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Molecular Docking Studies, Synthesis and Biological Evaluation of Substituted Pyrimidine-2,4-diamines as Inhibitors of *Plasmodium falciparum* Dihydrofolate Reductase

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A series of 5-[(phenethylamino)methyl]pyrimidine-2,4-diamines were assessed *in silico* as potential inhibitors of *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR), synthesised and tested for inhibitory activity against *Pf*DHFR *in vitro*. The compounds displayed promising inhibitory activity against both wild-type (K_i 1.3–243 nM) and quadruple mutant (K_i 13–208 nM) *Pf*DHFR in the biochemical enzyme assay, but were less potent in the whole-cell *P. falciparum* assay (IC_{50}(TM4/8.2) 0.4–28 μM; IC_{50}(V1S) 3.7–54 μM). Further investigation into the pharmacokinetic properties of these compounds may guide the development of more potent analogues.

Introduction

Malaria, a disease caused by protozoan parasites of the genus *Plasmodium*, is prevalent in sub-Saharan Africa. In 2020 an estimated 241 million cases of malaria, resulting in approximately 627 000 fatalities worldwide, was reported by the WHO.[1] As many as 96% of the reported fatalities occurred in Africa and were caused by *P. falciparum*, the predominant parasite species in this region. Despite interventions such as the use of preventative chemotherapy in children and pregnant women, and insecticides to control the mosquito vector, there has been a significant increase in both the number of observed malaria cases and reported fatalities since 2015.[2] Parasite resistance to current treatments, increasing mosquito resistance to insecticides, and service disruptions due to the COVID-19 pandemic are all contributing factors that threaten the progress made in the fight against malaria over the last two decades.

One of the few validated targets for malaria chemotherapy is dihydrofolate reductase (DHFR), an enzyme involved in folate metabolism. Folates are essential cellular cofactors required for a variety of key metabolic processes in living organisms.[3] DHFR, which exists as a bifunctional dimer with thymidylate synthase (DHFR-TS) in the parasite, is one of the enzymes responsible for maintaining cellular levels of folate derivatives.[4] Two of the original antifolate drugs that target DHFR, pyrimethamine (**1**) and cycloguanil (**2**) (Figure 1), are no longer used clinically due to widespread resistance caused by point mutations in the DHFR active site.[5] This has prompted the development of related compounds with inherent flexibility,

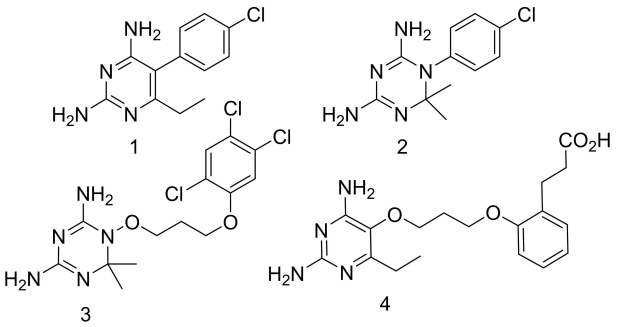

Figure 1. Known antifolates pyrimethamine (**1**), cycloguanil (**2**), WR99210 (**3**) and P218 (**4**).

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/cmdc.202200418

This article belongs to the Joint Special Collection "Biological and Medicinal Chemistry in Africa".

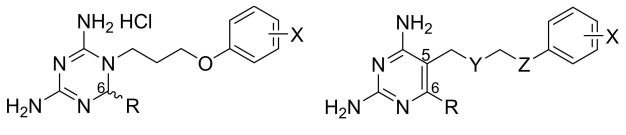
such as WR99210 (**3**) and P218 (**4**), that maintain good binding affinity to all mutant forms of *P. falciparum* DHFR.[6]

We have previously reported the synthesis and antiplasmodial activity of 6-aryl-1,6-dihydro-1,3,5-triazine-2,4-diamines **5** (Figure 2) as novel, flexible analogues of cycloguanil **2**.[7] These compounds, bearing a flexible linker of 4-atoms between the dihydrotriazine and phenyl rings, exhibited *in vitro* antiplasmodial activity in the low nanomolar range against both drug sensitive and drug resistant strains of *P. falciparum* and were shown to act as potent inhibitors of parasitic DHFR.[7] Despite the promising results *in vitro*, the synthesis of these analogues was low yielding and afforded a racemate. As a stereoselective synthesis was not feasible, we embarked on the synthesis of pyrimidine analogues **6** to eliminate the stereogenic centre (Figure 2).[8] Unfortunately, these analogues were found to be less potent than the dihydrotriazines **5** against the parasite *in vitro* with IC_{50} values in the low micromolar range.[8] This could be due to a change in the pharmacokinetic properties of the compounds, or the introduction of an inflexible biaryl axis now present between the 6-phenyl substituent and the pyrimidine ring in **6**, which was not present in the dihydrotriazines **5** as the phenyl substituent was bonded to an sp^3 hybridised carbon. However, we did not determine whether these compounds showed any inhibitory activity against *Pf*DHFR in a biochemical enzyme assay.

We now report our efforts to determine whether the 6-phenyl substituent present in pyrimidines **6** has an impact on inhibitory activity against *Pf*DHFR. In order to establish this, we have synthesised a series of analogues bearing a more flexible cyclohexyl substituent, or a smaller cyclopropyl substituent at the 6-position of the pyrimidine ring, and assessed their biological activity both against *Pf*DHFR and the parasite *in vitro*.

Results and Discussion

Initially, we attempted to prepare analogues of **6** bearing a non-aromatic substituent at the 6-position of the pyrimidine ring using our previously reported synthetic methodology.[8] However, this and other attempts to prepare these analogues were unsuccessful. We therefore identified an alternative approach which would afford analogues **7** with a modified side chain containing a nitrogen atom (Figure 2). In order to determine whether this modification of the side chain would affect binding in the *Pf*DHFR active site, a molecular modelling study was conducted. A series of compounds **7** bearing either a phenyl-, cyclopropyl- or cyclohexyl- substituent at the 6-position of the pyrimidine ring, and with Cl, F or OMe substituents X, was considered. Compounds **7** bearing a phenyl substituent could be directly compared with analogues **6** prepared previously, and would inform how modification of the side chain affects binding.



5 R = Ph or substituted Ph

6 Y = CH_2, Z = O, R = Ph
7 Y = NH, Z = CH_2, R = Ph, c-hex, c-prop

Figure 2. Dihydrotriazines **5**[7] and pyrimidines **6**[8] prepared previously and pyrimidines **7** considered in this work.

The induced fit docking (IFD) workflow from Schrödinger[9] was used to assess the binding ability of these compounds against quadruple mutant *Pf*DHFR (PDB structure 1J3K) bearing the following mutations: Asn51Ile, Cys59Arg, Ser108Asn and Ile164Leu. These mutations lead to resistance to known inhibitors cycloguanil and pyrimethamine as follows: i) the Ser108Asn and Cys59Arg mutations result in steric clashes with inhibitors in the DHFR active site and ii) the Asn51Ile and Ile164Leu mutations cause a shift in the chains around the active site, reducing the binding affinity of small inhibitors. For this study, docking constraints in the form of forced hydrogen bonds to the backbone of residues Ile14 and mutant Ile164, and to the side chain of Asp54, were added to ensure the ligands adhered to the known conserved binding mode. The default settings were used for the initial Glide docking and Prime refinement steps, while the more rigorous Glide XP algorithm was selected for the redocking step.

To assess the reliability of the docking procedures implemented, the generated poses for the co-crystalized ligand WR99210 (**3**) from the IFD experiment were compared to the original pose of this ligand as found in PDB structure 1J3K (see Supporting Information). A Root Mean Square Deviation (RMSD) value of 1.83 Å was calculated for the top scoring IFD pose for WR99210 when aligned to the original conformation (values of <3.0 angstroms are considered acceptable). Other lower scoring poses showed even better alignment, with the most similar yielding an RMSD value of 0.360 Å. This confirmed that the IFD method reliably replicates the binding mode of the ligand as found in the crystal structure.

Our series of compounds **7** was then subjected to the same IFD experiment, and compounds ranked according to several different docking scores, including the XP GScore, which is calculated for the final glide XP docking step, the Prime energy, which approximates the overall energy of the binding pose, and the IFDScore, which combines the Prime energy and docking score into a single term. The IFD results predict that the compounds bind with high affinity to the target enzyme, with IFD scores ranging between -2464.66 and -2471.58 cal mol^{-1}, comparing well with the known binders pyrimethamine (**1**), cycloguanil (**2**), WR99210 (**3**) and the natural substrate, dihydrofolate (DHF) (IFD scores of -2460.19 to -2470.90 cal mol^{-1}) (Table 1). Based on the moderate antiplasmodial data obtained previously for analogues of **6** bearing a 6-phenyl substituent, only the top binding compounds **7** bearing a 6-phenyl substituent have been included.

Several conserved binding interactions were observed, including strong pi-cation and pi-pi interactions with Phe58, and H-bonds to residues Cys15 and mutant Asn108, with the latter able to donate and accept H-bonds from the secondary amine present in the four-atom linker (Figure 3). At physiological pH, the

Table 1. Results of docking studies of proposed pyrimidines **7a–y** docked against 1J3K.

Compound	R[]]	X	XP GScore[a]	Prime energy[a]	IFD Score[a]
7a	Phenyl	2-OMe	−13.027	−49.1467	−2470.36
7b	Phenyl	3-OMe	−13.874	−49.1541	−2471.58
7c	Phenyl	4-OMe	−11.004	−49.1661	−2469.31
7d	Cyclopropyl	2-OMe	−11.028	−49.1597	−2469.01
7e	Cyclopropyl	3-OMe	−10.993	−49.1674	−2469.36
7f	Cyclopropyl	4-OMe	−11.231	−49.1596	−2469.21
7g	Cyclopropyl	2-F	−10.998	−49.1575	−2468.87
7h	Cyclopropyl	3-F	−11.356	−49.1319	−2467.95
7i	Cyclopropyl	4-F	−10.122	−49.1682	−2468.53
7j	Cyclopropyl	2-Cl	−10.192	−49.1645	−2468.42
7k	Cyclopropyl	3-Cl	−11.386	−49.1548	−2469.13
7l	Cyclopropyl	4-Cl	−10.726	−49.168	−2469.13
7m	Cyclopropyl	2,4-diCl	−11.483	−49.1672	−2469.84
7n	Cyclopropyl	3,4-diCl	−10.967	−49.1658	−2469.26
7o	Cyclohexyl	2-OMe	−9.542	−49.1875	−2468.92
7p	Cyclohexyl	3-OMe	−10.945	−49.1626	−2469.08
7q	Cyclohexyl	4-OMe	−11.692	−49.1493	−2469.16
7r	Cyclohexyl	2-F	−10.769	−49.1611	−2468.82
7s	Cyclohexyl	3-F	−11.283	−49.1446	−2468.51
7t	Cyclohexyl	4-F	−10.171	−49.1602	−2468.18
7u	Cyclohexyl	2-Cl	−10.358	−49.167	−2468.71
7v	Cyclohexyl	3-Cl	−11.239	−49.1677	−2469.62
7w	Cyclohexyl	4-Cl	−10.425	−49.162	−2468.53
7x	Cyclohexyl	2,4-diCl	−8.926	−49.1486	−2466.36
7y	Cyclohexyl	3,4-diCl	−11.196	−49.1554	−2468.96
DHF	Dihydrofolate		−15.891	−49.1001	−2470.90
1	Pyrimethamine		−8.827	−49.1487	−2466.26
2	Cycloguanil		−8.995	−49.0240	−2460.19
3	WR99210		−9.257	−49.0653	−2462.52

[a] All docking scores given in units cal mol^{-1}.

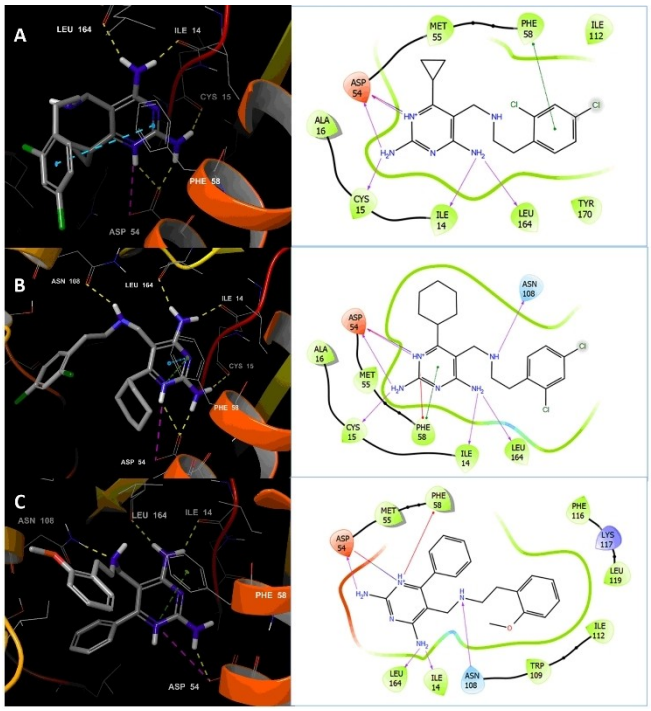


Figure 3. A: **7m**; B: **7x**, and C: **7a** bound in the active site of *Pf*DHFR (PDB: 1J3K) and the corresponding 2D interaction diagram in each case.

pyrimidine ring nitrogen in the 1 position can become protonated and engage in electrostatic interactions with nearby residues, with a salt-bridge interaction to the charged residue Asp54 being of particular importance (Figure 3).

A trend in the binding ability of the substituents X (**7**, Figure 2) was observed, with compounds bearing methoxy substituents on average giving the best IFD docking scores (lowest energy), followed by those substituted with 2,4- and 3,4-dichloro substituents, then chloro substituents and finally fluoro substituents. The position of the X-substituent on the ring was also found to be significant, with the 3-position clearly favoured, followed by the 4-position and then the 2-position. For the R substituents there was less of an obvious trend, however on average compounds bearing a cyclopropyl group in the 6-position gave the best IFD docking scores, followed by phenyl and cyclohexyl substituents.

The binding mode of compounds bearing cyclopropyl (**7d–7n**) and cyclohexyl (**7o–7y**) substituents at the 6-position of the pyrimidine ring was tightly conserved, with the position of the pyrimidine core virtually unchanged between ligands, while the linker and terminal rings showed greater variability. This is highlighted for **7e**, **7g**, **7j**, **7p** and **7w** in Figure 4A, which clearly shows the conserved binding of the pyrimidine core, and the variability in binding of the flexible side chain for compounds in these series. By comparison, compounds substituted with a phenyl group occupy a slightly offset binding position of the pyrimidine core, with slightly different binding interactions (Figure 4B). This is likely due to the rigid biaryl axis present in these compounds, and may explain the reduced docking scores observed for **7a–c**.

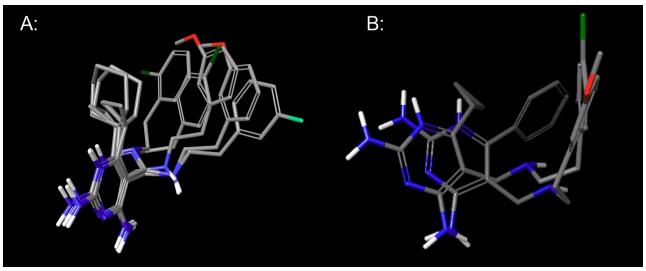


Figure 4. A) Superimposed binding positions of cyclopropyl (**7e**, **7g** and **7j**) and cyclohexyl (**7p** and **7w**) analogues. B) Binding position of **7i** superimposed with **7c**.

Many of the compounds in the cyclopropyl and cyclohexyl series engage in H-bonds to Cys15, while this residue is out of reach of compounds of the phenyl series (Figure 3). Additionally, the saturated analogues donate two H-bonds to the carbonyl of Asp54, while the phenyl analogues only make one H-bond to the carboxylate oxygen. Importantly, these compounds are still close enough to this group to engage in an electrostatic interaction.

Molecular dynamics (MD) simulations were run on one compound from each series: **7b**, **7i** and **7w**. The top scoring pose (IFD analysis) for each compound was selected as the starting structure for the simulations which were run for 100 ns, with frames captured every 100 ps for a total of 1000 frames. For each of these ligands, the RMSD values of the protein Cα atoms reached a steady state rapidly and fluctuated minimally at an RMSD of 2.4 Å from the initial position. For **7b**, the RMSD of the ligand reached a plateau shortly after 25 ns, with values ranging between 1.2 to 2.4 Å after this point. For **7i**, the RMSD values fluctuated between 1.2 and 3.2 Å for the first 67 ns of the simulation before adopting a more stable conformation with RMSD values between 1.4 and 2.2 Å, while **7w** maintained stable RMSD values between 1.0 and 2.3 Å for most of the simulation time (see Figure 5A for **7i** and Supporting Information). In each case, both ligand and protein RMSD plots show that the ligands are stable bound in the active site. Furthermore, the ligand Root Mean Square Fluctuation (RMSF) plots by atom position show that the relative position of the pyrimidine core is very constant (low RMSF values for atoms 1–8), while the positions of the linker and terminal ring display greater variability (higher RMSF values for these atoms, see Figure 5B for **7i** and Supporting Information).

The predicted binding mode of these compounds was also confirmed by the MD simulations. For all three analyses, key H-bonds to Leu164, Ile14, and Asp54 residues are preserved for more than 85% of the total simulation. Additional interactions are also observed with Phe58, Asn108 and Cys15, indicating a good correlation with the IFD results (see Figure 5C for **7i** and Supporting Information). While the IFD analysis indicated that compounds in the phenyl series were positioned too far away to interact with Cys15, the MD simulations show that a water molecule can facilitate this binding interaction. Finally, a prediction of the pharmacokinetic properties of the compounds

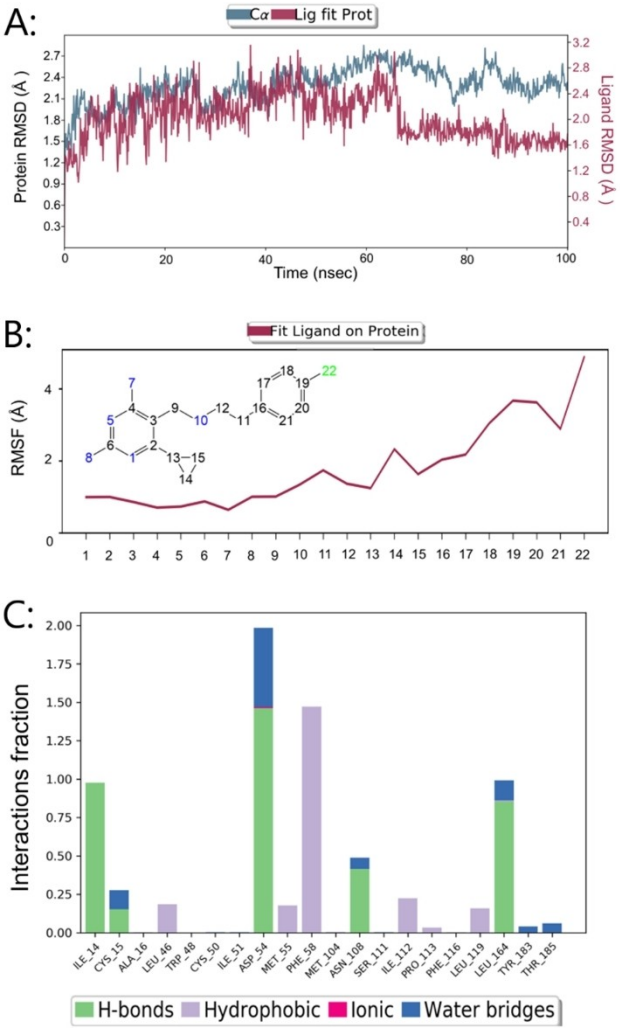
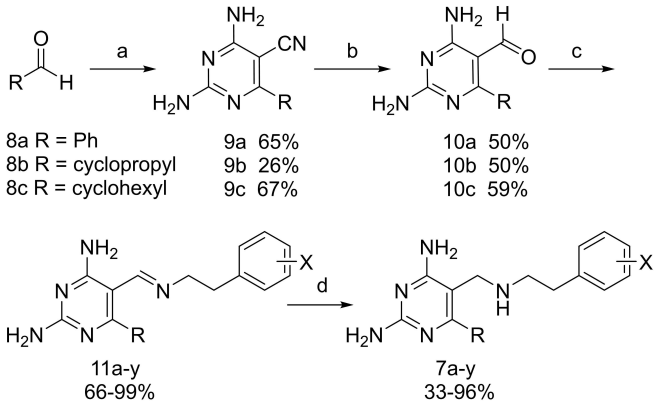


Figure 5. A) RMSD plot for **7i**. B) RMSF plot for **7i**. C) Protein-ligand interaction diagram for **7i**.

was done *in silico* using QikProp, with no adverse properties identified (see Supporting Information).

As the modelling study confirmed the potential for pyrimidines **7** to act as inhibitors of *Pf*DHFR, we embarked on the synthesis of analogues **7a**–**y** (Scheme 1). We adapted a reported multicomponent synthesis of 2,4-diaminopyrimidine-5-carbonitriles involving the reaction of guanidine hydrochloride and malononitrile and employing benzaldehyde **8a** (Scheme 1), to include the use of the non-aromatic aldehydes, cyclopropanecarbaldehyde **8b** and cyclohexanecarbaldehyde **8c** in the reaction.[10] As the synthesis of 2,4-diaminopyrimidine-5-carbonitriles by this approach has only been described for aromatic aldehydes,[10] the methodology was first tested using benzaldehyde **8a**.

In our hands the reported microwave method for the synthesis of pyrimidines **9** was not successful, but using conventional heating and guanidine carbonate, the products **9a**–**c** were isolated in moderate yields (26–67%). Each of the pyrimidine-5-carbonitriles **9a**–**c** prepared by this method was



Scheme 1. Synthesis of pyrimidines **7**. a) malononitrile, guanidine carbonate, NaOAc, H_2O/EtOH, reflux, 2.5–3 h; b) formic acid (81 %), Raney Ni, reflux, 1 h; c) 1,2-DCE, glacial AcOH, substituted phenethylamine, reflux, 3 h; d) 1,2-DCE/THF (1 : 2), glacial AcOH, $NaBH_4$, rt, 30 min.

then subjected to Raney nickel reduction under acidic conditions to afford the corresponding aldehydes **10a**–**c** in moderate yields. Other methods, including the use of Pd/C in the presence of formic acid were also successful, but often did not go to completion. This made purification of the aldehydes, as well as subsequent products in the synthetic sequence, difficult as the Rf values were very similar. Treatment of **10a**–**c** with suitably substituted commercially available phenethylamines under reductive amination conditions, either in a single reaction or in a two-step process via imines **11a**–**y**, gave pyrimidine analogues **7a**–**y** bearing both aromatic and non-aromatic substituents (Scheme 1). As both the yield and purity of the desired products improved when the imine formation and reduction reactions were decoupled, this is the route that was adopted for the compounds reported.

The series of compounds **7a**–**y** synthesised by this method were assessed for inhibitory activity against wild type (*Pf*DHFR-WT) and quadruple mutant (*Pf*DHFR-QM) *P. falciparum* DHFR, and human DHFR (Table 2). All of the compounds inhibited parasitic DHFR to a far greater extent than human DHFR. However, it can be seen that the phenyl substituent does negatively affect binding in the active site of *Pf*DHFR, with compounds **7a**–**c** bearing a 6-phenyl substituent displaying the weakest binding, with K_i values against wild type *Pf*DHFR in the range 86–243 nM and quadruple mutant *Pf*DHFR in the range 69–208 nM.

Compounds in the cyclopropyl series (**7d**–**n**) performed best against wild type *Pf*DHFR (K_i 1.3–15 nM) and, as predicted by the molecular modelling, against quadruple mutant *Pf*DHFR (K_i 13–31 nM), followed by compounds in the cyclohexyl series (**7o**–**y**; K_i(WT) 7.1–128 nM; K_i(QM) 28–136 nM).

Although none of the compounds inhibited the wild type enzyme to the same extent as the known inhibitor, pyrimethamine (K_i 0.3 nM), a number of compounds in the series inhibited the quadruple mutant to a similar extent (K_i 29 nM). Similar trends to what was predicted in the molecular modelling were observed for the X-substituents, with the exception of compounds bearing methoxy substituents where

Table 2. Results of DHFR enzyme inhibition studies *in vitro* antiplasmodial activity and *in vitro* cytotoxicity of compounds **7a**–**y**.

Entry	K_i [nM] *Pf*DHFR-WT[a]	K_i [nM] *Pf*DHFR-QM[b]	K_i [nM] *Hs*DHFR[c]	IC_{50} [μM] *Pf*TM4/8.2[d]	IC_{50} [μM] *Pf*V1S[e]	IC_{50} [μM] Vero[f]	IC_{50} [μM] KB[g]
7a	243 ± 21	208 ± 3	> 500 000	27 ± 3	54 ± 2	> 100	> 100
7b	107 ± 3	74 ± 1	> 500 000	27 ± 4	48 ± 1	> 100	> 100
7c	86 ± 13	69 ± 5	> 500 000	28 ± 5	34 ± 2	> 100	> 100
7d	15 ± 1	31 ± 2	1590 ± 29	25 ± 1	35 ± 4	> 100	> 100
7e	4.4 ± 0.2	26 ± 0.5	1040 ± 34	4.2 ± 0.2	13 ± 1	7.2 ± 2.0	74 ± 4
7f	3.5 ± 0.4	29 ± 0.6	647 ± 6	3.5 ± 0.2	15 ± 3	71 ± 7	72 ± 1
7g	8.2 ± 0.3	31 ± 2	205 ± 1	12 ± 5	25 ± 2	> 100	> 100
7h	10 ± 0.3	26 ± 2	458 ± 31	4.2 ± 0.3	20 ± 2	> 100	> 100
7i	5.7 ± 0.5	35 ± 2	212 ± 12	23 ± 3	48 ± 5	> 100	> 100
7j	9.3 ± 0.5	23 ± 0.6	304 ± 16	0.4 ± 0.01	17 ± 3	67 ± 2	83 ± 7
7k	2.5 ± 0.1	20 ± 0.9	345 ± 7	4.2 ± 0.9	14 ± 4	76 ± 4	82 ± 13
7l	2.5 ± 0.2	27 ± 0.1	142 ± 6	5.0 ± 0.6	18 ± 3	70 ± 2	70 ± 0.5
7m	2.0 ± 0.03	22. ± 2	131 ± 5	3.6 ± 0.2	6.3 ± 1.2	31 ± 7	66 ± 2
7n	1.3 ± 0.06	13 ± 0.04	202 ± 6	3.3 ± 0.1	4.4 ± 0.1	21 ± 1	23 ± 0.5
7o	128 ± 9	126 ± 9	> 500 000	3.7 ± 0.3	9.4 ± 1.9	21 ± 1	22 ± 0.3
7p	31 ± 5	56 ± 2	> 500 000	3.5 ± 0.2	4.1 ± 0.3	23 ± 2	23 ± 0.3
7q	32 ± 1	55 ± 2	> 500 000	1.8 ± 0.1	3.7 ± 0.2	21 ± 2	23 ± 0.2
7r	61 ± 1.5	83 ± 1	> 500 000	4.1 ± 0.7	16 ± 2	22 ± 1	48 ± 14
7s	76 ± 5	74 ± 2	> 500 000	3.0 ± 0.4	11 ± 2	25 ± 3	62 ± 3
7t	80 ± 9	136 ± 7	> 250 000	2.2 ± 0.1	7 ± 2	22 ± 1	56 ± 5
7u	22 ± 2	38 ± 1	> 250 000	3.3 ± 0.3	16 ± 1	21 ± 1	22 ± 0.05
7v	25 ± 1	36 ± 4	> 250 000	3.1 ± 0.4	4.0 ± 0.2	23 ± 2	23 ± 0.2
7w	22 ± 0. 5	60 ± 1	> 500 000	3.1 ± 0.2	4.5 ± 0.3	23 ± 2	23 ± 0.2
7x	7 ± 0.3	123 ± 8	> 250 000	2.5 ± 0.4	4.9 ± 0.3	16 ± 2	62 ± 3
7y	9 ± 0.7	28 ± 3	> 250 000	3.5 ± 0.3	4.0 ± 0.3	21 ± 1	22 ± 0.1
PYR	0.3 ± 0.02 0.3 ± 0.08[h]	29 ± 1.5 385 ± 163[14][h]	41 ± 1 28 ± 3[h]	0.05 ± 0.007	> 100	27 ± 2	60 ± 26
CYC	1.5 ± 0.3	420 ± 130	Nt	0.03 ± 0.005	92 ± 6	> 100	> 100

[a] Wild-type (WT) *Pf*DHFR. [b] Quadruple mutant (QM) *Pf*DHFR. [c] Human (Hs)DHFR. [d] WT *P. falciparum*. [e] *P. falciparum* with QM DHFR [f] African green monkey kidney epithelial cells. [g] Human epithelial carcinoma cells. [h] Time-dependent K_i, probably due to instability or conformation change of the enzyme.

the results were varied. In general, 2,4- and 3,4-dichloro-substituted compounds performed the best, followed by compounds substituted with chloro-substituents and finally fluoro-substituents in each series. The assay also confirmed that the position of the X-substituent on the ring is significant, with the 3-position favoured over the 4- and 2-positions. A weak correlation (R^2 value of 0.3404) was observed between the calculated prime energy and the experimentally obtained inhibition (see Supporting Information).

As many of the compounds in the series **7a**–**y** showed promising inhibitory activity against *Pf*DHFR, we assessed the antiplasmodial activity *in vitro* in a whole cell *P. falciparum* assay against a drug sensitive strain carrying WT *Pf*DHFR (TM4/8.2) and a multidrug resistant strain carrying QM *Pf*DHFR (V1S) of the parasite using a ^{3}H hypoxanthine incorporation assay (Table 2). Compounds were also assessed for cytotoxicity against both normal (Vero) and cancer (KB) cell lines.

Our pyrimidine compounds **7** showed moderate activity against the parasite *in vitro* (IC_{50}(WT) 0.4–28 μM; IC_{50}(QM) 3.7–54 μM). Once again, compounds **7a**-**c** bearing a 6-phenyl substituent were the least potent, with IC_{50} values against the drug sensitive strain (TM4/8.2) in the range 27–28 μM and against the multidrug resistant strain (V1S) in the range 3.8–54 μM. Interestingly, compounds in the cyclohexyl series (**7o**–**y**) generally performed best in the whole cell assay against both drug sensitive (IC_{50} 1.8–4 μM) and drug resistant strains (IC_{50} 3.7–16 μM), followed by compounds in the cyclopropyl series (**7d**–**n**; IC_{50}(WT) 0.4–25 μM; IC_{50}(QM) 4.4–48 μM). As was seen in the enzyme inhibition assay, none of the compounds **7a**–**y** were as potent against the drug sensitive strain as the known inhibitor pyrimethamine (IC_{50} 0.053 μM). However, all of the compounds were significantly more potent than pyrimethamine against the multidrug resistant strain (IC_{50} >100 μM). The cyclohexyl series of compounds (**7o**–**y**) also showed higher levels of cytotoxicity in the *in vitro* assays performed, than compounds from either the cyclopropyl or phenyl series. In order to better understand these data, further investigation of the pharmacokinetic properties of these compounds is warranted, in order to determine whether membrane permeability, solubility or binding to plasma proteins could have had an effect on their activity in the whole cell *P. falciparum* assay. The design of analogues with potentially improved pharmacokinetic properties is currently underway.

Conclusion

We have synthesized a series of 5-[(phenethylamino)methyl]pyrimidine-2,4-diamines with good inhibitory activity against both wild type (K_i 1.3–243 nM) and quadruple mutant (K_i 13–208 nM) *Pf*DHFR *in vitro*. While the observed enzyme inhibition did not correspond to potent antiplasmodial activity in a whole cell *P. falciparum* assay (IC_{50}(TM4/8.2) 0.4–28 μM; IC_{50}(V1S) 3.7–54 μM), further investigation into these compounds as potential antimalarial antifolates is warranted.

Experimental Section

Modelling

Maestro version 12.7.161, 2021-1 release by Schrödinger[9] was used for all computational experiments. The protein crystal structure 1J3K[11] of quadruple mutant *Pf*DHFR with resolution 2.10 Å, was downloaded from the protein data bank. This structure consists of four subunits, with the ligand WR99210 bound in the active site present in subunit, and was prepared using the protein preparation wizard. In this workflow non-explicit hydrogens were added, missing side chains and loops were incorporated using Prime, internal hydrogen bonds were optimized at a pH of 7, and the waters of crystallization were removed. The ligands selected for analysis consist of the natural substrate DHF, several known binders including pyrimethamine, cycloguanil and WR99210, and 33 relevant synthetic structures. Ligands were prepared using the LigPrep tool from Schrödinger; OPLS4 was selected as the force field for the energy minimization step, and Epik was used to generate possible states for the ligands under physiological conditions (pH 7 ± 2).

Prediction of pharmacokinetic properties was done using QikProp.[12]

Molecular dynamics (MD)[13] simulations were run using Desmond as part of the Schrödinger molecular modelling suite. Protein-ligand poses were taken from the previously run IFD experiments. Prior to analysis the other three subunits not containing the ligand bound in the active site were deleted. The Desmond system builder was used to prepare the protein - ligand complexes. An orthorhombic box shape was selected and the SPC solvent model used. 7 Cl^- ions were added to neutralize the system and a 0.15 M background NaCl concentration was also added. The OPLS_2005 force field was selected and a simulation time of 100 ns, with frames captured every 100 ps for a total of 1000 frames, was chosen. The NPT ensemble class was used at a temperature of 300 K and a pressure of 1.01 bar. The system was relaxed using the default procedures before simulation.

Chemistry

General

Reagents purchased from Sigma-Aldrich (Steinheim, Germany) Merck KGaA (South Africa) and MK Chemicals (South Africa) were of reagent grade and were used without any further purification unless specified. Ethyl acetate (EtOAc) and hexane used for chromatography or extractions were distilled prior to use. Tetrahydrofuran (THF) was distilled from sodium prior to use. Reactions were monitored by thin-layer chromatography (TLC) using precoated aluminium-backed plates (Merck silica gel 60 F254) visualised under UV light (λ = 254 nm). Intermediates and final compounds were purified by column chromatography on Macherey-Nagel silica gel 60 (particle size 0.063 mm to 0.200 mm) as well as aluminium oxide-neutral (0.063–0.200 mm). NMR spectra were acquired on a Bruker 300, 400 or 500 MHz spectrometer at room temperature, using the specified deuterated solvent. For those compounds soluble in deuterated chloroform ($CDCl_3$), the solvent contained tetramethyl silane (TMS, 0.05 % v/v) as internal standard. For others, the residual solvent signal was used for referencing. Data processing was done using MestreNova Software under license from Mestrelab Research, CA, USA. Infra-red spectra were recorded on a Bruker Tensor-27 Fourier Transform spectrometer. Mass Spectra (High Resolution) were recorded on a

Bruker Compact mass spectrometer. Melting points were determined on a Stuart SMP10 melting point apparatus and are uncorrected.

General procedure for the synthesis of 2,4-diamino-5-carbonitriles (9). A mixture of aldehyde **8** (23 mmol), malononitrile (1.0 eq) and NaOAc (1.0 eq) in H_2O (30 ml) and EtOH (15 ml) was stirred for 10 minutes, and then treated with guanidine carbonate (1.0 eq). The resulting mixture was heated at reflux for 2.5–3 h under an inert atmosphere and then cooled to room temperature. Where a precipitate formed, this was filtered off, or alternatively, the mixture was extracted with EtOAc (3×150 ml). The extracts were dried ($MgSO_4$), filtered through celite and concentrated *in vacuo*. The crude product was purified by recrystallization (EtOH). The following compounds were prepared by this method:

2,4-Diamino-6-phenylpyrimidine-5-carbonitrile (9a). Prepared from benzaldehyde **8a** (2.32 ml, 22.7 mmol), malononitrile (1.50 g, 22.7 mmol), sodium acetate (1.87 g, 22.7 mmol) and guanidine carbonate (2.05 g, 22.7 mmol), isolated as a yellow solid (3.14 g, 65%). m.p. 214–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.75 (dd, J = 7.3, 1.9 Hz, 2H), 7.54–7.46 (m, 3H), 7.19–6.88 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 169.4, 165.0, 163.0, 137.2, 130.2, 128.2, 128.1, 117.9, 75.9; IR (ν_{max}/cm^{-1}) 3375, 3159, 2206, 1610; HRMS (ESI): *m/z* calcd for $C_{11}H_{10}N_5^+$: 212.0931 $[M+H]^+$; found: 212.0929.

2,4-Diamino-6-cyclopropylpyrimidine-5-carbonitrile (9b). Prepared from cyclopropanecarbaldehyde **8b** (1.79 ml, 22.7 mmol), malononitrile (1.50 g, 22.7 mmol), sodium acetate (1.87 g, 22.7 mmol) and guanidine carbonate (2.04 g, 22.7 mmol), isolated as a yellow solid (1.02 g, 26%). m.p. 255–256 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.91 (s, 2H), 6.67 (s, 2H), 2.15–1.87 (m, 1H), 1.20–0.77 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 174.8, 163.9, 163.1, 117.7, 77.1, 15.0, 9.6; IR (ν_{max}/cm^{-1}) 3498, 3426, 2203, 1615, 1280, 1133; HRMS (ESI): *m/z* calcd for $C_8H_{10}N_5^+$: 176.0938 $[M+H]^+$; found: 176.0934.

2,4-Diamino-6-cyclohexylpyrimidine-5-carbonitrile (9c). Prepared from cyclohexanecarbaldehyde **8c** (2.30 mL, 18.9 mmol), malononitrile (1.25 g, 18.9 mmol), sodium acetate (1.55 g, 18.9 mmol) and guanidine carbonate (1.70 g, 18.9 mmol), isolated as a pale yellow solid (2.75 g, 67%). m.p. 223–224 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.90 (s, 2H), 6.77 (s, 2H), 2.66 (tt, J = 11.7, 3.5 Hz, 1H), 1.77 (dt, J = 12.9, 3.2 Hz, 2H), 1.73–1.62 (m, 3H), 1.60–1.43 (m, 2H), 1.30 (qt, J = 12.9, 3.2 Hz, 2H), 1.23–1.09 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 177.8, 164.4, 163.1, 117.3, 76.1, 43.9, 30.5, 25.7, 25.5; IR (ν_{max}/cm^{-1}) 3390, 2197, 2918, 1447, 890; HRMS (ESI): *m/z* calcd for $C_{11}H_{16}N_5^+$: 218.1400 $[M+H]^+$; found: 218.1406.

General procedure for the synthesis of 2,4-diaminopyrimidine-5-carbaldehydes (10). To a solution of 2,4-diamino-5-carbonitrile (**9**) in aqueous formic acid (81%) was added Raney Ni (3–6 eq by mass). The reaction flask was heated at reflux under a nitrogen atmosphere for 1 h. Upon complete consumption of starting material, the reaction mixture was filtered through celite (taking care not to filter to dryness) and the filtrate neutralised to pH 7 with solid $NaHCO_3$. The aqueous solution was then extracted with EtOAc (3×100 ml), and the combined organic layers were dried ($MgSO_4$), filtered through celite and concentrated *in vacuo*. The crude product was purified by column chromatography (50% EtOAc/hexane). The following compounds were prepared by this method:

2,4-Diamino-6-phenylpyrimidine-5-carbaldehyde (10a). Prepared from 2,4-diamino-6-phenylpyrimidine-5-carbonitrile (**9a**) (0.150 g, 0.71 mmol) in 81% aq. formic acid (4 ml) in the presence of Raney Nickel (0.600 g). Product isolated as a white solid (0.085 g, 56%). m.p. 237–238 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.67 (s, 1H), 8.76 (s, 1H), 7.83–7.39 (m, 5H), 5.56 (s, 1H), 5.36 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 190.6, 175.2, 164.2, 163.1, 136.5, 130.2, 129.6, 128.6, 104.7; IR (ν_{max}/cm^{-1}) 3431, 3314, 1610, 1546; HRMS (ESI): *m/z* calcd for $C_{11}H_{11}N_4O^+$: 215.0927 $[M+H]^+$; found: 215.0932.

2,4-Diamino-6-cyclopropylpyrimidine-5-carbaldehyde (10b). Prepared from 2,4-diamino-6-cyclopropylpyrimidine-5-carbonitrile (**9b**) (0.150 g, 0.86 mmol) in 81% aq. formic acid (4 ml) in the presence of Raney Nickel (0.600 g). as described, isolated as a white solid (0.077 g, 50%). m.p. 235–236 °C; ^{1}H NMR (500 MHz, MeOD) δ = 10.24 (s, 1H), 2.50 (tt, J = 8.4, 4.1 Hz, 2H), 1.23–1.12 (m, 2H), 1.04–0.93 (m, 2H); ^{13}C NMR (126 MHz, MeOD) δ = 189.3, 179.4, 165.3, 165.1, 105.3, 12.4, 10.5; IR (ν_{max}/cm^{-1}) 3221, 1747, 1598, 1260, 892; IR (ν_{max}/cm^{-1}) 3353, 1632, 2798, 1440; HRMS (ESI): *m/z* calcd for $C_8H_{11}N_4O^+$: 179.0927 $[M+H]^+$; found: 179.0924.

2,4-Diamino-6-cyclohexylpyrimidine-5-carbaldehyde (10c). Prepared from 2,4-diamino-6-cyclohexylpyrimidine-5-carbonitrile (**9c**) (0.200 g, 0.92 mmol) in 81% aq. formic acid (4 ml) in the presence of Raney Nickel (0.600 g) as described, isolated as a white solid (0.119 g, 59%). m.p. 211–214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 10.15 (s, 1H), 8.83 (s, 1H), 5.62 (s, 1H), 5.36 (s, 2H), 3.16 (tt, J = 11.4, 3.7 Hz, 1H), 2.04–1.81 (m, 2H), 1.80–1.50 (m, 5H), 1.45–1.10 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 188.0, 181.5, 164.1, 163.5, 103.1, 40.4, 31.9, 26.4, 26.0; IR ν_{max}/cm^{-1} 3462, 3370, 1551, 2928, 1470; HRMS (ESI): *m/z* calcd for $C_{11}H_{17}N_4O^+$: 221.1397 $[M+H]^+$; found: 221.1398.

General procedure for the synthesis of 6-substituted-2,4-diaminopyrimidine-5-phenethylimines (11). Carbaldehyde **10** was dissolved in 1,2-dichloroethane in the presence of glacial acetic acid (2 eq.). When fully dissolved, the substituted phenethylamine (1.3 eq.) was added with stirring and the resulting mixture was heated at reflux for 3 h under a nitrogen atmosphere. Upon complete reaction, the mixture was cooled to room temperature, diluted with water and extracted with EtOAc (3×100 ml). The organic layer was dried with magnesium sulfate or sodium sulfate and filtered through celite. The filtrate was concentrated *in vacuo* and the crude product was purified using aluminium oxide (neutral) column chromatography, using 50% EtOAc/Hexane as eluent. The following compounds were prepared by this method:

(*E*)-6-Phenyl-5-{[(2-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine (11a). Prepared from 2,4-diamino-5-carbaldehyde-6-phenylpyrimidine (**10a**) (0.080 g, 0.37 mmol), 2-methyoxyphenethylamine (0.071 ml, 0.48 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.043 ml, 0.75 mmol), isolated as a white solid (0.111 g, 85%). m.p. 154–156 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.86 (s, 1H), 7.97 (s, 1H), 7.42–7.28 (m, 3H), 7.28–7.15 (m, 3H), 7.06 (d, J = 7.4 Hz, 1H), 6.90–6.77 (m, 2H), 5.79 (s, 1H), 5.52 (s, 2H), 3.75 (s, 3H), 3.61 (t, J = 6.9 Hz, 2H), 2.90 (t, J = 6.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.5, 163.8, 161.7, 159.7, 157.7, 137.8, 130.9, 129.3, 128.9, 128.2, 127.5, 120.4, 110.3, 101.3, 61.1, 55.3, 32.4; IR (ν_{max}/cm^{-1}) 3267, 3105, 2922, 2882, 1647, 1245; HRMS (ESI): *m/z* calcd for $C_{20}H_{22}N_5O^+$: 348.1819 $[M+H]^+$; found: 348.1829.

(*E*)-6-Phenyl-5-{[(3-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine (11b). Prepared from 2,4-diamino-5-carbaldehyde-6-phenylpyrimidine (**10a**) (0.075 g, 0.35 mmol), 3-methyoxyphenethylamine (0.066 ml, 0.46 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.040 ml, 0.70 mmol), isolated as a white solid (0.108 g, 84%). m.p. 156–157 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.82 (s, 1H), 7.99 (s, 1H), 7.44–7.32 (m, 3H), 7.30–7.23 (m, 2H), 7.19 (t, J = 7.8 Hz, 1H), 6.80–6.68 (m, 3H), 5.61 (s, 1H), 5.27 (s, 2H), 3.77 (s, 3H), 3.64 (t, J = 6.9 Hz, 2H), 2.87 (t, J = 6.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.7, 163.9, 161.7, 160.1, 159.7, 141.7, 137.8, 129.4, 129.3, 129.2, 128.3, 121.6, 114.8, 111.6, 101.4, 62.7, 55.3, 37.9; IR (ν_{max}/cm^{-1}) 3168, 3073, 2924, 2863, 1645, 1256; HRMS (ESI): *m/z* calcd for $C_{20}H_{22}N_5O^+$: 348.1819 $[M+H]^+$; found: 348.1768.

(*E*)-6-Phenyl-5-{[(4-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine (11c). Prepared from 2,4-diamino-5-carbaldehyde-6-phenylpyrimidine (**10a**) (0.070 g, 0.33 mmol), 4-methyoxyphenethylamine (0.062 ml, 0.43 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid

(0.037 ml, 0.66 mmol), isolated as a white solid (0.108 g, 95%). m.p. 202–204 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.85 (s, 1H), 7.96 (s, 1H), 7.42–7.32 (m, 3H), 7.25 (d, *J* = 6.9 Hz, 2H), 7.05 (d, *J* = 8.2 Hz, 2H), 6.83 (d, *J* = 8.2 Hz, 2H), 5.48 (s, 1H), 5.03 (s, 2H), 3.79 (s, 3H), 3.61 (t, *J* = 6.8 Hz, 2H), 2.84 (t, *J* = 6.8 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.8, 163.9, 161.7, 160.1, 158.2, 137.9, 132.2, 130.2, 129.3, 129.2, 128.3, 113.9, 101.5, 63.1, 55.4, 37.0; IR (ν_{max}/cm^{-1}) 3269, 3103, 2920, 2883, 1617, 1240; HRMS (ESI): *m/z* calcd for $C_{20}H_{22}N_5O^+$: 348.1819 $[M+H]^+$; found: 348.1819.

(*E*)-6-Cyclopropyl-5-{[(2-methoxyphenethyl)imino]methyl}-pyrimidine-2,4-diamine (11 d). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.045 g, 0.25 mmol), 2-methyoxyphenethylamine (0.048 ml, 0.33 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.029 ml, 0.50 mmol), isolated as a colourless gel (0.065 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.83 (s, 1H), 8.66 (s, 1H), 7.23–7.11 (m, 2H), 6.91–6.82 (m, 2H), 5.53 (s, 1H), 5.00 (s, 2H), 3.82 (s, 3H), 3.77 (t, *J* = 7.2, 2H), 2.96 (t, *J* = 7.2 Hz, 2H), 2.11–2.02 (m, 1H), 1.12–1.06 (m, 2H), 0.90–0.82 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.5, 163.1, 161.9, 157.9, 157.7, 130.8, 128.4, 127.5, 120.4, 110.4, 101.8, 61.6, 55.3, 32.7, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3319, 3170, 2923, 1440, 1601, 1188, 908; HRMS (ESI): *m/z* calcd for $C_{17}H_{22}N_5O^+$: 312.1819 $[M+H]^+$; found: 312.1769.

(*E*)-6-Cyclopropyl-5-{[(3-methoxyphenethyl)imino]methyl}-pyrimidine-2,4-diamine (11 e). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.068 g, 0.38 mmol), 3-methyoxyphenethylamine (0.073 ml, 0.50 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.044 ml, 0.76 mmol) isolated as a yellow gel (0.109 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.78 (s, 1H), 8.65 (s, 1H), 7.20 (t, *J* = 7.8 Hz, 1H), 6.84–6.71 (m, 3H), 5.58 (s, 1H), 5.05 (s, 2H), 3.84–3.73 (m, 5H), 2.93 (*J* = 7.2 Hz, 2H), 2.06 (tt, *J* = 8.1, 4.8 Hz, 1H), 1.14–1.07 (m, 2H), 0.91–0.82 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.6, 163.1, 161.9, 159.7, 158.3, 141.8, 129.4, 121.5, 114.8, 111.6, 101.68, 63.1, 55.3, 38.2, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3325, 3192, 2901, 2833, 1601, 1189, 964; HRMS (ESI): *m/z* calcd for $C_{17}H_{22}N_5O^+$: 312.1819 $[M+H]^+$; found: 312.1772.

(*E*)-6-Cyclopropyl-5-{[(4-methoxyphenethyl)imino]methyl}-pyrimidine-2,4-diamine (11 f). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.073 g, 0.41 mmol), 4-methyoxyphenethylamine (0.078 ml, 0.53 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.047 ml, 0.82 mmol), isolated as a yellow gel that solidified upon standing (0.112 g, 88%). m.p. 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.80 (s, 1H), 8.62 (s, 1H), 7.16–7.07 (m, 2H), 6.88–6.78 (m, 2H), 5.69 (s, 1H), 5.17 (s, 2H), 3.81–3.66 (m, 5H), 2.89 (t, *J* = 7.1 Hz, 2H), 2.08–2.01 (m, 1H), 1.14–1.06 (m, 2H), 0.92–0.82 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.4, 163.1, 161.8, 158.2, 158.0, 132.2, 129.9, 113.8, 101.6, 63.4, 55.3, 37.2, 12.2, 9.2; IR (ν_{max}/cm^{-1}) 3346, 3216, 2904, 2833, 1608, 1241, 967; HRMS (ESI): *m/z* calcd for $C_{17}H_{22}N_5O^+$: 312.1819 $[M+H]^+$; found: 312.1772.

(*E*)-6-Cyclopropyl-5-{[(2-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 g). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.060 g, 0.34 mmol), 2-fluorophenethylamine (0.057 ml, 0.44 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.039 ml, 0.68 mmol), isolated as a colourless gel (0.070 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.72 (s, 1H), 8.65 (s, 1H), 7.23–7.13 (m, 2H), 7.08–6.95 (m, 2H), 5.56 (s, 1H), 5.03 (s, 2H), 3.79 (t, *J* = 7.1 Hz, 2H), 2.99 (t, *J* = 7.1 Hz, 2H), 2.10–1.99 (m, 1H), 1.13–1.03 (m, 2H), 0.90–0.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.7, 163.1, 162.6, 161.4 (d, $J_{C\text{-}F}$ = 244.7 Hz), 158.5, 131.5 (d, $J_{C\text{-}F}$ = 5.1 Hz), 128.0 (d, $J_{C\text{-}F}$ = 8.1 Hz), 127.0 (d, $J_{C\text{-}F}$ = 15.8 Hz), 124.0 (d, $J_{C\text{-}F}$ = 3.7 Hz), 115.3 (d, $J_{C\text{-}F}$ = 22.4 Hz), 101.6, 61.6, 31.5, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3324, 3176, 2901, 2837, 1644, 1540, 967; HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_5F^+$: 300.1619 $[M+H]^+$; found: 300.1577.

(*E*)-6-Cyclopropyl-5-{[(3-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 h). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.055 g, 0.31 mmol), 3-fluorophenethylamine (0.052 ml, 0.40 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.035 ml, 0.62 mmol), isolated as a colourless gel (0.061 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.70 (s, 1H), 8.66 (s, 1H), 7.29–7.18 (m, 1H), 7.03–6.82 (m, 3H), 5.54 (s, 1H), 5.01 (s, 2H), 3.79 (t, *J* = 7.1 Hz, 2H), 2.95 (t, *J* = 7.1 Hz, 2H), 2.06 (tt, *J* = 8.1, 4.7 Hz, 1H), 1.14–1.06 (m, 2H), 0.92–0.81 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.8, 163.2, 162.9 (d, $J_{C\text{-}F}$ = 245.0 Hz), 162.1, 158.6, 142.8 (d, $J_{C\text{-}F}$ = 7.3 Hz), 129.8 (d, $J_{C\text{-}F}$ = 8.4 Hz), 124.8 (d, $J_{C\text{-}F}$ = 2.6 Hz), 115.9 (d, $J_{C\text{-}F}$ = 20.5 Hz), 113.1 (d, $J_{C\text{-}F}$ = 20.9 Hz), 101.6, 62.8, 37.9, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3319, 3188, 2896, 2836, 1611, 1535, 964; HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_5F^+$: 300.1619 $[M+H]^+$; found: 300.1578.

(*E*)-6-Cyclopropyl-5-{[(4-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 i). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.045 g, 0.25 mmol), 4-fluorophenethylamine (0.043 ml, 0.33 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.029 ml, 0.50 mmol), product isolated as a white solid (0.056 g, 74%). m.p. 153–155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.72 (s, 1H), 8.62 (s, 1H), 7.03–6.92 (m, 2H), 7.19–7.10 (m, 2H), 5.35 (s, 1H), 4.81 (s, 2H), 3.77 (t, *J* = 7.1 Hz, 2H), 2.92 (t, *J* = 7.1 Hz, 2H), 2.09–1.98 (m, 1H), 1.09 (m, 2H), 0.91–0.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.8, 163.2, 161.6 (d, $J_{C\text{-}F}$ = 243.6 Hz), 162.1, 158.5, 135.8 (d, $J_{C\text{-}F}$ = 3.3 Hz), 130.5 (d, $J_{C\text{-}F}$ = 7.7 Hz), 115.2 (d, $J_{C\text{-}F}$ = 21.3 Hz), 101.7, 63.3, 37.4, 12.3, 9.3; IR (ν_{max}/cm^{-1}) 3319, 3274, 2901, 2838, 1615, 1551, 966; HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_5F^+$: 300.1619 $[M+H]^+$; found: 300.1573.

(*E*)-6-Cyclopropyl-5-{[(2-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 j). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.059 g, 0.28 mmol), 2-chlorophenethylamine (0.051 ml, 0.37 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.032 ml, 0.56 mmol), isolated as a colourless gel (0.059 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.74 (s, 1H), 8.65 (s, 1H), 7.38–7.33 (m, 1H), 7.24–7.11 (m, 3H), 5.54 (s, 1H), 5.01 (s, 2H), 3.81 (t, *J* = 7.1 Hz, 2H), 3.08 (t, *J* = 7.1 Hz, 2H), 2.11–1.99 (m, 1H), 1.12–1.06 (m, 2H), 0.90–0.82 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.7, 163.1, 161.9, 158.5, 137.7, 134.2, 131.4, 129.7, 127.8, 126.7, 101.7, 61.1, 35.9, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3319, 3201, 2901, 2860, 1615, 966, 748; HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_5Cl^+$: 316.1323 $[M+H]^+$;ek; found: 316.1272.

(*E*)-6-Cyclopropyl-5-{[(3-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 k). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.073 g, 0.41 mmol), 3-chlorophenethylamine (0.074 ml, 0.53 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.047 ml, 0.82 mmol), isolated as a colourless gel (0.116 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.68 (s, 1H), 8.67 (s, 1H), 7.27–7.13 (m, 3H), 7.08 (dt, *J* = 7.1, 1.7 Hz, 1H), 5.67 (s, 1H), 5.16 (s, 2H), 3.78 (t, *J* = 7.1 Hz, 2H), 2.92 (t, *J* = 7.1 Hz, 2H), 2.06 (tt, *J* = 8.1, 4.8 Hz, 1H), 1.13–1.06 (m, 2H), 0.91–0.84 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.8, 163.1, 162.1, 158.6, 142.3, 134.1, 129.6, 129.1, 127.3, 126.3, 62.8, 37.8, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3328, 3184, 2926, 2847, 1612, 966, 742; HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_5Cl^+$: 316.1323 $[M+H]^+$; found: 316.1275.

(*E*)-6-Cyclopropyl-5-{[(4-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 l). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.077 g, 0.43 mmol), 4-chlorophenethylamine (0.079 ml, 0.56 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.049 ml, 0.86 mmol), isolated as a white solid (0.132 g, 97%). m.p. 161–163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.70 (s, 1H), 8.62 (t, *J* = 1.2 Hz, 1H), 7.26–7.22 (m, 2H), 7.15–7.11 (m, 2H), 5.44 (s, 1H), 3.77 (td, *J* = 7.0, 1.2 Hz, 2H), 2.92 (t, *J* = 7.0 Hz, 2H), 2.09–1.97 (m, 1H), 1.12–1.05 (m, 2H), 0.92–0.83 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.9, 163.1, 162.0, 158.6, 138.7, 131.9, 130.5, 128.5, 101.6, 63.0, 37.5, 12.3, 9.3; IR (ν_{max}/cm^{-1}) 3208, 3090, 2903, 2833, 1609, 968, 797; HRMS (ESI): *m/z* calcd for $C_{16}H_{19}N_5Cl^+$: 316.1323 $[M+H]^+$; found: 316.1278.

(*E*)-6-Cyclopropyl-5-{[(2,4-dichlorophenethyl)imino]methyl}-pyrimidine-2,4-diamine (11 m). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.064 g, 0.36 mmol), 2,4-dichlorophe-

nethylamine (0.080 ml, 0.47 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.041 ml, 0.72 mmol), isolated as a white solid (0.121 g, 96%). m.p. 120–122 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.66 (s, 1H), 8.63 (t, *J* = 1.2 Hz, 1H), 7.35 (t, *J* = 1.3 Hz, 1H), 7.13 (d, *J* = 1.3 Hz, 2H), 5.68 (s, 1H), 5.15 (s, 2H), 3.77 (td, *J* = 7.0, 1.2 Hz, 2H), 3.03 (t, *J* = 7.1 Hz, 2H), 2.04 (tt, *J* = 8.2, 4.8 Hz, 1H), 1.12–1.05 (m, 2H), 0.91–0.81 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.8, 163.1, 162.1, 158.7, 136.3, 134.8, 132.6, 132.2, 129.3, 126.9, 101.5, 60.8, 35.2, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3216, 3092, 2917, 2834, 1612, 967, 744; HRMS (ESI): *m/z* calcd for $C_{16}H_{18}N_5Cl_2^+$: 350.0934 $[M+H]^+$; found: 350.0876.

(*E*)-6-Cyclopropyl-5-{[(3,4-dichlorophenethyl)imino]methyl}-pyrimidine-2,4-diamine (11 n). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclopropylpyrimidine (**10 b**) (0.067 g, 0.38 mmol), 3,4-dichlorophenethylamine (0.084 ml, 0.49 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.043 ml, 0.76 mmol), isolated as a white solid (0.113 g, 86%). m.p. 168–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.64 (s, 1H), 8.66 (s, 1H), 7.40–7.26 (m, 2H), 7.04 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.59 (s, 1H), 5.06 (s, 2H), 3.77 (t, 2H), 2.90 (t, *J* = 6.9 Hz, 2H), 2.11–2.00 (m, 1H), 1.09 (m, 2H), 0.94–0.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.9, 163.1, 162.1, 158.8, 140.5, 132.2, 131.0, 130.3, 130.1, 128.6, 101.5, 62.6, 37.2, 12.3, 9.2; IR (ν_{max}/cm^{-1}) 3312, 3162, 2946, 2844, 1609, 961, 740; HRMS (ESI): *m/z* calcd for $C_{16}H_{18}N_5Cl_2^+$: 350.0934 $[M+H]^+$; found: 350.0877.

(*E*)-6-Cyclohexyl-5-{[(2-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine (11 o). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.060 g, 0.27 mmol), 2-methyoxy-phenethylamine (0.051 ml, 0.35 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.031 ml, 0.54 mmol) as described above. Pure product isolated as a colourless gel (0.084 g, 88%).^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.86 (s, 1H), 8.37 (s, 1H), 7.21–6.94 (m, 2H), 6.94–6.64 (m, 2H), 5.32 (s, 1H), 4.82 (s, 2H), 3.82 (s, 3H), 3.77 (t, *J* = 7.3 Hz, 2H), 2.96 (t, *J* = 7.1 Hz, 2H), 2.86–2.71 (m, 1H), 1.86–1.53 (m, 7H), 1.42–1.18 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.5, 163.6, 162.0, 157.9, 157.8, 130.9, 128.4, 127.6, 120.5, 110.4, 100.1, 61.5, 55.4, 40.5, 32.6, 31.6, 26.6, 26.1; IR (ν_{max}/cm^{-1}) 3317, 3167, 2924, 2850, 1610, 1343, 1380, 826, 1240; HRMS (ESI): *m/z* calcd for $C_{20}H_{28}N_5O^+$: 354.2288 $[M+H]^+$; found: 354.2261.

(*E*)-6-Cyclohexyl-5-{[(3-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine (11 p). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.099 g, 0.45 mmol), 3-methyoxy-phenethylamine (0.086 ml, 0.58 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.051 ml, 0.90 mmol) as described above. Product isolated as a colourless gel (0.140 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.80 (s, 1H), 8.38 (s, 1H), 7.18 (t, *J* = 7.7 Hz, 1H), 6.85–6.65 (m, 3H), 5.51 (s, 1H), 5.04 (s, 2H), 3.81–3.74 (m, 5H), 2.92 (t, *J* = 6.9 Hz, 2H), 2.86–2.72 (m, 1H), 1.84–1.55 (m, 7H), 1.36–1.21 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.6, 163.6, 162.1, 159.7, 158.2, 141.7, 129.4, 121.5, 114.9, 111.6, 100.0, 63.1, 55.2, 40.5, 38.1, 31.6, 26.5, 26.0; IR (ν_{max}/cm^{-1}) 3324, 3187, 2922, 2850, 1608, 1341, 1380, 823, 1227; HRMS (ESI): *m/z* calcd for $C_{20}H_{28}N_5O^+$: 354.2288 $[M+H]^+$, found: 354.2271.

(*E*)-6-Cyclohexyl-5-{[(4-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine (11 q). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.091 g, 0.41 mmol), 4-methyoxyphenethylamine (0.079 ml, 0.54 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.047 ml, 0.83 mmol) as described, isolated as a white solid (0.108 g, 95%). m.p. 202–204 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.82 (s, 1H), 8.34 (s, 1H), 7.10 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.6 Hz, 2H), 5.28 (s, 1H), 4.78 (s, 2H), 3.80–3.71 (m, 5H), 2.89 (t, *J* = 6.9 Hz, 2H), 2.76 (tt, *J* = 10.5, 5.0 Hz, 1H), 1.86–1.50 (m, 7H), 1.39–1.16 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.6, 163.6, 162.0, 158.09, 158.06, 132.1, 130.1, 113.8, 99.9, 63.4, 55.3, 40.5, 37.1, 31.6, 26.5, 26.0; IR (ν_{max}/cm^{-1}) 3330, 3204, 2923, 2848, 1613, 1343, 1381, 1306, 830; HRMS (ESI): *m/z* calcd for $C_{20}H_{28}N_5O^+$: 354.2288 $[M+H]^+$;ek; found: 354.2285.

(*E*)-6-Cyclohexyl-5-{[(2-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 r). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.067 g, 0.30 mmol), 2-fluorophenethylamine (0.052 ml, 0.40 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.035 ml, 0.60 mmol) as described above. Product isolated as viscous yellow oil which solidified on standing (0.083 g, 80%). m.p. 141–142 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.98–9.55 (m, 1H), 8.38 (s, 1H), 7.23–7.11 (m, 2H), 7.09–6.96 (m, 2H), 5.43 (s, 1H), 4.97 (s, 2H), 3.79 (t, *J* = 7.0 Hz, 2H), 3.00 (t, *J* = 7.1 Hz, 2H), 2.90–2.65 (m, 1H), 1.94–1.48 (m, 7H), 1.40–1.14 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.7, 163.6, 162.6, 161.4 (d, $J_{C\text{-}F}$ = 244.9 Hz), 158.3, 131.6 (d, $J_{C\text{-}F}$ = 5.1 Hz), 128.0 (d, $J_{C\text{-}F}$ = 8.1 Hz), 127.0 (d, $J_{C\text{-}F}$ = 15.7 Hz), 124.0 (d, $J_{C\text{-}F}$ = 3.6 Hz), 115.3 (d, $J_{C\text{-}F}$ = 22.1 Hz), 100.0, 61.6, 40.5, 31.6, 31.4, 26.5, 26.1; IR (ν_{max}/cm^{-1}) 3312, 3178, 2920, 2848, 1611, 1362, 1385, 1419; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_5F^+$: 342.2089 $[M+H]^+$; found: 342.2069.

(*E*)-6-Cyclohexyl-5-{[(3-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 s). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.064 g, 0.29 mmol), 3-fluorophenethylamine (0.049 ml, 0.38 mmol), 1,2-dichloroethane (2 ml) and glacial acetic acid (0.033 ml, 0.58 mmol). Pure product isolated as a colourless gel (0.087 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.73 (s, 1H), 8.38 (s, 1H), 7.23 (q, *J* = 7.4 Hz, 1H), 6.96 (d, *J* = 7.7 Hz, 1H), 6.94–6.83 (m, 2H), 5.44 (s, 1H), 4.97 (s, 2H), 3.79 (t, *J* = 7.0 Hz, 2H), 2.95 (t, *J* = 7.0 Hz, 2H), 2.78 (tt, *J* = 10.2, 5.2 Hz, 1H), 1.84–1.50 (m, 7H), 1.44–1.12 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.8, 164.2, 163.0 (d, $J_{C\text{-}F}$ = 245.3 Hz), 162.2, 158.3, 142.73 (d, $J_{C\text{-}F}$ = 7.2 Hz), 129.82 (d, $J_{C\text{-}F}$ = 8.3 Hz), 124.86 (d, $J_{C\text{-}F}$ = 2.8 Hz), 115.92 (d, $J_{C\text{-}F}$ = 20.9 Hz), 113.10 (d, $J_{C\text{-}F}$ = 21.0 Hz), 99.9, 62.8, 40.6, 37.8, 31.6, 26.5, 26.1; IR (ν_{max}/cm^{-1}) 3312, 3178, 2920, 2848, 1611, 1362, 1385, 1419; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_5F^+$: 342.2089 $[M+H]^+$; found: 342.2063.

(*E*)-6-Cyclohexyl-5-{[(4-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 t). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.056 g, 0.25 mmol), 4-fluorophenethylamine (0.043 ml, 0.33 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.029 ml, 0.50 mmol). Pure product isolated as white solid (0.076 g, 87%). m.p. 151–152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.76 (s, 1H), 8.34 (s, 1H), 7.22–7.07 (m, 2H), 7.03–6.85 (m, 2H), 5.33 (s, 1H), 4.84 (s, 2H), 3.77 (t, *J* = 6.9 Hz, 2H), 2.92 (t, *J* = 6.9 Hz, 2H), 2.76 (tt, *J* = 10.3, 4.6 Hz, 1H), 1.89–1.46 (m, 7H), 1.34–1.17 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.7, 163.6, 162.1, 161.6 (d, $J_{C\text{-}F}$ = 243.8 Hz), 158.3, 135.8 (d, $J_{C\text{-}F}$ = 3.2 Hz), 130.6 (d, $J_{C\text{-}F}$ = 7.8 Hz), 115.20 (d, $J_{C\text{-}F}$ = 21.1 Hz), 100.0, 63.2, 40.6, 37.2, 31.6, 26.5, 26.1; IR (ν_{max}/cm^{-1}) 3315, 3189, 2921, 2846, 1610, 1361, 1384, 1474; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_5F^+$: 342.2089 $[M+H]^+$; found: 342.2087

(*E*)-6-Cyclohexyl-5-{[(2-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 u). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.075 g, 0.34 mmol), 2-chlorophenethylamine (0.062 ml, 0.44 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.039 ml, 0.68 mmol). Pure product isolated as colourless gel (0.087 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.76 (s, 1H), 8.38 (s, 1H), 7.44–7.31 (m, 1H), 7.22–7.07 (m, 3H), 5.43 (s, 1H), 4.97 (s, 2H), 3.82 (t, *J* = 6.9 Hz, 2H), 3.09 (t, *J* = 6.9 Hz, 2H), 2.88–2.68 (m, 1H), 1.92–1.49 (m, 7H), 1.39–1.12 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 175.7, 163.6, 162.1, 158.4, 137.6, 134.3, 131.5, 129.6, 127.8, 126.7, 99.9, 61.0, 40.5, 35.8, 31.6, 26.5, 26.1; IR (ν_{max}/cm^{-1}) 3297, 3133, 2923, 2851, 1612, 1345, 1268, 966, 830; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_5Cl^+$: 358.1793 $[M+H]^+$; found: 358.1587.

(*E*)-6-Cyclohexyl-5-{[(3-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11 v). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10 c**) (0.081 g, 0.37 mmol), 3-chlorophenethylamine (0.066 ml, 0.48 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.042 ml, 0.74 mmol) as described. Pure product isolated as a white solid (0.125 g, 95%). m.p. 132–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.72 (s, 1H), 8.39 (s, 1H), 7.24–7.14 (m, 3H), 7.13–7.01 (m, 1H), 5.33 (s,

1H), 4.84 (s, 2H), 3.79 (t, J=6.8 Hz, 2H), 2.93 (t, J=6.8 Hz, 2H), 2.83–2.70 (m, 2H), 1.94–1.50 (m, 7H), 1.27 (d, J=10.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=175.8, 163.6, 162.1, 158.4, 142.2, 134.2, 129.7, 129.3, 127.5, 126.5, 100.0, 62.8, 40.6, 37.7, 31.7, 26.6, 26.1; IR (ν_{max}/cm^{-1}) 3319, 3189, 2921, 2850, 1611, 1342, 1266, 964, 824; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_5Cl^+$: 358.1793 $[M+H]^+$; found: 358.1764.

(*E*)-6-Cyclohexyl-5-{[(4-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine (11w). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10c**) (0.091 g, 0.41 mmol), 4-chlorophenethylamine (0.075 ml, 0.54 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.047 ml, 0.83 mmol). Pure product isolated as a white solid (0.123 g, 83%). m.p. 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 9.74 (s, 1H), 8.33 (s, 1H), 7.24 (d, J=7.2 Hz, 3H), 7.11 (d, J=7.2 Hz, 2H), 5.35 (s, 1H), 4.88 (s, 2H), 3.77 (t, J=6.9 Hz, 2H), 2.92 (t, J=6.9 Hz, 2H), 2.81–2.69 (m, 1H), 1.87–1.51 (m, 7H), 1.27 (d, J=8.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=175.8, 163.6, 162.1, 158.4, 138.6, 132.1, 130.6, 128.5, 99.9, 63.0, 40.6, 37.4, 31.6, 26.5, 26.1; IR (ν_{max}/cm^{-1}) 3318, 3186, 2921, 2851, 1611, 1253, 1269, 963, 834; HRMS (ESI): *m/z* calcd for $C_{19}H_{25}N_5Cl^+$: 358.1793 $[M+H]^+$; found: 358.1777.

(*E*)-6-Cyclohexyl-5-{[(2,4-dichlorophenethyl)imino]methyl}-pyrimidine-2,4-diamine (11x). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10c**) (0.088 g, 0.40 mmol), 2,4-dichlorophenethylamine (0.089 ml, 0.52 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.046 ml, 0.80 mmol). Pure product isolated as a white solid (0.145 g, 92%). m.p. 151–152 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 9.70 (s, 1H), 8.35 (s, 1H), 7.37 (d, J=1.9 Hz, 1H), 7.19–6.98 (m, 2H), 5.47 (s, 1H), 5.01 (s, 2H), 3.79 (t, J=6.8 Hz, 2H), 3.05 (t, J=6.8 Hz, 2H), 2.77 (tt, J=10.5, 4.5 Hz, 1H), 1.90–1.51 (m, 7H), 1.39–1.17 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.8, 163.6, 162.2, 158.6, 136.2, 134.9, 132.8, 132.3, 129.4, 127.0, 99.8, 60.8, 40.6, 35.2, 31.6, 26.5, 26.0; IR (ν_{max}/cm^{-1}) 3514, 3341, 2914, 2850, 1645, 1340, 1312, 963, 815, 853; HRMS (ESI): *m/z* calcd for $C_{19}H_{24}N_5Cl_2^+$: 392.1403 $[M+H]^+$; found: 392.1363.

(*E*)-6-Cyclohexyl-5-{[(3,4-dichlorophenethyl)imino]methyl}-pyrimidine-2,4-diamine (11y). Prepared from 2,4-diamino-5-carbaldehyde-6-cyclohexylpyrimidine (**10c**) (0.077 g, 0.35 mmol), 3,4-dichlorophenethylamine (0.078 ml, 0.46 mmol), 1,2-dichloroethane (4 ml) and glacial acetic acid (0.040 ml, 0.70 mmol). Pure product isolated as a white solid (0.137 g, 99%). m.p. 126–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 9.68 (s, 1H), 8.37 (s, 1H), 7.49–7.28 (m, 2H), 7.01 (d, J=8.0 Hz, 1H), 5.37 (s, 1H), 4.89 (s, 2H), 3.77 (t, J=6.9 Hz, 2H), 2.90 (t, J=6.9 Hz, 2H), 2.82–2.65 (m, 1H), 1.98–1.41 (m, 7H), 1.42–1.04 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=175.9, 163.6, 162.2, 158.6, 140.5, 132.3, 131.1, 130.33, 130.26, 128.7, 99.8, 62.7, 40.6, 37.2, 31.7, 26.5, 26.1; IR (ν_{max}/cm^{-1}): 3319, 3183, 2927, 2850, 1611, 1344, 1266, 964, 806; HRMS (ESI): *m/z* calcd for $C_{19}H_{24}N_5Cl_2^+$: 392,1403 $[M+H]^+$; found: 392.1185.

General procedure for the synthesis of 6-substituted-2,4-diamino-pyrimidine-5-phenethylamines (7). In a round bottomed flask, the imine (**11**) was dissolved in a mixture of 1,2-dichloroethane and THF (1:2). Glacial acetic acid was added to the mixture until a pH of between 4 and 5. Once the desired pH was reached, sodium borohydride (6.5 eq) was added and the reaction was allowed to stir for 30 minutes under nitrogen atmosphere. Upon completion, the solution was diluted with water and neutralised to pH 7 using solid $NaHCO_3$. The organics were extracted with EtOAc (2×100 ml). The organic layer was dried with magnesium sulfate or sodium sulfate and filtered through celite/cotton wool. The solvent was removed *in vacuo* and the crude product was purified using silica gel column chromatography, using gradient elution of 50% ethyl acetate/hexane to 100% ethyl acetate. The following compounds were prepared by this method:

6-Phenyl-5-{[(2-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7a). Prepared from (*E*)-6-phenyl-5-{[(2-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11a** (0.098 g, 0.28 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.107 mg, 2.82 mmol), isolated as a colourless gel (0.044 g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.44–7.34 (m, 3H), 7.35–7.28 (m, 2H), 7.19 (ddd, J=8.1, 7.5, 1.8 Hz, 1H), 7.01 (td, J=8.0, 1.8 Hz, 1H), 6.90–6.82 (m, 2H), 6.37 (br s, 2H), 5.30 (s, 2H), 3.77 (s, 3H), 3.62 (s, 2H), 2.80–2.70 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=165.3, 164.4, 160.7, 157.6, 138.3, 130.5, 128.7, 128.4, 128.4, 128.0, 127.7, 120.6, 110.5, 102.8, 55.4, 48.2, 46.5, 30.5; IR (ν_{max}/cm^{-1}) 3174, 3062, 2939, 2836, 1613, 1242; HRMS (ESI): *m/z* calcd for $C_{20}H_{24}N_5O^+$: 350.1975 $[M+H]^+$; found: 350.1963.

6-Phenyl-5-{[(3-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7b). Prepared from (*E*)-6-phenyl-5-{[(3-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11b** (0.090 g, 0.26 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.098 mg, 1.68 mmol), isolated as a colourless gel (0.030 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.46–7.34 (m, 3H), 7.33–7.25 (m, 2H), 7.24–7.15 (m, 1H), 6.79–6.74 (m, 1H), 6.71–6.62 (m, 2H), 6.02 (s, 3H), 5.64 (s, 2H), 3.79 (s, 3H), 3.59 (s, 2H), 2.77 (t, J=6.2 Hz, 2H), 2.68 (t, J=6.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.0, 164.1, 160.4, 159.9, 141.2, 137.7, 129.6, 128.9, 128.4, 128.3, 121.1, 114.7, 111.6, 102.5, 55.3, 49.4, 46.4, 35.8; IR (ν_{max}/cm^{-1}) 3182, 3057, 2936, 2834, 1583, 1257; HRMS (ESI): *m/z* calcd for $C_{20}H_{24}N_5O^+$: 350.1975 $[M+H]^+$; found: 350.1967.

6-Phenyl-5-{[(4-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7c). Prepared from (*E*)-6-phenyl-5-{[(4-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11c** (0.088 g, 0.25 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.062 mg, 1.64 mmol), isolated as a colourless gel (0.055 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.43–7.35 (m, 3H), 7.35–7.27 (m, 2H), 7.05–6.98 (m, 2H), 6.85–6.76 (m, 2H), 6.09 (s, 2H), 4.92 (s, 2H), 3.78 (s, 3H), 3.59 (s, 2H), 2.75 (t, J=6.9 Hz, 2H), 2.64 (t, J=6.6 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=165.5, 164.9, 161.3, 158.2, 139.1, 131.8, 129.7, 128.5, 128.4, 128.3, 113.9, 103.1, 55.4, 49.9, 46.7, 35.1; IR (ν_{max}/cm^{-1}) 3302, 3152, 2932, 2834, 1610, 1242; HRMS (ESI): *m/z* calcd for $C_{20}H_{24}N_5O^+$: 350.1975 $[M+H]^+$; found: 350.1972.

6-Cyclopropyl-5-{[(2-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7d). Prepared from (*E*)-6-cyclopropyl-5-{[(2-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11d** (0.060 g, 0.19 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.047 mg, 1.25 mmol), isolated as a colourless gel (0.050 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ=7.19 (td, J=8.0, 1.7 Hz, 1H), 7.12 (dd, J=7.3, 1.7 Hz, 1H), 6.92–6.80 (m, 2H), 6.24 (s, 2H), 5.16 (s, 2H), 3.88 (s, 2H), 3.79 (s, 3H), 2.95–2.88 (m, 2H), 2.88–2.81 (m, 2H), 2.01 (s, 1H), 1.91 (tt, J=8.2, 4.8 Hz, 1H), 1.05–0.98 (m, 2H), 0.87–0.78 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ=166.6, 163.8, 160.6, 157.6, 130.5, 127.8, 127.7, 120.6, 110.5, 102.0, 55.3, 48.1, 44.7, 30.3, 12.7, 8.8; IR (ν_{max}/cm^{-1}) 3354, 3139, 2925, 2862, 1601, 1237; HRMS (ESI): *m/z* calcd for $C_{17}H_{24}N_5O^+$: 314.1975 $[M+H]^+$; found: 314.1962.

6-Cyclopropyl-5-{[(3-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7e). Prepared from (*E*)-6-cyclopropyl-5-{[(3-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11e** (0.105 g, 0.34 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.083 g, 2.19 mmol), isolated as a colourless gel (0.082 g, 78%). ^{1}H NMR (500 MHz, $CDCl_3$) δ=7.21 (t, J=7.8 Hz, 1H), 6.78 (d, J=7.8 Hz, 2H), 6.77–6.73 (m, 2H), 6.17 (s, 2H), 5.25 (s, 2H), 3.86 (s, 2H), 3.79 (s, 3H), 2.93 (t, J=6.8 Hz, 2H), 2.81 (t, J=6.8 Hz, 2H), 1.93 (tt, J=8.4, 4.2 Hz, 1H), 1.15–1.09 (m, 2H), 0.95–0.84 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=164.5 (2 C), 159.9 (2 C), 141.4, 129.7, 121.2, 114.8, 111.6, 103.0, 55.3, 49.7, 45.3, 36.2, 12.6, 8.9; IR (ν_{max}/cm^{-1}) 3330, 3192, 2936, 2835, 1602, 1257; HRMS (ESI): *m/z* calcd for $C_{17}H_{24}N_5O^+$: 314.1975 $[M+H]^+$; found: 314.1966.

6-Cyclopropyl-5-{[(4-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7f). Prepared from (*E*)-6-cyclopropyl-5-{[(4-meth-

oxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11 f** (0.110 g, 0.35 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.087 g, 2.29 mmol), isolated as a colourless gel (0.090 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.10 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 5.99 (s, 2H), 4.94 (s, 2H), 3.84 (s, 2H), 3.79 (s, 3H), 2.88 (t, *J* = 6.8 Hz, 2H), 2.75 (t, *J* = 6.8 Hz, 2H), 2.05 (s, 1H), 1.93 (tt, *J* = 8.2, 4.9 Hz, 1H), 1.11–1.04 (m, 2H), 0.90–0.82 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.4 (2 C), 158.3 (2 C), 131.8, 129.7, 114.1, 103.2, 55.4, 50.1, 45.3, 35.3, 12.6, 8.8; IR (ν_{max}/cm^{-1}) 3331, 3178, 2931, 2835, 1611, 1243; HRMS (ESI): *m/z* calcd for $C_{17}H_{24}N_5O^+$: 314.1975 $[M+H]^+$; found: 314.1966.

6-Cyclopropyl-5-{[(2-fluorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 g). Prepared from (*E*)-6-cyclopropyl-5-{[(2-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 g** (0.066 g, 0.22 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.054 g, 1.43 mmol), isolated as a colourless gel (0.045 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18 (t, *J* = 7.0 Hz, 2H), 7.08–6.98 (m, 2H), 6.04 (s, 2H), 5.04 (s, 2H), 3.85 (s, 2H), 2.94–2.88 (m, 2H), 2.88–2.81 (m, 2H), 2.03 (s, 1H), 1.94 (tt, *J* = 8.2, 4.9 Hz, 1H), 1.06–1.00 (m, 2H), 0.87–0.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.1, 164.1, 161.37 (d, J_{C-F} = 244.7 Hz), 160.7, 131.1 (d, J_{C-F} = 4.8 Hz), 128.2 (d, J_{C-F} = 8.1 Hz), 126.7 (d, J_{C-F} = 15.8 Hz), 124.2 (d, J_{C-F} = 3.7 Hz), 115.5 (d, J_{C-F} = 22.4 Hz), 102.9, 48.7, 45.2, 29.5, 12.6, 8.8; IR (ν_{max}/cm^{-1}) 3327, 3191, 3008, 2855, 1611, 1227; HRMS (ESI): *m/z* calcd for $C_{16}H_{21}N_5F^+$: 302.1776 $[M+H]^+$; found: 302.1767.

6-Cyclopropyl-5-{[(3-fluorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 h). Prepared from (*E*)-6-cyclopropyl-5-{[(3-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 h** (0.053 g, 0.18 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.044 g, 1.15 mmol), isolated as a colourless gel (0.040 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.29–7.20 (m, 1H), 7.00–6.85 (m, 3H), 6.09 (s, 2H), 5.07 (s, 2H), 3.84 (s, 2H), 2.90 (t, *J* = 6.3 Hz, 2H), 2.80 (t, *J* = 6.3 Hz, 2H), 2.03 (s, 1H), 1.92 (tt, *J* = 8.0, 4.8 Hz, 1H), 1.04–0.98 (m, 2H), 0.88–0.79 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 166.9, 164.3, 163.5 (d, J_{C-F} = 245.9 Hz), 161.0, 142.8 (d, J_{C-F} = 7.1 Hz), 130.5 (d, J_{C-F} = 8.2 Hz), 124.9 (d, J_{C-F} = 2.7 Hz), 116.1 (d, J_{C-F} = 20.9 Hz), 113.8 (d, J_{C-F} = 21.1 Hz), 103.0, 49.9, 45.6, 36.3, 13.1, 9.2; IR (ν_{max}/cm^{-1}) 3329, 3189, 3010, 2854, 1613, 1248; HRMS (ESI): *m/z* calcd for $C_{16}H_{21}N_5F^+$: 302.1776 $[M+H]^+$; found: 302.1766.

6-Cyclopropyl-5-{[(4-fluorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 i). Prepared from (*E*)-6-cyclopropyl-5-{[(4-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 i** (0.054 g, 0.18 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.044 g, 1.17 mmol), isolated as a colourless gel (0.040 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.16–7.10 (m, 2H), 7.00–6.94 (m, 2H), 5.96 (s, 2H), 4.89 (s, 2H), 3.83 (s, 2H), 2.87 (td, *J* = 6.7, 1.2 Hz, 2H), 2.77 (t, *J* = 6.8 Hz, 2H), 2.03 (s, 1H), 1.92 (tt, *J* = 8.1, 4.8 Hz, 1H), 1.04–0.98 (m, 2H), 0.85–0.78 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.7, 164.0, 161.6 (d, J_{C-F} = 243.9 Hz), 160.9, 135.5 (d, J_{C-F} = 2.9 Hz), 130.2 (d, J_{C-F} = 8.1 Hz), 115.4 (d, J_{C-F} = 21.3 Hz), 102.9, 49.9, 45.3, 35.5, 12.7, 8.7; IR (ν_{max}/cm^{-1}) 3318, 3191, 3006, 2845, 1620, 1216; HRMS (ESI): *m/z* calcd for $C_{16}H_{21}N_5F^+$: 302.1776 $[M+H]^+$; found: 302.1764.

6-Cyclopropyl-5-{[(2-chlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 j) Prepared from (*E*)-6-cyclopropyl-5-{[(2-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 j** (0.053 g, 0.27 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.041 g, 1.10 mmol), isolated as a colourless gel (0.040 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36–7.33 (m, 1H), 7.23–7.12 (m, 3H), 6.02 (s, 2H), 4.92 (s, 2H), 3.85 (s, 2H), 2.98–2.88 (m, 4H), 2.04 (s, 1H), 1.94 (tt, *J* = 8.1, 4.8 Hz, 1H), 1.04–0.98 (m, 2H), 0.86–0.79 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.8, 163.9, 160.8, 137.5, 134.2, 130.9, 129.8, 127.9, 126.9, 102.9, 48.3, 45.3, 34.0, 12.7, 8.8; IR (ν_{max}/cm^{-1}) 3326, 3194, 3007, 2857, 1615, 722; HRMS (ESI): *m/z* calcd for $C_{16}H_{21}N_5Cl^+$: 318.1480 $[M+H]^+$; found: 318.1468.

6-Cyclopropyl-5-{[(3-chlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 k). Prepared from (*E*)-6-cyclopropyl-5-{[(3-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 k** (0.106 g, 0.34 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.083 g, 2.18 mmol), isolated as a colourless gel (0.084 g, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.23–7.16 (m, 3H), 7.06 (d, *J* = 7.2 Hz, 1H), 5.80 (s, 2H), 4.77 (s, 2H), 3.83 (s, 2H), 2.90 (t, *J* = 6.8 Hz, 2H), 2.77 (t, *J* = 6.8 Hz, 2H), 1.97–1.90 (m, 1H), 1.04–0.98 (m, 2H), 0.86–0.77 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.5 (3 C), 141.9, 134.5, 129.9, 128.9, 127.1, 126.7, 103.1, 49.6, 45.4, 36.0, 12.6, 8.8; IR (ν_{max}/cm^{-1}) 3326, 3191, 3008, 2853, 1598, 782; HRMS (ESI): *m/z* calcd for $C_{16}H_{21}N_5Cl^+$: 318.1480 $[M+H]^+$; found: 318.1462.

6-Cyclopropyl-5-{[(4-chlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 l). Prepared from (*E*)-6-cyclopropyl-5-{[(4-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 l** (0.120 g, 0.38 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.093 g, 2.47 mmol), isolated as a colourless gel (0.084 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.24 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H), 5.91 (s, 2H), 4.90 (s, 2H), 3.82 (s, 2H), 2.87 (t, *J* = 6.7 Hz, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 1.92 (tt, *J* = 8.1, 4.7 Hz, 1H), 1.03–0.97 (m, 2H), 0.84–0.78 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.6, 164.3, 161.4, 138.5, 132.1, 130.2, 128.7, 103.1, 49.8, 45.4, 35.8, 12.6, 8.7; IR (ν_{max}/cm^{-1}) 3322, 3188, 3007, 2853, 1604, 807; HRMS (ESI): *m/z* calcd for $C_{16}H_{21}N_5Cl^+$: 318.1480 $[M+H]^+$; found: 318.1466.

6-Cyclopropyl-5-{[(2,4-dichlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 m). Prepared from (*E*)-6-cyclopropyl-5-{[(2,4-dichlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 m** (0.111 g, 0.32 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.078 g, 2.06 mmol), isolated as white semi-solid (0.081 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 (d, *J* = 2.0 Hz, 1H), 7.18 (dd, *J* = 8.2, 2.0 Hz, 1H), 7.14 (d, *J* = 8.2 Hz, 1H), 5.95 (s, 2H), 4.98 (s, 2H), 3.86 (s, 2H), 2.96–2.86 (m, 4H), 1.96 (tt, *J* = 8.2, 4.9 Hz, 1H), 1.12–1.04 (m, 2H), 0.92–0.84 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 164.5 (2 C), 136.2, 134.9, 132.9, 131.7, 129.6, 127.3, 103.1, 48.2, 45.4, 33.6, 12.6, 8.9; IR (ν_{max}/cm^{-1}) 3324, 3184, 3005, 2853, 1607, 814; HRMS (ESI): *m/z* calcd for $C_{16}H_{20}N_5Cl_2^+$: 352.1090 $[M+H]^+$; found: 352.1073.

6-Cyclopropyl-5-{[(3,4-dichlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7 n). Prepared from (*E*)-6-cyclopropyl-5-{[(3,4-dichlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11 n** (0.080 g, 0.23 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.056 g, 1.48 mmol), isolated as colourless gel (0.067 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35 (d, *J* = 8.2 Hz, 1H), 7.28 (d, *J* = 2.1 Hz, 1H), 7.02 (dd, *J* = 8.2, 2.1 Hz, 1H), 5.79 (s, 2H), 4.76 (s, 2H), 3.83 (s, 2H), 2.88 (td, *J* = 6.8, 0.9 Hz, 2H), 2.75 (t, *J* = 6.8 Hz, 2H), 2.30 (s, 1H), 1.94 (tt, *J* = 8.2, 4.8 Hz, 1H), 1.06–0.99 (m, 2H), 0.88–0.80 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.4, 164.3, 161.2, 140.3, 132.5, 130.8, 130.5, 130.4, 128.3, 102.9, 49.6, 45.4, 35.6, 12.6, 8.8; IR (ν_{max}/cm^{-1}) 3322, 3193, 3009, 2854, 1609, 814; HRMS (ESI): *m/z* calcd for $C_{16}H_{20}N_5Cl_2^+$: 352.1090 $[M+H]^+$; found: 352.1073.

6-Cyclohexyl-5-{[(2-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7 o). Prepared from (*E*)-6-cyclohexyl-5-{[(2-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11 o** (0.080 g, 0.23 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.056 g, 1.47 mmol), isolated as a colourless gel (0.074 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.22–7.15 (m, 1H), 7.10 (*J* = 7.5, 1.8 Hz, 1H), 6.91–6.81 (m, 2H), 6.37 (s, 2H), 5.61 (s, 2H), 3.79 (s, 3H), 3.69 (s, 2H), 2.89–2.78 (m, 4H), 2.69 (tt, *J* = 11.7, 3.6 Hz, 1H), 2.02 (s, 1H), 1.85–1.74 (m, 2H), 1.74–1.52 (m, 5H), 1.34–1.21 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.1, 164.9, 160.0, 157.6, 130.4, 127.9, 127.7, 120.6, 110.5, 101.6, 55.4, 48.3, 44.8, 40.8, 31.1, 30.6, 26.5, 25.8; IR (ν_{max}/cm^{-1}) 3327, 3193, 2923, 2851, 1623, 1241; HRMS (ESI): *m/z* calcd for $C_{20}H_{30}N_5O^+$: 356.2445 $[M+H]^+$; found: 356.2431.

6-Cyclohexyl-5-{[(3-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7p). Prepared from (*E*)-6-cyclohexyl-5-{[(3-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11p** (0.130 g, 0.37 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.090 g, 2.39 mmol), isolated as a colourless gel (0.118 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 (t, *J* = 7.6 Hz, 1H), 6.81–6.66 (m, 3H), 6.36 (s, 2H), 5.56 (s, 2H), 3.77 (s, 3H), 3.66 (s, 2H), 2.86 (t, *J* = 6.4 Hz, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 2.68 (tt, *J* = 11.7, 3.5 Hz, 1H), 2.02 (s, 1H), 1.84–1.76 (m, 3H), 1.73–1.60 (m, 2H), 1.55 (d, *J* = 11.8 Hz, 2H), 1.34–1.21 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.4, 165.1, 159.9, 159.8, 141.3, 129.6, 121.1, 114.6, 111.5, 101.8, 55.2, 49.6, 44.9, 40.6, 36.1, 31.2, 26.5, 25.7; IR (ν_{max}/cm^{-1}) 3321, 3184, 2923, 2851, 1601, 1257; HRMS (ESI): *m/z* calcd for $C_{20}H_{30}N_5O^+$: 356.2445 $[M+H]^+$; found: 356.2428.

6-Cyclohexyl-5-{[(4-methoxyphenethyl)amino]methyl}pyrimidine-2,4-diamine (7q). Prepared from (*E*)-6-cyclohexyl-5-{[(4-methoxyphenethyl)imino]methyl}pyrimidine-2,4-diamine **11q** (0.135 g, 0.38 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.094 g, 2.48 mmol), isolated as a colourless gel (0.114 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.08 (d, *J* = 8.6 Hz, 2H), 6.81 (d, *J* = 8.6 Hz, 2H), 6.51 (s, 2H), 5.74 (s, 2H), 3.75 (s, 3H), 3.66 (s, 2H), 2.83 (t, *J* = 6.9 Hz, 2H), 2.76–2.62 (m, 3H), 2.00 (s, 1H), 1.84–1.75 (m, 1H), 1.76–1.60 (m, 4H), 1.55 (d, *J* = 12.9 Hz, 2H), 1.35–1.18 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.6, 165.1, 159.6, 158.2, 131.5, 129.7, 114.0, 101.7, 55.3, 49.9, 44.9, 40.5, 35.0, 31.2, 26.4, 25.6; IR (ν_{max}/cm^{-1}) 3320, 3180, 2923, 2850, 1610, 1243; HRMS (ESI): *m/z* calcd for $C_{20}H_{30}N_5O^+$: 356.2445 $[M+H]^+$; found: 356.2427.

6-Cyclohexyl-5-{[(2-fluorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7r). Prepared from (*E*)-6-cyclohexyl-5-{[(2-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11r** (0.080 g, 0.23 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.058 g, 1.52 mmol), isolated as a colourless gel (0.069 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.22–7.12 (m, 2H), 7.09–6.98 (m, 2H), 6.25 (s, 2H), 5.56 (s, 2H), 3.69 (s, 2H), 2.91–2.86 (m, 2H), 2.86–2.80 (m, 2H), 2.69 (tt, *J* = 11.7, 3.6 Hz, 1H), 2.02 (s, 1H), 1.84–1.77 (m, 2H), 1.72–1.60 (m, 3H), 1.59–1.51 (m, 2H), 1.35–1.22 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.6, 164.8, 161.34 (d, $J_{C\text{-}F}$ = 244.7 Hz), 160.2, 131.01 (d, $J_{C\text{-}F}$ = 5.1 Hz), 128.2 (d, $J_{C\text{-}F}$ = 8.1 Hz), 126.6 (d, $J_{C\text{-}F}$ = 16.1 Hz), 124.2 (d, $J_{C\text{-}F}$ = 3.7 Hz), 115.5 (d, $J_{C\text{-}F}$ = 22.0 Hz), 101.6, 48.6, 44.9, 40.8, 31.2, 29.4, 26.5, 25.8; IR (ν_{max}/cm^{-1}) 3323, 3188, 2923, 2851, 1616, 1431; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_5F^+$: 344.2445 $[M+H]^+$; found: 344.2228.

6-Cyclohexyl-5-{[(3-fluorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7s). Prepared from (*E*)-6-cyclohexyl-5-{[(3-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11s** (0.084 g, 0.25 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.060 g, 1.60 mmol), isolated as a colourless gel (0.075 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.28–7.20 (m, 1H), 6.97–6.86 (m, 3H), 6.29 (s, 2H), 5.59 (s, 2H), 3.68 (s, 2H), 2.87 (t, *J* = 6.4 Hz, 2H), 2.79 (t, *J* = 6.4 Hz, 2H), 2.68 (tt, *J* = 11.7, 3.6 Hz, 1H), 2.02 (s, 1H), 1.85–1.74 (m, 2H), 1.74–1.61 (m, 3H), 1.61–1.50 (m, 2H), 1.36–1.20 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.6, 164.8, 163.03 (d, $J_{C\text{-}F}$ = 246.1 Hz), 160.1, 142.33 (d, $J_{C\text{-}F}$ = 7.3 Hz), 130.08 (d, $J_{C\text{-}F}$ = 8.4 Hz), 124.44 (d, $J_{C\text{-}F}$ = 2.6 Hz), 115.59 (d, $J_{C\text{-}F}$ = 20.9 Hz), 113.35 (d, $J_{C\text{-}F}$ = 20.9 Hz), 101.6, 49.5, 45.0, 40.9, 35.9, 31.2, 26.5, 25.8; IR (ν_{max}/cm^{-1}) 3324, 3193, 2924, 2852, 1616, 1431; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_5F^+$: 344.2445 $[M+H]^+$; found: 344.2228.

6-Cyclohexyl-5-{[(4-fluorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7t). Prepared from (*E*)-6-cyclohexyl-5-{[(4-fluorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11t** (0.072 g, 0.21 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.052 g, 1.37 mmol), isolated as a colourless gel (0.066 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.16–7.09 (m, 2H), 7.00–6.92 (m, 2H), 6.23 (s, 2H), 5.49 (s, 2H), 3.67 (s, 2H), 2.85 (t, *J* = 6.4 Hz, 2H), 2.75 (t, *J* = 6.7 Hz, 2H), 2.67 (tt, *J* = 11.7, 3.6 Hz, 1H), 2.02 (s, 1H), 1.85–1.75 (m, 3H), 1.74–1.58 (m, 2H), 1.54 (d, *J* = 13.7 Hz, 2H), 1.35–1.17 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.8, 164.8, 161.6 (d, $J_{C\text{-}F}$ = 243.9 Hz), 160.3, 135.4 (d, $J_{C\text{-}F}$ = 3.3 Hz), 130.1 (d, $J_{C\text{-}F}$ = 7.7 Hz), 115.39 (d, $J_{C\text{-}F}$ = 20.9 Hz), 101.6, 49.8, 45.1, 40.9, 35.4, 31.3, 26.5, 25.8; IR (ν_{max}/cm^{-1}) 3328, 3185, 2924, 2852, 1622, 1431; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_5F^+$: 344.2445 $[M+H]^+$; found: 344.2230.

6-Cyclohexyl-5-{[(2-chlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7u). Prepared from (*E*)-6-cyclohexyl-5-{[(2-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11u** (0.080 g, 0.22 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.055 g, 1.46 mmol), isolated as a colourless gel (0.077 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36–7.31 (m, 1H), 7.22–7.11 (m, 3H), 6.27 (s, 2H), 5.55 (s, 2H), 3.70 (s, 2H), 2.99–2.84 (m, 4H), 2.70 (tt, *J* = 11.7, 3.7 Hz, 1H), 2.03 (s, 1H), 1.81 (m, 2H), 1.66 (m, 2H), 1.56 (d, *J* = 13.8 Hz, 2H), 1.37–1.20 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.5, 164.8, 160.1, 137.4, 134.2, 130.9, 129.8, 127.9, 126.9, 101.7, 48.2, 45.1, 40.8, 33.9, 31.2, 26.5, 25.8; IR (ν_{max}/cm^{-1}) 3320, 3189, 2924, 2852, 1626, 729; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_5Cl^+$: 360.1950 $[M+H]^+$; found: 360.1944.

6-Cyclohexyl-5-{[(3-chlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7v). Prepared from (*E*)-6-cyclohexyl-5-{[(3-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11v** (0.115 g, 0.32 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.080 mg, 2.09 mmol), isolated as a colourless gel (0.097 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.23–7.10 (m, 3H), 7.04 (d, *J* = 7.1 Hz, 1H), 6.51 (s, 2H), 5.80 (s, 2H), 3.66 (s, 2H), 2.85 (t, *J* = 6.8 Hz, 2H), 2.79–2.75 (t, *J* = 6.8 Hz, 2H), 2.68 (td, *J* = 11.8, 5.9 Hz, 1H), 1.99 (s, 1H), 1.82–1.73 (m, 2H), 1.74–1.60 (m, 2H), 1.55 (d, *J* = 12.6 Hz, 2H), 1.32–1.21 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.6, 165.0, 159.6, 141.8, 134.3, 129.9, 128.8, 126.9, 126.6, 101.6, 49.5, 44.9, 40.5, 35.7, 31.2, 26.4, 25.6; IR (ν_{max}/cm^{-1}) 3316, 3182, 2921, 2850, 1636, 779; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_5Cl^+$: 360.1950 $[M+H]^+$; found: 360.1937.

6-Cyclohexyl-5-{[(4-chlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7w). Prepared from (*E*)-6-cyclohexyl-5-{[(4-chlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11w** (0.113 g, 0.32 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.078 mg, 2.05 mmol), isolated as a colourless gel (0.098 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.25–7.21 (m, 2H), 7.12–7.07 (m, 2H), 6.54 (s, 2H), 5.85 (s, 2H), 3.67 (s, 2H), 2.85 (t, *J* = 6.7 Hz, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 2.68 (tt, *J* = 11.9, 3.5 Hz, 1H), 2.01 (s, 1H), 1.84–1.75 (m, 2H), 1.75–1.60 (m, 2H), 1.55 (d, *J* = 12.7 Hz, 2H), 1.33–1.19 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.5, 165.0, 159.4, 138.1, 132.2, 130.1, 128.7, 101.6, 49.7, 44.9, 40.5, 35.4, 31.1, 26.5, 25.6; IR (ν_{max}/cm^{-1}) 3321, 3187, 2924, 2850, 1637, 807; HRMS (ESI): *m/z* calcd for $C_{19}H_{27}N_5Cl^+$: 360.1950 $[M+H]^+$; found: 360.1932.

6-Cyclohexyl-5-{[(2,4-dichlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7x). Prepared from (*E*)-6-cyclohexyl-5-{[(2,4-dichlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11x** (0.135 g, 0.40 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.085 mg, 2.24 mmol), isolated as a colourless gel (0.081 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35 (d, *J* = 2.2 Hz, 1H), 7.20–7.12 (m, 2H), 6.51 (s, 2H), 5.79 (s, 2H), 3.70 (s, 2H), 2.93–2.84 (m, 4H), 2.72 (tt, *J* = 11.7, 3.5 Hz, 1H), 2.02 (s, 1H), 1.87–1.78 (m, 3H), 1.75–1.62 (m, 2H), 1.57 (d, *J* = 12.6 Hz, 2H), 1.37–1.21 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.1 (2 C), 159.3, 135.9, 134.8, 132.9, 131.6, 129.5, 127.3, 101.7, 48.1, 45.0, 40.5, 33.4, 31.2, 26.5, 25.6; IR (ν_{max}/cm^{-1}) 3317, 3179, 2924, 2851, 1637, 817; HRMS (ESI): *m/z* calcd for $C_{19}H_{26}N_5Cl_2^+$: 394.1560 $[M+H]^+$; found: 360.1538.

6-Cyclohexyl-5-{[(3,4-dichlorophenethyl)amino]methyl}pyrimidine-2,4-diamine (7y). Prepared from (*E*)-6-cyclohexyl-5-{[(3,4-dichlorophenethyl)imino]methyl}pyrimidine-2,4-diamine **11y** (0.127 g, 0.32 mmol), 1,2-dichloroethane (3 ml), THF (6 ml) and sodium borohydride (0.080 mg, 2.10 mmol), isolated as a colourless gel (0.090 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.35 (d, *J* = 8.2 Hz, 1H), 7.27 (d, *J* =

2.1 Hz, 1H), 7.03 (dd, $J=8.2$, 2.1 Hz, 1H), 6.41 (s, 2H), 5.65 (s, 2H), 3.69 (s, 2H), 2.87 (t, $J=6.9$ Hz, 2H), 2.80–2.63 (m, 3H), 2.03 (s, 1H), 1.86–1.78 (m, 2H), 1.73–1.61 (m, 2H), 1.57 (d, $J=14.2$ Hz, 2H), 1.39–1.18 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) $\delta=167.2$, 164.9, 159.8, 140.1, 132.4, 130.7, 130.5, 130.4, 128.3, 101.7, 49.5, 45.0, 40.6, 35.3, 31.2, 26.5, 25.7; IR (ν_{max}/cm^{-1}) 3321, 3186, 2924, 2852, 1629, 784; HRMS (ESI): *m/z* calcd for $C_{19}H_{26}N_5Cl_2^+$: 394.1560 $[M+H]^+$; found: 360.1541.

Biology

Enzyme preparation and inhibition assays. *Pf*DHFRs and *Hs*DHFR enzymes were expressed under T7 promotor of pET17b plasmids in *E. Coli* BL21(DE3)[14] and purified from the crude bacterial lysate of IPTG (isopropyl-β-D-thiogalactopyranoside) induced cells by methotrexate-agarose affinity chromatography. Enzyme inhibition assay was determined in 96-well plates at 25 °C using Multiskan GO microplate spectrophotometer (Thermo Scientific) and the K_i-values were calculated as previously described.[15,16] Briefly, the DHFR inhibition assay contains 100 μM each of dihydrofolate (natural substrate) and reduced nicotinamide adenine dinucleotide phosphate (NADPH, the cofactor), various concentrations of an inhibitor, and PfDHFR. The reactions were monitored at 340 nm for depletion of NADPH by a kinetic plate reader (Labsystems, Finland). The enzyme inhibition constant or K_i values of each inhibitor against the wild-type and mutant enzymes were calculated by non-linear curve fitting using KaleidaGraph program.

Parasite culture, antimalarial and cytotoxicity testing *in vitro*. Two *P. falciparum* strains, TM4/8.2 (wild type *Pf*DHFR, CQ sensitive) and V1/S (QM *Pf*DHFR, CQ resistant) were cultured in human erythrocytes at 37 °C under 3% CO_2 in RPMI 1640 culture media containing 25 mM HEPES, 0.2% $NaHCO_3$, 40 μg/mL gentamicin and 8% human serum. *In vitro* antimalarial activity was determined by ^{3}H-hypoxanthine incorporation method.[16,17] Cytotoxicity of each compound was determined against two mammalian derived cell lines, Vero (African green monkey kidney epithelial cells) and KB (human epithelial carcinoma cells) by quantitative staining of cellular proteins using sulforhodamine B protein staining.[16,18]

Acknowledgements

KS, TDS, and TM thank the National Research Foundation (South Africa) for funding. KS and MM thank the University of the Witwatersrand for Post Graduate Merit Award funding. BIOTEC team led by SK thank Prof. S. Thaithong (Chulalongkorn University, Thailand), and MR4 (ATCC Manassas Virginia, contributed by Prof. D. Kyle) for Pf*TM4/8.2 and* Pf*V1S parasites, Bioassay Laboratory at BIOTEC (NSTDA, Thailand) for Vero and KB cells and NSTDA's Research Chair Grant (P1850116) for financial support. The authors acknowledge the Centre for High Performance Computing (CHPC), South Africa, for providing computational resources to this research project.*

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: biological activity · computational chemistry · dihydrofolate reductase · nitrogen heterocycles · *Plasmodium falciparum*

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Manuscript received: July 29, 2022
Revised manuscript received: October 3, 2022
Accepted manuscript online: October 4, 2022
Version of record online: November 2, 2022