Trisubstituted Pyrimidines as Efficacious and Fast-Acting Antimalarials

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Supporting Information

ABSTRACT: In this paper we describe the optimization of a phenotypic hit against Plasmodium falciparum, based on a trisubstituted pyrimidine scaffold. This led to compounds with good pharmacokinetics and oral activity in a P. berghei mouse model of malaria. The most promising compound (13) showed a reduction in parasitemia of 96% when dosed at 30 mg/kg orally once a day for 4 days in the P. berghei mouse model of malaria. It also demonstrated a rapid rate of clearance of the erythrocytic stage of P. falciparum in the SCID mouse model with an ED90 of 11.7 mg/kg when dosed orally. Unfortunately, the compound is a potent inhibitor of cytochrome P450 enzymes, probably due to a 4-pyridyl substituent. Nevertheless, this is a lead molecule with a potentially useful antimalarial profile, which could either be further optimized or be used for target hunting.

INTRODUCTION

Malaria is a devastating parasitic disease causing widespread mortality and morbidity across many parts of the developing world. Human malaria is caused by five Plasmodium species: P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi. P. falciparum causes the most mortality and is found in high levels in Africa, whereas P. vivax causes the most morbidity and is more commonly found across Asia and the Americas. In 2013, there were an estimated 198 million cases of malaria worldwide and 584 000 deaths, of which 453 000 were of children under 5 years, with 90% of all malaria deaths in the African region. Many medicines for the treatment of malaria such as chloroquine and pyrimethamine are failing due to increasing development of resistance. Furthermore, there are now cases of drug resistance to artemisinin-based combination therapies (ACTs), which are the mainstays for the World Health Organization (WHO) campaign against malaria. Currently, primaquine is the only drug in general use for radical cure of malaria due to P. vivax, preventing relapse, but this medicine has a prolonged dosing schedule and is toxic to individuals with glucose 6-phosphate deficiency. Therefore, new therapies for both treatment and prevention of this deadly disease across all of its life cycle stages are urgently needed. Efforts from...
academic groups and pharmaceutical companies to identify novel antimalarials are now beginning to bear fruit as novel therapies for the treatment of malaria are in clinical trials.\(^1\) However, the discovery of potential new antimalarials remains vital, given the high attrition rates in clinical development,\(^5\) the propensity of the parasite to develop resistance, and the need for additional indications (such as transmission blocking, chemoprevention, and radical cure of vivax malaria).\(^6\) Here, we report the design, synthesis, and biological evaluation of fast-acting and highly efficacious antimalarials, based on trisubstituted pyrimidines, which were discovered using a whole cell-based screening approach.

## RESULTS AND DISCUSSION

### Project Initiation.

A drug discovery program for the identification of novel antimalarials was initiated with the high throughput phenotypic screening (HTS) of an in-house library of protein kinase scaffolds (4731 compounds).\(^7\) This effort identified multiple structurally diverse chemical series that blocked asexual blood stage parasite viability, as measured by a SYBR green assay.\(^8,9\) In this paper, we describe a chemistry program based around one of these series, a trisubstituted pyrimidine, which displayed chemical tractability, nanomolar potency against \(P. falciparum\) cell line 3D7, and excellent selectivity over a mammalian cell line MRC-5 (Table 1). An initial example of this series was inactive against a panel of mammalian kinases up to a concentration of 10 \(\mu\)M.

### Lead Identification.

The initial hit from the screen, 1, was followed up by hit expansion through commercially available analogues. Systematic changes of functional groups at \(R_1, R_2,\) and \(R_3\) were carried out to try to improve potency and physicochemical properties. Analogues of our original screening hit (1) were also identified from published data from GSK\(^{10}\) and Novartis\(^9\) (Figure 1). Following resynthesis and screening in-house, compound 2 (reported by GSK and Novartis) provided a suitable chemical start point for further synthetic modifications. However, due to poor solubility (5 \(\mu\)M), compound 2 was not progressed any further than assessment at the in vitro (cellular) level for potency and absorption, distribution, metabolism, excretion, and toxicology (ADMET). Analogue design was then directed toward improving potency and solubility and reducing the number of aromatic rings, which can have a beneficial impact on overall development characteristics including solubility.\(^11,12\) Compound 2 has a high degree of planarity, so we sought further improvement by increasing the proportion of \(sp^3\) to \(sp^2\) carbon atoms, which is reported to increase the solubility.\(^13\)

We were concerned about the inhibition of cytochrome P450 isofrm CYP3A4, which we believed to be due to the 4-pyridyl group (see later for further discussion). Initial attempts to replace the 4-pyridyl functional group at \(R_1\) resulted in a significant loss of antimalarial activity (Table 2). Removal of the pyridine nitrogen at \(R_1\) or simply moving the nitrogen from the 4- to the 3-position resulted in >30-fold drop in potency. In addition, replacing the 4-pyridyl group with a morpholine group reduced potency by almost 60-fold, highlighting the importance of the pyridine nitrogen and suggesting that the vector of the lone pair donor was also crucial for activity. We decided therefore to investigate variations at \(R_2\) and \(R_3\) for improvements in potency, which would render the interaction with the 4-pyridyl less critical.

### Optimization of \(R_2\).

Removal of the tetrahydroisoquinoline (2) and replacement with an amino group (6) gave a 100-fold drop in activity, indicating the tetrahydroisoquinoline group has a significant effect on the potency. Replacement of the tetrahydroisoquinoline moiety of compound 2 with \(N-\)

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**Table 1. Hit Series Identified from Phenotypic Screening of Kinase-like Library**

| Series ID | MMV02 |
|-----------|-------|
| Compound ID | 1 |
| \(EC_{50}\) vs. \(P. falciparum\) 3D7 (\(\mu\)M) | 0.25 |
| \(EC_{50}\) vs. MRC5 (\(\mu\)M) | 31 |
| \(c\text{logP}\) | 3.2 |
| MWT | 343 |
| No. of examples with \(EC_{50} < 1\mu\)M | 3 |

**Table 2. Modifications at \(R_1\)**

| \(R\) | \(PY(3D7)\) | MRC5 |
|-------|-------------|-------|
| 2     | 0.1         | 14    |
| 3     | 3.1         | 30    |
| 4     | 3.4         | 49    |
| 5     | 6.5         | 24    |

*All parasite assays were run in duplicate.*
methylbenzylamine (7) resulted in a 10-fold loss of potency (Table 3), possibly suggesting that a degree of conformational restraint was necessary. Contracting the aliphatic ring size to a five-membered ring (8) led to a complete loss in activity. Replacing the phenyl ring in 2 with an imidazole (9) gave a 10-fold drop in activity (EC₅₀ = 1.7 μM). Interestingly, activity was retained when the phenyl was attached to a piperazine rather than being directly fused onto the piperidine ring (10, EC₅₀ = 0.3 μM), despite the different vector compared to compound 2.

Further work was undertaken to remove an aromatic ring, with a key aim being to increase solubility and improve the potential for clinical development. Replacing the phenyl ring found in 10 with piperidine gave a compound equipotent to the starting point (11, EC₅₀ = 0.1 μM). This compound had marginally improved aqueous solubility (56 μM, measured as the free base) and retained reasonably low microsomal turnover. Replacing the "terminal" piperidine with a morpholine gave a compound with similar activity (12, EC₅₀ = 0.3 μM) but with a significantly increased solubility (>100 μM), reduced clogP, and low microsomal turnover. It was also possible to add a flexible linker between the piperidine and the morpholine (13) with only a minimal effect on potency (EC₅₀ = 0.3 μM) and retaining low microsomal turnover but with a similar solubility (44 μM). It was possible to replace the piperidine of 13 with an alkyl linker to give 14. This compound had the same activity as 13 (EC₅₀ = 0.3 μM), but despite a lower clogP, showed a significantly higher microsomal turnover. Finally, a bicyclic aliphatic system, 15, also showed similar activity (EC₅₀ = 0.3 μM) and good solubility (>100 μM) but increased microsomal turnover. In summary, it is possible to reduce the number of aromatic rings and increase the proportion of sp³ carbon atoms which improves solubility and clogP without compromising potency and microsomal turnover.

**Optimization of R³.** Replacement of the planar aromatic 3-pyridyl unit at the R³ position with aliphatic substituents was investigated to both reduce the aromatic ring count and increase the sp³ nature. Small aliphatic groups such as the cyclopropyl group of 16 were not tolerated and resulted in around a 30-fold drop in potency (Table 4). Furthermore, replacement of the 3-pyridyl by the flexible aminoalkylmorpho-

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*Table 3. Modifications at R²*

| R²      | PI (3D7) EC₅₀ (μM) | MRC5 EC₅₀ (μM) | MWT | clogP¹ | Mouse microsomal Clint (ml/min/g) | Sol⁰ (μM) |
|---------|--------------------|----------------|-----|--------|----------------------------------|----------|
| 2       | 0.1                | 14             | 365 | 3.3    | 1.7                              | 5        |
| 6       | 17                 | 50             | 249 | 1.7    | ND                               | ND       |
| 7       | 1.4                | 28             | 353 | 3.4    | ND                               | ND       |
| 8       | 50                 | 50             | 351 | 3.0    | ND                               | ND       |
| 9       | 1.7                | 24             | 355 | 2.2    | ND                               | ND       |
| 10      | 0.3                | 50             | 394 | 3.4    | 1.4                              | 36       |
| 11      | 0.1                | 15             | 400 | 3.3    | 3.9                              | 56       |
| 12      | 0.3                | 36             | 402 | 2.3    | 1.2                              | >248     |
| 13      | 0.3                | 50             | 416 | 2.6    | 3                                | 106      |
| 14      | 0.3                | ND             | 390 | 2.1    | 13                               | >100     |
| 15      | 0.3                | 14             | 358 | 2.6    | 7.0                              | >100     |

¹clogP was calculated using StarDrop from Optibrium. Sol is solubility in water for the free base.
line (17) or aminoalkylamide (18) resulted in >90-fold drop in potency. In addition, the morpholine moiety 19 was completely inactive. Further examples are given in the Supporting Information. In summary, attempts to replace R3 with an aliphatic group or heteroaromatics such as the oxazole (20) were unsuccessful. Attempts to replace the pyridyl nitrogen atom with groups such as 3-fluorophenyl (21) or 4-fluorophenyl (22) lost around 10-fold activity and led to an increase in clogP. Furthermore, the addition of another nitrogen atom into the pyridyl unit to afford the pyrimidine 23 was less well tolerated (10-fold loss in potency). In summary, despite extensive investigation, we were unable to find a suitable replacement for the 3-pyridyl moiety at R3, and further changes were focused on different substitutions on the 3-pyridyl ring to improve activity and physicochemical properties (Table 5).

A variety of modifications were made at different positions around the 3-pyridyl ring. Small electron withdrawing and electron donating substituents meta to pyridyl nitrogen were tolerated (methoxy, 24; nitrile, 25; fluoro, 26). However, the aminomethyl analogue 27 had a 10-fold loss in activity, and the morpholine amide 28 was essentially inactive. Small functional groups ortho to the pyridine nitrogen such as amino (29) or methoxy (30) were tolerated, with only a 3-to 6-fold loss in activity compared to 12. However, larger groups at this position on the 3-pyridyl moiety, such as the methylamide (31) or morpholine (32), reduced activity by >10-fold. Furthermore, moving the methoxy from the meta-position of the pyridine (24) to the para-position (33) caused a 20-fold reduction in potency compared to 12. In summary, there appear to be limited opportunities for synthetic modification to enhance activity at the R3 position, based on the pyridyl moiety.

**In Vivo Efficacy.** Compounds 12 and 13 were selected for in vivo pharmacokinetic (PK) and efficacy studies, based on their overall profile of properties. Both compounds displayed suitable predicted physicochemical properties consistent with that of an oral drug. In addition, 12 and 13 demonstrated submicromolar potency in vitro and good aqueous solubility, were reasonably stable when incubated with mouse liver microsomes, and displayed low plasma protein binding. Unfortunately, 13 displayed some binding to the hERG ion channel (Table 6).

In vivo PK studies with 12 showed rapid absorption after oral administration (10 mg/kg) but with limited exposure and a short half-life, whereas 13 displayed an improved half-life with a 7-fold increase in AUC. Subsequently, in vivo efficacy experiments were carried out and mice were subjected to oral dosing of compounds 12 and 13 up to 30 mg/kg once a day for Table 4. Modifications at R3

| R3  | PT (3D7) EC50 (µM) | MRC5 EC50 (µM) | MWT | clogP |
|-----|-------------------|----------------|------|-------|
| 12  | 0.3               | 36             | 402  | 2.3   |
| 16  | 10                | ND             | 365  | 2.7   |
| 17  | 32                | ND             | 467  | 2.1   |
| 18  | >50               | ND             | 411  | 1.1   |
| 19  | >50               | ND             | 410  | 2.0   |
| 20  | 16                | ND             | 420  | 3.0   |
| 21  | 4.3               | ND             | 419  | 3.4   |
| 22  | 3.3               | ND             | 419  | 3.4   |
| 23  | 4.3               | ND             | 403  | 2.5   |

Table 5. Modifications at R3

| R3  | PT (3D7) EC50 (µM) | MRC5 EC50 (µM) | MWT | clogP |
|-----|-------------------|----------------|------|-------|
| 12  | 0.3               | 36             | 402  | 2.3   |
| 24  | 0.1               | ND             | 432  | 2.3   |
| 25  | 0.4               | ND             | 427  | 2.2   |
| 26  | 0.5               | ND             | 420  | 2.6   |
| 27  | 3.1               | ND             | 431  | 1.4   |
| 28  | >50               | ND             | 515  | 1.8   |
| 29  | 1.1               | ND             | 417  | 1.9   |
| 30  | 1.8               | ND             | 432  | 2.3   |
| 31  | 28                | ND             | 459  | 1.7   |
| 32  | 4.8               | ND             | 487  | 2.5   |
| 33  | 19                | ND             | 432  | 2.3   |
4 consecutive days using the *P. berghei* rodent model of infection (Peters’ test, Table 6). Compound 13 displayed superior efficacy compared with 12 with a 96% reduction in parasitemia (compared to 72% for 12), when dosed at 30 mg/kg, q.d., po. The early lead criteria, stipulated by MMV, required compounds to display both suppression of parasitemia and an ED50 < 50 mg/kg under this protocol.14 However, we were unable to obtain complete cures in the rodent model for either compound 12 or 13. For efficacy experiments with compound 12, all mice were euthanized by day 14. For compound 13, all mice were euthanized by day 11.

Compound 13 was also evaluated in vivo against *P. falciparum* parasites grown in the peripheral blood of NODscidIL2Rγnull mice (SCID), engrafted with human erythrocytes.15 Three days after infection, mice were dosed orally once a day with 13 for 4 days at concentrations up to 100 mg/kg (Figure 2a). The ED90 measured at day 7 = 11.7 mg/kg, and its equivalent estimated daily exposure in blood AUCED90 = 1.4 μg·h/mL. In vivo there was a rapid reduction of parasitemia at doses of ≥20 mg/kg or >7.96 μg·h·mL⁻¹ day⁻¹ in blood. With doses of ≥30 mg/kg, the parasites levels were reduced below detection limits within 2 days. The rate of parasite clearance in vivo was at least as fast as the artemisinins,16 and only pyknotic parasites are observed in peripheral blood of mice 48 h after treatment at 100 mg/kg (Figure 2c). Interestingly, the in vitro parasite reduction ratio (PRR)
assay\textsuperscript{17} identified 13 as a compound with a moderate rate of killing, displaying 99.9\% clearance of parasites in 52 h, when tested at 10 × EC\textsubscript{50} (Figure 2b). It is possible that the PRR assay would show a faster killing rate at higher concentrations of compound, more in-line with what is seen in vivo.

To assess the mode of action, given that the compound contained a potential heme binding moiety in the 4-pyridyl, the ability of compound 13 to block hemozoin (β-hematin) formation was also tested. It displayed relatively comparable activity to chloroquine in this assay (27 \(\mu\)M for 13 vs 6.6 \(\mu\)M for chloroquine). It was not known if the primary mode of action is through the same mechanism of action as chloroquine. However, when assayed against the chloroquine/pyrimethamine resistant (K1) lines, compound 13 displayed similar activity to sensitive cell lines, so it has a different profile to chloroquine.

**Reducing Affinity for Human CYP Isoforms.** Although the antimalarial properties of the compound series had been demonstrated in mouse models of malaria, further development of the series required compounds that had markedly reduced inhibition of the major CYP enzymes. Subsequent elaboration of 13 focused on reducing inhibition of human CYP isoforms 3A4 and 2D6. Previous work had not been successful in distinguishing the antimalarial activity and the inhibition of human CYP isoforms (Table 1), thought to be due to the 4-pyridyl group at the R\textsuperscript{1} position. Therefore, two approaches were investigated to reduce CYP inhibition. One approach involved replacement of the 4-pyridyl unit with functional groups that could have similar steric and H-bond acceptor properties (Table 7). In parallel, the possibility of modifying the 4-pyridyl unit with the addition of functional groups adjacent to the pyridine nitrogen was also investigated, which could potentially reduce binding to human CYP isoforms while retaining suitable affinity for the unknown target of interest (Table 8). The R\textsuperscript{2} and R\textsuperscript{3} positions were fixed with piperidine-morpholine and 3-pyridyl, respectively, to use as a reference point for changes in activity and with the view that if it were possible to optimize R\textsuperscript{1}, this should also work with other R\textsuperscript{2} and

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**Figure 2.** (a) In vivo efficacy data for compound 13 in *P. falciparum* infected SCID mice. (b) Levels of compound 13 in blood of the mice of the efficacy experiment during 23 h after the first oral dose. The symbols represent the same individuals depicted in plot a. (c) In vitro PRR data for compound 13 when parasites were treated at 10 × EC\textsubscript{50}. Comparator data for other standard drugs are included for reference (data previously reported\textsuperscript{17}). Compound 13 showed a similar rate of kill to pyrimethamine. (d) Comparison of morphology of parasitized human RBC in vehicle and compound 13 treated mice. Erythrocytes with only remnants of parasites showing nuclear condensation were seen following 2-day treatment with compound 13. Compound dosed as the fumarate salt.
Table 7. Modifications at R^1

| R^1 | R^1 | PF (3D7) EC_{50} (μM) | MRCS EC_{50} (μM) | MWT | clogP |
|-----|-----|------------------------|-------------------|------|-------|
| 10  |        |                        |                   |      |       |
| 12  |        | 0.3                    | 35.92             | 402  | 2.3   |
| 34  |        | 2.1                    | ND                | 426  | 3.1   |
| 35  |        | 5.3                    | ND                | 350  | 1.6   |
| 36  |        | 49                     | ND                | 458  | 1.3   |
| 37  |        | 24                     | ND                | 341  | 1.7   |
| 38  |        | 50                     | ND                | 423  | 1.6   |
| 39  |        | 30                     | ND                | 411  | 2.6   |

R^3 substituents (e.g., as found in 13). The key molecules prepared are summarized in the main text. Additional molecules prepared are presented in the Supporting Information.

Optimization of R^1. The initial focus was on placing a hydrogen bond acceptor (HBA) at the 4-position of the phenoxy ring to replace the 4-pyridyl moiety at the R^1 position (Table 7). Several nitro derivatives were prepared. The 4-cyanophenyl (34) gave a 7-fold reduction in potency (EC_{50} = 2.1 μM) from 12 (EC_{50} = 0.3 μM). This would place the HBA further from the pyrimidine than the pyridine nitrogen in 12. Therefore, it was decided to attach the nitro directly onto the pyrimidine ring (35), which gave a similar level of potency (EC_{50} = 5.3 μM) to the 4-cyanophenol analogue. Other HBAs such as sulfones (36) gave significantly reduced activity (EC_{50} = 49 μM). Direct attachment of a hydroxyl to the pyrimidine ring (37) also failed to increase activity (EC_{50} = 24 μM), although this may be in a different tautomeric form. Amide 38 was also inactive (EC_{50} = 50 μM). Finally, basic groups were investigated to determine if there was an interaction with an acidic group on the protein. None of these were active (e.g., 39, EC_{50} = 30 μM).

The original 4-pyridyl moiety at R^1 was then revisited with a focus on reducing binding to the human CYP450 isoenzymes with close analogues incorporating blocking groups adjacent to the pyridine nitrogen, to reduce the interaction with the heme iron (Table 8). Addition of two methyl groups in the 3- and 5-positions significantly reduced CYP inhibition across all five CYPs investigated (40), which confirmed involvement of the parent 4-pyridyl moiety. However, there was a 5-fold drop in activity (EC_{50} = 1.5 μM). Interestingly having just one methyl group in the 3-position (41, EC_{50} = 17 μM) led to a further 10-fold drop in potency compared to disubstitution. Other groups in the 3-position which would alter the electronics of the pyridine nitrogen were also inactive (e.g., the CF3 group 42, EC_{50} = 50 μM). The effects of both electron-donating and electron-withdrawing substituents (43 and 44) were also investigated, where both gave a 5- to 10-fold reduction in potency compared to the substituted pyridine 12. Changing the heterocycle to a pyrimidine, pyridone, or pyrazole (45–47) also led to a reduction in activity. Therefore, despite a variety of variations on the R^1 position, all modifications investigated led to a marked decrease in potency.

### CONCLUDING REMARKS AND FUTURE WORK

Compounds 12 and 13 both display suitable physicochemical properties for an oral drug lead, good cellular activity in vitro against P. falciparum parasites, and good selectivity in a mammalian counterscreen. Compound 13 also demonstrated excellent oral efficacy in vivo with a 96% reduction in levels of parasitemia (P. berghei, 4 × 30 mg/kg, q.d., po) and a fast kill rate in the P. falciparum SCID mouse model. Compound 13 was also further profiled in the liver-stage schizont assay (EC_{50} > 10 μM), and in a stage IV/V gametocyte assay (EC_{50} = 2.4 μM). Initial infection with malaria occurs when *Plasmodium* sporozoites injected by the mosquito invade the liver cells. The parasites then undergo a liver-stage life cycle that involves formation of liver schizonts. Compounds that can prevent liver schizont formation may have potential for chemoprevention. The data for compound 13 suggest that this is not likely to have chemopreventative activity. Blood-stage infection gives rise to the clinical symptoms of malaria. Some of the parasites involved in blood-stage infection differentiate into gametocytes, which are the form of the parasite that can infect a mosquito, completing the life cycle. Compounds that kill the gametocytes may be able to block transmission of the parasite to mosquitoes. The data for compound 13 suggest that these compounds may have transmission blocking activity. Additional studies would be required to assess this in detail.

Unfortunately, further development is hampered by the potent inhibition of major CYP enzymes, where involvement of the 4-pyridyl group has been demonstrated. Focus has now moved toward the identification of the biological target of 13 to see if this information can be used to scaffold-hop to compounds that do not inhibit human cytochrome P450s. Given the rapid development of parasite drug resistance to known antimalarials, the identification of an essential and druggable target associated with the rapid clearance of *P. falciparum* parasites would be significant.

### CHEMISTRY

Synthesis of 4-pyridylpyrimidines via a modified literature procedure was initially undertaken by condensation of 4-pyridylamine with dimethyl malonate using sodium methoxide as a base and refluxing in methanol for up to 3 days to afford 2-(pyridin-4-yl)pyrimidine-4,6-diol in 55% yield. However, by employing experiment design software Modde and transferring the process to a microwave reactor, we were able to rapidly optimize the reaction conditions, improving the reaction yield to 70% and shortening the reaction time from 3 days to 1 h (Scheme 1). Chlorination of diol 48 with phosphorus trichloride at 90 °C gave rise to 4,6-dichloro-2-(pyridin-4-yl)pyrimidine 49 with 58% yield. Nucleophilic displacement of one chlorine atom by an amine followed by a Suzuki cross-coupling reaction with a boronic acid or ester afforded pyrimidines 51, allowing us to investigate substituents at the R^2 and R^3 positions.
The synthetic route outlined in Scheme 1 is not amenable to explore the influence of changes at the $R^1$ position on antimalarial activity. Therefore, a number of synthetic routes that allowed the introduction of a diverse array of substituents...
Suzuki cross-coupling with 3-pyridylboronic acid allowed the introduction of aromatic R₁ substituents. The reaction was carried out at the 2-position as the minor product (Scheme 2).

Finally, the desired trisubstituted pyrimidines were obtained by Suzuki cross-coupling with 3-pyridylboronic acid at the 2-position. Aromatic and heteroaromatic substituents were introduced at C-2 position on the pyrimidine ring. For example, reactions using microwave irradiation were carried out in a Biotage Initiator microwave. Normal phase TLC was performed using precoated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (254 nm) and ninhydrin solution. Flash chromatography was performed using Combiflash Companion Rf (Teledyne ISCO) and prepacked silica gel columns purchased from Grace Davison Discovery Science or SiliCycle. Mass-directed preparative HPLC separations were performed using Teledyne ISCO and prepacked silica gel columns purchased from Grace Davison Discovery Science or SiliCycle.

EXPERIMENTAL SECTION

General. Reactions using microwave irradiation were carried out in a Biotage Initiator microwave. Normal phase TLC was performed using precoated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (254 nm) and ninhydrin solution. Flash chromatography was performed using Combiflash Companion Rf (Teledyne ISCO) and prepacked silica gel columns purchased from Grace Davison Discovery Science or SiliCycle. Mass-directed preparative HPLC separations were performed using a Waters HPLC (2545 binary gradient pumps, 3100 mass detector) and a Waters 2767 sample manager. HPLC chromatographic separations were performed with a Gilson 121 pump, 315 injection module, and a Waters 474 photodiode array detector. On both instruments, HPLC chromatographic separations were performed using a Waters XBridge C18 column, 1 mm × 100 mm.
mm, 5 μm particle size, using 0.1% ammonia in water (solvent A) and acetoneitrile (solvent B) as mobile phase. 1H NMR and 13C NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (1H at 500.1 MHz, 13C at 125 MHz, 19F at 470.5 MHz) or a Bruker Avance DPX 300 (1H at 300 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination thereof. Coupling constants (J) are quoted to the nearest 0.5 Hz. Low resolution electrospray (ES) mass spectra were recorded on a Bruker MicroTOF mass spectrometer, run in positive mode. High resolution mass spectrometry (HRMS) was performed using a Bruker MicroTOF mass spectrometer. LCMS analysis and chromatographic separation were conducted with a Bruker MicroTOF mass spectrometer or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 μm particle size) and the compounds were eluted with a gradient of 5−95% acetonicrile/ water + 0.1% ammonia. All compounds for in vitro and in vivo experiments displayed >95% purity by LCMS. Unless otherwise stated dry solvents were purchased in Sure/Seal bottles stored over molecular sieves.

**Synthetic Routes. See Schemes 1−4.**

*Preparation of Compounds. 2-(2,6-Di(pyridine-3-yl)pyrimidin-4-yl)-1,2,3,4-tetrahydroisoquinoline (3). To a solution of 2,4-trichloropyrimidin-3(2) (1 g, 5.45 mmol) in ethanol (12 mL) at 0 °C, a solution of 1,2,3,4-tetrahydroisoquinoline (0.68 mL, 5.45 mmol) in ethanol (5 mL) was added dropwise followed by triethylamine (1.14 mL, 8.19 mmol). Reaction mixture was stirred at 0 °C for 1.5 h. Solvents were removed under vacuum, and the reaction crude was partitioned between DCM (150 mL) and a saturated aqueous solution of NaHCO₃ (25 mL). The organic phase was washed with a saturated aqueous solution of NaHCO₃ (25 mL), dried over MgSO₄, and filtered, and solvents were removed under reduced pressure. The product was purified by column chromatography (25 g silica gel) using (A) hexane and (B) ethyl acetate as eluents and the following gradient: 3 min hold to 100% A, 15 min ramp to 100% B, 3 min hold at 100% B. Fractions containing product were pooled together and solvents were removed to obtain 4-(4-chloro-6-(3,4-dihydroisoquinolin-2(1H)-yl)pyrimidin-2-yl)morpholine as a white wax (1.25 g, 69% yield, 88% purity by LCMS) that was used for the next step without further purification.

To a stirred solution of 4-(4-chloro-6-(3,4-dihydroisoquinolin-2(1H)-yl)pyrimidin-2-yl)morpholine (0.15 g, 0.45 mmol) and 3- pyridylboronic acid (0.17 g, 1.4 mmol) in DMP (6 mL), a solution of potassium phosphate (0.30 g, 1.4 mmol) in water (2 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh₃)₄ (0.016 g, 0.01 mmol) was added. The reaction was heated at 120 °C under microwave irradiation for 30 min. The reaction crude was filtered through Celite and partitioned between DCM (2 × 50 mL) and saturated aqueous solution of NaHCO₃ (10 mL). The organics phase was dried over MgSO₄ before concentration to dryness. The product was purified by column chromatography (12 g silica gel) using (A) hexane and (B) ethyl acetate as eluents and the following gradient: 3 min hold to 100% A, 15 min ramp to 80% B, 2 min ramp to 100% B, 3 min hold to 100% B. The fractions containing product, first eluting peak, were pooled together and solvents were removed to obtain 5 as yellow solid (34 mg, 20% yield). Purity by LCMS (UV chromatogram, 190−450 nm) 95%. 1H NMR (500 MHz, CDCl3, δ 7.91 (bs, 1H), 6.86−6.85 (m, 1H), 5.34−5.33 (m, 1H), 4.10 (m, 1H), 3.81−3.79 (m, 4H), 2.97 (t, 2H, J = 5.9 Hz), LRMS (ES+) m/z 374 [M + H]+.

6-(Pyridyl-3-yl)-2-(pyridin-4-yl)pyrimidin-4-amine (6). In a sealed tube a solution of 4,6-dichloro-2-(pyridin-4-yl)pyrimidine (49) (0.13 g, 0.58 mmol) and ammonium hydroxide (2 mL) in methanol (2 mL) was heated at 80 °C for 5 h. Solvents were removed under reduced pressure, and the residue was partitioned between water (10 mL) and DCM (2 × 50 mL). The organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (12 g silica gel) using (A) DCM and (B) 20% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 100% B, 3 min hold at 100% B. The fractions containing product were pooled together and solvents were removed to obtain 6-chloro-2-(pyridin-4-yl)pyrimidin-4-amine as white solid (69 mg, 39% yield, 99% purity by LCMS). Product was used in the next step without further purification.

To a stirred solution of 6-chloro-2-(pyridin-4-yl)pyrimidin-4-amine (69 mg, 0.33 mmol) and 3-pyridylboronic acid (91 mg, 0.66 mmol) in DCM (3 mL), a solution of potassium phosphate (140 mg, 0.66 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh₃)₄ (20 mg, 0.017 mmol) was added. The reaction was heated at 120 °C under microwave irradiation for 30 min. Reaction crude was filtered through Celite, quenched with water (10 mL), and extracted with DCM (2 × 25 mL). The organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (4 g silica gel) using (A) DCM and (B) 20% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 50% B, 3 min hold at 50% B. The fractions containing product were pooled
N-Benzyl-N-methyl-6-(pyridine-3-yl)-2-(pyridine-4-yl)-pyrimidin-4-amine (7). 7 was prepared in an analogous four-step procedure as that of compound 12: To a stirred solution of N-benzyl-6-chloro-N-methyl-2-(pyridine-4-yl)pyrimidin-4-amine (0.18 g, 0.58 mmol) and 3-pyridylboronic acid (0.21 g, 1.69 mmol) in DMF (3 mL), a solution of potassium phosphate (0.35 g, 1.69 mmol) in water (3 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh3)4 (0.02 g, 0.014 mmol) was added. The reaction was heated at 120 °C under microwave irradiation for 30 min. Reaction crude was filtered through Celite, quenched with water (10 mL), and extracted with DCM (2 × 25 mL). The organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (12 g silica cartridge) using (A) DCM and (B) 20% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 50% B, 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 7 as a white solid (115 mg, 56% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.30 (d, 1H, J = 1.7 Hz), 8.75–8.74 (m, 2H), 8.72 (dd, 1H, J = 1.6, 4.8 Hz), 8.43–8.40 (m, 1H), 8.33–8.32 (m, 2H), 7.44 (ddd, 1H, J = 0.7, 4.8, 8.0 Hz), 7.09 (s, 1H), 6.98 (s, 1H), 6.92 (s, 1H), 4.97 (s, 2H), 4.93(s, 2H, J = 3.5 Hz), 4.20–4.18 (m, 2H), LRMS (ES+) m/z 356 [M + H]+.

4-(4-Phenylpiperazin-1-yl)-6-(pyridin-3-yl)-2-(pyridine-4-yl)pyrimidine (10). 10 was prepared in an analogous four-step procedure as that of compound 12: To a stirred solution of 4-chloro-6-(4-phenylpiperazin-1-yl)-2-(pyridine-4-yl)pyrimidine (0.18 g, 0.58 mmol) and 3-pyridylboronic acid (0.21 g, 1.69 mmol) in DMF (3 mL), a solution of potassium phosphate (0.35 g, 1.69 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh3)4 (0.02 g, 0.014 mmol) was added. The reaction was heated at 120 °C under microwave irradiation for 30 min. Reaction crude was filtered through Celite, quenched with water (10 mL), and extracted with DCM (2 × 25 mL). The organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (12 g silica cartridge) using (A) DCM and (B) 20% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 50% B, 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 10 as an off-white solid (261 mg, 91% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.30 (s, 1H), 8.77–8.74 (m, 3H), 8.47–8.45 (m, 1H), 8.37–8.36 (m, 2H), 7.46 (dd, 1H, J = 4.8, 7.7 Hz), 7.33–7.31 (m, 2H), 7.01–6.92 (s, 4H), 4.02 (broad peak, 4H), 3.73–3.35 (m, 4H); LRMS (ES+) m/z 395 [M + H]+.

1′-(6-(Pyridin-3-yl)-2-(pyridine-4-yl)pyrimidin-4-yl)-1,4′-bipiperidin-11. 11 was prepared in an analogous four-step procedure as that of compound 12: To a stirred solution of 1′-(6-(chloro-2-(pyridine-4-yl)pyrimidin-4-yl)-1,4′-bipiperidin-4-yl)-1′-(6-(Pyridin-3-yl)-2-(pyridine-4-yl)pyrimidin-4-yl)pyrimidine (0.52 g, 0.71 mmol) and 3-pyridylboronic acid (0.17 g, 1.43 mmol) in DMF (9 mL), a solution of potassium phosphate (0.45 g, 2.14 mmol) in water (3 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh3)4 (0.02 g, 0.014 mmol) was added. The reaction was heated at 120 °C under microwave irradiation for 30 min. Reaction crude was filtered through Celite, quenched with water (20 mL), and extracted with DCM (2 × 50 mL). The organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (12 g silica cartridge) using (A) DCM and (B) 20% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 100% B, 3 min hold at 100% B. The fractions containing product were pooled together and solvents were removed to obtain 11 as an off-white solid (261 mg, 91% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.21 (d, 1H, J = 1.8 Hz), 8.68–8.67 (m, 2H), 8.64 (dd, 1H, J = 1.6, 4.8 Hz), 8.36–8.34 (m, 1H), 8.27–8.26 (m, 2H), 7.76 (dd, 1H, J = 4.9, 7.8 Hz), 6.83 (s, 1H, J = 6.1, broad peak, 2H), 3.95–3.90 (m, 2H), 2.59–2.49 (m, 9H), 1.96–1.93 (m, 2H), 1.57–1.49 (m, 6H), 1.40–1.39 (m, 2H), LRMS (ES+) m/z 401 [M + H]+.

4-(1H-(6-(Pyridin-3-yl)-2-(pyridine-4-yl)pyrimidin-4-yl)pyrrolidin-4-yl)morpholine (12). Scheme 1 (Four-Step Procedure). Step 1: 2-(Pyridine-4-yl)pyrimidine-4,6-diol (48). A mixture of 4-amidinopyridine hydrochloride (0.5 g, 3.17 mmol) and N-methyl-2-pyrrolidone (10 mL) was prepared at rt and dimethylammonate (0.365 mL, 419 mg, 3.17 mmol) added followed by sodium methoxide (686 mg, 12.69 mmol) and the mixture heated in a microwave at 150 °C for 1 h. The mixture was then concentrated under reduced pressure, diluted with water (10 mL), and acidified to pH 6 with concentrated acetic acid. The resulting precipitate was then filtered and dried in vacuo to afford compound 48 (420 mg, 2.22 mmol, 70%) as an off-white solid. 1H NMR (500 MHz, DMSO-d6) δ 12.10 (bs, 2H), 8.76–8.75 (m, 2H), 8.02–8.03 (m, 2H), 5.56 (s, 1H); LRMS (ES+) m/z 190 [M + H]+.

Step 2: 4,6-Dichloro-2-(pyridine-4-yl)pyrimidine (49). A stirred solution of 2-(pyridine-4-yl)pyrimidine-4,6-diol (0.62 g, 3.28 mmol) in phosphorus oxychloride (6 mL) was heated at 90 °C for 3 h. The
reaction mixture was slowly added to ice-water, and 2.5 M NaOH was added to adjust to pH 7. The white precipitate was filtered. The filtrate was extracted with ethyl acetate (2 × 50 mL), and the organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. Precipitate and extracted product were combined to obtain 49 as a brown solid (421 mg, 58% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 90%. 1H NMR (500 MHz, DMSO-d6) δ 8.81–8.80 (m, 2H), 8.27–8.26 (m, 2H), 7.41 (s, 1H); LRMS (ES+) m/z 225 [M + H]+.

Step 3: 4-(1-(6-Chloro-2-(pyridin-4-yl)pyrimidin-4-yl)-piperidin-4-yl)morpholine. To a stirred solution of 6-chloro-2-(pyridin-4-yl)pyrimidin-4-yl)morpholine (0.13 g, 0.6 mmol) in anhydrous THF (5 mL), 4-morpholinopiperidine (0.11 g, 0.63 mmol) and disoprophyllamine (0.20 mL, 1.15 mmol) were added at room temperature, and the reaction mixture was stirred at room temperature overnight. Water (10 mL) was added, and the product was extracted with DCM (2 × 50 mL), the organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (12 g silica cartridge) using (A) DCM and (B) 10% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 50% B, 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-(1-(6-chloro-2-(pyridin-4-yl)pyrimidin-4-yl)-piperidin-4-yl)morpholine as white solid (38 mg, 24% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.47 (1H, d, J = 1.6 Hz), 8.74 (2H, d, J = 6.0 Hz), 8.71 (1H, dd, J = 1.3, 4.7 Hz), 8.67–8.64 (1H, m), 8.33 (2H, d, J = 6.0 Hz), 7.57 (1H, dd, J = 4.8, 8.0 Hz), 7.49 (1H, s), 6.60 (1H, s), 4.73–4.73 (2H, m), 3.79 (4H, dd, J = 4.0, 4.0 Hz), 3.04 (2H, t, J = 12.5 Hz), 2.35 (4H, s), 2.16 (2H, d, J = 7.3 Hz), 1.95–1.89 (1H, m), 1.86 (2H, d, J = 13.0 Hz), 1.67–1.08 (11H). LRMS (ES+) m/z 417 [M + H]+.

N-(4-Morpholinobutyl)-6-(pyridin-3-yl)-2-(pyridin-4-yl)pyrimidin-4-amine (14). 14 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-chloro-N-(4-morpholinobutyl)-2-(pyridin-4-yl)pyrimidin-4-amine (187 mg, 0.54 mmol) in DFM (4 mL) was prepared at rt and 3-phenylboronic acid (132, 1.08 mmol) added followed by potassium phosphate (228 mg, 1.08 mmol) in water (2 mL). Pd tetrakis (31 mg, 0.03 mmol) was added and the mixture heated in a microwave to 130 °C for 1 h. The mixture was diluted with dichloromethane (10 mL) and filtered through an SCX-2 column, washed with methanol (2 × 10 mL), and washed with 7 M ammonia in methanol (2 × 10 mL) and filtrate and extracted product was further purified by column chromatography (12 g silica cartridge) using (A) DCM and (B) 10% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 50% B, 3 min hold at 45% B. The fractions containing product were pooled together and solvents were removed under reduced pressure. The product was purified by preparative HPLC. The fractions containing product were pooled together, and solvents were removed to obtain compound 12 as off-white solid (38 mg, 24% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.27 (1H, J = 1.8 Hz), 8.75 (dd, 2H, J = 1.6, 4.5 Hz), 8.72 (dd, 1H, J = 1.6, 4.8 Hz), 8.45 (dt, 1H, J = 2.0, 9.9 Hz), 8.32 (d, 2H, J = 8.3 Hz), 7.45 (dd, 1H, J = 3.1, 7.3 Hz), 6.70 (s, 1H), 6.04 (bs, 1H), 3.78 (t, 4H, J = 4.4 Hz), 3.52 (bs, 2H), 2.49 (brs, 4H), 2.43 (t, 2H, J = 7.1 Hz), 1.80 (p, 2H, J = 6.8 Hz), 1.69 (p, 2H, J = 7.1 Hz); LRMS (ES+) m/z 389 [M + H]+.

(R)-2-(6-(Pyridin-3-yl)-2-(pyridine-4-yl)pyrimidin-4-yl)octahydropyrrolo[1,2-ajpyrazine (15). 15 was prepared in an analogous four-step procedure to that of compound 12: A stirred solution of (R)-2-(6-(pyridin-3-yl)-2-(pyridine-4-yl)pyrimidin-4-yl)octahydropyrrolo[1,2-ajpyrazine (0.14 g, 0.44 mmol) and 3-phenylboronic acid (0.16 g, 1.31 mmol) in DMF (4.5 mL), a solution of potassium phosphate (0.28 g, 1.31 mmol) in water (1.5 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh3)4 (0.015 g, 0.011 mmol) was added. The reaction was heated at 120 °C under microwave irradiation for 30 min. Reaction crude was filtered through Celite, quenched with water (20 mL), and extracted with DCM (2 × 50 mL). The organic phases were combined, dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by column chromatography (12 g silica cartridge) using (A) DCM and (B) 20% MeOH in DCM as eluents and the following gradient: 3 min hold at 100% A, 18 min ramp to 45% B, 3 min hold at 45% B. The fractions containing product were pooled together and solvents were removed to obtain 15 as white solid (119 mg, 75% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.22 (1H, s), 8.69–8.65 (3H, m), 8.37–8.35 (1H, m), 8.28–8.27 (2H, 2H, 7.37 (dd, 1H, J = 4.8, 7.8 Hz), 6.84 (s, 1H, 4.56 (br broad peak, 2H), 3.61–3.09 (3H, m), 2.78–2.74 (1H, m), 2.27–2.22 (1H, m), 2.18–2.13 (1H, m), 2.04–1.98 (1H, m), 1.94–1.82 (2H, m), 1.78–1.70 (1H, m), 1.54–1.45 (1H); LRMS (ES+) m/z 359 [M + H]+.

4-(1-(6-Cyclopentyl-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (16). 16 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-((1-(6-Chloro-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (0.050 g, 0.14 mmol), potassium phosphate (0.005 g, 0.04 mmol), cyclopentylboronic acid (0.012 g, 0.014 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in MeOH/DFM and purified by SCX 2 g column eluting with MeOH and then 2 M NH4 in MeOH. The fraction containing product was evaporated to dryness. The residue was dissolved in DMF and purified by mass directed HPLC 5–95% MeCN, basic, to afford 16 as a white solid (15 mg, 28% yield).
Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.68 (d, 2H, J = 5.5 Hz), 8.19 (d, 2H, J = 5.65 Hz), 6.42 (s, 1H), 4.50 (bs, 2H), 3.86–3.74 (broad peak, 4H), 2.96 (m, 2H), 2.76–2.51 (broad peak, 5H), 2.07–1.98 (broad peak, 2H), 1.88 (m, 1H), 1.65–1.52 (broad peak, 2H), 1.19 (m, 2H), 0.98 (m, 2H); LRMS (ES+) m/z 366 [M + H]+.

6-(4-Morpholinopiperidin-1-yl)-N-(3-morpholinopropyl)-2-(pyridin-4-yl)pyrimidin-4-amine (17). To a mixture of 4-[1H-chloro-2-(4-pyridyl)pyrimidin-4-yl]-piperidylmopholine (0.050 g, 0.14 mmol) in anhydrous NMP (1 mL) was added morpholine (0.050 g, 0.14 mmol), N,N-diisopropylethylamine (0.072 mL, 0.41 mmol), 3-aminopropanamide hydrochloride (0.052 g, 0.41 mmol) in anhydrous NMP (1 mL) was heated at 200 °C under microwave irradiation for 10 min. The cooled reaction mixture was purified by mass directed HPLC 5–95% MeCN, basic, to afford 17 as a yellow solid (38 mg, 55% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. LRMS (ES+) as a yellow solid (16 mg, 26% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.69–8.65 (m, 2H), 8.18–8.16 (m, 2H), 5.61 (bs, 1H), 5.43 (s, 1H), 4.50–4.45 (m, 2H), 3.80–3.70 (m, 8H), 3.44–3.38 (m, 2H), 2.90 (m, 2H), 2.62–2.44 (m, 1H), 1.97–1.94 (m, 2H), 1.87–1.80 (m, 2H), 1.58–1.48 (m, 2H); LRMS (ES+) m/z 468 [M + H]+.

3-((6-(4-Morpholinopiperidin-1-yl)-2-(pyridin-4-yl)pyrimidin-4-yl)aminopropanamide (18). A mixture of 4-[1H-chloro-2-(4-pyridyl)pyrimidin-4-yl]-piperidylmorpholine (0.050 g, 0.14 mmol), N,N-diisopropylethylamine (0.072 mL, 0.41 mmol), 3-aminopropanamide hydrochloride (0.052 g, 0.41 mmol) in anhydrous NMP (1 mL) was heated at 200 °C under microwave irradiation for 10 min. The cooled reaction mixture was purified by mass directed HPLC 5–95% MeCN, basic, to afford 18 as a yellow solid (16 mg, 26% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.67–8.64 (m, 2H), 8.15–8.12 (m, 2H), 7.33 (bs, 1H), 6.84–6.75 (broad peaks, 2H), 5.7 (s, 1H), 4.41–4.32 (m, 2H), 3.48–3.47 (m, 6H), 2.83 (m, 2H), 2.52–2.34 (m, 7H), 1.88–1.82 (m, 2H), 1.40–1.30 (m, 2H); LRMS (ES+) m/z 412 [M + H]+.

4-(1-(6-Morpholino-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-1-yl)morpholine (19). To a mixture of 4-[1H-chloro-2-(4-pyridyl)pyrimidin-4-yl]-piperidylmorpholine (0.050 g, 0.14 mmol) in anhydrous NMP (1 mL) was added morpholine (36 mg, 0.41 mmol), and the mixture was heated at 200 °C under microwave irradiation for 10 min. The cooled reaction mixture was purified by mass directed HPLC 5–95% MeCN, basic, to afford 19 as a white solid (30 mg, 57% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.69–8.67 (m, 2H), 8.20–8.18 (m, 2H), 5.61 (s, 1H), 4.55–4.49 (m, 2H), 3.83–3.80 (m, 4H), 3.68–3.71 (broad peak, 4H), 3.66–3.63, (m, 4H), 2.92 (m, 2H), 2.62–2.57 (broad peak, 5H), 2.00–1.93 (m, 2H), 1.62–1.52 (m, 2H); LRMS (ES+) m/z 411 [M + H]+.

4-(1-(6-(3,5-Dimethylisoxazol-4-yl)-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-1-yl)morpholine (20). To a mixture of 4-[1H-chloro-2-(4-pyridyl)pyrimidin-4-yl]-piperidylmorpholine (0.050 g, 0.14 mmol) in anhydrous NMP (1 mL) was added morpholine (36 mg, 0.41 mmol), and the mixture was heated at 200 °C under microwave irradiation for 10 min. The cooled reaction mixture was purified by mass directed HPLC 5–95% MeCN, basic, to afford 20 as a white solid (30 mg, 61% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.54–8.52 (m, 2H), 8.35–8.32 (m, 2H), 8.14–8.09 (m, 2H), 7.21–7.16 (m, 2H), 6.87 (s, 1H), 4.67 (broad peak, 2H), 3.84–3.77 (broad peak, 4H), 3.05 (m, 2H), 2.71–2.55 (broad peak, 5H), 2.10–2.00 (m, 2H), 1.67–1.52 (m, 2H), LRMS (ES+) m/z 420 [M + H]+.

4-(1-(2-(Pyridin-4-yl)[4,5-bipyrimidin]-6-yl)piperidin-4-yl)morpholine (23). 23 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-[1H-chloro-2-(4-pyridyl)pyrimidin-4-yl]-piperidylmorpholine (0.050 g, 0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd(PPh3)4 (0.005 g, 0.004 mmol), (4-fluorophenyl)boronic acid (0.22 g, 1.04 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in MeOH/DCM and purified by SCX 2 g column eluting with MeOH and then 2 M NH3 in MeOH. The fraction containing product was evaporated to dryness. The residue was dissolved in DMF and purified by mass directed HPLC 5–95% MeCN, basic, to afford 23 as a white solid (36 mg, 60% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.82 (d, 2H, J = 1.7 Hz). DOI: 10.1021/jacs.6b00028 J. Med. Chem. XXXX, XXX, XXX–XXX
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5-(6-(4-Morpholinopiperin-1-yl)-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)nicotinonitrile (25). 25 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-[1-[6-chloro-2-(4-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.050 g, 0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd[PPh3]4 (0.005 g, 0.004 mmol), morpholino(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)methanone (0.132 g, 0.41 mmol) in 1,4-dioxane (1.6 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in MeOH/DCM and purified by SCX 2 g column eluting with MeOH and then 2 M NH3 in MeOH. The fraction containing product was evaporated to dryness.

The residue was dissolved in DCM and purified by mass directed HPLC 5–95% MeCN, basic, to afford 28 as a light brown solid (52 mg, 68% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.97 (d, 1H, J = 1.94 Hz), 8.79–8.72 (m, 3H), 8.34–8.29 (m, 2H), 6.94 (s, 1H), 4.69 (double peak, 2H), 3.84–3.72 (double peak, 4H), 3.12 (m, 2H), 2.70–2.58 (double peak, 8H), 2.15–2.03 (m, 2H), 1.71–1.54 (double peak, 2H); LRMS (ES+) m/z 428 [M + H]+.

5-(6-(6-Methoxypyridin-3-yl)-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)methanamine (27). 27 was prepared in an analogous four-step procedure to that of compound 12: A solution of 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinamide (31). 31 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-[1-[6-chloro-2-(4-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.050 g, 0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd[PPh3]4 (0.005 g, 0.004 mmol), morpholino(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)methanone (0.132 g, 0.41 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in MeOH/DCM and purified by SCX 2 g column eluting with MeOH and then 2 M NH3 in MeOH. The fraction containing product was evaporated to dryness.

The residue was dissolved in DCM and purified by mass directed HPLC 5–95% MeCN, basic, to afford 29 as a white solid (42 mg, 68% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.92 (d, 1H, J = 2.05 Hz), 8.74–8.72 (m, 2H), 8.32–8.28 (m, 3H), 7.23 (s, 1H), 6.54 (d, 1H, J = 8.75 Hz), 6.48 (bs, 2H), 4.70 (bs, 2H), 3.59–3.55 (m, 4H), 3.04–2.97 (m, 2H), 2.55–2.48 (m, 2H), 1.94–1.88 (m, 2H), 1.45–1.36 (m, 2H); LRMS (ES+) m/z 418 [M + H]+.

5-(6-(6-Methoxypyridin-3-yl)-2-(pyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)methanamine (30). 30 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-[1-[6-chloro-2-(4-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.050 g, 0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd[PPh3]4 (0.005 g, 0.004 mmol), morpholino(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)methanone (0.132 g, 0.41 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness.

The residue was dissolved in MeOH/DCM and purified by SCX 2 g column eluting with MeOH and then 2 M NH3 in MeOH. The fraction containing product was evaporated to dryness.

The residue was dissolved in DCM and purified by mass directed HPLC 5–95% MeCN, basic, to afford 30 as a white solid (42 mg, 68% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.92 (d, 1H, J = 2.05 Hz), 8.74–8.72 (m, 2H), 8.32–8.28 (m, 3H), 7.23 (s, 1H), 6.54 (d, 1H, J = 8.75 Hz), 6.48 (bs, 2H), 4.70 (bs, 2H), 3.59–3.55 (m, 4H), 3.04–2.97 (m, 2H), 2.55–2.48 (m, 2H), 1.94–1.88 (m, 2H), 1.45–1.36 (m, 2H); LRMS (ES+) m/z 418 [M + H]+.

4-([1-[6-Chloro-2-(4-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholin-3-yl)pyridin-3-yl)methanamine (28). 28 was prepared in an analogous four-step procedure to that of compound 12: A mixture of 4-[1-[6-chloro-2-(4-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.050 g, 0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd[PPh3]4 (0.005 g, 0.004 mmol), morpholino(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)methanone (0.132 g, 0.41 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in MeOH/DCM and purified by SCX 2 g column eluting with MeOH and then 2 M NH3 in MeOH. The fraction containing product was evaporated to dryness.

The residue was dissolved in DCM and purified by mass directed HPLC 5–95% MeCN, basic, to afford 28 as a light brown solid (52 mg, 68% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 8.85–8.33 (m, 2H), 6.95 (s, 1H), 4.65 (bs, 2H), 4.04 (s, 2H), 3.76–3.70 (m, 4H), 3.09 (m, 2H), 2.62–2.56 (m, 5H), 2.07–1.99 (m, 2H), 1.65–1.54 (m, 2H); LRMS (ES+) m/z 432 [M + H]+.

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0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd(PPh3)4 (0.005 g, 0.004 mmol), N-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinamide (0.109 g, 0.41 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was evaporated to dryness. The residue was dissolved in MeOH/DCM and purified by SCX 2 g column eluting with MeOH and then 2 M NH3 in MeOH. The fraction containing product was evaporated to dryness. The residue was dissolved in DMF and purified by mass directed HPLC 5–95% MeCN, basic, to afford 31 as a white solid (22 mg, 32% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 99%. 1H NMR (500 MHz, CDCl3) δ 9.25 (m, 1H), 8.77 (bs, 2H), 8.35–8.49 (m, 1H), 7.59–8.11 (m, 8H), 4.00 (m, 2H), 1.92–2.60 (m, 9H), 1.32–1.52 (m, 2H); LRMS (ES+) 382 [M + H]+.

4-(1-(6-Chloro-2-(4-pyridyl)pyrimidin-4-yl)pyridin-2-yl)thiomorpholine (32). 32 was prepared in an analogous four-step procedure to that of compound 31. A mixture of 4-(1-[6-chloro-2-(4-pyridyl)pyrimidin-4-yl]piperidin-4-yl)phenylmorpholine (0.050 g, 0.14 mmol), potassium phosphate (0.088 g, 0.41 mmol), Pd(PPh3)4 (0.005 g, 0.004 mmol), 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrid-2-yl)phenylmorpholine (0.121 g, 0.41 mmol) in 1,4-dioxane (1.6 mL) and water (0.4 mL) was heated at 120 °C under microwave irradiation for 30 min. The cooled reaction mixture was filtered through a Celite cartridge (2.5 g), washing the cartridge with MeOH and then 50% MeCN, basic, to afford 32 as a white solid (46 mg, 72% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 99%. 1H NMR (500 MHz, CDCl3) δ 9.12–9.09 (m, 1H), 8.72 (d, 2H, J = 5.78 Hz), 8.48–8.42 (m, 1H), 8.30 (d, 2H, J = 5.93 Hz), 7.3 (s, 1H), 6.95 (d, 1H, J = 9.08 Hz), 4.65 (bs, 2H), 3.78–3.50 (m, 12H), 3.01 (m, 2H), 2.58–2.38 (m, 5H), 1.97–1.84 (m, 2H), 1.50–1.19 (m, 2H); LRMS (ES+) m/z 460 [M + H]+.

To a stirred solution of 6-chloro-4-(4-methoxypiperidin-1-yl)-1,6-dihydropyrimidine-2-carbonitrile (0.128 g, 0.41 mmol) and 3- pyridylboronic acid (0.103 g, 0.83 mmol) in DME (4 mL), an aqueous solution of sodium carbonate (2 M, 0.24 mL) and PdCl2(PPh3)2 (0.014 g, 0.02 mmol) were added. The reaction was heated at 120 °C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (5 mL) and applied to a SCX column (5 g) and the product was eluted with 2 M NH3 in MeOH. The product was further purified by preparative HPLC under acidic conditions. The fractions containing product were pooled together and the solvents were removed to obtain 35 as off-white solid (47 mg, 31% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 99%. 1H NMR (500 MHz, CDCl3) δ 9.14–9.13 (m, 1H), 8.72 (dd, 1H, J = 1.7, 4.8 Hz), 8.36–8.34 (m, 1H), 7.45 (ddd, 1H, J = 0.8, 4.8, 8.0 Hz), 6.97 (s, 1H), 4.39–4.56 (m, 2H), 3.76–3.74 (m, 4H), 3.10–3.05 (m, 2H), 2.63–2.57 (m, 5H), 2.04–2.02 (m, 2H), 1.60–1.52 (m, 2H); LRMS (ES+) m/z 351 [M + H]+.

4-(1-(6-Chloro-2-(4-pyridyl)pyrimidin-4-yl)phenyl)thiomorpholine 1,1-Dioxide (36). 36 was prepared in an analogous three-step procedure to that of compound 34: A solution of 4-(1-[6-chloro-2-(4-pyridyl)pyrimidin-4-yl]piperidyl)phenol (0.15 g, 0.36 mmol) in DME (4 mL), an aqueous solution of sodium carbonate (2 M, 0.24 mL) and PdCl2(PPh3)2 (0.014 g, 0.02 mmol) were added. The reaction was heated at 120 °C for 5 h. Reaction crude was diluted with methanol (5 mL) and applied to a SCX column (5 g) and the product was eluted with 2 M NH3 in MeOH. The product was further purified by preparative HPLC under acidic conditions. The fractions containing product were pooled together and the solvents were removed to obtain 35 as off-white solid (47 mg, 31% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 99%. 1H NMR (500 MHz, CDCl3) δ 9.14–9.13 (m, 1H), 8.72 (dd, 1H, J = 1.7, 4.8 Hz), 8.36–8.34 (m, 1H), 7.45 (ddd, 1H, J = 0.8, 4.8, 8.0 Hz), 6.97 (s, 1H), 4.39–4.56 (m, 2H), 3.76–3.74 (m, 4H), 3.10–3.05 (m, 2H), 2.63–2.57 (m, 5H), 2.04–2.02 (m, 2H), 1.60–1.52 (m, 2H); LRMS (ES+) m/z 351 [M + H]+.
(2 M, 0.5 mL) were added. The reaction was heated at 120 °C for 20 min under microwave irradiation. The reaction crude was diluted with methanol (10 mL) and applied to a SCX column (2 g), and the product was eluted with 2 M NH₄ in MeOH. The product was further purified by preparative HPLC under basic conditions. The fractions containing product were pooled together, and solvents were removed to obtain 36 as off-white solid (67 mg, 41% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 98%. 1H NMR (500 MHz, CDCl₃) δ 9.13 (dd, 1H, J = 0.7, 2.2 Hz), 8.66 (dd, 1H, J = 1.7, 4.8 Hz), 8.22–8.20 (m, 1H), 7.37 (dd, 1H, J = 0.8, 4.8, 8.0 Hz), 6.42 (s, 2H), 4.45–4.40 (m, 4H), 3.73–3.71 (m, 4H), 3.07–3.05 (m, 4H), 2.53–2.50 (m, 4H), 2.52–2.47 (m, 1H), 1.97–1.94 (m, 1H). LRMS (ES+) m/z 469 [M + H]+.

4-(4-Morpholino)piperidin-1-yl)-6-(pyridine-3-yl)pyrimidin-2-ol (37). To a stirred solution of 4-[1-(2,6-dichloropyrimidin-4-yl)-4-piperidyl]morpoline (0.60 g, 1.89 mmol) in THF (10 mL) in a 20 ℃ under microwave irradiation for 20 min. Reaction was further purified by preparative HPLC. The fractions containing product were pooled together and solvents were removed to obtain 38 as off-white solid (70 mg, 40% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 98%. 1H NMR (500 MHz, CDCl₃) δ 9.17 (dd, 1H, J = 0.7, 2.2 Hz), 8.67 (dd, 1H, J = 1.7, 4.8 Hz), 8.31–8.29 (m, 1H), 7.38 (dd, 1H, J = 0.8, 4.8, 8.0 Hz), 6.67 (bs, 1H), 6.41 (s, 1H), 4.52–4.49 (m, 4H), 4.16–4.15 (m, 2H), 3.16–3.13 (m, 4H), 2.99–2.94 (m, 2H), 2.60–2.59 (m, 4H), 2.54–2.48 (m, 1H), 1.98–1.95 (m, 1H), 1.57–1.49 (m, 2H); LRMS (ES+) m/z 425 [M + H]+.

N,N’-Dimethyl-N-(4-(4-morpholino)piperidin-1-yl)-6-(pyridine-3-yl)pyrimidin-2-yl)ethane-1,2-diamine (39). 39 was prepared by the analogous three-step procedure to that of compound 50. To a stirred suspension of N,N’-[4-(4-chloro-6-(4-morpholinopiperidin-1-yl))pyrimidin-2-yl]-N,N’-dimethyl-ethane-1,2-diamine (0.04 g, 0.11 mmol) and 3-pyridylboronic acid (0.41 g, 0.33 mmol) in DMF (3 mL), a solution of potassium phosphate (0.07 g, 0.33 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd[PPh₃]₄ (0.004 g, 0.003 mmol) was added. The reaction was heated at 130 ℃ under microwave irradiation for 20 min. Reaction was filtered through Celite and partitioned between DCM (10 mL) and a saturated aqueous solution of NaHCO₃ (5 mL). The organic phase was dried over MgSO₄ before concentration to dryness. The crude was then purified by preparative mass directed autoreactive HPLC (method: 5–95 basic). The fractions containing product were pooled together and solvents were removed to obtain 39 as a white solid (18 mg, 39% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 98%. 1H NMR (500 MHz, DMF-δ) δ 9.11 (s, 1H), 8.63 (dd, 1H, J = 1.6, 4.8 Hz), 8.24 (dd, 1H, J = 7.9 Hz), 7.55 (dd, 1H, J = 4.8, 7.9 Hz), 6.32 (s, 1H), 4.51–4.48 (m, 2H), 3.73–3.55 (m, 4H), 3.53–3.52 (m, 2H), 2.90 (t, 1H, J = 12.7 Hz), 2.58–2.54 (m, 4H), 2.53–2.51 (m, 2H), 2.49–2.44 (m, 1H), 2.27 (s, 6H), 1.94–1.91 (m, 2H), 1.54–1.45 (m, 2H); LRMS (ES+) m/z 412 [M + H]+.

4-(1-(2,6-Dimethylpyridin-4-yl)-6-(pyridine-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (40). To a stirred solution of 4-[1-(6-chloro-2-(2,6-dimethyl-4-pyridyl)pyrimidin-4-yl)-4-piperidyl]-morpoline (0.042 g, 0.11 mmol) and 3-pyridylboronic acid (0.40 g, 0.32 mmol) in DMP (3 mL), a solution of potassium phosphate (0.069 g, 0.32 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd[PPh₃]₄ (0.008 g, 0.005 mmol) was added. The reaction was heated at 130 ℃ under microwave irradiation for 20 min. The reaction crude was partitioned between DCM (15 mL) and saturated aqueous solution of NaHCO₃ (5 mL). The organic phase was dried over MgSO₄ before concentration to dryness. The crude was then purified by preparative HPLC. The fractions containing product were pooled together and solvents were removed to obtain 40 as off-white solid (8 mg, 17% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 98%. 1H NMR (500 MHz, DMF, δ) 9.27–9.26 (m, 1H), 8.72 (dd, 1H, J = 1.6, 4.8 Hz), 8.45–8.42 (m, 1H), 8.03 (bs, 2H), 7.46 (dd, 1H, J = 0.6, 4.8, 8.0 Hz), 6.93 (s, 1H), 4.69 (broad m, 2H), 3.78 (broad m, 4H), 3.12–3.06 (m, 2H), 2.67–2.61 (m, 1H), 2.09–2.07 (m, 2H), 1.67–1.57 (m, 2H); LRMS (ES+) m/z 412 [M + H]+.

4-(1-(2-Methylpyridin-4-yl)-6-(pyridine-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (41). 41 was prepared in an analogous three-step procedure to that of compound 43: In a sealed 5 mL microwave vial, a solution of 4-[1-(2-methylsulfonyl-6-(3-pyridyl)pyrimidin-4-yl)-4-piperidyl]morpoline (0.07 g, 0.20 mmol) in THF (4 mL) was degassed by bubbling argon through for 5 min. (2-Methyl-4-pyridyl)boronic acid (0.03 g, 0.20 mmol), thioephene-2-carboxyloxyester (0.06 g, 0.30 mmol), and Pd[PPh₃]₄ (0.02 g, 0.02 mmol) were added at room temperature. The reaction was heated in a sealed tube at 85 ℃ for 18 h. The reaction crude was filtered through Celite and partitioned between DCM (10 mL) and ammonium...(continued)
hydroxide (5 mL). The organic phase was dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by mass directed autopreparative HPLC under basic conditions (5–95 prep basic). The fractions containing product were pooled together and solvents were removed to obtain 41 as white solid (9 mg, 10% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 97%. 1H NMR (500 MHz, CDCl3) δ 9.26 (d, 1H, J = 2.0 Hz), 8.72–8.71 (m, 1H), 8.62 (d, 1H, J = 5.2 Hz), 8.45–8.42 (m, 1H), 8.19 (s, 1H), 8.13 (d, 1H, J = 5.1 Hz), 7.74 (dd, 1H, J = 4.8, 7.9 Hz), 6.92 (s, 1H), 4.67–4.66 (m, 2H), 3.75–3.73 (m, 4H), 3.10–3.04 (m, 2H), 2.67 (s, 3H), 2.61–2.54 (m, 3H), 2.05–2.00 (m, 2H), 1.63–1.55 (m, 2H), LRMS (ES+) m/z 417 [M + H]+.

4-(1-(6-(Pyridin-3-yl)-2-(trifluoromethyl)pyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (42). 42 was prepared in an analogous three-step procedure to that of compound 43: In a sealed 5 mL microwave vial, a solution of 4-(1-[2-methylsulfonyl-6-(3-pyridyl)pyrimidin-4-yl]-4-piperidyl)morpholine (0.10 g, 0.26 mmol) in 1,4-dioxane (4 mL) was degassed by bubbling argon through for 5 min. (3-Fluoro-4-pyridyl)boronic acid (0.076 g, 0.54 mmol), thiophene-2-carboxylic copper (0.102 g, 0.54 mmol), and Pd(PPh3)4 (0.031 g, 0.03 mmol) were added at room temperature. The reaction was heated under microwave irradiation at 130 °C for 1 h. The reaction crude was applied to a SCX column (2 g), and the product was eluted with 2 M NH3 in MeOH. The product was purified by mass directed autopreparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain 44 as off-white solid (10 mg, 9% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. 1H NMR (500 MHz, CDCl3) δ 9.32–9.31 (m, 1H), 8.58–8.51 (m, 1H), 8.96–8.52 (m, 2H), 8.55–8.54 (m, 1H), 8.49–8.47 (m, 1H), 8.10–8.08 (m, 1H), 7.51–7.47 (m, 1H), 7.00 (s, 1H), 4.88–4.84 (m, 2H), 3.41–3.38 (m, 2H), 4.03–3.99 (m, 2H), 3.42–3.36 (m, 3H), 3.08–2.95 (m, 4H), 2.47–2.45 (m, 2H), 2.00–1.91 (m, 2H); LRMS (ES+) m/z 421 [M + H]+.

4-(4-(4-Morpholino)piperidin-1-yl)-6-(3-pyridin-3-yl)pyrimidin-2-yl)pyridine-2-ol (45). 45 was prepared in an analogous three-step procedure to that of compound 46. In a sealed 5 mL microwave vial, a solution of 4-[1-[2-methylsulfonyl-6-(3-pyridyl)pyrimidin-4-yl]-4-piperidyl]morpholine (0.15 g, 0.40 mmol) in THF (8 mL) was degassed by bubbling argon through for 5 min. 444.5-S-Tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-ol (0.09 g, 0.40 mmol), thiophene-2-carboxylic copper (0.12 g, 0.61 mmol), and Pd(PPh3)4 (0.05 g, 0.04 mmol) were added at room temperature. The reaction was heated in a sealed tube at 85 °C for 16 h. The reaction crude was applied to a SCX column (2 g), and the product was eluted with 2 M NH3 in MeOH. Solvents were removed under reduced pressure, and the product was purified by mass directed autopreparative HPLC under basic conditions (5–95 prep basic). The fractions containing product were pooled together and solvents were removed to obtain 46 as white solid (10 mg, 66% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >97%. 1H NMR (500 MHz, DMSO-d6) δ 11.73 (bs, 1H), 9.43–9.42 (m, 1H), 8.70–8.69 (m, 1H), 8.61–8.60 (m, 1H), 7.56 (dd, 1H, J = 4.8, 8.0 Hz), 7.49 (s, 1H), 7.48 (d, 1H, J = 6.8 Hz), 7.32 (s, 1H), 7.13 (d, 1H, J = 6.9 Hz), 4.70–4.67 (m, 2H), 3.57–3.55 (m, 4H), 3.06–3.01 (m, 2H), 1.92–1.90 (m, 2H), 1.44–1.36 (m, 2H); LRMS (ES+) m/z 419 [M + H]+.

4-(1-(2-(Methyl-1H-pyrazol-4-yl)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (46). 46 was prepared in an analogous three-step procedure to that of compound 40. To a stirred solution of 4-[1-[2-methylpyrazol-4-yl]pyrimidin-4-yl]-4-piperidyl)morpholine (0.070 g, 0.19 mmol) and 3-pyridylboronic acid (0.071 g, 0.58 mmol) in DME (3 mL), a solution of potassium phosphate (0.122 g, 0.58 mmol) in water (1 mL) was added. The reaction mixture was degassed by bubbling argon through for 5 min, and then Pd(PPh3)4 (0.011 g, 0.010 mmol) was added. The reaction was heated at 130 °C under microwave irradiation for 20 min. Reaction was filtered through Celite and partitioned between DCM (15 mL) and a saturated aqueous solution of NaHCO3 (5 mL). The organics phase was dried over MgSO4 before concentration to dryness. The crude was then purified by preparative HPLC. The fractions containing product were pooled together and solvents were removed to obtain 46 as off-white solid (27 mg, 35% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 96%. 1H NMR (500 MHz, CDCl3) δ 9.26 (d, 1H, J = 2.0 Hz), 8.72–8.71 (m, 1H), 8.69–8.52 (m, 2H), 8.39–8.37 (m, 1H), 7.87 (d, 1H, J = 4.5 Hz), 7.43 (dd, 1H, J = 4.8, 7.8 Hz), 6.91 (s, 1H), 4.62–4.59 (m, 2H), 3.80–3.72 (m, 4H), 3.10–3.04 (m, 2H), 2.66 (s, 3H), 2.64–2.52 (m, 3H), 2.06–2.03 (m, 2H), 1.65–1.55 (m, 2H); LRMS (ES+) m/z 417 [M + H]+.
piperidyl)morpholine (0.075 g, 0.20 mmol) in THF (4 mL) was degassed by bubbling argon through for 5 min. Pyrimidin-4-ylboronic acid (0.037 g, 0.30 mmol), triphenylphosphine-carbonyldioperoxycopper (0.058 g, 0.30 mmol), and Pd(PPh3)4 (0.023 g,0.02 mmol) were added at room temperature. The reaction was heated in the sealed tube at 85 °C for 18 h. Reaction was filtered through Celite and partitioned between DCM (10 mL) and NH4 aq (5 mL). The organic phase was dried over magnesium sulfate, and solvents were removed under reduced pressure. The product was purified by the product was purified by mass directed autorepreparative HPLC under basic conditions. The fractions containing product were pooled together and solvents were removed to obtain 47 as off-white solid (10 mg, 12% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. H NMR (500 MHz, CDCl3) δ 9.45 (d, 1H, J = 1.3 Hz), 9.23 (dd, 1H, J = 0.7, 2.3 Hz), 8.72 (dd, 1H, J = 1.6, 4.8 Hz), 8.46–8.43 (m, 2H), 7.45 (dd, 1H, J = 0.8, 4.8, 8.0 Hz), 7.00 (s, 1H), 4.77–4.75 (m, 2H), 3.82–3.70 (m, 4H), 3.14–3.09 (m, 2H), 3.67–2.55 (m, 5H), 2.11–2.01 (m, 2H), 1.62–1.61 (m, 2H); LRMS (ES+) m/z 404 [M + H]+.

**Scheme 2 (General Procedure for Intermediates). 4-(1-(6-Chloro-2-(2,6-dimethylpyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (54).** To a solution of 2,4,6-trichloropyrimidine (10 g, 54.52 mmol) in ethanol (125 mL) at −5 °C (salt–ice bath), a solution of 4-(piperidyl)morpholine (9.28 g, 54.52 mmol) in ethanol (200 mL) was added dropwise. The reaction mixture was stirred at −5 °C for 4 h. A white precipitate was formed. Solvents were removed under vacuum, and the reaction crude was partitioned between DCM (300 mL) and a saturated aqueous solution of NaHCO3 (2 × 200 mL). The organic phase was dried over MgSO4, filtered, and washed with methanol and solvents were removed under reduced pressure. The product was purified by column chromatography (330 g silica cartridge) using (A) DCM and (B) 5% MeOH in DCM as eluents and the following gradient: 2 min hold to 100% A, 20 min ramp to 50% B, 3 min hold to 50% B. Fractions containing pure product were pooled together and solvents were removed to obtain intermediate 4-(1-(2,6-dichloropyrimidin-4-yl)piperidin-4-yl)morpholine as a white solid (5.6 g). Column fractions that contained a mixture of the desired product and a side product resulting from substitution at C-2 were pooled together and solvents removed under vacuum. The mixture was suspended in methanol, and DCM was added to obtain a clear solution that was left standing at −20 °C. The precipitate was filtered and dried to obtain 4-(1-(6-chloro-2-(2,6-dimethylpyridin-4-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine as a white solid (1.7 g). Both product fractions were mixed together (7.3 g, 42% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. H NMR (500 MHz, CDCl3) δ 8.64 (s, 1H), 4.41 (broad peak, 2H), 3.72 (broad peak, 4H), 2.01–2.97 (m, 2H), 2.55–2.47 (m, 5H), 1.97–1.94 (m, 2H), 1.54–1.46 (m, 2H); LRMS (ES+) m/z 317 [M + H]+.

To a stirred solution of 4-(1-(2,6-dichloropyrimidin-4-yl)piperidin-4-yl)morpholine (0.20 g, 0.63 mmol) and 2,6-dimethyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (0.16 g, 0.69 mmol) in 1,4-dioxane (6 mL), a solution sodium carbonate (0.20 g, 1.89 mmol) in water (2 mL) was added. The reaction mixture was stirred at room temperature for 18 h. Reaction mixture was stirred at room temperature for 3 h. A white precipitate was formed. Solvents were removed under reduced pressure, and the reaction crude was purified by filtration through a silica plug. First, impurities were removed with a mixture 1/1 of petroleum ether and ethyl acetate, and then the product eluted with methanol. The fractions containing product were pooled together and methanol was removed to obtain 4-[1-(6-chloro-2-methylsulfonyl-pyrimidin-4-yl)-4-piperidyl)morpholine (57) as off-white solid (7.17 g, 84% yield). Purity by LCMS (UV chromatogram, 190–450 nm) >98%. H NMR (500 MHz, CDCl3) δ 6.19 (s, 1H), 4.37 (broad peak, 2H), 3.76 (broad peak, 4H), 2.95–2.90 (m, 2H), 2.69–2.53 (m, 5H), 2.47 (s, 3H), 1.98–1.96 (m, 2H), 1.54–1.48 (m, 2H); LRMS (ES+) m/z 329 [M + H]+.

A solution of 57 (4.00 g, 12.16 mmol) and 3-arylboronic acid (2.99 g, 24.3 mmol) in 1,4-dioxane (60 mL) was divided equally into four 20 mL microwave vials. A solution of KO3PO4 (5.16 g, 24.3 mmol) in water (20 mL) was prepared, and an aliquot of the solution was added to each reaction vial. The reaction mixtures were degassed by bubbling argon through for 5 min. Then, Pd(PPh3)4 (0.70 mg, 0.61 mmol) was added and the reaction mixtures were heated under microwave irradiation at 130 °C for 30 min. The contents of the three vials were pooled together, and the reaction was filtered through Celite and partitioned between DCM (2 × 200 mL) and saturated aqueous solution of NaHCO3 (20 mL). The product was purified by column chromatography (120 g silica cartridge) using (A) DCM and (B) 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100% A, 20 min ramp to 50% B, 10 min hold at 50% B. The fractions containing product were pooled together and the solvents removed to obtain a dark color solid. The solid was dissolved in methanol (30 mL) and 3-mercaptopropyl ethyl sulfide silica (2 g, 60–200 μM, Phosphonic SPM-32) was added. The stirred suspension was heated at 50 °C overnight. The silica was filtered and washed with methanol (100 mL). Methanol was removed under reduced pressure to obtain the product as an off-white solid (2.24 g, 50% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 98%. H NMR (500 MHz, CDCl3) δ 9.14 (dd, 1H, J = 0.6, 2.2 Hz), 8.66 (dd, 1H, J = 1.7, 4.8), 8.32–8.29 (m, 1H), 7.37 (dd, 1H, J = 0.7, 4.8, 7.9 Hz), 6.61 (s, 1H), 4.49–4.41 (m, 2H), 3.73–3.71 (m, 4H), 3.00–2.94 (m, 2H), 2.58–2.56 (m, 7H), 2.53–2.46 (m, 1H), 1.97–1.95 (m, 1H), 1.52 (ddd, 2H, J = 4.3, 12.3, 24.2 Hz); LRMS (ES+) m/z 372 [M + H]+.

**Scheme 3 (General Procedure for Intermediates). 4-(1-(6-Chloro-2-(3-mercaptopropyl ethyl sulfide)-6-(pyridin-3-yl)pyrimidin-4-yl)piperidin-4-yl)morpholine (60).** To a stirred solution 4,6-dichloropyrimidin-2-amine (4.33 g, 25.8 mmol) and diiodomethane (6.91 g, 25.8 mmol) in anhydrous acetonitrile (36 mL) was added tert-butyl nitrite (11.97 g, 116.1 mmol) at room temperature under nitrogen. The reaction mixture was heated at 80 °C for 3 h and 30 min. The reaction crude was concentrated under reduced pressure and purified by column chromatography (80 g silica cartridge) using (A) Hex, (B) ethyl acetate as eluents and the following gradient: 5 min hold at 100% A, 10 min ramp to 20% B, 1 min hold at 20% B. Fractions containing product were pooled together and solvents removed under reduced pressure to obtain 12 as an off-white solid (4.49 g, 63% yield). Purity by LCMS (UV chromatogram, 190–450 nm) 98%. H NMR (500 MHz, CDCl3) δ 7.42 (m, 1H), 7.22–7.15 (m, 1H), 7.15–6.98 (m, 1H), 6.72 (m, 2H), 4.16–3.92 (m, 2H), 3.87–3.74 (m, 2H), 3.27–3.16 (m, 2H), 2.34–2.15 (m, 2H), 1.49–1.38 (m, 2H); LRMS (ES+) m/z 450 [M + H]+.
反应粗产物被柱色谱分离（80 g硅胶柱）。（A）异己烷；（B）乙酸乙酯作为洗脱剂，并根据下列梯度：1 min hold at 100% A, 10 min ramp to 50% B, and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidine-2-carbonitrile (62). In a stirred sealed tube a solution of 4-[1-(6-chloro-2-iodopyrimidin-4-yl)4-piperidyl]morpholine (61) (0.29 g, 0.72 mmol) and copper cyanide (0.077 g, 0.86 mmol) in NMP (3 mL) was heated at 120 °C for 5 h. Reaction crude was applied to a SCX cartridge (5 g), and the product was diluted with a solution of 2 N NH3 in methanol. The product was further purified by column chromatography (12 g silica gel) using (A) DCM, (B) 10% MeOH in DCM as eluents and the following gradient: 1 min hold at 100% A, 10 min ramp to 50% B, and 3 min hold at 50% B. The fractions containing product were pooled together and solvents were removed to obtain 4-chloro-6-(4-morpholinopiperidin-1-yl)pyrimidine-2-carbonitrile (62) as an off-white solid (139 mg, 62% yield). Purity by LCMS (UV chromatogram, compliance with EU Directive EU/2010/63). License applications will on University premises and is a subcommittee of the University Court, remit to develop and oversee policy on all aspects of the use of animals (ERC) before submission to the Home Office.

### Supporting Information

**Bioentry Materials and Methods.** This information is in the Supporting Information.

**Ethical Statements.** In vivo antimalarial efficacy studies in *P. berghei* carried out at the Swiss Tropical and Public Health Institute (Basel, Switzerland) adhere to local and national regulations of laboratory animal welfare in Switzerland (awarded permission no. 1731). Protocols are regularly reviewed and revised following approval by the local authority (Veterinäramt Basel Stadt).

In vivo antimalarial efficacy studies using *P. falciparum* in SCID mice carried out at GSK were approved by the Diseases of the Developing World Ethical Committee on Animal Research and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals. The animal studies were performed at DDW Laboratory Animal Science facilities accredited by AAALAC. The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents.

Mouse pharmacokinetics were carried out at the University of Dundee. All regulated procedures on living animals were carried out under the authority of a license issued by the Home Office under the Animals (Scientific Procedures) Act 1986, as amended in 2012 (and in compliance with EU Directive EU/2010/63). License applications will have been approved by the University’s Ethical Review Committee (ERC) before submission to the Home Office. The ERC has a general remit to develop and oversee policy on all aspects of the use of animals on University premises and is a subcommittee of the University Court, its highest governing body.

### Associated Content

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00028.

Synthetic details for all compounds, supplementary data tables, additional information on ADMET and pharmacology (PDF)

Molecular formula strings (CSV)

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**Notes**

The authors declare no competing financial interest.

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### Abbreviations Used

ACT, artemisinin-based combination therapy; MMV, Medicines for Malaria Venture; PRR, parasite reduction ratio; SCX, strong cation exchange; SCID, nonobese diabetic scid IL2RYγnull; WHO, World Health Organization

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