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Aminopyrazine CB1 receptor inverse agonists

David J. Wustrow,* George D. Maynard, Jun Yuan, He Zhao, Jianmin Mao, Qin Guo, Mark Kershaw, Jack Hammer, Robbin M. Brodbeck, Kristen E. Near, Dan Zhou, David S. Beers, Bertrand L. Chenard, James E. Krause and Alan J. Hutchison

Neurogen Corporation, 35 Northeast Industrial Road, Branford CT 06405, USA

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Abstract—A series of 5,6-diaryl-2-amino-pyrazines were prepared and found to have antagonist-like properties at the CB1 receptor. Subsequent SAR studies optimized both receptor potency and drug-like properties including solubility and Cytochrome-P450 inhibition potential. Optimized compounds were demonstrated to be inverse agonists and compared in vivo with rimonabant for their ability to inhibit food intake, to occupy central CB1 receptors and to influence hormonal markers associated with obesity.

The endocannabinoid system plays an important role in many physiological processes.[1] Key components of this system are CB1 receptors which are highly expressed in the central nervous system (CNS) and under certain conditions in peripheral tissues. Inverse agonists at CB1 receptors have been shown either clinically or preclinically, to decrease food intake,[2] influence energy utilization via glucose metabolism,[3] inhibit nicotine craving,[4] or other drug seeking behavior,[5] or block the fibrosis,[6] cardiomyopathy,[7] and hypotension[8] associated with liver cirrhosis. Most of these findings were enabled by the availability of the selective CB1 inverse agonist rimonabant (SR 141716A, **1**)[9] currently marketed as an antiobesity agent in Europe or its close derivative AM251 (**2**) (see Fig. 1).[10] Subsequently several medicinal chemistry strategies have been utilized to discover additional CB1 antagonists/inverse agonists with improved chemical, physical, and pharmacokinetic properties.[11]

One strategy that has proven to be successful for the discovery of novel CB1 inverse agonists is the introduction of alternatives to the pyrazole core found in rimonabant. Such a strategy was employed in the discovery of SLV319 (**3**)[12] and taranabant (**4**)[13] which are both currently undergoing clinical trials. Recently a scaffold hopping strategy that led to the discovery of the pyrazine analogue **5** was disclosed.[14] An analysis of structures **3** and **4** suggested that in addition to flexibility with regard to the core positioning of the two aryl moieties, there is also some flexibility around the position and nature of the hydrazide functionality found in rimonabant (**1**). As part of a program to prepare pyrazine CB1 antagonists with improved properties we undertook studies to determine if the amide functionality adjacent to a pyrazine core was a requirement for activity or if it could be either transposed to another position or replaced entirely. Herein, we describe these studies.

The syntheses used for the preparation of target compounds are outlined in Schemes 1–3. Reacting 2,6-dichloropyrazine (**6**) with amines **7a** or **7b** followed by regiospecific NBS bromination gave pyrazines **8a**–**b**. Aryl or heteroaryl functionality could be readily introduced by sequential Suzuki reactions in a regioselective manner to give the diaryl-pyrazines **9a**–**j** listed in Table 1. In a similar way dichloropyrazine **6** could be reacted with thiomorpholine **10**. The product was brominated with NBS and the thio group oxidized with mCPBA to give **11**. Compound **11** was subjected to sequential Suzuki conditions to give compound **12** (Scheme 2).

Alternatively, Suzuki coupling could be carried out first as shown in Scheme 3. 2-Chloro-6-aminopyrazine (**13**) was reacted with pyridyl-boronic acid **14**[15] and the resulting 6-(pyrid-4-yl)pyrazine was brominated giving compound **15**. This was smoothly reacted with

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* Corresponding author. Tel.: +1 203 315 3031; fax: +1 203 481 5290; e-mail addresses: dwustrow@nrgn.com; d.wustrow@comcast.net

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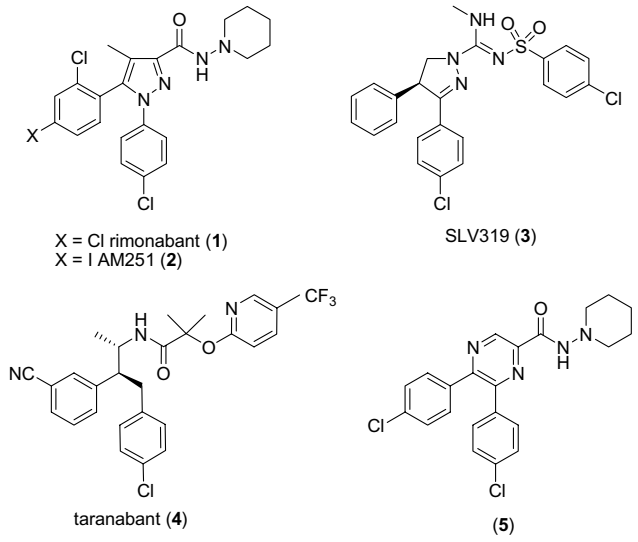
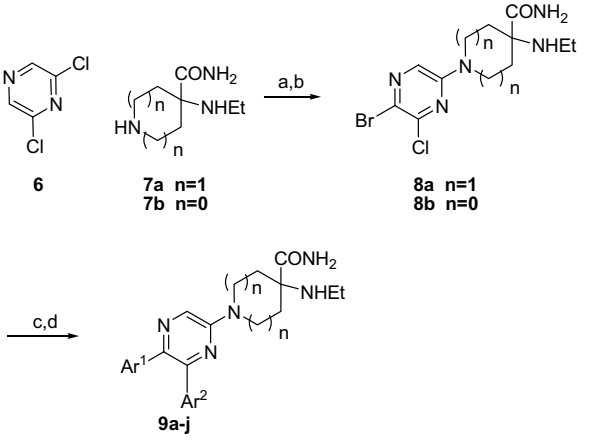
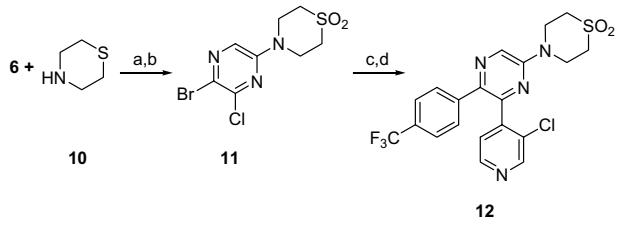


Figure 1. Selected CB1 Inverse Agonists.

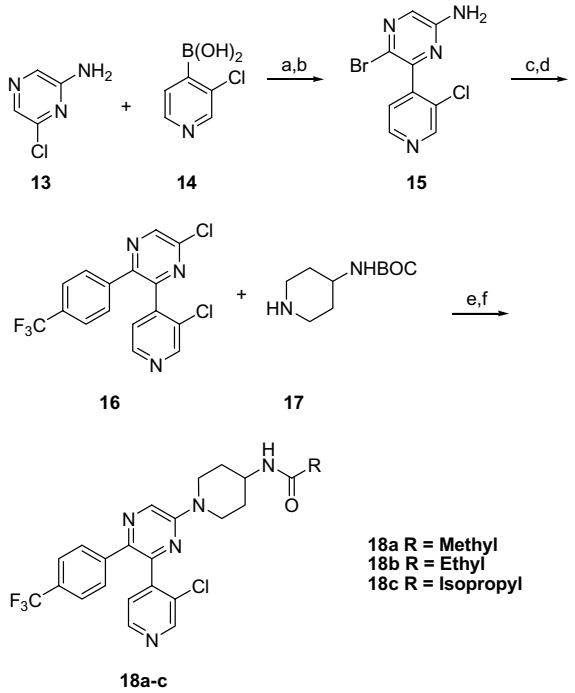


Scheme 1. Reagents for the syntheses of **9a**–**j**: (a) K_2CO_3, CH_3CN, 31–97%; (b) NBS, $CHCl_3$, 70–80%; (c) $Ar_1B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, dioxane, 75–90%; (d) $Ar_2B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, dioxane, 40–77%.



Scheme 2. Reagents for the synthesis of **12**: (a) K_2CO_3, CH_3CN; (b) i—NBS, $CHCl_3$, 46%; ii—*m*-CPBA, CH_2Cl_2, 36%; (c) 4-trifluoromethylphenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, dioxane, 76%; (d) 3-chloro-4-pyridyl-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, H_2O, dioxane, 50%.

4-trifluoromethylphenyl boronic acid and subsequently the 2-amino group was converted to the chloro derivative **16** using Sandmeyer conditions. Compound **16** was reacted with Boc-protected 4-aminopiperidine **17**.



Scheme 3. Reagents for the synthesis of **18a-c**: (a) K_2CO_3, $Pd(PPh_3)_4$, H_2O, dioxane, 31%; (b) NBS, $CHCl_3$, 66%; (c) 4-trifluoromethylphenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 43%, H_2O, dioxane; (d) $NaNO_2$, HCl, CuCl, 82%; (e) DMA, DMSO, KF 85%, (f) i—TFA, CH_2Cl_2, 16 h; ii—RCOCl, DIEA, CH_2Cl_2, 88–93%.

The resulting Boc derivative was deprotected under acidic conditions and the resulting amine was acylated to give the desired targets **18a**–**c**.

The compounds prepared were tested for their ability to inhibit GTPγ ^{35}S binding induced by the cannabinoid receptor agonist CP-55,940 in Sf9 membrane preparations expressing the hCB1 receptor along with Gαi2, Gβ1, and Gγ2 G-proteins. In addition to seeking compounds with good potency we desired compounds with improved solubility compared to rimonabant. To this end, a high throughput solubility assay was also used to guide SAR studies as shown in Table 2.

Initial studies with pyrazines **9a**–**c** having chlorophenyl substituents possessed excellent CB1 receptor potency (K_i <2 nM), however, these compounds showed little or no aqueous solubility in the high-throughput solubility assay. In order to find agents with improved aqueous solubility, a variety of aryl substituents were examined to find those that would impart both good potency inherent in compounds **9a**–**c** and have increased aqueous solubility.

Replacement of the 4-chloro substituent on the aryl ring at the 5 position of **9c** with an ethoxy substituent resulted in **9d** which is almost 10-fold weaker in hCB1 potency. Somewhat surprisingly however, the introduction of oxygen functionality into the *para* position of the phenyl ring at the 6 position of the pyrazine ring (compound **9e**) resulted in a substantial restoration of CB1 potency. While this did not improve solubility it did suggest that

Table 1. Substitution of aminopyrazines **9a–j**

Compound	Ar^1	Ar^2	*n*
9a	4-Chlorophenyl	2,4-Dichlorophenyl	1
9b	4-Fluorophenyl	2,4-Dichlorophenyl	1
9c	4-Chlorophenyl	2-Chlorophenyl	1
9d	4-Ethoxyphenyl	2-Chlorophenyl	1
9e	4-Ethoxyphenyl	4-Ethoxyphenyl	1
9f	4-Chlorophenyl	4-Cyanophenyl	1
9g	4-Chlorophenyl	4-Pyridyl	1
9h	4-Chlorophenyl	3-Chloro-4-pyridyl	1
9i	4-Trifluoromethylphenyl	3-Chloro-4-pyridyl	1
9j	4-Trifluoromethylphenyl	3-Chloro-4-pyridyl	0

Table 2. hCB1 antagonist potency, solubility, and CYP 3A4 inhibitory potential for **1**, **9a–j** , **12**, and **18a–c**

Compound	hCB1 GTPγS Inhibition K_i[a] (nM)	Solubility[b] (μg/mL)	% Inhib. CYP3A4 at 5 μM[c]
9a	0.91	0	<5
9b	1.75	0	20.5
9c	1.66	1.25	9.7
9d	14.96	1.2	10
9e	2.84	0	<5
9f	32.33	36	—
9g	9.81	74.9	71
9h	0.67	21.83	54
9i	0.87	25.5	<5
9j	1.2	38	<5
12	0.83	23	<5
18a	1.97	53	<5
18b	0.8	24	11
18c	0.24	4.5	<5
1	0.43	0.3	<5

[a] The antagonist mode K_i value is generated in the presence of the hCB1R agonist CP-55,940.

[b] Solubility was assessed by introducing 10 mM DMSO stock solutions directly into a pH 7.4 buffer solution at a concentration of 250 μM. A standard plate was created at the same concentration in DMSO. Precipitation from the solutions was removed by filtration. Analyses were carried out using a single point calibration based on UV detector peak areas.

[c] CYP3A4 inhibition was assessed by measuring inhibition of midazolam metabolism in human liver microsomes in the presence of test compounds.

the 4-position of the aryl ring in the 6 position of the pyrazine was an area tolerant of polar functionality.

To further explore the effect of hydrogen bond accepting functionality at the *para* position of the phenyl ring at the 6 position of the pyrazine ring, the cyano derivative **9f** was prepared. While **9f** had decreased hCB1 potency, this compound had improved aqueous solubility suggesting that investigation of alternative hydrogen bond accepting functionality at this position was warranted. To this end a 4-pyridinyl ring was introduced at the 6-position of the pyrazine core resulting in compound **9g** which had sub 10 nM potency and improved aqueous solubility compared to many of the biphenyl derivatives. Further enhancement of the CB1 potency was achieved by addition of a 3-chloro substituent to the pyridine ring (**9h**) which gave a nearly 15-fold improvement in hCB1 potency while retaining improved aqueous solubility compared to the initial chlorophenyl derivatives in the series.

Despite having good potency and acceptable solubility properties, **9h** was found to inhibit CYP3A4 as evidenced by its ability to block the metabolism of midazolam in human liver microsomes when co-incubated at a concentration of 5 μM (Table 2). However, it was found that substitution of the chlorine in the *para* position of the 5-phenyl ring of **9h** with a trifluoromethyl moiety resulted in compound (**9i**) with excellent potency, acceptable solubility and no evidence for CYP3A4 inhibition. With aryl rings identified that imparted excellent hCB1 potency and good drug-like properties to the aminopyrazine template, additional 2-aza substituents on the pyrazine ring were explored. In an effort to reduce molecular weight and decrease lipophilicity the azetidine analogue of **9i** was prepared. This compound (**9j**) showed similar potency and somewhat increased aqueous solubility while demonstrating a low potential for inhibiting CYP3A4.

The activity of thiomorpholine dioxide **12** suggested that one or both the sulfone oxygens could act as a hydrogen bond acceptor in a similar position to the amide carbonyl oxygen in analogue **9**. Like the best analogues such as **9j**, compound **12** displayed good potency as a hCB1 inverse agonist combined with acceptable solubility. Subsequent studies revealed that **12** had no CYP3A4 inhibition liability. To further explore the flexibility of substitution at the 1-amino position, analogues **18a–c** were evaluated for their potency at the hCB1 receptor. In this series it was determined that as the size and lipophilicity of the acyl substituent increased so did binding affinity. The isobutyric amide **18c** was found to have outstanding potency at the hCB1 receptor and retained a measurable level of aqueous solubility.

Having identified novel pyrazines with potent hCB1 inhibitory activity and improved solubility properties compared to rimonabant, we were motivated to further evaluate the properties of **9j**, **12**, and **18c**. These three compounds demonstrated hCB1 inverse agonist activity by decreasing GTPγS levels below those observed in the basal state of the Sf9 membrane preparation expressing hCB1 receptor plus G-proteins. The IC_{50}s in this assay are listed in Table 3. These

Table 3. Further in vitro characterization of selected compounds

Compound	hCB1 Inv. agonist IC_{50}[a] (nM)	hCB2 GTPγS inhibition K_i[b] (nM)	rCB1 GTPγS inhibition K_i[b] (nM)
9j	3.07	>50,000	2.06
12	4.2	1900	0.83
18c	0.54	4945	0.41
1	1.35	815	0.31

[a] Ability to block constitutive activity in Sf9 membrane preparations expressing hCB1 receptor.

[b] Both the hCB2 and rCB1 assays are virtually identical with the exception of the amino acid sequence of the receptor used to infect the Sf9 cells.

compounds also had greater than 2000-fold functional selectivity for the hCB1 receptor compared to their potency at inhibiting GTPγS interactions stimulated by CP-55,940 at hCB2 receptors. The compounds also showed appreciable functional inhibitory potency at the rat CB1 receptor. The compounds were initially evaluated in vivo for their anorectic potential in an overnight feeding model in diet-induced obesity (DIO) rats. After a single oral dose of 5 mg/kg food intake was evaluated for 24 h and compared to vehicle-treated controls. As shown in Table 4 all three compounds significantly decreased food intake with compounds **12** and **18c** causing a similar or slightly greater decrease than that observed with after a 5 mg/kg oral dose of rimonabant (**1**).

Compound **18c** and rimonabant (**1**) were studied in an ex vivo receptor occupancy assay to understand their relative abilities to access and bind to rat brain CB1 receptors after oral administration. First the relative binding affinities of **18c** and **1** were determined in a rat cerebellum membrane preparation using [^{3}H]CP-55940 as the radioligand. These studies revealed that rimonabant (**1**) had a higher binding affinity (K_i 1.1 nM) than **18c** (K_i 4.4 nM) in this assay. Compounds **18c** and **1** were next evaluated in an oral dose response ex vivo study that assessed occupancy of brain CB1 receptor sites 3 h after oral administration of the individual compounds. Both compounds prevent labeling of cerebellar homogenates with [^{3}H]CP-55,940, by occupying the hCB1 receptor after oral administration with **18c** and **1** having ID_{50} values of 0.3 and 0.5 mg/kg, respectively.

Because **18c** possessed oral activity and potency similar to rimonabant (**1**) in both the overnight feeding and receptor occupancy studies, the compounds were compared in an 18 day subchronic food intake study in DIO rats. Over the course of the study animals (10 per group) were dosed (po, qd) with 1 or 5 mg/kg of **18c** or 5 mg/kg of **1**. Rats were monitored for both food intake and body weight relative to a control group of animals receiving only vehicle. In addition changes to hormonal markers related to changes in obesity were measured in both the drug-treated and control animal groups.

The inhibition of food intake was greatest in the first part of the study with the food intake reduction observed with 5 mg/kg of **18c** remaining statistically significant throughout the course of the study (Fig. 2). Similarly the decreases in percentage of body weight in the treated groups relative to the control group were larger at the beginning of the study but appeared to have

Table 4. Overnight food intake inhibition study in DIO rats

Compound	Dose PO mpk	Overnight DIO % inhib. food intake
9j	10	58
12	10	71
18c	5	80
1	5	73

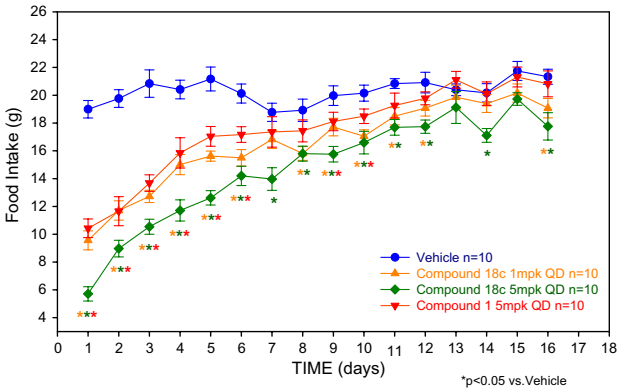


Figure 2. Food intake in subchronic DIO study.

leveled out by the end of the study (Fig. 3). The magnitude of effect on food intake and percentage of weight loss relative to control animals was similar between the group of animals dosed with 1 mg/kg of **18c** and 5 mg/kg of rimonabant (**1**). The group dosed with 5 mg/kg of **18c** showed a greater decrease in food intake and approximately twice the weight loss on a percentage basis compared to the group of animals dosed with 5 mg/kg **1**. (Table 5).

After the completion of the 18th dose, plasma and brain levels of compounds were assessed at T_{max} (1 h for **1**; 2 h for **18c**) and 17 h after the last dose (Table 6). These data suggest that roughly similar brain levels were achieved with the 5 mg/kg dose of **1** and the 1 mg/kg dose of **18c**. Compared to the 5 mg/kg dose of **1**, the 5 mg/kg dose of **18c** achieved a higher brain concentration which may account for the enhanced effect on food intake and

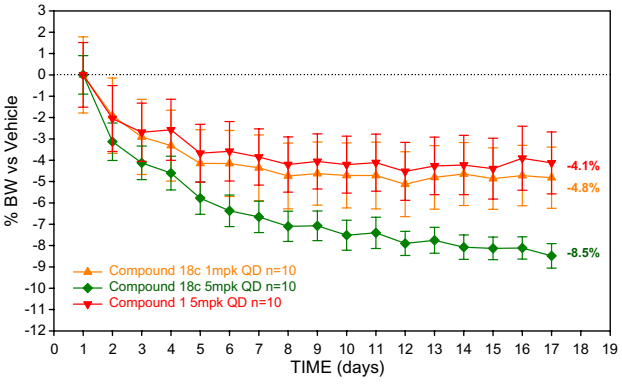


Figure 3. Percent of body weight versus vehicle in a subchronic DIO study.

Table 5. Cumulative decreases in food intake and percentage of body weight in the subchronic DIO study

Compound	Dose (mg/kg)	Cumulative food intake % reduction versus vehicle	Percentage of body weight versus vehicle
18c	1	18.8	4.8
18c	5	27.6	8.5
1	5	13.8	4.1

weight loss in these animals. This finding is also consistent with the lower oral ID_{50} observed for **18c** compared to **1** in the rat brain RO study despite **18c** being about fourfold weaker in receptor affinity (see Table 6).

On the day immediately prior to the start of the rat DIO feeding study and at the end of the 18th day of the study, the animals were tail-bled after a 8–10 h fast. Blood levels of glucose, insulin, and leptin were measured in the drug-treated and vehicle-treated groups. The groups of animals treated with **18c** and **1** showed a statistically significant decrease in blood glucose level (Fig. 4). Statistically significant decreases in insulin levels were also observed in all groups treated with either **18c** or **1** but not the vehicle-treated group (Fig. 5). The amount of decrease in each of the drug-treated groups showed similarly trending dose responses to that of inhibition of food intake and weight loss. Analysis of blood leptin levels revealed that both group of animals treated with **18c** had significant, dose dependent decreases in leptin levels, while those in the rimonabant-treated group showed a slightly smaller reduction in leptin levels (Fig. 6).

Table 6. Plasma and brain levels after 19th Dose

Compound	Dose (mg/kg)	T_{max} plasma/brain levels (ng/mL)	17 h plasma/brain levels (ng/mL)
18c	1	121/235	44.2/110
18c	5	346/630	142/253
1	5	34.9/318	3/65

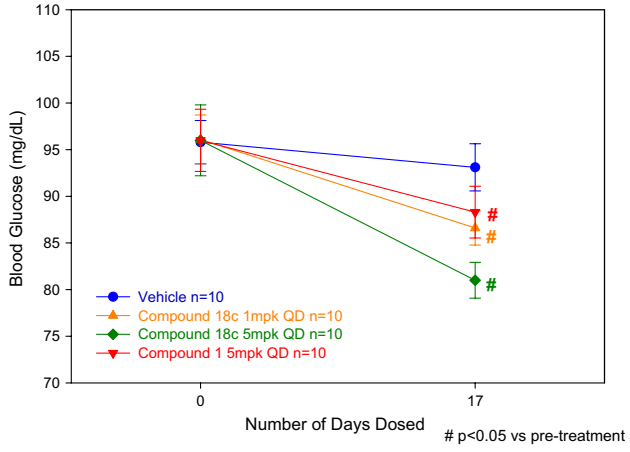


Figure 4. Fasting blood glucose levels.

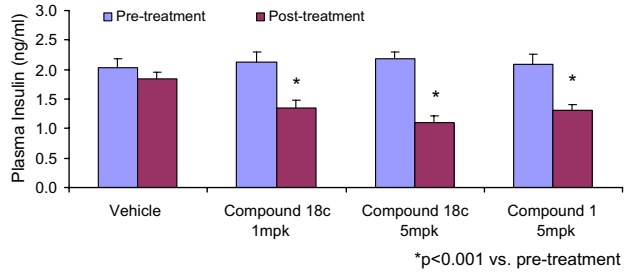


Figure 5. Blood insulin levels.

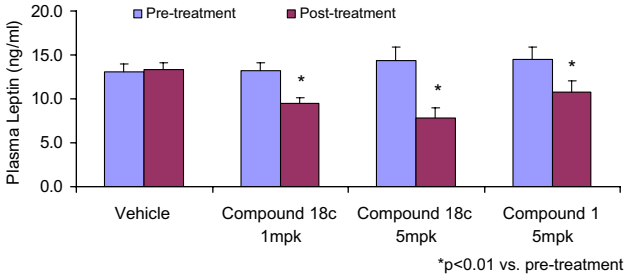


Figure 6. Fasting blood leptin levels.

In summary a class of novel pyrazine CB1 receptor inverse agonists was prepared. Simultaneous optimization of functional CB1 receptor potency, solubility, and CYP3A4 inhibition parameters rapidly led to the discovery of selective CB1 inverse agonists with good drug-like properties. Optimized compounds in the series such as **18c** had similar or better in vivo properties compared to the marketed antiobesity agent rimonabant in feeding and CB1 receptor occupancy studies. After dosing for 18 days, 1 mg/kg of **18c** had similar effects to 5 mg/kg of rimonabant (**1**) both in terms of inhibition of food intake and weight loss, while 5 mg/kg of **18c** caused approximately twice the amount of weight loss compared to animals treated with 5 mg/kg of rimonabant (**1**). Animals treated with the potent CB1 inverse agonist **18c** showed statistically significant reductions in glucose, insulin and leptin levels over the course of the subchronic study. Taken together these studies suggest that a compound such as **18c** could be a useful treatment in certain overweight and diabetic populations.

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Andrzej Kieltyka performed the ex vivo receptor occupancy experiments. Yung Lee and Danielle Lord participated in the PK study.

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