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Functionalized 6-(Piperidin-1-yl)-8,9-Diphenyl Purines as Peripherally Restricted Inverse Agonists of the CB1 Receptor

George Amato[#], Amruta Manke[#], Robert Wiethe, Vineetha Vasukuttan, Rodney Snyder, Yun Lan Yueh, Ann Decker, Scott Runyon and Rangan Maitra*

Discovery Science and Technology, RTI International, 3040 Cornwallis Rd., Research Triangle Park, NC 27709-2194, USA

* Corresponding author

# Equal contributors

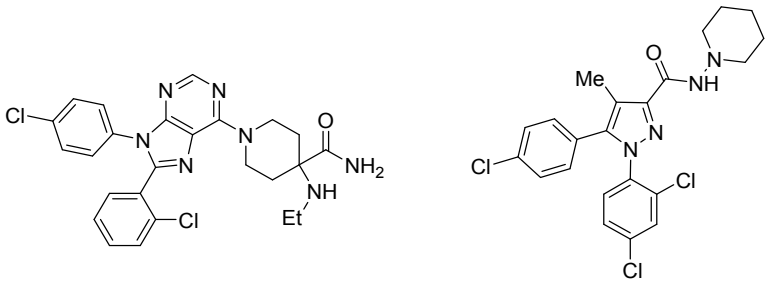
Key Words: *CB1, cannabinoid, peripheral, antagonist, otenabant, purine, CB2, MDCK, blood brain barrier*

ABSTRACT

Peripherally restricted CB1 receptor antagonists may be useful in treating metabolic syndrome, diabetes, liver diseases and gastrointestinal disorders. Clinical development of the centrally acting CB1 inverse agonist otenabant (**1**) was halted due to its potential of producing adverse effects. SAR studies of **1** are reported herein with the objective of producing peripherally restricted analogs. Crystal structures of hCB1 and docking studies with **1** indicate that the piperidine group could be functionalized at the 4-position to access a binding pocket that can accommodate both polar and nonpolar groups. The piperidine is studied as a linker, functionalized with alkyl, heteroalkyl, aryl and heteroaryl groups using a urea connector. Orally bioavailable and peripherally selective compounds have been produced that are potent inverse agonists of hCB1 with exceptional selectivity for hCB1 over hCB2. Compound **38** blocked alcohol-induced liver steatosis in mice and has good ADME properties for further development.

1. Introduction

The endocannabinoid system has many important physiological functions. There are two known cannabinoid receptors – CB1 and CB2. These GPCRs are expressed in many different tissues. The CB1 receptor is highly expressed in the central nervous system (CNS) and in metabolically important tissues including liver, skeletal muscles, adipocytes, and pancreas.[1] In contrast, CB2 receptor expression is mainly confined to immune cells.[2, 3] Selective antagonism of peripheral CB1 receptors has potential therapeutic applications in treating diabetes, metabolic syndrome, dyslipidemias and liver diseases such as steatosis or fatty liver disease.[4-9] Antagonism of CB1 receptors in the CNS, however, is linked to psychiatric disorders, precipitating the withdrawal of CNS-penetrating compounds such as otenabant (1) and rimonabant (2) from clinical development.[10] Presently, efforts are underway to develop compounds that selectively antagonize peripheral CB1 receptors.[8, 11-14] These compounds by virtue of being CNS-sparing are not expected to produce adverse effects seen with first-generation non tissue selective antagonists. Other approaches that may not require restriction from the CNS include development of neutral orthosteric antagonists and negative allosteric modulators.[15, 16]



1, Otenabant (CP-945,598) **2, Rimonabant (SR141716A)**

Figure 1. Examples of clinical CNS penetrating CB1 inverse agonists.

Identifying compounds that are both peripherally selective and orally bioavailable is possible by carefully managing physiochemical properties. Analyses of past research and advanced compounds therein suggest that a topological polar surface area (TPSA) of 80-140 Å, a molecular weight of 450-600 Da and 1-3 hydrogen bond donors improve the likelihood of identifying such compounds.[17, 18] To improve the

potential for good drug-like properties, it is also desirable to have a cLogP of less than 5. Compounds with a high cLogP may have poor aqueous solubility, which in turn can negatively impact absorption following an oral dose. Compound **1** has several properties that in theory could restrict this compound to the periphery including a TPSA of 102 Å, a cLogP of 4.4, 3 hydrogen bond donors and a molecular weight of >500 Da.[19] Compound **1** also has a polar and mildly basic 6-piperidinyl purine core that can aid in oral absorption. However, **1** can penetrate into the CNS due to intramolecular hydrogen bonding.[20] It was hypothesized that analogs of **1** with refined properties could lead to compounds that have limited penetration into the CNS.

We began such an effort with an investigation of isomeric replacements for the 4-aminopiperidine group [21] and employment of the piperazine group as a replacement.[22, 23] Early studies resulted in compounds **3-5**, possessing excellent hCB1 potency (Ke ~ 20 nM) and good selectivity (>50 fold) against hCB2 (**Figure 2**).[21, 22, 24] These compounds are peripherally selective and orally bioavailable, but their ADME properties were not optimized. Recent docking studies of **1** with the hCB1 crystal structure at the orthosteric binding site indicate that functionalization of the piperidine group at the 4-position could result in access to a binding pocket near the extracellular surface of the membrane.[22, 25, 26] This binding pocket may accommodate polar as well as nonpolar groups and differs significantly from hCB2.[27, 28] By targeting this region, we believe it is possible to identify compounds with better physical properties that have good hCB1 potency and excellent selectivity versus hCB2.

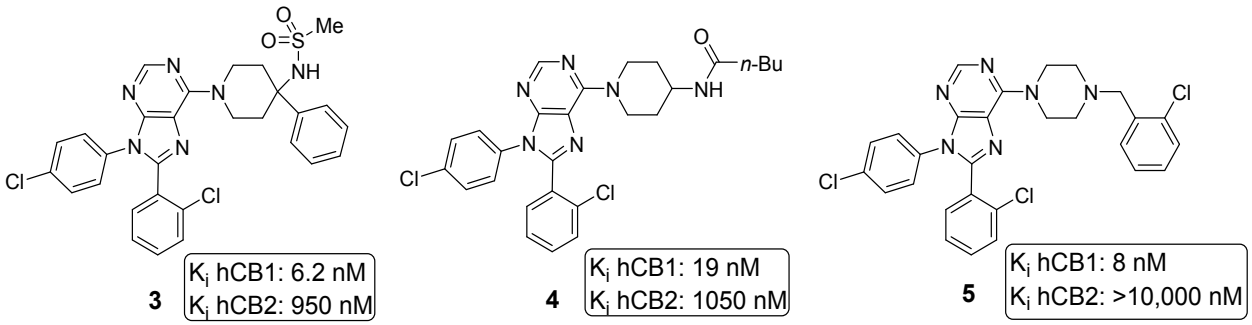
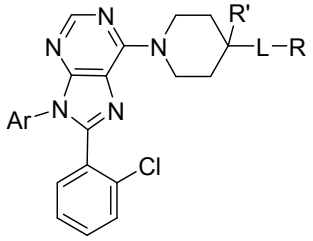


Figure 2. Early purine based peripherally selective CB1 inverse agonists.

2. Results and Discussion

2.1 Compound Design and Synthesis



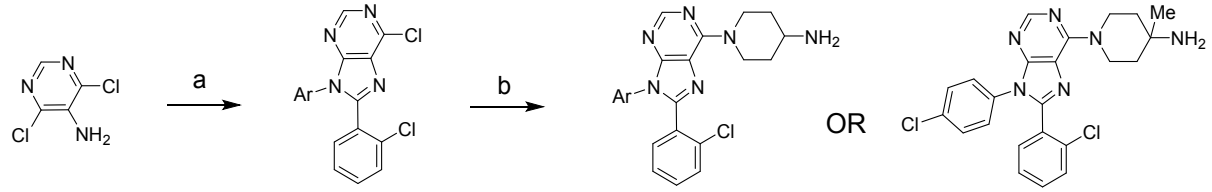
6

L = NHCONH, $NHCONHSO_2$,
R = Alkyl, Heteroalkyl, Aryl, Heteroaryl
R' = H, Me
Ar = 4-ClPh, Heteroaryl

Figure 3. General strategy for SAR studies

In this report, we disclose our findings in studying functionalization of the piperidine linker at the 4-position with a urea or a sulfonyl urea connector (**6, Figure 3**). We focused on alkyl, heteroalkyl, aryl and heteroaryl groups that are more likely to have good hCB1 potency. We also report on replacing the 4-chlorophenyl group of the 6-purine position with heterocycles, to increase TPSA and reduce cLogP. Finally, changing the R' from hydrogen to methyl was looked at to adjust in vitro ADME properties and favor higher hCB1 potency. Combined, these changes allowed us to design a variety of compounds with desirable calculated properties that are favorable for both peripheral selectivity and oral absorption.

To prepare most of the targeted compounds, we used the 4-aminopiperidine **8a** (**Scheme 1**) as a key intermediate, the preparation of which was previously described.[21] The 4-amino-4-methylpiperidine analog (**9**) and analogs of **8a** with replacements for the 4-chlorophenyl group of the purine ring were prepared in a similar way as shown in **Scheme 1** and detailed in the experimental section.

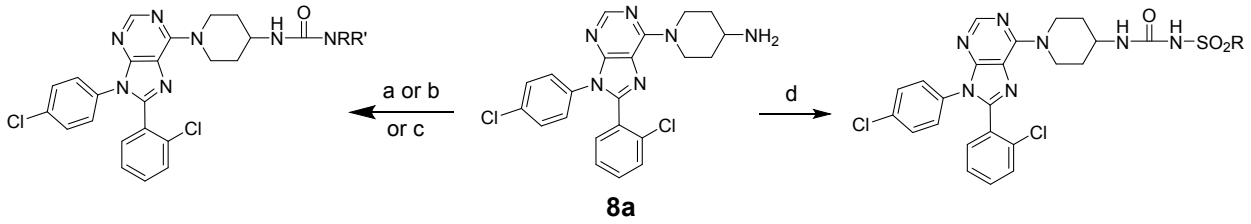


7(a-j) **8(a-j)** **9**

Scheme 1. Reagents and conditions: (a) (1) $ArNH_2$, 6 N HCl, *n*-BuOH, 80 °C; (2) 2-ClPhCHO, 15% $FeCl_3$/silica gel, dioxane, 95 °C, then DDQ, CH_2Cl_2, rt; (b) (1) 4-N-Boc-aminopiperidine or 4-N-Boc-

amino-4-methylpiperidine, K_2CO_3, NMP, 80 °C; (2) 6 N HCl, EtOH, 50 °C. **Ar** = 4-chlorophenyl (**7a, 8a**), 4-fluorophenyl (**7b, 8b**), 6-(trifluoromethyl)pyridin-3-yl (**7c, 8c**), 6-(difluoromethoxy)pyridin-3-yl (**7d, 8d**).

From intermediate **8a** we prepared a variety of ureas using standard procedures as shown in **Scheme 2** and provided in the experimental section. Intermediate **9** and analogs with a replacement for the 4-chlorophenyl group of the purine ring were functionalized in the same way.



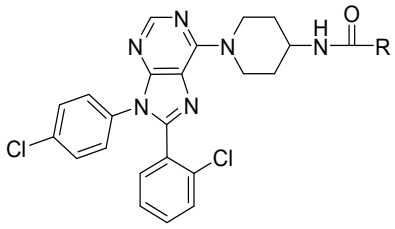
Scheme 2. Reagents and conditions: (a) (1) triphosgene, $NaHCO_3$, CH_2Cl_2, rt; (2) RR'NH, NEt_3, THF, rt; (b) (1) *p*-F-PhO_2CCl, NEt_3, DCE, rt; (2) RR'NH, dioxane, rt; (c) RNCO, NEt_3, THF, rt; (d) (1) *p*-NO_2-PhO_2CCl, NEt_3, DCE, rt; (2) RSO_2NH_2, NaHMDS, THF, rt.

2.2. Pharmacological Characterization

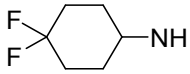
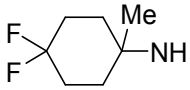
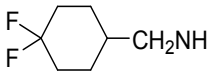
All target compounds were evaluated in a FLIPR-based calcium mobilization assay for hCB1 activity (**Tables 1-3**) as previously reported.[21, 29] In general, compounds displaying apparent antagonist dissociation equilibrium constant (Ke) < 50 nM were further tested for affinity to hCB receptors using radioligand displacement of [^{3}H]CP55940 in purified membrane fractions overexpressing either hCB1 or hCB2. Exceptions were made for certain interesting compounds. A selection of potent and selective compounds were tested for potential peripheral selectivity by calculating % apical (A) to basal (B) permeability in a MDCK-mdr1 monolayer permeability assay, which is predictive of brain penetration.[30] Lower values are predictive of less CNS penetration. To assess metabolic stability, some compounds were advanced to a human liver microsome (HLM) stability assay, determining half-life (HL) and clearance (Cl).

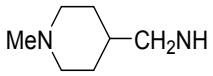
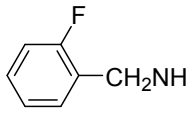
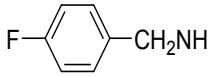
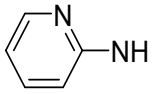
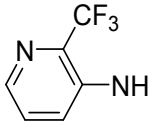
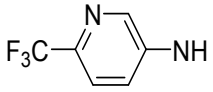
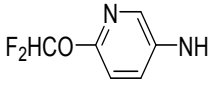
Ureas are good linkers for compounds targeting peripheral selectivity, as they contain one or two strong hydrogen bond donors and contribute three heteroatoms to assist in achieving higher TPSA values. **Table 1** contains in vitro data for a set of alkyl and aryl ureas. We previously reported that the *n*-propyl urea **10** and other ureas with small aliphatic groups have hCB1 activity, but the potency and selectivity versus hCB2 need further optimization.[24] To take advantage of the binding site to which the piperidine ring is neighboring, we tried larger groups that may result in an increase in both potency and selectivity.

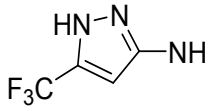
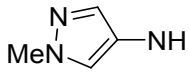
Table 1. In Vitro Data for CB1 Antagonists – Ureas



#	R	Ke hCB1 (nM± SEM)	Ki hCB1 (nM± SEM)[a]	Ki hCB2 (nM± SEM)[a]	Selectivity Ki CB2/CB1	MDCK-mdr1 A to B (%)[b]	HLM HL (mins); Cl (µL/min/mg)
1	Otenabant[c]	0.2	0.7	7700	11000	ND	ND
10[d]	*n*-PrNH	130	28	150	5	ND	ND
11	*n*-PrNMe	5±3	0.9±0.2	630±210	700	7	ND
12	*n*-Pr(CF_3CH_2)N	1.2±0.6	1.1±0.6	2800±600	2500	ND	ND
13	*i*-BuNMe	6±2	0.8±0.3	600±20	750	4	ND
14	*t*-BuCH_2NH	270±130	ND	ND	ND	ND	ND

15	$MeOCH_2CH_2NMe$	13±7	0.26±0.03	2300±200	8800	130	ND
16		190±110	ND	ND	ND	ND	ND
17		7±4	1.2±0.8	400±36	330	ND	ND
18		340±60	ND	ND	ND	ND	ND
19	*c*-PenNH	25±14	3±1	>10000	>3000	1	ND
20		12±8	6.3±0.5	>10000	>1500	0.1	HL: 3 Cl: 381
21	*c*-HexNH	35±10	1.4±0.8	4600±600	3300	0.3	HL: 21 Cl: 58
22		79±2	18±1	>10000	>500	ND	HL: 163 Cl: 8
23		0.4±0.3	5±3	7500±400	1500	ND	HL: 7 Cl: 178
24	*c*-PenCH_2NH	63±37	19±8	3200 ±1000	170	0.2	ND
25	*c*-HexCH_2NH	5±3	0.9±0	7600 ±4200	8400	0.1	HL: 25 Cl: 50
26		28±15	26±20	>10000	384	ND	HL: 30 Cl: 42

27		25000 ±12000	ND	ND	ND	ND	ND
28		71±7	2.4±0.2	220±100	92	ND	HL: 150 Cl: 8
29		19±6	3±1	>10000	>3000	ND	HL: 123 Cl: 10
30	PhNH	30±7	7±0	>10000	>1400	0.2	HL: 115 Cl: 11
31		150±50	ND	ND	ND	ND	ND
32	2-F-PhNH	58±7	4±1	>10000	>2500	0.1	HL: 97 Cl: 13
33	2,4-DiF-PhNH	4±1	14±4	>10000	>700	0.1	HL: 397 Cl: 3
34	3,4-DiF-PhNH	290±80	ND	ND	ND	ND	ND
35	4-F-PhNH	78±20	22±9	6500±600	300	1.3	ND
36		160±60	ND	ND	ND	ND	ND
37		37±16	51±32	>10000	>196	ND	HL: 39 Cl: 32
38		26±9	22±3	>10000	>263	0.1	HL: 423 Cl: 3

39		270±60	ND	ND	ND	ND	ND
40		28000 ±8000	ND	ND	ND	ND	ND
41	2-F-PhSO$_2$NH	280±130	380±40	ND	ND	ND	ND

[a]Displacement was measured using [^{3}H]CP55940 in HEK cell membrane preparations overexpressing hCB1 or hCB2 receptors.

[b]% transported from the apical side (A) to the basal side (B).

[c]See reference 21.

[d]See reference 22.

ND: not done as compound did not meet progression criterion/criteria.

Satisfyingly, adding a methyl to the primary urea of **10** (Ke = 130 nM, Ki hCB2/hCB1 = 5), provides the secondary urea **11** (Ke = 5 nM, Ki hCB2/hCB1 = 700) which showed an improvement in both hCB1 potency and binding selectivity versus hCB2. Similar results were obtained with other secondary ureas (see **12, 13, 15** & **17**). This prompted us to advance these compounds to the MDCK-mdr1 assay, in which we were seeking an A to B transport of <1%, values that we found to favor peripheral selectivity for these purine-based compounds. Unfortunately, the A to B values were generally >1%, indicating that they were unlikely to achieve the desired level of peripheral selectivity. This was true even in the case of **15**, which has a strong hydrogen bond donor and favorable calculated physical properties (MW = 554, TPSA = 88, cLogP = 4.3). It became evident to us, that for this class of compounds, two strong hydrogen bond donors would be needed to favor exclusion from the CNS. Based on previous docking studies of **1** on the hCB1 receptor, we anticipated that high polarity can be accepted close to the 4-position of the piperidine ring, where the binding pocket is more polar.[22] Groups attached to the 4-position that reach deeper into the pocket, however, need to be less polar. This led us to focus on primary ureas that have a combination of better calculated

physical properties and two strong hydrogen bond donors. We hypothesized that such compounds would have good peripheral selectivity, reasonable ADME and acceptable potency towards hCB1.

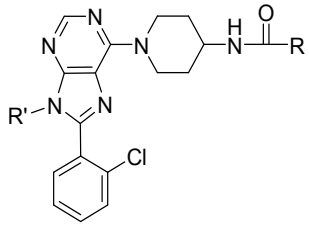
Primary ureas with cycloalkyl groups retained good potency, affinity and selectivity. The cyclohexyl urea **21**, for example, has good hCB1 potency and affinity (Ke = 35 nM, Ki = 1.4 nM) and excellent selectivity versus hCB2 (Ki hCB2/hCB1 = 3,300). In the MDCK-mdr1 assay, **21** and other cycloalkyl ureas had values for A to B transport of <1%, indicating the potential for peripheral selectivity. Several of these compounds were advanced to HLM stability studies, which revealed moderate clearance values at best. The cyclohexyl urea **21** for example, has a HL of 21 minutes and a Cl of 58 μL/min/mg. This led us to investigate fluorinated cycloalkyl groups as we hypothesized that the cycloalkyl group was susceptible to oxidative metabolism. While fluorinated analogs **20** and **26** were still metabolically unstable, the 4,4-difluorocyclohexyl urea **22** showed considerable improvement in the HLM assay with a HL of 270 minutes and a Cl of 5 μL/min/mg, meeting our goal of a HL >120 minutes and a Cl <10 μL/min/mg. We attempted to improve the hCB1 potency and affinity of **22** (Ke = 79 nM, Ki = 18 nM) by adding a methyl to the one position of the cyclohexyl ring. The resulting compound **23** showed a significant improvement in hCB1 potency and affinity (Ke = 0.4 nM, Ki = 5 nM), but its stability in HLM was poor (HL = 7 minutes). The benzyl ureas **28** and **29** were also tested and found to have good hCB1 binding (Ki = 2 nM and 3 nM, respectively), good selectivity versus hCB2 (Ki hCB2/hCB1 ~92 and ~1600, respectively) and good HLM stability (HL = 150 and 123 minutes, respectively).

Aryl ureas were pursued as options which would contain a more acidic urea hydrogen, a feature which may favor peripheral restriction. The unsubstituted aryl urea **30** has good hCB1 potency (Ke = 30 nM, Ki = 7 nM), good selectivity versus hCB2 (Ki hCB2/hCB1 ~1400) and in the MDCK-mdr1 assay, has an A to B transport ratio of 0.2%, indicating possible peripheral selectivity. The HLM stability was near our goal, with a HL of 115 minutes and a Cl of 11 μL/min/mg. To improve HLM stability, fluorinated analogs were pursued (**32-35**). The 2,4-difluorophenyl analog **33** had the desired profile. This compound has good hCB1 potency (Ke = 4 nM, Ki = 14 nM), excellent selectivity versus hCB2 (Ki hCB2/hCB1

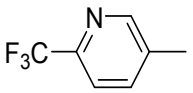
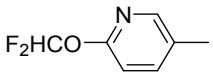
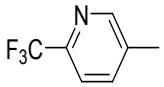
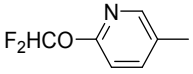
~700), is predicted to be peripherally selective (A to B <0.1%) and has excellent HLM stability (HL = 397 minutes, Cl = 3 μL/min/mg). We also investigated heteroaryl ureas, as these have more favorable calculated physical properties for solubility, which may result in bioavailability advantages. While most of these compounds possess significantly less hCB1 potency, the substituted 3-pyridyl analogs **37** and **38** have good hCB1 potency (Ke = 37 and 26 nM, respectively) and are highly selective versus hCB2 (~200-fold or greater selectivity). The difluoromethoxy analog **38** performed well in the HLM stability assay, with a HL of 423 minutes and a Cl of 3 μL/min/mg. In the MDCK-mdr1 assay, **38** was predicted to be peripherally restricted with an A to B transport ratio of 0.1%. The more acidic sulfonyl urea **41** was also investigated. This compound has a TPSA of 122, a cLogP of 4.9 and two strong hydrogen bond donors, attractive properties for a peripheral agent. Unfortunately, the hCB1 potency (Ke = 280 nM) was not good enough to progress the compound.

Replacement of the 4-chlorophenyl in the purine 9-position with a heterocycle was of interest as this may lead to more polar compounds with better pharmacokinetic properties. We investigated using the difluoromethoxypyridyl and trifluoromethylpyridyl groups, which were previously shown to be satisfactory replacements for the 4-chlorophenyl group when an amide or sulfonamide was used as the 4-piperidinyl linker in place of a urea (unpublished results). For the ureas, however, these groups led to significant reductions in hCB1 potency as shown in **Table 2** (see **25** vs. **42** & **43**; see **32** vs. **45** & **46**).

Table 2. In Vitro Data for CB1 Antagonists – 4-Chlorophenyl Replacements



#	R	R'	Ke hCB1 (nM ± SEM)	Ki hCB1 (nM± SEM)[a]	Ki hCB2 (nM± SEM)[a]	Selectivity Ki CB2/CB1

25	*c*-HexCH_2NH	4-Cl-Ph	5±3	0.9±0	7600±4200	8400
42	*c*-HexCH_2NH		76±34	730±210	ND	ND
43	*c*-HexCH_2NH		100±30	ND	ND	ND
32	2-F-PhNH	4-Cl-Ph	58±7	4±1	>10000	2500
44	2-F-PhNH	4-F-Ph	3800±1500	ND	ND	ND
45	2-F-PhNH		210±70	ND	ND	ND
46	2-F-PhNH		380±140	ND	ND	ND

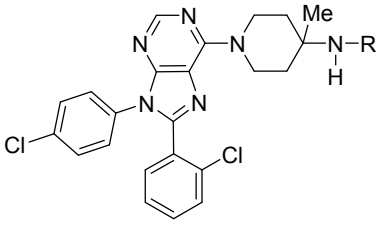
[a]Displacement was measured using [^{3}H]CP55940 in HEK cell membrane preparations overexpressing hCB1 or hCB2 receptors.

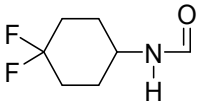
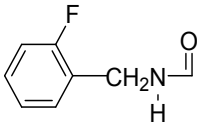
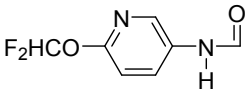
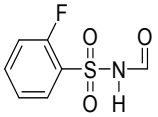
ND: not done as compound did not meet progression criterion/criteria.

Introduction of an aliphatic group in the 4-position of the piperidine ring can enhance hCB1 potency.[21] To enhance the activity of our molecules, while minimizing a reduction in polarity, we investigated the effect of a methyl group on the hCB1 potency of our ureas (**Table 3**). In general, a trend toward better Ke values was observed. Unfortunately, HLM stability was also generally lower. In one noteworthy exception, **48**, the methylated version of **22**, showed an improvement in hCB1 Ke from 79 nM to 2 nM. The selectivity versus hCB2 also remained high (Ki hCB2/hCB1 >550). Furthermore, this compound retained good HLM stability (HL = 279 minutes). Another noteworthy example is **53**, the methylated version of the sulfonyl urea **41**. The hCB1 Ke was again improved, from 280 nM to 7 nM. The

HLM stability for this compound was checked and found to be just below our goal of 120 minutes. Analog **54** is a sulfamide with good hCB1 potency (Ke = 17 nM), but the selectivity versus hCB2 was only 82-fold.

Table 3. In Vitro Data for CB1 Antagonists – 4-Methyl Piperidines



#	R	Ke hCB1 (nM± SEM)	Ki hCB1 (nM± SEM)[a]	Ki hCB2 (nM± SEM)[a]	Selectivity Ki CB2/CB1	HLM HL (mins) Cl (µL/min/mg)
47	*c*-HexNHCO	7±5	13±2	>10000	>769	ND
48		2±1	17±3	>10000	>588	HL: 279 Cl: 5
49		14±7	15±1	1100±700	73	ND
50	2-F-PhNHCO	1.6±1.1	4±2	>10000	>2500	HL: 77 Cl: 16
51	2,4-DiF-PhNHCO	9±4	6±1	>10000	>1600	HL: 49 Cl: 25
52		46±23	81±15	>10000	>120	HL: 51 Cl: 24
53		7±2	120±40	>10000	>80	HL: 84 Cl: 15

54	*c*-HexNHSO$_2$	17±3	9±3	740±120	82	ND

[a]Displacement was measured using [^{3}H]CP55940 in HEK cell membrane preparations overexpressing hCB1 or hCB2 receptors.
ND: not done as compound did not meet progression criterion/criteria.

While several interesting compounds were identified, based on the obtained data, compounds **33** and **38** were advanced into further studies. Compounds **1** and **2** are inverse agonists. Compounds **33** and **38** were further characterized using the calcium assay to establish whether these compounds are neutral

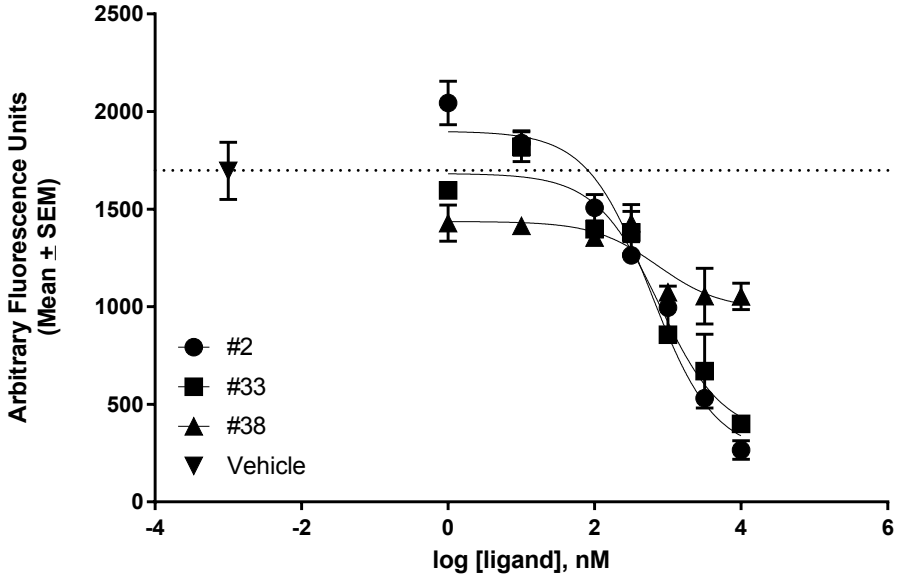


Figure 4. Compounds **33** and **38** are inverse agonists of hCB1. CHO-CB1 cells were loaded with calcium indicator dye for 60 min as described in Methods. Cells were then stimulated by various concentrations of each compound and fluorescence change recorded using FLIPR Tera (Molecular Devices) instrument. Data is reported as Mean ± SEM from 3 independent measurements.

antagonists or inverse agonists of the hCB1 receptor. As shown in **Figure 4**, these compounds suppress the basal signaling through hCB1 and are therefore inverse agonists. The EC_{50} for **33** and **38** were 765 and 718 nM, respectively compared to 585 nM for **2**. Compound **33** showed 88% of E_{max} observed with **2**, while **38** showed only a 34% change, closer to what might be described as a neutral antagonist.

We wanted to investigate whether these two lead compounds had the potential to significantly induce CYP enzymes, which might pose a liability in chronic use and polypharmacy situations. Isoform-specific CYP induction assays using real-time RT-PCR in HepaRG cells were performed to assess potential of the two lead compounds as inducers of CYP3A4, CYP1A2 and CYP2B6. These three isoforms are important within the context of human CYP-mediated drug metabolism of most common drugs. As indicated in **Table 5**, marginal potential for CYP3A4 induction was noted with **33** whereas **38** showed little to no activity. Some reported analogs of **1** were found to have hERG activity, which is a risk for arrhythmias.[19] These compounds were devoid of hERG channel interaction potential as assessed using displacement of radiolabeled astemizole in a radioligand displacement assay (**Table 5**).[31 19] Compound **38** was also characterized for off-target activity by testing at 10 μM in the Eurofins SafetyScreen44™ Panel. No off-target liability was detected after initial screening and follow-up studies (EC_{50} or IC_{50} >10 μM on all targets tested; data not shown).

Table 5. CYP induction potential and hERG interaction assessment

Compound	CYP1A2 induction* (% of omeprazole)	CYP2B6 induction* (% of phenobarbital)	CYP3A4 induction* (% of rifampicin)	hERG** (Ki, μM)
33	<1	<10	33	>10
38	<1	<10	<10	>10

*Data are expressed as % of positive control compound.

** Displacement was measured using [^{3}H]astemizole in HEK cell membrane preparations overexpressing hERG.

2.3 Pharmacokinetic Studies

Compounds **33** and **38,** with favorable in vitro properties, were tested in rodents to assess their ability to be orally absorbed and kept out of the brain. Compounds were dosed to mice and rats in multiple

time point studies to obtain plasma and brain maximum concentrations, which were used to calculate and compare brain/plasma levels. The main goals were to achieve good plasma levels (>100 ng/mL after an oral dose of 1 mg/kg or less) while keeping the ratio of maximum brain concentration to maximum plasma concentration as low as possible, preferably less than 0.05. To facility efficacy studies, a plasma half-life of at least 6 hours was targeted. As demonstrated in **Table 6** (mouse PK) and **Table 7** (rat PK), both lead compounds demonstrated high oral absorption upon dosing, very low brain exposure and good hepatic concentrations. Both compounds also possess a long plasma half-life and low clearance that would allow for once daily oral dosing. Adjusted for dose, there was good correlation between PK data

Table 6. Mouse PK Data for CB1 Antagonists at 0.3 mg/kg PO

#	Plasma Max. Conc. [a] (ng/mL)	Liver Max. Conc. [a] (ng/mg)	Brain Max. Conc. [a] (ng/mg)	Brain/ Plasma[b]	Plasma HL (hours) Plasma Cl (mL/h/kg)
33	1233 at 1 h	205 at 2 h	17 at 2 h	0.013	HL: 12 Cl: 19
38	940 at 2 h	338 at 4 h	10 at 4 h	0.011	HL: 11 Cl: 21

a. Time points used: 0.5, 1, 2, 4, 8 & 24 hours.

b. The maximum plasma and brain concentrations were used.

obtained in both species. The brain:plasma concentrations were very low in both species, accounting for ~1% of peak plasma values in un-perfused tissues. Since the volume of blood in un-perfused mouse brain is ~4-6% [32], these data indicate that these two lead compounds have little to no brain penetration.

Table 7. Rat PK Data for CB1 Antagonists at 1 mg/kg PO

#	Plasma Max. Conc. [a] (ng/mL)	Liver Max. Conc. [a] (ng/mg)	Brain Max. Conc. [a] (ng/mg)	Brain/ Plasma[b]	Plasma HL (hours) Plasma Cl

					(mL/h/kg)
33	3,450 at 2 h	480 at 2 h	37 at 2 h	0.011	HL: 12 Cl: 14
38	3,490 at 2 h	1020 at 4 h	46 at 2 h	0.014	HL: 9.9 Cl: 18

a. Time points used: 0.5, 1, 2, 4, 8 & 24 hours.

b. The maximum plasma and brain concentrations were used.

2.4. Effect of 38 on Alcohol Induced Liver Steatosis

The inverse agonist **2** has been previously shown to reduce alcohol consumption as well as alcoholic steatosis in preclinical models.[33, 34] While the former effect is potentially through centrally expressed CB1 receptors, our past studies confirm that hepatic CB1 receptors can be targeted to reduce alcoholic steatosis.[31] In alcoholic liver disease, paracrine activation of CB1 on liver hepatocytes by the full agonist endocannabinoid molecule 2-arachidonoylglycerol (2-AG), promotes alcohol-induced steatosis (AS) through a SREBP-1C regulated pathway.[34] Efficacy studies of **38** in a mouse model of AS were performed to assess effects on hepatic lipid accumulation. This compound was chosen over **33** due to its overall better ADME profile including reduced CYP3A4 induction potential. Female C57BL6 mice were maintained on a liquid Lieber-DeCarli diet containing ethanol or a matched control diet without alcohol for four weeks. To mice consuming ethanol, vehicle or compound **38** (0.03, 0.1 and 0.3 mg/kg) was administered by once daily oral gavage for the last two weeks of the study. **Figure 5A** shows representative photomicrographs depicting lipid accumulation in mouse livers. In the livers of animals on the control diet (no ethanol), lipid accumulation was minimal, as revealed through Oil Red O (ORO) staining (left panel in **Figure 5A**). The ethanol containing diet, in contrast, caused significant accumulation of lipid droplets (center panel in **Figure 5A**). In the absence of drug treatment, histology and ORO staining showed that the livers of ethanol diet-fed mice have microsteatosis and macrosteatosis. Administration of compound **38**

resulted in a significant reduction of hepatic lipid accumulation (right panel in **Figure 5A**). Quantification of liver droplets revealed statistically significant reduction of steatosis in the livers of mice treated with **38** at the two higher doses. Normalized liver weights were also lower in these mice with a statistically significant decrease noted in animals treated with the highest concentration of this agent. Collectively, these data indicate that **38** is a promising candidate for alcoholic steatosis.

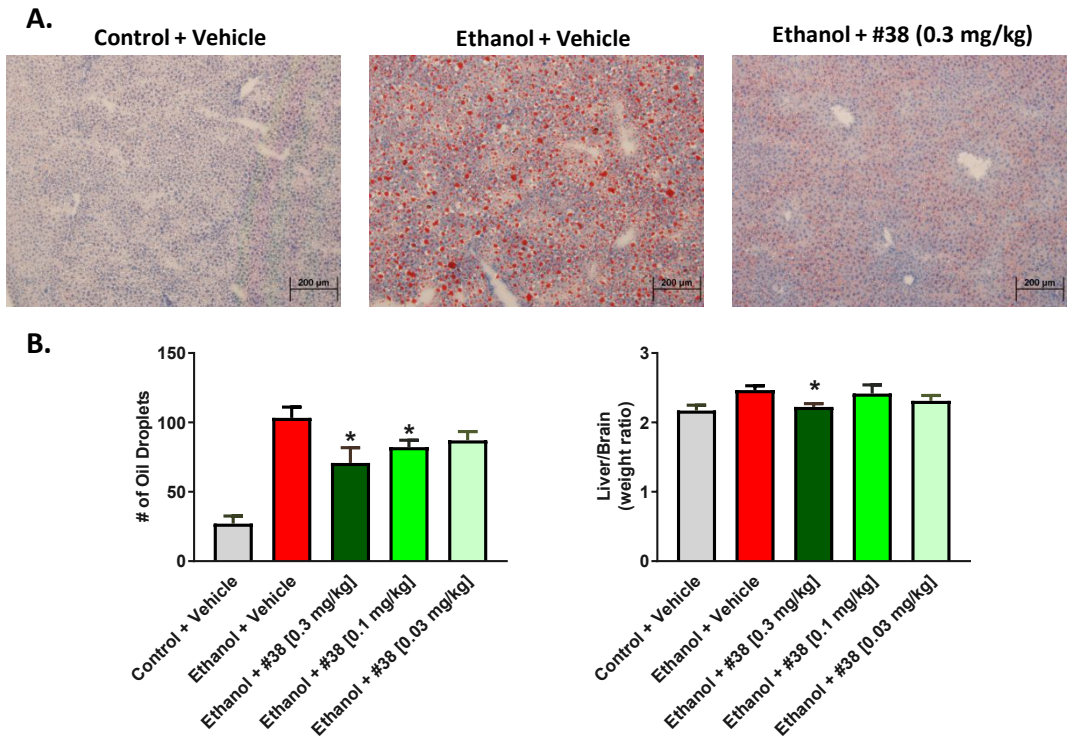


Figure 5. Oil Red O staining of liver sections indicates that compound 38 reduces steatosis. (A) Representative liver sections from mice receiving control diet without ethanol + vehicle (left panel), ethanol containing diet + vehicle (center panel) or ethanol containing diet + **38** (right panel). **(B)** ***Left:*** Quantification (Image J software) of lipid droplets in liver sections indicates treatment with **38** resulted in a statistically significant reduction of liver steatosis. ***Right:*** Reduced liver weights normalized to brain weights in animals treated with **38**. Data are reported as Mean ± S.E.M. from 6-10 animals per group. Statistical significance indicated as * (T-test, $p<0.05$ versus ethanol + vehicle).

3. Conclusions

Targeting peripheral populations of CB1 receptors is an important strategy for metabolic diseases as antagonism of this receptor in the CNS produces adverse psychological effects that could be life-threatening including suicidal ideation. Thus, efforts are underway to produce peripherally restricted antagonists of this receptor. Compound **1** is a selective CB1 inverse agonist developed by Pfizer, but its clinical development was terminated once CNS-related adverse effects were noted.[35] Recent docking studies of **1** with crystal structures of hCB1 indicate that the 4-postion of the piperidine group could be functionalized to access a binding pocket that may accommodate both polar and nonpolar groups. The same binding pocket differs significantly from that of hCB2 and hence could be used to obtain highly selective compounds. In this report, the piperidine linker was functionalized in the 4-position with alkyl, heteroalkyl, aryl and heteroaryl groups using a urea connector (**6**), further establishing the SAR needed to identify better compounds. Polarity was found to be best tolerated near to the 4-position of the piperidine ring, which is supported by docking of **1** with the crystal structure of the hCB1 receptor.[22] Compounds with a highly polar primary urea connector at the four position of the purine piperidine were found to be potent hCB1 antagonists with excellent selectivity versus hCB2.

Interesting compounds with requisite potency and selectivity were progressed into ADME studies. These studies led to the identification of compounds **33** and **38** as lead molecules that had little to no penetration across MDCK-mdr1 monolayers, long HLM half-lives, no hERG liability and little to no induction of three important P450 isoforms. Pharmacokinetic assessments were performed in mice and rats. High levels of peripheral restriction were observed with both compounds. Generally, <2% brain penetration was noted compared to plasma in both rats and mice. More importantly, both compounds had low clearance and long half-lives. These compounds are suitable for once daily oral dosing – an improvement over our previously reported lead structure that requires BID dosing.[31] The heteroaryl urea **38** was tested for efficacy in a model of alcoholic liver injury. Treatment with this compound reduced hepatic steatosis induced by

alcohol consumption. In summary, we have identified **38** as a potential clinical candidate pending further IND-enabling studies.

4. Experimental

Chemistry General. Purity and characterization of compounds were established by a combination of MS, NMR, HPLC and TLC analytical techniques, as described below. ^{1}H spectra were recorded on a Bruker Avance DPX-300 (300 MHz) spectrometer and were determined in chloroform-*d* (7.26 ppm) or methanol-d_4 (3.31 ppm) with TMS (0.00 ppm) or solvent peaks as the internal reference unless otherwise noted. Chemical shifts are reported in ppm relative to the solvent signal and coupling constant (*J*) values are reported in hertz (Hz). TLC was performed with EMD precoated silica gel 60 F254 plates. TLC spots were visualized with UV light or I_2 detection. Low-resolution mass spectra were obtained using a single quadrupole PE Sciex API 150EX (ESI). Unless stated otherwise, all test compounds were at least 95% pure as determined by HPLC. HPLC method: an Agilent-Varian system equipped with Prostar 210 pumps, a Prostar 335 Diode UV detector and a Phenomenex Synergi 4 μm Hydro RP 80A C18 250 x 4.6 mm column using a 20-min gradient elution of 5-95% solvent B at 1 mL/min followed by 5 min at 95% solvent B (solvent A, water with 0.1% TFA; solvent B, acetonitrile with 0.1% TFA and 5% water; absorbance monitored at 220 and 254 nm).

General Procedure A: Ureas of 8 or 9 from Isocyanates. To a solution of **8** or **9** (0.2 mmol, 1 equiv) in THF (1 mL) was added the isocyanate (0.24 mmol, 1.2 equiv), followed by TEA (0.24 mmol, 1.2 equiv). The mixture was stirred at rt for 15 h. Water (0.4 mL) was added, followed by ethyl acetate (3 mL) and then saturated $NaHCO_3$ solution (0.8 mL). After 10 min, the aqueous layer was removed. Celite (600 mg) was added to the organic layer and the solvent was evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the purified urea.

General Procedure B: Ureas of 8 or 9 from Amines. To a solution of the 4-fluorophenyl carbamate of **8** or **9** (0.2 mmol; prepared using general procedure F) in dioxane (1 mL) was added the amine (0.6 mmol, 3

equiv). The mixture was heated at 80-85 °C for 20 h. Ethyl acetate (3 mL) was added, followed by brine (1 mL) and 2 N NaOH (0.4 mL). After 10 min, the aqueous layer was removed, and the organic layer was washed with 0.8 M $NaHCO_3$ solution (1 mL). Celite (600 mg) was added to the organic layer and the solvent evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the purified urea.

General Procedure C: Ureas of 8 or 9 from Amines. To an ice-cold solution of **8** or **9** (0.2 mmol, 1 equiv) in DCM (1 mL) was added $NaHCO_3$ (0.6 mmol, 3 equiv), followed by saturated $NaHCO_3$ solution (0.2 mL). Triphosgene (0.2 mmol, 1 equiv) was added and after 10 min, the ice bath was removed, and the mixture was stirred at rt for 1 h (gas evolution). Saturated $NaHCO_3$ solution (0.8 mL) and water (0.4 mL) were added. After 10 min, the aqueous layer was removed, and the organic layer dried with sodium sulfate (20 min). The mixture was filtered, toluene (0.5 ml) was added and the solvent evaporated. THF (1 mL) was added, followed by the amine (0.4 mmol, 2 equiv) and then TEA (0.5 mmol, 2.5 equiv). The mixture was stirred at rt for 15 h. Ethyl acetate (3 mL) was added, followed by saturated $NaHCO_3$ solution (0.8 mL) and water (0.4 mL). After 10 min, the aqueous layer was removed. Celite (600 mg) was added to the organic layer and the solvent was evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the purified urea.

General Procedure D: Ureas of 8 or 9 from Amines. To an ice-cold solution of the amine (0.4 mmol, 2 equiv) in DCM (1 mL) was added $NaHCO_3$ (0.6 mmol, 3 equiv), followed by saturated $NaHCO_3$ solution (0.6 mL). Triphosgene (0.2 mmol, 1 equiv) was added and after 10 min, the ice bath was removed, and the mixture was stirred at rt for 1 h (gas evolution). Saturated $NaHCO_3$ solution (0.8 mL) and water (0.4 mL) were added. After 10 min, the aqueous layer was removed, and the organic layer dried with sodium sulfate (20 min). The mixture was filtered, toluene (0.5 ml) was added and most of the solvent evaporated. THF (1 mL) was added, followed by **8** or **9** (0.2 mmol, 1 equiv) and then TEA (0.4 mmol, 2 equiv). The mixture was stirred at rt for 15 h. Ethyl acetate (3 mL) was added, followed by saturated $NaHCO_3$ solution (0.8 mL) and water (0.4 mL). After 10 min, the aqueous layer was removed. Celite (600 mg) was added to the organic

layer and the solvent was evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the purified urea.

General Procedure E: Sulfonyl Ureas of 8 or 9 from Sulfonamides. To an ice-cold solution of the sulfonamide (0.4 mmol, 2 equiv) in THF (1 ml) was added drop-wise 1 M NaHMDS in THF (0.44 mL, 2.2 equiv). After 10 min, the 4-nitrophenyl carbamate of **8** or **9** (0.2 mmol; prepared using general procedure F) was added. After 10 min in the ice bath, the mixture was stirred at rt for 1 h. Brine (1 mL) was added, followed by 2 N HCl (0.3 mL, 3 equiv). Ethyl acetate (3 mL) was added and after 10 min, the aqueous layer was removed, and the organic layer was washed with brine (0.5 mL). Celite (600 mg) was added to the organic layer and the solvent evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the purified sulfonyl urea.

General Procedure F: Carbamates of 8 or 9 from Carbamoyl Chlorides. To an ice-cold solution of **8** or **9** (0.2 mmol, 1 equiv) in DCE (1 mL) was added TEA (0.24 mmol, 1.2 equiv), followed by slow addition of the chloroformate (0.24 mmol, 1.2 equiv). The ice bath was removed and stirring continued for 2 h. Ethyl acetate (3 mL) was added, followed by saturated $NaHCO_3$ solution (0.8 mL) and water (0.4 mL). After 10 min, the aqueous layer was removed. Celite (600 mg) was added to the organic layer and the solvent evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the purified carbamate.

General Procedure G: Preparation of 7 from Aryl Amines. To a heterogeneous mixture of 5-amino-4,6-dichloropyrimidine (1.6 g, 10 mmol) and an aryl amine (10 mmol, 1 equiv) in n-BuOH (20 mL) was added 6 N HCl (0.84 mL, 0.5 equiv). The mixture was heated at 100 °C for 15 h. At rt, ethyl acetate (20 mL) and 2 N NaOH (8 mL) were added and the mixture stirred vigorously for 15 min. Additional ethyl acetate (80 mL) and saturated $NaHCO_3$ solution (40 mL) were added. The aqueous layer was removed, and the organic layer washed with 0.8 M $NaHCO_3$ solution (25 mL). Celite (10 g) and toluene (10 mL) were added to the organic layer and most of the solvent evaporated. Purification by flash chromatography, using

EtOAc/hexanes, provided the intermediate 5-amino-4-arylamino-6-chloropyrimidine. To a solution of the intermediate (10 mmol, 1 equiv) and 2-chlorobenzaldehyde (2.3 mL, 2 equiv) in dioxane (30 mL) was added 15% $FeCl_3$/silica gel (6.0 g, 600 mg/mmol). The mixture was stirred at rt for 10 min and then at 95 °C for 20 h. At rt, the mixture was filtered using a sintered glass funnel (sand on sodium sulfate on celite with a medium frit) and washed with chloroform (30 mL). Toluene (3 mL) was added and most of the solvent evaporated. The resulting residue was dissolved in CH_2Cl_2 (30 mL) and cooled in an ice bath. DDQ (2.2 g, 1 equiv) was added and after 10 min, the ice bath was removed. The mixture was stirred at rt for 2 h and then most of the solvent was evaporated. Ethyl acetate (100 mL) was added and the resulting organic solution washed with water (2x 50 mL). Celite (10 g) was added and the solvent evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided purified **7**.

General Procedure H: Preparation of 8 or 9 from 7. To a solution of **7** (10 mmol, 1 equiv) and 4-N-boc-aminopiperidine (2.4 g, 1.2 equiv; for intermediates **8**) or 4-N-boc-amino-4-methylpiperidine (2.5 g, 1.2 equiv; for intermediate **9**) in NMP (20 mL) was added potassium carbonate (4.1 g, 3 equiv). The mixture was stirred at rt for 15 min and then at 80 °C for 15 h. Ethyl acetate (100 mL) was added, followed by brine (40 mL) and water (20 mL). The organic layer was washed with brine/water (3/1, 2x30 mL). Celite (10 g) was added to the organic layer and the solvent evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided the intermediate 9-aryl-8-(2-chlorophenyl)-6-(4-N-boc-aminopiperidin-1-yl)-9H-purine (for intermediates **8**) or 8-(2-chlorophenyl)-9-(4-chlorophenyl)-6-(4-N-boc-amino-4-methylpiperidin-1-yl)-9H-purine (for intermediate **9**). To a mixture of the intermediate (1 mmol, 1 equiv) and ethanol (2 mL) was added 6 N HCl (1 mL). The mixture was stirred at rt for 10 min and then at 50 °C for 3 h. The mixture was cooled in an ice bath and chloroform (6 mL) was added, followed by brine (2 mL). 6 N NaOH (1.1 mL) was added slowly and after 5 min, the bath was removed. After 10 min, the layers were separated and the aqueous layer was saturated with sodium chloride. The aqueous layer was extracted with chloroform (2x2 mL). The combined organic layers was dried (sodium sulfate for 20 min), filtered and evaporated. Toluene (1 mL) was added and the solvent evaporated to provide **8** or **9**.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-methyl-3-propylurea (11). The title compound was prepared by the general procedure B to provide 53 mg (90%) of a white crystalline solid, mp 218-219 °C. R_f = 0.32 (2%MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.31-7.46 (m, 5H), 7.20 (d, *J* = 8.5 Hz, 2H), 5.44 (br s, 2H), 4.19 (d, *J* = 7.5 Hz, 1H), 3.95-4.13 (m, 1H), 3.25-3.44 (m, 2H), 3.20 (t, *J* = 7.4 Hz, 2H), 2.86 (s, 3H), 2.11-2.22 (m, 2H), 1.39-1.60 (m, 4H), 0.90 (t, *J* = 7.4 Hz, 3H). MS (*m/z*) 538.3 (M+1). HPLC = >99% at 19.81 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-propyl-3-(2,2,2-trifluoroethyl)urea (12). The title compound was prepared by the general procedure C to provide 60 mg (83%) of a white crystalline solid, mp 212-213 °C. R_f = 0.48 (2%MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.30-7.45 (m, 5H), 7.20 (d, *J* = 8.7 Hz, 2H), 5.38-5.50 (m, 2H), 4.38 (d, *J* = 7.5 Hz, 1H), 4.01-4.15 (m, 1H), 3.94 (q, *J* = 9.0 Hz, 2H), 3.24-3.40 (m, 2H), 3.20 (d, *J* = 7.37 Hz, 2H), 2.10-2.22 (m, 2H), 1.40-1.72 (m, 4H), 0.91 (t, *J* = 7.4 Hz, 3H). MS (*m/z*) 606.6 (M+1). HPLC = >98% at 20.75 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-methyl-3-(2-methylpropyl)urea (13). The title compound was prepared by the general procedure B to provide 35 mg (58%) of a white crystalline solid, mp 194-195 °C. R_f = 0.43 (2%MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.31-7.46 (m, 5H), 7.20 (d, *J* = 8.5 Hz, 2H), 5.44 (br s, 2H), 4.19 (d, *J* = 7.5 Hz, 1H), 3.95-4.13 (m, 1H), 3.25-3.44 (m, 2H), 3.05 (d, *J* = 7.4 Hz, 2H), 2.86 (s, 3H), 2.11-2.22 (m, 2H), 1.85-1.92 (m, 1 H), 1.39-1.60 (m, 2H), 0.91 (d, *J* = 7.4 Hz, 6H). MS (*m/z*) 552.5 (M+1). HPLC = 97% at 20.33 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-(2,2-dimethylpropyl)urea (14). The title compound was prepared by the general procedure B to provide 25 mg (41%) of a white amorphous solid, mp 270-271 °C. R_f = 0.33 (2%MeOH/80% EtOAc/hexanes; blue with

UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.24-7.43 (m, 5H), 7.19 (d, *J* = 8.5 Hz, 2H), 5.42 (br s, 2H), 4.38 (t, *J* = 5.6 Hz, 1H), 4.27 (d, *J* = 7.5 Hz, 1H), 3.86-4.05 (m, 1H), 3.20-3.43 (m, 2H), 2.96 (d, *J* = 6.0 Hz, 2H), 2.02-2.26 (m, 2H), 1.35-1.56 (m, 2H),0.90 (s, 9H). MS (*m/z*) 552.5 (M+1). HPLC = >98% at 19.79 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-(2-methoxyethyl)-3-methylurea (15). The title compound was prepared by the general procedure B to provide 55 mg (90%) of an off white crystalline solid, mp 193-194 °C. R_f = 0.08 (2%MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.29-7.42 (m, 5H), 7.20 (d, *J* = 8.7 Hz, 2H), 5.39 (d, *J* = 6.8 Hz, 1H), 5.28 (br s, 2H), 3.90-4.08 (m, 1H), 3.44-3.65 (m, 4H), 3.27-3.44 (m, 5H), 2.93 (s, 3H), 2.00-2.25 (m, 2H), 1.39-1.58 (m, 2H). MS (*m/z*) 554.8 (M+1). HPLC = >99% at 19.09 min.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}pyrrolidine-1-carboxamide (16). The title compound was prepared by the general procedure B to provide 53 mg (90%) of a white amorphous solid, mp 134-136 °C. R_f = 0.17 (2% MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.59 Hz, 1H), 7.30-7.44 (m, 5H), 7.19 (d, *J*=8.67 Hz, 2H), 5.46 (br s, 2H), 3.92-4.22 (m, 3H), 3.19-3.52 (m, 5H), 2.07-2.22 (m, 2H), 1.69-1.88 (m, 2H), 1.37-1.59 (m, 2H). MS (*m/z*) 536.3 (M+1). HPLC = >99% at 19.36 min.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}piperidine-1-carboxamide (17). The title compound was prepared by the general procedure B to provide 60 mg (99%) of a white crystalline solid, mp 241-243 °C. R_f = 0.22 (2% MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.29-7.46 (m, 5H), 7.20 (d, *J* = 8.5 Hz, 2H), 5.45 (br s, 2H), 4.31 (d, *J* = 7.4 Hz, 1H), 3.93-4.20 (m, 1H), 3.16-3.44 (m, 6H), 2.11-2.22 (m, 2H), 1.40-1.75 (m, 8H). MS (*m/z*) 550.3 (M+1). HPLC = >99% at 19.93 min.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}morpholine-4-carboxamide (18). The title compound was prepared by the general procedure B to provide 49 mg (81%)

of a white crystalline solid, mp 210-211 °C. R_f = 0.14 (2% MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.50 (d, *J* = 6.59 Hz, 1H), 7.30-7.44 (m, 5H), 7.19 (d, *J* = 8.48 Hz, 2 H), 5.47 (br s, 2H), 4.30 (d, *J* = 7.35 Hz, 1H), 3.94-4.17 (m, 1H), 3.54-3.81 (m, 2H), 3.19-3.40 (m, 4H), 2.07-2.26 (m, 2H), 1.35-1.58 (m, 2H). MS (*m/z*) 552.5 (M+1). HPLC = 96% at 18.12 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-cyclopentylurea (19). The title compound was prepared by the general procedure A to provide 77 mg (100%) of a white crystalline solid, mp 223-224 °C. R_f = 0.11 (1%MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.51 (d, *J* = 6.40 Hz, 1H), 7.29-7.45 (m, 5H), 7.19 (d, *J* = 8.48 Hz, 2H), 5.40 (br s, 1H), 4.22-4.49 (m, 2H), 3.79-4.09 (m, 2H), 3.25-3.40 (m, 2H), 2.04-2.25 (m, 2H), 1.85-2.03 (m, 2H), 1.54-1.72 (m, 4H), 1.32-1.49 (m, 4H). MS (*m/z*) 550.1 (M+1). HPLC = 95% at 19.52 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-[1-(trifluoromethyl)cyclopentyl]urea (20). The title compound was prepared by the general procedure D to provide 32 mg (43%) of a white crystalline solid, mp 225-227 °C. R_f = 0.52 (2%MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.30-7.45 (m, 5H), 7.19 (d, *J* = 8.7 Hz, 2H), 5.35-5.48 (m, 2H), 4.29-4.44 (m, 2H), 3.85-4.04 (m, 1H), 3.25-3.37 (m, 2H), 2.01-2.24 (m, 6H), 1.68-1.93 (m, 4H), 1.35-1.55 (m, 2H). MS (*m/z*) 618.3 (M+1). HPLC = >98% at 20.44 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-cyclohexylurea (21). The title compound was prepared by the general procedure B to provide 58 mg (93%) of a white amorphous solid, mp 160-162 °C. R_f = 0.28 (2%MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.51 (d, *J* = 6.4 Hz, 1H), 7.29-7.45 (m, 5H), 7.19 (d, *J* = 8.3 Hz, 2H), 5.39 (br s, 2H), 4.20-4.43 (m, 2H), 3.96 (br s, 1H), 3.48 (br s, 1H), 3.25-3.41 (m, 2H), 2.01-2.24 (m, 2H), 1.84-2.01 (m, 2H), 1.53-1.82 (m, 4H), 1.22-1.53 (m, 4H), 0.96-1.22 (m, 2H). MS (*m/z*) 564.3 (M+1). HPLC = >98% at 19.49 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(4,4-difluorocyclohexyl)urea (22). The title compound was prepared by the general procedure B to provide 72 mg (100%) of an off white amorphous solid, mp 228-229 °C. R_f = 0.21 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.50 (d, *J* = 6.8 Hz, 1H), 7.29-7.43 (m, 5H), 7.18 (d, *J* = 8.5 Hz, 2H), 5.38 (br s, 1H), 4.35-4.53 (m, 2H), 3.83-4.04 (m, 1H), 3.62-3.81 (m, 1H), 3.23-3.42 (m, 2H), 1.67-2.22 (m, 8H), 1.32-1.59 (m, 4H). MS (*m/z*) 600.6 (M+1), 599.0 (M-1). HPLC = >99% at 19.41 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(4,4-difluoro-1-methylcyclohexyl)urea (23). The title compound was prepared by the general procedure C to provide 69 mg (94%) of an off white amorphous solid, mp 160-162 °C. R_f = 0.30 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.50 (d, *J* = 6.8 Hz, 1H), 7.29-7.44 (m, 5H), 7.18 (d, *J* = 8.7 Hz, 2H), 5.38 (br s, 2H), 4.54 (d, *J* = 7.9 Hz, 1H), 4.31 (s, 1H), 3.80-4.00 (m, 1H), 3.20-3.40 (m, 2H), 2.04-2.22 (m, 4H), 1.73-1.95 (m, 4H), 1.54-1.73 (m, 1H), 1.40-1.53 (m, 2H), 1.38 (s, 3H). MS (*m/z*) 614.4 (M+1). HPLC = >99% at 15.10 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(cyclopentylmethyl)urea (24). The title compound was prepared by the general procedure B to provide 59 mg (95%) of a white amorphous solid, mp 135-137 °C. R_f = 0.27 (2% MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.50 (d, *J* = 6.2 Hz, 1H), 7.24-7.44 (m, 5H), 7.18 (d, *J* = 8.1 Hz, 2H), 5.38 (br s, 2H), 4.43-4.78 (m, 2H), 3.96 (br s, 1H), 3.32 (br s, 2H), 2.95-3.17 (m, 2H), 1.87-2.24 (m, 3H), 1.70 (br s, 2H), 1.34-1.63 (m, 6H), 1.04-1.32 (m, 2H). MS (*m/z*) 564.4 (M+1). HPLC = >98% at 20.24 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(cyclohexylmethyl)urea (25). The title compound was prepared by the general procedure B to provide 65 mg (100%) of a white crystalline solid, mp 137-139 °C. R_f = 0.30 (2% MeOH/80% EtOAc/hexanes; blue

with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.50 (d, *J* = 6.2 Hz, 1H), 7.24-7.44 (m, 5H), 7.19 (d, *J* = 8.1 Hz, 2H), 5.38 (br s, 2H), 4.43-4.78 (m, 2H), 3.96 (br s, 1H), 3.32 (br s, 2H), 2.90-3.17 (m, 2H), 1.97-2.24 (m, 2H), 1.58-1.82 (m, 5H), 1.34-1.55 (m, 3H), 1.09-1.26 (m, 3H), 0.81-1.05 (m, 2H). MS (*m/z*) 578.5 (M+1). HPLC = 95% at 21.52 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[(4,4-difluorocyclohexyl)methyl]urea (26). The title compound was prepared by the general procedure C to provide 64 mg (87%) of an off white amorphous solid, mp 135-137 °C. R_f = 0.21 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.50 (d, *J* = 6.8 Hz, 1H), 7.29-7.44 (m, 5H), 7.18 (d, *J* = 8.5 Hz, 2H), 5.39 (br s, 2H), 4.54 (t, *J* = 5.8 Hz, 1H), 4.38 (d, *J* = 7.9 Hz, 1H), 3.82-4.04 (m, 1H), 3.19-3.45 (m, 2H), 3.08 (dd, *J* = 6.2, 6.2 Hz, 2H), 2.02-2.23 (m, 4H), 1.41-1.82 (m, 7H), 1.18-1.35 (m, 2H). MS (*m/z*) 614.5 (M+1). HPLC = >99% at 14.70 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[(1-methylpiperidin-4-yl)methyl]urea (27). The title compound was prepared by the general procedure B to provide 67 mg (100%) of a white amorphous solid, mp 121-122 °C. R_f = 0.10 (10% (20% NH_4OH/MeOH)/ EtOAc; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.51 (d, *J* = 6.4 Hz, 1 H), 7.24-7.44 (m, 5H), 7.18 (d, *J* = 7.9 Hz, 2H), 5.38 (br s, 2H), 4.67 (br s, 1H), 4.53 (d, *J* = 7.4 Hz, 1 H), 3.95 (br s, 1H), 3.32 (br s, 2H), 3.06 (br s, 2H), 2.78-2.88 (m, 2H), 2.24 (s, 3H), 1.99-2.17 (m, 2H), 1.81-1.96 (m, 2H), 1.61-1.73 (m, 2H), 1.16-1.51 (m, 5H). MS (*m/z*) 593.4 (M+1). HPLC = 95% at 16.93 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[(2-fluorophenyl)methyl]urea (28). The title compound was prepared by the general procedure C to provide 71 mg (100%) of a white amorphous solid, mp 210-212 °C. R_f = 0.25 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.49 (d, *J* = 6.2 Hz, 1H), 7.25-7.43 (m, 7H), 7.17 (d, *J* = 7.2 Hz, 2H), 7.00-7.10 (m, 1H), 6.86-7.00 (m, 1H), 5.30 (br s, 2H), 5.16-5.25 (m, 1H), 4.89 (d,

J = 7.2 Hz, 1H), 4.35 (d, *J* = 2.1 Hz, 2H), 3.82-3.98 (m, 1H), 3.26 (br s, 2H), 2.04 (br s, 2H), 1.28-1.48 (m, 2H). MS (*m/z*) 590.0 (M+1), 588.4 (M-1). HPLC = >99% at 19.68 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[(4-fluorophenyl)methyl]urea (29). The title compound was prepared by the general procedure C to provide 71 mg (100%) of a white amorphous solid, mp 184-185 °C. R_f = 0.47 (2% MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.50 (d, *J* = 6.6 Hz, 1H), 7.26-7.44 (m, 5H), 7.12-7.24 (m, 4H), 6.86-7.06 (m, 2H), 5.33 (br s, 2H), 4.98 (br s, 1H), 4.66 (d, *J* = 7.4 Hz, 1H), 4.27 (d, *J* = 5.3 Hz, 2H), 3.93 (br s, 1H), 3.23-3.35 (m, 2H), 2.09 (br s, 2H), 1.30-1.49 (m, 2H). MS (*m/z*) 590.2 (M+1), 588.5 (M-1). HPLC = >99% at 19.72 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-phenylurea (30). The title compound was prepared by the general procedure C to provide 29 mg (47%) of a tan crystalline solid, mp 160-162 °C. R_f = 0.37 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.49 (d, *J* = 6.4 Hz, 1H), 7.08-7.42 (m, 11H), 6.91-7.05 (m, 2H), 5.32 (br s, 2H), 5.01-5.19 (m, 1H), 3.91-4.10 (m, 1H), 3.26 (br s, 2H), 1.96-2.21 (m, 2H), 1.30-1.48 (m, 2H). MS (*m/z*) 558.3 (M+1). HPLC = 100% at 20.21 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(pyridin-2-yl)urea (31). The title compound was prepared by the general procedure C to provide 12 mg (18%) of a white crystalline solid, mp 233-234 °C. R_f = 0.14 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.52-9.74 (m, 1H), 8.40 (s, 1H), 8.03-8.18 (m, 2H), 7.48-7.70 (m, 2H), 7.23-7.43 (m, 5H), 7.20 (d, *J* = 8.1 Hz, 2H), 6.82-6.96 (m, 1H), 6.77 (d, *J* = 7.9 Hz, 1H), 5.25 (br s, 2H), 4.13-2.29 (m, 2H), 3.64 (br s, 2H), 2.15-2.29 (m, 2H), 1.54-1.87 (m, 2H). MS (*m/z*) 559.2 (M+1). HPLC = >99% at 17.59 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(2-fluorophenyl)urea (32). The title compound was prepared by the general procedure C to provide 55 mg (80%) of an off white amorphous solid, mp 152-154 °C. R_f = 0.50 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR

(300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 8.00 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 6.6 Hz, 1H), 7.23-7.43 (m, 5H), 7.18 (d, *J* = 8.1 Hz, 2H), 6.84-7.12 (m, 4H), 5.26-5.39 (m, 3H), 3.95-4.13 (m, 1H), 3.19-3.44 (m, 2H), 1.97-2.24 (m, 2H), 1.35-1.50 (m, 2H). MS (*m/z*) 576.2 (M+1). HPLC = >98% at 20.09 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(2,4-difluorophenyl)urea (33). The title compound was prepared by the general procedure C to provide 55 mg (77%) of an off white amorphous solid, mp 140-142 °C. R_f = 0.46 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^1H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.84-7.98 (m, 1H), 7.49 (d, *J* = 6.4 Hz, 1H), 7.23-7.43 (m, 5H), 7.17 (d, *J* = 7.9 Hz, 2H), 6.97 (s, 1H), 6.65-6.86 (m, 2H), 5.46 (d, *J* = 7.0 Hz, 1H), 5.32 (br s, 2H), 4.00 (br s, 1H), 3.32 (br s, 2H), 1.96-2.24 (m, 2H), 1.31-1.50 (m, 2H). MS (*m/z*) 594.3 (M+1). HPLC = >98% at 20.13 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(3,4-difluorophenyl)urea (34). The title compound was prepared by the general procedure C to provide 65 mg (91%) of an off white crystalline solid, mp 148-150 °C. R_f = 0.30 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^1H NMR (300 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.41-7.56 (m, 2H), 7.22-7.40 (m, 6H), 7.14 (d, *J* = 8.3 Hz, 2H), 6.86-7.02 (m, 1H), 6.81 (br s, 1H), 5.22-5.41 (m, 3H), 3.96 (br s, 1H), 3.16-3.36 (m, 2H), 2.05 (br s, 2H), 1.26-1.48 (m, 2H). MS (*m/z*) 594.2 (M+1). HPLC = >98% at 20.28 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(4-fluorophenyl)urea (35). The title compound was prepared by the general procedure C to provide 21 mg (33%) of a tan crystalline solid, mp 215-217 °C. R_f = 0.33 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^1H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.49 (d, *J* = 6.4 Hz, 1H), 7.22-7.44 (m, 5H), 7.08-7.22 (m, 4 H), 6.94 (t, *J* = 8.5 Hz, 2H), 6.75 (s, 1H), 5.35 (br s, 2H), 4.89 (d, *J* = 7.2 Hz, 1H), 3.92-4.10 (m, 1H), 3.22-3.36 (m, 2H), 1.97-2.23 (m, 2H), 1.34-1.48 (m, 2H). MS (*m/z*) 576.3 (M+1). HPLC = >98% at 19.79 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[2-(trifluoromethyl)pyridin-3-yl]urea (36). The title compound was prepared by the general procedure C to

provide 31 mg (41%) of an off-white crystalline solid, mp 249-251 °C. R_f = 0.49 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.66 (d, *J* = 8.5 Hz, 1H), 8.36 (s, 1H), 8.29 (d, *J* = 4.0 Hz, 1H), 7.42-7.60 (m, 2H), 7.31-7.42 (m, 5H), 7.20 (d, *J* = 8.5 Hz, 2H), 5.15-5.41 (m, 2H), 4.02 (br s, 1H), 3.34-3.58 (m, 2H), 2.08-2.24 (m, 2H), 1.45-1.68 (m, 2H). MS (*m/z*) 627.7 (M+1), 625.8 (M-1). HPLC = >99% at 19.76 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[6-(trifluoromethyl)pyridin-3-yl]urea (37). The title compound was prepared by the general procedure C to provide 42 mg (56%) of an off white crystalline solid, mp 140-142 °C. R_f = 0.36 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$-*d*) δ 8.37 (s, 1H), 8.34 (s, 1H), 8.22 (d, *J* = 8.3 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 7.49 (d, *J* = 6.8 Hz, 1H), 7.27-7.42 (m, 6H), 7.17 (d, *J* = 8.3 Hz, 2H), 5.25-5.50 (m, 2H), 5.03 (d, *J* = 7.7 Hz, 1H), 3.95 - 4.20 (m, 1H), 3.29-3.46 (m, 2H), 2.05-2.24 (m, 2H), 1.33-1.54 (m, 2H). MS (*m/z*) 627.5 (M+1). HPLC = >99% at 19.88 minutes.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[6-(difluoromethoxy)pyridin-3-yl]urea (38). The title compound was prepared by the general procedure C to provide 70 mg (93%) of an off white amorphous solid, mp 150-152 °C. R_f = 0.39 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.94 (d, *J* = 2.3 Hz, 1H), 7.85 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.49 (d, *J* = 6.8 Hz, 1H), 7.23-7.44 (m, 6H), 7.16 (d, *J* = 8.5 Hz, 2H), 7.03 (s, 1H), 6.81 (d, *J* = 8.8 Hz, 1H), 5.20-5.52 (m, 2H), 4.99 (d, *J* = 7.7 Hz, 1H), 3.88-4.05 (m, 1H), 3.24-3.40 (m, 2H), 2.03-2.25 (m, 2H), 1.31-1.55 (m, 2H). MS (*m/z*) 625.9 (M+1). HPLC = >99% at 19.68 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-[5-(trifluoromethyl)-1H-pyrazol-3-yl]urea (39). The title compound was prepared by the general procedure B to provide 42 mg (62%) of a white crystalline solid, mp 231-232 °C. R_f = 0.30 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.51 (d, *J* = 6.0 Hz, 1H), 7.29-7.45 (m, 6H), 7.20 (d, *J* = 7.9 Hz,

2H), 7.05 (d, *J* = 7.0 Hz, 1H), 5.41-5.73 (m, 4H), 4.04-4.20 (m, 1H), 3.36 (br s, 2H), 2.13-2.28 (m, 2H), 1.64-1.79 (m, 2H). MS (*m/z*) 616.5 (M+1). HPLC = >98% at 16.47 min.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-3-(1-methyl-1H-pyrazol-4-yl)urea (40). The title compound was prepared by the general procedure C to provide 65 mg (96%) of a tan amorphous solid, mp 138-140 °C. R_f = 0.11 (4% MeOH/80% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.44-7.58 (m, 2H), 7.24-7.42 (m, 5H), 7.17 (d, *J* = 8.5 Hz, 2H), 6.52 (br s, 1H), 5.36 (br s, 2H), 4.92 (d, *J* = 7.4 Hz, 1H), 3.91-4.08 (m, 1H), 3.82 (s, 3H), 3.21-3.38 (m, 2H), 2.02-2.23 (m, 2H), 1.32-1.54 (m, 2H). MS (*m/z*) 562.0 (M+1). HPLC = >98% at 17.73 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-[(2-fluorobenzene)sulfonyl]urea (41). The title compound was prepared by the general procedure E to provide 42 mg (55%) of a white crystalline solid, mp 201-203 °C. R_f = 0.31 (5% MeOH/75% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.89 (t, *J* = 7.1 Hz, 1H), 7.58-7.82 (m, 1H), 7.51 (d, *J* = 6.6 Hz, 1H), 7.17-7.44 (m, 10H), 6.56 (d, *J* = 7.4 Hz, 1H), 5.31 (br s, 2H), 4.00 (br s, 1H), 3.25-3.52 (m, 2H), 2.02 (br s, 2H), 1.47-1.62 (m, 2H). MS (*m/z*) 640.2 (M+1), 638.6 (M-1). HPLC = 99% at 14.44 min.

3-{1-[8-(2-Chlorophenyl)-9-[6-(trifluoromethyl)pyridin-3-yl]-9H-purin-6-yl]piperidin-4-yl}-1-(cyclohexylmethyl)urea (42). The title compound was prepared by the general procedure A to provide 62 mg (100%) of an off white crystalline solid, mp 230-232 °C. R_f = 0.25 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.51 (s, 1H), 8.36 (s, 1H), 7.96 (d, *J* = 7.4 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.62 (br s, 1H), 7.31-7.49 (m, 3H), 5.40 (br s, 2H), 4.49 (br s, 1H), 4.38 (d, *J* = 6.8 Hz, 1H), 3.88-4.06 (m, 1H), 3.33 (br s, 2H), 2.99 (br s, 2H), 2.06-2.22 (m, 2H), 1.62-1.78 (m, 7H), 1.36-1.54 (m, 2H), 1.05-1.32 (m, 2H), 0.83-0.98 (m, 2H). MS (*m/z*) 611.9 (M-1). HPLC = >99% at 20.88 min.

3-{1-[8-(2-Chlorophenyl)-9-[6-(difluoromethoxy)pyridin-3-yl]-9H-purin-6-yl]piperidin-4-yl}-1-(cyclohexylmethyl)urea (43). The title compound was prepared by the general procedure A to provide 63

mg (100%) of an off white crystalline solid, mp 141-143 °C. R_f = 0.23 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.04 (s, 1H), 7.71 (d, *J* = 7.2 Hz, 1 H), 7.57 (d, *J* = 6.2 Hz, 1H), 7.33-7.47 (m, 4H), 6.94 (d, *J* = 8.7 Hz, 1H), 5.40 (br s, 2H), 4.38 (br s, 1H), 4.25 (d, *J* = 7.4 Hz, 1H), 3.97 (br s, 1H), 3.25-3.41 (m, 2H), 2.92-3.06 (m, 2H), 2.06-2.22 (m, 2H), 1.65-1.80 (m, 5H), 1.36-1.55 (m, 3H), 1.07-1.33 (m, 3H), 0.79-1.03 (m, 2H). MS (*m/z*) 611.8 (M+1), 609.7 (M-1). HPLC = >99% at 20.45 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-fluorophenyl)-9H-purin-6-yl]piperidin-4-yl}-1-(2-fluorophenyl)urea (44). The title compound was prepared by the general procedure A to provide 67 mg (100%) of an off white amorphous solid, mp 138-140°C. R_f = 0.14 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (s, 1H), 8.00 (br s, 1H), 7.48 (d, *J* = 6.2 Hz, 1H), 7.15-7.40 (m, 6H), 6.80-7.08 (m, 5H), 5.62 (d, *J* = 7.1 Hz, 1H), 5.31 (br s, 2H), 4.01 (br s, 1H), 3.30 (br s, 2H), 2.05 (br s, 2H), 1.29-1.57 (m, 2H). MS (*m/z*) 560.1 (M+1), 558.2 (M-1). HPLC = >99% at 14.29 min.

3-{1-[8-(2-Chlorophenyl)-9-[6-(trifluoromethyl)pyridin-3-yl]-9H-purin-6-yl]piperidin-4-yl}-1-(2-fluorophenyl)urea (45). The title compound was prepared by the general procedure A to provide 63 mg (100%) of an off white amorphous solid, mp 227-228 °C. R_f = 0.17 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.36 (s, 1H), 7.86-8.12 (m, 2H), 7.75 (d, *J* = 8.1 Hz, 1H), 7.62 (d, *J* = 5.6 Hz, 1H), 7.30-7.51 (m, 3H), 6.80-7.15 (m, 4H), 5.24-5.38 (m, 3H), 3.90-4.22 (m, 1H), 3.33 (br s, 2H), 2.06-2.21 (m, 2H), 1.38-1.54 (m, 2H). MS (*m/z*) 611.5 (M+1), 609.9 (M-1). HPLC = >99% at 19.89 min.

3-{1-[8-(2-Chlorophenyl)-9-[6-(difluoromethoxy)pyridin-3-yl]-9H-purin-6-yl]piperidin-4-yl}-1-(2-fluorophenyl)urea (46). The title compound was prepared by the general procedure A to provide 62 mg (100%) of an off white amorphous solid, mp 140-142 °C. R_f = 0.14 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.90-8.15 (m, 2H), 7.69 (d, *J* = 7.0 Hz, 1H), 7.56 (d, *J* = 6.2

Hz, 1H), 7.29-7.48 (m, 4H), 6.80-7.17 (m, 5H), 5.25-5.45 (m, 3H), 3.90-4.12 (m, 1H), 3.25-3.39 (m, 2H), 2.04-2.27 (m, 2H), 1.35-1.52 (m, 2H). MS (*m/z*) 610.0 (M+1), 607.7 (M-1). HPLC = >99% at 19.51 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-cyclohexylurea (47). The title compound was prepared by the general procedure A to provide 69 mg (100%) of a solid, mp 155-157 °C. R_f = 0.12 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.50 (d, *J* = 6.6 Hz, 1H), 7.28-7.42 (m, 5H), 7.19 (d, *J* = 8.7 Hz, 2H), 4.37 (d, *J* = 7.6 Hz, 1H), 4.30 (s, 1H), 3.84 (br s, 2H), 3.35-3.58 (m, 2H), 2.10-2.30 (m, 2H), 1.85-1.99 (m, 2H), 1.52-1.81 (m, 6H), 1.46 (s, 3H), 1.22-1.39 (m, 2H), 1.00-1.21 (m, 2H). MS (*m/z*) 578.4 (M+1). HPLC = >99% at 15.48 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-(4,4-difluorocyclohexyl)urea (48). The title compound was prepared by the general procedure C to provide 71 mg (97%) of an off white amorphous solid, mp 175-177 °C. R_f = 0.21 (2% MeOH/60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.50 (d, *J* = 6.8 Hz, 1H), 7.30-7.43 (m, 5H), 7.19 (d, *J* = 8.7 Hz, 2H), 4.75 (br s, 2 H), 4.27 (d, *J* = 7.7 Hz, 1H), 4.20 (s, 1H), 3.87 (br s, 1H), 3.70 (br s, 2H), 2.10-2.23 (m, 2H), 1.82-2.08 (m, 4H), 1.68-1.80 (m, 4H), 1.34-1.50 (m, 5H). LC/MS (*m/z*) 616.6 (M+1), >97% at 2.76 min**.** HPLC = >99% at 15.02 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-[(2-fluorophenyl)methyl]urea (49). The title compound was prepared by the general procedure C to provide 74 mg (100%) of an off-white amorphous solid, mp 115-117 °C. R_f = 0.31 (60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.49 (d, *J* = 6.8 Hz, 1H), 7.26-7.42 (m, 7H), 7.18 (d, *J* = 8.3 Hz, 2H), 6.93-7.11 (m, 2H), 5.08 (t, *J* = 5.6 Hz, 1H), 4.78 (br s, 2H), 4.61 (s, 1H), 4.36 (d, *J* = 5.6 Hz, 2H), 3.77 (br s, 2H), 2.05-2.23 (m, 2H), 1.62-1.77 (m, 2H), 1.42 (s, 3H). MS (*m/z*) 604.3 (M+1). HPLC = >99% at 15.15 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-(2-fluorophenyl)urea (50). The title compound was prepared by the general procedure A to provide 71 mg (100%) of an off-white amorphous solid, mp 162-164 °C. R_f = 0.33 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.99 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.48 (d, *J* = 7.0 Hz, 1H), 7.24-7.41 (m, 5H), 7.18 (d, *J* = 8.7 Hz, 2H), 6.84-7.11 (m, 4H), 5.28 (s, 1H), 4.82 (br s, 2H), 3.77 (br s, 2H), 2.11-2.23 (m, 2H), 1.66-1.79 (m, 2H), 1.47 (s, 3H). MS (*m/z*) 590.4 (M+1), 588.4 (M-1). HPLC = 99% at 15.94 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-(2,4-difluorophenyl)urea (51). The title compound was prepared by the general procedure D to provide 47 mg (64%) of an off-white crystalline solid, mp 143-145 °C. R_f = 0.31 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.80-8.00 (m, 1H), 7.49 (d, *J* = 6.8 Hz, 1H), 7.28-7.41 (m, 5H), 7.19 (d, *J* = 7.7 Hz, 2H), 6.71-6.89 (m, 3H), 5.10 (s, 1H), 4.82 (br s, 2H), 3.80 (br s, 2H), 2.10- 2.26 (m, 2H), 1.67-1.85 (m, 2H), 1.48 (s, 3H). MS (*m/z*) 608.6 (M+1), 606.8 (M-1). HPLC = 99% at 16.04 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-[6-(difluoromethoxy)pyridin-3-yl]urea (52). The title compound was prepared by the general procedure C to provide 43 mg (56%) of an off white amorphous solid, mp 170-172 °C. R_f = 0.34 (60% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.82-8.01 (m, 2H), 6.99-7.56 (m, 10H), 6.81 (d, *J* = 7.4 Hz, 1H), 4.93 (s, 1H), 4.78 (br s, 2H), 3.80 (br s, 2H), 2.05-2.18 (m, 2H), 1.75 (br s, 2H), 1.47 (s, 3H). MS (*m/z*) 639.3 (M+1), 637.6 (M-1). HPLC = >99% at 15.54 min.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}-1-[(2-fluorobenzene)sulfonyl]urea (53). The title compound was prepared by the general procedure E to provide 45 mg (58%) of an off white amorphous solid, mp 111-113 °C. R_f = 0.44 (2% MeOH/60% EtOAc/Hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.92 (t, *J* = 7.2 Hz, 1H), 7.55-7.66 (m, 1H),

7.50 (d, *J* = 6.6 Hz, 1H), 7.16-7.44 (m, 10H), 6.57 (s, 1H), 4.99 (br s, 2H), 3.46 (br s, 2H), 2.16 (d, *J* = 13.8 Hz, 2H), 1.58-1.79 (m, 2H), 1.39 (s, 3H). MS (*m/z*) 654.5 (M+1), 652.6 (M-1). HPLC = 96% at 15.05 min.

N-Cyclohexyl({1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-methylpiperidin-4-yl}amino)sulfonamide (54). To a solution of **9** (55 mg, 0.12 mmol) in THF (1 mL) was added cyclohexylsulfamoyl chloride (36 mg, 1.5 equiv), followed by TEA (0.033 mL, 2 equiv). The mixture was stirred at rt for 15 h. Water (0.4 mL), ethyl acetate (3 mL) and then saturated $NaHCO_3$ solution (0.8 mL) were added. After 10 min, the aqueous layer was removed. Celite (600 mg) was added to the organic layer and the solvent was evaporated. Flash chromatography using silica gel with an EtOAc/hexanes gradient provided 72 mg (99%) of a white amorphous solid, mp 201-202 °C. R_f = 0.27 (40% EtOAc/hexanes; blue with UV). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 (s, 1H), 7.51 (d, *J* = 6.6 Hz, 1 H), 7.30-7.44 (m, 5H), 7.20 (d, *J* = 8.3 Hz, 2H), 4.61 (br s, 2H), 4.00-4.35 (m, 3H), 3.11-3.42 (m, 2H), 2.00-2.16 (m, 2H), 1.65-1.86 (m, 4H), 1.48-1.64 (s, 5H), 1.11-1.40 (m, 4H). MS (*m/z*) 614.3 (M+1), 612.6 (M-1). HPLC = >99% at 16.08 min.

Testing for Pan-assay Interference (PAIN)

The compounds synthesized were considered low risk for PAIN as they are analogs of a previously well-characterized diphenyl purine scaffold specific for hCB1.[19] Additionally, compounds were manually inspected to identify structural similarities related to known PAIN compounds [36-38] and all compounds were tested using the calcium mobilization assay in parental cells without hCB1 to ensure specificity of action.

Calcium mobilization and radioligand displacement assays

Each compound was biologically characterized using a functional fluorescent hCB1 activated $G\alpha q_{16}$-coupled intracellular calcium mobilization assay in CHO-K1 cells, as has been described in our previous publications and apparent affinity (Ke) values were determined.[21, 29] Briefly, CHO-K1 cells were engineered to co-express human CB1 and $G_{\alpha q16}$. Activation of CB1 by an agonist then leads to generation of inositol phosphatase 3 (IP_3) and activation of IP_3 receptors, which leads to mobilization of intracellular

calcium. Calcium flux was monitored in a 96-well format using the fluorescent dye Calcein-4 AM in an automated plate reader (Flexstation, Molecular Devices). The antagonism of a test compound was measured by its ability to shift the concentration response curve of the synthetic CB1 agonist CP55940 rightwards using the equation:

Ke = [Ligand]/[DR-1] where DR is the EC_{50} ratio of CP55940 in the presence or absence of a test agent.

For some assays, cells were loaded with Calcein-4 AM as described below and directly stimulated with various concentrations of a test agent for 90 seconds. Decrease in basal fluorescence was used in these assays to calculate EC_{50} values.

Further characterization of select compounds was performed using radioligand displacement of [^{3}H]CP55940 and equilibrium dissociation constant (Ki) values were determined as described previously.[21, 29] Selectivity of these compounds at hCB1 versus hCB2 was also determined by obtaining Ki values at either receptor in membranes of HEK cells over-expressing either receptor. Data reported are average values from 3-6 independent measurements typically with <30% standard error.

MDCK-mdr1 permeability assays

MDCK-mdr1 cells obtained from the Netherlands Cancer Institute were grown on Transwell type filters (Corning) for 4 d to confluence in DMEM/F12 media containing 10% fetal bovine serum and antibiotics. Compounds were added to the apical side at a concentration of 10 μM in a transport buffer comprising of 1X Hank's balanced salt solution, 25 mM D-glucose and buffered with HEPES to pH 7.4. Samples were incubated for 1 h at 37 °C and carefully collected from both the apical and basal side of the filters. Compounds selected for MDCK-mdr1 cell assays were infused on an Applied Biosystems API-4000 mass spectrometer to optimize for analysis using multiple reaction monitoring (MRM), as previously described.[39] The chromatography was conducted with an Agilent 1100 binary pump with a flow rate of 0.5 mL/min. Mobile phase solvents were 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B).

The solvent conditions were 10% B for 1 minute, followed by a gradient to 95% B over 5 min. Data reported are average values from 2-3 measurements.

Hepatic microsomal stability studies

Human microsomal stability assays were performed as described previously.[29] Briefly, test compounds were incubated at a 1 μM final concentration with 0.5 mg/ml pooled human liver microsomes from 200 unidentified donors (Xenotech, LLC, Lenexa, KS) in a 100 mM phosphate buffer (pH 7.4) containing 3 mM $MgCl_2$, 1 mM nicotinamide adenine dinucleotide phosphate (NADPH), 5 mM uridine diphosphate glucuronic acid (UDPGA), and 50 μg/ml alamethicin. Triplicate samples were incubated for up to 120 min. Samples were removed at regular intervals. Reactions were terminated by addition of 3 volumes of methanol and processed as described for the MDCK-mdr1 assays, but standard curves were prepared in blank matrix for each compound for quantitative assessment. Intrinsic clearance rate was calculated for each compound using the formula: Clint (μL/min/mg) = 0.693/($t_{1/2}$ X microsomal protein concentration). Data reported are average values from 3 measurements.

Radioligand Displacement Assay for hERG Interaction

Competition binding with [^{3}H]Astemizole was used to measure hERG interaction as has been previously described.[40] Preparations of membranes overexpressing hERG were purchased from PerkinElmer. The binding assays were performed for 60 min using 4 μg hERG expressing membranes, ~3 nM [^{3}H]Astemizole, and various concentrations of the test agent in a pH 7.4 buffer (10 mM HEPES, 130 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 1 mM NaEDTA, 10 mM glucose and 0.1% BSA). Binding was terminated by rapid filtration onto GF/B fiber filter mats (presoaked in 0.3% polyethyleneimine), followed by rapid washing (6x2 mL) with an ice-cold pH 7.4 buffer (25 mM Tris-HCl, 130 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 0.05 mM $CaCl_2$ and 0.1% BSA) using a Brandel harvester. Filters were dried and counted after addition of a scintillant. Data were analyzed using nonlinear regression (GraphPad Prism), and Ki

values. All experiments were performed at least twice in duplicate, and the data reported are the mean values.

Cytochrome P450 Induction Assay

Cryo-HepaRG cells (ThermoFisher Scientific) were used to measure cytochrome P450 induction. On day 1, cells were thawed and plated at 80,000 cells/well (125 μL/well) in a collagen (I) coated 96-well plate in Williams' Medium E supplemented with 1x GlutaMAX and HepaRG670 supplement. Plated cells were initially allowed to rest at rt for 10 min and then placed at 37 °C/5% CO_2 for 6 h, after which medium was removed and replaced with 125 μL of fresh medium. On day 4, compound solutions were prepared in serum-free induction medium (Williams' Medium E supplemented with 1x GlutaMAX and HepaRG650). Test compounds were prepared at 10 μM. Omeprazole (50 μM), phenobarbital (1 mM), and rifampicin (10 μM) were included as control inducers of CYP 1A2, CYP 2B6, and CYP 3A4, respectively. Induction medium containing 1% DMSO was used as the vehicle control. Medium was removed from the HepaRG cells and treatments were added. Cells were returned to 37° C/5% CO_2. After a 24-h induction period, cDNA was prepared from isolated RNA using Invitrogen's TaqMan Fast Advanced Cells-to-CT kit. For gene expression assays, established (validated) TaqMan assays were used for CYP 1A2, CYP 2B6, CYP 3A4, and GAPDH (housekeeping gene). Reactions were prepared according to the Cells-to-CT kit instructions and quantitative PCR was conducted on a LightCycler 96 (Roche). Relative fold gene expression was calculated using the ΔΔCt method.

Pharmacokinetic Testing

Female C57BL6 mice or SD rats were procured from Charles River Laboratories at 8 weeks of age and allowed to acclimate to the facility. Animals were dosed with compounds in a vehicle comprised of 1% NMP and 0.3% Tween 80 in 0.5% sodium carboxymethylcellulose (medium viscosity; deionized water). Animals were sacrificed at multiple time-points (0.5, 1, 2, 4, 8 & 24 h) and samples were removed. Pharmacokinetic analyses were performed as has been described in our previous publications using Phoenix WinNonlin (Certara).[21]

Alcohol Induced Hepatic Steatosis Study

Alcoholic steatosis of the liver was induced using the approach of Lieber and DeCarli.[41] Pathogen-free female C57BL/6J mice, aged 9 weeks (Jackson Laboratories, Bar Harbor, ME, USA) weighing 19-21 g were used in this study. Animals were housed in an "Association for Assessment and Accreditation of Laboratory Animal Care" (AAALAC)-accredited, specific-pathogen-free, environmentally controlled facility. All experimental procedures were conducted in accordance with a protocol approved by the Institutional Animal Care and Use Committee (IACUC). C57BL/6 mice were offered solid PicoLab Certified Rodent LabDiet® 5053 (pellet) *ad libitum* during the acclimation period following which, all animals were switched to a Lieber-DeCarli 82' Control (Catalog No: F1259SP, BioServ®) diet which was nutritionally complete. Control and ethanol (Catalog No: F1258SP, BioServ®) liquid diets were prepared daily as per instructions provided by the manufacturer. Once on the liquid diet for 3 d, ethanol was introduced progressively from 1% to 5% (vol/vol) over 15 d to scheduled animals. The animals were maintained on liquid diet for 4 additional weeks after reaching the final concentration of ethanol and administered the test article for the last two weeks to evaluate its efficacy in limiting/reversing alcoholic steatosis (AS). Compound **38** was formulated as a solution in 0.5% sodium carboxymethylcellulose with 1% N-methyl-2-pyrrolidone (NMP) and 0.3% Tween 80. Compound was administered to mice once daily by oral gavage. Control animals were administered the vehicle alone. Pairwise-feeding within groups was conducted to adjust for caloric intake between groups.

Following the final day of liquid diet/oral dosing administration, animals were euthanized and necropsied to collect tissue samples. The liver was removed and weighed, following which a section of the median lobe of the liver was embedded in optimal cutting temperature solution (OCT, Tissue-Plus®) and preserved in a base mold (24x24x5mm, Fisher brand™) for sectioning to examine degree of steatosis and monitor degree of liver damage and general cytotoxicity.

For histopathology studies, Oil Red O (ORO) staining was performed on harvested liver tissue to assess the degree of liver steatosis in the ethanol and control diet fed mouse groups. OCT embedded liver

sections were stained with ORO and examined under a light microscope at 10X magnification. Digitized images of mouse liver sections (10 μm in thickness) were assessed semi-quantitatively for degree of steatosis using Image J software (NIH, Bethesda, MD). Briefly, a 5 in^2 digital grid (Image J Command Menu: Plugin-Analyze-Grid) was placed on each image wherein lipid droplets in 6 grids per image were counted and the average number of oil droplets was recorded for each animal (n = 6, per group). Results have been reported as means ± SEM.

Author Information

* To whom correspondence should be addressed. Tel: 919-485-2668. Fax: 919-541-8868. Email: rmaitra@rti.org.

Abbreviations Used

BOP, (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; CB1, Cannabinoid Receptor 1; CB2, Cannabinoid Receptor 2; CHO-K1, Chinese Hamster Ovary Cells; CNS, central nervous system; EtOH, ethanol; Ke, apparent affinity constant, MDCK-mdr1, Madin-Darby canine kidney cells transfected with the human MDR1 gene; IP_3, Inositol Phosphatase 3; MeOH, methanol; MRM, Multiple Reaction Monitoring; NaHMDS, Sodium Bis(trimethylsilyl)amide; TEA, triethylamine.

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Ancillary Information

Supporting information availability:

1. Calculated properties of compounds – molecular weights, cLogP and TPSA

2. List of Molecular Formula Strings

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