Title Page

Nelotanserin, a Novel Selective Human 5-Hydroxytryptamine$_{2A}$ Inverse Agonist for the Treatment of Insomnia


Running Title Page

a) Running Title: Nelotanserin, a 5-HT2A inverse agonist for insomnia.

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c) Number of text pages: 43
   Number of tables: 4
   Number of figures: 8
   Number of references: 37
   Number of words in the Abstract: 250
   Number of words in Introduction: 676
   Number of words in the Discussion: 1393

d) Abbreviations: 5-HT, 5-Hydroxytryptamine (serotonin); IP, inositol phosphate; DOI, (+/−)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane; NREM, non-rapid eye movement; SWS, slow wave sleep; ST1, stage 1 non-rapid eye movement; ST2, stage 2 non-rapid eye movement; ST3, stage 3 non-rapid eye movement; ST4, stage 4 non-rapid eye movement; EEG, electroencephalogram; CNS, central nervous system; PK, pharmacokinetic; PD, pharmacodynamic; C_{max}, maximum plasma concentration; t_{max}, time to maximum plasma concentration; t_{1/2}, terminal phase plasma half-life; AUC, area under the plasma exposure curve; MRT, mean residency time; EMG, electromyogram; β, bregma; AP, anterioposterior; ML, mediolateral; λ, lambda; REM, rapid eye movement; PSG, polysomnogram; MCRT, Multiple Choice Reaction Time; CFFT, Critical Flicker Fusion Test; RAVLT, Rey Auditory Verbal Learning Test; ARCI-49, Addiction Research Center Inventory; VAS, Visual Analogue Scale; DSST, Digit Symbol Substitution Test; LSEQ, Leeds Sleep Evaluation Questionnaire; GPCR, G protein-coupled receptor; CL/F, clearance from plasma; Vz/F, volume of distribution.

e) Recommended section assignment is Neuropharmacology
Abstract

5-hydroxytryptamine (5-HT)2A receptor inverse agonists are promising therapeutic agents for the treatment of sleep maintenance insomnias. Among these agents is nelotanserin, a potent, selective 5-HT2A inverse agonist. Both, radioligand binding and functional inositol phosphate (IP) accumulation assays suggest nelotanserin has low nanomolar potency on the 5-HT2A receptor with at least 30- and 5000-fold selectivity compared to 5-HT2C and 5-HT2B receptors, respectively. Nelotanserin dosed orally prevented (+/−)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI; 5-HT2A agonist)-induced hypolocomotion, increased sleep consolidation, and increased total non-rapid eye movement (NREM) sleep time and deep sleep, the latter marked by increases in electroencephalogram (EEG) delta power. These effects on rat sleep were maintained following repeated sub-chronic dosing. In healthy human volunteers, nelotanserin was rapidly absorbed following oral administration and achieved maximum concentrations 1 hour later. EEG effects occurred within 2 to 4 hours after dosing, and were consistent with vigilance-lowering. A dose-response of nelotanserin was assessed in a post-nap insomnia model in healthy subjects. All doses (up to 40mg) of nelotanserin significantly improved measures of sleep consolidation, including decreases in the number of stage shifts, number of awakenings after sleep onset, microarousal index, and number of sleep bouts, concomitant with increases in sleep bout duration. Nelotanserin did not impact total sleep time, or sleep onset latency. Further, subjective pharmacodynamic effects observed the morning after dosing were minimal and had no functional consequences on psychomotor skills or memory. These studies point to an efficacy and safety profile for nelotanserin that might be ideally suited for the treatment of sleep maintenance insomnias.
Introduction

Serotonin has long been implicated in the regulation of sleep and wakefulness. Central serotonergic neurons of the dorsal raphe nucleus form part of the reticular activating system that innervates cortical and subcortical regions of the forebrain. As its name implies, this system modulates arousal in the central nervous system (CNS) and regulates sleep/wake states (Abrams et al., 2005). Within this system, 5-HT neurons are active during wakefulness, less active during non-REM sleep, and inactive during REM sleep.

5-HT activity in the CNS is mediated by multiple receptor subtypes that are classified into seven subfamilies (5-HT$_1$-5-HT$_7$) (Hoyer et al., 1994). The 5-HT$_2$ subfamily includes three subtypes: 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$. Unlike 5-HT$_{2B}$ receptors, which have limited CNS expression, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors are widely distributed in the CNS in areas that are implicated in the regulation of sleep and waking (Leysen, 2004). There is compelling preclinical and clinical evidence that supports a role for 5-HT$_{2A}$ antagonism in the treatment of sleep maintenance insomnias (Borbély et al., 1988; Monti and Jantos, 2006; Fish et al., 2005; Landolt et al., 1999; Popa et al., 2005). Indeed, several selective 5-HT$_{2A}$ inverse agonists have entered clinical development for the treatment of insomnia, these include eplivanserin, volinanserin, pruvanserin and nelotanserin (Teegarden et al., 2008).

Initial interest in 5-HT$_2$ receptors and their role in sleep arose from studies with selective 5-HT$_2$ modulators. For example, in both rodents and humans, slow wave sleep (SWS) is increased by the selective 5-HT$_2$ receptor antagonist ritanserin in a dose-dependent manner, in contrast, the selective 5-HT$_2$ receptor agonist mCPP produces the
opposite effect (Idzikowski et al., 1986). Additional evidence supporting a role for 5-HT2 receptors in sleep comes from the use of various antipsychotic agents known to promote SWS in both humans and rats (Sharpley et al., 1990; Dugovic and Wauquier, 1987; Monti and Monti, 2004). For example, the atypical antipsychotic drugs risperidone and olanzapine increase SWS in both schizophrenic and healthy subjects, respectively (Dursun et al., 1999; Yamashita et al., 2002; Sharpley et al., 2000). Nevertheless, as these agents lack selectivity, their sleep promoting effects cannot be attributed to inhibition of 5-HT2A receptors alone.

Studies with transgenic animals, specifically 5-HT2A and 5-HT2C receptor knockout mice, suggest that these receptors modulate sleep-wake states (Popa et al., 2005; Frank et al., 2002). Both knockout strains show a decrease in NREM sleep compared to wild type controls. This biologically induced decrease is in contrast to the increase in NREM sleep induced by antagonists to these two receptors. For example, the selective 5-HT2A inverse agonist volinanserin increases NREM sleep in wild type mice. This effects is absent in 5-HT2A receptor knockout mice, suggesting that volinanserin mediates its effects on sleep through this receptor site. These data, coupled with the baseline decreases in NREM sleep in the knockout mice, suggest possible adaptive mechanisms are at play in the constitutive 5-HT2A receptor mutants. Indeed, both 5-HT2C and 5-HT2B ligands induce different responses on sleep parameters in the 5-HT2A knockout compared to control strains (Popa et al., 2005).

The discovery of more selective 5-HT2A inverse agonists has confirmed the role of this receptor in sleep modulation. For example, eplivanserin, a selective 5-HT2A inverse agonist, increases SWS and slow wave EEG activity in humans and rats (Landolt...
et al., 1999; Francon et al., 2007). Similarly, volinanserin showed a dose dependent increase in sleep consolidation characterized by a decrease in the number of NREM sleep bouts and concomitant increase in NREM sleep bout length (Fish et al., 2005; Morairty et al., 2008).

We recently reported the discovery and synthesis of nelotanserin, a selective 5-HT$_{2A}$ inverse agonist (Teegarden et al., 2008). Nelotanserin induced dose-dependent sleep consolidation effects and increased markers of deep SWS in rats. Further, when tested in healthy volunteers, nelotanserin was well tolerated and induced increases in sleep consolidation effects. These findings confirm and extend earlier reports of 5-HT$_{2A}$ inverse agonist effects on sleep maintenance, and point to the potential clinical utility of nelotanserin in the treatment of insomnias. This manuscript details the preclinical, early clinical pharmacokinetic (PK) and pharmacodynamic (PD) results of nelotanserin.
Methods

Preparation of Nelotanserin

Nelotanserin was discovered and synthesized at Arena Pharmaceuticals, Inc. The chemical structure of nelotanserin (molecular weight 437.24) is displayed in Figure 1.

In Vitro Studies

Drugs and Cell Culture Reagents. The following reagents were purchased from commercial suppliers: HEK293 and COS7 cells (ATCC, Rockville, MD); fetal calf serum, Dulbecco’s Modified Eagle’s Medium, Optimem I, and lipofectamine (Invitrogen, Carlsbad, CA); [3H]-myoinositol and [125I]DOI (Perkin Elmer, Boston, MA). All 5-HT2 antagonists and inverse agonists were purchased from either RBI/Sigma (St. Louis, MO) or from Tocris (Ellisville, MO).

Cell Growth and Expression of Recombinant Human and Rat 5-HT2 Receptors. HEK293 cells stably expressing human 5-HT2A, 5-HT2B, and 5-HT2C (unedited INI version) receptors were generated and used for all [125I]DOI competition binding assays as previously described. The same HEK293 expressing human 5-HT2A and 5-HT2C, but not 5-HT2B receptors contained constitutive activity in the form of elevated basal IP accumulation, which allowed determination of potential inverse agonist activity of nelotanserin. Nelotanserin inverse agonist activity at 5-HT2B receptors was determined in HEK293 cells transiently transfected with the human 5-HT2B receptor.

Similarly, HEK293 cells stably expressing rat 5-HT2A receptor, or transiently expressing rat 5-HT2B or 5-HT2C were used for [125I]DOI competition studies as previously described (Thomsen et al., 2008). All functional IP accumulation studies for
rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were determined in HEK293 cells transiently expressing these receptors. Enhanced basal IP accumulation was not present in these cells, thus limiting our pharmacological evaluation of nelotanserin to antagonist mode (with an EC$_{80}$ concentration of 5-HT present in the assay).

**Preparation of HEK293 Cell Plasma Membranes from Cells Expressing Recombinant Human and Rat 5-HT$_2$ Receptors.** Crude plasma membranes from HEK293 cells stably or transiently expressing recombinant human or rat 5-HT$_2$ receptors were prepared as previously described (Thomsen et al., 2008). Crude membrane pellets were prepared 24-hours post transfection and stored at -80°C until they were used for radioligand binding competition assays.

**[125I]DOI Binding to Recombinant Human and Rat 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ Receptors.** Radioligand binding assays for human and rat 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors were performed as previously described (Thomsen et al., 2008). Each nelotanserin radioligand competition study tested eight to ten different concentrations (determined in triplicate).

**Radioligand Binding Assays for Additional Human 5-HT Receptors and Neurotransmitter Transporters.** Nelotanserin competition for radioligand binding to human 5-HT$_{1A}$, 5-HT$_3$, 5-HT$_{4C}$, 5-HT$_{5A}$, 5-HT$_6$, and 5-HT$_7$ receptors as well as dopamine, and norepinephrine transporters were performed at Cerep, Inc. (Poitiers, France).
5-HT$_2$ IP Accumulation Assays. Human and rat 5-HT$_2$ receptor IP accumulation assays were performed as previously described (Thomsen et al., 2008). Evaluations of potential antagonist activity were conducted in the presence of an EC$_{80}$ concentration of 5-HT. Nelotanserin was tested at eight different concentrations in triplicate.

In Vitro Data Analysis. For radioligand binding experiments, IC$_{50}$ values were obtained by fitting radioligand competition data to a sigmoidal function using a nonlinear least-squares program (GraphPad, San Diego, CA). In all cases, data produced a better fit to a single-site model than a two-site model (data not shown). The same nonlinear curve fitting program was used to fit $[^{125}I]$DOI, $[^{3}H]$ketanserin, and $[^{3}H]$mesulergine saturation data for 5-HT$_2$ receptors to a simple hyperbolic function for determination of K$_d$ and B$_{max}$ values (data not shown). K$_i$ values were calculated using the Cheng-Prusoff equation (Cheng and Prusoff, 1973). For IP accumulation, EC$_{50}$ values were also determined by fitting data to sigmoidal function (variable slope) using the same nonlinear least squares curve fitting program.

In Vivo Studies

Animal Subjects. Adult Male Sprague Dawley rats (Harlan, San Diego, CA) were used for all in vivo studies. These rats were maintained in an environment controlled for temperature and light (lights on: 1400h-0200h), and provided food and water ad-libitum.
Rat Pharmacokinetic Evaluation. Nelotanserin was formulated in 80% Tween 80 and 20% phosphate buffered saline and orally administered to rats at a dosing volume of 5 mL/kg. Heparinized plasma samples and brain extracts were collected and analyzed for nelotanserin using a selective LC/MS/MS method. Briefly, nelotanserin was separated from matrix proteins in rat plasma and brain homogenates with the addition of acetonitrile at 2-fold the tissue volume, followed by centrifugation. The supernatants collected from the processed plasma and brain samples were injected onto an HPLC system equipped with a Sciex API 3000 mass spectrometer (AME Biosciences, Toroed, Norway). Quantitation was performed with regression analyses of external calibration standards.

Noncompartmental PK analysis was performed with a commercial software package (WinNonlin Professional version 4.1.b., Pharsight, Mountain View, CA). The PK parameters evaluated include maximum plasma concentration (C_max), time to maximum plasma concentration (t_max), terminal phase plasma half-life (t_1/2), area under the plasma exposure curve from the time of dosing to the last sample collected (AUC_last), area under the plasma exposure curve from the time of dosing extrapolated to infinity (AUC_infinity), and mean residency time from the time of dosing to the last sample collected (MRT_last).

Inhibition of DOI-induced Decreases in Rearing. Prior to evaluation in rat sleep, nelotanserin was tested in a DOI screening assay. DOI is a 5-HT_2 partial agonist that induces a biphasic locomotor response (hypolocomotion followed by hyperlocomotion) in rats (Wing et al., 1990). The hypolocomotive response is readily measured by counting the number of times an animal rears within 10 minutes after DOI.
administration. Inhibition of this response with selected 5-HT\textsubscript{2A} inverse agonists suggests activity at central 5-HT\textsubscript{2A} receptors.

Nelotanserin or vehicle was administered first, followed (25 minutes later) by DOI. Ten minutes after DOI administration each rat was placed in a standard clear plastic cage surrounded by a stainless steel frame containing photocell beams across the long and short axis of the frame, which, when broken, register both fine and gross movement. Ten minutes of rearing activity was recorded for each animal dosed with vehicle or nelotanserin and DOI.

**Rat Sleep.** The effects of nelotanserin on rat sleep were evaluated in two studies. The first measured the acute effects of nelotanserin (vehicle, 1, 3 and 10 mg/kg), and the second measured the sub-chronic effects of nelotanserin (vehicle, 10mg/kg).

**Acute dose response study.** All rats used in the acute sleep studies were prepared with plastic implants designed to provide continuous EEG and electromyogram (EMG) recordings (Plastics One, Inc., Roanoke, VA). To this end, rats were anesthetized with a ketamine (80mg/kg)/xylazine(12mg/kg) cocktail, fitted into a stereotaxic apparatus, and a dorsal midline incision along the top of the head was used to expose the skull. Four stainless steel spring top screws (#000) were implanted so as to penetrate the skull and contact, but not disturb, the dura. These served as epidural electrodes to which the EEG leads were attached. Two of these screws were implanted on the left (bregma (\(\beta\)) -1.0mm anterioposterior (AP), \(\beta\) -3.0mm mediolateral (ML); and lambda (\(\lambda\)) +1.0mm AP, \(\lambda\) - 4.0mm) and used to record alpha and delta EEG. The remaining two screws were implanted on the right (\(\beta\) +3.0mm AP, \(\beta\) +1.0mm ML; and \(\lambda\) +4.0mm AP, \(\lambda\) +1.0mm)
and used to record theta waves. Finally, two electrodes for EMG recordings were implanted into dorsal neck muscles and secured with sutures. The plastic connector from which the six recording electrodes emanated was then secured to the skull with cement (3M Garant; Irvine, CA), and the scalp was sutured around the implant to allow protrusion of the lead connectors. All animals were treated with buprenex (0.3 mg/kg, intramuscular) for two days post operative recovery. Rats were allowed at least 2 weeks recovery before experimentation. At test, the rats were housed in plastic testing boxes (34cm x 24cm x 50cm; Dragonfly Inc., Ridgeley, WV) with free access to food and water. After box placement, the skull implants were connected to cable attached to a swivel system that allowed the rats to move freely within the cage, and transmitted data to a data collection system (Embla A10; Embla Systems; Broomfield, CO). EEG and EMG recordings were then digitized and analyzed using Somnologica Science Software (Embla Systems; Broomfield, CO).

The acute effects of nelotanserin on rat sleep were tested using a repeated measures design wherein each rat was dosed orally with either vehicle polyethylene glycol 400 or one of three concentrations of nelotanserin (1, 3, or 10mg/kg) administered three days apart in pseudo-random order. All doses were administered 2 hours prior to lights on. Sleep recordings were initiated immediately after dosing and continued for 24 hours.

Subchronic repeated dose study. The effects of repeated doses of nelotanserin on rat sleep were tested in animals implanted with telemetric transmitters (Data Sciences International, Model F50-EEE; St. Paul, MN). Similar to the tethered system described in the previous section, these transmitters contain six electrodes designed to transmit...
EEG and EMG signals. These electrodes were implanted using the same coordinates and procedures described above, with the exception that the telemetry transmitter was implanted subcutaneously in the flank region, and there was no need for a skull cap to accommodate hard wire connections. The rats were returned to their home cages (housed individually) for recovery and subsequent sleep study recordings. Telemetric data were captured using Data Quest 3.0 software (Data Sciences International, St. Paul, MN) and analyzed using Somnologica Science Software (Embla Systems; Broomfield, CO).

This study was designed to provide insight into the effects of repeated dosing on the sustained efficacy of nelotanserin. To this end, the effects of nelotanserin on rat sleep were compared between two groups which received once-a-day oral doses of either vehicle or nelotanserin (10mg/kg) for 6 consecutive days. All doses were administered 2 hours prior to lights on. Sleep recordings were initiated immediately after the first dose and continued for 7 consecutive days. Recordings from days 1, 3, 5, and 7 (recovery day) were scored and analyzed.

**Polysomnographic Data Analysis.** All polysomnogram (PSG) recordings were blinded prior to scoring to avoid experimenter bias. EEG and EMG data were scored visually in 10-second epochs for waking, rapid eye movement (REM) sleep, and NREM sleep. Scored data were analyzed and expressed as time spent in each state per half hour. Similarly, bout length and number of bouts for each state were clustered into half hour bins. A “bout” consisted of a minimum of two consecutive epochs of a given state. EEG delta power (1-4 Hz) within NREM sleep was also analyzed in half hour bins. The EEG spectra during NREM sleep were obtained offline with a fast Fourier transform algorithm on all epochs. Data were analyzed using ANOVA. When statistical significance was
found from our ANOVAs, t-tests were performed comparing all nelotanserin treatment groups to vehicle.

Evaluation of Nelotanserin in Healthy Volunteers

The single-dose nelotanserin PK and PD data were derived from two Phase 1 studies, nelotanserin-001 and nelotanserin-002. These studies were conducted in healthy volunteers with no pre-existing sleep disorders. The protocol and informed consent documents for each study were approved by an independent institutional review board. The research was conducted in accordance with the declaration of Helsinki and Good Clinical Practice guidelines.

**Nelotanserin-001 Study Design.** Forty five healthy male adults aged 18 to 45 years were randomized to a single-dose, double-blind, placebo-controlled, dose-escalation study. The doses of nelotanserin tested were 10, 20, 40, 80, and 160 mg. Within each dose cohort, 9 subjects were randomized in a 2:1 ratio to receive either nelotanserin or placebo. Standard PK and safety measurements were performed throughout the study. In addition, detailed PD measurements were performed and included: day-time EEG, Leeds psychomotor tests (Multiple Choice Reaction Time [MCRT] and Critical Flicker Fusion Test [CFFT]), memory test (Rey Auditory Verbal Learning Test [RAVLT]), vigilance and mood scales (Addiction Research Center Inventory [ARCI- 49 scale, Bond and Lader Visual Analogue Scale [VAS], Digit Symbol Substitution Test [DSST]), and subjective sleep assessment scale (Leeds Sleep Evaluation Questionnaire [LSEQ]).
Nelotanserin-002 Study Design. The second of the two Phase 1 studies was a randomized, single-dose, double-blind, placebo-controlled study with a 4-way crossover to assess the safety and pharmacological effects of nelotanserin. In this study, subjects aged 45-65 were randomized to receive a single dose of one of three doses of nelotanserin (10, 20, or 40 mg) or placebo in a random order and were then crossed over following a minimum 5-day washout period. To assess the effects of nelotanserin on sleep in healthy subjects, a post-nap insomnia model as described by Mathias et al. (2001) was used. During screening, subjects had 3 consecutive nights of PSG monitoring. The first night was to rule out any co-morbid sleep disorders such as obstructive sleep apnea and periodic limb movement disorder. The other screening nights were to establish baseline and also ensure that subjects were asleep for at least 30 minutes during the 2-hour nap period. During each of the four treatment periods, a single night of PSG recording was performed. Nelotanserin or placebo was administered at 22:30 hours, 30 minutes prior to the initiation of PSG recordings, which were carried through to 7:00 hours the following morning. In addition to PSG measurement, the LSEQ, Leeds psychomotor test, word-pair retrieval test, and Bond and Lader vigilance and mood scales were performed.
Results

In Vitro Pharmacology

Radioligand Binding and Functional Activity of Nelotanserin at Human and Rat 5-HT Receptors. Nelotanserin displays high affinity for recombinant human 5-HT\textsubscript{2A} receptors (K\textsubscript{i}=0.35 nM), moderate affinity for human 5-HT\textsubscript{2C} receptors (K\textsubscript{i}=100 nM), and low affinity for human 5-HT\textsubscript{2B} receptors (2,000 nM) stably expressed in HEK293 cells. A summary of mean K\textsubscript{i} values obtained from multiple determinations is provided in Table 1. The results suggest that nelotanserin has a 262-fold higher affinity for human 5-HT\textsubscript{2A} than 5-HT\textsubscript{2C} receptors and a 6,610-fold higher affinity for human 5-HT\textsubscript{2A} than 5-HT\textsubscript{2B} receptors. The K\textsubscript{i} of nelotanserin for rat 5-HT\textsubscript{2A} is approximately 6-fold higher than the K\textsubscript{i} for human 5-HT\textsubscript{2A} receptors, whereas the K\textsubscript{i} values of nelotanserin for rat 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} are similar to the K\textsubscript{i} values for human 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} receptors (Table 1). In addition to evaluating nelotanserin affinity using the 5-HT\textsubscript{2} agonist \textsuperscript{125}I\text{DOI} as radioligand, nelotanserin competition studies using antagonist/inverse agonist radioligands for human 5-HT\textsubscript{2A} ([^3]H\text{ketanserin}) and 5-HT\textsubscript{2C} ([^3]H\text{mesulergine}) receptors gave similar K\textsubscript{i} values (data not shown).

Agonist activation of human and rat 5-HT\textsubscript{2} receptors increase intracellular IP and calcium (Roth et al., 1998). Figure 2 shows representative nelotanserin inverse agonist dose response curves for human 5-HT\textsubscript{2} receptors. Results from IP accumulation assays suggest nelotanserin is a potent 5-HT\textsubscript{2A} full inverse agonist (IC\textsubscript{50}=1.7 nM), a moderately potent 5-HT\textsubscript{2C} partial inverse agonist (IC\textsubscript{50}=79 nM) (maximal response was 62% of the response obtained for the reference inverse agonist clozapine), and a weak 5-HT\textsubscript{2B} inverse agonist (IC\textsubscript{50}=791 nM). Nelotanserin inverse agonist values obtained from
multiple determinations provide mean IC$_{50}$ values of 0.9+/−0.1nM, 6,856+/−2,575nM, and 30+/−8nM for recombinant human 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors, respectively.

The functional potency of nelotanserin for rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors was evaluated in an IP accumulation assay in antagonist mode (in the presence of an EC$_{80}$ concentration of 5-HT present in the assay). Nelotanserin blocked 5-HT-mediated IP accumulation with a potency of 4.7+/−0.4 nM and 170+/−39 nM for rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively.

**Competition of Nelotanserin for Radioligand Binding to Several Other Human 5-HT Receptors and Neurotransmitter Transporters.** To further evaluate the selectivity of nelotanserin for human 5-HT$_{2A}$ relative to other additional human serotonin receptors and neurotransmitter transporters, nelotanserin was evaluated in radioligand competition studies performed at Cerep, Inc (Poitiers, France; summarized in supplementary tables 1 and 2). Nelotanserin, at a final concentration of 1 µM, does not appreciably compete for binding to any of the additional serotonin receptors or neurotransmitter transporters evaluated. The selectivity of nelotanserin for 5-HT$_{2A}$ receptor relative to 61 human G protein-coupled receptors (GPCR)s was also determined and nelotanserin did not appreciably inhibit radioligand binding to any of these GPCRs.

**In Vivo Studies**

**Rat PK.** Nelotanserin concentrations were determined in plasma derived from whole blood and brain tissue collected from male rats administered a single oral dose at 10 mg/kg. Nelotanserin absorption from the gastrointestinal tract into the systemic circulation resulted in a C$_{max}$ of 0.928 µg/mL (2.12 µM concentration) at 4 hours post-
dose, with a terminal elimination $t_{1/2}$ of 21.6 hours. The time-course for nelotanserin concentrations in the brain paralleled the plasma concentrations and reached almost 40% of plasma levels (rat PK data are summarized in Table 2 and Figure 3).

**Reversal of DOI Induced Hypolocomotion.** Nelotanserin dose dependently prevented DOI-induced decrease in rearing (Figure 4). DOI administered 10 minutes prior to locomotor testing induced a significant decrease in rearing compared to vehicle treated controls. This decrease was reversed by pre-treatment with nelotanserin 35 minutes prior to locomotor testing. Statistically significant reversal was observed at 3 and 10 mg/kg.

**Acute Effects of Nelotanserin on Rat Sleep.** The dose response established in the DOI reversal study, was used to evaluate acute effects of nelotanserin on rat sleep parameters. Although PSG recordings were collected 24 hours after compound administration, no differences between any of the groups were observed beyond the first 5 hours, therefore the analyses of these recordings were confined to the first 5 hours immediately after dosing. This time period encompasses 2 hours within the dark cycle followed by 3 hours in the light cycle. Analysis of the data showed no clear effects of cycle on any of the parameters measured, therefore the data for the first 5 hours after dosing are combined.

Compared to vehicle treatment, nelotanserin treatment had no effect on measures of sleep onset latency, which was measured as the time to initiation of the first continuous minute of sleep (6 x 10 second epochs; data not shown). Nelotanserin induced a dose-dependent increase in total NREM sleep time with a concomitant decrease in both REM sleep and total wakefulness.
Nelotanserin dose dependently increased sleep consolidation as defined by concomitant decreases in bout number and increases in bout duration. The most pronounced effects were observed for the NREM sleep state, during which nelotanserin induced statistically significant decreases in bout number in all three dosage groups, and statistically significant increases in bout duration for the two higher dosage groups (Figure 5). In addition to sleep consolidation, nelotanserin induced a dose-dependent statistically significant increase in EEG delta power (Figure 5).

**Sub-Chronic Effects of Nelotanserin.** The beneficial effects of nelotanserin on rat sleep parameters, observed following acute dosing, which included increases in sleep consolidation and promotion of deep sleep, were explored following repeated sub-chronic dosing. Nelotanserin maintained efficacy through the dosing period as measured on Days 1, 3, and 5. This efficacy did not carry through to Day 7, one day after dosing was suspended. The data reported here are confined to the most significant parameters observed with acute dosing, namely increases in NREM sleep consolidation and deep sleep (EEG delta power). Nelotanserin induced a statistically significant increase in NREM sleep consolidation, with significant decreases in the number of sleep bouts concomitant with significant increases in bout length scored on Days 1, 3, and 5 of treatment; this effect disappeared 24 hours after cessation of treatment on Day 7 (Figure 6). Further, nelotanserin induced significant increases in deep sleep as indicated by the increase in delta power observed on Days 1, 3, and 5. Again this effect was no longer apparent after cessation of treatment on Day 7, and importantly, there was no rebound in any of the parameters measured (Figure 7).
Evaluation of Nelotanserin in Healthy Volunteers

**PK Results.** The PK data from the nelotanserin-001 study are summarized in Table 3. Nelotanserin was rapidly absorbed following oral administration and $C_{\text{max}}$ was reached 1 to 1.3 hours after drug administration in all dose groups. The $t_{1/2}$ of nelotanserin in plasma was between 3.9 hours and 10.7 hours for the 10 mg to 40 mg doses, and 23 hours for the 80 mg and 160 mg doses. However, the exposure to nelotanserin was not dose proportional over the tested dose range (10 to 160 mg) with $AUC_{[0-t]}$ and $AUC_{[0-\infty]}$ increasing proportionally with doses from 10 to 80 mg, and $C_{\text{max}}$ increasing proportionally with doses from 10 to 40 mg. The $AUC_{[0-\infty]}$ was to 729 ng·h/mL following the highest dose of 160 mg, and appeared to be the same as the value obtained with the 80 mg dose (701 ng·h/mL). This suggests that there is dose-limited absorption of nelotanserin in humans at the 80 mg dose.

The apparent clearance from plasma ($\text{CL/F}$) ranged from 147 L/h to 264 L/h and the apparent volume of distribution ($Vz/F$) from 1229 L to 8799 L, with a moderate inter-subject variability in all dose groups. These data and the moderate to high inter-subject variability (% coefficients of variation up to 43% for $t_{1/2}$ and 107% for $C_{\text{max}}$) suggest that the PK variability between subjects is high.

**PD Results.** The duration of SWS was significantly increased by treatment with nelotanserin (Figure 8) based on the EEG results. Compared to placebo, the study showed that 10, 20, and 40 mg doses of nelotanserin all increased SWS, with the most marked effect observed with the 40 mg dose. However, no clear dose-effect relationship was evident. Further analyses of Stage 3 and 4 demonstrated that the most prominent
effects of nelotanserin on SWS were during the second quartile of the night (data not shown).

An indirect consequence of the SWS enhancement during the first part of the night was a significant increase in REM latency. Other REM sleep parameters were unchanged with treatment. Nelotanserin did not influence total sleep time, indicating that the increase in SWS occurred at the expense of other sleep stages.

Together with an increase in SWS, statistically significant treatment effects were observed for number of stage shifts (p<0.0001), number of awakenings after sleep onset (p<0.0001), microarousal index (p<0.01), as well as the number and duration of bouts of sleep (p<0.0001; Table 4). Analysis of each dose of nelotanserin as compared to placebo showed that all three doses significantly decreased the number of stage shifts, number of awakenings after sleep onset, microarousal index, and number of bouts of sleep whereas duration of bouts of sleep was significantly increased. No clear dose-relationship effect was seen on these parameters.

Results from the sleep EEG recordings showed that most of the EEG changes of nelotanserin occurred within the first 2 to 4 hours after dosing. The EEG changes that were most prominent as compared to placebo within this time window were observed with the 80 mg dose. Both in absolute and relative power analyses, this dose lowered the alpha-2 power, and enhanced the alpha-1 and theta powers. Consistently, the mean frequency was lower on 80 mg, indicating an overall slowing effect. The 40 mg dose lowered the relative alpha-2 power, had no effect on the alpha-1 power, but lowered the global alpha and enhanced the theta relative powers, though not significantly. This profile is in line with the other PD data, suggesting vigilance-lowering effects, which
could be related to dose in a bell-shaped manner. These EEG changes were considered modest, as there were no clear signs of major sedation.

Changes in the scores of the Leeds Psychomotor test subscales (i.e., CFFT and MCRT) and the RAVLT memory test suggest minimal drug effects on overall cortical arousal and memory following a single dose of nelotanserin in the morning at either 20, 40, 80, or 160 mg (data not shown). The effects were small and occurred without impairing alertness level. No drug effects were observed in the DSST, the Bond and Lader VAS tests, and the Memory Consolidation Test.

A slight impairment in long-term memory free recall was observed at 2.5 hours after administration in the 160-mg nelotanserin dose group, which is consistent with peak plasma concentration of nelotanserin. On the basis of the LSEQ scores, nelotanserin taken in the morning enhanced subjective sleep at night. It improved both the “getting to sleep” and “sleep quality” scores without any detrimental effect on scores assessing next-morning functioning suggesting that nelotanserin is devoid of next-day hangover effects.

Subjective and psychomotor testing was also performed in the nelotanserin-002 study. The LSEQ did not show any improvement. The CFFT decreased slightly with 20 mg (-0.89 ± 1.42) and 40 mg (-1.09 ± 1.44) doses compared with placebo (-0.11 ± 1.48) (p<0.05). No reaction times changes relative to baseline, or memory consolidation, or recall were observed for any dose of nelotanserin.
Safety Results. Administration of nelotanserin to healthy volunteers was well tolerated at all doses tested. There was no increase in CNS related adverse events and no hang-over effects from nelotanserin treatment. Moreover, no treatment related serious adverse events or deaths reported in the either studies.
Discussion

Nelotanserin, a highly selective 5HT$_{2A}$ inverse agonist, is a member of a novel therapeutic class under clinical investigation for the treatment of insomnia. Unlike the GABA$_A$ agonists, the 5HT$_{2A}$ inverse agonists are non-hypnotic and non-sedating. They promote sleep consolidation by increasing the amount of time spent in deep sleep and by decreasing the number of awakenings, sleep stage shifts and arousals (Landolt et al., 1999; Idzikowski et al., 1986). Our preclinical and clinical results suggest that nelotanserin increases sleep consolidation and slow wave deep sleep in both humans and rats after oral dosing.

The inverse agonist efficacy of nelotanserin on human 5-HT$_{2A}$ receptor showed comparable efficacy to clozapine, a well characterized inverse agonist on this receptor (Egan et al., 1998; Kenakin, 2004). Further, functional IP accumulation assay data from other laboratories using clozapine as a benchmark to compare inverse agonist efficacy on the human 5-HT$_{2A}$ receptor suggest that nelotanserin is comparable in efficacy to other selective inverse agonists that are currently in clinical testing including pimavanserin, volinanserin and eplivanserin (Vanover et al., 2006).

Oral doses of nelotanserin provided sufficient central exposure to prevent DOI (5-HT$_{2A}$ agonist)-induced hypolocomotion in rats. PK analysis suggests that a 10mg/kg nelotanserin dose provides maximum plasma exposure of approximately 1 $\mu$g/mL, with ~40% brain exposure. These values correspond to MRTs in plasma and brain of 5.6 and 4.4 hours, respectively (Table 2), which represent maximally efficacious exposures in rat sleep. Indeed, nelotanserin induced dose-dependent increases in sleep consolidation,
marked by decreases in sleep bout number with corresponding increases in sleep bout duration. Further, nelotanserin treatment increased total NREM sleep time and deep sleep, the latter marked by increases in EEG delta power. These findings are in agreement with previously published reports of the effects of selective 5-HT$_{2A}$ inverse agonists on rat sleep consolidation and delta power (Monti and Jantos, 2006; Fish et al., 2005; Morairty et al., 2008).

Contrary to our findings with nelotanserin, evidence from a recent report comparing the effects of volinanserin (a 5-HT$_{2A}$ selective inverse agonist) to zolpidem (a GABA hypnotic) on rat sleep, suggests that volinanserin significantly decreased sleep onset latency compared to vehicle treated control animals, although these effects were modest compared to zolpidem, and were not dose-dependent (Morairty et al., 2008). Similar studies with two other selective 5-HT$_{2A}$ inverse agonists, namely eplivanserin and pruvanserin, showed no changes in sleep onset latency when tested in rats (Monti and Jantos, 2006; Francon et al., 2007). Further, our data in healthy volunteers, which is in agreement with other reports of 5-HT$_{2A}$ inverse agonist activity in humans, suggests that this mechanism does not affect sleep onset latency (Landolt et al., 1999); in fact, nelotanserin induced a delay in sleep onset latency when dosed at 40mg in healthy volunteers. The primary benefits derived from this mechanism are observed in measures of sleep maintenance.

The most significant acute effects of nelotanserin on rat sleep parameters, namely sleep consolidation and EEG delta power, were maintained through repeated dosing. Nelotanserin (10 mg/kg) was administered for six consecutive days, during which statistically significant increases in NREM sleep consolidation, and EEG delta power
were maintained (as determined by PSG analysis on Days 1, 3, and 5 of dosing). These findings suggest that the efficacy of nelotanserin does not wane following repeated daily exposures. Further, there were no statistically significant changes in any baseline sleep parameters after cessation of treatment as measured on Day 7. The absence of both tolerance with repeated dosing and rebound following dosing cessation, suggests that nelotanserin and possibly the 5-HT$_2A$ mechanism are suitable for long term use in the clinic.

In healthy human volunteers, nelotanserin was rapidly absorbed following oral administration and achieved maximum concentrations 1 hour later. Within the dose range tested (10-160mg), nelotanserin had dose-limited absorption beginning at the 80 mg dose. The most significant EEG effects following daytime administration of nelotanserin occurred within 2 to 4 hours after dosing. Although the effects were mild, their pattern was consistent with vigilance-lowering. Further assessment of nelotanserin activity was evaluated with self rating psychometrics scales (ARCI-49, Bond and Lader VAS), vigilance and cognitive tasks (CFFT, DSST, RAVLT), and the LSEQ. Results from these scales suggest a decrease in vigilance and cortical arousal with doses of 40mg or greater. These decreases occurred without impairment of alertness level, and are clearly of smaller magnitude than those observed with benzodiazepines or related compounds given at therapeutic doses.

On the basis of the LSEQ scores, nelotanserin taken in the morning enhanced subjective sleep the same night. It improved both the “getting to sleep” and “sleep quality” scores without any detrimental effect on scores assessing next-morning functioning, which suggests that nelotanserin is devoid of next-day hangover effects.
In the second clinical trial, all three doses of nelotanserin significantly improved measures of sleep consolidation, including decreases in the number of stage shifts, number of awakenings after sleep onset, microarousal index, and number of sleep bouts, concomitant with increases in sleep bout duration. Nelotanserin did not impact total sleep time or sleep onset latency. Subjective pharmacodynamic effects observed the day after dosing were minimal and had no functional consequences on psychomotor skills (i.e., reaction time) or memory.

Studies with nelotanserin highlight important mechanistic differences between 5-HT$_{2A}$ inverse agonists and GABA agonists and their effects on sleep. The former selectively inhibit serotonergic pathways within arousal circuits of the reticular activating system (Abrams et al., 2005). This effect promotes sleep consolidation and deep SWS without affecting sleep onset latency, or total sleep time. The latter activate inhibitory circuits within the CNS and induce a more global suppression of central activity. This inhibition promotes a decrease in sleep onset latency, and an increase in total sleep time (Da Settimo et al., 2007). Based on these differences, drugs from the two classes might be ideally suited for treatment of different forms of insomnia, the first for sleep maintenance and the second for sleep onset insomnias (Roth et al., 2007).

Currently the most commonly used prescription medications for insomnia are non-benzodiazepine hypnotics, which bind the GABA$_A$ receptor, and produce strong sedative and hypnotic effects. These medications include zolpidem (Ambien), zaleplon (Sonata), and eszopiclone (Lunesta). These agents decrease sleep onset latency, and increase stage 2 and slow-wave sleep (Scharf et al., 1994; Nicholson and Pascoe, 1986; Walsh et al., 2000; Zammit et al., 2004; Krystal et al., 2003; Roth et al., 2005).
efficacy of these compounds is tied to their exposure, i.e. agents with longer half lives might be better suited to address sleep maintenance insomnias, but they also tend to have more significant next-day hangover effects that are associated with psychomotor and memory impairments (Drover, 2004). Agents like zaleplon, which have a very short half-life of approximately 1 hour, are very useful for sleep-onset insomnia and have no hangover effects, but they are not ideal for sleep maintenance (Walsh et al., 2000; Troy et al., 2000). Inverse agonism of the 5HT$_{2A}$ receptor appears to produce sleep maintenance without residual psychomotor impairment upon wakening. Nelotanserin administered at 10 and 40 mg has a half life of 3.9 and 10.7 hours, respectively. These longer half-lives appear to be of little consequence with respect to residual effects for this class of drug. As shown in the phase 1 studies, nelotanserin showed no clinically important psychomotor impairment even at peak blood levels.

To date, no 5-HT$_{2A}$ inverse agonist has been approved for insomnia, although, at least two are in late stage clinical testing, namely volinanserin and eplivanserin (information obtained from www.clinicaltrials.gov). Based on our studies, nelotanserin, which promotes sleep consolidation and slow wave deep sleep, might be ideal for the treatment of sleep maintenance insomnias, particularly since these effects are devoid of the psychomotor and memory impairments associated with the GABA hypnotics. Indeed, polled data compiled by the National Sleep Foundation suggest that the more common symptoms of insomnia are associated with sleep maintenance, including waking up feeling un-refreshed and waking up several times during the night. Less common symptoms include difficulty falling asleep, and/or waking up too early and not being able to return to sleep (National Sleep Foundation. 2005 Sleep in America Poll. Washington,
DC). These findings suggest a medical need that might be better met with 5-HT$_{2A}$ inverse agonists like nelotanserin, which hold the promise of improvement in both efficacy and safety profiles compared to traditional GABA hypnotics. This promise rests on the outcomes of ongoing and future clinical trials.
Acknowledgements

We thank James M. Willis for editorial assistance.
References


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


Egan CT, Herrick-Davis K, and Teitler M (1998) Creation of a constitutively activated
state of the 5-Hydroxytryptamine2A receptor by site-directed mutagenesis: Inverse

Fish LR, Gilligan MT, Humphries AC, Ivarsson M, Ladduwahetty T, Merchant KJ,
O'Connor D, Patel S, Philipps E, Vargas HM, Hutson PH, and MacLeod AM
(2005) 4-Fluorosulfonylpiperidines: selective 5-HT2A ligands for the treatment of

5: A3.

Frank MG, Stryker MP, and Tecott LH (2002) Sleep and sleep homeostasis in mice
lacking the 5-HT2C receptor. *Neuropsychopharmacology* **27**: 869-873.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, and
Humphrey PP (1994) International Union of Pharmacology classification of


Kenakin T (2004) Efficacy as a vector: the relative prevalence and paucity of


Footnotes

All financial support for this work was provided by Arena Pharmaceuticals Inc., San Diego, CA 92121.
Legends for Figures

Figure 1. Structure of Nelotanserin.

Figure 2. Nelotanserin-Mediated Inhibition of Elevated Basal IP Accumulation in HEK293 Cells Expressing Constitutively Active Human 5-HT$_{2A}$ and 5-HT$_{2C}$ Receptors. Representative competition curves were chosen for each human 5-HT$_2$ receptor. Each data point consists of the mean+/−SEM of triplicate determinations made at each concentration of nelotanserin IC$_{50}$ values of 1.7 and 79 nM were determined for human 5-HT$_{2A}$ and 5-HT$_{2C}$. The positive control for these experiments consisted of the addition of 10μM of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor inverse agonist clozapine.

Figure 3. Average Plasma and Brain Concentrations of Nelotanserin Following Oral Administration of 10 mg/kg to Male Sprague-Dawley Rats. Brain levels after 8hrs were below assay limits of quantitation (3ng/ml).

Figure 4. Nelotanserin Reversed DOI-Induced Hypolocomotion. DOI (1mg/kg) administered 10 minutes prior to locomotor testing induced a decrease in locomotor activity which was significantly reversed by a 35 minute pre-administration of nelotanserin. **p<0.01 vs. Veh/DOI; ##p<0.01 vs. Veh.
Figure 5. Dose Dependent Increases in NREM Sleep Consolidation and Delta Power. Nelotanserin dose-dependently reduced the average number and increased the average length of NREM sleep bouts per hour (A and B, respectively). Nelotanserin dose-dependently increased EEG delta power (C). * p<0.05 vs. Vehicle; ** p<0.01 vs. Vehicle.

Figure 6. Nelotanserin Increases NREM Sleep Consolidation Following Sub-chronic Dosing. Nelotanserin significantly decreased bout number (A), while significantly increasing bout length (C) scored on days 1, 3, and 5 of treatment, these effects disappeared 24 hours after cessation of treatment on day 7 (B and D). ** p<0.01 vs. Vehicle.

Figure 7. Nelotanserin Increases Deep Sleep Following Sub-chronic Dosing. Nelotanserin significantly increased delta power during treatment, as scored on days 1, 3, and 5 (A). This increase was not observed on day 7, 24 hours after treatment cessation. ** p<0.01 vs. Vehicle.

Figure 8. Effects of a Single Bedtime Dose of Nelotanserin on Sleep Architecture in the Afternoon Nap Model of Insomnia. Selected parameters are shown. Data are expressed as Mean ± SEM. *p<0.05; **p<0.01 as compared to placebo.
Table 1

Affinity and Functional Potency of Nelotanserin for Human and Rat 5-HT₂ Receptors

Values listed in this table comprise the mean +/- SEM of the indicated number of independent IC₅₀ (radioligand binding assay) and EC₅₀ (IP accumulation assay) determinations (in parentheses). Triplicate determinations were performed at each of eight to ten different test compound concentrations. Kᵢ values from radioligand competition studies were calculated from IC₅₀ values.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>[¹²⁵I]DOI RBA Kᵢ (nM)</th>
<th>IP Assay EC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human 5-HT₂A</td>
<td>0.4+/-0.07 (29)</td>
<td>0.9+/-0.1 (13)</td>
</tr>
<tr>
<td>Human 5-HT₂B</td>
<td>2,644+/-147 (26)</td>
<td>6,856+/-3,575 (5)</td>
</tr>
<tr>
<td>Human 5HT₂C</td>
<td>106+/-5 (27)</td>
<td>30+/-8 (4)</td>
</tr>
<tr>
<td>Rat 5-HT₂A</td>
<td>2.3+/-1.0 (3)</td>
<td>4.7+/-0.4 (3)</td>
</tr>
<tr>
<td>Rat 5-HT₂B</td>
<td>4,581+/-278 (2)</td>
<td>ND</td>
</tr>
<tr>
<td>Rat 5-HT₂C</td>
<td>221+/-23 (3)</td>
<td>170+/-39 (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup>antagonist assay with 5nM 5-HT (EC₈₀) added prior to compound addition.
Table 2

Plasma and Brain PK Parameters of Nelotanserin in Rat

Results following a single oral dose of 10mg/kg.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Brain</th>
<th>Brain/Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>21.6</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>$t_{\text{max}}$ (hr)</td>
<td>4.0</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>0.928</td>
<td>0.357</td>
<td>0.385</td>
</tr>
<tr>
<td>$t_{\text{last}}$ (hr)</td>
<td>40.0</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{\text{last}}$ (hr•µg/mL)</td>
<td>8.327</td>
<td>1.802</td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{0-\text{inf}}$ (hr•µg/mL)</td>
<td>8.386</td>
<td>3.091</td>
<td>0.369</td>
</tr>
<tr>
<td>$\text{MRT}_{\text{last}}$ (hr)</td>
<td>5.6</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>$\text{AUC}_{0-8\text{hr}}$ (hr•µg/mL)</td>
<td>5.478</td>
<td>1.802</td>
<td>0.329</td>
</tr>
</tbody>
</table>
Table 3

PK Parameters of Nelotanserin in Healthy Volunteers

<table>
<thead>
<tr>
<th>Parameters (%) CV</th>
<th>Nelotanserin Dose Group (N=6/group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>17.5 (62%)</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.0</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-120&lt;/sub&gt;(ng.h/mL)</td>
<td>48.9 (50%)</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-120&lt;/sub&gt;(ng.h/mL)</td>
<td>51.4 (53%)</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;(h)</td>
<td>3.9 (41%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MRT(h)</td>
<td>4.6 (17%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CL/F(L/h)</td>
<td>237.2 (14%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vz/F(L)</td>
<td>1332.6 (44%)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>N = 5; <sup>b</sup>median; <sup>c</sup>% CV = coefficient of variation (100·SD/mean).
### Table 4

**Effect of nelotanserin on sleep continuity parameters**

<table>
<thead>
<tr>
<th>PSG Parameter</th>
<th>Baseline</th>
<th>Placebo</th>
<th>10 mg</th>
<th>20 mg</th>
<th>40 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total sleep time (min)</strong></td>
<td>378.2 ± 7.7</td>
<td>375.8 ± 8.3</td>
<td>367.5 ± 10.0</td>
<td>376.6 ± 10.7</td>
<td>370.8 ± 10.5</td>
</tr>
<tr>
<td><strong>Shifts between stages</strong></td>
<td>109 ± 5.4</td>
<td>119.5 ± 4.6</td>
<td>92.1 ± 4.5***</td>
<td>99.9 ± 6.0***</td>
<td>93.7 ± 4.7***</td>
</tr>
<tr>
<td><strong>Awakenings after sleep onset</strong></td>
<td>27.5 ± 1.9</td>
<td>31.2 ± 1.8</td>
<td>20.7 ± 1.7***</td>
<td>23.3 ± 2.4***</td>
<td>21 ± 1.7***</td>
</tr>
<tr>
<td><strong>Microarousal index (# per hr)</strong></td>
<td>17.2 ± 1.5</td>
<td>17.3 ± 0.9</td>
<td>13.4 ± 1.4**</td>
<td>14.3 ± 1.6*</td>
<td>12.5 ± 1.1**</td>
</tr>
</tbody>
</table>

Differences between nelotanserin treatment groups and placebo were evaluated with an analysis of covariance. *p<0.05; **p<0.01, ***p<0.001 vs. placebo.
Figure 1
Figure 2

The graph shows the effect of nelotanserin on serotonin receptors. The x-axis represents the logarithm of the concentration of nelotanserin (M), while the y-axis represents the percentage of control. The different curves indicate the response of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors to varying concentrations of nelotanserin.
Figure 3

The graph illustrates the concentration of Nelotanserin (ng/ml or ng/g) over time (hr) in plasma and brain. The y-axis represents the concentration on a logarithmic scale, ranging from 1 to 10,000 ng/ml or ng/g. The x-axis represents time in hours, ranging from 0 to 50.

- **Plasma** is represented by solid black circles with error bars.
- **Brain** is represented by open circles.

The concentration peaks at around 10 hours, with a sharp decline thereafter in both plasma and brain. The concentration then stabilizes at a lower level.
Figure 4

![Bar graph showing rears with +DOI symbol]

- VEH
- VEH
- 1
- 3
- 10

Rears

0 25 50 75 100
Figure 5

A

NREM bouts (n)

VEH 1 3 10 Nelotanserin

*  **

B

NREM bout duration (min)

VEH 1 3 10 Nelotanserin

**

C

delta power

VEH 1 3 10 Nelotanserin

**
Figure 6

A

<table>
<thead>
<tr>
<th>Day</th>
<th>Vehicle</th>
<th>Nelotanserin</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Data" /></td>
<td><img src="image2" alt="Data" /></td>
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<td>3</td>
<td><img src="image3" alt="Data" /></td>
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<td>5</td>
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B

<table>
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<td>5</td>
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C

<table>
<thead>
<tr>
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<th>NREM bout duration (min)</th>
</tr>
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<tbody>
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D

<table>
<thead>
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<th>NREM bout duration (min)</th>
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<tbody>
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<tr>
<td>5</td>
<td><img src="image15" alt="Data" /></td>
</tr>
</tbody>
</table>
Figure 7

A

Vehicle

**

Nelotanserin

**

**

delta power

Day

1

3

5

B

delta power

Day

7

0.25

0.30

0.35

0.40

0.45

0.50