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

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Running Title: Pharmacology of a Novel, Fast-Dissociating D₂ Antagonist

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Abstract: 248 words

Introduction: 750 words

Discussion: 1483 words

Number of text pages: 48

Number of tables: 5

Number of figures: 8

Number of References: 31

Theme: Drug Discovery and Translational Medicine

Abbreviations: AE, adverse event; CAR, conditioned avoidance response; EPS, extrapyramidal symptoms; ESC, escape behavior; i.v., intravenous; s.c., subcutaneous
Abstract: (Word count: 248)

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 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, 5-HT2C) and for receptors that may interfere with the effects of D2 antagonism (D1, D3, 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 the first compound that allows 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 appears 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, olanzapine) and virtually absent with clozapine, which is considered the prototypical atypical antipsychotic. 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 fifteen years is the multi-receptor 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. While all major atypical antipsychotics fully occupy the serotonin 5-HT₂A 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, hypnosedation), α₂-adrenoceptors (tachycardia), histamine H₁ receptors (sedation, weight gain), and muscarinic receptors (blurred vision, dry mouth, constipation, 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. Interestingly, clozapine and quetiapine have the fastest rate of dissociation from dopamine D₂ receptors and they 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 to developing atypical antipsychotics with an improved tolerability profile. Therefore, we began screening compounds based on a fast rate of dissociation from D₂ 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 D₂ receptors in order 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 JNJ-37822681, a novel compound identified from this program. JNJ-37822681 has the chemical name N-[1-(3,4-difluorobenzyl)piperidin-4-yl]-6-(trifluoromethyl)pyridazin-3-amine (Figure 1). It was compared with 7 pharmacologically and chemically diverse reference antipsychotics: 3 tricyclics (the azapines - clozapine and olanzapine, and the thiapine - quetiapine), the benzisoxazole - risperidone, the benzothiazole - ziprasidone, the butyrophenone - haloperidol, and the dihydroquinolinone - aripiprazole (see Figure 1 for chemical structures).

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 greater than 95% using standard analytical methods. JNJ-37822681, clozapine, aripiprazole, haloperidol, ziprasidone, risperidone, olanzapine and quetiapine were acquired from internal sources and were dissolved in DMSO for in vitro studies. [3H]clozapine was purchased from ARC (American Radiolabeled Chemicals, Inc), [3H]prazosin, [3H]SCH23390, and [3H]pyrilamine from PerkinElmer, and [3H]spiperone, [125I]iodosulpride, [3H]mesulergine from Amersham. [125I]R091150 was custom made by Amersham; all other radioligands were synthesized in-house. The various compounds used to determine non-specific binding of these radioligands were also synthesized internally and dissolved in DMSO.

For in vivo prolactin release studies, JNJ-37822681 was dissolved in distilled water containing 1 equivalent of tartaric acid. For all other in vivo studies, JNJ-37822681 was dissolved in 10% hydroxypropyl-β-cyclodextrin in distilled water containing 1 equivalent of tartaric acid. The solutions were stored at room temperature in closed containers protected from light. The preparations were subcutaneously (s.c.) 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 Tris-HCl 50 mM, pH 7.4 using an Ultra Turrax.
homogenizer. The homogenate was centrifuged for 10 min at 23,500 g in a Sorvall RC-5B centrifuge (4°C). The cells were then suspended in 5 mM Tris-HCl, pH 7.4 and after re-centrifugation for 20 min at 30,000 g at 4°C, the pellet was homogenized in Tris-HCl 50 mM 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 µM 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<sub>2L</sub> Receptor**

**Indirect Dissociation.** The dissociation rate of compounds was evaluated using an indirect assay adapted from a method published by Josee E. Leysen and Walter Gommeren (Leysen and Gommeren, 1984). Membranes were prepared from Chinese Hamster Ovary (CHO) cells stably expressing the hD<sub>2L</sub> receptor, as described above. After thawing, membranes were homogenized using an UltraTurrax and suspended in ice-cold binding buffer containing Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl<sub>2</sub> 2 mM, CaCl<sub>2</sub> 1 mM (pH 7.6), 0.1% ascorbic acid and 10 µM pargyline.

After incubating hD<sub>2L</sub> membranes with 4 times IC<sub>50</sub> of compound for 1 hour at 25°C (final volume of 2 ml), incubation mixtures were poured on GF/C filters on top of a 40-well multividor. Vacuum was briefly applied to filter compound-membrane mixtures over the GF/C filters. 200 µl of buffer or 200 µl of 4 µM butaclamol (blanc; final concentration 2 µM) together with 200 µl of 2 nM [<sup>3</sup>H]spiperone was then added for 1, 3, 5, 7 or 10 min (final concentration [<sup>3</sup>H]spiperone 1 nM). Incubation was stopped by initiating full vacuum and immediate rinsing
with ice-cold buffer. After the addition of 3 ml Ultima Gold MV (Perkin Elmer), filter-bound radioactivity was measured in a liquid scintillation counter.

**Direct Dissociation.** The off rate of $[^3\text{H}]-\text{JNJ-37822681}$ was determined and compared with off rates for $[^3\text{H}]-$labeled reference antipsychotics by typical radioligand binding experiments. Membranes were prepared from CHO cells stably transfected with hD$_2$L receptor cDNA as described above. After thawing, membranes were homogenized using an UltraTurrax and suspended in ice-cold binding buffer containing Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl$_2$ 1 mM, CaCl$_2$ 2 mM, pH 7.7. Assay mixtures, containing 50 μg (for $[^3\text{H}]-\text{JNJ-37822681}$ and $[^3\text{H}]-\text{clozapine}$) or 10 μg (for $[^3\text{H}]-\text{haloperidol}$, $[^3\text{H}]-\text{risperidone}$, and $[^3\text{H}]-\text{paliperidone}$) of membranes were incubated for 1 h in a volume of 0.45 ml at RT and 37°C. Final concentrations of 10 nM radioligand were used for $[^3\text{H}]-\text{JNJ-37822681}$ and $[^3\text{H}]-\text{clozapine}$, whereas 2 nM was applied for the other $[^3\text{H}]-$labeled compounds. Dissociation kinetics were measured by adding 10 μM raclopride (50 μl) at different times before filtration. Filtration was performed using a 40-well multividor. In a parallel set of tubes, non-specific 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 seconds for $[^3\text{H}]-\text{clozapine}$ and $[^3\text{H}]-\text{JNJ-37822681}$ and 20, 30, 40, 60, 180, 600, 1200 and 3600 seconds for $[^3\text{H}]-\text{haloperidol}$, $[^3\text{H}]-\text{paliperidone}$ and $[^3\text{H}]-\text{risperidone}$).

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

Female Sprague-Dawley rats were used for the prolactin assay, male Lewis rats for the compound 48/80 lethality assay, Dunkin-Hartley-Pirbright guinea-pigs of both sexes for the histamine lethality assay and male Wiga Wistar rats for all other assays. The rats ranged in body weight between 175 and 275 g and the guinea-pigs between 300 and 500 g. All animals were
obtained from Charles River Breeding Laboratories (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 minutes after drug administration. The animals were decapitated 30 minutes after the [³H]raclopride injection. Brains were immediately removed and rapidly frozen in dry-ice cooled 2-methylbutane (-40°C). Twenty µm-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) for 12 hours. Digital autoradiograms were quantified using the Beta vision program (Biospace Lab, Paris). 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 D₂ receptors) and in the cerebellum (a brain area where D₂ 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 D₂ receptor. Apomorphine (1.0 mg/kg, i.v.)-induced
stereotypy (compulsive sniffing, licking, 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. Criterion for drug-induced inhibition of stereotypy: fewer than 6 scores of 3, fewer than 6 scores $\geq 2$, or fewer than 7 scores $\geq 1$ (0.14% false positives in $> 5000$ solvent-pretreated control rats). Criterion for drug-induced blockade: fewer than 2 scores of $\geq 1$ and 0 scores of $\geq 2$ (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: presence of diarrhea (3.2% false positive controls; $n = 154$). Clonidine antagonism reflects blockade of peripheral $\alpha_2$-adrenoceptors. In order to investigate whether intrinsic antidiarrheal activity might have masked the peripheral $\alpha_2$-adrenoceptor blockade, the ability of inactive compounds to block castor oil-induced diarrhea was also studied after injection of saline instead of clonidine. Criterion for antidiarrheal activity: 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 5-30 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: $> 240$ min survival
Histamine H₁ antagonists protect against compound 48/80-induced lethality.

**Conditioned One-Way Active Avoidance Test.** The apparatus consisted of an inner transparent box (length x width x height: 30 x 30 x 30 cm) with an open top and 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 inter-bar distance). Odd and even bars were connected with a source of alternative current (1.0 mA; Coulbourn Instruments Solid State Shocker/Distributor), which could be interrupted by a switch. The outer box (length x width x height: 40 x 40 x 36 cm) had also an open top and kept 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 edge of the outer and inner box served as a target for the rats on which to jump with fore- and hind-paws, respectively.

Rats were trained to avoid an electric shock during 5 sessions at 15-min time intervals during a 1-h period: the rat was placed on the non-electrified 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 in all the last 3 training sessions were included for further experiments, and received test compound or solvent immediately after the last training session.

The rats were tested 3 times, i.e. 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 is electrified) or escape (i.e., responding after the grid has been electrified; cut-off 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: > 120 min survival (0.6% false positive controls; n > 300). Histamine H₁ antagonists are active in this test.

**Locomotor Activity Assays.** Motor activity was measured in microprocessor-based motor activity cages (length x width x height: 43.5 x 43.5 x 41.5 cm; MED Associates) over a period of 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 a period of 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: total distance < 5000 cm for inhibition of d-amphetamine-induced hyperlocomotion (8.4% false positives in > 450 solvent-pretreated control rats) and total distance < 11000 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 H₁ antagonist 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, (0) absent. Criteria for drug-induced effects: score ≤ 1 for inhibition (7.1% false positives in controls; n = 162); score < 1 for blockade (0.6% false positives in controls). Cyanosis of the ears was scored (0, 0.5, 1) 5 min after the injection of compound 48/80. Score < 0.5 was adopted as a criterion for antagonism of cyanosis (0.0% false positives). Protection from gastric lesions and reversal of cyanosis is 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: duration = 0 min (2.4% false positive controls; n > 500). Centrally-acting $\alpha_2$-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 $\alpha_1$-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 (°C; using an esophageal thermistor probe) were assessed at hourly intervals over a
period of 8 h after administration of test compound or solvent. The scoring system was: for catalepsy: (3) pronounced, (2) moderate, (1) slight, and (0) absent; for palpebral opening: (5) exophthalmos, (4) wide open, (3) open for three-quarters, (2) half open, (1) open for one-quarter, (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: score 6 (not observed in controls). Criterion for drug-induced palpebral ptosis (assessed after manipulation): 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: $\geq 1.0$ °C decrease of temperature for the 1 h interval (not observed in controls) and $\geq 2.0$ °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: > 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: pupil diameter > 25 units for mydriasis (in controls: 2.4%), < 10 units for miosis (in controls: 0.5%). Protection against physostigmine-induced lethality is 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 decapitated. Blood was collected in Vacutainer SSTO tubes and centrifuged at 3000 rpm for 10 min. Serum was transferred into secondary tubes and subsequently frozen. Samples were kept at < -18 °C until analysis. Serum prolactin was measured with a commercially available radioimmunoassay, *viz.*, Rat Prolactin $[^{125}\text{I}]$ Assay System (Amersham, UK). The detection limit of the assay was 0.8
ng/ml. The inter-assay 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 ± SD; 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 min after injection of tryptamine. The direction of locomotion (backward, sideward, or forward) was also noted. The scoring system was: (A) for bilateral clonic seizures, and hunched back: (3) pronounced, (2) moderate, (1) slight, and (0) absent; (B) for palpebral opening: (5) exophthalmos, (4) wide open, (3) open for three-quarters, (2) half open, (1) open for one-quarter, (0) closed. Criteria for drug-induced inhibition or decrease: 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); 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: hyperemia of the ears (red ears; 0.0% false positives).

**In Vitro Data Analysis.** Data from radioligand competition binding experiments were calculated as percentage of total binding measured in the absence of test compound. Inhibition curves, plotting percentage of total binding versus the log concentration of the test compound were analyzed using non-linear 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 
were calculated from dissociation curves using the one-phase exponential decay equations in 
GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

**Determination of ED$_{50}$ Values.** The percentage of receptor occupancy was plotted against 
dosage and the sigmoidal log dose-effect curve of best fit was calculated by non-linear regression 
analysis, using the GraphPad Prism® software (Motulsky, 1999). From these dose-response 
curves, the ED$_{50}$s (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 doses range). ED$_{50}$s (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 ED$_{50}$ 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 
ED$_{50}$ values obtained in the two tests was studied by calculating Pearson correlation statistics and 
performing and graphing linear regression using GraphPad 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 D\(_{3}\) and serotonin 5-HT\(_{2A}\) receptors (Table 2) and did not interact with the human receptors dopamine D\(_{1}\), adrenergic \(\alpha_{1A}\), serotonin 5-HT\(_{2C}\), and histamine H\(_{1}\), up to the highest (10 \(\mu\)M) concentration tested. Further profiling at CEREP did not reveal any additional interactions except a high affinity to sigma 1 receptors (K\(_i\), 8.9 nM) (See supplemental data). Overall, JNJ-37822681 shows a high D\(_{2}\) specificity, especially compared with the second generation antipsychotics that display a moderate to weak affinity for the D\(_{2}\) receptor, such as olanzapine, clozapine and quetiapine.

Indirect Dissociation Assay with D\(_{2}\) Receptor. It is assumed that the faster a compound dissociates from D\(_{2}\) receptor after a 1-hour incubation period, the faster \[^{3}\text{H}]\text{spiperone}\) binds to the D\(_{2}\) receptor. JNJ-37822681 was initially selected after a 5-minute incubation with \[^{3}\text{H}]\text{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 \[^{3}\text{H}]\text{spiperone}\) was performed in the presence of JNJ-37822681 and reference antipsychotics. \[^{3}\text{H}]\text{spiperone}\) had a faster association to D\(_{2}\) receptor in the presence of JNJ-37822681 than in the presence of reference antipsychotics, including clozapine (Figure 2). This indicates that JNJ-37822681 is a fast-dissociating D\(_{2}\) ligand.

Direct Dissociation Assay with D\(_{2}\) Receptor using \[^{3}\text{H}]\text{JNJ-37822681}\). \[^{3}\text{H}]\text{JNJ-37822681}\) dissociated faster than \[^{3}\text{H}]\text{haloperidol}, \[^{3}\text{H}]\text{risperidone}\) and \[^{3}\text{H}]\text{paliperidone}\) and its dissociation
rate was similar to that of [³H]clozapine (Figure 3A, 3B, 3C). 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 20-35 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 due to differences in CNS 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 ED₅₀s for apomorphine antagonism was close to the ED₅₀s 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 regression line (Figure 4). Interestingly, the fast dissociating D₂ antagonist JNJ-37822681 is the only compound that achieved apomorphine antagonism at doses below 50% occupancy of the D₂ receptor whereas 50% or above is required for the reference antipsychotics (Figure 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 (Figure 5B).

**Antagonism of d-Amphetamine-induced Hyperlocomotion.** The ED$_{50}$s for inhibition of d-amphetamine-induced hyperlocomotion deviated somewhat from those obtained for apomorphine antagonism (Table 3, Figure 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 (Figure 4), suggesting that, apart from D$_2$ receptor blockade, other factors may be involved.

**Antagonism of Phencyclidine-induced Hyperlocomotion.** The ED$_{50}$s 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 (Figure 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 (Table 3 and Table 4; Figure 6A). Prolactin levels progressively increased with increase of dose (Figure 7). As a direct consequence, risperidone and quetiapine already induced pronounced prolactin release at doses below the ED$_{50}$. 
for apomorphine antagonism (Figure 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 (see inserts in Figure 7). 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 conditioned avoidance response (CAR), and, at slightly higher doses, also the escape response (ESC)(Table 4). The specificity margin between inhibition of CAR and apomorphine antagonism ranged between 0.19 for clozapine and 28 for aripiprazole (median: 5.3; Figure 6C). The smallest margin was obtained with non-specific 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 non-specific D$_2$ receptor blockers clozapine and risperidone and least readily with the specific D$_2$ receptor blockers (aripiprazole, JNJ-37822681 and haloperidol; Figure 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 (Figure 6E). All compounds also induced palpebral ptosis and hypothermia (Table 4). D$_2$ receptor blockers with associated $\alpha_1$-adrenocortceptor 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 (Figure 6F). A very similar profile was observed for the induction of hypothermia (Figure 6G).

**Additional Receptor Interactions**

**Serotonin 5HT<sub>2A</sub> Receptor Antagonism.** Relative to the ED<sub>50</sub> for apomorphine antagonism, risperidone and clozapine showed 5-HT<sub>2A</sub> antagonism already at > 10 fold lower doses, whereas haloperidol and JNJ-37822681 were devoid of 5-HT<sub>2A</sub> antagonism up to > 100 fold higher doses (Table 5A and Table 3; Figure 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<sub>2A</sub> antagonism, however, the inhibition of bilateral convulsions was in these cases probably related to behavioral depressant effects rather than to blockade of central 5-HT<sub>2A</sub> receptors. The wide specificity margin between central and peripheral 5-HT<sub>2A</sub> 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<sub>2C</sub> Receptor Antagonism.** Only clozapine and olanzapine showed antagonism of both 5HT<sub>2C</sub> effects and only with clozapine were these effects observed at doses below the ED<sub>50</sub> for apomorphine antagonism (Table 5A, Table 3; Figure 8B). Both compounds were the only ones that displayed higher affinity for 5-HT<sub>2C</sub> than for D<sub>2</sub> receptors (Table 2). Haloperidol and ziprasidone antagonized hunched back behavior only. No 5HT<sub>2C</sub> receptor-related effects could be demonstrated for JNJ-37822681.

**Histamine H<sub>1</sub> Receptor Antagonism.** Antihistaminergic activity was generally observed at comparable dose levels in both species (Table 5A; Figure 8C). However, aripiprazole was 10-fold more potent in guinea-pigs than in rats. JNJ-37822681 and haloperidol were devoid of
histamine H₁ antagonistic activity up to 100 times their apomorphine antagonistic dose (Table 5A and Table 3; Figure 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 a very high dose level only (28.3 mg/kg).

**α₁-Adrenoceptor Antagonism.** As 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 (Table 5B and Table 3; Figure 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 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 intrinsic constipating effects. Likewise, the antagonism of clonidine-induced mydriasis can 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 the effects of an α₂-adrenoceptor blocker. Interaction with peripheral α₂-adrenoceptors was observed from doses only slightly above the ED₅₀ for apomorphine antagonism (Table 5B and Table 3; Figure 8E). In order to obtain central α₂-adrenoceptor blocking activity, 10-fold higher doses were required, consistent with the poor
CNS 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. \( \alpha_2 \)-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 \( ED_{50} \) for apomorphine antagonism (Table 5B and Table 3; Figure 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 if we simply selected a low-affinity D₂ antagonist. However, fast dissociation does not necessarily mean low affinity only; 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 (Kᵢ, 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 affinity (e.g., olanzapine, clozapine and quetiapine).

In vivo, JNJ-37822681 was a relatively potent, centrally active D₂ antagonist as measured by 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 non-specific 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 to evaluate 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, in vivo D2 specificity). Our results demonstrate the specific blockade of central D2 receptors by JNJ-37822681 at moderate doses, comparable to 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 (Millan et al 2000, Natesan et al 2006; Olsen et al, 2006; Shannon et al, 1999; Wadenberg 1998). 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 inhibition of CAR by central D2 antagonism can be potentiated by e.g., 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 amongst 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 inhibition of CAR and central D2 receptor occupancy has previously been observed with aripiprazole (Natesan et al, 2006).
Although 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 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 due to 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 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, likely 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 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 i.v. 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 and corresponding high synaptic concentrations required for suppression of ESC, antagonist molecules that dissociate from the receptor are likely immediately replaced by other antagonist molecules rather than by dopamine. JNJ-37822681 showed a high margin between induction of catalepsy and apomorphine antagonism, exceeding that obtained for haloperidol and at least as high as measured for atypical antipsychotics (although exact values could not be determined for clozapine and quetiapine). As catalepsy in rats is generally considered to be predictive of EPS in man, this wide margin supports the hypothesis that fast dissociation from the D2 receptor decreases EPS liability and can make specific D2 antagonists behave similar to multireceptor atypical antipsychotics. Most dopamine antagonists induce palpebral ptosis in the rat, which generally relates well to $\alpha_1$-adrenergic blocking activity but poorly to apomorphine antagonism (Janssen, 1965; Niemegeers, 1974). However, butyrophenone dopamine antagonists (e.g., haloperidol), are virtually devoid of $\alpha_1$-adrenoceptor blocking activity and nevertheless induce palpebral ptosis due to their sedative liability. JNJ-37822681 is completely devoid of $\alpha_1$-adrenergic blocking activity and shows an even wider specificity margin between 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; Inoue et al., 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 to demonstrate 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 5HT2C, α1-adrenoceptors, α2-adrenoceptors, histamine H1, and muscarinic that are known or suspected to cause the major AEs associated with available antipsychotics. Finally, JNJ-37822681 is also devoid of relevant 5HT2A 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 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).
Acknowledgments: Dr. Christopher D. Pericone (Janssen Research & Development, LLC) provided writing assistance and Dr. Wendy P. Battisti (Janssen Research & Development, LLC) provided additional editor support for this manuscript.
Author Contributions:

Participated in study design: Langlois, Lavreysen, and Megens

Conducted experiments: te Riele, Peeters, Wouters, Vermeire and Hendrickx

Performed data analysis: Langlois, Lavreysen, and Megens

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


adrenoceptor antagonist prazosin and the dopamine D₂ receptor antagonist raclopride in rats. *J Neural Transm* 107: 1229-1238.


Footnotes

Study support: Funded by Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, N.J., USA. The sponsor also provided a formal review of this manuscript.

Disclosures: All authors are current or retired (Drs. De Bruyn and Vermeire) employees of Janssen Research & Development, a Division of Janssen Pharmaceutica NV, Beerse, Belgium and a Johnson & Johnson company.
Figure Legends

Figure 1. Structures of JNJ-37822681 (N-[1-(3,4-difluorobenzyl)piperidin-4-yl]-6-(trifluoromethyl)pyridazin-3-amine) and the 7 tested reference antipsychotics.

Figure 2. Evaluation of the Dissociation Speed of JNJ-37822681 and Reference Antipsychotics from hD_{2L} receptor. The level of [3H]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, risperidone, incubated at a concentration equal to 4 times their IC_{50}). NS = non-specific binding. Graph represents averaged data of three independent experiments for each compound. Data for olanzapine, risperidone and quetiapine were omitted for clarity.

Figure 2B: The percentage of total binding after incubation with [3H]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 D_{2} receptors.

Figure 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 hD2L 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 1 representative experiment. SB = Specific Binding

Figure 3A: Room temperature

Figure 3B: 37°C
Figure 3C: Dissociation from the human D2L receptor: half life ($t_{1/2}$) and off rate ($k_{off}$) for $[^3 \text{H}]$JNJ-37822681 and $[^3 \text{H}]$labeled reference antipsychotics. Data are mean $\pm$ S.D. (number of experiments indicated; each data point was performed in duplicate).

**Figure 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 using GraphPad Prism$^\text{®}$ software.

**Figure 5. Activity profile in tests related to antipsychotic activity.** ED$_{50}$s for D$_2$ 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 ED$_{50}$ for inhibition of apomorphine-induced stereotypy. The "^\text{\textasciigreek{a}}" sign above a bar indicate that the ED$_{50}$ is greater than ($>$) the value indicated by the height of that bar.

**Figure 6. Activity profile in tests related to D$_2$ receptor-related side effects.** ED$_{50}$s 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 ED$_{50}$ for inhibition of apomorphine-induced stereotypy. The "^\text{\textasciigreek{a}}" sign above a bar indicate that the ED$_{50}$ is greater than ($>$) the value indicated by the height of that bar.

**Figure 7. Dose-dependent prolactin release measured 1 h after s.c. injection of the test compounds.** The dotted horizontal line indicates the averaged prolactin concentration (given in the insert) at the ED$_{50}$ for apomorphine antagonism (dotted vertical line). A: JNJ-37822681; B: aripiprazole; C: clozapine; D: haloperidol; E: olanzapine; F: quetiapine; G: risperidone; H: ziprasidone.
Figure 8. Activity profile in tests related to interactions with various types of receptors. The ED₅₀s 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: Alpha₁ adrenoceptor antagonism; E: Alpha₂ adrenoceptor antagonism; F: muscarinic receptor antagonism. The "^" sign above a bar indicate that the ED₅₀ is greater than (> ) the value indicated by the height of that bar.
Table 1: Assay conditions for radioligand binding

<table>
<thead>
<tr>
<th>Receptor source</th>
<th>Assay conditions (incubation buffer, time and temp)</th>
<th>Radioligand</th>
<th>Non-specific binding</th>
</tr>
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<tbody>
<tr>
<td>hα1-adrenergic</td>
<td>CHO Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl2 1 mM, CaCl2 2 mM, BSA 0.1 % pH 7.7, RT, 20-24 h*</td>
<td>[3H]Prazosin, 0.5 nM</td>
<td>Aceperone, 1 µM</td>
</tr>
<tr>
<td>hD1</td>
<td>GH4C1 Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl2 1 mM, CaCl2 2 mM, pargyline 10 µM 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 Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl2 1 mM, CaCl2 2 mM pH 7.7, 37°C, 30 min</td>
<td>[3H]Spiperone, 0.2 nM</td>
<td>(+)Butaclamol, 1 µM</td>
</tr>
<tr>
<td>hD3</td>
<td>CHO Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl2 1 mM, CaCl2 2 mM, BSA 0.1 % pH 7.7, RT, overnight*</td>
<td>[125I]Iodosulpride, 0.2 nM</td>
<td>Risperidone, 1 µM</td>
</tr>
<tr>
<td>h5-HT2A</td>
<td>L929 Tris-HCl 50 mM, NaCl 120 mM, KCl 5 mM, MgCl2 1 mM, CaCl2 2 mM, BSA 0.1 % pH 7.4, 37°C, 60 min</td>
<td>[125I]R091150, 0.1 nM</td>
<td>BW501, 1 µM</td>
</tr>
<tr>
<td>h5-HT2C</td>
<td>Sf9 Tris-HCl 50 mM, CaCl2 4 mM pH 7.7, 37°C, 30 min</td>
<td>[3H]Mesulergine, 1 nM</td>
<td>Ritanserin, 1 µM</td>
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<tr>
<td>hH1</td>
<td>CHO Na-K phosphate 50 mM, BSA 0.05% pH 7.5, 25°C, 30 min</td>
<td>[3H]Pyrilamine, 2 nM</td>
<td>Astemizole, 1 µM</td>
</tr>
</tbody>
</table>

*SPA technology was used to quantify radioligand binding, whereas for the other assays filtration using a Packard Filtermate Harvester was done to separate bound from free radioligand.
Table 2: In vitro Binding Affinities (mean Ki values with indication of 95% confidence limits in brackets) of JNJ-37822681 and Reference Antipsychotics for Various Neurotransmitter Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>JNJ-37822681</th>
<th>Aripiprazole</th>
<th>Haloperidol</th>
<th>Ziprasidone</th>
<th>Risperidone</th>
<th>Olanzapine</th>
<th>Clozapine</th>
<th>Quetiapine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
<td>Ki (nM) n</td>
</tr>
<tr>
<td>D&lt;sub&gt;2L&lt;/sub&gt;</td>
<td>158 (132-190)</td>
<td>0.49 (0.2-1.2)</td>
<td>1.8 (1.3-2.3)</td>
<td>4.1 (2.8-5.9)</td>
<td>5.5 (5.1-5.9)</td>
<td>57 (35-93)</td>
<td>137 (112-168)</td>
<td>554 (419-733)</td>
</tr>
<tr>
<td>hD&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&gt; 3000 1</td>
<td>653 1</td>
<td>65 1</td>
<td>41 1</td>
<td>147 (117-186)</td>
<td>16 1</td>
<td>140 1</td>
<td>803 1</td>
</tr>
<tr>
<td>hD&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1159 1</td>
<td>4.5 1</td>
<td>5.1 6 (3.3-8)</td>
<td>23 3 (18-29)</td>
<td>17 15 (19)</td>
<td>55 6 (36-84)</td>
<td>231 4 (176-304)</td>
<td>844 10 (634-1123)</td>
</tr>
<tr>
<td>hα&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>&gt; 5000 3</td>
<td>211 (76-582)</td>
<td>122 (41-363)</td>
<td>32 3 (15-66)</td>
<td>4.8 2.6-9.5)</td>
<td>189 5 (100-356)</td>
<td>28 3 (19-41)</td>
<td>200 4 (172-234)</td>
</tr>
<tr>
<td>hH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4931 1</td>
<td>5.08 (3.5-7.4)</td>
<td>&gt; 5000 2</td>
<td>72 (45-116)</td>
<td>67 (61-74)</td>
<td>3.3 2.8-4.0)</td>
<td>1.5 1.2-2</td>
<td>7.4 2 (6.9-7.9)</td>
</tr>
<tr>
<td>h5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>2896 (2064-4062)</td>
<td>16 (15-17)</td>
<td>311 (159-609)</td>
<td>1.9 (0.9-4.1)</td>
<td>0.66 0.53-0.82)</td>
<td>3.8 2.6-5.7)</td>
<td>8.5 4.9-13</td>
<td>318 7 (161-627)</td>
</tr>
<tr>
<td>h5-HT&lt;sub&gt;2C&lt;/sub&gt;</td>
<td>&gt; 4000 2</td>
<td>14 (11-18)</td>
<td>&gt; 4000 1</td>
<td>7.4 (5.7-10)</td>
<td>13 (12-14)</td>
<td>12 (9.2-15)</td>
<td>12 (8.6-15)</td>
<td>2197 3 (1422-3395)</td>
</tr>
</tbody>
</table>
Table 3: ED₅₀ (95% confidence limits; mg/kg, s.c.) of JNJ-37822681 and 7 Reference Compounds for D₂ Receptor Occupancy and Inhibition of Stimulant-Induced Behavior 1 Hour After SC Administration.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>D₂ occupancy in vivo</th>
<th>Inhibition of apomorphine-induced stereotypy</th>
<th>Blockade of apomorphine-induced stereotypy</th>
<th>Inhibition of d-amphetamine hyperlocomotion</th>
<th>Inhibition of phencyclidine hyperlocomotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>0.39 (0.31-0.49)</td>
<td>0.19 (0.14-0.26)</td>
<td>8.1 (6.0-11)</td>
<td>1.0 (0.68-1.5)</td>
<td>4.7 (3.3-6.6)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>0.58 (0.44-0.76)</td>
<td>0.59 (0.43-0.80)</td>
<td>5.4 (4.0-7.3)</td>
<td>0.26 (0.16-0.41)</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 (1.5-2.8)</td>
<td>2.0 (2.4-5.3)</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.018 (0.014-0.022)</td>
<td>0.028 (0.023-0.035)</td>
<td>0.26 (0.19-0.35)</td>
<td>0.064 (0.048-0.087)</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 (0.75-1.4)</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 (3.1-7.0)</td>
<td>4.7 (12-18)</td>
<td></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>0.44 (0.36-0.55)</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>0.29 (0.19-0.35)</td>
<td>1.2 (0.78-1.8)</td>
</tr>
</tbody>
</table>
Table 4: ED50s (95% confidence limits; mg/kg, s.c.) of JNJ-37822681 and 7 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>Slight (Prolactin release)</th>
<th>Pronounced (Prolactin release)</th>
<th>Conditioned Avoidance Inhibition of Avoidance</th>
<th>Blockade of Escape</th>
<th>Catalepsy</th>
<th>Palpebral ptosis</th>
<th>Hypothermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>0.17</td>
<td>0.25</td>
<td>3.1</td>
<td>14</td>
<td>8.2</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>(0.11-0.25)</td>
<td>(0.82-1.9)</td>
<td>(2.1-4.6)</td>
<td>(12-17)</td>
<td>(5.5-12)</td>
<td>(10-26)</td>
<td>(11-24)</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>0.14</td>
<td>1.9</td>
<td>16</td>
<td>43</td>
<td>7.1</td>
<td>4.7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>(0.094-0.20)</td>
<td>(1.1-3.4)</td>
<td>(11-24)</td>
<td>(29-64)</td>
<td>(5.8-8.8)</td>
<td>(3.1-7.0)</td>
<td>(6.7-17)</td>
</tr>
<tr>
<td>Clozapine</td>
<td>11</td>
<td>≥ 160</td>
<td>3.1</td>
<td>5.4</td>
<td>&gt; 320</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>(7.3-16)</td>
<td></td>
<td>(1.9-5.0)</td>
<td>(3.6-8.0)</td>
<td>(0.86-1.6)</td>
<td>(0.73-1.9)</td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.030</td>
<td>0.127</td>
<td>0.13</td>
<td>2.0</td>
<td>0.34</td>
<td>1.0</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>(0.024-0.038)</td>
<td>(0.091-0.18)</td>
<td>(0.075-0.22)</td>
<td>(1.3-3.3)</td>
<td>(0.90-2.)</td>
<td>(0.68-1.5)</td>
<td>(3.0-5.5)</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>0.15</td>
<td>1.8</td>
<td>0.68</td>
<td>3.1</td>
<td>19</td>
<td>1.6</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>(0.11-0.21)</td>
<td>(1.3-2.5)</td>
<td>(0.39-1.2)</td>
<td>(2.3-4.2)</td>
<td>(12-28)</td>
<td>(1.0-2.3)</td>
<td>(1.5-3.8)</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>0.55</td>
<td>6.6</td>
<td>75</td>
<td>394(^a)</td>
<td>&gt; 160</td>
<td>1.8</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>(0.41-0.73)</td>
<td>(5.0-8.8)</td>
<td>(44-130)</td>
<td>(- - -)</td>
<td>(1.2-2.6)</td>
<td>(3.3-8.7)</td>
<td></td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.0046</td>
<td>0.028</td>
<td>0.51</td>
<td>1.8</td>
<td>8.1</td>
<td>0.065</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>(0.0036-0.0058)</td>
<td>(0.018-0.043)</td>
<td>(0.32-0.82)</td>
<td>(1.2-2.6)</td>
<td>(6.0-13)</td>
<td>(0.043-0.097)</td>
<td>(0.081-0.27)</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>0.14</td>
<td>0.78</td>
<td>2.7</td>
<td>7.1</td>
<td>6.2</td>
<td>2.0</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>(0.10-0.18)</td>
<td>(0.56-1.1)</td>
<td>(2.0-3.6)</td>
<td>(4.7-11)</td>
<td>(4.1-9.2)</td>
<td>(1.5-2.8)</td>
<td>(2.4-5.3)</td>
</tr>
</tbody>
</table>

\(^a\) Maximum 40% responders at 320 mg/kg. The ED\(_{50}\) has been estimated assuming that 100% responders would have been obtained if a higher dose of 640 mg/kg would have been tested.

\(^b\) The lowest ED\(_{50}\) obtained over the 8-h period is listed.
Table 5A: ED$_{50}$s 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>Antagonism of tryptamine cyanosis</th>
<th>Antagonism of compound 48/80 cyanosis</th>
<th>Inhibition of compound 48/80 gastric lesions</th>
<th>Blockade of tryptamine bilateral convulsions</th>
<th>Antagonism of tryptamine hunched back</th>
<th>Reversal of tryptamine backward locomotion</th>
<th>Antagonism of compound 48/80 lethality</th>
<th>Antagonism of histamine lethality (guinea-pigs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>14</td>
<td>11</td>
<td>(6.7-17)</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>28</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>≥ 40</td>
<td>8.2</td>
<td>(6.0-11)</td>
<td>&gt; 40</td>
<td>&gt; 40</td>
<td>9.4</td>
</tr>
<tr>
<td>Clozapine</td>
<td>0.34</td>
<td>0.67</td>
<td>0.89</td>
<td>0.34</td>
<td>(0.25-0.46)</td>
<td>2.4</td>
<td>2.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>≥ 10</td>
<td>&gt; 10</td>
<td>&gt; 10</td>
<td>0.89</td>
<td>(0.59-1.3)</td>
<td>5.4</td>
<td>&gt; 10</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Clozapine</td>
<td>0.037 (0.028-0.050)</td>
<td>0.085 (0.057-0.13)</td>
<td>0.097 (0.054-0.18)</td>
<td>0.13 (0.071-0.23)</td>
<td>(0.090-2.0)</td>
<td>1.3 (0.99-1.8)</td>
<td>0.15 (0.030-0.060)</td>
<td>0.043</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>0.0038 (0.0026-0.0056)</td>
<td>0.0041 (0.0027-0.0061)</td>
<td>0.0047 (0.0029-0.0076)</td>
<td>0.028 (0.019-0.042)</td>
<td>(14-32) (0.0027-0.0061)</td>
<td>(1.4-3.8) (0.0029-0.0076)</td>
<td>(18-39)</td>
<td>0.098</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.15 (0.011-0.20)</td>
<td>0.11 (0.092-0.14)</td>
<td>0.064</td>
<td>0.098</td>
<td>(0.043-0.097)</td>
<td>(0.043-0.097)</td>
<td>(3.2-7.7)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

*a* Estimated ED$_{50}$ 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 ED$_{50}$ of 16.2 (11.1-23.8) mg/kg onwards, possibly reflecting vasoconstriction.
Table 5B: ED$_{50}$s 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 $\alpha_1$</th>
<th>Peripheral $\alpha_2$</th>
<th>Central $\alpha_2$</th>
<th>Peripheral Muscarinic</th>
<th>Central Muscarinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ-37822681</td>
<td>&gt; 40</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>&gt; 20 $^b$</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>Clozapine</td>
<td>4.1 (3.0-5.5)</td>
<td>&gt; 2.0 $^a$</td>
<td>8.2 (5.1-13)</td>
<td>&gt; 1.25 $^b$</td>
<td>14 (12-18)</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>
</tr>
<tr>
<td>Olanzapine</td>
<td>7.1 (4.8-11)</td>
<td>&gt; 4.7 $^a$</td>
<td>9.3 (6.9-13)</td>
<td>&gt; 1.25 $^b$</td>
<td>&gt; 10</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 $^b$</td>
<td>&gt; 160</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</td>
<td>25 (16-37)</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>
</tr>
</tbody>
</table>

$^a$ ED$_{50}$ for inducing mydriasis per se; $^b$ Higher doses have intrinsic antidiarrheal activity; $^c$ Higher doses have intrinsic mydriatic activity.
Figure 2

---

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>
Figure 3

**A**

![Graph showing the percentage specific binding over time for various radioligands at room temperature.](image)

**B**

![Graph showing the percentage specific binding over time for various radioligands at 37°C.](image)

**C**

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>t_{1/2}(s)</th>
<th>RT</th>
<th>k_{off}(s^{-1})</th>
<th>t_{1/2}(s)</th>
<th>37°C</th>
<th>k_{off}(s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>[^{3}H]JNJ-37822681</td>
<td>6.5 ± 1.1</td>
<td></td>
<td>0.108 ± 0.016</td>
<td>3.7 ± 0.68</td>
<td></td>
<td>0.191 ± 0.038</td>
</tr>
<tr>
<td>[^{3}H]clozapine</td>
<td>14.5 ± 4.4</td>
<td></td>
<td>0.051 ± 0.016</td>
<td>5.8 ± 1.4</td>
<td></td>
<td>0.126 ± 0.037</td>
</tr>
<tr>
<td>[^{3}H]haloperidol</td>
<td>72.4 ± 5.8</td>
<td></td>
<td>0.01 ± 0.001</td>
<td>24.5 ± 1.72</td>
<td></td>
<td>0.028 ± 0.002</td>
</tr>
<tr>
<td>[^{3}H]risperidone</td>
<td>198 ± 20</td>
<td></td>
<td>0.004 ± 0</td>
<td>49.2 ± 14</td>
<td></td>
<td>0.015 ± 0.004</td>
</tr>
<tr>
<td>[^{3}H]paliperidone</td>
<td>239 ± 11</td>
<td></td>
<td>0.003 ± 0</td>
<td>46.5 ± 0.56</td>
<td></td>
<td>0.015 ± 0</td>
</tr>
</tbody>
</table>
Figure 6

A) Slight increase prolactin

B) Pronounced increase prolactin

C) Inhibition avoidance

D) Inhibition escape

E) Induction catalepsy

F) Induction palpebral ptosis

G) Induction hypothermia
Figure 7

A) JNJ-37822681

B) Aripiprazole

C) Clozapine

D) Haloperidol

E) Olanzapine

F) Quetiapine

G) Risperidone

H) Ziprasidone

Protein levels (mean ± SE, ng/ml) vs. Dose (mg/kg, s.c.).

- JNJ-37822681: 30 ng/ml
- Aripiprazole: 250 ng/ml
- Clozapine: 55 ng/ml
- Haloperidol: 35 ng/ml
- Olanzapine: 75 ng/ml
- Quetiapine: 400 ng/ml
- Risperidone: 575 ng/ml
- Ziprasidone: 95 ng/ml