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Novel Pyridopyrazine and Pyrimidothiazine Derivatives as FtsZ Inhibitors

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Abstract

A series of pyridopyrazine and pyrimidothiazine derivatives have been synthesized and their activity against FtsZ from *M. tuberculosis* (Mtb) and *in vitro* antibacterial activity against Mtb H₃₇Ra and Mtb H₃₇Rv are reported. Certain analogs described herein showed moderate to good inhibitory activity.

Keywords

Pyridopyrazine; Pyrimidothiazine; FtsZ inhibitor; Antitubercular

1. Introduction

Tuberculosis remains a high priority public health threat throughout the world, particularly in developing nations. The increasing prevalence of drug resistant strains of *Mycobacterium tuberculosis* from single (SDR) to multiple (MDR), extensively (XDR) and even totally (TDR) drug resistant strains underscores concerns within the public health network of the developed world and emphasizes the critical need for heightened disease surveillance and increased basic research, particularly into pathogen biology and new drug targets as well as greater emphasis on new drug discovery with the goal of developing more effective combination treatments.^{1,2}

Over the past decade, FtsZ (Filament temperature sensitive protein Z), the prokaryotic analog of tubulin, has received considerable attention as a new antibacterial drug target. This protein plays an essential role in bacterial cell division, and interruption of this process is a bactericidal event. Several inhibitors have been reported.^{3–10} Among these, an anti-malarial precursor 2-alkoxycarbonylaminopyridine, **1** (Fig. 1), was found to be a potent inhibitor of Mtb growth as well as selectively inhibiting *in vitro* polymerization of FtsZ relative to tubulin.^{11–13} A close pyrimidine analog **2** (Fig. 1) of the 2-alkoxycarbonylaminopyridines

has also been reported as an inhibitor of FtsZ polymerization, equipotent to **1** but less effective against Mtb growth *in vitro*.¹²

Herein, we report the synthesis of several pyridopyrazine and pyrimidothiazine analogs in order to further develop a structure–activity relationship (SAR) for the series against FtsZ with the eventual goal of improving potency against the target, increasing whole cell antibacterial activity, and reducing off target toxicity.

2. Chemistry

Lead compounds **1** and **2** were prepared as reported and were analytically identical with the original authentic samples.^{12,13} Synthetically, **6–10** and **17–25** are accessible through one common key intermediate **3**.¹⁴ The synthetic route for **6–10** and **17–25** is outlined in Scheme 1.

The pyridopyrazine analogs **6–10** were prepared by the displacement of the 4-chloro group of **3** with 2-amino-5-diethylaminopentane to form **4**.¹³ Reduction of the 5-nitro group of **4** yielded **5**, and direct coupling with different diketones gave the final targets **6–10** in good yields. Similarly for the synthesis of targets **17–25**, the displacement of the 4-chloro group of **3** with different amino compounds afforded **11–13**, and reduction of the 5-nitro group using Raney nickel and H₂ at atmospheric pressure and room temperature gave **14–16** in quantitative yields. Final coupling with benzil, 2,2'-thenil or furil provided the targets **17–25** in good yields.

The carbamate groups in **6** and **7** were hydrolyzed by treatment with KOH in ethanol under reflux to give **26** and **27** respectively in moderate yields (Scheme 2).¹⁴

The synthetic route for **37** and **38** is outlined in Scheme 3. Commercially available 2-amino-4,6-dichloropyrimidine was refluxed with oxalyl chloride in benzene followed by addition of ethyl alcohol to provide 4,6-dichloro-2-pyrimidyl urethane (**28**).^{12,15} Compound **28** was refluxed with either 2-amino-5-diethylaminopentane to yield **29** or 4-diethylaminobutylamine to give **30**, in quantitative yields. Compounds **29** and **30** were nitrated with sulfuric acid (conc.) and nitric acid (fum.) at 35 °C to provide the 5-nitropyrimidines (**31** and **32**).^{12,16} Displacement of the 4-chloro group in **31** and **32** by potassium thioacetate under reflux gave **33** and **34**.¹⁷ Reduction to the 5-aminopyrimidines **35** and **36** by refluxing with zinc in acetic acid at 80 °C followed by condensation with desyl chloride in the presence of sodium acetate under nitrogen atmosphere gave the target thiazines **37** and **38** respectively.¹⁷

3. Results and Discussion

3.1. In vitro cell studies

The minimum inhibitory concentration (MIC, the lowest concentration that completely inhibits growth) of all compounds for Mtb H₃₇Ra were determined using a colorimetric (Alamar blue) microdilution broth assay reported previously.¹¹ The compounds were also screened against Mtb H₃₇Rv to determine the IC₉₀ (the concentration that inhibits 90% of growth) as described previously and Vero cell cytotoxicity (CC₅₀).¹⁸ All the compounds were also examined for their ability to inhibit the target, Mtb FtsZ, and its mammalian homolog tubulin (reported as IC₅₀ values) as described previously.¹¹ Screening data are given in Table 1.

SAR substitutions were driven by screening results of available pyridopyrazines from the Southern Research repository versus Mtb H₃₇Rv and comparison to published anticancer and tubulin screening results.^{11,19} Through our initial comparison, it was clear that four

positions altered antitubercular and FtsZ selectivity versus Vero cells, mammalian cancer cells, and tubulin – see Figure 2.

Structure **39** represents the typical pyridopyrazine that would inhibit both mammalian cancer cells and tubulin as described by Temple.¹⁹ Certain features are important, if not crucial to anticancer and antimitotic activity, including a carbamate at R₁, smaller linear alkyl functions at R₂ (acyl and larger heteroalkyl are not tolerated), aryl functions (typically phenyl) at R₃, and smaller alkyl functions at R₄ and/or R_{4'}. Significant anticancer activity resides with the fused pyrazine systems (X₃ = N; O or S not tolerated), although increasing (diazepine) or decreasing (imidazole) the size of this fused pyrazine ring can also impact anticancer and tubulin activity with the best inhibitors in the pyridodiazepine systems.²⁰ Certain of these systems show significant antitubercular activity and select examples also can impact FtsZ polymerization *in vitro* (reference 11 and unpublished results). Beyond these overlaps in activity, there are clear alterations that significantly reduce tubulin and anticancer activity in the series including larger (alkyl or aryl) substituents in the carbamate group (R₁), sterically demanding R₂ groups (diphenylmethyl), as well as aromatization of the pyridopyrazine and inclusion of larger aryl functions at R₄ (see structures **1** and **2** and general structure **40** and discussion in reference 11). Thus, we chose to examine certain relatively facile substitutions that would scan the SAR pattern in these regions of structure **40** in order to optimize whole cell activity and FtsZ/tubulin selectivity.

Hence, we initially explored the SAR of compound **1** at the 6,7 positions with heterocycles such as furan, thiophene and pyridine, and small alkyl groups such as methyl and ethyl (**6–10**). These analogs were designed to evaluate the role of hydrophobic and steric differences in antitubercular activity. Among these five compounds, compound **6** with two furan-2-yl rings at the 6,7-positions of **1** was found to be modestly more FtsZ active than **1**. The MIC of **6** (3.6 μM) for H₃₇Ra was eightfold greater than the MIC of **1** (0.47 μM), although **6** appeared less active versus the virulent strain H₃₇Rv than the lead sample **1**. The thiophene-2-yl analog **7**, in contrast, had activity very similar to **1** in all the screening assays. The pyridine analog **8** was less active than **1** against H₃₇Ra and H₃₇Rv but showed good activity for FtsZ (ID₅₀ of 45.7 μM), suggesting that the more polar analog may have cell wall permeability issues. The less hydrophobic compounds **9** and **10**, with methyl and ethyl groups at the 6,7-positions respectively, were inactive against FtsZ and both bacterial strains *in vitro*.

As previously discussed, the C-4 position is a critical area for modification in terms of activity and selectivity. While a diphenylmethyl substitution at the 4-position of the bicyclic system showed significant and selective antitubercular and FtsZ activity in earlier screens, there were concerns regarding the medicinal properties of such hydrophobic and high molecular weight analogs. Hence, we chose to focus efforts on the 4-diamino alkyl substituted series that also showed relatively high activity and selectivity in our screens. Initially, efforts centered on the active 6,7-disubstituted phenyl, furan and thiophene scaffolds, and we explored variations in chain length [-N-(CH₂)₂-N versus -N-(CH₂)₄-N] and removal of the branched methyl group to ascertain the effects of this stereocenter on activity. Additionally, the terminal dialkylamino group was substituted with either a dimethyl or a diethyl substituent in these analogs. Compounds **17–25**, with N,N-diethylaminobutylamino group (**17–19** – these analogs lack chirality and the Me group), N,N-diethylaminoethylamino group (**20–22** – these analogs are shorter chain species and lack chirality) and N,N-dimethylaminoethylamino group (**23–25** – shorter chain analogs lacking chirality and substituting a dimethylamino for a diethylamino group in the lead compounds) at the 4-position, had activity against H₃₇Ra and H₃₇Rv similar to that of **1**, **6** and **7** with the exception of **18** and **19**, which were inactive against H₃₇Rv. Compounds **1** and **6** compare to analogs **17** and **19** respectively, which are basically the same compounds

minus the branched methyl group that produces a stereocenter in compounds **1** and **6**. In both cases it is notable that the two samples (**17** and **19**) are relatively comparable in all screens relative to the parent samples (**1** and **6**), although **19** is relatively less active versus avirulent Mtb and inactive versus Mtb H₃₇Rv. More interesting is the fact that both **17** and **19** show modest activity in the tubulin polymerization assay, possibly suggesting that the methyl group may play a role in selectivity against FtsZ. It is also interesting that the comparable disubstituted thiophen-2-yl analog **18** is significantly less potent than the methyl containing parent **7** in all screens as well as the tubulin polymerization assay. Compounds **20–22**, containing the shorter C-2 side chains all retained FtsZ polymerization activity, although only **21** and **22** were equipotent with the parent 6,7-difuran-2-yl and 6,7-dithiophen-2-yl analogs, suggesting that shorter chain analogs can, depending on other substitutions on the heterobicycle, retain target activity. It is notable that **20–22** all retain significant antitubercular activity, although **22** was virtually inactive against Mtb H₃₇Rv. Compounds **23–25** contained the C-2 -N-(CH₂)₂-NMe₂ and all samples showed relatively similar activity to **20–22** with this modest structural alteration. It is notable that **20–25** with the shortened C-2 side chain did not show any tubulin polymerization activity up to 100 μ M regardless of the fact that they did not contain the stereocenter of the lead compounds, giving synthetic options in designing inhibitors that do not have chirality/separation issues.

We also prepared a small set of 2-NH₂ analogs (**26–27**) in order to probe the requirement for a carbamate at the 2-position. These samples are comparable to the 6,7-difuran-2-yl and 6,7-dithiophen-2-yl analogs, **6** and **7** respectively. Compounds **26** and **27**, formed by the hydrolysis of **6** and **7**, were relatively comparable to **6** and **7** for FtsZ polymerization inhibition with IC₅₀ values of 18.6 and 29.5 μ M respectively. Neither of these samples showed tubulin polymerization activity, and both showed reduced activity in the whole bacterial growth assays, possibly a result of modestly reduced lipophilicity and reduced passive transport through the bacterial cell wall.

Finally, a modest set of two analogs were prepared to study the effect of substituting a thiazine ring for the pyrazine ring in **2**. These analogs were targeted to determine if insertion of sulfur for nitrogen would enhance target binding through an increase in the S-C bond lengths and alteration of planarity of the aromatic ring; such effects have been observed in tubulin binding with the deazapteridines.¹⁹ Furthermore, certain pyridodiazepines showed significant tubulin and cancer activity, and it was hypothesized by Temple that the larger non-planar diazepine ring allowed better interaction of the 6-Ph group with contact points in the tubulin structure. These analogs also showed significant antitubercular activity and inhibition of FtsZ polymerization.¹¹ Hence, we decided to prepare the S (thiazine) for N (pyrazine) substitution to determine the effects of interrupting the aromaticity of the pyrazine ring by adding the larger S atom that cannot participate equivalently with the trivalent N atom in the pyrazine π -system; it would be expected that this substitution might allow the proposed puckering of the thiazine ring resulting in the 6-Ph being out of plane and altering both the tubulin and possibly the FtsZ interaction. Both samples contain chiral centers; **37** has two stereocenters while **38** contains one chiral center at position 7. The MIC of **37** and **38** for Mtb H₃₇Ra were 49 μ M and 52 μ M, respectively. Compared to the MIC (H₃₇Ra) reported previously for **2** (3.8 μ M),¹² the MIC of **37** and **38** were 16-fold higher. Compounds **37** and **38** displayed IC₉₀ values of 21.7 and 18.5 μ M against H₃₇Rv, 10-fold less active than the lead compound **2**. In contrast, **37** and **38** were only slightly less potent than **2** in their inhibition of FtsZ polymerization (ID₅₀ of 46 μ M for both **37** and **38** compared to 38 μ M for **2**). It is notable that **38** lacks the Me group that generates a chiral center in the C-2 side chain, and that sample showed significant tubulin activity as was noted with other similar samples (**17** and **19**) that lacked the Me appendage at this position again suggesting that the Me group at this point may play some role in selectivity for FtsZ inhibition. Ideally, further pursuit of many of these structures, including both **37** and **38**,

would require separation of complex mixtures of stereoisomers in order to obtain conclusive SAR comparisons, and the relatively active shorter chain (and achiral) analogs at C-2 are attractive in that regard. A mammalian cell cytotoxicity screen was also run in order to assess relative selectivity of the reported compounds for bacterial inhibition. While there are interesting data points suggesting that FtsZ can be inhibited while not impacting tubulin polymerization, for the most part compounds that show antibacterial activity are also cytotoxic to Vero cells; this result lends further credence to the hypothesis that there are additional, off target activities of the class.

In summary, we report herein further SAR analysis of the pyridopyrazine lead series that shows significant antitubercular activity and inhibition of the novel target FtsZ.

3.2. In vivo animal studies

Concurrently with the SAR studies, both compounds **1** and **7** were selected for further evaluation *in vivo* in order to determine cytotoxicity and efficacy in a murine Mtb model to evaluate these scaffolds as further candidates for antitubercular drug discovery against FtsZ. The toxicity of compounds was studied in an acute maximum tolerated dose C57BL/6 mouse model (MTD) to evaluate toxicity and determine dose for safe use in mouse studies. Mice were administered escalation doses up to 300 mg/kg for 3 days daily by gavage with the compounds formulated in 0.5% methyl cellulose, and dosages were scaled back if any mortality or adverse effects were observed. Once an acceptable MTD value was determined, the compounds were tested for *in vivo* efficacy in a short term mouse *M. tuberculosis* model. This model uses the Interferon- γ gene-disrupted C57BL/6 mice (GKO) and was developed and extensively tested.²¹ Without the protective IFN- γ gene, these mice are highly susceptible to the *M. tuberculosis* infection and, therefore, the activity of a compound can be seen rapidly when compared to untreated controls. This sensitive mouse model requires only 9 days of treatment and a small number of animals, which makes it an ideal model for first line testing of TB compounds. The compounds were formulated in 0.5% methyl cellulose and administered daily by gavage. The viable counts were converted to logarithms, which were then evaluated by a one-way analysis of variance, followed by a multiple comparison analysis of variance by a one-way Tukey test (SigmaStat software program). Differences were considered significant at the 95% level of confidence. The lead compound of the pteridine series, compound **1**, was initially evaluated for *in vivo* toxicity using an acute toxicity mouse model. This compound showed lethality at 300 mg/kg and significant adverse effects at 100 and 30 mg/kg. The dosage selected for further *in vivo* efficacy studies of compounds in this series was therefore selected to be 10 mg/kg. In the short term GKO mouse model, **1** showed significant efficacy in the lungs by reducing the bacterial load by 0.86 Log 10CFU ($P < 0.05$) whereas the compound did not show any significant activity in the spleen (0.5 log 10 CFU reduction, $P > 0.05$). For compound **7**, there was no *in vivo* activity observed in lungs as well as spleens in the GKO mouse model after 9 days of treatment at 10 mg/kg (reduction of 0.51 Log10CFU in lungs, and 0.58 Log10CFU in spleens) ($P > 0.05$).

In conclusion, compound **1** showed efficacy at a very low dose of 10 mg/kg, which shows that the series might show some promise if the toxicity issue could be resolved. We have, however, deemphasized this class of compounds due to concern with the significant toxicity of the pyridopyrazine leads in the animal models. This fact, coupled with the relative lack of activity against tubulin suggests that there may be off target activities of the class that have yet to be determined. Furthermore, the relative difficulty of preparation, the high molecular weights of the active products and the poor dynamic range in the FtsZ SAR have led us to pursue other FtsZ inhibitors discovered through our screening programs, and these will be reported in due course.

4. Experimental

Anhydrous solvents and reagents from Aldrich were used without further drying. Reactions were monitored by thin-layer chromatography (TLC) on precoated E. Merck silica gel (60F254) plates (0.25 mm) and visualized using UV light (254 nm). Flash chromatography was carried out on Fischer silica gel G 60 (230–400 mesh). Melting points, determined with a Mel-Temp II capillary melting points apparatus, are uncorrected. ¹H NMR spectra were recorded on a Nicolet NT 300NB instrument at 300 MHz. The coupling constants (*J*) are reported in hertz, and chemical shifts are reported in ppm (δ) relative to residual solvent peak or internal standard. ESI-MS spectra were recorded on a BioTof-2 time-of-flight mass spectrometer. Mass spectra (HRMS) were recorded on a Agilent 6210 LCMS-TOF instrument. The purity of the synthesized compounds was determined on a Agilent 1100 hplc instrument using gradient system on a vydac column.

Ethyl-6-amino-4-[[4-(diethylamino)-butyl]-amino]-5-nitro-2-pyridinecarbamate hydrochloride (**11**)

4-Diethylaminobutylamine (91 mg, 0.63 mmol) was added to a stirred solution of **3** (150 mg, 0.57 mmol) in dry ethanol (5 mL) and the resulting solution was refluxed under nitrogen atmosphere. Progress of the reaction was monitored by TLC. After the complete consumption of the starting material **3**, the reaction mixture was cooled to room temperature and evaporated to dryness under reduced pressure. Diethyl ether was added drop wise to the residue and it was refrigerated for crystallization. The resulting solid was filtered and washed with ether (2 $\times$ 1 mL) and chilled ethanol (2 $\times$ 1 mL) to give **11** (110 mg, 47%) as a yellow solid. M.P. 178–180 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.38. ¹H NMR (300 MHz, CDCl₃): δ 9.18–9.10 (m, 1H, NH), 7.02 (s, 1H, NH), 6.76 (s, 1H, 5-H), 4.22 (q, 2H, *J* = 7.2 Hz, OCH₂), 3.38 (dd, 2H, *J* = 6.9 Hz, 12.6 Hz, NH-CH₂), 3.17–2.97 (m, 6H, 3 $\times$ NCH₂), 2.00–1.95 (m, 2H, CH₂), 1.84–1.78 (m, 2H, CH₂), 1.40 (t, 6H, *J* = 6.3 Hz, 2 $\times$ CH₃), 1.31 (t, 3H, *J* = 6.9 Hz, CH₃). MS (ES) *m/z* (M+H)⁺ 369.

Ethyl-6-amino-4-[[2-(diethylamino)-ethyl]-amino]-5-nitro-2-pyridinecarbamate (**12**)

The procedure was followed as detailed above using **3** (173 mg, 0.66 mmol) and N,N-diethylethylenediamine (0.38 mL, 2.65 mmol). The product was purified by silica gel column chromatography (0.5% MeOH:CHCl₃:1% NH₄OH) to afford **12** (200 mg, 88%) as a yellow solid. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.63. ¹H NMR (300 MHz, CDCl₃): δ 9.45 (bs, 1H, NH), 6.91 (s, 1H, NH), 6.74 (s, 1H, 3-H), 4.26 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.33–3.28 (m, 2H, NH-CH₂), 2.75 (t, 2H, *J* = 6.3 Hz, NH-CH₂-CH₂), 2.60 (q, 4H, *J* = 6.9 Hz, -NCH₂), 1.34 (t, 3H, *J* = 6.9 Hz, -OCH₂-CH₃), 1.07 (t, 6H, *J* = 7.2 Hz, -NCH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 341.

Ethyl-6-amino-4-[[4-(dimethylamino)-ethyl]-amino]-5-nitro-2-pyridinecarbamate (**13**)

The procedure was followed as detailed above for **11** using **3** (1.5 g, 5.7 mmol), N,N-dimethylethylenediamine (0.76 mL, 6.9 mmol) and methanol (20 mL). The title compound **13** (1.27 g, 71%) was obtained by silica gel column chromatography (0.5% MeOH:CHCl₃:1% NH₄OH) as a yellow solid. M.P. 238–240 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.63. ¹H NMR (300 MHz, DMSO-d₆): δ 9.97 (s, 1H, NH), 9.05 (t, 1H, *J* = 5.4 Hz, NH), 8.00 (bs, 2H, NH₂), 6.69 (s, 1H, 3-H), 4.17 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.65–3.62 (m, 2H, NH-CH₂), 3.29–3.26 (m, 2H, NH-CH₂-CH₂), 2.79 (bs, 6H, -NCH₃), 1.25 (t, 3H, *J* = 7.2 Hz, -OCH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 313.

General procedure for the synthesis of 5, 14–16

A solution of carbamate in ethanol was hydrogenated at room temperature and 1 atm pressure in the presence of Ra-Ni (washed $3 \times \text{H}_2\text{O}$ and $3 \times \text{EtOH}$) for 7–8 hours. Progress of the reaction was monitored by TLC. On completion of the reaction, the catalyst solution was filtered through celite and the filtrate was evaporated to dryness yielding a quantitative amount of diamine. These compounds were directly used in the next step without further purification.

Ethyl (8-((5-(diethylamino)pentan-2-yl)amino)-2,3-di(furan-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (6)

Furil (74 mg, 0.39 mmol) was added to a stirred solution of the diamine hydrochloride **5** (140 mg, 0.35 mmol) in ethanol (10 mL). The clear yellow solution was stirred at room temperature under nitrogen atmosphere for 24 h, and then refluxed for 16 h under the same conditions. The solvent was removed in *vacuo* leaving a residual solid, which was purified with silica gel column chromatography using 6% MeOH:CHCl₃:1% NH₄OH as eluant. The fractions containing compound were concentrated under *vacuum* to give **6** (120 mg, 66%, HPLC Purity: 96.3%) as yellow solid. M.P. 65–70 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.51. ¹H NMR (300 MHz, CDCl₃): δ 7.58 (dd, 1H, J = 0.9 Hz, 1.8 Hz, 5'-H), 7.53 (dd, 2H, J = 0.9 Hz, 1.8 Hz, 5'-H, NH), 7.45 (s, 1H, 6-H), 6.87 (dd, 1H, J = 0.9 Hz, 3.3 Hz, 3'-H), 6.73 (dd, 1H, J = 0.9 Hz, 3.3 Hz, 3'-H), 6.59 (dd, 1H, J = 1.8 Hz, 3.3 Hz, 4'-H), 6.54 (dd, 1H, J = 1.8 Hz, 3.3 Hz, 4'-H), 6.45 (d, 1H, J = 8.4 Hz, NH), 4.29 (q, 2H, J = 7.2 Hz, OCH₂), 3.82–3.75 (m, 1H, CH), 2.90–2.89 (m, 6H, N-CH₂), 1.84–1.70 (m, 4H, CH₂-CH₂), 1.42 (t, 3H, J = 6.3 Hz, -CH-CH₃), 1.35 (t, 3H, J = 6.9 Hz, -OCH₂-CH₃), 1.27 (t, 6H, J = 6.0 Hz, N-CH₂-CH₃). MS (ES) m/z (M+H)⁺ 507. HRMS calcd for C₂₇H₃₄N₆O₄ (M+H)⁺ 507.27143; found 507.27187.

Ethyl (8-((5-(diethylamino)pentan-2-yl)amino)-2,3-di(thiophen-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (7)

The procedure above was followed using 2,2'-thenil (95 mg, 0.42 mmol) and the diamine hydrochloride **5** (151 mg, 0.38 mmol). The title compound **7** (108 mg, 52%, HPLC Purity: 95.4%) was obtained as a yellow solid. M.P. 78–84 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.47. ¹H NMR (300 MHz, CDCl₃): δ 7.60 (bs, 1H, NH), 7.50 (dd, 1H, J = 1.2 Hz, 5.1 Hz, 5'-H), 7.48 (dd, 1H, J = 1.2 Hz, 5.1 Hz, 5'-H), 7.44 (s, 1H, 6-H), 7.29–7.24 (dd, 2H, 3'-H), 7.09 (dd, 1H, J = 3.9 Hz, 5.1 Hz, 4'-H), 6.99 (dd, 1H, J = 3.9 Hz, 5.1 Hz, 4'-H), 6.35 (d, 1H, J = 8.4 Hz, NH), 4.25 (q, 2H, J = 6.9 Hz, OCH₂), 3.79–3.76 (m, 1H, CH), 2.87–2.80 (m, 6H, N-CH₂), 1.80–1.71 (m, 4H, CH₂-CH₂), 1.39 (t, 3H, J = 6.9 Hz, -CH-CH₃), 1.35 (t, 3H, J = 7.2 Hz, -OCH₂-CH₃), 1.22 (t, 6H, J = 6.3 Hz, N-CH₂-CH₃). MS (ES) m/z (M+H)⁺ 539. HRMS calcd for C₂₇H₃₄N₆O₂S₂ (M+H)⁺ 539.22574; found 539.22571.

Ethyl (8-((5-(diethylamino)pentan-2-yl)amino)-2,3-di(pyridin-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (8)

The procedure was followed as detailed above using 2,2'-pyridil (106 mg, 0.5 mmol) and the diamine hydrochloride **5** (200 mg, 0.5 mmol). The title compound **8** (90 mg, 33%, HPLC Purity: 95.0%) was obtained as a yellow solid. M.P. 99–104 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.6. ¹H NMR (300 MHz, CDCl₃): δ 8.37–8.35 (m, 1H, 6'-H), 8.26–8.23 (m, 1H, 6'-H), 8.21 (d, 1H, J = 7.8 Hz, 3'-H), 7.89–7.80 (m, 3H, 3'-H, 5'-H), 7.58 (bs, 1H, NH), 7.52 (s, 1H, 6-H), 7.25–7.19 (m, 2H, 4'-H), 6.61 (d, 1H, J = 8.1 Hz, NH), 4.29 (q, 2H, J = 6.9 Hz, OCH₂), 3.89–3.80 (m, 1H, CH), 3.16–2.94 (m, 6H, N-CH₂), 2.03–1.91 (m, 2H, CH₂), 1.89–1.71 (m, 2H, CH₂), 1.45–1.27 (m, 12H, CH₃). MS (ES) m/z (M+H)⁺ 529. HRMS calcd for C₂₉H₃₆N₈O₂ (M+H)⁺ 529.30340; found 529.30355.

Ethyl (8-((5-(diethylamino)pentan-2-yl)amino)-2,3-dimethylpyrido[2,3-b]pyrazin-6-yl)carbamate (9)

The procedure was followed as detailed above using 2,3-butanedione (0.064 mL, 0.73 mmol) and the diamine hydrochloride **5** (190 mg, 0.48 mmol). The title compound **9** (70 mg, 36%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 181–184 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.3. ¹HNMR (300 MHz, CDCl₃): δ 7.60 (bs, 1H, NH), 7.39 (s, 1H, 6-H), 6.38 (d, 1H, *J* = 8.4 Hz, NH), 4.24 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.81–3.73 (m, 1H, CH), 3.07–2.85 (m, 6H, N-CH₂), 2.68 (s, 3H, 3-CH₃), 2.65 (s, 3H, 2-CH₃), 1.94–1.67 (m, 4H, CH₂-CH₂), 1.43–1.28 (m, 12H, CH₃). MS (ES) *m/z* (M+H)⁺ 403. HRMS calcd for C₂₁H₃₄N₆O₂ (M+H)⁺ 403.28160; found 403.28194.

Ethyl (8-((5-(diethylamino)pentan-2-yl)amino)-2,3-diethylpyrido[2,3-b]pyrazin-6-yl)carbamate (10)

The procedure was followed as detailed above using 3,4-hexanedione (0.05 mL, 0.4 mmol) and the diamine hydrochloride **5** (143 mg, 0.37 mmol). The title compound **10** (80 mg, 51%, HPLC Purity: 96.2%) was obtained as a yellow solid. M.P. 55–60 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.43. ¹HNMR (300 MHz, CDCl₃): δ 7.55 (bs, 1H, NH), 7.39 (s, 1H, 6-H), 6.38 (d, 1H, *J* = 8.1 Hz, NH), 4.27 (q, 2H, *J* = 7.2 Hz, OCH₂), 3.83–3.74 (m, 1H, CH), 3.02–2.83 (m, 10H, N-CH₂, 2-CH₂, 3-CH₂), 1.98–1.67 (m, 4H, CH₂-CH₂), 1.44–1.23 (m, 18H, CH₃). MS (ES) *m/z* (M+H)⁺ 431. HRMS calcd for C₂₃H₃₈N₆O₂ (M+H)⁺ 431.31290; found 431.31321.

Ethyl (8-((4-(diethylamino)butyl)amino)-2,3-diphenylpyrido[2,3-b]pyrazin-6-yl)carbamate (17)

The procedure was followed as detailed above using benzil (57 mg, 0.27 mmol) and the diamine hydrochloride **14** (92 mg, 0.24 mmol). The title compound **17** (30 mg, 24%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 155–158 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.27. ¹HNMR (300 MHz, CDCl₃): δ 7.57–7.28 (m, 12H, Ph-H, NH, 6-H), 6.57 (t, 1H, *J* = 5.4 Hz, NH), 4.31 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.51–3.46 (m, 2H, NH-CH₂), 3.00–2.71 (m, 6H, N-CH₂), 1.78–1.78 (m, 4H, CH₂-CH₂), 1.38 (t, 3H, *J* = 6.9 Hz, -OCH₂-CH₃), 1.23 (t, 6H, *J* = 6.9 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 513. HRMS calcd for C₃₀H₃₆N₆O₂ (M+H)⁺ 513.29725; found 513.29753.

Ethyl (8-((4-(diethylamino)butyl)amino)-2,3-di(thiophen-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (18)

The procedure was followed as detailed above using 2,2'-thenil (202 mg, 0.91 mmol) and the diamine hydrochloride **14** (257 mg, 0.69 mmol). The title compound **18** (170 mg, 47%, HPLC Purity: 91.9%) was obtained as a yellow solid. M.P. 125–129 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.16. ¹HNMR (300 MHz, CDCl₃): δ 7.60 (bs, 1H, NH), 7.49 (dd, 1H, *J* = 1.2 Hz, 5'-H), 7.47 (dd, 1H, *J* = 1.2 Hz, 5'-H), 7.44 (s, 1H, 6-H), 7.27–7.24 (m, 2H, 3'-H), 7.08 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 6.99 (dd, 1H, 3.9 Hz, 5.1 Hz, 4'-H), 6.61 (t, 1H, *J* = 5.4 Hz, NH), 4.30 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.47 (dd, 2H, *J* = 6.3 Hz, 12.6 Hz, NH-CH₂), 2.75–2.59 (m, 6H, N-CH₂), 1.81–1.69 (m, 4H, CH₂-CH₂), 1.37 (t, 3H, 7.2 Hz, -OCH₂-CH₃), 1.12 (t, 6H, *J* = 6.3 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 525. HRMS calcd for C₂₆H₃₂N₆O₂S₂ (M+H)⁺ 525.21009; found 525.20959.

Ethyl (8-((4-(diethylamino)butyl)amino)-2,3-di(furan-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (19)

The procedure was followed as detailed above using furil (172 mg, 0.91 mmol) and the diamine hydrochloride **14** (257 mg, 0.69 mmol). The title compound **19** (120 mg, 36%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 125–129 °C. TLC (10%

MeOH:CHCl₃:1% NH₄OH): *R_f* 0.42. ¹HNMR (300 MHz, MeOH-d₄): δ 7.80 (d, 1H, *J* = 1.5 Hz, 5'-H), 7.74 (d, 1H, *J* = 1.2 Hz, 5'-H), 7.07 (d, 1H, *J* = 3.0 Hz, 3'-H), 6.92 (d, 1H, *J* = 0.6 Hz, 3'-H), 6.71- 6.68 (m, 2H, 4'-H), 6.61 (bs, 1H, 6-H), 4.44 (q, 2H, *J* = 6.3 Hz, OCH₂), 3.62 (bt, 2H, NH-CH₂), 3.27-3.18 (m, 6H, NCH₂), 1.87-1.86 (m, 4H, CH₂-CH₂), 1.43 (t, 3H, *J* = 6.9 Hz, OCH₂-CH₃), 1.34 (t, 6H, *J* = 7.2 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 493. HRMS calcd for C₂₆H₃₂N₆O₄ (M+H)⁺ 493.25578; found 493.25638.

Ethyl (8-((2-(diethylamino)ethyl)amino)-2,3-diphenylpyrido[2,3-b]pyrazin-6-yl)carbamate (20)

The procedure was followed as detailed above using benzil (100 mg, 0.47 mmol) and the diamine **15** (147 mg, 0.47 mmol). The title compound **20** (113 mg, 49%, HPLC Purity: 97.1%) was obtained as a yellow solid. M.P. 158–164 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.75. ¹HNMR (300 MHz, CDCl₃): δ 7.59-7.50 (m, 6H, Ph-H), 7.45 (s, 1H, NH), 7.34-7.25 (m, 6H, Ph-H, 6-H, NH), 4.31 (q, 2H, *J* = 7.2 Hz, OCH₂), 3.41 (dd, 2H, *J* = 6.3 Hz, 11.7 Hz, NH-CH₂), 2.84 (t, 2H, *J* = 6.3 Hz, CH₂-CH₂-N), 2.64 (q, 4H, *J* = 6.9 Hz, N-CH₂), 1.38 (t, 3H, *J* = 7.2 Hz, OCH₂-CH₃), 1.09 (t, 6H, *J* = 7.2 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 485. HRMS calcd for C₂₈H₃₂N₆O₂ (M+H)⁺ 485.26595; found 485.26651.

Ethyl (8-((2-(diethylamino)ethyl)amino)-2,3-di(thiophen-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (21)

The procedure was followed as detailed above, using 2,2'-thenil (196 mg, 0.87 mmol) and the diamine **15** (228 mg, 0.73 mmol). The title compound **21** (250 mg, 69%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 145–148 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.84. ¹HNMR (300 MHz, CDCl₃): δ 7.52- 7.46 (m, 5H, 5'-H, 3'-H, NH, 6-H), 7.38 (d, 1H, *J* = 3.6 Hz, 3'-H), 7.05 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 7.02 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 4.29 (q, 2H, *J* = 6.9 Hz, OCH₂), 4.20-4.05 (m, 2H, NH-CH₂), 3.36 (t, 2H, *J* = 6.3 Hz, CH₂-CH₂-N), 3.25 (q, 4H, *J* = 6.6 Hz, N-CH₂), 1.45 (t, 6H, *J* = 6.9 Hz, N-CH₂-CH₃), 1.37 (t, 3H, *J* = 7.2 Hz, OCH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 497. HRMS calcd for C₂₄H₂₈N₆O₂S₂ (M+H)⁺ 497.17879; found 497.17947.

Ethyl (8-((2-(diethylamino)ethyl)amino)-2,3-di(furan-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (22)

The procedure was followed as detailed above using furil (300 mg, 1.6 mmol) and the diamine **15** (400 mg, 1.3 mmol). The title compound **22** (320 mg, 54%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 160–162 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.74. ¹HNMR (300 MHz, CDCl₃): δ 8.33 (bs, 1H, NH), 7.61-7.34 (m, 4H, 5'-H, 3'-H, 6-H), 6.89 (bs, 1H, 3'-H), 6.61-6.57 (m, 2H, 4'-H), 4.40-4.13 (m, 4H, OCH₂, NH-CH₂), 3.40-3.20 (m, 6H, NCH₂), 1.52-1.37 (m, 9H, CH₃). MS (ES) *m/z* (M+H)⁺ 465. HRMS calcd for C₂₄H₂₈N₆O₄ (M+H)⁺ 465.22448; found 465.22478.

Ethyl (8-((2-(dimethylamino)ethyl)amino)-2,3-diphenylpyrido[2,3-b]pyrazin-6-yl)carbamate (23)

The procedure was followed as detailed above using benzil (403 mg, 1.9 mmol) and the diamine **16** (446 mg, 1.58 mmol). The title compound **23** (260 mg, 36%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 203–205 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): *R_f* 0.7. ¹HNMR (300 MHz, DMSO-d₆): δ 10.63 (bs, 1H, NH), 8.75 (bs, 1H, NH), 7.59-7.37 (m, 10H, Ph-H), 7.28 (s, 1H, 6-H), 4.32 (q, 2H, *J* = 6.6 Hz, OCH₂), 3.87 (t, 2H, *J* = 6.9 Hz, NH-CH₂), 3.46 (t, 2H, *J* = 4.8 Hz, CH₂-CH₂-N), 2.89 (s, 6H, NCH₃), 1.33 (t, 3H, *J* = 7.2 Hz, OCH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 457. HRMS calcd for C₂₆H₂₈N₆O₂ (M+H)⁺ 457.23465; found 457.23562.

Ethyl (8-((2-(dimethylamino)ethyl)amino)-2,3-di(thiophen-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (24)

The procedure was followed as detailed above using 2,2'-thenil (157 mg, 0.71 mmol) and the diamine **16** (200 mg, 0.71 mmol). The title compound **24** (200 mg, 60%, HPLC Purity: 96.3%) was obtained as a yellow solid. M.P. 120–125 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.72. ¹H NMR (300 MHz, CDCl₃): δ 7.58 (s, 1H, NH), 7.48–7.46 (m, 2H, 5'-H), 7.42 (s, 1H, 6-H), 7.30–7.27 (m, 2H, 3'-H), 7.06 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 7.00 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 6.95 (t, 1H, *J* = 5.4 Hz, NH), 4.30 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.47 (dd, 2H, *J* = 6.0 Hz, 11.7 Hz, NH-CH₂), 2.70 (t, 2H, *J* = 6.0 Hz, CH₂-CH₂-N), 2.33 (s, 6H, NCH₃), 1.37 (t, 3H, *J* = 6.9 Hz, OCH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 469. HRMS calcd for C₂₂H₂₄N₆O₂S₂ (M+H)⁺ 469.14749; found 469.14839.

Ethyl (8-((2-(dimethylamino)ethyl)amino)-2,3-di(furan-2-yl)pyrido[2,3-b]pyrazin-6-yl)carbamate (25)

The procedure was followed as detailed above using furil (136 mg, 0.71 mmol) and the diamine **16** (200 mg, 0.71 mmol). The title compound **25** (95 mg, 31%, HPLC Purity: 100%) was obtained as a yellow solid. M.P. 153–156 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.66. ¹H NMR (300 MHz, CDCl₃): δ 7.59 (d, 2H, *J* = 18.0 Hz, 5'-H), 7.53 (bs, 1H, NH), 7.35 (s, 1H, 6-H), 7.14 (d, 1H, *J* = 3.0 Hz, 3'-H), 6.80 (d, 1H, *J* = 3.3 Hz, 3'-H), 6.56 (dd, 2H, *J* = 1.5 Hz, 12.6 Hz, 4'-H), 4.25 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.93 (bs, 2H, NH-CH₂), 3.40 (bs, 2H, CH₂-CH₂-N), 2.91 (s, 6H, NCH₃), 1.36 (t, 3H, *J* = 6.9 Hz, OCH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 437. HRMS calcd for C₂₂H₂₄N₆O₄ (M+H)⁺ 437.19318; found 437.19439.

N⁸-(5-(diethylamino)pentan-2-yl)-2,3-di(furan-2-yl)pyrido[2,3-b]pyrazine-6,8-diamine (26)

To a stirred solution of KOH (51 mg, 0.92 mmol) in EtOH (10 mL) was added **6** (100 mg, 0.19 mmol) under argon atmosphere. The reaction mixture was stirred at reflux temperature for 3 hrs. Excess ethanol was removed under reduced pressure. The dry residue was dissolved in 50% NaOH solution (10 mL) and extracted with diethyl ether (3 × 25 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford **26** (25 mg, 29%, HPLC Purity: 100%) as a yellow solid. M.P. 125–127 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.13. ¹H NMR (300 MHz, CDCl₃): δ 7.54 (dd, 1H, *J* = 0.6 Hz, 1.8 Hz, 5'-H), 7.48 (dd, 1H, *J* = 0.9 Hz, 1.8 Hz, 5'-H), 6.88 (dd, 1H, *J* = 0.9 Hz, 3.6 Hz, 3'-H), 6.61 (dd, 1H, *J* = 0.9 Hz, 3.6 Hz, 3'-H), 6.55 (dd, 1H, *J* = 1.8 Hz, 3.3 Hz, 4'-H), 6.51 (dd, 1H, *J* = 1.8 Hz, 3.3 Hz, 4'-H), 6.22 (d, 1H, *J* = 8.4 Hz, NH), 5.79 (s, 1H, 6-H), 4.89 (s, 2H, NH₂), 3.64–3.55 (m, 1H, CH), 2.58–2.41 (m, 6H, N-CH₂), 1.71–1.55 (m, 4H, CH₂-CH₂), 1.33 (d, 3H, *J* = 6.3 Hz, CH-CH₃), 1.03 (t, 6H, *J* = 7.2 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 435. HRMS calcd for C₂₄H₃₀N₆O₂ (M+H)⁺ 435.25030; found 435.25054.

N⁸-(5-(diethylamino)pentan-2-yl)-2,3-di(thiophen-2-yl)pyrido[2,3-b]pyrazine-6,8-diamine (27)

The procedure was followed as detailed above using **7** (185 mg, 0.34 mmol) and KOH (90 mg, 1.6 mmol). The title compound **27** (65 mg, 41%, HPLC Purity: 95.3%) was obtained as a yellow solid. M.P. 45–47 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.15. ¹H NMR (300 MHz, CDCl₃): δ 7.46 (dd, 1H, *J* = 1.2 Hz, 5.1 Hz, 5'-H), 7.43 (dd, 1H, *J* = 1.2 Hz, 5.1 Hz, 5'-H), 7.22 (dd, 1H, *J* = 1.2 Hz, 3.6 Hz, 3'-H), 7.20 (dd, 1H, *J* = 1.2 Hz, 3.9 Hz, 3'-H), 7.07 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 6.96 (dd, 1H, *J* = 3.6 Hz, 5.1 Hz, 4'-H), 6.16 (d, 1H, *J* = 8.4 Hz, NH), 5.77 (s, 1H, 6-H), 4.90 (s, 2H, NH₂), 3.64–3.56 (m, 1H, CH), 2.55–2.42 (m, 6H, N-CH₂), 1.69–1.53 (m, 4H, CH₂-CH₂), 1.31 (d, 3H, *J* = 6.3 Hz, CH-CH₃), 1.023 (t, 6H, *J* = 6.9 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 467. HRMS calcd for C₂₄H₃₀N₆S₂ (M+H)⁺ 467.20461; found 467.20412.

Ethyl (4-chloro-6-((5-(diethylamino)pentan-2-yl)amino)pyrimidin-2-yl)carbamate (29)

To a stirred solution of 4,6-dichloro-2-pyrimidinyl carbamic acid ethyl ester, **28** (1.0 g, 4.2 mmol) in dry methanol (20 mL), 2-amino-5-diethylaminopentane (0.84 mL, 4.2 mmol) was added. The mixture was refluxed for 3 h under nitrogen atmosphere. Excess methanol was evaporated under reduced pressure and the resulting residue was purified by silica gel column chromatography (3% MeOH:CHCl₃:1% NH₄OH) to afford **29** (1.22g, 80%) as a white foam. TLC (10% MeOH:CHCl₃): R_f 0.32. ¹H NMR (300 MHz, CDCl₃): δ 6.20-6.15 (m, 2H, NH, 5-H), 4.24-4.17 (m, 3H, OCH₂, CH), 3.15-2.82 (m, 6H, NCH₂), 2.02-1.56 (m, 4H, CH₂-CH₂), 1.43-1.20 (m, 12H, CH₃). MS (ES) *m/z* (M+H)⁺ 357. HRMS calcd for C₁₆H₂₈ClN₅O₂ (M+H)⁺ 358.2004; found 358.2008.

Ethyl (4-chloro-6-((4-(diethylamino)butyl)amino)pyrimidin-2-yl)carbamate (30)

The procedure was followed as detailed above using 4-diethylaminobutylamine (447 mg, 3.1 mmol) and 4,6-dichloro-2-pyrimidinyl carbamic acid ethyl ester, **28** (0.75 g, 3.1 mmol). The title compound **30** (1.0 g, 92%) was obtained as a syrup. TLC (10% MeOH:CHCl₃): R_f 0.17. ¹H NMR (300 MHz, CDCl₃): δ 6.00 (s, 1H, 5-H), 4.23 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.25 (bs, 2H, NH-CH₂), 2.59 (q, 4H, *J* = 6.0 Hz, N-CH₂-CH₃), 2.48 (t, 2H, *J* = 6.9 Hz, CH₂-CH₂-N), 1.68-1.50 (m, 4H, CH₂-CH₂), 1.32 (t, 3H, *J* = 7.2 Hz, OCH₂-CH₃), 1.07 (t, 6H, *J* = 7.2 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 344.

Ethyl (4-chloro-6-((5-(diethylamino)pentan-2-yl)amino)-5-nitropyrimidin-2-yl)carbamate (31)

To a stirred solution of **29** (0.32 g, 0.89 mmol) in 1.12 mL of concentrated H₂SO₄, 0.22 mL of fuming nitric acid (sp. gr 1.49) was added drop wise. The temperature during the addition was kept between 30–35 °C. After addition, the solution was stirred at 30–35 °C for 35 min. The mixture was added to crush ice with vigorous stirring. The pH was adjusted to 8–9 by dropwise addition of concentrated aqueous ammonia, keeping the temperature below 15 °C. The product separated as an oil, and the mixture was extracted with chloroform (3 × 25 mL). The organic extracts were dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo* providing 0.2 g of the crude mixture. The mixture was purified over a short pad of silica gel using 2% MeOH:CHCl₃:1% NH₄OH. The fractions containing pure compound were concentrated under *in vacuo* to give pure **31** (144 mg, 40%). TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.41. ¹H NMR (300 MHz, CDCl₃): δ 7.93 (d, 1H, *J* = 8.1 Hz, NH), 4.50-4.40 (m, 1H, CH), 4.25 (q, 2H, *J* = 6.9 Hz, OCH₂), 2.75-2.45 (m, 6H, NCH₂), 1.70-1.58 (m, 4H, CH₂-CH₂), 1.35-1.29 (m, 6H, OCH₂-CH₃, CH-CH₃), 1.07 (t, 6H, *J* = 7.2 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 403. HRMS calcd for C₁₆H₂₇ClN₆O₄ (M+H)⁺ 403.18551; found 403.18595.

Ethyl (4-chloro-6-((4-(diethylamino)butyl)amino)-5-nitropyrimidin-2-yl)carbamate (32)

The procedure was followed as detailed above using **30** (640 mg, 1.86 mmol). The title compound **32** (600 mg, 83%) was obtained as a syrup. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.26. MS (ES) *m/z* (M+H)⁺ 389.

Ethyl (4-((5-(diethylamino)pentan-2-yl)amino)-6-mercapto-5-nitropyrimidin-2-yl)carbamate (33)

A solution of **31** (160 mg, 0.39 mmol) and potassium thioacetate (66.8 mg, 0.585 mmol) in ethanol (5 mL) was refluxed for 2 h. Excess solvent was removed and the residue was dissolved in chloroform. The solution was washed with water, and the combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated and purified over a short pad of silica gel (70–230 mesh) using 3.5% MeOH:CHCl₃:1% NH₄OH. Selected fractions were collected and concentrated *in vacuo* to give pure **33** (80 mg, 50%). TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.14. ¹H NMR (300 MHz, CDCl₃): δ 9.46 (d, 1H, *J* = 7.5

Hz, NH), 4.24-4.19 (m, 3H, CH, OCH₂), 3.35-3.20 (m, 1H, NCH₂), 3.10-3.00 (m, 2H, NCH₂), 2.90-2.84 (m, 2H, NCH₂), 2.72-2.60 (m, 1H, NCH₂), 2.00-1.85 (m, 1H, CH₂), 1.84-1.65 (m, 2H, CH₂), 1.55-1.40 (m, 1H, CH₂), 1.32-1.23 (m, 12H, CH₃). MS (ES) *m/z* (M+H)⁺ 401. HRMS calcd for C₁₆H₂₈N₆O₄S (M+H)⁺ 401.19655; found 401.19751.

Ethyl 4-((4-(diethylamino)butyl)amino)-6-mercapto-5-nitropyrimidin-2-yl)carbamate (34)

The procedure was followed as detailed above using **32** (600 mg, 1.54 mmol) and potassium thioacetate (264 mg, 2.3 mmol). The title compound **34** (80 mg, 13%) was obtained as a syrup. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.21. MS (ES) *m/z* (M+H)⁺ 387.

Ethyl 5-amino-4-((5-(diethylamino)pentan-2-yl)amino)-6-mercaptopyrimidin-2-yl)carbamate (35)

To a stirred solution of **33** (80 mg, 0.2 mmol) in acetic acid (5 mL) was added zinc dust (0.4 g) portion wise over a period of 30 minutes at 80 °C under N₂ atmosphere. The reaction mixture was cooled, and the insoluble material was removed by filtration and washed with acetic acid (1 mL). The filtrate and wash were combined and evaporated to dryness, and the resulting brownish oil was triturated with an aqueous solution of 0.1 M K₂HPO₄ (3 mL). The mixture was extracted with chloroform (3 × 20 mL). The combined organic layer was separated, collected, dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to give **35** (60 mg, 81%). TLC (10% MeOH: CHCl₃: 1% NH₄OH): R_f 0.25. ¹H NMR (300 MHz, CDCl₃): δ 4.27-4.20 (m, 3H, CH, OCH₂), 2.57-2.35 (m, 6H, NCH₂), 1.52-1.47 (m, 4H, CH₂-CH₂), 1.33 (t, 3H, *J* = 7.2 Hz, OCH₂-CH₃), 1.20 (d, 3H, *J* = 6.6 Hz, CH-CH₃), 1.02 (t, 6H, *J* = 6.9 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 370.

Ethyl 5-amino-4-((4-(diethylamino)butyl)amino)-6-mercaptopyrimidin-2-yl)carbamate (36)

The procedure was followed as detailed above using **34** (80 mg, 0.2 mmol). The title compound **36** was obtained in 55% (40 mg) yield. TLC (10% MeOH: CHCl₃: 1% NH₄OH): R_f 0.07. ¹H NMR (300 MHz, CDCl₃): δ 5.84 (bs, 1H, NH), 4.34 (q, 2H, *J* = 6.9 Hz, OCH₂), 3.39 (m, 2H, NH-CH₂), 2.61-2.47 (m, 6H, NCH₂), 1.60-1.54 (m, 4H, CH₂-CH₂), 1.36 (t, 3H, *J* = 6.9 Hz, OCH₂-CH₃), 1.04 (t, 6H, *J* = 7.2 Hz, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 357.

Ethyl 4-((5-(diethylamino)pentan-2-yl)amino)-6,7-diphenyl-7H-pyrimido[4,5-b][1,4]thiazin-2-yl) carbamate (37)

To a solution of **35** (50 mg, 0.135 mmol) and sodium acetate (55mg, 0.67 mmol) in water-ethanol (1 mL, 1:1) was added desyl chloride (946 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated *in vacuo* and flash column chromatography of the residue (7% MeOH:CHCl₃) yielded **37** (56 mg, 77%, HPLC Purity: 95.6%) as a mixture of diastereomers. M.P. 130–135 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.43. ¹H NMR (300 MHz, CDCl₃): δ 7.89-7.85 (m, 2H, Ph-H), 7.45-7.38 (m, 3H, Ph-H, NH), 7.24-7.15 (m, 6H, Ph-H), 6.15-6.09 (m, 1H, NH), 5.39 (2s, 1H, S-CH-Ph), 4.28-4.27 (m, 1H, CH), 4.23 (q, 2H, *J* = 7.2 Hz, OCH₂), 2.58-2.46 (m, 6H, NCH₂), 1.62-1.55 (m, 4H, CH₂-CH₂), 1.32-1.25 (m, 6H, CH-CH₃, OCH₂-CH₃), 1.05-0.95 (m, 6H, N-CH₂-CH₃). MS (ES) *m/z* (M+H)⁺ 547. HRMS calcd for C₃₀H₃₈N₆O₂S (M+H)⁺ 547.28497; found 547.28484.

Ethyl 4-((4-(diethylamino)butyl)amino)-6,7-diphenyl-7H-pyrimido[4,5-b][1,4]thiazin-2-yl)carbamate (38)

The procedure was followed as detailed above using **36** (40 mg, 0.11 mmol). The title compound **38** (50 mg, 83%, HPLC Purity: 96.6%) was obtained as a yellow solid. M.P. 135–140 °C. TLC (10% MeOH:CHCl₃:1% NH₄OH): R_f 0.31 ¹H NMR (300 MHz, CDCl₃):

δ 7.90–7.87 (m, 2H, Ph-H), 7.44–7.38 (m, 2H, Ph-H), 7.25–7.17 (m, 6H, Ph-H), 7.15 (s, 1H, NH), 6.38 (t, 1H, $J = 5.7$ Hz, NH). 5.40 (s, 1H, S-CH-Ph), 4.23 (q, 2H, $J = 6.9$ Hz, OCH₂), 3.63–3.52 (m, 2H, NH-CH₂), 2.56–2.48 (m, 6H, NCH₂), 1.73–1.66 (m, 4H, CH₂-CH₂), 1.30 (t, 3H, $J = 7.2$ Hz, OCH₂-CH₃), 1.04 (t, 6H, $J = 6.9$ Hz, N-CH₂-CH₃). MS (ES) m/z (M+H)⁺ 533. HRMS calcd for C₂₉H₃₆N₆O₂S (M+H)⁺ 533.26932; found 533.26932.

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Figure 1.
Structures of Lead FtsZ Inhibitors 1 and 2

Figure 2.
General Structures of Heterocyclic Inhibitor Scaffolds

Scheme 1.

Synthetic pathways to analogs **6–10** and **17–25**. *Reagents and conditions:* (a) 2-amino-5-diethylaminopentane/4-diethylaminobutylamine/2-diethylaminoethylamine/2-dimethylaminomethylamine, MeOH/EtOH, reflux; (b) Raney Nickel, H₂, EtOH, r.t.; (c) Benzil/Furil/2,2'-Thenil/2,2'-Pyridil/2,3-butanedione/3,4-hexanedione, EtOH, reflux, N₂.

Scheme 2.

Synthetic pathway to **26–27**. *Reagents and conditions:* (a) KOH, EtOH, N₂, reflux.

Scheme 3.

Synthetic pathways to pyrimido[4,5-b][1,4]thiazines **37–38**. *Reagents and conditions:* (a) 2-amino-5-diethylaminopentane/4-diethylaminobutylamine, MeOH, reflux; (b) fuming HNO₃, concentrated H₂SO₄, 30–35 °C; (c) potassium thioacetate, EtOH, reflux; (d) Zn, AcOH, N₂, 80 °C; (e) desyl chloride, NaOAc, H₂O/EtOH, rt.

Table 1

Screening data for control **1** and **2**, pyridopyrazine analogs **6–10**, **17–25** and **26–27**, and pyrimidothiazines **37–38**.^a

Compounds	Mth FtsZ Polymerization ^b IC ₅₀ (μM)	Tubulin Polymerization ^b IC ₅₀ (μM)	Mth H ₃₇ Ra MIC μM	Mth H ₃₇ Rv IC ₉₀ (μM)	Vero Cytotoxicity CC ₅₀ (μM)
1	34.2 ± 2.5	>100 μM	0.47	<0.19	ND
2	38.1 ± 4.1 ^c	24% inhibition 100 μM	3.8	1.9	ND
6	26.8 ± 5.6	>100 μM	3.6	7.0	>40
7	34.3 ± 6.0	>100 μM	0.43	<0.19	>40
8	45.7 ± 3.6	>100 μM	54	>100	>40
9	>100 μM	>100 μM	>290	>100	>40
10	>100 μM	>100 μM	>290	>50	>40
17	52	40% inhibition 100 μM	0.24	<0.19	1.8
18	64% at 100 μM	>100 μM	0.23	>100	>40
19	16.4 ± 1.2	41 ± 5.9	3.6	>100	>40
20	59% at 100 μM	>100 μM	1.7	0.38	2.7
21	21.0 ± 2.0	>100 μM	≤7.9	0.92	6.3
22	34.8 ± 10.7	>100 μM	8.7	>100	5.5
23	54.6 ± 18.9	>100 μM	≤7.6	<0.19	0.7
24	27.3 ± 3.2	>100 μM	1.1	<0.19	1.6
25	34.1 ± 14.6	>100 μM	≤7.0	29	37
26	18.6 ± 2.3	>100 μM	150	>100	14
27	29.5 ± 14.5	>100 μM	17	4.1	5.9
37	46.0 ± 3.4	>100 μM	49	22	>40
38	46.2 ± 4.3	69.7 ± 3.4	52	18	>40

^aMethods are reported in references 11 and 18.

^bEach compound was analyzed at least three times per assay. For the polymerization assays, the mean ± the standard deviations are reported.

^cReported previously.¹² IC₅₀ = concentration to inhibit polymerization of either tubulin or FtsZ by 50%. MIC = minimum inhibitory concentration is the lowest concentration, using duplicate two-fold serial dilutions of drug, that completely inhibited growth as evidenced by a lack of metabolic dye reduction in the microplate Alamar blue assay – see reference 11. IC₉₀ is extrapolated from a 10 point dose response curve and is the concentration that inhibits bacterial growth by 90% - see reference 18. CC₅₀ (Cytotoxic Concentration) is extrapolated from a 10 point dose response curve and is the concentration that inhibits growth of Vero cells in culture by 50% - see reference 18.