Organic & Biomolecular Chemistry

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Cite this: *Org. Biomol. Chem.*, 2011, **9**, 3041

www.rsc.org/obc

PAPER

Synthesis and biological activity of 2-aminoimidazole triazoles accessed by Suzuki–Miyaura cross-coupling†

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Received 23rd October 2010, Accepted 3rd February 2011
DOI: 10.1039/c0ob00925c

A pilot library of 2-aminoimidazole triazoles (2-AITs) was synthesized and assayed against *Acinetobacter baumannii* and methicillin-resistant *Staphylococus aureus* (MRSA). Results from these studies show that these new derivatives have improved biofilm dispersal activities as well as antibacterial properties against *A. baumannii*. With MRSA biofilms they are found to possess biofilm inhibition capabilities at low micromolar concentrations.

Introduction

Biofilms are ubiquitous in nature.[1] They are formed when planktonic bacteria attach onto a surface, and create an encased bacterial community with an organized structure at an extracellular polymeric interface.[2] Biofilm formation is a serious medical concern as biofilm-associated infections account for nearly 80% of microbial infections in humans.[3] Biofilms are implicated in several diseases that range from cystic fibrosis[4,5] to tooth decay[6] and nosocomial infections.[7] Outside of medicine, bacteria enclosed within biofilms are the underlying cause of biofouling which is a serious problem for the shipping industry.[8] Biofilms also pose severe problems that are detrimental in food processing plants and agriculture products.[9]

Acinetobacter baumannii is a gram-negative bacterium that has been recognized as a human pathogen since the 1970s.[10] Recent reports show that there is an increasing number of infections attributed to *A. baumannii*, most notably in intensive care units.[11,12] *A. baumannii* infections also occur in victims of traumatic disasters such as earthquakes[13] and war.[14] The survival capability of this bacterium in hospital settings and other sterile environment is attributed to the ability of *A. baumannii* to form tenaciously persistent biofilms.[15]

Methicillin-resistant *Staphylococus aureus* (MRSA) infections, which are traditionally acquired from hospital settings, have been emerging outside long-term care facilities and have become common within society.[16] More of a concern is that risk factors that are typically associated with MRSA colonization are noticeably absent from recent reports of community-acquired MRSA infections.[17] The disturbing rate at which antibiotic-resistant strains are emerging necessitates the need for the development of new therapeutic strategies.[18]

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† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR scans and biofilm inhibition and dispersion and growth curves analyses. See DOI: 10.1039/c0ob00925c

While the threat of bacterial biofilms is alarming,[19] it has been only within the last decade that compounds have been developed to combat surface-associated bacteria.[20,21] Part of the difficulty in treating biofilm-associated infections is caused by the protective lifestyle that biofilms confer to the bacteria which is absent when bacteria are in planktonic form.[22,23]

Quorom sensing (QS) has been shown to be involved in biofilm development.[24] Baceterial cells communicate through this QS prcoess which is mediated by organic compounds secreted by the bacteria.[25] Most biofilm-modulating small molecules that have been reported have targeted quorum sensing machinery (Fig. 1).[26–28] Examples of such compounds include acylated homoserine lactones (AHL) and AHL mimics,[29,30] halogenated furanones,[31] and cyclic peptide derivatives.[32]

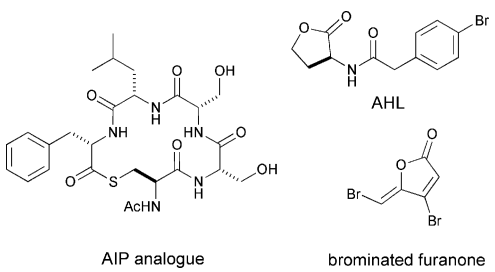


Fig. 1 Examples of compounds that interfere with quorum sensing.

Our group has turned to marine alkaloids for inspiration in designing compounds that have potential in modulating biofilms.[33,34] More specifically, we have focused on developing compound libraries that resemble bromoageliferin and oroidin, sponge metabolites that have been shown to inhibit biofilm formation and bacterial growth of the marine bacterium, *Rhodospirillum salexigens* (Fig. 2a).[20,35] We have shown that by retaining the 2-aminoimidazole (2-AI) moiety, simple synthetic derivatives are effective at inhibiting and dispersing bacterial and fungal biofilms.[36–39]

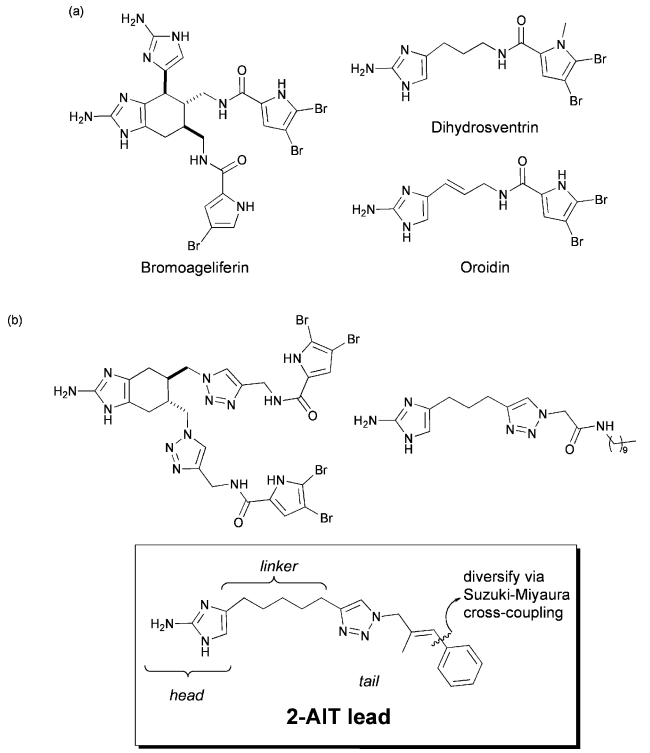


Fig. 2 (a) Anti-biofilm compounds from the oroidin family of alkaloids; (b) examples of 2-AIT analogues synthesized in our labs.

In our efforts to identify and develop novel 2-AI structures with antibiofilm activity, we have developed several classes of 2-aminoimidazole/triazole (2-AIT) libraries (Fig. 2b).[40,41] The lead 2-AIT from these libraries demonstrated biofilm inhibition and dispersal activities across bacterial order, class and phylum.[40] In another application, the lead 2-AIT was shown to work in concert with photodynamic inactivation to reduce biofilm mass population of *A. baumannii*,[42] as well as suppress antibiotic resistance in planktonic bacterial cultures.[43] Encouraged by the improved anti-pathogenic properties of these 2-AIT scaffolds, we have sought to synthesize 2-AIT analogues based on this lead 2-AIT structure and evaluate them for biofilm modulatory properties. In this paper, we report the chemical synthesis of such analogues and their antibiofilm and antibacterial activities against *A. baumannii* and MRSA.

Results and Discussion

Our design of 2-AIT analogues was focused on the structural modification of the aromatic appendage of the 2-AIT lead compound. This approach was driven by previous results from our studies suggesting that: (i) the 2-AI moiety is, at least in part, responsible for the observed antibiofilm activity; and (ii) a five carbon spacer for the linker is optimum for antibiofilm activity. Consequently, we have employed Suzuki–Miyaura coupling to introduce diversity in these structures.[44]

The requisite vinyl halide **2** was prepared *via* [3+2] cycloaddition of 2-AI-alkyne **3** and allyic azide **4** (Fig. 3). Compound **4** was accessed from allylic alcohol **5** (Scheme 1a).[45] The 2-AI precursor **7** was constructed *via* cyclization of α-bromoketone **6** and Boc-guanidine.[46–48] In contrast with the original synthesis of the lead

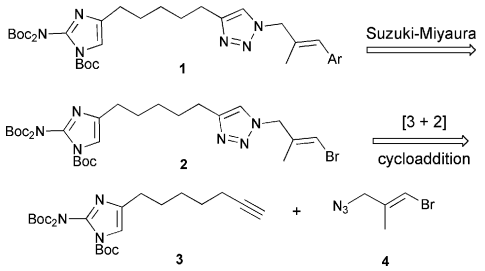
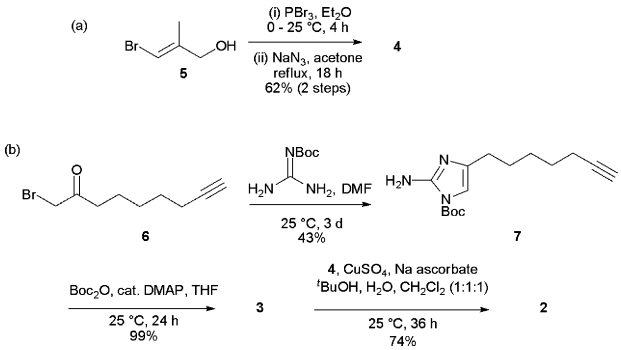


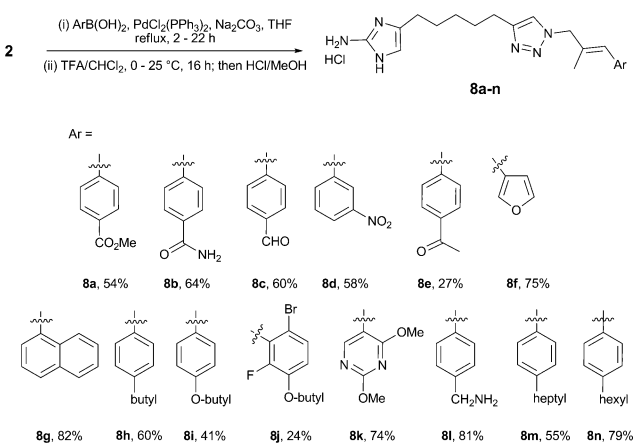
Fig. 3 Retrosynthetic approach to derivatizing the tail end of the lead 2-AIT *via* Suzuki–Miyaura cross-coupling.



Scheme 1 (a) Synthesis of allylic azide **4**; (b) synthesis of vinyl bromide **2**.

compound, this methodology, avoided both the use of Na/Hg and saturated NH_3–MeOH column chromatography[40] Cycloaddition of **7** with **4**, however, resulted in, disappointingly, low yields. This was circumvented by exhaustive Boc-protection of the exocyclic amine (Scheme 1b). The resulting tri-Boc-protected 2-AI-alkyne **3** enabled us to conduct the copper-catalyzed [3 + 2] cycloaddition on a larger scale, with higher yields, to furnish the corresponding vinyl bromide **2** needed for Suzuki–Miyaura couplings.

Compound **2** was reacted with various boronic acids using standard Suzuki–Miyaura conditions to furnish Boc-protected 2-AITs (Scheme 2). In general, most electron-rich boronic acids gave superior yields than those that are either electron-deficient or are sterically congested. Deprotection of the cross-coupling products



Scheme 2 Suzuki–Miyaura cross-couplings of vinyl bromide **2** and various boronic acids. Yields shown are after step (i). Yields after step (ii) are generally >95%.

Table 1 Biological activities of the most active compounds against *A. baumannii* and MRSA

	MIC *vs A. baumannii*			
Compound	μM	μg ml^{-1}	EC_{50}/μM *vs A. baumannii*	IC_{50}/μM *vs.* MRSA
8g	25	10.92	59.61 ± 7.28	9.86 ± 2.85
8h	6.25	2.77	53.90 ± 3.39	—[a]
8i	12.5	5.74	44.70 ± 2.25	8.55 ± 1.06
8j	12.5	6.95	49.38 ± 4.83	4.50 ± 0.53
8n	25	11.78	—[b]	—[b]

[a] Microbicidal. [b] Not active.

followed by salt exchange afforded the 2-AIT derivatives **8** as HCl salts.

To assess the biofilm modulating capabilities of the newly synthesized compounds, *A. baumannii* (ATCC 19606) was chosen as the initial bacteria for these studies. The impetus for chosing *A. baumannii* is that: (i) this strain forms robust biofilms on synthetic surfaces such as glass and polystyrene, and such synthetic surfaces are commonly used in production of medical devices;[15] and (ii) *A. baumannii* belongs to a cluster of *Acinetobacter* strains that are more prone to multi-drug resistance.[49] The compounds were first tested for biofilm inhibition at 100 μM using crystal violet (CV) reporter assay.[50] Compounds **8g–j** and **8n** were found to inhibit biofilm formation upwards of 94% at the initial concentration of 100 μM. Each of these compounds were then subjected to dose-response assays to determine IC_{50} values. Examining the data revealed an abrupt drop in activity at lower concentrations, indicating that the compounds may be acting as microbicides. Accordingly, the five compounds were subjected to microdilution susceptibility assays to find the minimum inhibitory concentration (MIC).[51] A 96-well microtiter plate with the top row having an initial concentration of 400 μM was subjected to 12 two-fold serial dilutions that resulted in a concentration range of 400–0.395 μM which were used for MIC determination.[52] After incubation at 37 °C for 16 h, the plates were visually inspected and the MICs determined as the lowest concentrations were no growth are observed. Compounds **8h**, **8i** and **8j** were found to have the most potent antibacterial activities from this library of 2-AITs (Table 1).

We then subjected these compounds to biofilm dispersion assays. Here, biofilms are allowed to form first before treatment with the compound. Initial biofilm dispersal activities at 200 μM revealed four compounds (**8g–j**) possessed the highest biofilm dispersal acitivities. Each of these compounds were then subjected to dose-response assays to determine the EC_{50} (effective concentration at which 50% of established biofilms are dispersed). Compound **8i** was found to be the most effective at dispersing preformed *A. baumannii* biofilms with an EC_{50} of 44.70 μM (Table 1). It is noteworthy to mention that compounds **8g–j** displayed better dispersal properties compared with the lead 2-AIT (EC_{50} = 130 μM).

All compounds were then tested against MRSA (ATCC BAA-44) biofilms in biofilm inhibition assays. As with *A. baumannii*, the same four 2-AITs were most active against MRSA. Follow up dose-response studies revealed IC_{50} values of 9.86, 8.55 and 4.50 μM for compounds **8g**, **8i** and **8j**, respectively. Subsequent analysis of both growth curves and colony counts indicated that **8g**, **8i** and **8j** were non-microbicidal at their respective IC_{50} concentrations. Compound **8h**, however, was found to be microbicidal and was determined to have an MIC of 6.25 μM (2.77 μg ml^{-1}) against MRSA.

Conclusions

In summary, we have reported an improved synthesis of the lead 2-AIT compound which is amenable to synthesizing a series of derivatives with antibiofilm as well as antibacterial properties. Derivatizing the aromatic appendage of the 2-AIT lead compound increases biofilm dispersal activities against *A. baumannii*. These results further support our recent report that subtle changes in the triazole appendage can have siginificant changes in biological activity of 2-AI-based antibiofilm agents.[55] These modifications also impart antibacterial capabilities to some of these 2-AITs. Analogues possessing a hydrophobic chain at the tail region proved to be the most active, with the n-butyl showing better bioactivities compared to the n-hexyl and n-heptyl. Compounds **8g–j** were shown to possess such antibacterial activities with biofilm dispersal capabilities against *A. baumannii*. Moreover, three (**8g**, **8i** and **8j**) of the aforementioned compounds were found to inhibit MRSA biofilm formation at low micromolar concentrations. These compounds demonstrate the ability of 2-AITs to control bacterial behavior and could potentially lead to new therapeutic strategies against bacterial infection.

Experimental

Chemistry

All chemicals and solvents used were purchased from commercial suppliers and used without further purification. Silica gel was used for column chromatography and was performed with 60-Å mesh standard grade silica gel from Sorbtech. ^{1}H and ^{13}C NMR spectra were performed using Varian Mercury 300 MHz and 400 MHz spectrometers. Compounds **8a–8n** are amorphous solids. Chemical shifts are reported in parts per million relative to $CDCl_3$ (δ 7.27), and DMSO-d_6 (δ 2.50) for ^{1}H NMR and relative to $CDCl_3$ (δ 77.0), and DMSO-d_6 (δ 39.99) for ^{13}C NMR. Abbreviations used for ^{1}H NMR splitting are as follows: s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet. High-resolution mass spectra were obtained at the North Carolina State Mass Spectrometry Laboratory for Biotechnology.

3-Bromo-2-methyl-prop-2-en-1-ol (5). Following literature procedure,[45] the title compound was obtained as colorless oil (8.71 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.23 (q, J = 1.38, 1H), 4.07 (s, 2H), 1.81 (d, J = 1.38, 3H). The proton NMR matched the one that was reported.

3-Azido-1-bromo-2-methyl-propene (4). A solution of **5** (3.02 g, 20 mmol) in 40 mL anhydrous Et_2O was cooled to 0 °C (ice bath). To this solution was added slowly, *via* syringe, PBr_3 (1.0 mL, 10 mmol). The colorless solution was stirred at 0 °C for 30 min, then at 25 °C for 4 h. At this time, the solution has turned yellow orange and the starting material has been consumed (TLC). The reaction was then quenched by slowly adding 70 mL aqueous K_2CO_3 solution (1 g/50 mL water). The resulting mixture was

extracted with 50 mL Et_2O. The layers were then separated and the organic layer dried over Na_2SO_4. Evaporation of the solvent under reduced pressure gave the allylic bromide intermediate as light yellow oil.

The foregoing allylic bromide intermediate (20 mmol, theoretical) was dissolved in 30 mL acetone. The resulting solution was then added to a stirring mixture of NaN_3 (6.5 g, 100 mmol) in 50 mL acetone and the mixture was refluxed under nitrogen atmosphere for 16 h. After this time, the mixture was allowed to cool to room temperature then 100 mL Et_2O and 80 mL water were added. The layers were separated and the water layer extracted with 40 mL Et_2O. The combined Et_2O layers were concentrated under reduced pressure and the residual mixture was taken in 100 mL CH_2Cl_2. The resulting solution was washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave the title compound as light yellow orange oil (2.188 g, 62%, 2 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.28 (q, J = 1.39, 1H), 3.79 (s, 2H), 1.87 (d, J = 1.39, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.4, 106.7, 56.8, 17.6. NMR data matched the one reported.[53]

1-Bromo-non-8-yn-2-one (6). A 200 mL ethylenediamine in a dry, 1 liter round bottom flask was cooled to 0 °C (ice bath). Sodium hydride (60% dispersion in mineral oil) (16 g, 397 mmol) was then added portion-wise into the flask. The white mixture was stirred under nitrogen atmosphere at 0 °C for 15 min, then at 25 °C for 1h. After this time, the mixture has turned to brownish purple. The mixture was then heated to 60 °C and was stirred at this temperature for 1 h. The temperature was then reduced to 40 °C before adding 3-octyn-1-ol (11.4 mL, 79.5 mmol). The mixture was stirred at 40 °C for 1.5 h before heating to 65 °C. The mixture was stirred at this temperature for 2 h then cooled to 0 °C. The reaction was quenched by adding 150 mL water and 150 mL 1 N HCl. The resulting mixture was extracted with 100 mL ether (3×) and 100 mL EtOAc (2×). The combined organic layers were washed with 200 mL brine and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the isomerized alkyne was purified *via* silica plug using 10% EtOAc/hexanes as solvent to give a colorless oil (8.62 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (t, J = 6.50, 2H), 2.20 (dt, J = 7.08, 2.65, 1H), 1.95 (dt, J = 2.65, 0.68, 2H), 1.62–1.51 (m, 4H), 1.48–1.38 (m, 4H). The proton NMR data matched the one reported from the literature.[54]

Jones reagent was prepared by adding 10 mL concentrated H_2SO_4 to a cold mixture of CrO_3 (3.1 g, 31.3 mmol) in 50 mL water. The reagent was cooled to 0 °C then a solution of the isomerized alkyne (3.3 g, 26.1 mmol) in 15 mL acetone was added dropwise *via* separatory funnel for 10 min. The reaction mixture was stirred at 0–25 °C for 18 h before diluting with 50 mL EtOAc. The layers were separated and the aqueous layer extracted with 20 mL EtOAc (3×). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue purified *via* column chromatography using 20% EtOAc/hexanes to give the acid as light yellow oil (2.22 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.70 (s, 1H), 2.35 (t, J = 7.48, 2H), 2.18 (dt, J = 6.82, 2.80, 2H), 1.93 (t, J = 2.80, 1H), 1.64 (quintet, J = 2.80, 2H), 1.57–1.41 (m, 4H); HRMS (ESI) calcd for $C_8H_{12}O_2$ (M + H) 141.0910, found 141.0906.

A solution of the acid-alkyne (3.2 g, 22.9 mmol) in anhydrous CH_2Cl_2 was cooled to 0 °C. Five drops of DMF were then added followed by dropwise addition of oxalyl chloride (6 mL, 71 mmol). The reaction was stirred at 0 °C for 1 h then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2/hexanes and the solvent removed under reduced pressure. This was done 3x before drying the acid chloride under high vacuum for 2 h.

The foregoing acid chloride was dissolved in 40 mL CH_2Cl_2 and the resulting solution was added dropwise, over 10 min, to a cooled solution of CH_2N_2 (66 mmol, 2.77 g) in 200 mL anhydrous Et_2O. The yellow orange turbid reaction was stirred at 0 °C for 1 h. After this time, 9 mL concentrated HBr was added dropwise (evolution of gas was observed) and stirring was continued for another 25 min. The reaction was quenched by adding 100 mL saturated $NaHCO_3$. The layers were separated and the organic layer was washed with 50 mL saturated $NaHCO_3$ (2×), 50 mL brine, before drying over Na_2SO_4. Evaporation of solvent under reduced pressure followed by column chromatography (10% EtOAc/hexanes) furnished the title compound as colorless oil (4.512 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 2H), 2.66 (t, J = 7.41, 2H), 2.18 (dt, J = 6.97, 2.71, 2H), 1.93 (t, J = 2.71, 1H), 1.68–1.57 (m, 2H), 1.56–1.49 (m, 2H), 1.45–1.39 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9, 84.2, 68.4, 39.5, 34.2, 28.0, 27.9, 23.2, 18.1; HRMS (ESI) calcd for $C_9H_{13}BrO$ (M + H) 217.0223, found 217.0228.

2-Amino-4-hept-6-ynyl-imidazole-1-carboxylic acid *tert*-butyl ester (7). To a solution of alpha-bromo ketone **6** (1.128 g, 5.2 mmol) in 12 mL DMF was added Boc-guanidine (2.5 g, 15.6 mmol) and NaI (0.78 g, 5.2 mmol). The reaction mixture was stirred for 3 days. The solvent was removed under reduced pressure and to the residue was added 100 mL EtOAc and 30 mL water. The layers were separated and the organic layer was washed with 30 mL water (3×). The combined aqueous washes were back extracted with 30 mL EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by column chromatography with 5% MeOH/CH_2Cl_2 gave the title compound as light yellow solid (0.619 g, 43%). NMR (300 MHz, $CDCl_3$) δ 6.51(s, 1H), 5.62 (s, 1H), 2.37 (t, J = 7.70, 2H), 2.20 (dt, J = 6.88, 2.76, 2H), 1.94 (t, J = 2.76), 1.65–1.60 (m, 2H), 1.56 (s, 9H), 1.54–1.42 (m, 4H); HRMS (ESI) calcd for $C_{15}H_{23}N_3O_2$ (M + H) 278.1863, found 278.1856.

2-[Bis(*tert*-butoxycarbonyl)amino]-4-(5-{1-[2-bromo-1-methyl-vinyl]-1*H*-1,2,3-triazol-4-yl}pentyl-1Himidazole-1-carboxylic acid methyl ester (2). To a solution of mono Boc-protected 2-AI 7 (0.750 g, 2.71 mmol) in 100 mL anhydrous THF was added DMAP (0.033 g, 0.271 mmol) and Boc anhydride (1.77 g, 8.13 mmol). The reaction was stirred under N_2 atmosphere at 25 °C for 48 h. After this time, TLC has indicated consumption of starting material. The reaction was diluted with 200 mL EtOAc and was washed with 50 mL 10% citric acid solution (2x). The organic layer was then dried over Na_2SO_4, concentrated under reduced pressure and dried under high vacuum to give the crude tri Boc-protected compound 3 which was carried over to the next step without further purification. Compound 3 (0.477 g, 1 mmol) and allylic azide 4 (0.180 g, 1 mmol) were dissolved in 5 mL CH_2Cl_2, 5 mL *tert*-BuOH and 5 mL water. The mixture was stirred vigorously then $CuSO_4$ (0.024 g, 0.15 mmol) was added followed by sodium ascorbate (0.059 g, 0.30 mmol). The reaction mixture was stirred at high speed for 5 h. After this time TLC indicated persistence of compound 3. Additional compound 4 (0.090 g, 0.5 mmol) was introduced and the reaction stirred for another 12 h. TLC after

this time indicated completion of the reaction. Ethyl acetate (50 mL) and water (50 mL) were added to the reaction mixture and the layers were separated. The organic layer was washed with 20 mL brine and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure, followed by purification *via* column chromatography afforded the title compound as thick viscous light yellow oil (0.481 g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) d 7.25 (s, 1H), 7.06 (s, 1H), 6.27 (q, J = 1.2, 1H), 4.90 (s, 2H), 2.68 (t, J = 7.8, 2H), 2.49 (t, J = 7.6, 2H), 1.73 (d, J = 1.2, 3H), 1.71–160 (m, 6H), 1.55 (s, 9H), 1.39 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) d 149.7, 149.1, 146.7, 140.5, 137.8, 136.5, 120.6, 114.1, 108.1, 85.7, 83.5, 55.9, 29.4, 28.8, 28.6, 28.4, 28.09, 28.07, 25.8, 17.4; HRMS (ESI) calcd for $C_{29}H_{45}BrN_6O_6$ (M + H) 653.2657, found 653.2638.

General Procedure for Suzuki-Miyaura Couplings and Deprotection. 4-(3-{4-[5-(2-Amino-1*H*-imidazol-4-yl)-pentyl]-[1,2,3]triazol-1-yl}-2-methyl-propenyl)-benzoic acid methyl ester hydrochloride (8a). A 20 mL vial was charged with vinyl bromide **2** (0.314 g, 0.480 mmol) and 4-methoxycarbonylphenylboronic acid (0.112 g, 0.62 mmol). The vial was flushed with nitrogen and 5 mL THF was added. $PdCl_2(PPh_3)_2$ (0.017 g, 0.024 mmol) and 0.72 mL 2 M Na_2CO_3 (1.44 mmol) were then added and the vial sealed with rubber septum. The reaction mixture was sonicated for 10 s and then refluxed for 2 h. TLC after this time indicated consumption of starting material. The reaction mixture was transferred into a separatory funnel and 30 mL EtOAc was added. The reaction was washed with 10 mL water and the organic layer was dried over Na_2SO_4. Evaporation of the solvent under reduced pressure followed by column chromatography (40% EtOAc/hexanes) gave the protected precursor as yellow oil (0.184 g, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 8.4, 2H), 7.33 (s, 1H), 7.3 (d, J = 8.4, 2H), 7.08 (s, 1H), 6.47 (s, 1H), 5.03 (s, 2H), 3.92 (s, 3H), 2.92 (t, J = 7.6, 2H) 2.52 (t, J = 7.4, 2H), 1.83 (d, J = 1.3, 3H), 1.75–1.64 (m, 6H), 1.57 (s, 9H), 1.41 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 149.8, 149.1, 146.7, 141.3, 140.5, 137.8, 134.8, 129.8, 129.1, 129.0, 128.8, 120.7, 114.1, 85.7, 83.5, 58.4, 52.3, 29.4, 28.9, 28.7, 28.4, 28.2, 28.1, 28.0, 27.9, 25.9, 16.0.

The protected cross-coupling product was taken up in 4.2 mL CH_2Cl_2 and then the solution was cooled to 0 °C. TFA (1.8 mL) was then added dropwise and the reaction was stirred from 0–25 °C for 18 h. After this time the solvent was evaporated under reduced pressure and the residue was taken up in CH_2Cl_2/hexanes and was concentrated under reduced pressure. This was done three times, after which the residue was dried under vacuum for 2 h. The foregoing TFA salt was removed from vacuum then 5 mL MeOH and a drop of concentrated HCl were added. The solution was concentrated under reduced pressure and then redissolved in 5 mL MeOH. The resulting solution was passed through a Whatman 0.2 micron polypropylene filter, concentrated under reduced pressure then dried under high vacuum to give the title compound as viscous yellow oil (0.110 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 11.69 (s, 1H), 7.90 (d, J = 8.1, 2H), 7.91, (s, 1H), 7.40 (d, J = 8.1, 2H), 6.83 (bs, 2H),6.50 (s, 1H), 6.47 (s, 1H), 5.06 (s, 2H), 3.81 (s, 3H), 2.60 (t, J = 7.6, 2H), 2.36 (t, J = 7.4, 2H), 1.73 (s, 3H), 1.59 (quintet, J = 7.2, 2H), 1.52 (quintet, J = 7.4, 2H), 1.31–1.24 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.6, 147.6, 147.4, 142.0, 136.3, 129.9, 129.7, 128.5, 127.9, 127.4, 123.1, 109.1, 57.8, 52.8, 29.2, 28.5, 28.0, 25.4, 24.5, 16.3; HRMS (ESI) calcd for $C_{22}H_{29}N_6O_2$ (M + H)$^+$ 409.2347, found 409.2346.

4-(3-{4-[5-(2-Amino-1*H*-imidazol-4-yl)-pentyl]-[1,2,3]triazol-1-yl}-2-methyl-propenyl)-benzamide hydrochloride (8b). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-aminocarbonylphenylboronic acid to furnish the protected precursor as yellow oil (0.231 g, 64%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.1, 2H), 7.344 (d, J = 8.1, 2H), 7.34 (s, 1H), 7.08 (s, 1H), 6.46 (s, 1H), 6.20 (bs, 1H), 5.67 (bs, 1H), 5.03, (s, 2H), 2.73 (t, J = 7.7, 2H), 2.51 (t, J = 7.4, 2H), 1.83 (s, 3H), 1.76–1.64 (m, 6H), 1.57 (s, 9H), 1.41 (s, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 149.5, 148.8, 146.5, 140.3, 140.2, 137.5, 134.3, 131.8, 129.1, 128.4, 127.4, 120.6, 113.9, 85.5, 83.3, 58.1, 29.2, 28.6, 28.4, 27.9, 27.86, 27.8, 25.6, 15.8.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.142 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 11.67 (s, 1H), 7.91 (s, 1H), 7.87 (d, J = 8.1, 2H), 7.35 (d, J = 8.1, 2H), 8.01 (bs, 2H), 6.54 (s, 1H), 6.48 (s, 1H), 5.07 (s, 1H), 2.63 (t, J = 7.6, 2H), 2.39 (d, J = 7.4, 2H),1.75 (s, 3H), 1.62 (quintet, J = 7.0, 2H), 1.55 (quintet, J = 7.4, 2H), 1.35–1.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.2, 147.6, 147.4, 140.0, 135.2, 133.2, 129.2, 128.24, 128.2, 127.4, 1, 123.0, 109.1, 57.8, 29.2, 28.5, 28.0, 25.5, 24.6, 16.3; HRMS (ESI) calcd for $C_{21}H_{28}N_7O_2$ (M + H)$^+$ 394.235, found 394.2357.

4-(3-{4-[5-(2-Amino-1*H*-imidazol-4-yl)-pentyl]-[1,2,3]triazol-1-yl}-2-methyl-propenyl)-benzaldehyde hydrochloride (8c). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-formylphenylboronic acid to furnish the protected precursor as yellow oil (0.219 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 1H), 7.82 (d, J = 8.0, 2H), 7.38 (d, J = 8.3, 2H), 7.33 (s, 1H), 7.04 (s, 1H), 6.43 (s, 1H), 5.00 (s, 2H), 2.68 (t, J = 7.8, 2H), 2.48 (t, J = 7.5, 2H), 1.80 (s, 3H), 1.71–1.58 (m, 6H), 1.53 (s, 9H), 1.36 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.5, 149.3, 148.7, 146.3, 142.6, 140.1, 137.4, 1335.3, 134.8, 129.5, 129.3, 128.1, 120.6, 113.7, 85.4, 83.1, 57.9, 29.0, 28.4, 28.3, 27.8, 27.72, 27.69, 25.2, 15.8.

After deprotection and salt exchange, the title compound was obtained as viscous yellow brown oil (0.133 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.21 (s, 1H), 11.73 (s, 1H), 7.92 (s, 1H), 7.29–7.72 (m, 4H), 6.53 (s, 1H), 6.45 (s, 1H), 5.05 (s, 2H), 2.63 (t, J = 7.6, 2H), 2.38 (t, J = 7.4, 2H), 1.72 (s, 3H), 1.61 (quintet, J = 7.3, 2H), 1.54 (quintet, J = 7.6, 2H), 1.33–1.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.5, 1474, 142.0, 133.1, 129.4, 129.2, 129.0, 128.2, 127.4, 127.1, 123.0, 109.1, 58.1, 29.2, 28.5, 28.0, 25.4, 24.6, 19.2; HRMS (ESI) calcd for $C_{21}H_{26}N_6O$ (M + H) 379.2241, found 379.2245

4-(5-{1-[2-Methyl-3-(3-nitro-phenyl)-allyl]-1*H*-[1,2,3]triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrocholoride (8d). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 3-nitrophenylboronic acid to furnish the protected precursor as yellow oil (0.157 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14–8.11 (m, 2H) 7.59–7.51 (m, 2H), 7.35 (s, 1H), 7.09 (s, 1H), 6.45 (s, 1H), 5.05 (s, 2H), 2.74 (t, J = 7.8, 2H), 2.52 (t, J = 7.6, 2H), 1.85 (d, J = 1.3, 3H), 1.77–1.65 (m, 6H), 1.58 (s, 9H), 1.42 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 148.9, 148.2, 146.5, 140.3, 138.1, 137.5, 135.6, 134.8, 129.3, 126.9, 123.6, 122.0, 120.7, 113.9, 85.5, 83.3, 57.8, 29.2, 28.6, 28.4, 27.9, 27.88, 27.85, 25.6, 15.7

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.97 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (s, 1H), 11.71 (s, 1H), 8.11 (d, J = 8.0, 1H),

8.07 (s, 1H), 7.98 (s, 1H), 7.74 (d, J = 7.8, 1H), 7.66 (t, J = 8.0, 1H), 7.25 (bs, 2H), 6.57 (s, 1H), 6.52 (s, 1H), 5.11 (s, 2H), 2.64 (t, J = 7.4, 2H), 2.39 (t, J = 7.2, 2H), 1.75 (s, 3H), 1.62 (quintet, J = 7.3, 2H), 1.55 (quintet, J = 7.4, 2H), 1.34–1.29 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.5, 147.5, 147.4, 138.8, 136.6, 135.9, 130.6, 127.4, 127.0, 123.7, 123.2, 122.4, 109.0, 57.7, 29.1, 28.5, 28.0, 25.4, 24.5, 16.1; HRMS (ESI) calcd for $C_{20}H_{26}N_7O_2$ (M + H)$^+$ 396.2142, found 396.2139.

1-[4-(3-{4-[5-(2-Amino-1*H*-imidazol-4-yl)-pentyl]-[1,2,3]triazol-1-yl}-2-methyl-propenyl)-phenyl]-ethanone hydrochloride (8e). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-acetylphenylboronic acid to furnish the protected precursor as yellow oil (0.070 g, 27%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.4, 2H), 7.36 (d, J = 8.1, 2H), 7.34 (s, 1H), 7.08 (s, 1H), 6.47 (s, 1H), 5.03 (s, 2H), 2.73 (t, J = 7.7, 2H), 2.52 (t, J = 7.0, 2H), 1.84 (s, 3H), 1.76–1.64 (m, 6H), 1.57 (s, 9H), 1.42 (s, 18H);^{13}C NMR (100 MHz, $CDCl_3$) δ 197.5, 149.5, 148.8, 146.5, 141.3, 140.3, 137.5, 135.6, 134.7, 129.0, 128.4, 128.3, 120.6, 113.9, 85.5, 83.3, 58.2, 29.2, 28.6, 28.4, 27.9, 27.87, 27.8, 26.6, 25.6, 15.8.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.043 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 11.64 (s, 1H), 7.95 (d, J = 8.2, 2H), 7.92 (s, 1H), 7.42 (d, J = 8.2, 2H), 7.31 (bs, 2H), 6.54 (s, 1H), 6.50 (s, 1H), 5.01 (s, 2H), 2.63 (t, J = 7.6, 2H), 2.34 (t, J = 7.4, 2H), 1.76 (s, 3H), 1.62 (quintet, J = 7.6, 2H), 1.55 (quintet, J = 7.8, 2H), 1.35–1.29 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 198.0, 147.7, 147.4, 141.9, 136.3, 135.8, 129.6, 129.0, 127.9, 127.5, 123.0, 109.1, 57.7, 29.2, 28.5, 28.0, 27.4, 25.5, 24.6, 16.4; HRMS (ESI) calcd for $C_{22}H_{29}N_6O$ (M + H)$^+$ 393.2397, found 393.2401.

4-{5-[1-(3-Furan-3-yl-2-methyl-allyl)-1*H*-[1,2,3]triazol-4-yl]-pentyl}-1*H*-imidazol-2-ylamine hydrochloride (8f). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 3-furanylboronic acid to furnish the protected precursor as yellow oil (0.139 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (s, 1H), 7.42 (t, J = 1.7, 1H), 7.29 (s, 1H), 7.08 (s, 1H), 6.48 (dd, J = 1.7, 0.8, 1H), 6.26 (s 1H), 4.98 (s, 2H), 2.71 (t, J = 8.0, 2H), 2.52 (t, J = 7.5, 2H), 1.81 (d, J = 1.3, 3H), 1.75–1.62 (m, 6H), 1.57 (s, 6H), 1.41 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 148.8, 146.5, 143.0, 141.5, 140.3, 137.5, 131.0, 121.8, 120.3, 120.1, 113.9, 110.6, 85.5, 83.3, 58.5, 29.2, 28.6, 28.4, 27.9, 27.87, 27.86, 25.7, 16.0.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.081 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 11.68 (s, 1H), 7.87, (s, 1H), 7.78 (s, 1H), 7.68(s, 1H), 7.34 (bs, 2H), 6.60 (s, 1H), 6.54(s, 1H), 6.23 (s, 1H), 5.00 (s, 1H), 2.62 (t, J = 7.6, 2H), 2.38 (t J = 7.4, 2H), 1.71 (s, 3H), 1.61 (quintet, J = 8.0, 2H), 1.56–1.50 (m, 2H), 1.32–1.26 (2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.5, 147.4, 144.1, 142.4, 132.2, 127.4, 122.9, 122.4, 119.6, 111.5, 109.1, 58.0, 29.2, 28.5, 28.0, 25.4, 24.5, 16.7; HRMS (ESI) calcd for $C_{18}H_{25}N_6O$ (M + H)$^+$ 341.2084, found 341.2093.

4-{5-[1-(2-Methyl-3-naphthalen-1-yl-allyl)-1*H*-[1,2,3]triazol-4-yl]-pentyl}-1*H*-imidazol-2-ylamine hydrochloride (8g). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 1-napthylboronic acid to furnish the protected precursor as yellow oil (0.177 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83–7.80 (m, 3H), 7.73 (s, 1H), 7.49–7.46 (m, 2H), 7.40 (dd, J = 8.5, 1.7, 1H), 7.37 (s, 1H), 7.08 (s, 1H), 6.64 (s, 1H), 5.06 (s, 2H), 2.73 (t, J = 7.9, 2H), 2.52 (t, J = 7.2, 2H), 1.88 (d, J = 1.3, 3H), 1.77–1.63 (m, 6H), 1.57 (s, 6H), 1.41 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 149.0, 146.7, 140.5, 137.8, 134.2, 133.4, 132.9, 132.6, 130.0, 128.2, 128.13, 128.07, 127.8, 127.2, 126.5, 126.3, 120.7, 114.1, 85.7, 83.5, 58.8, 29.5, 28.9, 28.7, 28.2, 28.11, 28.09, 25.9, 16.0.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.109 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.18 (s, 1H), 11.70 (s, 1H), 7.96 (s, 1H), 7.92–7.88 (m, 3H), 7.81 (s, 1H), 7.50–7.48 (m, 2H), 7.44, (d, J = 8.4, 1H), 7.33(bs, 2H), 6.62 (s, 1H), 6.53 (s, 1H), 5.12 (s, 2H), 2.654 (t, J = 7.4, 2H), 2.39 (t, J = 7.4, 2H), 1.82 (s, 3H), 1.63 (quintet, J = 7.0, 2H), 1.55 (quintet, J = 7.2, 2H), 1.35–1.30 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.6, 147.4, 134.7, 134.2, 133.5, 132.5, 129.0, 128.6, 128.4, 128.14, 128.1, 127.7, 127.4, 127.0, 126.8, 123.1, 109.1, 58.1, 29.2, 28.5, 28.0, 25.4, 24.6, 16.4; HRMS (ESI) calcd for $C_{24}H_{29}N_6$ (M + H)$^+$ 401.2448, found 401.2447.

4-(5-{1-[3-(4-Butyl-phenyl)-2-methyl-allyl]-1*H*-[1,2,3] triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrochloride 8h. Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-butylphenylboronic acid to furnish the protected precursor as yellow oil (0.062 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (s, 1H), 7.22–7.16 (m, 4H), 7.08 (s, 1H), 6.49 (s, 1H), 5.00 (s, 2H), 2.72 (t, J = 8.0, 2H), 2.61 (t, J = 7.6, 2H), 2.52 (t, J = 7.4, 2H), 1.81 (d, J = 1.2, 3H), 1.75–1.66 (m, 6H), 1.58 (s, 9H), 1.42 (s, 18H), 1.40–1.33 (m, 4 H), 0.93 (t, J = 7.3, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 148.7, 146.5, 142.1, 140.3, 137.5, 133.7, 131.4, 129.8, 128.8, 128.3, 120.3, 113.9, 85.4, 83.2, 58.7, 35.3, 33.5, 29.2, 28.6, 28.4, 27.9, 27.86, 27.8, 25.7, 22.3, 15.7, 13.9.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.101 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (s, 1H), 11.76 (s, 1H), 9.60 (bs, 2H), 8.0, 7.18–7.13 (m, 4H), 6.51 (s, 1H), 6.46 (s, 1H), 2.63 (t, J = 7.4, 2H), 2.52 (t, J = 7.5, 2H), 2.57 (t, J = 7.2, 2H), 1.71 (s, 3H), 1.61 (quintet, J = 7.3, 2H), 1.57–1.46 (m, 4H), 1.31–1.22 (m, 4H), 0.84 (t, J = 7.3, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.3, 146.9, 141.7, 134.2, 132.2, 129.2, 129.17, 128.7, 127.1, 123.3, 108.8, 58.3, 35.0, 33.5, 28.3, 28.2, 27.7, 25.0, 24.3, 22.2, 16.0, 14.2; HRMS (ESI) calcd for $C_{24}H_{35}N_6$ (M + H)$^+$ 407.2918, found 407.2918.

4-(5-{1-[3-(4-Butoxy-phenyl)-2-methyl-allyl]-[1,2,3] triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrochloride (8i). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-butoxyphenylboronic acid to furnish the protected precursor as yellow oil (0.091 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.27–7.23 (m, 2H), 7.08 (s, 1H), 6.85 (d, J = 7.1, 1H), 6.81–6.80 (m, 2H), 6.46 (s, 1H), 5.00 (s, 2H), 3.96(t, J = 6.5, 2), 2.72 (t, J = 7.8, 2H), 2.52 (t, J = 7.6, 2H), 1.81 (d, J = 1.3, 3H), 1.79–1.64 (m, 6H), 1.58 (s, 9H), 1.52–1.47 (m, 4H), 1.41 (s, 18H), 0.98 (t, J = 7.5, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 149.5, 148.8, 140.3, 137.7, 132.4, 129.7, 129.2, 121.2, 120.4, 115.1, 113.9, 113.2, 85.5, 83.3, 67.6, 58.5, 31.3, 29.2, 28.6, 28.4, 27.9, 27.86, 27.8, 25.7, 19.2, 15.7, 13.8.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.057 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.24 (s, 1H), 11.74 (s, 1H), 7.97 (s, 1H), 7.26–7.22 (m, 2H), 6.92 (bs, 2H), 6.84–6.79 (m, 2H), 6,52 (s, 1H), 6.44 (s, 1H), 5.05 (s, 2H), 3.94 (t, J = 6.2, 2H), 2.63 (t, J = 7.2, 2H), 2.38

(t, J = 7.0, 2H), 1.72 (s, 3H), 1.69–1.60 (m, 4H), 1.57–1.51 (m, 2H), 1.40 (q, J = 7.2, 2H), 1.34–1.26 (m, 2H), 0.90 (t, J = 7.2, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.4, 147.3, 133.6, 130.0, 129.2, 127.3, 123.3, 121.7, 115.4, 113.8, 109.0, 67.7, 58.2, 31.4, 29.11, 28.5, 28.0, 25.3, 24.6, 19.4, 16.3, 14.4; HRMS (ESI) calcd for C24H35N6O (M + H)$^+$ 423.2867, found 423.2868.

4-(5-{1-[3-(2-Bromo-4-butoxy-6-fluoro-phenyl)-2-methyl-allyl]-1*H*-[1,2,3]triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrochloride (8j). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 2-bromo-4-butoxy-5-fluorophenylboronic acid to furnish the protected precursor as yellow oil (0.068 g, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 7.26 (dd, J = 8.8, 1.7, 1H), 7.07 (s, 1H), 6.80 (t, J = 8.8, 1H), 6.16 (s, 1H), 5.05 (s, 2H), 4.00 (t, J = 6.7, 2H), 2.70 (t, J = 7.6, 2H), 2.50 (t, J = 7.3, 2H), 1.82–1.75 (m, 2H), 1.73–1.56 (m, 4H), 1.56 (s, 9H), 1.53 (s, 3H), 1.51–1.43 (4H), 1.40 (s, 18H), 0.96 (t, J = 7.4, 3H);^{13}C NMR (100 MHz, $CDCl_3$) δ 151.2, 149.7, 149.1, 148.7, 147.2, 146.7, 140.5, 137.8, 137.4, 127.5, 126.0, 123.2, 120.6, 114.8, 114.1, 85.7, 83.5, 69.6, 57.4, 31.3, 29.5, 28.8, 28.6, 28.2, 28.1, 25.9, 19.3, 16.4, 16.36, 14.0.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.046 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 11.70 (s, 1H), 7.85 (s, 1H), 7.4 (d, J = 8.5, 1H), 7.31 (bs, 2H), 7.09 (t, J = 8.5, 1H), 6.52 (s, 1H), 6.05 (s, 1H), 5.14, (s, 2H), 4.02 (t, J = 6.0, 2H), 2.64 (t, J = 7.2, 2H), 2.38 (t, J = 7.0, 2H), 1.68 (quintet, J = 7.1, 2H), 1.65–1.53 (m, 4H), 1.42 (s, 3H), 1.41–1.38 (m, 2H), 1.35–1.26 (m, 2H), 0.90 (t, J = 7.2, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.6, 147.4, 147.1, 138.7, 128.3, 128.7, 127.4, 126.0, 122.9, 121.5, 115.8, 113.9, 109.1, 69.3, 56.3, 31.2, 29.2, 28.5, 28.0, 25.4, 24.6, 19.3, 16.8, 14.3; HRMS (ESI) calcd for $C_{24}H_{33}BrFN_6O$ (M + H)$^+$ 519.1878, found 519.1881.

4-(5-{1-[3-(2,4-Dimethoxy-pyrimidin-5-yl)-2-methyl-allyl]-1*H*-[1,2,3]triazol-4-yl}-pentyl)-1*H*-imidazol-2 -ylamine hydrochloride (8k). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 2,4-dimethoxy-5-pyrimidinylboronic acid to furnish the protected precursor as yellow oil (0.148 g, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 1H), 8.07 (s, 1H), 7.28 (s, 1H), 7.01 (s, 1H), 6.22 (s, 1H), 4.95 (2, H), 3.93 (s, 3H), 3.927 (s, 3H), 2.65 (t, J = 7.9, 2H), 2.45 (t, J = 7.5, 2H), 1.67 (d, J = 1.3, 3H), 1.65–157 (m, 6H), 1.50 (s, 9H), 1.34 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 164.1, 157.2, 149.3, 148.5, 146.3, 140.1, 137.4, 133.7, 120.3, 120.0, 113.7, 111.1, 85.3, 83.1, 57.8, 54.6, 53.9, 29.0, 28.4, 28.2, 28.0, 27.7, 27.65, 25.5, 15.6.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.092 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (s, 1H), 11.66 (s1H), 8.23 (s, 1H), 7.88 (s, 1H), 7.31 (bs, 2H), 6.34 (s, 1H), 6.17 (s, 1H), 5.07 (s, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 2.63 (t, J = 7.2, 2H), 2.39 (t, J = 7.2, 2H), 1.67 (s, 3H), 1.65–1.58 (m, 2H), 1.55 (quintet, J = 7.4, 2H), 1.34–1.27 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.2, 158.8, 157.6, 147.5, 147.4, 135.6, 127.5, 123.1, 119.1, 111.8, 109.1, 57.3, 55.4, 54.8, 29.2, 28.5, 28.0, 25.4, 24.5, 16.3; HRMS (ESI) calcd for $C_{20}H_{29}N_8O_2$ (M + H)$^+$ 413.2408, found 413.2412.

4-(5-{1-[3-(4-Aminomethyl-phenyl)-2-methyl-allyl]-1*H*-[1,2,3]-triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrochloride (8l). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-{[*N*-(*tert*-butoxycarbonyl)amino]-methyl}phenylboronic acid to furnish the protected precursor as yellow oil (0.163 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.29–7.23 (m, 4H), 7.08 (s, 1H), 6.41 (s, 1H), 5.00 (s, 2H), 4.87 (bs, 1H), 4.32 (d, J = 5.6, 2H), 2.72 (t, J = 7.6, 2H), 2.52 (t, J = 7.4, 2H), 1.80 (d, J = 1.2, 3H), 1.71–1.64 (m, 6H), 1.59 (s, 9H), 1.49 (s, 9H), 1.41 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 148.8, 146.5, 140.3, 137.9, 137.5, 135.5, 132.3, 129.3, 129.1, 127.4, 127.1, 120.4, 113.9, 85,5, 83.3, 83.2, 58.5, 44.3, 29.7, 29.2, 28.7, 28.4, 27.9, 27.8, 25.7, 15.6, 14.2.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.099 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 11.69 (s1H), 8.53 (bs, 2H), 7.91 (s, 1H), 7.47 (d, J = 8.1, 2H), 7.31 (d, J = 8.1, 2H), 6.53 (s, 1H), 6.46 (s, 1H), 5.06 (s, 2H), 4.02 (d, J = 6.0, 2H), 2.63 (t, J = 7.5, 2H), 2.39 (t, J = 7.5, 2H), 1.73 (s, 3H), 1.62 (quintet, J = 7.6, 2H), 1.55 (quintet, J = 7.4, 2H), 1.34–1.29 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.3, 147.1, 136.9, 134.0, 133.1, 129.3, 129.2, 128.0, 127.1, 122.7, 108.8, 57.5, 42.2, 28.9, 28.2, 27.7, 25.2, 24.3, 15.9; HRMS (ESI) calcd for $C_{21}H_{30}N_7$ (M + H)$^+$ 380.2557, found 380.2558.

4-(5-{1-[3-(4-Heptyl-phenyl)-2-methyl-allyl]-1*H*-[1,2,3]triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrochloride (8m). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-heptylphenylboronic acid to furnish the protected precursor as yellow oil (0.058 g, 55%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.22–7.14 (m, 4H), 7.08 (s, 1H), 6.48 (s, 1H), 5.00 (s, 2H), 2.71 (t, J = 7.7, 2H), 2.59 (t, J = 7.8, 2H), 2.51 (t, J = 7.5, 2H), 1.81 (d, J = 1.3, 3H), 1.75–1.60 (m, 6H), 1.57 (s, 9H), 1.41 (s, 18H), 1.36–1.19 (m, 10H), 0.87 (t, J = 7.0, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 148.9, 146.7, 142.4, 140.5, 137.8, 133.9, 131.5, 130.2, 129.1, 128.6, 120.7, 114.1, 85.8, 83.6, 59.0, 35.9, 32.0, 31.6, 29.5, 29.4, 29.38, 28.8, 28.6, 28.1, 28.08, 28.07, 25.8, 22.9, 15.9, 14.3.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.037 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 11.64 (s1H), 7.88 (s, 1H), 7.31 (bs, 2H), 7.20–7.16 (m, 4H), 6.54 (s, 1H), 6.43 (s, 1H), 5.03 (s, 2H), 2.62 (t, J = 7.3, 2H), 2.56–2.52 (m, 2H), 2.39 (t, J = 6.9, 2H), 1.72 (s, 3H), 1.61 (quintet, J = 7.4, 2H), 1.58–1.51 (m, 4H), 1.32–1.23 (m, 8H), 0.84 (t, J = 6.7,3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.6, 147.4, 141.8, 134.5, 132.8, 129.4, 129.0, 128.9, 127.5, 122.8, 109.1, 58.1, 35.5, 31.9, 31.6, 29.3, 29.23, 29.2, 28.5, 28.0, 25.5, 24.6, 22.8, 16.2, 14.6; HRMS (ESI) calcd for $C_{27}H_{41}N_6$ (M + H)$^+$ 449.3387, found 449.3398.

4-(5-{1-[3-(4-Hexyl-phenyl)-2-methyl-allyl]-1*H*-[1,2,3]triazol-4-yl}-pentyl)-1*H*-imidazol-2-ylamine hydrochloride (8n). Following the procedure for **8a**, tri Boc-protected compound **2** was coupled with 4-hexylphenylboronic acid to furnish the protected precursor as yellow oil (0.209 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 1H), 7.22–7.16 (m, 4H), 7.08 (s, 1H), 6.49 (s, 1H), 5.00 (s, 2H), 2.71 (t, J = 7.6, 2H), 2.60 (t, J = 7.8, 2H), 2.52 (t, J = 7.4, 2H), 1.81 (d, J = 1.3), 1.74–1.64 (m, 6H), 1.58 (s, 9H), 1.41 (s, 18H), 1.39–1.26 (m, 8H), 0.88 (t, J = 6.9, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 149.0, 146.7, 142.4, 140.5, 134.0, 131.6, 130.1, 129.5, 129.1, 128.6, 120.6, 114.1, 85.7, 83.5, 59.0, 35.9, 32.0, 31.9, 31.6, 29.5, 28,9, 28.7, 28.2, 28.11, 28.0, 25.9, 22.8, 15.9, 14.3.

After deprotection and salt exchange, the title compound was obtained as viscous yellow oil (0.132 g, 99%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.14 (s, 1H), 11.67 (s, 1H), 7.89 (s, 1H), 7.31 (bs, 2H), 7.20–7.16 (m, 4H), 6.53 (s, 1H), 6.43(s, 1H), 5.03 (s, 2H), 2.62 (t, J = 7.6, 2H), 2.54 (t, J = 7.6, 2H), 2.38 (t, J = 7.4, 2H), 1.72 (s, 3H), 1.61 (quintet, J = 7.3, 2H), 1.58–1.51 (m, 4H), 1.36–1.22, (m, 6H), 0.84 (t, J = 6.7, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 147.6, 147.4 141.9, 134.5, 132.8, 129.4, 129.0, 128.9, 127.4, 122.9,109.1, 58.1, 35.5, 31.8, 31.5, 29.2, 29.0, 28.5, 28.0, 25.5, 24.6, 22.7, 16.2, 14.6; HRMS (ESI) calcd for $C_{26}H_{39}N_6$ $(M + H)^+$ 435.231, found 435.3227.

Biology

Biofilm Screening. Compounds **8a–n** were dissolved in DMSO, filtered through Whatman 13 mm polypropylene case filter with 0.2 micron pore sized and stored 100 mM solutions at –20 °C until needed for biological study. The DMSO used in these biofilm screens did not exceed 1% by volume and had no effect on bacterial growth or biofilm formation. *Acinetobacter baumannii* (ATCC 19606) and MRSA (BAA-44) were purchased from ATCC. *A. baumannii* was grown in LB media during the course of this study for inhibition assay and dispersion assays. MRSA was grown in Tryptic Soy Broth supplemented with 0.5% glucose for biofilm inhibition assays.

Biofilm Inhibition Assay. An overnight culture of bacterial strain was subcultured at an OD_{600} of 0.01 into the media used depending on bacterial strain. This was pipetted into test tubes along with a predetermined concentration of test compound. The contents of the test tubes were then poured into tilted Petri dishes and 100 mL of medium, bacteria and compound were then transferred into 96-well PVC microtiter plates. These microtiter plates were then covered with a plastic lid, wrapped in Saran™ wrap and incubated at either room temperature or 37 °C for 24 h. After incubation, the medium was discarded and the plates were thoroughly washed two or three times with water. The remaining biofilm was stained with 100 mL of a 0.1% crystal violet solution and allowed to sit at room temperature for 30 min. After 30 min, the crystal violet was discarded and washed thoroughly again with water. The remaining crystal violet which stained the biofilm on the inside of the wells was solubilized with 200 mL of 95% ethanol. The quantitation of biofilm formation was accomplished by transferring 125 mL of the ethanol solution into a polystyrene microtiter plate and reading the absorbance at 540 nm (A_{540}) using a Biotek ELX808 plate reader After subtracting the background from each well, a percent inhibition could be calculated by subtracting from a hundred, the ratio of the amount of crystal violet stain in the wells that contained compound by the amount of crystal violet stain in wells that contained bacteria only. Each concentration reported during the course of this study was repeated two to four times with each biofilm inhibition assay being done in 6 or 8 replicates each. The inhibition effectiveness was plotted to generate a 5 to 9 point curve of the percent inhibition of biofilm formation *versus* the concentration of compound tested. This curve was used to determine IC_{50} values.

Biofilm Dispersion Assay. Biofilm dispersion is similar to biofilm inhibition assay, but here bacterial biofilms are allowed to first form in 96-well PVC plates using a sub-cultured bacterial strain at an OD_{600} of 0.05. A 96-well microtiter plate was filled with 100 µL of inoculated medium and was incubated at 25 °C for 24 h. After this time, the medium was discarded and the plates were thoroughly washed two or three times with water. The plates were shaken off gently to remove excess water and then refilled with 110 µL of media with test compound at a pre-determined concentration. The plates were then covered with the lid and wrapped in Saran™ wrap and incubated at 37 °C for 24 h. After this time, the plate's contents were discarded, washed and stained with crystal violet as before (see biofilm inhibition assay above). Each concentration reported during the course of this study was repeated two to four times with each biofilm dispersion assay being done in 6 or 8 replicates each. The dispersion effectiveness was plotted to generate a 5 to 7 point curve of the percent inhibition of biofilm formation *versus* the concentration of test compound. This curve was used to determine EC_{50} values.

Microdilution susceptibility testing. All antibiotic susceptibility testing was completed with a starting inoculum of 5×10^5 CFU mL^{-1} according to NCCLS standards and incubated for 16–20 h at 37 °C. After this time bacterial growth was visually inspected and the lowest concentration at which no observable bacterial growth or turbidity was observed was considered to be the MIC value. Pellets that formed at the bottom of 96 well microtiter plates were considered growth and although there was essentially no turbidity, the pellet was considered bacterial growth in these studies. Microdilutions were made in the 96-well microtiter plates from a starting concentration of 400 µM with the addition of 200 µL to a predetermined well. From this, two-fold serial dilutions were made by transferring 100 µL of the initially treated well (of 400 µM) into the next well and mixing once using a multichannel pipette, this next well then contained 200 µL of bacterial, media and test 2-AIT compound at a concentration of 200 µM. The transfer was done in succession for a total of 12 two-fold serial dilutions giving a range of 400–3.91 µM for tested 2-AIT in 96 well microtiter plates. After the final dilution, the wells in this row then had 200 µL (of the 3.91 µM wells), 100 µL of which werer pipetted out and thrown away. The plates were then covered by a plastic lid and then were wrapped in Saran™ wrap and placed in a humidified chamber and incubated for 16–20 h at 37 °C. After the incubation time, the wells were observed for bacterial growth and MICs were determined accordingly (described above).

Growth Curves. An overnight culture of bacterial strain was subcultured into its respective media at an OD_{600} of 0.01. The inoculated media (3 ml) was added to two test tubes, one that would not be treated, to serve as a control for bacterial growth, and one that would be treated with 8 g, 8i or 8j at the respective IC_{50} value determined in biofilm inhibition assays. The test tubes were shaken while incubated and optical densities were taken after 2, 4, 6, 8 and 24 h. These experiments were done from two different overnight cultures from two different bacterial colonies in single replicate.

Acknowledgements

The authors would like to thank the North Carolina Biotechnology Center for funding this work.

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