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Article

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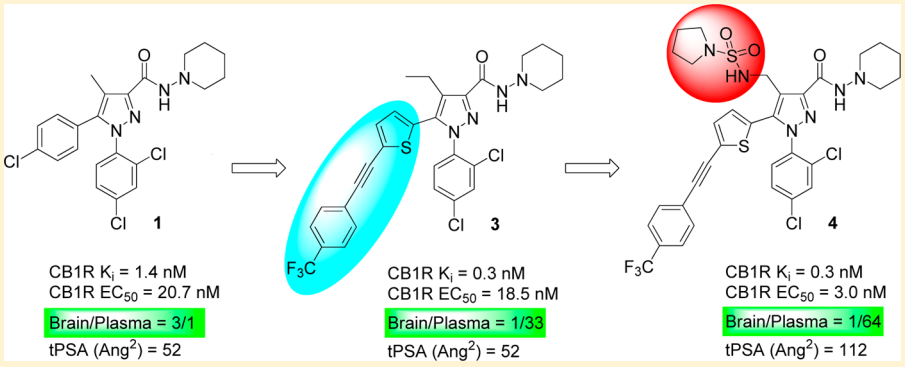
Discovery of 1-(2,4-Dichlorophenyl)-*N*-(piperidin-1-yl)-4-((pyrrolidine-1-sulfonamido)methyl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide as a Novel Peripherally Restricted Cannabinoid-1 Receptor Antagonist with Significant Weight-Loss Efficacy in Diet-Induced Obese Mice

Chun-Ping Chang,[†] Chien-Huang Wu,[†] Jen-Shin Song, Ming-Chen Chou, Ying-Chieh Wong, Yinchiu Lin, Teng-Kuang Yeh, Amit A. Sadani, Ming-Hung Ou, Kun-Hung Chen, Pei-Hsuan Chen, Po-Chu Kuo, Chen-Tso Tseng, Kuei-Hua Chang, Shi-Liang Tseng, Yu-Sheng Chao, Ming-Shiu Hung,* and Kak-Shan Shia*

Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli County 35053, Taiwan, R.O.C.

Supporting Information



ABSTRACT: After extensive synthetic efforts, we found that many structurally diverse bioisosteres could be generated via derivatizing the C-4 alkyl chain on the pyrazole ring of compound **3** (B/P = 1/33) with different electronegative groups. Especially when a sulfonamide or sulfamide moiety was added, resulting compounds exhibited not only potent CB1R activity but also a desired tPSA value over 90 Å^2, a threshold considered to possess a low probability to cross BBB, leading to the identification of compound **4** (B/P = 1/64) as a peripherally restricted CB1R antagonist. Apart from its significant weight-loss efficacy in DIO mice, compound **4** also displays 163 clean off-target profiles and is currently under development for treating obesity and the related metabolic syndrome.

INTRODUCTION

Cannabinoid-1 receptor (CB1R) is one of the most abundant neuroregulatory receptors in the brain and involved mainly in regulating feeding and appetite.[1] In addition to the brain, this receptor is also expressed in the peripheral organs, such as adipose tissues, muscle, and liver.[2] Distinct from CB1R, cannabinoid-2 receptor (CB2R) is mostly expressed in the immune system and primarily associated with immune regulation and neurodegeneration.[3,4] Clinically, a CB1R-targeted antiobesity agent known as rimonabant (**1**) or SR141716A was launched in Europe in 2006; however, it was soon withdrawn in 2008 due to severe central nervous system (CNS) adverse effects, including depression, anxiety, and stress disorders (Figure 1). Consequently, antiobesity agents acting on brain CB1R, such as taranabant (MK-0364) and otenabant (CP-945598), were all terminated in the late stages of development.

On the other hand, great efforts have been made particularly on the development of peripherally selective CB1R antagonists in that accumulating evidence from obese animal models indicated that peripheral CB1R antagonists could also cause weight loss and improve related metabolic disorders without additional CNS-mediated behavioral effects.[5−7] Encouragingly, 7TM Pharma has successfully completed a phase I clinical trial of TM38837, a peripherally restricted CB1R antagonist claimed for the treatment of obesity and type 2 diabetes, and clearly demonstrated this CB1R restricted antagonist is lack of CNS

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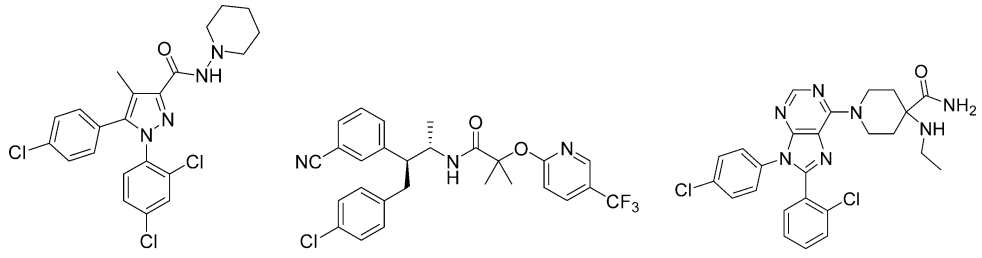

Figure 1. Typical brain CB1R-acting antagonists reported in the literature.

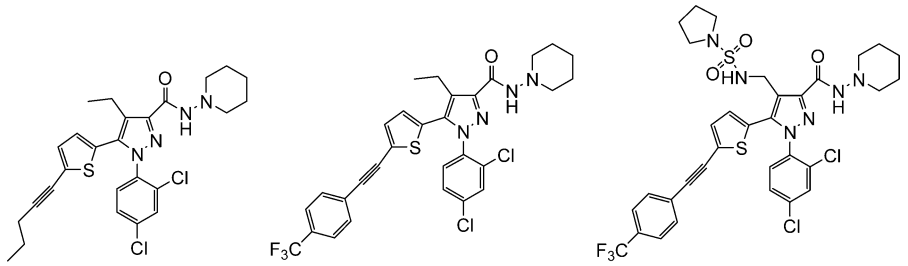

Figure 2. Compounds **2**–**4** progressing from mainly targeting brain to targeting peripheral CB1R.

side effects as observed with **1**.[8] In fact, International Diabetes Federation clearly points out that there is an urgent medical need for pharmacological agents suitable for treating coexistent type 2 diabetes and obesity because some common antidiabetic agents, including thiazolidinediones (TZDs), insulin, and sulfonylureas (SUs), are associated with undesired weight gain.[9] Thus, peripheral CB1R antagonists, which are able to improve hyperglycemia and induce weight loss simultaneously, might meet with this particular purpose and is worth further development. To date, three chronic treatment drugs, namely orlistat, lorcaserin (belviq), and qsymia, have been approved by FDA (US) for the treatment of obesity. Mechanistically, orlistat serves as a pancreatic lipase inhibitor with the significant efficacy to reduce the intestinal absorption of fat, but unpleasant side effects have resulted in little success.[10,11] Lorcaserin is a selective serotonin 5-HT_{2C} agonist, and qsymia is a combination of two old drugs phentermine and topiramate.[12−14] However, on the basis of side effects observed in clinical trials, these two recently approved antiobesity drugs might have a long-term safety issue after chronic treatment.

During the past decade, tremendous efforts have been dedicated to seeking antiobesity agents targeting CB1R in our laboratories, and several novel series of CB1R antagonists have been found.[15−23] Representative compounds **2**–**4** are highlighted (Figure 2) in chronological order to demonstrate the evolution of our structure-based design from mainly targeting brain CB1R to targeting peripheral CB1R. Experimentally, assessing brain-to-plasma ratio (B/P) appears to be an effective and practical approach to pursue desired peripheral CB1R antagonists. Along this axis (Table 1), structural modifications started with agent **1** (B/P = 3/1) through second-generation derivatives **2** (B/P = 1/6) and **3** (B/P = 1/33), leading to the identification of a potential drug candidate **4** (B/P = 1/64) with a significant improvement in the B/P ratio by ∼200-fold relative to the initial model **1**.

We serendipitously discovered that installing a sulfamide or sulfonamide functionality at the C-4 position of the pyrazole ring of lead **3** resulted in a 2-fold increase in the topological polar surface area (tPSA), and more importantly, activities toward CB1R were retained at the nanomolar level during the current synthetic tailoring. As demonstrated by many historical cases,[24−36] in general compounds with a tPSA value higher than 90 Å^2 are considered to have a low probability to permeate the blood–brain barrier (BBB). Indeed, this synthetic strategy is commonly applied to develop peripheral CB1R antagonists with low brain penetration during structural optimization. Detailed description on the design, synthesis, structure–activity relationships (SAR) of the newly developed compounds, and animal studies for the potential candidate are presented as follows.

Table 1. Brain-to-Plasma Ratio (B/P) and Calculated tPSA of Compounds 1–4

compd	tPSA (Å^2)	B/P[a,d]	hCB1R K_i (nM)
1	52	3/1[b]	1.4 ± 0.4
2	52	1/6	2.0 ± 0.6
3	52	1/33	0.3 ± 0.1
4	112	1/64 (1/120)[c]	0.3 ± 0.1

[a]Nonperfused brain samples were assayed 2 h after oral dosing (20 mg/kg) in C57BL/6 mice (n = 4). [b]Nonperfused brain samples were assayed 2 h after oral dosing (2 mg/kg) in C57BL/6 mice (n = 4). [c]Nonperfused brain samples were assayed 2 h after oral dosing (20 mg/kg) in DIO mice (n = 4). [d]Vehicle formulation: DMSO/Tween 80/H_2O (1:1:8, v/v/v)

CHEMISTRY

Test compounds **4**, **12**, and **14**–**32** in Table 2 were prepared according to a general synthetic method shown in Scheme 1 using compounds **4** and **12**, respectively, as a typical example.

Starting material **6** was readily synthesized based on the modified synthetic procedures reported by our laboratories.[17] Radical bromination of **6** was effected under treatment with NBS and AIBN in CCl_4 at 80 °C to afford dibromo ester **7** (92%), which in turn was treated with silver nitrate in acetone/

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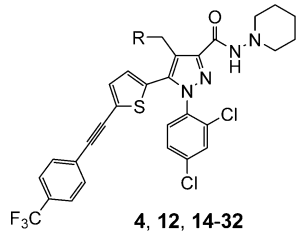
Table 2. Biological Evaluations of 5-(5-Alkynylthiophene-2-yl)pyrazole Derivatives on CB1 and CB2 Receptors



4, 12, 14-32

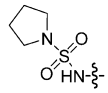
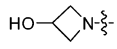
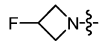
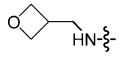
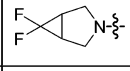
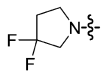
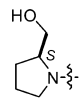
Cpd.	R	hCB1R K_i (nM)[a,c]	hCB2R K_i (nM)[a,c]	hCB2R /hCB1R	hCB1R EC_{50} (nM)[b,c]	IP (10 μM) (% efficacy)[d]	tPSA (Å^2)[e]	Hypothermia[f]	Analgesia[f]
1		1.4 ± 0.4	500.4 ± 105.4	357	20.7 ± 0.6	−32	52	+++	+++
4		0.3 ± 0.1	21.0 ± 4.7	70	3.0 ± 0.2	−31	112	−	−
12		0.6 ± 0.1	1760.6 ± 185.6	2933	5.1 ± 1.7	−26	78	+	−
14		3.6 ± 1.1	153.9 ± 6.8	43	13.1 ± 1.5	−41	78	ND	ND
15		3.6 ± 0.5	436.4 ± 13.1	121	5.8 ± 1.5	−25	79	+	+
16		0.8 ± 0.1	23.9 ± 6.0	30	82.4 ± 14.0	−39	79	ND	ND
17		5.4 ± 2.0	1123.3 ± 215.9	207	15.8 ± 3.2	−24	65	−	+
18		13.7 ± 3.9	327.6 ± 52.3	24	28.9 ± 3.6	−28	65	ND	ND
19		0.2 ± 0.1	214.0 ± 17.8	1070	229.1 ± 30.1	−28	54	ND	ND
20		0.3 ± 0.1	222.4 ± 2.4	741	80.3 ± 3.5	−36	54	+	+
21		3.5 ± 0.8	163.4 ± 26.6	47	10.0 ± 0.8	−14	78	−	+
22		1.3 ± 0.3	995.3 ± 48.8	765	3.1 ± 1.2	−31	77	++	++
23		0.9 ± 0.1	45.8 ± 14.3	51	6.1 ± 0.8	−25	109	−	+
24		7.2 ± 0.6	22.8 ± 5.3	3	7.5 ± 1.4	−36	109	ND	ND
25		0.1 ± 0.1	14.9 ± 2.2	149	7.4 ± 0.4	−51	109	−	−

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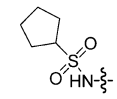
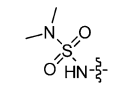
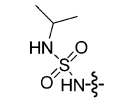
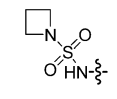
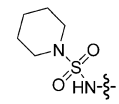
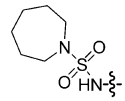
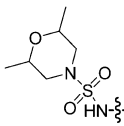
Table 2. continued

Cpd.	R	hCB1R K_i (nM)[a,c]	hCB2R K_i (nM)[a,c]	hCB2R /hCB1R	hCB1R EC_{50} (nM)[b,c]	IP (10 μM) (% efficacy)[d]	tPSA (Å^2)[e]	Hypothermia[f]	Analgesia[f]
26		0.8 ± 0.1	24.7 ± 1.5	31	7.4 ± 2.5	−48	109	−	−
27		0.2 ± 0.1	14.9 ± 4.7	75	3.3 ± 0.1	−32	112	−	−
28		1.4 ± 0.3	3.3 ± 0.7	2	5.3 ± 0.6	−34	122	+	+
29		0.4 ± 0.1	1.9 ± 0.5	5	12.0 ± 1.6	−47	112	+	+
30		1.0 ± 0.4	6.2 ± 0.3	6	14.8 ± 0.3	−47	112	+	+
31		0.2 ± 0.1	13.7 ± 3.0	69	16.5 ± 1.3	−35	112	−	−
32		0.4 ± 0.1	10.4 ± 1.2	26	4.2 ± 0.4	−19	126	−	−

[a]Binding affinity determined by inhibition of [^{3}H]-CP-55940 binding to hCB1R or hCB2R-transfected HEK 293 membrane is expressed as K_i. [b]Functional activity determined by inhibition of Eu-GTP binding to hCB1R-transfected HEK 293 membrane is expressed as EC_{50}. [c]Data are expressed as the mean ± SEM of at least three independent experiments. [d]IP: intrinsic property for inverse agonist efficacy. [e]tPSA: topological polar surface area. [f]Tetrad model response (n = 4/group) (CP-55940 100%; "−": >90% (weakly or not reversed); "+": 50% < X ≤ 90%; "++": 10% < X ≤ 50%; "+++": ≤10% (strongly reversed)). Test compound (50 mg/kg) was orally dosed 1 h prior to ip treatment with agonist CP-55940 (1 mg/kg) in the CB1R agonist-induced hypothermia or analgesia model.

water (1:1) to give hydroxyl bromide **8** in good yield (76%). We also attempted to brominate both the thiazole ring and 4-methyl group of the precursor of bromide **6** in one step using the reaction conditions of Scheme 1. However, a complex mixture was formed, and product **7** was isolated in a yield much lower than that of the current two-step process. Under catalysis with aluminum chloride, compound **8** was reacted with 1-aminopiperidine efficiently to furnish the corresponding amide **9** in quantitative yield (97%). Amide **9** thus obtained was coupled with 1-ethynyl-4-(trifluoromethyl)benzene in the presence of $Pd(PPh_3)_2Cl_2$ and CuI as catalyst to give compound **10** in 87% yield, which in turn underwent bromination with PBr_3 to furnish bromide **11** in quantitative yield (98%). Compound **11** was sequentially subjected to S_N2 substitution (NaN_3) and Staudinger reduction (PPh_3/H_2O) to afford the corresponding primary amine **13** in 66% over two steps. Finally, amine **13** was reacted with pyrrolidine-1-sulfonyl chloride in the presence of triethylamine to accomplish the desired target **4** in 87% yield. Similarly, intermediate **13** was allowed to couple individually with different sulfonyl and sulfamoyl chlorides to give the corresponding sulfonamide and sulfamide derivatives **23**−**32** in moderate to good yields (45−89%). In addition, bromide **11** directly underwent S_N2 displacement with 3-hydroxyazetidine to afford target **12** in 68% yield. Thus, following the similar synthetic operation, intermediate **11** was coupled with selected primary and secondary amines, respectively, to afford another series of analogues **14**−**22** in moderate to good yields (56−88%). As evidenced by HPLC analysis in the Experimental Section, all test compounds exhibited more than 95% purity prior to in vitro assays and animal studies.

RESULTS AND DISCUSSION

It is widely accepted that installing an electronegative group could dramatically increase the tPSA value of a molecule. In general, compounds with a tPSA over 90 Å^2 are able to significantly lower the probability to penetrate BBB. However, prior to this strategy-oriented optimization, it is necessary to identify the synthetically tolerated position in the initial lead, thus allowing us to generate structurally diverse analogues without compromising the potency and activity toward the target of interest. Compound **3**, having a low brain-to-plasma partition (B/P = 1/33), was considered an ideal lead for seeking novel analogues with further enhancement in peripheral

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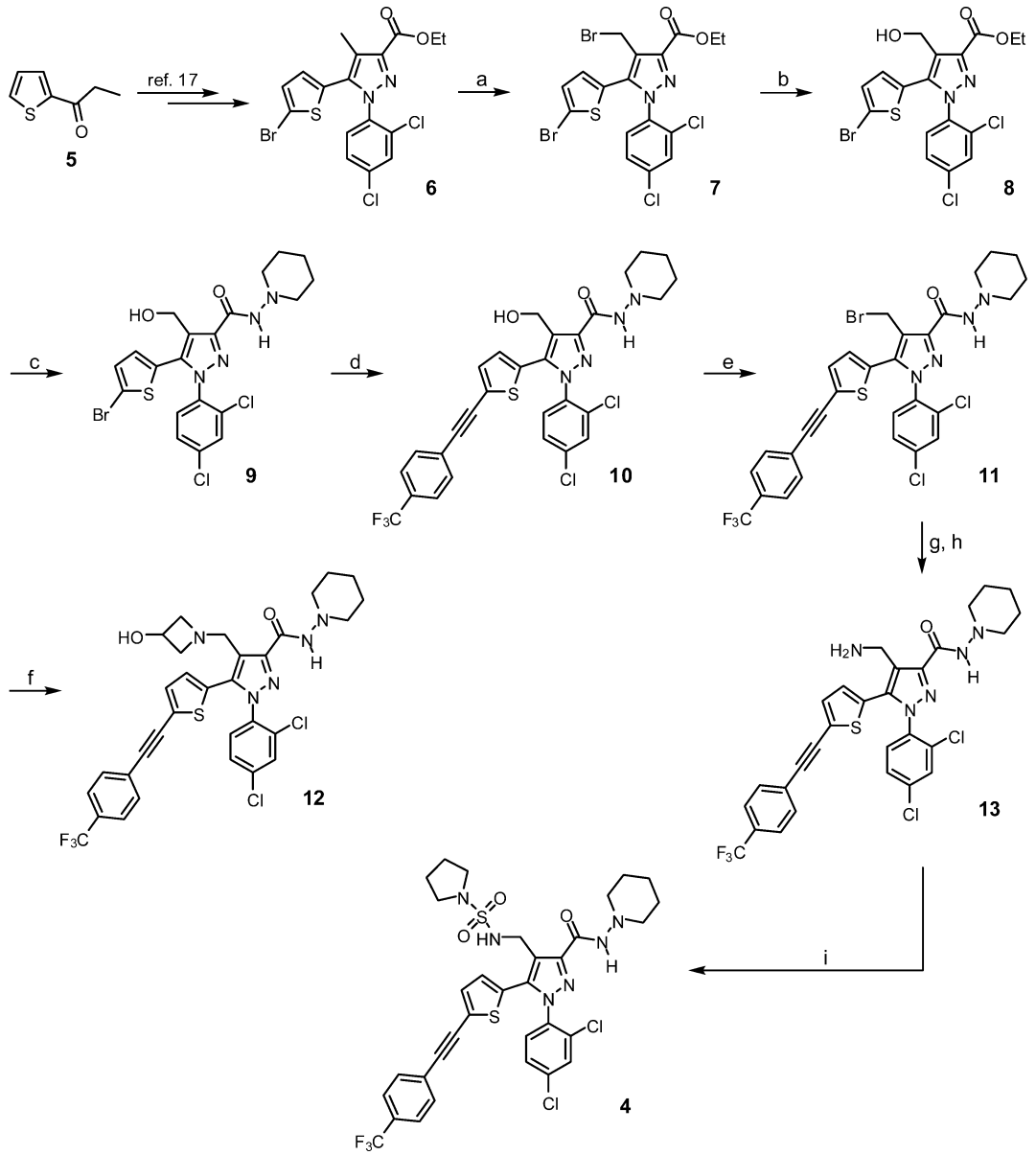
Scheme 1. Synthesis of Sulfamide and Sulfonamide Derivatives Using Compounds 4 and 12 as an Example[a]



[a]Reagents and conditions: (a) NBS, AIBN, CCl_4, 80 °C, 16 h, 92%; (b) $AgNO_3$, acetone/water = 1/1, 60 °C, 16 h, 76%; (c) $AlCl_3$, 1-aminopiperidine, DCE, rt, 16 h, 97%; (d) $PdCl_2(PPh_3)_2$, CuI, 1-ethynyl-4-(trifluoromethyl)benzene, 2-ethanolamine, and THF in a sealed pressure vessel, 80 °C, 12 h, 87%; (e) PBr_3, CH_2Cl_2, 0 °C to rt, 2 h; 98%; (f) 3-hydroxyazetidine hydrochloride, Et_3N, DMF, 60 °C, 3 h, 68%; (g) NaN_3, DMF, rt, 3 h; (h) PPh_3, THF/H_2O = 1/1, rt, 16 h, 66% over two steps; (i) pyrrolidine-1-sulfonyl chloride, Et_3N, DMF, 0 °C to rt, 16 h, 87%.

selectivity over brain. After extensive structural modifications on its C-3, C-4, and C-5 positions on the pyrazole ring, respectively, the C-4 alkyl group was found to be a well tolerated position for structural variation. As listed in Table 2, many bioisosteres with a tPSA value higher than that of parent hit **3** (52 Å^2) were obtained through appending a variety of heteroatom-containing moieties on the C-4 side chain.

Structurally, these potential peripheral-acting CB1R antagonists can be divided into two main groups as represented by sulfamide **4** (CB1R: K_i = 0.3 ± 0.1 nM; EC_{50} = 3.0 ± 0.2 nM; tPSA = 112 Å^2) and sulfonamide **26** (CB1R: K_i = 0.8 ± 0.1 nM; EC_{50} = 7.4 ± 2.5 nM; tPSA = 109 Å^2), respectively. Compounds **4**, **25**−**27**, **31**, and **32** showed a dual negative response on CB1R agonist-induced hypothermic and analgesic effects at a dose of 50 mg/kg, an in-house threshold to select compounds potentially with limited BBB penetration, and meanwhile their tPSA values were all greater than 90 Å^2. Compounds **28**−**30**, though possessing a high tPSA value (112−122 Å^2) and potent CB1R activity (K_i = 0.4−1.4 nM), were found to have positive effects on tetrad responses, presumably due to the fact that under physiological conditions, many other factors, including solubility, absorption, and metabolic issues, might dominate the final consequences. Compounds **12**, **15**, **17**, and **20**−**22** with tPSA values (54−79 Å^2) less than 90 Å^2 were also selected for testing. As expected, they all failed to give a dual negative response in both hypothermia and analgesia tests (Table 2). As such, compounds **14**, **16**, **18**, and **19** with tPSA falling in between 54 Å^2 and 78 Å^2 were not elected for further tetrad-response tests. Substitution of the conventional alkyl group with an electronegative moiety

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at C-4 position could dramatically increase the tPSA value, but this structural alteration somehow resulted in a loss of CB2/1 selectivity as demonstrated with compounds **24** and **28**−**30** (CB2/1 = 2−6) relative to parent hit **3** (CB2/1 = 167).[19] Intriguingly, though compounds **4** and **29**−**32** are structurally closely related analogues, there is no clear relationship linking their CB2/1 selectivity to the ring size at the C-4 side chain. Further structural design resulted in compound **32** with an excellent biological activity (CB1R: K_i = 0.4 ± 0.1 nM; EC_{50} = 4.2 ± 0.4 nM) and a high tPSA (126 Å^2). Apparently, inserting an extra oxygen in the terminal C-4 ring is responsible for such a substantial increase in tPSA as compared to the corresponding compounds **4**, **29**, **30**, and **31** (tPSA = 112 Å^2). However, compound **32** shows poorer water solubility (<0.005 ng/mL) than **4** (0.014 ng/mL) though it has an additional oxygen. In terms of intrinsic properties, all test compounds induced a significant decrease in Eu-GTP binding ranging from −14% to −51%, suggesting that they should behave as the CB1R inverse agonist as with parent **3** (Table 2).[19]

In light of the lowest B/P ratios observed in both normal and diet-induced obese (DIO) mice after 2 h acute treatment (Table 1), compound **4** was chosen for further in vivo studies. As illustrated in Figures 3 and 4, compound **4** did not reverse

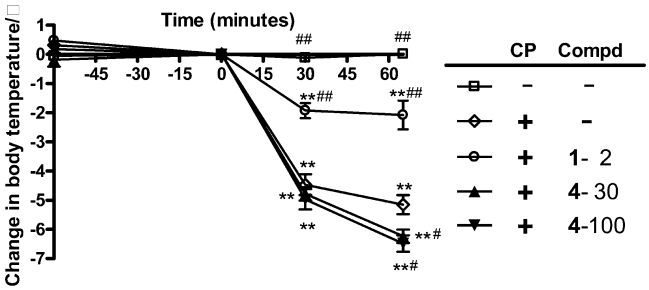

Figure 3. Compound **4** (30 and 100 mg/kg, po) and control **1** (2 mg/kg, po) were administrated, respectively, 1 h prior to the treatment with agonist CP-55940 (1 mg/kg, ip) in the CB1R agonist-induced hypothermia model. Body temperature was measured at a time point of 30 and 65 min, respectively, after agonist dosing. (P < 0.05, #vs CP-55940 group; P < 0.01, **vs vehicle control, ##vs CP-55940 group).

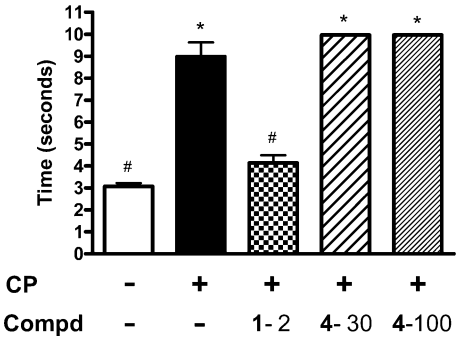

Figure 4. Compound **4** (30 and 100 mg/kg, po) and control **1** (2 mg/kg, po) were administrated, respectively, 1 h prior to the treatment with agonist CP-55940 (1 mg/kg, ip) in the CB1R agonist-induced analgesia model. Tail flick response was measured at a time point of 35 min after agonist dosing. (P < 0.01, *vs vehicle control, #vs CP-55940 group).

the tetrad responses at an oral dose up to 100 mg/kg, whereas the positive control **1**, a typical brain CB1R-acting inverse agonist, was found to cross the BBB easily and counteract significantly both temperature-lowering and analgesic effects at an oral dose as low as 2 mg/kg.

Compound **4** appears to be a promising peripherally restricted CB1R inverse agonist. A long-term treatment of compound **4** was then carried out to evaluate the weight-loss efficacy in DIO mice. As depicted in Figure 5A, reduction of

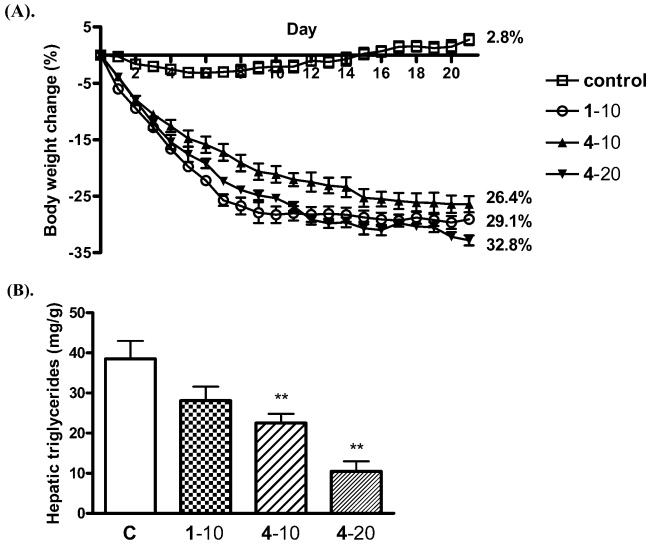

Figure 5. Efficacy in the DIO mouse model following oral administration of compound **4** (10 and 20 mg/kg, po qd) compared to that in ref 1 (10 mg/kg, po qd) in the 22-day chronic study (n = 6/group). (A) Body weight change was measured daily during compound treatment. Data are expressed as mean ± standard error. (B) The hepatic triglyceride level was measured after 22-day chronic treatment of compounds. Statistical significance is analyzed by one-way ANOVA followed by Dunnett's post test (**P < 0.01).

body weight occurred gradually and persistently in a dose-dependent manner throughout the 22-day period with a relative weight-loss rate of 26.4% and 32.8% for 10 and 20 mg/kg groups, respectively, indicating that this compound is as effective as its brain-acting counterpart **1** (10 mg/kg; 29.1% weight loss) in weight-reduction efficacy, again supporting that instead of brain CB1R, peripheral CB1R could be a potential target for treating obesity. In addition, a significant decrease in hepatic triglyceride level was observed in a dose-dependent manner after the above chronic treatment (Figure 5B), suggesting that compound **4** might have great potential to ameliorate this related metabolic syndrome.[37]

More encouragingly, after 22-day repeated dosing, only trace amounts of compound **4** were found in the mouse brain (4.3 ± 0.4 and 7.2 ± 0.3 ng/g for 10 and 20 mg/kg groups). These amounts were even lower than the amount detected in brain, 15.9 ± 3.2 ng/g, after 2 h acute treatment at an oral dose of 20 mg/kg, indicating that no severe drug accumulation occurred in the CNS (Table 3). In sharp contrast, the positive control **1** was detected at a high brain concentration of 125.6 ± 4.2 ng/g (vs 4.3 ± 0.4 ng/g of **4**) over the same period (22 days) at an oral dose of 10 mg/kg. However, whether such a low concentration in brain is owing to P-glycoprotein (P-gp) efflux needs to be addressed. The P-gp ATPase assay was then performed to clarify this issue and indicated that compound **4** was not a P-gp substrate, in that ATPase activity resulted in a

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Table 3. Determination of Brain Concentration of Compounds 1 and 4

compound[a]	dose (mg/kg)	brain (ng/g)
1	10	125.6 ± 4.2
4	10	4.3 ± 0.4[b]
4	20	7.2 ± 0.3[b]

[a]Compound 4 (10 and 20 mg/kg, po qd) compared to ref 1 (10 mg/kg, po qd) after chronic treatment for 22 days in DIO mice (n = 6/group). Data are presented as mean ± standard error; P < 0.01. [b]Versus ref 1.

significant decrease in the single-to-noise ratio from 3.34 (verapamil-treated, positive control) to 0.87.[38]

The title compound, 1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-4-((pyrrolidine-1-sulfonamido)methyl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (**4**), is currently under preclinical development for treating obesity and related metabolic syndrome in the model of type 2 diabetes. The 163 off-target standard assays were conducted, and the clean off-target profiles are displayed in Supporting Information.[39] Accordingly, besides $GABA_A$ (K_i = 2.99 μM), sodium channel, vanilloid, and angiotensin AT1 (50−57% inhibition at 10 μM), compound **4** showed low affinity (<50% inhibition at 10 μM) toward the other 159 off-targets. As well, no hERG liability was observed at a concentration up to 30 μM in the patch-clamp assay.

In conclusion, a combination of the calculated tPSA value and experimental B/P ratio appears to be a practical approach to modulate biopharmaceutical properties of a molecule, such as BBB permeability, in the development of peripherally acting derivatives. The incorporation of an electronegative fragment is usually an effective way to significantly increase the overall tPSA of a molecule and thereof might result in low BBB penetration. Nevertheless, an appropriate location must be identified in the lead molecule during synthetic tailoring to avoid compromising any fundamental elements, such as potency and activity toward the target of interest. Current results demonstrate that compound **4** is not a P-gp substrate and is considered a peripherally restricted CB1R inverse agonist as evidenced by its negative tetrad responses, low B/P partition, and trace amount in brain after chronic treatment.

EXPERIMENTAL SECTION

General. Unless otherwise stated, all materials used were commercially available and used as supplied. Reactions requiring anhydrous conditions were performed in flame-dried glassware and cooled under an argon or nitrogen atmosphere. Unless otherwise stated, reactions were carried out under argon or nitrogen and monitored by analytical thin layer chromatography performed on glass-backed plates (5 × 10 cm) precoated with silica gel 60 F_{254} as supplied by Merck. Visualization of the resulting chromatograms was performed by looking under an ultraviolet lamp (λ = 254 nm) followed by dipping in an ethanol solution of vanillin (5% w/v) containing sulfuric acid (3% v/v) or phosphomolybdic acid (2.5% w/v) and charring with a heat gun. Solvents for reactions were dried and distilled under an argon or nitrogen atmosphere prior to use as follows: THF, diethyl ether (ether), and DMF from a dark blue solution of sodium benzophenone ketyl; toluene, dichromethane, and pyridine from calcium hydride. Flash chromatography was used routinely for purification and separation of product mixtures using silica gel 60 of 230−400 mesh size as supplied by Merck. Eluent systems are given in volume/volume concentrations. Melting points were determined using an Electrothermal IA9000 digital melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-300 (300 MHz) and a Varian Mercury-400 (400 MHz). Chloroform-*d* or dimethyl sulfoxide-d_6 was used as the solvent and TMS (δ 0.00 ppm) as an internal standard. Chemical shift values are reported in ppm relative to the TMS in delta (δ) units. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), and m (multiplet). Coupling constants (J) are expressed in hertz. Electrospray mass spectra (ESMS) were recorded as m/z values using an Agilent 1100 MSD mass spectrometer. All test compounds displayed more than 95% purity as determined by a Hitachi 2000 series HPLC system using a phenyl column (Waters XBridge 5 μm, 4.6 mm × 150 mm). Mobile phase A: acetonitrile; mobile phase B: 10 mM ammonium acetate aqueous solution containing 0.1% formic acid. The gradient system started from A:B (10%:90%) to A:B (90%:10%) with a flow rate of 0.5 mL/min, and the injection volume was 5 μL. The system was operated at 25 °C. Peaks were detected at 254 nm. IUPAC nomenclature of compounds was determined with ACD/Name Pro software.

1-(2,4-Dichlorophenyl)-*N*-(piperidin-1-yl)-4-((pyrrolidine-1-sulfonamido)methyl)-5-(5-((4-(trifluoromethyl)phenyl)-ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (4). To a magnetically stirred solution of **13** (1.15 g, 1.86 mmol) and Et_3N (0.38 g, 3.72 mmol) in DMF (10 mL) at 0 °C was added pyrrolidine-1-sulfonyl chloride (0.47 g, 2.79 mmol) dropwise. The resulting mixture was allowed to warm to room temperature for 16 h and then quenched with water. The aqueous phase was extracted with ethyl acetate (2 × 20 mL), and the combined organic extracts were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to give the crude residue, which was purified by flash chromatography with *n*-hexane/ethyl acetate (1:1) as an eluting solvent to afford the desired product **4** (1.21 g, 87%) as a white solid: mp 163.5−164.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.71 (br s, 1H), 7.60−7.58 (m, 4H), 7.53 (d, J = 2.0 Hz, 1H), 7.38 (dd, J = 8.4 and 2.0 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.23 (d, J = 3.6 Hz, 1H), 7.22 (d, J = 3.6 Hz, 1H), 6.50 (t, J = 6.8 Hz, 1H), 4.34 (d, J = 6.8 Hz, 2H), 3.28−3.25 (m, 4H), 2.87−2.81 (m, 4H), 1.87−1.85 (m, 4H), 1.79−1.77 (m, 4H), 1.48−1.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.2, 144.3, 137.4, 136.7, 134.8, 133.3, 132.7, 131.3, 130.6, 130.2, 130.1, 130.0 (q, J_{C-F} = 33.0 Hz), 128.5, 127.9, 125.9, 125.6, 125.0 (q, J_{C-F} = 4.0 Hz), 123.5 (q, J_{C-F} = 270.5 Hz), 119.7, 93.4, 83.7, 56.9, 47.7, 37.2, 25.3, 25.0, 22.9; ESMS m/z: 751.0 (M + 1); HPLC purity = 99.21%, t_R = 46.15 min.

Ethyl 4-(Bromomethyl)-5-(5-bromothiophene-2-yl)-1-(2,4-dichlorophenyl)-1*H*-pyrazole-3-carboxylate (7). To a magnetically stirred solution of bromo ester **6** (2.20 g, 4.78 mmol) in CCl_4 (22 mL) were sequentially added NBS (1.1 g, 6.21 mmol) and AIBN (0.05 g, 0.33 mmol) in one portion. The resulting mixture was heated under reflux for 16 h and then cooled to room temperature. The reaction was quenched with saturated aqueous sodium thiosulfate and extracted with CH_2Cl_2 (2 × 40 mL). The organic layers were combined, washed with water, brine, dried over Na_2SO_4, filtered, and concentrated to give the crude product, which was passed through a layer of silica gel in a sintered-glass funnel and washed with CH_2Cl_2. The filtrate was concentrated to give dibromide **7** (2.52 g, 92%) as a pale yellow solid: mp 128.1−129.7 °C; ^{1}H NMR ($CDCl_3$) δ 7.48 (d, J = 2.0 Hz, 1H), 7.38−7.32 (m, 2H), 7.01 (d, J = 3.6 Hz, 1H), 6.99 (d, J = 3.6 Hz, 1H), 4.77 (s, 2H), 4.48 (q, J = 7.2 Hz, 2H), 1.43 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.5, 142.2, 138.4, 137.1, 135.0, 133.8, 130.8, 130.5, 130.3, 130.2, 128.2, 128.0, 120.6, 116.4, 61.6, 22.4, 14.3; ESMS m/z: 540.8 (M + 1).

Ethyl 5-(5-Bromothiophene-2-yl)-1-(2,4-dichlorophenyl)-4-(hydroxymethyl)-1*H*-pyrazole-3-carboxylate (8). To a vigorously stirred solution of silver nitrate (3.25 g, 19.12 mmol) in 100 mL of aqueous acetone (1:1) was added crude compound **7** (2.52 g) in one portion. The resulting mixture was heated at 60 °C for 16 h and then cooled to room temperature. The suspension solution was filtered, and the filtrate was concentrated under reduced pressure to remove acetone. The residue was extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a crude residue, which was purified

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by flash chromatography eluting with *n*-hexane/ethyl acetate (2:1) to afford hydroxy ester **8** (1.67 g, 76%) as a white solid: mp 99.2−100.4 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (d, J = 1.2 Hz, 1H), 7.33−7.32 (m, 2H), 6.96 (d, J = 3.6 Hz, 1H), 6.76 (d, J = 3.6 Hz, 1H), 4.71 (d, J = 7.2 Hz, 2H), 4.48 (q, J = 7.2 Hz, 2H), 3.76 (t, J = 7.2 Hz, 1H), 1.42 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 163.3, 142.9, 137.3, 136.8, 135.0, 133.7, 130.7, 130.4, 130.3, 130.1, 128.4, 127.8, 124.2, 115.8, 61.9, 54.5, 14.2; ESMS m/z: 498.9 (M + 23).

5-(5-Bromothiophene-2-yl)-1-(2,4-dichlorophenyl)-4-(hydroxymethyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (9). To a mixture of hydroxy ester **8** (4.40 g, 9.24 mmol) and aluminum trichloride (2.46 g, 18.48 mmol) in dichloroethane (88 mL) was added 1-aminopiperidine (3.70 g, 36.96 mmol) slowly at 0 °C under an atmosphere of argon. The resulting mixture was allowed to warm to room temperature, was stirred overnight, and then was quenched with ice−water. The aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic extracts were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a crude residue, which was purified by flash chromatography with *n*-hexane/ethyl acetate (1:1) as eluant to afford compound **9** (4.75 g, 97%) as a white solid: mp 113.5−115.2 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.35 (dd, J = 8.4, 2.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.95 (d, J = 4.0 Hz, 1H), 6.69 (d, J = 4.0 Hz, 1H), 5.17 (t, J = 7.2 Hz, 1H), 4.67 (d, J = 7.2 Hz, 2H), 2.83 (m, 4H), 1.78−1.73 (m, 4H), 1.43−1.41 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.0, 144.7, 136.9, 136.6, 135.0, 133.6, 130.7, 130.4, 130.3, 130.2, 128.6, 128.1, 124.0, 115.8, 57.1, 54.7, 25.3, 23.2; ESMS m/z: 529.1 (M + 1).

1-(2,4-Dichlorophenyl)-5-(5-(2-(4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-4-(hydroxymethyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (10). A mixture of bromothiophene **9** (1.20 g, 2.26 mmol), $PdCl_2(PPh_3)_2$ (0.16 g, 0.23 mmol), CuI (0.06 g, 0.28 mmol), and 2-ethanolamine (0.5 M(aq), 14 mL, 6.78 mmol) in THF (50 mL) was stirred in a pressure vessel and degassed with argon for 10 min, at which time 1-ethynyl-4-(trifluoromethyl)benzene (0.58 g, 3.39 mmol) was added in one portion. The resulting mixture was heated at 80 °C in an oil bath for 12 h. After cooling to room temperature, the reaction mixture was poured into water (20 mL) and the aqueous layer was extracted with ethyl acetate (2 × 40 mL). The combined organic extracts were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a crude residue, which was purified by flash chromatography with *n*-hexane/ethyl acetate (1:1) to afford compound **10** (1.22 g, 87%) as a white solid: mp 154.3−155.6 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (br s, 1H), 7.58−7.56 (m, 4H), 7.50 (d, J = 2.0 Hz, 1H), 7.36 (dd, J = 8.4, 2.0 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 4.0 Hz, 1H), 6.84 (d, J = 4.0 Hz, 1H), 5.21 (t, J = 7.2 Hz, 1H), 4.73 (d, J = 7.2 Hz, 2H), 2.85−2.81 (m, 4H), 1.77−1.75 (m, 4H), 1.44−1.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.0, 144.8, 136.8, 136.6, 135.1, 133.6, 132.6, 131.5, 130.7, 130.3, 130.2 (q, J_{C-F} = 32.6 Hz), 129.7, 129.1, 128.0, 126.1, 125.7, 125.3 (q, J_{C-F} = 3.6 Hz), 124.0, 123.7 (q, J_{C-F} = 270.9 Hz), 93.6, 83.7, 57.1, 54.8, 25.3, 23.1; ESMS m/z: 619.1 (M + 1).

4-(Bromomethyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (11). To a magnetically stirred solution of compound **10** (0.30 g, 0.48 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added PBr_3 (0.26 g, 0.92 mmol) dropwise. The resulting mixture was warmed to room temperature for 2 h and then quenched with ice−water. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give the crude residue, which was purified with flash chromatography with *n*-hexane/ethyl acetate (4:1) to give bromide **11** (0.32 g, 98%) as a pale yellow solid: mp 207.3−208.6 °C ; ^{1}H NMR ($CDCl_3$) δ 7.59−7.57 (m, 4H), 7.52 (m, 1H), 7.38−7.37 (m, 2H), 7.23 (d, J = 4.0 Hz, 1H), 7.17 (d, J = 4.0 Hz, 1H), 4.92 (s, 2H), 2.86−2.84 (m, 4H), 1.77−1.71 (m, 4H), 1.43−1.41 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.5, 143.5, 138.4, 137.0, 134.9, 133.6, 132.8, 131.5, 130.7, 130.4, 130.3, 130.2 (q, J_{C-F} = 37.0 Hz), 129.4, 128.8, 128.1, 126.0, 125.8, 125.2 (q, J_{C-F} = 3.7 Hz), 123.5 (q, J_{C-F} = 270.2 Hz), 119.9, 93.8, 83.7, 56.9, 25.3, 23.1, 22.4; ESMS m/z: 681.1 (M + 1).

1-(2,4-Dichlorophenyl)-4-((3-hydroxyazetidin-1-yl)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (12). To a magnetically stirred solution of bromide **11** (0.33 g, 0.48 mmol) in DMF (3 mL) at room temperature were sequentially added 3-hydroxyazetidine hydrochloride (0.08 g, 0.72 mmol) and diisopropylethylamine (0.12 g, 0.92 mmol) in one portion. The resulting mixture was heated to 60 °C for 16 h, and then cooled to room temperature and quenched with water. The aqueous phase was extracted with ethyl acetate (2 × 10 mL). The combined organic extracts were washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a crude residue, which was subjected to purification by flash chromatography on silica gel with *n*-hexane/ethyl acetate (1:1) to afford the desired product **12** (0.22 g, 68%) as a white solid: mp 174.0−175.3 °C; ^{1}H NMR ($CDCl_3$) δ 9.38 (br s, 1H), 7.59−7.57 (m, 4H), 7.46 (d, J = 2.4 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.4 and 2.4 Hz, 1H), 7.18 (d, J = 4.0 Hz, 1H), 7.04 (d, J = 4.0 Hz, 1H), 4.41 (br s, 1H), 3.83 (s, 2H), 2.86−2.82 (m, 4H), 1.77−1.73 (m, 4H), 1.43−1.41 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.1, 146.1, 138.0, 136.5, 135.4, 133.6, 132.7, 131.6, 130.8, 130.3 (q, J_{C-F} = 32.2 Hz), 130.2, 130.1, 129.5, 127.9, 126.1, 125.6, 125.3 (q, J_{C-F} = 3.6 Hz), 123.7 (q, J_{C-F} = 270.8 Hz), 117.1, 93.6, 83.8, 62.9, 62.0, 57.2, 50.5, 25.2, 23.3; ESMS m/z: 674.1 (M + 1); HPLC purity = 97.78%, t_R = 37.81 min.

4-(Aminomethyl)-1-(2,4-dichlorophenyl)-5-(5-(2-(4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (13). To a magnetically stirred solution of compound **11** (1.1 g, 1.62 mmol) in DMF (10 mL) was added NaN_3 (0.53 g, 8.08 mmol) at room temperature for 3 h. The reaction mixture was poured into water (10 mL), and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with water and brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to yield the corresponding azide. This azido compound, without purification, was further treated with PPh_3 (0.51 g, 1.94 mmol) in THF/H_2O (1/1) (20 mL) at room temperature for 16 h to afford compound **13** (0.66 g, 66% over two steps) as a pale yellow solid after chromatographic purification (CH_2Cl_2:MeOH = 9:1): ^{1}H NMR ($CDCl_3$) δ 7.85 (br s, 1H), 7.58−7.56 (m, 4H), 7.51 (d, J = 1.8 Hz, 1H), 7.38−7.32 (m, 2H), 7.19 (d, J = 3.9 Hz, 1H), 6.91 (d, J = 3.9 Hz, 1H), 3.99 (s, 2H), 2.87−2.79 (m, 4H), 2.01 (s, 1H), 1.74−1.76 (m, 4H), 1.49−1.38 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.2, 144.6, 136.9, 136.6, 135.2, 133.5, 132.7, 131.5, 130.7, 130.2 (q, J_{C-F} = 32.4 Hz), 130.1, 129.5, 129.4, 127.9, 126.0, 125.4, 125.2 (q, J_{C-F} = 4.0 Hz), 124.1, 123.7 (q, J_{C-F} = 270.4 Hz), 93.5, 83.8, 57.0, 35.4, 25.2, 23.2; ESMS m/z: 618.0 (M + 1).

1-(2,4-Dichlorophenyl)-4-((3-fluoroazetidin-1-yl)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (14). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.10 g, 0.15 mmol), 3-fluoroazetidine hydrochloride (0.03 g, 0.23 mmol), and diisopropylethylamine (0.05 mL, 0.3 mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **14** (82 mg, 83%) as a white solid: mp 186.2−187.9 °C; ^{1}H NMR ($CDCl_3$) δ 8.83 (br s, 1H), 7.61−7.58 (m, 4H), 7.48 (d, J = 2.1 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.35 (dd, J = 8.4, 2.1 Hz, 1H), 7.19 (d, J = 3.9 Hz, 1H), 7.07 (d, J = 3.9 Hz, 1H), 5.10 (dtt, J = 57.3, 5.1, 4.8 Hz, 1H), 3.89 (s, 2H), 3.65 (m, 2H), 3.44−3.32 (m, 2H), 2.86−2.84 (m, 4H), 1.84−1.71 (m, 4H), 1.46−1.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.9, 145.5, 138.3, 136.5, 135.3, 133.6, 132.6, 131.5, 130.7, 130.2 (q, J_{C-F} = 34.8 Hz), 130.1, 130.0, 129.6, 127.9, 126.0, 125.4, 125.2 (q, J_{C-F} = 4.0 Hz), 123.6 (q, J_{C-F} = 270.8 Hz), 117.6, 93.5, 83.8, 82.3 (d, J_{C-F} = 203.8 Hz), 60.2 (d, J_{C-F} = 20.9 Hz), 57.1, 50.1, 25.1, 23.2; ES-MS (M + 1): 676.1; HPLC purity = 96.76%, t_R = 40.11 min.

1-(2,4-Dichlorophenyl)-4-((oxetan-3-ylamino)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (15). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.15 g, 0.22 mmol) and oxetan-3-amine (0.02 g, 0.33

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mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **15** (113 mg, 76%) as a white solid: mp 115.2−116.3 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.63−7.61 (m, 4H), 7.52 (d, J = 2.4 Hz, 1H), 7.36 (dd, J = 8.4 and 2.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.20 (d, J = 4.0 Hz, 1H), 6.91 (d, J = 4.0 Hz, 1H), 4.69 (t, J = 6.8 Hz, 2H), 4.49 (t, J = 6.8 Hz, 2H), 4.00 (quintet, J = 6.8 Hz, 1H), 3.86 (s, 2H), 2.85−2.83 (m, 4H), 1.81−1.76 (m, 4H), 1.68−1.59 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.1, 144.6, 137.5, 136.7, 135.1, 133.5, 132.6, 131.5, 130.6, 130.3, 130.2 (q, J_{C-F} = 32.0 Hz), 129.6, 129.3, 128.0, 126.0, 125.6, 125.3 (q, J_{C-F} = 4.0 Hz), 123.7 (q, J_{C-F} = 272.0 Hz), 121.9, 93.7, 83.7, 79.5, 57.2, 53.4, 40.6, 25.2, 23.9; ES-MS (M + 1): 674.1; HPLC purity = 97.84%, t_R = 38.15 min.

1-(2,4-Dichlorophenyl)-4-((oxetan-3-ylmethylamino)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (16). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.12 g, 0.18 mmol) and (oxetan-3-yl)methanamine (0.03 g, 0.27 mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **16** (90 mg, 73%) as a white solid: mp 96.3−97.9 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (br s, 1H), 7.59−7.57 (m, 4H), 7.50 (d, J = 2.0 Hz, 1H), 7.37−7.31 (m, 2H), 7.19 (d, J = 4.0 Hz, 1H), 6.94 (d, J = 4.0 Hz, 1H), 4.79 (dd, J = 7.6, 6.4 Hz, 2H), 4.41 (dd, J = 6.4, 6.0 Hz, 2H), 3.87 (s, 2H), 3.12 (m, 1H), 2.97 (d, J = 7.6 Hz, 2H), 2.82−2.80 (m, 4H), 1.77−1.72 (m, 4H), 1.41−1.39 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.2, 144.8, 137.9, 136.7, 135.2, 133.6, 132.7, 131.6, 130.6, 130.3, 130.2 (q, J_{C-F} = 32.6 Hz), 129.6, 129.5, 128.0, 126.0, 125.6, 125.3 (q, J_{C-F} = 4.0 Hz), 123.7 (q, J_{C-F} = 270.9 Hz), 121.4, 93.6, 83.8, 76.1, 57.2, 52.6, 42.9, 35.1, 25.3, 23.2; ES-MS (M + 1): 688.1; HPLC purity = 97.23%, t_R = 37.94 min.

4-((Cyclopropylamino)methyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (17). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.16 g, 0.23 mmol) and cyclopropanamine (0.05 g, 0.92 mmol) in THF (3 mL) was stirred at room temperature for 16 h to afford product **17** (132 mg, 84%) as a pale yellow solid: mp 85.5−87.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.59−7.56 (m, 5H), 7.48 (d, J = 1.6 Hz, 1H), 7.36 (dd, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.4, 1.6 Hz, 1H), 7.18 (d, J = 4.0 Hz, 1H), 7.01 (d, J = 4.0 Hz, 1H), 3.94 (s, 2H), 2.83−2.80 (m, 4H), 2.32−2.29 (m, 1H), 1.75−1.72 (m, 4H), 1.47−1.37 (m, 2H), 0.78−0.48 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 159.5, 144.5, 138.3, 136.7, 135.0, 133.4, 132.7, 131.5, 130.7, 130.2 (q, J_{C-F} = 32.6 Hz), 130.1 (q, J_{C-F} = 4.0 Hz), 128.8, 127.9, 126.0, 125.7, 125.2, 123.6 (q, J_{C-F} = 272.0 Hz), 119.1, 93.5, 83.7, 57.0, 42.0, 29.7, 25.1, 23.0, 5.1; ES-MS (M + 1): 658.1; HPLC purity = 99.29%, t_R = 40.71 min.

1-(2,4-Dichlorophenyl)-4-((isopropylamino)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (18). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.27 g, 0.40 mmol) and propan-2-amine (0.12 g, 2.0 mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **18** (189 mg, 72%) as a pale yellow solid: mp 110.9−112.2 °C; ^{1}H NMR ($CDCl_3$) δ 8.15 (br s, 1H), 7.59−7.57 (m, 4H), 7.49 (d, J = 2.0 Hz, 1H), 7.34 (dd, J = 8.4, 2.0 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 3.6 Hz, 1H), 6.99 (d, J = 3.6 Hz, 1H), 3.88 (s, 2H), 2.90−2.78 (m, 5H), 1.77−1.73 (m, 4H), 1.45−1.39 (m, 2H), 1.10 (d, J = 6.0 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 159.2, 145.1, 137.6, 136.6, 135.4, 133.7, 132.7, 131.6, 131.5, 130.8, 130.3 (q, J_{C-F} = 32.2 Hz), 130.2, 129.7, 128.0, 126.1, 125.4, 125.3 (q, J_{C-F} = 3.6 Hz), 123.7 (q, J_{C-F} = 270.9 Hz), 121.4, 93.5, 83.9, 57.3, 48.4, 40.4, 29.6, 25.3, 22.6; ES-MS (M + 1): 660.1; HPLC purity = 97.14%, t_R = 40.22 min.

1-(2,4-Dichlorophenyl)-4-((6,6-difluoro-3-azabicyclo[3.1.0]hexan-3-yl)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (19). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.10 g, 0.15 mmol), 6,6-difluoro-3-azabicyclo[3.1.0]hexane hydrochloride (0.03 g, 0.23 mmol), and diisopropylethylamine (0.05 mL, 0.3 mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **19** (88 mg, 83%) as a white solid: mp 130.0−131.2 °C; ^{1}H NMR ($CDCl_3$) δ 8.78 (br s, 1H), 7.58−7.56 (m, 4H), 7.46 (d, J = 2.0 Hz, 1H), 7.40−7.32 (m, 2H), 7.18 (d, J = 4.0 Hz, 1H), 7.11 (d, J = 4.0 Hz, 1H), 3.78 (s, 2H), 3.12 (d, J = 10.0 Hz, 2H), 2.93−2.83 (m, 6H), 2.13 (d, J = 12.8 Hz, 2H), 1.76−1.73 (m, 4H), 1.43−1.41 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.1, 145.7, 138.3, 136.6, 135.4, 133.7, 132.7, 131.5, 130.8, 130.5, 130.2 (q, J_{C-F} = 32.6 Hz), 130.1, 129.7, 127.9, 126.1, 125.4, 125.3 (q, J_{C-F} = 3.7 Hz), 123.7 (q, J_{C-F} = 270.5 Hz), 118.5, 115.4 (dd, J_{C-F} = 270.5 and 302.4 Hz), 93.5, 83.9, 56.6, 51.4, 46.7, 26.9 (t, J_{C-F} = 12.4 Hz), 25.3, 23.2; ES-MS (M + 1): 720.2; HPLC purity = 98.35%, t_R = 43.21 min.

1-(2,4-Dichlorophenyl)-4-((3,3-difluoropyrrolidin-1-yl)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (20). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.10 g, 0.15 mmol), 3,3-difluoropyrrolidine hydrochloride (0.03 g, 0.23 mmol), and diisopropylethylamine (0.05 mL, 0.3 mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **20** (89 mg, 86%) as a white solid: mp 75.5−76.9 °C; ^{1}H NMR ($CDCl_3$) δ 8.55 (br s, 1H), 7.59−7.57 (m, 4H), 7.48 (d, J = 2.4 Hz, 1H), 7.41−7.34 (m, 2H), 7.18 (d, J = 4.0 Hz, 1H), 7.07 (d, J = 4.0 Hz, 1H), 3.86 (s, 2H), 3.01 (t, J = 12.8 Hz, 2H), 2.86−2.78 (m, 6H), 2.27 (tt, J = 14.4 and 7.2 Hz, 2H), 1.77−1.71 (m, 4H), 1.46−1.38 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.0, 145.4, 138.7, 136.7, 135.4, 133.6, 132.6, 131.5, 130.7, 130.3 (q, J_{C-F} = 32.6 Hz), 130.2, 130.1, 129.7, 129.6 (t, J_{C-F} = 247.5 Hz), 128.0, 126.1, 125.6, 125.3 (q, J_{C-F} = 4.1 Hz), 123.7 (q, J_{C-F} = 270.8 Hz), 118.0, 93.6, 83.9, 60.6 (t, J_{C-F} = 28.9 Hz), 57.1, 50.8, 46.7, 35.8 (t, J_{C-F} = 24.6 Hz), 25.3, 23.2; ES-MS (M + 1): 708.1; HPLC purity = 97.39%, t_R = 45.04 min.

1-(2,4-Dichlorophenyl)-4-(((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (21). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.16 g, 0.24 mmol) and ((*S*)-pyrrolidin-2-yl)methanol (0.12 g, 1.2 mmol) in DMF (3 mL) was heated at 60 °C for 16 h to afford product **21** (95 mg, 56%) as a pale yellow solid: mp 125.8−126.0 °C; ^{1}H NMR ($CDCl_3$) δ 9.96 (br s, 1H), 7.60−7.55 (m, 4H), 7.45 (d, J = 2.0 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.32 (dd, J = 8.4, 2.1 Hz, 1H), 7.16 (d, J = 4.0 Hz, 1H), 6.84 (d, J = 4.0 Hz, 1H), 4.18 (d, J = 12.8 Hz, 1H), 3.74 (dd, J = 11.6, 2.4 Hz, 1H), 3.55 (d, J = 12.8 Hz, 1H), 3.40 (dd, J = 11.6, 2.4 Hz, 1H), 3.12−3.03 (m, 4H), 2.86 (m, 1H), 2.70 (m, 1H), 2.22 (m, 1H), 1.89−1.83 (m, 2H), 1.75−1.60 (m, 6H), 1.43−1.41 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 160.0, 145.8, 137.7, 136.5, 135.4, 133.6, 132.5, 131.5, 130.7, 130.3 (q, J_{C-F} = 32.6 Hz), 130.1, 129.9, 129.7, 127.9, 126.1, 125.6, 125.3 (q, J_{C-F} = 3.7 Hz), 123.7 (q, J_{C-F} = 270.9 Hz), 119.7, 93.6, 83.8, 65.3, 62.1, 55.8, 54.0, 46.9, 27.1, 25.7, 23.6, 23.1; ES-MS (M + 1): 702.1; HPLC purity = 95.00%, t_R = 39.70 min.

4-((1*H*-1,2,4-Triazol-1-yl)methyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (22). Following the similar coupling-reaction and workup procedures for **12**, a mixture of bromide **11** (0.16 g, 0.24 mmol) and 1*H*-1,2,4-triazole, sodium salt (0.11 g, 1.2 mmol) in DMF (5 mL) was heated at 60 °C for 16 h to afford product **21** (142 mg, 88%) as a pale yellow solid: mp 218.0−218.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.68 (s, 1H), 7.91 (s, 1H), 7.74 (d, J = 4.0 Hz, 1H), 7.61 (s, 1H), 7.58 (m, 4H), 7.83 (d, J = 2.0 Hz, 1H), 7.37 (dd, J = 8.4 and 2.0 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 4.0 Hz, 1H), 5.59 (s, 2H), 2.81−2.79 (m, 4H), 1.77−1.72 (m, 4H), 1.43−1.41 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.8, 151.3, 144.7, 143.8, 139.8, 137.1, 134.9, 133.7, 132.9, 131.6, 131.4, 130.6, 130.4, 130.3 (q, J_{C-F} = 32.2 Hz), 128.2, 128.1, 126.4, 126.0, 125.3 (q, J_{C-F} = 4.0 Hz), 123.7 (q, J_{C-F} = 270.8 Hz), 116.8, 93.9, 83.7, 57.3, 42.7, 25.1, 23.1; ES-MS (M + 1): 670.1; HPLC purity = 98.27%, t_R = 50.79 min.

1-(2,4-Dichlorophenyl)-4-(methylsulfonamidomethyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (23). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.10 g, 0.16 mmol), triethylamine (0.04 mL, 0.24 mmol), and methanesulfonyl chloride (0.02 mL, 0.24 mmol) in CH_2Cl_2 (3 mL) at 0 °C was allowed to react for 1 h, leading to product **23** (95 mg, 84%) as a white solid: mp 110.2−111.9 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (br s, 1H), 7.59−7.57 (m, 4H), 7.54 (d, J = 2.0 Hz, 1H), 7.38 (dd,

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J = 8.4 and 2.0 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.25 (d, J = 3.9 Hz, 1H), 7.20 (d, J = 3.9 Hz, 1H), 6.49 (t, J = 6.3 Hz, 1H), 4.41 (d, J = 6.3 Hz, 2H), 2.94 (s, 3H), 2.84 (m, 4H), 1.78−1.75 (m, 4H), 1.45−1.43 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.5, 144.1, 138.0, 137.1, 134.9, 133.6, 133.0, 131.6, 130.7, 130.5, 130.4, 130.3 (q, J_{C-F} = 32.5 Hz), 128.1, 128.2, 126.2, 126.0, 125.3 (q, J_{C-F} = 3.4 Hz), 123.7 (q, J_{C-F} = 270.4 Hz), 119.1, 93.8, 83.7, 57.1, 40.3, 37.5, 25.1, 22.9; ES-MS (M + 1): 696.0; HPLC purity = 98.84%, t_R = 43.93 min.

1-(2,4-Dichlorophenyl)-4-((1-methylethylsulfonamido)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (24). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.10 g, 0.16 mmol), triethylamine (0.04 mL, 0.24 mmol), and propane-2-sulfonyl chloride (0.04 g, 0.24 mmol) in CH_2Cl_2 (3 mL) at 0 °C was allowed to react for 1 h, leading to product **24** (60 mg, 52%) as a white solid: mp 105.2−106.9 °C; ^{1}H NMR ($CDCl_3$) δ 7.59−7.57 (m, 4H), 7.52 (d, J = 2.0 Hz, 1H), 7.39 (dd, J = 8.4 and 2.0 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.24−7.21 (m, 2H), 6.33 (br s, 1H), 4.41 (s, 2H), 3.20−3.01 (m, 5H), 1.94−1.88 (m, 4H), 1.59−1.44 (m, 2H), 1.36 (d, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 159.7, 143.8, 137.7, 137.1, 134.9, 133.6, 133.0, 131.6, 130.8, 130.6, 130.4 (q, J_{C-F} = 32.6 Hz), 130.3, 128.3, 128.2, 126.2, 126.1, 125.3 (q, J_{C-F} = 4.0 Hz), 123.7 (q, J_{C-F} = 270.4 Hz), 119.9, 93.8, 83.8, 56.9, 53.5, 37.4, 24.8, 22.6, 17.2; ES-MS (M + 1): 724.1; HPLC purity = 98.63%, t_R = 45.66 min.

4-(Cyclopropanesulfonamidomethyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (25). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.07 g, 0.11 mmol), triethylamine (0.03 mL, 0.23 mmol), and cyclopropanesulfonyl chloride (0.03 g, 0.17 mmol) in CH_2Cl_2 (3 mL) at −30 °C was allowed to react for 1 h, leading to product **25** (58 mg, 71%) as a white solid: mp 105.5−106.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.60−7.58 (m, 4H), 7.53 (d, J = 1.5 Hz, 1H), 7.39 (dd, J = 8.4 and 1.5 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.26−7.24 (m, 2H), 6.58 (t, J = 6.6 Hz, 1H), 4.45 (d, J = 6.6 Hz, 2H), 2.86−2.84 (m, 4H), 2.35 (m, 1H), 1.81−1.75 (m, 4H), 1.48−1.46 (m, 2H), 1.15 (m, 2H), 0.91 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.3, 144.5, 138.5, 137.7, 134.9, 133.6, 133.0, 131.6, 130.7, 130.4, 130.3, 130.2 (q, J_{C-F} = 33.0 Hz), 128.4, 128.1, 126.1, 125.5, 125.3 (q, J_{C-F} = 3.5 Hz), 123.7 (q, J_{C-F} = 272.0 Hz), 119.7, 93.7, 83.8, 57.2, 37.6, 30.2, 25.2, 22.6, 5.3; ES-MS (M + 1): 722.1; HPLC purity = 98.54%, t_R = 46.01 min.

4-(Cyclopentanesulfonamidomethyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (26). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.09 g, 0.15 mmol), triethylamine (0.04 mL, 0.30 mmol), and cyclopentanesulfonyl chloride (0.04 g, 0.23 mmol)in CH_2Cl_2 (3 mL) at −30 °C was allowed to react for 1 h, leading to product **26** (86 mg, 76%) as a white solid: mp 111.5−112.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (br s, 1H), 7.63−7.61 (m, 4H), 7.53 (d, J = 1.8 Hz, 1H), 7.40 (dd, J = 8.4 and 1.8 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.30−7.23 (m, 2H), 6.50 (t, J = 6.6 Hz, 1H), 4.42 (d, J = 6.6 Hz, 2H), 3.37 (quintet, J = 7.5 Hz, 1H), 2.85−2.83 (m, 4H), 2.01−1.99 (m, 4H), 1.80−1.76 (m, 6H), 1.58−1.54 (m, 2H), 1.47−1.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.4, 144.5, 137.6, 137.0, 135.0, 133.6, 133.1, 131.6, 130.7, 130.6, 130.5 (q, J_{C-F} = 32.0 Hz), 130.4, 128.4, 128.2, 126.1, 126.0, 125.3 (q, J_{C-F} = 4.6 Hz), 123.8 (q, J_{C-F} = 272.0 Hz), 120.0, 93.7, 83.7, 62.0, 57.3, 37.4, 28.0, 25.9, 25.2, 23.2; ES-MS (M + 1): 750.1; HPLC purity = 97.72%, t_R = 47.70 min.

1-(2,4-Dichlorophenyl)-4-((*N*,*N*-dimethylsulfamoylamino)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (27). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.10 g, 0.16 mmol), triethylamine (0.04 mL, 0.32 mmol), and dimethylsulfamoyl chloride (0.03 mL, 0.24 mmol) in DMF (3 mL) at room temperature was allowed to react for 16 h, leading to product **27** (90 mg, 78%) as a white solid: mp 104.6−105.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.99 (br s, 1H), 7.63−7.61 (m, 4H), 7.50 (m, 1H), 7.40−7.32 (m, 2H), 7.22 (d, J = 3.9 Hz, 1H), 7.16 (d, J = 3.9 Hz, 1H), 6.48 (t, J = 6.3 Hz, 1H), 4.31 (d, J = 6.3 Hz, 2H), 2.94−2.92 (m, 4H), 2.74 (s, 6H), 1.80−1.78 (m, 4H), 1.46−1.44 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.5, 144.2, 137.7, 136.9, 135.0, 133.6, 132.9, 131.6, 130.7, 130.5, 130.4, 130.3 (q, J_{C-F} = 33.0 Hz), 129.1, 128.6, 126.1, 126.0, 125.3 (q, J_{C-F} = 3.5 Hz), 123.7 (q, J_{C-F} = 272.0 Hz), 119.9, 93.7, 83.8, 57.1, 38.0, 37.5, 25.1, 22.9; ES-MS (M + 1): 725.1; HPLC purity = 99.66%, t_R = 45.43 min.

1-(2,4-Dichlorophenyl)-4-((*N*-isopropylsulfamoylamino)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (28). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.10 g, 0.16 mmol), triethylamine (0.04 mL, 0.32 mmol), and isopropylsulfamoyl chloride (0.04 g, 0.24 mmol) in DMF (3 mL) at room temperature was allowed to react for 16 h, leading to product **28** (54 mg, 45%) as a white solid: mp 104.1−105.2 °C; ^{1}H NMR ($CDCl_3$) δ 8.85 (br s, 1H), 7.63−7.61 (m, 4H), 7.58 (d, J = 2.1 Hz, 1H), 7.40−7.34 (m, 2H), 7.22 (d, J = 3.9 Hz, 1H), 7.11 (d, J = 3.9 Hz, 1H), 6.10 (br s, 1H), 4.30 (s, 2H), 4.10 (m, 1H), 3.55−3.37 (m, 5H), 2.06−1.97 (m, 4H), 1.67−1.58 (m, 2H), 1.18 (d, J = 6.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 159.4, 144.2, 137.7, 136.9, 134.9, 133.5, 132.9, 131.5, 130.7, 130.4, 130.3, 130.2 (q, J_{C-F} = 32.6 Hz), 128.6, 128.1, 126.1, 125.9, 125.3 (q, J_{C-F} = 3.5 Hz), 123.7 (q, J_{C-F} = 272.0 Hz), 119.8, 93.7, 83.8, 57.1, 46.0, 37.2, 25.1, 23.7, 22.9; ES-MS (M + 1): 739.1; HPLC purity = 99.18%, t_R = 45.57 min.

4-((Azetidine-1-sulfonamido)methyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (29). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.07 g, 0.11 mmol), triethylamine (0.03 mL, 0.22 mmol), and 1-azetidinesulfonyl chloride (0.03 g, 0.17 mmol) in DMF (3 mL) at room temperature was allowed to react for 16 h, leading to product **29** (53 mg, 65%) as a white solid: mp 97.3−98.3 °C; ^{1}H NMR ($CDCl_3$) δ 7.71 (br s, 1H), 7.60−7.58 (m, 4H), 7.52 (d, J = 1.8 Hz, 1H), 7.39 (dd, J = 8.4, 1.8 Hz, 1H), 7.35 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 3.9 Hz, 1H), 7.23 (d, J = 3.9 Hz, 1H), 6.58 (t, J = 6.6 Hz, 1H), 4.33 (d, J = 6.6 Hz, 2H), 3.80 (t, J = 7.8 Hz, 4H), 2.85−2.83 (m, 4H), 2.12 (quint, J = 7.8 Hz, 2H), 1.80−1.76 (m, 4H), 1.50−1.40 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.4, 144.4, 137.7, 136.9, 135.0, 133.5, 133.0, 131.5, 130.7, 130.4, 130.3, 130.2 (q, J_{C-F} = 32.0 Hz), 128.7, 128.1, 127.3 (q, J_{C-F} = 270.2 Hz), 126.1, 125.9, 125.3 (q, J_{C-F} = 3.4 Hz), 123.7 (q, J_{C-F} = 271.5 Hz), 120.1, 93.6, 83.9, 57.2, 50.3, 37.4, 25.2, 23.1, 22.6; ES-MS (M + 1): 737.0; HPLC purity = 99.06%, t_R = 46.22 min.

1-(2,4-Dichlorophenyl)-*N*-(piperidin-1-yl)-4-((piperidine-1-sulfonamido)methyl)-5-(5-((4-(trifluoromethyl)phenyl)-ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (30). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.07 g, 0.11 mmol), triethylamine (0.03 mL, 0.22 mmol), and 1-piperidinesulfonyl chloride (0.03 g, 0.17 mmol) in DMF (3 mL) at room temperature was allowed to react for 16 h, leading to product **30** (63 mg, 73%) as a white solid: mp 113.5−114.6 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (br s, 1H), 7.62−7.60 (m, 4H), 7.53 (d, J = 2.0 Hz, 1H), 7.38 (dd, J = 8.4 and 2.0 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 4.0 Hz, 1H), 7.21 (d, J = 4.0 Hz, 1H), 6.54 (t, J = 6.8 Hz, 1H), 4.32 (d, J = 6.8 Hz, 2H), 3.16−3.13 (m, 4H), 2.86−2.84 (m, 4H), 1.80−1.78 (m, 4H), 1.65−1.60 (m, 2H), 1.58−1.56 (m, 4H), 1.49−1.42 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.4, 144.5, 137.6, 136.9, 135.1, 133.7, 133.0, 131.6, 130.7, 130.5, 130.4, 130.3 (q, J_{C-F} = 32.0 Hz), 128.7, 128.1, 126.1, 126.0, 125.3 (q, J_{C-F} = 4.0 Hz), 123.8 (q, J_{C-F} = 270.0 Hz), 120.1, 93.7, 83.9, 57.3, 46.9, 37.6, 25.3, 25.2, 23.7, 23.2; ES-MS (M + 1): 765.1; HPLC purity = 98.65%, t_R = 48.29 min.

4-((Azepane-1-sulfonamido)methyl)-1-(2,4-dichlorophenyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)-thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (31). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.07 g, 0.11 mmol), triethylamine (0.03 mL, 0.22 mmol), and azepane-1-sulfonyl chloride (0.03 g, 0.17 mmol) in DMF (3 mL) at room temperature was allowed to react for 16 h, leading to product **31** (70 mg, 80%) as a white solid: mp 110.2−110.7 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (br s, 1H), 7.59−7.57 (m, 4H), 7.55 (d, J = 1.8 Hz, 1H), 7.38 (dd, J = 8.4, 1.8 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 4.0 Hz, 1H), 7.18 (d, J = 4.0 Hz, 1H), 6.40 (t, J = 6.6 Hz, 1H), 4.27 (d, J = 6.6 Hz, 2H), 3.33−3.25 (m, 4H), 2.89−2.87 (m, 4H), 1.81−

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1.77 (m, 4H), 1.76−1.67 (m, 4H), 1.66−1.55 (m, 4H), 1,54−1.40 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 159.4, 144.3, 137.7, 137.0, 135.0, 133.7, 133.0, 131.6, 130.7, 130.5, 130.4, 130.3 (q, J_{C-F} = 32.2 Hz), 128.7, 128.1, 126.1, 126.0, 125.3 (q, J_{C-F} = 3.5 Hz), 123.8 (q, J_{C-F} = 272.0 Hz), 120.0, 93.7, 83.8, 57.2, 48.3, 37.2, 29.0, 27.0, 25.2, 23.0; ES-MS (M + 1): 798.8; HPLC purity = 98.86%, t_R = 48.40 min.

1-(2,4-Dichlorophenyl)-4-((2,6-dimethylmorpholine-4-sulfonamido)methyl)-*N*-(piperidin-1-yl)-5-(5-((4-(trifluoromethyl)phenyl)ethynyl)thiophene-2-yl)-1*H*-pyrazole-3-carboxamide (32). Following the similar coupling-reaction and workup procedures for **4**, a mixture of amine **13** (0.07 g, 0.11 mmol), triethylamine (0.03 mL, 0.22 mmol), and 2,6-dimethyl-4-morpholine-sulfonyl chloride in DMF (3 mL) at room temperature was allowed to react for 16 h, leading to product **32** (80 mg, 89%) as a white solid: mp 169.8−170.6 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (br s, 1H), 7.59−7.57 (m, 4H), 7.51 (d, J = 2.1 Hz, 1H), 7.37 (dd, J = 8.4, 2.1 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 4.0 Hz, 1H), 7.13 (d, J = 4.0 Hz, 1H), 6.68 (t, J = 6.6 Hz, 1H), 4.33 (d, J = 6.6 Hz, 2H), 3.57−3.50 (m, 2H), 3.40 (d, J = 11.1 Hz, 2H), 2.84 (m, 4H), 2.40 (t, J = 11.4 Hz, 2H), 1.79−1.75 (m, 4H), 1.45−1.43 (m, 2H), 1.14 (d, J = 6.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 159.4, 144.4, 137.5, 137.0, 135.0, 133.6, 132.9, 131.6, 130.7, 130.4, 130.3 (q, J_{C-F} = 32.0 Hz), 130.2, 128.6, 128.1, 126.1, 125.5, 125.3 (q, J_{C-F} = 4.0 Hz), 123.7 (q, J_{C-F} = 272.0 Hz), 120.0, 93.8, 83.7, 71.3, 57.2, 51.0, 37.7, 25.3, 23.1, 18.7; ES-MS (M + 1): 795.0; HPLC purity = 97.95%, t_R = 47.19 min.

Establishing Human CB1R (hCB1R) and CB2R (hCB2R) Stable Cell Lines and Membrane Purification. The hCB1R cDNA tagged with Flag at the N-terminus or hCB2R cDNA was subcloned into the pIRES2-EGFP vector (Clontech Laboratories, Inc., Mountain View, CA). After transfection to HEK 293 cells, clones that stably expressed either hCB1 or hCB2 were selected by GFP and G418 sulfate and maintained in DMEM supplemented with 10% fetal bovine serum and 0.5 mg/mL of G418 sulfate under 5% CO_2 at 37 °C. For membrane purification, cells were homogenized in ice-cold buffer A (50 mM Tris, 5 mM $MgCl_2$, 2.5 mM EDTA, pH 7.4, 10% sucrose) with 1 mM PMSF. The homogenate was centrifuged for 15 min at 2000g at 4 °C. The resulting supernatant was centrifuged for another 30 min at 43 000g at 4 °C. The final pellet was resuspended in buffer A and stored at −80 °C.

Radioligand Binding Assay. The radioligand binding assay was performed according to Felder et al.[40] with minor modification. An amount of 0.2−8 μg of the purified membrane was incubated with 0.75 nM [^{3}H]-CP-55940 and compounds of interest in the incubation buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.3% BSA, pH 7.4). The nonspecific binding was defined in the presence of 1 μM of CP-55940. The reactions were incubated for 1.5 h at 30 °C in Multiscreen microplates (Millipore Corp., Billerica, MA). The reactions were terminated by manifold filtration and washed with ice-cold wash buffer (50 mM Tris, pH 7.4, 0.25% BSA) four times. The radioactivity of [^{3}H]-CP-55940 bound to the filters was measured by Topcount (PerkinElmer Inc., Waltham, MA). For estimation of K_d and B_{max} of hCB1R and hCB2R, the nonspecific binding was defined in the presence of 50 μM SR141716A and 30 μM Win 552122, respectively. K_i values were determined by the concentration of compounds required to inhibit 50% of the specific binding of [^{3}H]-CP-55940 and calculated by nonlinear regression (GraphPad software, San Diego, CA).

Eu-GTP Binding Assay. The Eu-GTP binding assay, an in vitro functional assay, was performed using the DELFIA Eu-GTP binding kit (Perkin-Elmer Inc., Waltham, MA) based on methods developed by Frang et al.[41,42] with minor modifications as described in the following: 1−4 μg of purified membrane was incubated with compounds of interest and 25 nM of CP-55940 in assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 100 μg/mL saponin, 5 mM $MgCl_2$, 2 μM GDP, 0.5% BSA) at 30 °C for 60 min in acroplates (Pall Life Sciences, Ann Arbor, MI). Following the addition of Eu-GTP and incubation for 30 min at 30 °C, the assay was terminated by washing four times in washing buffer provided in the kit. The fluorescence signal of Eu-GTP was determined by Victor 2 multilabel reader (Perkin-Elmer Inc., Waltham, MA). EC_{50} values were analyzed by increasing concentrations of test compounds after activation with 25 nM of CP-55940 and were determined by nonlinear regression analysis using the GraphPad Prism program (GraphPad Software, San Diego, CA). The intrinsic property for inverse agonist efficacy was assessed by using test compounds alone in the absence of agonist CP-55940; the percentage change of the induced Eu-GTP binding is defined as [(Eu-GTP binding in the presence of test compound at 10 μM − basal Eu-GTP binding)/basal Eu-GTP binding] × 100% and expressed as % efficacy.

Measurement of Body Temperature and Tail-Flick Response. Core body temperature was measured using a TH-5 thermometer and a lubricated RET-3 rectal probe (Physitemp, Clifton, NJ) inserted into the rectum to a constant depth of 2 cm of C57BL/6J mice. The tail-flick response was measured by an analgesia meter RS232 (Columbus Instruments, Columbus, OH), and the intensity of the heat source was adjusted to produce tail-flick latency of 2 to 3 s with a cutoff at 10 s to avoid possible tissue damage. In the CB1R agonist-induced hypothermia and analgesia model, the body temperature and the tail-flick baseline latency were measured before drug administration. One hour after drug administration, agonist CP-55940 (1 mg/kg, Tocris Cookson, UK) was injected intraperitoneally (ip). Body temperatures were recorded at a time point of 30 and 65 min, respectively, after agonist dosing. The tail-flick response was measured at a time point of 35 min after agonist dosing. Data were analyzed by one-way ANOVA with Dunnett's post test. A P value (*) less than 0.05 was considered significant.

Quantitation of Brain and Plasma Concentration. Compounds dissolved in DMSO/Tween 80/H_2O (1:1:8, v/v/v) were administered orally (10 mL/kg) to 9- to 13-week-old C57BL/6 mice fed ad libitum (n = 3−4/group). At defined time points, mice were decapitated after they were sacrificed. Blood samples and brain tissues with cerebellum removed were collected and frozen at −80 °C before analysis. Brain tissues were weighed and homogenized with two volumes (v/w) of acetonitrile containing 500 ng/mL of the internal standard on ice bath and vortexed for 30 s. After centrifugation at 14 000g for 20 min at room temperature, the supernatant was transferred to a clean tube for LC-MS/MS analysis. The chromatographic system was composed of an Agilent 1100 series LC system (Palo Alto, CA, USA) and an Agilent ZORBAX Eclipse XDB-C8 reverse-phase column (5 μm, 3.0 mm × 150 mm) interfaced with an AB Sciex API 3000 tandem mass spectrometer with an ESI in the positive scanning mode (Applied Biosystems, Foster City, CA). Mobile phase A: water containing 0.1% formic acid; mobile phase B: acetonitrile. The gradient system started from A:B (30%:70%) to A:B (97%:3%) with a flow rate of 0.5 mL/min. Data were analyzed by one-way ANOVA with Dunnett's post test. A P value (*) less than 0.05 was considered significant.

Diet-Induced Obese (DIO) Mouse Model. Six-week-old C57BL/6 mice were given high-fat diet of 4.73 kcal/g energy density (Research Diet D 12451; 45% fat, 20% protein, and 35% carbohydrate) for 16 weeks before the drug treatment. Weight-matched mice were assigned to different groups and orally gavaged once daily with vehicle (10% DMSO/10% Tween 80/80% H_2O) or compounds at defined dosage for 22 days. Body weight was measured daily. At the end of the study, blood samples after animals had fasted for 5 h were collected and brain tissues were dissected and frozen at −80 °C before being analyzed. The B/P partition in DIO mice was determined using the same procedure described previously except that DIO mice, instead of C57BL/6 mice, were used.[43]

Aqueous Solubility. Solubility of compound **4** and **32** were determined by suspending 10 mg of test article in double distilled water (1 mL), followed by shaking the mixture at room temperature for 24 h. After 24 h shaking, the mixture was filtered to remove undissolved test article, and the amount of test article dissolved in the filtrate was determined by HPLC. Calibration standard curves were prepared by dissolving known concentrations of the test article in DMSO and then diluting with acetonitrile to conduct peaks areas vs concentrations obtained from HPLC analysis. HPLC conditions were the same as those used for the purity determination of the test compounds.

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P-gp ATPase Assay. The P-gp ATPase assay procedures were conducted according to the manufacturer's protocol. The assay is based on the determination of ATPase activity by measuring the release of inorganic phosphate resulting from substrate-stimulated ATP using a colorimetric method. In brief, human P-gp/MDR1 membrane or P-gp/MDR1-negative control membrane was preincubated at 37 °C for 5 min in the reaction buffer containing compound **4** in the presence or absence of 10 mM Na_3VO_4 in a 96-well microplate. The ATPase reaction was initiated by the addition of 20 μL of MgATP solution. The reaction was stopped after 30 min by addition of 30 μL of 10% (w/v) sodium dodecyl sulfate solution, and the amount of phosphate was determined immediately by adding 200 μL of detection color regent solution and incubated at 37 °C for 20 min in the dark. The amount of inorganic phosphate complex was determined by measuring the absorbance value at 800 nm wavelength. Verapamil was used as the positive control in these experiments.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Results of 163 off-target and patch-clamp assays, conducted by Ricerca Biosciences, for compound **4**, which was renumbered as BPRCB1184 for further development purposes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +886-37-246-166 ext. 35709. Fax: + 886-37-586-456. E-mail: ksshia@nhri.org.tw.

*Tel: +886-37-246-166 ext. 35721. Fax: + 886-37-586-456. E-mail: mhung@nhri.org.tw.

Author Contributions

†These authors contributed equally.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CB, cannabinoid; CNS, central nervous system; B/P, brain-to-plasma ratio; BBB, blood−brain barrier; tPSA, topological polar surface area; SAR, structure−activity relationships; DIO, diet-induced obese; AIBN, azobis(isobutyronitrile); P-gp, P-glycoprotein; Eu-GTP, europium guanosine 5′-triphosphate; ESMS, electrospray mass spectra

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