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1. Title page

Translational evaluation of JNJ-18038683, a 5-HT_7 receptor antagonist, on REM sleep and in major depressive disorder

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2. Running title page.

a) A running title: Translational evaluation of a 5-HT_7 receptor antagonist.

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Number of words in the Introduction: 703

Number of words in the Discussion: 1362

d) List of nonstandard abbreviations: 5-CT, 5-carboxamidotryptamine; 5-HT, serotonin; AUC, area under the curve; DA, dopamine; DDI, drug-drug interaction; DLT, dose limiting toxicity; EEG, electroencephalogram; EMG, electromyogram; FLIPR, fluorescence imaging plate reader; JNJ-18038683, (3-(4-chlorophenyl)-1,4,5,6,7,8-hexahydro-1-(phenylmethyl)pyrazolo[3,4-d]azepine 2-hydroxy-1,2,3-propanetricarboxylate); MDD, major depressive disorder; MTD, maximally tolerated dose; NE, norepinephrine; NREM, non REM sleep; PSG, polysomnogram; REM, rapid eye movement; SB-269970, (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]-pyrrolidine; SSRI, selective serotonin reuptake inhibitor.

e) Recommended section assignment: Drug Discovery and Translational Medicine

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3. Abstract

In rodents, 5-HT_7 receptor blockade has been shown to be effective in models of depression and to increase the latency to REM sleep and decrease REM duration. In the clinic, the REM sleep reduction observed with many antidepressants may serve as a biomarker. We report here the preclinical and clinical evaluation of a 5-HT_7 receptor antagonist, JNJ-18038683. In rodents, JNJ-18038683 increased the latency to REM sleep and decreased REM duration and this effect was maintained after repeated administration for 7 days. The compound was effective in the mouse tail suspension test. JNJ-18038683 enhanced serotonin transmission, antidepressant-like behavior, and REM sleep suppression induced by citalopram in rodents. In healthy human volunteers, JNJ-18038683 prolonged REM latency and reduced REM sleep duration demonstrating that the effect of 5-HT_7 blockade on REM sleep translated from rodents to humans. Like in rats, JNJ-18038683 enhanced REM sleep suppression induced by citalopram in humans, although a drug-drug interaction could not be ruled out. In a double blind, active- and placebo-controlled clinical trial in 225 patients suffering from major depressive disorder, neither treatment with pharmacologically active doses of JNJ-18038683 or citalopram separated from placebo indicating a failed study lacking assay sensitivity. A post hoc analyses using an enrichment window strategy, where all the efficacy data from sites with an implausible high placebo response (placebo group MADRS <= 12) and from sites with no placebo response (MADRS >=28) are removed, there was a clinically meaningful and statistically significant difference between JNJ-18038683 and placebo. Further clinical studies are required to characterize the potential antidepressant efficacy of JNJ-18038683.

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4. Introduction

Since its identification, the 5-HT_7 receptor has been the subject of intense research efforts driven by its presence in functionally relevant regions of the brain and by the discovery that several non- selective antipsychotics and antidepressants currently on the market display high affinity for this receptor (Stahl, 2010; Leopoldo et al., 2011; Matthys et al., 2011). The 5-HT_7 receptor is positively coupled to adenylate cyclase with a pharmacological profile distinct from all other 5-HT receptor subtypes. In the central nervous system, 5-HT_7 receptor is most abundant in cortex, hippocampus, thalamus and hypothalamus of both humans and rodents (Varnas et al., 2004). Important physiological roles for the 5-HT_7 receptor have been established in circadian rhythmicity and thermoregulation (Lovenberg et al., 1993; Hagan et al., 2000; Hedlund et al., 2003). Early indications of an involvement of 5-HT_7 receptors in mood disorders came from a study showing downregulation of 5-HT_7 receptor expression after chronic treatment with various antidepressants (Mullins et al., 1999). More recent preclinical studies using selective 5-HT_7 receptor antagonist tool compounds or mice lacking the 5-HT_7 receptor further support a role for 5-HT_7 receptors in depression. For instance, SB-269970 (a selective 5-HT_7 receptor antagonist), like classical selective serotonin reuptake inhibitors (SSRIs), decreased immobility in the tail suspension and forced swim tests, two tests that are widely used as predictors of antidepressant activity (Hedlund et al., 2005; Wesolowska et al., 2006; Bonaventure et al., 2007; Wesolowska et al., 2007; Mnie-Filali et al., 2011). In agreement with these pharmacological data, 5-HT_7 knockout mice showed reduced immobility in both the tail suspension and forced swim tests (Hedlund et al., 2005). In addition, a recent study showed that a 1-week treatment with SB-269970 did not alter 5-HT firing activity, but desensitized cell body 5-HT autoreceptors,

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enhanced the hippocampal cell proliferation, and counteracted the depressive-like behavior in olfactory bulbectomized rats (Mnie-Filali et al., 2011).

Interestingly, both SSRIs and 5-HT_7 receptor antagonists have been shown to induce changes in sleep parameters in rats in a pattern opposite from those in patients with clinical depression. More specifically, in rats, selective 5-HT_7 receptor-selective antagonists and SSRIs, when administered at the beginning of the sleep phase, increased the latency to rapid eye movement (REM) sleep and decreased the amount of time spent in REM sleep (Hagan et al., 2000). Consistent with these observations, 5-HT_7 knockout mice spent less time in and had less frequent episodes of REM sleep (Hedlund et al., 2005).

To the best of our knowledge, no systematic clinical evaluation of the effectiveness of selective 5-HT_7 blockade has been conducted. We report here the preclinical and clinical evaluation of a novel selective 5-HT_7 receptor antagonist, JNJ-18038683 (Supplemental Figure 1). After *in vitro* characterization, the functional 5-HT_7 antagonist activity of JNJ-18038683 was assessed using the 5-CT-induced hypothermia model in conscious rats. It has been shown that mice lacking the 5-HT_7 receptors do not experience either 5-HT or 5-CT-induced hypothermia (Hedlund et al., 2003), and experimental data suggest this hypothermia is a centrally mediated effect (Guscott et al., 2003). JNJ-18038683 was then evaluated for its acute and chronic effect on EEG sleep architecture and then tested in the mouse tail suspension test. In a previous study performed with SB-269970, we had shown that blockade of 5-HT_7 receptor enhanced 5-HT transmission, REM sleep suppression and antidepressant-like behavior induced by citalopram in rodents (Bonaventure et al., 2007). Consistent with these pharmacological data we also demonstrated that 5-HT_7 receptor deletion enhanced REM sleep suppression induced by selective serotonin reuptake inhibitors (Shelton et al., 2009). In the present study, we examined

the effect of a combination of individually sub-efficacious doses of citalopram and JNJ-18038683 on REM sleep, immobility time in tail suspension test and 5-HT release in the prefrontal cortex of freely moving rats.

Following appropriate 1-month GLP toxicological experiments in rat and dog, we received Investigational New Drug approval to conduct a safety, tolerability and pharmacokinetic study in healthy human volunteers. Two polysomnography studies were carried out in healthy subjects to evaluate the effect of the 5-HT_7 receptor antagonist on REM sleep by itself or in combination with citalopram. Following this translational study, a multicentered, randomized, double-blind, placebo and active-controlled, parallel-arm study was run to assess the efficacy of JNJ-18038683 in patients with major depressive disorder.

5. Methods.

All the preclinical studies have been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health.

All clinical studies were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and are consistent with Good Clinical Practices and applicable regulatory requirements. After receiving a complete description of the study and understanding the potential risks and benefits, all healthy subjects/patients gave a signed informed consent.

Animals

The tail suspension test and locomotor activity measurement were performed in male C57BL/6J mice (Jackson Laboratories) weighing 22-30 g. The rat telemetry study (blockade of 5-CT induced hypothermia) were performed in female Sprague-Dawley rats weighing 300-325 g (Charles Rivers, Wilmington, MA). The microdialysis, and sleep/EEG experiments were performed in male Sprague-Dawley rats (Charles River, Wilmington, MA for microdialysis and Harlan, Indianapolis, IN for sleep EEG) weighing 280-350 g (microdialysis) or 400-500 g (sleep EEG).

Animals were allowed to acclimate for at least 7 days prior to investigations after receipt in the facility, were housed in accordance with institutional standards, provided food and water ad libitum, and were maintained on a 12-hour light dark cycle (lights on: 6:00 to 18:00).

Chemicals

JNJ-18038683 was synthesized and prepared as a salt at Janssen Research & Development, LLC. [^{3}H]5-CT was purchased from Amersham (GE Healthcare, Buckinghamshire, UK). 5-HT and SB-269970 were obtained from Tocris Cookson (Ellisville, MO). Citalopram (HBr salt) was purchased from Sigma (Saint Louis, MO). For in vitro assays, JNJ-18038683 and SB-269970 was dissolved in dimethyl sulfoxide (stock solution at 10 mM) and further diluted in assay buffer.

For the preclinical studies JNJ-18038683 was formulated in hydroxylpropyl methylcellulose (telemetry blockade of 5-CT induced hypothermia and sleep EEG, oral administration), 0.9% saline (telemetry blockade of 5-CT induced hypothermia and tail suspension test, intraperitoneal administration) or in 5% Pharmasolve and 95% D5W (5% dextrose solution) (sleep EEG and microdialysis, subcutaneous administration). Citalopram was formulated in 0.9 % saline for the tail suspension test (intraperitoneal administration) or 5% Pharmasolve and 95% D5W (5% dextrose solution) for sleep EEG and microdialysis (subcutaneous administration). For in vivo preclinical work, SB-269970 was formulated in 5% dextrose.

For clinical studies, JNJ-18038683 was formulated as an oral suspension in 0.5% weight/weight (w/w) hypromellose solution.

Primary in vitro pharmacology: 5-HT$_7$ receptor binding and 5-HT$_7$ receptor antagonism

The affinity of JNJ-18038683 for the rat 5-HT$_7$ recombinant receptors was determined by competition binding assays using [^{3}H]5-CT as previously described (Hagan et al., 2000; Thomas et al., 2000). The assays were performed on membranes prepared from HEK-293 cells stably transfected with the rat 5-HT$_{7a}$ receptor or on membranes prepared from rat thalamus tissue.

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Sigmoidal inhibition curves were generated and fitted by nonlinear regression analysis (GraphPad Prism). IC_{50} values (concentration producing 50 % inhibition of specific radioligand binding) were calculated. K_i values were derived according to Cheng and Prussoff (Cheng and Prusoff, 1973). Experiments were conducted in triplicate and at least three separate experiments were run. SB-269970, a standard literature compound was included for comparison purpose. Results were later confirmed against the human 5-HT_7 receptor.

In vitro selectivity of JNJ-18038683 versus a panel of monoamine receptors and neurotransmitter uptake sites was performed using standard radioligand assays as previously described (Schotte et al., 1996). In addition, the selectivity of JNJ-18038683 (1 μM) was also evaluated on a commercially available panel of 50 ion-channels, transporters and receptor-binding assays at CEREP (Celles L'Evescault, France). Experimental conditions for these assays are described on line at www.cerep.fr.

The potency of JNJ-18038683 on the rat 5-HT_7 recombinant receptor was determined with the HitHunter cAMP Assay kit (DiscoveRx, Fremont, CA). Briefly, HEK-293 cells stably expressing the rat 5-HT_7 receptor were pre-incubated for 10 min with seven concentrations of JNJ-18038683 (10 μM – 100 pM) followed by incubation with 100 nM of the agonist (5-HT). cAMP measurements were determined according to protocol described in HitHunter kit. SB-2699970 was used as the reference compound. Results were later confirmed against the human 5-HT_7 receptor. Antagonistic potency values were converted to apparent pK_B values using a modified Cheng Prusoff correction. Apparent pK_B = - log IC_{50}/1+[conc agonist/EC_{50}].

In addition, *in vitro* functional assays were run for h5- HT_{1B} (cAMP measurement), h5-HT_{1D} (cAMP measurement), h5-HT_{2A} (calcium release using FLIPR), h5-HT_{2B} (calcium release using

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FLIPR), h5-HT_{2C} (calcium release using FLIPR) as described in the literature (Lesage et al., 1998; Porter et al., 1999).

Primary in vivo pharmacology: Blockade of 5-CT induced hypothermia in rat

Rat telemetry experiments were performed as previously described (Hedlund et al., 2004). Briefly, telemetric device were implanted in the peritoneal cavity of rats. After a 7-day recovery period, body temperature was measured noninvasively by radiotelemetry. On the day of the experiment, the baseline was monitored for 60 min prior to drug injection. Rat core temperature was continuously recorded, before and after injection, and averaged over each 2-min collection period. JNJ-18038683 (0.09, 0.9 and 9.0 mg/kg) was administered orally 6 hours before 5-CT (0.1 mg/kg, i.p.) administration or intraperitoneally (0.027, 0.09, 0.18, 0.27, 0.9 mg/kg) 20 min before 5-CT (0.1 mg/kg, i.p.) administration (n = 5 in each group). Change in core body temperature was calculated for all animals by comparing baseline prior to dosing to the minimal temperature reached following drug administration. In order to quantify any statistical difference, one-way ANOVA followed by Dunnett's test was conducted using Prism software (GraphPad, San Diego, CA) and the level of significance was $P < 0.05$.

Sleep EEG in rat

Sleep EEG experiments in rats were performed as previously described (Bonaventure et al., 2007). Animals were chronically implanted with electrodes for the recording of electroencephalogram (EEG) and electromyogram (EMG) signals using telemetric devices (Data

Sciences International, St. Paul, MN). Polysomnographic waveforms were analyzed per 10-sec epoch and classified as wake, non-rapid eye movement (NREM) or REM sleep using the computer program SleepSign (Kissei Comtec, Nagano, Japan). For each experiment, EEG and EMG signals were recorded for up to 16 h following administration of the tested compounds at the beginning of the light phase (2 h after light onset). Results were then averaged and expressed as mean ± S.E.M. in defined time intervals.

The dose-response experiment with JNJ-18038683 was carried out in 32 animals which were randomly assigned to four treatment conditions (three doses and vehicle, n=8 per condition). Subsequent statistic analysis of the obtained data was conducted using Wilcoxon-Mann-Whitney rank sum tests in comparison with the vehicle group. The drug combination experiment (co-administration of citalopram and JNJ-18038683) was performed in a separate group of 9 animals which received the four treatment conditions (the two compounds or their corresponding vehicle). To determine if differences were significant between the four conditions, a one-way ANOVA followed by a Neuman-Keuls post hoc test was conducted. Differences were determined to be significant if $P < 0.05$.

The repeated dosing experiment with JNJ-18038683 for 7 days was performed in a separate group of 6 animals. A paired one-way ANOVA followed by a Dunnett's Multiple Comparison post hoc test was executed to assess differences between vehicle and compound treatment for each day.

Tail suspension tests in mice

Mice were dosed intraperitoneally 30 min before testing (n= 8 in each group). Experiments were performed as previously described (Bonaventure et al., 2007). The time spent immobile was totaled for the last four minutes of the six minute test for each animal, averaged for the dose group, and then compared. Statistics were calculated using Prism software (GraphPad, San Diego, CA). The data were presented as the mean ± SEM and evaluated by one-way ANOVA followed by Newman-Keuls multiple comparison test. The level of significance was $P < 0.05$.

Effect of JNJ-18038683 on locomotor activity was also measured as previously described (Bonaventure et al., 2007). In order to quantify any statistical difference, one-way ANOVA followed by Neuman-Keuls test was conducted using Prism software (GraphPad, San Diego, CA) and the level of significance was $P < 0.05$.

Microdialysis in rat

Microdialysis in the prefrontal cortex (incisor bar, –3.5 mm, +3.2 mm anterior, 0.8 mm lateral and 1 mm ventral to Bregma, (Paxinos and Watson, 1997) of freely moving rats (n = 4 in each group) was performed as previously described (Bonaventure et al., 2007). Dialysis samples were analyzed for serotonin, norepinephrine and dopamine by high-performance liquid chromatography (HPLC) with electrochemical detection (ECD). Statistical analyses were performed on the area under the curve (AUC) values by a one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test. The level of significance was $P < 0.05$. Data was graphed and statistics were calculated using Prism software (GraphPad, San Diego, CA).

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Clinical studies

Polysomnogram studies

Two clinical studies were performed to investigate the effect of JNJ-18038683 on the human polysomnogram (PSG): Study 1 was a blinded, placebo- and comparator-controlled study to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of JNJ-18038683 in healthy male subjects and Study 2 was a double-blinded, placebo-controlled, randomized, parallel group, multiple dose study to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of JNJ-18038683 concomitantly administered with citalopram in healthy male subjects. Both studies were approved by the appropriate authorities and local ethics committees prior to the initiation of any study-related procedure. In total, 48 healthy male subjects who had signed the Informed Consent Form were included.

Study 1

Study 1 was performed at Guy's Drug Research Unit, Quintiles Ltd, London, UK. Initially, 12 subjects (Cohort 1) were included who received a 100 mg loading dose of JNJ-18038683 on day 1 followed by 20 mg JNJ-18038683 on Days 2 and 3, 20 mg citalopram on days 1 – 3, and Placebo on Days 1 – 3 in a 3-way crossover study. Twelve additional subjects were included in Cohort 2 to investigate the effect of JNJ-18038683 at up to 4-fold lower dose levels. Cohort 2 results are not further described but suggest that lower JNJ-18038683 dose levels are efficacious. PSG was recorded thrice on separate study days using Medilog ambulatory recorders using silver/silver chloride stick on electrodes placed according to the international 10-20 system. PSG recordings were reviewed by an EEG study scientist while blind to treatment allocation. Results obtained overnight following the last dose administration (Day 3 to 4) were considered primary effect parameters. Between study periods, there was a washout period of at least 14 days.

REM sleep after dosing was analyzed by repeated measures ANOVA on Day 1/2 and Day 3/4.

Study 2

Study 2 was performed at QPS Netherlands, Groningen, The Netherlands. Twenty-four subjects received 20 mg citalopram once daily (*qd*) from Day 1 to 17. On Day 8, subjects were randomized to receive either 20 mg JNJ-18038683 *qd* (n=12) or placebo (n=12) in addition to citalopram. PSG was recorded overnight on Day -1 to 1 (baseline), Day 1 to 2, Day 7 to 8, Day 10 to 11, and Day 17 to 18. PSG was recorded using TMS Refa ambulatory recordings using silver/silver chloride stick electrodes placed according to the international 10-20 system. PSG recordings were reviewed by an EEG study scientist while blind to treatment allocation. Subjects were hospitalized from Day 8 until Day 22. While hospitalized, regular assessments of safety and tolerability were obtained. Blood samples were collected for the measurement of JNJ-18038683 plasma concentrations at regular time-points on Days 8 and Day 17 and for the measurement of S and R citalopram on Day 17. The following pharmacokinetic parameters were derived from JNJ-18038683 and citalopram plasma concentrations: plasma concentration measured immediately before dosing ($C_{predose}$); observed maximum plasma concentration after first (Day 8, JNJ-18038683 only) and last dose (C_{max}); area under the plasma concentration-time curve from 0 to 24 hours after the first (Day 8, JNJ-18038683 only) and last dose dose (AUC_{0-24h}); the ratio of the C_{max} and AUC_{0-24h} of the last dose over the first dose multiplied by 100% (F_{rel}; JNJ-18038683 only).

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REM onset latencies after dosing were analyzed by ANOVA on Day 10/11 and Day 17/18. The ANOVA used treatment, day, and treatment-by-day interaction as factors and Day 7/8 REM latency as the baseline covariate.

Major Depressive Disorder study

A multi-center, double-blind, randomized, double-dummy, placebo- and active controlled, parallel-design study in subjects with moderate to severe MDD was conducted to evaluate the safety, tolerability and efficacy of JNJ-18038683 as an antidepressant. A total of 230 men and women diagnosed with moderate to severe MDD (based on a HAMD-17 score ≥ 25), aged 18 to 60 years, and who met the inclusion and exclusion criteria participated in the study. This study consisted of a screening period (up to 1 week), a washout period (7 days or 5 half-lives of the drug to be washed out, whichever was shorter), 7 weeks of treatment, a 1-week follow-up period during which subjects who received citalopram received a reduced dose and subjects who received JNJ-18038683 received placebo, and a follow-up visit at the end of the follow-up period.

Subjects were randomly assigned to receive a treatment regimen of 20 mg of JNJ-18038683, 20 mg of citalopram, or placebo in a 1:1:1 fashion. For the JNJ-18038683 treatment group, subjects started at doses of 10 mg once daily for the first week (Week 1), which was increased to 20 mg once daily for the remaining 6 weeks (Weeks 2-7). The JNJ-18038683 treatment group received placebo during the follow-up period (Week 8).

In the citalopram treatment group, subjects started dosing at 10 mg once daily for the first week (Week 1), and during the second week (Week 2), the dose increased to 20 mg once daily for the

remaining 6 weeks (Weeks 2-7). During Week 8, the citalopram treatment group received doses of 10 mg once daily in order to taper the medication in accordance with the citalopram label.

The efficacy analysis set for all analyses was based on the intent-to-treat population, including all randomized subjects who received at least 1 dose of study treatment and had at least 1 post-baseline MADRS total score measurement.

The sample size was calculated based on the primary endpoint (change in MADRS total score from Baseline to Week 7). The desired sample size for each arm was approximated using a 2-sample t-test with a 2-sided alpha level equal to 0.05, in order to produce an 80% power to detect a between-group difference (to placebo) of 4 with a standard deviation of 8.4. Assuming a drop-out rate of 5% by the end of Week 1 (titration period), a total of 225 (75 per arm) subjects were to be enrolled to ensure the availability of approximately 71 subjects per arm with at least 1 post-baseline efficacy measurement.

The primary efficacy variable was change from baseline to Week 7 in MADRS total score. Key secondary efficacy endpoints included change from baseline to Week 7 in CGI-S and Week 7 proportion of MADRS responders (≥50% improvement from baseline in MADRS total score). Other secondary efficacy endpoints included change from baseline to Week 7 in MADRS-6, HAMD-17, HAMD-6 (O'Sullivan et al., 1997), Epworth Sleepiness Scale (ESS), Global Assessment of Function (GAF), Sleep Assessment (SA) scores (Items 1-4), as well as the Week 7 proportion of HAMD-17 responders (subjects who had at least a 50% improvement from baseline).

Safety assessments included reported adverse events, clinical laboratory tests, vital signs measurements, physical examinations, electrocardiogram (ECG) findings, suicide monitoring using the Suicide Tracking Scale (STS), and the Arizona Sexual Experience Scale (ASEX). All safety analyses were performed based on the safety analysis set, which includes all subjects who were randomly assigned to a treatment and received at least 1 dose of a study drug.

The statistical significance level for the primary endpoint comparison of JNJ-18038683 versus placebo was at 5% (2-sided test). The comparison of escitalopram versus placebo (5%, 2-sided) based on the primary endpoint was carried out for the purpose of assay sensitivity; therefore, no multiplicity adjustment was required for these 2 comparisons.

To control the Type-I error inflation introduced by the 2 key secondary efficacy endpoints, a step-down procedure was used without further adjustment of test multiplicity: the statistical test based on the Week-7 change in CGI-S score was performed given the significance of the test using the primary endpoint (Week 7 change in MADRS score), and the statistical test based on MADRS responders was performed given the significance of the test using the Week-7 change in CGI-S score. In addition, for exploratory purposes, both key secondary efficacy endpoints and other secondary endpoints were compared with placebo separately, and no multiplicity adjustment was performed.

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6. Results.

JNJ-18038683 is a high affinity relatively selective 5-HT$_7$ receptor antagonist

The affinity of JNJ-18038683 for the rat and human 5-HT$_7$ receptor binding site was evaluated by competition radioligand binding assay using [^{3}H]5-CT as a radioligand (Table 1). JNJ-18038683 displaced, with high affinity, specific [^{3}H]5-CT binding sites from rat and human 5-HT$_7$ receptor expressed in HEK-293 cells (pK_i = 8.19 ± 0.02 and 8.20 ± 0.01, respectively). Similar values were obtained on the native 5-HT$_7$ in membranes from rat thalamus (pK_i = 8.50 ± 0.20). Hill slope values were close to unity suggesting one site competitive binding.

Antagonist potency of JNJ-18038683 was determined by measurement of adenylate cyclase activity in HEK-293 cells expressing the human or rat 5-HT$_7$ receptor (Table 1). 5-HT stimulated adenylyl cyclase activity in rat and human 5-HT$_7$/HEK293 cells with a pEC_{50} of 8.09 and 8.12 respectively. JNJ-18038683 produced a concentration dependent decrease of 5-HT (100 nM) stimulated adenylyl cyclase. The pK_B values determined for JNJ-18038683 were in good agreement with the corresponding K_i determined from [^{3}H]5-CT binding studies (Table 1).

SB-269970, which was also included for comparison purpose, showed affinity and antagonist potency values similar to JNJ-18038683 (Table 1).

In vitro selectivity of JNJ-18038683 versus a panel of monoamine receptors and neurotransmitter uptake sites was performed using standard radioligand binding and functional assays (Supplemental Table 1). JNJ-18038683 showed 10-fold selectivity over h5-HT$_6$ receptor, 15-fold selectivity over rat adrenergic α_1 receptor, 14-25 fold selectivity over the h5-HT$_2$ receptor

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subtypes and 20-fold selectivity over h5-HT_{1B} receptor. JNJ-18038683 exhibited greater than 30-fold selectivity versus all the other receptors tested in this study. JNJ-18038683 was further tested for its functional properties on h5-HT_{1B}, h5-HT_{2A}, h5-HT_{2B}, h5-HT_{2C} and hα_{1B} receptors. JNJ-18038683 behaved as an antagonist on all these receptor with moderate potency (pK_B : h5-HT_{1B} = 6.6 ± 0.1; h5-HT_{2A} = 6.6 ± 0.1, h5-HT_{2B} < 6, h5-HT_{2C} = 6.5 ± 0.1 and hα_{1B} = 6.6 ± 0.3). JNJ-18038683 had no agonistic activity on any of the receptor tested in this study (up to 10 μM).

JNJ-18038683 blocks 5-CT induced hypothermia in rats

The functional 5-HT_7 antagonist activity of JNJ-18038683, given orally or intraperitoneally, was assessed using the 5-CT-induced hypothermia model in conscious rats (Supplemental Figure 2A and 2B). Pharmacokinetic studies in rats had shown that, when given orally, JNJ-18038683 exhibited slow absorption characteristics with a maximum concentration (C_{max}) observed around 6 hours after dosing (data not shown). Thus, JNJ-18038683 (0.09 to 9.0 mg/kg) was administered orally 6 hours before 5-CT (0.1 mg/kg, i.p.) administration. JNJ-18038683 antagonized the 5-CT-induced hypothermia in a dose-related manner following either oral

or intraperitoneal administration (Supplemental Figure 2A and 2B). In this assay, JNJ-18038683 was equipotent (ED_{50} = 0.40 ± 0.2 mg/kg) to SB-269970 (ED_{50} = 0.3 ± 0.2 mg/kg, not shown). The plasma concentration of JNJ-18038683 following oral or intraperitoneal administration of the 0.9 mg/kg dose which produced a near full blockade of 5-CT induced hypothermia was approximately 20-40 ng/mL as determined in parallel studies (data not shown).

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The results of the *in vitro* binding studies show that JNJ-18038683 has some affinity for α_1 adrenergic and 5-HT_{2A} receptors (Supplemental Table 1). The functional consequences of this were evaluated *in vivo*, in phenylephrine-induced mydriasis in mice (a model of α_1 adrenergic activity) and antagonism of (±)-2,5-dimethoxy 4 iodoamphetamine (DOI)-induced head-twitches in mice (a standard test for 5- HT_{2A} antagonism). At plasma concentrations that nearly fully blocked 5-CT induced hypothermia in rats, JNJ-18038683 did not have any effect on phenylephrine-induced mydriasis or DOI induced-head twitches (data not shown). At higher doses which corresponded to a plasma concentration >50-fold above the plasma exposure required to elicit action in the 5-CT induced rat hypothermia model JNJ-18038683 had moderate *in vivo* α_1 adrenergic and 5-HT_{2A} activity (~ 40 % inhibition of phenylephrine-induced mydriasis or DOI induced head twitches).

JNJ-18038683 preclinical profile as an antidepressant

REM sleep suppressive effect in the rat

Dose-response effects of JNJ-18038683 (1, 3 and 10 mg/kg) on sleep-wake parameters were evaluated in rats orally dosed at the beginning of the light phase. As illustrated in Figure 1, JNJ-18038683 dose-dependently suppressed REM sleep mainly during the first 4 h following the treatment. The duration of REM sleep was significantly decreased from the dose of 1 mg/kg onwards ($P < 0.05$) during the first 4 h after oral administration (Figure 1A). Concomitantly, the REM sleep latency tended to be prolonged in a dose-related manner with a significant increase in REM latency occurring only at the highest dose tested (10 mg/kg, $P < 0.05$, Figure 1B). These alterations in REM sleep appeared to be state-specific since no major changes in the duration of

either NREM sleep or wake were observed (data not shown). JNJ-18038683 did not induce changes in spontaneous activity, heart rate, blood pressure, or body temperature in rats at doses that clearly affected sleep-wake organization (data not shown).

A separate study was conducted to determine if repeated administration of JNJ-18038683 for seven days would result in an adaptation of the EEG sleep response in particular on REM sleep in rats during the course of the treatment and after its discontinuation. JNJ-18038683 was administered for 7 consecutive days (1 mg/kg s.c. per day) at two hours into the light phase. EEG and EMG signals were recorded on vehicle control day, on days 1, 3, 5 and 7 of compound administration, and on two consecutive recovery days (R1 and R2 following vehicle injection). On the first day of treatment, JNJ-18038683 produced a significant decrease in the time spent in REM sleep during the first 8 hours following the injection (Figure 1C) and a prolongation of the REM sleep latency (Figure 1D). The REM sleep latency was increased during the 7-day repeated treatment, and was normalized on the first recovery day after cessation of treatment. The significant decrease in REM sleep time was maintained during the 7-day repeated treatment, with a rebound occurring on the first recovery day after treatment discontinuation. The NREM sleep latency and the total NREM sleep time were not affected during the entire treatment (data not shown).

Antidepressant-like activity in the mouse tail suspension test

Antidepressant-like activity was investigated in the tail suspension test (Figure 2). JNJ-18038683 administered intraperitoneally at doses of 0.3, 0.5 and 1 mg/kg significantly decreased the immobility time compared with vehicle treated mice by 31% ($P < 0.05$ versus vehicle), 39%

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(P < 0.01 versus vehicle) and 59% (P < 0.01 versus vehicle), respectively (Figure 2). JNJ-18038683 (1 mg/kg i.p.) did not significantly change locomotor activity compared with vehicle (P > 0.05 versus vehicle, data not shown).

Effect of combination of sub-efficacious doses of citalopram and JNJ-18038683 on REM sleep, 5-HT transmission and antidepressant-like behavior

In a previous study performed with SB-269970 we had shown that blockade of 5-HT_7 receptor enhanced REM sleep suppression, antidepressant-like behavior and 5-HT transmission induced by citalopram in rodents (Bonaventure et al., 2007). Therefore, we evaluated the effect of a combination of individually sub-efficacious doses of citalopram and JNJ-18038683 on REM sleep, immobility time in tail suspension test and 5-HT release in the prefrontal cortex of freely moving rats (Figure 3).

To determine the sub-efficacious dose of test compounds on REM sleep latency and duration, a pilot study was conducted with citalopram (1 mg/kg, s.c.) and JNJ-18038683 (0.3 mg/kg, s.c.). These doses were found to elicit minimal effects on EEG sleep parameters in rats. Citalopram induced a moderate but significant REM sleep inhibition as evidenced by an increase in REM sleep latency (P <0.05, Figure 3A) and a decrease in REM sleep duration (P <0.05, Figure 3B) during the first 8 h after administration compared with vehicle treatment. JNJ-18038683 had no effect when injected alone but did potentiate the REM sleep suppressive effect of citalopram. Compared with citalopram alone, the combination of citalopram with JNJ-18038683 produced an additional delay of the latency to REM sleep (+60 min, P < 0.001, Figure 3A) and further reduced the time spent in REM sleep (-12 min, P <0.001, Figure 3B). At the doses tested in this

study, citalopram and JNJ-18038683 injected either alone or in combination did not influence the NREM sleep latency and duration or the time spent in wake.

A tail suspension test experiment was performed with a combination of sub-efficacious dose of citalopram and JNJ-18038683 (Figure 3C). Citalopram (1 mg/kg, i.p.) or JNJ-18038683 (0.1 mg/kg, i.p.) alone did not significantly change immobility time compared with vehicle treated mice (Figure 3C). In contrast, co-administration of citalopram (1 mg/kg, i.p.) and JNJ-18038683 (0.1 mg/kg, i.p) significantly decreased the immobility time versus vehicle ($P < 0.001$). The combination of JNJ-18038683 and citalopram had no effect on locomotor activity.

The effect of various doses of citalopram alone (1, 3, 5, 10 mg/kg, i.p.) or in combination with a fixed dose of JNJ-18038683 (1 mg/kg, i.p.) on immobility time was also tested (data not shown). The effect of citalopram on immobility time was significantly enhanced by co-administration of 1 mg/kg JNJ-18038683 (21% versus 49%, $P < 0.001$; 36% versus 70%, $P < 0.001$; 55% versus 80 %, $P < 0.001$; 74% versus 84%, $P < 0.05$).

The effect of a combination of sub-efficacious dose of citalopram and JNJ-18038683 on 5-HT release in cortex was tested using microdialysis in freely moving rats (Figure 3D). A dose response of citalopram was tested and 0.05 mg/kg was determined to be the sub-efficacious dose on prefrontal 5-HT release. Absolute basal levels of serotonin, dopamine and norepinephrine in dialysate from the rat frontal cortex (without adjusting for probe recovery) were 0.048 ± 0.003 pg/µL (n=16), 0.089 ± 0.005 pg/µL (n=16), 0.231 ± 0.016 pg/µL (n=16), respectively.

Subcutaneous injection of JNJ-18038683 (1 mg/kg) did not increase extracellular 5-HT concentration compared with vehicle treated rats (Figure 3D). Co-administration of an ineffective dose of citalopram (0.05 mg/kg, s.c.) with JNJ-18038683 (1 mg/kg, s.c.) resulted in a

significant increase in extracellular concentration of 5-HT (Figure 3D, $P < 0.001$). Neither citalopram (0.05 mg/kg, s.c.), JNJ-18038683 (1 mg/kg, s.c.) or co-administration of citalopram and JNJ-18038683 induced significant change in extracellular concentration of dopamine or norepinephrine (data not shown).

Good Laboratory Practice toxicological studies were conducted in rat and dog and indicated that JNJ-18038683 has a suitable safety profile to allow testing in humans. JNJ-18038683 was well tolerated in single and multiple dose studies of up to 13 weeks duration. In both rats and dogs, the primary toxicities were neurologic (ptosis and clinical signs of sedation) or gastrointestinal (decreased food consumption, salivation, emesis, fecal changes). In standard tests to assess embryo-fetal safety, genotoxicity, and phototoxicity potential, JNJ-18038683 was also shown to be well tolerated.

During the single and multiple ascending-dose studies, no serious adverse events were reported and most adverse events were mild indicating that JNJ-18038683 is well tolerated in healthy volunteers.

JNJ-18038683 Reduces REM Sleep Duration in Healthy Human Volunteers

Study 1

In the pre-clinical pharmacology models, the dose for maximal inhibition of REM sleep was measured at plasma concentrations of 20-40 ng/mL. Pharmacokinetic modeling indicated that a loading dose of 100 mg of JNJ-18038683 on day one followed by 2 maintenance doses of 20 mg

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on day two and three predicted a plasma concentrations > 20ng/mL on average throughout the dosing interval.

Twelve subjects (19 – 41 years) eligible by medical history and screening examination who signed the informed consent form were included in Cohort 1. All subjects completed the study. Study treatments were overall well tolerated and the nature of treatment emergent adverse events was not different between the different treatment groups.

Both JNJ-18038683 (100/20/20 mg) and citalopram (20 mg) significantly increased the time to the first REM sleep episode relative to placebo (Table 2). Total REM sleep time was also significantly reduced both following JNJ-18038683 and citalopram (Table 2).

The following JNJ-18038683 plasma pharmacokinetic parameters were measured on day 3 following administration of a loading dose of 100 mg on day 1 followed by 20 mg on days 2 and 3: T_{max} = 6 hours, C_{max} = 56.7 ± 14.6 ng/mL and $AUC_{0\text{-}24h}$ = 1,222 ± 343 ng.h/mL.

Study 2

Twenty-four healthy subjects (18 – 55 years) eligible by medical history and screening examination who signed the informed consent for were included. All subjects completed the study. Overall, the incidence and nature of the adverse events were not significant between the two treatment groups and were mild to moderate in intensity.

Plasma concentrations of JNJ-18038683 accumulated over the 10-day dosing period (C_{max}: 4-fold and $AUC_{0\text{-}24h}$: 4.8-fold). Day 17 JNJ-18038683 plasma exposures were comparable to those measured following administration of 100/20/20 mg JNJ-18038683 in Study 1. The peak – trough variation on Day 17 was approximately 1.6-fold. In the presence of JNJ-18038683 C_{max}

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and $AUC_{0\text{-}24h}$ values for S- and R-citalopram appeared relatively increased compared to the placebo condition (Table 3).

REM onset latency in subjects treated with placebo plus 20 mg citalopram (Group I) increased from 86 ± 10.44 (mean ± S.E.M) minutes at baseline to maximally 176.18 ± 16.40 minutes on Day 7/8 (Figure 4A). Relative to Day 7/8, the REM onset latency was somewhat decreased on Days 10/11 and 17/18 with -19. 4 and 33.4 minutes, respectively. In subjects treated with 20 mg JNJ-18038683 plus 20 mg citalopram (Group II), the increase in REM onset latency from baseline to Day 7/8 (Citalopram only treatment) was comparable to subjects assigned to Group I: from 69.9 ± 7.56 on Day -1/1 to 177.17 ± 20.73 minutes on Day 7/8. However, in contrast to subjects assigned to Group I, REM onset latency further increased to 200.43 ± 25.79 on Day 10/11 and 214.88 ± 32.27 on Day 17/18 in Group II subjects (Figure 4A). Compared to Group I, the relatively larger increase in REM onset latency on Day 17/18 in subjects treated with 20 mg JNJ-18038683 + 20 mg citalopram was borderline significant (P = 0.0678).

In both groups of subjects, total REM time (Figure 4B) was reduced relatively to baseline on Day 1/2 (-42.1 minutes) with a rebound on Day 7/8 (during which period only citalopram was administrated): Group I [placebo plus 20 mg citalopram]: 103.18 ± 6.36 (Day -1/1), 58.73 ± 3.40 (Day 1/2), and 93 ± 5.56 (Day 7/8); Group II [20 mg JNJ-18038683 plus 20 mg placebo]: 103.3 ± 8.59 (Day -1/1), 53.1 ± 5.14 (Day 1/2), and 80.18 ± 6.66 (Day 7/8). Compared to combined treatment with placebo plus 20 mg citalopram, treatment with 20 mg JNJ-18038683 plus 20 mg citalopram significantly suppressed total REM duration on Days 10/11 (-52.46 minutes; P = 0.0071) and 17/18 (-49.5 minutes; P = 0.0136).

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Depression study: No significant difference from placebo for either JNJ-18038683 or citalopram

Neither JNJ-18038683 nor the active comparator, citalopram, demonstrated a statistically significant improvement over placebo in the primary efficacy endpoint (Figure 5). The mean change from Baseline to Week 7 in total MADRS score was –13.8 for the placebo group, -15.2 for the JNJ-18038683, and –13.5 for the citalopram group. Additionally, neither JNJ-18038683 nor citalopram achieved statistical significance over placebo in any secondary measure of efficacy (Supplemental data Table 2). This was a failed study due to lack of assay sensitivity, rather than a negative study.

Nearly half of all trials in major depression fail and a high placebo response is the most common reason for these failures (Khin et al., 2011). Recent studies have suggested that the high placebo response maybe from a few sites (Merlo-Pich et al 2010). A posthoc analysis was performed using an enrichment window strategy (Merlo-Pich et al., 2010) to learn about the role of placebo response specific by site. Lower boundary was defined as: MADRS score of less than or equal to 12 (an implausible high reduction for any treatment group) at end point in placebo group and sites where these is less than a 10% reduction in the placebo arm (MADRS >=28) (Merlo Pich, 2011). Using this band pass approach, the data showed a clinically meaningful difference between JNJ-18038683 and placebo that trended towards a statistical significance (p=0.057) (Table 4 and Supplemental Table 3). The difference between citalopram and placebo was not significant (p= 0.353).

A total of 6 serious adverse events (2 in the placebo group and 4 in the citalopram group) were reported during the study. A total of 10 subjects (3 in the placebo group, 4 in the JNJ-18038683

group, and 3 in the citalopram group) were withdrawn from the study prematurely due to adverse events. Adverse events that were reported in more than 5% of subjects in any treatment group included headache, nausea, nasopharyngitis, insomnia, somnolence, dyspepsia, dizziness, dry mouth, constipation, diarrhea, vomiting, back pain, upper respiratory tract infection, palpitations, and fatigue. The only reported case of euphoric mood occurred in the placebo group.

There were no clinically significant changes in laboratory values or physical examination findings in the 3 treatment groups.

Mean heart rate was slightly elevated in the JNJ-18038683 group as measured by both vital signs and ECG. Mean supine-to-standing diastolic blood pressure decreased slightly in the JNJ-18038683 group, but no subject in the JNJ-18038683 group met the criteria for orthostatic hypotension as predefined in the statistical analysis plan (decrease in systolic blood pressure >20 mmHg or diastolic BP >10 mmHg and an increase in heart of >15 bpm) at any timepoint.

Mean changes from Baseline in Suicide Tracking Score scores were similar for all treatment groups. Mean (ASEX) scores for men and women in the placebo and JNJ-18038683 treatment groups were decreased compared to Baseline, and mean ASEX scores for men and women in the escitalopram group were increased compared with Baseline (higher score indicates increased sexual dysfunction).

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7. Discussion.

Recently, it has been proposed that selective blockade of 5-HT_7 receptors may be a novel therapeutic approach for achieving antidepressant action (Stahl, 2010). Here, we report the preclinical and clinical characterization of JNJ-18038683, the first relatively selective small molecule 5-HT_7 receptor antagonist with suitable drug-like properties for examination in humans. In rodents, JNJ-18038683 increased the latency to REM sleep and decreased REM duration. The compound was also effective in the mouse tail suspension test. In addition, JNJ-18038683 enhanced 5-HT transmission, antidepressant-like behavior, and REM suppression induced by citalopram in rodents. Nonclinical safety studies indicated that JNJ-18038683 has a suitable safety profile to allow testing in humans. JNJ-18038683 was well tolerated in humans and the effect of 5-HT_7 blockade on REM sleep translated from rodent to humans demonstrating target engagement. The augmentation of citalopram effect on REM sleep by JNJ-18038683 was also observed in human but citalopram plasma concentration was also increased. Finally the compound was evaluated in a depression clinical trial but the lack of separation observed with the positive control group indicated a failed study, thus rendering interpretation of the lack of separation in the JNJ-18038683 group data inconclusive.

The recent availability of selective 5-HT_7 receptor antagonists and of 5-HT_7 receptor knockout mice has considerably advanced the understanding of the physiological function of this receptor in rodents (Leopoldo et al., 2011; Matthys et al., 2011). SB-269970 is the most commonly used compound in the literature but it has a relatively short half-life in preclinical species and poor drug-like properties (Hagan et al., 2000). We set out to identify a suitable 5-HT_7 receptor antagonist for clinical studies. Following a high throughput screening campaign and a medicinal chemistry effort, JNJ-18038683 was synthesized and characterized. *In vitro*, the JNJ-18038683

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has similar affinity/potency for the human and rat 5-HT_7 receptor compared to SB-269970. *In vivo*, JNJ-18038683 profile in rodent was very similar to SB-269970. The functional 5-HT_7 antagonist activity of JNJ-18038683 was demonstrated using the 5-CT-induced hypothermia model in conscious rats. JNJ-18038683 efficacy appears to correlate well with the C_{max}, thus demonstrating a pharmacokinetic / pharmacodynamic relationship. JNJ-18038683 was equipotent to SB-269970 after intraperitoneal administration. JNJ-18038683 was also found to be efficacious after oral administration of a low dose in this model. The results of the *in vitro* binding studies show that JNJ-18038683 has moderate affinity for α_1 adrenergic 5-HT_{2A} and 5-HT_6 receptors. However, the *in vivo* functional consequences of this cross reactivity at α_1 and 5-HT_{2A} receptor were minimal and a margin greater than 50-fold was established using the antagonism of phenylephrine-induced mydriasis and antagonism of DOI-induced head-twitches models, respectively. The 5-HT_6 receptor has not been associated with any clinical liability, and several selective 5-HT_6 receptor ligands entered the clinical development as potential anti-dementia, antipsychotic and anti-obese drugs (Marazziti et al., 2011). A noteworthy example, RO-4368554, a selective 5-HT_6 receptor antagonist has no effect on REM sleep in rats (Morairty et al., 2008).

Despite some encouraging preclinical data, there is currently no suitable 5-HT_7 PET tracer available for clinical studies (Andries et al., 2011). Therefore we decided to use the effect of JNJ-18038683 on REM sleep as a biomarker for target engagement in humans. Consistent with the data reported for SB-269970 and other 5-HT_7 receptor antagonists (Hagan et al., 2000; Thomas et al., 2003), JNJ-18038683 was also found to increase REM sleep latency and decrease REM duration after oral administration in rats. In agreement with our data obtained with SB-269970 (Bonaventure et al., 2007) and by using 5-HT_7 knockout mice (Shelton et al., 2009), JNJ-

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18038683 was also found to enhance REM suppression induced by citalopram in rodents. Furthermore, total REM sleep time was decreased during the 7-day repeated treatment in rats, with a rebound on the first recovery day after treatment cessation. Similarly, REM sleep rebound occurs following SSRI abrupt discontinuation in volunteers and depressed patients (Sharpley and Cowen, 1995). In healthy volunteers, JNJ-18038683 was found to increase REM latency and to decrease REM sleep duration demonstrating that the effect of 5-HT_7 blockade on REM sleep translated from rodent to humans similarly to other antidepressants, in particular SSRIs (Sharpley and Cowen, 1995). The 5-HT_7 receptor antagonist was more effective in decreasing REM sleep duration than increasing REM latency in both rats (Figure 1A and 1B) and humans (Table 2). JNJ-18038683 also enhanced REM suppression induced by citalopram in human again with a more pronounced effect on REM sleep time than on REM latency (Figure 4). However, JNJ-18038683 was also found to increase plasma concentration of both S- and R-citalopram. Citalopram is metabolized by CYP3A4, 2C19, and 2D6 with a somewhat more efficient conversion of the S enantiomer (Olesen and Linnet, 1999; von Moltke et al., 2001). *In vitro*, JNJ-18038683 was characterized as a potential inhibitor of CYP2D6 with negligible effects on CYP3A4 and CYP2C19 (IC_{50} > 30 µM; *data on file*). However, JNJ-18038683 was demonstrated to be a mechanism-based inhibitor of CYP2C19, which is estimated to account for approximately 40% of the intrinsic clearance of citalopram (Olesen and Linnet, 1999; von Moltke et al., 2001). Mechanism based enzyme inhibition is associated with irreversible or quasi-irreversible loss of enzyme function, requiring synthesis of new enzyme before activity is restored. Conceivably, the effect of JNJ-18038683 on R- and S-citalopram levels was related to time-dependent inactivation of CYP2C19. An effect on CYP2C19 polymorphisms on citalopram metabolism has also been demonstrated clinically (Noehr-Jensen et al., 2009). Investigations of

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the 5-HT transporter occupancy using positron emission tomography (PET) have demonstrated an average 5-HT transporter occupancy after a single dose of 20 mg citalopram of seventy percent whereas 20 mg escitalopram led to a 5-HT transporter occupancy of seventy five percent (Klein et al., 2006). The E_{max} was slightly higher after administration of citalopram (84%) than escitalopram (79%). Thus, 5-HT transporter occupancy in our study is expected to have been near maximal with limited additional effect on the increased R- and S-citalopram levels in the JNJ-18038683 interaction arm. Therefore, although the observed drug-drug interaction (DDI) may have implications for the safety and tolerability of both drugs when administered in combination, it is less likely that the increase in R- and S-citalopram plasma concentrations is responsible for the pharmacodynamic interaction. Indeed, our interaction studies in rodents with SB-269970 showed that rat plasma and brain concentration of citalopram were not affected by coadministration of SB-269970 (Bonaventure et al., 2007) and 5-HT_7 receptor deletion enhanced REM sleep suppression induced by selective serotonin reuptake inhibitors (Shelton et al., 2009).

The previous studies argue for a pharmacodynamic rather than pharmacokinetic mechanism.

Regardless of the drug-drug interaction finding, the effect observed on REM sleep after oral administration of JNJ-18038683 demonstrates target engagement. Therefore, a multi-center, double dummy, placebo and active controlled parallel-design study in subjects with moderate to severe MDD was conducted. Overall, 20 mg of JNJ-18038683 was safe and well tolerated. The depression study was a failed study i.e. lacked assay sensitivity, with neither the JNJ-18038683 nor citalopram treatments demonstrating statistical significance over placebo in all efficacy measures. Therefore, it is not possible to establish a definitive conclusion regarding the efficacy of JNJ-18038683 in the treatment of MDD. A posthoc analysis using an enrichment window strategy, where all the efficacy data from sites with an implausible high placebo response

(MADRS <=12) is removed and also efficacy data from sites with no placebo response (<10% improvement, MADRS >=28) showed a clinically meaningful difference between JNJ-18038683 and placebo that trended towards statistical significance (p=0.057) (Table 4). This methodology cannot be included in a protocol prospectively as it will introduce operational bias in that scheme. Once known it may affect behavior of the sites. However it is very useful as a post hoc analyses, especially in cases of failed studies as it is expected to improve signal detection in proof of concept studies and may help prevent discontinuation of promising compounds in early development (Merlo-Pich et al., 2010).

In conclusion, we successfully demonstrated that the effect of JNJ-18038683 on REM sleep translated from rodent to human. Because of the failed MDD study due to assay sensitivity, it is not possible to establish a definitive conclusion regarding the efficacy of JNJ-18038683 in the treatment of MDD. More clinical studies are needed to assess the clinical potential of 5-HT_7 antagonism.

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11. Legends for figures.

Figure 1: Dose-dependent effect of JNJ-18038683 (1, 3 and 10 mg/kg) on (A) REM sleep duration and (B) REM sleep latency during the first 4 hours following the oral administration in rats. Effects of repeated administration of JNJ-18038683 (1 mg/kg s.c. per day) on REM sleep. (C) Total duration of REM sleep during the first 8 hours and (D) REM sleep latency for the seven days of treatment (D1 to D7) and two recovery days following vehicle injection (R1, R2) Results are expressed in minutes and represent means ± S.E.M. of n=8 animals (acute dose response) or n=6 animals (repeated administration). * $P < 0.05$ based on Wilcoxon-Mann-Whitney rank sum tests in comparison with the vehicle group (acute dose response). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ versus vehicle based on one-way ANOVA followed by a Dunnett's Multiple Comparison post hoc test (repeated administration).

Figure 2: Dose-dependent effects of JNJ-18038683 in the tail suspension test in mice. Drugs were administered i.p., 30 min before the test. Citalopram at 5 mg/kg (i.p.) was included for comparison. Data bars represents the means ± S.E.M., n = 8, * $P < 0.05$, ** $P < 0.01$, versus vehicle group.

Figure 3: Effects of coadministration of citalopram (CIT) and JNJ-18038683 ('683) on REM sleep, antidepressant-like behavior and 5-HT transmission at doses (mg/kg s.c.) defined for each experiment. (A) REM sleep latency and (B) duration during the first 8 hours in rats are expressed in minutes and represent means ± S.E.M. of 9 animals. (C) Immobility time in the mouse tail suspension test is expressed in minutes and bars represent means ± S.E.M. of 8 animals per

group. (D) Extracellular 5-HT release in rats is represented by area under the curve values (means ± S.E.M. of 4 animals per group). * $P < 0.05$, ** $P < 0.01$ and *** $P< 0.001$ based on one-way ANOVA followed by Newman-Keuls post hoc test for all experiments.

Figure 4: Effect of 20 mg citalopram and the combined treatment of 20 mg citalopram and placebo (open squares) or 20 mg citalopram and 20 mg JNJ-18038683 (filled squares) on REM sleep onset latency (minutes ± SE) (A) and total REM sleep time (B) in healthy subjects (n =12). All subjects received 20 mg citalopram from Day 1 – 7 and were randomized to concurrently receive for 10 days placebo or 20 mg JNJ-18038683 on Day 8. ** $P < 0.01$ by ANOVA on Day 10/11 and Day 17/18. The ANOVA used treatment, day, and treatment-by-day interaction as factors and Day 7/8 REM latency as the baseline covariate.

Figure 5: Primary efficacy endpoint from MDD study, longitudinal profile of mean decrease from baseline in MADRS total score (placebo n = 71, JNJ-18038683 n = 72, citalopram n = 75).

12. Tables.

Table 1: In vitro affinity and antagonist potency values of JNJ-18038683 compared to SB-269970 at human and rat 5-HT_7 receptor. Affinity values were determined by radioligand binding using [^{3}H]5-CT. Antagonist potency values were determined using inhibition of 5-HT stimulated cAMP activity. Data are the mean ± S.E.M from at least three separate experiments.

Receptor	JNJ-18038683 In Vitro Binding pK_i	JNJ-18038683 In Vitro Functional pK_B	SB-269970 In Vitro Binding pK_i	SB-269970 In Vitro Functional pK_B
h5-HT_7 (recombinant)	8.20 ± 0.01	8.01 ± 0.2	8.21 ± 0.17	8.57 ± 0.30
r5-HT_7 (recombinant)	8.19 ± 0.02	7.99 ± 0.42	8.13 ± 0.18	8.31 ± 0.03
r5-HT_7 (native, thalamus)	8.50 ± 0.20	ND	ND	ND

ND: not determined, h: human, r: rat.

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Table 2: Duration of REM sleep onset and total time spent in REM sleep following treatment with placebo, citalopram (20 mg), and JNJ-18038683 (100/20/20 mg) for 3 days measured in healthy subjects (n = 12). All data are mean ± S.E.M. (minutes). For all data points, n = 12.

Treatment Group	REM onset latency (min)	Total REM time (min)
Pre dose		
Placebo	63.83 ± 4.51	112.33 ± 6.25
Citalopram	93.83 ± 15.15	98.33 ± 7.24
JNJ-18038683	63.25 ± 5.11	104.21 ± 7.81
Day 1/2		
Placebo	60.58 ± 3.61	113.00 ± 7.69
Citalopram	154.83 ± 22.77 (P = 0.0003)	62.5 ± 5.62 (P < 0.0001)
JNJ-18038683	101.33 ± 16.14 (P = 0.0207)	68.08 ± 6.71 (P < 0.0001)
Day 3/4		
Placebo	57.67 ± 5.64	115.58 ± 8.09
Citalopram	188 ± 28.65 (P < 0.0001)	69.73 ± 10.62 (P = 0.0001)
JNJ-18038683	85.17 ± 7.07 (P<0.0097)	90.75 ± 7.57 (P = 0.0256)

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Table 3: Key JNJ-18038683 and R- and S-citalopram plasma levels measured on Day 8 (JNJ-18038683) and Day 17 in healthy control subjects (n = 12, citalopram 20 mg; JNJ-18038683, 20 mg). For C_{max} (ng/mL), AUC_{0-24h} (ng.h/mL), and F_{rel} mean values are provided. T_{max} values are median values ± S.E.M.

	Day 8			Day 17			F_{rel} (Day 17/Day1)	
	C_{max}	T_{max}	AUC_{0-24h}	C_{max}	T_{max}	AUC_{0-24h}	C_{max}	AUC_{0-24h}
JNJ-18038683 (citalopram + JNJ-18038683)	16.0 ± 1.13	4.5	248 ± 8.35	60.7 ± 3.35	6	1182 ± 69.65	4.03 ± 0.35	4.8 ± 0.29
S-citalopram (citalopram + Placebo)	--	--	--	12.2 ± 1.49	3	168 ± 25.43	--	--
S-citalopram (citalopram + JNJ-18038683)	--	--	--	28.8 ± 1.98	3	499 ± 39.59	--	--
R-citalopram (citalopram + Placebo)	--	--	--	36.2 ± 3.79	3	693 ± 70.81	--	--
R-citalopram (citalopram + JNJ-18038683)	--	--	--	51.7 ± 2.67	3	1026 ± 52.60	--	--

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Table 4:

MADRS total score - change from baseline to end point (LOCF)

excluding sites with mean MADRS total score in the placebo arm less than or equal to 12 or greater than or equal to 28 at end point.

	Placebo (n = 48)	JNJ-18038683 (n = 45)	Citalopram (n =53)
Baseline			
Mean (SD)	32.8 (4.12)	32.7 (4.30)	31.7 (4.57)
End Point			
Mean (SD)	20.2 (9.84)	16.4 (8.86)	17.8 (9.58)
Change from Baseline			
Mean (SD)	-12.6 (9.35)	-16.2 (10.42)	-13.9 (8.67)
P-value (minus placebo) (a)		0.057	0.353
Difference of LS Means (SE)		-4.0 (2.07)	-1.8 (1.97)

(a) Based on Analysis of covariance (ANCOVA) model with treatment, sex, and center as factors, and baseline value as a covariate.

Note: Negative change in score indicates improvement.

Figure 1

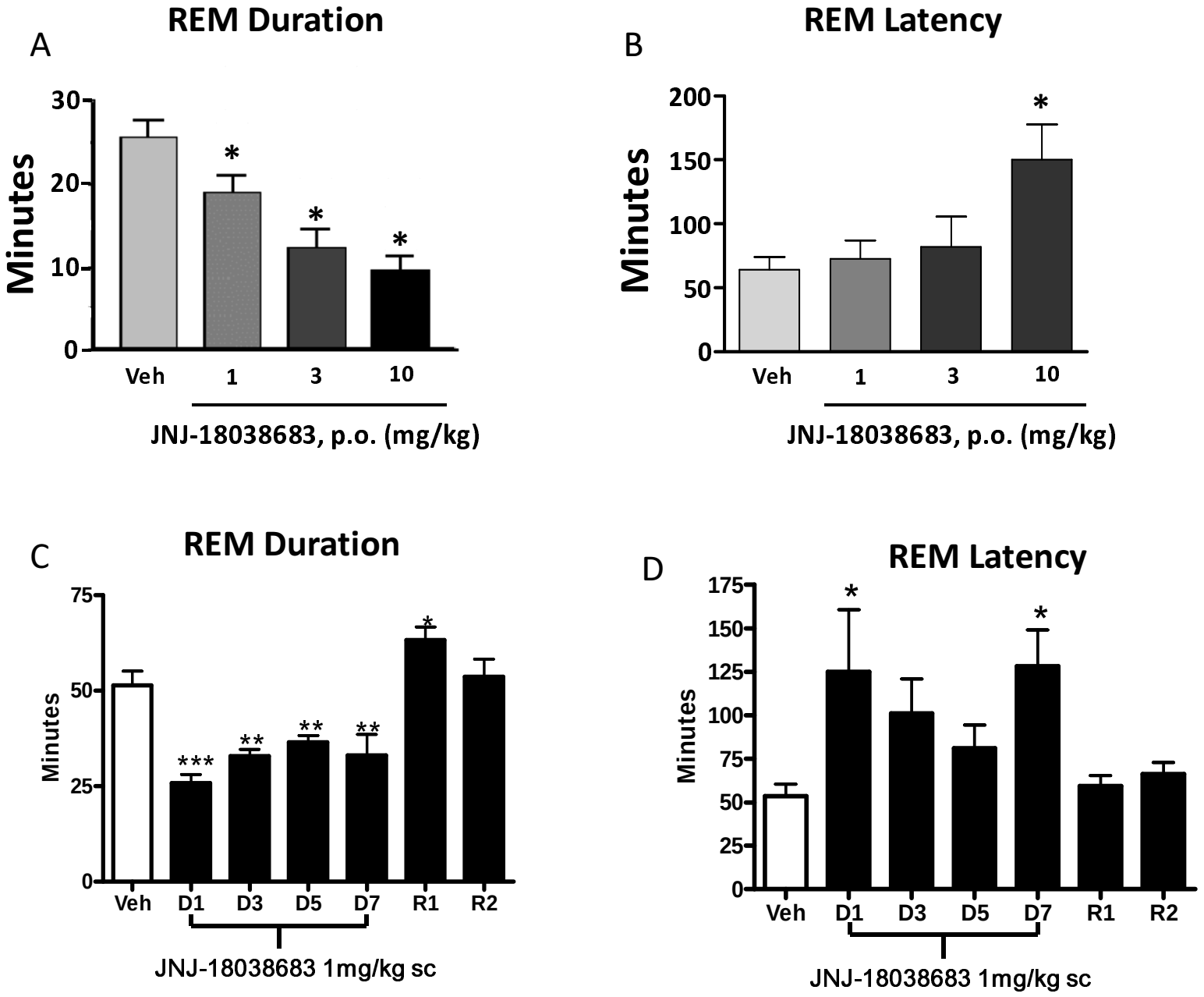


Figure 2

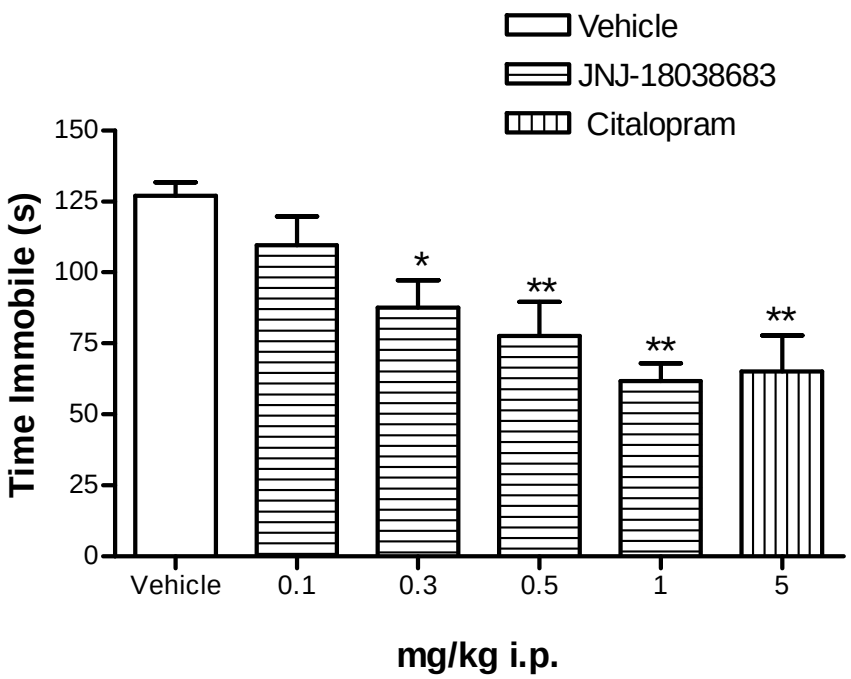


Figure 3

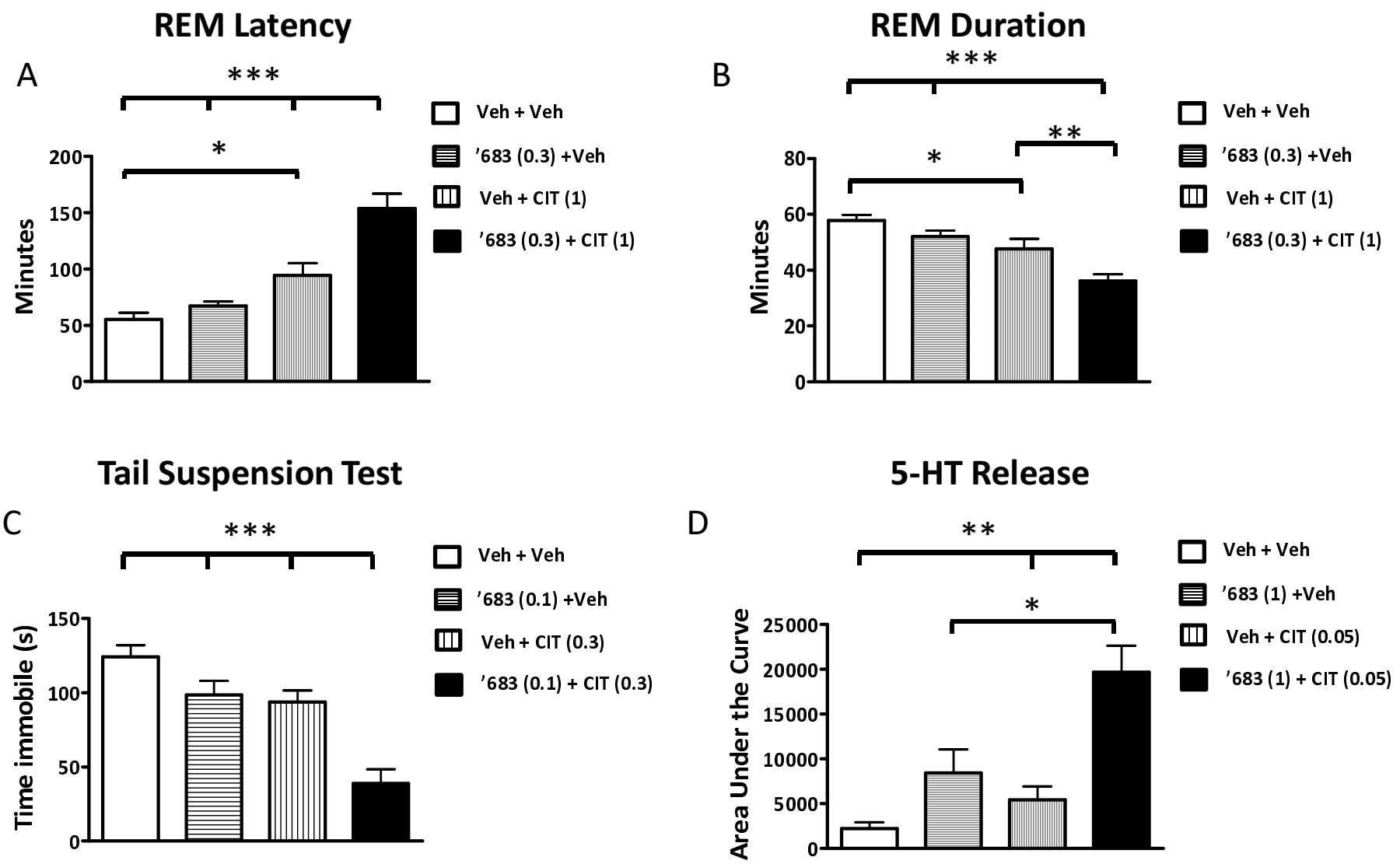


Figure 4A

B

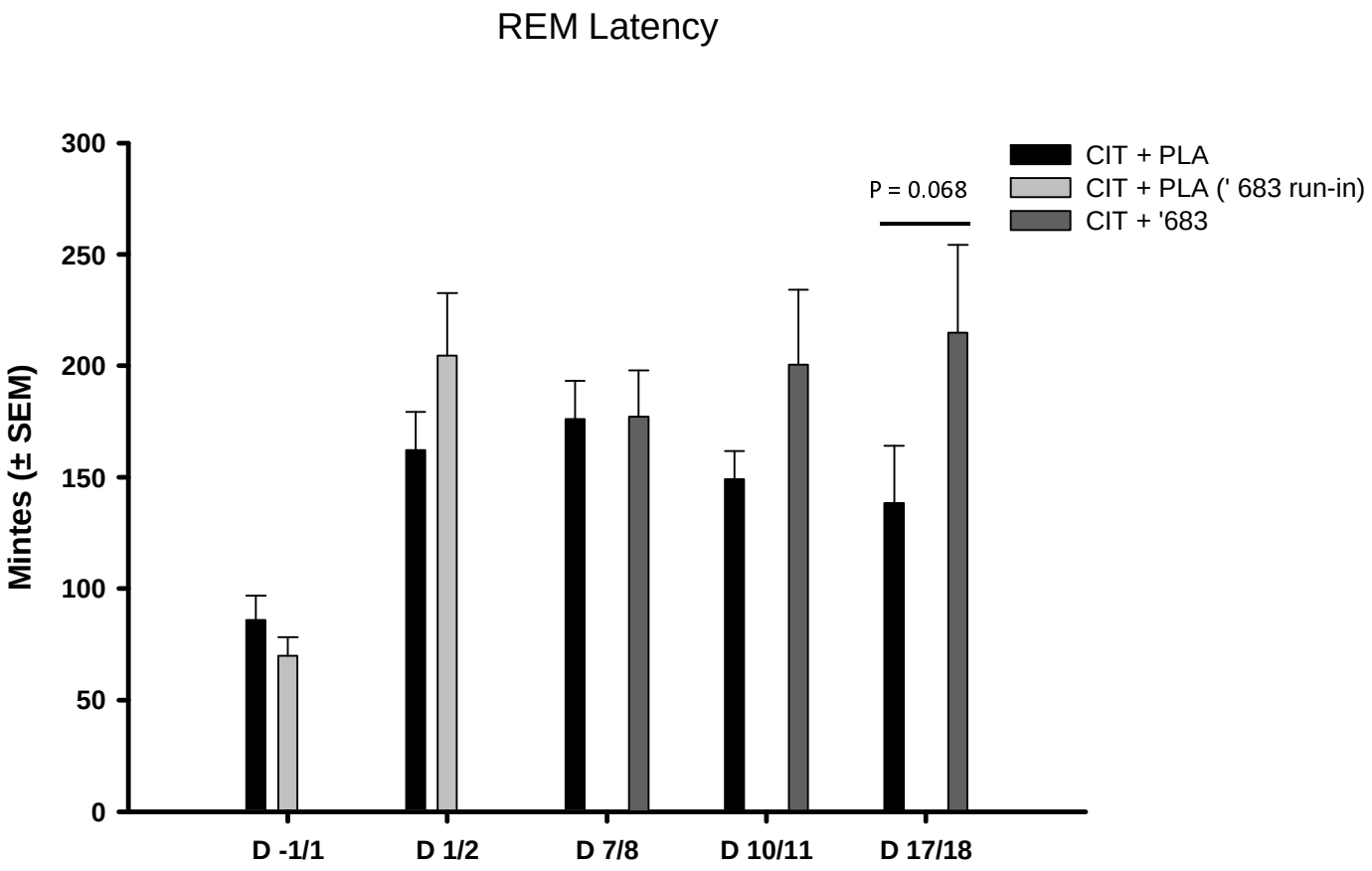


Figure 4B

A

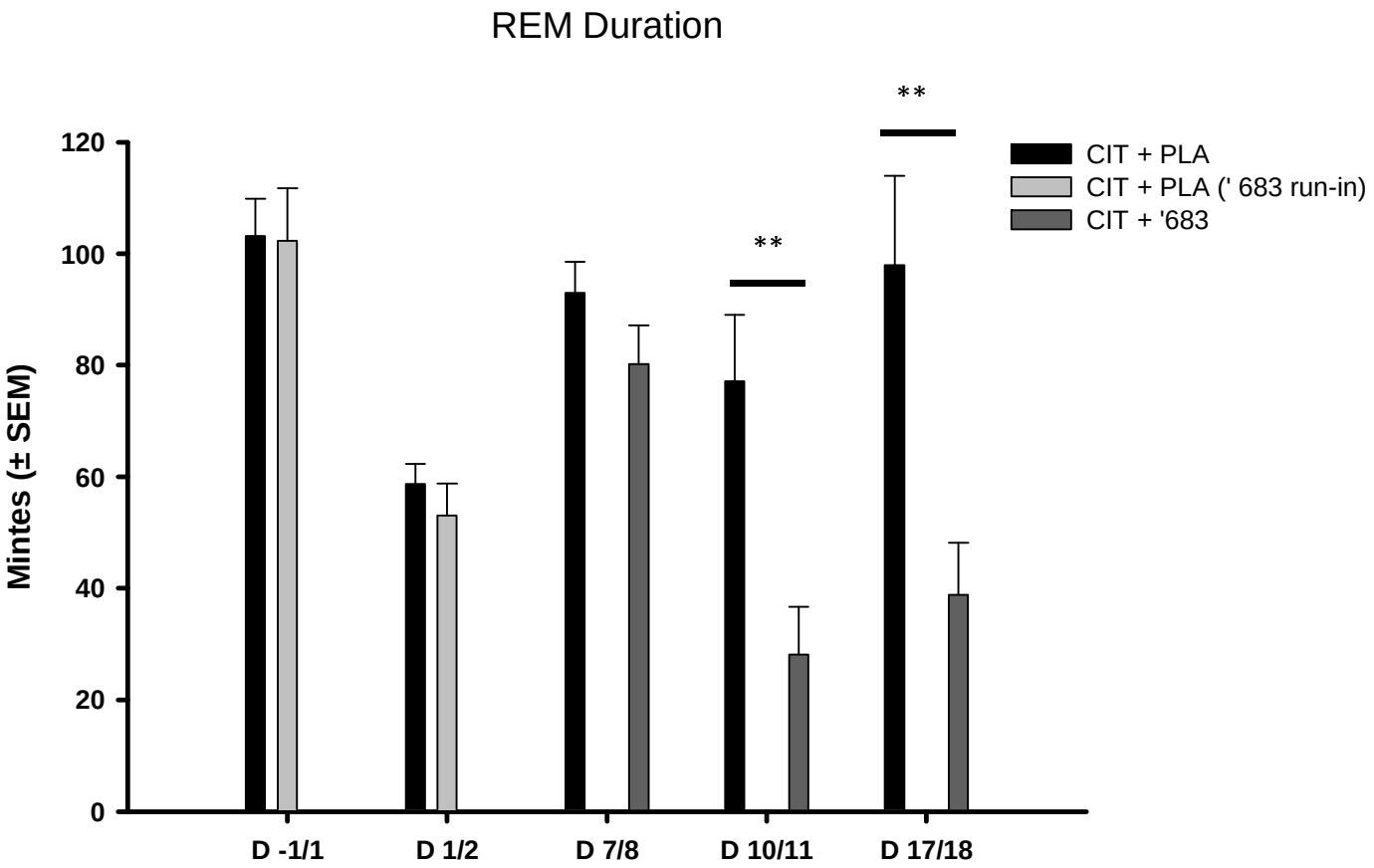
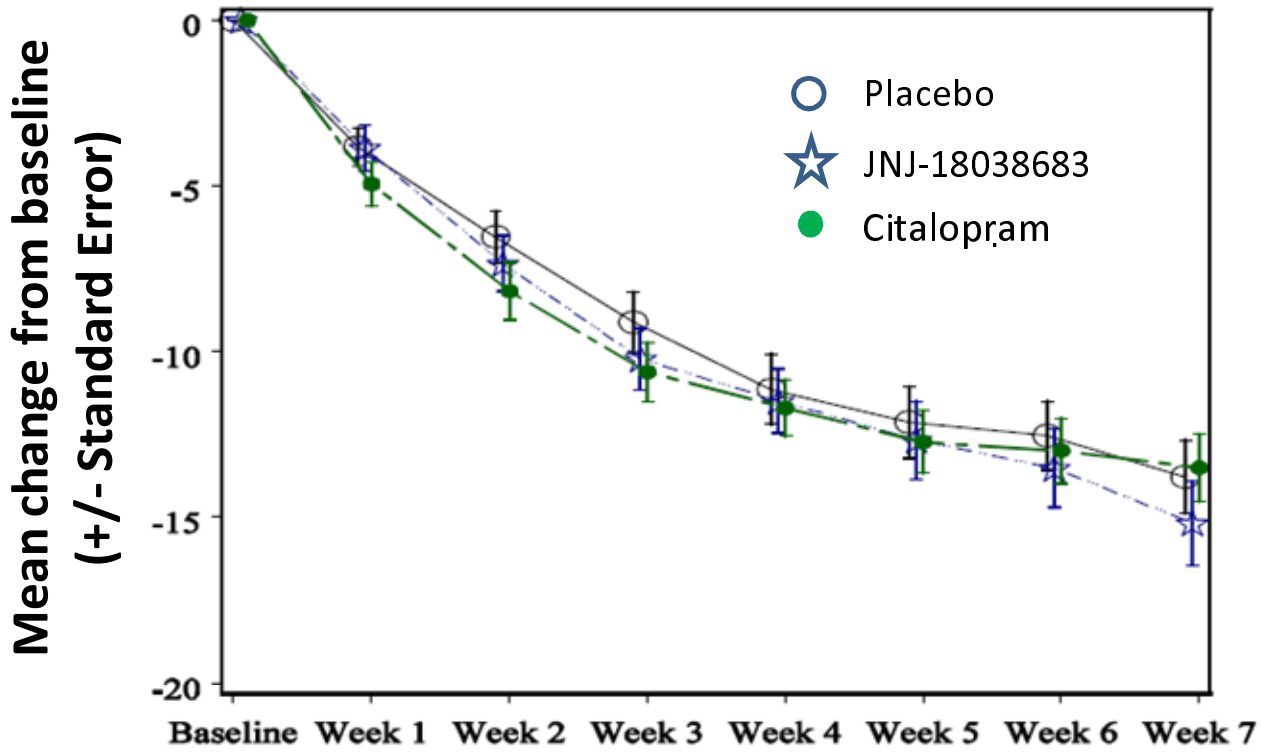


Figure 5



Translational evaluation of JNJ-18038683, a 5-HT_7 receptor antagonist, on REM sleep and in major depressive disorder

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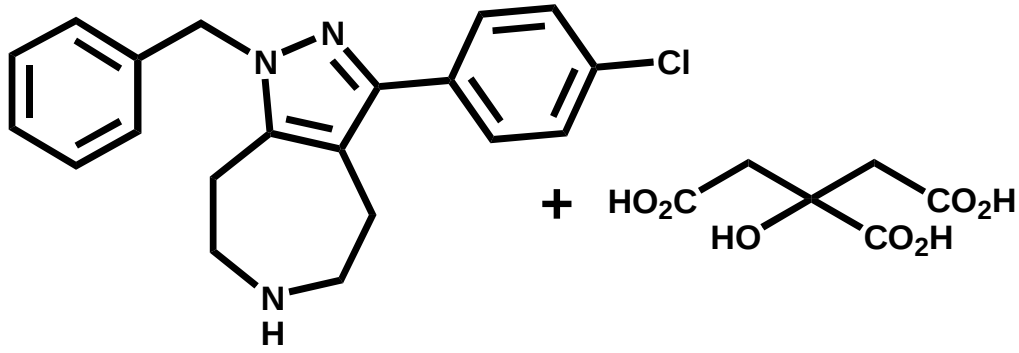
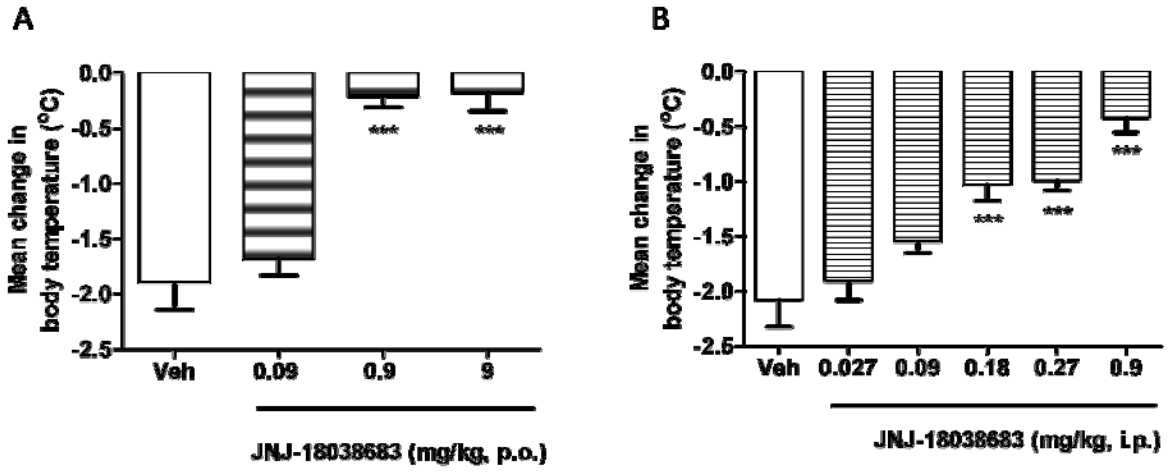
Supplemental data files JPET#193995

Supplemental Figure 1: Chemical structure of JNJ-18038683(3-(4-chlorophenyl)-1,4,5,6,7,8-hexahydro-1-(phenylmethyl)pyrazolo[3,4-d]azepine 2-hydroxy-1,2,3-propanetricarboxylate).



Supplemental Figure 2: Effect of oral administration (A) and intraperitoneal (B) of JNJ-18038683 on 5-CT-induced hypothermia in rats. JNJ-18038683 was administered orally 6 hours before 5-CT administration or intraperitoneally 20 minutes before 5-CT administration. 5-CT was administered intraperitoneally (0.1 mg/kg). Data bars represent the mean ± S.E.M. (n = 5). *** P < 0.001 compared to 5-CT alone group (Veh = vehicle).



Supplemental Table 1: In vitro selectivity profile of JNJ-18038683. Data were obtained using standard radioligand binding assays. Data are the mean ± S.E.M from at least three separate experiments unless indicated by an asterisk.

r = rat; h = human, c = canine. * Indicate single value

	In vitro binding pK_i	Fold selectivity versus h5-HT_7
Serotonin receptors		
r5-HT_{1A}	6.39 ± 0.38	65
h5-HT_{1A}	6.50 ± 0.28	50
h5-HT_{1B}	6.90 ± 0.14	20
r5-HT_{1B}	6.54*	46
c5-HT_{1D}	6.20*	100
r5-HT_{2A}	6.50 ± 0.08	50
h5-HT_{2A}	7.03 ± 0.03	14
h5-HT_{2B}	6.81 ± 0.16	25
h5-HT_{2C}	7.06 ± 0.23	14
h5-HT_3	<5	>1000
r5-HT_4	<5	>1000
h5-HT_{5A}	5.50*	>100
h5-HT_6	7.20*	10
Dopamine receptors		
rD_1	6.06 ± 0.21	>100

hD_1	6.15*	>100
rD_2	6.69*	32
hD_2	<5	>1000
<u>Adrenergic receptors</u>		
$r\alpha_1$	7.04 ± 0.13	15
$h\alpha_{1B}$	7.00*	15
$r\alpha_2$	<5	>1000
<u>Histamine receptors</u>		
hH_1	<5	>1000
hH_3	<5	>1000
<u>Transporters</u>		
hNE uptake	<5	>1000
hDAT uptake	<5	>1000
r5-HT uptake	5.52*	>100
h5-HT uptake	< 5	>1000
<u>CEREP pannel</u>		
adenosine (A1,A2A,A3), angiotensin (AT1), bradykinin (B2), cholecystokinin (CCKA), galanin (GAL2), melatonin ML1), muscarinic (M1, M2, M3), neurotensin (NT1), neurokinin (NK2, NK3), opiate (μ, κ, δ), somatostatin, vasopressin (V1a), ion channels (sodium, calcium, potassium and chloride)	< 50% inh at 1 μM	>100

Supplemental Table 2: Summary of secondary efficacy results from the MDD study at week 7 (placebo n = 71, JNJ-18038683 n = 72, escitalopram n = 75). CI = confidence interval.

Efficacy endpoints	JNJ-18038683 vs. Placebo		Escitalopram vs. Placebo	
	Mean (95% CI)	p-value[a, b, c]	Mean (95% CI)	p-value[a, b, c]
CGI-S	-0.3 (-0.71,0.14)	0.192	-0.2 (-0.59,0.25)	0.432
MADRS Responder %	4.9 (-11.46,21.28)	0.931	-1.2 (-17.32,15.03)	0.896
MADRS-6	-1.1 (-3.37,1.14)	0.332	-0.6 (-2.83,1.60)	0.583
HAMD-17	-0.9 (-3.48,1.77)	0.522	-1.1 (-3.69,1.46)	0.394
HAMD-17 Responder %	-4.9 (-21.23,11.49)	0.538	-4.0 (-20.25,12.17)	0.820
HAMD-6	-0.4 (-1.85,1.03)	0.575	-0.5 (-1.90,0.92)	0.492
ESS	0.4 (-1.10,1.83)	0.622	1.1 (-0.31,2.56)	0.124
GAF	2.1 (-2.17,6.42)	0.330	1.0 (-3.21,5.20)	0.642
SA Item-1 (Sleep Latency)	-3.5 (-25.65,18.69)	0.757	9.4 (-12.60,31.44)	0.400
SA Item-2 (# of Awakenings)	-0.2 (-0.74,0.30)	0.401	-0.1 (-0.59,0.42)	0.744
SA Item-3 (Sleep Time)	-18.8 (-50.28,12.61)	0.239	-5.8 (-36.54,24.90)	0.709
SA Item-4 % (Sleep Quality)[d]	6.3 (-9.93,22.65)	0.733	3.0 (-13.01,19.17)	0.569

(a): For CGI-S, MADRS-6, HAMD-17, HAMD-6, ESS, GAF, SA Items 1-3, analysis based on an ANCOVA model with treatment, (pooled) center and sex as factors and baseline as covariate.

(b): For MADRS responder, HAMD-17 responder and SA Item-4, analysis based on the generalized Cochran-Mantel-Haenszel test for row mean scores differ, with (pooled) center and sex as stratification factors.

(c): Pairwise comparison without adjustment of multiplicity.

(d): Mean and CI given based on subcategories ‘Excellent’ and ‘Good’, p-values given based on all 4 subcategories.

Supplemental Table 3: HAMD-17 total score - change from baseline to end point (LOCF) excluding sites with mean HAMD-17 total score in the placebo arm less or equal to10 at end point.

	Placebo (n = 52)	JNJ-18038683 (n = 53)	Escitalopram (n = 57)
Baseline			
Mean (SD)	27.5 (2.31)	27.1 (1.92)	27.1 (1.99)
End Point			
Mean (SD)	18.2 (7.53)	14.1 (8.71)	14.7 (7.47)
Change from Baseline			
Mean (SD)	-9.4 (7.33)	-13.1 (8.45)	-12.4 (7.03)
P-value (minus placebo) (a)		0.0125	0.0291
Difference of LS Means (SE)		-4.0 (1.56)	-3.3 (1.51)

(a) Based on Analysis of covariance (ANCOVA) model with treatment, sex, and center as factors, and baseline value as a covariate.

Note: Negative change in score indicates improvement.