1. Title page

Characterization of JNJ-42847922, a Selective Orexin-2 Receptor Antagonist, as a Clinical Candidate for the Treatment of Insomnia

Pascal Bonaventure, Jonathan Shelton, Sujin Yun, Diane Nepomuceno, Steven Sutton, Leah Aluisio, Ian Fraser, Brian Lord, James Shoblock, Natalie Welty, Sandra R. Chaplan, Zuleima Aguilar, Robin Halter, Anthony Ndifor, Tatiana Koudriakova, Michele Rizzolio, Michael Letavic, Nicholas Carruthers, Timothy Lovenberg and Christine Dugovic

Janssen Research & Development, LLC
2. Running title page

a) Running title: Characterization of JNJ-42847922, a Selective Orexin-2 Receptor Antagonist

b) Corresponding author: Pascal Bonaventure, Janssen R&D, LLC, 3210 Merryfield Row, CA 92121 San Diego. Pbonave1@its.jnj.com and Christine Dugovic, Janssen R&D, LLC, 3210 Merryfield Row, CA 92121 San Diego. CDugovic@its.jnj.com

c) The number of Text pages: 43
   Tables: 4
   Figures: 8
   References: 27
   Words in the Abstract: 248
   Words in the Introduction: 660
   Words in the Discussion: 1264

d) List of nonstandard abbreviations: DORA, dual orexin receptor antagonist; EEG, electroencephalogram; EMG, electromyogram; i.p., intraperitoneal; KO, knockout; NREM, non-REM; OX1R, orexin 1 receptor; OX2R, orexin 2 receptor; p.o., per os; REM, rapid eye movement; s.c., subcutaneous; SORA, selective orexin receptor antagonist; WT, wild-type

e) Recommended section assignment: Drug Discovery and Translational Medicine
3. Abstract

Dual orexin receptor antagonists have been shown to promote sleep in various species, including human. Emerging research indicates that selective orexin-2 receptor (OX2R) antagonists may offer specificity and a more adequate sleep profile by preserving normal sleep architecture. Here we characterized JNJ-42847922 ([5-(4,6-Dimethyl-pyrimidin-2-yl)-hexahydro-pyrrolo[3,4-c]pyrrol-2-yl)-(2-fluoro-6-[1,2,3]triazol-2-yl-phenyl)-methanone), a high affinity/potent OX2R antagonist. JNJ-42847922 had an approximate 2-log selectivity ratio versus the human orexin-1 receptor (OX1R). Ex vivo receptor binding studies demonstrated that JNJ-42847922 quickly occupied OX2R binding sites in the rat brain after oral administration and rapidly cleared from the brain. In rats, single oral administration of JNJ-42847922 (3-30 mg/kg) during the light phase, dose-dependently reduced the latency to non-rapid eye movement (NREM) sleep and prolonged NREM sleep time in the first 2 hours, whereas REM sleep was minimally affected. The reduced sleep onset and increased sleep duration were maintained upon 7-day repeated dosing (30 mg/kg) with JNJ-42847922, then all sleep parameters returned to baseline levels following discontinuation. While the compound promoted sleep in wild-type mice, it had no effect in OX2R knockout mice, consistent with a specific OX2R mediated sleep response. JNJ-42847922 did not increase dopamine release in rat nucleus accumbens, or produce place preference in mice after subchronic conditioning, indicating that the compound lacks intrinsic motivational properties in contrast to zolpidem. In a single ascending dose study conducted in healthy subjects, JNJ-42847922 increased somnolence and displayed a favorable pharmacokinetic and
safety profile for a sedative/hypnotic, thus emerging as a promising candidate for further clinical development for the treatment of insomnia.
4. Introduction

Orexin-A and orexin-B, also known as hypocretin-1 and hypocretin-2, are neuropeptides produced by perifornical and lateral hypothalamic neurons that project throughout the brain and bind two different G protein coupled receptors, orexin-1 receptor (OX1R) and orexin 2 receptor (OX2R) (de Lecea et al., 1998; Sakurai et al., 1998). Impairment of the orexin system at the OX2R level in an inherited canine model of narcolepsy was reported one year after its initial discovery (Lin et al., 1999). At the same time, a narcoleptic phenotype was observed in mice lacking the gene coding for orexin, identifying orexin as a key mediator for the maintenance of wakefulness (Chemelli et al., 1999). The following year, Peyron and colleagues demonstrated that there was a near complete loss of central orexin production in human narcolepsy, as measured by orexin immunoreactivity in post-mortem brain slices (Peyron et al., 2000). The salient narcoleptic phenotype of orexin knockout (KO) mice and the altered orexin signaling in narcoleptic patients provided both genetic and clinical proof of the critical role of OXRs for sleep regulation. It was then hypothesized that pharmacological blockade of the OXRs would be a novel approach for the treatment of insomnia. Less than 10 years after the elucidation of the role of orexin system, the first clinical data on almorexant, a dual OXR antagonist (DORA), were disclosed and confirmed the validity of this new mechanism of action for the treatment of insomnia (Brisbare-Roch et al., 2007). Only a few years later, at least three other DORAs (SB-649868, suvorexant and filorexant) reached clinical Phase 2 for the treatment of sleep related disorders (reviewed in (Hoyer and Jacobson, 2013). In August 2014, the U.S. Food and Drug Administration approved Belsomra® (suvorexant) for the treatment of primary insomnia.
Taking advantage of newly developed selective OX1R and OX2R antagonists, we dissected the contribution of each OXR and demonstrated that in rodents antagonism of OX2R alone was sufficient to induce and prolong sleep (Dugovic et al., 2009). In agreement with these data, Mang and colleagues reported that almorexant did not induce sleep in mice lacking the OX2R (Mang et al., 2012). Furthermore, we showed that the addition of OX1R blockade to OX2R antagonism elicited a dysregulation of rapid eye movement (REM) sleep by shifting the balance in favor of non-REM (NREM) sleep at the expense of NREM sleep that may increase the risk of narcoleptic-like events (Dugovic et al., 2014).

Based on these preclinical data, we developed a selective OX2R antagonist with suitable drug-like properties for clinical evaluation. We optimized the compound for selectivity over the OX1R (greater than 50 fold), oral bioavailability, rapid absorption and short half-life to avoid next day residual effects. A separate paper describes the new OX2R antagonist JNJ-42847922 (Figure 1) synthesis, structure activity relationship, pharmacokinetics in preclinical species and acute dose-response effects on sleep in rats during the dark phase (Letavic et al., in press). Here, we report a comprehensive characterization of JNJ-42847922, a novel selective OX2R antagonist.

In vitro affinity and potency for the human and rat OX2R were determined by radioligand binding and in vitro functional assays. In vivo target engagement was measured in rat brain using ex vivo receptor occupancy after oral administration. To confirm the mechanism of action, the acute dose-response effects of the OX2R antagonist on sleep were tested in rats, and in OX2R KO and corresponding wild-type (WT) mice. The maintenance of the sleep response upon repeated administration of JNJ-42847922
was also assessed in rats. The effect of the compound on motor coordination and on alcohol induced ataxia was investigated in rats using the Rotarod test. Potential abuse liability was evaluated by measuring changes in nucleus accumbens dopamine levels using microdialysis in freely moving rats, and by using the conditioned place preference test in mice. Following appropriate 1-month GLP toxicological studies in the rat and dog, we conducted a single ascending dose to evaluate the safety, tolerability and pharmacokinetics of JNJ-42847922 in healthy subjects. Sleepiness was assessed by the Stanford Sleepiness Scale.
5. Materials and Methods

All animal procedures performed in this study were in accordance with the Guide Care for and Use of Laboratory Animals adopted by the US National Institutes of Health (NIH Publication no. 80-23 revised 1996) and the guidelines of the Institutional Animal Care and Use Committee. Animals were housed individually under controlled conditions with a 12/12 h light/dark schedule and temperature of 22 ± 2°C. Food and water were provided ad libitum. Experiments were performed after animals had acclimated for at least one week unless stated otherwise.

In vitro radioligand binding assays:

Human or rat OX2R binding was measured in competitive radioligand binding assays using [3H]EMPA, N-ethyl-2-[(6-methoxy-pyridin-3-yl)-(toluene-2-sulfonyl)-amino]-N-pyridin-3-ylmethyl-acetamide (2 nM, specific activity 27 Ci/mmol) (Malherbe et al., 2009a). Cell membranes were prepared from a stable pool of HEK-293 cells transfected with the human OX2R or CHO-K1 cells transfected with the rat OX2R. Dilutions of test compounds were made in Dulbecco’s PBS from 10 mM stocks dissolved in DMSO. After a 60-minute incubation at room temperature, binding reactions were filtered. The membranes were counted in a scintillation counter. Non-specific binding was determined in the presence of 10 μM almorexant ((2R)-2-[(1S)-2-[(4-(trifluoromethyl)phenyl)ethyl]-3,4-dihydroisoquinolin-2(1H)-yl]-N-methyl-2-phenylethanamide).

Affinities of compounds for the human or rat OX1R were measured using [3H]SB-674042 (1-(5-(2-fluoro-phenyl)-2-methyl-thiazol-4-yl)-1-((S)-2-(5-phenyl-
(1,3,4)oxadiazol-2-ylmethyl)-pyrrolidin-l-yl)-methanone) as a tracer (4 nM, specific activity 35 Ci/mmol) (Langmead et al., 2004). Cell membranes were prepared from clonal CHO K1 cells transfected with the human OX1R or clonal HEK-293 cells transfected with the rat OX1R. Non-specific binding was determined with 10 μM almorexant. The Kᵢ of the test compounds was calculated based on nonlinear regression (one site competition) using Graphpad Prism.

The selectivity of JNJ-42847922 was evaluated in a large panel of binding assays including adenosine (A₁, A₂A, A₃), adrenergic (α₁, α₂, α₄), angiotensin (AT₁), dopamine (D₁, D₂), bradykinin (B₂), cholecystokinin (CCKₐ), galanin (GAL₂), melatonin (ML₁), muscarinic (M₁, M₂, M₃), neurotensin (NT₁), neurokinin (NK₂, NK₃), opiate (μ, κ, δ), serotonin (5-HT₁A, 5-HT₁B, 5-HT₂A, 5-HT₃, 5-HT₅A, 5-HT₆, 5-HT₇), somatostatin, vasopressin (V₁a), norepinephrine transporter, dopamine transporter and ion channels (sodium, calcium, potassium and chloride). These assays were performed by Eurofins (Celles L’Evescault, France).

In vitro functional assays (calcium mobilization assays):

Functional antagonism of OXR has been shown in cellular assays by monitoring changes in intracellular calcium. The human OX2R functional assay used PFSK-1 cells, which are a human neuroectodermal cell line that innately expresses the OX2R. The rat OX2R functional assay was performed using Sk-N-Mc cells stably expressing the rat OX2R (Sk-N-Mc-rOX2R). Stably transfected CHO-K1 cells for the human OX1R or HEK-293 cells for the rat OX1R were used for the in vitro functional assays.
Since the intracellular calcium response is transient and not consistent with equilibrium assumptions, the assays were performed by giving a standard, EC$_{80}$ dose of the OXR agonist and calculating a pK$_b$ from inhibition of the agonist response by a dose range of the antagonists. The cells were plated in black 96 well tissue culture plates with clear bottoms at 50,000 cells/well and grown overnight at 37°C in 5% carbon dioxide (CO$_2$). Dilutions of the antagonist were prepared in Hanks Balanced Salt Solution (HBSS) from 10 mM DMSO stocks, while dilutions of OX peptides (OX-A for OX1R assays, OX-B for OX2R assays) were prepared in HBSS + 0.1% bovine serum albumin. On the day of the assay, a 2X dye-loading solution (BD Calcium Assay Kit) was added to the cells and incubated for 45 minutes at 37°C in 5% CO$_2$. Dilutions of the test compounds were added and the cells were incubated at room temperature for 15 minutes. The cell plate was then transferred to the Molecular Devices Fluorometric Imaging Plate Reader (FLIPR) Tetra instrument, which adds the OX agonist and monitors changes in fluorescence reflecting intracellular calcium levels.

Results were calculated using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Raw data from the FLIPR Tetra were exported as the difference between maximum and minimum fluorescence observed for each well. A non-linear regression was used to determine the agonist EC$_{50}$ and antagonist IC$_{50}$ for each plate, then the antagonist K$_b$ was calculated according to Cheng and Prusoff (Cheng and Prusoff, 1973).

**Ex vivo receptor occupancy assays in rats:**

Experiments were performed as previously described (Dugovic et al., 2009) in male Sprague-Dawley rats (300-400 g, Charles River Laboratories, San Diego, CA).
Animals were treated orally with JNJ-42847922 or vehicle. Time and dose dependency were evaluated. The animals were euthanized using CO₂ and decapitated at different time points after drug administration (n = 3 per time point or dose regimen). Brains were rapidly frozen on powdered dry ice and stored at −80°C before sectioning. Plasma samples were also collected for bioanalysis (LC-MS/MS). Twenty micron thick tissue sections at the level of the frontal cortex were prepared for autoradiography. OX2R radioligand binding autoradiography was determined at room temperature with 3 nM [³H]EMPA. Sections were incubated for 10 min to minimize dissociation. Nonspecific binding was determined in the presence of 10 μM JNJ-10397049 (McAtee et al., 2004). Sections were allowed to dry before acquisition with β-Imager (BioSpace, Paris, France) for 16 h. Quantitative analysis was performed using the β-imager TRacer. Ex vivo receptor labelling was expressed as the percentage of receptor labelling in corresponding brain areas (i.e. cortex) of vehicle-treated animals. The percentage of receptor occupancy was plotted against time or dosage using GraphPad Prism (GraphPad Software, San Diego, CA, USA). Percentage of receptor occupancy was also plotted against drug plasma or brain concentration. Pharmacokinetic parameters were analyzed by applying a non-compartmental model using the software package WinNonlin Version 4.0.1. (Pharsight, Palo Alto, CA).

Sleep recording and analysis in rats and mice:

Sleep experiments were conducted in male Sprague-Dawley rats (350-450 g, Harlan Laboratories, Livermore, CA) and in male C57Bl6 OX2R KO and corresponding wild-type (WT) mice (30-35 g, Charles River Laboratories, San Diego, CA) as described
previously (Dugovic et al., 2009). Animals were chronically implanted with telemetric devices (F40-EET, Data Sciences International, St. Paul, MN) for the recording of electroencephalogram (EEG), electromyogram (EMG), locomotor activity and body temperature. EEG and EMG signals were digitized at a sampling rate of 100 Hz on an IBM PC-compatible computer using Dataquest A.R.T. software (version 3.1, Data Sciences International, St. Paul, MN). Polysomnographic waveforms were divided into individual 10-s epochs that were then visually assigned as wake, non-rapid eye movement (NREM) or REM sleep by using the computer software program SleepSign (version 2.6.3.807, Kissei Comtec, Nagano, Japan). EEG activity within specific vigilance states was determined by power spectral analysis using the Fast Fourier Transform within a frequency range of 1-30 Hz. Values for power spectra were divided into four frequency bands: delta (1-4 Hz), theta (4-10 Hz), sigma (10-15 Hz) and beta (15-30 Hz). For each experiment, all signals were recorded for up to 6 hours after administration of the tested compounds.

Analysis of sleep parameters included latency to NREM sleep (defined as the time interval to the first six consecutive NREM epochs) and REM sleep (the first two consecutive REM epochs post-treatment), the duration of NREM and REM sleep and bout analysis (number and duration) for each vigilance state. Locomotor activity counts and body temperature were analyzed into 1- and 15-min bins, respectively, and averaged into 2-h periods for each animal. Results were averaged and expressed as the mean ± S.E.M. in defined time intervals. To determine whether differences were significant at a given interval, one-way analysis of variance (ANOVA) followed by Dunnett’s multiple
comparison post-hoc test was performed for experiments in rats, and Student’s t test was performed for experiments in mice. Significance threshold of $p < 0.05$ was used.

*In vivo microdialysis in rats:*

Experiments were performed in male Sprague-Dawley rats (280-320 g) from Charles Rivers (San Diego, CA) as previously described (Galici et al., 2011) using a guide cannula (Eicom, Kyoto, Japan) in the nucleus accumbens core (incisor bar set at –3.5 mm, nucleus accumbens: +1.8 mm anterior, 1.6 mm lateral and 6.0 mm ventral to Bregma) (Paxinos and Watson, 2005). Microdialysis probes (artificial cellulose; molecular weight cut off, 50,000; 2 mm active membrane length, Eicom) were implanted the afternoon prior to sample collection and perfused with artificial cerebral spinal fluid (147 mM NaCl, 4.0 mM KCl, 0.85 mM MgCl$_2$, 2.3 mM CaCl$_2$) at a rate of 1 μl/min. The following morning, three 30-min baseline samples were collected prior to drug injections. Animals were then treated with JNJ-42847922 (30 mg/kg, p.o.) or vehicle. Samples were collected every 30 min for 240 min post-dosing into a 96-well plate containing 7.5 μl of antioxidant (0.1 M acetic acid, 1 mM oxalic acid, and 3 mM L-cysteine in ultra-pure water) maintained at 4°C. One hour prior to the end of the experiment ($t = 180$ min), each animal received an injection of amphetamine (0.3 mg/kg s.c.) as a positive control. Microdialysis samples were analyzed for dopamine by HPLC with electrochemical detection (Eicom) as previously described (Galici et al., 2011). Following the experiment, the brains were removed and sliced to verify the probe placement.

The concentration of dopamine in each sample was calculated from the peak area of the chromatographic signal and the slope from the corresponding standard curve. The
percent change from baseline value was calculated from the mean basal value of each neurotransmitter for each animal and presented in the figures as mean ± S.E.M. Data were graphed using GraphPad Prism (GraphPad Software, San Diego, CA, USA) and statistical analysis was performed using unpaired t-test using a significance threshold of p < 0.05.

Conditioned place preference in mice:

Experiments were conducted in male DBA/2 mice (30-35 g, Jackson Laboratory, Sacramento, CA). The conditioning apparatus consisted of two chambers, identical in size and texture, divided by a plexiglass partition with a door that could be opened or closed. One of the chambers was decorated on all four walls and floor with a black and white cow pattern and scented with lemon extract (200 μl, Kroger Co., Cincinnati, Ohio) as an olfactory cue. The other chamber was decorated on all four walls and floor with a black and white checker pattern and the scent of almond extract (200 μl, Kroger Co., Cincinnati, Ohio). The conditioning apparatus automatically recorded the location of the animal via a grid of photobeams (8 photobeams along its length and 4 along its width, evenly spaced). On the first day, during habituation to the apparatus, animals were placed in one of the chambers (chosen randomly) and given free access to both chambers (door open) for 30 min. Over the next 4 days, animals were conditioned twice a day. In the morning, animals received a treatment of vehicle, and were immediately confined to the initially most preferred conditioning chamber (as determined during the habituation) with door closed for 30 min. In the afternoon, animals received either JNJ-42847922 (10 mg/kg, i.p.), zolpidem (10 mg/kg, i.p.), or vehicle (20% or 45% hydroxypropyl β-
cycloheximide, 10 ml/kg, i.p) as a control, and were immediately confined to the opposite conditioning chamber (door closed) for 30 min. For historical reason the i.p. route of administration was used for this test. OX2R occupancy after administration of a 10 mg/kg i.p. of JNJ-42847922 was also determined in mice to demonstrate target engagement. Animals received the same treatment each afternoon. As a positive control, an additional group of animals was conditioned in a similar manner, receiving saline (10 ml/kg, i.p) in the morning and amphetamine (2 mg/kg, i.p) in the afternoon. On the 6th day, animals received a test for conditioned place preference. Animals were placed in one of the chambers (chosen randomly), with the door open, and the time spent in each chamber was recorded over 15 min.

The time spent in the drug-paired chamber was analyzed with a two-way analysis of variance for test (comparing time during the habituation session to time during the conditioned place preference test) and group, with repeated measures on time. A Dunnett’s post-hoc test with a significance threshold of p < 0.05 was used to compare each group to the time spent by the vehicle group in the drug-paired chamber during the place preference test.

Rotarod test in rats:

Male Sprague-Dawley rats (400-450g, Harlan Laboratories, Livermore, CA) were trained on the Rotarod (Stoelting, Wood Dale, IL) a day prior to testing. On the day of the experiment, JNJ-42847922, zolpidem, or vehicle (30% SBE-β-CD) was administered orally, and ethanol was co-administered intraperitoneally, then the test was conducted 15 minutes post-treatment. The time that the animals were able to remain on the rotating
drum was recorded. For Rotarod testing, the latencies were given a 60-second cut-off time. Statistical analysis was performed using a Kruskal-Wallis test with Dunnett’s multiple comparisons using a significance threshold of p < 0.05.

*Single ascending dose study in humans:*

The study was conducted in accordance with the Declaration of Helsinki and approved by a local Ethics Committee. A double-blind, randomized, placebo-controlled, single ascending dose study was conducted at a Phase 1 unit in Germany between May and August 2011. Eligible subjects were healthy males between 18 and 55 years of age, without sleep apnea, heavy snoring, or regular use of sleep medications. Subjects in each cohort were randomized to receive a single dose of either JNJ-42847922, formulated as an oral suspension, or placebo. Subjects slept in the unit the evening prior to dosing, were fasted overnight, and received study drug the morning of the following day. A starting dose of 10 mg was administered to 3 subjects (JNJ-42847922 n=2, placebo n=1). Subsequent cohorts (n=6 active, 3 placebo) received doses of 10, 20, 40, and 80 mg, based upon review of safety, tolerability, and plasma pharmacokinetic data for each cohort. Assessments were performed while subjects remained in the clinic through at least 72 hours after dosing, as well as at a follow up visit 1 to 2 weeks after dosing. Safety assessments included collection of adverse events, physical and neurological examinations, vital signs, 12-lead and continuous electrocardiograms (ECGs), pulse oximetry, and blood and urine safety laboratories. Venous blood samples were collected for determination of plasma JNJ-42847922 levels at various time points through 72 hours after dosing. Plasma samples were analyzed using a validated, specific, and sensitive
liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS) method. The Stanford Sleepiness Scale (SSS) was used to collect subjects’ ratings of somnolence at 2, 8, and 12 hours after dosing.

Chemicals:

Almorexant, EMPA, SB-674042, JNJ-10397049 and JNJ-42847922 were synthetized at Janssen Research & Development LLC. Zolpidem tartrate was obtained from Aarti Drugs Ltd., Tarapur. Peptides were obtained from Bachem (Torrance, CA).
6. Results

**JNJ-42847922 is a selective high affinity OX2R antagonist:**

JNJ-42847922 showed high affinity binding to the human and rat OX2R, with pKᵢ values of 8.0 and 8.1, respectively (Table 1). The binding selectivity of JNJ-42847922 at the OX2R compared with the OX1R was substantial (approximately 2 log units). In a panel of 50 receptors, ion channels and transporters assays, JNJ-42847922 at 1 μM had no significant affinity for any receptor/transporter/ion channel (< 50 % inhibition at 1 μM) other than the OX2R. The functional antagonism of JNJ-42847922 for the human or rat OX2R assessed by measuring changes in intracellular calcium in cell culture assays in response to an EC₈₀ dose of OX-A showed that the high affinity OX2R binding of JNJ-42847922 was reflected in potent functional activity (Table 1). The pKᵦ values correlated well with the pKᵢ values for the human and rat OX2R. The binding selectivity of JNJ-42847922 at the OX2R compared to the OX1R was confirmed at the functional level (Table 1).

**JNJ-42847922 crosses the blood brain barrier and occupies the OX2R in rat brain after oral administration:**

Time-dependency and dose-dependency of in vivo occupancy of OX2R assessed by ex vivo receptor binding autoradiography of [³H]EMPA in rat cortex brain tissue sections after oral dosing are shown in Figure 2. Corresponding plasma and brain concentrations are presented in Table 2.

Orally administered JNJ-42847922 (30 mg/kg) inhibited [³H] EMPA binding to the rat cortex, indicating sufficient oral bioavailability and brain penetration. JNJ-
42847922 OX2R occupancy reached maximal at 60 minutes (74 ± 6%), followed by a rapid decline to 40% at 4 hours, and no occupancy at 24 hours (Figure 2A). Overall, there was a strong relationship between receptor occupancy level and plasma/brain concentration, i.e. receptor occupancy levels dropped when plasma/brain levels decreased. In vivo OX1R occupancy was also determined in the same experiment to ensure selectivity for the OX2R. Oral administration of JNJ-42847922 (30 mg/kg) minimally inhibited [3H] SB674042 binding to the rat tenia tecta, indicating low OX1R occupancy (< 20%, data not shown).

For the dose-response analysis, ex vivo receptor occupancy was measured 15 minutes after dosing (Figure 2B). This time point was the apparent time to maximum plasma drug concentration (tₘₐₓ) of JNJ-42847922 attained after the 30 mg/kg dose (Table 2). The measured ED₅₀ was 3 mg/kg, and OX2R occupancy was approximately 74% at the highest dose of 60 mg/kg (Figure 2B). The ED₅₀ dose of 3 mg/kg in this study corresponded to a calculated plasma exposure of 171 ng/mL. Occupancy of the OXR1 by JNJ-42847922 was negligible (< 20 % at 30 mg/kg) (data not shown). The unbound plasma concentration of JNJ-42847922 (rat plasma unbound fraction of 0.056, Letavic et al., in press) corresponding to 50% receptor occupancy in the rat was 9.58 ng/mL. In humans, JNJ-42847922 has a similar affinity for OXR2 compared to rats. Therefore, based on a mean unbound plasma fraction of 0.029, the predicted total human plasma concentration for 50% level of OX2R occupancy would be 330 ng/mL.

OX2R occupancy was also determined in mice to demonstrate target engagement (data not shown). After i.p. injection of a 10 mg/kg dose, maximal OX2R occupancy (66 ± 17%) was observed at 30 minutes. The plasma concentration for maximal receptor
occupancy was determined to be 1965 ± 142 ng/mL. JNJ-42847922 achieved greater than 40% OX2R occupancy for 1 hour; by 2 hours, there was no significant receptor occupancy for the remainder of the time course.

**JNJ-42847922 dose-dependently induces and prolongs sleep in rats:**

Acute effects of JNJ-42847922 on sleep-wake patterns in rats were investigated either during the dark (active) phase or the light (rest) phase in two separate studies. In the first study (Letavic et al., in press), rats were orally dosed at the onset of the dark phase (1, 3, 10 and 30 mg/kg) and in the second study (present data), rats were orally dosed at 2 hours into the light phase (3, 10 and 30 mg/kg). Based on our observation that the lowest dose tested was not effective in the dark phase (when the hypnotic activity of OX antagonists is preferentially revealed), this dose of 1 mg/kg was not evaluated in the light phase. Oral administration of JNJ-42847922 either during the dark phase or the light phase produced a dose-dependent reduction in NREM sleep latency and an increase of NREM sleep time in the first 2 hours (dark phase effects shown in Letavic et al., in press; light phase effects shown in Figure 3). JNJ-42847922 displayed efficacy from the dose of 3 mg/kg onwards for both sleep induction (decreased NREM sleep latency) \([F_{(3, 21)} = 11.45, p < 0.001]\) (Figure 3A) and sleep promotion (increased NREM sleep time) \([F_{(3, 21)} = 7.89, p = 0.001]\) (Figure 3C). While the dose-response effects were more apparent during the dark phase than during the light phase, the time spent in NREM sleep was still gradually prolonged from 3 to 30 mg/kg. The increased NREM sleep time that was measured with the highest dose tested (30 mg/kg) during the light phase was the result of a prolongation of NREM bout duration (compound: 2.83 ± 0.16 min vs. vehicle:
2.25 ± 0.14 min, p < 0.05 based on paired Student’s t test) with no change in the number of NREM bouts (compound: 28.9 ± 1.9 vs. vehicle: 29.4 ± 2.4), suggesting an enhanced sleep consolidation. There was no significant effect on REM sleep latency and REM sleep duration at doses up to 30 mg/kg (Figure 3B, D). REM sleep latency was significantly reduced and REM sleep time was significantly increased only at the dose of 60 mg/kg tested during the dark phase (data not shown). Sleep architecture was preserved at all doses tested in the light phase, as indicated by the maintenance of the REM/total sleep ratio at 3 mg/kg (12.1%), 10 mg/kg (11.0%), and 30 mg/kg (10.9%) as compared to vehicle treatment (10.8%). Power spectral densities in NREM and REM sleep, an index of sleep intensity, were not altered, specifically in regard to NREM delta power (1-4 Hz) or REM theta power (4-10 Hz) (data not shown). The sleep effects lasted for 2 hours following JNJ-42847922 administration either during the light phase or the dark phase. Concomitantly, the animals displayed a moderate reduction in locomotor activity and a small decrease in body temperature (data not shown).

Sleep-promoting effects of JNJ-42847922 are maintained upon 7-day repeated dosing in rats:

At 2 hours into the light phase, animals were daily dosed orally with vehicle for two consecutive days (Veh1 and Veh2), then with JNJ-42847922 (30 mg/kg) for 7 days (D1 to D7) and again with vehicle for two consecutive recovery days (R1 and R2). Sleep-wake patterns, locomotor activity and body temperature were recorded daily except on D2, D3 and D6. Results are shown for the first 2 hours following the treatment (Figure 4). The reduced sleep onset (NREM latency) \[F(5, 40) = 18.70, p < 0.001\] (Figure 4A) and
the increased NREM sleep duration \([F(5, 40) = 32.14, p < 0.001]\) (Figure 4C) were maintained upon 7-day repeated dosing with JNJ-42847922. The prolongation of NREM sleep time was due to a significant increase in NREM bout duration throughout the treatment period assessed on D1 and D7 \([F(5, 40) = 3.65, p = 0.008]\) (Figure 4E) while the number of NREM bouts was not affected (data not shown). REM sleep was only marginally affected on D4 of treatment resulting in a small but significant reduction in REM sleep latency \([F(5, 40) = 8.57, p < 0.001]\) (Figure 4B) and an increase in REM sleep duration \([F(5, 40) = 7.71, p < 0.001]\) (Figure 4D). Power spectral analysis indicates that the increased sleep time did not impact sleep intensity as evidenced by consistent NREM delta power and REM theta power across vehicle and compound treatment days (data not shown). Concomitantly, locomotor activity was significantly diminished only during the JNJ-42847922 treatment period \([F(5, 40) = 6.25, p < 0.001]\) (Figure 4F). The values of all sleep parameters and locomotor activity returned to control levels after discontinuation of the treatment.

*JNJ-42847922 has no effect on sleep parameters in mice lacking the OX2R:*

First, spontaneous sleep-wake states were evaluated in two groups of OX2R KO and corresponding WT mice during a 24-h baseline recording period, and the results are presented in Table 3. As compared to WT, OX2R KO mice spent less time in wake (- 64 min) and more time in NREM sleep (+ 56 min) during the dark phase only. REM sleep amounts were slightly higher during the dark phase (+ 8 min) but lower during the light phase (- 7 min). Next, the effects of JNJ-42847922 (30 mg/kg) on sleep parameters were investigated in WT and OX2R KO mice after oral administration at the onset of the dark
phase (Figure 5). In accordance with the differences observed in baseline conditions, OX2R KO mice showed a shorter NREM latency and higher amounts of NREM sleep as compared to WT following vehicle treatment. In WT mice, JNJ-42847922 displayed efficacy for both sleep induction (decreased NREM sleep latency) and sleep promotion (increased NREM sleep time) in the first 2 hours after compound administration (Figure 5A, C). These effects on NREM sleep in mice were similar to those observed in rats. As in rats, administration of JNJ-42847922 in WT mice had little impact on REM sleep latency and REM sleep duration (Figure 5E, G). In contrast, JNJ-42847922 did not affect any sleep parameters in OX2R KO mice. Specifically, there were no significant differences between compound and vehicle conditions on NREM sleep latency or duration (Figure 5B, D) and on REM sleep latency or duration (Figure 5F, H). Interestingly, both the NREM latency and the NREM sleep duration were comparable/similar in vehicle-treated OX2R KO mice and in WT mice treated with the OX2R antagonist. These results demonstrate that the mechanism of action for the sleep promotion elicited by JNJ-42847922 is mediated via the selective blockade of the OX2R.

JNJ-42847922 does not affect dopamine release in the rat nucleus accumbens:

In vivo microdialysis was used to assess the effect of JNJ-42847922 on extracellular concentrations of dopamine in the nucleus accumbens of freely moving rats (Figure 6A). Oral administration of 30 mg/kg of JNJ-42849722 had no effect on extracellular dopamine release in rat nucleus accumbens (p > 0.05). Amphetamine, a common drug of abuse known to increase dopamine release in the nucleus accumbens was administered as a positive control at the end of the study. Administration of
amphetamine (0.3 mg/kg s.c.) increased dopamine release by approximately 300% above baseline levels (Figure 6A).

*JNJ-42847922 does not produce place preference in mice after sub-chronic conditionings:*

Mice were conditioned to zolpidem (10 mg/kg, i.p.), JNJ-42847922 (10 mg/kg, i.p.) or vehicle using a place preference model (Figure 6B). Amphetamine (2 mg/kg i.p.) was also included as a positive control. A two-way analysis of variance revealed a significant interaction between test and group ($F_{(3,56)}$=4.43, $p=0.007$). Dunnett’s post-hoc tests revealed that both the zolpidem and amphetamine groups spent significantly more time in the drug-paired chamber during the place preference test than the vehicle group ($p<0.05$), indicating a place preference for these drugs. JNJ-42847922 was not significantly different from the vehicle group ($p=0.4$), indicating a lack of place preference or aversion. OX2R occupancy was determined in mice after i.p. administration of JNJ-42847922 to demonstrate target engagement (see above). The level of receptor occupancy and plasma exposure values obtained after i.p. administration (10 mg/kg) are similar to the values measured in rat after administration of a 30 mg/kg p.o. dose.

*JNJ 42847922 has no effect on motor coordination or on alcohol-induced ataxia in rats:*

The effects of JNJ-42847922 on ataxia in rats were evaluated using the Rotorod apparatus. Animals were trained to stay on the Rotorod apparatus for 60 seconds (ie, acquisition criteria) for a maximum of 3 trials. When tested 24 hours later, JNJ-42847922
(30 mg/kg, p.o.) had no effect on motor coordination in rats at a dose that induced sleep (Figure 6C) (p < 0.05).

The effects of JNJ-42847922 on alcohol-induced ataxia in rats were also evaluated using the Rotorod apparatus. JNJ-42847922 (30 mg/kg, p.o.) did not modify the ataxic effects of alcohol (1 g/kg, i.p.) while zolpidem (10 mg/kg, p.o.) exacerbated the effect of alcohol (Figure 6D). These data indicate that JNJ-42847922 had no myorelaxant effect and no effect on alcohol-induced ataxia.

Toxicology:

Toxicological studies up to 1 month in duration under Good Laboratory Practice conditions were conducted in rats and dogs and indicate that JNJ-42847922 had a suitable safety profile to allow testing in humans. In both species, JNJ-42847922 was well tolerated in single and multiple dose studies of up to 1-month duration. JNJ-42847922 had no genotoxicity potential in the standard genotoxicity test battery and was well tolerated in a dog cardiovascular safety study.

JNJ 42847922 displays a suitable pharmacokinetic profile in humans and promotes somnolence in a First-in human trial:

Plasma pharmacokinetics of JNJ-42847922 were characterized by rapid absorption and followed by an apparently monophasic decline, with mean $t_{\text{max}}$ for each dose ranging from 0.33 to 0.5 hour and a half-life of approximately 2 hours. The $C_{\text{max}}$ and $\text{AUC}_{\infty}$ values were dose-dependent but changed in somewhat less than dose-proportional manner (Figure 7, Table 4).
Evidence of somnolence, the expected pharmacodynamic effect of JNJ-42847922, was apparent at all doses. Somnolence was reported as an adverse event for 22 of 26 subjects (85%) who received JNJ-42847922, compared to 3 of 13 subjects (23%) who received placebo. The incidence of somnolence appeared to be dose-dependent, reaching 100% at 40 mg (Figure 8). The time of onset of the first somnolence adverse events ranged from 10 to 40 minutes after dosing, with a mean of 20 minutes for both actively-treated and placebo subjects. Additionally, actively-treated subjects in all cohorts had significantly higher SSS scores, reflecting greater sleepiness, compared to placebo at the first assessment time point of 2 hours after dosing (ANCOVA with baseline as covariate, p=0.023 for 10 mg, p=0.017 for 20 mg, p<0.0001 for 40 and 80 mg). No significant treatment differences were observed at 8 and 12 hours after dosing.

There were no deaths, serious adverse events, or discontinuations due to an adverse event, and all events were considered mild or moderate in severity. Overall, 24 of 26 subjects (92%) who received JNJ-42847922 and 8 of 19 placebo-treated subjects (42%) had at least one adverse event, including somnolence. The most frequently reported adverse events other than somnolence were headache (12% for JNJ-42847922 versus 0% for placebo), and dizziness/postural dizziness (12% for JNJ-42847922 versus 8% for placebo). One subject administered 80 mg JNJ-42847922 experienced a single brief episode of sleep paralysis (awareness of environment with inability to move, while transitioning from wakefulness to sleep) shortly after dosing. There were no clinically significant observations in any other safety evaluations for actively-treated subjects.
7. Discussion

In the present report we characterized a novel selective OX2R antagonist as a clinical candidate for the treatment of insomnia. We demonstrated that the compound had excellent in vitro affinity/potency/selectivity for the OX2R and promoted sleep after oral administration in rodent models. In healthy subjects, JNJ-42847922 displayed a favorable pharmacokinetic and safety profile as well as a strong pharmacodynamic effect.

Only a few selective OX2R antagonists have been reported in the literature. The first one to be reported was TCS OX229, a tetrahydroisoquinoline with moderate affinity but good selectivity for the OX2R (Hirose et al., 2003). Our evaluation of this molecule revealed poor pharmacokinetic properties and lack of target engagement after systemic administration in rodents (unpublished data). A year later, we reported the synthesis of JNJ-10397049, a phenyl-dioxanyl urea (McAtee et al., 2004). JNJ-10397049 crossed the blood brain barrier, engaged the OX2R and induced sleep in rats after systemic administration (Dugovic et al., 2009). However, further development of that molecule was stopped due to poor drug-like properties including low oral bioavailability, poor solubility and cytochrome P450 interactions. EMPA, an acetamide, was disclosed by Roche in 2009 and was shown to be a highly selective molecule for OX2R (Malherbe et al., 2009b). This molecule was also used as a radioligand for the OX2R. Eli Lily has also recently reported the development of LSN2424100, a sulfonamide (Fitch et al., 2014). Interestingly, this selective OX2R antagonist displayed antidepressant-like activity in rodents (Fitch et al., 2014). Lastly, MK-1064 and MK-3697 were disclosed by Merck and are potential clinical candidates (Roecker et al., 2014a; Roecker et al., 2014b).
In the present study, we conducted an extensive preclinical characterization of this novel OX2R antagonist. *In vitro*, JNJ-42847922 was a high affinity OX2R antagonist with an approximately 2-log selectivity ratio versus the OX1R. It had a remarkable intrinsic selectivity for the OX2R over a panel of receptors and enzymes. Taking advantage of the OX2R KO mice we clearly demonstrated that the mechanism of action for the sleep promoting effect occurred via selective blockade of OX2R. We also showed that both the reduced sleep onset and the increased sleep duration were maintained upon 7-day repeated dosing, and that upon discontinuation there was no rebound effect on any of the sleep parameters measured.

Various DORAs have been shown to promote both NREM and REM sleep in animals and humans (Hoyer and Jacobson, 2013; Winrow and Renger, 2014). However, emerging preclinical data indicate that selective blockade of OX2R is sufficient to initiate and prolong sleep (Dugovic et al., 2009; Mang et al., 2012). In the present study, oral administration of JNJ-42847922 in rats dose-dependently reduced NREM latency and increased NREM sleep duration whereas REM sleep was minimally affected. The lowest effective dose was 3 mg/kg administered either at the beginning of the light phase (present study) or at dark onset (Letavic et al., in press). This dose corresponds to the ED$_{50}$ value in the receptor occupancy evaluation, indicating that 50% OX2R occupancy was sufficient to promote sleep. Sleep efficacy was obtained at the same degree of receptor occupancy with the OX2R antagonist JNJ-10397049 at the dose of 3 mg/kg, s.c. (Dugovic et al., 2009). In contrast, higher levels of receptor occupancy were required for sleep-promoting effects with DORAs, 90% OX2R occupancy with almorexant at 30 mg/kg dosage (Dugovic et al., 2009) and 70-80% receptor occupancy with suvorexant.
(Winrow and Renger, 2014). At all doses of JNJ-42847922 tested in acute conditions, small non-significant REM sleep-promoting effects were observed. However, significant levels were reached only on day 4 during the sub-chronic treatment. Similarly, a newly developed selective OX2R antagonist, compound 1m, has been reported to primarily increase NREM sleep time with minimal effects on REM sleep when administered in mice during the dark phase (Etori et al., 2014). Efficacy on both NREM and REM sleep has been shown with a number of selective OX2R antagonists from Merck: compound 18 (Mercer et al., 2013), MK-1064 (Roecker et al., 2014a), MK-3697 (Roecker et al., 2014b), compound PE-6 (Raheem et al., 2015) and 2-SORA 19 (Roecker et al., 2015) in rodent models. However, dose-response effects have not been reported with these compounds, and the single dose tested might have been high enough to promote both sleep states. While JNJ-42847922 did achieve mainly NREM sleep efficacy at doses up to 30 mg/kg, we found a significant reduction in REM sleep latency and increased REM sleep time at the high dose of 60 mg/kg administered at dark onset in rats with which 74% receptor occupancy was attained. These data confirm that selective OX2R blockade primarily promotes NREM sleep, as opposed to the DORAs SB-649868 and suvorexant which have been reported to predominantly promote REM sleep (Dugovic et al., 2014; Etori et al., 2014). Importantly, specificity of OX2R antagonists may favorably preserve sleep architecture as evidenced by the maintenance of the REM/total sleep ratio with JNJ-42847922 (present study) and JNJ-10397049 (Dugovic et al., 2009) even at doses that also promote REM sleep, in contrast to several dual OX1/2R antagonists (Hoyer and Jacobson, 2013; Dugovic et al., 2014; Etori et al., 2014).
We also evaluated the potential abuse liability of this mechanism of action by using neurochemistry and conditioned placed preference. The neurochemistry experiment showed that JNJ-42847922 did not increase dopamine release in nucleus accumbens. In the conditioned place preference test, JNJ-42847922 did not produce place preference unlike zolpidem, indicating that the compound lacks intrinsic motivational properties. The Rotarod test was also used to assess the effect of JNJ-42847922 on motor coordination. At 30 mg/kg, a dose 10-fold higher than the minimal effective dose to induce sleep, the compound did not alter motor coordination. In addition, the compound did not potentiate the ataxic effect of alcohol in this test. This is in sharp contrast to the well-known zolpidem-induced ataxia, highlighting the safety advantage of this new mechanism of action versus traditional GABAergic mechanism. Therefore, JNJ-42847922, like other OXR antagonists promotes sleep without causing motor impairment or alcohol interaction (reviewed in (Hoyer and Jacobson, 2013; Winrow and Renger, 2014).

JNJ-42847922 did show great promise as a development candidate for the treatment of insomnia. A clear, consistent, and rapid hypnotic-like effect was observed with single dose administration of 10 to 80 mg in healthy subjects. JNJ-42847922 was safe and well tolerated, and had a favorable pharmacokinetic profile for a sedative/hypnotic, characterized by rapid absorption and a short half-life of approximately 2 hours. The human pharmacokinetic profile is in line with the prediction based on the pharmacokinetic properties in mouse, rat, dog and monkey (Letavic et al., in press). Specifically, the compound had a relatively short duration of action in all preclinical species, and moderate to low bioavailability. Remarkably, the plasma
concentration obtained in humans at the 20 mg dose (~550 ng/mL) is in the range of the plasma concentration required to occupy 50% of OX2R in rat brain occupancy studies (~400 ng/mL). Preliminary EEG polysomnography data obtained in a phase 1b study showed that treatment with JNJ-42847922 resulted in significant improvement in both sleep onset and total sleep duration in patients with comorbid insomnia related to major depressive disorder (http://ir.minervaneurosciences.com/). Further details will be disclosed in a future manuscript.

In conclusion, JNJ-42847922 displayed a favorable pharmacokinetic and safety profile in concert with a strong pharmacodynamic effect on sleep induction. The data indicate multiple points of differentiation from non-benzodiazepine sedative-hypnotics such as zolpidem, as well as clear distinction on sleep architecture and achievable efficacy from DORAs such as suvorexant. Thus, JNJ-42847922 is a prototype of the Selective Orexin-2 Receptor Antagonists (SORAs) emerging as a promising new class of non-sedative hypnotics for further clinical development in the treatment of insomnia.
8. Acknowledgment

The assistance of Dr. K. Sharp and his staff at Janssen Research & Development L.L.C. (San Diego, CA) is gratefully acknowledged.

9. Authorship Contributions

Participated in research design: Pascal Bonaventure, Sandra Chaplan, Zuleima Aguilar, Timothy Lovenberg, Christine Dugovic, Tatiana Koudriakova, Anthony Ndifor.

Conducted experiments: Jonathan Shelton, Sujin Yun, Diane Nepomuceno, Steven Sutton, Leah Aluisio, Ian Fraser, Brian Lord, James Shoblock, Natalie Welty.

Contributed new reagents or analytic tools: Michael Letavic, Nicholas Carruthers, Michele Rizzolio.

Performed data analysis: Christine Dugovic, Jonathan Shelton, Sujin Yun, Diane Nepomuceno, Brian Lord, Leah Aluisio, James Shoblock, Robin Halter, Sandra Chaplan.

Wrote or contributed to the writing of the manuscript: Pascal Bonaventure, Robin Halter, Christine Dugovic.
10. References


Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem Pharmacol* **22**:3099-3108.


Etori K, Saito YC, Tsujino N and Sakurai T (2014) Effects of a newly developed potent orexin-2 receptor-selective antagonist, compound 1 m, on sleep/wakefulness states in mice. *Front Neurosci* **8**:8.


11. Figure Legends

**Figure 1**: Chemical structure of JNJ-42847922 ([5-(4,6-Dimethyl-pyrimidin-2-yl)\-hexahydro-pyrrolo[3,4-c]pyrrol-2-yl]-(2-fluoro-6-[1,2,3]triazol-2-yl-phenyl)-methanone).

**Figure 2**: Ex vivo OX2R occupancy with JNJ-42847922 in rat cortex: Time-dependency (30 mg/kg) (A) and dose-dependency (B) after oral administration. OX2R occupancy was measured 15 minutes after drug administration. N = 3 per time point or dose. Data are represented as means ± S.E.M.

**Figure 3**: Dose-response effects of JNJ-42847922 (3, 10, and 30 mg/kg, p.o.) on sleep parameters during the light phase in rats. NREM latency (A), REM latency (B), NREM duration (C) and REM duration (D) were determined for the 2-hour period after compound or vehicle administration. Data are expressed in minutes and are represented as means ± S.E.M. of the same 8 animals per dose. ** p < 0.01 and *** p < 0.001 versus vehicle, based on one-way ANOVA followed by Dunnett’s multiple comparison post hoc test.

**Figure 4**: Effects of 7-day repeated dosing (D1 to D7) with JNJ-42847922 (30 mg/kg, p.o./day) on sleep parameters during the light phase in rats. NREM latency (A), REM latency (B), NREM duration (C) REM duration (D), NREM bout duration (E) and locomotor activity (F) were determined for the 2-hour period after compound or vehicle...
administration. Data are expressed in minutes except for locomotor activity (counts) and are represented as means ± S.E.M. of the same 9 animals per condition. * p < 0.05, ** p < 0.01 and *** p < 0.001 versus vehicle 2 (Veh2) condition, based on one-way ANOVA followed by Dunnett’s multiple comparison post hoc test.

**Figure 5:** Effects of JNJ-42847922 (30 mg/kg, p.o.) on sleep parameters in WT and OX2R KO mice. NREM latency (A, B), NREM duration (C, D), REM latency (E, F), and REM duration (G, H) were determined for the 2-hour period after compound or vehicle administration. Data are expressed either in minutes (A, B, C, D, E, F) or in seconds (G, H) and are represented as means ± S.E.M. of the same number of animals (N= 7 WT; N = 5 OX2R KO) per condition. Vehicle represented as open bar and JNJ-42847922 as closed bar. * P<0.05 and ** P<0.01 versus vehicle, based on paired Student’s t test.

**Figure 6:** Nonclinical secondary pharmacodynamic studies performed with JNJ-42847922. All data are represented as means ± S.E.M. (A) Effects of JNJ-42847922 (30 mg/kg, p.o) on extracellular dopamine levels from rat nucleus accumbens (N = 5). Amphetamine (0.3 mg/kg, s.c.) was administered as a positive control at the end of the experiment. (B) Effects of JNJ-42847922 (10 mg/kg, i.p., N = 17), vehicle (N = 13) or zolpidem (10 mg/kg, i.p., N = 18) in the conditioned place preference model in mice, amphetamine (2 mg/kg i.p., N = 12) was administered as a positive control. (C) Effects of JNJ-42847922 (30 mg/kg, p.o.) or vehicle on rat motor coordination in the Rotarod apparatus (N = 8 animals per group). (D) Effects of JNJ 42847922 (30 mg/kg, p.o.), vehicle or zolpidem (10 mg/kg, p.o.) coadministered with alcohol (1 g/kg, i.p.) on motor
coordination in the Rotarod apparatus (N = 8 animals per group). * p<0.05 compared to vehicle.

**Figure 7:**
Plasma concentrations of JNJ-42847922 (means ± S.E.M.) at various time points after a single oral dose in humans. Doses were 10 mg (●), 20 mg (■), 40 mg (○) and 80 mg (□). Inset: The Y- axis depicts the natural log of each subject’s C\text{max} (□) and AUC (●) value for each dose, plotted versus the natural log of each dose on the X-axis. Linear regression with 99% confidence limits was performed for C\text{max} and AUC. R\textsuperscript{2} values for goodness of fit were 0.86 and 0.71, respectively.

**Figure 8:**
Percent of subjects with somnolence captured as an adverse event after a single oral dose of JNJ-42847922 in humans. PBO: Placebo. N: PCB = 13, 10 mg = 8, 20 mg = 6, 40 mg = 6, 80 mg = 6.
Tables

**Table 1:** *In vitro* binding affinity (pKᵢ values) and *in vitro* pKᵦ values for intracellular calcium responses of JNJ-42847922 at human and rat OX1R and OX2R. The values are expressed as average ± S.D., and the number of triplicate experiments is indicated in parentheses. OX1R = orexin-1 receptor; OX2R = orexin-2 receptor, h: human; r: rat.

<table>
<thead>
<tr>
<th></th>
<th>Affinity</th>
<th>Potency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pKᵢ</td>
<td>pKᵦ</td>
</tr>
<tr>
<td>hOX2R</td>
<td>8.0 ± 0.1 (23)</td>
<td>8.8 ± 0.2 (11)</td>
</tr>
<tr>
<td>hOX1R</td>
<td>6.1 ± 0.2 (23)</td>
<td>6.3 ± 0.3 (3)</td>
</tr>
<tr>
<td>rOX2R</td>
<td>8.1 ± 0.1 (14)</td>
<td>8.0 ± 0.1 (4)</td>
</tr>
<tr>
<td>rOX1R</td>
<td>6.2 ± 0.1 (4)</td>
<td>&lt;6.0 ± 0.01 (3)</td>
</tr>
</tbody>
</table>
**Table 2**: Ex vivo OX2R occupancy with JNJ-42847922 (30 mg/kg, p.o.) in rat cortex: plasma and brain exposure at the various time points. Results are means ± S.E.M (N = 3). BLLOQ = below the lowest level of quantification; OX2R = orexin-2 receptor; RO = receptor occupancy.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>% OX2R RO</th>
<th>Brain Concentration (ng/mL)</th>
<th>Plasma Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08</td>
<td>49 ± 5</td>
<td>205 ± 30</td>
<td>1119 ± 296</td>
</tr>
<tr>
<td>0.25</td>
<td>62 ± 1</td>
<td>646 ± 153</td>
<td>2330 ± 443</td>
</tr>
<tr>
<td>0.5</td>
<td>68 ± 1</td>
<td>512 ± 106</td>
<td>1844 ± 298</td>
</tr>
<tr>
<td>1</td>
<td>74 ± 6</td>
<td>1040 ± 388</td>
<td>2107 ± 109</td>
</tr>
<tr>
<td>2</td>
<td>43 ± 4</td>
<td>98 ± 9</td>
<td>379 ± 44</td>
</tr>
<tr>
<td>4</td>
<td>40 ± 4</td>
<td>42 ± 7</td>
<td>167 ± 24</td>
</tr>
<tr>
<td>6</td>
<td>23 ± 4</td>
<td>15 ± 3</td>
<td>105 ± 45</td>
</tr>
<tr>
<td>24</td>
<td>0 ± 0</td>
<td>BLLOQ</td>
<td>BLLOQ</td>
</tr>
</tbody>
</table>
Table 3: Basal duration of wake, NREM and REM sleep during the light phase and dark phase in WT and OX2R KO mice. Data are means ± S.E.M. (N=10 WT, N=10 OX2 KO) expressed in minutes (min). * P<0.05 and ** P<0.01 versus WT based on unpaired Student’s t test.

<table>
<thead>
<tr>
<th></th>
<th>Wake duration (min)</th>
<th>NREM duration (min)</th>
<th>REM duration (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light phase</td>
<td>Dark phase</td>
<td>Light phase</td>
</tr>
<tr>
<td>OX2R WT</td>
<td>233.4       ± 12.6</td>
<td>472.5       ± 14.7</td>
<td>431.3       ± 11.6</td>
</tr>
<tr>
<td>OX2R KO</td>
<td>255.0       ± 5.8</td>
<td>408.6       ± 11.9 **</td>
<td>416.2       ± 6.4</td>
</tr>
</tbody>
</table>
Table 4: Plasma pharmacokinetics of JNJ-42847922 after single oral doses of 10 to 80 mg in the morning in healthy male subjects. Mean ± S.D.

<table>
<thead>
<tr>
<th>Dose</th>
<th>N</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;inf&lt;/sub&gt; (ng.h/mL)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg</td>
<td>8</td>
<td>309 ± 74</td>
<td>0.33</td>
<td>802 ± 275</td>
<td>2.02 ± 0.26</td>
</tr>
<tr>
<td>20 mg</td>
<td>6</td>
<td>556 ± 104</td>
<td>0.42</td>
<td>1616 ± 607</td>
<td>2.11 ± 0.47</td>
</tr>
<tr>
<td>40 mg</td>
<td>6</td>
<td>743 ± 149</td>
<td>0.42</td>
<td>1807 ± 533</td>
<td>2.02 ± 0.30</td>
</tr>
<tr>
<td>80 mg</td>
<td>6</td>
<td>1208 ± 291</td>
<td>0.50</td>
<td>3655 ± 1064</td>
<td>2.44 ± 0.46</td>
</tr>
</tbody>
</table>
Figure 1
Figure 2

A

Time (h)

0 2 4 6 8 10 12 14 16 18 20 22 24 26

% OX2R Occupancy

0 10 20 30 40 50 60 70 80 90 100

B

Dose (mg/kg, p.o.)

0.01 0.1 1 10 100

% OX2R Occupancy

0 10 20 30 40 50 60 70 80 90 100
Figure 3

A. NREM Latency

B. REM Latency

C. NREM Duration / 2h

D. REM Duration / 2h
Figure 4

A. NREM Latency

B. REM Latency

C. NREM Duration / 2h

D. REM Duration / 2h

E. NREM Bout Duration / 2h

F. Locomotor Activity / 2h
Figure 5

**WT**

A. NREM Latency

B. NREM latency

C. NREM Duration / 2h

D. NREM Duration / 2h

**OX2 KO**

E. REM Latency

F. REM Latency

G. REM Duration / 2h

H. REM Duration / 2h
Figure 6

A. Dopamine release

B. Conditioned place preference

C. Ataxia

D. Alcohol-induced ataxia