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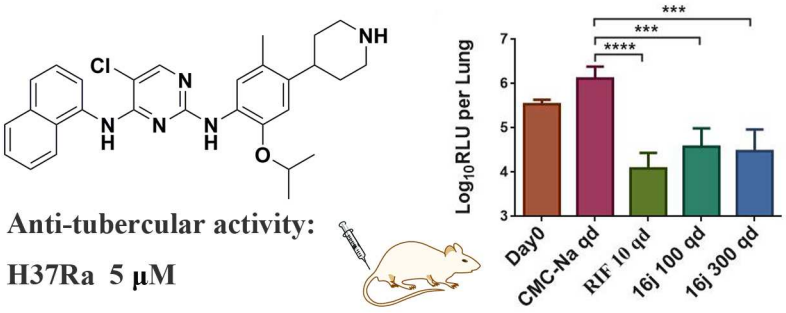

Anti-tubercular activity:

H37Ra 5 μM

Design and synthesis of novel pyrimidine derivatives as potent antitubercular agents

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Abstract

The emergence of various drug-resistant *Mycobacterium tuberculosis* (*Mtb*) strains has necessitated the exploration of new drugs that lack cross-resistance with existing therapeutics. By screening the Med Chem Express bioactive compound library, **ceritinib** was identified as a compound with activity against *Mtb* H37Ra. Ceritinib had a MIC value of 9.0 μM *in vitro* and demonstrated *in vivo* efficacy in a BALB/c mouse model infected with autoluminescent H37Ra. Then, 32 novel ceritinib derivatives were synthesized, and their antimycobacterial activities were evaluated *in vitro*. The antimycobacterial activities of the synthesized compounds were drastically affected by substitutions at position 4 of the pyrimidine nucleus and were enhanced by the presence of 2-isopropoxy-5-methyl-4-(piperidin-4-yl)aniline at position 2 of the pyrimidine nucleus. The *in vivo* antitubercular activities of the three most potent compounds were evaluated. 5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)

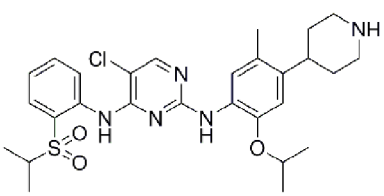
phenyl)-N^4-(naph thalen-1-yl) pyrimidine-2,4-diamine (**16j**) remarkably reduced the *Mtb* burden of mice. This result suggested the potential of **16j** as a novel drug with superior antitubercular activities. The results of experiments on the combination of sulfamethoxazole with **16j** and *in silico* modeling suggest that dihydrofolate reductase is the potential molecular target of **16j**.

Key words: Antimycobacterial; ceritinib; pyrimidine derivatives; dihydrofolate reductase inhibitors.

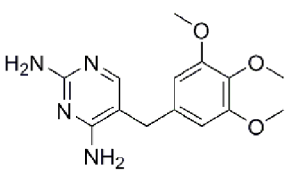
1. Introduction

Tuberculosis (TB), an infection caused by *Mycobacterium tuberculosis* (*Mtb*), remains a serious health problem worldwide [1]. In 2017, approximately 10.0 million new cases of TB were reported, and 1.3 million patients died of TB [2]. Treatment with a combination of first-line antitubercular drugs, including rifampicin, isoniazid, ethambutol, and pyrazinamide requires 6–9 months to complete and results in several side effects. Hence, new drugs and therapeutic strategies are urgently required to improve the efficacy of TB treatment [3]. Nevertheless, the development of drug-resistant *Mtb* strains has complicated the treatment of TB [4-5]. Efforts to discover antitubercular drugs have increased, and various candidates for new antitubercular drugs have begun to emerge [6-8].

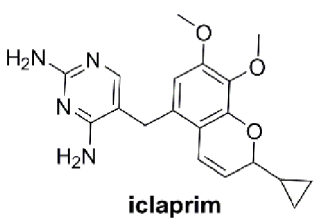
In our ongoing effort to discover new molecules with antitubercular activities and potentially novel mechanisms of action, we preliminarily screened commercially available libraries for compounds with antimycobacterial activities. Ceritinib, a pyrimidine compound (**Figure 1**), was identified as a potential antitubercular agent, and its antimycobacterial activity was confirmed through biological evaluation. Ceritinib showed moderate efficacy *in vitro* with an MIC of 9.0 μM. Given this result, ceritinib is a potentially novel scaffold or pharmacophore with antitubercular activity.



ceritinib



trimethoprim



iclaprim

Figure 1. Chemical structures of ceritinib, trimethoprim, and iclaprim.

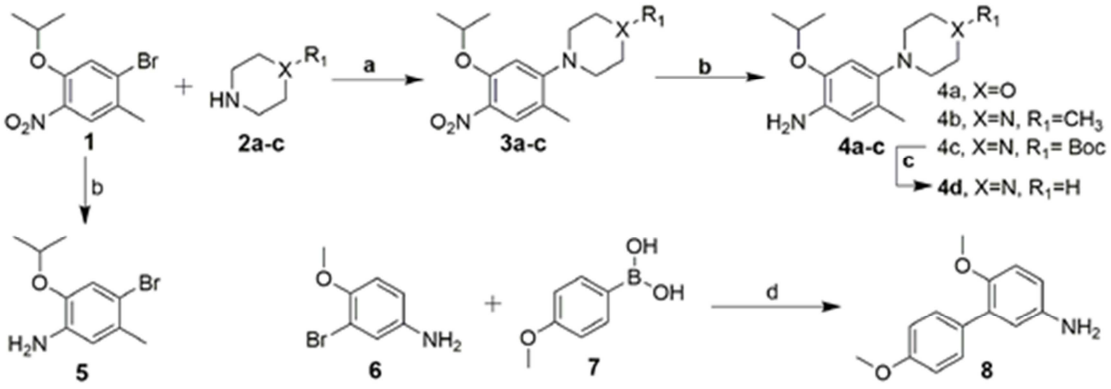
Therefore, we designed and synthesized a series of ceritinib analogues. We then attempted to ascertain the structure–activity relationships (SARs) of ceritinib that determine the antimycobacterial activities of the synthesized ceritinib derivatives. Most ceritinib derivatives displayed encouraging antimycobacterial activities *in vitro*. We selected and evaluated the *in vivo* antitubercular activities of the three most potent compounds. Our results indicate that 5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(naphthalen-1-yl)pyrimidine-2, 4-diamine (**16j**) is an ideal lead compound for future investigation. We predicted the potential target of **16j** by using PharmMapper [9-10], a large-scale reverse pharmacophore mapping strategy, to elucidate its antimycobacterial mechanism. We failed, however, to predict the targets of **16j** in *Mtb* via this strategy (**Figure S1**). We noticed that the structural units of our synthesized compounds are the same as those of the nonclassical dihydrofolate reductase (DHFR) inhibitors of the antibacterial drugs **iclaprim** and **trimethoprim** (**Figure 1**) [11-12]. DHFR is a promising drug target for the treatment of mycobacterial infections [13-14]. Therefore, we compared the amino acid sequences of *Mtb* DHFR with those of the top 10 potential targets predicted by PharmMapper. We found that DHFR is a putative target of **16j** (**Figure S2**). Sulfamethoxazole (SMX) targets the folate biosynthesis pathway by inhibiting dihydropteroate synthase (DHPS) [15]. DHPS and DHFR are the rate-limiting enzymes of the folate biosynthesis pathway. Thus, we explored the fractional inhibitory concentration index (FICI) of SMX and potential DHFR inhibitors in *Mtb* H37Ra. Finally, we investigated the specific interactions of these active compounds with the *Mtb* DHFR enzyme (PDB ID: 1DG5) [16] by performing molecular docking experiments with Discover Studio 3.1. Our results indicate that the antimycobacterial activity of these compounds may partly originate from the inhibition of the *Mtb* DHFR enzyme and provide leads for the further development of novel antitubercular agents.

2. Results and discussion

2.1 Chemistry

Various aminobenzenes (**4a–d**, **5**, **8**) for the synthesis of target molecules were initially synthesized through different routes as outlined in **Scheme 1**. Anilines **4a–c** were prepared through Pd-catalyzed cross-coupling between 1-bromo-5-isopropoxy-

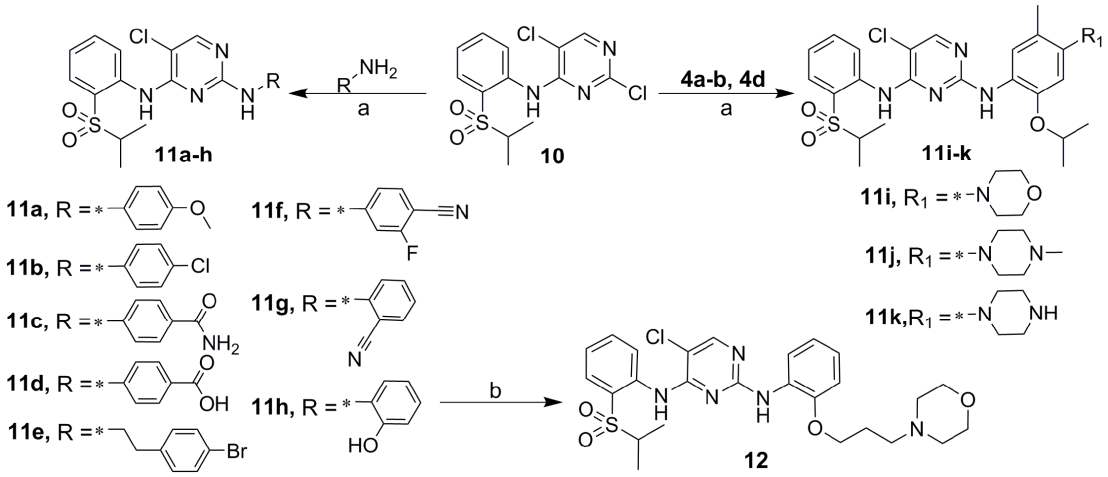
2-methyl-4-nitrobenzene (**1**) and morpholine (**2a**) or 6-substituted piperazine (**2b–c**) followed by nitro group reduction. Piperazine intermediate **4d** was obtained from N-Boc material **4c** in accordance with a previously reported synthetic strategy [17]. Aniline **5** could be easily prepared from compound **1** through nitro group reduction in the presence of Fe powder. Aniline **8** was prepared through Suzuki coupling between bromide **6** and boronic acid **7**.



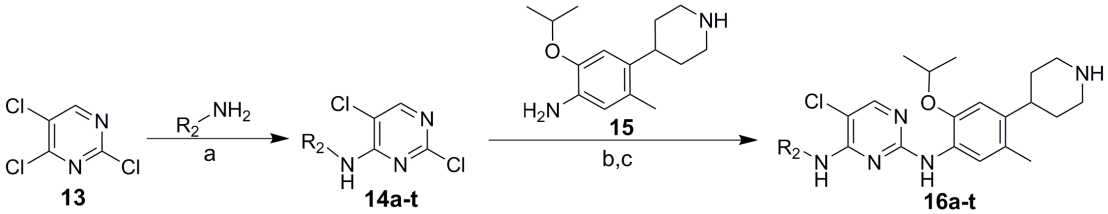
Scheme 1. Reagents and conditions: (a) $Pd(AcO)_2$, xantphos, Cs_2CO_3, dioxane, 80 □. (b) Fe, NH_4Cl, $EtOH/H_2O$, 80 □. (c) TFA, CH_2Cl_2, rt. (d) $Pd(dppf)Cl_2$, Cs_2CO_3, Dioxane, 80 □.

The final products were prepared through coupling between various amines and intermediate compounds (**10** or **14a–v**) under acidic conditions. As shown in **Scheme 2**, under acidic conditions, compound **10** and various anilines produced good yields of the first class of coupling products **11a–k**. Compound **12** was obtained by treating **11h** with 4-(3-chloropropyl)morpholine at 60 □ in the presence of K_2CO_3 as the base and DMF as the solvent. As shown in **Scheme 3**, the synthesis of analogues **16a–t** proceeded through key 4-aminopyrimidine intermediates (**14a–t**), which were obtained by heating corresponding amines (commercially available or prepared in accordance with published procedures) for several hours at 80 □ with 2,4,5-trichloropyrimidine **13** in the presence of DIPEA as the base and isopropanol as the solvent. The reaction of **14a–t** with 2-isopropoxy-5-methyl-4-(piperidin-4-yl)aniline **15** under acidic conditions provided the second class of 2,4-diaminopyrimidines (**16a**–**t**) in moderate to good yields. These compounds were finally prepared into corresponding salts in ether hydrochloride solution. All novel compounds were validated through ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. The

results of these analyses fully validated the chemical structures of the obtained compounds.



Scheme 2. Reagents and conditions: (a) HCl, IPA, 85 □. (b) 4-(3-chloropropyl)morpholine, K_2CO_3, DMF, 60 □.



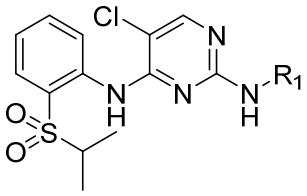
Scheme 3. Reagents and conditions: (a) DIPEA, IPA, 85 □. (b) HCl, IPA, 85 □. (c) HCl, ether, rt.

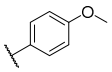
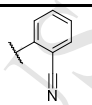
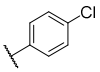
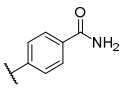
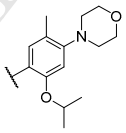
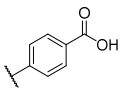
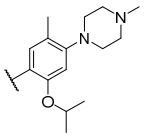
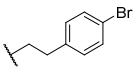
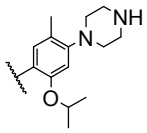
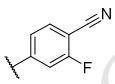
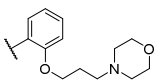
2.2. Antimycobacterial activity *in vitro*

The results of the evaluation of the *in vitro* antimycobacterial activities of the compounds are presented in **Tables 1** and **2**. Most compounds from the first class of 2, 4-diaminopyrimidines (**11a–k**, **12**) failed to show activity in the preliminary screening as summarized in **Table 1**. Only compounds **11j** and **11k** demonstrated antimycobacterial activity with MIC values of 18 and 9 μM, respectively. Differences between the antimycobacterial activity of compounds **11j** and **11k** illustrate that the substitution of the piperazine group with the *N*-methylpiperazine group decreased antimycobacterial activity by 2-fold. Notably, the presence of the morpholine ring resulted in the inactivity of compound **11i** against *Mtb* H37Ra. We speculated that NH at position 4 of the piperazine ring is highly favorable for antimycobacterial activity. The inactivity of most derivatives obtained using the first class of compounds also

highlights the importance of the 2-isopropoxy-5-methyl-4-(piperidin-4-yl)aniline moiety for antimycobacterial activity given that the activity of compounds **11j–k** against *Mtb* H37Ra was superior to those of compounds **11a–i** and **12.**

Table 1. Activities of the first structural type of novel pyrimidines against *Mtb* H37Ra.

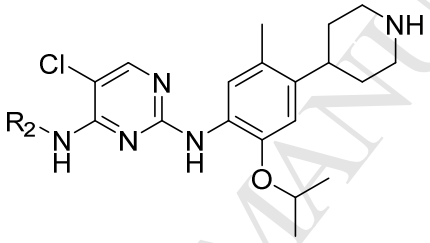


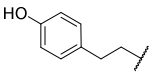
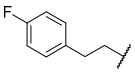
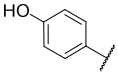
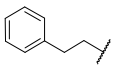
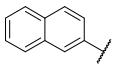
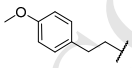
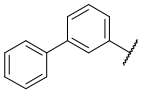
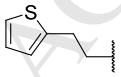
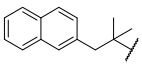
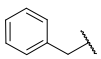
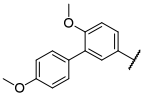
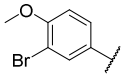
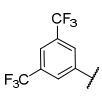
Compd	R_1	MIC (μM)	LogP	**Compd**	R_1	MIC (μM)	LogP
11a		>200	4.94	**11g**		>200	4.51
11b		>200	5.74	**11h**		>200	4.44
11c		>200	3.55	**11i**		>150	5.75
11d		>200	4.78	**11j**		18	6.19
11e		>150	6.15	**11k**		9	5.74
11f		>200	4.65	**12**		>150	5.37

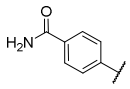
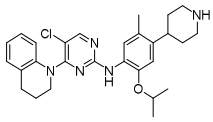
As shown in **Table 2**, all compounds exhibited promising activities against *Mtb* H37Ra and possessed MIC values of less than 20 μM. Compounds with amantadine at position 4 (**16s**), however, were less potent than compounds with aromatic nuclei. Poor potency may be related to the negative influence of the cyclic hydrocarbon attached to position 4. We observed compounds **16b** and **16d** to identify the influence of an electron-withdrawing group (F atom) and electron donor (methoxy group) at position 4 of phenyl on antimycobacterial activity. Substitution with the electron donor group improved antitubecular activity. By using substances **16c** and **16f** as

examples, we found that the linkage between an aromatic nucleus and position 4 of pyrimidine remarkably influenced the antimycobacterial activity of the compounds. Specifically, the presence of this linkage weakened the antimycobacterial activity of **16c** and **16f** by 1-fold. Compounds **16n** and **16p** were less potent than other compounds, such as **16k** and **16q**. The poor potency of **16n** and **16p** may be ascribed to the negative influence of the bulky group substituent attached to the side phenyl moiety. Compounds with phenethylamine, 4-methoxyphenylethylamine, or naphthylamine substitutions at position 4 (**16c–d** and **16j**) exhibited remarkable antimycobacterial activities with MIC values of 5 μM.

Table 2. Activities of the second structural type of novel pyrimidines against *Mtb* H37Ra.



Compd	R_2	MIC (μM)	LogP	**Compd**	R_2	MIC (μM)	LogP
16a		10	6.75	**16k**		≤10	6.48
16b		10	7.56	**16l**		10	6.48
16c		5	7.42	**16m**		10	8.32
16d		5	7.33	**16n**		19	9.04
16e		10	7.06	**16o**		18	9.30
16f		10	6.77	**16p**		17	8.33
16g		10	7.08	**16q**		9	7.77
16h		17	8.97	**16r**		11	1.10

16i		20	5.68	**16s**		20	7.67
16j		5	8.32	**16t**		20	7.84

Compounds with high lipophilicity can easily permeate the cell walls of *Mtb* [18]. Hence, we used Chemdraw Ultra 15.0 trial to calculate the LogP of all compounds and surveyed the correlation between lipophilicity and antimycobacterial activity (**Tables 1** and **2**). Although all compounds were highly lipophilic, the first class of substances failed to exhibit any antimycobacterial activity. Moreover, the antimycobacterial activities of compounds with high lipophilicity was not superior to those of compounds with low lipophilicity, such as **16r**. Thus, the antitubercular activities of the compounds are related to structure factors rather than lipophilicity.

2.3. Antitubercular activity *in vivo*

We evaluated the *in vivo* antitubercular activities of **16c**, **16d**, **16j**, and **ceritinib**. We performed a dose escalation efficacy assay with ceritinib in BALB/c mice infected with *Mtb*. Compared with treatment with the CMC-Na (solvent) control, treatment with 300 mg/kg ceritinib resulted in a 1.0 $\log_{10}$ relative light unit (RLU) reduction in total lung bacterial burden (**Figure 2A**) but not spleen bacterial burden (**Figure 2D**) in surviving mice. We also noted intense drug toxicity over the 6-day treatment period because only one mouse survived to day 6 (**Figure 3**).

We then determined the efficacy of **16c**, **16d**, and **16j** in BALB/c mice infected with *Mtb*. In this assay, **16d** demonstrated high toxicity (**Figure 3**) and no activity when administered at the dose of 200 mg/kg. By contrast, **16c** (**Figures 2B** and **2E)** demonstrated weaker *in vivo* activity in lungs and spleens than CMC-Na after 6 days of treatment at the dose of 200 mg/kg. **16j** (**Figures 2C** and **2F**) demonstrated the same efficacy at two dosages (100mg/kg and 300mg/kg). Treatment with **16j** resulted in a 2.0 and 0.5 $\log_{10}$ relative light unit (RLU) reduction in total lung and spleen bacterial burden when compared to the control group, respectively. The *in vivo* efficacy of 100 mg/kg **16j** was superior to that of 200 mg/kg **16c** and 300mg/kg ceritinib. Compounds **16c** and **16j** did not exert toxic effects (**Figure 3**).

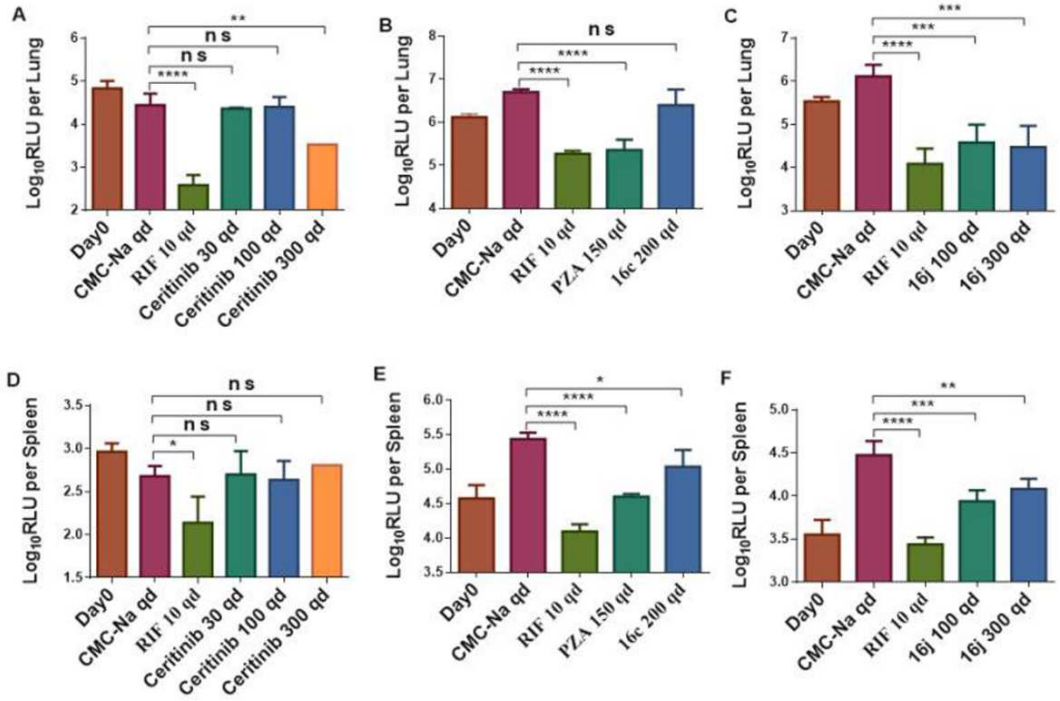


Figure 2. *In vivo* efficacy assay showing the lung and spleen bacterial burdens (log_{10} RLU) of mice infected with *Mtb*. Lung (**A**) and spleen (**D**) bacterial burdens of mice treated with **ceritinib**. Lung (**B**) and spleen (**E**) bacterial burdens of mice treated with **16c**. Lung (**C**) and spleen (**F**) bacterial burdens of mice treated with **16j**. Mice received treatment for 6 days. Each treatment group comprised four mice. Dosage (mg/kg). Data were presented as mean ± SD. ns, insignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

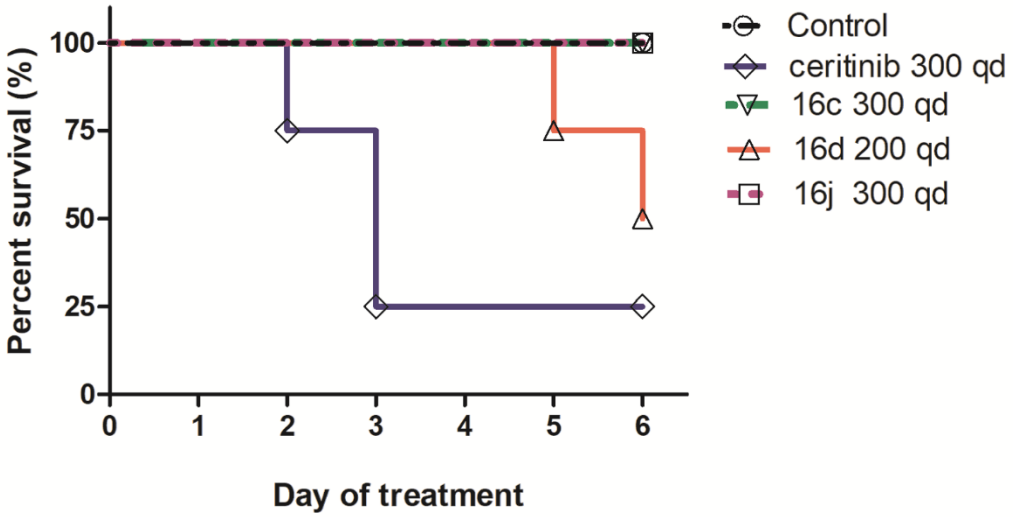


Figure 3. Percent survival of mice treated with **ceritinib**, **16c**, **16d**, or **16j**. Mice received treatment for 6 days. Each treatment group comprised four mice. All mice were surveyed for death events. Dosage (mg/kg). Black line + circle, control group; blue line + rhombus, **ceritinib** group; green line + inverted triangular, **16c** group; red line + triangle, 1**6d** group; purple line + square, **16j** group.

2.4. SAR study of 2,4-diaminopyrimidines

The presence of 2-isopropoxy-5-methyl-4-(piperidin-4-yl)aniline at position 2 is required for antimycobacterial activities. In contrast to derivatives that lacked isopropoxy-5-methyl-4-(piperidin-4-yl)aniline at position 2, all members of the second class of derivatives showed antimycobacterial activity. In addition, the change from the morpholine group to a piperazine group increased antimycobacterial activity by up to 16-fold, as demonstrated by **11i** (MIC > 150 μM) and **11k** (MIC: 9 μM). Notably, the activity of **11j** (MIC: 18 μM) was 2-fold lower than that of **11k** given the addition of a methyl in position 4 of the piperazin ring in the former. The linker of the amino moiety at position 4 of the pyrimidine ring influences *in vivo* activity. To illustrate, **16d** exhibited good activity *in vitro* (MIC: 5.0 μM) but did not exhibit activity *in vivo*. Furthermore, compounds with an aromatic amine at position 4 (**16b**, **16e**, and **16g**, MIC: 10 μM) displayed better antimycobacterial activity than compounds with an aliphatic amine at position 4 of the pyrimidine ring (**16s**, MIC: 20 μM). Meanwhile, the presence of electron-withdrawing substituents in the aromatic amine at position 4 of the pyrimidine ring (MIC for **16h:** 17μM, MIC for **16i**: 20 μM)) may adversely affect antimycobacterial activity. The SARs of these series of novel substances are summarized in **Figure 4**.

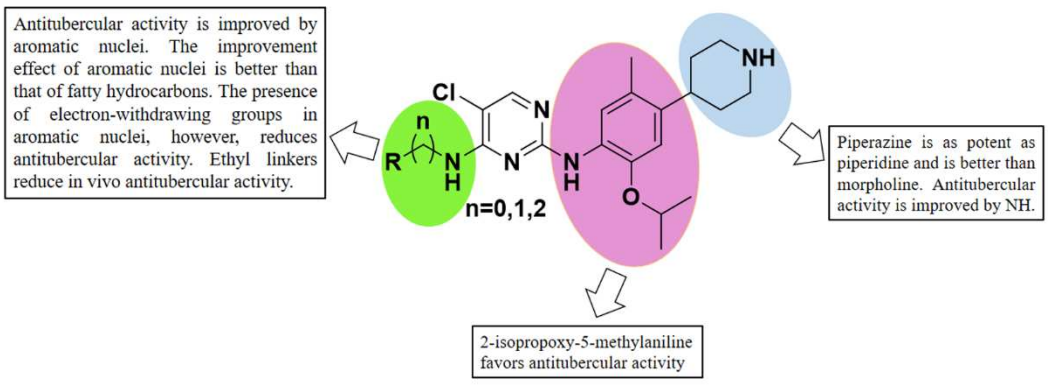


Figure 4. Structure–activity relationship.

2.5. Activity of the derivatives in combination with SMX against *Mtb* H37Ra

The *in vitro* activities of the derivatives in combination with SMX against *Mtb* H37Ra are reported in **Table 3**. Ceritinib, 16c, and 16j but not 16b exhibited partial synergism (FICI = 0.74) [19] with SMX. DHPS catalyzes the addition of dihydropterindiphosphate to P-aminobenzoic acid (PABA). SMX, a structural analogue of PABA, inhibits DHPS activity. 7,8-Dihydropteroate (DHP), a product of DHPS, reacts with glutamate to form dihydrofolate (DHF). DHFR then reduce DHF to tetrahydrofolate (THF) [15]. Ceritinib, 16c, and 16j exhibited partial synergism with SMX. Thus, we inferred that DHFR may be a potential target of the three compounds.

Table 3. MICs of individual derivatives and in combination (FICs) with SMX against *Mtb* H37Ra.

Compd	MIC (μM)	FICI
SMX	67.0	-
Ceritinib	9.0	-
16c	5.0	-
16d	5.0	-
16j	5.0	-
Ceritinib/SMX	4.5/15.8	0.74
16c/SMX	2.5/15.8	0.74
16d/SMX	5.0/15.8	1.24
16j/SMX	2.5/15.8	0.74

2.6. Molecular docking and simulation

We docked **ceritinib**, **16c,** and **16j** against the *Mtb* DHFR enzyme (PDB ID: 1DG5). The structure of the *Mtb* DHFR enzyme was obtained from the Protein Data Bank. The ligand-binding energies of the drug–receptor complexes were obtained and presented in **Table 4**. We generated 3D diagrams (**Figure 5**) to show the binding sites of all the ligands within the receptor.

Table 4. Docking results for **ceritinib** and its analogues **16c**, **16d**, and **16j** with the *Mtb* DHFR enzyme (PDB ID: 1DG5).

Compd	Absolute Energy (kcal/mol)	Relative Energy (kcal/mol)	LibDock Score
Ceritinib	96.39	11.52	130.03
16c	80.66	0.99	153.62
16d	88.82	16.75	160.00
16j	117.78	18.92	144.59

As shown in **Table 4**, the absolute binding energies of **16c**, **16d**, and **16j** ranged from 80.66 kcal/mol to 117.78 kcal/mol. The LibDock scores of these compounds were higher than those of ceritinib. In the ceritinib ligand, the pyrimidine ring interacted with receptors by forming π-alkyl with ARG45 at the distance of 4.1Å (**Figure 6A**). Moreover, the O atom of sulfuryl in ceritinib formed hydrogen bond with SER49 (3.1Å). The NH of the piperidine ring in 16j interacted with enzyme active sites by forming hydrogen bond with ARG67 at the distance of 2.5 Å (**Figure 6B**). Furthermore, a π-alkyl type of interaction was observed between the aromatic ring of 16j and ARG45 at the distance 6.5 Å. The NH of the piperidine ring in 16d (**Figure 6C**) interacted through conventional hydrogen bond with ARG45 at the distance of 3.2 Å. In ligand 16c, the aromatic ring interacted with the receptor by forming π-sigma bonds with ARG45 at the distance of 3.7 Å (**Figure 6D**).

Molecular docking results revealed that **ceritinib**, **16j**, **16d**, and **16c** can interact with different amino acid residues and fit into the active site of DHFR through completely different approaches. ARG45 always interacted with different parts of molecules. We speculated that this residue is the key position for the interaction between the derivatives and DHFR. In addition, the hydrogen bond between DHFR and the NH of the piperidine ring provided evidence that the piperidine ring is important for antimycobacterial activities. The results provided by docking simulations reveal that the antimycobacterial activity of our derivatives may partly

originate from the inhibition of the *Mtb* DHFR enzyme. Given these results, our derivatives are potential antitubercular DHFR inhibitors that warrant further development.

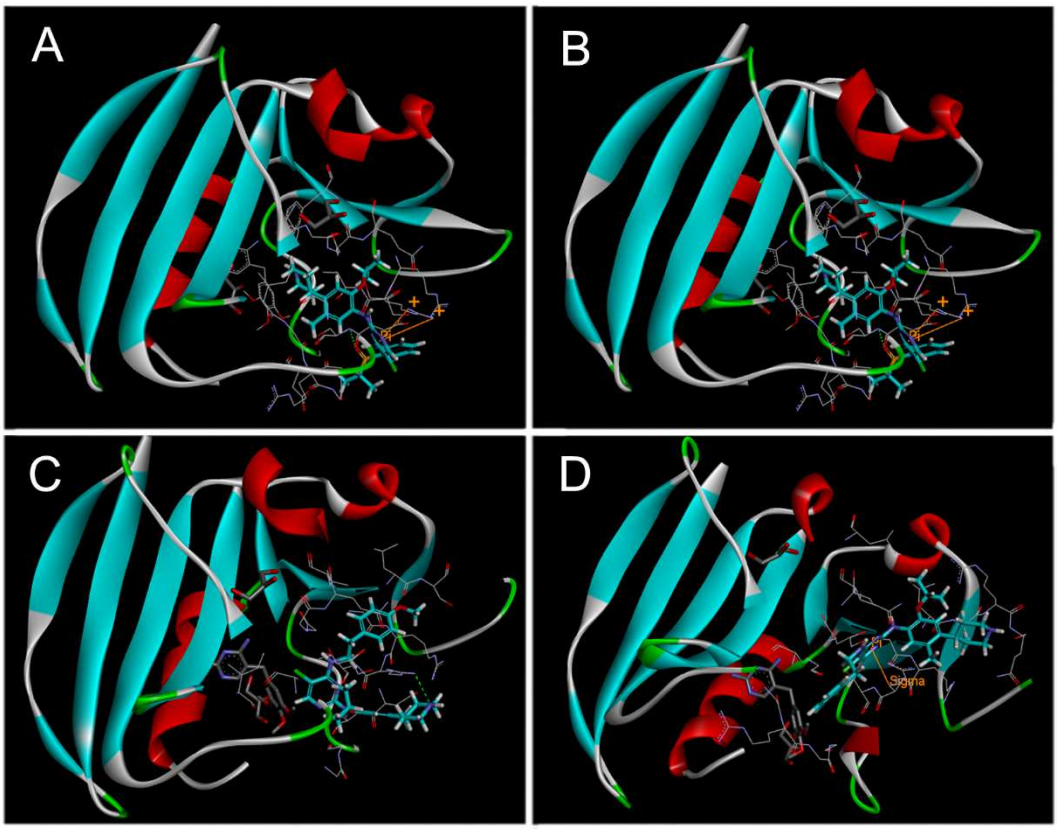


Figure 5. 3D model of ligands bonded with the active site of the *Mtb* DHFR enzyme. A) **Ceritinib**, B) **16j**, C) **16d**, and D) **16c.**

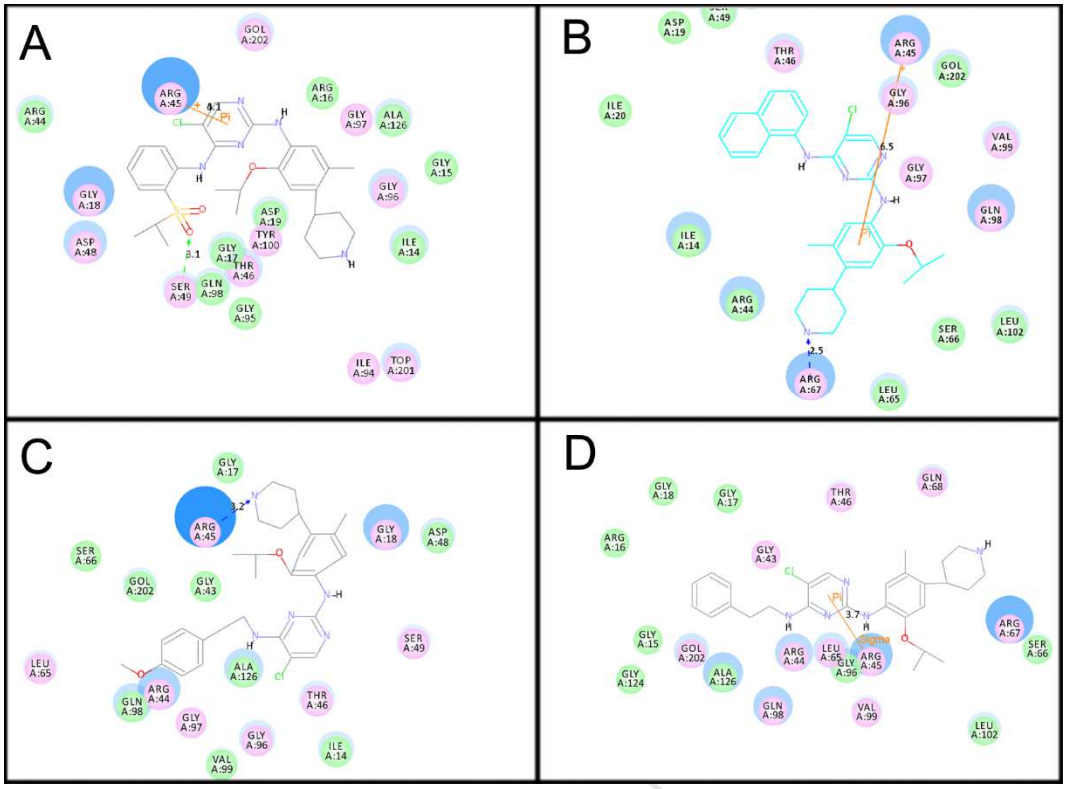


Figure 6. 2D model of the interactions between amino acid residues and ligands with different bond types and lengths. A) **Ceritinib**, B) **16j**, C) **16d**, D) **16c**.

3. Conclusion

We attempted to synthesize ceritinib analogues with drug-like properties as potential highly effective antitubercular agents. We successfully synthesized 32 pyrimidine derivatives and evaluated their inhibitory activities against *Mtb* H37Ra *in vitro*. The antimycobacterial activities of the second class of 2, 4-diaminopyrimidines (**16a–t**) were superior to that of the first class of compounds (**11a-i** and **12**). This characteristic indicates that the 2-isopropoxy-5-methyl-4-(piperidin-4-yl)aniline moiety is important for the antimycobacterial activity of the second class of compounds. Substitutions at position 4 of the pyrimidine ring improved the antitubercular activity and pharmaceutical properties of the compounds. We evaluated the antitubercular activities of **ceritinib, 16c, 16d,** and **16j** against *Mtb* H37Ra *in vivo* and found that **16j** is more hypotoxic and potent than ceritinib. The results of synergistic studies and molecular docking simulation indicate that **16c, 16d,** and **16j** are potential *Mtb* DHFR inhibitors. We selected **16j** as the lead candidate compound for the development of antitubercular drugs.

4. Experimental section

4.1 Chemistry

All chemicals and solvents were acquired from commercial suppliers and used without further purification. Compounds were purified through silica gel column chromatography using petroleum ether/ethyl acetate or DCM/methanol solvent mixtures as eluents. NMR data were obtained using Bruker Fourier 400 MHz, and TMS was used as an internal standard. Mass spectra (HRMS) were obtained using Agilent 6110 (ESI).

*4.1.1. General protocol for the synthesis of aminobenzenes **4a–c** and related compound **4d***

Intermediate products were obtained as described in previous reports [4]. We only obtained the crude products **4a–c** and **4d**, which were directly used for the synthesis of the final products through the following reactions.

A solution of 1-bromo-5-isopropoxy-2-methyl-4-nitrobenzene **1** (2.2 mmol) and amines **2a–c** (2.0 mmol) in 10.0 mL of dioxane was prepared with palladium acetate (0.2 mmol), xantphos (0.4 mmol), and cesium carbonate (6.0 mmol). The mixture was stirred under nitrogen and heated to 100 □ for 10 h. The reaction mixture was then filtered, concentrated, and purified through silica gel chromatography to afford crude products **3a–c**, which were directly used in the following nitro reduction reactions to obtain **4a–c**.

NH_4Cl (320.9 mg, 6.0 mmol) and Fe powder (112.0 mg, 2.0 mmol) were added to a solution of nitro compounds **3a–c** (0.4 mmol) in 5 mL of 5:1 EtOH/H_2O. After 4 h of stirring at 80 □, the reaction mixture was filtered, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The resulting crude product was then purified through column chromatography on silica gel to obtain the crude amine products **4a–c**. Meanwhile, a mixture of the crude product **4c** (0.19 mmol) in 4 mL of 1:3 TFA/CH_2Cl_2 was stirred for 3 h at room temperature. After the removal of solvent under reduced pressure, the crude product **4d** was obtained through simple purification on silica gel.

4.1.2. Synthesis of 4-bromo-2-methoxy-5-methylaniline **5**

Compound 5 was synthesized with 65% overall yield (76.9 mg, 0.356 mmol) in accordance with a previously reported procedure. Compound 5 was synthesized from 150 mg (0.547 mmol) of 1-bromo-5-isopropoxy-2-methyl-4-nitrobenzene 1.

Purple powder; yield, 65.0%; 1H NMR (400 MHz, DMSO-d6) δ 6.92 (s, 1H), 6.60 (s, 1H), 4.72 (s, 2H), 4.44 (dt, J = 12.1, 6.0 Hz, 1H), 2.14 (s, 3H), 1.23 (d, J = 6.0 Hz, 6H). HRMS for C8H18BrNO calcd, 214.9946; found, 215.9957(M + H+).

4.1.3. Alternative protocol for the synthesis of 4',6-dimethoxy-[1, 1'-biphenyl]-3-amine ***8***

First, 3-bromo-4-methoxyaniline 6 (2.2 mmol) was dissolved in dioxane (10 mL). N_2 was bubbled through the solution for 10 min. Next, [1, 1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.2 mmol) and cesium carbonate (6.0 mmol) were added to the solution under N_2 protection. The reaction vessel was sealed and heated overnight at 85 °C. The mixture was filtered, the solvent was removed under reduced pressure, and the residue was purified through silica gel chromatography to obtain the desired aminobenzene **8**.

White powder; yield, 60.3%; 1H NMR (400 MHz, DMSO-d6) δ 7.38 (d, J = 8.8 Hz, 2H), 6.94 (d, J = 8.8 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 6.65 – 6.41 (m, 2H), 4.67 (s, 2H), 3.76 (s, 3H), 3.59 (s, 3H). 13C NMR (101 MHz, DMSO-d6) δ 158.55, 148.18, 143.10, 131.58, 130.67, 130.59 (2C), 116.80, 114.19, 113.82 (2C), 56.70, 55.47. HRMS for C14H15NO2 calcd, 229.1103; found, 230.1204($M + H^+$).

4.1.4. General protocol for the synthesis of 4-amino-2, 5-dichloropyrimidines **14a–t**

Method A.

A mixture of 2,4,5-trichloropyrimidine (3.3 mmol), DIPEA (3.0 mmol), and amine (3.0 mmol) in isopropanol (5 mL) was stirred for 3 h at 80 □. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (10 mL × 4). Organic layers were dried over anhydrous Na_2SO_4, filtered, concentrated, and purified through column chromatography on silica gel to afford **14a–t**. The following compounds were prepared in accordance with a previously described protocol.

4-(2-((2, 5-Dichloropyrimidin-4-yl)amino)ethyl)phenol (**14a**).

Light yellow powder; yield, 75.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.14 (s, 1H), 7.93 (t, J = 6.0 Hz, 1H), 7.01 (d, J = 8.0 Hz, 2H), 6.69 (d, J = 8.0 Hz, 2H), 3.52 (dd, J = 16.0, 6.0 Hz, 2H), 2.84 – 2.65 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.03, 157.91, 156.20, 154.11, 129.99 (2C), 129.44, 115.63（2C）, 113.29, 42.95, 33.93. HRMS for $C_{12}H_{11}Cl_2N_3O$ calcd, 283.0279; found, 306.0223 ($M + Na^+$).

2, 5-Dichloro-N-(4-fluorophenethyl)pyrimidin-4-amine (**14b**).

Light yellow powder; yield, 90.5%; ^{1}H NMR (400 MHz, CDCl3) δ 8.01 (s, 1H), 7.18 (dd, J = 8.0, 6.0 Hz, 2H), 7.02 (t, J = 8.0 Hz, 2H), 3.76 (dd, J = 12.0, 6.0 Hz, 2H), 2.92 (t, J = 8.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d6) δ 160.15, 159.03, 157.91, 154.02, 135.57 (2C), 130.85 (2C), 115.40 (2C), 113.29, 42.95, 33.93. HRMS for $C_{12}H_{10}Cl_2FN_3$ calcd, 285.0236; found, 308.0176 ($M + Na^+$).

2, 5-Dichloro-N-phenethylpyrimidin-4-amine (**14c**).

Light yellow powder; yield, 74.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.97 (t, *J* = 5.6 Hz, 1H), 7.37 – 7.14 (m, 5H), 3.64 (dd, *J* = 14.4, 6.0 Hz, 2H), 2.91 (t, *J* = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.03, 157.97, 154.00, 139.46, 129.09 (2C), 128.75 (2C), 126.60, 113.33, 42.64, 34.81. HRMS for $C_{12}H_{11}Cl_2N_3$ calcd, 267.0330; found, 290.0185 ($M + Na^+$).

2, 5-Dichloro-N-(4-methoxyphenethyl)pyrimidin-4-amine (**14d**).

Light yellow powder; yield, 75.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.13 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 8.0 Hz, 2H), 5.54 (s, 1H), 3.80 (s, 3H), 3.74 (q, 8.0 Hz, 2H), 2.88 (t, J = 4.0 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.02, 158.22, 157.96, 153.99, 131.29, 130.03 (2C), 114.21 (2C), 113.31, 55.38, 42.87, 33.91. HRMS for $C_{13}H_{13}Cl_2N_3O$ calcd, 297.0436; found, 320.0332 ($M + Na^+$).

2, 5-Dichloro-N-(2-(thiophen-2-yl)ethyl)pyrimidin-4-amine (**14e**).

Light yellow powder; yield, 89.2%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.19 (dd, *J* = 5.2, 1.2Hz, 1H), 6.97 (dd, *J* = 4.8, 3.2 Hz, 1H), 6.91 – 6.84 (m, 1H), 5.67 (s, 1H), 3.81 (q, *J* = 8.0 Hz, 2H), 3.17 (t, *J* = 6.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.71, 158.46, 153.51, 140.45, 127.21, 125.69, 124.33, 113.37, 42.45, 29.35. HRMS for $C_{10}H_9Cl_2N_3S$ calcd, 272.9894; found, 295.9798 ($M + H^+$).

N-benzyl-2, 5-dichloropyrimidin-4-amine (**14f**).

Light yellow powder; yield, 90.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (t, *J* = 6.0 Hz, 1H), 8.18 (s, 1H), 7.41 – 7.29 (m, 4H), 7.25 (dt, *J* = 8.0, 2.0 Hz, 1H), 4.64 (d, *J* = 6.0Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.14, 157.91, 154.33, 138.94, 128.76 (2C), 127.78 (2C), 127.41, 113.40, 44.25. HRMS for $C_{11}H_9Cl_2N_3$ calcd, 253.0174; found, 276.0073 ($M + H^+$).

(R)-2, 5-dichloro-N-(1-phenylethyl)pyrimidin-4-amine (**14g**).

Light yellow powder; yield, 87.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.38 (d, J = 4.0 Hz, 4H), 7.34 – 7.27 (m, 1H), 5.45 – 5.32 (m, 1H), 1.62 (d, J = 8.0 Hz, 3H). HRMS for $C_{12}H_{11}Cl_2N_3$ calcd, 267.0330; found, 268.0428($M + H^+$).

N-(3, 5-bis(trifluoromethyl)phenyl)-2,5-dichloropyrimidin-4-amine (**14h**).

White powder; yield, 72.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.57 (s, 1H), 8.06 – 7.85 (m, 2H), 7.77 (dd, J = 8.8, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.19, 156.92, 156.80, 140.34, 130.88, 127.73, 125.02, 122.71, 122.31, 119.60, 117.36, 114.76. HRMS for $C_{12}H_5Cl_2F_6N_3$ calcd, 374.9765; found, 375.9841($M + H^+$).

2-((2, 5-Dichloropyrimidin-4-yl)amino)phenol (**14k**).

Light yellow powder; yield, 87.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.93 (s, 1H), 8.82 (s, 1H), 8.35 (s, 1H), 7.60 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.09 (td, *J* = 8.0, 1.6 Hz, 1H), 6.94 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.86 (td, *J* = 7.6, 1.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.87, 157.87, 157.58, 157.58, 155.10, 155.10, 150.87, 150.87, 127.03,

127.03, 125.47, 125.20, 119.52, 119.52, 116.10, 116.10, 114.13, 114.13. HRMS for $C_{10}H_7Cl_2N_3O$ calcd, 254.9966; found, 277.9868 ($M + Na^+$).

4-((2, 5-Dichloropyrimidin-4-yl)amino)phenol (**14l**) .

Purple powder; yield, 87. 5%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.38 (d, J = 4.0 Hz, 4H), 7.34 – 7.27 (m, 1H), 5.45 – 5.32 (m, 1H), 1.62 (d, J = 8.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.95, 157.56, 155.59, 155.20, 128.95 (2C), 126.18 (2C), 115.56 (2C), 113.62. HRMS for $C_{10}H_7Cl_2N_3O$ calcd, 254.9966; found, 277.9868 ($M + Na^+$).

2, 5-Dichloro-N-(naphthalen-2-yl)pyrimidin-4-amine (**14m**).

Purple powder; yield, 79.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 8.22 (d, J = 2.0 Hz, 1H), 7.85 (dd, J = 16.0, 8.0 Hz, 3H), 7.62 (dd, J = 8.0, 2.0 Hz, 1H), 7.54 – 7.43 (m, 2H), 7.41 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.26, 156.42, 154.61, 134.30, 133.67, 130.97, 128.99, 127.77, 127.67, 126.79, 125.59, 120.79, 118.21, 113.86. HRMS for $C_{14}H_9Cl_2N_3$ calcd, 289.0174; found, 327.9818($M + K^+$).

N-([1,1'-biphenyl]-3-yl)-2, 5-dichloropyrimidin-4-amine (**14n**).

Light yellow powder; yield, 78.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.60 (s, 1H), 8.41 (s, 1H), 7.92 (s, 1H), 7.68 (d, *J* = 7.6 Hz, 2H), 7.63 (s, 1H), 7.50 (S, 4H), 7.39 (t, *J* = 7.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.63, 157.37, 155.94, 140.99, 140.28, 138.53, 129.58, 129.45 (2C), 128.11, 127.08 (2C), 123.63, 122.60, 122.11, 114.18. HRMS for $C_{16}H_{11}Cl_2N_3$ calcd, 315.0330; found, 338.0225 ($M + H^+$).

2, 5-Dichloro-N-(2-methyl-1-(naphthalen-2-yl)propan-2-yl)pyrimidin-4-amine (**14o**).

Light yellow powder; yield, 68.1%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.81 (dd, *J* = 6.0, 3.6 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.53 (s, 1H), 7.50 – 7.40 (m, 2H), 7.19 (dd, *J* = 8.0, 1.6 Hz, 1H), 5.39 (s, 1H), 3.33 (s, 2H), 1.55 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.38, 157.81, 153.32, 134.75, 133.33, 132.30, 128.94, 128.69,

127.80, 127.61, 127.57, 126.15, 125.69, 113.65, 56.15, 45.24, 27.11(2C). HRMS for $C_{18}H_{17}Cl_2N_3$ calcd, 345.0800; found, 368.0703 ($M + H^+$).

2, 5-Dichloro-N-(4', 6-dimethoxy-[1,1'-biphenyl]-3-yl)pyrimidin-4-amine (**14p**).

Light yellow powder; yield, 81.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.33 (s, 1H), 7.53 – 7.42 (m, 4H), 7.12 (d, *J* = 8.8 Hz, 1H), 7.01 – 6.96 (m, 2H), 3.79 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.95, 158.30, 156.72, 154.35, 154.14, 130.81, 130.59 (2C), 130.01, 129.71, 124.61, 121.79, 113.61 (2C), 113.55, 111.72, 55.91, 55.31. HRMS for $C_{18}H_{15}Cl_2N_3O_2$ calcd, 375.0541; found, 398.0437($M + H^+$).

N-(3-bromo-4-methoxyphenyl)-2, 5-dichloropyrimidin-4-amine (**14q**).

Light yellow powder; yield, 86.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.73 (d, J = 2.4 Hz, 1H), 7.59 (dd, J = 8.8, 2.8 Hz, 1H), 7.11 (s, 1H), 6.94 (d, J = 8.8 Hz, 1H), 3.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.69, 149.85, 140.24, 131.33, 131.04, 130.53, 118.17, 114.70, 113.52, 113.34, 56.47. HRMS for $C_{18}H_8BrCl_2N_3O$ calcd, 346.9228; found, 346.2268(M^+).

2, 5-Dichloro-N-cyclohexylpyrimidin-4-amine (**14r**).

Light yellow powder; yield, 80.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 5.35 (s, 1H), 4.13 – 3.93 (m, 1H), 2.15 – 1.89 (m, 2H), 1.83 – 1.71 (m, 2H), 1.71 – 1.62 (m, 1H), 1.52 – 1.38 (m, 2H), 1.29 – 1.23 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.48, 157.96, 153.29, 112.98, 49.73, 32.69 (2C), 25.42, 24.64 (2C). HRMS for $C_{10}H_{13}Cl_2N_3$ calcd, 245.0487; found, 246.0563($M + H^+$).

N-((3s, 5s, 7s)-adamantan-1-yl)-2, 5-dichloropyrimidin-4-amine (**14s**).

Light yellow powder; yield, 72.3%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 5.25 (s, 1H), 2.14 (s, 9H), 1.72 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.07, 157.54, 153.13, 113.22, 53.86, 41.12, 41.12 (6C), 36.23 (6C), 29.47 (3C). HRMS for $C_{14}H_{17}Cl_2N_3$ calcd, 297.0800; found, 320.0700 ($M + Na^+$).

1-*(2,5-Dichloropyrimidin-4-yl)-1, 2, 3, 4-tetrahydroquinoline* (**14t**).

Yellow powder; yield, 75.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 7.20 (d, J = 6.8 Hz, 1H), 7.14 – 6.99 (m, 2H), 6.84 (d, J = 7.6 Hz, 1H), 3.83 (t, J = 6.4 Hz, 2H), 2.76 (t, J = 6.4 Hz, 2H), 2.07 – 1.81 (m, 2H). 13C NMR (101 MHz, DMSO-d_6) δ 159.91, 159.53, 157.47, 138.93, 130.79, 128.98, 126.14, 124.52, 121.15, 118.81, 47.59, 26.36, 23.82. HRMS for $C_{13}H_{11}Cl_2N_3$ calcd, 279.0330; found, 317.9968 (M + K^+). Chemical Formula:

4.1.5. General protocol for the synthesis of the final products **11a–k** Method B.

Hydrochloric acid (35% aq., 1.0 eq.) was dripped under stirring into a mixture of 2, 5-dichloro-N-(2-(isopropylsulfonyl)phenyl)pyrimidin-4-amine **10** (0.36 mmol) and aniline (0.3 mmol) in isopropanol (2.0 mL). The mixture was heated to 85 □ overnight, neutralized with saturated aqueous $NaHCO_3$, extracted with EtOAc (10 mL × 4), washed with saturated aqueous NaCl, and dried and purified through column chromatography on silica gel with petroleum ether/ethyl acetate as the eluent.

5-Chloro-N^4-(2-(isopropylsulfonyl)phenyl)-N^2-(4-methoxyphenyl)pyrimidine-2,4-diamine (**11a**).

Purple powder; yield, 26.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 9.36 (s, 1H), 8.62 (s, 1H), 8.25 (s, 1H), 7.84 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 (dd, J = 16.0, 4.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.42 – 7.33 (m, 1H), 6.86 (d, J = 8.0 Hz, 2H), 3.73 (s, 3H), 3.44 (dt, J = 12.0 Hz, 1H), 1.17 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.40, 155.72, 155.25, 155.17, 138.57, 135.34, 133.48, 131.37, 124.64, 124.18, 123.94, 122.18 (2C), 114.11 (2C), 104.61, 55.67, 55.37, 15.33 (2C). HRMS for $C_{20}H_{21}ClN_4O_3S$ calcd, 432.1023; found, 455.0926 (M + Na^+).

5-Chloro-N^2-(4-chlorophenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**11b**).

Yellow powder; yield, 53.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 9.47 (s, 1H), 8.54 (d, J = 8.0Hz, 1H), 8.32 (s, 1H), 7.87 (dd, J = 8.0, 1.6 Hz, 1H), 7.84 – 7.78 (m, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.43 (dd, J = 12.0, 4.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 3.45 (dt, J = 12.0, 8.0 Hz, 1H), 1.16 (d, J = 8.0Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.81, 155.58, 155.49, 139.57, 138.35, 135.38, 131.42, 128.67 (2C), 125.71, 125.39, 124.76, 124.40, 121.20 (2C), 105.69, 55.31, 15.31 (2C). HRMS for $C_{19}H_{18}Cl_2N_4O_2S$ calcd, 436.0528; found, 459.0433 (M + Na^+).

4-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)benzamide (**11c**).

White powder; yield, 20.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.49 (s, 1H), 8.55 (s, 1H), 8.36 (s, 1H), 7.89 (dd, J = 8.0, 1.6 Hz, 1H), 7.84 (t, J = 8.0 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 2H), 7.45 (t, J = 8.0 Hz, 1H), 7.19 (s, 1H), 3.47 (dt, J = 12.0, 8.0 Hz, 1H), 1.17 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.97, 157.74, 155.68, 155.58, 143.33, 138.30, 135.41, 131.45, 128.60 (2C), 127.49, 125.65, 125.03, 124.58, 118.41 (2C), 105.93, 55.29, 15.33 (2C). HRMS for $C_{20}H_{20}ClN_5O_3S$ calcd, 445.0975; found, 468.0872(M + Na^+).

3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)benzoic acid (**11d**).

Light yellow powder; yield, 47.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.94 (s, 1H), 9.48 (s, 1H), 8.52 (d, J = 8.0 Hz, 1H), 8.37 (s, 1H), 7.89 (dd, J = 8.0, 1.6 Hz, 1H), 7.86 – 7.82 (m, 1H), 7.79 (d, J = 12.0 Hz, 2H), 7.73 (d, J = 12.0 Hz, 2H), 7.47 (t, J = 8.0 Hz, 1H), 3.48 (td, J=12, 4.0 Hz, 1H), 1.16 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.56, 157.59, 155.66, 155.57, 144.85, 138.27, 135.34, 131.45, 130.57 (2C), 125.87, 125.21, 124.66, 123.68, 118.42 (2C), 106.29, 55.27, 15.31 (2C). HRMS for $C_{20}H_{19}ClN_4O_4S$ calcd, 446.0816; found, 485.0449 (M + K^+).

N^2-(4-Bromophenethyl)-5-chloro-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine (**11e**).

Light yellow powder; yield, 37.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 8.66 (d, J = 83.4 Hz, 1H), 8.11 (s, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.71 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 2H), 7.36 (s, 2H), 7.26 – 6.98 (m, 2H), 3.43 (dt, J = 12.0, 8.0Hz, 3H), 2.77 (s, 2H), 1.17 (d, J = 4.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.17, 156.34, 155.15, 139.51, 138.83, 137.22, 135.67, 131.58 (2C), 131.40 (2C), 126.82, 125.67, 124.94, 119.57, 115.3, 55.41, 55.07, 34.86, 15.34 (2C). HRMS for $C_{21}H_{22}BrClN_4O_2S$ calcd, 508.0335; found, 531.0261 ($M + Na^+$).

4-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)-2-fluoro benzonitrile **(11f).**

Light yellow powder; yield, 40.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 9.49 (s, 1H), 8.41 (s, 2H), 7.95 – 7.87 (m, 2H), 7.84 (t, J = 8.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 7.48 (dd, J = 16.0, 8.0 Hz, 2H), 3.47 (dt, J = 16.0, 8.0 Hz, 1H), 1.16 (d, J = 8.0 Hz, 6H).^{13}C NMR (101 MHz, DMSO-d_6) δ 164.89, 162.39, 157.02, 155.96, 155.52, 147.32, 137.98, 135.51, 134.01, 131.54, 126.75, 125.68, 125.23, 115.24, 107.37, 105.16, 91.20 55.17, 15.29 (2C). HRMS for $C_{20}H_{17}ClFN_5O_2S$ calcd, 445.0776; found, 468.0668 ($M + Na^+$).

3-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl) amino)benzonitrile **(11g).**

Light yellow powder; yield, 40.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 (s, 1H), 8.63 (s, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.19 (t, J = 8.0 Hz,1H) 3.45 (dt, J = 12.0, 8.0 Hz, 1H), 1.14 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.49, 155.66, 155.12, 146.18, 137.82, 135.22, 135.00, 134.29, 131.44, 130.75, 128.30, 124.14, 124.12, 122.48, 116.97, 112.60, 110.39, 55.51, 15.30 (2C). HRMS for $C_{20}H_{18}ClN_5O_2S$ calcd, 428.0870; found, 429.0769 ($M + H^+$).

2-((5-Chloro-4-((2-(isopropylsulfonyl)phenyl)amino)pyrimidin-2-yl)amino)phenol

(11h).

Light yellow powder; yield, 50.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.80 (s, 1H), 8.71 (s, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 8.33 (s, 1H), 7.86 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.74 – 7.65 (m, 1H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.45 – 7.37 (m, 1H), 7.14 (d, *J* = 16.0 Hz, 8.0 Hz, 1H), 6.96 (d, *J* = 3.6 Hz, 2H), 6.81 – 6.64 (m, 1H), 3.46 (dt, *J* = 6.8 Hz, 1H), 1.16 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (101 MHz, DMSO) δ 156.86, 156.14, 152.63, 149.35, 137.96, 135.37, 131.43, 128.59, 126.67, 126.0, 125.00, 124.79, 123.52, 119.27, 115.96, 105.02, 55.29, 15.30 (2C). HRMS for $C_{19}H_{19}ClN_4O_3S$ calcd, 418.0866; found, 419.0768 (M + H^+).

5-Chloro-N^2-(2-isopropoxy-5-methyl-4-morpholinophenyl)-N^4-(2-(isopropyl sulfonyl)phenyl)pyrimidine-2,4-diamine **(11i).**

Purple powder; yield, 23.8%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.51 (s, 1H), 8.57 (d, *J* = 8.0 Hz, 1H), 8.14 (s, 1H), 8.01 (s, 1H), 7.93 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.68 – 7.57 (m, 1H), 7.49 (s, 1H), 7.30 – 7.22 (m, 1H), 6.65 (s, 1H), 4.54 (dt, *J* = 12.0, 8.0 Hz, 1H), 3.91 – 3.78 (m, 4H), 3.36 – 3.15 (m, 1H), 2.94 – 2.81 (m, 4H), 2.15 (s, 3H), 1.38 (d, *J* = 4.0 Hz, 6H), 1.32 (d, *J* = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.24, 155.42, 45.72, 145.10, 138.44, 134.64, 131.28, 124.96, 124.69, 123.68, 123.19, 121.61, 105.66, 105.60, 71.85, 67.47 (2C), 55.49, 52.56 (2C), 22.26 (2C), 17.37, 15.37 (2C). HRMS for $C_{27}H_{34}ClN_5O_4S$ calcd, 559.2020; found, 560.2098 (M + H^+).

5-Chloro-N^2-(5-isopropoxy-2-methyl-4-(4-methylpiperazin-1-yl)phenyl)-N^4-(2-(isopropylsulfonyl)phenyl)pyrimidine-2,4-diamine **(11j).**

Light yellow powder; yield, 40.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 10.14 (s, 1H), 9.35 (s, 1H), 8.51 (s, 1H), 8.08 (d, J = 7.2 Hz, 1H), 7.94 (dd, J = 8.0, 1.6 Hz, 1H), 7.76 (dd, J = 12.0, 4.0 Hz, 1H), 7.57 (t, J = 7.2 Hz, 1H), 7.33 (s, 1H), 6.71 (s, 1H), 4.68 – 4.58 (m, 3H), 3.56 – 3.38 (m, 3H), 3.24 – 3.01 (m, 6H), 2.81 (d, J = 4.6 Hz, 3H), 1.96 (s, 3H), 1.26 (t, J = 12.0 Hz, 6H), 1.14 (dd, J = 14.0, 6..0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.40, 155.72, 155.25, 155.17, 138.57, 135.34, 133.48, 131.37, 124.64, 124.18, 123.94, 122.18, 114.11, 104.61, 72.03, 54.86, 53.22

(2C), 48.56 (2C), 25.96, 22.13 (2C), 17.57, 15.22 (2C). HRMS for $C_{28}H_{37}ClN_6O_3S$ calcd, 572.2336; found, 573.2411 ($M + H^+$).

5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperazin-1-yl)phenyl)-N^4-(2-(isopropyl sulfonyl) phenyl)pyrimidine-2,4-diamine **(11k).**

Light yellow powder; yield, 53.6%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (s,2H), 8.54 (d, J = 8.0 Hz, 1H), 8.16 (s, 1H), 8.05 (s, 1H), 7.93 (dd, J = 8.0,4.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.65 – 7.57 (m, 1H), 7.54 (s, 1H), 7.30 – 7.21 (m, 1H), 6.65 (s, 1H), 4.53 (dt, J = 12.0, 8.0 Hz, 1H), 3.43 (s,4H), 3.26 (dt, J = 12.0, 8.0 Hz, 1H), 3.18 (d, J = 4.0 Hz, 4H), 2.11 (s, 1H), 1.36 (d, J = 8.0 Hz, 6H), 1.32 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.59, 155.85, 155.31, 146.68, 138.54, 135.27, 131.41, 128.57, 125.97, 125.62, 125.31, 124.88, 124.19, 124.02, 106.64, 104.64, 71.46, 55.31, 49.26 (2C), 44.09 (2C), 22.36 (2C), 17.40, 15.32 (2C). HRMS for $C_{27}H_{35}ClN_6O_3S$ calcd, 558.2180; found, 559.2259 ($M + H^+$).

4.1.6. Synthesis of 5-chloro-N4-(2-(isopropylsulfonyl)phenyl)-N^2-(2-(3-morpholino propoxy)phenyl)pyrimidine-2,4-diamine (**12**)

First, 100 mg (0.24 mmol) of **11d**, 100 mg (0.72 mmol) of K_2CO_3, 33 mg (0.2 mmol) of 4-(3-chloropropyl)morpholine, and 5.0 mL of DMF were added to a flask. The reaction medium was stirred for 8 h at 60 □. The resulting suspension was cooled to room temperature and filtered. Then, the solvent was removed under reduced pressure, and the residue was purified through silica gel chromatography to obtain the desired product.

Light Yellow powder; yield, 53.5%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.47 (d, J = 8.0 Hz, 1H), 8.42 (s, 1H), 8.25 (s, 1H), 7.81 (dd, J = 8.0, 1.6 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.55 (t, J = 4.0 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 7.15 – 7.04 (m, 2H), 6.94 – 6.85 (m, 1H), 4.01 (t, J = 6.0 Hz, 2H), 3.52 – 3.46 (m, 4H), 3.46 – 3.40 (m, 1H), 2.29 – 2.17 (m, 6H), 1.85 – 1.74 (m, 2H), 1.16 (d, J =4.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.86, 155.83, 155.23, 151.26, 138.60, 135.15, 131.39, 128.73, 125.09, 124.25, 124.17, 123.74, 123.49, 120.69, 112.65, 104.91, 66.71, 66.57(2C),

55.43, 55.41, 53.78(2C), 26.26, 15.32(2C). HRMS for $C_{26}H_{32}ClN_5O_4S$ calcd, 545.1864; found, 546.1935 ($M + H^+$).

4.1.7. General synthesis of the final derivatives **16a–t**

The final derivatives were obtained from 4-amino-2,5-dichloropyrimidines **16a–t** (0.36 mmol) and amine (0.3 mmol) after 16 h of stirring (Method A). Then, the desired products were dissolved in dichloromethane and treated with ether hydrochloride solution for transformation into the corresponding salt.

*4-(2-((5-Chloro-2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)benzyl)pyrimidin-4-yl) amino)ethyl)phenol (***16a**).

Light yellow powder; yield, 46.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.91 (s, 1H), 7.44 (s, 1H), 7.26 (t, J = 5.7 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 6.80 (s, 1H), 6.67 (d, J = 8.4 Hz, 2H), 4.58 (dt, J = 12.1, 6.0 Hz, 1H), 3.64 – 3.50 (m, 2H), 3.05 (d, J = 11.5 Hz, 2H), 2.76 (dd, J = 16.0, 6.0Hz, 2H), 2.64 (dd, J = 20.0, 10.0 Hz, 2H), 2.12 (s, 3H), 1.64 – 1.48 (m, 4H), 1.29 (d, J = 6.0 Hz, 6H).^{13}C NMR (101 MHz, DMSO-d_6) δ 158.25, 157.94, 156.14, 153.49, 144.88, 138.46, 129.90 (2C), 129.79, 128.15, 126.80, 121.32, 115.58(2C), 111.93, 104.06, 71.48, 47.17, 42.97, 38.50, 34.47, 33.75, 22.43 (2C), 19.07. HRMS for $C_{27}H_{34}ClN_5O_2$ calcd, 495.2401; found, 496.2477 ($M + H^+$).

*5-Chloro-N^4-(4-fluorophenethyl)-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl) pyrimidine-2,4-diamine hydrochloride (***16b**).

Light yellow powder; yield, 55.7%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 9.23 (d, J = 16.0 Hz, 2H), 8.91 (s, 1H), 8.34 (s, 1H), 7.76 (s, 1H), 7.17 (dd, J = 8.0, 6.0 Hz, 2H), 7.08 (t, J = 8.0 Hz, 2H), 6.89 (s, 1H), 4.65 – 4.51 (m, 1H), 3.66 (dd, J = 16.0, 8.0 Hz,2H), 3.34 (d, J = 12.0 Hz, 2H), 3.12 – 2.95 (m, 3H), 2.89 (t, J =8.0 Hz, 2H), 2.15 (s, 3H), 2.06 – 1.88 (m, 2H), 1.79 (d, J = 12.0 Hz, 2H), 1.30 (d, J =8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.60, 160.20, 158.74, 151.38, 140.53, 135.21, 130.89 (2C), 130.81, 127.40, 124.78, 115.61 (2C), 115.40, 112.40, 104.38,

71.94, 44.05, 43.40, 35.37, 33.55, 29.13, 22.21 (2C), 18.80. HRMS for $C_{27}H_{33}ClFN_5O$ calcd, 497.2358; found, 498.2439(M + H^+).

*5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-phenethylpyrimidine-2,4-diamine (***16c***).*

Light yellow powder; yield, 59.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (s, 1H), 7.93 (s, 1H), 7.49 (s, 1H), 7.37 (t, *J* = 5.6 Hz, 1H), 7.31 - 7.27 (m, 2H), 7.23 -7.19 (m, 3H), 6.81 (s, 1H), 4.58 (dt, *J* = 12.0, 6.0 Hz, 1H), 3.71 – 3.60 (m, 1H), 3.11 (d, *J* = 12.0 Hz, 1H), 2.96 – 2.85 (m, 1H), 2.81 – 2.64 (m, 2H), 2.12 (s, 2H), 1.71 – 1.57 (m, 2H), 1.30 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.21, 157.97, 153.85, 144.90, 139.81, 137.39, 129.05 (2C), 128.78 (2C), 128.46, 127.04, 126.60, 121.35, 111.81, 104.16, 71.61, 45.53 (2C), 42.61, 36.77, 35.27, 31.38 (2C), 22.42 (2C), 19.05. HRMS for $C_{27}H_{34}ClN_5O$ calcd, 479.2452; found, 480.2527 (M + H^+).

*5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(4-methoxyphenethyl)pyrimidine-2,4-diamine (***16d***).*

Light yellow powder; yield, 59.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (s, 1H), 7.91 (s, 1H), 7.46 (s, 1H), 7.31 (t, *J* = 5.6 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 6.79 (s, 1H), 4.56 (dt, *J* = 12.0, 6.0 Hz, 1H), 3.71 (s, 3H), 3.59 (dd, *J* = 14.8, 6.0 Hz, 2H), 3.10 (d, *J* = 11.6 Hz, 2H), 2.87 – 2.78 (m, 2H), 2.78 – 2.65 (m, 3H), 2.12 (s, 3H), 1.60 (d, *J* = 13.5 Hz, 4H), 1.29 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.23, 158.16, 157.96, 153.47, 144.88, 137.88, 131.67, 129.98 (2C), 128.32, 126.92, 121.34, 114.19 (2C), 111.86, 104.12, 71.54, 55.43 (2C), 46.25, 42.82, 37.64, 34.38 (2C), 32.54, 22.40 (2C), 19.05. HRMS for $C_{28}H_{36}ClN_5O_2$ calcd, 509.2558; found, 510.2676 (M + H^+).

*5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(2-(thiophen-2-yl)ethyl)pyrimidine-2,4-diamine hydrochloride (***16e***).*

6-Light yellow powder; yield, 89.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.38 – 9.09 (m, 1H), 9.02 (s, 1H), 8.37 (s, 1H), 7.71 (s, 1H), 7.43 – 7.30 (m, 1H), 6.95

(dd, *J* = 5.0, 3.4 Hz, 1H), 6.88 (s, 1H), 6.82 (d, *J* = 2.8 Hz, 1H), 4.58 (dt, *J* = 12.0, 6.0 Hz, 1H), 3.71 (dd, *J* = 13.6, 6.9 Hz, 1H), 3.33 (d, *J* = 11.8 Hz, 1H), 3.13 (t, *J* = 7.4 Hz, 1H), 3.09 – 2.93 (m, 1H), 2.15 (s, 1H), 1.97 (dd, *J* = 23.8, 11.3 Hz, 1H), 1.78 (d, *J* = 12.6 Hz, 1H), 1.29 (d, *J* = 6.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.82, 151.42, 140.85, 140.66, 127.53, 127.46, 125.79, 124.81, 112.43, 104.45, 71.89, 45.89 (2C), 44.09, 35.41, 29.09 (2C), 22.22 (2C), 18.77, 8.90. HRMS for $C_{25}H_{32}ClN_5OS$ calcd; 485.2016, found, 486.2093 (M + H^+).

N^4-Benzyl-5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidine-2,4-diamine (**16f**).

Light yellow powder; yield, 43.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 (s, 2H), 7.94 (d, *J* = 120 Hz, 2H), 7.87 (t, *J* = 6.0 Hz, 1H), 7.44 (s, 1H), 7.30 (s, 4H), 7.22 (d, *J* = 6.0 Hz, 1H), 6.74 (s, 1H), 4.55 (d, J = 4.0 Hz), 4.50 (dt, J = 12.0, 8.0 Hz, 1H)3.27 (s, 2H), 3.09 – 2.82 (m, 3H), 2.06 (s, 3H), 1.86 (dd, *J* = 20.0, 8.0 Hz, 2H), 1.76 (d, *J* = 12.0 Hz, 2H), 1.27 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.19, 156.70, 151.30, 146.46, 142.11, 128.59, 127.98, 127.51, 127.08 (2C), 125.95, 125.28, 115.79 (2C), 111.93, 104.44, 71.97, 44.05 (2C), 35.34, 29.08, 25.96, 22.18 (2C), 18.71. HRMS for $C_{26}H_{32}ClN_5O$ calcd, 465.2295; found, 466.2373 (M + H^+).

(R)-5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(1-phenylethyl) pyrimidine-2,4-diamine (***16g***).

Light yellow powder; yield, 67.9%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (s, 1H), 7.93 (d, *J* = 9.4 Hz, 1H), 7.40 (dd, *J* = 14.6, 6.2 Hz, 3H), 7.29 (t, *J* = 7.5 Hz, 2H), 7.20 (t, *J* = 7.3 Hz, 1H), 6.78 (d, *J* = 16.8 Hz, 1H), 5.36 (p, *J* = 7.0 Hz, 1H), 4.51 (tt, *J* = 12.0, 6.2 Hz, 1H), 3.00 (dd, *J* = 23.8, 12.0 Hz, 3H), 2.23 (s, 2H), 1.84 (dd, *J* = 35.5, 12.2 Hz, 3H), 1.54 (dd, *J* = 18.2, 7.3 Hz, 2H), 1.27 (dd, *J* = 5.9, 1.3 Hz, 4H).^{13}C NMR (101 MHz, DMSO-d_6) δ 158.06, 157.30, 153.87, 136.61, 128.66 (2C), 128.59, 127.23, 127.12, 126.60 (2C), 121.62, 111.74, 104.15, 71.76, 50.00, 44.21 (2C), 35.28, 29.34, 22.55, 22.42 (2C), 22.38 (2C), 19.05. HRMS for $C_{27}H_{34}ClN_5O$ calcd, 479.2452; found, 480.1165 (M + H^+).

N^4-(3,5-bis(trifluoromethyl)phenyl)-5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidine-2,4-diamine (**16h**).

Light yellow powder; yield, 23.6%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.46 (s, 1H), 8.45 (s, 2H), 8.29 (s, 1H), 7.85 – 7.67 (m, 3H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.76 (s, 1H), 4.53 (dt, *J* = 12.0, 6.0 Hz, 1H), 3.15 – 2.90 (m, 3H), 2.29 (s, 2H), 2.09 (s, 3H), 1.81 (s, 4H), 1.25 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.76, 156.06, 155.90, 146.04, 145.72, 141.45, 138.19, 137.41, 130.97, 130.65, 127.96, 127.37, 125.12, 122.40, 111.85, 105.25, 71.56, 44.33 (2C), 35.31, 29.35, 22.24 (2C), 21.24, 18.84. HRMS for $C_{27}H_{28}ClF_6N_5O$ calcd, 587.1887; found, 588.1962 ($M + H^+$).

3-((5-Chloro-2-((2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)amino)pyrimidin-4-yl)amino)benzamide (**16i**).

Light yellow powder; yield, 25.0%; 1H NMR (400 MHz, DMSO-d6) δ 9.03 (s, 1H), 8.16 (s, 1H), 7.91 (s, 1H), 7.85 (d, J = 8.7 Hz, 2H), 7.80 (s, 1H), 7.71 (d, J = 8.0 Hz, 2H), 7.66 (s, 1H), 7.25 (s, 1H), 6.79 (s, 1H), 4.51 (dt, J = 12.0, 6.0 Hz, 1H), 3.32 (s, 2H), 3.08 – 2.89 (m, 3H), 2.08 (s, 3H), 2.01 – 1.85 (m, 2H), 1.80 (d, J = 12.0 Hz, 2H), 1.27 (d, J = 6.0 Hz, 6H). 13C NMR (101 MHz, DMSO-d6) δ 167.76, 158.16, 156.16, 155.67, 146.13, 141.96, 137.64, 129.41, 128.45 (2C), 128.04, 127.34, 123.04, 122.16 (2C), 111.89, 104.53, 71.59, 44.17 (2C), 35.29, 29.34 (2C), 22.42 (2C), 18.78. HRMS for $C_{26}H_{31}ClN_6O_2$ calcd, 494.2197; found, 495.2273 ($M + H^+$).

5-*Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(naphthalen-1-yl)pyrimidine-2,4-diamine* (**16j**).

Light yellow powder; yield, 54.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.84 (s, 2H), 8.13 (s, 1H), 8.00 (d, *J* = 7.2 Hz, 1H), 7.93 (d, *J* = 7.6 Hz, 1H), 7.86 (d, *J* = 7.6 Hz, 1H), 7.65 – 7.44 (m, 4H), 7.36 (s, 1H), 7.12 (s, 1H), 6.60 (s, 1H), 4.52 – 4.37 (m, 1H), 3.28 (d, *J* = 11.6 Hz, 2H), 2.93 (t, *J* = 11.6 Hz, 2H), 2.78 (s, 1H), 1.75 (d, *J* = 12.0 Hz, 2H), 1.65 (d, *J* = 12.4 Hz, 2H), 1.59 (s, 3H), 1.22 (d, *J* = 5.6 Hz, 6H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 158.52 (s), 157.79 (s), 154.96 (s), 144.10 (s), 135.83 (s), 135.06 (s), 134.52 (s), 130.71 (s), 128.67 (s), 128.18 (s), 127.33 (s), 127.09 (s), 126.68 (s), 126.60 (s), 126.30 (s), 125.71 (s), 123.83 (s), 120.16 (s), 111.11 (s), 104.02 (s), 71.62 (s), 44.17 (s), 35.14 (s), 29.25 (s), 22.32 (s), 18.48 (s). HRMS for $C_{29}H_{32}ClN_5O$ calcd, 501.2295; found, 502.2367 ($M + H^+$).

*2-((5-Chloro-2-((2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)amino)pyrimidin-4-yl)amino)phenol (***16k***).*

Light yellow powder; yield, 50.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 8.10 (s, 1H), 7.93 (d, *J* = 8.0 Hz, 1H), 7.72 (s, 1H), 7.63 (s, 1H), 6.94 (dt, *J* = 16.0, 8.0 Hz, 2H), 6.78 (s, 1H), 6.74 (t, *J* = 8.0 Hz, 1H), 4.53 (dt, *J* = 12.0, 6.0 Hz, 2H), 3.05 (d, *J* = 12.0 Hz, 2H), 2.67 (dt, *J* = 20.0, 12.0 Hz, 3H), 2.11 (s, 3H), 1.68 – 1.42 (m, 4H), 1.25 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.49, 155.87, 154.46, 149.56, 146.08, 139.28, 127.58, 126.99, 126.89, 124.80, 123.07, 122.59, 118.85, 115.48, 112.00, 104.39, 71.35, 46.18, 38.21, 33.28, 22.41 (2C), 18.83. HRMS for $C_{25}H_{30}ClN_5O_2$ calcd, 467.2088; found, 468.2163 ($M + H^+$).

4-((5-Chloro-2-((2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)amino)pyrimidin-4-yl)amino)phenol hydrochloride hydrochloride (**16l**).

Light yellow powder; yield, 55.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 9.68 (s, 1H), 9.27 (s, 1H), 9.10 (d, J = 9.2 Hz, 1H), 8.96 (d, J = 9.9 Hz, 1H), 8.42 (s, 1H), 7.50 (s, 1H), 7.27 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 9.0 Hz, 3H), 4.56 (dt, J = 12.0, 6.0 Hz, 1H), 3.33 (d, J = 12.0 Hz, 2H), 3.00 (dt, J = 24.0, 12.0 Hz, 3H), 2.03 (s, 3H), 1.90 (dd, J = 24.0, 12.0 Hz, 2H), 1.77 (d, J = 12.0 Hz, 2H), 1.28 (d, J = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.14, 158.08, 153.73, 144.89, 139.96, 136.58, 128.68 (2C), 128.54, 127.41 (2C), 127.19, 127.13, 121.38, 111.72, 104.15, 71.74, 44.14 (2C), 35.31, 29.30 (2C), 22.40 (2C), 18.84. HRMS for $C_{25}H_{30}ClN_5O_2$ calcd, 467.2088; found, 468.2162 ($M + H^+$).

5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(naphthalen-2-yl)pyrimidine-2,4-diamine hydrochloride (**16m**).

Light yellow powder; yield, 74.8%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 9.57 (s, 1H), 9.26 (d, J = 16.0 Hz, 2H), 8.56 (s, 1H), 8.08 (s, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.78 (d, J = 4.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.54 (d, J = 3.6 Hz, 2H), 7.37 (s, 1H), 6.81 (s, 1H), 4.67 – 4.42 (m, 1H), 3.29 (d, J = 12.0 Hz, 2H), 2.96 (d, J = 8.0 Hz, 2H), 2.86 (t, J = 12.0 Hz, 1H), 1.92 (d, J = 12.0 Hz, 2H), 1.67 (d, J = 12.0 Hz, 2H), 1.54 (s, 3H), 1.27 (d, J = 4.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.37, 151.78, 134.87, 133.40, 132.00, 128.85, 123.01, 112.10, 104.68, 71.96, 43.97 (2C), 35.24, 29.01 (2C), 22.18 (2C), 18.09. HRMS for $C_{29}H_{32}ClN_5O$ calcd, 501.2295; found, 502.2376 ($M + H^+$).

N^4-([1,1'-biphenyl]-3-yl)-5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidine-2,4-diamine hydrochloride (**16n**).

Light yellow powder; yield, 69.1%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.48 (s, 1H), 9.27 (d, J = 24.0 Hz, 2H), 8.54 (s, 1H), 7.82 (s, 1H), 7.68 – 7.54 (m, 4H), 7.51 (s, 2H), 7.40 (d, J = 4.0 Hz, 2H), 7.36 (d, J = 4.0 Hz, 1H), 6.80 (s, 1H), 4.56 (s, 1H), 3.28 (d, J = 9.8 Hz, 2H), 3.05 – 2.75 (m, 2H), 1.90 (d, J = 11.6 Hz, 1H), 1.84 (s, 2H), 1.65 (d, J = 12.2 Hz, 2H), 1.29 (d, J = 5.3 Hz, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.23, 141.06, 139.79, 137.86, 129.81, 129.39 (2C), 128.18, 127.33, 127.01 (2C), 125.36, 124.90, 124.41, 123.91, 123.55, 112.01, 104.71, 71.94, 43.98, 35.30, 28.99, 22.18 (2C), 18.62. HRMS for $C_{31}H_{34}ClN_5O$ calcd, 527.2452; found, 528.2530 ($M + H^+$).

5-Chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N^4-(2-methyl-1-(naphthalen-2-yl)propan-2-yl)pyrimidine-2,4-diamine hydrochloride (**16o**).

Light yellow powder; yield, 38.2%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 9.20 (s, 1H), 9.11 (s, 1H), 8.34 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 2H), 7.57 (s, 1H), 7.54 (s, 1H), 7.48 (s, 2H), 7.18 (d, J =8.0 Hz, 1H), 6.97 (s, 1H), 6.90 (s, 1H), 4.61 – 4.45 (m, 1H), 3.33 (d, J = 12.0 Hz, 2H), 3.23 (s, 2H), 3.04 (s, 3H),

2.25 (s, 3H), 1.97 (d, J = 12.0 Hz, 2H), 1.80 (d, J = 12.0 Hz, 2H), 1.39 (s, 6H), 1.26 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.46, 135.38, 133.29, 132.25, 129.30, 129.12, 127.91, 127.85, 127.73, 127.68, 126.53, 126.06, 113.01, 104.80, 71.97, 57.32, 56.49, 44.04, 35.47 (2C), 29.16 (2C), 26.78 (2C), 22.31 (2C), 18.52. HRMS for $C_{33}H_{40}ClN_5O$ calcd, 557.2921; found, 558.3000 ($M + H^+$).

5-Chloro-N^4-(4',6-dimethoxy-[1,1'-biphenyl]-3-yl)-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidine-2,4-diamine hydrochloride (**16p**).

Light yellow powder; yield, 78.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 9.50 (s, 1H), 9.20 (s, 2H), 8.51 (s, 1H), 7.53 (s, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 9.0 Hz, 3H), 7.15 (d, J = 8.7 Hz, 1H), 6.91 (d, J = 8.0 Hz, 2H), 6.82 (s, 1H), 4.67 – 4.45 (m, 1H), 3.79 (d, J = 20.0 Hz, 6H), 3.29 (d, J = 10.8 Hz, 2H), 3.07 – 2.82 (m, 3H), 1.89 (s, 5H), 1.69 (d, J = 12.0 Hz, 2H), 1.30 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.85, 158.59, 155.16, 150.95, 150.93, 146.19, 141.81, 139.81, 130.68 (2C), 129.99, 129.84, 128.16, 127.33, 125.87, 125.33, 123.11, 113.92 (2C), 112.33, 111.99, 104.55, 72.05, 56.32, 55.62, 43.98 (2C), 35.24, 29.02 (2C), 22.14 (2C), 18.60. HRMS for $C_{33}H_{38}ClN_5O_3$ calcd, 587.2663; found, 588.2745 ($M + H^+$).

N^4-(3-bromo-4-methoxyphenyl)-5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidine-2,4-diamine hydrochloride (**16q**).

Light yellow powder; yield, 45.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.25 (s, 1H), 9.50 (s, 1H), 9.34 – 9.09 (m, 2H), 8.52 (s, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.51 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.45 (s, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 6.83 (s, 1H), 4.58 (dt, *J* = 12.0, 6.0 Hz, 1H), 3.88 (s, 3H), 3.31 (d, *J* = 12.0 Hz, 2H), 3.10 – 3.01 (m, 3H), 1.90 (d, J=12.0 Hz, 2H), 1.74 (d, *J* = 12.0 Hz, 2H), 1.29 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.59, 160.19, 158.73, 151.30, 140.75, 140.41, 135.20, 135.17, 130.88, 130.80, 127.37, 125.00, 115.61, 115.40, 112.41, 104.36, 71.95, 44.03 (2C), 35.37, 33.55, 29.10 (2C), 22.20 (2C), 18.82. HRMS for $C_{26}H_{31}BrClN_5O_2$ calcd, 559.1350; found, 560.1427 ($M + H^+$).

5-Chloro-N^4-cyclohexyl-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl) pyrimidine-2,4-diamine hydrochloride (**16r**).

Light yellow powder; yield, 61.0%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 9.24 (d, J = 28.0 Hz, 2H), 8.51 (d, J = 4.0 Hz, 1H), 8.35 (s, 1H), 7.79 (s, 1H), 6.91 (s, 1H), 4.66 – 4.54 (m, 1H), 3.99 (d, J = 8.0 Hz, 1H), 3.33 (d, J = 8.0 Hz, 2H), 3.01 (d, J = 12.0 Hz, 3H), 2.30 (s, 3H), 2.08 – 1.89 (m, 2H), 1.81 (s,5H), 1.62 (t, J = 12.0 Hz, 1H), 1.52 (dd, J = 20.0, 12.0 Hz, 2H), 1.30 (t, J = 4.0 Hz, 6H), 1.27 – 1.17 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.79, 151.27, 127.14, 112.25, 104.19, 71.91, 56.48, 51.87, 44.06 (2C), 35.44, 31.56 (2C), 29.10 (2C), 25.60 (2C), 22.21 (2C), 19.12. HRMS for $C_{25}H_{36}ClN_5O$ calcd, 457.2608; found, 458.2685 ($M + H^+$).

N^4-((3s, 5s, 7s)-adamantan-1-yl)-5-chloro-N^2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidine-2,4-diamine hydrochloride (**16s**).

Light yellow powder; yield, 38.3%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.77 (s, 1H), 9.27 (s, 2H), 8.28 (s, 1H), 7.18 (s, 1H), 6.99 (s, 1H), 6.88 (s, 1H), 4.55 – 4.40 (m, 1H), 3.33 (d, J = 12.0 Hz, 2H), 3.03 (s, 3H), 2.23 (s, 3H), 2.10 – 1.97 (m, 2H), 1.95 (s, 5H), 1.90 (s, 3H), 1.77 (d, J = 12.0 Hz, 2H), 1.51 (d, J = 12.0 Hz, 3H), 1.36 (d, J = 8.0 Hz, 3H), 1.32 – 1.26 (m, 1H), 1.23 (d, J = 8.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.10, 151.97, 150.42, 142.63, 140.97, 129.44, 127.64, 124.14, 113.04, 104.39, 71.65, 56.48, 54.97 (2C), 44.04 (2C), 35.89 (3C), 35.49, 29.25 (3C), 28.99, 22.32 (3C), 18.48. HRMS for $C_{29}H_{40}ClN_5O$ calcd, 509.2921; found, 510.2997 ($M + H^+$).

5-Chloro-4-(3, 4-dihydroquinolin-1(2H)-yl)-N-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)pyrimidin-2-amine (**16t**)

Light yellow powder; yield, 53.4%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24 (s, 1H), 7.89 (s, 1H), 7.82 (s, 1H), 7.16 (d, J = 7.2 Hz, 1H), 7.06 (t, J = 7.2 Hz, 1H), 7.00 – 6.91 (m, 1H), 6.80 (s, 1H), 6.76 (d, J = 8.0 Hz, 1H), 4.56 (dt, J = 12.0, 6.0 Hz, 1H), 3.82 (t, J = 6.0 Hz, 2H), 3.33 (d, J = 12.0 Hz, 2H), 2.98 (dd, J = 23.6, 11.2 Hz, 3H), 2.78 (t, J = 6.4 Hz, 2H), 2.16 (s, 3H), 2.04 – 1.85 (m, 4H), 1.78 (d, J = 12.8 Hz, 2H), 1.30 (d, J = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d6) δ 159.59, 158.55, 158.27,

145.39, 140.23, 137.29, 129.15 (2C), 128.00, 127.23, 126.21, 122.89, 122.05, 120.62, 111.66, 110.03, 71.58, 47.57, 44.20 (2C), 35.38, 29.37 (2C), 26.64, 23.68, 22.38 (2C), 19.01.HRMS for $C_{28}H_{34}ClN_5O$ calcd, 491.2452; found, 492.2495($M + H^+$).

4.2 Antimycobacterial activity evaluation *in vitro*

Selectable marker-free autoluminescent *Mtb* H37Ra（UAlRa）[17] were cultured in a 250 mL flask containing 30 mL Middlebook 7H9 medium plus 0.05% Tween 80, 10% V/V oleic acid albumin dextrose catalase (OADC) supplement (7H9-OADC-Tw). When OD_{600} reached 0.5-0.8, relative light unit (RLU) value was measured by injecting 200 μL broth culture into 1.5 mL tube and putting it on the detection hole of the luminometer. When the RLU of broth culture reached over 5 million/mL, the activities of compounds were assayed with a concentration range of 2-fold decreasing from 100 μg/mL to 0.625 μg/mL prepared. UAlRa broth culture was diluted to 10,000-50,000 RLU/mL in 7H9 broth without Tween80. In the different groups, DMSO (2%) was used as negative controls and rifampicin (RIF, 1μg/mL) was used as positive controls. RLU values were determined every day until day 6. MIC is defined as the lowest drug concentration that can achieve the ratio (RLU_{drug}/RLU_{DMSO}) less than 10%.

4.3. Antitubercular activity evaluation *in vivo*

UAlRa isolated on plates were cultured in a 250 mL flask containing 50 mL 7H9-OADC-Tw with sterile glass beads. When culture OD_{600} reached 0.5-1.0 and 10,000 RLU/mL, the culture was used to infect 4-to-5-week-old female BALB/c mice by tail vein injection [20]. The day after infection (day 0), RLU counts were determined in live mice and organs. Firstly the mice were narcotized by isoflurane inhalation, then putting the breast of mice on the detection hole of the luminometer and detecting RLU for 3 seconds for twice. Mice with similar RLU values were randomly assigned to treatment groups. Four mice each group and individually marked. The treatment dosages by gavage: 0.5% CMC-Na (solvent) alone as a negative control, RIF (10 mg/kg) and PZA (150mg/kg) [21] as positive controls.

Treatment was administrated daily from day 0 to day 5. Mice were sacrificed at day0 as a base line and at day 6 for activity comparison. The lungs and spleens of mice were ground and RLUs were detected by luminometer.

4.4. Activity of the derivatives in combination with SMX against *Mtb* H37Ra

UAlRa was used to determine FICIs of compounds combined with SMX. UAlRa was grown in 7H9-OADC-Tw medium to exponential phase. The compound concentrations used were in a range of 2-fold decreasing from 2×MICs to MIC/16 prepared in UAlRa broth culture diluted in 7H9 broth without Tween80 with 10,000-50,000 RLU/mL. Each concentration of compounds was combined with SMX at 16, 8, 4, 2 μg/mL, respectively. DMSO (2%) was used as a negative control. RLU values were determined after incubation for 7 daysat 37□. The MIC was defined as the lowest drug concentration that can achieve the ratio (RLU_{drug}/RLU_{DMSO}) less than 10%. FICI was calculated as follows: FICI = ($MIC_{drug\ A\ in\ combination}/MIC_{drug\ A\ alone}$) + ($MIC_{drug\ B\ in\ combination}/MIC_{drug\ B\ alone}$). Combinations were considered synergistic if the FICI was ≤ 0.5, partially synergistic if the FICI was > 0.5 and < 1.0, additive if FICI was =1.0, indifferent if FICI was > 1.0 and ≤ 4.0, or antagonistic if the FICI was > 4.0 [19].

4.5. Molecular docking

The molecular docking experiment was performed with Discovery Studio 3.1. The crystal structure of DHFR (PDB ID: 1DG5) was retrieved from PDB. The protein structure was prepared for docking simulation by using the prepare protein tool implemented in DS 3.1 under the CHARMM force field. The binding site was defined as a sphere with a radius maintained within 15 Å from the binding site of TMP [15]. We also used the LibDock module to observe the intermolecular H bonds or π–π interactions to investigate the potential binding modes among ceritinib, 16c, 16d, 16j and DHFR.

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Highlights

- A series of novel pyrimidine derivatives was designed and synthesized.
- Evaluation of anti-mycobacterial activity against *Mtb* H37Ra *in vitro.*
- Evaluation of anti-tubercular activity *in vivo*.
- Identification of compound **16j** as a promising lead for further investigation.