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Identification and hit-to-lead optimization of a novel class of CB1 antagonists

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ABSTRACT

The discovery, synthesis and preliminary structure–activity relationships (SARs) of a novel class of CB1 antagonists is described. Initial optimization of benzimidazole-based screening hit **4** led to the identification of 'inverted' indole-based lead compound **18c** with improved properties versus compound **4** including reduced *A* log *P*, improved microsomal stability and improved aqueous solubility. Compound **18c** demonstrates in vivo CB1 antagonist efficacy (CB1 agonist induced hypothermia model) and is orally bioavailable in rat.

A large number of CB1 antagonists and inverse agonists have been reported in the literature as potential therapeutic agents for the treatment of obesity.[1] In fact, the archetypical CB1 inverse agonist Rimonabant was approved in Europe for this indication, although its marketing was recently discontinued by the European Medicines Agency (EMEA) due to an increased risk of psychiatric disorders.[2] Many CB1 antagonists reported to date are similar in structure to Rimonabant, sharing the common structural motif of two adjacent phenyl groups (usually chloro-substituted) on a five or six-membered heterocyclic scaffold. Most of these CB1 antagonists are highly lipophilic, with calculated log *P*'s >5 (i.e., Rimonabant *A* log *P* = 7.02).[3]

Recent reports of efforts to reduce the log *P* –aimed at the improvement of pharmaceutical properties of CB1 antagonists– have appeared in the literature.[4] Some of these efforts have met with limited success in that improvements in lowering log *P* have resulted in lower in vitro[4a,4b] and/or in vivo[4a] potency versus the initial, highly lipophilic lead molecule. Herein is described a similar effort to improve physicochemical properties (including reduction of log *P*) during the course of hit-to-lead (HtL) optimization around a novel series of CB1 antagonists which are structurally distinct from most known ligands.

In a program aimed at the identification of a novel and structurally distinct class of CB1 antagonists, high-throughput screening (HTS) of a diverse set of combinatorial screening libraries, prepared using ECLiPS™ technology,[5] was undertaken. Multiple active structures, or hits, were identified in this HTS campaign. Among these was compound **4** (Fig. 1) which was chosen as a starting point for a HtL program, with the task of evaluating this hit class in terms

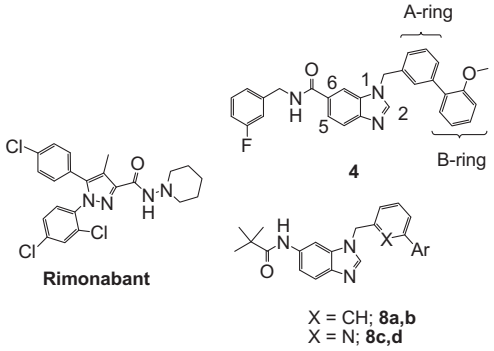

Figure 1. Structures of Rimonabant, and benzimidazole-based CB1 antagonists **4** and **8a–d.**

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of both target independent (e.g., physicochemical properties) and target dependent (e.g., potency) properties.[6] The main objective of this HtL program was to deliver a reasonably potent lead compound (IC_{50} <100 nM) with appropriate drug-like physicochemical properties[7] to serve as the basis for further lead optimization efforts. Importantly, we sought to minimize log *P* (*A* log *P* <5) and achieve modest aqueous solubility (>10 μg/mL).

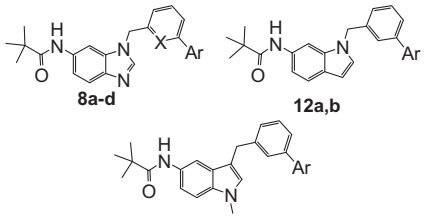
The compounds appearing in Tables 1 and 2 were synthesized via the general schemes depicted in Schemes 1–3. As illustrated in Scheme 1, benzimidazole **4** was prepared in four steps from 3-fluoro-4-nitrobenzoic acid. Benzimidazoles **8a–d**, which contain a retroamide at C(6), were also prepared from the same starting material with a key step being a Curtius rearrangement of compound **1** to give intermediate **5**. Boc-deprotection followed by acylation gave intermediate **6**. Finally, an S_NAr_2 reaction of intermediate **6** with either 3-bromobenzylamine or (6-bromopyridin-2-yl)methanamine, to give intermediates **7a** or **7b**, followed by Suzuki coupling with the appropriate arylboronic acids gave compounds **8a–d**. Indoles **12a,b** and **13a–d** were prepared in four steps from commercially available 6-nitroindole **9** (Scheme 2). Lastly, 'inverted' indoles **17a,b** and **18a–d** were prepared from 5-nitroindole **14** with the key steps being reaction of **14** with either 3-bromobenzyaldehyde or 2-bromo-5-formylpyridine followed by reduction with triethylsilane in the presence of trifluoroacetic acid to give intermediates **15a,b** (Scheme 3).

The compounds in Tables 1 and 2 were evaluated for CB1 antagonistic activity in a luciferase-based CB1 reporter assay.[8,9] In addition, metabolic stability was determined in both human liver microsome (HLM) and mouse liver microsome (MLM) assays.[10] The optimization effort around benzimidazole **4** initially entailed exploration of SAR around both the A and B-rings of the biaryl moiety and around the C(6) amide functionality. This effort gave rise to a series of retroamides exemplified by structures **8a,b** (A-ring = phenyl) and **8c,d** (A-ring = 2,6-disubstituted pyridyl) (see Table 1).

Interestingly, compound **8c** was not only 5-fold more potent than compound **4**, but it also had modestly improved microsomal stability. The salient SAR of compounds **8a–d** and related analogs regarding potency are the following: (1) *meta*-substitution of the A-ring in the biaryl moiety is highly preferred; (2) substitution at the 2-position of the benzimidazole scaffold is not tolerated; (3) amido substitution at C(6) versus C(5) is preferred with sterically bulky amides (e.g., pivalylamide) being optimal; (4) a secondary amide is preferred over tertiary amide (i.e., N-H>N-Me); and, (5) *ortho* and/or *meta*-substitution of the B-ring is preferred versus *para*-substitution, with alkoxy substituents preferred versus alkyl, halo, etc.

One concern which arose about these, and related, benzimidazole-based structures was the lack of microsomal stability. Although compounds **8a** and **8c** represented a modest improvement over compound **4**, we sought further improvement in this re-

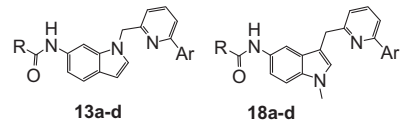
Table 1
In vitro potency, *A* log *P* values, and microsomal stability of benzimidazoles **4** and **8a–d**, indoles **12a,b** and 'inverted' indoles **17a,b**



Compds	X	Ar	IC_{50} (μM)[a]	*A* log *P*	HLM/MLM (% rem. @ 0.5 h)
4			0.104 (±.007)	5.63	2/0
8a	CH		0.064 (±.003)	5.09	35/2
8b	CH		0.269 (±.122)	5.51	ND
8c	N		0.021 (±.006)	4.58	28/17
8d	N		0.073 (±.001)	5.00	14/1
12a			0.051 (±.018)	5.31	72/65
12b			0.058 (±.023)	5.73	51/58
17a			0.037 (±.002)	5.70	52/29
17b			0.045 (±.001)	6.12	70/72

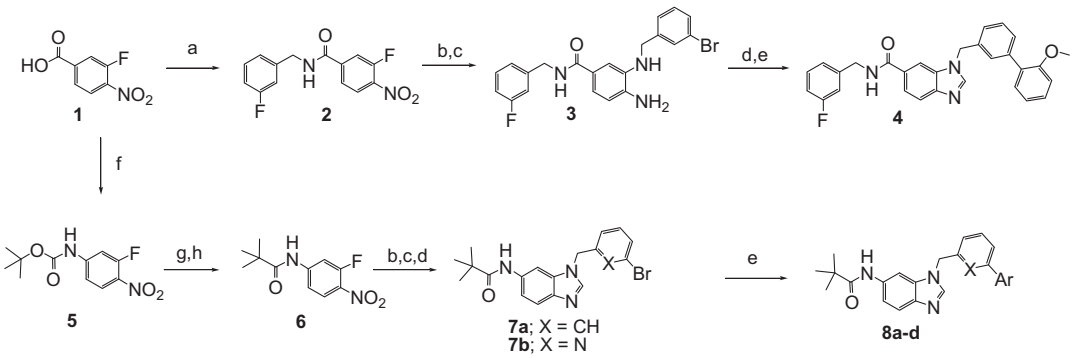
[a] Values are means of at least two experiments, standard deviation is given in parentheses.

Table 2
In vitro potency, *A* log *P* values, microsomal stability and aqueous solubility of indoles **13a–d** and 'inverted' indoles **18a–d**



Compds	R	Ar	IC_{50} (μM)[a]	*A* log *P*	HLM/MLM (% rem. @ 0.5 h)	aq sol (μg/mL)
13a			0.007 (±.002)	3.71	62/52	3.2
13b			0.045 (±.014)	4.13	45/54	11.7
13c			0.003 (±.001)	3.42	51/53	<1
13d			0.023 (±.010)	3.84	35/44	17.9
18a			0.007 (±.001)	3.90	56/16	<1
18b			0.027 (±.010)	4.32	47/5	2.0
18c			0.005 (±.001)	3.60	56/36	14.9
18d			0.019 (±.006)	4.02	41/14	10.6

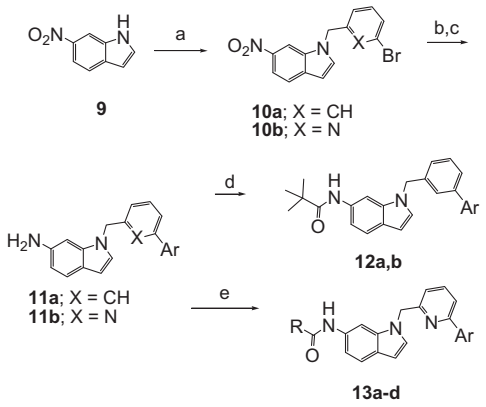
[a] Values are means of at least two experiments, standard deviation is given in parentheses.



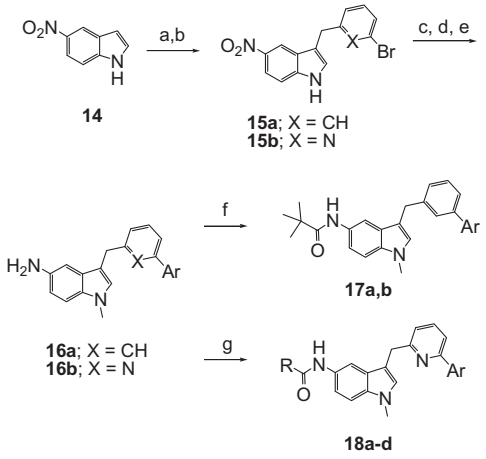
Scheme 1. Reagents and conditions: (a) *i*-butylchloroformate, NMM, THF, 0 °C, 90 s; 3-fluorobenzylamine; (b) 3-bromobenzylamine or (6-bromopyridin-2-yl)methanamine, DIPEA, ACN, 40 °C; (c) Fe, EtOH/H_2O/AcOH, reflux; (d) triethylorthoformate, TsOH·H_2O (cat.); (e) Ar-$B(OH)_2$, PS-PPh_3-Pd, 1 N K_2CO_3 (aq), EtOH, microwave @ 120 °C, 5 min; (f) DPPA, TEA, *t*-BuOH, dioxane, reflux; (g) 50% TFA/DCM; (h) trimethylacetylchloride, TEA, DCM.

gard. Part of our strategy for further improvements in properties, including microsomal stability, was to employ the concept of 'scaffold-hopping'. It was discovered that replacement of the benzimidazole scaffold with either indole (structures **12a,b**) or 'inverted' indole (structures **17a,b**) led to improvement in microsomal stability, while maintaining good potency.

While these indoles and 'inverted' indoles demonstrated adequate potency and microsomal stability they lacked aqueous solubility (i.e., measured aqueous solubility was <1 μg/mL)[11] and were more lipophilic than desired (i.e., *A* log *P*'s >5). In an attempt to lower *c* log *P* and improve aqueous solubility, while maintaining adequate potency, the introduction of polar hydroxyl functionality into the C(6) amido substituent of both indoles and 'inverted' indoles was examined (Table 2). It had been demonstrated in our earlier HtL efforts that the steric bulk of the pivalylamide substituent at C(6) was important for potency. Therefore, we chose to incorporate either 3-hydroxy-3-methylbutanamide or 3-hydroxy-2,2-dimethyl-propionamide substituents at C(6) because of their similar steric demand relative to the pivalylamide substituent contained in potent analogs **12a,b** and **17a,b**. To minimize *c* log *P*, a 2,6-disubstituted pyridyl ring, which was demonstrated to be an adequate surrogate for phenyl in the benzimidazole series (e.g., compare **8a** to **8c** in Table 1), was incorporated at the A-ring position. Both 4-benzo[1,3]dioxolyl and 2-methoxy-4-fluoro phenyl



Scheme 2. Reagents and conditions: (a) 1-bromo-3-(bromomethyl)benzene or 2-bromo-6-(bromomethyl)pyridine, K_2CO_3, acetone, reflux; (b) Ar-B$(OH)_2$, 1 N Na_2CO_3 (aq), Pd$(PPh_3)_4$, dioxane, reflux; (c) raney Ni, H_2 (1 atm), THF; (d) trimethylacetylchloride, TEA, DCM; (e) 3-hydroxy-3-methylbutyric acid or 3-hydroxy-2,2-dimethylpropanoic acid, DIPEA, HATU, DCM.



Scheme 3. Reagents and conditions: (a) 3-bromobenzyaldehyde or 2-bromo-5-formylpyridine, NaOMe, MeOH; (b) TFA, Et_3SiH, DCM; (c) NaH, THF; add CH_3I; (d) Ar-B$(OH)_2$, 1 N Na_2CO_3 (aq), Pd$(PPh_3)_4$, dioxane, reflux; (e) raney Ni, H_2 (1 atm), THF; (f) trimethylacetylchloride, TEA, DCM; (g) 3-hydroxy-3-methylbutyric acid or 3-hydroxy-2,2-dimethylpropanoic acid, DIPEA, HATU, DCM.

were incorporated at the B-ring position as these substituents were earlier demonstrated to be optimal for potency.

Four out of eight of the analogs in Table 2 met our requirement for aqueous solubility (>10 μg/mL). Of these, compound **18c** had the best potency in the luciferase assay (5 nM), and it also had adequate HLM stability (i.e., >50% remaining @ 0.5 h). In addition, this compound is substantially less lipophilic than highly lipophilic CB1 antagonists such as Rimonabant (i.e., Rimonabant *A* log *P* = 7.02 versus compound **18c** *A* log *P* = 3.60).[3]

Because it had the best balance of potency, microsomal stability and aqueous solubility, compound **18c** was further profiled in vivo including an in vivo efficacy model (i.e., ability to reverse WIN 55,212-2 induced mouse hypothermia)[12] and rat pharmacokinetics (PK). Compound **18c** demonstrated a partial reversal of hypothermia at a dose of 30 μmol/kg and nearly full reversal of hypothermia at a dose of 100 μmol/kg (compound dosed sc) resulting in an ED_{50} of ~30 μmol/kg. In comparison, Rimonabant demonstrated an ED_{50} of 0.8 μmol/kg in this model (Table 3).[13] The relatively weak activity of **18c** in the mouse hypothermia model is perhaps due to lower affinity of this compound at the mouse CB1 receptor versus the human CB1 receptor.[14] Rat PK data for compound **18c** is summarized in Table 4.

Table 3
Effect of compound **18c** and Rimonabant on WIN 55,212-2 induced hypothermia in mice

Dose (μmol/kg, sc)	Percent (±sem) reversal of WIN 55,212-2 (10 μmol/kg, sc) induced hypothermia	
	18c	Rimonabant
0.01	—	−0.2 ± 4.6
0.1	—	13.8 ± 5.7
0.3	—	14.7 ± 14.9
1	—	60.2 ± 15.3
3	—	116.7 ± 3.8
10	—	113.4 ± 4.8
30	46.8 ± 10.7	100.4 ± 2.2
100	92.5 ± 2.4	—
ED_{50} (95% Cl)	~30	0.8 (0.5–1.5)

Table 4
Pharmacokinetic parameters for **18c** in male Wistar rats at 5 mpk po and 1 mpk iv[a]

$AUC_{po\ 0-7.5\ h}$	1292 ng h/mL
F_{po} (%)	37
CL_{iv} (mL/min/kg)	19
V_{ss} (L/kg)	1.2
$T_{1/2,\ iv}$ (h)	1.0

[a] Vehicle for po dosing is 0.5% gelatin in 5% mannitol (aq) and for iv dosing is 20% *N*,*N*-dimethylacetamide in saline.

Compound **18c** had reasonable oral bioavailability (*F* = 37%), moderate clearance (CL = 19 mL/min/kg) and volume of distribution (V_{ss} = 1.2 L/kg) and a half-life of 1 h.

In conclusion, we report a novel series of CB1 antagonists that were developed from a benzimidazole-based screening hit. Replacement of the benzimidazole scaffold with isosteric indole and 'inverted' indole, and incorporation of a hydroxyl moiety into the amide side-chain, led to structures with improved properties including lower log *P*, enhanced aqueous solubility, and better stability to both human and mouse liver microsomes. In addition, compound **18c** demonstrated in vivo efficacy in a CB1 agonist induced hypothermia model and was orally bioavailable in rat.

Acknowledgements

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8. CB1 reporter assay. Chinese hamster ovary cells expressing the human CB1 receptor and containing a luciferase gene under the regulatory control of seven AP1 response element repeats were grown at 37 °C and 5% CO_2 in DMEM/F12 (Cellgro) containing 10% FBS (Fetalclone II, Hyclone Cat. #SH30066.03), 0.2 mg/mL Geneticin, 0.5 mg/mL Hygromycin, 1 μg/mL Fungizone, 5 mL Penicillin/Streptomycin (Invitrogen Cat. #15070-063) and 2 mM GlutaMax (GIBCO Cat. #35050-061). The cells were seeded into white 96-well plates at a density of 3×10^4 cells per well in 100 μL/well DMEM/F12 without Phenol Red containing Penicillin/Streptomycin and Fungizone and incubated at 37 °C overnight. Test compounds were serially diluted in DMEM/F12 containing 3% bovine serum albumin, and 10 μL of each compound dilution was transferred to the appropriate wells of the cell plate. After 5 min, 10 μL of 10^{-6} M CP-55940 in DMEM/F12 without BSA were added to all wells of the assay plate. After 5 h at 37 °C, detection was performed by adding 100 μL/well Bright-Glo, incubating the assay plate at RT for a further 5 min and counting for 0.5 s/well in a Wallac Microbeta Trilux.
9. The CB1 receptor affinity (displacement of specific binding of [^{3}H]CP-55,940 in commercially available membranes prepared from Sf9 cells expressing the human CB1 receptor) of certain compounds was assessed and found to be in good agreement with the data obtained in the luciferase-based CB1 reporter assay. For example, compounds **8b**, **12b** and **17b** had IC_{50} values of 20 nM, 13 nM and 8 nM, respectively, in the CB1 binding assay.
10. Liver microsome stability assays were performed as follows: Assay mixtures typically contained human or mouse microsomes (300 nM cytochrome P450, BD Gentest, Woburn, MA), phosphate buffer (pH 7.4), 1 μM test compound, 1 mM NADPH in a final assay volume of 0.1 mL. Incubations were for 30 min at 37 °C and were terminated by the addition of 0.2 ml of acetonitrile. Samples were centrifuged at 2000*g* and analyzed by LC/MS. The percentage of compound remaining at 30 min was calculated.
11. Aqueous solubilities were determined using a medium-throughput adaptation of a shake-flask methodology. A 10 mM solution of the test compound in DMSO was added to 0.05 M phosphate buffered saline pH 7.4 such that the final concentration of DMSO was 2%. The resultant mixture was then vortex mixed (1500 rpm) for 24 ± 0.5 h at 21 ± 2 °C. After mixing, the resultant solution/suspension was filtered under vacuum using a filter plate (Millipore Multiscreen HTS, 0.4 μM). The concentration of the compound in the filtrate was determined by High Performance Liquid Chromatography (HPLC) running a generic acid gradient method with UV detection at 230 nm. Peak areas from analysis of the diluted filtrates were quantified by comparison to a calibration line prepared by injecting onto the HPLC three different volumes of a 50 μM solution of the test compound in DMSO. Solubilities were determined in duplicate for each test compound and average values reported.
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13. WIN 55,212-2 induced hypothermia test. The antagonist or vehicle (5% mulgofen in saline, 10 ml/kg) was administered sc 75 min before rectal temperature was measured. WIN 55,212-2 mesylate (10 μmol/kg, 10 ml/kg) was administered sc 60 min prior to the rectal temperature measurement. The 60 min pre-treatment with WIN 55,212-2 mesylate corresponded to the maximal hypothermia attained by this agonist. Rectal temperature was measured using a metal probe with a Fluke 51 K/J thermometer. The probe was covered in a lubricant (vaseline) and was inserted approximately 1.5 cm into the rectum.– The highest temperature stable for 10 s was recorded. Following completion of the test animals were humanely terminated.
14. Compound **18c** and other compounds from this series were demonstrated to have significantly lower affinity at the mouse CB1 receptor versus the human CB1 receptor. For example, compound **18c** was found to be ~14-fold less potent at the mouse CB1 receptor than at the human CB1 receptor as determined in a mouse brain membrane binding displacement assay (unpublished data).