The Triple Uptake Inhibitor (1R,5S)-(+)-1-(3,4-Dichlorophenyl)-3-azabicyclo[3.1.0]Hexane Hydrochloride (DOV 21947) Reduces Body Weight and Plasma Triglycerides in Rodent Models of Diet-Induced Obesity

Joseph P. Tizzano, D. Sloan Stribling, Diego Perez-Tilve, Alison Strack, Andrea Frassetto, Richard Z. Chen, Tung M. Fong, Lauren Shearman, Philip A. Krieter, Matthias H. Tschöp, Phil Skolnick, and Anthony S. Basile

Department of Preclinical Pharmacology, DOV Pharmaceutical, Inc., Somerset, New Jersey (J.P.T., P.A.K., P.S., A.S.B.); Department of Psychiatry, Obesity Research Center and Genome Research Institute, University of Cincinnati College of Medicine, Cincinnati, Ohio (D.P.-T., M.H.T.); and Departments of Metabolic Disorders (A.F., R.Z.C., T.M.F.) and Pharmacology (D.S.S., A.S., L.S.), Merck Research Laboratories, Rahway, New Jersey

Received November 5, 2007; accepted December 17, 2007

ABSTRACT

Selective inhibitors of biogenic amine (e.g., serotonin, norepinephrine, and dopamine) uptake exhibit varying degrees of safety and efficacy as antiobesity agents. Moreover, preclinical findings suggest that the combined inhibition of monoamine neurotransmitter transporters synergistically enhances antiobesity activity. (1R,5S)-(+)-1-(3,4-Dichlorophenyl)-3-azabicyclo[3.1.0]hexane hydrochloride (DOV 21947) inhibits norepinephrine, 5-hydroxytryptamine, and dopamine uptake, and it reduces body weight in rodent models of diet-induced obesity (DIO). DIO rats treated orally with DOV 21947 for 1 to 24 days showed significantly lower body weights than vehicle-treated DIO rats. The decrease in body weight resulted specifically from a loss of retroperitoneal and mesenteric depots of white adipose tissue. DOV 21947 also reduced daily food intake in DIO rats, but consumption returned to control levels after 11 days of treatment. With the exception of a decrease in triglyceride levels, blood chemistry was unaltered after 24 days of DOV 21947 treatments. DOV 21947 had no effect on motor activity. Although DOV 21947 increased respiratory rate and decreased the tidal volume of normal rats, it did not alter the minute volume. In addition, DOV 21947 did not significantly affect blood pressure, heart rate, electrocardiographic indices or body temperature in telemeterized dogs. However, it caused a sustained, but reversible reduction in the rate of body weight gain for as long as 6 months in normal rats, and for up to 1 year in normal dogs. In summary, DOV 21947 is effective in causing a sustained and selective reduction in fat content and triglyceride levels in animal models of obesity without significantly altering vital organ function.

The monoamine neurotransmitters 5-hydroxytryptamine (serotonin; 5-HT), norepinephrine (NE), and dopamine (DA) play significant roles in regulating behaviors relevant to food intake and metabolism (Hoebel et al., 1989). Serotonin levels in the ventromedial hypothalamus increase during feeding, and they act as a satiety signal (Hoebel et al., 1989; Leibowitz, 1992). Decreased 5-HT levels are often associated with eating disorders, such as binge-eating, bulimia, and obesity (Ericson et al., 1996; Hainer et al., 2006). Stimulation of central noradrenergic pathways in the lateral hypothalamus can suppress food intake (Meguid et al., 2000), whereas increased NE release resulting from activation of sympathetic

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); NE, norepinephrine; DA, dopamine; phen-fen, phentermine (2-methyl-1-phenylpropan-2-amine and 2-methyl-amphetamine) and fenfluramine [N-ethyl-1-[3-(trifluoromethyl)-phenyl]propan-2-amine]; DEXAscan, dual-energy X-ray absorptiometer; CNS, central nervous system; SERT, serotonin transporter; NET, norepinephrine transporter; DAT, dopamine transporter; AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; DOV 21947, (1R,5S)-(+)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane hydrochloride; DIO, diet-induced obesity; AFIS, automated food intake monitoring system; AM251, 1-(2,4-dichlorophenyl)-5-[4-iodophenyl]-4-methyl-N-[1-piperidyl]pyrazole-3-carboxamide; ANOVA, analysis of variance; BW, body weight; AUC, area under the time-concentration curve.
pathways can elevate glucose and lipid catabolism (Nonogaki, 2000). In addition, the mesocorticolimbic dopaminergic pathways arising from the ventral tegumentum and terminating in the nucleus accumbens and prefrontal cortex are involved in mediating the reinforcing and rewarding aspects of food (Wise, 2006), whereas nigrostriatal pathways terminating in the dorsal striatum contribute to the arousal and somatosensory aspects of feeding and the motor activity related to acquiring and consuming food (Kelley et al., 2005). Dopamine levels in the ventromedial hypothalamus rise during feeding (Hoebel et al., 1989), and they act to reduce the number and duration of feeding bouts (Meguid et al., 2000).

Thus, dopaminergic systems play an integral role in controlling appetitive behavior, the initiation of feeding, and the reward qualities of food. Deficits in dopaminergic neurotransmission contribute to a “reward deficiency syndrome” (Comings and Blum, 2000), which may be manifested by hyperphagia and increased body mass (Wang et al., 2001). Together, these neurotransmitter pathways provide targets for treating eating disorders and obesity.

Obesity in particular constitutes a significant public health problem, predisposing individuals to increased risk of type 2 diabetes, cardiovascular disease, cholecystitis, osteoarthritis, and sleep and mental disorders (Mokdad et al., 2003). Agents that activate DA, NE, and 5-HT pathways may be useful in treating obesity (Hainer et al., 2006; Nelson and Gehlert, 2006). Classical antiobesity agents, such as d-amphetamine or the mixture of phentermine and fenfluramine (phen-fen), activate combinations of noradrenergic, serotonergic, or dopaminergic pathways by inhibiting neurotransmitter transporters and increasing neurotransmitter release. These sympathomimetics not only transiently suppress appetite and moderately enhance metabolism but also cause significant CNS stimulation and cardiovascular side effects (e.g., systemic and pulmonary hypertension, tachycardia, stroke, and valvulopathy) (Sachdev et al., 2002). In addition, tolerance develops to their actions, which may also be associated with a substantial abuse liability (Gehlert et al., 1998; Sachdev et al., 2002). Compared with these agents, selective 5-HT (SERT) (Yen and Fuller, 1992) or NE (NET) (Gehlert et al., 1998) transport inhibitors are relatively free of serious side effects, and they have little or no abuse potential. Effective in treating specific eating abnormalities (McElroy et al., 2007), they produce only moderate, transient weight loss in obese subjects (Li et al., 2005; Gadde et al., 2006; Hainer et al., 2006). Agents that inhibit both NET and dopamine transport (DAT), such as mazindol (Smith et al., 1975) or bupropion (Gadde and Xiong, 2007), synergistically suppress food intake and increase thermogenesis in preclinical models (Meguid et al., 2000; Billes and Cowley, 2007), suggesting enhanced clinical efficacy as antiobesity agents. In contrast, inhibitors of both SERT and NET show variable efficacy as antiobesity agents (Nelson and Gehlert, 2006). Phen-fen inhibits both NE and 5-HT uptake, but additional mechanisms, including enhanced release of neurotransmitters, contribute to its antiobesity properties. In contrast, neither of the dual uptake inhibitors duloxetine nor venlafaxine causes substantial weight loss (Kraus et al., 2002; Wise et al., 2006). Moreover, the antiobesity agent 1-(4-chlorophenyl)-N,N-dimethyl-α-(2-methylpropyl)-cyclobutanemethanamine (sibutramine), originally described as a low-affinity SERT and NET inhibitor (Heal et al., 1998), is rapidly converted to metabolites that potently inhibit SERT, NET, and DAT (Heal et al., 1998; Balicgolu and Wurtman, 2000). This triple uptake inhibitor profile may account for the ability of sibutramine to cause sustained body weight loss in humans (Li et al., 2005). Nonetheless, sibutramine has sympathomimetic actions, causing small but significant elevations in blood pressure (Arterburn et al., 2004; Woolard et al., 2004), and it is registered as a controlled substance in the United States. Given the importance of NE, DA, and 5-HT pathways in regulating feeding behavior, and the demonstrated utility of multiple reuptake inhibitors in suppressing food intake, the development of an intrinsic triple uptake inhibitor, with inactive metabolites and no sympathomimetic actions could be a useful antiobesity agent. We now report on the sustained antiobesity actions of the triple uptake inhibitor DOV 21947 (Skolnick et al., 2003) in models of diet-induced obesity (DIO), and in normal animals in the absence of significant long-term toxicity.

Materials and Methods

Pharmacokinetics. Pharmacokinetic studies were performed by WIL Research Laboratories (Ashland, OH), and they were approved by its Animal Care and Use Committee. Male Sprague-Dawley rats were obtained from Charles River Laboratories (Raleigh, NC), and they were maintained in accordance with the Guide for the Care and Use of Laboratory Animals in AAALAC-accredited facilities. Light times were set to provide a 12-h light/dark cycle (lights on, 6:00 AM), with an average daily temperature of 22 ± 3°C and 50 ± 20% relative humidity with 10 room air changes/h of 100% fresh air. Rodent LabDiet 5002 (PMI Nutrition International, Richmond, IN) and water were supplied ad libitum throughout the study period.

In the study of the absolute bioavailability of DOV 21947, each rat received a single dose in either by oral gavage (10 mg/kg) or i.v. via the tail vein (5 mg/kg). DOV 21947 is freely soluble in aqueous solutions (93 mg/ml water), so the vehicle used in this study was 0.9% saline. Blood samples (two/rat) of approximately 1 ml each were collected via retro-orbital puncture from 0.25 to 24 h after dose (three rats/time point), while the rats were under isoflurane anesthesia. In multiple-dose administration studies, DOV 21947 was administered orally using deionized water as the vehicle, and blood samples were collected as described above from 1 to 24 h after dose. Plasma concentrations of DOV 21947 were determined using a validated liquid chromatography-tandem mass spectrometry assay with a calibration range of 10 to 2000 ng/ml. Aliquots (0.2 ml) of plasma were transferred to glass tubes containing 50 µl of 0.5 M KOH, 25 µl (120 ng) of internal standard [1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane hydrochloride], and 300 µl of control plasma. Diethyl ether (8 ml) was added, and the tubes were extracted for 15 min. After centrifugation, the organic layer was transferred to a clean tube and dried under a stream of nitrogen. The samples were reconstituted in 200 µl of methanol and transferred to autosampler vials. They were injected onto a Hypersil C18-BDS column (50 × 4.6 mm; 3-µm particle size) with a C18 guard column (Agilent Technologies, Santa Clara, CA) equilibrated at 25°C. The mobile phase was 30% (v/v) acetonitrile/35% methanol/0.5% formic acid/34.5% 5 mM ammonium acetate, and the flow rate was 0.4 ml/min. The instrumentation was a 2695 liquid chromatograph (Waters, Milford, MA) equipped with an autosampler, a Micromass tandem quadrupole Quattro Micro mass spectrometer equipped with an APCI+ interface, and Mass Lynx software, version 3.5 (Waters). Multiple reaction monitoring was performed for DOV 21947 (m/z 228 → m/z 160 and m/z 228 → m/z 187) and the internal standard (m/z 174 → m/z 133). Pharmacokinetic parameters were determined using either WinNonlin, version 4.0.1 (Pharsight, Mountain View, CA) or Excel 97 (Microsoft, Redmond, WA).
Models of Diet-Induced Obesity. Male Sprague-Dawley rats (Charles River Laboratories) and male C57BL/6 mice (Taconic Farms, Germantown, NY) were used to establish the diet-induced models of obesity. The animals were housed and fed according to the Guide for the Care and Use of Laboratory Animals in AAALAC-accredited facilities maintained on a standard 12-h light/dark cycle (lights on, 6:00 AM; lights off, 6:00 PM) at a room temperature of 19.5 to 24.5°C and relative humidity of 45 to 65%. All animals had free access to water. At 4 weeks of age, the rats were made obese by switching to the moderately high-fat diet (D12266B; Research Diets, New Brunswick, NJ) in pellet form. The rats were housed in a group environment until 1 week before the study (body weight approx. 50–625 g) when they were singly housed in cages with an automated food intake monitoring system (AFIS), where consumption of a milled pellet form of the same diet was measured for the duration of the studies. Likewise, the mice had free access to a high-fat diet (12451i; Research Diets), reaching a body weight of 50 to 52 g when the study was initiated. Mice were switched to the high-fat diet at 4 weeks of age. All the experimental groups (n = 6–7 rats and n = 10 mice) were matched on the basis of total body mass and fat content.

Body composition analyses in DIO rats (14 days of administration), and mice were performed using a dual-energy X-ray absorptiometer (DEXAscan; Hologic, Bedford, MA) at baseline and after 8 days of treatment in mice, or at the end of a 14-day treatment cycle in rats. In the DIO rats treated for 21 days with DOV 21947 (20 and 40 mg/kg/day), body composition was assessed using a whole body nuclear magnetic resonance (Echo-MRI, Waco, TX) at both the beginning and at the end of the dosing cycle. Each animal was placed into a clear Plexiglas tube and scanned for 45 s. Body fat distribution in DI rats was determined by dissection and weighing of rat epidermal, retroperitoneal, and mesenteric white adipose tissue stores after the 14-day administration cycle. Blood chemistry was performed on samples obtained by cardiac puncture 18 h after the last dose using a Roche P Modular Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Rat motor activity was monitored in the home cages of 12 rats over a 24-h period using a video-based behavior analysis system (HomeCageScan; Clever Sys, Reston, VA). Data are presented as bouts (number of episodes of each behavior) and frames (duration of the behavior, at 30 frames/s).

Safety Pharmacology. The effects of DOV 21947 on CNS and pulmonary function were determined in male Sprague-Dawley rats (Charles River Breeding Laboratories, Portage, MI) at WIL Research Laboratories. The rats were approximately 57 days of age at receipt, and they were housed for a minimum of 15 days to acclimate. Otherwise, the rats were maintained as described under Pharmacokinetics.

For the pulmonary studies, the rats were randomized into separate treatment groups and moved to a separate room where the ventilation rate was reduced to five room changes/h to avoid interference with the pressure transducers. The rats were then condition to nose-only restraint tubes on four different occasions for periods of 1 to 6 h. Pulmonary function was measured using a pneumotach to detect the airflow in and out of the head-out, neck-sealed plethysmograph in response to the thoracic movement of an animal. Signals were amplified and recorded by the PONEMAH Physiology platform, 3.322-SP2 (LDS Test and Measurement, Herts, UK), and the respiratory frequency, tidal volume, and calculated minute volume were recorded. One day before dosing, the rats were placed in the plethysmograph for collection of baseline pulmonary function parameters over a 60-min period. On the following day, animals were dosed, and then they were placed into the plethysmograph. Data were collected for a 6-h period. For statistical evaluations, a single, 15-min average of each parameter was obtained from the 1-h baseline period, whereas eight episodes were averaged over the 0- to 120-min period after dosing, and four episodes representing the last 15 min of each hour between 2 and 6 h after dosing were taken. Results from the 0- to 60-min period after dosing are presented in Table 4.

The CNS safety studies were performed using functional observation batteries (Moser, 2000), which were performed by trained technicians in sound-attenuated rooms containing a white noise generator operating at 70 ± 10 db. All animals were observed for the following parameters: 1) home cage activity: posture, convulsions, biting, ptosis, and feces consistency; 2) handling observations: ease of removal from cage, laceration, piloerection, palpebral closure, eye prominence, red/crusty deposits, ease of handling, salivation, fur appearance, respiratory rate, color of eyes, mucus membranes, skin, and muscle tone; 3) open-field activity: mobility, rearing, convulsions, grooming, stereotypies, time to first step (seconds), gait, arousal, urination/defecation, gait score, backing, and locomotor activity (ambulatory and fine motor activity); 4) sensory observations: approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch response, eye blink response, hindlimb extension, and olfactory orientation; 5) neuromuscular observations: hindlimb extensor strength, hindlimb foot splay, grip strength, and rotated performance; and 6) physiological observations: catalepsy, body temperature, and body weight. These parameters were scored using criteria and procedures established by WIL Research Laboratories.

The effects of DOV 21947 on canine cardiovascular function were determined at WIL Research Laboratories. Five male and five female beagle dogs of approximately 7 months of age (~8-kg females and 10-kg males) were received from Ridglan Farms, Inc. (Mt. Horeb, WI). The animals were housed in individual stainless steel cages that were cleaned daily and maintained in accordance with the Guide for the Care and Use of Laboratory Animals in AAALAC-accredited facilities. Light timers were set to provide a 12-h light/dark cycle (lights on, 6:00 AM), with an average daily temperature of 20 ± 3°C and 50 ± 20% relative humidity. The dogs had ad libitum access to chew toys and water, and they received approximately 400 g of certified Canine LabDiet 5007 (PMI Nutrition International).

All dogs were implanted with a radiotransmitter system (Data Sciences International, St. Paul, MN), consisting of large animal transmitter (TL11M2-D70-PCT; arterial pressure and ECG waveforms) and a data exchange matrix that relays information from receivers to a computer. After the recovery period from surgical implantation and before dosing, the telemetry signals were monitored for patency and accuracy. Baseline recordings were obtained from all animals for 30-s intervals every 10 min for 1 h before dosing. After dosing, the heart rate (derived from arterial waveforms), blood pressure (systolic, diastolic, and calculated mean), body temperature, and ECG waveform intervals (PR, QRS, RR, QT and corrected QTcV; Van de Water et al., 1989) were acquired using the above-mentioned parameters for 24 h. Data from the first hour after dosing are presented in Tables 5 and 6. Arterial waveform, blood pressure, and body temperature data were recorded and analyzed using Dataquest ART Gold 2.2 and Physiostat ECG 3.2 (DSI, Overland Park, KS) software.

All experimental compounds and vehicle (10% Tween 80 and H2O) were made fresh daily, and they were administered orally to rodents by gastric intubation in a volume of 2 ml/kg (rats) or 0.2 ml (mice). Dogs received DOV 21947 orally in a number 12 gelatin capsule, with an empty capsule used as placebo. For daily dosing regimens, animals received vehicle in the morning and test compound in the evening approximately 6 h apart and 1 h before the dark phase (rats, 1–30 days; mice, 12 days). In b.i.d. administration, the rats received test material in the morning and evening, 6 h apart, with the evening dose 1 h before the start of the dark phase for 14 days. Before initiating the study, rats were adapted to gavage for 7 days with vehicle, whereas mice received daily gavages for 3 days before starting the study. DOV 21947 was synthesized by Cambridge Major Diagnostics, Indianapolis, IN). Rat motor activity was monitored in the home cages of 12 rats over a 24-h period using a video-based behavior analysis system (HomeCageScan; Clever Sys, Reston, VA). Data are presented as bouts (number of episodes of each behavior) and frames (duration of the behavior, at 30 frames/s).
Data were analyzed using either a two-tailed t test, a one-way ANOVA followed by Dunnett’s post-hoc comparison test, or a two-way ANOVA followed by a Bonferroni adjusted post-hoc comparison matrix, or Dunnett’s test, where appropriate.

Results

The plasma pharmacokinetics of DOV 21947 were determined following single doses administered via the oral and i.v. routes (Fig. 1; Table 1). Single doses of DOV 21947 (5 mg/kg i.v. and 10 mg/kg p.o.) provided good exposure (AUC_{0–48} = 3524 and 5117 ng·h/mL, respectively), with an elimination half-life of approximately 1.6 h after i.v. administration, and for slightly longer (2.6 h) after oral administration (Table 1). DOV 21947 showed high absolute bioavailability (77%), with a volume of distribution of 2.6 l/kg, indicating distribution throughout body tissues. There were no significant changes in the C_{max} and AUC values after repeated oral administration of DOV 21947 at 10 mg/kg/day, for as long as 26 weeks (data not shown), consistent with a lack of autoinduction. The primary metabolite of DOV 21947 is the lactam 5-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexan-2-one. This metabolite is a low-potency inhibitor of [3H]DA, [3H]NE, and [3H]βHT uptake by rat synaptosomes (IC_{50} = 3.7, 7.5, and 20 μM, respectively).

Single doses of DOV 21947 (6–40 mg/kg p.o.) dose-dependently reduced the body weight (BW) of DIO rats over an 18-h period (Fig. 2). Thus, vehicle-treated animals gained approximately 4 g of BW overnight (0.4 ± 0.2% of total BW), whereas 2-, 6-, 20-, and 40-mg/kg doses of DOV 21947 caused a 3-g gain and losses of 3, 10, and 15 g, respectively, over an 18-h period (0.5 ± 0.2, −0.4 ± 0.4, −1.6 ± 0.3, and −2.5 ± 0.2% of total BW, respectively). The cannabinoid receptor inverse agonist AM251 (3 mg/kg) and dexfenfluramine (1 mg/kg), which were used as positive controls, caused reductions in BW of 6 and 4 g, respectively. In parallel with the decreases in BW, DOV 21947 (6–40 mg/kg) significantly reduced cumulative food intake during the 18-h monitoring period after dosing (Fig. 3A). Although the anorectic effect of 2 and 6 mg/kg DOV 21947 was manifested only within the first 2 h after administration, the 20- and 40-mg/kg doses of DOV 21947 significantly suppressed food consumption for 8 to 12 h after administration, during the dark cycle (Fig. 3B). In contrast, the anorectic effect of AM251 (3 mg/kg) was confined to the first 2 h after administration, whereas the actions of dexfenfluramine (1 mg/kg) were observed for up to 8 h after treatment, into the dark cycle (Fig. 3B).

During this acute administration study, the effects of DOV 21947 on the kinetics of food consumption were analyzed (Fig. 4). DOV 21947 (20 mg/kg, 40 mg/kg, and 6 mg/kg b.i.d. p.o.), AM251, and dexfenfluramine significantly decreased the amount of high-fat diet consumed during a feeding episode compared with control-treated DIO rats (Fig. 4A). The percentage of suppression of food consumed by the DOV 21947-treated rats (49.9, 75.1, and 86.6% at 6 mg/kg b.i.d. and 20 and 40 mg/kg, respectively) was generally higher than observed in the AM251 (44.7%) and dexfenfluramine (63.8%) groups. In contrast, none of the drug treatments had a significant effect on the time to the first feeding bout (Fig. 4B), the interbout interval (Fig. 4C), the duration of the feeding bouts (Fig. 4D), or the number of feeding episodes (Fig. 4E). The exception to this observation was the DOV 21947 40-mg/kg group, which showed significant increases in both the time to the first feeding bout (928%; Fig. 4B) and the interbout interval (376%; Fig. 4C), with a reduced duration of feeding bouts (63%; Fig. 4D).

Given the acute effects of DOV 21947 on BW and food consumption in the DIO rat, the maintenance of its activity after a longer duration of administration was investigated. Daily oral administration of DOV 21947 (6 mg/kg) to DIO rats significantly reduced BW relative to vehicle-treated controls, beginning at 6 days of administration, an effect sustained through day 13 of administration (Fig. 5, A and B). The average decrease in BW over this period was 12 ± 0.6 g, or 2.9 ± 0.1%. The b.i.d. administration of 6 mg/kg DOV 21947 resulted in a similar magnitude of weight reduction (15 ± 0.9 g; 12 ± 0.1%). The onset of this effect occurred earlier than observed with daily dosing, because significant decreases in BW were observed by the third day of administration. However, daily administration of 20 mg/kg DOV 21947 resulted in a rapid onset of activity, with a significant reduction in BW after 2 days of administration. In addition, a higher average reduction in BW was observed over the 13-day period of significant weight loss (33 ± 3.2 g; 5.9 ± 0.5%), with a maximum sustained loss of BW of 7.7 ± 0.1% between days 10 and 14 (Fig. 5B). Comparable changes in the onset and extent of BW loss were observed after the daily administration of AM251 (20 ± 1.5 g; 3.9 ± 0.3%), dexfenfluramine (27 ± 1.8 g; 4.8 ± 0.3%), and sibutramine (25 ± 1.4 g; 4.5 ± 2.9%) over the 2 to 14-day period of administration.

DOV 21947 altered food intake over a 14-day administration period (Fig. 6). Although DOV 21947 (6 mg/kg/day) transiently decreased food intake on the second day of administration, DOV 21947 (6 mg/kg b.i.d. and 20 mg/kg/day) significantly reduced daily food intake on the first day of treatment, an effect that lasted to day 4 of treatment for the 6-mg/kg b.i.d. dose, and to days 11 and 13 with the 20 mg/kg/day dose regimen (Fig. 6A). The percentage of suppression of daily food intake induced by DOV 21947 (20 mg/kg/day) over days 1 to 11 averaged 47 ± 3.7% (Fig. 6B). Although the effects of DOV 21947 (6 mg/kg b.i.d. and 20 mg/kg/day) on indices of daily food consumption were of limited duration, eventually returning to levels not significantly different from the vehicle control groups, significant reductions in cumulative food intake (Fig. 6, C and D) and feeding efficiency (Fig. 6E) were maintained for the duration of the study. Of the reference agents tested, AM251 significantly decreased daily

![Fig. 1. Plasma concentrations after a single dose of DOV 21947. DOV 21947 (5 mg/kg i.v., 10 mg/kg p.o., □) was administered as a single dose to male rats.](https://example.com/fig1.png)
food intake on days 1 to 3, 7, and 8 of administration, whereas dexfenfluramine and sibutramine significantly decreased daily food intake on days 1 to 7, which significantly affected cumulative food intake starting from days 2 to 6 and lasting to the end of the study (Fig. 6, A and E). AM251 (3 mg/kg/day) was least effective in reducing both daily and cumulative feeding efficiency. Dexfenfluramine and sibutramine significantly decreased daily feeding efficiency throughout the study, causing cumulative feeding efficiency to fall between days 1 to 7. AM251 variably decreased daily feeding efficiency on days 7 to 10 and days 12 to 14 of the study, although cumulative feeding efficiency was significantly reduced over the course of the investigation (Fig. 6E).

At the end of the 14-day regimen, the effects of drug treatment on body mass relative to baseline body mass values (measured 8 days before dosing) were determined using DEXAscan (Fig. 7). Fourteen days of treatment with DOV 21947 (6 and 20 mg/kg/day and 6 mg/kg b.i.d.), AM251, dexfenfluramine, and sibutramine significantly reduced total body mass (Fig. 7A). None of the treatments significantly altered either the lean mass or bone mineral content (data not shown), although there was a trend toward reduced lean mass in the AM251-treated group. In contrast, fat mass was

![Fig. 2. Dose-response curve of the effect of acutely administered DOV 21947 on the body weight of DIO rats. Vehicle (●) or the antiobesity agents DOV 21947 (○), d-fenfluramine (◇), or AM251 (□) were administered orally to adult DIO rats 1 h before initiation of the dark cycle in the vivarium. Body weights were determined before administration of the test agents and 18 h after administration, and the differences were determined. DOV 21947 (2–40 mg/kg p.o.) produced a significant, dose-dependent reduction in body weight compared with predosing weights ($F_{6,369} = 9.16; P < 0.01$), as did dexfenfluramine ($t_{14} = 3.628$) and AM251 ($t_{14} = 4.535$). $n = 6$ to 7 rats/group. Baseline weight, determined immediately before dosing: 614 ± 8.5 g, $n = 48$. Final weight, vehicle-treated group: 593 ± 26 g, $n = 6$, **$P < 0.01$, significantly different from predose weight, paired t test. All values represent the mean ± S.E.M. of observations from six to seven rats/group.

![Fig. 3. Food intake is significantly reduced by acutely administered DOV 21947 in DIO rats. A, administration of DOV 21947 (6, 20, and 40 mg/kg), AM251 (3 mg/kg), or dexfenfluramine (d-Fen; 1 mg/kg) significantly reduced cumulative food intake as measured over 18 h after administration (A). $F_{6,41} = 22.8; P < 0.01$ (B). **$P < 0.01$, significantly different from predose weight, one-way ANOVA followed by Dunnett’s test. **$P < 0.01$, significantly different from contemporaneous vehicle control, two-way ANOVA followed by Bonferroni adjusted post-hoc test. All values represent the mean ± S.E.M. of observations from seven rats/group.

### Table 1

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>$C_{max}$ (ng/ml)</th>
<th>$T_{max}$ (h)</th>
<th>AUC$_{0-t}$ (ng · h/ml)</th>
<th>AUC$_{inf}$ (ng · h/ml)</th>
<th>$t_{1/2}$ (h)</th>
<th>$V_{d}$/kg</th>
<th>Absolute Bioavailability (%)</th>
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<td>i.v. (5 mg/kg)</td>
<td>3524</td>
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<td>3551</td>
<td>1.6</td>
<td>2.6</td>
<td>77</td>
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</tr>
<tr>
<td>p.o. (10 mg/kg)</td>
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<td>1.0</td>
<td>5117</td>
<td>5992</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
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</table>

AUC$_{inf}$ area under the time-concentration curve, from 0 to 24 h; AUC$_{inf}$ area under the time-concentration curve, from 0 to infinity.
cantly reduced the normalized weight of the retroperitoneal (124 ± 20 and 130 ± 12 g, respectively) and mesenteric (75 ± 15 and 92 ± 11 g, respectively), but not epididymal fat stores relative to vehicle-treated DIO rats (Fig. 7B).

The effects of antiobesity treatments on the blood chemistry of DIO rats were assessed at the termination of 14 days of treatment. None of the treatments caused significant changes in plasma glucose, total cholesterol, total protein, blood urea nitrogen, or creatinine (Table 2). However, plasma triglyceride levels were significantly decreased relative to the vehicle group by 47, 63, and 50% after a course of treatment with DOV 21947 (6 mg/kg b.i.d. and 20 mg/kg/day) and sibutramine, respectively. DOV 21947 (6 mg/kg b.i.d.) significantly decreased plasma creatine kinase levels by 64%; however, this effect of DOV 21947 was not dose-dependent (Table 3), and its significance is unclear. Otherwise, none of the antiobesity treatments tested significantly altered liver function tests (Table 3) or plasma electrolyte levels (data not shown).

The ability of DOV 21947 to induce a sustained decrease in the body weight of DIO rats was examined in a 24-day treatment protocol. DOV 21947 (20 and 40 mg/kg/day) significantly reduced the total BW of DIO rats between days 8 and 24 of administration by an average of 43 ± 1.2 and 64 ± 3.0 g, respectively (Fig. 8A). The decrease in BW seemed to stabilize between 18 and 24 days of administration of DOV 21947 (20 mg/kg/day) and at 20 to 24 days of administration of DOV 21947 (40 mg/kg/day). This amounted to a maximum loss of 8.8 ± 2.1 and 12 ± 4.2% of total body weight (Fig. 8B). Although DOV 21947 (20 and 40 mg/kg/day) significantly decreased food consumption between days 1 and 7 and 1 and 10 of administration, respectively, food consumption returned to levels that were not significantly different from control between 11 and 21 days of administration (Fig. 8C).
Nonetheless, DOV 21947 treatment had a significant affect on the cumulative food intake between days 15 and 21 of administration (Fig. 8D). After 21 days of administration, DOV 21947 (20 and 40 mg/kg/day) significantly and selectively reduced fat mass (Fig. 8E) by an average of 40 and 38%, respectively, without altering lean body mass (Fig. 8F).

By monitoring DIO rats in their home cages during the administration of DOV 21947 (20 mg/kg), its effects on motor activity could be determined (data not shown). On day 1 of administration, DOV 21947 had no significant effect on motor activity, with two exceptions. A significant, 70% increase in the number of episodes of fidgeting was observed, with fidgeting defined as a composite of stationary behaviors, such as twitching, stretching, and sniffing. DOV 21947 also increased the number of sniffing episodes by 86%. However, the duration of these, and other motor behaviors, was not significantly different from vehicle-treated animals. It is noteworthy that no significant changes in either the number or duration of sleep episodes were observed.

The antiobesity activity of these agents was tested in a second model of obesity, the DIO mouse (Fig. 9). DOV 21947 (6 mg/kg/day) initially decreased BW in these mice, but this effect waned after 4 days of administration (Fig. 9A). In contrast, DOV 21947 (20 mg/kg/day) significantly decreased BW after the first day of administration. An additional decrease was manifested by the second day of administration, which was sustained for the duration of the study. AM251, dexfenfluramine, and sibutramine had a similar effect. Treatment: F(7, 720) = 53.7, P < 0.01; time: F(14, 720) = 15.1, P < 0.01; and interaction: F(98, 720) = 11.3, P < 0.01. Baseline weight, determined immediately before dosing: 614 ± 64.4 g, n = 56. Final weight of vehicle-treated group: 614 ± 34 g, n = 7. a, body weight lost on day 2 of treatment with either DOV 21947 (20 mg/kg/day), AM251, dexfenfluramine, or sibutramine was significantly different from vehicle control, P < 0.05, two-way ANOVA, Bonferroni adjusted post-hoc test. **, significantly different from vehicle over the range of days indicated for DOV 21947 (6 mg/kg b.i.d. and 20 mg/kg/day), AM251, dexfenfluramine, or sibutramine treatment groups, two-way ANOVA, Bonferroni adjusted post-hoc test. B, DOV 21947 dose-dependently increased the percentage of total body weight lost relative to vehicle-treated DIO rats, and it was comparable with the weight loss induced by treatment with positive controls. All values represent the mean of observations from seven rats/group. Variance values not presented for visual clarity.

Safety pharmacology studies were performed investigating the propensity of DOV 21947 to significantly alter CNS and pulmonary function in normal rats, and cardiovascular function in normal beagle dogs with implanted transponders for telemetry measurements. In the CNS safety studies, DOV 21947 (10, 30, and 100 mg/kg) had no effect on home cage, handling, neuromuscular, or sensory performance (data not shown) of treated rats. An increased incidence (three of eight male and three of eight female rats) of hunched posture was observed after administration of 100 mg/kg DOV 21947. A decrease in the number of rearing episodes were noted in the open field after doses of 30 (67% reduction) and 100 mg/kg (56.5% reduction) DOV 21947. DOV 21947 had no effect on male rat body temperature measured 1 h after oral admin-
Fig. 6. Effects of DOV 21947 on food intake and feeding efficiency in DIO rats over 14 days of daily administration. The effects of DOV 21947 (6 mg/kg/day, □; 6 mg/kg b.i.d., ▲; and 20 mg/kg/day, ▼), AM251 (3 mg/kg/day, ○), dexfenfluramine (3 mg/kg/day, ▼) and sibutramine (2 mg/kg/day, △) on daily food intake (A), suppression of food intake (B), cumulative food intake (C), suppression of cumulative food intake (D), and cumulative feeding efficiency (E) were determined relative to vehicle-treated animals (■). A, DOV 21947 dose-dependently reduces the amount of food consumed on a daily basis. Treatment: $F_{(7,672)} = 72.9; P < 0.01$; time: $F_{(13,672)} = 32.64; P < 0.01$; and interaction: $F_{(91,672)} = 2.49; P < 0.01$.* significantly different from vehicle-treated rat values, DOV 21947 (6 mg/kg b.i.d., days 1–4). a, significantly different from vehicle-treated rat values, DOV 21947 (20 mg/kg/day, days 1–11), dexfenfluramine (days 1–7), and sibutramine (days 1–7). b, significantly different from vehicle-treated rat values, DOV 21947 (6 mg/kg b.i.d., days 1–4). B, DOV 21947 (20 mg/kg/day) suppresses daily food consumption by a greater percentage and a longer duration relative to vehicle than any other treatment. Treatment: $F_{(6,588)} = 60.9; P < 0.01$; time: $F_{(13,588)} = 26.3; P < 0.01$; and interaction: $F_{(78,588)} = 2.38; P < 0.01$.* significantly different from vehicle-treated rat values, DOV 21947 (6 mg/kg/day, day 2), AM251 (days 1–3, 7, and 8). a, significantly different from vehicle-treated rat values, DOV 21947 (6 mg/kg b.i.d., days 1, 2, and 4). b, significantly different from vehicle-treated rat values, AM251 (days 6–14). C, effect of DOV 21947 on cumulative food intake by DIO rats. Treatment: $F_{(7,672)} = 221; P < 0.01$; time: $F_{(13,672)} = 941; P < 0.01$; and interaction: $F_{(91,672)} = 3.17; P < 0.01$.* significantly different from vehicle-treated rat values, dexfenfluramine, sibutramine (days 2 and 3). a, significantly different from vehicle-treated rat values, DOV 21947 (6 mg/kg b.i.d. and 20 mg/kg/day), dexfenfluramine, and sibutramine (days 4–14). b, significantly different from vehicle-treated rat values, AM251 (days 6–14). D, cumulative suppression of food intake relative to vehicle-treated DIO...
nature of the changes in respiratory frequency and tidal volume yielded no net effect on the minute volume of respiration (Table 4). Administration of DOV 21947 at 1, 3, and 10 mg/kg p.o. to telemeterized dogs caused no physiologically meaningful or statistically significant changes in blood pressure, heart rate, body temperature (Table 5), or electrocardiographic indices (Table 6). Finally, DOV 21947 inhibited the human ether-a-go-go-related gene channel current, with an IC_{50} = 4.6 μM.

In addition to the acute safety studies, the effects of chronic administration DOV 21947 on the BW and food consumption of normal male rats and male dogs was investigated over a period of 26 and 52 weeks, respectively (Figs. 10 and 11). The rate of BW gain by male rats (starting weight ~250 g; Fig. 10A) was significantly decreased between 10 and 26 weeks of administration of 25 mg/kg/day p.o. DOV 21947, with the BW of treated rats averaging 9.8% lower than vehicle controls. Further insight into the effect of DOV 21947 on weight gain by normal rats was obtained by measuring the cumulative weight gain (the change in weight from day 0; Fig. 10B). DOV 21947 at 25 mg/kg/day p.o. significantly reduced the rate of cumulative weight gain of normal rats between weeks 10 and 26 of treatment by an average of 17% over this period. The decreases in the rate of increase in both absolute and cumulative BW normalized within 4 weeks after the administration of DOV 21947 was halted. DOV 21947 at 25 mg/kg/day transiently (week 0–1) decreased food consumption by individual rats by 8.3% (Fig. 10C). However, food intake quickly normalized, trending toward increased levels of consumption by DOV 21947-treated rats. Cumulative food consumption was sporadically increased relative to controls only in the DOV 21947 25 mg/kg/day p.o. group (Fig. 10D).

A similar decrease in the rate of BW gain was observed in normal male dogs after 1 year of treatment with DOV 21947 (Fig. 11). The absolute BW of dogs treated with DOV 21947 at 15 mg/kg/day p.o. trended toward being lower than their vehicle-treated controls (Fig. 11A). The effects of DOV 21947 at 15 mg/kg/day p.o. on normal dogs are best illustrated as the cumulative change in BW, where a significant decrease relative to control in the cumulative BW of DOV 21947-treated dogs, averaging 49.4%, was observed between 10 and 52 weeks of administration (Fig. 11B). The differential in both absolute and cumulative BW normalized within 4 weeks after the cessation of DOV 21947 administration. DOV 21947 (15 mg/kg/day p.o.) transiently decreased food consumption by dogs by 28.1% over weeks 0 to 4 of administration (Fig. 11C). After this initial time period, there was a trend toward increased food consumption in the DOV 21947-treated groups.

**Discussion**

DOV 21947 is a potent inhibitor of 5-HT, NE, and DA uptake in vitro, and it is orally active in preclinical models predictive of antidepressant activity (Skolnick et al., 2003).

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**Fig. 7.** Changes in body mass characteristics of DIO rats after 14 days of administration of DOV 21947. A, DOV 21947 (6 mg/kg b.i.d., downward hatch; 20 mg/kg/day, vertical stripe), AM251 (boxed), dexfenfluramine (cross-hatch), and sibutramine (closed box) significantly reduced total body mass [F(6,42) = 6.55; P < 0.01]. A selective loss of fat mass was observed after DOV 21947 (20 mg/kg/day) and sibutramine treatment [F(6,42) = 2.81; P < 0.01]. Final, total body weight of vehicle-treated group: 664 ± 67 g, n = 7. Total, lean, and fat mass determined by DEXAscan. B, measurement of the distribution of white adipose tissue indicated that DOV 21947 (20 mg/kg/day) and sibutramine selectively reduced the mass of retroperitoneal [F(6,42) = 2.32; P < 0.05] and mesenteric [F(6,42) = 2.92; P < 0.05], but not epididymal [F(6,42) = 0.91; not significantly different] fat deposits. Regional fat masses determined after manual dissection, and normalized to total body mass, ∗, P < 0.05 and ∗∗, P < 0.01, significantly different from vehicle-treated animal values, one-way ANOVA, Dunnett’s post-hoc test.

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**Table 4.** Administration of DOV 21947 to animals by dogs caused no physiologically meaningful or statistically significant changes in blood pressure, heart rate, body temperature, or electrocardiographic indices. DOV 21947 significantly decreased the cumulative weight gain by normal rats during weeks 10 to 26 of treatment. Cumulative food consumption by normal male dogs treated with DOV 21947 transiently decreased by 28.1% over weeks 0 to 4 of administration. After this initial time period, there was a trend toward increased food consumption in the DOV 21947-treated groups.
TABLE 2
Effects of antiobesity agents on blood glucose, lipids, and organic nitrogen levels in male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>Total Cholesterol (g/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total Protein (g/dl)</th>
<th>Blood Urea Nitrogen (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Blood Urea Nitrogen/Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>151 ± 7</td>
<td>110 ± 6</td>
<td>147 ± 24</td>
<td>7.3 ± 0.2</td>
<td>12.1 ± 0.6</td>
<td>0.3 ± 0.0</td>
<td>38.5 ± 2.0</td>
</tr>
<tr>
<td>DOV 21947 6 mg/kg</td>
<td>150 ± 4</td>
<td>99 ± 8</td>
<td>75 ± 14**</td>
<td>7.2 ± 0.1</td>
<td>11.1 ± 1.2</td>
<td>0.4 ± 0.0</td>
<td>28.7 ± 2.5</td>
</tr>
<tr>
<td>6 mg/kg b.i.d.</td>
<td>146 ± 2</td>
<td>110 ± 10</td>
<td>120 ± 23</td>
<td>7.4 ± 0.2</td>
<td>10.8 ± 0.3</td>
<td>0.3 ± 0.0</td>
<td>32.3 ± 2.4</td>
</tr>
<tr>
<td>AM251, 3 mg/kg</td>
<td>152 ± 4</td>
<td>85 ± 7</td>
<td>101 ± 10*</td>
<td>7.0 ± 0.2</td>
<td>11.0 ± 0.3</td>
<td>0.3 ± 0.0</td>
<td>39.4 ± 2.7</td>
</tr>
</tbody>
</table>

*P < 0.05 and **P < 0.01, significantly different from vehicle-treated animal levels by one-way ANOVA and Dunnett’s test [F(7,47) = 2.501; P = 0.029].

TABLE 3
Effects of antiobesity agents on liver function tests and plasma creatine kinase values in male rats

A significant decrease in creatine kinase levels was observed only after the administration of DOV 21947 (6 mg/kg b.i.d. p.o.). None of the other treatments caused significant changes in the measured parameters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alkaline Phosphatase (U/l)</th>
<th>Alanine Aminotransferase (g/l)</th>
<th>Aspartate Aminotransferase (g/l)</th>
<th>Lactate Dehydrogenase (g/l)</th>
<th>Albumen (mg/dl)</th>
<th>Globulin (mg/dl)</th>
<th>Albumen/Globulin</th>
<th>Total Bilirubin (mg/dl)</th>
<th>Creatine Kinase (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>105 ± 6</td>
<td>37 ± 4</td>
<td>116 ± 14</td>
<td>499 ± 115</td>
<td>4.4 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>1.7 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>364 ± 78</td>
</tr>
<tr>
<td>DOV 21947 6 mg/kg</td>
<td>111 ± 11</td>
<td>45 ± 5</td>
<td>115 ± 22</td>
<td>574 ± 191</td>
<td>4.6 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>249 ± 48</td>
</tr>
</tbody>
</table>

*P < 0.05, significantly different from vehicle levels, one-way ANOVA and Dunnett’s post hoc test [F(7,47) = 1.333; P = 0.26].

Although this behavioral activity indicates that DOV 21947 is orally bioavailable and crosses the blood-brain barrier, the current studies validate its high oral bioavailability in rats (77%), indicate that it is widely distributed throughout the body, and that it has a t½ value of approximately 90 min. Repeated dosing of DOV 21947 in male rats does not induce its metabolism, and the lactam metabolite of DOV 21947 is approximately 40, 300, and 1600 times less potent as an inhibitor of DA, NE, and 5-HT uptake, respectively, compared with the parent compound.

DOV 21947 dose-dependently decreased DIO rat BW within 18 h of administration. This effect was due, in part, to a significant decrease in food consumption over 18 h, spanning both dark and light cycles. The quantity of food consumed during feeding episodes decreased after DOV 21947 (6 mg/kg b.i.d. and 20 and 40 mg/kg), similar to doses active in behavioral despair models. These results suggest that DOV 21947 may reduce the satiety and reward set points, consistent with an enhancement of serotoninergic and dopaminergic neurotransmission. At the highest dose tested, 40 mg/kg, DOV 21947 also reduced the number and duration of feeding episodes. Although this may reflect an enhancement of the reinforcing attributes of food and a reduction in the drive to obtain food, it may also reflect the development of negative reinforcement, reductions in palatability, or gastrointestinal upset. Further studies are required to investigate these potential confounds.

The reduction in BW of DIO rats produced by DOV 21947 could be sustained by daily dosing for as long as 24 days. The decrease in body mass was specific for white adipose tissue (as determined using three different techniques in two models, after 14–21 days of treatment) residing in the retroperitoneal and mesenteric spaces. These fat depots represent the most difficult stores to deplete, and they contribute the most to sustaining a state of obesity, with its detrimental effects on cardiac function and insulin sensitivity (Gabriely et al., 2002; Gasteiger and Tremblay, 2002). In contrast, the positive controls, particularly AM251, decreased both lean and fat mass, a physiologically undesirable condition. Although no significant changes in plasma electrolytes, liver function tests or blood glucose levels were observed after 14 days of DOV 21947 administration, a significant decrease in blood triglycerides was noted. Food intake was also decreased over the first week of administration of DOV 21947, which probably contributed to the decrease in total BW after single doses of DOV 21947. Tolerance developed to the anorectic actions of DOV 21947, with the amount of food consumed returning to control levels by 11 days of administration of DOV 21947, as did the cumulative feeding efficiency. A similar profile of weight loss, transient decrease in food consumption, and selective decrease in fat mass was observed in the DIO mouse model after DOV 21947 administration. Although tolerance to the DOV 21947-induced decrease in food intake seems to be consistent in two animal models of obesity, the decrease in plasma triglycerides and BW was maintained, suggesting that DOV 21947 has a sustained effect on lipid metabolism, as opposed to merely reducing calorie consumption.

It is unlikely that the sustained decrease in BW results solely from a thermogenic effect of DOV 21947, because there was no detectable change in body temperature in either normal rats or dogs after DOV 21947 administration. Moreover, it is not likely that the actions of DOV 21947 are due to
Fig. 8. Effect of DOV 21947 on body weight, food intake, and body composition in DIO rats after 21 to 24 days of administration. A, both doses of DOV 21947 (20 mg/kg/day, ■; 40 mg/kg/day, ▲) significantly decreased the total body weight (grams) of DIO rats from days 10 to 24 (20 mg/kg/day) and 7 to 24 (40 mg/kg/day) of administration \(F(2,600) = 37.96; P < 0.01\; \text{and}\; F(2,600) = 20.95; P < 0.01\); and interaction: \(F(46,600) = 12.4; P < 0.01\). Baseline weight, determined immediately before dosing: 651 ± 5.7 g, \(n = 29\). Final weight of vehicle-treated group: 657 ± 12 g, \(n = 10\). B, daily treatments of DIO rats with DOV 21947 resulted in substantial percentage decreases in body weight relative to vehicle-treated rats. Significant decreases in daily food consumption (C) were observed after the first day of DOV 21947 administration \(F(3,804) = 130.8; P < 0.01\; \text{and}\; F(22,804) = 10.39; P < 0.01\); and interaction: \(F(66,804) = 3.03; P < 0.01\). However, daily food consumption levels normalized after 7 to 11 days of DOV 21947 administration. The DOV 21947 induced decrease in cumulative food intake (D) was manifested later in the administration period (15–21 days). Both doses of DOV 21947 (20 and 40 mg/kg/day) significantly decreased fat mass (E) after 21 days of administration \(F(2,52) = 6.354; P < 0.01\; \text{and}\; F(2,52) = 28.02; P < 0.01\); and interaction: \(F(2,52) = 6.034; P < 0.01\). However, 21 days of administration of DOV 21947 had no significant effect on lean mass (F). **, \(P < 0.01\), significantly different from vehicle levels for both DOV 21947 dosage groups, two-way ANOVA, Bonferroni adjusted post-hoc analysis.
increased locomotor activity in rats, because there is no evidence for significant increases in home cage activity, with the exception of the fidgeting and sniffing behaviors. Although fidgeting contributes to the maintenance of normal body weight (Levine et al., 1999, Heinrichs, 2003), it may also represent increased stereotypic activity or stimulation consistent with dopaminergic activation in the striatum. However, there is no alteration in grooming activity or orofacial stereotypies, nor are there decreases in the number of episodes or duration of sleeping behavior induced by DOV 21947. In previous investigations (Skolnick and Basile, 2007), there was no evidence of increased motor or stereotypic activity in the open field for up to 2 h after the administration of DOV 21947. Together, these results suggest that the antiobesity actions of DOV 21947 do not result from psychomotor stimulation.

**TABLE 4**

Pulmonary safety pharmacology of DOV 21947 in male rats

Values represent mean ± S.E.M. of n = 8 observations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Respiratory Frequency</th>
<th>Tidal Volume</th>
<th>Minute Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>0 to 120 min after Dose</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>breaths/min</td>
<td>ml</td>
<td>ml</td>
</tr>
<tr>
<td>Vehicle</td>
<td>119 ± 6.41</td>
<td>174 ± 5.90</td>
<td>1.84 ± 0.07</td>
</tr>
<tr>
<td>DOV 21947</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg p.o.</td>
<td>120 ± 6.26</td>
<td>192 ± 3.72**</td>
<td>1.76 ± 0.48</td>
</tr>
<tr>
<td>30 mg/kg p.o.</td>
<td>127 ± 7.03</td>
<td>198 ± 3.78**</td>
<td>1.85 ± 0.12</td>
</tr>
<tr>
<td>100 mg/kg p.o.</td>
<td>129 ± 3.91</td>
<td>225 ± 4.64**</td>
<td>1.65 ± 0.13</td>
</tr>
</tbody>
</table>

* P < 0.05 and ** P < 0.01, significantly different from baseline values by two-way ANOVA and Bonferroni adjusted post-hoc test.
### TABLE 5
Cardiovascular safety pharmacology of DOV 21947 in male dogs
Values represent mean ± S.E.M. of n = 6 to 8 observations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart Rate</th>
<th>Systolic Blood Pressure</th>
<th>Diastolic Blood Pressure</th>
<th>Mean Blood Pressure</th>
<th>Body Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 h after Administration</td>
<td>Baseline</td>
<td>1 h after Dose</td>
<td>Baseline</td>
</tr>
<tr>
<td>Vehicle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>115 ± 5.80</td>
<td>107 ± 5.18</td>
<td>146 ± 5.39</td>
<td>141 ± 5.26</td>
<td>91.1 ± 4.01</td>
<td>87.6 ± 3.31</td>
</tr>
<tr>
<td>DOV 21947</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg p.o.</td>
<td>122 ± 5.13</td>
<td>110 ± 5.59</td>
<td>142 ± 3.01</td>
<td>139 ± 2.27</td>
<td>90.7 ± 3.04</td>
</tr>
<tr>
<td>3 mg/kg p.o.</td>
<td>112 ± 6.74</td>
<td>106 ± 7.51</td>
<td>144 ± 3.77</td>
<td>142 ± 4.20</td>
<td>89.6 ± 4.1</td>
</tr>
<tr>
<td>10 mg/kg p.o.</td>
<td>114 ± 3.97</td>
<td>111 ± 4.40</td>
<td>142 ± 4.41</td>
<td>150 ± 7.04</td>
<td>90.3 ± 2.82</td>
</tr>
</tbody>
</table>

### TABLE 6
Effect of DOV 21947 on the canine electrocardiogram
Values represent mean ± S.E.M. of n = 8 observations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PR Interval</th>
<th>QRS Interval</th>
<th>RR Interval</th>
<th>QT Interval</th>
<th>QTcV Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 h after Administration</td>
<td>Baseline</td>
<td>1 h after Administration</td>
<td>Baseline</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.113 ± 0.003</td>
<td>0.115 ± 0.003</td>
<td>0.031 ± 0.001</td>
<td>0.031 ± 0.001</td>
<td>0.557 ± 0.023</td>
</tr>
<tr>
<td>DOV 21947</td>
<td>0.119 ± 0.003</td>
<td>0.115 ± 0.003</td>
<td>0.032 ± 0.001</td>
<td>0.031 ± 0.001</td>
<td>0.497 ± 0.021</td>
</tr>
<tr>
<td>1 mg/kg p.o.</td>
<td>0.117 ± 0.003</td>
<td>0.116 ± 0.004</td>
<td>0.032 ± 0.001</td>
<td>0.031 ± 0.001</td>
<td>0.554 ± 0.033</td>
</tr>
<tr>
<td>3 mg/kg p.o.</td>
<td>0.116 ± 0.003</td>
<td>0.117 ± 0.003</td>
<td>0.032 ± 0.001</td>
<td>0.031 ± 0.001</td>
<td>0.529 ± 0.024</td>
</tr>
<tr>
<td>10 mg/kg p.o.</td>
<td>0.116 ± 0.003</td>
<td>0.117 ± 0.003</td>
<td>0.032 ± 0.001</td>
<td>0.031 ± 0.001</td>
<td>0.529 ± 0.024</td>
</tr>
</tbody>
</table>
Although DOV 21947 may activate the sympathetic nervous system to expend calories, this does not result in deleterious changes in cardiovascular function. DOV 21947, at antiobesity doses, did not affect blood pressure, heart rate, or electrocardiographic parameters of normal dogs at $T_{\text{max}}$ and over the subsequent 24 h. The affinity of DOV 21947 for the $\alpha_1$ adrenergic receptor ($K_i = 730\ \text{nM}$ against $[^3\text{H}]$prazosin), and its antagonist potency ($IC_{50} = 1\ \mu\text{M}$; phenylephrine-induced rabbit aorta contractions) may contribute to its lack of cardiovascular activity. Additional interactions of DOV 21947 with adrenergic receptor subtypes that may suppress cardiovascular activation are under investigation.

The unique antiobesity actions of DOV 21947 may result from a number of its characteristics. Thus, NE, 5-HT, and DA have established roles in regulating metabolism, appetitive behaviors, and food-related reward and satiety, and they provide multiple targets for intervention by a potential antiobesity agent. A triple uptake inhibitor can simultaneously exploit these targets. Not only would activation of any of these pathways serve as an effective mechanism for an antiobesity agent but also when combined could synergistically reduce weight loss (Billes and Cowley, 2007). Unlike the NET/DAT inhibitors bupropion and mazindol, the addition of the SERT inhibitory component may result in regionally
limited elevations in synaptic DA levels (Navailles et al., 2006; Alex and Pehek, 2007). This would enhance satiety signals without requiring full DAT blockade, limiting the extent of CNS activation. In addition to its transporter inhibition profile, the interactions of DOV 21947 with secondary targets may contribute not only to the management of its side effect profile but also to its antiobesity efficacy. Activation of hypothalamic pathways subserved by 5-HT2c receptors suppresses feeding behaviors, and it has an antiobesity effect (Nilsson, 2006). DOV 21947 is a partial agonist at the 5-HT2c receptor \( K_i = 47 \text{nM} \) against \([\text{H}]\text{mesulergine}, EC_{50} \) value of \([\text{H}]\text{guanosine 5'-O-(3-thio)triphosphate} \) binding \( = 190 \text{nM} \), and \( E_{\text{max}} = 52\% \), which may further enhance its antiobesity actions. Thus, an antiobesity agent that enhances the function of NE, DA and 5-HT pathways through multiple mechanisms may not be influenced by neuroadaptive processes at the cellular and molecular levels in those circuits that regulate feeding behaviors (Horvath, 2005), resulting in sustained efficacy.

There are numerous examples of antiobesity agents that are either ineffective after chronic administration or that have serious side effects. Monoamine uptake inhibitors/releasers robustly activate both the sympathetic nervous system and CNS, but possess a significant abuse liability (Nelson and Gehlert, 2006). As a result, sibutramine was found to be an effective antiobesity agent, but its active metabolites complicate dosing, and its ability to cause small but significant elevations in blood pressure limits its application to subpopulations of obese individuals that can tolerate these changes (Arterburn et al., 2007). More recently, the cannabinoid receptor inverse agonist rimonabant, although an effective antiobesity agent (Patel and Pathak, 2007), causes significant adverse psychiatric effects (Christensen et al., 2007). Although the complete pharmacologic and toxicologic profile of DOV 21947 remains to be elucidated, structurally related triple uptake inhibitors (DOV 216303 and bicifadine) have been safely administered in the clinic for as long as 1 year. Consistent with the preclinical findings presented here, DOV 21947 produced a significant reduction in both body weight and plasma triglyceride levels in an 8-week safety and tolerability study using healthy volunteers (body mass index at entry ranged from 25 to 35) (www.dovpharm.com). DOV 21947 was safe and well tolerated in this study, and plasma levels were within the range eliciting weight loss in DIO rats and mice (our unpublished observations). Moreover, the data from preclinical models predictive of antidepressant activity suggest that DOV 21947, instead of causing adverse psychiatric effects (Christensen et al., 2007), may be effective in treating depression with comorbid obesity (Ericsson et al., 1996), uncomplicated obesity, and other eating disorders.

A

<figure>

**Fig. 11.** Effects of chronic administration of DOV 21947 on the body weight and food consumption of normal dogs. DOV 21947 (2, 6, or 15 mg/kg) was orally administered on a daily basis to male beagle dogs for a total of 52 weeks. After 52 weeks, drug administration ceased and the dogs were allowed to recover for 4 weeks (vertical dotted line). Absolute body weight (A) of DOV 21947 (15 mg/kg/day p.o.)-treated dogs trended toward being lower than vehicle-treated controls \( [\text{treatment: } F_{(3,972)} = 90.54; P < 0.01; \text{time: } F_{(54,972)} = 13.9; P < 0.01] \). However, DOV 21947 (15 mg/kg/day p.o.)-treated dogs showed significantly lower cumulative body weight indices between 10 and 52 weeks of administration relative to controls \( [\text{treatment: } F_{(3,954)} = 179.6; P < 0.01; \text{time: } F_{(49,954)} = 22.0; P < 0.01] \). The differential in both absolute and cumulative body weight were normalized within 4 weeks after cessation of DOV 21947 administration. DOV 21947 (15 mg/kg/day p.o.) only transiently decreased food consumption \( [\text{treatment: } F_{(3,954)} = 42.24; P < 0.01; \text{time: } F_{(54,954)} = 3.957; P < 0.01] \), \( * P < 0.05 \) and \( ** P < 0.01 \), significantly different from contemporaneous control group, two-way ANOVA with Bonferroni’s test.

B

C
References


Address correspondence to: Dr. Anthony S. Basile, DOV Pharmaceutical, Inc., 150 Fierce St., Somerset, NJ 08873-4185. E-mail: abasile@dovpharm.com