Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

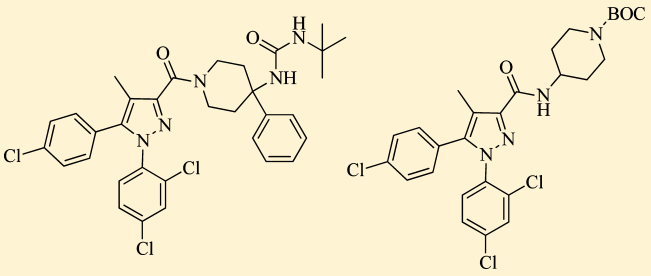
Design and Synthesis of Cannabinoid Receptor 1 Antagonists for Peripheral Selectivity

Alan Fulp, Katherine Bortoff, Herbert Seltzman, Yanan Zhang, James Mathews, Rodney Snyder, Tim Fennell, and Rangan Maitra*

Discovery Sciences, Research Triangle Institute, 3040 Cornwallis Road, P.O. Box 12194, Research Triangle Park, North Carolina 27709, United States

Supporting Information

ABSTRACT: Antagonists of cannabinoid receptor 1 (CB1) have potential for the treatment of several diseases such as obesity, liver disease, and diabetes. Recently, development of several CB1 antagonists was halted because of adverse central nervous system (CNS) related side effects observed with rimonabant, the first clinically approved CB1 inverse agonist. However, recent studies indicate that regulation of peripherally expressed CB1 with CNS-sparing compounds is a viable strategy to treat several important disorders. Our efforts aimed at rationally designing peripherally restricted CB1 antagonists have resulted in compounds that have limited blood–brain barrier (BBB) permeability and CNS exposure in preclinical in vitro and in vivo models. Typically, compounds with high topological polar surface areas (TPSAs) do not cross the BBB passively. Compounds with TPSAs higher than that for rimonabant (rimonabant TPSA = 50) and excellent functional activity with limited CNS penetration were identified. These compounds will serve as templates for further optimization.

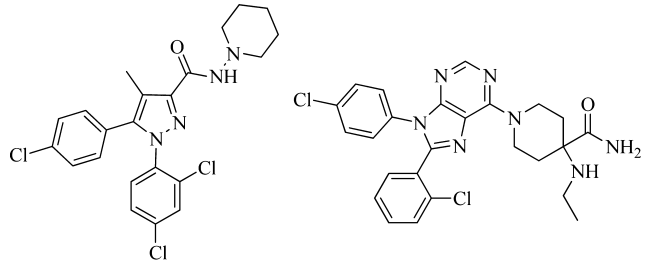


INTRODUCTION

The endocannabinoid system (ECS) consists of receptors, transporters, endocannabinoids, and the enzymes involved in synthesis and degradation of endocannabinoids.[1] There have been two cannabinoid receptors (CBRs) identified to date, CB1 and CB2. CB1 and CB2 are both G-protein-coupled receptors (GPCRs), and their primary function is to activate inhibitory G proteins (Gi/o).[1,2] The ECS is responsible for many important physiological processes, and regulation of these processes holds promise for the treatment of several diseases. ECS components are under evaluation for the treatment of obesity, liver disease, diabetes, pain, and inflammation.[2]

The CB1 receptor is expressed throughout the body; however, it is found at much greater concentrations in the central nervous system (CNS). There has been great interest in the use of CB1 antagonists for the treatment of metabolic disorders, such as obesity and diabetes. Rimonabant (SR141716A, **1**, Figure 1), a potent and selective CB1 inverse agonist/antagonist, was clinically approved to treat obesity in Europe. Unfortunately, **1** produced serious CNS-related side effects such as anxiety, depression, and suicidal ideation in patients, leading to its withdrawal from European markets and denial of approval in the United States.[3] Upon discovery of rimonabant's side effects, several other CB1 antagonists, such as taranabant, otenabant (**2**), and ibipinabant, were pulled from development because of regulatory concerns.[4]

A strategy to take advantage of the therapeutic potential of CB1 antagonism and avoid the CNS-related adverse effects is to generate CB1 antagonists that do not cross the blood–brain barrier (BBB). This strategy is being pursued by several groups,



1 (rimonabant) (SR141716A) **2 (otenabant) (CP-945,598)**

Figure 1. CB1 antagonists.

and a small set of CB1 antagonists that do not cross the BBB have been reported (**3**–**6**, Figure 2).[5] However, none of these peripherally restricted CB1 antagonists have been fully characterized or their efficacy demonstrated clinically.

Our group has pursued a two-pronged strategy to develop peripherally restricted CB1 antagonists. The first strategy involved development of CB1 antagonists that have a permanent charge. Charged compounds do not normally cross the BBB unless they are acted upon by a transporter.[6] Results for this strategy have been previously reported.[7] The second strategy was to target compounds with high topological polar surface areas (TPSAs). It has been shown that compounds with higher TPSAs have lower

Received: December 22, 2011
Published: February 28, 2012

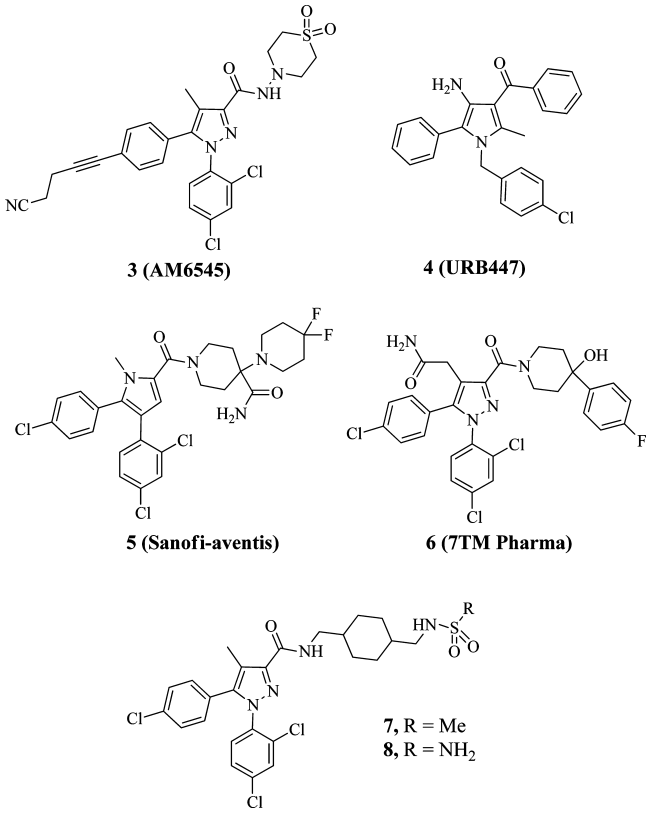


Figure 2. Reported CB1 antagonists that are selective for the periphery.

permeability into the CNS.[8] Higher TPSAs can be achieved by adding polar groups, such as sulfonamide or sulfamide, or by replacing existing functional groups with more polar functional groups. This strategy led to the identification of compounds **7** and **8** that we have previously reported. While these compounds were promising, their selectivity for CB1 over CB2 was only modest. Here we describe our ongoing efforts toward designing peripherally restricted CB1 antagonists with improved properties. This study evolved to include a more empirical approach that was based more on SAR than on computational parameters. We have been able to design, synthesize, and characterize highly selective CB1 antagonists that appear to be peripherally restricted.

RESULTS

Ligand Design and Pharmacological Characterization.

Compounds were synthesized, purified, characterized, and tested as has been described in the "Experimental Section". All compounds were tested in vitro as antagonists using a calcium mobilization assay as has been previously described.[7] The ability of each compound to antagonize functional activation of CB1 was quantitatively measured and expressed as its apparent dissociation affinity constant (K_e). Compounds that were found to be potent ($K_e \approx 100$ nM or less) using the functional assay were subsequently characterized using radioligand displacement of either [^{3}H]SR141716A or [^{3}H]CP55940 at CB1 and CB2. Equilibrium dissociation constant (K_i) values were determined at each receptor.

During our studies of charged compounds, carboxylic acids were examined because they are negatively charged at the physiological pH. Around the same time, carboxylic acid **9** (Figure 3) was reported by another group to be a CB1 antagonist.[5d] This finding led to the synthesis and evaluation of carboxylic acid **10**

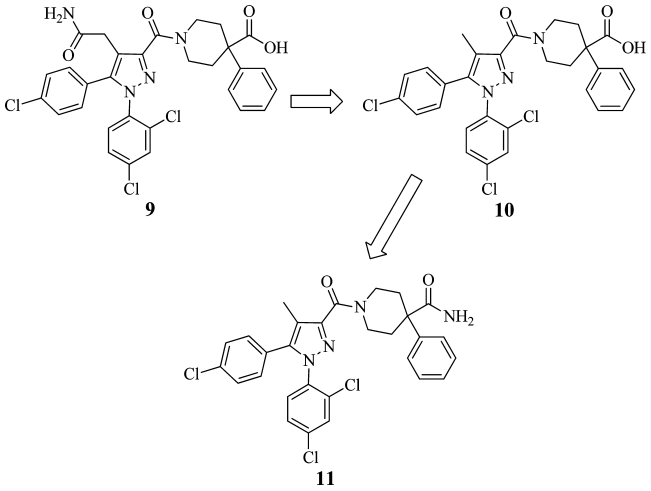


Figure 3. Design of compound **11**.

(Table 1). Compound **10** was only moderately active (K_e = 1170 nM). However, examination of the structure of **2** revealed a primary amide at the 4-position of the piperdine ring (Figure 1). This amide was in a similar position as the carboxylic acid functionality of compound **10**, leading to the decision to convert carboxylic acid **10** to amide **11** (Figure 3). Amide **11** lacked the charged nature of a carboxylic acid, but it did have hydrogen bonding ability that could lower its permeability into the CNS. Compound **11** was found to be a potent CB1 antagonist having a K_e of 0.44 nM and was also highly selective for CB1 over CB2 (CB2/CB1 of 1600). Interestingly, the 4-phenylpiperidine-4-carboxamide group was also reported on a closely related pyrrole scaffold.[5c] Compound **11** was advanced into Madin–Darby canine kidney cells transfected with the human MDR1 gene (MDCK-mdr1) model of BBB penetration, which is widely used to predict in vivo permeability of compounds.[9] The potency, selectivity, and relatively low permeability of compound **11** across the MDCK-mdr1 cells (apical (A) to basal (B), 8%) made it an interesting starting point for further modifications toward designing potent and selective CB1 antagonists that do not cross the BBB.

Compound **11** served as a starting point for several modifications to the amine portion of the pyrazole C-3 carboxamide (Figure 4, Table 2). One of the first modifications of compound **11** studied was the replacement of the phenyl group. Compound **12** was targeted because it represented a hybrid of compounds **11** and **2**. Compound **12** also closely resembles the Sanofi-Aventis compound **5**. However, compound **12**'s potency (K_e(CB1) = 91 nM) and selectivity (ratio CB2/CB1 of 28.3) did not warrant further investigation of this compound. Next, the reversal of the primary amide of compound **11** became of interest. To realize this, both compounds **13** and **14** needed to be synthesized as precursors of reverse amide compound **15**. However, both compounds **13** and **14** were interesting in their own right. Compound **13** added the additional functionality of a carbamate; this was a functionality that had not been pursued in our laboratory, and it also had good potency (K_e(CB1) = 20.2 nM) and selectivity (ratio CB2/CB1 of ~50). Compound **14** was of interest because it replaced the primary amide of **11** with a primary amine. This maintained the possibility of hydrogen bonding and increased the basicity of the molecule. This amine group proved to be detrimental to potency (K_e(CB1) = 485 nM). However, the amine group in compound **14** also allowed for the introduction of different functionalities. The amine group of compound **14** was used to make the reverse amide compound **15**

dx.doi.org/10.1021/jm201731z | J. Med. Chem. 2012, 55, 2820–2834

Table 1. Pharmacological Assessment of Compound 11 with Rimonabant 1 and Otenabant 2

			K_i (nM)				
compd	TPSA	K_e (nM) CB1	CB1 [^{3}H]SR141716 (**1**)	CB1 [^{3}H]CP55940	CB2 [^{3}H]CP55940	CB2/CB1	MDCK-mdr1[a] (%)
1	50	1.1		6.2	313	50.6	15
2		8.7					90
10	75	1170					
11	81	0.4	0.4	3.4	5504	1600	8

[a]Compound's permeability was measured from apical to basal sides of the membrane.

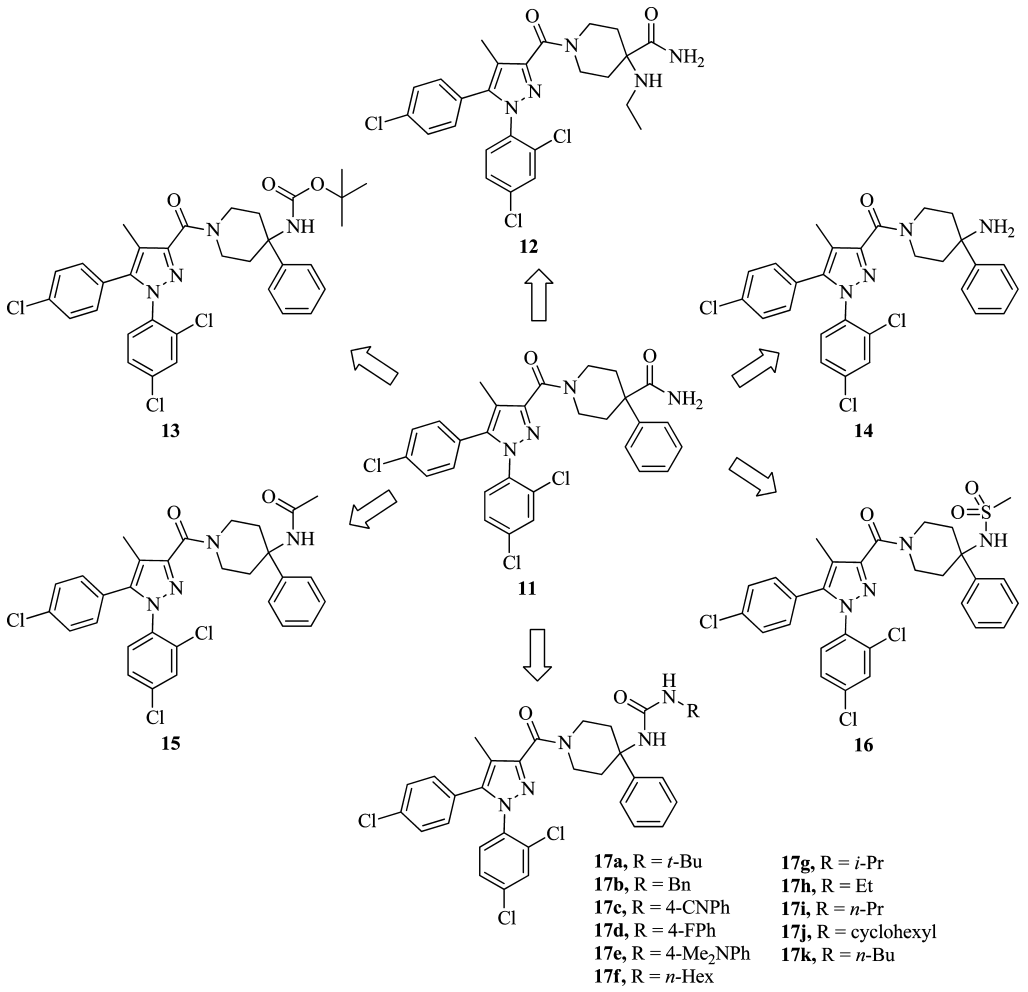


Figure 4. Ligand design around compound **11**.

which was only weakly active (K_e(CB1) = 201 nM). Sulfonamide **16**, which was also made from amine **14**, had higher TPSA compared to compound **11** and was potent but only moderately selective for CB1 over CB2 (K_e(CB1) = 3.5 nM, ratio of CB2/CB1 of 5.64). Finally, amine **14** also allowed for the synthesis of urea **17a**. Urea **17a** proved to be a potent CB1 antagonist (K_e(CB1) = 2.4 nM) and had good selectivity against CB2 (ratio CB2/CB1 of ~425). Compound **17a** was advanced into the in vitro model of BBB permeability (MDCK-mdr1, apical to basal) and was predicted not to cross the BBB (Table 2, <1% transported). These results spawned the synthesis of a small library of ureas **17b–k**, which had potencies (K_e) ranging from 0.5 to >10000 nM against CB1. Several of these compounds were very selective with 5 of the 10 compounds being over 100-fold selective for CB1 over CB2 (Table 2).

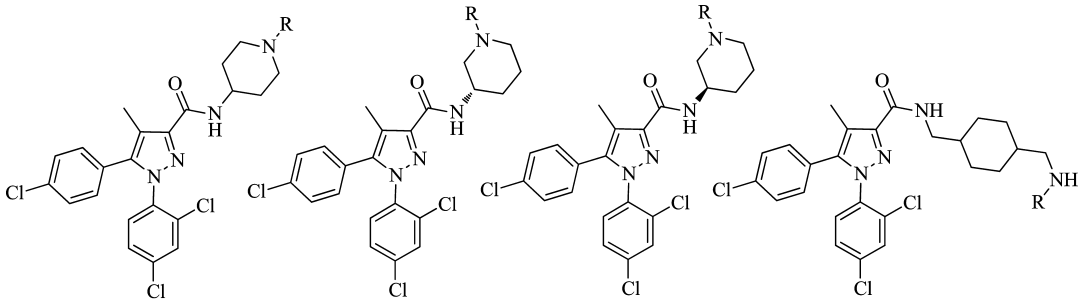
The positive results for compound **17a** also led to the exploration of 4- and 3-aminopiperdine and cyclohexylamides as different spacers between the pyrazole amide and their polar functionality (Figure 5, Table 3). Structurual series **18** was chosen because it offered similar spacing to compound **17a** and presented the opportunity for rapid derivatization off the piperidine nitrogen. Because of the positive results observed for compounds **18a–l** (7 out of the 12 compounds had K_e < 100 nM against CB1, and 4 analogues had CB2/CB1 ratios greater than 100), other aminopiperidine linkers were explored. The 1,3-disubstituted aminopiperdine series **19** and **20** were used to explore the importance of the effect of an alternative juxtaposition of substituents and the introduction of stereochemistry versus the 4-aminopiperdine linker of structurual series **18**. Both enantiomers were explored to examine the effect of chirality on potency and selectivity. Positive results were obtained with compounds **19a–j**, with 3 of the 10 analogues having K_e values below 100 nM at CB1, and two

dx.doi.org/10.1021/jm201731z | J. Med. Chem. 2012, 55, 2820–2834

Table 2. Pharmacological Assessment of Analogues of Compound 11

compd	TPSA	K_e (nM) CB1	K_i (nM) CB1 [^{3}H]CP55940	K_i (nM) CB2 [^{3}H]CP55940	CB2/CB1	MDCK-mdr1[a] (%)
12	93	91	78.4	2217	28.3	
13	76	20.2	42.3	2110	49.9	<1
14	64	485	104	1127	10.8	
15	67	201	62.6	214	3.4	
16	93	3.5	7.3	41	5.6	3
17a	79	2.4	47.1	20000	424.6	<1
17b	79	2.1	43	17126	398	<1
17c	103	>10000				
17d	79	89	614	20000	33	
17e	82	264	1489	16289	11	
17f	79	0.5	38.8	2414	62	<1
17g	79	0.7	13.5	4914	364	
17h	79	10.8	15	182	12	
17i	79	0.4	7.6	293	39	
17j	79	12	792	20000	25	
17k	79	0.4	15.5	2760	178	<1

[a]Compound's permeability was measured from apical to basal sides of the membrane.



18a, R = Boc
18b, R = H
18c, R = Ac
18d, R = SO_2Me
18e, R = SO_2NH_2
18f, R = CONH*t*-Bu
18g, R = $CONH_2$
18h, R = CONHEt
18i, R = CONH*i*-Pr
18j, R = CONH*n*-Pr
18k, R = CONH*n*-Bu
18l, R = CO_2Et

19a, R = Boc
19b, R = H
19c, R = Ac
19d, R = SO_2Me
19e, R = CONHEt
19f, R = CONH*i*-Pr
19g, R = CONH*n*-Pr
19h, R = CONH*n*-Bu
19i, R = CONH*t*-Bu
19j, R = CO_2Et

20a, R = Boc
20b, R = H
20c, R = Ac
20d, R = SO_2Me
20e, R = CONHEt
20f, R = CONH*i*-Pr
20g, R = CONH*n*-Pr
20h, R = CONH*n*-Bu
20i, R = CONH*t*-Bu
20j, R = CO_2Et

21a, R = CONHEt
21b, R = CONH*i*-Pr
21c, R = CONH*n*-Pr
21d, R = CONH*n*-Bu
21e, R = CONH*t*-Bu

Figure 5. Exploration of different spacers.

analogues were over 100-fold selective for CB1 versus CB2. Compounds **20a–j** were also of interest with 3 of the 10 analogues having K_e(CB1) less than 30 nM. In addition 3 of the 10 analogues were over 100-fold selective for CB1 vs CB2. Finally, since sulfonamide **7** and sulfamide **8** have been found to be potentially useful in the development of periphery restricted CB1 antagonists,[7] ureas of structure **21** were targeted. In general these compounds were only weakly active, and compounds **21a–e** were not pursued further.

Synthesis. Compound **10** (Scheme 1) was prepared by first making the acid chloride of the readily available acid **22**.[10] Acid **22** was treated with oxalyl chloride and a catalytic amount of dimethylformamide (DMF) in dichloromethane to form the desired acid chloride. This acid chloride was then treated with amino acid **23** in the presence of triethylamine to yield compound **10** in 67% yield. Carboxylic acid **10** was converted to amide **11** by the use of benzotriazol-1-yloxytris-(dimethylamino)phosphonium hexafluorophosphate (BOP), triethylamine, and ammonium chloride in 46% yield.

Aminoamide **12** was made by reacting acid **22** with commercially available piperidine **24** under BOP coupling conditions, and this reaction produced compound **12** in 46% yield (Scheme 2). The protected amine **13** was made by the coupling of acid **22** and readily available amine **25**.[11] The Boc group of compound **13** was removed using 30% trifluoroacetic acid (TFA) in dichloromethane to yield amine **14** in 87% yield. Amine **14** was used as a common intermediate for compounds **15**, **16**, and **17a–k**. Acetylamide **15** was made by reacting amine **14** with acetic anhydride and pyridine in 71% yield. Sulfonamide **16** was formed by the reaction of **14** with methanesulfonyl chloride and triethylamine in 65% yield. Urea **17a** was synthesized in 45% yield by reacting amine **14**, *tert*-butyl isocyanate, and triethylamine at 40 °C in THF. Synthesis of ureas **17b–k** was accomplished by reacting amine **14**, the

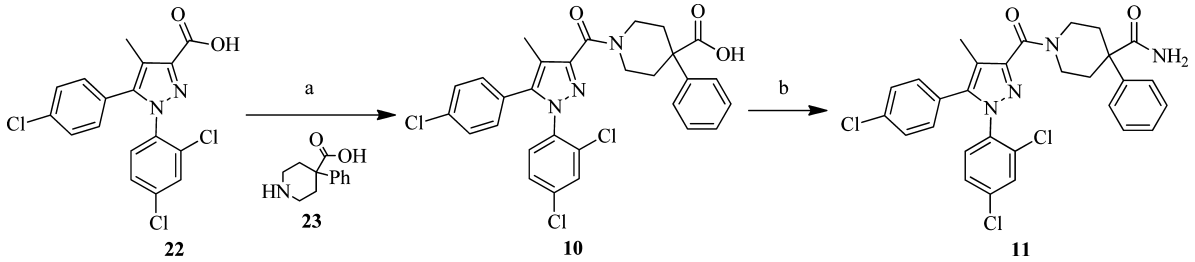
dx.doi.org/10.1021/jm201731z | *J. Med. Chem.* 2012, 55, 2820–2834

Table 3. Pharmacological Assessment of Different Spacers and Functional Groups

compd	TPSA	K_e (nM) CB1	K_i (nM) CB1 [^{3}H]CP55940	K_i (nM) CB2 [^{3}H]CP55940	CB2/CB1	MDCK-mdr1[a] (%)
18a	76	4.7	2.9	2510	877.6	<1
18b	59	5115				
18c	67	195	65.3	2236	34.2	
18d	93	269				
18e	119	592				
18f	79	78	14.7	3349	227.8	<1
18g	93	4097				
18h	79	20.5	165	5693	35	
18i	79	16.7	86	9791	114	14
18j	79	66.5	184	8459	46	
18k	79	78.1	187	8721	47	
18l	76	3	9.2	2997	326	<1
19a	76	842				
19b	58	1879				
19c	67	86	39.1	4350	111.3	14
19d	93	62	77.9	10893	140	<1
19e	79	280				
19f	79	265				
19g	79	70	271	7795	29	
19h	79	230				
19i	79	206				
19j	76	75	65.5	5420	83	<1
20a	76	52	22.3	6720	302	<1
20b	58	1174				
20c	67	530				
20d	93	7	11.84	1248	105.5	<1
20e	79	171				
20f	79	31.3	97	4135	43	
20g	79	27	134	3090	23	
20h	79	118	125	3031	24	
20i	79	268				
20j	76	10.4	17.5	2507	143	<1
21a	88	135				
21b	88	351				
21c	88	486				
21d	88	274				
21e	88	150				

[a]Compound's permeability was measured from apical to basal sides of the membrane.

Scheme 1. Synthesis of Compound 11[a]



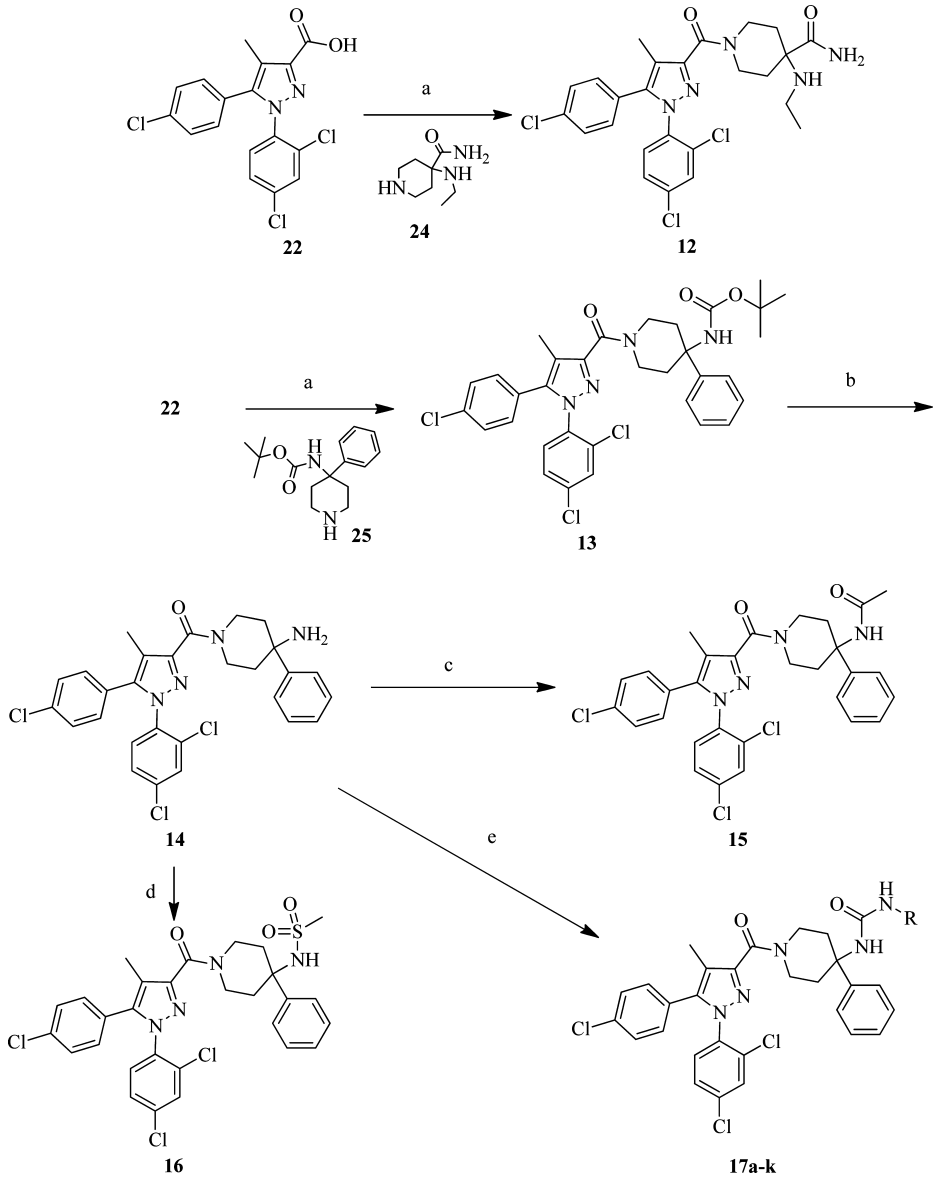
[a]Reagents and conditions: (a) (1) oxalyl chloride, DMF (cat.), dichloromethane, (2) **23**, triethylamine, dichloromethane; (b) benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), triethylamine, ammonium chloride, THF.

appropriate isocyanate, and triethylamine in tetrahydrofuran (THF) at room temperature. These reactions proceeded in yields that ranged from 58% to 74%.

Compounds **18a**, **19a**, and **20a** were synthesized by the coupling of the appropriate commercially available amine to acid **22** in the presence of BOP and triethylamine in yields ranging from 88% to 96% (Scheme 3). Treatment of compounds **18a**, **19a**, and **20a** with trifluoroacetic acid in dichloromethane produced the amines **18b**, **19b**, and **20b**. The amines **18b**, **19b**, and **20b** were reacted with acetic anhydride

dx.doi.org/10.1021/jm201731z | J. Med. Chem. 2012, 55, 2820−2834

Scheme 2. Synthesis of Analogues of Compound 11[a]



[a]Reagents and conditions: (a) amine, BOP, triethylamine, THF; (b) 30% TFA in dichloromethane; (c) acetic anhydride, pyridine; (d) methanesulfonyl chloride, triethylamine, dichloromethane; (e) isocyanate, triethylamine, THF, rt or 40 °C.

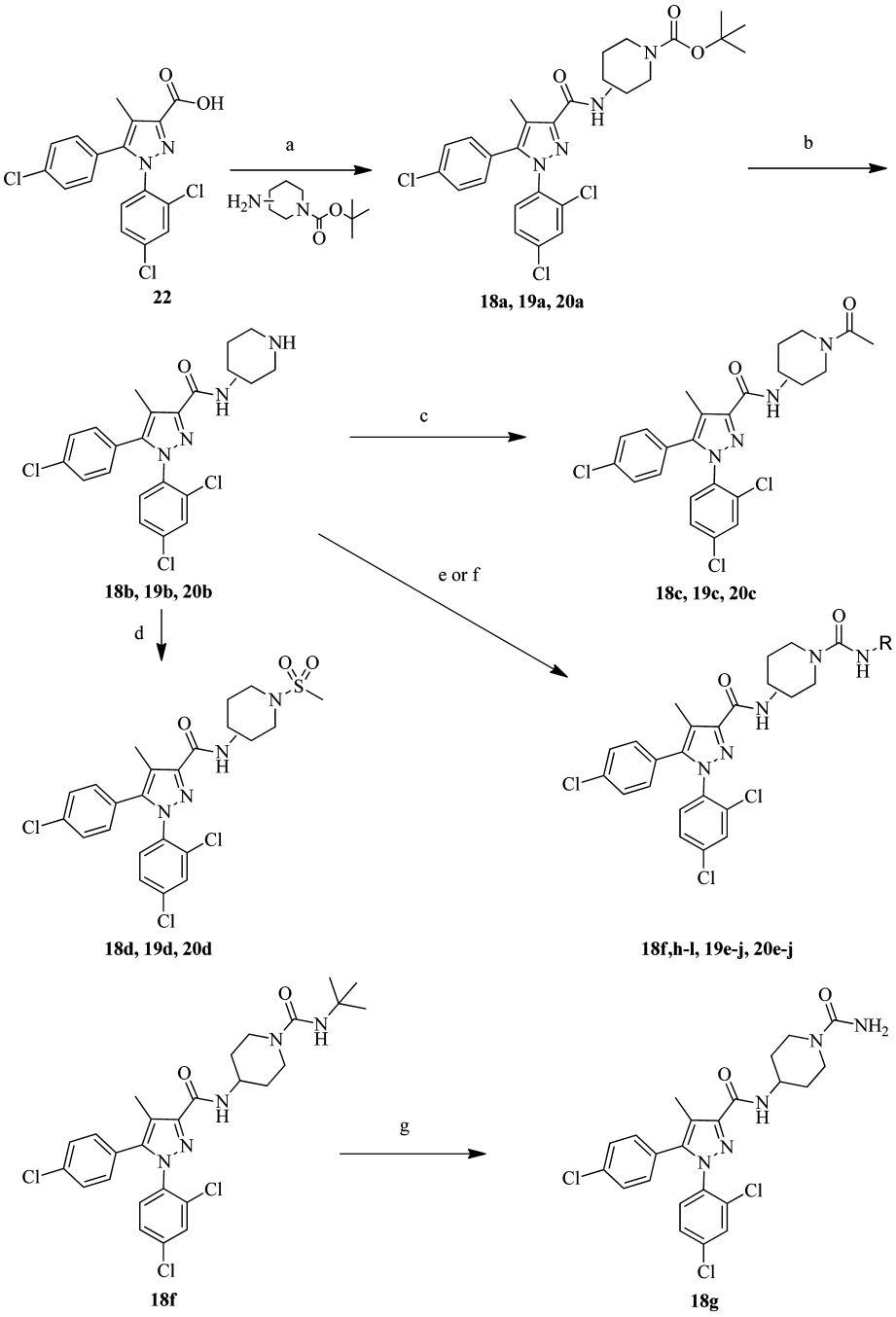
and pyridine to produce amides **18c**, **19c**, and **20c**, respectively, in yields ranging from 81% to 87%. Sulfonamides **18d**, **19d**, and **20d** were made by reacting amines **18b**, **19b**, and **20b** with methanesulfonyl chloride and triethylamine in THF. Ureas **18f,h–l**, **19e–j**, and **20e–j** were made by reacting the appropriate amine with the appropriate isocyanate in dichloromethane or THF. The *N-tert*-butylpiperidinecarboxamide **18f** was converted to the unsubstituted piperidine carboxamide **18g** in 81% yield by stirring **18f** in 50% TFA in dichloromethane.[12] The sulfamide **18e** was synthesized from the reaction of **18b** with excess sulfamide in dioxane at 90 °C in 67% yield (Scheme 4).[7] Ureas **21a–e** were made as a mixture of cis/trans isomers from the previously described amine **26** in yields ranging from 71% to 98%.[7]

In Vitro Metabolic Stability and in Vivo Evaluation of Brain Penetration. A small set of compounds that were potent, selective, and predicted not to penetrate the CNS as determined using the MDCK-mdr1 assay were tested for in vitro metabolic stability (Table 4). Stability was measured in human plasma and human hepatic S9 fractions to gauge any metabolic liabilities that might be present with these compounds. All compounds tested had good stability in plasma. Stabilities of compounds in S9 fractions were more variable than stabilities in plasma. However, all compounds except **17b** displayed metabolic stabilities similar to or greater than compound **1**.

Compounds were prioritized and progressed into in vivo experiments in mice for analysis of brain penetration (Table 5). Compound **13** was not progressed because of its relatively low selectivity compared with other compounds found in Table 4, and compound **17b** was not progressed because of its relatively low stability in S9 fractions. Ureas **17a** and **18f** along with carbamate **18a** were chosen and evaluated in vivo. Urea **17a** was bioavailable with either oral or intraperitoneal (ip) dosing. Brain levels of **17a** were below the lower limit of quantitation when dosed orally, and its brain to plasma ratio was 0.03 with ip

dx.doi.org/10.1021/jm201731z | J. Med. Chem. 2012, 55, 2820–2834

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



[a]Reagents and conditions: (a) amine, BOP, triethylamine, THF; (b) 30% TFA in dichloromethane; (c) acetic anhydride, pyridine; (d) methanesulfonyl chloride, triethylamine, THF; (e) isocyanate, triethylamine, THF, rt; (f) ethyl chloroformate, triethylamine, THF; (g) 50% TFA in dichloromethane.

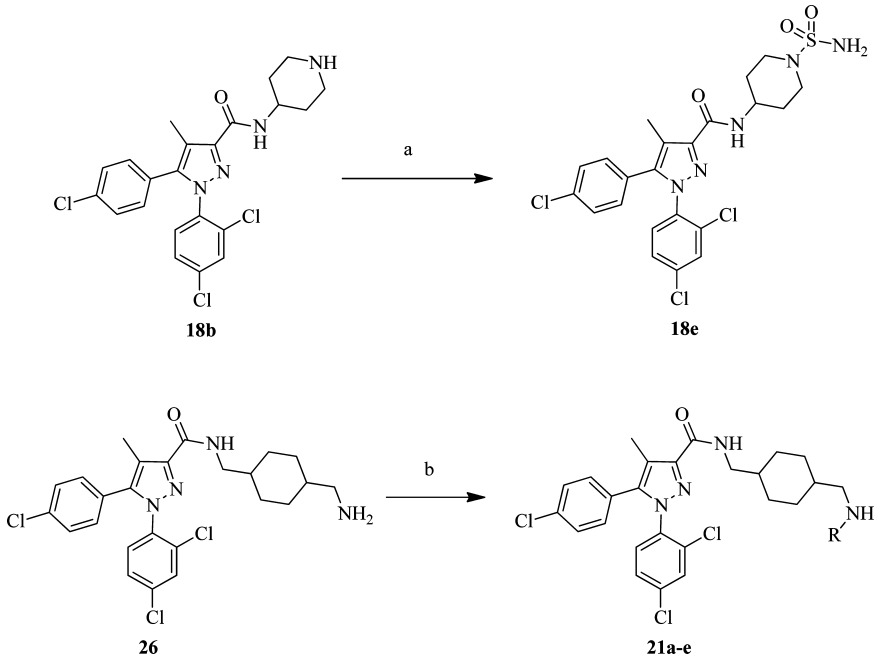
dosing at 1 h. Carbamate **18a** was also bioavailable with either oral or ip dosing. When dosed by ip injection, carbamate **18a** had a brain to plasma ratio of 0.02 at 1 h. Urea **18f** was also bioavailable with oral or intraperitoneal dosing. However, brain to plasma ratios for urea **18f** were 0.16 with oral dosing at 1 h and were 0.38 with intraperitoneal dosing at 1 h. Since unperfused brains were examined and because the volume of blood in the unperfused brain is ∼2−4%,[13] these promising results indicated that **17a**and **18a** had little to no permeability into the brain as expected while **18f** was not selective for the periphery.

DISCUSSION

In this publication we report our ongoing efforts to produce peripherally selective CB1 antagonists that may be useful in treating a wide range of clinical indications. We have now identified several highly selective CB1 antagonists that are metabolically stable with limited oral bioavailability. The addition of polarity to the 3-carboxamide position has been found to be advantageous. Sulfonamide **7** came out of our efforts to synthesize CB1 antagonists that had high TPSAs.[7] Those efforts focused on compounds that contained sulfonamide and sulfamide functionality because of the

dx.doi.org/10.1021/jm201731z | J. Med. Chem. 2012, 55, 2820−2834

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



[a]Reagents and conditions: (a) sulfamide, dioxane, 90 °C; (b) isocyanate, triethylamine, THF, rt.

Table 4. Pharmacological Assessment of Select Compounds in for *in Vitro* Metabolic Stability

					in vitro metabolic stability	
compd	TPSA	K_e (nM) CB1	CB2/CB1 [^{3}H] CP55940	MDCK-mdr1 A to B (%)	S9 (% remaining, 120 min)	plasma (% remaining, 60 min)
1	50	1.1	50.6	15	47	>90
7	101	113	16	<1	37	>90
8	127	106	17	<1	62	>90
13	76	20.2	49.9	<1	88	>90
17a	79	2.4	424.6	<1	88	>90
17b	79	2.09	398	<1	18	82
18a	76	4.7	877.6	<1	>90	>90
18f	79	78	227.8	<1	67	71

relatively large TPSA for these functional groups. Continued efforts along those lines have yielded more positive results, including two new sulfonamides **19d** and **20d** that are more selective than **7**. Sulfonamide **7** demonstrated modest selectivity for CB1 versus CB2 (16-fold), but both **19d** and **20d** demonstrated over 100-fold selectivity. This improvement in selectivity came with an increase in potency as well; sulfonamide **20d** is 16-fold more potent than **7**.

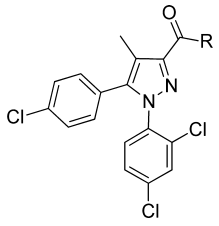
Sulfonamides and sulfamides were not the only polar functionalities to be utilized. Amides, ureas, and carbamates were also synthesized to increase the polarity. Examples of all three have been found to be potent and selective. However, polar groups caused a loss of activity unless they were accompanied by additional lipophilicity. This is best demonstrated by comparing **18g** to **18h**. The unsubstituted piperidinecarboxamide **18g** contained no additional lipophilicity and had poor activity at CB1 (**18g**, K_e(CB1) = 4097 nM). With the addition of an ethyl group, such as found in compound **18h**, the activity was significantly increased (**18h**, K_e(CB1) = 20.5 nM). The enhancing effects of lipophilicity on potency at CB1 could be also seen in compounds **13**−**17k**.

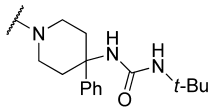
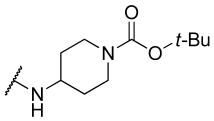
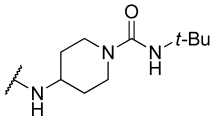
The shape of the functional group seemed to have impact on potency. The linear (4-aminopiperdine) linker seemed to be favored over the bent (3-aminopiperidine) linker for potency. Structure **18** was found to be the most potent analogue in 6 out of the 10 examples where structures **18**−**20** possessed the same substituent. However, those six analogues were all ureas or carbamates. Of the analogues that favored the bent (3-aminopiperidine) linker only one contained a urea (**20g**). Amine (**19b** and **20b**), amide (**19c**), and sulfonamide (**20d**) substituents favored the 3-aminopiperdine linker. Of the two bent (**19**, (3*S*)-3-aminopiperdine; **20**, (3*R*)-3-aminopiperdine) linkers, the *R* enantiomer (**20**) was the most favored for activity at CB1. Analogues of structure **20** were found to be more potent than their corresponding analogues of structure **19** in 7 out of 10 times. The 4-amino-4-phenylpiperidine linker present in compounds **13**−**17k** was by far the most potent linker tested. However, it was difficult to determine if the improved potency observed with the 4-amino-4-phenylpiperidine linker was due to shape or the greater lipophilicity present with this linker.

While maintaining a desirable profile in the MDCK-mdr1 assay, which is predictive of brain penetration, gains were made in selectivity over our previously reported sulfonamide **7**. Fifteen compounds with over 100-fold selectivity (CB1/CB2 K_i vs CP55940) have been identified. These compounds, at least in part, were designed to increase the TPSA over currently known CB1 antagonists in hopes of limiting exposure to the CNS. They were found to have limited permeability (<1%) in the MDCK-mdr1 permeability assay, which serves as an in vitro measure of CNS permeability. On the basis of data from the MDCK-mdr1 permeability assay, a set of compounds were chosen for both plasma and metabolic stability studies in human plasma and hepatic S9 fractions. These studies demonstrated that most compounds tested were at least as

dx.doi.org/10.1021/jm201731z | *J. Med. Chem.* 2012, 55, 2820−2834

Table 5. In Vivo Evaluation of Select Compounds



Comd	R	Route of administration	Sacrifice Time (min)	Plasma Conc. (ng/mL)	Brain Conc. (ng/mL)	Brain:Plasma
17a		Oral	30	3.72	NA	NA
		Oral	60	10.3	LOQ[a]	NA
		ip	60	386	12.4	0.0320
18a		Oral	30	LOQ[a]	NA[b]	NA[b]
		Oral	60	13.2	LOQ[a]	NA[b]
		ip	60	197	4.20	0.0214
18f		Oral	30	5.08	NA	NA
		Oral	60	28.0	4.46	0.160
		ip	60	67.4	25.5	0.379

[a]LOQ: below limit of quantitation. [b]NA: not applicable.

stable as **1** with low loss of the parent molecule even after 2 h of incubation. Further evidence that some of these compounds do not penetrate the CNS was seen in an in vivo pharmacokinetics (PK) assay on compounds **17a**, **18a**, and **18f**. Of these, **17a** and **18a** had little to no CNS penetration as demonstrated by a very low brain/plasma ratio. Further, both compounds demonstrated limited but clearly detectable oral absorption. Future studies will be aimed at improving the oral bioavailability of this class of compounds as well as exploring other scaffolds. Efficacy studies in disease models where these compounds may be useful are being planned as are more detailed PK studies to establish compound half-lives and dosing regimens.

In conclusion, a series of highly potent and selective CB1 antagonists were synthesized and evaluated leading to the identification of two compounds with limited brain penetration. These compounds will serve as templates for further refinement to enhance their oral bioavailability and to examine the role of peripheral CB1 receptors in various diseases such as obesity, liver fibrosis, and diabetes.

EXPERIMENTAL SECTION

Compound Synthesis and Characterization. Chemistry. Reactions were conducted under N_2 atmosphere using oven-dried glassware. All solvents and chemicals used were reagent grade. Anhydrous tetrahydrofuran, dichloromethane, and *N*,*N*-dimethylformamide (DMF) were purchased from Aldrich and used as such. Unless otherwise mentioned, all reagents and chemicals were purchased from commercial vendors and used as received. Flash column chromatography was carried out on a Teledyne ISCO CombiFlash Companion system using RediSep Rf prepacked columns. 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 constants (*J*) 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 mm × 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.

1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidine-4-carboxylic Acid (10). A 2 M solution of oxalyl chloride in dichloromethane (3 equiv, 0.19 mL, 0.377 mmol) was added to 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (**22**) (1 equiv, 48 mg, 0.126 mmol) in dichloromethane (5 mL). Next, 2 drops of anhydrous *N*,*N*-dimethylformamide was added, and the mixture was stirred for 2 h. The mixture was concentrated in vacuo. The reaction mixture was dissolved in dichloromethane (5 mL). Triethylamine (3 equiv, 0.05 mL, 0.377 mmol) and 4-carboxy-4-phenylpiperidin-1-ium chloride (**23**) (1.5 equiv, 45.7 mg, 0.189 mmol) was added, and the mixture was stirred for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−10% methanol/dichloromethane with 1% acetic acid to yield pure desired product (**10**) (48 mg, 67%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.87−2.09 (m, 2 H), 2.15 (s, 3 H), 2.61 (t, *J* = 16.18 Hz, 2 H), 3.21 (t, *J* = 12.03 Hz, 1 H), 3.47 (t, *J* = 11.94 Hz, 1 H), 4.26 (d, *J* = 13.61 Hz, 1 H), 4.57 (d, *J* = 13.56 Hz, 1 H), 7.05 (d, *J* = 8.34 Hz, 2 H), 7.12−7.45 (m, 10 H); $[M + H]^+$ 568.4.

1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidine-4-carboxamide (11). 1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidine-4-carboxylic acid (**22**) (1 equiv, 12.7 mg, 0.024 mmol), ammonium chloride (10 equiv, 12.7 mg, 0.24 mmol), benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (1 equiv, 10.5 mg, 0.024 mmol), and triethylamine (10.1 equiv, 0.03 mL, 0.024 mmol) were stirred in tetrahydrofuran (5 mL) for 3 days. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel

dx.doi.org/10.1021/jm201731z | *J. Med. Chem.* 2012, 55, 2820−2834

column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**11**) (6 mg, 44%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.92−2.31 (m, 5 H), 2.46 (d, *J* = 13.94 Hz, 2 H), 3.65 (t, *J* = 10.36 Hz, 1 H), 3.75−3.90 (m, 1 H), 4.02 (d, *J* = 13.38 Hz, 1 H), 4.23 (d, *J* = 13.00 Hz, 1 H), 5.24 (br s, 2 H), 7.07 (d, *J* = 8.38 Hz, 2 H), 7.12−7.20 (m, 1 H), 7.20−7.49 (m, 9 H); $[M + H]^+$ 567.5.

1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-(ethylamino)piperidine-4-carboxamide (12). 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (**22**) (1 equiv, 20 mg, 0.052 mmol), triethylamine (3 equiv, 0.02 mL, 0.157 mmol), 4-(ethylamino)-4-piperidinecarboxamide (1 equiv, 9 mg, 0.052 mmol), and benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (1 equiv, 23 mg, 0.052 mmol) were stirred in tetrahydrofuran (5 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% CMA 80 (chloroform, methanol, ammonium hydroxide 80:18:2)/ethyl acetate and precipitated from ethyl acetate with hexane to yield pure desired product (**12**) (13 mg, 46%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.10 (t, *J* = 6.97 Hz, 3 H), 1.61−1.80 (m, 2 H), 2.08−2.26 (m, 5 H), 2.45−2.63 (m, 2 H), 3.68 (td, *J* = 8.85, 4.43 Hz, 2 H), 3.96−4.19 (m, 2 H), 5.40 (br s, 1 H), 7.07 (d, *J* = 8.29 Hz, 2 H), 7.12−7.19 (m, 1 H), 7.20−7.36 (m, 3 H), 7.44 (d, *J* = 1.98 Hz, 1 H); $[M + H]^+$ 534.5.

***tert*-Butyl *N*-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)carbamate (13).** 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (**22**) (1 equiv, 201 mg, 0.53 mmol), triethylamine (3 equiv, 0.22 mL, 0.157 mmol), *tert*-butyl *N*-(4-phenylpiperidin-4-yl)carbamate (**25**) (1 equiv, 146 mg, 0.53 mmol), and benzotriazole-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (1 equiv, 233 mg, 0.53 mmol) were stirred in tetrahydrofuran (10 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**13**) (295 mg, 87%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.37 (br s, 9 H), 2.11 (dd, *J* = 12.67, 4.00 Hz, 2 H), 2.21 (s, 3 H), 2.24−2.34 (m, 1 H), 2.34−2.56 (m, 1 H), 3.26 (t, *J* = 12.01 Hz, 1 H), 3.56 (t, *J* = 12.39 Hz, 1 H), 4.32 (d, *J* = 13.75 Hz, 1 H), 4.64 (d, *J* = 13.56 Hz, 1 H), 4.96 (br s, 1 H), 7.08 (d, *J* = 8.38 Hz, 2 H), 7.13−7.49 (m, 10 H); $[M + H]^+$ 639.7.

1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-amine (14). *tert*-Butyl *N*-(1-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)carbamate (**13**) (1 equiv, 243 mg, 0.380 mmol) was stirred in dichloromethane (7 mL) and trifluoroacetic acid (3 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−50% CMA 80/ethyl acetate to yield pure desired product (**14**) (178 mg, 87%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.64−1.95 (m, 2 H), 2.10−2.29 (m, 5 H), 3.50−3.66 (m, 1 H), 3.70−3.88 (m, 1 H), 4.03−4.19 (m, 1 H), 4.42 (d, *J* = 13.28 Hz, 1 H), 7.04−7.10 (m, 2 H), 7.13−7.20 (m, 1 H), 7.20−7.40 (m, 6 H), 7.40−7.50 (m, 3 H); $[M + H]^+$ 539.4.

***N*-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)acetamide (15).** 1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-amine (**14**) (1 equiv, 35.3 mg, 0.066 mmol) was stirred in a mixture of acetic anhydride (2 mL) and pyridine (2 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**15**) (27 mg, 71%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.01 (s, 3 H), 2.05−2.19 (m, 2 H), 2.21 (s, 3 H), 2.34 (d, *J* = 14.60 Hz, 1 H), 2.66 (d, *J* = 13.85 Hz, 1 H), 3.15−3.34 (m, 1 H), 3.52 (t, *J* = 11.68 Hz, 1 H), 4.26 (d, *J* = 13.75 Hz, 1 H), 4.54 (d, *J* = 13.75 Hz, 1 H), 6.10 (s, 1 H), 7.03−7.11 (m, 2 H), 7.14−7.19 (m, 1 H), 7.19−7.41 (m, 8 H), 7.44 (d, *J* = 2.17 Hz, 1 H); $[M + H]^+$ 581.0.

***N*-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-methanesulfonamide (16).** 1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-amine (**14**) (1 equiv, 36.5 mg, 0.068 mmol), methanesulfonyl chloride (2 equiv, 0.01 mL, 0.135 mmol), and triethylamine (3 equiv, 0.03 mL, 0.203 mmol) were stirred in tetrahydrofuran for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**16**) (27 mg, 65%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.18 (d, *J* = 4.52 Hz, 6 H), 2.21−2.37 (m, 2 H), 2.39−2.61 (m, 2 H), 3.65 (t, *J* = 10.69 Hz, 1 H), 3.86 (t, *J* = 10.93 Hz, 1 H), 4.07−4.20 (m, 2 H), 4.29 (d, *J* = 13.75 Hz, 1 H), 5.30 (s, 1 H), 7.03−7.11 (m, 2 H), 7.15−7.21 (m, 1 H), 7.21−7.38 (m, 4 H), 7.38−7.47 (m, 2 H), 7.47−7.55 (m, 2 H); $[M + H]^+$ 617.3.

3-*tert*-Butyl-1-(1-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)urea (17a). 1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-amine (**14**) (1 equiv, 39.3 mg, 0.073 mmol), *tert*-butyl isocyanate (1.5 equiv, 0.013 mL, 0.109 mmol), and triethylamine (3.0 equiv, 0.03 mL, 0.218 mmol) were stirred in dichloromethane (5 mL) for 16 h. Next, tetrahydrofuran (5 mL) and an additional 0.02 mL of *tert*-butyl isocyanate were added, and the mixture was stirred for 16 h. Finally, the mixture was heated to 40 °C for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**17a**) (21 mg, 45%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.15 (s, 9 H), 1.93−2.17 (m, 4 H), 2.20 (s, 3 H), 2.42 (br s, 1 H), 3.13−3.35 (m, 1 H), 3.58 (br s, 1 H), 4.25 (br s, 1 H), 4.44 (s, 1 H), 4.52−4.69 (m, 1 H), 5.13 (s, 1 H), 7.03−7.10 (m, 2 H), 7.13−7.37 (m, 7 H), 7.38−7.46 (m, 3 H); $[M + H]^+$ 638.7.

1-Benzyl-3-(1-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)urea (17b). Amine **14** (1 equiv, 26.1 mg, 0.048 mmol), benzyl isocyanate (1.5 equiv, 9.7 mg, 0.073 mmol), and triethylamine (3.0 equiv, 0.02 mL, 0.145 mmol) were stirred in THF (2 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield **17b** (24 mg, 74%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.79−2.13 (m, 3 H), 2.17 (s, 3 H), 2.53 (br s, 1 H), 3.13 (br s, 1 H), 3.48 (br s, 1 H), 4.21 (d, *J* = 5.75 Hz, 3 H), 4.49 (d, *J* = 13.47 Hz, 1 H), 5.24 (br s, 1 H), 5.53 (s, 1 H), 6.94−7.49 (m, 17 H); $[M + H]^+$ 672.4.

3-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-1-(4-cyanophenyl)urea (17c). Following a procedure similar to the preparation of **17b**, **17c** was obtained from **14** and the appropriate isocyanate in 74% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.98 (br s, 2 H), 2.11−2.41 (m, 4 H), 2.87 (d, *J* = 13.47 Hz, 1 H), 3.40 (br s, 1 H), 3.64 (br s, 1 H), 3.97−4.24 (m, 1 H), 4.58 (d, *J* = 13.19 Hz, 1 H), 6.79 (s, 1 H), 6.90−7.52 (m, 16 H), 8.56 (br s, 1 H); $[M + H]^+$ 683.8.

3-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-1-(4-fluorophenyl)urea (17d). Following a procedure similar to the preparation of **17b**, **17d** was obtained from **14** and the appropriate isocyanate in 67% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.95 (br s, 2 H), 2.08−2.34 (m, 4 H), 2.83 (d, *J* = 13.37 Hz, 1 H), 3.23−3.41 (m, 1 H), 3.58 (t, *J* = 12.29 Hz, 1 H), 4.21 (d, *J* = 13.56 Hz, 1 H), 4.58 (d, *J* = 13.28 Hz, 1 H), 6.31 (br s, 1 H), 6.82 (t, *J* = 8.62 Hz, 2 H), 6.93−7.48 (m, 14 H), 7.78 (br s, 1 H); $[M + H]^+$ 676.3.

3-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-1-[4-(dimethylamino)phenyl]urea (17e). Following a procedure similar to the preparation of **17b**, **17e** was obtained from **14** and the appropriate isocyanate in 58% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.86−2.15 (m, 3 H), 2.21 (s, 3 H), 2.62 (d, *J* = 13.47 Hz, 1 H), 2.90 (s, 6 H), 3.11 (br s, 1 H), 3.47 (br s, 1 H), 4.29 (d, *J* = 13.37 Hz, 1 H), 4.59 (d, *J* = 13.37 Hz, 1 H), 5.40 (br s, 1 H), 6.50 (br s, 1 H), 6.66 (d, *J* = 8.67 Hz, 2 H), 6.94−7.49 (m, 14 H); $[M + H]^+$ 701.6.

1-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-3-hexylurea

(17f). Following a procedure similar to the preparation of **17b**, **17f** was obtained from **14** and the appropriate isocyanate in 72% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.84 (t, J = 6.78 Hz, 3 H), 1.04−1.38 (m, 8 H), 1.95−2.17 (m, 3 H), 2.17−2.25 (m, 3 H), 2.51 (br s, 1 H), 2.90−3.11 (m, 2 H), 3.24 (br s, 1 H), 3.55 (br s, 1 H), 4.28 (d, J = 13.56 Hz, 1 H), 4.48−4.65 (m, 2 H), 5.18−5.38 (m, 1 H), 6.96−7.51 (m, 12 H); $[M - H]^-$ 666.8.

1-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-3-(propan-2-yl)urea (17g). Following a procedure similar to the preparation of **17b**, **17g** was obtained from **14** and the appropriate isocyanate in 73% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.82−1.03 (m, 6 H) 1.94−2.24 (m, 6 H) 2.46 (d, J = 13.47 Hz, 1 H) 3.26 (t, J = 11.26 Hz, 1 H) 3.56 (t, J = 12.10 Hz, 1 H) 3.68−3.85 (m, 1 H) 4.16−4.37 (m, 2 H) 4.59 (d, J = 13.47 Hz, 1 H) 5.05 (s, 1 H) 6.94−7.49 (m, 12 H); $[M + H]^+$ 624.7.

1-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-3-ethylurea (17h). Following a procedure similar to the preparation of **17b**, **17h** was obtained from **14** and the appropriate isocyanate in 73% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.78−1.04 (m, 3 H), 1.92−2.25 (m, 6 H), 2.51 (d, J = 13.56 Hz, 1 H), 2.98−3.15 (m, 2 H), 3.24 (br s, 1 H), 3.55 (br s, 1 H), 4.29 (br s, 1 H), 4.44−4.68 (m, 2 H), 5.23 (s, 1 H), 6.90−7.49 (m, 12 H); $[M + H]^+$ 610.1.

1-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-3-propylurea (17i). Following a procedure similar to the preparation of **17b**, **17i** was obtained from **14** and the appropriate isocyanate in 71% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.74 (t, J = 7.39 Hz, 3 H), 1.21−1.40 (m, 2 H), 2.05−2.29 (m, 6 H), 2.35 (br s, 1 H), 3.02 (q, J = 6.72 Hz, 2 H), 3.28 (br s, 1 H), 3.57 (br s, 1 H), 4.07 (t, J = 5.27 Hz, 1 H), 4.34 (d, J = 13.66 Hz, 1 H), 4.63 (d, J = 14.32 Hz, 1 H), 4.74 (s, 1 H), 7.00−7.54 (m, 12 H); $[M + H]^+$ 624.8.

3-(1-{[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)-1-cyclohexylurea (17j). Following a procedure similar to the preparation of **17b**, **17j** was obtained from **14** and the appropriate isocyanate in 69% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.78−0.98 (m, 2 H), 1.06 (d, J = 9.89 Hz, 1 H), 1.16−1.35 (m, 2 H), 1.50 (d, J = 8.76 Hz, 3 H), 1.73 (d, J = 10.83 Hz, 2 H), 2.00−2.27 (m, 6 H), 2.43 (d, J = 13.56 Hz, 1 H), 3.26 (br s, 1 H), 3.37−3.66 (m, 2 H), 4.15−4.40 (m, 2 H), 4.62 (br s, 1 H), 4.99 (s, 1 H), 6.90−7.55 (m, 12 H); $[M + H]^+$ 664.9.

3-Butyl-1-(1-{[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl]carbonyl}-4-phenylpiperidin-4-yl)urea (17k). Following a procedure similar to the preparation of **17b**, **17k** was obtained from **14** and the appropriate isocyanate in 71% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.76−0.86 (m, 3 H), 1.08−1.21 (m, 2 H), 1.28 (dq, J = 14.40, 7.10 Hz, 2 H), 1.94−2.26 (m, 6 H), 2.50 (d, J = 13.47 Hz, 1 H), 3.04 (q, J = 6.56 Hz, 2 H), 3.15−3.32 (m, 1 H), 3.55 (t, J = 12.15 Hz, 1 H), 4.27 (d, J = 13.56 Hz, 1 H), 4.48−4.74 (m, 2 H), 5.33 (s, 1 H), 6.98−7.49 (m, 12 H); $[M + H]^+$ 638.6.

***tert*-Butyl 4-[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (18a).** Benzotriazole-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (1 equiv, 490 mg, 1.11 mmol) was added to a solution of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (**22**) (1 equiv, 422 mg, 1.11 mmol), *tert*-butyl 4-amino-1-piperidinecarboxylate (1 equiv, 222 mg, 1.11 mmol), and triethylamine (3 equiv, 0.46 mL, 3.32 mmol) in tetrahydrofuran (5 mL). The reaction mixture was stirred for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**18a**) (548 mg, 88%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.33−1.51 (m, 9 H), 1.93−2.10 (m, 2 H), 2.37 (s, 3 H), 2.91 (t, J = 11.82 Hz, 2 H), 3.89−4.23 (m, 2 H), 6.84 (d, J = 8.19 Hz, 1 H), 7.00−7.12 (m, 2 H), 7.19−7.36 (m, 4 H), 7.43 (d, J = 1.32 Hz, 1 H); $[M + H]^+$ 563.6.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (18b). *tert*-Butyl 4-[5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (**18a**) (1 equiv, 531 mg, 0.941 mmol) was stirred in dichloromethane (4 mL) and trifluoroacetic acid (1 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% CMA 80/ethyl acetate to yield pure desired product (**18b**) (415 mg, 95%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.44 (qd, J = 11.81, 3.86 Hz, 2 H), 1.92−2.08 (m, 2 H), 2.37 (s, 3 H), 2.64−2.85 (m, 2 H), 2.98−3.21 (m, 2 H), 3.92−4.19 (m, 1 H), 6.85 (d, J = 8.29 Hz, 1 H), 7.06 (d, J = 8.38 Hz, 2 H), 7.28 (s, 4 H), 7.43 (s, 1 H); $[M + H]^+$ 463.5.

***N*-(1-Acetylpiperidin-4-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (18c).** 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (**18b**) (1 equiv, 34 mg, 0.073 mmol) was stirred in pyridine (1 mL) and acetic anhydride (1 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**18c**) (30 mg, 81%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.28−1.49 (m, 2 H), 1.87−2.13 (m, 5 H), 2.30 (s, 3 H), 2.62−2.82 (m, 1 H), 3.05−3.24 (m, 1 H), 3.75 (d, J = 13.56 Hz, 1 H), 3.98−4.25 (m, 1 H), 4.49 (d, J = 13.37 Hz, 1 H), 6.80 (d, J = 8.01 Hz, 1 H), 6.99 (d, J = 8.48 Hz, 2 H), 7.13−7.29 (m, 4 H), 7.36 (d, J = 1.51 Hz, 1 H); $[M + H]^+$ 505.5.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(1-methanesulfonylpiperidin-4-yl)-4-methyl-1*H*-pyrazole-3-carboxamide (18d). Methanesulfonyl chloride (2 equiv, 0.01 mL, 0.15 mmol) was added to 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (**18b**) (1 equiv, 35 mg, 0.076 mmol) and triethyamine (3 equiv, 0.03 mL, 0.227 mmol) in tetrahydrofuran (2 mL). The mixture was stirred for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**18d**) (36 mg, 87%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.53−1.78 (m, 2 H), 2.06−2.22 (m, 2 H), 2.37 (s, 3 H), 2.67−3.00 (m, 5 H), 3.82 (d, J = 12.24 Hz, 2 H), 4.01−4.17 (m, 1 H), 6.88 (d, J = 8.01 Hz, 1 H), 7.07 (s, 2 H), 7.19−7.36 (m, 4 H), 7.43 (d, J = 1.70 Hz, 1 H); $[M + H]^+$ 543.6.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(1-sulfamoylpiperidin-4-yl)-1*H*-pyrazole-3-carboxamide (18e). 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (**18b**) (1 equiv, 38 mg, 0.082 mmol) and sulfamide (5 equiv, 39 mg, 0.41 mmol) were heated to 90 °C in dioxane (2 mL) for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield desired product (**18e**) (30 mg, 67%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.60−1.78 (m, 2 H), 2.07−2.21 (m, 2 H), 2.37 (s, 3 H), 2.86 (t, J = 10.83 Hz, 2 H), 3.74 (d, J = 12.24 Hz, 2 H), 4.00−4.16 (m, 1 H), 4.37 (s, 2 H), 6.87 (d, J = 7.91 Hz, 1 H), 7.06 (d, J = 8.38 Hz, 2 H), 7.20−7.37 (m, 4 H), 7.43 (d, J = 1.32 Hz, 1 H); $[M + H]^+$ 542.7.

1-*N*-*tert*-Butyl-4-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,4-diamido (18f). 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-4-yl)-1*H*-pyrazole-3-carboxamide (**18b**) (1 equiv, 38 mg, 0.082 mmol), *tert*-butyl isocyanate (1.5 equiv, 0.014 mL, 0.123 mmol), and triethylamine (3 equiv, 0.034 mL, 0.246 mmol) were stirred in dichloromethane for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield pure desired product (**18f**) (42 mg, 91%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.27 (d, J = 7.16 Hz, 9 H), 1.40−1.73 (m, 2 H), 2.05 (s, 2 H), 2.37 (s, 2 H), 2.92 (t, J = 11.44 Hz, 2 H), 3.87 (d, J = 13.37 Hz, 2 H), 4.00−4.18 (m, 1 H), 4.33 (s, 1 H), 6.84 (d, J = 7.91 Hz, 1 H), 7.05 (d, J = 8.48 Hz, 2 H), 7.20−7.35 (m, 4 H), 7.42 (s, 1 H); $[M + H]^+$ 562.4.

4-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,4-diamido (18g). 1-*N*-*tert*-Butyl-4-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,4-diamido (**18f**) (1 equiv, 33 mg, 0.059 mmol) was stirred in dichloromethane (2 mL) and trifluoroacetic acid (2 mL) overnight. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100%

dx.doi.org/10.1021/jm201731z | *J. Med. Chem.* 2012, 55, 2820−2834

CMA 80/ethyl acetate to yield pure desired product (**18g**) (24 mg, 81%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.41−1.60 (m, 2 H), 1.99−2.12 (m, 2 H), 2.37 (s, 3 H), 3.01 (t, *J* = 11.77 Hz, 2 H), 3.94 (d, *J* = 13.09 Hz, 2 H), 4.03−4.24 (m, 1 H), 4.65 (br s, 2 H), 6.90 (d, *J* = 7.91 Hz, 1 H), 7.06 (d, *J* = 8.38 Hz, 2 H), 7.22−7.37 (m, 4 H), 7.43 (s, 1 H); $[M + H]^+$ 506.4.

4-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-ethylpiperidine-1,4-diamido (18h). Following a procedure similar to the preparation of **17b**, **18h** was obtained from **18b** and the appropriate isocyanate in 97% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.15 (t, *J* = 7.21 Hz, 3 H), 1.35−1.60 (m, 2 H), 1.93−2.14 (m, 2 H), 2.38 (s, 3 H), 2.97 (t, *J* = 11.54 Hz, 2 H), 3.16−3.38 (m, 2 H), 3.94 (d, *J* = 13.47 Hz, 2 H), 4.04−4.25 (m, 1 H), 4.46 (br s, 1 H), 6.87 (d, *J* = 8.01 Hz, 1 H), 7.08 (s, 2 H), 7.23−7.37 (m, 4 H), 7.44 (d, *J* = 1.22 Hz, 1 H); $[M + H]^+$ 534.4.

4-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-(propan-2-yl)piperidine-1,4-diamido (18i). Following a procedure similar to the preparation of **17b**, **18i** was obtained from **18b** and the appropriate isocyanate in 99% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.16 (d, *J* = 6.50 Hz, 6 H), 1.49 (dd, *J* = 11.68, 3.11 Hz, 2 H), 1.98−2.11 (m, 2 H), 2.38 (s, 3 H), 2.95 (t, *J* = 11.68 Hz, 2 H), 3.86−4.02 (m, 3 H), 4.11 (dd, *J* = 13.70, 6.92 Hz, 1 H), 4.27 (d, *J* = 7.16 Hz, 1 H), 6.86 (d, *J* = 7.91 Hz, 1 H), 7.07 (d, *J* = 8.38 Hz, 2 H), 7.25−7.36 (m, 4 H), 7.44 (s, 1 H); $[M + H]^+$ 548.5.

4-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-propylpiperidine-1,4-diamido (18j). Following a procedure similar to the preparation of **17b**, **18j** was obtained from **18b** and the appropriate isocyanate in 95% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.86−0.98 (m, 3 H), 1.43−1.60 (m, 4 H), 1.94−2.13 (m, 2 H), 2.38 (s, 3 H), 2.97 (t, *J* = 11.77 Hz, 2 H), 3.20 (q, *J* = 6.69 Hz, 2 H), 3.94 (d, *J* = 13.37 Hz, 2 H), 4.09 (d, *J* = 6.78 Hz, 1 H), 4.51 (br s, 1 H), 6.87 (d, *J* = 8.01 Hz, 1 H), 7.07 (d, *J* = 8.29 Hz, 2 H), 7.23−7.37 (m, 4 H), 7.44 (s, 1 H); $[M + H]^+$ 548.6.

1-*N*-Butyl-4-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,4-diamido (18k). Following a procedure similar to the preparation of **17b**, **18k** was obtained from **18b** and the appropriate isocyanate in 76% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.94 (t, *J* = 7.16 Hz, 3 H), 1.28−1.41 (m, 2 H), 1.42−1.58 (m, 4 H), 1.95−2.12 (m, 2 H), 2.38 (s, 3 H), 2.97 (t, *J* = 12.24 Hz, 2 H), 3.16−3.33 (m, 2 H), 3.94 (d, *J* = 13.37 Hz, 2 H), 4.03−4.23 (m, 1 H), 4.47 (br s, 1 H), 6.86 (d, *J* = 7.91 Hz, 1 H), 7.07 (d, *J* = 8.29 Hz, 2 H), 7.23−7.36 (m, 4 H), 7.44 (s, 1 H); $[M + H]^+$ 562.4.

Ethyl 4-[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (18l). Following a procedure similar to the preparation of **20j**, **18l** was obtained from **18b** and ethyl chloroformate in 76% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.27 (d, *J* = 14.22 Hz, 3 H), 1.47 (dd, *J* = 11.63, 3.44 Hz, 2 H), 1.93−2.14 (m, 2 H), 2.38 (s, 3 H), 2.98 (br s, 2 H), 4.14 (q, *J* = 6.97 Hz, 4 H), 6.86 (d, *J* = 8.10 Hz, 1 H), 7.06 (s, 2 H), 7.22−7.38 (m, 4 H), 7.44 (d, *J* = 1.51 Hz, 1 H); $[M + H]^+$ 535.3.

***tert*-Butyl (3*S*)-3-[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (19a).** Following a procedure similar to the preparation of **18a**, **19a** was obtained from **22** and an appropriate amine in 96% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.31 (s, 9 H), 1.49 (dd, *J* = 12.62, 5.84 Hz, 1 H), 1.65 (d, *J* = 5.09 Hz, 2 H), 1.82 (d, *J* = 9.04 Hz, 1 H), 2.29 (s, 3 H), 3.32 (br s, 3 H), 3.48−3.73 (m, 1 H), 3.93−4.14 (m, 1 H), 6.99 (d, *J* = 8.38 Hz, 3 H), 7.12−7.27 (m, 4 H), 7.33 (s, 1 H); $[M + H]^+$ 563.4.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-[(3*S*)-piperidin-3-yl]-1*H*-pyrazole-3-carboxamide (19b). Following a procedure similar to the preparation of **18b**, **19b** was obtained from **19a** in >99% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.42−1.95 (m, 4 H), 2.26 (s, 3 H), 2.86 (dd, *J* = 12.15, 8.76 Hz, 2 H), 3.09 (d, *J* = 12.81 Hz, 1 H), 3.40 (dd, *J* = 12.20, 2.97 Hz, 1 H), 4.13−4.32 (m, 1 H), 6.99 (s, 2 H), 7.09−7.28 (m, 5 H), 7.33 (s, 1 H); $[M + H]^+$ 463.7.

***N*-[(3*S*)-1-Acetylpiperidin-3-yl]-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (19c).** Following a procedure similar to the preparation of **18c**, **19c** was obtained from **19b** in 87% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.62 (s, 4 H), 2.01−2.08 (m, 3 H), 2.30 (s, 3 H), 3.10−3.29 (m, 2 H), 3.81 (d, *J* = 13.19 Hz, 2 H), 4.00 (d, *J* = 6.59 Hz, 1 H), 6.86 (d, *J* = 7.06 Hz, 1 H), 6.99 (d, *J* = 8.38 Hz, 2 H), 7.15−7.28 (m, 4 H), 7.36 (s, 1 H); $[M + H]^+$ 505.6.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-[(3*S*)-1-methanesulfonylpiperidin-3-yl]-4-methyl-1*H*-pyrazole-3-carboxamide (19d). Following a procedure similar to the preparation of **18d**, **19d** was obtained from **19b** in 73% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.58−1.73 (m, 2 H), 1.83 (d, *J* = 10.46 Hz, 2 H), 2.29 (s, 3 H), 2.74 (s, 3 H), 2.99−3.25 (m, 3 H), 3.45 (dd, *J* = 11.73, 3.16 Hz, 1 H), 4.24 (br s, 1 H), 6.84−7.11 (m, 3 H), 7.14−7.28 (m, 4 H), 7.35 (s, 1 H); $[M + H]^+$ 543.5.

(3*S*)-3-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-ethylpiperidine-1,3-diamido (19e). Following a procedure similar to the preparation of **17b**, **19e** was obtained from **19b** and the appropriate isocyanate in 80% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.04 (t, *J* = 7.39 Hz, 3 H), 1.55 (d, *J* = 7.44 Hz, 3 H), 1.91 (br s, 1 H), 2.29 (s, 3 H), 2.95−3.30 (m, 4 H), 3.60 (br s, 1 H), 3.97 (br s, 1 H), 4.63 (br s, 1 H), 6.90 (d, *J* = 6.97 Hz, 1 H), 6.99 (d, *J* = 8.38 Hz, 2 H), 7.15−7.29 (m, 4 H), 7.35 (s, 1 H); $[M - H]^-$ 534.4.

(3*S*)-3-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-(propan-2-yl)piperidine-1,3-diamido (19f). Following a procedure similar to the preparation of **17b**, **19f** was obtained from **19b** and the appropriate isocyanate in 78% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.01 (d, *J* = 6.50 Hz, 3 H), 1.07 (d, *J* = 6.50 Hz, 3 H), 1.41−1.66 (m, 3 H), 1.88 (d, *J* = 6.40 Hz, 1 H), 2.30 (s, 3 H), 3.10−3.27 (m, 2 H), 3.40−3.61 (m, 2 H), 3.87 (d, *J* = 6.59 Hz, 1 H), 3.93−4.04 (m, 1 H), 4.43 (d, *J* = 7.06 Hz, 1 H), 6.81−7.05 (m, 3 H), 7.10−7.28 (m, 4 H), 7.35 (s, 1 H); $[M - H]^-$ 548.7.

(3*S*)-3-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-propylpiperidine-1,3-diamido (19g). Following a procedure similar to the preparation of **17b**, **19g** was obtained from **19b** and the appropriate isocyanate in 78% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.71−0.87 (m, 3 H), 1.35−1.47 (m, 2 H), 1.48−1.63 (m, 3 H), 1.90 (br s, 1 H), 2.29 (s, 3 H), 2.95−3.27 (m, 4 H), 3.58 (br s, 2 H), 3.98 (d, *J* = 6.69 Hz, 1 H), 4.68 (br s, 1 H), 6.86−7.05 (m, 3 H), 7.12−7.29 (m, 4 H), 7.35 (s, 1 H); $[M + H]^+$ 548.6.

(3*S*)-1-*N*-Butyl-3-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,3-diamido (19h). Following a procedure similar to the preparation of **17b**, **19h** was obtained from **19b** and the appropriate isocyanate in 85% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.74−0.94 (m, 3 H), 1.09−1.32 (m, 2 H), 1.33−1.46 (m, 2 H), 1.46−1.63 (m, 3 H), 1.90 (br s, 1 H), 2.29 (s, 3 H), 3.05−3.21 (m, 4 H), 3.58 (t, *J* = 14.32 Hz, 2 H), 3.84−4.06 (m, 1 H), 4.65 (br s, 1 H), 6.90 (d, *J* = 6.88 Hz, 1 H), 6.99 (d, *J* = 8.38 Hz, 2 H), 7.16−7.29 (m, 4 H), 7.35 (s, 1 H); $[M - H]^-$ 562.5.

(3*S*)-1-*N*-*tert*-Butyl-3-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,3-diamido (19i). Following a procedure similar to the preparation of **17b**, **19i** was obtained from **19b** and the appropriate isocyanate in 77% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.07−1.29 (m, 9 H), 1.42−1.60 (m, 2 H), 1.66 (s, 1 H), 1.80−1.95 (m, 1 H), 2.30 (s, 3 H), 3.10−3.31 (m, 2 H), 3.46 (d, *J* = 2.45 Hz, 2 H), 3.99 (br s, 1 H), 4.51 (s, 1 H), 6.99 (d, *J* = 8.29 Hz, 3 H), 7.15−7.31 (m, 4 H), 7.34 (s, 1 H); $[M + H]^+$ 562.4.

Ethyl (3*S*)-3-[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (19j). Following a procedure similar to the preparation of **20j**, **19j** was obtained from **19b** and ethyl chloroformate in 67% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.01−1.20 (m, 3 H), 1.40−1.75 (m, 3 H), 1.91 (br s, 1 H), 2.30 (s, 3 H), 3.16 (br s, 2 H), 3.47−3.64 (m, 1 H), 3.81 (d, *J* = 10.93 Hz, 1 H), 3.93−4.17 (m, 3 H), 6.88 (d, *J* = 8.01 Hz, 1 H), 6.99 (d, *J* = 8.38 Hz, 2 H), 7.15−7.28 (m, 4 H), 7.35 (s, 1 H); $[M + H]^+$ 535.5.

***tert*-Butyl (3*R*)-3-[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (20a).** Following a procedure similar to the preparation of **18a**, **20a** was obtained from **22** and an appropriate amine in 89% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.28−1.42 (m, 9 H), 1.47−1.61 (m, 1 H), 1.70 (d, *J* = 5.18 Hz, 2 H), 1.87 (d, *J* = 8.95 Hz, 1 H), 2.35 (s, 3 H), 3.38 (br s, 3 H), 3.60 (br s, 1 H), 3.95−4.21 (m, 1 H), 7.04 (d, *J* = 8.38 Hz, 3 H), 7.19−7.33 (m, 4 H), 7.39 (s, 1 H); $[M + H]^+$ 563.3.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-[(3*R*)-piperidin-3-yl]-1*H*-pyrazole-3-carboxamide (20b). Following a procedure similar to the preparation of **18b**, **20b** was obtained from **20b** in >99% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.54−2.01 (m, 4 H), 2.33 (s, 3 H), 2.80−3.00 (m, 2 H), 3.17 (d, *J* = 12.62 Hz, 1 H), 3.38−3.57 (m, 1 H), 4.22−4.41 (m, 1 H), 6.50 (br s, 2 H), 7.06 (d, *J* = 8.38 Hz, 2 H), 7.18−7.35 (m, 5 H), 7.41 (s, 1 H); $[M + H]^+$ 463.7.

***N*-[(3*R*)-1-Acetylpiperidin-3-yl]-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (20c).** Following a procedure similar to the preparation of **18c**, **20c** was obtained from **20b** in 87% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.72 (br s, 4 H), 2.11−2.17 (m, 3 H), 2.38 (s, 3 H), 3.18−3.37 (m, 2 H), 3.89 (d, *J* = 13.19 Hz, 2 H), 4.09 (d, *J* = 6.59 Hz, 1 H), 6.95 (d, *J* = 7.06 Hz, 1 H), 7.08 (d, *J* = 8.29 Hz, 2 H), 7.20−7.40 (m, 4 H), 7.44 (s, 1 H); $[M + H]^+$ 505.5.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-[(3*R*)-1-methanesulfonylpiperidin-3-yl]-4-methyl-1*H*-pyrazole-3-carboxamide (20d). Following a procedure similar to the preparation of **18d**, **20d** was obtained from **20b** in 78% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.68−1.82 (m, 2 H), 1.91 (d, *J* = 10.36 Hz, 2 H), 2.38 (s, 3 H), 2.83 (s, 3 H), 3.11−3.34 (m, 3 H), 3.53 (dd, *J* = 11.73, 3.06 Hz, 1 H), 4.33 (br s, 1 H), 7.00−7.19 (m, 3 H), 7.23−7.38 (m, 4 H), 7.44 (s, 1 H); $[M - H]^-$ 541.6.

(3*R*)-3-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-ethylpiperidine-1,3-diamido (20e). Following a procedure similar to the preparation of **17b**, **20e** was obtained from **20b** and the appropriate isocyanate in 76% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.06−1.21 (m, 3 H), 1.49−1.74 (m, 3 H), 1.99 (br s, 1 H), 2.38 (s, 3 H), 3.04−3.34 (m, 4 H), 3.57−3.79 (m, 2 H), 4.07 (d, *J* = 6.50 Hz, 1 H), 4.72 (br s, 1 H), 6.98 (d, *J* = 6.97 Hz, 1 H), 7.07 (d, *J* = 8.38 Hz, 2 H), 7.23−7.36 (m, 4 H), 7.44 (s, 1 H); $[M + H]^+$ 534.5.

(3*R*)-3-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-(propan-2-yl)piperidine-1,3-diamido (20f). Following a procedure similar to the preparation of **17b**, **20f** was obtained from **20b** and the appropriate isocyanate in 69% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.09 (d, *J* = 6.50 Hz, 3 H), 1.17 (s, 3 H), 1.51−1.78 (m, 3 H), 1.98 (br s, 1 H), 2.38 (s, 3 H), 3.18−3.41 (m, 2 H), 3.63 (dd, *J* = 13.47, 2.73 Hz, 2 H), 3.95 (d, *J* = 6.59 Hz, 1 H), 4.07 (d, *J* = 6.78 Hz, 1 H), 4.51 (d, *J* = 7.16 Hz, 1 H), 6.90−7.14 (m, 3 H), 7.21−7.37 (m, 4 H), 7.43 (s, 1 H); $[M + H]^+$ 548.6.

(3*R*)-3-*C*-5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-1-*N*-propylpiperidine-1,3-diamido (20g). Following a procedure similar to the preparation of **17b**, **20g** was obtained from **20b** and the appropriate isocyanate in 87% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.81 (t, *J* = 7.44 Hz, 3 H) 1.36−1.47 (m, 2 H), 1.48−1.65 (m, 3 H), 1.82−1.96 (m, 1 H), 2.29 (s, 3 H), 2.98−3.24 (m, 4 H), 3.46−3.68 (m, 2 H), 3.86−4.09 (m, 1 H), 4.68 (t, *J* = 4.99 Hz, 1 H), 6.91 (d, *J* = 6.97 Hz, 1 H), 6.99 (d, *J* = 8.38 Hz, 2 H), 7.15−7.28 (m, 4 H), 7.35 (s, 1 H); $[M + H]^+$ 548.8.

(3*R*)-1-*N*-Butyl-3-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,3-diamido (20h). Following a procedure similar to the preparation of **17b**, **20h** was obtained from **20b** and the appropriate isocyanate in 72% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.82 (t, *J* = 7.16 Hz, 3 H) 1.24 (dd, *J* = 14.93, 7.21 Hz, 2 H), 1.32−1.45 (m, 2 H), 1.47−1.64 (m, 3 H), 1.90 (br s, 1 H), 2.29 (s, 3 H), 2.95−3.26 (m, 4 H), 3.42−3.70 (m, 2 H), 3.98 (d, *J* = 6.69 Hz, 1 H), 4.65 (br s, 1 H), 6.90 (d, *J* = 6.88 Hz, 1 H), 6.98 (d, *J* = 8.38 Hz, 2 H), 7.14−7.28 (m, 4 H), 7.35 (s, 1 H); $[M + H]^+$ 562.3.

(3*R*)-1-*N*-*tert*-Butyl-3-*C*-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-piperidine-1,3-diamido (20i). Following a procedure similar to the preparation of **17b**, **20i** was obtained from **20b** and the appropriate isocyanate in 70% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.22−1.38 (m, 9 H), 1.61 (d, *J* = 8.01 Hz, 2 H), 1.71−1.79 (m, 1 H), 1.89−2.04 (m, 1 H), 2.38 (s, 3 H), 3.23−3.37 (m, 2 H), 3.54 (d, *J* = 2.73 Hz, 2 H), 4.07 (d, *J* = 6.78 Hz, 1 H), 4.59 (s, 1 H), 6.94−7.13 (m, 3 H), 7.20−7.39 (m, 4 H), 7.43 (s, 1 H); $[M + H]^+$ 562.5.

Ethyl (3*R*)-3-[5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-amido]piperidine-1-carboxylate (20j). Amine **20b** (1.0 equiv, 19.2 mg, 0.041 mmol), ethyl chloroformate (1.5 equiv, 6.7 mg, 0.062 mmol), and triethyamine (3.0 equiv, 0.02 mL, 0.124 mmol) were stirred in THF (2 mL) at room temperature for 16 h. The mixture was concentrated in vacuo. The crude reaction material was then purified by silica gel column chromatography using 0−100% ethyl acetate/hexane to yield **20j** (15 mg, 68%). ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.08−1.22 (m, 3 H), 1.42−1.74 (m, 3 H), 1.91 (br s, 1 H), 2.30 (s, 3 H), 3.17 (br s, 2 H), 3.56 (d, *J* = 13.19 Hz, 1 H), 3.82 (d, *J* = 10.74 Hz, 1 H), 3.94−4.15 (m, 3 H), 6.88 (d, *J* = 7.91 Hz, 1 H), 6.94−7.04 (m, 2 H), 7.15−7.28 (m, 4 H), 7.36 (s, 1 H); $[M + H]^+$ 535.4.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-[(4-{[(ethylcarbamoyl)amino]methyl}cyclohexyl)methyl]-4-methyl-1*H*-pyrazole-3-carboxamide (21a). Following a procedure similar to the preparation of **17b**, **21a** was obtained from **26** and the appropriate isocyanate in 91% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.01−1.12 (m, 3 H), 1.21−1.63 (m, 8 H), 1.72 (br s, 2 H), 2.29 (s, 3 H), 2.94 (br s, 1 H), 3.04 (br s, 1 H), 3.09−3.23 (m, 2 H), 3.30 (t, *J* = 6.73 Hz, 1 H), 4.48 (br s, 2 H), 6.85−7.06 (m, 3 H), 7.15−7.27 (m, 4 H), 7.36 (s, 1 H); $[M + H]^+$ 576.6.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-{[4-({[(propan-2-yl)carbamoyl]amino}methyl)cyclohexyl]methyl}-1*H*-pyrazole-3-carboxamide (21b). Following a procedure similar to the preparation of **17b**, **21b** was obtained from **26** and the appropriate isocyanate in 84% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.07 (d, *J* = 6.40 Hz, 6 H), 1.25−1.61 (m, 8 H), 1.64−1.78 (m, 2 H), 2.30 (s, 3 H), 2.93 (br s, 1 H), 3.04 (br s, 1 H), 3.16−3.24 (m, 1 H), 3.25−3.36 (m, 1 H), 3.77 (br s, 1 H), 4.21 (br s, 1 H), 4.38 (br s, 1 H), 6.81−7.06 (m, 3 H), 7.12−7.28 (m, 4 H), 7.36 (s, 1 H); $[M + H]^+$ 590.5.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-[(4-{[(propylcarbamoyl)amino]methyl}cyclohexyl)methyl]-1*H*-pyrazole-3-carboxamide (21c). Following a procedure similar to the preparation of **17b**, **21c** was obtained from **26** and the appropriate isocyanate in 84% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.78−0.89 (m, 3 H), 1.25−1.53 (m, 10 H), 1.71 (br s, 2 H), 2.29 (s, 3 H), 2.94 (br s, 1 H), 3.05 (br s, 3 H), 3.19 (s, 1 H), 3.29 (t, *J* = 6.73 Hz, 1 H), 4.49 (br s, 2 H), 6.82−7.05 (m, 3 H), 7.12−7.28 (m, 4 H), 7.35 (s, 1 H); $[M + H]^+$ 590.4.

***N*-[(4-{[(Butylcarbamoyl)amino]methyl}cyclohexyl)methyl]-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21d).** Following a procedure similar to the preparation of **17b**, **21d** was obtained from **26** and the appropriate isocyanate in 98% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 0.69−0.91 (m, 3 H), 1.08−1.62 (m, 12 H), 1.64−1.84 (m, 2 H), 2.29 (s, 3 H), 2.94 (s, 1 H), 2.99−3.13 (m, 3 H), 3.19 (s, 1 H), 3.29 (t, *J* = 6.73 Hz, 1 H), 4.47 (d, *J* = 5.56 Hz, 2 H), 6.81−7.06 (m, 2 H), 7.13−7.29 (m, 4 H), 7.36 (s, 1 H); $[M + H]^+$ 604.6.

***N*-[(4-{[(*tert*-Butylcarbamoyl)amino]methyl}cyclohexyl)methyl]-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21e).** Following a procedure similar to the preparation of **17b**, **21e** was obtained from **26** and the appropriate isocyanate in 71% yield. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 1.34 (d, *J* = 2.07 Hz, 9 H), 1.38−1.66 (m, 8 H), 1.82 (br s, 2 H), 2.38 (s, 3 H), 2.98 (br s, 1 H), 3.09 (br s, 1 H), 3.28 (s, 1 H), 3.38 (t, *J* = 6.78 Hz, 1 H), 4.25 (br s, 2 H), 6.93−7.12 (m, 3 H), 7.23−7.37 (m, 4 H), 7.44 (s, 1 H); $[M + H]^+$ 604.6.

Calcium Mobilization and Radioligand Displacement Assays. Each compound was pharmacologically characterized using a functional fluorescent CB1 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 (K_e) values were determined.[7] Briefly, CHO-K1 cells were engineered to coexpress human CB1 and $Gq_{i\alpha16}$. Activation of CB1 by an agonist then leads to generation of inositol phospahatase 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

$$K_e = [\text{ligand}]/[DR - 1]$$

where DR is the EC_{50} ratio of CP55940 in the presence or absence of a test agent.[14]

Further characterization of select compounds was performed using radioligand displacement of [^{3}H]SR141716, and equilibrium dissociation constant (K_i) values were determined as has been described previously.[7,15] 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-K1cells overexpressing either receptor. Data reported are average values from three to six measurements.

MDCK-mdr1 Permeability Assays. MDCK-mdr1 cells obtained from The Netherlands Cancer Institute were grown on transwell type filters (Corning) for 4 days to confluence in DMEM/F12 medium containing 10% fetal bovine serum and antibiotics as has been described previously.[7] Compounds were added to the apical side at 3.16 μM in a transport buffer comprising 1× Hank's balanced salt solution and 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 sides 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 two to three measurements.

In Vitro Stability Testing. 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. Identities of the donors were 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 regeneration system comprising 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 volumes 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 of 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.

Evaluation of Compounds in Vivo. Male Sprague–Dawley rats aged 7–8 weeks at time of dosing were acquired from Charles River Laboratories and were dosed by two routes: ip and oral. Oral doses were formulated in corn oil, and ip doses were formulated in 1:1:18 ethanol/cremophor/saline, both at 10 mg/kg. Plasma and brain were taken from all rats at 1 h postdose. At 30 min postdose, tail vein blood was collected only from rats dosed orally.

Samples were prepared and analyzed as follows: Plasma (50 μL) was mixed with 10 μL of internal standard, reserpine (1 μg/mL), 10 μL of acetonitrile, and 300 μL of acetonitrile, vortexed, and centrifuged at 9000g for 5 min. Supernatant, (100 μL) was mixed with 900 μL of 50:50 methanol/water in autosampler vials. For 30 min plasma samples, the supernatant was injected without dilution. The left lobe of the brain was 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 internal standard, reserpine (1 μg/mL), 10 μL of acetonitrile, and 300 μL of acetonitrile, vortexed, and centrifuged at 9000g for 5 min. Supernatant was transferred to 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.

■ ASSOCIATED CONTENT

Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 919-541-6795. Fax: 919-541-8868. Email: rmaitra@rti.org.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Ann Gilliam for performing the binding assays, and Sherry Black and Purvi Patel for help with metabolic stability studies. The authors also express gratitude to the NIDA drug supply program for providing radiolabeled probes and to Dr. Brian Thomas for supplying the CB1 cells. This research was funded by Research Grants 1R21AA019740-01 and 1R03AA017514-01 to R.M. from NIAAA.

■ ABBREVIATIONS USED

CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CNS, central nervous system; BBB, blood–brain barrier; TPSA, topological polar surface area; ECS, endocannabinoid system; CBR, cannabinoid receptor; K_e, apparent affinity constant; MDCK-mdr1, Madin–Darby canine kidney cells transfected with the human MDR1 gene; A, apical; B, basal; BOP, benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; CHO-K1, Chinese hamster ovary cell; IP_3, inositol phospahatase 3; MRM, multiple reaction monitoring; LOQ, below limit of quantitation; NA, not applicable

■ REFERENCES

(1) Pacher, P.; Batkai, S.; Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* **2006**, *58*, 389–462.

(2) (a) Bouaboula, M.; Bianchini, L.; McKenzie, F. R.; Pouyssegur, J.; Casellas, P. Cannabinoid receptor CB1 activates the Na^+/H^+ exchanger NHE-1 isoform via Gi-mediated mitogen activated protein kinase signaling transduction pathways. *FEBS Lett.* **1999**, *449*, 61–65. (b) Bouaboula, M.; Perrachon, S.; Milligan, L.; Canat, X.; RinaldiCarmona, M.; Portier, M.; Barth, F.; Calandra, B.; Pecceu, F.;

Lupker, J.; Maffrand, J. P.; LeFur, G.; Casellas, P. A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1: evidence for a new model of receptor/ligand interactions. *J. Biol. Chem.* **1997**, *272*, 22330−22339.

(3) (a) Janero, D. R.; Makriyannis, A. Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis. *Expert Opin. Emerging Drugs* **2009**, *14*, 43−65. (b) Di Marzo, V. CB(1) receptor antagonism: biological basis for metabolic effects. *Drug Discovery Today* **2008**, *13* (23−24), 1026−1041.

(4) Lee, H. K.; Choi, E. B.; Pak, C. S. The current status and future perspectives of studies of cannabinoid receptor 1 antagonists as anti-obesity agents. *Curr. Top. Med. Chem.* **2009**, *9*, 482−503.

(5) (a) Tam, J.; Vemuri, V. K.; Liu, J.; Batkai, S.; Mukhopadhyay, B.; Godlewski, G.; Osei-Hyiaman, D.; Ohnuma, S.; Ambudkar, S. V.; Pickel, J.; Makriyannis, A.; Kunos, G. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity (vol 120, pg 2953, 2010). *J. Clin. Invest.* **2010**, *120*, 3735−3735. (b) Tarzia, G.; LoVerme, J.; Duranti, A.; Tontini, A.; Spadoni, G.; Mor, M.; Rivara, S.; Stella, N.; Xu, C.; Piomelli, D. Synthesis and characterization of a peripherally restricted CB(1) cannabinoid antagonist, URB447, that reduces feeding and body-weight gain in mice. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 639−643. (c) Barth, F.; Hortala, L.; Rinaldi-Carmona, M.; Congy, C.; Boulu, L.; Sadoun, F.; Fabre, G.; Finance, O. Rational design of a novel peripherally-restricted, orally active CB(1) cannabinoid antagonist containing a 2,3-diarylpyrrole motif. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4573−4577. (d) Hogberg, T.; Receveur, J. M.; Murray, A.; Linget, J. M.; Norregaard, P. K.; Cooper, M.; Bjurling, E.; Nielsen, P. A. Conversion of 4-cyanomethyl-pyrazole-3-carboxamides into CB1 antagonists with lowered propensity to pass the blood−brain-barrier. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 453−457. (e) Hogberg, T.; Cooper, M.; Receveur, J. M.; Bjurling, E.; Norregaard, P. K.; Nielsen, P. A.; Skold, N. Exploring SAR features in diverse library of 4-cyanomethyl-pyrazole-3-carboxamides suitable for further elaborations as CB1 antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 26−30. (f) Sasmal, P. K.; Reddy, D. S.; Talwar, R.; Venkatesham, B.; Balasubrahmanyam, D.; Kannan, M.; Srinivas, P.; Kumar, K. S.; Devi, B. N.; Jadhav, V. P.; Khan, S. K.; Mohan, P.; Chaudhury, H.; Bhuniya, D.; Iqbal, J.; Chakrabarti, R. Novel pyrazole-3-carboxamide derivatives as cannabinoid-1 (CB1) antagonists: journey from non-polar to polar amides. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 562−568.

(6) Chen, R. Z.; Frassetto, A.; Lao, J. Z.; Huang, R. R. C.; Xiao, J. C.; Clements, M. J.; Walsh, T. F.; Hale, J. J.; Wang, J. Y.; Tong, X. C.; Fong, T. M. Pharmacological evaluation of LH-21, a newly discovered molecule that binds to cannabinoid CB1 receptor. *Eur. J. Pharmacol.* **2008**, *584*, 338−342.

(7) Fulp, A.; Bortoff, K.; Zhang, Y.; Seltzman, H.; Snyder, R.; Maitra, R. Towards rational design of cannabinoid receptor 1 (CB1) antagonists for peripheral selectivity. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5711−5714.

(8) Clark, D. E.; Pickett, S. D. Computational methods for the prediction of "drug-likeness". *Drug Discovery Today* **2000**, *5*, 49−58.

(9) Garberg, P.; Ball, M.; Borg, N.; Cecchelli, R.; Fenart, L.; Hurst, R. D.; Lindmark, T.; Mabondzo, A.; Nilsson, J. E.; Raub, T. J.; Stanimirovic, D.; Terasaki, T.; Oberg, J. O.; Osterberg, T. In vitro models for the blood-brain barrier. *Toxicol. in Vitro* **2005**, *19*, 299−334.

(10) Seltzman, H. H.; Carroll, F. I.; Burgess, J. P.; Wyrick, C. D.; Burch, D. F. Tritiation of SR141716 by metallation−iodination−reduction: tritium−proton nOe study. *J. Labelled Compd. Radiopharm.* **2002**, *45*, 59−70.

(11) Tulshian, D.; Ho, G. D.; Silverman, L. S.; Matasi, J. J.; McLeod, R. L.; Hey, J. A.; Chapman, R. W.; Bercovici, A.; Cuss, F. M. High Affinity Ligands for Nociceptin Receptor ORL-1. US7094787, 2006.

(12) Randolph, J. T.; Flentge, C. A.; Huang, P. P.; Hutchinson, D. K.; Klein, L. L.; Lim, H. B.; Mondal, R.; Reisch, T.; Montgomery, D. A.; Jiang, W. W.; Masse, S. V.; Hernandez, L. E.; Henry, R. F.; Liu, Y. Y.; Koev, G.; Kati, W. M.; Stewart, K. D.; Beno, D. W. A.; Molla, A.; Kempf, D. J. Synthesis and biological characterization of B-ring amino analogues of potent benzothiadiazine hepatitis C virus polymerase inhibitors. *J. Med. Chem.* **2009**, *52*, 3174−3183.

(13) (a) Doran, A.; Obach, R. S.; Smith, B. J.; Hosea, N. A.; Becker, S.; Callegari, E.; Chen, C.; Chen, X.; Choo, E.; Cianfrogna, J.; Cox, L. M.; Gibbs, J. P.; Gibbs, M. A.; Hatch, H.; Hop, C. E.; Kasman, I. N.; Laperle, J.; Liu, J.; Liu, X.; Logman, M.; Maclin, D.; Nedza, F. M.; Nelson, F.; Olson, E.; Rahematpura, S.; Raunig, D.; Rogers, S.; Schmidt, K.; Spracklin, D. K.; Szewc, M.; Troutman, M.; Tseng, E.; Tu, M.; Van Deusen, J. W.; Venkatakrishnan, K.; Walens, G.; Wang, E. Q.; Wong, D.; Yasgar, A. S.; Zhang, C. The impact of P-glycoprotein on the disposition of drugs targeted for indications of the central nervous system: evaluation using the MDR1A/1B knockout mouse model. *Drug Metab. Dispos.* **2005**, *33*, 165−174. (b) Polli, J. W.; Olson, K. L.; Chism, J. P.; John-Williams, L. S.; Yeager, R. L.; Woodard, S. M.; Otto, V.; Castellino, S.; Demby, V. E. An unexpected synergist role of P-glycoprotein and breast cancer resistance protein on the central nervous system penetration of the tyrosine kinase inhibitor lapatinib (*N*-{3-chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethy l]amino}methyl)-2-furyl]-4-quinazolinamine; GW572016). *Drug Metab. Dispos.* **2009**, *37*, 439−442.

(14) Kosterlitz, H. W.; Lees, G. M.; Wallis, D. I.; Watt, A. J. Non-specific inhibitory effects of morphine-like drugs on transmission in the superior cervical ganglion and guinea-pig isolated ileum. *Br. J. Pharmacol.* **1968**, *34*, 691−692.

(15) Zhang, Y.; Gilliam, A.; Maitra, R.; Damaj, M. I.; Tajuba, J. M.; Seltzman, H. H.; Thomas, B. F. Synthesis and biological evaluation of bivalent ligands for the cannabinoid 1 receptor. *J. Med. Chem.* **2010**, *53*, 7048−7060.