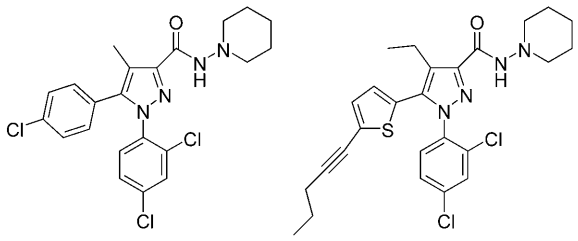
DOI: 10.1002/cmdc.201000246

Discovery of 1-(2,4-Dichlorophenyl)-4-ethyl-5-(5-(2-(4-(trifluoromethyl)phenyl)ethynyl)thiophen-2-yl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide as a Potential Peripheral Cannabinoid-1 Receptor Inverse Agonist

Ming-Shiu Hung, Chun-Ping Chang, Ting-Chieh Li, Teng-Kuang Yeh, Jen-Shin Song, Yinchiu Lin, Chien-Huang Wu, Po-Chu Kuo, Prashanth K. Amancha, Ying-Chieh Wong, Wen-Chi Hsiao, Yu-Sheng Chao, and Kak-Shan Shia*[a]

Cannabinoid-1 receptor (CB1R) is one of the most abundant neuroregulatory receptors in the brain, and it is involved in regulating feeding and appetite.[1] In addition to expression in brain, this receptor is also found in the peripheral organs, such as adipose tissues, muscle, and liver.[2] In sharp contrast, the structurally closely related cannabinoid-2 receptor (CB2R) is expressed almost exclusively in the immune system and is primarily involved in immune regulation and neurodegeneration.[3] The therapeutic potential of CB1R antagonists has been extensively reviewed,[4] and at least one compound (**1**; also called rimonabant or SR141716A) has shown clinical evidence of weight reducing action. However, after its launch in 2006, it was subsequently withdrawn (2008) in Europe due to severe psychiatric effects including depression, anxiety and stress disorders. Currently, only two drugs, orlistat and sibutramine, are available for the long-term treatment of obesity; however, both have met with moderate success because of their limited weight-loss efficacy and many accompanying adverse effects, including high blood pressure and flatulence.[5]



1 **3**

As such, there is still an urgent medical need for the safe and effective treatment of obesity in modern society. It is widely accepted that peripherally acting CB1R antagonists might avoid adverse central nervous system (CNS) side effects, as observed with brain-acting CB1R antagonists (e.g., **1**). This hypothesis, along with increasing evidence that weight loss and significant reduction in insulin and triglyceride levels might be achieved as demonstrated with several probable peripheral CB1R antagonists fed in diet-induced obese (DIO) mice,[6–10] made up the foundation of proposing peripheral CB1R as a potential therapeutic molecular target. However, more in-depth studies on the peripheral CB1R-ligand axis are required before it can gain firm grounds as a therapeutic target in treating obesity or other metabolic disorders such as type II diabetes.[11] Toward this end, discovery of non-brain-penetrating chemical entities with substantial CB1R binding affinity and potency will have a top priority.

Herein, we wish to report that based on the skeleton of a proven antiobesity agent (**3**) with significant weight-loss efficacy in DIO mice reported previously,[12] a novel series of aryl alkynylthiophene derivatives has been developed, leading to the identification of compound **8** as a highly promising CB1 peripheral inverse agonist with excellent potency (EC_{50} = 8.5 nM) and poor brain permeability as indicated by a low brain-to-plasma ratio (B/P = 1/33). The design, synthesis, and preliminary structure–activity relationship (SAR) data of the series are described below.

Aryl alkynylthiophenes **4**–**13** were synthesized in good to high yield (65–92%) mainly through Pd-mediated coupling of an appropriate alkyne with the common intermediate **2**, readily prepared following the synthetic procedure reported in the previous literature,[12] under modified Sonogashira coupling conditions (Scheme 1).[13] As a typical example, a mixture of compound **2** and 1-ethynyl-4-(trifluoromethyl)benzene dissolved in THF in the presence of $PdCl_2(PPh_3)_2$/CuI and 2-ethanolamine was stirred in a sealed pressure vessel, immersed in an oil bath at 100 °C, overnight to afford product **8** in 86% yield after chromatographic purification. Compounds thus obtained were subjected to various biological evaluations toward CB1R and CB2R, the results of which are compiled in Table 1.

As indicated, our synthetic efforts were mainly focused on replacing the linear alkyl linker of **3** with an array of aryl moieties. An initial analogue **4** (IC_{50} = 25.8 nM; EC_{50} = 89.8 nM; B/P = 1/23), obtained by substituting the propyl side chain with a benzene ring, was found to have fourfold improvement in plasma exposure as compared to **3** (IC_{50} = 6.1 nM; EC_{50} = 13.8 nM; B/P = 1/6), suggesting that a significant impact on increasing plasma exposure might be easily achieved through appropriate chemical modifications. Along this line, the R

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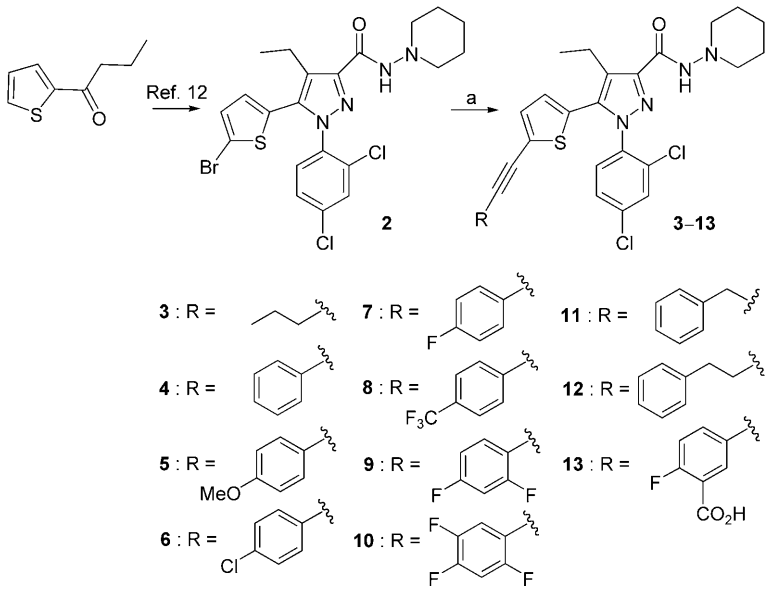
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201000246.



Scheme 1. *Reagents and conditions*: a) various alkynes, $PdCl_2(PPh_3)_2$, CuI, 2-ethanolamine, THF in a sealed pressure vessel immersed in an oil bath at 100 °C, 16 h, 65–92%.

group was changed to a *p*-methoxy benzene ring to provide **5** (IC_{50} = 21.9 nM; EC_{50} = 241.9 nM; B/P = 1/26); again, the blood-to-plasma ratio was greatly enhanced relative to parent **3**. Though analogues **4** and **5** resulted in losing their CB1 binding affinity and potency by three- to fourfold and six- to 18-fold, respectively, structural modifications directed toward enhancing plasma exposure by altering substituents on the benzene ring appeared to be on the right track.

The introduction of an electron-withdrawing substituent was then carried out to afford the *p*-chloride derivative **6** (IC_{50} = 7.2 nM; EC_{50} = 126.6 nM; B/P = 1/18), in which a significant improvement in binding affinity and potency was immediately observed as compared to **5** with a classical electron-donating *p*-methoxy moiety. Encouraged by these findings, compounds **7**–**10**, possessing a substituent(s) with stronger electron-withdrawing ability on the benzene ring, were synthesized. Accordingly, the halogenated analogues thus generated, without exception, exhibited strong binding affinities and potency toward CB1R (IC_{50} = 1.1–8.5 nM; EC_{50} = 7.2–38.5 nM); however, most of them incurred a certain degree of loss in CBR2/CB1R selectivity, and this drawback will be addressed in the future structural modifications. An attempt to install an extra carboxylic group, which is supposed to increase polar surface area (PSA) significantly and reduce blood–brain barrier (BBB) permeability of the global molecule,[14] was also made on the potent compound **7** (IC_{50} = 6.6 nM; EC_{50} = 38.5 nM; PSA = 50.16 Å^2), of which the benzene ring already bears a fluorine atom in the *para* position. Unfortunately, the resultant carboxylic acid **13** (IC_{50} = 2274.8 nM; EC_{50} = 1407.6 nM; PSA = 90.29 Å^2) exhibited a complete loss of CB1R activity and potency, though the PSA value, as expected, was practically doubled. This result appears to be not too surprising in that, as manifested by many historical cases,[9,15] a hydrophobic pocket consisting of a series of aromatic residues (Trp 255/Tyr 275/Phe 278) as illustrated in Figure 1, is proposed around the pyrazole-5 substitution, presumably making it extremely sensitive to the presence of the polar functionalities (i.e., –COOH).

Thus, approaches to increase polarity by introducing a polar group(s) on the 5-position were abandoned. More interestingly, when the benzene ring of **4** was extended by two methylene units, the resulting compound **12** (IC_{50} = 4.4 nM; EC_{50} = 10.3 nM; CB2R/CB1R = 395; B/P = 1/31) possessed, not only excellent biological activities as with parent **3**, but a desirable plasma exposure and CB1R selectivity over CB2R. Also noteworthy is the finding that the intrinsic property appears to vary with the length of the linker as indicated with **12** and its counterparts **4** and **11**, wherein the former behaves as a neutral antagonist and the latter as inverse agonists (Figure 2).[12] Inspired by favorable biological properties conferred on **12**, its structurally closely related derivatives, which are being designed to become large enough not to cross the BBB but remain small enough to bind tightly to the CB1R, are under active pursuit in

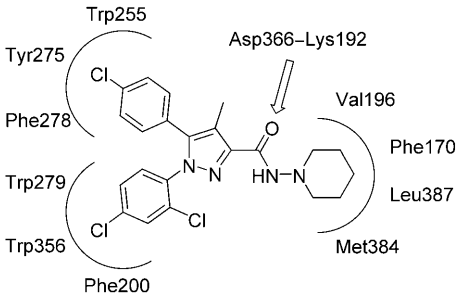


Figure 1. The binding mode of rimonabant (**1**) in the proposed CB1R homology model.

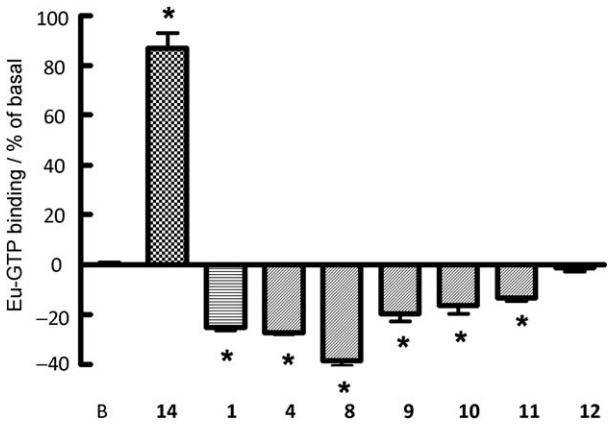
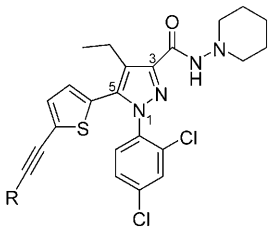
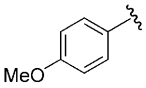
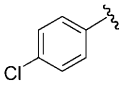
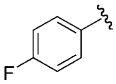
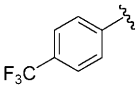
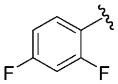
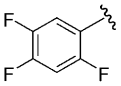
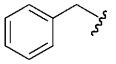
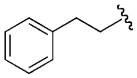
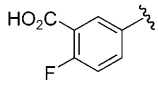


Figure 2. Eu-GTP binding assays of selected alkynylthiophenes **4**, **8**–**12**, and a reference inverse agonist (**1**) and agonist (**14**) were conducted at a concentration of 10 μM (see section B3 in the Supporting Information). Data are expressed as the mean ± SD of at least three experiments performed in duplicate. Statistical significance was assessed by unpaired two-tailed *t*-test using the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). $p < 0.05$ (*) was considered significant. Compound **12** with the induced Eu-GTP binding intensity around the basal level was assigned as a neutral antagonist (NA); compounds **4** and **8**–**11** with a significant decrease in the binding intensity relative to the basal level were defined as inverse agonists (IA), as was reference **1**.

Table 1. Biological evaluation of 5-(5-alkynyl-thiophen-2-yl)pyrazole derivatives on human CB1R and CB2R.



Compd	R	IC_{50} [nM][a,c] CB1R	EC_{50} [nM][b,c] CB1R	IC_{50} [nM][a,c] CB2R	CB2R/CB1R	IP[d]	B/P[e]
1		14.6 ± 1.8	17.3 ± 1.6	1939.8 ± 131.1	130	IA	3/1
3[f]		6.1 ± 2.6	13.8 ± 4.4	919.6 ± 53.1	151	IA	1/6
4		25.8 ± 10.0	89.8 ± 22.1	1093.7 ± 182.4	42	IA	1/23
5		21.9 ± 2.4	241.9 ± 14.1	395.5 ± 51.0	18	IA	1/26
6		7.2 ± 0.3	126.6 ± 48.0	183.7 ± 85.3	26	IA	1/18
7		6.6 ± 1.5	38.5 ± 12	119.0 ± 28.8	18	IA	1/12
8		8.5 ± 0.3	18.5 ± 8.0	604.9 ± 101.1	71	IA	1/33
9		4.4 ± 1.6	20.8 ± 3.7	964.8 ± 39.9	219	IA	1/12
10		1.1 ± 0.2	7.2 ± 1.5	36.6 ± 13.3	33	IA	1/22
11		6.6 ± 1.3	54.0 ± 0.6	536.4 ± 150.9	81	IA	1/15
12		4.4 ± 0.5	10.3 ± 3.6	1738.2 ± 74.2	395	NA	1/31
13		2274.8 ± 356.5	1407.6 ± 242.3	> 10000	> 4	ND[g]	ND[g]

[a] The binding affinity, determined by 50% inhibition of agonist [^{3}H]-CP55940 ([^{3}H]-**14**) binding to human CB1R or human CB2R, is expressed as the IC_{50} value. [b] Functional activity, determined by 50% inhibition of Eu-GTP binding to CB1R-overexpressing membrane, is expressed as the EC_{50} value. [c] Data are expressed as the mean ± SD of at least three independent experiments. [d] Intrinsic property (IP) of each compound was identified at a concentration of 10 μM as illustrated in Figure 2.[12] [e] The brain (B) to plasma (P) ratio was determined at a time point of 2 h after oral dosing (see section B4 in the Supporting Information). [f] This compound has previously been structurally characterized.[12]. [g] Not determined (ND) because of poor CB1 activity.

our laboratories. Nevertheless, a structurally metabolic liability for **12**, such as benzylic oxidation which could occur easily and exclude it from BBB penetration, could not be ignored.

Compound **8** (IC_{50} = 8.5 nM; EC_{50} = 18.5 nM; CB2R/CB1R = 71; B/P = 1/33), which behaves as an inverse agonist with strong binding affinity and high potency as well as a low blood-to-plasma ratio, appears to be a good candidate for testing the peripheral CB1R-ligand interaction. As such, **8** was initially evaluated for its central effects in the CB1R agonist-induced hypothermia model, one of four tests known as "tetrad responses".[16] Accordingly, treatment with a CB1R agonist, such as CP55940 (**14**), alone would induce a reduction in body temperature by acting on CB1R in the hypothalamus; this effect can be reversed by addition of brain-acting CB1R antagonists (e.g., inverse agonist **1**). As indicated in Figure 3, a negative central effect was observed for **8** at a dose of 20 mg kg^{-1} after treatment with agonist **14** (1 mg kg^{-1}), and a slight reversal, but not a significant change, of the body temperature reduction was detected at a dose of up to 50 mg kg^{-1}, suggesting that compound **8** remains primarily in the circulating system and has much poorer BBB permeability than the positive control **1**, in which a significant reversal of the temperature lowering effect was observed at a dose as low as 2 mg kg^{-1}. Similar conclu-

sions could also be derived through another tetrad-response test, a CB1R agonist-induced analgesia model.

As illustrated in Figure 4, compound **8** was administered at a dose of 20 and 50 mg kg^{-1}, and was found unable to counteract the analgesic effect induced by agonist **14** (1 mg kg^{-1}). On the contrary, a significant reversal of the tail-flick response was seen with brain-acting **1** at a dose of 2 mg kg^{-1}. Accordingly, compound **8** was assumed to be a potential peripheral CB1R candidate, and its pharmacokinetic study was conducted prior to the subsequent long-term efficacy studies, results of which are compiled in Table 2.

Table 2. Pharmacokinetic studies of compounds **1** and **8** in fasted male Sprague Dawley rats.[a]

Compd	Route	Dose [mg kg^{-1}]	t_{max} [h]	C_{max} [ng mL^{-1}]	$t_{1/2}$ [h]	$AUC_{(0-\infty)}$ [h × ng mL^{-1}]	BA [%]
1	oral	10	2.8 ± 1.5	230 ± 52	5.3 ± 0.6	801 ± 389	24
8	oral	10	1.3 ± 0.6	3000 ± 920	6.8 ± 0.4	12810 ± 4330	28

[a] Values indicate the mean ± SD ($n = 3$). The experimental protocol is detailed in section B4 of the Supporting Information. Abbreviations: maximum plasma concentration of drug (C_{max}); time taken to reach C_{max} (t_{max}); half-life ($t_{1/2}$); area under the curve (AUC); oral bioavailability (BA).

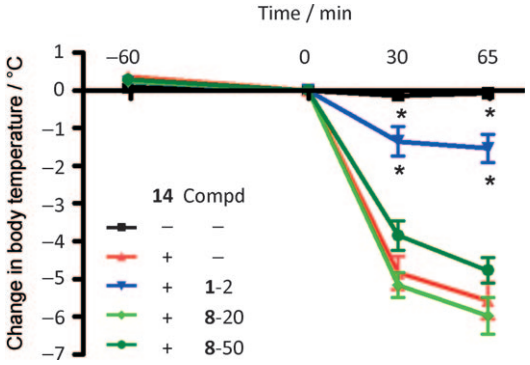


Figure 3. Compounds **8** (20 and 50 mg kg^{-1}) and **1** (2 mg kg^{-1}) were administered (po) 1 h prior to ip treatment with agonist **14** (1 mg kg^{-1}) in the CB1 agonist-induced hypothermia model. Body temperature was measured at 30 and 65 min after agonist dosing. $p < 0.05$; * vs **14**. See section B5 of the Supporting Information for full details.

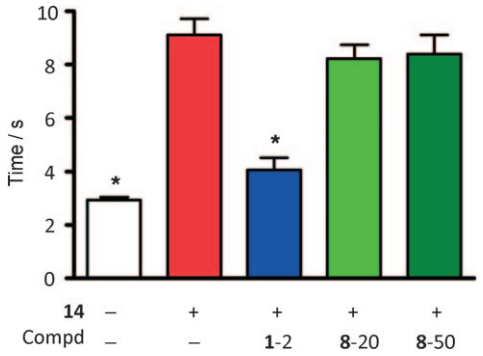


Figure 4. Compounds **8** (20 and 50 mg kg^{-1}) and **1** (2 mg kg^{-1}) were administrated (po) 1 h prior to ip treatment with agonist **14** (1 mg kg^{-1}) in the CB1 agonist-induced analgesia model. Tail flick response was measured 35 min after agonist dosing. $p < 0.05$; * vs **14**. See section B5 of the Supporting Information for full details.

As listed, it was found that, in addition to displaying similar in vitro biological activities as described previously, both inverse agonists **1** ($t_{1/2} = 5.3$ h; BA = 24%) and **8** ($t_{1/2} = 6.8$ h; BA = 28%) also had very similar pharmacokinetic profiles such as half-life ($t_{1/2}$) and oral bioavailability except for a distinct difference in plasma exposure as evidenced by C_{max} and AUC values, thus rendering them ideal compounds, for comparison purposes, to address the peripheral CB1R-acting hypothesis.

In summary, a novel class of aryl alkynylthiophenes has been designed to target peripheral CB1R to eliminate/minimize CNS side effects as observed with **1**, a typical brain CB1R-acting agent. The titled compound, 1-(2,4-dichlorophenyl)-4-ethyl-5-(5-(2-(4-(trifluoromethyl)phenyl)ethynyl)thiophen-2-yl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (**8**), is tentatively recognized as a peripheral inverse agonist based on its negative central effects in CB1R agonist-induced hypothermia and analgesia models, and low brain exposure (B/P = 1/33) measured at a time point of 2 h following oral administration. The chronic study of **8** in DIO mice is currently underway. A full account of the in vivo studies, with particular emphasis on addressing issues of central drug accumulation and peripheral metabolic benefits, as well as further SAR studies on the series, will be reported in due course.

Experimental Section

See the Supporting Information for experimental details on chemical syntheses, biological assays and pharmacokinetic studies.

All experiments involving animals were approved by the Institutional Animal Care and Use Committee of National Health Research Institutes (Taiwan) and performed accordingly.

Acknowledgements

We are grateful to the National Health Research Institutes and National Science Council of the Republic of China (grant NSC 96-2323-B-400-001) for financial support.

Keywords: blood–brain barrier · brain-to-plasma ratio · cannabinoid-1 receptor · central nervous system · diet-induced obesity

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Received: June 10, 2010
Published online on July 22, 2010

