

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 [α_1 , α_2 , H₁, muscarinic, and 5-hydroxytryptamine (5-HT) type 2C] and for receptors that may interfere with the effects of D₂ antagonism (D₁, D₃, and 5-HT_{2A}). 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-en-

hancing 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 antipsy-

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ABBREVIATIONS: AE, adverse effect; CAR, conditioned avoidance response; EPS, extrapyramidal symptoms; ESC, escape behavior; 5-HT, 5-hydroxytryptamine; hD₂, human D₂; CHO, Chinese hamster ovary; PCP, phencyclidine; BSA, bovine serum albumin; JNJ-37822681, *N*-[1-(3,4-difluorobenzyl)piperidin-4-yl]-6-(trifluoromethyl)pyridazin-3-amine; SCH23390, 7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; R037617, (5-[[4-(diphenylmethyl)piperazin-1-yl]methyl]-1-methyl-1*H*-benzimidazol-2-yl)methanol; R091150, 4-amino-*N*-{1-[3-(4-fluorophenoxy)propyl]-4-methylpiperidin-4-yl}-2-methoxybenzamide.

chotic. Currently available antipsychotics are also well known to cause prolactin release. Hyperprolactinemia can cause a number of AEs (e.g., menstrual disturbances, galactorrhoea, 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 D_2 receptors, in particular with serotonin 5-HT_2 receptors (Meltzer et al., 1989). In contrast, typical antipsychotics, like haloperidol, bind more specifically to D_2 receptors. Although all major atypical antipsychotics fully occupy the serotonin 5-HT_{2A} 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 5HT_{2C} receptors (weight gain), α_1 -adrenoceptors (orthostatic hypotension, reflex tachycardia, and hypnosedation), α_2 -adrenoceptors (tachycardia), histamine H_1 receptors (sedation and weight gain), and muscarinic receptors (blurred vision, dry mouth, constipation, and cognitive impairment).

As an alternative to the “balanced serotonin 5-HT_{2A} -dopamine D_2 ” hypothesis, it has been proposed that the rates at which they dissociate from dopamine D_2 receptors may better distinguish atypical from typical antipsychotics (Kapur and Seeman, 2001). Fast dissociation from the D_2 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 D_2 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 D_2 antagonists. Thus, identifying new drugs based on their rate of dissociation from the D_2 receptor could be a valid strategy for developing atypical antipsychotics with an improved tolerability profile. Therefore, we began screening compounds based on a fast rate of dissociation from D_2 receptors. Results of this screening campaign and the methodology used to evaluate the speed of dissociation in a screening mode have recently been published (Tresadern et al., 2011). An additional goal was to combine fast-dissociating properties with specificity for D_2 receptors to avoid the AEs related to the multiple receptor interactions of current atypical antipsychotics. We also wanted to avoid other interactions (such as 5-HT_{2A} and D_3) that could explain atypicality and exclude D_1 antagonism as an interfering factor in the interpretation of the results. We report here the in vitro and in vivo pharmacological profile of *N*-[1-(3,4-difluorobenzyl)piperidin-4-yl]-6-(trifluoromethyl)pyridazin-3-amine (JNJ-37822681), a novel compound identified from this program. JNJ-37822681 (Fig. 1) was compared with seven pharmacologically and chemically diverse reference antipsychotics: three tricyclics (the azapines clozapine and olanzapine and the thiapine quetiapine), the benzisoxazole risperidone, the benzothiazole ziprasidone, the butyrophenone haloperidol, and the dihydroquinolinone aripiprazole (see Fig. 1 for chemical structures).

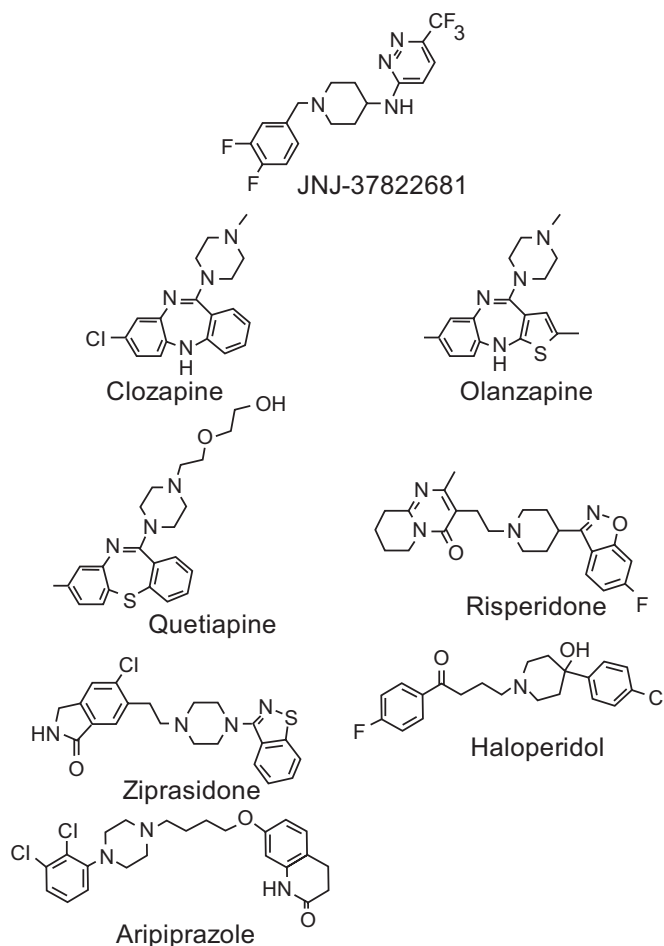


Fig. 1. Structures of JNJ-37822681 and the seven tested reference antipsychotics.

JNJ-37822681 is a specific, centrally active, and fast-dissociating D_2 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, [^{125}I]iodosulpride, and [^3H]mesulergine were from GE Healthcare, Chalfont St. Giles, Buckinghamshire, UK. [^{125}I]-4-amino-*N*-[1-[3-(4-fluorophenoxy)propyl]-4-methylpiperidin-4-yl]-2-methoxybenzamide (R091150) 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 subcutaneously injected in volumes of 10 ml/kg. Solvent was also tested to control for solvent-related effects.

In Vitro Binding Affinity for hD_{2L} 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 IKA Werke T25 Basic (GmbH & Co, Staufen, Germany). The homogenate was centrifuged for 10 min at 23,500g in a Sorvall RC-5C Plus centrifuge (Goffin Meyvis, Overijse, Belgium) (4°C). The cells were then suspended in 5 mM Tris-HCl, pH 7.4, and after recentrifugation for 20 min at 30,000g at 4°C, the pellet was homogenized in 50 mM Tris-HCl, pH 7.4, aliquoted, and stored at -80°C.

Binding assays were carried out under incubation conditions as summarized in Table 1. JNJ-37822681 was also tested at a concentration of 1 μ M by CEREP (Celle L'Evescault, France) for its inhibition of radioligand binding to a battery of other neurotransmitter receptors, peptide receptors, and neurotransmitter transporters.

Dissociation Rate from the hD_{2L} Receptor

Indirect Dissociation. The dissociation rate of compounds was evaluated by using an indirect assay adapted from a previously described method (Leysen and Gommeren, 1984). Membranes were prepared from Chinese hamster ovary (CHO) cells stably expressing the hD_{2L} receptor 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, 2 mM MgCl₂, 1 mM CaCl₂, pH 7.6, 0.1% ascorbic acid, and 10 μ M pargyline.

After incubating hD_{2L} 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 multivibrator. Vacuum was briefly applied to filter compound-membrane mixtures over the GF/C filters. Two hundred microliters of buffer or 200 μ l of 4 μ M butaclamol (blanc; final concentration 2 μ M) together with 200 μ l 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 initi-

ating 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.

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 hD_{2L} 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 μ g (for [³H]JNJ-37822681 and [³H]clozapine) or 10 μ g (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 μ M raclopride (50 μ l) at different times before filtration. Filtration was performed by using a 40-well multivibrator. In a parallel set of tubes, nonspecific binding was determined in the presence of 10 μ M 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 (Sulzfeld, Germany) and housed under standard laboratory conditions (21 \pm 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 μ Ci/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
Assay conditions for radioligand binding

	Receptor Source	Assay Conditions (Incubation Buffer, Time, and Temperature)	Radioligand	Nonspecific Binding
h α_1 -Adrenergic	CHO	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl ₂ , 2 mM CaCl ₂ , 0.1% BSA, pH 7.7, room temperature, 20–24 h*	[³ H]prazosin, 0.5 nM	Aceperone, 1 μ M
hD ₁	GH4C1	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl ₂ , 2 mM CaCl ₂ , 10 μ M pargyline, pH 7.7, 25°C, 60 min	[³ H]SCH23390, 1 nM	Piflutixol, 1 μ M
hD _{2L}	CHO	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl ₂ , 2 mM CaCl ₂ , pH 7.7, 37°C, 30 min	[³ H]spiperone, 0.2 nM	(+)Butaclamol, 1 μ M
hD ₃	CHO	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl ₂ , 2 mM CaCl ₂ , 0.1% BSA, pH 7.7, room temperature, overnight*	[¹²⁵ I]iodosulpride, 0.2 nM	Risperidone, 1 μ M
h5-HT _{2A}	L929	50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl ₂ , 2 mM CaCl ₂ , 0.1% BSA, pH 7.4, 37°C, 60 min	[¹²⁵ I]R091150, 0.1 nM	BW501, 1 μ M
h5-HT _{2C}	Sf9	50 mM Tris-HCl, 4 mM CaCl ₂ , pH 7.7, 37°C, 30 min	[³ H]mesulergine, 1 nM	Ritanserin, 1 μ M
hH ₁	CHO	50 mM Na-K phosphate, 0.05% BSA, pH 7.5, 25°C, 30 min	[³ H]pyrilamine, 2 nM	Astemizole, 1 μ M

* 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.

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 [3 H]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 [3 H]raclopride in striatum of drug-treated animals was expressed as the percentage of the binding potential of [3 H]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 [3 H]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 first 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 ≥ 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 action 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: presence 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_1 antagonists protect against compound 48/80-induced lethality.

Conditioned One-Way Active Avoidance Test

The apparatus consisted of an inner transparent box (length \times width \times height: 30 \times 30 \times 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 \times width \times height: 40 \times 40 \times 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_1 antagonists were active in this test.

Locomotor Activity Assays

Motor activity was measured in microprocessor-based motor activity cages (length \times width \times height: 43.5 \times 43.5 \times 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 2.0 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 phencyclidine (PCP; 1.25 mg/kg i.v.) 1 h later. Locomotion was measured over 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

lesions and reversal of cyanosis was obtained with peripheral serotonin 5HT_{2A} antagonists.

Medetomidine-Induced Loss of Righting

The duration of medetomidine (0.10 mg/kg i.v.)-induced loss of righting was recorded. Criterion for drug-induced reversal was: duration = 0 min (2.4% false positive controls; $n > 500$). Centrally acting α_2 -adrenoceptor antagonists or behavioral stimulants antagonize the loss of righting; sedative compounds may result in prolongation.

Norepinephrine-Induced Lethality

Survival time after norepinephrine (0.63 mg/kg i.v.) was recorded up to 1 h after challenge. Survival times > 60 min were considered to reflect significant norepinephrine antagonism (0% false positives in controls; $n = 175$). Protection against norepinephrine lethality evaluates blockade of peripheral α_1 -adrenoceptors.

Norepinephrine-Induced Mydriasis

The pupil diameter of the right eye was measured (in 1/24 mm units) with a graduated microscope (Gant type 55017) 1, 2, 3, 4, and 5 min after the norepinephrine (0.08 mg/kg i.v.) challenge. A pupil diameter < 25 units at 1 min after norepinephrine challenge was used as all-or-none criterion for inhibition of the norepinephrine-induced mydriasis (3.7% false positives in > 200 solvent-pretreated control rats).

Observation Test

Catalepsy, palpebral opening (before and after manipulation), and body temperature ($^{\circ}\text{C}$; using an esophageal thermistor probe) were assessed at 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. Evaluations of catalepsy and palpebral opening were based on the sum of the scores from two independent observers. Criterion for drug-induced catalepsy was: score 6 (not observed in controls). Criteria for drug-induced palpebral ptosis (assessed after manipulation) were: score < 8 for slight ptosis (in controls: 0.8%) and score < 4 for pronounced ptosis (not observed in controls). Criteria for drug-induced hypothermic effects were: $\geq 1.0^{\circ}\text{C}$ decrease of temperature for the 1-h interval (not observed in controls) and $\geq 2.0^{\circ}\text{C}$ decrease of temperature for other time intervals (0% false positives).

Physostigmine-Induced Lethality

Physostigmine (1.0 mg/kg i.v.)-induced lethality was recorded up to 120 min after challenge. Criterion for drug-induced protection was: > 120 min survival (0.0% false positives in > 200 solvent-pretreated control rats). Immediately before the physostigmine injection, the pupil diameter of the rats was measured with a microscopic micrometer (1 unit = 1/24 mm). Criteria for drug-induced effects were: pupil diameter > 25 units for mydriasis (in controls: 2.4%) and < 10 units for miosis (in controls: 0.5%). Protection against physostigmine-induced lethality was observed with centrally acting antimuscarinics. Mydriasis is an expression of peripheral antimuscarinic activity.

Prolactin Release

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 and subsequently frozen. Samples were kept at $< -18^{\circ}\text{C}$ until analysis. Serum prolactin was measured with a commercially available radioimmunoassay (Rat Prolactin [¹²⁵I] Assay System; GE Healthcare). The detection limit of the assay was 0.8 ng/ml. The interassay

TABLE 2

In vitro binding affinities (mean K_i values with indication of 95% confidence limits in parentheses) of JNJ-37822681 and reference antipsychotics for various neurotransmitter receptors

Receptor	JNJ-37822681		Aripiprazole		Haloperidol		Ziprasidone		Risperidone		Olanzapine		Clozapine		Quetiapine	
	K_i	n	K_i	n	K_i	n	K_i	n	K_i	n	K_i	n	K_i	n	K_i	n
D _{2L}	158 (132–190)	5	0.49 (0.2–1.2)	7	1.8 (1.3–2.3)	10	4.1 (2.8–5.9)	7	5.5 (5.1–5.9)	133	57 (35–93)	9	137 (112–168)	9	554 (419–733)	6
hD ₁	> 3000	1	653	1	65	1	41	1	147 (117–186)	12	16	1	140	1	803	1
hD ₃	1159	1	4.5	1	5.1 (3.3–8)	6	23 (18–29)	3	17 (15–19)	30	55 (36–84)	6	231 (176–304)	4	844 (634–1123)	10
h α_1A	> 5000	3	211 (76–582)	2	122 (41–363)	4	32 (15–66)	3	4.8 (2.6–9.5)	8	189 (100–356)	5	28 (19–41)	3	200 (172–234)	4
hH ₁	4931	1	5.08 (3.5–7.4)	3	> 5000	2	72 (45–116)	2	67 (61–74)	13	3.3 (2.8–4.0)	2	1.5 (1.2–2)	3	7.4 (6.9–7.9)	2
h5-HT _{2A}	2896 (2064–4062)	2	16 (15–17)	2	31.1 (159–609)	6	1.9 (0.9–4.1)	6	0.66 (0.53–0.82)	26	3.8 (2.6–5.7)	12	8.5 (4.9–13)	8	318 (161–627)	7
h5-HT _{2C}	> 4000	2	14 (11–18)	2	< 4000	1	7.4 (5.7–10)	3	1.3 (1.2–1.4)	30	12 (9.2–15)	4	12 (8.6–15)	5	2197 (1422–3395)	3

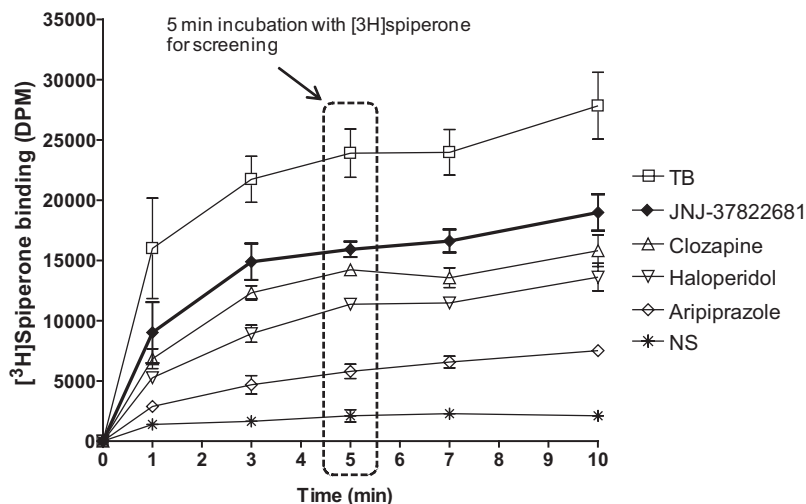
coefficient of variation was 9.7% at 25 ng/ml and 14% at 192 ng/ml. In solvent-pretreated control rats, the average prolactin level was 3.8 ± 5.7 ng/ml (mean \pm S.D.; $n = 200$), ranging from 0.8 to 35 ng/ml. The following all-or-none criteria for drug-induced effects on prolactin release were adopted: 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 [3 H]spiperone binding. Dissociation rates of [3 H]JNJ-37822681 and [3 H]reference compounds were calculated from dissociation curves by using



	% of total binding after a 5 min incubation with [3 H]spiperone
JNJ37822681	67.0 ± 4.6 ($n = 3$)
Aripiprazole	24.2 ± 2.2 ($n = 3$)
Clozapine	59.7 ± 2.5 ($n = 3$)
Haloperidol	47.9 ± 3.9 ($n = 3$)
Olanzapine	48.9 ± 1.2 ($n = 3$)
Quetiapine	50.5 ± 5.8 ($n = 2$)
Risperidone	44.1 ± 4.6 ($n = 2$)

the one-phase exponential decay equations in Prism (GraphPad Software, Inc., San Diego, CA).

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 ($n \geq 5$ in the relevant doses range). ED₅₀ values (the doses producing 50% responders to criterion) and corresponding 95% confidence limits were determined according to the modified Spearman-Kärber 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 D_{2L} receptor (K_i 158 nM), similar to olanzapine and clozapine. JNJ-37822681 displayed a weak affinity for the human dopamine D₃ and serotonin 5-HT_{2A}

Fig. 2. Evaluation of the dissociation speed of JNJ-37822681 and reference antipsychotics from hD_{2L} receptor. A, the level of [3 H]spiperone binding to membranes from CHO cells stably expressing the hD_{2L} receptor was measured in the absence (TB) or presence of drugs (JNJ-37822681, aripiprazole, clozapine, haloperidol, olanzapine, quetiapine, and risperidone, incubated at a concentration equal to four times their IC₅₀). NS, nonspecific binding. Graph represents averaged data of three independent experiments for each compound. Data for olanzapine, risperidone, and quetiapine were omitted for clarity. B, the percentage of total binding after incubation with [3 H]spiperone for every drug for 5 min, which is the time point used in the dissociation screening assay (Tresadern et al., 2011). Higher numbers indicate faster drug dissociation from D₂ receptors.

weak affinity for the D₂ receptor, such as olanzapine, clozapine, and quetiapine.

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. 2). This indicates that JNJ-37822681 is a fast-dissociating D₂ ligand.

Direct Dissociation Assay with D₂ Receptor using [³H]JNJ-37822681

[³H]JNJ-37822681 dissociated faster than [³H]haloperidol, [³H]risperidone, and [³H]paliperidone, and its dissociation rate was similar to that of [³H]clozapine (Fig. 3). These data indicate that [³H]JNJ-37822681 is a fast-dissociating D₂ antagonist, confirming the indirect dissociation assay data.

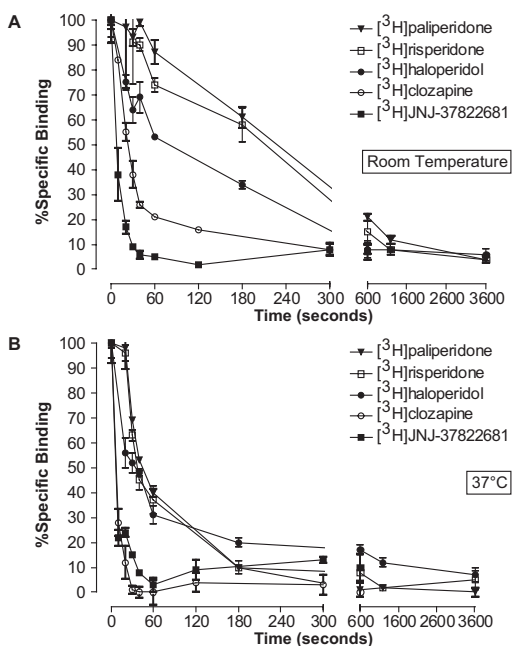
D₂ Receptor Occupancy

The ED₅₀ for JNJ-37822681 (ED₅₀ 0.39 mg/kg) was similar to those obtained for aripiprazole, olanzapine, risperidone, and ziprasidone, approximately 20 times greater than that obtained for haloperidol and 20 to 35 times smaller than those of clozapine and quetiapine (Table 3). The order of potencies in this occupancy assay differs from that in the affinity assay, presumably because of differences in central nervous system penetration and time of peak effect. Considering the moderate in vitro affinity of JNJ-37822681, the relative high potency for occupying D₂ receptor indicates its high brain disposition.

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 ED₅₀ values for apomorphine antagonism



Radioligand	RT		37°C	
	$t_{1/2}$ (s)	k_{off} (s ⁻¹)	$t_{1/2}$ (s)	k_{off} (s ⁻¹)
[³ H]JNJ-37822681	6.5 ± 1.1 (n=3)	0.108 ± 0.016 (n=3)	3.7 ± 0.68 (n=3)	0.191 ± 0.038 (n=3)
[³ H]clozapine	14.5 ± 4.4 (n=3)	0.051 ± 0.016 (n=3)	5.8 ± 1.4 (n=4)	0.126 ± 0.037 (n=4)
[³ H]haloperidol	72.4 ± 5.8 (n=2)	0.01 ± 0.001 (n=2)	24.5 ± 1.72 (n=3)	0.028 ± 0.002 (n=3)
[³ H]risperidone	198 ± 20 (n=2)	0.004 ± 0 (n=2)	49.2 ± 14 (n=2)	0.015 ± 0.004 (n=2)
[³ H]paliperidone	239 ± 11 (n=2)	0.003 ± 0 (n=2)	46.5 ± 0.56 (n=2)	0.015 ± 0 (n=2)

Fig. 3. Dissociation of [³H]JNJ-37822681 and ³H-reference antipsychotics from hD_{2L} receptor. Drugs were incubated for 1 h with membranes from CHO cells stably expressing the hD_{2L} receptor. Then, an excess of raclopride was added, followed by rapid filtration at the time indicated for each data point. Values are mean ± S.D. of duplicate determinations and are from one representative experiment. A, room temperature. B, 37°C. C, dissociation from the human D_{2L} receptor: half-life ($t_{1/2}$) and off rate (k_{off}) for [³H]JNJ-37822681 and [³H]-labeled reference antipsychotics. RT, room temperature. Data are mean ± S.D. (number of experiments indicated; each data point was performed in duplicate).

receptors (Table 2) and did not interact with the human receptors dopamine D₁, adrenergic α_{1A} , serotonin 5-HT_{2C}, and histamine H₁, up to the highest (10 μ M) concentration tested. Further profiling at CEREP did not reveal any additional interactions except a high affinity to σ_1 receptors (K_i 8.9 nM) (see Supplemental Data). Overall, JNJ-37822681 shows a high D₂ specificity, especially compared with the second-generation antipsychotics that display a moderate to

TABLE 3

ED₅₀ values (milligram/kilogram, subcutaneously; 95% confidence limits are in parentheses) of JNJ-37822681 and seven reference compounds for D₂ receptor occupancy and inhibition of stimulant-induced behavior 1 h after subcutaneous administration

Test Compound	ED ₅₀				
	D ₂ Occupancy In Vivo	Inhibition of Apomorphine-Induced Stereotypy	Blockade of Apomorphine-Induced Stereotypy	Inhibition of <i>n</i> -Amphetamine Hyperlocomotion	Inhibition of Phencyclidine Hyperlocomotion
JNJ-37822681	0.39 (0.31–0.49)	0.19 (0.14–0.26)	8.1 (6.0–11)	1.0 (0.68–1.5)	4.7 (3.3–6.6)
Aripiprazole	0.58 (0.44–0.76)	0.59 (0.43–0.80)	5.4 (4.0–7.3)	0.26 (0.16–0.41)	3.6 (2.0–6.5)
Clozapine	8.5 (6.2–12)	16 (12–22)	>160	2.0 (1.5–2.8)	3.6 (2.4–5.3)
Haloperidol	0.018 (0.014–0.022)	0.028 (0.023–0.035)	0.26 (0.19–0.35)	0.064 (0.048–0.087)	0.112 (0.075–0.17)
Olanzapine	0.15 (0.13–0.17)	0.22 (0.15–0.33)	7.1 (5.2–9.6)	1.0 (0.75–1.4)	1.0 (0.68–1.5)
Quetiapine	13 (9.6–17)	12 (8.3–18)	>160	4.7 (3.1–7.0)	14 (12–18)
Risperidone	0.11 (0.09–0.14)	0.26 (0.16–0.40)	2.7 (2.0–3.6)	0.44 (0.36–0.55)	0.51 (0.32–0.83)
Ziprasidone	0.24 (0.18–0.36)	0.28 (0.20–0.38)	4.7 (3.4–6.3)	0.29 (0.19–0.35)	1.2 (0.78–1.8)

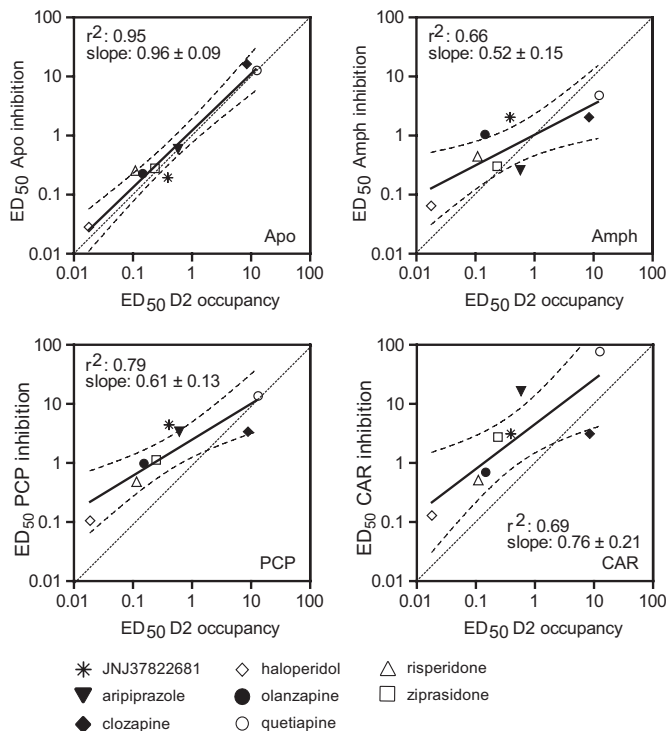


Fig. 4. Linear relationship between the ED_{50} for D_2 occupancy and various in vivo tests. Pearson correlation statistics were calculated for the inter-relationship between the ED_{50} values obtained in the two tests, and linear regressions were graphed by using Prism software. Apo, apomorphine; Amph, amphetamine.

were close to the ED_{50} values for D_2 receptor occupancy (Table 3), the ED_{50} 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 regression line (Fig. 4). It is noteworthy that the fast dissociating D_2 antagonist JNJ-37822681 is the only compound that achieved apomorphine antagonism at doses below 50% occupancy of the D_2 receptor, whereas 50% or above is required for the reference antipsychotics (Fig. 5A). It should also be mentioned that all compounds selected in this drug discovery program and further profiled all share this property (results not shown). Complete blockade of the apomorphine-induced stereotypy was less readily obtained with JNJ-37822681 than with the other compounds (Fig. 5B).

Antagonism of D-Amphetamine-Induced Hyperlocomotion. The ED_{50} values for the inhibition of D-amphetamine-induced hyperlocomotion deviated somewhat from those obtained for apomorphine antagonism (Table 3; Fig. 5C). The specificity margin ranged from 0.12 for clozapine to 5.3 for JNJ-37822681, although the median was close to 1.0 (1.4). The correlation with D_2 receptor occupancy was less than in the case of apomorphine stereotypy, and the slope of the linear regression line was far from unity (Fig. 4), suggesting that, apart from D_2 receptor blockade, other factors may be involved.

Antagonism of Phencyclidine-Induced Hyperlocomotion. The ED_{50} values for inhibition of PCP-induced hyperlocomotion deviated from those obtained for apomorphine antagonism (Table 3). The specificity margin varied highly from 0.22 for clozapine to 24 for JNJ-37822681

(median factor 4.1). Although the correlation with D_2 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 D_2 receptor blockade, other factors may be involved.

Adverse Effects Related to D_2 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 ED_{50} 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 ED_{50} for apomorphine antagonism (Fig. 6B). At the ED_{50} 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 D_2 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 D_2 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 D_2 receptor blockers clozapine and risperidone and least readily with the specific D_2 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). D_2 receptor blockers with associated α_1 -adrenoreceptor blocker activity (clozapine, quetiapine, and risperidone; see below) already induced palpebral ptosis at doses below the ED_{50} for apomorphine antagonism, whereas specific D_2 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 5HT_{2A} Receptor Antagonism. Relative to the ED_{50} for apomorphine antagonism, risperidone and clozapine showed 5-HT_{2A} antagonism at >10-fold lower doses, whereas haloperidol and JNJ-37822681 were devoid of 5-HT_{2A} 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

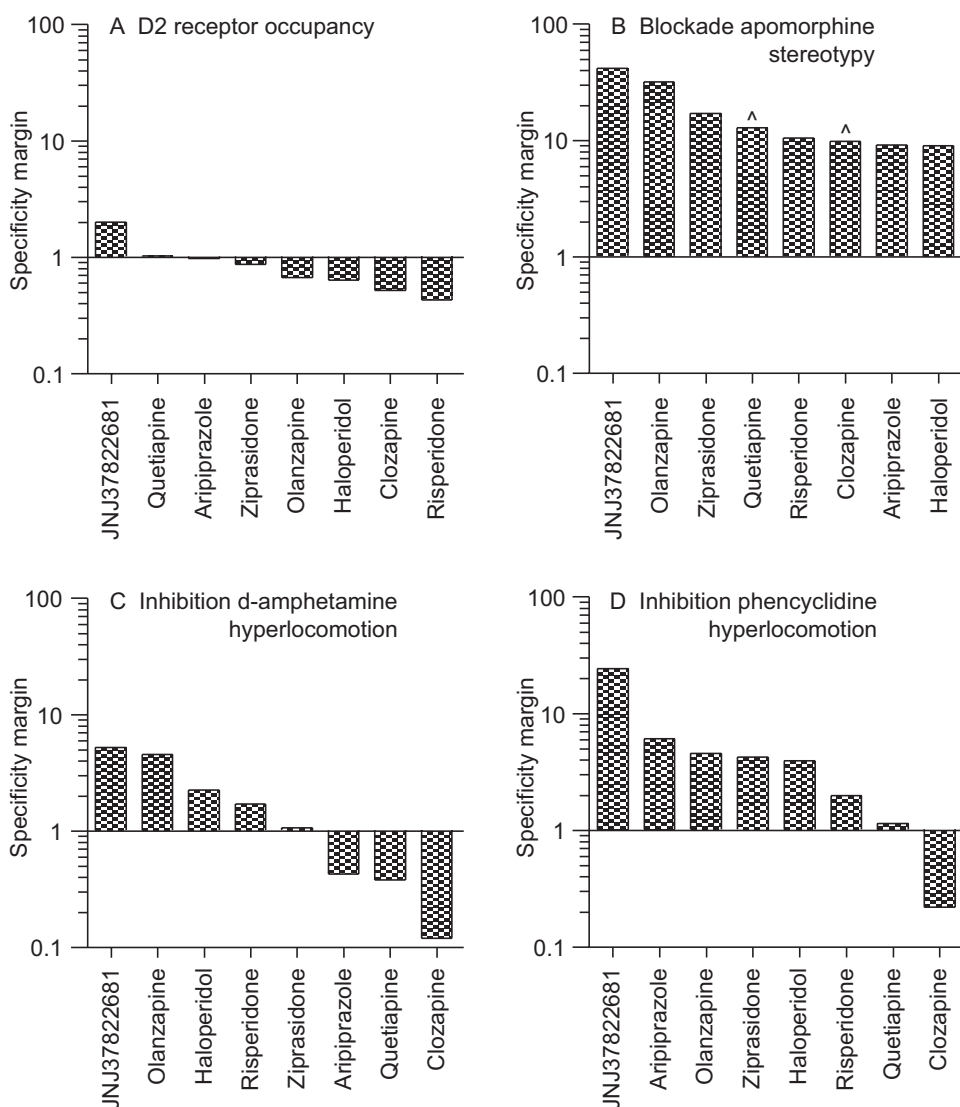


Fig. 5. Activity profile in tests related to antipsychotic activity. ED₅₀ values for D₂ receptor occupancy (A), blockade of apomorphine stereotypy (B), and inhibition of d-amphetamine (C) or PCP-induced hyperlocomotion (D) are shown as ratios over the ED₅₀ for inhibition of apomorphine-induced stereotypy. ^ above a bar indicates that the ED₅₀ is greater than the value indicated by the height of that bar.

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 ED₅₀ 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 D₂ receptors (Table 2). Haloperidol and ziprasidone antagonized only hunched back behavior. No 5HT_{2C} receptor-related effects could be demonstrated for JNJ-37822681.

Histamine H₁ 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 H₁ an-

tagonistic 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 H₁ 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 α₁-adrenoceptors at doses slightly below those required for apomorphine antagonism (Tables 3 and 6; Fig. 8D). JNJ-37822681 was completely devoid of α₁-adrenoceptor blocking activity up to the highest dose tested.

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

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 hypothemia

Test Compound	Prolactin Release, 1 h		Conditioned Avoidance, 1–2 h		Observation Test, 1–8 h ^a		
	Slight, >20 ng/ml	Pronounced, >300 ng/ml	Inhibition of Avoidance	Blockade of Escape	Catalepsy	Palpebral Ptosis	Hypothemia
JNJ-37822681	0.17 (0.11–0.25)	0.25 (0.82–1.9)	3.1 (2.1–4.6)	14 (12–17)	8.2 (5.5–12)	16 (10–26)	16 (11–24)
Aripiprazole	0.14 (0.094–0.20)	1.9 (1.1–3.4)	16 (11–24)	43 (29–64)	7.1 (5.8–8.8)	4.7 (3.1–7.0)	11 (6.7–17)
Clozapine	11 (7.3–16)	≥ 160	3.1 (1.9–5.0)	5.4 (3.6–8.0)	>320	1.2 (0.86–1.6)	1.2 (0.73–1.9)
Haloperidol	0.030 (0.024–0.038)	0.127 (0.091–0.18)	0.13 (0.075–0.22)	2.0 (1.3–3.3)	0.34 (0.30–2.0)	1.0 (0.68–1.5)	4.1 (3.0–5.5)
Olanzapine	0.15 (0.11–0.21)	1.8 (1.3–2.5)	0.68 (0.39–1.2)	3.1 (2.3–4.2)	19 (12–28)	1.6 (1.0–2.3)	2.4 (1.5–3.8)
Quetiapine	0.55 (0.41–0.73)	6.6 (5.0–8.8)	75 (44–130)	394 ^b	>160	1.8 (1.2–2.6)	5.4 (3.3–8.7)
Risperidone	0.0046 (0.0036–0.0058)	0.028 (0.018–0.043)	0.51 (0.32–0.82)	1.8 (1.2–2.6)	8.1 (6.0–13)	0.065 (0.043–0.097)	0.15 (0.081–0.27)
Ziprasidone	0.14 (0.10–0.18)	0.78 (0.56–1.1)	2.7 (2.0–3.6)	7.1 (4.7–11)	6.2 (4.1–9.2)	2.0 (1.5–2.8)	3.6 (2.4–5.3)

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

^b Maximum 40% responders at 320 mg/kg. The ED₅₀ has been estimated by assuming that 100% responders would have been obtained if a higher dose of 640 mg/kg would have been tested.

intrinsic constipating effects. Likewise, the antagonism of clonidine-induced mydriasis could be evaluated for olanzapine and clozapine only up to the doses that induce mydriasis per se (both compounds have associated antimuscarinic activity; see below).

Risperidone was the only compound showing all of the effects of an α_2 -adrenoceptor blocker. Interaction with peripheral α_2 -adrenoceptors was observed from doses only slightly above the ED₅₀ for apomorphine antagonism (Tables 3 and 6; Fig. 8E). To obtain central α_2 -adrenoceptor blocking activity, 10-fold higher doses were required, consistent with the poor central nervous system disposition of this compound. For clozapine, reversal of medetomidine-induced loss of righting occurred at doses close to that required for apomorphine antagonism. The intrinsic constipating and mydriatic activity of this antimuscarinic compound hampered evaluation of the other two effects. α_2 -Adrenoceptor blocking activity was not detected for any other compound.

Muscarinic Receptor Antagonism. Olanzapine and clozapine were the only compounds showing both pharmacological characteristics of an antimuscarinic, and clozapine was the only compound doing so at doses below the ED₅₀ for apomorphine antagonism (Tables 3 and 6; Fig. 8F). Quetiapine protected against physostigmine-induced lethality but did not induce mydriatic effects.

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 D_{2L} receptor (K_i 158 nM), similar to olanzapine and clozapine, displayed a weak affinity for dopamine D₃ and serotonin 5-HT_{2A} receptors, and did not interact with dopamine D₁, adrenergic α_{1A} , serotonin 5-HT_{2C}, and histamine H₁ receptors, up to the highest (10 μ M) concentration tested. JNJ-37822681 showed a remarkably high D₂ selectivity and specificity, especially compared with antipsychotics that display a moderate to weak D₂ affinity (e.g., olanzapine, clozapine, and quetiapine).

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,

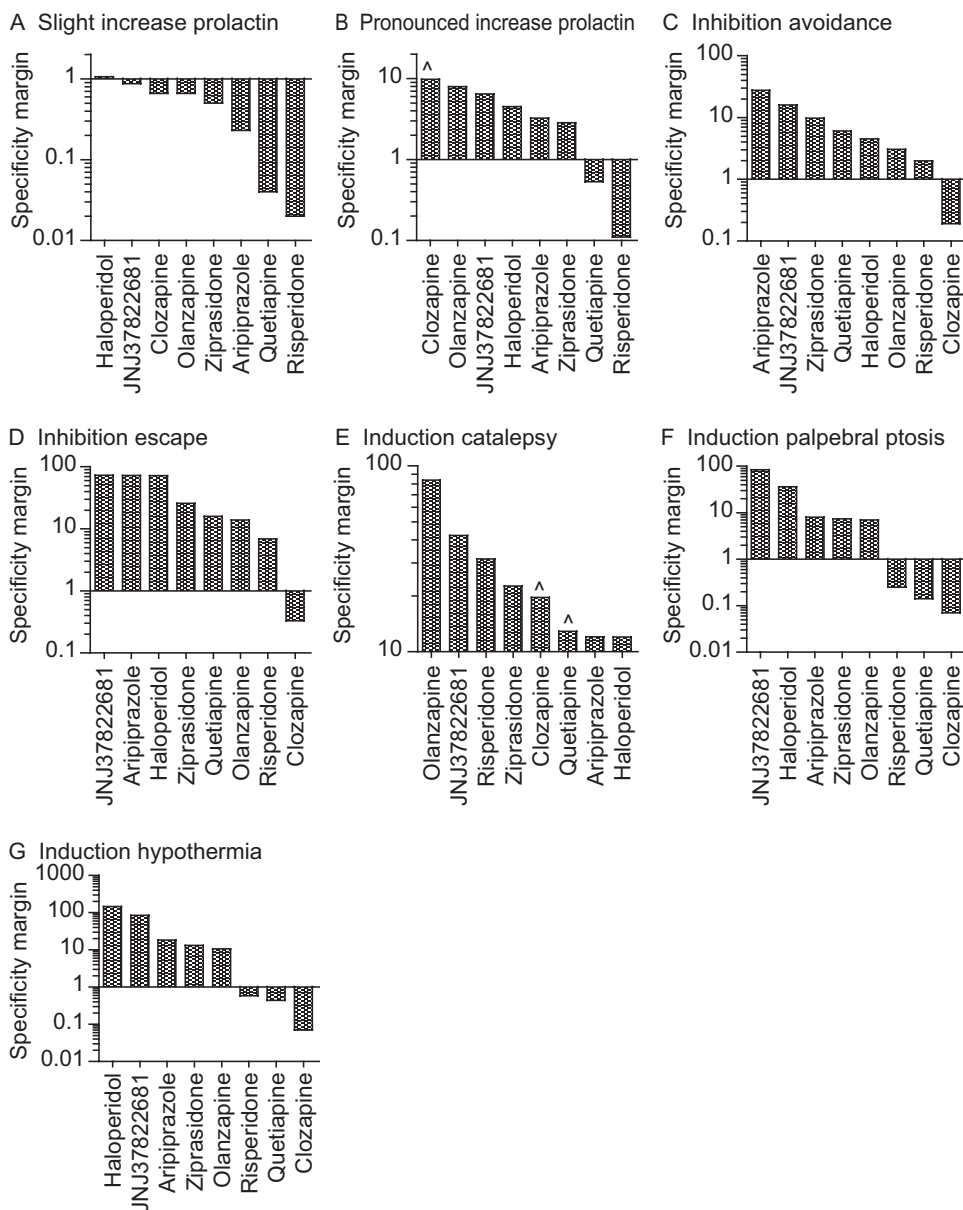


Fig. 6. Activity profile in tests related to D₂ receptor-related side effects. ED₅₀ values for slight and pronounced prolactin release (A and B, respectively), inhibition of avoidance and escape behavior (C and D, respectively), and induction of catalepsy (E), palpebral ptosis (F), and hypothermia (G) are shown as ratios over the ED₅₀ for inhibition of apomorphine-induced stereotypy. ^ above a bar indicates that the ED₅₀ is greater than the value indicated by the height of that bar.

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-HT_{2A} antagonism, α_1 -adrenoceptor blockade, and 5-HT_{1A} 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

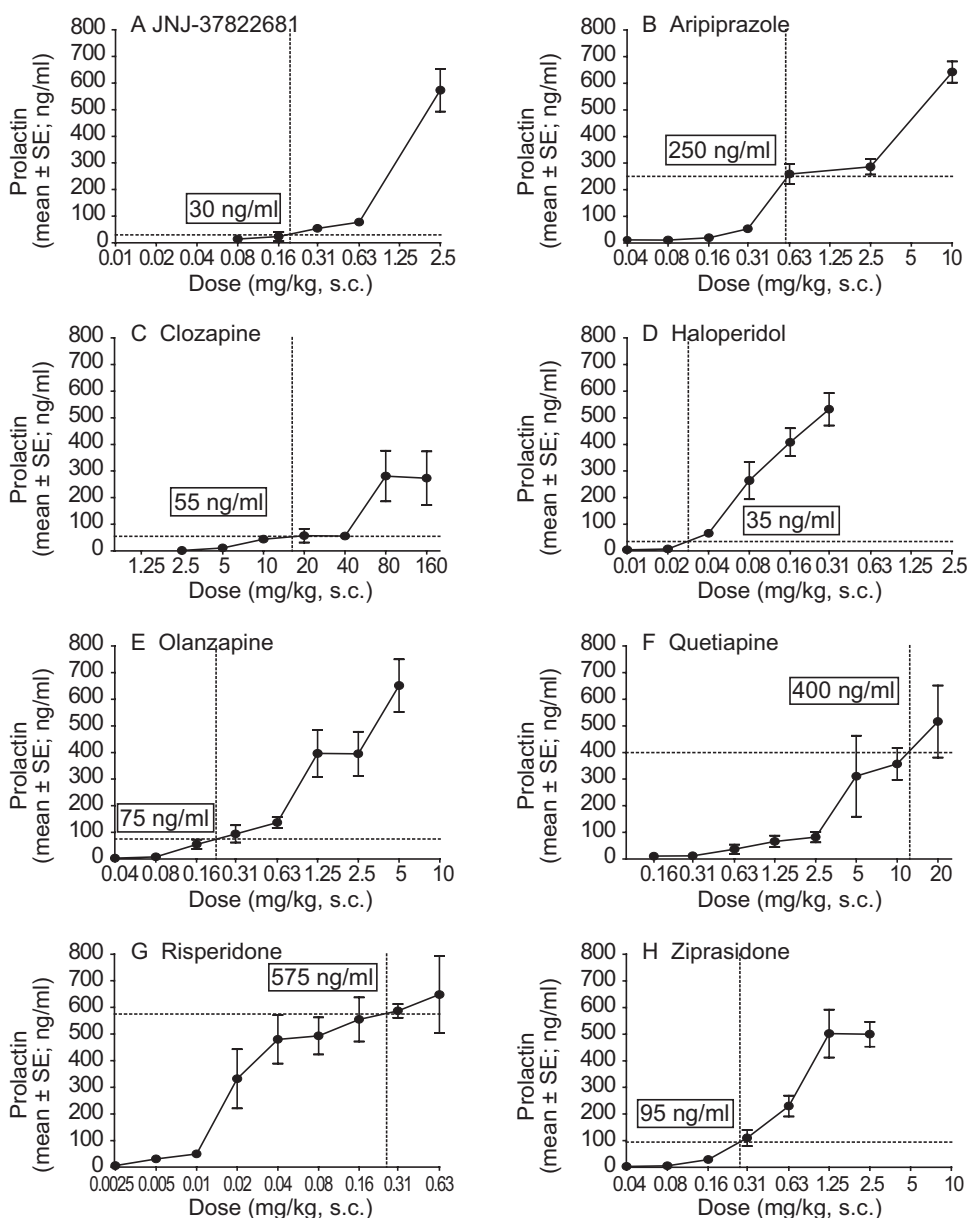


Fig. 7. Dose-dependent prolactin release measured 1 h after subcutaneous injection of the test compounds. The dotted horizontal lines indicate the averaged prolactin concentration (boxed values) at the ED₅₀ for apomorphine antagonism (dotted vertical lines). A, JNJ-37822681. B, aripiprazole. C, clozapine. D, haloperidol. E, olanzapine. F, quetiapine. G, risperidone. H, ziprasidone.

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 sud-

den 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 5

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

Test Compound	ED ₅₀					
	Peripheral 5-HT _{2A}		Central 5-HT _{2A}		Peripheral H ₁	
	Antagonism of Tryptamine Cyanosis	Antagonism of Compound 48/80 Cyanosis	Inhibition of Compound 48/80 Gastric Lesions	Blockade of Tryptamine Bilateral Convulsions	Antagonism of Tryptamine Hunched Back	Reversal of Tryptamine Backward Locomotion
JNJ-37822681	>40	>40	14	11 (6.7–17)	>40	>40
Aripiprazole	>40	>40	≥40	8.2 (6.0–11)	>40	>40
Clozapine	0.34 (0.25–0.46)	0.67 (0.50–0.91)	0.89 (0.59–1.33)	0.34 (0.25–0.46)	2.4 (1.4–4.0)	9.4 (6.9–13)
Haloperidol	≥10	>10	>10	0.89 (0.59–1.3)	5.4 (3.6–8.0)	0.032 (0.022–0.048)
Olanzapine	0.037 (0.028–0.050)	0.085 (0.057–0.13)	0.097 (0.054–0.18)	0.13 (0.071–0.23)	1.3 (0.90–2.0)	0.043 (0.030–0.060)
Quetiapine	32 ^a	22 (14–32)	2.4 (1.4–3.8)	26 (18–39)	>160	0.15 (0.098–0.22)
Risperidone	0.0038 (0.0026–0.0056)	0.0041 (0.0027–0.0061)	0.0047 (0.0029–0.0076)	0.028 (0.019–0.042)	>10	0.0082 (0.0051–0.013)
Ziprasidone	0.15 (0.11–0.20)	0.11 (0.092–0.14)	0.064 (0.043–0.097)	0.098 (0.073–0.13)	5.0 (3.2–7.7)	0.38 (0.25–0.59)
						0.51 (0.38–0.69)
						0.17 (0.12–0.23)
						0.13 (0.095–0.17)
						>5.0

^a Estimated ED₅₀ value (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.

and corresponding high synaptic concentrations required for the suppression of ESC, antagonist molecules that dissociate from the receptor are probably immediately replaced by other antagonist molecules rather than by dopamine.

JNJ-37822681 showed a high margin between the induction of catalepsy and apomorphine antagonism, exceeding that obtained for haloperidol and at least as high as that measured for atypical antipsychotics (although exact values could not be determined for clozapine and quetiapine). Because catalepsy in rats is generally considered to be predictive of EPS in humans, this wide margin supports the hypothesis that fast dissociation from the D₂ receptor decreases EPS liability and can make specific D₂ antagonists behave similarly to multireceptor atypical antipsychotics.

Most dopamine antagonists induce palpebral ptosis in the rat, which generally relates well to α₁-adrenergic blocking activity but poorly to apomorphine antagonism (Janssen et al., 1965; Niemegeers, 1974). However, butyrophenone dopamine antagonists (e.g., haloperidol) are virtually devoid of α₁-adrenoceptor blocking activity and nevertheless induce palpebral ptosis because of their sedative liability. JNJ-37822681 is completely devoid of α₁-adrenergic blocking activity and shows an even wider specificity margin between the induction of palpebral ptosis and apomorphine antagonism than haloperidol, which may relate to the fast dissociation of JNJ-37822681 from the D₂ receptor. The same may apply for the exceptionally high margin obtained for the induction of hypothermia.

JNJ-37822681, clozapine, olanzapine, haloperidol, and ziprasidone induced slight prolactin release from doses slightly below or equal to those required for central D₂ antagonism and induced pronounced prolactin release only at approximately 10-fold higher doses. In contrast, aripiprazole, quetiapine and, in particular, risperidone induced slight prolactin release at doses much below those required for central D₂ antagonism and pronounced prolactin release at the dose required for central D₂ antagonism. The apparent preferential action of these compounds at peripheral over central D₂ receptors is consistent with results from ex vivo occupancy studies (Kapur et al., 2002). The aripiprazole-induced prolactin release measured in the present study is at variance with published data showing a smaller increase or even a decrease in prolactin levels in rats (Inoue et al., 1996, 1998). Although a D₂ agonistic component of aripiprazole would be expected to reduce the prolactin release originating from its D₂ receptor blockade (Marchese et al., 2002), we could not establish this. Fast dissociation from the D₂ receptor has been proposed to explain the low prolactin release observed with certain antipsychotics, like clozapine and quetiapine (Kapur and Seeman, 2001). However, the present data instead support the hypothesis that improved brain disposition explains the lower incidence of prolactin release by these compounds.

Because of the moderate D₂ affinity of JNJ-37822681, in vitro binding affinity assays may be less appropriate for demonstrating the compound's high specificity. However, in vivo tests showed that over a wide dose range JNJ-37822681 is devoid of relevant interactions with receptors such as 5HT_{2C}, α₁-adrenoceptors, α₂-adrenoceptors, histamine H₁, and muscarinic receptors that are known or suspected to cause the major AEs associated with available antipsychotics. Finally, JNJ-37822681 is also devoid of relevant 5HT_{2A}

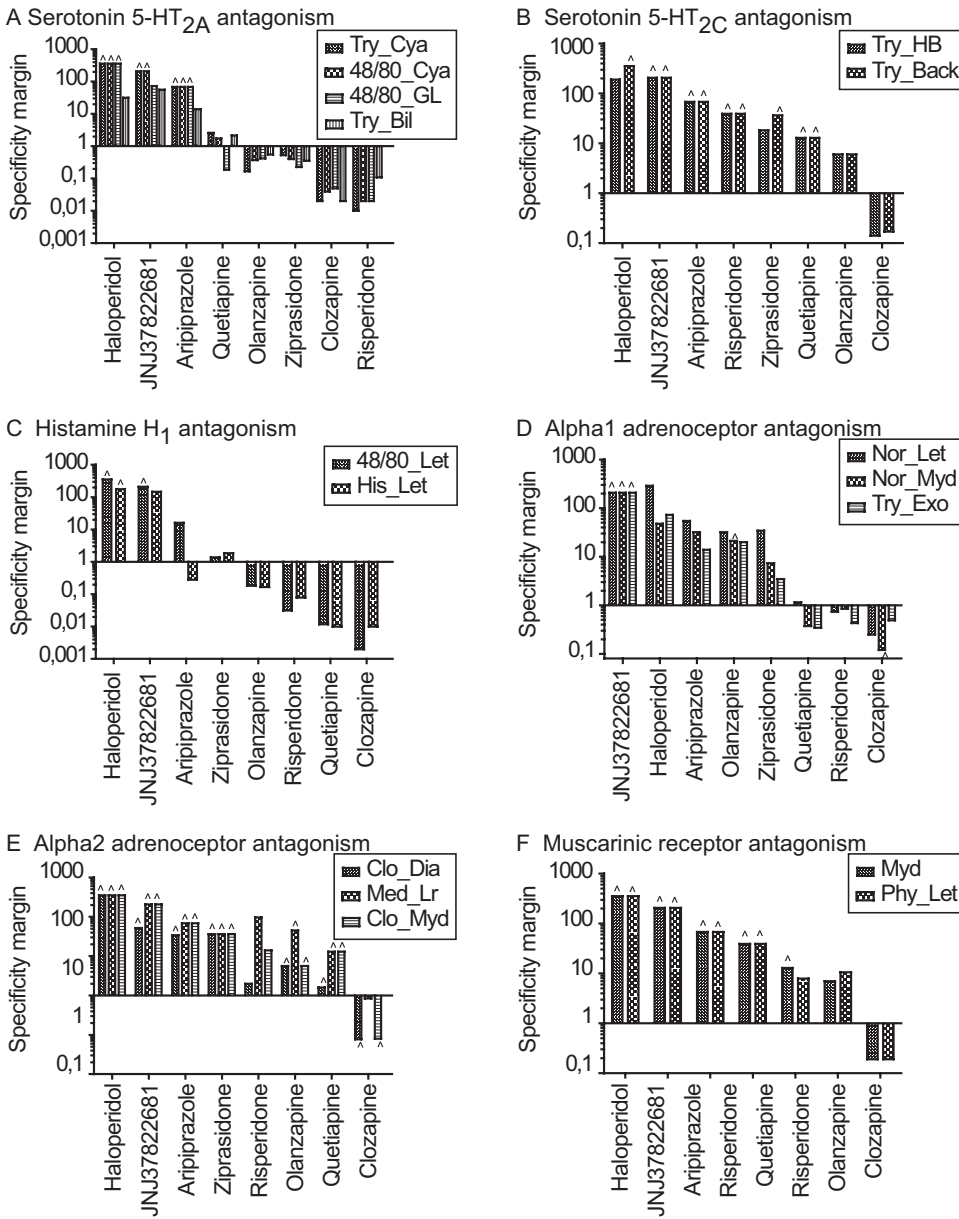


Fig. 8. Activity profile in tests related to interactions with various types of receptors. The ED₅₀ values in the various tests have been expressed as ratios over the ED₅₀ for inhibition of apomorphine-induced stereotypy. A, serotonin 5-HT_{2A} antagonism. B, serotonin 5-HT_{2C} antagonism. C, histamine 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 bilateral 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, norepinephrine-induced lethality; Nor_Myd, norepinephrine-induced mydriasis; Try_Exo, tryptamine-induced exophthalmos; Clo_Dia, clonidine-induced antiarrhythmic effect; Med_Lr, medetomidine-induced loss of righting; Clo_Myd, clonidine-induced mydriasis; Myd, mydriasis; Phy_Let, physostigmine-induced lethality.

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

Test Compound	ED ₅₀							
	Peripheral α ₁		Peripheral α ₂		Central α ₂		Peripheral Muscarinic	Central Muscarinic
	Antagonism of Norepinephrine Lethality	Antagonism of Norepinephrine Mydriasis	Antagonism of Tryptamine Exophthalmos	Antagonism of Antidiarrheal Effect of Clonidine	Antagonism of Medetomidine Loss of Righting	Antagonism of Clonidine Mydriasis	Antagonism of Physostigmine Lethality	Antagonism of Physostigmine Lethality
JNJ-37822681	>40	>40	>40	>10 ^a	>40	>40	>40	>40
Aripiprazole	32 (22–49)	19 (12–28)	8.2 (4.8–14)	>20 ^a	>40	>40	>40	>40
Clozapine	4.1 (3.0–5.5)	>2.0 ^b	8.2 (5.1–13)	>1.25 ^a	14 (12–18)	>1.25 ^c	3.1 (2.1–4.6)	3.1 (2.3–4.2)
Haloperidol	8.1 (5.4–12)	1.35 (0.84–2.2)	2.0 (1.4–3.0)	>10	>10	>10	>10	>10
Olanzapine	7.1 (4.8–11)	>4.7 ^b	9.3 (6.9–13)	>1.25 ^a	>10	>1.25 ^c	1.6 (0.96–2.5)	2.4 (1.6–3.5)
Quetiapine	14 (9.5–21)	4.7 (2.9–7.6)	4.4 (3.3–5.8)	>20 ^b	>160	>160	>160	98 (61–159)
Risperidone	0.19 (0.14–0.26)	0.22 (0.15–0.33)	0.11 (0.085–0.15)	0.51 (0.34–0.76)	25 (16–37)	3.6 (2.9–4.4)	>10	>10
Ziprasidone	9.4 (6.9–13)	2.0 (1.5–2.8)	0.95 (0.52–1.7)	>10	>10	≥10	>10	>10

^a Higher doses have intrinsic antiarrhythmic activity.

^b ED₅₀ for inducing mydriasis per se.

^c Higher doses have intrinsic mydriatic activity.

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 5HT_{2A} 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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Authorship Contributions

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Wrote or contributed to the writing of the manuscript: Langlois, Megens, Lavreysen, Atack, Cik, te Riele, Peeters, Wouters, Vermeire, Hendrickx, Macdonald, and De Bruyn.

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