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WAY-163909 ((7b*R*,10a*R*)-1,2,3,4,8,9,10,10a-octahydro-7b*H*-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole); A Novel 5-HT_{2C} Receptor Selective Agonist with Anorectic Activity

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5-HT, 5-Hydroxytryptamine (serotonin);

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Abstract

The pharmacological profile of WAY-163909 ((7b*R*,10a*R*)-1,2,3,4,8,9,10,10a-octahydro-7b*H*-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole), a novel 5-HT_{2C} receptor selective agonist is presented. WAY-163909 displaced [^{125}I]-DOI binding from human 5-HT_{2C} receptor sites, in CHO cell membranes, with a Ki value of 10.5 ± 1.1 nM. Binding affinities determined for the human 5-HT_{2A} and 5-HT_{2B} receptor subtypes were 212 and 485 nM, respectively. In functional studies, WAY-163909 stimulated the mobilization of intracellular calcium in CHO cells stably expressing the human 5-HT_{2C} receptor with an EC_{50} value of 8 nM, and Emax relative to 5-HT of 90%. WAY-163909 failed to stimulate calcium mobilization in cells expressing the human 5-HT_{2A} receptor subtype (EC_{50} >> 10 μM) and was a 5-HT_{2B} receptor partial agonist (EC_{50} 185 nM, Emax 40%). WAY-163909 exhibited negligible affinity (<50% inhibition at 1 μM) for other receptor sites examined including human 5-HT_{1A}, D2, D3 receptors, and the 5-HT transporter binding site in rat cortical membranes. WAY-163909 exhibited weak affinity for the human D4 (245 nM) and 5-HT_7 (343 nM) receptor subtypes and the α1 binding site in rat cortical membranes (665 nM). WAY-163909 produced a dose-dependent reduction in food intake in normal Sprague-Dawley rats (ED_{50} = 2.93 mg/kg), an effect blocked by a 5-HT_{2C} receptor antagonist, but not by a 5-HT_{2A} or 5-HT_{2B} receptor antagonist. Additionally, WAY-163909 decreased food intake in obese Zucker rats and diet-induced obese mice with ED_{50}s of 1.4 and 5.19 mg/kg i.p., respectively, consistent with the potential utility of 5-HT_{2C} receptor agonists as anti-obesity agents.

Introduction

At least 14 distinct 5-HT receptor subtypes have been cloned and their classification has been based on both sequence similarity and common signal transduction pathways (see review by Barnes and Sharp, 1999). The 5-HT_2 receptor sub-family currently accommodates three subtypes designated 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} and these receptors belong to the large family of seven transmembrane domain G-protein coupled receptors. They display high sequence homology with each other and common signal transduction (Baxter et al., 1995) principally via the activation of phospholipase C. Alterations or dysfunction of the 5-HT_{2C} receptor has been implicated in a wide variety of conditions including obesity, anxiety, depression, obsessive compulsive disorder, schizophrenia, migraine and erectile dysfunction (Fozard and Gray, 1989; Kennett and Curzon, 1991; Sanders-Bush and Breeding, 1991; Gibson et al., 1994; Berendsen et al., 1995; Bos et al., 1997; Millan et al., 1997) and as a consequence has received considerable attention as a target for drug discovery.

With specific reference to obesity, evidence from both transgenic mice with a targeted deletion of the 5-HT_{2C} receptor subtype (Tecott et al., 1995) and pharmacological studies employing 5-HT_{2C} receptor ligands (reviewed in Bickerdike, 2003) support a potential therapeutic utility of 5-HT_{2C} receptor agonists as anti-obesity agents. 5-HT_{2C} receptor knockout mice are obese, hyperphagic, have impaired satiety, have elevated insulin and leptin levels and have impaired glucose utilization (Tecott et al., 1995; Heisler et al., 1998; Nonogaki et al., 1998). Moreover, these mice are insensitive to the hypophagic effects of the non-selective 5-HT_2 receptor agonist mCPP (Tecott et al., 1995) . mCPP, the most widely studied agonist at 5-HT_{2C} receptors, decreases food intake in several

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species including humans (Samanin et al., 1979; Kennett et al., 1987; Walsh et al., 1994; Cowen et al., 1995; Sargent et al., 1997). Studies using 5-HT antagonists that differ in selectivity among the 5-HT receptor subtypes have provided evidence supporting a role for the 5-HT_{2C} receptor in the regulation of this mCPP response (Kennett and Curzon, 1988, 1991). More recently described 5-HT_{2C} agonists such as Ro 60-0175 ((S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine), WAY-161503 ((4aR)-8,9-dichloro-2,3,4,4a-tetrahydro-1H-pyrazino[1,2-a]quinoxalin-5(6H)-one), VER-3323 ((2S)-1-[6-bromo-2,3-dihydroindolyl)]-2-propylamine), PNU-22394 (6-methyl-1,2,3,4,5,6-hexahydroazepino[4,5-b]indole), YM348 ((S)-2-(7-ethyl-1Hfuro[2,3-g]indazol-1-yl)-1-methylethylamine) and WAY-629 (1,2,3,4,8,9,10,11-octahydro[1,4]diazepino[6,5,4-jk]cabazole) have been reported to reduce food intake and body weight in animal models (Martin et al., 1988; Rosenzweig-Lipson et al., 2000; Vickers et al., 2000; Welmaker et al., 2000; McCall et al., 2001; Bickerdike et al., 2002; Vickers et al., 2003; Hayashi et al., 2004; Kimura et al., 2004; Sabb et al., 2004). Conversely, pharmacological agents exhibiting 5-HT_{2C} antagonist activity such as neuroleptics increase food intake in rodents and cause weight gain in humans (Kennett and Curzon, 1988, Allison et al., 1999; Masand, 2000; Whitaker, 2000). Taken together, these results clearly support the therapeutic potential of 5-HT_{2C} receptor agonists as anti-obesity drugs.

In the course of our efforts to identify novel 5-HT_{2C} receptor agonists we discovered a new heterocyclic ring system initially exemplified by WAY-162545. Chiral resolution of this racemic compound yielded the eutomer WAY-163909 ((7b*R*,10a*R*)-1,2,3,4,8,9,10,10a-octahydro-7b*H*-cyclopenta-[b][1,4]diazepino[6,7,1hi]indole), a potent 5-HT_{2C} receptor selective agonist. In this report we describe the in vitro pharmacological

profile of WAY-163909. In addition, we demonstrate that WAY-163909 reduces food intake in a number of animal models consistent with a potential therapeutic utility of 5-HT_{2C} receptor agonists as anti-obesity agents.

Materials and Methods

$5HT_{2C}$ Receptor Radioligand Binding. Stable Chinese hamster ovary (CHO) cell lines expressing each of the human 5-HT_2 receptor subtypes were generated in-house using standard protocols. 5-HT_{2C} receptor binding affinities were determined using the displacement of agonist ([^{125}I]DOI; 2,5-dimethoxy-4-iodoamphetamine) or antagonist ([^{3}H]mesulergine) radioligand binding to human 5-HT_{2C} receptor sites in CHO cell membranes. Cell membrane suspensions were prepared in 50 mM Tris-HCl containing 0.1% ascorbic acid, 10 μM pargyline and 4 mM $CaCl_2$ and stored at a protein concentration of 1-2 mg/ml. Binding experiments were performed in 96-well microtiter plates in a total volume of 200 μl 50 mM Tris-HCl buffer containing 4 mM $CaCl_2$ (pH 7.4) in the presence of a concentration of radioligand equivalent to the dissociation constant; 0.4 and 0.8 nM for [^{125}I]DOI and [^{3}H]mesulergine, respectively, as determined by saturation binding analysis (data not shown). Reactions were initiated by the addition of 100 μl membrane suspension (50 μg protein final) and incubated for 60 ([^{125}I]DOI) or 120 ([^{3}H]mesulergine) min at room temperature followed by rapid filtration to terminate. Non-specific binding was determined in the presence of 1 μM DOI and 1 μM mianserin, respectively. Filter plates were dried and radioactivity determined after addition of 40 μl Microscint-20 using a Packard TopCount. Analysis of binding data was performed by non-linear regression using GraphPad Prism.

$5HT_{2A/B}$ Receptor Radioligand Binding. 5-HT_{2A} and 5-HT_{2B} receptor binding affinities were determined using the displacement of agonist ([^{125}I]DOI and [^{3}H]5-HT, respectively) radioligand binding to human 5-HT_{2A} or 5-HT_{2B} receptor sites in Chinese

hamster ovary (CHO) cell membranes. Binding experiments were performed in 96-well microtiter plates in a total volume of 200 µl. Incubation buffer for 5-HT_{2A} receptor binding studies comprised 50 mM Tris HCl buffer containing 4 mM $CaCl_2$ (pH 7.4) in the presence of 0.5 nM [^{125}I]DOI (approximate Kd concentration). 5-HT_{2B} receptor binding studies employed 50 mM Tris-HCl containing 4 mM $CaCl_2$, 0.1% ascorbate, 10 µM pargyline and 20 nM [^{3}H]5-HT (approximate Kd). Non-specific binding was determined in the presence of 1 µM DOI and 10 µM 5-HT for 5-HT_{2A} and 5-HT_{2B}, respectively, and assays were processed as described above for the 5-HT_{2C} protocol.

Functional Studies. Stable CHO cell lines expressing either the human 5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C} receptor subtype were used for functional studies employing the measurement of agonist-stimulated mobilization of intracellular calcium with the fluorometric imaging plate reader (FLIPR). Cells were maintained and passaged upon reaching approximately 80% confluence. Cells were plated 24 hours prior to the experiment in poly-D-lysine coated 96 well plates at a density of approximately 60,000 cells per well. In preparation of the assay, the confluent monolayer of cells was washed twice with Hank's Buffered Saline Solution (HBSS) supplemented with 20 mM Hepes and 2.5 mM probenecid (FLIPR buffer), then the cells were loaded by adding 4 µM Fluo-4 AM (Molecular Probes, Eugene, OR) in FLIPR buffer for 1 hour at 37°C. Following loading, the cells were then rinsed twice with FLIPR buffer and intracellular calcium increases following agonist application were detected by measuring increases in fluorescence with the FLIPR. For evaluation of antagonist activity compounds were included during the dye-loading step and subsequently stimulated by the addition of an EC_{80} concentration of 5-HT.

Concentration-response data was fit to a four-parameter logistic function for generation of EC_{50} and IC_{50} values using Kaleidagraph.

Ancillary Binding Studies. Selectivity binding assays for the human 5-HT_{1A}, 5-HT_7 and dopamine D2, D3 and D4 receptors were performed using similar protocols to those described above for 5-HT_2 receptor binding studies. In all cases membranes derived from clonal CHO cell lines were used as receptor source. Radioligands and displacers for non-specific binding were as follows; 5-HT_{1A}, [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), 10 µM 5-HT; 5-HT_7, [^{3}H]LSD (lysergic acid diethylamide), 10 µM methiothepin; D2 and D3, [^{3}H] spiperone, 1 µM d-butaclamol; D4, [^{3}H]spiperone, 10 µM clozapine. Affinity for the 5-HT transporter and the α1 receptor was evaluated by measuring the displacement of ligand ([^{3}H]paroxetine and [^{3}H]WB-4101, respectively) from these sites labeled in rat cortical membrane preparations.

Food Intake Studies in Rats. Male Sprague-Dawley rats weighing 290 – 370 g or obese Zucker rats weighing 670 – 985 g were individually housed in wire hanging cages. Each cage was equipped with a water spigot attached to an automated watering system which allow free access to water at all times. Animals were acclimated for a 2-week period to powdered chow (Purina rat chow) prior to experiments. Food cups containing powdered chow were removed from the rats' home cage for 24 hours prior to 2-hour test sessions. Rats were usually fasted on Monday and Thursday nights and rats were tested for effects of compounds on food intake on Tuesday and Friday. For the ip studies, vehicle and 3 doses of WAY-163909 (1-10 mg/kg, ip) in Sprague-Dawley rats (n=8) and 0.3-3 mg/kg,

ip in obese Zucker rats (n=8) were evaluated in a group of rats in pseudorandom order. For the po studies, vehicle and 3 doses of WAY-163909 (3-30 mg/kg, po) were administered in separate groups (n=6 per group) of rats. WAY-163909 was dissolved in 0.9% saline and was administered ip in a volume of 1 ml/kg or po in a volume of 2 ml/kg immediately before the food cups were replaced in the home cage. Dose calculations were based on active moiety. Data were analyzed using either a one-way analysis of variance (ANOVA) or a repeated measures ANOVA as appropriate. Post hoc tests comparing vehicle to doses of WAY-163909 were conducted using contrasts in a least squares model. Where appropriate, ED_{50} values (dose decreasing food intake to 50% of vehicle values) were calculated using nonlinear regression models.

Food Intake Antagonism Studies in Rats. 4 different dose combinations were evaluated. These included: 1) vehicle + vehicle; 2) vehicle + 10 mg/kg WAY-163909; 3) antagonist + vehicle; 4) antagonist + 10 mg/kg WAY-163909. Doses, pretreatment times and route of administration for antagonists were determined based on literature (SB-242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl 3-oxy)pyrid-5-yl carbamoyl]indoline) – Kennett et al., 1997, a selective 5-HT_{2C} receptor antagonist; SB-215505 (6-chloro-5-methyl-1-(5-quinolyl carbamoyl) indoline) - Kennett et al., 1988, a selective 5-HT_{2B} receptor antagonist) or other in-house studies (3 mg/kg ketanserin (a non-selective 5-HT_{2A} receptor antagonist) completely blocks the anorectic effects of 1 mg/kg of the 5-HT_{2A} receptor agonist DOI; data not shown). For the studies with SB 242084 (1 mg/kg, ip, 30’ pre) or ketanserin (3 mg/kg, ip, 30’ pre), vehicle and WAY-163909 (10 mg/kg, ip, 0’ pre) were evaluated in Sprague-Dawley rats (n=8) in pseudorandom order. For the

studies with SB 215505 (3 mg/kg, po, 60' pre), 4 different groups of rats (n=5 per group) were used. SB 242084 was dissolved in 10% Tween / 0.5% methylcellulose, SB 215505 was dissolved in 5% dextrose, and ketanserin was dissolved in sterile water. Dose calculations were based on active moiety. Data were analyzed using either a one-way ANOVA or a repeated measures ANOVA as appropriate. Post hoc tests comparing the groups were conducted using contrasts in a least squares model.

Food Intake Studies in Mice. Male C57Bl6 mice weighing (54 – 69 g) had been maintained on a high fat diet for a period of greater than 1 year and were considered to be obese. The mice were individually housed in wire hanging cages. Each cage was equipped with a water spigot attached to an automated watering system which allow free access to water at all times. Animals were acclimated for a 2-week period to powdered high fat chow prior to experiments. Food cups containing powdered chow were removed from the mouses' home cage for 24 hours prior to 2-hour test sessions. Mice were usually fasted on Monday and Thursday nights and were tested for effects of compounds on food intake on Tuesday and Friday. In all studies, vehicle and 3 doses of WAY-163909 (3-30 mg/kg, ip) were evaluated in a group of mice in pseudorandom order. WAY-163909 was dissolved in 0.9% saline and was administered ip in a volume of 10 ml/kg immediately before the food cups were replaced in the home cage. Dose calculations were based on active moiety. Data were analyzed using a repeated measures ANOVA. Post hoc tests comparing vehicle to doses of WAY-163909 were conducted using contrasts in a least squares model. Where appropriate, ED_{50} values (dose

decreasing food intake to 50% of vehicle values) were calculated using nonlinear regression models.

Chronic Study in Rats. Male Sprague-Dawley rats weighing 195-225 g at the start of the study were individually housed in stainless steel cages and were acclimated for 10 days. Animals were weighed and assigned to dosage groups in a manner that minimized mean body weight differences among dosage groups. Food and water were available ad libitum. WAY-163909 (3-30 mg/kg, po) was administered once daily and food intake and body weight were monitored for 10 days. 24 hr food intake was evaluated on day 10. Body weights were taken on days 4, 7, and 10. At the end of the study, blood samples were drawn and triglyceride levels were determined. Data were analyzed using either a one-way ANOVA or repeated measures ANOVA as appropriate. Post hoc tests comparing the groups were conducted using contrasts in a least squares model.

Results

Radioligand Binding Studies. [^{125}I]DOI and [^{3}H]mesulergine represent agonist and antagonist radioligands, respectively, suitable for 5-HT$_{2C}$ receptor binding studies in cells transfected with the 5-HT$_{2C}$ receptor in the absence of 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor sites also labeled by these agents. Figure 1 illustrates the structures of WAY-162545, the originally identified racemic compound and the enantiomer WAY-163909, generated via chiral HPLC methodology. Potent 5-HT$_{2C}$ receptor binding affinity was determined for WAY-162545 for the displacement of [^{125}I]DOI (Ki 6.6 nM), while the compound was >50-fold less potent is displacing [^{3}H]mesulergine binding (Table 1). Demonstration of potent binding affinity for WAY-162545 prompted the chiral separation toward the enantiomers to evaluate stereospecificity. 5-HT$_{2C}$ receptor binding affinities for the enantiomers are shown in Table 1 indicating that WAY-163909 represents the more active enantiomer, while WAY-163907 was found to be a relatively weak 5-HT$_{2C}$ receptor agonist (Ki in micromolar range). As observed for WAY-162545, WAY-163909 was 20-fold less potent in displacing [^{3}H]mesulergine binding compared with [^{125}I]DOI binding.

Primary selectivity determinations for the 3 compounds using 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor radioligand displacement studies are presented in Table 2. In each case the compounds exhibited weaker affinity for the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor binding sites; e.g., in the case of WAY-163909 the compound was found to display 20- and 46-fold higher affinity toward the 5-HT$_{2C}$ receptor compared to the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor, respectively. It was further observed that the stereospecificity observed in 5-HT$_{2C}$ receptor binding affinity was retained in both the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor binding

studies with WAY-163909 representing the more potent enantiomer (Table 2). Ancillary binding studies addressing selectivity of WAY-163909 toward a number of biogenic amine binding sites (Table 3) and a broad spectrum of receptor, transporter and ion channel targets (Supplemental Data, Table 1) failed to reveal potent binding affinity for any of the targets examined indicating the compound to be highly selective for the 5-HT_{2C} receptor subtype.

Functional Studies. Further pharmacological characterization of WAY-163909, WAY-162545 and WAY-163907 was performed using measurements of the stimulation of intracellular calcium mobilization in stable CHO cell lines expressing each of the human 5-HT_2 receptor subtypes. Figure 2 illustrates the concentration-response curves for WAY-162545 (A), WAY-163909 (B) and WAY-163907 (C) -stimulated calcium signaling. WAY-163909 exhibited potent (EC_{50}, 8 nM) and highly efficacious and best described as a full agonist (Emax, 90 ± 6%) 5-HT_{2C} receptor agonism, weak (EC_{50}, 185 nM) 5-HT_{2B} receptor partial agonism (Emax, 40%) and failed to activate 5-HT_{2A} receptors. A similar functional profile was observed for the racemate WAY-162545 albeit with slightly weaker potency for activation of 5-HT_{2C} receptors, while WAY-163907 was a relatively weak 5-HT_{2C} receptor agonist, consistent with the weaker binding affinity and stereospecificity (Table 4). Notably, WAY-163907 exhibited 5-HT_{2B} receptor partial agonist activity, yet failed to significantly displace agonist radioligand binding.

Lack of 5-HT_{2A} agonism exhibited by WAY-163909 prompted us to evaluate its ability to antagonize 5-HT-stimulated calcium mobilization in 5-HT_{2A} receptor-expressing cells. Results are presented in Figure 3 indicating that the compound failed to antagonize 5-HT-

stimulated calcium signaling while potent antagonism was observed with the selective 5-HT_{2A} receptor antagonist MDL100907 ((±)2,3-dimethoxyphenyl-1-[2-4-(piperidine)-methanol]) (IC_{50}, 0.15 nM). The inability to demonstrate a functional interaction of WAY-163909 with 5-HT_{2A} receptors in light of its displacement of [^{3}H]5-HT binding to these receptor sights is an obvious discrepancy unexplained at the present time.

Food Intake Studies. WAY-163909 (1-10 mg/kg, ip) produced dose-dependent decreases in 2-hour food intake in Sprague-Dawley rats ($F(3,28)= 29.04$, $p<0.0001$ (Figure 4). Food intake was significantly decreased by 52% ($p=0.0001$) and 96% ($p<0.0001$) at 3 and 10 mg/kg, ip, respectively. Similarly, oral administration of WAY-163909 (3-30 mg/kg, po) produced dose-dependent decreases in 2-hour food intake intake in Sprague-Dawley rats ($F(3,23)= 5.56$, $p=0.0061$ (Figure 4). Food intake was significantly decreased by 72% ($p<0.0001$) at 30 mg/kg, po. The ED_{50} values were 2.93 mg/kg (95% CI: 2.1-4.1 mg/kg) and 19.6 mg/kg (95% CI: 11.0-34.8 mg/kg) following ip and po administration, respectively.

In diet-induced obese mice, WAY-163909 (3-30 mg/kg, ip) produced dose-dependent decreases in 2-hour food intake $F(3,36)=12.78$, $p<0.0001$ (Figure 5, left). Food intake was significantly decreased by 46% ($p=0.0028$) and 59% ($p=0.0008$) and 84% ($p<0.0001$) at 3,10 and 30 mg/kg, ip, respectively. The ED_{50} value was 5.19 mg/kg (95% CI: 2.4-11.4 mg/kg).

In obese Zucker rats, WAY-163909 (0.3-3 mg/kg, ip) produced dose-dependent decreases in 2-hour food intake $F(3,28)=8.99$, $p=0.0002$ (Figure 5, right). Food intake was significantly decreased by 73% ($p<0.0001$) at 3 mg/kg, ip. The ED_{50} value was 1.4mg/kg (95% CI: 0.84-2.4 mg/kg).

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Vehicle + WAY-163909 (10 mg/kg, ip) decreased 2 hour food intake in the studies with the 5-HT_{2C} (SB 242084; 6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline), 5-HT_{2A} (ketanserin), and 5-HT_{2B} (SB 215505; 6-chloro-5-methyl-1-(5-quinolyl carbamoyl) indoline) antagonists ($p<0.001$ in all three studies; Figure 6 left, center, right). SB 242084 (3 mg/kg, ip; Figure 3 left), but not SB 215505 (3 mg/kg, po; Figure 3 center) or ketanserin (3 mg/kg, ip; Figure 6 right) blocked the effects of WAY-163909 (10 mg/kg, ip) on 2 hour food intake ($p<0.001$).

Chronic study. 24 hr food intake was evaluated at day 10 following once daily dosing with WAY-163909 (3-30 mg/kg, po). As illustrated in Figure 7 WAY-163909 produced a dose-dependent decrease in food intake $F(3,35)=7.37$, $p=0.0007$. Post hoc tests revealed that both 10 mg/kg (-12%; $p=0.022$) and 30 mg/kg (-21%; $p=0.0001$), significantly decreased food intake. WAY-163909 also produced decreases in body weight gain $F(3,32)=10.24$, $p<0.0001$ (Figure 8). Following vehicle administration, rats gained 62.6 g over the 10 day study whereas following 30 mg/kg WAY-163909, rats gained only 28.6 g (46% relative to controls). Post hoc tests revealed that 30 mg/kg of WAY-163909 decreased body weight gain relative to vehicle on days 4, 7, and 10. Triglyceride levels were also decreased by WAY-163909 ($p<0.01$; Table 5). Following vehicle administration, triglyceride levels were 263.11 ± 41.58 mg/dL. WAY-163909 at the 30 mg/kg dose produced a statistically significant ($p<0.01$) reduction in triglyceride levels by 40% to 157.56 ± 8.35 mg/dL.

Discussion

In the present study, we provide a pharmacological characterization of WAY-163909 as a novel and selective 5-HT_{2C} receptor agonist. The novel heterocyclic ring system represented in WAY-163909 and WAY-162545 evolved from structure-activity relationship studies with compounds identified in a focused screening effort using a pharmacophore model based on compounds with 5-HT_{2C} receptor agonist activity. WAY-163909 exhibited good binding selectivity compared with the closely related 5-HT_{2A} and 5-HT_{2B} receptor subtypes and excellent binding selectivity across a broad spectrum of targets including both related biogenic amine receptor subtypes and unrelated receptor, ion channel and enzyme targets. In functional studies using the agonist-stimulated mobilization of intracellular calcium WAY-163909 also exhibited excellent functional selectivity. This separation is critically important given the postulated side effect liabilities associated with 5-HT_{2A} and 5-HT_{2B} receptor activation. Specifically, activation of 5-HT_{2A} receptors has been linked to the hallucinogenic properties of LSD and under certain conditions 5-HT_{2B} receptor activation has been implicated in primary pulmonary hypertension and cardiac valvulopathy (Fitzgerald et al., 2000; Rothman et al., 2000, Launey et al., 2002). WAY-163909 was observed to be a relatively weak and low intrinsic activity partial 5-HT_{2B} receptor agonist and was devoid of functional activity at the 5-HT_{2A} receptor subtype. With respect to both 5-HT_{2C} binding and functional receptor selectivity WAY-163909 appears to be the most selective agent identified to date compared with other compounds reported in the literature including Org 37684 (Leyson and Kelder, 1998), Ro 60-0175 (Martin et al., 1998), WAY-161503 (Rosenzweig-Lipson et al., 2000; Welmaker et al., 2000), PNU-22394 (McCall et al., 2001), VER-3323

(Bickerdike et al., 2002), YM348 (Kimura et al., 2004) and WAY-629 (Sabb et al., 2004). Specifically although many of the newly reported agents have equivalent low nanomolar affinity for the 5-HT_{2C} receptor they have similar high affinity and most significantly equipotent functional activity at the 5-HT_{2B} receptor subtype. WAY-163909 has distinct advantages over reported 5-HT_{2C} agonist compounds with respect to both binding and functional selectivity and decreased intrinsic activity at the 5-HT_{2A} and 5-HT_{2B} receptors. These enhanced properties would suggest that WAY-163909 will have a low propensity for 5-$HT_{2A/B}$ receptor mediated side effects.

From the early pharmacological studies demonstrating anorectic effects of mCPP in multiple species (Samanin et al., 1979; Kennett and Curzon, 1988: Kennett and Curzon, 1991; Walsh et al., 1994; Cowen et al., 1995; Sargent et al., 1997), to more recent data with novel 5-HT_{2C} agonists which demonstrate effects on both food intake and body weight (Martin et al., 1998; Rosenzweig-Lipson et al., 2000; Welmaker et al., 2000; Vickers et al., 2000; Vickers et al., 2003; Hayashi et al., 2004; Sabb et al., 2004), the pharmacological data supporting a role for 5-HT_{2C} agonists in obesity is compelling. The pharmacological data is supported by studies in mice lacking the 5-HT_{2C} receptor. 5-HT_{2C} knockout mice are obese, hyperphagic, have impaired satiety, have elevated insulin and leptin levels and have impaired glucose utilization (Tecott et al., 1995; Heisler et al., 1998; Nonogaki et al., 1998). Moreover, these mice are insensitive to the hypophagic effects of the serotonergic agonist mCPP (Tecott et al., 1995). The present studies investigated the effects of WAY-163909 on food intake in fasted animals. WAY-163909 produced dose-dependent decreases in food intake in fasted male Sprague-Dawley rats following both intraperitoneal and oral administration with ED_{50} values of 2.93 and 19.6

mg/kg, respectively. These results confirm previous findings demonstrating anorectic effects of 5-HT_{2C} agonists and extend these findings to a class of compounds that is structurally distinct from all 5-HT_{2C} agonists. The pharmacological specificity of the effects of WAY-163909 was demonstrated by the selective antagonism of the anorectic effects of WAY-163909 by the 5-HT_{2C} receptor antagonist SB-242084. In contrast antagonists at the 5-HT_{2A} receptor (ketanserin) or 5-HT_{2B} receptor (SB-215505) were not able to block the effects of WAY-163909. In additional studies, the anorectic effects of WAY-163909 were also shown in two animal models of obesity (obese Zucker rats and diet-induced obese mice).

Several studies have investigated the effects of 5-HT_{2C} agonists following repeated administration in rats. mCPP, Ro 60-0175, WAY-161503 and YM348 decrease food intake and/or body weight gain in either lean or obese rats (Vickers et al., 2000; Rosenzweig-Lipson et al., 2000; Hayashi et al., 2004). Following chronic administration, WAY-163909 produced decreases in both food intake and body weight gain and we observed a 40% reduction in triglyceride levels at the end of the study. Similar decreases in food consumption were observed during days 1-5 (-23%) and days 6-10 (-21%), suggesting that there was no tolerance to the anorectic effects of WAY-163909. Over a 10 day period, body weight gain was reduced by 56%. By comparison, in the chronic studies conducted with Ro 60-0175 (Vickers et al., 2000) and YM-348 (Hayashi et al., 2004) the anorectic effect of both these agents was lost by day 10 of the study, in contrast to our observations with WAY-163909, and the reductions in body weight gain were more modest in the region of 20-25% compared to 56% for WAY-163909.

In conclusion, WAY-163909 is a novel 5-HT_{2C} receptor selective agonist. The anorectic effects of WAY-163909 in multiple animal models coupled with the decreased body weight and the decreased triglyceride levels provide compelling rationale for the potential utility of this compound in the treatment of obesity.

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Footnotes

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Legends for figures

Figure 1. Chemical structures of WAY-162545 (racemic), and WAY-163909 ((7b*R*,10a*R*)-1,2,3,4,8,9,10,10a-octahydro-7b*H*cyclopenta[b][1,4]diazepino [6,7,1hi]indole).

Figure 2. Effects of WAY-162545 (A), WAY-163909 (B) and WAY-163907 (C) on calcium signaling in stable CHO cells expressing human 5-HT_2 receptor subtypes. Agonist-stimulated calcium signaling was measured in cells loaded with the fluorescent calcium indicator dye Fluo-4. Data are expressed as percent of 10 μM 5-HT maximum response and represent the mean ± SEM from 3 independent experiments.

Figure 3. Effects of WAY-163909 and MDL100907 pre-treatment on 5-HT stimulated calcium signaling in a stable CHO cell line expressing the 5-HT_{2A} receptor subtype. 5-HT (100 nM) stimulated calcium was measured following a 60 min preincubation with WAY-163909 or MDL100907. Data are expressed as percent of 100 nM 5-HT response measured in cells not pre-treated with ligand and represent the mean ± SEM from 3 independent experiments.

Figure 4. Effect of WAY-163909 on 2 hr food intake in 24 hr fasted normal Sprague-Dawley rats. WAY-163909 was evaluated using i.p. or p.o. routes of administration immediately prior to placement of food cups in the home cage. Data are expressed as g

food consumed in 2 hr period and represent mean ± SEM (n = 8 and 6, respectively, for i.p. and p.o. routes).

Figure 5. Effect of WAY-163909 on 2 hr food intake in 24 hr fasted diet-induced obese mice and obese Zucker rats. WAY-163909 was evaluated using i.p. route of administration immediately prior to placement of food cups in the home cage. Data are expressed as mg (mice) or g (rats) food consumed in 2 hr period and represent mean ± SEM (n = 8).

Figure 6. Effect of 5-HT_2 receptor antagonists on the WAY-163909 mediated reduction in 2 hr food intake in 24 hr fasted normal Sprague-Dawley rats. Antagonists were administered i.p. 30 min (SB242084 and ketanserin) or 60 min (SB215505) prior to treatment with 10 mg/kg i.p. WAY-163909 was administered coincident with the introduction of food cups for 2 hr. Data are expressed as g food consumed in 2 hr period and represent mean ± SEM (n = 8).

Figure 7. Effect of WAY-163909 on 24 hr food intake on day 10 of administration of WAY-163909 in normal Sprague-Dawley rats. WAY-163909 was evaluated following 10 days of p.o. administration. Data are expressed as g food consumed in 24 hr period and represent mean ± SEM (n = 9 per group).

Figure 8. Effect of daily WAY-163909 administration on body weight in normal Sprague-Dawley rats. WAY-163909 was administered once daily and body weight was

evaluated on days 4, 7 and 10 of p.o. administration. Data are expressed as g body weight and represent mean ± SEM (n = 9 per group).

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Table 1.

5-HT_{2C} receptor binding affinities determined using agonist and antagonist radioligand displacement. The numbers in parentheses represent the number of independent experiments (n) used to determine the mean ± SEM.

	Ki, nM	
	[^{125}I]DOI	[^{3}H]mesulergine
WAY-162545	6.6 ± 0.5 (3)	385 ± 112 (3)
WAY-163909	10.5 ± 1.1 (8)	221 ± 29 (8)
WAY-163907	2021 ± 332 (5)	10351 ± 2318 (5)

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Table 2.

5-HT_{2A} and 5-HT_{2B} receptor binding affinities determined using agonist radioligand displacement. The numbers in parentheses represent the number of independent experiments (n) used to determine the mean ± SEM.

	Ki, nM	
	5-HT_{2A}	5-HT_{2B}
WAY-162545	136 ± 18 (3)	2101 ± 803 (3)
WAY-163909	212 ± 29 (7)	485 ± 49 (3)
WAY-163907	4766 ± 1406 (3)	> 10,000 (3)

Table 3.

Ancillary receptor binding affinities determined for WAY-163909. Data are average values obtained in two independent experiments using human receptor subtypes (except *rat cortical membranes) expressed in stable CHO cell lines.

Receptor	Ki, nM
5-HT_{1A}	> 1000
5-HT_7	343
D2	> 1000
D3	> 1000
D4	245
5-HT-T*	> 1000
α-1*	665

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Table 4.

Curve fit parameters for agonist-stimulated calcium signaling in stable CHO cell lines expressing human 5-HT_2 receptor subtypes. Data are mean ± SEM from 3 independent experiments. NE, no effect.

	5-HT_{2C}		5-HT_{2A}		5-HT_{2B}	
	EC_{50}, nM	Emax, %	EC_{50}, nM	Emax, %	EC_{50}, nM	Emax, %
WAY-162545	39±16	85±3	NE	NE	563±166	40±3
WAY-163909	8±3	90±6	NE	NE	185±105	40±3
WAY-163907	1651±375	60±6	NE	NE	47±21	20±6

Table 5.

Triglyceride levels following 10 day administration of WAY-163909 in male Sprague-Dawley rats. Data are mean ± SEM.

	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Mean	263.11	257.33	244.11	157.56
SEM	41.58	20.34	24.44	8.35

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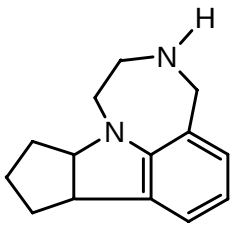
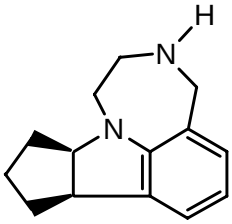


WAY-162545



WAY-163909

Figure 1

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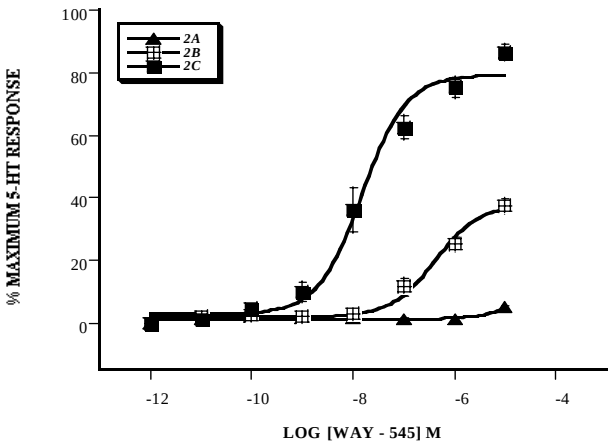
A.



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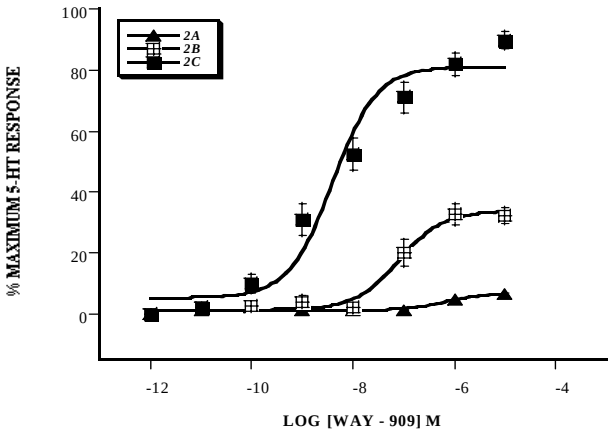

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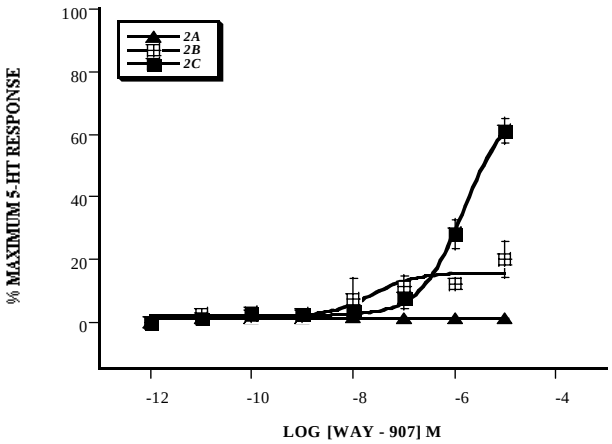

Figure 2

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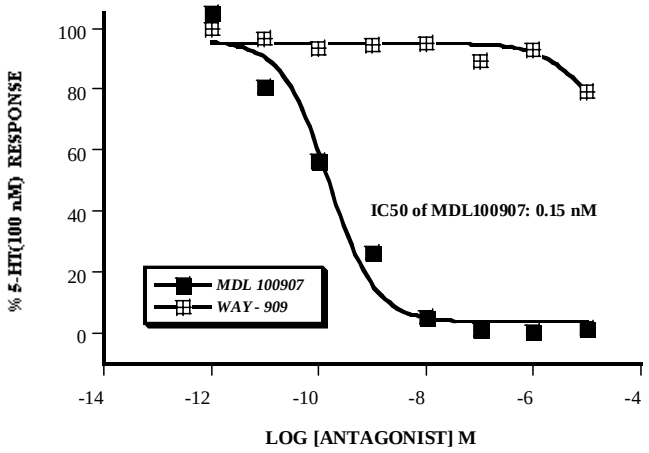

Figure 3

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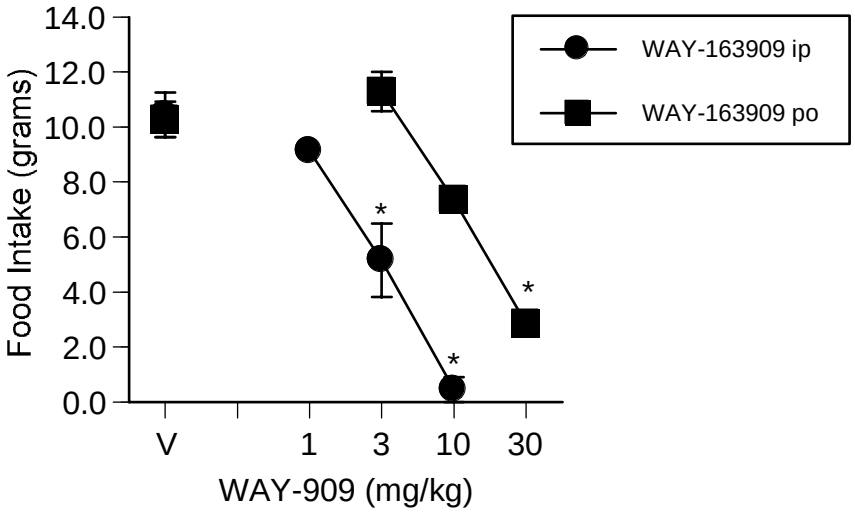

Figure 4

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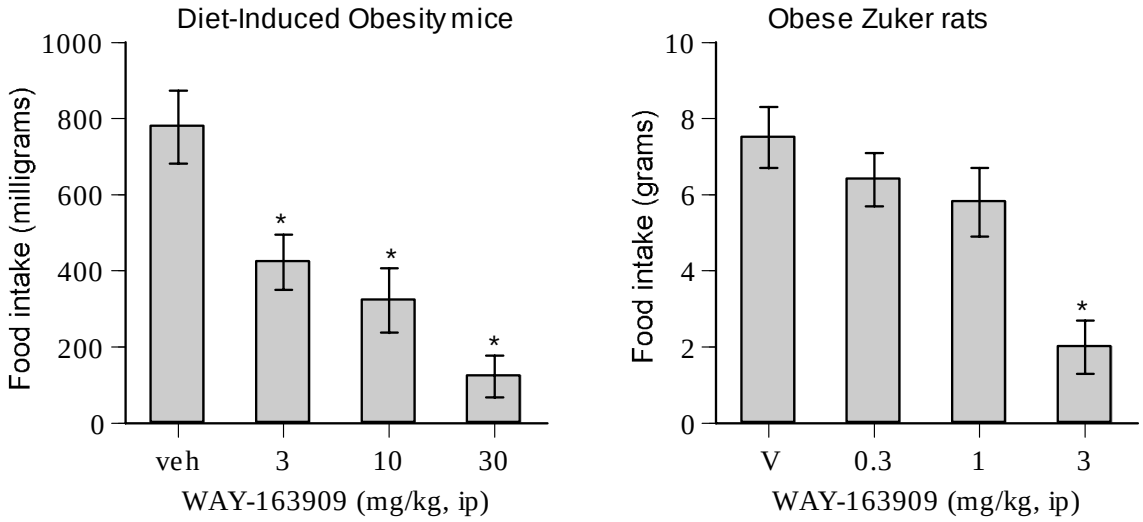

Figure 5

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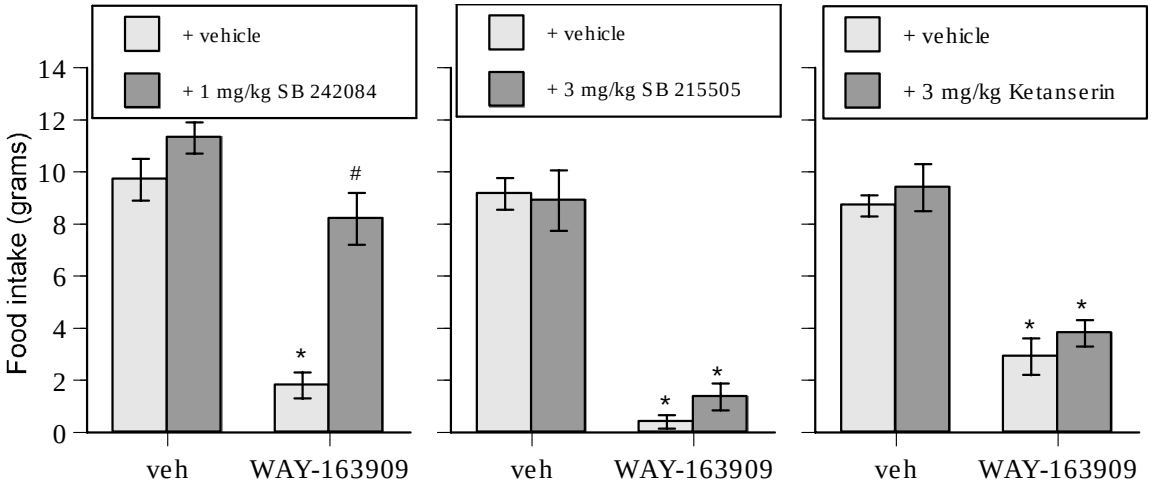

Figure 6

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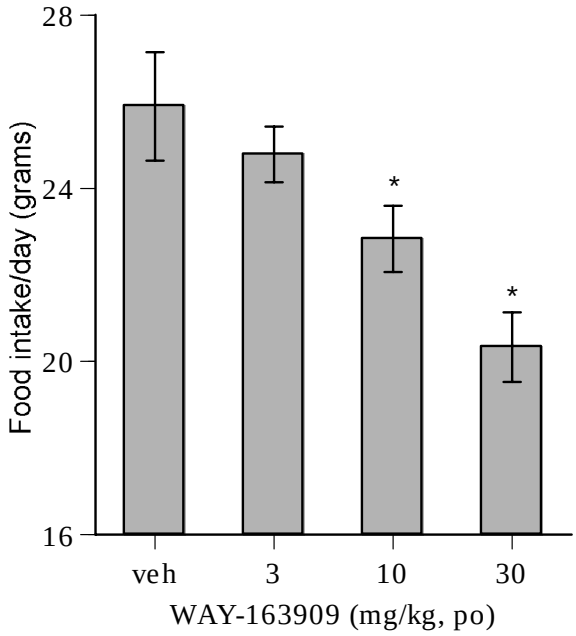

Figure 7

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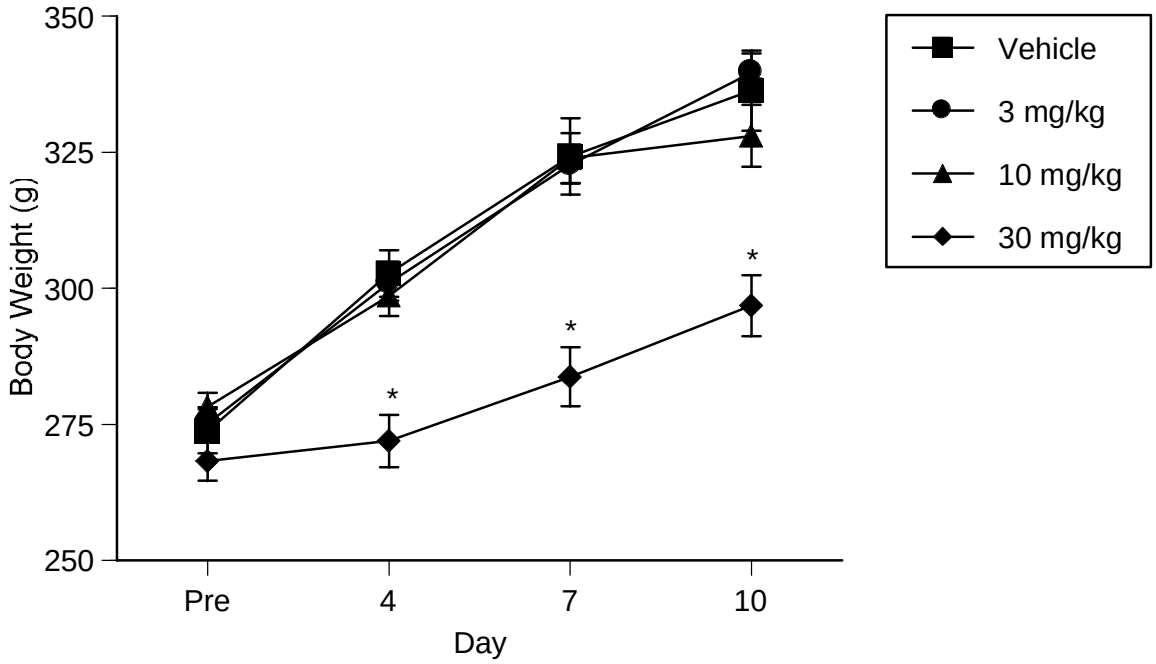

Figure 8