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Selective Cannabinoid Receptor Type 2 (CB2) Agonists: Optimization of a Series of Purines Leading to the Identification of a Clinical Candidate for the Treatment of Osteoarthritic Pain

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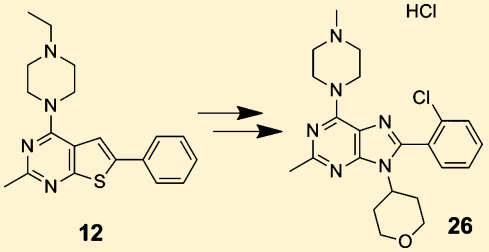
ABSTRACT: A focused screening strategy identified thienopyrimidine **12** as a cannabinoid receptor type 2 agonist (hCB2) with moderate selectivity over the hCB1 receptor. This initial hit suffered from poor in vitro metabolic stability and high in vivo clearance. Structure−activity relationships describe the optimization and modification to a new more polar series of purine CB2 agonists. Examples from this novel scaffold were found to be highly potent and fully efficacious agonists of the human CB2 receptor with excellent selectivity against CB1, often having no CB1 agonist activity at the highest concentration measured (>100 μM). Compound **26** is a centrally penetrant molecule which possesses good biopharmaceutical properties, is highly water-soluble, and demonstrates robust oral activity in rodent models of joint pain. In addition, the peripherally restricted molecule **22** also demonstrated significant efficacy in the same analgesic model of rodent inflammatory pain.



INTRODUCTION

Cannabinoids belong to a class of compounds which were originally isolated from marijuana (*Cannabis sativa* L.) and partially act at two well characterized cannabinoid receptors, CB1[1] and CB2,[2] both of which are G-protein coupled receptors (GPCRs). The medicinal and psychoactive properties of cannabinoids are well documented, and marijuana has been used for centuries as a therapeutic and a recreational drug. It has also been used for a variety of treatments including lack of appetite, pain, emesis, spasticity, and rheumatism.[3] The primary active and most abundant constituent of marijuana is Δ^9-tetrahydrocannabinol **1** (THC, Figure 1), which was isolated and synthesized in 1964.[4] The medical use of THC (and other cannabinoids) has been limited by the undesirable psychotropic side effects and potential addictive properties associated with these compounds.[5]

The two human CB1 and CB2 receptors share 44% overall homology and exhibit different pharmacologic profiles.[6] Both receptors are composed of seven transmembrane proteins and belong to the class A rhodopsin-like GPCRs coupling to inhibitory $G_{i/o}$ proteins. Human and rat CB1 and CB2 receptors show 97% and 81% amino acid sequence identity across species, repectively.[7]

The CB2 receptor was thought to be expressed primarily in peripheral immune cells[8] such as B-cells, T-cells, monocytes, macrophages, and in organs such as the spleen, pancreas, thymus, lung, and tonsil. More recently, expression in the central nervous system (CNS) has been demonstrated albeit to a much lesser extent[9] and activation of the CB2 receptor has been implicated in the analgesic activity of marijuana. Conversely, the CB1 receptor is expressed abundantly in the central nervous system (CNS) with the highest density in hippocampus, cerebellum, basal ganglia, and striatum[10] and

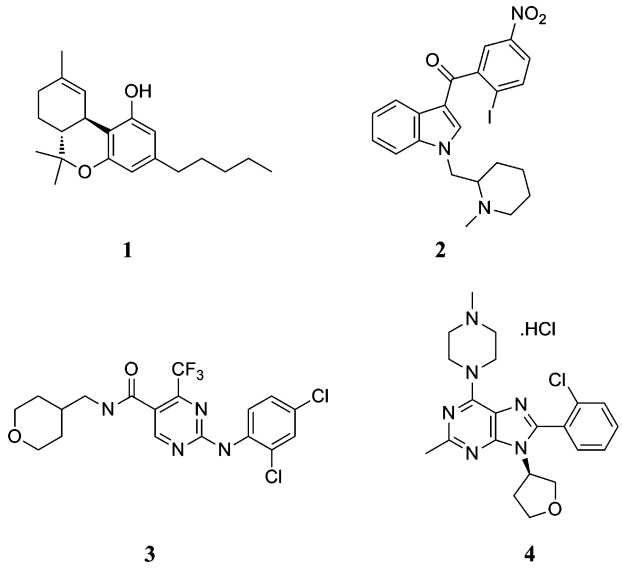


Figure 1. Structures of **1** (Δ^9-tetrahydrocannabinol, THC), **2** (AM1241), **3** (GW842166X), and **4**.

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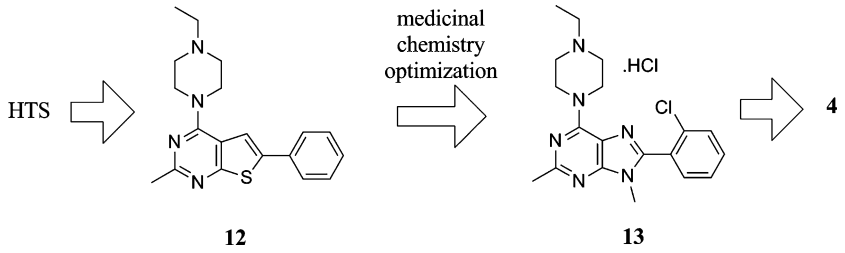
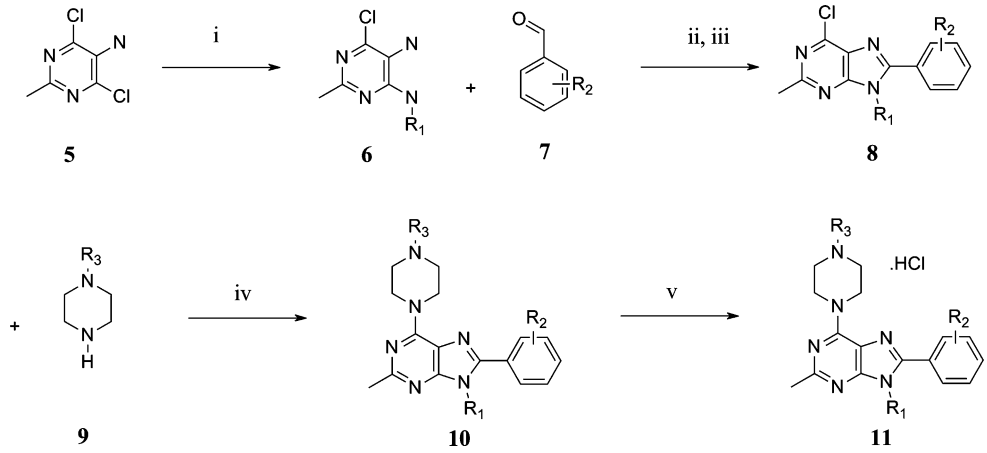


Figure 2. Optimization of screening hit **12** and elaboration to purine scaffold as exemplified by **13** and **4**.

Scheme 1. General Synthesis of Purine Analogues[a]



[a]Reagents and conditions: (i) R1-NH_2, IPA, heat; (ii) 15% $FeCl_3$ on SiO_2, dioxane, heat; (iii) DDQ, DCM; (iv) amine, EtOH−THF (4:1 v/v), heat; (v) acetone, 1 M aqueous HCl.

CB1 activation is believed to be responsible for the unwanted side effects such as ataxia, hypothermia, and euphoria.

CB2 is upregulated "on demand" in the CNS and periphery during early inflammatory events and under pathological pain conditions[8,9a,11] A recent study[12] also provides the first evidence for the localization of CB2-receptor like immunoreactivity in human DRG sensory neurons in vitro and, importantly, colocalization with TRPV1 receptors in injured nerve fibres in human osteoathritic synovium. There are several lines of evidence to suggest CB2 receptors in tissues such as skin and the synovial membrane surrounding the knee joint are expressed in small and medium diameter nociceptive fibers. The CB2 receptor is upregulated at these locations in painful or inflammatory conditions and it has been shown that CB2 agonists block the activation of these nerve fibres,[12] suggesting this may be a useful target for the treatment of osteoarthritic (OA) pain.

Additionally, cannabinoid CB2 agonists have been shown to suppress nociceptive transmission in rodent models of chronic pain with the effects being reversed by selective CB2 antagonists or absent in CB2 knockout mice.[11,13−15] Importantly, CB2-selective agonists lack the centrally mediated side effects associated with activation of CB1 receptors including hypoactivity, hypothermia, and catalepsy.[16,17] It has been hypothesized therefore that a selective CB2 agonist could be therapeutically useful in the treatment of pain but devoid of the unwanted CB1-mediated CNS side effects.

In support of this hypothesis, there have been a growing number of reports from various academic and industrial groups on the development and in vivo evaluation of selective CB2 agonists and this subject has been the subject of several reviews.[18−23] Historically these ligands have tended to be inherently lipophilic in nature with poor pharmacokinetic (PK) properties, but the compounds which are the subject of more recent disclosures have much improved properties (for example lower log *P*) and also encompass a high degree of structural diversity.[22] Despite this progress, no selective CB2 agonist has been approved for the treatment of pain. It remains unclear from preclinical studies whether agonism of the central CB2 receptor represents a clinically useful approach.[24−29]

Two examples of well characterized CB2 agonists include indole **2** (AM1241), which has demonstrated efficacy in inflammatory and neuropathic pain models,[30,31] and pyrimidinecarboxamide **3** (GW842166X),[15] which was entered into clinical trials for the treatment of acute inflammatory pain following third molar extraction (Figure 2); however, this drug candidate has since been discontinued due to lack of efficacy.[32] One potential reason for this failure which was offered by the authors related to the duration of drug exposure and occupancy at the CB2 receptor. There remains then the need for further research into the role of CB2 agonists in the treatment of different chronic pain conditions and the provision of CNS penetrant molecules that are highly selective for CB2 over CB1. The purine molecule **4** (Figure 1) represents a previously reported example from our own internal research efforts directed toward the discovery of such molecules.[33] This compound **4** penetrates into the brain and shows no measurable CB1 agonist activity in vitro and demonstrates robust oral activity in rodent models of joint pain. In this paper, we present the SAR and optimization of this series of purine molecules to identify candidate molecules competent to test the utility of selective CB2 agonists in clinical studies.

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CHEMISTRY

The main synthetic route to obtain the desired purine analogues in Tables 1−2 has been previously described[33] and is briefly outlined in Scheme 1.

Typically, the commercial starting material 5-amino-4,6-dichloro-2-methyl pyrimidine **5** is reacted with an amine (R1-NH_2) to afford the monoamino substituted intermediates **6**. Subsequent reaction with aldehydes **7** is followed by cyclization promoted by $FeCl_3$−SiO_2 according to the procedure of Dang et al.[34] This was typically followed by DDQ oxidation to ensure complete conversion to the desired purines **8**. Subsequent reaction with amines **9** gave the desired products **10**. Those analogues containing basic amine functionality could then be converted to the corresponding HCl salt **11** if required.

RESULTS AND DISCUSSION

Our internal efforts to identify unique CB2 agonists commenced with the screening of a 130K compound collection (biased toward known GPCR ligands) in both human CB1 and CB2 binding assays. We selected actives on the basis of affinity for hCB2 (K_i < 1 μM), >10× selectivity over hCB1, a lack of promiscuity against the panel of GPCRs, and finally CNS drug-like properties.[35] The actives from this screen were then evaluated for agonist activity in a functional GTPγS hCB2 binding assay validated in SF9 cells or CHO cell homogenates expressing hCB2 (Perkin-Elmer, Boston, MA) using a scintillation proximity method.[36] Selectivity over CB1 was subsequently determined in a corresponding GTPγS hCB1 functional assay. These assays were used to drive the structure−activity relationship (SAR) determination described in this paper. Relative potency and selectivity across species was determined from displacement of [^{3}H]-CP-55,940 (a non-selective CB1/2 agonist) from CHO cell homogenates transiently expressing the rat or human, CB2 or CB1 receptors.[37]

This line of investigation led to the initial discovery of thienopyrimidine **12** (Figure 2) which displayed high human and rat affinity (h and r CB2 K_i = 82 and 16 nM, respectively) and potent agonist activity (hCB2 EC_{50} = 24.8 nM), albeit with modest selectivity over CB1 (104 fold).[33] Historically, most cannabinoid receptor ligands tend to be highly lipophilic and we were encouraged that **12** contained a basic center which conferred very good aqueous solubility (>200 μM, simulated GI fluid pH 6.8) but did not have an affinity for the hERG ion channel (IC_{50} > 10 μM, as measured in a displacement assay using hERG channels stably expressed in HEK293 cells and radiolabeled [^{3}H]-dofetilide).[38] Compound **12** possessed a high oral bioavailability in rat (F = 91%), however the rate of clearance was high (CL = 90 mL/min/kg, iv dose of 1 mg/kg). In addition, **12** inhibited CYP2D6 activity with an IC_{50} = 3 μM and also showed moderate serotonin binding most notably to 5HT1A and 1B (K_is ∼ 1 μM).

Compound **12** demonstrated in vivo efficacy in a rodent monoiodoacetic acid (MIA) model[39] of osteoarthritic (OA) pain and significantly reduced pain compared to vehicle at a dose of 10 mg/kg po (see Figure 3A).

This effect was reversed upon treatment of a selective CB2 antagonist SR-144528,[40] confirming that this analgesic effect is mediated by CB2 receptors (Figure 3B). Overall, compound **12** offered an attractive starting point for optimization with the potential for improving in vitro CB2 potency, selectivity over CB1, and in vivo clearance characteristics.

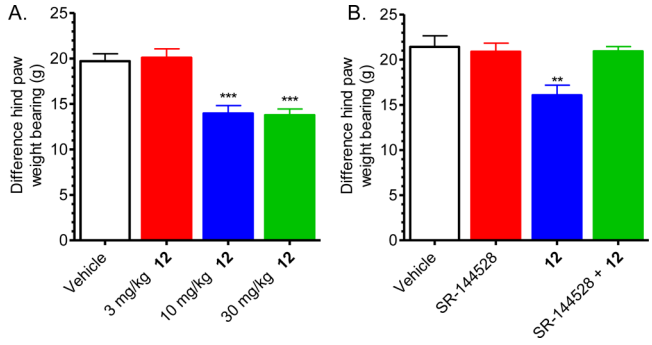

Figure 3. (A) Effect of CB2 agonist **12** (dosed po 2 h prior to testing) and (B) blockade of the response by a CB2 selective antagonist[40] (SR-144528, dosed ip 30 min prior to dosing with **12**) in the MIA model of pain.[39] Values are the mean ± SEM and group size N = 5−6. (**/*** p < 0.01/p < 0.001 One-way ANOVA followed by Dunnett's test versus vehicle).

The major routes of metabolism of **12** were established to be *N*-dealkylation **A**, hydroxyethylation **B**, and aromatic oxidation **C** by metabolic ID studies (Figure 4). An initial SAR focus was to reduce the lipophilicity of the scaffold (while also improving CB2 efficacy and selectivity against CB1) in an early attempt to reduce hepatic microsomal metabolism (94%, 73%, and 97% metabolized in rat, human, and dog species, respectively[41]) before removing or blocking the labile sites.[42]

A number of more polar scaffolds were investigated as alternatives to the thienopyrimidine, and this approach led to the identification of the purine core as exemplified initially by **13** (see Figure 2) as an alternate structural motif. Compound **13** has a lower measured log P (3.1) when compared with **12** (log P = 3.8) and was a 5-fold more potent CB2 agonist (hCB2 EC_{50} = 4.6 nM) relative to **12** (see Table 1). This modification also made 2-fold improvement in the selectivity over the CB1 receptor (CB1/2 = 206). Additionally, this compound displayed high human and rat affinity as determined from displacement of [^{3}H]-CP-55,940 from CHO cell homogenates transiently expressing human or rat CB2 receptors (h and r CB2 K_i = 8.6 and 8.2 nM, respectively).[37] Encouragingly, **13** retained excellent aqueous solubility characteristics (>100 μM in simulated GI fluid pH 6.8) and an IC_{50} > 10 μM against a panel of CYP enzymes. The K_i against the hERG ion channel was >10 μM and displayed an improved metabolic profile (7%, 30%, and 88% metabolized in human, rat, and dog species, respectively)[41] as compared directly with **12** (see Table 1).

As outlined in our previous publication, a substituent in the ortho position on the phenyl ring was established early in our SAR studies to be optimal for CB2 potency.[33] This group was retained during our initial SAR studies on the purine scaffold (Table 1). The next focus for SAR investigation was the R1 substituent on the purine nitrogen. Removal of this R1 substituent gave compound **14**, which maintained CB2 potency and a moderate selectivity over CB1. We considered the introduction of alternative R1 substituents in a further attempt to reduce the log P (and which would also hopefully impact metabolism and in vivo clearance). This tactic and specific changes in the R1 substituent was found to be a very effective way to modulate selectivity over CB1 and also the pharmacokinetic (PK) properties of the target molecules. For example, the alcohol **15** maintained excellent potency and encouragingly also increased the separation between CB2 and CB1 agonism (CB1/CB2 = 760). The related methyl ether **16**

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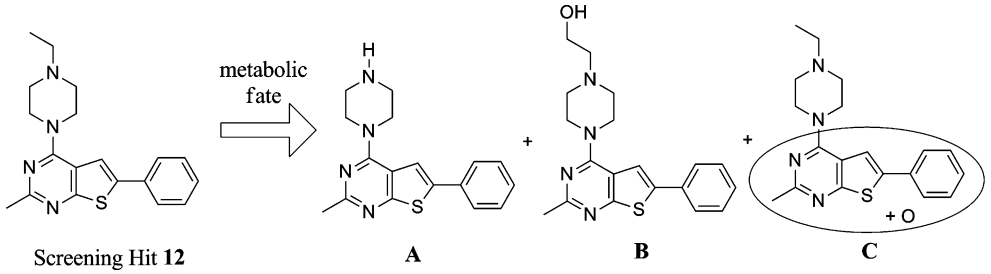

Figure 4. Metabolic analysis of pooled plasma samples of **12** (10 mg/kg po, 2 h time point).

(R1 = CH_2CH_2OMe, R3 = Et) was a potent CB2 agonist (EC_{50} = 6.8 nM) and showed no measurable CB1 activity at the highest concentration measured (CB1 EC_{50} > 100000 nM) and translated into a >14700-fold selectivity over CB1. Other substituents at R1 were also investigated including an amide (**20**), lactam (**21**), and sulphone (**22**). CB2 potency was reduced, however metabolic stability was generally improved across species, resulting in an improvement in oral exposure. Although accomplishing a reduction in clogP and the desired improvement in metabolism and clearance, we found that these R1 groups generally reduced brain penetration and in some cases made the analogues substrates for the PgP efflux transporter (see discussion below for compound **22**).

We established that R3 = H was inactive (data not shown), and so the R3 group was modified with the goal of stabilizing this site to metabolism. Compound **16** and the fluoroethyl analogue **17** possessed similar CB2 potency and excellent CB1 selectivity (CB2 EC_{50} = 4.5 nM, CB1 EC_{50} > 100000 nM, CB1/CB2 > 22222). This change did not improve in vitro metabolic stability, and when both compounds were dosed orally to rats at 3 mg/kg po, AUC values were similarly low (<50 ng·h/mL). The N−Ac analogue **18** interestingly was a potent CB2 agonist but behaved as an inverse agonist at CB1, illustrating a subtlety in the functional effects at this position. Metabolism however was not impacted by this change and the bicyclic amide **19** prepared as a yet more stable replacement similarly did not affect metabolic stability.

Having identified R1 as a position to impact PK and CB1 selectivity, we continued investigating additional substitutions at this position (Table 1). The cyclopropyl compound **23** had excellent CB2 potency (hCB2 EC_{50} = 4.1 nM) and very high selectivity over CB1 (CB1 EC_{50} > 100000 nM, CB1/CB2 > 24390). Oral activity in the MIA pain model was demonstrated at doses as low as 0.1 mg/kg po although the increase in lipophilicity (clogP = 4.0) resulted in a high measured clearance in rat and a high volume of distribution (CL = 83 mL/min/kg, V_d = 12.4 L/kg, 1.0 mg/kg iv). We next prepared cyclic ethers **4**, **24**−**26**. The pair of 3-amino-tetrahydrofuryl enantiomers (**4** and **24**) were evaluated, and the (*R*)-isomer **4** was found to be more potent than the corresponding (*S*)-isomer **24**. Thus compound **4** had a hCB2 EC_{50} = 8.8 nM and CB1/CB2 selectivity > 11364 with moderate metabolic turnover across rat, human, and dog species. Relative to **23**, the clearance and volume of distribution values of **25** in rat were much improved (CL = 30 mL/min/kg, V_d = 5.0 L/kg) and oral bioavailability was moderate (*F* = 27%, dosed at 1 mg/kg). For compounds **25** and **26**, excellent CB2 agonist activity (hCB2 EC_{50} = 11.9 and 20.1 nM, respectively) and selectivity over CB1 were observed (both hCB1 EC_{50} > 100000 nM). Compound **25** demonstrated high clearance in rat (CL = 47 mL/min/kg) and low bioavailability (<20%); however, **26** exhibited very good PK parameters across species and the further profiling of this compound will be discussed below.

With the R1 and R3 groups optimized in compound **26**, we returned to an investigation of the phenyl substitution R2 (compounds **27**−**37**), and results are summarized in Table 2. In general, the ring was insensitive to the electron donating or withdrawing character of the substituent. Preferred substituents in the 2-position include Cl, CF_3, Me, and Et with F, OCH_3, and CN being less active. All substitutions at the 3-or 4-positions reduced activity.

The initial screening hit **12** and optimized compounds **22** and **26** have been evaluated in vivo for efficacy in pain models (see below for compounds **22** and **26**), and confirmation of low/no activity as CB1 agonist or antagonists in a rat native tissue assay was desirable. Thus these three compounds were tested for stimulation or inhibition of CP-55,940 induced GTPγS binding in rat cerebellar homogenates[43] that has previously been characterized as a CB1-mediated activity. As observed in the human clonal cell lines, compounds **12**, **22**, and **26** were all inactive in stimulating GTPγS (EC_{50} > 100000 nM) or inhibiting CP-55,940 stimulated GTPγS binding (IC_{50} > 10000 nM). Thus these compounds appear to be highly selective for the CB2-receptor in both human and rat.

Cross Reactivity of Target Compounds. We had originally noted some off-target cross reactivity with our initial screening hit **12**, most notably against 5HT1A and 1B receptors (K_i = 1.4 and 1.2 μM, respectively). However, the logical progression of our SAR as described toward molecules having a lower clogP also tended to provide compounds which were largely clean against a broad panel of GPCR (including 5HT), enzyme, and ion channel targets (data not shown).[44]

Pharmacokinetic Profiles in Rat and Dog. As summarized in Table 3, compounds **26** and **22** displayed moderate clearance (CL = 43 and 25 mL/min/kg, respectively) and half-lives (2.4−7.4 h) in male SD rats when dosed iv and which translated to acceptable oral bioavailability (67 and 43%, respectively). In male beagle dogs, compound **26** had a low clearance (18 mL/min/kg) and long half-life (7.4 h). Clearance was moderate for **22** (35 mL/min/kg) and also had a moderately long half-life (5 h). Both compounds had excellent solubility as the hydrochloride salts (>100 μM in simulated GI fluid at pH6.8) and measured IC_{50}s at a panel of CYP enzymes were >10 μM. However, these compounds varied in their levels of CNS penetration, making them interesting tools to explore analgesic efficacy. Compound **26** is not a substrate for PgP[45] and is highly brain penetrant (brain/plasma = 0.9), with 17% free fraction in rat plasma and 3% free fraction in brain. In contrast, compound **22** is a substrate for PgP[46] and brain exposure is negligible, limited by PgP mediated efflux.

Profile of Compounds in in Vivo Animal Models. Compound **26** was shown to be potent and efficacious in rat

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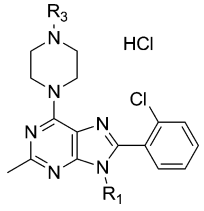
Table 1. Functional GTPγS and Binding Data for CB2 and CB1 Receptors and in Vitro Microsomal Stability for Compounds 4 and 13−26[a]



Compound number	Substitutions		c log P[b]	CB2 and CB1 GTPγS assays[36]					CB2 binding assay[37]		microsomal stability[41]		
	R_1	R_3		hCB2 EC_{50} (nM)	hCB2 Eff (%)	hCB1 EC_{50} (nM)	hCB1 Eff (%)	ratio CB1/2	hCB2 K_i (nM)	rCB2 K_i (nM)	hum %	rat %	dog %
13	Me	Et	3.9 (3.1)	4.6±0.8[c]	90.1±2.9[c]	948±69.0[d]	89.8±5.2[d]	206	8.6±1.3[c]	8.2±1.4[d]	7	30	88
14	H	Et	3.5 (3.7)	4.7±0.5[c]	97.6±3.4[c]	1,430±404[d]	95.4±2.0[d]	304	15.8	4.3	ND[e]	ND[e]	ND[e]
15	CH_2CH_2OH	Me	2.9	8.1±1.5[c]	96.9±3.7[c]	6,160±1,280[c]	66.2±3.3[c]	760	ND[e]	48.4	13	6	99
16	CH_2CH_2OMe	Et	3.8	6.8±0.3[d]	97.9±1.0[d]	>100,000[d]		>14,700	13.0	10.7	31	53	86
17	CH_2CH_2OMe	CH_2CH_2F	3.6	4.5±0.5[c]	95.4±1.6[c]	>100,000[d]		>22,222	9.2	16.0	34	75	62
18	CH_2CH_2OMe	COMe	2.8	10.4±1.4[d]	103±6.3[d]	512±138[d,f]			20.4	39.7	35	95	34
19	CH_2CH_2OMe		3.0	8.4±2.9[d]	99.0±4.9[d]	4,770±2,180[d]	30.4±2.5[d]	567	5.3±3.1[d]	41.2±3.8[d]	32	91	35
20	$CH_2CH_2NHCOMe$	Me	2.7	53.8±7.7[d]	96.1±6.9[d]	4,660±785[d]	26.2±10.6[d]	86	ND[e]	ND[e]	0	0	40
21		Me	3.1	62.0±7.7[d]	92.0±3.6[d]	>100,000[d]		>1,613	459	459	13	28	60
22	$CH_2CH_2SO_2Me$	Me	2.4	20.3±2.6[c]	88.6±4.3[c]	13,300±7,170[c]	52.4±16.3[c]	655	37.5±4.8[d]	87.4±7.7[d]	7	20	36
23		Me	4.0	4.1±0.4[d]	92.52±.5[d]	>100,000[d]		>24,390	13.7	19.4	31	44	98
24		Me	3.4	22.8±3.8[c]	82.8±5.0[c]	>100,000[d]		>4,386	48.2±22.0[d]	83.7±50.9[d]	32	58	80
4		Me	3.4 (3.2)	8.8±0.9[c]	85.2±1.5[c]	>100,000[c]		>11,364	19.9±0.6[d]	24.0±5.0[d]	31	61	55
25		Et	4.3 (3.4)	11.9±1.7[c]	104±3.8[c]	>100,000[d]		>8,403	52.8±5.9[d]	23.1±4.5[d]	41	80	76
26		Me	3.5 (3.1)	20.1±3.2[c]	86.9±2.0[c]	>100,000[c]		>4,975	40.3±6.9[c]	36.7±2.7[c]	29	65	52

[a] [^{35}S]-GTPγS and binding data results represented are the mean values ± SEM. Data without ± SEM are an n of 1. [b] Number in parentheses is the measured log P. [c] Values and SEM are a result of n = 4 or more experiments. [d] Values and SEM are a result of n = 2−3 experiments. [e] ND = not determined. [f] Inverse agonist

models of knee-joint related chronic pain induced by intra-articular monoiodoacetic acid (MIA).[39] A dose related reversal of pain is observed in this MIA pain model (see Figure 5), with a 0.3 mg/kg po dose of **26** demonstrating an equivalent degree of efficacy to the NSAID diclofenac, a standard of care for osteoarthritic pain. As observed with compound **12**, the efficacy seen with a 1 mg/kg dose of **26** was completely blocked by pretreatment with the CB2 selective antagonist SR-144528[40] (data not shown).

In support of a lack of central CB1 activity oral administration of **26** did not induce hypothermia or behavioral impairment in a rotorod study[47] in rats up to 30 mg/kg po. In contrast, the nonselective cannabinoid agonist CP-55,940 (0.5 mg/kg ip) tested as a positive control, caused a significant hypothermia and impaired performance on the rotorod,

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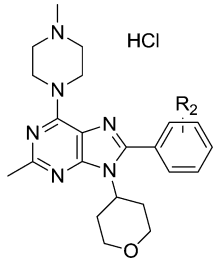
Table 2. Functional GTPγS and Binding Data for CB2 and CB1 Receptors and in Vitro Microsomal Stability for Compounds 27−37[a]



			CB2 and CB1 GTPγS assays[36]					CB2 binding assay[37]		microsomal stability[41]		
compd no.	R_2	clogP	hCB2 EC_{50} (nM)	hCB2 eff (%)	hCB1 EC_{50} (nM)	hCB1 eff (%)	ratio CB1/2	hCB2 K_i (nM)	rCB2 K_i (nM)	hum %	rat %	dog %
27	3-Cl	3.5	71.4 ± 1.1	91.3 ± 4.6	3010 ± 180	67.0 ± 2.0	42.2	ND[b]	ND[b]	25	45	45
28	2-CF3	3.8	13.6 ± 7.1	77.3 ± 2.9	>100000 ± 0		>7353	62.8	40.4	24	38	61
29	3-CF3	3.8	53.1 ± 7.1	91.6 ± 4.1	16900 ± 12000	74.0 ± 13.7	318	ND[b]	ND[b]	19	28	44
30	2-OCH3	2.7	97.4 ± 9.5	57.3 ± 2.8	12200 ± 4880	61.1 ± 9.4	125	ND[b]	ND[b]	22	35	36
31	3-OCH3	2.7	108 ± 11.5	86.7 ± 3.4	22900 ± 16800	67.8 ± 17.9	212	ND[b]	ND[b]	40	30	46
32	2-CN	2.8	106 ± 27.1	65.7 ± 1.4	>1000000 ± 0		>943	ND[b]	ND[b]	20	21	11
33	2-F	3.1	82.7 ± 2.2	74.9 ± 2.0	6050 ± 5080	51.5 ± 18.7	73.1	ND[b]	ND[b]	10	30	21
34	2-CycPro	3.7	21.3 ± 4.1	59.3 ± 10.5	>100000 ± 0		>4695	56.3	21.8	33	67	69
35	2-Me	3.4	15.9 ± 5.2	90.7 ± 1.4	>100000 ± 0		>6289	104	87.1	18	39	39
36	2-Et	3.9	14.1 ± 1.3	94.2 ± 1.7	>100000 ± 0		>7092	31.8	60	33	51	74
37	2-Me-4-OMe	3.2	82.4 ± 22.5	41.5 ± 9.1	>100000 ± 0		>1213	ND[b]	ND[b]	21	24	41

[a][^{35}S]-GTPγS, binding data and ± SEM results were calculated based on at least three experiments. Data without ± SEM are for n = 1. [b]ND = not determined.

Table 3. Pharmacokinetic (PK) Properties for Compounds 26 and 22

compd	species	CL (mL/min/kg)	V_d (L/kg)	$T_{1/2}$ po (h)	AUC (ng·h/mL)	C_{max} (ng/mL)	bioavailability (%)
26	rat PK[a]	43	6	2.4	265	60	67
	dog PK[b]	18	5.5	7.4	1,940	352	76
22	rat PK[a]	25	1.9	1.4	312	76	43
	dog PK[b]	35	9.2	5	286	62	60

[a]Rat (Male Sprague−Dawley) 1 mg/kg iv, 1 mg/kg po. [b]Dog (Beagle) 1 mg/kg iv, 3 mg/kg po.

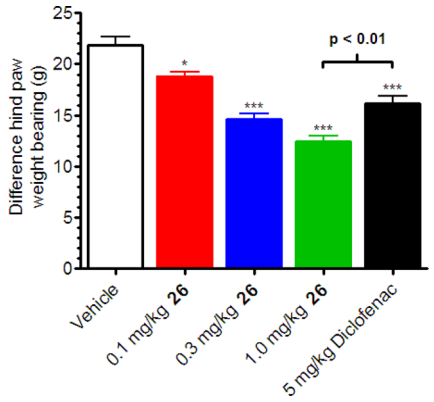


Figure 5. Dose dependent effect of **26** (dosed by oral gavage 1 h prior to testing) in the MIA model.[39] Compound **26** at 0.1, 0.3, and 1 mg/kg significantly reduced weight bearing deficits compared to vehicle as did diclofenac at 5 mg/kg. The 1 mg/kg dose of **26** was significantly different from diclofenac whereas the 0.1 and 0.3 mg/kg doses of **26** were not. Values are presented as mean ± SEM, N = 5. (*/***p < 0.05/0.001 vs vehicle, One-Way ANOVA followed by Tukey HSD.

thereby confirming the high selectivity for CB2 in vivo (data not shown).[48]

We were interested in testing the peripherally restricted CB2 agonist **22** in the in vivo MIA model of rat joint pain and were surprised to find that a 0.3 mg/kg po dose of **22** demonstrated an equivalent degree of efficacy (see Figure 6). Similarly, **22** also did not induce hypothermia[48] in rats up to 30 mg/kg po (data not shown).

While this data may suggest that CNS penetration is not required for efficacy of a CB2 agonist in the MIA model of osteoarthritic pain, whether this would similarly be true in any other preclinical models of pain or spinal pain transmission will require further study and relevance to any human pain condition remains unknown at this time.

CONCLUSIONS

From the initial starting thienopyrimidine **12**, we have discovered and optimized a novel series of purines as orally bioavailable, selective CB2 agonists. The results have demonstrated that the selectivity against CB1 could be modulated by a judicious choice of R1 substituent, and several highly selective CB2 agonists were prepared (many having no measurable in vitro CB1 agonist activity up to 100 μmol). Finally, the in vitro and in vivo clearance characteristics of this series of purines were optimized, and compound **26** emerged as a leading molecule demonstrating good CNS penetration and

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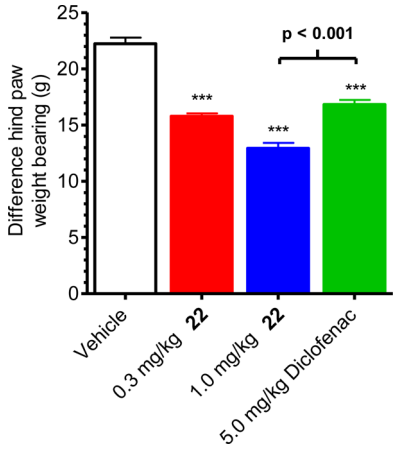

Figure 6. Dose dependent effect of CB2 agonist **22** (dosed by oral gavage 1 h prior to testing) in the MIA model.[39] Both doses of compound **22** significantly reduced weight bearing deficits and the 1 mg/kg doses was significantly more efficacious than 5 mg/kg diclofenac. Values are the mean ± SEM, $N = 6$ (*** $p < 0.001$ vs vehicle, One-Way ANOVA followed by Tukey–Kramer HSD).

potent oral activity in a preclinical model of joint pain. This compound showed no central CB1 agonist effects as measured in preclinical models and demonstrated an overall safety profile, allowing evaluation in clinical studies of OA pain. The results of these studies will be reported in subsequent communications from these laboratories.

EXPERIMENTAL SECTION

Radioligand Binding Assays. Displacement of [^{3}H]-CP-55,940 from human and rat CB2 receptors. The methods of Felder et al. were utilized with minor modifications.[37] Specifically, membrane homogenates from cells stably or transiently expressing the human or rat CB2 receptor were washed by centrifugation and diluted into a 50 mM Tris HCl (pH 7.4), 5 mM $MgCl_2$, 2.5 mM EDTA, and 0.1% BSA 20 buffer. Specific binding of [^{3}H]-CP-55,940 was defined with 1 μM CP-55,940. The ability of compounds to displace specific [^{3}H]-CP-55,940 binding was tested over a range of concentrations in the Tris, $MgCl_2$, EDTA, and BSA buffer in the presence of 1% dimethyl sulfoxide by incubating at room temperature for 90 min in a volume of 300 μL. Unifiltered 96-well microplates pretreated with 0.5% polyvinylpyrrolidone, 0.1% 25 polysorbate 20 in water were washed three times with cold Tris buffer. The reaction mixture was then transferred to the filter plate immediately before terminating the incubation by rapid filtration, and three 200 μL washes with cold Tris buffer. After the filter plates were dried, microscint 20 (Perkin-Elmer, Boston, MA) was added to each well, the plate sealed, and counted for determination of disintegrations per minute. The displacement curves were graphed and the resulting K_i values determined utilizing Graphpad Prism.

CB1 and CB2 in Vitro Functional Assays. Exemplified compounds are tested for agonist activity against cloned human CB1 and CB2 receptors using a SPA (scintillation proximity assay) based [^{35}S]-GTPγ binding assay.[36] All assay components were prepared in assay buffer made up of 20 mM HEPES (pH 7.4), 100 mM NaCl, 5 mM $MgCl_2$, and 0.125% bovine serum albumin. [^{35}S]-GTPγ binding was measured in a 96-well format using a whole membrane capture technique for the CB1 assay and a modified antibody capture technique previously described for the CB2 assay.[36] Human CB1-CHO membranes (Applied Cell Sciences, Rockville, MD), GDP (1 μM final, Sigma, St Louis, MO), and saponin (10 μg/mL final, Sigma, St Louis, MO) were added to assay buffer and homogenized with a Brinkman KINEMATICA AG homogenizer. Diluted compounds, [^{35}S]-GTPγ (500 pM final, PerkinElmer Life Sciences, Boston, MA), and membranes were added to the assay plate and incubated for 30 min at room temperature. Then 1 mg/well Wheatgerm Agglutinin SPA bead (GE Healthcare, Piscataway, NJ) was added, and the plates were sealed, vortexed, and incubated for an additional hour. Plates were then centrifuged at 700g for 10 min and amount of bound [^{35}S] using a Wallac MicroBeta TriLux scintillation counter (PerkinElmer, Boston, MA). Similarly, hCB2-Sf9 membranes (Perkin-Elmer, Boston, MA) and GDP (1 μM final, Sigma, St Louis, MO) were added to assay buffer above and homogenized with a polytron. Diluted compounds and membranes were added to the assay plate and preincubated for 15 min at room temperature. This was followed by addition of [^{35}S]-GTPγ (500 pM final) for an additional 35 min. Subsequently, a mixture containing Nonidet P40 detergent (0.2% final, Roche, Indianapolis, IN), anti-Gi antibody (final dilution of 1:362; Covance, Princeton, NJ), and 1.25 mg antirabbit antibody scintillation proximity assay beads (GE Healthcare, Piscataway, NJ) was added. The plates were then sealed, vortexed, and incubated for an additional 2 h before centrifugation and determination of [^{35}S] bound as above. Percent agonist effect was determined by normalizing the agonist/inverse agonist dose response data to the response seen with the CB1/2 agonist (methanandamide). Analysis of the resulting sigmoidal shaped curve using a four-parameter logistic reduced fit with Activity Base and XLFit3 (IDBS, Emeryville, CA) resulted in the reported EC_{50} and percent relative efficacy. Ability to stimulate [^{35}S]-GTPγ binding or inhibit CP-55,940 stimulated [^{35}S]-GTPγ binding in rat cerebellar homogenates was determined using the methods of Griffin, et al.[43]

Monoiodoacetate (MIA) Model.[39] For all studies, male Lewis rats of approximately 8 weeks of age at the time of MIA injection were used to measure pain in the MIA model. The rats were housed in groups of 2 or 3 per cage and maintained in a constant temperature and on a 12 h light/12 h dark cycle. Animals had free access to food and water at all times except during data collection.

In the MIA model, the right knees of each rat were injected with 0.3 mg of MIA in 50 μL of saline and the left knees with 50 μL of saline. Pain was measured at varying times after MIA injection (not normally before 10 day post MIA injection) using incapacitance testing. This measured the difference in hind paw weight bearing between the MIA and saline injected knees, and each measurement was the average of three separate measurements each measured over 1 s.

For studies with CB2 agonists, rats were randomized into dose groups ($n = 5$ or 6) and then dosed once with the compound under investigation. Dosing was staggered by 15 min for each rat and at a predetermined time postdose (usually corresponding to the T_{max}), pain measured using incapacitance testing. Studies were routinely run with fourgroups, vehicle (1% carboxy methyl cellulose in water plus 0.25% polysorbate 80), and three compound groups which can be either single compounds at a single dose or the same compound at three doses. Results were reported as the difference in weight bearing between saline and MIA injected knees and statistical comparisons were made between vehicle treated and compound treated animals to assess the effect of compounds on knee pain in the model.

Chemistry: General Methods. All reagents and anhydrous solvents were obtained from commercial sources and used without further purification unless noted otherwise. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer. ^{1}H NMR chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$ 7.26 ppm, methanol-d_4 3.31 ppm, DMSO-d_6 2.49 ppm).

Optical rotations were measured with a 341 polarimeter (Perkin-Elmer) at 20 °C and at 589 nm (sodium lamp).

Compounds were analyzed for purity by HPLC and HPLC-MS and purities of synthesized compounds were all found to be >95% by methods 1 and/or 2, as specified below.

HPLC-MS Method 1. Agilent 1100 HPLC, Phenomenex Gemini-NX 2 mm × 50 mm × 3 μm C18 110A column; solvent A, 95/5 water/acetonitrile with 10 mM ammonium bicarbonate pH = 10; solvent B, acetonitrile; gradient 5–100% B in 3 min with 0.75 min hold of 100% B. Flow rate 1.0 mL/min. Atmospheric pressure ionization-electrospray (API-ES) mass spectra data were acquired with an Agilent Technologies MSD single quadrupole coupled to an HP1100 LC system. DAD 200–400 nM scan.

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HPLC-MS Method 2. Agilent 1100 HPLC, Phenomenex Gemini-NX 2 mm × 50 mm × 3 μm C18 110A column; solvent A, water with 0.1% formic acid; solvent B, acetonitrile with 0.1% formic acid; gradient 5−100% B in 3 min with 0.75 min hold of 100% B. Flow rate 1.0 mL/min. Atmospheric pressure ionization-electrospray (API-ES) mass spectra data were acquired with an Agilent Technologies MSD single quadrupole coupled to an HP1100 LC system. DAD 200−400 nM scan.

Chiral HPLC Method 1. HPLC, Chiralpak AD-H 4.6 mm × 150 mm eluting with 20% methanol (containing 0.2% *iso*-propyl amine) in CO_2. Flow rate 5 mL/min. λ = 225 nM.

Chiral HPLC Method 2. HPLC, Chiralpak AS-H 4.6 mm × 150 mm eluting with 0.2% *N*,*N*-dimethylethylamine in methanol. Flow rate 1.0 mL/min. λ = 225 nM.

8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-[(3*R*)-tetrahydrofuran-3-yl]purine Hydrochloride (4). The title compound was prepared by the general methods A−D (see below). HPLC = 100% at 2.30 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 413 (M + 1). Chiral HPLC = 96% at 2.68 min by chiral HPLC method 2. ^{1}H NMR (400 MHz, CD_3OD) 7.48−7.64 (m, 4H), 5.54−5.64 (2H, br m), 4.70−4.77 (m, 1H), 4.30 (q, *J* = 7.6 Hz, 1H), 4.14−4.24 (m, 1H), 3.95−4.04 (br, 1H), 3.86 (q, *J* = 7.4 Hz, 1H), 3.53−3.73 (br m, 4H), 3.19−3.31 (br m, 2H), 2.93 (s, 3H), 2.62 (s, 3H), 2.52−2.63 (br m, 1H), 2.27−2.39 (br m, 1H). Specific rotation = $[\alpha]^{20}_{D}$ + 12.8 (*c* 10, MeOH).

SNAr, General Procedure A: 6-Chloro-*N*4,2-dimethyl-pyrimidine-4,5-diamine (6, R1 = Me). As an example, 40% monomethylamine in water (1 equiv, 11.2 mmol) was added to a solution of 2-amino-4,6-dichloro-2-methylpyrimidine **5** (1 equiv, 11.2 mmol) and diisopropylethylamine (1 equiv, 11.2 mmol) in isopropyl alcohol (36 mL). The mixture was heated in a sealed tube at 150 °C for 4 h. The reaction mixture was cooled and directly adsorbed onto silica and purified by flash chromatography on silica gel eluting with 30−50% acetone in hexanes to afford the title compound **6** (R1 = Me) (1.5 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) 4.85 (br s, 1H), 3.18 (br s, 2H), 3.01 (d, *J* = 5.3 Hz, 3H), 2.42 (s, 3H).

Purine Formation, General Procedure B: 6-Chloro-8-(2-chlorophenyl)-2,9-dimethyl-purine (8, R1 = Me, R2 = *ortho*-Cl). As an example, 6-chloro-*N*4,2-dimethyl-pyrimidine-4,5-diamine **6** (R1 = Me) (1 equiv, 40.0 mmol), 2-chloro benzaldehyde **7** (R2 = *ortho*-Cl) (2 equiv, 80.0 mmol), and 15% $FeCl_3$ on SiO_2 (21g)[34] were combined in dioxane (90 mL) and heated to 100 °C for 16 h. The mixture was cooled and filtered through silica. The filtrates were evaporated and the residue dissolved in dichloromethane (50 mL). The mixture was cooled in an ice-bath, and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.0 equiv, 40 mmol) was added. The cooling bath was removed, and the reaction mixture was then stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane and sequentially washed with 1N aqueous sodium hydroxide solution, water, and brine. The organic layer was dried over anhydrous sodium sulfate and filtered and concentrated to afford a residue which was purified on silica gel eluting with 15% ethyl acetate in hexanes to provide the title compound **8** (R1 = Me, R2 = *ortho*-Cl) (7.0 g, 59%) as a white solid. HPLC = 100% at 2.07 min by HPLC-MS method 2, mass spectrum (*m*/*z*): 293 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$) 7.50−7.60 (m, 3H), 7.40−7.42 (m, 1H), 3.68 (s, 3H), 2.80 (s, 3H).

SNAr, General Procedure C: 8-(2-Chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2,9-dimethyl-purine (10, R1 = Me, R2 = *ortho*-Cl, R3 = Et). As an example, 6-chloro-8-(2-chlorophenyl)-2,9-dimethyl-purine **8** (R1 = Me, R2 = *ortho*-Cl) (1 equiv, 1.7 mmol), *N*-ethyl piperazine **9** (R3 = Et) (1.1 equiv, 1.8 mmol), and triethylamine (1.1 equiv, 1.8 mmol) were combined together in ethanol (5 mL) and heated at 90 °C for 16 h. The reaction mixture was cooled and concentrated under reduced pressure. The residue was dissolved in dichloromethane and sequentially washed with saturated sodium bicarbonate solution, water, and brine. The organics were dried over anhydrous sodium sulfate and filtered and concentrate to provide a residue which was purified on silica gel column using 2% methanol in dichloromethane as eluent to afford the title compound **10** (R1 = Me, *ortho*-Cl, R3 = Et) as a white solid (0.5 g, 78%). HPLC = 100% at 2.1 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 371 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.52−7.60 (m, 3H), 7.44−7.48 (m, 1H), 4.22−4.32 (br, 4H), 3.54 (s, 3H), 2.55 (t, 4H), 2.51 (s, 3H), 2.44 (q, *J* = 7.35 Hz, 2H), 1.10 (t, *J* = 7.25 Hz, 3H).

Hydrochloride Salt Formation, General Procedure D: 8-(2-Chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2,9-dimethyl-purine Hydrochloride (13). As an example, 8-(2-chlorophenyl)-6-(4-ethyl-piperazin-1-yl)-2,9-dimethyl-purine **10** (R1 = Me, *ortho*-Cl, R3 = Et) (118 mg) was dissolved in a 1:1 mixture of acetone−acetonitrile (6 mL), and a 1 M solution of HCl in ether (1.1 equiv, 0.4 mL) was added. The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed. The residue was dissolved in water (1 mL) and lyophilized overnight to afford the title compound **13** (0.13 g, 100%) as a white solid. HPLC = 100% at 2.16 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 371 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.47−7.62 (m, 4H), 5.60−5.68 (br m, 2H), 3.64−3.70 (br m, 2H), 3.58 (s, 3H), 3.42−3.56 (br m, 2H), 3.22 (q, *J* = 7.2 Hz, 2H), 3.12−3.19 (br m, 2H), 2.58 (s, 3H), 1.36 (t, *J* = 7.2 Hz, 3H).

8-(2-Chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2-methyl-9*H*-purine Hydrochloride (14). The title compound was prepared by the general methods A−D. HPLC = 100% at 1.86 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 357 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.77 (dd, *J* = 1.9, 7.6 Hz, 1H), 7.43−7.58 (m, 3H), 5.62−5.78 (br s, 2H), 3.62−3.78 (br s, 2H), 3.50−3.62 (br s, 2H), 3.24 (q, *J* = 7.2 Hz, 2H), 3.15−3.26 (br s, 2H), 2.54 (s, 3H), 1.37 (t, *J* = 7.3 Hz, 3H).

2-[8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-purin-9-yl]ethanol Hydrochloride (15). The title compound was prepared by the general methods A−D. HPLC = 100% at 1.66 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 387 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.57−7.62 (m, 3H), 7.47−7.54 (m, 1H), 5.61−5.74 (br, 2H), 4.20 (t, *J* = 5.42 Hz, 2H), 3.67 (t, *J* = 5.35 Hz, 2H), 3.55−3.68 (m, 4H), 3.21−3.33 (m, 2H), 2.93 (s, 3H), 2.65 (s, 3H).

8-(2-Chlorophenyl)-6-(4-ethylpiperazin-1-yl)-9-(2-methoxyethyl)-2-methyl-purine Hydrochloride (16). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.12 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 415 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) 7.56−7.65 (m, 3H), 7.48−7.52 (m, 1H), 5.25−5.50 (br, 2H), 4.11 (t, *J* = 5.5 Hz, 2H), 3.46−3.60 (br, 4H), 3.44 (t, *J* = 5.5 Hz, 2H), 2.96−3.13 (br, 4H), 2.94 (s, 3H), 2.47 (s, 3H), (br t, 3H).

8-(2-Chlorophenyl)-6-[4-(2-fluoroethyl)piperazin-1-yl]-9-(2-methoxyethyl)-2-methyl-purine Hydrochloride (17). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.07 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 433 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$) 7.39−7.53 (m, 4H), 5.54−6.14 (br, 2H), 5.09−5.14 (m, 1H), 4.97−5.02 (m, 1H), 4.37−4.68 (m, 2H), 4.01−4.31 (m, 2H), 3.65−3.82 (m, 2H), 3.59 (br t, *J* = 4.70 Hz, 2H), 3.38−3.46 (m, 1H), 3.31−3.38 (m, 1H), 3.07−3.24 (m, 2H), 3.06 (s, 3H), 2.86 (s, 3H).

1-[4-[8-(2-Chlorophenyl)-9-(2-methoxyethyl)-2-methyl-purin-6-yl]piperazin-1-yl]ethanone Hydrochloride (18). The title compound was prepared by the same general methods A−D. HPLC = 100% at 1.82 min by HPLC-MS method 1, mass spectrum (*m*/*z*): 429 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.51−7.67 (m, 4H), 4.40−4.80 (br, 4H), 4.34 (t, *J* = 5.05 Hz, 2H), 3.78−3.84 (m, 4H), 3.51 (t, *J* = 5.05 Hz, 2H), 3.12 (s, 3H), 2.74 (s, 3H), 2.16 (s, 3H).

Isomer 2,2-[8-(2-Chlorophenyl)-9-(2-methoxyethyl)-2-methyl-purin-6-yl]-1,3,4,7,8,8a-hexahydropyrrolo[1,2-*a*]pyrazin-6-one Hydrochloride (19). The racemic title compound (as the free base) was prepared by the general methods A−C. This material (694 mg) was separated into enantiomers by supercritical fluid chromatography (SFC) utilizing a Berger Multigram Supercritical Fluid chromatograph with a Chiralpak AD-H column (2.1 cm × 25 cm × 5 μm). The components were eluted with 20% methanol (containing 0.2% DMEA) in CO_2 at 70 mL/min monitoring at λ = 225 nM. Fractions containing each separated enantiomer were collected and evaporated to yield isomer 1 (230 mg) and isomer 2 (220 mg). Data for isomer 2: chiral HPLC = 98% at 1.95 min by chiral HPLC method 1. Isomer 2 (220 mg) was directly converted into the corresponding

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hydrochloride salt using general procedure D to provide the title compound **19** (240 mg) as a white solid. HPLC = 100% at 3.15 min by HPLC-MS method 1, mass spectrum (m/z): 441 (M + 1). Chiral HPLC method 1 = 99% at 2.10 min. ^{1}H NMR (400 MHz, CD_3OD) 7.58−7.64 (m, 3H), 7.49−7.54 (m, 1H), 5.45−5.80 (br, 2H), 4.32 (t, J = 5.0 Hz, 2H), 4.13 (dd, J = 2.8, 12.9 Hz, 1H), 3.80−3.89 (m, 1H), 3.48 (t, J = 5.0 Hz, 2H), 3.24−3.27 (m, 1H), 3.10 (s, 3H), 3.02−3.16 (m, 2H), 2.72 (s, 3H), 2.40−2.46 (m, 2H), 2.25−2.33 (m, 1H), 1.72−1.82 (m, 1H). Specific rotation = $[\alpha]^{20}_D$ − 19.4 (c 10, EtOH).

***N*-[2-[8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)purin-9-yl]ethyl]acetamide Hydrochloride (20).** The title compound was prepared by the same general methods A−D. HPLC = 100% at 1.59 min by HPLC-MS method 1, mass spectrum (m/z): 428 (M + 1). ^{1}H NMR (CD_3OD) 7.58−7.63 (m, 3H), 7.49−7.54 (m, 1H), 5.59−5.69 (br, 2H), 4.20 (t, 2H, J = 5.5 Hz), 3.54−3.68 (m, 4H), 3.39 (t, 2H, J = 5.65 Hz), 3.20−3.27 (m, 2H), 2.93 (s, 3H), 2.64 (s, 3H), 1.67 (s, 3H).

1-[2-[8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)purin-9-yl]ethyl]pyrrolidin-2-one Hydrochloride (21). The title compound was prepared by the same general methods A−D. HPLC = 100% at 0.935 min by HPLC-MS method 1, mass spectrum (m/z): 454 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.61−7.69 (m, 3H), 7.53−7.58 (m, 1H), 5.55−5.71 (m, 2H), 4.34 (t, J = 5.4 Hz, 2H), 3.60−3.78 (m, 4H), 3.48 (t, J = 5.40 Hz, 2H), 3.29−3.38 (m, 2H), 3.16 (t, J = 7.10 Hz, 2H), 2.95 (s, 3H), 2.72 (s, 3H), 2.09 (t, J = 8.20 Hz, 2H), 1.76−1.86 (m, 2H).

8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-(2-methylsulfonylethyl)purine Hydrochloride (22). The title compound was prepared by the same general methods A−D. HPLC = 100% at 1.86 min by HPLC-MS method 1, mass spectrum (m/z): 449 (M + 1). High resolution mass spectrum: calcd 448.1448, found 448.1460. ^{1}H NMR (400 MHz, CD_3OD) 7.58−7.65 (m, 3H), 7.49−7.53 (m, 1H), 5.56−5.67 (br m, 2H), 4.57 (t, J = 6.6 Hz, 2H), 3.56−3.68 (br m, 4H), 3.55 (t, J = 6.5 Hz, 2H), 3.21−3.30 (br m, 2H), 2.93 (s, 3H), 2.82 (s, 3H), 2.65 (s, 3H).

8-(2-Chlorophenyl)-9-cyclopropyl-2-methyl-6-(4-methylpiperazin-1-yl)purine Hydrochloride (23). The title compound was prepared by the same general methods A−D. HPLC = 100% at 2.23 min by HPLC-MS method 1, mass spectrum (m/z): 383 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$) 7.38−7.52 (m, 4H), 5.59−5.80 (br, 2H), 3.94−4.10 (br, 2H), 3.42−3.62 (br, 3H), 2.90−3.07 (br, 2H), 2.81 (s, 3H), 2.77 (s, 3H), 0.97−1.04 (br, 2H), 0.67−0.72 (br, 2H).

8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-[(3*S*)-tetrahydrofuran-3-yl]purine Hydrochloride (24). The title compound was prepared by the same general methods A−D. HPLC = 100% at 2.24 min by HPLC-MS method 1, mass spectrum (m/z): 413 (M + 1). Chiral HPLC = 99% at 3.71 min by chiral HPLC method 2. ^{1}H NMR (400 MHz, CD_3OD) 7.49−7.64 (m, 4H), 5.59 (br d, J = 13.6 Hz, 2H), 4.69−4.76 (m, 1H), 4.30 (q, J = 7.6 Hz, 1H), 4.15−4.22 (br, 1H), 3.95−4.05 (br, 1H), 3.86 (q, J = 7.4 Hz, 1H), 3.50−3.66 (br m, 4H), 3.18−3.27 (br m, 2H), 2.93 (s, 3H), 2.60 (s, 3H), 2.52−2.63 (br m, 1H), 2.27−2.39 (br m, 1H). Specific rotation = $[\alpha]^{20}_D$ − 13.6 (c 10, MeOH).

8-(2-Chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2-methyl-9-(tetrahydropyran-4-ylmethyl)purine Hydrochloride (25). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.17 min by HPLC-MS method 1, mass spectrum (m/z): 455 (M + 1). ^{1}H NMR (400 MHz, $CDCl_3$) 7.44−7.57 (m, 4H), 5.53−5.78 (m, 2H), 3.91−4.06 (m, 4H), 3.78−3.88 (m, 2H), 3.60 (d, J = 11.5 Hz, 2H), 3.22 (t, J = 11.5 Hz, 2H), 3.05−3.14 (m, 2H), 2.79−2.92 (m, 2H), 2.61 (s, 3H), 2.00−2.13 (m, 1H), 1.52 (t, J = 7.34 Hz, 3H), 1.25−1.35 (m, 2H), 1.04−1.16 (m, 2H).

8-(2-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (26). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.31 min by HPLC-MS method 1, mass spectrum (m/z): 427 (M + 1). High resolution mass spectrum: calcd 426.1935, found 426.1947. ^{1}H NMR (400 MHz, DMSO-d_6) 7.56−7.67 (m, 3H), 7.48−7.52 (m, 1H), 5.20−5.50 (br, 2H), 3.83−4.04 (m, 5H), 3.39−3.50 (m, 4H), 3.20 (t, J = 11.4 Hz, 2H), 3.00−3.11 (m, 2H), 2.72 (s, 3H), 2.48 (s, 3H), 1.59−1.76 (m, 2H).

8-(3-Chlorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (27). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.31 min by HPLC-MS method 1, mass spectrum (m/z): 427 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.69 (s, 1H), 7.54−7.64 (m, 3H), 5.52−5.65 (br, 2H), 4.47−4.56 (m, 1H), 4.02 (dd, J = 11.78, 4.52 Hz, 2H), 3.61−3.78 (m, 4H), 3.41 (t, J = 11.61 Hz, 2H), 3.27−3.36 (m, 2H), 2.94 (s, 3H), 2.86 (qd, J = 12.35, 4.60 Hz, 2H), 2.68 (s, 3H), 1.78−1.84 (m, 2H).

2-Methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-8-[2-(trifluoromethyl)phenyl]purine Hydrochloride (28). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.20 min by HPLC-MS method 1, mass spectrum (m/z): 461 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.92−7.97 (m, 1H), 7.80−7.85 (m, 2H), 7.60−7.65 (m, 1H), 5.48−5.66 (m, 2H), 4.10 (td, J = 12.25, 4.10 Hz, 1H), 3.96 (dd, J = 11.80, 4.35 Hz, 2H), 3.55−3.76 (m, 4H), 3.29 (t, J = 12.3 Hz, 2H), 2.93 (s, 3H), 2.70−2.96 (m, 4H), 2.68 (s, 3H), 1.55−1.8 (m, 2H).

2-Methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-8-[3-(trifluoromethyl)phenyl]purine Hydrochloride (29). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.38 min by HPLC-MS method 1, mass spectrum (m/z): 461 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.95 (s, 1H), 7.87−7.91 (m, 2H), 7.76−7.80 (m, 1H), 5.55−5.67 (m, 2H), 4.42−4.51 (m, 1H), 4.02 (dd, J = 11.66, 4.42 Hz, 2H), 3.51−3.69 (m, 4H), 3.39 (t, J = 12.1 Hz, 2H), 3.19−3.29 (m, 2H), 2.94 (qd, J = 12.50, 4.70 Hz, 2H), 2.93 (s, 3H), 2.62 (s, 3H), 1.76−1.83 (m, 2H).

8-(2-Methoxyphenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (30). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.03 min by HPLC-MS method 1, mass spectrum (m/z): 423 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.55−7.60 (m, 1H), 7.42 (dd, J = 7.50, 1.75 Hz, 1H), 7.19 (d, J = 8.2 Hz, 1H), 7.11 (td, J = 7.5, 0.9 Hz, 1H), 5.46−5.62 (br, 2H), 4.08−4.17 (m, 1H), 3.95−4.03 (m, 2H), 3.80 (s, 3H), 3.45−3.67 (m, 4H), 3.28−3.38 (m, 2H), 3.14−3.28 (m, 2H), 2.92 (s, 3H), 2.76−2.93 (br, 2H), 2.60 (s, 3H), 1.55−1.84 (br, 2H).

8-(3-Methoxyphenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (31). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.11 min by HPLC-MS method 1, mass spectrum (m/z): 423 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.49 (t, J = 7.35 Hz, 1H), 7.15−7.19 (m, 3H), 5.49−5.63 (br, 2H), 4.53−4.63 (m, 1H), 4.02 (dd, J = 12.10, 4.61 Hz, 2H), 3.84 (s, 3H), 3.64−3.85 (m, 4H), 3.29−3.43 (m, 4H), 2.95 (s, 3H), 2.82 (qd, J = 12.55, 4.55 Hz, 2H), 2.72 (s, 3H), 1.78−1.84 (m, 2H).

2-[2-Methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purin-8-yl]benzonitrile Hydrochloride (32). The title compound was prepared by the general methods A−D. HPLC = 100% at 1.90 min by HPLC-MS method 1, mass spectrum (m/z): 418 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.96−7.98 (m, 1H), 7.89 (td, J = 7.56, 1.5 Hz, 1H), 7.71−7.79 (m, 2H), 5.56−5.70 (br, 2H), 4.32 (tt, J = 12.05, 4.19 Hz, 1H), 3.98 (dd, J = 11.7, 4.50 Hz, 2H), 3.49−3.66 (m, 4H), 3.37 (td, 12.1, 1.65 Hz, 2H), 3.15−3.27 (m, 2H), 2.91 (s, 3H), 2.87 (qd, J = 12.35, 4.45 Hz, 2H), 2.60 (s, 3H), 1.77−1.84 (m, 2H).

8-(2-Fluorophenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (33). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.09 min by HPLC-MS method 1, mass spectrum (m/z): 411 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.55−7.66 (m, 2H), 7.29−7.40 (m, 2H), 7.54−7.64 (m, 2H), 4.22 (td, J = 12.05, 4.02 Hz, 1H), 3.99 (dd, J = 11.78 Hz, 2H), 3.43−3.63 (m, 4H), 3.36 (td, J = 12.10, 1.72 Hz, 2H), 3.14−3.24 (m, 2H), 2.91 (s, 3H), 2.88 (qd, J = 12.40, 4.50 Hz, 2H), 2.57 (s, 3H), 1.67−1.73 (m, 2H).

8-(2-Cyclopropylphenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (34). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.26 min by HPLC-MS method 1, mass spectrum (m/z): 433 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.45−7.50 (m, 1H), 7.26−7.32 (m,

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2H), 7.02 (d, J = 8.01 Hz, 1H), 5.50−5.61 (br, 2H), 4.18 (tt, J = 12.20, 4.10 Hz, 1H), 3.90−4.03 (m, 2H), 3.47−3.67 (m, 4H), 3.17−3.34 (m, 4H), 2.83−3.07 (m, 2H), 2.92 (s, 3H), 2.60 (s, 3H), 1.61−1.81 (br, 2H), 1.52−1.60 (m, 1H), 0.59−0.89 (m, 4H).

2-Methyl-6-(4-methylpiperazin-1-yl)-8-(o-tolyl)-9-tetrahydropyran-4-yl-purine Hydrochloride (35). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.13 min by HPLC-MS method 1, mass spectrum (m/z): 407 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.44−7.49 (m, 1H), 7.38−7.41 (m, 1H), 7.30−7.36 (m, 2H), 5.45−6.62 (br, 2H), 4.13 (tt, J = 12.05, 4.20 Hz, 1H), 3.95 (dd, J = 11.60, 4.25 Hz, 2H), 3.34−3.62 (m, 4H), 3.29 (td, J = 12.10, 1.41 Hz, 2H), 3.11−3.25 (m, 2H), 2.91 (s, 3H), 2.76−2.89 (m, 2H), 2.55 (s, 3H), 2.17 (s, 3H), 1.65−1.69 (m, 2H).

8-(2-Ethylphenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (36). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.29 min by HPLC-MS method 1, mass spectrum (m/z): 421 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.50−7.55 (m, 1H), 7.45−7.47 (m, 1H), 7.30−7.38 (m, 2H), 5.50−5.63 (m, 2H), 4.10 (td, J = 12.15, 4.10 Hz, 1H), 3.96 (dd, J = 11.45, 4.32 Hz, 2H), 3.51−3.68 (m, 4H), 3.29 (td, J = 12.30, 1.50 Hz, 2H), 3.19−3.29 (m, 2H), 2.92 (s, 3H), 2.69−2.88 (m, 2H), 2.64 (s, 3H), 2.45−2.57 (m, 2H), 1.65−1.74 (m, 2H), 1.11 (t, J = 7.50 Hz, 3H).

8-(4-Methoxy-2-methyl-phenyl)-2-methyl-6-(4-methylpiperazin-1-yl)-9-tetrahydropyran-4-yl-purine Hydrochloride (37). The title compound was prepared by the general methods A−D. HPLC = 100% at 2.13 min by HPLC-MS method 1, mass spectrum (m/z): 437 (M + 1). ^{1}H NMR (400 MHz, CD_3OD) 7.23 (d, J = 8.40 Hz, 1H), 6.94 (d, J = 2.42 Hz, 1H), 6.89 (dd, J = 2.64, 8.30 Hz, 1H), 4.20−4.75 (br, 2H), 4.10−4.19 (m, 1H), 3.96 (dd, J = 4.40, 11.55 Hz, 2H), 3.83 (s, 3H), 3.20−3.35 (m, 8H), 2.84 (s, 3H), 2.78−2.88 (m, 2H), 2.53 (s, 3H), 2.14 (s, 3H), 1.61−1.67 (m, 2H).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ANOVA, analysis of variance; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CHO, Chinese hamster ovary; CL, clearance; CNS, central nervous system; DDQ, 2,3-dichloro-5,6-dicyanobenzoquinone; DRG, dorsal root ganglia; GDP, guanosine diphosphate; GI, gastrointestinal; GPCR, G-protein coupled receptor; GTPγS, guanosine 5′-O-[γ-thio]-triphosphate; hERG, human ether-à-go-go-related gene; HSD, honest significant difference; 5HT, 5-hydroxytryptamine receptor; ip, intraperitoneal; iv, intravenous; MIA, monosodium iodoacetate; NSAID, nonsteroidal anti-inflammatory drugs; NT, not tested; OA, osteoarthritis; PgP, P-glycoprotein 1; po, per os (by mouth); SAR, structure−activity relationship; SEM, standard error of the mean; THC, tetrahydrocannabinol; THP, tetrahydropyran; $T_{1/2}$, half-life; TRPV1, transient receptor potential cation channel subfamily V member 1

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