screening, where these three compounds displayed high affinity for MAO A (92, 63, and 18 nM for **14**, **23**, and **24**, respectively) but lacked essential affinity for dopamine D_2 receptors (Table 1). The most striking effect was the replacement of the sulfur in 3-benzothiophene **13** (a dopamine D_2 receptor antagonist lacking affinity for MAO A, 13 990 nM; Table 1) with oxygen (3-benzofuran **14**), which resulted in loss of much of the dopamine D_2 receptor affinity but increased the affinity for MAO A by 1000-fold (92 nM). From a SAR perspective this is a very unexpected finding, especially given that oxygen and sulfur belong to the same atom "family". Similarly, it was surprising to discover that moving the position of attachment for the naphthalene ring from position 1 (**22**) to 2 (**23**) switched the selectivity from dopamine D_2 receptor to MAO A. This is easier to understand from a SAR perspective, since this relates to geometrical aspects and it seems that

Table 2. In Vivo Data for Compounds 9 and 11−24 and Reference Compounds in Rats

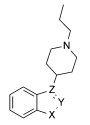
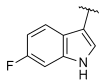
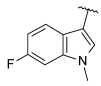
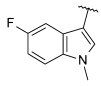
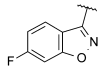
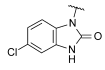
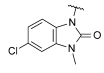
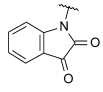
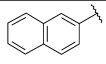
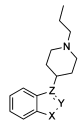
Compound		clogP[f]	ED_{50} DOPAC (μmol/kg)[d]	Dose (μmol/kg)	DOPAC % of control ± SEM[a]	5-HIAA % of control ± SEM[b]	LMA % of control ± SEM[c]
9		3.5	4.1	33	299 ± 9 *	93 ± 2.6	2.8 ± 2.2 *
11		4.3	33	100	264 ± 18 *	95 ± 4.8	27 ± 5
12		4.2	3.0	33	275 ± 15 *	87 ± 6.2	9 ± 2.1 *
13		4.4	54	100	290 ± 25 *	79 ± 4.8 *	48 ± 24
14		4.3	n.c.[g]	100	35 ± 3.4 *	84 ± 3.3	75 ± 34
15		3.2	4.0	33	350 ± 17 *	111 ± 0.7	8.3 ± 2.9 *
16		2.5	55	33	227 ± 7.7 *	105 ± 4.9	7 ± 4.7 *
17		4.4	126	100	227 ± 17 *	87 ± 8.8	92 ± 43
18		3.0	IA	100	111 ± 8.6	98 ± 3.4	20 ± 8 *
19		3.0	7.0	33	334 ± 18 *	101 ± 1	54 ± 13
20		3.8	47	100	253 ± 4.3 *	113 ± 7.5	27 ± 9
21		2.4	IA	100	101 ± 2.9	95 ± 2.7	118 ± 48
22		4.5	103	100	218 ± 11 *	76 ± 6.3 *	62 ± 16
23		4.6	n.c.[g]	100	32 ± 1.4 *	70 ± 2.5 *	22 ± 8 *

Table 2. continued

Compound		clogP[f]	ED_{50} DOPAC (μmol/kg)[d]	Dose (μmol/kg)	DOPAC % of control ± SEM[a]	5-HIAA % of control ± SEM[b]	LMA % of control ± SEM[c]
24		4.7	n.c.[g]	100	28 ± 3.7 *	76 ± 9 *	37 ± 7
2[e]		2.21	81	300	298 *	106	200
3		2.36	63	100	260±15*[h]	100±1.8 *	215±62[h]
Risperidone (**5**)		2.7	0.5	2.4	347 ± 8 *	115 ± 4.8 *	11 ± 4.8 *
Ziprasidone (**6**)		4.2	1.2	6.4	311 ± 10 *	98 ± 0.5	13 ± 4.9 *
Pimozide (**7**)		4.4	1.6	5.8	416 ± 18 *	101 ± 5	7.3 *
Citalopram		n.c.	IA	25	101 ± 2.3	69 ± 2 *	50 ± 20
Moclobemide		n.c.	n.c.[g]	37	18 ± 0.4 *	81 ± 1.7 *	164 ± 48

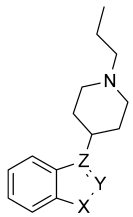
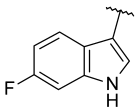
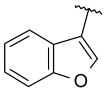
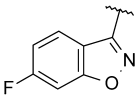
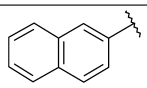
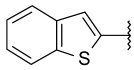
[a]Post-mortem neurochemistry analysis of striatal DOPAC levels compared with saline control (n = 4). [b]Post-mortem neurochemistry analysis striatal 5-HIAA levels compared with saline control (n = 4). [c]LMA 15−60 min after subcutaneous injection, measured at 25 Hz, compared with saline control. To compare the LMA of different compounds, the lowest dose required to produce a maximal DOPAC response was selected. [d]Calculated using methodology described by Ponten et al.[4] [e]Data from Ponten et al.[4] [f]Calculated logarithm of the compound's partition coefficient between n-octanol and water with Advanced Chemistry Development (ACD), version 12 (Toronto, Canada). [g]Not calculated because of decreasing values in dose response. [h]Data from Pettersson et al.[3] *, P <0.05 using Student's t test. Abbreviations: n.c., not calculated; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; LMA, locomotor activity; IA, inactive.

substitution in the "para" position of the aromatic ring (methine carbon in **23**) is not tolerated by dopamine D_2 receptors but is by MAO A. This is also in agreement with what has been reported by Pettersson et al. for para-substituted phenylpiperidines[86] and is further supported by the finding of 2-benzothiophene **24**. Compound **24** also mimicked the reported MAO A inhibitor brofaromine (**35**, Figure 5).[85,87]

Relationship between in Vivo DOPAC and in Vitro Dopamine D_2 Receptor and MAO A. Once released, dopamine is metabolized primarily by MAO A to its main metabolite DOPAC, and an increased release of dopamine therefore leads to higher levels of DOPAC. However, if MAO A is inhibited, this metabolism is blocked, and in agreement with this, inhibitors of MAO A (e.g., moclobemide) reduce striatal DOPAC levels. MAO A inhibition and dopamine D_2 receptor antagonsim have opposing effects on DOPAC levels. As such, we investigated whether these two counteracting effects could explain why some compounds did not produce the maximal increase (350−400%) in DOPAC levels that would be expected from dopamine D_2 receptor antagonism. We also examined whether this could explain why some compounds (e.g., 1-naphthalene **22**) had less potent effects on DOPAC levels compared with their in vitro potency. Finally, the effects of 2*H*-indazole **16** on DOPAC levels (which plateau at a maximum 227% of control rather than the expected 350−400%) may be explained by such a counteracting mechanism. The effect on DOPAC levels was modeled against the binding affinity for dopamine D_2 receptors, MAO A, and SERT, using partial least-squares (PLS) regression.[88−90] Compounds for which it was not possible to measure a K_i value have been excluded from the modeling. A two-component model with Q^2 of 0.85 and a R^2Y of 0.93 was obtained (Figure 6), in which binding to MAO A and dopamine D_2 receptors modeled very well the effect on observed DOPAC levels. The affinity for SERT had a minor contribution to the model and is therefore hard to interpret. On the basis of this result, we conclude that the overall effect of any particular compound on DOPAC levels results from a combination of its ability to antagonize dopamine D_2 receptors and inhibit MAO A, which both counteract each other. Compounds **22** and **16** fit well with this model and support the hypothesis that less potent effects on DOPAC levels are due to inhibition of MAO A. It is also worth mentioning that the MAO A inhibitor moclobemide displays low affinity for MAO A (K_i = 11 500 nM) but is still able to inhibit the enzyme efficiently. As such, the low affinity observed for some of the new compounds may at first glance appear insufficient to explain the effects on DOPAC levels; however, the supporting evidence indicates that low affinity is an important contributor to the net effect on DOPAC.

Effects on Affinity for SERT and 5-HIAA Levels in Vivo. The compounds were also screened for their effects on 5-HIAA levels in the rat striatum, which can be decreased by direct stimulation of serotonin 5-HT_{1A} receptors (e.g., by agonists such as (+)-8-OH-DPAT[91,92]) or indirectly by increased synaptic levels of 5-HT (e.g., by selective serotonin reuptake inhibitors, SSRIs, such as citalopram, Table 2).[93,94] In general, there was no effect on 5-HIAA levels among the compounds tested, with the exceptions of 1-naphthalene **22** and 3-benzothiophene **13**, which induced a partial but statistically significant decrease in 5-HIAA levels that was comparable with the effects of citalopram. These effects also correlated with the affinity for the SERT protein (Table 1). In addition, 2-naphthalene **23** and 2-benzothiophene **24** induced a statistically significant decrease in 5-HIAA, although these two compounds were also potent MAO A inhibitors (moclobemide is also known to induce a decrease in 5-HIAA levels)[84,85] (Table 2). The reason for the decrease in 5-HIAA levels following treatment with **23** and **24** can therefore be inhibition of MAO A or SERT or a combination of the two. Furthermore, the low in vitro binding affinities of 1,2-benzisoxazole **15**, 1*H*-indazole **16**, benzimidazolones **19** and **20**, and isatin **21** for SERT indicate that these structural motifs

Table 3. In Vitro Selectivity Data for Compounds 9, 14, 15, 23, and 24

		5-HT_{1A}[a] (%)	5-HT_{2A}[a] (%)	α_2[a,b] (%)	D_3[a] (%)	D_4[a] (%)	DAT[a] (%)
9		14	100	91	NT	NT	1
14		49	NT	NT	28	26	34
15		17	NT	NT	83	37	15
23		41	91	20	NT	NT	47
24		23	95	21	NT	NT	67

[a]Inhibition of control specific binding at 1 μM reported with [^{3}H]8-OH-DPAT as ligand for 5-HT_{1A} (ag) (h), [^{3}H]ketanserin as ligand for 5-HT_{2A} (ant.) (h), [^{3}H]UK 14.304 as ligand for α_2 (nonselective) (ag) (h), [^{3}H]7-OH-DPAT as ligand for D_3 (ag) (h), [^{3}H]methylspiperone as ligand for D_4 (ant.) (h), and [^{3}H]BTCP as ligand for DAT (ant.) (h). [b]α_2 nonselective binding. Abbreviations: [^{3}H]8-OH-DPAT, [^{3}H]8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; [^{3}H]LSD, [^{3}H]lysergic acid diethylamide; [^{3}H]UK 14.304, 5-bromo-6-(imidazoline-2-yl)aminoquinoxaline tartrate; [^{3}H]7-OH-DPAT, [^{3}H]7-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; [^{3}H]BTCP, [^{3}H]*N*-[1-(2-benzo(*b*)thiophenyl)cyclohexyl]piperidine; 5-HT, serotonin; α_2, adrenergic receptor; D_3, dopamine type 3 receptor; D_4, dopamine type 4 receptor; DAT, dopamine transporter protein; ag, agonist; ant., antagonist; h, human; IC_{50}, half maximal inhibitory concentration; K_i, inhibition constant; NT, not tested.

are not tolerated in the interaction with the SERT protein (Table 1). From a SAR perspective, it is interesting to note that these five compounds have a heteroatom in the 2-position of the five-membered ring while remaining compounds that display affinity for SERT have a methine carbon in the 2-position.

Compound 19 Additional in Vivo and in Vitro Data. Despite not being classified as a dopaminergic stabilizer, compound **19** showed an interesting pharmacological profile for further characterization in animal models of psychoses. Compound **19** was found to counteract both *d*-amphetamine- and MK-801-induced hyperlocomotion, which were reduced to 4% and 42% of control, respectively (see Table 4S). The effects were comparable with the effects observed for typical/atypical antipsychotic drugs and dopaminergic stabilizers such as **2**.[3,4] **19** was also found to be selective for dopamine D_2 receptors compared with dopamine D_3 (6-fold), D_4, serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and adrenergic α_{2A} receptors, DAT and SERT transporters, and MAO A enzyme (Tables 1 and 4). In summary, **19** demonstrated selective dopamine D_2 receptor antagonism and indicated a low propensity to induce EPS in patients, which warrants further characterization in different animal models.

CONCLUSION

Scaffold jumping among five- and six-membered bicyclic aryl rings attached to the piperidine ring had a marked impact on the effects of compounds on the dopaminergic and serotonergic systems. Potent and selective dopamine D_2 receptor antagonists were achieved using 3-indoles, 3-benzoisoxazoles, 3-benzimidazol-2-one, and 3-benzothiophenes, although 3-isatin and 3-benzimidazole were devoid of activity. In contrast, 3-benzofuran was a potent and selective MAO A inhibitor. Effects on DOPAC levels correlated very well with affinity for dopamine D_2 receptors and MAO A. This correlation may explain why some compounds did not reach a full dopamine D_2 receptor antagonist effect in vivo, since MAO A inhibition would have counteracted the increase in DOPAC levels following dopamine D_2 receptor blockade. It is clear that none of the new compounds mimicked the behavioral effects of the dopaminergic stabilizer pridopidine, most likely because of retention in high affinity for both dopamine D_2^{High} and D_2^{Low}. This prevents rapid responsiveness to synaptically released dopamine, hampering subsequent increases in behavioral activity. Thus, previous development of dopaminergic stabilizers from dopamine agonist motifs seems to offer an advantage with respect to the interaction with the dopamine D_2 receptor. Among the compounds tested, **19** was the most interesting, demonstrating efficacy in several animal

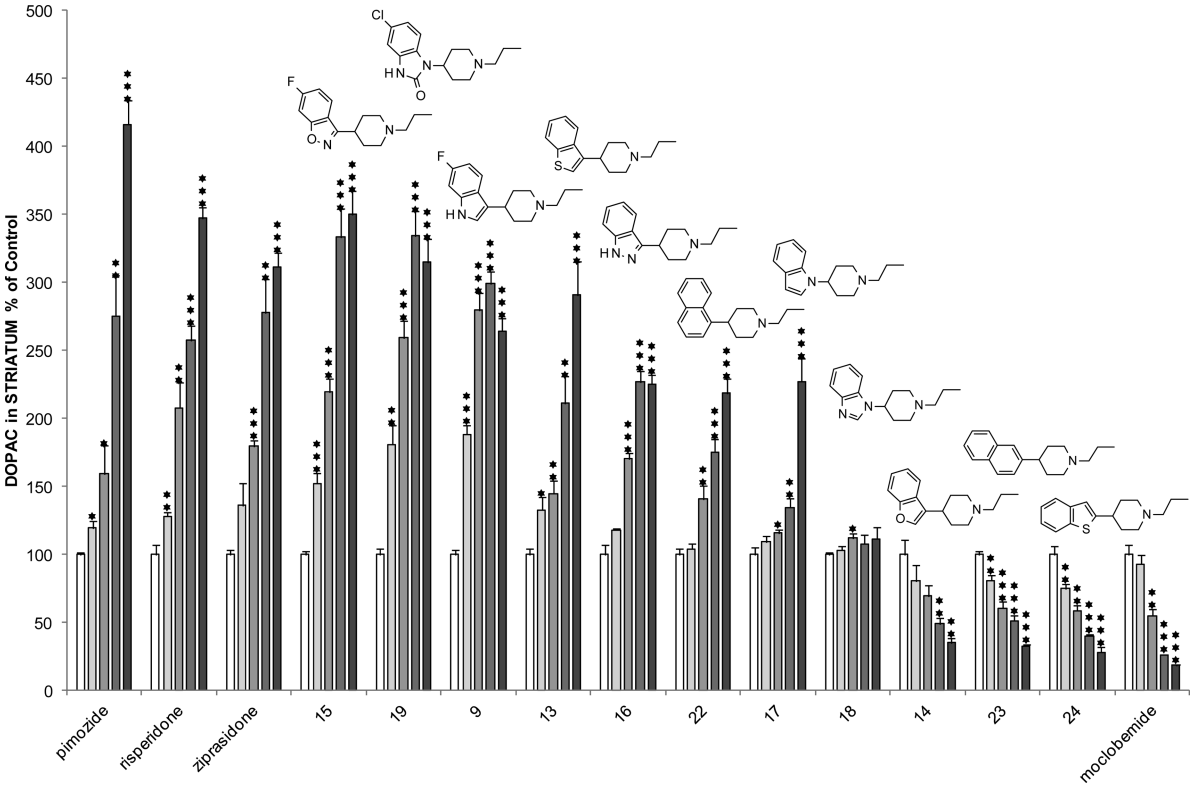
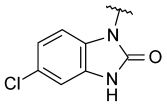


Figure 4. In vivo DOPAC (% of control) dose response in rat striatum for selected compounds and reference compounds. Controls are indicated with a white bar. **9**, **13**, **14**, **16**−**19**, **22**−**24** in doses 3.7, 11, 33, and 100 μmol/kg. **15**: 1.2, 3.7, 11, and 33 μmol/kg. Ziprasidone: 0.2, 0.7, 2.1, and 6.4 μmol/kg. Pimozide: 0.2, 0.6, 1.9, and 5.8 μmol/kg. Risperidone: 0.07, 0.2, 0.7, and 2.4 μmol/kg. Moclobemide: 1.4, 4, 12, and 37 μmol/kg. Statistical significance is assessed using *t* test (two-tailed) versus controls: (∗) $P < 0.05$, (∗∗) $P < 0.01$, (∗∗∗) $P < 0.001$. Error bars indicate standard error of the mean (SEM).

Table 4. In Vitro Selectivity Data for Compound 19

		K_i 5-HT$_{1A}$[a] (nM)	K_i 5-HT$_{2A}$[a] (nM)	K_i 5-HT$_6$[a] (nM)	K_i α_{2A}[a] (nM)	K_i D$_3$[a] (nM)	D$_4$[b] (%)	DAT[b] (%)
19[c]		5709	21570	>580000[d]	17170	2520	17	15

[a]Binding affinities (apparent K_i) with [^{3}H]8-OH-DPAT as ligand for 5-HT$_{1A}$ (ag) (h), [^{3}H]ketanserin as ligand for 5-HT$_{2A}$ (ant.) (h), [^{3}H]LSD as ligand for 5-HT$_6$ (ag) (h), [^{3}H]UK 14.304 as ligand for α_{2A} (ag) (h), and [^{3}H]7-OH-DPAT as ligand for D$_3$ (ag) (h). [b]Inhibition of control specific binding at 1 μM reported with [^{3}H]methylspiperone as ligand for D$_4$ (ant.) (h) and [^{3}H]BTCP as ligand for DAT (ant.) (h). [c]Confidence intervals are reported in Supporting Information. [d]IC$_{50}$ less than 25% displacement at the highest concentration tested (1.0×10^{-4} M). Abbreviations: [^{3}H]8-OH-DPAT, [^{3}H]8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; [^{3}H]7-OH-DPAT, [^{3}H]7-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; [^{3}H]BTCP, [^{3}H]*N*-[1-(2-benzo(*b*)thiophenyl)cyclohexyl]piperidine; [^{3}H]LSD, [^{3}H]lysergic acid diethylamide; [^{3}H]UK 14.304, 5-bromo-6-(imidazoline-2-yl)-aminoquinoxaline tartrate; 5-HT, serotonin; α_{2A}, adrenergic type 2A receptor; D$_3$, dopamine type 3 receptor; D$_4$, dopamine type 4 receptor; DAT, dopamine transporter protein; ag, agonist; ant., antagonist; h, human; IC$_{50}$, half-maximal inhibitory concentration; K_i, inhibition constant.

models of psychosis with only a partial reduction of spontaneous LMA, indicating it may have very low propensity to induce EPS in patients.

■ EXPERIMENTAL SECTION

Chemistry. General. ^{1}H and ^{13}C NMR spectra were recorded in CD$_3$OD, CDCl$_3$, or DMSO-d_6 at 300 and 75 MHz, respectively, using a Varian XL 300 spectrometer (Varian, Darmstadt, Germany), or at 400 and 100 MHz, respectively, using a Mercury Plus 400 spectrometer (Varian, Darmstadt, Germany). Chemical shifts are reported as δ values (ppm) relative to an internal standard (tetramethylsilane). Low-resolution mass spectra were recorded on a HP 5970A instrument (Agilent Technologies, Stockholm, Sweden) operating at an ionization potential of 70 eV. The mass detector was interfaced with a HP5700 gas chromatograph (Agilent Technologies, Stockholm, Sweden) equipped with a fused silica column (11 m, 0.22 mm i.d.) coated with cross-linked SE-54 (film thickness 0.3 mm, He gas, flow 40 cm/s). Electrospray

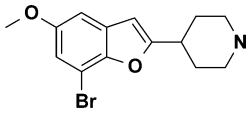


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Figure 5. Chemical structure of monoamine oxidase A (MAO A) inhibitor brofaromine.

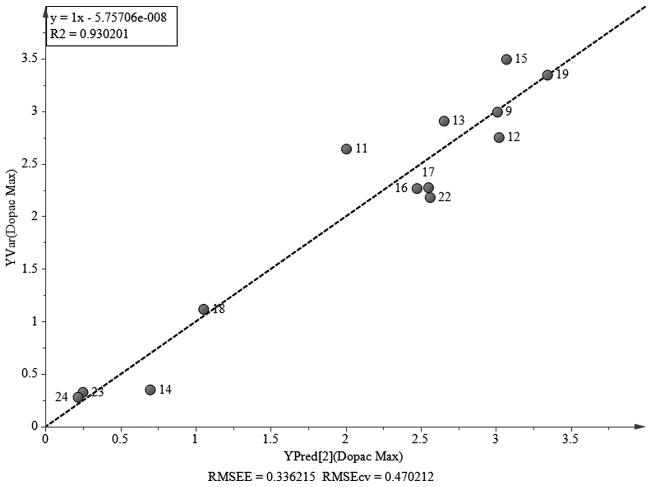

Figure 6. Partial least-squares (PLS) regression on 3,4-dihydroxyphenylacetic acid (DOPAC) was modeled against the binding affinity for dopamine D_2 receptors, monoamine oxidase A (MAO A) and serotonin transporter protein (SERT).

ionization mass spectra were recorded on Agilent 1200 series liquid chromatography/mass selective detector (Agilent Technologies, Stockholm, Sweden). The microwave heating was performed in a Smith synthesizer single-mode microwave cavity producing continuous irradiation at 2450 MHz (Personal Chemistry AB, Uppsala, Sweden). For further instructions see Alterman et al.[95] Elemental analyses were performed by MikroKemi AB (Uppsala, Sweden). Melting points were determined with Büchi 545 instrument (Kebo Lab, Goteborg, Sweden) and are uncorrected. For flash chromatography, silica gel 60 (0.040−0.063 mm, VWR, no. 109385) was used. The amine products were converted to the corresponding salts by dissolving the free base in methanol or ethanol and adding 1 equiv of oxalic acid or ethanolic HCl solution. The solvent was removed and azeotroped with absolute ethanol in vacuo followed by recrystallization from appropriate solvents. Purity of all target compounds was assessed as greater than 95% by elemental analysis (C, H, N).

General Procedure for the Alkylation of the 4-Arylpiperidines/piperidenes (9, 10, 14, 15, 17−19, 22, 23, 32, 34). The 4-arylpiperidine (1 equiv, 2.5 mmol) was dissolved in ACN (50 mL), and iodopropane (1.2 equiv) and K_2CO_3 (3 equiv) were added. The mixture was refluxed for 15 h, cooled to ambient temperature, and K_2CO_3 was filtered off and subsequently washed with ACN (2 × 50 mL). The combined organic phases were concentrated in vacuo. The residue was purified with flash chromatography using an ethyl acetate (EtOAc)−MeOH or CH_2Cl_2−MeOH gradient to give the title compounds.

General Procedure for the Methylation of 1-Propyl-4-arylpiperidine Core NH Position (11, 12, and 20). 1-Propyl-4-arylpiperidine (1 equiv, 1 mmol) was dissolved in 2 mL of anhydrous DMF at 0 °C, and NaH at 60% dispersion in mineral oil (1.1 equiv) was added. The mixture was stirred under N_2 at ambient temperature until evolution of H_2 gas ceased (∼30 min). Iodomethane (1.1 equiv) was added, and the mixture was allowed to stir for 2 h at room temperature. Brine was added, and the reaction mixture was extracted with EtOAc (2 × 50 mL). The organic portion was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (isooctane/EtOAc/MeOH gradient) to give the title compounds.

4-(Benzothiophen-3-yl)-1-propylpiperidine (13). To a solution of **32** (0.26 g, 1.01 mmol) in MeOH (10 mL) were added concentrated HAc (1 mL) and Pd/C (0.26 g) under N_2. The reaction mixture was hydrogenated under H_2 (50 psi) for 30 h. Filtration and evaporation of the filtrate afforded the crude product as the HAc salt. Aqueous Na_2CO_3 (10%, 50 mL) and EtOAc (50 mL) were added and the phases separated. The water layer was extracted with EtOAc (2 × 50 mL), and the combined organic phases were dried ($MgSO_4$) and evaporated to dryness in vacuo. The residue was purified by flash column chromatography with EtOAc/MeOH gradient to give the title compound in 38% yield (0.10 g, 0.38 mmol). MS *m/z* (relative intensity, 70 eV) 259 (M^+, 27), 231 (17), 230 (bp), 115 (28), 98 (38). ESIMS: *m/z* 260.0 $(M + H)^+$. ^1H NMR (DMSO-d_6, 300 MHz) δ = 0.96 (t, *J* = 7.20 Hz, 3H), 1.55−1.82 (m, 2H), 1.84−2.04 (m, 2H), 2.08−2.31 (m, 2H), 2.53 (br s, 2H), 2.90−3.18 (m, 2H), 3.20−3.35 (m, 1H), 3.55 (d, *J* = 11.23 Hz, 2H), 7.43 (quin, *J* = 7.14 Hz, 2H), 7.52 (s, 1H), 7.99 (dd, *J* = 16.36, 7.57 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ = 10.95, 17.14, 29.01, 32.91, 51.84, 57.37, 121.15, 121.73, 123.00, 123.98, 124.38, 137.74, 138.97, 139.81. The amine was converted to the oxalate salt and recrystallized in MeOH/diethyl ether, mp 182−184 °C. Anal. ($C_{16}H_{21}NS\cdot{}^4/_3C_2H_2O_4$).

4-(Benzothiophen-2-yl)-1-propylpiperidine (24). To a solution of **34** (0.37 g, 1.43 mmol) in MeOH (10 mL) were added concentrated HCl (1 mL) and Pd/C (0.6 g) under N_2. The reaction mixture was hydrogenated under H_2 (50 psi) for 15 h. Filtration and evaporation of the filtrate afforded 0.3 g of crude product as the HCl salt. Aqueous Na_2CO_3 (10%, 50 mL) and EtOAc (50 mL) were added and the phases separated. The water layer was extracted with EtOAc (2 × 50 mL), and the combined organic phases were dried ($MgSO_4$) and evaporated to dryness in vacuo. The residue was purified by flash column chromatography with EtOAc/MeOH gradient to give the title compound in 22% yield (0.083 g, 0.31 mmol). MS *m/z* (relative intensity, 70 eV) 259 (M^+, 30), 231 (17), 230 (bp), 115 (22), 98 (15). ESIMS: *m/z* 260.0 $(M + H)^+$. ^1H NMR (CD_3OD, 300 MHz) δ = 0.92 (t, *J* = 7.45 Hz, 3H), 1.53 (dd, *J* = 15.87, 7.57 Hz, 2H), 1.81 (dd, *J* = 12.21, 2.44 Hz, 2H), 1.92−2.18 (m, 4H), 2.29 (m, 2H), 2.84 (t, *J* = 11.84 Hz, 1H), 2.99 (d, *J* = 11.96 Hz, 2H), 7.05 (s, 1H), 7.16−7.46 (m, 2H), 7.55−7.94 (m, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ = 12.29, 20.65, 34.64, 39.14, 54.63, 61.90, 120.04, 123.06, 124.00, 124.65, 125.16, 140.07, 141.46, 151.87. The amine was converted to the oxalate salt and recrystallized in MeOH, mp 200−201 °C. Anal. ($C_{16}H_{21}NS\cdot C_2H_2O_4$) C, H, N.

4-(Benzothiophen-3-yl)-1,2,3,6-tetrahydropyridine (31). To a solution of 3-bromobenzothiophene (4.0 g, 18.8 mmol) in dry diethyl ether (10 mL) at −78 °C, *n*-butyllithium in hexane (2.5 M, 8.24 mL, 20.6 mmol) was added. The mixture was stirred at −78 °C under an N_2 atmosphere for 20 min. 1-Boc-4-piperidone (3.74 g, 9.38 mmol) in dry diethyl ether (10 mL) was added via syringe. The solution was stirred for an additional 1 h. The reaction mixture was then diluted with aqueous NH_4Cl, and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 100 mL) and the combined organic phase was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give (4.54 g, 72%) of crude *tert*-butyl 4-(benzothiophen-3-yl)-4-hydroxypiperidine-1-carboxylate. MS *m/z* (relative intensity, 70 eV) 333 (M^+, 13), 233 (47) 188 (52), 162 (55), 57 (bp). ESIMS: *m/z* 356.0 $(M + Na)^+$. The residue was dissolved in CH_2Cl_2 (100 mL). TFA (8 mL) was added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was poured out on ice, basified with aqueous 10% Na_2CO_3 to pH 10, and extracted with EtOAc (3 × 100 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo to give crude product, 2.34 g. The residue was purified by flash chromatography using EtOAc/MeOH gradient as eluent, affording **31** (1.42 g, 35%). MS *m/z* (relative intensity, 70 eV) 215 (M^+, bp), 214 (54) 186 (64), 171 (37), 147 (26). ESIMS: *m/z* 216.0 $(M + H)^+$. ^1H NMR (CD_3OD, 300 MHz) δ = 2.78 (br s, 2H), 3.43 (t, *J* = 6.0 Hz, 2H), 3.84 (d, *J* = 2.70 Hz, 2H), 6.06 (t, *J* = 1.80 Hz, 1H), 7.31−7.42 (m, 2H), 7.51 (s, 1H), 7.86 (d, *J* = 6.9 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H). ^{13}C NMR

(CD_3OD, 75 MHz) δ = 27.12, 42.35, 43.35, 120.22, 123.90, 123.92, 124.64, 125.46, 125.59, 132.80, 137.67, 138.29, 141.94.

4-(Benzothiophen-3-yl)-1-propyl-3,6-dihydro-2*H*-pyridine (32). **31** was alkylated by the above general procedure and was obtained in 100% yield. MS *m/z* (relative intensity, 70 eV) 257 (M^+, 74), 256 (33), 228 (bp), 185 (36), 147 (28). ESIMS: *m/z* 258.0 $(M + H)^+$. ^1H NMR (CD_3OD, 300 MHz) δ = 0.89 (t, *J* = 7.32 Hz, 3H), 1.50 (sxt, *J* = 7.62 Hz, 2H), 2.29 (d, *J* = 8.06 Hz, 2H), 2.48 (d, *J* = 1.95 Hz, 4H), 3.01 (s, 2H), 5.92 (br s, 1H), 6.78−7.56 (m, 3H), 7.78 (d, *J* = 7.81 Hz, 1H), 7.90 (d, *J* = 7.57 Hz, 1H). ^{13}C NMR (CD_3OD, 75 MHz) δ = 12.35, 20.73, 30.56, 51.15, 53.54, 61.31, 123.16, 123.85, 124.15, 124.51, 125.16, 125.31, 132.18, 138.68, 138.92, 141.94.

4-(Benzothiophen-2-yl)-1,2,3,6-tetrahydropyridine (33). To a solution of benzothiophene (0.5 g, 3.72 mmol) in dry THF (10 mL) at −78 °C, *n*-buthyllithium in hexane (2.5 M, 1.63 mL, 4.09 mmol) was added. The mixture was stirred at −78 °C under an N_2 atmosphere for 2 h and then allowed to warm to room temperature for 2 h. 1-Boc-4-piperidone (0.74 g, 3.72 mmol) in dry THF (5 mL) was added via syringe. The solution was stirred for an additional 30 min. The reaction mixture was then diluted with aqueous NH_4Cl, and the phases were separated. The aqueous phase was extracted with ethyl acetate (3 × 50 mL) and the combined organic phase was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 1.26 g (95%) of crude *tert*-butyl 4-(benzothiophen-2-yl)-4-hydroxypiperidine-1-carboxylate. MS *m/z* (relative intensity, 70 eV) 333 (M^+, 13), 233 (63), 188 (34), 162 (37), 57 (bp). ESIMS: *m/z* 356.0 $(M + Na)^+$. The residue was dissolved in CH_2Cl_2 (50 mL), and TFA (2 mL) was added. The mixture was stirred at room temperature for 15 h. The reaction mixture was poured out on ice, basified with aqueous 10% Na_2CO_3 to pH 10, and extracted with EtOAc (3 × 50 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo to give 0.43 g of crude **33**. The residue was purified by flash chromatography using EtOAc/MeOH gradient as eluent, affording **33** (0.32 g, 39%). MS *m/z* (relative intensity, 70 eV) 215 (M^+, bp), 214 (67), 186 (49), 147 (25), 115 (22). ESIMS: *m/z* 216.0 $(M + H)^+$. ^1H NMR (CD_3OD, 300 MHz) δ = 2.36 (br s, 2H), 2.87 (t, *J* = 5.74 Hz, 2H), 3.29 (d, *J* = 2.69 Hz, 2H), 6.09 (br s, 1H), 7.07 (s, 1H), 7.15−7.38 (m, 2H), 7.65 (dd, *J* = 14.16, 6.35 Hz, 2H). ^{13}C NMR (CD_3OD, 75 MHz) δ = 27.71, 43.29, 45.47, 119.58, 122.92, 124.50, 125.36, 125.52, 131.58, 139.61, 141.70, 146.42.

4-(Benzothiophen-2-yl)-1-propyl-3,6-dihydro-2*H*-pyridine (34). **33** was alkylated by the above general procedure, and 100% yield was obtained. MS *m/z* (relative intensity, 70 eV) 257 (M^+, 77), 256 (33), 228 (bp), 185 (29), 147 (28). ESIMS: *m/z* 258.0 $(M + H)^+$. ^{13}C NMR (CD_3OD, 75 MHz) δ = 12.15, 20.59, 28.10, 50.79, 53.48, 61.00, 120.19, 122.85, 123.59, 124.52, 125.36, 125.58, 131.41, 139.73, 141.69, 145.51.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Statistical data on in vitro binding, raw data for in vivo DOPAC, PLS methods, linear regression of ED_{50}, biological methods, and experimental details of the synthesis of **9−12**, **14−23**, **28−30**; plot of ED_{50} vs pK_i in a separate pdf file. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

EPS, extrapyramidal symptoms; LMA, locomotor activity; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 3-MT, 3-metoxytyramine; MAO A, monoamine oxidase enzyme A; D_2, dopamine type 2 receptor; D_3, dopamine type 3 receptor; D_4, dopamine type 4 receptor; α_{2A}, adrenergic type 2A receptor; L-DOPA, L-3,4-dihydroxyphenylalanine; ACN, acetonitrile; ag, agonist; ant., antagonist; IA, inactive; NT, not tested; SEM, standard error of the mean; clogP, calculated log of the partition coefficient; PLS, partial least-squares; CHO, Chinese hamster ovary; G-protein, guanine nucleotide-binding protein; D_{2S}, dopamine D_2 short; D_{2L}, dopamine D_2 long; D_2^{High}, dopamine D_2 high affinity state; D_2^{Low}, dopamine D_2 low affinity state; nc, not calculated; [^{3}H]8-OH-DPAT, [^{3}H]8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; [^{3}H]7-OH-DPAT, [^{3}H]7-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; [^{3}H]UK 14.304, 5-bromo-6-(imidazoline-2-yl)-aminoquinoxaline tartrate; Ro 41-1049, *N*-(2-aminoethyl)-5-(*m*-fluorophenyl)-4-thiazolecarboxamide HCl; [^{3}H]LSD, [^{3}H]-lysergic acid diethylamide; [^{3}H]BTCP, [^{3}H]*N*-[1-(2-benzo(*b*)-thiophenyl)cyclohexyl]piperidine

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