Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2706–2711
Bioorganic & Medicinal Chemistry Letters

Optimization of imidazole amide derivatives as cannabinoid-1 receptor antagonists for the treatment of obesity

Roger A. Smith,[a,*] Zahra Fathi,[b] Furahi Achebe,[a] Christiana Akuche,[a] 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] Ning Su,[a] Christy Taing,[b] Susan Tomlinson,[a] Theresa Tritto,[b] Gan Wang,[a] Stephan-Nicholas Wirtz,[d] Wai Wong,[a] Xiao-Fan Yang,[a] Shihong Ying[a] 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*
[d]*Bayer HealthCare AG, Pharma Research, D-42096 Wuppertal, Germany*

Received 12 January 2007; revised 1 March 2007; accepted 1 March 2007
Available online 12 March 2007

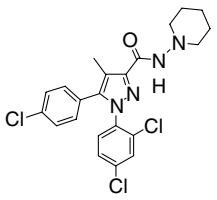
Abstract—Several imidazole-based cyclohexyl amides were identified as potent CB-1 antagonists, but they exhibited poor oral exposure in rodents. Incorporation of a hydroxyl moiety on the cyclohexyl ring provided a dramatic improvement in oral exposure, together with a ca. 10-fold decrease in potency. Further optimization provided the imidazole 2-hydroxy-cyclohexyl amide **45**, which exhibited hCB-1 K_i = 3.7 nM, and caused significant appetite suppression and robust, dose-dependent reduction of body weight gain in industry-standard rat models.

Obesity and excessive body weight are now recognized as serious health concerns, as these conditions are associated with decreased life span and several medical complications such as diabetes, hyperlipidemia, coronary artery disease, osteoarthritis, and some cancers.[1] Furthermore, with the prevalence of obesity increasing rapidly and current therapies being considered largely inadequate,[2] obesity has been declared one of the most significant health problems faced by mankind.[3] During the last decade, antagonism of the cannabinoid type 1 receptor (CB-1) has been pursued as a highly promising strategy for the treatment of obesity.[4] To date, one CB-1 antagonist, the 1,5-diaryl-pyrazole hydrazide rimonabant (**1**, SR-141716, Sanofi-Aventis), has been approved in the European Union for the treatment of obese or overweight patients with associated risk factors, such as type 2 diabetes or dyslipidemia.[5]

Of the various structure classes reported as CB-1 antagonists, the majority incorporate a central core fragment substituted by two aromatic rings and a hydrogen bond donor/acceptor functionality such as a carboxamide group.[4] Indeed, the pyrazole core exemplified in **1** has been effectively replaced with other 5-membered heterocycles such as dihydropyrazole,[6] triazole,[7–9] thiazole,[8,10] pyrrole,[11,12] and imidazole.[7,8,13–15] For example, the 1,2-diaryl-imidazole hydrazide **2** was reported by researchers at Neurocrine Biosciences[7] and Solvay[8] to exhibit human CB-1 K_i values of 85 and 23 nM, respectively. In our assay, hCB-1 K_i = 7.8 nM was determined for **2**.[14] The related cyclohexyl amide **3** was investigated as an isosteric analog by Neurocrine Biosciences and was found to have somewhat superior potency[7] (hCB-1 K_i = 3.9 nM was obtained for **3** in our assay). In this report, we describe our investigation and optimization of 1,2-diaryl-imidazole amides related to **3** as effective CB-1 antagonists.

Keywords: Cannabinoid; Antagonist; Obesity; Appetite suppressant.
* Corresponding author at present address: Angiotech Pharmaceuticals, Inc., 1618 Station Street, Vancouver, BC, Canada V6A-1B6. Tel.: +1 604 221 6927; e-mail: rosmith@angio.com

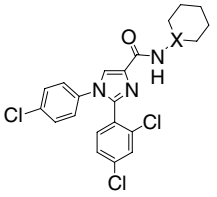
0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.03.011



1 rimonabant

(SR-141716, Acomplia™)

hCB-1 Ki = 5.6 nM,[5d] 25 nM,[6] 12 nM[7]



2, X=N: hCB-1 Ki = 85 nM,[7] 23 nM,[8]

7.8 nM (this report)

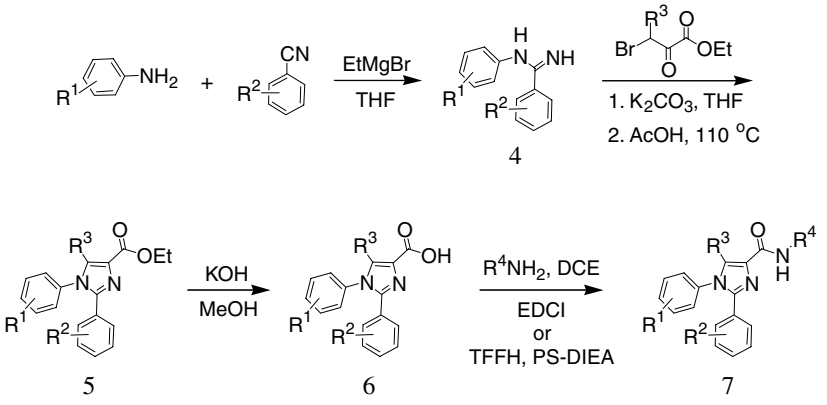
3, X=CH: hCB-1 Ki = 48 nM,[7]

3.9 nM (this report)

The 1,2-diaryl-imidazole amides such as **3** were efficiently synthesized by the route summarized in Scheme 1, which is similar to the methods described previously.[7,8] In our protocol,[14] an aniline was reacted with an aromatic nitrile in the presence of ethylmagnesium bromide to give the amidine intermediate **4**, that was then condensed with a bromopyruvate.[16] The resulting imidazole ester **5** was hydrolyzed to the corresponding carboxylic acid **6**, which was then coupled with an amine building block through the use of any of a variety of coupling agents, such as EDCI (*N*-ethyl-*N*′-dimethylaminoethyl-carbodiimide). For effective and versatile parallel syntheses, the carboxylic acid **6** was converted to the corresponding acyl fluoride by treatment with TFFH (fluoro-*N*,*N*,*N*′,*N*′-tetramethylformamidinium hexafluorophosphate)[17] in the presence of PS-DIEA (polystyrene-supported tertiary amine), followed by reaction in situ with an amine R^4NH_2 to provide the desired product **7** generally in high purity.[18]

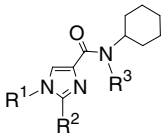
In our in vitro hCB-1 (human CB-1) binding assay,[19] the imidazole amide **3** was determined to have hCB-1 K_i = 3.9 nM; for comparison, the binding affinity observed in our assay for **1** was K_i = 1.1 nM. Amide **3** was also found to function as an antagonist,[21] with hCB-1 K_b = 18 nM, and to be selective for CB-1 over CB-2 (hCB-2 K_i = 240 nM). A variety of additional imidazole cyclohexyl amides were investigated, and several were found to exhibit hCB-1 K_i values < 10 nM (Table 1). Some of the observed structure–activity relationships (SAR) were similar to those reported for pyrazole hydrazides related to **1**,[23] imidazole hydrazides related to **2**,[7,8] and constrained pyrrolopyridinone analogs.[24] The chlorine atom of the 4-chlorophenyl group in **3** (R^1 in Table 1) could be replaced with other small substituents without substantial loss in potency (**8**–**10**). In contrast, replacement of the 2,4-dichlorophenyl substituent in **3** (R^2 in Table 1) with 2,5-dichlorophenyl caused a significant decrease in binding affinity (**11**), and replacement with unsubstituted phenyl resulted in a further marked decrease in potency (**12**). The 2,4-dichlorophenyl group could be effectively replaced with a 2,4-dimethylphenyl group (**13**), but not with a 2,4-difluorophenyl group (**14**), presumably due to insufficient steric bulk at the 2-position. Similar to the SAR reported for constrained pyrrolopyridinone analogs,[24] removal of the *para*-chloro substituent in **3** to provide **15** resulted in comparable or slightly improved CB-1 binding affinity. Replacement of the *ortho*-chloro in **15** with other substituents caused a decrease in potency (**19**–**21**). Finally, replacement of the phenyl ring at R^1 in **16** with a cyclohexyl ring as in **22**, substitution on the amide nitrogen in **15** with a methyl group (**23**), and replacement of the R^1 substituent in **15** with a 3-pyridinyl group (**24**) all resulted in significant decreases in binding affinity (Table 1).

The most potent imidazole cyclohexyl amide compounds were evaluated by pharmacokinetics screening in male Wistar rats,[25] and unfortunately all of these



Scheme 1. Synthesis of 1,2-diaryl-imidazole amides **7**.

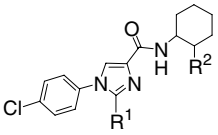
Table 1. Binding affinities of imidazole cyclohexyl amides to the human CB-1 and CB-2 receptors



Compound	R^1	R^2	R^3	hCB-1 K_i[a] (nM)
3	4-Cl–Ph	2,4-Cl_2–Ph	H	3.9[b]
8	4-CH_3–Ph	2,4-Cl_2–Ph	H	1.9
9	4-CH_3O–Ph	2,4-Cl_2–Ph	H	4.9
10	4-F–Ph	2,4-Cl_2–Ph	H	8.2
11	4-Cl–Ph	2,5-Cl_2–Ph	H	25
12	4-Cl–Ph	Ph	H	130
13	4-Cl–Ph	2,4-Me_2–Ph	H	7.2
14	4-Cl–Ph	2,4-F_2–Ph	H	36
15	4-Cl–Ph	2-Cl–Ph	H	2.2[c]
16	4-CH_3–Ph	2-Cl–Ph	H	4.6
17	4-CH_3O–Ph	2-Cl–Ph	H	9.0
18	4-F–Ph	2-Cl–Ph	H	20
19	4-Cl–Ph	2-Me–Ph	H	21[d]
20	4-Cl–Ph	2-Et–Ph	H	39
21	4-Cl–Ph	2-MeO–Ph	H	95
22	4-Me-cyHex	2-Cl–Ph	H	17
23	4-Cl–Ph	2-Cl–Ph	CH_3	34
24	3-Pyridinyl	2-Cl–Ph	H	1100

[a] hCB-1 = 1.1 nM was determined for **1** in our assay.
[b] hCB-1 K_b = 18 nM; hCB-2 K_i = 240 nM.
[c] hCB-1 K_b = 11 nM; hCB-2 K_i = 2300 nM.
[d] hCB-1 K_b = 7.3 nM; hCB-2 K_i > 1000 nM.

Table 2. Binding affinities of imidazole amino- and hydroxyl-substituted cyclohexyl amides to the human CB-1 and CB-2 receptors



Compound	R^1	R^2	Cyclohexane isomer	hCB-1 K_i (nM)
25	2,4-Cl_2–Ph	NH_2	*S,S-trans*	270
26	2,4-Cl_2–Ph	NH_2	*R,R-trans*	940
27	2,4-Cl_2–Ph	$NHCH_3$	*S,S-trans*	620
28	2,4-Cl_2–Ph	$NHCH_3$	*R,R-trans*	920
29	2,4-Cl_2–Ph	OH	*trans–rac*	22[a]
30	2-Cl–Ph	OH	*S,S-trans*	29[b]
31	2-Cl–Ph	OH	*R,R-trans*	97[c]

[a] hCB-1 K_b = 4.4 nM; hCB-2 K_i = 1100 nM.
[b] hCB-1 K_b = 7.1 nM; hCB-2 K_i = 5300 nM.
[c] hCB-1 K_b = 22 nM.

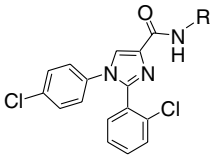
compounds were found to provide quite poor plasma exposure from oral dosing (10 mg/kg po, typically in 30% cyclodextrin suspension). For example, imidazole amide **3** exhibited $C_{max} \sim 50$ nM. Consistent with this finding, **3** was also inactive in the fasted–refed rat model for appetite suppression[26] (10 mg/kg po, 30% CD). In contrast, the corresponding imidazole hydrazide **2** exhibited plasma $C_{max} = 210$ nM and caused 50 to 70% reduction in food intake at 0.5–4.0 h time points in the fasted–refed rat model (10 mg/kg po, 30% CD).

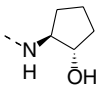
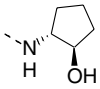
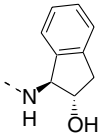
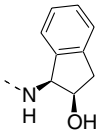
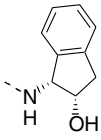
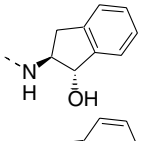
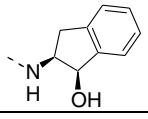
We considered that the introduction of a basic nitrogen or polar moiety such as a hydroxyl group to the cyclohexyl ring might improve the plasma exposure of this series of compounds. However, incorporation of either an amino or methylamino moiety was found to give a substantial reduction in CB-1 binding affinity (**25**–**28**, Table 2). Incorporation of a hydroxyl group was less detrimental, causing approximately a 10-fold decrease in potency (**29** vs **3**, **30** vs **15**). The (*S,S*)-*trans* isomers of these derivatives were found to be somewhat more potent than the (*R,R*)-*trans* isomers (Table 2). From Wistar rat pharmacokinetics screening, the 2-hydroxy-cyclohexyl amide **30** was found to provide dramatically improved plasma exposure levels, with $C_{max} = 1.64$ μM (10 mg/kg po, PEG400/25 mM methanesulfonic acid (80:20) suspension (PEG/MSA)).

Brain exposure levels for **30** at 2 h were determined to be 110 nM. Consistent with these results, compound **30** was found to have a significant effect in the fasted–refed rat model, providing 34–62% reduction in food intake measured at 0.5–4.0 h time points (10 mg/kg po). Likewise, **29** was active in this model, causing 21–29% reduction in food intake at 0.5–4.0 h time points (10 mg/kg po).

A diverse variety of additional hydroxyl-substituted amides was therefore explored,[14] with the goal of achieving greater CB-1 binding affinity while maintaining significant plasma (and brain) exposure. Selected examples of these derivatives are listed in Table 3. The 2-hydroxy-cyclopentyl amide **32** was found to be equipotent to the related cyclohexyl analog **30**, it exhibited similar exposure levels (plasma $C_{max} = 2.11$ μM, brain $C_{(2h)} = 330$ nM; 10 mg/kg po in PEG/MSA) in PK screening studies, and it caused a significant reduction (26–56%) in food intake in the fasted–refed rat model. Fusion of an aryl ring to the cyclopentyl group resulted in significantly improved CB-1 binding affinity for analog **35**; however, this compound and other promising aryl alkyl compounds were found to exhibit low plasma exposure and/or insufficient activity in the fasted–refed rat model to warrant further study.

Table 3. Binding affinities of imidazole hydroxyl-substituted alkyl and arylalkyl amides to the human CB-1 and CB-2 receptors



Compound	–NHR	Cyclopentane isomer	hCB-1 K_i (nM)
32		*S,S-trans*	29[a]
33		*R,R-trans*	75
34		*trans–rac*	22
35		*S,R-cis*	6.3[b]
36		*R,S-cis*	18[c]
37		*trans–rac*	41
38		*cis–rac*	17

[a] hCB-1 $K_b = 9.7$ nM; hCB-2 $K_i = 6300$ nM.
[b] hCB-1 $K_b = 2.2$ nM; hCB-2 $K_i = 1200$ nM.
[c] hCB-1 $K_b = 9.2$ nM; hCB-2 $K_i = 5100$ nM.

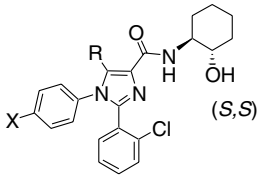
Finally, alkyl substitutions were incorporated at the imidazole 5-position. Introduction of methyl to *n*-propyl groups provided single-digit nanomolar hCB-1 K_i values (**39**–**41**), while larger substituents were less effective (Table 4). Finally, replacement of the 4-chlorophenyl group with a 4-bromophenyl group provided an apparent further improvement in potency (**45**, hCB-1 K_i = 3.7 nM).

While compound **45** was found to be equipotent in the hCB-1 binding assay to the cyclohexyl amide lead **3**, it exhibited considerably greater rat plasma exposure ($C_{max} = 2.10$ μM, 10 mg/kg po in PEG/MSA) and robust activity in the fasted–refed Wistar rat model. In this study, the percent reduction in food intake caused by **45** (10 mg/kg po in PEG/MSA) at 0.5, 1.0, 1.5, 3.0, and 4.0 h time points was 63, 64, 62, 52, and 35%, respectively.[26]

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.[27] Following the determination that **45** causes a significant suppression of appetite, we also investigated its effect in this Zucker rat model.[28]

In the Zucker rat model, imidazole amide **45** was dosed at 1, 5, and 10 mg/kg qd po, and was observed to cause a significant and dose-dependent reduction in body weight gain as compared to vehicle-treated rats (Fig. 1). The effect on day 13 was −1.9% (not statistically significant), −6.1%, and −10.6% at 1, 5, and 10 mg/kg, respectively.

Table 4. Binding affinities of imidazole 2-hydroxy-cyclohexyl amides to the human CB-1 and CB-2 receptors



Compound	R	X	hCB-1 K_i (nM)
39	Me	Cl	6.9
40	Et	Cl	5.0[a]
41	*n*Pr	Cl	5.7[b]
42	*n*Bu	Cl	10
43	*i*Pr	Cl	18
44	Br	Cl	19
45	Et	Br	3.7[c]

[a] hCB-1 $K_b = 0.9$ nM; hCB-2 $K_i = 2300$ nM.
[b] hCB-1 $K_b = 1.4$ nM; hCB-2 $K_i = 2700$ nM.
[c] hCB-1 $K_b = 13$ nM; hCB-2 $K_i = 2700$ nM.

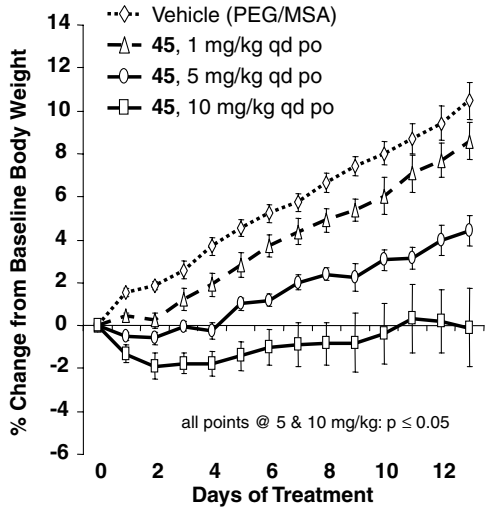


Figure 1. Effect of the imidazole 2-hydroxycyclohexyl amide **45** on body weight in genetically-obese Zucker *fa/fa* rats, upon dosing at 1, 5, and 10 mg/kg po qd as a suspension in PEG/20 mM methanesulfonic acid (80:20).

In a separate study, **45** and rimonabant (**1**) were both dosed at 10 mg/kg qd po for eight days, and the compounds were observed to cause 5.6% and 6.5% reduction in body weight gain, respectively. The difference in the effects observed for **45** and **1** in this study was not statistically significant.

In summary, imidazole amides were investigated and optimized as CB-1 antagonists. SAR studies for a series of cyclohexyl amides highlighted the importance of an *ortho* substituent such as chloro or methyl on the phenyl group at the imidazole 2-position. Although several very potent examples of these analogs could be identified, they lacked sufficient plasma exposure from oral dosing to enable in vivo efficacy. Incorporation of a hydroxyl moiety on the cyclohexyl ring provided a dramatic improvement in oral exposure, together with a ca. 10-fold decrease in in vitro potency. However, further optimization of substituents provided the imidazole 2-hydroxycyclohexyl amide **45,** which exhibited hCB-1 K_i = 3.7 nM, a significant anorexigenic effect in the fasted–refed Wistar rat model, and robust, dose-dependent reduction in body weight gain in the chronic Zucker rat model.

Acknowledgments

We would like to thank Romulo Romero for assistance with chromatographic separations, Anthony Paiva for LC–MS analyses, and members of the Analytical Chemistry Group for NMR 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 R. Schoenleber, D. Campbell, C. Mahle, and S. Gardell for helpful discussions.

References and notes

1. Bray, G. A. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2583.
2. Stein, C. J.; Colditz, G. A. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2522.
3. (a) Centers for Disease Control website, http:/www.cdc.gov/nccdphp/dnpa/obesity; (b) Flier, J. S. *Cell* **2004**, *116*, 337.
4. For recent reviews, see (a) Muccioli, G. G.; Lambert, D. M. *Expert Opin. Ther. Pat.* **2006**, *16*, 1405; (b) Antel, J.; Gregory, P. C.; Nordheim, U. *J. Med. Chem.* **2006**, *49*, 4008; (c) Lange, J. H. M.; Kruse, C. G. *Drug Discovery Today* **2005**, *10*, 693; (d) Smith, R. A.; Fathi, Z. *IDrugs* **2005**, *8*, 53; (e) Hertzog, D. L. *Expert Opin. Ther. Pat.* **2004**, *14*, 1435; (f) Lange, J. H. M.; Kruse, C. G. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 498.
5. (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; (d) 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.
6. 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. J.; Adolfs, T. J. P.; Hoogendoorn, J.; Kruse, C. G. *J. Med. Chem.* **2004**, *47*, 627.
7. Dyck, B.; Goodfellow, V. S.; Phillips, T.; Grey, J.; Haddach, M.; Rowbottom, M.; Naeve, G. S.; Brown, B.; Saunders, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1151.
8. Lange, J. H.; van Stuivenberg, H. H.; Coolen, H. K.; Adolfs, T. J.; McCreary, A. C.; Keizer, H. G.; Wals, H. C.; Veerman, W.; Borst, A. J.; de Looff, W.; Vermeer, P. C.; Kruse, C. G. *J. Med. Chem.* **2005**, *48*, 1823.
9. Jagerovic, N.; Hernandez-Folgado, L.; Alkorta, I.; Goya, P.; Navarro, M.; Serrano, A.; de Fonseca, F. R.; Dannert, M. T.; Alsasua, A.; Suardiaz, M.; Pascual, D.; Martin, M. I. *J. Med. Chem.* **2004**, *47*, 2939.
10. Berggren, A. I. K.; Bostrom, S. J.; Elebring, S. T.; Fallefors, L.; Wilstermann, J. M.; Greasley, P. PCT Int. Appl. WO2004058255, 2004.
11. Smith, R. A.; Kluender, H. C.; Su, N.; Lavoie, R.; Fan, J. PCT Int. Appl. WO2003027069, 2003.
12. Mayweg, A.; Marty, H. P.; Mueller, W.; Narquizian, R.; Neidhart, W.; Pflieger, P.; Roever, S. PCT Int. Appl. WO2004060870, 2004.
13. Plummer, C. W.; Finke, P. E.; Mills, S. G.; Wang, J.; Tong, X.; Doss, G. A.; Fong, T. M.; Lao, J. Z.; Schaeffer, M. T.; Chen, J.; Shen, C. P.; Stribling, D. S.; Shearman, L. P.; Strack, A. M.; Van der Ploeg, L. H. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1441.
14. Smith, R. A.; O'Connor, S. J.; Wirtz, S. N.; Wong, W. C.; Choi, S.; Kluender, H. C.; Su, N.; Wang, G.; Achebe, F.; Ying, S. PCT Int. Appl. WO2003040107, 2003.
15. Hagmann, W. K.; Qi, H.; Shah, S. K. PCT Int. Appl. WO2003063781, 2003.
16. Tsuji, K.; Nakamura, K.; Konishi, N.; Tojo, T.; Ochi, T.; Senoh, H.; Matsuo, M. *Chem. Pharm. Bull.* **1997**, *45*, 987.
17. (a) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401; (b) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.
18. General procedure for parallel syntheses: In a 20-mL screw-cap vial, 0.5 mmol of 1,2-diaryl-imidazole-4-carboxylic acid **6**, 145 mg (0.55 mmol) TFFH (e.g., Advanced Chemtech, Louisville, KY), and 5.0 equiv PS-DIEA (loading level: 3.50 mmol/g, 716 mg, 2.5 mmol) (e.g., Argonaut Technologies Inc., San Carlos, CA) were heated in 10 mL 1,2-dichloroethane at 35 °C overnight. The formation of acyl fluoride was monitored by LC–MS. To the mixture, 1.1 equiv (0.55 mmol) of amine building

block R^4NH_2 was added and the reaction was continued at 35 °C overnight. The mixture was filtered through a filter tube (polypropylene frit), and the filtrate was evaporated under reduced pressure to provide the product, typically in >70% purity. For compounds of particular interest, the crude product was redissolved in 1 mL MeOH and purified by preparative HPLC. The methodology was also successfully applied to library synthesis (10 μmol scale) by using 2-mL 96-well Robbins FlexChem™ reaction blocks and Robbins rotator ovens.

19. The hCB-1 and hCB-2 data reported in this article are the average of two or more triplicate determinations for purified and characterized (^{1}H NMR, LC–MS) samples. Cannabinoid receptor binding assays were performed with cell membranes from human CB-1 or human CB-2 receptor expressing HEK 293 cells using [3H]CP 55940 as radioligand. Membrane pellets were suspended in ice-cold binding buffer (50 mM Tris, pH 7.4, 2.5 mM EDTA, 5 mM $MgCl_2$, 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 [3H]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.[20]
20. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
21. 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 (a) 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; (b) Recombinant cells expressing human CB-1 were used to examine cell-based efficacy of compounds as antagonists, that is, ability to block receptor agonist-stimulated inhibition of forskolin-stimulated cAMP levels. Cannabinoid receptor inhibition of adenylyl cyclase activity was determined in CHO K1 cells stably transfected with cDNA encoding the human CB-1 receptor (Euroscreen). Cells were maintained at 37 °C and 5% CO_2 in Ham's F12 medium supplemented with 20 mM HEPES (pH 7.4), 2 mM L-glutamine, 400 μg/mL G418, and 5% FBS. CB-1 receptor expressing CHO K1 cells grown to 90% confluence in 150 cm^2 flasks were washed with 10 mL PBS. Sigma cell dissociation solution (5 mL; Cat# C5914) was added to flasks and the cells collected in Ham's F12 media and centrifuged for 5 min at 500*g*. The cell pellet was resuspended in assay buffer (Dulbecco's PBS containing 0.1 mM 3-isobutyl-1-methylxanthine and 0.1 mM Ro20-17241). The cells (110,000/well) were incubated (250 μL, final volume) for 20 min at 37 °C with various concentrations of cannabinoid receptor agonist and forskolin (3 μM, final concentration) in the presence or absence of a fixed concentration of CB-1 receptor antagonist. The cell suspension was centrifuged at 1000*g* for 5 min in a table top centrifuge (4 °C). The supernatant was aspirated and 100 μL of cell lysis buffer added to each well. The cyclic AMP content of the supernatant was measured by scintillation proximity assay (cAMP SPA Biotrak Direct Screening Assay System, Amersham Biosciences, UK) according to the manufacturer's instructions. Apparent dissociation constants of antagonists (K_b values) were calculated by using the formula $pK_b = \log_{10}(CR-1) - \log_{10}B$, where B is the concentration of antagonist used and CR (concentration ratio) is the ratio of agonist EC_{50} measured in the presence of antagonist over that measured in the absence of antagonist.[22]
22. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
23. Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769.
24. Smith, R. A.; Fathi, Z.; Brown, S.; Choi, S.; Fan, J.; Jenkins, S.; Kluender, H. C. E.; Konkar, A.; Lavoie, R.; Mays, R.; Natoli, J.; O'Connor, S. J.; Ortiz, A. A.; Podlogar, B.; Taing, C.; Tomlinson, S.; Tritto, T.; Zhang, Z. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 673.
25. In pharmacokinetics screening studies, plasma exposure levels were determined typically at 0.5, 1, and 2 h time points following a 10 mg/kg oral dose to Wistar rats. As the CB-1 receptor is a CNS target, brain exposure levels at 2 h after compound dosing were also determined for selected compounds. However, insufficient data were collected to permit an interpretation of the SAR for brain/plasma exposure ratio.
26. 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 were collected for 10 rats per treatment group.
27. Vickers, S. P.; Webster, L. J.; Wyatt, A.; Dourish, C. T.; Kennett, G. A. *Psychopharmacology* **2003**, *167*, 103.
28. 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 five 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 1 represent means ± SEM for 9–10 rats per treatment group.