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Rational design of a novel peripherally-restricted, orally active CB_1 cannabinoid antagonist containing a 2,3-diarylpyrrole motif

Laurent Hortala [a], Murielle Rinaldi-Carmona [a], Christian Congy [a], Laurent Boulu [a], Freddy Sadoun [b], Gérard Fabre [b], Olivier Finance [c], Francis Barth [a,*]

[a] Exploratory Unit, Sanofi-aventis R&D, 371 rue du Professeur Joseph Blayac, 34184 Montpellier, France
[b] Disposition, Safety and Animal Research, Sanofi-aventis R&D, 371 rue du Professeur Joseph Blayac, 34184 Montpellier, France
[c] Prospective and Strategic Initiative, Sanofi-aventis R&D, 371 rue du Professeur Joseph Blayac, 34184 Montpellier, France

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ABSTRACT

A new series of 2,3-diarylpyrroles have been prepared and evaluated as CB_1 antagonists. Modulation of the topological polar surface area allowed the identification of high affinity peripherally-restricted CB_1 antagonists. Compound **11**, obtained after further optimization of the metabolic profile displayed very low brain penetration, yet was able to reverse CP55940-induced gastrointestinal transit inhibition following oral administration.

CB_1 cannabinoid receptor antagonists are a promising approach in the treatment of metabolic disorders. Their efficacy was recently demonstrated not only in animal models,[1] but also in several clinical studies targeting different patient populations including obese dyslipidemic[2] and diabetic[3] patients. However, rimonabant[4] (Fig. 1), the first CB_1 antagonist to reach the market, was withdrawn in 2008 by the European Medicines Agency. The agency considered that the experience with rimonabant had indicated that the incidence of serious psychiatric disorders (mainly depression) may be more common than in the clinical trials and concluded that the benefits of rimonabant no longer outweighed its risks.

One attractive approach to the development of safer CB_1 antagonists would be the identification of peripherally-restricted drugs. While psychiatric side effects are most likely centrally mediated, several lines of evidence indicate that the mechanism of action of CB_1 antagonists on metabolic disorders involved both central and peripheral pathways.[5,6] CB_1 receptors expressed in adipocytes,[7] in the liver[8] but also in skeletal muscle[9] and in pancreatic β-cells[10] maybe involved in the effects of CB_1 antagonists on body weight, dyslipidemia, hepatic steatosis, and glucose metabolism.

At least three putative CB_1 peripherally-restricted antagonists have been described in the literature. The triazole derivative LH-21 (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole) was first described as a peripherally acting neutral CB_1 antagonist.[11] However, it was recently demonstrated that the compound was actually brain penetrant and acted on body weight by a non-CB_1 mechanism.[12]

Recently, URB447 ([4-amino-1-[(4-chlorophenyl) methyl]-2-methyl-5-phenyl-1H-pyrrol-3-yl] phenyl-methanone) was characterized as a mixed CB_1 antagonist (IC_{50} = 313 nM)/CB_2 agonist (IC_{50} = 41 nM). URB447 was not detected in brain following systemic acute administration (20 mg/kg ip) but effectively reduced food intake and body weight gain in genetically obese ob/ob mice following ip administration over a 14 day period.[13]

More recently a more potent CB_1 antagonist (IC_{50} = 0.19 nM)/inverse agonist (IC_{50} = 0.10 nM), (5-(4-chloro-phenyl)-1-(2-chloro-phenyl)-3-[4-(4-fluoro-phenyl)-4-hydroxy-piperidine-1-carbonyl]-1H-pyrazole-4-carboxamide) was identified with higher polar surface and lower lipophilicity than rimonabant. A pharmacokinetic study demonstrated that in mice, 6 h after oral administration a 10-fold higher concentration in plasma than brain was reached. Interestingly, this compound showed effects on body weight loss comparable to rimonabant after subchronic oral administration (10 mg/kg) in the DIO (diet induced-obese) mouse model. However, some contribution of central effect could not be definitively ruled out.[14]

However, the need for a potent, selective and orally active peripherally-restricted CB_1 antagonist remains clear.

The 2,3-diarylpyrrole scaffold was chosen as a starting point for our rational design approach to peripherally-restricted CB_1 antagonists. This new scaffold was discovered during our extensive

* Corresponding author.
E-mail address: francis.barth@sanofi-aventis.com (F. Barth).

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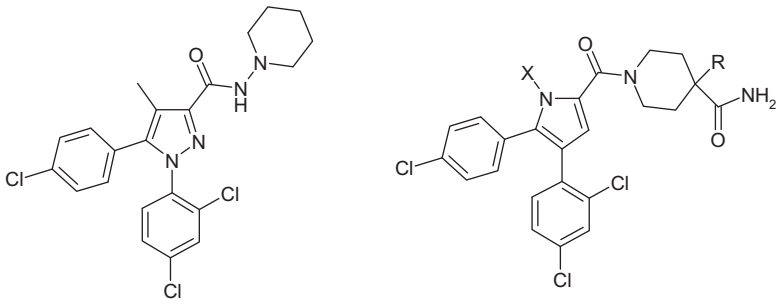


Figure 1. Structure of rimonabant and polar pyrrole bioisostere compounds **2**–**11**.

efforts of scaffold hopping in which the pyrazole ring of SR141716 was replaced by various heterocyclic five-membered rings.[15,16] 1,5-Diarylpyrroles CB_1 antagonists have also been described in the patent literature.[17] However, the 2,3-diarylpyrrole series appeared as an ideal starting point to design CB_1 peripherally-restricted antagonists because high potency (sub-nanomolar activity) could be obtained in the series. This was important because a loss of affinity was expected to occur during the optimization to a less brain-penetrant compound. Moreover the nitrogen substituent could be easily modulated via different types of N-alkylation reaction, affording an additional modification point in the SAR exploration.

Based on several studies conducted over the years, a picture of the molecular determinants of blood–brain barrier (BBB) permeation is now available. In brief, increasing hydrogen bonding capacity, molecular weight and polar surface area (PSA), introducing acidic function or decreasing lipophilicity ($c \log P < 1$) are all associated with decreased brain penetration.[18,19] Unfortunately, increasing polarity, molecular charge and lowering $\log P$ is also usually associated with a decrease in affinity towards the CB_1 receptor. Furthermore several of the factors which decrease blood–brain barrier permeation also decrease intestinal barrier permeation and therefore negatively impact oral bioavailability. We decided to focus on the topological polar surface area (TPSA), a molecular determinant which can be modulated by simple structural changes, is easily predicted using molecular polar surface calculation,[20] and has been shown to be a dominating determinant for brain penetration.[21]

Our strategy was based on a conservation of the positions of three substituents (chlorophenyl, dichlorophenyl, and carbonyl group) connected around the pyrrole core.

Compound **2**[15] (Table 1) was selected for further modifications with the objective of increasing TPSA. During our optimization process, we routinely used brain ex vivo binding to roughly assess brain penetration. The compound was administered iv (10 mg/kg) to mice 30 min before they were killed by decapitation. Then the inhibition of [^{3}H]-CP55940 binding was performed in homogenized brain (without the cerebellum) as previously described.[22] In this assay, compound **2** inhibited 86% of CP55940 binding, indicating a good brain penetration, in accordance with its calculated TPSA of 68 Å^2.

The compounds **2–11** were synthesized as shown in Scheme 2 from the key intermediate pyrrole **B**.[23] The latter was easily accessible by a Suzuki coupling with 4-iodo-2,3-dihydropyrrole **A** using 2,4-dichlorophenyl boronic acid followed by a one pot detosylation and aromatization with two equivalents of 1,8-diaza-bicyclo[5.4.0]undecene (DBU) in hot dimethyl formamide (DMF) as indicated in Scheme 1. The 4-iodo-2,3-pyrrole intermediate **A** was accessible from homopropargylic tosylamide according to a brief and straightforward approach described in the litterature.[24]

N-Methyl compounds **2, 3, and 4** were synthesized by alkylation of the pyrrole **B** with methyl iodine in the presence of potassium carbonate in dichloromethane (DCM) followed by hydrolysis of the ester group in standard basic condition, acidification and coupling with 4-substituted-4-carboxamidopiperidine in the presence of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) in DMF. On the opposite, carboxylic acids **6** and **7** were obtained by conversion of the ester **B** in the corresponding amide as described above, alkylation of the pyrrole nitrogen by methyl acrylate using catalytic amount of TritonB as a base for compound **6**, or by alkylation with methyl 5-chloro-2,2-dimethyl valerate in refluxing acetone in the presence of potassium carbonate in the case of **7**. Mild hydrolysis conditions of the esters were required to generate the carboxylic acids in the last step.

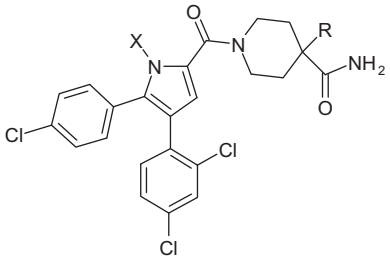
For the introduction of the sulfonyl group, methyl-vinylsulfone was used as a Michael acceptor in the same conditions as described for the acrylate reagent above; mild hydrolysis of the ester was performed with lithium hydroxide in a THF/water mixture at 90 °C followed by the usual coupling conditions to afford compound **8**. Similarly, compound **5** was obtained when acrylonitrile was used as Michael acceptor. To prepare the methylsulfonamides 9–**11**, the intermediate cyanoester **C** was selectively reduced to aminoester using sodium borohydride in presence of Cobalt(II) chloride hexahydrate.[25] The sulfonamides **9**–**11** were finally obtained by mesylation of the amino group followed by the standard sequence hydrolysis/acidification/coupling.

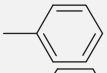
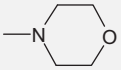
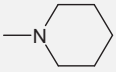
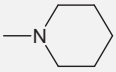
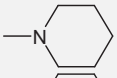
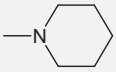
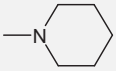
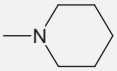
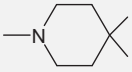
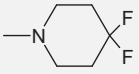
In vitro, the affinity of compounds **2–11** for mice brain CB_1 receptors was evaluated as described by Rinaldi-Carmona et al.,[4] while brain penetration was assessed using the ex vivo binding in mice described above. The results are reported in Table 1, together with calculated TPSA.

Starting from the high affinity compound **2** we decided first to replace the phenyl substituent of the piperidine ring by less lipophilic groups (morpholine, piperidinyl)[27]; those modifications afforded diversely active compounds. Introduction of a morpholine ring (compound **3**) increased TPSA but reduced the CB_1 affinity (about 50-fold). Interestingly, the piperidinyl analog **4** was even more potent than compound **2**. In line with its similar TPSA, the compound was brain penetrant too.

In order to further increase TPSA, we decided to introduce additional heteroatoms in the molecule by substituting the pyrrole nitrogen with alkylene groups bearing various polar substituents. Introduction of the ethylcyano substituent afforded a reasonably potent compound **5** with a moderate brain penetration, in line with its TPSA of 83 Å^2. Introduction of carboxylic acids groups afforded even more polar compounds (TPSA above 100 Å^2) but unfortunately to the detriment of affinity. Interestingly, increasing chain length and steric bulk (*gem*-dimethyl group) around the acid function as in compound **7** does not alter the binding compared to **6**.

Table 1
rCB_1 and rCB_2 in vitro binding, ex vivo rat brain binding and TPSA by representative 1-[5-(4-Chloro-phenyl)-4-(2,4-dichloro-phenyl)-1H-pyrrole-2-carbonyl]-piperidine-4-carboxamides



Compound	R	X	rCB_1 binding IC_{50}[a] (nM)	rCB_2 binding % inhibition at 1 μM[a]	Ex vivo brain binding % inhibition[b]	TPSA[c] (Å^2)
1	(SR141716)	—	2.5	66%	100%	50
2		Me	0.7	17%	86%	68
3		Me	34	42%	32%	81
4		Me	0.2	48%	96%	72
5		–$(CH_2)_2CN$	8	18%	39%	83
6		–$(CH_2)_2CO_2H$	180	14%	nt	109
7		–$(CH_2)_3C(CH_3)_2CO_2H$	160	52%	nt	109
8		–$(CH_2)_2SO_2Me$	0.4	26%	19%	114
9		–$(CH_2)_3NHSO_2Me$	1.4	16%	18%	126
10		–$(CH_2)_3NHSO_2Me$	4.3	27%	33%	126
11		–$(CH_2)_3NHSO_2Me$	1.0	39%	28%	126

nt, not tested.
[a] rCB_1 from rat brain synaptosomal membranes, rCB_2 from rat spleen, as described in Ref. 4.
[b] At 10 mg/kg, iv. Determined as described in Ref. 22.
[c] Calculated TPSA using ACDv9 software.[26]

Introducing the sulfonyl and amino sulfonyl groups further increased the TPSA to 126 Å^2 and afforded the most interesting compounds. Compounds **8** and **9** are both very potent, and display very low ex vivo brain binding compared to **1**.

Oral activity of compound **9** was then assessed in the intestinal transit test[22] (Table 2). Compared to rimonabant, **9** was only moderately active in this assay (Table 2). In vitro microsome metabolism studies allowed us to pin-point an important oxidation on the piperidine rings.[28] To prevent this metabolic pathway, we attempted to block the 4 position of the piperidyl group by introducing *gem*-dimethyl or *gem*-difluoro groups.

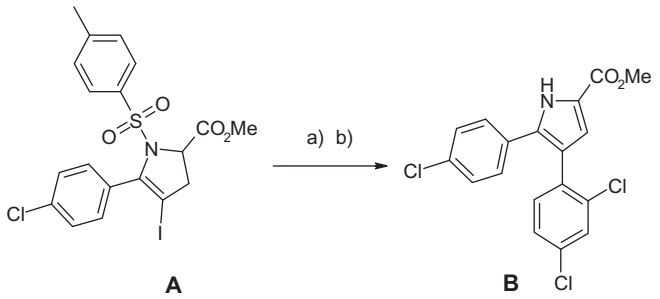
Compared to analog **9** the respective resulting compounds **10** and **11** have increased microsomal stability while retaining high affinity and low brain penetration. Both were significantly more active following oral administration in the intestinal transit test.

We decided to focus on the most metabolically stable compound **11** to assess brain penetration. Drug levels were measured in plasma and brain after systemic administration of compound **11** (10 mg/kg po) to male Swiss CD1 mice. Plasma levels peaked 2 h after administration (C_{max} = 570 ng/mL), while the maximal concentration in brain was obtained after 6 h and did not exceed 6.4 ng/g of tissue. Brain to plasma $AUC_{(0-24h)}$ ratio was 0.01. Combined with the intravenous route (3 mg/kg), the oral bioavailability of compound **11** was assessed at 37% in Tween 80/DMSO/water (0.5:10:89.5; v/v/v).

In line, with this low brain penetration, and to the contrary of rimonabant, compound **11** was also ineffective in reversing CP55940-induced hypothermia in mice (Table 2).

Compound **11** was also a potent ligand for human recombinant CB_1 receptors expressed in CHO cells (IC_{50} = 1.4 nM) and was selective versus human and rat CB_2 receptors (IC_{50} > 1 μM). Compound **11** also dose-dependently antagonized CP55940-induced inhibition of forskolin-stimulated cAMP accumulation in U373-MG cells[29] with an EC_{50} of 15 nM.

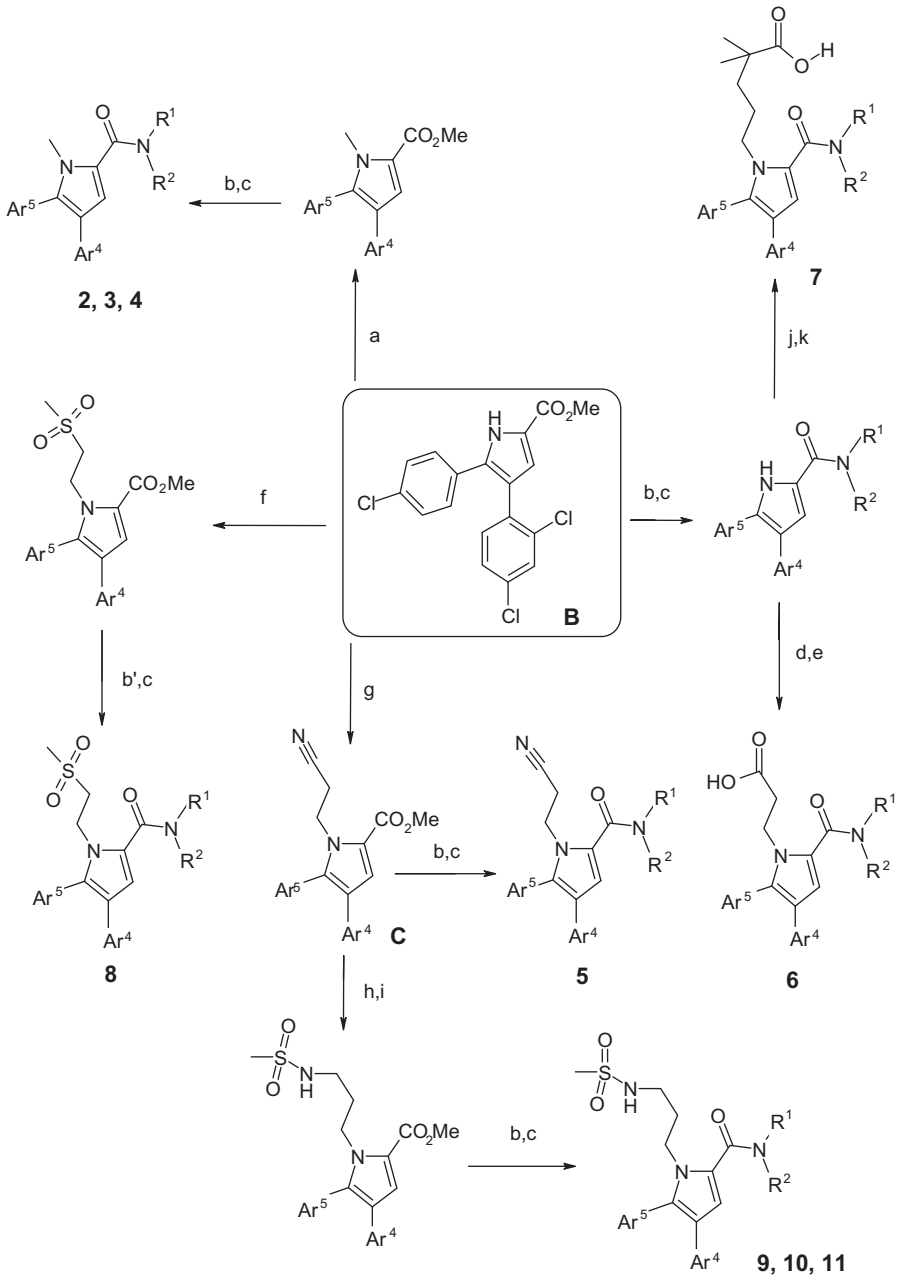
Complementary in vitro functional test using CHO cells co-expressing hCB_1 receptor and a cAMP response element (CRE)-



Scheme 1. Reagents and conditions: (a) 1 equiv 2,4-dichlorophenyl boronic acid, Pd Tetrakis, 3 equiv Na_2CO_3 aq, toluene/MeOH, 70 °C; (b) 2 equiv DBU, DMF, 110 °C.

luciferase reporter gene[30] showed that compound **11** behaved as a weak CB_1 inverse agonist (10% stimulation at 1 μM).

In conclusion, we presented a successful strategy around a pyrrole core leading to a series of high affinity analogs with significantly increased TPSA compared to rimonabant. Our investigations of different types of functionalities attached to a variable carbon linker allowed us to identify a sulfonamide diarylpyrrole **11**, presenting a non-brain penetrant CB_1 antagonist profile with a 100-fold higher exposure in plasma than brain. In order to assess the potential therapeutic value of the peripheral compound **11** versus rimonabant, complementary studies are under investigation in several models of metabolic disorders.



Scheme 2. Reagents and conditions: (a) 2 equiv MeI, 3 equiv K_2CO_3, DCM, rt; (b) 10 equiv KOH, MeOH/water, reflux, HCl aq; (c) 1.3 equiv 4-substituted-4-acetamidopiperidine, DiPEA, HBTU, HOBt, DMF, rt; (d) 5 equiv methyl acrylate, TritonB, dioxane, reflux; (e) 10 equiv KOH, MeOH/water, reflux; HCl aq; (f) 5 equiv methyl vinylsulfone, TritonB, dioxane,70 °C; (g) 5 equiv acrylonitrile, TritonB, dioxane, reflux; (h) 5 equiv $NaBH_4$, 2 equiv $CoCl_2 6H_2O$, THF/water, 0 °C to rt; (i) 1.1 equiv MsCl, 2 equiv NEt_3, DCM, rt; (j) methyl-5-chloro-2,2-dimethyl valerate, K_2CO_3, acetone, reflux; (k) 10 equiv LiOH, THF/water, reflux, HCl aq.

Table 2
Reversion of CP-induced intestinal transit inhibition and hypothermia

Compound	Intestinal transit[a] DE_{50} po mg/kg	Hypothermia[b] DE_{50} po mg/kg
1 (SR141716)	1	0.6
9	51% at 10 mg/kg	1% at 30 mg/kg
10	4.1	0% at 30 mg/kg
11	2.4	0% at 30 mg/kg

nt, not tested.
[a] Reversion of CP55940 (0.15 mg/kg, ip) induced inhibition of intestinal transit.[22]
[b] Reversion of CP55940 (0.3 mg/kg, ip) induced hypothermia.[22]

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28. The metabolism of compound **9** was investigated in vitro using hepatic microsomal fractions prepared from mouse, rat and humans following in house procedure:
Microsomal proteins concentration = 1 mg/mL, substrate concentration = 5 μM, incubation duration = 10 min. Cytochrome P-450 (CYP) and Flavin-containing monooxygenases (FMO) cofactor = 1 mM NADPH. Chromatographic analysis of the supernatant fluids was performed after removal of precipitated proteins using an HPLC (column:YMC-Pack J sphere H80) coupled with UV (254 nm) and mass spectrometry detection.
The main metabolic pathway observed was hydroxylation (mouse species) on the piperidine moiety, di and tetra-dehydrogenation on the bi-piperidinyl moiety (rat and humans species).
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