Design, synthesis, and biological evaluation of novel alkenylthiophenes as potent and selective CB1 cannabinoid receptor antagonists†

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A novel class of (5-(pent-1-enyl)thiophen-2-yl)pyrazole antagonists was discovered, many of which exhibited potent CB1 activity and good CB1/2 selectivity, suggesting that along with a 1,3-transposition of the carbonyl of the pyrazole 3-carboxamide, bioisosteric replacement of the conventional pyrazole 5-aryl group with a thienyl ring substituted with an appropriate alkenyl moiety is viable.

The therapeutically beneficial effects of cannabinoid-related agents have caught the attention of researchers, particularly following the identification of two distinct G protein-coupled cannabinoid receptors (GPCRs), namely CB1 and CB2.[1] CB1 receptors are predominantly expressed in several brain areas, including the hippocampus, hypothalamus, cerebral cortex, cerebellum and basal ganglions, while CB2 receptors are mainly associated with immune cells.[2–4] Activation of cannabinoid receptors by binding to endogenous (*e.g.*, arachidonoylglycerol), plant-derived (*e.g.*, Δ^9-tetrahydrocannabinol) or synthetic cannabinoids (*e.g.*, CP55940) has been shown to be involved in numerous physiological processes, including analgesia, decrease in intestinal motility, and attenuation of vomiting and nausea.[2] Among the pharmacological effects elicited by cannabinoids, reduction of food intake, owing to antagonizing CB1 receptors, is one of the most important medicinal properties.[5] Further studies in this area verified that selective CB1 receptor antagonists[6] could significantly reduce obesity/overweightness in both animals and humans,[5,7,8] and are thus suggested to have potential therapeutic utility as appetite suppressants for the treatment of obesity. Abundant evidence indicates that obesity is a risk factor for noncommunicable diseases such as hypertension, stroke, type 2 diabetes, cancer and arthritis.[9] Obesity has become a global pandemic and is recognized as a serious health concern by the World Health Organization. To date, only two anti-obesity agents, namely Orlistat and Sibutramine, have been successfully marketed for long-term obesity treatment. However, due to their limited efficacy in weight reduction and significant accompanying adverse effects, these pharmacological approaches have only met with moderate success. As a result, enormous attention has been paid to new drug discovery in the treatment of obesity acting on novel molecular targets, such as cannabinoid 1 receptors (CB1),[10] 5-hydroxytryptamine 2C receptors (5-HT2c),[11] melanocortin-4 receptors (MC4R),[12] and melanin-concentrating hormone-1 receptors (MCHR-1).[13]

Along these lines, the first successful clinical candidate SR141716A (**1**) (Fig. 1), behaving as a potent CB1 receptor inverse agonist with excellent CB1/2 selectivity,[14] was discovered and launched in Europe in 2006.

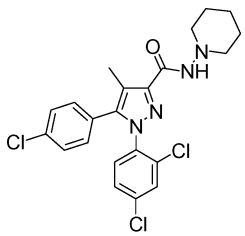

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Fig. 1 SR141716A (Rimonabant, Acomplia™).

After Rimonabant was identified as the first selective CB1 antagonist in 1994, a wide variety of mimetics, mainly generated by replacing the central pyrazole scaffold with analogous heteroaromatic rings, have been extensively explored.[15] However, taking into account these structural modifications, limited efforts have been devoted to the modification of the pyrazole 5-position (Fig. 1).[16] In order to investigate the pharmacological effects exerted by C5 substitution, a strategy of bioisosterism[17] was then applied to generate novel Rimonabant-mimicking molecules. Accordingly, the vinylene unit (–CH=CH–) in the aromatic rings of drug molecules can be replaced with groups such as S, O, Se, and NH, resulting in aromatic rings with equivalent steric and electronic properties. As such, the thiophene ring, recognized as the most popular bioisostere of the phenyl ring,[17c] was adopted as the initial model to this concept. In conjunction with above bioisosteric replacement, 1,3-transposition of the carbonyl at the pyrazole C3 atom was also implemented to explore the impact of hydrogen bonding on the pyrazole 3-carboxamide group, wherein the NH was proposed to play an essential role in forming intramolecular hydrogen bonding and the carbonyl serves as a hydrogen bond acceptor to stabilize the Asp366–Lys192 salt bridge in CB1 receptors.[18] In this communication, we wish to report that a novel series of (5-(pent-1-enyl)thiophen-2-yl)pyrazoles have been designed and experimentally realized, many of which were found to behave as potent CB1 receptor antagonists[6] with good CB1/2 selectivity.

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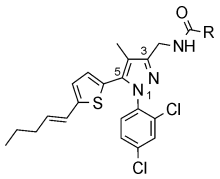
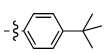
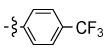
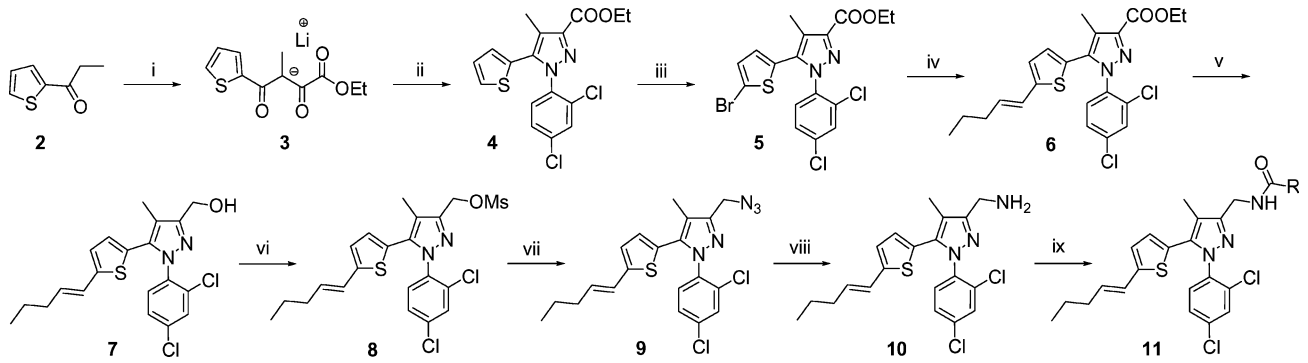
As illustrated in Table 1, an array of pent-1-enylthiophenes **11a–p** were readily prepared† through a nine-step synthetic sequence outlined in Scheme 1 starting from 1-(thiophen-2-yl)-1-propanone (**2**). Commercially available **2** was first subjected to Claisen condensation with diethyl oxalate using LHMDS as a base for deprotonation to afford lithium salt **3** in 85% yield, which without purification was treated with 2,4-dichlorophenyl hydrazine hydrochloride in ethanol at room temperature for 24 h, followed by intramolecular cyclization under refluxing acetic acid to give ester **4** in 52% yield over two steps. Subsequent bromination of compound **4** was smoothly effected using NBS in acetonitrile to afford bromo ester **5** in excellent yield (95%). The common 1-pentenyl group on the thiophene ring was successfully introduced by a typical Suzuki cross-coupling reaction,[19] wherein bromo ester **5** was mixed with (*E*)-pent-1-enylboronic acid and $Pd(PPh_3)_4$ with stirring in DME at 80 °C for 3 h to produce the desired thiophene

Table 1 Biological evaluations of (5-(pent-1-enyl)thiophen-2-yl)pyrazoles on hCB1 and hCB2 receptors



		IC_{50}/nM [a,b]			
Compound	R	hCB1	hCB2	EC_{50}/nM [a,c]	Selectivity (hCB2/hCB1)
11a		30.3 ± 5.4	3040.0 ± 185.3	55.4 ± 10.5	100
11b		25.2 ± 10.1	2045.3 ± 503.5	93.8 ± 6.8	81
11c		24.4 ± 3.3	3837.5 ± 636.1	37.0 ± 6.9	157
11d		58.0 ± 3.9	2428.0 ± 463.1	123.8 ± 46.9	41
11e		15.1 ± 4.0	5866.7 ± 1492.0	68.0 ± 34.7	391
11f		117.3 ± 22.5	8674.0 ± 1646.1	66.4 ± 11.0	74
11g		21.3 ± 5.3	2298.0 ± 628.2	22.2 ± 8.0	109
11h		23.2 ± 9.4	1382.9 ± 497.0	51.1 ± 8.8	60
11i		58.0 ± 12.2	2547.7 ± 704.3	93.7 ± 16.5	43
11j		8.7 ± 3.0	5097.7 ± 512.9	26.8 ± 6.8	637
11k		7.1 ± 0.8	1384.0 ± 241.5	17.3 ± 2.4	197
11l		4.0 ± 1.4	1101.3 ± 389.3	23.3 ± 3.9	275
11m		703.7 ± 36.0	3004.1 ± 555.8	261.2 ± 41.9	4
11n		327.5 ± 35.6	4143.4 ± 444.1	205.0 ± 18.3	12
11o		57.2 ± 22.0	837.9 ± 469.9	35.5 ± 3.0	14
11p		41.8 ± 4.4	1333.7 ± 163.4	170.3 ± 74.8	32
SR141716A		16.6 ± 1.71	2533.7 ± 464.6	18.0 ± 3.8	158

[a] Data are expressed as the mean ± SEM of at least three independent experiments. [b] Binding affinity determined by inhibition of [^{3}H]-CP55940 binding to hCB1 or hCB2-transfected HEK 293 membrane is expressed as IC_{50}. [c] Functional activity determined by inhibition of Eu-GTP binding to hCB1-transfected HEK 293 membrane is expressed as EC_{50}.



Scheme 1 *Reagents and conditions*: (i) LHMDS, diethyl oxalate, THF–Et_2O, −78 °C to r.t., 20 h, 85%; (ii) 2,4-dichlorophenylhydrazine hydrochloride, EtOH, r.t., 24 h; then AcOH, 120 °C, 24 h, 52%; (iii) NBS, CH_3CN, 0 °C to r.t., 24 h, 95%; (iv) $Pd(PPh_3)_4$, (*E*)-pent-1-enylboronic acid, Cs_2CO_3, DME, 80 °C, 3 h, 80%; (v) LAH, THF, 0 °C, 30 min, 87%; (vi) MsCl, Et_3N, THF, 0 °C to r.t., 8 h, 96%; (vii) NaN_3, DMF, 75 °C, 3 h, 82%; (viii) PPh_3, THF–H_2O, r.t., 48 h, 71%; (ix) RCOCl or RNCO, CH_2Cl_2, Et_3N, 0 °C to r.t., 8 h, 51–78%.

6 (80%). Intermediate **6** thus obtained was reduced with LAH to provide alcohol **7** (87%), which in turn underwent mesylation using mesyl chloride/triethylamine in THF to give compound **8** in virtually quantitative yield (96%). The mesyl group of compound **8** was sequentially displaced with sodium azide, followed by Staudinger reduction[20] to provide the primary amine **10** in 58% yield over two steps. Compound **10** thus generated was allowed to couple with various acid chlorides or isocyanates to afford final products **11a–11p** in moderate to good yields (51–78%).

With these compounds in hand, biological evaluations were conducted, and preliminary results are compiled in Table 1. The initial results with simple *N*-alkylcarboxamides **11a–c**, which exhibit strong CB1 binding affinities with IC_{50} values of 30.3, 25.2 and 24.4 nM, respectively, are encouraging in that they are only slightly inferior to the parent compound **1** and appear to validate the bioisosteric hypothesis, namely, that in conjunction with 1,3-transposition of the C-3 carbonyl, the C-5 phenyl ring could be replaced with an appropriately substituted thiophene moiety. Moreover, among various aliphatic substituents on the thiophene ring, it was found that alkenylthiophenes were superior to the corresponding alkyl or alkynyl counterparts in binding affinity towards CB1 receptors.[21] As such, the 1-pentenyl substituent, one of the most promising aliphatic units observed, was selected as a fixed motif for all subsequently synthesized derivatives. A series of cyclic amides **11d–h** with an increasing order of ring size were obtained, wherein cyclobutyl amide **11e** (IC_{50} = 15.1 nM; CB2/1 = 391) and cyclohexyl amide **11g** (IC_{50} = 21.3 nM; CB2/1 = 109) not only exhibited CB1 binding affinity comparable to **1** (IC_{50} = 16.6 nM; CB2/1 = 158) but also good selectivity for CB1 over CB2. Also encouraging was the finding that **11g** (EC_{50} = 22.2 nM) showed improvement in functional activity by 5-fold relative to **11d** (EC_{50} = 123.8 nM), presumably due to an increase in hydrophobic interaction with CB1 receptors as the ring size increases from a three- to a six-membered ring.

The above findings inspired us to further explore the receptor–ligand interaction by replacing the cycloalkyl rings with aromatic groups. Representative arylcarboxamide derivatives **11j–l** were synthesized and showed significant improvement in *in vitro* biological activities compared to **1**. 4-*tert*-Butylbenzamide **11j** exhibited unexpectedly more potent CB1 binding affinity (IC_{50} = 8.7 nM) and higher CB1/2 selectivity (637-fold) than **11b** (IC_{50} = 25.2 nM; CB2/1 = 81), indicating that apart from a required hydrophobic interaction provided by the *tert*-butyl group itself, an additional aromatic stacking interaction between the phenyl ring and CB1 receptors is needed for effective binding capability. Similar results with particular enhancement in both binding affinity and selectivity were also observed for analogues **11k** (IC_{50} = 7.1 nM; CB2/1 = 197) and **11l** (IC_{50} = 4.0 nM; CB2/1 = 275). In addition, more importantly, compounds **11j–l** also showed potent functional activity for CB1 receptors with EC_{50} values of 26.8, 17.3, and 23.3 nM, respectively. Taken together, the arylcarboxamides are considered to be the most promising candidates for the potential treatment of obesity in the system examined; as such, compounds **11j–l** will be selected for further SAR, ADME and *in vivo* efficacy studies.[21]

Water solubility and permeability are two key physicochemical properties for oral absorption, which might be estimated/predicted by calculated Clog *P* values. The Clog *P* values of the most potent and selective compounds **11j–l** were 10.7, 9.9, and 10.1, respectively, indicating that they are less water-soluble than SR141716A (Clog *P* = 6.5), and that a more hydrophobic formula is needed to dissolve the series when orally administered. Heterocyclic amides **11m** (IC_{50} = 703.7 nM; EC_{50} = 261.2 nM) and **11n** (IC_{50} = 327.5 nM; EC_{50} = 205.0 nM) were found to exhibit weak binding affinity and poor functional activity towards CB1 receptors, presumably due to the presence of the N or S atom leading to the disruption of the π–π stacking interaction. Urea-type compounds, such as **11o** and **11p**, were also produced for biological evaluation; in comparison with the corresponding compounds **11a** and **11g**, not only do they show decreased biological activity but also lower CB1/2 selectivity, indicating that the extra NH unit of the ureas might counteract the intramolecular hydrogen bonding capability between the pyrazole N2 and the original carboxamide NH, and/or reduce the required hydrophobic interaction available for CB1 receptors.

In summary, based on the concept of bioisosteric replacement of SR141716A (**1**) with 1,3-transposition of carbonyl and the thiophene ring, respectively, at the pyrazole 3- and 5-positions, a novel class of (5-(pent-1-enyl)thiophen-2-yl)pyrazole antagonists was discovered, many of which exhibited potent biological activity towards CB1 receptors along with good CB1/2 selectivity. Among them, the arylcarboxamide series (**11j–l**), in terms of potency

and selectivity, appear to be the most promising candidates for further development as anti-obesity agents. Moreover, we have also disclosed in this study that the receptor–ligand interaction significantly decreases when a heterocyclic ring is attached to the amide group, presumably due to the disruption of π–π stacking interaction in the presence of the heteroatom.

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Notes and references

1 V. Di Marzo, M. Bifulco and L. De Petrocellis, *Nat. Rev. Drug Discovery*, 2004, **3**, 771.
2 A. C. Howlett, F. Barth, T. I. Bonner, G. Cabral, P. Casellas, W. A. Devane, C. C. Felder, M. Herkenham, K. Mackie, B. R. Martin, R. Mechoulam and R. G. Pertwee, *Pharmacol. Rev.*, 2002, **54**, 161.
3 M. Herkenham, A. B. Lynn, M. D. Little, M. R. Johnson, L. S. Melvin, B. R. de Costa and K. C. Rice, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 1932.
4 A. B. Lynn and M. Herkenham, *J. Pharmacol. Exp. Ther.*, 1994, **268**, 1612.
5 G. Colombo, R. Agabio, G. Diaz, C. Lobina, R. Reali and G. L. Gessa, *Life Sci.*, 1998, **63**, 113.
6 Although the general term "antagonist" is used here, it is understood that many CB1 receptor antagonists function as inverse agonists. Biologically, a more accurate term—"modulator"—is used to describe ligands with unknown functions. As for known-function ligands, they can be mainly classified into agonists, antagonists and inverse agonists.
7 C. R. Trillou, M. Arnone, C. Delgorge, N. Gonalons, P. Keane, J. P. Maffrand and P. Soubrie, *Am. J. Physiol.: Regul., Integr. Comp. Physiol.*, 2003, **284**, 345.
8 L. F. Van Gaal, A. M. Rissanen, A. J. Scheen, O. Ziegler and S. Rossner, *Lancet*, 2005, **365**, 1389.
9 (*a*) *Overweight and Obesity*, from the webpage for the Centers for Disease Control and Prevention: http://www.cdc.gov/nccdphp/dnpa/obesity/; (*b*) S. D. Malnick and H. Knobler, *Q. J. Med.*, 2006, **99**, 579.
10 J. Antel, P. C. Gregory and U. Nordheim, *J. Med. Chem.*, 2006, **49**, 4008.
11 B. M. Nilsson, *J. Med. Chem.*, 2006, **49**, 4023.
12 R. P. Nargund, A. M. Strack and T. M. Fong, *J. Med. Chem.*, 2006, **49**, 4035.
13 A. L. Handlon and H. Zhou, *J. Med. Chem.*, 2006, **49**, 4017.
14 M. Rinaldi-Carmona, F. Barth, M. Heaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Neliat, D. Caput, P. Ferrara, P. Soubrie, J. C. Breliere and G. Le Fur, *FEBS Lett.*, 1994, **350**, 240.
15 For recent reviews, see: (*a*) J. H. M. Lange and C. G. Kruse, *Drug Discovery Today*, 2005, **10**, 693; (*b*) G. G. Muccioli and D. M. Lambert, *Expert Opin. Ther. Pat.*, 2006, **16**, 1405.
16 M. Xia, F. Liotta, M. Pan, M. P. Wachter, and H. Lu, *World Pat.* WO 2007/061948.
17 (*a*) L. M. Lima and E. J. Barreiro, *Curr. Med. Chem.*, 2005, **12**, 23; (*b*) G. A. Patani and E. J. LaVoie, *Chem. Rev.*, 1996, **96**, 3147; (*c*) A. Burger, *Prog. Drug Res.*, 1991, **37**, 287.
18 (*a*) D. Hurst, U. Umejiego, D. Lynch, H. Seltzman, S. Hyatt, M. Roche, S. McAllister, D. Fleischer, A. Kapur, M. Abood, S. Shi, J. Jones, D. Lewis and P. Reggio, *J. Med. Chem.*, 2006, **49**, 5969; (*b*) J. Y. Shim, W. J. Welsh, E. Cartier, J. L. Edwards and A. C. Howlett, *J. Med. Chem.*, 2002, **45**, 1447; (*c*) D. P. Hurst, D. L. Lynch, J. Barnett-Norris, S. M. Hyatt, H. H. Seltzman, M. Zhong, Z. H. Song, J. Nie, D. Lewis and P. H. Reggio, *Mol. Pharmacol.*, 2002, **62**, 1274.
19 K. Kobayashi, A. Sugie, M. Takahashi, K. Masui and A. Mori, *Org. Lett.*, 2005, **7**, 5083.
20 F. L. Lin, H. M. Hoyt, H. V. Halbeek, R. G. Bergman and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2005, **127**, 2686.
21 Among various aliphatic substituents on the thiophene ring examined, the alkenyl group was found to be the most appropriate choice in this system. A full account of this work, including further SAR, ADME and *in vivo* efficacy studies of the best and most selective alkenylthiophenes, will be reported elsewhere in due course.

