Discovery of APD371: Identification of a Highly Potent and Selective CB₂ Agonist for the Treatment of Chronic Pain

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Supporting Information

ABSTRACT: The discovery of a novel, selective and fully efficacious CB₂ agonist with satisfactory pharmacokinetic and pharmaceutical properties is described. Compound **6** was efficacious in a rat model of osteoarthritis pain following oral administration and, in contrast to morphine, maintained its analgesic effect throughout a 5-day subchronic treatment paradigm. These data were consistent with our hypothesis that full agonist efficacy is required for efficient internalization and recycling of the CB₂ receptor to avoid tachyphylaxis. Based on its overall favorable preclinical profile, **6** (APD371) was selected for further development for the treatment of pain.

KEYWORDS: cannabinoids, cannabinoid receptor type 2 (CB₂), CB₂ agonist, tachyphylaxis, APD371, chronic pain

Endocannabinoids interact with a receptor system that presently consists of two Class A G-protein coupled receptors (GPCR), namely, cannabinoid receptor type 1 (CB₁) and cannabinoid receptor type 2 (CB₂), which regulate a wide range of pharmacological processes.¹ More recently, GPR55 has been proposed as a third member of the family, but this has yet to be universally accepted. The ability to modulate the endocannabinoid system may offer opportunities to control symptoms of pain arising from numerous clinical disorders. The CB₁ receptor, which is abundantly expressed in the peripheral and central nervous system,² can elicit multiple actions, but the therapeutic utility of agonists of this receptor is limited by the psychotropic effects associated with CB₁ activation in the central nervous system. The CB₂ receptor is mainly expressed on the cells of the immune system.³ Preclinical studies have consistently demonstrated that activation of CB₂ receptors reverses pain responses in a wide range of acute and chronic pain models.^{4–9} A lack of selectivity inherent in some of the pharmacological tools used in these studies, however, has plagued the field and doubts remain as to the true potential of selective CB₂ agonists for pain management. The precise mechanism of action underlying antinociceptive effects mediated by CB₂ receptor activation remains elusive too, despite several proposed hypotheses.^{10–13} Importantly though, this mode of intervention could avoid issues such as tolerance and addiction as well as some of the severe side effects associated with current pain therapies. The pharmaceutical industry has made considerable efforts to identify potent and selective CB₂ agonists for the treatment of pain,¹⁴ and several companies have developed CB₂ selective molecules for evaluation in human trials, so far without success.¹⁵

We recently disclosed a tricyclic 3–5–5-fused pyrazole 3-carboxamide series (e.g., **1**) as a novel CB₂ receptor chemotype.¹⁶ Despite the discovery of potent and selective CB₂ agonists that demonstrated robust acute efficacy in several pain models in rodents, we failed with this first iteration to identify a molecule that produced a sustained response upon repeated dosing. This potential for *in vivo* desensitization of the CB₂ receptor has not previously been critically examined. Whereas it is often assumed that agonists of GPCRs may induce tachyphylaxis, it is known that agonist-induced endocytosis regulates many GPCRs by a specific membrane trafficking pathway.¹⁷ Typically, the rapid recycling of agonist-induced internalized receptors back to the plasma membrane can facilitate the maintenance of the cellular response to the agonist during long-term agonist exposure. In the case of the μ -opioid receptor, for example, activation of the receptor with morphine, a partial μ -opioid receptor agonist, results in desensitization of the response due to a weak (or partial) internalization response. This lack of receptor recycling results in the receptor being essentially removed from the cell surface and thus unavailable to the agonist ligand.¹⁸ In contrast, activation with the full agonists etorphin or DAMGO allows for efficient receptor recycling and no desensitization is observed.

The intrinsic efficacy (E_{max}) of **1** is significantly attenuated in both human and rat CB₂ receptor assays relative to the nonselective cannabinoid receptor full agonist CP-55,940 (Table 1). In addition, **1** failed to induce the same level of

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Table 1. *In Vitro* EC₅₀ Data and Liver Microsomal Stability

compd	stereochemistry	R ^a	R ¹ /R ^{2a}	β -arrestin, EC ₅₀ (nM) ^b				LM stability ^c (h, r, m)
				hCB ₂ (E _{max})	hCB ₁	rCB ₂ (E _{max})	rCB ₁	
1	(R,R)-	2,4-difluoro-Ph	CH ₃ /CH ₃	64 (83)	>10,000	70 (46)	>10,000	>60, >60, 13
2	(R,R)-	2-pyrazinyl	CH ₃ /CH ₃			320 (85)	>10,000	>60, >60, >60
3	(R,R)-	2-pyrazinyl	(S)- <i>t</i> -butyl/H	26 (104)	>10,000	21 (78)	>10,000	>60, 38, 9
4	(S,S)-	2-pyrazinyl	(R)- <i>t</i> -butyl/H	560 (78)	>10,000			
5	(S,S)-	2-pyrazinyl	(S)- <i>t</i> -butyl/H	59 (107)	>10,000	53 (95)	>10,000	>60, >60, 24
6	(S,S)-	4-oxo-2-pyrazinyl	(S)- <i>t</i> -butyl/H	6.2 (106)	>10,000	7.6 (100)	>10,000	>60, >60, >60

^aR, R¹, and R² refer to substituents in Figure 1 for the general structure above the arrow. ^bEC₅₀ values are the mean of three or more independent experiments with logSD < 0.4. Intrinsic activity (E_{max}) was determined relative to CP-55,940 (100). ^cHalf-life (t_{1/2}, min).

internalization of rat CB₂ receptors compared to CP-55,940, as measured by time-resolved flow cytometry (~68% relative to CP-55,940, Figure S6). We hypothesized that this inefficient internalization by **1** may be responsible for the tachyphylaxis observed *in vivo* by limiting the receptor endocytic recycling pathway in a similar way to the morphine- μ -opioid receptor interaction.¹⁸ Furthermore, we wanted to investigate whether internalization efficiency could be improved by increasing agonist efficacy, which could then reduce or eliminate the tachyphylaxis effect.

We therefore focused on identifying a highly selective and fully efficacious CB₂ agonist to test our hypothesis. With this goal in mind we further explored aryl group substitution in our pyrazole N-1,3-carboxamide series, as well as stereoisomers of the tricyclic core (Figure 1) using our previously described synthetic

efficacy relative to **2**. Although the intrinsic efficacy for the human receptor was already at a maximum level, the (S,S) analogue **5** further improved intrinsic efficacy at the rat CB₂ receptor while retaining good potency. The stereochemistry of the *tert*-butyl group also played a crucial role in engaging the receptor as demonstrated by the weaker effect of the (R)-*tert*-butyl analogue **4**. Finally, an additional modest decrease in lipophilicity by incorporation of a pyrazine N-oxide into the scaffold provided the highly potent and fully efficacious CB₂ agonist **6** (cLogP = -0.65). To focus further on this promising scaffold, an additional series of 3-carboxamide analogues (**10–21**) were prepared starting from commercially available and optically pure (S)-2-(but-3-en-1-yl)oxirane and utilizing the synthetic sequence illustrated in Scheme 1. The significant

Scheme 1. General Synthesis of Tricyclo Pyrazole-3-carboxamides^a

Figure 1. SAR approach to the discovery of **6**.

^aReagents and conditions: (a) lithium 2,2,6,6-tetramethylpiperidine, MTBE; (b) TEMPO (2.5 mol %), NaOCl, 67% for a and b; (c) *t*-BuOK, diethyl oxalate; (d) 2-hydrazinylpyrazine, EtOH, 73% two steps; (e) LiOH, 100%; (f) MCPBA, 81%; (g) HATU, NH₂CR³R⁴, 67% for **6**.

approaches.¹⁶ An additional objective was to improve the drug-like properties of our initial series, which was rather lipophilic and had only marginal solubility, and to identify compounds that maintained full intrinsic efficacy regardless of species. To assess potency and efficacy, we employed a DiscoverX PathHunter β -arrestin assay, which was reported to exhibit a much more robust signal for CB₂ ligands than a homogeneous time-resolved fluorescence (HTRF) cyclase assay.¹⁹ Indeed, the β -arrestin assay proved to be more discerning in identifying small differences in intrinsic efficacy of our novel series than the cAMP assay, although EC₅₀ values in this platform were generally five- to 10-fold right-shifted. In this assay (Table 1), **1** appeared even more partial relative to the full agonist CP-55,940 (rCB₂ E_{max} = 46) than in the cAMP platform (rCB₂ E_{max} = 80). Incorporation of a pyrazine to give **2** both significantly decreased the lipophilicity of the molecule (cLogP for **1** = 2.1; for **2** = -0.36) and increased the intrinsic efficacy for rat CB₂ albeit with a loss of potency. Replacing the *gem*-dimethyl feature with a sterically bulky group, (S)-*tert*-butyl (**3**), resulted in dramatically improved CB₂ potency without any meaningful change in

reduction in the lipophilicity of the core now allowed for the use of more lipophilic amines in the exploration of this series, while still maintaining a good balance of physical properties. (1S,5R)-Bicyclo[3.1.0]hexan-2-one (**7**) was prepared through a catalytic intramolecular cyclopropanation of the oxirane, followed by a TEMPO/bleach oxidation.²⁰ A facile one-pot, two-step synthesis involving acylation of ketone **7** with diethyl oxalate followed by condensation with 2-hydrazinylpyrazine yielded the tricyclic pyrazole core **8**.²¹ The resultant ethyl ester was hydrolyzed with LiOH to an acid intermediate, which was then selectively oxidized with mCPBA to form the N-oxide acid **9**. We found that hydrolysis of the ester followed by oxidation provided better control over the regioselectivity of the oxidation step than using the reverse sequence. Compound **9** was then converted to the

Table 2. 3-Carboxamide SAR

compd	R ³ /R ⁴	R ⁵	β -arrestin, EC ₅₀ nM ^a				LM stability ^b (h, r)
			hCB ₂ (E _{max})	hCB ₁ (E _{max})	rCB ₂ (E _{max})	rCB ₁ (E _{max})	
6	(S)- <i>t</i> -butyl/H	-CH ₂ OH	6.2 (106)	>10,000	7.6 (100)	>10,000	>60, >60
10	(S)-isopropyl/H	-CH ₂ OH	46 (103)	>10,000	55 (88)	>10,000	>60, >60
11	(S)-phenyl/H	-CH ₂ OH	105 (96)	>10,000	60 (72)	>10,000	>60, >60
12	(S)-CF ₃ /H	-CH ₂ OH	17.2 (119)	>10,000	35 (107)	>10,000	>60, >60
13	(R)-CF ₃ /H	-CH ₂ OH	280 (98)	>10,000	530 (85)	>10,000	>60, >60
14	-cyclopentyl- ^c	-CH ₂ OH	5.6 (112)	>10,000	6.8 (106)	>10,000	>60, >60
15	(S)- <i>t</i> -butyl/H	-CH ₂ F	1.2 (107)	1100 (105)	1.5 (97)	840 (107)	>60, >60
16	(S)-isopropyl/H	-CH ₂ F	3.8 (98)	6100 (96)	6.4 (87)	2700 (74)	>60, >60
17	CH ₃ /CH ₃	-CF ₃	2.3 (108)	>10,000	6.3 (98)	>10,000	>60, >60
18	-cyclobutyl- ^c	-CF ₃	1.2 (115)	>10,000	1.6 (106)	>10,000	>60, >60
19	-cyclopropyl- ^c	-CF ₃	5.9 (108)	>10,000	14.5 (103)	>10,000	>60, >60
20	-cyclopropyl- ^c	-CH ₃	91 (102)	>10,000	290 (81)	>10,000	>60, >60

^aEC₅₀ values are the mean of three or more independent experiments with logSD < 0.4. Intrinsic activity (E_{max}) was determined relative to CP-55,940 (100). ^bHalf-life (t_{1/2}, min). ^cR³ and R⁴ are connected to form a carbocycle.

target compounds via a HATU-mediated amide coupling. Screening data for selected examples prepared in this manner are shown in Table 2.

In general, the carboxamides maintained excellent stability in both human and rat liver microsomes (LM), but CB₂ potency and efficacy were somewhat sensitive to variations in R³, R⁴, and R⁵. For example, replacing the *tert*-butyl with less sterically hindered groups, including fluoro-alkyl groups (e.g., 10, 12–13) or phenyl (11) led to a decrease in CB₂ potency. Although the stereochemistry of a single substituent had a significant impact on CB₂ potency (e.g., 12 vs 13 or 4 vs 5), achiral analogues such as cyclopentane (14) were well tolerated, affording an excellent potency and efficacy profile. However, further characterization of 14 later showed it did not have a significantly improved pharmacokinetic profile in rodents compared to 6 (Table S4). Isosteric replacement of -OH with -F (e.g., 15–16) resulted in improved potency for CB₂, but reduced selectivity against CB₁. However, incorporating the lipophilic CF₃ group into either the *gem*-dimethyl array (17) or small ring systems (e.g., 18–19) allowed us to achieve an outstanding balance of potency and selectivity. Compound 17 showed favorable rat PK albeit with a somewhat elongated T_{max} (Table S5) and was selected for evaluation in several *in vivo* rodent pain models. Compound 17 displayed good efficacy in these models after oral administration.

However, 17 had relatively high CNS penetration (b/p ratio = 0.83 following a 3 mg/kg, PO dose, Table S6). Despite this profile, 17 was dosed up to 100 mg/kg in rats without inducing any change on body temperature and locomotor activity, which are known to be characteristic of CB₁ activation. This apparent lack of CB₁ engagement *in vivo* is consistent with the *in vitro* data that shows no measurable activity at the rat CB₁ receptor. However, as a further mitigation strategy with respect to potential central CB₁ activity in the clinic, we focused our further efforts on 6, which was equally selective compared to 17, and in CNS partitioning studies had significantly lower brain exposure in the rat (b/p ratio = 0.04 at 3 mg/kg, PO, Table S3).

A more comprehensive *in vitro* profile of 6 (see Table S10) showed that single digit nanomolar potency and full intrinsic efficacy were maintained in all species assessed and that 6 was highly selective for CB₂ over CB₁ in both binding and functional assays. Furthermore, 6 induced efficient receptor internalization (~106% relative to the CB_{1/2} agonist CP55,940, Figure S6) in CHO cells expressing HA-tagged rat CB₂ suggesting that,

according to our hypothesis, 6 would be able to drive agonist-induced receptor recycling.

Compound 6 had a favorable overall ADME profile (see Table S1 for parameters). It was stable in mouse, rat, dog, monkey, and human liver microsomes and showed moderate plasma protein binding (PPB; ~10 to 20% unbound fraction) in all five species examined. *In vivo* pharmacokinetic studies in multiple preclinical species showed that 6 had good plasma exposure, moderate systemic clearance, low steady-state volume of distribution across species, and good oral bioavailability in rodents and dogs. Compound 6 had a relatively short half-life in most species, something that we regarded as a potential advantage for pain treatments if it were to translate into humans. The outlying species in terms of oral bioavailability was the cynomolgus monkey. Additional studies to address this species difference suggested that cytosolic aldehyde oxidases (AO), which are highly expressed in monkeys,²² were likely responsible for the *in vivo* biotransformation of the *N*-oxide to the reduced form 5. These data (not shown) were consistent with the high concentrations of 5 observed in the plasma of monkeys (AUC_{last} ratio 5/6 = 4.89). In contrast, the AUC_{last} ratio between 5 and 6 in rats and dogs were only 0.17 and 0.12, respectively, after a dose of 10 mg/kg PO, reflecting the much lower expression of cytosolic aldehyde oxidases in these species. We were concerned that the generation of an active metabolite, even at low levels, may result in undesirable *in vivo* activity, particularly if it lacked the optimized CB₂ efficacy, selectivity, and partitioning profile of the parent. However, although it was a less potent agonist at the CB₂ receptor, 5 maintained full agonist efficacy, along with high selectivity against CB₁ and in a broader panel (Table S12). In addition, 5 was largely peripherally restricted in abbreviated CNS studies in the rat (b/p ratio ≈ 0.1, Table S7). Thus, with a pharmacokinetic and metabolic profile suitable for *in vivo* testing, the acute analgesic effect of 6 was evaluated in the monosodium iodoacetate (MIA) model of osteoarthritis (OA) pain in the rat.²³

In this model, knee cartilage damaged by MIA injection into the joint precipitates osteoarthritis and an associated hyperalgesia that can be measured by von Frey hair stimulation. Eighteen days post-MIA injection, MIA treated rats received an oral dose of 6 (0.3–30 mg/kg). Von Frey paw withdrawal thresholds were measured 1 h postdose to assess the analgesic effect in MIA-induced late phase hypersensitivity. Compound 6 significantly increased paw withdrawal thresholds at doses ≥3

mg/kg PO ($ED_{50} = 2.3$ mg/kg; Figure 2a), an effect that was not statistically different from that observed with 3 mg/kg IP of

Figure 2. (a) Dose–response in the MIA-induced model of OA pain. (b) Blocking experiments with a CB_2 antagonist or a CB_1 antagonist. Doses: **6** (3 mg/kg, IP), AR401733 (10 mg/kg, PO), and rimonabant (Rim, 10 mg/kg, PO).

morphine (data not shown). In a separate experiment, a single dose of **6** (10 mg/kg, PO) inhibited paw withdrawal threshold for up to 4 h after administration. Based on the PK data for this dose, there appeared to be a good correlation between serum drug concentrations and pharmacological response over this time period. Separately, the analgesic effects of **6** were shown to be highly likely mediated via activity at CB_2 receptors since the effects were blocked by a novel CB_2 inverse agonist AR401733, a much more selective CB_2 inverse agonist in the rat than the literature antagonists AM-630 and SR144528 (Figure S1), but not by the selective CB_1 antagonist rimonabant (Figure 2b). Being from the same scaffold as **6**, it is possible that AR401733 has equal and opposite functional activity via another target. However, we regard this possibility as remote as the analgesic effect of **1**, with the same tricyclic core as both AR401733 and **6**, was reported to be blocked by AM-630,¹⁶ while less selective for CB_2 over CB_1 has significant structural differences.

With encouraging acute *in vivo* data, we investigated the effect of subchronic treatment with **6** in the OA pain model (Figure 3).

Figure 3. Five-day subchronic study: **6** (3 mg/kg, BID, IP) and morphine (10 mg/kg, BID, IP).

Because of its short half-life and moderate C_{max} after PO administration, **6** was dosed IP twice daily for 5 days. This route gave greater acute exposure (~ 2 -fold higher C_{max} and 5-fold higher AUC after dose correction; Table S2) and was calculated to have the best chance of maintaining receptor coverage over the dosing period. Reversal of MIA-induced hypersensitivity was assessed 1 h postdose following the morning dose on days 1, 3,

and 5. An effect comparable to the acute drug response was retained over the duration of the study at each time point tested, suggesting that dosing with **6** does not induce tachyphylaxis in this model at least under this dosing paradigm. In contrast, animals treated with morphine using the same dosing regimen exhibited a significant decrease in drug response after 3 or more days of dosing.

Having achieved a good *in vivo* analgesic effect in our preclinical models, the potential candidate was further profiled. An X-ray crystal structure showed the absolute stereochemistry and the position of the *N*-oxide function to be consistent with the original assignments from 1H -NMR (Figure S4). Solid form characterization revealed **6** to have excellent physicochemical properties, including high aqueous solubility (~ 2 mg/mL @ pH 2 to 7), a high melting point ($164^\circ C$), and excellent stability. Compound **6** showed no significant inhibition of major CYP P450 enzymes (i.e., 1A2, 2C9, 2C19, 2D6, 3A4; $IC_{50} > 50 \mu M$) indicating a low risk for drug–drug interactions. Compound **6** had no effect on the hERG channel in either [3H]-Astemizole binding or patch clamp assays ($IC_{50} > 30 \mu M$) and showed no cytotoxic potential in an Essential Cell Function (ECF) panel measuring cellular parameters in live HepG2 cells up to 1000 μM . It was negative for mutagenic activity in the Ames bacterial reverse mutation assay in the presence and absence of rat liver S9 fraction. Additionally, both **6** and its predominant metabolite **5** showed only weak or no off-target activity (at 10 μM) in *in vitro* binding assays that cover a broad range of receptors, ion channels, and transporters (Eurofins/CEREP panel, Tables S11 and S12).

In vivo, **6** did not induce any change in core body temperature (CBT) up to a dose of 100 mg/kg PO, whereas CP-55940 showed a clear decrease in CBT at 0.3 mg/kg (Figure S7). These data are consistent with an absence of measurable functional activity at the CNS expressed CB_1 receptor ($EC_{50} > 30 \mu M$) and/or the peripheral restriction observed *in vivo* (b/p ratio = 0.04). A dose-range finding 14-day toxicity study was conducted in male and female Sprague–Dawley rats using 0, 30, 100, and 300 mg/kg (PO) dose groups. Compound **6** was well tolerated at all doses, and only at the highest dose of 300 mg/kg was transient lethargy observed on the first day of dosing, which resolved with subsequent doses. No treatment related changes in hematology and histopathological abnormalities were observed.

In summary, we have discovered a selective and fully efficacious CB_2 agonist with an overall satisfactory pharmacokinetic profile, good pharmaceutical properties, and that met our preclinical efficacy and safety criteria. Compound **6** was as efficacious as morphine in a rat model of OA pain following oral administration and, unlike morphine, maintained its analgesic effect throughout a subchronic treatment paradigm without tachyphylaxis. These data are consistent with our developing hypothesis that a full agonist is required for efficient internalization and recycling of the CB_2 receptor, which we believe may be a viable approach to avoiding tachyphylaxis. Based on its favorable profile, **6** (APD371) was selected for clinical development for the treatment of pain. The outcome of further studies will be disclosed in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.7b00396.

Chemistry methods, full characterization data for APD371, *in vitro* screening and *in vivo* pharmacology methods, and additional functional and pharmacokinetic data for compounds discussed in the text (PDF)

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

CB₂, cannabinoid receptor-2; CB₁, cannabinoid receptor-1; cLogP, calculated LogP; hERG, human ether-à-go-go-related gene; CYP, cytochrome P450; CHO cell, Chinese hamster ovary cell; CNS, central nervous system; HA-tag, hemagglutinin fragment tag; PO, per oral; IP, intraperitoneal; SC, subcutaneous; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; mCPBA, meta-chloro perbenzoic acid; PK, pharmacokinetics; ADME, adsorption, distribution, metabolism, and excretion; MIA, monoiodoacetate; MTBE, methyl *tert*-butyl ether; OA, osteoarthritis; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; PPB, plasma protein binding; AUC, area under the curve; DAMGO, D-Ala², N-MePhe⁴, Gly-ol]-enkephalin.

REFERENCES

- (1) Di Marzo, V.; Bifulco, M.; De Petrocellis, L. The Endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discovery* **2004**, *3*, 771–784.
- (2) Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; De Costa, B. R.; Rice, K. C. Cannabinoid receptor localization in brain. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 1932–1936.
- (3) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993**, *365*, 61–65.
- (4) Ibrahim, M. M.; Deng, H.; Zvonok, A.; Cockayne, D. A.; Kwan, J.; Mata, H. P.; Vanderah, T. W.; Lai, J.; Porreca, F.; Makriyannis, A.; Malan, T. P., Jr. Activation of CB₂ Cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 10529–10533.
- (5) Malan, T. P., Jr.; Ibrahim, M. M.; Deng, H.; Liu, Q.; Mata, H. P.; Vanderah, T. W.; Porreca, F.; Makriyannis, A. CB₂ cannabinoid receptor-mediated peripheral anti-nociception. *Pain* **2001**, *93*, 239–245.
- (6) Nackley, A. G.; Makriyannis, A.; Hohmann, A. G. Selective activation of cannabinoid CB₂ receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neuroscience* **2003**, *119*, 747–757.
- (7) Quartilho, A.; Mata, H. P.; Ibrahim, M. M.; Vanderah, T. W.; Porreca, F.; Makriyannis, A.; Malan, T. P. Inhibition of inflammatory hyperalgesia by activation of peripheral CB₂ cannabinoid receptors. *Anesthesiology* **2003**, *99*, 955–960.
- (8) Yao, B. B.; Hsieh, G. C.; Frost, J. M.; Fan, Y.; Garrison, T. R.; Daza, A. V.; Grayson, G. K.; Zhu, C. Z.; Pai, M.; Chandran, P.; Salyers, A. K.

Wensink, E. J.; Honore, P.; Sullivan, J. P.; Dart, M. J.; Meyer, M. D. In vitro and In vivo characterization of A-796260: a selective cannabinoid CB₂ receptor agonist exhibiting analgesic activity in rodent pain models. *Br. J. Pharmacol.* **2008**, *153*, 390–401.

(9) Guidetti, R.; Astles, P. C.; Sanderson, A. J.; Hollinshead, S. P.; Johnson, M. P.; Chambers, M. G. The SAR development of substituted purine derivatives as selective CB₂ agonists for the treatment of chronic pain. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5572–5575.

(10) Ibrahim, M. M.; Porreca, F.; Lai, J.; Albrecht, P. J.; Rice, F. L.; Khodorova, A.; Davar, G.; Makriyannis, A.; Vanderah, T. W.; Mata, H. P.; Malan, T. P., Jr. CB₂ cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 3093–3098.

(11) Anand, U.; Otto, W. R.; Sanchez-Herrera, D.; Facer, P.; Yiangou, Y.; Korchev, Y.; Birch, R.; Benham, C.; Bountra, C.; Chessell, I. P.; Anand, P. Cannabinoid receptor CB₂ localization and agonist-mediated inhibition of capsaicin responses in human sensory neurons. *Pain* **2008**, *138*, 667–680.

(12) Wilkinson, J. D.; Kendall, D. A.; Ralevic, V. Δ⁹-Tetrahydrocannabinol inhibits electrically evoked CGRP release and capsaicin-sensitive sensory neurogenic vasodilatation in the rat mesenteric arterial bed. *Br. J. Pharmacol.* **2007**, *152*, 709–716.

(13) Nagarkatti, P.; Pandey, R.; Rieder, S. A.; Hegde, V.; Nagarkatti, M. Cannabinoids as novel anti-inflammatory drugs. *Future Med. Chem.* **2009**, *1*, 1333–1349.

(14) (a) Han, S.; Thatte, J.; Buzard, D. J.; Jones, R. M. Therapeutic utility of cannabinoid receptor type 2 (CB₂) selective agonists. *J. Med. Chem.* **2013**, *56*, 8224–8256. (b) Morales, P.; Hernandez-Folgado, L.; Goya, P.; Jagerovic, N. Cannabinoid receptor 2 (CB₂) agonists and antagonists a patent update. *Expert Opin. Ther. Pat.* **2016**, *26*, 843–856.

(15) Gilron, I.; Dickenson, A. H. Emerging drugs for neuropathic pain. *Expert Opin. Emerging Drugs* **2014**, *19*, 329–41.

(16) Han, S.; Thoresen, L.; Zhu, X.; Narayanan, S.; Jung, J.-K.; Strahl-Pleynt, S.; Decaire, M.; Choi, K.; Xiong, Y.; Yue, D.; Semple, G.; Thatte, J.; Solomon, M.; Fu, L.; Whelan, K.; Al-Shamma, H.; Gatlin, G.; Chen, R.; Dang, H.; Pride, C.; Gaidarov, I.; Unett, D. J.; Behan, D. P.; Sadeque, A.; Usmani, K. A.; Chen, C.; Edwards, J.; Morgan, M.; Jones, R. M. Discovery of 1a,2,5,5a-tetrahydro-1*H*-2,3-diaza-cyclopropa[*a*]pentalen-4-carboxamides as potent and selective CB₂ receptor agonists. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 322–326.

(17) Ferguson, S. S. G. Evolving concepts in G protein-coupled receptor endocytosis: The role in receptor desensitization and signaling. *Pharmacol. Rev.* **2001**, *53*, 1–24.

(18) Cox, B. M. Agonists at μ-opioid receptors spin the wheels to keep the action going. *Mol. Pharmacol.* **2005**, *67*, 12–14.

(19) McGuinness, D.; Malikzay, A.; Visconti, R.; Lin, K.; Bayne, M.; Monsma, F.; Lunn, C. A. Characterizing Cannabinoid CB₂ receptor ligands using DiscoverX PathHunter™ β-Arrestin assay. *J. Biomol. Screening* **2009**, *14* (1), 49–58.

(20) Alorati, A. D.; Bio, M. M.; Brands, K. M. J.; Cleator, E.; Davies, A. J.; Wilson, R. D.; Wise, C. S. A practical and scalable synthesis of 1*R*,5*S*-bicyclo[3.1.0]hexan-2-one: The development of a catalytic lithium 2,2,6,6-tetramethylpiperidide (LTMP) mediated intramolecular cyclopropanation of (*R*)-1,2-epoxyhex-5-ene. *Org. Process Res. Dev.* **2007**, *11*, 637–641.

(21) Boatman, P. D.; Schrader, T. O.; Kasem, M.; Johnson, B. R.; Skinner, P. J.; Jung, J.-K.; Xu, J.; Cherrier, M. C.; Webb, P. J.; Semple, G.; Sage, C. R.; Knudsen, J.; Chen, R.; Taggart, A. K.; Carballo-Jane, E.; Richman, J. G. Potent tricyclic pyrazole tetrazole agonists of the nicotinic acid receptor (GPR109a). *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2797–2800.

(22) Pryde, D. C.; Dalvie, D.; Hu, Q.; Jones, P.; Obach, R. S.; Tran, T.-D. Aldehyde Oxidase: An Enzyme of Emerging Importance in Drug Discovery. *J. Med. Chem.* **2010**, *53*, 8441–8460.

(23) Pomonis, J. D.; Boulet, J. M.; Gottshall, S. L.; Phillips, S.; Sellers, R.; Bunton, T.; Walker, K. Development and pharmacological characterization of a rat model of osteoarthritis pain. *Pain* **2005**, *114*, 339–346.