Diaryl Dihydropyrazole-3-carboxamides with Significant In Vivo Antiobesity Activity Related to CB1 Receptor Antagonism: Synthesis, Biological Evaluation, and Molecular Modeling in the Homology Model†

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A number of analogues of diaryl dihydropyrazole-3-carboxamides have been synthesized. Their activities were evaluated for appetite suppression and body weight reduction in animal models. Depending on the chemical modification of the selected dihydropyrazole scaffold, the lead compounds—the bisulfate salt of (±)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid morpholin-4-ylamide **26** and the bisulfate salt of (−)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid morpholin-4-ylamide **30**—showed significant body weight reduction in vivo, which is attributed to their CB1 antagonistic activity and exhibited a favorable pharmacokinetic profile. The molecular modeling studies also showed interactions of two isomers of (±)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid morpholin-4-ylamide **9** with CB1 receptor in the homology model similar to those of *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide (rimonabant) **1** and 4*S*-(−)-3-(4-chlorophenyl)-*N*-methyl-*N*′-[(4-chlorophenyl)-sulfonyl]-4-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamidine (SLV-319) **2**.

Introduction

Obesity is one of the greatest health threats of this century.[1] Obesity is usually defined using the body mass index [BMI = weight (kg)/height $(m)^2$]. People with BMI of 30 and above (27 and above for Chinese and Asian people) possess great risk of co-morbid diseases such as hypertension, type 2 diabetes, and dyslipidaemia.[2]

The worldwide explosion of obesity and related disorders are generally attributed to Western dietary habits (high sugar, high fat diet). The benefits of a controlled dietary intake can be profound for the management of obesity. Hence, it has been a goal of research to develop antiobesity drugs that are effective and safe in targeting appetite suppression.[3]

The endocannabinoid system (ECB) has an important conserved evolutionary role in regulation of energy balance.[4] The ECB system comprises of at least two cannabinoid receptors (CB1 and CB2), endogenous lipid-like ligands (endocannabinoids), and the enzymes involved in their formation and breakdown.[5]

CB1 receptors are found primarily in brain and neuronal tissues, while CB2 receptors are found predominantly in immune cells and tissues. Both cannabinoid receptors belong to the G-protein coupled receptor family, sharing approximately 48% homology in their amino acid sequences. Both CB1 and CB2 receptors are coupled to mitogen-activated protein kinase and adenylyl cyclase signaling systems.[5]

It has been found clinically and experimentally that the endocannabinoid system is hyperactive in obese subjects.[6] Both exogenous and endogenous cannabinoids stimulate food intake, which is attenuated by pretreatment with CB1 antagonist/inverse agonist in humans and rodents, indicating the role of CB1 receptor in regulation of energy balance.[4,6] Thus, CB1 receptor antagonists/inverse agonists represent a promising new approach for reducing body weight and decreasing the co-morbidities associated with excessive adiposity.[7]

Rimonabant hydrochloride **1** (Figure 1) is the first therapeutically relevant, potent, and selective CB1 receptor inverse agonist, recently approved in Europe as an antiobesity drug, which belongs to the diaryl pyrazole family.[8]

Since the discovery of rimonabant, several classes of CB1 receptor antagonist with diverse chemical structures have been disclosed.[9−11] Solvay pharmaceuticals has disclosed the 3,4-diaryl dihydropyrazole class of compound **2** (Figure 1) as a CB1 antagonist, which has elicited potent in vitro[12] and in vivo[13] activities.

More recently, there has been a surge of interest in this area, and several research groups are actively working with a view to generate diverse structures with desired activity. These studies have provided more insight into pivotal receptor−ligand interactions and eventually led to the diverse chemical structures,[10] although very limited in vivo studies of body weight regulation by these CB1 antagonists are published. 8-Chloro-1-(2,4-dichlorophenyl)-*N*-piperidin-1-yl-1,4,5,6-tetrahydrobenzo-[6,7]-cyclohepta-[1,2-*c*]-pyrazole-3-carboxamide **3** (Figure 1) was found to be a very potent CB1 antagonist in cell-based in vitro assays and ex vivo screens.[14] However, the molecule had poor in vivo efficacy and oral bioavailability.[15] Demonstration of in vivo efficacy in appropriate animal models of obesity and appetite suppression is an important criterion for selection of CB1 antagonists for further development.

Recently Lange et al. have emphasized bioisosteric replacement in the cannabinoid research,[16] wherein the thiazoles, triazoles, and imidazoles have replaced the pyrazole moiety of rimonabant. The resulting imidazole compounds have shown impressive potency in pharmacological in vivo activities in both

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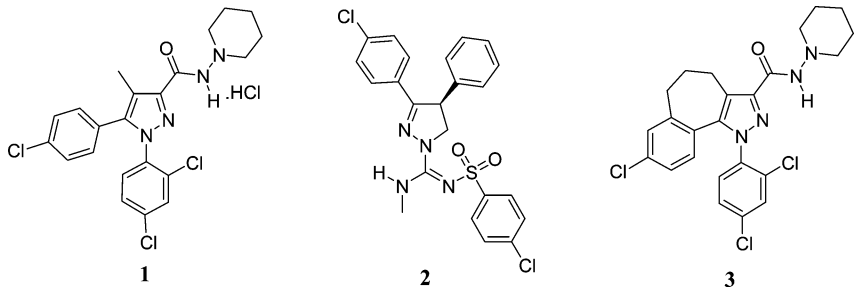


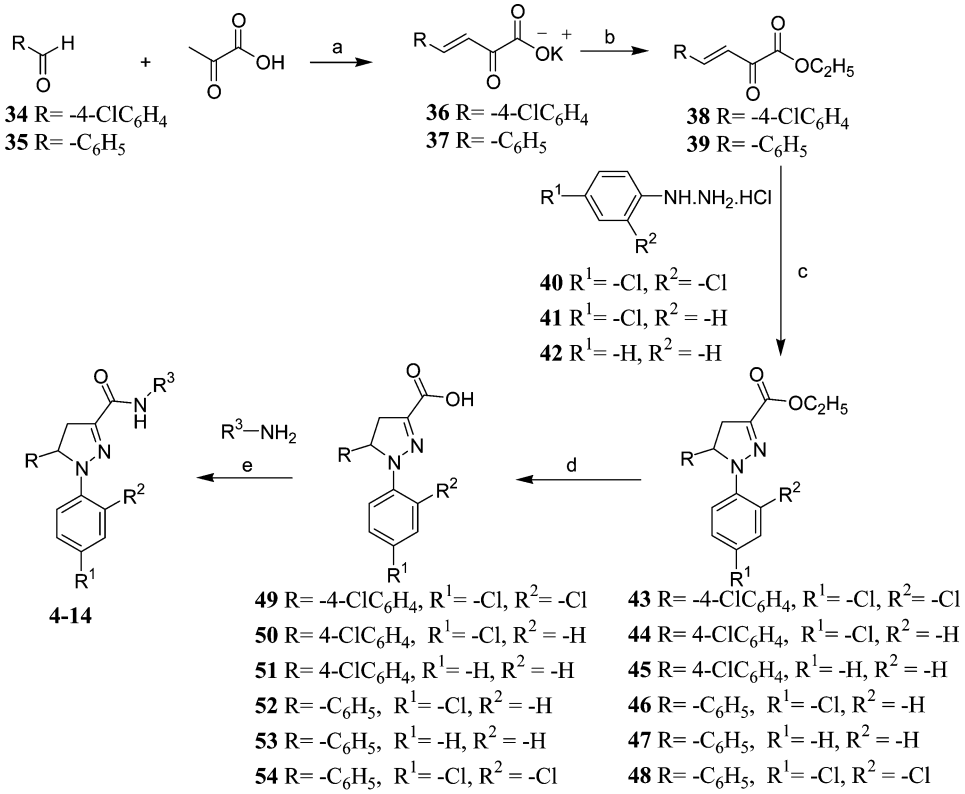
1 **2** **3**

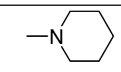
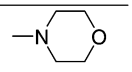
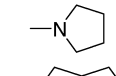
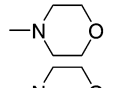
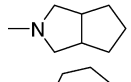
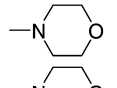
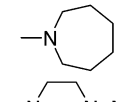
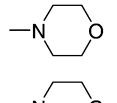
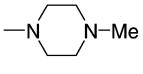
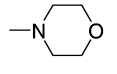
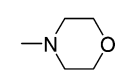
Figure 1.

a CB agonist-induced hypertension model and a CB agonist-induced hypothermia model.[16]

Similarly, Christopher et al. have published an imidazole surrogate related to the pyrazole rimonabant and an optimized series for compounds that are orally bioavailable with good pharmacokinetic properties and that have shown efficacy in obese rats both by reducing food intake and overall body weight.[17]

Scheme 1[a]



Compd.	R	R^1	R^2	R^3	Compd.	R	R^1	R^2	R^3
4	4-ClC_6H_4	-Cl	-Cl		**10**	4-ClC_6H_4	-Cl	-H	
5	4-ClC_6H_4	-Cl	-Cl		**11**	4-ClC_6H_4	-H	-H	
6	4-ClC_6H_4	-Cl	-Cl		**12**	-C_6H_5	-Cl	-H	
7	4-ClC_6H_4	-Cl	-Cl		**13**	-C_6H_5	-H	-H	
8	4-ClC_6H_4	-Cl	-Cl		**14**	-C_6H_5	-Cl	-Cl	
9	4-ClC_6H_4	-Cl	-Cl						

[a] Reagents and conditions: (a) KOH, MeOH, 0−10 °C, 10−15 h; (b) EtI, DMF, 72−75 °C, 4−6 h; (c) EtOH, 26−28 °C, 2−4 h, AcOH, 108−110 °C, 18−22 h; (d) KOH(aq), MeOH, 65−68 °C, 2−3 h; (e) R^3NH_2, $HOBt{\cdot}H_2O$, EDC·HCl, NEt_3, CH_2Cl_2, 26−28 °C, 30−50 min.

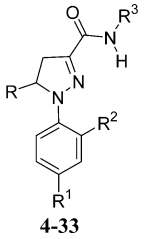


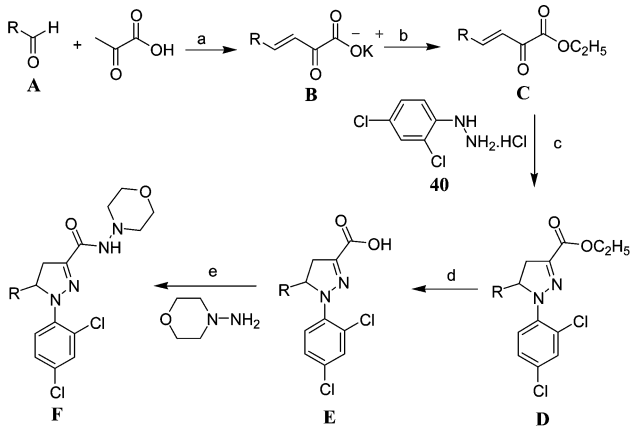
Figure 2.

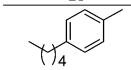
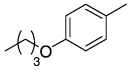
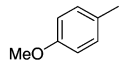
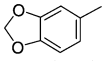
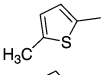
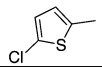
Using bioisosteric replacement in a rational approach, in conjunction with molecular modeling studies, we synthesized several diaryl dihydropyrazole carboxamide derivatives[18] **4**−**33** (Figure 2) and evaluated their efficacy in a 5% sucrose solution intake model at a single dose. A few selected compounds were tested for efficacy with a chronic dose for food consumption and body weight reduction in genetically obese rodent models. The pharmacokinetic parameters and tissue distribution of selected compound were also evaluated. The selected compounds were used for molecular modeling studies in the homology model.[19]

Chemistry

A series of proprietary compounds **4**−**33** based on the structural similarities with rimonabant **1** were synthesized in convergent approach as shown in Schemes 1−4. The dihydro analogues **4**−**14** were prepared by condensing the corresponding acids **49**−**54** with cyclic amines R^3-NH_2 under usual peptide (amide) bond formation chemistry using [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] hydrochloride and 1-hydroxybenzotriazole hydrate. The synthesis of acids **49**−**54** involved usual basic hydrolysis of corresponding esters **43**−**48** which in turn were prepared by condensing α,*β*-unsaturated keto esters **38** and **39** with suitably substituted phenyl hydrazines **40**−**42** under acidic conditions. The construction of α,*β*-unsaturated keto esters involved the usual condensation of corresponding aldehydes **34** and **35** with pyruvic acid under basic conditions followed by esterification with ethyl iodide. The formation of a diversity of compounds **15**−**24** (Formula F, Scheme 2) in this series was ensued by varying aldehydes **55**−**64** (Formula A, Scheme 2). The aldehydes **34** and **35** were procured from Sigma-Aldrich, and aldehydes **55**−**64** were synthesized as per literature procedure.[20] Although there is sufficient literature evidence available for synthesis of α,*β*-unsaturated keto esters, the present

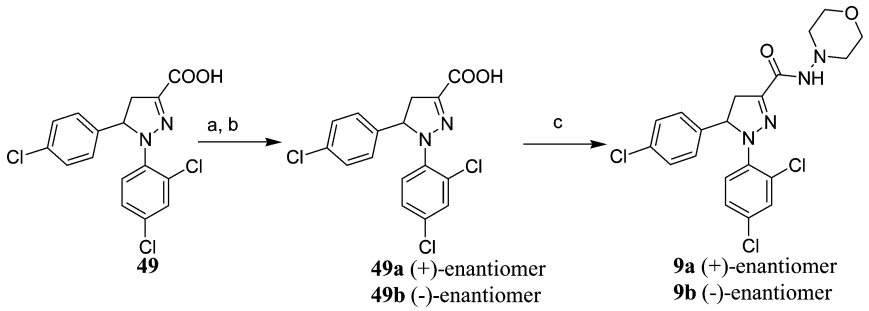
Scheme 2[a]



R	Formula **A**	Formula **B**	Formula **C**	Formula **D**	Formula **E**	Formula **F**
	**55**	**65**	**75**	**85**	**95**	**15**
	**56**	**66**	**76**	**86**	**96**	**16**
	**57**	**67**	**77**	**87**	**97**	**17**
	**58**	**68**	**78**	**88**	**98**	**18**
	**59**	**69**	**79**	**89**	**99**	**19**
	**60**	**70**	**80**	**90**	**100**	**20**
	**61**	**71**	**81**	**91**	**101**	**21**
	**62**	**72**	**82**	**92**	**102**	**22**
	**63**	**73**	**83**	**93**	**103**	**23**
	**64**	**74**	**84**	**94**	**104**	**24**

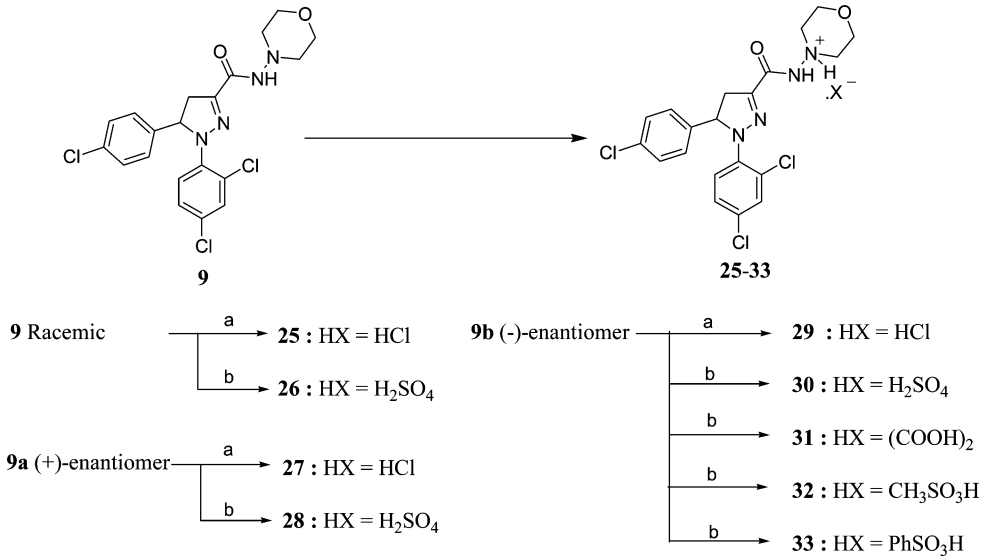
[a] Reagents and conditions: (a) KOH, MeOH, 0−10 °C, 10−15 h; (b) EtI, DMF, 72−75 °C, 4−6 h; (c) EtOH, 26−28 °C, 2−4 h, AcOH, 108−110 °C, 18−22 h; (d) KOH(aq), MeOH, 65−68 °C, 2−3 h; (e) R^3NH_2, $HOBt{\cdot}H_2O$, EDC·HCl, NEt_3, CH_2Cl_2, 26−28 °C, 30−50 min.

Scheme 3[a]



[a] Reagents and conditions: (a) for (+)-enantiomer: *S*-(−)-α-methyl benzylamine, CH_3CN:$(CH_3)_2CO$ (1:1), 26−28 °C, 2−4 h; for (−)-enantiomer: *R*-(+)-α-methyl benzylamine, CH_3CN:$(CH_3)_2CO$ (1:1), 26−28 °C, 2−4 h; (b) dil. HCl, 5−10 °C, 5−10 min; (c) morpholin-4-ylamine, HOBt·H_2O, EDC·HCl, NEt_3, CH_2Cl_2, 26−28 °C, 30−50 min.

Scheme 4[a]



[a] Reagents and conditions: (a) ethereal HCl, MeOH, 0−15 °C, 10−15 min; (b) HX, MeOH, 60−65 °C, 30−40 min.

set of conditions afforded compounds **38**, **39**, and **75**−**84** in better yields and are scalable.[21−28]

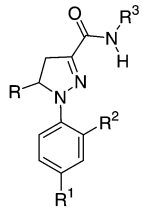
As dihydropyrazole analogues **4**−**24** contained a chiral center at position 5, the above synthesis provides a target compound in the form of racemic mixture. To further explore the stereochemical (chiral) requirements/consequences for binding to the CB1 receptor, the key compound **9** was synthesized as a chiraly pure moiety by introducing resolution at suitable stage. Thus dihydropyrazole-3-carboxylic acid **49** was resolved by employing *S*-(−)-α-methyl benzylamine and *R*-(+)-α-methyl benzylamine to furnish the corresponding diastereomeric salts. Removal of resolving agent by 10% HCl solution afforded the corresponding chiraly pure carboxylic acid as two chiral antipodes (+)-carboxylic acid **49a** and (−)-carboxylic acid **49b**, respectively (Schemes 3). Chiral purity of these carboxylic acids was confirmed by chiral HPLC and specific optical rotation. Subsequently various salts **25**−**33**, e.g., hydrochloride, bisulfate, oxalate, mesylate, and besylate, were prepared with a view to examine the pharmacodynamic and pharmacokinetic profiles.

Results and Discussion

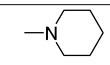
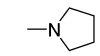
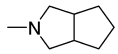
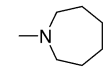
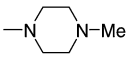
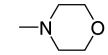
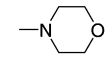
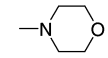
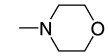
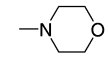
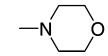
The present synthetic design was based on the bioisosteric replacement of the pyrazole moiety of rimonabant **1** by dihydropyrazole. In this context, we synthesized several diaryl dihydropyrazoles **4**−**33**. Our strategy in evaluating newly synthesized cannabinoid antagonists was first to determine their ability to reduce the consumption of sucrose solution in genetically obese Zucker fa/fa rats. The cannabinoid agonists are reported to enhance sucrose consumption in rodents.[29] On the other hand, the selective central CB1 receptor antagonist markedly and selectively reduces sucrose feeding and drinking in rodents.[30] Obese Zucker rats have elevated hypothalamic levels of the endocannabinoid 2-arachidonoyl glycerol as compared to lean controls.[31,32] The higher endocannabinoid tone in these obese animals may result in higher sensitivity for a CB1 blockade.

N-Aminopiperidine derivative **4**, which structurally mimics rimonabant, exerted moderate appetite suppression in a single-dose 5% sucrose solution intake model in genetically obese Zucker fa/fa rats (Table 1). We have replaced the *N*-amino-piperidine side chain of compound **4** by various amino derivatives such as pyrrolidine derivative **5**, azabicyclo derivative **6**, homopiperidine derivative **7**, *N*-methyl piperazine derivative **8**, and morpholine derivative **9**. Aminopyrrolidine **5** having one carbon less than the six-membered aminopiperidine was found to have greatly reduced efficacy. A similar trend was seen for azabicyclo derivative **6** as well as by seven-membered homo-piperidine derivative **7**. For the carboxamide derivative with one more heteroatom, i.e., *N*-amino-*N*-methyl piperazine **8**, the efficacy was revived to some extent whereas the *N*-aminomor-pholine derivative **9** exhibited an efficacy closer to that shown by rimonabant **1** (Table 1).

Table 1. In Vivo Efficacy of Dihydro Diarylpyrazoles **4**−**14** in 5% Sucrose Solution Intake Model in Female Zucker fa/fa Rats at a Single Oral Dose of 10 mg/kg



4-14

Compd.	R	R^1	R^2	R^3	% Inhibition *vs.* Control [a]
4	4-ClC_6H_4	-Cl	-Cl		23.6 ± 5.5
5	4-ClC_6H_4	-Cl	-Cl		2.5 ± 8.5*
6	4-ClC_6H_4	-Cl	-Cl		3.6 ± 3.2*
7	4-ClC_6H_4	-Cl	-Cl		8.4 ± 1.4*
8	4-ClC_6H_4	-Cl	-Cl		22.6 ± 3.6
9	4-ClC_6H_4	-Cl	-Cl		34.2 ± 2.8
10	4-ClC_6H_4	-Cl	-H		8.1 ± 4.3*
11	4-ClC_6H_4	-H	-H		0.5 ± 7.4*
12	-C_6H_5	-Cl	-H		5.6 ± 2.2*
13	-C_6H_5	-H	-H		11.9 ± 6.7*
14	-C_6H_5	-Cl	-Cl		31.9 ± 3.8
1					36.7 ± 5.3

[a] Values indicate mean ± SEM for $n = 6$ in 4 h. *$P < 0.05$, when compared with compound **1**, one-way ANOVA followed by Dunnett's multiple comparison test.

After optimizing the R^3 substituent (Table 1), we changed the substituents R, R^1, and R^2 on the phenyl ring at positions 1 and 5 (Table 1). The removal of 2-chloro from the phenyl ring at position N-1 gave compound **10** with low efficacy. The removal of both the chlorine atoms gave compound **11**, and it was found to be inactive in the 5% sucrose solution intake model (Table 1). Subsequently, the removal of the chlorine atom from the phenyl ring at position 5 and removal of the 2-chloro from the phenyl ring at position N-1 showed a drastic drop in efficacy for compound **12** (Table 1). Furthermore, with removal of the chlorine atom of the phenyl ring at position 5 and both chlorine atoms from the phenyl ring at position 1, **13** also did not improve the efficacy. Similarly removal of the chlorine atom of the phenyl ring at position 5 and substitution of 2,4-dichlorophenyl on N-1 resulted in compound **14**, which showed percent inhibition of sucrose solution comparable to compound **1**. Since the 2,4-dichlorophenyl substitution on N-1 of dihydropyrazole and *N*-aminomorpholine appeared to be optimal for in vivo efficacy, we made some more changes at position 5 of the dihydropyrazole system such as 4-pentylphenyl derivative **15**, 4-butoxyphenyl derivative **16**, 4-methoxyphenyl derivative **17**, benzo-[1,3]dioxol-5-yl derivative **18**, 4-bromophenyl and derivative **19**, which elicited much inferior in vivo efficacy than that of rimonabant **1** (Table 2). Replacement of the aryl ring at position 5 of dihydropyrazole by heterocycles such as pyridin-3-yl derivative **20**, thiophen-2-yl analogue **21**, furan-2-yl derivative **22**, 5-methylthiophen-2-yl analogue **23**, and 5-chlorothiophen-2-yl derivative **24** gave compounds whose efficacies tend to remain inferior to rimonabant **1** as well as **9** (Table 2).

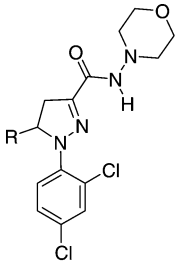
None of the compounds **15**−**24** were found suitable for imparting efficacy comparable to **1** in the 5% sucrose solution intake model in female Zucker fa/fa rats (Table 2). On the basis of the efficacy observed in the 5% sucrose solution intake model in female Zucker fa/fa rats, the compound **9** was selected as a reasonably good candidate for further studies.

The resolution of **9** was done at the pyrazole carboxylic acid stage **49** (Scheme 3). The racemic compound **9** and both the enantiomers **9a** and **9b** in the form of their hydrochloride salts **25**, **27**, and **29**, respectively, were compared in the 5% sucrose solution intake model in female Zucker fa/fa rats (Table 3). The hydrochloride salt of (−)-enantiomer **29** showed superior efficacy to **1**, **9**, **25**, and **27**. Since different pharmaceutically acceptable salts do exhibit different kinetics, few other salts such as bisulfate, oxalate, methane sulfonate, and benzene sulfonate salts were synthesized for **9b** and evaluated (Scheme 4 and Table 3) for differences in their efficacy profile in the 5% sucrose model. The oxalate salt **31**, methane sulfonate salt **32**, and benzene sulfonate salt **33** exhibited lower potency as compared to **1** in female Zucker fa/fa rats. The bisulfate salt of racemic compound **26** was found to be equiefficacious to **1**, while the bisulfate salt of (−)-enantiomer **30** was found to be much superior than the bisulfate salt of (±)-**26**, the bisulfate salt of (+)-enantiomer **28**, and **1**, demonstrating that bisulfate salt is probably more bioavailable than its hydrochloride salt. On the basis of the superior efficacy shown by **30** (Table 3), the compound was studied for a long term (60 days) repeat dose regime. The results (Figure 3) indicate a favorable response by **30** without developing tolerance, and a similar trend has been observed for the cumulative food intake by the animals (Figure 4). Furthermore, the inhibitory effect on body weight gain was accompanied by a significant reduction in serum triglyceride levels (Figure 5).

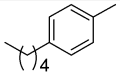
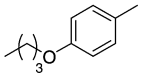
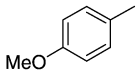
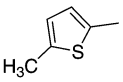
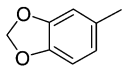
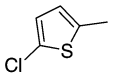
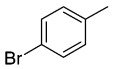
The pharmacokinetic profile of compound **30** was evaluated in fasted female Zucker fa/fa rats and compared with **1** (Table 4). The $AUC_{0-\infty}$ for **30** was found to be inferior to **1**. The tissue and plasma distribution of compound **30** (Table 5), when compared to compound **1**, revealed a higher availability of drug in the brain (1.61 ± 0.20 μg/mL) for **30** as compared to **1** (0.15 ± 0.01 μg/mL). Similarly the concentration of compound **30** was found to be higher in white adipose tissues (2.10 ± 0.40 μg/mL) as compared to **1** (1.40 ± 0.22 μg/mL). The in vitro binding affinity[33] to hCB1 receptor for the compound **30** was confirmed using CHO cells stably transfected with hCB1 receptors (Table 6). The highest CB1 receptor affinity (0.150 μM) was found for the bisulfate salt of (−)-enantiomer **30**. The (+)-enantiomer **28** showed threefold less affinity than **30**, indicating that these chiral ligands bind stereoselectively to the CB1 receptor. The results from Table 6 reveal that highest CB1/CB2 receptor selectivity (∼163) was found in the most active compound **30**, which is even fourfold higher than the CB1/ CB2 selectivity (42) for compound **1**. The CB1 antagonistic activity of **30** was further evaluated using mouse tetrad models[34,35] to confirm its efficacy through the cannabinoid mechanism (Table 7). Compound **30** showed a dose dependent CB1 antagonism in this model.

Since the dihydropyrazole class of compounds exhibited a significant potency and efficacy we have studied the binding

Table 2. In Vivo Efficacy of Dihydro Diarylpyrazoles **15**−**24** in 5% Sucrose Solution Intake Model in Female Zucker fa/fa Rats at a Single Oral Dose of 10 mg/kg



15-24

Compd.	R	% Inhibition *vs.* Control [a]	Compd.	R	% Inhibition *vs.* Control [a]
15		13.1 ± 2.8*	**21**		3.0 ± 13.5*
16		18.1 ± 2.9*	**22**		15.1 ± 5.9*
17		9.4 ± 7.0*	**23**		9.3 ± 10.6*
18		17.0 ± 6.7*	**24**		16.7 ± 7.7*
19		24.2 ± 9.1*	**1**		36.7 ± 5.3
20		4.0 ± 6.0*			

[a] Values indicate mean ± SEM for $n = 6$ in 4 h. *$P < 0.05$, when compared with compound **1**, one-way ANOVA followed by Dunnett's multiple comparison test.

Table 3. In Vivo Efficacy of Different Salts of Dihydro Diarylpyrazoles **9** and Its Enantiomers in 5% Sucrose Solution Intake Model in Female Zucker fa/fa Rats at a Single Oral Dose of 10 mg/kg

compd	salts prepared	% inhibition vs control[a]
25	HCl	34.5 ± 2.4
26	H_2SO_4	36.4 ± 1.2
27	HCl	30.1 ± 5.8
28	H_2SO_4	15.4 ± 2.6*
29	HCl	42.2 ± 7.1
30	H_2SO_4	58.6 ± 6.8*
31	$(COOH)_2$	20.4 ± 1.9
32	CH_3SO_3H	19.2 ± 2.3*
33	$PhSO_3H$	17.1 ± 3.2*
9	free base	34.2 ± 2.8
1		36.7 ± 5.3

[a] Values indicate mean ± SEM for $n = 6$ in 4 h. *$P < 0.05$, when compared with compound **1**, one-way ANOVA followed by Dunnett's multiple comparison test.

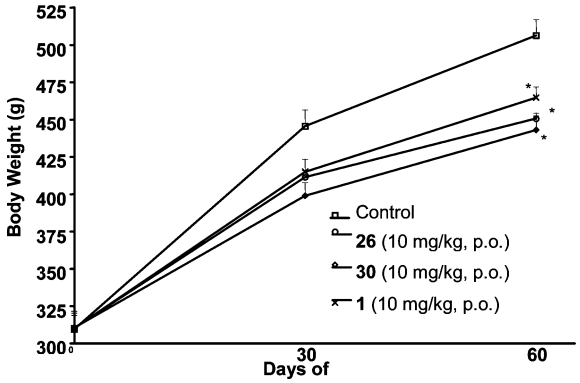


Figure 3. Effect of Compounds **26**, **30**, and **1** on body weight gain in female obese Zucker fa/fa rats. Values represent the mean and standard deviation for six animals in each group. *$P < 0.01$. Significant differences with the control group were found.

mode of **9** using modeling techniques. Automated docking of energy-minimized structures of **1** and **2** was performed using Ligandfit Software in the active site formed by aromatic residues in transmembrane helices (TMH) 3-4-5-6.[19] It was observed that the oxygen atom of group SO_2 in **2** forms a H bond with Asp 366-Lys 192 and an additional bond with Ser 383 (Figure 7). Taking a clue from these modeling studies we have taken two isomers *R* and *S* (**9a**, **9b**) of diaryl dihydropyrazole **9** for CB1 receptor interaction and studied them in silico using the same homology model (Figure 8). On the basis of the reconstructed model (in order to reproduce the interaction pattern described in the literature) for the binding of **1** in the CB1 receptor (Figure 6), the target compounds **2** and both enantiomers *R* and *S* (**9a**, **9b**) were automatically docked into the receptor using Monte Carlo simulations implemented in DS SBD.[19] Binding of **1** was characterized by aromatic stacking interactions between the two aromatic rings of **1** and the aromatic residue rich region in TMH 3-4-5-6.[36] A general CB1 inverse agonist pharmacophore model required for crucial receptor−ligand interaction has been proposed on the basis of the CB1 receptor modeling.[10] However, it is possible that our docked CB1-ligand model may vary from other published CB1 models[37−39] due to the possible differences and/or similarities of modeling techniques and different conformations of the CB1 ligands.[40−42]

In the Figures 6−8 the resulting docked conformations of **2** and both enantiomers *R* and *S* (**9a**, **9b**) of the compound **9** have slightly different orientations as compared with that of **1**. They exhibit a hydrogen bond interaction with Lys192, and the framework of aromatic rings forming aromatic stacking interactions with aromatic residues in the binding pocket of the receptor is nicely retained. Binding of **9a** and **9b** is further enhanced by

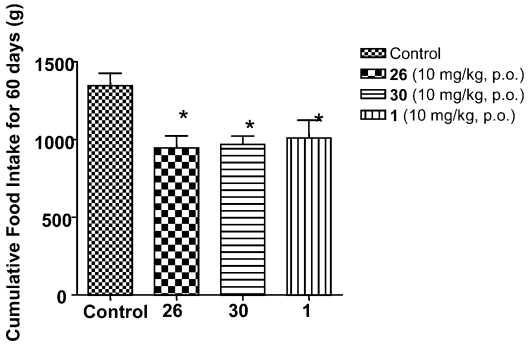


Figure 4. Effect of compounds **26**, **30**, and **1** on food intake in female obese Zucker fa/fa rats. Values represent the mean and standard deviation for six animals in each group. *P < 0.01. Significant differences with the control group (n = 6) were found.

Table 4. Mean Pharmacokinetic Parameters of **30** and **1** in Fasted Female Zucker fa/fa Rats[a]

compd	route	dose (mg/kg)	T_{max} (h)	C_{max} (μg/mL)	$T_{1/2}$ (h)	AUC(0−∞) (h·μg/mL)
30	oral	30	3.70 ± 1.10	0.51 ± 0.14	17.61 ± 6.10	10.00 ± 1.40
1	oral	30	2.60 ± 1.30	1.92 ± 0.31	26.55 ± 7.23	41.20 ± 3.65

[a] Values indicate mean ± SD for n = 6 rats.

Table 5. Tissue and Plasma Distribution of **30** and **1** in Fasted Female Zucker fa/fa Rat at 3 h after Oral Dose of 30 mg/kg[a]

	drug concn in brain		drug concn in white adipose tissue		drug concn in plasma
compd	μg/mL	μg/g	μg/mL	μg/g	μg/mL
30	1.61 ± 0.20	4.86 ± 0.55	2.10 ± 0.40	6.31 ± 1.20	1.40 ± 0.10
1	0.15 ± 0.01	0.45 ± 0.03	1.40 ± 0.22	4.25 ± 0.70	0.40 ± 0.05

[a] Values indicate mean ± SEM for n = 6 rats.

Table 6. Radioligand Binding Data of Compounds **26**, **28**, **30**, and **1**[33]

compd	CB1 K_i (μM)[a]	CB2 K_i (μM)[a]
26	0.334	21.7
28	0.475	26.4
30	0.150	24.5
1	0.047	1.99

[a] Values indicate the mean of at least two independent experiments performed.

an additional intramolecular H-bond involving the hydrogen of the amidic nitrogen with the nitrogen atom of the dihydropyrazole ring as shown in Figure 7. In the absence of crystallized receptor−ligand complexes, our model still gave valuable information on the receptor−ligand interactions.

Conclusion

The optimization of the diaryl dihydropyrazol-3-carboxamides **4**−**33** has led to the compound **30** as a potent cannabinoid CB1 receptor antagonist with a significant antiobesity effect in animal models. In the dihydropyrazole motif, the *N*-aminomorpholine is the optimal side chain, and bisulfate salt is more bioavailable

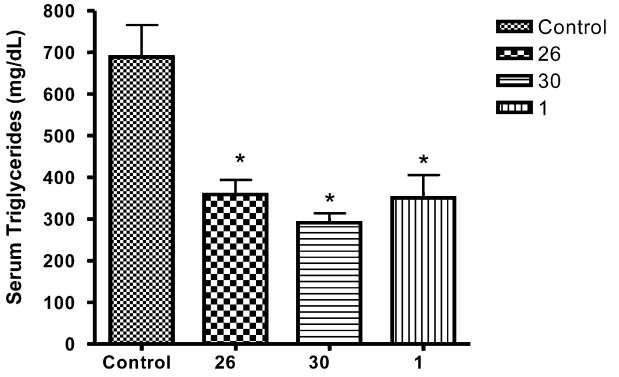


Figure 5. Effect of compounds **26**, **30**, and **1** on serum triglycerides level after 60 days treatment in female Zucker fa/fa rats. *P < 0.01, ANOVA followed by Dunnett's test. Significant differences with the same-day control group (n = 6) were found.

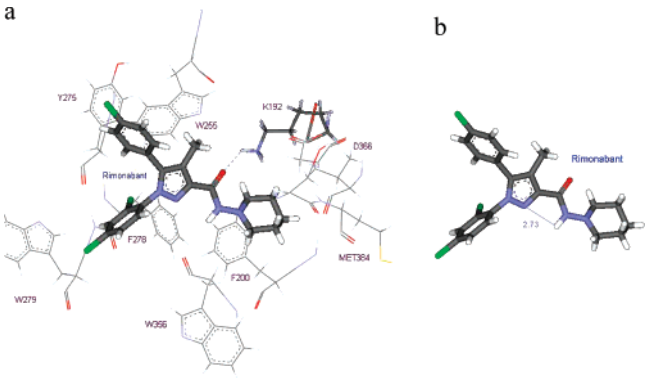


Figure 6. Docking of **1** in the homology model of CB1: (a) key interactions such as H bond with Lys 192 and aromatic stacking of the phenyl rings with Phe 200, Trp 255, Tyr 275, Phe 278, Trp 279, and Trp 356; (b) intramolecular H bond in the docked conformer.

for imparting the antiobesity effect. Similar interactions of diaryl dihydropyrazol-3-carboxamides have been observed in the homology models of CB1 receptor as those with **1** and **2**. The diaryl dihydropyrazole-3-carboxamide class of compounds possesses a promising therapeutic potential as a CB1 receptor antagonist to treat obesity and needs further exploration.

Experimental Section

Chemistry. Melting points were recorded on a scientific melting point apparatus and are uncorrected. IR spectra were recorded as neat (for oils) or on KBr pellet (for solid) on FT-IR 8300 Shimadzu and are expressed in ν (cm^{-1}). All ^{1}H spectral data are recorded on a 300 MHz ^{1}H NMR spectrometer (M-300) using DMSO-d_6, $CDCl_3$, or D_2O as solvent with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ downfield from tetramethylsilane. Multiplicities are recorded as a s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), or m (multiplet). Coupling constants (J) are expressed in hertz. Mass spectra are recorded on Perkin-Elmer Sciex API 3000. HPLC analysis were carried out at λ_{max} 220 nm using column

Table 7. Effect of Compound **30** in the Mouse Tetrad Model[a]

compd	compd, **30** (mg/kg, po)	locomotor activity[b]	change in body temp (°C)	catalepsy[c]	analgesic effect[d]
WIN-55212-2 (1 mg/kg, iv)	0 (control)	7.60 ± 7.00	−4.30 ± 0.35	86.10 ± 2.80	74.20 ± 8.60
	1	0.40 ± 0.40	−4.24 ± 0.25	85.20 ± 3.04	24.00 ± 6.20
	3	3.00 ± 2.00	−2.10 ± 0.30*	33.75 ± 7.00*	12.00 ± 1.50*
	10	13.25 ± 5.30	−0.15 ± 0.40*	14.20 ± 7.30*	−0.15 ± 1.43*
	30	25.00 ± 3.00*	0.15 ± 0.25*	18.23 ± 7.00*	11.50 ± 4.32*

[a] Values indicate the mean ± SEM for n = 6. [b] Number of squares crossed in 5 min. [c] Percent immobility. [d] Percent MPE on hot plate. *P < 0.05. Significant differences with the WIN 55212-2-treated control group.

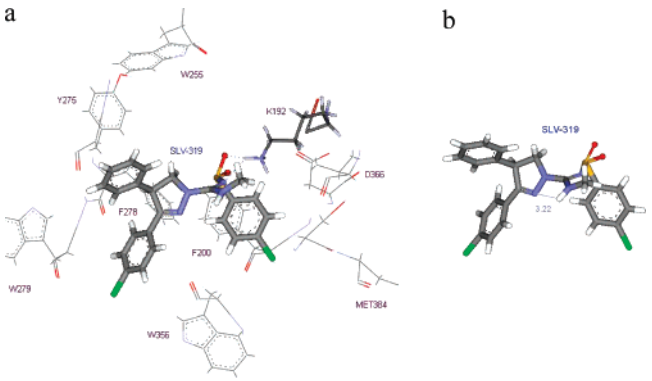


Figure 7. Docking of **2** in the homology model of CB1 receptor: (a) key interactions such as H bond with Lys 192 and aromatic stacking of the phenyl rings with Phe 200, Trp 255, Tyr 275, Phe 278, Trp 279, and Trp 356; (b) intramolecular H bond in the docked conformer.

ODS C-18, 150 mm × 4.6 mm × 4 μm on AGILENT 1100 series. Chiral HPLC analysis were carried out at λ_{max} 220 nm using columns CHIRALCEL OJ-H, 250 mm × 4.6 mm × 5 μm on SHIMADZU LCυp 10Aυp. Optical rotations ($[\alpha]_D$) were measured on an a JASCO P-1030 polarimeter. Specific rotations are given as deg/dm; the concentration values are reported as g/100 mL of the specified solvent and were recorded at 25 °C. Elemental analyses were performed on a Thermo Quest EA 1110 CHNS. All reactions involving air or moisture sensitive compounds were performed under argon atmosphere. Thin layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel 60 F_{254}, and spots were visualized with UV light. Flash chromatography (FC) was performed using silica gel 230−400 mesh.

Synthesis of Potassium 4-(4-Chlorophenyl)-2-oxo-but-3-enoate (36). To 4-chlorobenzaldehyde (117 g, 832.1 mmol) in methanol (117 mL) was added pyruvic acid (146.4 g, 166.2 mmol), and the mixture was cooled to 10 °C under the atmosphere of argon. To this was added a solution of KOH (109.8 g, 166.2 mmol) in methanol (234 mL) dropwise at 15−20 °C over a period of 30 min. The temperature of the reaction mixture increased from 20 °C to 35−40 °C. The reaction mixture was stirred at this temperature for 3 h and then maintained at 10 °C for 10 h. The solid separated out was filtered on a Buchner funnel under suction and washed with chilled methanol (330 mL) followed by diethyl ether (330 mL) to afford **36** as a yellow solid (161 g, 77%): 95.92% purity by HPLC; mp 280 °C; IR (KBr) 3338, 3066, 1676, 1604, 1587 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.68 (d, J = 8.52 Hz, 2H), 7.45 (d, J = 8.48 Hz, 2H), 7.36 (d, J = 16.39 Hz, 1H), 6.73 (d, J = 16.35 Hz, 1H); ESI-MS 211.5 $[M + H]^+$.

Potassium 2-Oxo-4-phenyl-but-3-enoate (37). Compound **37** was prepared in 43% yield from benzaldehyde and pyruvic acid by the same procedure as described for **36**: 97.61% purity by HPLC; mp 248 °C; ESI-MS 214.9 $[M + H]^+$.

Potassium 2-Oxo-4-(4-pentylphenyl)-but-3-enoate (65). Compound **65** was prepared in 48% yield from 4-*n*-pentylbenzaldehyde and pyruvic acid by the same procedure as described for **36**: 97.26% purity by HPLC; mp 217 °C; ESI-MS 285.2 $[M + H]^+$.

Potassium 4-(4-Butoxyphenyl)-2-oxo-but-3-enoate (66). Compound **66** was prepared in 72% yield from 4-butoxybenzaldehyde and pyruvic acid by the same procedure as described for **36**: 95.39% purity by HPLC; mp 220 °C; ESI-MS 286.8 $[M + H]^+$.

Potassium 4-(4-Methoxyphenyl)-2-oxo-but-3-enoate (67). Compound **67** was prepared in 58% yield from 4-methoxybenzaldehyde and pyruvic acid by the same procedure as described for **36**: 95.16% purity by HPLC; mp 248 °C; ESI-MS 244.8 $[M + H]^+$.

Potassium 4-Benzo[1,3]dioxol-5-yl-2-oxo-but-3-enoate (68). Compound **68** was prepared in 70% yield from 4-piperonal and pyruvic acid by the same procedure as described for **36**: 95.69% purity by HPLC; mp 250 °C; ESI-MS 258.7 $[M + H]^+$.

Potassium 4-(4-Bromophenyl)-2-oxo-but-3-enoate (69). Compound **69** was prepared in 42% yield from 4-bromobenzaldehyde and pyruvic acid by the same procedure as described for **36**: 99.24% purity by HPLC; mp 233 °C; ESI-MS 294.9 $[M + H]^+$.

Potassium 2-Oxo-4-pyridin-3-yl-but-3-enoate (70). Compound **70** was prepared in 34% yield from 3-pyridinecarboxaldehyde and pyruvic acid by the same procedure as described for **36**: 95.78% purity by HPLC; mp 239 °C; ESI-MS 216.1 $[M + H]^+$.

Potassium 2-Oxo-4-thiophen-2-yl-but-3-enoate (71). Compound **71** was prepared in 69% yield from 2-thiophenecarboxaldehyde and pyruvic acid by the same procedure as described for **36**: 98.18% purity by HPLC; mp 260.8 °C; ESI-MS 220.8 $[M + H]^+$.

Potassium 4-Furan-2-yl-2-oxo-but-3-enoate (72). Compound **72** was prepared in 33% yield from furfuraldehyde and pyruvic acid by the same procedure as described for **36**: 95.86% purity by HPLC; mp 240 °C; ESI-MS 204.8 $[M + H]^+$.

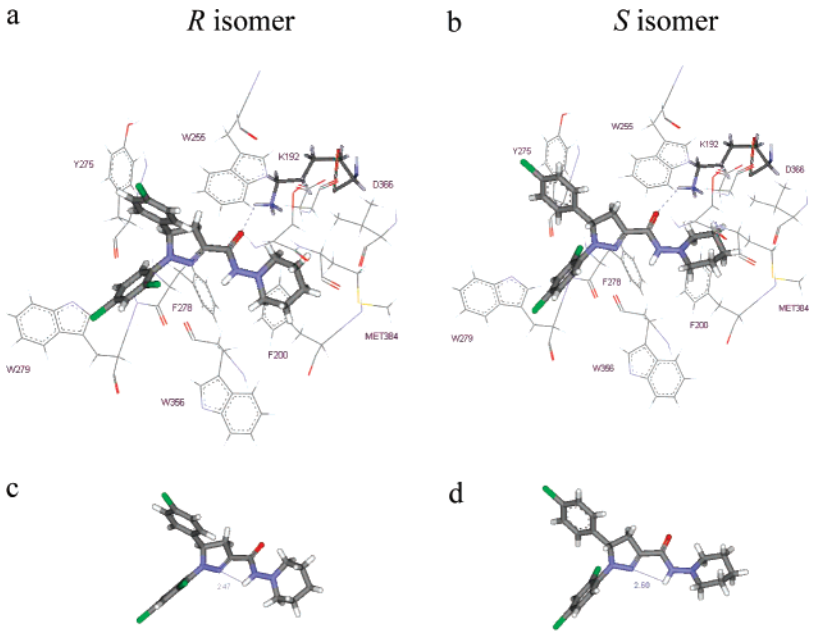


Figure 8. Docking of the both isomers *R* and *S* (**9a**, **9b**) for compound **9** in the homology model of the CB1 receptor: (a, b) key interactions of *R* and *S* isomers, respectively, such as the H bond with Lys 192 and aromatic stacking of the phenyl rings with Phe 200, Trp 255, Tyr 275, Phe 278, Trp 279, and Trp 356; (c, d) intramolecular H bond in the docked conformer of both *R* and *S* isomers.

Potassium 4-(5-Methylthiophen-2-yl)-2-oxo-but-3-enoate (73). Compound **73** was prepared in 64% yield from 5-methyl-2-thiophenecarboxaldehyde and pyruvic acid by the same procedure as described for **36**: 98.47% purity by HPLC; mp 242 °C; ESI-MS 234.6 $[M + H]^+$.

Potassium 4-(5-Chlorothiophen-2-yl)-2-oxo-but-3-enoate (74). Compound **74** was prepared in 71% yield from 5-chloro-2-thiophenecarboxaldehyde and pyruvic acid by the same procedure as described for **36**: 97.49% purity by HPLC; mp 280 °C; ESI-MS 255.6 $[M + H]^+$.

Synthesis of 4-(4-Chlorophenyl)-2-oxo-but-3-enoic Acid Ethyl Ester (38). To a solution of **36** (30 g, 120 mmol) in dimethylformamide (100 mL) was added ethyl iodide (16.5 mL, 204 mmol), and the mixture was stirred at 72−75 °C for 4 h under the argon atmosphere. The progress of the reaction was monitored by TLC using 40% EtOAc in petroleum ether as a mobile phase. The reaction mixture was cooled to 27−28 °C, diluted with water (400 mL), and extracted with ethyl acetate (3 × 100 mL). The ethyl acetate layer was separated and washed with water (2 × 200 mL) followed by aqueous $NaHCO_3$ solution (2 × 200 mL). The organic layer was separated and dried over anhydrous Na_2SO_4, and solvents were evaporated on a rotary evaporator under reduced pressure. The solid obtained was further triturated in di-isopropyl ether (100 mL), filtered on a Buchner funnel under suction, and dried to afford **38** as a pale yellow solid (8.7 g, 30.1%): 95.49% purity by HPLC; mp 69−71 °C; IR (KBr) 3371, 1720, 1689 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, $J = 16.11$ Hz, 1H), 7.57 (d, $J = 8.11$ Hz, 2H), 7.38 (d, $J = 8.52$ Hz, 2H), 7.29 (d, $J = 14.67$ Hz, 1H), 4.43−4.36 (q, 2H), 1.41 (t, 3H); ESI-MS 239.1 $[M + H]^+$.

2-Oxo-4-phenyl-but-3-enoic Acid Ethyl Ester (39). Compound **39** was prepared from **37** in 94% yield by the same procedure as described for **38**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a yellow oil: 97.58% purity by HPLC; ESI-MS 205.1 $[M + H]^+$.

2-Oxo-4-(4-pentylphenyl)-but-3-enoic Acid Ethyl Ester (75). Compound **75** was prepared from **65** in 62% yield by the same procedure as described for **38**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 95.27% purity by HPLC; ESI-MS 274.9 $[M + H]^+$.

4-(4-Butoxyphenyl)-2-oxo-but-3-enoic Acid Ethyl Ester (76). Compound **76** was prepared from **66** in 84% yield by the same procedure as described for **38**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 94.28% purity by HPLC; ESI-MS 277.1 $[M + H]^+$.

4-(4-Methoxyphenyl)-2-oxo-but-3-enoic Acid Ethyl Ester (77). Compound **77** was prepared from **67** in 77% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as a pale yellow solid: 96.39% purity by HPLC; mp 49−51 °C; ESI-MS 234.8 $[M + H]^+$.

4-Benzo[1,3]dioxol-5-yl-2-oxo-but-3-enoic Acid Ethyl Ester (78). Compound **78** was prepared from **68** in 78% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as an orange solid: 98.66% purity by HPLC; mp 73−75 °C; ESI-MS 248.8 $[M + H]^+$.

4-(4-Bromophenyl)-2-oxo-but-3-enoic Acid Ethyl Ester (79). Compound **79** was prepared from **69** in 65% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as an orange solid: 95.19% purity by HPLC; mp 70 °C; ESI-MS 283.7 $[M + H]^+$.

2-Oxo-4-pyridin-3-yl-but-3-enoic Acid Ethyl Ester (80). Compound **80** was prepared from **70** in 89% yield by the same procedure as described for **38**: The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as an orange solid: 96.27% purity by HPLC; mp 70−72 °C; ESI-MS 206.0 $[M + H]^+$.

2-Oxo-4-thiophen-2-yl-but-3-enoic Acid Ethyl Ester (81). Compound **81** was prepared from **71** in 74% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as an orange solid: 95.38% purity by HPLC; mp 49−50 °C; ESI-MS 210.8 $[M + H]^+$.

4-Furan-2-yl-2-oxo-but-3-enoic Acid Ethyl Ester (82). Compound **82** was prepared from **72** in 89% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as a pale yellow solid: 96.58% purity by HPLC; mp 56−57 °C; ESI-MS 194.8 $[M + H]^+$.

4-(5-Methylthiophen-2-yl)-2-oxo-but-3-enoic acid Ethyl Ester (83). Compound **83** was prepared from **73** in 88% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as an orange solid: 98.49% purity by HPLC; mp 42−43 °C; ESI-MS 224.8 $[M + H]^+$.

4-(5-Chlorothiophen-2-yl)-2-oxo-but-3-enoic Acid Ethyl Ester (84). Compound **84** was prepared from **74** in 97% yield by the same procedure as described for **38**. The solid obtained was triturated in di-isopropyl ether. It was filtered on a Buchner funnel under suction and dried to afford the title compound as a pale yellow solid: 96.89% purity by HPLC; mp 79−81 °C; ESI-MS 245.8 $[M + H]^+$.

Synthesis of 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (43). To **38** (20.0 g, 83.79 mmol) in ethanol (200 mL) was added 2,4-dichlorophenyl hydrazine hydrochloride **40** (17.9 g, 83.79 mmol), and the reaction mixture was stirred at 28−31 °C over a period of 2 h under argon atmosphere. To the reaction mixture was added acetic acid (50 mL), and the mixture refluxed for 18 h. The progress of the reaction was monitored by TLC using 10% EtOAc in petroleum ether as a mobile phase. The reaction mixture was cooled to 27−28 °C, poured into water (300 mL), and extracted with ethyl acetate (3 × 100 mL). The combined ethyl acetate layers were washed with water (2 × 200 mL) followed by aqueous $NaHCO_3$ solution (2 × 200 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvents were evaporated on a rotatory evaporator under reduced pressure. The residue obtained was triturated in methanol (100 mL) to afford a solid which was filtered on a Buchner funnel under suction and dried to afford **43** as a yellow solid (4.3 g, 57%): 96.79% purity by HPLC; mp 120−122 °C; IR (KBr) 2981, 1737, 1706, 1575, 1556, 1479 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, $J = 8.27$ Hz, 2H), 7.21 (d, $J = 2.29$ Hz, 1H), 7.08−7.05 (dd, $J = 8.47$ and 2.29 Hz, 4H), 5.88−5.82 (dd, $J = 12.54$ and 5.94 Hz, 1H), 4.40−4.33 (q, 2H), 3.74−3.64 (dd, $J = 18.05$ and 12.841 Hz, 1H), 3.30−3.22 (dd, $J = 18.05$ and 5.94 Hz, 1H), 1.38 (t, 3H); ESI-MS 398.3 $[M + H]^+$.

1,5-Bis-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (44). Compound **44** was prepared from **38** and 4-chlorophenyl hydrazine hydrochloride **41** in 87% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 99.67% purity by HPLC; ESI-MS 364.0 $[M + H]^+$.

5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (45). Compound **45** was prepared from **38** and phenylhydrazine hydrochloride **42** in 69% yield by the same procedure as described for **43**. The residue obtained was triturated in methanol to afford a solid, which was filtered on a Buchner funnel under suction and dried to afford the title compound as a yellow solid: 97.88% purity by HPLC; mp 120−122 °C; ESI-MS 329.3 $[M + H]^+$.

1-(4-Chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (46). Compound **46** was prepared from **39** and 4-chlorophenyl hydrazine hydrochloride **41** in 70% yield by the same procedure as described for **43**. The residue obtained

was triturated in methanol to afford a solid, which was filtered on a Buchner funnel under suction and dried to afford the title compound as a yellow solid: 98.56% purity by HPLC; mp 109−111 °C; ESI-MS 329.3 $[M + H]^+$.

1,5-Diphenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (47). Compound **47** was prepared from **39** and phenyl hydrazine hydrochloride **42** in 77% yield by the same procedure as described for **43**. The residue obtained was triturated in methanol to afford a solid, which was filtered on a Buchner funnel under suction and dried to afford the title compound as an off-white solid: 97.25% purity by HPLC; mp 83−85 °C; ESI-MS 295.0 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (48). Compound **48** was prepared from **39** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 63% yield by the same procedure as described for **43**. The residue obtained was triturated in methanol to afford a solid, which was filtered on a Buchner funnel under suction and dried to afford the title compound as a yellow solid: 99.67% purity by HPLC; mp 65−67 °C; ESI-MS 364.1 $[M + H]^+$

1-(2,4-Dichlorophenyl)-5-(4-pentylphenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (85). Compound **85** was prepared from **75** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 73% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 96.88% purity by HPLC; ESI-MS 434.0 $[M + H]^+$.

5-(4-Butoxyphenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (86). Compound **86** was prepared from **76** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 73% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 97.92% purity by HPLC; ESI-MS 437.3 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (87). Compound **87** was prepared from **77** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 45% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a yellow oil: 97.47% purity by HPLC; ESI-MS 393.8 $[M + H]^+$.

5-Benzo[1,3]dioxol-5-yl-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (88). Compound **88** was prepared from **78** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 86% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 98.38% purity by HPLC; ESI-MS 408.1 $[M + H]^+$.

5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (89). Compound **89** was prepared from **79** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 78% yield by the same procedure as described for **43**. The residue obtained was triturated in methanol to afford a solid, which was filtered on a Buchner funnel under suction and dried to afford the title compound as a yellow solid: 95.92% purity by HPLC; mp 117−118 °C; ESI-MS 442.9 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-pyridin-3-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (90). Compound **90** was prepared from **80** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 81% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 97.25% purity by HPLC; ESI-MS 365.0 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-thiophen-2-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (91). Compound **91** was prepared from **81** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 72% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a brown oil: 99.08% purity by HPLC; ESI-MS 370.4 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-furan-2-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (92). Compound **92** was prepared from **82** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 82% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a yellow oil: 95.79% purity by HPLC; ESI-MS 354.3 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(5-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (93). Compound **93** was prepared from **83** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 67% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a yellow oil: 99.34% purity by HPLC; ESI-MS 384.9 $[M + H]^+$.

5-(5-Chlorothiophen-2-yl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Ethyl Ester (94). Compound **94** was prepared from **84** and 2,4-dichlorophenyl hydrazine hydrochloride **40** in 69% yield by the same procedure as described for **43**. The residue was purified by column chromatography using petroleum ether/EtOAc (9.5:1.5) as an eluent to afford the title compound as a yellow oil: 97.38% purity by HPLC; ESI-MS 378.9 $[M + H]^+$.

Synthesis of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (49). To a solution of **43** (4.2 g, 10.57 mmol) in methanol (35 mL) was added a solution of KOH (1.183 g, 21.14 mmol) in water (35 mL), and the mixture was refluxed at 65−68 °C for 2 h. The progress of the reaction was monitored by TLC using 5% MeOH in $CHCl_3$ as a mobile phase. The reaction mixture was cooled to 27−28 °C, poured into ice cold water (150 mL), acidified to pH 4 using 10% HCl solution, and extracted with ethyl acetate (3 × 50 mL). The ethyl acetate layers were pooled and washed with water (100 mL). The organic layer was separated, dried over anhydrous Na_2SO_4, and the solvents were evaporated on a rotatory evaporator under reduced pressure. The residue was triturated in petroleum ether (25 mL) to afford a solid. The solid was filtered on a Buchner funnel under suction and dried to afford **49** as a yellow solid (2.9 g, 74%): 99.23% purity by HPLC; mp 99−102 °C; IR (KBr) 3433, 2927, 2582, 1685, 1571, 1479 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 13.21 (s, 1H), 7.49 (d, J = 1.98 Hz, 1H), 7.41−7.38 (m, 1H), 7.35−7.32 (m, 3H), 7.25−7.20 (m, 2H), 5.90−5.84 (dd, J = 12.03 and 6.66 Hz, 1H), 3.72−3.62 (dd, J = 17.91 and 12.44 Hz, 1H), 3.08−3.01 (dd, J = 18.0 and 6.75 Hz, 1H); ESI-MS 370.1 $[M + H]^+$.

1,5-Bis-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (50). Compound **50** was prepared from **44** in 79% yield by the same procedure as described for **49**: 97.18% purity by HPLC; mp 165−167 °C; ESI-MS 336.0 $[M + H]^+$.

5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (51). Compound **51** was prepared from **45** in 98% yield by the same procedure as described for **49**: 98.93% purity by HPLC; mp 179−181 °C; ESI-MS 301.4 $[M + H]^+$.

1-(4-Chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (52). Compound **52** was prepared from **46** in 85% yield by the same procedure as described for **49**: 99.68% purity by HPLC; mp 173−175 °C; ESI-MS 301.3 $[M + H]^+$.

1,5-Diphenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (53). Compound **53** was prepared from **47** in 78% yield by the same procedure as described for **49**: 99.35% purity by HPLC; mp 195−197 °C; ESI-MS 267.0 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (54). Compound **54** was prepared from **48** in 80% yield by the same procedure as described for **49**: 99.09% purity by HPLC; mp 248−250 °C; ESI-MS 336.2 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(4-pentylphenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (95). Compound **95** was prepared from **85** in 91% yield by the same procedure as described for **49**: 97.84% purity by HPLC; mp 175−176 °C; ESI-MS 406.0 $[M + H]^+$.

5-(4-Butoxyphenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (96). Compound **96** was prepared from

86 in 82% yield by the same procedure as described for **49**: 99.47% purity by HPLC; mp 108−110 °C; ESI-MS 408.1 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (97). Compound **97** was prepared from **87** in 79% yield by the same procedure as described for **49**: 97.63% purity by HPLC; mp 178−180 °C; ESI-MS 366.0 $[M + H]^+$.

5-Benzo[1,3]dioxol-5-yl-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (98). Compound **98** was prepared from **88** in 89% yield by the same procedure as described for **49**: 96.78% purity by HPLC; mp 157−158 °C; ESI-MS 380.0 $[M + H]^+$.

5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (99). Compound **99** was prepared from **89** in 84% yield by the same procedure as described for **49**: 99.49% purity by HPLC; mp 92−93 °C; ESI-MS 415.7 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-pyridin-3-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (100). Compound **100** was prepared from **90** in 75% yield by the same procedure as described for **49**: 98.29% purity by HPLC; ESI-MS 336.8 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-thiophen-2-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (101). Compound **101** was prepared from **91** in 86% yield by the same procedure as described for **49**: 99.37% purity by HPLC; mp 137−138 °C; ESI-MS 342.1 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-furan-2-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (102). Compound **102** was prepared from **92** in 89% yield by the same procedure as described for **49**: 97.38% purity by HPLC; mp 140−141 °C; ESI-MS 326.2 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(5-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (103). Compound **103** was prepared from **93** in 84% yield by the same procedure as described for **49**: 99.94% purity by HPLC; mp 148−150 °C; ESI-MS 355.7 $[M + H]^+$.

5-(5-Chlorothiophen-2-yl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (104). Compound **104** was prepared from **94** in 81% yield by the same procedure as described for **49**: 99.06% purity by HPLC; mp 82−84 °C; ESI-MS 377.4 $[M + H]^+$.

Typical Procedure for the Resolution of 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (49). (−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (49b). To a solution of **49** (64 g, 0.17 mole) in acetone (320 mL) was added *R*-(+)-α-methyl benzylamine (20.9 mL, 0.17 mol) at 26−28 °C, and the mixture was stirred for 10−15 min. To this was added acetonitrile (320 mL), and the mixture was stirred at 26−28 °C for another 2 h. The solid salt separated out was filtered on a Buchner funnel under suction and washed with chilled acetonitrile to afford the *R*-(+)-α-methyl benzylamine salt of (−)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid as an off-white solid salt (34 g, 40%): 99.3% purity by HPLC, 99.8% chiral purity by HPLC (99.6% ee), $[\alpha_D] = -407°$, $c = 0.2$, DMSO; mp 164−165 °C; IR (KBr) 3413, 2977, 1569, 1477, 1307 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.45 (d, $J = 6.93$ Hz, 2H), 7.40−7.37 (m, 3H), 7.35−7.31 (m, 2H), 7.27 (d, $J = 8.43$ Hz, 2H), 7.21−7.19 (dd, $J = 8.76$ and 2.34 Hz, 1H), 7.12 (d, $J = 8.43$ Hz, 2H), 5.71−5.65 (dd, $J = 11.34$ and 6.63 Hz, 1H), 4.33−4.27 (q, 1H), 3.56−3.47 (dd, $J = 17.82$ and 11.82 Hz, 1H), 2.99−2.91 (dd, $J = 17.88$ and 5.43 Hz, 1H), 1.45 (d, 3H) ESI-MS 492.7 $[M + H]^+$.

The *R*-(+)-α-methyl benzylamine salt of (−)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid (34.0 g) was taken in ice cold water (100 mL) and acidified to pH 3 using 10% HCl solution. The solution was extracted with dichloromethane (2 × 200 mL), and the organic layer was washed with water (3 × 200 mL). The organic layer was separated and dried over anhydrous Na_2SO_4, and the solvents were evaporated on a rotatory evaporator under reduced pressure. The residue was triturated in petroleum ether to get a solid. The solid was filtered on a Buchner funnel under suction and dried to afford **49b** as an off-white solid (20 g, 31%): 99.90% purity by HPLC, 99.8% chiral purity by HPLC (99.6% ee), $[\alpha_D] = -187°$, $c = 0.2$, DMSO; mp 144−146 °C; IR (KBr) 3413, 2925, 1685, 1571, 1479 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 2H), 7.21 (d, $J = 1.93$ Hz, 1H), 7.19 (s, 1H), 7.09 (d, $J = 8.65$ Hz, 3H), 5.95−5.89 (dd, $J = 12.68$ and 6.14 Hz, 2H), 3.77−3.67 (dd, $J = 18.06$ and 12.14 Hz, 1H), 3.32−3.24 (dd, $J = 18.10$ and 6.18 Hz, 1H); ESI-MS 370.9 $[M + H]^+$.

(+)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (49a). The resolution of **49** to (+)-enantiomer **49a** was done using the same procedure described for compound **49b**. Compound **49** was reacted with *S*-(−)-α-methyl benzylamine to get the *S*-(−)-α-methyl benzylamine salt of (+)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid as an off-white solid (18 g, 35%): 99.95% purity by HPLC, 99.6% chiral purity by HPLC (99.2% ee), $[\alpha_D] = +407°$, $c = 0.2$, DMSO; mp 160−161 °C; IR (KBr) 3033, 2943, 2881, 1571, 1475 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 7.45−7.41 (m, 2H), 7.38−7.33 (m, 5H), 7.23 (d, $J = 8.38$ Hz, 2H), 7.20−7.16 (dd, $J = 8.74$ and 2.33 Hz, 1H), 7.11 (d, $J = 8.41$ Hz, 2H), 5.69−5.64 (dd, $J = 11.29$ and 4.95 Hz, 1H), 4.36−4.29 (q, 1H), 3.55−3.45 (dd, $J = 17.19$ and 12.22 Hz, 1H), 2.97−2.90 (dd, $J = 17.01$ and 5.07 Hz, 1H), 1.43 (d, 3H) ESI-MS 492.7 $[M + H]^+$.

The *S*-(−)-α-methyl benzylamine salt of (+)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic acid was converted into free acid **49a** as an off-white solid (11.5 g, 28%): 99.86% purity by HPLC, 99.66% chiral purity by HPLC (99.2% ee), $[\alpha_D] = +187°$, $c = 0.2$, DMSO; mp 180−181 °C; IR (KBr) 3425, 1689, 1575, 1555 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 13.01 (br s, 1H), 7.48 (d, $J = 2.19$ Hz, 1H), 7.35−7.28 (m, 4H), 7.20 (d, $J = 8.49$ Hz, 2H), 5.90−5.84 (dd, $J = 12.15$ and 6.69 Hz, 2H), 3.72−3.62 (dd, $J = 17.91$ and 12.57 Hz, 1H), 3.08−3.01 (dd, $J = 18.03$ and 6.78 Hz, 1H); ESI-MS 370.9 $[M + H]^+$.

Synthesis of 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (9). To a solution of **49** (2.5 g, 6.66 mmol) in dichloromethane (30 mL) were added 1-hydroxybenzotriazole hydrate (1.825 g, 13.32 mmol) and [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] hydrochloride (1.945 g, 9.99 mmol), and the mixture was stirred for 5−7 min at 26−27 °C. To this mixture *N*-aminomorpholine (0.755 g, 7.326 mmol) was added followed by triethylamine (1.78 mL, 13.32 mmol). The reaction mixture was stirred at 28−29 °C for 30 min. The progress of the reaction was monitored by TLC using 5% MeOH in $CHCl_3$ as a mobile phase. The reaction mixture was poured in water (80 mL) and extracted with dichloromethane (2 × 50 mL). The dichloromethane layer was separated and dried over anhydrous Na_2SO_4, and the solvents were evaporated on a rotatory evaporator under reduced pressure to get crude brown oil. The crude oil was purified through flash column chromatography petroleum ether:EtOAc (9: 1) as an eluent. The fractions were pooled and solvents were evaporated on a rotatory evaporator under reduced pressure to afford oil. The oil was triturated in petroleum ether (15 mL) to afford **9** as a white solid (1.1 g, 34%): 99.94% purity by HPLC, $[\alpha_D] = +0.28°$, $c = 0.2$, DMSO; mp 172−174 °C; IR (KBr) 3421, 3246, 1662, 1583, 1477 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.18 (d, $J = 8.40$ Hz, 2H), 7.12 (d, $J = 8.61$ Hz, 2H), 7.09−7.04 (m, 3H), 5.76−5.69 (dd, $J = 12.18$ and 6.36 Hz, 1H), 3.87−3.84 (br s, 4H), 3.74−3.63 (dd, $J = 18.33$ and 12.46 Hz, 1H), 3.37−3.29 (dd, $J = 18.36$ and 6.36 Hz, 1H), 2.95−2.92 (br s, 4H); ESI-MS 454.9 $[M + H]^+$.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Piperidin-1-ylamide (4). Compound **4** was prepared from **49** and *N*-aminopiperidine in 38% yield by the same procedure as described for **9**: 99.46% purity by HPLC, $[\alpha_D] = -0.78°$, $c = 0.2$, DMSO; mp 176−177 °C; IR (KBr) 3422, 3219, 1649, 1587, 1475 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (s, 1H), 7.25 (d, $J = 3.82$ Hz, 1H), 7.17 (d, $J = 8.47$ Hz, 2H), 7.11 (d, $J = 8.65$ Hz, 1H), 7.08−7.04 (m, 3H), 5.74−5.68 (dd, $J = 12.12$ and 6.24 Hz, 1H), 3.73−3.63 (dd, $J = 18.34$ and 12.63 Hz, 1H), 3.37−3.29 (dd, $J = 18.34$ and 6.28 Hz, 1H), 2.85−2.82 (br s, 4H), 1.79−1.75 (br s, 4H), 1.46−1.42 (br s, 2H); ESI-MS 452.6 $[M + H]^+$.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Pyrrolidin-1-ylamide (5). Compound

5 was prepared from **49** and *N*-aminopyrrolidine in 30% yield by the same procedure as described for **9**: 99.14% purity by HPLC, $[\alpha_D] = +0.63°$, $c = 0.2$, DMSO; mp 140−142 °C; IR (KBr) 3425, 3220, 1654, 1595, 1490 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 1H), 7.26−7.24 (m, 2H), 7.18 (d, $J = 8.43$ Hz, 2H), 7.12 (d, $J = 8.67$ Hz, 1H), 7.08−7.04 (m, 2H), 5.74−5.68 (dd, $J = 12.09$ and 6.21 Hz, 1H), 3.74−3.64 (dd, $J = 18.33$ and 12.45 Hz, 1H), 3.36−3.30 (dd, $J = 18.34$ and 6.22 Hz, 1H), 2.99−2.96 (br s, 4H), 1.92−189 (br s, 4H); ESI-MS 438.8 $[M + H]^+$.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (Hexahydrocyclopenta[*c*]pyrrol-2-yl)-amide (6). Compound **6** was prepared from **49** and 3-amino-3-azabicyclo[3.3.0]octane hydrochloride in 34% yield by the same procedure as described for **9**: 99.69% purity by HPLC, $[\alpha_D] = +0.28°$, $c = 0.2$, DMSO; mp 80 °C (fuses); IR (KBr) 3409, 3234, 1671, 1585, 1477 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.26−7.24 (m, 2H), 7.16 (d, $J = 8.43$ Hz, 2H), 7.12 (d, $J = 7.06$ Hz, 1H), 7.07−7.03 (m, 2H), 5.74−5.68 (dd, $J = 12.12$ and 6.15 Hz, 1H), 3.73−3.63 (dd, $J = 18.33$ and 12.58 Hz, 1H), 3.38−3.31 (dd, $J = 18.35$ and 6.17 Hz, 1H), 3.29−3.27 (m, 2H), 2.71−2.68 (br s, 2H), 2.46−2.35 (m, 2H), 1.68−1.63 (m, 3H), 1.56−1.50 (m, 3H); ESI-MS 478.9 $[M + H]^+$.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Azepan-1-ylamide (7). Compound **7** was prepared from **49** and *N*-aminohomopiperidine in 9% yield by the same procedure as described for **9**: 98.25% purity by HPLC, $[\alpha_D] = -1.18°$, $c = 0.2$, DMSO; mp 190−191 °C; IR (KBr) 3424, 3224, 1649, 1585, 1490 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.26−7.24 (m, 2H), 7.18 (d, $J = 8.37$ Hz, 2H), 7.08−7.04 (m, 3H), 5.74−5.68 (dd, $J = 12.09$ and 6.24 Hz, 1H), 3.73−3.63 (dd, $J = 18.30$ and 12.35 Hz, 1H), 3.37−3.29 (dd, $J = 18.33$ and 6.27 Hz, 1H), 3.14−3.11 (br s, 4H), 1.75−1.72 (br s, 4H), 1.67−1.64 (br s, 4H); ESI-MS 466.2 $[M + H]^+$.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid (4-Methylpiperazin-1-yl)-amide (8). Compound **8** was prepared from **49** and *N*-amino-4-methylpiperazine in 26% yield by the same procedure as described for **9**: 99.18% purity by HPLC, $[\alpha_D] = +0.02°$, $c = 0.2$, DMSO; mp 80 °C (fuses); IR (KBr) 3425, 3261, 1658, 1583, 1480 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41 (s, 1H), 7.25 (s, 1H), 7.18 (d, $J = 8.30$ Hz, 2H), 7.10 (d, $J = 7.36$ Hz, 1H), 7.09−7.03 (m, 3H), 5.76−5.70 (dd, $J = 12.42$ and 6.45 Hz, 1H), 3.73−3.63 (dd, $J = 18.43$ and 12.57 Hz, 1H), 3.37−3.29 (dd, $J = 18.33$ and 6.21 Hz, 1H), 2.98−2.95 (br s, 4H), 2.72−2.69 (br s, 4H), 2.37 (s, 3H); ESI-MS 468 $[M + H]^+$.

1,5-Bis-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (10). Compound **10** was prepared from **50** and *N*-aminomorpholine in 29% yield by the same procedure as described for **9**: 99.76% purity by HPLC, $[\alpha_D] = +0.32°$, $c = 0.2$, DMSO; mp 216−217 °C; IR (KBr) 3438, 3235, 1737, 1651, 1598, 1476 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.31 (d, $J = 8.44$ Hz, 2H), 7.19−7.12 (m, 4H), 6.89 (d, $J = 8.91$ Hz, 2H), 5.30−5.24 (dd, $J = 12.87$ and 7.38 Hz, 1H), 3.87−3.84 (br s, 4H), 3.76−3.73 (dd, $J = 18.45$ and 12.45 Hz, 1H), 3.09−3.06 (dd, $J = 18.48$ and 7.41 Hz, 1H), 2.85−2.82 (br s, 4H); ESI-MS 419 $[M + H]^+$.

5-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (11). Compound **11** was prepared from **51** and *N*-aminomorpholine in 10% yield by the same procedure as described for **9**: 98.89% purity by HPLC, $[\alpha_D] = +0.28°$, $c = 0.2$, DMSO; mp 201−202 °C; IR (KBr) 3424, 3228, 1687, 1596, 1468 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 1H), 7.39 (d, $J = 7.43$ Hz, 2H), 7.23 (d, $J = 7.90$ Hz, 2H), 7.17 (s, 2H), 7.04 (d, $J = 6.61$ Hz, 2H), 6.82−6.79 (m, 1H), 5.57−5.49 (dd, $J = 11.69$ and 5.57 Hz, 1H), 3.65−3.62 (br s, 4H), 3.51−3.49 (dd, $J = 18.05$ and 12.02 Hz, 1H), 3.16−3.13 (dd, $J = 18.07$ and 5.77 Hz, 1H), 2.89−2.86 (br s, 4H); ESI-MS 385 $[M + H]^+$.

1-(4-Chlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (12). Compound **12** was prepared from **52** and *N*-aminomorpholine in 29% yield by the same procedure as described for **9**: 99.35% purity by HPLC, $[\alpha_D] = -0.63°$, $c = 0.2$, DMSO; mp 197−198 °C; IR (KBr) 3433, 3222, 1739, 1651, 1596, 1494 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (s, 1H), 7.33−7.30 (m, 3H), 7.18 (d, $J = 6.49$ Hz, 2H), 7.13 (d, $J = 8.95$ Hz, 2H), 6.92 (d, $J = 8.94$ Hz, 2H), 5.34−5.27 (dd, $J = 12.94$ and 7.35 Hz, 1H), 3.88−3.85 (br s, 4H), 3.81−3.70 (dd, $J = 18.45$ and 12.49 Hz, 1H), 3.15−3.06 (dd, $J = 18.45$ and 7.38 Hz, 1H), 2.97−2.94 (br s, 4H); ESI-MS 385 $[M + H]^+$.

1,5-Diphenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (13). Compound **13** was prepared from **53** and *N*-aminomorpholine in 62% yield by the same procedure as described for **9**: 99.09% purity by HPLC, $[\alpha_D] = -0.18°$, $c = 0.2$, DMSO; mp 212−214 °C; IR (KBr) 3421, 3213, 1737, 1651, 1598, 1498 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.26−7.23 (m, 2H), 7.21−7.08 (br s, 4H), 7.06−7.01 (br s, 2H), 6.87 (br s, 1H), 5.38−5.31 (dd, $J = 11.95$ and 6.95 Hz, 1H), 3.87−3.84 (br s, 4H), 3.73−3.70 (dd, $J = 17.7$ and 12.28 Hz, 1H), 3.12−3.09 (dd, $J = 17.70$ and 6.96 Hz, 1H), 2.97−2.94 (br s, 4H); ESI-MS 247.3 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (14). Compound **14** was prepared from **54** and *N*-aminomorpholine in 28% yield by the same procedure as described for **9**: 99.49% purity by HPLC, $[\alpha_D] = -0.48°$, $c = 0.2$, DMSO; mp 184 °C; IR (KBr) 3411, 3228, 1685, 1647, 1569, 1481 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.54 (s, 1H), 7.51 (d, $J = 8.77$ Hz, 1H), 7.43 (d, $J = 2.33$ Hz, 1H), 7.29−7.25 (dd, $J = 8.83$ and 2.21 Hz, 1H), 7.23−7.19 (m, 3H), 7.14−7.11 (m, 2H), 5.84−5.77 (dd, $J = 12.23$ and 6.15 Hz, 1H), 3.72−3.62 (br s, 4H), 3.40−3.37 (dd, $J = 18.75$ and 12.78 Hz, 1H), 3.06−3.01 (dd, $J = 18.75$ and 5.76 Hz, 1H), 2.90−2.87 (br s, 4H); ESI-MS 421 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(4-pentylphenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (15). Compound **15** was prepared from **95** and *N*-aminomorpholine in 33% yield by the same procedure as described for **9**: 99.18% purity by HPLC, $[\alpha_D] = -0.33°$, $c = 0.2$, DMSO; mp 210 °C; IR (KBr) 3309, 3136, 1691, 1577, 1479 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.47 (s, 1H), 7.47 (d, $J = 8.76$ Hz, 1H), 7.41 (d, $J = 2.28$ Hz, 1H), 7.26−7.25 (dd, $J = 8.73$ and 2.21 Hz, 1H), 7.07−7.01 (br s, 4H), 5.78−5.72 (dd, $J = 11.67$ and 5.55 Hz, 1H), 3.77−3.64 (br s, 5H), 3.09−3.01 (dd, $J = 18.01$ and 12.51 Hz, 1H), 2.87−2.84 (br s, 4H), 2.43−2.40 (br s, 2H), 1.49−1.42 (m, 2H), 1.22−1.13 (br s, 4H), 0.80 (t, 3H); ESI-MS 491 $[M + H]^+$.

5-(4-Butoxyphenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (16). Compound **16** was prepared from **96** and *N*-aminomorpholine in 30% yield by the same procedure as described for **9**: 99.75% purity by HPLC, $[\alpha_D] = -0.18°$, $c = 0.2$, DMSO; mp 195 °C; IR (KBr) 3423, 3254, 1685, 1510, 1479 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.52 (s, 1H), 7.47 (d, $J = 8.74$ Hz, 1H), 7.42 (d, $J = 2.28$ Hz, 1H), 7.28−7.24 (dd, $J = 8.72$ and 2.29 Hz, 1H), 7.01 (d, $J = 8.55$ Hz, 2H), 6.73 (d, $J = 8.59$ Hz, 2H), 5.76−5.71 (dd, $J = 11.70$ and 5.33 Hz, 1H), 3.83 (t, 2H), 3.60−3.57 (br s, 4H), 3.40−3.37 (br s, 1H), 3.08−3.01 (dd, $J = 17.43$ and 12.13 Hz, 1H), 2.88−2.85 (br s, 4H), 1.62−1.57 (m, 2H), 1.39−1.21 (m, 2H), 0.87 (t, 3H); ESI-MS 492.1 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (17). Compound **17** was prepared from **97** and *N*-aminomorpholine in 7% yield by the same procedure as described for **9**: 98.46% purity by HPLC, $[\alpha_D] = +0.58°$, $c = 0.2$, DMSO; mp 176−178 °C; IR (KBr) 3423, 1685, 1610, 1577 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (s, 1H), 7.11−7.02 (m, 5H), 6.70 (d, $J = 8.28$ Hz, 2H), 5.73−5.67 (dd, $J = 11.76$ and 6.03 Hz, 1H), 3.86−3.79 (br s, 4H), 3.72 (s, 3H), 3.66−3.60 (dd, $J = 18.16$ and 11.95 Hz, 1H), 3.39−3.31 (dd, $J = 18.15$ and 6.0 Hz, 1H), 2.96−2.85 (br s, 4H); ESI-MS 450.2 $[M + H]^+$.

5-Benzo[1,3]dioxol-5-yl-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (18). Compound **18** was prepared from **98** and *N*-aminomorpholine in 45% yield by the same procedure as described for **9**: 99.48% purity by HPLC, $[\alpha_D] = +0.62°$, $c = 0.2$, DMSO; mp 174−176 °C; IR (KBr)

3429, 3236, 1683, 1548, 1485 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.52 (d, J = 8.73 Hz, 1H), 7.45 (d, J = 2.26 Hz, 1H), 7.31−7.27 (dd, J = 8.72 and 2.33 Hz, 1H), 6.73 (d, J = 7.91 Hz, 1H), 6.65 (s, 1H), 6.60 (d, J = 7.89 Hz, 1H), 5.93 (d, J = 4.22 Hz, 2H), 5.74−5.68 (dd, J = 11.65 and 5.56 Hz, 1H), 3.64−3.61 (br s, 4H), 3.49−3.43 (br s, 1H), 3.07−2.99 (dd, J = 18.02 and 11.94 Hz, 1H), 2.86−2.83 (br s, 4H); ESI-MS 464.1 $[M + H]^+$.

5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (19). Compound **19** was prepared from **99** and *N*-aminomorpholine in 39% yield by the same procedure as described for **9**: 98.88% purity by HPLC, $[\alpha_D] = +0.66°$, c = 0.2, DMSO; mp 156−157 °C; IR (KBr) 3417, 3234, 1645, 1573, 1475 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.41 (s, 1H), 7.50 (d, J = 8.73 Hz, 1H), 7.42 (d, J = 8.17 Hz, 3H), 7.29 (d, J = 8.69 Hz, 1H), 7.08 (d, J = 8.16 Hz, 2H), 5.81−5.76 (dd, J = 11.64 and 5.82 Hz, 1H), 3.66−3.60 (br s, 5H), 3.10−3.02 (dd, J = 18.14 and 11.86 Hz, 1H), 2.85−2.82 (br s, 4H); ESI-MS 498.8 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-pyridin-3-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (20). Compound **20** was prepared from **100** and *N*-aminomorpholine in 27% yield by the same procedure as described for **9**: 98.99% purity by HPLC, $[\alpha_D] = -0.38°$, c = 0.2, DMSO; mp 107 °C (fuses); IR (KBr) 3421, 3254, 1654, 1560, 1490 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.68−6.65 (br s, 2H), 7.98−7.81 (m, 2H), 7.69−7.50 (m, 2H), 7.33 (s, 1H), 6.10−5.98 (dd, J = 12.17 and 6.90 Hz, 1H), 3.66−3.63 (br s, 4H), 3.56−3.51 (dd, J = 18.76 and 12.46 Hz, 1H), 3.37−3.29 (dd, J = 18.76 and 6.90 Hz, 1H), 2.89−2.86 (br s, 4H); ESI-MS 421.5 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-thiophen-2-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (21). Compound **21** was prepared from **101** and *N*-aminomorpholine in 83% yield by the same procedure as described for **9**: 98.87% purity by HPLC, $[\alpha_D] = +0.18°$, c = 0.2, DMSO; mp 168−170 °C; IR (KBr) 3435, 3209 1670, 1651, 1579, 1473 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.31 (d, J = 1.62 Hz, 1H), 7.11−7.04 (m, 3H), 6.79−6.75 (m, 2H), 6.05−6.04 (dd, J = 11.19 and 4.92 Hz, 1H), 3.88−3.85 (br s, 4H), 3.69−3.60 (dd, J = 18.01 and 11.74 Hz, 1H), 3.55−3.47 (dd, J = 18.15 and 4.86 Hz, 1H), 2.99−2.94 (br s, 4H); ESI-MS 426.3 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-furan-2-yl-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (22). Compound **22** was prepared from **102** and *N*-aminomorpholine in 38% yield by the same procedure as described for **9**: 99.18% purity by HPLC, $[\alpha_D] = +0.38°$, c = 0.2, DMSO; mp 203−205 °C; IR (KBr) 3417, 3238, 1687, 1533, 1477 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H), 7.51 (d, J = 2.05 Hz 1H), 7.42 (s, 1H), 7.39 (d, J = 8.69 Hz 1H), 7.28−7.25 (dd, J = 8.67 and 2.11 Hz, 1H), 6.20 (s, 2H), 5.83−5.78 (dd, J = 11.07 and 4.53 Hz, 1H), 3.67−3.64 (br s, 4H), 3.56−3.46 (dd, J = 17.75 and 11.83 Hz, 1H), 3.29−3.21 (dd, J = 17.86 and 4.62 Hz, 1H), 2.90−2.87 (br s, 4H); ESI-MS 410.2 $[M + H]^+$.

1-(2,4-Dichlorophenyl)-5-(5-methylthiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (23). Compound **23** was prepared from **103** and *N*-aminomorpholine in 53% yield by the same procedure as described for **9**: 99.16% purity by HPLC, $[\alpha_D] = +0.34°$, c = 0.2, DMSO; mp 135−138 °C; IR (KBr) 3423, 3230, 1664, 1579, 1475 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.31 (d, J = 2.13 Hz, 1H), 7.15−7.06 (m, 2H), 6.57 (d, J = 3.40 Hz, 1H), 6.39−6.38 (dd, J = 3.37 and 1.01 Hz, 1H), 6.00−5.94 (dd, J = 11.12 and 4.87 Hz, 1H), 3.87−3.84 (br s, 4H), 3.64−3.54 (dd, J = 18.08 and 11.56 Hz, 1H), 3.50−3.42 (dd, J = 18.09 and 4.92 Hz, 1H), 2.95−2.92 (br s, 4H), 2.32 (s, 3H); ESI-MS 440.4 $[M + H]^+$.

5-(5-Chlorothiophen-2-yl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (24). Compound **24** was prepared from **104** and *N*-aminomorpholine in 39% yield by the same procedure as described for **9**: 98.36% purity by HPLC, $[\alpha_D] = +0.47°$, c = 0.2, DMSO; mp 76−79 °C (fuses); IR (KBr) 3409, 3244, 1664, 1581, 1477 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.33 (d, J = 1.86 Hz, 1H), 7.19−7.09 (m, 2H), 6.58 (s, 2H), 5.97−5.91 (dd, J = 11.31 and 4.80 Hz, 1H), 3.87−3.84 (br s, 4H), 3.67−3.57 (dd, J = 18.21 and 11.90 Hz, 1H), 3.49−3.41 (dd, J = 18.24 and 4.83 Hz, 1H), 2.95−2.92 (br s, 4H); ESI-MS 461 $[M + H]^+$.

(+)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (9a). Compound **9a** was prepared from **49a** and *N*-aminomorpholine in 46% yield by the same procedure as described for **9**: 99.15% purity by HPLC, 99.8% chiral purity by HPLC, $[\alpha_D] = +265°$, c = 0.2, DMSO; mp 164−165 °C (fuses); IR (KBr) 3410, 3236, 1632, 1568, 1549 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.49 (d, J = 8.73 Hz, 1H), 7.44 (d, J = 2.17 Hz, 1H), 7.29 (d, J = 8.36 Hz, 3H), 7.15 (d, J = 8.35 Hz, 2H), 5.82−5.76 (dd, J = 11.81 and 5.90 Hz, 1H), 3.65−3.62 (br s, 4H), 3.39−3.35 (dd, J = 18.08 and 12.14 Hz, 1H), 3.10−3.02 (dd, J = 18.03 and 5.91 Hz, 1H), 2.85−2.82 (br s, 4H); ESI-MS 455.2 $[M + H]^+$.

(−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (9b). Compound **9b** was prepared from **49b** and *N*-aminomorpholine in 56% yield by the same procedure as described for **9**: 99.85% purity by HPLC, 100% chiral purity by HPLC $[\alpha_D] = -265°$, c = 0.2, DMSO; mp 181−183 °C (fuses); IR (KBr) 3388, 3238, 1635, 1571, 1550 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.49 (d, J = 8.73 Hz, 1H), 7.44 (d, J = 2.17 Hz, 1H), 7.29 (d, J = 8.36 Hz, 3H), 7.15 (d, J = 8.35 Hz, 2H), 5.82−5.73 (dd, J = 11.81 and 5.90 Hz, 1H), 3.63−3.60 (br s, 4H), 3.44−3.38 (dd, J = 18.09 and 12.21 Hz, 1H), 3.10−3.02 (dd, J = 18.03 and 5.91 Hz, 1H), 2.85−2.82 (br s, 4H); ESI-MS 455.2 $[M + H]^+$.

Procedure for the Preparation of Hydrochloride Salt. Hydrochloride Salt of 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (25). The compound **9** (11.7 g, 25.80 mmol) was taken in methanol (45 mL), and ethereal HCl (12 mL) was added at 0−5 °C dropwise. The reaction mixture was stirred at 10−15 °C for 10−15 min. Solvent was evaporated on a rotatory evaporator under reduced pressure. The residue obtained was triturated in EtOAc (55 mL) at 60−70 °C for 10−15 min to afford an off-white solid which was filtered on a Buchner funnel under suction and dried to afford **25** as an off-white solid (10.60 g, 84%): 99.89% purity by HPLC, $[\alpha_D] = -1.9°$, c = 0.2, DMSO; mp 184−185 °C (with decomposition); IR (KBr) 3423, 2987, 1687, 1571, 1481 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.51 (s, 1H), 7.50 (d, J = 8.75 Hz, 1H), 7.45 (d, J = 2.32 Hz, 1H), 7.29 (d, J = 6.96 Hz, 3H), 7.15 (d, J = 8.47 Hz, 2H), 5.83−5.77 (dd, J = 11.88 and 5.80 Hz, 1H), 3.70−3.60 (br s, 5H), 3.10−3.02 (dd, J = 18.12 and 5.95 Hz, 1H), 2.87−2.84 (br s, 4H); ESI-MS 455 $[M + H - HCl]^+$.

Hydrochloride Salt of (+)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (27). Compound **27** was prepared from **9a** in 83% yield by the same procedure as described for **25**: 99.81% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = +233°$, c = 0.2, DMSO; mp 207−208 °C (with decomposition); IR (KBr) 3413, 2858, 1683, 1571, 1483 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.50 (s, 1H), 7.49 (d, J = 8.74 Hz, 1H), 7.45 (d, J = 2.24 Hz, 1H), 7.28 (d, J = 8.22 Hz, 3H), 7.14 (d, J = 8.40 Hz, 2H), 5.83−5.77 (dd, J = 11.81 and 5.79 Hz, 1H), 3.74−3.61 (br s, 5H), 3.10−3.02 (dd, J = 18.05 and 6.05 Hz, 1H), 2.87−2.84 (br s, 4H); ESI-MS 454.8 $[M + H - HCl]^+$.

Hydrochloride Salt of (−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (29). Compound **29** was prepared from **9b** in 90% yield by the same procedure as described for **25**: 99.92% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = -233°$, c = 0.2, DMSO; mp 211−212 °C (with decomposition); IR (KBr) 3419, 3229, 1683, 1571, 1550 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.45 (s, 1H), 7.50 (d, J = 8.73 Hz, 1H), 7.45 (d, J = 2.24 Hz, 1H), 7.28 (d, J = 8.47 Hz, 3H), 7.14 (d, J = 8.42 Hz, 2H), 5.83−5.77 (dd, J = 11.91 and 5.82 Hz, 1H), 3.64−3.55 (br s, 5H), 3.10−3.02 (dd, J = 17.91 and 5.94 Hz, 1H), 2.85−2.82 (br s, 4H); ESI-MS 454.9 $[M + H - HCl]^+$.

Procedure for Preparation of Salts. Preparation of Bisulfate Salt of 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (26). To a solution of **9** (5.4 g, 11.2 mmol) in methanol (108 mL) was added concentrated H_2SO_4 (0.54 mL) at 10−15 °C over period of 5−7 min. The reaction mixture was refluxed at 60−65 °C for 30−40 min. The solvents were evaporated on a rotatory evaporator under reduced pressure to get a solid. The solid was stirred in diethyl ether (32 mL) at 26−28 °C for 20−25 min and filtered on a Buchner funnel under suction to afford **26** as a light yellow solid (6.51 g, 99%), 99.98% purity by HPLC, $[\alpha_D] = +0.3°$, $c = 0.2$, DMSO; mp 135−137 °C (fuses); IR (KBr) 3438, 2927, 1679, 1571, 1477 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.58 (s, 1H), 7.49 (d, $J = 8.73$ Hz, 1H), 7.44 (d, $J = 2.17$ Hz, 1H), 7.29 (d, $J = 8.36$ Hz, 3H), 7.15 (d, $J = 8.35$ Hz, 2H), 5.83−5.77 (dd, $J = 11.81$ and 5.90 Hz, 1H), 3.71−3.61 (br s, 5H), 3.11−3.03 (dd, $J = 18.03$ and 5.92 Hz, 1H), 2.89−2.86 (br s, 4H); ESI-MS 454.9 $[M+H-H_2SO_4]^+$.

Bisulfate Salt of (+)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (28). Compound **28** was prepared from **9a** in 99% yield by the same procedure as described for **26**: 99.87% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = +157°$, $c = 0.2$, DMSO; mp 162−164 °C (with decomposition); IR (KBr) 3390, 3259 1670, 1577, 1490 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.54 (br s, 1H), 7.32−7.11 (m, 4H), 7.09−7.04 (m, 3H), 5.76−5.70 (dd, $J = 12.21$ and 6.36 Hz, 1H), 3.88−3.85 (br s, 4H), 3.74−3.63 (dd, $J = 18.30$ and 12.68 Hz, 1H), 3.37−3.28 (dd, $J = 18.31$ and 6.37 Hz, 1H), 2.99−2.96 (br s, 4H); ESI-MS 454.9 $[M + H - H_2SO_4]^+$.

Bisulfate Salt of (−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (30). Compound **30** was prepared from **9b** in 96% yield by the same procedure as described for **26**: 99.77% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = -157°$, $c = 0.2$, DMSO; mp 149−151 °C (with decomposition); IR (KBr) 3427, 3219 1685, 1575, 1551 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (br s, 1H), 7.36 (d, $J = 8.67$ Hz, 1H), 7.23−7.17 (dd, $J = 7.92$ and 2.31 Hz, 2H), 7.12 (d, $J = 2.31$ Hz, 1H), 7.09 (d, $J = 8.40$ Hz, 3H), 5.85 (dd, $J = 12.54$ and 5.91 Hz, 1H), 4.02−3.99 (br s, 4H), 3.75−3.65 (dd, $J = 18.09$ and 12.79 Hz, 1H), 3.57−3.54 (br s, 4H), 3.32−3.24 (dd, $J = 18.15$ and 6.01 Hz, 1H); ESI-MS 454.9 $[M + H - H_2SO_4]^+$.

Oxalate Salt of (−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (31). Compound **31** was prepared from **9b** and oxalic acid in 60% yield by the same procedure as described for **26**: 99.74% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = -213.3°$, $c = 0.2$, DMSO; mp 170 °C (with decomposition); IR (KBr) 3390, 3249, 1670, 1577, 1554 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.17 (d, $J = 8.45$ Hz, 3H), 7.14 (s, 1H), 7.09−7.04 (m, 3H), 5.76−5.70 (dd, $J = 11.38$ and 6.34 Hz, 1H), 3.88−3.85 (br s, 4H), 3.74−3.64 (dd, $J = 18.33$ and 11.86 Hz, 1H), 3.37−3.29 (dd, $J = 18.34$ and 6.34 Hz, 1H), 2.95−2.92 (br s, 4H); ESI-MS 454.4 $[M + H - C_2H_2O_4]^+$.

Methane Sulfonate Salt of (−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (32). Compound **32** was prepared from **9b** and methanesulfonic acid in 95% yield by the same procedure as described for **26**: 99.09% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = -207.2°$, $c = 0.2$, DMSO; mp 145−147 °C (with decomposition); IR (KBr) 3425, 3219, 1685, 1575, 1551 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.68 (br s, 1H), 7.40 (d, $J = 8.71$ Hz, 1H), 7.19 (d, $J = 7.99$ Hz, 3H), 7.12 (d, $J = 8.71$ Hz, 1H), 7.03 (d, $J = 8.40$ Hz, 2H), 5.95 (dd, $J = 12.66$ and 5.46 Hz, 1H), 4.04−3.99 (br s, 4H), 3.89 (br s, 3H), 3.74−3.64 (dd, $J = 18.01$ and 12.78 Hz, 1H), 3.27−3.20 (dd, $J = 18.09$ and 5.46 Hz, 1H), 2.96−2.93 (br s, 4H); ESI-MS 454.1 $[M + H - CH_3SO_3H]^+$.

Benzene Sulfonate Salt of (−)-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4,5-dihydro-1*H*-pyrazole-3-carboxylic Acid Morpholin-4-ylamide (33). Compound **33** was prepared from **9b** and benzene sulfonic acid in 63% yield by the same procedure as described for **26**: 99.80% purity by HPLC, 100% chiral purity by HPLC, $[\alpha_D] = -201.6°$, $c = 0.2$, DMSO; mp 190−192 °C (with decomposition); IR (KBr) 3398, 1685, 1654, 1637, 1544 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.68 (br s, 1H), 7.90 (d, $J = 6.60$ Hz, 2H), 7.45−7.39 (m, 4H), 7.21−7.17 (m, 3H), 7.06−7.02 (m, 3H), 5.99−5.92 (dd, $J = 12.66$ and 5.46 Hz, 1H), 4.05−3.93 (br s, 4H), 3.74−3.63 (dd, $J = 18.03$ and 12.31 Hz, 1H), 3.27−3.19 (dd, $J = 18.03$ and 5.52 Hz, 1H), 2.48−2.45 (br s, 4H); ESI-MS 612.7 $[M + C_6H_5SO_3H]^+$.

Molecular Modeling. All computational experiments were conducted on a HP Windows workstation. Human CB1 sequence was retrieved from the SWISS-PROT protein sequence database,[44] and structure construction, optimization, and visualization were carried out using the molecular modeling packages including the Modeler module of Discovery Studio 1.1.[36] The conformations of the input structures **1** (the conformational properties of **1** are readily modeled with constraints), **2**, and of both enantiomers *R* and *S* (**9a**, **9b**) for the compound **9** were generated using corina 3D and cosmic module of TSAR[36] followed by minimization using the PM3 method. The PM3 method has been reported to give the best approximation of the angle in comparison with other methods for CB1 receptor antagonists.[12]

The coordinates of the inactive R-state of the human CB1 cannabinoid receptor were modeled using the 2.8 Å crystal structure of bovine rhodopsin.[45] The residues were aligned and mutated, and the helices were constructed as mentioned in the experiments earlier.[46−48] The loops were omitted. This model was minimized with CFF force field, holding the TMH backbones fixed. Subsequently, the kink in TMH6 at Pro358[48] was introduced to enable the salt bridge between Lys192 and Asp366, and the model was further minimized. The stereochemical quality of the resulting protein structure was tested with the PROCHECK program.[49]

The ligand **1** was automatically docked into the receptor, using Ligandfit module of DS SBD 1.2.[36] It performs docking of flexible ligands into proteins with partial flexibility in the neighborhood of the active site. To take protein flexibility into account, the complex were then refined using molecular dynamics (CFF force field, dielectric constant = 4, 300 °K). The energy minimization process consists of two sequential steps: the Steepest Descent algorithm, reaching a final convergence of 10.0 kcal mol^{-1} $Å^{-1}$, followed by the Conjugate Gradient algorithm to reach a final convergence of 0.01 kcal mol^{-1} $Å^{-1}$. Constraints including the distance and torsion angles of aromatic residues and as well as range-constraint of 2.5−3.0 Å on the N-atom of Lys192 were applied during the run so that hydrogen-bond formation is favored as reported in earlier models. The other ligands **2** and both enantiomers *R* and *S* (**9a**, **9b**) for the compound **9** were then automatically docked in the binding site defined by the docked posed of ligand **1.** The poses were ranked with PLP-1, PLP-1, and Dockscore, and the conformations with the best scores were checked visually.

In Vivo Pharmacological Studies. Animals. All the animals used in the study were procured from the Animal Breeding Facility of Zydus Research Center. Institutional Animal Ethical Committee approved all the study protocols. Female Zucker fa/fa rats (age of 10−12 weeks and 300−350 g of weight) were used for the 5% sucrose solution intake as well as long term study. The pharmacokinetic parameters were also assessed in the obese overnight-fasted female Zucker fa/fa rats. For the mouse tetrad model, 10−12 weeks old female Swiss Albino Mice (25−30 g) were used.

Drug Preparation and Administration. For in vivo experiments, compounds were suspended with 0.01% Tween 80 plus 5% DMSO in saline (NaCl 0.9%) for intravenous administration (particularly for the CB1 agonist WIN-55212-2) or with 0.5% carboxymethylcellulose sodium salt in distilled water for oral route. The test compounds were administered at the dose of 10 mg/kg. All the compounds were administered by oral route in a volume of 2 mL/kg body weight for rats and 10 mL/kg body weight for mice. WIN-55212-2 was injected intravenous at a dose of 1 mg/kg in mice in a volume of 10 mL/kg body weight.

5% Sucrose Solution Intake in Zucker fa/fa Rats. The obese Zucker fa/fa rats were housed individually and subjected to training

for consuming 5% sucrose solution over 4 h, by allowing access to the 5% sucrose solution in the bottles. Food and water were withdrawn during this time. This training was given for six consecutive days, at the same time of the day. On seventh day, the animals were randomized into groups of six animals each and treated with the test compounds. After 1 h of treatment, the animals were exposed to the 5% sucrose solution for 4 h as that of the training schedule. The primary pharmacokinetic measurements indicated the $T_{1/2}$ of 2−3 h for all the tested compounds (Data not shown). Accordingly, this duration was selected for sucrose consumption studies, so that the $T_{1/2}$ of the compound falls exactly in between of the study duration. The amount of sucrose solution consumed by each animal was calculated. Difference between the control and treatment groups were analyzed by performing one-way ANOVA followed by Dunnett's test on sucrose solution consumption using Graph pad Prism software.

Long-Term Effect on Body Weight and Serum Biochemistry. Female obese Zucker fa/fa rats were individually housed and divided into groups of six animals each. All treatments were given orally, 1 h before the start of the dark phase. Body weight was recorded daily for 60 days. On day 61, blood samples were collected from retro-orbital sinus of the animals in the morning, and the serum was analyzed for triglyceride using Pointe Scientific kit using SpectraMax190 microplate reader. The difference between groups was analyzed by performing one-way ANOVA followed by Dunnett's test of control and treated groups using Graphpad Prism software.

Mouse Tetrad Model for Cannabinoid Receptor 1 Antagonist Activity. Female Swiss albino mice were used for this test. The test compounds were administered orally, and after 1 h of test compound administration, WIN-55212-2 (1 mg/kg) was given by intravenous route. Thirty minutes later, the four tests typically comprising of tetrad were carried out. Temperature was measured for evaluation of hypothermia using a lubricated thermometer inserted into the rectum twice: before and after the treatment. The hot plate test was carried out using a hot plate at 55 °C as nociceptive stimulus, immediately after testing the temperature. The latency was measured before and after the treatment, and analgesia was quantified as the percentage of maximum possible effect (% MPE) with a cutoff time of 30 s. The formula for % MPE is as follows: (latency after treatment − control latency)/(cutoff time − control latency) × 100. Catalepsy was measured using the "ring test" 30 min after drug injection for a duration of 5 min.[35] The data is expressed as an immobility index defined as percentage of total time spent on a ring during which the animal remained motionless. The Locomotor activity was evaluated after 35 min of drug injection in a squared open field. The total number of squares crossed in 5 min was taken as the mobility index.

Pharmacokinetic of Antiobesity Compound. Pharmacokinetic parameters of **1** and **30** were evaluated by administration of 30 mg/kg dose orally to female obese Zucker fa/fa rats. The oral suspension of both the compounds was prepared in 0.5% carboxymethylcellulose sodium salt in distilled water. A group of six female obese Zucker fa/fa rats were kept for an overnight fasting and then administered with a dose of 30 mg/kg orally. Serial blood samples were collected from retro-orbital of animals at various time points, i.e., 10, 20,and 40 min and 1.0, 2.0, 4.0, 6.0, 8.0, 24.0, and 48.0 h. The blood samples were subjected to centrifugation for collection of the plasma.

For tissue distribution study, a group of six female rats were administered with a single dose of 30 mg/kg orally. After 3 h, the samples of blood, brain, and adipose tissues were collected from each rat of the group. The tissue samples were washed with saline and homogenized in Tris buffer (pH 7.4) using laboratory homogenizer. The plasma and tissue homogenates were extracted through liquid−liquid extraction method. The concentration of compound was determined using LC-MS. The samples were analyzed on ACE, Cyano, 100 mm × 4.6 mm × 5 μm HPLC column using the mobile phase of 0.4 mM ammonium acetate containing 0.06% trifluoroacetic acid and acetonitrile (30:70% v/v) with flow rate of 0.3 mL/min. The mass spectrometry detector parameter was put on voltage 1.8 kV, Block and CDL temperature was 200 °C, and Nebulizer gas was 1.5 L/min. The quantitation was performed using the known concentration versus response curve. The calibration standards were obtained by spiking a known concentration in blank matrix with internal standard; they were processed and analyzed as for the samples.

The pharmacokinetic parameters, T_{max}, C_{max}, half-life, and $AUC_{(0-\infty)}$ were calculated using WinNolin software version 5.0.1. The brain to plasma and adipose to plasma ratios were calculated using plasma (μg/mL) concentration over brain/adipose (μg/g) concentration.

Radioligand Binding Assay for CB1 and CB2. The radioligand binding assays were performed at MDS Pharma Services Taiwan Ltd. (Taiwan, ROC). The measurements were done in duplicate, and values indicate the mean of at least two independent experiments performed. The CB1 assay was performed using human recombinant HEK-293 cells. The membranes were incubated at 37 °C with 0.5 nM [^{3}H] CP-55940 in 1 mL of buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 3 mM MgO_2, 0.5% BSA). The K_i values were calculated from the equation of Cheng and Prusoff using a fixed K_d value (1.3 nM) for WIN-55212-2, obtained from independent experimental assays. For the CB2 binding assay, a similar procedure was followed using human recombinant CHO-K1 cells with 2.4 nM [^{3}H] WIN-55212-2 in 1 mL of buffer (20 mM HEPES, pH 7.0, 5 mg/mL BSA). K_i values were calculated from the equation of Cheng and Prusoff using a fixed K_d value (4.9 nM) for WIN-55212-2. IC_{50} values were determined by a nonlinear, least-square regression analysis using Data Analysis Toolbox (MDL Information Systems, San Leandro, CA).

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Supporting Information Available: Table of spectroscopic data (IR and ^{1}H NMR) of compounds **37**, **39**, **44**−**48**, **50**−**54**, and **65**−**104** and a table of microanalytical data of compounds **4**−**33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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