Discovery of 4,6-bis(2-((*E*)-benzylidene)hydrazinyl) pyrimidin-2-Amine with Antibiotic Activity

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Robenidine (*E*)-*N*′-((*E*)-1-(4-chlorophenyl)ethylidene)-2-(1-(4-chlorophenyl)ethylidene)hydrazine-1-carboximidhydrazide displays methicillin-resistant *Staphyoccoccus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) MICs of 2 µg mL^{-1}. Herein we describe the structure-activity relationship development of a novel series of guanidine to 2-aminopyrimidine isosteres that ameliorate the low levels of mammalian cytotoxicity in the lead compound while retaining good antibiotic activity. Removal of the 2-NH$_2$ pyrimidine moiety renders these analogues inactive. Introduction of a central 2-NH$_2$ triazine moiety saw a 10-fold activity reduction. Phenyl to cyclohexyl isosteres were inactive. The 4-BrPh and 4-CH$_3$Ph with MIC values of 2 and 4 µg mL^{-1}, against MRSA and VRE respectively, are promising candidates for future development.

Introduction

Bacteria resistant to polymyxin have been reported, this marks the advent of an era where bacteria resistant to all current antibiotics have been observed.[1] The importance of developing new antibiotics has been highlighted by the World Health Organization, the Centre for Disease Control, the Infectious Disease Society of America and the European Centre for Disease Control.[2–5] The drive to produce novel antibiotics has received a global call over a significant threat to human life by bacteria with current estimates citing > 50,000 deaths in the USA and Europe alone as a consequence of antibiotic resistance.[4,5]

Of the antibiotics brought to market in the past 30 years, most have been derivatives of existing drugs.[3,6] These next generation antibiotics are typically a response to resistance emerging to the prior generation. It is unclear how long this cycle of next generation – resistance – new generation antibiotics within the same class of compounds can be perpetuated. Of equal concern is that the Food and Drug Administration (FDA) only approved one new antibiotic in 2015, Avycaz® (avibabctam/ceftazidine) for the treatment of complicated intra-abdominal infections.[3] This lack of innovation, and investment, has meant that a number of multidrug resistant bacterial strains, particularly the "ESKAPE" pathogens: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species, are extremely challenging to treat and in some cases require complex antibiotic cocktails.[3,5–10]

Our critical reliance on antibiotics has engendered government initiatives and global strategies to rejuvenate the antibiotic pipeline, such as the Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) initiative and "The 10×20 Initiative" seek to combat this crisis and has the ambitious target of ten new antibacterial drugs by 2020.[11–13] Whilst these ambitious targets have stimulated a resurgence in antibacterial research at the academic level, this research has failed to translate into new antibiotics with novel mechanisms of action.[14–19] Of particular concern is the lack of efficacious compounds which treat Gram-negative bacteria, owing to the poor drug penetration of the outer membrane and the efficient efflux systems widespread within this group of microbes, making these pathogens extremely challenging to treat.[6] Both the Infectious Diseases Society of America and the European Centre for Disease Control have announced that only a handful of potential drugs which target Gram-negative bacteria in clinical trials offer significant benefits over current clinically used antibiotics.[15,16] Clearly there is a pressing need to develop new antibiotic classes, especially those with lower inherent resistance susceptibility.[15–23]

We recently reported the development of robenidine based analogues with antibiotic activity against clinically relevant strains of MRSA and VRE.[24–26] These prior efforts included the identification and biological evaluation of a pyrimidine based robenidine analogue.[25] Herein we explore the structure activity relationship data and design characteristics that led to the

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identification of a novel guanidine bioisostere,[27–30] and the discovery of a family of benzylidinehydrazylpyrimidin-2-amines displaying modest to good levels of antibiotic activity against MRSA and VRE.

Results and Discussion

Our earlier studies revealed that **1**, displayed good levels of activity against methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE) with MIC values of 2 µg mL^{-1} against both bacteria.[24,25] However, some of our parent robenidine analogues displayed moderate levels mammalian cell cytotoxicity.[24] We were thus keen to explore possible isosteric modifications that would enable retention or enhancement of antibiotic activity while ameliorating this low level of cytotoxicity further.

We envisaged that replacement of the central guanidine core could be accomplished through the installation of a diaminopyrimidine nucleus with retention of the key binding features of the lead, **1** (Figure 1). As such we targeted the development of a small focused library of diaminopyrimidine based analogues of **1** (Scheme 1).
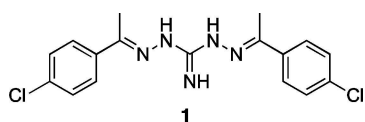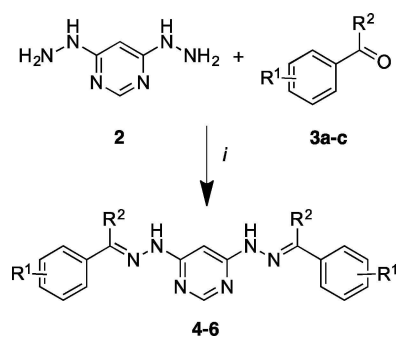

Figure 1. Guanidine based lead, **1**, with MIC values of 2 µg mL^{-1} against both MRSA and VRE.

Scheme 1. *Reagents and Conditions*: i) EtOH, reflux, 16 h.

In a typical synthesis 4,6-dihydrazinylpyrimidine **2** was refluxed with a phenone and/or aldehydes **3 a–c** for 16 h, which after reaction work up (see experimental) gave pyrimidines **4–6** in good (**4**, 68 %) to excellent yields (**6**, 91 %). These analogues were screened for activity against the Gram-positive MRSA and VRE and the Gram-negative *E. coli* and *Pseudomonas*. These data are presented in Table 1. The antibiotic activity screening was conducted in Luria Bertani (LB) broth as the robenidine has been shown to chelate Ca^{2+} ions.[24] It is not known, nor explored here, if all robenidine analogues do so. The use of LB broth ensured assay to assay comparison consistency. In

Table 1. Inhibition of *MRSA, VRE, E. coli* and *Pseudomonas* growth by pyrimidines **4–6**.

| Compound | R^1 | R^2 | MIC mode ^{a} (µg mL^{-1}) at 24 h | | | |
			MRSA	VRE	E. coli	P. aeruginosa
4	4-Cl	Me	– ^{b}	–	–	–
5	4-Cl	CH$_2$OH	–	128	–	–
6	4-Cl	H	–	–	–	–

^{a} MIC value among all observations that occurs at the greatest frequency, ^{b} no activity at 128 µg mL^{-1} compound concentration.

subsequent *in vivo* studies, no effect of potential Ca^{2+} sequestration was observed.[25]

Disappointingly, only **5** returned any sign of activity, and then only low levels limited to the inhibition of VRE. Re-examination of our initial bioisosteric modification suggested that the lack of an exocyclic NH moiety may have been the cause of such low levels of antibiotic activity. The modelled structures of **1** and **4** highlight the change in the position of the nitrogen moieties and the lack of a pendant NH capable of H-bonding interactions. Based on this, we examined the corresponding 2-NH$_2$ pyrimidine analogue, **7**, and in this case preliminary modelling analysis supported the overlay of the key residues relative to **1** (Figure 2).
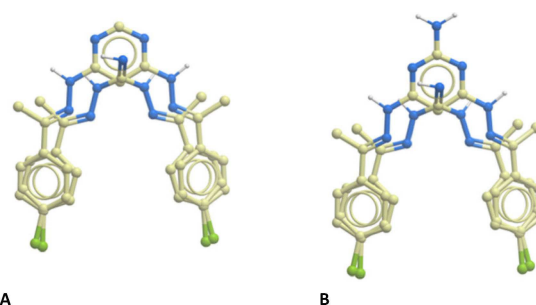
Figure 2. A. Superimposition of **1** and **4**; and **B**. Superimposition of **1** and **7**, showing the introduction of the exocyclic NH$_2$ moiety capable of the similar H-bonding interactions as the central guanidine NH of **1**.

As such we synthesised a focused library based on the 2-aminopyrimidine core using the same approach as outlined in Scheme 1 commencing from pyrimidine-2,4,6-triamine. As anticipated the condensation of aldehydes and phenones occurred exclusively at the 4,6-amino moieties to afford analogues **7–23** (see Table 2 for detail), which were subsequently screened for activity against MRSA, VRE, *E. coli* and *Pseudomonas*. No Gram-negative activity was observed, Gram-positive data are presented in Table 2.

Examination of the antibacterial data presented in Table 2 reveals good levels of activity with the aminopyrimidine isostere **7** of our initial lead **1** retaining high levels of activity against MRSA (4 µg mL^{-1}) and VRE (8 µg mL^{-1}). This activity was enhanced through the introduction of a 4-Br **9** or a 4-CH$_3$

Table 2. Inhibition of *MRSA* and *VRE* growth by aminopyrimidines **7–23**.

Compound	R1	R2	MRSA	VRE	Compound	R1	R2	MRSA	VRE
			MIC mode [a] (µg mL^{-1}) at 24 h					MIC mode (µg mL^{-1}) at 24 h	
7	4-Cl-phenyl	H	4	8	**16**	4-HO-phenyl	H	32	64
8	2-Cl-phenyl	H	8	64	**17**	3-HO-phenyl	H	8	8
9	4-Br-phenyl	H	2	4	**18**	2-OH-phenyl	H	8	8
10	4-F-phenyl	H	16	32	**19**	4-H$_3$CO-phenyl	H	–	–
11	4-F$_3$C-phenyl	H	4	64	**20**	4-NMe$_2$-phenyl	H	32	
12	phenyl	H	4	–[b]	**21**	4-NHAc-phenyl	H	–	–
13	4-methyl-phenyl	H	2	4	**22**	cyclohexyl	H	32	32
14	4-t-Bu-phenyl	H	–	–	**23**	4-Cl-phenyl	CH$_3$	8	64
15	4-Ph-phenyl	H	–	–					

[a] MIC value among all observations that occurs at the greatest frequency, [b] no activity at 128 µg mL^{-1} compound concentration.

moiety **13**. In this Library, good tolerance for a 4-substituent was noted with **7–13**, and **16–18** returning MIC values <64 µg mL^{-1} against MRSA or VRE or both bacteria. Only bulky groups appear to be disfavoured with the 4-t-Bu **14**, 4-Ph **15** and 4-OCH$_3$ **19** analogues inactive. In most cases where activity was observed, each analogue was more potent against either MRSA or VRE, e.g. **12** with an MRSA MIC of 4 µg mL^{-1}, but inactive against VRE (MIC >128 µg mL^{-1}). Introduction of the acetyl moiety **21** effectively removed all antibiotic activity whereas the replacement of the phenyl moiety with a cyclohexyl moiety **22** retained modest activity against MRSA and VRE. Introduction of a methyl moiety at the hydrazone carbon (C=N–NH), with **23**, afforded good MRSA activity (MIC 8 µg mL^{-1}), but only modest VRE activity (MIC 64 µg mL^{-1}). In all cases no Gram-negative activity was observed.

We have reported a more detailed *in vivo* biochemical evaluation of the aminopyrimidine isostere analogue,[25] **13**, wherein we noted that this compound displayed potent bactericidal activity against *Streptococcus pneumoniae* and *Staphylococcus aureus* by disrupting the cell membrane potential. Critically this guanidine to aminopyrimidine isosteric modification gave analogues with lower levels of mammalian cell toxicity (3.5-fold less toxic to MCF-7 (breast), Hel299 (lung) and MDBK (kidney) tumour cell lines relative to **1**); low metabolic degradation rates in human and mouse liver microsomes; high plasma concentrations after 5 mg/kg i.v. dosing, and low plasma clearance rates in mice relative to the guanidine equivalent analogue.[25]

Having successfully introduced an aminopyrimidine guanidine isostere as the core linker with retention, and modest potency enhancement with a reduction in cytotoxicity, we explored further modifications through the installation of 1,3,5-triazine moiety through the synthesis of **24–32**. These analogues were synthesised as per Scheme 1 from 1,3,5-triazin-2-amine and screened for antibiotic activity as before and these data are presented in Table 3.

Despite the promising activity observed with the equivalent aminopyrimidine analogues (Table 2), the installation of the 1,3,5-triazin-2-amine moiety essentially abolished antibiotic activity with only 2-OH **24**, 4-CF$_3$ **27** and 4-Br **29** displaying modest levels of activity with MIC values of 16–64 µg mL^{-1}. Even in these instances' activity was only observed against either MRSA or VRE, but not both Gram positive bacteria. No Gram-negative activity was observed.

Table 3. Inhibition of *MRSA* and *VRE* growth by aminotriazines **24–32**.

Compound	R¹	R²	MRSA	VRE	Compound	R¹	R²	MRSA	VRE
			MIC mode [a] (μg mL^{-1}) at 24 h					MIC mode (μg mL^{-1}) at 24 h	
24	2-hydroxyphenyl (OH)	H	32	–[b]	**29**	4-bromophenyl (Br)	H	–	16
25	chlorophenyl (Cl)	CH₃	–	–	**30**	phenyl	H	–	–
26	hydroxyphenyl (HO)	H	–	–	**31**	cyclohexyl	H	–	–
27	4-(trifluoromethyl)phenyl (F₃C)	H	–	64	**32**	4-methylphenyl	H	–	–
28	4-hydroxyphenyl (HO)	H	–	–					

[a] MIC value among all observations that occurs at the greatest frequency, [b] no activity at 128 μg mL^{-1} compound concentration.

Increasing the complexity and/or the number of substituents on the phenyl moiety had mixed outcomes (these data are presented in Table 4). A phosphate ester **33** was inactive, whereas di- and tri- OH substitution was only effective with the 2,3-di-OH **37** (8 μg mL^{-1}) against both MRSA and VRE and the 2,4-di-OH **39** (64 μg mL^{-1}) against only MRSA. Naphthyl substituted **40** and **41** returned good activity at 8/64 and 8/32 μg mL^{-1} against MRSA and VRE, respectively. This suggests a preference for aromatic moieties in this region, noting that the 4-*t*-Bu **14** and cyclohexyl **22** were inactive. Introduction of a spacer unit between the phenyl and guanidine moieties with **42** and **43** was detrimental to activity, especially against VRE with both analogues inactive. Taking this into consideration we next evaluated the introduction of a simple pyridyl moiety with **44**–**47**. Favourable outcomes were noted with **44** and **45** with a marked preference for the 3-pyridyl moiety in effecting antibiotic activity with **44** more active than 2-pyridyl **45**. The 4-pyridyl **46** showed low VRE activity and the 4-Cl-3-pyridyl **47** was inactive.

Conclusions

Attempts to introduce a 4,6-dihydrazinylpyrimidine moiety as a guanidine bioisostere to robenidine based antibiotic lead compounds were unsuccessful with the pyrimidine analogue **4** inactive whereas the parent guanidine lead **1** displayed an MIC of 2 μg mL^{-1} against both MRSA and VRE bacterial strains. Examination of the potential 3-dimenstional conformation of **1** and **4** highlighted the probable lack of a key hydrogen bonding moiety – the guanidine NH, which potentially explained the abrogation of activity. Subsequent use of a 2-aminopyrimidine afforded a library of guanidine to 2-aminopyrimidine isosteres

that returned good to excellent MIC values against both MRSA and VRE. Of most note were the 4-BrPh **9** and 4-CH₃Ph **13** with MIC values of 2 and 4 μg mL^{-1} against MRSA and VRE respectively. The presence of the pendant aromatic ring was critical to activity, the equivalent cyclohexyl analogue, **22**, was inactive. The central nature of the 2-aminopyrimidine isostere brooked limited modification with all exemplars **24**–**32** of the triazine bioisosteres effectively inactive or showing only low levels of activity against either MRSA (**24**, MIC 16 μg mL^{-1}) or VRE (**29**, 32 μg mL^{-1}).

The introduction of more complex aromatic moieties in conjunction with the 2-aminopyrimidine moiety had mixed outcomes. Of the OH substituted analogues only the 2,3-di-OH **37** showed noteworthy activity with a MRSA and VRE MIC of 8 μg mL^{-1}. Bulky aromatic systems were tolerated with the 1- and 2-naphthyl **40** and **41** 8 μg mL^{-1} active against MRSA, with reduced activity against VRE. The introduction of a spacer between the aromatic moiety and 2-aminopyrimidine with **42** and **43** was better tolerated with MRSA than VRE, with good to modest activity. A heteroatom, viz the pyridyl analogues **44**–**46**, showed moderate to good activity in the absence of a 4-substituent, with the 4-Cl-3-pyridyl analogue inactive. No analogue displayed Gram-negative activity against the *E. coli* and *Ps. Aeruginosa* strains examined.

A more extensive biochemical evaluation of aminopyrimidine, **13**, was consistent with this class of compounds being promising chemical leads for on-going medicinal chemistry development. Combined these data suggest that **9** and **13** are excellent candidates for further development, and we will report on this in due course.

Table 4. Inhibition of *MRSA* and *VRE* growth by aminopyrimidines **33–47**.

Compound	R¹	R²	MIC mode[a] (µg mL⁻¹) at 24 h		Compound	R¹	R²	MIC mode (µg mL⁻¹) at 24 h	
			MRSA	*VRE*				*MRSA*	*VRE*
33	(EtO)₂P(O)O-phenyl	H	–[b]		**41**	naphthalen-2-yl	H	8	32
34	3,4-difluorophenyl	H	–	–	**42**	styryl	H	16	–
35	2,4-dihydroxyphenyl	H	–	–	**43**	phenethyl	H	32	–
36	3,4-dihydroxyphenyl	H	–	–	**44**	pyridin-3-yl	H	8	16
37	2,3-dihydroxyphenyl	H	8	8	**45**	pyridin-2-yl	H	16	32
38	3,4,5-trihydroxyphenyl	H	–	–	**46**	pyridin-4-yl	H	–	64
39	2,4-dihydroxyphenyl	H	64	–	**47**	6-chloropyridin-3-yl	H	–	–
40	naphthalen-1-yl	H	8	64					

[a] MIC value among all observations that occurs at the greatest frequency, [b] no activity at 128 µg mL⁻¹ compound concentration.

Experimental

Chemistry – General Methods

All reagents were purchased from Sigma-Aldrich, AK Scientific, Matrix Scientific or Lancaster Synthesis and were used without purification. All solvents were re-distilled from glass prior to use.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance™ AMX 400 at 400.13 and 100.62 MHz, respectively and Advance™ AMX 600 at 600.21 and 150.92 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured relative to the internal standards. Coupling constants (J) are expressed in hertz (Hz). Mass spectra were recorded on a Shimadzu LCMS 2010 EV and Agilent 6100 series single quadrupole LCMS using a mobile phase of 1:1 acetonitrile :H₂O with 0.1% formic acid. The University of Wollongong, Australia, Mass Spectrometry User resource & Research Facility analysed samples for High Resolution Mass Spectrometry. The spectra were acquired on the VG Autospec-oa-tof tandem high resolution mass spectrometer using CI (chemical ionization), with methane as the carrier gas and PFK (perfluorokerosene) as the reference. HRMS Analytical HPLC traces were obtained using a Shimadzu system possessing a SIL-20A auto-sampler, dual LC-20AP pumps, CBM-20A bus module, CTO-20A column heater, and a SPD-20A UV/vis detector. This system was fitted with an Alltima™ C₁₈ 5 µm 150 mm×4.6 mm column with solvent A: 0.06% trifluoro-

acetic acid (TFA) in water and solvent B: 0.06% TFA in CH₃CN–H₂O (90:10). In each case HPLC traces were acquired at a flow rate of 2.0 mL min⁻¹, gradient 10–100 (%B), over 15.0 min, with detection at 220 nm and 254 nm. All samples returned satisfactory analyses. Compound purity was confirmed by a combination of LC-MS (HPLC), micro and/or high resolution mass spectrometry and NMR analysis. All analogues are ≥ 95% purity.

Melting points were recorded on a Büchi Melting Point M-565 instrument. IR spectra were recorded on a PerkinElmer Spectrum Two™ FTIR Spectrometer with the UATR accessories. Thin layer chromatography (TLC) was performed on Merck 60 F254 pre-coated aluminium plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230–400 mesh).

Microbiology

Antimicrobial Agents

Robenidine (**1**, NCL812) was provided by Neoculi Pty. Ltd. Ampicillin used in this study for quality control of susceptibility testing was sourced from Sigma Aldrich.

Bacterial Isolates

Isolates used in initial screening assay were sourced as follows: SCCmec type IV MRSA (n = 2), VRE (n = 2), multidrug-resistant *E. coli* (n = 2) and *P. aeruginosa* (n = 2) clinical isolates were kindly provided by Prof Mary Barton, University of South Australia. MSSA strains of *S. aureus* ATCC 25923 and 29213 were obtained from the American Type Culture Collection together with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Susceptibility Testing

The MIC of all analogues was determined using a slightly modified microdilution method according described to in CLSI guidelines as follows: Luria Bertani (LB) broth was used instead of CAMHB as it has been previously shown that **1** can chelate calcium ions.[24] In addition, the antimicrobial dilutions of all analogues were completed in 100% DMSO, with 1 µL added to each well in the challenge plate, as the compounds are hydrophobic. The assay was performed in a total volume of 100 µL with test concentration increasing 2-fold from 0.25 µg.mL^{-1} to 128 µg.mL in 96 well plates. MIC tests involving ampicillin were performed according to CLSI guidelines in CAMHB. Plates were incubated for 24 hours at $35 \pm 2\,°C$ before determination of the MIC.

Control reference strains, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, were tested against the test and control antimicrobials to ensure MIC values were within range according to CLSI documents.[25]

Synthesis

4,6-Bis(2-((E)-1-(4-chlorophenyl)ethylidene)hydrazinyl) pyrimidine (4)

A suspension of 4,6-dihydrazinylpyrimidine (65 mg, 0.465 mmol) and 4-chloroacetophenone (182 mg, 1.175 mmol, 2.53 eq.) in EtOH (2.3 mL) was heated at reflux for 16 h. Upon cooling to ambient temperature the resulting precipitate was collected and washed with Et$_2$O to afford the pyrimidine (131 mg, 68%) as an off-white amorphous solid. MP 251–252 °C.

^{1}H NMR (DMSO-d_6) δ 10.17 (s, 2H), 8.24 (s, 1H), 7.83 (d, J = 8.6 Hz, 4H), 7.50 (d, J = 8.6 Hz, 4H), 6.97 (s, 1H), 2.32 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 162.5, 157.4, 145.0, 137.5, 133.2, 128.4, 127.3, 83.2, 13.4; MS: LRMS 412.65; HRMS calculated for M+H: C$_{20}$H$_{19}$Cl$_2$N$_6$, 413.1043; found 413.1049.

(2Z,2'Z)-2,2'-(pyrimidine-4,6-diylbis(hydrazin-2-yl-1-ylidene)) bis(2-(4-chlorophenyl)ethan-1-ol) (5)

A suspension of 4,6-dihydrazinylpyrimidine (309 mg, 2.203 mmol) and 1-(4-chlorophenyl)-2-hydroxyethanone (1.148 g, 6.7319 mmol, 3.06 eq.) in EtOH (10 mL) was heated at reflux for 16 h. The heterogeneous reaction mixture changed to a bright yellow colour and the precipitate filtered hot, washing with Et$_2$O (20 mL) to afford the pyrimidine (681 mg, 69%) as a yellow amorphous powder. MP 232 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.62 (s, 2H), 8.22 (s, 1H), 7.79 (d, J = 8.5 Hz, 4H), 7.51 (d, J = 8.5 Hz, 4H), 6.93 (s, 1H), 5.85 (t, J = 5.0 Hz, 2H), 4.74 (d, J = 5.0 Hz, 4H); ^{13}C NMR (DMSO-d_6) δ 161.9, 157.7, 146.8, 135.7, 133.3, 128.5, 127.8, 82.2, 57.0; MS: LRMS 444.6; HRMS calculated for M+H: C$_{20}$H$_{19}$Cl$_2$N$_6$O$_2$, 445.0941; found 445.0943.

4,6-Bis(2-((E)-4-chlorobenzylidene)hydrazinyl)pyrimidine (6)

A suspension of 4,6-dihydrazinylpyrimidine (146 mg, 1.042 mmol) and 4-chlorobenzaldehyde (365 mg, 2.599 mmol, 2.49 eq.) in EtOH (20 mL) was heated at reflux for 16 h. Upon cooling to 40 °C the resulting precipitate was collected and washed with Et$_2$O (30 mL) to afford the pyrimidine (374 mg, 93%) as a white amorphous solid. MP 350 °C (decomp).

^{1}H NMR (DMSO-d_6) δ 11.20 (s, 2H), 8.17 (s, 1H), 8.09 (s, 2H), 7.72 (d, J = 8.5 Hz, 4H), 7.54 (d, J = 8.5 Hz, 4H), 6.83 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 161.6, 157.8, 140.4, 133.8, 133.5, 129.0, 127.9, 81.4; MS: LRMS ESI +ve 385 (M+1); HRMS calculated for M+H: C$_{18}$H$_{15}$Cl$_2$N$_6$, 385.0730; found 385.0737.

4,6-Bis(2-((E)-4-chlorobenzylidene)hydrazinyl) pyrimidin-2-amine (7)

A suspension of 2-amino-4,6-dihydrazinylpyrimidine (67 mg, 0.434 mmol) and 4-chlorobenzaldehyde (199 mg, 1.414 mmol, 3.26 eq.) in EtOH (25 mL) was heated at reflux for 16 h. After this time, the condenser was removed and the solution concentrated to approx. 1 mL and the resulting precipitate filtered hot and washed with Et$_2$O (10 mL) to afford the aminopyrimidine (43 mg, 25%) as an off-white amorphous powder. MP 275 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.70 (s, 2H), 8.02 (s, 2H), 7.67 (d, J = 8.4 Hz, 4H), 7.52 (d, J = 8.4 Hz, 4H), 6.28 (s, 1H), 5.85 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.6, 138.7, 134.1, 133.1, 128.9, 127.6, 73.5; MS: LRMS 399.8; HRMS calculated for M+H: C$_{18}$H$_{16}$Cl$_2$N$_7$, 400.0839; found 400.0844.

4,6-Bis(2-((E)-2-chlorobenzylidene)hydrazinyl) pyrimidin-2-amine (8)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (88 mg, 0.568 mmol) and 2-chlorobenzaldehyde (0.15 mL, 190 mg, 1.3 mmol, 2.3 eq.) in EtOH (25 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature, diluted with Et$_2$O (30 mL) and concentrated in vacuo to ca. 5 mL before collecting the precipitate to afford the pyrimidine (24 mg, 11%) as an off-white powder. MP 244–246 °C.

^{1}H NMR (DMSO-d_6) δ 10.91 (s, 2H), 8.41 (s, 2H), 7.98 (d, J = 7.5 Hz, 2H), 7.50–7.35 (m, 6H), 6.34 (s, 1H), 5.93 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.75, 162.68, 136.0, 132.4, 131.9, 130.1, 129.9, 127.6, 126.2, 73.7; MS: LRMS ESI +ve 400.1 (M+1); HRMS calculated for M+H: C$_{18}$H$_{16}$Cl$_2$N$_7$, 400.0839; found 400.0840.

4,6-Bis(2-((E)-4-bromobenzylidene)hydrazinyl) pyrimidin-2-amine (9)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (66 mg, 0.423 mmol) and 4-bromobenzaldehyde (186 mg, 1.005 mmol, 2.38 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting precipitate and washing with ice cold EtOH (10 mL) and Et$_2$O (10 mL) to afford the pyrimidine (133 mg, 64%) as a white crystalline solid. MP 274 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.71 (s, 2H), 8.00 (s, 2H), 7.63 (dd, J = 24.3, 8.6 Hz, 8H), 6.27 (s, 1H), 5.86 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.6, 138.8, 134.5, 131.8, 127.9, 121.7, 73.5; MS: LRMS ESI +ve 488.1 (M+1); HRMS calculated for M+H: C$_{18}$H$_{16}$Br$_2$N$_7$, 487.9828; found 487.9830.

4,6-Bis(2-((E)-4-fluorobenzylidene)hydrazinyl) pyrimidin-2-amine (10)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (109 mg, 0.704 mmol) and 4-fluorobenzaldehyde (0.16 mL, 180 mg, 1.5 mmol, 2.13 eq.) in EtOH (10 mL) was heated at reflux for 16 h. The reaction mixture was filtered hot, washing with Et_2O (10 mL), to afford the pyrimidine (110 mg, 42%) as a tan powder. MP 262 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.61 (s, 2H), 8.03 (s, 2H), 7.70 (dd, J=8.8, 5.6 Hz, 4H), 7.29 (t, J=8.8 Hz, 4H), 6.27 (s, 1H), 5.82 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.8, 162.6, 162.3 (d, J=246.0 Hz), 138.9, 131.8 (d, J=3.0 Hz), 128.0 (d, J=8.3 Hz), 115.9 (d, J=21.8 Hz), 73.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ −112.57. MS: LRMS ESI +ve 368.2 (M+1); HRMS calculated for M+H: $C_{18}H_{16}F_2N_7$, 368.1430; found 368.1431.

4,6-Bis(2-((E)-4-(trifluoromethyl)benzylidene)hydrazinyl) pyrimidin-2-amine (11)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (112 mg, 0.7224 mmol) and 4-(trifluoromethyl)benzaldehyde (0.21 mL, 270 mg, 1.5 mmol, 2.08 eq.) in EtOH (11 mL) was heated at reflux for 16 h. The reaction mixture was concentrated using a compressed air stream before suspending the resulting crude material in Et_2O (10 mL) and collected, washing with Et_2O (10 mL), to afford the pyrimidine (19 mg, 5%) as a brown powder. MP 261 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.89 (s, 2H), 8.11 (s, 2H), 7.84 (dd, J=20.5, 8.4 Hz, 8H), 6.34 (s, 1H), 5.93 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.6, 139.1, 138.4, 128.4 (q, J=31 Hz), 126.9 (q, J=263 Hz) * 126.5, 125.8 (q, J=3.8 Hz)*, 74.1. * poorly resolved quartet; ^{19}F NMR (376 MHz, DMSO-d_6) δ −60.90. MS: LRMS ESI +ve 468.2 (M+1); HRMS calculated for M+H: $C_{20}H_{16}F_6N_7$, 468.1366; found 468.1371.

4,6-Bis(2-((E)-benzylidene)hydrazinyl)pyrimidin-2-amine (12)

To a slurry of 2-amino-4,6-dihydrazinopyrimidine (49 mg, 0.316 mmol) and benzaldehyde (0.100 mL, 104 mg, 0.980 mmol, 3.10 eq.) was added EtOH (10 mL) and the solution heated at reflux for 16 h. Upon cooling the resulting precipitate was collected, washing with Et_2O (5 mL) to afford the target compound (23 mg, 22%) as a white powder. MP 242–244 °C.

^{1}H NMR (DMSO-d_6) δ 10.60 (s, 2H), 8.04 (s, 2H), 7.66 (d, J=7.5 Hz, 4H), 7.45 (t, J=7.1 Hz, 4H), 7.38–7.34 (m, 2H), 6.30 (s, 1H), 5.82 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 163.3, 163.1, 140.5, 135.7, 129.3, 129.2, 126.5, 73.9; MS:LRMS 331.65; HRMS calculated for M+H: $C_{18}H_{18}N_7$, 332.1618; found 332.1619.

4,6-Bis(2-((E)-4-methylbenzylidene)hydrazinyl) pyrimidin-2-amine (13)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (59 mg, 0.380 mmol) and 4-methylbenzaldehyde (0.10 mL, 100 mg, 0.832 mmol, 2.19 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the pellet-like precipitate, washing with Et_2O (20 mL). The 'pellets' were then crushed and the solid further washed with Et_2O (10 mL) to afford the pyrimidine (86 mg, 63%) as a white 'fluffy' powder. MP 274–276 °C.

^{1}H NMR (DMSO-d_6) δ 10.51 (s, 2H), 8.00 (s, 2H), 7.54 (d, J=8.0 Hz, 4H), 7.26 (d, J=8.0 Hz, 4H), 6.27 (s, 1H), 5.78 (s, 2H), 2.34 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 162.8, 162.6, 140.1, 138.4, 132.5, 129.4, 126.0,

21.0; MS: LRMS ESI +ve 360.2 (M+1); HRMS calculated for M+H: $C_{20}H_{22}N_7$, 360.1931; found 360.1939.

4,6-Bis(2-((E)-4-(tert-butyl)benzylidene)hydrazinyl) pyrimidin-2-amine (14)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (56 mg, 0.363 mmol) and 4-(tert-butyl)benzaldehyde (0.13 mL, 130 mg, 0.80 mmol, 2.20 eq.) in EtOH (12 mL) was heated at reflux for 6 h. The reaction mixture was cooled to in a freezer for 1 h before collecting the precipitate, washing with Et_2O (50 mL), to afford the pyrimidine (6 mg, 4%) as a white powder

^{1}H NMR (DMSO-d_6) δ 10.51 (s, 2H), 8.02 (s, 2H), 7.58 (d, J=8.3 Hz, 4H), 7.47 (d, J=8.3 Hz, 4H), 6.26 (s, 1H), 5.77 (s, 2H), 1.31 (s, 18H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.5, 151.4, 140.2, 132.5, 125.9, 125.6, 73.2, 34.5, 31.0 MS: LRMS ESI +ve 444.3 (M +1); HRMS calculated for M+H: $C_{26}H_{34}N_7$, 444.2870; found 444.2873.

4,6-Bis(2-((E)-[1,1'-biphenyl]-4-ylmethylene)hydrazinyl) pyrimidin-2-amine (15)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (200 mg, 1.286 mmol) and biphenyl-4-carboxaldehyde (520 mg, 2.852 mmol, 2.22 eq.) in EtOH (10 mL) was heated at reflux for 16 h. The solution was cooled to ambient temperature and the resulting precipitate was collected, washing with EtOH (20 mL) and Et_2O (20 mL) to afford the bis-hydrazone (267 mg, 43%) as a yellow powder. MP 292 °C (decomp).

^{1}H NMR (DMSO-d_6) δ 10.69 (s, 2H), 8.09 (s, 2H), 7.77 (s, 8H), 7.71 (d, J=7.5 Hz, 4H), 7.47 (t, J=7.6 Hz, 4H), 7.37 (t, J=7.3 Hz, 2H), 6.42 (s, 1H), 5.87 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.8, 162.6, 140.2, 139.7, 139.5, 134.3, 129.0, 127.6, 127.1, 126.7, 126.6, 73.5; MS LRMS ESI +ve 484.3 (M+1); HRMS calculated for $C_{30}H_{26}N_7$, 484.2244; found 484.2248.

4,4′-((1E,1′E)-(2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))diphenol (16)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (70 mg, 0.454 mmol) and 4-hydroxybenzaldehyde (140 mg, 1.149 mmol, 2.53 eq.) in EtOH (3 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate, washing with Et_2O (25 mL), to afford the pyrimidine (91 mg, 55%) as an off-white powder. MP 298 °C (Decomp).

^{1}H NMR (CDCl$_3$) δ 10.31 (s, 2H), 9.74 (s, 2H), 7.94 (s, 2H), 7.48 (d, J=8.6 Hz, 4H), 6.83 (d, J=8.6 Hz, 4H), 6.20 (s, 1H), 5.70 (s, 2H); ^{13}C NMR (CDCl$_3$) δ 162.7, 162.5, 158.3, 140.5, 127.7, 126.3, 115.7, 73.0; MS: LRMS 364.2 (M+1); HRMS calculated for M+H: $C_{18}H_{18}N_7O_2$, 364.1516; found 364.1519.

3,3′-((1E,1′E)-(2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))diphenol (17)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (66 mg, 0.427 mmol) and 3-hydroxybenzaldehyde (207 mg, 1.693 mmol, 3.96 eq.) in EtOH (3 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate, washing with Et_2O (25 mL), to afford the pyrimidine (19 mg, 12%) as a white powder. MP 256 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.51 (s, 2H), 9.55 (s, 2H), 7.95 (s, 2H), 7.22 (t, J=7.9 Hz, 2H), 7.11–7.04 (m, 4H), 6.76 (d, J=8.4 Hz, 2H), 6.23 (s, 1H), 5.80 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.8, 162.6, 157.7, 140.4, 136.4,

129.9, 117.4, 116.1, 112.4, 73.3; MS: LRMS ESI +ve 364.2 (M+1); HRMS calculated for M+H: C₁₈H₁₈N₇O₂, 364.1516; found 364.1512.

2,2′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis(hydrazin-2-yl-1-ylidene))bis(methanylylidene))diphenol (18)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (66 mg, 0.425 mmol) and 2-hydroxybenzaldehyde (110 mg, 0.900 mmol, 2.1 eq.) in EtOH (3 mL) was subject to microwave irradiation for 20 minutes at 120 °C. The reaction mixture was cooled to ambient temperature before collecting the precipitate, washing with Et₂O (25 mL), to afford the pyrimidine (65 mg, 42 %) as a white powder. MP 240 °C (Decomp).

¹H NMR (DMSO-d_6) δ 10.72 (s, 2H), 10.56 (s, 2H), 8.28 (s, 2H), 7.55 (d, J=7.1 Hz, 2H), 7.24–7.19 (m, 2H), 6.92–6.88 (m, 4H), 5.98 (s, 1H), 5.88 (s, 2H). ¹³C NMR (DMSO-d_6) δ 162.7, 162.2, 156.2, 140.0, 130.0, 127.4, 120.2, 119.4, 116.1, 72.7; MS: LRMS ESI +ve 364.3 (M+1); HRMS calculated for M+H: C₁₈H₁₈N₇O₂, 364.1516; found 364.1522.

4,6-Bis(2-((E)-4-methoxybenzylidene)hydrazinyl)pyrimidin-2-amine (19)

To a refluxing solution of 2-amino-4,6-dihydrazinopyrimidine (645 mg, 4.159 mmol) in EtOH (40 mL) was added p-anisaldehyde (1.15 mL, 1.290 g, 9.47 mmol, 2.28 eq.) and the solution heated at reflux for 62 h, whilst protecting the flask from light. The reaction mixture was filtered warm (ca. 30 °C) and washed with Et₂O (100 mL) to afford the pyrimidine (1.120 g, 49 %) as a white crystalline powder. MP 234–236 °C.

¹H NMR (DMSO-d_6) δ 10.41 (s, 2H), 7.98 (s, 2H), 7.59 (d, J=8.9 Hz, 4H), 7.02 (d, J=8.9 Hz, 4H), 6.23 (s, 1H), 5.73 (s, 2H), 3.80 (s, 6H); ¹³C NMR (DMSO-d_6) δ 162.7, 162.5, 159.8, 140.1, 127.8, 127.5, 114.3, 73.1, 55.2; MS: LRMS 394.65; HRMS calculated for M+H: C₂₀H₂₂N₇O₂, 392.1829; found 392.1836.

4,4′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis(hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis(N,N-dimethylaniline) (20)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (46 mg, 0.296 mmol) and 4-(dimethylamino)benzaldehyde (100 mg, 0.673 mmol, 2.27 eq.) in EtOH (5 mL) was heated at reflux for 6 h. The reaction mixture was cooled to ambient temperature and the precipitate filtered and washed with Et₂O (20 mL). Subsequent recrystallisation from EtOH afforded the pyrimidine (47 mg, 38 %) as a yellow powder. MP 152 °C (Decomp).

¹H NMR (DMSO-d_6) δ 10.20 (s, 2H), 7.92 (s, 2H), 7.47 (d, J=8.5 Hz, 4H), 6.77 (d, J=8.5 Hz, 4H), 6.19 (s, 1H), 5.64 (s, 2H), 2.95 (s, 12H).; ¹³C NMR (DMSO-d_6) 162.6, 162.4, 150.7, 141.2, 127.3, 122.9, 112.1, 72.8, 39.9; MS ;LRMS 417.75; HRMS calculated for M+H: C₂₂H₂₈N₉, 418.2462; found 418.2464.

N,N′-(((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis(hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis(4,1-phenylene))diacetamide (21)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (86 mg, 0.5439 mmol) and N-(4-formylphenyl)acetamide (210 mg, 1.287 mmol, 2.37 eq.) in EtOH (11 mL) was heated at reflux for 16 h. After cooling, the reaction precipitate was collected and washed with Et₂O (15 mL) to afford the pyrimidine (164 mg, 66 %) as a tan powder. MP 268 °C (Decomp).

¹H NMR (DMSO-d_6) δ 10.47 (s, 2H), 10.07 (s, 2H), 7.97 (s, 2H), 7.65 (d, J=8.7 Hz, 4H), 7.58 (d, J=8.7 Hz, 4H), 6.24 (s, 1H), 5.75 (s, 2H), 2.06 (s, 6H); ¹³C NMR (DMSO-d_6) δ 168.4, 162.7, 162.5, 140.0, 139.8, 129.9, 126.7, 119.1, 73.2, 24.1; MS: LRMS ESI +ve 446.2 (M+1); HRMS calculated for M+H: C₂₂H₂₄N₉O₂, 446.2047; found 446.2057.

4,6-Bis(2-((E)-cyclohexylmethylene)hydrazinyl)pyrimidin-2-amine (22)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (145 mg, 0.94 mmol) and cyclohexanecarboxaldehyde (264 mg, 0.25 mL, 2.2 eq.) in EtOH (3 mL) was subject to microwave irradiation for 20 minutes at 120 °C. The reaction was concentrated in vacuo before column chromatography (hexanes:EtOAc gradient). The resulting solid was collected and slurried with Et₂O (10 mL) the pyrimidine as an off-white powder. MP 205 °C (Slow Decomp).

¹H NMR (DMSO-d_6) δ 9.86 (s, 2H), 7.18 (d, J=5.7 Hz, 2H), 5.90 (s, 1H), 5.56 (s, 2H), 2.18 (dd, J=10.2, 3.2 Hz, 2H), 1.74–1.60 (m, 10H), 1.34–1.16 (m, 10H). ¹³C NMR (DMSO-d_6) δ 162.8, 162.4, 147.7, 72.6, 40.0, 30.2, 25.5, 25.0; MS: LRMS 343.75; HRMS calculated for M+H: C₁₈H₃₀N₇, 344.2557; found 344.2561.

4,6-Bis(2-((E)-1-(4-chlorophenyl)ethylidene)hydrazinyl)pyrimidin-2-amine (23)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (74 mg, 0.479 mmol) and 4′-chloroacetophenone (0.14 mL, 170 mg, 1.1 mmol, 2.30 eq.) in i-PrOH (10 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate and washing with Et₂O (10 mL) to afford the pyrimidine (83 mg, 40 %) as a tan powder. MP 203 °C (Slow Decomp).

¹H NMR (DMSO-d_6) δ 9.58 (s, 2H), 7.81 (d, J=8.3 Hz, 4H), 7.49 (d, J=8.3 Hz, 4H), 6.44 (s, 1H), 5.74 (s, 2H), 2.27 (s, 6H). ¹³C NMR (DMSO-d_6) δ 163.5, 162.5, 143.3, 137.7, 132.9, 128.3, 127.2, 75.2, 13.0; MS: LRMS 428.0; HRMS calculated for M+H: C₂₀H₂₀Cl₂N₇, 428.1122; found 428.1155.

2,2′-((1E,1′E)-((6-amino-1,3,5-triazine-2,4-diyl)bis(hydrazin-2-yl-1-ylidene))bis(methanylylidene))diphenol (24)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (88 mg, 0.566 mmol) and salicylaldehyde (0.13 mL, 150 mg, 1.2 mmol, 2.12 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate, washing with Et₂O (20 mL), to afford the triazine (100 mg, 48 %) as a white powder. MP 282 °C (Decomp).

¹H NMR (DMSO-d_6) δ 11.43 (s, 2H), 11.05 (s, 2H), 8.31 (s, 2H), 7.42 (s, 2H), 7.29–7.18 (m, 2H), 6.89 (t, J=7.7 Hz, 4H), 6.74 (s, 2H); ¹³C NMR (DMSO-d_6) δ 167.20, 164.23, 157.02, 143.32, 130.24, 129.10, 119.21, 119.12, 116.27; MS: LRMS ESI +ve 365.2 (M+1); HRMS calculated for M+H: C₁₇H₁₇N₈O₂, 365.1469; found 365.1474.

4,6-Bis(2-((E)-1-(4-chlorophenyl)ethylidene)hydrazinyl)-1,3,5-triazin-2-amine (25)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (115 mg, 0.738 mmol) and 4′-chloroacetophenone (0.21 mL, 250 mg, 1.6 mmol, 2.17 eq.) in n-BuOH (10 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate and washing with Et₂O (10 mL) to afford

the triazine (151 mg, 48%) as a pale pink powder. MP 264 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 9.63 (s, 1H), 7.81 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 8.6 Hz, 2H), 6.73 (s, 2H), 2.28 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.9, 165.3, 146.5, 137.6, 133.2, 128.2, 127.8, 13.5; MS: LRMS ESI +ve 429.1 (M+1); HRMS calculated for M+H: $C_{19}H_{19}Cl_2N_8$, 429.1104; found 429.1108.

3,3′-((1E,1′E)-((6-amino-1,3,5-triazine-2,4-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))diphenol (26)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (48 mg, 0.305 mmol) and 3-hydroxybenzaldehyde (161 mg, 1.321 mmol, 4.33 eq.) in EtOH (3 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate, washing with Et$_2$O (20 mL), to afford the triazine (24 mg, 21%) as a white powder. MP 306 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.75 (s, 1H), 9.55 (s, 1H), 8.04 (s, 1H), 7.21 (t, J = 7.8 Hz, 1H), 7.06 (s, 1H), 7.02 (d, J = 7.6 Hz, 1H), 6.84–6.57 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 167.4, 164.6, 157.6, 142.6, 136.4, 129.7, 117.9, 116.4, 112.5; MS: LRMS ESI −ve 363.1 (M-1); HRMS calculated for M+H: $C_{17}H_{17}N_8O_2$, 365.1469; found 365.1472.

4,6-Bis(2-((E)-4-(trifluoromethyl)benzylidene) hydrazinyl)-1,3,5-triazin-2-amine (27)

A suspension of 4,6-dihydrazinyl-1,3,5-triazin-2-amine (186 mg, 1.19 mmol) and 4-trifluoromethylbenzaldehyde (0.36 mL, 2.62 mmol, 2.2 eq.) in EtOH (20 mL) was heated at reflux for 6 h. After cooling, the emulsified mixture was diluted with Et$_2$O (15 mL) absorbed on to silica for column chromatography. Flash chromatography was performed via the reveleris system using a gradient method comprising of 100% DCM to 10% MeOH in DCM. Concentration of the relevant fraction under vacuum gave the triazine (97 mg, 17%) as a white solid. MP 297–300 °C.

^{1}H NMR (DMSO-d_6) δ 11.16 (s, 2H), 8.22 (s, 2H), 7.84–7.79 (m, 8H), 6.96 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.4, 164.7 (2 C), 140.7 (2 C), 139.0 (2 C), 128.7 (q, J = 31.7 Hz, 2 C), 126.9 (4 C), 125.6 (q, J = 4.0 Hz, 4 C), 124.3 (q, J = 272.0 Hz, 2 C).^{19}F NMR (376 MHz, DMSO-d_6) δ −60.96. HRMS calculated for M+H: $C_{19}H_{15}F_6N_8$, 469.1318; found 469.1324.

4,4′-((1E,1′E)-((6-amino-1,3,5-triazine-2,4-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))diphenol (28)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (87 mg, 0.556 mmol) and 4-hydroxybenzaldehyde (190 mg, 1.557 mmol, 2.80 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the precipitate, washing with Et$_2$O (20 mL), to afford the triazine (120 mg, 59%) as a white powder. MP 310 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.56 (s, 1H), 9.78 (s, 1H), 8.02 (s, 1H), 7.45 (d, J = 8.6 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 6.62 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 167.3, 164.5, 158.5, 142.7, 128.0, 126.1, 115.6; MS: LRMS ESI +ve 365.2 (M+1). HRMS calculated for M+H: $C_{17}H_{17}N_8O_2$, 365.1469; found 365.1474.

4,6-Bis(2-((E)-4-bromobenzylidene) hydrazinyl)-1,3,5-triazin-2-amine (29)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (57 mg, 0.368 mmol) and 4-bromobenzaldehyde (175 mg, 0.943 mmol,

2.56 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting precipitate and washing with Et$_2$O (10 mL) to afford the triazine (116 mg, 64%) as a yellow powder. MP 302 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.94 (s, 2H), 8.10 (s, 2H), 7.67–7.60 (m, 4H), 7.61–7.54 (m, 4H), 6.80 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 164.6, 141.1, 134.3, 132.0, 131.7, 128.2, 122.1; MS: LRMS ESI +ve 491 (M+1); HRMS calculated for M+H: $C_{17}H_{15}Br_2N_8$, 488.9781; found 488.9784.

4,6-Bis(2-((E)-benzylidene)hydrazinyl)-1,3,5-triazin-2-amine (30)

A suspension of 4,6-dihydrazinyl-1,3,5-triazin-2-amine (67 mg, 0.430 mmol) and benzaldehyde (0.10 mL, 100 mg, 0.94 mmol, 2.19 eq.) in EtOH (5 mL) was heated at reflux for 6 h. After cooling, the resulting precipitate was filtered, washed with Et$_2$O (2 × 20 mL) to afford the triazine (50 mg, 35%) as a white powder. MP 284–287 °C.

^{1}H NMR (DMSO-d_6) δ 10.84 (s, 1H), 8.14 (s, 1H), 7.63 (d, J = 7.4 Hz, 2H), 7.46–7.33 (m, 3H), 6.76 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 167.4, 164.6, 142.3, 135.1, 129.0, 128.7, 126.4; MS: LRMS ESI +ve 333.2 (M+1); HRMS calculated for M+H: $C_{17}H_{17}N_8$, 333.1571; found 333.1572.

4,6-Bis(2-((E)-cyclohexylmethylene) hydrazinyl)-1,3,5-triazin-2-amine (31)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (59 mg, 0.380 mmol) and cyclohexanecarboxaldehyde (0.10 mL, 93 mg, 0.83 mmol, 2.2 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction was concentrated in vacuo before dilution with Et$_2$O (10 mL) and hexanes (10 mL) and the resulting precipitate was collected afford the triazine (52 mg, 39%) as a white powder. MP 120 °C (Slow Decomp).

^{1}H NMR (DMSO-d_6) δ 10.34 (br s, 2H), 7.27 (d, J = 5.7 Hz, 2H), 6.66 (br s, 2H), 2.15 (br s, 2H), 1.70–1.63 (m, 10H), 1.29–1.17 (m, 10H); ^{13}C NMR (DMSO-d_6) δ 150.27, 150.12, 40.2, 30.2, 25.4, 25.0, 24.5; MS: LRMS ESI +ve 345.3 (M+1); HRMS calculated for M+H: $C_{17}H_{29}N_8$, 345.2510; found 345.2516.

4,6-Bis(2-((E)-4-methylbenzylidene) hydrazinyl)-1,3,5-triazin-2-amine (32)

A suspension of 2-amino-4,6-dihydrazino-1,3,5-triazine (49 mg, 0.311 mmol) and 4-methylbenzaldehyde (0.10 mL, 100 mg, 0.832 mmol, 2.67 eq.) in EtOH (4 mL) was heated at reflux for 16 h. The reaction mixture was cooled to ambient temperature before collecting the yellow precipitate, washing with Et$_2$O (20 mL) to afford the pyrimidine (67 mg, 60%) as a yellow powder. MP 318 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.75 (s, 1H), 8.09 (s, 1H), 7.52 (d, J = 8.1 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 6.71 (s, 1H), 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.4, 164.6, 142.4, 138.6, 132.4, 129.3, 126.4, 21.0; MS: LRMS ESI +ve 361.2 (M+1); HRMS calculated for M+H: $C_{19}H_{21}N_8$, 361.1884; found 361.1887.

4-((E)-(2-(2-amino-6-(2-((E)-4-((diethoxyphosphoryl)oxy) benzylidene)hydrazinyl)pyrimidin-4-yl)hydrazono)methyl) phenyl diethyl phosphate (33)

Diethyl (4-formylphenyl) phosphate: To a stirring suspension of 4-hydroxybenzaldehyde (412 mg, 3.372 mmol) in CH$_2$Cl$_2$ (10 mL) was

added diethyl phosphorochloridate (0.53 mL, 640 mg, 3.7 mmol, 1.1 eq.) followed by triethylamine (0.50 mL, 360 mg, 3.6 mmol, 1.1 eq.). The solution as stirred at ambient temperature for 16 h before being diluted with H_2O (10 mL), 1 M NaOH (10 mL) and CH_2Cl_2 (30 mL). The organics were partitioned and washed with 1 M HCl (20 mL) and 1 M NaOH (20 mL) before drying over $MgSO_4$ and concentrating in vacuo to afford the phosphate ester (555 mg, 64%) as a colourless oil.

To a suspension of 2-amino-4,6-dihydrazinopyrimidine (151 mg, 0.9729 mmol) in THF (16 mL) was added diethyl (4-formylphenyl) phosphate (555 mg, 2.148 mmol, 2.21 eq.) and the solution heated at reflux for 48 h. The cooled reaction mixture was filtered to remove unreacted 2-amino-4,6-dihydrazinopyrimidine and resulting filtrate was concentrated over a stream of compressed air. The resulting crude material was triturated with EtOAc (10 mL) to afford the pyrimidinyl phosphate (117 mg, 19%) as an orange/brown powder. MP 235 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.81 (s, 2H), 8.06 (s, 2H), 7.71 (d, $J = 8.5$ Hz, 4H), 7.29 (d, $J = 8.1$ Hz, 4H), 6.24 (s, 1H), 4.17 (dq, $J = 14.2$, 7.1 Hz, 8H), 1.28 (td, $J = 7.1$, 0.6 Hz, 12H).; ^{13}C NMR (DMSO-d_6) δ 150.7, 139.7, 127.8, 120.4, 120.4, 73.1, 64.44, 64.38, 15.93, 15.87; ^{31}P NMR (162 MHz, DMSO-d_6) δ −6.54. MS: LRMS ESI +ve 636.3 (M+1); HRMS calculated for M+H: $C_{26}H_{36}N_7O_8P_2$, 636.2095; found 636.2100.

4,6-bis(2-((E)-3,4-difluorobenzylidene)hydrazinyl) pyrimidin-2-amine (34)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (127 mg, 0.8178 mmol) and 3,4-difluorobenzaldehyde (0.19 mL, 240 mg, 1.7 mmol, 2.08 eq.) in EtOH (11 mL) was heated at reflux for 16 h. After cooling, the reaction precipitate was collected and washed with Et_2O (15 mL) to afford the pyrimidine (144 mg, 44%) as a brown powder. MP 261 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.76 (s, 2H), 7.99 (s, 2H), 7.70 (td, $J = 1.7$, 9.2 Hz, 2H), 7.54–7.41 (m, 4H), 6.30 (s, 1H), 5.87 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.8, 162.6, 149.9 (dd, $J = 247.9$, 15.3 Hz), 149.6 (dd, $J = 249.6$, 14.8 Hz), 137.6, 133.2 (dd, $J = 5.7$, 4.0 Hz), 123.2 (dd, $J = 5.5$, 3.6 Hz), 117.9 (dd, $J = 14.7$, 3.1 Hz), 114.0 (d, $J = 16.8$ Hz), 73.7; ^{19}F NMR (376 MHz, DMSO-d_6) δ −138.16 (q, J = 22.1 Hz). MS: LRMS 403.6; HRMS calculated for M+H: $C_{18}H_{14}F_4N_7$, 404.1241; found 404.1246.

2,2′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis (benzene-1,4-diol) (35)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (137 mg, 0.8797 mmol) and 2,5-dihydroxybenzaldehyde (278 mg, 2.012 mmol, 2.29 eq.) in EtOH (10 mL) was heated at reflux for 16 h. The resulting suspension was filtered hot and the precipitate washed with Et_2O (20 mL) to afford the bis-hydrazone (180 mg, 50%) as a yellow powder. MP 279 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.61 (s, 2H), 9.78 (s, 2H), 8.84 (s, 2H), 8.19 (s, 2H), 6.92 (d, $J = 2.7$ Hz, 2H), 6.71 (d, $J = 8.7$ Hz, 2H), 6.64 (dd, $J = 8.7$, 2.8 Hz, 2H), 5.91 (s, 1H), 5.84 (s, 2H) ^{13}C NMR (DMSO-d_6) δ 162.7, 162.2, 149.9, 149.1, 140.2, 120.4, 117.4, 116.7, 112.6, 72.7; MS: LRMS ESI +ve 396.2 (M+1); HRMS calculated for M+H: $C_{18}H_{18}N_7O_4$, 396.1415; found 396.1419.

4,4′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis (benzene-1,2-diol) (36)

2-Amino-4,6-dihydrazinopyrimidine (122 mg, 0.7843 mmol) and 3,4-dihydroxybenzaldehyde (253 mg, 1.828 mmol, 2.32 eq.) were suspended in EtOH (10 mL) and heated at reflux for 16 h. The resulting solution was concentrated to ca 5 mL by boiling at ambient pressure and the resulting precipitate was filtered hot, washing with Et_2O (20 mL) to afford the bis-hydrazone (725 mg, 71%) as a tan powder. MP > 400 °C (Discolours to Black).

^{1}H NMR (DMSO-d_6) δ 10.25 (s, 2H), 9.18 (s, 4H), 7.87 (s, 2H), 7.11 (d, $J = 1.4$ Hz, 2H), 6.90 (dd, $J = 8.1$, 1.4 Hz, 2H), 6.78 (d, $J = 8.1$ Hz, 2H), 6.14 (s, 1H), 5.69 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.64, 162.61, 146.8, 145.6, 141.2, 126.6, 118.9, 115.8, 112.7, 72.8; MS: LRMS 395.85; HRMS calculated for M+H: $C_{18}H_{18}N_7O_4$, 396.1415; found 396.1416.

3,3′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis (benzene-1,2-diol) (37)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (200 mg, 1.286 mmol) and 2,3-dihydroxybenzaldehyde (516 mg, 3.738 mmol, 2.91 eq.) in EtOH (10 mL) was heated at reflux for 16 h. The resulting yellow precipitate was collected and washed with Et_2O (20 mL) to afford the bis-hydrazone (405 mg, 61%) as a yellow powder. MP 256 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.67 (s, 2H), 9.96 (s, 2H), 9.28 (s, 2H), 8.26 (s, 2H), 7.00 (d, $J = 7.3$ Hz, 2H), 6.84–6.75 (m, 2H), 6.71 (t, $J = 7.8$ Hz, 2H), 5.94 (s, 1H), 5.84 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.2, 145.5, 144.8, 140. 7, 120.6, 119.2, 118.0, 116.0, 72.8; MS: LRMS ESI +ve 396.2 (M+1); HRMS calculated for M+H: $C_{18}H_{18}N_7O_4$, 396.1415; found 396.1421.

5,5′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis (benzene-1,2,3-triol) (38)

2-Amino-4,6-dihydrazinopyrimidine (371 mg, 2.387 mmol) and 3,4,5-trihydroxybenzaldehyde (939 mg, 6.093 mmol, 2.55 eq.) were suspended in EtOH (20 mL) and heated at reflux for 16 h. The resulting suspension was filtered hot, washing with Et_2O (50 mL) to afford the bis-hydrazone (725 mg, 71%) as a pale yellow powder. > 400 °C (Discolours to Black).

^{1}H NMR (DMSO-d_6) δ 10.20 (s, 2H), 9.00 (s, 4H), 8.43 (s, 2H), 7.80 (s, 2H), 6.59 (s, 4H), 6.07 (s, 1H), 5.67 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.4, 146.2, 141.7, 134.7, 125.5, 105.5, 72.8.MS: LRMS ESI + ve 428.2 (M+1); HRMS calculated for M+H: $C_{18}H_{18}N_7O_6$, 428.1313; found 428.1319.

4,4′-((1E,1′E)-((2-aminopyrimidine-4,6-diyl)bis (hydrazin-2-yl-1-ylidene))bis(methanylylidene))bis (benzene-1,3-diol) (39)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (301 mg, 1.940 mmol) and 2,4-dihydroxybenzaldehyde (601 mg, 4.350 mmol, 2.24 eq.) in EtOH (5.2 mL) was heated at reflux for 16 h. The resulting precipitate was collected, washed with EtOH (10 mL) and Et_2O (25 mL) and dried to afford the bis-hydrazone (450 mg, 59%) as a tan powder. MP 270 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.47 (s, 4H), 9.81 (s, 2H), 8.14 (s, 2H), 7.29 (d, $J = 8.4$ Hz, 2H), 6.40–6.24 (m, 4H), 5.78 (s, 3H); ^{13}C NMR (DMSO-d_6) δ

162. 7, 162.1, 159.6, 158.0, 141.6, 129.3, 111.8, 107.6, 102.6, 72.1. MS: LRMS ESI + ve 396.2 (M + 1); HRMS calculated for M + H: $C_{18}H_{18}N_7O_4$, 396.1415; found 396.1420.

4,6-Bis(2-((E)-naphthalen-1-ylmethylene)hydrazinyl) pyrimidin-2-amine (40)

2-Amino-4,6-dihydrazinopyrimidine (104 mg, 0.6715 mmol) and 1-naphthaldehyde (0.20 mL, 230 mg, 1.5 mmol, 2.2 eq.) were suspended in EtOH (10 mL) and heated at reflux for 3 h. The resulting suspension was filtered hot and the resulting material washed with Et$_2$O (20 mL) to afford the bis-hydrazone (83 mg, 29 %) as a white powder. MP 246 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.75 (s, 2H), 8.83–8.78 (m, 2H), 8.74 (s, 2H), 8.05–8.00 (m, 2H), 7.98 (d, J = 8.2 Hz, 2H), 7.92 (d, J = 7.0 Hz, 2H), 7.67–7.53 (m, 6H), 6.51 (s, 1H), 5.92 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.9, 162.7, 139.7, 133.7, 130.5, 129.9, 129.3, 128.8, 126.9, 126.1, 126.0, 125.6, 124.0, 73.5; MS: LRMS ESI + ve 432.2 (M + 1); HRMS calculated for M + H: $C_{26}H_{22}N_7$, 432.1931; found 432.1936.

4,6-bis(2-((E)-naphthalen-2-ylmethylene)hydrazinyl) pyrimidin-2-amine (41)

2-Amino-4,6-dihydrazinopyrimidine (170 mg, 1.094 mmol) and 2-naphthaldehyde (382 mg, 2.447 mmol, 2.24 eq.) were suspended in EtOH (7.5 mL) and heated at reflux for 16 h. The resulting suspension was diluted with EtOH (10 mL), boiled and filtered hot. The resulting material was slurried with EtOH (2 × 10 mL), washed with Et$_2$O (2 × 20 mL) to afford the bis-hydrazone (285.8 mg, 61 %) as a white powder. MP 255 °C (decomp).

^{1}H NMR (DMSO-d_6) δ 10.78 (s, 2H), 8.23 (s, 2H), 8.10–7.87 (m, 10H), 7.56 (s, 4H), 6.46 (s, 1H), 5.88 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.8, 162.7, 140.1, 133.2, 133.1, 133.0, 128.5, 128.1, 127.8, 127.0, 126.7, 126.5, 122.3, 73.6; MS: LRMS ESI + ve 432.2 (M + 1); HRMS calculated for M + H: $C_{26}H_{22}N_7$, 432.1931; found 432.1934.

4,6-Bis(2-((1E,2E)-3-phenylallylidene)hydrazinyl) pyrimidin-2-amine (42)

2-Amino-4,6-dihydrazinopyrimidine (104 mg, 0.6715 mmol) and cinnamaldehyde (0.15 mL, 160 mg, 1.2 mmol, 1.8 eq.) were suspended in EtOH (10 mL) and heated at reflux for 3 h. The resulting suspension was filtered hot and the resulting material washed with Et$_2$O (10 mL) to afford the bis-hydrazone (33 mg, 16 %) as a tan crystalline powder. MP 258 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.55 (s, 2H), 7.87 (d, J = 9.2 Hz, 2H), 7.59 (d, J = 7.4 Hz, 4H), 7.37 (t, J = 7.5 Hz, 4H), 7.29 (t, J = 7.3 Hz, 2H), 7.01 (dd, J = 16.1, 9.3 Hz, 2H), 6.84 (d, J = 16.0 Hz, 2H), 6.13 (s, 1H), 5.78 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.5, 142.7, 136.4, 135.2, 128.8, 128.2, 126.7, 126.0, 73.4; MS: LRMS ESI + ve 384.2 (M + 1); HRMS calculated for M + H: $C_{22}H_{22}N_7$, 384.1931; found 384.1939.

4,6-Bis(2-((E)-3-phenylpropylidene)hydrazinyl) pyrimidin-2-amine (43)

A suspension of 2-amino-4,6-dihydrazinopyrimidine (226 mg, 1.457 mmol) and 3-phenylpropionaldehyde (444 mg, 3.305 mmol, 2.27 eq.) in EtOH (10 mL) was heated at reflux for 16 h. The resulting solution was concentrated to ca. 3.5 mL at ambient pressure and stood at ambient temperature for 16 h to afford a suspension. This suspension was collected, washing with ice cold

Et$_2$O (25 mL) to afford the bis-hydrazone (147 mg, 26 %) as a tan powder. MP 160–161 °C

^{1}H NMR (DMSO-d_6) δ 9.98 (s, 2H), 7.42–7.07 (m, 12H), 5.97 (d, J = 25.2 Hz, 1H), 5.59 (s, 2H), 2.79 (t, J = 7.6 Hz, 4H), 2.57–2.44 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.5, 143.1, 141.2, 128.4, 128.3, 125.9, 72.6, 33.6, 32.4; MS: LRMS ESI + ve 388.2 (M + 1); HRMS calculated for M + H: $C_{22}H_{26}N_7$, 388.2244; found 388.2251.

4,6-Bis(2-((E)-pyridin-3-ylmethylene)hydrazinyl) pyrimidin-2-amine (44)

To a suspension of 2-amino-4,6-dihydrazinopyrimidine (208 mg, 1.339 mmol) in EtOH (50 mL) was added 3-pyrimidinecarbaldehyde (0.30 mL, 340 mg, 3.2 mmol, 2.4 eq.) and the solution heated at reflux for 16 h. The resulting suspension was filtered hot, washing with Et$_2$O (20 mL) to afford the bis-hydrazone (111 mg, 25 %) an off-white powder. MP 280 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.82 (s, 2H), 8.80 (d, J = 1.6 Hz, 2H), 8.54 (dd, J = 4.7, 1.5 Hz, 2H), 8.11–8.03 (m, 4H), 7.46 (dd, J = 7.9, 4.8 Hz, 2H), 6.33 (s, 1H), 5.91 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.6, 149.4, 147.7, 137.2, 132.6, 131.0, 124.0, 73.6; MS: LRMS 333.7; HRMS calculated for M + H: $C_{16}H_{16}N_9$, 334.1523; found 334.1530.

4,6-Bis(2-((E)-pyridin-2-ylmethylene)hydrazinyl) pyrimidin-2-amine (45)

To a suspension of 2-amino-4,6-dihydrazinopyrimidine (259 mg, 1.670 mmol) in EtOH (50 mL) was added 2-pyridinecarboxaldehyde (0.40 mL, 450 mg, 4.2 mmol, 2.5 eq.) and the solution heated at reflux for 16 h. The resulting suspension was filtered hot and the precipitate washed with Et$_2$O (20 mL) to afford the bis-hydrzone (197 mg, 35 %) as a bright yellow powder. MP 260 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.92 (s, 2H), 8.56 (s, 2H), 8.10 (s, 2H), 7.91 (dd, J = 26.3, 6.7 Hz, 4H), 7.33 (s, 2H), 6.36 (s, 1H), 5.95 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.72, 162.68, 154.0, 149.4, 140.8, 136.7, 123.2, 119.1, 73.7; MS: LRMS 333.8; HRMS calculated for M + H: $C_{16}H_{16}N_9$, 334.1523; found 334.1528.

4,6-Bis(2-((E)-pyridin-4-ylmethylene)hydrazinyl) pyrimidin-2-amine (46)

To a suspension of 2-amino-4,6-dihydrazinopyrimidine (355 mg, 2.288 mmol) in EtOH (50 mL) was added 4-pyridinecarboxaldehyde (0.50 mL, 570 mg, 5.3 mmol, 2.3 eq.) and the solution heated at reflux for 16 h. The resulting suspension was filtered hot and the precipitate washed with Et$_2$O (20 mL) to afford the bis-hydrazone (279 mg, 37 %) as a yellow powder. 295 °C (decomp).

^{1}H NMR (DMSO-d_6) δ 11.03 (s, 2H), 8.62 (d, J = 3.3 Hz, 4H), 8.01 (s, 2H), 7.60 (d, J = 3.2 Hz, 4H), 6.38 (s, 1H), 6.00 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.7, 150.2, 142.2, 137.5, 120.2, 74.0; MS: LRMS 333.7; HRMS calculated for M + H: $C_{16}H_{16}N_9$, 334.1523; found 334.1527.

4,6-Bis(2-((E)-(6-chloropyridin-3-yl)methylene)hydrazinyl) pyrimidin-2-amine (47)

To a suspension of 2-amino-4,6-dihydrazinopyrimidine (266 mg, 1.711 mmol) in EtOH (10 mL) was added 2-chloro-5-pyridinecarbaldehyde (581 mg, 4.101 mmol, 2.40 eq.) and the suspension heated at reflux for 16 h. The resulting suspension was filtered hot, washing with ice cold EtOH (10 mL) and Et$_2$O (20 mL) before drying

to afford the hydrazone (414 mg, 60 %) as an off white powder. MP 243 °C (Decomp).

^{1}H NMR (DMSO-d_6) δ 10.92 (s, 2H), 8.61 (d, $J = 1.7$ Hz, 2H), 8.14 (dd, $J = 8.3$, 2.0 Hz, 2H), 8.05 (s, 2H), 7.59 (d, $J = 8.3$ Hz, 2H), 6.30 (s, 1H), 5.93 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 162.7, 162.6, 149.7, 147.9, 135.9, 130.6, 124.6, 73.6; MS: LRMS 402; HRMS calculated for M + H: $C_{16}H_{14}Cl_2N_9$, 402.0744; found 402.0750.

Conflict of Interest

Dr Stephen Page is Director of Neoculi Pty Ltd who funded this study

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