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Two distinct classes of novel pyrazolinecarboxamides as potent cannabinoid CB_1 receptor agonists

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ABSTRACT

The synthesis and SAR of 3-alkyl-4-aryl-4,5-dihydropyrazole-1-carboxamides **1**–**23** and 1-alkyl-5-aryl-4,5-dihydropyrazole-3-carboxamides **24**–**27** as two novel cannabinoid CB_1 receptor agonist classes were described. The target compounds elicited high affinities to the CB_1 as well as the CB_2 receptor and were found to act as CB_1 receptor agonists. The key compound **19** elicited potent CB_1 agonistic and CB_2 inverse agonistic properties in vitro and showed in vivo activity in a rodent model for multiple sclerosis after oral administration.

Cannabinoids constitute an area of intensive research since the endocannabinoid system plays an important role in many physiological processes.[1,2] Cannabinoid CB_1 receptor agonists have good prospects for the treatment of various disorders such as (neuropathic) pain, inflammation, multiple sclerosis, glaucoma, gastrointestinal disorders, and chemotherapy-induced nausea and vomiting.[3,4] The cannabinoid receptor has a high density in several brain areas and is also found in peripheral tissues including the eye, gastrointestinal tract, liver, pancreas, prostate, testis, adipose tissue, and heart. Both CB_1 and CB_2 receptor belong to the class A, G-protein-coupled receptor (GPCR) superfamily.

Both naturally occurring CB_1 receptor agonists (e.g., the endocannabinoid anandamide and the herbal Δ^9-tetrahydrocannabinol (dronabinol)) and synthetic cannabinoids (e.g., CP-55,940, WIN 55,212-2, and nabilone) have been disclosed[5] (Fig. 1).

Previous research efforts concentrated on diarylpyrazoline derivatives as CB_1/CB_2 subtype selective CB_1 receptor antagonists/inverse agonists.[6–10] However, pyrazoline-based CB_1 receptor agonists have hitherto not been reported. This prompted the introduction of the pyrazoline heterocyclic template in CB_1 receptor agonist drug design. It has been reported that the CB_1 receptor pharmacophore incorporates a vicinal diaryl substitution pattern.[11,12] Since many CB_1 receptor agonists contain a flexible alkyl chain (cf. Fig. 1) it was anticipated that replacement of one of the vicinal aryl groups by a flexible alkyl chain in our 3,4-diarylpyrazoline antagonist chemotype might lead to a functional switch from CB_1 antagonism to CB_1 receptor agonism.

These considerations resulted in the design of the target compounds **1**–**27**. The synthesis of the target compounds **1**–**21** is depicted in Scheme 1.

Ketones **28**–**32** are either commercially available or easily accessible, for example, via the reaction of the appropriate Weinreb amide with a Grignard reagent $ArCH_2MgCl$ in THF at 0 °C, followed by acidification in 4 N HCl. The enones **33**–**37** were obtained from the corresponding ketones **28**–**32**, respectively, using a Mannich reaction/elimination sequence[6] and further cyclocondensed in the presence of hydrazine·H_2O into the pyrazolines **38**–**42**. Subsequent isocyanate addition at room temperature delivered the target compounds **1**–**13** (Route 1). Alternatively, the pyrazoline **38** was converted in situ to the corresponding carbonyl chloride **43** by treatment with diphosgene at ambient temperature. Subsequent treatment with the appropriate amines R^1–NH_2 in the presence of Hünig's base furnished the target compounds **14**–**18** (Route 2). Preparative HPLC separation of the *endo*-[1*R*,2*S*,4*R*]-bornyl derivative **9** produced the pure diastereomers (−)-**19** and (+)-**20**, respectively. The 1-naphthoyl substituted pyrazoline **21** was prepared from **38** in moderate chemical yield.

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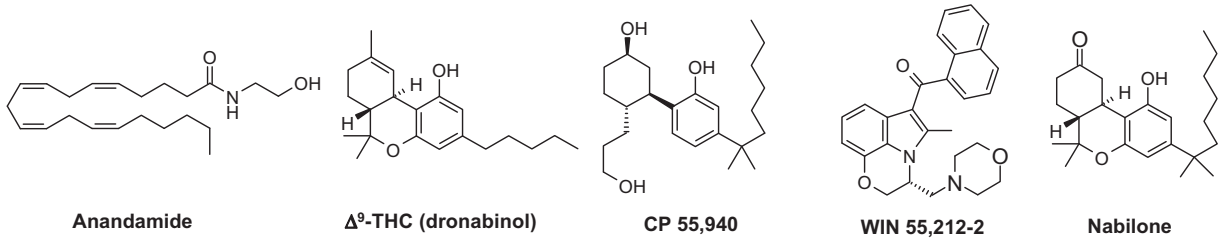
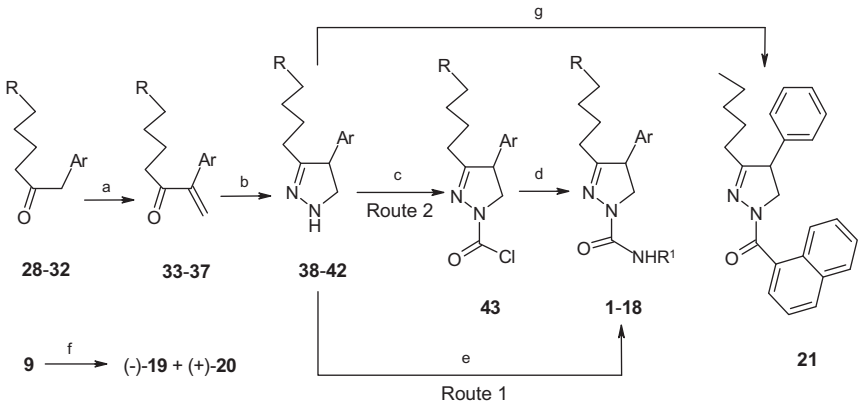


Figure 1. Representative naturally occurring and synthetic CB_1 receptor agonists.

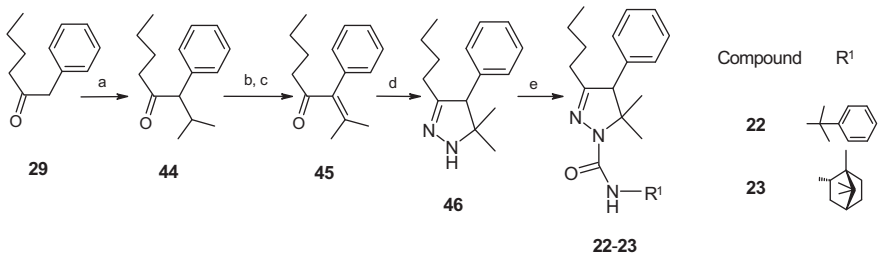


Compound	R	Ar	Route	R^1
1	CH_3	C_6H_5	1	
2	CH_3	C_6H_5	1	
3	CH_3	C_6H_5	1	
4	CH_3	C_6H_5	1	
5	CH_3	C_6H_5	1	
6	CH_3	C_6H_5	1	
7	CH_3	C_6H_5	1	
8	CH_3	C_6H_5	1	
9	CH_3	C_6H_5	1	
10	H	C_6H_5	1	
11	H	3-F-C_6H_4	1	
12	H	2-F-C_6H_4	1	
13	H	3-thienyl	1	
14	CH_3	C_6H_5	2	
15	CH_3	C_6H_5	2	
16	CH_3	C_6H_5	2	
17	CH_3	C_6H_5	2	
18	CH_3	C_6H_5	2	

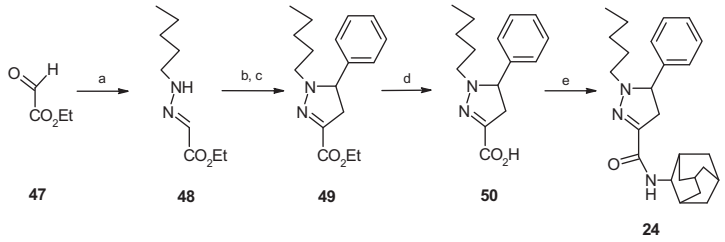
Scheme 1. Reagents and conditions: (a) CH_2O (35% aq), HOAc, piperidine, MeOH, 55 °C, 60 h (92%); (b) $H_2NNH_2{\cdot}H_2O$, EtOH, N_2, reflux, 4 h; (c) triphosgene (0.33 mol equiv), DIPEA, CH_2Cl_2, 30 °C, 1 h; (d) R^1–NH_2, DIPEA, CH_2Cl_2, 30 °C, 18 h; (e) R^1–N=C=O, Et_3N (cat.), benzene, rt, 16 h (60–70%); (f) Chiral preparative HPLC separation, stationary phase: Chiralpak® AD 20 μm, mobile phase: acetone/methanol (95:5 (v/v), flow rate 2 ml/min; (g) 1-naphthoyl chloride, toluene, rt, 16 h (53%).

The target compounds **22** and **23** had to be prepared by an alternative route since the Mannich reaction/elimination sequence as outlined above (Scheme 1)—but with the enolisable acetone instead of formaldehyde—was unsuccessful. This alternative route is depicted in Scheme 2.

Addition of the isopropyl group to the ketone **29** under basic conditions[13] provided **44** in a moderate yield. Subsequent radical bromination of **44** with *N*-bromosuccinimide, followed by an elimination of HBr at elevated temperature in dimethylformamide furnished the desired enone **45** which was converted in the presence



Scheme 2. Reagents and conditions: (a) 2-iodopropane, $NaOCH_3$, N_2, reflux, 1 h (40%); (b) NBS, dibenzoyl peroxide (cat.), CCl_4, reflux, 6 h, (~100%); (c) LiCl, DMF, N_2, 130 °C, 90 min (46%); (d) $H_2NNH_2{\cdot}H_2O$, EtOH, N_2, reflux, 4 h; (e) R^1–N=C=O, Et_3N (cat.), toluene, rt, 16 h (55–58%).



Scheme 3. Reagents and conditions: (a) *n*-pentylhydrazine, EtOH, 80 °C, 16 h (93%); (b) NCS, EtOAc, N_2, 60 °C, 1 h; (c) styrene, $NaHCO_3$, H_2O, 70 °C, 16 h (22%); (d) LiOH, THF, H_2O, 70 °C, 1 h, followed by acidification (Et_2O, 1 N HCl) (74%); (e) 2-adamantamine·HCl, DIPEA, CIP, CH_2Cl_2, N_2, rt, 16 h (67%).

of $H_2NNH_2{\cdot}H_2O$ to the pyrazoline **46** as outlined above for **38**–**42**. Reaction with the appropriate isocyanates smoothly yielded the target compounds **22** and **23**.

Besides the 3-alkyl-4-aryl-4,5-dihydropyrazole-1-carboxamides **1**–**23** we were also interested in the structurally related—but distinct—1-alkyl-5-aryl-4,5-dihydropyrazole-3-carboxamides **24**–**27**. The synthesis of **24** is depicted in Scheme 3.

The commercially available oxoacetic acid ester **47** was reacted with *n*-pentylhydrazine[14] to give **48**. Subsequent chlorination with *N*-chlorosuccinimide, followed by cyclization with styrene[10] afforded the ester **49** which was saponified into the corresponding acid **50**. Target compound **24** was obtained by amidating **50** with 2-adamantamine·HCl in the presence of the coupling reagent CIP in 67% yield.

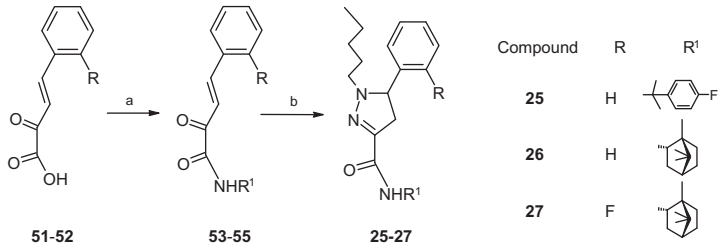
The synthesis of the target compounds **25**–**27** is shown in Scheme 4. Amidation of the carboxylic acids[15] **51** and **52** in the presence of the coupling reagent HBTU led to the amides **53**–**55**, respectively. Treatment of **53**–**55** with *n*-pentylhydrazine in ethanol produced the target compounds **25**–**27** in moderate yields.

The pharmacological results of the reference cannabinoid receptor agonists WIN 55,212-2,[16,17] CP-55,940,[16,17] nabilone,[18,19] and the target compounds **1**–**27** are given in Table 1. They were evaluated in vitro at the human CB_1 and CB_2 receptor, stably expressed into Chinese hamster ovary (CHO) cells,[6] utilizing radioligand binding studies (displacement of the specific binding of [^{3}H]-CP-55,940). CB_1 receptor agonism was measured using a CP-55,940 induced arachidonic acid release functional assay,[6] using the same recombinant cell line. CB_1 agonist stimulation leads to activation of PLA_2 followed by release of [^{3}H]arachidonic acid into the medium.

The compounds **1**–**27** elicited moderate to high CB_1 receptor affinities. In the 3-alkyl-4-aryl-4,5-dihydropyrazole-1-carboxamides series (target compounds **1**–**23**), the compounds bearing a bulky and apolar carboxamide N-substituent—such as **2**, **7**, **19**, and **22**—showed single digit nanomolar CB_1 receptor affinities comparable to the values obtained for nabilone. In accordance with this SAR trend, the alternative 1-alkyl-5-aryl-4,5-dihydropyrazoline series (target compounds **24**–**27**) contained three of such high CB_1 receptor binders, viz. the 2-adamantyl substituted **24** and the *endo*-bornyl substituted **26** and **27**.

The compounds **1**–**27** also elicited moderate to high CB_2 receptor affinities. Again, the target compounds bearing a bulky and apolar carboxamide N-substituent—such as **2**, **4**, **9**, **19**, **23**, **24**, and **27**—showed single digit nanomolar CB_2 receptor affinities comparable to the values obtained for nabilone. It should be noted that the degree of observed CB_1/CB_2 receptor subtype selectivity of the target compounds **1–27** is modest, their values ranging from 0.21 to 5.4. These CB_1/CB_2 selectivity values are in the same range as those obtained for CP-55,940 and nabilone.

In general, the target compounds were found to behave as CB_1 receptor agonists. The target compounds **9**, **19**, and **24**–**27** showed pA_2 values ranging from 8.1 to 9.2 which are comparable to the observed values of nabilone and CP-55,940, respectively.



Scheme 4. Reagents and conditions: (a) R^1–NH_2, DIPEA, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), CH_2Cl_2, rt, 16 h (60–70%); (b) *n*-pentylhydrazine, HOAc, EtOH, N_2, 60 °C, 8 h (40–50%).

Table 1
Pharmacological in vitro results of the reference compounds WIN 55,212-2, CP-55,940, nabilone, and the target compounds **1**–**27**

Compound	K_i (CB_1),[a] (nM)	pEC_{50} (CB_1),[b]	K_i (CB_2),[c] (nM)	CB_1/CB_2 selectivity[d]
WIN 55,212-2	94.5 ± 18	7.5 ± 0.4	9.1 ± 3.4	0.1
CP-55,940	1.36 ± 0.18	8.9 ± 0.1	0.62 ± 0.09	0.46
Nabilone	5.1 ± 1.6	8.4 ± 0.1	17.6[g]	3.5
1	68 ± 32	6.4[e]	14.9 ± 5.9	0.22
2	6.4 ± 2.1	7.6[e]	6.3 ± 3.4	1.0
3	15.6 ± 11.5	5.7[e]	13.3 ± 3.3	0.85
4	16.1 ± 2.1	5.7 ± 0.4	8.0 ± 2.3	0.50
5	15.3 ± 2.9	n.d.[f]	53 ± 16	3.5
6	51 ± 13	6.4[e]	260 ± 60	5.1
7	7.4 ± 0.5	5.8[e]	36.6 ± 8.9	4.9
8	3.26 ± 1.27	8.4[e]	17.6[f]	5.4
9	28.5 ± 14.1	9.2 ± 0.1	7.4 ± 3.3	0.26
10	14.2 ± 3.0	8.4[e]	35.6 ± 19.7	2.5
11	44.9 ± 16.4	7.0 ± 0.1	74 ± 11	1.6
12	32.0 ± 8.1	6.9 ± 0.2	89 ± 19	2.8
13	19.3 ± 15.5	7.4 ± 0.2	62 ± 17	3.2
14	52 ± 19	n.d.[f]	79[g]	1.5
15	15.3 ± 8.7	n.d.[f]	19.8 ± 6.8	1.3
16	11.9 ± 4.9	n.d.[f]	26.6 ± 6.6	2.2
17	33.1 ± 15.9	n.d.[f]	22.5 ± 4.7	0.68
18	70 ± 44	n.d.[f]	15.0 ± 5.0	0.21
19	8.3 ± 3.4	8.4 ± 0.4	5.3 ± 0.8	0.64
20	46.8 ± 5.7	6.8 ± 0.3	36.0 ± 3.7	0.77
21	164 ± 118	7.5 ± 0.5	103[g]	0.63
22	9.0 ± 3.7	8.1[e]	11.9 ± 3.1	1.3
23	16.1 ± 2.1	7.4 ± 0.3	3.4 ± 0.4	0.21
24	9.2 ± 2.6	8.1 ± 0.1	7.1 ± 1.5	0.77
25	118 ± 68	8.4 ± 0.4	121 ± 23	1.0
26	4.7 ± 1.9	8.3 ± 0.2	20.1 ± 9.2	4.3
27	6.1 ± 1.8	8.4 ± 0.2	9.1 ± 3.1	1.5

[a] Displacement of specific CP-55,940 binding in CHO cells stably transfected with human CB_1 receptor, expressed as K_i ± SEM (nM). The values represent the mean result based on at least three independent experiments, unless otherwise noted.
[b] [^{3}H]Arachidonic acid release in CHO cells expressed as pEC_{50} ± SEM values. The values represent the mean result based on at least three independent experiments, unless otherwise noted.
[c] Displacement of specific CP-55,940 binding in CHO cells stably transfected with human CB_2 receptor, expressed as K_i ± SEM (nM). The values represent the mean result based on three independent experiments, unless otherwise noted.
[d] CB_1/CB_2 selectivity values are provided as single values instead of their ranges based on the underlying CB_1 and CB_2 receptor affinity SEM values.
[e] Result of single measurement.
[f] n.d., not determined.
[g] Result of duplicate measurement.

It is interesting to note that **19** showed significantly higher CB_1 and CB_2 receptor affinities and a higher CB_1 agonistic potency than its diastereomeric counterpart **20**, indicating that these chiral ligands bind to some extent stereoselectively to the CB_1 and CB_2 receptor.

Reference compounds such as anandamide, Δ^9-tetrahydrocannabinol, WIN 55,212-2, CP-55,940, HU210, O-2545, and nabilone have been reported to act as agonists at both the CB_1 receptor and the CB_2 receptor. Intriguingly, compound **19** elicited a potent antagonistic effect (dose-dependent antagonism of the selective CB_2 receptor agonist JWH133) on human CB_2 receptor mediated adenylate cyclase activity in vitro (pA_2 = 9.2), which nicely corresponded to the obtained value (pA_2 = 9.4) in our human CB_2 cAMP accumulation assay.[20,21] Furthermore, our CB_2 cAMP accumulation assay revealed significant inverse agonistic properties of **19** (pEC_{50} = 8.5 ± 0.3) in the absence of JWH133 at the constitutively active[20] CB_2 receptor. It can be concluded that **19** has a distinct cannabinoid in vitro profile as compared to the $CB_{1/2}$ agonistic reference compounds mentioned hereinabove.

The Experimental Autoallergic Encephalomyelitis (EAE) model[22] is regarded as a key pharmacological model for multiple sclerosis.[23–25] It has been reported[26] in animal studies that the cannabinoid system is neuroprotective[27] during EAE. In such reported experiments the administration of the CB_1 receptor agonist was started from day one of the EAE experiment. By using such a dosing regime it was observed that **19** by oral administration in the male Lewis rat reduced the EAE induced motor deficits in line with the reported results of other CB_1 receptor agonists (data not shown).

More interestingly, it was decided to test the effect of our CB_1 receptor agonist **19** on the disease course of acute EAE by oral administration in the male Lewis rat starting the day after the appearance of the first paralysis symptoms in the entire cohort (day 11). The results are depicted in Figure 2. Compound **19** (20 mg/kg po, once daily) significantly ($p < 0.0001$) reduced the EAE induced motor deficits by reducing the intensity of the paralysis (AUC and average score).

At the end of the EAE experiment, defined as the day all animals had completely recovered, plasma and brains were sampled at several time points (0.5, 1, 3, 7, 14, and 24 h) after the last administration of **19** to determine the exposure levels and CNS/plasma ratio after repeated administration (Fig. 3). These data revealed that the levels of **19** in CNS as well as plasma returned to the basis within 24 h and thereby corroborate the absence of accumulation of **19** during the course of the EAE experiment. In addition, these data showed a significant CNS exposure of **19**. It is interesting to note that the levels of **19** at the end of the experiment were comparable to the values obtained during day 1 (data not shown) which is an indication that the degree of its metabolic breakdown remained unaffected during the course of the EAE experiment.

In general, cannabinoid CB_1 receptor agonists are known as highly lipophilic compounds. Despite the high calculated log *D* value of **19** (5.7 at pH 7.4), both its relatively low molecular weight and its low polar surface area (calculated PSA = 45 Å^2)

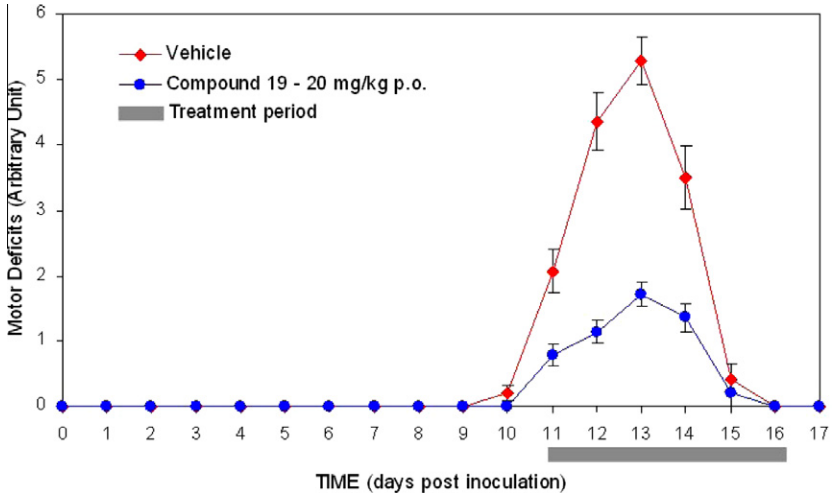


Figure 2. In vivo results of **19** in the EAE model.

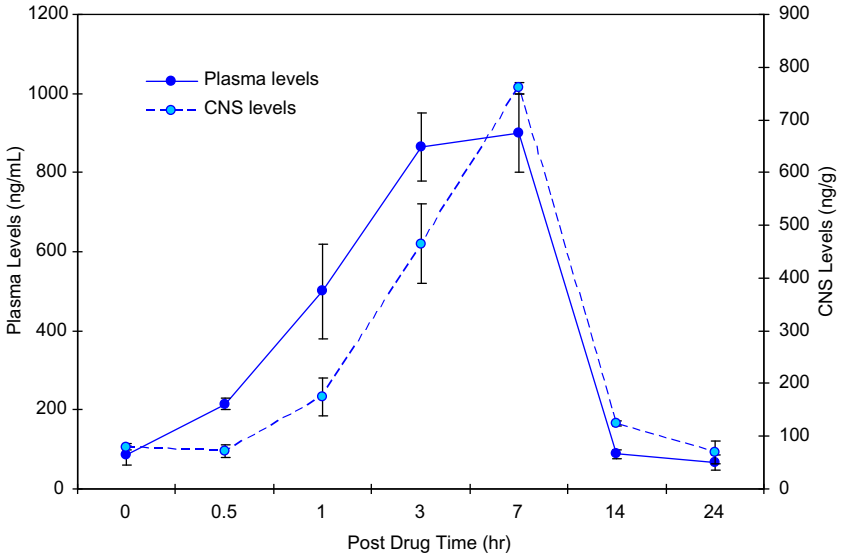


Figure 3. Plasma and CNS levels at different time points after the last administration of **19** in the EAE experiment.

were anticipated to favorably contribute to its CNS penetrability.

In conclusion, two structurally related pyrazoline classes[28] were presented as a novel CB_1 receptor agonist chemotype. The target compounds **1**–**27** elicited high affinities to the CB_1 and CB_2 receptor and were in general found to act as CB_1 receptor agonists. The key compound **19** showed oral in vivo activity in a rodent model for multiple sclerosis (EAE), even if treatment was started a day after the first symptom was observed.

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28. Yields refer to isolated pure products unless otherwise noted and were not maximized. Coupling constants (*J*) are expressed in Hz. Flash chromatography was performed using silica gel 60 (0.040–0.063 mm, Merck). Sepacore chromatographic separations were carried out using Supelco equipment, VersaFLASH™ columns, VersaPak™ silica cartridges, Büchi UV monitor C-630, Büchi pump module C-605, Büchi fraction collector C-660, and Büchi pump manager C-615. Selected data for target compounds **3**, **13**, **19**–**22**, **24**, **25**, and **27**, the protocols for the in vitro cannabinoid-CB_2 receptor antagonism assay and the acute EAE assay. *Synthesis of compound* ***3***: To a magnetically stirred solution of hexanoic acid methoxymethylamide (12.2 g, 77 mmol) at 0 °C in THF was slowly added BnMgCl (20% in THF, 90 ml, 116 mmol) and the resulting mixture was reacted for 2 h. The reaction mixture was poured in excess HCl (4 N) and extracted with *tert*-butyl-methyl ether (MTBE). Concentration, followed by flash chromatographic purification (heptane/EtOAc = 40:1 (v/v)) gave **28** (11.6 g, 79% yield) as an oil; ^{1}H NMR (300 MHz, $CDCl_6$) δ 0.86 (t, *J* = 7, 3H), 1.20–1.27 (m, 4H), 1.52–1.60 (m, 2H), 2.40–2.46 (m, 2H), 3.68 (s, 2H), 7.18–7.33 (m, 5H). To a magnetically stirred solution of **28** (11.6 g, 61 mmol) in MeOH (100 ml) was added piperidine (1 ml) and HOAc (1 ml), followed by formalin (20 ml (35% aq.), 226 mmol) and the resulting mixture was stirred at 55 °C for 60 h. The reaction mixture was cooled to rt, concentrated and taken up in a mixture of MTBE and water. The organic layer was collected, dried over Na_2SO_4, filtered and concentrated to give **33** (11.4 g, 92% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (t, *J* = 7, 3H), 1.18–1.30 (m, 4H), 1.54–1.63 (m, 2H), 2.65 (t, *J* = 7, 2H), 5.80 (s, 1H), 6.02 (s, 1H), 7.20–7.32 (m, 5H). To a magnetically stirred solution of **33** (5 g, 24.7 mmol) in EtOH (30 ml) was added hydrazine·H_2O (2.46 ml, 50.7 mmol) and the resulting solution was heated at reflux temperature for 4 h. The resulting solution was allowed to attain rt, concentrated and taken up in a mixture of MTBE and water. The organic layer was collected, dried over Na_2SO_4, filtered and concentrated to give crude **38** (4.8 g) as an impure oil which was used immediately in the subsequent step. Compound **38** (2.2 g, 10.3 mmol) was dissolved in benzene (25 ml) and treated with *cis*-myrtanylisocyanate (2.12 g, 11.8 mmol)—which was prepared from (−)-*cis*-myrtanylamine (CAS 38235-68-6) and diphosgene in CH_2Cl_2 at 0 °C—and five drops of Et_3N and the resulting solution was stirred at rt for 16 h. The solution was concentrated, followed by flash chromatographic purification (heptane/EtOAc = 6:1 (v/v)) to give **3** as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85–0.95 (m, 4H), 1.06 (s, 3H), 1.19–1.31 (m, 7H), 1.38–1.60 (m, 3H), 1.82–2.41 (m, 9H), 3.22–3.40 (m, 2H), 3.83–3.90 (m, 1H), 4.12 (dd, *J* = 12 and 7, 1H), 4.18–4.26 (m, 1H), 5.92–5.96 (m, 1H), 7.15 (br d, *J* = 8, 2H), 7.25–7.37 (m, 3H). *Compound* ***10***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7, 3H), 1.21–1.33 (m, 2H), 1.38–1.54 (m, 2H), 1.75 (s, 3H), 1.77 (s, 3H), 2.04–2.22 (m, 2H), 3.82 (dd, *J* = 9.7 and 5.6, 1H), 4.07–4.20 (m, 2H), 6.38 (br s, 1H), 7.13–7.36 (m, 8H), 7.48 (br d, *J* = 8, 2H). *Compound* ***13***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7, 3H), 1.21–1.57 (m, 4H), 1.74 (s, 3H), 1.77 (s, 3H), 2.05–2.25 (m, 2H), 3.79 (dd, *J* = 11 and 7, 1H), 4.08–4.13 (m, 1H), 4.28 (dd, *J* = 11 and 7, 1H) 6.36 (br s, 1H), 6.91 (dd, *J* = 6 and 2, 1H), 7.06–7.08 (m, 1H), 7.19–7.24 (m, 1H), 7.30–7.37 (m, 3H), 7.45–7.49 (m, 2H); HRMS exact mass calcd for $C_{21}H_{28}N_3OS$ *m/z* 370.1953 $[MH]^+$, found 370.1963. *Compound* ***19***: $([\alpha]_D)$ −85 (*c* 1.55 g/100 ml, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80–0.94 (m, 10H), 0.97 (s, 3H), 1.20–1.69 (m, 10H), 1.74–1.83 (m, 1H), 2.00–2.22 (m, 2H), 2.33–2.45 (m, 1H), 3.83–3.89 (m, 1H), 4.09–4.27 (m, 3H), 6.02 (br d, *J* = 10, 1H), 7.16 (br d, *J* = 8, 2H), 7.27–7.37 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.73, 13.93, 18.73, 20.00, 22.31, 25.75, 28.03, 28.26, 28.46, 31.36, 38.12, 44.99, 48.00, 49.37, 53.34, 53.62, 54.41, 127.56, 127.68, 129.06, 139.71, 155.78, 158.83; HRMS exact mass calcd for $C_{25}H_{38}N_3O$ *m/z* 396.3015 $[MH]^+$, found 396.3028. *Compound* ***20***: $([\alpha]_D)$ +124 (*c* 1.3 g/100 ml, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80–0.92 (m, 10H), 0.97 (s, 3H), 1.20–1.69 (m, 10H), 1.74–1.83 (m, 1H), 2.00–2.22 (m, 2H), 2.33–2.45 (m, 1H), 3.83–3.89 (m, 1H), 4.09–4.27 (m, 3H), 6.02 (br d, *J* = 10, 1H), 7.16 (br d, *J* = 8, 2H), 7.27–7.37 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.74, 13.93, 18.74, 20.00, 22.32, 25.76, 28.05, 28.27, 28.45, 31.35, 38.20, 44.97, 47.99, 49.29, 53.30, 53.58, 54.42, 127.54, 127.64, 129.05, 139.67, 155.87, 158.88. *Compound* ***21***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80–0.90 (m, 3H), 1.02–1.40 (m, 6H), 1.92–2.11 (m, 2H), 4.21–4.30 (m, 2H), 4.57–4.65 (m, 1H), 7.20 (d, *J* = 8, 2H), 7.29–7.55 (m, 6H), 7.66 (d, *J* = 8, 1H), 7.84–7.94 (m, 2H), 8.03 (br d, *J* = 8, 1H); HRMS exact mass calcd for $C_{25}H_{27}N_2O$ *m/z* 371.2123 $[MH]^+$, found 371.2142. *Synthesis of compound* ***22***: To an ice-cold magnetically stirred mixture of **29** (7.04 g, 0.04 mol) and $NaOCH_3$ (4.32 g, 0.08 mol) was added dropwise 2-iodopropane (15 ml) in a N_2 atmosphere. The resulting mixture was heated for 1 h at reflux temperature. The obtained mixture was allowed to attain rt and concentrated. The resulting residue was taken up in Et_2O and water. The Et_2O layer was separated and successively washed with an aqueous $Na_2S_2O_3$ solution and water. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was further purified using Sepacore equipment: (petroleum ether/Et_2O = 19:1 (v/v)) to give **44** (3.52 g) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.66 (d, *J* = 7, 3H), 0.81 (t, *J* = 7, 3H), 0.96 (d, *J* = 7, 3H), 1.10–1.24 (m, 2H), 1.36–1.54 (m, 2H), 2.29–2.47 (m, 3H), 3.30 (d, *J* = 10 Hz, 1H), 7.20–7.33 (m, 5H). To a magnetically stirred solution of **44** (4.31 g, 0.02 mol) in CCl_4 (40 ml) was added a catalytic amount of dibenzoyl peroxide and NBS (6.56 g). The resulting mixture was heated for 6 h at reflux temperature. The obtained mixture was allowed to attain rt. The formed precipitate was removed by filtration. The filtrate was concentrated to give crude 2-methyl-3-bromo-3-phenyloctan-4-one as a dark-colored oil (6.77 g) which was dissolved in anhydrous DMF (35 ml) under a N_2 atmosphere. LiCl (3.2 g, 0.075 mol) was added and the resulting mixture was heated at 130 °C for 90 min. The resulting mixture was allowed to attain rt and was subsequently poured into water and extracted with Et_2O. The organic layer was separated and washed with water (four portions). The organic layer was dried over Na_2SO_4, filtered and concentrated. The obtained residue was purified using Sepacore equipment: (petroleum ether/Et_2O = 98:2 (v/v)) to give **45** (1.96 g, 46% yield) as a pale yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (d, *J* = 7, 3H), 1.13–1.24 (m, 2H), 1.43–1.52 (m, 2H), 1.66 (s, 3H), 2.00 (s, 3H), 2.25 (t, *J* = 7, 2H), 7.15 (br d, *J* = 8, 2H), 7.20–7.39 (m, 3H). To a magnetically stirred solution of **45** (1.96 g, 9.07 mmol) in abs EtOH (15 ml) was added hydrazine·hydrate (0.88 ml, 18.14 mmol) and the resulting solution was heated at reflux temperature for 4 h under a N_2 atmosphere. The resulting solution was allowed to attain rt, concentrated and taken up in a mixture of MTBE and water. The organic layer was collected, dried over $MgSO_4$, filtered and concentrated to give crude **46** (2.06 g) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80–0.89 (m, 6H), 1.23–1.37 (m, 5H), 1.42–1.54 (m, 2H), 2.06–2.35 (m, 2H), 3.52 (s, 1H), 4.90 (br s, 1H), 7.07 (br d, *J* = 8 Hz, 2H), 7.19–7.38 (m, 3H). Crude **46** (1.03 g, ~4.48 mmol) was dissolved in toluene (10 ml) and treated with 1-methyl-1-phenyl-ethylisocyanate (0.72 g, 4.48 mmol) and two drops of Et_3N and the resulting solution was stirred at rt for 16 h. The solution was concentrated and purified using Sepacore equipment: (petroleum ether/diethyl ether = 85:15 (v/v)) to give **22** as a colorless oil (0.97 g, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7, 3H), 1.08 (s, 3H), 1.26–1.39 (m, 2H), 1.44 (s, 3H), 1.45–1.56 (m, 2H), 1.70 (s, 3H), 1.76 (s, 3H), 2.09–2.18 (m, 1H), 2.25–2.35 (m, 1H), 3.70 (s, 1H), 6.59 (br s, 1H), 7.03 (br d, *J* = 8 Hz, 2H), 7.20 (br t, *J* = 8 Hz, 1H), 7.26–7.36 (m, 5H), 7.44 (br d, *J* = 8 Hz, 2H); HRMS exact mass calcd for $C_{25}H_{34}N_3O$ *m/z* 392.2702 $[MH]^+$, found 392.2711. *Synthesis of compound* ***24***: To a magnetically stirred solution of **47** (35.08 ml, 177 mmol; 50% solution in toluene) in EtOH (450 ml) was added *n*-pentylhydrazine (21.7 g, 212 mmol) and the resulting solution was heated at 80 °C for 16 h. The obtained mixture was allowed to attain rt and concentrated. The resulting residue was taken up in EtOAc and water. The organic layer was separated and subsequently dried over $MgSO_4$, filtered and concentrated to give **48** (32.2 g, 93% yield) as a purple colored oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87–0.94 (m, 3H), 1.25–1.42 (m, 7H), 1.55–1.68 (m, 2H), 3.17–3.23 (m, 1H), 3.35–3.45 (m, 1H), 4.28 (q, *J* = 7, 2H), 6.51 (br s, 1H), 6.72 (s, 1H). To a magnetically stirred solution of **48** (35.16 g, 179 mmol) in EtOAc (450 ml) was added NCS (26.34 g, 197 mmol) and the resulting mixture was heated at 60 °C for 1 h in a N_2 atmosphere. To the reaction mixture was added styrene (41.1 ml, 359 mmol) and $KHCO_3$ (89.8 g, 897 mmol) and water (8 ml). The resulting mixture was heated at 70 °C for 16 h. The resulting mixture was allowed to attain rt, concentrated and the resulting residue was chromatographically separated using Sepacore equipment (CH_2Cl_2/MeOH = 98:2 v/v) to give **49** (12.1 g, 22% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (t, *J* = 7, 3H), 1.13–1.28 (m, 4H), 1.35 (t, *J* = 7, 3H), 1.53–1.67 (m, 2H), 2.89 (dd, *J* = 16 and 13, 1H), 3.01–3.09 (m, 1H), 3.14–3.22 (m, 1H), 3.41 (dd, *J* = 16 and 12, 1H), 4.31 (double (diastereotopic) quartet, *J* = 7, 2H), 4.63 (dd, *J* = 13 and 12, 1H), 7.27–7.39 (m, 5H). To a magnetically stirred solution of **49** (11.76 g, 38.74 mmol) in THF (100 ml) and water (100 ml) was added LiOH (1.86 g, 77.5 mmol) and the resulting mixture was heated at 70 °C for 1 h. The reaction mixture was allowed to attain rt and Et_2O (200 ml) and concentrated HCl (7 ml) were added. The organic layer was separated, washed three times with water and with brine and subsequently dried over Na_2SO_4, filtered and concentrated to give **50** (7.9 g, 74% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, *J* = 7, 3H), 1.15–1.28 (m, 4H), 1.53–1.65 (m, 2H), 2.92 (dd, *J* = 17 and 13, 1H), 3.02–3.11 (m, 1H), 3.18–3.27 (m, 1H), 3.44 (dd, *J* = 17 and 13, 1H), 4.75 t, *J* = 13, 1H), 7.31–7.41 (m, 5H), 7.42–9.00 (br s, 1H). To a magnetically stirred solution of **50** (0.70 g, 2.55 mmol) in CH_2Cl_2 (40 ml) was successively added 2-adamantanamine.HCl (480 mg, 2.55 mmol), DIPEA (1.78 ml, 10.22 mmol) and 2-chloro-1,3-dimethylimidazolinium hexafluorophosphate (CIP) (853 mg, 3.07 mol) and the resulting mixture was reacted at rt for 16 h in a N_2 atmosphere. The reaction mixture was successively washed twice with water, twice with aqueous citric acid (0.5 M), twice with $NaHCO_3$ (5% aqueous solution) and brine, and subsequently dried over Na_2SO_4, filtered and concentrated to give crude **24** (1.26 g) as an orange oil. Further chromatographic purification using Sepacore equipment (petroleum ether/Et_2O = 85:15 (v/v)) gave **24** (750 mg, 67% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7, 3H), 1.21–1.30 (m, 4H), 1.55–1.65 (m, 2H), 1.65–1.70 (m, 2H), 1.76 (br s, 2H), 1.75–1.92 (m, 8H), 1.97–2.01 (m, 2H), 2.82 (dd, *J* = 17 and 14, 1H), 2.92–2.97 (m, 2H), 3.42 (dd, *J* = 17 and 11, 1H), 4.09–4.14 (m, 1H), 4.40 (dd, *J* = 14 and 11, 1H), 6.99–7.07 (m, 1H), 7.28–7.38 (m, 5H); HRMS exact mass calcd for $C_{25}H_{36}N_3O$ *m/z* 394.2858 $[MH]^+$, found 394.2880. *Compound* ***25***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7, 3H), 1.20–1.31 (m, 4H), 1.54–1.67 (m, 2H), 1.74 (s, 3H), 1.75 (s, 3H), 2.77 (dd, *J* = 17 and 14, 1H), 2.90–2.97 (m, 2H),

3.35 (dd, J = 17 and 11, 1H), 4.38 (dd, J = 14 and 11, 1H), 6.94 (br s, 1H), 6.98–7.04 (m, 2H), 7.27–7.43 (m, 7H); HRMS exact mass calcd for $C_{24}H_{31}FN_3O$ m/z 396.2451 $[MH]^+$, found 396.2451. *Synthesis of compound* ***27***: To a magnetically stirred solution of **55** in EtOH (50 ml) was successively added AcOH (660 ml, 11.58 mmol) and *n*-pentylhydrazine (1.45 ml, 9.65 mmol) and the resulting mixture was reacted in a N_2 atmosphere at 60 °C for 8 h in an oil bath. The reaction mixture was allowed to attain rt and concentrated. The residue was dissolved in CH_2Cl_2, washed with water and subsequently dried over $MgSO_4$, filtered and concentrated. Further chromatographic purification using Sepacore equipment (petroleum ether/EtOAc = 95:5 (v/v)) gave **27** (940 mg, 46% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83–0.94 (m, 10H), 1.20–1.85 (m, 14H), 2.32–2.42 (m, 1H), 2.74–2.85 (m, 1H), 2.91–3.02 (m, 2H), 3.43–3.54 (m, 1H), 4.26–4.36 (m, 1H), 4.69–4.80 (m, 1H), 6.63–6.70 (m, 1H), 7.02–7.09 (m, 1H), 7.12–7.19 (m, 1H), 7.25–7.31 (m, 1H), 7.46–7.54 (m, 1H); HRMS exact mass calcd for $C_{25}H_{37}FN_3O$ m/z 414.2921 $[MH]^+$, found 414.2891. *Compound* ***55***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84–1.00 (m, 10H), 1.21–1.30 (m, 1H), 1.38–1.48 (m, 1H), 1.53–1.62 (m, 1H), 1.70–1.87 (m, 2H), 2.34–2.44 (m, 1H), 4.21–4.30 (m, 1H), 7.10–7.25 (m, 3H), 7.38–7.45 (m, 1H), 7.70–7.75 (m, 1H), 7.85 (d, J = 16, 1H), 8.12 (d, J = 16, 1H). *In vitro cannabinoid-CB_2 receptor antagonism*. Functional activity of **19** at the CB_2 receptor was assessed using a forskolin-stimulated cAMP accumulation assay in Chinese ovarian hamster (CHO) K_1 cells expressing human CB_2 receptor. CHO cells were grown in a CHO-S-SFM-II culture medium, supplemented with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 400 μg/ml Hygromycine B and 500 μg/ml G418 at 37 °C in 93% air/5% CO_2. For incubation with test compounds, confluent cultures grown in 24-well plates were used. Each condition or substance was routinely tested in quadruplicate. Cells were loaded with 1 mCi [^{3}H]adenine in 0.5 ml medium per well. After 2 h, cultures were washed with 0.5 ml PBS containing 1 mM IBMX and incubated for 20 min with 0.5 ml PBS containing 1 mM IBMX and 3×10^{-7} M forskolin with or without the test compound. Antagonistic effects of test compounds were determined as inhibition of 0.1 μM JWH133-decreased [^{3}H]cAMP formation. After aspiration, the reaction was stopped with 1 ml trichloroacetic acid (5% w/v). The [^{3}H]ATP and [^{3}H]cAMP formed in the cellular extract were assayed as follows: a volume of 0.8 ml of the extract was passed over Dowex (50WX-4200-400 mesh) and aluminum oxide columns, eluted with water and 0.1 M imidazole (pH 7.5). Eluates were mixed with 7 ml Ultima-Flo [AP] and the β-radioactivity was counted with a liquid scintillation counter. The conversion of [^{3}H]ATP into [^{3}H]cAMP was expressed as the ratio in percentage radioactivity in the cAMP fraction as compared to the combined radioactivity in both cAMP and ATP fractions, and basal activity was subtracted to correct for spontaneous activity. Compounds were studied in a concentration range of 10^{-10}–10^{-6} M. pEC_{50} and pA_2 values were calculated according to Cheng–Prusoff equation. Two independent experiments were performed in triplicate. *Induction of Acute EAE in the Lewis rat*. Male Lewis rats (Harlan Laboratories B.V., the Netherlands) kept under normal housing conditions with water and food ad libitum and weighing between 175 and 225 g at the start of the experiment, were inoculated on day 0 as previously described.[22] Briefly, a 100 μL saline based emulsion containing 50 μL Complete Freund Adjuvant H37 RA (CFA, Difco Laboratories, Detroit, MI), 500 μL *Mycobacterium tuberculosis* type H37RA (Difco) and 20 μg guinea pig myelin basic protein (MBP) was injected subcutaneously in the pad of left hind paw of isoflurane anaesthetized animals. Animals were group housed per 3 and cages were randomized across treatments. Disease symptoms and weights of all animals were recorded daily. The following scores for motor dysfunctions were used: 0, healthy animal with normal curling reflex at the tail; 1, paralysis of the tip of the tail; 2, loss of muscle tone at the base of the tail; 3, low posture of hind limbs; 4, instability at hips; 5, partial hind limb paralysis; 6, complete hind limb paralysis; 7, paralysis include midriff; 8, quadriplegia; 9, moribund; 10, death due to EAE. All experimental procedures were approved by Abbott's Institutional Animal Care and Use Committee.