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Structure Guided Lead Generation toward Nonchiral *M. tuberculosis* Thymidylate Kinase Inhibitors

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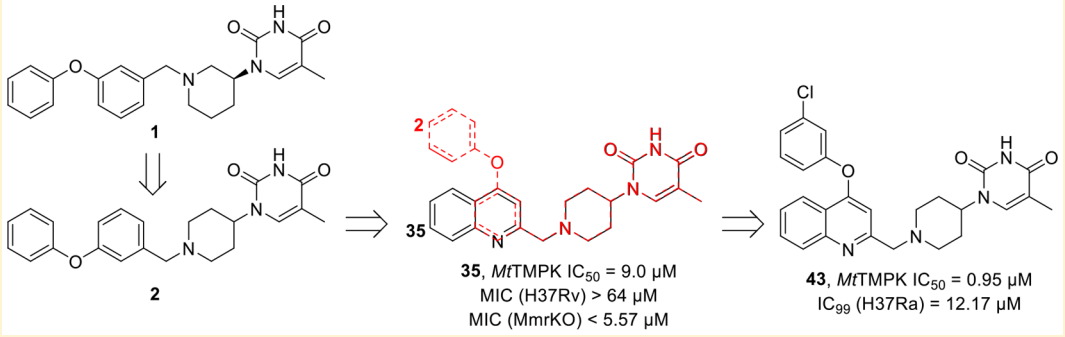
Supporting Information



ABSTRACT: In recent years, thymidylate kinase (TMPK), an enzyme indispensable for bacterial DNA biosynthesis, has been pursued for the development of new antibacterial agents including against *Mycobacterium tuberculosis*, the causative agent for the widespread infectious disease tuberculosis (TB). In response to a growing need for more effective anti-TB drugs, we have built upon our previous efforts toward the exploration of novel and potent *Mycobacterium tuberculosis* TMPK (*Mt*TMPK) inhibitors, and reported here the design of a novel series of non-nucleoside inhibitors of *Mt*TMPK. The inhibitors display hitherto unexplored interactions in the active site of *Mt*TMPK, offering new insights into structure−activity relationships. To investigate the discrepancy between enzyme inhibitory activity and the whole-cell activity, experiments with efflux pump inhibitors and efflux pump knockout mutants were performed. The minimum inhibitory concentrations of particular inhibitors increased significantly when determined for the efflux pump *mmr* knockout mutant, which partly explains the observed dissonance.

INTRODUCTION

Tuberculosis (TB) has been ranked as the most fatal infectious disease worldwide and constitutes the leading cause of death among people with HIV/AIDS.[1] As one of the most ancient human infectious diseases, it revives with immigration, increased homelessness, and the emergence of HIV/AIDS. It declines with improved public health practices, the development of and access to antituberculosis drugs and antibiotics, and immunization with *M. bovis* BCG vaccine.[2−4] At the early stage of TB infection, people with a low immunity directly develop the active disease, which is called primary or primary-progressive TB. Most people, however, progress into asymptomatic disease, which is referred to as latent tuberculosis.[5] On the basis of the tuberculin skin test reactivity, it is estimated that one-third of the world's population is infected with latent TB, which may be activated upon aging, malnutrition, treatment with immunosuppressive agents, HIV coinfection, etc.[1] It is estimated that 10.4 million new TB cases occurred in 2015.[1] South-East Asian and Western Pacific

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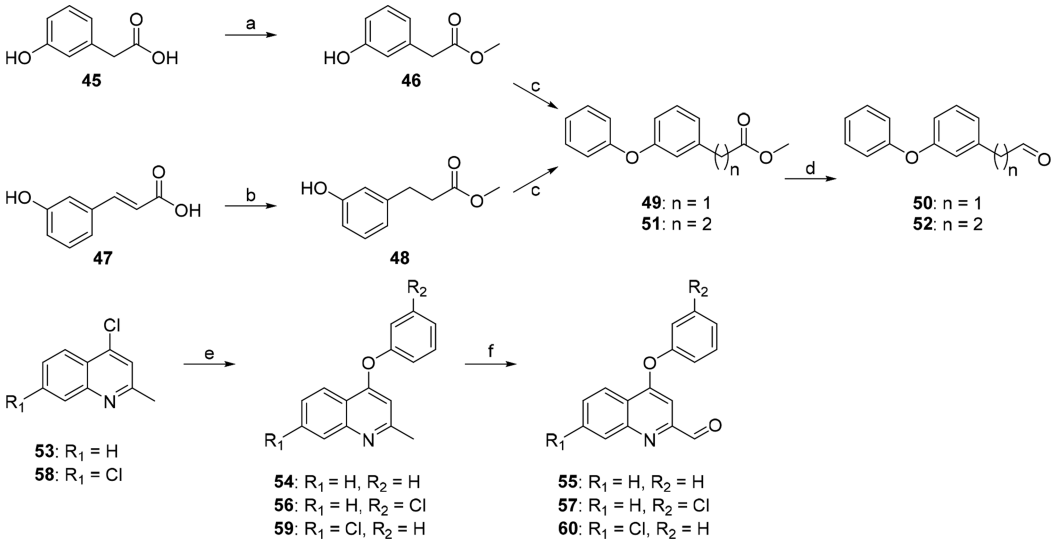
Scheme 1. Synthesis of the Required Aldehyde Building Blocks[a]



[a]Reagents and conditions: (a) MeOH, cat. H_2SO_4, 0 °C to reflux, overnight; (b) (i) MeOH, cat. H_2SO_4, 0 °C to reflux, overnight; (ii) H_2, Pd/C, AcOEt, rt, 2 h; (c) $Cu(OAc)_2$, molecular sieves, pyridine, rt, overnight; (d) (i) $LiAlH_4$, dry THF, 0 °C to rt, 20 min; (ii) Dess−Martin reagent, CH_2Cl_2, 0 °C to rt, 8 h; (e) Cs_2CO_3, *N*,*N*-dimethylglycine·HCl, CuI, dry 1,4-dioxane, 90 °C, 24 h; (f) SeO_2, 1,4-dioxane, 100 °C, 1.5−2 h.

regions accounted for 58% of these cases, Africa for 28%, while about 3% of the cases were allocated in high-income countries.[1] The currently used regime for drug-susceptible TB consists of four first-line drugs to be taken almost daily for six months or longer: isoniazid, rifampicin, ethambutol, and pyrazinamide. This lengthy treatment period with an associated risk of poor patient compliance increases the risk of drug-susceptible TB to develop into multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB).[6,7] Treatment of MDR-TB and XDR-TB is much longer, more expensive, less effective, and causes more severe side effects than that for drug-susceptible TB. Although the new anti-TB drugs bedaquiline and delamanid have been recommended for the treatment of MDR-TB by WHO, resistant isolates were reported as early as 2014.[8,9] Therefore, there is a pressing need to develop novel anti-TB drugs that allow shortening of the treatment period. Drugs exerting their effect via novel mechanisms of action are preferred, as they are believed to be less susceptible to generate cross-resistance with already existing antituberculosis drugs and, hence, could be useful for treating MDR-TB and XDR-TB.[10]

Human TB is caused by *Mycobacterium tuberculosis* (Mtb).[8] The intrinsic resistance of Mtb to common antibiotics is due to the combination of several factors, including a highly lipid-rich cell wall that forms a permeability barrier, and the activity of efflux pumps that transport noxious agents out of the cell.[11−14] Moreover, Mtb manages to survive inside macrophages (the first-line bodyguard of the human immune system) and hides itself by building granulomas inside the deep lung tissue (latent TB). Once the host immune system is weakened, the bacterium starts replicating and invading the neighboring tissue of the lung.[11,15,16] The granuloma creates heterogeneous microenvironments including a different blood supply, diverse pH, and various extent of caseum.[17,18] As a result, it is challenging for anti-TB drugs to reach and kill all bacilli.[19]

Mtb thymidylate kinase (*Mt*TMPK), a key enzyme for the synthesis of thymidine triphosphate as a DNA building block, is essential for the survival of Mtb.[20] Its known crystal structure and druggable active site make it an attractive target for drug design.[21−23] In this paper, we describe our attempts to optimize the *Mt*TMPK inhibitory activity of the earlier identified non-nucleoside hit **1** via scaffold screening, ligand efficiency exploration, and structure-based drug design.[24]

The envisioned modifications were inspired by the cocrystal structure of *Mt*TMPK in complex with **1** (cf. infra), indicating that the thymine ring (ring A) forms a primary binding motif with the enzyme that is very similar to that observed in the crystal structures of substrate-like ligands,[20] including a $\pi-\pi$ stacking interaction with Phe70 and hydrogen bonds between O^4 and N^3 and Arg74 and Asn100, respectively. Rings B, C, and D, however, mainly formed hydrophobic interactions with the enzyme. Therefore, we decided to investigate if the meta-piperidine ring (ring B) could be substituted by either a para-piperidine (**2**), an azetidine (**7**), or an aminoalkyl moiety (**11** and **12**). We also explored variations in the inter-ring space by adding a methylene unit (analogues **3**, **8**, and **9**) or a carbonyl group (analogue **13**) between rings B and C or a methylene group between A and B (analogue **5**). We also investigated the effect of moving the terminal phenoxy ring from the meta- to the para-position (as in **4**, **6**, **10**, and **14**). Next, we tried to optimize the ligand efficiency by removing the distal D-ring, while replacing the ring C with different hetero(aryl) moieties or a cyclohexyl group (analogues **15**−**41**). Quinolin-2-yle analogue **35**, which resulted from this effort, was further optimized to afford a potent *Mt*TMPK inhibitor with a new chemotype (analogues **42**−**44**).

RESULTS AND DISCUSSION

Synthesis. Generally, the synthesis of the envisioned analogues comprised reductive amination of an azaheterocyclic or aminoalkyl N3-BOM-protected thymine derivative with an appropriate aldehyde. Final acidic cleavage of the BOM-group afforded the desired final compounds. The azaheterocyclic (substituted azetidine or piperidine) and aminoalkyl thymine intermediates were prepared via alkylation of thymine with the appropriate mesylates.[24]

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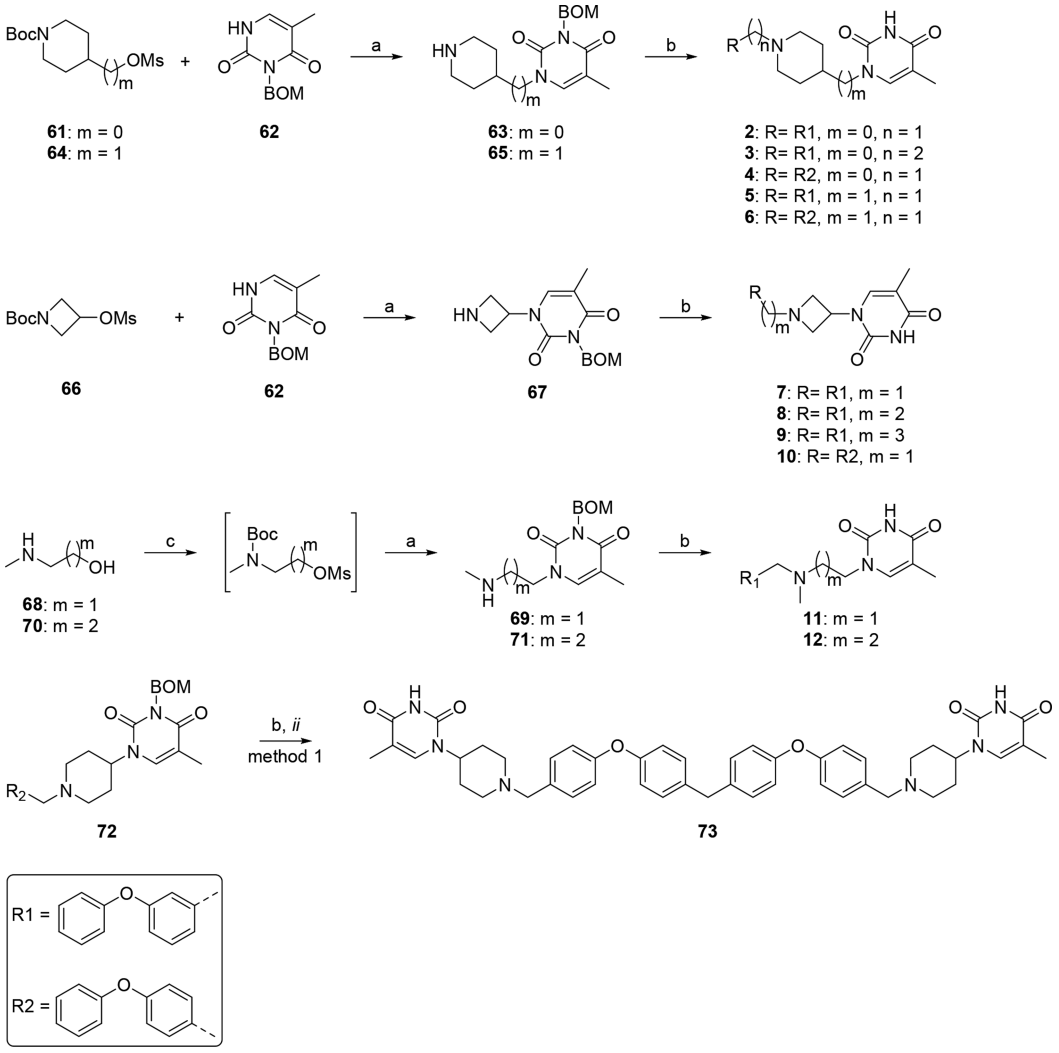
Scheme 2. Synthesis of Azetidinyl, para-Piperidinyl, and Aminoalkyl Analogues and the Structure of Compound 73[a]



[a]Reagents and conditions: (a) (i) K_2CO_3, dry DMF, 80 °C, 3 h (**71**), overnight (**65**), 48 h (**67** and **69**), 4 days (**63**); (ii) 10% TFA/CH_2Cl_2, 2−4 h, rt; (b) (i) substituted aromatic aldehydes, $NaBH(OAc)_3$, dry 1,2-dichloroethane, rt, overnight; (ii) method 1 TFA, 72 °C, 30 min to 1 h; method 2 80% TFA/H_2O, 0.25−0.27 M cysteine, 72 °C, 3−12 h; (c) (i) $(Boc)_2O$, Et_3N, CH_2Cl_2, rt, 2 h; (ii) MsCl, Et_3N, dry dichloromethane, 0 °C, 1 h.

When the required aldehydes were not commercially available, they were prepared as described in Scheme 1. The preparation of aldehyde **50** started with Fischer esterification of 3-hydroxyphenylacetic acid **45**. Chan−Lam coupling with phenylboronic acid and reduction of the resulting product **49** with $LiAlH_4$ and subsequent reoxidation with Dess−Martin furnished the desired aldehyde **50**. Esterification of *m*-coumaric acid **47**, followed by catalytic hydrogenation, yielded propionic ester **48**, which was converted to the required aldehyde **52** via an analogous method. The synthesis of aldehyde **55** was first attempted via Chan−Lam and Ullmann coupling using 2-methylquinolin-4-ol, with either phenylboronic acid or iodobenzene, but both reactions failed to give the desired product. The failure in the Ullmann coupling might be due to the poor solubility of 2-methylquinolin-4-ol, as well as the low nucleophilic character of the quinolone compared to a phenol. However, Ullmann coupling between 4-chloro-2-methylquinoline **53** and phenol generated the substituted phenoxyquinoline **54**, which upon oxidation with selenium dioxide afforded aldehyde **55**. Aldehydes **57** and **60** were synthesized using similar conditions.

Compounds **2**−**12** were synthesized as depicted in Scheme 2. The final TFA catalyzed deprotection step[24] was found to produce the desired products in varying yields. Purification of the final compounds was complicated by the presence of undesired side products with a molecular mass of 2 M + 12. (M represents the exact mass of the deprotected substrates.) Attempts to optimize this deprotection step by lowering the temperature or diminishing the TFA concentration failed. 2D-NMR analysis allowed us to identify the main side product as dimer **73**, which is probably formed by a double electrophilic aromatic substitution reaction of formaldehyde released during the deprotection reaction.[25] To circumvent this problem, we explored the use of cysteine as a formaldehyde scavenger.[26] By using a 0.25 M solution of L-cysteine·HCl in 80% TFA/H_2O, little to no byproduct formation was observed.

The method to access amides **13** and **14** was similar as described earlier for the meta-analogues (Scheme 3).[24]

Enzymatic Evaluation and Structural Analysis. At the onset of this study, we sought to obtain structural information about the possible binding mode of inhibitor **1** into *Mt*TMPK in order to fuel further inhibitor development. To this end, we

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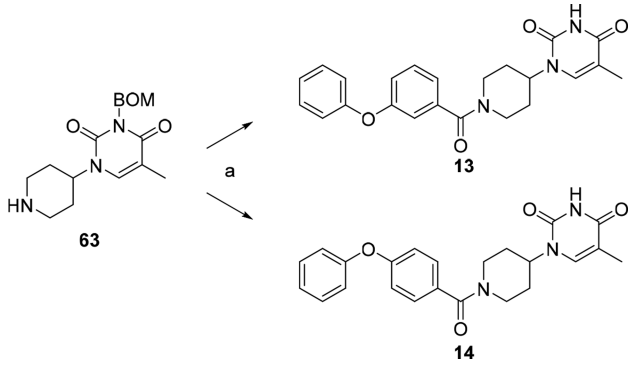
Scheme 3. Synthesis of Amide-Containing para-Piperidinylthymine Analogues[a]



[a]Reagents and conditions: (a) (i) EDC, 4-DMAP, dry CH_2Cl_2, overnight; (ii) H_2, Pd/C, EtOH, 6 h, rt; (iii) THF/H_2O, rt, 4 h.

determined the cocrystal structure of *Mt*TMPK in complex with **1** at 2.85 Å resolution (PDB code: 5NQ5), which reveals the key interaction features. Consistent with its competitive mode of inhibition against the natural substrate dTMP, compound **1** is bound with a high occupancy in the active site of *Mt*TMPK (Figure 1).

Compound **1** adopts a surprising bent conformation that places rings A and B into the active site pocket and rings C and D protruding outward at a right angle (Figure 1A). The thymidine ring (ring A) reaches deep into the catalytic pocket where it is stabilized via hydrophobic interactions between its 5-methyl group and Arg95, Pro37, and Phe36 side chains (Figure 1A and 1B). By analogy to the interactions of thymidine-like TMPK inhibitors,[20] compound **1** also displays $\pi-\pi$ electron stacking interactions between ring A and Phe70 and additional anchoring via hydrogen bonds linking the O^4 and N^3 groups of the thymine ring with Arg74 and Asn100, respectively (Figure 1A and 1B).

The meta-piperidine ring (ring B) adopting a chair conformation, protrudes out of the active site and positions as the deoxyribose ring of dTMP would to establish a π-alkyl interaction with the aromatic side chain of Tyr103 (Figure 1C and 1D) Of interest, the phenyl ring (ring C) unexpectedly bends away from the substrate channel and binds into a newly formed hydrophobic pocket establishing a CH-π electron interaction with Phe70. Binding of the inhibitor is possible through a displacement of the α-helix (α2) bearing Leu52, and this residue adopts a new rotameric conformation to enable hydrophobic interactions between the phenyl ring and neighboring hydrophobic side chains of Ala48, Ala49, and Leu52. The *S*-enantiomer, which was shown to be a more potent inhibitor than the *R*-enantiomer, provides the correct geometry for the ligand to adopt this bent conformation.[24] The diphenyl ether group is further anchored through ring D, which occupies a new binding pocket obtained after the reorientation of the Arg107 side chain and is sandwiched between residues Met66 and Arg107 establishing a cation-π interaction with the guanidinium group of Arg107.

Next our efforts were directed toward the identification of *Mt*TMPK inhibitors with new chemotypes, to increase chances of identifying analogues with whole-cell activity. First we focused on altering ring B, i.e., the meta-substituted piperidine ring (Figure 2).

Regioisomer **2**, obtained by repositioning the thymine ring of **1** from the meta- to the para-position of the piperidine ring,

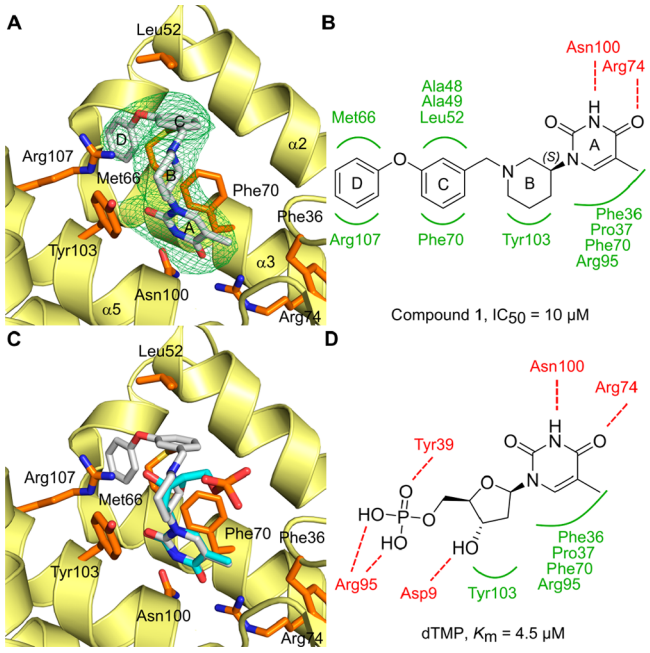


Figure 1. Structural characterization of the binding mode of compound **1** and overlay of compound **1** binding pose with dTMP in the active site of *Mt*TMPK. (A) Cocrystal structure of compound **1** bound to *Mt*TMPK at 2.85 Å resolution. Protein is shown in a pale yellow cartoon representation. Compound **1** (stick representation, carbon atoms in white) and the side chains of *Mt*TMPK interacting residues (stick representation, carbon atoms in orange) are depicted. The corresponding unbiased Fo-Fc difference electron density (contoured to +3 sigma) calculated before adding the ligand in the refinement process is shown as a green mesh. (B) Schematic drawing of compound **1** binding site displaying key *Mt*TMPK interacting residues. (Hydrogen bonds are represented with a red dashed line.) Compound **1** comprises four rings named A–D. (C) Cocrystal structure of *Mt*TMPK in complex with compound **1** showing the binding of the inhibitor (stick representation, carbon atoms in white) in the *Mt*TMPK active site. Protein is depicted in a pale yellow cartoon representation. Crystallographic pose of dTMP (stick representation, carbon atoms in cyan) in *Mt*TMPK (PDB code 1G3U) has been overlaid with compound **1** cocrystal structure for comparison. The superimposition shows a strict conservation of the thymidine core position. (D) Schematic drawing of dTMP binding site displaying key *Mt*TMPK interacting residues. (Hydrogen bonds are represented with a red dashed line.)

displayed a slightly better *Mt*TMPK inhibitory activity than compound **1**. This result is encouraging since compound **2** possesses internal symmetry and is consequently easier to prepare. Moving the terminal phenoxy ring of **2** to the para-position of ring C (compound **4**) increases the IC_{50} by a factor 5. Homologation of the linker between the B- and C-ring afforded compound **3**, which exhibited a marginally decreased inhibitory activity, while adding a methylene between the thymine and piperidine rings led to inactive derivatives **5** and **6**. Replacement of the piperidine by an azetidin-3-yl ring, as in **7**–**10**, generally led to a dramatic drop in inhibitory potency.

Compound **11**, which could be considered as a ring opened form of the meta-piperidine analogue **1**, gave a 7-fold drop in the IC_{50} value, while compound **12**, an acyclic variant of the para-piperidine analogue **2**, was drastically impaired in its inhibitory potency.

Changing the methylene group between rings B and C of inhibitors **2** and **4** by a carbonyl group to produce more rigid

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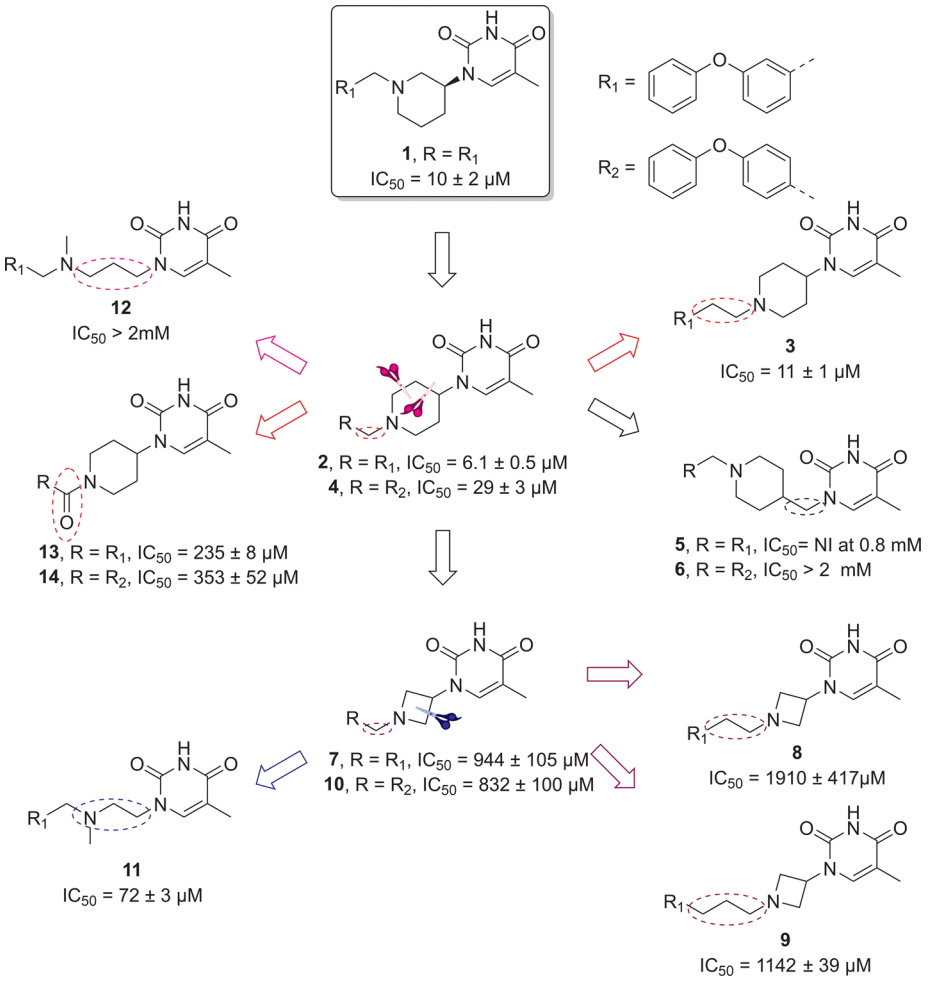

Figure 2. *Mt*TMPK inhibitory activities of the analogues with alternative scaffolds for the meta-substituted B-ring of compound **1**.

amides **13** and **14** was also at the expense of the inhibitory potency.

In an attempt to identify *Mt*TMPK inhibitors with an improved ligand efficiency (LE), we decided to synthesize truncated analogues of the para-substituted piperidine analogue **2** in which the D-ring was omitted and in which we explored alternative C-rings (Figure 3). From this exercise, we deduce that deletion of the terminal phenoxy group decreases the inhibitory potency with a factor 22 (compound **15**). As shown in Figure 3, a planar C-ring is necessary for the enzyme potency (pairs **15**/**32**); however, big aryl rings with improved inhibitory potencies do not yield a better ligand efficiency (pairs **15**/**34**, **29**/**41**). Branching the methylene between rings B and C had no influence on the activity (pairs **15**/**31**).[27] We observed that the inhibitory potency benefits from the introduction of electron-withdrawing substituents on the C-ring (analogues **20**−**22**/**15**). Consistently, electron-poor heterocycles are also favorable (e.g., analogues **27** and **35**). Comparing with other modifications, these electron-deficient substituents also generated active analogues with a high LE value (0.25−0.29), out of which compound **21** emerged as the best.

Using the cocrystal structure of *Mt*TMPK in complex with **1**, modeling studies indicated that introducing a carboxylic acid functionality at C-3 of ring C (**28**) could afford a beneficial electrostatic interaction with Arg107. As shown in Figure 4, the position of Arg107 in the model points to the carbonyl group of ring A instead of the expected carboxylic group of ring C, compared to the position obtained from the cocrystal structure. Analogues that have a similar group or an isosteric tetrazole ring at the C-4 position were also tried (**23** and **24**). However, the introduction of these acidic substituents tends to lower the inhibitory potency and LE (0.20−0.21). This might be due to conformational changes of the active site, which are induced by the carboxylic group and lead to weaker binding.

Since the effect of all of the truncated analogues described in Figure 3 were found to be vastly inferior to **2**, ring D is deemed necessary for good *Mt*TMPK inhibition. Taking into account the synthetic feasibility and the fact that quinolines and quinolones have emerged as promising scaffolds for developing antimycobacterial agents,[28] quinoline derivative **35**, which has a favorable LE of 0.27, was selected for further structural elaboration.

An overlay of the docking poses of compounds **2** and **35** in *Mt*TMPK is represented in Figure 5A. As expected, the thymine and piperidine moieties of **2** and **35** occupy similar positions. The aromatic tails of **2**, however, point toward an additional pocket in the enzyme. This led us to explore if this additional pocket could be occupied by introducing a phenoxy group at position 4 of the quinolone moiety of **35**. The structures of the envisaged 4-phenoxyquinoline derivatives are shown in Figure 5B. Indeed, compound **42** shows a 5-fold improved *Mt*TMPK inhibitory potency compared to analogue **2** and an 8-fold improvement compared to the parent quinoline **35**. Introduction of a chlorine substituent at two different

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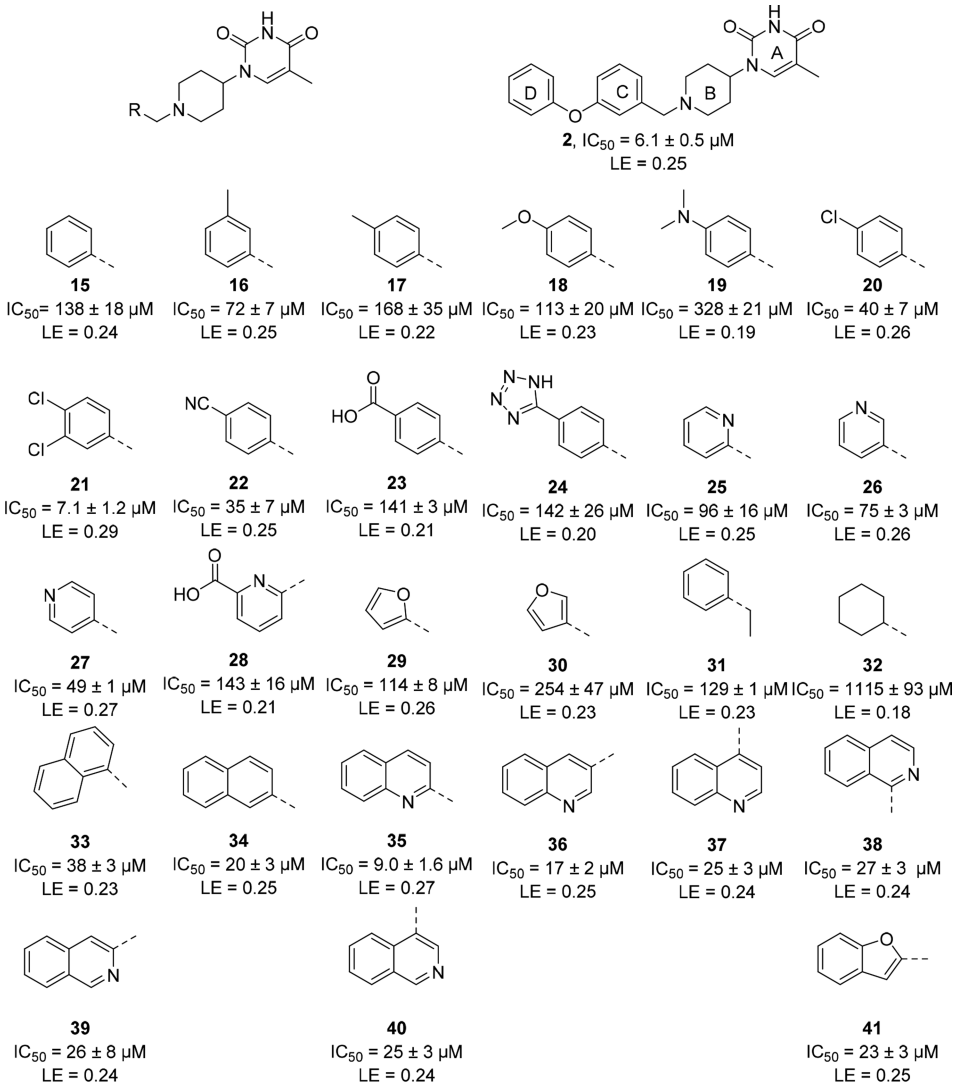

Figure 3. SAR and LE exploration of ring C based on para-piperidinylthymine **2**.

positions of **42** afforded analogues **43** and **44**, of which **43** represents the most potent *Mt*TMPK inhibitor identified so far, bearing this particular scaffold.

The cocrystal structure of *Mt*TMPK in complex with compound **43** at 2.35 Å resolution (PDB code: 5NR7) reveals the key interaction features involved in binding of compound **43** to the enzyme and is consistent with the predicted binding configuration obtained from the docking experiments. Inhibitor **43**, which is bound in the active site of the enzyme, was placed in the residual Fobs-Fcalc electron density and adopts an L shaped conformation (Figure 6).

Consistently with the cocrystal structure of **1**-*Mt*TMPK, ring A adopts an identical pose to that observed in the crystal structures of substrate-like ligands. The para-substituted piperidine ring (ring B) establishes a similar π-alkyl interaction with Tyr103 as observed in the cocrystal structure of **1** but displays a slightly different binding pose. Interestingly, the quinoline aromatic group protrudes linearly to ring B out of the catalytic pocket and is located in a new binding spot establishing a π-alkyl interaction with Leu52 and an edge-to-face π-stacking interaction with His53. Despite the weak ligand electron density, which does not completely support the positioning of ring D in the model, the ether group can be placed with confidence supporting the orientation of ring D that bends in an opposite direction than the one observed for **1**. The chlorobenzene ring is involved in hydrophobic interactions with Ala49 and could establish a parallel-displaced π-stacking interaction with Tyr39.

Antimicrobial Activity and Enzymatic Inhibitory Activity. Next to their ability to inhibit *Mt*TMPK, all final compounds were tested for *M. tuberculosis* growth inhibitory activity. The antimycobacterial activity of meta-piperidinylthymine **1** reported in a previous study turned out to be nonrepeatable (IC_{50} > 64 μM, H37Ra),[24,29] but we found that analogue **42** had a 4-fold stronger antituberculosis activity than compound **1** (H37Rv, Table 1). This result is in line with the enzymatic inhibitory activity, which is 8-fold higher for **42** compared to **1**. Analogue **43** is 33-fold more potent than derivative **42**, which represents the most potent compound in this study so far (H37Ra). All analogues did not display severe cytotoxicity, with only **42** and **43** having some cytotoxicity against MRC-5 fibroblasts. The analogues not included in Table 1 were found to have no antibacterial activity for the H37Ra strain up to 64 μM.

We hypothesized that the discrepancy between the potent enzyme inhibitory activity and the whole-cell antimycobacterial

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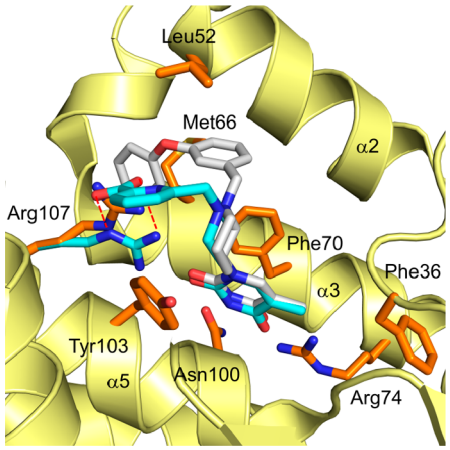

Figure 4. Structure overlay of compound **1** (stick representation, carbon atoms in white) and docking pose of compound **28** (carbon atoms in cyan) in the *Mt*TMPK active site. Protein is shown in a pale yellow cartoon representation. Key *Mt*TMPK interacting residues (stick representation, carbon atoms in orange) with compound **1** are shown: in particular, Arg107 is in close proximity to the ether bridge between rings C and D. For compound **28**, the envisaged favorable electrostatic interaction is shown in a red dashed line between the carboxyl moiety and Arg107 (in stick representation, carbon atoms in cyan) in the docked structure.

activity could be due to poor mycobacterial uptake or due to efflux.

To explore this hypothesis, the MIC of the selected analogues was determined first in the presence of efflux pump inhibitors (at one-fourth of their MIC) and, second, by using a series of *M. tuberculosis* mutants defective in the production of efflux pumps, along with the wild type strain H37Rv. From Table 2, it can be observed that in the presence of the efflux inhibitor verapamil, a calcium channel blocker, the MIC of two compounds (**21** and **35**) markedly decreased in comparison to that for the reference strain H37Rv. In addition, the *M. tuberculosis* mutant defective in the Mmr efflux pump also showed a significant (more than 4-fold change) decrease in the MICs of these two analogues. These results strongly suggest that active efflux plays an important role in the transport of compounds **21** and **35**, so this could be the reason for the discrepancy between antituberculosis activity and enzyme inhibitory activity. For compounds **1**, **42**, and **43**, the decrease in the MICs in the presence of efflux inhibitors is moderate, and no significant change could be detected in the efflux defective mutants. We can conclude that in these cases efflux may play a moderate role in the resistance to these compounds, and in any case, this would not be mediated by Tap or Mmr efflux pumps. Finally, we could not detect any effect of either addition of efflux inhibitors or deletion of gene encoding efflux pumps for compound **24**, indicating that this compound may have retarded uptake due to its negative charged tetrazole ring.

CONCLUSION

Starting from the previously identified hit **1**, scaffold screening and ligand efficiency exploration resulted in two novel chemical series: one series represented by the phenoxylbenzyl analogue **2** and a truncated series represented by quinolin-2-yl analogue **35**. Structure-guided scaffold morphing between compound **2** and **35** furnished phenoxylquinolin-2-yl derivatives with a potent *Mt*TMPK inhibitory activity and improved antibacterial activity. A potential explanation for the observed discrepancy between IC_{50} and MIC values is provided by assessing the antimycobacterial activity in the presence of efflux pump inhibitors or by using mutant *M. tuberculosis* strains deficient in efflux pumps. In particular, the cellular activity of **35** was potentiated 128-fold when tested on the *mmr* efflux pump knockout strain. These observations provide possible directions for further inhibitor development, e.g., by combining the optimized *Mt*TMPK inhibitors with an efflux pump inhibitor, by designing hybrid compounds that feature both an *Mt*TMPK as well as an efflux pump inhibitor motif, or, ideally, by looking for *Mt*TMPK inhibitors that feature structural motifs that facilitate mycobacterial uptake.

EXPERIMENTAL SECTION

Expression and Purification of *Mycobacterium tuberculosis* Thymidylate Kinase (*Mt*TMPK). *Mt*TMPK was expressed and purified as previously described with minor modifications.[21] In short, the pHL50 vector containing the coding sequence of wild-type *Mt*TMPK was used to transform *E. coli* BLi5 competent cells. Transformed cells were selected on LB agar plates containing 100 μg/mL of carbenicillin and 34 μg/mL of chloramphenicol and directly

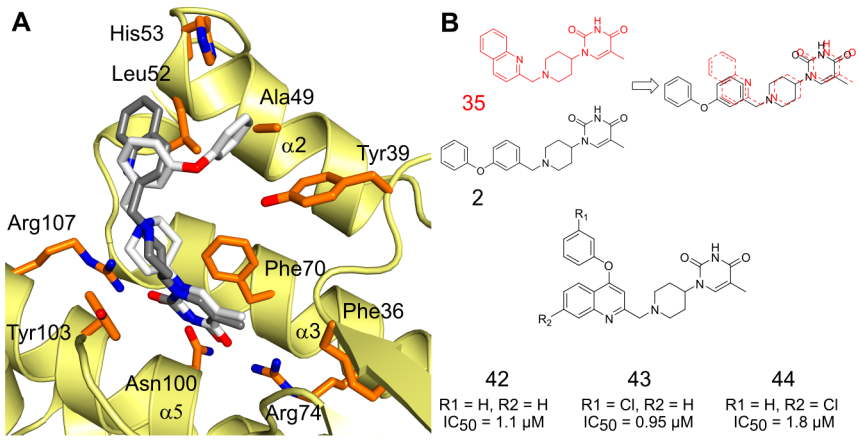

Figure 5. Docking poses of compounds **2** and **35** in the *Mt*TMPK active site. (A) Structural overlay of inhibitors **2** (in stick representation, carbon atoms in white) and **35** (in stick representation, carbon atoms in gray) docked poses in the *Mt*TMPK catalytic pocket. Protein is depicted in a pale yellow cartoon representation. Key interacting residues are represented in stick conformation and colored in orange for carbon atoms. (B) Design of the phenoxyl quinoline series and their corresponding inhibition constants.

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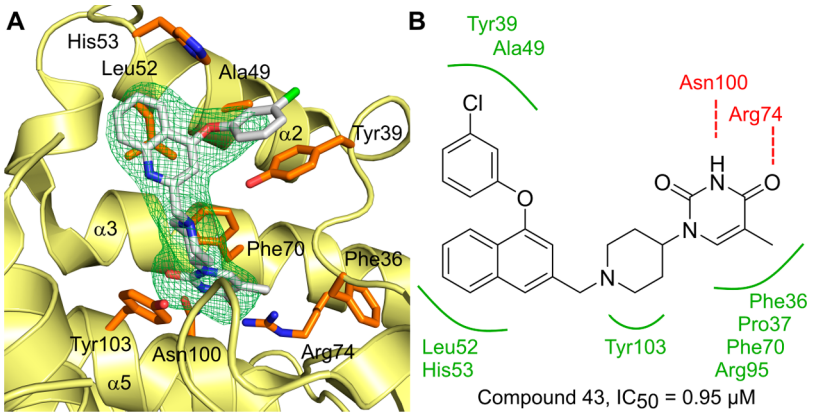

Figure 6. Cocrystal structure of *Mt*TMPK in complex with compound **43** and key interactions with the enzyme. (A). Compound **43** and the side chains of interacting residues of *Mt*TMPK are represented as sticks for carbon atoms and colored in white and orange, respectively. The corresponding unbiased Fo-Fc electron density (contoured to +3 sigma) calculated before adding the ligand in the refinement process is shown as a green mesh. Protein is shown in a pale yellow cartoon representation. (B) Schematic drawing of the compound **43** binding site displaying key *Mt*TMPK residues in direct contact with **43**. (Hydrogen bonds are represented with a red dashed line.)

Table 1. Inhibitory Activity of Compounds against *Mt*TMPK and *M. tuberculosis* cells (H37Ra and H37Rv Strains) and Toxicity against MRC-5 Human Cell Line

compound	IC_{50} (μM) *Mt*MTPK	MIC[a] H37Rv	IC_{50}[b] (μM) H37Ra	IC_{50}[b] (μM) MRC-5
1	10	318.50 (125)	>64	29.5
21	7.1	678.87 (>250)	60.45	47.85
24	142	650.29 (>250)	>64	>64
35	9.0	678.87 (>250)	>64	>64
42	1.1	35.25−70.62 (15.6−31.25)	32.51	8.35
43	0.95	16.35−32.70 (7.8−15.6)	0.96[c]	6.47

[a]MIC values are given in μM, and between brackets they are expressed in μg/mL. [b]IC_{50} values were determined graphically using the plot of the % viability as a function of compound concentration. [c]IC_{99} = 12.17 μM.

used to inoculate 1 L of 2XYT medium supplemented with antibiotics. The cells were allowed to grow at 37 °C, and protein production was induced with 1 mM isopropyl-1-thio-β-D-thiogalactoside when the culture reached an absorbance of 1.5 at 600 nm. After 3 h of incubation at 37 °C, cells were harvested by centrifugation at 8000*g* at 4 °C and stored at −80 °C before proceeding with purification. Cells from 1 L of culture were resuspended in 50 mL of cold lysis buffer containing 50 mM Tris HCl pH 8.0 supplemented with an antiprotease cocktail (Roche) and disrupted by sonication. After centrifugation at 20000*g* for 30 min at 4 °C, the filtered bacterial lysate was injected on a 5 mL Blue-Sepharose column pre-equilibrated with lysis buffer. The column was washed with lysis buffer and most of the protein was eluted with elution buffer containing 50 mM Tris HCl pH 8.0, 1 M NaCl. A second elution was performed using elution buffer containing 50 mM Tris HCl pH 8.0, 2 M NaCl. Fractions containing *Mt*TMPK were pooled and injected on a HiLoad Superdex 75 16/600 column equilibrated with 20 mM Tris HCl pH 7.4, 1 mM EDTA as polishing and desalting steps. *Mt*TMPK fractions were pooled and Tris(2-carboxyethyl)-phosphine (TCEP) was added to 1 mM final concentration before flash-freezing the protein samples in liquid nitrogen.

Enzymatic Assay. The compounds were dissolved in DMSO. The assays were performed at fixed concentrations of ATP (0.5 mM) and dTMP (0.05 mM) and at varying concentrations of tested compound (between 0.0008 and 0.6 mM) using the spectrophotometric assay described by Blondin et al.[30] The reaction medium contains 50 mM Tris-HCl, pH 7.4, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate, and 2 units each of coupling enzymes (lactate dehydrogenase, pyruvate kinase and nucleoside diphosphate kinase). IC_{50} values were calculated using KaleidaGraph. First the data points were plotted and then best-fit concentrations were generated using the following equation: $y = (m_2)/(1 + (x/m_3)^{m_4})$; where y = % inhibition; $m_2 = y_{max}$; $m_3 = IC_{50}$; m_4 = slope of the curve at the midpoint; x = % inhibition.

In Table S1 (Supporting Information), for each compound, column 2 indicates the IC_{50} values in μM, column 3 the number of data points taken into account for the fit, column 4 the number of compound concentrations used to measure the residual TMPKmt activity, column 5 the maximum concentration tested, column 6 the minimum concentration tested, and column 8 the coefficient of determination (R^2).

Protein Crystallization. Cocrystallization conditions of *Mt*TMPK-inhibitor complexes were screened at 20 °C by the sitting-drop vapor-diffusion method using a 1:1 protein/solution volume ratio and available commercial screens. Before each cocrystallization experiment, *Mt*TMPK samples were thawed and concentrated to 6−8 mg/mL. Compound **1** was initially dissolved in

Table 2. Impact of Efflux on Antituberculosis Activity of *Mt*TMPK Inhibitors

	MIC (μg/mL)				
M. tuberculosis strain	H37Rv	H37Rv	H37Rv	KO-Tap	KO-Mmr
efflux inhibitor	none	verapamil	PAβN	none	none
compound **1**	125−31.25	15.6	62.5−31.25	31.25−62.5	31.25−15.63
compound **21**	>250	31.25	>250	>31.25	15.63
compound **24**	>250	>250	>250	>250	>250
compound **35**	250	62.5−31.25	250	125	<1.95
compound **42**	31.25−15.6	7.8	7.8	31.25−15.63	15.63
compound **43**	7.8−15.6	3.9−7.8	7.8−15.6	7.8−15.6	7.8

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pure DMSO at a concentration of 100 mM and diluted to 50 mM using pure isopropanol. Compound **43** was less soluble and initially dissolved in pure DMSO at a concentration of 50 mM and diluted to 25 mM using pure isopropanol. Isopropanol appeared to be crucial in improving solubility of compounds after dilution in protein buffer. Diluted compounds were then added to a concentrated protein stock at a final concentration of 1 mM, and the protein/inhibitor sample was incubated on ice for 1 h before setting up crystallization experiments.

For the *Mt*TMPK/**1** complex, the ligand stock solution at 50 mM in 50/50 (v/v) pure DMSO/pure isopropanol solution was added to the concentrated protein solution stock to reach a final inhibitor concentration of 1 mM. To further increase the solubility of **1**, a 50/50 (v/v) *Mt*TMPK buffer/pure isopropanol solution was added to the protein/inhibitor sample to increase the final isopropanol concentration from 1% (v/v) to 1.5% (v/v). An initial hit leading to bipyramidal crystals was obtained in condition G6 (condition 30) of the crystal screen II (0.1 M HEPES pH 7.5, 10% (w/v) PEG 6000 and 5% (v/v) MPD). The crystals were grown for a few weeks at room temperature and exposed to a cryoprotection buffer obtained by the combination of mother liquor supplemented with 1 mM compound **1** and 10% v/v PEG 400 before being mounted and flash frozen in liquid nitrogen.

Initial efforts to obtain *Mt*TMPK/**43** cocrystals led to crystals with very limited diffraction power. Better crystals were obtained using the cross-seeding approach starting from in-house grown *Mt*TMPK crystals obtained with compound **43** related inhibitors. In short, *Mt*TMPK cocrystals initially grown in 0.1 M HEPES pH 6.8, 4.40 M NaCl were crushed in 50 μL of a stabilizing solution (0.1 M HEPES pH 6.8, 4.50 M NaCl, 1% (v/v) DMSO, 1% (v/v) isopropanol, 0.5 mM compound **43**) using the seed bead kit (Hampton Research), and serial dilutions of seeds with a stabilizing solution were performed. The *Mt*TMPK/**43** sample was prepared as previously described for compound **1** complex but using a lower protein concentration (6.0 mg/mL) and an initial ligand solution at 25 mM in 50/50 (v/v) pure DMSO/pure isopropanol. Crystallization trials were setup at room temperature using the contemporary seeding approach by mixing 0.3 μL of *Mt*TMPK/compound **43** sample with 0.2 μL of a crystallization solution and 0.1 μL of an undiluted or diluted seed stock. The best diffracting crystal was obtained after a few weeks at room temperature using the undiluted seed stock and crystallization solution consisting of 0.1 M HEPES pH 6.8, 4.35 M NaCl. This crystal was exposed to a cryoprotection buffer (mother liquor supplemented with 1 mM compound **43** and 10% v/v ethylene glycol) before being mounted and flash frozen in liquid nitrogen.

Data Collection, Structure Determination, and Refinement. Data collection of *Mt*TMPK/**1** cocrystal was achieved on Proxima1 beamline at Soleil synchrotron (Paris, France) at a wavelength of 0.978 57 Å and a temperature of 100 K on a Dectris Pilatus 6 M pixel detector. Diffraction data from *Mt*TMPK/**43** cocrystal were collected at beamline P14 operated by EMBL Hamburg at the PETRA III storage ring (DESY, Hamburg, Germany) at a wavelength of 0.9763 Å and a temperature of 100 K on a Dectris Pilatus 6 M pixel detector.

All data sets were indexed, processed, and scaled using XDS/XSCALE, and mtz conversion was performed with XDSCONV.[31] Five percent of the randomly selected reflections were kept apart for cross-validation and used for the calculation of Rfree. All structures were solved by molecular replacement with PDB input files 4UNP for *Mt*TMPK/**1** and 4UNR for *Mt*TMPK/**43** complex using MOLREP from the CCP4 suite (Table S1).[32] Structures were further refined by one cycle of rigid-body refinement in BUSTER[33] followed by positional and individual isotropic B-factor refinement in BUSTER and PHENIX.[34] Models were manually improved during the course of the crystallographic refinement using the program COOT.[35] For the *Mt*TMPK/**43**, torsion NCS restraints where used throughout the refinement. Ligand coordinates were generated with the grade Web Server (http://grade.globalphasing.org), which was also used for energetic minimization and restraint generation. Ligand molecules were modeled in sigma-weighted Fo-Fc difference electron density maps in the course of the refinement. TLS refinement with one TLS group definition per chain was applied in the latest stage of the refinement procedure after having all atoms B-factor reset to the Wilson B-factor value. The real-space correlation coefficient (RSCC) is reported as an objective measure of the fit of inhibitor coordinates to electron density and was calculated using the Twilight program.[36] Discovery studio visualizer version 16.1.0 (Dassault Systèmes BIOVIA, San Diego: Dassault Système) was used to analyze and describe the binding mode of inhibitors within the protein. The quality of the final crystal structures was assessed with MOLPROBITY[37] prior to deposition at the PDB database under the codes 5NQ5 (*Mt*TMPK/**1**) and 5NR7 (*Mt*TMPK/**43**). Molecular images were generated with PyMOL (The PyMOL Molecular Graphics System, Schrödinger, LLC). Data collection and refinement statistics are presented in Table S1 (Supporting Information).

Computational Studies. *Ligand Efficiency (LE).* According to eq 1, where HA stands for the number of non-hydrogen atoms of the ligand and pK_i for the negative logarithmic values of its corresponding inhibition constant, ligand efficiency (average binding energy per atom, Δg) values were calculated by using an Excel spreadsheet (Supporting Information).[38,39]

$$\mathrm{LE} = (1.37/\mathrm{HA}) \times \mathrm{pIC}_{50} \quad (1)$$

Docking. All molecular modeling was calculated using the software packages AutoDock 4.2 on Windows Cygwin and AutodockTools-1.5.6. The X-ray structure of compound **1** in complex with *Mt*TMPK (PDB entry 5NQ5) was used in all docking experiments. The 2D chemical structures and PDB files of the ligands were drawn and created using ChemDraw 15 and ChembioDraw 15 separately. The PDBQT file of the ligands and receptor were prepared by AutodockTools-1.5.6, which include atomic partial charges, atom types, and the information on the ligand torsional degrees. For the docking, a default grid spacing of 0.375 Å and 60 × 60 × 60 number of grid points were used, which centered the box on residue Phe70, at the active site of *Mt*TMPK (the coordinates *x*, *y*, *z* were −21.132, 31.018, −12.924 correspondingly). Arg107 and Leu52 surrounding the biphenyl ether tail (ring C and ring D) were set as flexible residues. The other settings such as search parameters and docking parameters were adopted as default. A total of 50 possible conformations were given by Autodock 4.2 for each docking. A manual selection procedure, which combines visual inspection in AutodockTools-1.5.6 guided by Chimera together with the predicted free energy found for each conformation, was used to validate the docked conformations.

In Vitro Antituberculosis Activity. *Mycobacterium tuberculosis* strains used were the reference strain H37Rv,[40,41] and the knockout mutants, KO-Tap and KO-Mmr, were deleted, respectively, in genes encoding the efflux pumps Tap[42] and Mmr.[43]

Mycobacterium tuberculosis cultures were routinely grown at 37 °C in Middlebrook 7H9 broth supplemented with 10% ADC (albumin, dextrose, catalase supplement, Becton-Dickinson) and 0.05% Tween 80. The knockout mutant strains (KO-TAP and KO-Mmr) were grown in the same medium supplemented with hygromycin at 50 μg/mL. The minimum inhibitory concentration (MIC) was determined using the Resazurin Microtiter Assay Plate.[44] In 96-well plates, double of the desired concentrations of the compounds were added in 100 μL of Middlebrook 7H9 broth supplemented with 10% ADC and 0.5% glycerol in a series of 2-fold dilutions, starting from 500 μg/mL. When required, efflux pumps inhibitors were added to the medium, to a final concentration of 40 μg/mL for verapamil, and 15 μg/mL in the case of PAßN. Bacteria were inoculated by adding 100 μL of a suspension of 10^5 cfu/mL, as determined by optical density, and prepared from a culture in the exponential growth phase. Following inoculation, the final concentration of the compounds ranged from 250 μg/mL to 1.95 μg/mL. After 6 days of incubation at 37 °C, 30 μL of resazurin (0.01% w/v) was added to each well, incubated for a further 48 h at 37 °C, and assessed for color change. A change from blue to pink indicated bacterial growth. The MIC is defined as the lowest drug concentration that prevents this color change. Changes in the MIC between strains or experimental conditions were considered as significant when there was at least a 4-fold variation with the reference strain or condition.

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In vitro antimycobacterial activity of the 1,3-diaryltriazenides was further evaluated by a luminometric assay based on a *M. tuberculosis* H37Ra laboratory strain (ATCC 25177) transformed with a pSMT1 luciferase reporter plasmid (H37Ra-*lux*). A 2-fold serial dilution of each compound was made in Middlebrook 7H9 broth and 10% OADC (complete 7H9 broth) with final concentrations ranging from 128 μM to 0.5 μM. Volumes of 100 μL of the serial dilutions were added in triplicate to black, flat-bottomed 96-well plates. As a positive control, isoniazid, a first-line antimycobacterial drug, was included. The mycobacterial suspension was made by thawing a frozen glycerol-stock of H37Ra-*lux* and, subsequently, diluting it in complete 7H9 broth to obtain a suspension with 10.000 relative light units (RLU)/mL. A volume of 100 μL of bacteria was added to each well. All of the outer-perimeter wells were filled with 200 μL of sterile deionized water to minimize evaporation of the medium in the test wells during incubation. After 7 days, the bacterial replication was analyzed by luminometry. To evoke a luminescent signal, 25 μL of 1% *n*-decanal in ethanol was added to each well, where after light emission was measured using a luminometer (Promega Discover).

In Vitro Cytotoxicity Assay. In vitro cytotoxicity on the MRC-5 *Homo sapiens* long fibroblast cell line (ATCC CCL-171) was assessed for each analogue by a NRU assay. The MRC-5 cells were cultured in a 75 cm^2 sterile Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum in a 5% CO_2 atmosphere at 37 °C. When a semiconfluent layer of cells was formed, the cells were trypsinized, washed with sterile PBS, seeded into a transparent, flat-bottomed 96-well plate at a density of 4 × 104 cells per well, and left for recovery at 37 °C, 5% CO_2. For each compound, a 2-fold serial dilution was made in complete DMEM with final concentrations ranging from 128 μM to 0.5 μM. Subsequently, the MRC-5 cells were exposed to the compounds (in triplicate) by adding 100 μL of the serial dilutions to the wells. Test plates were incubated for 24 h in an atmosphere of 5% CO_2 at 37 °C. For the NRU assay, the cells were washed 2 times with 200 μL of PBS and 100 μL of the neutral red working solution was added per well. Subsequently, the plates were left for incubation at 37 °C, 5% CO_2 for 3 h. After incubation, the wells were washed 2 times with 200 μL of PBS and 150 μL of an ethanol/acetic acid mixture was added per well. The plates were left shaking until the color became homogeneous purple, and the optical density was measured at 530 and 620 nm (reference wavelength) using a Promega Discover plate reader.

CHEMISTRY

General. Solvents were purchased from standard commercial sources and were of analytical grade. Building blocks and reagents were used as received without any further purification. TLC analysis was performed using precoated Alugram Silica Gel F254 plates (Machery-Nagel). Spots were examined under ultraviolet light at 254 nm. Column chromatography was carried out on a Reveleris X2 (Grace) automated flash unit using the corresponding disposable silica gel cartridges. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 300/75 MHz spectrometer. Chemical shifts are given in parts per million (ppm δ), δ relative to the residual solvent peak or TMS for ^{1}H and ^{13}C. Structural assignment was confirmed with COSY, HSQC, and HMBC. Exact mass measurements (HRMS) were performed on a Waters LCT Premier XETM time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/H_2O (1:1) mixture at 100 μL/min. Preparative reversed phase HPLC chromatography was carried out using a Phenomenex Luna C-18 (21.2 mm × 250 mm) column using a linear gradient from 10% MeCN to 100% MeCN in a 10 mM ammonium bicarbonate solution over 20 min and a flow rate of 17.5 mL/min. The purity of all final compounds was determined by LC-MS analyses on a Waters Alliance 2695 XE separation module using a Phenomenex Kinetex EVO C18, 5um 100 mm × 2.1 mm column and a gradient system of HCOOH in H_2O (0.1%, v/v)/HCOOH in CH_3CN (0.1%, v/v) at a flow rate of 0.6 mL/min, 05:95 to 0:100 (5 to 100% CH_3CN) in 8 min. All final compounds showed >95% purity.

General Procedure for the Synthesis of Final Compounds. A suspension of azaheterocyclic or aminoalkyl thymine (1 equiv), substituted aromatic aldehyde (1−2 equiv), and sodium triacetoxyborohydride (1.5−4 equiv) in dry 1,2-dichloroethane (~0.03 M) was stirred at room temperature under argon overnight or 48 h. The reaction mixture was evaporated and dried with an oil pump for 0.5 h. The residue was purified by column chromatography (10% ethyl acetate/hexane and 0.8% Et_3N−100% ethyl acetate and 0.8% Et_3N or 100% CH_2Cl_2−10% MeOH/CH_2Cl_2 or 100% ethyl acetate−5% MeOH/ethyl acetate in a linear gradient elution) to afford the pure intermediate, followed by BOM-deprotection. There are two methods for the BOM-deprotection step. For method 1, according to a literature procedure,[45] the intermediate was dissolved with TFA (~0.05 M) under argon. The reaction mixture was stirred at 72 °C for 30 min to 1 h. The reaction progress was monitored by HRMS. Once the starting material was consumed completely, the reaction mixture was cooled to room temperature, concentrated in a vacuum, and dried with an oil pump. The residue was dissolved with the solvent mixture (1−2 mL, MeCN/*t*-BuOH/H_2O, v/v/v = 1/1/1 or acetic acid or H_2O) and purified with preparative HPLC. After lyophilization, all of the products were obtained as white powders. For method 2, the intermediate was dissolved with 80% TFA/H_2O (~0.01 M), and L-cysteine hydrochloride (0.25 or 0.27 M) was added to the reaction mixture. The reaction mixture was stirred at 72 °C for 5−16 h. HRMS was used to monitor the reaction progress. Once the starting material was consumed completely, the reaction mixture was cooled to room temperature and evaporated. The residue was purified with preparative HPLC.

*5-Methyl-1-(1-(3-phenoxybenzyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**2**).* Following the General Procedure for the Synthesis of Final Compounds (method 1), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (86.3 mg, 0.262 mmol), 3-phenoxybenzaldehyde (77.9 mg, 0.393 mmol), and sodium triacetoxyborohydride (111.1 mg, 0.524 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with TFA (8 mL), yielded compound **2** (39.6 mg, 39%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (d, J = 9.38 Hz, 2H, piperidin-3a-yl, piperidin-5a-yl), 1.73−1.93 (m, 5H, piperidin-3b-yl, piperidin-5b-yl, 5-CH_3), 1.98−2.12 (m, 2H, piperidin-2a-yl, piperidin-6a-yl), 2.90 (d, J = 11.43 Hz, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.49 (s, 2H, CH_2, 1-methylene), 4.25 (ddd, J = 12.02, 8.06, 4.25 Hz, 1H, piperidin-4-yl), 6.87 (dd, J = 8.06, 2.49 Hz, 1H, Ph), 6.97−7.03 (m, 3H, Ph), 7.06−7.16 (m, 2H, Ph), 7.30−7.43 (m, 3H, Ph), 7.63 (s, 1H, H-6), 11.18 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00 (5-CH_3), 29.98 (2C, piperidin-3-yl, piperidin-5-yl), 52.33 (3C, piperidin-2-yl, piperidin-6-yl, piperidin-4-yl), 61.23 (1-methylene), 108.93 (C-5), 117.17 (Ph), 118.47 (2C, Ph), 118.78 (Ph), 123.35 (Ph), 123.86 (Ph), 129.75 (Ph), 130.06 (2C, Ph), 137.69 (C-6), 140.92 (Ph), 150.82 (C-2), 156.57 (Ph), 156.75 (Ph), 163.68 (C-4). HRMS (ESI): calcd for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found, 392.1974.

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*5-Methyl-1-(1-(3-phenoxyphenethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**3**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (93.9 mg, 0.285 mmol), 2-(3-phenoxyphenyl)acetaldehyde **50** (120.0 mg, 0.57 mmol), and sodium triacetoxyborohydride (151.0 mg, 0.713 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.88 mmol) to the reaction mixture yielded compound **3** (59.0 mg, 51%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.59−1.69 (m, 2H), 1.72−1.91 (m, 5H), 2.05 (t, J = 10.69 Hz, 2H), 2.53−2.61 (m, 2H), 2.67−2.79 (m, 2H), 3.02 (d, J = 11.72 Hz, 2H), 4.16−4.32 (m, 1H), 6.82 (dt, J = 8.13, 1.21 Hz, 1H), 6.89−6.94 (m, 1H), 6.96−7.05 (m, 3H), 7.09−7.16 (m, 1H), 7.29 (t, J = 7.91 Hz, 1H), 7.34−7.43 (m, 2H), 7.61 (d, J = 1.17 Hz, 1H), 11.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.01, 29.99 (2C), 32.77, 52.39 (2C), 52.48, 59.00, 108.88, 116.12, 118.47 (2C), 118.99, 123.26, 123.86, 129.75, 129.99 (2C), 137.64, 142.83, 150.80, 156.49, 156.75, 163.67. HRMS (ESI): calcd for $[C_{24}H_{27}N_3O_3 + H]^+$, 406.2125; found, 406.2175.

*5-Methyl-1-(1-(4-phenoxybenzyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**4**).* Following the General Procedure for the Synthesis of Final Compounds (method 1), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (80.0 mg, 0.243 mmol), 4-phenoxybenzaldehyde (72.3 mg, 0.365 mmol), and sodium triacetoxyborohydride (103.0 mg, 0.486 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with TFA (10 mL), yielded compound **4** (34.5 mg, 36%) and the corresponding dimer **73** (20.3 mg). Compound **4**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (d, J = 9.37 Hz, 2H, piperidin-3a-yl, piperidin-5a-yl), 1.72−1.92 (m, 5H, piperidin-3b-yl, piperidin-5b-yl, 5-CH_3), 2.04 (t, J = 10.84 Hz, 2H, piperidin-2a-yl, piperidin-6a-yl), 2.91 (d, J = 11.72 Hz, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.46 (s, 2H, CH_2, 1-methylene), 4.19−4.33 (m, 1H, piperidin-4-yl), 6.93−7.01 (m, 4H, Ph), 7.08−7.15 (m, 1H, Ph), 7.26−7.41 (m, 4H, Ph), 7.63 (d, J = 1.17 Hz, 1H, H-6), 11.18 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.99 (5-CH_3), 29.96 (2C, piperidin-3-yl, piperidin-5-yl), 52.26 (2C, piperidin-2-yl, piperidin-6-yl), 52.35 (piperidin-4-yl), 61.01 (CH_2, 1-methylene), 108.92 (C-5), 118.37 (2C, Ph), 118.46 (2C, Ph), 123.33 (Ph), 130.02 (4C, Ph), 130.43 (Ph), 137.70 (C-6), 150.81 (C-2), 155.54 (Ph), 156.78 (Ph), 163.66 (C-4). HRMS (ESI): calcd for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found, 392.1972. Dimer **73**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (m, 4H, piperidin-3a-yl, piperidin-5a-yl), 1.75−1.88 (m, 10H, piperidin-3b-yl, piperidin-5b-yl, 5-CH_3), 2.05 (t, J = 10.70 Hz, 4H, piperidin-2a-yl, piperidin-6a-yl), 2.91 (d, J = 11.73 Hz, 4H, piperidin-2b-yl, piperidin-6b-yl), 3.46 (s, 4H, CH_2, 1-methylene), 3.91 (s, 2H, methylene), 4.19−4.33 (m, 2H, piperidin-4-yl), 6.90−6.97 (m, 8H, Ph), 7.22−7.31 (m, 8H, Ph), 7.63 (d, J = 0.88 Hz, 2H, H-6), 11.18 (s, 2H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00 (2C, 5-CH_3), 29.95 (4C, piperidin-3-yl, piperidin-5-yl), 40.00 (1C, methylene), 52.25 (4C, piperidin-2-yl, piperidin-6-yl), 52.36 (2C, piperidin-4-yl), 61.00 (2C, CH_2, 1-methylene), 108.91 (2C, C-5), 118.10 (4C, Ph), 118.68 (4C, Ph), 130.13 (4C, Ph), 130.39 (4C, Ph), 136.42 (2C, Ph), 137.69 (2C, C-6), 150.80 (2C, C-2), 154.91 (2C, Ph), 155.82 (2C, Ph), 163.65 (2C, C-4). HRMS (ESI): calcd for $[C_{47}H_{50}N_6O_6 + H]^+$, 795.3865; found, 795.3862.

*5-Methyl-1-((1-(3-phenoxybenzyl)piperidin-4-yl)methyl)-pyrimidine-2,4(1H,3H)-dione (**5**).* Following the General Procedure for the Synthesis of Final Compounds (method 1), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-ylmethyl)-pyrimidine-2,4(1*H*,3*H*)-dione **65** (144.2 mg, 0.42 mmol), 3-phenoxybenzaldehyde (166.5 mg, 0.84 mmol), and sodium triacetoxyborohydride (267.1 mg, 1.26 mmol) in dry 1,2-dichloroethane (15 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with TFA (10 mL), yielded compound **5** (68.1 mg, 40%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.08−1.26 (m, 2H, piperidin-3a-yl, piperidin-5a-yl), 1.50 (d, J = 12.01 Hz, 2H, piperidin-3b-yl, piperidin-5b-yl), 1.57−1.71 (m, 1H, piperidin-4-yl), 1.74 (s, 3H, 5-CH_3), 1.88 (t, J = 11.13 Hz, 2H, piperidin-2a-yl, piperidin-6a-yl), 2.78 (d, J = 11.13 Hz, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.44 (s, 2H, CH_2, 1-methylene), 3.50 (d, J = 7.03 Hz, 2H, CH_2, *N*-methylene), 6.82−7.09 (m, 5H, Ph), 7.09−7.18 (m, 1H, Ph), 7.27−7.44 (m, 3H, Ph), 7.48 (s, 1H, H-6), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.94 (5-CH_3), 29.03 (2C, piperidin-3-yl, piperidin-5-yl), 34.91 (piperidin-4-yl), 52.36 (CH_2, *N*-methylene), 52.56 (2C, piperidin-2-yl, piperidin-6-yl), 61.68 (CH_2, 1-methylene), 108.11 (C-5), 117.02 (Ph), 118.47 (Ph), 118.56 (2C, Ph), 123.37 (Ph), 123.64 (Ph), 129.70 (Ph), 130.03 (2C, Ph), 141.00 (Ph), 141.87 (C-6), 151.03 (C-2), 156.63 (Ph), 156.68 (Ph), 164.25 (C-4). HRMS (ESI): calcd for $[C_{24}H_{27}N_3O_3 + H]^+$, 406.2125; found, 406.2130.

*5-Methyl-1-((1-(4-phenoxybenzyl)piperidin-4-yl)methyl)-pyrimidine-2,4(1H,3H)-dione (**6**).* Following the General Procedure for the Synthesis of Final Compounds (method 1), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-ylmethyl)-pyrimidine-2,4(1*H*,3*H*)-dione **65** (171.7 mg, 0.50 mmol), 4-phenoxybenzaldehyde (198.2 mg, 1.0 mmol), and sodium triacetoxyborohydride (317.9 mg, 1.5 mmol) in dry 1,2-dichloroethane (15 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with TFA (10 mL), yielded compound **6** (38.0 mg, 19%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.12−1.31 (m, 2H, piperidin-3a-yl, piperidin-5a-yl), 1.54 (d, J = 11.13 Hz, 2H, piperidin-3b-yl, piperidin-5b-yl), 1.61−1.80 (m, 4H, piperidin-4-yl, 5-CH_3), 1.86−2.09 (m, 2H, piperidin-2a-yl, piperidin-6a-yl), 2.85 (d, J = 10.54 Hz, 2H, piperidin-2b-yl, piperidin-6b-yl), 3.51 (d, J = 6.74 Hz, 4H, 2CH_2, 1-methylene, *N*-methylene), 6.97 (t, J = 9.08 Hz, 4H, Ph), 7.13 (t, J = 7.32 Hz, 1H, Ph), 7.19−7.44 (m, 4H, Ph), 7.48 (H-6), 11.21 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.94 (5-CH_3), 28.74 (2C, piperidin-3-yl, piperidin-5-yl), 34.77 (piperidin-4-yl), 52.25 (CH_2, *N*-methylene), 52.34 (2C, piperidin-2-yl, piperidin-6-yl), 61.18 (CH_2, 1-methylene), 108.15 (C-5), 118.39 (2C, Ph), 118.50 (2C, Ph), 123.35 (Ph), 130.03 (Ph), 130.54 (Ph), 141.84 (C-6), 151.06 (C-2), 155.66 (Ph), 156.75 (Ph), 164.25 (C-4). HRMS (ESI): calcd for $[C_{24}H_{27}N_3O_3 + H]^+$, 406.2125; found, 406.2142.

*5-Methyl-1-(1-(3-phenoxybenzyl)azetidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**7**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)-methyl)-1-(azetidin-3-yl)-3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **67** (90.4 mg, 0.3 mmol), 3-phenoxybenzaldehyde (118.9 mg, 0.6 mmol), and sodium triacetoxyborohydride (127.2 mg, 0.6 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with

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80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **7** (46.9 mg, 43%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.77 (d, J = 0.59 Hz, 3H), 3.19 (t, J = 7.18 Hz, 2H), 3.53 (t, J = 7.76 Hz, 2H), 3.62 (s, 2H), 4.75 (quin, J = 6.66 Hz, 1H), 6.83−6.93 (m, 2H), 6.95−7.07 (m, 3H), 7.08−7.17 (m, 1H), 7.27−7.43 (m, 3H), 7.66 (d, J = 0.88 Hz, 1H), 11.23 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.12, 46.05, 58.89 (2C), 61.49, 108.99, 117.07, 117.94, 118.73 (2C), 123.20, 123.48, 129.86, 130.04 (2C), 137.78, 140.42, 150.69, 156.54, 156.78, 163.85. HRMS (ESI): calcd for $[C_{21}H_{21}N_3O_3 + H]^+$, 364.1656; found, 364.1675.

*5-Methyl-1-(1-(3-phenoxyphenethyl)azetidin-3-yl)-pyrimidine-2,4(1H,3H)-dione (**8**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-1-(azetidin-3-yl)-3-((benzyloxy)-methyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **67** (114.5 mg, 0.38 mmol), 2-(3-phenoxyphenyl)acetaldehyde **50** (120.0 mg, 0.57 mmol), and sodium triacetoxyborohydride (161.1 mg, 0.76 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−methanol eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.32 g, 8.37 mmol) to the reaction mixture yielded compound **8** (38.0 mg, 26%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.79 (d, J = 0.88 Hz, 3H), 2.54−2.62 (m, 2H), 2.63−2.71 (m, 2H), 3.11−3.20 (m, 2H), 3.49−3.59 (m, 2H), 4.74 (quin, J = 6.52 Hz, 1H), 6.81 (ddd, J = 8.05, 2.49, 0.88 Hz, 1H), 6.89−6.93 (m, 1H), 6.95−7.04 (m, 3H), 7.12 (tt, J = 7.36, 1.13 Hz, 1H), 7.29 (t, J = 7.76 Hz, 1H), 7.33−7.43 (m, 2H), 7.69 (d, J = 1.17 Hz, 1H), 11.25 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.10, 33.38, 46.04, 59.03 (2C), 59.50, 108.98, 116.15, 118.42 (2C), 119.05, 123.26, 123.90, 129.67, 129.98 (2C), 137.78, 142.45, 150.69, 156.43, 156.75, 163.85. HRMS (ESI): calcd for $[C_{22}H_{23}N_3O_3 + H]^+$, 378.1812; found, 378.1829.

*5-Methyl-1-(1-(3-(3-phenoxyphenyl)propyl)azetidin-3-yl)-pyrimidine-2,4(1H,3H)-dione (**9**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-1-(azetidin-3-yl)-3-((benzyloxy)-methyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **67** (98.4 mg, 0.327 mmol), 3-(3-phenoxyphenyl)propanal **52** (147.7 mg, 0.653 mmol), and sodium triacetoxyborohydride (138.4 mg, 0.653 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−methanol eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.32 g, 8.37 mmol) to the reaction mixture yielded compound **9** (40.0 mg, 31%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.57 (quin, J = 7.25 Hz, 2H), 1.80 (s, 3H), 2.43 (br s, 2H), 2.58 (t, J = 7.62 Hz, 2H), 3.12 (br s, 2H), 3.53 (t, J = 6.59 Hz, 2H), 4.74 (quin, J = 6.44 Hz, 1H), 6.80 (dd, J = 7.91, 2.05 Hz, 1H), 6.85 (t, J = 1.76 Hz, 1H), 6.95−7.03 (m, 3H), 7.09−7.17 (m, 1H), 7.29 (t, J = 7.91 Hz, 1H), 7.34−7.43 (m, 2H), 7.69 (d, J = 1.17 Hz, 1H), 11.26 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.10, 28.76, 32.56, 46.07, 57.61, 58.98 (2C), 108.96, 115.95, 118.50 (2C), 118.55, 123.29, 123.48, 129.80, 129.98 (2C), 137.81, 144.33, 150.71, 156.57, 156.72, 163.87. HRMS (ESI): calcd for $[C_{23}H_{25}N_3O_3 + H]^+$, 392.1969; found, 392.1962.

*5-Methyl-1-(1-(4-phenoxybenzyl)azetidin-3-yl)pyrimidine-2,4(1H,3H)-dione (**10**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-1-(azetidin-3-yl)-3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **67** (81.4 mg, 0.27 mmol), 4-phenoxybenzaldehyde (107.1 mg, 0.54 mmol), and sodium triacetoxyborohydride (114.5 mg, 0.54 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **10** (39.4 mg, 40%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.81 (d, J = 1.17 Hz, 3H), 3.22 (t, J = 7.32 Hz, 2H), 3.53−3.61 (m, 2H), 3.63 (s, 2H), 4.77 (quin, J = 6.74 Hz, 1H), 6.92−7.03 (m, 4H), 7.09−7.17 (m, 1H), 7.27−7.34 (m, 2H), 7.34−7.43 (m, 2H), 7.71 (d, J = 1.17 Hz, 1H), 11.26 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.10, 46.11, 58.85 (2C), 61.30, 108.98, 118.44 (2C), 118.52 (2C), 123.35, 129.87 (2C), 130.01 (2C), 133.14, 137.84, 150.71, 155.58, 156.74, 163.88. HRMS (ESI): calcd for $[C_{21}H_{21}N_3O_3 + H]^+$, 364.1656; found, 364.1661.

*5-Methyl-1-(3-(methyl(3-phenoxybenzyl)amino)propyl)-pyrimidine-2,4(1H,3H)-dione (**11**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(2-(methylamino)-ethyl)pyrimidine-2,4(1*H*,3*H*)-dione **69** (95.2 mg, 0.3 mmol), 3-phenoxybenzaldehyde (118.9 mg, 0.6 mmol), and sodium triacetoxyborohydride (127.2 mg, 0.6 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.88 mmol) to the reaction mixture yielded compound **11** (71.3 mg, 65%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.66−1.80 (m, 5H), 2.10 (s, 3H), 2.32 (t, J = 6.00 Hz, 2H), 3.44 (br s, 2H), 3.61 (t, J = 7.03 Hz, 2H), 6.85−7.03 (m, 4H), 7.04−7.17 (m, 2H), 7.29−7.42 (m, 3H), 7.43−7.48 (m, 1H), 11.18 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.94, 26.01, 41.53, 45.66, 53.47, 61.01, 108.24, 117.08, 118.56, 118.64 (2C), 123.40, 123.74, 129.72, 130.03 (2C), 141.43, 141.57, 150.82, 156.68 (2C), 164.30. HRMS (ESI): calcd for $[C_{22}H_{25}N_3O_3 + H]^+$, 380.1969; found, 380.1962.

*5-Methyl-1-(2-(methyl(3-phenoxybenzyl)amino)ethyl)-pyrimidine-2,4(1H,3H)-dione (**12**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(3-(methylamino)-propyl)pyrimidine-2,4(1*H*,3*H*)-dione **71** (91.0 mg, 0.3 mmol), 3-phenoxybenzaldehyde (118.9 mg, 0.6 mmol), and sodium triacetoxyborohydride (127.2 mg, 0.6 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.88 mmol) to the reaction mixture yielded compound **12** (61.1 mg, 56%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.72 (3H), 2.24 (br s, 3H), 2.57 (br s, 2H), 3.55 (br s, 2H), 3.75 (br s, 2H), 6.79−7.05 (m, 5H), 7.12 (t, J = 7.32 Hz, 1H), 7.28 (t, J = 7.76 Hz, 1H), 7.32−7.47 (m, 3H), 11.17 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.91, 41.87, 44.51, 54.72, 60.77, 107.75, 117.29, 118.44 (2C), 118.90, 123.29, 123.74, 129.67, 129.99 (2C), 141.93 (2C), 150.91, 156.54, 156.75, 164.28. HRMS (ESI): calcd for $[C_{21}H_{23}N_3O_3 + H]^+$, 366.1812; found, 366.1801.

*5-Methyl-1-(1-(3-phenoxybenzoyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**13**).* According to a literature procedure,[24] to the reaction mixture of 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (148.2 mg, 0.45 mmol) and 3-phenoxybenzoic acid (144.6 mg, 0.675 mmol) in dichloromethane (10 mL) were added 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (139.7 mg,

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0.90 mmol) and 4-dimethylaminopyridine (1.5 mg) at room temperature under argon. The reaction mixture was stirred overnight and diluted with CH_2Cl_2 (50 mL). The mixture was washed with water (50 mL) and brine (50 mL) sequentially. The organic layer was dried over Na_2SO_4 and concentrated in a vacuum. The residue was purified by column chromatography (25% ethyl acetate/hexane−65% ethyl acetate/hexane in a linear gradient elution) to give the amide intermediate, which was subsequently dissolved in EtOH (10 mL), and Pd/C (0.15 g) was added. The reaction was stirred under hydrogen for 6 h; the suspension was filtered, and the filtrate evaporated and was dried with an oil pump vacuum for 0.5 h. The residue was dissolved in a mixture of THF/H_2O (10 mL, v/v = 2/1) and stirred for 4 h. After evaporation, the residue was purified with column chromatography (1% MeOH/CH_2Cl_2−10% MeOH/CH_2Cl_2 in a linear gradient elution). After lyophilization, the desired product **13** was obtained as a white powder (45.0 mg, 25%). ^{1}H NMR (300 MHz, DMSO-d_6, 80 °C): δ 1.63−1.87 (m, 7H, piperidin-3-yl, piperidin-5-yl, 5-CH_3), 2.89−3.02 (m, 2H, piperidin-2a-yl, piperidin-6a-yl), 4.00−4.29 (m, 2H, piperidin-2b-yl, piperidin-6b-yl), 4.47−4.64 (m, 1H, piperidin-4-yl), 7.01−7.12 (m, 4H, Ph), 7.13−7.21 (m, 2H, Ph), 7.37−7.50 (m, 3H, Ph), 7.56 (d, J = 1.17 Hz, 1H, H-6), 10.91 (br s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 60 °C): δ 11.60 (5-CH_3), 29.72 (2C, piperidin-3-yl, piperidin-5-yl), 51.81 (piperidin-4-yl), 108.76 (C-5), 116.29 (Ph), 118.78 (2C, Ph), 119.00 (Ph), 121.19 (Ph), 123.61 (Ph), 129.86 (2C, Ph), 129.93 (Ph), 137.29 (C-6), 137.81 (Ph), 150.48 (C-2), 155.99 (Ph), 156.66 (Ph), 163.32 (C-4), 167.85 (CO). C (piperidin-2-yl) and C (piperidin-6-yl) could not be found. HRMS (ESI): calcd for $[C_{23}H_{23}N_3O_4 + H]^+$, 406.1761; found, 406.1752.

*5-Methyl-1-(1-(4-phenoxybenzoyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**14**).* According to a literature procedure,[24] to 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (148.2 mg, 0.45 mmol) and 4-phenoxybenzoic acid (144.6 mg, 0.68 mmol) in dry dichloromethane (CH_2Cl_2, 10 mL) were added EDC (139.7 mg, 0.90 mmol) and 4-DMAP (1.5 mg) at room temperature under argon. The desired compound **14** was obtained as a white powder (170.0 mg, 93%). ^{1}H NMR (300 MHz, DMSO-d_6, 50 °C): δ 1.74−1.92 (m, 7H), 3.02 (t, J = 11.57 Hz, 2H), 4.19 (br s, 2H), 4.51−4.66 (m, 1H), 7.01−7.11 (m, 4H), 7.15−7.23 (m, 1H), 7.38−7.51 (m, 4H), 7.64 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.61, 30.33 (2C), 30.97 (br s) 44.06 (2C), 52.65, 77.23, 111.39, 118.00 (2C), 119.71 (2C), 124.21, 129.20 (2C), 129.49, 129.98 (2C), 135.69, 150.71, 156.04, 159.29, 163.15, 170.28. HRMS (ESI): calcd for $[C_{23}H_{23}N_3O_4 + H]^+$, 406.1761; found, 406.1764.

*1-(1-Benzylpiperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**15**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), benzaldehyde (84.9 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (28 mL), and the addition of L-cysteine hydrochloride (1.19 g, 7.59 mmol) to the reaction mixture yielded compound **15** (75.1 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (d, J = 9.37 Hz, 2H), 1.73−1.94 (m, 5H), 1.97−2.13 (m, 2H), 2.90 (d, J = 11.42 Hz, 2H), 3.49 (s, 2H), 4.26 (tt, J = 12.08, 3.88 Hz, 1H), 7.21−7.37 (m, 5H), 7.65 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 30.01 (2C), 52.36, 52.40 (2C), 61.75, 108.94, 126.94, 128.15 (2C), 128.80 (2C), 137.70, 138.47, 150.82, 163.68. HRMS (ESI): calcd for $[C_{17}H_{21}N_3O_2 + H]^+$, 300.1707; found, 300.1705.

*5-Methyl-1-(1-(3-methylbenzyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (**16**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), 3-methylbenzaldehyde (96.1 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.10 mmol) to the reaction mixture yielded compound **16** (75.2 mg, 60%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (d, J = 9.37 Hz, 2H), 1.74−1.92 (m, 5H), 1.96−2.10 (m, 2H), 2.30 (s, 3H), 2.90 (d, J = 11.42 Hz, 2H), 3.44 (s, 2H), 4.17−4.34 (m, 1H), 7.03−7.13 (m, 3H), 7.17−7.24 (m, 1H), 7.66 (d, J = 1.17 Hz, 1H), 11.19 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.98, 21.00, 29.99 (2C), 52.40 (3C), 61.79, 108.91, 125.92, 127.55, 128.01, 129.38, 137.17, 137.70, 138.36, 150.82, 163.67. HRMS (ESI): calcd for $[C_{18}H_{23}N_3O_2 + H]^+$, 314.1863; found, 314.1859.

*5-Methyl-1-(1-(4-methylbenzyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (**17**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.40 mmol), 4-methylbenzaldehyde (96.1 mg, 0.80 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.80 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (28 mL), and the addition of L-cysteine hydrochloride (1.19 g, 7.59 mmol) to the reaction mixture yielded compound **17** (101.0 mg, 81%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.62 (d, J = 9.37 Hz, 2H), 1.71−1.90 (m, 5H), 1.94−2.08 (m, 2H), 2.27 (s, 3H), 2.88 (d, J = 11.42 Hz, 2H), 3.42 (s, 2H), 4.24 (tt, J = 12.01, 3.66 Hz, 1H), 7.08−7.21 (m, 4H), 7.64 (d, J = 1.17 Hz, 1H), 11.18 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.89, 20.59, 29.90 (2C), 52.19 (2C), 52.30, 61.38, 108.81, 128.61 (2C), 128.68 (2C), 135.22, 135.84, 137.60, 150.69, 163.56. HRMS (ESI): calcd for $[C_{18}H_{23}N_3O_2 + H]^+$, 314.1863; found, 314.1869.

*1-(1-(4-Methoxybenzyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**18**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (140.0 mg, 0.425 mmol), 4-methoxybenzaldehyde (115.7 mg, 0.85 mmol), and sodium triacetoxyborohydride (180.2 mg, 0.85 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (36 mL), and the addition of L-cysteine hydrochloride (1.53 g, 9.72 mmol) to the reaction mixture yielded compound **18** (99.4 mg, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.63 (d, J = 9.37 Hz, 2H), 1.73−1.91 (m, 5H), 1.94−2.07 (m, 2H), 2.89 (d, J = 11.42 Hz, 2H), 3.41 (s, 2H), 3.74 (s, 3H), 4.17−4.33 (m, 1H), 6.84−6.92 (m, 2H), 7.17−7.25 (m, 2H), 7.64 (d, J = 0.88 Hz, 1H), 11.19 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 29.99 (2C), 52.22 (2C), 52.43, 55.00, 61.15, 108.91, 113.51, 130.03 (2C), 130.22 (2C), 137.70,

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150.82, 158.27, 163.68. HRMS (ESI): calcd for $[C_{18}H_{23}N_3O_3 + H]^+$, 330.1812; found, 330.1806.

*1-(1-(4-(Dimethylamino)benzyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**19**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), 4-(dimethylamino)benzaldehyde (119.4 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (33 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.91 mmol) to the reaction mixture yielded compound **19** (93.0 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.63 (d, J = 9.08 Hz, 2H), 1.71−1.89 (m, 5H), 1.91−2.05 (m, 2H), 2.80−2.95 (m, 8H), 3.35 (s, 2H), 4.24 (tt, J = 11.97, 3.99 Hz, 1H), 6.67 (d, J = 8.79 Hz, 2H), 7.09 (d, J = 8.49 Hz, 2H), 7.64 (s, 1H), 11.18 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 30.03 (2C), 40.25 (2C), 52.19 (2C), 52.50, 61.43, 108.90, 112.15 (2C), 125.63, 129.74 (2C), 137.72, 149.59, 150.82, 163.67. HRMS (ESI): calcd for $[C_{19}H_{26}N_4O_2 + H]^+$, 343.2129; found, 343.2118.

*1-(1-(4-Chlorobenzyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**20**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), 4-chlorobenzaldehyde (112.5 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.10 mmol) to the reaction mixture yielded compound **20** (82.4 mg, 62%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (d, J = 9.67 Hz, 2H), 1.73−1.94 (m, 5H), 2.07 (t, J = 11.42 Hz, 2H), 2.89 (d, J = 11.42 Hz, 2H), 3.49 (s, 2H), 4.26 (tt, J = 12.05, 3.77 Hz, 1H), 7.30−7.42 (m, 4H), 7.64 (d, J = 1.17 Hz, 1H), 11.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 29.95 (2C), 52.27 (2C), 52.31, 60.72, 108.93, 128.12 (2C), 130.54 (2C), 131.45, 137.54, 137.67, 150.80, 163.67. HRMS (ESI): calcd for $[C_{17}H_{20}ClN_3O_2 + H]^+$, 334.1317; found, 334.1319.

*1-(1-(3,4-Dichlorobenzyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**21**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), 3,4-dichlorobenzaldehyde (140.0 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (33 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.91 mmol) to the reaction mixture yielded compound **21** (94.4 mg, 64%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (d, J = 9.37 Hz, 2H), 1.74−1.96 (m, 5H), 2.01−2.16 (m, 2H), 2.89 (d, J = 11.42 Hz, 2H), 3.51 (s, 2H), 4.19−4.34 (m, 1H), 7.31 (dd, J = 8.20, 1.76 Hz, 1H), 7.55−7.62 (m, 2H), 7.66 (d, J = 0.88 Hz, 1H), 11.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.03, 29.99 (2C), 52.30 (3C), 60.11, 108.99, 128.99, 129.41, 130.38, 130.42, 130.91, 137.73, 140.02, 150.85, 163.73. HRMS (ESI): calcd for $[C_{17}H_{19}Cl_2N_3O_3 + H]^+$, 368.0927; found, 368.0917.

*4-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-piperidin-1-yl)methyl) benzointrile (**22**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), 4-formylbenzonitrile (105.0 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.10 mmol) to the reaction mixture yielded compound **22** (91.5 mg, 71%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (d, J = 9.37 Hz, 2H), 1.74−1.96 (m, 5H), 2.03−2.17 (m, 2H), 2.88 (d, J = 11.72 Hz, 2H), 3.59 (s, 2H), 4.27 (tt, J = 12.08, 3.88 Hz, 1H), 7.49−7.57 (m, 2H), 7.65 (d, J = 1.17 Hz, 1H), 7.77−7.84 (m, 2H), 11.19 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 29.98 (2C), 52.24, 52.37 (2C), 60.95, 108.94, 109.71, 118.91, 129.48 (2C), 132.15 (2C), 137.66, 144.73, 150.83, 163.70. HRMS (ESI): calcd for $[C_{18}H_{20}N_4O_2 + H]^+$, 325.1659; found, 325.1667.

*4-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-piperidin-1-yl)methyl)benzoic acid (**23**).* 4-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)piperidin-1-yl)methyl)-benzonitrile **22** (90.0 mg, 0.28 mg) was dissolved in 0.5 M NaOH (11.1 mL) at room temperature. The reaction mixture was heated at 90 °C for 3.5 h. After the mixture cooled to room temperature, acetic acid (0.64 mL) was added to neutralize the solution. After evaporation and drying with an oil pump, the residue was purified with preparative HPLC to yield compound **23** (50.3 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (d, J = 9.37 Hz, 2H), 1.74−1.96 (m, 5H), 2.01−2.16 (m, 2H), 2.91 (d, J = 11.72 Hz, 2H), 3.56 (s, 2H), 4.27 (ddt, J = 11.94, 7.98, 3.95, 3.95 Hz, 1H), 7.42 (d, J = 8.49 Hz, 2H), 7.66 (d, J = 1.17 Hz, 1H), 7.91 (d, J = 8.20 Hz, 2H), 10.86−11.45 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.98, 29.99 (2C) 52.30, 52.43 (2C), 61.30, 108.93, 128.61 (2C), 129.22 (2C), 130.45, 137.70, 143.35, 150.80, 163.67, 167.41. HRMS (ESI): calcd for $[C_{18}H_{21}N_3O_4 + H]^+$, 343.1532; found, 343.1530.

*1-(1-(4-(1H-Tetrazol-5-yl)benzyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**24**).* According to the procedure introduced by Hye Yeon Sagong,[46] the compound 4-((4-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)piperidin-1-yl)methyl)benzonitrile **22** (103.4 mg, 0.319 mmol), NaN_3 (82.9 mg, 1.275 mmol), and acetic acid (32 μL) in dry DMF (5 mL) yielded the crude product, which was purified by preparative HPLC to yield compound **24** (49.1 mg, 40%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.66−2.01 (m, 7H), 2.25 (t, J = 10.98 Hz, 2H), 3.01 (d, J = 11.42 Hz, 2H), 3.69 (s, 2H), 4.31 (ddd, J = 11.94, 8.27, 3.81 Hz, 1H), 7.54 (d, J = 8.20 Hz, 2H), 7.63 (d, J = 0.88 Hz, 1H), 8.02 (d, J = 8.20 Hz, 2H), 11.22 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.03, 29.49, 52.13 (3C), 60.75, 108.98, 124.56, 126.73 (2C), 129.83 (2C), 137.66, 140.02, 150.79, 156.04, 163.67. HRMS (ESI): calcd for $[C_{18}H_{21}N_7O_2 + H]^+$, 368.1829; found, 368.1828.

*5-Methyl-1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**25**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), picolinaldehyde (85.7 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the methanol−ethyl acetate eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (22 mL), and the addition of L-cysteine hydrochloride (0.94 g, 5.94 mmol) to the reaction mixture yielded compound **25** (57.2 mg, 48%). ^{1}H NMR (300 MHz, D_2O): δ 1.73−1.95

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(m, 7H), 2.27−2.44 (m, 2H), 3.09 (d, J = 12.30 Hz, 2H), 4.31−4.47 (m, 1H), 7.41 (ddd, J = 7.62, 5.13, 1.03 Hz, 1H), 7.50 (d, J = 7.91 Hz, 1H), 7.57 (d, J = 1.17 Hz, 1H), 7.89 (td, J = 7.76, 1.76 Hz, 1H), 8.50 (dt, J = 4.98, 0.88 Hz, 1H). ^{13}C NMR (75 MHz, D_2O): δ 12.07, 30.03 (2C), 52.76 (2C), 54.02, 63.26, 111.72, 124.13, 125.87, 138.82, 139.84, 149.02, 153.11, 155.99, 167.33. HRMS (ESI): calcd for $[C_{16}H_{20}N_4O_2 + H]^+$, 301.1659; found, 301.1656.

*5-Methyl-1-(1-(pyridin-3-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**26**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (164.7 mg, 0.5 mmol), nicotinaldehyde (107.1 mg, 1 mmol), and sodium triacetoxyborohydride (212.0 mg, 1 mmol) in dry 1,2-dichloroethane (15 mL) using the methanol−ethyl acetate eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.36 g, 8.64 mmol) to the reaction mixture yielded compound **26** (61.4 mg, 41%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (d, J = 9.37 Hz, 2H), 1.72−1.93 (m, 5H), 2.00−2.15 (m, 2H), 2.89 (d, J = 11.72 Hz, 2H), 3.52 (s, 2H), 4.26 (tt, J = 12.05, 4.06 Hz, 1H), 7.36 (ddd, J = 7.76, 4.83, 0.88 Hz, 1H), 7.64 (d, J = 1.17 Hz, 1H), 7.70 (dt, J = 7.76, 1.83 Hz, 1H), 8.47 (dd, J = 4.69, 1.76 Hz, 1H), 8.51 (d, J = 1.76 Hz, 1H), 10.46 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.03, 29.96 (2C), 52.27 (2C), 52.31, 58.81, 108.96, 123.40, 133.83, 136.53, 137.70, 148.31, 150.04, 150.85, 163.71. HRMS (ESI): calcd for $[C_{16}H_{20}N_4O_2 + H]^+$, 301.1659; found, 301.1656.

*5-Methyl-1-(1-(pyridin-4-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**27**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), isonicotinaldehyde (85.7 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the methanol−ethyl acetate eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.10 mmol) to the reaction mixture yielded compound **27** (58.4 mg, 49%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (d, J = 10.54 Hz, 2H), 1.72−1.99 (m, 5H), 1.99−2.21 (m, 2H), 2.88 (d, J = 11.13 Hz, 2H), 3.53 (s, 2H), 4.27 (t, J = 12.01 Hz, 1H), 7.33 (d, J = 5.27 Hz, 2H), 7.63 (s, 1H), 8.51 (d, J = 5.27 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.06, 30.01 (2C), 52.28, 52.46 (2C), 60.30, 108.99, 123.72 (2C), 137.67, 147.72, 149.55 (2C), 150.92, 163.82. HRMS (ESI): calcd for $[C_{16}H_{20}N_4O_2 + H]^+$, 301.1659; found, 301.1658.

*6-((4-(5-Methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-piperidin-1-yl)methyl)picolinic Acid (**28**).* According to a literature procedure,[47] the suspension of methyl 6-(hydroxymethyl)picolinate (0.42 g, 2.50 mmol) and manganese(IV) dioxide (4.35 g, 50.00 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 3 h. After filtration with Celite, the filtrate was evaporated under reduced pressure, and the residue was purified by column chromatography (100% hexane−30% ethyl acetate/hexane in a linear gradient elution) to offer methyl 3-formylbenzoate as a colorless gel (255.8 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.07 (s, 3H), 8.06 (td, J = 7.76, 0.88 Hz, 1H), 8.13−8.18 (m, 1H), 8.36 (dd, J = 7.62, 1.17 Hz, 1H), 10.19 (d, J = 0.88 Hz, 1H). HRMS (ESI): calcd for $[C_8H_7NO_3 + H]^+$, 166.0499; found, 166.0482.

Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (88.4 mg, 0.268 mmol), methyl 3-formylbenzoate (66.6 mg, 0.403 mmol), and sodium triacetoxyborohydride (113.6 mg, 0.536 mmol) in dry 1,2-dichloroethane (6 mL) using the ethyl acetate−methanol eluent system obtained the picolinate intermediate, which was dissolved in THF (8 mL). NaOH (53.6 mg, 2.68 mmol) in H_2O (4 mL) was added to the reaction mixture at room temperature. The solution was stirred at room temperature for 1 h. Two M HCl was used to neutralize the pH to 3. After extraction with CH_2Cl_2 (20 mL × 5), the combined organic layer was concentrated to offer the picolinic acid intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and L-cysteine hydrochloride (1.28 g, 8.1 mmol) was added to the reaction mixture to yield compound **28** (54.8 mg, 51%). ^{1}H NMR (300 MHz, D_2O): δ 1.79 (s, 3H), 2.01−2.32 (m, 4H), 3.28 (t, J = 11.72 Hz, 2H), 3.61 (d, J = 12.30 Hz, 2H), 4.46 (s, 2H), 4.56−4.65 (m, 1H), 7.43 (s, 1H), 7.50 (dd, J = 5.57, 2.34 Hz, 1H), 7.87−7.96 (m, 2H). ^{13}C NMR (75 MHz, D_2O): δ 10.64, 26.15 (2C), 50.30, 51.46 (2C), 59.38, 110.64, 123.46, 125.26, 137.92, 138.67, 147.95, 151.31, 152.21, 165.65, 171.38. HRMS (ESI): calcd for $[C_{17}H_{20}N_4O_4 + H]^+$, 345.1557; found, 345.1554.

*1-(1-(Furan-2-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**29**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (140.0 mg, 0.425 mmol), furan-2-carbaldehyde (81.7 mg, 0.85 mmol), and sodium triacetoxyborohydride (180.2 mg, 0.85 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.10 mmol) to the reaction mixture yielded compound **29** (84.0 mg, 68%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.64 (dd, J = 11.57, 2.20 Hz, 1H), 1.74−1.92 (m, 5H), 2.02−2.13 (m, 1H), 2.92 (d, J = 11.72 Hz, 2H), 3.51 (s, 2H), 4.23 (tt, J = 12.12, 3.99 Hz, 1H), 6.28 (dd, J = 3.08, 0.73 Hz, 1H), 6.40 (dd, J = 3.22, 1.76 Hz, 1H), 7.58 (dd, J = 2.05, 0.88 Hz, 1H), 7.64 (d, J = 1.17 Hz, 1H), 11.19 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.03, 29.87 (2C), 51.99 (2C), 52.21, 53.67, 108.58, 108.93, 110.29, 137.72, 142.33, 150.82, 151.93, 163.68. HRMS (ESI): calcd for $[C_{15}H_{19}N_3O_3 + H]^+$, 290.1499; found, 290.1495.

*1-(1-(Furan-3-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**30**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), furan-3-carbaldehyde (76.9 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (25 mL), and the addition of L-cysteine hydrochloride (1.06 g, 6.75 mmol) to the reaction mixture yielded compound **30** (63.1 mg, 55%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.57−1.70 (m, 2H), 1.73−1.92 (m, 5H), 1.94−2.07 (m, 2H), 2.93 (d, J = 11.72 Hz, 2H), 3.34 (s, 2H), 4.16−4.30 (m, 1H), 6.41 (dd, J = 1.76, 0.59 Hz, 1H), 7.56 (d, J = 0.88 Hz, 1H), 7.61 (t, J = 1.61 Hz, 1H), 7.62−7.65 (m, 1H), 7.63 (d, J = 1.17 Hz, 1H), 8.62−9.54 (m, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.01, 29.93 (2C), 51.79, 52.13 (2C), 52.40, 108.90, 111.45, 121.68, 137.70,

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140.89, 143.28, 150.82, 163.67. HRMS (ESI): calcd for $[C_{15}H_{19}N_3O_3 + H]^+$, 290.1499; found, 290.1494.

*5-Methyl-1-(1-(1-phenylethyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (**31**).* According to a modified literature procedure,[48] a mixture of 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (148.2 mg, 0.45 mmol) and acetophenone (64.9 mg, 0.54 mmol) in dry THF (5 mL) at room temperature was treated with Ti(O*i*-Pr)$_4$ (255.8 mg, 0.9 mmol) under argon. The reaction mixture was heated at 75 °C overnight. After the mixture cooled to room temperature, $NaBH_4$ (68.95 mg, 1.35 mmol) was added to the reaction mixture, and it was stirred further at room temperature for 24 h, followed by dilution with CH_2Cl_2 (20 mL) and filtration. The filtrate was evaporated and purified by column chromatography (0.8% Et_3N/10% ethyl acetate/hexane−0.8% Et_3N/50% ethyl acetate/hexane) to offer the intermediate as a yellow gel (160.0 mg, 82%), which followed the general BOM-deprotecting procedure. (See the General Procedure for the Synthesis of Final Compounds, method 2.) The obtained intermediate was dissolved with 80% TFA/H_2O (32 mL), and L-cysteine hydrochloride (1.36 g, 8.64 mmol) was added to the reaction mixture to offer compound **31** (92.5 mg, 80%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.30 (d, J = 6.74 Hz, 3H), 1.55−2.07 (m, 9H), 2.85 (dd, J = 11.13, 1.76 Hz, 1H), 3.06 (d, J = 10.84 Hz, 1H), 3.51 (q, J = 6.74 Hz, 1H), 4.08−4.25 (m, 1H), 7.19−7.28 (m, 1H), 7.29−7.37 (m, 4H), 7.64 (d, J = 0.88 Hz, 1H), 11.17 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.98, 18.94, 30.21, 30.25, 49.00, 49.58, 52.59 (s, 1C), 63.09, 108.90, 126.77, 127.34 (2C), 128.09 (2C), 137.70, 143.41, 150.80, 163.67. HRMS (ESI): calcd for $[C_{18}H_{23}N_3O_2 + H]^+$, 314.1863; found, 314.1861.

*1-(1-(Cyclohexylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**32**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (167.6 mg, 0.5 mmol), cyclohexanecarbaldehyde (112.2 mg, 1 mmol), and sodium triacetoxyborohydride (212.0 mg, 1 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.36 g, 8.64 mmol) to the reaction mixture yielded compound **32** (90.0 mg, 59%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.73−0.91 (m, 2H), 1.07−1.29 (m, 3H), 1.36−1.53 (m, 1H), 1.56−1.99 (m, 14 H), 2.02−2.14 (m, 2H), 2.89 (d, J = 11.13 Hz, 2H), 4.22 (tt, J = 11.64, 3.88 Hz, 1H), 7.63 (d, J = 1.17 Hz, 1H), 11.17 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.97, 25.54 (2C), 26.39, 30.15 (2C), 31.28 (2C), 34.83, 52.54, 53.04 (2C), 64.46, 108.91, 137.69, 150.82, 163.67. HRMS (ESI): calcd for $[C_{17}H_{27}N_3O_2 + H]^+$, 306.2176; found, 306.2155.

*5-Methyl-1-(1-(naphthalen-1-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**33**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (164.7 mg, 0.5 mmol), 1-naphthaldehyde (156.2 mg, 1 mmol), and sodium triacetoxyborohydride (212.0 mg, 1 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.10 mmol) to the reaction mixture yielded compound **33** (85.0 mg, 49%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.57−1.69 (m, 2H), 1.70−1.89 (m, 5H), 2.07−2.22 (m, 2H), 2.99 (d, J = 11.72 Hz, 2H), 3.89 (s, 2H), 4.28 (tt, J = 12.05, 3.77 Hz, 1H), 7.40−7.47 (m, 2H), 7.47−7.58 (m, 2H), 7.60 (d, J = 0.88 Hz, 1H), 7.79−7.87 (m, 1H), 7.87−7.95 (m, 1H), 8.25 (dd, J = 8.49, 1.17 Hz, 1H), 11.18 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.94, 30.06 (2C), 52.56, 52.71 (2C), 59.85, 108.90, 124.77, 125.17, 125.63, 125.81, 127.35, 127.72, 128.24, 132.03, 133.46, 134.16, 137.69, 150.82, 163.67. HRMS (ESI): calcd for $[C_{21}H_{23}N_3O_2 + H]^+$, 350.1863; found, 350.1864.

*5-Methyl-1-(1-(naphthalen-2-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**34**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), 2-naphthaldehyde (124.9 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.36 g, 8.64 mmol) to the reaction mixture yielded compound **34** (100.0 mg, 72%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.66 (d, J = 9.67 Hz, 2H), 1.75−1.98 (m, 5H), 2.03−2.19 (m, 2H), 2.96 (d, J = 11.72 Hz, 2H), 3.65 (s, 2H), 4.29 (tt, J = 12.01, 3.95 Hz, 1H), 7.44−7.55 (m, 3H), 7.67 (d, J = 1.17 Hz, 1H), 7.80 (s, 1H), 7.85−7.93 (m, 3H), 11.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 30.03 (2C), 52.42, 52.48 (2C), 61.87, 108.93, 125.63 (2C), 126.06, 127.09, 127.28, 127.51, 127.66, 132.27, 132.86, 136.22, 137.70, 150.82, 163.67. HRMS (ESI): calcd for $[C_{21}H_{23}N_3O_2 + H]^+$, 350.1863; found, 350.1862.

*5-Methyl-1-(1-(quinolin-2-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**35**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), quinoline-2-carbaldehyde (125.7 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the methanol−ethyl acetate eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (36 mL), and the addition of L-cysteine hydrochloride (1.53 g, 9.72 mmol) to the reaction mixture yielded compound **35** (85.3 mg, 61%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.60−1.71 (m, 2H), 1.73−2.00 (m, 5H), 2.14−2.30 (m, 2H), 2.95 (d, J = 11.42 Hz, 2H), 3.79 (s, 2H), 4.30 (tt, J = 12.19, 4.06 Hz, 1H), 7.53−7.61 (m, 1H), 7.62−7.69 (m, 2H), 7.73 (ddd, J = 8.49, 6.88, 1.32 Hz, 1H), 7.91−8.01 (m, 2H), 8.34 (d, J = 8.20 Hz, 1H), 11.20 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.01, 30.04, 52.28, 52.75 (2C), 64.00, 108.94, 121.00, 126.16, 126.97, 127.80, 128.53, 129.46, 136.31, 137.70, 147.00, 150.83, 159.64, 163.70. HRMS (ESI): calcd for $[C_{20}H_{22}N_4O_2 + H]^+$, 351.1816; found, 351.1825.

*5-Methyl-1-(1-(quinolin-3-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**36**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (98.8 mg, 0.3 mmol), quinoline-3-carbaldehyde (94.3 mg, 0.6 mmol), and sodium triacetoxyborohydride (127.2 mg, 0.6 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−methanol eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **36** (46.7 mg, 44%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.67 (d, J = 9.37 Hz, 2H), 1.74−1.97 (m, 5H),

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2.08−2.23 (m, 2H), 2.97 (d, J = 11.42 Hz, 2H), 3.72 (s, 2H), 4.21−4.37 (m, 1H), 7.58−7.64 (m, 1H), 7.66 (d, J = 1.17 Hz, 1H), 7.74 (m, 1H), 7.97 (dd, J = 8.20, 1.17 Hz, 1H), 8.02 (d, J = 8.49 Hz, 1H), 8.22 (d, J = 1.76 Hz, 1H), 8.88 (d, J = 2.05 Hz, 1H), 11.19 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.00, 29.96 (2C), 52.36 (3C), 59.06, 108.91, 126.68, 127.45, 127.83, 128.67, 129.11, 131.38, 135.12, 137.69, 146.92, 150.80, 151.98, 163.65. HRMS (ESI): calcd for $[C_{20}H_{22}N_4O_2 + H]^+$, 351.1816; found, 351.1826.

*5-Methyl-1-(1-(quinolin-4-ylmethyl)piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**37**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1*H*,3*H*)-dione **63** (92.2 mg, 0.28 mmol), isoquinoline-4-carbaldehyde (88.0 mg, 0.56 mmol), and sodium triacetoxyborohydride (118.7 mg, 0.56 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **37** (51.0 mg, 52%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.67 (d, J = 9.67 Hz, 2H), 1.73−1.95 (m, 5H), 2.17−2.30 (m, 2H), 3.00 (d, J = 11.42 Hz, 2H), 3.98 (s, 2H), 4.23−4.40 (m, 1H), 7.52 (d, J = 4.39 Hz, 1H), 7.59−7.68 (m, 2H), 7.77 (m, 1H), 8.04 (dd, J = 8.20, 0.88 Hz, 1H), 8.29 (dd, J = 8.49, 0.88 Hz, 1H), 8.86 (d, J = 4.39 Hz, 1H), 11.21 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.95, 30.03 (2C), 52.37, 52.79 (2C), 58.10, 108.91, 121.42, 124.61, 126.32, 127.13, 129.12, 129.43, 137.66, 143.95, 147.90, 150.14, 150.82, 163.67. HRMS (ESI): calcd for $[C_{20}H_{22}N_4O_2 + H]^+$, 351.1816; found, 351.1807.

*1-(1-(Isoquinolin-1-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-diimine (**38**).* According to a literature procedure,[49] the suspension of 1-methylisoquinoline (286.4 mg, 2 mmol) and SeO_2 (443.8 mg, 4 mmol) in 1,4-dioxane (10 mL) was heated at 80 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered through Celite. The filtrate was evaporated and purified with column chromatography (100% hexane−10% ethyl acetate/hexane) to offer compound isoquinoline-1-carbaldehyde as a white solid (220.0 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73−7.80 (m, 2H), 7.88−7.95 (m, 2H), 8.77 (d, J = 5.57 Hz, 1H), 9.30−9.37 (m, 1H), 10.38−10.42 (m, 1H). HRMS (ESI): calcd for $[C_{10}H_7NO + H]^+$, 158.0600; found, 158.0604.

Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (82.3 mg, 0.25 mmol), isoquinoline-1-carbaldehyde (78.6 mg, 0.5 mmol), and sodium triacetoxyborohydride (106.0 mg, 0.5 mmol) in dry 1,2-dichloroethane (5 mL) using the ethyl acetate−methanol eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **38** (37.7 mg, 43%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.58−1.86 (m, 7H), 2.14−2.31 (m, 2H), 3.01 (d, J = 11.72 Hz, 2H), 4.07 (s, 2H), 4.21−4.36 (m, 1H), 7.59 (d, J = 1.17 Hz, 1H), 7.65−7.73 (m, 1H), 7.74−7.81 (m, 2H), 7.96 (d, J = 7.91 Hz, 1H), 8.41 (d, J = 5.86 Hz, 1H), 8.48 (d, J = 7.91 Hz, 1H), 11.19 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.89, 30.01 (2C), 52.43, 52.85 (2C), 62.28, 108.87, 120.41, 126.44, 126.96, 127.08, 127.16, 130.10, 135.86, 137.67, 141.25, 150.80, 157.81, 163.64. HRMS (ESI): calcd for $[C_{20}H_{22}N_4O_2 + H]^+$, 351.1816; found 351.1823.

*1-(1-(Isoquinolin-3-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**39**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (92.2 mg, 0.28 mmol), isoquinoline-3-carbaldehyde (88.0 mg, 0.56 mmol), and sodium triacetoxyborohydride (118.7 mg, 0.56 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **39** (44.2 mg, 45%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.67 (d, J = 9.37 Hz, 2H), 1.75−2.03 (m, 5H), 2.21 (t, J = 10.98 Hz, 2H), 3.03 (d, J = 11.72 Hz, 2H), 3.79 (s, 2H), 4.22−4.38 (m, 1H), 7.60−7.70 (m, 2H), 7.73−7.84 (m, 2H), 7.91−7.98 (m, 1H), 8.11 (d, J = 7.91 Hz, 1H), 9.27 (s, 1H), 11.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.01, 30.06 (2C), 52.39, 52.60 (2C), 63.24, 108.91, 118.36, 126.32, 126.99, 127.23, 127.46, 130.56, 135.67, 137.72, 150.82, 151.76, 151.90, 163.67. HRMS (ESI): calcd for $[C_{20}H_{22}N_4O_2 + H]^+$, 351.1816; found 351.1829.

*1-(1-(Isoquinolin-4-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**40**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (92.2 mg, 0.28 mmol), isoquinoline-4-carbaldehyde (88.0 mg, 0.56 mmol), and sodium triacetoxyborohydride (118.7 mg, 0.56 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **40** (49.1 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.65 (d, J = 9.00 Hz, 2H), 1.71−1.89 (m, 5H), 2.18 (t, J = 10.84 Hz, 2H), 3.00 (d, J = 11.42 Hz, 2H), 3.89 (s, 2H), 4.21−4.38 (m, 1H), 7.61 (s, 1H), 7.66−7.74 (m, 1H), 7.78−7.87 (m, 1H), 8.14 (d, J = 8.20 Hz, 1H), 8.29 (d, J = 8.49 Hz, 1H), 8.42 (s, 1H), 9.25 (s, 1H), 11.19 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.92, 29.99 (2C), 52.48, 52.60 (2C), 56.88, 108.87, 124.00, 127.19, 127.92, 128.09, 130.38, 134.57, 137.67, 143.41, 150.80, 152.43, 163.65. HRMS (ESI): calcd for $[C_{20}H_{22}N_4O_2 + H]^+$, 351.1816; found, 351.1817.

*1-(1-(Benzofuran-2-ylmethyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**41**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (131.8 mg, 0.4 mmol), benzofuran-2-carbaldehyde (116.9 mg, 0.8 mmol), and sodium triacetoxyborohydride (169.6 mg, 0.8 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (33 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.91 mmol) to the reaction mixture yielded compound **41** (120.0 mg, 88%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.66 (d, J = 9.67 Hz, 2H), 1.76 (d, J = 1.17 Hz, 3H), 1.88 (qd, J = 12.16, 3.66 Hz, 2H), 2.09−2.25 (m, 2H), 3.00 (d, J = 11.72 Hz, 2H), 3.70 (s, 2H), 4.26 (ddd, J = 12.16, 8.20, 3.95 Hz, 1H), 6.78 (d, J = 0.88 Hz, 1H), 7.17−7.31 (m, 2H), 7.51−7.62 (m, 2H), 7.66 (d, J = 1.17 Hz, 1H), 10.46 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 11.92, 29.80 (2C), 52.04, 52.11 (2C), 53.99, 105.22, 108.81, 110.82, 120.73, 122.61, 123.83, 127.89, 137.64, 150.69, 154.22, 155.14, 163.56. HRMS (ESI): calcd for $[C_{19}H_{21}N_3O_3 + H]^+$, 340.1656; found, 340.1661.

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*5-Methyl-1-(1-((4-phenoxyquinolin-2-yl)methyl)piperidin-4-yl)pyrimidine-2,4(1H,3H)-dione (**42**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), the suspension of 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (114.5 mg, 0.35 mmol), 4-phenoxyquinoline-2-carbaldehyde **55** (130.0 mg, 0.52 mmol), and sodium triacetoxyborohydride (259.7 mg, 1.23 mmol) in dry 1,2-dichloroethane (5 mL) was stirred at room temperature for 48 h (ethyl acetate−hexane eluent system) to give the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **42** (75.6 mg, 49%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.52−1.85 (m, 7H), 2.09−2.23 (m, 2H), 2.88 (d, J = 11.42 Hz, 2H), 3.69 (s, 2H), 4.17−4.32 (m, 1H), 6.83 (s, 1H), 7.27−7.39 (m, 3H), 7.49−7.67 (m, 4H), 7.81 (m, 1H), 8.00 (d, J = 8.20 Hz, 1H), 8.26 (dd, J = 8.35, 1.03 Hz, 1H), 11.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.03, 29.86 (2C), 52.33, 52.53 (2C), 63.93, 104.12, 108.90, 120.15, 120.55 (2C), 121.35, 125.49, 126.07, 128.51, 130.30, 130.54 (2C), 137.54, 148.65, 150.77, 154.33, 160.77, 161.04, 163.64. HRMS (ESI): calcd for $[C_{26}H_{26}N_4O_3 + H]^+$, 443.2078; found, 443.2071.

*1-(1-((4-(3-Chlorophenoxy)quinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4(1H,3H)-dione (**43**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (82.3 mg, 0.25 mmol), 4-(3-chlorophenoxy)quinoline-2-carbaldehyde **57** (141.9 mg, 0.5 mmol), and sodium triacetoxyborohydride (106.0 mg, 0.5 mmol) in dry 1,2-dichloroethane (10 mL) using the ethyl acetate−methanol eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (30 mL), and the addition of L-cysteine hydrochloride (1.28 g, 8.1 mmol) to the reaction mixture yielded compound **43** (32.3 mg, 27%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.55−1.89 (m, 7H), 2.16 (t, J = 10.84 Hz, 2H), 2.91 (d, J = 11.72 Hz, 2H), 3.72 (s, 2H), 4.19−4.33 (m, 1H), 6.92 (s, 1H), 7.29 (ddd, J = 8.20, 2.34, 0.88 Hz, 1H), 7.42 (ddd, J = 7.98, 1.98, 0.88 Hz, 1H), 7.46−7.67 (m, 4H), 7.78−7.85 (m, 1 H) 8.01 (d, J = 7.91 Hz, 1H), 8.21 (dd, J = 8.35, 1.03 Hz, 1H), 11.21 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.06, 29.96 (2C), 52.21, 52.48 (2C), 63.75, 104.69, 108.90, 119.16, 120.09, 120.65, 121.29, 125.48, 126.22, 128.54, 130.41, 131.93, 134.27, 137.43, 148.72, 150.76, 155.35, 160.40, 160.95, 163.62. HRMS (ESI): calcd for $[C_{26}H_{25}ClN_4O_3 + H]^+$, 477.1688; found, 477.1682.

*1-(1-((7-Chloro-4-phenoxyquinolin-2-yl)methyl)piperidin-4-yl)-5-methylpyrimidine-2,4 (1H,3H)-dione (**44**).* Following the General Procedure for the Synthesis of Final Compounds (method 2), 3-((benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)pyrimidine-2,4(1*H*,3*H*)-dione **63** (90.0 mg, 0.273 mmol), 7-chloro-4-phenoxyquinoline-2-carbaldehyde **60** (120.0 mg, 0.423 mmol), and sodium triacetoxyborohydride (115.7 mg, 0.546 mmol) in dry 1,2-dichloroethane (8 mL) using the ethyl acetate−hexane eluent system to obtain the intermediate, which was dissolved with 80% TFA/H_2O (32 mL), and the addition of L-cysteine hydrochloride (1.40 g, 8.88 mmol) to the reaction mixture yielded compound **44** (54.0 mg, 41%). ^{1}H NMR (300 MHz, pyridine-d_5): δ 1.73−1.89 (m, 4H), 2.03 (d, J = 0.88 Hz, 3H), 2.14 (td, J = 11.50, 2.78 Hz, 2H), 2.89 (d, J = 11.72 Hz, 2H), 3.79 (s, 2H), 4.60−4.75 (m, 1H), 7.14 (s, 1H), 7.24−7.37 (m, 4H), 7.44−7.52 (m, 2H), 7.62 (dd, J = 8.79, 2.05 Hz, 1H), 8.31−8.38 (m, 2H), 13.18 (br s, 1H). ^{13}C NMR (75 MHz, pyridine-d_5): δ 13.14, 31.26 (2C), 53.21, 53.51 (2C), 65.25, 105.29, 110.64, 120.19, 121.46 (2C), 124.34, 126.28, 127.23, 128.75, 131.18 (2C), 136.40, 137.23, 150.71, 152.46, 155.38 (s, 1C), 162.54, 163.59, 165.13. HRMS (ESI): calcd for $[C_{26}H_{25}ClN_4O_3 + H]^+$, 477.1688; found, 477.1690.

*Methyl 2-(3-Phenoxyphenyl)acetate (**49**).* According to a literature procedure,[50] to the solution of 2-(3-hydroxyphenyl)-acetic acid **45** (5.08 g, 33.4 mmol) in MeOH (20 mL) was added concentrated H_2SO_4 (0.5 mL) to offer the desired intermediate methyl 2-(3-hydroxyphenyl)acetate **46** (4.94 g, 89%). According to a literature procedure,[51] compound **46** (0.53 g, 3.2 mmol), phenylboronic acid (1.17 g, 9.6 mmol), $Cu(OAc)_2$ (1.16 g, 6.4 mmol), molecular sieves (0.60 g), and pyridine (0.77 mL, 9.6 mmol) in 1,2-dichloroethane (10 mL) yielded compound **49** as a colorless gel (0.25 g, 33%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.60 (s, 2H), 3.69 (s, 3H), 6.88−6.93 (m, 1H), 6.93−6.96 (m, 1H), 6.99−7.04 (m, 3H), 7.11 (tt, J = 7.32, 1.17 Hz, 1H), 7.24−7.28 (m, 1H), 7.30−7.37 (m, 2H). HRMS (ESI): calcd for $[C_{15}H_{14}O_3 + H]^+$, 243.1016; found, 243.1024.

*2-(3-Phenoxyphenyl)acetaldehyde (**50**).* According to a literature procedure,[47] methyl 2-(3-phenoxyphenyl)acetate **49** (0.73 g, 3.01 mmol) was reduced by $LiAlH_4$ (285.4 mg, 7.52 mmol) in dry THF (20 mL) to offer the alcohol, which was dissolved with dry CH_2Cl_2 (8 mL). Dess−Martin reagent (1.53 g, 3.61 mmol) was added to the reaction mixture at 0 °C under argon. The reaction mixture was stirred at room temperature for 8 h, followed by dilution with CH_2Cl_2 (100 mL), and washed with the mixture of a saturated $Na_2S_2O_3$ solution (50 mL) and saturated $NaHCO_3$ solution (50 mL) and brine (100 mL) sequentially. The organic layer was dried over Na_2SO_4 and purified with column chromatography (100% hexane−10% ethyl acetate/hexane in a linear gradient solution) to offer compound **50** (0.36 g, 56%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.66 (d, J = 2.34 Hz, 2H), 6.86−6.89 (m, 1H), 6.91−6.97 (m, 2H), 7.00−7.05 (m, 2H), 7.13 (tt, J = 7.43, 1.06 Hz, 1H), 7.31−7.39 (m, 3H), 9.74 (t, J = 2.20 Hz, 1H). HRMS (ESI): calcd for $[C_{14}H_{12}O_2 + H]^+$, 213.0910; found, 213.0904.

*Methyl 3-(3-Phenoxyphenyl)propanoate (**51**).* According to a literature procedure,[50] to the solution of 3-Hydroxycinnamic acid **47** (11.49 g, 70 mmol) in MeOH (40 mL) was added concentrated H_2SO_4 (1.15 mL) to offer compound methyl 3-(3-hydroxyphenyl)acrylate as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 3H), 6.25 (br s, 1H), 6.41 (d, J = 15.82 Hz, 1H), 6.91 (ddd, J = 8.05, 2.49, 1.17 Hz, 1H), 7.02−7.10 (m, 2H), 7.21−7.28 (m, 1H), 7.65 (d, J = 16.11 Hz, 1H). HRMS (ESI): calcd for $[C_{10}H_{10}O_3 + H]^+$, 179.0703; found, 179.0710. The suspension of methyl 3-(3-hydroxyphenyl)-acrylate **47** (1.00 g, 5.612 mmol) and 10% Pd/C in ethyl acetate was stirred at room temperature under H_2 for 2 h. After filtration through Celite, the filtrate was evaporated and the crude intermediate methyl 3-(3-hydroxyphenyl)propanoate **48** was obtained (0.91 g, 90%). According to a literature procedure,[51] the suspension of methyl 3-(3-hydroxyphenyl)-propanoate **48** (0.58 g, 3.2 mmol), phenylboronic acid (1.17 g, 9.6 mmol), $Cu(OAc)_2$ (1.16 g, 6.4 mmol), molecular sieves (0.60 g), and pyridine (0.77 mL, 9.6 mmol) in 1,2-dichloroethane (10 mL) yielded compound **51** as a light yellow gel (0.41 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.58−2.65 (m, 2H), 2.89−2.96 (m, 2H), 3.66 (s, 3H), 6.82−6.88 (m, 2H), 6.91−6.96 (m, 1H), 6.97−7.03 (m, 2H), 7.06−7.13 (m, 1H), 7.20−7.28 (m, 1H), 7.29−7.38 (m, 2H). HRMS (ESI): calcd for $[C_{16}H_{16}O_3 + H]^+$, 257.1172; found, 257.1178.

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*3-(3-Phenoxyphenyl)propanal (**52**).* Following the procedure described for compound **50**, methyl 3-(3-phenoxyphenyl)-propanoate **51** (0.40 g, 1.57 mmol) was first reduced by $LiAlH_4$ (148.6 mg, 3.91 mmol) in dry THF (10 mL) to offer intermediate 3-(3-phenoxyphenyl)propan-1-ol, which was dissolved with dry CH_2Cl_2 (3 mL). Dess–Martin reagent (0.80 g, 1.88 mmol) was added to the reaction mixture at 0 °C under argon. The desired compound **52** (147.7 mg, 45%) was obtained as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.72–2.81 (m, 2H), 2.73–2.80 (m, 2H), 2.90–2.98 (m, 2H), 6.81–6.87 (m, 2H), 6.90–6.96 (m, 1H), 6.97–7.03 (m, 2H), 7.11 (tt, J = 7.32, 1.17 Hz, 1H), 7.21–7.28 (m, 1H), 7.31–7.38 (m, 2H), 9.81 (t, J = 1.32 Hz, 1H). HRMS (ESI): calcd for $[C_{15}H_{14}O_2 + H]^+$, 227.1067; found, 227.1065.

*2-Methyl-4-phenoxyquinoline (**54**).* According to a modified literature procedure,[52] the suspension of 4-chloro-2-methylquinoline **53** (0.53 g, 3 mmol), phenol (0.85 g, 9 mmol), Cs_2CO_3 (2.93 g, 9 mmol), *N*,*N*-dimethylglycine hydrochloride (125.6 mg, 0.9 mmol), and CuI (57.1 mg, 0.3 mmol) in dry 1,4-dioxane (5 mL) under air with a drying tube was stirred at 90 °C for 24 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered. The filtrate was washed with CH_2Cl_2 (100 mL), and the combined organic layer was evaporated; the residue was purified with column chromatography (100% toluene–90% toluene/ethyl acetate) to yield compound **54** as a white solid (158.8 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.61 (s, 3H), 6.44 (s, 1H), 7.17–7.22 (m, 2H), 7.28–7.35 (m, 1H), 7.46–7.56 (m, 3H), 7.74 (ddd, J = 8.57, 6.96, 1.46 Hz, 1H), 8.06 (d, J = 7.91 Hz, 1H), 8.31 (dd, J = 8.35, 1.03 Hz, 1H). HRMS (ESI): calcd for $[C_{16}H_{13}NO + H]^+$, 236.1070; found, 236.1079.

*4-Phenoxyquinoline-2-carbaldehyde (**55**).* According to a literature procedure,[49] 2-methyl-4-phenoxyquinoline **54** (100.0 mg, 0.43 mmol) and SeO_2 (94.3 mg, 0.85 mmol) were dissolved with 1,4-dioxane (6 mL). The suspension was heated at 80 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered through Celite. The filtrate was evaporated and purified with column chromatography (100% hexane–10% ethyl acetate/hexane) to offer compound **55** as a white solid (80.0 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.16 (s, 1H), 7.17–7.23 (m, 2H), 7.35 (tt, J = 7.47, 1.17 Hz, 1H), 7.47–7.54 (m, 2H), 7.73 (ddd, J = 8.27, 6.96, 1.17 Hz, 1H), 7.87 (ddd, J = 8.42, 6.81, 1.46 Hz, 1H), 8.25 (d, J = 8.49 Hz, 1H), 8.46 (dd, J = 8.35, 1.32 Hz, 1H), 10.11 (s, 1H). HRMS (ESI): calcd for $[C_{16}H_{12}NO_2 + H]^+$, 250.0863; found, 250.0870.

*4-(3-Chlorophenoxy)quinoline-2-carbaldehyde (**57**).* Following the Ullmann coupling procedure described for compound **54**, the suspension of 4-chloro-2-methylquinoline **53** (0.44 g, 2.5 mmol), 3-chlorophenol (0.96 g, 7.5 mmol), Cs_2CO_3 (2.44 g, 7.5 mmol), *N*,*N*-dimethylglycine hydrochloride (104.7 mg, 0.75 mmol), and CuI (47.6 mg, 0.25 mmol) in dry 1,4-dioxane (8 mL) under air with a drying tube yielded 4-(3-chlorophenoxy)-2-methylquinoline **56** (210.0 mg, 31%). According to a modified literature procedure,[49] the suspension of compound **56** (210.0 mg, 0.7 mmol) and SeO_2 (150.5 mg, 1.4 mmol) in 1,4-dioxane (8 mL) was heated at 80 °C for 1.5 h. After cooling to room temperature, the reaction mixture was filtered through Celite. The filtrate was evaporated and purified with column chromatography (100% hexane–10% ethyl acetate/hexane in a linear gradient elution) to offer compound **57** as a white solid (181.0 mg, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.11 (ddd, J = 8.20, 2.34, 1.17 Hz, 1H), 7.19 (s, 1H), 7.23 (t, J = 2.20 Hz, 1H), 7.34 (ddd, J = 8.13, 1.98, 1.03 Hz, 1H), 7.44 (t, J = 8.05 Hz, 1H), 7.72–7.78 (m, 1H), 7.89 (ddd, J = 8.49, 6.88, 1.61 Hz, 1H), 8.29 (dd, J = 8.35, 1.03 Hz, 1H), 8.38–8.43 (m, 1H), 10.15 (s, 1H). HRMS (ESI): calcd for $[C_{16}H_{10}ClNO_2 + H]^+$, 284.0473; found, 284.0479.

*7-Chloro-4-phenoxyquinoline-2-carbaldehyde (**60**).* Following the Ullmann coupling procedure described with compound **54**, the suspension of 4,7-dichloro-2-methylquinoline **58** (0.53 g, 2.5 mmol), phenol (0.71 g, 7.5 mmol), Cs_2CO_3 (2.44 g, 7.5 mmol), *N*,*N*-dimethylglycine hydrochloride (104.7 mg, 0.75 mmol), and CuI (47.6 mg, 0.25 mmol) in dry 1,4-dioxane (5 mL) under air with a drying tube yielded 4-(3-chlorophenoxy)-2-methylquinoline as a yellow solid, 7-chloro-2-methyl-4-phenoxyquinoline **59** (156.0 mg, 23%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.58 (s, 3H), 6.41 (s, 1H), 7.15–7.18 (m, 1H), 7.20 (q, J = 1.86 Hz, 1H), 7.29–7.36 (m, 1H), 7.45–7.53 (m, 3H), 8.03 (d, J = 1.76 Hz, 1H), 8.24 (d, J = 9.08 Hz, 1H). HRMS (ESI): calcd for $[C_{16}H_{12}ClNO + H]^+$, 270.0680; found, 270.0615. According to a literature procedure,[49] the suspension of compound **59** (146.0 mg, 0.54 mmol) and SeO_2 (120.1 mg, 1.08 mmol) in 1,4-dioxane (5 mL) was heated at 80 °C for 2 h. After cooling to room temperature, the reaction mixture was filtered through Celite. The filtrate was evaporated and purified with column chromatography (100% hexane–10% ethyl acetate/hexane in a linear gradient elution) to offer compound **60** as a white solid (138.2 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13 (s, 1H), 7.16–7.22 (m, 2H), 7.32–7.39 (m, 1H), 7.47–7.55 (m, 2H), 7.67 (dd, J = 9.08, 2.05 Hz, 1H), 8.24 (d, J = 2.05 Hz, 1H), 8.39 (d, J = 8.79 Hz, 1H), 10.07 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 99.99, 121.11 (2C), 121.36, 123.66, 126.42, 128.81, 129.54, 130.64 (2C), 137.29, 149.76, 153.50, 154.66, 163.24, 193.16. HRMS (ESI): calcd for $[C_{16}H_{10}ClNO_2 + H]^+$, 284.0473; found, 284.0468.

*tert-Butyl 4-((Methylsulfonyl)oxy)piperidine-1-carboxylate (**61**).* According to a modified literature procedure,[53] a solution of *tert*-butyl 4-hydroxypiperidine-1-carboxylate (10.06 g, 50 mmol) and triethylamine (10.81 mL, 77.5 mmol) in CH_2Cl_2 (200 mL) at 0–5 °C was treated with methanesulfonyl chloride (4.64 mL, 60 mmol). After 2 h, the reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated $NaHCO_3$ (100 mL) and brine (100 mL) sequentially, followed by drying over Na_2SO_4. After evaporation, the residue was purified by column chromatography (95% CH_2Cl_2/MeOH) to offer compound **61** as a white solid (11.31 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 9H), 1.74–1.89 (m, 2H), 1.89–2.04 (m, 2H), 3.04 (s, 3H), 3.30 (m, 2H), 3.71 (m, 2H), 4.88 (tt, J = 7.76, 3.81 Hz, 1H). HRMS (ESI): calcd for $[C_{11}H_{21}NO_5S + H]^+$, 280.1213; found, 280.1210.

*3-((Benzyloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**62**).* According to a literature procedure,[54] **62** was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78 (d, J = 1.17 Hz, 3H), 4.58 (s, 2H), 5.31 (s, 2H), 7.23–7.37 (m, 6H), 10.94 (br s, 1H). HRMS (ESI): calcd for $[C_{13}H_{14}N_2O_3 + H]^+$, 247.1077; found, 247.1080.

*3-((Benzyloxy)methyl)-5-methyl-1-(piperidin-4-yl)-pyrimidine-2,4(1H,3H)-dione (**63**).* According to a literature procedure,[24] a suspension of 3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione (**62**, 0.29 g, 1.18 mmol), *tert*-butyl 4-((methylsulfonyl)oxy)piperidine-1-carboxylate (**61**, 0.33 g, 1.18 mmol), and potassium carbonate (0.32 g, 2.31 mmol) in dry DMF (5 mL) was stirred at 80 °C for 36 h under argon. Additional **61** (0.33 g, 1.18 mmol) and potassium

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carbonate (0.17 g, 1.23 mmol) were added to the reaction mixture, and the mixture was stirred at 80 °C for 24 h. Additional **61** (0.33 g, 1.18 mmol) and potassium carbonate (0.16 g, 1.16 mmol) were added, and the mixture was stirred at 80 °C for another 24 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (50 mL), following by washing with water (50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4. After evaporation, the residue was purified by column chromatography (50% hexane/ethyl acetate) to give the intermediate, which was dissolved with CH_2Cl_2 (10 mL), and TFA (1 mL) was added to the solution. The reaction mixture was stirred at room temperature for 3 h, followed by evaporation in vacuo. The residue was dissolved in a saturated $NaHCO_3$ solution (15 mL) and extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were washed with brine (50 mL × 2) and dried over sodium sulfate. The water layer can be re-extracted again if the product is found by TLC. After evaporation, the residue was purified by column chromatography (0.1% Et_3N/4% MeOH/CH_2Cl_2) to afford compound **63** as a colorless gel (259.8 mg, 67%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.58−1.73 (m, 2H), 1.80−1.90 (m, 2H), 1.92 (d, J = 1.17 Hz, 3H), 2.76 (td, J = 12.16, 2.34 Hz, 2H), 3.15−3.28 (m, 2H), 4.54−4.68 (m, 1H), 4.71 (s, 2H), 5.51 (s, 2H), 7.03 (d, J = 1.17 Hz, 1H), 7.23−7.39 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.31, 31.19 (2C), 45.57 (2C), 52.97, 70.89, 72.28, 110.27, 127.65 (2C), 128.27 (3C), 134.93, 138.06, 151.50, 163.23. HRMS (ESI): calcd for $[C_{18}H_{23}N_3O_3 + H]^+$, 330.1812; found, 330.1810.

*3-((Benzyloxy)methyl)-5-methyl-1-(piperidin-4-ylmethyl)-pyrimidine-2,4(1H,3H)-dione (**65**).* Following a literature procedure,[55] to the solution of *tert*-butyl 4-(hydroxymethyl)-piperidine-1-carboxylate (1.08g, 5.00 mmol) in CH_2Cl_2 (15 mL) were added triethylamine (0.70 mL, 5.00 mmol), 4-DMAP (190.9 mg, 1.56 mmol), and methanesulfonyl chloride (0.44 mL, 5.63 mmol) to yield the mesylate **64** (1.43 g, 97%). According to a modified procedure,[24] the mixture of **62** (0.42 g, 1.20 mmol), compound **64** (0.53 g, 1.8 mmol), and potassium carbonate (0.33 g, 2.4 mmol) in dry DMF (10 mL) was stirred at 80 °C overnight to yield the *N*-Boc-protected intermediate, which was deprotected with 10% TFA/CH_2Cl_2 (20 mL) to yield compound **65** as a colorless gel (0.41 g, 77%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.21 (qd, J = 12.20, 4.10 Hz, 2H), 1.65 (d, J = 12.30 Hz, 2H), 1.84−1.97 (m, 4H), 2.42 (br s, 1H), 2.59 (td, J = 12.23, 2.49 Hz, 2H), 3.07−3.16 (m, 2H), 3.56 (d, J = 7.03 Hz, 2H), 4.71 (s, 2H), 5.51 (s, 2H), 6.89 (d, J = 1.17 Hz, 1H), 7.21−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.01, 30.21 (2C), 35.54, 45.69 (2C), 55.40, 70.72, 72.26, 109.56, 127.63 (3C), 128.26 (2C), 138.04, 139.75, 151.63, 163.76. HRMS (ESI): calcd for $[C_{19}H_{25}N_3O_3 + H]^+$, 344.1969; found, 344.1970.

*tert-Butyl 3-((Methylsulfonyl)oxy)azetidine-1-carboxylate (**66**).* According to a modified literature procedure,[53] a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (2.60 g, 15 mmol) and triethylamine (3.24 mL, 23.25 mmol) in CH_2Cl_2 (75 mL) at 0−5 °C was treated with methanesulfonyl chloride (1.39 mL, 18 mmol). After 1 h, the reaction mixture was diluted with CH_2Cl_2 (75 mL) and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL) sequentially, followed by drying over Na_2SO_4. After evaporation, the residue was purified by column chromatography (95% CH_2Cl_2/MeOH) to offer compound **66** as a white solid (3.58 g, 95%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H), 3.06 (s, 3H), 4.06−4.13 (m, 2H), 4.24−4.31 (m, 2H), 5.20 (tt, J = 6.66, 4.17 Hz, 1H). HRMS (ESI): calcd for $[C_9H_{17}NO_5S + H]^+$, 251.0827; found, 251.0824.

*1-(Azetidin-3-yl)-3-((benzyloxy)methyl)-5-methylpyrimidine-2,4(1H,3H)-dione (**67**).* According to a modified literature procedure,[24] the reaction mixture of **66** (0.75 g, 3 mmol), **62** (0.49 g, 2 mmol), and potassium carbonate (0.55 g, 4 mmol) in dry DMF (10 mL) was stirred at 80 °C for 24 h. **66** (1 equiv) and potassium carbonate (1 equiv) were added to the reaction mixture. After 24 h, the reaction mixture was worked up as described in the literature to offer compound **67** as a colorless gel (0.26 g, 44%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.98 (d, J = 1.17 Hz, 3H), 2.27 (br s, 1H), 3.67−3.77 (m, 2H), 4.12 (t, J = 8.49 Hz, 2H), 4.70 (s, 2H), 5.24−5.36 (m, 1H), 5.50 (s, 2H), 7.21−7.39 (m, 5H), 7.51 (d, J = 1.17 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.23, 50.86, 53.14 (2C), 70.75, 72.26, 110.92, 127.65 (3C), 128.26 (2C), 135.67, 138.00, 151.31, 163.37. HRMS (ESI): calcd for $[C_{16}H_{19}N_3O_3 + H]^+$, 302.1499; found, 302.1501.

*3-((Benzyloxy)methyl)-5-methyl-1-(2-(methylamino)-ethyl)pyrimidine-2,4(1H,3H)-dione (**69**).* According to a literature procedure,[56] to the mixture of 2-(methylamino)-ethan-1-ol **68** (4.13 g, 55 mmol) and triethylamine (9.6 mL, 69 mmol) in CH_2Cl_2 (100 mL) was added $(Boc)_2O$ (10 g, 45.82 mmol) at 0 °C. The mixture was stirred at room temperature for 2 h and quenched by 1 M HCl (200 mL). The organic layer was washed with 1 M HCl (100 mL) and brine (100 mL) sequentially. After the residue was dried over Na_2SO_4, the organic solvent was evaporated to offer the intermediate *tert*-butyl (2-hydroxyethyl)(methyl)carbamate as a colorless gel (8.67 g, 90%). To the solution of *tert*-butyl (2-hydroxyethyl)-(methyl)carbamate (1.4 g, 8 mmol) in CH_2Cl_2 (20 mL) were added triethylamine (1.46 mL, 10.4 mmol) and methanesulfonyl chloride (0.66 mL, 8.4 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and quenched with 1 M HCl (20 mL). The organic layer was washed with 1 M HCl (20 mL × 2) and brine (40 mL). After the residue was dried over Na_2SO_4, the organic layer was evaporated to offer the crude intermediate 2-((tert-butoxycarbonyl)(methyl)amino)-ethylmethanesulfonate, which was unstable and used for next step directly. According to a modified procedure,[24] the reaction mixture of the mesylate (1.01 g, 4 mmol), 3-((benzyloxy)-methyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione **62** (0.49 g, 2 mmol), and potassium carbonate (0.55 g, 4 mmol) in dry DMF (5 mL) was stirred at 80 °C for 24 h. Mesylate (1.01 g, 4 mmol) was added to the reaction mixture, which was stirred at 80 °C for 24 h to offer the *N*-Boc-protected intermediate. The intermediate was dissolved with 10% TFA/DCM (20 mL) at room temperature to offer the desired product **69** as a colorless oil (140.0 mg, 23%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.54 (br s, 1H), 1.92 (d, J = 1.17 Hz, 3H), 2.45 (s, 3H), 2.87 (t, J = 6.00 Hz, 2H), 3.80 (t, J = 6.00 Hz, 2H), 4.71 (s, 2H), 5.51 (s, 2H), 7.01 (q, J = 1.17 Hz, 1H), 7.22−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.04, 36.30, 49.08, 50.16, 70.68, 72.23, 109.59, 127.58 (2C), 127.68 (2C), 128.26, 138.10, 139.77, 151.69, 163.85. HRMS (ESI): calcd for $[C_{16}H_{21}N_3O_3 + H]^+$, 304.1656; found, 304.1634.

*3-((Benzyloxy)methyl)-5-methyl-1-(3-(methylamino)-propyl)pyrimidine-2,4(1H,3H)-dione (**71**).* Following the procedure described for compound **69**, 3-(methylamino)propan-1-ol **70** (5.05g, 56.7 mmol) was treated with $(Boc)_2O$, followed by the preparation of mesylate. The alkylation between the mesylate (1.07 g, 4 mmol), compound **62** (0.49 g, 2 mmol), and potassium carbonate (0.55 g, 4 mmol) in dry DMF (5 mL)

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offered compound **71** as a colorless oil (440 mg, 69%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.88−1.96 (m, 5H), 2.46 (s, 3H), 2.65 (t, J = 6.74 Hz, 2H), 2.81 (br s, 1H), 3.82 (t, J = 6.88 Hz, 2H), 4.71 (s, 2H), 5.51 (s, 2H), 7.04 (q, J = 1.17 Hz, 1H), 7.21−7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.99, 28.30, 35.87, 46.99, 47.87, 70.68, 72.23, 109.95, 127.61 (2C), 127.65 (2C), 128.27, 138.04, 139.45, 151.69, 163.79. HRMS (ESI): calcd for $[C_{17}H_{13}N_3O_3 + H]^+$, 318.1812; found, 318.1810.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b01570.

Details on the IC_{50} determination, data collection and refinement statistics, ligand efficiency calculations, data for compounds **1** and **43** in complex with *M. tuberculosis* thymidylate kinase (*Mt*TMPK) and physicochemical properties for *Mt*TMPK inhibitors, and NMR spectra (PDF)

Molecular formula strings of all final compounds (CSV)

Accession Codes

The authors will release the atomic coordinates and experimental data upon article presentation: *Mt*TMPK-**1** (PDB code: 5NQ5); *Mt*TMPK-**43** (PDB code: 5NR7).

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ADC, albumin, dextrose and catalase supplement; ATCC, American type culture collection; BCG, Bacillus Calmette-Guérin; BOM, benzyloxymethyl; cfu/mL, colony forming units per milliliters; DMEM, Dulbecco's modified Eagle's medium; dTMP, thymidine monophosphate; EDC, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; *h*TMPK, human thymidylate kinase; LB, lysogeny broth; MDR-TB, multidrug-resistant tuberculosis; KO-Mmr, H37Rv knockout for *mmr*; MPD, 2-methyl-2,4-pentanediol; Mtb, *Mycobacterium tuberculosis*; *Mt*TMPK, *Mycobacterium tuberculosis* thymidylate kinase; NCS, noncrystallographic symmetry; NRU, neutral red uptake; PaβN, phenylalanine-arginine β-naphthylamide; RSCC, real-space correlation coefficient; RLU, relative light units; TCEP, tris(2-carboxyethyl)-phosphine; TLS, translation-libration-screw; TMPK, thymidylate kinase; XDR-TB, extensively drug-resistant TB

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