Bioorganic & Medicinal Chemistry Letters 22 (2012) 6173–6180

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

JD-5006 and JD-5037: Peripherally restricted (PR) cannabinoid-1 receptor blockers related to SLV-319 (Ibipinabant) as metabolic disorder therapeutics devoid of CNS liabilities

Robert J. Chorvat *, Jennifer Berbaum, Kristine Seriacki, John F. McElroy

Jenrin Discovery, 2515 Lori Lane North, Wilmington, DE 19810, USA

ARTICLE INFO

Article history:
Received 28 July 2012
Accepted 1 August 2012
Available online 20 August 2012

Keywords:
CB1 receptor inverse agonist
Metabolic disorders
Diabetes
Liver diseases
Dyslipidemias
Obesity
JD-5006
JD-5037
SLV-319
Peripherally restricted cannabinoid-1 receptor blockers
Rimonabant
CB1 antagonist

ABSTRACT

Analogs of SLV-319 (Ibipinibant), a CB1 receptor inverse agonist, were synthesized with functionality intended to limit brain exposure while maintaining the receptor affinity and selectivity of the parent compound. Structure activity relationships of this series, and pharmacology of two lead compounds, **16** (JD-5006) and **23** (JD-5037) showing little brain presence as indicated by tissue distribution and receptor occupancy studies, are described. Effects with one of these compounds on plasma triglyceride levels, liver weight and enzymes, glucose tolerance and insulin sensitivity support the approach that blockade of peripheral CB_1 receptors is sufficient to produce many of the beneficial metabolic effects of globally active CB_1 blockers. Thus, PR CB_1 inverse agonists may indeed represent a safer alternative to highly brain-penetrant agents for the treatment of metabolic disorders, including diabetes, liver diseases, dyslipidemias, and obesity.

The endocannabinoid system, comprised of the cannabinoid receptors (CB1 and CB2) and their endogenous ligands (primarily anandamide and 2-AG), plays a prominent role in the control of food intake[1] and energy metabolism.[2] Stimulation of this system triggers metabolic processes leading to weight gain, lipogenesis, insulin resistance, dyslipidemias and impaired glucose homeostasis. Consistent with these actions is the presence of CB1 receptors widely expressed in brain, (cortex, hippocampus, amygdala, pituitary and hypothalamus) as well as numerous peripheral organs and tissues (thyroid, adrenals, reproductive organs, adipose tissue, liver, kidney, muscle, pancreas, and gastrointestinal tract[3,4]) which can mediate these pharmacological consequences. CB2 receptors are mainly localized in blood and immune cells[2] including macrophages, and regulate the inflammatory responses in various settings.[5] Evidence does exist, however, that these receptors may also be present in the CNS.[6]

The ability of the CB1 endocannabinoid system to regulate food intake and energy expenditure has prompted extensive work on the utilization of blockers of these receptors as potential therapeutic agents. As a result of these investigations, it has been found that apart from their ability to control food intake through interaction with CB1 brain receptors, an association supported by numerous studies,[2,7] a variety of inverse agonists have been shown to influence energy utilization through modulation of glucose metabolism, as well as improving cardiometabolic risk factors associated with obesity such as HDL-cholesterol and triglycerides.[2] The effects of these inverse agonists are most likely mediated through both central and peripheral CB1 receptor interactions.[3,4,8,9] Indeed, clinical trials with Rimonabant, the first marketed inverse agonist, showed improvement in glycemic control and lipid profile in type 2 diabetic patients (SERENADE),[10] and loss of visceral, and hepatic fat in abdominally obese patients (ADAGIO).[11]

The ability of CNS-penetrant CB1-antagonists/inverse agonists to suppress food intake is generally associated with interactions that target CB1 receptors in hypothalamus and mesolimbic regions that control appetite.[12] However, CB1 receptor blockers have equal access in brain to non-targeted receptors that have little if any role in appetite control, binding to which can often lead to unwanted results.[2–4,13] Indeed, the CB1 inverse agonists Rimonabant and taranabant produce psychiatric and neurological side effects — headache, irritability, insomnia, dizziness, anxiety, seizures, depressed mood, and suicidality — which are dose-related, and appear most pronounced at doses most efficacious in reducing

* Corresponding author. Tel.: +1 484 356 3303; fax: +1 832 550 3120.
E-mail address: r.chorvat@jenrindiscovery.com (R.J. Chorvat).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.08.004

weight (see Chart 1).[14,15] The occurrence of therapeutic efficacy (appetite suppression) and side effects over the same dose range strongly suggested that both effects are mediated through concurrent interaction of CB1 receptors in both 'targeted' (hypothalamus) and 'non-targeted' (frontal cortex, hippocampus and amygdale) brain regions.[16]

To overcome the unwanted CNS liabilities associated with brain-penetrant CB1 antagonists/inverse agonists, we developed a strategy to incorporate functionality that would eliminate or diminish passive diffusion of compounds into the brain, or increase the chances that these molecules would be substrates for efflux transporters.[17] One of these active transport systems, Pgp, has been responsible for the limited brain exposure of a number of currently marketed drugs that are substrates for this transport system.[18] Utilizing the structural feature information of low brain presence agents, as well as in a reverse fashion, the conventional rules that guide medicinal chemists in producing brain-penetrant compounds,[19] we investigated the compatibility of various structural changes in different regions of the chemical lattice of the SLV-319 (Ibipinibant) molecule on receptor affinity/selectivity, as well the concomitant effects on lipophilicity, polar surface area (PSA), and brain penetration potential. To support the strategy that a CB1 receptor blocker with low brain exposure will have therapeutic potential for alleviating cardiometabolic risk in obese patients, it has recently been shown that a CB1 receptor neutral antagonist (AM 6545), largely restricted to the periphery, did not affect behavioral responses mediated by brain CB1 receptors.[20] This agent did cause weight-independent improvements in glucose homeostasis, fatty liver, and plasma lipid profile in genetic or diet-induced obese mice. Other reports of potential non-brain penetrant CB1 receptor antagonists/inverse agonists have been reported, though the absence of brain presence with concomitant effects on metabolic parameters is lacking.[24]

The calculated relative lipophilicity values of compounds were used as an initial guide because of the role this property may have in passive diffusion through the blood brain barrier as well as retaining compounds within the brain,[21] and overall PK and safety.[22] Polar surface area (PSA), defined as the surface sum over all polar atoms, (usually oxygen and nitrogen), including attached hydrogens, is also a common metric used for the estimation of passive molecular transport through membranes.[23] This property has been reported to be a basis for identifying various analogs of Rimonabant with a lowered propensity to pass the blood–brain-barrier.[24] Molecules with a PSA of >140 Å squared are usually believed to be poor at permeating cell membranes. For molecules to penetrate the blood–brain-barrier (and thus interact with receptors in the central nervous system), PSA is suggested to be less than 60 Å squared.[25] This property, along with *c*Log*P*, hydrogen bond donors and molecular weight, all factors contributing to the likelihood of a molecule penetrating the blood brain barrier and/or being absorbed from the GI tract, were collectively used as guides for identification of suitable analogs of SLV-319 for pharmacological characterization. Thus, this work was intended to identify potential therapeutic agents with desirable biological properties mediated through peripheral CB1 receptor blockade with an absence of the untoward CNS side effects that have accompanied first generation, brain-penetrant compounds currently terminated from further clinical development or removed from the market.[26]

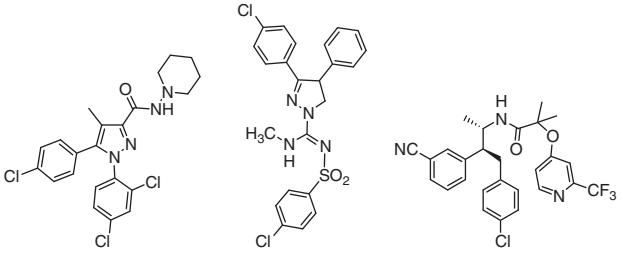
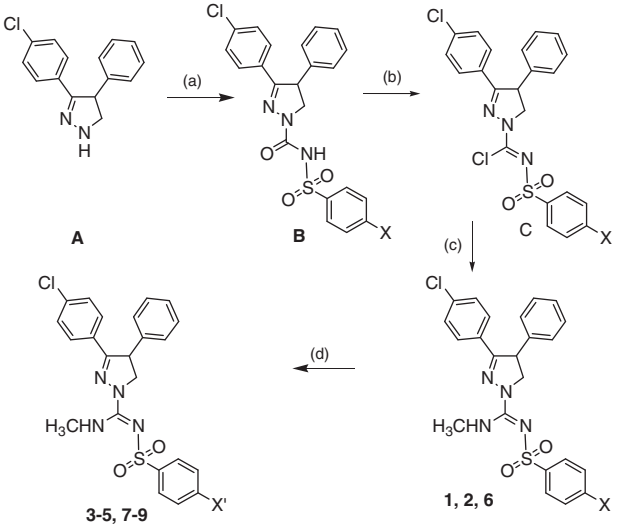


Chart 1.

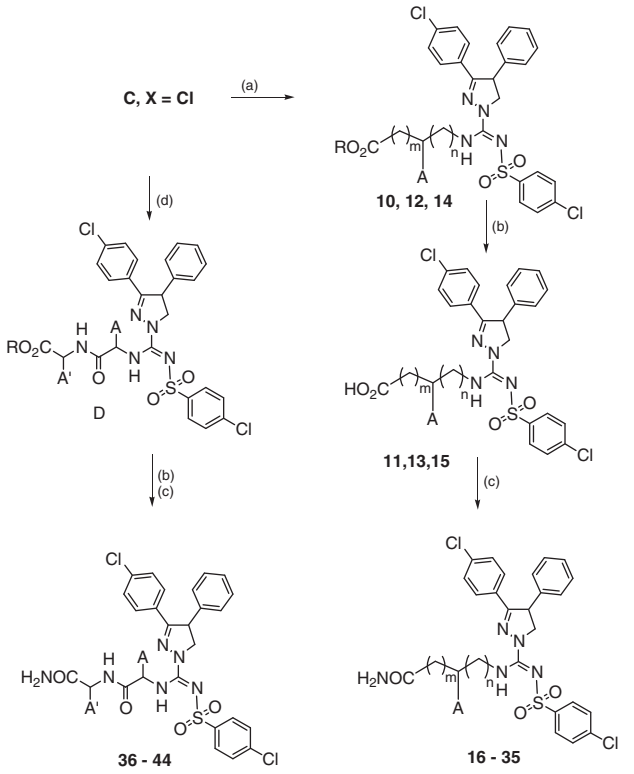
The initial target compounds, prepared to determine the effect of incorporating H-bond donor/polar groups as substituents on the phenylsulfonyl group, were synthesizd according to the sequence in Scheme 1. The diarylpyrazine **A** was prepared as previously described,[27] and treatment with various sulfonylated carbamic acid esters, which were obtained in a straightforward manner from the corresponding aryl sulfonamides and methyl chloroformate, provided the sulfonylated pyrazine-1-carboxamides **B**. Conversion of **B** to the imidoyl chlorides **C** with PCl_5 was followed by methylamine treatment to afford the sulfonylated carboxamidines **1**–**9**. Ester substituents on the phenyl ring produced the acids (**3** and **6**) upon routine hydrolysis, and conventional amination reactions gave the carboxamides and hydroxamic acids (**4**, **5** and **7**). The nitro analog was reduced to the aniline using Fe-filings in hydrochloric acid solution, and then converted to the acetamide or carbamate (**8** and **9**).

Analogs with modifications on the amino methyl group of SLV-319 could be conveniently prepared from the imidoyl chloride **C** using a large variety of single or di-amino acid esters (see Scheme 2). Further conversions of these esters of sulfonylated pyrazoline-1-carboxamidines to the amino carboxamide compounds, were achieved through conventional amino acid/carboxamide transformation methodology (compounds **10**–**44**).

Compounds were initially evaluated for their binding affinity to cannabinoid receptor sites by their ability to displace a [^{3}H] ligand from human recombinant CB1- and CB2-protein.[28] Results from these tests are shown in Tables 1–5. Compounds which showed affinity ⩾ the 100 nM screening concentration were not evaluated

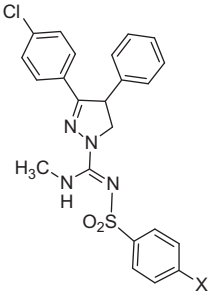


Scheme 1. Reagents and conditions: (a) $ArylSO_2NHCO_2CH_3$, toluene, reflux, 3–6 h, 30–95%; (b) PCl_5, chlorobenzene, reflux, 2–4 h, 50–90%; (c) $NH_2Me.HCl$, Et_3N, CH_2Cl_2, ice-bath to rt, 16–20 h, 30–70%; (d) X = CO_2Et/X′ = CO_2H: LiOH, H_2O/THF(1:3), 50%; X′ = CO_2H/X′ = $CONH_2$, (1) IBCF, NMM, CH_2Cl_2, (2) NH_3/THF, 60%; X′ = CO_2H/X′ = CONHOH, (1) IBCF, NMM, CH_2Cl_2, (2) $NH_2OH{\cdot}HCl$, NMM, CH_2Cl_2, 40%; X = OCH_2CO_2Et/OCH_2CO_2H: LiOH, H_2O/THF(1:3), rt, 50%; X′ = OCH_2CO_2H/X′ = OCH_2CONH_2: (1) IBCF, NMM, CH_2Cl_2, (2) NH_3/THF, 30%; X = NO_2:/X′ = NHC-OCH_3: (1) Fe/HCl, EtOH, H_2O, reflux 1–2 h, 90%; (2) Ac_2O, pyr, CH_2Cl_2, rt, 8 h, 90%; X = NO_2:/X′ = $NHCO_2Et$: (1) Fe/HCl, EtOH, H_2O, reflux 1–2 h, 90%; (2) $ClCO_2Et$, pyr, CH_2Cl_2, 0 °C to rt, 8 h, 90%.



Scheme 2. Reagents and conditions: (a) amino acid ester. HCl, Et_3N, CH_2Cl_2, 0 °C to rt, 20–90%; (b) LiOH, H_2O/THF(1:3), 30–90%; (c) 1) IBCF, NMM, CH_2Cl_2, (2) NH_3/THF, 60%; (d) diamino acid ester, Et_3N, CH_2Cl_2, 0 °C to rt, 20–90%. *m*,*n* = 0,1.

Table 1
CB1 receptor binding affinities of compounds **1–9**

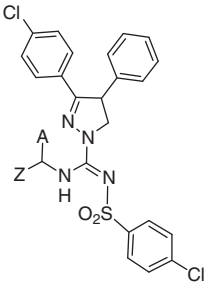


Compound	X	CB_1 IC_{50}[a] (nM)
SLV-319[c]	Cl	22[b]
1	OMe	~100
2	CO_2Et	~100
3	CO_2H	>5000
4	$CONH_2$	>5000
5	CONHOH	>5000
6	OCH_2CO_2H	>5000
7	OCH_2CONH_2	>5000
8	$NHCOCH_3$	>1000
9	$NHCO_2Et$	~300

[a] Initial screening performed at two concentrations run in duplicate; values ⩾100 nM are estimated.
[b] IC_{50} value determined from four point concn, curves in duplicate.
[c] All compounds racemic unless designated otherwise.

further. Certain compounds with high affinity for the CB1-receptor, and good selectivity over the CB2-receptor were also evaluated in the kidney cell screen that assesses membrane permeability potential by the bi-directional passage across MDR1-MDCK mono-layers

Table 2
CB1 receptor binding affinities of compounds **10–15**



Compound	A	Z	CB_1 IC_{50} (nM)
10	H	CO_2Me	88%[d]
11	H	CO_2H	>1000
12[e]	CH_3	CO_2Me	96%
13[e]	CH_3	CO_2H	>1000
14[e]	H	$CH_2CHOHCO_2Et$	53% [d]
15[e]	H	$CH_2CHOHCO_2H$	>1000

[d] Per cent inhibition at 100 nM.
[e] (*S*)-Amino acid side chain.

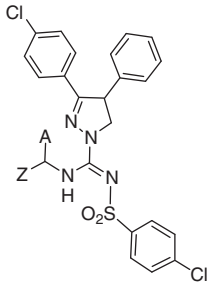
(Table 7).[29] Based on properties from these assays, two compounds JD-5006 (**16**) and JD-5037 (**23**), were selected for further in vivo characterization which included blood and brain level determinations, brain CB1 receptor occupancy, and therapeutic efficacy studies.

Incorporation of a variety of more hydrophilic, hydrogen bonding substituents in place of the chloro group at the 4-position of the phenyl-sulfonyl ring showed diminished affinity for the CB1 receptor, especially with those groups with hydrogen-bond donor properties (Table 1). Based on these early disappointing results, we focused our attention on other regions in the molecule where these types of functionalities might be compatible with high receptor affinity.

Amino ester adducts at the central (*N*-methyl) region of the SLV-319 molecule proved to be more promising since, in many cases, these neutral compounds retained the high binding affinity of the parent molecule, though the corresponding ionizable acids possessed significantly diminished affinity for the CB1 receptor (Table 2), a property shared by others series with this functionality.[24] Since these esters were expected to readily convert to the low-affinity acids in vivo, they were of no interest as drug candidates, and accurate IC_{50} values were not determined. They did, however, demonstrate the compatibility of branched-chain pendants at this position with high receptor affinity. Our attention then turned to other non-ionizable functionality that would provide properties associated with decreased brain penetration due to a reduction in passive diffusion, or participation with efflux transporters.[18,30] One of these functionalities was the carboxamido group, an obvious analog of our amino acid adducts, and a group indeed found in certain drugs, e. g., atenolol and labetolol, with low brain presence.[29] In addition, high-affinity carboxamide analogs would also provide several other physicochemical properties that should contribute to limited membrane permeability, for example, lower lipophilicity, higher polar surface area,[23] a greater number of H-bond donors,[31] and a concomitant increase in the number of solvated water molecules (desolvation energy).[32]

Table 3 shows a number of these single amino carboxamido adducts with a variety of side chains. Several of these racemic (glycine, dimethyl-gly) or diastereomeric (natural L-amino acid derived, unless indicated) molecules have binding affinities comparable to, or higher than parent, SLV-319. Of note are those high-affinity analogs with *c*Log*P*s significantly lower than the parent structure (**16**, **17**, **26**, **28**), and one, **23**, with a *c*Log*P* comparable

Table 3
CB1 receptor binding affinities and physicochemical properties of compounds **16–29**



Compound[e]	A	Z	*c*Log*P*[f]	TPSA[g] Ang[2]	CB_1 IC_{50} [a] (nM)	CB_2 IC_{50} (nM)
SLV–319[c]	H	H	5.88	74	22[b]	>5000
16	H	$CONH_2$	4.64	117	18	5000
16a	H	$CONH_2$	4.64	117	32	>5000
16b	H	$CONH_2$	4.64	117	>1000	>1000
17	H	CH_2CONH_2	4.79	117	32	>5000
18	H	$CH(CH_3)CONH_2$	5.28	117	26	>1000
19[e]	CH_3	$CONH_2$	5.13	117	20	>1000
20	$(CH_3)_2$	$CONH_2$	5.52	117	8.5	>1000
21[e]	CH_2CONH_2	$CONH_2$	3.70	—	>100	>5000
22[e]	$CH_2C_6H_5$	$CONH_2$	6.80	—	61%[d]	>5000
23[e]	$CH(CH_3)_2$	$CONH_2$	6.01	117	2	>1000
23a[h]	$CH(CH_3)_2$	$CONH_2$	6.01	117	1.5	>1000
23b[h]	$CH(CH_3)_2$	$CONH_2$	6.01	117	>1000	—
24[e]	$C(CH_3)_3$	$CONH_2$	6.55	117	2.0	>5000
25[e]	*c*-C_4H_6	$CONH_2$	5.58	117	8.3	>1000
26[e]	(2*S*,3*R*)$CHOHCH_3$	$CONH_2$	4.59	138	7	>5000
27	(2*R*,3*S*)$CHOHCH_3$	$CONH_2$	4.59	138	>100	>5000
28[e]	CH_2OH	$CONH_2$	4.27	138	13	>5000
29[e]	H	$CH_2CHOHCONH_2$	4.25	—	66	>5000

[a] Initial screening performed at two concentrations run in duplicate; values $\geqslant$100 nM are estimated.
[b] IC_{50} value determined from four point concn, curves in duplicate.
[c] All compounds racemic unless designated otherwise.
[d] Per cent inhibition at 100 nM.
[e] (S)-Amino acid side chain.
[f] Crippen's fragmentation: *J. Chem. Inf. Comput. Sci.*, **1987**, *27*, 21.
[g] Ertl, P., Rohde, B., Selzer, P., *J. Med. Chem.* **2000**, *43*, 3714.
[h] Single diastereomer: **23a** (S, ring, S, side-chain); **23b** (R, ring, S, side-chain).

to SLV-319, but with a CB1 receptor affinity more than an order of magnitude greater than SLV-319. The enantiomers of one racemic compound, **16**, were separated via chiral column chromatography, and as expected the high receptor affinity resides with a single compound (**16a**).[33] Also in the case of **23**, where L-amino acid pendant afforded a pair of diastereomers, the two components could fortuitously be separated via silica gel column chromatography and recrystallization to afford the potent diastereomer **23a** which was shown to have the same absolute configuration (S) of SLV-319 at the chiral carbon of the pyrazoline ring.[34]

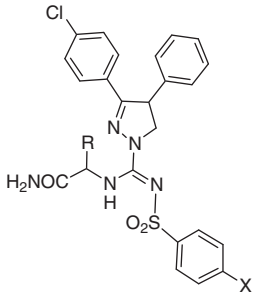
Table 4 shows analogs with other substituents on the phenylsulfonyl group of forementioned adducts with CB1 receptor affinities $\geqslant$SLV-319. In these cases, replacement of the 4-chloro-substituent with a methoxyl or hydroxyl group, regardless of the central amino acid pendant, diminished CB1 receptor binding affinity. In this limited study, the optimal substituent on the phenyl sulfonyl group of SLV-319 also seems best in the case of the amino carboxamide adducts.

Table 5 shows the binding affinities for a number of di-amino carboxamide adducts. Little difference in binding affinity was indicated from the parent compound and the gly–gly, ala–gly, and ala–ala carboxamides. The ala-ser **39** compound did show higher binding affinity, an improvement also shared with the thr-ser adduct **44**. The high PSA values of these two analogs, however, suggest general membrane permeability may be an issue.

Certain compounds were then evaluated in the MDR-MDCK cell monolayer assay. This kidney cell line has been shown to be a reasonable predictor of brain permeability, as well as an indicator of compounds that are actively participating in P-gp mediated efflux processes.[29] Generally, CNS penetrant drugs show absorptive permeability coefficients in going from apical to basal layers (Papp A–B) $\geqslant 3.0 \times 10^{-6}$ cm/s. Those drugs that have been found to have low CNS penetration show a Papp A–B of $<1.0 \times 10^{-6}$ cm/s. Compounds with intermediate values are likely to have low brain presence if the efflux rate Papp B–A exceeds the Papp A–B by more than three-fold (efflux ratio $\geqslant$ 3). Of the compounds evaluated in this assay (Table 6), we early on selected **16** and **23** as agents with potential for low brain exposure based on their Papp A–B coefficients (1.8 and 1.1, respectively), as well as high efflux ratios (34 and 15, respectively) and PSA values (both, 117 angstroms squared). They were evaluated in vivo for plasma and brain exposure, brain CB1-receptor occupancy, and therapeutic efficacy in pharmacological assays that reflect potential therapy for the regulation of metabolic dysfunction.

Racemic **16** was also characterized as an inverse agonist in forskolin-stimulated CHO cells over- expressing CB1-receptors by its ability to affect cAMP as shown in Figure 1. Its IC_{50} of 46 nM was comparable with that which we also determined for SLV-319, IC_{50} = 139 nM and Rimonabant, IC_{50} = 51 nM. Establishing this property with our lead compounds allowed us to characterize non-brain penetrant versions of clinically effective compounds,[35] and also determine if an inverse agonist would have equivalent or better properties in certain pharmacological assays than those of the neutral antagonist previously reported.[20]

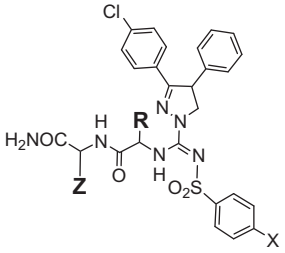
Table 4
CB1 receptor binding affinities and physicochemical properties of compounds **30–35**



Compound	R	X	*c*Log *P*[d]	TPSA[e]Ang[2]	CB_1 IC_{50} (nM)	CB_2 IC_{50} (nM)
16	H	Cl	4.64	117	18[b]	>1000
30	H	OCH_3	3.95	127	~100[a]	>5000
31	H	OH	3.68	139	>100	.5000
32[c]	CH_3	Cl	5.13	117	20	>1000
33[c]	CH_3	OCH_3	4.44	127	70	>5000
34[c]	CH_3	OH	—	139	>500	>5000
23a	$CH(CH_3)_2$ (*S*,*S*)	Cl	6.01	117	0.5	>1000
35[c]	$CH(CH_3)_2$	3-OCH_3	5.33	127	4	>1000

[a] Initial screening performed at two concentrations run in duplicate; values ⩾100 nM are estimated.
[b] IC_{50} value determined from four point concn, curves in duplicate.
[c] (S)-Amino acid side chain.
[d] Crippen's fragmentation: *J. Chem. Inf. Comput. Sci.*, **1987**, *27*, 21.
[e] Ertl, P., Rohde, B., Selzer, P., *J. Med. Chem.* **2000**, *43*, 3714.

Table 5
CB1 receptor binding affinities and physicochemical properties of compounds **36–44**



Compound	X	R[c]	Z[c]	*c*Log *P*[d]	TPSA[e]Ang[2]	CB_1 IC_{50} (nM)	CB_2 IC_{50} (nM)
36	Cl	H	H	3.49	146	25[b]	>5000
37[c]	Cl	CH_3	H	3.98	148	10	>1000
38[c]	Cl	CH_3	CH_3	4.47	—	27	>5000
39[c]	Cl	CH_3	CH_2OH	3.48	167	8	>5000
40[c]	OMe	CH_3	CH_2OH	3.62	—	>100[a]	>5000
41[c]	Cl	$CH(CH_3)_2$	H	4.87	—	>100	>1000
42[c]	OMe	$CH(CH_3)_2$	H	4.18	—	>100	>5000
43[c]	Cl	$CH(CH_3)_2$	CH_3	5.36	—	~100	>5000
44[c]	Cl	$CH(CH_3)OH$	CH_2OH	3.08	187	7	>1000

[a] Initial screening performed at two concentrations run in duplicate; values ⩾100 nM are estimated.
[b] IC_{50} value determined from four point concn, curves in duplicate.
[c] (S)-Amino acid side chain.
[d] Crippen's fragmentation: *J. Chem. Inf. Comput. Sci.*, **1987**, *27*, 21.
[e] Ertl, P., Rohde, B., Selzer, P., *J. Med. Chem.* **2000**, *43*, 3714.

Plasma and brain levels of **16a**,[36] **23**[37] and SLV-319 in chow fed mice were determined after oral administration of 100 mg/kg doses of compound over a 3 day period.[38] As shown in Table 7, high plasma levels of administered compound were detected when analysis was performed 1 h after administration of the last dose. While the plasma levels of **16** are even greater than SLV-319 at this dose, the total brain levels are only a fraction of that observed for the parent compound. Similarly with **23**, plasma levels also approach that of the parent at this high dose, but brain level remains below the level of detection (LOD) in this assay. Tissue distribution levels of Rimonabant are also included, though it was administered at a lower (20 mg/kg) dose. As has been previously reported, Rimonabant shows greater brain pentrance relative to SLV-319.[39]

To confirm negligible if any brain presence of these compounds, they were also evaluated for CB1 receptor occupancy in hippocampal and cerebellular regions of mice after administering doses of 3 or 30 mg/kg, PO, over a 3 day period with tissues collected 1 h after the last dose. In this study, shown in Table 8, SLV-319 and Rimonabant showed total displacement of [^{3}H] SR-141716A at 30 mg/kg in both brain regions, whereas, **16a**, and **23a** showed little displacement of radio ligand in these two brain sections under these conditions at this dose. The variability of this assay (5 animals

Table 6
MDR-MDCK mono layer cell transport of selected compounds.

Compound	R	Papp($\times 10^{-6}$ cm/s) [c]		Efflux ratio
		A–B	B–A	
SLV319[a]	CH_3	9.45	10.0	~1
16	CH_2CONH_2	1.80	60.5	34
36	$CH_2CONHCH_2CONH_2$	0.20	22.3	112
17	$CH_2CH_2CONH_2$	0.88	58,0	66
26[b]	$CHCHOHCH_3CONH_2$	<0.9	18.6	>20
24[b]	$CH[C(CH_3)_3]CONH_2$	0.7	<0.1	–
19[b]	$CHCH_3CONH_2$	2.44	9.25	3.8
23[b]	$CH[CH(CH_3)_2]CONH_2$	1.08	16.6	15
38[b]	$CHCH_3CONHCHCH_3CONH_2$	0.3	nt	–

[a] All compounds racemic unless designated otherwise.
[b] (S)-Amino acid side chain.
[c] Ref. 29.

Table 7
100 mg/kg of SLV-319, **16a** and **23** and 20 mg/kg of Rimonabant PO for 3 consecutive days in Chow-fed Mice

Compound	PO dose (mg/kg)	Avg plasma(ng/mL)	Avg brain(ng/gm)	% Brain versus plasma
SLV-319	100	5132	2083	41
16a	100	16693	685	4
23	100	3849	<LOD	<1
Rimonabant	20	1203	1075	89

per dose) accounts for the displacement seen at the 3 mg/kg dose in the hippocampal regions of the brain.

We further evaluated compound **16** in vivo to determine efficacy on a number of cardiometabolic parameters. Figure 2 shows the effects of SLV-319 and **16** on triglyceride levels after dosing orally at 3, 10 and 30 mg/kg for 21 days in DIO mice. In this assay both compounds showed maximal efficacy in normalizing triglyceride levels to vehicle controls at the lowest dose tested. Paralleling this effect was a concomitant normalization in terminal liver weights with a maximal effect again achieved at the lowest dose tested as shown in Figure 3. The salutary effects on liver were also indicated by normalization of the terminal ALT (alanine amino transferase) levels after this dosing period as indicated in Figure 4. ALT is an enzyme that often reflects abnormal liver function due to liver cell damage, or conditions predisposing the liver toward this state. In all figures, *p*-values for the various doses are indicated in each bar or column.

Rimonabant and **16** were also evaluated in a model of diabetes where mice, maintained on a high fat diet for 14 weeks, were administered PO 20 mg/kg of test compound for seven days then challenged with a 2 gm/kg of body-weight dose of glucose. The ability of these animals to tolerate this glucose challenge was significantly improved by the more rapid elimination of glucose from the animals treated with both **16** and Rimonabant (Fig. 5). In addition, in this same test group of animals both compounds enhanced insulin sensitity as indicated by the diminshed levels of insulin that were present during the glucose tolerance testing (Fig. 6).

In summary, we have prepared novel SLV-319 analogs with functionality that can diminish brain exposure while maintaining high potency and selectivity for the CB1 receptor. Based on physicochemical properties, we chose to study two analogs to establish whether a compound with little or no brain exposure would mimic the pharmacological profile of known brain penetrant CB1 inverse agonists without the potential to cause neuropsychiatric side effects. Tissue concentration and CB_1 receptor occupancy studies showed minimal brain levels of **16** and **23**[40] when compared to the known brain penetrant compounds. However, in spite of this lack of direct brain receptor interaction, **16** was shown to normalize triglyceride levels, liver mass and ALT as well demonstrating a beneficial effect on glycemic control. Our results support the hypothesis that blockade of peripheral CB_1 receptors may be

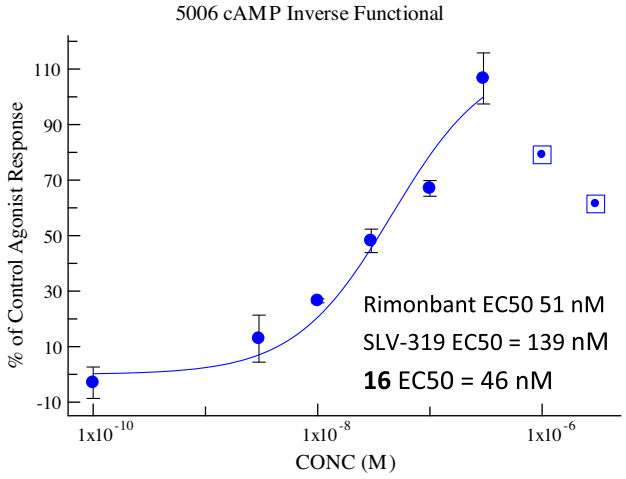


Figure 1. Inverse agonist activity of **16**, Rimonabant and SLV-319.

Table 8
CB1 Receptor Occupancy in Mouse Brain of Rimonbant, SLV-319, **16a** and **23a**

Compound	Brain region	% Occupancy	
		3 mg/kg	30 mg/kg
Rimonabant	Hippocampus	10[a]	100
SLV-319	Hippocampus	15	100
16a	Hippocampus	10[a]	3.0
23a	Hippocampus	10[a]	0
Rimonabant	Cerebellum	12	87
SLV-319	Cerebellum	15	102
16a	Cerebellum	0	0
23a	Cerebellum	0	0

Chow fed mice were administered compounds once daily at 3 or 30 mg/kg PO for 3 consecutive days, and euthanized one hour after the last dose. Thirty minutes before euthanization, animals were given an injection of [3H]SR141716A (Amersham Biosciences; 2 μCi in 0.1 ml water) via the lateral tail vein.[40]
[a] Indicative of the variability of this assay with only 5 animals per dose.

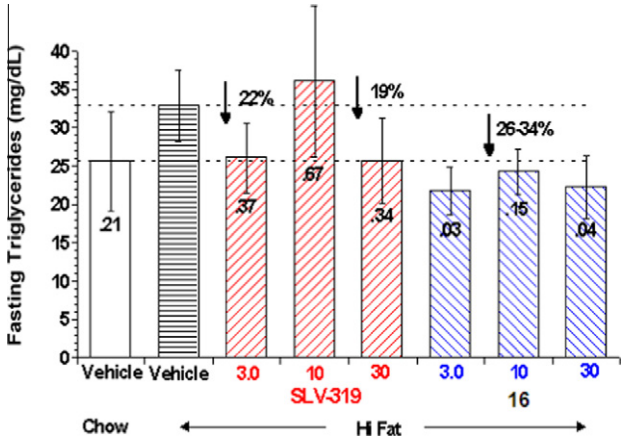


Figure 2. Terminal fasting triglyceride levels after oral PO dosing of SLV-319 and **16** for 21 consecutive days at 3, 10 and 30 mg/kg in DIO mice.

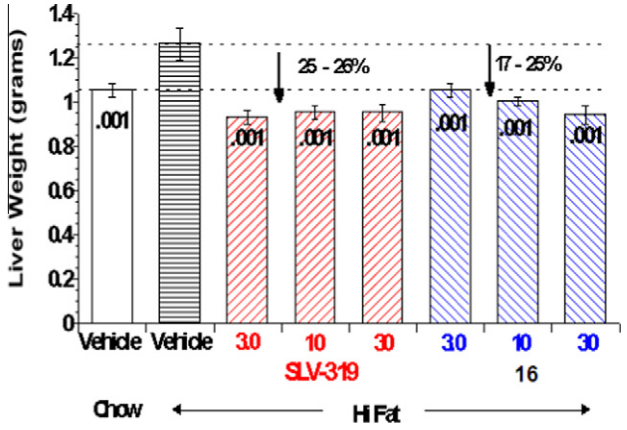


Figure 3. Terminal liver weights after after PO dosing of SLV-319 and **16** for 21 consecutive days at 3, 10 and 30 mg/kg in DIO mice.

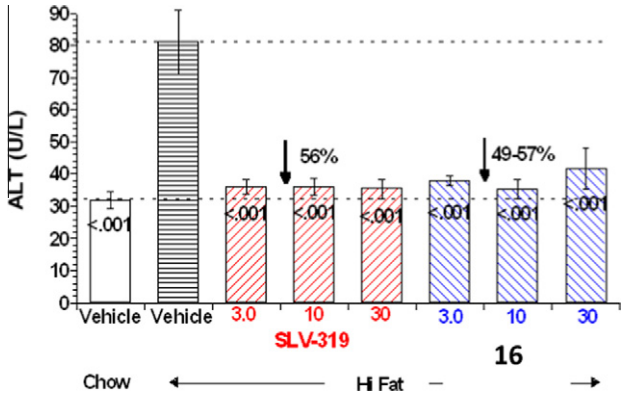


Figure 4. Terminal ALT (alanine aminotransferase) levels after PO dosing of SLV-319 and **16** for 21 consecutive days at 3, 10 and 30 mg/kg in DIO mice.

sufficient to produce many of the beneficial metabolic effects of globally active CB_1 inverse agonists. PS CB_1 inverse agonists with little or no access to the brain may indeed represent a safer alternative to highly brain-penetrant CB_1 inverse agonists for the treatment of metabolic disorders, including diabetes, dyslipidemias, liver diseases, and obesity.[41]

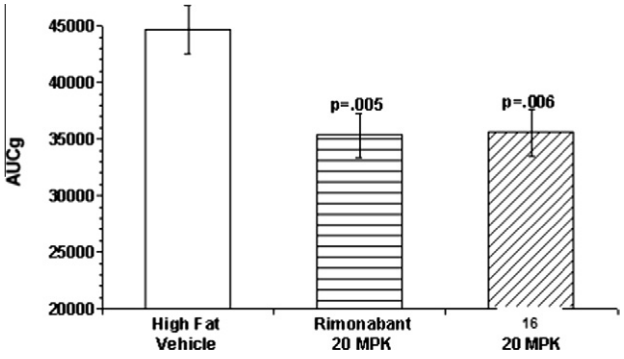


Figure 5. Effects of Rimonabant and **16** on glucose tolerance.

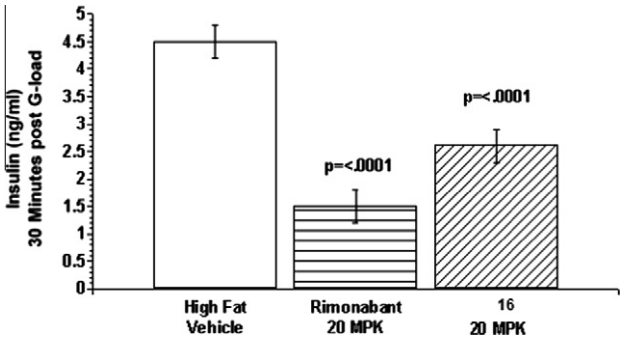


Figure 6. Effects of Rimonabant and **16** on insulin sensitivity.

Acknowledgments

We wish to acknowledge and thank the chemists at SAR Life Sciences, Hangzhou, China, for compound synthesis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.004.

References and notes

1. (a) Salamone, J. D.; McLaughlin, P. J.; Sink, K.; Makriyannis, A.; Parker, L. A. *Physiol. Behav.* **2007**, *91*, 383; (b) Di Marzo, V. *Pharmacol. Res.* **2009**, *60*, 77.
2. Pagotto, U.; Marsicano, G.; Cota, D.; Lutz, B.; Pasquali, R. *Endocr. Revs.* **2006**, *27*, 73.
3. Di Marzo, V. *Drug Discovery Today* **2008**, *13*, 1026.
4. Akbas, F.; Gasteyger, C.; Sjodin, A.; Astrup, A.; Larsen, T. M. *Obes. Rev.* **2009**, *10*, 58.
5. Klein, T. W. *Nat. Rev. Immunol.* **2005**, *5*, 400.
6. (a) Mackie, K. J. *Neuroendocrinology* **2008**, *20*, 10; (b) Onaivi, E. S.; Ishiguro, H.; Gong, J.-P.; Patel, S.; Perchuk, A.; Meozzi, P. A.; Myers, L.; Mora, Z.; Tagliaferro, Z.; Gardner, P. E.; Brusco, A.; Askinshola, B. E.; Liu, Q.-R.; Hope, B.; Iwasaki, S.; Arinami, T.; Teasenfitz, L.; Uhl, G. R. *Ann. N.Y. Acad. Sci.* **2006**, *1074*, 514; (c) Gong, J.-P.; Onaivi, E. S.; Ishiguro, H.; Liu, Q.-R.; Tagliaferro, P. A.; Brusco, A.; Uhl, G. R. *Brain Res.* **2006**, *1071*, 10.
7. Lee, H.-K.; Choi, E. B.; Pak, C. S. *Curr. Top. Med. Chem.* **2009**, *9*, 482.
8. Scheen, A. J.; Paquot, N. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 103.
9. Samaha, F. F.; Chou, C. M. *Obesity* **2008**, *17*, 220.
10. Rosenstock, J.; Hollander, P.; Chevalier, S.; Iranmanesh, A. *Diabetes Care* **2008**, *31*, 2169.
11. Després, J.-P.; Ross, R.; Boka, G.; Alméras, N.; Lemieux, I. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 416.
12. Ravinet Trillou, C.; Arnone, M.; Delgorge, C.; Gonalons, N.; Keane, P.; Maffrand, J. P.; Soubrié, P. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284*, R345.

13. Moreira, F. A.; Greib, M.; Lutz, B. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 133.
14. Pi-Sunyer, F. X.; Aronne, L. J.; Heshmati, H. M.; Devin, J.; Rosenstock, J. *JAMA* **2006**, *295*, 761.
15. Addy, C.; Wright, H.; Van Laere, K.; Gantz, I.; Erondu, N.; Musser, B. J.; Lu, K.; Yuan, J.; Sanabria-Bohórquez, S. M.; Stoch, A.; Stevens, C.; Fong, T. M.; De Lepeleire, I.; Cilissen, C.; Cote, J.; Rosko, K.; Gendrano, I. N.; Nguyen, A. M.; Gumbiner, B.; Rothenberg, P.; De Hoon, J.; Bormans, G.; Depré, M.; Eng, W.; Ravussin, E.; Klein, S.; Blundell, J.; Herman, G. A.; Burns, H. D.; Hargreaves, R. J.; Wagner, J.; Gottesdiener, K.; Amatruda, J. M.; Heymsfield, S. B. *Cell Metab.* **2008**, *7*, 68.
16. Viveros, M. P.; Marco, E. M.; Llorente, R.; López-Gallardo, M. *Neural Plast.* **2007**, 52908.
17. Schinkel, A. H.; Wagenaar, E.; Mol, C. A.; van Deemter, L. *J. Clin. Invest.* **1996**, *97*, 2517.
18. Chen, C.; Hanson, E.; Watson, J. W.; Lee, J. S. *Drug Metab. Dispos.* **2003**, *31*, 312.
19. (a) Hitchcock, S. A.; Pennington, L. D. *J. Med. Chem.* **2006**, *49*, 7559; (b) Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. *ACS Chem. Neurosci.* **2010**, *1*, 420.
20. Tam, J.; Kiran Vemuri, V.; Liu, J.; Batkai, S.; Mukhopadhyay, B.; Godlewski, G.; Osei Hyiaman, D.; Ohnuma, S.; Ambudkar, S. V.; Pickel, J.; Makriyannis, A.; Kunos, G. *J. Clin. Invest.* **2010**, *120*, 2953.
21. Liu, X.; Chen, C.; Smith, B. J. *J. Pharm. Exp. Ther.* **2008**, *325*, 349.
22. (a) Price, D. A.; Blagg, J.; Jones, L.; Greene, N.; Wagner, T. *Expert Opin. Drug Metabol. Tox.* **2009**, *5*, 921; (b) Hughes, J. D.; Blagg, J.; Price, D. A.; Bailey, S.; DeCrescenzo, G. A.; Devra, R. V.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Greene, N.; Huang, E.; Krieger-Burke, T.; Loesel, J.; Wager, T.; Whiteley, L.; Zhang, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4872.
23. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
24. (a) Cooper, M.; Receveneur, J.-M.; Bjurling, E.; Norregaard, P. K.; Nielsen, P. A.; Skold, N.; Hogberg, T. *Biorg. Med. Chem. Lett.* **2010**, *20*, 26; (b) Receveur, J.-M.; Murray, A.; Linget, J.-M.; Nørregaard, P. K.; Cooper, M.; Bjurling, E.; Nielsen, P. A.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 453; (c) Hortala, L.; Rinaldi-Carmona, M.; Congy, C.; Boulu, L.; Sadoun, F.; Fabre, G.; Finance, O.; Bart, F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4573; (d) Sasmal, P. K.; Reddy, D. S.; Talwar, R.; Ventkatesham, B.; Balasubrahmanyam, D.; Kannan, M.; Srinivas, P.; Kumar, K. S.; Devi, B. N.; Jadhav, V. P.; Khan, S. K.; Mohan, P.; Chaudhury, H.; Bhuniya, D.; Iqbal, J.; Chakrabarti, R. *Biorg. Med. Chem. Lett.* **2011**, *21*, 562; (e) Fulp, A.; Bortoff, K.; Seltzman, H.; Zhang, Y.; Mathews, J.; Snyder, R.; Fennel, T.; Maitra, R. *J. Med. Chem.* **2012**, *55*, 2820.
25. Palm, K.; Stenberg, P.; Luthman, K.; Artursson, P. *Pharm. Res.* **1997**, *14*, 568.
26. Jones, D. *Nat. Revs./Drug Discov.* **2008**, *7*, 961.
27. Lange, J. H.; Coolen, H. K.; van Stuivenberg, H. H.; Dijksman, J. A.; Herremans, A. H.; Ronken, E.; Keizer, H. G.; Tipker, K.; McCreary, A. C.; Veerman, W.; Wals, H. C.; Stork, B.; Verveer, P. C.; den Hartog, A. P.; de Jong, N. M.; Adolfs, T. J.; Hoogendoorn, J.; Kruse, C. G. *J. Med. Chem.* **2004**, *47*, 627.
28. (a) Rinaldi-Carmona, M.; Calandra, B.; Shire, D.; Bouaboula, M.; Oustric, D.; Barth, F.; Casellas, P.; Ferrara, P.; Le Fur, G. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 871; (b) Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
29. Wang, Q.; Rager, J. D.; Weinstein, K.; Kardos, P. S.; Dobson, G. L.; Li, J.; Hidalgo, I. J. *Int. J. Pharm.* **2005**, *288*, 349.
30. Feng, B.; Mills, J. B.; Davidson, R. E.; Mireles, R. J.; Janiszewski, J. S.; Troutman, M. D.; de Morais, S. M. *Drug Metab. Dispos.* **2008**, *36*, 268.
31. Lipinski, C. A. *Drug Discovery Today: Technol.* **2004**, *1*, 337.
32. Chikhale, E. G.; Ng, K.-Y.; Burton, P. S.; Borchardt, T. R. *Pharm. Res.* **1999**, *11*, 412.
33. Separation was performed by Daicel Chiral Technologies, Inc., (China) with a ChiralPak 1A column using methanol as the mobile phase; HPLC: Shimadzu LC 20 with UV detector SPD-20A. Peak separation was >99%.
34. Absolute configuration of **23a** (S,S) was determined by Jeffrey R. Deschamps, Naval Research Laboratory, Washington, DC, via single-crystal X-ray diffraction data which has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 891288.
35. Felder, C. C.; Joyce, K. E.; Briley, E. M.; Glass, M.; Mackie, K. P.; Fahey, K. J.; Cullinan, G. J.; Hunden, D. C.; Johnson, D. W.; Chaney, M. O.; Koppel, G. A.; Brownstein, M. J. *Pharm. Exp. Therap.* **1998**, *284*, 29.
36. *Analytical data for 3-(4-chlorophenyl)-N′-[(4-chlorophenyl)sulfonyl]-N-(aceetamide)-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamidine **(16a)**:* ^{1}H NMR (400 MHz, $CDCl_3$) 3.99 (1H, dd, CH), 4.40 (2H, m, CH_2), 4.45 (1H, t, CH), 4.65 (1H, dd, CH), 5.80 (1H, br d s, NH), 6.40 (1H, br d s, NH), 7.09–7.85 (13H, aromatic Hs).
37. *Analytical data for 3-(4-chlorophenyl)-N′-[(4-chlorophenyl)sulfonyl]-N-[(isopropyl)acetamide)-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamidine **(23a)**:* ^{1}H NMR (400 MHz, CD_3OD) 1.02 (6H, d, i-Pr), 2.26 (1H, br d s), 4.15 (1H, br d m), 4.48 (1H, br d m), 4.67 (1H, br d m), 4.89 (1H, br d m), 7.18–7.88 (13H, aromatic Hs); ESI$^+$ MS exact mass calculated for $C_{27}H_{27}Cl_2N_5O_3S$, 571.12; found 572.1.
38. Plasma calibration samples were prepared by five-fold serial dilutions in DMSO followed by dilution to final concentrations in either plasma or brain homogenate (starting at 1000 nM going to 1.6 nM). Immediately following dilution of the samples after thawing on ice, the protein was precipitated from the samples by the addition of a 3× volume of ice cold acetonitrile containing internal standard (IS). These samples containing precipitated protein were centrifuged at 3750 RPM, for 15 min, at 4 °C. The supernatants were then analyzed by LC/MS/MS using either an Agilent 6410 mass spectrometer coupled with an Agilent 1200 HPLC and a CTC PAL chilled autosampler, all controlled by MassHunter software (Agilent), or an ABI2000 mass spectrometer coupled with an Agilent 1100 HPLC and a CTC PAL chilled autosampler, all controlled by Analyst Software (ABI). After separation on a C18 reverse phase HPLC column (Agilent, Waters, or equivalent) using an acetonitrile–water gradient system, peaks were analyzed by mass spectrometry (MS) using ESI ionization in MRM mode.
39. Need, A. B.; Davis, R. J.; Alexander-Shacko, J, T.; Eastwood, B.; Chernet, E.; Phebus, L. A.; Sindelar, D. K.; Nomikos, G. G. Psychopharmacology (Berlin, Ger.) **2006**, *184*, 26.
40. Animals were sacrificed by decapitation and a blood sample (0.2–0.4 ml) taken at the time of sacrifice, mixed with EDTA and centrifuged to obtain plasma. The hippocampus, cerebellum, brain stem and a sample of liver were dissected out, weighed and digested in 0.5 ml Solvable™. Once digested, the tissue samples to be counted were mixed with 10 ml scintillation cocktail (Ultima Gold™) and counted in a liquid scintillation counter. Tails were digested overnight in 1 M NaOH and a sample of the digested tissue counted to confirm successful injection of the radiotracer. *Data analysis:* Hippocampus and cerebellum (receptor regions) and brain stem (reference region) counts from the scintillation counter were converted to DPM/mg. The CB1 receptor binding potential was obtained for each animal as Receptor region (DPM/mg)–Reference region (DPM/mg)/Reference region (DPM/mg). Once the data from all animals was collected, an estimate of receptor occupancy for each drug treatment was obtained from the binding potential values as follows: Receptor occupancy (%)* = 100× (CB1 binding potential in vehicle animals − CB1 binding potential in drug animals)/(CB1 binding potential in vehicle animals).
41. A manuscript describing a more comprehensive profile of the pharmacology and mechanism of action of **23a** has recently been published: Tam, Joseph; Cinar, Resat; Liu, Jie; Godlewski, Gregorz; Wesley, Daniel; Jourdan, Tony; Szanda, Gergo; Mukhopadhyay, Bani; Chedester, Lee; Liow, Jeih.-San; Innis, Robert. B.; Cheng, Kejun; Rice, Kenner. C.; Deschamps, Jeffrey. R.; Chorvat, Robert. J.; McElroy, John. F.; Kunos, George *Cell Metabolism* **2012**, *16*, 1–13. http://dx.doi.org/10.1016/j.cmet.2012.07.002.