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Structure–activity relationships of 2,4-diphenyl-1*H*-imidazole analogs as CB2 receptor agonists for the treatment of chronic pain

Shu-Wei Yang [a,*], Jennifer Smotryski [a], Julius Matasi [a], Ginny Ho [a], Deen Tulshian [a], William J. Greenlee [a], Rossella Brusa [b], Massimiliano Beltramo [b], Kathleen Cox [c]

[a] Department of Chemical Research—CV & CNS, Merck Research Laboratory, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA
[b] Neurobiology Department of Biological Research, Merck Research Laboratory, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA
[c] Department of Drug Metabolism, Merck Research Laboratory, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

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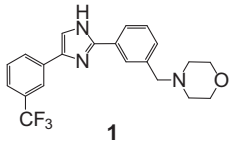
ABSTRACT

A series of 2,4-diphenyl-1*H*-imidazole analogs have been synthesized and displayed potent human CB2 agonist activity. Many of these analogs showed high functional selectivity over human CB1 receptors. The syntheses, structure–activity relationships, and selected pharmacokinetic data of these analogs are described.

Cannabinoid receptors, members of the superfamily of G protein-coupled receptors, mediate many of the CNS effects of Δ9-tetrahydrocannabinol (Δ9-THC), the active component of *Cannabis sativa* (marijuana).[1] The medical and psychotropic properties of marijuana have been known for centuries. Up to now, two cannabinoid receptors, CB1 and CB2, have been cloned and characterized.[2] Although both receptors and their ligands have been implicated in pain perception, CB1 is expressed predominantly in the brain and its activation is linked to the CNS effects of cannabinoids.[3] In contrast, CB2 is mainly found in peripheral immune cells, sensory afferents, and the spinal cord. Therefore selective CB2 agonists have potential to retain analgesic efficacy with fewer adverse effects associated with CB1 receptors, such as catalepsy, ataxia, sedation, and undesired psychotropic effects.

Selective CB2 agonists have been reported to be efficacious in rodent models of neuropathic or inflammatory pain.[4] Some CB2 agonists including Hu-308,[5] AM1241,[6] GW405833,[7] GW842166X,[8] and JWH133[9] have been investigated in various acute and chronic pain models. The analgesic effects of these selective CB2 agonists were blocked by a CB2 antagonist, but not by a CB1 antagonist. The analgesic effects of AM1241 and GW405833 were not observed in CB2 knockout mice.[6,7] These evidences demonstrated that the analgesic effect against inflammatory pain was mediated by CB2. Although CB2 receptors are considered an attractive analgesic target, more studies are needed to elaborate their clinical potential.

During high-throughput screening of our chemical library, some analogs with the 2,4-diphenyl-1*H*-imidazole skeleton were identified to display high human CB2 (hCB2) affinity and good selectivity against human CB1 (hCB1). Among them, compound **1** displayed high hCB2 affinity (K_i 42 nM) and potent agonist activity (EC_{50} 9 nM, E_{max} 80%) in the cAMP assay. Compound **1** displayed decent selectivity over hCB1. In the pharmacokinetic assay, **1** showed reasonable plasma exposure after oral administration in rats (AUC (0–6 h) 866 nM h, 10 mg/kg oral administration).



1

hCB2 EC_{50}: 9 nM (E_{max} 80%)
hCB2 Ki: 42 nM
hCB1 EC_{50}: >1 uM (E_{max} 67%)
hCB1 Ki: 1 uM
rat PK: AUC (0-6 h) 866 nM.h
(oral admin., 10 mg/kg, 6 h)

In this Letter, we will describe syntheses and structure–activity relationships of this series of 2,4-diphenyl-1*H*-imidazole analogs. The pharmacokinetic data of selected compounds will be discussed.

The synthetic route to the 2,4-diphenyl-1*H*-imidazole analogs is summarized in Scheme 1. The commercially available substituted acetophenone was brominated with bromine and then cyclized with formamide to form 4-phenyl-1*H*-imidazole **2**.[10] Subsequent

* Corresponding author. Tel.: +1 908 740 7291; fax: +1 908 740 7164.
E-mail address: shu-wei.yang@merck.com (S.-W. Yang).

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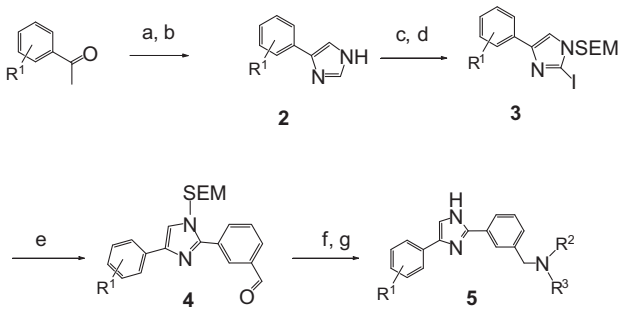


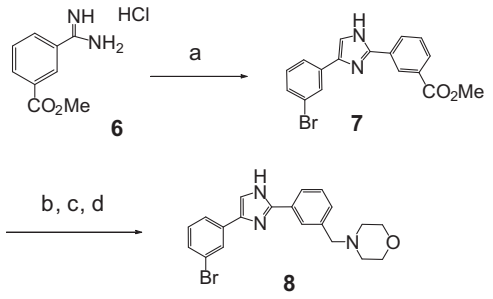
Scheme 1. Reagents and conditions: (a) Br_2, $CHCl_3$, rt; (b) $HCONH_2$, neat, 185 °C, 2 h; (c) SEM-Cl, NaH, THF, rt; (d) *n*-BuLi, I_2, THF, −78 °C to rt; (e) 3-(CHO)Ph-B$(OH)_2$, Pd$(dppf)_2Cl_2$, dioxane–H_2O, 95 °C; (f) amine, NaBH$(OAc)_3$, DCE, rt; (g) 6 N HCl, MeOH, 80 °C.

N-protection with 2-(trimethylsilyl)ethoxymethyl (SEM) chloride and iodination using iodine were achieved under basic conditions to afford **3**. The iodo displacement with 3-phenyl aldehyde was conducted under Suzuki coupling condition using Pd$(dppf)_2Cl_2$ as the catalyst to give **4**. Reductive amination and final removal of the SEM yielded the target compounds (**5**).[11]

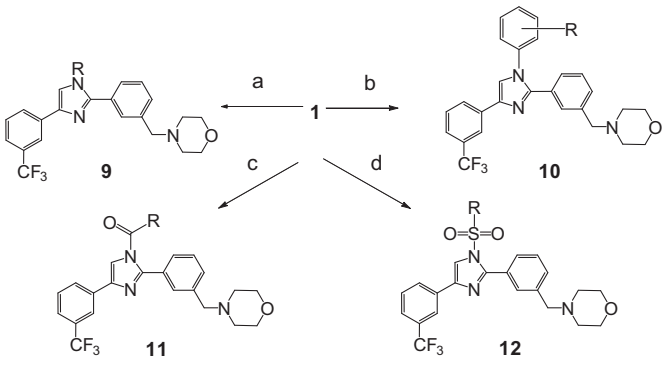
When R_1 was bromine, Suzuki coupling (step e, in Scheme 1) was not successful with **3**. Both bromine and iodine atoms could be displaced with the phenyl group, and this made it difficult to obtain the desired target. Therefore, an alternative route was implemented to prepare target **5** (Scheme 2). Commercially available methyl 3-carbamimidoylbenzoate hydrochloride (**6**) was coupled with 3-bromophenacyl bromide to give the 2,4-diphenyl-1*H*-imidazole moiety in one step. The methyl ester was reduced to the alcohol and then oxidized to aldehyde. Reductive amination then afforded target **8**.

Several N-1 substituted analogs of **1** were synthesized as described in Scheme 3. Alkylation of **1** using alkyl halide and sodium hydride gave **9**. Compound **1** was coupled with phenyl boronic acid using Cu$(OAc)_2$ to generate **10**. Acylation or sulfonylation of **1** afforded analogs **11** and **12**, respectively.

Target compounds were tested for their affinity at the cloned hCB1 or hCB2 receptors expressed in CHO cell membranes by measuring their ability to compete with [H^3]-CP55,940, a non-selective CB1 and CB2 agonist. The functional activities of all synthesized compounds were evaluated by their ability to inhibit forskolin-mediated cAMP accumulation, using CHO cells expressing recombinant hCB1 or hCB2 receptors. Functional efficacy (EC_{50}) and maximum percent activation (E_{max}) of hCB2 were used to evaluate the compound's potency and agonist activity. WIN55212-2 was employed as a reference compound with an EC_{50} value of 4 nM for hCB2 agonist activity.



Scheme 2. Reagents and conditions: (a) 3-bromophenacyl bromide, Na_2CO_3, DMF, rt; (b) $LiAlH_4$, THF, 0 °C; (c) Dess–Martin, DCM, rt; (d) morpholine, NaBH$(OAc)_3$, DCE, rt.

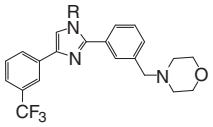


Scheme 3. Reagents and conditions: (a) NaH, X-R, DMF, 100 °C; (b) R-Ph-B$(OH)_2$, Cu$(OAc)_2$, 4 Å mol. sieve, pyridine, DCM, rt; (c) Ac_2O, pyridine, 0 °C; (or) Ph-COCl, Et_3N, DCM, rt; (d) $PhSO_2Cl$, Et_3N, DCM, rt.

The first SAR study focused on N-1 substitution of the middle imidazole ring to identify whether the N-1 proton is important for hCB2 agonist activity. Alkyl substitution of N-1 led to significantly reduced hCB2 functional activity. Examples including the *i*-propyl, *c*-propylmethyl, benzyl, and phenyl analogs (**13**–**16**) are shown in Table 1. The acylated analogs (**17** and **18**) were evaluated for the CB2 agonist activity, and displayed a similar potency to that of **1**. However, both acetyl and benzoyl analogs (**17** and **18**) were unstable under aqueous acidic conditions (1 N HCl, 37 °C). The acyl group was hydrolyzed, and parent **1** was detected. Therefore, these analogs were not further evaluated because of their instability. The N-1 substitution with a phenyl-sulphonyl group (**19**) totally eradicated the hCB2 functional activity. From these SAR data, the NH group was identified to be critical for the hCB2 agonist activity, and preferred not to be substituted.

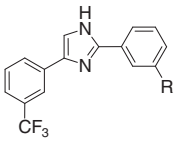
The following SAR was focused on morpholine side chain replacement, and the data are summarized in Table 2. Replacement of the morpholine with thio-morpholine (**20**) retained the hCB2 agonist activity (EC_{50} 6 nM, E_{max} 100%). Methyl substitution on the 2- or 3-position of the morpholine ring led to slightly and significantly reduced functional activity (25 and 169 nM for **21** and **22**, respectively). The piperidinyl analog (**23**) displayed potent full hCB2 agonist activity (EC_{50} 14 nM, E_{max} 104%), indicating that the morpholine oxygen atom was not crucial for potent activity. Hence a series of substituted piperidinyl analogs were prepared for the SAR study. Compound **24** with the 4-methyl piperidinyl moiety displayed similar hCB2 agonist activity compared to that of **23**. In contrast, the 3-methyl piperidinyl analog (**25**) showed a ~fivefold reduction in the hCB2 agonist activity, compared to that of **23**. Analogs **26** and **27** with the 4-fluoro or 4,4-di-fluoro piperidinyl moiety retained potent CB2 functional activity with EC_{50} values of 9 and

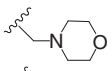
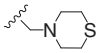
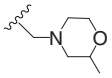
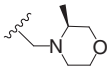
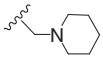
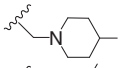
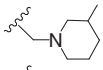
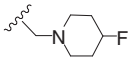
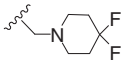
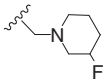
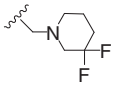
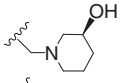
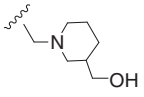
Table 1
SAR of N-1 substituted analogs of **1**



	R	hCB2, EC_{50} (nM) (E_{max})	hCB1, EC_{50} (nM)
13	*i*-Propyl	218	>1000
14	Cyclopropyl-methyl	412 (68%)	>1000
15	Benzyl	>1000	>1000
16	Phenyl	>1000	ND
17	Ac	9 (102%)	>1000
18	Benzoyl	11 (101%)	ND
19	$-SO_2Ph$	>1000	ND

Table 2
SAR of analogs with morpholine, piperidine or thio-morpholine side chain

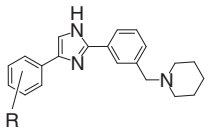


	R	hCB2, EC_{50} (nM) (E_{max})	hCB1, EC_{50} (nM)
1		8 (80%)	>10,000
20		6 (100%)	>1000
21		25 (97%)	>10,000
22		169 (78%)	ND
23		14 (104%)	>10,000
24		21 (97%)	>10,000
25		70 (87%)	ND
26		9 (107%)	2290
27		5 (100%)	2550
28		16 (98%)	ND
29		25 (98%)	ND
30		113 (92%)	>10,000
31		61 (91%)	>1000

5 nM, respectively. Fluoro substitution at the 3-position of the piperidine led to less potent analogs (**28** and **29**). Hydroxyl or hydroxylmethyl substitution at the 3-position of piperidine (**30** and **31**) resulted in an ~eightfold or fourfold decrease in potency, respectively, compared to that of **23**. Overall, the methyl (**24**) or fluoro substitution (**26**–**29**) at the 4-position of piperidine was allowed and retained potent hCB2 agonist activity and good selectivity over hCB1.

The SAR of substitution on the 4-phenyl ring was further investigated. The hCB2 functional activity of these piperidinyl analogs (**32**–**36**) were compared to that of **23** (Table 3). Replacement of the 3-trifluoromethyl group of **23** with a chlorine atom retained potent hCB2 agonist activity (**32**, EC_{50} 10 nM). Additional chlorine substitution on 2-position of the phenyl did not affect the potency (**33**, EC_{50} 8 nM). However the 2,4-dichloro analog (**34**) showed a threefold reduction in the hCB2 agonist activity, compared to that of **33**. When the substitution was changed to 2-fluoro and 5-methoxy groups on the 4-phenyl ring, functional activity was improved to EC_{50} 4 nM (**35**, E_{max} 108%). However hCB2/hCB1 selectivity of **35** declined to ~80-fold. Increasing the size of the alkoxyl group from methoxy to ethoxy group resulted in a significant reduction of the hCB2 activity (**36**, EC_{50} 87 nM). In this preliminary study, variation

Table 3
SAR of substituted 4-phenyl piperidinyl analogs



	R	hCB2, EC_{50} (nM) (E_{max})	hCB1, EC_{50} (nM)
32	3-Cl	10 (114%)	1055
33	2,3-di-Cl	8 (115%)	12,550
34	2,4-di-Cl	24 (104%)	1800
35	2-F, 5-OMe	4 (108%)	320
36	2-F, 5-OEt	87 (102%)	8500

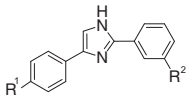
of the 3-trifluoromethyl group was tolerated and retained potent CB2 agonist activity.

The biological data of some selected potent hCB2 agonists from hybrid molecules are outlined in Table 4. The bromo analog (**8**) of **1** displayed similar potent CB2 agonist activity. Two 3-chloro analogs (**37** and **38**) retained potent hCB2 agonist activity. Conversely, the selectivity ratio of hCB1/hCB2 was significantly reduced to only ~16-fold for **38**. All three 2,3-dichloro-substituted piperidinyl analogs (**39**–**41**) exhibited potent and selective hCB2 agonist activity. In the 2-fluoro-(4 or 5)-methoxy series (**42**–**45**), potent hCB2 agonist activity was retained, but selectivity over hCB1 was diminished (~5–35-fold).

Several ortho-substituted 2-phenyl analogs were synthesized using the same methods described in Scheme 1 to compare with the meta-substituted analogs. In general, hCB2 functional activity was significantly reduced for ortho-substituted analogs. Two examples (**46** and **47**) are shown in Table 5.

Because compound **1** did not possess high plasma exposure (AUC 866 nM h) in rats, selected potent analogs were investigated for their pharmacokinetic properties, mainly AUC (0–6 h) in the rat

Table 4
SAR of compounds with hCB2 agonist activities <25 nM



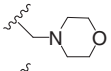
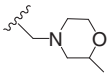
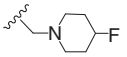
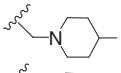
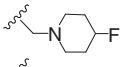
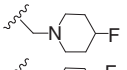
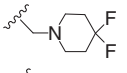
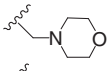
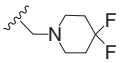
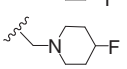
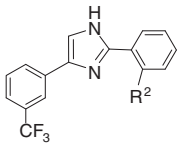
	R^1	R^2	hCB2, EC_{50} (nM) (E_{max})	hCB1, EC_{50} (nM)
8	3-Br		5 (112%)	665
37	3-Cl		14 (102%)	3250
38	3-Cl		8 (107%)	130
39	2,3-di-Cl		25 (107%)	>10,000
40	2,3-di-Cl		5 (119%)	1294
41	2,4-di-Cl		10 (107%)	2450
42	2-F, 4-OMe		3 (107%)	39
43	2-F, 5-OMe		4 (109%)	140
44	2-F, 5-OMe		5 (107%)	25
45	2-F, 5-OMe		5 (106%)	87

Table 5
SAR of ortho-substituted phenyl analogs



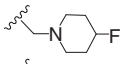
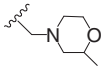
	R^2	hCB2, EC_{50} (nM) (E_{max})	hCB1, EC_{50} (nM)
46		130 (108%)	>10,000
47		510 (102%)	>10,000

Table 6
Rat pharmacokinetic data (AUC) of selected compounds

	AUC, (nM h), rat 0–6 h
1	866
8	158
20	42
21	1989
23	537
24	881
25	1411
26	524
27	1111
28	682
29	851

model with 10 mg/kg oral administration. Their AUC data are listed in Table 6. Replacing the 3-CF_3 group with a bromine atom (**8**) or replacing the oxygen atom with a sulfur atom (**20**) led to reduced plasma exposure (AUC 158 and 42 nM h, respectively). Implementing a methyl group next to the morpholine oxygen atom (**21**) resulted in a twofold enhancement of the plasma exposure (AUC: 1989 nM h), compared to that of **1**. This may be due to the blockage of the metabolic potential site of the morpholine ring. The piperidinyl analog (**23**) displayed slightly reduced AUC (537 nM h) in comparison to that of **1**. However, compounds (**24** and **25**) with methyl substitution at the 4- or 3-position of the piperidine ring improved the exposure in rats (AUC: 881 and 1411 nM h, respectively), relative to that of **23**. Four fluoro-substituted piperidinyl analogs (**26**–**29**) were evaluated for their pharmacokinetic properties. Their AUC values were comparable or slightly better than that of **1**. In general, methyl substitution at the 2-position of the morpholine ring or 3- or 4-position of the piperidine ring could increase the compound exposure in rats, compared to those of the unsubstituted analogs (**1** and **23**).

In conclusion, a novel series of potent and selective CB2 receptor agonists based on the 2,4-diphenyl-1*H*-imidazole scaffold were discovered. The preliminary SAR studies are summarized as follows. The N-1 substitution was not desirable. The morpholine ring could be substituted with a methyl group or replaced with a (un)substituted piperidine ring. Changing the substitution group or pattern of 4-phenyl group of **1** was tolerated. However, some disubstituted 4-phenyl analogs (**42**–**45**) displayed diminished selectivity over hCB1. Moving the saturated-heterocyclic methyl moiety from the meta to the ortho position led to reduced hCB2 agonist activity, and was not desired. During efforts to improve the pharmacokinetics of **1**, our screening results indicated that the methyl substitution at the 2-position of the morpholine ring could enhance compound exposure to twofold in rats, compared to that of **1**. Several analogs from this 2,4-diphenyl-1*H*-imidazole series exhibited efficacy in neuropathic pain models in rodents. These results will be published in due course.

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11. Analytical data for compound **1**: ^{1}H NMR ($CDCl_3$): δ 2.50 (m, 4H), 3.58 (s, 2H), 3.74 (t, J = 4.6 Hz, 4H), 7.36 (d, J = 7.7 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.46 (br s, 1H), 7.51 (m, 2H), 7.82 (d, J = 7.7 Hz, 1H), 7.92 (s, 1H), 8.03 (m, 1H), 8.11 (m, 1H), 9.70 (br s, NH). ESI-MS: m/z 388 $[M+H]^+$, $C_{21}H_{20}F_3N_3O$.