Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 2552

www.rsc.org/obc

PAPER

2-Aminopyrimidine as a novel scaffold for biofilm modulation†

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Received 7th November 2011, Accepted 21st December 2011
DOI: 10.1039/c2ob06871k

An efficient synthetic route to a series of substituted 2-aminopyrimidine (2-AP) derivatives has been developed. Subsequent biofilm screening has allowed comparison between the biological activity of these new derivatives and that of the 2-aminoimidazole class of anti-biofilm compounds. Several derivatives displayed the ability to modulate bacterial biofilm formation, exhibiting greater activity against Gram-positive strains than Gram-negative strains. Additionally some 2-aminopyrmidines were able to suppress MRSA resistance to conventional antibiotics.

Antibiotic resistance has become one of the most pressing issues in biomedical science. The ability to exert control over bacterial life processes that contribute to pathogenesis and drug resistance, such as biofilm formation, is one promising route toward dealing with infections that stem from multidrug resistant (MDR) bacteria. A bacterial biofilm can be defined as a surface accreted community of bacteria that are encased in an extracellular matrix.[1] Once in the biofilm state, bacteria become significantly more resilient to conventional antimicrobial treatments. In some cases bacteria within a biofilm are 1000 times more resistant to antibiotic therapies than their planktonic counterparts.[2] It is estimated that 80% of all bacterial infections are biofilm mediated.[3] Biofilms are thought to be responsible for the chronic infections of indwelling medical devices,[4,5] degenerative tooth decay *via* plaque formation, as well as the mortality and morbidity of cystic fibrosis patients.[6,7]

Despite the widespread problems caused by biofilms, there exist few small molecule scaffolds capable of modulating the biofilm life cycle.[8] These scaffolds include homoserine lactones,[9] brominated furanones,[10,11] phenethyl-carbamates,[12,13] and flustramine derivatives.[14] One of the most successful classes of biofilm modulators has been the 2-aminoimidazole (2-AI) class based on the marine natural products oroidin and bromoageliferin.[15–22] The next logical heterocycle to investigate in an effort to identify novel biofilm modulating scaffolds was the 2-aminopyrimidine (2-AP, Fig. 1). The replacement of a hydrogen bond donor (N–H) with a hydrogen bond acceptor (C=N) allows us to probe the importance of this hydrogen bonding site in the context of biofilm modulation. The 2-AP ring is of similar size to the 2-AI ring, with only a one carbon homologation between the two heterocycles. Many 2-aminopyrimidine analogues exhibit a host of interesting biological functions.[23,24] One series of analogues, the imidazo[1,2-*a*]pyrimidinium salts, have been shown to inhibit biofilm formation of both *Salmonella typhimurium* and *Pseudomonas aeruginosa*, however it is hypothesized that these compounds are cleaved *in vivo* to the corresponding 2-aminoimidazole.[25] Therefore, to explore the potential for 2-aminopyrimidine derivatives themselves to serve as biofilm modulators, we were interested in testing 2-aminopyrimidine analogues that could not be cleaved *in vivo* to the corresponding 2-aminoimidazole derivative.

Due to the success of derivatives **1**, a class of 2-aminopyrimidine amides **2** and **3** were envisioned to test the hypothesis that suitably functionalized 2-aminopyrimidines could also modulate biofilm formation (Fig. 2). The synthetic approach to access these compounds is outlined in Scheme 1. Standard Boc-protection of 2-amino-5-iodopyrimidine yielded di-Boc protected 2-amino-5-iodopyrimidine **4**, which was coupled with various propargylic alkyne amides under standard Sonogashira coupling conditions. The resulting alkyne 2-aminopyrimidines were then deprotected (TFA/DCM) and subjected to counter ion exchange (Cl^- for trifluoroacetate) to deliver 2-aminopyrimidines **9–12** for biological testing. The effect of side chain unsaturation was also investigated. The intermediate Boc-protected alkyne 2-aminopyrimidines were fully reduced *via* hydrogenation, which, following deprotection and counter ion exchange, delivered 2-aminopyrimidines **13–16**.

With compounds **9–16** in hand, biological screening was undertaken against two representative Gram-negative bacterial

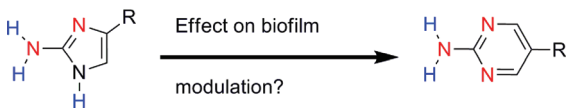

Fig. 1 Comparison of H-bond donor/acceptor profiles between generic 2-AI and 2-AP rings.

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†Electronic supplementary information (ESI) available: Experimental protocols for the synthesis and biological assays, characterization data for new compounds, and representative biofilm inhibition dose response curves and bacterial growth curves are displayed. See DOI: 10.1039/c2ob06871k

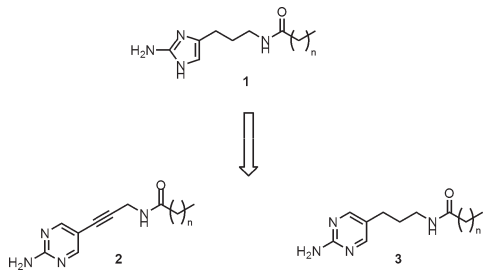
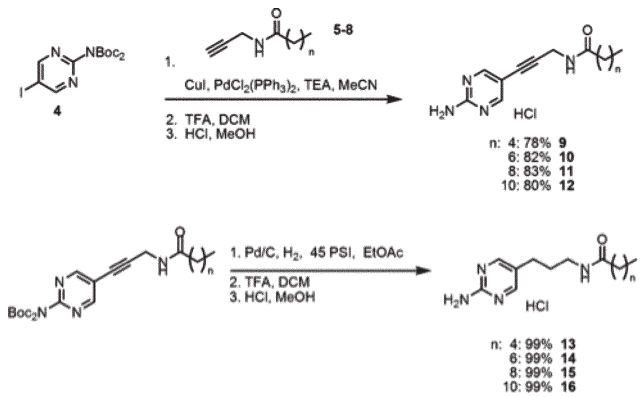


Fig. 2 Evolution of the 2-aminopyrimidine class of biofilm modulators.



Scheme 1 Preparation of 2-aminopyrimidines *via* Sonogashira coupling.

strains (*Acinetobacter baumannii* and *P. aeruginosa*) and two distinct isolates of one representative Gram-positive bacteria: methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA). Each compound was screened at 200 μM for the ability to inhibit bacterial biofilm formation using the crystal violet reporter assay.[26] The effect of 2-aminopyrimidine (2-AP) itself was also investigated. These results are summarized in Table 1.

The data revealed that compounds **10** and **15** are the most active compounds from this initial class of 2-aminopyrimidine amides. Compounds **10** and **15** were able to inhibit MRSA biofilm formation by 80.1% and 85.5% respectively at 200 μM. 2-Aminopyrimidine **10** was also able to inhibit MSSA biofilm formation by 83.9%, while **15** showed only marginal activity. Compound **10** was able to inhibit 52.5% *P. aeruginosa* biofilm formation, while **15** was ineffective at 200 μM. None of the 2-aminopyrimidines inhibited *A. baumannii* biofilm formation substantially, with the most active compound **10**, showing only 40.4% inhibition at 200 μM. As expected, 2-aminopyrimidine itself showed no noteworthy biofilm inhibition activity at 200 μM against the four strains tested.

Both **10** and **15** were then subjected to dose-response studies in an attempt to determine their IC_{50} values (concentration at which 50% of the biofilm formation is inhibited). Compound **10** displayed IC_{50} values of 200 μM, 128 μM, and 84 μM against *P. aeruginosa*, MSSA, and MRSA, respectively (Table 2). Compound **15** returned IC_{50} values of 344 μM and 72 μM against MSSA and MRSA, respectively (Table 2). Growth curve analysis was then performed at the IC_{50} concentrations of **10** and **15** to assess the effect on planktonic cell growth. The discovery of

Table 1 Percent inhibition of biofilm formation at 200 μM

Cmpd	*A. baumannii*	*P. aeruginosa*	MSSA	MRSA
9	23.1 ± 4.6[a]	57.4 ± 22.0*	58.8 ± 7.7*	72.9 ± 24.4*
10	40.4 ± 5.2	52.5 ± 11.0	83.9 ± 8.4	80.1 ± 7.7
11	34.2 ± 2.4	12.4 ± 20.9*	15.5 ± 24.8*	30.7 ± 19.9
12	17.9 ± 1.5	30.6 ± 9.7*	10.6 ± 5.4*	0.2 ± 3.9
13	10.7 ± 18.9	22.0 ± 19.0*	30.1 ± 9.7*	29.7 ± 14.4*
14	26.3 ± 8.0	3.5 ± 1.6	5.7 ± 16.9	11.7 ± 9.4
15	33.1 ± 5.2	4.9 ± 12.5	30.2 ± 7.9	85.5 ± 3.5
16	33.0 ± 17.9	32.6 ± 16.2*	35.5 ± 14.6*	65.0 ± 10.6*
2-AP	8.08 ± 4.0	19.0 ± 3.6*	12.2 ± 0.7	15.4± 7.2

[a] ± Indicates one standard deviation; * Indicates biofilm promotion.

Table 2 Summary of IC_{50} values for the 2-aminopyrimidine scaffold (μM)

Cmpd	MSSA	MRSA
10	128	84
15	344	72
23	137	>200
26	67	>200
37	114	>200

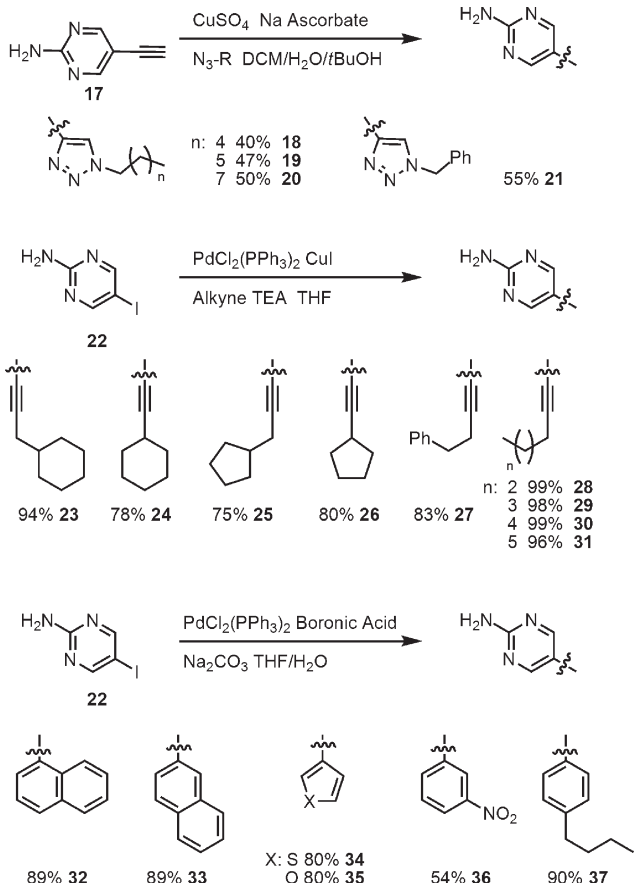
novel non-toxic biofilm modulators is of utmost importance, in order to reduce the evolutionary pressures and therefore limit the development of resistance.

Against our MRSA strain, both **10** and **15** were shown to be non-toxic biofilm inhibitors. Both however, were eliciting their behavior in a toxic manner against MSSA. The ability of compound **10** to inhibit *P. aeruginosa* biofilms was also occurring *via* a toxic mechanism. Alkyne **10** was shown to be a more potent biofilm inhibitor against both *P. aeruginosa* and MSSA, as compared to **15**. Both **10** and **15** were each able to equally inhibit MRSA biofilms

With the success of the 2-aminopyrimidine amides **10** and **15**, additional analogues of the 5-substituted 2-aminopyrimidine scaffold were explored to potentially identify further biofilm inhibitors. In an effort to discover additional non-toxic biofilm modulators we focused on three classes of molecules: 2-aminopyrimidine-triazoles, 2-aminopyrimidine-alkyls, and 2-aminopyrimidine-aryls. These classes were once again based on previously active 2-aminoimidazole scaffolds.[27–29]

The synthetic route to each class is outlined in Scheme 2. The 2-aminopyrimidine-triazoles were accessed through the use of terminal alkyne **17**[30] and the Cu catalyzed [3 + 2] cycloaddition. The 2-aminopyrimidine-alkynes were synthesized utilizing the Sonogashira coupling reaction. The final class of 2-aminopyrimidines prepared was the 2-aminopyrimidine-aryl class, which was synthesized using the Suzuki coupling reaction.

These second generation classes of 2-aminopyrimidines were then screened for their ability to inhibit *P. aeruginosa*, MSSA, and MRSA biofilm formation (See Table 3). Activity against *A. baumannii* was not examined due to the low activity displayed by the 2-aminopyrimidine amides. The 2-aminopyrimidine terminal alkyne, **17**, and triazole class **18–21** exhibited little ability to inhibit biofilm formation at the highest concentration tested (200 μM) against any of the three strains. 5-Iodo-2-aminopyrimidine (**22**) was able to inhibit MSSA biofilm formation by 65.5% at 200 μM. Unfortunately, when **22** was screened at lower



Scheme 2 Preparation of 2nd generation 2-aminopyrimidines.

concentrations a precipitous drop in activity was observed (*i.e.*, 65.5% at 200 μM, 8% at 150 μM), typical of a toxic mechanism.

The 2-aminopyrimidine-aryl class, **32–37** exhibited interesting behavior against MSSA biofilms; however they were ineffective against both MRSA and *P. aeruginosa*. Compound **37** was able to inhibit MSSA biofilm formation by 64% at 200 μM. Compound **37** was then subjected to dose-response assays, in order to determine the IC_{50} value for the inhibition of MSSA biofilms, and an IC_{50} of 114 μM was obtained (Table 2). When assayed at 200 μM, the naphthyl-derivative **32** promoted MSSA biofilm formation by 68%. Compound **32** returned dose-response data indicating that at 61 μM it induced 50% more biofilm formation than the control. Growth curve analysis confirmed that both **32** and **37** act *via* non-toxic mechanisms.

The 2-aminopyrimidine-alkyne class of analogues also provided two biofilm modulators. Compounds **23** and **26** demonstrated the ability to inhibit MSSA biofilm formation by 66% and 80% at 200 μM and returned IC_{50} values of 137 μM and 67 μM respectively (Table 2). Growth curve analysis of **23** and **26** proved that the inhibition of MSSA biofilm formation occurs through a non-toxic mechanism.

Given that the 2-aminopyrimidine classes were able to inhibit *S. aureus* biofilm formation we turned our attention to the dispersion of pre-formed biofilms. Unfortunately, unlike the 2-AI class of anti-biofilm compounds, none of the 2-aminopyrimidines were able to successfully disperse MSSA biofilms at the highest concentration tested (200 μM). The dispersion of biofilms formed by Gram-negative strains was not investigated.

Table 3 Percent inhibition of biofilm formation at 200 μM for the 2nd generation 2-aminopyrimidines

Cmpd	*P. aeruginosa*	MSSA	MRSA
17	41.7 ± 4.0[a*]	18.3 ± 8.5	20.3 ± 3.7
18	6.98 ± 6.0*	10.2 ± 12.6	7.2 ± 1.0
19	14.0 ± 7.6	0.8 ± 10.0	20.4 ± 17.2
20	1.9 ± 1.6*	24.5 ± 20.2*	9.4 ± 12.1
21	11.6 ± 5.1	34.1 ± 5*	0.6 ± 5.9
22	15.8 ± 4.0*	2.5 ± 4.1	39.6 ± 9.4
23	25.3 ± 9.4*	65.5 ± 4.0	11.9 ± 8.8*
24	20.2 ± 1.3*	3.6 ± 10.2*	2.1 ± 21
25	24.2 ± 4.3*	22.0 ± 13.1	14.7 ± 3.7
26	21.1 ± 3.1*	80.2 ± 2.8	14.5 ± 26.4
27	3.3 ± 0.9*	12.7 ± 3.0	4.6 ± 3.3*
28	23.8 ± 9.9*	3.0 ± 4.1	16.9 ± 8
29	32.6 ± 4.3*	30.3 ± 2	40.7 ± 10.9
30	32.6 ± 5.4*	6.4 ± 10.8	32.9 ± 4.1
31	12.9 ± 7.8*	43.1 ± 8.8*	27.6 ± 5.2
32	0.5 ± 7.9	68.1 ± 6.6*	19.4 ± 7.1
33	22.0 ± 5.0*	27.0 ± 11.4	14.7 ± 11.1
34	14.3 ± 8.7*	27.2 ± 9.4*	10.8 ± 13.5
35	3.6 ± 2.7	29.5 ± 17.7*	9.2 ± 9.9*
36	18.6 ± 4.3*	18.4 ± 18.1	13.3 ± 3.7
37	26.7 ± 6.6*	63.6 ± 3.0	32.0 ± 23.5

[a] ± Indicates one standard deviation; * Indicates biofilm promotion.

We have recently reported synergistic effects between a 2-aminoimidazole derivative and β-lactam antibiotics against MRSA and multi-drug resistant *A. baumannii*.[31] We therefore investigated whether 2-aminopyrimidine derivatives were also able to suppress resistance of MRSA to penicillin G. The minimum inhibitory concentration (MIC) of penicillin G against MRSA (ATCC BAA-44) was determined using a standard broth microdilution assay[32] in media supplemented with each 2-aminopyrimidine derivative at a concentration of 50 μM. The MIC of penicillin G was reduced by four-fold (32 μg mL^{-1} to 8 μg mL^{-1}) in the presence of 2-aminopyrimidines **16** and **37** when compared to penicillin G only controls. Both **16** and **37** were shown by growth curve analysis to have no effect on bacterial growth at 50 μM.

Conclusions

In conclusion, we have developed a novel scaffold of biofilm modulators based upon the 2-aminopyrimidine architecture. The library of 2-aminopyrimidines synthesized in this study has provided three non-toxic as well as two toxic inhibitors of MSSA biofilms (*i.e.*, non-toxic compounds **23**, **26**, and **37**; toxic compounds **10** and **15**). Both **10** and **15** were also shown to inhibit MRSA biofilms in a non-toxic fashion. 2-Aminopyrimidine **10** was also able to inhibit *P. aeruginosa* biofilm formation, *via* a toxic mechanism. Interestingly, **32** displayed the ability promote *S. aureus* biofilm formation in a non-toxic manner. Two members of this 2-aminopyrimidine class of compounds were also able to suppress resistance of MRSA to penicillin G.

Experimental section

All reagents used for chemical synthesis were purchased from commercially available sources and used without further

purification. Chromatography was performed using 60 Å mesh standard grade silica gel from Sorbtech. NMR solvents were obtained from Cambridge Isotope Labs and used as is. All ^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded at 25 °C on Varian Mercury spectrometers. Chemical shifts (δ) are given in ppm relative to tetramethylsilane or the respective NMR solvent; coupling constants (J) are in hertz (Hz). Abbreviations used are s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, bt = broad triplet, qt = quartet, m = multiplet, bm = broad multiplet, p = pentet, and br = broad. Mass spectra were obtained at the NCSU Department of Chemistry Mass Spectrometry Facility. Funding was obtained from the North Carolina Biotechnology Center and the NCSU Department of Chemistry. Infrared spectra were obtained on a FT/IR-4100 spectrophotometer (ν_{max} in cm^{-1}). UV absorbance was recorded on a Genesys 10 scanning UV/visible spectrophotometer (λ_{max} in nm).

Di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate (4)

A mixture of 2-amino-5-iodopyrimidine (5 g, 22.6 mmol) and di-*tert*-butyl dicarbonate (14 g, 64.1 mmol), was dissolved in 40 mL of pyridine. The reaction was placed under a N_2 atmosphere, and heated to 70 °C overnight. The reaction was then cooled to room temperature, and diluted with ethyl acetate. The mixture was then washed with water, followed by brine. The aqueous layer was then back extracted two times with ethyl acetate. The combined organic layers were then dried with Na_2SO_4 and concentrated *in vacuo*. The residue was then purified using silica gel column chromatography, to yield di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate as an orange solid (7.62 g, 80%, m.p. = 134–139 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 2H), 1.43 (s, 18H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 157.6, 150.6, 90.0, 84.1, 28.0 ppm; IR ν_{max} (cm^{-1}) 2943, 1798, 1754, 1657, 1525, 1442, 1296 1164, 1104; HRMS (ESI) calcd for $C_{14}H_{20}IN_3O_4$ (M^+) 444.0391, found 444.0397.

General procedure for the Sonogashira coupling of di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate

Di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate (0.5 mmol) was added to a flame dried vial, followed by the addition of $PdCl_2(PPh_3)_2$ (0.05 mmol) and CuI (0.05 mmol). This mixture was then dissolved in 5 mL of dry acetonitrile. To this the appropriate alkyne (1.1 mmol) was added to the reaction mixture. The reaction was placed under a N_2 atmosphere and triethylamine (3 mmol) was then added to the reaction mixture dropwise. Reaction mixtures were allowed to stir until completion *via* TLC analysis. The solvents were then removed *in vacuo*. The mixture was then taken up in 50 mL of ethyl acetate, and washed with water and brine. The organic layer was then dried using $MgSO_4$, filtered and concentrated. The mixture was then purified *via* silica gel column chromatography.

Di-*tert*-butyl 5-(3-hexanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate

Following the general procedure for the Sonogashira coupling of di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate, di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate was reacted with N-(prop-2-ynyl)hexanamide to produce di-*tert*-butyl 5-(3-hexanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate (0.19 g, 86% yield) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 2H), 5.82 (bs, 1H), 4.29 (d, J = 5.6 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.642–1.274 (m,25H), 0.87 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 160.7 156.9, 150.5, 116.7, 92.8, 84.0, 76.3, 36.6, 31.6, 29.9, 28.0, 25.4, 22.6, 14.1 ppm; IR ν_{max} (cm^{-1}) 3367, 2943, 1798, 1754, 1657, 1525, 1442, 1296 1164; UV (λ_{max} nm) 251; HRMS (ESI) calcd for $C_{23}H_{34}N_4O_5$ (M^+) 469.2421, found 469.2420.

Di-*tert*-butyl 5-(3-octanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate

Following the general procedure for the Sonogashira coupling of di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate, di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate was reacted with N-(prop-2-ynyl)octanamide to produce di-*tert*-butyl 5-(3-octanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate (0.20 g, 85% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 2H), 5.95 (bs, 1H), 4.28 (d, J = 5.6 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.65–1.24 (m, 28H), 0.84 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 160.7, 156.8, 150.5, 116.7, 92.9, 84.0, 76.1, 36.6 31.8, 29.9, 29.4, 29.2, 28.0, 25.7, 22.7, 14.3 ppm; IR ν_{max} (cm^{-1}) 3318, 2943, 2867, 1797, 1762, 1657, 1533, 1428, 1303, 1115; UV (λ_{max} nm) 251; HRMS (ESI) calcd for $C_{25}H_{38}N_4O_5$ (M^+) 497.2734, found 497.2743.

Di-*tert*-butyl 5-(3-decanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate

Following the general procedure for the Sonogashira coupling of di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate, di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate was reacted with N-(prop-2-ynyl) decanamide to produce di-*tert*-butyl 5-(3-decanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate (0.22 g, 87% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 2H), 5.75 (bs, 1H), 4.30 (d, J = 5.2 Hz, 2H), 2.21 (t, J = 7.6 Hz, 2H), 1.65–1.23 (m, 32H), 0.85 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 160.7, 156.9, 150.6, 116.7, 92.8, 84.0, 76.3, 36.7, 32.1, 30.0, 29.7, 29.6, 29.5. 29.4, 28.1, 25.8, 22.9, 14.3 ppm; IR ν_{max} (cm^{-1}) 3377, 2929, 2853, 1797, 1762, 1655, 1536, 1432, 1374, 1291, 1252, 1154, 1115; UV (λ_{max} nm) 251; HRMS (ESI) calcd for $C_{27}H_{42}N_4O_5$ (M^+) 525.3047, found 525.3049.

Di-*tert*-butyl 5-(3-dodecanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate

Following the general procedure for the Sonogashira coupling of di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate, di-*tert*-butyl 5-iodopyrimidin-2-ylcarbamate was reacted with N-(prop-2-ynyl) dodecanamide to produce di-*tert*-butyl 5-(3-dodecanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate (0.22 g, 82% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 2H), 5.80 (bs, 1H), 4.29 (d, J = 5.2 Hz, 2H), 2.21 (t, J = 7.2 Hz, 2H), 1.65–1.23 (m, 36H), 0.85 (J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz,

$CDCl_3$) δ 173.1, 160.7, 156.9, 150.6, 116.7, 92.8, 84.0, 76.3, 36.7, 32.1, 29.9, 28.8, 29.8, 29.7, 29.6, 29.5, 28.0, 25.7, 22.9, 14.3 ppm; IR ν_{max} (cm^{-1}) 3377, 2923, 2853, 1797, 1761, 1660, 1532, 1443, 1373, 1293, 1241, 1153, 1108 UV (λ_{max} nm) 249; HRMS (ESI) calcd for $C_{27}H_{42}N_4O_5$ (M^+) 553.336, found 553.334.

General procedure for alkyne reduction

The appropriate pyrimidine containing alkyne (0.25 mmol) was dissolved in 5 mL ethyl acetate. The reaction mixture was cooled to 0 °C and charged with 10% Pd/C. The reaction mixture was then placed under a 65 psi atmosphere of H_2. The reaction was allowed to stir for 16 h. Upon completion the reaction mixture was filtered through Celite and concentrated *in vacuo*.

Di-*tert*-butyl 5-(3-hexanamidopropyl)pyrimidin-2-ylcarbamate

Following the general procedure for alkyne reduction, di-*tert*-butyl 5-(3-hexanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give di-*tert*-butyl 5-(3-hexanamidopropyl)pyrimidin-2-ylcarbamate (0.12, 99%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 2H), 5.61 (bs, 1H), 3.30 (q, J = 6.8 Hz, 2H), 2.64 (t, J = 7.2 Hz, 2H), 2.15 (t, J = 7.6 Hz, 2H), 1.82 (p, J = 7.6 Hz, 2H), 1.61 (p, J = 7.2 Hz, 2H), 1.39 (s, 18H), 1.27 (m, 4H), 0.86 (t, J = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 158.5, 157.2, 151.1, 132.6, 83.6, 39.0, 37.0, 31.7, 31.2, 28.1, 27.5, 25.6, 22.6, 14.2 ppm; IR ν_{max} (cm^{-1}) 3399, 2951, 2860, 1791, 1757, 1652, 1543, 1429, 1370, 1290, 1255, 1159, 1105; UV (λ_{max} nm) 225; HRMS (ESI) calcd for $C_{23}H_{38}N_4O_5$ (M^+) 473.2734, found 473.2737.

Di-*tert*-butyl 5-(3-octanamidopropyl)pyrimidin-2-ylcarbamate

Following the general procedure for alkyne reduction, di-*tert*-butyl 5-(3-octanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give di-*tert*-butyl 5-(3-octanamidopropyl)pyrimidin-2-ylcarbamate (0.12 g, 99%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 2H), 5.65 (bs, 1H), 3.29 (q, J = 6.4 Hz, 2H), 2.62 (t, J = 7.6 Hz, 2H), 2.14 (t, J = 7.6 Hz, 2H), 1.81 (p, J = 7.6 Hz, 2H), 1.61–1.24 (m, 24H), 0.84 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 158.5, 157.2, 151.1, 132.6, 83.6, 39.0, 37.0, 31.9, 31.1, 29.4, 29.2, 28.1, 27.5, 26.0, 22.8, 14.2 ppm; IR ν_{max} (cm^{-1}) 3398, 2956, 2853, 1791, 1750, 1644, 1559, 1434, 1361, 1304, 1247, 1161, 1113; UV (λ_{max} nm) 227; HRMS (ESI) calcd for $C_{25}H_{42}N_4O_5$ (M^+) 501.3047, found 501.3044.

Di-*tert*-butyl 5-(3-decanamidopropyl)pyrimidin-2-ylcarbamate

Following the general procedure for alkyne reduction, di-*tert*-butyl 5-(3-decanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give di-*tert*-butyl 5-(3-decanamidopropyl)pyrimidin-2-ylcarbamate (0.13 g, 99%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 2H), 5.55 (bs, 1H), 3.31 (q, J = 7.2 Hz, 2H), 2.64 (t, J = 8 Hz, 2H), 2.15 (t, J = 7.2 Hz, 2H), 1.82 (p, J = 7.6 Hz, 2H), 1.60 (m, 2H), 1.42 (m, 18H), 1.26 (m, 12H), 0.84 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 158.5, 157.2, 151.1, 132.6, 83.7, 39.0, 37.0, 32.0, 31.2, 29.6, 29.6, 29.5, 29.5, 28.1, 27.5, 26.0, 22.9, 14.3 ppm; IR ν_{max} (cm^{-1}) 3419, 2956, 2853, 1793, 1734, 1655, 1560, 1455, 1433, 1372, 1288, 1252, 1163, 1121; UV (λ_{max} nm) 228; HRMS (ESI) calcd for $C_{27}H_{46}N_4O_5$ (M^+) 529.336, found 529.3363.

Di-*tert*-butyl 5-(3-dodecanamidopropyl)pyrimidin-2-ylcarbamate

Following the general procedure for alkyne reduction, di-*tert*-butyl 5-(3-dodecanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give di-*tert*-butyl 5-(3-dodecanamidopropyl)pyrimidin-2-ylcarbamate (0.13 g, 99%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (s, 2H), 5.96 (t, J = 5.7 Hz, 1H), 3.25 (q, J = 6.6 Hz, 2H), 2.60 (t, J = 7.5 Hz, 2H), 2.11 (t, J = 7.5 Hz, 2H), 1.78 (p, J = 7.5 Hz, 2H), 1.58–1.51 (m, 2H), 1.44–1.18 (m, 34H), 0.80 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 158.4, 157.0, 150.9, 132.7, 83.5, 38.8, 36.8, 32.0, 31.0, 29.7, 29.6, 29.5, 29.4, 29.4, 28.0, 27.4, 25.9, 22.8, 14.2 ppm; IR ν_{max} (cm^{-1}) 3288, 3364, 2929, 2853, 1788, 1733, 1650, 1554, 1436, 1367, 1297, 1153, 1111 UV (λ_{max} nm) 226; HRMS (ESI) calcd for $C_{29}H_{50}N_4O_5$ (M^+) 535.3854, found 535.3851.

General procedure for Boc-deprotection

The appropriate Boc-protected amine was dissolved in a 1 : 20 trifluoroacetic acid : dichloromethane mixture and stirred for 16 h. Upon completion, the reaction mixture was concentrated *in vacuo* and then left on a high vacuum overnight. Methanol supplemented with HCl was added to the product forming the HCl salt of the deprotected product and then concentrated *in vacuo*. The resulting residue was washed with pentane and then placed on a high vacuum overnight.

N-(3-(2-Aminopyrimidin-5-yl)prop-2-ynyl)hexanamide hydrochloride (9)

Following the general Boc-deprotection procedure di-*tert*-butyl 5 di-*tert*-butyl 5-(3-hexanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)prop-2-ynyl)hexanamide hydrochloride (0.05 g, 99%) as a tan solid (m.p. = 150–152 °C). ^{1}H NMR (300 MHz, CD_3OD) δ 8.66 (s, 2H), 4.20 (s, 2H), 2.23 (t, J = 7.6 Hz, 2H), 1.62 (p, J = 6.9 Hz, 2H), 1.34 (m, 4H), 0.90 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 176.3, 160.0, 156.1, 108.7, 92.4, 74.2, 36.9, 32.6, 30.2, 26.7, 23.6, 14.5 ppm; IR ν_{max} (cm^{-1}) 3296, 3170, 2921, 2850, 1693, 1643, 1602, 1533, 1504, 1467, 1413, 1396, 1348, 1300, 661; HRMS (ESI) calcd for $C_{13}H_{18}N_4O$ (M^+) 247.1553, found 247.1555.

N-(3-(2-Aminopyrimidin-5-yl)prop-2-ynyl)octanamide hydrochloride (10)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-octanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)prop-2-ynyl)octanamide hydrochloride (0.05 g, 99%) as a tan solid (m.p. = 154–156 °C).

^{1}H NMR (400 MHz, DMSO-d6) δ 8.35 (s, 2H), 4.4–3.8 (bs, 6H + H_2O), 2.08 (t, J = 7.2 Hz, 2H), 1.48 (p, J = 7.2 Hz, 2H), 1.23 (bs, 8H), 0.84 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d6) δ 172.0, 159.5, 158.3, 105.9, 90.7, 75.1, 35.1, 31.3, 28.7, 28.6, 28.5, 25.2, 22.1, 14.0 ppm; IR ν_{max} (cm^{-1}) 3296, 3168, 2921, 2850, 1693, 1643, 1602, 1533, 1504, 1467, 1413, 1396, 1348, 1300, 661; HRMS (ESI) calcd for $C_{15}H_{22}N_4O$ (M^+) 275.1866, found 275.1868.

N-(3-(2-Aminopyrimidin-5-yl)prop-2-ynyl)decanamide hydrochloride (11)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-decanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)prop-2-ynyl)decanamide hydrochloride (0.04 g, 99%) as a tan solid (m.p. = 154–156 °C). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.41 (s, 2H), 4.66 (bs, 4H), 4.30 (d, J = 5.2 Hz, 2H), 2.08 (t, J = 7.6 Hz, 2H), 1.48 (m, 2H), 1.22 (m, 12H), 0.84 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d6) δ 172.0, 159.8, 159.7, 105.9, 90.2, 75.8, 35.1, 31.3, 29.0, 28.8, 28.7, 28.7, 28.5, 25.2, 22.1, 14.0 ppm; IR ν_{max} (cm^{-1}) 3295, 2921, 2850, 1693, 1643, 1533, 1467, 1396, 1348, 1226, 1195, 661; HRMS (ESI) calcd for $C_{17}H_{26}N_4O$ (M^+) 303.2179, found 303.2179.

N-(3-(2-Aminopyrimidin-5-yl)prop-2-ynyl)dodecanamide hydrochloride (12)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-dodecanamidoprop-1-ynyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)prop-2-ynyl) dodecanamide hydrochloride (0.04 g, 99%) as a tan solid (m.p. = 154–156 °C). ^{1}H NMR (300 MHz, DMSO-d6) δ 8.38 (s, 2H), 4.49 (bs, 4H), 4.08 (d, J = 5.7 Hz, 2H), 2.08 (t, J = 7.2 Hz, 2H), 1.48 (p, J = 6.9 Hz, 2H), 1.22 (s, 12H), 0.84 (t, J = 6.3 Hz, 3H) ppm; ^{13}C NMR (100 MHz, DMSO-d6) δ 172.0, 160.2, 160.0 105.9, 90.0, 76.0, 35.1, 31.3, 29.1, 29.0, 28.8, 28.8, 28.7, 28.6, 25.2, 22.1, 14.1 ppm; IR ν_{max} (cm^{-1}) 3297, 3176, 2919, 2850, 1693, 1641, 1602, 1553, 1504, 1467, 1415, 661; HRMS (ESI) calcd for $C_{19}H_{30}N_4O$ (M^+) 331.2492, found 331.2943.

N-(3-(2-Aminopyrimidin-5-yl)propyl)hexanamide hydrochloride (13)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-hexanamidopropyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)propyl)hexanamide hydrochloride (.04, 99%) as a tan solid (m.p. = 138–142 °C). ^{1}H NMR (400 MHz, CD_3OD) δ 8.54 (s, 2H), 3.34 (m, 2H), 2.65 (t, J = 8 Hz, 2H), 2.375 (t, J = 7.2 Hz, 2H), 1.88 (p, J = 7.2 Hz, 2H), 1.65 (p, J = 8 Hz), 1.39–1.30 (m, 4H), 0.92 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 178.5, 157.7, 156.5, 124.8, 40.9, 35.8, 32.5, 30.2, 27.0, 26.9, 23.4, 14.4 ppm; IR ν_{max} (cm^{-1}) 3303, 3158, 2921, 2850, 1702, 1658, 1637, 1538, 1467, 1396; HRMS (ESI) calcd for $C_{13}H_{22}N_4O$ (M^+) 251.1866, found 251.1869.

N-(3-(2-Aminopyrimidin-5-yl)propyl)octanamide hydrochloride (14)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-octanamidopropyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)propyl)octanamide hydrochloride (0.05 g, 99%) as a tan solid (m.p. = 140–142 °C). ^{1}H NMR (400 MHz, CD_3OD) δ 8.50 (s, 2H), 3.24 (t, J = 6.8 Hz, 2H), 2.62 (t, J = 8 Hz, 2H), 2.25 (t, 8 Hz, 2H), 1.83 (p, J = 8 Hz, 2H), 1.62 (p, J = 8 Hz, 2H), 1.33–1.31 (m, 8H), 0.90 (t, J = 8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 177.6, 157.7, 156.5, 125.0, 40.1, 36.5, 32.9, 30.5, 30.3, 30.1, 27.3, 27.0, 23.7, 14.5 ppm; IR ν_{max} (cm^{-1}) 3303, 3156, 2922, 2850, 1701, 1658, 1636, 1539, 1467, 1395; HRMS (ESI) calcd for $C_{15}H_{26}N_4O$ (M^+) 279.2179, found 279.2181.

N-(3-(2-Aminopyrimidin-5-yl)propyl)decanamide hydrochloride (15)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-decanamidopropyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)propyl)decanamide hydrochloride (0.03 g, 99%) as a tan solid (m.p. = 144–148 °C). ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, 2H), 3.20 (t, J = 6.4 Hz, 2H), 2.60 (t, J = 8 Hz, 2H), 2.19 (t, J = 8 Hz, 2H), 1.81 (p, J = 8 Hz, 2H), 1.60 (p, J = 8 Hz, 2H), 1.32–1.29 (m, 12H), 0.89 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, CD_3OD) δ 175.3, 156.4, 155.4, 123.8, 37.8, 36.0, 31.9, 29.8, 29.4, 29.3, 29.2, 29.2, 25.9, 25.7, 22.6, 13.3 ppm; IR ν_{max} (cm^{-1}) 3303, 3158, 2921, 2850, 1702, 1658, 1637, 1538, 1467, 1396, 1211; HRMS (ESI) calcd for $C_{17}H_{30}N_4O$ (M^+) 307.2492, found 307.2498.

N-(3-(2-Aminopyrimidin-5-yl)propyl)dodecanamide hydrochloride (16)

Following the general Boc-deprotection procedure di-*tert*-butyl 5-(3-dodecanamidopropyl)pyrimidin-2-ylcarbamate was reacted to give *N*-(3-(2-aminopyrimidin-5-yl)propyl)dodecanamide hydrochloride (0.04 g, 99%) as a tan solid (m.p. = 145–147 °C). ^{1}H NMR (400 MHz, CD_3OD) δ 8.50 (s, 2H), 3.21 (t, J = 8 Hz, 2H), 2.61 (t, J = 8 Hz, 2H), 2.20 (t, J = 8 Hz, 2H), 1.81 (p, J = 8 Hz, 2H), 1.61 (p, J = 8 Hz, 2H), 1.32–1.29 (m, 16H), 0.90 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d6) δ 172.2, 156.5, 155.3, 122.9, 37.2, 35.4, 31.3, 29.8, 29.0, 29.0, 28.8, 28.8, 25.3, 22.1, 14.0 ppm; IR ν_{max} (cm^{-1}) 3301, 3159, 2925, 2855, 1704, 1659, 1632, 1535, 1467, 1391; HRMS (ESI) calcd for $C_{19}H_{34}N_4O$ (M^+) 335.2805, found 335.2805.

5-Ethynylpyrimidin-2-amine (17)

Was synthesized following the general procedure by H. Zhao *et al.*[33]

General procedure for click reactions

The terminal alkyne (1.0 mmol.) was dissolved in a 1 : 1 : 1 mixture of *tert*-butanol, water and methylene chloride (*ca.* 3 mL per 0.200 g of terminal alkyne). To this mixture, the

appropriate azide (1.2 mmol.) was added while stirring vigorously at room temperature. Copper(II) sulfate (15 mol%) and sodium ascorbate (45 mol%) were then added sequentially to the solution. Reaction mixtures were allowed to stir until completion *via* TLC analysis (12–24 h). The solvents were then removed *in vacuo* in which the resulting residue was purified *via* silica gel column chromatography.

5-(1-Hexyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (18)

Following the general procedure for the click reaction 5-ethynylpyrimidin-2-amine was reacted with 1-azidohexane to give 5-(1-hexyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (0.10 g, 40%) as a white solid (m.p. = 164–166 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 2H), 7.70 (s, 1H), 5.60 (bs, 2H), 4.37 (t, J = 6.8 Hz, 2H), 1.92 (m, 2H), 1.30 (bs, 6H), 0.86 (t, J = 6.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 155.8, 142.9, 118.7, 115.7, 50.7, 31.3, 30.4, 26.3, 22.6, 14.1 ppm; IR ν_{max} (cm^{-1}) 3455, 3305, 3160, 2954, 2923, 2854, 2684, 1737, 1666, 1643, 1563, 1548, 1488, 1348, 1224, 798; HRMS (ESI) calcd for $C_{12}H_{18}N_6$ (M^+) 47.1666, found 247.1673.

5-(1-Heptyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (19)

Following the general procedure for the click reaction 5-ethynylpyrimidin-2-amine was reacted with 1-azidoheptane to give 5-(1-heptyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (0.12 g, 47%) as a white solid (m.p. = 168–170 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 2H), 7.66 (s, 1H), 5.26 (bs, 2H), 4.38 (t, J = 6.8 Hz, 2H), 1.93 (m, 2H), 1.33–1.26 (m, 8H), 0.86 (t, J = 7.2 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 155.8, 142.8, 118.7, 115.7, 50.7, 31.7, 30.5, 28.8, 26.6, 22.7, 14.2 ppm; IR ν_{max} (cm^{-1}) 3455, 3305, 3160, 2954, 2923, 2854, 2684, 1737, 1666, 1643, 1563, 1548, 1488, 1348, 1224, 798; HRMS (ESI) calcd for $C_{13}H_{20}N_6$ (M^+) 261.1822, found 261.1832.

5-(1-Nonyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (20)

Following the general procedure for the click reaction 5-ethynylpyrimidin-2-amine was reacted with 1-azidononane to give 5-(1-nonyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (0.14 g, 50%) as a white solid (m.p. = 178–180 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 2H), 7.67 (s, 1H), 5.18 (bs, 2H), 4.38 (t, J = 7.2 Hz, 2H), 1.92 (m, 2H), 1.66 (m, 2H), 1.33–1.24 (m, 12H), 0.85 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 155.9, 143.0, 118.7, 116.0, 50.8, 32.0, 30.6, 29.5, 29.4, 29.2, 26.7, 22.8, 14.3 ppm; IR ν_{max} (cm^{-1}) 3455, 3305, 3160, 2954, 2923, 2854, 2684, 1737, 1666, 1643, 1563, 1548, 1488, 1348, 1224, 798; HRMS (ESI) calcd for $C_{15}H_{24}N_6$ (M^+) 289.2148, found 289.2144.

5-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (21)

Following the general procedure for the click reaction 5-ethynylpyrimidin-2-amine was reacted with benzylazide to give 5-(1-benzyl-1*H*-1,2,3-triazol-4-yl)pyrimidin-2-amine (0.14 g, 55%) as a white solid (m.p. = 195–196 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 2H), 7.58 (s, 1H), 7.39–7.29 (m, 5H), 5.56 (s, 2H), 5.18 (s, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 155.8, 143.5, 134.6, 129.5, 129.2, 128.3, 118.7, 116.0, 54.6 ppm; IR ν_{max} (cm^{-1}) 3455, 3305, 3160, 2954, 2923, 2854, 2684, 1737, 1666, 1643, 1563, 1548, 1488, 1348, 1224, 798; HRMS (ESI) calcd for $C_{13}H_{12}N_6$ (M^+) 253.1196, found 253.1203.

General procedure for the Sonogashira coupling of 2-amino-5-iodpyrimidine

2-Amino-5-iodpyrimidine (0.5 mmol) was added to a flame dried vial, followed by the addition of $PdCl_2(PPh_3)_2$ (0.05 mmol) and CuI (0.05 mmol). This mixture was then dissolved in 5 mL of dry THF. To this the appropriate alkyne (1.1 mmol) was added to the reaction mixture. The reaction was placed under a N_2 atmosphere and triethylamine (3 mmol) was then added to the reaction mixture dropwise. The reaction mixture was then heated to reflux and allowed to stir until completion *via* TLC analysis. The solvents were then removed *in vacuo*. The mixture was then taken up in 50 mL of ethyl acetate, and washed with water and brine. The organic layer was then dried using $MgSO_4$, filtered and concentrated. The mixture was then purified *via* silica gel column chromatography.

5-(3-Cyclohexylprop-1-ynyl)pyrimidin-2-amine (23)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with prop-2-ynylcyclohexane to yield 5-(3-cyclohexylprop-1-ynyl)pyrimidin-2-amine (0.10 g, 94%) as a tan solid (m.p. = 144–145 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 2H), 5.34 (bs, 2H), 2.28 (d, J = 6.8 Hz, 2H) 1.85–1.64 (m, 5H), 1.57–1.49 (m,1H), 1.32–0.98 (m, 5H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.6, 110.0, 92.8, 72.5, 37.6, 33.0, 27.5, 26.4, 26.3 ppm; IR ν_{max} (cm^{-1}) 3328, 3180, 2948, 2863, 2719, 1664, 1596, 1544, 1527, 1506, 798; HRMS (ESI) calcd for $C_{13}H_{17}N_3$ (M^+) 216.1495, found 216.1502

5-(Cyclohexylethynyl)pyrimidin-2-amine (24)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with ethynylcyclohexane to yield 5-(cyclohexylethynyl)pyrimidin-2-amine (0.08 g, 78%) as a tan solid (m.p. = 192–196 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 2H0, 5.19 (bs, 2H), 2.56 (septet, J = 4.8 Hz, 1H), 1.85 (bs, 4H), 1.76–1.72 (m, 2H), 1.56–1.45 (m, 2H), 1.36–1.33 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.6, 110.2, 98.0, 74.6, 32.8, 30.0, 26.1, 25.2 ppm; IR ν_{max} (cm^{-1}) 3328, 3180, 2948, 2863, 2719, 1664, 1596, 1544, 1527, 1506, 798; HRMS (ESI) calcd for $C_{12}H_{15}N_3$ (M^+) 202.1339, found 202.1343.

5-(3-Cyclopentylprop-1-ynyl)pyrimidin-2-amine (25)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with prop-2-ynylcyclopentane to yield 5-(3-cyclopentylprop-1-ynyl)pyrimidin-2-amine (0.08 g, 75%) as a tan solid (m.p. = 145–147 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 2H), 5.24 (bs, 2H), 2.39 (d, J = 6.4 Hz, 2H), 2.11 (septet, J = 7.2 Hz, 1H), 1.86–1.78 (m, 2H), 1.69–1.52

(m, 4H), 1.35–1.25 (m, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0,160.6, 110.1, 93.5, 74.7, 39.2, 32.3, 25.4 ppm; IR ν_{max} (cm^{-1}) 3328, 3180, 2948, 2863, 2719, 1664, 1596, 1544, 1527, 1506, 798; HRMS (ESI) calcd for $C_{12}H_{15}N_3$ (M^+) 202.1339, found 202.1346.

5-(Cyclopentylethynyl)pyrimidin-2-amine (26)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with ethynylcyclopentane to yield 5-(cyclopentylethynyl)pyrimidin-2-amine (0.08 g, 80%) as a tan solid (m.p. = 204–208 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 2H), 5.31 (bs, 2H), 2.79 (p, J = 7.6 Hz, 1H), 2.00–1.96 (m, 2H), 1.83–1.58 (m, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 160.6, 110.1, 98.1, 74.2, 34.0, 31.0, 25.4 ppm; IR ν_{max} (cm^{-1}) 3328, 3180, 2948, 2863, 2719, 1664, 1596, 1544, 1527, 1506, 798; HRMS (ESI) calcd for $C_{11}H_{13}N_3$ (M^+) 188.1182, found 188.1187.

5-(4-Phenylbut-1-ynyl)pyrimidin-2-amine (27)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with but-3-ynylbenzene to yield 5-(4-phenylbut-1-ynyl)pyrimidin-2-amine (0.09 g, 83%) as a tan solid (m.p. = 187–192 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 2H), 7.32–7.21 (m, 5H), 5.15 (bs, 2H), 2.89 (t, J = 7.2 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 160.6, 140.6, 128.7, 128.6, 126.6, 109.8, 93.0, 75.6, 35.2, 21.9 ppm; IR ν_{max} (cm^{-1}) 3328, 3183, 3027, 2962, 2927, 2857, 2717, 1666, 1596, 1542, 1508, 1454, 1220, 696; HRMS (ESI) calcd for $C_{14}H_{13}N_3$ (M^+) 224.1182, found 224.1178.

5-(Hex-1-ynyl)pyrimidin-2-amine (28)

Was synthesized following the general procedure by Balzarini *et al.*[34]

5-(Hept-1-ynyl)pyrimidin-2-amine (29)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with 1-heptyne to yield 5-(hept-1-ynyl)pyrimidin-2-amine (0.09 g, 99%) as a tan solid (m.p. = 147–149 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 2H), 5.14 (bs, 2H), 2.36 (t, J = 7.6 Hz, 2H), 1.57 (p, J = 7.2 Hz, 2H), 1.43–1.30 (m, 4H), 0.90 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 160.5, 110.1, 93.6, 74.7, 31.3, 28.5, 22.4, 19.6, 14.2 ppm; IR ν_{max} (cm^{-1}) 3334, 3181, 2931, 2867, 1656, 1594, 1542, 1527, 1504, 1375, 1222, 939, 798, 665, 565, 520, 453; HRMS (ESI) calcd for $C_{11}H_{15}N_3$ (M^+) 190.1339, found 190.1338.

5-(Oct-1-ynyl)pyrimidin-2-amine (30)

Was synthesized following the general procedure by Odashima *et al.*[35]

5-(Non-1-ynyl)pyrimidin-2-amine (31)

Following the general Sonogashira coupling procedure, 2-amino-5-iodpyrimidine was reacted with 1-nonyne to yield 5-(non-1-ynyl)pyrimidin-2-amine (0.09 g, 99%) as a tan solid (m.p. = 136–138 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 2H), 5.24 (bs, 2H), 2.38, (t, J = 6.8 Hz, 2H), 1.58 (p, J = 7.2 Hz, 2H), 1.42 (m, 2H), 1.30 (m, 6H), 0.89 (t, J = 6.8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 160.6, 110.1, 94.1, 74.6, 31.9, 29.1, 29.0, 28.8, 22.8, 06.7, 14.3 ppm; IR ν_{max} (cm^{-1}) 3335, 3181, 2930, 2867, 1658, 1595, 1544, 1527, 1504, 1375, 1222, 941, 796, 665, 567, 520, 455; HRMS (ESI) calcd for $C_{13}H_{19}N_3$ (M^+) 218.1652, found 218.1661.

General procedure for the Suzuki coupling of 2-amino-5-iodpyrimidine

2-Amino-5-iodpyrimidine (0.5 mmol) was added to a vial, followed by the addition of $PdCl_2(PPh_3)_2$ (0.025 mmol). This mixture was then dissolved in 5 mL of THF. To this the appropriate boronic acid (1.0 mmol) was added to the reaction mixture, followed by K_2CO_3(2 M, 2 mmol). The reaction was placed under a N_2 atmosphere, heated to reflux, and allowed to stir until completion *via* TLC analysis. The solvents were then removed *in vacuo*. The mixture was then taken up in 50 mL of ethyl acetate, and washed with saturated $NaHCO_3$, water, and brine. The organic layer was then dried using $MgSO_4$, filtered and concentrated. The mixture was then purified *via* silica gel column chromatography.

5-(Naphthalen-1-yl)pyrimidin-2-amine (32)

Was synthesized following the general procedure by Murali *et al.*[36]

5-(Naphthalen-2-yl)pyrimidin-2-amine (33)

Was synthesized following the general procedure by Mattay *et al.*[37]

5-(Thiophen-3-yl)pyrimidin-2-amine (34)

Was synthesized following the general procedure by Pallavi *et al.*[38]

5-(Furan-3-yl)pyrimidin-2-amine (35)

Following the general Suzuki coupling procedure, 2-amino-5-iodpyrimidine was reacted with 3-furylboronic acid to 5-(furan-3-yl)pyrimidin-2-amine (0.06 g, 80%) as a tan solid (m.p. = 138–140 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 2H), 7.64 (s, 1H), 7.48 (d, J = 1.6 Hz, 1H), 6.59 (d, J = 1.6 Hz, 1H), 5.05 (bs, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 1162.2, 155.8, 144.3, 137.8, 120.7, 117.2, 108.4 ppm; IR ν_{max} (cm^{-1}) 3315, 3170, 1670, 1612, 1579, 1513, 1160, 1056, 1012, 771; HRMS (ESI) calcd for $C_8H_7N_3O$ (M^+) 162.0662, found 162.0663.

5-(3-Nitrophenyl)pyrimidin-2-amine (36)

Was synthesized following the general procedure by Pallavi *et al.*[38]

5-(4-Butylphenyl)pyrimidin-2-amine (37)

Following the general Suzuki coupling procedure, 2-amino-5-iodpyrimidine was reacted with 4-butylphenylboronic acid to 5-(4-butylphenyl)pyrimidin-2-amine (.10 g, 90%) as a tan solid (m.p. = 162–167 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 2H), 7.39 (d, J = 8 Hz, 2H), 7.26 (d, J = 8 Hz, 2H), 5.30 (bs, 2H), 2.65 (t, J = 8 Hz, 2H), 1.62 (p, J = 8 Hz, 2H), 1.37 (sextet, J = 8 Hz, 2H), 0.94 (t, J = 8 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.2, 156.6, 142.8, 132.7, 129.5, 126.2, 125.2, 35.5, 33.9, 22.6, 14.2 ppm; IR ν_{max} (cm^{-1}) 3451, 3305, 3170, 3019, 2954, 2925, 2856, 1671, 1643, 1600, 1569, 1550, 1533, 1500, 829; HRMS (ESI) calcd for $C_{14}H_{17}N_3$ (M^+) 228.1495, found 228.15.

Procedure to determine the inhibitory effect of test compounds on *S. aureus*, PA14, and *A. baumannii* biofilm formation

Inhibition assays were performed by taking an overnight culture of bacterial strain and subculturing it at an OD_{600} of 0.01 into the necessary medium (tryptic soy broth with a 0.5% glucose supplement (TSBG) for MRSA (ATCC # BAA-44) and *S. aureus* (ATCC # 29213), Luria-Bertani (LB) medium for *A. baumannii* (ATCC # 19606) and Luria-Bertani medium without NaCl (LBNS) for PA14. Stock solutions of predetermined concentrations of the test compound were then made in the necessary medium. These stock solutions were aliquoted (100 μL) into the wells of the 96-well PVC microtiter plate. Sample plates were then wrapped in GLAD Press n' Seal® followed by an incubation under stationary conditions for 24 h at 37 °C (6 h for PA14). After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 110 μL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 μL of 95% ethanol. A sample of 125 μL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantitated by measuring the OD_{540} of each well and calculated as a percentage of the control (no compound), a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

Procedure to determine the dispersal effect of test compounds on *S. aureus* pre-formed biofilms

Dispersion assays were performed by taking an overnight culture of *S. aureus* (ATCC # 29213) and subculturing it at an OD_{600} of 0.01 into TSBG. The resulting bacterial suspension was aliquoted (100 μL) into the wells of a 96-well PVC microtiter plate. Plates were then wrapped in GLAD Press n' Seal® followed by an incubation under stationary conditions at 37 °C to establish the biofilms. After 24 h, the medium was discarded from the wells and the plates were washed thoroughly with water. Stock solutions of predetermined concentrations of the test compound were then made in the necessary medium. These stock solutions were aliquoted (100 μL) into the wells of the 96-well PVC microtiter plate with the established biofilms. Medium alone was added to a subset of the wells to serve as a control. Sample plates were then incubated for 24 h at 37 °C. After incubation, the medium was discarded from the wells and the plates were washed thoroughly with water. Plates were then stained with 110 μL of 0.1% solution of crystal violet (CV) and then incubated at ambient temperature for 30 min. Plates were washed with water again and the remaining stain was solubilized with 200 μL of 95% ethanol. A sample of 125 μL of solubilized CV stain from each well was transferred to the corresponding wells of a polystyrene microtiter dish. Biofilm inhibition was quantitated by measuring the OD_{540} of each well and calculated as a percentage of the control (no compound), a negative control lane wherein no biofilm was formed served as a background and was subtracted out.

Procedure to determine the effect of test compounds at IC_{50} concentrations on planktonic viability *via* growth curve analysis

Growth curves were performed by taking an overnight culture of bacterial strain and subculturing it at an OD_{600} of 0.01 into the necessary medium (LB for *A. baumannii*, LBNS for PA14, TSBG for *S. aureus* and MRSA. The resulting bacterial suspension was then aliquoted (3.0 mL) into culture tubes. The test compound was then added at a predetermined concentration. Controls were employed in which no test compound was added to the bacterial suspension. Samples were then placed in an incubator at 37 °C and shaken at 200 rpm. The OD_{600} of the samples was measured at time intervals starting at 2 h and ending at 24 h.

Broth microdilution method for antibiotic resensitization

Mueller–Hinton broth (MHB) was inoculated (5×10^5 CFU/mL) with MRSA (BAA-44). Aliquots (4 mL) of the resulting bacterial suspension were distributed to culture tubes and compound, from 100 mM DMSO stock, was added to give the final testing concentration. Bacteria not treated with compound served as the control. After sitting for 30 min at room temperature, 1 mL of each sample was transferred to a new culture tube and penicillin G sodium salt was added from 128 mg mL^{-1} H_2O stock to give a final concentration of 128 μg mL^{-1}. Rows 2–12 of a 96-well microtiter plate were filled (100 μL per well) from the remaining 3 mL bacterial subcultures, allowing the concentration of compound to be kept uniform throughout the antibiotic dilution procedure. After standing for 10 min, aliquots (200 μL) of the samples containing antibiotic were distributed to the corresponding first-row wells of the microtiter plate. Row 1 wells were mixed six to eight times, and then 100 μL were transferred to row 2. Row 2 wells were mixed six to eight times, followed by a 100 μL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate, with the exception of the final row, to which no antibiotic was added (to check for growth of bacteria in the presence of

compound alone). The plate was then covered and incubated under stationary conditions at 37 °C. After 16 h, MIC values were recorded as the lowest concentration of antibiotic at which no visible growth of bacteria was observed. To ensure that the compounds were non-toxic growth curve analysis was performed at the tested concentrations in MHB as described above, from a starting inoculation of 5×10^5 CFU mL^{-1}.

Acknowledgements

The authors would like to thank the NIH (GM GM055769) for financial support.

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