Pharmacology of JNJ-37822681, a Specific and Fast-Dissociating D2 Antagonist for the Treatment of Schizophrenia

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ABSTRACT

All marketed antipsychotics act by blocking dopamine D2 receptors. Fast dissociation from D2 receptors may be one of the elements contributing to the lower incidence of extrapyramidal symptoms (EPS) exhibited by newer antipsychotics. Therefore, we screened for specific D2 receptor blockers with a fast rate of dissociation. Radioligand binding experiments identified N-[1-(3,4-difluorobenzyl)piperidin-4-yl]-6-(trifluoromethyl)pyridazin-3-amine (JNJ-37822681) as a fast-dissociating D2 ligand. Its D2 receptor specificity was high compared with atypical antipsychotics, with little activity at receptors associated with unwanted effects [α1, α2, H1, muscarinic, and 5-hydroxytryptamine (5-HT) type 2C] and for receptors that may interfere with the effects of D2 antagonist (D1, D3, and 5-HT2A). JNJ-37822681 occupied D2 receptors in rat brain at relatively low doses (ED50 0.39 mg/kg) and was effective in animal models of psychosis (e.g., inhibition of apomorphine-induced stereotypy or d-amphetamine/phencyclidine-induced hyperlocomotion). Prolactin levels increased from an ED50 (0.17 mg/kg, peripheral D2 receptors) close to the ED50 required for apomorphine antagonism (0.19 mg/kg, central D2 receptors), suggesting excellent brain disposition and minimal prolactin release at therapeutic doses. JNJ-37822681 induced catalepsy and inhibited avoidance behavior, but with a specificity margin relative to apomorphine antagonism that was larger than that obtained for haloperidol and similar to that obtained for olanzapine. This larger specificity margin (compared with haloperidol) may reflect lower EPS liability and less behavioral suppression after JNJ-37822681. JNJ-37822681 is a novel, potent, specific, centrally active, fast-dissociating D2 antagonist with optimal brain disposition, and it is the first compound that allows the evaluation of the potential value of fast D2 antagonism for the treatment of schizophrenia and bipolar disorder.

Introduction

Schizophrenia is a severe and chronic mental illness. The etiology of the disease is still unknown, but aberrant neurotransmitter activity has been hypothesized to underlie the symptoms of schizophrenia. The dopaminergic hypothesis is the one that is most widely accepted; it proposes that hyperactivity of dopaminergic transmission is responsible for the positive symptoms observed in schizophrenic patients. This hypothesis is based on the observation that dopamine-enhancing drugs, such as amphetamine or cocaine, may induce psychosis and on the correlation that exists between clinical doses of antipsychotics and their potency in blocking dopamine D2 receptors (Kapur and Mamo, 2003). All marketed antipsychotics mediate their therapeutic efficacy against positive symptoms through blockade of the dopamine D2 receptor (Seeman, 2006). Apart from the clinical efficacy, it seems that the major adverse effects (AEs) of antipsychotics, such as extrapyramidal symptoms (EPS) and tardive dyskinesia, are also related to dopamine antagonism. Those debilitating effects appear most frequently with “typical” (first-generation) antipsychotics (e.g., haloperidol). They are less pronounced with the “atypical” (second-generation) antipsychotics (e.g., risperidone and olanzapine) and virtually absent with clozapine, which is considered to be the prototypical atypical antipsychotics.
chotic. Currently available antipsychotics are also well known to cause prolactin release. Hyperprolactinemia can cause a number of AEs (e.g., menstrual disturbances, galactorrhea, sexual dysfunction, decreased fertility, movement disorders, and behavioral disturbances) (Dickson and Glazer, 1999).

Among the different theories proposed for explaining the lower incidence of EPS observed with atypical antipsychotics, the one that has received the most attention during the last 15 years is the multireceptor hypothesis (Meltzer, 2000). Receptor binding studies showed that many atypical antipsychotics interact with various other neurotransmitter receptors in addition to dopamine D₂ receptors, in particular with serotonin 5-HT₂ receptors (Meltzer et al., 1989). In contrast, typical antipsychotics, like haloperidol, bind more specifically to D₂ receptors. Although all major atypical antipsychotics fully occupy the serotonin 5-HT₂ receptors at clinically relevant dosages, they differ in their propensity to induce motor side effects. Moreover, they show interactions with additional receptors, some of which may be responsible for undesirable effects, such as 5HT₂C receptors (weight gain), α₁-adrenoceptors (orthostatic hypotension, reflex tachycardia, and hypnosedation), α₂-adrenoceptors (tachycardia), histamine H₁ receptors (sedation and weight gain), and muscarinic receptors (blurred vision, dry mouth, constipation, and cognitive impairment).

As an alternative to the “balanced serotonin 5-HT₂A-dopamine D₂” hypothesis, it has been proposed that the rates at which they dissociate from dopamine D₂ receptors may better distinguish atypical from typical antipsychotics (Kapur and Seeman, 2001). Fast dissociation from the D₂ receptor would allow more physiological dopamine transmission, permitting an antipsychotic effect with fewer adverse motor effects. It is noteworthy that clozapine and quetiapine have the fastest rate of dissociation from dopamine D₂ receptors and carry the lowest risk of inducing EPS in humans. Conversely, typical antipsychotics associated with a high prevalence of EPS are the slowest-dissociating dopamine D₂ antagonists. Thus, identifying new drugs based on their rate of dissociation from the D₂ receptor could be a valid strategy for developing atypical antipsychotics with an improved tolerability profile.

Fig. 1. Structures of JNJ-37822681 and the seven tested reference antipsychotics.

JNJ-37822681 is a specific, centrally active, and fast-dissociating D₂ antagonist with optimal brain disposition and has potential therapeutic value for the treatment of schizophrenia and bipolar disorder. Some of these data were previously presented at the 23rd Congress of the European College of Neuropsychopharmacology (Langlois et al., 2010). A phase IIb trial of JNJ-37822681 in schizophrenia has recently been completed, confirming antipsychotic efficacy and the preclinical findings of atypicality (low EPS and prolactin side-effect liability) (Schmidt et al., 2012).

Materials and Methods

Preparation of Test Article and Controls and Sources

The purity of all batches used in pharmacological studies was assessed to be equal to or more than 95% by using standard analytical methods. JNJ-37822681, clozapine, aripiprazole, haloperidol, ziprasidone, risperidone, olanzapine, and quetiapine were acquired from internal sources and dissolved in dimethyl sulfoxide for in vitro studies. [³H]clozapine was purchased from American Radiolabeled Chemicals (St. Louis, MO); [³H]prazosin, [³H]-7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol (SCH23390), and [³H]pyrilamine were from PerkinElmer Life and Analytical Sciences (Waltham, MA); and [³H]spiperone, [³H]iodosulpride, and [³H]mesulergine were from GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK. [¹⁰⁰⁵]-[³H]-4-aminoo-N-[1-[3-[4-fluoroxy]propyl]-4-methylpiperidin-4-yl]-2-methoxybenzamide (8091150) was custom made by GE Healthcare; all other radioligands were synthesized in-house. The various compounds used
to determine nonspecific binding of these radioligands were also synthesized internally and dissolved in dimethyl sulfoxide.

For in vivo prolactin release studies, JNJ-37822681 was dissolved in distilled water containing one equivalent of tartaric acid. For all other in vivo studies, JNJ-37822681 was dissolved in 10% hydroxypropyl-β-cyclodextrin in distilled water containing one equivalent of tartaric acid. The solutions were stored at room temperature in closed containers protected from light. The preparations were subsequently injected in volumes of 10 ml/kg. Solvent was also tested to control for solvent-related effects.

**In Vitro Binding Affinity for hD2L Receptor and Specificity Profile**

Unlabeled JNJ-37822681, clozapine, aripiprazole, haloperidol, ziprasidone, risperidone, olanzapine, and quetiapine were used in different radioligand competition binding assays to assess their affinity for a set of receptors. First, membranes expressing the different receptors of interest were prepared as follows. Cells were transfected with cloned human receptor cDNA, collected by scraping and homogenized in 50 mM Tris-HCl, pH 7.4 by using an Ultra Turrax homogenizer. The homogenate was centrifuged for 10 min at 23,500 g in a Sorvall RC-5C Plus centrifuge (Goffin Meyvis, Overijse, Belgium) (4°C). The pellets were then homogenized by using an Ultra Turrax and suspended in ice-cold binding buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 2 mM CaCl₂, pH 7.7. Assay mixtures containing 50 μg (for [³H]JNJ-37822681 and [³H]clozapine) or 10 μg (for [³H]haloperidol, [³H]risperidone, and [³H]paliperidone) of membranes were incubated for 1 h in a volume of 0.45 ml at room temperature and 37°C. Final concentrations of 10 nM radioligand were used for [³H]JNJ-37822681 and [³H]clozapine, whereas 2 nM was applied for the other [³H]-labeled compounds. Dissociation kinetics were measured by adding 10 μM raclopride (50 μl) at different times before filtration. Filtration was performed by using a 40-well multivisor. In a parallel set of tubes, nonspecific binding was determined in the presence of 10 μM butaclamol. Time intervals were chosen to provide an optimal estimate of the rate of dissociation (time points were 10, 20, 30, 40, 60, 120, 300, and 600 s for [³H]clozapine and [³H]JNJ-37822681 and 20, 30, 40, 60, 180, 600, 1200, and 3600 s for [³H]haloperidol, [³H]paliperidone, and [³H]risperidone).

**Animals (Species, Weight, and Sex)**

Female Sprague-Dawley rats were used for the prolactin assay; male Lewis rats were used for the compound 48/80 lethality assay; Dunkin-Hartley-Pirbright guinea pigs of both sexes were used for the histamine lethality assay; and male Wiga Wistar rats were used for all other assays. The rats ranged in body weight between 175 and 275 g, and the guinea pigs were between 300 and 500 g. All animals were obtained from Charles River Breeding Laboratories (Sulzburg, Germany) and housed under standard laboratory conditions (21 ± 2°C; 45–65% relative humidity; light/dark cycle set at 12 h). Except for the occupancy assay, the animals were fasted overnight before the start of the experiments (tap water remained available ad libitum). During the test period, they were housed in individual cages. The local Ethical Committee in compliance with the Declaration of Helsinki approved all studies.

**D₂ Receptor Occupancy**

Rats were treated subcutaneously with vehicle or test compounds at five to eight dosages ranging from 0.0025 to 40 mg/kg body weight. Three to six animals were used per dose of compound. [³H]Raclopride (8 μCi/animal) was injected intravenously 30 min after drug administration. The animals were decapitated 30 min after the [³H]Raclopride injection. Brains were immediately removed and rapidly frozen.

**TABLE 1**

<table>
<thead>
<tr>
<th>Receptor Source</th>
<th>Assay Conditions (Incubation Buffer, Time, and Temperature)</th>
<th>Radioligand</th>
<th>Nonspecific Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>hD₁-Adrenergic</td>
<td>CHO 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1% BSA, pH 7.7, room temperature, 20–24 h*</td>
<td>[³H]prazosin, 0.5 nM</td>
<td>Aceperone, 1 μM</td>
</tr>
<tr>
<td>hD₂</td>
<td>GH4C1 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1% BSA, pH 7.7, 25°C, 60 min</td>
<td>[³H]SCH23390, 1 nM</td>
<td>Piflutixol, 1 μM</td>
</tr>
<tr>
<td>hD₂L</td>
<td>CHO 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 μM pargyline, pH 7.7, 25°C, 30 min</td>
<td>[³H]spiperone, 0.2 nM</td>
<td>(+)butaclamol, 1 μM</td>
</tr>
<tr>
<td>hD₃</td>
<td>CHO 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1% BSA, pH 7.7, 25°C, 30 min</td>
<td>[¹²⁵I]iodosulpride, 0.2 nM</td>
<td>Risperidone, 1 μM</td>
</tr>
<tr>
<td>h-HT₁A</td>
<td>L929 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1% BSA, pH 7.4, 37°C, 30 min</td>
<td>[¹²⁵I]BRO91150, 0.1 nM</td>
<td>BW501, 1 μM</td>
</tr>
<tr>
<td>h-HT₁C</td>
<td>SB 50 mM Tris-HCl, 4 mM CaCl₂, pH 7.7, 37°C, 30 min</td>
<td>[³H]mesulergine, 1 nM</td>
<td>Ritanserin, 1 μM</td>
</tr>
<tr>
<td>h-HT₄</td>
<td>CHO 50 mM Na-K phosphate, 0.05% BSA, pH 7.5, 25°C, 30 min</td>
<td>[³H]pyrilamine, 2 nM</td>
<td>Astemizole, 1 μM</td>
</tr>
</tbody>
</table>

* Scintillation proximity assay technology was used to quantify radioligand binding, whereas for the other assays filtration using a Packard Filtermate Harvester Hewlett Packard (Palo Alto, CA) was done to separate bound from free radioligand.

**Direct Dissociation.** The off rate of [³H]JNJ-37822681 was determined and compared with off rates for [³H]-labeled reference antipsychotics by typical radioligand binding experiments. Membranes were prepared from CHO cells stably transfected with hD₂L receptor cDNA as described above. After thawing, membranes were homogenized by using an UltraTurrax and suspended in ice-cold binding buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 2 mM CaCl₂, pH 7.7. Assay mixtures containing 50 μg (for [³H]JNJ-37822681 and [³H]clozapine) or 10 μg (for [³H]haloperidol, [³H]risperidone, and [³H]paliperidone) of membranes were incubated for 1 h in a volume of 0.45 ml at room temperature and 37°C. Final concentrations of 10 nM radioligand were used for [³H]JNJ-37822681 and [³H]clozapine, whereas 2 nM was applied for the other [³H]-labeled compounds. Dissociation kinetics were measured by adding 10 μM raclopride (50 μl) at different times before filtration. Filtration was performed by using a 40-well multivisor. In a parallel set of tubes, nonspecific binding was determined in the presence of 10 μM butaclamol. Time intervals were chosen to provide an optimal estimate of the rate of dissociation (time points were 10, 20, 30, 40, 60, 120, 300, and 600 s for [³H]clozapine and [³H]JNJ-37822681 and 20, 30, 40, 60, 180, 600, 1200, and 3600 s for [³H]haloperidol, [³H]paliperidone, and [³H]risperidone).

**Indirect Dissociation.** The dissociation rate of compounds was evaluated by using an indirect assay adapted from a previously described method (Lysen and Gommeren, 1984). Membranes were prepared from Chinese hamster ovary (CHO) cells stably expressing the hD₂L receptor as described above. After thawing, membranes were homogenized by using an Ultra Turrax and suspended in ice-cold binding buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, pH 7.6, 0.1% ascorbic acid, and 10 μM pargyline.

After incubating hD₂L membranes with four times IC₅₀ of compound for 1 h at 25°C (final volume of 2 ml), incubation mixtures were poured on GF/C filters on top of a 40-well multivisor. Vacuum was briefly applied to filter compound-membrane mixtures over the GF/C filters. Two hundred microliters of buffer or 200 μl of 4 μM butaclamol (final concentration 2 μM) together with 200 μl of 2 nM [³H]spiperone was then added for 1, 3, 5, 7, or 10 min (final concentration 1 nM [³H]spiperone). Incubation was stopped by initiating full vacuum and immediate rinsing with ice-cold buffer. After the addition of 3 ml of Ultima Gold MV (PerkinElmer Life and Analytical Sciences), filter-bound radioactivity was measured in a liquid scintillation counter.
in dry ice-cooled 2-methylbutane (−40°C). Twenty-micrometer-thick frozen sections were thaw-mounted on slides. Two striatal sections and one cerebellum section were collected per slide. Brain sections were loaded in a β-imager (Biospace Lab, Paris, France) for 12 h. Digital autoradiograms were quantified by using the Beta vision program (Biospace Lab). The binding potential of $[^3H]$raclopride was given as the difference between the radioligand binding quantified in the striatum (a brain area showing a high density of D$_2$ receptors) and the cerebellum (a brain area where D$_2$ receptors are virtually absent). The binding potential of $[^3H]$raclopride in striatum of drug-treated animals was expressed as the percentage of the binding potential of $[^3H]$raclopride in vehicle-treated animals. Percentages of receptor occupancy by the drug administered to the animal correspond to 100% minus the percentage of the binding potential of $[^3H]$raclopride in the treated animal.

**Apomorphine-Induced Stereotypy**

Apomorphine is a dopamine receptor stimulant and mimics the agonistic action of dopamine at the D$_2$ receptor. Apomorphine (1.0 mg/kg i.v.)-induced stereotypy (compulsive sniffing, licking, and chewing) was scored every 5 min over the hour after injection of apomorphine. The score system was: 3, pronounced; 2, moderate, 1, slight; and 0, absent. Criteria for drug-induced inhibition of stereotypy were: fewer than six scores of 3, fewer than six scores of 2, or fewer than seven scores ≥1 (0.14% false positives in >5000 solvent-pretreated control rats). Criteria for drug-induced blockade were: fewer than two scores of ≥1 and zero scores of ≥2 (0.0% false positives).

**Clonidine-Induced Antidiarrheal Activity**

Clonidine (0.02 mg/kg i.v.)-induced antidiarrheal activity in rats challenged simultaneously with castor oil (1 ml p.o.) was assessed 120 min later (modified after Megens et al., 1986). Criterion for drug-induced reversal was: absence of diarrhea (3.2% false positive controls; n = 154). Clonidine antagonism reflects blockade of peripheral α$_2$-adrenoceptors. To investigate whether intrinsic antidiarrheal activity might have masked the peripheral α$_2$-adrenoceptor blockade, the ability of inactive compounds to block castor oil-induced diarrhea was also studied after the injection of saline instead of clonidine. Criterion for antidiarrheal activity was: absence of diarrhea (6.7% false positive controls; n = 194).

**Clonidine-Induced Mydriasis**

The pupil diameter of the right eye was measured with a graduated microscope (Gant type 55017; 1 unit = 1/24 mm) just before administration of the test compound or solvent, immediately before injection of clonidine (0.16 mg/kg i.v.), and at 5, 15, and 30 min after the clonidine challenge. The median pupil diameter over the 5- to 30-min interval after clonidine challenge was used for further evaluation. A pupil diameter <25 units after clonidine (occurrence in 0.8% of the control rats) was adopted as all-or-none criterion for inhibition of clonidine-induced mydriasis.

**Compound 48/80-Induced Lethality**

Compound 48/80 (0.30 mg/kg i.v.)-induced lethality was recorded up to 240 min after injection. Criterion for drug-induced protection was: >240 min survival (in controls: 1.2%; n = 750). Histamine H$_2$ antagonists protect against compound 48/80-induced lethality.

**Conditioned One-Way Active Avoidance Test**

The apparatus consisted of an inner transparent box (length × width × height: 30 × 30 × 30 cm) with an open top surrounded by an outer box. The inner box was equipped with a grid floor made of 15 pairs of iron bars (2-mm diameter; 6-mm interbar distance). Odd and even bars were connected with a source of alternative current [1.0 mA; Coulbourn Instruments (Allentown, PA) solid-state shocker/distributor], which could be interrupted by a switch. The outer box (length × width × height: 40 × 40 × 36 cm) had an open top and was a distance of 5 cm from the inner box at all sides. Only the front wall of the outer box was transparent to allow inspection of the animal during the test. The upper edges of the outer and inner boxes served as targets for the rats on which to jump with fore- and hind-paws, respectively.

Rats were trained to avoid an electric shock during five sessions at 15-min time intervals during a 1-h period: the rat was placed on the nonelectrified grid floor and the grid was electrified 10 s later for not more than 30 s, if the rat did not jump out of the box. Only rats that showed a correct conditioned avoidance response (CAR) in the last three training sessions were included for further experiments and received test compound or solvent immediately after the last training session.

The rats were tested three times, at 60, 90, and 120 min after the injection of test compound or solvent. Latency to avoidance (i.e., responding within the 10-s interval before the grid was electrified) or escape (i.e., responding after the grid had been electrified; cutoff time, 10 s) was recorded. The median avoidance response and the maximum escape response obtained over the three experimental sessions per rat were used. A median avoidance latency ≥8 s occurred in only 1.8% of solvent-pretreated control rats (n > 400) and was selected as an all-or-none criterion for drug-induced inhibition of avoidance. A maximum escape response ≥10 s over the three trials never occurred in these control rats and was adopted as an all-or-none criterion for inhibition of escape behavior.

**Histamine-Induced Lethality in Guinea Pigs**

Histamine (1.25 mg/ml/kg i.v.)-induced lethality was recorded in guinea pigs up to 120 min after the histamine challenge. Criterion for drug-induced protection was: >120 min survival (0.6% false positive controls; n > 300). Histamine H$_2$ antagonists were active in this test.

**Locomotor Activity Assays**

Motor activity was measured in microprocessor-based motor activity cages (length × width × height: 43.5 × 43.5 × 41.5 cm; MED Associates, St. Albans, VT) over 30 min. The distance traveled was measured by light beam interruptions [32 infrared light beams (1.3 cm apart) were located in two arrays perpendicular to each other in a horizontal plane at 2.9 cm above the floor]. Rats were pretreated with test compound or solvent (10 ml/kg s.c.) and placed in individual cages. The rats were challenged with either D-amphetamine (1.25 mg/kg s.c.) 30 min later or phenycyclidine (PCP; 1.25 mg/kg i.v.) 1 h later. Locomotion was measured after 30 min in motor activity cages starting 1 h after test compound administration (i.e., 30 min after D-amphetamine and immediately after PCP challenge). All-or-none criteria for drug-induced inhibition were: total distance <5000 cm for inhibition of D-amphetamine-induced hyperlocomotion (8.4% false positives in >450 solvent-pretreated control rats) and total distance <11,000 cm for inhibition of PCP-induced hyperlocomotion (4.3% false positives in >600 solvent-pretreated control rats).

**Mast Cell Serotonin-Induced Gastric Lesions**

Compound 48/80 (1.0 mg/kg i.v.)-induced gastric lesions were scored 4 h after challenge in rats (175–275 g) that were protected against lethality by injection, 1 h earlier, of the histamine H$_1$ antagonist (5-[4-(diphenylmethyl)piperazin-1-yl]methyl-1-methyl-1H-benzimidazol-2-yl)methanol (R037617) (10 mg/kg s.c.). The scoring system was: 3, red areas covering more than half the glandular tissue; 2, large red areas covering less than half the glandular tissue; 1, at least one distinct red area; 0.5, traces of superficial erosion; and 0, absent. Criterion for drug-induced effects were: score ≤1 for inhibition (7.1% false positives in controls; n = 162) and score <1 for blockade (0.6% false positives in controls). Cyanosis of the ears was scored (0, 0.5, and 1) 5 min after the injection of compound 48/80. Scores <0.5 were adopted as criteria for antagonism of cyanosis (0.0% false positives). Protection from gastric
Rats were treated with test compound or solvent, and 1 h later they were decapitated. Blood was collected in Vacutainer SST tubes (Becton Dickinson, Plymouth, United Kingdom) and centrifuged at 3000 rpm for 10 min. Serum was transferred into secondary tubes (Becton Dickinson, Plymouth, United Kingdom) and centrifuged at 2500 rpm for 10 min. Serum protease inhibitor cocktail and 0.1% SDS were added to every sample. Samples were kept at -80°C until use. The detection limit of the assay was 0.8 ng/ml. The interassay variation was 7.5%.

**Physostigmine-Induced Lethality**

Physostigmine (1.0 mg/kg i.v.)-induced lethality was recorded up to 120 min survival (0% false positives in 200 solvent-pretreated control rats). Immediately before the physostigmine injection, the pupil diameter of the rats was measured with a micrometer. Mydriasis is an expression of peripheral antimuscarinic activity. Mydriasis is an expression of peripheral antimuscarinic activity. Mydriasis was assessed as all-or-none criterion for inhibition of the norepinephrine-induced mydriasis (3.7% false positives in 200 solvent-pretreated control rats).

**Physostigmine-Induced Ptosis**

Evaluations of catalepsy and palpebral opening were based on the following scale: score 5, exophthalmos; 4, wide open; 3, open for three-quarters; 2, half open; 1, open for one-quarter; and 0, closed. The scoring system for catalepsy was: 3, pronounced; 2, moderate; 1, slight; and 0, absent. The scoring system for palpebral opening was: 5, exophthalmos; 4, wide open; 3, open for three-quarters; 2, half open; 1, open for one-quarter; and 0, closed.

**Norepinephrine-Induced Miosis**

Evaluations blockade of peripheral 2-adrenoceptors. Mydriasis is an expression of peripheral antimuscarinic activity. Antimuscarinic activity.

**Norepinephrine-Induced Mydriasis**

Catecholamine, pupil opening (before and after manipulation), and body temperature (°C; using an esophageal thermistor probe) were assessed hourly intervals over 8 h after the administration of test compound or solvent. The scoring system for catalepsy was: 3, pronounced; 2, moderate; 1, slight; and 0, absent. The scoring system for palpebral opening was: 5, exophthalmos; 4, wide open; 3, open for three-quarters; 2, half open; 1, open for one-quarter; and 0, closed.

**Norepinephrine-Induced Lethality**

Survival time after norepinephrine (0.63 mg/kg i.v.) was recorded up to 120 min survival (0% false positives in 200 solvent-pretreated control rats). The detection limit of the assay was 0.8 ng/ml. The interassay variation was 7.5%.

**Medetomidine-Induced Loss of Righting**

Medetomidine (0.10 mg/kg i.v.)-induced loss of righting was recorded. Criteria for drug-induced reversal was duration of caudal trunk on the back: score 6 (not observed in controls). The detection limit of the assay was 0.8 ng/ml. The interassay variation was 7.5%.

**Medetomidine-Induced Loss of Righting**

Medetomidine (0.10 mg/kg i.v.)-induced loss of righting was recorded. Criteria for drug-induced reversal was duration of caudal trunk on the back: score 6 (not observed in controls). The detection limit of the assay was 0.8 ng/ml. The interassay variation was 7.5%.
coefficient of variation was 9.7% at 25 ng/ml and 14% at 192 ng/ml. In solvent-pretreated control rats, the average prolactin level was 3.8 ± 5.7 ng/ml (mean ± S.D.; n = 200), ranging from 0.8 to 35 ng/ml. The following all-or-none criteria for drug-induced effects on prolactin release were adopted: prolactin concentration >20 ng/ml for a slight increase (4.0% false positives) and prolactin concentration >300 ng/ml for a pronounced increase (0.0% false positives).

Tryptamine-Induced Behavior

Tryptamine (25.0 mg/kg i.v.)-induced bilateral clonic seizures of the forepaws and hunched back and palpebral opening were scored the first minute after the injection of tryptamine. The direction of locomotion (backward, sideward, or forward) was also noted. The scoring system for bilateral clonic seizures and hunched back was: 3, pronounced; 2, moderate; 1, slight; and 0, absent. The scoring system for palpebral opening was: 5, exophthalmos; 4, wide open; 3, open for three-quarters; 2, half open; 1, open for one-quarter; and 0, closed. Criteria for drug-induced inhibition or decrease were: bilateral clonic seizures, score <3 for inhibition (1.5% false positives; n = 300), score <2 for blockade (0.0% false positives); palpebral opening, score <4 for decrease (0.0% false positives), score <3 for hunched back (0.0% false positives); and locomotion, sideward or forward direction for reversal of backward locomotion (0.0% false positives). Tryptamine-induced hyperemia or cyanosis of the ears, an expression of serotonin-induced vascular congestion, was evaluated 2 min after the injection of tryptamine. Criterion for reversal of cyanosis was: hyperemia of the ears (red ears; 0.0% false positives).

In Vitro Data Analysis

Data from radioligand competition binding experiments were calculated as the percentage of total binding measured in the absence of test compound. Inhibition curves, plotting the percentage of total binding versus the log concentration of the test compound, were analyzed by using nonlinear regression analysis for one- or two-site curve fitting (Becker and Chambers, 1984). Data from indirect dissociation assays were expressed as a percentage of total [3H]spiperone binding versus the log concentration of the test compound, were calculated as the percentage of total binding measured in the absence of [3H]spiperone.

Determination of ED50 Values

The percentage of receptor occupancy was plotted against dosage, and the sigmoidal log dose-effect curve of best fit was calculated by nonlinear regression analysis, using Prism software (Motulsky, 1999). From these dose-response curves, the ED50 values (the doses producing 50% occupancy) with their 95% confidence limits were calculated. For the other in vivo studies, all-or-none criteria for significant (p < 0.05) effects were defined by analyzing a frequency distribution of a series of historical control data. The fraction of animals responding to these criteria was determined per dose level (n = 5 in the relevant dose range). ED50 values (the doses producing 50% responders to criterion) and corresponding 95% confidence limits were determined according to the modified Spearman-Kaerber estimate, using theoretical probabilities instead of empirical ones (Tsutakawa, 1982). This modification allows the determination of the ED50 and its confidence interval as a function of the slope of the log dose-response curve (Lewi et al., 1977).

Spearman Correlation and Linear Regression Statistics

The inter-relationship between the ED50 values obtained in the two tests was studied by calculating Pearson correlation statistics and performing and graphing linear regression with Prism software (Motulsky, 1999).

Results

Receptor Binding Affinity

JNJ-37822681 and reference antipsychotics were tested in radioligand competition binding experiments to investigate their affinity for various monoaminergic neurotransmitter receptors (Table 2). JNJ-37822681 had a moderate binding affinity for the dopamine D2L receptor (Ki, 158 nM), similar to olanzapine and clozapine. JNJ-37822681 displayed a weak affinity for the human dopamine D3 and serotonin 5-HT2A

![Fig. 2](evaluating-the-dissociation-speed-of-jnj-37822681-and-reference-antipsychotics-from-h-d2l-receptor.png)

5 min incubation with [3H]spiperone for screening

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of total binding after a 5 min incubation with [3H]spiperone</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ37822681</td>
<td>67.0 ± 4.6 (n = 3)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>24.2 ± 2.2 (n = 3)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>59.7 ± 2.5 (n = 3)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>47.9 ± 3.9 (n = 3)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>48.9 ± 1.2 (n = 3)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>50.5 ± 5.8 (n = 2)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>44.1 ± 4.6 (n = 2)</td>
</tr>
</tbody>
</table>

The one-phase exponential decay equations in Prism (GraphPad Software, Inc., San Diego, CA).
A pharmacological study was conducted to evaluate the properties of JNJ-37822681, a novel fast-dissociating D2 antagonist. The study aimed to investigate its dissociation from the human D2L receptor, in vitro and in vivo activities, and selectivity against other receptors.

### In Vitro Assays

#### Direct Dissociation Assay with D2 Receptor

It was observed that the faster a compound dissociates from the D2 receptor after a 1-h incubation period, the faster \([^3H]\)JNJ-37822681 binds to the D2 receptor (Fig. 2). JNJ-37822681 was initially selected after a 5-min incubation with \([^3H]\)spiperone (data not shown), which is the time point used in our dissociation screening assay (Tresadern et al., 2011). To further characterize this property, an association experiment of \([^3H]\)spiperone was performed in the presence of JNJ-37822681 and reference antipsychotics. \([^3H]\)spiperone had a faster association to D2 receptor in the presence of JNJ-37822681 than in the presence of reference antipsychotics, including clozapine (Fig. 3). This indicates that JNJ-37822681 is a fast-dissociating D2 ligand.

#### Indirect Dissociation Assay with D2 Receptor

It is assumed that the faster a compound dissociates from the D2 receptor after a 1-h incubation period, the faster \([^3H]\)spiperone binds to the D2 receptor. JNJ-37822681 was initially selected after a 5-min incubation with \([^3H]\)spiperone (data not shown), which is the time point used in our dissociation screening assay (Tresadern et al., 2011). To further characterize this property, an association experiment of \([^3H]\)spiperone was performed in the presence of JNJ-37822681 and reference antipsychotics. \([^3H]\)spiperone had a faster association to D2 receptor in the presence of JNJ-37822681 than in the presence of reference antipsychotics, including clozapine (Fig. 3). These data indicate that \([^3H]\)JNJ-37822681 is a fast-dissociating D2 antagonist, confirming the indirect dissociation assay data.

### Models Predictive for Antipsychotic Activity in Rats

The antipsychotic-like activity of JNJ-37822681 was evaluated in several established animal models.

#### Antagonism of Apomorphine-Induced Stereotypy

Inhibition of apomorphine-induced stereotypy directly reflects the ability of the compounds to block central D2 receptors and thereby inhibit the D2 agonistic action of apomorphine. Indeed, the ED_{50} values for apomorphine antagonism

### Table 3

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>D_{50} Occupancy In Vivo</th>
<th>Inhibition of Apomorphine-Induced Stereotypy</th>
<th>Blockade of Apomorphine-Induced Hyperlocomotion</th>
<th>Inhibition of Phencyclidine Hyperlocomotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>0.29 (0.31–0.49)</td>
<td>0.19 (0.14–0.26)</td>
<td>8.1 (6.0–11)</td>
<td>4.7 (3.3–6.6)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>0.58 (0.44–0.76)</td>
<td>0.50 (0.43–0.80)</td>
<td>5.4 (4.0–7.3)</td>
<td>3.6 (2.0–6.5)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>8.5 (6.2–12)</td>
<td>16 (12–22)</td>
<td>&gt;160</td>
<td>3.6 (2.4–5.3)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.018 (0.014–0.022)</td>
<td>0.025 (0.023–0.035)</td>
<td>0.26 (0.19–0.35)</td>
<td>0.112 (0.075–0.17)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.15 (0.13–0.17)</td>
<td>0.22 (0.15–0.33)</td>
<td>7.1 (5.2–9.6)</td>
<td>1.0 (0.68–1.5)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>13 (9.6–17)</td>
<td>12 (8.3–18)</td>
<td>&gt;160</td>
<td>4.7 (3.1–7.0)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.11 (0.09–0.14)</td>
<td>0.26 (0.16–0.40)</td>
<td>2.7 (2.0–3.6)</td>
<td>0.51 (0.32–0.83)</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>0.24 (0.18–0.36)</td>
<td>0.28 (0.20–0.38)</td>
<td>4.7 (3.4–6.3)</td>
<td>1.2 (0.78–1.8)</td>
</tr>
</tbody>
</table>

Volatility in the test conditions and errors were obtained from the percentage difference from the species to the species.
were close to the ED50 values for D2 receptor occupancy (Table 3), the ED50 ratio between both tests ranging from 0.52 for JNJ-37822681 to 2.3 for risperidone (median factor 1.3). There was an excellent correlation between both tests, with a slope close to unity for the corresponding linear regressions. The ED50 values for the inhibition of D2 receptor occupancy was quite high (r^2 0.79), the slope of the linear regression line was far from unity (Fig. 4), suggesting that, apart from D2 receptor blockade, other factors may be involved.

**Adverse Effects Related to D2 Receptor Blockade**

**Prolactin Release.** Whereas most compounds started to increase prolactin release from doses slightly below or equal to those required for antagonism of apomorphine-induced behavior, risperidone, quetiapine, and aripiprazole were already active in this respect at 56, 22, and 4.3 times below the ED50 for apomorphine antagonism (Tables 3 and 4; Fig. 6A). Prolactin levels progressively increased with increase of dose (Fig. 7). As a direct consequence, risperidone and quetiapine already induced pronounced prolactin release at doses below the ED50 for apomorphine antagonism (Fig. 6B). At the ED50 for apomorphine antagonism, prolactin levels were approximately 575 ng/ml for risperidone, 400 ng/ml for quetiapine, 250 ng/ml for aripiprazole, 95 ng/ml for ziprasidone, 75 ng/ml for olanzapine, 55 ng/ml for clozapine, 35 ng/ml for haloperidol, and 30 ng/ml for JNJ-37822681 (Fig. 7 Insets). Thus, JNJ-37822681 induces minimal prolactin release at the lowest doses required for central D2 receptor blockade.

**Inhibition of Conditioned Avoidance Response and Escape Behavior.** All compounds dose-dependently inhibited the CAR, and, at slightly higher doses, also the escape response (ESC) (Table 4). The specificity margin between the inhibition of CAR and apomorphine antagonism ranged between 0.19 for clozapine and 28 for aripiprazole (median 5.3; Fig. 6C). The smallest margin was obtained with nonspecific D2 receptor antagonists, such as clozapine and risperidone, whereas the largest specificity was obtained with aripiprazole and JNJ-37822681. Blockade of ESC was also most readily obtained with the nonspecific D2 receptor blockers clozapine and risperidone and least readily with the specific D2 receptor blockers (aripiprazole, JNJ-37822681, and haloperidol; Fig. 6D).

**Catalepsy, Palpebral Ptosis, and Hypothermia.** With the exceptions of clozapine and quetiapine within the dose range tested, all compounds induced catalepsy (Table 4). Relative to antagonism of apomorphine-induced stereotypy, the largest specificity margin was obtained for olanzapine and JNJ-37822681, whereas haloperidol induced catalepsy most readily (Fig. 6E). All compounds also induced palpebral ptosis and hypothermia (Table 4). D2 receptor blockers with associated \( \alpha_1 \)-adrenecoptor blocker activity (clozapine, quetiapine, and risperidone; see below) already induced palpebral ptosis at doses below the ED50 for apomorphine antagonism, whereas specific D2 receptor blockers such as JNJ-37822681 and haloperidol were devoid of effect on palpebral, opening at up to >30-fold higher dose levels (Fig. 6F). A very similar profile was observed for the induction of hypothermia (Fig. 6G).

**Additional Receptor Interactions**

**Serotonin 5HT2A Receptor Antagonism.** Relative to the ED50 for apomorphine antagonism, risperidone and clozapine showed 5-HT2A antagonism at >10-fold lower doses, whereas haloperidol and JNJ-37822681 were devoid of 5-HT2A antagonism up to >100-fold higher doses (Tables 3 and 5; Fig. 8A). The other compounds showed an intermediate profile. Note that all compounds were able to block the tryptamine-induced bilateral...
convulsions. In the absence of the effects related to peripheral 5-HT$_{2A}$ antagonism, however, the inhibition of bilateral convulsions was in these cases probably related to behavioral depressant effects rather than to the blockade of central 5-HT$_{2A}$ receptors. The wide specificity margin between central and peripheral 5-HT$_{2A}$ antagonism observed for risperidone confirms its less optimal brain disposition already evidenced above by the specificity margin between apomorphine antagonism and prolactin release.

**Serotonin 5HT$_{2C}$ Receptor Antagonism.** Only clozapine and olanzapine showed antagonism of both 5HT$_{2C}$ effects, and only with clozapine were these effects observed at doses below the ED$_{50}$ for apomorphine antagonism (Tables 3 and 5; Fig. 8B). Both compounds were the only ones that displayed higher affinity for 5-HT$_{2C}$ than for D$_2$ receptors (Table 2). Haloperidol and ziprasidone antagonized only hunched back behavior. No 5HT$_{2C}$ receptor-related effects could be demonstrated for JNJ-37822681.

**Histamine H$_1$ Receptor Antagonism.** Antihistaminergic activity was generally observed at comparable dose levels in both species (Table 5; Fig. 8C). However, aripiprazole was 10-fold more potent in guinea pigs than in rats. JNJ-37822681 and haloperidol were devoid of histamine H$_1$ antagonistic activity up to 100 times their apomorphine antagonistic dose (Tables 3 and 5; Fig. 8C). Conversely, olanzapine, risperidone and, in particular, quetiapine and clozapine were potent antihistamines. JNJ-37822681 was completely devoid of histamine H$_1$ antagonism up to 40 mg/kg in rats and protected against histamine-induced lethality in guinea pigs at only a very high dose level (28.3 mg/kg).

**α1-Adrenoceptor Antagonism.** Because olanzapine and clozapine have associated antimuscarinic activity (see below) and induce mydriasis per se, the antagonism of norepinephrine-induced mydriasis could be tested only up to the doses having intrinsic mydriatic activity. Risperidone, quetiapine, and clozapine showed antagonistic activity at α$_1$-adrenoceptors at doses slightly below those required for apomorphine antagonism (Tables 3 and 6; Fig. 8D). JNJ-37822681 was completely devoid of α$_1$-adrenoceptor blocking activity up to the highest dose tested.

**α2-Adrenoceptor Antagonism.** Reversal of the antidiarrheal effect of clonidine reflects an interaction with peripheral α$_2$-adrenoceptors, whereas reversal of medetomidine-induced loss of righting and antagonism of clonidine-induced mydriasis are central mediated. Reversal of the antidiarrheal effect of clonidine could be tested only at doses devoid of

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**Fig. 5.** Activity profile in tests related to antipsychotic activity. ED$_{50}$ values for D$_2$ receptor occupancy (A), blockade of apomorphine stereotypy (B), and inhibition of d-amphetamine hyperlocomotion (C) or PCP-induced hyperlocomotion (D) are shown as ratios over the ED$_{50}$ for inhibition of apomorphine-induced stereotypy. ^ above a bar indicates that the ED$_{50}$ is greater than the value indicated by the height of that bar.
TABLE 4
ED50 values (milligram/kilogram, subcutaneously; 95% confidence limits are in parentheses) of JNJ-37822681 and seven reference compounds for slight and pronounced prolactin release, inhibition of avoidance and escape behavior, and induction of catalepsy, palpebral ptosis, and hypothermia.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Conditioned Avoidance, 1–2 h</th>
<th>Inhibition of Escape Catalepsy Palpebral Ptosis Hypothermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>0.17 (0.11–0.25)</td>
<td>3.1 (2.1–4.6)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>0.14 (0.094–0.20)</td>
<td>16 (11–16)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>11 (7.3–16)</td>
<td>3.1 (2.3–3.3)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.030 (0.024–0.038)</td>
<td>0.027 (0.017–0.037)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.15 (0.11–0.21)</td>
<td>0.083 (0.058–0.093)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>0.55 (0.41–0.73)</td>
<td>1.8 (1.2–2.6)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.0046 (0.0036–0.0058)</td>
<td>0.027 (0.017–0.037)</td>
</tr>
</tbody>
</table>

The lowest ED50 obtained over the 8-h period is listed.

Discussion

After selection with an indirect assay, the dissociation rate of JNJ-37822681 from the D2 receptor was directly measured and found to be similar to that of clozapine and faster than that of representative first- and second-generation antipsychotics (e.g., haloperidol and risperidone), indicating that JNJ-37822681 is a fast-dissociating D2 ligand. Because an inverse correlation between dissociation speed and affinity is generally assumed, one might ask whether we simply selected a low-affinity D2 antagonist. However, fast dissociation does not necessarily mean only low affinity; properties other than D2 receptor affinity (e.g., lipophilicity and molecular weight) influence dissociation speed (Tresadern et al., 2011). In fact, JNJ-37822681 had moderate in vitro binding affinity for the human dopamine D2L receptor (Kd 158 nM), similar to olanzapine and clozapine, displayed a weak affinity for dopamine D3 and serotonin 5-HT2A receptors, and did not interact with dopamine D1, adrenergic α1A, serotonin 5-HT2C, and histamine H1 receptors, up to the highest (10 μM) concentration tested. JNJ-37822681 showed a remarkably high D2 selectivity and specificity, especially compared with antipsychotics that display a moderate to weak D2 activity. This finding is consistent with the hypothesis that a D2 antagonist has a relatively similar D2 and D2 receptor affinity.

In vivo, JNJ-37822681 was a relatively potent, centrally active D2 antagonist as measured by the occupancy of central D2 receptors and antagonism of apomorphine-induced stereotypy in rats. JNJ-37822681 was more potent against apomorphine-induced stereotypy than against stimulant-induced hyperlocomotion (similar to haloperidol), whereas the reverse was found for nonspecific D2 antagonists such as clozapine and, to a lesser extent, quetiapine. Across compounds, the two hyperlocomotion models showed less correlation with D2 receptor occupancy than the apomorphine-induced stereotypy model. The effects obtained in the hyperlocomotion models depend on central D2 antagonism, but associated,
nondopaminergic effects (e.g., sedation, cardiovascular complications, and altered energy consumption) undoubtedly affect hyperlocomotion to a larger extent than stereotypy. This suggests that antagonism of dopamine agonist-induced stereotypy is more reliable than antagonism of stimulant-induced hyperlocomotion for evaluating specific blockade of central D2 receptors. Thus, antagonism of apomorphine-induced stereotypy was used as a basis for calculating specificity margins for all effects discussed below (CAR, catalepsy, and in vivo D2 specificity). Our results demonstrate the specific blockade of central D2 receptors by JNJ-37822681 at moderate doses, comparable with those required for most atypical antipsychotics.

Inhibition of CAR closely correlates with clinical antipsychotic potency (Janssen et al., 1965; Wadenberg et al., 2000b). JNJ-37822681 dose-dependently inhibited CAR and ESC, as did all test compounds, in excellent agreement with published data (Wadenberg et al., 1997; Shannon et al., 1999; Millan et al., 2000; Natesan et al., 2006; Olsen et al., 2006). Inhibition of CAR was generally obtained at doses exceeding several times the ED50 for apomorphine antagonism. The nonspecific compounds clozapine and risperidone were relatively more potent than the specific D2 antagonists. Indeed, it has been reported that the inhibition of CAR by central D2 antagonism can be potentiated by 5-HT2A antagonism, α1-adrenoceptor blockade, and 5-HT1A agonism (Wadenberg and Hicks, 1999; Wadenberg et al., 2000a). JNJ-37822681 and aripiprazole showed the widest specificity margin among the tested compounds. Therefore, fast dissociation from the D2 receptor (JNJ-37822681) and functional-selective actions at different D2 receptor signaling pathways for aripiprazole (Urban et al., 2007) may differentiate these compounds from haloperidol. A wide specificity margin between the inhibition of CAR and central D2 receptor occupancy has previously been observed with aripiprazole (Natesan et al., 2006).

Although the inhibition of CAR is considered predictive for antipsychotic activity, inhibition of an appropriate response to danger may be analogous to EPS, which include retarded...
initiation of motor movements. Thus, the large specificity margin between the inhibition of CAR and apomorphine antagonism may be consistent with a high neurological safety margin for JNJ-37822681 and aripiprazole. Relative to apomorphine antagonism, ESC was less readily suppressed with specific D2 antagonists such as JNJ-37822681, aripiprazole, and haloperidol than with nonspecific D2 antagonists (Wadenberg et al., 2000a). The specificity margin obtained with specific D2 antagonists is apparently maximal and independent from the nature of the interaction with the D2 receptor (fast dissociation for JNJ-37822681; functional selectivity for aripiprazole). Nonspecific compounds may cause a more potent inhibition of ESC because of their additional AEs. Therefore, the wide margin obtained with JNJ-37822681 attests to its high specificity as a central D2 antagonist.

The larger specificity margin between the inhibition of CAR and apomorphine antagonism with JNJ-37822681 than with haloperidol suggests that a fast-dissociating D2 antagonist allows the organism to respond more readily to a sudden rise in dopamine levels than a slowly dissociating D2 antagonist, thereby sparing CAR (which is a rapid process) at moderate doses. A large margin was not obtained with the fast-dissociating compounds quetiapine and clozapine, probably because their nonspecific nature counteracts the beneficial effect of their fast dissociation. Fast dissociation from the D2 receptor may also explain the wide margin between the inhibition and blockade of apomorphine-induced behavior observed with JNJ-37822681. Similar and even wider margins were consistently observed with other fast-dissociating D2 antagonists within this drug discovery program. JNJ-37822681 is rapidly displaced from the D2 receptor by the rapidly increasing synaptic apomorphine concentrations after the intravenous injection of apomorphine, but is considerably more potent in counteracting the declining apomorphine concentrations during the final stage of the apomorphine-induced behavior.

In contrast to CAR, ESC is apparently not affected by the rate of dissociation from the D2 receptor. At the high doses...
<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Antagonism of Tryptamine Cyanosis</th>
<th>Antagonism of Compound 48/80 Cyanosis</th>
<th>Antagonism of Compound 48/80 Gastric Lesions</th>
<th>Backward Convulsions</th>
<th>Lethality, Guinea Pigs</th>
<th>Location</th>
<th>Locomotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>40</td>
<td>40</td>
<td>14</td>
<td>11 (6.7–17)</td>
<td>100</td>
<td>28 (20–17)</td>
<td>50 (36–68)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>0.34 (0.25–0.46)</td>
<td>0.38 (0.25–0.46)</td>
<td>0.097 (0.05–0.19)</td>
<td>0.019 (0.012–0.03)</td>
<td>1.14 (0.7–1.8)</td>
<td>2.8 (2.0–1.3)</td>
<td>0.08 (0.05–0.11)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.67 (0.34–0.91)</td>
<td>0.89 (0.34–0.91)</td>
<td>0.971 (0.05–0.19)</td>
<td>0.021 (0.012–0.03)</td>
<td>1.14 (0.7–1.8)</td>
<td>2.8 (2.0–1.3)</td>
<td>0.08 (0.05–0.11)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.0038 (0.0026–0.0056)</td>
<td>0.0041 (0.0027–0.0061)</td>
<td>0.0047 (0.0029–0.0067)</td>
<td>0.0047 (0.0029–0.0067)</td>
<td>1.14 (0.7–1.8)</td>
<td>2.8 (2.0–1.3)</td>
<td>0.08 (0.05–0.11)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>0.15 (0.11–0.20)</td>
<td>0.11 (0.082–0.14)</td>
<td>0.011 (0.0027–0.02)</td>
<td>0.011 (0.0027–0.02)</td>
<td>1.14 (0.7–1.8)</td>
<td>2.8 (2.0–1.3)</td>
<td>0.08 (0.05–0.11)</td>
</tr>
</tbody>
</table>

*Estimated ED50 values (bell-shaped dose-response relation). The evaluation of the color of the ears after quetiapine was highly hampered by a decreased intensity of the color of the ears from an ED50 of 16.2 (11.1–23.8) mg/kg onward, possibly reflecting vasoconstriction.*
various in vivo models looking at interaction with peripheral and central receptors. The ED50 values in the various tests have been expressed as ratios over the ED50 for inhibition of apomorphine-induced stereotypy. A, serotonin 5-HT2A antagonism. B, serotonin 5-HT2C antagonism. C, histamine H1 antagonism. D, α1 adrenoceptor antagonism. E, α2 adrenoceptor antagonism. F, muscarinic receptor antagonism. ** above a bar indicates that the ED50 is greater than the value indicated by the height of that bar. Try_Cya, tryptamine-induced cyanosis; 48/80_Cya, compound 48/80-induced cyanosis; 48/80_GL, compound 48/80-induced gastric lesions; Try_Bil, tryptamine-induced bilaterally convulsions; Try_HB, tryptamine-induced hunched back; Try_Back, tryptamine-induced backward locomotion; 48/80_Let, compound 48/80-induced lethality; His_Let, histamine-induced lethality; Nor_Let, noradrenaline-induced lethal; Nor_Myd, noradrenaline-induced mydriasis; Clo_Dia, clonidine-induced antidiarrheal effect; Clo_Myd, clonidine-induced mydriasis; Med_Lr, medetomidine-induced loss of righting; Phy_Let, physostigmine-induced lethality; Phy_Myd, physostigmine-induced mydriasis.

**TABLE 6**

ED50 values (milligram/kilogram, subcutaneously; 95% confidence limits are in parentheses) of JNJ37822681 and reference antipsychotics in various in vivo models looking at interaction with peripheral and central receptors.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Peripheral α2 Antagonism of Norepinephrine Lethality</th>
<th>Peripheral α2 Antagonism of Tryptamine Exophthalmos</th>
<th>Antagonism of Antidiarrheal Effect of Clonidine</th>
<th>Antagonism of Medetomidine Loss of Righting</th>
<th>Antagonism of Clonidine Lethality</th>
<th>Peripheral Muscarinic Antagonism of Physostigmine Lethality</th>
<th>Central Muscarinic Antagonism of Physostigmine Lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;10*</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>32 (22–49)</td>
<td>19 (12–28)</td>
<td>8.2 (4.8–14)</td>
<td>20*</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Clozapine</td>
<td>4.1 (3.0–5.5)</td>
<td>&gt;2.0*</td>
<td>8.2 (5.1–13)</td>
<td>&gt;1.25*</td>
<td>14 (12–18)</td>
<td>3.1 (2.3–4.2)</td>
<td>3.1 (2.3–4.2)</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>8.1 (5.4–12)</td>
<td>1.35 (0.84–2.2)</td>
<td>2.0 (1.4–3.0)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>7.1 (4.8–11)</td>
<td>&gt;4.7*</td>
<td>9.3 (6.9–13)</td>
<td>&gt;1.25*</td>
<td>&gt;10</td>
<td>1.6 (0.96–2.5)</td>
<td>2.4 (1.6–3.5)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>14 (9.5–21)</td>
<td>4.7 (2.9–7.6)</td>
<td>4.4 (3.3–5.8)</td>
<td>&gt;20*</td>
<td>&gt;160</td>
<td>&gt;160</td>
<td>98 (61–159)</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.19 (0.14–0.26)</td>
<td>0.22 (0.15–0.33)</td>
<td>0.11 (0.085–0.15)</td>
<td>0.51 (0.34–0.76)</td>
<td>25 (16–37)</td>
<td>3.6 (2.9–4.4)</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>9.4 (6.9–13)</td>
<td>2.0 (1.5–2.8)</td>
<td>0.95 (0.52–1.7)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

* Higher doses have intrinsic antidiarrheal activity.
** ED50 for inducing mydriasis per se.
*** Higher doses have intrinsic mydriatic activity.

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**Fig. 8.** Activity profile in tests related to interactions with various types of receptors. The ED50 values in the various tests have been expressed as ratios over the ED50 for inhibition of apomorphine-induced stereotypy. A, serotonin 5-HT2A antagonism. B, serotonin 5-HT2C antagonism. C, histamine H1 antagonism. D, α1 adrenoceptor antagonism. E, α2 adrenoceptor antagonism. F, muscarinic receptor antagonism. ** above a bar indicates that the ED50 is greater than the value indicated by the height of that bar. Try_Cya, tryptamine-induced cyanosis; 48/80_Cya, compound 48/80-induced cyanosis; 48/80_GL, compound 48/80-induced gastric lesions; Try_Bil, tryptamine-induced bilaterally convulsions; Try_HB, tryptamine-induced hunched back; Try_Back, tryptamine-induced backward locomotion; 48/80_Let, compound 48/80-induced lethality; His_Let, histamine-induced lethality; Nor_Let, noradrenaline-induced lethal; Nor_Myd, noradrenaline-induced mydriasis; Clo_Dia, clonidine-induced antidiarrheal effect; Clo_Myd, clonidine-induced mydriasis; Med_Lr, medetomidine-induced loss of righting; Phy_Let, physostigmine-induced lethality; Phy_Myd, physostigmine-induced mydriasis.
antagonism, in contrast to most atypical neuroleptics, and devoid of affinity for D3 receptors. Thus, any atypical properties that might be observed with this compound cannot be attributed to 5HT2A or D3 antagonism. JNJ-37822681 is the first potent, specific, fast-dissociating D2 antagonist characterized, and it shows optimal brain disposition. It achieves an atypical profile without binding to receptors other than dopamine D2. It is relatively devoid of dopaminergic and non-dopaminergic AEs in animal models. Moreover, fast dissociation from the D2 receptor may result in more flexible levels of D2 receptor blockade, allowing D2 receptors to react rapidly to rising dopamine levels in response to environmental stimuli. A recent phase IIb trial of JNJ-37822681 in schizophrenia confirms antipsychotic efficacy and preclinical findings of atypicality (low EPS and prolactin liability) (Schmidt et al., 2012).

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