2,4-Diaminopyrimidine Derivatives as Potent Growth Hormone Secretagogue Receptor Antagonists

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Ghrelin, a gut-derived orexigenic hormone, is an endogenous ligand of the growth hormone secretagogue receptor (GHS-R). Centrally administered ghrelin has been shown to cause hunger and increase food intake in rodents. Inhibition of ghrelin actions with ghrelin antibody, peptidyl GHS-R antagonists, and antisense oligonucleosides resulted in weight loss and food intake decrease in rodents. Here we report the effects of GHS-R antagonists, some of which were potent, selective, and orally bioavailable. A structure−activity relationship study led to the discovery of **8a**, which was effective in decreasing food intake and body weight in several acute rat studies.

Introduction

Obesity is a mounting worldwide problem. The cost of medical care and productivity lost due to diseases associated with obesity is estimated to be greater than $100 billion per year in the United States alone.[1,2] Obesity is associated with a variety of chronic disorders including insulin resistance, diabetes, dyslipidemia, hypertension, coronary heart disease, ischemic stroke (cerebrovascular and thromboembolic disease), pulmonary disease, gout, osteoarthritis, cancer, neurologic diseases, cataracts, gastrointestinal disease, and in women, genitourinary disease. Weight loss has been shown to reverse or slow the progression of these disorders.[3]

Caloric intake must be balanced with the energy expenditure in order to maintain body weight.[3] Diet and exercise are crucial to weight control, but they might be assisted by an oral agent that would reduce appetite. This combination could be beneficial for those attempting to lose weight and maintain their lower weight in the fight against obesity.

Ghrelin is a 28-amino acid orexigenic hormone that is produced in the stomach and hypothalamus.[4] It was found to be an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), a G-protein-coupled receptor (GPCR) expressed primarily in the pituitary gland, the brain, and to a lesser extent in the periphery. Active ghrelin contains an octanoyl group at the Ser-3 hydroxyl group. This unique post-translational acylation is essential for its orexigenic function and is considered necessary for its penetration of the blood−brain barrier.[4,5] Intracerebroventricular (icv) administration of ghrelin increased food intake in rats, and intravenous (iv) administration of ghrelin increased appetite and food intake in human subjects.[6−10] Ghrelin levels in the blood appear to be regulated by nutrient intake. Carbohydrates are slightly more effective in lowering ghrelin levels than proteins, which are considerably more effective than lipids.[11] The weight gain induced by ghrelin has been observed to be primarily from adipose tissue and not muscle or bone mass.[12] Smith and co-workers have demonstrated that mice lacking the GHS-R gene are immune to the orexigenic effects of ghrelin.[13] Rats dosed icv with antisense mRNA for GHS-R were found to have lower body weights and significantly decreased food intake compared to controls from weeks 3 to 9 in this study.[14] Antagonizing the GHS-R receptor with a peptide ([D-Lys-3]-GHRP-6) was shown to decrease food intake and body weight gain.[9,12] Small molecule GHS-R antagonists are expected to have similar beneficial effects.

Previously we have reported the discovery of isoxazole carboxamide and tetralin carboxamide based GHS-R antagonists.[15−18] These two chemically distinct series of GHS-R antagonists effectively displaced ^{125}I-ghrelin binding as well as antagonizing ghrelin-induced intracellular Ca^{2+} flux in our functional assay. However, these compounds did not exhibit an effect when tested in in vivo animal models. In searching for efficacious GHS-R antagonists, 2,4-diaminopyrimidine derivatives identified from a high throughput screen of the Abbott Laboratories compound collection were explored.[19] The prototype compound, **7a** (Table 1), demonstrated a binding IC_{50} of 310 nM for GHS-R and an intracellular Ca^{2+} release functional IC_{50} of 1.38 μM.

Chemistry

The general synthesis of the 2,4-diaminopyrimidine core began with acylation of 4-nitrophenylacetonitrile (**1a**) or 4-cyanophenylacetonitrile (**1b**) with an acid chloride (**2a**−**c**, Scheme 1). The resulting aryl-β-ketonitriles (**3a**−**e**) were treated with either trimethylsilyldiazomethane or diazomethane solution in diethyl ether to give the corresponding enol ethers (**4a**−**e**). Diaminopyrimidines **5a**−**e** were obtained by addition of guanidine to enol ethers **4a**−**e** followed by elimination of methanol.[20] The nitro group in **5a**, **5c**, and **5e** was reduced to provide anilines **6a**, **6b**, and **6c** (Scheme 2).[21] Compounds **7a**−**j**, **8a**−**o**, **9**, and intermediates for **12a**, **12b**, and **13** were then synthesized via a simple reductive amination reaction between **6a**, **6b**, and **6c** with the corresponding aldehydes.[22] Intermediates for compounds **10a** and **10b** were synthesized by diazotization of aniline **6a** with aqueous workup to form the phenol. The intermediate phenol

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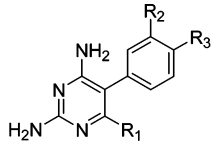
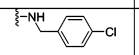
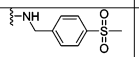
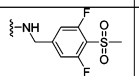
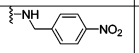
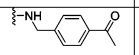
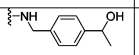
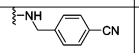
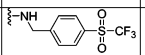
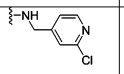
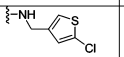
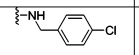
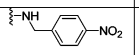
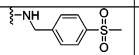
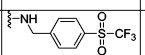
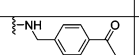
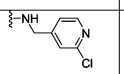
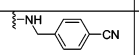
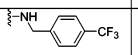
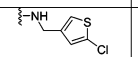
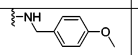
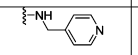
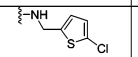
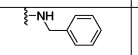
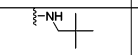
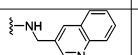
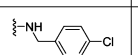
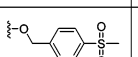
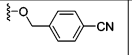
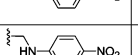
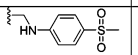
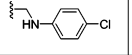
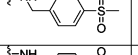
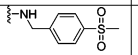
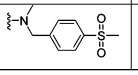
Table 1. IC_{50} Values of the Diaminopyrimidines against GHS-R



Compound	R_1	R_2	R_3	Binding IC_{50} ± SEM (nM)	Ca^{2+} Flux IC_{50} ± SEM (nM)
7a	Et	H		310 ± 42	1380 ± 600
7b	Et	H		7.4 ± 2.3	12 ± 8.8
7c	Et	H		2.4	45 ± 43
7d	Et	H		11	23 ± 5.6
7e	Et	H		57 ± 7.9	110 ± 66
7f	Et	H		13	110 ± 31
7g	Et	H		160	780 ± 390
7h	Et	H		21	690 ± 560
7i	Et	H		110	220 ± 220
7j	Et	H		290	790 ± 300
8a	-CH_2OCH_2Ph	H		19 ± 11	180 ± 28
8b	-CH_2OCH_2Ph	H		0.2 ± 0.1	5.8 ± 2.6
8c	-CH_2OCH_2Ph	H		0.3 ± 0.1	7.2 ± 0.5
8d	-CH_2OCH_2Ph	H		1.0	85 ± 89
8e	-CH_2OCH_2Ph	H		1.2 ± 0.2	10 ± 0.7
8f	-CH_2OCH_2Ph	H		1.5	3.6 ± 0.9
8g	-CH_2OCH_2Ph	H		2.8 ± 0.1	19 ± 3.9

Compound	R_1	R_2	R_3	Binding IC_{50} ± SEM (nM)	Ca^{2+} Flux IC_{50} ± SEM (nM)
8h	-CH_2OCH_2Ph	H		2.9	97 ± 19
8i	-CH_2OCH_2Ph	H		6.3	170 ± 110
8j	-CH_2OCH_2Ph	H		7.7	90 ± 7
8k	-CH_2OCH_2Ph	H		21	86 ± 58
8l	-CH_2OCH_2Ph	H		43	320 ± 410
8m	-CH_2OCH_2Ph	H		50	980 ± 1060
8n	-CH_2OCH_2Ph	H		67	700 ± 1240
8o	-CH_2OCH_2Ph	H		130	780 ± 540
9		H		3170 ± 660	3350
10a	-CH_2OCH_2Ph	H		6.9	48 ± 77
10b	-CH_2OCH_2Ph	H		83	3610 ± 2490
11a	-CH_2OCH_2Ph	H		0.8 ± 0.2	16 ± 1.3
11b	Et	H		9.2	190 ± 190
11c	Et	H		9.8 ± 1.0	120 ± 110
11d	Et	H		300	3150 ± 645
12a	Et	Cl		4.2 ± 1.5	11 ± 3.5
12b	Et	Br		5.7	42 ± 14
13	Et	H		3.7	32 ± 18

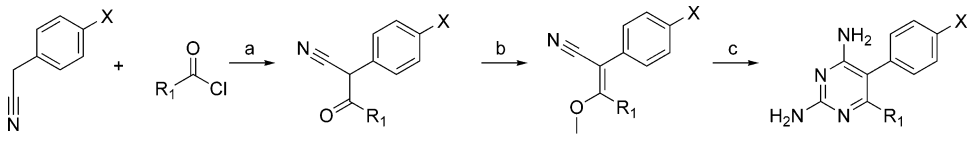
was deprotonated with KOEt and treated with the corresponding benzyl bromides to form compounds **10a** and **10b**. Halogenated analogues **12a** and **12b** were made by reacting **7b** with *N*-chlorosuccinimide (NCS), acetic acid, and $HClO_4$ in methanol/dichloromethane[23] or bromine in acetic acid,[24] respectively (Scheme 3). *N*-Methylbenzylamine **13** was obtained by reductive amination of **7b** with formaldehyde.[25]

The cyano groups in **5b** and **5d** were reduced to yield the corresponding benzylamines by hydrogenation (Scheme 2).[26] Compounds **11a**−**d** were obtained by displacement of substituted aryl fluorides with the aforementioned benzylamines.[27]

Results and Discussion

We began our structure−activity relationship (SAR) exploration by extending the diversity of the benzylamine moiety in **7a** because of the easy synthetic accessibility of these analogues. The compounds were first run in the GHS-R binding assay, which was used primarily to triage the compounds synthesized. Those compounds with an IC_{50} value under 2 μM were tested in an intracellular Ca^{2+} flux activity assay.[15] Functionally pure antagonists are compounds capable of blocking ghrelin-induced intracellular Ca^{2+} flux and do not cause any Ca^{2+} flux in the absence of ghrelin. Since the binding assay was used to decide

Scheme 1. General Synthesis of a Common Diaminopyrimidine Core[a]



1a-b **2a-c** **3a-e** **4a-e** **5a-e**

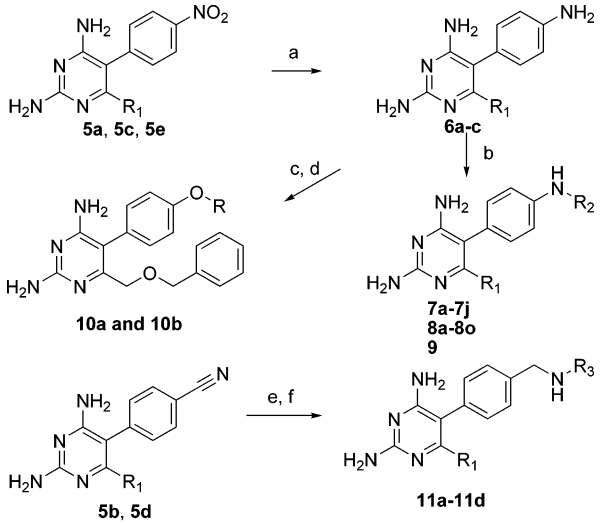
1a X = NO_2
1b X = CN

2a R_1 = Et
2b R_1 = CH_2OCH_2Ph
2c R_1 = CH_2CH_2Ph

3,4, and **5a** X = NO_2, R_1 = Et
3,4, and **5b** X=CN, R_1 = Et
3,4, and **5c** X = NO_2, R_1 = CH_2OCH_2Ph
3,4, and **5d** X = CN, R_1 = CH_2OCH_2Ph
3,4, and **5e** X = NO_2, R_1 = CH_2CH_2Ph

[a] Reagents and conditions: (a) Et_3N, DMAP, CH_2Cl_2; (b) $TMSCHN_2$, CH_2Cl_2; (c) guanidine, EtOH, reflux.

Scheme 2. General Synthesis of Diaminopyrimidine GHS-R Antagonists **7**−**11**[a]

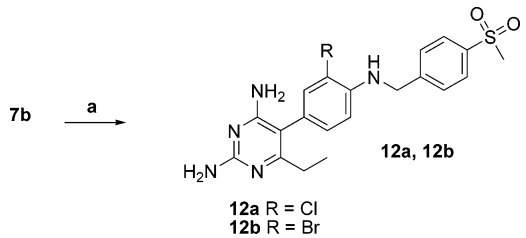


5a, **5c**, **5e** **6a-c** **10a and 10b** **7a-7j** **8a-8o** **9** **5b**, **5d** **11a-11d**

6a R_1 = Et
6b R_1 = CH_2OCH_2Ph
6c R_1 = CH_2CH_2Ph
7a-7j R_1 = Et
8a-8o R_1 = CH_2OCH_2Ph
9 R_1 = CH_2CH_2Ph
10a R = 4-methylsulfonylbenzyl
10b R = 4-cyanobenzyl
11a R_1 = CH_2OCH_2Ph R_3 = 4-nitrophenyl
11b R_1 = Et R_3 = 4-nitrophenyl
11c R_1 = Et R_3 = 4-methylsulfonylphenyl
11d R_1 = Et R_3 = 4-chlorophenyl

[a] Reagents and conditions: (a) H_2, $Pd(OH)_2$, MeOH; (b) aldehyde, $NaBH_3CN$, MeOH, HOAc/NaOAc buffer; (c) $NaNO_2$, H_2SO_4 (aq); (d) KOEt, EtOH, substituted benzyl bromides; (e) H_2, Raney nickel, NH_3/MeOH; (f) arylfluoride, NMP.

Scheme 3. General Synthesis of Phenyl Substituted Benzylamine Diaminopyrimidines **12a** and **12b**[a]



7b **12a, 12b**

12a R = Cl
12b R = Br

[a] Reagents and conditions: (a) NCS, $HClO_4$, HOAc, DCM, MeOH (for **12a**), or Br_2, HOAc (for **12b**).

which compounds would be tested in the activity assay, the majority of the compounds were only tested once to maximize the throughput. A large number of aromatic ring systems were examined, and the representative analogues are shown in Table 1. Exploration of the SAR about the phenyl ring revealed that benzylamines or benzyl ethers at the 4-position were optimal for GHS-R blockade. The benzyl groups in turn were most potent when substituted in the 4-position, and these analogues are shown in Table 1. Generally, small hydrophilic groups at this position gave favorable binding potency and Ca^{2+} flux activity. For example, 4-methylsulfonyl, 3,5-difluoro-4-methylsulfonyl, and 4-nitro analogues **7b**, **7c**, and **7d** demonstrated increased binding potency (28- to 130-fold over **7a**) and improved Ca^{2+} flux activity (30- to 115-fold over **7a**). Closely related methylketo group (i.e., **7e**) or cyano group (i.e., **7g**) appeared less effective than **7b**, **7c**, or **7d**. Interestingly, 1-hydroxyethyl analogue **7f** showed binding potency similar to **7b**, but it showed a 9-fold decrease in Ca^{2+} flux activity. A larger and more hydrophobic 4-trifluoromethylsulfonyl analogue, **7h**, exhibited a 58-fold decrease in IC_{50} value in the Ca^{2+} flux activity assay with only a slight decrease (3-fold) in the binding IC_{50} value compared to **7b**. Chlorinated heterocyclic compounds **7i** and **7j** demonstrated IC_{50} values that are within 2-fold of those for **7e** and **7g**, respectively, in the Ca^{2+} flux assay.

In the course of SAR exploration based on the hit compound **7a**, a significant enhancement in binding potency was observed when the ethyl side chain at the 6-position of the diaminopyrimidine ring was replaced with a benzyloxymethyl group (e.g., **8a**, Table 1). This modification led to a greater than 16-fold improvement in binding and an 8-fold improvement of the intracellular Ca^{2+} flux assay IC_{50} values. Later SAR results revealed the etheral oxygen atom in **8a** played a key role in receptor binding. A structurally similar compound, phenethyl analogue **9** (Table 1), exhibited greater than a 160-fold loss in binding potency compared to **8a** and a 20-fold loss of activity in the Ca^{2+} flux assay. The similarity in structure and lack of potency with compound **9** made it a good negative control compound for in vivo studies. Analogues **8a**−**e** exhibited roughly similar SAR trends when directly compared with the corresponding 6-ethyldiaminopyrimidine modifications. 6-Benzyloxydiaminopyrimidine compounds **8f** and **8g** demonstrated a great improvement over **8a** in binding and intracellular Ca^{2+} flux assay IC_{50} values. The improvement of **8f** and **8g** over **8a** was greater than that seen with 6-ethyldiaminopyrimidines **7i** and **7g**, respectively, compared to analogue **7a**. These results showed that the SAR was not completely transparent between the 6-ethyldiaminopyrimidines and the 6-benzyloxymethyldiaminopyrimidines. The Ca^{2+} flux assay IC_{50} values of the chlorothiophene analogue **8i** were similar to the parent compound **8a**. 4-Trifluoromethyl analogue **8h**, 4-methoxy analogue **8j**, and 4-pyridyl analogue **8k** showed comparable intracellular Ca^{2+} flux assay IC_{50} values to 4-chloro analogue **8a**. Introduction of a chloro group in the pyridine ring in **8f** further improved both binding potency and intracellular Ca^{2+} flux activity over that of **8k**. 5-Chlorothiophen-2-yl analogue **8l** demonstrated a 2-fold decrease in intracellular Ca^{2+} flux IC_{50} values below that of its isomeric analogue, **8i**. Both **8i** and **8l** were less active

Table 2. PK Properties and Brain Plasma Ratios (B/P) of Selected Compounds in Rats

#	AUC[a−c]	CL_p[a,d]	$t_{1/2}$ (h)[a]	C_{max}[a,c,e]	F (%)[a,c,f]	B/P levels at 1 h[a] (μg/g/μg/mL)	B/P levels at 8 h[a] (μg/g/μg/mL)
7a	2.2	1.8	5.2	0.17	76	2.74/0.25	0.60/0.11
7b	2.8	0.94	6.6	0.32	52	0.05/1.02	n.d.[g]
7d	1.6	2.6	5.5	0.14	68	0.53/0.27	n.d.
7e	0.68	3.7	2.4	0.14	51	0.42/0.12	n.d.
8a	0.12	2.2	n.d.	0.054	5.5	1.07/0.35	0.05/0.03
8a (ip dose)	1.14	2.2	3.9 (ip)	0.26 (ip)	68 (ip)	0.33/0.50[h]	0.14/0.19[h]
9	n.d.	n.d.	n.d.	n.d.	n.d.	1.34/0.80[h]	0.40/0.09[h]
12a	2.7	1.6	8.7	0.16	78	0.03/1.04	n.d.
13	2.7	0.99	3.8	0.43	53	0.04/1.28	n.d.

[a] 5 mg/kg dose, mean for $n = 3$. [b] μg·h/mL, iv. [c] Oral unless otherwise specified. [d] L/h·kg, iv. [e] μg/mL, iv. [f] Oral bioavailability unless otherwise specified. [g] n.d. = not determined. [h] 30 mg/kg, ip.

than **8f**. Replacements of the 4-chlorobenzyl group in **8a** with a benzyl, *tert*-butyl, or quinolin-3-yl all resulted in less potent compounds (analogues **8m**, **8n**, and **8o**).

Although the replacement of the benzylamine nitrogen atom with an oxygen atom (e.g. **10a**) resulted in 23-fold weaker binding potency and a 7-fold decrease in intracellular Ca^{2+} flux activity compared to **8c**, **10a** is still a very potent compound. The modification from 4-methylsulfonyl benzyl ether **10a** to cyano benzyl ether **10b**, however, demonstrated an even further loss of binding potency and a tremendous loss in intracellular Ca^{2+} flux IC_{50}.

Juxtaposition of the nitrogen atom and the benzylic carbon atom of the benzylamine moiety in **7a** or **8a** generally resulted in equipotent antagonists. For example, **11a** and **11d** (Table 1) exhibited similar binding potencies and a 3-fold loss in intracellular Ca^{2+} flux activities compared to **8b** and **7a**, respectively. This demonstrates that the sequence of these two atoms is not critical to the receptor binding. They might also function as a scaffold group that holds the diaminopyrimidine ring and the phenyl ring in the right orientation.

Halogenation of the central phenyl ring in **7b** (i.e. **12a** and **12b**, Table 1) resulted in minimal changes in binding potency and intracellular Ca^{2+} flux activity. Methylation of the benzylamine nitrogen in **7b** to form **13** gave a 2-fold improvement in binding potency and an identical intracellular Ca^{2+} flux activity. These data, in addition to compounds **10a** and **10b**, suggests that the nitrogen atom of the benzylamine moiety does not act as a significant hydrogen bond donor.

Overall, the SAR indicated that a variety of changes were tolerated. Compounds containing hydrogen bonding or electron withdrawing groups at the 3- and 4-positions exhibited the best binding potency and intracellular Ca^{2+} flux activity, whereas lipophilic groups at the same positions diminished binding potency and intracellular Ca^{2+} flux activity. This is interesting since polar interactions are generally more difficult to identify in the lead optimization process. Antagonists **8b** and **8c** represent the most potent of our GHS-R binders with IC_{50} values of 0.21 and 0.30 nM, respectively, in the ^{125}I-ghrelin displacement assay. Antagonists **8b** and **8f** represent the most active antagonists in the functional assay with intracellular Ca^{2+} flux IC_{50} values of 4−6 nM.

Compound **8a** (Table 1) and several structurally similar analogues were tested against a panel of GPCRs and were found to be selective against adrenergic ($IC_{50} > 33$ μM), histaminergic ($IC_{50} > 33$ μM), muscarinic ($IC_{50} > 5.5$ μM), dopaminergic ($IC_{50} > 33$ μM), opioid like-1 ($IC_{50} > 33$ μM), and serotonergic/5HT2C ($IC_{50} > 33$ μM) receptors. Compound **8a** was also tested against several targets that have been implicated in appetite control, such as cannabinoid-1 receptor (CB_1) and melanin-concentrating hormone receptor (MCH). In a Cerep screen, compound **8a** was demonstrated to be inactive against CB_1 (−1% inhibition at 10 μM). The compound also demonstrated an IC_{50} of >10 μM against MCH in an intracellular Ca^{2+} flux activity assay.

The pharmacokinetic (PK) properties were studied for a number of analogues in this series (Table 2). Compound **7a**, although not extremely potent, exhibited an excellent PK profile. More active analogues **7b**−**e**, **12a**, and **13** demonstrated very similar PK profiles. For example, **7b** showed a rat oral bioavailability of 52%, half-life of 6.6 h, and a C_{max} of 0.32 μg/mL. Compound **7b** was studied in a 14-day diet induced obesity mouse model study, and severe toxicity was observed during the second week of the study with half of the animals (the other half appeared healthy). This may be due to the fact that **7b** is an extremely potent dihydrofolate reductase (DHFR) inhibitor ($K_i = 0.5$ nM).[28]

Some of the current literature suggests that central nervous system penetration is necessary for both ghrelin and its antagonists to exert their orexigenic effects.[4] GHS receptors have also been found in the periphery, but we were interested in GHS-R antagonists that penetrate the blood−brain barrier (BBB). Brain and plasma levels were measured on selected compounds (Table 2). For example, antagonist **7a** demonstrated brain drug levels of 2.74 μg/g at 1 h (Table 2), indicating that **7a** crosses the BBB. Methylsulfonyl analogue **7b** exhibited much lower brain drug levels, probably due to its increased molecular size and polarity. 4-Chlorobenzyl analogue **8a** exhibited significantly greater brain drug levels than those of the other benzyl ether side chain analogues, **12a** and **13**.

With this information we chose to utilize compound **8a** (Table 1) in in vivo studies to determine its effects on food intake and body weight in free-fed and fasted Sprague−Dawley rats. Antagonist **8a** was administered intraperitoneally (ip) for these studies since the bioavailability was considerably higher when dosed ip (68%) than after oral (5%) dosing.

In the first experiment, compound **8a** (10 and 30 mg/kg, ip) caused a dose-dependent decrease in cumulative food intake at 1 and 24 h after a single dose in free-fed rats, and at 24 h in 16 h (overnight) fasted rats (Figure 1). The decrease in food intake 24 h after the 30 mg/kg dose was significant and comparable in both free-fed and overnight fasted rats (29% and 24%, respectively). Additionally, body weight in the 30 mg/kg ip free-fed group was significantly reduced 24 h after dosing (Figure 2). However, a statistically significant decrease was not observed in 1 h food intake in overnight fasted rats. The small molecule antagonist induced decrease in food intake seen here is consistent with that seen previously with the peptide antagonist [D-Lys-3]-GHRP-6[12] and an anti-ghrelin antibody.[9]

Our next goal was to determine if the effects on food intake caused by compound **8a** are attributable to functional GHS-R blockade. DHFR inhibition was a primary off-target selectivity concern due to the diaminopyrimidine pharmacophore common

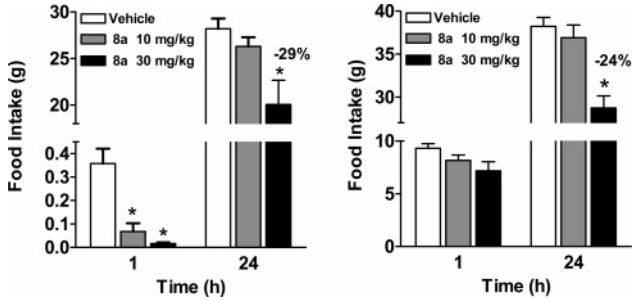


Figure 1. Effect of **8a** (given a single dose of 10 and 30 mg/kg, ip) on food intake in free-fed (left) and overnight-fasted (16 h) (right) Sprague−Dawley rats, mean values ± SEM for $n = 10$/group, $p < 0.01$ via Anova post hoc Dunnett.

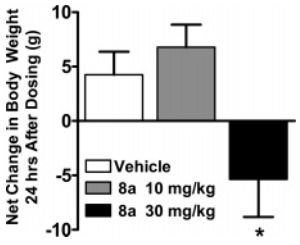


Figure 2. Effect of **8a** (single dose of 10 and 30 mg/kg, ip) on body weight in free-fed Sprague−Dawley rats, mean values ± SEM for $n = 10$/group, $P < 0.05$ via Anova post hoc Dunnett.

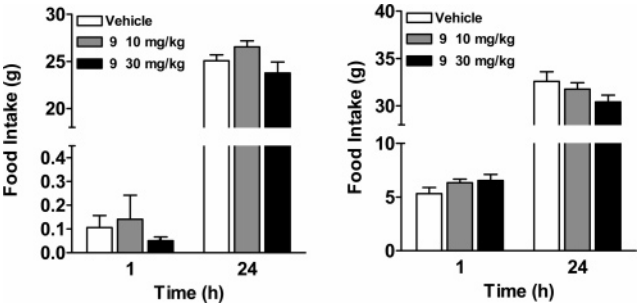


Figure 3. Effect of compound **9** (single dose of 10 and 30 mg/kg, ip) on food intake in free-fed (left) and overnight-fasted (16 h) (right) Sprague−Dawley rats, mean values ± SEM for $n = 10$/group, Anova post hoc Dunnett.

to known DHFR inhibitors (e.g. pyrimethamine or methotrexate.[29] Compound **9** was advanced to in vivo food intake studies for comparison to **8a**. Compound **9** is a structurally similar analogue that is at least equipotent at inhibiting DHFR (90% vs 75% inhibition at 1 μM for **9** and **8a**, respectively) but binds GHS-R with >160-fold lower affinity (Table 1). In contrast to results obtained with compound **8a**, compound **9** did not cause a significant decrease in food intake at any time point (Figure 3). Analysis of brain and plasma levels in like-treated animals from the same experiment indicates that brain levels of compound **9** were higher than those of **8a** throughout the study. Plasma levels of compound **9** were greater than **8a** through 2 h (Figure 4). These data taken together support the notion that the compound **8a** induced reduction of acute food intake is not due to DHFR inhibition. In addition, all rats dosed with compound **8a** and **9** appeared normal after dosing with no injection site irritation. More specifically, no observed behavioral effects consistent with nonhomeostatic effects such as visceral illness were observed.

Additional mechanistic insight was gained by administering compound **8a** (30 mg/kg ip) to overnight (16 h) fasted GHS-R null (KO) and wild-type (WT) mice (Figure 5). At each time point, food intake in vehicle treated WT mice exceeded

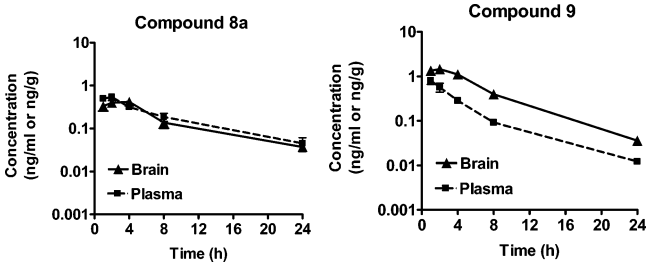


Figure 4. Plasma and brain exposures of **8a** and **9** (given a single dose of 30 mg/kg, ip), mean values ± SEM for $n = 5$/timepoint.

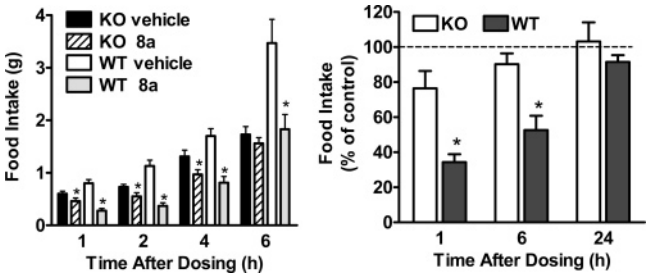


Figure 5. Pharmacological effect of **8a** (given a single dose of 30 mg/kg, ip) on food intake in GHS-R knockout and wild-type mice, mean values ± SEM for $n = 7-8$/group, $P < 0.01$ via Tukey−Kramer.

that in KO mice, which is consistent with ghrelin acting via GHS-R contributing to fasting-induced food intake. Figure 5 illustrates that compound **8a** caused a relatively greater reduction of food intake in WT than KO mice, suggesting that most of the anorectic efficacy of this compound is mediated through GHS-R.

Conclusion

Many diaminopyrimidine GHS-R antagonists, some of which were potent, selective, and orally bioavailable, were identified. Antagonist **8a**, which was initially used for proof-of-principle studies, has demonstrated robust efficacy in blocking food intake and body weight increase in several animal models. The observed activity of the antagonist appears to be mainly due to the antagonism of the GHS-R as evidenced by the lack of efficacy of an inactive but structurally similar compound **9** and by the diminished efficacy of antagonist **8a** in GHS-R knockout mice. These compounds showed some cross activity toward DHFR, a key enzyme in DNA synthesis. Compound **9** is a much weaker binder to the ghrelin receptor, a more potent inhibitor of DHFR than **8a**, and has similar plasma and brain exposures. Rats dosed with compound **9** did not show a decrease in acute food intake. This indicates that the effects of DHFR inhibition are not responsible for the efficacy of **8a**. Compound **8a** was tested in a GPCR selectivity panel, a CB_1 assay, and MCH activity assay and was found to be inactive against each of these targets. The optimization efforts that lead to the discovery of potent and DHFR-selective diaminopyrimidine GHS-R antagonists will be discussed in a following paper.

Experimental Methods

General Information. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. All reactions were performed under nitrogen atmosphere unless specifically noted. Flash chromatography was performed using silica gel (230−400 mesh) from E. M. Science. ^{1}H NMR spectra were recorded on a Varian Mercury 300 (300 MHz) or Varian UNITY 400 (400 MHz) spectrometer and are reported in ppm (δ) from tetramethylsilane (TMS: δ 0.0 ppm).

Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets), coupling constants (Hz), integration. Mass spectral analyses were accomplished on a Finnigan SSQ7000 GC/ MS mass spectrometer using different techniques, including desorption chemical ionization (DCI), atmospheric pressure chemical ionization (APCI), and electrospray ionization (ESI), as specified for individual compounds. Exact mass measurement was performed on a Finnigan FTMS Newstar T70 mass spectrometer. The compound is determined to be "consistent" with the chemical formula if the exact mass measurement is within 4.0 ppm relative mass error (RME) of the exact monoisotopic mass.

Analytical HPLC for selected compounds were performed on two systems. System A: Samples were analyzed with a Gilson analytical HPLC system, using an YMC C-8 column (50 × 4.6 mm i.d., S-5 um, 120 Å). Solvent system used was acetonitrile/ 0.1% aqueous NH_4OAc, gradient 0−70% over 15 min at 2 mL/ min. System B: Samples were analyzed by Agilent Analytical HPLC 1100 Series, SB-C8 column (Zorbax 3.5 um, 4.6 × 75 mm). Solvent system used was acetonitrile/0.1% aqueous TFA, gradient 10−70% over 10 min at 1.5 mL/min. Using two analytical HPLC methods, the purity of the compounds was determined to be >95% by UV detection.

Preparative reverse phase HPLC was performed on an automated Gilson HPLC system, using a SymmetryPrep Shield RP18 prep cartridge, 25 mm × 100 mm i.d., S-7 μm, and a flow rate of 25 mL/min; λ = 214, 245 nm; mobile phase A, 5% CH_3CN and 0.1% TFA or 10 mM NH_4OAc in H_2O; mobile phase B, linear gradient 0−70% of B in 19 min. The purified fractions were evaporated to dryness with a ThermoSavant SpeedVac.

General Procedure for Synthesis of the Diaminopyrimidine Compounds 7a−j, 8a−o, 9, 10a−b, 11a−d, 12a−b, and 13. 4-Benzyloxy-2-(4-nitro-phenyl)-3-oxo-butyronitrile (**3c**). To a solution of 4-nitrophenylacetonitrile (**1a**, 10.0 g, 61.7 mmol), triethylamine (14.5 g, 144 mmol), and 4-(dimethylamino)pyridine (800 mg, 6.56 mmol) in CH_2Cl_2 (150 mL) at 0 °C was added benzyloxyacetyl chloride (2b, 12.0 g, 64.8 mmol) over 30 min. The resulting mixture was warmed to room temperature, stirred for 2 h, and concentrated in vacuo. The mixture was extracted in ethyl acetate (1 × 150 mL) and washed with saturated $NaHCO_3$ (1 × 80 mL), aqueous HCl (1 × 80 mL), and brine (1 × 80 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to provide crude 4-benzyloxy-2-(4-nitro-phenyl)-3-oxo-butyronitrile (**3c**, 19.6 g) as a red-brown solid which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.24 (d, J = 9.2 Hz, 2H), 7.96 (d, J = 8.8 Hz, 2H), 7.25−7.46 (m, 5H), 4.60 (s, 2H), and 4.48 (s, 2H). MS (APCI) m/z = 311 $[M + H]^+$, 309 $[M - H]^-$.

4-Benzyloxy-3-methoxy-2-(4-nitro-phenyl)-but-2-enenitrile (4c). To a solution of 4-benzyloxy-2-(4-nitro-phenyl)-3-oxo-butyronitrile (**3c**, 9.72 g, 31.4 mmol) in CH_2Cl_2 (80 mL) was slowly added a solution of 2 M trimethylsilyldiazomethane ($TMSCHN_2$) in diethyl ether (30 mL, 60 mmol). The reaction was monitored by TLC using an aliquot to which had been added 3 M HOAc and was extracted in EtOAc. Glacial HOAc (5 mL) was added dropwise until the excess $TMSCHN_2$ was consumed as indicated by the absence of N_2 evolution. The mixture was concentrated in vacuo, extracted with EtOAc (1 × 100 mL), and washed with 1 M HOAc (1 × 150 mL) and brine (1 × 100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to provide 4-benzyloxy-3-methoxy-2-(4-nitro-phenyl)-but-2-enenitrile (**4c**, 10.2 g) which was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (d, J = 9.2 Hz, 2H), 7.87 (d, J = 9.2 Hz, 2H), 7.29−7.45 (m, 5H), 4.69 (s, 2H), 4.66 (s, 2H), 4.09 (s, 3H). MS (APCI) m/z = 325 $[M + H]^+$.

6-Benzyloxymethyl-5-(4-nitro-phenyl)-pyrimidine-2,4-diamine (5c). To a solution of 4-benzyloxy-3-methoxy-2-(4-nitro-phenyl)-but-2-enenitrile (**4c**, 10.2 g, 31.4 mmol) in EtOH (60 mL) was added a premixed solution of guanidine hydrochloride (3.605 g, 37.5 mmol) in EtOH (60 mL) and 2.65 M NaOEt in EtOH (14.2 mL, 37.6 mmol). The resulting dark, purple solution was heated at reflux for 3 h, concentrated in vacuo, extracted with EtOAc (1 × 150 mL), and washed with 0.5 M NaOH (2 × 100 mL). The mixture was stirred vigorously, then the resulting precipitate was filtered, providing 6-benzyloxymethyl-5-(4-nitro-phenyl)-pyrimidine-2,4-diamine (**5c**, 8.78 g, 80% over three steps) as a light brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 9.2 Hz, 2H), 7.18−7.31 (m, 3H), 7.05−7.17 (m, 2H), 6.13 (s, 2H), 5.90 (s, 2H), 4.30 (s, 2H), 3.99 (s, 2H). MS (ESI) m/z = 352 $[M + H]^+$, 350 $[M - H]^-$.

5-(4-Amino-phenyl)-6-benzyloxymethyl-pyrimidine-2,4-diamine (6b). To a solution of 20 wt % $Pd(OH)_2$/C (wet, 600 mg) in MeOH (140 mL) was added 6-benzyloxymethyl-5-(4-nitro-phenyl)-pyrimidine-2,4-diamine (**5c**, 5.00 g, 14.25 mmol) in a heavy walled high pressure reaction vessel. The vessel was charged with H_2 (60 psi), and the mixture was stirred at room temperature for 14 h. The reaction mixture was filtered through a nylon membrane, and the solution was concentrated in vacuo followed by trituration with MeOH (20 mL). 5-(4-Aminophenyl)-6-benzyloxymethyl-pyrimidine-2,4-diamine (**6b**, 4.34 g, 95%) was obtained as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.16−7.37 (m, 5H) 6.85 (d, J = 8.1 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 5.86 (s, 2H), 5.49 (s, 2H), 5.12 (s, 2H), 4.35 (s, 2H), 3.97 (s, 2H). MS (ESI) m/z = 322 $[M + H]^+$.

6-Benzyloxymethyl-5-[4-(4-chloro-benzylamino)-phenyl]-pyrimidine-2,4-diamine (8a). To a solution of 5-(4-amino-phenyl)-6-benzyloxymethyl-pyrimidine-2,4-diamine (**6b**, 1.62 g, 5.05 mmol) in 1 M NaOAc/HOAc in MeOH (80 mL, pH 4) was added 4-chlorobenzaldehyde (851 mg, 6.06 mmol). The resulting mixture was stirred for 15 min followed by slow addition of $NaBH_3CN$ (375 mg, 6.06 mmol). The reaction mixture was stirred for 16 h at 25 °C. The product was diluted with EtOAc (80 mL) and washed with 1.2 M HCl (75 mL), 2 M NaOH (2 × 100 mL), and brine (100 mL). The crude material was purified by silica gel chromatography (EtOAc to 10% MeOH in EtOAc gradient) providing 6-benzyloxymethyl-5-[4-(4-chloro-benzylamino)-phenyl]-pyrimidine-2,4-diamine (**8a**, 1.56 g, 70%). Unless otherwise noted analogues mentioned hereafter were purified by preparative reverse phase HPLC (0−70% CH_3CN in 0.1% TFA (aq)/5% CH_3CN or 10 mM NH_4OAc (aq)/ 5% CH_3CN). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33−7.47 (m, 4H), 7.21−7.30 (m, 3H), 7.14−7.20 (m, 2H), 6.89 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 6.37 (t, J = 5.9 Hz, 1H), 5.87 (s, 2H), 5.48 (s, 2H), 4.32 (s, 2H), 4.27 (d, J = 6.1 Hz, 2H), 3.94 (s, 2H). MS (ESI) m/z = 446 $[M + H]^+$, 444 $[M - H]^-$. Anal. ($C_{25}H_{24}ClN_5O$·0.2 CH_3OH) C, H, N.

5-[4-(4-Chloro-benzylamino)-phenyl]-6-ethyl-pyrimidine-2,4-diamine (7a). Title compound **7a** was synthesized by the general procedure described for **8a** substituting propionyl chloride for benzyloxyacetyl chloride to provide compound **3a** in the first step. Yield, 36%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.40 (m, 4H), 6.85 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 6.34 (t, J = 5.9 Hz, 1H), 5.76 (s, 2H), 5.31 (s, 2H), 4.26 (d, J = 6.1 Hz, 2H), 2.11 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 354 $[M + H]^+$, 352 $[M - H]^-$. Anal. ($C_{19}H_{20}N_5Cl$·0.19H_2O) C, H, N.

6-Ethyl-5-(4-{[4-(methylsulfonyl)benzyl]amino}phenyl)-pyrimidine-2,4-diamine (7b). Title compound **7b** was synthesized by the general procedure described for **7a** substituting 4-methanesulfonylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.5 Hz, 2H), 6.54 (t, J = 5.9 Hz, 1H), 6.21 (s, 2H), 5.86 (s, 2H), 4.40 (d, J = 6.1 Hz, 2H), 3.20 (s, 3H), 2.14 (q, J = 7.6 Hz, 2H), 0.97 (t, J = 7.6 Hz, 3H). MS (ESI) m/z = 398 $[M + H]^+$. Anal. ($C_{20}H_{23}N_5O_2S$·0.1CF_3CO_2H) C, H, N.

5-(4-{[3,5-Difluoro-4-(methylsulfonyl)benzyl]amino}phenyl)-6-ethylpyrimidine-2,4-diamine (7c). To a solution of 3,4,5-trifluorobenzaldehyde (250 mg, 1.76 mmol) in DMSO (2 mL) was added sodium methanesulfinate (198 mg, 1.94 mmol). The reaction vessel was purged with N_2, and the reaction mixture was heated in a microwave reactor for 20 min at 185 °C. The reaction was diluted with water (5 mL), and then the mixture was extracted with EtOAc

(10 mL). The organic phase was washed with water (1 × 20 mL) and then brine (1 × 20 mL), dried over Na_2SO_4, and concentrated in vacuo to give 3,5-difluoro-4-methanesulfonylbenzaldehyde (200 mg, 70%), which was used directly without any further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.0 (t, J = 1.7 Hz, 1H), 7.58 (s, 1H), 7.56 (s, 1H), 3.36 (s, 3H). MS (DCI) m/z = 238 $(M + NH_4)^+$.

Title compound **7c** was synthesized by the general procedure described for **7a** substituting 3,5-difluoro-4-methanesulfonylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 65%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33 (d, J = 10.2 Hz, 2H), 6.88 (d, J = 8.5 Hz, 2H), 6.61 (d, J = 8.5 Hz, 2H), 6.50 (t, J = 6.3 Hz, 1H), 5.72 (s, 2H), 5.27 (s, 2H), 4.39 (d, J = 6.1 Hz, 2H), 3.41 (s, 3H), 2.11 (q, J = 7.6 Hz, 2H), 0.94 (t, J = 7.6 Hz, 3H). MS (ESI) m/z = 434 $[M + H]^+$. Anal. ($C_{20}H_{21}F_2N_5O_2S \cdot 1.0CF_3CO_2H$) C, H, N. Calcd for C, 48.26; H, 4.05; N, 12.79. Found: C, 47.96; H, 4.46; N, 12.36.

6-Ethyl-5-{4-[(4-nitrobenzyl)amino]phenyl}pyrimidine-2,4-diamine (7d). Title compound **7d** was synthesized by the general procedure described for **7a** substituting 4-nitro-benzaldehyde for 4-chlorobenzaldehyde. Yield, 56%.^{1}H NMR (300 MHz, DMSO-d_6) δ 8.22 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 6.51 (t, J = 6.1 Hz, 1H), 5.71 (s, 2H), 5.23 (bs, 2H), 4.44 (d, J = 6.1 Hz, 2H), 2.10 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 365 $[M + H]^+$. Anal. ($C_{19}H_{20}N_6O_2 \cdot 0.15CF_3CO_2H$) C, H, N. Calcd for C, 60.76; H, 5.32; N, 22.03. Found: C, 60.92; H, 5.49; N, 21.47.

1-(4-{[4-(2,4-Diamino-6-ethyl-pyrimidin-5-yl)-phenylamino]-methyl}-phenyl)-ethanone (7e). Title compound **7e** was synthesized by the general procedure described for **7a** substituting 4-acetylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.93 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.1 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 6.43 (t, J = 5.9 Hz, 1H), 5.73 (s, 2H), 5.27 (s, 2H), 4.36 (d, J = 6.1 Hz, 2H), 2.56 (s, 2H), 2.10 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.6 Hz, 3H). MS (ESI) m/z = 362 $[M + H]^+$. HRMS calcd for ($C_{21}H_{24}N_5O$) 362.1981. Found: 362.1977.

1-(4-{[4-(2,4-Diamino-6-ethyl-pyrimidin-5-yl)-phenylamino]-methyl}-phenyl)-ethanol (7f). To a solution of **7e** (76 mg, 0.21 mmol) in 3:1 MeOH/THF (1.5 mL) at room temperature was added $NaBH_4$ (32 mg, 0.84 mmol) slowly, then the reaction mixture was stirred for 1 h. The reaction mixture was quenched with 1 M HCl, filtered, and purified by preparative RP-HPLC to give 1-(4-{[4-(2,4-diamino-6-ethyl-pyrimidin-5-yl)-phenylamino]-methyl}-phenyl)-ethanol (55 mg, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.36−7.26 (m, 4H), 6.84 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 8.8 Hz, 2H), 6.25 (t, J = 5.9 Hz, 1H), 5.72 (s, 2H), 5.26 (s, 2H), 5.08 (s, 1H), 4.69 (q, J = 6.3 Hz, 1H), 4.23 (d, J = 5.8 Hz, 2H), 2.11 (q, J = 7.8 Hz, 2H), 1.30 (d, J = 6.4 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 364 $[M + H]^+$. Anal. ($C_{21}H_{25}N_5O \cdot 0.15CF_3CO_2H$) C, H, N.

4-({[4-(2,4-Diamino-6-ethylpyrimidin-5-yl)phenyl]amino}-methyl)benzonitrile (7g). Title compound **7g** was synthesized by the general procedure described for **7a** substituting 4-cyano-benzaldehyde for 4-chlorobenzaldehyde. Yield, 73%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (d, J = 8.1 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 6.61 (d, J = 8.4 Hz, 2H), 6.44 (t, J = 5.9 Hz, 1H), 5.77 (s, 2H), 5.32 (s, 2H), 4.38 (d, J = 5.9 Hz, 2H), 2.11 (q, J = 7.6 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 345 $[M + H]^+$. Anal. ($C_{20}H_{20}N_6 \cdot 0.05CF_3CO_2H$) C, H, N.

6-Ethyl-5-[4-(4-trifluoromethanesulfonyl-benzylamino)-phenyl]-pyrimidine-2,4-diamine (7h). Title compound **7h** was synthesized by the general procedure described for **7a** substituting (4-trifluoromethanesulfonyl)-benzaldehyde[30] for 4-chlorbenzaldehyde. Yield, 71%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 8.5 Hz, 2H), 6.54 (t, J = 6.1 Hz, 1H), 5.72 (s, 2H), 5.27 (bs, 2H), 4.50 (d, J = 6.1 Hz, 2H), 2.11 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 452 $[M + H]^+$, 450 $[M - H]^-$. Anal. ($C_{20}H_{20}F_3N_5O_2S \cdot 0.1CF_3CO_2H$) C, H, N.

5-{4-[(2-Chloro-pyridin-4-ylmethyl)-amino]-phenyl}-6-ethyl-pyrimidine-2,4-diamine (7i). Title compound **7i** was synthesized by the general procedure described for **7a** substituting 2-chloro-pyridine-4-carboxaldehyde[31] for 4-chlorobenzaldehyde. Yield, 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (d, J = 5.1 Hz, 1H), 7.49 (s, 1H), 7.42 (d, J = 5.1 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 6.46 (t, J = 6.1 Hz, 1H), 5.72 (s, 2H), 5.27 (bs, 2H), 4.36 (d, J = 6.1 Hz, 2H), 2.11 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 455 $[M + H]^+$. Anal. ($C_{18}H_{19}ClN_6 \cdot 0.05CF_3CO_2H$) C, H, N.

5-{4-[(5-Chloro-thiophen-3-ylmethyl)-amino]-phenyl}-6-ethyl-pyrimidine-2,4-diamine (7j). Title compound **7j** was synthesized by the general procedure described for **7a** substituting 5-chloro-2-thiophenecarboxaldehyde for 4-chlorobenzaldehyde. Yield, 30%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.28 (d, J = 1.6 Hz, 1H), 7.09 (d, J = 1.9 Hz, 1H), 6.87 (d, J = 8.7 Hz, 2H), 6.66 (d, J = 8.7 Hz, 2H), 6.16 (t, J = 5.8 Hz, 1H), 5.28 (bs, 2H), 5.73 (s, 2H), 4.18 (d, J = 5.6 Hz, 2H), 2.12 (q, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 360 $[M + H]^+$. HRMS calcd for ($C_{17}H_{19}ClN_5S$) 360.1050. Found: 360.1044.

6-[(Benzyloxy)methyl]-5-{4-[(4-nitrobenzyl)amino]phenyl}-pyrimidine-2,4-diamine (8b). Title compound **8b** was synthesized by the general procedure described for **8a** substituting 4-nitro-benzaldehyde for 4-chlorobenzaldehyde. Yield, 27%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20 (ddd, J = 9.1, 2.5, 2.3 Hz, 2H), 7.66 (d, J = 8.8 Hz, 2H), 7.25 (m, 3H), 7.16 (dd, J = 7.5, 2.0 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 6.55 (t, J = 6.3 Hz, 1H), 5.97 (s, 2H), 5.61 (s, 2H), 4.44 (d, J = 6.1 Hz, 2H), 4.32 (s, 2H), 3.95 (s, 2H). MS (ESI) m/z = 457 $[M + H]^+$, 455 $[M - H]^-$. HRMS calcd for ($C_{25}H_{25}N_6O_3$) 457.1988. Found: 457.1984.

6-[(Benzyloxy)methyl]-5-(4-{[4-(methylsulfonyl)benzyl]amino}-phenyl)pyrimidine-2,4-diamine (8c). Title compound **8c** was synthesized by the general procedure described for **8a** substituting 4-methanesulfonylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 67%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.31−7.15 (m, 5H), 6.90 (d, J = 8.5 Hz, 2H), 6.61 (d, J = 8.8 Hz, 2H), 6.52 (t, J = 6.3 Hz, 1H), 6.01 (s, 2H), 5.64 (s, 2H), 4.41 (d, J = 6.1 Hz, 2H), 4.33 (s, 2H), 3.96 (s, 2H), 3.18 (s, 3H). MS (ESI) m/z = 490 $[M + H]^+$. Anal. ($C_{26}H_{27}N_5O_3S \cdot 0.53CF_3CO_2H$) C, H, N. Calcd for C, 59.09; H, 5.04; N, 12.73. Found: C, 59.23; H, 5.48; N, 12.29.

6-[(Benzyloxy)methyl]-5-[4-({4-[(trifluoromethyl)sulfonyl]-benzyl}amino)phenyl]pyrimidine-2,4-diamine (8d). Title compound **8d** was synthesized by the general procedure described for **8a** substituting 4-trifluoromethanesulfonylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.11 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 8.5 Hz, 2H), 7.30−7.15(m, 5H), 6.92 (d, J = 8.5 Hz, 2H), 6.61 (d, J = 8.5 Hz, 2H), 6.57 (t, J = 6.1 Hz, 1H), 5.89 (s, 2H), 5.51 (bs, 2H), 4.51(d, J = 6.1 Hz, 2H), 4.31 (s, 2H), 3.94 (s, 2H). MS (ESI) m/z = 544 $[M + H]^+$, 542 $[M - H]^-$. Anal. ($C_{26}H_{24}F_3N_5O_3S \cdot 0.1CF_3CO_2H$) C, H, N.

1-(4-{[4-(2,4-Diamino-6-benzyloxymethyl-pyrimidin-5-yl)-phenylamino]-methyl}-phenyl)-ethanone (8e). Title compound **8e** was synthesized by the general procedure described for **8a** substituting 4-acetylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8.5 Hz, 2H), 7.30−7.12 (m, 5H), 6.89 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 6.46 (t, J = 6.1 Hz, 1H), 5.88 (s, 2H), 5.48 (s, 2H), 4.37 (d, J = 5.8 Hz, 2H), 4.31 (s, 2H), 3.94 (s, 2H), 2.55 (s, 2H), 1.89 (s, 3H). MS [ESI] m/z = 454 $[M + H]^+$. Anal. ($C_{27}H_{27}N_5O_2 \cdot 0.2CF_3CO_2H$) C, H, N.

6-[(Benzyloxy)methyl]-5-(4-{[(2-chloropyridin-4-yl)methyl]-amino}phenyl)pyrimidine-2,4-diamine (8f). Title compound **8f** was synthesized by the general procedure described for **8a** substituting 2-chloro-pyridine-4-carboxaldehyde[32] for 4-chlorobenzaldehyde. Yield, 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.34 (d, J = 5.1 Hz, 1H), 7.49 (s, 1H), 7.42 (d, J = 5.1 Hz, 1H), 7.30−7.15 (m, 5H), 6.91 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 6.49 (t, J = 6.5 Hz, 1H), 5.87 (s, 2H), 5.50 (bs, 2H), 4.36 (d, J = 6.5 Hz, 2H), 4.31 (s, 2H), 3.93 (s, 2H). MS (ESI) m/z = 447 $[M + H]^+$, 445 $[M - H]^-$. Anal. ($C_{24}H_{23}ClN_6O \cdot 0.15CH_3CO_2NH_4 \cdot 0.6CH_3CN$) C, H, N.

4-{[(4-{2,4-Diamino-6-[(benzyloxy)methyl]pyrimidin-5-yl}-phenyl)amino]methyl}benzonitrile (8g). Title compound **8g** was synthesized by the general procedure described for **8a** substituting 4-cyanobenzaldehyde for 4-chlorobenzaldehyde. Yield, 54%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.80 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H), 7.28−7.15 (m, 5H), 6.90 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 8.5 Hz, 2H), 6.48 (t, J = 6.1 Hz, 1H), 5.87 (s, 2H), 5.47 (bs, 2H), 4.39 (d, J = 6.1 Hz, 2H), 4.31 (s, 2H), 3.93 (s, 2H). MS (ESI) m/z = 502 [M + Na]$^+$, 480 [M + H]$^+$, 478 [M − H]$^-$. Anal. ($C_{26}H_{24}N_6O\cdot0.05CF_3CO_2H$) C, H, N.

6-Benzyloxymethyl-5-[4-(4-trifluoromethyl-benzylamino)-phenyl]-pyrimidine-2,4-diamine (8h). Title compound **8h** was synthesized by the general procedure described for **8a** substituting 4-trifluoromethylbenzaldehyde for 4-chlorobenzaldehyde. Yield, 37%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.69 (d, J = 8.1 Hz, 2H), 7.61 (d, J = 8.1 Hz, 2H), 7.21−7.29 (m, 3H), 7.13−7.19 (m, 2H), 6.90 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.8 Hz, 2H), 6.47 (t, J = 6.1 Hz, 1H), 5.87 (s, 2H), 5.48 (s, 2H), 4.39 (d, J = 5.8 Hz, 2H), 4.31 (s, 2H), 3.94 (s, 2H). MS (ESI) m/z = 437 [M + H]$^+$, 435 [M − H]$^-$. Anal. ($C_{26}H_{24}F_3N_5O\cdot0.05CF_3CO_2H$) C, H, N.

6-Benzyloxymethyl-5-{4-[(5-chloro-thiophen-3-ylmethyl)-amino]-phenyl}-pyrimidine-2,4-diamine (8i). To a suspension of *N*-chlorosuccinimide (0.28 g, 2.2 mmol) in 5 mL of CH_2Cl_2 was added thiophene-3-carboxaldehyde (0.18 mL, 2 mmol) followed by few drops of $HClO_4$. The mixture was stirred at room temperature overnight, after which it was filtered to remove the white precipitate. The filtrate was washed with water, then dried over $MgSO_4$, and concentrated to give a mixture of 5-chloro-3-thiophenecarboxaldehyde and starting material as an oil which was used without further purification. Title compound **8i** was synthesized by the general procedure described for **8a** substituting the 5-chloro-3-thiophenecarboxaldehyde mixture for 4-chlorobenzaldehyde. Yield, 66%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15−7.32 (m, 6H), 7.10 (d, J = 1.7 Hz, 1H), 6.91 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 6.22 (t, J = 5.9 Hz, 1H), 5.54 (bs, 2H), 5.91 (s, 2H), 4.33 (s, 2H), 4.19 (d, J = 6.1 Hz, 2H), 3.96 (s, 2H). MS (ESI) m/z = 452 [M + H]$^+$, 450 [M − H]$^-$. Anal. ($C_{23}H_{22}ClN_5OS\cdot0.1CF_3CO_2H$) C, H, N.

6-[(Benzyloxy)methyl]-5-{4-[(4-methoxybenzyl)amino]phenyl}-pyrimidine-2,4-diamine (8j). Title compound **8j** was synthesized by the general procedure described for **8a** substituting 4-methoxybenzaldehyde for 4-chlorobenzaldehyde. Yield, 30%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.32 (d, J = 8.5 Hz, 2H), 7.28−7.15 (m, 5H), 6.90 (d, J = 8.5 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 6.22 (t, J = 5.8 Hz, 1H), 5.87 (s, 2H), 5.47 (bs, 2H), 4.33 (s, 2H), 4.19 (d, J = 5.8 Hz, 2H), 3.95 (s, 2H), 3.72 (s, 3H). MS (ESI) m/z = 442 [M + H]$^+$, 440 [M − H]$^-$. Anal. ($C_{26}H_{27}N_5O_2$) C, H, N.

6-[(Benzyloxy)methyl]-5-{4-[(pyridin-4-ylmethyl)amino]-phenyl}pyrimidine-2,4-diamine (8k). Title compound **8k** was synthesized by the general procedure described for **8a** substituting pyridine 4-carbaldehyde for 4-chlorobenzaldehyde. Yield, 23%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (m, 2H), 7.38 (d, J = 5.8 Hz, 2H), 7.26 (m, 3H), 7.17 (m, 2H), 6.90 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 8.5 Hz, 2H), 6.45 (t, J = 5.9 Hz, 1H), 5.87 (s, 2H), 5.49 (s, 2H), 4.33 (d, J = 7.1 Hz, 2H), 4.32 (s, 2H), 3.93 (s, 2H). MS (ESI) m/z = 413 [M + H]$^+$, 411 [M − H]$^-$. Anal. ($C_{24}H_{24}N_6O\cdot1.35CH_3CO_2H$) C, H, N.

6-Benzyloxymethyl-5-{4-[(5-chloro-thiophen-2-ylmethyl)-amino]-phenyl}-pyrimidine-2,4-diamine (8l). Title compound **8l** was synthesized by the general procedure described for **8a** substituting 5-chloro-2-thiophenecarboxaldehyde for 4-chlorobenzaldehyde. Yield, 63%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.14−7.30 (m, 5H), 6.89−6.98 (m, 4H), 6.67 (d, J = 8.8 Hz, 2H), 6.39 (t, J = 5.9 Hz, 1H), 5.88 (s, 2H), 5.49 (bs, 2H), 4.41 (d, J = 6.1 Hz, 2H), 4.32 (s, 2H), 3.94 (s, 2H). MS (ESI) m/z = 452 [M + H]$^+$, 450 [M − H]$^-$. Anal. ($C_{23}H_{22}ClN_5OS\cdot0.1CF_3CO_2H$) C, H, N.

5-[4-(Benzylamino)phenyl]-6-[(benzyloxy)methyl]pyrimidine-2,4-diamine (8m). Title compound **8m** was synthesized by the general procedure described for **8a** substituting benzaldehyde for 4-chlorobenzaldehyde. Yield, 36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.40 (m, 2H), 7.34 (m, 2H), 7.26 (m, 4H), 7.18 (dd, J = 7.8, 1.7 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 6.63 (d, J = 8.6 Hz, 2H), 6.29 (t, J = 6.0 Hz, 1H), 5.85 (s, 2H), 5.48 (s, 2H), 4.33 (s, 2H), 4.28 (d, J = 5.8 Hz, 2H), 3.95 (s, 2H). MS (ESI) m/z = 412 [M + H]$^+$, 410 [M − H]$^-$. Anal. ($C_{25}H_{25}N_5O\cdot0.35H_2O$) C, H, N.

6-[(Benzyloxy)methyl]-5-[4-(neopentylamino)phenyl]pyrimidine-2,4-diamine (8n). Title compound **8n** was synthesized by the general procedure described for **8a** substituting trimethylacetaldehyde for 4-chlorobenzaldehyde. Yield, 48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.30 (m, 3H), 7.22 (m, 2H), 6.89 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 6.29 (s, 2H), 6.04 (s, 2H), 5.57 (t, J = 5.8 Hz, 1H), 4.38 (s, 2H), 4.03 (s, 2H), 2.83 (d, J = 5.8 Hz, 2H), 0.97 (s, 9H). MS (ESI) m/z = 392 [M + H]$^+$, 390 [M − H]$^-$. Anal. ($C_{23}H_{29}N_5O\cdot0.1CF_3CO_2H$) C, H, N.

6-Benzyloxymethyl-5-{4-[(quinolin-3-ylmethyl)-amino]-phenyl}-pyrimidine-2,4-diamine (8o). Title compound **8o** was synthesized by the general procedure described for **8a** substituting quinoline-3-carboxaldehyde for 4-chlorobenzaldehyde. Yield, 22%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.97 (d, J = 2.4 Hz, 1H), 8.30 (d, J = 1.4 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.94 (d, J = 8.5 Hz, 1H), 7.69−7.75 (m, 1H), 7.59 (t, J = 7.0 Hz, 1H), 7.12−7.26 (m, 5H), 6.91 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 6.50 (t, J = 5.9 Hz, 1H), 5.86 (s, 2H), 5.48 (s, 1H), 4.51 (d, J = 5.8 Hz, 2H), 4.30 (s, 2H), 3.93 (s, 2H), 3.29 (s, 1H), 3.17 (d, J = 5.1 Hz, 1H). MS (ESI) m/z = 463 [M + H]$^+$. Anal. ($C_{28}H_{26}N_6O\cdot0.4CF_3CO_2H\cdot0.5CH_3CN$) C, H, N.

5-(4-Nitro-phenyl)-6-phenethyl-pyrimidine-2,4-diamine (5e, Scheme 1). Title compound **5e** was synthesized by the general procedure described for **5c** substituting 3-phenyl-propionyl chloride for benzyloxyacetyl chloride and used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20 (d, J = 8.5, 2H), 7.30 (d, J = 8.8 Hz, 2H), 7.11−7.23 (m, 3H), 6.95 (d, J = 6.4 Hz, 2H), 6.04 (s, 2H), 5.72 (s, 2H), 2.76−2.81 (m, 2H), 2.34−2.40 (m, 2H). MS (ESI) m/z = 336 [M + H]$^+$, 334 [M − H]$^-$.

5-(4-Amino-phenyl)-6-phenethyl-pyrimidine-2,4-diamine (6c, Scheme 2). Title compound **6c** was synthesized by the general procedure described for **6b** and used without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12−7.20 (m, 3H), 6.97 (d, J = 6.8 Hz, 2H), 6.73 (d, J = 7.8 Hz, 2H), 6.60 (d, J = 7.5 Hz, 2H), 5.82 (s, 2H), 5.34 (s, 2H), 5.09 (s, 2H), 2.72−2.77 (m, 2H), 2.36−2.41 (m, 2H). MS (ESI) m/z = 306 [M + H]$^+$, 304 [M − H]$^-$.

5-[4-(4-Chloro-benzylamino)-phenyl]-6-phenethyl-pyrimidine-2,4-diamine (9). Title compound **9** was prepared according to the general procedure for **8a** using **6c** in place of **6b** for the final reductive amination (Scheme 2). Yield, 49%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.31−7.43 (m, 4H), 7.10−7.21 (m, 3H), 6.91−6.93 (d, J = 6.4 Hz, 2H), 6.77 (d, J = 8.5 Hz, 2H), 6.59 (d, J = 8.5 Hz, 2H), 6.35 (t, J = 5.8 Hz, 1H), 5.86 (s, 2H), 5.39 (s, 2H), 4.27 (d, J = 5.8 Hz, 2H), 2.69−2.74 (m, 2H), 2.33−2.39 (m, 2H). MS (ESI) m/z = 430 [M + H]$^+$, 428 [M − H]$^-$. Anal. ($C_{25}H_{24}ClN_5$) C, H, N.

4-[4-(2,4-Diamino-6-benzyloxymethyl-pyrimidin-5-yl)-phenoxymethyl]-benzonitrile (10b). To 5-(4-amino-phenyl)-6-benzyloxymethyl-pyrimidine-2,4-diamine (654 mg, 2.03 mmol) was added 1 M H_2SO_4 (aq, 7 mL). After being stirred at ambient temperature until the aniline had dissolved, the solution was cooled to 0 °C. (Some precipitate formed after cooling the solution.) To this mixture was added a solution of $NaNO_2$ (168 mg, 2.43 mmol) dissolved in a minimum amount of water. The reaction was stirred at 0 °C for 10 min, warmed to ambient temperature over 10 min, then heated at reflux for 40 min. The reaction was cooled to ambient temperature followed by addition of EtOAc (10 mL) and of 1.2 M $NaHCO_3$ (15 mL). A gummy precipitate formed that was dissolved in a small amount of methanol and then partitioned between the EtOAc and aqueous layers to aid dissolution. The layers were separated followed by extraction of the aqueous layer with EtOAc (2 × 10 mL). The combined EtOAc layers were back extracted with brine (1 × 10 mL), dried over $MgSO_4$, filtered, and concentrated to a foam. This was taken up in methanol and reconcentrated to 600 mg (92%) of 4-(2,4-diamino-6-benzyloxymethyl-pyrimidin-5-yl)-

phenol which was used without further purification in the next step. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.43 (s, 1H), 7.24 (m, 5H), 6.99 (m, 2H), 6.79 (m, 2H), 5.92 (s, 2H), 5.56 (s, 2H), 4.33 (s, 2H), 3.95 (s, 2H). MS (ESI) m/z = 323 [M + H]$^+$, 321 [M − H]$^-$.

To 4-cyanobenzyl bromide (30.4 mg, 0.16 mmol) was added 4-(2,4-diamino-6-benzyloxymethyl-pyrimidin-5-yl)-phenol (50 mg, 0.15 mmol) followed by 0.31 M KOEt in EtOH (0.5 mL). The reaction was shaken at ambient temperature for 8 h, diluted with H_2O (1 mL), and filtered. The precipitate was washed with H_2O and then diethyl ether and recrystallized from EtOH/H_2O to give **10b** as a solid (40 mg, 59%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.88 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 8.1 Hz, 2H), 7.25 (m, 3H), 7.16 (m, 4H), 7.04 (m, 2H), 5.95 (s, 2H), 5.60 (m, 2H), 5.24 (s, 2H), 4.32 (s, 2H), 3.94 (s, 2H). MS (ESI) m/z = 438 [M + H]$^+$, 436 [M − H]$^-$. Anal. ($C_{26}H_{23}N_5O_2$·0.27H_2O) C, H, N.

6-[(Benzyloxy)methyl]-5-(4-{[4-(methylsulfonyl)benzyl]oxy}-phenyl)pyrimidine-2,4-diamine (10a). Title compound **10a** was synthesized according to the procedure described for **10b** substituting 4-methanesulfonylbenzyl bromide for 4-cyanobenzyl bromide. Yield, 64%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.5 Hz, 2H), 7.30−7.04 (m, 9H), 5.95 (s, 2H), 5.59 (s, 2H), 5.26 (s, 2H), 4.32 (s, 2H), 3.39 (s, 2H), 3.22 (s, 3H). MS (ESI) m/z = 491 [M + H]$^+$, 489 [M − H]$^-$. Anal. ($C_{26}H_{26}N_4O_4S$·0.4H_2O) C, H, N.

6-[(Benzyloxy)methyl]-5-(4-{[(4-nitro-phenyl)amino]methyl}-phenyl)pyrimidine-2,4-diamine (11a). To a stirred suspension of **5d** (600 mg, 1.8 mmol) in 1.0 N NH_3/MeOH was added Raney Ni (75 mg, prewashed with MeOH and THF). The reaction flask was charged with hydrogen (1 atm) and hydrogenated at 60 °C for 4 h. The solution was cooled to ambient temperature, filtered through Celite, and concentrated under reduced pressure to provide 5-(4-aminomethyl-phenyl)-6-benzyloxymethyl-pyrimidine-2,4-diamine as a beige solid (450 mg, 74%). ^{1}H NMR (300 MHz, DMSO-d_6), δ 7.36 (d, J = 8.1 Hz, 2H), 7.34−7.12 (m, 5H), 7.15 (d, J = 8.1 Hz, 2H), 5.97 (s, 2H), 5.55 (s, 2H), 4.44 (br m, 2H), 4.33 (s, 2H), 3.96 (s, 2H). MS (ESI) m/z = 336 [M + H]$^+$.

To a mixture of 5-(4-aminomethyl-phenyl)-6-benzyloxymethyl-pyrimidine-2,4-diamine (30 mg, 0.089 mmol), excess diisopropylethylamine (150 μL), and 1-fluoro-4-nitrobenzene (19 μL, 0.18 mmol) was added NMP (1.0 mL). The mixture was heated at 200 °C for 20 min in a microwave reactor, concentrated in vacuo, and then purified by preparative TLC to provide the title compound as a light yellow solid (10 mg, 24%).^{1}HNMR (300 MHz, DMSO-d_6), δ 7.98 (d, J = 9.5 Hz, 2H), 7.86 (t, J = 6.1 Hz, 1H), 7.37 (d, J = 8.1 Hz, 2H), 7.30−7.09 (m, 9H), 6.7 (d, J = 9.5 Hz, 2H), 6.01 (s, 2H), 5.58 (s, 2H), 4.46 (d, J = 6.1 Hz, 2H), 4.29 (s, 2H), 3.94 (s, 2H). MS (ESI) m/z = 457 [M + H]$^+$, 455 [M − H]$^-$. HRMS calcd for ($C_{25}H_25N_6O_3$) 457.1983. Found: 457.1984.

6-Ethyl-5-(4-{[(4-nitro-phenyl)amino]methyl}phenyl)-pyrimidine-2,4-diamine (11b). Title compound **11b** was prepared according to the procedure described for **11a** substituting **5b** for **5d** in the reduction. Yield, 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (d, J = 9.0 Hz, 2H), 7.83 (t, J = 6.0 Hz, 1H), 7.41 (d, J = 9.0 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 8.5 Hz, 2H), 5.86 (s, 2H), 5.37 (s, 2H), 4.46 (d, J = 6.0 Hz, 2H), 2.10 (q, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 365 [M + H]$^+$, 363 [M − H]$^-$. Anal. ($C_{19}H_{20}N_6O_2$·0.35CF_3CO_2H) C, H, N.

6-Ethyl-5-[4-({[4-(methylsulfonyl)phenyl]amino}methyl)phenyl]-pyrimidine-2,4-diamine (11c). Title compound **11c** was prepared according to the procedure described for **11b** substituting 1-fluoro-4-methanesulfonylbenzene for 1-fluoro-4-nitrobenzene. Yield, 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.56 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 9.0 Hz, 2H), 7.26 (t, J = 6.0 Hz, 1H), 7.16 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 8.5 Hz, 2H), 5.87 (s, 2H), 5.38 (s, 2H), 4.40 (d, J = 6.0 Hz, 2H), 2.11 (q, J = 7.5 Hz, 2H), and 0.96 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 398 [M + H]$^+$, 396 [M − H]$^-$. Anal. ($C_{20}H_{23}N_5O_2S$·1.05CF_3CO_2H) C, H, N.

6-Benzyloxymethyl-5-{4-[(4-chloro-phenylamino)-methyl]-phenyl}-pyrimidine-2,4-diamine (11d). Title compound **11d** was prepared according to the procedure described for **11b** substituting 1-fluoro-4-methanesulfonylbenzene for 1-fluoro-4-nitrobenzene. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.41 (d, J = 8.1 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 7.07 (d, J = 9.2 Hz, 2H), 6.62 (d, J = 8.8 Hz, 2H), 6.44 (t, J = 6.1 Hz, 1H), 6.19 (s, 2H), 5.81 (s, 2H), 4.29 (d, J = 5.8 Hz, 2H), 2.12 (q, J = 7.5 Hz, 2H), 0.97 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 354 [M + H]$^+$, 352 [M − H]$^-$. Anal. ($C_{19}H_{20}ClN_5$·0.05CF_3CO_2H) C, H, N.

5-[3-Chloro-4-(4-methanesulfonyl-benzylamino)-phenyl]-6-ethyl-pyrimidine-2,4-diamine (12a). To a mixture of **7b** (40 mg, 0.1 mmol), acetic acid (20 μL), and $HClO_4$ (500 μL) in 2:1 dichloromethane/methanol (1.5 mL) was added *N*-chlorosuccinimide (13 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 48 h and was partitioned between ethyl acetate (10 mL) and aqueous $NaHCO_3$ (10 mL). The organic phase was washed with brine, dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel column with ethyl acetate/methanol (10:1) to provide **12a** (16 mg, 37%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 2.0 Hz, 1H), 6.84 (dd, J = 8.1, 2.0 Hz, 1H), 6.57(d, J = 8.1 Hz, 1H), 6.35 (t, J = 6.1 Hz, 1H), 5.80 (s, 2H), 5.42 (bs, 2H), 4.53 (d, J = 6.1 Hz, 2H), 3.19 (s, 3H), 2.09 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 432 [M + H]$^+$, 430 [M − H]$^-$. Anal. ($C_{20}H_{22}ClN_5O_2S$) C, H, N.

5-[3-Bromo-4-(4-methanesulfonyl-benzylamino)-phenyl]-6-ethyl-pyrimidine-2,4-diamine (12b). To a mixture of **7b** (80 mg, 0.2 mmol) in a mixed solvent of acetic acid (400 μL) and MeOH (1 mL) was added bromine (10 μL, 0.2 mmol). The reaction mixture was stirred at ambient temperature for 2 h. It was partitioned between ethyl acetate (10 mL) and saturated $NaHCO_3$ (aq, 15 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude material was triturated with CH_3CN to provide **12b** as a white solid (68 mg, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.35 (d, J = 5.1 Hz, 1H), 7.49 (s, 1H), 7.42 (d, J = 5.1 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 6.46 (t, J = 6.1 Hz, 1H), 5.72 (s, 2H), 5.27 (bs, 2H), 4.36 (d, J = 6.1 Hz, 2H), 2.11 (q, J = 7.5 Hz, 2H), 0.94 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 455 [M + H]$^+$. Anal.($C_{20}H_{22}N_5O_2SBr$) C, H, N.

6-Ethyl-5-(4-{methyl[4-(methylsulfonyl)benzyl]amino}phenyl)-pyrimidine-2,4-diamine (13). To a mixture of **7b** (40 mg, 0.1 mmol) and formaldehyde (37%, 11 μL, 0.15 mmol) in acetic acid (20 μL) and MeOH (500 μL) was added $NaBH_3CN$ (10 mg, 0.15 mmol). The reaction mixture was stirred at ambient temperature for 2 h followed by partitioning between ethyl acetate (10 mL) and saturated $NaHCO_3$ (aq, 15 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude material was triturated with CH_3CN to provide **13** as a white solid (34 mg, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 5.74 (s, 2H), 5.28 (bs, 2H), 4.69(s, 2H), 3.20 (s, 3H), 3.07 (s, 3H), 2.12 (q, J = 7.5 Hz, 2H), 0.95 (t, J = 7.5 Hz, 3H). MS (ESI) m/z = 412 [M + H]$^+$, 410 [M − H]$^-$. Anal. ($C_{21}H_{25}N_5O_2S$·0.15H_2O) C, H, N.

GHS-R Binding Assay. GHS-R binding assays were performed with membrane preparations. CHO-K cells expressing human ghrelin receptor (Euroscreen) were suspended in sucrose buffer (0.25−0.025% bacitracin) and disrupted by sonication using a vibra cell (Sonics and Materials Inc.) on 70% duty cycle in 15 s pulses on ice for 2.5 min. The homogenate was centrifuged at 60000*g* for 60 min. Pellets were suspended in tris buffer (20 mM tris pH 7.4, 5 μg/mL pepstatin-A, 0.1 mM PMSF, and 3 mM EDTA). Binding reactions contained 1 μg membrane as determined by BCA protein assay (Pierce), 0.1 nM [^{125}I]-(25 mM Hepes pH 7.4, 1 mM $CaCl_2$, 5 mM $MgSO_4$. and 0.5% protease free BSA). Incubations were carried out at room temperature for 2 h and were terminated by filtration using Filtermate Harvester (Perkin-Elmer) onto GF/C filter plates (Millipore) previously soaked in 0.5% polyethylenimine for 2 h. Filter plates were washed a minimum of 3× with cold wash buffer (binding buffer w/o BSA, supplemented with 0.5 M NaCl) to remove unbound counts, after which the filter plate bottoms were sealed and 50 μL of MicroScint 20 was added to each well. Bound

[^{125}I]-ghrelin was then determined by scintillation counting using Top Count NXT (Perkin-Elmer). The effects of compound were expressed as percent inhibition of [^{125}I]-ghrelin binding. Sigmoidal curves were fitted by Assay Explorer (MDL) software, and IC_{50} values determined.

GHS-R Ca^{2+} Flux Assay. CHO-K cells expressing human GHS-R (Euroscreen) were cultured in Ultra-CHO medium from BioWhittaker supplemented with 1% dialyzed FCS, 1% penicillin/streptomycin/fungizone, and 400 μg/mL G418 (all from Life Technologies) at 37 °C in a humidified cell incubator containing 5% CO_2. Cells were plated in black 96-well plates with clear bottom (Costar) and cultured to confluency overnight. Prior to assay, cells were incubated in 100 μL of Dulbecco's phosphate-buffered saline (DPBS) containing 1000 mg/L D-glucose, 36 mg/L sodium pyruvate, without phenol red (Life Technologies), with 1.14 mM Fluo-4 AM (Molecular Probes), and 0.25 M probenecid (Sigma) for 1−3 h in the dark at room temperature. The dye solution was aspirated, and the cells were washed twice with DPBS using the EL-450X cell washer (BioTech). After the last wash, 100 μL of DPBS was added to each well. Cell plates were then transferred to the FLIPR unit (Molecular Probes). Compound additions were 50 μL in duplicate or triplicate of 4× final concentration in DPBS containing 0.1% BSA and 4% DMSO. Fluorescence emissions from 96 wells were measured simultaneously at excitation and emission wavelength of 488 and 520 nm, respectively, for 3 min in 1 s intervals for the first minute and 5 s intervals thereafter. During this time, agonist responses, if any, were recorded in the absence of ghrelin. Next, 50 μL in duplicate or triplicate of 4× final concentration of ghrelin in DPBS containing 0.1% BSA and 4% DMSO were delivered within 1 s by an integrated 96-well pipettor. Fluorescence emissions were measured for another 3 min as above. During this time the antagonist effects of compounds on ghrelin-stimulated calcium flux were recorded. Sigmoidal curves were fitted, and IC_{50} and EC_{50} values were determined by GraphPad Prism software. Ghrelin shows an EC_{50} of 0.2 nM in this assay.

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Supporting Information Available: Elemental analysis data for compounds **7a**,**b**, **7f**−**i**, **8a**, **8d**−**o**, **9**, **10a**,**b**, **11b**−**d**, **12a**,**b**, and **13**, procedures for in vivo assays, and derivation of GHS-R −/− mice. This material is available free of charge via the Internet at http://pubs.acs.org.

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