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Discovery of 2-(4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (GCC2680) as a potent, selective and orally efficacious cannabinoid-1 receptor antagonist

Jinhwa Lee *, Hee Jeong Seo, Suk Ho Lee, Jeongmin Kim, Myung Eun Jung, Sung-Han Lee, Kwang-Seop Song, Junwon Lee, Suk Youn Kang, Min Ju Kim, Mi-Soon Kim, Eun-Jung Son, MinWoo Lee, Ho-Kyun Han

Research Center, Green Cross Corporation, 303 Bojeong-dong, Giheung-gu, Yongin 446-770, Republic of Korea

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ABSTRACT

Structure–activity relationship studies in a series of diarylpyrazolyl thiadiazoles identified cannabinoid-1 receptor antagonists with excellent potency and selectivity. Based on its exceptional in vivo efficacy in animal models and its favorable pharmacokinetic and toxicological profiles, 2-(4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (GCC2680) was selected as a preclinical candidate for the treatment of obesity.

1. Introduction

The prevalence of obesity has rapidly increased over the last decade. Obesity is not simply a cosmetic concern, but a serious health problem. Thus, the World Health Organization (WHO) recently declared that obesity has become a global epidemic.[1,2] Obesity is characterized by an excess of body fat and includes a pro-inflammatory state, eventually resulting in type 2 diabetes, coronary heart disease, and hypertension. Furthermore, obesity elevates the relative risk of mortality due to cardiovascular disease,[3] and obesity now ranks as the second leading cause of preventable death after smoking in the United States.[4] Treatment of obesity involves a combination of diet, exercise and pharmacotherapy. However, there is growing evidence that short-term dietary changes or exercise alone cannot adequately address obesity as a chronic disease. Only two drugs are currently approved for chronic weight loss treatment: orlistat and sibutramine. However, both of these agents have different adverse event profiles that limit more widespread use.[5]

Numerous studies on the causes of obesity have been conducted to identify new potential targets that could be exploited to create novel types of anti-obesity drugs.[14] Eventually, the discovery was made that modulation of the endocannabinoid system by specifically blocking the cannabinoid receptor 1 (CB1) in both the brain and periphery can provide a novel target for the treatment of obesity.[6a] The endocannabinoid system includes endogenous ligands (such as anandamide and 2-AG)[6b] and two cannabinoid receptor subtypes, CB1 and CB2. CB1 and CB2 belong to the G-protein coupled receptor superfamily and were first cloned in 1990 and 1993, respectively.[7–9] The CB1 receptors are mainly expressed in several brain areas including the limbic system (amygdala and hippocampus), hypothalamus, cerebral cortex, cerebellum, and basal ganglia. It is known that CB1 receptors, especially in the limbic system-hypothalamus axis cannabinoids, have an important role in the control of appetite. In contrast, CB2 receptors are almost exclusively expressed in cells of the immune system.[10,11] Considering the important impact of obesity on public health, its increasing incidence worldwide, and the lack of highly efficient and well-tolerated drugs for treatment, it is not surprising that CB1 antagonism or inverse agonism is a subject of considerable interest.[12]

Although a few CB1 receptor inverse agonists/antagonists were recently withdrawn from clinical development, including 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, **1** (rimonabant, SR141716A),[22] *N*-((2*S*,3*S*)-4-(4-chlorophenyl)-3-(3-cyanophenyl)butan-2-yl)-2-methyl-2-(5-(trifluoromethyl)pyridin-2-yloxy)propanamide, **2** (taranabant, MK-0364),[23] and 1-(8-(2-chlorophenyl)-9-(4-chlorophenyl)-9*H*-purin-6-yl)-4-(ethylamino)piperidine-4-carboxamide, **3** (otenabant, CP-945,598),[24] many research groups and pharmaceu-

* Corresponding author. Tel.: +82 31 260 9892; fax: +82 31 260 9870.
E-mail addresses: jinhwa_2_lee@hotmail.com, jinhwalee@greencross.com (J. Lee).

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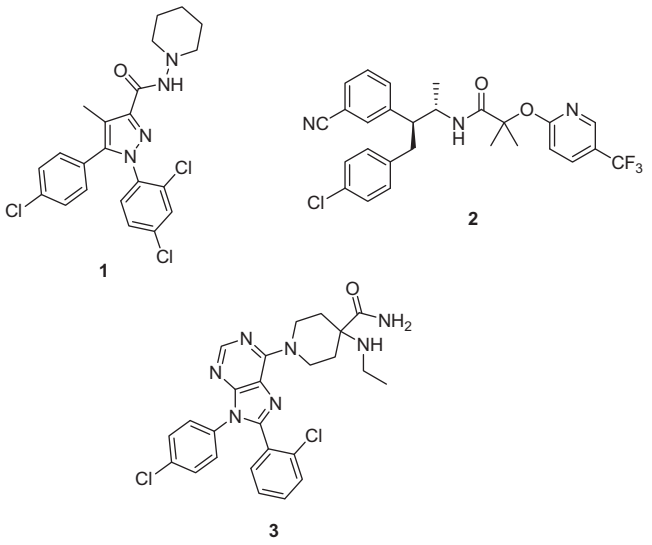


Figure 1. Structures of CB1 receptor antagonists/inverse agonists.

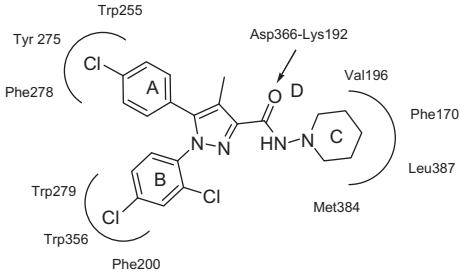


Figure 2. Compound **1** and its receptor–ligand interaction.

tical companies are still seeking novel CB1 antagonists that display improved physicochemical properties and decreased adverse effects involving depression, anxiety or suicidality (Fig. 1).[26]

The main objective of this study was to find novel CB1 receptor antagonists. A pharmacophore model for the binding of a low energy conformation of **1** in the CB1 receptor has been well-documented.[11,13] The key receptor–ligand interaction is known to be a hydrogen bond between the carbonyl group of **1** and the Lys192-Asp366 residues of the CB1 receptor, which stabilizes the Lys192-Asp366 salt bridge as shown in Figure 2.[11]

In our previous studies,[17,18] we investigated a series of diarylpyrazolyl oxadiazole derivatives as antagonists for the cannabinoid CB1 and CB2 receptors. A few of the compounds (e.g., **4**, Fig. 3) in this series exhibited better binding affinity than known CB1 antagonists, validating the hypothesis that a 1,3,4-oxadiazole[15,16] could act as a CB1 bioisostere of the amide moiety in **1**. Along these lines,

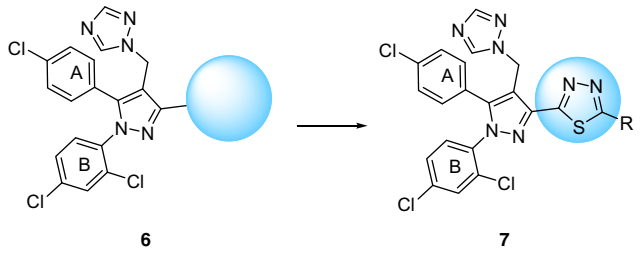


Figure 4. Exploration of 1,2,4-triazole-containing diarylpyrazolyl thiadiazole series **7**.

both imidazole[19] and tetrazole[20] replacements for the amide functionality have been reported. Subsequently, we introduced a triazole ring onto the C4 methyl group of the pyrazole scaffold via a methylene linker (**5**, Fig. 3) to afford a potent CB1 receptor antagonist with a significant anti-obesity effect in an animal model. A subsequent modeling study revealed that the N2 of the triazole substituent forms a bidentate H-bond with the side chain OH and backbone NH of Thr 197, which is an interaction exclusively observed in the binding model of **5**.[17,21]

As shown in Figure 4, we envisioned that incorporation of the 1,2,4-triazolylmethyl moiety onto a diarylpyrazolyl thiadiazole could lead to more potent CB1 receptor antagonists based on the encouraging observation made in our previous studies.[17,21] Modifications into thiadiazole could provide more efficacious series of compounds not to mention securing superior intellectual property (IP) position to other existing pyrazole series of CB1 receptor ligands.

Herein, we describe the design, synthesis and biological evaluation of diarylpyrazolyl thiadiazole analogues as novel CB1 receptor antagonists. Through a process of extensive lead optimization, we successfully identified 2-(4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole, **17s** (GCC2680), as a candidate for development as an anti-obesity agent.

2. Chemistry

As shown in Scheme 1, the synthesis of the diarylpyrazolyl thiadiazole analogs commenced with the generic acid **8**.[23,25] Compounds of the general structure **11** were prepared by (i) reaction of carboxylic acid **8** with a hydrazide compound **9** in the presence of coupling reagents (EDCI, DMAP) and (ii) thionation–cyclization of the resulting product **10** using Lawesson's reagent[28] to obtain a 1,3,4-thiadiazole **11**. Alternatively, the acylhydrazide intermediate **10** was also available through the coupling of the hydrazide **13** with a corresponding acid **14** mediated by coupling reagents such as DMAP, EDCI or EDCI, HOBt, NMM. For this sequence, the requisite hydrazide **13** was prepared by treating the known ester **12** with hydrazine in refluxing EtOH, as shown in Scheme 1.

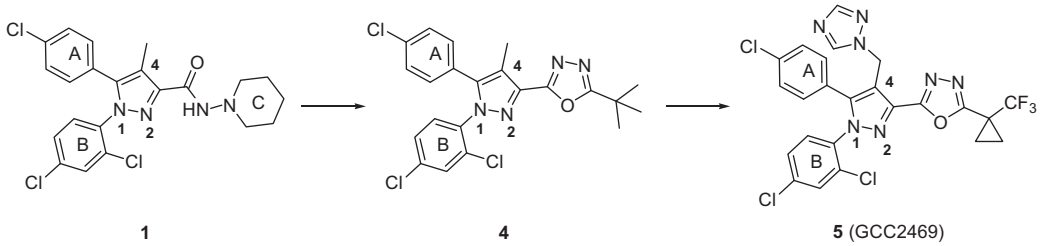
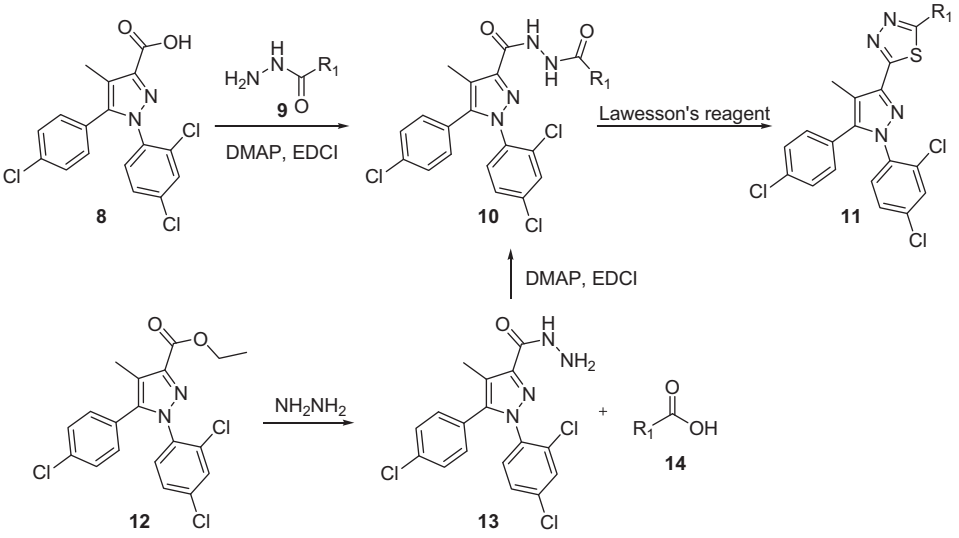
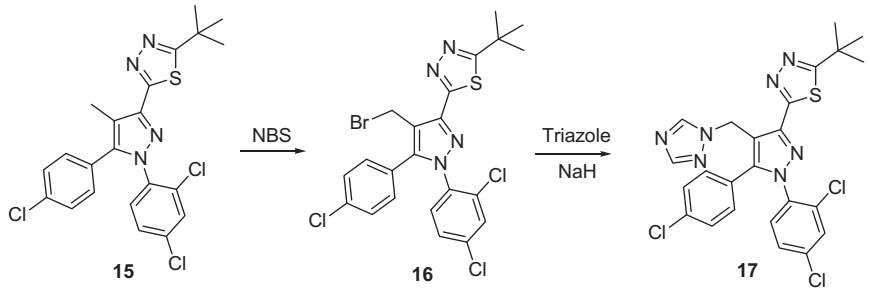


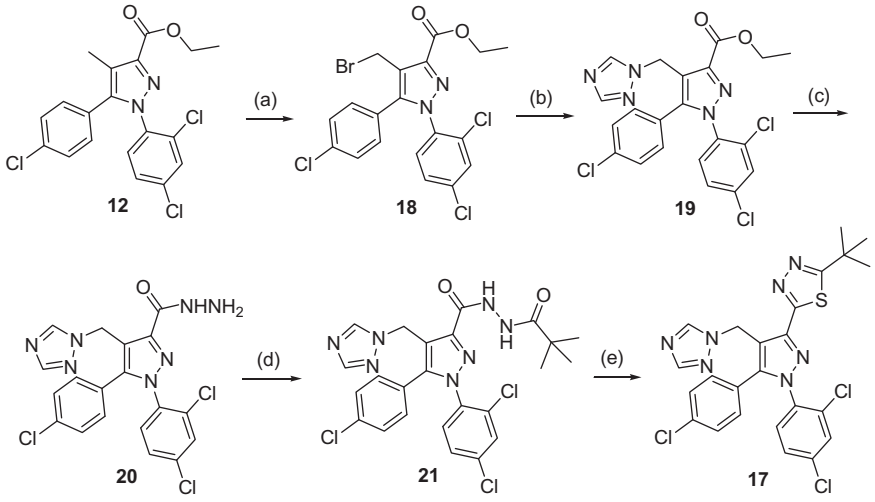
Figure 3. The conceptual development of antagonist **5** from Rimonabant (**1**) and its bioisostere **4**.



Scheme 1. Preparation of thiadiazole **11**.



Scheme 2. Preparation of triazole **17**.

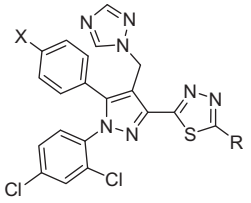


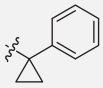
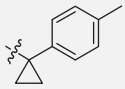
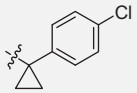
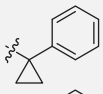
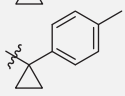
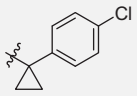
Scheme 3. Preparation of thiadiazole **17**. Reagents and conditions: (a) NBS, AIBN, CCl_4; (b) 1,2,4-triazole·Na, DMF; (c) hydrazine·H_2O, EtOH, 65 °C; (d) pivalic acid, EDCI, HOB*t*, NMM, DMF; (e) Lawesson's reagent, THF, reflux or microwave.

The preparation of triazole **17** was initiated with the activated 4-pyrazole intermediate **16**, which was prepared from **15** via a benzylic bromination-type reaction[27] as illustrated in Scheme 2. The triazole group of **17** was then introduced by treating bromide **16** with triazole in the presence of a suitable base such as sodium hydride or cesium carbonate.

The compounds containing the 1,2,4-triazole could also be obtained by a reaction sequence involving the key intermediate bromide **18**. Thus, bromide **18**, obtained by reaction of pyrazole **12** with NBS in the presence of catalytic AIBN, was reacted with 1,2,4-triazole sodium derivatives to provide **19**. Hydrazinolysis of ester **19** produced the corresponding hydrazide **20**, which could

Table 1
Structures and binding affinities of selected ligands to rat CB1 and human CB2 receptors and hCB2/rCB1 selectivity of the ligands[a,b]



Entry	X	R	Compound	Receptor affinity (IC_{50}, nM)		hCB2/rCB1 selectivity
				rCB1	hCB2	
1	Cl		**1**	4.53	1760	389
2	Cl	*t*-Bu	**17**	1.73	1140	659
3	Cl		**17a**	2.40	–	–
4	Cl		**17b**	2.29	3631	1586
5	Cl		**17c**	2.89	505	175
6	Cl		**17d**	1.79	–	–
7	Cl		**17e**	5.74	–	–
8	Cl		**17f**	2.49	–	–
9	Br	*t*-Bu	**17g**	1.54	430	279
10	Br		**17h**	2.91	–	–
11	Br		**17i**	0.991	–	–
12	Br		**17j**	3.27	–	–
13	Br		**17k**	2.23	–	–
14	Br		**17l**	2.74	–	–
15	Br		**17m**	0.877	–	–

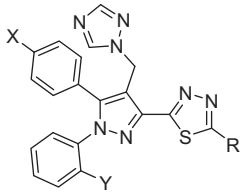
[a] These data were obtained by single determinations.
[b] Compound **1** was estimated to have CB1 binding affinity via in-house assay.

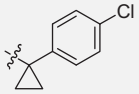
then be coupled with an acid to give acylhydrazide **21**. Alternatively, hydrolysis of ester **19** and activation of the acid followed by coupling with a hydrazide in the presence of triethylamine then afforded acylhydrazide **21**. Thionation–cyclization was then performed using Lawesson's reagent under microwave irradiation to give thiadiazole **17**, as illustrated in Scheme 3.

3. Results and discussion

The target analogs were evaluated in vitro in a rat CB1 binding assay,[30] and the results are shown in Table 1. We focused on branched aliphatic groups or surrogates, including *t*-butyl, trifluoromethylcyclopropyl, trifluoromethylcyclobutyl and phenylcyclopropyl, for the groups connected to the thiadiazole because our previous findings indicated that these lipophilic or fluorine-containing substituents favorably affected the biological activity of the examined diarylpyrazolyl oxadiazoles.[17] A methylcyclopropyl substituent (**17a**) was chosen for the direct comparison with *t*-butyl thiadiazole **17** (IC_{50} = 1.73 nM). Compound **17a** showed a slight decrease in rCB1 receptor binding affinity (IC_{50} = 2.40 nM). Trifluoromethylcyclopropyl derivative **17b**, which should provide lower lipophilicity and possibly enhanced water solubility, exhibited an increase relative to **17a** in binding affinity against the rCB1 receptor, albeit to a low degree. Importantly, compound **17b** also showed the highest hCB2/rCB1 receptor selectivity (~1586), as noted in Table 1. When the chlorine at the 4-position of one of the aryl substituents (entries 2–8) was replaced with bromine (entries 9–15), most of the resulting compounds displayed improved binding affinity against the rCB1 receptor, implying that increased lipophilicity can improve in vitro activity. Notably, a couple of compounds were shown to reach subnanomolar rCB1 receptor binding affinity (IC_{50} = 0.991 nM for **17i**; IC_{50} = 0.877 nM for **17m**).

Table 2
Structures and binding affinities of selected ligands to rat CB1 and human CB2 receptors and hCB2/rCB1 selectivity of the ligands[a,b]



Entry	X	Y	R	Compound	Receptor affinity (IC_{50}, nM)		hCB2/rCB1 selectivity
					rCB1	hCB2	
1				**1**	4.53	1760	389
2	Cl	Cl	*t*-Bu	**17n**	1.93	–	–
3	Cl	Cl		**17o**	1.66	–	–
4	Cl	Cl		**17p**	1.01	–	–
5	Cl	CF_3		**17q**	6.48	76.8	11.85
6	Cl	Cl		**17r**	0.966	265	274
7	Br	Cl	*t*-Bu	**17s**	0.681	549	807
8	Br	Cl		**17t**	0.992	124	125
9	Br	Cl		**17u**	0.848	73.6	87
10	Br	CF_3		**17v**	4.38	27.6	6.30

[a] These data were obtained by single determinations.
[b] Compound **1** was estimated to have CB1 binding affinity via in-house assay.

Next, in order to test the effect of modifying the substituents on the diphenylpyrazole, one of the chlorine atoms on the N1 phenyl group was removed. As shown in Table 2, removal of the chlorine at the 4-position of the N1 phenyl ring actually improved the rCB1 receptor binding affinity, as exemplified by compounds **17p** (IC_{50} = 1.01 nM) and **17r** (IC_{50} = 0.966 nM). This effect is further boosted by replacement of chlorine with bromine at the position marked X, as exemplified by compounds **17s–17u** in Table 2. It is worth noting that pyrazolyl-*t*-butyl thiadiazole **17s** (IC_{50} = 0.681 nM) was shown to possess the highest binding affinity against the rat CB1 receptor of the pyrazolyl thiadiazole compounds prepared to date. Compound **17s** also showed high hCB2/rCB1 receptor selectivity (~807) due to lower hCB2 receptor binding affinity. Interestingly, introduction of a trifluoromethyl group at the position marked Y decreased the CB2/CB1 receptor selectivity by increasing the hCB2 receptor binding affinity, as exemplified by **17q** (~11.85) and **17v** (~6.30) in Table 2. Some selected promising compounds have been tested on animal models for in vivo efficacy.

In vivo efficacy was evaluated on high fat diet induced obese (DIO) mice and rats.[31] All animals were housed 1 per cage on a 12/12-h light/dark cycle, were fed food (high fat diet) and water, and were experimentally naive before testing. The samples of reference compounds **1** and **2** and the newly designed compounds **17**, **17c**, **17g**, and **17s** were prepared fresh daily by dissolving them in de-ionized water containing 10% DMSO at a volume of 10 mg/kg.

Figure 5 shows that all of our tested compounds **17–17s** are substantially more efficacious than **1** in the in vivo efficacy study using the DIO mice model. The compounds prepared in this study exhibited reductions in body weight after 14 days ranging from

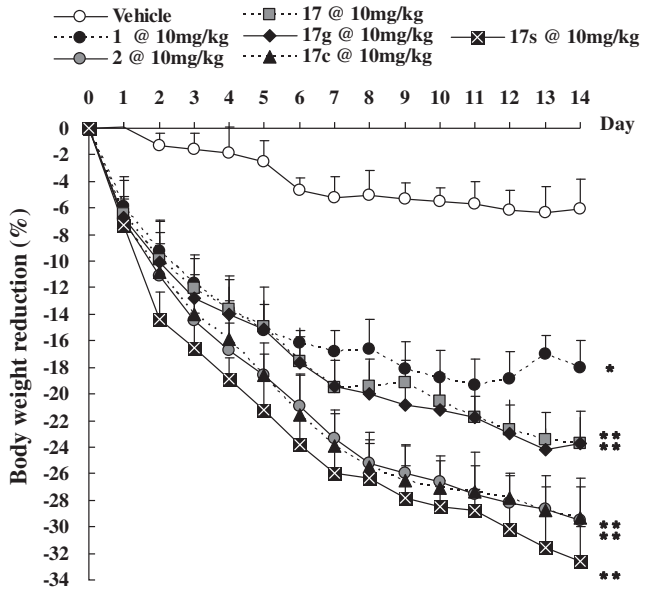


Figure 5. DIO mouse 2-week efficacy following daily administration of compounds **1**, **2**, **17**, **17c**, **17g** and **17s** at 10 mg/kg. Each value is the mean ± S.E.M. of 6 mice. $^{*}P$ <0.05, $^{**}P$ <0.01 versus corresponding vehicle (ANOVA with Dunnett's test).

23.68 ± 2.58% up to 32.55 ± 2.58%, whereas **1** reduced body weight moderately by comparison (18.02 ± 2.05%). Moreover, **17s** turned out to be more efficacious than **2** (32.55 ± 2.58% vs 29.55 ± 2.54%).

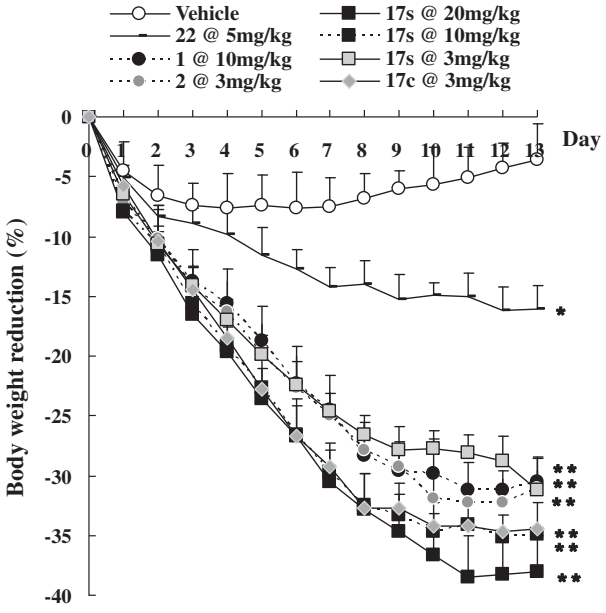


Figure 6. DIO mouse 13 day efficacy following daily administration of **17s** at 3, 10, or 20 mg/kg, shown together with data from administration of sibutramine (**22**) at 5 mg/kg, **1** at 10 mg/kg, **2** at 3 mg/kg, and **17c** at 3 mg/kg. Each value is the mean ± S.E.M. of six mice. $^{*}P$ <0.05, $^{**}P$ <0.01 versus corresponding Vehicle (ANOVA with Dunnett's test).

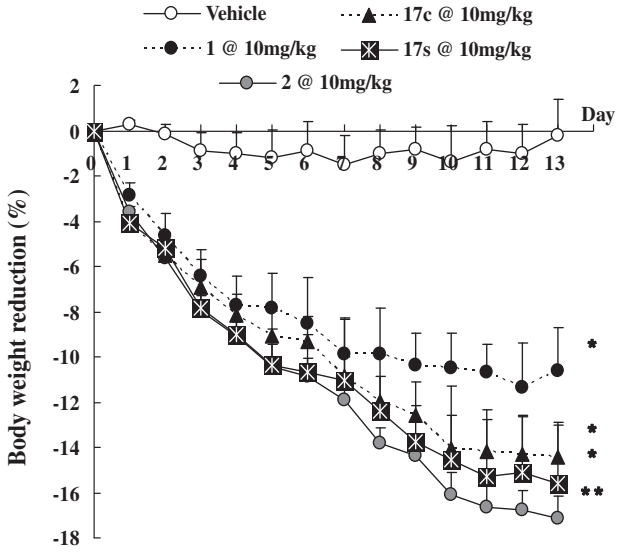


Figure 8. Effects of treatment with tested compounds on body weight in DIO rat. All rats were fed HFD for 12 wk and then treated for 14 days with compound at 10 mg/kg/day. All results are expressed as mean ± S.E.M. of six rats. The statistical analysis was performed using a one-way ANOVA followed by Dunnett's post hoc test. $^{*}P$ <0.05, $^{**}P$ <0.01 versus corresponding vehicle.

Chronic evaluation of **17s** was carried out with oral administration for 13 days in a DIO mouse in order to test its dose dependency. As indicated by the data in Figure 6, **17s** showed a dose-dependent response in its ability to affect body weight. Dosing at 10 or 20 mg/kg significantly reduced body weights by 34.90 ± 1.89 and 38.06 ± 3.01%, respectively. Dosing **17s** at 3 mg/kg proved as effective as reference compound **2** at the same dose. Compound **17c** is also included in Figure 6 for comparison. Interestingly, dosing **17c** at 3 mg/kg reduced body weight (34.45 ± 1.45%) as much as dosing **17s** at 10 mg/kg (34.90 ± 1.89%). The mean cell diameter of white adipocytes in vehicle-treated DIO mice were large and exhibited heterogeneous sizes, while adipocytes in **17s**-treated animals were dose-dependently smaller than those in vehicle-treated animals (Fig. 7).

For comparison of efficacy in a different animal model, chronic evaluation of **17c** and **17s** along with reference compounds **1** and **2** was performed by oral administration at 10 mg/kg for 13 days in DIO rats. Dosing **17c** and **17s** at 10 mg/kg each significantly reduced body weight (14.43 ± 1.56%, 15.59 ± 2.56%) compared with **1** (10.61 ± 1.89%), while **2** demonstrated superior efficacy in DIO rats (17.16 ± 1.89%).

Compound **17s** also displayed significant anti-diabetic activity through oral administration at 10 mg/kg in various animal models including *ob*/*ob* mice, *db*/*db* mice, and DIO mice (Fig. 8).[32]

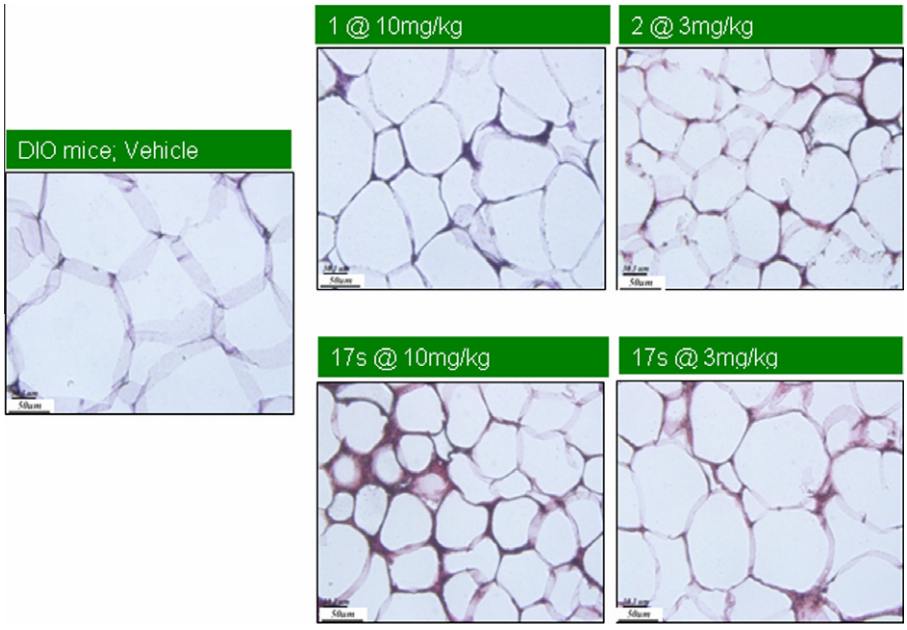


Figure 7. Effects of treatment with tested compounds on white adipocyte (WAT) phenotype. WAT from mice that were treated 10 mg/kg/d or 3 mg/kg/d compounds for 14 days were stained H&E. The magnification is 400X.

Table 3
Pharmacokinetic profiles of **1**, **2**, and **17s** in rats

Pharmacokinetic parameters	**1**		**2**		**17s**	
	po (5 mg/kg, $n = 3$) Mean ± SD	iv (1 mg/kg, $n = 3$) Mean ± SD	po (5 mg/kg, $n = 3$) Mean ± SD	iv (1 mg/kg, $n = 3$) Mean ± SD	po (5 mg/kg, $n = 3$) Mean ± SD	iv (1 mg/kg, $n = 3$) Mean ± SD
T_{max} (h)	0.50 ± 0.00	—	1.00 ± 0.00	—	0.50 ± 0.00	—
C_{max} (μg/mL)	0.29 ± 0.14	0.30 ± 0.08	1.53 ± 0.47	2.50 ± 1.11	0.36 ± 0.01	0.47 ± 0.06
C_{last} (μg/mL)	0.00 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.05 ± 0.04	0.02 ± 0.00	0.01 ± 0.00
$T_{1/2}$ (h)	8.23 ± 3.29	3.09 ± 0.32	7.11 ± 0.06	5.30 ± 1.40	14.80 ± 0.31	2.76 ± 0.38
CL/F	154.10 ± 36.63	—	13.70 ± 2.12	—	59.77 ± 1.00	—
CL (mL/min/kg)	—	82.40 ± 20.47	—	10.70 ± 7.07	—	40.83 ± 5.26
$AUC_{0-\infty}$ (min/μg/mL)	33.40 ± 7.99	12.59 ± 2.74	369.72 ± 56.37	119.90 ± 79.27	83.64 ± 1.44	24.75 ± 3.09
Bioavailability (%)	**53.1**		**61.7**		**67.6**	

Table 4
Pharmacological data of compounds **17g**, **17c**, **17s** along with reference **1**, **2**

Compound	CYP 450 (IC_{50}, nM)				hERG (IC_{50}, HM)	Ames assay	Comet assay
	1A2	2C9	2D6	3A4			
1	>20.0	5.3	>20.0	>20.0	4.8	Non-mutagenic	neg*
2	>20.0	0.93	0.59	>20.0	14.2	Non-mutagenic	neg
I7g	2.7	2.6	>20.0	>20.0	>10.0	Non-mutagenic	+
17c	>20.0	>20.0	>20.0	>20.0	>10.0	Non-mutagenic	neg
17s	>20.0	>20.0	>20.0	>20.0	10.5	Non-mutagenic	neg

Table 5
Single-dose toxicity in rats and 7-day repeat toxicity in rats

Compound	Single-dose			7-day repeat		
	mg/kg, po	Mortality/total animal number	LD_{50} (mg/kg)	mg/kg × day, po	Mortality/total animal number	NOAEL (mg/kg)
17s	2000	0/5	>2000	1000 × 7	0/2	125
				500 × 7	0/5	
	1500	0/5		250 × 7	0/5	
				125 × 7	0/5	

The pharmacokinetic properties of **17s** along with **1** and **2** were measured in male SD rats (Table 3). After oral administration of 5 mg/kg of **1**, **2**, and **17s**, compound **17s** had slightly higher exposure ($F = 67.6\%$) than reference compound **1** ($F = 53.1\%$) or **2** ($F = 61.7\%$) in rats with 14.80 ± 0.31 h $T_{1/2}$. The clearance of **17s** was determined from iv PK studies (CL = 40.83 ± 5.26 mL/min/kg) and showed a value in between reference compounds **1** and **2**. Compound **17s** also showed a similar brain/plasma ratio (58%) to that of reference **2** (52%).

The off-target activity of CB1 ligands including **17s** was also evaluated as shown in Table 4. Both **17c** and **17s** were demonstrated not to be significant inhibitors of the cytochrome p450 enzymes (IC_{50} >20 μM). This observation is in sharp contrast to the activity shown by reference **1** (IC_{50} = 5.3 μM) and **2** (IC_{50} = 0.93 μM) against CYP2C9. The activity of **17s** in the hERG potassium channel assay was determined to be 10.5 μM for IC_{50}. The comet assay[33] and Ames assay[34] were used for detection of mutagenicity of the compounds. Neither **17c** nor **17s** proved to be mutagenic. In addition, when the cytotoxicity[35] of **17c** and **17s** was tested on a panel of cells (Huh-7, Vero, HCT116, and HEK-293), both **17c** and **17s** demonstrated their safety by showing >100 μM for EC_{50}.

Subsequently, in vivo toxicity studies were performed for compound **17s** to examine critical safety issues. Acute toxicity studies were performed on oral administration of 2000 mg/kg or 1500 mg/kg in rats ($n = 5$). As shown in Table 5, there was no mortality of the animals tested up to 2000 mg/kg. Thus, the estimated LD_{50} is greater than 2000 mg/kg. Table 5 also describes results of preliminary 7-day repeat toxicity studies. Every animal in 4 different dosing cohorts survived on oral administration q.d. for 7 days. NOAEL (No adverse effect level) is roughly estimated to be 125 mg/kg. Based on its favorable toxicity studies, its excellent in vivo efficacy in animal models, and a satisfactory pharmacokinetic profile, compound **17s** was selected as a development candidate.

4. Conclusion

Structure–activity relationship studies in a series of diarylpyrazolyl thiadiazoles identified cannabinoid-1 receptor antagonists with excellent potency and selectivity. Modifications of previous oxadiazole ring into the corresponding thiadiazole ring appears to provide more efficacious series of compounds, not to mention securing superior intellectual property (IP) position to other existing pyrazole series of CB1 receptor ligands. Based on its exceptional in vivo efficacy in animal models and favorable pharmacokinetic and toxicological profiles, 2-(4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (GCC2680) was selected as a preclinical candidate for the treatment of obesity.

5. Experimental section

All references to ether are to diethyl ether; brine refers to a saturated aqueous solution of NaCl. Unless otherwise indicated, all temperatures are expressed in °C (degrees Centigrade). All reactions were conducted under an inert atmosphere at room temperature

unless otherwise noted, and all solvents were of the highest available purity unless otherwise indicated. Microwave reactions were conducted with a Biotage microwave reactor. ^{1}H NMR and ^{13}C NMR spectra were recorded on 400 MHz Fourier Transform-Nuclear Magnetic Resonance; Varian, 400-MR. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants are in units of Hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), or br (broad). Mass spectra were obtained with either a Micromass, Quattro LC Triple Quadrupole Tandem Mass Spectrometer, ESI or an Agilent, 1200LC/MSD, ESI. High-resolution mass spectra were determined by a Jeol, JMS-700 Mstation. For preparative HPLC, ca. 100 mg of a product was injected in 1 mL of DMSO onto a SunFire™ Prep C18 OBD 5 μm 30 × 100 mm column with a 10-min gradient from 10% CH_3CN to 90% CH_3CN in H_2O. A Biotage® SP1 Flash purification system was used for normal phase column chromatography with ethyl acetate and hexane. Flash chromatography was carried out using Merck Silica Gel 60 (230–400 mesh). Most of the reactions were monitored by thin-layer chromatography on 0.25 mm E. Merck silica gel plates (60F-254), visualized with UV light using a 5% ethanolic phosphomolybdic acid or *p*-anisaldehyde solution. All tested compounds were found to be greater than 95% pure by LC/MS, except as otherwise indicated.

5.1. Ethyl 4-(bromomethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (18)

To a solution of ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate (**12**) (10.0 g, 22.0 mmol) in CCl_4 (150 mL) was added *N*-bromosuccinimide (4.7 g, 26.4 mmol) and AIBN (36 mg, 0.2 mmol). The reaction mixture was heated for 8 h at 95 °C. Reaction mixture was filtered. Filtrate was washed with H_2O and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to provide 10.5 g of desired bromide as a pale yellow solid. The obtained bromide was used without further purification. ESI-MS *m*/*z*: 487 $[M+H]^+$.

5.2. Ethyl 4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (19)

To a solution of ethyl 4-(bromomethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (**18**) (10.5 g, 19.7 mmol) in DMF (50 mL) was added 1,2,4-triazole sodium derivatives (1.97 g, 21.7 mmol). The resulting mixture was concentrated, diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica column chromatography (Biotage SP1™) and the desired compound was obtained as a white solid (6.7 g, 58% 2-steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 8.09 (s, 1H), 7.41–7.27 (m, 7H), 5.48 (s, 2H), 4.44 (quartet, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H); ESI-MS *m*/*z*: 476 $[M+H]^+$.

5.3. 4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carbohydrazide (20)

To a solution of ethyl 4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (**19**) (14.0 g, 29.4 mmol) in EtOH (400 mL) was added hydrazine monohydrate (40 mL). The reaction mixture was refluxed for 2 h at 65 °C. The reaction mixture was diluted with EtOAc and washed with saturated NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to provide 13.0 g (28.1 mmol, 95.6%) as a white solid. The obtained hydrazide was used without further purification. ESI-MS *m*/*z*: 462 $[M+H]^+$.

5.4. 4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-*N*'-pivaloyl-1*H*-pyrazole-3-carbohydrazide (21)

To a solution of 4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carbohydrazide (**20**, 5.5 g, 11.9 mmol), pivalic acid (1.6 g, 15.5 mmol), EDCI (3.4 g, 17.9 mmol), HOBt (3.2 g, 23.8 mmol) in DMF (100 mL) was added NMM (4.0 mL, 36.4 mmol). The resulting mixture was concentrated, diluted with CH_2Cl_2 and washed with 1*N*-HCl solution. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica column chromatography (Biotage SP1™) and the desired compound was obtained as a white solid (7.0 g, 99%). ESI-MS *m*/*z*: 546 $[M+H]^+$.

5.5. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (17)

To a solution of 4-((1*H*-1,2,4-triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-*N*'-pivaloyl-1*H*-pyrazole-3-carbohydrazide (**21**) (244 mg, 0.45 mmol) and Lawesson's reagent (453 mg, 1.12 mmol) in THF (10 mL) was irradiated in a microwave reactor (Biotage Initiator™) for 50 minutes at 150 °C The resulting mixture was concentrated, diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (Biotage SP1™ FLASH Purification System was used for normal phase column chromatography with ethyl acetate and hexane) and the desired compound was obtained as a white solid (167 mg, 68.8%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.99 (s, 1H), 7.45–7.42 (m, 3H), 7.39–7.34 (m, 4H), 5.64 (s, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.9, 160.5, 150.7, 145.3, 143.8, 142.9, 136.0, 135.6, 134.8, 132.6, 131.1, 130.1, 130.0, 129.0, 127.7, 125.2, 113.4, 44.4, 37.9, 32.9; ESI-MS *m*/*z*: 544 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 544.0647 $[M+H]^+$ (calcd for $C_{24}H_{20}Cl_3N_7S$: 544.0645).

5.5.1. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-methylcyclopropyl)-1,3,4-thiadiazole (17a)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.16 (s, 1H), 7.48–7.29 (m, 7H), 5.68 (s, 2H), 1.65 (s, 3H), 1.45–1.39 (m, 2H), 1.16–1.10 (m, 2H); ESI-MS *m*/*z*: 542 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 542.0486 $[M+H]^+$ (calcd for $C_{24}H_{18}Cl_3N_7S$: 542.0488).

5.5.2. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl-)cyclopropyl)-1,3,4-thiadiazole (17b)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.01 (s, 1H), 7.48–7.28 (m, 7H), 5.65 (s, 2H), 1.83–1.61 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 162.0, 150.6, 145.5, 143.7, 142.3, 136.1, 135.8, 134.6, 132.6, 131.0, 130.1, 130.0, 128.9, 127.7, 125.0, 124.9 (q, *J* = 266 Hz), 113.4, 44.4, 27.0, 16.1; ESI-MS *m*/*z*: 596 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 596.0208 $[M+H]^+$ (calcd for $C_{24}H_{15}Cl_3F_3N_7S$: 596.0206).

5.5.3. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclobutyl)-1,3,4-thiadiazole (17c)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H), 8.28 (s, 1H), 7.47–7.45 (m, 1H), 7.43–7.31 (m, 6H), 5.72 (s, 2H), 2.84 (d, *J* = 8.0 Hz, 4H), 2.20 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 163.9, 151.6, 146.2, 144.5, 143.0, 136.6, 136.2, 135.1, 133.0, 131.4, 130.5, 130.4, 129.2, 128.0, 126.1 (q, *J* = 277 Hz), 125.3, 113.6, 48.2 (q, *J* = 30 Hz), 43.0,

28.8, 15.8; ESI-MS *m*/*z*: 610 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 610.0365 $[M+H]^+$ (calcd for $C_{25}H_{17}Cl_3F_3N_7S$: 610.0362).

5.5.4. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-phenylcyclopropyl)-1,3,4-thiadiazole (17d)

^1H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.05 (s, 1H), 7.50–7.46 (m, 2H), 7.42–7.21 (m, 10H), 5.63 (s, 2H), 1.98–1.90 (m, 2H), 1.61–1.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.9, 160.1, 150.7, 145.2, 143.8, 142.8, 141.5, 135.9, 135.6, 134.7, 132.6, 131.0, 130.1, 130.0, 129.8, 128.8, 128.7, 127.9, 127.6, 125.2, 113.2, 44.4, 29.1, 21.3; ESI-MS *m*/*z*: 604 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 604.0646 $[M+H]^+$ (calcd for $C_{29}H_{20}Cl_3N_7S$: 604.0645).

5.5.5. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-*p*-tolylcyclopropyl)-1,3,4-thiadiazole (17e)

^1H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.04 (s, 1H), 7.47–7.22 (m, 9H), 7.18–7.10 (m, 2H), 5.62 (s, 2H), 2.33 (s, 3H), 1.95–1.88 (m, 2H), 1.56–1.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 160.1, 150.7, 145.1, 143.8, 142.8, 138.7, 137.5, 135.9, 135.6, 134.8, 132.6, 131.0, 130.1, 130.0, 129.7, 129.3, 128.8, 127.6, 125.2, 113.2, 44.4, 28.7, 23.1, 21.4; ESI-MS *m*/*z*: 618 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 618.0807 $[M+H]^+$ (calcd for $C_{30}H_{22}Cl_3N_7S$: 618.0801).

5.5.6. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(4-chlorophenyl)-cyclopropyl)-1,3,4-thiadiazole (17f)

^1H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.13 (s, 1H), 7.44–7.24 (m, 11H), 5.29 (s, 2H), 1.99–1.88 (m, 2H), 1.59–1.51 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 160.2, 150.7, 145.2, 143.8, 142.6, 139.9, 136.0, 135.7, 134.7, 133.7, 132.6, 131.2, 131.0, 130.1, 130.0, 128.9, 128.8, 127.7, 125.1, 113.2, 44.4, 28.6, 21.4; ESI-MS *m*/*z*: 638 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 638.0254 $[M+H]^+$ (calcd for $C_{29}H_{19}Cl_4N_7S$: 638.0255).

5.5.7. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (17g)

^1H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 7.96 (s, 1H), 7.53–7.50 (m, 2H), 7.45–7.44 (m, 1H), 7.39–7.36 (m, 2H), 7.35–7.30 (m, 2H), 5.63 (s, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3, 161.5, 151.5, 146.0, 144.5, 143.5, 136.5, 135.2, 133.0, 132.1, 131.7, 130.5, 130.4, 128.0, 125.9, 124.5, 113.4, 43.0, 36.3, 31.3; ESI-MS *m*/*z*: 588 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 588.0130 $[M+H]^+$ (calcd for $C_{24}H_{20}BrCl_2N_7S$: 588.0140).

5.5.8. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-methylcyclopropyl)-1,3,4-thiadiazole (17h)

^1H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.02 (s, 1H), 7.54–7.51 (m, 2H), 7.46–7.44 (m, 1H), 7.38–7.33 (m, 2H), 7.32–7.28 (m, 2H), 5.64 (s, 2H), 1.63 (s, 3H), 1.40 (dd, *J* = 6.8 Hz, 4.4 Hz, 2H), 1.10 (dd, *J* = 6.8 Hz, 4.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 160.4, 151.5, 145.9, 144.5, 143.5, 136.4, 135.2, 133.1, 132.1, 131.6, 130.5, 130.4, 128.0, 125.9, 124.5, 113.3, 42.9, 23.2, 20.3, 17.7; ESI-MS *m*/*z*: 586 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 585.9984 $[M+H]^+$ (calcd for $C_{24}H_{18}BrCl_2N_7S$: 585.9983).

5.5.9. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclopropyl)-1,3,4-thiadiazole (17i)

^1H NMR (400 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.25 (s, 1H), 7.58–7.54(m, 2H), 7.47 (d, *J* = 1.6 Hz, 1H), 7.39–7.31 (m, 4H), 5.69 (s, 2H), 1.77–1.65 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.6, 163.0, 151.6, 146.1, 144.4, 142.9, 136.6, 135.1, 133.0, 132.1, 131.6, 130.5, 130.4, 128.0, 125.7, 125.1 (q, *J* = 272 Hz), 124.6, 113.5, 42.9, 25.2, 14.1; ESI-MS *m*/*z*: 640 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 639.9705 $[M+H]^+$ (calcd for $C_{24}H_{15}BrCl_2F_3N_7S$: 639.9700).

5.5.10. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclobutyl)-1,3,4-thiadiazole (17j)

^1H NMR (400 MHz, $CDCl_3$) δ 9.2 (s, 1H), 8.12 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.45–7.33 (m, 7H), 5.63 (s, 2H), 2.87–2.74 (m, 4H), 2.20–2.11 (m, 2H); ESI-MS *m*/*z*: 654 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 653.9851 $[M+H]^+$ (calcd for $C_{25}H_{17}BrCl_2F_3N_7S$: 653.9857).

5.5.11. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-phenylcyclopropyl)-1,3,4-thiadiazole (17k)

^1H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.04 (s, 1H), 7.58–7.45 (m, 4H), 7.42–7.23 (m, 8H), 5.62 (s, 2H), 1.95 (dd, *J* = 6.8 Hz, 4.4 Hz, 2H), 1.57 (dd, *J* = 6.8 Hz, 4.4 Hz, 2H); ESI-MS *m*/*z*: 648 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 648.0132 $[M+H]^+$ (calcd for $C_{29}H_{20}BrCl_2N_7S$: 648.0140).

5.5.12. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-*p*-tolylcyclopropyl)-1,3,4-thiadiazole (17l)

^1H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.08 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.42–7.23 (m, 7H), 7.15 (d, *J* = 8.0 Hz, 2H), 5.63 (s, 2H), 2.33 (s, 3H), 1.92 (dd, *J* = 6.8 Hz, 4.4 Hz, 2H), 1.54 (dd, *J* = 6.8 Hz, 4.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 161.1, 151.5, 145.8, 144.4, 143.5, 139.2, 138.0, 136.4, 135.2, 133.0, 132.1, 131.6, 130.4, 130.3, 130.0, 129.6, 127.9, 125.9, 124.4, 113.1, 42.9, 26.9, 21.1, 19.4; ESI-MS *m*/*z*: 662 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 662.0299 $[M+H]^+$ (calcd for $C_{30}H_{22}BrCl_2N_7S$: 662.0296).

5.5.13. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(4-chlorophenyl)-cyclopropyl)-1,3,4-thiadiazole (17m)

^1H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.18 (s, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.46–7.40 (m, 2H), 7.38–7.24 (m, 8H), 5.67 (s, 2H), 2.05–1.93 (m, 2H), 1.59–1.52 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.0, 162.7, 153.0, 147.4, 145.9, 144.7, 141.9, 137.9, 136.6, 135.6, 134.5, 133.5, 133.1, 133.0, 132.9, 131.8, 130.6, 129.4, 127.3, 125.9, 114.6, 44.3, 28.2, 20.9; ESI-MS *m*/*z*: 682 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 681.9741 $[M+H]^+$ (calcd for $C_{29}H_{19}BrCl_3N_7S$: 681.9750).

5.5.14. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-1-(2-chlorophenyl)-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (17n)

^1H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 8.18 (s, 1H), 7.47–7.31 (m, 8H), 5.71 (s, 2H), 1.54 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 161.7, 151.5, 145.8, 144.5, 143.2, 136.6, 135.9, 132.2, 131.5, 131.0, 130.5, 129.8, 129.0, 127.6, 125.7, 113.1, 43.1, 36.3, 31.1; ESI-MS *m*/*z*: 510 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 510.1032 $[M+H]^+$ (calcd for $C_{24}H_{21}Cl_2N_7S$: 510.1034).

5.5.15. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-1-(2-chlorophenyl)-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclopropyl)-1,3,4-thiadiazole (17o)

^1H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.18 (s, 1H), 7.45–7.38 (m, 4H), 7.36–7.32 (m, 3H), 5.68 (s, 2H), 1.75–1.67 (m, 2H), 1.65–1.63 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 162.2, 150.8, 145.4, 143.7, 142.0, 136.0, 135.5, 131.7, 131.1, 130.7, 130.2, 129.4, 128.7, 127.4, 125.3, 124.9 (q, *J* = 266 Hz), 113.3, 44.5, 26.8 (q, *J* = 35 Hz), 16.1; ESI-MS *m*/*z*: 562 $[M+H]^+$; positive HR-FAB-MS *m*/*z*: 562.0588 $[M+H]^+$ (calcd for $C_{24}H_{16}Cl_2F_3N_7S$: 562.0595).

5.5.16. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-1-(2-chlorophenyl)-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclobutyl)-1,3,4-thiadiazole (17p)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.10 (s, 1H), 7.45–7.31 (m, 7H), 5.69 (s, 2H), 2.83 (t, J = 8.0 Hz, 4H), 2.23–2.14 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 164.1, 151.6, 146.1, 144.5, 142.7, 136.5, 136.0, 132.1, 131.5, 131.1, 130.6, 129.7, 129.0, 127.7, 126.1 (q, J = 278 Hz), 125.5, 113.4, 48.2 (q, J = 30 Hz), 43.0, 28.8, 15.8; ESI-MS m/z: 576 $[M+H]^+$; positive HR-FAB-MS m/z: 576.0756 $[M+H]^+$ (calcd for $C_{25}H_{18}Cl_2F_3N_7S$: 576.0752).

5.5.17. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-chlorophenyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)cyclobutyl)-1,3,4-thiadiazole (17q)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.92 (s, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.56 (quint, J = 7.2 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 7.6 Hz, 1H), 5.63 (s, 2H), 2.84 (t, J = 8.4 Hz, 4H), 2.21–2.17 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 164.0, 151.6, 146.0, 144.5, 142.5, 136.2, 136.1, 132.6, 131.7, 130.1, 129.2, 128.1, 128.0 (q, J = 5 Hz), 127.7, 126.2 (q, J = 280 Hz), 125.4, 122.7 (q, J = 273 Hz), 113.8, 48.2 (q, J = 30 Hz), 43.1, 28.8, 15.8; ESI-MS m/z: 610 $[M+H]^+$; positive HR-FAB-MS m/z: 610.1021 $[M+H]^+$ (calcd for $C_{26}H_{18}ClF_6N_7S$: 610.1015).

5.5.18. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-1-(2-chlorophenyl)-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(4-chlorophenyl)-cyclopropyl)-1,3,4-thiadiazole (17r)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H), 8.20 (s, 1H), 7.44–7.28 (m, 11H), 5.68 (s, 2H), 1.96–1.93 (m, 2H), 1.56–1.53 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 160.4, 150.7, 145.1, 143.7, 142.4, 140.0, 136.0, 135.4, 133.7, 131.7, 131.2, 131.0, 130.6, 130.1, 129.4, 128.8, 128.6, 127.3, 125.4, 112.9, 44.5, 28.6, 21.3; ESI-MS m/z: 604 $[M+H]^+$; positive HR-FAB-MS m/z: 604.0652 $[M+H]^+$ (calcd for $C_{29}H_{20}Cl_3N_7S$: 604.0645).

5.5.19.1. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-*tert*-butyl-1,3,4-thiadiazole (17s)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.13 (s, 1H), 7.50 (d, J = 8.4 Hz, 2H), 7.45–7.31 (m, 8H), 5.69 (s, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 160.6, 150.7, 145.2, 143.8, 142.6, 136.1, 131.7, 131.5, 131.3, 130.6, 130.2, 129.4, 127.3, 125.9, 124.0, 113.1, 44.5, 37.9, 32.9; ESI-MS m/z: 554 $[M+H]^+$; positive HR-FAB-MS m/z: 554.0530 $[M+H]^+$ (calcd for $C_{24}H_{21}BrClN_7S$: 554.0529).

5.5.20. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclopropyl)-1,3,4-thiadiazole (17t)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.24 (s, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.46–7.38 (m, 3H), 7.36–7.32 (m, 3H), 5.70 (s, 2H), 1.77–1.75 (m, 2H), 1.67–1.64 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.5, 163.2, 151.6, 146.1, 144.4, 142.7, 136.5, 132.1, 132.0, 131.7, 131.1, 130.6, 129.7, 127.7, 126.0, 125.1 (q, J = 271 Hz), 124.4, 113.3, 43.0, 25.0 (q, J = 36 Hz), 14.1; ESI-MS m/z: 606 $[M+H]^+$; positive HR-FAB-MS m/z: 606.0097 $[M+H]^+$ (calcd for $C_{24}H_{16}BrClF_3N_7S$: 606.0090).

5.5.21. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)-cyclobutyl)-1,3,4-thiadiazole (17u)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.21 (s, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.45–7.30 (m, 6H), 5.71 (s, 2H), 2.87–2.81 (m, 4H), 2.23–2.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.2, 165.6, 153.2, 147.6, 146.0, 144.3, 138.0, 133.6, 133.5, 133.2, 132.7, 132.1, 131.2, 129.2, 127.7 (q, J = 277 Hz), 127.5, 125.9, 114.9, 49.5 (q, J = 30 Hz), 44.6, 30.3, 17.4; ESI-MS m/z: 620 $[M+H]^+$; positive HR-FAB-MS m/z: 620.0259 $[M+H]^+$ (calcd for $C_{25}H_{18}BrClF_3N_7S$: 620.0247).

5.5.22. 2-(4-((1*H*-1,2,4-Triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-pyrazol-3-yl)-5-(1-(trifluoromethyl)cyclobutyl)-1,3,4-thiadiazole (17v)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H), 7.94 (s, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.58 (quint, J = 6.8 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 7.6 Hz, 1H), 5.64 (s, 2H), 2.86 (t, J = 8.4 Hz, 4H), 2.24–2.16 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 164.0, 151.6, 146.0, 144.5, 142.5, 136.1, 132.6, 132.1, 131.9, 130.1, 128.1, 128.0 (q, J = 5.0 Hz), 127.7, 126.1 (q, J = 278 Hz), 125.8, 124.5, 122.7 (q, J = 273 Hz), 113.8, 48.2 (q, J = 30 Hz), 43.0, 28.8, 15.8; ESI-MS m/z: 654 $[M+H]^+$; positive HR-FAB-MS m/z: 654.0499 $[M+H]^+$ (calcd for $C_{26}H_{18}BrF_6N_7S$: 654.0510).

5.6. CB1 and CB2 receptor binding assay[29,30]

For CB1 receptor binding studies, rat cerebellar membranes were prepared as previously described by the methods of Kuster et al. Male Sprague-Dawley rats (200–300 g) were sacrificed by decapitation, and the cerebella were rapidly removed. The tissue was homogenized in 30 volumes of TME buffer (50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, pH 7.4) using a Dounce homogenizer. The crude homogenates were immediately centrifuged (48,000g) for 30 min at 4 °C. The resultant pellets were resuspended in 30 volumes of TME buffer, the protein concentration was determined by the method of Bradford, and the pellets were stored at −70 °C until use.[31]

For CB2 receptor binding studies, CHO K-1 cells were transfected with human CB2 receptor as previously described, and cell membranes were prepared as described above.[32]

Competitive binding assays were performed as described.[33] Briefly, approximately 10 μg of rat cerebellar membranes (containing CB1 receptor) or cell membranes (containing CB2 receptor) were incubated in a 96-well plate with TME buffer containing 0.5% essentially fatty acid free bovine serum albumin (BSA).

Three nM [^{3}H]**23** (for CB2 receptor, NEN; specific activity 50–80 Ci/mmol) or 3 nM **24** ([^{3}H]CP55,940, [^{3}H]2-[(1*S*,2*R*,5*S*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol, for CB1 receptor, NEN; specific activity 120–190 Ci/mmol) and various concentrations of the synthesized cannabinoid ligands in a final volume of 200 μL. The assays were incubated for 1 h at 30 °C and then immediately filtered over GF/B glass fiber filter (PerkinElmer Life and Analytical Sciences, Boston, MA) that had been soaked in 0.1% PEI for 1 h by a cell harvester (PerkinElmer Life and Analytical Sciences, Boston, MA). Filters were washed five times with ice-cold TBE buffer containing 0.1% essentially fatty acid free BSA, followed by oven-drying for 60 min and then were placed in 5 ml of scintillation fluid (Ultima Gold XR; PerkinElmer Life and Analytical Sciences, Boston, MA), and radioactivity was quantitated by liquid scintillation spectrometry. In the CB1 and CB2 receptor competitive binding assay, nonspecific binding was assessed using 1 μM **1** and 1 μM **23** (WIN55,212-2), respectively. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 μM concentrations of **1** or **23** and was 70–80% of the total binding. IC_{50} was determined by nonlinear regression analysis using GraphPad PRISM. All data were collected in triplicate, and IC_{50} was determined from three independent experiments.

5.7. Body weight reduction in diet induced obese (DIO) mice and rat

Male C57BL/6 mice and Sprague-Dawley rats were purchased by Charles River Laboratory (Gyeonggi-Do, Korea). All animals were housed individually with food and water freely available and were maintained at room temperature under on a 12/12 h light-dark cycle. Seven-week old mice were fed a high-fat diet (Rodent Diet with 45Kcal%Fat, Research Diet), and rats were fed a high-fat diet (Rodent Diet with 32%Kcal and high in sucrose 25%Kcal, Research Diet) ad libitum for 12 weeks. Thereafter, DIO mice and DIO rats were treated with either vehicle or compounds orally in distilled water with 0.1% Tween 80 for 14 days. Drugs were administered at 1 h before the dark cycle and their body weight was recorded daily before drug treatment. After sacrifice, white adipose tissue was isolated, weighed and fixed in formalin-alcohol for 48 h and paraffin-embedded. Plasma glucose levels were determined with a Blood Chemistry System (ADIVA 1650, USA). All procedures were performed in accordance with the experimental guidelines for animal care and use in the Humane Care and Use of Laboratory Animals (NIH Guide for the Care and Use of Laboratory Animals).

5.8. Histology of white adipose tissue in DIO mice

Paraffin embedded white adipose tissues were stained with hematoxylin and eosin and processed with routine immunohistological analysis technique. An adipocyte of these sections was analyzed using Olympus microscope with a high-power lens (400X).

5.9. Pharmacokinetics and BBB permeability study

Male Sprague-Dawley rats (200–230 g) were purchased from Orient Bio Inc. (Gyeonggi-Do, Korea). Two days before dosing, the femoral artery and vein (intravenous [iv] only) were cannulated using PE-26 tubing (Becton Dickinson, Lincoln Park, NJ, USA). For intravenous administration, prepared dosing solution was injected via the femoral vein. The rats were fasted overnight before drug administration and until 6 h after dosing. For the po experiment, rats (three in each group) were given a single dose of 5 mg/kg, and heparinized samples of blood (0.3 ml) were collected at 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600 min and 24 h post-dose. For the iv experiment, rats (three in each group) were given a single 1 mg/kg dose, and blood samples were collected at 5, 10, 20, 40, 60, 90, 120, 180, 240, 360 and 480 min post-dose. Plasma was harvested after centrifugation and stored frozen at −20 °C until analyzed. The concentrations of compounds in plasma were determined by LC/MS/MS (API3200). The results are shown as the maximum plasma concentration (C_{max}), the time to reach peak plasma concentration (T_{max}), terminal half-life ($t_{1/2}$) and the area under the plasma concentration-time curve from zero to time infinity ($AUC_{0-\infty}$). For the BBB permeability assay, brains were taken out of the cranium after heart perfusion with PBS at 30, 60, 120, 240 and 360 min.

5.10. Cytochrome P450 inhibition assay

Incubation mixtures consisted of 0.2 mg human liver microsomes, GCC2680 (0.2, 1, 2, 10 and 20 μM), substrate mixture and an NADPH-generating system (0.1 M glucose-6-phosphate, 10 mg/ml β-$NADP^+$ and 1 U/ml glucose-6-phosphate dehydrogenase) in a total volume of 200 μL potassium phosphate buffer (0.1 M, pH 7.4). Reactions were initiated by the addition of NADPH-generating system and continued in a water bath at 37 °C. After the incubation for 30 min, the reaction was stopped by addition of 400 μL of 0.1% acetic acid, and then internal standard was added (16 μM terfenadine). Two volumes of acetonitrile were added to each sample and mixed. After centrifugation, the supernatants (5 μL) were injected onto an HPLC column for LC/MS/MS analyses.

5.11. Comet and Ames assay

For the comet assay, 8×10^5 cells were seeded into 12 well plates (Falcon 3043) and then treated with the compounds. The compounds were tested at three concentration points at a range of concentrations up to 50 μM. After treatment with the compounds for 2 h, cells were centrifuged for 3 min at 100*g* (about 1200 rpm) and gently resuspended with PBS, and 100 μL of the cell suspension was immediately used for the test. Cells were mixed with 0.1 ml of 1% low melting point agarose (LMPA, Life Technologies, MD, USA) and added to a fully frosted slide (Fisher Scientific, PA, USA) that had been covered with a bottom layer of 100 μL of 1% normal melting agarose (Amresco, OH, USA). The cell suspension was immediately covered with cover glass, and the slides were then kept at 4 °C for 5 min to allow solidification. After removing the cover glass gently, the slides were covered with a third layer of 100 μL of 0.5% LMPA by using a cover glass and then the slide were kept again at 4 °C for 5 min. The Ames assay was performed essentially as described by Ames et al. (1973, 1975). Media and positive control chemicals were obtained from commercial sources and were the purest grades available. Strain TA 100 was chosen as the representative tester strain because of its high spontaneous reversion rate. Spontaneous revertant numbers were counted and plotted against the dose of the test chemical to produce a survival curve for the his^+ genotype. The mutagenicity assay was performed by mixing one of the tester strains that was cultured overnight with the test substance in the presence and in the absence of S9 mixture condition, where sodium phosphate buffer was added instead of S9 mixture both in negative and positive controls in the test tube. Then, the mixture was incubated in a water bath for 30 min at 37 °C, and after incubation, the mixture was mixed with top agar containing a minimal amount of histidine and then poured onto the surface of a r-ray sterile Petri dish (Falcon, USA) containing 25 ml of solidified bottom agar. The finished plates were incubated for 48 h at 37 °C, and revertant colonies were counted later. Negative control plates containing no added test chemical, while positive control plates containing appropriate amounts of chemicals known to be active were included with each tester strain. Sodium azide (SA) and 2-aminoanthracene (2-AA) were used as positive control substances. Compounds were tested at seven concentration points in duplicate at a range of concentrations up to 2500 μg/plate. A response was considered to be positive in our criteria if there was a dose-dependent increase in revertants per plate resulting in at least a doubling of the background reversion rate for strains TA 100.

5.12. hERG assay

A three pulse protocol was applied by stepping from a holding potential of −80 mV to +40 mV for 2 s to inactivate hERG channels. The membrane voltage was then stepped back to −50 mV for 2 s to evoke a tail current prior to returning to the holding potential for 1 s. This sequence was repeated a further two times. This voltage protocol was applied prior to drug treatment (pre-compound) and after 600 s in the presence of the drug (post-compound).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.013. These data include MOL files and InChiKeys of the most important compounds described in this article.

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