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Development and evaluation of 2,4-disubstituted-5-aryl pyrimidine derivatives as antibacterial agents

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Abstract

Designing novel candidates as potential antibacterial scaffolds has become crucial due to the lack of new antibiotics entering the market and the persistent rise in multidrug resistance. Here, we describe a new class of potent antibacterial agents based on a 5-aryl-N^2,N^4-dibutylpyrimidine-2,4-diamine scaffold. Structural optimization focused on the 5-aryl moiety and the bioisosteric replacement of the side chain linker atom. Screening of the synthesized compounds focused on a panel of bacterial strains, including gram-positive *Staphylococcus aureus* strains (Newman MSSA, methicillin- and vancomycin-resistant), and the gram-negative *Escherichia coli* (ΔAcrB strain). Several compounds showed broad-spectrum antibacterial activity with compound **12**, bearing a 4-chlorophenyl substituent, being the most potent among this series of compounds. This frontrunner compound revealed a minimum inhibitory concentration (MIC) value of 1 µg/mL against the *S. aureus* strain (Mu50 methicillin-resistant *S. aureus*/vancomycin-intermediate *S. aureus*) and an MIC of 2 µg/mL against other tested strains. The most potent derivatives were further tested against a wider panel of bacteria and evaluated for their cytotoxicity, revealing further potent activities toward *Streptococcus pneumoniae*, *Enterococcus faecium*, and *Enterococcus faecalis*. To explore the mode of action, compound **12** was tested in a macromolecule inhibition assay. The obtained data were supported by the safety profile of compound **12**, which possessed an IC_{50} of 12.3 µg/mL against HepG2 cells. The current results hold good potential for a new class of extended-spectrum antibacterial agents.

KEYWORDS
2,4-diaminopyrimidines, antibacterial agents, antibiotic resistance

1 | INTRODUCTION

The introduction of antibiotics to treat infectious diseases revolutionized the practice of medicine in the 20th century.[1] However, the significant increase in multidrug-resistant (MDR) bacteria remains a threat that calls for an urgent search for new antimicrobial agents.[2,3] Many MDR bacterial strains such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Enterococcus faecium*, and *Enterococcus faecalis*, which cause serious infections, are often referred to as "superbugs." Superbugs are microbial strains with multiple mutations that lead to antibiotic resistance to most antibiotics.[4]

Methicillin-resistant *S. aureus* (MRSA) is recognized as the most common cause of severe infections. In a recent study, MRSA was shown to cause more than 100,000 deaths attributable to AMR in 2019.[5] This, in large part, is due to the practical ineffectiveness of most ß-lactam antibiotics regarding these virulent strains.[6] While vancomycin remains a basic treatment for drug-resistant MRSA infections, the rise of nonsusceptible *S. aureus* strains (vancomycin-intermediate *S. aureus* [VISA] or vancomycin-resistant *S. aureus* [VRSA]) has not complimented its frequent use.[7] Indeed, broad-spectrum antibiotics are widely used to treat both gram-positive and gram-negative bacterial infections. Nevertheless, their use is regarded as a last resort attributed to the high probability of severe adverse effects.[8]

It was anticipated by the World Health Organization (WHO) almost a decade ago that the world is heading toward a postantibiotic era where common infections will no longer be therapeutically manageable and thus fatal once more.[9] In 2019, the WHO declared antimicrobial resistance (AMR) as one of the top 10 threats to world health due to its impact on human health. [10] Hence, a profound solution to this never-ending battle of emerging antibacterial resistance would be the search and design of novel scaffolds to combat bacterial infections with an unprecedented mode of action.[11]

On such grounds, we investigated the potential antibacterial activity of a novel class of pyrimidine-derived compounds. The complex 2,4-pyrimidinediamine scaffold is of particular interest as it constitutes a composite part of the FDA-approved antimetabolite trimethoprim (TMP) (Figure 1).[12,13] In addition to the not yet approved iclaprim, which is a more potent drug with expanded coverage against TMP-resistant pathogens.[14]

Seleem et al. explored the potency of a pyrimidine-containing phenylthiazole antibacterial class against MRSA. Compound **I** (Figure 1) showed a submicromolar MIC value of 0.4 µg/mL.[15] Moreover, Seenaiah et al. disclosed a pyrimidinyl bis-methylthio benzimidazole compound **II** (Figure 1) as a potential lead for novel antimicrobial agents with a reported minimum inhibitory concentration (MIC) value of 12.5 µg/mL against *S. aureus*, in addition to 50 and 12.5 µg/mL against *E. coli* and *P. aeruginosa*, respectively.[16] Bai et al. also demonstrated the significance of a pyrimidine derivative bearing a benzyloxyphenyl (compound **III**, Figure 1) in fighting bacterial infections.[17] Another study evaluated a novel pyrimidine-containing compound **IV** (Figure 1) with reported MIC values of 21.2 µM versus *S. aureus*, 10.6 µM versus *B. subtilis* and 42.3 µM versus *E. coli*.[18]

We, among others, have previously reported a novel class of quinazoline derivatives (**V**) with broad-spectrum antibacterial activity.[19–21] Herein, we applied a diversity-oriented synthesis (DOS) approach through skeletal (scaffold) modification to design novel molecules with promising antibacterial activity.[22,23] To this end, we aimed to dissect the quinazoline scaffold (**V**) to the 5-arylpyrimidine core (**VI**), keeping a butylamino chain at positions 2 and 4 that showed high potency within the quinazoline series.[20,21] Structural modifications included the 5-aryl moiety besides the bioisosteric replacement of the linker heteroatom (Figure 2).

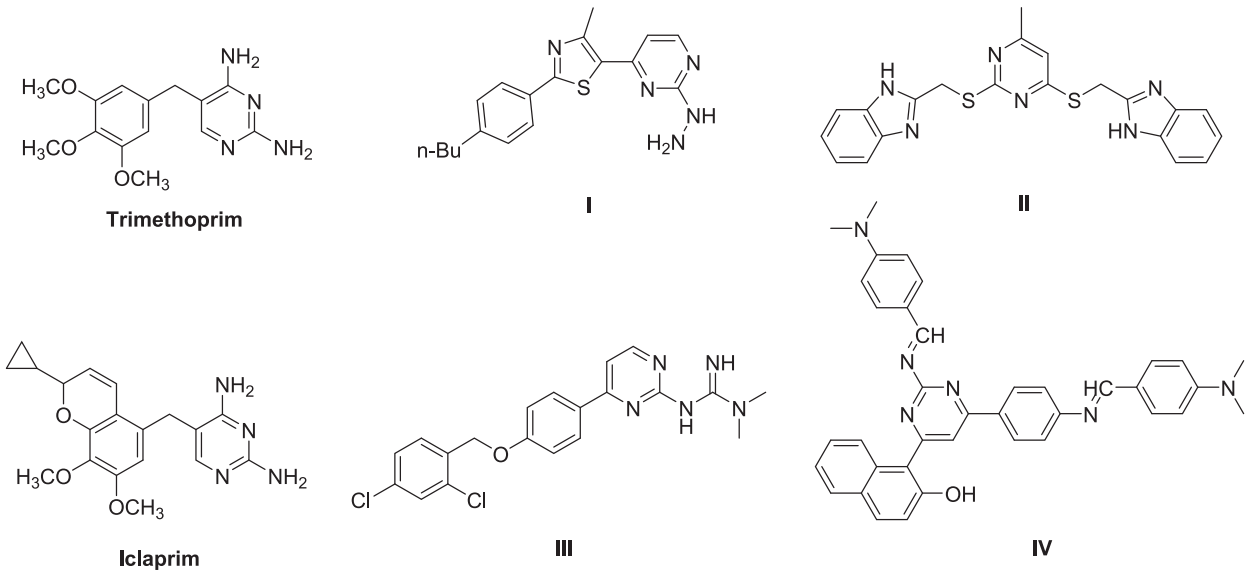


FIGURE 1 Reported pyrimidine derivatives as antibacterial agents.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The 2,4-diamino substitution in (Scheme 1) was achieved by S_NAr reaction of the commercially available 5-bromo-2,4-dichloropyrimdine with excess butylamine to afford 5-bromo-N^2,N^4-dibutylpyrimidine-2,4-diamine (**A**) in good yield. This was followed by palladium-catalyzed Suzuki–Miyaura cross-coupling of the resulting intermediate with various arylboronic acids in the presence of a base to give 5-aryl-N^2,N^4-dibutylpyrimidine-2,4-diamines (**1**–**28**). Similarly, symmetric substitution at positions 2 and 4 of the pyrimidine core with 1-butanol or 1-butanethiol afforded intermediates **B** and **C** (Scheme 2). Next, the intermediates were reacted with selected arylboronic acids to form the 5-aryl-2,4-disubstituted pyrimidine derivatives (**29**–**34**). Selective substitution occurred in position 4 of the pyrimidine (Scheme 3), providing 5-bromo-2-chloro-*N*-butylpyrimidine-4-amine (**D1**).[24] Subsequently, position 2 of the scaffold was isosterically substituted by either 2-butoxy or 2-butylthio derivatives (**D2**–**D3**) as depicted in Scheme 3. Subsequently, the synthesized intermediates were reacted with the appropriate arylboronic acids to yield the coupled products (**35**–**37**). Alkylation of 5-bromouracil was performed through its reaction with n-butylbromide to give 5-bromo-1,3-dibutylpyrimidine-2,4-(1*H*,3*H*)-dione (**E**) in good yield, which was consequently coupled with substituted arylboronic acids to form compounds (**38**–**40**) (Scheme 4).

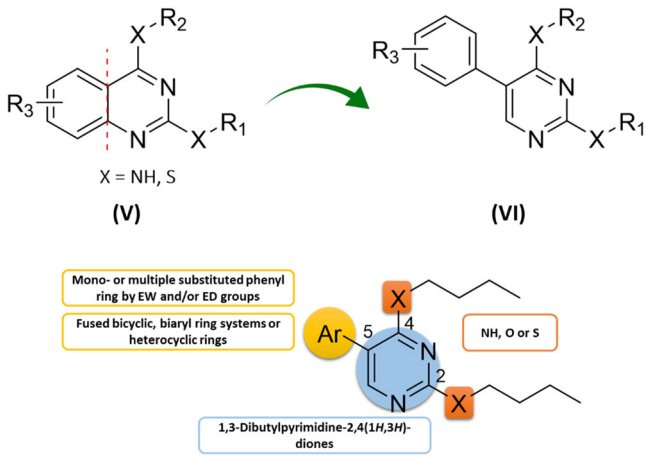


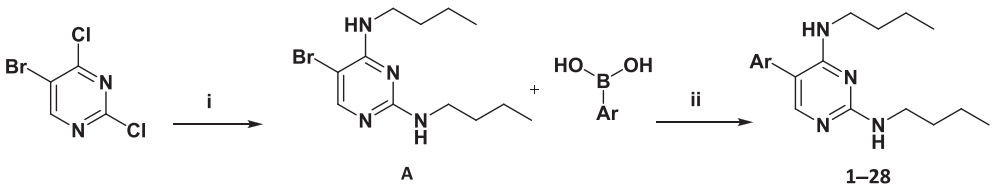
FIGURE 2 Design strategy and modification plan.

2.2 | Biological evaluation

All synthesized compounds were screened against a panel of bacterial strains, including gram-positive *S. aureus* strains; (Newman MSSA, N315 MRSA/VSSA, and Mu50 MRSA/VISA), and gram-negative *E. coli* ΔacrB (null AcrB *E. coli* isolate is devoid of the multidrug efflux transporter AcrB), at concentrations ranging from 0.06 to 128 μg/mL using linezolid and vancomycin as positive controls. The results are shown in Tables 1–4.

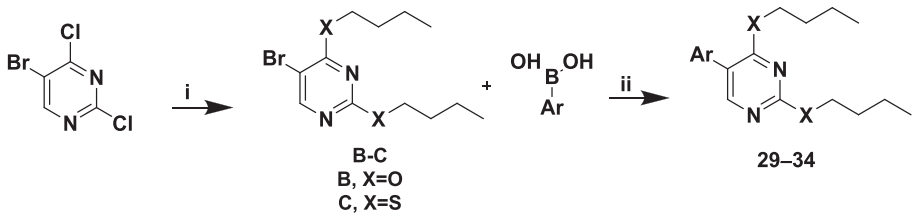
2.3 | Structure–activity relationships (SAR)

Initially, compound **1** was synthesized as a prototype pyrimidine-analog to evaluate the N^2,N^4-dibutylpyrimidine-2,4-diamine as a potential antibacterial scaffold. Compound **1** showed good potency against *S. aureus* strains; (Newman MSSA, N315 MRSA/VSSA), especially against Mu50 MRSA/VISA (MIC = 2 μg/mL). Also, compound **1** exhibited promising activity against *E. coli* ΔacrB having an MIC value of 4 μg/mL (Table 1). These preliminary findings corroborated the use of the 2,4-disubstituted pyrimidine motif as a template scaffold and encouraged us to proceed with further structural modifications to improve the antibacterial activity and explore the novel scaffold.



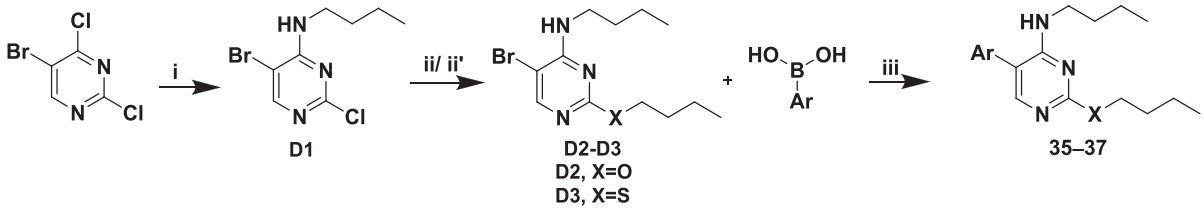
Cpd No.	Ar	Cpd No.	Ar	Cpd No.	Ar	Cpd No.	Ar
1	phenyl	8	4-methylphenyl	15	3-cyanophenyl	22	3-fluoro-5-methoxyphenyl
2	2-methoxyphenyl	9	3-fluorophenyl	16	3,4-dimethoxyphenyl	23	3-chloro-4-methoxyphenyl
3	3-methoxyphenyl	10	4-fluorophenyl	17	3,4-dimethylphenyl	24	4-methoxy-3,5-dimethylphenyl
4	4-methoxyphenyl	11	3-chlorophenyl	18	3,4-difluorophenyl	25	4-(benzyloxy)phenyl
5	3-ethoxyphenyl	12	4-chlorophenyl	19	3,5-difluorophenyl	26	naphthalen-2-yl
6	3-hydroxyphenyl	13	3-(trifluoromethoxy)phenyl	20	4-methoxy-3-methylphenyl	27	thiophen-3-yl
7	3-methylphenyl	14	3-nitrophenyl	21	3-fluoro-4-methoxyphenyl	28	furan-3-yl

SCHEME 1 Synthesis of compounds **1**–**28**. Reagents and conditions: (i) excess n-BuNH_2, dioxane, MW, 150°C, 30 min, yield 93.6%; (ii) arylboronic acid, [Pd(dppf)Cl_2], Na_2CO_3, dioxane/H_2O (4:1), MW, 150°C, 30 min, yield 10.5%–84.6%. MW, microwave.



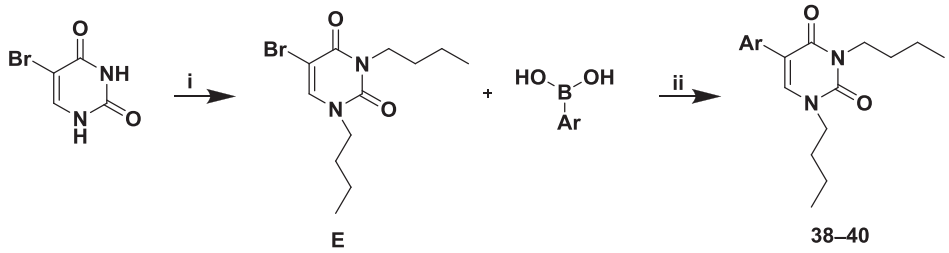
Cpd No.	X	Ar	Cpd No.	X	Ar
29	O	4-chlorophenyl	32	S	4-chlorophenyl
30	O	4-methoxy-3,5-dimethylphenyl	33	S	4-methoxy-3,5-dimethylphenyl
31	O	4-(benzyloxy)phenyl	34	S	naphthalen-2-yl

SCHEME 2 Synthesis of compounds **29–34**. Reagents and conditions: (i) Na, excess n-BuOH, 0°C, 20 min, then RT, overnight (**B**, X=O, yield 98%), or Na, THF, 0°C, 1-butanethiol, 20 min, then RT, overnight (**C**, X=S, yield 89%); (ii) arylboronic acid, $[Pd(dppf)Cl_2]$, Na_2CO_3, dioxane/H_2O (4:1), MW, 150°C, 30 min, yield 10.6%–42.7%. MW, microwave; RT, room temperature; THF, tetrahydrofuran.



Cpd No.	X	Ar
35	O	4-chlorophenyl
36	O	4-methoxy-3,5-dimethylphenyl
37	S	4-chlorophenyl

SCHEME 3 Synthesis of compounds **35–37**. Reagents and conditions: (i) excess n-$BuNH_2$, dioxane, 0°C, 0 min, yield 82.8%; (ii) NaH, excess n-BuOH, 0°C, 30 min, then add **D1**, MW, 150°C, 30 min → (**D2**, X=O, yield 73%); (ii') Na, THF, 0°C, 1-butanethiol, 20 min, then add **D1**, RT, overnight → (**D3**, X=S, yield 66.7%); (iii) arylboronic acid, $[Pd(dppf)Cl_2]$, Na_2CO_3, dioxane/H_2O (4:1), MW, 150°C, 30 min, yield 14.4%–49%. MW, microwave, RT; room temperature; THF, tetrahydrofuran.



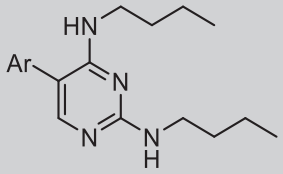
Cpd No.	Ar
38	4-chlorophenyl
39	3-methylphenyl
40	4-(benzyloxy)phenyl

SCHEME 4 Synthesis of compounds **38–40.** Reagents and conditions: (i) K_2CO_3, DMF, 5-bromouracil, 30 min, RT, n-BuBr, 70°C, overnight, yield 75%; (ii) arylboronic acid, $[Pd(dppf)Cl_2]$, Na_2CO_3, dioxane/H_2O (4:1), microwave, 150°C, 30 min, 26.5%–75.6%. DMF, dimethylformamide; MW, microwave; RT, room temperature.

2.3.1 | Position 5: Aryl group modifications

Initial efforts focused on the optimization of the 5-aryl ring system. Hence, we synthesized a pool of diversified 5-substituted aryl pyrimidine analogs bearing electron-donating (ED) and/or electron-withdrawing (EW) groups, hydrophobic or polar groups, bulkier diaryl ring systems or heteroaromatic rings (compounds **2–28**) and evaluated for their antibacterial potency.

TABLE 1 Minimum inhibitory concentration (MIC) results of 5-(substituted)aryl-N^2,N^4-dibutylpyrimidine-2,4-diamine series.



1–28

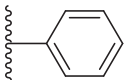
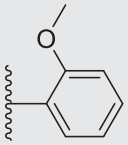
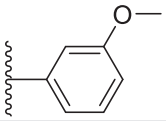
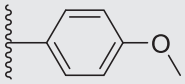
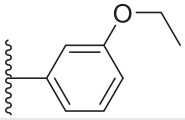
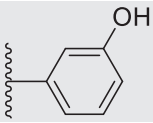
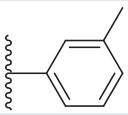
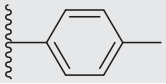
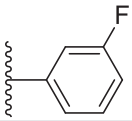
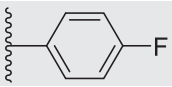
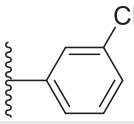
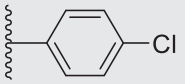
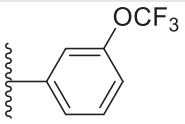
Compound	Ar	MIC (μg/mL) *Staphylococcus aureus* Newman MSSA	*Staphylococcus aureus* N315 MRSA/VSSA	*Staphylococcus aureus* Mu50 MRSA/VISA	*Escherichia coli* ΔacrB
1		8	8	2	4
2		16	8	8	8
3		8	8	4	8
4		8	8	8	4
5		16	16	8	64
6		16	16	8	16
7		4	8	4	4
8		4	4	2	2
9		8	8	2	16
10		8	8	4	8
11		4	4	2	4
12		2	2	1	2
13		4	4	2	32

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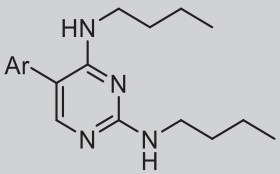
TABLE 1 (Continued)



1–28

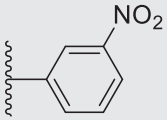
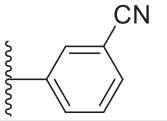
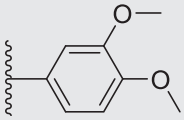
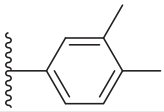
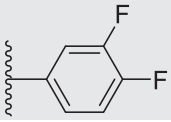
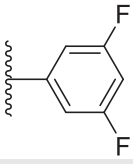
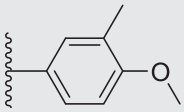
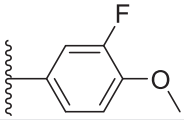
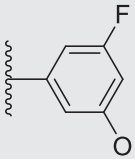
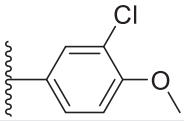
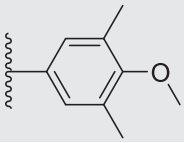
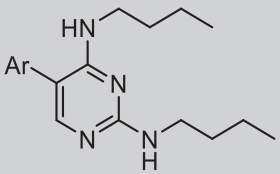
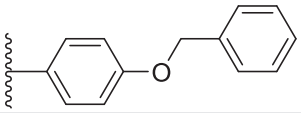
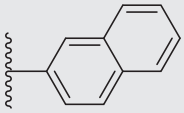
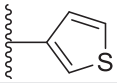
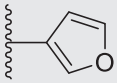
Compound	Ar	MIC (μg/mL) *Staphylococcus aureus* Newman MSSA	N315 MRSA/VSSA	Mu50 MRSA/VISA	*Escherichia coli* ΔacrB
14		16	8	8	16
15		16	16	8	16
16		64	64	64	32
17		4	4	2	4
18		8	16	8	16
19		8	16	8	8
20		4	4	4	4
21		16	16	8	8
22		8	8	4	8
23		8	8	8	8
24		4	4	4	4

TABLE 1 (Continued)



1–28

Compound	Ar	MIC (μg/mL) *Staphylococcus aureus* Newman MSSA	N315 MRSA/VSSA	Mu50 MRSA/VISA	*Escherichia coli* ΔacrB
25		4	4	4	128
26		4	4	4	4
27		8	8	2	8
28		16	16	16	16
-	Linezolid	1	2	2	16
-	Vancomycin	0.5	1	8	>64

Note: Results shown are the mean of at least two independent experiments, SD ≤10%.

First, we investigated the effect of monosubstitution with the slightly polar, ED methoxy group on the phenyl at the *ortho*-, *meta*- and *para*-positions (compounds **2**, **3**, and **4**, respectively). All three compounds were found to have promising activity and coverage over the tested strains. Notably, the activity of compounds **3** and **4** superseded that of the *ortho*-substituted analog (compound **2**). Therefore, further exploratory modifications focused more on the *meta* and *para* positions. Next, extending the ether side chain of compound **3** was considered, where an ethoxy group was introduced (compound **5**). This elicited a twofold decrease in activity against the three *S. aureus* strains and abolished the efficacy against the gram-negative *E. coli* ΔacrB. Switching the ether functionality to the more polar hydroxyl group (compound **6**) exhibited a similar anti-gram-positive activity to the 3-ethoxy analog but recovered a moderate antibacterial activity against *E. coli* ΔacrB (MIC = 16 μg/mL).

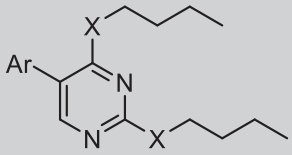
We observed a noteworthy improvement in activity by using the slightly lipophilic, yet ED methyl group at positions 3 or 4 (compounds **7** and **8**, respectively). Compound **8** was found to be slightly more potent than its positional isomer **7**, with MIC values ranging from 2 to 4 μg/mL, which gave us a preliminary idea that the *para*-substituted phenyl might be more favorable for the antibacterial activity.

On the other hand, mono-substitution of the phenyl ring with the lipophilic, EW fluorine at positions 3 or 4 (compounds **9** and **10**, respectively) showed a decent spectrum of activity versus the four strains. The overall activity was favored with the 4-fluorophenyl analog (compound **10**), which further confirmed the previous observation that the *p*-substituted analogs were superior. In an attempt to further increase the potency by tuning at the phenyl ring substitution, we synthesized compounds **11** and **12**, bearing a mono-chloro substitution, at the same positions. It was obvious that compound **12** (with the *para*-chlorophenyl substituent) prevailed, further confirming the enhanced activity manifested by the *p*-substituted analogs. Compound **12** demonstrated strong antibacterial activity against the most virulent *S. aureus* strain (Mu50 MRSA/VISA) with an MIC of 1 μg/mL.

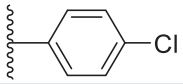
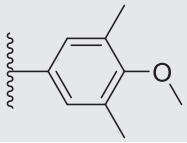
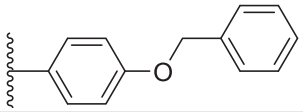
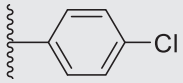
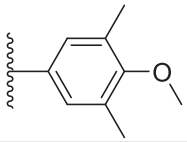
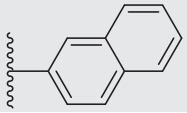
On the other hand, compounds **14** and **15**, bearing an EW 3-nitro function, and the more hydrophilic 3-cyano group, respectively, significantly reduced the activity compared to other analogs with EW-substituted phenyl rings (compounds **9**–**12**). Introduction of the trifluoromethoxy group (compound **13**) preserved a potent activity against the gram-positive strains, but displayed reduced activity toward the *E. coli* ΔacrB (MIC = 32 μg/mL).

In light of these findings, the idea of combining favorable groups was conceivable for a possible synergistic effect on bacterial growth inhibition. We began by conjoining similar ED or EW groups. A 3,4-dimethoxy substitution was attempted (compound **16**). Rather unexpectedly, the inhibitory activity against the three gram-positive strains was abolished, and only little activity was preserved toward both *E. coli* ΔacrB (MIC = 32 μg/mL). On the contrary, when the 3,4-dimethyl groups were used (compound **17**), the MIC values were restored to ranges from 2 to 4 μg/mL, suggesting that lipophilic

TABLE 2 Minimum inhibitory concentration (MIC) results of 2,4-di-*O*-butyl and 2,4-di-S-butyl analogs.



29–34

Compound	X	Ar	MIC (μg/mL) *Staphylococcus aureus* Newman MSSA	N315 MRSA/VSSA	Mu50 MRSA/VISA	*Escherichia coli* ΔacrB
29	O		>64	>64	>64	>64
30	O		32	>128	>128	>128
31	O		>128	>128	>128	>128
32	S		>64	>64	>64	>64
33	S		>128	>128	>128	>128
34	S		>128	>128	>128	>128
-	-	Linezolid	1	2	2	16
-	-	Vancomycin	0.5	1	8	>64

Note: Results shown are the mean of at least two independent experiments, SD ≤10%.

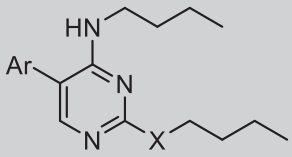
groups are better tolerated in terms of activity. However, the added lipophilicity in compound **17** did not offer an extra advantage with respect to the single substituted 4-methylphenyl analog (compound **8**), which had similar MIC values. Similarly, introduction of two fluorine substituents in compounds **18** and **19** did not improve the activity relative to the mono-substituted analogs (compounds **9** and **10**).

Combinations of a halogen along with a methoxy group with different patterns (compound **21**: 3-fluoro-4-methoxyphenyl, compound **22**: 3-fluoro-5-methoxyphenyl, and compound **23**: 3-chloro-4-methoxyphenyl) significantly decreased the overall activity, whereas mono-substitution using each group alone was better tolerated. Finally, dissimilar di- as well as tri-substitution with ED groups was displayed in compounds **20** and **24**, proved to have broad-spectrum antibacterial activity with an MIC value of 4 μg/mL against both the gram-positive and -negative strains, but again the mono-substituted compound **8** superseded these combinations as well.

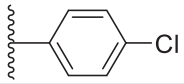
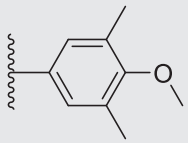
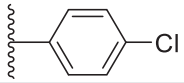
The use of fused or extended aromatic ring systems (compounds **25** and **26**) sustained a good activity of 4 μg/mL versus all three gram-positive strains. In contrast to compound **26** (the 2-naphthyl analog), the 4-benzyloxyphenyl in compound **25** was deleterious to the activity toward *E. coli* ΔacrB.

Next, we decided to test the impact of isosteric replacement of the 5-phenyl moiety with heteroaryl rings by introducing thiophen-3-yl and furan-3-yl instead (compounds **27** and **28**, respectively). Compound **27** showed similar potency to compound **1**; however, both compounds displayed superior activity to the furanyl analog with doubled potency against Newman MSSA, N315 MRSA/VSSA, and ΔacrB, an eightfold enhanced effect against the virulent Mu50 MRSA/VISA strain (MIC = 2 μg/mL). Nevertheless, for compounds **27** and **28**, multiple analogs with a 5-substituted phenyl ring system were superior to the heteroaryls in terms of the antibacterial activity. Notably, 15 of the synthesized derivatives in this series exhibited a broad range of

TABLE 3 Minimum inhibitory concentration (MIC) results of selected N-/O- or N-/S- cross analogs.

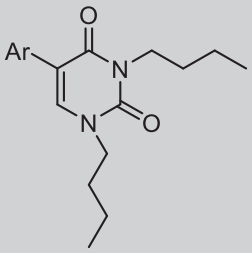


35–37

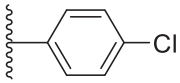
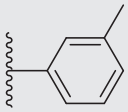
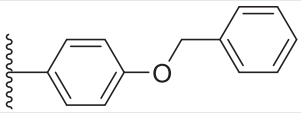
Compound	X	Ar	MIC (μg/mL) *Staphylococcus aureus* Newman MSSA	N315 MRSA/VSSA	Mu50 MRSA/VISA	*Escherichia coli* ΔacrB
35	O		>64	>64	>64	>64
36	O		8	8	4	>128
37	S		>128	>128	>128	>128
-	-	Linezolid	1	2	2	16
-	-	Vancomycin	0.5	1	8	>64

Note: Results shown are the mean of at least two independent experiments, SD ≤10%.

TABLE 4 Minimum inhibitory concentration (MIC) results of pyrimidine-2,4-diones scaffold representatives.



38–40

Compound	Ar	MIC (μg/mL) *Staphylococcus aureus* Newman MSSA	N315 MRSA/VSSA	Mu50 MRSA/VISA	*Escherichia coli* ΔacrB
38		>64	>64	>64	>64
39		32	>128	>128	>128
40		>128	>128	>128	>128
-	Linezolid	1	2	2	16
-	Vancomycin	0.5	1	8	>64

Note: Results shown are the mean of at least two independent experiments, SD ≤10%.

antibacterial activity against all four tested strains with MIC values ranging between 1 and 8 μg/mL.

Altogether, the following conclusions were drawn from this set of synthesized compounds: (1) a mono-substituted phenyl ring at position 5 is superior to an unsubstituted or a multiply-substituted phenyl in terms of the inhibitory activity. (2) The *para*- position was found to be optimal for activity in both gram-positive and -negative strains. (3) Bulkier groups displayed a better activity against the gram-positive strains than the gram-negative one, (4) In general; MRSA was more sensitive to most of the synthesized analogs compared to *E. coli* ΔacrB.

2.3.2 | The importance of the 2,4-diamino groups

Next, we tested the effect of isosteric replacement of the NH groups at position 2 and/or 4 on potency. The use of di-*O*-butyl or di-*S*-butyl side chains as isosteric replacements for the N^2,N^4-dibutyl substitution seemed plausible. The replacement of the NH was found to be deleterious to the antibacterial activity irrespective of the aryl substituent present in position 5 as observed in compounds **29–34** (Table 2). A slight deviation was observed with compound **30**, featuring the di-*O*-butyl side chains and the 5-(3,5-dimethyl-4methoxyphenyl), which merely preserved minimum activity against the *S. aureus* strain (MIC = 32 μg/mL). These results suggested that the presence of a H-bond donor might be essential for activity. This directed us to the next step, where only the N^2-butyl was replaced with an *O*-butyl or *S*-butyl, whilst position 4 was conserved with a butylamino side chain and the 4-chlorophenyl substituent at position 5 (compounds **35** and **37** in Table 3). However, the antibacterial activity was not restored. Conversely, when replacing the 4-chlorophenyl in compound **35** with a 3,5-dimethyl-4-methoxyphenyl (compound **36**) the potency was preserved. It showed MIC values of 4–8 μg/mL against the three tested gram-positive strains but failed to show any activity against *E. coli* ΔacrB (MIC = >128 μg/mL). Hence, a new prospect for potential scaffold modification has emerged, affording a strong antibacterial efficacy selectively toward *S. aureus* strains.

2.3.3 | Exploration of other core scaffolds

We synthesized a small series of 1,3-dibutylpyrimidine-2,4(1*H*,3*H*)-diones as a second maneuver to prove the essentiality of the 2,4-diamino pyrimidine scaffold (Table 4). Consistent with our hypothesis, the activity of substituted pyrimidinediones was entirely wiped out amongst the three representative analogs synthesized (compounds **38–40**) from all four tested strains, further corroborating the necessity of 2,4-pyrimidinediamine core.

In general, the gram-positive *S. aureus* strains were more sensitive to most of the tested analogs. As a result, we extended our investigation of the most potent ones toward a wider panel of gram-positive strains, including two strains of *S. pneumoniae* (DSM20566 and DSM11865 penicillin-resistant *S. pneumoniae*), *E. faecium*, *E. faecalis* in addition to the *E. coli* wild type, at concentrations from 0.06 to 128 μg/mL (Table 5), to determine the extent of antibacterial coverage against other pathogenic bacteria.

In consensus with our hypothesis, all candidates displayed good coverage against all of the tested gram-positive strains with the exception of compound **25** with the bulky 4-benzyloxyphenyl substituent. Compounds **11**, **12**, **17**, and **20** were found to have consistent antibacterial activity against these challenging strains, especially against *E. faecium* and *E. faecalis*, which are categorized by the WHO among the threatening high priority level bacterial strains to human health and require urgent development of new antibiotics to treat their infections.[25,26] Compound **11** showed the best inhibitory profile against the resistant strains in-question with comparable efficacy to broad-spectrum antibiotic linezolid. Finally, upon investigating the compounds against wild-type *E. coli*, none of

TABLE 5 Minimum inhibitory concentration (MIC) results of some of the most promising analogs against *Streptococcus pneumonia*, *Enterococcus faecium*, *Enterococcus faecalis*, and *Escherichia coli* wild type.

	MIC (μg/mL)				
	S. pneumoniae				
Compound	DSM20566	DSM11865 PRSP	*E. faecium* DSM20477	*E. faecalis* DSM20478	*E. coli* wild type BW25113
7	2	8	32	128	>128
8	64	128	4	4	>128
11	4	2	4	4	>128
12	4	16	8	4	>128
17	8	32	2	2	>128
20	16	8	4	16	>128
25	64	>128	>128	>128	>128
Linezolid	0.5	2	4	2	>64
Vancomycin	0.5	1	1	0.5	>64

Note: Results shown are the mean of at least two independent experiments, SD ≤10%.

them achieved any antibacterial activity unlike their potent effect against *E. coli* ΔacrB, suggesting that these compounds are effluxed from *E. coli*. In all respects, the novel synthesized analogs could serve as potential leads for the design and development of new broad-spectrum antibiotics with minimum antibacterial resistance.

The cytotoxicity of the most potent analogs identified amongst this series with promising antibacterial activity was evaluated by testing them against HepG2 cells (Table 6). Although they exhibited toxicity against a eukaryotic cell line, compounds **7**, **11**, **12**, and **17** with a substituted phenyl at position 5 showed a significantly improved safety profile (six- to sevenfold) when compared to the parent compound **1** bearing an unsubstituted phenyl. They showed an IC_{50} of 12.3–13.8 μg/mL against the HepG2 cells rather than an IC_{50} of 2.1 μg/mL as in the case of compound **1**, with compound **12** showing the best safety margin relative to its MIC values. Overall, these findings pave the way for future improvement of the analogs.

2.4 | Mode-of-action investigation

To explore the mode of resistance of *S. aureus* to the present series and to potentially reveal a molecular target, we aimed to generate mutants for one of the most active compounds, compound **12**. However, we were unable to select spontaneous resistant mutants on a solid medium containing the antibiotic, which could be attributed to the moderate solubility of compound **12**. Thus, we studied the effect of compound **12** on the synthesis of cellular constituents by measuring the incorporation of [^{3}H] labeled precursors into the major macromolecules DNA (thymidine), RNA (uridine), proteins (glutamine), and cell wall (glucosamine). In contrast to mutant generation experiments, only short time exposure to (sub-)inhibitory concentrations is needed. The incorporation of the precursors was studied in the absence and presence of compound **12** (at 1.5× the MIC) as well as two reference antibiotics responsible (or not) for inhibition of each synthetic pathway in bacteria (as positive controls: rifampicin for RNA-synthesis inhibition, ciprofloxacin for DNA-synthesis inhibition, chloramphenicol for protein-synthesis inhibition and meropenem for cell-wall-synthesis inhibition).

TABLE 6 Growth inhibitory activity against HepG2 cells.

Compound	HepG2 cells IC_{50} (μg/mL)
1	2.1 ± 0.9
7	13.8 ± 2.3
11	13.8 ± 2.3
12	12.3 ± 0.9
17	13.8 ± 1.6
Doxorubicin	0.2 ± 0.52

Note: Results shown are the mean of at least two independent experiments.

As shown in Figure 3, while the reference antibiotics clearly reduced the synthesis of the different macromolecules compared with the DMSO control, the effect of compound **12** was almost comparable to chloramphenicol and even stronger than meropenem in inhibiting protein and peptidoglycan synthesis respectively. On the other hand, **12** had no effect on DNA synthesis and some effect on RNA synthesis. The effect seen on protein and RNA synthesis could be due to inhibition of peptidoglycan synthesis,[27] hence further investigations about the mode of action are needed to be done.

3 | CONCLUSIONS

In this study, we adopted a systemic design and synthesis of 40 compounds having a 2,4-disubstitutedpyrimidine core as a scaffold. We started our modifications from the prototype compound **1**, which showed good potency. The use of various aryls at position 5 led to the recognition of the *p*-chlorophenyl analog (compound **12**) as a hit compound with the optimum substituent in terms of both potency (MIC vs. MRSA = 1 μg/mL) and safety profile with an IC_{50} of more than 12-fold higher than its MIC in this series. The data presented from the macromolecular synthesis determinations showed that compound **12** inhibits both peptidoglycan- and protein synthesis and to a lesser extent RNA synthesis. On the basis of the results of the present study, it could be hypothesized that the conversion of the quinazoline scaffold into the relatively flexible 5-aryl pyrimidine had a positive impact with regard to activity and safety to mammalian cells. These findings suggest that the novel synthesized 2,4-diamino-5-aryl pyrimidines merit optimization for their development as a new broad-spectrum class of antibacterial agents.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Solvents and reagents were obtained from commercial suppliers and used without purification. Mass spectrometric analysis (HPLC-ESI-MS) was performed on an MSQ plus (Thermo Fisher Scientific) instrument equipped with an ESI source and a single quadrupole mass detector. Column chromatography was performed on silica gel (230–400 mesh). Flash chromatography was carried out using a CombiFlash® NextGen 300+, by means of disposable RediSep® normal-phase silica flash columns, (230–400 mesh). All tested compounds had a purity of at least 95% verified by means of HPLC coupled with mass spectrometry. A Bruker DRX 500 spectrometer was used to obtain ^{1}H-NMR and ^{13}C-NMR spectra, in some cases a Varian 400 spectrometer was used. Chemical shifts (δ) were reported in parts per million (ppm) by reference to the hydrogenated residues of deuterated solvent as internal standard ($CDCl_3$: $\delta = 7.260$ ppm

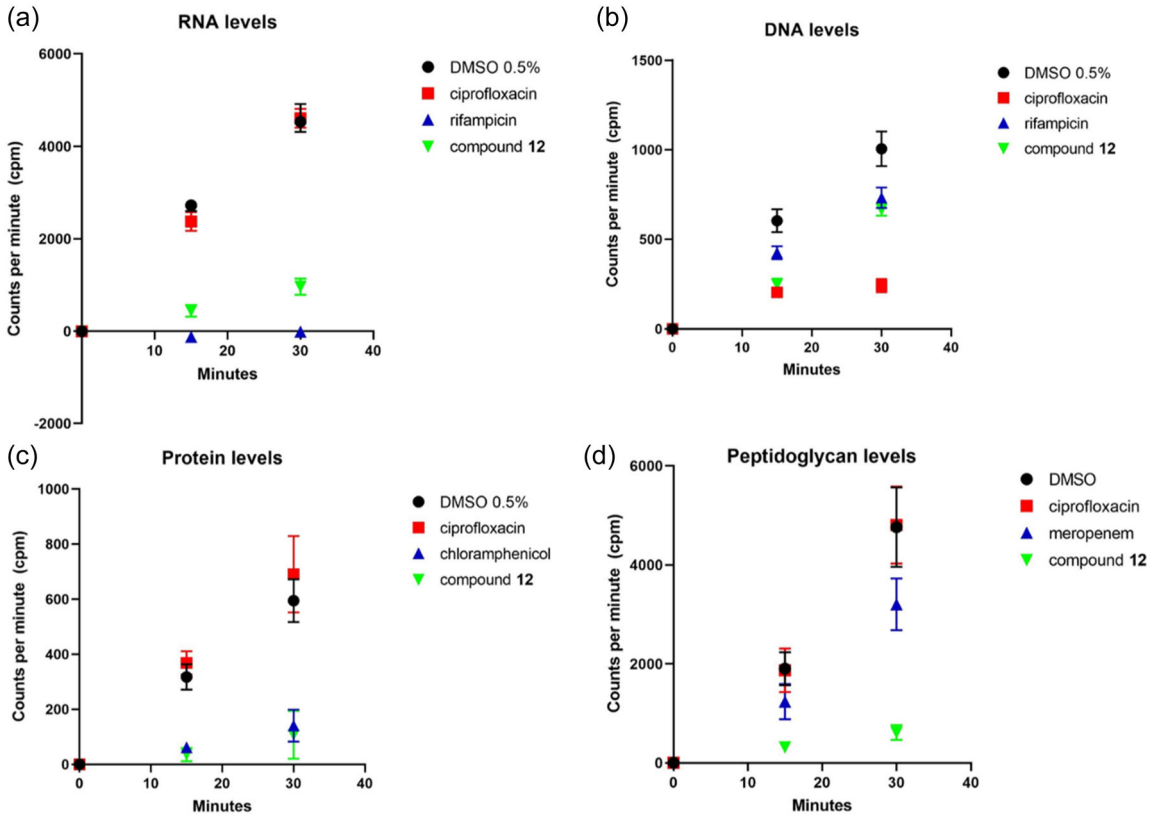


FIGURE 3 Effect of compound **12** and reference antibiotics (one positive control and one negative control used per experiment) on the synthesis of RNA (a), DNA (b), protein (c), and peptidoglycan (d) in *Bacillus subtilis* ssp. The amounts of synthesized macromolecule were quantified after 0, 15, and 30 min. All experiments were performed in duplicate.

(^{1}H NMR) and δ = 77.160 ppm (^{13}C NMR), MeOD: δ = 3.31 ppm (^{1}H NMR) and δ = 49.00 ppm (^{13}C NMR), $(CD_3)_2CO$: δ = 2.050 ppm (^{1}H NMR) and δ = 29.84 ppm (^{13}C NMR), DMSO δ = 2.50 ppm (^{1}H NMR) and δ = 39.52 ppm (^{13}C NMR)). All coupling constants (*J*) are given in Hz. Melting points were measured using a Stuart Scientific SMP30 melting point apparatus.

The NMR spectra of the investigated compounds as well as their InChI codes, together with some biological activity data, are provided as Supporting Information.

4.1.2 | Synthesis of 5-bromo-N^2,N^4-dibutylpyrimidine-2,4-diamine (**A**)

To an ice-cooled solution of 5-bromo-2,4-dichloropyrimidine (0.5 g, 1 equiv) in dioxane (1 mL), an excess of butylamine was added. The reaction was performed in a microwave synthesizer at 150°C for 30 min. Upon completion, the solvent was removed under reduced pressure. Water was then added to the residue, and the aqueous layer was with EtOAc (3 × 50 mL), the organic layers were combined, dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude was then purified by column chromatography (DCM/MeOH 100:1) to give intermediate **A** as a yellow oily liquid (93.6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1H), 5.12 (s, 1H), 4.89 (s, 1H), 3.42 (dd, *J* = 13.0, 6.8 Hz, 2H), 3.35–3.30 (m, 2H), 1.57 (tdd, *J* = 14.8, 10.9, 7.5 Hz, 4H), 1.43–1.34 (m, 4H), 0.94 (dt, *J* = 10.7, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.2, 158.3, 155.5, 92.0, 41.5, 40.7, 31.9, 31.7, 20.2, 20.2, 13.9, 13.9. MS (ESI) *m/z* = 301.1 $[M+H]^+$.

4.1.3 | Synthesis of 5-bromo-2,4-dibutoxypyrimidine (**B**)

Na (0.069 g, 3 equiv) was added to butanol (20 mL) at 0°C and stirred for 20 min. Then, 5-bromo-2,4-dichloropyrimidine (0.23 g, 1 equiv) was added to the reaction mixture and stirred at room temperature overnight. Afterward, butanol was evaporated under reduced pressure. The residue was partitioned between EtOAc (50 mL) and water (20 mL), and then the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were filtered over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The product was purified by flash chromatography (petroleum benzene 40–60/EtOAc) to give intermediate **B** as a colorless oily liquid (98% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.24 (s, 1H), 4.39 (t, *J* = 6.6 Hz, 2H), 4.28 (t, *J* = 6.7 Hz, 2H), 1.80–1.71 (m, 4H), 1.51–1.38 (m, 4H), 0.94 (q, *J* = 7.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 164.1, 159.1, 98.1, 68.0, 67.7, 30.9, 30.6, 19.2, 19.2, 13.8. MS (ESI) *m/z* = 302.97 $[M+H]^+$.

4.1.4 | Synthesis of 5-bromo-2,4-bis(butylthio) pyrimidine (**C**)

To an ice-cooled suspension of Na (0.14 g, 3 equiv) in tetrahydrofuran (20 mL), butanethiol (3 equiv) was added. The reaction mixture was stirred at 0°C for 20 min. This was followed by the addition of 5-bromo-2,4-dichloropyrimidine (0.46 g, 1 equiv), and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. Water was then added to the residue, and the aqueous layer was extracted with DCM (3 × 50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product was purified by flash chromatography (petroleum benzene 40–60/DCM) to give intermediate **C** as a colorless oily liquid (89% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H), 3.19–3.15 (m, 2H), 3.13–3.08 (m, 2H), 1.75–1.67 (m, 4H), 1.51–1.43 (m, 4H), 0.95 (td, J = 7.4, 5.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8, 169.4, 155.4, 113.1, 31.4, 31.2, 31.0, 30.3, 22.2, 13.8, 13.8. MS (ESI) m/z = 334.97 $[M+H]^+$.

4.1.5 | Synthesis of 5-bromo-*N*-butyl-2-chloropyrimidin-4-amine (**D1**)

To an ice-cooled solution of 5-bromo-2,4-dichloropyrimidine (0.5 g, 1 equiv) in dioxane (0.5 mL), an excess of butylamine was added. The reaction occurred instantaneously. The solvent was then removed under reduced pressure. Water was added to the residue, and the aqueous layer was extracted with DCM (3 × 50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product was purified using flash chromatography (petroleum benzene 40–60/EtOAc) to give intermediate **D1** as a yellow oily liquid (82.8% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 5.50 (s, 1H), 3.51 (dd, J = 12.9, 7.1 Hz, 2H), 1.62 (dt, J = 14.9, 7.3 Hz, 2H), 1.40 (dt, J = 14.7, 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.5, 159.5, 156.2, 103.0, 41.3, 31.3, 20.1, 13.8. MS (ESI) m/z = 263.84 $[M+H]^+$.

4.1.6 | Synthesis of 5-bromo-2-butoxy-*N*-butylpyrimidin-4-amine (**D2**)

To excess butanol, NaH (0.05 g, 2 equiv) was added and stirred at 0°C for 30 min. Following this, intermediate **D1** (0.25 g, 1 equiv) was added to the reaction mixture. The reaction was then performed in a microwave synthesizer at 150°C for 30 min. Butanol was then removed under reduced pressure, and the residue was partitioned between 50 mL of DCM and 20 mL of water. The aqueous layer was extracted with DCM (3 × 20 mL), and the combined organic extracts were filtered over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified by flash chromatography (petroleum benzene 40–60/EtOAc) to give intermediate **D2** as a yellow oily liquid (73% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (s, 1H), 5.30 (s, 1H), 4.25 (t, J = 6.8 Hz, 2H), 3.48 (td, J = 7.2, 5.7 Hz, 2H), 1.79–1.71 (m, 2H), 1.60 (dt, J = 14.9, 7.6 Hz, 2H), 1.45 (dd, J = 15.1, 7.5 Hz, 2H), 1.40 (dd, J = 15.1, 7.4 Hz, 2H), 0.94 (td, J = 7.4, 3.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.3, 159.6, 156.1, 96.7, 67.5, 41.0, 31.5, 31.0, 20.1, 19.2, 13.9, 13.9. MS (ESI) m/z = 302.00 $[M+H]^+$.

4.1.7 | Synthesis of 5-bromo-*N*-butyl-2-(butylthio) pyrimidin-4-amine (**D3**)

To excess butanethiol, Na (0.05 g, 2 equiv) was added and stirred at 0°C for 30 min. Afterwards, intermediate **D1** (0.25 g, 1 equiv) was added to the reaction mixture, and the reaction was stirred at room temperature overnight. The solvent was removed under reduced pressure. Water was added to the residue, and the aqueous layer was extracted with DCM (3 × 50 mL). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (DCM) to give intermediate **D3** as a yellow oily liquid (66.7% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 5.34 (s, 1H), 3.49 (dd, J = 13.0, 7.1 Hz, 2H), 3.10–3.02 (m, 2H), 1.74–1.66 (m, 2H), 1.64–1.58 (m, 2H), 1.49–1.43 (m, 2H), 1.43–1.35 (m, 2H), 0.95 (dt, J = 12.9, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.2, 157.7, 154.9, 99.7, 41.0, 31.7, 31.5, 31.1, 22.2, 20.2, 13.9, 13.8. MS (ESI) m/z = 317.98 $[M+H]^+$.

4.1.8 | Synthesis of 5-bromo-1,3-dibutylpyrimidine-2,4(1*H*,3*H*)-dione (**E**)

To a suspension of K_2CO_3 (0.36 g, 2 equiv) in dimethylformamide (2 mL), 5-bromouracil (0.25 g, 1 equiv) was added. The reaction mixture was stirred for 30 min, followed by the addition of butyl bromide (0.36 mL, 2.5 equiv). The mixture was refluxed at 70°C overnight. Afterward, the suspension was cooled to room temperature and extracted using EtOAc and water, and the combined organic layers were thoroughly washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure, and the resulting residue was purified by flash chromatography (petroleum benzene 40–60/EtOAc) to give intermediate **E** as a white solid (75% yield). Melting point 66.1–66.8°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (s, 1H), 4.01–3.93 (m, 2H), 3.77–3.70 (m, 2H), 1.71–1.64 (m, 2H), 1.63–1.56 (m, 2H), 1.40–1.31 (m, 4H), 0.94 (dt, J = 14.7, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.3, 150.7, 141.8, 96.1, 50.0, 42.7, 31.2, 29.5, 20.2, 19.8, 13.8, 13.7. MS (ESI) m/z = 303.00 $[M+H]^+$.

4.1.9 | Procedure C: General procedure for biaryl compound synthesis through palladium-catalyzed Suzuki-Miyaura cross coupling to synthesize compounds **1–40**

Intermediates of Schemes 1–4 were suspended in a mixture of dioxane and water in ratio (4:1, respectively); this was followed by

the addition of the appropriate boronic acid (1.2 equiv), Na_2CO_3 (4 equiv) and [Pd(dppf)Cl_2] (0.05 equiv). The reaction was performed in a microwave synthesizer at 150°C held for 30 min. After completion, the solvent was evaporated under reduced pressure. A small amount of water was added, extraction of the aqueous layer was carried out with DCM (3 × 50 mL). The combined organic layers were filtered over anhydrous $MgSO_4$, filtered and the solvent was removed under reduced pressure. The final product was purified either by column chromatography on silica gel or flash chromatography to afford compounds **1–40**. Several final compounds required further purification after flash chromatography via semi-preparative HPLC. HPLC methods: eluent A (water + 0.05% formic acid), eluent B (acetonitrile + 0.05% formic acid); gradient of B (5%–100%) in 60 min/or gradient of B (40%–100%) in 60 min; flow 5 mL/min.

N^2,N^4-Dibutyl-5-phenylpyrimidine-2,4-diamine (**1**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with phenylboronic acid. The product was purified by column chromatography (DCM/MeOH 100:2) to give compound **1** as an orange oily liquid (40.3% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H), 7.43 (t, J = 7.7 Hz, 2H), 7.33 (dd, J = 10.5, 4.5 Hz, 3H), 4.96 (s, 1H), 4.89 (s, 1H), 3.46–3.36 (m, 4H), 1.64–1.57 (m, 2H), 1.56–1.49 (m, 2H), 1.43 (dq, J = 14.6, 7.4 Hz, 2H), 1.34 (dq, J = 14.6, 7.3 Hz, 2H), 0.94 (dt, J = 17.5, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.5, 160.2, 154.0, 135.8, 129.3, 129.0, 127.4, 110.0, 41.3, 40.4, 32.1, 31.7, 20.3, 20.3, 14.0, 14.0. MS (ESI) m/z = 299.11 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{18}H_{27}N_4^+$ $[M+H]^+$: 299.2230 found 299.2228.

N^2,N^4-Dibutyl-5-(2-methoxyphenyl)pyrimidine-2,4-diamine (**2**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 2-methoxybenzene boronic acid. The product was purified by column chromatography (DCM/MeOH 100:2) to give compound **2** as a yellow solid (30% yield). Melting point 70.1–70.6°C. ^{1}H NMR (500 MHz, DMSO) δ 7.43 (s, 1H), 7.34 (td, J = 8.4, 1.7 Hz, 1H), 7.10 (dd, J = 7.4, 1.7 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.98 (td, J = 7.4, 0.7 Hz, 1H), 6.51 (s, 1H), 5.72 (s, 1H), 3.73 (s, 3H), 3.29 (s, 2H), 3.24 (dd, J = 13.3, 6.7 Hz, 2H), 1.52 (dd, J = 14.4, 7.1 Hz, 2H), 1.49–1.44 (m, 2H), 1.33 (dd, J = 14.9, 7.4 Hz, 2H), 1.27 (dd, J = 14.9, 7.4 Hz, 2H), 0.89 (dt, J = 11.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 160.9, 159.6, 157.0, 153.9, 131.4, 128.8, 124.1, 120.7, 111.4, 106.1, 55.0, 40.3, 40.1, 31.5, 31.2, 19.7, 19.5, 13.8, 13.8. MS (ESI) m/z = 329.19 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{29}N_4O^+$ $[M+H]^+$: 329.2336 found 329.2333.

N^2,N^4-Dibutyl-5-(3-methoxyphenyl)pyrimidine-2,4-diamine (**3**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-methoxybenzene boronic acid. The product was purified by column chromatography (EtOAc/hexane 1:2) to give compound **3** as a yellow oily liquid (52.1% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.33 (t, J = 7.9 Hz, 1H), 6.89 (d, J = 7.6 Hz, 1H), 6.88–6.85 (m, 1H), 6.85–6.81 (m, 1H), 5.41 (s, 1H), 5.02 (s, 1H), 3.81 (s, 3H), 3.40 (dt, J = 12.6, 5.1 Hz, 4H), 1.60 (dt, J = 14.8, 7.4 Hz, 2H), 1.52 (dt, J = 14.8, 7.4 Hz, 2H), 1.41 (tt, J = 10.1, 5.1 Hz, 2H), 1.34 (dq, J = 14.6, 7.4 Hz, 2H), 0.93 (dt, J = 14.7, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.1, 160.3, 160.2, 152.9, 137.0, 130.3, 121.2, 114.5, 112.9, 109.8, 55.3, 41.3, 40.5, 32.0, 31.6, 20.2, 20.2, 14.0, 13.9. MS (ESI) m/z = 329.17 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{29}N_4O^+$ $[M+H]^+$: 329.2336 found 329.2334.

N^2,N^4-Dibutyl-5-(4-methoxyphenyl)pyrimidine-2,4-diamine (**4**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 4-methoxybenzene boronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1.5) to give compound **4** as a yellow solid (37.6% yield). Melting point 81.7–82.4°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.23 (d, J = 8.5 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 4.88 (s, 1H), 4.79 (s, 1H), 3.83 (s, 3H), 3.39 (ddd, J = 16.1, 9.3, 6.9 Hz, 4H), 1.63–1.56 (m, 2H), 1.51 (dt, J = 14.8, 7.3 Hz, 2H), 1.42 (dq, J = 14.6, 7.3 Hz, 2H), 1.33 (dq, J = 14.5, 7.3 Hz, 2H), 0.93 (dt, J = 17.3, 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.7, 160.5, 159.0, 154.2, 130.3, 128.0, 114.7, 109.7, 55.4, 41.3, 40.4, 32.1, 31.8, 20.3, 20.3, 14.0, 13.9. MS (ESI) m/z = 329.20 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{29}N_4O^+$ $[M+H]^+$: 329.2336 found 329.2333.

N^2,N^4-Dibutyl-5-(3-ethoxyphenyl)pyrimidine-2,4-diamine (**5**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-ethoxybenzene boronic acid. The product was purified by column chromatography (EtOAc/hexane 1:2) followed by semipreparative HPLC; gradient of B (5%–100%) in 60 min to give compound **5** as a white oily liquid (12.7% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.33 (t, J = 7.9 Hz, 1H), 6.90–6.85 (m, 2H), 6.85–6.81 (m, 1H), 5.20 (s, 1H), 5.01 (s, 1H), 4.05 (q, J = 7.0 Hz, 2H), 3.45–3.37 (m, 4H), 1.61 (dt, J = 14.8, 7.4 Hz, 2H), 1.53 (dt, J = 14.8, 7.4 Hz, 2H), 1.47–1.39 (m, 5H), 1.33 (dt, J = 14.6, 7.4 Hz, 2H), 0.94 (dt, J = 15.6, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.2, 159.7, 137.0, 130.4, 121.1, 115.1, 113.6, 110.0, 63.6, 41.3, 40.5, 32.1, 31.7, 20.3, 14.9, 14.0, 13.9. MS (ESI) m/z = 343.19 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{20}H_{31}N_4O^+$ $[M+H]^+$: 343.2492 found 343.2491.

3-[2,4-Bis(butylamino)pyrimidin-5-yl]phenol (**6**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-hydroxybenzene boronic acid. The product was purified by column chromatography (EtOAc/hexane 2:1) to give compound **6** as a yellow solid (26.8% yield). Melting point 95.9–96.7°C. ^{1}H NMR (500 MHz, DMSO) δ 9.48 (s, 1H), 7.52 (s, 1H), 7.20 (dd, J = 8.7, 7.6 Hz, 1H), 6.74–6.66 (m, 3H), 6.49 (s, 1H), 6.01 (s, 1H), 3.30 (dd, J = 13.3, 6.6 Hz, 2H), 3.23 (dd, J = 13.4, 6.7 Hz, 2H), 1.54–1.44 (m, 4H), 1.32 (dd, J = 13.8, 6.3 Hz, 2H), 1.27 (dd, J = 13.8, 6.3 Hz, 2H), 0.89 (td, J = 7.3, 5.8 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.3, 159.3, 157.7, 154.4, 137.2, 129.9, 119.1, 115.3, 113.7, 108.5, 40.3, 40.1, 31.6, 31.2, 19.7, 19.7, 13.8. MS (ESI) m/z = 315.07 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{18}H_{27}N_4O^+$ $[M+H]^+$: 315.2179 found 315.2177.

N^2,N^4-Dibutyl-5-(*m*-tolyl)pyrimidine-2,4-diamine (**7**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with *m*-tolylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:2) to give compound **7** as a yellow oily liquid (23.6% yield). ^{1}H NMR (500 MHz, DMSO) δ 7.53 (s, 1H), 7.29 (t, J = 7.8 Hz, 1H), 7.14–7.05 (m, 3H), 6.59–6.42 (m, 1H), 6.06 (s, 1H), 3.30 (dd, J = 13.4, 6.7 Hz, 2H), 3.23 (dd, J = 13.4, 6.7 Hz, 2H), 2.33 (s, 3H), 1.54–1.44 (m, 4H), 1.37–1.23 (m, 4H), 0.92–0.85 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.3, 159.3, 154.6,

138.0, 135.9, 129.2, 128.8, 127.2, 125.5, 109.1, 40.3, 40.1, 31.6, 31.1, 21.1, 19.7, 19.7, 13.8, 13.8. MS (ESI) *m/z* = 313.17 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{19}H_{29}N_4^+$ $[M+H]^+$: 313.2387 found 313.2382.

N^2,N^4-Dibutyl-5-(*p*-tolyl)pyrimidine-2,4-diamine (**8**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with *p*-tolylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1) to give compound **8** as a yellow solid (35.7% yield). Melting point 50.6–51.4°C. ^{1}H NMR (500 MHz, DMSO) δ 7.53 (s, 1H), 7.24 (d, *J* = 7.7 Hz, 2H), 7.19 (d, *J* = 7.9 Hz, 2H), 6.86 (s, 1H), 6.52 (s, 1H), 3.31 (s, 2H), 3.25 (s, 2H), 2.33 (d, *J* = 4.1 Hz, 3H), 1.49 (d, *J* = 6.1 Hz, 4H), 1.30 (ddd, *J* = 25.7, 14.5, 7.2 Hz, 4H), 0.94–0.80 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 159.6, 151.1, 136.1, 132.1, 129.5, 128.6, 108.8, 40.3, 40.1, 31.4, 31.0, 20.7, 19.7, 13.7. MS (ESI) *m/z* = 313.17 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{19}H_{29}N_4^+$ $[M+H]^+$: 313.2387 found 313.2383.

N^2,N^4-Dibutyl-5-(3-fluorophenyl)pyrimidine-2,4-diamine (**9**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-fluorophenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:2) followed by semi-preparative HPLC; gradient of B (5%–100%) in 60 min to give compound **9** as a white solid (11% yield). Melting point 65–65.7°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.39 (dt, *J* = 14.4, 4.0 Hz, 1H), 7.10 (dd, *J* = 7.6, 1.0 Hz, 1H), 7.02 (dd, *J* = 12.3, 5.8 Hz, 2H), 5.05 (s, 1H), 4.89 (s, 1H), 3.41 (dd, *J* = 13.2, 6.5 Hz, 4H), 1.64–1.56 (m, 2H), 1.57–1.49 (m, 2H), 1.46–1.38 (m, 2H), 1.34 (dq, *J* = 14.6, 7.4 Hz, 2H), 0.94 (ddd, *J* = 13.7, 7.3, 3.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.3 (d, $^1J_{C-F}$ = 247.3 Hz), 161.5, 160.0, 154.0, 138.1 (d, $^3J_{C-F}$ = 8.0 Hz), 130.9 (d, $^3J_{C-F}$ = 8.5 Hz), 124.6 (d, $^4J_{C-F}$ = 2.7 Hz), 115.8 (d, $^2J_{C-F}$ = 21.1 Hz), 114.3 (d, $^2J_{C-F}$ = 21.1 Hz), 108.9, 41.3, 40.5, 32.0, 31.7, 20.3, 14.0, 14.0. MS (ESI) *m/z* = 317.16 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{26}FN_4^+$ $[M+H]^+$: 317.2136 found 317.2134.

N^2,N^4-Dibutyl-5-(4-fluorophenyl)pyrimidine-2,4-diamine (**10**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 4-fluorophenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1.5) followed by semi-preparative HPLC; gradient of B (5%–100%) in 60 min to give compound **10** as a white solid (30% yield). Melting point 81.1–81.8°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.30–7.26 (m, 2H), 7.14–7.09 (m, 2H), 4.97 (s, 1H), 4.74 (s, 1H), 3.45–3.36 (m, 4H), 1.64–1.56 (m, 2H), 1.56–1.48 (m, 2H), 1.43 (dq, *J* = 14.6, 7.3 Hz, 2H), 1.33 (dq, *J* = 14.6, 7.3 Hz, 2H), 0.94 (dt, *J* = 16.2, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.2 (d, $^1J_{C-F}$ = 247.1 Hz), 161.6, 160.3, 154.0, 131.7 (d, $^4J_{C-F}$ = 3.4 Hz), 130.9 (d, $^3J_{C-F}$ = 7.9 Hz), 116.3 (d, $^2J_{C-F}$ = 21.3 Hz), 109.1, 41.3, 40.5, 32.1, 31.7, 20.3, 20.3, 14.0, 13.9. MS (ESI) *m/z* = 317.16 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{26}FN_4^+$ $[M+H]^+$: 317.2136 found 317.2134.

N^2,N^4-Dibutyl-5-(3-chlorophenyl)pyrimidine-2,4-diamine (**11**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-chlorobenzene boronic acid. The product was purified using flash chromatography (cyclohexane:EtOAc) to give compound **11** as a yellow oily liquid (84.1% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.30 (t, *J* = 1.7 Hz, 1H), 7.30–7.27 (m, 1H), 7.20 (d, *J* = 7.5 Hz, 1H), 5.02 (s, 1H), 4.81 (s, 1H), 3.40 (dd, *J* = 13.0, 6.9 Hz, 4H), 1.59 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.55–1.48 (m, 2H), 1.41 (dq, *J* = 14.6, 7.4 Hz, 2H), 1.33 (dq, *J* = 14.6, 7.4 Hz, 2H), 0.93 (dt, *J* = 12.3, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 159.9, 154.7, 138.0, 135.0, 130.5, 129.0, 127.4, 127.1, 108.6, 41.2, 40.4, 32.1, 31.7, 20.2, 14.0, 13.9. MS (ESI) *m/z* = 333.12 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{26}ClN_4^+$ $[M+H]^+$: 333.1841 found 333.1838.

N^2,N^4-Dibutyl-5-(4-chlorophenyl)pyrimidine-2,4-diamine (**12**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 4-chlorophenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:2) to give compound **12** as a yellowish white solid (19.9% yield). Melting point 68.4–69°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.25 (d, *J* = 8.4 Hz, 2H), 4.97 (s, 1H), 4.76 (s, 1H), 3.44–3.34 (m, 4H), 1.64–1.57 (m, 2H), 1.56–1.48 (m, 2H), 1.42 (dq, *J* = 14.6, 7.3 Hz, 2H), 1.34 (dq, *J* = 14.6, 7.3 Hz, 2H), 0.94 (dt, *J* = 14.6, 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.8, 160.1, 154.4, 134.4, 133.3, 130.4, 129.5, 108.8, 41.3, 40.5, 32.1, 31.7, 20.3, 14.0, 13.9. MS (ESI) *m/z* = 333.14 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{26}ClN_4^+$ $[M+H]^+$: 333.1841 found 333.1838.

N^2,N^4-Dibutyl-5-[3-(trifluoromethoxy)phenyl]pyrimidine-2,4-diamine (**13**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-(trifluoromethoxy)benzene boronic acid. The product was purified by column chromatography (DCM/MeOH 100:2) to give compound **13** as a yellow solid (41.7% yield). Melting point 55.9–56.5°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.27 (s, 1H), 7.19 (d, *J* = 10.0 Hz, 2H), 5.14 (s, 1H), 4.84 (s, 1H), 3.43 (d, *J* = 6.0 Hz, 4H), 1.62 (dt, *J* = 14.7, 7.2 Hz, 2H), 1.55 (dt, *J* = 14.7, 7.4 Hz, 2H), 1.44 (dq, *J* = 14.9, 7.5 Hz, 2H), 1.35 (dt, *J* = 14.6, 7.4 Hz, 2H), 0.96 (dt, *J* = 14.4, 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.6, 160.0, 154.2, 149.9 (d, $^3J_{C-F}$ = 1.4 Hz), 138.0, 130.8, 127.3, 121.3, 120.5 (q, $^1J_{C-F}$ = 257.6 Hz), 119.7, 108.6, 41.3, 40.5, 32.1, 31.6, 20.3, 20.2, 14.0, 13.9. MS (ESI) *m/z* = 383.14 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{19}H_{26}F_3N_4O^+$ $[M+H]^+$: 383.2053 found 383.2050.

N^2,N^4-Dibutyl-5-(3-nitrophenyl)pyrimidine-2,4-diamine (**14**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-nitrophenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1) to give compound **14** as a yellow oily liquid (35.1% yield). ^{1}H NMR (500 MHz, DMSO) δ 8.14–8.11 (m, 1H), 8.10 (t, *J* = 1.9 Hz, 1H), 7.77–7.73 (m, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.65 (s, 1H), 6.71 (s, 1H), 6.58 (s, 1H), 3.31–3.28 (m, 2H), 3.25 (dd, *J* = 13.4, 6.7 Hz, 2H), 1.51 (dt, *J* = 14.3, 7.2 Hz, 4H), 1.31 (dt, *J* = 21.1, 7.4 Hz, 4H), 0.89 (td, *J* = 7.4, 4.0 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.7, 159.2, 155.5, 148.1, 137.9, 135.2, 130.3, 123.2, 121.1, 106.0, 40.3, 40.1, 31.5, 31.1, 19.7, 13.8. MS (ESI) *m/z* = 344.17 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{26}N_5O_2^+$ $[M+H]^+$: 344.2081 found 344.2079.

3-[2,4-Bis(butylamino)pyrimidin-5-yl]benzonitrile (**15**): The title compound was prepared according to procedure C by the reaction of

intermediate **A** (0.25 g, 1 equiv) with 3-cyanophenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1) followed by semi-preparative HPLC; gradient of B (5%–100%) in 60 min to give compound **15** as a yellow solid (57.9% yield). Melting point 76.3–77°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.61 (t, J = 3.6 Hz, 2H), 7.58–7.51 (m, 2H), 5.07 (s, 1H), 4.70 (s, 1H), 3.41 (dd, J = 13.2, 6.8 Hz, 4H), 1.60 (dt, J = 14.9, 7.3 Hz, 2H), 1.57–1.50 (m, 2H), 1.42 (dq, J = 14.6, 7.4 Hz, 2H), 1.34 (dq, J = 14.6, 7.4 Hz, 2H), 0.94 (dt, J = 12.2, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 159.9, 154.8, 137.6, 133.5, 132.4, 130.9, 130.2, 118.6, 113.6, 107.8, 41.3, 40.6, 32.0, 31.7, 20.3, 20.3, 14.0, 13.9. MS (ESI) m/z = 324.15 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{26}N_5^+$ $[M+H]^+$: 324.2184 found 324.2180.

N^2,N^4-Dibutyl-5-(3,4-dimethoxyphenyl)pyrimidine-2,4-diamine (**16**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3,4-dimethoxyphenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 3:1) to give compound **16** as a white solid (59.5% yield). Melting point 94.9–95.6°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (s, 1H), 6.93 (d, J = 8.2 Hz, 1H), 6.86 (dd, J = 8.1, 2.0 Hz, 1H), 6.80 (d, J = 1.9 Hz, 1H), 5.26 (s, 1H), 4.96 (s, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 3.45–3.36 (m, 4H), 1.64–1.57 (m, 2H), 1.56–1.49 (m, 2H), 1.43 (dq, J = 14.6, 7.4 Hz, 2H), 1.34 (dq, J = 14.6, 7.3 Hz, 2H), 0.94 (dt, J = 14.7, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.8, 160.6, 149.6, 148.6, 127.8, 121.4, 112.3, 111.9, 110.0, 56.1, 56.0, 41.3, 40.5, 32.0, 31.7, 20.3, 14.0, 13.9. MS (ESI) m/z = 359.20 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{20}H_{31}N_4O_2^+$ $[M+H]^+$: 359.2442 found 359.2438.

N^2,N^4-Dibutyl-5-(3,4-dimethylphenyl)pyrimidine-2,4-diamine (**17**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3,4-dimethylphenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1.5) to give compound **17** as a yellow solid (37.2% yield). Melting point 75.6–76.3°C. ^{1}H NMR (500 MHz, DMSO) δ 7.50 (s, 1H), 7.16 (d, J = 7.8 Hz, 1H), 7.06 (s, 1H), 7.00 (dd, J = 7.7, 1.7 Hz, 1H), 6.45 (s, 1H), 5.97 (s, 1H), 3.31–3.26 (m, 2H), 3.23 (dd, J = 13.4, 6.7 Hz, 2H), 2.23 (d, J = 2.0 Hz, 6H), 1.54–1.44 (m, 4H), 1.29 (ddd, J = 22.7, 15.0, 7.4 Hz, 4H), 0.89 (q, J = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.3, 159.4, 154.4, 136.6, 134.5, 133.3, 130.0, 129.7, 125.9, 108.4, 40.3, 40.1, 31.6, 31.1, 19.7, 19.7, 19.5, 19.0, 13.8, 13.8. MS (ESI) m/z = 327.21 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{20}H_{31}N_4^+$ $[M+H]^+$: 327.2543 found 327.2541.

N^2,N^4-Dibutyl-5-(3,4-difluorophenyl)pyrimidine-2,4-diamine (**18**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3,4-difluorophenylboronic acid. The product was purified by column chromatography (DCM/MeOH 100:1) to give compound **18** as a yellow solid (11.3% yield). Melting point 64.3–64.8°C. ^{1}H NMR (500 MHz, DMSO) δ 7.56 (s, 1H), 7.46 (dt, J = 10.9, 8.6 Hz, 1H), 7.34 (ddd, J = 11.7, 7.9, 2.0 Hz, 1H), 7.15–7.10 (m, 1H), 6.80 (s, 1H), 6.53 (s, 1H), 3.31–3.27 (m, 2H), 3.24 (dd, J = 13.0, 6.5 Hz, 2H), 1.49 (dd, J = 13.7, 6.6 Hz, 4H), 1.32 (dd, J = 13.8, 6.2 Hz, 2H), 1.27 (dd, J = 13.7, 6.3 Hz, 2H), 0.91–0.86 (m, 6H). ^{13}C NMR (126 MHz, DMSO) δ 160.8, 159.4, 153.4, 149.4 (dd, $^1J_{C-F}$, $^2J_{C-F}$ = 245.6, 12.5 Hz), 148.5 (dd, $^1J_{C-F}$, $^2J_{C-F}$ = 244.9, 12.5 Hz), 133.2 (dd, $^3J_{C-F}$, $^4J_{C-F}$ = 5.6, 2.7 Hz), 125.7 (dd, $^3J_{C-F}$, $^4J_{C-F}$ = 5.9, 3.0 Hz), 118.0–117.7 ($^2J_{C-F}$, $^3J_{C-F}$ m), 106.9, 40.3, 40.1, 31.4, 31.0, 19.7, 19.7, 13.8. MS (ESI) m/z = 335.13 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{18}H_{25}F_2N_4^+$ $[M+H]^+$: 335.2042 found 335.2039.

N^2,N^4-Dibutyl-5-(3,5-difluorophenyl)pyrimidine-2,4-diamine (**19**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3,5-difluorophenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:3) to give compound **19** as a yellow oily liquid (10.5% yield). ^{1}H NMR (500 MHz, DMSO) δ 7.62 (s, 1H), 7.12 (ddd, J = 11.4, 7.6, 2.1 Hz, 1H), 7.03–6.98 (m, 2H), 6.68 (s, 1H), 6.51 (s, 1H), 3.29 (dd, J = 13.9, 7.1 Hz, 2H), 3.23 (dd, J = 13.2, 6.7 Hz, 2H), 1.55–1.45 (m, 4H), 1.30 (dt, J = 22.0, 7.4 Hz, 4H), 0.89 (td, J = 7.4, 1.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 162.6 (dd, $^1J_{C-F}$, $^3J_{C-F}$ = 242.2, 10.5 Hz), 161.6, 159.0, 155.3, 139.8 (dd, $^3J_{C-F}$, $^3J_{C-F}$ = 9.4, 6.3 Hz), 111.5 (dd, $^2J_{C-F}$, $^4J_{C-F}$ = 19.3, 5.1 Hz), 101.7 (dd, $^2J_{C-F}$, $^2J_{C-F}$ = 25.8, 9.3 Hz), 101.5, 40.3, 40.1, 31.5, 31.1, 19.7, 19.7, 13.8. MS (ESI) m/z = 335.15 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{18}H_{25}F_2N_4^+$ $[M+H]^+$: 335.2042 found 335.2039.

N^2,N^4-Dibutyl-5-(4-methoxy-3-methylphenyl)pyrimidine-2,4-diamine (**20**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 4-methoxy-3-methylphenylboronic acid. The product was purified using flash chromatography (cyclohexane/EtOAc) to give compound **20** as a yellow solid (27.8% yield). Melting point 82.8–83.5°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.10 (dd, J = 8.2, 2.1 Hz, 1H), 7.07 (s, 1H), 6.87 (d, J = 8.3 Hz, 1H), 4.92 (s, 1H), 4.86 (s, 1H), 3.85 (s, 3H), 3.43–3.35 (m, 4H), 2.24 (s, 3H), 1.60 (dt, J = 14.8, 7.4 Hz, 2H), 1.55–1.48 (m, 2H), 1.42 (dq, J = 14.6, 7.3 Hz, 2H), 1.33 (dt, J = 14.6, 7.4 Hz, 2H), 0.94 (dt, J = 15.1, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.5, 160.5, 157.2, 153.8, 131.5, 127.6, 127.5, 127.5, 110.6, 109.9, 55.5, 41.3, 40.4, 32.1, 31.7, 20.3, 20.3, 16.4, 14.0, 13.9. MS (ESI) m/z = 343.19 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{21}H_{31}N_4O^+$ $[M+H]^+$: 343.2492 found 343.2491.

N^2,N^4-Dibutyl-5-(3-fluoro-4-methoxyphenyl)pyrimidine-2,4-diamine (**21**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-fluoro-4-methoxyphenylboronic acid. The product was purified using flash chromatography (DCM/MeOH) to give compound **21** as a yellow solid (43.5% yield). Melting point 61.5–70.2°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (s, 1H), 7.05 (d, J = 1.6 Hz, 1H), 7.03–6.99 (m, 2H), 5.74 (s, 1H), 4.89 (s, 1H), 3.92 (d, J = 3.8 Hz, 3H), 3.40 (dd, J = 12.8, 6.3 Hz, 4H), 1.60 (dd, J = 8.8, 5.9 Hz, 2H), 1.53 (dd, J = 8.8, 5.8 Hz, 2H), 1.45–1.38 (m, 2H), 1.37–1.31 (m, 2H), 0.98–0.90 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.9, 160.3, 152.6 (d, $^1J_{C-F}$ = 247.5 Hz), 151.7, 147.2 (d, $^2J_{C-F}$ = 10.8 Hz), 128.2 (d, $^3J_{C-F}$ = 5.7 Hz), 125.0 (d, $^3J_{C-F}$ = 3.3 Hz), 116.8 (d, $^2J_{C-F}$ = 18.2 Hz), 114.3 (d, $^4J_{C-F}$ = 2.0 Hz), 108.6, 56.4, 41.3, 40.6, 32.0, 31.7, 20.3, 20.2, 14.0, 13.9. MS (ESI) m/z = 347.16 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{28}FN_4O^+$ $[M+H]^+$: 347.2242 found 347.2240.

N^2,N^4-Dibutyl-5-(3-fluoro-5-methoxyphenyl)pyrimidine-2,4-diamine (**22**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-fluoro-5-methoxyphenylboronic acid. The product was purified by column chromatography (DCM/MeOH 100:2) to give compound **22** as a yellow oily liquid (32.6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H), 6.64 (d, J = 2.2 Hz, 1H), 6.61 (dd, J = 7.6, 6.1 Hz, 1H), 6.57 (dt, J = 10.6, 2.3 Hz, 1H), 4.97 (s, 1H), 4.93 (s, 1H), 3.81 (s, 3H), 3.40 (dd, J = 13.1, 6.9 Hz, 4H), 1.59 (dd, J = 14.7, 7.5 Hz, 2H), 1.54 (dd, J = 14.7, 7.2 Hz, 2H), 1.42 (dd, J = 15.0, 7.4 Hz, 2H), 1.35 (dd, J = 15.0, 7.4 Hz, 2H), 0.94 (dt, J = 11.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.1 (d, $^1J_{C-F}$ = 246.1 Hz), 161.8, 161.6 (d, $^3J_{C-F}$ = 11.9 Hz), 159.9, 154.4, 138.6 (d, $^3J_{C-F}$ = 10.2 Hz), 110.5 (d, $^4J_{C-F}$ = 2.6 Hz), 109.0, 107.9 (d, $^2J_{C-F}$ = 21.8 Hz), 100.5 (d, $^2J_{C-F}$ = 25.1 Hz), 55.7, 41.3, 40.5, 32.1, 31.7, 20.3, 14.0, 13.9. MS (ESI) m/z = 347.13 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{28}FN_4O^+$ $[M+H]^+$: 347.2242 found 347.2241.

N^2,N^4-Dibutyl-5-(3-chloro-4-methoxyphenyl)pyrimidine-2,4-diamine (**23**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-chloro-4-methoxyphenylboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:1) to give compound **23** as a yellow oily liquid (84.6% yield). ^{1}H NMR (500 MHz, DMSO) δ 7.51 (s, 1H), 7.29 (d, J = 1.9 Hz, 1H), 7.21 (dd, J = 8.5, 2.0 Hz, 1H), 7.18 (d, J = 8.5 Hz, 1H), 6.51 (s, 1H), 6.17 (s, 1H), 3.87 (s, 3H), 3.28 (dd, J = 13.3, 6.8 Hz, 2H), 3.22 (dd, J = 13.4, 6.7 Hz, 2H), 1.54–1.45 (m, 4H), 1.31 (dd, J = 14.0, 6.6 Hz, 2H), 1.27 (dd, J = 14.0, 6.6 Hz, 2H), 0.89 (td, J = 7.4, 5.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.4, 159.5, 154.7, 153.3, 130.0, 129.3, 128.7, 121.2, 113.2, 107.8, 56.1, 40.3, 40.1, 31.6, 31.1, 19.7, 19.7, 13.8. MS (ESI) m/z = 363.12 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{19}H_{28}ClN_4O^+$ $[M+H]^+$: 363.1946 found 363.1945.

N^2,N^4-Dibutyl-5-(4-methoxy-3,5-dimethylphenyl)pyrimidine-2,4-diamine (**24**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3,5-dimethyl-4-methoxyphenylboronic acid. The product was purified by column chromatography (DCM/MeOH 100:2) to give compound **24** as a yellow solid (20.5% yield). Melting point 50.8–51.5°C. ^{1}H NMR (500 MHz, DMSO) δ 7.49 (s, 1H), 6.93 (s, 2H), 6.45 (s, 1H), 6.03 (s, 1H), 3.66 (s, 3H), 3.29 (dd, J = 13.4, 6.6 Hz, 2H), 3.22 (dd, J = 13.4, 6.7 Hz, 2H), 2.23 (s, 6H), 1.53–1.46 (m, 4H), 1.32 (dd, J = 14.1, 6.6 Hz, 2H), 1.27 (dd, J = 14.1, 6.7 Hz, 2H), 0.89 (td, J = 7.4, 4.8 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.2, 159.4, 155.4, 154.3, 131.2, 130.6, 129.0, 108.0, 59.2, 40.3, 40.1, 31.6, 31.2, 19.7, 19.7, 15.9, 13.8, 13.8. MS (ESI) m/z = 357.23 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{21}H_{33}N_4O^+$ $[M+H]^+$: 357.2649 found 357.2647.

5-[4-(Benzyloxy)phenyl]-N^2,N^4-dibutylpyrimidine-2,4-diamine (**25**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 4-(benzyloxy) phenylboronic acid. The product was purified by column chromatography (DCM/MeOH 100:2) to give compound **25** as a yellow solid (48.3% yield). Melting point 71.7–72.6°C. ^{1}H NMR (500 MHz, DMSO) δ 7.50 (s, 1H), 7.47 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 7.22 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.7 Hz, 2H), 6.73 (s, 1H), 6.42 (s, 1H), 5.13 (s, 2H), 3.30 (dd, J = 13.1, 6.5 Hz, 2H), 3.27–3.22 (m, 2H), 1.54–1.46 (m, 4H), 1.32 (dd, J = 14.9, 7.5 Hz, 2H), 1.27 (dd, J = 14.9, 7.4 Hz, 2H), 0.89 (dd, J = 16.1, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 159.7, 157.4, 151.5, 137.1, 130.0, 128.4, 127.8, 127.6, 115.2, 108.7, 69.2, 40.3, 40.1, 31.4, 31.0, 19.7, 13.8. MS (ESI) m/z = 405.16 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{25}H_{33}N_4O^+$ $[M+H]^+$: 405.2649 found 405.2650.

N^2,N^4-Dibutyl-5-(naphthalen-2-yl)pyrimidine-2,4-diamine (**26**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 2-naphthaleneboronic acid. The product was purified by column chromatography (EtOAc/hexane 1:2) to give compound **26** as a yellow oily liquid (12% yield). ^{1}H NMR (500 MHz, DMSO) δ 7.95 (s, 1H), 7.91 (dd, J = 7.2, 6.6 Hz, 2H), 7.82 (s, 1H), 7.67 (s, 1H), 7.54–7.47 (m, 2H), 7.45 (dd, J = 8.4, 1.7 Hz, 1H), 6.58 (s, 1H), 6.29 (s, 1H), 3.32 (dd, J = 13.2, 6.6 Hz, 2H), 3.27 (dd, J = 13.4, 6.7 Hz, 2H), 1.52 (dq, J = 14.6, 7.2 Hz, 4H), 1.34 (dd, J = 15.3, 7.7 Hz, 2H), 1.31–1.26 (m, 2H), 0.90 (dt, J = 11.1, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, DMSO) δ 161.4, 159.5, 155.0, 133.6, 133.4, 131.8, 128.2, 127.8, 127.4, 127.2, 126.9, 126.1, 125.7, 118.3, 108.1, 40.3, 40.1, 31.6, 31.1, 19.7, 19.7, 13.8, 13.8. MS (ESI) m/z = 349.20 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{22}H_{29}N_4^+$ $[M+H]^+$: 349.2387 found 349.2387.

N^2,N^4-Dibutyl-5-(thiophen-3-yl)pyrimidine-2,4-diamine (**27**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with thiophene-3-boronic acid. The product was purified using flash chromatography (DCM/MeOH) to give compound **27** as a yellow oily liquid (57.9% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.43–7.40 (m, 1H), 7.20–7.18 (m, 1H), 7.09 (d, J = 4.9 Hz, 1H), 4.95 (s, 2H), 3.40 (tt, J = 10.7, 5.2 Hz, 4H), 1.63–1.56 (m, 2H), 1.53 (dd, J = 14.8, 7.6 Hz, 2H), 1.42 (dt, J = 14.9, 7.4 Hz, 2H), 1.34 (dt, J = 14.6, 7.4 Hz, 2H), 0.94 (dd, J = 16.1, 7.5 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.6, 160.4, 154.1, 136.1, 128.0, 126.9, 122.1, 105.0, 41.3, 40.5, 32.1, 31.7, 20.3, 20.3, 14.0, 13.9. MS (ESI) m/z = 305.06 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{16}H_{25}N_4S^+$ $[M+H]^+$: 305.1794 found 305.1794.

N^2,N^4-Dibutyl-5-(furan-3-yl)pyrimidine-2,4-diamine (**28**): The title compound was prepared according to procedure C by the reaction of intermediate **A** (0.25 g, 1 equiv) with 3-furylboronic acid. The product was purified using flash chromatography (cyclohexane/EtOAc) to give compound **28** as a yellow solid (52.2% yield). Melting point 50.7–51.4°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.50 (t, J = 1.6 Hz, 1H), 7.47–7.44 (m, 1H), 6.45 (dd, J = 1.7, 0.8 Hz, 1H), 4.87 (s, 2H), 3.43–3.35 (m, 4H), 1.62–1.56 (m, 2H), 1.56–1.50 (m, 2H), 1.45–1.38 (m, 2H), 1.35 (dt, J = 14.6, 7.4 Hz, 2H), 0.93 (td, J = 7.4, 4.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 160.5, 154.3, 143.9, 139.4, 119.7, 110.8, 100.5, 41.3, 40.4, 32.1, 31.7, 20.3, 20.2, 14.0, 13.9. MS (ESI) m/z = 289.11 $[M+H]^+$. HRMS (ESI+) m/z calcd. for $C_{16}H_{25}N_4O^+$ $[M+H]^+$: 289.2023 found 289.2022.

2,4-Dibutoxy-5-(4-chlorophenyl)pyrimidine (**29**): The title compound was prepared according to procedure C by the reaction of intermediate **B** (0.2 g, 1 equiv) with 4-chlorophenylboronic acid. The product was purified using flash chromatography (DCM/MeOH) to give compound **29** as a yellow oily liquid (36.4% yield). ^{1}H NMR

(500 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.42–7.39 (m, 2H), 7.36–7.33 (m, 2H), 4.39 (t, *J* = 6.6 Hz, 2H), 4.35 (t, *J* = 6.7 Hz, 2H), 1.79 (dt, *J* = 14.6, 6.8 Hz, 2H), 1.71 (dt, *J* = 14.5, 6.7 Hz, 2H), 1.48 (dq, *J* = 15.0, 7.5 Hz, 2H), 1.40 (dq, *J* = 14.8, 7.5 Hz, 2H), 0.93 (dt, *J* = 16.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.7, 164.4, 157.4, 133.4, 132.0, 129.9, 128.5, 114.7, 67.5, 66.8, 30.9, 30.7, 19.3, 19.2, 13.8, 13.8. MS (ESI) *m/z* = 335.10 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{24}ClN_2O_2^+$ $[M+H]^+$: 335.1521 found 335.1519.

2,4-Dibutoxy-5-(4-methoxy-3,5-dimethylphenyl)pyrimidine (**30**): The title compound was prepared according to procedure C by the reaction of intermediate **B** (0.2 g, 1 equiv) with 3,5-dimethyl-4-methoxyphenylboronic acid. The product was purified using flash chromatography (cyclohexane/DCM) to give compound **30** as a colorless oily liquid (10.6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.15 (s, 2H), 4.42 (t, *J* = 6.6 Hz, 2H), 4.36 (t, *J* = 6.8 Hz, 2H), 3.75 (s, 3H), 2.31 (s, 6H), 1.81 (dt, *J* = 14.7, 6.9 Hz, 2H), 1.75 (dt, *J* = 14.4, 6.7 Hz, 2H), 1.54–1.43 (m, 4H), 0.97 (dd, *J* = 14.1, 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 164.0, 157.3, 156.5, 130.8, 129.3, 128.9, 115.6, 67.5, 66.7, 59.8, 31.0, 30.7, 19.3, 19.2, 16.2, 13.9, 13.8. MS (ESI) *m/z* = 359.18 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{21}H_{31}N_2O_3^+$ $[M+H]^+$: 359.2329 found 359.2325.

5-[4-(Benzyloxy)phenyl]-2,4-dibutoxypyrimidine (**31**): The title compound was prepared according to procedure C by the reaction of intermediate **B** (0.2 g, 1 equiv) with 4-(benzyloxy)phenylboronic acid. The product was purified by column chromatography (DCM/hexane 1:3) to give compound **31** as a yellowish white solid (26.6% yield). Melting point 54.9–55.5°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.48–7.37 (m, 6H), 7.36–7.31 (m, 1H), 7.05–7.03 (m, 1H), 7.03–7.01 (m, 1H), 5.11 (s, 2H), 4.42 (t, *J* = 6.7 Hz, 2H), 4.39–4.35 (m, 2H), 1.82 (dt, *J* = 14.6, 6.8 Hz, 2H), 1.78–1.72 (m, 2H), 1.55–1.47 (m, 2H), 1.47–1.40 (m, 2H), 0.97 (dt, *J* = 15.2, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.9, 164.0, 158.4, 157.1, 137.0, 130.0, 128.7, 128.1, 127.6, 126.2, 115.6, 114.8, 70.1, 67.5, 66.8, 31.1, 30.8, 19.4, 19.3, 13.9, 13.9. MS (ESI) *m/z* = 407.12 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{25}H_{31}N_2O_3^+$ $[M+H]^+$: 407.2329 found 407.2327.

2,4-Bis(butylthio)-5-(4-chlorophenyl)pyrimidine (**32**): The title compound was prepared according to procedure C by the reaction of intermediate **C** (0.2 g, 1 equiv) with 4-chlorophenylboronic acid. The product was purified using flash chromatography (petroleum benzene 40–60/DCM) to give compound **32** as a yellowish white solid (42.6% yield). Melting point 81.1–82°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 3.17 (dt, *J* = 9.1, 7.5 Hz, 4H), 1.79–1.72 (m, 2H), 1.70–1.62 (m, 2H), 1.54–1.46 (m, 2H), 1.46–1.39 (m, 2H), 0.98–0.91 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.4, 168.5, 153.8, 134.7, 133.1, 130.6, 129.0, 127.4, 31.6, 31.1, 30.8, 29.7, 22.2, 22.2, 13.8, 13.8. MS (ESI) *m/z* = 367.07 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{24}ClN_2S_2^+$ $[M+H]^+$: 367.1064 found 367.1063.

2,4-Bis(butylthio)-5-(4-methoxy-3,5-dimethylphenyl)pyrimidine (**33**): The title compound was prepared according to procedure C by the reaction of intermediate **C** (0.2 g, 1 equiv) with 3,5-dimethyl-4-methoxyphenylboronic acid. The product was purified by column chromatography (DCM) to give compound **33** as a yellow oily liquid (42.7% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.04 (s, 2H), 3.77 (s, 3H), 3.21–3.12 (m, 4H), 2.32 (s, 6H), 1.80–1.72 (m, 2H), 1.71–1.63 (m, 2H), 1.51 (dt, *J* = 15.1, 7.5 Hz, 2H), 1.43 (dt, *J* = 14.8, 7.5 Hz, 2H), 0.95 (dt, *J* = 14.7, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5, 168.5, 157.3, 153.8, 131.4, 130.0, 129.6, 128.4, 59.8, 31.6, 31.2, 30.8, 29.7, 22.3, 22.2, 16.3, 13.8, 13.8. MS (ESI) *m/z* = 391.08 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{21}H_{31}N_2OS_2^+$ $[M+H]^+$: 391.1872 found 391.1871.

2,4-Bis(butylthio)-5-(naphthalen-2-yl)pyrimidine (**34**): The title compound was prepared according to procedure C by the reaction of intermediate **C** (0.2 g, 1 equiv) with 2-naphthaleneboronic acid. The product was purified by column chromatography (DCM/hexane 1:1) followed by semi-preparative HPLC; gradient of B (40%–100%) in 60 min to give compound **34** as a yellow oily liquid (24.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.90–7.86 (m, 3H), 7.54 (d, *J* = 2.8 Hz, 1H), 7.54–7.51 (m, 2H), 3.24–3.20 (m, 2H), 3.20–3.16 (m, 2H), 1.83–1.75 (m, 2H), 1.71–1.64 (m, 2H), 1.57–1.48 (m, 2H), 1.48–1.39 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.93 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.0, 168.8, 154.0, 133.3, 133.1, 132.2, 128.6, 128.5, 128.4, 128.3, 127.9, 126.8, 126.7, 126.6, 31.6, 31.2, 30.9, 29.7, 22.2, 22.2, 13.8, 13.8. MS (ESI) *m/z* = 383.10 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{22}H_{27}N_2S_2^+$ $[M+H]^+$: 383.1610 found 383.1609.

2-Butoxy-*N*-butyl-5-(4-chlorophenyl)pyrimidin-4-amine (**35**): The title compound was prepared according to procedure C by the reaction of intermediate **D2** (0.2 g, 1 equiv) with 4-chlorophenylboronic acid. The product was purified using flash chromatography (petroleum benzene 40–60/EtOAc) to give compound **35** as a yellow oily liquid (49% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80–7.71 (m, 1H), 7.43 (dd, *J* = 8.8, 4.6 Hz, 2H), 7.32–7.19 (m, 2H), 4.91 (s, 1H), 4.37–4.23 (m, 2H), 3.44 (d, *J* = 5.3 Hz, 2H), 1.78 (s, 2H), 1.59–1.40 (m, 4H), 1.33 (s, 2H), 1.01–0.81 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.8, 161.3, 154.7, 134.1, 133.1, 130.4, 129.7, 129.2, 117.1, 112.6, 67.0, 40.8, 31.5, 31.1, 20.2, 19.3, 13.9. MS (ESI) *m/z* = 334.11 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{25}ClN_3O^+$ $[M+H]^+$: 334.1681 found 334.1680.

2-Butoxy-*N*-butyl-5-(4-methoxy-3,5-dimethylphenyl)pyrimidin-4-amine (**36**): The title compound was prepared according to procedure C by the reaction of intermediate **D2** (0.2 g, 1 equiv) with 3,5-dimethyl-4-methoxyphenylboronic acid. The product was purified using flash chromatography (cyclohexane/EtOAc) to give compound **36** as a yellow oily liquid (14.4% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 6.95 (s, 2H), 5.01 (t, *J* = 5.1 Hz, 1H), 4.31 (t, *J* = 6.8 Hz, 2H), 3.76 (s, 3H), 3.44 (td, *J* = 7.1, 5.8 Hz, 2H), 2.31 (s, 6H), 1.79 (dt, *J* = 14.6, 6.9 Hz, 2H), 1.56–1.44 (m, 4H), 1.34 (dq, *J* = 14.6, 7.4 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H), 0.92 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.6, 161.6, 156.8, 154.4, 132.1, 130.1, 129.4, 113.6, 66.9, 59.8, 40.7, 31.6, 31.2, 20.2, 19.3, 16.3, 14.0, 13.9. MS (ESI) *m/z* = 358.20 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{21}H_{32}N_3O_2^+$ $[M+H]^+$: 358.2489 found 358.2489.

N-Butyl-2-(butylthio)-5-(4-chlorophenyl)pyrimidin-4-amine (**37**): The title compound was prepared according to procedure C by the reaction of intermediate **D3** (0.2 g, 1 equiv) with 4-chlorophenylboronic acid. The product was purified using flash chromatography (DCM/MeOH) to give compound **37** as a yellow solid (33.8% yield). Melting point 82.4–83°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.46–7.44 (m, 1H), 7.44–7.42 (m, 1H), 7.29–7.27 (m, 1H), 7.26 (t, *J* = 2.3 Hz, 1H),

4.93 (s, 1H), 3.45 (td, *J* = 7.1, 5.9 Hz, 2H), 3.16–3.09 (m, 2H), 1.79–1.70 (m, 2H), 1.55 (dd, *J* = 14.7, 7.3 Hz, 2H), 1.48 (dd, *J* = 15.1, 7.6 Hz, 2H), 1.34 (dq, *J* = 14.7, 7.4 Hz, 2H), 0.95 (t, *J* = 6.3 Hz, 3H), 0.92 (t, *J* = 6.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 170.8, 159.1, 153.4, 134.4, 133.0, 130.3, 129.8, 113.9, 40.8, 31.9, 31.6, 22.3, 20.2, 13.9, 13.8. MS (ESI) *m/z* = 350.10 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{25}ClN_3S^+$ $[M+H]^+$: 350.1452 found 350.1454.

1,3-Dibutyl-5-(4-chlorophenyl)pyrimidine-2,4(1*H*,3*H*)-dione (**38**): The title compound was prepared according to procedure C by the reaction of intermediate **E** (0.2 g, 1 equiv) with 4-chlorophenylboronic acid. The product was purified by flash chromatography (petroleum benzene 40–60/DCM) to give compound **38** as a yellow oily liquid (75.6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.45 (dd, *J* = 8.7, 1.9 Hz, 2H), 7.35 (t, *J* = 5.4 Hz, 2H), 7.26 (s, 1H), 4.04–3.98 (m, 2H), 3.84–3.77 (m, 2H), 1.75–1.68 (m, 2H), 1.64 (tt, *J* = 7.8, 6.6 Hz, 2H), 1.43–1.34 (m, 4H), 0.96 (dt, *J* = 13.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 161.9, 150.9, 139.9, 133.7, 131.6, 129.6, 128.7, 113.3, 49.9, 41.8, 31.3, 29.7, 20.4, 19.9, 13.9, 13.8. MS (ESI) *m/z* = 335.09 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{18}H_{24}ClN_2O_2^+$ $[M+H]^+$: 335.1521 found 335.1517.

1,3-Dibutyl-5-(*m*-tolyl)pyrimidine-2,4(1*H*,3*H*)-dione (**39**): The title compound was prepared according to procedure C by the reaction of intermediate **E** (0.2 g, 1 equiv) with m-tolylboronic acid. The product was purified using column chromatography (EtOAc/hexane 1:6) to give compound **39** as a yellow oily liquid (26.5% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.34 (s, 1H), 7.27 (d, *J* = 5.0 Hz, 2H), 7.25 (s, 1H), 7.16–7.11 (m, 1H), 4.05–3.99 (m, 2H), 3.83–3.78 (m, 2H), 2.38 (s, 3H), 1.76–1.69 (m, 2H), 1.65 (tt, *J* = 7.7, 6.7 Hz, 2H), 1.44–1.35 (m, 4H), 0.96 (dt, *J* = 11.4, 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 162.1, 151.0, 139.8, 138.1, 133.1, 129.1, 128.6, 128.4, 125.4, 114.6, 49.8, 41.7, 31.3, 29.7, 21.5, 20.4, 19.9, 13.9, 13.8. MS (ESI) *m/z* = 315.06 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{19}H_{27}N_2O_2^+$ $[M+H]^+$: 315.2067 found 315.2065.

5-[4-(Benzyloxy)phenyl]-1,3-dibutylpyrimidine-2,4(1*H*,3*H*)-dione (**40**): The title compound was prepared according to procedure C by the reaction of intermediate **E** (0.2 g, 1 equiv) with 4-(benzyloxy) phenylboronic acid. The product was purified using column chromatography (EtOAc/hexane 1:5) to give compound **40** as a yellow oily liquid (26.6% yield). ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 7.48–7.40 (m, 4H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.1 Hz, 1H), 7.21 (s, 1H), 6.99 (d, *J* = 8.6 Hz, 2H), 5.08 (s, 2H), 4.05–3.96 (m, 2H), 3.79 (t, *J* = 7.3 Hz, 2H), 1.71 (dt, *J* = 15.0, 7.5 Hz, 2H), 1.67–1.60 (m, 2H), 1.39 (dq, *J* = 14.7, 7.2 Hz, 4H), 0.96 (dt, *J* = 14.6, 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 162.3, 158.5, 151.0, 139.0, 136.9, 129.6, 128.6, 128.0, 127.5, 125.8, 114.9, 114.1, 70.1, 49.7, 41.7, 31.3, 29.7, 20.3, 19.9, 13.9, 13.7. MS (ESI) *m/z* = 407.09 $[M+H]^+$. HRMS (ESI+) *m/z* calcd. for $C_{25}H_{31}N_2O_3^+$ $[M+H]^+$: 407.2329 found 407.2327.

4.2 | Biology

4.2.1 | MIC determination

All bacterial isolates were handled according to standard procedures or were part of our internal collection and were cultured under conditions recommended by the depositor. *S. aureus* strains Newman, Mu50 and N315 were acquired from the Institute of Medical Microbiology, Zurich, Switzerland, and were generously supplied by Brigitte Berger-Bächi. Single colonies of *S. aureus*, *E. faecium*, *E. faecalis*, *S. pneumoniae*, and *E. coli* were inoculated into cation-adjusted Mueller Hinton Broth, to obtain a final inoculum of 10^5 colony-forming units (CFU)/mL. The tested derivatives were prepared as DMSO stocks (10 mg/mL). Serial dilutions of derivatives in the respective growth medium (0.06–128 μg/mL) were prepared in sterile 96-well plates and the bacterial suspensions were added. Growth inhibition was assessed after incubation at 37°C. MIC was determined as the lowest compound concentration where no visible growth was observed.

4.2.2 | Cytotoxicity evaluation

HepG2 cells (human hepatoblastoma cell line; ACC 180, DSMZ) were cultured under conditions recommended by the depositor and cells were propagated in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum. For determining the antiproliferative activity of test compounds, cells were seeded at 6×10^3 cells per well of 96-well plates in 120 μL complete medium. After 2 h of equilibration, compounds were added in serial dilution in 60 μL complete medium. Compounds, as well as the solvent control and doxorubicin as reference, were tested in duplicate in two independent experiments. After 5 days incubation, 20 μL of 5 mg/mL MTT (thiazolyl blue tetrazolium bromide) in PBS was added per well, and cells were further incubated at 37°C for 2 h. The medium was then discarded and cells were washed with 100 μL PBS before adding 100 μL 2-propanol/10 N HCl (250:1) to dissolve formazan granules. The absorbance at 570 nm was measured using a microplate reader (Tecan Infinite M200Pro), and cell viability was expressed as percentage relative to the respective solvent control. IC_{50} values were determined by sigmoidal curve fitting using GraphPad PRISM 8 (GraphPad Software).

4.2.3 | Macromolecule synthesis assay

B. subtilis ssp. was cultured in lysogeny broth (LB) medium. ^{3}H-labeled precursors (1 μCi/mL for uridine, glucosamine and glutamine; 1.25 μCi/mL for thymidine) were added separately to the bacteria during their logarithmic growth phase and several min (3 min for uridine and thymidine, 5 min for glucosamine, 12 min for glutamine) before the addition of compound **12** at 1.5 times its MIC. For DNA, RNA, peptidoglycan and protein synthesis, 300 μL of the cultured bacteria were harvested 0, 15 and 30 min after addition of the inhibitors and supplemented with 450 μL of 10% TCA. After 45 min at 4°C, the precipitates were collected by centrifugation and washed 1x with 10% TCA and 2x with 95% ethanol in 96-well glass fiber filter plates (Multiscreen GFB). After adding Optiphase Supermix (Perkin Elmer), the quantification of radioactivity was performed using a Wallac MicroBeta TriLux system (Perkin Elmer). For each set of experiments, two antibiotics known to (or not) specifically inhibit the

pathway of interest were included as positive and negative controls (also at 1.5 times their MIC values). Before starting the experiment, MIC values of compounds were determined to be 9.5 µM (compound **12**), 0.17 µg/mL (rifampicin), 0.094 µg/mL (ciprofloxacin), 25.5 µg/mL (chloramphenicol), and 0.14 µg/mL (meropenem). The evaluation and presentation of the data were performed with GraphPad Prism 9.4.0 (GraphPad Software).

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