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Synthesis and characterization of selective dopamine D_2 receptor antagonists

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Abstract—A series of indole compounds have been prepared and evaluated for affinity at D_2-like dopamine receptors using stably transfected HEK cells expressing human D_2, D_3, or D_4 dopamine receptors. These compounds share structural elements with the classical D_2-like dopamine receptor antagonists, haloperidol, *N*-methylspiperone, and benperidol. The compounds that share structural elements with *N*-methylspiperone and benperidol bind non-selectively to the D_2 and D_3 dopamine receptor subtypes. However, several of the compounds structurally similar to haloperidol were found to (a) bind to the human D_2 receptor subtype with nanomolar affinity, (b) be 10- to 100-fold selective for the human D_2 receptor compared to the human D_3 receptor, and (c) bind with low affinity to the human D_4 dopamine receptor subtype. Binding at sigma (σ) receptor subtypes, σ_1 and σ_2, was also examined and it was found that the position of the methoxy group on the indole was pivotal in both (a) D_2 versus D_3 receptor selectivity and (b) affinity at σ_1 receptors. Adenylyl cyclase studies indicate that our indole compounds with the greatest D_2 receptor selectivity are neutral antagonists at human D_2 dopamine receptor subtypes. With stably transfected HEK cells expressing human D_2 (hD_2-HEK), these compounds (a) have no intrinsic activity and (b) attenuated quinpirole inhibition of adenylyl cyclase. The D_2 receptor selective compounds that have been identified represent unique pharmacological tools that have potential for use in studies on the relative contribution of the D_2 dopamine receptor subtypes in physiological and behavioral situations where D_2-like dopaminergic receptor involvement is indicated.

1. Introduction

There are three dopaminergic pathways that are of fundamental importance to the function of the normal brain: the nigrostriatal pathway, the mesocorticolimbic pathway and the tuberoinfundibular pathway. These neuronal systems are involved in movement coordination, cognition, emotion, affect, memory, and the regulation of prolactin secretion by the pituitary. Alterations in the dopaminergic pathways are thought to be involved in the pathogenesis of neurological, neuropsychiatric, and hormonal disorders, including Parkinson's disease, schizophrenia, and hyperprolactinemia.[1–8] In addition, modulation of the dopaminergic pathways is thought to occur as a consequence of acute and chronic abuse of psychostimulants, including cocaine and amphetamines.[9–11]

Molecular genetic studies of G-protein coupled receptors have defined two types of dopamine receptors, the D_1-like (D_1 and D_5 receptor subtypes) and D_2-like (D_2, D_3, and D_4 receptor subtypes) receptors based upon structural and pharmacological similarities. For example, D_1-like receptors are structurally similar and positively linked to the activation of adenylyl cyclase via coupling to the Gs/Golf class of G-proteins.[12] Stimulation of the D_2-like receptors results in coupling with the Gi/Go class of G-proteins, leading to the inhibition of adenylyl cyclase activity.[13–15]

The D_2 and D_3 dopamine receptors have approximately 46% amino acid homology. However, the transmembrane spanning (TMS) regions of the D_2 and D_3

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receptors, which are thought to construct the ligand binding site, share 78% homology.[16] Despite the similarities in the structure of the D_2 and D_3 receptors, the D_2 and D_3 receptors differ in their (a) neuroanatomical localization,[17] (b) levels of receptor expression, (c) efficacy in response to agonist stimulation,[8] and (d) regulation and desensitization.[18] Because of the high degree of homology between D_2 and D_3 receptor binding sites, the pharmacologic properties of these two receptor subtypes are similar and it has been difficult to obtain compounds that can bind selectively to either the D_2 or the D_3 dopamine receptor subtypes.[8,19–21] However, D_2 or D_3 dopamine receptor selective agonists and antagonists would be useful pharmacologic tools to precisely define the role of these two D_2-like receptor subtypes in a variety of experimental physiological and behavioral situations, including the reinforcing and toxic properties of cocaine,[22,23] socialization, memory, and the regulation of interneuronal activity in the basal ganglia.[24]

A previous study reported indole analogs, represented by compound **1**, as having a high affinity for dopamine D_4 versus D_2 and D_3 receptors.[25] These compounds were developed as potential atypical antipsychotic agents based on the observation that clozapine has a moderate affinity for D_4 receptors and a relatively low affinity for D_2 and D_3 receptors.[26] An interesting observation noted in the paper of Kulagowski et al.,[25] but not explored in greater detail, was the high affinity and selectivity of compound **2** for dopamine D_2 versus D_3 and D_4 receptors (Fig. 1). In this communication, we report the synthesis and in vitro binding of congeners of **2** with nanomolar affinity at D_2 receptors and range from 10- to 100-fold selective for D_2 compared to the D_3 receptor subtype. In vitro studies indicate that these compounds are neutral antagonists at D_2-like dopamine receptors.

2. Chemistry

The synthesis of the target compounds is outlined in Schemes 1 and 2. Gramine and 5-methoxygramine are commercially available. Only 4-methoxygramine was made in this study in quantitative yield by treatment of 4-methoxyindole, **3**, with *N*,*N*-dimethylmethyleneammonium iodide (Eschenmoser's salt) as outlined in Scheme 1. Reaction of **4a**–**c** with the corresponding secondary amines gave the corresponding indoleamine analogs in moderate yield (Scheme 2). The synthesis of the 4′-methylthio analogs, **20** and **21**, required a different synthetic route (Scheme 3), which involved the synthesis of the 4-piperidone intermediates, **18a** and **18c**, followed by treatment with the Grignard reagent, **19**, to give the

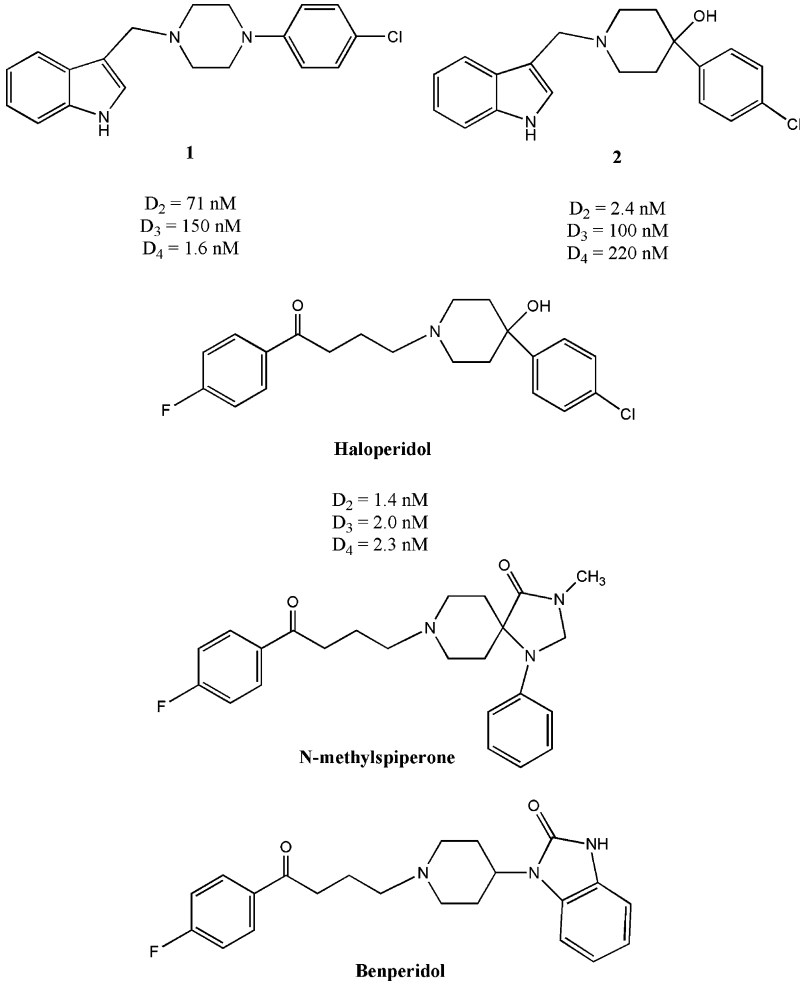
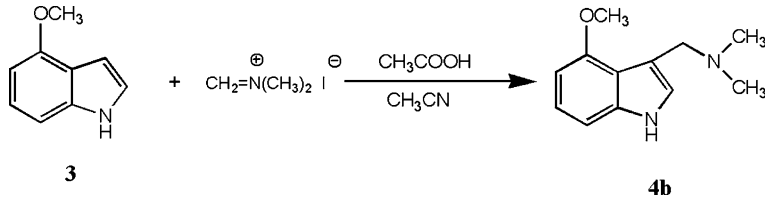
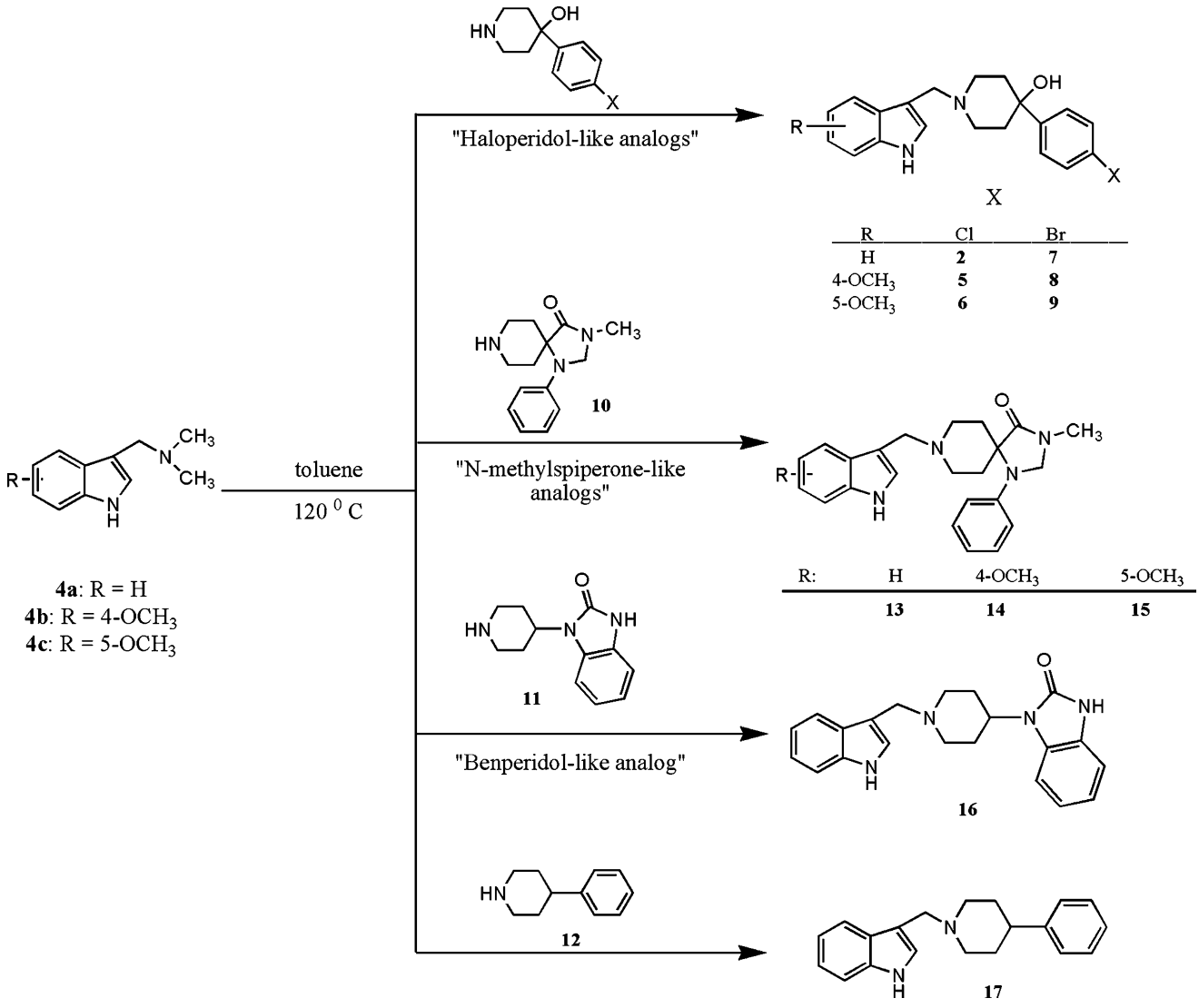


Figure 1. Structures of the lead compounds for the current study. The in vitro binding data are K_i values reported in Ref. 25.



Scheme 1.



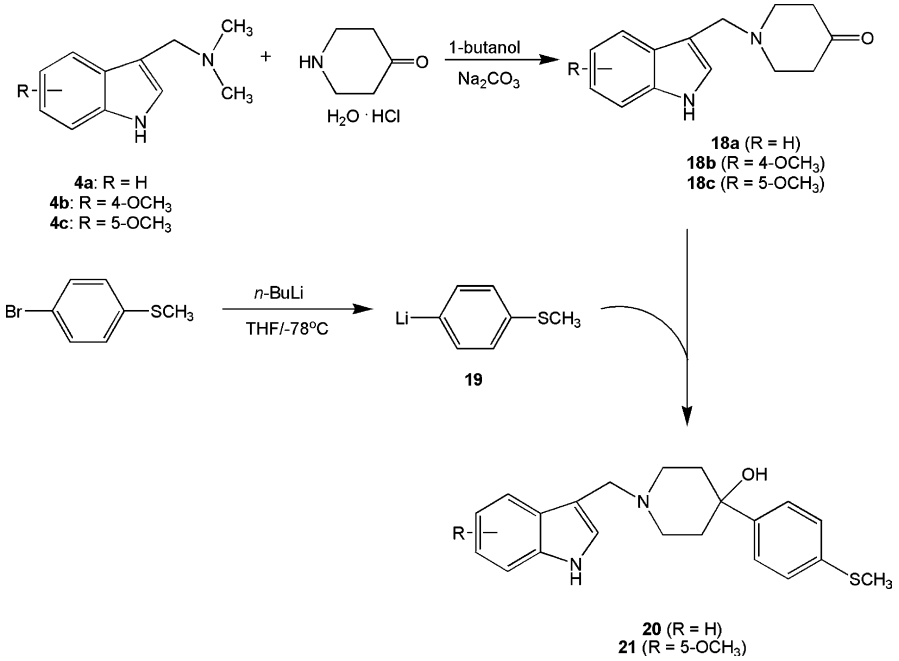
Scheme 2.

target compounds in moderate yield. We were unable to make the corresponding 4-methoxy analog due to the chemical instability of the intermediate, **18b**.

3. Radioligand binding studies at dopamine receptors

Competitive radioligand binding studies were performed to determine the equilibrium dissociation constants of each compound at human D_2, D_3, and D_4 dopamine receptors (Table 1). For these studies tissue homogenates from stably transfected HEK 293 cells were used in conjunction with the radioligand ^{125}I-IABN. We have previously reported that the benzamide ^{125}I-IABN binds with high affinity and selectively to D_2-like dopamine receptors, but it binds non-selectively to the D_2 and D_3 dopamine receptor subtypes.[27]

First, when a comparison is made of the affinity at D_2 and D_3 dopamine receptors of the unsubstituted indoles which have structural elements similar to the butyrophenones *N*-methylspiperone (**13**) and haloperidol (**2**) or benperidol (**16**), only **2** exhibits pharmacological selectivity at D_2 receptors. Compound **2** was reported by



Scheme 3.

Table 1. In vitro binding data for the indole analogs

Compound	K_i[a] (nM)						
	D_2[b]	D_3[c]	D_4[d]	D_3:D_2 ratio[e]	σ_1	σ_2	Log P[f]
2	10 ± 2.5	104 ± 19	449 ± 123[g]	10	19.3 ± 3.6	1811 ± 569	1.54
5	4.2 ± 0.4	250 ± 6	>2000	59	>5000	1246 ± 234	2.10
6	4.2 ± 0.8	204 ± 60	>3500	49	242 ± 68	1900 ± 241	2.16
7	4.8 ± 0.3	110 ± 39	652 ± 7	28	12.1 ± 2.5	2134 ± 356	1.72
8	2.3 ± 0.7	190 ± 34	840 ± 197	82	2557 ± 334	943 ± 64	2.34
9	2.5 ± 0.7	96.9 ± 6.1	700 ± 80	39	108 ± 17	2702 ± 696	2.28
20	23.9 ± 5.5	638 ± 159	319 ± 58[g]	27	39.6 ± 7.1	884 ± 32	1.33
21	5.5 ± 0.1	580 ± 92	567 ± 140	106	236 ± 13	>10,000	1.91
13	134 ± 27	200 ± 125	>5000	1.5	>5000	>10,000	0.46
14	101 ± 2	150 ± 38	>5000	1.5	3147 ± 1290	>10,000	1.17
15	20 ± 5	128 ± 18	>5000	6.4	>5000	>10,000	1.08
16	34 ± 3	38 ± 11	756 ± 243	1.1	>5000	>10,000	1.83
17	203 ± 18	153 ± 36	133 ± 24	0.8	331 ± 21	1374 ± 159	1.75

[a] Mean ± SEM, K_i values were determined by at least three experiments.
[b] K_i values for D_2 receptors were measured on human $D_{2(long)}$ expressed in HEK cells using [^{125}I]IABN as the radioligand.
[c] K_i values for D_3 receptors were measured on human D_3 expressed in HEK cells using [^{125}I]IABN as the radioligand.
[d] K_i values for D_4 receptors were measured on human $D_{4.4}$ expressed in HEK cells using [^{125}I]IABN as the radioligand.
[e] K_i for D_3 receptor/K_i for D_2 receptor.
[f] Calculated value using the program Clog P.
[g] Reported to be 220 nM in Kulagowski et al., 1996.

Kulagowski et al.,[25] to be 40-fold selective for the human D_2 compared to the human D_3 receptor subtype. We found essentially the same affinity for this compound at the D_3 receptor subtype, but a fourfold lower affinity at D_2 receptors. Therefore, our experiments indicate that compound **2** is 10-fold selective at D_2 receptors compared to D_3 dopamine receptors.

Based upon those results, modifications of the indole group were prepared to determine if the addition of a methoxy group to **2** at the 4- or 5-position (**5** and **6**, respectively) would alter D_2/D_3 dopamine receptor selectivity. We found that the addition of the methoxy group resulted in a 2- to 2.5-fold decrease in affinity at D_3 receptors with a concomitant 2-fold increase in affinity at D_2 receptors, resulting in compounds that were 50- to 60-fold selective at D_2 receptors. A similar modification of the 5-position on an unsubstituted indole structurally related to *N*-methylspiperone (**15**) was also found to increase D_2 receptor selectivity, indicating that this type of substitution was important in obtaining a compound that could discriminate pharmacologically between D_2 and D_3 dopamine receptor subtypes. Surprisingly, substitution of a methoxy group at the 4-position (**14**) did not appear to have an effect on D_2-like receptor affinity or D_3:D_2 selectivity.

A comparison of unsubstituted indoles structurally related to haloperidol was made in which a Br (**7**) or a SCH_3 (**20**) was substituted for the Cl moiety (**2**). The substitution with these bulkier groups increased selectivity at D_2 receptors 2- to 3-fold. Although there was an increase in D_2 receptor selectivity, the substitution with the Br group (**7**) increased affinity at D_2 receptors 2-fold (K_i = 5 nM), whereas the substitution with SCH_3 group (**20**) resulted in a 2-fold decrease in affinity.

Our analysis of the indole compounds structurally similar to haloperidol indicated that addition of a methoxy group to the indole and the substitution of a Cl group increased the D_3:D_2 affinity ratio. Therefore, a panel of methoxy indole compounds was prepared and evaluated which substituted either a Br (**8** and **9**) or a SCH_3 group (**21**) for the Cl. This combination of modifications led to the identification of two compounds (**8** and **21**) which exhibited >80-fold selectivity at D_2 receptors compared to D_3 dopamine receptors with nanomolar affinity at D_2 receptors (K_i values of 2.3 and 5.5 nM, respectively).

Finally, we evaluated the compounds with the highest selectivity for D_2 receptors, **2**, **6**, **8**, and **21**, for binding activity at D_1 receptors. Concentration-dependent competitive binding studies were performed using (a) the D_1 receptor selective radioligand ^{3}H-SCH 23390, (b) rat caudate tissue, which is known to express high levels of D_1 dopamine receptors, and (c) the four indoles with the greatest selectivity at D_2 receptors or non-radioactive SCH 23390, which was included as a positive control. While SCH 23390 competed for radioligand binding with high affinity, each of the four indoles exhibited low affinity (IC_{50} > 10 μM) binding at rat D_1 dopamine receptors (data not shown).

4. Adenylyl cyclase studies with D_2-like receptors

The pharmacological properties of a select panel of substituted indoles with the highest selectivity for D_2 dopamine receptors (**5**, **6**, **8**, and **21**) were further evaluated using a whole cell adenylyl cyclase assay to determine if they are agonists or antagonists at D_2 dopamine receptors (Fig. 2). The data shown in Figure 2 are representative of three independent sets of experiments. For these cells, 80–90% inhibition of forskolin-dependent stimulation (100 μM) of adenylyl cyclase activity can be achieved at high concentrations (100 nM) of the D_2-like receptor full agonist quinpirole (**Quin**). For these studies 10 nM quinpirole was used. Although the extent of the inhibition by agonist of forskolin (**Fsk**) stimulation was less than maximal, these experimental conditions were more optimal for determining if the test compound was capable of blocking agonist activity. Although the full agonists, such as quinpirole or dopamine, are able to attenuate forskolin-dependent stimulation of adenylyl cyclase in HEK cells expressing human D_2 receptors (hD_2-HEK cells), essentially no intrinsic activity was observed for any of the four compounds that were evaluated. In addition, each compound was able to block the forskolin-dependent inhibition of quinipirole at D_2 dopamine receptors. These results indicate that these compounds are antagonists at D_2 dopamine receptor.

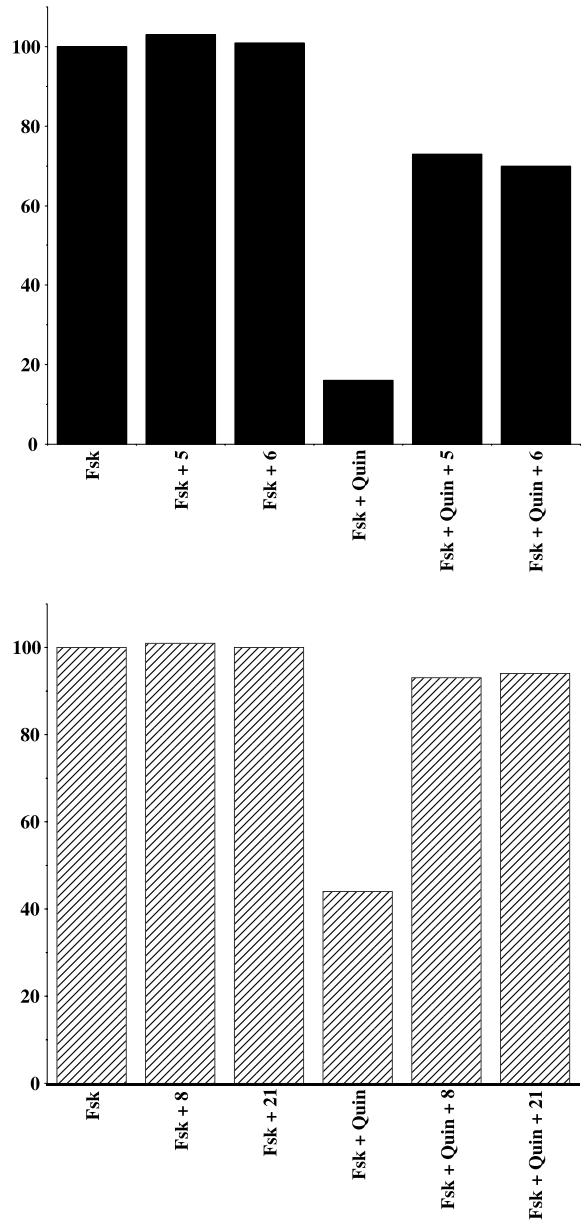


Figure 2. Evaluation of intrinsic activity of D_2 receptor selective indoles at human D_2 dopamine receptors expressed in HEK 293 cells. The top panel presents representative data for compounds **5** and **6**, while the bottom panel depicts data for compounds **8** and **21**. The data presented are representative of three independent experiments for each compound tested. The values for the bar graph are the mean of triplicates for a single experiment.

Finally, we selected compound **5** for further evaluation. Dose–response curves were performed using increasing concentrations of **5** with a constant amount of forskolin (100 μM) and quinpirole (10 nM). In Figure 3, a composite of three independent inhibition studies is shown in which **5** competitively inhibits the effect of quinpirole on forskolin-stimulated hD_2-HEK cells. Under the conditions of these experiments quinpirole inhibited approximately 70% of the forskolin-dependent activation

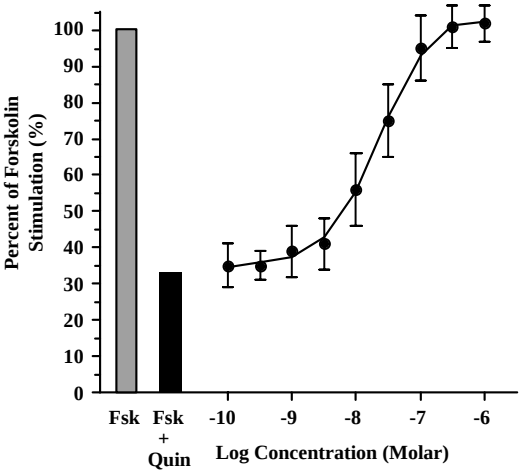


Figure 3. Dose-dependent attenuation of the inhibition of forskolin-dependent stimulation of adenylyl cyclase by **5**. The concentration-dependent mean attenuation of quinpirole inhibition of forskolin-dependent stimulation of adenylyl cyclase by compound **5** using hD_2-HEK cells is shown. The open bar is the normalized stimulation of adenylyl cyclase activity in hD_2-HEK cells using 100 μM forskolin (**Fsk**). The solid bar is the mean percent inhibition (67 ± 4% of maximum) of forskolin-dependent stimulation using 10 nM quinpirole (**Fsk** + **Quin**). The concentration-dependent curve is the mean percent stimulation of adenylyl cyclase activity relative to the normalized forskolin stimulation as a function of the concentration of **5**. The mean values and the error bars (SEM) are for three independent experiments. The IC_{50} value for these composite data is 21 nM and was fit to a one-site fit model using a non-linear regression analysis ($r^2 = 0.99$) where the values for maximum percent stimulation and maximum inhibition of percent stimulation were constrained to experimental values.

of adenylyl cyclase. Analysis of these composite data indicates that **5** attenuated the quinpirole inhibition with an IC_{50} value equal to 21 nM and was able to completely surmount the effect of quinpirole. Similar results were found for compound **6** (data not shown). These studies provide further evidence that these indole analogs are neutral antagonists at D_2 dopamine receptors.

5. Radioligand binding studies at sigma receptors

We also investigated the pharmacological properties of these indole compounds by determining the K_i values of these compounds at sigma-1 (σ_1) and sigma-2 (σ_2) receptors. The tissue source for the σ_1 assay was guinea pig brain and the radioligand used to measure σ_1 receptor affinity was [^{3}H](+)-pentazocine. The tissue source for the σ_2 receptor assay was rat liver and the binding studies were conducted using [^{3}H]DTG as the radioligand in the presence of 100 nM unlabeled (+)-pentazocine to mask σ_1 sites.[38] Although haloperidol is a dopaminergic antagonist that is used clinically as a neuroleptic, its high affinity at sigma receptors has precluded its usefulness as a radioligand for in vitro or in vivo radioligand binding studies. First, all of the compounds in this study bound with low affinity (>800 nM) at σ_2 receptors. The affinity at σ_1 receptors varied from 12 nM to >5000 nM. Second, all of the unsubstituted indole analogs that are structurally similar to haloperidol were found to have affinity at σ_1 receptors ranging from K_i values of 12–19 nM, irrespective of whether they had a Cl, Br, or SCH_3 group. However, clearly the addition and position of the methoxy groups on the indole moiety contributed to both the D_2/D_3 dopamine receptor subtype selectivity of the compounds and the affinity at σ_1 receptors. For example, the affinity of the unsubstituted indole compound **2** at σ_1 receptors was found to be equal to 19 nM. Addition of the methoxy group at the 5-position (**6**) decreased the affinity greater than 10-fold ($K_i = 242$), whereas the addition of a methoxy at the 4-position decreased σ_1 receptor affinity >100-fold. Similar effects were found for the indoles containing a Br moiety. Addition of a methoxy to the unsubstituted indole (**7**) at the 5-position resulted in an 8-fold decrease in affinity at σ_1 receptors and addition of a methoxy at the 4-position reduced the affinity at σ_1 receptors to a K_i value of >2000 nM. These data indicate that substitution of the indole ring in either the 4- or 5-position with a methoxy group has the beneficial effect of both (a) increasing affinity at dopamine D_2 receptors and (b) decreasing affinity at σ_1 receptors. However, the most advantageous strategy to reduce the σ_1 receptor affinity of the substituted indoles would be to incorporate a methoxy moiety at the 4-position on the indole ring.

6. Discussion

The D_2 and D_3 dopamine receptor subtypes are structurally and pharmacologically similar.[16] Both receptor subtypes regulate adenylyl cyclase, extracellular acidification, mitogenesis, and dopamine release.[28–32] However, the neuroanatomical localization and the levels of expression of these two receptor subtypes are quite different. In addition, these two subtypes differ in terms of the magnitude of the second messenger response following agonist stimulation.[29] Studies by Kim et al.,[33] indicated that D_2 and D_3 receptor expression may be regulated in a different manner and that the intracellular trafficking properties of these two receptor subtypes may also be different. Src, phosphatidylinositol 3-kinase, and atypical protein kinase C are commonly involved in D_2R-/D_3R-mediated ERK activation. However, ERK activation-mediated by D_3 receptors, but not D_2 receptors, can be blocked by ARK-CT, AG1478 epidermal growth factor receptor (EGFR) inhibitor, and by dominant negative mutants of Ras and Raf, suggesting the involvement of the different G-proteins.[34] Recent studies also demonstrate that both D_2 and D_3 dopamine receptors couple to inward rectifier potassium channels (GIRK). However, the shape of the termination current and the desensitization of the response are different for these two D_2-like dopamine receptor subtypes.[18]

In one sense, the D_2 receptor is the predominate CNS D_2-like dopamine receptor subtype because it (a) is expressed at the highest levels in the CNS and (b) was the first of the D_2-like dopamine receptor subtypes to be cloned and characterized pharmacologically. It has been documented that a pharmacologic property common to the majority of clinically used antipsychotics is

that they are antagonists at D_2 dopamine receptors. However, more recently it is becoming clear that the D_3 receptor subtype may also play a role in the development of pharmacotherapeutic strategies for the treatment of neuropsychiatric and neurological disorders, as well as for the rehabilitation of individuals who abuse psychostimulants.[8] The precise physiological role of each of these two structurally and pharmacologically related receptor subtypes can be more precisely defined only when selective agonists and antagonists at each of the three D_2-like dopamine receptors become available to the neuroscience community.

Over the past five years there have been a number of structurally diverse compounds reported having a high affinity and selectivity for dopamine D_3 versus D_2 receptors.[8,40] However, the same cannot be stated for compounds having a high affinity and selectivity for D_2 versus D_3 receptors. As part of our ongoing effort to identify compounds that have D_2-like receptor subtype selectivity, we have evaluated the pharmacological properties of a panel of indoles that are structurally related to the D_2-like dopamine receptor butyrophenone antagonists, haloperidol, and spiperone. It has been reported previously that both spiperone and haloperidol exhibited 5- to 10-fold higher affinity at rat D_2 when compared to rat D_3 receptor subtype,[10,16] and that an indole analog structurally related to haloperidol exhibited selective binding at the D_2 dopamine receptor subtype.[25] This information served as the rationale for the design of the indole analogs described in this report.

The indole analogs that we prepared were initially evaluated for binding selectivity at human D_2, D_3, and D_4 dopamine receptor subtypes. We identified two methoxy substituted indoles, **8** and **21**, which (a) are structurally related to haloperidol, (b) bind at D_2 receptors with nanomolar affinity, and (c) have >80-fold selectivity for human D_2 receptors compared to the human D_3 dopamine receptor subtype. These two analogs were also found to bind with low affinity at the D_4 and D_1 dopamine receptor subtypes, as well as σ_1 and σ_2 receptors.

Four of the indole analogs with the greatest D_2 receptor selectivity were evaluated for intrinsic activity at human D_2 receptors using a whole cell adenylyl cyclase assay. None of the four compounds that were evaluated exhibited agonist activity in that assay. In addition, all compounds that were tested were shown to be able to attenuate the quinpirole inhibition of forskolin-dependent stimulation of adenylyl cyclase, indicating that this series of compounds are neutral antagonists at D_2 dopamine receptors.

The potential utility of these compounds to distinguish pharmacologically between the D_2 and D_3 receptor subtypes in in vitro and in vivo assays remains to be established. However, our preliminary pharmacological analysis suggests that these D_2 receptor selective compounds may be valuable tools to study the expression and regulation of both D_2 and D_3 dopamine receptors. The lipophilicity ($\log P$) of **8** and **21** (Table 1) also suggests that they will readily cross the blood–brain barrier. These pharmacological properties make **8** and **21** good candidates for the development of D_2 receptor selective imaging agents for the functional imaging technique, positron emission tomography (PET).

In addition, we have identified several structurally related compounds which bind with essentially the same affinity at D_2 receptors but with varying affinity at σ_1 receptors. These compounds may prove to be valuable for pharmacologically dissecting the role of σ_1 and D_2 dopamine receptors in a variety of behavioral paradigms in which both D_2 and σ_1 receptors have been implicated, including cocaine-dependent locomotor activity and toxicity.[35]

7. Conclusion

Several unsubstituted and methoxy substituted indole analogs that bind selectively and with high affinity to D_2 dopamine receptor subtypes have been identified. These compounds appear to be neutral antagonists at human D_2 receptors. These compounds bind with low affinity at D_1, D_4, and σ_2 receptors, but with varying affinity at σ_1 receptors. In conjunction with other dopaminergic radiolabeled reagents, this family of compounds may have utility in studies designed to quantitate the expression and regulation of the two pharmacologically related D_2-like dopamine receptor subtypes, the D_2 and D_3 dopamine receptors. They may also prove to be useful in more precisely defining the role of σ_1 and D_2 dopamine receptors in a variety of (a) in vitro assays and (b) in vivo behavioral paradigms.

8. Experimental

8.1. Chemical analysis

^{1}H NMR spectra were recorded on a Varian 300 MHz NMR spectrometer. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane (TMS). The following abbreviations are used for multiplicity of NMR signals : br s = broad singlet, d = doublet, dd = doublet of doublets, m = multiplet, s = singlet, and t = triplet. Melting points were determined with an electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA, and were within ±0.4% of the calculated values. All reactions were carried out under an inert atmosphere of nitrogen. Gramine and 5-methoxygramine were purchased from Sigma–Aldrich Chemicals (St. Louis, MO); 4-methoxygramine was synthesized using the method described by Ley and Priour.[36] Lipophilicity measurements of the compounds were estimated using the computational program, C log *P* (Advanced Chemistry Development, Inc., Toronto, Canada).

8.2. General procedure for the synthesis of the *N*-indolylmethyl analogs

A mixture of gramine derivatives (1.0–7.0 mmol) and appropriate amines (1.2–8.4 mmol, 1.2 equiv) in toluene

(5–25 mL) was stirred at reflux overnight. The volatile components were evaporated and the resulting residue was purified by silica gel column chromatography (dichloromethane–methanol–NH_4OH, 90:10:0.5) to afford the target compounds. The oxalate salt was prepared using 1 equiv of oxalic acid in ethyl acetate.

8.3. 1-((1*H*-Indol-3-yl)methyl)-4-(4-chlorophenyl)piperidin-4-ol oxalate (2)

Yield 95% from gramine and 4-(4-chlorophenyl)-4-hydroxypiperidine. Conversion to the oxalate salt gave **2** as a white powder, mp 164–165 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.20 (br s, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.11–7.44 (m, 8H), 3.81 (s, 2H), 2.88–2.92 (m, 2H), 2.46–2.55 (m, 2H), 2.07–2.17 (m, 2H), 1.78 (br s, 1H), 1.67–1.73 (m, 2H). Anal. ($C_{20}H_{21}ClN_2O{\cdot}C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

8.4. 4-(4-Chlorophenyl)-1-((4-methoxy-1*H*-indol-3-yl)methyl)piperidin-4-ol oxalate (5)

Yield 66% from 4-methoxygramine and 4-(4-chlorophenyl)-4-hydroxypiperidine. Conversion to the oxalate salt gave **5** as a white powder, mp 127–129 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.64 (br s, 1H), 6.96–7.42 (m, 7H), 6.51 (d, J = 7.8 Hz, 1H), 4.11 (s, 2H), 3.92 (s, 3H), 2.98–3.02 (m, 2H), 2.61–2.70 (m, 2H), 2.13–2.23 (m, 2H), 1.66–1.71 (m, 2H). Anal. ($C_{21}H_{23}ClN_2O_2{\cdot}C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

8.5. 4-(4-Chlorophenyl)-1-((5-methoxy-1*H*-indol-3-yl)methyl)piperidin-4-ol oxalate (6)

Yield 95% from 5-methoxygramine and 4-(4-chlorophenyl)-4-hydroxypiperidine. Conversion to the oxalate salt gave **6** as a white powder, mp 167–168 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.23 (br s, 1H), 7.40–7.44 (m, 2H), 7.14–7.32 (m, 5H), 6.86 (dd, J = 8.5 and 2.4 Hz, 1H), 3.87 (s, 3H), 3.77 (s, 2H), 2.89–2.92 (m, 2H), 2.46–2.54 (m, 2H), 2.07–2.17 (m, 2H), 1.68–1.74 (m, 2H). Anal. ($C_{21}H_{23}ClN_2O_2{\cdot}C_2H_2O_4$) C, H, N.

8.6. 1-((1*H*-Indol-3-yl)methyl)-4-(4-bromophenyl)piperidin-4-ol oxalate (7)

Yield 66% from gramine and 4-(4-bromophenyl)-4-hydroxypiperidine. Conversion to the oxalate salt gave **7** as a tan powder, mp 149–151 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.19 (br s, 1H), 7.77 (d, J = 7.8 Hz, 1H), 7.36–7.46 (m, 4H), 7.12–7.26 (m, 4H), 3.81 (s, 2H), 2.89–2.93 (m, 2H), 2.47–2.55 (m, 2H), 2.08–2.18 (m, 2H), 1.81 (br s, 1H), 1.68–1.72 (m, 2H). Anal. ($C_{20}H_{21}BrN_2O{\cdot}C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

8.7. 4-(4-Bromophenyl)-1-((4-methoxy-1*H*-indol-3-yl)methyl)piperidin-4-ol oxalate (8)

Yield 84% from 4-methoxygramine and 4-(4-bromophenyl)-4-hydroxypiperidine. Conversion to the oxalate salt gave **8** as a white powder, mp 135–136 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.19 (br s, 1H), 7.43–7.47 (m, 2H), 7.35–7.39 (m, 2H), 6.96–7.12 (m, 3H), 6.51 (d, J = 7.6 Hz, 1H), 4.04 (s, 2H), 3.93 (s, 3H), 2.95–2.99 (m, 2H), 2.52–2.60 (m, 2H), 2.09–2.19 (m, 2H), 1.68–1.72 (m, 2H). Anal. ($C_{21}H_{23}BrN_2O_2{\cdot}C_2H_2O_4$) C, H, N.

8.8. 4-(4-Bromophenyl)-1-((5-methoxy-1*H*-indol-3-yl)methyl)piperidin-4-ol oxalate (9)

Yield 72% from 5-methoxygramine and 4-(4-bromophenyl)-4-hydroxypiperidine. Conversion to the oxalate salt gave **9** as a white powder, mp 158–159 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.12 (br s, 1H), 7.17–7.47 (m, 7H), 6.87 (d, J = 8.3 Hz, 1H), 3.87 (s, 3H), 3.79 (s, 2H), 2.90–2.94 (m, 2H), 2.49–2.65 (m, 2H), 2.11–2.18 (m, 2H), 1.65–1.68 (m, 2H). Anal. ($C_{21}H_{23}BrN_2O_2{\cdot}C_2H_2O_4$) C, H, N.

8.9. 8-((1*H*-Indol-3-yl)methyl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (13)

Yield 95% from gramine and 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **10**. Conversion to the oxalate salt gave **13** as a white powder, mp 193–194 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.33 (br s, 1H), 7.85 (d, J = 7.5 Hz, 1H), 7.35–7.37 (m, 1H), 7.12–7.24 (m, 5H), 6.80–6.91 (m, 3H), 4.65 (s, 2H), 3.82 (s, 2H), 2.98 (s, 3H), 2.66–2.94 (m, 5H), 1.61–1.73 (m, 3H). Anal. ($C_{23}H_{26}N_4O{\cdot}C_2H_2O_4{\cdot}0.25H_2O$) C, H, N.

8.10. 8-((4-Methoxy-1*H*-indol-3-yl)methyl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (14)

Yield 24% from 4-methoxygramine and 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **10**. Conversion to the oxalate salt gave **14** as a tan powder, mp 154–155 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 9.00 (br s, 1H), 7.42 (s, 1H), 7.26–7.32 (m, 2H), 6.82–7.11 (m, 5H), 6.53 (d, J = 7.5 Hz, 1H), 4.68 (s, 2H), 4.65 (s, 2H), 3.97 (s, 3H), 3.77–3.81 (m, 2H), 3.45–3.48 (m, 2H), 3.06–3.17 (m, 2H), 3.01 (s, 3H), 1.71–1.76 (m, 2H). Anal. ($C_{24}H_{28}N_4O_2{\cdot}C_2H_2O_4{\cdot}H_2O$) C, H, N.

8.11. 8-((5-Methoxy-1*H*-indol-3-yl)methyl)-3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one oxalate (15)

Yield 57% from 5-methoxygramine and 3-methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one, **10**. Conversion to the oxalate salt gave **15** as a white powder, mp 190–191 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.12 (br s, 1H), 7.22–7.31 (m, 5H), 6.80–6.92 (m, 4H), 4.66 (s, 2H), 3.90 (s, 3H), 3.79 (s, 2H), 2.99 (s, 3H), 2.71–2.93 (m, 6H), 1.62–1.67 (m, 2H). Anal. ($C_{24}H_{28}N_4O_2{\cdot}C_2H_2O_4$) C, H, N.

8.12. 1-(1-((1*H*-Indol-3-yl)methyl)piperidin-4-yl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one oxalate (16)

Yield 97% from gramine and 4-(2-keto-1-benzimidazolinyl)piperidine, **11**. Conversion to the oxalate salt gave **16** as a white powder, mp 207–209 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 9.74 (s, 1H), 8.28 (s, 1H), 7.79 (d, J = 7.3 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.14–7.24 (m, 4H), 7.00–7.08 (m, 3H), 4.32–4.40 (m, 1H), 3.81 (s,

2H), 3.17 (d, J = 11.7 Hz, 2H), 2.43–2.54 (m, 2H), 2.19–2.27 (m, 2H), 1.78–1.81 (m, 2H). Anal. ($C_{21}H_{22}N_4O\cdot C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

8.13. 1-(1-(1*H*-Indol-3-yl)methyl)-4-phenylpiperidine oxalate (17)

Yield 89% from gramine and 4-phenylpiperidine, **12**. Conversion to the oxalate salt gave **17** as a white powder, mp 147–149 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.18 (br s, 1H), 7.77 (d, J = 7.9 Hz, 1H), 7.12–7.38 (m, 9H), 3.79 (s, 2H), 3.11–3.14 (m, 2H), 2.42–2.52 (m, 1H), 2.11–2.20 (m, 2H), 1.77–1.83 (m, 4H). Anal. ($C_{20}H_{22}N_2 C_2H_2O_4\cdot 0.25H_2O$) C, H, N.

8.14. 1-((1*H*-Indol-3-yl)methyl)-4-(4-(methylthio)phenyl)piperidin-4-ol oxalate (20)

A solution of 4-piperidone monohydrate hydrochloride (970 mg, 6.3 mmol) in 10% aqueous sodium carbonate (1 mL) was added to a solution of gramine (1.0 g, 5.7 mmol) in 1-butanol (20 mL). The reaction mixture was stirred at 100 °C overnight and then concentrated. The crude residue was purified by column chromatography on silica gel (dichloromethane–methanol–NH_4OH, 90:10:0.5) to give 1-((1*H*-indol-3-yl)methyl)piperidin-4-one, **18a** (350 mg, 27%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 8.14 (br s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.12–7.26 (m, 3H), 3.84 (s, 2H), 2.81 (t, J = 6.3 Hz, 4H), 2.45 (t, J = 6.3 Hz, 4H).

A solution of 4-bromothioanisole (685 mg, 3.4 mmol) in dry THF (4 mL) was added to a solution of *n*-butyllithium (3.2 mL, 5.06 mmol, 1.6 M in hexane) in dry THF (10 mL) at −70 °C. After stirring at −70 °C for 1 h, a solution of **18a** (350 mg, 1.53 mmol) in dry THF (5 mL) was added. The reaction mixture was stirred at −70 °C for 4 h and then concentrated. Water (10 mL) was added and then extracted with dichloromethane. Purification by column chromatography on silica gel (dichloromethane–methanol–NH_4OH, 90:10:0.5) gave **20** (319 mg, 59%). Conversion to the oxalate salt gave the compound as a tan powder, mp 118–119 °C (dec); ^{1}H NMR (free base, $CDCl_3$) δ 8.23 (br s, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.36–7.43 (m, 3H), 7.12–7.26 (m, 5H), 3.82 (s, 2H), 2.89–2.93 (m, 2H), 2.50–2.58 (m, 2H), 2.46 (s, 3H), 2.10–2.20 (m, 2H), 1.70–1.74 (m, 2H). Anal. ($C_{21}H_{24}N_2OS\cdot C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

8.15. 1-((5-Methoxy-1*H*-indol-3-yl)methyl)-4-(4-(methylthio)phenyl)piperidin-4-ol oxalate (21)

The reaction was carried out according to the procedure for **20** using 5-methoxygramine (1.6 g, 7.8 mmol) to give **18c** as a pale brown powder (630 mg, 31%). ^{1}H NMR ($CDCl_3$) δ 8.05 (br s, 1H), 7.27 (d, J = 8.8 Hz, 1H), 7.23 (d, J = 2.4 Hz, 1H), 7.13 (d, J = 2.4 Hz, 1H), 6.88 (dd, J = 8.8 and 2.4 Hz, 1H), 3.87 (s, 3H), 3.80 (s, 2H), 2.81 (t, J = 6.1 Hz, 4H), 2.45 (t, J = 6.1 Hz, 4H). Treatment with **19** gave **21** in modest yield (530 mg, 57%). Conversion to the oxalate salt gave the compound as a tan powder, mp 128–129 °C (dec). ^{1}H NMR (free base, $CDCl_3$) δ 8.12 (br s, 1H), 7.41 (d, J = 8.5 Hz, 2H), 7.15–7.27 (m, 5H), 6.87 (dd, J = 8.8 and 2.4 Hz, 1H), 3.87 (s, 3H), 3.77 (s, 2H), 2.87–2.93 (m, 2H), 2.48–2.57 (m, 2H), 2.47 (s, 3H), 2.08–2.19 (m, 2H), 1.71–1.75 (m, 2H). Anal. ($C_{22}H_{26}N_2O_2S\cdot C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

9. Radioligand binding and functional assays

9.1. Dopamine receptor binding assay

The method for the iodination of ^{125}I-IABN using peracetic acid has been previously described.[27] For radioligand binding studies, membrane homogenates from stably transfected HEK 293 cells expressing either the human D_2, D_3, and D_4 receptors were prepared using a polytron tissue homogenizer (Brinkman Instruments, Westbury, NY). The tissue was suspended in 50 mM Tris–HCl, 150 mM NaCl, and 1 mM EDTA at pH 7.5 to approximately 5–20 μg of protein per 50 μL prior to the assay. Assays were performed in a total volume of 150 μL. Binding reactions were carried out for 60 min at 37 °C and the reaction was terminated by rapid filtration over glass fiber filters (Schleicher and Schuell, No. 32 filters). After washing filters with buffer, the radioactivity of the ^{125}I-labeled ligand was quantitated using a Packard Cobra gamma counter with an efficiency of 75%. Protein concentrations were determined using a BCA reagent (Pierce) with bovine serum albumin as the protein standard.

For competition curves using a transfected cell line expressing D_2, D_3 or D_4 dopamine receptors, experiments were performed in triplicate with two concentrations of inhibitor per decade over at least five orders of magnitude. The concentration of the radioligand was approximately equal to the K_d values. Controls containing either no inhibitor or 2 μM (+)-butaclamol were used to define total binding and non-specific binding, respectively. For competition curves using dissected rat brain tissue and the D_1 receptor selective radioligand ^{3}H-SCH 23390, two independent experiments were performed in triplicate with two concentrations of inhibitor per decade. Controls containing either no inhibitor or 10 μM (+)-butaclamol were used to define total binding and non-specific binding, respectively. Since low levels of D_1 receptor binding were observed for the indoles, a competition curve using non-radioactive SCH 23390 was performed simultaneously as a positive control for the validity of the assay. Competition data for D_2-like dopamine receptors were modeled for a single-site fit using the TableCurve program (Jandel) and the IC_{50} values for the competitive inhibitors were converted to K_i values using the Cheng and Prusoff corrections.[37] A similar analysis was performed for SCH 23390 binding to D_1 receptors expressed in rat caudate, but the low level of binding of the test compounds to rat striatal D_1 receptors precluded obtaining accurate IC_{50} values.

9.2. Sigma receptor binding assays

The σ_1 receptor binding assay was conducted using guinea pig brain membrane homogenates (100 μg protein). Membrane homogenates were incubated with

3 nM [^{3}H](+)-pentazocine (31.6 Ci/mmol) in 50 mM Tris–HCl (pH 8.0) at 25 °C for either 120 or 240 min. Test compounds were dissolved in ethanol and then diluted in buffer for a total incubation volume of 0.5 mL. Test compounds were added in concentrations ranging from 0.005 to 1000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris–HCl (pH 8.0) followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL ice-cold buffer. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals; Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%.

The σ_2 receptor binding assay was conducted using rat liver membrane homogenates (35 μg of protein). Membrane homogenates were incubated with 3 nM [^{3}H]DTG (38.3 Ci/mmol) in the presence of 100 nM (+)-pentazocine to block σ_1 sites. Incubations were carried out in 50 mM Tris–HCl (pH 8.0) for 120 min at 25 °C in a total incubation volume of 0.5 mL. Test compounds were added in concentrations ranging from 0.005 to 1000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris–HCl (pH 8.0) followed by rapid filtration through Whatman GF/B glass fiber filters (presoaked in 0.5% polyethylenimine) using a Brandel cell harvester (Gaithersburg, MD). Filters were washed twice with 5 mL ice-cold buffer. Nonspecific binding was determined in the presence of 5 μM DTG. Liquid scintillation counting was carried out in EcoLite(+) (ICN Radiochemicals; Costa Mesa, CA) using a Beckman LS 6000IC spectrometer with a counting efficiency of 50%.

The IC_{50} values at sigma sites were generally determined in triplicate from non-linear regression of binding data as analyzed by JMP (SAS Institute; Cary, NC), using eight concentrations of each compound. K_i values were calculated using the method of Cheng and Prusoff[37] and represent mean values ± SEM. All curves were best fit to a one-site fit. The K_d value used for [^{3}H]DTG in rat liver was 17.9 nM and was 4.8 nM for [^{3}H](+)-pentazocine in guinea pig brain.[38]

9.3. Whole cell adenylyl cyclase assay

The accumulation of ^{3}H-cyclic AMP in HEK cells was measured by a modification of the method of Shimizu et al.[39] Transfected HEK cells were treated with serum-free medium containing 2,8-^{3}H-adenine (ICN) and cells were incubated at 37 °C for 75 min. The media were then replaced with serum-free media containing 0.1 mM 3-isobutyl-1-methylxanthine (Sigma). Cells and drugs were mixed to give a final volume of 500 μL and cells were incubated for 20 min at 37 °C. The reaction was stopped by addition of 500 μL of 10% trichloroacetic acid and 1 mM cyclic AMP. After centrifugation, the supernatants were fractionated using Dowex AG1-X8 and neutral alumina to separate the ^{3}H-ATP and the ^{3}H-cyclic AMP. Individual samples were corrected for column recovery by monitoring the recovery of the cyclic AMP using spectrophotometric analysis at OD 259 nm.[27,39] For concentration-dependent experiments, competition data were modeled for a single-site fit using the TableCurve program (Jandel) and the IC_{50} values for the competitive inhibitors.

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Supplementary data

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