Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.6b01448 • Publication Date (Web): 06 Jan 2017

ACS Publications

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Pyrimidinyl Biphenylureas: Identification of New Lead Compounds as Allosteric Modulators of the Cannabinoid Receptor CB_1

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ABSTRACT

The allosteric modulator 1-(4-chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (PSNCBAM-1, **2**) bound the cannabinoid receptor 1 (CB_1) and antagonized G protein coupling. This compound demonstrated potent anorectic effects similar to the CB_1 antagonist rimonabant that once was marketed for the treatment of obesity suggesting a new chemical entity for the discovery of anti-obesity drugs. To increase structural diversity of this class of CB_1 ligands, we designed and synthesized two classes of novel analogs, in which the pyridine ring of **2** was replaced by a pyrimidine ring. These positively modulate the binding of the CB_1 orthosteric agonist CP55,940, while exhibiting an antagonism of G-protein coupling activity. Interestingly, compounds **7d** and **8d** demonstrated ERK1/2 phosphorylation mediated via β-arrestin unlike the orthosteric CP55,940 that does so in a G protein-dependent manner. These can serve as new lead compounds for the future development of CB_1 allosteric modulators that show biased agonism and potentially anti-obesity behavior via a new mechanism.

INTRODUCTION

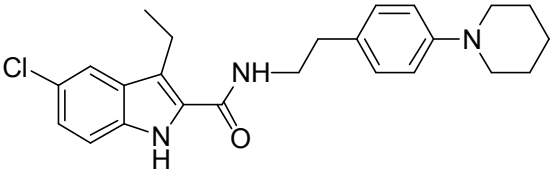
The endocannabinoid system (ECS) consists of two well-characterized receptor subtypes, the cannabinoid receptor 1 (CB_1) and the cannabinoid receptor 2 (CB_2), the eicosanoid ligands synthesized in the body called endocannabinoids and several metabolic proteins (e.g. fatty acid amide hydrolase, monoacylglycerol lipase).[1] Both the receptors are activated by Δ^9-tetrahydrocannabinol (THC), the psychoactive component of marijuana (*Cannabis sativa*) and belong to class 1A rhodopsin-like G-protein coupled receptors (GPCRs). Moreover, CB_1 is one of the most abundant GPCRs expressed in the central nervous system (CNS).[2,3] Upon activation, the CB_1 receptor primarily couples to $G_{i/o}$ protein, which causes downstream inhibition of adenylyl cyclase. The CB_1 receptor can also activate inwardly rectifying and A-type outward potassium channels[4,5] and inhibit N-type and P/Q type of calcium channels.[6,7] The CB_1 receptor can also activate different members of mitogen-activated protein kinases including p44/42 MAP kinase, p38 kinase and JUN-terminal kinase through G-protein mediated pathways or pathways independent of G-proteins via β–arrestins.[8,9] Due to its complex signaling network, CB_1 has been implicated in the pathology of many disorders and thus is a promising therapeutic target for ameliorating diseases including nausea, obesity, neurodegenerative disorders, pain and substance abuse disorders.[8,10] However, despite extensive efforts to generate cannabinoid-based therapeutics, only a few medications from cannabinergic compounds are commercially available due to the extensive side effects associated with CB_1 orthosteric ligands.[11] Another CB1 ligand, SR141716A (rimonabant), which was initially developed as an anti-obesity drug, was withdrawn from the market due to its psychiatric side effects.[12]

To overcome challenges posed by orthosteric ligands (ligands which compete with the endogenous ligands for the same site), recent research in GPCRs including CB_1 has shifted focus

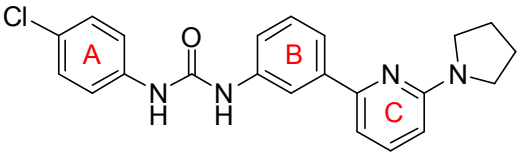
to allosteric modulators that bind to a topographically distinct site called the allosteric site. Allosteric modulators offer many therapeutic advantages over their orthosteric counterparts. First, due to less evolutionary pressure, allosteric binding pockets have amino-acid sequences which are not highly conserved like the orthosteric sites and thus are more specific for each receptor subtype. Second, allosteric modulators have a ceiling effect due to their limited allosteric cooperativity and thus can be used to generate titrated pharmacological responses.[13,14] Third, allosteric modulators can be used to fine-tune endogenous signaling without affecting the spatial and temporal aspects of endogenous ligand-receptor signaling.[15]

Allosteric modulators, upon binding to a receptor, can induce an array of distinct conformations that can be very different from those stabilized by orthosteric ligands and thus have expanded the spectrum of biological conformations among the inactive and active states. Several classes of allosteric modulators of CB_1 have been identified (Figure 1), which include 5-chloro-3-ethyl-*N*-(4-(piperidin-1-yl)phenethyl)-1*H*-indole-2-carboxamide (**1**, ORG27569),[16] 1-(4-chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)pyridin-2-yl)phenyl)urea (**2**, PSNCBAM-1),[17] 3-(4-chlorophenyl)-5-(8-methyl-3-p-tolyl-8-azabicyclo[3.2.1]octan-2-yl)isoxazole (**3**, RTI-371),[18] the endogenous ligand (5S,6R,9E,11Z,13E,15S)-5,6,15-trihydroxyicosa-9,11,13-trienoic acid (**4**, lipoxin A4)[19] and 6-methyl-3-(2-nitro-1-(thiophen-2-yl)ethyl)-2-phenyl-1*H*-indole (**5**, ZCZ011).[20] These ligands are positive allosteric modulators (PAMs) of orthosteric agonist binding at the CB_1 receptor. A family of peptide endocannabinoids (Pepcans) represented by pepcan-12 (**6**) has recently been reported to exhibit negative allosteric modulation (NAM) of the CB_1 receptor at the level of regulating orthosteric agonist binding as well as the signaling functions of CB_1 receptor.[21] Since allosteric modulators can induce distinct conformations than those induced by orthosteric ligands, they offer potential to generate pharmacological responses

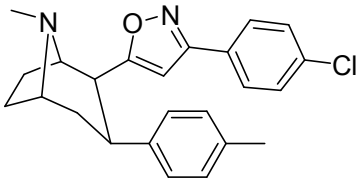
that may be difficult to achieve with orthosteric ligands alone. Furthermore, the different receptor states induced by allosteric modulators can be biased for coupling to different G proteins or β-arrestin likely due to the different conformations they induce. This can impact some intracellular signaling pathways as is seen with some allosteric modulators including **1** for the CB_1 receptor and consequently offer promising avenues to develop subtype-specific and pathway-specific therapeutics.[22,23]



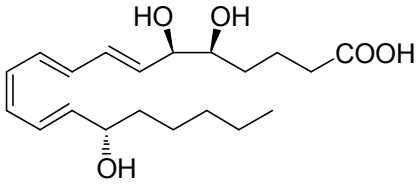
1 (ORG27569)



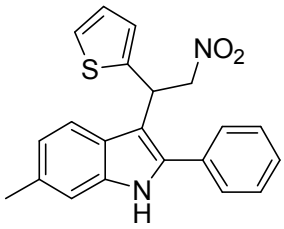
2 (PSNCBAM-1)



3 (RTI-371)



4 (Lipoxin A4)



5 (ZCZ011)

Primary sequence:

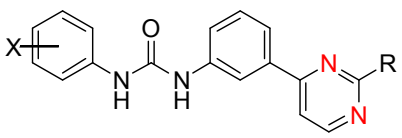
RVDPVNFKLLSH

6 (Pepcan-12)

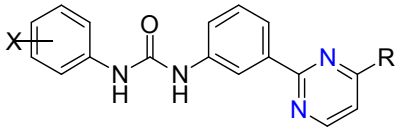
Figure 1. Structures of representative allosteric modulators of the CB_1 receptor.

Some *in vivo* pharmacological assessments have indicated that these allosteric modulators can generate therapeutic effects relevant to various clinical therapies. For instance, **1** was demonstrated to be effective in inhibiting reinstatement of drug-seeking behavior[24] and **4** was found to reduce β-amyloid-provoked neurotoxicity.[19] The positive CB_1 allosteric modulator **5** was shown to generate antinociceptive effects without psychoactive effects typically found with orthosteric ligands of the CB_1 receptor.[20] Interestingly, **2** was found capable of reducing food intake and body weight in an acute feeding study.[17] Thus, this scaffold holds promise for the development of anti-obesity drugs by targeting CB_1 allosteric sites. A recent SAR study of **2** has revealed that two structural variations can preserve its activity. These include the replacement of the chloro group on the ring A with either a fluoro (F) or a cyano (CN) group and the replacement of the pyrrolidine ring with a *N,N*-dimethyl amino group. These analogues exhibited a dose-dependent reduction of E_{max} values with the agonist CP55,940 in a calcium mobilization assay, as expected for CB_1 G protein NAMs.[25] In order to increase the structural diversity from this scaffold, we designed novel analogs with the hypothesis that a pyrimidinyl ring can be employed to replace the pyridine ring of **2**. This hypothesis was supported by two considerations. First, many allosteric modulators of various GPCRs possess cyclic moieties that contain multiple nitrogen atoms.[26] The pyrimidine moiety has been found in a number of allosteric modulators of different GPCRs such as for the glucagon-like peptide 1 (GLP-1) receptor,[27] the metabotropic glutamate receptor 5 ($mGluR_5$),[28] and the γ-aminobutyric $acid_B$ ($GABA_B$) receptor[29] among others.[30] Second, in comparison with pyridine, the pyrimidine structure offers more synthetic versatility. The π-electron density is decreased to an even greater extent than in pyridine.[31] Therefore, nucleophilic aromatic substitution is facilitated. Synthetically, the electron-deficient nature of pyrimidine accounts for better opportunities when

nucleophilic aromatic substitution chemistry is employed in functionalization of the electron-deficient heteroaromatic ring.[31] Based on the above features, we synthesized and assessed novel analogs **7a-7h** and **8a-8t** whose structures are shown in Figure 2.



7



8

7a. X = *p*-Cl, R = *N*-pyrrolidinyl
7b. X = *p*-Br, R = *N*-pyrrolidinyl
7c. X = *p*-F, R = *N*-pyrrolidinyl
7d. X = *p*-CN, R = *N*-pyrrolidinyl
7e. X = *p*-F, R = *N,N*-dimethyl
7f. X = *p*-CN, R = *N,N*-dimethyl
7g. X = *p*-I, R = *N,N*-dimethyl
7h. X = *m*-F, R = *N,N*-dimethyl

8a. X = *p*-Cl, R = *N*-pyrrolidinyl
8b. X = *p*-Br, R = *N*-pyrrolidinyl
8c. X = *p*-F, R = *N*-pyrrolidinyl
8d. X = *p*-CN, R = *N*-pyrrolidinyl
8e. X = *p*-F, R = *N,N*-dimethyl
8f. X = *p*-CN, R = *N,N*-dimethyl
8g. X = *p*-I, R = *N,N*-dimethyl
8h. X = *m*-F, R = *N,N*-dimethyl
8i. X = *p*-CF_3, R = *N*-pyrrolidinyl
8j. X = *p*-$CH_3C(O)$, R = *N*-pyrrolidinyl
8k. X = p-EtOC(O), R = *N*-pyrrolidinyl

8l. X = *p*-COOH, R = *N*-pyrrolidinyl
8m. X = *p*-OCH3, R = *N*-pyrrolidinyl
8n. X = *p*-OH, R = *N*-pyrrolidinyl
8o. X = *m*-CN, R = *N*-pyrrolidinyl
8p. X = *p*-CN, R = *N*-azetidinyl
8q. X = *p*-CN, R = *N*-piperidinyl
8r. X = *p*-CN, R = $N(Et)_2$
8s. X = *p*-CN, R = $CH_3CH_2NCH_3$
8t. X = *p*-CN, R = *N*-cyclopropyl amino

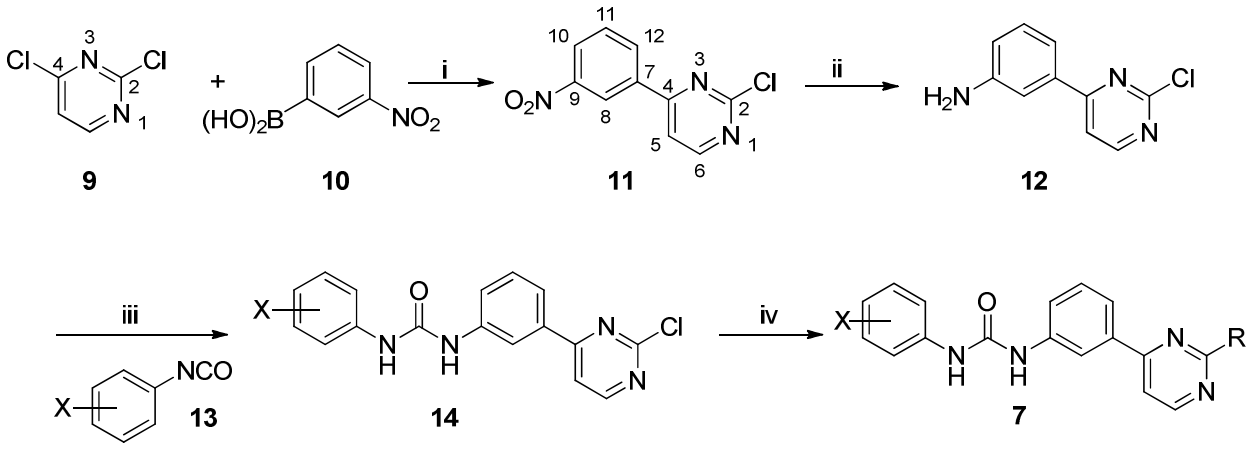
Figure 2. The designed and synthesized pyrimidinyl biphenyl ureas.

CHEMISTRY

The syntheses of the target compounds **7** and **8** were achieved through the routes illustrated by Scheme 1 and 2, respectively. To obtain compounds **7a-7h**, the synthesis of intermediate 3-(2-chloropyrimidin-4-yl)aniline **12** was critical. Coupling of the commercially available 2,4-dichloropyrimidine **9** with (3-nitrophenyl)boronic acid **10** under typical Suzuki reaction conditions exclusively gave the 2-chloro-4-(3-nitrophenyl)pyrimidine **11**. We found that this Suzuki coupling reaction only took place at the 4-chloro position of the starting material **9**, whilst its 2-chloro group was spared. The structure of the Suzuki coupling product **11** was confirmed by NOESY signals between the protons H-8 and H-12 with proton H-5 of **11** (see

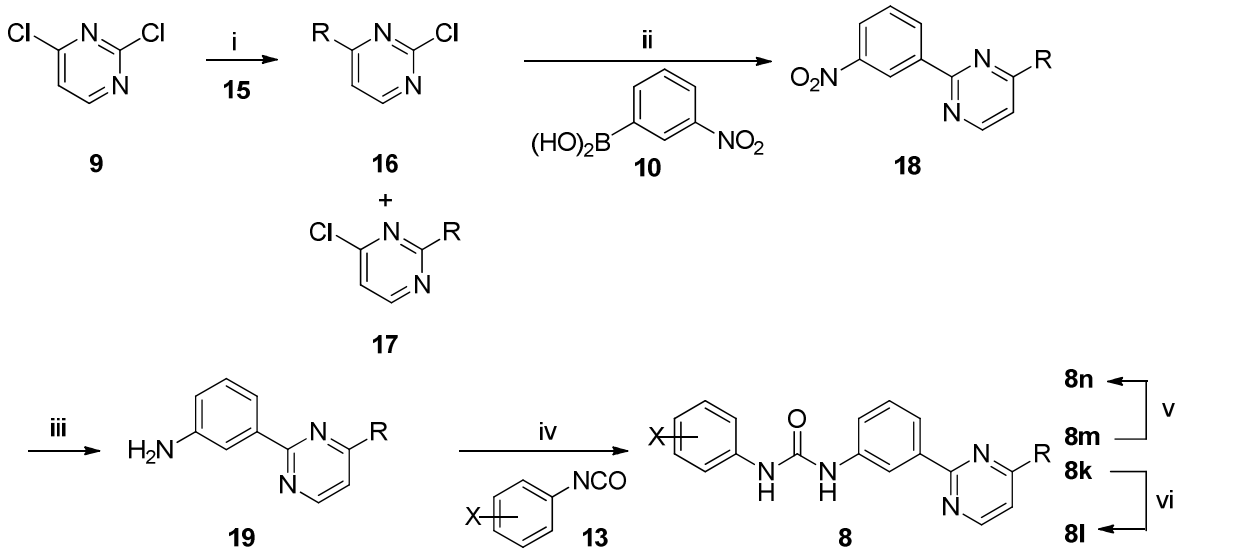
supporting information Figure S3 for 2D ^{1}H NMR of **11**). Upon reduction of **11** catalyzed with stannous chloride dihydrate, the desired intermediate 3-(2-chloropyrimidin-4-yl)aniline **12** was obtained in an acceptable yield (40%). Reaction of the pyrimidinyl aniline **12** with properly substituted isocyanate (**13a-13f**) yielded the key intermediates **14a-14f**. Thereafter, amination of the pyrimidinyl ring of individual **14** with either pyrrolidine or dimethyl amine in THF generated the final compounds **7a-7h**.

Scheme 1. Synthesis of Pyrimidinyl Biphenyl Ureas **7a-7h**[a]



[a]Reagents and conditions: (i) Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O; 90 °C, 7 h, 61%; (ii) $SnCl_2{\cdot}2H_2O$, DCM/MeOH (1:1), 0 °C followed by reflux at 60 °C, 4 h, 40%; (iii) DCM, 0 °C to rt, 2 h, 40-86%; (iv) pyrrolidine (for **7a-7d**) or dimethyl amine (for **7e-7h**), THF, reflux, 2-4 h, 35-81%.

Scheme 2. Synthesis of Pyrimidinyl Biphenyl Ureas **8a-8t**[a]



16a-19a. R = *N*-pyrrolidinyl
16b-19b. R = dimethyl amino
16c-19c. R = $N(CH_3)(C_2H_5)$
16d-19d. R = diethyl amino
16e-19e. R = *N*-piperidinyl
16f-19f. R = *N*-cyclopropyl amino
16g-19g. R = *N*-azetidinyl

13g. X = *p*-CF_3
13h. X = *p*-$CH_3C(O)$
13i. X = *p*-EtOC(O)
13j. X = *p*-OCH3
13k. X = *m*-CN

8a. X = *p*-Cl, R = *N*-pyrrolidinyl
8b. X = *p*-Br, R = *N*-pyrrolidinyl
8c. X = *p*-F, R = *N*-pyrrolidinyl
8d. X = *p*-CN, R = *N*-pyrrolidinyl
8e. X = *p*-F, R = $N(CH_3)_2$
8f. X = *p*-CN, R = $N(CH_3)_2$
8g. X = *p*-I, R = $N(CH_3)_2$
8h. X = *m*-F, R = $N(CH_3)_2$
8i. X = *p*-CF_3, R = *N*-pyrrolidinyl
8j. X = *p*-$CH_3C(O)$, R = *N*-pyrrolidinyl
8k. X = *p*-EtOC(O), R = *N*-pyrrolidinyl

8l. X = *p*-COOH, R = *N*-pyrrolidinyl
8m. X = *p*-OCH3, R = *N*-pyrrolidinyl
8n. X = *p*-OH, R = *N*-pyrrolidinyl
8o. X = *m*-CN, R = *N*-pyrrolidinyl
8p. X = *p*-CN, R = *N*-azetidinyl
8q. X = *p*-CN, R = *N*-piperidinyl
8r. X = *p*-CN, R = $N(CH_2CH_3)_2$
8s. X = *p*-CN, R = $N(CH_3)(C_2H_5)$
8t. X = *p*-CN, R = *N*-cyclopropyl amino

[a]Reagents and conditions: (i) amine **15** (**15a**: pyrrolidine; **15b**: $NH(CH_3)_2$; **15c**: MeNHEt; **15d**: $NH(Et)_2$; **15e**: piperidine; **15f**: cyclopropylamine; **15g**: azetidine), THF, rt, 2 h, 46%-80%; (ii) Na_2CO_3, $Pd(PPh_3)_4$, 1,4-dioxane, H_2O (4:1), 110 °C, 10 h, 46-75%; (iii) 10% Pd/C, H_2, EtOAc or

ethanol, rt, 4 h, 38-97%; (iv) DCM, 0 °C-rt, 2 h, 25-86%; (v) BBr_3, DCM, 0 °C-rt; (vi) LiOH, THF/H_2O, rt.

Since the Suzuki coupling reaction between **9** and **10** could not lead to the intermediate that can provide the desired amine **19**, we took the route illustrated in Scheme 2 and aminated the 2,4-dichloropyrimidine **9** first. This yielded two regioisomers **16** and **17**. The 4-amino pyrimidine **16** was obtained as the major product whilst the 2-amino pyrimidine **17** was the minor product. The structures of individual isomers (i.e. **16a-16g** and **17a-17g**) were either verified by comparison with the NMR data reported in the literature or determined by 2D ^{1}H NMR (see supporting information). Coupling the 4-amino pyrimidine **16a-16g**, respectively, with (3-nitrophenyl)boronic acid **10** under Suzuki reaction conditions yielded the desired 2-(3-nitrophenyl)pyrimidin-4-amines **18a-18g** in acceptable yields (46%-75%). Hydrogenation of **18a-18g** produced the 2-(3-aminophenyl)-pyrimidin-4-amines **19a-19g** in good yield (87-97%). Coupling the selected isocyanates **13g-13k** with amines **19a-19g** yielded the final compounds **8a**-8**k**, **8m** and **8o-t**. The carboxylic acid **8l** was further derived from **8k** through hydrolysis, and **8n** was obtained from demethylation of **8m**.

RESULTS AND DISCUSSION

The synthesized analogs were evaluated for allosteric modulation of CB_1, using the orthosteric agonist CP55,940, and two key parameters were determined: K_B, the equilibrium dissociation constant of the allosteric modulator which reflects the affinity of the allosteric modulator for the receptor and α, the binding cooperativity factor which defines the magnitude and direction of the allosteric effect on the binding of the orthosteric ligand, when both occupy

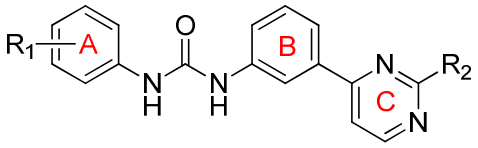
the receptor. Modulators with an $\alpha>1$ promote orthosteric ligand binding and are classified as positive allosteric modulators (PAMs) whereas modulators with $\alpha<1$ inhibit orthosteric ligand binding and are classified as negative allosteric modulators (NAMs). Compounds with no allosteric activity on orthosteric ligand binding have an $\alpha = 1$.

To evaluate the scaffolds **7** and **8**, we first compared **7a** and **8a** with the lead compound **2**. The results, shown in Tables 1 and 2 (entry 2 and 10), suggested that replacing the pyridine ring of **2** with a pyrimidine ring reduced the binding affinity relative to the parent compound while the modulation on orthosteric ligand binding was enhanced (i.e. the α values are increased). This promoted our further investigation of the two new scaffolds by optimizing the substituents. We synthesized and assessed compounds **7b-7h** and their counterpart compounds **8b-8h**. The results shown in Table 1 (entry 3-9) and Table 2 (entry 11-17) revealed that the scaffolds **7** and **8** do not show significant difference in binding affinity (K_B) and binding cooperativity factor (α) when compounds with the same substitutions on the two different scaffolds are compared. However, the binding affinity is significantly influenced by the substitution on the phenyl ring A and the amino substituent on the pyrimidine ring C. We found that a *para*-cyano group on the phenyl ring A and a pyrrolidinyl group on the pyrimidine ring C provided a compound (i.e. **8d**) that showed activity comparable to the lead compound **2**. It also appears that a dimethyl amino substituent on the pyrimidinyl ring C is suboptimal than a pyrrolidinyl group within each series of compounds from scaffolds **7** and **8**.

A cyano group is a strong electron-withdrawing group (EWG). To investigate if other substituents on the phenyl ring A can increase the binding affinity and cooperativity, we synthesized and assessed the compounds **8i-8n** (Table 2, entry 18-23), which bear either an EWG such as CF_3 (**8i**), acetyl (**8j**), ethoxyacyl (**8k**) and COOH (**8l**) or an electron-donating group

(EDG) such as OMe (**8m**) and OH (**8n**). However, none of them surpassed the cyano-substituted compound **8d**. Introducing EDGs on the phenyl ring A (i.e. compound **8m** and **8n**) significantly reduced the binding affinity to the allosteric site. Some of the EWGs are able to retain some binding affinity to the allosteric site (i.e. **8i** and **8k**). These suggested that variation of the electron density of the phenyl ring A alone is unable to enhance the binding affinity. It seems that the electron-withdrawing cyano group is not only to polarize the adjacent electron density on the ring A, but is also likely involved in the molecular recognition process. The multiple biological functions of a cyano group have been well recognized.[32] Its functions include serving as carbonyl and halogen bioisosteres, a hydroxyl and carboxyl surrogate, an inducer of non-specific dipole interactions with amino acids and metal ions, and a functionality able to replace a conserved water molecule from the binding domain.[32] Expulsion of a conserved water molecule from the binding domain by a cyano group has been indicated to provide additional entropic improvement of binding affinity.[33,34] Additionally, we found that the position of the cyano substitution impacts the allosteric activity drastically. When the cyano group on the ring A of **8** was shifted from the *para*-position to the *meta*-position, the binding affinity to the allosteric site and the allosteric modulation of orthosteric ligand binding were nearly abolished (i.e. **8d** vs **8o,** Table 2).

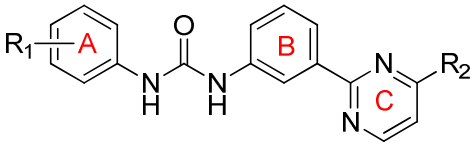
Table 1. Allosteric parameters of analogs derived from scaffold **7** (**7a-7h**)



Entry	Compd	R_1	R_2	K_B (nM)[a]	α[b]
1	**2**	*p*-Cl	*N*-Pyrrolidinyl	54.3 (21.9-134.6)	7.3
2	**7a**	*p*-Cl	*N*-Pyrrolidinyl	226.9 (61.8-832.2)	11.6
3	**7b**	*p*-Br	*N*-Pyrrolidinyl	394.9 (79.3-1964)	16.8
4	**7c**	*p*-F	*N*-Pyrrolidinyl	195.6 (113.8-336.1)	3.5
5	**7d**	*p*-CN	*N*-Pyrrolidinyl	167.8 (57.1-492.1)	10.5
6	**7e**	*p*-F	$N(CH_3)_2$	1116 (506.6-2459)	7.3
7	**7f**	*p*-CN	$N(CH_3)_2$	416.6 (308.1-563.2)	6.1
8	**7g**	*p*-I	$N(CH_3)_2$	965.3 (629.0-1481)	2.7
9	**7h**	*m*-F	$N(CH_3)_2$	967.4 (540.3-1722)	2.5

[a]K_B: equilibrium dissociation constant of a potential allosteric ligand. [b]α: binding cooperativity factor for the tested allosteric modulator. The two allosteric parameters were tested using [^{3}H]CP55,940 as the orthosteric ligand.

Table 2. Allosteric parameters of analogs derived from scaffold **8** with variation of substituents on the phenyl ring A (**8a-8o**)



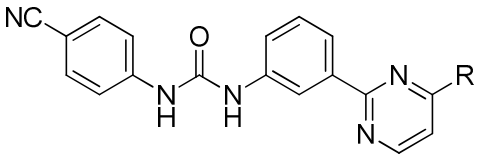
Entry	Compd	R_1	R_2	K_B (nM)[a]	α[b]
1	**2**	*p*-Cl	*N*-Pyrrolidinyl	54.3 (21.9-134.6)	7.3
10	**8a**	*p*-Cl	*N*-Pyrrolidinyl	264.4 (61.9-1129)	18.1
11	**8b**	*p*-Br	*N*-Pyrrolidinyl	101.9 (32.8-316.9)	6.1
12	**8c**	*p*-F	*N*-Pyrrolidinyl	222.9 (154.2-322.1)	4.9
13	**8d**	*p*-CN	*N*-Pyrrolidinyl	49.1 (27.9-86.3)	4.6
14	**8e**	*p*-F	$N(CH_3)_2$	1221 (849.6-1755)	5.2
15	**8f**	*p*-CN	$N(CH_3)_2$	313.3 (216.2-454.2)	4.5
16	**8g**	*p*-I	$N(CH_3)_2$	997.1 (480.7-2068)	2.5
17	**8h**	*m*-F	$N(CH_3)_2$	669.8 (173.6-2584)	2.0
18	**8i**	*p*-CF_3	*N*-Pyrrolidinyl	144.2 (85.8-242.4)	3.8
19	**8j**	*p*-$CH_3C(O)$	*N*-Pyrrolidinyl	2108 (1299-3422)	2.5
20	**8k**	*p*-EtOC(O)	*N*-Pyrrolidinyl	463.0 (51.8-4132)	1.6
21	**8l**	*p*-COOH	*N*-Pyrrolidinyl	NB[c]	NB[c]
22	**8m**	*p*-OCH_3	*N*-Pyrrolidinyl	1826	3.8

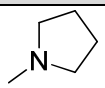
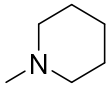
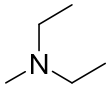
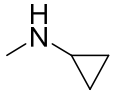
				(991.7-3364)	
23	**8n**	*p*-OH	*N*-Pyrrolidinyl	NB[c]	NB[c]
24	**8o**	*m*-CN	*N*-Pyrrolidinyl	1980 (388.1-10100)	1.5

[a]K_B: equilibrium dissociation constant of a potential allosteric ligand. [b]α: binding cooperativity factor for the tested allosteric modulator. The two allosteric parameters were tested using [^{3}H]CP55,940 as the orthosteric ligand. [c]NB: no detectable binding or modulation of [^{3}H]CP55,940 with the receptor using up to 32 μM of test compound.

Following the identification of **8d**, we investigated if other amino substituents on the pyrimidine ring can enhance activity (Table 3). We reduced the ring size to a 4-membered azetidinyl ring (**8p**). This led to a decline in K_B. When the ring size was increased to a 6-membered piperidinyl ring (**8q**), it showed a K_B comparable to **8d** with slightly reduced binding cooperativity factor (α). This promoted our synthesis of analogs with acyclic amino substituents of the pyrimidinyl ring (i.e. **8r**-**8t**). The results (entry 27-29, Table 3) indicated that only the *N*,*N*-diethyl amino group (**8r**) preserve the activity of **8d**. The results from **8r to 8t** suggested that a dialkyl amino group is preferred as the substituent on the pyrimidinyl ring C and each of the alkyl substitutions should be greater than methyl.

Table 3. Allosteric parameters of analogs derived from scaffold **8** (**8p-8t**)



entry	Compd	R	K_B (nM)[a]	α[b]
13	**8d**		49.1 (27.9-86.3)	4.6
25	**8p**		211.0 (100.7-442.0)	4.5
26	**8q**		66.4 (24.9-176.4)	3.2
27	**8r**		69.6 (20.1-240.3)	4.8
28	**8s**		206.9 (110.3-388.1)	4.5
7	**8f**		313.3 (216.2-454.2)	4.4
29	**8t**		97.7 (13.4-711.0)	1.7

[a]K_B: equilibrium dissociation constant of a potential allosteric ligand. [b]α: binding cooperativity factor for the tested allosteric modulator. The two allosteric parameters were tested using [^{3}H]CP55,940 as the orthosteric ligand.

The allosteric binding parameters of the compounds suggest using pyrimidine in lieu of the pyridine ring led to two compounds (i.e. **8d** and **8r**) showing similar binding affinity (K_B) to an allosteric site as the lead compound **2**. Their allosteric effects on orthosteric agonist binding

(i.e. the α values) are comparable to **2**. To further investigate the value of these novel allosteric modulators, compounds **7a** and **8a, 7d** and **8d**, and **8r** were compared with **2** via [^{35}S]GTPγS binding activity indicative of G protein coupling. The results are shown in Figure 3. All of these tested compounds showed a concentration-dependent inhibition of CP55,940-induced GTPγS binding. In this functional assay, compounds **7a** and **8a** are weaker than **2** in reducing the G-protein coupling to the CB1 receptor. In contrast, compounds **7d** (D, Figure 3) and **8d** (E, Figure 3) showed a potency comparable to **2** in reducing the G-protein coupling. Notably, although **7d** has a weaker binding affinity for the allosteric site than **8d** and **8r**, it displayed a reduction in G protein coupling comparable to **8d** and **2** in the functional assay. This was indicated by their relative reduction in G protein coupling at the 0.3 μM testing concentration in the [^{35}S]GTPγS binding assay. In each assay in Figure 3, cells not transfected and transfected with the CB1 receptor treated with the inverse agonist SR141716A are also given. As expected, the later reduces the [^{35}S]GTPγS activity below the basal (constitutive activity in the absence of ligand) level of coupling activity. In combination with the allosteric binding parameters, we conclude that compounds **7d** and **8d** are viable CB_1 allosteric modulators showing a binding profile and functional activity similar to **2**.

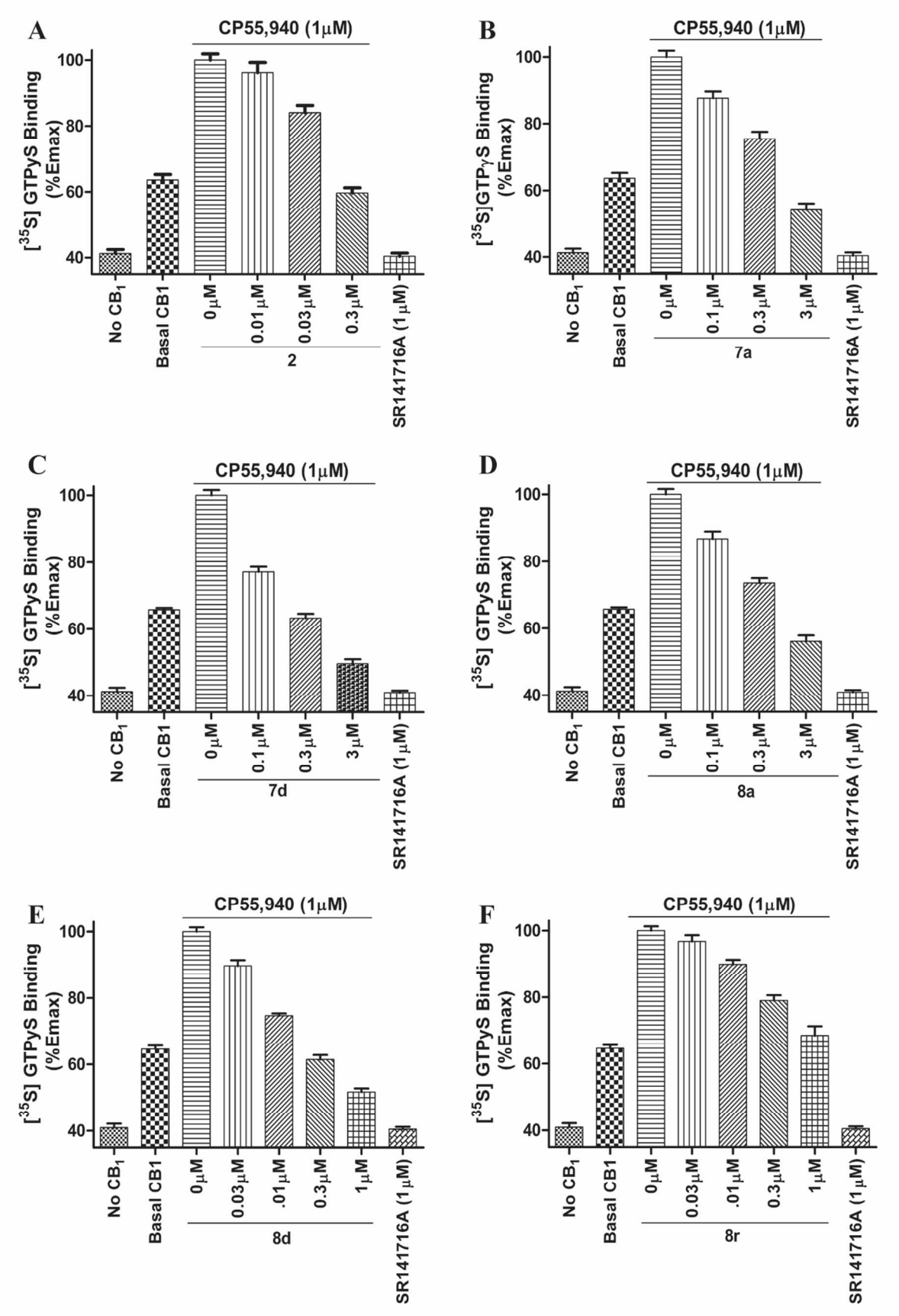

A
CP55,940 (1μM)
[35S] GTPyS Binding (%Emax)
100
80
60
40
No CB1
Basal CB1
0μM
0.01μM
0.03μM
0.3μM
2
SR141716A (1μM)
B
CP55,940 (1μM)
[35S]GTPγS Binding (%Emax)
100
80
60
40
No CB1
Basal CB1
0μM
0.1μM
0.3μM
3μM
7a
SR141716A (1μM)
C
CP55,940 (1μM)
[35S] GTPyS Binding (%Emax)
100
80
60
40
No CB1
Basal CB1
0μM
0.1μM
0.3μM
3μM
7d
SR141716A (1μM)
D
CP55,940 (1μM)
[35S] GTPyS Binding (%Emax)
100
80
60
40
No CB1
Basal CB1
0μM
0.1μM
0.3μM
3μM
8a
SR141716A (1μM)
E
CP55,940 (1μM)
[35S] GTPyS Binding (%Emax)
100
80
60
40
No CB1
Basal CB1
0μM
0.03μM
.01μM
0.3μM
1μM
8d
SR141716A (1μM)
F
CP55,940 (1μM)
[35S] GTPyS Binding (%Emax)
100
80
60
40
No CB1
Basal CB1
0μM
0.03μM
.01μM
0.3μM
1μM
8r
SR141716A (1μM)

Figure 3. CP55,940-induced GTPγS levels in the presence and absence of varying concentrations of test allosteric modulators. HEK293 cell membranes expressing the CB_1 receptor were tested for GTPγS levels in the absence and presence of compounds (A) **2**, (B) **7a**, (C) **7d**, (D) **8a**, (E) **8d**, (F) **8r** at the indicated concentrations in the presence of CP55,940 (1 μM). The basal levels of [^{35}S]GTPγS binding was also measured in the absence of any orthosteric and allosteric ligand (Basal CB_1), and its inhibition was tested by treatment with SR141716A (1 μM). Data are presented as a percentage of the GTPγS levels in the presence of CP55,940 (1μM). Each data point represents the mean ± SE (error bars) of at least three independent experiments performed in duplicate. Non-CB_1-mediated GTPγS levels were measured by obtaining [^{35}S]GTPγS binding to membrane preparations transfected with vector PCDNA3.1 not incorporating the gene for CB_1 (No CB_1).

Since the compounds tested antagonize the CP55,940-induced G protein coupling, we determined if signaling via β-arrestin, instead of G protein, was utilized. For this purpose, we used ERK1/2 phosphorylation as a good earmark of cellular signaling that can exhibit both G-protein dependent and G-protein independent phosphorylation.[35,36] Consistent with previous observations, ERK1/2 phosphorylation induced by treatment with the orthosteric agonist CP55,940 alone for 5 min was substantially reduced by G protein-sensitive pertussis toxin (PTX). However, knockdown of β-arrestin 1 or 2 did not impact ERK1/2 phosphorylation by CP55940, collectively indicating phosphorylation by this compound alone is G_i-mediated (Figure 4).[9] However, treatment with the allosteric modulators **7d** and **8d** alone demonstrated ERK1/2 phosphorylation was insensitive to treatment by PTX, indicating that phosphorylation of ERK1/2 induced by these allosteric modulators is not G_i-mediated. This is consistent with the results

showing inhibition of G-protein coupling shown for these allosteric modulators (Figure 3 above). Interestingly, knockdown of β-arrestin-1 with siRNA attenuated ERK1/2 phosphorylation by the modulators **7d** and **8d**. Compound **2** exhibited a similar response shown for comparison.[37, 38] These results, and the enhancement in binding of the orthosteric agonist CP55,940, suggest that these compounds are PAMs and promote an active state of the receptor that may be biased to signal via β-arrestin-1 (at least for ERK1/2 phosphorylation) and not G protein.

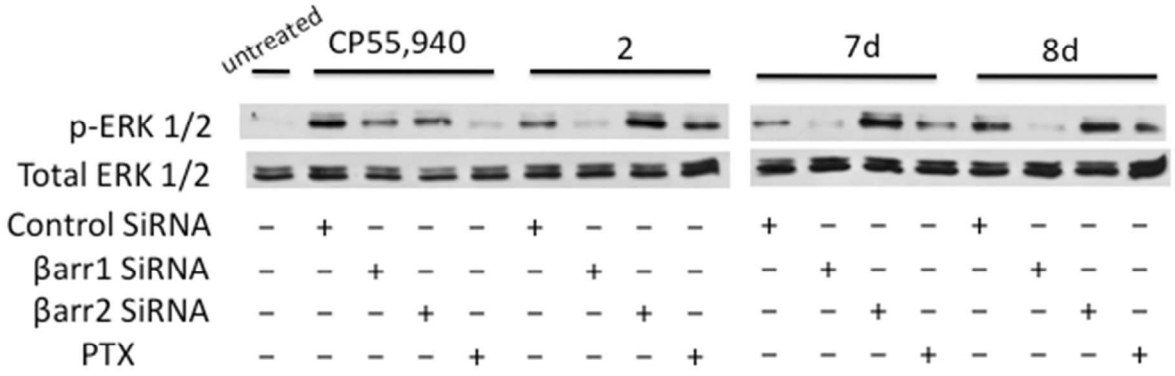


Figure 4. Impact of compound **7d** and **8d** on ERK1/2 phosphorylation. HEK293 cells expressing the CB_1 receptor were untreated (DMSO vehicle only) or treated with CP55,940 (0.5 μM) or **2** (10μM), **7d** (10 μM), **8d** (10 μM) for 5min. Control siRNA, or β-arrestin 1 siRNA, or β-arrestin 2 siRNA or PTX (5 ng/mL) were used to treat the cells as indicated. Protein from cell lysates were separated on SDS-PAGE and analyzed by Western blots probed with phosph-ERK1/2 (p-ERK1/2). The total level of ERK1/2 detected is shown for comparison. Noted that the two bands correspond to the predominant isoforms, p42 (ERK2) and p44 (ERK1), for ERK1/2.

CONCLUSIONS

The recent identification of CB_1 allosteric modulators indicated a new approach for developing safer therapeutics based on regulating the pharmacologically important CB_1 receptor. Unlike CB_1 orthosteric ligands, which typically present undesired psychotropic and psychiatric side effects, allosteric modulators hold promise as therapeutics with lesser side effects. This is because allosteric modulation of the receptor can be more finely regulated. Furthermore, some allosteric modulators may utilize biased signaling pathways such that signal transduction is more pathway-specific. In contrast to CB_1 orthosteric ligands, the structural diversity of CB_1 allosteric modulators is fairly limited. This work identified novel lead compounds from the biphenyl ureas that possess a pyrimidine ring in their structures. The new compounds bearing a pyrimidine moiety showed biological activities comparable to the well-established CB_1 allosteric modulator **2** that has a pyridine moiety. The two new scaffolds (i.e. **7** and **8**) offered broader opportunity for structural optimization due to the chemical nature of pyrimidine ring. Collectively, the newly identified pyrimidinyl biphenyl urea analogs provide new chances for the development of novel and potent CB_1 allosteric modulators. Additionally, the bias exhibited by these allosteric modulators for β-arrestin mediated signaling can confer selective fine-tuning of CB_1-impacted pathways.[39] In many other GPCRs, β-arrestin biased ligands have been shown to have therapeutically relevant benefits. Carvedilol ((±)-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy)ethyl]amine), for example, is a β-arrestin biased ligand for both β_1AR and β_2AR which acts as an antagonist for G-protein mediated signaling and promotes β-arrestin mediated signaling that promotes epidermal growth factor transactivation to confer cardioprotection.[40] Thus, biased CB_1 allosteric modulators offer unique opportunities to develop therapeutics.

EXPERIMENTAL SECTION

Compounds. The compound **2** was purchased from Tocris Bioscience. All other tested compounds were synthesized in our synthetic laboratory at Texas A&M University.

Synthesis. All chemical reagents and solvents were purchased from Sigma-Aldrich Chemical Co. unless specified otherwise and used without further purification. All anhydrous reactions were performed under a static argon atmosphere in dried glassware using anhydrous solvents. Organic phases in work up were dried over anhydrous Na_2SO_4, and removed by evaporation under reduced pressure. The crude compounds were purified by a Combiflash R_f chromatography system (Teledyne Technologies, Inc., Thousand Oaks, CA) unless specified otherwise. Purities of the intermediates were established by several analytical methods including thin-layer chromatography (TLC), melting point, ^{1}H NMR and mass spectrometry. Analytical thin-layer chromatography (TLC) was run on pre-coated silica gel TLC aluminum plates (Whatman®, UV_{254}, layer thickness 250 μm), and the chromatograms were visualized under ultraviolet (UV) light. Melting points were determined on a capillary Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra of some intermediates and all final compounds were recorded on a Bruker Avance DPX-300 spectrometer operating at 300 MHz. The 2D ^{1}H NMR data of some intermediates were recorded on a Bruker Ascend-600 spectrometer operating at 600 MHz. All NMR spectra were recorded using $CDCl_3$ or DMSO-d_6 as solvent unless otherwise specified. The chemical shifts are reported in ppm (parts per million) relative to tetramethylsilane (TMS) as an internal standard. Multiplicities are indicated as brs (broad singlet), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplets), dt (doublet of triplets), ddd (doublet of doublet of doublets) and coupling constants (*J*) are reported in hertz (Hz). Low and high resolution mass spectra were obtained at either the Laboratory for Biological Mass Spectrometry of Texas A&M University

for APCI ionization method or at the School of Chemical Sciences, University of Illinois at Urbana−Champaign for ESI ionization method. The purities of biologically tested compounds were analyzed either by combustion elemental analysis performed in Roberson Microlit laboratories (Madison, NJ) or by the combination of HRMS and HPLC. The results of combustion elemental analysis indicated by the symbols of the elements were within ±0.4% of the calculated values for the proposed formula. The purity of each tested compound was determined to be greater than 95% either by combustion elemental analyses (C, H, N) or by HPLC with two different mobile phase systems.

General Procedure A: Preparation of biphenyl ureas (**14a-14f** and **8a-8t**)**.** To the solution of 0.5 mmol of amine **12** (for synthesis of **14a-14f**) or amine **19** (for synthesis of **8a-8t**) in anhydrous dichloromethane (5-8 mL) was added the selected isocyanate **13** (0.75-0.95 mmol, 1.2-1.9 equiv) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min and then at room temperature for 2 h. The reaction was monitored by TLC (30% acetone in hexane or 50-70% ethyl acetate in hexane). After completion of the reaction, if white suspension was formed, it was filtered. The filtered solid was washed with dichloromethane (2 mL) and diethyl ether (5 mL) successively, and then dried in vacuum oven. If no solid was precipitated out upon the completion of the reaction, the reaction mixture was condensed *in vacuo*. The resultant crude compound was purified by Combiflash chromatography (0 to 50-80% ethyl acetate in hexane) to obtain the pyrimidinyl biphenyl urea compounds **14a-14f** and **8a-8t**.

General Procedure B: Preparation of pyrimidinyl biphenyl ureas 7a-7d. To the solution of biphenyl urea **14** (0.1 mmol) in anhydrous tetrahydrofuran (2-10 mL), pyrrolidine (7-13 equiv) was added dropwise at room temperature. The reaction mixture was refluxed for 2-4 h. The progress of reaction was monitored by TLC (40 % acetone in hexane). Upon completion of the

reaction, the mixture was cooled to room temperature and condensed under reduced pressure. The residue was purified by Combiflash chromatography to obtain the target compounds **7a-7d**.

General Procedure C: Preparation of pyrimidinyl biphenyl ureas 7e-7h. To the solution of biphenyl urea **14** (0.3 mmol, 1.0 equiv) in anhydrous tetrahydrofuran (2-10 mL) in a pressure tube was added dimethylamine (2-10 equiv, 2.0 M in methanol) at room temperature. The reaction mixture was refluxed for 2-4 h. The progress of the reaction was monitored by TLC (50% ethyl acetate in hexane). Upon completion of the reaction, it was cooled to room temperature and condensed under reduced pressure. The residue was partitioned between water (15 mL) and ethyl acetate (3 x 10 mL). The organic layer was separated and was washed with water, brine and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent under reduced pressure provided the crude compound, which was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to obtain the desired compounds **7e-7h**.

1-(4-Chlorophenyl)-3-(3-(2-(pyrrolidine-1-yl)pyrimidine-4-yl)phenyl)urea (7a). The compound **7a** was prepared from the biphenyl urea **14a** (28 mg, 0.078 mmol) and pyrrolidine (76 mg, 1.06 mmol) in anhydrous THF (2 mL) according to the general procedure B. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to provide product **7a** (14 mg, 46%) as a white solid; mp 171-174 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.87 (d, J = 3.9 Hz, 2H), 8.41 (d, J = 4.8 Hz, 1H), 8.18 (s, 1H), 7.73-7.64 (m, 2H), 7.52-7.32 (m, 5H), 7.07 (d, J = 5.1 Hz, 1H), 3.58 (s, 4H), 1.96 (s, 4H). MS (ESI): m/z = 394.1 $[M+H]^+$, 396.1 $[M+H+2]^+$. Anal. Calcd for ($C_{21}H_{20}N_5OCl$): C, 64.04; H, 5.12; N, 17.78. Found: C, 63.89; H, 5.37; N, 17.42.

1-(4-Bromophenyl)-3-(3-(2-(pyrrolidine-1-yl)pyrimidine-4-yl)phenyl)urea (7b). The compound **7b** was prepared from the biphenyl urea **14b** (40 mg, 0.1 mmol) and pyrrolidine (83

mg, 1.2 mmol) according to the general procedure B. The product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to provide product **7b** (19 mg, 47%) as a white solid; mp 180-183 °C. ^{1}HNMR (300 MHz, DMSO-d_6): δ 8.87 (d, J = 3.6 Hz, 2H), 8.40 (d, J = 5.1 Hz, 1H), 8.17 (s, 1H), 7.72 (d, J = 7.5 Hz, 1H), 7.66 (d, J = 8.1 Hz, 1H), 7.45-7.38 (m, 5H), 7.07 (d, J = 4.8 Hz, 1H), 3.57 (s, 4H), 1.96 (s, 4H). MS (ESI): m/z = 438.1 $[M+H]^+$, 440.1$[M+H+2]^+$. Anal. Calcd for ($C_{21}H_{20}N_5OBr \cdot H_2O$): C, 56.39; H, 4.73; N, 15.66. Found: C, 56.46; H, 4.35; N, 15.50.

1-(4-Fluorophenyl)-3-(3-(2-(pyrrolidine-1-yl)pyrimidine-4-yl)phenyl)urea (7c). The compound **7c** was prepared from the biphenyl urea **14c** (0.12 g, 0.35 mmol) and pyrrolidine (0.17 g, 2.45 mmol) in anhydrous THF (12 mL) according to the general procedure B. The crude product was isolated by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield **7c** (98 mg, 74%) as a white solid; mp 206-208 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.84 (s, 1H), 8.75 (s, 1H), 8.40 (d, J = 5.1 Hz, 1H), 8.17 (s, 1H), 7.71-7.64 (m, 2H), 7.50-7.38 (m, 3H), 7.16-7.06 (m, 3H), 3.57 (s, 4H), 1.96 (s, 4H). MS (ESI): m/z = 378.2 $[M+H]^+$. Anal. Calcd for ($C_{21}H_{20}FN_5O \cdot ¼H_2O$): C, 66.04; H, 5.41; N, 18.34. Found: C, 66.16; H, 5.34; N, 18.12.

1-(4-Cyanophenyl)-3-(3-(2-(pyrrolidine-1-yl)pyrimidine-4-yl)phenyl)urea (7d). The compound **7d** was prepared from the biphenyl urea **14d** (30 mg, 0.085 mmol) and pyrrolidine (84 mg, 1.18 mmol) in anhydrous THF (2 mL) according to the general procedure B. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to obtain product **7d** (17 mg, 51%) as a white solid; mp 153-156 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.25 (s, 1H), 9.03 (s, 1H), 8.41 (d, J = 5.1 Hz, 1H), 8.19 (s, 1H), 7.76-7.64 (m, 6H), 7.43 (t, J = 7.9 Hz, 1H), 7.08 (d, J = 5.1 Hz, 1H), 3.58 (s, 4H), 1.96 (s, 4H). MS (ESI): m/z = 385.2 $[M+H]^+$.

Anal. Calcd for ($C_{22}H_{20}N_6O \cdot H_2O$): C, 65.66; H, 5.51; N, 20.88. Found: C, 65.51; H, 5.26; N, 20.76.

1-(3-(2-(Dimethylamino)pyrimidin-4-yl)phenyl)-3-(4-fluorophenyl)urea (7e). The compound **7e** was prepared from the biphenyl urea **14c** (0.1 g, 0.3 mmol) and dimethyl amine (1 mL, 2.0 mmol, 2.0 M in MeOH) in anhydrous THF (10 mL) according to the general procedure C. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to obtain product **7e** (80 mg, 78%) as a white solid; mp 182-184 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H), 8.76 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 8.19 (t, J = 1.8 Hz, 1H), 7.72-7.69 (m, 1H), 7.66-7.63 (m, 1H), 7.50-7.38 (m, 3H), 7.16-7.07 (m, 3H), 3.21 (s, 6H). MS (ESI): *m/z* = 352.1 $[M+H]^+$. Anal. Calcd for ($C_{19}H_{18}FN_5O$): C, 64.95; H, 5.16; N, 19.93. Found: C, 64.85; H, 5.26; N, 19.79.

1-(4-Cyanophenyl)-3-(3-(2-(dimethylamino)pyrimidin-4-yl)phenyl)urea (7f). The compound **7f** was prepared from the biphenyl urea **14d** (0.10 g, 0.286 mmol) and dimethylamine (0.275 mL, 0.55 mmol, 2.0 M in MeOH) in anhydrous THF (10 mL) according to the general procedure C. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to provide **7f** (0.069 g, 67.6%) as a white solid; mp 132-134 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.27 (s, 1H), 9.05 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 8.22 (t, J = 1.8 Hz, 1H), 7.77- 7.73 (m, 3H), 7.67-7.63 (m, 3H), 7.43 (t, J = 7.8 Hz, 1H), 7.09 (d, J = 5.1 Hz, 1H), 3.21 (s, 6H). MS (ESI): *m/z* = 359.2 $[M+H]^+$. Anal. Calcd for ($C_{20}H_{18}N_6O \cdot ½H_2O$): C, 65.38; H, 5.21; N, 22.87. Found: C, 65.75; H, 4.99; N, 22.58.

1-(3-(2-(Dimethylamino)pyrimidin-4-yl)phenyl)-3-(4-iodophenyl)urea (7g). The compound **7g** was prepared from the biphenyl urea **14e** (0.10 g, 0.22 mmol) and dimethylamine (1.2 mL, 2.4 mmol, 2.0 M in MeOH) in anhydrous THF (15 mL) according to the general procedure C.

The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to provide **7g** (35 mg, 35%) as a white solid; mp 230-232 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1H), 8.85 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 8.19 (s, 1H), 7.73-7.59 (m, 4H), 7.44-7.31 (m, 3H), 7.08 (d, J = 5.1 Hz, 1H), 3.21 (s, 6H). MS (ESI): m/z = 460.1 $[M+H]^+$. Anal. Calcd for ($C_{19}H_{18}IN_5O$): C, 49.69; H, 3.95; N, 15.25. Found: C, 50.01; H, 4.02; N, 14.81.

1-(3-(2-(Dimethylamino)pyrimidin-4-yl)phenyl)-3-(3-fluorophenyl)urea (7h). The compound **7h** was prepared from the biphenyl urea **14f** (0.12 g, 0.35 mmol) and dimethylamine (1.22 mL, 2.44 mmol, 2.0 M in MeOH) in anhydrous THF (15 mL) according to the general procedure C. The crude compound was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to provide **7h** (0.10 g, 81%) as a white solid; mp 195-198 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.98 (s, 1H), 8.94 (s, 1H), 8.42 (d, J = 5.1 Hz, 1H), 8.20 (s, 1H), 7.74-7.64 (m, 2H), 7.53-7.28 (m, 3H), 7.16-7.08 (m, 2H), 6.83-6.76 (m, 1H), 3.21 (s, 6H). MS (ESI): m/z = 352.2 $[M+H]^+$. Anal. Calcd for ($C_{19}H_{18}FN_5O$): C, 64.95; H, 5.16; N, 19.93. Found: C, 64.91; H, 5.27; N, 19.72.

1-(4-Chlorophenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)-phenyl)urea (8a). The title compound **8a** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (0.07 g, 0.29 mmol) and 4-chlorophenyl isocyanate **13a** (0.044 g, 0.29 mmol) in anhydrous CH_2Cl_2 (5 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield the product **8a** (0.085 g, 74.5%) as a white solid; mp 192-195 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.88 (s, 1H), 8.83 (s,1H), 8.38 (s, 1H), 8.26 (d, J = 6.3 Hz, 1H), 7.97 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 8.7 Hz, 2H), 7.40-7.32 (m, 3H), 6.44 (d, J = 6.0 Hz, 1H), 3.61 (brs, 2H), 3.33 (brs, 2H), 1.98 (s,

4H). MS (ESI): *m/z* = 394.2 $[M+H]^+$, 396.1 $[M+H+2]^+$. Anal. Calcd for ($C_{21}H_{20}N_5OCl \cdot \frac{1}{3}H_2O$): C, 63.08; H, 5.21; N, 17.51. Found: C, 63.20; H, 5.38; N, 17.15.

1-(4-Bromophenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)-phenyl)urea (8b). The title compound **8b** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (0.07 g, 0.3 mmol) and 4-bromo phenyl isocyanate **13b** (0.086 g, 0.43 mmol) in anhydrous CH_2Cl_2 (5 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield product **8b** (0.105 g, 82.6%) as a white solid; mp 223-225 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H), 8.79 (s,1H), 8.39 (s, 1H), 8.25 (d, *J* = 6.0 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.46 (s, 4H), 7.36 (t, *J* = 7.8 Hz, 1H), 6.42 (d, *J* = 6.0 Hz, 1H), 3.62 (brs, 2H), 3.39 (brs, 2H), 1.98 (s, 4H). MS (ESI): *m/z* = 438.1 $[M+H]^+$, 440.1 $[M+H+2]^+$. Anal. Calcd for ($C_{21}H_{20}N_5OBr$): C, 57.54; H, 4.60; N, 15.98. Found: C, 57.73; H, 4.53; N, 15.78.

1-(4-Fluorophenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)-phenyl)urea (8c). The title compound **8c** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (0.075 g, 0.31 mmol) and 4-fluorophenyl isocyanate **13c** (0.051 g, 0.37 mmol) in anhydrous CH_2Cl_2 (5 mL) according to the general procedure A. The white suspension was filtered. The solid was washed with dichloromethane (3 mL), then dried in vacuum oven to yield the compound **8c** (0.079 g, 67 %) as a white solid; mp 185-187 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.80 (s, 1H), 8.68 (s, 1H), 8.39 (s, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.50-7.46 (m, 2H), 7.36 (t, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 9.0 Hz, 2H), 6.42 (d, *J* = 6.0 Hz, 1H), 3.64 (brs, 2H), 3.39 (brs, 2H), 1.99 (s, 4H). HRMS (ESI) *m/z* for $C_{21}H_{21}FN_5O$ $[M+H]^+$: Calcd 378.1730; Found: 378.1727.

1-(4-Cyanophenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)-phenyl)urea (8d). The compound **8d** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (0.07 g, 0.3 mmol) and 4-cyanophenyl isocyanate **13d** (0.05 g, 0.36 mmol) in anhydrous CH_2Cl_2 (5 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield the title compound **8d** (0.064 g, 57.6%) as a white solid; mp 219-221 °C. ^{1}HNMR (300 MHz, DMSO-d_6): δ 9.19 (s, 1H), 9.0 (s, 1H), 8.42 (s, 1H), 8.26 (d, J = 6.0 Hz, 1H), 8.0 (d, J = 7.5 Hz, 1H), 7.76-7.73 (d, J = 8.4 Hz, 2H), 7.67-7.62 (m, 3H), 7.39 (t, J = 7.9 Hz, 1H), 6.42 (d, J = 6.0 Hz, 1H), 3.61 (brs, 2H), 3.39 (brs, 2H), 1.98 (s, 4H). MS (ESI): *m/z* = 385.2 $[M+H]^+$. Anal. Calcd for ($C_{22}H_{20}N_6O$): C, 68.73; H, 5.24; N, 21.86. Found: C, 68.59; H, 5.46; N, 21.51.

1-(3-(4-(Dimethylamino)pyrimidin-2-yl)phenyl)-3-(4-fluorophenyl)urea (8e). The title compound **8e** was prepared from amine **19b** (0.1 g, 0.47 mmol) and 4-fluorophenyl isocyanate **13c** (0.076 g, 0.55 mmol) as described in the general procedure A. The crude compound was purified by Combiflash chromatography (0-80% ethyl acetate in hexane) to yield the compound **8e** (0.127 g, 78%) as a white solid; mp 202-205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.82 (s, 1H), 8.69 (s,1H), 8.41 (t, J = 1.8 Hz, 1H), 8.28 (d, J = 6.3 Hz, 1H), 7.99-7.96 (dt, J = 7.8 Hz, 1.2 Hz, 1H), 7.64-7.60 (m, 1H), 7.51- 7.46 (m, 2H), 7.37 (t, J = 7.8 Hz, 1H), 7.13 (t, J = 9.0 Hz, 2H), 6.62 (d, J = 6.0 Hz, 1H), 3.16 (s, 6H). MS (ESI): *m/z* = 352.1 $[M+H]^+$. Anal. Calcd for ($C_{19}H_{18}FN_5O$): C, 64.95; H, 5.16; N, 19.93. Found: C, 64.68; H, 5.31; N, 19.86.

1-(4-Cyanophenyl)-3-(3-(4-(dimethylamino)pyrimidin-2-yl)phenyl)urea (8f). The title compound **8f** was prepared from the amine **19b** (0.1 g, 0.47 mmol) and 4-cyanophenyl isocyanate **13d** (0.08 g, 0.56 mmol) as described in the general procedure A. The crude compound was purified by Combiflash chromatography (0-80% ethyl acetate in hexane) to yield

the title compound **8f** (0.133 g, 79.6 %) as a white solid; mp 146-149 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (s, 1H), 9.02 (s,1H), 8.44 (s, 1H), 8.28 (d, J = 6.0 Hz, 1H), 8.0 (d, J = 7.8 Hz, 1H), 7.76-7.73 (m, 2H), 7.67- 7.61 (m, 3H), 7.39 (t, J = 7.8 Hz, 1H), 6.62 (d, J = 6.3 Hz, 1H), 3.16 (s, 6H). MS (ESI): m/z = 359.1 $[M+H]^+$. Anal. Calcd for ($C_{20}H_{18}N_6O\cdot H_2O$): C, 63.82; H, 5.36; N, 22.33. Found: C, 63.91; H, 5.27; N, 22.18.

1-(3-(4-(Dimethylamino)pyrimidin-2-yl)phenyl)-3-(4-iodophenyl)urea (8g). The title compound **8g** was prepared from the amine **19b** (75 mg, 0.35 mmol) and 4-iodophenyl isocyanate **13e** (0.15 g, 0.61 mmol) was added at 0 °C as described in the general procedure A. The crude compound was purified by Combiflash chromatography (0-60% ethyl acetate in hexane) to yield the compound **8g** (0.112 g, 70 %) as a white solid; mp 230-232 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H), 8.77 (s, 1H), 8.41 (s, 1H), 8.28 (d, J = 6.3 Hz, 1H), 8.0 (d, J = 7.8 Hz, 1H), 7.63-7.59 (m, 3H), 7.39-7.31 (m, 3H), 6.61 (d, J = 6.0 Hz, 1H), 3.15 (s, 6H). MS (ESI): m/z = 460.1 $[M+H]^+$. Anal. Calcd for ($C_{19}H_{18}IN_5O$): C, 49.69; H, 3.95; N, 15.25. Found: C, 49.69; H, 3.90; N, 15.02.

1-(3-(4-(Dimethylamino)pyrimidin-2-yl)phenyl)-3-(3-fluorophenyl)urea (8h). The title compound **8h** was prepared from the amine **19b** (45 mg, 0.21 mmol) and 3-fluorophenyl isocyanate **13f** (35 mg, 0.26 mmol) as described in the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield the compound **8h** (44 mg, 60 %) as a white solid; mp 116-119 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 2H), 8.42 (s, 1H), 8.28 (d, J = 6.3 Hz, 1H), 8.0 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.54-7.48 (m, 1H), 7.40-7.27 (m, 2H), 7.15 (d, J = 8.1 Hz, 1H), 6.82-6.76 (m, 1H), 6.62 (d, J = 6.0 Hz, 1H), 3.16 (s, 6H). HRMS (ESI) m/z for $C_{19}H_{19}FN_5O$ $[M+H]^+$: Calcd 352.1574; Found: 352.1571.

1-(3-(4-(Pyrrolidin-1-yl)pyrimidin-2-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (8i). The title compound **8i** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (70 mg, 0.29 mmol) and the commercially available 4-(trifluoromethyl)phenyl isocyanate **13g** (98 mg, 0.52 mmol) in anhydrous CH_2Cl_2 (8 mL) according to the general procedure A. Upon completion of reaction, the solution was filtered to obtain the white solid (TLC: R_f= 0.15, 70% ethyl acetate in hexane) after washed with ether (5 mL), dichloromethane (3 mL). The solid was dried in vacuum oven to yield the title compound **8i** (70 mg, 56%) as a white solid; mp 185-188 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.95 (s, 1H), 8.42 (s, 1H), 8.26 (d, J = 6.0 Hz, 1H), 8.0 (d, J = 7.8 Hz, 1H), 7.70-7.62 (m, 5H), 7.38 (t, J = 7.9 Hz, 1H), 6.43 (d, J = 6.0 Hz, 1H), 3.65 (brs, 2H), 3.39 (brs, 2H), 1.98 (s, 4H). MS (ESI): m/z = 428.2 $[M+H]^+$. Anal. Calcd for ($C_{22}H_{20}F_3N_5O$): C, 61.82; H, 4.72; N, 16.39. Found: C, 61.49; H, 4.66; N, 16.28.

1-(4-Acetylphenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)phenyl)urea (8j). The compound **8j** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (70 mg, 0.29 mmol) and the commercially available 4-acetylphenyl isocyanate **13h** (84 mg, 0.52 mmol) in anhydrous CH_2Cl_2 (8 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield the compound **8j** (30 mg, 25%) as an off-white solid; mp 163-166 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.10 (s, 1H), 8.96 (s, 1H), 8.42 (s, 1H), 8.26 (d, J = 6.0 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.65-7.59 (m, 3H), 7.38 (t, J = 7.9 Hz, 1H), 6.43 (d, J = 6.0 Hz, 1H) 3.65 (brs, 2H), 3.35 (brs, 2H), 2.52 (s, 3H), 1.98 (s, 4H). HRMS (ESI) m/z for $C_{23}H_{24}N_5O_2$ $[M+H]^+$: Calcd 402.1930; Found: 402.1920.

Ethyl 4-(3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)phenyl)ureido)benzoate (8k). The title compound **8k** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (70 mg,

0.29 mmol) and ethyl 4-isocyanatobenzoate **13i** (100 mg, 0.52 mmol) in anhydrous CH_2Cl_2 (8 mL) according to the general procedure A. Upon completion of reaction, the suspension was filtered to afford the white solid (TLC: R_f = 0.3, 80% ethyl acetate in hexane), which was washed with dichloromethane (5 mL). The solid was dried in vacuum oven to yield the title compound **8k** (80 mg, 64%) as a white solid; mp 116-119 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.09 (s, 1H), 8.95 (s, 1H), 8.42 (s, 1H), 8.26 (d, J = 6.0 Hz, 1H), 8.0 (d, J = 7.5 Hz, 1H), 7.9 (d, J = 8.4 Hz, 2H), 7.65-7.60 (m, 3H), 7.38 (t, J = 7.9 Hz, 1H), 6.43 (d, J = 6.0 Hz, 1H), 4.28 (q, J = 7.2 Hz, 6.9 Hz, 2H), 3.65 (brs, 2H), 3.35 (brs, 2H), 1.98 (s, 4H), 1.31 (t, J = 7.0 Hz, 3H). MS (ESI): m/z = 432.2 $[M+H]^+$. Anal. Calcd for ($C_{24}H_{25}N_5O_3 \cdot ¼H_2O$): C, 66.12; H, 5.90; N, 16.06. Found: C, 65.85; H, 5.80; N, 15.89.

4-(3-(3-(4-(Pyrrolidin-1-yl)pyrimidin-2-yl)phenyl)ureido)benzoic acid (8l). To a solution of compound **8k** (55 mg, 0.127 mmol) in methanol (1 mL) and THF (2 mL) was added the solution of lithium hydroxide monohydrate (64 mg, 1.53 mmol) in 2 mL water at room temperature. The reaction mixture was stirred overnight. The reaction was monitored by TLC (50% acetone in hexane). Upon completion of reaction, the solution was acidified with 2 N HCl to pH 5. The white solid formed was filtered and washed with water (2 mL) and hexane. The solid was dried in vacuum oven to obtain the title compound **8l** (35 mg, 68%) as a white solid; mp 221-223 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.64 (brs, 1H), 9.66 (s, 1H), 9.55 (s, 1H), 8.38 (s, 1H), 8.29 (d, J = 7.2 Hz, 1H), 7.88 (d, J = 8.7 Hz, 3H), 7.76-7.72 (m, 1H), 7.60-7.49 (m, 3H), 6.76 (d, J = 7.2 Hz, 1H), 3.78 (brs, 2H), 3.58 (brs, 2H), 2.03 (brs, 4H). HRMS (ESI) m/z for C_{22} $H_{22}N_5O_3$ $[M+H]^+$: Calcd 404.1723; Found: 404.1716.

1-(4-Methoxyphenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)phenyl)urea (8m). The compound **8m** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (70 mg,

0.29 mmol) and 4-methoxyphenyl isocyanate **13j** (78 mg, 0.52 mmol) in anhydrous CH_2Cl_2 (8 mL) according to the general procedure A. Upon completion of reaction, the suspension was filtered to obtain the white solid (TLC: R_f= 0.2, 70% ethyl acetate in hexane), which was washed with dichloromethane (5 mL). The solid was dried in a vacuum oven to provide compound **8m** (83 mg, 73 %) as a white solid; mp 219-221 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.72 (s, 1H), 8.44 (s, 1H), 8.37 (s, 1H), 8.25 (d, J = 5.7 Hz, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.39-7.32 (m, 3H), 6.87 (d, J = 8.4 Hz, 2H), 6.42 (d, J = 6.0 Hz, 1H) 3.72 (s, 3H), 3.64 (brs, 2H), 3.35 (brs, 2H), 1.98 (s, 4H). MS (ESI): m/z = 390.2 $[M+H]^+$. Anal. Calcd for ($C_{22}H_{23}N_5O_2 \cdot ¼H_2O$): C, 67.07; H, 6.01; N, 17.78. Found: C, 66.90; H, 6.05; N, 17.72.

1-(4-Hydroxyphenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)phenyl)urea (8n). To the suspension of compound **8m** (0.6 g, 0.15 mmol) in 20 mL CH_2Cl_2, BBr_3 (1.65 mL, 1.65 mmol, 1.0 M in hexane) was added at 0 °C. The mixture was stirred overnight at room temperature. The reaction was monitored by TLC. Upon completion of the reaction, 10 mL of water was slowly added to quench the reaction. The reaction mixture was treated with saturated Na_2CO_3 aqueous solution to pH 9. Then it was condensed *in vacuo* to remove CH_2Cl_2. The residue was extracted with ethyl acetate (3 x10 mL). The organic layer was separated and washed with water, brine and dried over Na_2SO_4. Filtration and removal of solvent provided the crude product, which was purified by Combiflash chromatography (0-10% methanol in dichloromethane) to yield **8n** (23 mg, 41%) as a white solid; mp 229-231 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.68 (s, 1H), 8.36-8.24 (m, 3H), 7.94 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 8.1Hz, 1H), 7.34 (t, J = 7.8 Hz, 1H), 7.23 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 2H), 6.42 (d, J = 5.7 Hz, 1H), 3.61 (brs, 2H), 3.39 (brs, 2H), 1.97 (s, 4H). HRMS (ESI) m/z for $C_{21}H_{22}N_5O_2$ $[M+H]^+$: Calcd 376.1773; Found: 376.1771.

1-(3-Cyanophenyl)-3-(3-(4-(pyrrolidin-1-yl)pyrimidin-2-yl)-phenyl)urea (8o). The compound **8o** was synthesized from 3-(4-(pyrrolidine-1-yl)pyrimidin-2-yl)aniline **19a** (0.1 g, 0.42 mmol) and 3-cyanophenyl isocyanate **13k** (0.089 g, 0.62 mmol) in 5 mL anhydrous dichloromethane according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield title compound **8o** (0.08 g, 50 %) as a white solid; mp 203-205 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.01 (d, J = 5.4 Hz, 2H), 8.41 (t, J = 1.8 Hz, 1H), 8.26 (d, J = 6.0 Hz, 1H), 8.01-7.98 (m, 2H), 7.71-7.67 (m, 1H), 7.64-7.61 (m, 1H), 7.53-7.35 (m, 3H), 6.43 (d, J = 6.0 Hz, 1H), 3.66 (brs, 2H), 3.40 (brs, 2H), 1.99 (s, 4H). HRMS (ESI) m/z for $C_{22}H_{21}N_6O$ $[M+H]^+$: Calcd 385.1777; Found: 385.1774.

1-(3-(4-(Azetidin-1-yl)pyrimidin-2-yl)phenyl)-3-(4-cyanophenyl)urea (8p). The compound **8p** was prepared from 3-(4-(azetidin-1-yl)pyrimidin-2-yl)benzenamine **19g** (148.8 mg, 0.66 mmol) and 4-isocyanatobenzonitrile **13d** (109.8 mg, 0.72 mmol) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-80% ethyl acetate in hexane) to afford the product **8p** (210.4 mg, 86.37%) as a white solid; mp 140–143 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.18 (s, 1H), 9.02 (s, 1H), 8.37–8.36 (m, 1H), 8.27 (d, J = 5.8 Hz, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.74 (d, J = 8.8 Hz, 2H), 7.66–7.61 (m, 3H), 7.38 (t, J = 7.9 Hz, 1H), 6.31 (d, J = 6.0 Hz, 1H), 4.10 (t, J = 7.5 Hz, 4H), 2.40 (t, J = 7.7 Hz, 2H). HRMS (ESI) m/z for $C_{21}H_{19}N_6O$ $[M+H]^+$: Calcd 371.1620; Found: 371.1620.

1-**(4-Cyanophenyl)-3-(3-(4-(piperidin-1-yl)pyrimidin-2-yl)phenyl)urea (8q)**. The compound **8q** was prepared from amine **19e** (80 mg, 0.31 mmol) and 4-cyanophenyl isocyanate **13d** (54 mg, 0.37 mmol) in anhydrous dichloromethane (8 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield the title compound **8q** (95 mg, 76%) as a white solid; mp 120-123 °C. ^{1}H NMR (300 MHz,

DMSO-d_6): δ 9.20 (s, 1H), 9.03 (s, 1H), 8.36 (t, J = 1.5 Hz, 1H), 8.27 (d, J = 6.3 Hz, 1H), 7.99-7.96 (dt, J = 9.0 Hz, 1.2 Hz, 1H), 7.75 (m, 2H), 7.67-7.64 (m, 3H), 7.39 (t, J = 7.8 Hz, 1H), 6.76 (d, J = 6.3 Hz, 1H), 3.73 (s, 4H), 1.67 (s, 2H), 1.57 (s, 4H). MS (ESI): m/z = 399.2 $[M+H]^+$. Anal. Calcd for ($C_{23}H_{22}N_6O \cdot H_2O$): C, 66.33; H, 5.81; N, 20.18. Found: C, 66.63; H, 5.61; N, 19.68.

1-(4-Cyanophenyl)-3-(3-(4-(diethylamino)pyrimidin-2-yl)phenyl)urea (8r). The compound **8r** was synthesized from amine **19d** (80 mg, 0.33 mmol) and 4-cyanophenyl isocyanate **13d** (57 mg, 0.40 mmol) in anhydrous dichloromethane (8 mL) as described in the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield the product **8r** (80 mg, 63%) as a white solid; mp 139-141 °C.^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (s, 1H), 9.02 (s,1H), 8.42 (t, J = 1.5 Hz, 1H), 8.25 (d, J = 6.0 Hz, 1H), 7.99-7.96 (m, 1H), 7.76-7.73 (d, J = 9.0 Hz, 2H), 7.67-7.64 (d, J = 9.0 Hz, 2H), 7.62-7.61 (m,1H), 7.40 (t, J = 7.9 Hz, 1H), 6.58 (d, J = 6.3 Hz, 1H), 3.59 (brs, 4H), 1.18 (t, J = 7.0 Hz, 6H). MS (ESI): m/z = 387.2 $[M+H]^+$. Anal. Calcd for ($C_{22}H_{22}N_6O \cdot H_2O$): C, 65.33; H, 5.98; N, 20.78. Found: C, 65.04; H, 5.78; N, 20.71.

1-(4-Cyanophenyl)-3-(3-(4-(ethyl(methyl)amino)pyrimidin-2-yl)phenyl)urea (8s). The compound **8s** was synthesized from the amine **19c** (60 mg, 0.26 mmol) and 4-cyanophenyl isocyanate **13d** (45 mg, 0.31 mmol) in anhydrous dichloromethane (8 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield the product **8s** (62 mg, 64%) as a white solid; mp 120-123 °C.^{1}H NMR (300 MHz, DMSO-d_6): δ 9.20 (s, 1H), 9.02 (s, 1H), 8.42 (t, J = 1.6 Hz, 1H), 8.27 (d, J = 6.0 Hz, 1H), 8.0-7.97 (dt, J = 7.8 Hz, 1.4 Hz, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 9.0 Hz, 2H), 7.61 (m, 1H), 7.39 (t, J = 7.8 Hz, 1H), 6.60 (d, J = 6.3 Hz, 1H), 3.65 (brs, 2H), 3.11 (s,

3H), 1.15 (t, J = 7.0 Hz, 3H). MS (ESI): m/z = 373.2077 $[M+H]^+$. Anal. Calcd for ($C_{21}H_{20}N_6O \cdot H_2O$): C, 64.60; H, 5.68; N, 21.52. Found: C, 64.48; H, 5.58; N, 21.50.

1-(4-Cyanophenyl)-3-(3-(4-(cyclopropylamino)pyrimidin-2-yl)phenyl)urea (8t). The compound **8t** was prepared from the amine **19f** (80 mg, 0.35 mmol) and 4-cyanophenyl isocyanate **13d** (61 mg, 0.42 mmol) in anhydrous dichloromethane (8 mL) according to the general procedure A. The crude compound was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to yield the product **8t** (87 mg, 67%) as a white solid; mp 180-182 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.19 (s, 1H), 9.0 (s, 1H), 8.44 (t, J = 1.5 Hz, 1H), 8.28 (brs, 1H), 7.97 (d, J = 7.5 Hz, 1H), 7.76-7.73 (d, J = 9.0 Hz, 2H), 7.66-7.64 (m, 3H), 7.56 (d, J = 7.2 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 6.55 (brs, 1H), 2.66 (brs, 1H), 0.82-0.75 (m, 2H), 0.55-0.49 (m, 2H). MS (ESI): m/z = 371.2 $[M+H]^+$. Anal. Calcd for ($C_{21}H_{18}N_6O \cdot ½H_2O$): C, 66.48; H, 5.05; N, 22.15. Found: C, 66.21; H, 5.01; N, 21.72.

2-Chloro-4-(3-nitrophenyl)pyrimidine (11). In a pressure tube, argon gas was bubbled through a mixture of the boronic acid **10** (0.672 g, 4.0 mmol), 2,4-dichloropyrimidine **9** (0.50 g, 3.4 mmol), Na_2CO_3 (1.07 g, 10.1 mmol), 1.5 mL of water, and 15 mL of dimethoxyethane for 20 min. The catalyst $Pd(PPh_3)_4$ (387 mg, 0.34 mmol) was added to the reaction mixture. Then the mixture was saturated with argon gas for 5 min and heated in the pressure tube for 7 h at 90 °C. It was then cooled to room temperature and filtered through a celite padded funnel. The filtrate was mixed with water (15 mL) and extracted with ethyl acetate (2 x 20 mL). The combined organic layer was washed with water and brine, and then dried over Na_2SO_4. Filtration and removal of solvent under reduced pressure provided the crude product, which was purified by chromatography (0-30% ethyl acetate in hexane) to afford 0.350 g (43.8%) of target compound **11** as off-white solid; mp 138-141 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.93 (t, J = 2.1 Hz, 1H,

H8), 8.77 (d, J = 5.2 Hz, 1H, H6), 8.49 (ddd, J = 7.9 Hz, 1.5 Hz, 1.0 Hz, 1H, H10), 8.41 (ddd, J = 8.4 Hz, 2.3 Hz, 1.1 Hz, 1H, H12), 7.77-7.73 (m, 2H, H5 and H11). MS (ESI): m/z = 236.0 $[M+H]^+$. The ^{1}H NMR data were in agreement with the reported data.[41]

3-(2-Chloropyrimidin-4-yl)aniline (12). To the solution of compound **11** (1.38 g, 5.87 mmol) in anhydrous dichloromethane (20 mL) and methanol (20 mL) was added stannous chloride dihydrate (6.23 g, 27.6 mmol) at 0 °C. The reaction mixture was then stirred at rt overnight. The reaction was monitored by TLC (30% acetone in hexane). After completion of the reaction, solvents were removed under reduced pressure. Saturated Na_2CO_3 aqueous solution (20 mL) was added to the residue. The suspension was filtered. The filtrate was extracted with ethyl acetate twice (2 x 20 mL). The organic phase was separated and washed with water, brine and dried over Na_2SO_4. Filtration and removal of solvent under reduced pressure provided the crude product, which was purified by Combiflash chromatography (0-40% ethyl acetate in hexane) to afford 0.48 g (40%) of amine **12** as yellow solid; mp 137-140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (d, J = 5.1 Hz, 1H), 7.60 (d, J = 5.4 Hz, 1H), 7.48 (t, J = 1.9 Hz, 1H), 7.40-7.37 (ddd, J = 7.8 Hz, 1.5 Hz, 1.2 Hz, 1H), 7.31-7.26 (m, 1H), 6.87-6.83 (m, 1H), 3.86 (s, 2H). MS (ESI): m/z = 206.0 $[M+H]^+$.

1-(4-Chlorophenyl)-3-(3-(2-chloropyrimidin-4-yl)phenyl)urea (14a). The biphenyl urea **14a** was synthesized from 3-(2-chloropyrimidin-4yl)aniline **12** (0.06 g, 0.29 mmol) and 4-chlorophenyl isocyanate **13a** (0.067 g, 0.43 mmol) in 5 mL of CH_2Cl_2 according to the general procedure A. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield **14a** (40 mg, 40%) as a white solid; mp 227-229 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.05 (s, 1H), 8.85-8.82 (m, 2H), 8.33 (s, 1H), 8.09 (d, J = 5.4 Hz, 1H), 7.81-

7.78 (d, J = 7.8 Hz, 1H), 7.69-7.66 (m, 1H), 7.53-7.46 (m, 3H), 7.35 (s, 1H), 7.32 (s, 1H). MS (ESI): m/z = 359.0 $[M+H]^+$, 361.0 $[M+H+2]^+$.

1-(4-Bromophenyl)-3-(3-(2-chloropyrimidin-4-yl)phenyl)urea (14b). The biphenyl urea **14b** was synthesized from 3-(2-chloropyrimidin-4-yl)aniline **12** (0.07 g, 0.34 mmol) and 4-bromophenyl isocyanate **13b** (0.1 g, 0.5 mmol) in 5 mL of CH_2Cl_2 according to the general procedure A. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to provide **14b** (55 mg, 41%) as a white solid; mp 235-237 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.88 (s, 1H), 8.83 (d, J = 5.4 Hz, 1H), 8.34 (s, 1H), 8.09 (d, J = 5.1 Hz, 1H), 7.80 (d, J = 7.8 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.51-7.46 (m, 5H). MS (ESI): m/z = 403.0 $[M+H]^+$, 405.0 $[M+H+2]^+$.

1-(3-(2-Chloropyrimidin-4-yl)phenyl)-3-(4-fluorophenyl)urea (14c). The biphenylurea **14c** was synthesized from 3-(2-chloropyrimidin-4-yl)aniline **12** (0.1 g, 0.49 mmol) and 4-fluorophenyl isocyanate **13c** (0.12 g, 0.88 mmol) in 10 mL of CH_2Cl_2 according to the general procedure A. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) and yielded **14c** (0.145 g, 86.7 %) as a white solid; mp 223-225 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.83 (d, J = 5.4 Hz, 1H), 8.73 (s, 1H), 8.33 (t, J = 1.8 Hz, 1H), 8.08 (d, J = 5.4 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.69-7.66 (m, 1H), 7.51-7.46 (m, 3H), 7.17-7.11 (t, J = 9.0 Hz, 2H). MS (ESI): m/z = 343.1 $[M+H]^+$, 345.1 $[M+H+2]^+$.

1-(3-(2-Chloropyrimidin-4-yl)phenyl)-3-(4-cyanophenyl)urea (14d). The biphenyl urea **14d** was synthesized from 3-(2-chloropyrimidin-4yl)aniline **12** (0.1 g, 0.49 mmol) and 4-cyanophenyl isocyanate **13d** (84 mg, 0.58 mmol) in 5 mL of CH_2Cl_2 according to the general procedure A. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield **14d** (120 mg, 70%) as a white solid; mp 237-239 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ

9.24 (s, 1H), 9.20 (s,1H), 8.83 (d, J = 5.1 Hz, 1H), 8.34 (s, 1H), 8.09 (d, J = 5.1 Hz, 1H), 7.83-7.65 (m, 6H), 7.51 (t, J = 7.9 Hz, 1H). MS (ESI): m/z = 350.1 $[M+H]^+$, 352.1 $[M+H+2]^+$.

1-(3-(2-Chloropyrimidin-4-yl)phenyl)-3-(4-iodophenyl)-urea (14e). The biphenyl urea **14e** was synthesized from 3-(2-chloropyrimidin-4yl)aniline **12** (0.07 g, 0.34 mmol) and 4-iodophenyl isocyanate **13e** (0.125 g, 0.51 mmol) in 5 mL of CH_2Cl_2 according to the general procedure A. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield **14e** (0.11 g, 71%) as a white solid; mp 226-229 °C.^{1}H NMR (300 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.83 (d, J = 5.1 Hz, 2H), 8.33 (t, J = 1.6 Hz, 1H), 8.09 (d, J = 5.4 Hz, 1H), 7.80 (d, J = 8.1 Hz, 1H), 7.68-7.65 (m, 1H), 7.63- 7-60 (m, 2H), 7.51-7.46 (m, 1H), 7.33 (d, J = 8.7 Hz, 2H). MS (ESI): m/z = 451.0 $[M+H]^+$, 453.0 $[M+H+2]^+$.

1-(3-(2-Chloropyrimidin-4-yl)phenyl)-3-(3-fluorophenyl)urea (14f). The biphenyl urea **14f** was synthesized from 3-(2-chloropyrimidin-4yl)aniline **12** (0.12 g, 0.59 mmol) and 3-fluorophenyl isocyanate **13f** (0.144 g, 1.1 mmol) in CH_2Cl_2 (15 mL) according to the general procedure A. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to yield product **14f** (150 mg, 75%) as a white solid; mp 216-219 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.08 (s, 1H), 8.94 (s, 1H), 8.83 (d, J = 5.1 Hz, 1H), 8.33 (t, J = 1.8 Hz, 1H), 8.09 (d, J = 5.4 Hz, 1H) 7.82-7.79 (m, 1H), 7.70-7.67 (m, 1H), 7.54-7.47 (m, 2H), 7.36-7.29 (m, 1H), 7.19-7.14 (m, 1H), 6.84-6.78 (m, 1H). MS (ESI): m/z = 343.1 $[M+H]^+$, 345.1 $[M+H+2]^+$.

2-Chloro-4-(pyrrolidine-1-yl)pyrimidine (16a) and 4-chloro-2-(pyrrolidin-1-yl)pyrimidine (17a). To the solution of 2,4-dichloropyrimidine **3** (4 g, 26.8 mmol) in anhydrous THF (35 mL), pyrrolidine **15a** (3.82 g, 53.69 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 2 h. The reaction was monitored by TLC (30% ethyl acetate in hexane). Upon

completion of the reaction, solvent was removed under reduced pressure. The crude compound was purified by column chromatography (0-30% ethyl acetate in hexane) to yield 2-chloro-4-(pyrrolidine-1-yl)pyrimidine **16a** (R_f = 0.5, 3.8 g, 77.3%) as a white solid and 4-chloro-2-(pyrrolidin-1-yl)pyrimidine **17a** (R_f = 0.75, 800 mg, 16%) as a white solid. **16a**: mp 111-114 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 7.98 (d, J = 6.0 Hz, 1H), 6.18 (d, J = 6.0 Hz, 1H), 3.63 (brs, 2H), 3.34 (brs, 2H), 2.05-2.00 (brs, 4H). MS (ESI): m/z = 184.1 $[M+H]^+$, 186.1 $[M+H+2]^+$. **17a:** mp 65-67 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.16 (d, J = 5.1 Hz, 1H), 6.47 (d, J = 5.1 Hz, 1H), 3.57 (t, J = 6.3 Hz, 4H), 1.99 (s, 4H). MS (ESI): m/z = 184.1 $[M+H]^+$, 186.1 $[M+H+2]^+$. The ^{1}H NMR data of **16a** and **17a** were in agreement with the reported data.[42]

2-Chloro-*N*,*N*-dimethylpyrimidine-4-amine (16b) and 4-Chloro-*N*,*N*-dimethylpyrimidin-2-amine (17b). 2,4-Dichloropyrimidine **9** (1.5 g, 10.068 mmol) was dissolved in dimethylamine **15b** (15 mL, 30 mmol, 2.0 M in MeOH). The reaction mixture was stirred at room temperature for 2 h and monitored by TLC (**16b:** R_f = 0.17, **17b** R_f = 0.77, 30% ethyl acetate in hexane). Upon completion of the reaction, the solvent was removed under reduced pressure. 10 mL of water was added to the residue and the organic products were extracted with ethyl acetate (3x10 mL). The combined organic layer was washed with water, brine, and dried over Na_2SO_4. Filtration and removal of solvent *in vacuo* provided the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to yield 2-chloro-*N,N*-dimethylpyrimidine-4-amine **16b** as a white solid (1.26 g, 80%) and 4-chloro-*N,N*-dimethylpyrimidine-2-amine **17b** as a white solid (100 mg, 9.5%). **16b**: mp 71-73 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01 (d, J = 6.0 Hz, 1H), 6.31 (d, J = 6.0 Hz, 1H), 3.12 (s, 6H). MS (ESI): m/z = 158.0 $[M+H]^+$. **17b**: mp 33-35 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, J = 5.1 Hz, 1H),

6.47 (d, J = 5.1 Hz, 1H), 3.18 (s, 6H). MS (ESI): m/z = 158.0 $[M+H]^+$. Their ^{1}H NMR data were in agreement with the reported data.[43,44]

2-Chloro-*N*-ethyl-*N*-methylpyrimidin-4-amine (16c) and 4-Chloro-*N*-ethyl-*N*-methylpyrimidin-2-amine (17c). 2,4-Dichloropyridimidine **9** (2.5 g, 16.78 mmol) was dissolved in anhydrous THF (25 mL) under argon atmosphere. *N*-methylethanamine **15c** (1.56 mL, 17.07 mmol) was added to the reaction mixture at room temperature. The mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC (**16c**: R_f = 0.3, **17c**: R_f = 0.83, 60% hexane in ethyl acetate). Upon completion of the reaction, the solution was diluted with 50 mL dichloromethane. The mixture was treated with 30 mL saturated $NaHCO_3$ aqueous solution to pH = 9. The aqueous layer was extracted with ethyl acetate twice. The organic phase was separated and dried over anhydrous magnesium sulfate. Filtration and removal of the solvent afforded the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to obtain the product 2-chloro-*N*-ethyl-*N*-methylpyrimidin-4-amine **16c** (1.70 g, 59%) as a colorless liquid and 4-chloro-*N*-ethyl-*N*-methylpyrimidin-2-amine **17c** (0.414g, 14,4%) as a colorless liquid. **16c:** ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (dd, J = 6.2, 1.3 Hz, 1H), 6.30 (d, J = 6.2 Hz, 1H), 3.56 (brs, 2H), 3.07(s, 3H), 1.18 (t, J = 7.1Hz, 3H); MS (APCI): m/z 171.8 $[M+H]^+$. **17c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14 (d, J = 5.1 Hz, 1H), 6.45 (d, J = 5.0 Hz, 1H), 3.66 (dd, J = 7.1 Hz, 2H), 3.13 (s, 3H), 1.17 (t, J = 7.1 Hz, 3H); MS (APCI): m/z = 171.8 $[M+H]^+$. The ^{1}H NMR data of the two isomers were in agreement with the data reported in the literature.[44]

2-Chloro-*N*,*N*-diethylpyrimidin-4-amine (16d) and 4-Chloro-*N*,*N*-diethylpyrimidin-2-amine (17d). 2,4-dichloropyridimidine **9** (2.5 g, 16.78 mmol) was added to diethylamine **15d** (10 mL, 96.68 mmol) at room temperature under argon. The reaction was stirred for 30 min. The reaction

was monitored by TLC (**16d**: R_f = 0.43, **17d:** R_f = 0.9, 60% hexane in ethyl acetate). Upon completion of the reaction, the solution was diluted with 100 mL ethyl acetate. Filtration and removal of the solvent afforded the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to obtain the product 2-chloro-*N*,*N*-diethylpyrimidin-4-amine **16d** (2.085 g, 67%) as a colorless liquid and 4-chloro-*N*,*N*-diethylpyrimidin-2-amine **17d** (0.414g, 0.87%) as a colorless liquid. **16d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, J = 6.3 Hz, 1H), 6.28 (d, J = 6.3 Hz, 1H), 3.85–3.14 (brs, 4H), 1.20 (t, J = 7.1 Hz, 6H); MS (APCI): m/z = 185.8 $[M+H]^+$. **17d** : ^{1}H NMR of (300 MHz, $CDCl_3$): 8.13 (d, J = 4.8 Hz, 1H), 6.44 (d, J = 4.8 Hz, 1H), 3.60 (dd, J = 7.2, 6.9 Hz, 4H), 1.18 (t, J = 7.2 Hz, 6H); MS (APCI): m/z = 185.85 $[M+H]^+$. The ^{1}H NMR data of the two isomers were in agreement with the reported data.[45]

2-Chloro-4-(piperidin-1-yl) pyrimidine (16e) and 4-Chloro-2-(piperidin-1-yl)pyrimidine (17e). 2,4-dichloropyridimidine **9** (2.5 g, 16.78 mmol) was dissolved in anhydrous 1,4-dioxane (16 mL) under argon. Triethylamine (2.33 mL, 16.78 mmol) was added dropwise to the reaction mixture at room temperature. Piperidine **15e** (1.56 mL, 17.081 mmol) was added dropwise into the reaction mixture, which was cooled with cold water-bath. The reaction mixture was stirred at room temperature for 3 h. The reaction was monitored by TLC (**16e:** R_f = 0.23, **17e:** R_f = 0.80, 60% ethyl acetate in hexane). Upon completion of the reaction, removal of solvent under reduced pressure afforded the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to yield the product 2-chloro-4-(piperidin-1-yl)pyrimidine **16e** (2.40 g, 72.4%) as a white solid; mp 81- 83 °C and 4-chloro-2-(piperidin-1-yl)pyrimidine **17e** (0.62 g, 18.77%) as a yellow solid; mp 45-47 °C. **16e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.99 (d, J = 6.0 Hz, 1H), 6.37 (d, J = 6.0 Hz, 1H), 3.62 (s, 4H), 1.79–1.47 (m, 6H). MS (APCI): m/z = 197.8

$[M+H]^+$. **17e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, J = 5.1 Hz, 1H), 6.43 (d, J = 5.1 Hz, 1H), 3.77 (t, J = 5.2 Hz, 4H), 1.80–1.44 (m, 6H). MS (APCI): m/z = 197.8 $[M+H]^+$. The ^{1}H NMR data of **16e** and **17e** were in agreement with the reported data.[46]

2-Chloro-*N*-cyclopropylpyrimidin-4-amine (16f) and 4-Chloro-*N*-cyclopropylpyrimidin-2-amine (17f). 2,4-dichloropyridimidine **9** (2.5 g, 16.78 mmol) was dissolved in anhydrous 1,4-dioxane (25 mL) under argon. Triethylamine (3.5 mL, 25.17 mmol) was added dropwise into the reaction mixture at room temperature. Cyclopropanamine **15f** (1.2 mL, 16.78 mmol) was added dropwise to the reaction mixture, which was cooled with cold water-bath. The reaction mixture was stirred at room temperature overnight. It was monitored by TLC (**16f**: R_f = 0.22, **17f**: R_f = 0.41, 60% hexane in ethyl acetate). Upon completion of the reaction, the solvent was removed under reduced pressure to afford the crude product, which was partitioned between 50 mL dichloromethane and 20 mL water. The organic layer was separated and the aqueous phase was extracted with dichloromethane twice. The combined organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. Filtration and removal of solvent *in vacuo* provided the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to obtain the product 2-chloro-*N*-cyclopropylpyrimidin-4-amine **16f** (1.81 g, 63.7%) as a white solid; mp 126-128 °C and 4-chloro-*N*-cyclopropylpyrimidin-2-amine **17f** (0.643 g, 22.7%) as a yellow solid; mp 80-83 °C. **16f**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.14 (d, J = 5.8 Hz, 1H, H6), 6.61 (d, J = 5.8 Hz, 1H, H5), 5.84 (s, 1H, H7), 2.57 (s, 1H, H8), 0.90–0.87 (m, 2H, H9 and H10), 0.63–0.61 (m, 2H, H9 and H10). MS (ESI): m/z = 170.1 $[M+H]^+$. **17f**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.20 (d, J = 5.2 Hz, 1H, H6), 6.61 (d, J = 5.2 Hz, 1H, H5), 5.59 (s, 1H, H7), 2.80-2.77 (m, 1H, H8), 0.87-0.83 (m, 2H, H9 and H10), 0.56-0.55 (m, 2H, H9 and H10). MS (ESI): m/z =

170.1 $[M+H]^+$. The structures of **16f** and **17f** were assigned by their 2D ^{1}H NMR data (see supporting information Figure S6 and S9).

4-(Azetidin-1-yl)-2-chloropyrimidine (16g) and 2-(Azetidin-1-yl)-4-chloropyrimidine (17g). 2,4-dichloropyridimidine **9** (5.21 g, 35.03 mmol) was dissolved in anhydrous methanol (53 mL) under argon. Triethylamine (5.1 mL, 36.78 mmol) was added into the reaction mixture at room temperature. Azetidine **15g** (2.3 mL, 35.03 mmol) was added dropwise into the reaction mixture, which was cooled with cold water-bath. The reaction mixture was stirred at room temperature overnight. The reaction was monitored by TLC (**16g**, R_f = 0.14; **17g**, R_f = 0.39, 60% hexane in ethyl acetate). Upon completion of the reaction, removal of solvent *in vacuo* afforded a residue, which was partitioned between 50 mL dichloromethane and 20 mL water. The organic layer was separated and the aqueous phase was extracted with dichloromethane twice. The combined organic layer was washed with water, brine and dried over anhydrous Na_2SO_4. Filtration and removal of solvent provided the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to afford the product 4-(azetidin-1-yl)-2-chloropyrimidine **16g** (2.755 g, 46.5%) as a white solid; mp 93- 95 °C and the 2-(azetidin-1-yl)-4-chloropyrimidine **17g** (0.63 g, 10.8%) as a white solid; mp 85-87 °C. **16g**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.97 (d, J = 5.8 Hz, 1H, H6), 6.03 (d, J = 5.8 Hz, 1H, H5), 4.15 (brs, 4H, H8 and H10), 2.50-2.44 (m, 2H, H9). MS (ESI): m/z = 170.1 $[M+H]^+$. **17g**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.14 (d, J = 5.2 Hz, 1H, H6), 6.51 (d, J = 5.2 Hz, 1H, H5), 4.18 (t, J =7.8Hz, 4H, H8 and H10), 2.41-2.36 (m, 2H, H9). MS (ESI): m/z = 170.1 $[M+H]^+$. The structures of **16g** and **17g** were assigned by their 2D ^{1}H NMR data (see supporting information Figure S12 and S15).

2-(3-Nitrophenyl)-4-(pyrrolidin-1-yl)pyrimidine (18a). In a pressure tube, argon gas was bubbled through a mixture of 3-nitrophenylboronic acid **10** (0.68 g, 4.09 mmol), 2-chloro-4-

amino-pyrimidine **16a** (0.5 g, 2.73 mmol), Na_2CO_3 (0.87 g, 8.18 mmol), 1,4-dioxane (12 mL), H_2O (3 mL) for 20-25 min. Then catalyst $Pd(PPh_3)_4$ (315 mg, 0.27 mmol) was added and the reaction mixture was refluxed overnight. Upon completion of the reaction, the mixture was allowed to cool to room temperature and was filtered through a small celite pad. The filtrate was washed with water (20 mL) and the organic compound was extracted with ethyl acetate (3x 15 mL). The combined organic layers were washed with water, brine and dried over Na_2SO_4. Filtration and removal of solvent *in vacuo* provided the crude product, which was purified by Combiflash chromatography (0- 30% ethyl acetate in hexane) to afford the compound **18a** (0.46 g, 62.4%) as a yellow solid; mp 147-149 °C. ^{1}HNMR (300 MHz, $CDCl_3$): δ 9.25 (s, 1H), 8.75 (d, J = 7.8 Hz, 1H), 8.28 (d, J = 6.0 Hz, 2H), 7.60 (t, J = 8.1 Hz, 1H), 6.26 (d, J = 6.0 Hz, 1H), 3.78 (brs, 2H), 3.41 (brs, 2H), 2.07 (s, 4H). MS (ESI): m/z = 271.1 $[M+H]^+$.

***N*,*N*-Dimethyl-2-(3-nitrophenyl)pyrimidin-4-amine (18b).** Argon gas was bubbled through the mixture of the commercially available 3-nitrophenylboronic acid **10** (0.637 g, 3.815 mmol), 2-chloro-*N,N*-dimethylpyrimidin-4-amine **16b** (0.5 g, 3.18 mmol), Na_2CO_3 (1.012 g, 9.55 mmol) for 10 min. Then $Pd(PPh_3)_4$ (0.367 g, 0.317 mmol) was charged into the reaction vessel. The reaction was carried as described in the procedure for compound **18a**. The crude compound was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to yield compound **18b** (0.42 g, 64%) as a pale yellow solid; mp 146-149 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.25 (t, J = 1.9 Hz, 1H), 8.76-8.73 (dt, J = 7.8 Hz, 1.2 Hz, 1H), 8.32-8.26 (m, 2H), 7.61 (t, J = 7.8 Hz, 1H), 6.41 (d, J = 6.3 Hz, 1H), 3.22 (s, 6H). MS (ESI): m/z = 245.1 $[M+H]^+$.

***N*-Ethyl-*N*-methyl-2-(3-nitrophenyl)pyrimidin-4-amine (18c).** In a pressure tube, argon gas was bubbled through the mixture of 3-nitrophenylboronic acid **10** (1.181 g, 7.07 mmol), 2-chloro-*N*-ethyl-*N*-methylpyrimidin-4-amine **16c** (1.0 g, 5.89 mmol), Na_2CO_3 (1.874 g, 17.68

mmol), in the mixture solvent of water (6.5 mL) and dimethoxyethane (26 mL) for 10 min. Then $Pd(PPh_3)_4$ (681 mg, 0.5893 mmol) catalyst was added to the mixture. The reaction mixture was refluxed in the pressure tube for 7 h. The reaction was monitored by TLC (30% acetone in hexane). Upon completion of the reaction, the mixture was cooled to room temperature and filtered through a short celite pad. The filtrate was concentrated and ethyl acetate was added. The solution was washed with water, brine and dried over anhydrous Na_2SO_4. Filtration and removal of the solvent provided the crude product, which was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to afford the title compound **18c** (0.788 g, 51.8%) as a yellow solid; mp 57-60 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.24 (s, 1H), 8.74 (d, *J* = 7.8, 1.4 Hz, 1H), 8.37 – 8.19 (m, 2H), 7.61 (t, *J* = 7.9 Hz, 1H), 6.39 (d, *J* = 6.1 Hz, 1H), 3.69 (s, 2H), 3.17 (s, 3H), 1.25 (t, *J* = 7.1 Hz, 3H). MS (APCI): m/z = 258.9 $[M+H]^+$.

***N*,*N*-Diethyl-2-(3-nitrophenyl) pyrimidin-4-amine (18d).** Argon gas was bubbled through the mixture of the commercially available 3-nitrophenylboronic acid **10** (1.353 g, 8.1045 mmol), 2-chloro-*N*,*N*-diethylpyrimidin-4-amine **16d** (1.0 g, 5.403 mmol), Na_2CO_3 (1.145 g, 10.806 mmol), in the mixture of water (10.8 mL) and 1,2- dimethoxyethane (25 mL) for 10 min. Then $Pd(PPh_3)_4$ (313 mg, 0.2702 mmol) was added to the reaction mixture. The reaction was carried through the procedure as described in the method for compound **18a**. The crude compound was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to afford *N*,*N*-diethyl-2-(3-nitrophenyl)pyrimidin-4-amine **18d** (0.974 g, 66.2%) as a yellow solid; mp 65-67 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.23 (s, 1H), 8.72 (d, *J* = 7.8 Hz, 1H), 8.28 (d, *J* = 6.6 Hz, 2H), 7.61 (t, *J* = 6.0 Hz, 1H), 6.37 (d, *J* = 8.0 Hz, 1H), 3.62 (s, 4H), 1.27 (t, *J* = 7.1 Hz, 6H). MS (APCI): m/z = 272.9 $[M+H]^+$.

2-(3-Nitrophenyl)-4-(piperidin-1-yl) pyrimidine (18e). Argon gas was bubbled through the mixture of the commercially available 3-nitrophenylboronic acid **10** (1.271 g, 7.612 mmol), 2-chloro-4-(piperidin-1-yl) pyrimidine **16e** (1.0 g, 5.074 mmol), Na_2CO_3 (1.076 g, 10.148 mmol), in the mixture of water (10.1 mL) and 1,2- dimethoxyethane (25 mL) for 10 min. Then $Pd(PPh_3)_4$ (313 mg, 0.2702 mmol) was added to the reaction mixture. The reaction was carried through the procedure as described in the method for compound **18a**. The crude compound was purified by flash column chromatography (0-30% ethyl acetate in hexane) to yield 2-(3-nitrophenyl)-4-(piperidin-1-yl) pyrimidine **18e** (0.664 g, 46.08%) as a yellow solid; mp 85 - 88°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.22 (s, 1H), 8.73 (d, *J* = 7.5 Hz, 1H), 8.41–8.15 (m, 2H), 7.61 (t, *J* = 8.0 Hz, 1H), 6.48 (d, *J* = 6.3 Hz, 1H), 3.74 (s, 4H), 1.71 (m, 7H). MS (APCI): m/z = 285.0 $[M+H]^+$.

***N*-Cyclopropyl-2-(3-nitrophenyl)pyrimidin-4-amine(18f).** Argon gas was bubbled through the mixture of the commercially available 3-nitrophenylboronic acid **10** (1.482 g, 8.874 mmol), 2-chloro-*N*-cyclopropylpyrimidin-4-amine **16f** (1.0 g, 5.92 mmol), Na_2CO_3 (1.254 g, 8.87 mmol), in the mixture of water (12.5 mL) and 1,2- dimethoxyethane (30 mL) for 10 min. Then $Pd(PPh_3)_4$ (684 mg, 0.5916 mmol) was added to the reaction mixture. The reaction was carried through the procedure as described in the method for compound **18a**. The crude product was purified by flash chromatography (0-30% ethyl acetate in hexane) to yield **18f** (1.434 g, 75.47%) as a yellow solid; mp 150 - 152 °C . ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.20 (t, *J* = 2.0 Hz, 1H), 8.69 (dt, *J* = 7.9, 1.4 Hz, 1H), 8.42 (d, *J* = 6.0 Hz, 1H), 8.28 (ddd, *J* = 8.2, 2.4, 1.1 Hz, 1H), 7.60 (t, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 5.7 Hz, 1H), 5.53 (s, 1H), 2.63 (brs, 1H), 1.00 – 0.80 (m, 2H), 0.76 – 0.53 (m, 2H). MS (ESI): m/z = 257.1$[M+H]^+$.

4-(Azetidin-1-yl)-2-(3-nitrophenyl)pyrimidine (18g). Argon gas was bubbled through the mixture of the commercially available 3-nitrophenylboronic acid **10** (1.382 g, 8.2734 mmol), 4-(azetidin-1-yl)-2-chloropyrimidine **16g** (1.0 g, 5.916 mmol), Na_2CO_3 (1.254 g, 11.8314 mmol), in the mixture solvent of water (12.5 mL) and 1,2- dimethoxyethane (30 mL) for 10 min. Then $Pd(PPh_3)_4$ (684 mg, 0.5916 mmol) was added to the reaction mixture. The reaction was carried through the procedure as described in the method for compound **18a**. The crude product was purified by Combiflash chromatography (0-30% ethyl acetate in hexane) to yield **18g** (0.779g, 51.42%) as a yellow solid; mp 123-125 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.22 (t, *J* = 1.5, 1H), 8.73 (dt, *J* = 7.8, 1.5 Hz, 1H), 8.48 – 8.17 (m, 2H), 7.60 (t, *J* = 8.0 Hz, 1H), 6.14 (d, *J* = 5.9 Hz, 1H), 4.20 (t, *J* = 7.6 Hz, 4H), 2.50 (m , 2H). MS (ESI): m/z = 257.1 $[M+H]^+$.

3-(4-(Pyrrolidine-1-yl)pyrimidin-2-yl)aniline (19a). To the solution of compound **18a** (0.29 g, 1.073 mmol) in ethyl acetate (15 mL) was added catalytic amount (5% equiv) of 10% Pd/C. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 3-4 h. It was monitored by TLC. After the starting material was consumed, the reaction mixture was filtered through a small celite pad. The filtrate was concentrated *in vacuo*. The crude product was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to obtain title compound **19a** (0.23 g, 90.5 %) as an off-white solid; mp 158-161 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (d, *J* = 6.3 Hz, 1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.78 (s, 1H), 7.26-7.21 (m, 1H), 6.78 (d, *J* = 7.5 Hz, 1H), 6.20 (d, *J* = 6.3 Hz, 1H), 3.91-3.20 (brs, 6H), 2.04 (s, 4H). MS (ESI): *m/z* = 241.1 $[M+H]^+$.

2-(3-Aminophenyl)-*N*,*N*-dimethylpyrimidine-4-amine (19b). To the solution of compound **18b** (0.35 g, 1.433 mmol) in ethyl acetate (30 mL) was added catalytic amount (5% equiv) of 10% Pd/C. The reaction mixture was stirred at room temperature under hydrogen atmosphere for

3-4 h and monitored by TLC. After the starting material was consumed, the reaction mixture was filtered through a small celite pad. The filtrate was concentrated *in vacuo* to afford the crude product, which was purified by Combiflash chromatography (0-50% ethyl acetate in hexane) to obtain pure amine **19b** (0.3 g, 97%) as an off-white solid; mp 137-140 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (d, *J* = 6.3 Hz, 1H), 7.84-7.80 (m, 1H), 7.76 (t, *J* = 1.8 Hz, 1H), 7.27-7.21 (m, 1H), 6.80-6.76 (m, 1H), 6.33 (d, *J* = 6.3 Hz, 1H), 4.73 (brs, 2H), 3.18 (s, 6H). MS (ESI): *m/z* = 215.1 $[M+H]^+$.

2-(3-Aminophenyl)-*N*-ehtyl-*N*-methylpyrimidin-4-amine (19c). To the solution of nitro compound **18c** (0.70 g, 2.712 mmol) in ethyl acetate (40 mL), catalytic amount (5% equiv) of 10% Pd/C was added. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 3-4 h. The reaction was monitored by TLC (70% ethyl acetate in hexane). After the starting material was consumed, the reaction mixture was filtered through a small celite pad. The filtrate was concentrated *in vacuo*. The crude product was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to obtain pure amine **19c** (0.25 g, 38 %) as a viscous pale yellow gum. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.26 (d, *J* = 6.0 Hz, 1H), 7.83-7.80 (dt, *J* = 7.8 Hz, 1.3 Hz, 1H), 7-76-7.75 (dd, *J* = 3.6Hz, 1.8 Hz, 1H), 7.26-7.21 (m, 1H), 6.79-6.75 (ddd, *J* = 7.8 Hz, 1.1 Hz, 1H), 6.31 (d, *J* = 6.0 Hz, 1H), 3.67 (d, *J* = 6.9 Hz, 2H), 3.13 (s, 3H), 1.22 (t, *J* = 7.2 Hz, 3H). MS (ESI): *m/z* = 229.1505 $[M+H]^+$.

2-(3-Aminophenyl)-*N*,*N*-diethylpyrimidin-4-amine (19d). To the solution of nitro compound **18d** (0.35 g, 1.286 mmol) in ethyl acetate (20 mL) catalytic amount (5% equiv) of 10% Pd/C was added. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 4 h. It was monitored by TLC (70% ethyl acetate in hexane). After the starting material was consumed, the reaction mixture was filtered through a small celite pad. The filtrate was

concentrated *in vacuo* and was dried in vacuum oven to obtain amine **19d** (0.25 g, 80%) as a viscous pale yellow gum. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (d, *J* = 6.0 Hz, 1H), 7.82-7.79 (m, 1H), 7.75 (m, 1H), 7.26-7.22 (m, 2H), 6.79-6.75 (m, 1H), 6.29 (d, *J* = 6.3 Hz, 1H), 3.60 (m, 4H), 1.24 (t, *J* = 7.0 Hz, 6H). MS (ESI): *m/z* = 243.2 $[M+H]^+$.

3-(4-(Piperidin-1-yl)pyrimidin-2-yl)benzenamine (19e). To the solution of nitro compound **19e** (0.45 g, 1.583 mmol) in ethyl acetate (20 mL), catalytic amount (5% equiv) of 10% Pd/C was added. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 3-4 h. It was monitored by TLC (70% ethyl acetate in hexane). After the starting material was consumed, the reaction mixture was filtered through a small celite pad. The filtrate was concentrated *in vacuo*. The crude product was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to obtain pure amine **19e** (0.36 g, 89%) as an off-white solid; mp 118-120 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (d, *J* = 6.3 Hz, 1H), 7.80-7.77 (m, 1H), 7.73 (m, 1H), 7.25-7.20 (m, 1H), 6.78-6.74 (m, 1H), 6.39 (d, *J* = 6.3 Hz, 1H), 3.70 (s, 6H), 1.66 (s, 6H). MS (ESI): *m/z* = 255.2 $[M+H]^+$.

2-(3-Aminophenyl)-*N*-cyclopropylpyrimidin-4-amine (19f). To the solution of compound **18f** (0.5 g, 1.952 mmol) in ethyl acetate (30 mL), catalytic amount (5% equiv) of 10% Pd/C was added. The reaction mixture was stirred at room temperature under hydrogen atmosphere for 12 h. It was monitored by TLC (70% ethyl acetate in hexane). Upon completion of reaction, the reaction mixture was filtered through a small celite pad. The filtrate was concentrated *in vacuo*. The crude product was purified by Combiflash chromatography (0-70% ethyl acetate in hexane) to obtain pure amine **19f** (0.335 g, 76%) as a pale yellow solid; mp 90-92 °C. ^{1}HNMR (300 MHz, $CDCl_3$): δ 8.38 (d, *J* = 6.0 Hz, 1H), 7.74-7.70 (m, 1H), 7.67 (m, 1H), 7.22 (d, *J* = 7.8 Hz,

1H), 6.79-6.75 (ddd, *J* = 7.8 Hz, 1.2 Hz, 1H), 6.60 (d, *J* = 6.0 Hz, 1H), 5.43 (brs, 1H), 2.58 (brs, 1H), 0.89-0.83 (m, 2H), 0.64-0.59 (m, 2H). MS (ESI): *m/z* = 227.1 $[M+H]^+$.

3-(4-(Azetidin-1-yl)pyrimidin-2-yl)benzenamine (19g). 4-(Azetidin-1-yl)-2-(3-nitrophenyl)pyrimidine **18g** (200 mg, 0.78 mmol) was dissolved in 10 mL methanol. 100 mg of 10% Pd/C was added to the solution. The reaction mixture was stirred at room temperature for 1 h and was monitored by TLC. Upon complete consumption of the starting material, the solution was filtered. The filtration was evaporated under reduced pressure to afford the product **19g** (79.9 mg, 90.49%) as a white solid; mp 168-170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.22 (d, *J* = 5.8 Hz, 1H), 7.57 (t, *J* = 2.0 Hz, 1H), 7.49 (dt, *J* = 7.2, 1.2 Hz, 1H), 7.07 (t, *J* = 7.8 Hz, 1H), 6.64 (ddd, *J* = 7.9, 2.5, 1.1 Hz, 1H), 6.25 (d, *J* = 5.8 Hz, 1H), 5.14 (s, 2H), 4.07 (t, *J* = 7.5 Hz, 4H), 2.38 (m, 2H). MS (ESI): m/z = 227.1 $[M+H]^+$.

Cell Line and Culture Conditions. Human embryonic kidney cells (HEK293T) was a kind gift from Dr. Randall Walikonis (Department of Physiology and Neurobiology, University of Connecticut). The cell line was cultured in DMEM medium with 10% FBS (v/v) and glucose (3.5 mg/ml). Cultures were maintained at 37 °C in humidified incubators with 5% CO_2/ 95% air.

CB_1 Expression and Membrane Preparation. HEK293T cells were seeded at approximately 800,000 cells/100 mm dishes and transiently transfected using the calcium-phosphate precipitation method. Membrane preparation was made as described previously.[47] Briefly, 24 hours post transfection, the cells were harvested and washed with phosphate buffered saline (PBS). The cells were resuspended in PBS containing mammalian protease inhibitor cocktail ((4-2-aminoethyl) benzene-sulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin, and aprotinin) (Sigma-Aldrich, St. Louis, MO) followed by lysis by nitrogen cavitation at 750 psi for 5 min using a Parr cell disruption bomb. The cell lysate was spun at 500 *g* for 10 min at 4ºC to remove

nuclei, cell debris and intact cells. The supernatant was collected and spun at 100,000 g for 45 min at 4ºC. The membrane containing pellet was resuspended in TME buffer (25 mM Tris-HCl, 5 mM $MgCl_2$, and 1 mM EDTA, pH 7.4) containing 7% w/v sucrose. The protein concentration was determined using Bradford assay.[48] The membrane preparation was then stored at -70 ºC.

Radioligand Binding Assay. To determine the allosteric parameters K_B and α, ligand binding assays were performed as previously described.[37] Membrane preparation expressing the CB_1 receptor was incubated with at least nine different concentrations (ranging between 100 pM and 100 μM) of unlabeled allosteric compound, in the presence of 0.5 nM [^{3}H]CP55,940 (141.2 Ci/mmol, PerkinElmer Life Sciences (Boston, MA)) which is an orthosteric agonist of CB_1. TME containing 0.2% fatty acid-free BSA was used as a buffer to make a final volume of 200 μl. Nonspecific binding was determined by incubating the membranes with a high concentration of unlabeled CP55,940 (1 μM). The reaction was terminated by adding 300 μl TME buffer containing 5% BSA and subsequent filtration through Whatman GF/C filter paper with a Brandel cell harvester. This was followed by washing with ice cold TME buffer and collecting the filter paper sections corresponding to each sample. Radioactivity was measured using liquid scintillation counting.

[^{35}S]GTPγS Evaluation. To evaluate the impact of the test compounds on the G-protein coupling efficiency of the CB_1 receptor, GTPγS assays were performed as described previously.[30] Briefly, 7.5 μg membrane preparation expressing the CB_1 receptor was incubated with a saturating concentration of CP55,940 (1 μM), 0.1 nM [^{35}S]GTPγS (1250 Ci/mmol; PerkinElmer Life Sciences, Boston, MA), 5 μM GDP (Sigma, St. Louis, MO) and 0.1% (w/v) BSA in the absence and presence of varying concentrations of the test allosteric modulators. GTPγS binding assay buffer (50 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA, and 100

mM NaCl) was used to make a total volume of 200 μL and the membranes were incubated at 30°C for an hour. Nonspecific binding was determined with 10 μM unlabeled GTPγS (Sigma, St. Louis, MO). For the controls, membrane preparations expressing CB_1 receptor were treated with DMSO or a high concentration of the inverse agonist SR141716A (1 μM). Membrane preparations from the same cells not transfected with CB_1 were also evaluated for the G-protein coupling levels to determine non-CB_1 mediated GTPγS binding. The reaction was terminated by filtration through Whatman GF/C filter papers followed by washing with cold TME buffer. The filter paper sections were collected and radioactivity was measured by liquid scintillation counting.

Data Analysis. All ligand binding and GTPγS assays were carried out in duplicate and at least three independent experiments were performed for each curve. For the ligand binding assays, data are presented as a mean with the corresponding 95% confidence limits. Data were analyzed by nonlinear regression using Prism 6.0 (Graphpad Software Inc., San Diego, CA) as previously described.[30]

Immunoblotting Studies. Cells expressing CB_1 and siRNA targeting β-arrestin 1, β-arrestin 2 or non-silencing RNA duplex were washed twice with PBS and treated with CP55,940 (0.5μM) or allosteric modulator **2** (10μM), **7d** (10μM), **8d** (10μM) for 5 min. To determine if there is a G protein component to ERK1/2 phosphorylation, cells were treated with 5ng/mL PTX for 12 hour at 37 °C before compound treatment. Subsequently the cells were washed with ice-cold PBS and lysed with ice-cold lysis buffer (150 nM NaCl, 1.0% IGEPAL CA-630, 0.5%sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 7.5) plus protease inhibitor cocktail (4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), pepstain A, E-64, bestatin, leupeptin, and aprotinin; Sigma, St Louis, MO). Cell extracts were centrifuged at 18500g for 15 min at 4 °C, and the supernatant

stored at -20 °C. After heating at 95 °C for 3min, 13 μg of total protein was resolved by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane. After washing with blocking reagent (Fisher Scientific, Pittsburgh, PA), the membrane was incubated for 1 h at rt with the primary antibody (1:3000 phospho- p44/42 and p44/42 antibodies; Cell signal technology, Danvers, MA). The membrane was then washed with PBS and incubated with antirabbit peroxidase-conjugated secondary antibody (1:5000; Cell Signaling Technology, Danvers, MA) for 1 h at rt. Detection of reactive proteins employed the SuperSignal West Femto chemiluminescent substrate system (Thermo Fisher Scientific).

ASSOCIATED CONTENT

The spectra of 1D and 2D ^{1}H NMR of compounds **11**, **16f**, **17f**, **16g** and **17g**; and the methods and results of HPLC analysis of compounds **8c**, **8h**, **8j**, **8l**, **8n**, **8o**, and **8p** can be found in the supporting information. These materials are available free of charge via the internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported in part by National Institutes of Health Grant DA039942 (to D.A.K. and D.L.) and Faculty Development Fund of Texas A&M Health Science Center (to D.L.). We thank the Laboratory for Biological Mass Spectrometry of Texas A&M University for assistance

obtaining mass data. We thank Dr. Kumar Kaluarachchi of M.D. Anderson Cancer Center for help recording 2D NMR spectra.

ABBREVIATIONS

CB_1: cannabinoid receptor type 1; CB_2: cannabinoid receptor type 2; GPCR: G-protein coupled receptor; SAR: structure activity relationship; [^{35}S]GTPγS: guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate; ERK: extracellular signal-regulated kinases; cAMP: 3'-5'-cyclic adenosine monophosphate; HEK293: human embryonic kidney 293 cells; BSA: bovine serum albumin; TME: Tris-Mg^{2+}- EDTA; EGTA: ethylene glycol tetraacetic acid; PTX: pertussis toxin. SR141716A: 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide; CP55,940: 2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol.; APCI: Atmospheric pressure chemical ionization; E-64: (1*S*,2*S*)-2-(((*S*)-1-((4-Guanidinobutyl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)cyclopropane-carboxylic acid.

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