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Title

# Regulation of ingestive behaviors in the rat by GSK1521498, a novel mu-opioid receptor-selective inverse agonist

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Running Title Page

a) Regulation of ingestive behaviors by GSK1521498 in the rat

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d) Abbreviations: $AUC_{0\text{-}24}$, area under the plasma concentration-time curve for 24 hours; $AUC_{inf}$, area under the plasma concentration-time curve extrapolated to infinite time; CHO, Chinese Hamster Ovary; CL, plasma clearance; $C_{max}$, maximum plasma concentration; DAMGO, ([D-Ala2, N-MePhe4, Gly-ol]-enkephalin); Deltorphin II, (2-D-Ala)-[tyrosyl-3, 5-$^{3}$H]; DIO, diet-induced obese; DOR, delta opioid receptor; DPDPE, D-Pen$^{2}$, dPen$^{5}$; F, oral bioavailability; fpKi, negative log of the functional Ki, FRx, fixed-ratio schedule where each lever press is x rewards; GSK, GlaxoSmithKline; GSK1521498, (N-{[3,5-difluoro-3'-(1H-1,2,4-triazol-3-yl)-4-biphenylyl]methyl}-2,3-dihydro-1H-inden-2-amine phosphate (1:1); KOR, kappa opioid receptor; LY255582, (3R,4R)-3,4-Dimethyl-1-[(3S)-3- hydroxy-3-cyclohexyl-propyl]-4-(3-hydroxyphenyl)piperidine; MOR, mu opioid receptor; PK, pharmacokinetic; $T_{max}$, time of maximum plasma concentration;$V_{ss}$, steady-state volume of distribution

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Abstract

Mu opioid receptor (MOR) agonism induces palatable food consumption principally through the modulation of rewarding properties of food. GSK1521498, (N-{[3,5-difluoro-3'-(1H-1,2,4-triazol-3-yl)-4-biphenylyl]methyl}-2,3-dihydro-1H-inden-2-amine phosphate (1:1), is a novel opioid receptor inverse agonist that, based on in vitro affinity assays, is greater than 10-fold or 50-fold selective for human or rat MOR, respectively, compared with kappa opioid receptors (KOR) and delta opioid receptors (DOR). Similarly, preferential MOR occupancy versus KOR and DOR was observed by autoradiography in brain slices from Long Evans rats dosed orally with the drug. GSK1521498 suppressed nocturnal food consumption of standard or palatable chow in lean and diet-induced obese (DIO) Long Evans rats. Both the dose-response relationship and time course of efficacy in lean rats fed palatable chow correlated with mu receptor occupancy and the plasma concentration profile of the drug. Chronic oral administration of GSK1521498 induced body weight loss in DIO rats which was comprised of fat mass reduction. The reduction in body weight was equivalent to the cumulative reduction in food consumption, thus, the effect of GSK1521498 on body weight is related to inhibition of food consumption. GSK1521498 suppressed the preference for sucrose-containing solutions in lean rats. In operant response models also using lean rats, GSK1521498 reduced the reinforcement efficacy of palatable food reward and enhanced satiety. In conclusion, GSK1521498 is a potent, MOR-selective inverse agonist that modulates hedonic aspects of ingestion and, therefore, could represent a pharmacological treatment for obesity and binge-eating disorders.

Introduction

Obesity is a worldwide epidemic for which there are few pharmacologic treatment options. In the US alone, two-thirds of the adult population is classified as overweight or obese. The etiology of the relatively recent surge in incidence is thought to be due to availability of relatively inexpensive, highly palatable foods and sedentary lifestyles. The imbalance between consumption of high fat and high sugar food of limited nutritional value versus energy expenditure results in a chronic positive energy state. Behavioral and cognitive factors, including binge eating, cue-induced craving and cognitive control of food seeking, are likely to contribute to obesity in many subjects and are the proximal targets of centrally acting anti-obesity drugs [for reviews see Hill, 2006; Morton 2006; Ogden, 2006; Rolls, 2007].

The mu, kappa, and delta opioid receptors (MOR, KOR and DOR) are localized in brain regions mediating food intake and reward [Mansour, 1995]. While all three receptors have been implicated in regulation of food intake, MOR is thought to play the predominant role, as MOR knockout mice display reduced motivation to eat [Papaleo, 2007] and are resistant to diet-induced obesity [Tabarin, 2005]. In addition, elevated MOR levels are observed in diet-induced obese Wistar rats [Smith, 2002] and in Osbourne-Mendel rats which are susceptible to diet-induced obesity [Barnes, 2006]. While MOR is present in areas of the brain which regulate consummatory behavior, such as the hypothalamus and nucleus tractus solitarus, the expression is more prevalent in the limbic system (nucleus accumbens shell and ventral pallidum) [Smith and Berridge, 2007] implicating MOR in the regulation of hedonic aspects of food consumption. Morphine administered into the nucleus accumbens amplifies positive affective reactions to sucrose taste [Peciña and Berridge, 2000]. Furthermore, intraaccumbens administration of the MOR-selective agonist, DAMGO (([D-Ala$^2$, N-Me-Phe$^4$, Gly-ol] enkephalin), induces hyperphagia and enhances preference for high fat and sugar content [Mucha and Iverson, 1986; Will, 2003; Zhang and Kelley, 1997; Zhang, 1998]. MOR agonism in the nucleus accumbens communicates with other

centers important to motivation, metabolic and autonomic regulation of food consumption as evidenced by the activation of hypothalamic, midbrain and brainstem regions after DAMGO infusion into the ventrolateral striatum [Zhang and Kelley, 2000]. Recently, MOR polymorphisms have been associated with obesity in humans [Xu, 2008].

It is now generally accepted that G protein-coupled receptor antagonists are either neutral antagonists, which only block agonist-induced effects, or inverse agonists, which reduce spontaneous constitutive activity as well as agonist effects [Kenakin, 1995]. Clinical experience with neutral opioid receptor antagonists, such as naltrexone, demonstrates that while suppression of short-term food intake is observed [Drewnowski, 1997], significant impact on body weight is not [Atkinson, 1985; Malcolm, 1985]. It has been suggested that opioid receptor inverse agonists may be more effective than neutral antagonists in the treatment of obesity. Indeed, the opioid receptor inverse agonist, LY255582, inhibited weight gain in obese Zucker rats over 30 days while the effect of naltrexone was only observed for 7 days [Shaw, 1993]. It should be noted that opioid receptor antagonists/inverse agonists appear to modulate the incentive value of palatable food and satiety and, therefore, these agents may behave differently than anorectics such as phentermine.

GSK1521498 (N-{[3,5-difluoro-3'-(1H-1,2,4-triazol-3-yl)-4-biphenylyl]methyl}-2,3-dihydro-1H-inden-2-amine phosphate (1:1)) (GlaxoSmithKline, Research Triangle Park, NC), is currently in clinical development for treatment of obesity and disorders characterized by compulsive eating. This report describes the preclinical characterization of opioid receptor pharmacology and selectivity profile and the pharmacokinetic properties of GSK1521498. The regulation of ingestive behaviors by GSK1521498 is explored in several paradigms as well as the translation of effects on consumption to reduction of body weight and fat mass.

Methods

Drugs and Materials

*In vitro* and *in vivo* studies were conducted with the dihydrochloride salt of GSK1521498, which was synthesized in the GSK Department of Medicinal Chemistry in Research Triangle Park, NC [Cowan, 2010]. GSK1521498 was prepared in 0.5% hydroxypropyl methylcellulose/0.1% Tween 80, which served as the vehicle for oral administration for all studies. Vehicle and drug were administered orally in a volume of 2 ml/kg body weight. Dose calculations were based on the dihydrochloride salt for all pharmacology studies.

DAMGO, [Tyrosyl-3, 5-$^{3}$H(N)] and Deltorphin II (2-D-Ala)-[tyrosyl-3,5-$^{3}$H] were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA), [$^{3}$H]CI-977 was from GE Healthcare (Buckinghamshire, UK) and naloxone-HCl was from Biomol (Plymouth Meeting, PA). Naltrexone HCl was from Sigma-Aldrich (St Louis, MO).

Operant response chambers (Med Associates, St. Albans, VT) were custom built to contain the following: 2-retractable arm levers, 2-food reward pellet dispensers, 1-house light, 1-tone cue, 1-light cue for each lever, 1-head entry detector, and 1-pellet detector. All programs were written to provide both the tone and light cues for one second concurrent with the delivery of the food reward. The 45-mg palatable food rewards were custom made (#F06162, Bio-Serv, Frenchtown, NJ) and contained the following ingredients: dextrate 341 g/kg, m-dextrin 26 g/kg, casein 200 g/kg, cystine 3 g/kg, vitamin mix 10 g/kg, choline bit 2 g/kg, salt mix 35 g/kg, sucrose 100 g/kg, fiber 50 g/kg, hydrogenated vegetable oil 213 g/kg, soybean oil 20 g/kg.

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Opioid Antagonist GTPγS Assay

The assay was designed to measure the GDP-GTP exchange that occurs on the trimeric G-proteins using [$^{35}$S]GTPγS, a poorly hydrolyzed, labeled analogue of GTP with high affinity for the G protein. Addition of an agonist promotes an increased GDP exchange at the G-protein such that more [$^{35}$S]GTPγS is bound to the G-protein. For quantification of antagonist activity, the addition of a 4*$EC_{50}$ final concentration of agonist, DAMGO, dynorphin A or Met-Enkephalin for MOR, KOR and DOR, respectively, was added. A separate plate with 6 curves of the standard agonist compound was run in the agonist mode in parallel with the antagonist assay. The $EC_{50}$ value and slope of agonist was used to calculate the fpKi.

Reactions were performed in white 384-well polystyrene low-volume plates in a final volume of 10 µl. Membranes expressing receptors and Wheat Germ Agglutinin Leadseeker bead solutions in assay buffer (20 mM HEPES, 10 mM $MgCl_2$ and 100 mM NaCl, pH7.4 with KOH) , were combined and shaken at room temperature for 30 minutes. GDP (10 µM final) was added to the mixture; then GTPγS solution containing 4*$EC_{50}$ final agonist concentration. To quantify compound potencies, the mixture was added (10µL) using a Multidrop Combi (Thermo Inc, Waltham, MA) to the compound plates containing 100 nL of test compound dissolved in 100% $Me_2SO$. Compounds under analysis were dissolved in $Me_2SO$ to 3.0 mM and serially diluted 1 to 4 with $Me_2SO$ through 11 dilutions. 0.1 µL of each concentration was transferred to the corresponding well of an assay plate. This created a final compound concentration range from 0.000007 to 30 µM. The plates were sealed and centrifuged at 500 × g for 2 minutes, incubated at 20°C for 5 hours and GTPγS binding was then imaged using a Viewlux Plus instrument (PerkinElmer, Waltham, MA).

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For assessment of affinity, opioid receptors were expressed at levels that did not result in measurable constitutive activity. Human MOR, KOR and DOR were stably expressed in Chinese Hamster Ovary (CHO-K1) cells (ATCC, Rockville, MD) and rat MOR, KOR and DOR were expressed in CHO-Gam E1A cells using the BacMam virus expression system previously described [Condreay, 1999]. CHO-Gam E1A cells have improved BacMam transduction capabilities and were derived from CHO-K1 cells at GlaxoSmithKline (Research Triangle Park, NC). For assessment of inverse agonism, CHO-GamE1A cell membranes transduced using the BacMam system to overexpress human or rat MOR, KOR and DOR were utilized. Altering the stoichiometry of receptor and G protein by enhancing receptor expression then increases the number of active state receptors in the system allowing for the assessment of constitutive activity [Chen, 1999].

Data analysis was performed with Abase software package (IDBS, version 5.4, Surrey, UK), raw counts from the View Lux CCD imager (Perkin Elmer, Waltham, MA) were normalized and expressed as % inhibition using the formula: % Response = 1-([U–C1]/[C2–C1]) * 100 where U is the unknown value, C1 is the average of the low signal (0%) control wells and C2 is the average of the high signal (100%) control wells. Curve fitting was performed using the equation: $Y=A+([B-A]/[1+[10^{x}/10^{C}]^{D})$ where A is minimum response, B is maximum response, C is $\log_{10}*XC_{50}$, D is slope factor, and x is $\log_{10}$ compound concentration. The results were recorded as $pIC_{50}$ values (-C in above equation) and converted to fpKi by the equation: $fpKi=-\log(IC_{50}/((2+([L]/EC_{50})^{n})^{1/n-1})$ where [L] is the concentration of agonist used for the antagonist assay ($=4*EC_{50}$ final assay concentration where the $EC_{50}$ was determined from historical agonist curve data), $EC_{50}$ is determined on assay day using a separate agonist plate containing >6 agonist curves, and n is the slope of the agonist curve. fpKi is the negative log of the Ki.

Animals

All studies were conducted after review by the GSK Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals, the Animal Welfare Act (US Department of Agriculture), and the 1996 Guide for Care and Use of Laboratory Animals (National Institutes of Health). Lean and diet-induced obese (DIO) Long Evans rats were purchased from Harlan Sprague Dawley, Inc (Indianapolis, IN). Male rats were used unless otherwise noted. Rats were housed at 72ºF and 50% relative humidity with a 12 hour light and dark cycle. For all studies except nocturnal food consumption, the rats were habituated in cages at GlaxoSmithKline facilities for at least one week before experimentation. Rats were given *ad libitum* access to water and either standard laboratory chow (Lab Diet 5001, PMI Nutrition International, Brentwood, MO) for lean rats or high fat chow (TD95217, Harlan Laboratories, Madison, WI) for DIO rats. For nocturnal food consumption studies, rats were housed individually in chambers of a computerized feeding system from Accuscan Instruments Inc. (Columbus, OH). The rats were given *ad libitum* access to water and either powdered Lab Diet 5001 or D12266B, a purified diet intended to mimic sweetened condensed milk (Research Diets, New Brunswick, NJ). DIO rats were provided with TD95217 at Harlan facilities starting at 4 weeks of age and were maintained on that chow for 8 weeks. The rats were then transferred to GlaxoSmithKline where they were maintained on TD95217 until the average weight of the rats was approximately 500 grams and the body weight gain rate was slowing.



Plasma Pharmacokinetic Analysis

For intravenous administration, the dose formulation was administered to each animal via femoral vein cannula (1 ml/kg of 2.6 mg/ml GSK1521498). Following dose administration, the cannula was flushed with approximately 0.5 ml of heparinized saline. Blood samples (approximately 0.3 ml each) were collected from each animal at 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr post-dose. For oral administration, the dose formulation was administered via oral gavage (2 ml/kg of 0.5 mg/ml

GSK1521498). Blood samples (approximately 0.3 ml each) were collected from each animal from a jugular vein cannula using a syringe at 0.25, 0.5, 1, 2, 4, 8 and 24 hrs post-dose and transferred into labeled collection tubes containing tripotassium EDTA and chilled at 4°C. Plasma was harvested by centrifugation (4000 × g for 20 min at 4°C) within 10 minutes of blood collection and stored at -20°C until analysis.

For the brain distribution study, 30 mg/kg was administered via oral gavage (5 ml/kg of 6 mg/ml GSK1521498). Blood (approximately 0.3 m each) and brain samples were collected from each animal at 1.5 and 8 hrs post-dose. Plasma was harvested as described above and brain samples were stored at -20°C until analysis.

To analyze for levels of GSK1521498, rat plasma samples were thawed at room temperature and vortex-mixed. Brain samples were homogenized in 5 volumes of water. Aliquots (10 µl of plasma or 20 µl of brain homogenate) of the study samples were extracted by protein precipitation with acetonitrile (200 µL) containing an internal standard (GSK1631668, 100 ng/ml) in 96-well plates. After the samples were vortexed for 40 seconds, and centrifuged at 2304 × g at 4°C for 15 min, the acetonitrile supernatants (30 µL) were transferred to a clean 96-well plate containing 210 µL of water. The samples were then vortexed for 1 minute, placed into a chilled auto-injector and injected (10 µL) onto a 50 x 2 mm, 3µ, Varian Polaris C18 Ether high performance liquid chromatography column (Lake Forest, CA) using a gradient method. After an initial 0.5 minute hold, the gradient increased linearly from 60% 5.2 mM ammonium acetate (pH 6) with 2% acetonitrile (A) 40% methanol (B) to 95% B over 1 minute, then held at 95% B for 1.5 minute. The analytical column was then re-equilibrated at 40% B over the final minute. The flow rate was 0.6 ml/min. The flow was diverted to waste for the first 0.5 minutes, to the mass spectrometer for the next 3 minutes and back to waste for the remaining run time. The total run time was 4 minutes. Analytes were detected using positive turboionspray. GSK1521498 and the

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internal standard GSK1631668 were detected by multiple reaction monitoring of the transitions m/z 403.2 to m/z 269.9 and m/z 341.2 to m/z 208.0, respectively. GSK1521498 and GSK1631668 eluted at retention times of 2.0 and 1.7 minutes, respectively.

Triplicate plasma calibration curves for GSK1521498 ranged from 1 to 5000 ng/ml at 12 concentration levels and 36 of 36 standards were within acceptable accuracy limits of ± 20% of the nominal concentration. The correlation coefficient was 0.9973 using linear regression with 1/x2 weighting. Plasma quality control samples were analyzed as 3 samples at each of 4 concentrations (10, 400, 1000, 4000 ng/ml plasma) and 12 of 12 were within acceptable accuracy limits of ± 20% of the nominal concentration.

The brain homogenate calibration curve for GSK1521498 ranged from 4 to 10,000 ng/g at 11 concentration levels and 11 of 11 standards were within acceptable accuracy limits of ± 20% of the nominal concentration. The correlation coefficient was 0.9953 using quadratic regression with 1/x2 weighting.

The liquid chromatography tandem mass spectroscopy system consisted of a CTC HTS PAL auto-injector (Leap, Carrboro, NC), an Agilent 1100 binary pump (Palo Alto, CA), a Valco switching valve (Houston, TX), and an Applied Biosystems API4000 triple quadrupole mass spectrometer (Concord, ON). Data were acquired, analyzed, and quantified using Analyst software version 1.4.1 (Applied Biosystems MDS/SCIEX, Concord, ON).

Noncompartmental pharmacokinetic parameters (terminal plasma half life [$t_{1/2}$], maximum plasma concentration [$C_{max}$], time of maximum plasma concentration [$T_{max}$], plasma clearance [CL], steady-state volume of distribution [$V_{ss}$], area under the plasma concentration-time curve extrapolated to infinite time [$AUC_{inf}$], and area under the plasma concentration-time curve for 24 hours [$AUC_{0-24}$] were

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calculated based on the individual plasma concentration-time data using WinNonlin Professional 4.1 (Pharsight, Mountain View, CA). Oral bioavailability [F] was calculated using the following equation: $F(\%)=([AUC_{inf,oral}/\text{oral dose}]/[AUC_{inf,\ IV}/\text{IV dose}])*100$ where $AUC_{inf,oral}$ was the individual $AUC_{inf}$ following oral administration and $AUC_{inf,IV}$ was the mean $AUC_{inf}$ following IV administration.

Nocturnal Food Consumption Protocol

Rats were acclimatized to Accuscan chambers for between 4 and 11 days and to oral gavage with vehicle for 3 days before the study. Experiments were limited to 4 groups of 8 rats as the Accuscan apparatus contained 32 cages. The average food consumption over the 2 days prior to the study was used as baseline food consumption for each rat. Oral administration of vehicle or GSK1521498 was completed approximately 1-2 hours before the commencement of the dark cycle. Food hopper weight was measured every hour for 24 hours by the computerized system. Manual weights of food in hoppers at the start of the study and end of study were taken. Rats were, on occasion, removed from the study either because of individual apparatus failure or because the difference between the manual and computer measures was greater than 3 grams. Food spillage was monitored daily, and adjustments were made accordingly. Lean rats weighed approximately 320 g while DIO rats weighed approximately 530 g at the time of the study.

Receptor Occupancy in Frozen Brain Slices

Rats were weighed, dosed orally with GSK1521498 (0.3 to 30 mg/kg) or vehicle at 8 to 9 AM, and tissues was collected 1.5, 8, or 24 hours after administration of compound. To harvest brains, the rats were asphyxiated with $CO_2$ and decapitated; skulls were opened and brains removed, immersed in precooled isopentane for 5 to 8 seconds and stored at -80ºC. MOR, KOR, and DOR occupancy was measured in frozen brain sections by *ex vivo* autoradiography [Poisnel, 2006]. Coronal sections (20 µm)

were taken through the nucleus accumbens, dorsal endopiriform nucleus, or caudate/putamen for mu, kappa or delta receptor autoradiography, respectively, using a cryostat (Leica Microsystems, Inc, Bannockburn, IL). The sections were thaw mounted onto Superfrost Plus slides and stored at -80ºC until use.

For autoradiography, slides were thawed to room temperature under a gentle stream of cool air. A barrier was formed between sections designated for total and nonspecific binding by applying a line of DPX mountant and allowing to fully dry. The sections were covered with 0.2 to 0.3 ml of 50 mM Tris-HCl, 5 mM $MgCl_2.6H_2O$), pH 7.4, protease inhibitors, and 0.2% bovine serum albumin (Assay Buffer) containing either 5 nM [$^3$H]-DAMGO (MOR-selective agonist), 1.3nM [$^3$H]-CI-977 (KOR-selective agonist), or 12 nM [$^3$H]-Deltorphin II (DOR-selective agonist) ± 10 μm naloxone and incubated at room temperature for 10 (mu, delta) or 20 (kappa) minutes in the dark. Excess cold naloxone was included to assess nonspecific binding. For assessment of ligand specificity, 100 nM DAMGO, norbinaltorphimine HCl (norBNI) or [d-Pen$^2$,d-Pen$^5$]-enkephalin (DPDPE) were utilized to assess nonspecific binding for MOR, KOR, or DOR, respectively. The slides were washed in ice cold Assay Buffer (no ligands) for 30 seconds then immersed in 50 mM Tris-HCl, 5 mM $MgCl_2.6H_2O$), pH 7.4 (Wash buffer) for 3 x 30 second washes. The sections were analyzed by removing all traces of DPX from the slides, allowing the slides to dry completely, covering the back of the slides with metallic electrical tape (as per instrument manufacturer instructions) and removing all dust with clean, compressed air. The slides were imaged in a beta-imager (Biospace Lab, Cambridge, MA) for 12 to 24 hours. Beta vision software was used to measure binding in the region of interest, captured as cpm and cpm/mm$^2$. Specific binding was calculated by subtracting nonspecific binding from total binding of radioligand. The vehicle group represented maximal possible occupancy for each radioligand and the ability of GSK1521498 to

displace radioligand binding in *vivo* was expressed as percentage DAMGO binding calculated as (specific binding for drug-treated rat/ mean of specific binding for vehicle group) x 100.

Weight Loss Protocol

Three days prior to study, rats were weighed and body composition was assessed by Quantitative Magnetic Resonance (QMR) (Echo-MRI 100 QMR, Echomedical Systems, Houston, TX). Rats were randomized into groups based on % body fat such that average % body fat was not different between groups (8 rats per group). DIO rats weighed approximately 500 g while lean control weighed approximately 440 g at the start of the study.

Following randomization, rats were habituated to handling and oral gavage for 3 days and baseline food consumption was measured before the treatment regimen commenced. Rats were dosed once daily for 11 days, 1 hour before the dark cycle commenced with vehicle or GSK1521498. Body weight was measured 3 days a week on Monday, Wednesday, and Friday. Food hoppers were weighed daily to estimate daily consumption. Body composition was assessed again 18 hours after the last dose, body weights were measured and the rats were then rendered unconscious via $CO_2$ inhalation. Blood was collected by cardiac puncture and serum prepared by allowing clot formation at room temperature for 20 minutes followed by centrifugation for 10 minutes at 3000 rpm at 5ºC.

Sucrose Preference

The experimental protocol was derived from methods reported in previous publications [Hommell, 2006; Bolanos, 2003]. At the start of the study, each rat was given 2 bottles of ultrapure water and dosed with vehicle by oral gavage. The weight of each water bottle was recorded prior to provision to rats in order to assess overnight water intake. On Day 2, the water bottles were weighed and then the positions of the bottles were switched prior to oral gavage with vehicle. Alternating the

bottle positions controls for position bias. On the third day, the bottles were weighed and then emptied. The rats were then dosed with either vehicle or GSK1521498 by oral gavage and were provided with a bottle of ultrapure water and a bottle of 0.25% sucrose water. After the bottles were weighed, the ultrapure water and 0.25% sucrose-filled bottle were place on the left and right sides of the cage, respectively. As before, the bottle weights were recorded the following day, GSK1521498 or vehicles were delivered by oral gavage and the position of the bottles were switched. The bottle weights were recorded again the next day, the bottles were emptied and refilled with either fresh ultrapure or 0.5% sucrose water and the process described above was repeated. The procedure was then repeated a third and final time with 1.0% sucrose water. Bottle weight assessment and dosing occurred at approximately 2:00 PM each day. To calculate the sucrose preference score for each animal at each sucrose concentration, the volume consumed of the sucrose-water solution was divided by the total liquid intake (sucrose-water or water alone) over a 24-hour period. Since each sucrose concentration was available for 2 days, the two 24-hour periods were averaged together to get the final preference percentage.

Operant Response Protocol

After one week of habituation, food intake was measured and recorded over a 3-day period and the average food intake per rat/day was determined. A fixed-ratio schedule of reinforcement was used where each lever press resulted in one reward (FR1). The palatable food reward was a 45 mg pellet containing 23.3% fat and 10% sucrose by mass. The rats were then put on a 50% food restriction diet for 3 days to promote lever acquisition. This resulted in a temporary weight loss of about 10% body weight, which animals regained before drug testing began. The second day of the food restriction began Day 1 of the operant response lever training. During the time of food restriction and training, animals could obtain additional food by learning to press the operant response lever. After Day 4 of training,

25% of the rats, those with the lowest average number of rewards received from Day 1 to Day 4 of FR1 training, were eliminated from the experiment. Rats were trained during all 3 food-restricted days, but then allowed *ad libitum* access to standard chow in their home cage for the remainder of the experiments. Although animals were given *ad libitum* access to standard chow, they still were willing to press the lever to obtain the more palatable food reward. During training, animals were placed in the operant response chambers for approximately 30 minutes or until 120 food rewards were obtained, whichever came first. Total training time was approximately 3 weeks and rats were trained on FR1 and were then switched to FR3 followed by FR5 and finally to the progressive-ratio. Fixed-ratio training consisted of 5 sessions of FR1, 2 sessions of FR3, and a minimum of 5 sessions of FR5 to stabilize responding before testing began. Progressive-ratio training consisted of 5 sessions of FR1, 2 sessions of FR3, 2 sessions of FR5, and a minimum of 7 sessions of PR to stabilize the responding before testing began. After the animals stabilized on the progressive-ratio, compound testing began. Animals were given at least 7 days between experimental blocks. For the fixed ratio study, on the test day rats were dosed approximately 1.5 hours before being placed in the chambers for a maximum of 30 minutes and could work to obtain up to 120 food rewards on an FR1 schedule. The auditory and visual cues were presented concurrently with each food reward. For the progressive ratio protocol, on test day, rats were dosed approximately 1.5 hours before being placed in chambers and allowed to press on a progressive ratio. The schedule for the first through eighth reward was 2, 4, 7, 12, 17, 25, 38, and 50 presses. If the animals failed to obtain the reward within 10 minutes, they were considered to have reached their "breakpoint", which caused the levers to retract and houselight to turn off. On average, animals only remained in the cages for approximately 30 minutes. All doses were counterbalanced over time, such that any specific trial contained all doses of drug, including controls. In addition, each group of animals was distinct, thus eliminating the risk of carry-over effects.

Statistical Analysis

Statistical analyses were performed using the JMP statistical software packages (Release 8.0.2, SAS Institute, Cary, NC). In general, data are presented as means ± SEM with N indicating the number of animals per group. Each of the reported studies was planned to compare specific doses of GSK1521498 to a control group via ANOVA or ANCOVA with post-hoc t-tests, and the studies were powered accordingly. In this case, retrospective application of a procedure for testing multiple comparisons suggested by the data, e.g. Bonferroni, is not necessary and would decrease the power of the studies to detect the differences for which they were planned. Thus, all studies were evaluated via post-hoc t-tests following ANOVA or ANCOVA with the results reported as p-values $< 0.05$, $<0.01$, and $<0.001$ to show the strength of evidence for each comparison. Differences between groups were considered significant when a value of $P < 0.05$ was achieved. One-way ANOVA followed by post-hoc t-test was used to analyze nocturnal food consumption ((Figure 3 and 6) and body composition (Table 4). Two-way ANOVA with dose and time factors followed by post-hoc t-tests was used to analyze the receptor occupancy data, expressed as % of vehicle response (Figure 5). A square root transformation was applied to the DAMGO binding data prior to analysis in order to meet the assumptions of ANOVA. One -way analysis of variance (ANOVA) with repeated measures followed by post-hoc t-test was used for weight loss (Figure 7a), sucrose preference (Figure 8) and operant response (Figure 9) experiments. For the analysis of balance of food intake versus body weight loss (Figure 7b), the mean loss of weight minus mean reduction in food consumption for each group was tested to determine if the value was different from zero.

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Results

GSK1521498

The chemical structure of the dihydrochloride salt of GSK1521498, (N-{[3,5-difluoro-3'-(1H-1,2,4-triazol-3-yl)-4-biphenylyl]methyl}-2,3-dihydro-1H-inden-2-amine phosphate (1:1) is presented in **Figure 1**. This salt form of GSK1521498 was used in all studies described herein. The pharmacokinetic characteristics of GSK1521498 after intravenous and oral administration to Long Evans rats are presented in **Table 1**. The excellent oral bioavailability and access to brain tissue as well as the low volume of distribution and clearance supported once daily oral administration and optimal targeting of a central nervous system receptor.

Receptor Pharmacology

The potency of GSK1521498 at human and rat MOR, KOR, and DOR was tested in a GTPγS assay using membranes prepared from CHO-K1 cells expressing human opioid receptors or CHO-Gam E1A cells which were Bacmam-transduced with rat opioid receptors (**Table 2**). GSK1521498 is 14- and 20-fold selective for human MOR over KOR and DOR and is 52- and 66-fold selective for rat MOR over KOR and DOR. Naltrexone was less potent and selective for MOR compared with KOR and DOR. Naltrexone was approximately 4- and 10-fold selective at human MOR versus KOR and DOR and 20- and 25-fold selective at rat MOR compared with KOR and DOR.

Cell membranes from CHO-Gam E1A cells in which human and rat opioid receptors were expressed at a level that resulted in constitutive activity were used to assess inverse agonism of GSK1521498. As shown in **Figures 2A** and **2B**, GSK1521498 behaved as an inverse agonist at human and rat MOR while naltrexone displayed activity characteristic of a neutral antagonist or partial agonist in this system. Similarly, GSK1521498 functioned as an inverse agonist at the human and rat KOR and

DOR, whereas naltrexone was a neutral antagonist at DOR and a neutral antagonist/partial agonist at KOR (data not shown).

Correlation of Effect of GSK1521498 on Nocturnal Food Consumption with Receptor Occupancy and Plasma Exposure

The interrelationship between GSK1521498 brain receptor occupancy and plasma concentration and the effect of GSK1521498 on food consumption is shown in **Figure 3**, **Figure 5,** and **Table 3**. GSK1521498 dose-dependently suppressed nocturnal food consumption in lean male rats provided with palatable chow (**Figure 3**). In the vehicle-treated group, the rats began to eat when lights were extinguished at the 2 hour time point which was approximately 2 hours after rats were dosed. Food consumption reached a plateau 12 hours later when the light cycle began (14 hr time point). At early time points, all three doses of GSK1521498 suppressed food consumption to the same extent, however, the effect waned in a dose-dependent manner until lights were turned on and the rats entered the sleep cycle. The efficacy of GSK1521498 was not impacted by gender since results similar to those in **Figure 3** were also observed in intact or ovariectomized female rats fed palatable chow (data not shown). The suppression of food consumption by 30 mg/kg GSK1521498 in this model was of similar magnitude to a maximally efficacious oral dose (30 mg/kg) of rimonabant, a cannabinoid receptor 1 inverse agonist previously marketed for treatment of obesity (data not shown).

The time course of the plasma drug concentrations after administration of oral doses of 1 and 10 mg/kg GSK1521498 is shown in **Table 3.** Maximal plasma concentrations for both doses were achieved by approximately two hours post-dosing. The maximal plasma concentration of the 10 mg/kg dose was approximately ten-fold higher than that of the 1 mg/kg dose indicating the escalation was linear in this dose range. At 8 hours, the plasma drug levels were starting to decline which correlates with the timing of the separation of the efficacy curves for the 0.3, 3 and 30 mg/kg doses in **Figure 3**. By 24 hours,

plasma concentrations at both doses had fallen to 5% of the maximum levels, however, the plasma concentration in the 10 mg/kg dose group was still approximately 25% of the maximal exposure of the 1 mg/kg dose.

The displacement of radioligands specific for MOR, KOR and DOR by GSK1521498 in brain regions enriched for each receptor was assessed in order to compare the time course and dose response of receptor occupancy to that of food consumption. The specificity of the radioligands [$^3$H]-DAMGO, [$^3$H]-CI-977, and [$^3$H]-Deltorphin II for MOR, KOR and DOR, respectively, was assessed in nucleus accumbens, dorsal endopiriform nucleus, and caudate/putamen, respectively (**Figure 4**). Each radioligand was highly selective for its respective receptor subtype as indicated by the similarity of total binding to specific binding in the presence of high concentrations of receptor-selective ligands for either of the other two opioid receptors. As shown in **Figure 5A**, GSK1521498 dose-dependently displaced the MOR occupancy of [$^3$H]-DAMGO. The maximum [$^3$H]-DAMGO displacement at each dose was present at the first time point (1.5 hours after dosing). Maximal suppression of food consumption was noted during this timeframe with the 0.3 mg/kg dose for which approximately 60% displacement was observed**.** Diminished signal was observed for all doses at 8 hours which is consistent with the plasma drug level time course and the divergence of the efficacy curves from one another in the food consumption study. At the 24 hour time point, [$^3$H]-DAMGO displacement was not different from vehicle for the 0.3, 1, and 3 mg/kg doses and had decreased in a dose-dependent manner in the 10 and 30 mg/kg groups. The signal at 24 hours in the 10 mg/kg dose group is consistent with the remaining plasma drug levels at this time point. [$^3$H]-DAMGO displacement did not fall below 60% for the 30 mg/kg dose group even at the 24 hour time point which is consistent with the sustained efficacy observed at the end of the dark cycle (14 hours) in the food consumption study. Displacement of radioligand at KOR and DOR was not different from vehicle at the 0.3 and 3 mg/kg doses at any time

point (**Figures 5B and C)** and these doses were fully efficacious in the early hours of the nocturnal food consumption study. Although the 1 mg/kg dose at the 8 hour time point was statistically different (p=0.03) from vehicle at KOR, this result was not consistent with either the 1.5 hour time point or the other effect at this time point for the 0.3, 3 or 10 mg/kg doses, suggesting that the mild significance may be due to random variability and the small sample size (N=3). Statistically significant displacement of radioligand at KOR and DOR was observed in the 10 and 30 mg/kg dose groups at the 1.5 hour time point, however, the magnitude of displacement observed at MOR was not observed.

Reduction of Food Consumption and Translation to Weight Loss in DIO rats

GSK1521498 (0.3, 3, and 30 mg/kg) inhibited palatable food consumption in a dose-dependent manner in DIO rats fed palatable chow (**Figure 6A**) . The same group of DIO rats was then given *ad lib* access to standard chow instead of palatable chow for a week. During the 24 hours preceding the experiment, the rats were presented with an amount of food representing 30% caloric restriction. Rats were dosed with GSK1521498 and then were given *ad lib* access to standard chow (**Figure 6B**). Under these conditions, the $ED_{50}$ for inhibition of food intake is 6.2 mg/kg, compared to an $ED_{50}$ of only 1.6 mg/kg in rats with access to palatable chow (**Figure 6A**).

Oral administration of 3, 10, or 30 mg/kg of GSK1521498 to DIO rats once daily for 12 days resulted in dose-responsive induction of weight loss (**Figure 7A**). As shown in **Table 4**, there was a dose-dependent reduction of fat mass and the effects of the 10 and 30 mg/kg doses were different from vehicle (p = 0.05 for 3 mg/kg group). At the highest dose (30 mg/kg), body weight loss reached a plateau at a fat mass level above that of lean rats fed standard chow. Lean mass was not different from vehicle in any dose group, thus, the body weight reduction was predicated on reduction of fat mass. The linear correlation of mean body weight loss and mean fat loss across the treatment groups was highly positive (0.98) with $p<0.05$. In contrast, the correlation between body weight loss and lean loss (0.32)

was not significantly different from zero (p=0.7). These correlation coefficients align with the analyses which showed significant differences in body weight loss and fat mass loss across treatment without a significant reduction in lean mass.

The amount of body weight loss was compared to the cumulative suppression of food consumption during the study **(Figure 7B).** The loss in body weight for each rat was converted to kilocalories (Kcal) using a conversion factor of 9 Kcal per gram body weight since the body weight loss was comprised of fat mass. The weight loss in Kcal was not different from the reduction in food intake expressed in Kcal suggesting that body weight loss is related to reduced caloric intake.

GSK15214 98 Regulates Sucrose Preference, Food Reinforcement Efficacy and Satiety

An escalating concentration, 2 bottle choice sucrose preference paradigm was employed to demonstrate the effects of GSK1521498 on carbohydrate preference (**Figure 8**). Preference peaked at approximately 80-90% for the highest sucrose concentration (1%) in the vehicle group. Rimonabant (30 mg/kg) was used as a positive control since it has been previously demonstrated to be effective at reducing sucrose preference. Sucrose preference at both 0.5% and 1% concentration was significantly suppressed by 1 and 10 mg/kg GSK1521498 and the effect was similar to that of 30 mg/kg of rimonabant.

Food reinforcement efficacy was assessed in a progressive ratio model where the number of lever presses required for delivery of a palatable food reward increased with each successive reward. GSK1521498 significantly suppressed the number of lever presses on the first day of dosing in a progressive ratio paradigm at doses of 1, 3, and 10 mg/kg (**Figure 9A**). While the trend was still present on days 2 and 3 of dosing, the effect of all three doses was diminished. The difference from the vehicle group was not statistically significant with the exception of the 10 mg/kg dose on test day 3 although p = 0.05 on test day 2 for this dose. The initial time course of lever-pressing was not affected

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by GSK1521498, which indicates that the rats are not compromised by drug treatment such that they are unable to perform the task (data not shown). The effect of GSK1521498 on satiety was assessed using a fixed-ratio schedule of reinforcement. In the FR1 interval paradigm, doses of 1, 3, and 10 mg/kg GSK1521498 inhibited the total number of lever presses by 50% to 75% over a period of 3 consecutive days relative to the vehicle group (**Figure 9B**). The vehicle group averaged between 86 and 95 lever presses during the 3 test days, the 1 mg/kg dose group achieved 40 to 43 lever presses per day while the 3 and 10 mg/kg groups achieved 24 to 37 lever presses per day.

Secondary Pharmacology

Adverse neurological effects were not observed in an Irwin screen where male Sprague Dawley rats were dosed orally with 5 and 50 mg/kg GSK1521498 once daily for 3 days. GSK1521498 treatment did not cause significant changes in arterial blood pressure, core body temperature, or activity following 3 daily oral doses of 3 or 30 mg/kg/day of telemetered Sprague Dawley rats.

Discussion

The endogenous opioid system was first implicated in the control of ingestion by the discovery that morphine could promote food intake [Martin, 1963]. Numerous subsequent studies have demonstrated that opioids primarily regulate ingestive behavior related to palatability of foods (i.e. incentive value) rather than subsistence intake and that the effects are mediated through central reward systems [Kelley and Berridge, 2002; Levine and Billington, 1997]. MOR plays a critical role in modulating hedonic and motivational components of palatable food intake [Pecina and Berridge, 2000; Will, 2003; Zhang and Kelley, 1997; Zhang, 1998].

LY255582 was the first opioid inverse agonist to be developed for the treatment of obesity. This compound has been carefully studied for its ability to regulate palatable food consumption and body weight in rats [Mitch, 1993; Shaw, 1993; Statnick, 2003]. LY255582 has subnanomolar potency for rat MOR and 5- and 13-fold selective over KOR and DOR, respectively, thus, it is considered relatively nonselective. In DIO Long Evans rats, the maximal suppression of 24 hour food intake was approximately 50-60% compared to vehicle. Striatal opioid receptor occupancy by LY255582 correlated with the reduction of food consumption, however, both the radioligand used to assess occupancy (diprenorphine) and LY255582 are not selective so the receptor(s) mediating the efficacy could not be identified. A subsequent study which studied localization of LY255582 in mouse brain by autoradiography suggested that the effects on food intake and body weight were mediated by the combined effect at mu, kappa, and delta receptors [Gackenheimer, 2005].

The goal of our program was to identify a MOR-selective molecule in order to avoid undesired adverse events that might be elicited by inverse agonism at KOR and DOR. For instance, increased levels of anxiety and depressive phenotype are observed in DOR knockout mice and KOR knockout mice experience increased nociception for specific pain responses [Gaveriaux-Ruff and Kieffer, 2002].

GSK1521498 appeared to have similar potency for rat MOR compared with LY255582, however, it was more selective for rat MOR versus KOR and DOR (greater than 50-fold). It should be noted that the potency and selectivity of the two compounds were assessed by different methodologies. LY255582 was assessed in a binding assay using rat brain homogenates while GSK1521498 was tested in a functional system using membranes from cells expressing recombinant rat receptors. Both GSK1521498 and LY255582 suppressed 24-hour food consumption in the DIO Long Evans rat model, however, the maximal reduction achieved with LY255582 was 50% while that of GSK1521498 was greater than 85%. A possible explanation for the disparity is that MOR receptor occupancy in the maximally efficacious LY255582 dose groups (10 and 20 mg/kg) was less than that observed with GSK1521498 (10 and 30 mg/kg).

The data in **Figure 3, Figure 5,** and **Table 3** show consistency between the patterns of MOR occupancy, plasma drug concentrations, and the time course and dose-response relationship of palatable food consumption. An oral dose as low as 0.3 mg/kg dose was sufficient to maximally suppress food intake over the first few hours of the nocturnal cycle coincident with maximal plasma drug levels and approximately 60% MOR occupancy. Neither KOR nor DOR occupancy by GSK 1521498 were observed at this dose which suggests that maximal efficacy in the food consumption model can be mediated solely by MOR occupancy. Strong suppression of food intake was present throughout the nocturnal cycle for the 30 mg/kg dose group in which MOR occupancy did not fall below 60% for the 24 hour period. The 0.3 mg/kg dose groups exhibited similar efficacy in the food intake model to the 30 mg/kg group at early time points when occupancy was approximately in the range of 60%. We conclude that at least 60% MOR occupancy is sufficient for robust efficacy in this model. The correlation of MOR occupancy with efficacy indicates that techniques such as PET imaging can be used

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to estimate efficacious dose and plasma drug levels in humans. In addition, plasma drug levels should serve as an easily measurable surrogate for MOR occupancy in the clinic.

The suppression of nocturnal intake of palatable food observed in DIO rats translated into induction of weight loss. Weight loss reached a plateau before the fat mass level was reduced to that of lean rats fed standard chow. This pattern has been observed in clinical studies with antiobesity agents such as rimonabant. The plateau effect was also observed with 30 mg/kg dose of rimonabant in this model and the maximal percentage weight loss from baseline was similar to that observed with 30 mg/kg GSK1521498 (data not shown). Since the cumulative reduction in food consumption was equivalent to the loss of weight, we conclude that GSK1521498 induced fat mass loss through the suppression of food consumption. It is not possible to compare the magnitude of weight loss efficacy for GSK1521498 with LY255582 as the models utilized were inherently different [Shaw, 1993; Statnick 2003]. Obese Zucker rats continue to gain weight and treatment of the DIO Long Evans rats started early in the weight gain curve, thus, LY255582 inhibited the progressive weight gain compared with the vehicle group. The DIO Long Evans rats used in the present study were permitted to become heavier before the start of the experiment such that the rate of gain had slowed and induction of weight loss could be measured. While GSK1521498 strongly suppressed palatable food consumption in DIO rats, the same rats that were then provided with standard chow and subjected to caloric restriction during the 24 hours before the nocturnal food consumption experiment were less sensitive to GSK1521498. In fact, the lowest dose of 0.3 mg/kg was no longer active in this model. This observation may have implications for how this mechanism should be best assessed in clinical studies with regard to the composition of diet and restriction of calories.

Opioid receptor antagonists/inverse agonists are thought to modulate satiety and motivation or desire to eat palatable food. A progressive ratio model was used to assess the reinforcement efficacy for

food reward while a fixed-ratio schedule was employed to assess effects on satiety when food reward was obtained with a minimal amount of work [Maccioni, 2008; Nair, 2008]. GSK1521498 inhibited reinforcement efficacy in the progressive ratio model and suppressed performance in an FR1 schedule compared to vehicle. The efficacy in the FR1 paradigm suggests that GSK1521498 enhanced the development of satiety. The amount of lever-pressing in the GSK1521498-treated rats FR1 paradigm was far greater than that observed for the progressive ratio model, therefore, the efficacy of GSK1521498 in the latter model cannot be explained simply by an effect on satiety but rather suppression of the motivation to work for reward or the sensitivity to reward. Durability of efficacy over three days of treatment especially at the lower doses was greater in the FR1 schedule than in the progressive ratio paradigm. Interestingly, we have observed that rimonabant (30 mg/kg) reduced lever pressing in the progressive ratio schedule to the same extent as GSK1521498 on day one of drug testing, but a significant difference from vehicle on days 2 and 3 of dosing was not observed (data not shown). There may be compensatory mechanism which limits the persistence of the effect on reinforcement, whereas, the effect on satiety is sustained. In the operant response setting, initiation of lever pressing was not disrupted, thus, the rats were not neurologically impaired such that they were unable to work for rewards. Neurologic function was also not found to be impaired in an Irwin screen in rats treated with GSK1521498 (data not shown). Finally, GSK1521498 altered the choice to imbibe palatable food as demonstrated by the diminished preference for sucrose-containing solutions.

Currently, there are few pharmacotherapeutic options for obesity therapy. GSK1521498 may be clinically useful for the treatment of obesity and other disorders characterized by compulsive eating including binge-eating disorder, since it robustly inhibits ingestion of palatable food under normal conditions of nocturnal consumption, induces satiety, and inhibits the preference for and desire to seek palatable food. This molecule may, therefore, be effective in modulating choice of nutrients and desire

for fat and sugar in the clinic. Since GSK1521498 also suppressed consumption of standard chow, we expect that binge eating of any type of food could be suppressed.

GSK1521498 is currently in clinical development for obesity therapy and has indeed been shown to significantly reduce hedonic taste preference and caloric intake particularly for high fat/high sugar foods in subjects with body mass index between 25 and 35 $kg/m^2$ [Nathan, 2011]. In a human functional magnetic resonance imaging study, GSK1521498 attenuated palatable food reward-related amygdala activation while naltrexone was inactive (unpublished observations), thus, this novel mu-selective inverse agonist may have a different clinical profile from naltrexone when used for treatment of obesity and binge-eating disorders.

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Authorship Contribution

Participated in research design: DMI, JDH, SDS, JDS, ASG, TWS, AHE

Conducted experiments: KNN, TAB, JCF, LHC, AES, SDS, JDH, ALL

Contributed new reagents or analytic tools: ALL, JCF

Performed data analysis and/or interpretation: DMI, JDH, KNN, TAB, JCF, LHC, AES, JDS, TWS, JAB, ASG, AHE

Wrote or contributed to the writing of the manuscript: DMI, JDH, JAB, ASG, AHE, TWS

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Footnotes

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Legends for Figures

**Figure 1** Chemical structure of the dihydrochloride salt of GSK1521498

**Figure 2** GSK1521498 acts as an inverse agonist. GTPγS binding was assessed in membrane preparations constitutively expressing (A) human or (B) rat MOR in the presence of increasing concentrations of mu-specific agonist DAMGO (closed circles); opioid antagonist naltrexone (closed squares); and GSK 1521498 (closed triangles). Results are presented as percent maximal response to DAMGO vs. molar concentration of test compound.

**Figure 3** GSK1521498 inhibits nocturnal consumption of palatable chow in lean rats. Lean rats were dosed by oral gavage with vehicle or the indicated doses of GSK1521498. Dosing was completed at time = 0 approximately two hours prior to extinguishing the lights and food consumption was measured hourly thereafter. N= 8 rats per group. All drug-treated groups were significantly different from vehicle from 3 hours to 24 hours. For the 3 and 30 mg/kg dose groups, $p < 0.001$ from 3 to 24 hours except $p<0.01$ at 3 hours for the 3 mg/kg group. For the 0.3 mg/kg group, $p<0.05$ at hours 11 and 13, $p<0.01$ at hour 3 and 7, $p<0.005$ at hour 9, 10, 14, 18 and 20-24, and $p<0.001$ at hours 4-6, 8, 12, 15-17, and 19. The comparison with vehicle at the 24 hour time point is shown in the figure (*$p<0.005$, **$p<0.001$).

**Figure 4** Autoradiography showing specificity of MOR, KOR and DOR radioligands. Coronal brain slices were incubated with [$^3$H]DAMGO; [$^3$H]-CI-977; or [$^3$H]-Deltorphin II (Delt II) to determine total binding to the MOR, KOR or DOR, respectively. Slices were incubated with 100 nM DAMGO, norBNI, and DPDPE to assess nonspecific binding for MOR, KOR, and DOR, respectively.

**Figure 5** Displacement of opioid receptor-specific ligands by GSK1521498. Lean rats were dosed with vehicle or GSK1521498 by oral gavage and brains were removed 1.5, 8, or 24 hours after dosing.

Coronal brain slices were incubated in the presence of 10 μM naloxone to determine nonspecific binding. Nucleus accumbens (A), dorsal endopiriform nucleus (B) and caudate/putamen (C) were scanned by beta imaging to assess MOR, KOR and DOR receptor occupancy. Percent specific binding of radioligand in each GSK1521498 dose group compared with vehicle group is presented. N = 3 rats per group. *p<0.05, **p<0.005, ***p<0.001.

**Figure 6** Inhibition of food consumption in DIO rats. (A) Palatable chow consumption was monitored for 24-hour period post oral gavage with vehicle or the indicated doses of GSK1521498. Timing of dosing was as described in figure 3 legend. Effects in the 3 and 30 mg/kg groups were statistically significant versus vehicle from 4 to 24 hours (p<0.001) except p<0.005 for the 3 mg/kg group at 4 hours. At 3 hours, p<0.05 for the 3 mg/kg group and p<0.005 for 30 mg/kg group. For the 0.3 mg/kg group, p<0.005 or 0.001 for all points after 2 hours except p<0.01 at 22 and 23 hours and p<0.05 at 3 and 24 hours. (B) The same group of DIO rats was then fed standard chow *ad libitum* for one week after which the amount of food presented during the 24 hours preceding the experiment represented 30% caloric restriction. The rats were then dosed with vehicle or GSK1521498 as in part A. The 30 mg/kg dose group was significantly different from vehicle at every time point with p<0.001 except p<0.05 at 1 hour. The 3 mg/kg group was different from vehicle p<0.001 at 7 and 10 -24 hrs, p<0.005 at 8 and 9 hours, p<0.05 hours 1-6 except NS at 4 hours. The 0.3 mg/kg group was not different from vehicle at most time points except p<0.05 at 6, 7, and 9 hours. For both experiments, n=6-8/group. The difference from vehicle is shown at the 24 hours time point (*p<0.05, *p<0.001).

**Figure 7** Induction of body weight and fat mass loss by GSK1521498. Vehicle or GSK1521498 at the indicated doses were orally administered to DIO rats for 11 days. (A) Body weight loss expressed as a percentage of baseline body weight over the 11-day period, (B) Grams of body weight loss and

cumulative food consumption reduction for each dose group were converted to Kcal. N = 8 rats per group. * $p < 0.0005$, **$p<0.0001$

**Figure 8** GSK1521498 suppresses sucrose preference. Vehicle, GSK1521498 (1, 10 mg/kg) or rimonabant (30 mg/kg) were orally administered to rats which were then tested in an escalating percentage sucrose solution, two bottle assay. The volume consumed of the sucrose-containing solutions was monitored and expressed as a percentage of total liquid intake. N = 10 rats per group. * $p < 0.05$, **$p<0.001$

**Figure 9** GSK1521498 suppresses reinforcement efficacy and satiety. (A) Lever presses in trained rats were measured in the progressive operant response reinforcement model in rats dosed orally with vehicle or GSK1521498 at indicated doses. (B) Lever pressing in rats dosed orally with vehicle or GSK1521498 was tested in a fixed-ratio operant response model in which 1 food pellet was released with each lever press. N = 10 rats per group in both experiments. *$p < 0.05$, **$p<0.005$, ***$p<0.001$.

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Tables

**Table 1**

**Pharmacokinetic Characteristics of GSK1521498**

Rats were dosed with GSK1521498 at 1 and 10 mg/kg orally and 3 mg/kg intravenously. Plasma and brain samples were obtained at various time points to assess drug concentration.

| Parameter | Value |
|---|---|
| Clearance (CL) | 5.6 ml/min/kg |
| Steady-state volume of distribution ($V_{ss}$) | 1.8 l/kg |
| Half-life ($T_{1/2}$ IV) | 4.4 hr |
| Peak plasma concentration ($C_{max}$) | 2060 + 410 ng/ml |
| Time to peak plasma concentration ($T_{max}$) | 2-4 hrs |
| Bioavailability (F) at 10 mg/kg | 74 $\pm$ 10% |
| Brain:Plasma ratio at 3 mg/kg | 1.1 – 1.6 |

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**Table 2**

**Affinity of GSK1521498 and Naltrexone for Human and Rat MOR, KOR and DOR**

| **Human** | GSK1521498 | | | Naltrexone | | |
|---|---|---|---|---|---|---|
| | fpKi[a] | SD[b] | N | fpKi | SD | N |
| *Mu* | 8.82 | 0.09 | 17 | 8.33 | 0.10 | 4 |
| *Delta* | 7.52 | 0.24 | 14 | 7.38 | 0.14 | 4 |
| *Kappa* | 7.69 | 0.17 | 14 | 7.70 | 0.14 | 4 |
| **Rat** | GSK1521498 | | | Naltrexone | | |
| | fpKi | SD | N | fpKi | SD | N |
| *Mu* | 9.14 | 0.22 | 12 | 8.83 | 0.17 | 4 |
| *Delta* | 7.32 | 0.24 | 8 | 7.45 | 0.26 | 4 |
| *Kappa* | 7.42 | 0.17 | 10 | 7.55 | 0.21 | 4 |

[a] Negative log(Ki)

[b] Standard deviation

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**Table 3**

**Plasma Concentrations of GSK1521498 after Oral Administration**

GSK1521498 was administered by oral gavage at the indicated doses and plasma concentration was determined over a 24 hour period.

| | *plasma concentration in ng/*ml | | | |
|---|---|---|---|---|
| | **1 mg/kg**[a] | | **10 mg/kg**[a] | |
| **Time (h)** | Mean | SD[b] | Mean | SD |
| 0.25 | 58 | 39 | 387 | 61 |
| 0.5 | 110 | 57 | 1120 | 240 |
| 1 | 198 | 97 | 1690 | 80 |
| 2 | 271 | 58 | 2060 | 410 |
| 4 | 305 | 23 | 1750 | 170 |
| 8 | 172 | 22 | 1110 | 180 |
| 24 | 15 | 6 | 66 | 8 |

[a] single administered dose

[b] Standard deviation

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**Table 4**
**Composition of Body Weight in DIO rats after Oral Administration of GSK1521498**

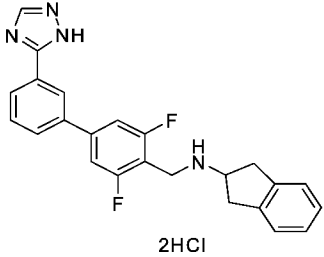
GSK1521498 was administered by oral gavage at the indicated doses and composition of body weight was determined after 12 days.

| | Fat mass (grams) | | Lean mass (grams) | |
|---|---|---|---|---|
| **Treatment** | Mean | SD[a] | Mean | SD[a] |
| Vehicle | 114.6 | 15.0 | 324.0 | 22.2 |
| GSK1521498 | | | | |
| 3 mg/kg | 98.8 | 12.4 | 327.3 | 22.2 |
| 10 mg/kg | 88.9* | 19.0 | 337.4 | 14.9 |
| 30 mg/kg | 83.7** | 19.7 | 306.3 | 15.0 |
| Lean rats | 68.8 | 11.1 | 329.7 | 20.9 |

* P<0.005 and **P<0.0005

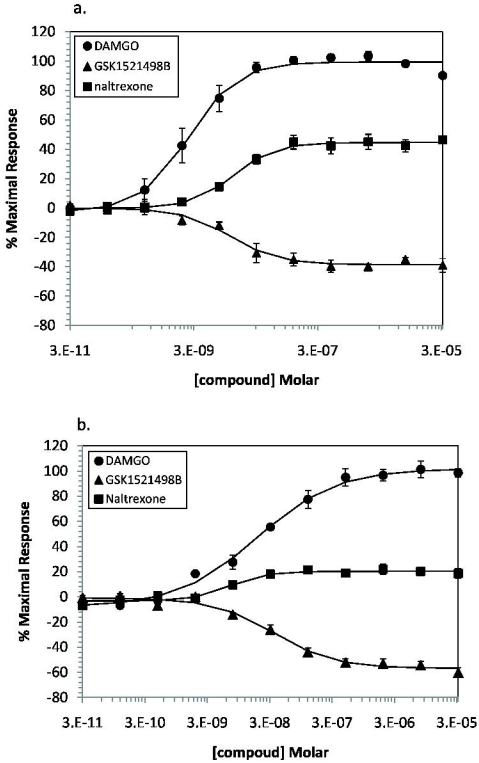
[a] Standard deviation

Figure 1




2HCl

# Figure 2


a.
DAMGO
GSK1521498B
naltrexone
% Maximal Response
120
100
80
60
40
20
0
-20
-40
-60
-80
3.E-11
3.E-09
3.E-07
3.E-05
[compound] Molar
b.
DAMGO
GSK1521498B
Naltrexone
% Maximal Response
120
100
80
60
40
20
0
-20
-40
-60
-80
3.E-11
3.E-10
3.E-09
3.E-08
3.E-07
3.E-06
3.E-05
[compoud] Molar

# Figure 3

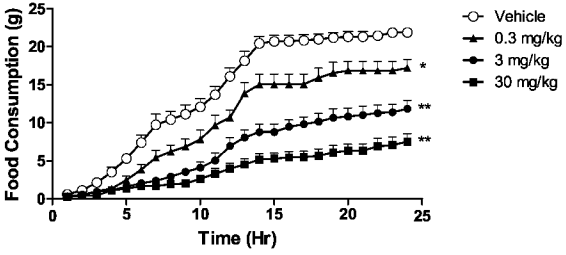
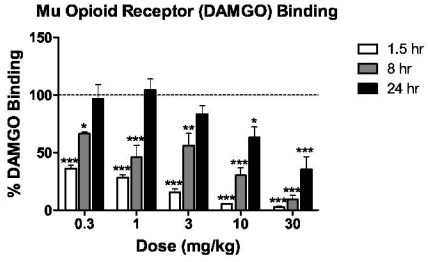

Food Consumption (g)
25
20
15
10
5
0
0
5
10
15
20
25
Time (Hr)
Vehicle
0.3 mg/kg
3 mg/kg
30 mg/kg
*
**
**

Figure 4

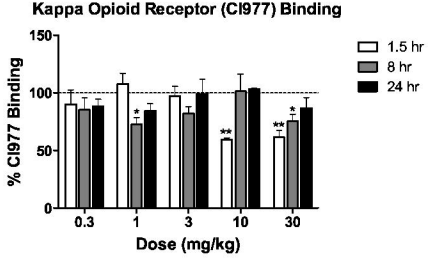
| Radioligand Treatment | $^3$H-DAMGO (μ) 2nM ($K_d$) | $^3$H-CI-977 (κ) 0.8nM ($K_d$) | $^3$H-Delt II (δ) 12nM ($K_d$) |
|---|---|---|---|
| Total Binding |  |  |  |
| NSB 10uM Naloxone |  |  |  |
| 100nM DAMGO (μ) |  |  |  |
| 100nM norBNI (κ) |  |  |  |
| 100nM DPDPE (δ) |  |  |  |

# Figure 5

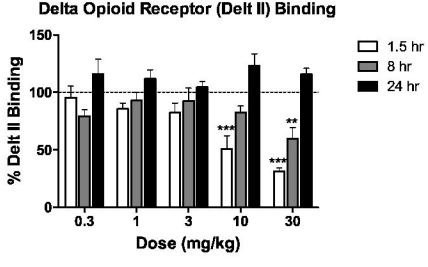
a.


Mu Opioid Receptor (DAMGO) Binding
% DAMGO Binding
150
100
50
0
0.3
1
3
10
30
Dose (mg/kg)
1.5 hr
8 hr
24 hr

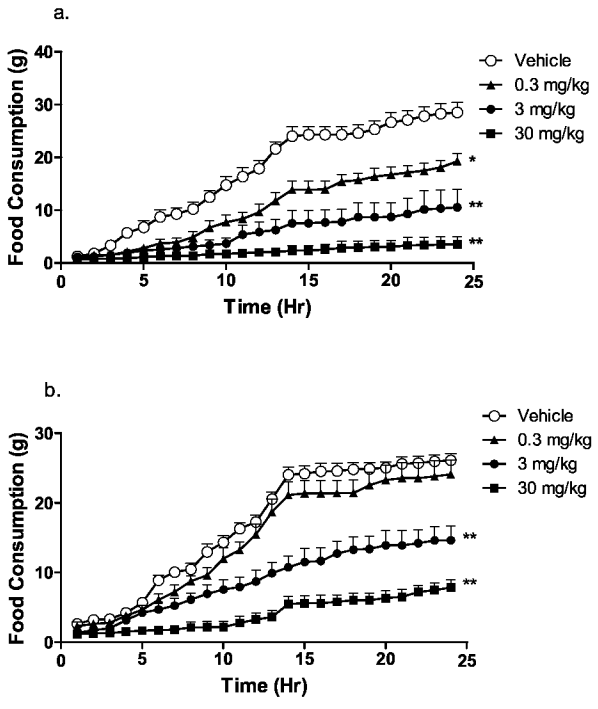
b.


Kappa Opioid Receptor (CI977) Binding
% CI977 Binding
150
100
50
0
0.3
1
3
10
30
Dose (mg/kg)
1.5 hr
8 hr
24 hr

c.

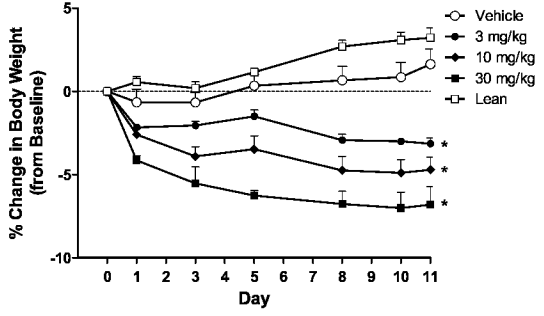

Delta Opioid Receptor (Delt II) Binding
% Delt II Binding
150
100
50
0
0.3
1
3
10
30
Dose (mg/kg)
1.5 hr
8 hr
24 hr

# Figure 6

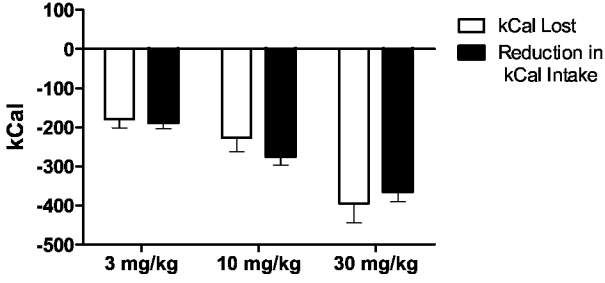

a.
Food Consumption (g)
Time (Hr)
Vehicle
0.3 mg/kg
3 mg/kg
30 mg/kg
*
**
**
b.
Food Consumption (g)
Time (Hr)
Vehicle
0.3 mg/kg
3 mg/kg
30 mg/kg
**
**

# Figure 7

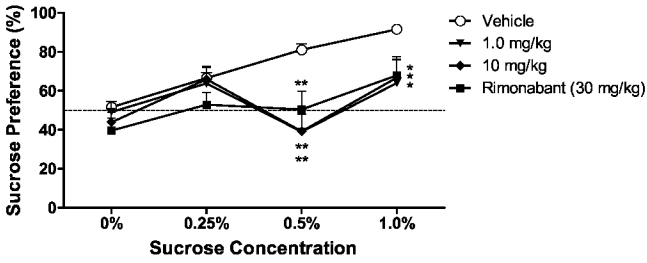
a.


% Change In Body Weight (from Baseline)
5
0
-5
-10
0 1 2 3 4 5 6 7 8 9 10 11
Day
Vehicle
3 mg/kg
10 mg/kg
30 mg/kg
Lean
*
*
*

b.

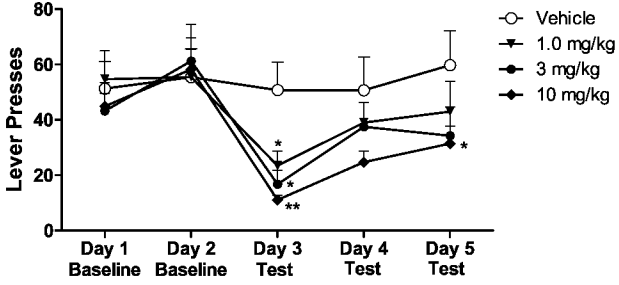

kCal
100
0
-100
-200
-300
-400
-500
3 mg/kg
10 mg/kg
30 mg/kg
kCal Lost
Reduction in kCal Intake

# Figure 8

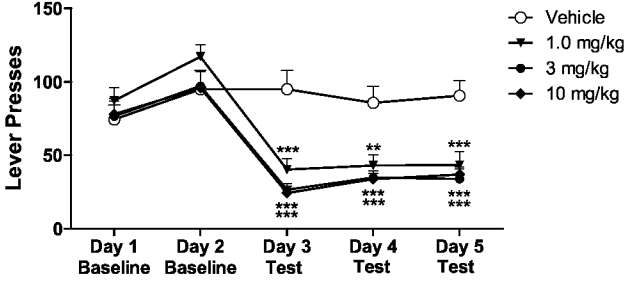

Sucrose Preference (%)
100
80
60
40
20
0
0%
0.25%
0.5%
1.0%
Sucrose Concentration
**
**
**
**
**
**
Vehicle
1.0 mg/kg
10 mg/kg
Rimonabant (30 mg/kg)

# Figure 9

a.


Lever Presses
80
60
40
20
0
Day 1 Baseline
Day 2 Baseline
Day 3 Test
Day 4 Test
Day 5 Test
Vehicle
1.0 mg/kg
3 mg/kg
10 mg/kg
*
*
**
*

b.


Lever Presses
150
100
50
0
Day 1 Baseline
Day 2 Baseline
Day 3 Test
Day 4 Test
Day 5 Test
Vehicle
1.0 mg/kg
3 mg/kg
10 mg/kg
***
***
**
***
***
***
***