WS-50030 [7-{4-[3-(1H-inden-3-yl)propyl]piperazin-1-yl}-1,3-benzoazol-2(3H)-one]: A Novel Dopamine D₂ Receptor Partial Agonist/Serotonin Reuptake Inhibitor with Preclinical Antipsychotic-Like and Antidepressant-Like Activity


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ABSTRACT

The preclinical characterization of WS-50030 [7-{4-[3-(1H-inden-3-yl)propyl]piperazin-1-yl}-1,3-benzoazol-2(3H)-one] is described. In vitro binding and functional studies revealed highest affinity to the D₂ receptor (D₂L, Kᵢ, 4.0 nM) and serotonin transporter (Kᵢ, 7.1 nM), potent D₂ partial agonist activity (EC₅₀, 0.38 nM; Eₘₐₓ, 30%), and complete block of the serotonin transporter (IC₅₀, 56.4 nM). Consistent with this in vitro profile, WS-50030 (10 mg/kg/day, 21 days) significantly increased extracellular 5-HT in the rat medial prefrontal cortex, short-term WS-50030 treatment blocked apomorphine-induced climbing (ID₅₀, 0.51 mg/kg) in a dose range that produced minimal catalepsy in mice and induced low levels of contralateral rotation in rats with unilateral substantia nigra 6-hydroxydopamine lesions (10 mg/kg i.p.), a behavioral profile similar to that of the D₂ partial agonist aripiprazole. In a rat model predictive of antipsychotic-like activity, WS-50030 and aripiprazole reduced conditioned avoidance responding by 42 and 55% at 10 mg/kg, respectively. Despite aripiprazole’s reported lack of effect on serotonin transporters, long-term treatment with aripiprazole or WS-50030 reversed olfactory bulbectomy-induced hyperactivity at doses that did not reduce activity in sham-operated rats, indicating antidepressant-like activity for both compounds. Despite possessing serotonin reuptake inhibitory activity in addition to D₂ receptor partial agonism, WS-50030 displays activity in preclinical models predictive of antipsychotic- and antidepressant efficacy similar to aripiprazole, suggesting potential efficacy of WS-50030 versus positive and negative symptoms of schizophrenia, comorbid mood symptoms, bipolar disorder, major depressive disorder, and treatment-resistant depression. Furthermore, WS-50030 provides a tool to further explore how combining these mechanisms might differentiate from other antipsychotics or antidepressants.

Schizophrenia is a debilitating disease that affects approximately 1% of the world population. The symptoms of schizophrenia can be loosely categorized into three main groups: positive symptoms, such as hallucinations and delusions; negative symptoms, such as social withdrawal and anhedonia; and disorganized symptoms, such as poor attention and disorganized speech.
nia; and cognitive symptoms, such as deficits in attention and higher-order thought processing (Meltzer, 1999). The neurotransmitter dopamine is known to play a pivotal role in schizophrenia, and an excellent correlation exists between the potency of compounds for antagonizing postsynaptic dopamine D2 receptors and their efficacy in treating the positive symptoms of schizophrenia (Seeman et al., 1976; Kapur et al., 2000). It is also known, however, that excessive D2 receptor antagonism in areas of the brain responsible for motor control results in extrapyramidal side effects such as Parkinson’s-like movement disorders, a property common to all conventional and several atypical antipsychotic agents (Sanberg, 1980; Kapur et al., 2000). In addition, dopamine has been shown to play a significant role in the reward process and pleasure, such that excessive D2 receptor blockade would be expected to have a less-than-optimal effect on the negative symptoms of schizophrenia (Bressan and Crippa, 2005). Indeed, currently marketed D2 receptor full antagonists lack efficacy in the treatment of the negative symptoms of schizophrenia.

A dopamine D2 partial agonist, however, can exhibit a dynamic range of functional activity depending upon regional receptor reserve and local endogenous levels of dopamine (Stahl, 2001; Tamminga, 2002; Tamminga and Carlsson, 2002). A D2 partial agonist would be expected to behave as an agonist at presynaptic autoreceptors where receptor reserve is high and levels of the neurotransmitter are relatively low, effectively reducing dopamine transmission. A D2 partial agonist could function postsynaptically as either an agonist or an antagonist depending upon its level of intrinsic activity and local dopaminergic tone. A partial agonist should modulate—as opposed to completely block—dopaminergic transmission, thereby controlling positive symptoms with reduced potential for motoric side effects. The potential benefits of a D2 partial agonist are also important considering evidence that the positive and negative symptoms of schizophrenia may result from dopamine hyperactivity and hypoactivity in mesolimbic and mesocortical brain regions, respectively (Weinberger and Lipska, 1995), such that a modulator of dopamine, instead of a complete blocker, seems appropriate. The clinical benefits of a D2 receptor partial agonist have been realized with the currently marketed antipsychotic agent aripiprazole (Abilify). Moreover, in addition to treating positive symptoms, clinical data suggest that aripiprazole significantly improves various negative symptom rating scales and has reduced extrapyramidal side-effect liability (Kane et al., 2002; Swainston and Perry, 2004; Cassano et al., 2007).

In addition to the positive, negative, and cognitive symptoms of schizophrenia, there exists a high incidence of comorbidity of depressive symptoms in patients with schizophrenia (Siris et al., 2001). Serotonin reuptake inhibitors (SRIs) are commonly used in the clinic to treat both depression and anxiety, and there is mounting evidence to support the value of SRI augmentation in the treatment of the negative symptoms of schizophrenia (Silver, 2003; Mazeh et al., 2004). SRIs such as fluoxetine, fluvoxamine, citalopram, and sertraline have been combined with antipsychotics to alleviate these often-refractory symptoms. Therefore, a D2 receptor partial agonist with low intrinsic activity coupled with serotonin reuptake inhibitory activity would be expected to have benefit in the treatment of the positive symptoms as well as the comorbid depressive, and possibly the negative, symptoms of schizophrenia. In addition to their use in treating comorbid depression in schizophrenia, SRIs are often coadministered with antipsychotics for the treatment of bipolar disorder (Emilien et al., 2007). Therefore, a combined D2 receptor partial agonist/SRI may have utility in the treatment of bipolar disorder. The present studies describe the in vitro and in vivo preclinical characterization of WS-50030 [7-{4-[3-(1H-inden-3-yl)propyl]piperazin-1-yl}-1,3-benzoxazol-2(3H)-one] (Fig. 1), a dopamine D2 receptor partial agonist with serotonin reuptake inhibitor activity. In addition, WS-50030 is compared with the D2 receptor partial agonist aripiprazole in in vivo functional and efficacy models.

### Materials and Methods

#### Subjects

Male CF-1 mice (22–32 g; Charles River Laboratories, Wilmington, MA) were used in the antagonism of apomorphine-induced behaviors and cataleptogenic potential assays. Male Sprague-Dawley rats (Charles River Laboratories) were used for conditioned avoidance test (350–500 g of body weight), in vivo microdialysis studies (280–350 g of body weight), and olfactory bulbectomy studies (200–225 g of body weight). Male Wistar rats (400–500 g; Harlan, Horst, The Netherlands) were used for unilaterally 6-OHDA-lesioned rat studies. All animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. The animals were maintained on a 12-h light/dark cycle (lights on at 6:00 AM). Animals were group-housed with food and water available ad libitum, except for rats used in conditioned avoidance studies, which were housed individually with ad libitum access to water but were food restricted to maintain their weight at approximately 80% of their free feeding body weight (15–20 g of rodent chow per day). All studies conducted at Wyeth Research were previously approved by the Institutional Animal Care and Use Committee and were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). All studies conducted at Solvay Pharmaceuticals were in accordance with Dutch law and conformed to local animal care and use committee stipulations.

#### Drugs

WS-50030 was prepared by Solvay Pharmaceuticals (Weesp, The Netherlands). WAY-106635 and aripiprazole (except for rotational behavior studies) were prepared by Wyeth Research (Princeton, NJ). WAY-106635 and aripiprazole used for rotational behavior studies were prepared by Solvay Pharmaceuticals (Weesp, The Netherlands). Desmethyl-imipramine and 6-hydroxydopamine were purchased from Sigma-Aldrich Chemicals (Zwijndrecht, The Netherlands), Apomorphine and d-amphetamine were obtained from Sigma (St. Louis, MO). Drugs were dissolved in saline (apomorphine, d-amphetamine), 2% Tween 80/0.5% methylcellulose (aripiprazole) or 2% Tween 80/0.5% methylcellulose/5% mannitol (WS-50030), and

![Fig. 1. Chemical structure of WS-50030.](image-url)
solutions were administered at a volume of 10 ml/kg to mice and 1 to 5 ml/kg to rats unless otherwise noted. Dose calculations were based on active moiety. All other materials were analytical grade and were purchased from Sigma-Aldrich Chemicals (Milwaukee, WI).

**Procedures**

### In Vitro Receptor/Transporter Binding

**D₂** receptor binding. Affinity for the long form of the human D₂ receptor (D₂L) was measured in a membrane preparation of CHO-D₂L, cells by binding studies using [³H]spiperone as a ligand. The membrane preparation was prepared from CHO-K1 cells transfected with the D₂L receptor (CHO-hD₂L cells, obtained from Dr. D. Grandy, Vollum Institute, Portland, OR) (Bunzow et al., 1988). After thawing, the cells were centrifuged for 10 min at 2000g at 4°C. Cell pellets were resuspended in 50 mM Tris-HCl, pH 7.7. Cell membranes were prepared by two cycles of homogenization (10 strokes, 600 rpm; Potter-Elvehjem) and centrifugation (40,000g for 10 min, 4°C). After the second centrifugation step, the pellets were resuspended in assay buffer (50 mM Tris-HCl, pH 7.7, 4 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 0.1% ascorbic acid). The assay was established so as to achieve steady state conditions and to optimize specific binding. Compounds were incubated with 0.2 nM [³H]spiperone at 37°C for 15 cycles of homogenization (10 strokes, 600 rpm; Potter-Elvehjem) and centrifugation (40,000g for 10 min, 4°C). After the second centrifugation step, the pellets were resuspended in assay buffer (50 mM Tris-HCl, pH 7.7, 4 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 0.1% ascorbic acid). Membranes from 5 × 10⁶ cells and test compounds were incubated with 0.2 nM [³H]spiperone at 37°C for 15 min. Nonspecific binding was determined using 10⁻² M haloperidol. Assays were terminated by rapid vacuum filtration through glass fiber filters (GF/B). Total and bound radioactivity was determined by liquid scintillation counting using MicroScint (PerkinElmer Life and Analytical Sciences, Waltham, MA). Greater than 90% specific binding was achieved in each of these assays. Compounds were tested at a 4-log concentration range; all determinations were performed in triplicate.

**Receptor selectivity binding.** Human 5-HT₁A receptor binding was determined by the displacement of [³H]8-hydroxy-2-dipropylaminotetralin from human 5-HT₁A receptor-transfected CHO cells (Dunlop et al., 1998). Human 5-HT₂A receptor binding was determined via the displacement of 4-(1²Cl)lido-(2,5-dimethoxy)phenylisopropylamine from human 5-HT₂A transfected CHO cells (Rosenzweig-Lipson et al., 2006). Human 5-HT₆A receptor binding was determined via the displacement of [³H]5-HT from human 5-HT₆A-transfected CHO cells (Rosenzweig-Lipson et al., 2006). Cortical membranes prepared from male Sprague-Dawley rats incubated with [³H]prazosin (0.2 nM) were employed to determine binding to the adrenergic α₁ receptor (U’Prichard and Snyder, 1980). Membranes from stably transfected HEK293 cells expressing the human histamine H₁ receptor incubated with 3 nM pyridinyl-5-[³H]pyrilamine were employed to determine binding. CHO cells expressing the human adrenergic α₂A receptor incubated with 2 nM [³H]MK912 were used for binding analysis (Pettibone et al., 1989). IC₅₀ values were determined by nonlinear regression analysis, and inhibition constants (Kᵢ) were calculated using the equation Kᵢ = IC₅₀/(1 + ([L]/Kₐₕ)), where [L] is the concentration of radioligand in the assay, and Kₐₕ is the affinity of the radioligand for the receptor (Cheng and Prusoff, 1973). Results are expressed as mean Kᵢ (± S.E.M.) from at least three separate experiments.

**Transporter binding.** Binding to crude membrane preparations from HEK293 cells expressing the human serotonin transporter (hSERT) was determined as described by competition with [³H]citalopram (Deecher et al., 2006). Binding to crude membrane preparations from Madin-Darby canine kidney-Net6 cells, stably transfected with the human norepinephrine transporter was determined by competition with [³H]Hnisoxetine (Deecher et al., 2006). Binding to crude membrane preparations from CHO cells expressing the human dopamine transporter was determined as described by competition with [³H]WIN-35,428 (Deecher et al., 2006).

### In Vitro Functional Assays

**Human D₂L.** WS-50030 was tested for functional agonist/antagonist activities using stably expressed recombinant receptors with a published Bmax of 1.30 ± 0.17 (S.D.) and Kᵦ of 0.064 ± 0.011 (S.D.) (Oak et al., 2001). CHO-K1 cells transfected with the human D₂L receptor were grown in Dulbecco’s modified Eagle’s medium supplemented with 2 mM glutamine, 200 µg/ml G418, and 10% heat-inactivated fetal bovine serum in 93% air/7% CO₂. Cells were cultured two times per week. For the cAMP assay, CHO-hD₂L cells were trypsinized and transferred into 96-well plates. Cells were grown for 48 h to produce a 100% confluent monolayer.

Functional cAMP assays were performed to assess WS-50030 activity on D₂L receptors. Test compounds were diluted in Dulbecco’s modified Eagle’s medium containing 1 mM 3-isobutyl-1-methylxanthine and 0.3 µM forskolin. Incubations were initiated by addition of medium in presence or absence of test compound and performed at 37°C for 20 min. Nucleotides were extracted with lysis-buffer (63% acetonitrile with 10 mM NH₄HCO₃, pH 9) and plates were sealed and stored at 4°C until analysis. Samples were analyzed using HPLC-tandem mass spectrometry. After separation of ATP and cAMP using hydrophilic interaction liquid chromatography column, analytes were quantified using a linear ion trap mass spectrometer (LTQ; Thermo Fisher Scientific, Waltham, MA). The mass spectrometer was operated in negative electrospray, and peaks were quantified using extracted ion chromatography of the daughter ion, m/z 328 and 134 for cAMP and m/z 506 and 408 for ATP. The conversion of ATP into cAMP was calculated as the ratio of cAMP compared with combined cAMP and ATP. Data were expressed as percentage of control values (forskolin-stimulated cAMP accumulation). The maximal forskolin-induced conversion was taken as maximum and the maximal inhibition (1 µM quinpirole) as minimum. Basal cAMP levels (± S.E.M.) were 1.4 ± 0.4 nM, whereas 0.3 µM forskolin-elevated cAMP levels (± S.E.M.) were 9.5 ± 1.0 nM, producing a suitable assay window. Agonistic effects of compounds are expressed as mean EC₅₀ ± S.E.M. Antagonistic effects of compounds were determined as percentage inhibition of 1 µM quinpirole-inhibited cAMP production and the pA₂ was calculated according to the Cheng-Prusoff equation (Cheng and Prusoff, 1973). Compounds were tested at a 5-log concentration range, in duplicate, and were performed in three independent experiments.

**Human 5-HT₁A.** Assays of 5-HT₁A receptor agonism were conducted as described previously (Hirst et al., 2008). Intracellular cAMP levels were measured in human 5-HT₁A CHO cells, grown in 96-well plates. Cells were preincubated at 37°C for 15 min in Krebs’ solution. Functional activity of WS-50030 was assessed by treating the cells with forskolin (final concentration, 10 µM) followed immediately by the test compound at concentrations ranging from 0.1 to 10,000 nM. The cells were incubated for an additional 10 min at 37°C, and the assay was terminated by the addition of 0.5 M perchloric acid. The plates were stored at -20°C before the assessment of cAMP formation by a cAMP scintillation proximity assay according to manufacturers instructions (GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK). Data were generated in singlet within each experiment, and each experiment was carried out at least three times. Curve-fitting of the mean data was generated by a four-parameter logistic equation using GraphPad 5.0 (GraphPad Software, Inc., San Diego, CA), and results were reported as the mean ± S.E.M.

**SERT uptake.** [³H]5-HT functional uptake assays using the human placental chorionicarcinoma JAR cell line were conducted as described previously (Deecher et al., 2006). On day 1, cells were plated at 75 × 10⁵ cells/well in 96-well plates containing growth medium (RPMI 1640 with 10% fetal bovine serum and 1% penicillin/streptomycin) and 40 nM staurosporine to increase the expression of the 5-HT transporter. On day 2, growth medium was replaced with 200 µl of assay buffer (25 mM HEPES, 120 mM NaCl, 5 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, and 2 mg/ml glucose, pH 7.4, at 37°C) containing 0.2 mg/ml ascorbic acid and 1 µM pargyline. A stock solution of fluoxetine was prepared in DMSO (10 mM) and delivered to duplicate wells containing cells for a final test concentration of 10 µM. Data from these wells were used to define nonspecific 5-HT.
uptake (minimal 5-HT uptake in the presence of a 5-HT reuptake inhibitor). Test compounds were prepared in DMSO and diluted in assay buffer according to the test range (1 nM–10 µM). Twenty-five microliters of assay buffer (maximal 5-HT uptake) or test compound was added directly to duplicate wells containing cells in 200 µl of assay buffer. The cells were incubated with the test compound for 5 min (37°C). To initiate the reaction, [3H]hydroxytryptamine creatine sulfate diluted in assay buffer was delivered in 25-µl aliquots to each well for a final test concentration of 12.5 nM. The cells were incubated with the reaction mixture for 5 min at 37°C. Removing the supernatant from the plates terminated the reaction. The cells were washed twice with 200 µl of assay buffer (37°C) to remove free radioligand. The plates were air-dried for a minimum of 2 h. Subsequently, the cells were lysed in 25 µl of 0.25 N NaOH (4°C), placed on a shaker table and shaken vigorously for 5 min. After cell lysis, 75 µl of scintillation cocktail was added to the wells. The plates were sealed with film tape and counted in a TopCount scintillation counter to collect the raw counts-per-minute data. Data were generated in duplicate within each experiment, and each experiment was carried out at least three times. Curve fitting of the mean data were generated by a four-parameter logistic equation using Prism 5.0 (GraphPad Software Inc., San Diego, CA), and results were reported as the mean ± S.E.M.

Human 5-HT2B. WS-50030 was tested up to 10 µM in 5-HT2B receptor functional assays that were conducted as described previously (Dunlop et al., 2005). Stable CHO cell lines expressing the human 5-HT2B receptor subtype were used for functional studies (Dunlop et al., 2005). Stable CHO cell lines expressing the human 5-HT2B receptor subtype were used for functional studies. Curve fitting of the mean data were generated by a four-parameter logistic equation using Prism 5.0 (GraphPad Software Inc.), and results were reported as the mean ± S.E.M.

In Vivo Microdialysis. Rats were dosed intraperitoneally with vehicle or WS 50030 (10 mg/kg) once a day for 20 days. On day 21, animals were anesthetized with isoflurane (2–3%) to allow stereotaxic implantation of microdialysis guide canulae. Two microdialysis guide canulae (CMA/12; CMA Microdialysis, Solna, Sweden) were implanted above the medial prefrontal cortex (mPFC) anterior +3.2 mm and lateral +0.5 mm from bregma; ventral, −1.8 mm from dura) and the nucleus accumbens (anterior +2.2 mm and lateral −2.8 mm from bregma, ventral −6.1 mm at a 10° angle; Paxinos and Watson, 1986). Guide canulae were secured to the skull with two stainless steel screws (Small Parts, Roanoke, VA) and dental acrylic (Plastics One, Roanoke, VA). After recovery from surgery, animals were administered the 21st dose of the long-term treatment and then housed individually in Plexiglas cages (45 × 45 cm) with free access to food and water overnight before the microdialysis experiment. The day after surgery (day 22), microdialysis studies were performed.

As described previously (Beyer et al., 2002), concentric-style microdialysis probes (CMA/12, 20-kDa cut-off, CMA Microdialysis) with a 4 mm (mPFC) or a 2 mm membrane (nucleus accumbens) were perfused with artificial cerebrospinal fluid (125 mM NaCl, 3 mM KCl, 0.75 mM MgSO4 and 1.2 mM CaCl2, pH 7.4) at a flow rate of 1 µl/min. Microdialysis probes were inserted, via the guide canulae, and allowed to stabilize for 3 h. Dialysis samples were then collected every 20 min. Initially, six dialyse samples were taken to determine the baseline neurotransmitter levels. After the sixth baseline sample, animals were dosed with vehicle or WS 50030 (10 mg/kg i.p.), and dialysis samples were collected for the subsequent 3 h. The three treatment groups were: vehicle on days 1 to 21 + vehicle on day 22 (control); vehicle on days 1 to 21 + WS-50030 on day 22 (single administration); WS-50030 on days 1 to 21 + WS-50030 on day 22 (multiple administration). Group sizes were as follows: 5-HT in nucleus accumbens: vehicle, 3; single administration WS-50030, 4; multiple administration WS-50030, 3. DA in nucleus accumbens: vehicle, 7; single administration WS-50030, 6; multiple administration WS-50030, 4. 5-HT in mPFC: vehicle, 3; single administration WS-50030, 3; multiple administration WS-50030, 5. DA in mPFC: vehicle, 5; single administration WS-50030, 5; multiple administration WS-50030, 5.

After completion of microdialysis experiments, rats were euthanized to confirm correct probe placement. For the nucleus accumbens studies, probe placement did not discriminate between core and shell. Rats with incorrect probe placement were excluded from the analysis.

Neurochemical analysis. The outlet tubing of the microdialysis probes was connected directly to an ANTEC (Zoeterwoude, The Netherlands) HPLC electrochemical detection system. Twenty microliters of dialysate containing serotonin and dopamine was separated by HPLC (C18 ODS3 column, 150 × 3.0 mm; Metachem, Torrance, CA) and detected using an ANTEC electrochemical detector set at a potential of 0.65 V versus a silver/AgCl reference electrode. Mobile phase (0.15 M NaH2PO4, 0.25 mM EDTA, 1.75 mM 1-octane sulfonic acid, 2% isopropanol, and 4% methanol, pH 4.6) was delivered by an HPLC pump (PU1580; Jasco Ltd., Great Dunmow, Essex, UK) at a flow rate of 0.5 ml/min. Neurochemical data were compared with an external standard curve, and all data were acquired using the Atlas software package (Thermo Fisher Scientific) for the PC. The mean neurotransmitter levels (femtomolar concentrations) of the baseline samples were calculated, and all sample values were expressed as a percentage change from this preinjection baseline value. Concentrations of neurochemicals were not corrected for probe recovery. Area under the curve (percentage basal) was calculated using the trapezoidal equation. area-under-the-curve data were analyzed using one-way analysis of variance (ANOVA) with post hoc least significant difference (LSD) test. All statistical calculations were performed using SAS for Excel (SAS Institute, Cary, NC).

Antagonism of Apomorphine-Induced Climbing and Stereotypy in Mice. Male CF-1 mice were aclimated to wire cages (5 × 5.5-inch mesh; cylindrical dimensions 4.5 inches deep × 5.5 inches high) for at least 30 min before testing. WS-50030 (0.03–30 mg/kg) or aripiprazole (0.1–1 mg/kg) was administered intraperitoneally to 6 to 12 mice per dose level. Separate control groups for each study were run simultaneously with drug-treated groups and received vehicle at equal volumes. Thirty minutes later, experimental and control animals were challenged with 1 mg/kg s.c. apomorphine. Five minutes after the apomorphine injection, stereotyped behavior (sniffing-licking-gnawing syndrome; 0, absent; 1, present) and climbing behavior (0, all four feet on ground; 1, two feet up on wire cage; 2, all four feet on wire cage) induced by apomorphine were scored and recorded for each animal. Readings were repeated every 5 min during a 30-min test session. Scores for each animal were totaled over the 30-min test session for each syndrome (stereotypy behavior and climbing). Mean climbing and stereotypy scores were then expressed as a percentage of control values observed in vehicle-treated mice that received apomorphine. ID50 values were calculated using non-linear regression.
Cataleptogenic Potential in Mice. WS-50030 (0.3–10 mg/kg i.p.), aripiprazole (0.1–3 mg/kg i.p.), or vehicle was administered to six male CF-1 mice per dose level. Every 30 min for 2 h after dosing, the animal’s forelegs were draped over a thin horizontal rod 1.75 inches high. The amount of time (in seconds) for which the animal maintained this awkward position was recorded (60 s maximum). Maintenance of this position is considered catalepsy. Mean seconds spent in the catalepsy position for each dose at each time point was calculated, and data were analyzed by two-way repeated measures ANOVA with post hoc LSD test. The time point at which peak catalepsy was observed was expressed graphically. Severity of catalepsy is defined as follows: maintenance of awkward position for 20 s or less, minimal to no catalepsy; 21 to 40 s, moderate catalepsy, and 41 to 60 s, high catalepsy.

Rotational Behavior in Unilaterally 6-OHDA-Lesioned Rats. Surgery. Unilateral 6-OHDA lesions of the substantia nigra zona compacta were performed on male Wistar rats using a stereotaxic procedure. One hour before surgery, desmethylimipramine (20 mg/kg i.p.) was administered to protect the noradrenergic neurons. Rats were anesthetized with a 3% halothane gas mixture was adjusted to 1.75 to 2% halothane, 0.6 liter/min N2O and 0.6 liter/min O2. The incisor bar of the stereotaxic instrument (David Kopf Instruments, Tujunga, CA) was set at 3.3 mm anterior from the interaural line, +1.8 mm lateral from the midline, and −8.2 mm ventral from the skull surface.

Apparatus. Eight commercially available (TSE Systems, Bad Homburg, Germany) “rotameter” units (transparent plastic bowls; 57 × 55 × 52 cm) were used for testing. The rats were harnessed and tethered to a rotation sensor interfaced to an IBM-compatible personal computer (using the TSE Rotameter Software v. 1.11; TSE Systems) that registered clockwise or counterclockwise movement. An internal software rotation filter of 10 was used. A minimum of 10 to 14 days after surgery, the rats were habituated (twice) to the apparatus. The latency of the 6-OHDA lesion was checked by testing their sensitivity to direct and indirect dopamine agonism. After recovery from surgery, rats were challenged with apomorphine (0.1 mg/kg s.c.), amphetamine (0.25 mg/kg s.c.), and a second apomorphine challenge (0.1 mg/kg s.c.), with 1 week between each challenge. Only those rats that elicited >20 ipsilateral turns after amphetamine in a 5-min time period beginning 25 min after administration and >20 contralateral turns per 5 min measured over 1 hour in the second apomorphine challenge were used in WS-50030 and aripiprazole studies. In addition, regular testing with apomorphine (0.1 mg/kg s.c.) was carried out to ensure the reliability of the animals in this procedure.

Protocol. After statistical randomization to the treatment groups, rats were treated with WS-50030 (10 mg/kg i.p.; n = 8), aripiprazole (10 mg/kg i.p.; n = 8), apomorphine (0.1 mg/kg s.c.; n = 16), or vehicle (intraperitoneal; n = 5) and placed in rotameters for 60 min. Rotational behavior both ipsilateral and contralateral to the side of lesion were recorded, and data are displayed graphically as mean number of contralateral minus ipsilateral turns ± S.E.M. for the 15-min period 45 to 60 min after drug administration. Data were subjected to a one-way ANOVA with post hoc LSD test.

Conditioned Avoidance Responding in Rats. Shuttlebox test chambers (MED Associates, St. Albans, VT) divided into two compartments by an archway were used. Each chamber floor half was composed of 13 stainless steel grid rods wired for the presentation of an electric foot shock (0.5 mA). In addition, each side of the chamber was equipped with a stimulus light, tone generator, and two infrared beamsource/detectors used to locate the rat within the chamber. Male Sprague-Dawley CD rats trained to avoid the foot shock were placed in the chambers for a 4-min habituation period followed by 50 trials presented on a 15-s variable interval schedule (range, 7.5–22.5 s). Each trial consisted of a 10-s warning tone and stimulus light (conditioned stimulus) followed by a 10-s shock (unconditioned stimulus), presented through the grid floor on the side where the rat was located, in the presence of the tone and light. If an animal crossed through the archway during the initial 10 s of the trial, thereby breaking the beam located 13 cm from the center of the archway, the tone and light were terminated and the response was considered an avoidance response. If an animal crossed through the archway after a foot shock was initiated, the tone, light, and shock were terminated and the response was considered an escape response. If a response was made during an intertrial interval, the animal was punished with a 0.5-s shock (0.5 mA). A MED Associates computer with MEDSTATE NOTATION software controlled the test session and counted the number of trials in which the animal avoided shock, escaped shock, and did not respond. The rats used in these studies were part of a trained colony used repeatedly for antipsychotic screening. Only animals displaying stable performance (approximately 90% avoidance responding on the training session before test day) were considered “trained” and included on test day. Training was maintained by at least one nondrug test session each week. On test days, rats were treated with WS-50030 (3 or 10 mg/kg i.p.; 10 mg/kg p.o.) or aripiprazole (1, 3, or 10 mg/kg i.p.; 17 or 30 mg/kg p.o.). Rats were tested 30 min after i.p. dosing and 60 min after p.o. dosing (within subject design, n = 7–8, separate cohorts for each drug and each route of administration). Doses were administered in ascending order. Data were analyzed using repeated measures ANOVA with post hoc LSD test.

Olfactory Bullectomy. Surgery. Isoflurane anesthesia (2–3%) was administered before animals were secured in a stereotaxic frame with ear and incisor bars (David Kopf Instruments). Using aseptic techniques, an incision was made and the skin was retracted to reveal bregma. Bilateral burr holes (2-mm diameter) were drilled above the olfactory bulbs (8.1 mm anterior from bregma; ±2.0 mm lateral from midline), and olfactory bulbs were removed by suction with a vacuum pump attached to a Pasteur pipette. Burr holes were then filled with gel foam (Harvard Apparatus Inc., Holliston, MA). For animals receiving sham surgery (Sham), burr holes were drilled and filled, but olfactory bulbs were not removed. An antibiotic solution, cefazolin (0.05 ml), was applied locally before closing the incision with surgical clips. After surgery, animals were injected s.c. with 5 ml of 0.9% sterile saline to maintain hydration and were placed in an incubator (85–90°C) until fully awake. Animals were then housed with another animal of the same surgery group (e.g., Bulb with Bulb or Sham with Sham) for the duration of the experiment. The day after surgery, rats were administered the analgesic buprenorphine hydrochloride (Buprenex; 6 μg/rat s.c.). All animals were allowed to recover for 14 days after surgery and were handled daily throughout the recovery period. Surgical clips were removed 14 days after surgery.

Drug treatment. Immediately after the 14-day recovery period, animals were each assigned to treatment groups (n = 10 bullectomized and 8 sham-operated rats/treatment group) and received WS-50030 (3–10 mg/kg i.p.) or aripiprazole (1–5.6 mg/kg i.p.) or vehicle (intraperitoneal) once daily for 2 weeks. Doses were selected based on previous studies using these compounds and limited to a range that produced <20% reduction in locomotor activity over the short term (data not shown).

Locomotor activity. Four hours after administration of the final dose, rats were placed in an open field (90 × 90-cm Plexiglas box with 30-cm walls) where locomotor activity was recorded for 5 min using a video camera suspended over the open field and Ethovision tracking software (Noldus Ethovision, Leesburg, VA). Total distance (centimeters) moved in the 5-min period in vehicle-treated sham-operated rats was compared with vehicle-treated bullectomized rats using a t test (p < 0.05) for each experiment. Subsequently, separate one-way ANOVAs compared the effect of dose of drug on total dis-
Results

Receptor Binding Profile. WS-50030 binds with high affinity to the serotonin transporter (mean $K_i \pm \text{S.E.M.}, 7.1 \pm 0.1 \text{nM}$) and the dopamine $D_2$ receptor (D2L, $4.0 \pm 0.1 \text{nM}$). In addition, WS-50030 had moderate affinity for the serotonin 5-HT$_{1A}$ (22.2 $\pm$ 7.7 nM), 5-HT$_{2A}$ (85.5 $\pm$ 23.5 nM), and 5-HT$_{3A}$ (38 $\pm$ 23 nM) receptors, adrenergic $a_1$ receptor (15.4 $\pm$ 5.1 nM), and histamine $H_1$ receptor (98.8 $\pm$ 34.5 nM) and low affinity for human norepinephrine transporter (3.6 $\pm$ 0.1 $\mu$M) and human dopamine transporter (2.9 $\pm$ 0.1 $\mu$M).

In Vitro Functional Activity. The functional activities of WS-50030 against the primary target proteins ($D_2$, SERT) were assessed in vitro. WS-50030 displayed potent $D_2$ partial agonist activity (mean EC$_{50} \pm$ S.E.M., 0.38 $\pm$ 0.02 nM; $E_{max}$, 30 $\pm$ 3%) by attenuating forskolin-induced accumulation of cAMP in CHO cells expressing D2L (Fig. 2A), whereas quinpirole exhibited full agonist activity (EC$_{50}$, 10 nM; data not shown). In a corresponding antagonist assay (Fig. 2A), WS-50030 partially attenuated quinpirole-induced blockade of forskolin-stimulated cAMP accumulation in a dose-dependent manner (mean IC$_{50}$ $\pm$ S.E.M., 2.2 $\pm$ 0.2 nM ($p_A$, 10.7); $I_{max}$, 73 $\pm$ 3%). Complete serotonin transporter blockade was exhibited by WS-50030 (IC$_{50}$, 56.4 $\pm$ 0.2 nM) in $[^3H]5-HT$ uptake assays using Jar cells (Fig. 2B). In cAMP accumulation assays using CHO cells transfected with the human serotonin 5-HT$_{1A}$ receptor, WS-50030 was found to be an agonist (EC$_{50}$, 67 $\pm$ 1.1 nM; $E_{max}$, 90 $\pm$ 3%; Fig. 2C). WS-50030 (10 $\mu$M) displayed no detectable 5-HT$_{2A}$ agonist activity in measurements of intracellular calcium accumulation (data not shown). In FLIPR-based functional agonist and antagonist assays using the human 5-HT$_{3A}$ receptor expressed recombinantly in HEK293 cells, WS-50030 was determined to be an antagonist ($p_A$, 6.65 $\pm$ 0.15). There was no detectable 5-HT$_{1A}$ agonist activity.

In Vivo Microdialysis. Basal levels of 5-HT and dopamine (mean $\pm$ S.E.M.) in either the mPFC or nucleus accumbens were not affected by long-term WS-50030 treatment (10 mg/kg i.p. daily for 22 days): mPFC: 5-HT, 679.78 $\pm$ 280.84 pM vehicle; 482.13 $\pm$ 106.69 pM long-term WS-50030; dopamine, 84.06 $\pm$ 22.89 pM vehicle; 175.12 $\pm$ 50.92 pM long-term WS-50030. Nucleus accumbens: 5-HT, 105.99 $\pm$ 41.54 pM vehicle; 186.07 $\pm$ 20.98 pM long-term WS-50030; dopamine, 901.31 $\pm$ 234.44 pM vehicle; 898.83 $\pm$ 109.67 pM long-term WS-50030. These findings are consistent with previous studies showing that long-term treatment with an SRI (Dawson et al., 2000) or aripiprazole (Jordan et al., 2004) does not significantly change basal levels of 5-HT or dopamine in the rat frontal cortex. WS-50030 treatment significantly elevated extracellular levels of 5-HT in the rat mPFC (Fig. 3A) [F(2,10) = 5.02, $p < 0.05$]. After 21 days of WS-50030 treatment (10 mg/kg i.p.), a challenge injection of WS-50030 (10 mg/kg i.p.) elicited a significant increase in the extracellular levels of 5-HT in the rat mPFC ($p = 0.01$ relative to vehicle treatment). Additional post hoc analyses revealed that in rats treated continuously with vehicle, a short-term injection of WS-50030 (10 mg/kg i.p.) elicited a nonsignificant trend ($p = 0.096$) toward elevating 5-HT relative to vehicle. In addition, no significant differences were observed in sham and bulbectomized subjects, followed by Dunnett’s post hoc comparison with the vehicle treated group ($p < 0.05$).
observed between rats that received short- or long-term treatment with WS-50030 (p = 0.40). In contrast, neither short-term nor long-term treatment with WS-50030 altered levels of dopamine in the mPFC after a dose of 10 mg/kg (Fig. 3B) [F(2,13) = 0.01, p = 0.98]. After 21 days of treatment with either vehicle or WS-50030 (10 mg/kg i.p.), a challenge injection of WS-50030 (10 mg/kg i.p.) elicited no significant changes in levels of 5-HT [F(2,10) = 0.01, p = 2.52] or dopamine [F(2,14) = 1.84, p = 0.83] in the rat nucleus accumbens (Fig. 4, A and B).

Antagonism of Apomorphine-Induced Climbing and Stereotypy in Mice. Figure 5 shows the effects of WS-50030 and aripiprazole on apomorphine-induced climbing (effects on apomorphine-induced stereotypy not shown). Apomorphine (1 mg/kg s.c.) induced consistent levels of climbing and stereotypy in vehicle-treated mice [climbing (mean ± S.E.M.), 10.89 ± 0.21; stereotypy, 5.78 ± 0.22]. WS-50030 (0.03–30 mg/kg i.p.) produced a dose-dependent decrease in apomorphine-induced climbing more potently than apomorphine-induced stereotypy [ID₅₀ climbing, 0.51 mg/kg (95% CI, 0.39–0.66); ID₅₀ stereotypy, 1.02 mg/kg (95% CI, 0.83–1.27)]. Likewise, aripiprazole (0.1–1 mg/kg i.p.) produced a dose-dependent decrease in both apomorphine-induced behaviors [ID₅₀ climbing, 0.14 mg/kg (95% CI, 0.06–0.30); ID₅₀ stereotypy, 0.58 mg/kg (95% CI, 0.48–0.71)].

Cataleptogenic Potential in Mice. Although WS-50030 (0.3–10 mg/kg i.p.) demonstrated statistically significant catalepsy [minimal effective dose, 3 mg/kg at 120-min time point; treatment × time interaction F(12,75) = 2.15; p < 0.05, repeated-measures ANOVA; p < 0.01, LSD post hoc, Table 1], it was minimal (20 s). The peak catalepsy exhibited was at the 10 mg/kg dose at 90 min after dosing, when mice displayed a mean ± SEM of 14.33 ± 7.29 s in the catalepsy position of a maximum possible 60 s (Fig. 6). Aripiprazole (0.1–3 mg/kg i.p.) also demonstrated statistically significant catalepsy [minimal effective dose, 1 mg/kg at 120-min time point; treatment × time interaction F(12,75), 2.67; p < 0.01, repeated-measures ANOVA; p < 0.05, LSD post hoc, Table 1]. Peak catalepsy after aripiprazole administration was also exhibited at the 90-min test point, when the 1 and 3 mg/kg i.p. dose groups displayed means ± SEM of 16.83 ± 9.25 and 30.33 ± 9.06 s in the catalepsy position.
Fig. 5. Antagonism of apomorphine-induced behaviors in mice. A, mean climbing scores (± S.E.M.) are expressed as a percentage of control values observed in vehicle-treated mice that received apomorphine. ID50 values were calculated using nonlinear regression. WS-50030 (0.03–30 mg/kg s.c.) produced a dose-dependent decrease in apomorphine-induced (1 mg/kg s.c.) climbing (ID50, 0.51 mg/kg (95% CI, 0.39–0.67); n = 12 for vehicle and 1 mg/kg groups, n = 6 for all other treatment groups. Aripiprazole (0.1–1 mg/kg i.p.) produced a dose-dependent decrease in apomorphine-induced (1 mg/kg s.c.) climbing (ID50, 0.14 mg/kg (95% CI, 0.06–0.30); n = 6 per treatment group. ■, WS-50030; ●, aripiprazole.

respectively, of a maximum possible 60 s (minimal and moderate catalepsy, respectively).

Rotational Behavior in Unilaterally 6-OHDA-Lesioned Rats. Contralateral minus ipsilateral rotations recorded in the 15-min period occurring 45 to 60 min after dosing are displayed in Fig. 7. Apomorphine (0.1 mg/kg s.c.) produced an expected amount of contralateral rotation [contralateral-ipsilateral rotations (mean ± S.E.M.), 74.59 ± 13.24]. WS-50030 (10 mg/kg s.c.) produced contralateral rotation that was significantly less than that produced by apomorphine (WS-50030 contralateral-ipsilateral rotations, 22.23 ± 5.38; post hoc p = 0.01 versus apomorphine). Aripiprazole produced a similar amount of contralateral rotation (contralateral-ipsilateral rotations, 37.21 ± 17.55) to WS-50030, demonstrating a nonsignificant trend to be less than that produced by apomorphine (post hoc p = 0.52 versus WS-50030; post hoc p = 0.07 versus apomorphine). Mean ± S.E.M. of contralateral and ipsilateral rotations were as follows: apomorphine: 78.24 ± 13.40 contralateral, 3.65 ± 1.53 ipsilateral; WS-50030: 23.62 ± 5.39 contralateral, 1.39 ± 0.55 ipsilateral; aripiprazole: 38.86 ± 17.38 contralateral, 1.65 ± 0.39 ipsilateral.

Conditioned Avoidance Responding in Rats. Aripiprazole and WS-50030 both significantly decreased avoidance responding after intraperitoneal and oral dosing with no effect on the number of trials in which escape failures occurred. WS-50030 at 3 and 10 mg/kg i.p. produced 17 and 42% reductions in avoidance responding, respectively [F(2,14) = 8.82; p < 0.01; Fig. 8]. After oral administration, 10 mg/kg WS-50030 produced a 40% reduction in avoidance [F(1,7) = 13.10; p < 0.01; data not shown]. Aripiprazole produced 5, 36, and 55% reductions in avoidance responding at 1, 3, and 10 mg/kg i.p. [F(3,18) = 26.33; p < 0.001; Fig. 8], respectively, and 21 and 49% reductions in avoidance at 17 and 30 mg/kg p.o. [F(2,14) = 14.41; p < 0.001; data not shown], respectively.

Table 1

Table 1. Catalepsy induced by WS-50030 or aripiprazole in mice: effect of dose and pretreatment interval

Data are mean ± S.E.M. in male CF-1 mice evaluated repeatedly at different intervals (minutes) after the intraperitoneal administration of test compound (n = 6 per dose level). Time spent (in seconds) with forelimbs positioned on an elevated bar was measured with a maximum cut-off of 60 s. Severity of catalepsy is defined as follows: maintenance of awkward position for 20 s or less, minimal to no catalepsy; 21 to 40 s, moderate catalepsy; and 41 to 60 s, high catalepsy.

<table>
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<th>Pretreatment</th>
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<td>WS-50030</td>
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<td>Aripiprazole</td>
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<td>90 min</td>
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* p < 0.05 vs. vehicle-treated mice at same time point by post hoc LSD test.
The effect of surgery on locomotor activity was observed in both experiments as a significant increase in locomotor activity in the vehicle-treated bullectomized rats compared with the vehicle-treated sham-operated rats (WS-50030: $t = 2.677$, df = 12, $p < 0.05$; aripiprazole: $t = 3.650$, df = 16, $p < 0.01$; Fig. 9). Administration of WS-50030 for 14 days completely reversed bullectomy-induced hyperactivity [F(3,31) = 3.65, $p < 0.01$] while producing a smaller effect in sham subjects [F(3,25) = 6.28, $p < 0.01$]. Post hoc analysis revealed a selective effect of WS-50030 at 3 mg/kg i.p. to normalize bullectomy-induced hyperactivity without significantly affecting locomotor activity.

**Olfactory Bullectomy.** The effect of surgery on locomotor activity was observed in both experiments as a significant increase in locomotor activity in the vehicle-treated bullectomized rats compared with the vehicle-treated sham-operated rats (WS-50030: $t = 2.677$, df = 12, $p < 0.05$; aripiprazole: $t = 3.650$, df = 16, $p < 0.01$; Fig. 9). Administration of WS-50030 for 14 days completely reversed bullectomy-induced hyperactivity [F(3,31) = 3.65, $p < 0.01$] while producing a smaller effect in sham subjects [F(3,25) = 6.28, $p < 0.01$]. Post hoc analysis revealed a selective effect of WS-50030 at 3 mg/kg i.p. to normalize bullectomy-induced hyperactivity without significantly affecting locomotor activity.
in sham subjects (Fig. 9A). The 5.6 and 10 mg/kg doses of WS-50030 also reversed bullectomy-induced hyperactivity relative to vehicle-treated bullectomized subjects; however, these doses also caused a significant reduction in activity in sham-operated animals. Administration of aripiprazole (1–5.6 mg/kg i.p.) for 14 days also significantly reversed bullectomy-induced hyperactivity [F(3,35) = 9.76, p < 0.0001] without significantly reducing activity in sham-operated rats [F(3, 24) = 0.963, p > 0.05] (Fig. 9B). Administration of 1 mg/kg i.p. aripiprazole for 14 days partially reversed the bullectomy-induced hyperactivity (66% reversal versus vehicle-treated bullectomized; Fig. 9B). Higher doses of aripiprazole (3 and 5.6 mg/kg i.p.) completely reversed bullectomy-induced hyperactivity without reducing locomotor activity in sham-operated animals.

Discussion

Our objective was to develop a novel antipsychotic agent with broad therapeutic efficacy by combining D₂ receptor partial agonist activity with SRI properties in a single molecule. We have characterized WS-50030 binding and function in vitro as well as in a variety of in vivo functional and efficacy models. When appropriate, we compared the effects of WS-50030 directly with the D₂ receptor partial agonist, aripiprazole, which reportedly lacks significant SRI activity.

The present data indicate that WS-50030 is a combined D₂ receptor partial agonist and serotonin reuptake inhibitor. WS-50030 demonstrates both antipsychotic- and antidepressant-like activity in preclinical in vivo models with a low propensity for extrapyramidal side effects as measured by mouse catalepsy, a profile similar to aripiprazole.

Receptor binding studies demonstrate that WS-50030 binds with high affinity to the human D₂ receptor (Kᵢ, 4.0 nM) and human serotonin transporter (Kᵢ, 7.1 nM) and with moderate affinity to other receptors. In vitro functional studies demonstrated that WS-50030 behaved as a D₂ receptor partial agonist, partially attenuating forskolin-induced accumulation of cAMP in CHO cells expressing the long form of the dopamine D₂ receptor and partially attenuating forskolin-induced accumulation of cAMP. This in vitro characterization of partial agonist activity in cells recombinantly expressing the D₂L receptor has the potential to overestimate agonist activity, because receptor density is higher than might be expected in vivo. Therefore, it is important to evaluate D₂ agonist-like and antagonist-like activity in vivo (see below).

WS-50030 exhibited complete serotonin transporter blockade in [³H]5-HT uptake assays using JAR cells and behaved as an agonist in CHO cells expressing the human 5-HT₁A receptor. Whereas WS-50030 exhibited moderate binding affinity to 5-HT₂B receptors (Kᵢ, 38 nM), no detectable agonist activity was observed, thereby limiting the likelihood of cardiovascular effects that have been demonstrated to occur in response to 5-HT₂B receptor agonists (Huang et al., 2009).

Aripiprazole is reportedly a D₂ receptor partial agonist as well as a partial agonist at 5-HT₁A receptors and an antagonist at 5-HT₂A receptors (Stark et al., 2007). Consistent with preliminary in house data (not shown), literature reports demonstrate that aripiprazole binds more potently to the long form of the D₂ receptor (Kᵢ, 0.74 nM; Shapiro et al., 2003) than WS-50030. Unlike WS-50030, which binds equipotently at D₂ receptors and the serotonin transporter, aripiprazole binds with much greater selectivity for D₂ receptors (serotonin transporter Kᵢ, 1080 nM; Shapiro et al., 2003).

To examine the effects of WS-50030 on extracellular levels of serotonin and dopamine, in vivo dual probe microdialysis studies were performed in the rat. These studies were designed to investigate the neurochemical effects of WS-50030 in both the mPFC and nucleus accumbens in the same animal. Consistent with the serotonin reuptake properties of this molecule, long-term treatment with WS-50030 elicited a significant increase in the extracellular levels of serotonin in the rat mPFC. This response was qualitatively similar in nature to that previously reported for long-term treatment with the SRI fluoxetine (Dawson et al., 2000). A similar trend toward increased serotonin was also observed after short-term WS-50030 in this brain region; however, this latter effect did not reach statistical significance. It is likely that additional receptor functional efficacy, such as agonism at 5-HT₁A receptors, acts to limit the short-term effects of WS-50030 on this neurotransmitter; upon repeated administration, these receptors desensitize, allowing higher levels of serotonin to occur. In contrast, neither short-term nor long-term treatment with WS-50030 altered levels of serotonin or dopamine in the rat nucleus accumbens, nor dopamine in the mPFC. The lack of dopaminergic effects found in both of these brain regions is consistent with the D₂ receptor partial agonist activity of WS-50030. For instance, the D₂ receptor partial agonist aripiprazole does not alter the extracellular levels of serotonin (Zocchi et al., 2005) or dopamine in the rat mPFC or striatum after short-term treatment (Jordan et al., 2004). Likewise, Jordan et al. (2004) demonstrated that long-term aripiprazole treatment—unlike long-term olanzapine treatment—failed to significantly alter extracellular concentrations of 5-hydroxyindole acetic acid (a metabolism product of serotonin) or dopamine in the rat striatum. These in vivo microdialysis studies support the conclusion that WS-50030 modulates serotonin transporters whereas aripiprazole does not.

In vivo behavioral studies showed that both WS-50030 and aripiprazole antagonize dopamine agonist-induced behaviors in mice, namely, climbing and stereotypy. As expected based on in vitro potency at D₂ receptors, aripiprazole was slightly more potent than WS-50030 in this model. However, neither compound elicited a strong cataleptogenic response in mice, consistent with a D₂ partial agonist profile and predictive of low extrapyramidal side effects. Other receptor activities of aripiprazole and WS-50030, such as 5-HT₁A agonism, may also contribute to their low extrapyramidal side-effect liability. In vivo dopamine agonist activity was demonstrated using rats with unilateral 6-OHDA-lesions of the substantia nigra zona compacta. In lesioned rats, postsynaptic dopamine receptors become supersensitive on the side of the lesion. Systemic injection of a postsynaptic agonist such as apomorphine will induce rotational behavior contralateral to the side of the lesion. Both WS-50030 and aripiprazole produced modest yet significant contralateral rotations in 6-OHDA-lesioned rats. As expected given the low intrinsic activity of WS-50030, the amount of contralateral rotation induced by WS-50030 was significantly less than that produced by the full D₂ receptor agonist apomorphine. Thus it can be surmised that WS-50030 demonstrated less in vivo D₂...
agonist activity than the corresponding full agonist. Although aripiprazole produced contralateral rotational behavior that was similar in magnitude to WS-50030, it did not differ significantly from that of apomorphine. This result is inconsistent with previous studies in 6-OHDA-lesioned rats by Kikuchi et al. (1995), in which no contralateral rotation was observed after short-term aripiprazole administration (10 mg/kg p.o.).

Overall, the results described thus far support the conclusion that WS-50030 demonstrates in vitro and in vivo D₂ receptor partial agonism and in vivo functional effects on serotonin reuptake inhibition, whereas the literature characterizes aripiprazole as a D₂ receptor partial agonist. WS-50030 and aripiprazole were therefore compared in in vivo models predictive of antipsychotic and antidepressant efficacy. WS-50030 demonstrated, at the same dose (10 mg/kg), an effect similar in magnitude to that of aripiprazole in the rat conditioned avoidance test, a standard screening model for antipsychotic efficacy (Arnt, 1982). In this model, all clinically active antipsychotics reportedly reduce the number of trials in which a rat will avoid a footshock by crossing to the opposite side of the test chamber. In our hands, aripiprazole produced a 55% reduction in avoidance responding and WS-50030 a 42% reduction in avoidance responding (both at 10 mg/kg), and both increased escape responding without producing escape failures. These data suggest that WS-50030 may provide antipsychotic efficacy similar to that of aripiprazole.

As previously stated, there exists a high incidence of comorbidity of depressive symptoms in patients with schizophrenia (Siris et al., 2001), and there is mounting evidence to support the value of SRI augmentation in the treatment of the negative symptoms of schizophrenia (Silver, 2003; Mazeh et al., 2004). Therefore, doses of WS-50030 that were active in rat conditioned avoidance and increased extracellular 5-HT were evaluated in a preclinical model of depression, specifically the olfactory bulbectomy-induced hyperactivity model in rats. It has previously been shown that a bilateral olfactory bulbectomy induces increased locomotor activity, compared with sham-operated rats, that is reversed by long-term administration of SRIs (Kelly et al., 1997). Long-term treatment with WS-50030 (3 mg/kg once daily for 14 days) was efficacious in this model, completely reversing bulbectomy-induced hyperactivity without reducing locomotor activity in sham-operated rats. Efficacy in this model suggests that WS-50030 may provide benefit in the treatment of mood disorders associated with schizophrenia and comorbid depression.

We were surprised to find that long-term treatment with aripiprazole was also effective in this model, behaving similarly to WS-50030 despite a reported lack of effect on serotoninn transporters. Therefore, WS-50030 failed to distinguish itself from aripiprazole using the olfactory bulbectomy model. In preliminary studies, aripiprazole has been reported to be active in the olfactory bulbectomy model (Pistovcakova and Sulcova, 2008), and although antidepressant efficacy of aripiprazole treatment alone has yet to be demonstrated in the clinic, clinical data indicate that aripiprazole augments the effectiveness of standard antidepressant treatment in patients with major depressive disorder and treatment-resistant depression (Pae et al., 2008). In addition, aripiprazole has been reported to produce improvement on various clinical negative symptom rating scales in patients with schizophrenia (Swainston and Perry, 2004; Cassano et al., 2007).

Given the similar results in the olfactory bulbectomy-induced hyperactivity model with WS-50030 and aripiprazole, further studies are required to definitively elucidate the mechanisms of action responsible for their effect in the model and to determine the degree to which serotonin reuptake inhibition activity may be contributing to the efficacy of WS-50030 in this model. Mechanisms other than serotonin reuptake inhibition have been reported to normalize olfactory bulbectomy-induced hyperactivity (Kelly et al., 1997). It may be that the D₂ receptor partial agonist properties of WS-50030 and aripiprazole are sufficient to demonstrate efficacy in this model. Millan et al. (2004) have reported preclinical efficacy with the D₂/D₃ receptor agonists ropinirole and S32504 in depression models such as the forced swim test, learned helplessness, and chronic mild stress models that were blocked by D₂ but not D₃ receptor antagonists.

In summary, despite possessing serotonin reuptake inhibitory activity in addition to D₂ receptor partial agonism, WS-50030 displays activity in preclinical models predictive of antipsychotic and antidepressant efficacy similar to aripiprazole, thus suggesting potential efficacy of WS-50030 versus positive and negative symptoms of schizophrenia, comorbid mood symptoms, and bipolar disorder as well as major depressive disorder and treatment resistant depression. Furthermore, WS-50030 provides a tool to further explore how combining these mechanisms might differentiate other antipsychotics or antidepressants.

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