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Metabolic Profiling of CB1 Neutral Antagonists

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Abstract

PIMSR is among the first neutral antagonists for the CB1R and was demonstrated pharmacologically to bind to the CB1R, yet not alter calcium flux. It was further shown computationally to be able to stabilize both the active and inactive states of CB1R revealing the molecular interactions that mechanistically afford the property of neutral antagonism. PIMSR shows dramatic positive effects in reducing weight, food intake, and adiposity as well as in improving glycemic control and lipid homeostasis in high-fat diet-induced obese mice, but also shows increased ALT and liver weight as markers of liver injury with chronic administration. Further, in a separate study, 3-day administration of PIMSR in C57BL/6J mice, hepatic steatosis from an acute administration of high of ethanol was significantly reduced. Also, it partially prevented alcohol-induced increases in ALT, AST, and LDH. The differences in ALT levels in obese and nonobese mice under different test paradigms are unlikely to be due to neutral antagonism itself since other neutral antagonists (AM6545) do not exhibit liver injury. The brain levels of low micromolar would support significant brain CB1 receptor occupancy (re: $K_i = 17\text{nM}$), thus potentially including both CNS and peripheral influences on the observed weight loss. Overall, these studies suggest that marked improvements in aspects of metabolic disease and alcoholic steatosis can be realized with CB1R neutral antagonists and hence warrants the exploration of further members of this class of cannabinoid ligands.

1. INTRODUCTION

Endogenous, plant, and chemist designed cannabinoids have widespread pharmacological and physiological effects that are exhibited through their receptors (Pertwee, 2005b; Szabo

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& Schlicker, 2005). The cannabinoid receptors are G protein-coupled primarily activating inhibitory G-coupled proteins (Gi/o) (Pacher, Batkai, & Kunos, 2006) and are considered to be constitutively active (Fong, 2014). The two currently identified cannabinoid receptor subtypes are CB1R, found mostly in neurons throughout the body, and CB2R, mostly expressed outside the CNS in immune cells (Howlett et al., 2002). The seven transmembrane helix CB1R receptor is associated with numerous physiological effects including pain, appetite, metabolism, and mood. Cannabinoid agonists of the CB1R block pain (Hohmann, 2002) stimulate appetite, influence energy balance (Andre & Gonthier, 2010; Cota, 2008), and lipidogenesis (Alonso et al., 2012; Cota et al., 2003), and typically elevate mood. Potential medical applications of activating CB1R response, especially as regards pain, obesity, seizures, and mood (e.g., PTSD), are inviting (Pacher et al., 2006) but the psychomimetic effects (euphoria and subsequent paranoia) have been a significant impediment to the development of cannabinoid agonists as drugs.

Alternatively, the potential applications of blocking endogenous agonist effects relating to appetite (Tam et al., 2012) and energy balance via lipogenesis as well as appetitive conditions such as drug, alcohol, and tobacco use, prompted much research on CB1R antagonists. It was found that the first and preeminent CB1R antagonist Rimonabant (SR141716) reduced food intake and altered metabolism with beneficial effects on weight loss (Silvestri & Di Marzo, 2012). This had significant implications regarding the epidemic levels of obesity and the downstream consequences of resultant diabetes. After Rimonabant came to the market (as AcompliaTM), the psychomimetic effects of mood alteration (Beyer et al., 2010) including suicidal ideation (Christensen, Kristensen, Bartels, Bliddal, & Astrup, 2007) had begun to be observed which led to its removal from the market and a pull-back from this line of research by pharmaceutical companies (Lee, Choi, & Pak, 2009). Rimonabant, it was discovered, was not simply an antagonist, but rather it was an inverse agonist (Pan, Ikeda, & Lewis, 1998).

Other classes of CB1R regulators in the form of neutral antagonists, that do not change basal CB1R activity, have been revealed (Pan et al., 1998) and are the subject of study (Hurst et al., 2002, 2006; Pertwee, 2005a; Reggio, 2009; Silvestri & Di Marzo, 2012) and development (Alonso et al., 2012; Argueta & DiPatrizio, 2017; Bostroem et al., 2010; Brents et al., 2012; Franks et al., 2014; Fride et al., 2007; Greig & Ross, 2010; Kangas et al., 2013; Makriyannis & Vemuri, 2008; Ruiiu et al., 2003; Vela Hernandez & Yenes Minguez, 2009; Wargent et al., 2013; Wiley et al., 2011).

In this chapter, we will present our research on a potent and selective neutral antagonist, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-[(*E*)-piperidinoiminomethyl]-1H-pyrazole (PIMSR) (Fig. 1), which interacts with the G protein-coupled cannabinoid type-1 receptor CB1R in a mechanistically different way than Rimonabant affording metabolic and functional benefits without the potential downside consequences of inverse agonism at CB1R (Beyer et al., 2010; Christensen et al., 2007). This will start with defining constitutive activity and its relation to neutral antagonism vs inverse agonism, continue with a molecular modeling description of the interaction of relevant ligands with the CB1R, and its role in designing neutral antagonists, and then present the effects of PIMSR in vivo and in vitro and methods for those studies.

2. BACKGROUND

2.1 Constitutive Activity of CB1R

The CB1R exhibits constitutive activity, which is the activity of the activated, or on-state, of the receptor in the absence of an agonist; that is, the intrinsic activity that results from the structure (constitution) of the receptor (Fong, 2014). In brief, the ligand-free receptor exists in an equilibrium between the active (on) state and the inactive (off) state and likely a few intermediate states. A constitutively active receptor equilibrates at a basal state wherein there is a portion of the receptors in the active state that result in downstream physiological effects (Kenakin, 2005).

When an agonist ligand binds to the receptor it shifts the equilibrium to the active state resulting in functional activity. In contrast, an inverse agonist ligand shifts the equilibrium from the basal state to the inactive state. Many inverse agonists were earlier described as antagonists, the latter typically defined as having no efficacy. But a reduction in basal levels of activation caused by an inverse agonist does have physiological consequences, hence the evolved description (Pan et al., 1998; Pertwee, 2005a). A neutral or silent antagonist is one that does not shift the equilibrium from the basal level of the activation of the receptor and hence does not change observed activity. Holding basal activity, or restoring an imbalanced constitutive activity could have therapeutic potential in disease states that are the result of excessive receptor activation (Beltramo, Brusa, Mancini, & Scandroglio, 2010).

3. COMPUTATIONAL MODELING

Rational design of CB1R neutral antagonists requires an understanding of the interaction of cannabinoid ligands with the receptor as regards how the interaction induces the changes in conformation and the resulting coupling of the receptor to G proteins when stabilized by agonists, neutral antagonists, and inverse agonists. A powerful method of obtaining such an understanding is the use of computational modeling of the receptor, which is developed from its amino acid sequence, the X-ray structure of a similar GPC receptors as a starting point, and computational refinement. Computationally docking tested ligands in the receptor model suggests amino acid side chains that play a key role in the receptor activity. Point mutations of these key amino acids of the wild-type receptor and testing the receptor function in the presence of ligands of known function before and after point mutations identifies regions of the receptor associated with its activity. The nature of the ligand–receptor interaction (H-bonding, ionic interaction, Van Der Waals forces, aromatic stacking, steric interactions, etc.) can then be inferred and calculated. From the proposed interactions, modified ligands can be designed to test the proposed interaction in vitro.

From such studies of the CB1R, a model was developed that proposed that a hydrogen bond between the inactive state of the receptor and the inverse agonist Rimonabant (SR141716) was critical. In this model, the lysine (K) on helix-3 at residue 28, where the most conserved residue of any helix is number 50, i.e., K3.28, contributed a hydrogen bond (donor) to the oxygen of the C-3 hydrazide moiety of the ligand SR141716 (H-bond acceptor). To test this, the H-bonding capacity of the donor (lysine) and/or the acceptor (C-3 hydrazide oxygen) is eliminated by mutating the lysine to non donating alanine (K3.28A) and by synthesizing

nonaccepting desoxy-analogs (X'SR) of Rimonabant (described later). If the H-bonding is a direct interaction, then eliminating it would reduce the affinity in (1) the K3.28A–Rimonabant pair, (2) the K3.28–X'SR pair, and (3) the K3.28A–X'SR pair, relative to (4) the wild-type K3.28–Rimonabant pair, since all three preclude H-bonding. If mutations K3.28A and X'SR operated independently of one another, i.e., did not participate in the same H-bond, then the decrease in affinity brought about by these mutations would be additive in pairing (3) (Hurst et al., 2002).

In affinity assays with cloned human wild-type CB1 or the mutant receptors and with SR141716 or X'SR ligand analogs (Fig. 1) vs [³H]SR141716, the following was observed for X'SR=VCHSR, the vinylcyclohexyl analog of SR141716 lacking H-bonding at the C-3 substituent. First, eliminating the H-bond donor moiety caused the affinity of SR141716 for the mutant K3.28A receptor (Alonso et al., 2012) to be reduced to $39.6 \pm 10.5 \text{ nM}$ from $2.3 \pm 1.1 \text{ nM}$ for the SR141716 wild-type K3.28 receptor (Beltramo et al., 2010) supporting a strong interaction, such as a H-bonding, between SR141716 and the 3.28 Lysine. Second, elimination the H-bond acceptor moiety caused a similar reduction of binding affinity between the wild-type receptor K3.28 and the mutant ligand VCHSR (Andre & Gonthier, 2010) to $31.3 \pm 9.6 \text{ nM}$ supporting the strong H-bonding interaction. Third, when both H-bonding donor and acceptor moieties were lacking, such as in the K3.28A–VCHSR pair (Argueta & DiPatrizio, 2017), the binding affinity was similarly reduced to $35.2 \pm 1.4 \text{ nM}$, indicating the absence of an additive effect of both mutations supporting that they participate in the same H-bond interaction (Hurst et al., 2002).

In addition to the H-bond between the C-3 hydrazide moiety and the wild-type receptor's 3.28 lysine contributing to the strength of the ligands affinity for the receptor binding site, there are also aromatic stacking interactions that enable all these ligands to bind with nanomolar affinity and to stabilize receptor conformations (Hurst et al., 2002, 2006; Reggio, 2009).

PIMSR (Fig. 1) another variant mutation was also prepared and tested (Hurst et al., 2006). It too adopts the low energy conformations of SR141716 and VCHSR and also shows reduced affinity, by nearly an order of magnitude, for the wild-type K3.28 receptor ($K_i = 17 \text{ nM}$) supporting the identification of the oxygen acceptor as the H-bonding site. However, this is about twice the affinity of the VCHSR ligand ($K_i = 31.3 \text{ nM}$) suggesting the possibility that one of the nitrogens of the PIMSR C-3 moiety is participating in bonding. Indeed, the computational modeling shows that the imino nitrogen ($\text{C}=\text{N}-$) supports a weak H-bond with the receptor's C7.42 cysteine (but not K3.28) which restores some additional binding affinity.

Testing these mutant ligands in the calcium flux functional assay for the CB1R (Pan et al., 1998; Hurst et al., 2006), both VCHSR and PIMSR as well as the other ligands lacking the C-3 substituent oxygen exhibited no change in calcium flux, an indication of neutral antagonism, which is in contrast to SR141716 and other C-3 carbonyl analogs that exhibit an increase in calcium flux typical of an inverse agonist. Collectively, this points to these deoxygenated analogs of SR141716 as being among the first CB1 neutral antagonists and

suggests their further pharmacological and physiological study to probe for potential therapeutic benefit.

4. CHEMISTRY AND STRUCTURE PROOF AND STABILITY Ki CB1 CB2

The synthesis, structure proof including the *trans*-geometry of the double bond, and the demonstrated stability of PIMSR in aqueous media necessary for pharmacological studies were discussed in a prior report (Hurst et al., 2006).

5. IN VIVO METABOLIC EFFECTS

The encouraging result that PIMSR was shown to be free of dysphoric effects in electrical brain stimulation reward studies, led to a study of its potential as a regulator of metabolic disease-related effects. This study reports the effect of PIMSR in high-fat diet (HFD)-induced obese mice on body weight, food intake, glycemic control, and lipid homeostasis. Tissue distribution and markers of liver condition and function are also reported.

5.1 Methods

5.1.1 Animals—Animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the NIAAA, NIH. Male 5–6-week-old C57Bl/6J mice were obtained from Jackson Laboratory. Mice were maintained under a 12-h light/dark cycle and fed ad libitum. To generate diet-induced obesity (DIO) (body weight > 42g), mice were fed HFD (Research Diet, NJ; D12492; 60% of calories from fat, 20% from protein, and 20% from carbohydrates) for 14 weeks.

5.1.2 Experimental Protocol—HFD-fed obese mice received vehicle or PIMSR [10mg/kg, intraperitoneal (ip)] daily for 28 days. Food intake and body weight were measured daily. Mice were sacrificed by cervical dislocation, the brain, liver, and combined fat pads were removed, weighed, and snap-frozen, and trunk blood was collected for determining endocrine and biochemical parameters. Adiposity index was defined as the combined weight of the epididymal, retroperitoneal, and inguinal fat pads, expressed as % of total body weight.

5.1.3 Tissue Levels of PIMSR—Mice received a single ip dose (10mg/kg) of PIMSR and were sacrificed 1h later. Blood was collected, and the mice were perfused with phosphate-buffered saline for 1min to remove drug from the intravascular space before removing the brain adipose tissue and liver. Tissues and plasma were extracted as described (Tam et al., 2012) and drug levels were determined by liquid chromatography/tandem mass spectrometry using an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies) coupled with an Agilent 1200 LC system (Agilent Technologies). Chromatographic and mass spectrometer conditions were set as described (Tam et al., 2012). Levels of PIMSR were analyzed by multiple reactions monitoring. The molecular ion and fragments for PIMSR were measured as follows: m/z 447.1 $\rightarrow$ 364 and 447.1 $\rightarrow$ 84.1 (collision-induced dissociation-energy: 24 and 36V, respectively). The acquisition and quantitation of analytes were achieved using MassHunter Workstation LC/QQQ Acquisition and MassHunter Workstation Quantitative Analysis softwares, respectively (Agilent

Technologies). The amounts of PIMSR in the samples were determined against standard curves. Values are expressed as $\mu\text{g/g}$ or $\mu\text{g/mL}$ in wet tissue weight or plasma volume, respectively.

5.1.4 Blood Chemistry—Blood was collected at the time the mice were sacrificed. Serum alanine transaminase (ALT), aspartate transaminase (AST), cholesterol, HDL- and LDL-cholesterol, and triglycerides were quantified using AMS Vegasys Chemistry Analyzer (Diamond Diagnostics, MA). Blood glucose was determined using the Elite glucometer (Bayer, PA). Serum insulin was determined using the Ultra-Sensitive Mouse Insulin ELISA kit (Crystal Chem Inc., IL).

5.1.5 Glucose Tolerance (ipGTT) and Insulin Sensitivity Tests (ipIST)—Mice fasted overnight were injected with glucose (1.5g/kg ip), followed by tail blood collection at 0, 15, 30, 45, 60, 90, and 120min. Blood glucose levels were determined using the Elite glucometer (Bayer, PA). On the following day, mice were fasted for 6h before receiving insulin (0.75U/kg, ip; Eli Lilly, DC), and blood glucose levels were determined at the same intervals as earlier.

5.1.6 Hepatic Triglyceride (TG) Content—Liver tissue was extracted as described previously (Knani et al., 2016) and its triglyceride content determined using EnzyChrom™ Triglyceride Assay Kit (BioAssay Systems, CA).

5.1.7 Statistics—Values are expressed as mean $\pm$ SEM. Unpaired two-tailed Student's *t*-test was used to determine differences between vehicle- and drug-treated groups. Time-dependent variables were analyzed and results in multiple groups were compared by ANOVA followed by Bonferroni test. Significance was at $P < 0.05$.

5.2 Results

5.2.1 PIMSR Is a Brain-Penetrant Neutral CB1R Antagonist—Tissue distribution of PIMSR following an acute ip dosing of 10mg/kg revealed a marked reduction in its brain penetrance ($1.24 \pm 0.09 \mu\text{g/g}$), as reflected by a brain: plasma concentration ratio of 0.24 (Fig. 2). Yet, the brain levels of this compound are more than two orders of magnitude higher than the K_i (17nM) required for its binding to CB1 receptor. Extremely high levels of PIMSR were found in the liver ($21.72 \pm 2.22 \mu\text{M}$, 414% of plasma) and fat tissue ($18.06 \pm 4.19 \mu\text{M}$, 345% of plasma).

5.2.2 PIMSR Improves Metabolic Profile in Diet-Induced Obese Mice—The metabolic profile of PIMSR was examined in mice with DIO. Male C57Bl/6J mice fed a HFD for 14 weeks became obese and were then started on daily ip injections of vehicle or PIMSR (10mg/kg/day) for an additional 28 days. The overweight and increased adiposity of mice on HFD were significantly reduced by PIMSR (Fig. 3A–C). These reductions were associated with a transient (14 days) reduction in food intake by PIMSR (Fig. 3D).

HFD-induced obesity is related to reduce glucose tolerance (Fig. 4A) decreased sensitivity to the hypoglycemic effect of insulin (Fig. 4B), hyperglycemia (Fig. 4C), and hyperinsulinemia (Fig. 4D). All of these changes were reduced (improved) by PIMSR.

Further metabolic improvements were noted by the ability of PIMSR to reduce serum triglyceride and cholesterol levels (Fig. 5A and B), and increase the ratio between HDL and LDL cholesterol (Fig. 5C).

PIMSR did not ameliorate the HFD-induced fatty liver, as reflected in by its inability to reduce the liver weight (Fig. 6A), serum ALT and AST levels (Fig. 6B and C), and hepatic triglyceride content (Fig. 6D). In fact, PIMSR increased the hepatocellular damage, as indicated by increased liver weight and serum ALT levels (Fig. 6A and B), which might be attributed to its extremely high liver content (Fig. 2). This is likely not a consequence of CB1R occupancy and hence similarly not due to neutral antagonism, but rather is due to the chemical properties and pharmacokinetics of this particular analog. Overall, this study suggests that marked improvements in aspects of metabolic disease can be realized with CB1R neutral antagonists and hence warrants the exploration of further members of this class of cannabinoid ligands.

6. PIMSR AND BINGE ALCOHOLIC HEPATIC STEATOSIS

Liver disease from alcohol abuse is a major problem (Mathurin & Deltenre, 2009). A new threat to the physical welfare of alcoholics stems from a marked increase in rates of binge drinking, particularly in adolescents. While considerable data exist on effects of chronic alcohol consumption on liver, the pathological effects of binge drinking remain poorly understood. However, epidemiological data point to a drastic increase in liver cirrhosis and mortality rates in British binge drinkers and similar outcomes may be expected in other countries (Pincock, 2003). Brief intervention strategies using behavioral and/or pharmacological strategies may be useful in dealing with binge alcoholism and related pathologies. In this report, further in vivo studies were performed with PIMSR in a model of alcoholic steatosis (AS) induced by a single acute challenge with ethanol. Data presented herein indicate that PIMSR can significantly reduce AS induced by binge alcoholism.

6.1 Materials and Methods

6.1.1 Binge Alcohol-Induced Liver Steatosis—AS was induced in inbred C57BL/6J mice (Charles River Laboratories, Raleigh, NC) at 9 weeks of age by a single dose of 6g/kg ethanol. Control animals received sucrose. For the efficacy study with PIMSR, the drug was solubilized in a 1:1:18 ethanol/cremaphor/saline (vehicle) solution and administered once daily as an ip injection at 30mg/kg. Control animals were given vehicle only via ip injections. After three daily doses of PIMSR or vehicle, animals were challenged with ethanol or sucrose 1h after the last injection.

Serum samples from each animal were analyzed for AST, ALT, and lactate dehydrogenase (LDH) levels by an independent laboratory (Antech Diagnostics, Morrisville, NC) using good laboratory practice. Tissue sections (10 µm) from frozen livers were prepared and analyzed following hematoxylin–eosin staining and Oil Red O staining using standard procedures. Images were acquired using an Olympus inverted microscope. Three to six animals were used per group. Data were graphed using Prism software (GraphPad, La Jolla, CA) and statistical analyses were performed using the same program.

6.2 Results

6.2.1 Acute Alcohol-Induced Hepatic Steatosis and Prevention by PIMSR—

Binge alcoholism involves acute episodic consumption of fairly large quantities of alcohol with blood alcohol levels of $>300\text{ nM}$ routinely noted in patients. Thus, a single dose model of acute alcohol (6g/kg) induced steatosis in C57BL/6J mice was used for these studies.

For these studies, PIMSR was given to mice daily for 3 days at a 30mg/kg dose by ip injection. Control mice were injected with the vehicle alone. An hour after the last injection, mice were challenged with alcohol at 6g/kg. Animals were sacrificed after 16h and the rest of the studies was completed as described in the preceding section. As demonstrated in Fig. 7A, PIMSR was able to significantly reduce hepatic steatosis as demonstrated through Oil Red O staining (~60% reduction). Furthermore, PIMSR partially prevented alcohol-induced increases in levels of AST, ALT, and LDH (Fig. 7B). Thus, administration of PIMSR was an effective intervention/prevention strategy to combat hepatic steatosis induced by acute alcohol consumption. Unfortunately, it is now known that chronic administration of this compound produces a non-CB1R-associated liver toxicity. However, these studies followed an acute regimen. Therefore, it is assumed the short-term beneficial effect of CB1R blockade is reflected in these studies. Taken together, this class of compounds may be promising agents in the treatment of alcoholic liver injury in agreement with past results (Trebecka et al., 2011).

7. CONCLUSIONS

PIMSR is among the first neutral antagonists for the CB1R and was demonstrated pharmacologically to bind to the CB1R yet not alter calcium flux. It was further shown computationally to be able to stabilize both the active and inactive states of CB1R revealing the molecular interactions that mechanistically afford the property of neutral antagonism. PIMSR shows dramatic positive effects in reducing weight, food intake, and adiposity as well as in improving glycemic control and lipid homeostasis in HFD-induced obese mice, but also shows increased ALT and liver weight as markers of liver injury with chronic administration. Further, in a separate study, 3-day administration of PIMSR in C57BL/6J mice, hepatic steatosis from an acute administration of high of ethanol was significantly reduced. Also, it partially prevented alcohol-induced increases in ALT, AST, and LDH. The differences in ALT levels with obese and nonobese mice under different test paradigms are unlikely to be due to neutral antagonism itself since other neutral antagonists (AM6545) do not exhibit liver injury. Rather, this suggests that other biochemistry of PIMSR is responsible for hepatic damage such as extremely high levels of PIMSR measured in DIO mice. The brain levels of low micromolar would support significant brain CB1R occupancy (re: $K_i = 17\text{ nM}$), thus potentially including both CNS and peripheral influences on the observed weight loss. Overall, these studies suggest that marked improvements in aspects of metabolic disease and AS can be realized with CB1R neutral antagonists and hence warrants the exploration of further members of this class of cannabinoid ligands.

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ABBREVIATIONS

ALT	alanine transaminase
AS	alcoholic steatosis
AST	aspartate transaminase
DIO	diet-induced obesity
HFD	high-fat diet
ip	intraperitoneal
LDH	lactate dehydrogenase
PIMSR	5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-[(<i>E</i>)-piperidinoiminomethyl]-1H-pyrazole

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Fig. 1.

Inverse agonist (SR141716) and neutral antagonists (VCHSR, PIMSR, VPSR) of CB1R.

Fig. 2.

Drug levels in plasma (αg/mL), brain (αg/g), liver (αg/g), and fat (αg/g) 1 h after acute ip administration. Concentrations of PIMSR after ip dose of 10 mg/kg. Data represent mean ± SEM from three animals.

Fig. 3. Effect of PIMSR on body weight and food intake. Mice on HFD for 14 weeks were treated with ip of either vehicle or PIMSR (10 mg/kg/day) for 28 days. Note that PIMSR reduced body weight (A and B), adiposity (C), and daily food intake (D). Data represent mean $\pm$ SEM from six animals per group.

Fig. 4. Effect of PIMSR on glycemic control. Mice on HFD for 14 weeks were treated with ip of either vehicle or PIMSR (10mg/kg/day) for 28 days. Note that PIMSR improves glucose intolerance (A) and insulin sensitivity (B), reduced the basal hyperglycemia (C), and hyperinsulinemia (D). Data represent mean $\pm$ SEM from six animals per group.

Fig. 5.

Effect of PIMSR on lipid homeostasis. Mice on HFD for 14 weeks were treated with ip of either vehicle or PIMSR (10 mg/kg/day) for 28 days. Note that PIMSR reduced serum triglycerides (A), cholesterol (B), and improved HDL/LDL cholesterol (C). Data represent mean $\pm$ SEM from six animals per group.

Fig. 6.

Effect of PIMSR on hepatic parameters. Mice on HFD for 14 weeks were treated with ip either vehicle or PIMSR (10 mg/kg/day) for 28 days. Note that PIMSR increased liver weight (A) and serum ALT levels (B), without affecting serum AST levels (C), and hepatic triglyceride content (D). Data represent mean $\pm$ SEM from six animals per group.

Fig. 7.

Effect of PIMSR1 on binge alcoholic steatosis. PIMSR or vehicle was administered to male C57BL6 mice by ip injection for 3 days followed by a single challenge of 6g/kg alcohol. Matched controls received vehicle alone and equicaloric sucrose instead of alcohol. Animals were sacrificed 16 h after the alcohol challenge. (A) PIMSR pre-treatment decreased steatosis and preserved liver architecture as determined by Oil Red O (*bottom panel*) and H&E staining (*top panel*) compared to animals receiving alcohol only. (B) Biomarkers of liver damage were increased upon alcohol challenge including AST, ALT, and LDH. Pretreatment with PIMSR reduced liver damage. Data were statistically significant (Student's *t*-test, $P < 0.01$) between vehicle-treated animals receiving alcohol or sucrose and between alcohol challenged mice treated with PIMSR or vehicle only.