Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 673–678
Bioorganic & Medicinal Chemistry Letters

Constrained analogs of CB-1 antagonists: 1,5,6,7-Tetrahydro-4H-pyrrolo[3,2-c]pyridine-4-one derivatives

Roger A. Smith,[a,*] Zahra Fathi,[b] Su-Ellen Brown,[b] Soongyu Choi,[a] Jianmei Fan,[a] Susan Jenkins,[c] Harold C. E. Kluender,[a] Anish Konkar,[b] Rico Lavoie,[a] Ronald Mays,[c] Jennifer Natoli,[b] Stephen J. O'Connor,[a] Astrid A. Ortiz,[b] Brent Podlogar,[a] Christy Taing,[b] Susan Tomlinson,[a] Theresa Tritto[b] and Zhonghua Zhang[a]

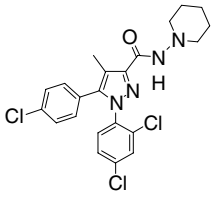
[a]*Department of Chemistry Research, Bayer HealthCare, Pharmaceuticals Division, West Haven, CT 06516, USA*
[b]*Department of Metabolic Disorders Research, Bayer HealthCare, Pharmaceuticals Division, West Haven, CT 06516, USA*
[c]*Department of Research Technologies, Bayer HealthCare, Pharmaceuticals Division, West Haven, CT 06516, USA*

Received 29 September 2006; revised 27 October 2006; accepted 30 October 2006
Available online 2 November 2006

Abstract—A series of pyrrolopyridinones was designed and synthesized as constrained analogs of the pyrazole CB-1 antagonist rimonabant. Certain examples exhibited very potent hCB-1 receptor binding affinity and functional antagonism with K_i and K_b values below 10 nM, and with high selectivity for CB-1 over CB-2 (>100-fold). A representative analog was established to cause significant appetite suppression and reduction in body weight gain in industry-standard rat models used to develop new therapeutics for obesity.

The prevalence of excessive body weight and obesity has dramatically increased over the last 30 years, apparently the result of an increasingly sedentary lifestyle, as well as the plentiful availability of energy-rich food.[1] Indeed, the World Health Organization has declared human obesity to be one of the most significant health problems facing mankind, with more than 250 million obese patients (body mass index (BMI) $\geqslant$ 30) around the world, including epidemic proportions in the US and Europe. During the last decade, antagonism of the cannabinoid type 1 receptor (CB-1) has emerged as a highly promising strategy for the treatment of obesity.[2] The 1,5-diarylpyrazole rimonabant (**1**, SR-141716, Sanofi-Aventis) is the most advanced CB-1 antagonist, and this compound was recently approved in the European Union for the treatment of obese or overweight patients with associated risk factors, such as type 2 diabetes or dyslipidemia.[3]

A large proportion of the structure classes investigated as CB-1 antagonists consist of a five- or six-membered heterocyclic core, substituted by two aromatic rings and a hydrogen bond donor/acceptor functionality such as a carboxamide group.[2] 1,5-Diarylpyrazoles, as exemplified by rimonabant (**1**), represent the first series of CB-1 antagonists to be disclosed having this pharmacophore.[4] Several research groups have reported compounds that incorporate conformational constraints into the antagonist structure. Such compounds have the potential for increased binding affinity resulting from an entropic advantage for the bioactive conformation, and improved pharmacokinetics due to altered resistance to metabolizing enzymes. Tricyclic derivatives

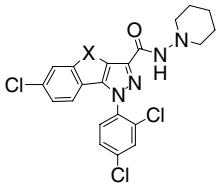


1 rimonabant
(SR-141716, Acomplia™)
hCB-1 Ki = 5.6 nM[4a], 25 nM[5a]
mCB-1 Ki = 1.8 nM[5d]

Keywords: Cannabinoid; Antagonist; Obesity; Appetite suppressant.
* Corresponding author. Tel.: +1 203 812 2154; fax: +1 203 812 6182; e-mail: roger.smith.b@bayer.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.10.095

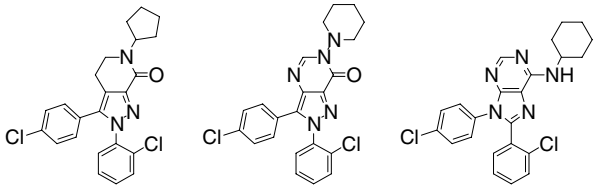
of **1** having a constraint between the pyrazole core and the 5-aryl group have been reported, for example (e.g., **2a**–**c**).[5] Compounds having a bicyclic core that effectively incorporates a constraint between the pyrazole core C-4 position and the hydrogen bond donor/acceptor moiety have also been described (e.g., **3**–**5**).[6]



2a, X = CH_2, mCB-1 Ki = 2050 nM[5d]

2b, X = $(CH_2)_2$, mCB-1 Ki = 14.8 nM[5d]

2c, X = $(CH_2)_3$, mCB-1 Ki = 0.35 nM[5d],
hCB-1 Ki = 126 nM[5a]

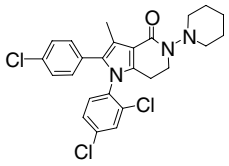


3 **4** **5**

4: hCB-1 Ki = 12 nM[6b]

As part of our research investigations on CB-1 antagonists, we conducted computational studies on **1** and concluded that the preferred conformation would have a *trans*-amide with the carboxamide oxygen nearly coplanar with the pyrazole ring and oriented in the same direction as the pyrazole C-4 methyl group (Fig. 1a).[7] Further modeling studies indicated that a constrained analog of this conformation could be readily achieved by linking the pyrazole *N*-2 position via an ethylene bridge to the carboxamide nitrogen. An overlay of the low-energy conformation of **1** with this type of constrained derivative (**6**), having a 1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one scaffold, is represented in Figure 1b.

Subsequent to our studies, an X-ray structure and conformational studies of **1** were reported,[8] and these were consistent with the low-energy conformation that we had determined (Fig. 1a). Furthermore, computational studies reported for **1** docked to a model of the CB-1 receptor protein suggested that this same conformation represented the bioactive conformation of **1**, with a key hydrogen bond from the carboxamide oxygen in **1** to a lysine residue at protein position K 3.28.[8,9] Also of interest, this model indicated that the carboxamide nitrogen in **1** was not involved in a hydrogen bond—thereby supporting the design of our constrained analog **6**.



6

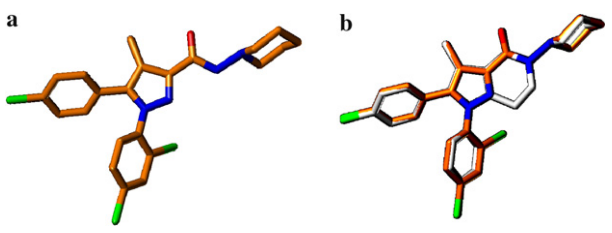
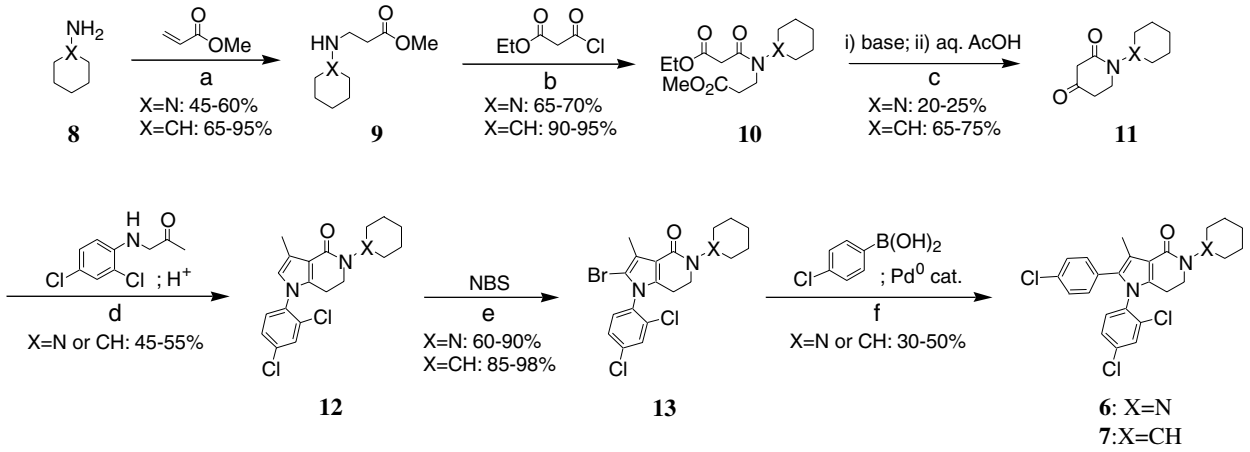


Figure 1. (a) Low-energy conformation of **1**. (b) Overlay of the low-energy conformation of **1** with the 1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one derivative **6**.

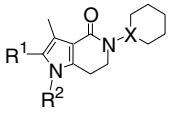
A novel and efficient synthesis of 1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-ones such as **6** and the related *N*-cyclohexyl amide **7** was developed, as depicted in Scheme 1. This protocol involved the Michael addition of an amine or hydrazine (**8**) to an acrylic ester to provide the 3-amino ester (**9**), followed by *N*-acylation with a malonyl chloride in the presence of base to afford a diester amide (**10**). This diester amide was cyclized by a Dieckmann condensation to give an intermediate[10] that was hydrolyzed and decarboxylated under acidic conditions to give a ketopiperidinone (**11**). Condensation with an amino ketone provided the key bicyclic pyrrolopyridinone intermediate **12**. The introduction of a substituent on the pyrrole core group was accomplished by bromination to give **13**, followed by a palladium-catalyzed coupling reaction with an organoborane. This methodology was successfully applied with some slight modifications to produce more than fifty pyrrolopyridinone derivatives.[11] For a variety of constrained amide analogs (e.g., **7**) the overall synthesis yield was typically 10–15% (6 steps), whereas for the related hydrazides (e.g., **6**) the overall yield was considerably lower (1–3%) (Scheme 1).

In an in vitro hCB-1 (human CB-1) binding assay, the target hydrazide analog **6** was determined to have hCB-1 K_i = 2.2 nM (Table 1).[12] This potency is very similar to that observed in our assay for **1** (K_i = 1.1 nM), validating the design of **6** as a constrained mimic of the bioactive conformation of **1**. The potency of **6** is also consistent with the rimonabant/CB-1 binding model,[7,8] which indicates that the carboxamide NH is not required for H-bonding to the receptor. Analog **6** was also found to function as an antagonist or inverse agonist,[15] with hCB-1 K_b = 12 nM, and was also determined to be highly selective for CB-1 over CB-2 (hCB-2 K_i = 1300 nM). The constrained cyclohexyl amide analog **7** was also found to be a potent hCB-1 antagonist, with hCB-1 K_i = 7.5 nM and K_b = 6.5 nM, and exhibiting high selectivity against CB-2 (hCB-2 K_i = 8300 nM). In



Scheme 1. Synthesis of 1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-ones such as **6** and **7**. Reagents and conditions: (a) MeOH, 0 °C, then rt, 16 h; (b) Et_3N, CH_2Cl_2, 0 °C, then rt, 16 h; (c) i—X = N: DMF/THF, Cs_2CO_3, 77 °C, 48 h; X = CH: NaH, cyHex/toluene, reflux, 3 h; ii—10% aq AcOH, reflux, 1–2 h; (d) toluene, TsOH, H_2O trap, 6–24 h; (e) *N*-bromosuccinimide (NBS), DMF, 0 °C, 1 h; (f) 0.1 equiv $Pd(PPh_3)_4$, DME, aq Na_2CO_3, 60 °C, 16–18 h.

Table 1. Binding affinities of pyrrolopyridinone compounds to the human CB-1 receptor



Compound	R^1	R^2	X	hCB-1 K_i (nM)[a]
6	4-Cl–Ph	2,4-Cl_2–Ph	N	2.2 ± 0.40
7	4-Cl–Ph	2,4-Cl_2–Ph	CH	7.5 ± 0.97
14	4-Cl–Ph	4-Cl–Ph	CH	>2500
15	2,4-Cl_2–Ph	4-Cl–Ph	CH	>2500
16	Ph	2,4-Cl_2–Ph	CH	16 ± 3.5[b]
17	4-F–Ph	2,4-Cl_2–Ph	CH	6.0 ± 0.35
18	4-CH_3–Ph	2,4-Cl_2–Ph	CH	10 ± 2.6
19	4-CF_3–Ph	2,4-Cl_2–Ph	CH	4.1 ± 0.60
20	4-CH_3O–Ph	2,4-Cl_2–Ph	CH	7.9 ± 1.6[b]
21	4-CH_2=CH–Ph	2,4-Cl_2–Ph	CH	9.8 ± 2.8[b]
22	4-NH_2–Ph	2,4-Cl_2–Ph	CH	920 ± 77
23	4-HOOC–Ph	2,4-Cl_2–Ph	CH	>2500
24	4-CH_3S–Ph	2,4-Cl_2–Ph	CH	>2500
25	3-CH_3–Ph	2,4-Cl_2–Ph	CH	17 ± 0.50[b]
26	3-CH_3O–Ph	2,4-Cl_2–Ph	CH	6.8 ± 1.6[b]
27	3,4-$(CH_3O)_2$–Ph	2,4-Cl_2–Ph	CH	5.5 ± 1.5
28	3,4-(OCH_2O)–Ph	2,4-Cl_2–Ph	CH	12 ± 3.1[b]
29	3-CF_3–Ph	2,4-Cl_2–Ph	CH	67 ± 13[b]
30	3-NH_2–Ph	2,4-Cl_2–Ph	CH	450 ± 108
31	3-Thienyl	2,4-Cl_2–Ph	CH	37 ± 16[b]
32	2-Thienyl	2,4-Cl_2–Ph	CH	19 ± 9.6[b]
33	2-Furyl	2,4-Cl_2–Ph	CH	20 ± 1.0[b]
34	2-Benzothienyl	2,4-Cl_2–Ph	CH	23 ± 16[b]
35	3-Pyridinyl	2,4-Cl_2–Ph	CH	390 ± 87
36	H	2,4-Cl_2–Ph	CH	240 ± 45
37	4-Cl–Ph	2-Cl–Ph	CH	2.8 ± 0.85
38	4-Cl–Ph	2,6-Cl_2–Ph	CH	200 ± 31
39	4-CH_3O–Ph	2-Cl–Ph	CH	7.5 ± 0.70
40	4-CH_3O–Ph	2-CH_3–Ph	CH	22 ± 4.7[b]
41	4-CF_3–Ph	2-CH_3–Ph	CH	9.3 ± 0.60[b]
42	4-F–Ph	2-CH_3–Ph	CH	40 ± 20[b]
43	4-Cl–Ph	2-Cl–Ph	N	3.5 ± 1.2
44	4-CH_3O–Ph	2-Cl–Ph	N	20 ± 3.7
45	4-CH_3O–Ph	2-CH_3–Ph	N	74 ± 1.0

[a] Data are expressed as K_i ± SEM (nM); hCB-1 K_i = 1.1 ± 0.04 nM was determined for **1** in our assay.
[b] IC_{50} value; incomplete displacement of receptor ligand was observed at 1–10 μM, likely due to compound solubility limitations.

sharp contrast to **7**, when the 2-chloro substituent was removed from the 2,4-dichlorophenyl group as in **14**, or when the 4-chlorophenyl and 2,4-dichlorophenyl groups were interchanged as in **15**, a substantial loss in hCB-1 binding affinity was observed (Table 1). These key structure–activity relationships (SAR) are consistent with those reported for the 1,5-diarylpyrazole series related to **1**,[4b] as well as for related 1,2-diarylimidazole and 4,5-diarylthiazole analogs.[16] However, the effect on potency by these structural changes is more profound for the constrained analog **7**.

The SAR for a diverse set of substituents at the 2-position of the pyrrolopyridinone scaffold were investigated (R^1, Table 1). For a variety of substituted phenyl groups, it was found that relatively small groups at the 4-position are preferred, such as 4-F, Cl, CH_3, CH_3O, and CF_3, providing compounds with K_i values in the 4–10 nM range (**7** and **17**–**20**, Table 1). Acidic or basic groups at the 4-position result in a substantial loss in potency (**22**, **23**), as do certain bulky substitutions (**24**). Substituents at the 3-position of phenyl groups are reasonably well tolerated (**25**–**29**), although a basic amino moiety once again provides a loss in potency (**30**). Replacement of the 4-chlorophenyl group in **7** with heterocycles such as thienyl, furyl, and benzothienyl results in a 3- to 5-fold decrease in hCB-1 binding affinity (**31**–**34**), whereas replacement with a pyridyl group (**35**) or a hydrogen atom (**36**) causes a 50- or 30-fold loss in potency, respectively.

With respect to the *N*1-position of the pyrrolopyridinone scaffold (R^2, Table 1), the *ortho*-chloro substituent on the 2,4-dichlorophenyl group is important for potent activity, as described above (**7** vs **14**, Table 1). On the other hand, removal of the *para*-chloro substituent in **7** provides analog **37**, exhibiting comparable or slightly increased CB-1 binding affinity. Addition of a second *ortho*-chloro substituent as in **38**, however, results in a substantial drop in potency. The 2-chlorophenyl group can be replaced with a 2-methylphenyl group without a substantial loss in binding affinity (**39** vs **40**).

Finally, hydrazide analogs related to **6** were investigated (X = N, Table 1). Replacement of the 2,4-dichlorophenyl group in **6** with a 2-chlorophenyl group provided **43**, with similar potency against hCB-1. Compound **43** also exhibited functional activity (hCB-1 K_b = 3.2 nM) and high selectivity against human CB-2 (hCB-2 K_i = 1100 nM). Two hydrazide derivatives with a 4-methoxyphenyl group at the R^1 position (**44**, **45**) were found to be somewhat less potent than **6** or **43**. Compound **44** also exhibited hCB-1 K_b = 32 nM and hCB-2 K_i = 850 nM.

From pharmacokinetics screening in male Wistar rats, the constrained cyclohexyl amides were generally found to give quite low plasma exposure after oral dosing (e.g., C_{max} < 50 nM at 10 mg/kg for **7**), whereas certain related hydrazides provided improved exposure levels. For example, **44** provided C_{max} = 0.62 μM and $AUC_{(0–2h)}$ = 0.58 μM·h at 10 mg/kg p.o.

1,5-Diarylpyrazole hydrazides such as **1** have been demonstrated to reduce food intake in rodents in a variety of studies.[2c] Likewise, **44** was investigated as a representative pyrrolopyridinone hydrazide in a fasted-refed rat model for appetite suppression,[17] and was found to cause a significant anorexigenic effect (36% cumulative reduction in food intake at 4 h, Fig. 2). In comparison, compound **7** caused no effect in this model (data not shown), which is consistent with the very poor plasma exposure determined for this compound.

The genetically obese Zucker *fa/fa* rat has been used for evaluating compound efficacy for the reduction of body weight, including compounds such as rimonabant (**1**) that have been shown to be effective in the management of body weight in obese humans.[18] Following the determination that **44** causes a significant suppression of appetite (Fig. 2), we also investigated its effect in this Zucker rat model.[19]

In the Zucker rat model, pyrrolopyridinone hydrazide **44** was dosed at 5 mg/kg qd p.o., and was observed to cause a significant reduction in body weight gain as compared to vehicle-treated rats (Fig. 3). The effect on day 9 was −3.0%, which is approximately half the effect determined for rimonabant (**1**) (−5.6%, 5 mg/kg po qd) included in the study as a reference standard.[20]

In conclusion, a series of pyrrolopyridinones was successfully designed and efficiently synthesized as constrained analogs of the pyrazole CB-1 antagonist rimonabant. Certain examples exhibited very potent CB-1 binding affinity and functional antagonism, with K_i and K_b values below 10 nM. As well, these analogs were found to be highly selective for CB-1 over CB-2 (>100-fold). Structure–activity relationships were generally consistent with SAR reported for the pyrazole series of CB-1 antagonists. A representative analog (**44**) was established to cause a significant anorexigenic effect in the fasted-refed Wistar rat model, and a significant reduction in body weight gain in the chronic Zucker rat model. The efficacy established in these industry-

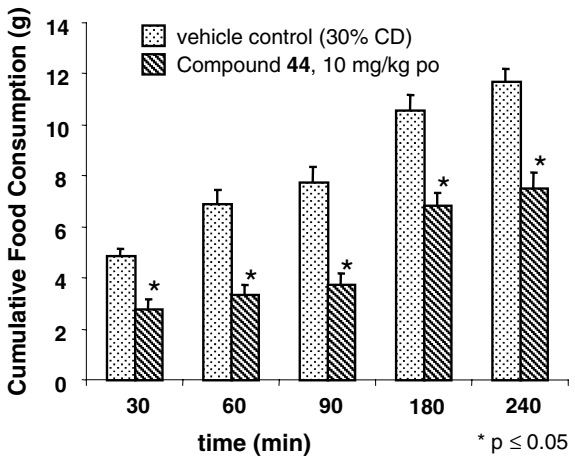


Figure 2. Effect of the pyrrolopridinone hydrazide **44** on food intake in overnight-fasted Wistar rats, after dosing at 10 mg/kg p.o. as a suspension in 30% cyclodextrin.

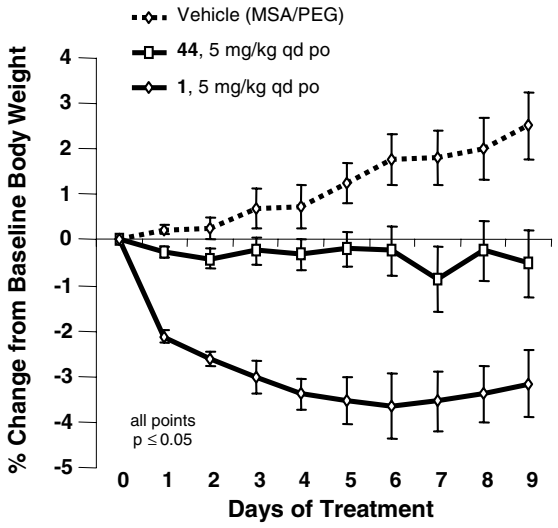


Figure 3. Effect of the pyrrolopridinone hydrazide **44** on body weight in genetically obese Zucker *fa/fa* rats, upon dosing at 5 mg/kg po qd as a suspension in PEG/20 mM methanesulfonic acid (80:20).

standard models validates this series of analogs as promising leads for the potential treatment of obesity.

Acknowledgments

We thank Romulo Romero for assistance with chromatographic separations and the Analytical Chemistry Group for NMR and LC-MS analyses. We are grateful to J. Evenski, S. Hudgins, D. Kelly-Sullivan, L. Milardo, and C. Ralphalides for assistance with in vivo pharmacology studies. We also thank W. Wong, R. Schoenleber, D. Campbell, C. Mahle, and S. Gardell for helpful discussions.

References and notes

1. (a) Centers for Disease Control website, http://www.cdc.gov/nccdphp/dnpa/obesity/; (b) Flier, J. S. *Cell* **2004**, *116*, 337; (c) Stein, C. J.; Colditz, G. A. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2522.
2. For recent reviews, see (a) Antel, J.; Gregory, P. C.; Nordheim, U. *J. Med. Chem.* **2006**, *49*, 4008; (b) Lange, J. H. M.; Kruse, C. G. *Drug Discov. Today* **2005**, *10*, 693; (c) Smith, R. A.; Fathi, Z. *IDrugs* **2005**, *8*, 53; (d) Hertzog, D. L. *Expert Opin. Ther. Pat.* **2004**, *14*, 1435; (e) Muccioli, G. G.; Lambert, D. M. *Exp. Opin. Ther. Pat.* **2006**, *16*, 1405; (f) Lange, J. H. M.; Kruse, C. G. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 498.
3. (a) Pharma Marketletter, June 23, **2006**; (b) Scrip, June 26, **2006**; (c) Fernandez, J. R.; Allison, D. B. *Curr. Opin. Invest. Drugs* **2004**, *5*, 430.
4. (a) Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubrie, P.; Breliere, J. C.; Le Fur, G. *FEBS Lett.* **1994**, *350*, 240; (b) Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769.
5. (a) Stoit, A. R.; Lange, J. H.; den Hartog, A. P.; Ronken, E.; Tipker, K.; Stuivenberg, H. H.; Dijksman, J. A.; Wals, H. C.; Kruse, C. G. *Chem. Pharm. Bull. (Tokyo)* **2002**, *50*, 1109; (b) Mussinu, J. M.; Ruiu, S.; Mulè, A. C.; Pau, A.; Carai, M. A.; Loriga, G.; Murineddu, G.; Pinna, G. A. *Bioorg. Med. Chem.* **2003**, *11*, 251; (c) Murineddu, G.; Ruiu, S.; Mussinu, J.-M.; Loriga, G.; Grella, G. E.; Carai, M. A. M.; Lazzari, P.; Pani, L.; Pinna, G. A. *Bioorg. Med. Chem.* **2005**, *13*, 3309; (d) Murineddu, G.; Ruiu, S.; Loriga, G.; Manca, I.; Lazzari, P.; Reali, R.; Pani, L.; Toma, L.; Pinna, G. A. *J. Med. Chem.* **2005**, *48*, 7351; (e) Francisco, M. E. Y.; Burgess, J. P.; George, C.; Bailey, G. S.; Gilliam, A. F.; Seltzman, H. H.; Thomas, B. F. *Magn. Reson. Chem.* **2003**, *41*, 265.
6. (a) Carpino, P. A.; Dow, R. L. U.S. Pat. Appl. US2004214855, **2004**; (b) Carpino, P. A.; Griffith, D. A.; Sakya, S.; Dow, R. L.; Black, S. C.; Hadcock, J. R.; Iredale, P. A.; Scott, D. O.; Fichtner, M. W.; Rose, C. R.; Day, R.; Dibrino, J.; Butler, M.; DeBartolo, D. B.; Dutcher, D.; Gautreau, D.; Lizano, J. S.; O'Connor, R. E.; Sands, M. A.; Kelly-Sullivan, D.; Ward, K. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 731; (c) Carpino, P. A.; Griffith, D. A. U.S. Pat. Appl. US2004214838, **2004**; (d) Griffith, D. A. PCT Int. Appl. WO2004037823, **2004**.
7. Conformational analysis was carried out by molecular mechanics computations, using Sybyl® 6.6 software and the Tripos force field (Tripos, Inc., St. Louis, MO).
8. Hurst, D. P.; Lynch, D. L.; Barnett-Norris, J.; Hyatt, S. M.; Seltzman, H. H.; Zhong, M.; Song, Z. H.; Nie, J.; Lewis, D.; Reggio, P. H. *Mol. Pharmacol.* **2002**, *62*, 1274.
9. McAllister, S. D.; Rizvi, G.; Anavi-Goffer, S.; Hurst, D. P.; Barnett-Norris, J.; Lynch, D. L.; Reggio, P. H.; Abood, M. E. *J. Med. Chem.* **2003**, *46*, 5139.
10. Micovic, I. V.; Roglic, G. M.; Ivanovic, M. D.; Dosen-Micovic, L.; Kiricojevic, V. D.; Popovic, J. B. *J. Chem. Soc., Perkin Trans. 1* **1996**, *16*, 2041.
11. Smith, R. A.; O'Connor, S. J.; Wong, W. W.; Choi, S.; Kluender, H. C. E.; Fan, J.; Zhang, Z.; Lavoie, R.; Podlogar, B. U.S. Pat. 7,071,207, **2006**.
12. The data in Table 1 are the average of two or more triplicate determinations for purified and characterized (^{1}H NMR, LC-MS) samples. Hydrazide derivatives **6** and **43–45** were isolated as the HCl salts. Cannabinoid receptor binding assays were performed with cell membranes from human CB-1 or human CB-2 receptor expressing HEK 293 cells using [^{3}H]CP 55940 as radioligand.[10] Membrane pellets were suspended in ice-cold binding buffer (50 mM Tris, pH 7.4, 2.5 mM EDTA, 5 mM $MgCl_2$, and 0.1% fatty acid free BSA) and immediately used for determining protein content (Bio-Rad Assay) and binding assays. Competition binding assays were performed in triplicate by incubating cell membranes (corresponding to 3.3 μg protein) with 0.3 nM [^{3}H]CP 559440 and varying concentrations of competing compounds. Reactions were carried out in a final volume of 200 μL binding buffer in polypropylene plates with constant shaking at 30 °C for 90 min. Non-specific binding was determined in the presence of unlabeled 10 μM WIN 55212-2. The binding reaction was terminated by filtration through pretreated (50 mM Tris, pH 7.4) Millipore GF/C filter plates using a vacuum manifold. Filters were washed seven times with ice-cold 50 mM Tris (pH 7.4). Microscint O (25 μL) was added to each well and radioactivity bound to the filters was measured using a Wallac 1450 MicroBeta Trilux liquid scintillation counter. All competition binding and concentration-response curves were analyzed using nonlinear regression with Prism software (GraphPad Software, San Diego, CA). K_i values were calculated from IC_{50} values according to the Cheng and Prusoff formula.[13] Antagonist potencies were determined mathematically by evaluation of pK_b values ($pK_b = -\log$ [antagonist

(M)] + log (DR − 1)) where DR is the agonist concentration-ratio between the EC_{50} for WIN55212-2 in the presence and absence of antagonist.[14]
13. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
14. Van den Brink, F. G. Kinetics of Drug Action. In Van Rossum, J. M., Eds.; Springer-Verlag, Berlin, 1977, pp. 69–254.
15. Rimonabant has been demonstrated to behave as a CB-1 receptor inverse agonist, that is, it decreases the constitutive level of CB-1 receptor activation in the absence of an agonist: Bouaboula, M.; Perrachon, S.; Milligan, L.; Canat, X.; Rinaldi-Carmona, M.; Portier, M.; Barth, F.; Calandra, B.; Pecceu, F.; Lupker, J.; Maffrand, J.-P.; Le Fur, G.; Casellas, P. *J. Biol. Chem.* **1997**, *272*, 22330.
16. Lange, J. H. M.; van Stuivenberg, H. H.; Coolen, H. K. A. C.; Adolfs, T. J. P.; McCreary, A. C.; Keizer, H. G.; Wals, H. C.; Veerman, W.; Borst, A. J. M.; de Looff, W.; Verveer, P. C.; Kruse, C. G. *J. Med. Chem.* **2005**, *48*, 1823.
17. Male Wistar rats were individually housed in suspended cages with a mesh floor and were kept in standard animal rooms under controlled temperature and humidity and a 12-h/12-h light/dark cycle for a minimum of week before the start of the experiment. Rats were fasted overnight (18 h) and then dosed orally with vehicle or test compound at 10 mg/kg in a volume of 2 mL/kg, 1 h before re-feeding. Cumulative food intake was recorded 30, 60, 90, 180, and 240 min after the return of the pre-weighed food jar, taking food spillage into account. Data shown in Fig. 2 represent means ± SEM for 10 rats per treatment group.
18. Vickers, S. P.; Webster, L. J.; Wyatt, A.; Dourish, C. T.; Kennett, G. A. *Psychopharmacology* **2003**, *167*, 103.
19. Genetically obese male Zucker *falfa* rats (average 550 g body weight at start of study) were kept in standard animal rooms under controlled temperature and humidity and a reversed 12-h/12-h light/dark cycle. Water and food were continuously available. Rats were single-housed in large rat shoeboxes containing grid floor. Animals were adapted to the grid floors and sham dosed with vehicle (PEG/25 mM methanesulfonic acid (80:20)) for at least 5 days before the recording of two-days baseline measurement of body weight and 24 h food and water consumption. Using the baseline body weight data, rats were weight-matched and assigned to the different treatment groups. Rats received a daily oral dose of vehicle or test compound in a volume of 2 mL/kg, before their feeding phase (nocturnal cycle). Body weights were recorded daily during the treatment period. Data shown in Figure 3 represent means ± SEM for 9–10 rats per treatment group.
20. The superior in vivo potency exhibited by rimonabant in this study is presumably due, at least in part, to its greater CB-1 binding affinity (hCB-1 K_i = 1.1 nM in our assay). However, interpretation of the in vivo efficacy SAR for these agents is complicated by factors such as oral absorption, metabolic half-life, and brain penetration of the compounds administered.