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Diphenyl Purine Derivatives as Peripherally Selective Cannabinoid Receptor 1 Antagonists

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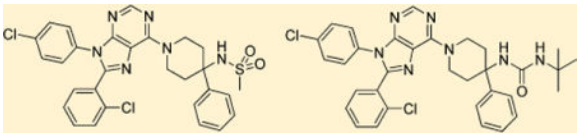
Abstract

Cannabinoid receptor 1 (CB1) antagonists are potentially useful for the treatment of several diseases. However, clinical development of several CB1 antagonists was halted due to central nervous system (CNS)-related side effects including depression and suicidal ideation in some users. Recently, studies have indicated that selective regulation of CB1 receptors in the periphery is a viable strategy for treating several important disorders. Past efforts to develop peripherally selective antagonists of CB1 have largely targeted rimonabant, an inverse agonist of CB1. Reported here are our efforts toward developing a peripherally selective CB1 antagonist based on the otenabant scaffold. Even though otenabant penetrates the CNS, it is unique among CB1 antagonists that have been clinically tested because it has properties that are normally associated with peripherally selective compounds. Our efforts have resulted in an orally absorbed compound that is a potent and selective CB1 antagonist with limited penetration into the CNS.



INTRODUCTION

Cannabinoid receptors belong to the endocannabinoid (EC) system, which consists of receptors, transporters, endocannabinoids, and the enzymes involved in synthesis and degradation of endocannabinoids. To date, two receptors have been identified — CB1 and CB2.[1] Both CB1 and CB2 are G protein-coupled receptors (GPCRs) primarily activating inhibitory G proteins ($G_{i/o}$).[2] Of the two, CB1 is prominently expressed in the central nervous system (CNS).[1] However, it is also expressed peripherally in a number of peripheral tissues. The CB2 receptor is nominally expressed in the brain. However, it is highly expressed in cells of the immune system.[3] The CB1 receptor is also a prominent target of

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Supporting Information

HPLC and melting point data of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Notes

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drugs of abuse including (−)-Δ^9-tetrahydrocannabinol (THC), the main psychoactive constituent of marijuana.[4]

In recent years, CB1 antagonists have received attention in the treatment of disorders that have a central nervous system (CNS)-related craving component, including alcoholism.[5] Further, CB1 is a validated drug target to treat obesity, metabolic syndrome, liver disease, diabetes, and dyslipidemias through both CNS (anorexic) and peripheral (metabolic) effects.[6] Despite the therapeutic promise of CB1 antagonists, this drug class has been limited by adverse effects including anxiety and depression, and the first clinically approved CB1 antagonist (inverse agonist) for weight loss, rimonabant (**1**, SR141716A) (Figure 1), was withdrawn from Europe.[7] Consequently, development or clinical trials of several CB1 antagonists, such as taranabant, otenabant (**2**), and ibipinabant, were halted due to regulatory concerns generated by **1**.[8]

An alternative strategy to target this receptor is to develop antagonists that are peripherally restricted by virtue of not being able to cross the blood–brain barrier (BBB) and thus avoiding CNS-mediated adverse effects noted with nontissue selective compounds. A similar strategy has been successful in the development of peripherally selective opioids.[9] Several groups are currently pursuing this strategy.[10] A few peripherally selective CB1 antagonists have been reported (Figure 2), and their further validation and characterization are underway. It should be noted almost all reported efforts (e.g., **3**–**7**) at designing peripherally restricted antagonists of CB1 to date have focused on compounds that closely resemble **1**.

Our group has been involved in the development of peripherally selective CB1 antagonists based on amide variations of compound **1** using two different approaches. First, charged compounds were produced based on the observation that such compounds cross the BBB only when transported by a specific transporter.[11] However, the compounds synthesized to date using this approach have low potency as reported in our previous publication.[11b] The second strategy involved generation of compounds with high topological polar surface areas (TPSAs). Past studies indicate that compounds with high TPSA, usually >100, have lower permeability into the CNS.[12] This strategy was more successful and led to the identification of **7** that we have previously reported.[13] While this compound was promising, having a brain to plasma ratio of less than 4% in a simple pharmacokinetic experiment, oral absorption of this compound was less than optimal.[13]

In this paper, we report our efforts toward the synthesis and characterization of peripherally selective CB1 antagonists based on **2**. This compound is a highly selective CB1 receptor antagonist developed by Pfizer that was later abandoned due to CNS-related adverse effects during phase 3 clinical trials. This compound is unique among CB1 antagonists that have been clinically tested because it has properties that would be normally associated with a peripherally selective compound, including a TPSA of 102, three hydrogen bond donors, and formula weight >500.[14] However, **2** is CNS permeable. It was suggested that the intramolecular H-bonding observed in the X-ray structure between the primary amide and the ethyl amine portion of the molecule effectively lowered the polarity of this compound, allowing for penetration into the CNS.[14] Thus upon examination, this scaffold appeared to

be more favorable for peripheralization as synthesis of compounds based on 2 but incapable of forming intramolecular H-bonding would be expected to be peripherally selective. This innovative approach to ligand design has now provided us with several promising compounds for further refinement.

RESULTS

Approaches to Ligand Design and Testing

Synthesis, purification, and characterization of compounds are described in the Experimental Section. Antagonism of each compound toward CB1 was quantitatively tested using an *in vitro* calcium mobilization assay for CB1 as has been previously described,[13,15] and this antagonism is expressed as an apparent dissociation affinity constant (K_e). Compounds that demonstrated K_e < 100 nM were further characterized using competitive radioligand displacement of [^{3}H]CP55940 at CB1 and CB2 overexpressing membrane preparations as described previously.[13] Displacement data were quantified and expressed as an equilibrium dissociation constant (K_i) value at each receptor. Potent and selective compounds were progressed into an *in vitro* model of brain penetration in monolayers of MDCK-mdr1cells as described previously.[11b,13] Certain compounds were also characterized for metabolic stability in human hepatic microsomal fractions (S9). Finally, *in vivo* testing was conducted in Sprague–Dawley (SD) rats to measure CNS permeability of these compounds.

Compound Development Strategy and Results

During our pursuit of peripherally selective CB1 antagonists, 8 (Figure 3) was synthesized and found to be highly potent, selective, and to have relatively low permeability in the MDCK-mdr1 model.[13] These results along with our interest in CB1 antagonists that possess a purine core, like **2**, moved us to make purine **9**. Purine **9** is a potent (K_e = 9 nM) and selective (CB2/CB1 selectivity ratio ∼ 3077) hybrid of **2** and **8** with a TPSA of 90. Despite having a TPSA that is lower than that of **2**, **9** cannot form intramolecular hydrogen bonds, and therefore, was predicted to be more peripherally selective than **2**. This hypothesis was tested and confirmed (Table 1) using the MDCK-mdr1 permeability assay,[13] where **9** was only 6% permeable (A–B), while **2** was ∼40% permeable (apical to basal, A–B) in the same assay. These positive results warranted further SAR exploration around **9**.

To further improve upon **9**, the amide was changed to more polar functionalities such as sulfonamide and urea (Figure 4, Table 2). Carbamate **10** and amine **11** were first synthesized, the latter of which also serves as a common synthetic intermediate for other analogues. Interestingly, carbamate **10** was very potent (K_e = 3.9 nM), selective (CB2/CB1 = 1959), and poorly permeable in the MDCK-mdr1 model (A to B <1%). Similarly, amine **11** was also very potent (K_e = 15.9 nM), selective (CB2:CB1 = 151), and poorly permeable in the MDCK-mdr1 model (A to B <1%). With amine **11** in hand, sulfonamide **12** and a series of ureas **13a–g** were synthesized. Sulfonamide **12** was especially interesting because it has high potency (K_e = 2.9 nM), selectivity (CB2/CB1 = 153), poor permeability in the MDCK-mdr1 model (A to B is <1%), and a high TPSA (101), similar to **2** (TPSA = 102); it also lacks the ability to hydrogen bond in an intramolecular fashion. Two members of

structural series **13** were found to have interesting pharmacological profiles. Urea **13a** was potent (K_e = 19.2 nM), selective (CB2/CB1 = 20000), and poorly permeable in the MDCK-mdr1assay (A to B is <1%). Urea **13e** was also found to be potent (K_e = 62 nM) and selective (CB2/CB1 = 74) as well.

The positive result observed with compounds **10–13g** led to further exploration of the SAR around purines (Figure 5). The 4-amino-4-phenylpiperidine linker of compounds **10–13g** was replaced with 4- and 3-aminopiperidine linkers to test the importance of the juxtaposition of the terminal polar groups of compounds **10–13g**. These changes also had the benefit of reducing the molecular weight of analogues that were synthesized by removing the phenyl group present in **10–13g**. Structural series **14**, **15**, **16**, and **17** were all made and evaluated pharmacologically. The data (Table 3) for these series show that the 4-aminopiperidine linker of structural series **17** was superior to the linkers in structural series **14**, **15**, and **16** for all direct comparisons of the R group. However, in almost every case the 4-amino-4-phenylpiperidine linker of **10–13g** was more potent than its direct analogues in structural series **14–17**.

However, several interesting compounds were identified in structural series **14–17**. The *tert*-butoxycarbonyl (BOC) analogue **14a** was potent (K_e = 92 nM) and selective (CB2/CB1 = 568). In general, compounds from structural series **15** and **16** were weakly active against CB1 in the calcium mobilization assay and the compounds were not characterized further. Several analogues in structural series **17** were very interesting. The BOC analogue **17a** was very potent (K_e = 11 nM) and selective (CB2/CB1 = 1142). The urea analogues **17c–f** all showed good activity (K_e ranging from 49 to 149 nM). Selectivity (CB2/CB1) for ureas **17c–f** ranged from 5 to 175 fold. Amides and sulfonamides **17g–k** were generally active with 3 of the 5 analogues having Ke < 10 nM. One of these compounds, 17k, was found to be highly selective (CB2/CB1 = 4888).

In Vitro Metabolic Stability and In Vivo Evaluation of Brain Penetration

A small set of potent and selective compounds were progressed into *in vitro* models of metabolic stability (Table 4). Stability in human plasma and human hepatic S9 fractions was measured to gauge potential metabolic liabilities of these compounds. All compounds were stable in plasma up to 1 h. The stabilities in S9 fractions for these compounds were more variable. All compounds displayed acceptable metabolic stabilities; however, **13a** did show significant metabolism in S9 fraction and was deemed unsuitable for further development.

Several compounds showed excellent *in vitro* properties, including compounds **17a–k**. Because of limited resources only sulfonamide **12** was advanced into an *in vivo* experiment to evaluate its brain penetration in SD rats upon oral dosing. Future studies are planned with additional compounds. Sulfonamide **12** was dosed orally in SD rats at 10 mg/kg, and both plasma and brain levels were analyzed out to 8 h post dose (Table 5). The compound showed slow absorption and did not reach a maximum concentration until 240 min post dose. Sulfonamide **12** also showed diminished brain penetration with the brain to plasma ratio ranging from 0.05 to 0.11. It should be noted that unperfused brain tissues, which are expected to contain 2–4% blood, were examined.[16] Thus, it is concluded that **12** has limited

brain penetration. Furthermore, **12** demonstrated excellent oral absorption and favorable clearance upon oral dosing in rats. Thus, this compound may be suitable for further development.

Synthesis

Compound **9** (Scheme 1) was prepared from commercially available piperidine **19** and the readily available chloro-purine **18**.[14] Chloro-purine **18**, piperidine **19**, and triethylamine were heated in ethanol at 55 °C for 3 days yielding **9** in 63% yield. In a similar manner chloro-purine **18** was reacted with piperidine **20** and triethylamine in ethanol at 80 °C for 16 h to yield **10** in 84% yield (Scheme 2). The BOC group of **10** was removed using 20% trifluoroacetic acid (TFA) in dichloromethane to yield the primary amine **11** in 99%. Sulfonamide **12** was prepared by reacting **11**, methanesulfonyl chloride, and triethylamine in tetrahydrofuran (THF) in 82% yield. The ureas **13a–g** were made by reacting amine **11**, triethylamine, and the appropriate isocyanate in THF in yields ranging from 69 to 92%.

Chloro-purine **18** was converted to **14a**, **15a**, and **16a** in yields ranging from 86 to 95% by reaction with the appropriate amine and triethylamine in ethanol at 80 °C for 16 h (Scheme 3). The BOC groups were removed from **14a**, **15a**, and **16a** to form amines **14b**, **15b**, and **16b** using 30% TFA in dichloromethane in yields ranging from 65 to 88%. Amines **14b**, **15b**, and **16b** were reacted with the appropriate isocyanate and triethylamine in THF to generate ureas **14c–f**, **15c**, and **16c** in yields ranging from 65 to 95%. Amines **15b** and **16b** were reacted with acetic anhydride in pyridine to yield amides **15d** and **16d** in 87% and 81% respectively. Sulfonamides **14g**, **15e**, and **16e** were made from **14b**, **15b**, and **16b** by reaction with methanesulfonyl chloride and triethylamine in THF in yields ranging from 51 to 91%.

Chloro-purine **18** and piperidine **21** were heated in ethanol at 80 °C for 16 h yielding **17a** in 99% yield (Scheme 4). The remaining chemistry, to generate **17b–k**, was performed in a similar manner to reactions discussed above.

DISCUSSION

CB1 receptor antagonism is a validated strategy to treat several important disorders including diabetes, obesity, metabolic syndromes, liver diseases, and dyslipidemias. However, non-tissue selective antagonists of this receptor are not clinically useful due to adverse CNS effects. CB1 receptors are also expressed in peripheral organs and targeting of these peripheral receptors has produced promising data in experimental models of important diseases. Thus, several groups are trying to develop peripherally selective antagonists of CB1 for therapeutic use. While some progress has been made in this direction, currently disclosed CB1 antagonists that are peripherally selective need further improvement and validation prior to clinical use. Furthermore, most of these compounds are closely related to **1**. In this report, we disclose our efforts to develop peripherally selective antagonists of CB1 based on **2**, which we believe is a promising scaffold for optimization. This is because **2** has several chemical properties that would indicate poor brain penetration. However, intramolecular hydrogen bonding may effectively lower the polarity of **2** and this has been suggested by Griffith and co-workers.[14] Thus, we hypothesized that optimized design and

synthesis of compounds based on **2** that do not have the ability to form intramolecular hydrogen bonds could lead to CB1 antagonists with limited brain penetration.

Several potent and selective CB1 antagonists have been reported in this paper including nine compounds that have K_e < 100 nM and selectivity >100-fold versus CB2. Potency at CB1 has been obtained with a variety of functional groups on the terminus of the aminopiperidine in structures **10–17k**. Carbamates, amines, sulfonamides, ureas, and amides have all been well tolerated at this position. The 4-amino-4-phenylpiperidine linker present in compounds **10–13g** was the most potent linker noted in this study. 4-Aminopiperidine derivatives were more potent that 3-aminopiperidine analogues. However, of the 1–3 aminopiperidine compounds in structural series **15** and **16**, the R enantiomers (**15**) were generally more potent at CB1 (four out of five examples) than the S enantiomers (**16**) in head to head comparisons. Of the 4-aminopiperidine linker compounds, lacking a phenyl group on the piperidine ring, in structural series **14** and **17**, the analogues where the piperidine nitrogen was bonded to the purine core (structural series **17**) were favored over the isomers where the 4-amino group was bonded to the core (structural series **14**). Compounds with branched terminal R groups were found to be more selective for CB1 over CB2 than compounds with less sterically demanding R groups. This trend was especially prevalent in ureas **13a–d** and **17c–f**. In both of these series, **13a–d** and **17c–f**, the compounds with the most branched R group have the highest selectivity ratio for CB2/CB1. This selectivity is achieved not by gains in CB1 binding affinity, but by lowering the binding affinity for CB2. While **2** is an inverse agonist at CB1, it is currently not known if the compounds described in this publication act as inverse agonist or neutral antagonists at the present time. Those studies will be performed in the future.

The removal of the intramolecular hydrogen bond found in 2 allowed us to synthesize compounds with significantly lower permeability in the MDCK-mdr1 assay. This can be demonstrated by comparing **2** and **12**. While **2** and **12** have almost identical TPSAs (102 vs 101), **12** which lacks the ability to hydrogen bond in an intramolecular fashion has vastly reduced permeability in the MDCK-mdr1 assay. Thus, confirming Griffith and co-worker's hypothesis.[14]

Compounds **9**, **12**, and **13a** were identified for further evaluation. All of these compounds showed reasonable stability to human plasma. Both **9** and **12** had good stability in human S9 fraction with over 90% of compound remaining after 120 min of incubation. The stability of **13a** to S9 fraction was acceptable, but it did show significant metabolism in the assay. Compound **12** was advanced into *in vivo* models to assess its brain penetration. In a rat PK assay, **12** showed brain to plasma ratios ranging from 0.05 to 0.11, indicating limited brain penetration. Generally, compounds that have <10% brain penetration are considered peripherally selective. This is also a significant reduction in brain penetration in comparison to **2** in SD rats, which has ∼33% brain exposure at 2–3 h postdose.[17]

In conclusion, through our studies, we have discovered novel analogues of **2** with vastly reduced brain penetration. Of the compounds reported, **12** had excellent potency, selectivity, and oral absorption in rats. This compound has low levels of brain penetration as well. This compound will be evaluated in proof of concept studies for diseases such as obesity, liver

fibroses, and diabetes in the future. Furthermore, sulfonamide **12** will also serve as a starting point for refinement to further lower the brain penetration of future compounds, if necessary. However, any such endeavor must be dictated by the proposed use, and an acceptable balance between therapeutic benefits and undesirable effects must be achieved. For example, antiobesity drugs should have a very favorable profile as far as adverse effects are concerned. By contrast, in other diseases such as liver disease or diabetes, a more permissive profile of adverse effects could be tolerated.

EXPERIMENTAL SECTION

Compound Synthesis and Characterization

Chemistry General—Purity and characterization of compounds were established by a combination of HPLC, TLC, and NMR analytical techniques described below. ^{1}H and ^{13}CNMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz) spectrometer and were determined in $CHCl_3$-*d* or MeOH-d_4 with tetramethylsilane (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). Thin-layer chromatography (TLC) was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light or I_2 detection. Low-resolution mass spectra were obtained using a Waters Alliance HT/Micromass ZQ system (ESI). All test compounds were greater than 95% pure as determined by HPLC on an Agilent 1100 system using an Agilent Zorbax SB-Phenyl, 2.1 × 150 mm, 5 μm column with gradient elution using the mobile phases (A) H_2O containing 0.05% CF_3COOH and (B) methanol. A flow rate of 1.0 mL/min was used. All compounds were isolated as white or off-white solids.

1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phe-nylpiperidine-4-carboxamide (9)—To a solution of 6-chloro-8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purine (**9**) (23.1 mg, 0.062 mmol, 1 equiv) in 2 mL of ethanol was added 4-carbamoyl-4-phenylpiperidin-1-ium trifluoroacetate (**19**) (25 mg, 0.123 mmol, 2 equiv) and triethylamine (0.03 mL, 0.19 mmol, 3 equiv). The reaction was heated to 55 °C for 3 days. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 21 mg (63%) of desired product. ^{1}H NMR (300 MHz, methanol-d_4) δ ppm 2.02–2.21 (m, 2 H) 2.62 (d, J = 13.37 Hz, 2 H) 3.82 (br. s., 2 H) 4.98 (d, J = 15.54 Hz, 2 H) 7.07–7.67 (m, 13 H) 8.02–8.29 (m, 1 H), $[M + H]^+$ 543.6.

tert-Butyl N-{1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}carbamate (10)—To a solution of **18** (50 mg, 0.133 mmol, 1 equiv) in 2 mL of ethanol was added tert-butyl N-(4-phenylpiperidin-4-yl)carbamate (**20**) (44 mg, 0.16 mmol, 1.2 equiv) and triethylamine (0.03 mL, 0.20 mmol, 1.5 equiv). The reaction was heated to 80 °C for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 69 mg (84%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.31–1.49 (m, 9 H) 2.09–2.28 (m, 2 H) 2.43 (br. s., 2 H) 3.62 (br. s., 2 H) 4.96 (s, 1 H) 5.30 (br. s., 2 H) 7.03–7.61 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 615.4.

1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phe-nylpiperidin-4-amine (11)—A solution of **10** (2.65 g, 4.3 mmol) was stirred in dichloromethane (32 mL) and trifluoroacetic acid (8 mL) for 1.5 h. The reaction was concentrated *in vacuo*. The crude product was dissolved in ethyl acetate and washed with 3.8 M NaOH. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with brine and dried with $MgSO_4$ to yield 2.21 g (99%) of pure desired product. 1H NMR (300 MHz, chloroform-*d*) δ ppm 1.87 (d, J = 13.56 Hz, 2 H) 2.15–2.39 (m, 2 H) 3.82–4.11 (m, 2 H) 5.10 (br. s., 2 H) 7.11–7.62 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 515.8.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}methanesulfonamide (12)—To a solution of **11** (8.4 mg, 0.016 mmol, 1 equiv) in 2 mL of THF was added methanesulfonyl chloride (0.01 mL) and triethylamine (0.02 mL). The mixture was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 8 mg (82%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.22 (s, 3 H) 2.30–2.44 (m, 2 H) 2.47–2.66 (m, 2 H) 4.20 (br. s., 2 H) 4.75 (s, 2 H) 7.08–7.64 (m, 13 H) 8.38 (s, 1 H), $[M + H]^+$ 593.3.

General Procedure for Making Ureas from 1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-amine (11)—To a solution of **11** (19 mg, 0.036 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.015 mL, 0.108 mmol, 3 equiv) and the appropriate isocyanate (1.5 equiv). The reaction was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield pure compound.

3-tert-Butyl-1-{1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}urea (13a)—Reaction proceeded in 81% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.12 (s, 9 H) 2.13–2.27 (m, 2 H) 2.27–2.43 (m, 2 H) 3.67 (br. s., 2 H) 3.96 (br. s., 1 H) 4.78 (br. s., 1 H) 5.10–5.68 (m, 2 H) 7.07–7.63 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 614.7.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}-3-ethylurea (13b)—Reaction proceeded in 81% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.85–1.01 (m, 3 H) 2.11–2.29 (m, 2 H) 2.30–2.47 (m, 2 H) 2.96–3.20 (m, 2 H) 3.64 (br. s., 2 H) 4.10–4.24 (m, 1 H) 5.00 (s, 1 H) 5.32 (d, J = 14.41 Hz, 2 H) 7.10–7.60 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 586.8.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}-3-(propan-2-yl)urea (13c)—Reaction proceeded in 79% yield. ^{1}H NMR (300 MHz, chloroform-d) δ ppm 0.94 (d, J = 6.50 Hz, 6 H) 2.13–2.29 (m, 2 H) 2.29–2.42 (m, 2 H) 3.51–3.76 (m, 2 H) 3.95 (d, J = 7.82 Hz, 1 H) 4.93 (s, 1 H) 5.32 (d, J = 13.56 Hz, 2 H) 7.07–7.63 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 600.7.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}-3-propylurea (13d)—Reaction proceeded in 88% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.64–0.77 (m, 3 H) 1.19–1.37 (m, 2 H) 2.10–2.27 (m, 2 H) 2.29–2.45

(m, 2 H) 2.87–3.08 (m, 2 H) 3.63 (br. s., 2 H) 4.28 (br. s., 1 H) 5.11 (s, 1 H) 5.36 (br. s., 2 H) 7.10–7.60 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 600.5.

3-Butyl-1-{1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}urea (13e)—Reaction proceeded in 82% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.74–0.86 (m, 3 H) 1.11 (dq, *J* = 14.79, 7.22 Hz, 2 H) 1.20–1.31 (m, 2 H) 2.11 – 2.27 (m, 2 H) 2.29–2.41 (m, 2 H) 3.05 (q, *J* = 6.59 Hz, 2 H) 3.63 (br. s., 2 H) 4.22 (t, *J* = 5.13 Hz, 1 H) 5.07 (s, 1 H) 5.34 (br. s., 2 H) 7.12–7.56 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 614.7.

Ethyl 2-[({1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}carbamoyl)amino]acetate (13f)—Reaction proceeded in 92% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.18–1.28 (m, 3 H) 2.11–2.29 (m, 2 H) 2.33–2.52 (m, 2 H) 3.64 (br. s., 2 H) 3.82–3.92 (m, 2 H) 4.09–4.24 (m, 2 H) 5.13 (br. s., 1 H) 5.23–5.52 (m, 2 H) 5.60 (s, 1 H) 7.05–7.59 (m, 13 H) 8.39 (s, 1 H), $[M + H]^+$ 644.4.

3-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-4-phenylpiperidin-4-yl}-1-cyclohexylurea (13g)—Reaction proceeded in 69% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.77–1.13 (m, 3 H) 1.15–1.36 (m, 3 H) 1.36–1.54 (m, 3 H) 1.56–1.76 (m, 1 H) 2.12–2.28 (m, 2 H) 2.29–2.42 (m, 2 H) 3.34–3.55 (m, 1 H) 3.65 (br. s., 2 H) 3.96–4.20 (m, 1 H) 4.96 (s, 1 H) 5.34 (br. s., 2 H) 7.06–7.61 (m, 13 H) 8.40 (s, 1 H), $[M + H]^+$ 640.5.

tert-Butyl 4-{[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}piperidine-1-carboxylate (14a)—To a solution of **18** (151 mg, 0.403 mmol, 1 equiv) in 4 mL of ethanol was added *tert*-butyl 4-aminopiperidine-1-carboxylate (96 mg, 0.483 mmol, 1.2 equiv) and triethylamine (0.08 mL, 0.6 mmol, 1.5 equiv). The reaction was heated to 80 °C for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 203 mg (94%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.38–1.55 (m, 11 H) 2.14 (d, *J* = 12.34 Hz, 2 H) 2.98 (t, *J* = 12.10 Hz, 2 H) 4.00–4.21 (m, 2 H) 4.28–4.52 (m, 1 H) 5.83 (d, *J* = 7.63 Hz, 1 H) 7.17–7.27 (m, 2 H) 7.31–7.44 (m, 5 H) 7.50 (d, J = 6.88 Hz, 1 H) 8.44 (s, 1 H), $[M + H]^+$ 539.4.

8-(2-Chlorophenyl)-9-(4-chlorophenyl)-N-(piperidin-4-yl)-9H-purin-6-amine (14b)—A solution of **14a** (180 mg, 0.33 mmol) was stirred in dichloromethane (9 mL) and trifluoroacetic acid (4 mL) for 16 h. The reaction was concentrated *in vacuo*. The crude product was dissolved in ethyl acetate and washed with saturated $NaHCO_3$. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with brine and dried with $MgSO_4$. The crude material was purified by silica gel column chromatography using 0–100% CMA 80 (80% chloroform, 18% methanol, and 2% ammonium hydroxide)/ethyl acetate to yield 129 mg (88%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.45 (qd, *J* = 11.66, 3.81 Hz, 2 H) 2.10 (d, *J* = 11.87 Hz, 2 H) 2.64–2.88 (m, 2 H) 3.10 (d, *J* = 12.62 Hz, 2 H) 4.27 (br. s., 1 H) 5.80 (d, *J* = 6.31 Hz, 1 H) 7.01–7.55 (m, 8 H) 8.37 (s, 1 H), $[M + H]^+$ 439.6.

General Procedure for Making Ureas from 8-(2-chlorophenyl)-9-(4-chlorophenyl)-N-(piperidin-4-yl)-9H-purin-6-amine (14b)—To a solution of **14b** (18.4 mg, 0.042 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.02 mL, 0.126 mmol, 3 equiv) and the appropriate isocyanate (1.5 equiv). The reaction is stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% CMA 80/ethyl acetate to yield pure compound.

4-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}-N-ethylpiperidine-1-carboxamide (14c)—Reaction proceeded in 86% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.09 (t, *J* = 7.21 Hz, 3 H) 1.34–1.60 (m, 2 H) 2.11 (d, *J* = 12.34 Hz, 2 H) 2.83–3.06 (m, 2 H) 3.13–3.32 (m, 2 H) 3.92 (d, *J* = 13.37 Hz, 2 H) 4.40 (d, *J* = 4.90 Hz, 2 H) 5.80 (d, *J* = 7.82 Hz, 1 H) 7.04–7.51 (m, 8 H) 8.37 (s, 1 H), $[M + H]^+$ 510.4.

4-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}-N-(propan-2-yl)piperidine-1-carboxamide (14d)—Reaction proceeded in 91% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.18 (d, *J* = 6.50 Hz, 6 H) 1.46–1.68 (m, 2 H) 2.19 (d, *J* = 10.55 Hz, 2 H) 3.03 (t, *J* = 11.59 Hz, 2 H) 3.89–4.10 (m, 3 H) 4.29 (d, *J* = 7.16 Hz, 1 H) 4.42 (br. s., 1 H) 5.88 (d, *J* = 7.82 Hz, 1 H) 7.13–7.64 (m, 8 H) 8.45 (s, 1 H), $[M + H]^+$ 524.7.

4-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}-N-propylpiperidine-1-carboxamide (14e)—Reaction proceeded in 95% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.86–1.01 (m, 3 H) 1.44–1.67 (m, 4 H) 2.19 (d, *J* = 10.64 Hz, 2 H) 3.05 (t, *J* = 11.73 Hz, 2 H) 3.22 (q, *J* = 6.59 Hz, 2 H) 3.92–4.08 (m, 2 H) 4.43 (br. s., 1 H) 4.54 (t, *J* = 5.04 Hz, 1 H) 5.88 (d, *J* = 7.72 Hz, 1 H) 7.09–7.58 (m, 8 H) 8.45 (s, 1 H), $[M + H]^+$ 524.8.

N-Butyl-4-{[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}piperidine-1-carboxamide (14f)—Reaction proceeded in 91% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.79–0.92 (m, 3 H) 1.29 (dq, *J* = 14.75, 7.20 Hz, 2 H) 1.36–1.58 (m, 4 H) 2.11 (d, *J* = 10.64 Hz, 2 H) 2.96 (t, *J* = 11.73 Hz, 2 H) 3.17 (q, *J* = 6.75 Hz, 2 H) 3.81–4.01 (m, 2 H) 4.22–4.51 (m, 2 H) 5.79 (d, *J* = 7.54 Hz, 1 H) 7.01–7.53 (m, 8 H) 8.37 (s, 1 H), $[M + H]^+$ 538.4.

8-(2-Chlorophenyl)-9-(4-chlorophenyl)-N-(1-methanesulfonylpiperidin-4-yl)-9H-purin-6-amine (14g)—To a solution of **14b** (18.4 mg, 0.042 mmol, 1 equiv) in 2 mL of THF was added methanesulfonyl chloride (0.005 mL, 0.063 mmol, 1.5 equiv) and triethylamine (0.02 mL, 0.126 mmol, 3 equiv). The mixture was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 20 mg (91%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.58–1.76 (m, 2 H) 2.21 (d, *J* = 10.64 Hz, 2 H) 2.76 (s, 3 H) 2.90 (t, *J* = 10.93 Hz, 2 H) 3.77 (d, *J* = 12.15 Hz, 2 H) 4.33 (br. s., 1 H) 5.84 (br. s., 1 H) 7.04–7.51 (m, 8 H) 8.36 (s, 1 H), $[M - H]^-$ 515.7.

tert-Butyl (3R)-3-{[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}piperidine-1-carboxylate (15a)—To a solution of **18** (100 mg, 0.267 mmol, 1 equiv) in 3 mL of ethanol was added *tert*-butyl (3R)-3-aminopiperidine-1-carboxylate (64

mg, 0.32 mmol, 1.2 equiv) and triethylamine (0.06 mL, 0.4 mmol, 1.5 equiv). The reaction was heated to 80 °C for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 124 mg (86%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.33 (br. s., 9 H) 1.49–1.67 (m, 2 H) 1.74 (d, *J* = 6.97 Hz, 1 H) 2.00 (d, *J* = 2.83 Hz, 1 H) 3.20 (br. s., 2 H) 3.50 (br. s., 1 H) 3.88 (br. s., 1 H) 4.32 (br. s., 1 H) 5.87 (d, *J* = 7.44 Hz, 1 H) 7.02–7.54 (m, 8 H) 8.38 (s, 1 H), $[M + H]^+$ 539.3.

8-(2-Chlorophenyl)-9-(4-chlorophenyl)-N-[(3R)-piperidin-3-yl]-9H-purin-6-amine (15b)—A solution of *tert*-butyl **15a** (109 mg, 0.202 mmol) was stirred in dichloromethane (7 mL) and trifluoroacetic acid (3 mL) for 16 h. The reaction was concentrated *in vacuo*. The crude product was dissolved in ethyl acetate and washed with saturated $NaHCO_3$. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with brine and dried with $MgSO_4$. The crude material was purified by silica gel column chromatography using 0–100% CMA 80/ethyl acetate to yield 70 mg (79%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.43–1.82 (m, 4 H) 1.91–2.03 (m, 1 H) 2.67 (dt, *J* = 11.68, 7.16 Hz, 2 H) 2.76–2.94 (m, 1 H) 3.21 (dd, *J* = 11.82, 2.87 Hz, 1 H) 4.28 (br. s., 1 H) 6.11 (d, *J* = 6.12 Hz, 1 H) 6.98–7.57 (m, 8 H) 8.36 (s, 1 H), $[M + H]^+$ 439.6.

(3R)-3-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-amino}-N-ethylpiperidine-1-carboxamide (15c)—To a solution of **15b** (20 mg, 0.046 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.02 mL, 0.137 mmol, 3 equiv) and ethyl isocyanate (0.005 mL, 0.068 mmol, 1.5 equiv). The reaction is stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 15 mg (65%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.03–1.34 (m, 3 H) 1.56–1.96 (m, 4 H) 2.18 (br. s., 1 H) 3.07 (br. s., 2 H) 3.32 (br. s., 1 H) 3.73–4.42 (m, 3 H) 5.05 (br. s., 1 H) 6.02 (d, *J* = 5.84 Hz, 1 H) 7.09–7.63 (m, 8 H) 8.45 (br. s., 1 H), $[M + H]^+$ 510.3.

1-[(3R)-3-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-amino}piperidin-1-yl]ethan-1-one (15d)—A solution of 15b (20 mg, 0.046 mmol, 1 equiv) in 1 mL of acetic anhydride and 1 mL of pyridine was stirred for 16 h. The reaction was concentrated in vacuo. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 19 mg (87%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.53–1.92 (m, 3 H) 2.07 (br. s., 4 H) 2.84–3.37 (m, 2 H) 3.86–4.59 (m, 3 H) 5.88 (br. s., 1 H) 7.04–7.60 (m, 8 H) 8.37 (br. s., 1 H), $[M + H]^+$ 481.3.

8-(2-Chlorophenyl)-9-(4-chlorophenyl)-N-[(3R)-1-methanesulfonylpiperidin-3-yl]-9H-purin-6-amine (15e)—To a solution of **15b** (20 mg, 0.046 mmol, 1eq.) in 2 mL of dichloromethane was added methanesulfonyl chloride (0.007 mL, 0.091 mmol, 2 equiv) and triethylamine (0.02 mL, 0.137 mmol, 3 equiv). The mixture was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 12 mg (51%) of desired

product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.81–2.18 (m, 4 H) 2.77–2.94 (m, 3 H) 3.07–3.29 (m, 2 H) 3.42 (br. s., 1 H) 3.82 (d, *J* = 9.89 Hz, 1 H) 4.68 (br. s., 1 H) 6.13 (br. s., 1 H) 7.12–7.65 (m, 8 H) 8.48 (s, 1 H), $[M + H]^+$ 517.8.

tert-Butyl (3S)-3-{[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]amino}piperidine-1-carboxylate (16a)—To a solution of 18 (100 mg, 0.267 mmol, 1 equiv) in 3 mL of ethanol was added *tert*-butyl (3S)-3-aminopiperidine-1-carboxylate (64 mg, 0.32 mmol, 1.2 equiv) and triethylamine (0.06 mL, 0.4 mmol, 1.5 equiv). The reaction was heated to 80 °C for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 137 mg (95%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.43 (br. s., 9 H) 1.60–1.76 (m, 2 H) 1.77–1.93 (m, 1 H) 2.08–2.22 (m, 1 H) 3.30 (br. s., 2 H) 3.60 (br. s., 1 H) 3.98 (br. s., 1 H) 4.42 (br. s., 1 H) 5.96 (d, *J* = 7.54 Hz, 1 H) 7.14–7.68 (m, 8 H) 8.48 (s, 1 H), $[M + H]^+$ 539.4.

8-(2-Chlorophenyl)-9-(4-chlorophenyl)-N-[(3S)-piperidin-3-yl]-9H-purin-6-amine (16b)—A solution of **16a** (120 mg, 0.22 mmol) was stirred in dichloromethane (7 mL) and trifluoroacetic acid (3 mL) for 16 h. The reaction was concentrated *in vacuo*. The crude product was dissolved in ethyl acetate and washed with saturated $NaHCO_3$. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with brine and dried with $MgSO_4$. The crude material was purified by silica gel column chromatography using 0–100% CMA 80/ethyl acetate to yield 64 mg (65%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.55–1.86 (m, 4 H) 2.01–2.14 (m, 1 H) 2.66–2.85 (m, 2 H) 2.87–3.04 (m, 1 H) 3.32 (dd, *J* = 11.77, 2.35 Hz, 1 H) 4.39 (br. s., 1 H) 6.20 (d, *J* = 5.84 Hz, 1 H) 7.12–7.66 (m, 8 H) 8.47 (s, 1 H), $[M + H]^+$ 439.6.

(3S)-3-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-amino}-N-ethylpiperidine-1-carboxamide (16c)—To a solution of **16b** (16 mg, 0.036 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.015 mL, 0.109 mmol, 3 equiv) and ethyl isocyanate (0.004 mL, 0.055 mmol, 1.5 equiv). The reaction is stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 16 mg (86%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.06–1.34 (m, 3 H) 1.53–1.95 (m, 4 H) 2.12–2.29 (m, 1 H) 3.07 (br. s., 2 H) 3.32 (br. s., 1 H) 3.72–4.41 (m, 3 H) 5.06 (br. s., 1 H) 6.02 (d, *J* = 6.12 Hz, 1 H) 7.08–7.66 (m, 8 H) 8.45 (br. s., 1 H), $[M + H]^+$ 510.3.

1-[(3S)-3-{[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-amino}piperidin-1-yl]ethan-1-one (16d)—A solution of **16b** (15.7 mg, 0.036 mmol, 1 equiv) in 1 mL of acetic anhydride and 1 mL of pyridine was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 14 mg (81%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.48–1.91 (m, 3 H) 2.07 (s, 4 H) 2.90–3.39 (m, 2 H) 3.96–4.57 (m, 3 H) 5.83 (d, *J* = 6.50 Hz, 1 H) 7.04–7.56 (m, 8 H) 8.37 (s, 1 H), $[M + H]^+$ 481.3.

8-(2-Chlorophenyl)-9-(4-chlorophenyl)-N-[(3S)-1-methanesulfonylpiperidin-3-yl]-9H-purin-6-amine (16e)—To a solution of **16b** (15.3 mg, 0.035 mmol, 1 equiv) in 2 mL of THF was added methanesulfonyl chloride (0.005 mL, 0.07 mmol, 2 equiv) and triethylamine (0.015 mL, 0.11 mmol, 3 equiv). The mixture was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 16 mg (89%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.63–2.02 (m, 4 H) 2.77 (s, 3 H) 2.98–3.19 (m, 2 H) 3.31 (br. s., 1 H) 3.71 (d, *J* = 10.55 Hz, 1 H) 4.56 (br. s., 1 H) 6.01 (d, *J* = 7.54 Hz, 1 H) 6.97–7.56 (m, 8 H) 8.37 (s, 1 H), $[M + H]^+$ 517.4.

tert-Butyl N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}carbamate (17a)—To a solution of **18** (309 mg, 0.824 mmol, 1 equiv) in 10 mL of ethanol was added *tert*-butyl N-(piperidin-4-yl)carbamate (21) (329 mg, 1.65 mmol, 2 equiv). The reaction was heated to 80 °C for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexanes to yield 442 mg (99%) of desired product. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.40–1.53 (m, 11 H) 2.12 (d, *J* = 11.40 Hz, 2 H) 3.32 (t, *J* = 11.63 Hz, 2 H) 3.80 (br. s., 1 H) 4.41–4.68 (m, 1 H) 5.40 (br. s., 2 H) 7.16–7.24 (m, 2 H) 7.26–7.43 (m, 5 H) 7.51 (d, *J* = 6.59 Hz, 1 H) 8.38 (s, 1 H), $[M + H]^+$ 539.2.

1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-amine (17b)—A solution of **17a** (380 mg, 0.705 mmol) was stirred in dichloromethane (7 mL) and trifluoroacetic acid (3 mL) for 16 h. The reaction was concentrated *in vacuo*. The crude product was dissolved in ethyl acetate and washed with saturated $NaHCO_3$. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with brine and dried with $MgSO_4$. The crude material was purified by silica gel column chromatography using 0–100% CMA 80/ethyl acetate to yield 294 mg (95%) of the desired compound. Compound was determined to be 95% pure by ^{1}H NMR and was carried forward into further chemistry. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.31–1.54 (m, 2 H) 1.92–2.08 (m, 2 H) 2.92–3.12 (m, 1 H) 3.27 (t, *J* = 11.87 Hz, 2 H) 5.42 (br. s., 2 H) 7.07–7.61 (m, 8 H) 8.38 (s, 1 H), $[M + H]^+$ 439.4.

General Procedure for Making Ureas from 1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-amine (17b)—To a solution of **17b** (20 mg, 0.046 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.02 mL, 0.136 mmol, 3 equiv) and the appropriate isocyanate (1.5 equiv). The reaction was stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexane to yield pure compound.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}-3-ethylurea (17c)—Reaction proceeded in 56% yield. ^{1}H NMR (300 MHz, chloroform-d) δ ppm 1.13 (t, *J* = 7.21 Hz, 3 H) 1.33–1.57 (m, 2 H) 2.04–2.20 (m, 2 H) 3.14–3.25 (m, 2 H) 3.32 (t, *J* = 12.06 Hz, 2 H) 3.86–4.05 (m, 1 H) 4.23–4.45 (m, 2 H) 5.39 (br. s., 2 H) 7.03–7.59 (m, 8 H) 8.37 (s, 1 H), $[M + H]^+$ 510.2.

1-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}-3-(propan-2-yl)urea (17d)—Reaction proceeded in 84% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.14 (d, *J* = 6.50 Hz, 6 H) 1.35–1.55 (m, 2 H) 2.12 (d, *J* = 10.46 Hz, 2 H) 3.32 (t, *J* = 12.10 Hz, 2 H) 3.84 (dd, *J* = 13.66, 6.69 Hz, 1 H) 3.89–4.04 (m, 1 H) 4.12–4.36 (m, 2 H) 5.39 (br. s., 2 H) 7.19 (d, *J* = 8.67 Hz, 2 H) 7.28–7.43 (m, 5 H) 7.51 (d, *J* = 6.69 Hz, 1 H) 8.37 (s, 1 H), $[M + H]^+$ 524.6.

1-{1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}-3-propylurea (17e)—Reaction proceeded in 71% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.83–0.98 (m, 3 H) 1.37–1.59 (m, 4 H) 2.13 (d, *J* = 10.27 Hz, 2 H) 3.12 (q, *J* = 6.66 Hz, 2 H) 3.32 (t, *J* = 12.01 Hz, 2 H) 3.83–4.07 (m, 1 H) 4.20–4.47 (m, 2 H) 5.40 (br. s., 2 H) 7.13–7.24 (m, 2 H) 7.28–7.44 (m, 5 H) 7.51 (d, *J* = 6.69 Hz, 1 H) 8.37 (s, 1 H), $[M + H]^+$ 524.1.

3-Butyl-1-{1-[8-(2-chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-yl}urea (17f)—Reaction proceeded in 69% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.84–0.99 (m, 3 H) 1.28–1.55 (m, 6 H) 2.04–2.20 (m, 2 H) 3.15 (q, *J* = 6.75 Hz, 2 H) 3.32 (t, *J* = 12.15 Hz, 2 H) 3.83–4.08 (m, 1 H) 4.18–4.39 (m, 2 H) 5.40 (br. s., 2 H) 7.08–7.43 (m, 7 H) 7.51 (d, *J* = 6.69 Hz, 1 H) 8.37 (s, 1 H), $[M + H]^+$ 538.4.

General Procedure for Making Amides from 1-[8-(2-Chlorophen-yl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-amine (17b)—To a solution of **17b** (21 mg, 0.048 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.02 mL, 0.143 mmol, 3 equiv) and the appropriate anhydride (2 equiv). The reaction is stirred for 16 h. The reaction was concentrated *in vacuo*. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexane to yield pure compound.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}acetamide (17g)—Reaction proceeded in 65% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.42–1.56 (m, 2 H) 1.99 (s, 3 H) 2.12 (d, *J* = 10.08 Hz, 2 H) 3.30 (t, *J* = 12.24 Hz, 2 H) 4.09–4.25 (m, 1 H) 5.41 (d, *J* = 8.01 Hz, 3 H) 7.13–7.43 (m, 7 H) 7.51 (d, *J* = 6.78 Hz, 1 H) 8.38 (s, 1 H), $[M + H]^+$ 481.4.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}-2,2,2-trifluoroacetamide (17i)—Reaction proceeded in 39% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.62 (qd, J = 11.99, 3.86 Hz, 2 H) 2.18 (d, *J* = 10.83 Hz, 2 H) 3.31 (t, *J* = 12.43 Hz, 2 H) 4.11–4.29 (m, 1 H) 5.53 (br. s., 2 H) 6.19 (d, *J* = 7.06 Hz, 1 H) 7.01–7.57 (m, 8 H) 8.40 (s, 1 H), $[M + H]^+$ 535.4.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}benzamide (17j)—Reaction proceeded in 19% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.82–1.97 (m, 2 H) 2.77 (d, *J* = 12.34 Hz, 2 H) 3.08 (br. s., 2 H) 3.46–3.62 (m, 1 H) 3.66–3.80 (m, 1 H) 4.01–4.13 (m, 1 H) 5.26–5.46 (m, 1 H) 7.12–7.59 (m, 13 H) 8.38 (s, 1 H), $[M + H]^+$ 543.6.

General Procedure for Making Sulfonamides from 1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]piperidin-4-amine (17b)—To a solution of **17b** (21 mg, 0.048 mmol, 1 equiv) in 2 mL of THF was added triethylamine (0.02 mL, 0.143 mmol, 3 equiv) and the appropriate sulfonyl chloride (2 equiv). The reaction is stirred for 16 h. The reaction was concentrated in vacuo. The crude material was purified by silica gel column chromatography using 0–100% ethyl acetate/hexane to yield pure compound.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}methanesulfonamide (17h)—Reaction proceeded in 32% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.55–1.67 (m, 2 H) 2.18 (d, J = 12.53 Hz, 2 H) 3.03 (s, 3 H) 3.36 (t, J = 12.29 Hz, 2 H) 3.59–3.80 (m, 1 H) 4.31 (d, J = 7.44 Hz, 1 H) 5.43 (d, J = 9.89 Hz, 2 H) 7.13–7.44 (m, 7 H) 7.51 (d, J = 6.78 Hz, 1 H) 8.39 (s, 1 H), $[M + H]^+$ 517.6.

N-{1-[8-(2-Chlorophenyl)-9-(4-chlorophenyl)-9H-purin-6-yl]-piperidin-4-yl}benzenesulfonamide (17k)—Reaction proceeded in 53% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.43–1.59 (m, 2 H) 1.94 (d, J = 10.46 Hz, 2 H) 3.30 (t, J = 11.96 Hz, 2 H) 3.41–3.66 (m, 1 H) 4.62 (d, J = 7.54 Hz, 1 H) 5.26 (d, J = 10.08 Hz, 2 H) 7.07–7.69 (m, 10 H) 7.92 (d, J = 7.35 Hz, 2 H) 8.35 (s, 1 H), $[M + H]^+$ 579.4.

Calcium Mobilization and Radioligand Displacement Assays

Each compound was pharmacologically characterized using a functional fluorescent CB1 activated Gαq$_{16}$-coupled intracellular calcium mobilization assay in CHO-K1 cells, as has been described in our previous publications and apparent affinity (K_e) values were determined.[13] Briefly, CHO-K1 cells were engineered to coexpress human CB1 and Gαq$_{16}$. Activation of CB1 by an agonist then leads to generation of inositol phospahatase 3 (IP3) and activation of IP3 receptors, which leads to mobilization of intracellular calcium. Calcium flux was monitored in a 96-well format using the fluorescent dye Calcein-4 a.m. in an automated platereader (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:

K_e = [Ligand]/[DR-1] where DR is the EC_{50} ratio of CP55940 in the presence or absence of a test agent. Standard errors were between 5 and 25% of mean in most cases and have been left out for clarity.

Further characterization of select compounds was performed using radioligand displacement of [^{3}H]SR141716 and equilibrium dissociation constant (K_i) values were determined as described previously.[13] Selectivity of these compounds at CB1 versus CB2 was also determined by obtaining K_i values at either receptor using displacement of [^{3}H]CP55940 in membranes of CHO-K1 cells overexpressing either receptor. Data reported are average values from 3 to 6 measurements. The standard errors for most measurements were between 5 and 25% of mean and have been left out from the tables and figures for clarity.

MDCK-mdr1 Permeability Assays

MDCK-mdr1 cells permeability assays were preformed as previously described.[11b,13] MDCK-mdr1 cells obtained from Netherlands Cancer Institute were grown on Transwell type filters (Corning) for 4 days to confluence in DMEM/F12 media containing 10% fetal bovine serum and antibiotics as has been described previously.[13] Compounds were added to the apical side at a concentration of 10 μM in a transport buffer comprising of 1× 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). Flow injection analysis was also conducted to optimize for mass spectrometer parameters. Samples from the apical and basolateral side of the MDCK cell assay were dried under nitrogen on a Turbovap LV. The chromatography was conducted with an Agilent 1100 binary pump with a flow rate of 0.5 mL/min. Mobile phase solvents were A, 0.1% formic acid in water, and B, 0.1% formic acid in methanol. The initial solvent conditions were 10% B for 1 min, then a gradient was used by increasing to 95% B over 5 min, then returning to initial conditions. Data reported are average values from 2 to 3 measurements.

In Vitro Stability Testing

Stability of compounds to plasma and S9 fraction was preformed as previously described.[13] *In vitro* testing for metabolic stability was conducted in pooled samples of mixed gender human plasma from BioChemed Services, Winchester, VA and human mixed gender pooled hepatic S9 fraction supplied by Xenotech, LLC, Lenexa, KS. Identity of the donors was unknown.

For the hepatic S9 metabolism studies, all samples were tested at 10 μM final concentration in a 1 mL volume containing 1 mg/mL S9. Samples were incubated in a buffer containing 50 mM potassium phosphate, pH 7.4 with 3 mM $MgCl_2$ and a NADPH regeration system comprising of NADP (1 mM), glucose-6-phosphate (5 mM) and glucose-6-phosphate dehydrogenase (1 unit/mL). Triplicate samples were incubated for 0, 15, 30, 60, and 120 min. Reactions were terminated by addition of 3 vol of acetonitrile and processed as described for the MDCK-mdr1 assays, but standard curves were prepared in blank matrix for each compound for quantitative assessment.

The plasma stability studies were conducted at 37 °C in a volume of 1 mL plasma per sample. All compounds were tested at 10 μM final concentration at 0, 30, and 60 min after a 5 min preincubation. Reactions were terminated by addition of acetonitrile and analyzed as described above.

Pharmacokinetic Evaluation in Vivo

Male Sprague–Dawley rats aged 8 weeks at time of dosing were acquired from Charles River Laboratories and were dosed orally. Three to four animals were used per time point. Dose was formulated in food-grade corn oil at 10 mg/kg. Plasma, liver, and brain were taken from all rats at 0.5, 1, 2, 4, and 8 h postdose. Samples were prepared and analyzed as

follows: Plasma (50 μL) was mixed with 10 μL of 10 μL of acetonitrile, and 300 μL of acetonitrile, vortexed, and centrifuged at 9000g for 5 min. Supernatants were transferred to autosampler vials with low-volume inserts and injected without dilution. Brains were homogenized with 50:50 ethanol/water (3:1, v/v) using a Potter Elvehjem type homogenizer. Homogenate (50 μL) was mixed with 10 μL of acetonitrile, and 300 μL of acetonitrile, vortexed and centrifuged at 9000g for 5 min. Supernatant was transferred to autosampler vials with low-volume inserts and injected without dilution. Standards were prepared as above for each compound in blank plasma, blank liver homogenate, and blank brain homogenate. Standards used were within 15% of nominal; except for 20% at LOQ. Compounds for LC-MS/MS analyses were supplied at 1 mg/mL in methanol. The stock solutions were further diluted to ∼100 ng/mL. The 100 ng/mL solutions were used to optimize the mass spectrometer for MRM transitions and mass spectrometer parameters. Infusion and flow injection optimization were also performed. LC-MS/MS was conducted using an Applied Biosystems API 4000 coupled with an Agilent 1100 HPLC system. Chromatography was performed with a Phenomenex Luna C18 (50 × 2 mm, 5 μm) column. Mobile phases were 0.1% formic acid and 10 mM ammonium formate in water (A), and 0.1% formice acid and 10 mM ammonium formate in methanol. Initial conditions were 10% B and held for one minute, followed by a linear gradient to 90% B over 5 min. 90% B was held for 2 min before returning to initial conditions. Compound 12 was analyzed with multiple reaction monitoring in the positive mode with a transition of 595.28 → 370.0. The following parameters were used, DP = 116, CE = 45, CXP = 36, CAD = 4, CUR = 10, GS1 = 40, GS2 = 60, IS = 4000, and TEM = 650.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS USED

CB1	cannabinoid receptor 1
CB2	cannabinoid receptor 2
CMA 80	80% chloroform, 18% methanol, and 2% ammonium hydroxide
CNS	central nervous system
BBB	blood–brain barrier
TPSA	topological polar surface area
ECS	endocannabinoid system
CBR	cannabinoid receptors

Ke	apparent affinity constant
MDCK-mdr1	Madin-Darby canine kidney cells transfected with the human MDR1 gene
A	apical
B	basal
BOP	benzotriazole-1-yl-oxytris(dimethylamino)-phosphonium hexafluorophosphate
CHO-K1	Chinese hamster ovary cells
IP3	inositol phospahatase 3
MRM	multiple reaction monitoring
LOQ	below limit of quantitation
NA	not applicable

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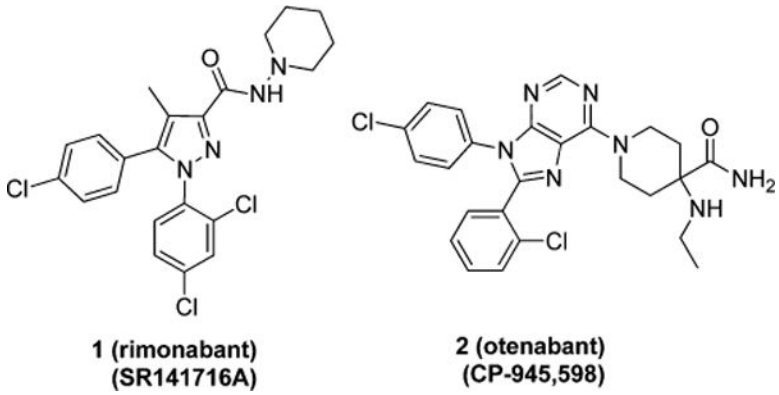


Figure 1.
Examples of CB1 antagonists.

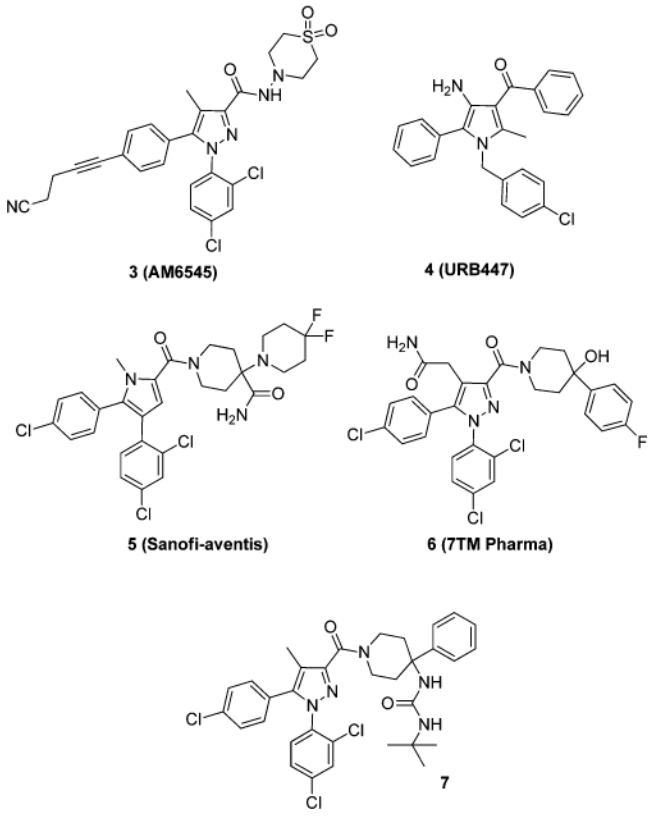


Figure 2.
CB1 antagonists that are reported to be selective for the periphery.

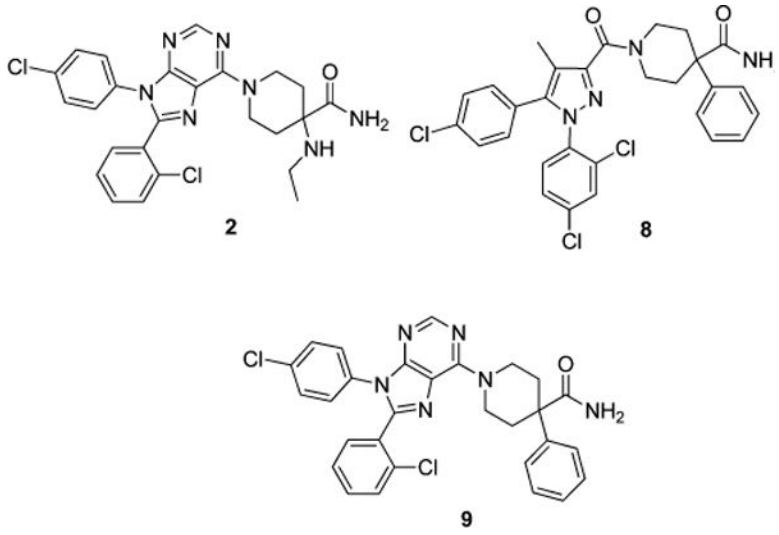


Figure 3.
Design of compound **9**; a hybrid of compounds **2** and **8**.

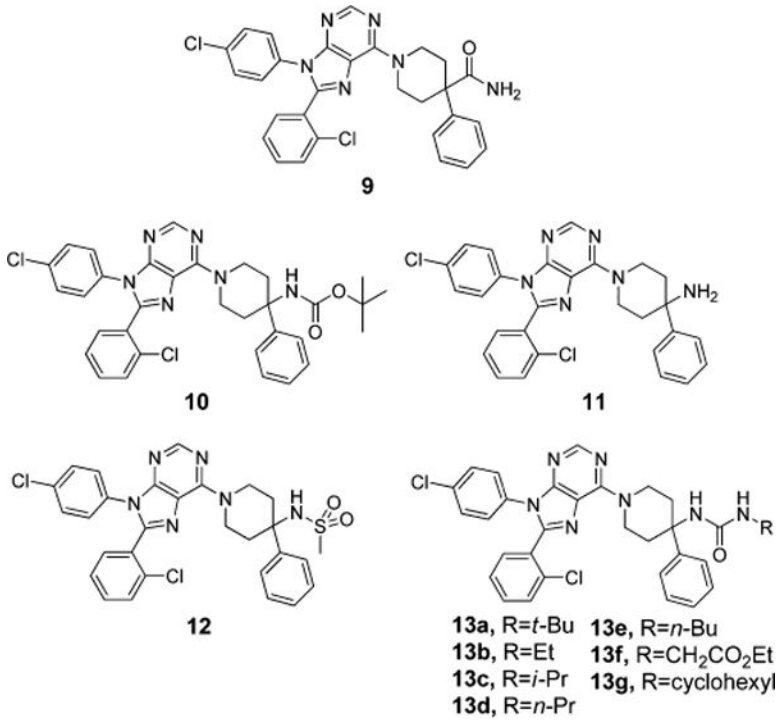


Figure 4.

Ligand design around compound **9**, replacement of the amide functionality.

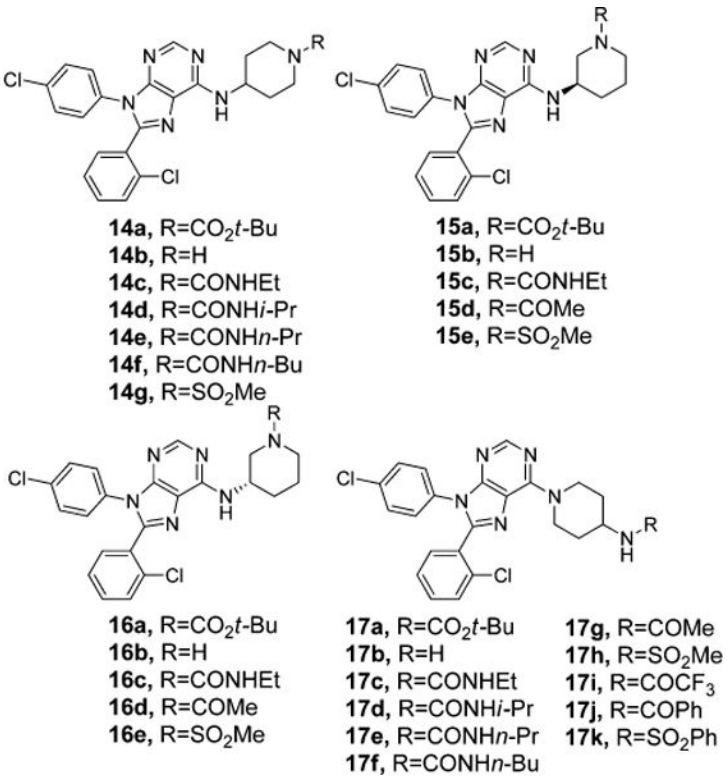
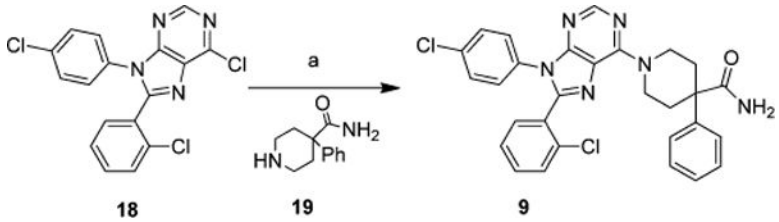
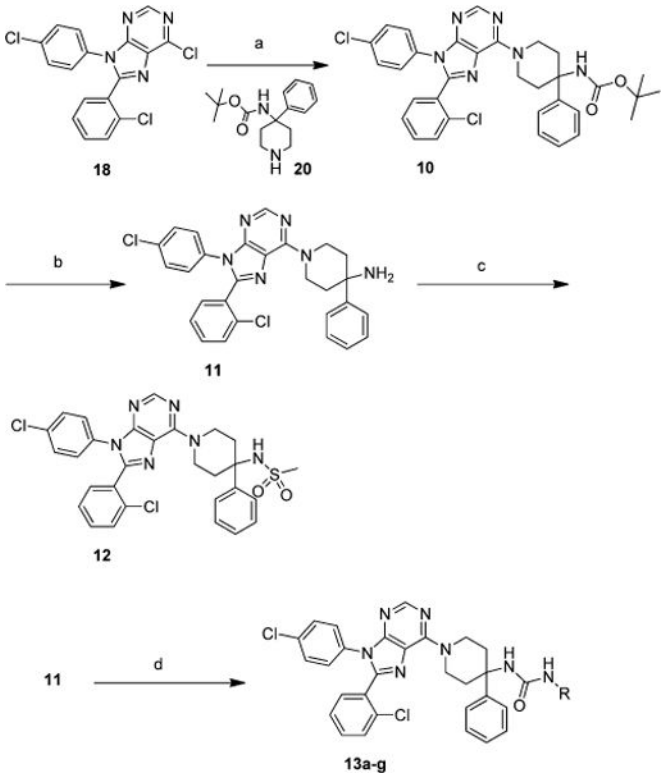


Figure 5.
Exploration of different spacers.



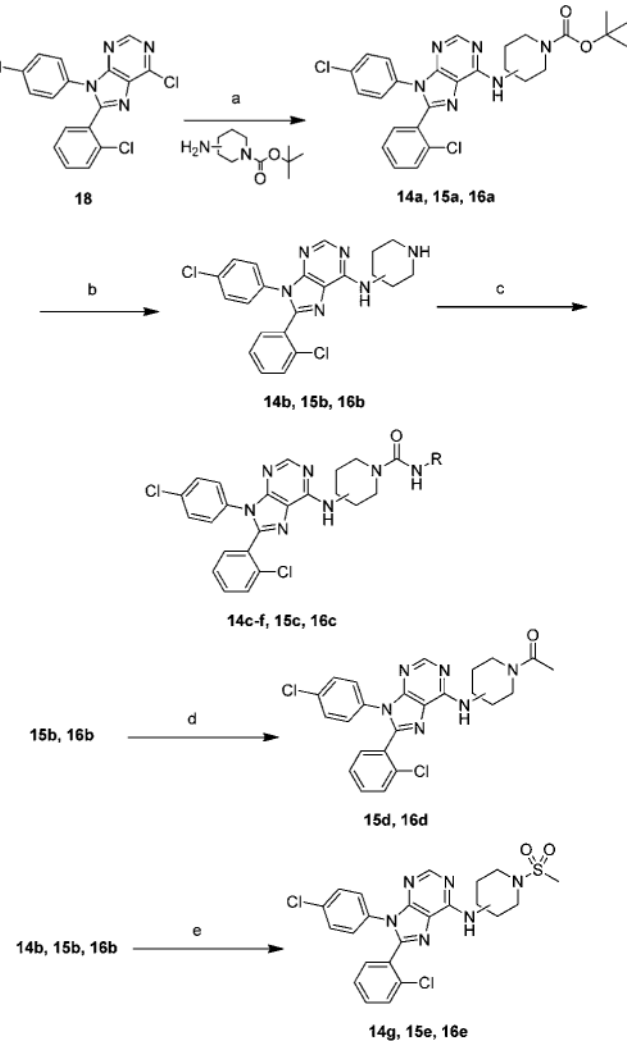
Scheme 1. Synthesis of Compound 9[a]

[a]Reagents and conditions: (a) **19**, triethylamine, ethanol, 55 °C, 3 days.



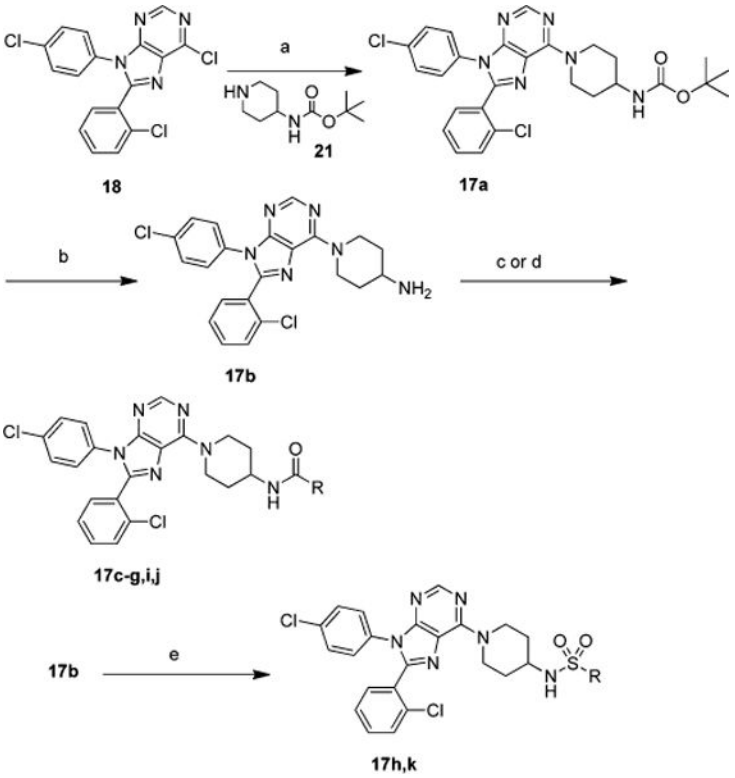
Scheme 2. Synthesis of Analogues of Compound 9[a]

[a]Reagents and conditions: (a) **20**, triethylamine, ethanol, 80 °C, 16 h; (b) 20% TFA in dichloromethane; (c) methanesulfonyl chloride, triethylamine, THF; (d) appropriate isocyanate, triethylamine, THF.



Scheme 3. Synthesis of Compounds with Different Spacers and Functional Groups[a]

[a]Reagents and conditions: (a) triethylamine, ethanol, 80 °C, 16 h; (b) 30% TFA in dichloromethane; (c) appropriate isocyanate, triethylamine, THF, rt; (d) acetic anhydride, pyridine; (e) methanesulfonyl chloride, triethylamine, THF.



Scheme 4. Synthesis of Compounds with Different Spacers and Functional Groups Continued[a]

[a]Reagents and conditions: (a) **21**, ethanol, 80 °C, 16 h; (b) 30% TFA in dichloromethane; (c) appropriate isocyanate, triethylamine, THF; (d) appropriate anhydride, triethylamine, THF; (e) appropriate sulfonyl chloride, triethylamine, THF.

Table 1

Functional Assessment of Compound 9

compd	TPSA	K_e CB1 (nM)	K_i CB1 (nM) [^{3}H] CP55940	K_i CB2 (nM) [^{3}H] CP55940	CB2/CB1 K_i ratio	MDCK-mdr1 A to B (%)[a]
2	102	8.7				40
8	81	0.45	3.44	5504	1600	8
9	90	9	1.79	5507	3077	6

[a]Compound's permeability was measured from apical to basal (**A** to **B**) sides of the membrane.

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Table 2

Functional Assessment of Analogues of Compound 9

compd	TPSA	K_e CB1 (nM)	K_i CB1 (nM) [^{3}H] CP55940	K_i CB2 (nM) [^{3}H] CP55940	CB2/CB1 K_i ratio	MDCK-mdr1 A to B (%)[a]
10	85	3.9	7.1	13947	1959	<1
11	73	15.9	11	1657	151	<1
12	101	2.9	6.2	948	153	<1
13a	88	19.2	25.5	20000	784	<1
13b	88	443	195	4645	24	
13c	88	46	337	8946	27	
13d	88	99	220	7779	35	
13e	88	62	269	>20000	>74	
13f	114	171				
13g	88	199				

[a] Compound's permeability was measured from apical to basal (**A** to **B**) sides of the membrane.

Table 3

Functional Assessment of Different Spacers and Functional Groups

compd	TPSA	K_e CB1 (nM)	K_i CB1 (nM) [^{3}H] CP55940	K_i CB2 (nM) [^{3}H] CP55940	CB2/CB1 K_i ratio
14a	85	92	24	14000	568
14b	68	5706			
14c	88	1148			
14d	88	495			
14e	88	1964			
14f	88	71	3343	>20000	>5
14g	101	1645			
15a	85	326			
15b	68	5546			
15c	88	1500			
15d	76	920			
15e	101	346			
16a	85	3927			
16b	68	8406			
16c	88	1153			
16d	76	4449			
16e	101	6044			
17a	85	11	1.5	1713	1142
17c	88	149			
17d	88	133	14	2453	175
17e	88	132	28	153	5
17f	88	49	25	1548	62
17g	76	631			
17h	101	71	29	1073	37
17i	76	3.2			
17j	76	0.7			
17k	101	0.3	1.2	5865	4888

Table 4

Functional Assessment of Select Compounds in for *in Vitro* Metabolic Stability

					in vitro metabolic stability	
compd	**TPSA**	**K_e CB1 (nM)**	**CB2/CB1 K_i CP55940**	**MDCKmdr1 A to B (%)**	**S9% remaining 120 min**	**plasma % remaining 60 min**
9	90	0.45	3077	6	>90	>90
12	101	2.9	153	<1	>90	>90
13a	88	19.2	784	<1	43	>90

Table 5

Pharmacokinetic Analyses of 12

compd	dose mg/kg oral	sacrifice time (min)	plasma conc. (ng/mL)	brain conc. (ng/mL)	brain/plasma ratio
12	10	30	752	38	0.05
		60	767	58	0.08
		120	1188	122	0.10
		240	1653	184	0.11
		480	914	89	0.10

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